The Influence of Substrate and Temperature on Biological Nitrogen Removal in Wastewater Treatment Systems

by

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Abstract

Previous evaluations have indicated that savings in operating costs of greater than 25% can be realized if denitrification is added to a system already achieving nitrification. Energy requirements are reduced because organics are consumed under both aerobic and anoxic conditions. Reduced expenditures for energy, and lower sludge handling and disposal costs due to decreased biosolids production make nitrogen removal an economically attractive proposition. A number of recent articles in the literature suggest nitrogen removal is not as well understood as it once was believed. Conflicting reports of sludge production rates for nitrogen removal systems and systems operating under low temperatures are particularly interesting because they call into question the current economic evaluations of nitrogen removal.

This research examined the influence of substrate and temperature on biological nitrogen removal. Proteins were chosen as model substrates because they account for approximately 28% of the COD in raw sewage. In this study, sequencing batch reactors (SBRs) achieving aerobic carbon removal and anoxic carbon removal were run in parallel to assess and compare the overall efficacy of the two processes. Areas of process performance considered were effluent quality and biosolids production.

It was found that aerobic and anoxic systems produced an effluent of acceptable quality. Biosolids production under anoxic conditions was found to be 24% and 32% higher than the aerobic reactors when operating at temperatures of 20 and 14 °C, respectively. Batch rate studies using cultures generated from the SBR examined the effect of using complex substrates. Proteins because of their high molecular weight require enzymatic degradation before the cell can use the substrate for growth. Batch testing proceeded with: one half of a stock culture sample being fed a protein hydrolysate (PH) while the remaining portion was fed a whole protein. Ovalbumin, α-casein and lactalbumin were chosen for further study on the basis of their molecular weight and physical properties.

Results show ovalbumin and α-casein were used at a rate comparable to that of the PH under many of the test conditions. Lactalbumin was metabolized at a much slower rate than the PH under both

aerobic and anoxic conditions and at temperatures of 14 and 20°C. The molecular weight of lactalbumin (18kd) is comparable to α-casein (22-25 kd) and is less than that of ovalbumin (45 kd).

Based on the results of this study it is proposed that the overall structure of the protein may be important in defining the rate of uptake and hydrolysis. Proteins containing a large number of cross-links may undergo uptake and hydrolysis very slowly while proteins with minimal cross-linking appear to be utilized more rapidly. The overall structure of the protein will play a role in determining the protein's solubility. Lactalbumin, an example of a cross-linked protein, forms settleable particles in solution. Ovalbumin and α-casein are soluble proteins with minimal cross-linking.

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I would also like to acknowledge three special people for the role they have played: Ljuba Simovic, Dr. Keith Murphy and Gerry Roeland. Ljuba has provided encouragement and advice for the last decade. It is with her encouragement that I undertook this degree. Keith posed the "what ifs" that developed into a quest for knowledge and led to this dissertation. It is with the love and patience of Gerry, my husband, that this dream has become reality.

To Keith, Ljuba and Gerry for giving me my wings.

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1. Introduction

There is a distinct possibility that the Municipal and Industrial Strategic Abatement Program, given past requirements, will require municipal sewage treatment plants (STP) to produce a non-toxic effluent. The effluent of many of Ontario's STPs is toxic to fish due to the presence of unionized ammonia (Beak Consultants Ltd. and Canviro Consultants Ltd., 1988). Due to this toxicity, it may become mandatory to include nitrification in the treatment train. The cost of the necessary upgrades was estimated at $1.4 billion by the Ontario Ministry of the Environment and Energy (1992$). Complete nitrogen removal can be achieved if denitrification is incorporated into the proposed treatment train. At this time, it would be prudent to assess the incremental costs of doing so and define the efficacy of the overall process.

The environmental benefits of achieving nitrogen removal cannot be disputed. Nitrogen discharged to receiving waters, either as ammonia nitrogen or nitrate nitrogen (NO_3-N), will cause accelerated rates of eutrophication (U.S. EPA, 1993). Nitrate present in drinking water at sufficiently high concentrations leads to methemoglobinemia (i.e., commonly referred to as "blue baby syndrome") and carcinogenesis (U.S. EPA, 1993). The U.S. Environmental Protection Agency (EPA) interim drinking standard for nitrate is 10 mg/L as NO_3-N (Bitton, 1994). In Ontario, contamination of drinking water is a secondary concern since many of the STPs discharge into rivers and the Great Lakes where significant dilution occurs. With increased urbanization, situations may arise where ground water is both recharged with STP effluent and used as a drinking water source. Under these circumstances, the presence of nitrate nitrogen in high concentrations would be cause for alarm.

Nitrogen removal systems have to accomplish nitrification and denitrification as well as producing an effluent low in organics and suspended solids. Removal of organics is carried out by heterotrophic organisms growing under anoxic and aerobic conditions. Heterotrophic growth in the presence of nitrate is defined as denitrification because nitrate nitrogen is consumed and nitrogen gas is produced. Nitrification is carried out by autotrophs that convert ammonia nitrogen into nitrate. These bacteria use dissolved carbon dioxide as a source of carbon for cell synthesis rather than using organics present in the wastewater.

Current evaluations have indicated that savings in operating costs of greater than 25% can be realized if denitrification is added to a system already achieving nitrification (McClintock *et al.*, 1988; Ip *et al.*, 1987). Reduced expenditures for energy, and lower sludge handling and disposal costs due to decreased sludge production make nitrogen removal an economically attractive proposition. The economic evaluation performed by McClintock *et al.* (1988) was based on sludge production being 25% less under anoxic conditions than it is under aerobic conditions. Energy requirements are reduced because organics are consumed partly under anoxic conditions.

The savings generated by decreases in sludge production will be dependent on the disposal method. In Southern Ontario, many municipalities (e.g. Hamilton, Burlington and Toronto) have to landfill or incinerate their stabilized sludge because the industrial component makes it unsuitable for land application. The Region of Waterloo is fortunate because the sludge produced by the Waterloo Sewage Treatment Plant is of good quality and can be land farmed. Sludge haulage accounts for 20% of the treatment plant operating budget (Schyff, 1996). If the approximately 55,000 m^3 of unconditioned digested sludge produced yearly (Schyff, 1996) had to be landfilled at a cost of $50/tonne (Waterloo, 1996) then, denitrification would become an even more attractive proposition.

A number of recent articles in the literature suggest that nitrogen removal is not as well understood as it was once believed. Conflicting reports of sludge production rates for nitrogen removal systems and systems operating under low temperatures are noteworthy (Oleszkiewicz and Berquist, 1988; Ketchen, 1994; McClintock *et al.*, 1988; Symth, 1994) because they call into question the current economic evaluations of nitrogen removal. By developing a better understanding of the underlying biotransformations, it may be possible to rationalize these conflicting reports.

The conceptual framework of the IAWQ Activated Sludge Model No. 2 (ASM2) (Gujer *et al.*, 1995), a mathematical model of bacterial growth in the activated sludge process, provides a good starting point. The model's merits have been widely debated in the literature but it is used extensively in the field of wastewater treatment. The model can describe organic removal under either aerobic or anoxic (i.e. denitrification) conditions, nitrification, and biological phosphorus removal. Within the conceptual framework of ASM2, heterotrophic metabolism includes: hydrolysis (enzymatic degradation of substrate outside of the

cell), growth (oxidation of substrate and concurrent generation of new microorganisms) and cell lysis (loss of metabolic activity, lysis of the cell wall and predation). Worthy of particular note is the exclusion of substrate uptake and storage in the list of significant biotransformations. It was decided that this process was essentially immediate in discussions held before the model's formulation (Dold *et al.*, 1980).

Nitrogen removal without doubt provides real environmental benefits. As real as those benefits are, it is unlikely that nitrogen removal will be implemented in Ontario unless it is economically feasible. The majority of projected cost savings are due to decreased sludge production. Conflicting reports of sludge production puts the current economic evaluations into question. Real gains in understanding the differences in sludge production rates can be made only if the "black box" is opened up and the underlying biotransformations are examined.

1.1. Overall Objective

The objectives of this research are to measure and evaluate rates of substrate removal obtained during denitrification and to compare them to equivalent rates for aerobic treatment operating under parallel conditions. This research will also assess the effects of substrate complexity and temperature on the rates of substrate and nitrate reduction and on biosolids production.

1.2. Principal Research Goals

1. To quantify and compare biosolids production rates for sequencing batch reactors (SBRs) achieving carbon removal under aerobic or alternately anoxic conditions as a function of temperature.
2. Determine if substrate uptake and hydrolysis is a rate limiting step under aerobic and anoxic conditions at both 14°C or 20 °C. Determine if the rate of hydrolysis is dependent on the molecular weight of the protein or its overall structure.
3. Evaluate acridine orange direct counts (AODC) as a method of enumerating the number of bacteria in a mixed microbial population. Using this technique, the fraction of reactor biosolids composed of cells will be determined as a function of temperature and electron acceptor.

4. Determine if there are any significant differences in the specific substrate utilization rates for aerobic and anoxic cultures based on electron equivalents. The impact of temperature on the utilization rate will be determined.
5. Determine if there are significant differences in the observed yield for aerobic and anoxic cultures under conditions of active substrate removal.
6. Compare the effluent quality of two different carbon removal systems (aerobic and anoxic) in the areas of effluent solids and COD. The effect of temperature on the consumptive ratio will also be quantified.

1.3. Commentary on Research Methodology

1.3.1. Choice of Substrate

This research focuses on the effect of temperature and the impact of protein complexity on process performance. Metabolism of proteins was chosen for further study for a number of reasons:

a) Proteins contribute 28% of the total COD of raw sewage (Raunkjer *et al.*, 1994).

b) Most proteins are slowly biodegradable substrates by virtue of their high molecular weight.

c) Overall protein as well as molecular weight could play a role in determining how they are metabolized.

d) Studying carbohydrate metabolism is less desirable because many carbohydrates such as cellulose are resistant to bacterial degradation and Ketchen (1994) and San Pedro *et al.* (1994) have already looked at aspects of carbohydrate metabolism using starch.

e) Lipids are extremely difficult to study because they may assume a micellar nature.

1.3.2. Experiment Types and Sequence

An overview of the experimental program is presented in Figure 1.1 and 1.2. For a denitrifying system, process performance was measured in terms of effluent quality, biosolids production and nitrate reduction. Biosolids production which is the summation of solids lost through intentional and unintentional wastage was of particular interest. An aerobic reactor achieving carbon removal was operated in parallel to the denitrifying reactor to provide a basis of comparison in the areas of effluent quality and biosolids production. Substrate uptake and hydrolysis and the kinetics and stoichiometry of growth will have an impact on biosolids production.

A denitrifying system was chosen in preference to a nitrogen removal system because its ecology is simpler and fewer biotransformations are occurring. A nitrogen removal system will have a mixed microbial population composed of heterotrophic organisms, which may be either facultative or obligate aerobes and autotrophic organisms. The contribution of each group to the total population will depend on process parameters such as sludge age, carbon to nitrogen loading, and hydraulic residence time (HRT). Such a system does not easily lend itself to studying the underlying biotransformations because of the possible interactions between autotrophs and heterotrophs and within the heterotrophic population itself. With a denitrifying system it is possible to quantify biosolids production and examine aspects of heterotrophic metabolism concurrently.

SBRs operating under aerobic and anoxic conditions were used to quantify biosolids production and provided conditioned mixed liquor needed subsequently for batch rate experiments. The SBRs were fed a sewage and protein mixture in a 1:2 volume ratio on a daily basis. Sewage was included in the daily feed to ensure that a diverse microbial population existed since using proteins as a sole substrate would have resulted in an enriched culture.

Substrate uptake and hydrolysis were chosen as biotransformations warranting further investigation. Slow rates of hydrolysis may lead to the accumulation of proteins and hence increased biosolids production rates. Rate studies examined the rate of hydrolysis as influenced by protein type, terminal electron acceptor and temperature.

Not all substrates require hydrolysis. If a biodegradable substrate is sufficiently simple, it does not have to be degraded into smaller fragments before its use as a growth substrate. These substrates are referred to as rapidly biodegradable while those requiring hydrolysis are classified as slowly biodegradable (Henze, 1992). While the two types of biodegradable substrates are clearly defined, in practice distinguishing between them is difficult. Many different protocols exist for differentiating the two components (Xu and Hasselblad, 1996; Ekama *et al.*, 1986; Mamais *et al.*, 1993).

Before proteins could be selected for this phase of the experimental program, an operational definition of a slowly biodegradable substrates was required. After considering the physiology of Gram-negative bacteria, biodegradable substrates greater than 1000 Da were operationally defined as slowly biodegradable. The initial expectation was that molecular weight would be correlated to the rate of hydrolysis.

As the experimental program evolved, this hypothesis was discounted. It became clear that even within the slowly biodegradable category, proteins required further classification. The dependency of hydrolysis on the overall shape and solubility of the protein was therefore investigated.

Growth kinetics impact biosolids production in much the same way as hydrolysis does. Even if the proteins are broken into small fragments, a fraction will remain as part of the floc structure if the cell does not metabolize the fragments as quickly as they are produced. If this is the case there still will be an increase in the biosolids production since undegraded organic material is accumulating within the floc.

The observed yield under conditions of active substrate removal was investigated because of the possible impact on biosolids production. Under conditions of active substrate removal, the observed yield will approach the true yield. This is because the maintenance energy demands are much less than the energy used for synthesis (Grady and Lim, 1977). On a theoretical basis, the growth yield of heterotrophic microorganisms using nitrate as a terminal electron acceptor was expected to be 77% or less of that realized under aerobic conditions because of electron transport system considerations (Payne, 1981). If this was the only consideration and all other factors were equal, then biosolids production would be higher for the aerobic reactor. An activated sludge system has a complex ecology with many species of microorganisms present and thus, the observed yield for the system will reflect the observed yields of all individual species of microorganisms present.

1.3.3. Biomass Measurements

AODC allows the total number of bacteria in the system to be enumerated. The RNA and DNA of the bacteria interact with acridine orange making the bacteria visible under fluorescent light of the correct wavelength. AODC is widely used in the areas of aquatic and soil microbiology. AODC for enumerating the bacterial population of activated sludge has been rarely used. In some circumstances but not process control it should offer significant advantages over MLVSS, a surrogate parameter, because only the bacterial component of the sludge floc will be enumerated. Non-biodegradable organic material, partially degraded material, higher life forms (e.g. protozoa, rotifers, and metazoa) and bacteria will be present in activated sludge. Rate data normalized to the total number of bacteria may be preferable to that normalized on MLVSS as it allows comparisons to be made between systems not operating under comparable SRT and carbon loadings. An

evaluation of AODC was carried out under the growth regimes of chronic starvation during SBR operation, active metabolism during the rate studies and declining growth.

The integration of the data and ideas derived through the experimental program and the literature review will provide insight into the effect of temperature on reaction rates, the extent of biosolids production; and the impact of substrate complexity on process performance as measured by substrate and nitrate removal. The knowledge gained from this work will also be used to propose modifications to the structure of the ASM2 (Gujer *et al.*, 1995).

Figure 1.1 Overview of Experimental Program

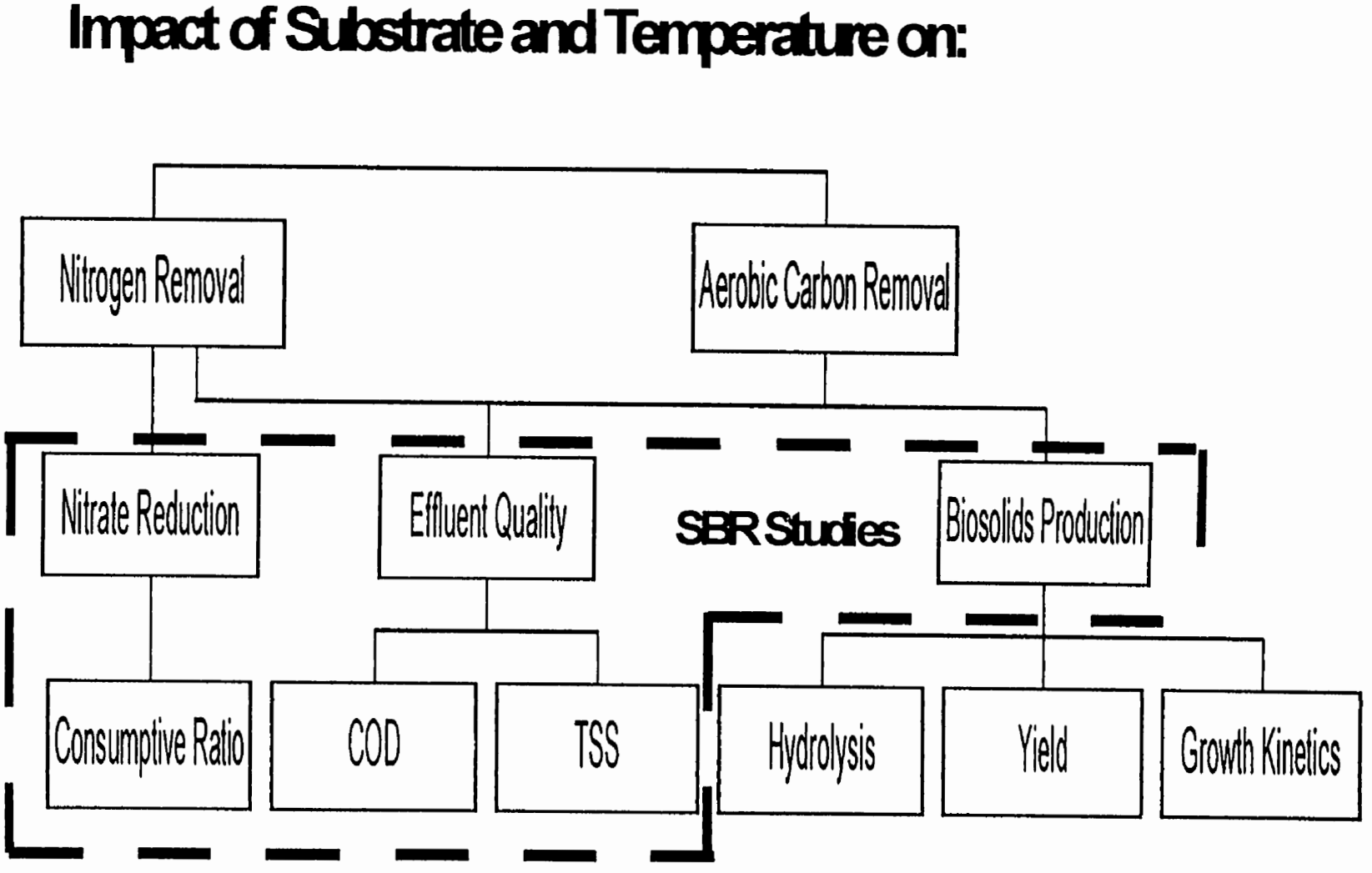
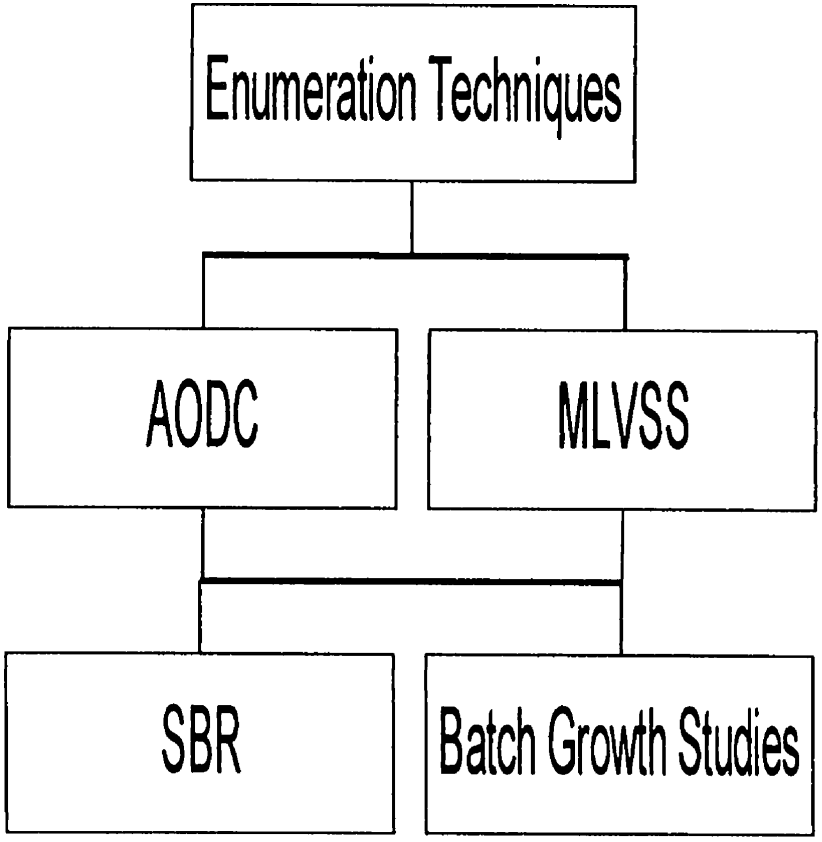


Figure 1.2 Overview of AODC Evaluation



2. Literature Review

2.1. Introduction

In 1987, a task group of the International Association on Water Pollution and Control Research (later renamed IAWQ) developed an activated sludge model, Activated Sludge Model 1 (ASM1). This model mathematically describes nitrification, denitrification, substrate removal and biomass growth. According to Gujer *et al.* (1995), the model was brought forth because a good understanding of nitrification and denitrification existed, but a comprehensive model describing these processes did not. Because the model was internationally recognized, it became widely used by both researchers and practicing engineers. As time progressed, biological phosphorous removal became more widely implemented. This led to Activated Sludge Model No. 2 (ASM2), which was based largely on ASM1, but incorporated biological phosphorous removal (Gujer *et al.*, 1995). Introduction of the model has given engineers worldwide the opportunity to describe the activated sludge process using a common framework.

The merits of ASM2 and its predecessor ASM1 have been extensively debated in the past and will be the subject of continuing discussion. At this point, the concepts incorporated in the model represent the current consensus of the engineering community. Some of the ideas integral to the model are the focus of on-going research since they stand now without rigorous proof.

Real gains in understanding the differences in biosolids production rates in activated sludge systems, particularly under anoxic conditions and low temperatures, can be made only if the 'black box' is opened and the underlying biotransformations are examined. Those biotransformations are mathematically described in ASM2. Discussing the findings of this research using the language and concepts of ASM2 will make it easier to communicate the results.

In the first part of this Literature Review, the significant features and the depiction of heterotrophic metabolism under aerobic and anoxic conditions used for ASM2 will be stated. The second portion of the

Literature Review examines more closely the biology of the biotransformations depicted by ASM2. The last segment of this Review surveys the literature dealing specifically with nitrogen removal.

2.2. Activated Sludge Model No. 2 -A Conceptual Framework

ASM2 can mathematically describe heterotrophic growth under aerobic, anoxic and anaerobic conditions as well as the growth of autotrophs. Anoxic within the context of ASM2 refers to the absence of oxygen and the presence of nitrate. ASM1 has been used extensively because the interactions between various types of heterotrophic organisms and between heterotrophic and autotrophic organisms can be seen. Within the model's framework, the biotransformations mediated by heterotrophic organisms include: hydrolysis (enzymatic degradation of substrate outside of the cell by exoenzymes), growth (oxidation of substrate and an increased mass of microorganisms), fermentation and lysis. Worthy of particular note is the exclusion of substrate uptake and storage in the list of significant biotransformations. In the discussions leading up to the formulation of ASM1, it was believed that this process was essentially immediate and non-energy consuming; therefore, this process did not merit inclusion in the model (Dold *et al.*, 1980). Phosphorous-accumulating organisms, in addition to the above biotransformations, can take up phosphorus and internally store polyhydroxyalkanotes and polyhydroxybutyrates. Possible transformations for autotrophic organisms would include growth and lysis.

The IAWQ Task group that formulated ASM2, acknowledged that there were limitations and restrictions to the model (IAWQ Task Group, 1995). ASM2 will simulate activated sludge systems with solids retention times of up to approximately 30 d. It does not describe secondary clarification or anaerobic digestion. The requirement of a constant pH between 6.3-7.8 and unchanging rate coefficients and stoichiometry is typical of a structured model. Further restrictions and limitations placed on individual processes will be stated as the individual processes are described. The IAWQ Task group has identified two areas where there is the greatest need for research: fermentation and anaerobic hydrolysis.

2.2.1. Distinguishing Features of ASM2

a) Treatment of Carbonaceous and Nitrogenous Material

Reactions mediated by microbes are oxidation-reduction reactions that involve the transfer of electrons from a donor, which is generally the substrate being oxidized, to an acceptor (Grady and Lim, 1980). In reactions occurring under aerobic and anoxic conditions, the electron acceptors are oxygen and nitrate, respectively. Of the several methods available to quantify the amount of carbonaceous substrate present, the use of chemical oxygen demand (COD) is preferable. COD is the only link between electron equivalents in the organic substrate, with those of the biomass and the electron acceptor (Gaudy and Gaudy, 1971). Without this link, mathematically describing the behaviour of a process possibly existing under alternating aerobic, anoxic and anaerobic conditions would be difficult.

Carbonaceous material found in the influent can be classified as either non-biodegradable or biodegradable (Figure 2.1). Non-biodegradable organics pass through the activated sludge system unchanged while biodegradable organics are oxidized during the synthesis of microorganisms (Henze *et al.*, 1986). Non-biodegradable carbonaceous material depending on its physical properties will be either present in the reactor effluent or accumulate as biologically inert material within the reactor (IAWQ Task Group, 1995). In ASM2, biodegradable organics are defined by their fate. Non-biodegradable organics in the effluent are referred to as soluble. Those accumulated in the floc are referred to as particulate regardless of whether they are particulate or soluble organics adsorbed onto the floc.

In ASM1, biodegradable carbon was classified as either readily or slowly biodegradable (Ekama *et al.*, 1986). Slowly biodegradable substrates require degradation by enzymes external to the cell (i.e., hydrolysis) before becoming available growth substrates, whereas readily biodegradable substrates can be used directly by the cell. In ASM2, readily biodegradable substrates are further characterized as fermentable readily biodegradable substrate, and fermentation products considered to be acetate. Since fermentation is included in the biological processes, fermentation products must be modelled separately from other soluble organic materials. For stoichiometric computations, fermentation products are considered to be acetate even though a whole spectrum of fermentation products is possible.

Fermentable readily biodegradable substrate is generated by the hydrolysis of slowly biodegradable substrate. Under anaerobic conditions, fermentable readily biodegradable substrate undergoes fermentation to produce fermentation products considered to be acetate. Microorganism growth can proceed on either the fermentable readily biodegradable substrate or the fermentation products.

Influent organic nitrogen measured, as total Kjeldahl nitrogen (TKN), can be classified as either biodegradable or non-biodegradable depending on whether it is associated with a biodegradable or non-biodegradable carbonaceous species (Dold and Marais, 1986). As shown in Figure 2.1, the four types of organic nitrogen correspond with: fermentable readily biodegradable, slowly biodegradable, particulate non-biodegradable and soluble non-biodegradable. No nitrogen is associated with fermentation products considered to be acetate. The fate of the organic nitrogen depends on its organic counterpart.

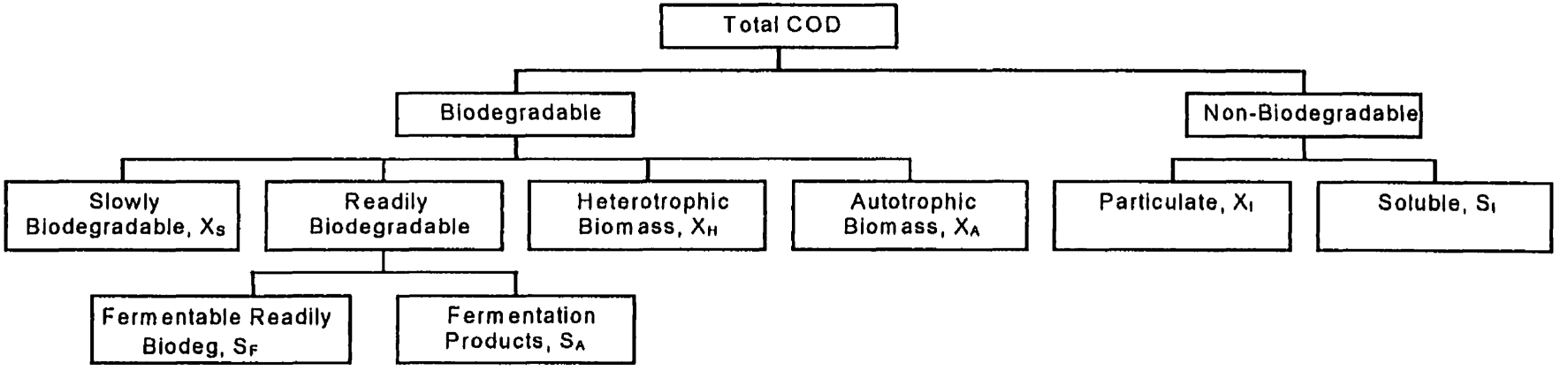
b) Substrate Utilization

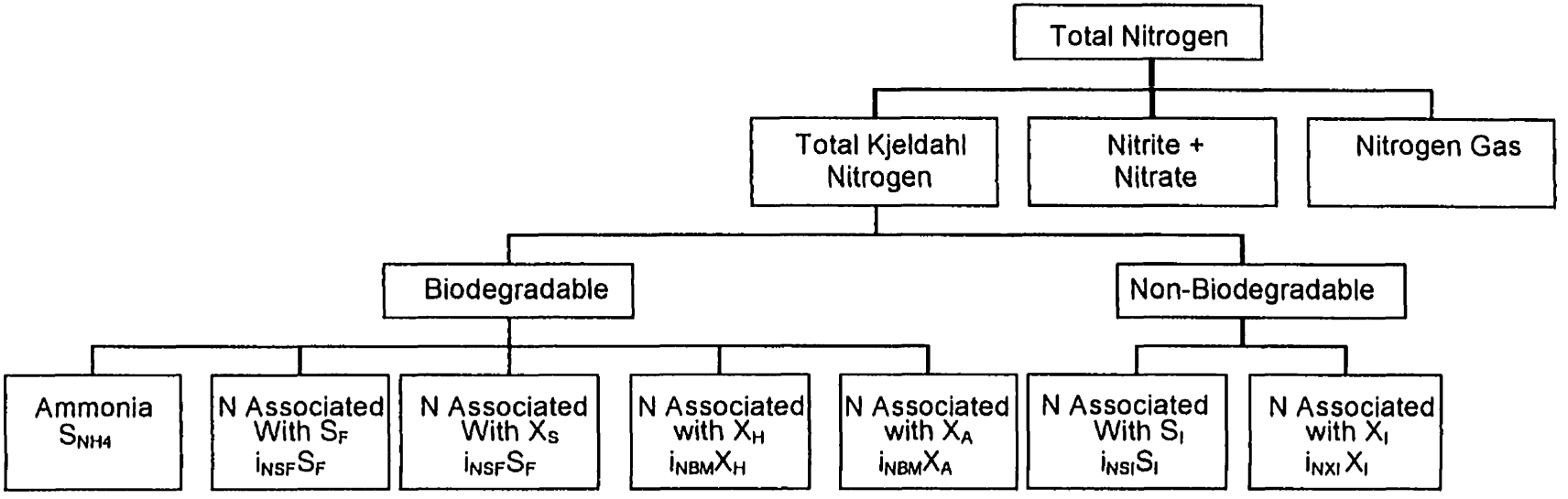
Within the framework of ASM2, fermentable readily biodegradable COD, and fermentation products are the only growth substrates available for heterotrophic growth under either aerobic or anoxic conditions. Slowly biodegradable organics are not available growth substrates before hydrolysis. Readily biodegradable fermentable COD may be present initially in the wastewater or generated through the hydrolysis of slowly biodegradable organics. Fermentation products considered to be acetate are generated by fermenting fermentable readily biodegradable COD.

c) Death-regeneration

Two existing theories, endogenous respiration and death-regeneration, have been used to describe the pathway of energy generated by the death of organisms and its use by the remaining microorganism population (Dold *et al.*, 1980). Prior to the formulation of ASM1, there was much debate on which of two theories should be incorporated into the model. Incorporated in ASM1 and its successor ASM2 is the death-regeneration approach. Because it is an important and distinguishing feature of ASM1 and ASM2, the two approaches will be discussed in further detail.

Figure 2.1 Influent Characterization as Depicted by ASM2





In the presence of an external substrate, a portion of the substrate is oxidized to fulfill maintenance energy requirements. In the absence of an external substrate, a fraction of the viable organism mass is used directly as an energy source to satisfy the maintenance requirements of the remaining living organisms (Herbert, 1958; Pirt, 1965). Endogenous respiration describes the net effect as the mass that disappears is utilized directly by live organisms (i.e. replicating and non-replicating organisms). Under zero substrate conditions, the rate of change in live mass due to endogenous respiration is proportional to the rate of oxygen or nitrate utilization.

To allow the energy to be used by the respiring biomass, an exogenous electron acceptor such as oxygen or nitrate must be present. Under anaerobic conditions, organism death continues without an exogenous acceptor present and a portion of the organic substrate acts as an endogenous acceptor with little or no reduction in COD. Experimentally, it has been found that effluents discharged from an anaerobic reactor to a following anoxic or aerobic reactor causes a higher than expected oxygen or nitrate demand. The traditional endogenous respiration approach fails to explain this (Ekama *et al.*, 1979).

An alternative to the endogenous respiration is the death-regeneration cycle as incorporated in ASM1 (Henze *et al.*, 1986). In ASM1, death is referred to as the loss of organisms due to autolysis. In ASM2, death was replaced with lysis which includes all processes that lead to a loss of microorganisms including: predation, autolysis, and loss of cell wall integrity. The IAWQ Task Group (IAWQ Task Group, 1995) in their discussion of ASM2 do not note any differences between the death-regeneration and the lysis-regeneration processes.

The rates of death were thought to be independent of the nature or concentration of the electron acceptor present. In the death-regeneration cycle, a fraction of the active biomass lyses to produce endogenous particulate products (McKinney and Ooten, 1969) and slowly biodegradable COD. Endogenous particulate products formed are degraded at a sufficiently slow rate so that may be considered inert at sludge retention times normally encountered (Grady *et al.*, 1986). The slowly biodegradable COD generated is degraded by hydrolysis and used in the synthesis of new microorganisms. The rate of hydrolysis and subsequent synthesis depends on the type and quantity of electron acceptor present. The regenerated mass will be less than the initial mass due to energy losses incurred during synthesis and the formation of endogenous products.

Replacing endogenous respiration with the death-regeneration approach allows the behaviour found in anaerobic reactors to be integrated consistently with those under aerobic or anoxic conditions. Under aerobic conditions, the two approaches can be used interchangeably contingent on the storage of COD being negligible (i.e. hydrolysis or synthesis is not rate limiting) (Dold *et al.*, 1980). Under anoxic and anaerobic conditions, the response characteristics become dissimilar (Dold and Marais, 1986).

2.2.2. Heterotrophic Metabolism in the context of ASM2

Within the model's framework, heterotrophic processes occurring under aerobic and anoxic conditions, as shown in Figure 2.2, are: hydrolysis, growth, and cell lysis. A brief discussion of each of the processes is to follow with the equations governing these processes outlined in Table 2.1.

Figure 2.2 Overview of the Processes occurring in Heterotrophic Metabolism

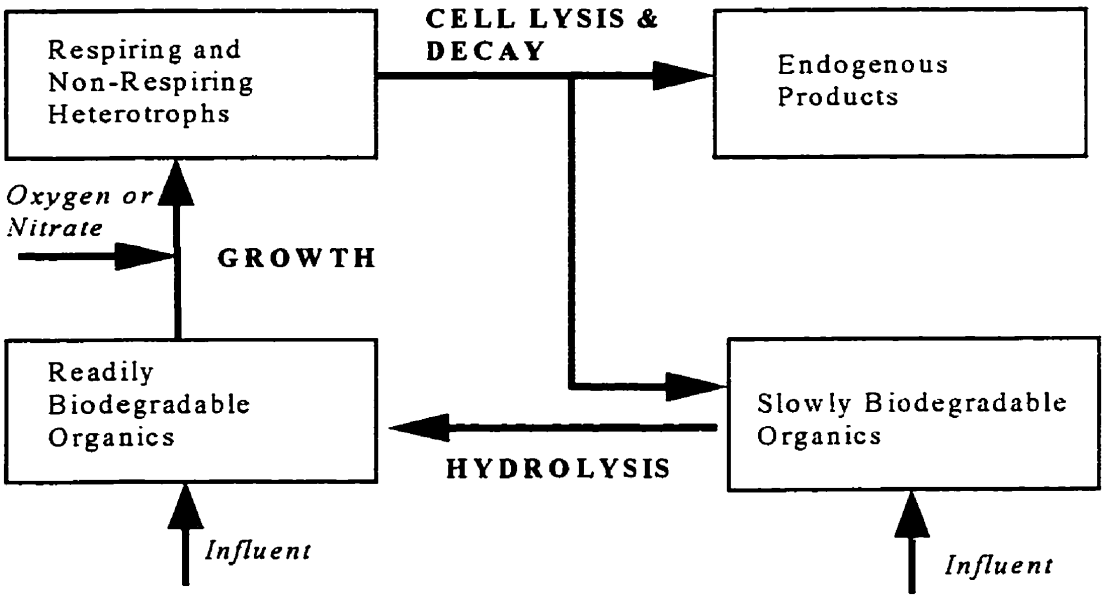


Table 2.1 Stoichiometric Matrix for Selected Components of Activated Sludge Model No. 2

Process	S_A	S_F	S_I	S_{NH4}	S_{O2}	S_{NO3}	S_{N2}	X_H	X_S	X_I	Rate Expression
1. Aerobic Hydrolysis		$1-f_{SI}$	f_{SI}	$\gamma_{1,NH4}NH_4$					-1		ρ_1
2. Anoxic Hydrolysis		$1-f_{SI}$	f_{SI}	$\gamma_{2,NH4}NH_4$					-1		ρ_2
3. Aerobic Growth on S_F		$-\frac{1}{Y}$			$1-\frac{1}{Y}$			1			ρ_3
4. Aerobic Growth on S_A	$-\frac{1}{Y}$				$1-\frac{1}{Y}$			1			ρ_4
5. Anoxic Growth on S_F		$-\frac{1}{Y}$				$\frac{1-Y}{2.86Y}$	$\frac{1-Y}{2.86Y}$	1			ρ_5
6. Anoxic Growth on S_A	$-\frac{1}{Y}$					$\frac{1-Y}{2.86Y}$	$\frac{1-Y}{2.86Y}$	1			ρ_6
7. Lysis								-1	$1-f_{XI}$	f_{XI}	ρ_7

Process	Rate Expression
1. Aerobic Hydrolysis	$\rho_1=K_h \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \frac{\frac{X_S}{X_H}}{K_X+\frac{X_S}{X_H}} X_H$
2. Anoxic Hydrolysis	$\rho_2=K_h\, \eta_{NO3} \frac{K_{O2}}{K_{O2}+S_{O2}} \frac{\frac{X_S}{X_H}}{K_X+\frac{X_S}{X_H}} X_H$
3. Growth on Fermentable Substrate S_F	$\rho_3=\mu_H \frac{S_{O2}}{K_{O2}+S_{O2}} \frac{S_F}{S_F+S_A} \frac{S_F}{K_F+S_F} \frac{S_{NH4}}{K_{NH4}+S_{NH4}} X_H$
4. Growth on Fermentation Products S_A	$\rho_4=\mu_H \frac{S_{O2}}{K_{O2}+S_{O2}} \frac{S_A}{S_F+S_A} \frac{S_A}{K_A+S_A} \frac{S_{NH4}}{K_{NH4}+S_{NH4}} X_H$
5. Denitrification on Fermentable Substrates S_F	$\rho_5=\mu_H\, \eta_{NO3} \frac{K_{O2}}{K_{O2}+S_{O2}} \frac{S_F}{S_F+S_A} \frac{S_F}{K_F+S_F} \frac{S_{NO3}}{K_{NO3}+S_{NO3}} X_H$
6. Denitrification on Fermentation Products S_A	$\rho_6=\mu_H\, \eta_{NO3} \frac{S_A}{S_F+S_A} \frac{S_A}{K_A+S_A} \frac{S_{NO3}}{K_{NO3}+S_{NO3}} X_H$
7. Lysis	$\rho_7=b_H X_H$

b) **Growth of Heterotrophs**

There are several different types of heterotrophs in the activated sludge population. Obligate aerobes; aerobes with denitrifying capabilities; aerobes with the capacity to ferment substrate under anaerobic conditions, but incapable of denitrifying; and anaerobes may all be present. Heterotrophs as defined in ASM2 can grow under aerobic, anoxic and anaerobic conditions. The growth kinetics for each of these three processes are unique. The kinetics reflect that only a portion of the total active heterotrophic population is capable of carrying out transformations in the presence of a specified electron acceptor.

Balanced growth is often described by Monod kinetics. The Monod kinetics incorporated in ASM2 are only applicable when sufficient nitrogen, phosphorus and other micronutrients are present.

i) Aerobic Growth of Heterotrophs

In activated sludge systems, the growth of heterotrophs under aerobic conditions is responsible for the majority of carbon removal. Within the context of ASM2, only forms of readily biodegradable COD can be used as a growth substrate. For each unit of readily biodegradable COD metabolized, Y units of microorganisms are produced with the remainder of the COD (1-Y) being oxidized. The rate of growth is expressed as Monod kinetics and is proportional to the rate of oxygen utilization. The rate of growth is governed by the concentration of readily biodegradable COD and oxygen.

A microbial population can be classified into four physiological groups: replicating, non-replicating, dormant or dead (Mason *et al.*, 1986). Replicating bacteria are respiring bacteria that can actively assimilate substrate, increase in mass and replicate. Similarly, non-replicating bacteria respire and consume substrate, but they are incapable of forming new organisms under the current growth conditions. Dormant microorganisms are capable of becoming either actively respiring microbes or undergoing death and subsequent lysis depending on subsequent substrate availability. Dead bacteria are intact organisms devoid of any metabolic activity.

In ASM2, the organisms carrying out substrate transformations are referred to as "active" organisms. The model does not define the active microbial population. It will be assumed for this research that the active mass would include the actively respiring population which includes replicating and non-replicating

bacteria. The possible presence of a dormant population has not been mentioned at any stage of the development of ASM1 or ASM2.

ii) Anoxic Growth of Heterotrophs

Anoxic growth within the context of ASM2 refers to growth occurring in the presence of nitrate. As with the aerobic growth of heterotrophs, the rate of anoxic growth is expressed in terms of Monod kinetics and is proportional to the rate of nitrate consumed. Heterotrophic growth under anoxic conditions can proceed under a dual substrate limitation; rapidly biodegradable COD and nitrate. The model suggests that the yield of denitrifying organisms is the same as that of aerobic heterotrophs on an electron equivalent basis. Only a fraction of the entire heterotrophic population is capable of denitrification. As previously stated, substrate transformations are carried out by active organisms.

c) Hydrolysis

Slowly biodegradable organics require: uptake, storage and enzymatic degradation (i.e. hydrolysis) before being used as a growth substrate. Neither uptake and storage of slowly biodegradable organics nor hydrolysis is considered an energy consuming process (Dold *et al.*, 1980). The amount of substrate stored within the floc is restricted by the active mass concentration (Dold *et al.*, 1980).

As storage and uptake occur rapidly (Gujer (1980) as cited by Grady *et al.* (1986)), the rate governing step is hydrolysis. Hydrolysis of entrapped organics provides fermentable readily biodegradable COD that is used in the growth of heterotrophs. Hydrolysis is thought to be a surface meditated reaction; thus the rate expression is "Monod-like" in form and dependent on the ratio of slowly biodegradable organics to active organisms.

van Haandel *et al.* (1981) hypothesized that hydrolysis under anoxic conditions proceeds at a much slower rate than it does under aerobic conditions. Experimentally, the observed rate of nitrate removal (as equivalent oxygen) in anoxic reactors using slowly biodegradable substrates was not as rapid as the simulated oxygen utilization rate. This suggested that the rate of utilization of particulate slowly biodegradable substrate in the anoxic environment is lower. The task group developing ASM2 accepted this hypothesis. They go on to

state that hydrolysis under anaerobic conditions still requires study. but probably the rate realized under these conditions is slower than under aerobic conditions (IAWQ Task Group, 1995).

The rate of hydrolysis of slowly biodegradable organic nitrogen is proportional to the rate at which slowly biodegradable substrate undergoes hydrolysis. Ammonia nitrogen is the product of slowly biodegradable organic nitrogen hydrolysis.

d) Cell Lysis and Subsequent Use of Decay Products

Within the framework of the death-regeneration approach. the rate of loss of active organism mass due to death or lysis is proportional to the active organism mass and proceeds at a constant rate regardless of the electron acceptor. Cell lysis results in endogenous residue and slowly biodegradable organics. Organic nitrogen produced by lysis is distributed between the endogenous residue and the slowly biodegradable organics.

2.3. Heterotrophic Metabolism

Microbial growth under nutrient limited conditions is defined as chronic starvation (Chesbro *et al.*, 1990). When necessary growth nutrients are exhausted, the microbial population is placed in a starvation state (Siegele *et al.*, 1993; Chesbro *et al.*, 1990). Bacteria of the conventional activated sludge system are chronically starved because they exist under carbon-limited conditions. Chronically starved and starved bacteria undergo changes in their metabolic behaviour and morphology that allows them to survive under adverse conditions.

The transition between chronic starvation and starvation can be rapid. A chronically starved population of *Escheria coli* NF161 became starved within 30 minutes of a recycling fermentor being shut off as indicated by a 30% decrease in viable counts. Approximately 62 hours later, 99% of the population were found to be non-viable under laboratory conditions (Chesbro *et al.*, 1990).

Under starvation conditions, a significant portion of the population is in a dormant state. Stevenson (1978) suggested that this is true for most aquatic systems. When *Pseudomonas sp.*, an aquatic bacterium, was placed under starvation conditions the population of actively respiring cells was 10 times the

number of culturable cells. It took the culture approximately 15-18 days before a number of measures of metabolic activity became relatively constant (Kurath and Morita, 1983). The existence of a significant population of actively respiring non-culturable microorganisms has been noted for cultures of *E. coli* and *Salmonella enteritidis* under starvation conditions (Roszak and Colwell, 1987).

In a conventional activated sludge plant there is a limited supply of biodegradable organics. Kinetic coefficients representative of an activated sludge system are: μ_m=0.55 h^{-1}, K_s=120 mg BOD_5/L (Jordan, 1971). Using an effluent 5 day biochemical oxygen demand (BOD_5) of 5 mg/L , μ/μ_m is 4%. The bacterial population is most likely in a state of chronic starvation. A Literature Review of the metabolic changes occurring under starvation conditions will show the importance of considering the growth state when interpreting data from this system.

2.3.1. Growth

a) <u>Aerobic Growth</u>

Substrate utilization, for reasons of simplicity, is typically described using an empirical Monod expression (Table 2.1, Eqn 3-4). Diauxic growth is seen under batch conditions and in the presence of high concentrations of different carbon substrates suitable for growth. With diauxic growth, the better substrate (i.e. capable of supporting the highest maximum specific growth rate) is consumed preferentially. Diauxic growth is not normally seen with activated sludge systems.

Under carbon limited conditions, simultaneous use of multiple carbon-energy sources occurs (Egli *et al.*, 1993). *E. coli B*, grown under nutrient sufficient conditions in a minimal medium containing glucose, lactose and L-tryptophan, was capable of metabolizing only glucose. The ability to use multiple carbon substrates simultaneously is advantageous when carbon is limiting as it is under conditions of chronic starvation or starvation.

The effect of temperature on the constants describing biological reactions, can be described in the form proposed by Phelps (1944) (Eq. 2.1) to be applicable over moderate temperature ranges (0-40°C) (Grady and Lim, 1980).

$$k_1 = k_2 \theta^{T_1 - T_2}$$ Eq. (2.1)

where k_1 = temperature dependent rate coefficient at T_1
k_2 = temperature dependent rate coefficient at T_2
θ = modified Arrhenius coefficient
T_1, T_2 = temperature (°C)

b) **Yield**

Cell yield measurements vary with growth rate. This has been explained by incorporating the concept of maintenance energy. Maintenance energy is the energy required for specific functions such as: continual degradation and repair of cell components, maintenance of concentration gradients between the cell and its exterior, and cell motility (Pirt, 1975). Energy derived from substrate oxidation is used for both maintenance functions and growth. If maintenance energy requirements are negligible or zero, the achieved yield will be the true growth yield. Under conditions where this is not true, the achieved yield is referred to as the observed yield.

The maintenance energy demand (MED) as a fraction of the cellular energy flux is dependent on the growth state of the bacterium (McCarty *et al.*, 1969). Under nutritionally sufficient conditions, the MED will be minimal. Under chronic starvation, the MED can account for 50% of the cell's energy flux. Starvation will become more severe once internal energy reserves are depleted. Under these conditions the MED will account for 75% of the energy flux (Chesbro *et al.*, 1990).

In the absence of an external substrate, a fraction of the viable organism mass is used directly as an energy source to satisfy the maintenance requirements of the remaining living organisms (Herbert, 1958). Often referred to as endogenous respiration it is mathematically described as:

$$\frac{dX}{dt} = (\mu - a)X$$ Eq. (2.2)

where $\frac{dX}{dt}$ = rate of microorganism growth ($mg \cdot d^{-1} \cdot L^{-1}$)
μ = specific rate of growth (d^{-1})
a = specific maintenance rate (d^{-1})
X = cell concentration ($mg \cdot L^{-1}$)

Pirt (1987) extended the concept of endogenous respiration to systems growing at rates less than 10% of the maximum specific growth rate. Activated sludge systems can be considered slow growing as defined by Pirt (1987) because the growth rate is typically 4% of the maximum obtainable. The slow growth

rate is due to the lack of available substrate. Pirt hypothesized that, in slow growing systems, a fraction of the cell population differentiates into a "dormant state". This state is characterized by the cessation of growth and substrate uptake for growth and maintenance. The differentiating microorganisms derive energy through endogenous metabolism resulting in a loss of biomass. It is assumed that the microorganism's uptake for growth and maintenance ceases once it becomes dormant. When the differentiating fraction is small, then the growth of heterotrophic organisms can be described as:

$$\frac{dX}{dt} = \mu \alpha X \qquad \text{Eq. (2.3)}$$

where dX/dt = rate of microorganism growth ($mg \cdot d^{-1} \cdot L^{-1}$)
X = cell concentration ($mg \cdot L^{-1}$)
α = the growing fraction
μ = the specific rate of growth (d^{-1})

For pure cultures, yield is thought to be a weak function of temperature. With a pure culture, the energy required for a certain rate of growth becomes greater as one deviates from the optimum growth temperature (Inniss and Ingraham, 1978). This finding is consistent with the results of a number of studies employing pure cultures (Topiwala and Sinclair, 1971; Mennett and Nakayama, 1971; Harder and Vedkamp, 1967).

Muck and Grady (1974) grew a mixed population under carbon limited conditions. The maximum true yield of 0.6 g COD/g COD was obtained at a temperature of approximately 20 °C. At temperatures of 10 and 40 °C, the yield was 0.45 g COD/g COD and 0.48 g COD/g COD, respectively. Medronho and Russo (1983) found the sensitivity of yield to temperature to be dependent on the COD concentration. Yield increased with higher temperatures with the amount of increase depending on the substrate concentration.

Tian *et al.* (1994) found an increased observed yield at lower temperatures regardless of the influent volatile suspended solids concentration for an activated sludge culture (Table 2.2). Influent VSS was considered important because it would include nonbiodegradable organics which would accumulate in the reactor. The study made use of two SBRs run in parallel but at different temperatures (8 and 20°C). In the first phase of the experiment, concentrated sewage was used as a substrate. In the second phase, sewage supplemented with nutrient broth was used.

Table 2.2 Observed Yields for a Carbon Removal Reactor (Tian *et al., 1994)*

Phase	Influent VSS (mg VSS/l)	20 °C (gΔVSS/gΔCOD)	8 °C (gΔVSS/gΔCOD)
1	69	0.253	0.309
2	17	0.230	0.292

With a mixed microbial population, changes in the observed yield could reflect changes in the microbial population. At low temperatures both pyschrophiles and psychrotrophic bacteria will be present. Pyschrophiles have an optimum temperature for growth of 15°C or lower and a maximum temperature for growth of 20°C. Psychrotrophs are cold tolerant organisms having a maximum temperature for growth above 20°C (Morita, 1975). Microbial competition at temperatures less than 10 °C will favour psychrophiles over psychrotrophic bacteria when all growth factors are in excess. Under conditions of carbon and energy limitations (i.e. starvation), it was suggested that the half velocity coefficient (K_s) plays a role in defining dominance (Harder and Veldkamp, 1971).

c) **Anoxic Growth of Heterotrophs**

i) Microbiology of Denitrification

Many common sewage bacteria are capable of denitrification (Table 2.3). The denitrifying population will be defined by the composition of the influent wastewater and other environmental factors such as pH, temperature and sludge age. If the organics present in the influent wastewater are insufficient to complete denitrification, an exogenous carbon source can be used. However, use of a particular carbon source may cause selective enrichment of certain denitrifying species.

Table 2.3 Denitrifying Bacteria Abundant in Sewage (after Christensen and Harremoës (1977))

Genera	Transformation Performed	
	$NO_3^- \rightarrow N_2$	$NO_3^- \rightarrow NO_2^-$
Achromobacter	X	
Aerobacter		X
Alcaligenes	X	
Flavobacterium		X
Micrococcus	X	
Proteus		X
Pseudomonas	X	

The concept of selective enrichment was investigated by Blaszczyk *et al.* (1983). They examined the effect of nitrites or nitrates in conjunction with a number of exogenous substrates on a denitrifying population. The denitrifying bacteria, initially isolated from a wastewater holding tank near a fertilizer plant, were grown in Winogradsky's medium supplemented with the carbon source mentioned. Denitrifying bacteria accounted for 5-100% of the total bacterial population as indicated by plate counts depending on the carbon source. The results of Table 2.4 are after 6 days of incubation at 26°C.

Table 2.4 Dominant Denitrifying Bacteria Isolated after Culture in Noted Media (after Blaszczyk *et al.*(1983))

Carbon Source	Form of Nitrogen			
	Nitrite Nitrogen		Nitrate Nitrogen	
	% Denitrifying Bacteria	Composition of Denitrifiers %[1]	% Denitrifying Bacteria	Composition of Denitrifiers %[1]
Methanol	71	*P. fluorescens* (78.9) *P. mendocina* (5.3) Others (15.8)	36	*P. fluorescens*(91.2) *P. aeruginosa* (5.6) Others (3.2)
Ethanol	96	*P. mendocina* (96.8) *P. fluorescens* (2.1) *P. aeruginosa* (1.1)	100	*P. mendocina* (88.9) *P. aeruginosa* (11.1)
Acetic Acid	100	*P. fluorescens* (92.8) *P. mendocina* (2.4) Others (4.8)	42	*P. aeruginosa* (78.3) *P. fluorescens* (13.0) *P. stutzeri* (4.4) Others (4.4)
Glucose	84	*A. faecalis* (100.0)	5	*A. faecalis* (100.0)

Composition of denitrifiers is based on representative sampling of the culture and identification.

Mycielski *et al.* (1985) investigated the selection of denitrifying bacteria on a quantitative basis when starch was used as a substrate. In this instance, the cultures were grown in a chemostat at temperatures ranging from 18-22°C yielding a population of obligate and facultative pyschrophiles. Even though the mixed liquor composition changed with the starch to nitrate-nitrogen ratio, *Alcaligenes faecalis*, *Paracoccus denitrificans* and *Pseudomonas mendocina* consistently predominated. This is surprising considering these bacteria are unable to hydrolyze starch. The investigators suggested that these bacteria may be using either the products of starch hydrolysis or carbohydrate fermentation products generated by other members of the microbial population.

ii) Denitrification Kinetics

In the absence of oxygen, the specific rate of denitrification is dependent on the concentration of biodegradable carbonaceous material and nitrate as seen in Table 2.1, Eqn. 5-6. The rate expression applies under anoxic conditions and pHs between 6.0-8.0 (U.S. EPA, 1993).

iii) Yield of Denitrification

The growth yield of heterotrophic microorganisms using nitrate as a terminal electron acceptor is expected to be 77% or less of that realized under aerobic conditions because of electron transport chain considerations (Payne, 1981). Table 2.5 shows that the realized yield under anoxic conditions in comparison to aerobic conditions is variable depending on the substrate. The yield published by Koike and Hattori (1975) is suspect because the yield on a COD basis must be less than 1.

Table 2.5 Yield of Denitrifiers

Culture	Anoxic / Aerobic	Anoxic Yield g COD / g COD	Temp (°C)	Carbon Source	Investigator
Theoretical	0.77				Payne (1981)
Paracoccus denitrificans	0.65			Various	Stouthamer *et al.* (1982)
Pseudomonas denitrificans	0.4	0.64	20	Glutamate	Koike and Hattori (1975)
Pseudomonas denitrificans		1.01	20	Glutamate	Koike and Hattori (1975)
Pseudomonas denitrificans		0.64	20	Ethanol	Wang *et al.* (1995)

d) Summary

1. Under carbon limited conditions, simultaneous use of multiple carbon energy sources occurs. Under conditions where the culture is in a state of either chronic starvation or starvation conditions, diauxic growth should not be seen. Diauxic growth occurs only at high substrate concentrations and thus will only be seen during batch growth. It will not be seen under the carbon limited conditions typically seen in activated sludge systems.
2. Yield is thought to be a function of temperature. For a pure culture, energy requirements for a certain rate of growth become greater with large deviations away from the optimum growth temperature. For a mixed culture, changes in the observed yield may reflect changes in the microbial population. Microbial

competition at temperatures of less than 10°C will favour psychrophiles over psychrotrophic bacteria when all growth factors are in excess. Under conditions of carbon and energy limitations this may not be true.

3. Often an exogenous carbon source is used when denitrification occurs under carbon limitations. Selective enrichment of a particular denitrifying species may occur if a exogenous carbon source is used.
4. When the electron transport chain is considered, the growth yield of heterotrophic microorganisms achieved using nitrate as a terminal electron acceptor is expected to be less than 77% of that obtained under aerobic conditions. A number of studies comparing yield under anoxic conditions in comparison to aerobic conditions found it to be variable depending on the substrate.

2.3.2. Hydrolysis

a) Introduction

Hydrolysis of proteins involves the cleavage of a peptide bond and the subsequent incorporation of water (Stryer, 1975). When complex proteins are the growth substrate, hydrolysis is necessary before a microorganism can derive any benefit from the protein. Within the context of activated sludge systems, hydrolysis is the one of the most poorly understood growth associated processes. The ideas incorporated in the ASM2 as detailed by Gujer *et al.*(1995), represent our current understanding of the process and are summarized below:

1. There exists a number of protocols for differentiating between readily and slowly biodegradable substrate. Their existence implies that hydrolysis is a rate limiting step.
2. Hydrolysis occurs even in the presence of high concentrations of rapidly biodegradable substrate.
3. The rate of hydrolysis is expected to be different under aerobic, anoxic and anaerobic conditions.

Enzyme types, concentrations and specific activities play an important role in determining the overall rate of hydrolysis. The rate of protein hydrolysis also can be influenced by temperature and the presence of competitive and non-competitive inhibitors.

Enzymes external to the cell, exoenzymes, can be further classified as either ectoenzymes or extracellular enzymes. Ectoenzymes are secreted across the cytoplasmic membrane and remain in communication with their producing cell (Karnovsky, 1986). These inducible enzymes react with polymeric

or enzymes produced by cell lysis. Causes for cell lysis would include autolysis and predation. They may be dissolved in water or adsorbed to solid surfaces (Chróst, 1990). If hydrolysis is a rate limiting process, then identifying the dominant enzyme type may be important because ectoenzymes and extracellular enzymes are produced and their activity is modulated in different ways. Studies determining whether either ectoenzymes or extracellular enzymes are dominant in activated sludge systems are lacking. The Literature Review will show that either enzyme type is possible given our current understanding of the activated sludge system.

b) Enzyme Location and Type

The location and type of enzymes carrying out hydrolysis have not been addressed by many researchers working with activated sludge. If the bacteria secrete enzymes outside the cell during starvation conditions, then ectoenzymes or extracellular enzymes (i.e. ectoenzymes no longer associated with their producer) may carry out hydrolysis. If this is not so, then the enzymes mediating hydrolysis are produced due to cell lysis or protozoa grazing. From an engineering perspective, the distinction is important as culture conditions will impact on hydrolysis in different ways depending on the mode of exoenzyme generation.

Bacteria producing exoenzymes can degrade polymeric organic material and make use of the products. This gives them a significant competitive advantage when easy to metabolize energy and nutrient sources become limiting. Direct secretion of enzymes by Gram-negative bacteria is not as frequent as for Gram-positive bacteria (Chróst, 1991). The microorganisms most frequently known to be proteolytic are members of the genera: *Pseudomonas, Flavobacterium, Xanthomonas, Aeromonas, Vibrio, Acinetobacter, Cytphaga, Bacillus* and *Enterobacter* (Jones, 1971). Lighthart and Oglesby (1969) determined the presence of bacteria of the following genera: *Pseudomonas, Flavobacterium, Aeromonas* and *Vibrio* in raw wastewater. These bacteria are all Gram-negative and less likely to secrete enzymes directly.

Pseudomonas sp. accounts for 96-100% of the microbial population when methanol, ethanol or acetic acid is used (Table 2.4). Fairbairn and Law (1987) examined proteinase production by *Pseudomonas fluorescens NCDO 2085.* Proteinase production occurred during in the exponential phase of growth when the culture was grown on protein substrates, but not until the early stationary phase during growth on amino acids. Gügi *et al.* (1991) obtained similar results using *P. fluorescens MFO* cultured in a minimal salts media

culture was grown on protein substrates, but not until the early stationary phase during growth on amino acids. Gügi *et al.* (1991) obtained similar results using *P. fluorescens MFO* cultured in a minimal salts media augmented with 10% skim milk. Myhara and Skura (1990) found that proteinase production in *Pseudomonas fragi* commences during the late logarithmic and early stationary phases of growth.

A study by Albertson *et al.* (1990) examined extracellular protease production during different stages of starvation of *Pseudomonas S9* and *Vibrio S14*. Hide powder azure (HPA), a non-toxic insoluble dye-labeled protein, was used as a substrate in this study. With both species, exoprotease activity as indicated by the rate of HPA degradation was higher 4-5 h after the onset of starvation than at its initiation. After 24 h of starvation, *Pseudomonas sp.* continued to degrade the HPA at a similar rate. The investigators questioned whether increases in activity with starvation were the result of increased ectoenzyme production or extracellular enzymes generated by cell lysis. This was addressed by washing the starved cells and subdividing them into two. One portion of the culture served as a control while the other received chloroamphenicol, a chemical suppressing *de novo* protein synthesis. After 10 h of additional starvation, the treated culture exhibited no exoprotease activity suggesting that ectoenzyme production was responsible for extracellular protease activity.

The detection of exoenzymes in activated sludge systems is difficult due to the flocculent nature of the microorganisms. Extracellular enzymes may be produced, but not detected in the bulk liquid due to diffusional limitations or reactions within the sludge floc. In a study of bovine serum albumin (BSA) degradation by acclimated activated sludge, McLoughlin and Crombie-Quilty (1983) found proteolytic enzymes in the supernatant during BSA degradation. During the investigation, there was never a rise in the levels of trichloroacetic acid (TCA) soluble protein in the cell free supernatant; therefore, protein degradation byproducts did not accumulate in the supernatant. The investigators identified two possible reasons. The BSA degradation products could be readily assimilated by the activated sludge following degradation in the supernatant. Alternatively, the protein could be degraded close to the activated sludge matrix. In this case, the resulting degradation products would not be released into the supernatant. The rate of proteolytic activity was found to be correlated with the oxygen utilization rate.

This is in contrast to the findings of Boczar *et al.* (1992) who noted an absence of enzyme activity in the supernatant. Analysis of homogenized mixed liquor samples detected the following enzymes in

significant quantities: alkaline phosphatase (APase), amino peptidase (protein hydrolysis) and glucosyl hydrolase (carbohydrate hydrolysis). APase catalyzes the hydrolysis of phosphate esters including the esters of primary and secondary alcohols, sugar alcohols, cyclic alcohols, phenols, and polyphosphates. The enzymes detected in the mixed liquor would include: secreted enzymes, enzymes associated with dead cells, and possibly intracellular enzymes whose substrates would pass through the cell membrane. Dold *et al.* (1991) using a starch substrate, studied the rate of hydrolysis for α-1,4 glucan bonds. In this instance, the enzymes were associated with the microbial floc and no enzymatic activity was observed in the bulk solution.

Frolund *et al.* (1995) compared full sludge enzyme activity to that of a batch grown culture. The extracellular polymeric substance (EPS) was extracted from the activated sludge culture using cation exchange. The sludge bacteria were taken from the same treatment plant and grown for 3 days at room temperature in a mineral salts media augmented with yeast extract. The activity of the activated sludge bacteria was 32 and 18 times greater for esterase and leucine aminopeptidase activity normalized on a per cell basis, respectively, when compared to a culture of sludge bacteria. The investigators suggested that perhaps the role of EPS is not only to combine the activated sludge components, but also to act as a sink for immobilized exoenzymes.

The bacteria present in activated sludge are either a slow growing (i.e. stationary growth phase) or a chronically starved culture. It is under these conditions that ectoenzyme production has been noted in pure cultures (Albertson *et al.*, 1990; Skura, 1990; Fairbairn and Law, 1987). Some of the bacteria present in activated sludge have the ability to produce ectoenzymes (Jones, 1971). All investigators, except for McLoughlin and Crombie-Quilty (1983), have suggested that enzymes are present within the activated sludge floc since their absence has been noted in the bulk liquid. The enzymes present in the floc may be either extracellular enzymes or ectoenzymes. As ectoenzymes may play a role in hydrolysis, it is important to understand their production and factors governing the overall enzyme activity.

c) Ectoenzyme production and activity

Temperature, pH, oxygen concentration, and ionic strength are environmental parameters known to influence the production levels of ectoenzymes (Everleigh and Montenecourt, 1979). Ectoenzyme production is also governed by the presence of inducers and repressors. Inducers stimulate ectoenzyme production and

already expressed (Priest, 1984). The action of the end product will depend on the particular end product, its concentration and the bacterial species.

An in-depth study by Fairbairn and Law (1987) sought to determine if nutritional factors in milk serve to induce or repress proteinase production by *Pseudomonas fluorscens*. As part of this investigation, the effect of 22 different amino acids on proteinase production was examined. Amino acids and peptides are produced by proteinases and therefore amino acids may induce or suppress enzyme production. Proteinase production was determined by examining HPA degradation by cell free samples. The amino acids were either the sole carbon source, the sole nitrogen source or provided both carbon and organic nitrogen. Sodium pyruvate and ammonium sulphate provided the non-amino acid source of carbon and nitrogen, respectively, when appropriate. From the results of their studies (Table 2.6), the investigators concluded proteinase production is a balance between induction by low concentrations of low molecular weight degradation products and sensitivity to end products. They further suggested that the function of proteinases is to ensure a supply of carbon rather than amino acids for protein synthesis. If sufficient organic carbon is available, the cell can synthesize the needed amino acids. A number of studies examining the role of suppressors and inducers in ectoenzyme production for cultures of bacteria indigenous to sewage are summarized in Table 2.7.

Table 2.6 Amino acids inducing proteinase production in *Pseudomonas fluorescens* after 96 h of growth at 20°C in minimal medium supplemented as a sole carbon, nitrogen or carbon and nitrogen source (after Fairbairn and Law (1987))

As carbon source	As nitrogen source	As carbon/nitrogen source
Isoleucine (Ile)	Alanine (Ala)	Isoleucine (Ile)
Proline (Pro)	Valine (Val)	Tryptophan (Trp)
Tryptophan (Trp)	Isoleucine (Ile)	Asparagine (Asn)
Phenylalanine (Phe)	Methionine (Met)	Glutamine (Gln)
Asparagine (Asn)	Tryptophan (Trp)	Arginine (Arg)
Glutamine (Gln)	Asparagine (Asp)	Aspartic acid (Asp)
Tyrosine (Tyr)	Glutamine (Gln)	Glutamic acid (Glu)
Aspartic acid (Asp)	Arginine (Arg)	
Glutamic acid (Glu)	Histidine (His)	
	Ornithine	
	Aspartic acid (Asp)	

Table 2.7 The Effect of End Product Addition on Enzyme Production and Activity

Culture	End Product Added	Concentration	Enzyme	Enzyme Production	Overall Activity	Investigator
Pseudomonas sp. S9	glucose	4 g/L			N.C.	Albertson *et al.* (1990)
	Casamino Acids	4 g/L			Decr.	
	cAMP	1.99 mM			Incr.	
Vibrio sp. S14	glucose	4 g/L			Decr.	Albertson *et al.* (1990)
	Casamino Acids	4 g/L			N.C.	
	cAMP	1.99 mM			Incr.	
Pseudomonas fluorscens	citrate	0.05% w/v	Proteinase	Repress	Decr.	Fairbairn and Law (1987)
	glucose				Decr.	
	pyruvate				Decr.	
	lactose	1% w/v		N.C.	N.C.	
Pseudomonas sp	Casitone	30µg/L	Proteases		Incr.	Little *et al.* (1979)
Flavobacterium sp.	Casamino acids	30µg/L			Incr.	
Other aquatic bacteria	sodium caseinate	30µg/L			Incr.	
	yeast extract	30µg/L			Incr.	
	gelatin	30µg/L			N.C.	
	glutathione	30µg/L			N.C.	
	methionyl glycine	30µg/L			N.C.	
	glycyl glycine	30µg/L			N.C.	
	phenylalanine	30µg/L			N.C.	

Note: Overall activity takes into consideration both enzyme levels and enzyme activity.
Decr - decreased Incr - increased NC - no change

Modification of existing enzymes has been reported by (Halemejko and Chrost, 1986). The kinetics of amino-peptidase and endopeptidase as modified in the presence of proteins were considered in this study. The function of aminopeptidase is to degrade a large number of peptides and amino acid amides of the L-configuration. Leucine was found to be a competitive inhibitor of amino-peptidase activity. BSA was also inhibitory to amino-peptidase, but the mechanism of inhibition was unclear. The addition of BSA resulted in lowered Michaelis Menten maximum velocity (μ_M) and higher Michaelis Menten half saturation coefficients (K_M) values for amino-peptidase. Albumin was a non-competitive inhibitor of endopeptidase activity. This experimental study seems to indicate that the role of amino acids and proteins on enzyme activity is specific to the substrate-enzyme system.

Chróst (1991) as part of a study on aquatic systems examined the modulation of already expressed alkaline phosphatase (APase) and β-glucosidase due to the presence of a number of different end products. β-glucosidase catalyzes the hydrolysis of β-linked dissaccharides of glucose, celluhexose and carboxymethylcellulose. The effect of these is summarized in Table 2.8. This study also examined modulation of leucine aminopeptidase after the addition of the end products. Specific enzyme activity, bacterial production and the apparent K_M were observed at 4, 20 and 44 h for leucine aminopeptidase, in the presence of acetate, glucose and amino acids (Figures 2.3-2.5). Addition of amino acids to the culture precipitated the fastest change with lower specific activity, higher bacterial production and slightly higher Michaelis constants occurring within 4 hours. After 44 h incubation, amino acids and acetate supplemented cultures gave comparable growth rates while the growth rate of the glucose augmented culture was 44-55% higher. At this time, the specific activity of the enzyme normalized on the number of cells was approximately 10% of the control culture after 44 h of incubation. For the control culture, and those cultures supplemented with acetate and glucose, the Michaelis remained relatively constant. The culture supplemented with amino acids after 44 h of incubation had a Michaelis Menten constant approximately double that of the control culture.

Table 2.8 Specific activities and Michaelis constants of APase and β-glucosidase in water supplemented with various organic compounds (after Chróst (1991))

Substance Added	APase		β-glucosidase	
	Activity ($fmol \cdot cell^{-1} \cdot h^{-1}$)	K_m ($\mu mol \cdot L^{-1}$)	Activity ($fmol \cdot cell^{-1} \cdot h^{-1}$)	K_m ($\mu mol \cdot L^{-1}$)
cAMP	Increased	Decreased	No Change	Increased
ATP	Increased	Decreased	No Change	Increased
Glucose-6-P	Increased	Decreased	No Change	Increased
Glucose	Decreased	Decreased	Decreased	Increased
Cellobiose	Decreased	Decreased	Increased	Decreased
Amino acids	Decreased	Decreased	Decreased	Increased

Figure 2.3 Effect of the Addition of Low MW Substrates on Specific Activity of Leu-Amp (after Chróst (1991))

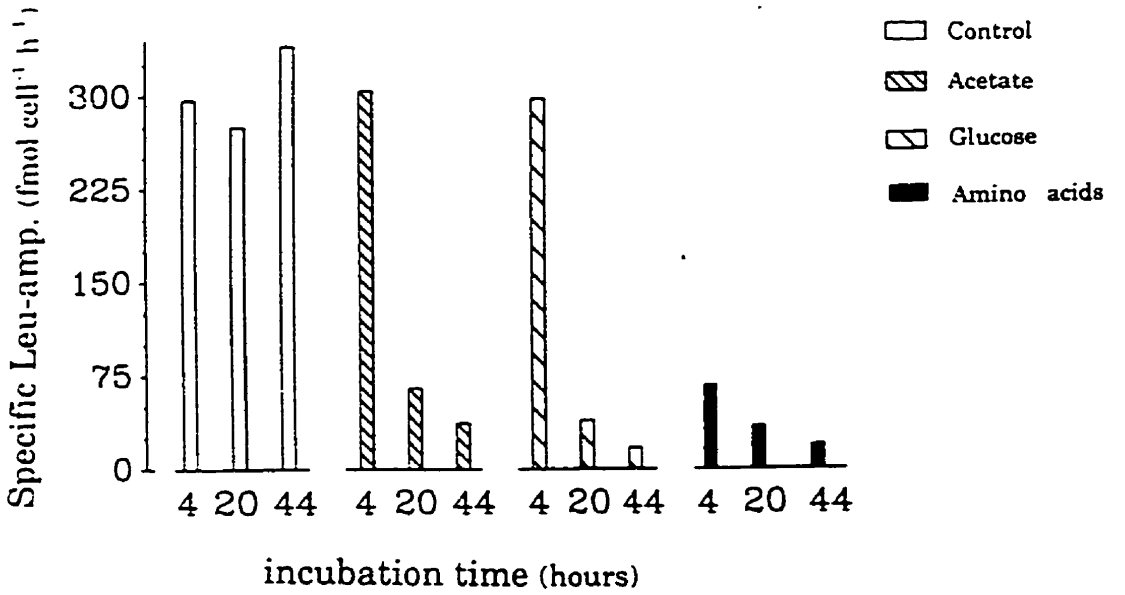


Figure 2.4 Bacterial cell Production and Growth Rates in Water Samples Supplemented with Readily Utilizable Substrates (after Chróst (1991))

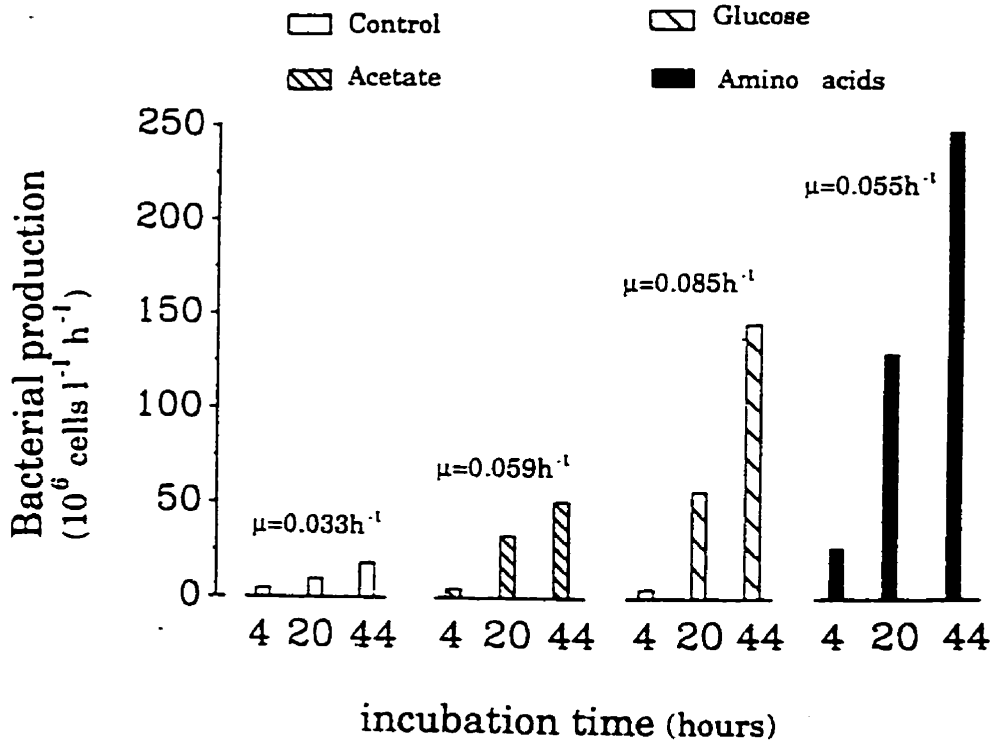
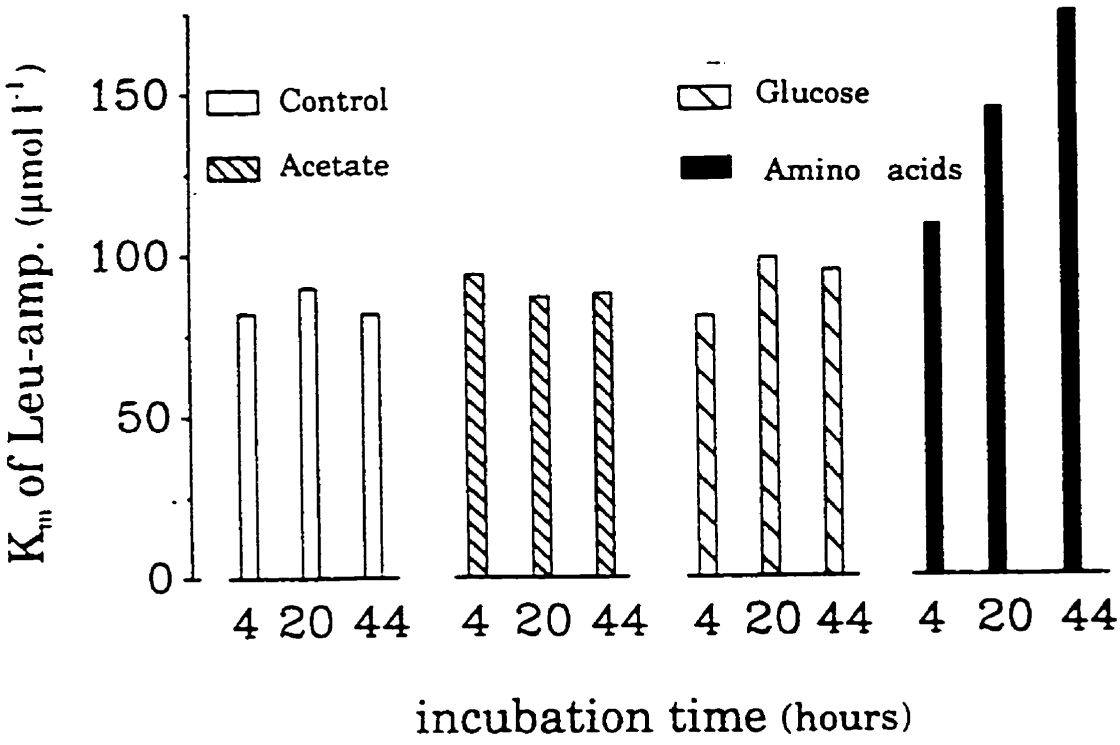


Figure 2.5 Michaelis constants (K_m) of Bacterial Leu-amp in Water Samples Supplemented with Readily Utilizable Substrates (after Chróst (1991))



Further investigation of this enzyme system with Leu-Leu, Ala-Ser, and albumin showed the effects of supplementing with dipeptides and proteins. Supplementing lake water with dipeptides resulted in a slightly decreased specific activity of the enzyme synthesized by bacteria in comparison to the control samples. In control samples, specific (per cell) activity slightly increased after 24 and 48 h of incubation. Addition of albumin (molecular weight (MW) approx. 66,000) to samples resulted in an accelerated rate of growth and rapid induction of leu-amp synthesis. The newly formed enzymes had lower K_M. The investigator concluded enzyme production and modulation is controlled by different mechanisms. Chróst (1991) cited other research that shows that in general the presence of readily utilizable substrates or amino acids represses enzyme activity. In contrast, the research of Daatselaar and Harder (1974), Litchfield and Prescott (1976) and Chróst (1991) show amino acids, peptides and proteins induce aminopeptidase synthesis in some bacteria. As stated previously, the action of the end product will depend on the particular end product, its concentration and the bacterial species.

d) <u>The Rate Kinetics of Hydrolysis</u>

Use of a particulate biodegradable substrate requires (1) uptake and storage, (2) extracellular enzymatic breakdown of the complex organic and (3) synthesis by the organism (Dold *et al.*, 1980). Soluble, slowly biodegradable substrates may also require these processes for utilization. Uptake and storage of substrate are considered to be essentially immediate (Dold *et al.*, 1980). Enzymatic breakdown of the complex organics (hydrolysis) has been mathematically described in ASM2. This is the formulation accepted by most investigators.

$$\frac{dXs}{dt} = K_H \frac{Xs/X_H}{K_X + Xs/X_H} X_H \qquad \text{Eq. (2.4)}$$

where dX_S/dt = rate of hydrolysis of slowly biodegradable substrate (mg COD·L^{-1} ·d^{-1})

K_H = maximum specific rate of hydrolysis (d^{-1})

X_S = concentration of slowly biodegradable substrate (mg COD·L^{-1})

X_H = concentration of heterotrophs (mg COD·L^{-1})

K_X = half velocity coefficient for hydrolysis (mg COD·L^{-1})

The coefficients are unique for hydrolysis under aerobic, anoxic, and anaerobic conditions. Given the dynamic nature of enzyme systems, it seems reasonable that enzyme activity, and thus the rate of hydrolysis, would change in the presence of different electron acceptors.

Kappeler and Gujer (1992) utilized batch tests with iterative curve fitting to calculate K_H of Equation 2.4 and quantify the amount of slowly biodegradable substrate present. After inhibiting nitrification, the oxygen utilization rate was measured as a function of time. The oxygen utilization rate indicates the rate of hydrolysis only if growth is limited by the concentration of readily biodegradable substrate. At 14 °C a value for K_H of 4 d^{-1} was determined. The accuracy of the determination is dependent on having accurate values for the death and decay coefficients, and the heterotrophic yield.

Comparisons can be made between the work of Kappeler and Gujer (1992) and Wanner *et al.* (1992). The latter investigators used mathematical optimization and 25 sets of time series data of oxygen utilization rates to determine the rate of hydrolysis. Initial estimates were updated using predictive-corrective techniques. Experiments were divided into two groups: those having a sludge age between 7.3 and 11.1 days; and a second group where sludge ages ranged between 1.8 - 2.8 days. Comparing K_H values given by Wanner *et al.* (1992) to those derived by Kappeler and Gujer (1992) produces estimates that differ by a ratio of 2 to 1. For estimates of the initial concentration of the slowly biodegradable organic matter this ratio is 1 to 1.7 (i.e. the inverse ratio). Comparable overall hydrolysis rates were obtained from these two studies.

One of the few studies to specifically look at the rate of hydrolysis used activated sludge (AS), acclimated to the polymer, peptone or dextrin, to investigate the role of hydrolysis at 20°C (Ubukata, 1992). The polymer-acclimated AS was fed the corresponding monomer, amino acids or glucose, as a part of rate testing. The rate of removal was determined by measuring the concentration of filtered total organic carbon in the reactor over time. For both substrates it was concluded that the removal rate of monomer by the polymer-acclimated AS was higher than that of the polymers. The only difference between the polymer and the monomer is the existence of a glycosidic or peptide bond. Ubukata suggested that the rate of hydrolysis of polymer to monomer was the rate limiting step.

In a second slightly more limited study, the hydrolysis of α-1,4 glycosidic bonds (i.e., the bond predominantly found in starches) by activated sludge was examined (Dold *et al.*, 1991). The activated sludge was taken from a full scale treatment plant achieving aerobic carbon removal. In this investigation, the rate of hydrolysis was significantly altered by the availability of an electron acceptor (oxygen or nitrate). At 20°C, the rate under anoxic conditions (i.e., in the presence of nitrate and absence of dissolved oxygen) was more than double that observed under aerobic conditions. In this instance, hydrolysis under anoxic conditions may be carried out by both the obligate aerobes and the denitrifying organisms present in the mixed liquor. The mixed liquor had never been exposed to anoxic conditions before testing. The type of enzymes initially present and their overall activity would be characteristic of an aerobic culture and not an anoxic culture. Use of an anoxic culture may lead to very different results.

e) Hydrolysis of Nitrogenous Compounds

Henze and Mladenovski (1991) used ammonia production rates to indicate the rate of hydrolysis of nitrogenous compounds by activated sludge. Raw sewage having the following characteristics: 450-600 mg COD/L, 40-60 mg TKN/L and 30-40 mg NH_3-N/L was added to the MLVSS to give a final concentration of 1900 mg VSS/L. The MLVSS and sewage were taken from the same plant so acclimation was not considered a problem. Nitrification was inhibited so ammonia nitrogen was not further metabolized by autotrophs. Tests were carried out at temperatures of 12.3, 15 and 20 °C. The experimental data suggested that the hydrolysis of nitrogenous compounds is significantly affected by the available electron acceptor. Under aerobic conditions the rate of ammonia nitrogen production was 5 g NH_3-N/mg TKN·h while in the presence of nitrate the rate was 1.25 g NH_3-N/mg TKN·h. The investigators found that the ratio between the hydrolysis rates under aerobic and anoxic conditions was similar to the respiration rates expressed in terms electron equivalents.

f) Summary

1. A number of Gram-negative bacteria found in activated sludge have been noted to produce ectoenzymes. Although the production of ectoenzymes in relation to growth phase seems to be species and substrate dependent, the general trend is for enzymes to be produced during the late logarithmic stages and beyond.

2. Studies of pure cultures and aquatic systems have shown that ectoenzymes are present. The presence or absence of ectoenzymes has not been investigated in activated sludge systems. It has been suggested that the activated sludge floc matrix most likely immobilizes and accumulates large amounts of enzymes. In this instance, the role of the extracellular polymer is not only to combine the activated sludge components, but also to act as a sink for immobilized exoenzymes.
3. Temperature, pH, oxygen concentration, and ionic strength all are environmental parameters known to influence the production levels of ectoenzymes. The production and specific activity of ectoenzymes is also governed by the presence or absence of end products. In general, high concentrations of end products decreases enzyme activity either through decreased production or by changing the activity of existing enzymes.
4. Hydrolysis as carried out by the microorganisms of activated sludge is poorly understood. Within the framework of ASM2, the rate of hydrolysis is independent of the concentration of readily biodegradable substrate even under conditions when the level of readily biodegradable substrates far exceeds that required for growth. If ectoenzymes are responsible for hydrolysis then this hypothesis would be contradictory to the current understanding of these enzyme systems gained by examining other systems. If extracellular enzymes produced by cell lysis are responsible for hydrolysis, then culture conditions such as the presence of readily biodegradable substrates may have minimal impact on hydrolysis kinetics.

2.3.3. Cell Lysis and Subsequent Use of Decay Products

a) Introduction

Microbial death results from irreversible damage to the deoxyribonucleic acid (DNA). If sufficient damage is incurred by the cell wall/membrane, then both death and lysis occur. Agents precipitating microbial death include: cell age, chemical damage (including antibiotics), low temperature, and freezing. Autolysins can cause the loss of cell wall/membrane integrity.

Autolysins play an important role in cell function as they are responsible for: daughter cell separation, protein turnover, morphological differentiation, and expansion of cell wall polymers (Mason *et al.*, 1986). These are the constructive roles they play. In general, if the synthesis of autolysins is prevented because

of either nutritional limitations or the presence of antibiotics then lysis of bacterial cells will be observed (Shockman and Höltje, 1994). The capacity of bacterium to autolyse is at a maximum or near maximum during the exponential growth phase (Shockman and Höltje, 1994). There are many reports that cells do not lyse during starvation (Harrison and Lawrence, 1963; Gronlund and Campbell, 1963; Novitsky and Morita, 1977).

b) Cryptic Growth

Cryptic growth refers to the use of organics that have leaked from a dying bacterium as a growth substrate (Postgate, 1976). A more inclusive definition would include substrate generated through lysis of microorganisms as well as substrate generated by cell leakage. It should be noted that cryptic growth does not appear to occur during the starvation survival process. Kurath and Morita (1983) came to this conclusion after observing the endogenous respiration rate of a *Pseudomonas sp.* culture transferred from a spent culture to a buffered medium. Since a drop in respiration rate did not occur, the investigators reasoned that the leaching of organic material from the non-viable cells is not a major factor in the survival of the other cells. It was not ascertained whether this was due to minimal lysis or if the biopolymers generated through lysis were in a form that limited their use as a growth substrate. Gronlund and Campbell (1963) found increases in the ultraviolet absorbing material, largely RNA degradation products, as starvation proceeded with pure cultures of *Pseudomonas aeruginosa.*

Gaudy *et al.* (1971) used sonicated sludge to simulate cryptic growth in an activated sludge system at 16 °C. A 50% reduction in the COD of the supernatant was evident in the first 15 minutes with an over 90% reduction occurring after 25 hours. The sonicate was composed of 5-15% carbohydrate and 40-65% protein on a COD basis. King and Forester (1990) have shown that sonication power influences the concentrations of both soluble carbohydrate and protein. Even though Gaudy *et al.* (1971) used lower power levels over longer periods of time, the results of King and Forester would suggest that some degradation of long chain organics did occur.

Banks and Bryers (1990) extended the studies on cryptic growth using pure cultures of *Pseudomonas putida* and *Hyphomicrobium sp.* The pure cultures of *P. putida* and *Hyphomicrobium sp.* were

grown on C^{14} labeled glucose and methanol to produce radiolabelled cell debris which could be sonicated and used as a substrate. The growth of *P. putida* and *Hyphomicrobium sp.* on the sonicated cell debris was followed by monitoring the radioactivity of soluble (<0.22 μm) and particulate material; and by performing epifluorescent cell counts. *P. putida* metabolized 30-60% of the soluble organic material released by sonication of the radiolabeled cells whereas *Hyphomicrobium sp.* could metabolize only 20-30% of the soluble organic material released regardless of its origin.

Use of the particulate debris (>0.2 μm) remaining after sonication as a growth substrate for either species produced no noticeable increase in epifluorescence cell counts. No significant increase in soluble organic carbon labeled with C^{14} was observed. The investigators believe that hydrolysis of labeled cell debris did not occur. As mentioned above, sonicating cells can lead to the degradation of long chain organics.

Death and decay and subsequent cryptic growth are conceptually equivalent to the death-regeneration concept as currently depicted. From pure culture studies of Banks and Bryers (1990), the biodegradability of the growth substrate derived from cell lysis seems to be dependent on the microorganism carrying out the degradation. The accuracy of most of the current information is suspect because the sonication used to lyse the cell can degrade proteins and carbohydrates.

c) **Endogenous Respiration**

In the absence of an external source of substrate, the cell has to make changes to its metabolic functions to ensure continued survival. Energy needed to sustain maintenance functions is derived through endogenous metabolism. Endogenous metabolism is the summation of all metabolic reactions that occur when a cell is deprived of compounds which can serve as a substrate. The ability to reduce the endogenous metabolic rate rapidly to save dwindling energy reserves, may be a prerequisite for surviving starvation (Dawes, 1976). After 2 days of starvation, the rate of endogenous respiration decreased by 80% for Ant-300 (i.e., a marine *Vibrio*) (Novitsky and Morita, 1977).

Under carbon starvation, energy is derived from the consumption of carbon reserves (e.g. polyglucans, poly-β-hyroxybutyric acid polymers (PHB), and polyvolutins). In the case of *Vibrio* S14, the depletion of PHB reserves developed during growth could be followed as starvation progressed (Rodriguez *et*

al., 1992). Malmcrona-Friberg *et al.* (1986) noted PHB reserves were depleted 3 hours after the onset of carbon starvation in an unidentified marine species. In *E. coli*, depletion of glycogen reserves occurred within 10 hours of being placed under conditions of chronic starvation (Chesbro *et al.*, 1990). High levels of reserve materials do not necessarily guarantee a bacterium's survival. One having a lower content of reserve material may have an equal chance of survival depending on the rate of endogenous metabolism (Dawes, 1976).

The degradation of ribosomal ribonucleic acid (RNA) can be retarded by the presence of intracellular carbon reserves or extracellular products such as polysaccharides. It has been reported that under prolonged starvation up to 85% of the net RNA reserves will be consumed even though RNA synthesis continues (Burleigh and Dawes, 1967; Boylen and Ensign, 1970). While it is thought that in most bacteria the DNA of the microbe remains stable under prolonged starvation, there appear to be exceptions (Siegele and Kolter, 1992). For example, the DNA levels of *Ant-300* cells decreased rapidly during the first 14 days of starvation and then declined only slightly over the rest of the study period (Novitsky and Morita, 1977; Moyer and Morita, 1989).

Activated sludge systems exist as chronically starved systems. Starvation causes significant changes in the cell's metabolic behaviour. A number of the starvation-induced changes discussed above are significant to the general understanding of activated sludge systems. For instance, a substantial portion of the microbial population may exist in a dormant state brought on by starvation. Under nutrient sufficient conditions, the dormant bacteria will again become actively replicating and respiring bacteria. If the number of dormant bacteria was significant, then the existence of dormant population should be incorporated in a revised conceptual model.

A number of researchers have suggested that cryptic growth does not occur under starvation conditions. If this was true, the death-regeneration approach would not be valid under starvation conditions such as those found under batch digestion (Novitsky and Morita, 1977; Dawes, 1976). Rapid decreases in the rate of endogenous respiration over time have been noted for a variety of marine bacteria. Decreased endogenous respiration is a survival tactic employed by microorganisms. Declines in the oxygen utilization rate (OUR) were seen during the transition between chronic starvation, starvation and dormancy. The rate of decline is not a good measure of the rate of heterotrophic death and decay under chronically starved conditions.

This is contrary to the suggestions of Marais and Ekama (1976). A better understanding of energy requirements under starvation conditions does cast doubt on the current conceptual model. Declines in OUR with time derived through batch digestion of biosolids are representative of a system undergoing a transition and not necessarily one operating under chronic starvation.

2.4. Nitrogen Removal

2.4.1. Introduction

Biological nitrogen removal is a two step process. In this process, nitrification is followed by denitrification. During nitrification, ammonia nitrogen is converted into nitrate by autotrophic bacteria under aerobic conditions. The nitrate produced is then reduced by heterotrophic bacteria under anoxic conditions to produce gaseous molecular nitrogen (dinitrogen) in a process called denitrification or dissimilatory nitrate reduction. Based on the experimental work of McCarty *et al.* (1969) when methanol is used as a growth substrate, denitrification proceeds as follows:

$$NO_3^- + 1.08\ CH_3OH + H^+ \rightarrow 0.065\ C_5H_7O_2N + 0.47\ N_2 + 2.44\ H_2O + 0.76CO_2 \qquad \text{Eq. (2.5)}$$

There are two practical ways to achieve denitrification. Predenitrification utilizes organics present in the influent wastewater. With this configuration, denitrification precedes aerobic growth/nitrification with nitrate being recycled back to the anoxic reactor. The stability of the process depends on sufficient nitrate being recycled back to the anoxic reactor. With post denitrification, denitrification follows nitrification. This process has the advantage of increased process stability; however, additional costs are incurred in providing an exogenous source of carbon. Organics are added in excess of stoichiometric requirements to ensure complete denitrification. Residual organic substrate will always be present in the plant effluent unless additional carbon removal facilities are available. In the case of low organic strength wastes or if denitrification proceeds under hydrolysis limited conditions, use of a supplementary carbon source is required for both pre-denitrification and post-denitrification configurations.

2.4.2. Carbon Limited Growth

For most systems achieving denitrification, the organic carbon to nitrogen ratio is a critical variable. Lyngå and Balmér (1992) suggest that for COD/N ratios above 15, nitrate removal is complete or close to complete. At COD/N ratios below 15, nitrate removal is highly variable. There are three possible sources of soluble biodegradable organics: the influent wastewater, the products of endogenous respiration and exogenous carbon sources (e.g. methanol, fermented primary sludge and brewery wastewater).

In a study using influent wastewater as a carbon source for denitrification, van Haandel *et al.* (1981) reported the occurrence of two distinct phases with characteristic rates when activated sludge was mixed with influent wastewater under batch conditions. The initial phase proceeds until the rapidly biodegradable COD present in the influent is depleted. During this phase, the rate of denitrification is relatively fast. During the second phase, slowly biodegradable organics are hydrolysized and consumed in denitrification. The rate of denitrification proceeds at a much slower rate than that realized during the initial phase. The rate of denitrification realized under these conditions was similar to those produced by post-denitrification plug flow systems. This is consistent with the findings of Barnard (1975) who found three distinctive and successive zero-order rates of nitrate reduction. The third rate overlapped with the second and corresponded to the period when the contribution of organics generated by endogenous respiration became significant.

Henze *et al.* (1993) proposed the existence of three distinct fractions for biodegradable substrates present in wastewater: directly metabolizable, easily degradable and slowly degradable. An example of a directly metabolizable substrate is acetic acid. Higher volatile fatty acids, lower alcohols, lower amino acids and simple carbohydrates are all considered examples of easily degradable substrates. Most slowly degradable substrates are complex organics having a higher molecular weight. Directly metabolizable substrates do not require the participation of exoenzymes. Easily degradable and slowly degradable both require hydrolysis but easily degradable substrates require that far fewer bonds be broken. Denitrification rates for comparable cultures using the various fractions are presented in Table 2.9.

Table 2.9 Denitrification Rates at 20°C for Various fractions of COD in Municipal Wastewater (Henze *et al.* (1993))

Fraction	Denitrification Rate (mg N/(g VSS·h))
Directly metabolizable	10-20
Easily degradable	2-4
Slowly degradable	0.2-0.5

Different rates of denitrification are realized for denitrifying and biological phosphorous removal systems during phase two as described by van Haandel *et al.* (1981). Griffths (1994) suggested the presence of two distinct groups of anoxic heterotrophs in nitrogen removal systems. The first group has a competitive advantage as they are able to utilize rapidly biodegradable organics anoxically; however, they are unable to hydrolyse slowly biodegradable organics (SBCOD) anoxically. The second group is capable of hydrolysing SBCOD under anoxic conditions. This group retains and utilizes the hydrolysis products. Novak (1995) in a comment on Griffths proposal suggested that the profile described by van Haandei *et al.* (1981) and Barnard (1975) involves four phases. They consist of the elimination of: directly metabolizable, easily degradable, slowly biodegradable and organics derived by cell lysis. The breakpoints between phase one and phase two may or may not be visible depending on the substrate quality. He further explains it might be difficult to distinguish between the end of phase three and the beginning of phase four. The difficulty results from the wide range of molecular weights of slowly biodegradable organics that probably translates into different rates of hydrolysis.

Several researchers have evaluated the feasibility of using exogenous organic substrates. A comprehensive listing of studies on various exogenous substrates has been compiled by Mateju *et al.* (1992).

2.4.3. Effect of Temperature on Nitrogen Removal Kinetics

The efficacy of nitrogen removal at low temperatures is a concern for wastewater treatment plants in Southern Ontario as well as much of Canada and parts of the United States. A profile of the average aeration basin temperatures for Waterloo STP is presented in Table 2.10.

Table 2.10 Temperature Profile Waterloo STP (after Whitehead (1980)).

Month	Average Temperature °C	Month	Average Temperature °C
January	13.2	May	15.3
February	13.2	June	19.6
March	12.6	July	20.4
April	12.8	August	22.1

Overall process performance is a function of organic carbon source, temperature and the growth kinetics of denitrifying organisms (Lewandoswki, 1982). van Haandel *et al.* (1981) found denitrification rate coefficients (mg NO_3-N/(g active organisms · h)) to be independent of sludge age, influent COD, sludge concentration and recycle ratios over the temperature range of 14-20 °C.

Oleszkiewicz and Berquist (1988) have shown nitrogen removal to be a viable process down to temperatures as low as 2 °C. In this study, SBRs were fed raw sewage augmented with a high strength pharmaceutical wastewater. The resulting influent had a COD:TKN ratio varying between 2.3-2.7. To achieve denitrification and ultimately nitrogen removal in a SBR, aeration was turned off for a portion of the react cycle. The investigators found denitrification could be achieved by not supplying air for 2 h of the 8 h react cycle or alternately 2 h for a 12 h react cycle. Although the rate of denitrification was comparable for the two cycles in the 7-15°C range, higher efficiencies where realized with a 8 h cycle when the temperature where in the 2-7 °C range. The rate of change of denitrification with temperature was discontinuous at 7 °C. Temperature correction factors for the unit rate of denitrification (g NO3-N/ (gVSS·h)) were discontinuous. Over the 7-15 °C range, the modified Arrenhius coefficient for denitrification (θ_{DN}) was 1.06. Over the 2-7°C range, θ_{DN} is 1.30. The discontinuity mentioned at 7 °C may reflect a change in the mechanism controlling denitrification.

Barnard (1975) studied biological nitrogen removal over a more moderate range of 17-25 °C. Four reactors under alternating anoxic and aerobic conditions were run in series. Raw sewage (COD:TKN ratio of approximately 10-13) entered the first basin and contacted with the mixed liquor from the second basin which was recycled at a rate of three to four times the average flow rate. Almost complete denitrification occurred in the first basin where 70-80% of the nitrogen removal occurred. Total nitrogen removals of 94% were achieved. The denitrification rates at 20°C were 3-6 and 1-3 mg NO_3-N/ (g MLSS · h) for the first and third basin, respectively. The corresponding θ values were 1.09 and 1.20, respectively over the temperature range of 17-25 °C.

van Haandel *et al.* (1981) determined the temperature sensitivity of each of the three rate coefficients using a plug flow reactor at temperatures between 14-20 °C. The coefficients for phases one, two

and three were 1.20, 1.08 and 1.03, respectively. The data showed wide variation (±25%) with values being either extremely high or extremely low. The investigators rationalized the fluctuations as being batch to batch variation in the composition of the wastewater.

The effect of temperature on microbial kinetics is difficult to discern when influent wastewater is the carbon source, since often substrate and temperature effects are confounded. Methanol is a widely used carbon source in denitrification. Sutton *et al.* (1978) performed one of the few studies where both raw sewage and methanol were independently used as substrates under similar environmental conditions. The organics present in raw sewage produced comparable denitrification rates when compared to methanol. Although generalizations cannot be made, two recent studies suggest appreciable gains in the rate of denitrification can be made when methanol is substituted for raw sewage (Tam *et al.*, 1992a; 1992b).

Use of methanol as a substrate led to the development of modified Arrhenius coefficients for sludge ages of 3, and 6 days (Sutton *et al.*, 1974). Pooling of data derived from batch and continuous testing led to modified Arrenhius coefficients of 1.093 and 1.097 for sludge ages of 3 and 6 days, respectively. At a temperature of 7 °C and an anoxic SRT of 7 d, the unit rate of denitrification where raw sewage was the carbon source (0.026 mg NO3-N+NO2-N/(mg MLVSS- d)) was comparable to that derived using methanol (0.023 mg NO3-N+NO2-N/(mg MLVSS- d)), suggesting similar low temperature performance when either pre-denitrification or post-denitrification configurations are used.

Lewandoswki (1982) evaluated the performance of denitrification using: acetic acid, acetone, endogenous sources and methanol. The investigator reported a linear rate between 5-35 °C. Over the 2.5-5°C range, the temperature response became much different which is similar to that noted by Oleszkiewicz and Berquist (1988). Although the rates of denitrification were dependent on the chosen substrate, no significant differences in the modified Arrhenius coefficient derived for each of the substrates was noted.

Halmo and Eimhjellen (1981) considered the efficiency of two denitrifying sludges enriched at 5 and 20 °C using methanol as an organic carbon source. The investigators wanted to develop cultures with distinctive psychrophilic and mesophilic character. From batch studies over the temperature range 0-8 °C, the low temperature sludge had 3-4 times higher rate of denitrification than the one grown at higher temperatures.

At the higher temperatures (18-20 °C), the rate of denitrification of the predominantly psychrophilic sludge was comparable to that of the sludge grown at warm temperatures. The maximum rate of denitrification for this sludge was obtained at a temperature of approximately 14 °C.

Continuous culture experiments produced some unexpected results. The high temperature sludge operating at 22 °C gave 96-98% nitrate removal down at a HRT of 2 hours. Comparable performance was achieved with the low temperature sludge at 2 °C and a HRT of 3 hours. Exposure to temperature changes of up to 10 °C did not seem to influence the denitrification characteristics of any of the sludge types. The low temperature sludge was exposed to 17 °C for some time. Once the temperature was restored to 2 °C, the behaviour reverted to normal. After 4 weeks of maintaining the culture at 2 °C, there was a significant loss of denitrification capacity. Further testing with a three-stage process evaluated the feasibility of biological nitrogen removal at 5 °C. When complete nitrification was achieved, 94-97% of influent inorganic nitrogen was removed. The investigators made no attempt to characterize the temperature response of the biological nitrogen system.

2.4.4. Biomass Production in Nitrogen Removal Systems

As indicated above, yield under anoxic conditions has been found to be significantly less than that realized under aerobic conditions for pure cultures of denitrifiers. The behaviour of the mixed microbial population present in activated sludge may deviate markedly from pure cultures. A number of investigations comparing sludge production of denitrifying, and nitrogen removal systems to that of aerobic carbon removal systems have been done and are summarized in Table 2.11.

Table 2.11 Biomass Production in Nitrogen Removal Systems

SRT (d)	Anoxic Aerobic (g/g)	Pre-denit Aerobic (g/g)	Temperature (°C)	Carbon Source	Investigator
<10	0.6-0.7		24-26	Nutrient Broth	McClintock et. al. (1992)
8-12	≈1		20	Primary Effluent	Symth (1994)
13		0.85-0.89	6-20	Glucose	Ketchen (1994)
13		0.70	20	Glucose/Starch	Ketchen (1994)
13		0.69	13	Glucose/Starch	Ketchen (1994)
13		0.92	6	Glucose/Starch	Ketchen (1994)

The goal of experimental studies carried out by Symth (1994) was to observe and compare sludge production and COD removal efficiency in aerobic and anoxic systems under steady-state conditions. The experimental program used primary effluent from Mid Halton STP as a feed source for a number of fed batch reactors operated at 20°C. The study concluded that sludge production from an anoxic system is very similar to that produced by an equivalent aerobic system and may even be higher. Sludge production did not seem to vary much with sludge age over the range of 8-12 days.

Symth (1994) went on to reevaluate the work of McClintock (1992). The original investigation found sludge production to be approximately 40% less for anoxic conditions in comparison to aerobic conditions at sludge ages of 10 days or less. Reductions in sludge production were attributed to lower true yields and endogenous rate coefficients and higher maximum substrate utilization rates. Further analysis by Smyth suggested that the accumulation of endogenous products might account for differences in the results. She further suggested that no reliable comparison between the aerobic and anoxic activated sludge parameters could occur until a more extensive data base was available.

Ketchen (1994) considered biomass production of nitrogen removal systems in comparison to those achieving carbon removal/nitrification. Two SBRs were operated in parallel at temperatures of 6, 13 and 20°C. Glucose, a rapidly biodegradable substrate, not requiring hydrolysis was used as a feed stock. Sludge production for both reactors increased with decreasing temperatures. This same general trend of increasing sludge production with decreasing temperatures has been reported for both carbon (Tian *et al.*, 1994; Sollfrank *et al.*, 1992) and nitrogen removal systems (Oleszkiewicz and Berquist, 1988). The pre-denitrification reactor generally had a lower observed yield than the one achieving strictly carbon removal. This is in stark contrast with the findings of Symth (1994).

2.4.5. Summary

The following key points can be summarized from the literature review of nitrogen removal:

1. There are conflicting reports of sludge production under aerobic, anoxic or alternating anoxic/aerobic conditions for activated sludge systems. Some researchers suggest equal sludge production under anoxic and aerobic conditions, but this is not in keeping with pure culture and theoretical considerations.

2. The overall rate of denitrification is dependent on the type of organic substrate present. Much higher rates of denitrification are realized when rapidly biodegradable organics are used instead of slowly biodegradable substrates suggesting hydrolysis may be an important factor in the denitrification process.
3. Current rates of denitrification are based on the rate of removal per unit of volatile suspended solids making the rate of removal a function of sludge composition. Unless similar carbon loadings and sludge are maintained; the results of one study are not directly comparable to the results of another. The best way to overcome this is through the use of enumeration techniques that will define the underlying microbial population. Another alternative is to use a mathematical model to estimate the growth kinetics (μ_m, K_s). In turn, the growth kinetics of the different systems can be directly compared.

3. Method Development

3.1. Wastewater Characterization

3.1.1. Introduction

Within the context of Activated Sludge Model 1 (ASM1) and Activated Sludge Model 2 (ASM2), slowly biodegradable substrates require degradation by enzymes external to the cell (i.e., hydrolysis) before becoming available growth substrates. Readily biodegradable substrates can be used directly by the cell (Ekama *et al.*, 1986). The conceptual distinction between rapidly and slowly biodegradable substrate is simple; however, developing an operational protocol capable of distinguishing between the two is not. Many protocols characterizing the wastewaters into slowly and rapidly biodegradable fractions assume that hydrolysis controls the overall rate of growth but this has not yet been proven to be the case. It was also shown in the literature review, that the rate of hydrolysis was dependent on a number of conditions such as growth phase, temperature, and the presence of substrates which induce or repress enzyme production.

A characterization protocol based on metabolic response has a number of disadvantages. Ekama *et al.* (1986) suggested that characterization of an influent could be achieved by monitoring the OUR of the influent wastewater combined with an appropriate quantity of microorganisms in a batch reactor. With a suitable initial substrate to microorganism ratio, the OUR should remain constant for 1-3 hours, then precipitously decrease. Ekama *et al.* (1986) hypothesized that the initial high OUR results from the metabolism of rapidly degradable organics while the second lower plateau can be attributed to the slowly biodegradable organics which remains relatively constant until these latter organics are exhausted. Wastewaters of identical composition may be characterized differently using this protocol because of variability in metabolic response which may have been induced by factors other than substrate complexity. A protocol based on physical characteristics is desirable because it allows the character of wastewaters of different origins to be easily compared.

An alternative protocol exists based on the premise that removal of the colloidal (0.001-1μm) and supra colloidal (1-100μm) material will yield a solution principally composed of soluble organics (Mamais *et al.*, 1993). The COD exerted by these organics is believed to represent the rapidly biodegradable COD present. In reality, it overestimates the rapidly biodegradable COD. Soluble long chain organics requiring hydrolysis will be erroneously included in this measurement.

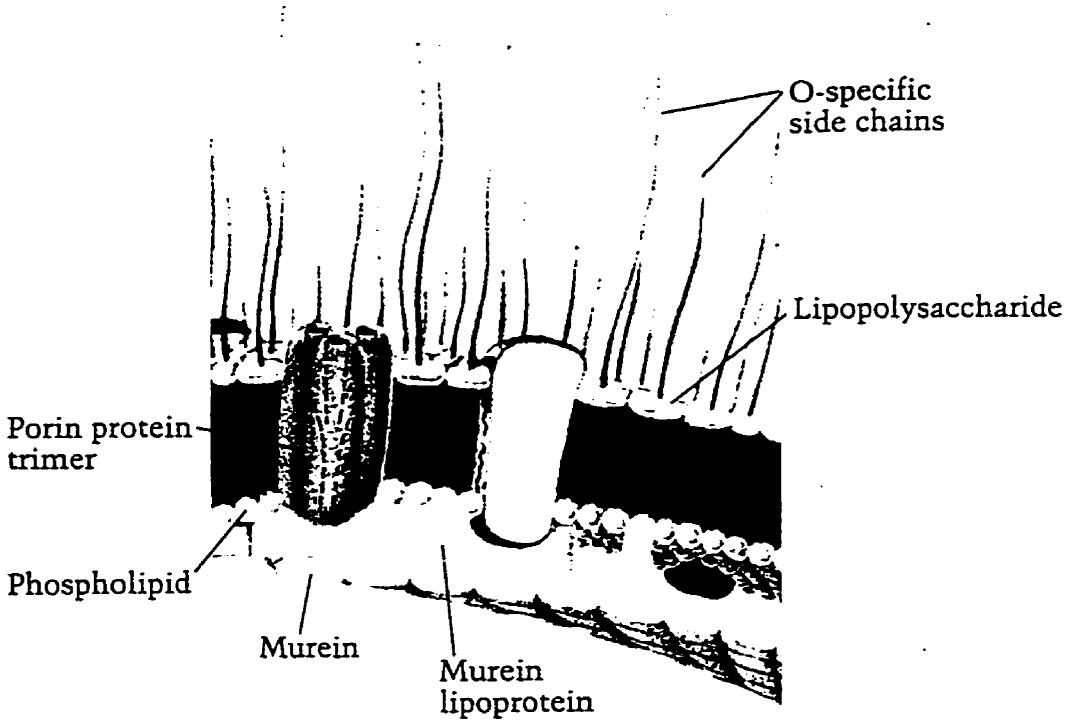
3.1.2. A New Operational Definition

None of the previous protocols have considered the mechanism of substrate transport through the cell wall. When considering substrate transport, it is important to recognize that the bacteria of activated sludge are predominantly Gram-negative (Hawkes, 1963). The mechanism of substrate transport differs significantly in Gram-negative and Gram-positive bacteria. Many Gram-negative bacteria exclude many hydrophobic, amphiphilic and hydrophilic molecules above a given size limit (Hancock, 1991).

Gram-negative bacteria possess water filled protein channels, porins, that allow small hydrophilic molecules to diffuse through the phospholipid and lipopolysaccharide layers of the cell wall (Figure 3.1). Most porins are non-selective which distinguishes them from many other transport processes. Diffusion through non-selective porins is generally limited to compounds with sizes less than the porin exclusion limit of 600-1400 Da (Jeanteur *et al.*, 1994). Factors affecting substrate permeability include size (chain length and steric factors), shape, hydrophobicity and electrical charge.

Selective porins permit the diffusion of specified growth factors larger than the porin exclusion limit. Selective porins have been noted for: maltose and maltodextrins, Vitamin B12, and at least four proteins specific for iron uptake (Benz, 1985). Expression of some of the highly selective porins is often regulated by environmental conditions such as osmolarity, phosphate concentration, and temperature (Benz, 1985). Considering the physiology of Gram-negative bacteria, this study proposes using a MW of 1000 Da to operationally distinguish between rapidly and slowly biodegradable organics. Slowly biodegradable substrates require hydrolysis before becoming available for growth. Rapidly biodegradable substrates are present in a useable form.

Figure 3.1. Major Features of the Cell Envelope of a Gram-negative bacterium (after Neidhardt *et al.*, 1990)



3.2. Enumeration of the Microbial Population

3.2.1. Enumeration Techniques

a) Plate Counts

Plate counts substantially underestimate the total number of bacteria present in environmental samples (Oliver, 1993). This is to be anticipated since there is no single universal medium capable of supporting the growth of all organisms. Thus, a portion of the population may exist in a viable but non-culturable (VBNC) state. Viable bacteria would include replicating, and dormant organisms. The four physiological states that a bacteria may have was defined previously in Section 2.2.b.i. VBNC bacteria are metabolically active, while being incapable of undergoing the sustained cellular division required for growth in or on a medium normally supporting growth (Oliver, 1993; Nilsson *et al.*, 1991).

The VBNC state can be induced by changes in temperature, salt levels, nutrient levels, light, aeration, temperature, cell washing and the physiological age of the culture (Nilsson *et al.*, 1991; Oliver, 1993).

Of particular interest is the response to temperature reductions. Nilsson *et al.* (1991) found that *Vibrio vulnificus* would respond to a temperature downshift from 20°C to 5 °C by assuming a VBNC state. An upshift in temperature (5 to 20°C) resuscitated the cells giving plate counts comparable to those prior to the temperature change. The response to subsequent repeated temperature shifts was identical. Typically activated sludge cultures are older cultures that have been subjected to repeated temperature shifts. These conditions would promote the existence of bacteria in a VBNC state.

b) Electron Transport Activity

The activity of the electron transport system (ETS) or the quantity of energy produced as adenosine triphosphate levels, is often used as an indicator of microbial activity. The ETS is mediated by the action of several dehydrogenase enzymes and thus, measurement of dehydrogenase activity is a reliable indication of ETS activity. Dehydrogenase activity can be indirectly assessed using redox dyes (e.g. methylene blue, resazurin) or tetrazolium salts.

The respiring population would include replicating, non-replicating and dormant organisms. In these organisms, tetrazolium salts compete with oxygen for electrons with the resulting reduced salts forming insoluble formazan compounds. Reduction of the tetrazolium salts occurs at the inner surface of the cytoplasmic membrane (Sedar and Burde, 1965). Salts commonly used include 2,3,5-triphenyltetrazolium chloride (TTC), 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT). After incubation with the tetrazolium salt, the cells are disrupted and the formazan solublized. After centrifuging, the spectral absorbance measurements of the supernatant are made. This method requires an active ETS with a minimum level of activity. The ETS of dormant bacteria operates at reduced levels so this method may or may not detect dormant bacteria.

The success of this method is dependent on the bacterial species and the tetrazolium salt being used. Not all bacterial species are capable of tetrazolium salt reduction; thus, the level of total bacterial activity may be underestimated (Trevors, 1984). When stains were applied to a wide variety of microorganisms under comparable conditions, methylthiazolydiphenyltetrazolium (MTT) and INT were taken up by a wider range of organisms than NBT (nitroblue) (Thom *et al.*, 1993). It has also been noted that sufficiently high

concentrations of the salts may suppress the ETS (Lopez *et al.*, 1986; Trevors, 1984). Further inaccuracies result from dissolved oxygen competing with the chosen salt as an electron acceptor (Miksch, 1983). Despite these possible limitations, INT reduction has shown good correlation with the OUR, an independent measurement of culture metabolic activity, by a number of investigators (Trevors, 1984; Urbain *et al.*, 1993).

c) **Total Direct Counts**

The use of acridine orange direct counts (AODC) to enumerate aquatic bacteria is well established. For this technique, cells are counted on a polycarbonate filter after the filtration of a known sample volume. The cells are stained using acridine orange which stains nucleic acids. Stained DNA fluoresces green while RNA fluoresces orange (Hobbie *et al.*, 1977; ASTM, 1994; Bitton *et al.*, 1993). It has been suggested that orange cells could be deemed viable due to the excess of RNA relative to DNA resulting from active metabolism whereas dead cells fluoresce green (Carillo *et al.*, 1985; Lopez-Torres *et al.*, 1987). Differentiation between active and inactive bacterium by colour is inappropriate because the moisture content of the filter will influence the colour of fluorescence (Bitton *et al.*, 1993). A number of stains can be substituted for acridine orange; these include ethidium bromide, 4,6-diamidino-2-phenyl indole (DAPI), acriflavine, and bisbenzimide (Bergström *et al.*, 1986).

d) **Determination of Total and Respiring Bacteria**

The number of total and respiring bacteria can be enumerated by using the AODC protocol in conjunction with the use of tetrazolium salts and microscopic examination. In the ASTM protocol for aquatic bacteria, Method 4454-85 (ASTM, 1994), the sample is incubated with INT prior to being stained with acridine orange. Tetrazolium salts like INT are reduced by an active electron transport system to form an insoluble formazan crystal. There are two patterns of formazan deposition either combined extracellular and cell-associated deposits or exclusively cell-associated deposits. Mixed deposits were observed with MTT and INT, whereas NBT and NT produced exclusively cell-associated deposits (Thom *et al.*, 1993).

If INT is used, the crystal produced can be identified under direct bright field microscopic examination as a bright red spot. The sites of dehydrogenase and INT-formazan deposits may not be necessarily identical. INT seems to coalesce after its reduction to form a typical larger formazan deposits in the

cytoplasm (Zimmermann *et al.*, 1978). Bacteria lacking formazan spots maybe in a state of activity which is below the level of detection (Zimmermann *et al.*, 1978). Recognition of formazan deposits as a bright-field image is difficult with bacteria less than 0.4 μm in diameter (Zimmermann *et al.*, 1978).

Although incubation with INT and later microscopic examination is considerably more accurate than either plate counts or activity measurements under some conditions, the technique may significantly underestimate the number of respiring cells. Whereas only 3% of the cells exhibited detectable INT-formazan deposits, 100% of these same cells were platable following room temperature resuscitation (Nilsson *et al.*, 1991).

e) Applicability to Field of Wastewater Treatment

As shown in Table 3.1, the population enumerated will depend on the technique employed. None of the conventionally employed techniques can enumerate the respiring bacterial population. Mason (1986) after a literature review suggests of the techniques evaluated, incubation with INT and further microscopic examination in conjunction with AODC provides the most comprehensive results. Advanced staining techniques such as INT incubation/microscopic evaluation and AODC have been used only on a limited basis in the field of wastewater engineering (Table 3.2). The current research evaluated the value of using incubation with INT and AODC as an enumeration technique. Preliminary evaluations of this technique showed it was difficult to see the INT deposits so only AODC counts were done. AODC were used extensively in the experimental program and the protocol for this enumeration technique is found in the following section. The applicability of the cell counts will be discussed in Chapter 6.

3.2.2. Experimental Protocol

a) Homogenization and Dilution

Counting the number of bacteria present in environmental samples is challenging in part because of the high concentrations of particulates. The bacteria are often colonizing the surface of these particulates making it difficult to visualize the bacteria. Even when the bacteria are dislodged from the surface of the particles, the particles may land on the membrane surface during filtration and cover free suspended bacteria and other particles. With activated sludge, the majority of the bacteria are contained within the sludge floc.

Table 3.1 Evaluation of Microbial Enumeration Techniques (after Mason *et al.* (1986))

Method	Environment of Test	Cell Replication Required	Cell Types Enumerated	Differentiation Between Cell Types	Accuracy
Plate Count	Modified	Yes	(Rep), (Dorm)	Yes	Low
MPN	Modified	No	Rep, Non-Rep, (Dorm)	(Yes)	Low
ETS Incubation with Tetrazolium Salts/Microscopic Examination	Original/ Modified	No	Rep, Non-Rep,(Dorm)	No	High
AODC	Original/ Modified	No	All	No	High

Rep, Non-Rep and Dorm have been defined in Section 2.3
All refers to replicating, non-replicating, dead and dormant cells.
Parentheses imply that only a part of this population was enumerated.

Table 3.2 Advanced Enumeration Techniques Employed in the Field of Wastewater Treatment

Technique	Protocol	Study
ETS	Resazurin Reduction	Liu (1983)
	INT	Urbain *et al.* (1993)
		Lopez *et al.* (1986)
	TTC	Miksch (1983)
Tetrazolium Salts/ Direct Microscopic Examination	CTC/Microscopic Examination	Rodriguez *et al.* (1992)
	Malachite Green	Dutton *et al.* (1983)
	INT/MicroscopicExamination	Dutton *et al.* (1983)
Direct Count	AODC	Rodriguez *et al.* (1992)
		Urbain *et al.* (1993)

Thus, the number of bacteria counted are representative of the total population only when the floc is disrupted and all fluorescing cells are viewed. Preliminary work undertaken in this research using acridine orange on intact floc showed individually fluorescing cells within the floc. The floc structure appeared to be open enough to allow for diffusion of the dye through the matrix.

AODC enumerates bacterial having stainable DNA and RNA. Theoretically, this would include replicating, non-replicating, dormant and dead bacteria. Sub-lethal injury to the bacteria caused by shear or heat are not important. The counts will be altered only if the DNA and RNA of the bacteria are no longer intact or if the DNA and RNA are denatured in a way which changes their interaction with the dye. Plate counts or measurements examining electron transport activity could be affected by sub-lethal injury.

Mixed liquor samples augmented with Tween 80, a surfactant, were homogenized using a Waring blender. It was believed that Tween 80 would limit the restabilization of particles after homogenization had been completed. Camper *et al.* (1985) found homogenization using a Waring blender at 16 000 rpm to be the best way to desorb bacteria from granular activated carbon. The investigators reported that the blender produced a rapid temperature increase; the final temperature was approximately 30°C after 3 minutes of blending. Heterotrophic plate counts of *Escheria coli* did not decrease because of blending until a temperature of 45°C was exceeded, but some injury did result. Injury is described as the percent difference between counts on two different types of media.

Samples were previously preserved by adding 37% formaldehyde (Sigma Chemical, St. Louis, Mo., F-1268) for a final concentration of 0.1 mL/10 mL. Samples were homogenized using the following protocol. The high cell concentrations in the original samples and the short interval between homogenization and staining made it possible to proceed without aseptic conditions.

1. 1 mL of a 0.1% solution of Tween 80 (Polyoxyethylene 20 Sorbitan Monooleate) was added to 50 mL of the mixed liquor; surfactant was obtained from Fisher Scientific Company, Fairtown, USA. The stock solution was filtered using a 0.2 μm syringe filter prior to use to remove any bacteria that may have grown in the solution.
2. The solution was placed in the Waring Blender where it was homogenized for 45 s.

3. The mixed liquor was left for 5 minutes to allow the surfactant time to diffuse through the floc after which the mixed liquor was blended for an additional 3 minutes.

Direct microscopic examination of samples stained with acridine orange (See below for staining procedure) showed very few floc particles under 125 x and 500 x magnification. Under 1250x magnification, some small flocs were seen.

Duplicate 1 mL samples were taken of the homogenate and diluted in 9 mL of filtered sterilized water. The dilution water was prepared with Milli-Q water filtered through a 0.2 μm filter (Sartorius, Mississauga, Cat No. SM-2007). Two or three samples of the MLVSS were taken through the dilution, staining and counting procedure independently of each other. Samples were diluted so that between 20 and 200 cells were seen in the square of the eyepiece micrometer. Aerobic samples typically required 10^3 dilution while the anoxic samples required a 10^4 dilution.

b) **Staining**

1. Acridine Orange Stain

10 mg of Acridine Orange (3,6 bis [Dimethyl-amino]acridine) (Sigma Chemical, St. Louis, Cat. No. A-6014) was dissolved in 100 mL of Milli-Q water. Approximately 40 mL of the stain was filtered using a sterile 0.2 μm cellulose acetate syringe filter (Corning, Corning NY. Cat. No. 21052-25) and syringe. The solution was stored in a sterile amber glass vial in the refrigerator for up to three weeks.

Herbert (1990) suggest acridine orange can be stored for up to 2 months prior to use at room temperature. To check the sterility of each new batch of acridine orange, filter sterile water was stained with acridine orange and taken through the procedure outlined below. If bacteria were detected in the reagent blank, then either the dilution water or the stain was contaminated. Bacterial contamination would bias the results of the enumeration.

2. An autoclaved Nucleopore® (Costar Scientific Corporation, Cambridge MA) black regular polycarbonate filter 25 mm (0.2 μm pore size) and a 25 mL filter apparatus (Sartorius, Mississauga, Cat No. SM16306) was used for collecting the bacteria from the stained sample.

3. To the final sample dilution, 1 mL of acridine orange stain was added. The volume of the final dilution may be 9 or 10 mL depending on which dilution in the series was used. The final concentration of acridine orange was 9-10 μg/mL.
4. After a minimum of 3 minutes, the contents of the test tube were added to the filter tower. Incubation times slightly longer than this did not affect the enumeration number.
5. The vacuum was turned on and the contents of the filter tower were filtered. With the vacuum on, the sides of the filter tower were rinsed with filter sterilized water.
6. The membrane was removed from the membrane filter support and allowed to air dry.
7. After drying the membrane was placed on a clean microscope slide to which had been added 2 drops of Cargille Type FF immersion oil (Cargille Lab, Cedar Grove NJ, Cat. No. 16212). Another drop of immersion oil was placed on top of the membrane and the cover slip was applied.
8. The filter tower was reassembled. To reestablish sterility it was rinsed initially with 95% ethanol and then later with filter sterilized water.

c) **Viewing and Counting**

Filters were viewed using a Nikon Microscope with Episcopic-Fluorscent Attachment EF-D. The filter cube used had a 400 - 440 nm excitation wavelength and a barrier filter with a lower cut off of 470 nm. A eyepiece micrometer with a 10x10 square was used for counting cells. This micrometer was calibrated for this specific microscope using a stage micrometer. All counts were done at approximately 1250x magnification. At this magnification, the eyepiece micrometer measured 60μm^2. Fields for counting were chosen from different areas of the filter. To eliminate the natural tendency of choosing those fields with high concentrations of cells, the field of view was chosen with the shutter closed. The same segment of the graticule was consistently used for counts on a single filter.

The precision of the count may depend on both the number of fields counted and the counts per field. Herbert (1990) stated that most investigators have found bacteria on membrane filters to be represented by a Poisson distribution. With this distribution the precision of the count depends on the number of bacteria counted and not the number of fields. Most researchers count at least 400 bacteria per filter.

With samples coming from natural environments, a lack of homogeneity is often a problem. Montagna (1982) examined replication of counts as determined by borehole core, filter and microscope field. The results of his analysis suggest the more reliable results are determined by counting five fields of at least 30 bacteria on four replicate filters rather than by counting 20 fields on one filter. Kirchman *et. al* (1982) in a similar study with water, recommended counting 25 bacteria per field and two replicate filters.

Both of these studies underlined the need for counts on replicate filters. In this experimental program, 10 fields containing at least 20 bacteria were counted for at least two replicate filters. The total number of bacteria counted per unit volume is calculated using:

$$N_b = \frac{AnD}{a_{net}V} \qquad \text{Eq. (3.1)}$$

N_b	=	number of bacteria per mL
A	=	wetted area of 25-mm filter (μm^2)
n	=	average number of bacteria per net micrometer field; cells per 100 squares
D	=	dilution factor
a_{net}	=	area of the net micrometer field (640 μm^2)
V	=	volume of sample (mL)

d) **Limitations of AODC**

The AODC protocol can only detect bacteria larger than 0.2μm, the size of the pores in the polycarbonate membrane. Increases in AODC for cultures actively metabolizing substrate are due to replication or by bacteria increasing to a size larger than 0.2μm or by a combination of these two processes. The condition which prevails depends on the initial size of the bacteria and the conditions under which growth proceeds.

e) **Analysis of Results**

An Analysis of Variance (ANOVA) was done to determine if the counts for replicate filters were significantly different at the 95% confidence level. Prior to analysis, the number of bacteria per unit volume was log transformed. If the ANOVA suggested that there was no significant difference between the filters, the data was pooled. Approximate upper and lower 95% confidence limits on the cell counts were developed from the pooled data set. If the ANOVA did find significant differences in the filters, a third replicate was counted. Each replicate underwent dilution, staining and counting independently.

4. Materials and Methods

4.1. Overview

The experimental program was undertaken in two distinctive phases: Phase 1 (Sept. 1995-May 1996) and Phase 2 (July 1996-Aug. 1996). The majority of the goals of each phase were unique to that particular phase; however, there was some replication. In Phase 1, the SBRs used casein and albumin as principal protein substrates. In Phase 2, lactalbumin was substituted for these two proteins. With a culture acclimated to lactalbumin, it was possible to repeat some of the experiments of Phase 1 to determine if the underlying cause of the observed behaviour was related to culture conditions. It should be noted that at the end of Phase 1, the culture was discarded. Phase 2 began with a new inoculum of mixed liquor from the aeration tanks of Waterloo STP.

4.2. Phase 1

4.2.1. Sequencing Batch Reactors

a) Introduction

This laboratory study employed two reactors run in parallel. Operation of the reactors were identical in all respects with the exception of the electron acceptor. One reactor was run under aerobic conditions while the second reactor denitrified. Use of SBRs allowed for a more precise COD measurements as the problem of accounting for daily changes in the clarifier solids inventory was eliminated.

Temperature levels of 14 and 20 °C were chosen because of the historical data of the Waterloo STP presented in Table 2.10. The lower temperature is approximately, the average of the monthly temperatures for January - May. The average for July and August is 21°C. A slightly lower temperature than this was chosen because most studies reported in the literature were done at 20°C. Batch testing was conducted concurrently with on-going SBR experiments.

Each 10 L reactor was fed 8 L/d of substrate resulting in a HRT of 1.25 d. The operating cycle of the reactor is outlined in Table 4.1. The cycle began with feed being added to 2 L of agitated mixed liquor. The react portion of the cycle began after 12 h of feeding. During the wastage portion of the cycle, 1/10th of the reactor contents were wasted resulting in a target SRT of 10 d. The realized SRT calculated using Eq. 4.1 was less than 10 d due to unintentional solids losses in the effluent.

$$SRT = \frac{VX}{Q_e X_e + Q_w X} \qquad \text{Eq. (4.1)}$$

where SRT = solids retention time (d)
V = reactor volume (L)
Q_E = effluent volume (L)
E_x = effluent solids concentration (mg VSS/L)
Q_w = wastage volume (L)
X = reactor mixed liquor concentration (mg VSS/L)

Table 4.1 Sequencing batch reactor (SBR) operating cycle

Operation	Hours	Volume (L)	Time of Initiation
Fill	12	2.0-10	11:00 a.m.
React	10.25	10	11:00 p.m.
Waste	0	10-9	9:00 a.m.
Settle	1.25	9	9:15 a.m.
Draw	0.25	2.0	10:30 a.m.
Idle	0.25	2.0	10:45 a.m.

b) Substrate Selection

Selection of substrates for use in the SBR experiments was done with the needs of the batch studies in mind (Table 4.2). When the research was initiated, one of the hypotheses to be tested was that hydrolysis was dependent on the molecular weight of the substrate. This was to be addressed specifically in batch studies using refined proteins with a narrow molecular weight distribution. These refined proteins are expensive and thus unsuitable for use on a large scale so crude proteins containing the protein of interest were used. Use of the crude proteins as a daily feedstock for the SBRs ensured that the biosolids generated were acclimated to the refined proteins. With casein and lactalbumin being of similar molecular weight, it was decided to use only one of the two proteins; thus the proteins selected as a SBR feedstock were casein and albumin.

Table 4.2 Protein Selection Criteria

	Criteria			
Purified Protein	Purified Protein	α-casein	ovalbumin	lactalbumin
	Molecular Weight	22,000-25,000	45,000	NA
	Cost of Purified Protein	$26/g	$8/g	NA
Bulk Form	Bulk Protein	casein	albumin	lactalbumin
	Cost of Bulk Protein	$20/kg	$39/kg	$18/kg
	Level of Purity	90% protein, 0.2% lactose	54% ovalbumin	80% protein 4% lactose
	Contaminants Present	β-casein, κ-casein, milk sugars	avidin, conalbumin, lysozyme, ovomucoid	

Technical grade casein from bovine milk contains 90% protein and 0.2% lactose. Whole milk at pH 4.6 and 20°C has two protein fractions, casein which precipitates and whey proteins which are soluble. Whole casein is composed of α-,β-,γ-casein in the following proportions of 75%, 22% and 3%, respectively. Caseins are considered to have several unique structural characteristics, low molecular weight phosphoproteins (15-26 kilodaltons), phosphate esters covalently bonded to serine and threonine, negligible sulphydryl groups and minimal structural dependence upon disulfide bonding (Barraquio and van de Voort, 1988).

The second feed protein, albumin, is derived from dried chicken egg whites. Egg white in its natural state is 88% water, 11.0% protein and 0.5% lipid. Egg whites may contain as many as 40 different proteins but more than half of these are minor components. Ovalbumin, the protein used in this study, accounts for 54% of the egg albumin (Table 4.3). The manufacturer places the ovalbumin content at 60-80% of the total protein. Lysozyme which is present as 3.5% of the total solids is of particular interest because it is an enzyme capable of disrupting certain bacteria by cleaving the polysaccharide component of their cell walls (Stryer, 1975). A number of other enzymes have been also detected in small quantities. Some of these include α-mannosidase, β-galactosidase, β-glucuronidase, β-N-acetylglucosaminidase, catalase, and mono- and diphosphoesterase.

Table 4.3 Chemical Composition of Egg Albumin (after Vaderia and Nath, 1973)

Protein	% Solids
Ovalbumin	54.0
Ovotransferrin	13.0
Ovomucoid	11.0
Ovomucin	1.5-3.0
Globulins	11.5
Ovoinhibitor	0.1-1.5
Unidentified	8.0

Note: Only proteins contributing more than 1% are listed

c) **Feed Preparation and pH Control**

The feed for the reactors was a protein solution and raw sewage in a 2:1 ratio on a volume basis. The protein feed stock was prepared twice weekly on Tuesdays and Fridays. A new batch of sewage from Waterloo STP (Waterloo, ON) was obtained every Tuesday. The protein solution and sewage were stored in different feed tanks at 4°C.

One of the goals of this phase of the experimental program was to quantify biosolids production measured as Volatile Suspended Solids (VSS) as a function of electron acceptor, protein type and temperature. An accumulation in the reactor of VSS present in the sewage may have masked biosolids production making it difficult to detect differences in production rates. For this reason, the raw sewage was filtered through glass wool. This reduced both the total COD and the VSS of the sewage. If bacteria are associated with the particulate matter; then there also would be a reduction in the total number of bacteria. Filtered raw sewage was used in preference to the primary effluent because Waterloo STP directs it waste mixed liquor back to the primary clarifier. If the waste mixed liquor contained filamentous organisms, they would be present in the primary effluent.

The composition of a typical domestic wastewater is outlined in Table 4.4. The composition of the synthetic feed was made to approximate medium strength domestic wastewater. One of the goals of this phase of the experimental work is to quantify and compare solids production under anoxic and aerobic conditions. The solids production rates are more precisely defined with higher substrate concentrations. When the reactors were operated at 20°C, protein was added at a concentration of 0.5 g/L to produce a synthetic feed with a strength of approximately 500 mg COD/L. Prior to changing the temperature to 14°C, it was decided that

adjusting the protein strength to accommodate fluctuations in sewage strength was a better operating strategy. A consistent feed strength is one way to increase process stability and promote pseudo-steady state behaviour. The target total feed strength (i.e., sewage and synthetic substrate) for the 14°C study was also approximately 500 mg COD/L.

Table 4.4 Typical Characteristics of Domestic Wastewater (after Metcalf and Eddy (1991))

	Concentration (mg/L)		
Parameter	Strong	Medium	Weak
COD	1000	500	220
Organic Nitrogen	35	15	8
NH_3-N	50	25	12
Total N	85	40	20
Total P	15	8	4
Total Solids	1,200	720	350
Suspended Solids	350	220	100
Dissolved Solids	850	500	250

The proteins in the feed were technical grade casein from bovine milk (Sigma Chemical, St. Louis, MO., C-7078) and albumin Grade III Crude (Sigma Chemical, St. Louis, MO., A-5253). The protein feed was added to a mineral salts media made up in deionized water (Table 4.5). Sodium bicarbonate ($NaHCO_3$) and potassium dihydrogen phosphate (KH_2PO_4) was added to deionized water to increase the ionic strength of the solution. Using these two salts, the dissolved solids in the feed were approximately 400 mg/L. When nitrification inhibitor was added to the feed, the dissolved solids were approximately 1000 mg/L. Potassium dihydrogen phosphate was used in preference to sodium hydrogen phosphate because it increased the acidity of the solution. Alkalinity produced by protein deamination and denitrification caused the pH to rise to undesirable levels. The addition of acidity would consume some of the alkalinity thereby decreasing the pH.

High concentrations of dissolved solids in the protein feed was used as a precautionary measure. If protein addition occurred independent of sewage due to mechanical failure, the dissolved solids would minimize the osmotic shock experienced by the bacteria. The addition of salts also facilitated the dissolution of proteins in deionized water. Although the proteins and the sewage contained phosphorus, the addition of phosphate ensured that this macro nutrient was not growth limiting.

Table 4.5 Composition of the Synthetic Feed and Resulting Reactor Concentration-Phase 1

Component	Feed Concentration	Reactor Concentration
KH_2PO_4 (mg P/L)	95	50
$NaHCO_3$ (mg $CaCO_3$/L)	69	31
Hach Nitrification Inhibitor (mg/L)	530	300
Dissolved Solids	500	
Sodium Nitrate* (mg NO_3-N/L)		82.5
Weight % Albumin in Protein Feed	26-29%	

* Note: This was added to the anoxic reactor only; for rationale see below.

Heterotrophic metabolism was of interest so it was desirable not to have nitrification occurring. At 20°C and pH 7.2, concentrations of ammonia and ammonium nitrogen in excess of 20 mg/L will inhibit *Nitrobacter sp.* (Anthonisen *et al.*, 1976). Under these same conditions, inhibition of *Nitrosomonas sp.* will occur when ammonia and ammonium nitrogen concentrations exceed 1000 mg/L (Anthonisen *et al.*, 1976). Ammonium nitrogen was initially present from the wastewater and subsequently derived from the deamination of the proteins. Concentrations of total ammonium nitrogen in the reactor effluent were well in excess of 20 mg/L. In the presence of high concentrations of ammonia nitrogen, ammonia nitrogen would be used as a nitrogen source for cell synthesis in preference to nitrate.

As an added precaution, Hach Nitrification Inhibitor Formula 2533 (Hach, Loveland Co.) was added to the reactors on a daily basis. N-Serve (2-chloro-6-(trichloromethyl)-pyridine), the active ingredient, is less than 5% of the total mass of the nitrification inhibitor based on data given in the product's material safety data sheet. The reactor concentration was approximately 15 mg/L of N-Serve. Henninger and Bollag (1976) have shown that concentrations of less than 25 mg/L of N-Serve do not affect denitrification in pure culture studies of *Pseudomonas sp.*.

To maintain comparable conditions in the two reactors, nitrate was added only to the anoxic reactor. The presence of approximately 30 mg NO_3-N/L in the anoxic reactor would have had only a modest impact on the ionic strength of the reactor contents; whereas, the additional 82.5 mg NO_3-N/L in the aerobic reactor would have changed the ionic strength to a much larger extent. A stock solution of approximately 13.2 g $NaNO_3$-N /L was added to the anoxic reactor at a rate of 45-50 mL per day to maintain a final reactor concentration of 10-30 mg NO_3-N/L.

Proteins in the feed are converted to peptides or amino acids which undergo either oxidative (Eq. 4.2) or reductive deamination (Eq. 4.3) (Bitton, 1994). High concentrations of ammonia nitrogen in equilibrium with distilled water will produce a solution with a pH of 9.2. Thus the production of ammonia will tend to increase the pH of the reactor. Denitrification will also increase the pH because bicarbonate alkalinity is produced and carbonic acid concentrations are reduced. For every mg of nitrate nitrogen reduced to nitrogen gas 3.57 mg $CaCO_3$ of alkalinity is produced (U.S. EPA, 1993). To maintain the pH at 7.2, the optimum for denitrification, pH control was implemented (U.S. EPA, 1993).

$$R\text{-}CH(NH_2)\text{-}COOH + 0.5\ O_2 + 2H^+ \rightarrow R\text{-}CO\text{-}COOH + NH_4^+ \qquad \text{Eq. (4.2)}$$

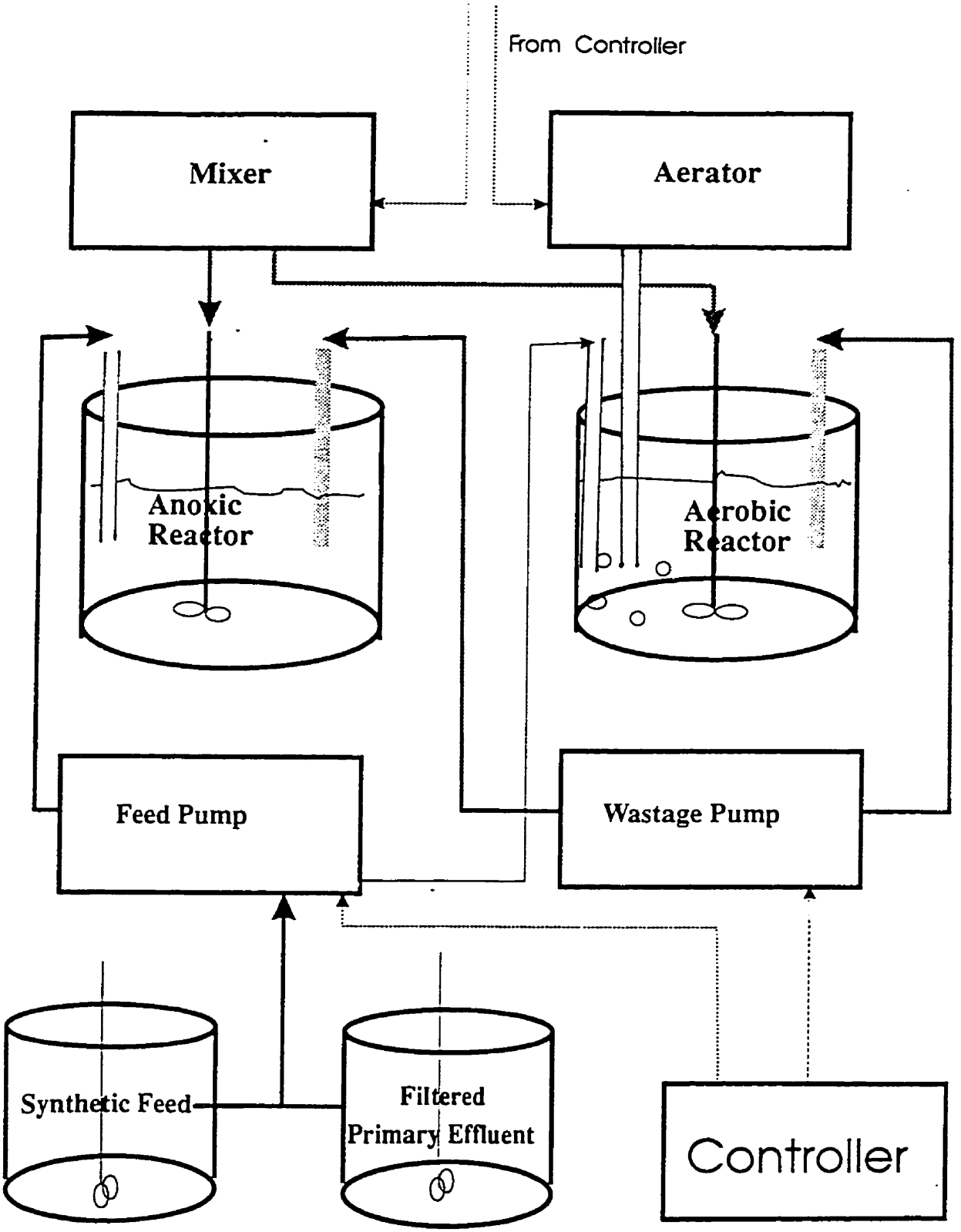
$$R\text{-}CH(NH_2)\text{-}COOH + 2H^+ \rightarrow R\text{-}CH_2\text{-}COOH + NH_4^+ \qquad \text{Eq. (4.3)}$$

The rates of deamination for the aerobic and anoxic reactors were not identical so two pH controllers were used to add 0.15 N HCl acid to maintain the pH at 7.2. Approximately 100-200 mL of the acid was added to the reactors daily.

d) Equipment Requirements and Operation

A general description of the experimental setup is given here with details of the equipment in Appendix A. The reactors and their auxiliary equipment (Figure 4.1) were set up under controlled temperature conditions in two separate environmental chambers. The first chamber contained the reactors, various pumps, control equipment (i.e. timers and pH controllers) and reservoirs containing acid and nitrate. A second environmental chamber with independent temperature control was adjacent to the first. Feed tanks were placed in this second chamber which was maintained at 4 °C. Feed lines were run through pipe chases placed in the common wall. Waste and effluent containers were placed in the 4 °C chamber with lines being run in a similar fashion.

Figure 4.1 Experimental Setup



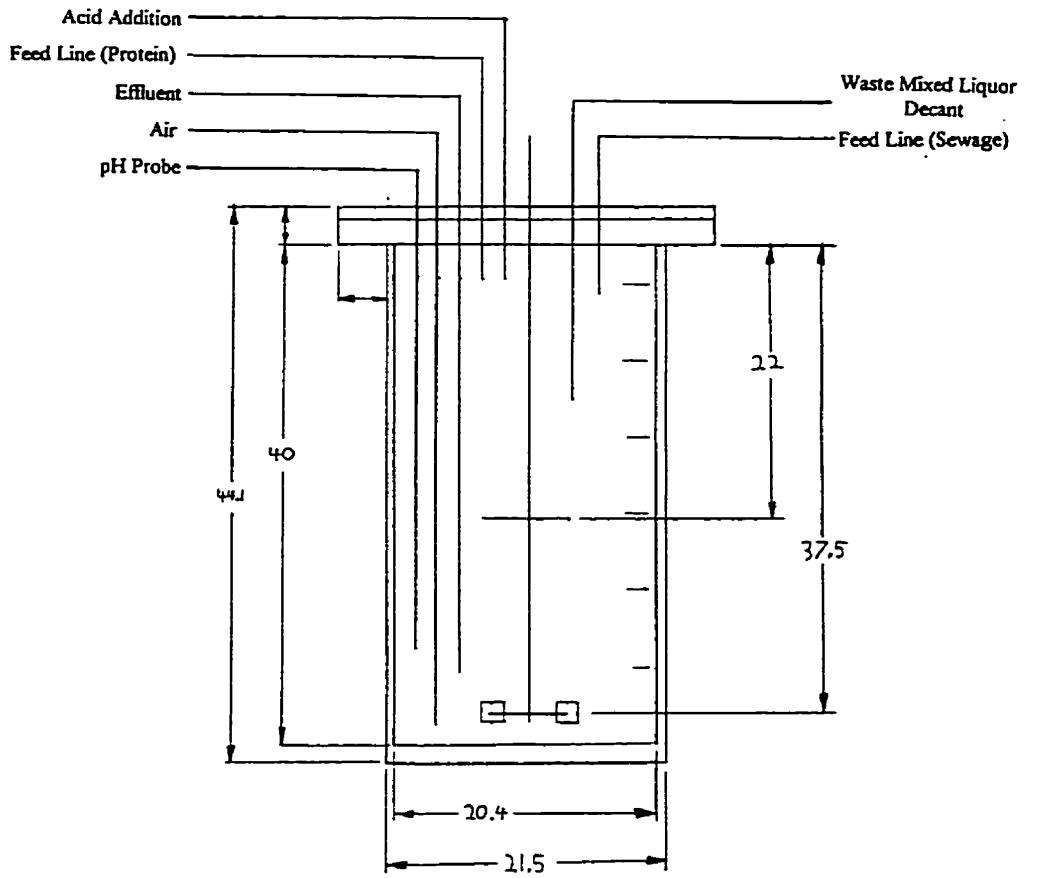
The temperature in the environmental room was monitored over 3.5 d and found to vary between 12.9 and 14.5 °C. During the 14°C experiments, the aerobic SBR was followed over a 22 h feed and react cycle. The temperature remained within the range of 13.6-14.4 °C. In a similar experiment done with a setpoint of 20°C, the maximum and minimum temperature of the environmental chamber was 20.7 and 18.4°C, respectively. The SBR temperature ranged between 20.2 and 18.1°C with an average temperature of 19.4°C. The lower temperatures were seen during the fill portion of the reactor cycle.

Each reactor consisted of a 20 cm ID flanged acrylic column complete with a lid and a total liquid volume of 14 L (Figure 4.2). Mixing was accomplished using a motor driven mixer coupled to a shaft on which two impellers were mounted. The bottom and top impellers were disk flat-blade turbine and marine turbine, respectively. The disk flat blade impeller had 6 blades with each blade measuring 1.8 cm wide by 1.6 cm high. The overall diameter of the impeller was 7.2 cm. The blades of the marine impeller measured 3.8 cm wide by 4.5 cm long with a pitch of 15°. The lid of the reactor contained fittings for connecting the flexible tubing for the waste, effluent and feed lines to their stainless steel reactor counterparts.

Tygon™ tubing served as an air line and was fed through a hole in the lid of the reactor. It was fastened to a piece of stainless steel tubing for support. An aquarium aeration stone attached to the Tygon™ tubing was used as a surrogate fine bubble diffuser. Air was supplied by an aquarium pump which drew air from within the environmental chamber. To minimize evaporative effects, the air was humidified using a gas washing bottle filled with deionized water. Feed for the reactors was drawn from two well mixed 40 L high density polyethylene tanks. One tank contained the protein substrate while the other contained filtered raw sewage.

The rate of substrate addition was 444 mL/h and 221 mL/h for the protein and sewage, respectively. Substrate addition over such a long period (12 hours) and small delivery volumes required these pumps to operate at low speeds with small diameter tubing. The sewage contained particulates which could settle out and clog the tubing. To minimize the risk of this occurring, the velocity through the tubing was increased by cycling the feed pumps off and on every 5 min using an interval timer. This permitted them to operate at twice the original speed.

Figure 4.2 Reactor Configuration



Dimensions in centimetres

To eliminate any negative effect high nitrate concentrations may have had on the aerobic culture, the aerobic culture was not exposed to nitrate. A separate nitrate pump was used to add nitrate to the anoxic culture. The nitrate pump was cycled at 5 min. intervals over the 12 h of the feeding period.

e) **Reactor and Auxiliary Equipment Maintenance**

When continuously running an aerobic mixed culture under carbon limitations, there is a risk of the culture being overtaken by filaments. Conditions in the feed, effluent and waste lines are ideal for the growth of filaments. To minimize the number of filaments introduced into the reactor, these lines were cleaned on a regular basis. Feed lines were autoclaved on a twice per week basis and effluent and waste lines were autoclaved weekly. The Masterflex® pump head tubing used in the feed pump was flushed twice weekly and discarded after a week. The Masterflex® pump head tubing in the other pumps was flushed and discarded as

necessary. Growth on the walls of the reactors was scraped off daily using a rubber spatula and returned to the bulk liquid.

The sewage feed tank was emptied and the wall growth was removed on a weekly basis for the same reason. The tanks were rinsed with hot water but not washed. The protein feed tank was cleaned on a weekly basis in a similar fashion.

pH meters were cleaned with 0.1 N HCl solution on a weekly basis to remove any microbial growth and calibrated with pH 7 and 4 buffers at this time. The pH meters were checked with pH 7 buffer once during the week and inspected daily when the wall growth was removed. The aeration stone was cleaned with 0.15 N HCl at this time as well.

f) Reactor Characterization

i) Solids Production

To determine the solids production rate and define the sludge age of the reactors, the quantity of solids produced had to be quantified. During most of the monitoring period, the total suspended solids (TSS) and VSS concentrations of the mixed liquor and effluent were measured daily; however, during the rate study period, mixed liquor and effluent samples were composited for a two day period and the TSS and VSS determinations were done on the composites. The analytical protocol is outlined in Appendix A. The volume of mixed liquor wasted on a daily basis was measured using a 250 mL graduate cylinder. Effluent volumes were determined by measuring the weight of effluent and assuming the density of the effluent was the same as water.

To characterize the VSS of the filtered raw sewage, a number of VSS measurements were done on the filtered sewage. Total and filtered COD were also measured on these same samples. Rather than measuring the VSS of the sewage on a continuous basis, it was estimated using the average particulate COD/VSS ratio and the measured particulate COD.

ii) Chemical Analysis

Three composites a week were generated by the reactors for Monday and Tuesday; Wednesday, Thursday and Friday; and Saturday and Sunday. The volume of nitrate stock solution delivered to the reactor was determined by a measuring the weight of stock solution delivered to the reactor and assuming the density of the solution was similar to water. Measurements were made at the beginning and ending of the composite period. During the 20°C phase, acid consumption was determined in an similar fashion. Listed in Table 4.6 is the sample frequency during the non-rate study period while in Table 4.7 is the sample frequency during a rate study period. Increased analysis was done during the rate study period so that the culture could be better characterized. Details of sample preservation and analysis are found in Appendix A.

Table 4.6 Sampling Schedule Conventional Parameters - Non-Rate Study Period

Sample Point	Total COD	Filtered COD	NO_x-N
Filtered Primary Effluent	Tr	Tr	Tr
Synthetic Feed	Tr	Tr	
Mixed Liquor			
Reactor Effluent	Tr	Tr	Tr
Nitrate Stock			W

Table 4.7 Sampling Schedule Conventional Parameters - Rate Study Period

Sample Point	Total COD	Filtered COD	Total TKN	Filtered TKN	NH_3-N	NO_x-N
Filtered Primary Effluent	Tr	Tr	Tr	Tr	Tr	Tr
Synthetic Feed	Tr	Tr	Tr	Tr	Tr	
Mixed Liquor			Tr	Tr		
Reactor Effluent	Tr	Tr	Tr	Tr	Tr	Tr
Nitrate Stock						W

Tr-Thrice Weekly W-Weekly

Note: COD analysis were done in triplicate
Total TKN done in duplicate

4.2.2. Batch Rate Studies

a) Introduction

The experiments outlined in Table 4.8 were concurrent with the appropriate testing phases of the SBR studies and represented a replicated $(2 \times 2)^2$ factorial design. Replicated experiments were not carried out simultaneously because one rate study required essentially all the mixed liquor produced each day. At 20°C only one of the either aerobic or the anoxic studies was performed per day because of the demanding sampling

schedule. At 14°C, a less demanding sampling schedule and some additional help allowed many aerobic and anoxic studies to be done concurrently.

Table 4.8 Experimental Levels for Batch Studies

Growth Conditions	Substrate Pair	Temperature (°C)
Aerobic, Anoxic	α-casein/N-Z Amine A(CAA)	20 and 14
Aerobic, Anoxic	ovalbumin/ N-Z Amine A(CAA)	20 and 14
Aerobic, Anoxic	lactalbumin/ N-Z Amine A(CAA)	20 and 14
	lactalbumin/lactalbumin enzymatic hydrolysate(LEH)	20 and 14

The substrate pairs were made up of a slowly and rapidly biodegradable substrate. The slowly biodegradable substrate was a protein. The rapidly biodegradable substrate was the protein hydrolysate(PH) of either casein or lactalbumin. Enzymatic hydrolysates are composed of amino acids and peptides and thus do not possess either a secondary or tertiary structure.

It would have been preferential to use the whole protein and its corresponding enzymatic hydrolysate but this was not possible for a number of reasons. The enzymatic hydrolysate of α-casein and ovalbumin are not commercially available. An enzymatic hydrolysate of albumin can be purchased from Sigma Chemical but its molecular weight distribution has not been characterized. The enzymatic hydrolysates of lactalbumin (LEH) and casein (CAA), were used as a PH.

Batch studies used mixed liquor produced by the SBR during the wastage portion of the reactor cycle as a stock culture. SBR operation was unaffected by batch studies. Wastage occurred 10 h after feeding was stopped which allowed the bacteria time to metabolize slowly biodegradable substrate present in with the feed. Biosolids produced during the wastage portion of the operating cycle would contain a minimum amount of enmeshed slowly biodegradable substrate.

The mixed liquor was also composed of microorganisms, endogenous particles, accumulated non-biodegradable particulates. The microbial population of the aerobic biomass consisted of both bacterial and other microorganisms (e.g. protozoa and rotifiers); whereas, the anoxic biomass was principally composed of bacteria. Non-bacterial microorganisms require dissolved oxygen which was present in excess in the aerobic reactor.

b) Substrate Selection

i) Introduction

Proteins were chosen on the basis of their MW and characteristics of their overall structure. It was assumed that overall structure would influence how a protein is taken up into the floc and degraded by extracellular enzymes. Protein solubility may influence whether or not the protein is adsorbed onto the surface of the floc or enmeshed into the floc. Table 4.9 details a classification systems for proteins based on their solubility.

Table 4.9 Classification of Proteins Based on Solubility (Rodwell, 1988)

Classification	Characteristics
Albumins	Soluble in water and salt solutions. No distinctive amino acids
Globulins	Sparingly soluble in water but soluble in salt solutions. No distinctive amino acids.
Prolamines	Soluble 70-80% ethanol but insoluble in water and absolute ethanol. Arginine-rich.
Histones	Soluble in salt solutions.
Scleroproteins	Insoluble in water or salt solutions. Rich in Gly, Ala and Pro.

Proteins maybe classified as one of two types depending on their axial ratio (ratio of length to breadth). Globular proteins generally have an axial ratio of 3-4 and are characterized as compactly folded and coiled polypeptide chains (Rodwell, 1988). Examples are insulin, plasma albumins and globulins. Fibrous proteins have an axial ratio greater than 10. They are characterized by groups of polypeptide chains coiled in a spiral or helix and cross-linked covalently or by hydrogen bonds (Rodwell, 1988). Examples are keratin, myosin, collagen and fibrin.

A disulfide bond between 2 cysteine residues is an example of covalent bond linking two portions of a polypeptide chain. The disulfide bond is strong and is resistant to the usual conditions which cause protein denaturation (Rodwell, 1988). Branden and Tooze (1991) suggest the presence of a disulfide bridge as a way to increase protein stability. They further suggest that inclusion of the bridges in a manner which maximizes the length of the loop between cysteine residues increases the stability of the protein. The overall shape could influence the mechanism of substrate uptake into the floc and the accessibility of the interior of the protein to exoenzymes. The physical properties of the selected proteins are outlined in Table 4.10.

Table 4.10 Selected Proteins and Hydrolysates

Protein	Average MW (Da)	Solubility	Catalogue No.
Lactalbumin	21,000	Scleroprotein	L-7252
β-Lactoglobulin			
α-Lactalbumin			
α-Casein	22,000	Globulins	C-7891
Ovalbumin	45,000	Globulins	A-5378
LEH	302		L-0375
CAA	247		C-0626

Note: All proteins and PH were purchased from Sigma Chemical, St. Louis, Mo.

ii) Globulins and Their Hydrolysates

α-Casein

α-casein is composed of two major components: α_S-casein and κ-casein. The α-casein used in the batch experiments was a lyophilized powder containing 10-20% NaCl , 60% α-casein as determined by electrophoresis and the balance primarily β-casein and κ-casein.

Like most of the other caseins, α_S-casein has strongly hydrophobic and charged residues that are not uniformly distributed along the polypeptide chain (Swaisgood, 1982). This investigator went on to suggest that the protein contains domains of rather unstable structure, particularly the polar domain which approach random coil behaviour. The protein's marginal stability and amphipathic nature gives rise to molecular dimensions that are sensitive to ionic strength (Swaisgood and Timasheef, 1968). For this reason Swaisgood went on to state that this structural instability means that at any instant an appreciable fraction of the molecular backbone and side chains will be exposed to water.

κ-casein is a glycoprotein (Wheelock and Sinkinson, 1969) containing disulphide bonds (MacKinlay and Wake, 1965) and accounts for approximately 15% of whole bovine casein (MacKinlay and Wake, 1964). Schmidt (1982) postulated that casein in solution will form sub-micelles consisting of a hydrophobic core surrounded by unevenly distributed κ-casein molecules linked by $Ca_9(PO_4)_6$ clusters to form micelles.

N-Z Amine A (CAA)

N-Z Amine A is a mixture of small peptides and amino acids derived enzymatically from casein. It is produced using a proprietary process of Sheffield Ltd. so details of the process are lacking. The amino acid and peptide molecular weight distribution ranges from 100-1000 Da with the average molecular weight being 247 Da (personal communication; Sigma).

Ovalbumin

The ovalbumin used was approximately 95% pure. Ovalbumin is a monomeric, nearly spherical globular phosphoglycoprotein that exists in three distinct forms differing only in phosphorous content (Osuga and Feeney, 1977). A glycoprotein is a protein to which a carbohydrate is covalently attached (Spiro, 1973). In this instance, the carbohydrate chain is a single branched chain carbohydrate composed of D-mannose and N-acetyl glucosamine (Osuga and Feeney, 1977). Ovalbumin is not a very highly cross linked protein as it contains only one disulfide bond per molecule of 45,000 Da (Osuga and Feeney, 1977).

iii) Scleroproteins and Their Hydrolysates

Lactalbumin

Lactalbumin, the whole protein of whey, is normally produced during the manufacture of lactose from cheese whey or casein whey. It is a mixture of proteins produced by adjusting the pH of skim milk to 4.6 by the addition of acid followed by heat coagulation. Barraquio and Van de Voort (1988) suggest that as a consequence of applying heat, whey proteins will be denatured resulting in a loss of their solubility and related functionality. β-lactoglobulin is the milk protein most susceptible to denaturation.

The major components of lactalbumin are: 50% β-lactoglobulin (MW 18,000), 12% α-lactalbumin (MW 16,000) and 5% serum albumin (MW 65,000) (Barraquio and van de Voort, 1988). β-lactoglobulin is a dimer (approx. 36,000 Da) cross-linked by two disulfide bonds with each monomer having a intramolecular disulfide and one sulfhydryl group. The presence of the two disulfide bonds and a thiol group are important features of the primary structure. The thiol group must be located in a structural region which limits its

accessibility to reagents (Swaisgood, 1982). At the pH of milk and at room temperature the protein will have a axial ratio of 2.

α-lactalbumin is a compact, nearly spherical single chain globulin with no sulfhydryl groups. The disulfides of α-lactalbumin (4 per monomer) are not involved in bridging or cross linking between the strands. The hydrodynamic properties of α-lactalbumin indicate that it is a nearly spherical, very compact globular protein (Swaisgood, 1982).

Lactalbumin Enzymatic Hydrolysate (LEH)

Lactalbumin enzymatic hydrolysate (LEH) is prepared from lactalbumin using a pancreatic extract to hydrolyze the protein followed by heat inactivation. The residual colour of the solution is removed by the addition of charcoal. No further details were available because this is a propietary process owned by Sheffield Ltd.

The LEH is a mixture of small peptides and amino acids. The MW distribution ranges from 100-1000 Da with the average being 302 Da (Sigma, personal communication). The amino acid composition of the LEH is unknown because it is derived using a proprietary process with the specificity of the pancreatic enzymes dictating the nature of the peptides and the free amino acids found in the hydrolysate. Mullally *et al.* (1994) examined six different pancreatic protease preparations of lactalbumin. All of the hydrolsates, with the exception of one, were found deficient in asparate, glutamate, glycine, tryptophan and proline.

c) Substrate Preparation

Many factors normally important when working with a synthetic feed could be ignored because sewage was part of the original culture media. Trace micro-nutrients (i.e. iron, zinc, and maganese) needed for growth did not have to be included in the media because of their presence in the sewage. The buffering capacity of the media was neglected because of the small volume of media being introduced and the implementation of pH control.

Proteins and protein hydrolysates (PH) were dissolved in a salt solution. The composition of the salt solution is outlined in Table 4.11. The final concentration of the proteins and hydrolysates was 200 mg/100mL.

Table 4.11 Composition of Salt Solution - Batch Studies

Component	Concentration in Units Specified
Na_2HPO_4 (mg P/L)	52
KH_2PO_4 (mg P/L)	45
$NaHCO_3$ (mg $CaCO_3$/L)	31
Dissolved Solids (mg/L)	470

d) Equipment Details and Experimental Protocol

i) Experimental Protocol for Casein/Ovalbumin

Biosolids generated by the SBR were split into two equal portions and fed whole protein or alternately PH (see Appendix A4 for protein-PH pairs). The concentration of protein and hydrolysate added to the respective reactors was 0.4 g dry weight/L; this produced a reactor concentration of 400-500 mg COD/L. This is comparable to a medium strength sewage (Table 4.4). The half saturation coefficient is often quoted as 5-20 mg COD/L (Gibson and Dold, 1991; Henze *et al.*, 1986). At the initial substrate level used in these experiments, it was anticipated that zero order growth kinetics would be applicable initially. Differences in the rate of consumption of the terminal electron acceptor, either oxygen or nitrate as appropriate, between the protein and the hydrolysate would be attributed to substrate uptake and hydrolysis. Experiments done using each protein-hydrolysate pair were replicated (see Appendix A4).

Under normal operating conditions, waste mixed liquor was sent to the environmental room maintained at 4°C. On the day of a batch study, the waste mixed liquor was kept within the reactor environmental chamber to avoid temperature shock. Of the approximately 1000 mL of waste mixed liquor, 100 mL was used for both solids measurements and AODC. The remaining 800 mL was divided equally between two 600 mL beakers and placed on stir plates. A 1 cm piece of Styrofoam was placed on the stir plate to minimize heat transfer. During the lactalbumin rate studies performed on April 26th-April 29th (See Section 4.2.4 b ii), the temperature of the aerobic culture was monitored over a 7 h period. The culture had been aerated for approximately 2 d when temperature monitoring began so the system was at thermal equilibrium. The equilibrium temperature of the culture was 15.0 °C. In a more typical batch study where the biosolids were being generated at a temperature of 13.6-14.4 °C, the culture was at a temperature of 13.6-15°C.

During wastage, the anoxic culture was exposed to air and the aerobic culture may have experienced oxygen limitations. Prior to using these cultures, it was necessary to return to them to their initial environmental conditions. The beakers were sparged with humidified air or nitrogen as appropriate for a minimum of 30 min before making any measurements. Sparging the anoxic culture with nitrogen was continued for the duration of the rate study. The gas was drawn from a cylinder in the environmental room and bubbled through distilled water equilibrated to the test temperature to minimize evaporative losses. Air was supplied by an aquarium pump drawing air from with within the environmental chamber. These efforts were taken to minimize heat transfer.

Gas sparging also caused the pH to rise well beyond 7.2 in the absence of pH control. With the optimal rate of denitrification occurring at pH 7.2 (U.S. EPA, 1993) it was necessary to implement pH control. As only one pH controller was available for two experiments, the following approach was taken: (1) the pH of the culture supplemented with protein was maintained at 7.2 by the addition of 0.2 N HCl acid, (2) an equal amount of acid was added to the culture augmented with hydrolysate and (3) the pH of the this culture was monitored and logged. Details of the pH controllers, pH meters and other equipment are available in Appendix A-Table 2.

Prior to adding the protein, one half of the split aerobic culture was transferred to a BOD bottle and the OUR was determined. This was repeated with the second portion of the aerobic culture. To keep the mixed liquor in suspension the bottle was placed on a Styrofoam insulated stir plate. The average OUR for the two samples was defined as the basal OUR (OUR_B), since at this point, the culture was in a starved condition. The basal OUR is considered to be the sum of the oxygen demands of the non-bacterial population (e.g. protozoa and rotifers) and the bacterial population.

The OUR of the bacterial population includes respiration associated with maintenance energy demands and utilization of enmeshed slowly biodegradable substrate. Utilization of enmeshed slowly biodegradable substrate was expected to account for a small portion of the OUR demand. First, the biosolids were produced at a time when enmeshed slowly biodegradable substrate was expected to be a minimum. Second, if the slowly biodegradable substrate was not completely degraded over the previous 10-22 h, then it being consumed very slowly.

The nitrate concentration at the beginning of the rate study had to be sufficiently high that nitrate did not become rate limiting over the course of the experiment. A literature survey by U.S. EPA (1993) reported values for the half saturation constant for nitrate to be 0.1-0.2 mg NO_3-N/L. They further suggest at concentrations greater than 1-2 mg NO_3-N/L, nitrate should not limit the rate of denitrification. Extraplolating the rate of nitrate utilization (NUR), the starting mixed liquor nitrate concentration had to be in excess of 30 mg NO_3-N/L.

The slowly biodegradable protein solution (100 mL) was added at this time to the beaker and the pH monitored. Approximately 30–45 minutes later, the rapidly biodegradable substrate was added to the second beaker which was subject to pH control. Approximately eight samples were taken over the 4 or 6 h period after feeding at 20°C or 14°C, respectively. For the aerobic and anoxic cultures 20 and 25 mL of the mixed liquor were removed per sample, respectively. After centrifuging at 34,540 x g at 8°C for 17 min, the supernatant was filtered through 0.45 μm surfactant free cellulose acetate filters using a syringe filter (Nalgene, Rochester NY). Subsamples of the original samples were preserved as specified in Appendix A and later analyzed for ammonia nitrogen and COD. If the sample was anoxic, an additional sample was placed in a vial and analyzed for nitrate, nitrite, and ammonia nitrogen. Details of the analysis procedures are found in Appendix A. At the end of the rate study, the cultures were preserved for cell counts.

With the aerobic reactors, the OUR was determined concurrently with sampling for chemical analysis. Both dissolved oxygen meters and pH meters/controllers were calibrated prior to initiating the batch experiments. A single dissolved oxygen meter (Orion Model 810 Dissolved Oxygen Meter) was used for both the slowly and rapidly biodegradable culture. The dissolved oxygen meter in conjunction with a personal computer was used to log the dissolved oxygen (DO) every 15 s.

On those occasions when enzyme assays were done, the intact mixed liquor was taken from the batch reactors 75 minutes after substrate addition. The mixed liquor was homogenized using a hand driven Porter tissue homogenizer. Details of the enzyme assay are found in Appendix A.

ii) Variances on Ovalbumin/Casein Protocol for Lactalbumin

After completing the initial set of rate studies at 20°C, it became clear that the bacterial response to lactalbumin was much slower than for casein and albumin. The possibility existed that the chronically starved bacteria of the SBRs did not have sufficient energy for lactalbumin hydrolysis.

This possible limitation was taken into account when the second set of rate studies using lactalbumin were performed at 14°C. Anoxic and aerobic rate experiments run in parallel were initiated on April 8, 1996. The experimental protocol outlined above was used in this case with the following exceptions:

1. The culture using hydrolysate was augmented with LEH and not CAA. LEH was chosen in preference to CAA for two reasons. The true yield for lactalbumin and its enzymatic hydrolysate should be similar. If for some reason synthesis, not hydrolysis, was rate limiting due to the composition of amino acids and peptides derived from lactalbumin, then this would become evident by choosing LEH.
2. For both the aerobic and anoxic cultures, the 1000 mL of biosolids generated by the SBR was split into three portions: 200 mL for both MLVSS and AODC, 270 mL for the culture using hydrolysate, and 540 mL for the culture using lactalbumin. The 540 mL volume for the culture augmented with lactalbumin was later used in two rate studies. The first rate study occurred on the day the biosolids were generated. A second rate study using this culture was done after several days of exposure to lactalbumin.
3. With the aerobic culture, a minimum of 300 mL of augmented culture was required on both days because the dissolved oxygen was measured using a dissolved oxygen probe in a BOD bottle. The anoxic culture was treated in an identical manner to the aerobic culture 70 and 140 mL of substrate was added to the cultures using LEH and lactalbumin, respectively. The mass loadings of protein were unchanged from those in Section 4.2.2.d.i.
4. With the aerobic culture, 300 mL of the culture was held in reserve so continued dissolved oxygen measurements could be made. For this reason, only 1-2 mL of mixed liquor was taken per sample for both the aerobic and anoxic cultures instead of the typical volumes of 20-25 mL. Approximately 10 samples were required over the 6 hour study period. The samples were centrifuged using a Sorvall Microcentrifuge at 10,000 r.p.m. for 2 min to remove the majority of biosolids and later syringe filtered through a 0.2 μm

syringe filter (Nalgene, Rochester NY). Filter sterilizing the samples with the 0.2 μm filter ensured that microbial degradation did not occur prior to analysis.

If the samples were not analysed immediately, 20 μl of formaldehyde was added to the sample. The presence of formaldehyde affected the ammonia nitrogen determination so this analysis was not done with these samples. The formaldehyde did not affect the accuracy of the nitrate and nitrite nitrogen determination. The smaller sample volumes meant that the COD of the bulk liquid could not be determined. Earlier experiments showed that the COD of the bulk liquid fell to the lower detection level of the test (25 mg COD/L) very early in the experiment (approximately 1 h) and thereby provided little useful information.

5. At the end of the rate study, the cultures augmented with lactalbumin were maintained with pH control and gas sparging. This culture was allowed to acclimate to the lactalbumin for four days before testing was resumed.

On April 13th, the above procedure was repeated on the culture acclimated to lactalbumin. At the end of the rate study, the lactalbumin culture was spiked with LEH and a number of measurements were made. So a second rate experiment was initiated on April 29th similar to that of April 18th on an acclimated culture.

4.3. Phase 2

4.3.1. Sequencing Batch Reactor Operation

Two reactors were operated at 20°C. One was operating under aerobic conditions and the other under anoxic conditions. The operation of these reactors has been previously detailed for Phase 1 in Section 4.2.1(b) with some modifications. The working volume of the reactors was 5 L rather than 10 L as used in Phase 1. With reduced volumes the pumps used previously were no longer suitable. The volumes required were small enough that the feed could be made-up batchwise. As in Phase 1, the reactor operated at a HRT of 1.25 d. As outlined in Table 4.12, 1/10th of the reactor contents were wasted daily resulting in a target SRT of 10 d. As before the realized SRT calculated using Eq. 4.1 was less than 10 d due to unintentional solids losses in the effluent.

Table 4.12 SBR operating cycle

Operation	Hours	Volume (L)
Fill	0	1.0-5.0
React	22.25	5.0
Waste	0	5.0-4.5
Settle	1.25	4.5
Draw	0.25	4.5-1.0
Idle	0.25	1.0

In both Phase 1 and 2, the reactors were operated as SBRs; however, the feeding regime differed significantly. In Phase 2, the feed was added to the reactors at the beginning of the cycle; whereas, in Phase 1 feed was introduced into the reactor over a 12 h period. The objective in this phase was to determine whether or not lactalbumin was degradable and to provide biosolids for further testing. The feed was composed of sewage and protein in a 1:2 ratio by volume. The sewage was raw sewage from Waterloo STP filtered through glass wool and stored at 4°C. The protein substrate was made up in deionized water and its composition is outlined in Table 4.13. Feed concentrations are different from those in Table 4.5 because they were calculated on the total feed volume rather than on the volume of the synthetic substrate. A small quantity of Hach Nitrification Inhibitor 2533 (Hach, Loveland CO) was added to both the aerobic and anoxic reactors. The inhibitor was added to prevent nitrification in the aerobic reactor. To ensure nitrate was in excess in the anoxic reactor approximately 25 mL/d of a sodium nitrate stock solution (13.2 g NO_3-N/L) was added.

Table 4.13 Composition of the Synthetic Feed and Resulting Reactor Concentration-Phase 2

Component	Feed Concentration	Reactor Concentration
Na_2PO_4 (mg P/L)*	330	270
Na HCO_3 (mg $CaCO_3$/L)	43	35
Hach Nitrification Inhibitor (mg/L)	375	305
% Lactalbumin in Supplemented Protein	100%	

The sampling schedule for this phase of the experimental program is outlined in Table 4.5. Details of sample preservation and analytical procedures can be found in Appendix A.

Table 4.14 Sampling Schedule SBR-Phase 2

Source	Parameter	Sampling Frequency
Reactor Mixed Liquor	Total Suspended Solids	Daily
	Volatile Suspended Solids	Daily
Effluent	Total Suspended Solids	Daily
	Volatile Suspended Solids	Daily
	COD	Daily
Influent	COD	Composite of Two Days

4.3.2. Batch Rate Studies

a) Batch Studies-Protein Utilization

The goals of this portion of the experimental program were the same as batch study as described for Phase I in Section 4.2.2.d.i. What differentiated this study from the one of Section 4.2.2 was that the biosolids were generated using lactalbumin as the principle protein substrate rather than a mixture of albumin and casein. The lactalbumin-lactalbumin enzymatic hydrolysate was the protein pair which was evaluated at 20°C under aerobic and anoxic conditions. The experimental protocol followed was the one of April 13. 1996 (Section 4.2.2.d.ii).

5. Results and Discussion

5.1. Introduction

The results of this experimental program are discussed under the headings of: **Sequencing Batch Reactor Operation, Growth Kinetics and Stoichiometry**, and **Substrate Uptake and Hydrolysis** (see Figures 1.1 and 1.2 for a pictorial overview of the experimental program).

Sequencing Batch Reactor Operation details the performance of the aerobic and anoxic reactors during Phases 1 and 2 of the experimental program. During Phase 1, the reactors were fed sewage, albumin and casein. It was during this phase, that long term performance data at 14 and 20°C were obtained. Biosolids production, effluent quality and consumptive ratios were derived using this data. During Phase 2, the SBRs were maintained on a feed of sewage and lactalbumin. The reactors were operated at 20°C for approximately one month. They provided a culture acclimated to the lactalbumin which could be used in rate studies. Performance data from the reactors confirmed the biodegradability of lactalbumin.

Batch rate studies experiments were used to study growth kinetics and stoichiometry and substrate uptake and hydrolysis. Rate studies using a protein hydrolysate (PH) were conducted concurrently with those using a protein. Rate studies using the PH were designed to quantify the impact of temperature and terminal electron acceptor on growth kinetics and stoichiometry. Protein hydrolysates are simple substrates not requiring hydrolysis. From these studies, specific substrate utilization rates and observed yields under conditions of active substrate metabolism were determined. In those rate studies where proteins were used, growth kinetics were influenced by the kinetics of substrate uptake and hydrolysis. A comparison of substrate utilization rates for cultures using proteins and PH cultures lead to a better understanding of substrate uptake and hydrolysis. The role of molecular weight and overall protein structure on the rate of hydrolysis was investigated.

Results and Discussion presented under each of the three broad headings follows the same general format. After a brief introduction, a presentation of results follows. In each of the sections, the pertinent aspects of the 20°C data is given followed by the 14°C data. For instance for SBR operation, trends in the following are

presented: influent strength, effluent COD, reactor MLVSS and nitrate consumption. Relationships between presented trends are discussed for each temperature level separately. An interpretation of the trends and the effect of the temperature and reactor has follows. Each section is concluded with a number of summary statements.

5.2. Sequencing Batch Reactor Operation

In Phases 1 and 2, SBRs were run using a sewage-protein feedstock. In Phase 1, the reactors were run for over eight months on a feed of sewage, casein and albumin. In Phase 2, the SBRs were fed sewage and lactalbumin as a substrate for a period of twenty eight days.

5.2.1. Phase 1 - Casein and Albumin Proteins

a) Introduction

The experimental work of Phase 1 was carried out to meet the following objectives:

a) To quantify and compare solids production rates for SBRs achieving carbon removal under aerobic or alternately anoxic conditions as a function of temperature.

b) To evaluate acridine orange direct counts (AODC) as a method of enumerating the number of bacteria in a mixed microbial population. Using this technique, the fraction of reactor biosolids composed of cells will be determined as a function of temperature and electron acceptor.

c) To compare the effluent quality of two different carbon removal systems (aerobic and anoxic) in the areas of effluent COD and solids. The effect of temperature on the consumptive ratio will also be quantified.

d) To generate biosolids acclimated to α-casein and ovalbumin for use in rate studies producing a culture with a well-defined history.

Aerobic and anoxic systems were operated in parallel to facilitate comparisons between the systems. Operating the reactors under comparable experimental conditions does not guarantee equivalent performance because these living systems may have different responses to the same event. The two reactors received the same quantity and quality of substrate and were operated with the same operating protocol. As to be discussed in Section 5.2.1.b-Solids Retention Time, they operated under different SRTs because of unintentional biomass

losses caused by differences in settling prior to decanting. Each reactor could therefore possess a unique ecology that is dictated in part by the sludge age. The microorganisms present and their physiological state will define the overall process efficacy.

Much of the feed's organic strength resulted from the protein mixture. In domestic wastewater treatment, protein degradation may or may not be responsible for the reported increases in biosolids production under low temperatures or anoxic conditions. For this reason, caution should be exercised in applying biosolids production rates presented here to other systems.

b) Presentation of Experimental Results

i) 20°C Cultures

Introduction

Before the monitoring phase began at 20°C, the culture was acclimated for 4 months. On Nov. 12, a feed pump was inadvertently left on resulting in an additional 4 L of substrate being added to each reactor. The reactors were settled and the extra feed was decanted. There was no obvious loss of biosolids but a change in the physiological state of the bacteria could have occurred. Higher than normal substrate loadings may place the bacteria in a different growth state. A ten day period (Nov. 13 - Nov. 24) was allowed for the system to come back to pseudo-steady state before monitoring was reinitiated.

Feed Strength and Composition

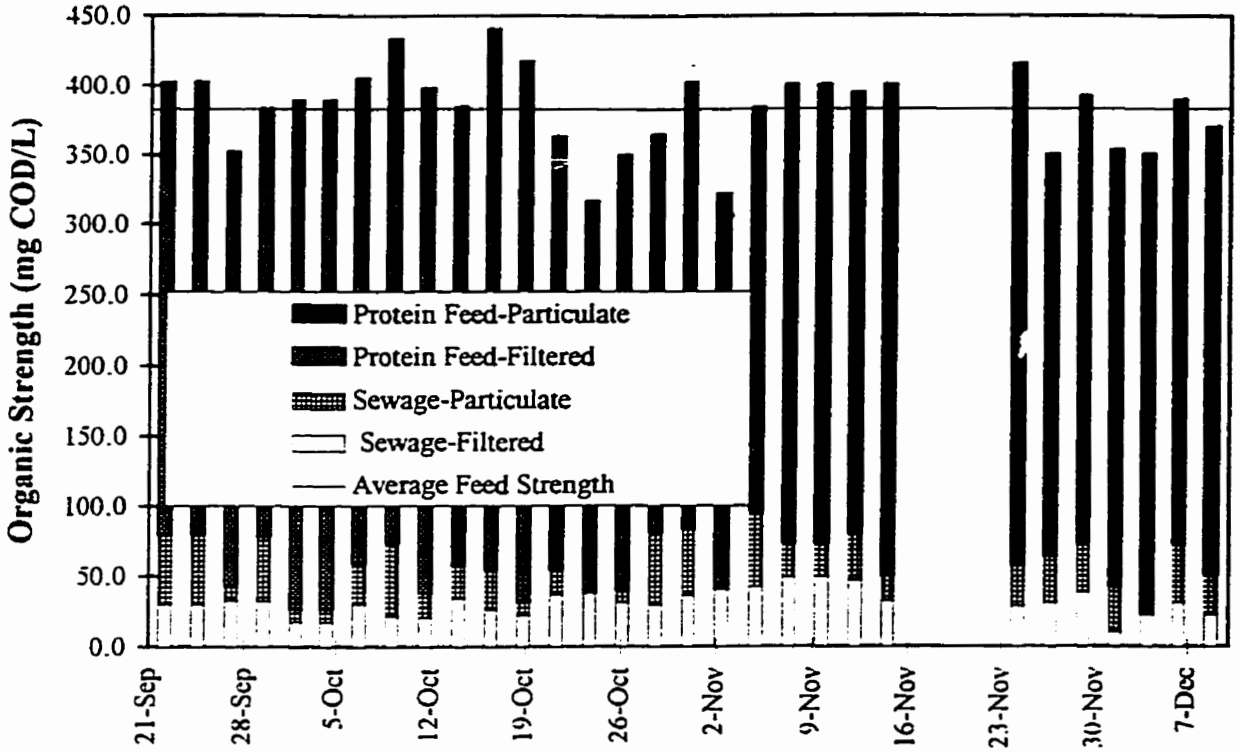
Figure 5.1 illustrates the variability in feed strength and composition over the eighty day Phase I monitoring period. For the periods of Nov. 4 - 5, Nov. 6 - 7 and Dec. 9 - 10, only total COD data for the synthetic feed were available. The total COD was used to calculate the COD of the particulate fraction on these days. Only the filtered COD of the sewage was available on Oct. 21 - 22 and Nov. 1 - 2.

Much of the feed's organic strength was derived from the synthetic protein substrate. Fluctuations in the organic strength of the sewage were not easily seen from the figure because of its limited contribution to the feed. It is reasonable to expect that the microbial population in sewage will change with influent strength

and season. Sewage with a low organic strength will minimally affect the organic loading; however it could have a very significant impact on the numbers and species of microorganisms in the reactor.

The average total COD for the 80 day period was approximately 381 mg/L with a standard deviation of 30 mg COD/L. However, there appears to be a general trend toward reduced COD with time. The average feed strength prior to Oct. 19 was 393 mg/L; this was not considered to be a substantial difference.

Figure 5.1 Influent Feed Concentration and Composition - 20°C

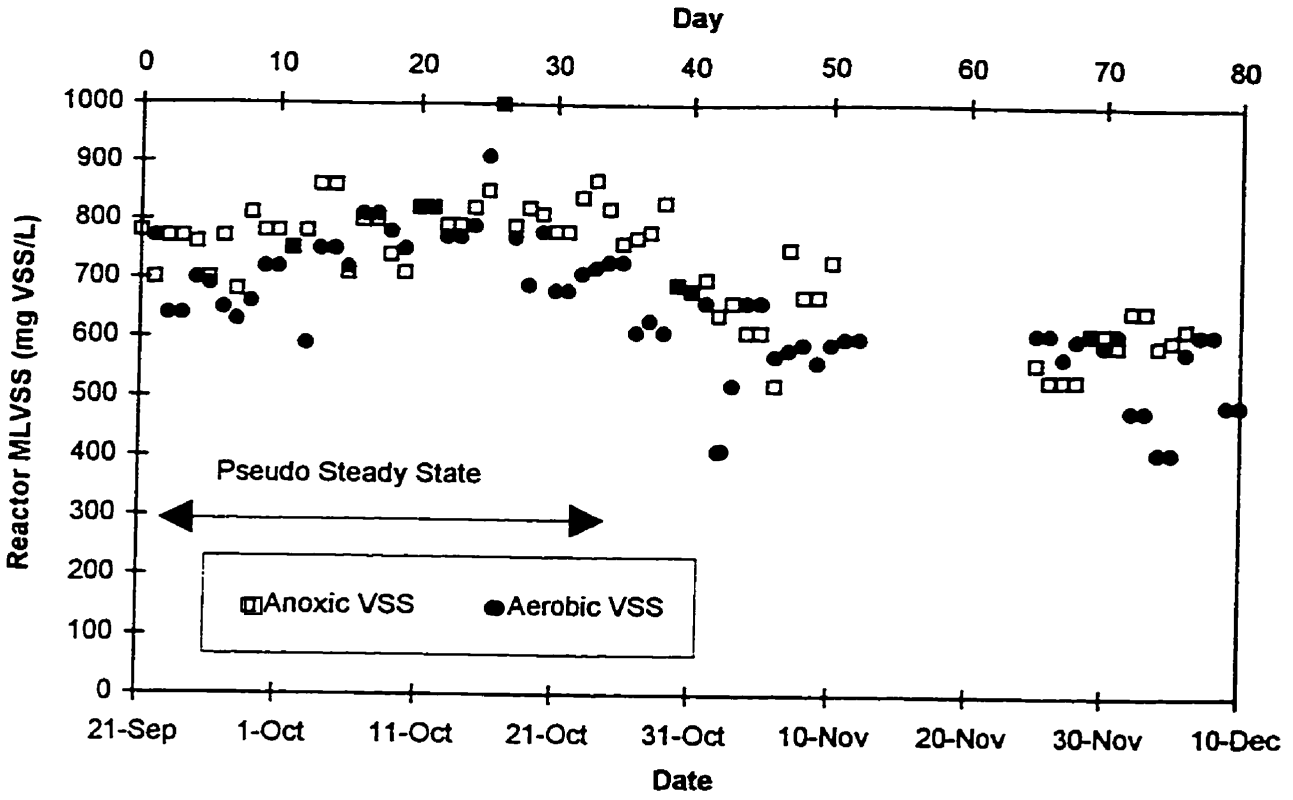


MLVSS Profile

At 20°C, similar trends were seen in the mixed liquor volatile solids (MLVSS) profiles for the aerobic and anoxic reactors (Figure 5.2). The data were scattered but fairly consistent. For the first 35 days of the period, the MLVSS ranged between 700-800 mg VSS/L and then declined to the 500-600 mg VSS/L range.

During the period of Oct. 19 - Nov. 2, the influent feed strength was lower possibly causing the decreases in the MLVSS seen for both the aerobic and anoxic reactors as of Oct. 26th. With a SRT approaching ten days, decreases in feed strength would take some time before they would be reflected as a decreased MLVSS. This concept was supported by decreases in MLVSS occurring in both aerobic and anoxic reactors.

Figure 5.2 Reactor MLVSS - 20°C



Bacteria Counts

Bacteria count data were available for the cultures during the later part of the 20°C period (Table 5.1). The confidence limits for the cell counts were derived in the manner specified in Section 3.2.2.d . The purpose of the experiment was to compare the number of bacteria in the aerobic and anoxic reactor at the same instant and to relate cell counts to MLVSS measurements. Time and resource constraints made it impossible to perform bacteria counts for the entire period. Instead samples were taken from the aerobic and anoxic reactor over the same ten day period.

There was insufficient data at 20°C to determine if statistically a correlation existed between the bacteria counts and the reactor MLVSS concentration; however, visual inspection of the data indicated, no correlation between reactor MLVSS and bacteria counts. A more extensive bacteria count data set at 14°C allowed for more complete analysis.

Table 5.1 Bacteria Counts and Reactor MLVSS for SBR at 20°C

Reactor	Date	Bacteria Count Data (cell /L)x 10^{11}			Reactor MLVSS (mg VSS/L)	Bacteria per MLVSS (Cell/MLVSS)x 10^{8}
		Mean	Upper 95% CL	Lower 95% CL		
Aerobic	Nov. 27	7.04	8.58	5.78	570	12.4
Aerobic	Nov. 29	23.2	25.1	21.5	610	38.0
Aerobic	Dec. 6	4.82	5.81	4.00	580	8.31
Anoxic	Dec. 1	13.3	15.3	11.5	590	22.5
Anoxic	Dec. 4	14.0	17.4	11.3	590	23.7
Anoxic	Dec. 7	5.77	7.02	4.74	620	9.38

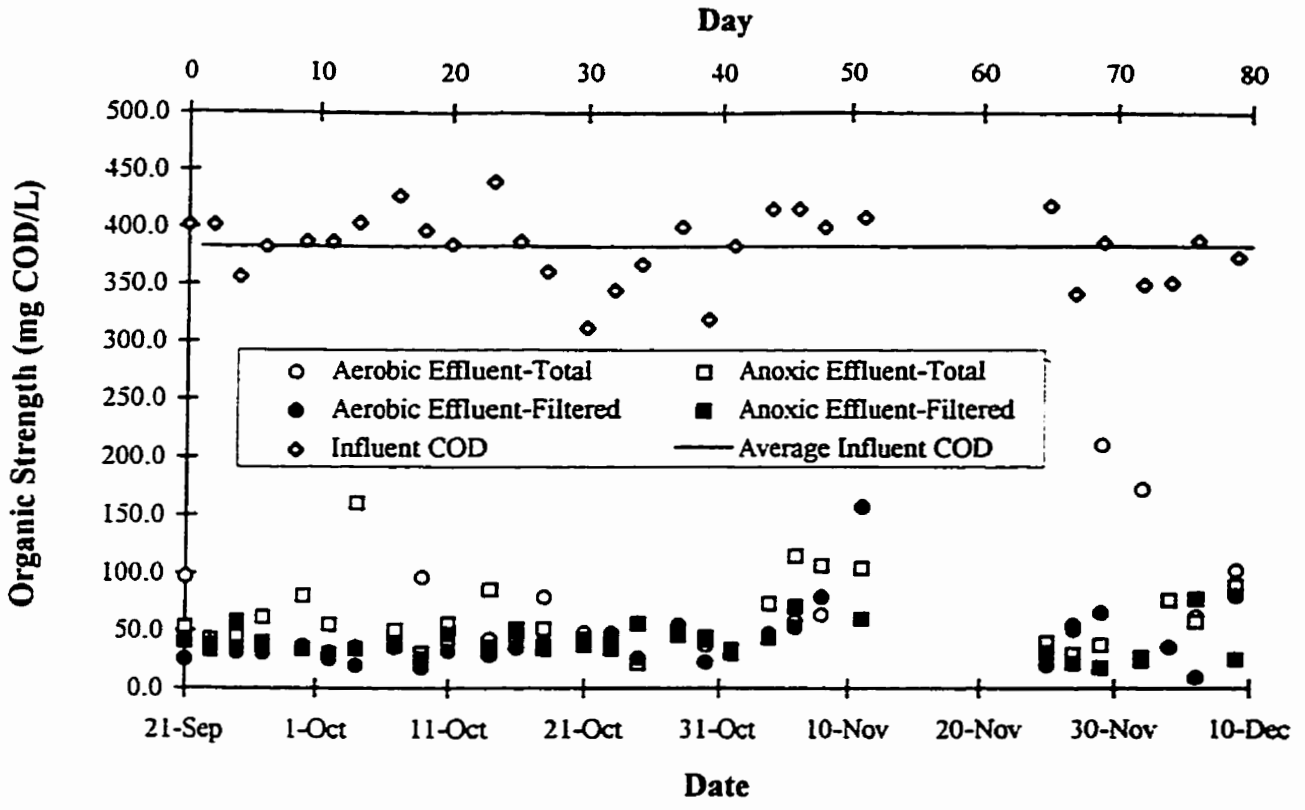
Effluent COD

Figure 5.3 shows both the total and filtered effluent COD for the aerobic and anoxic reactors and influent strength. Generally, good COD removal was obtained for both reactors regardless of the influent COD concentration.

The filtered effluent concentration was typically 40 - 50 mg COD/L. The organics included in the filtered effluent COD could have been soluble non-biodegradable organics present in the sewage, soluble refractory organics generated during the process and possibly undegraded biodegradable organics. With municipal wastewaters, refractory organics can account for 50% of the effluent COD (Henze, 1992). There were some fluctuations in the effluent's total COD but with consistent filtered COD values, these fluctuations may arise as a result of reduced settability.

There was a period of higher total and filtered COD during the period of Oct. 31 - Nov. 10 for both aerobic and anoxic reactors. This corresponds to a period of decreasing MLVSS due to biosolids lost in the effluent (Figure 5.2). Total effluent concentrations of the aerobic reactor were exceptionally high (150-200 mg COD/L) for Nov. 29 - Dec. 3 suggesting reduced settability. This is consistent with the marked decrease in the MLVSS in the aerobic reactor for this same period (Figure 5.2). Microscopic examination of the samples from early December showed the presence of filamentous growth. When these are present in excess amounts, the result is sludge separation problems known as bulking and foaming often occur.

Figure 5.3 Influent and Effluent CODs - 20°C



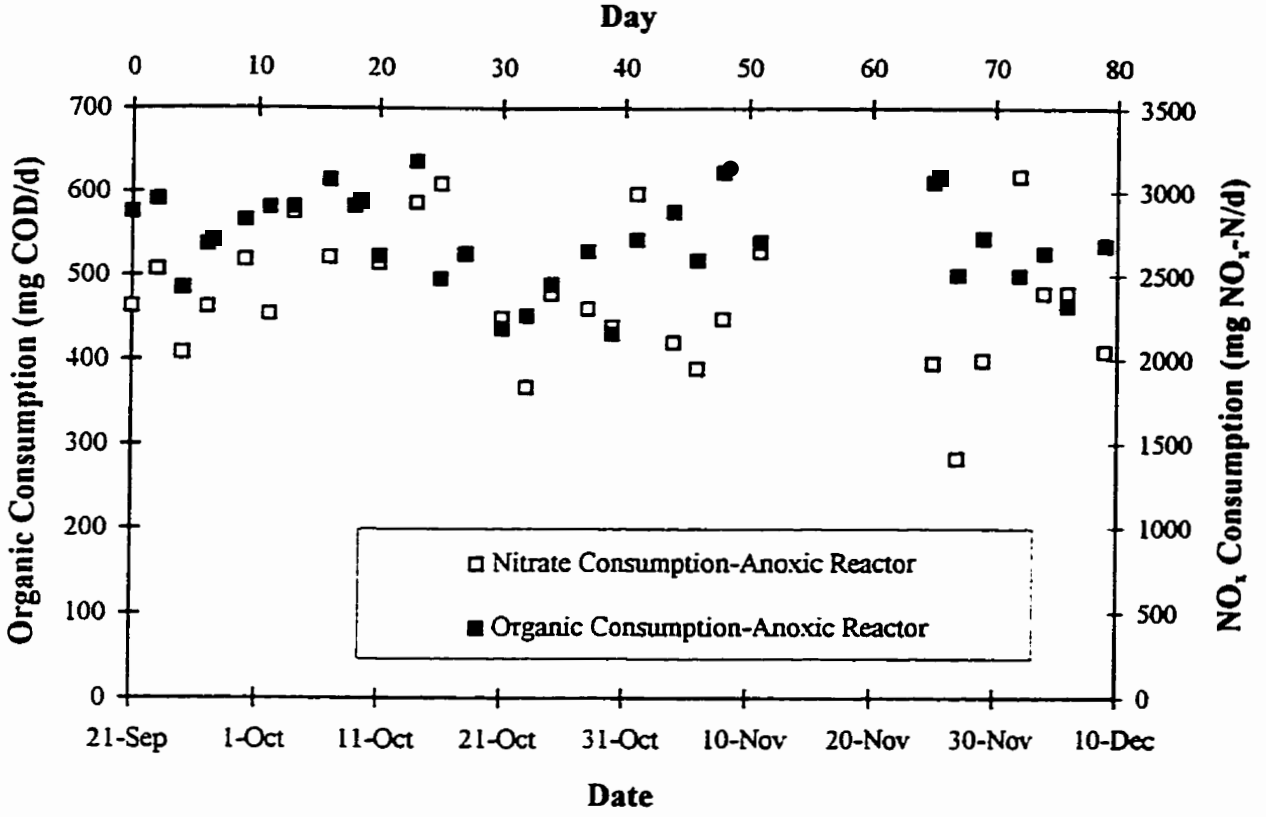
Nitrate Removal

If nitrate concentrations are greater than 1-2 mg NO_3-N/L, then nitrate should not limit the rate of denitrification (U.S. EPA, 1993). Nitrate determinations were done on acidified samples so nitrate cannot be differentiated from nitrite. NO_X will be used to designate nitrate determined on acidified samples. The median effluent nitrate concentration was 14.8 mg NO_X-N/L suggesting denitrification proceeded under carbon limited conditions.

Figure 5.4 illustrates the amount of organic substrate and nitrate consumed per day over the eighty day period. The amount of denitrification realized should closely follow the amount of organic consumed. As seen from the graph, the patterns of carbon and nitrate utilization do not correspond with each other. Organic consumption was based on the influent organic strength less the filtered effluent COD. This calculation would accurately reflect organic consumption if substrate had not accumulated within the floc or the reactor was at pseudo-steady state. These conditions may not apply to this system. A further discussion of the ratio of nitrate to organic substrate consumption is presented in Section 5.2.2.b-Consumptive Ratio. The amount of nitrate

nitrogen consumed was 400-600 mg NO_x-N/d or alternately 40-60 mg NO_x-N/L. During the period from Sept. 21 to Oct. 21, nitrate consumption was higher than during the rest of the period. An influent with higher than average strength as seen in Figure 5.1 could be responsible for this behaviour.

Figure 5.4 Organic and Nitrate Consumption for an Anoxic SBR - 20°C

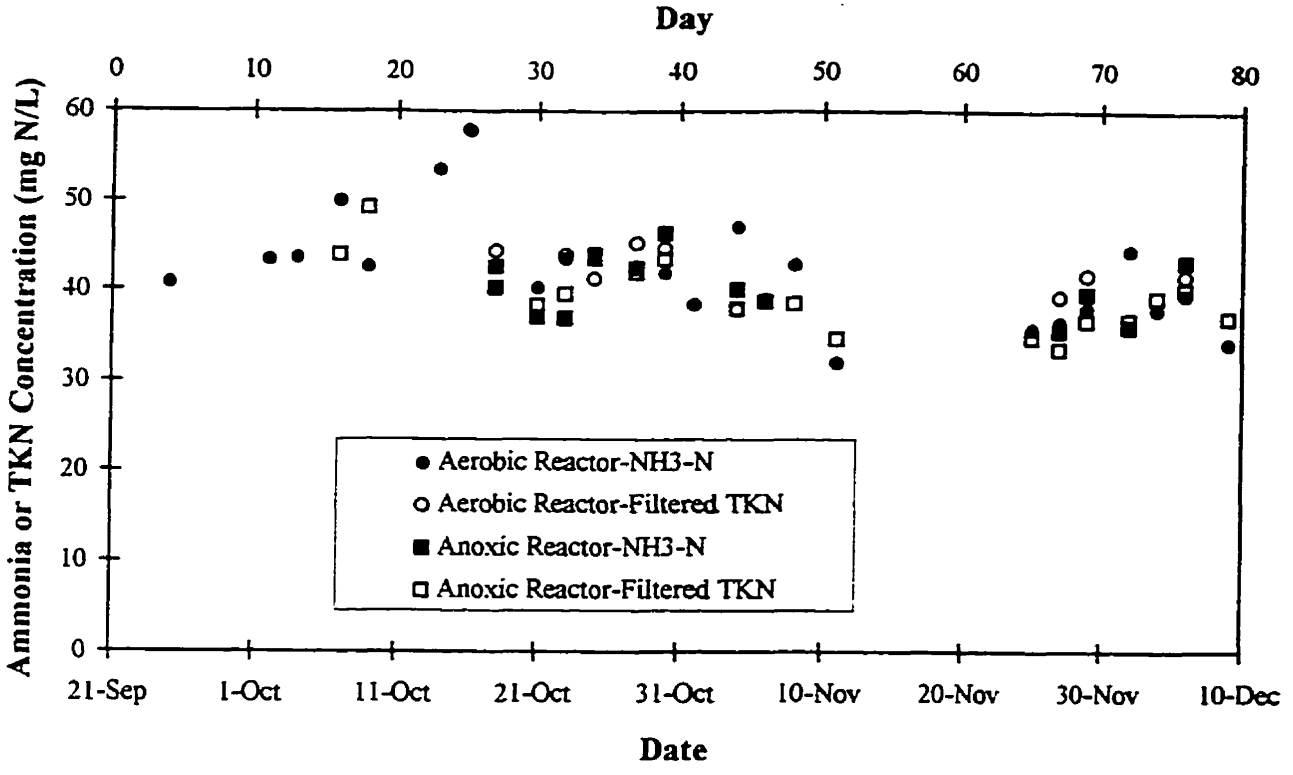


Effluent Nitrogen

The degradation of influent biodegradable TKN produces ammonia nitrogen which can be used in cell synthesis. In this particular instance, most of the influent TKN was derived from the protein feed since no other nitrogen source was added apart from small amounts present in the sewage. Ammonia nitrogen in excess of the requirements of cell synthesis was present in the effluent. Filtered TKN measurements included both the ammonia nitrogen and soluble organic nitrogen. Theoretically, the TKN should be greater than the ammonia nitrogen. If the difference between the actual TKN and ammonia nitrogen is only 1-2 mg N/L, then the measured TKN may be less than the ammonia nitrogen because of the errors incurred in the digestion step of the TKN determination. These measurements are presented for the SBRs at 20°C in Figure 5.5. For a number of

days, the ammonia nitrogen value exceed the measured TKN. As shown by the figure effluent ammonia nitrogen or TKN is typically greater for the aerobic reactor than it is for the anoxic reactor.

Figure 5.5 Effluent Filtered TKN and Ammonia Nitrogen - 20°C



Effluent ammonia nitrogen is related to the organic strength of the feed and the amount of ammonia nitrogen present in the sewage. Nitrification was inhibited so ammonia nitrogen was consumed only for cell synthesis. Figure 5.5 shows the same general trend as does the feed strength (Figure 5.1).

A paired Student's t test using mixed liquor TKN values presented in Table 5.2 indicates the aerobic reactor has a significantly lower TKN than the aerobic reactor (Raw Data- Appendix B). The t value of 2.41 with 10 degrees of freedom (df) was compared to a $t_{0.95}$ of 2.22.

Further analysis was done to determine if this was a consequence of higher MLVSS concentrations or a higher TKN/MLVSS ratio. An accumulation of partially degraded proteins in the mixed liquor would have resulted in a higher TKN/MLVSS ratio. Differences in this ratio for the aerobic and anoxic reactor would suggest an accumulation of proteins in the reactor having the higher ratio. A paired Student's t-test on the data

of Table 5.3 showed that was no significant difference between the TKN/MLVSS ratios of aerobic and anoxic reactors. A t-value of -0.286 on 10 df was less than the critical value, $t_{0.95}$, of 2.22.

ii) 14°C Cultures

Feed Strength and Composition

The average influent strength for the 14°C period was 386.0 mg COD/L (Figure 5.6). This is comparable to the influent strength of the 20°C period of 382.9 mg COD/L (Figure 5.1). The standard deviation for the 14°C period was 48.5 mg COD/L while for the 20°C period it was 30.7 mg COD/L. The influent strength at 14°C was more variable than it was at 20°C. The influent feed for Mar. 4 - 8 had a much higher organic strength than the rest of the period due to an error in making up the protein feed. Both the total and filtered protein samples for the periods of Mar. 23 - 25 and Mar. 26 - 27 were lost after the glass sample container cracked in the freezer. The strength of the feed was generally higher in the first third of the experimental period. In the last two thirds of the experimental program, the feed strength tended to be more variable. Some of this variation was caused by changes in sewage strength caused by rain and ground water infiltration.

MLVSS Profiles

The temperature of the reactors was lowered to 14°C on January 30, 1996. Monitoring of reactor performance began on Mar. 4. Two events that occurred during the interim are noteworthy. On Feb. 12th, the anoxic reactor's pH controller failed leading to the addition of excess acid. The pH was as low as 2 for a period of approximately fifteen minutes. The waste MLVSS of the aerobic reactor was added to the anoxic reactor for the next three days to hasten the recovery. On Feb. 16, the power was out for five hours causing the temperature of the environmental chamber to rise as high as 22°C.

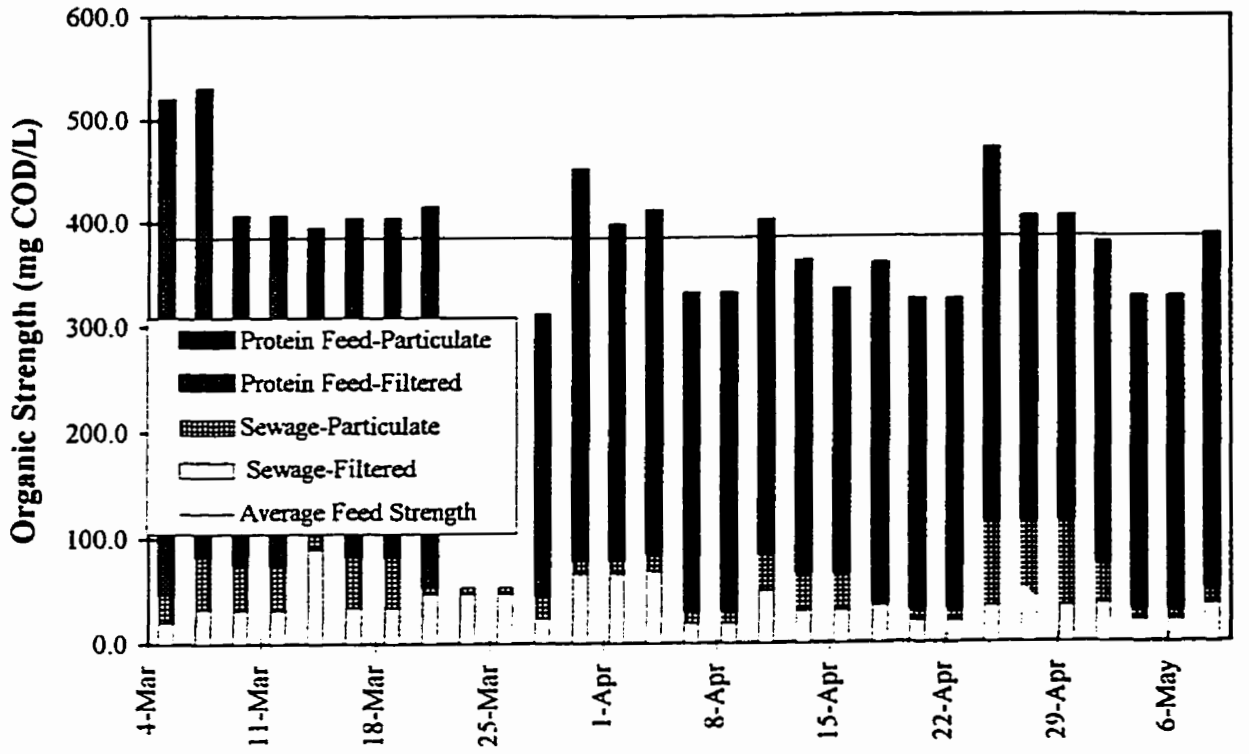
Table 5.2 Influent and Effluent Total TKN - 20°C

Starting Date	Ending Date	Feed			Aerobic Reactor		Anoxic Reactor	
		Sewage TKN (mg N/L)	Protein TKN (mg N/L)	Average Feed TKN (mg N/L)	Effluent TKN (mg N/L)	Mixed Liquor TKN (mg N/L)	Effluent TKN (mg N/L)	Mixed Liquor TKN (mg N/L)
18-Oct	20-Oct	36.2	57.7	50.5	49.2	114.3	34.9	132.0
21-Oct	22-Oct	39.0	58.7	52.1	42.3	123.0	37.0	136.1
23-Oct	24-Oct	24.5	65.8	52.0	41.4	123.3	36.8	134.8
25-Oct	27-Oct	46.9	68.8	61.4	43.0	119.1	40.3	138.5
28-Oct	29-Oct	45.3	65.7	58.8	46.1	118.0	44.9	142.1
30-Oct	31-Oct	48.2	61.9	57.3	45.8	83.7	47.2	123.9
4-Nov	5-Nov	37.6	70.0	59.1	40.1	116.1	44.2	111.7
27-Nov	28-Nov	31.5	62.4	52.0	42.1	117.9	35.5	108.7
29-Nov	1-Dec	28.0	63.8	51.8	49.2	119.9	36.2	104.1
2-Dec	3-Dec	25.0	55.0	45.0	44.4	96.7	33.7	127.4
6-Dec	8-Dec	37.6	73.7	61.6	44.4	125.9	44.4	134.3

Table 5.3 Nitrogen Content of Mixed Liquor

Starting Date	Ending Date	Aerobic Reactor			Anoxic Reactor		
		Average MLVSS (mg VSS/L)	Mixed Liquor TKN (mg N/L)	TKN / MLVSS (mg N/mg VSS)	Average MLVSS (mg VSS/L)	Mixed Liquor TKN (mg N/L)	TKN / MLVSS (mg N/mg VSS)
18-Oct	20-Oct	747	114.3	0.153	807	132	0.164
21-Oct	22-Oct	680	123	0.181	780	136.1	0.174
23-Oct	24-Oct	715	123.3	0.172	855	134.8	0.158
25-Oct	27-Oct	690	119.1	0.173	783	138.5	0.177
28-Oct	29-Oct	620	118	0.190	805	142.1	0.177
30-Oct	31-Oct	685	83.7	0.122	685	123.9	0.181
4-Nov	5-Nov	660	116.1	0.176	610	111.7	0.183
27-Nov	28-Nov	585	117.9	0.202	530	108.7	0.205
29-Nov	1-Dec	603	119.9	0.199	603	104.1	0.173
2-Dec	3-Dec	480	96.7	0.201	650	127.4	0.196
6-Dec	8-Dec	590	125.9	0.213	620	134.3	0.217

Figure 5.6 Influent Feed Concentration and Composition - 14°C



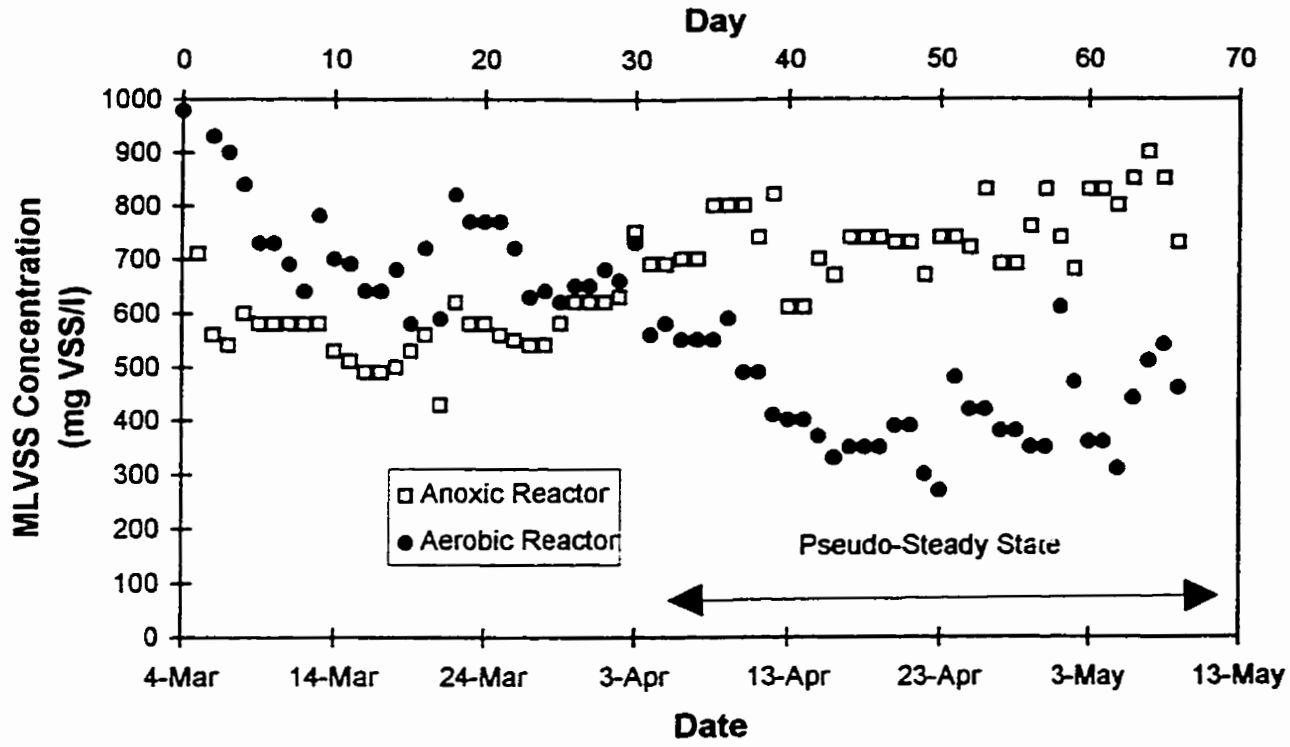
Three sludge ages are generally considered to be a sufficiently long time for acclimation to occur (Benedict and Carlson, 1973). With a target sludge age of ten days, thirty days of acclimation time should be sufficient based on three sludge ages criteria. An acclimated culture should have been produced by Mar. 16. The data suggests that this may not have been the case given the MLVSS profiles shown in Figure 5.7. In the early part of the monitoring period, the aerobic reactor had a higher MLVSS than the anoxic reactor. By the end of the period, the converse was true. As a result, only the Apr. 5 - May 9 data will be considered to be acclimated and at pseudo-steady state.

Bacteria Counts

Bacteria counts for the SBRs at 14°C were determined as described in Section 3.22.d and are presented in Table 5.4. As mentioned previously, bacteria counts on samples taken from the aerobic and anoxic reactor on the same day would have been ideal but time constraints made this impossible. On some occasions, the samples were taken two or three days apart.

Table 5.4 shows that the anoxic reactor consistently had more bacteria than the aerobic reactor. The cell counts in the aerobic reactor range from 4.58 - 12.1 x 10^{11} bact/L while the counts of the anoxic reactor range between 13.5 - 25.0 x 10^{11} bact/L. A significant linear correlation does not exist between bacteria counts and MLVSS for the aerobic reactor. The calculated correlation coefficient (r) value was 0.436 while the critical value at the 95% CL with 4 df was 0.811. For the anoxic reactor, the MLVSS was correlated with the bacteria counts. The calculated r value was 0.811 while the critical value at the 95% CL was 0.707 with 6 df.

Figure 5.7 Reactor MLVSS - 14°C



Effluent COD

Figure 5.8 shows that excellent substrate removal occurred with the filtered effluent remaining constant at 40 - 50 mg COD/L over the seventy day period in spite of variation in the COD of the feed and major changes in the MLVSS. There was no apparent correlation between the influent and effluent filtered COD. From Mar. 4 - 24, the anoxic reactor has total CODs that ranged between 50 - 130 mg COD/L and filtered CODs of 50 mg COD/L or less. This would suggest that the anoxic reactor was experiencing settling problems during this period. The same comments are applicable to the aerobic reactor's performance during the Mar. 24 - Apr. 8

period. Microscopic examination of the aerobic mixed liquor during the Apr. 9 - Apr. 12 period did not indicate the presence of filamentous growth which could have resulted in the poor settling characteristics of the biomass.

Table 5.4 Bacteria Counts for SBRs - 14°C

Reactor	Date	Bacteria Count Data (bact /L)x10^{11}			Reactor MLVSS (mg VSS/L)	Bacteria per MLVSS (Bact/MLVSS)x 10^{8}
		Mean	Upper 95% CL	Lower 95% CL		
Aerobic	Apr. 9	4.58	5.08	4.13	590	7.76
Aerobic	Apr. 12	6.83	7.68	6.06	410	16.7
Aerobic	Apr. 26	12.1	14.1	10.4	420	28.8
Aerobic	May 5	4.09	4.54	3.69	310	13.1
Aerobic	May 7	5.08	5.94	4.34	510	9.96
Aerobic	May 9	5.90	7.43	4.69	460	12.8
Anoxic	Mar. 29	25.0	28.5	22.0	580	43.1
Anoxic	Apr. 9	13.5	14.9	12.3	800	17.4
Anoxic	Apr. 12	13.9	16.0	12.2	820	16.9
Anoxic	Apr. 24	21.0	23.5	18.8	740	28.3
Anoxic	Apr. 26	19.3	21.8	17.0	820	23.5
Anoxic	Apr. 29	16.0	19.6	13.1	760	21.0
Anoxic	May 5	23.3	25.9	20.9	800	29.1
Anoxic	May 9	14.5	18.3	11.5	730	19.9

Nitrate Removal

As mentioned previously, nitrate was added in excess so the rate of the denitrification was limited by the amount of organics present. Figure 5.9 illustrates the amount of organic and nitrate consumed per day over the seventy day period. The ratio of these two factors, the consumptive ratio, indicates the amount of exogenous substrate required in a post-denitrification reactor. Ideally, the ratio should be a constant but because of the difficulties in accurately measuring the amount of substrate consumed it often is not. The consumptive ratio will be calculated later in this section. Excluding the data from Mar. 4 - Mar. 24, 400 - 700 mg NO_XN/d were consumed. The equivalent conversion on a volume basis would be 50 - 87 mg NO_X-N/L. During this period, nitrate consumption does not follow any trend even though the reactor MLVSS was increasing over this period. The variability in the nitrate removal may be due in part to the variability in the influent organic strength.

Figure 5.8 Influent and Effluent COD for SBRs - 14°C

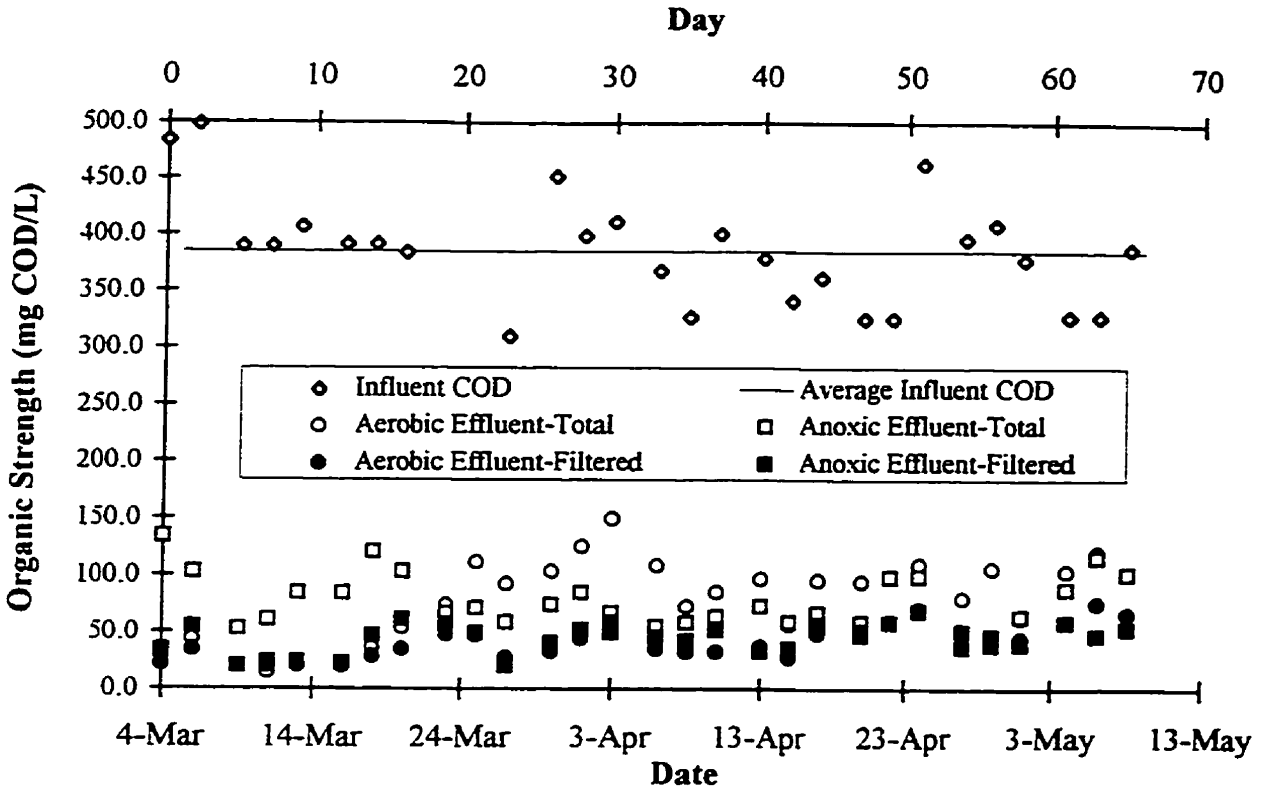
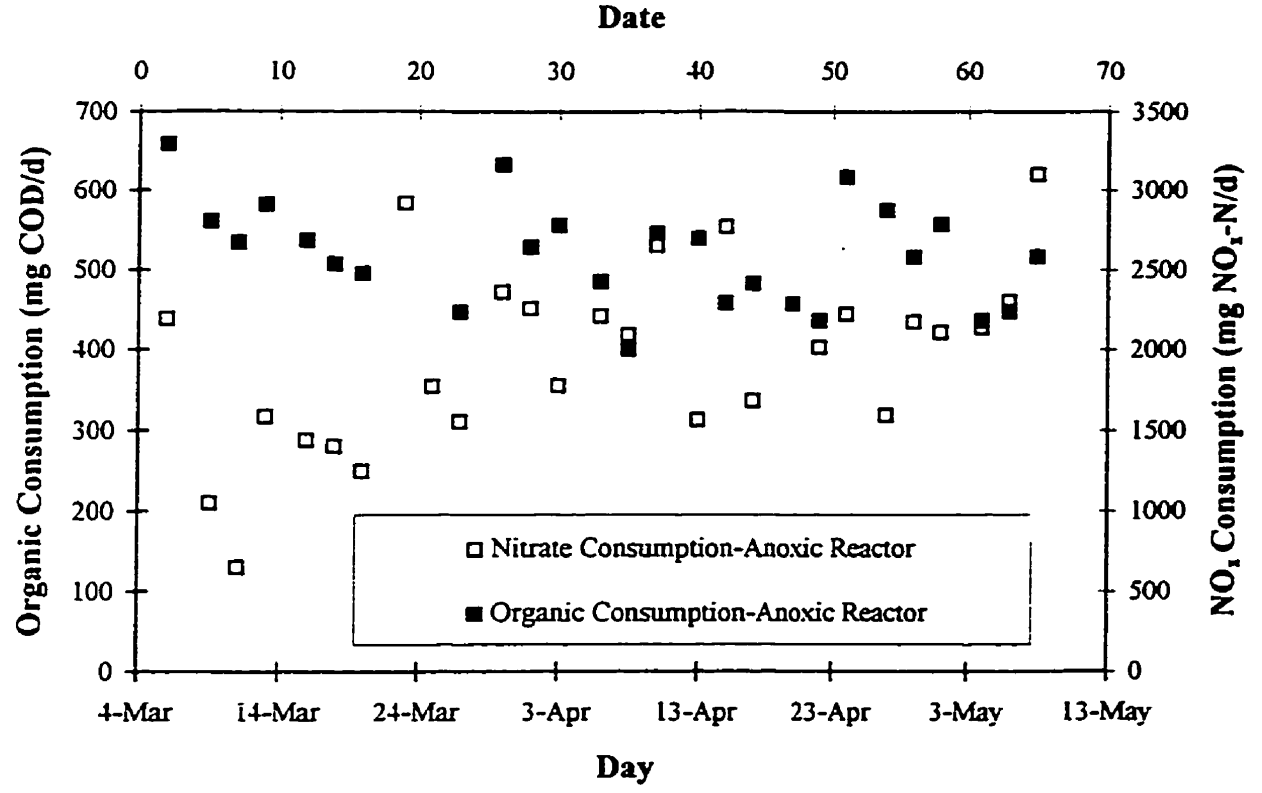


Figure 5.9 Organic and Nitrate Consumption for Anoxic SBR- 14°C



Effluent Nitrogen

Effluent filtered ammonia levels were affected principally by the concentration of proteins in the feed. Figure 5.10 shows the same general trend as does the feed strength (Figure 5.6). Filtered TKNs and effluent ammonia nitrogen range from 28 - 42 mg N/L over the period of Mar. 30 to May 10 (Figure 5.10). For the same reasons as discussed previously, there are occasions when the measured ammonia nitrogen exceeds the filtered TKN. By visual inspection, the filtered TKN and ammonia nitrogen concentration appears to be highest for the aerobic reactor.

Table 5.5 presents the influent, effluent total TKN, and mixed liquor TKN for a number of composite samples (Raw data - Appendix B). The mixed liquor TKN values for the anoxic reactors is significantly higher for the anoxic reactor than it is for the aerobic reactor. A paired t-test gave a value of 5.80 on 13 df while the critical value, $t_{0.95}$ was 2.16. These same trends were seen for the 20°C cultures.

Figure 5.10 Effluent Filtered TKN and Ammonia Nitrogen

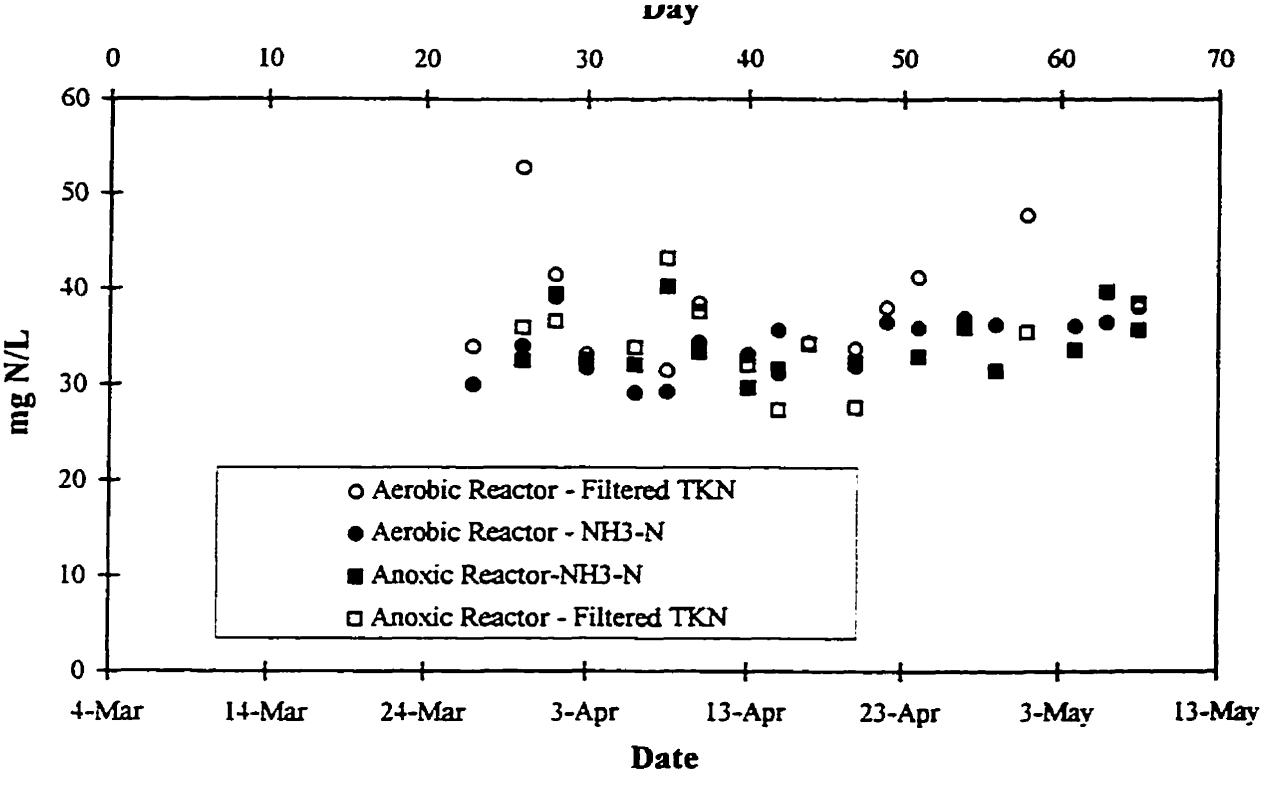


Table 5.5 Influent and Effluent Total TKN - 14°C

Starting Date	Ending Date	Feed			Aerobic		Anoxic	
		Sewage TKN (mg N/L)	Protein TKN (mg N/L)	Feed TKN (mg N/L)	Effluent TKN (mg N/L)	Mixed Liquor TKN (mg N/L)	Effluent TKN (mg N/L)	Mixed Liquor TKN (mg N/L)
30-Mar	31-Mar	28.7	63.1	51.6	42.7	103	39.0	112
1-Apr	2-Apr	28.7	63.1	51.6	45.9	87.9	36.6	119
3-Apr	5-Apr	28.2	52.9	44.6	44.9	103	36.9	131
6-Apr	7-Apr	28.5	73.3	58.3	39.6	101	36.4	131
8-Apr	9-Apr	28.5	73.3	58.3	40.3	104	42.3	137
10-Apr	12-Apr	30.0	65.9	53.9	42.8	101	39.6	119
13-Apr	14-Apr	25.5	57.6	46.9	38.7	84.5	37.6	127
15-Apr	16-Apr	25.5	50.2	41.9	36.5	108	30.1	105
17-Apr	19-Apr	25.4	58.5	47.4	40.8	70.8	**34.2**	123
20-Apr	21-Apr	25.4	58.5	47.4	34.2	63.1	27.6	115
22-Apr	23-Apr	21.5	55.0	43.8	**38.0**	57.3	33.3	117
24-Apr	26-Apr	28.7	70.6	56.6	45.9	86.0	38.8	145
1-May	2-May	31.1	61.3	51.2	**47.7**	78.8	38.3	111
7-May	9-May	25.7	71	55.8	42.4	131.2	42.4	131

Note: Bolded Values are Filtered TKN values.

Table 5.6 Nitrogen Content of Mixed Liquor

Starting Date	Ending Date	Aerobic Reactor			Anoxic Reactor		
		Average MLVSS (mg VSS/L)	Mixed Liquor TKN (mg N/L)	TKN / MLVSS (mg N/mg VSS)	Average MLVSS (mg VSS/L)	Mixed Liquor TKN (mg N/L)	TKN / MLVSS (mg N/mg VSS)
30-Mar	31-Mar	650	103	0.158	620	112	0.180
1-Apr	2-Apr	670	87.9	0.131	620	119	0.192
3-Apr	5-Apr	645	103	0.160	710	131	0.185
6-Apr	7-Apr	550	101	0.183	700	131	0.187
8-Apr	9-Apr	570	104	0.182	800	137	0.171
10-Apr	12-Apr	490	101	0.207	787	119	0.151
13-Apr	14-Apr	400	84.5	0.211	610	127	0.209
15-Apr	16-Apr	350	108	0.307	685	105	0.153
17-Apr	19-Apr	350	70.8	0.202	740	123	0.167
20-Apr	21-Apr	390	63.1	0.162	730	115	0.157
22-Apr	23-Apr	265	57.3	0.216	705	117	0.167
24-Apr	26-Apr	450	86	0.191	763	145	0.190
1-May	2-May	540	78.8	0.146	710	111	0.156

The mixed liquor TKN was normalized on MLVSS to determine if there was a difference in the nitrogen content of the MLVSS (Table 5.6). A higher nitrogen content in the MLVSS of the anoxic reactor would suggest an accumulation of proteins. A paired Student's t-test comparing the nitrogen content in the MLVSS showed no significant difference between the aerobic and the anoxic reactors. The t-value was 1.02 while the critical value, $t_{0.95}$, was 2.18 with 12 df. Higher mixed liquor TKNs for the anoxic reactor were the direct result of higher MLVSS concentrations.

c) Discussion of Experimental Data

i) Solids Retention Time

Defining the average age of microorganisms in the reactor or the sludge age is challenging because they are generated by growth and lost by predation and cell lysis. The traditionally used solids retention time (SRT) as expressed in Equation 5.1 was derived from a steady state mass balance on a stirred tank reactor with recycle. Rarely are activated sludge systems at steady state because of changes in influent strength. The steady state assumption, on which the definition of SRT is based on, often is not applicable. In practice, there is a minimum SRT at which the reactor can operate before washout occurs; however there is no limit to the value of SRT. If little or no wastage occurs in a SBR, the SRT continues to increase. In contrast, if a large amounts of MLVSS are lost from the system, the SRT will become very short. Various averaging strategies have been developed to account for the variability in SRT which occur under continuous operation.

$$SRT = \frac{VX}{Q_e X_e + Q_w X} \qquad \text{Eq. (5.1)}$$

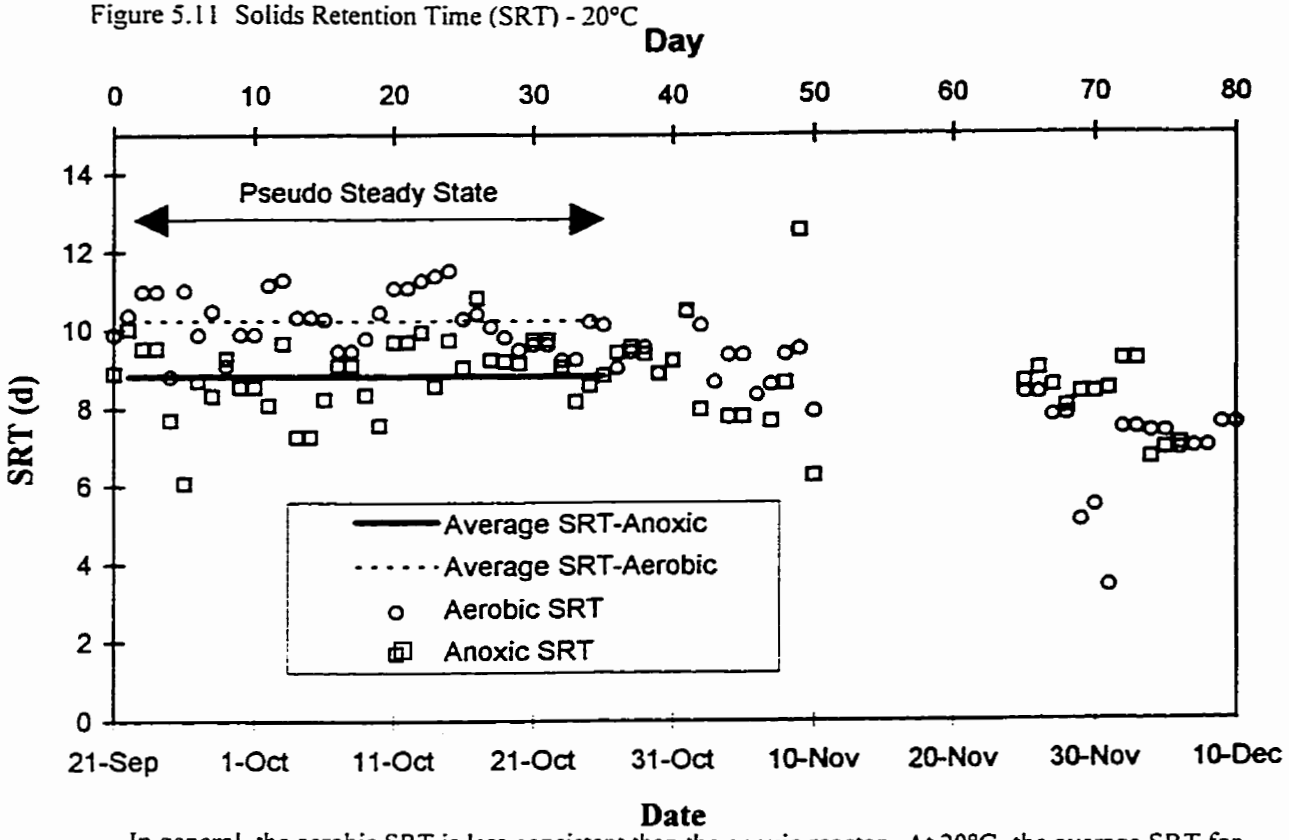
where SRT = solids retention time (d)
V = reactor volume (L)
Q_e = discharge volume (L)
X_e = discharge solids concentration (mg VSS/L)
Q_w = wastage volume (L)
X = reactor MLVSS concentration at the time of wastage (mg VSS/L)

On a theoretical basis, the current experimental system should have a SRT of 10 d because 10% of the mixed reactor volume was wasted daily. Loss of solids in the effluent, will further reduce the SRT. The

SRT calculations do not take into consider changes in the inventory of biosolids in the reactor when calculating the biosolids produced. They are not considered because of the pseudo-steady state assumption.

Figure 5.11 shows the SRT for the system at 20°C. The lines on the figure represent the mean SRT for a particular time period. At 20°C, the aerobic reactor had two of sixty eight data points outside the range of 0 - 15 days. They have been excluded from further calculations. The scale of the graph was limited to this range for easy comparison of the aerobic and anoxic reactors.

Figure 5.11 Solids Retention Time (SRT) - 20°C

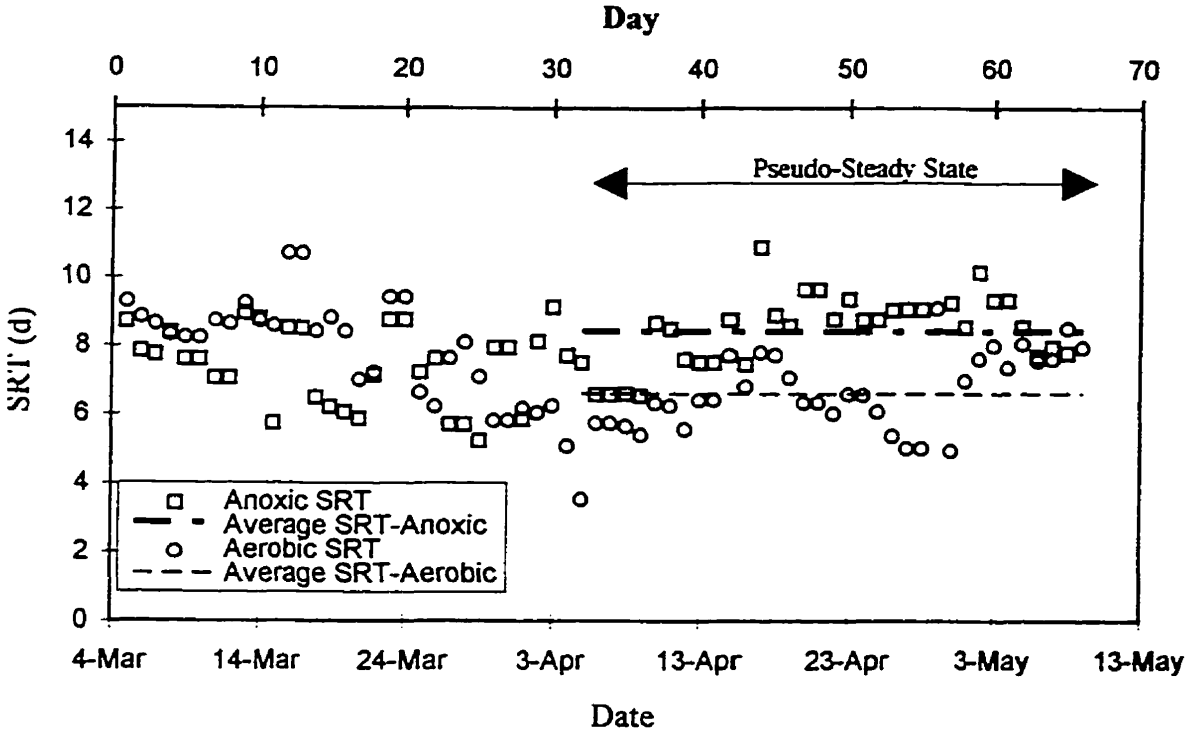


In general, the aerobic SRT is less consistent than the anoxic reactor. At 20°C, the average SRT for sixty five days of aerobic and anoxic reactor operation was 10 and 8.8 d, respectively. The aerobic data are more variable with the average SRT of 9.9 d for the first 50 days. This is greater than the average of 7.7 d which was calculated for days sixty five to eighty. The standard deviations for the aerobic and anoxic reactors are 4.7 and 1.9 d, respectively. A one day difference in SRT between the aerobic and anoxic reactors is not significant given the standard deviations in the SRT of the anoxic reactor.

A plot of SRT versus time for the reactors at 14°C appears to show more scatter than was seen at 20°C (Figure 5.12). There was a gradual decrease in the SRT of the aerobic reactor over the first thirty days of

period. The SRT of the anoxic reactor increased slowly over this same period. Over the 14°C period, the average SRT for the aerobic and anoxic reactors was 6.6 and 8.4 days, respectively. At 14°C. the mean SRT for the aerobic reactor was 3.4 days lower than it was at 20°C. In contrast, the anoxic reactor showed no real change in SRT with the lower temperature.

Figure 5.12 Solids Retention Time (SRT) - 14°C



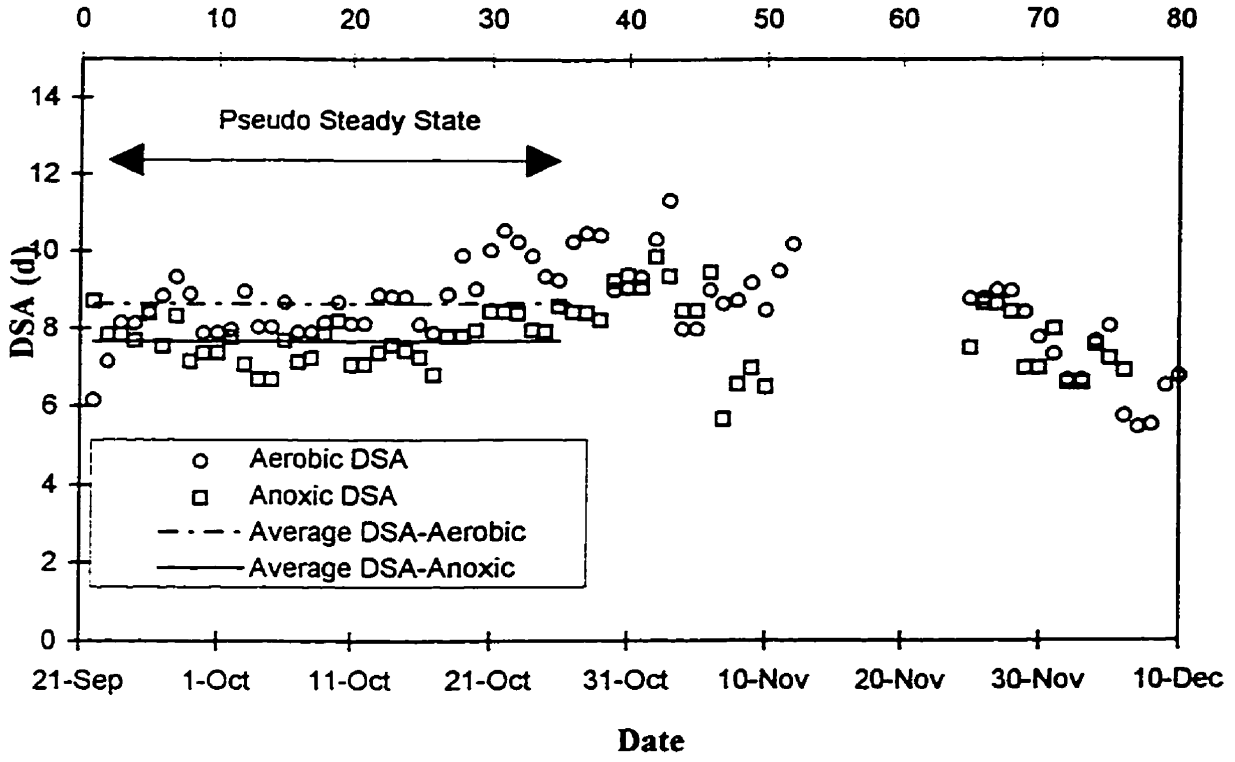
ii) Dynamic Sludge Age

The dynamic sludge age (DSA) was proposed by Vaccari *et al.* (1985) as an alternative way of calculating sludge age. The DSA, the mean of the biomass age distribution, is applicable to non-steady state systems. Wastage is assumed to occur from each age distribution in proportion to its contribution to the total biomass. If 95% of the reactor biomass was wasted, the mean of the age distribution remains unchanged. Although the amount of biomass has decreased dramatically, the age distribution is not affected because losses from each age group occur in proportion to its contribution to the total biomass. The DSA examines the production of biomass taking into account increases and decreases in the solid inventory. If there is no net production of biomass, a culture will age one day per day regardless of how much wastage occurs. The DSA

was calculated using the methodology and equations found in Appendix D and is presented in Figures 5.13 and 5.14.

The aerobic reactor at 20°C has an average DSA of 8.5 days with a standard deviation of 1.2 days over the 80 day period (Figure 5.13). Similarly for the anoxic reactor at 20°C, the average DSA and the associated standard deviation are 7.8 and 0.8 days, respectively. The DSA for both the aerobic and anoxic reactors decreased over the last 20 days of operation.

Figure 5.13 Dynamic Solids Age (DSA) - 20°C



Over the first 31 days of the 14°C period, the DSA for the aerobic and anoxic reactors decreases because of reductions in the MLVSS of the reactors (Figure 5.14). The average DSA for the 30 to 65 day period of the 14°C work was 5.7 days for both aerobic and anoxic reactors. The standard deviations for aerobic and anoxic reactors in this latter period were 0.9 and 1.2 days, respectively. These are comparable to those at 20°C.

The DSA differs from the SRT in two respects when Figures 5.13 and 5.14 were compared to Figures 5.11 and 5.12.

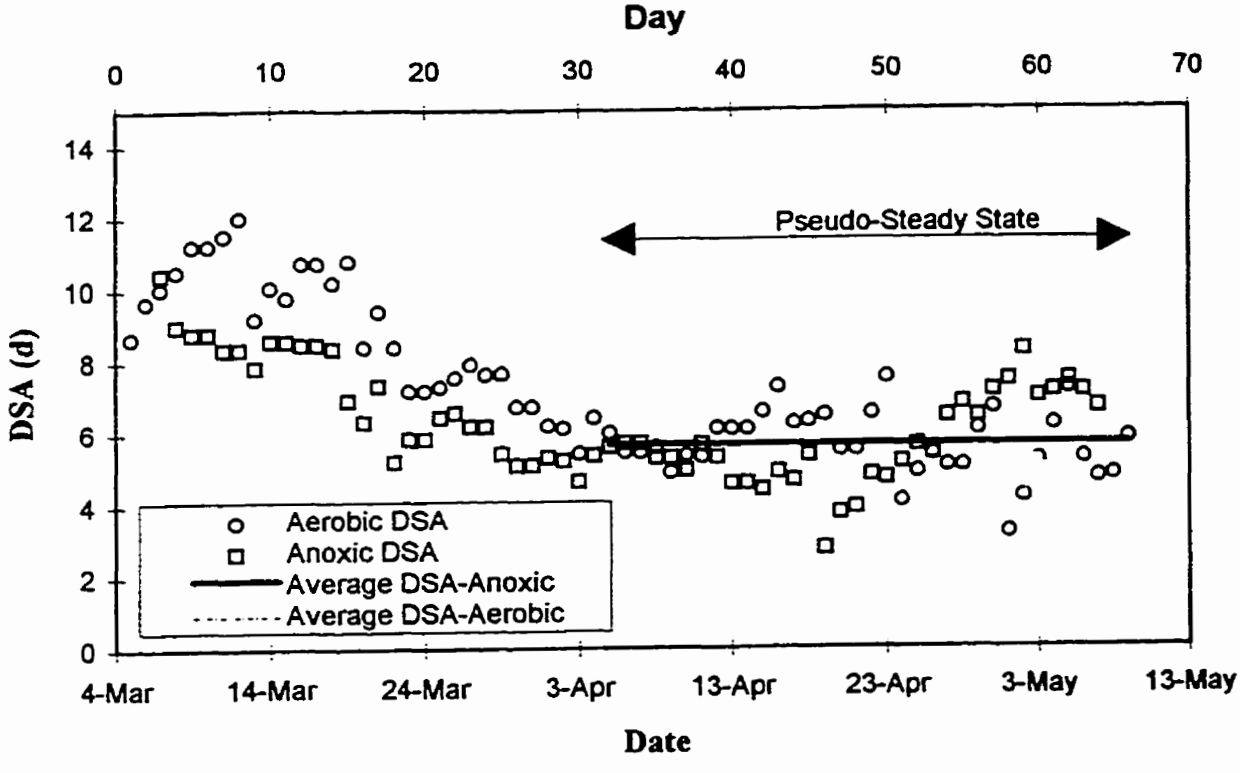
1. Changes in the reactor solids inventory at 14°C (Figure 5.7) were reflected in both the DSA and SRT (Figure 5.12) but it were more evident in the DSA (Figure 5.14). The DSA takes changes in solids inventory

into account whereas the SRT does not because of the steady state assumption. For the 20°C period, there was also a downward trend in the DSA of the aerobic reactor over the 15 days of the period. During this same period, the SRT was relatively constant; however there were three days where the SRT ranged between 3 and 6 days (Figure 5.11).

2. The DSA was generally lower than the SRT. The DSA can only increase one day per day regardless of the amount of wastage that has occurred because the age distribution remains unchanged. A large influx of organic material will decrease the DSA because microorganisms are generated causing the mean of the age distribution to decrease.

Neither the traditionally used SRT nor the newer DSA can completely characterize a reactor. SRT is useful in estimating the accumulated inerts and solid levels in the reactor because it was derived from a mass balance. The DSA better characterizes the age distribution of the bacterial culture because it is a true residence time distribution. Both concepts are helpful in characterizing the operating conditions of the anoxic and aerobic reactors.

Figure 5.14 Dynamic Solids Age (DSA) - 14°C



iii) Biosolids Production

Biosolids production was investigated for carbon removal under anoxic and aerobic conditions using reactors run in parallel. Biosolids production refers to the mass of organic matter produced through either intentional wastage or solids lost in the effluent. To ensure the aerobic and anoxic reactors received the same quality and quantity of feed, the same feed pumps were used for substrate delivery. Target effluent and wastage volumes for the reactors were the same. Effluent and wastage volumes were measured on a daily basis and totaled for the pseudo-steady state periods of 14°C and 20°C. At 14°C and 20°C, the difference between the aerobic and anoxic reactors in output volumes is 0.8% and 2.7%, respectively.

Under essentially identical loading conditions, the aerobic and anoxic reactors did not have equivalent performance as seen in Figure 5.2 or Figure 5.7. As discussed above, the SRTs which include solids lost in the effluent, were not equal at either 20°C or 14°C. For an equitable comparison of biosolids production rates, reactor performance meeting the following criteria were used: pseudo-steady state, and comparable SRTs and DSAs. In the context of this work, pseudo-steady state refers to a period of reactor performance where effluent COD values show no obvious trend, and changes in MLVSS are less than 200 mg VSS/L. The standard deviation of the SRT for the four data sets selected for analysis in order of decreasing value are: 2.5, 1.6, 1.3 and 1.2. On this basis, a difference of less than 1.5 d was the criterion for comparable SRTs or DSAs. Reactor performance meeting these criteria was found between Sept. 21 - Oct. 27 and Apr. 5 - May 9 for the systems at 20°C and 14°C, respectively.

Biosolids production accounted for solid losses through wastage and carry over in the effluent. The accumulated production was calculated using Eq. 5.2. The accumulated production was calculated for each day and is plotted in Figure 5.15 - 5.16.

$$P = \sum_{i=1}^{n} (Q_{e,i} X_{e,i} + Q_{w,i} X_i) \qquad \text{Eq. (5.2)}$$

where P = accumulated production (mg VSS)
$Q_{e,i}$ = effluent discharge rate on the ith day (L/d)
$X_{e,i}$ = effluent solids concentration (mg VSS/L)
$Q_{w,i}$ = wastage discharge rate on the ith day (L/d)
X_i = reactor MLVSS at time of wastage on ith day (mg VSS/L)

Figure 5.15 Accumulated Biosolids Production - 20°C

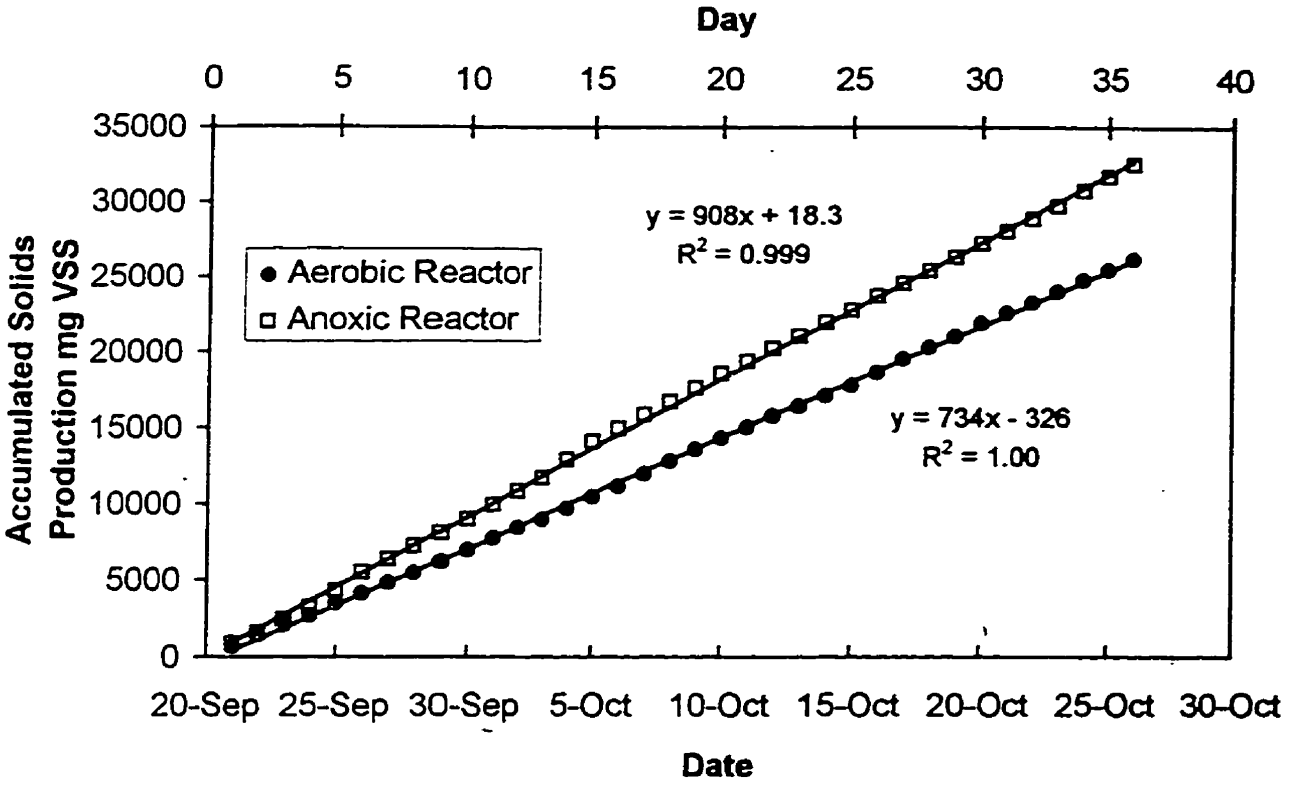
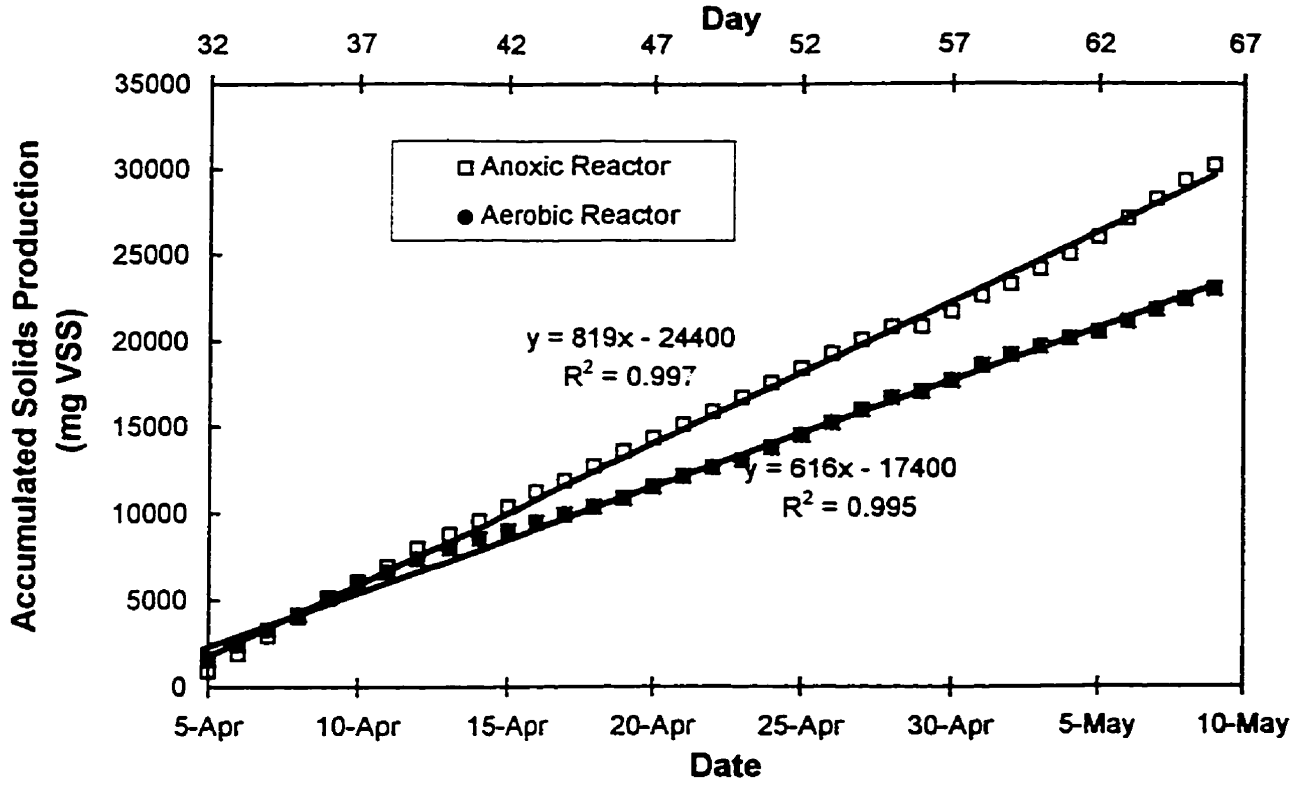


Figure 5.16 Accumulated Biosolids Production - 14°C



Figures 5.15 - 5.16 show that the accumulated solids production was highly linear and increased with time. The slope of these graphs represents the average daily rate of biosolids production. An average rate is preferable to a daily biosolids production rate because changes in the influent strength could have a substantial impact on the daily production rates.

Regression lines were determined for Figures 5.15 and 5.16. Average daily production rates were analyzed to see if there was any significant difference at either 14°C or 20°C. A value of F higher than the critical value indicates that the regression lines are unique at the 95% confidence level and cannot be described by a single regression equation. This analysis showed the anoxic reactor to produce more biosolids than the aerobic reactor at the 95% confidence level at both 14 and 20°C (Table 5.7). Details of the statistical analysis are found in Appendix C.

Table 5.7 Analysis of Average Daily Solids Production Rates as a function of Reactor

Temperature (°C)	Degrees of Freedom	F	$F_{0.95}$
20	(1,68)	1620	3.99
14	(1,66)	527	3.99

Changes in the initial and final reactor MLVSS concentration were not included in the average daily production rates. It was assumed that the overall change would be small in comparison to the total accumulated solids. As shown in Table 5.8, this assumption resulted in an error of <1% to 5%. Typically MLVSS determinations were done in triplicate. The average standard deviation for this measurement for aerobic and anoxic biomass were 25 and 33 mg VSS/L, respectively. Differences in MLVSS concentrations of up to 66 mg/L, approximately 2 standard deviations, may be the result of analytical error rather than actual changes in the reactor MLVSS. For these reasons, the changes in the concentration of reactor solids were considered insignificant in comparison to the biosolids production accumulated over 35 days. If the changes in the solids inventory were included in the calculations, then solids production rates for the aerobic reactor would be lower.

Table 5.8 Changes in Solids Inventory as % of Accumulated Solids

Temperature (°C)	Reactor	Initial MLVSS (mg VSS/L)	Final MLVSS (mg VSS/L)	Change in MLVSS (mg VSS)	% of Accumulated VSS
20	Aerobic	730	620	- 1100	4
20	Anoxic	780	760	- 200	<1
14	Aerobic	580	460	- 1200	5
14	Anoxic	690	730	+ 400	1

iv) Observed Yield

Observed yield is the mass of bacteria formed per mass of COD removed and takes into account maintenance energy requirements. When MLVSS is used as a surrogate for the mass of bacteria, the observed yield becomes the biosolids production divided by the mass of COD consumed.

The observed yields for the current experimental systems were calculated using Equation 5.3 are listed in Table 5.9. Differences in the reactor MLVSS between the first and last day were included in the observed yield calculations.

$$Y_{obs} = \frac{\sum_{i=1}^{n} Q_{e,i} X_{e,i} + Q_{w,i} X_i}{\sum_{i=1}^{n} (Q_{e,i} + Q_{w,i})(S_i - S_{e,i})} + \Delta MLVSS \qquad \text{Eq. (5.3)}$$

where Y_{obs} = observed yield (mg VSS/mg COD)
$Q_{e,i}$ = discharge rate on the ith day (L/d)
$X_{e,i}$ = discharge solids concentration (mg VSS/L)
$Q_{w,i}$ = wastage flow rate on the ith day (L/d)
X_i = reactor solids on ith day (mg VSS/L)
S_i = influent COD concentration on ith day (mg COD/L)
$S_{e,i}$ = discharge filtered COD concentration on ith day (mg COD/L)
ΔMLVSS = change in reactor MLVSS inventory (mg VSS)

Non-biodegradable organics present in the influent can accumulate within the reactor and increase the observed yield. For the current experimental system, an average VSS/COD ratio of 1.52 was calculated for sewage. Using the non-filterable COD of sewage and the previously mentioned ratio, the quantity of influent organics was calculated. The average influent VSS in the sewage was 20 and 43 mg VSS/L for 20 and 14°C, respectively. The concentration of influent VSS in the reactor feed is less because sewage was only one component of the feed.

Comparisons between aerobic and anoxic reactors run at the same temperature lead to the same conclusion as before. Observed yields were higher for the anoxic reactors than they were for the aerobic reactors. The observed yield for the aerobic reactor decrease by 4% with decreasing temperature. In contrast, the anoxic yield increased by 8% with decreasing temperature.

Table 5.9 Observed Yield

Temperature °C	Reactor	Influent VSS	Observed Yield (gΔVSS/gΔCOD)
20	Aerobic	7	0.258
20	Anoxic	7	0.350
14	Aerobic	14	0.248
14	Anoxic	14	0.378

The observed yields of this experimental study are compared to those of Tian *et al.* (1994). The experimental yield of this study was comparable to the value of 0.258 gΔVSS/gΔCOD given by Tian *et al.* (1994) for an influent VSS of 69 mg VSS/L. In contrast to the work of Tian *et al.* (1994), the observed yield for the aerobic reactor of this experimental study does not increase with decreasing temperature. The temperature difference of this study, 6°C, may not be sufficient to see the same trends as seen with a 12°C difference.

v) Bacterial Population and MLVSS Composition

A factorial analysis was completed to examine the significance of the electron acceptor system (EAS), and temperature on the number of bacteria per unit MLVSS. This analysis would provide information on factors influencing the composition of the mixed liquor. The analysis shows that neither temperature nor the EAS or any interaction between the factors had a significant impact on the fraction of MLVSS composed of bacteria (Table 5.10).

A second factorial analysis was done to examine whether or not the same factors influenced the number of bacteria in the reactor. The analysis indicated that temperature and EA were not significant. Further inspection of the complete data set suggested that the bacteria count of Nov. 29 was suspect. It was 300-400% higher than the other two values at this temperature. With these data points excluded, the analysis was repeated and is presented in Table 5.10. The analysis shows, that on average, there were significantly more bacteria in the anoxic reactors than there were in the aerobic reactors.

Table 5.10 Factorial Analysis of MLVSS Composition and Number of Bacteria

	Bacteria per MLVSS x 10^8		Bacteria Counts x 10^{12}	
Effect	Estimated Effect	t value	Estimated Effect	t value
EAS	2.25	0.124	**4.23**	2.37
Temp.	-0.400	-0.022	-0.265	1.10
EAS x Temp.	-2.80	0.154	-1.70	0.955

MLVSS effects - 14 df and $t_{0.95}$ of 2.14.
Bacteria Count effects - 13 df and $t_{0.95}$ of 2.16.
Bolded effects are significant at the 95% CL Data presented in Table 5.1 and 5.4

Given the experimental evidence outlined, the hypothesis that predation is in part responsible for lower net biosolids production in the aerobic reactor is put forth. Microscopic examination of the aerobic mixed liquor found a number of stalked protozoa to be present. The absence of protozoa was noted for the anoxic culture upon microscopic examination.

Henry and Jones (1972) examined the effect of temperature on the bacterial population of a chemostat using a synthetic waste. The number of bacteria present was a maximum at 1°C. The culture exhibited a large increase in the number of bacteria from 1 to 4°C. The number of bacteria present in the reactor decreased rapidly when the reactors were operated at temperatures between 5 and 18°C. The investigators felt that the decline in bacteria at temperatures above 4°C was due partly to endogenous respiration but primarily to the removal of bacteria by the growing protozoan population.

The role of predators has not often been considered in evaluating biomass production under aerobic and anoxic conditions. Symth (1994) found comparable sludge production in aerobic and anoxic systems under steady-state when operating fed-batch reactors using primary effluent. These reactors were not blanketed so it is possible that the DO concentration in the anoxic reactor was sufficient to support predators. The reactors of the current experimental system were covered and had a height to diameter ratio of 3:2. This configuration would minimize oxygen ingress. This in part may explain the differences in the yield of this study and that reported by Symth.

vi) Effluent Quality

An anoxic reactor must have performance comparable to an aerobic reactor in several key areas if denitrification is going to be implemented. Effluent organic strength (i.e. typically specified in terms of BOD_5) and solids requirements are usually specified in a treatment plant's operating permit. During the experimental program, the effluent solids and COD were tracked. These numbers will be higher than the BOD_5 because both biodegradable and non-biodegradable organics are measured. A third parameter, the organic substrate to nitrogen ratio, required for the design of an anoxic system, was calculated. This information is used to calculate the amount of supplementary organic substrate required if post-denitrification was implemented.

The effluent quality of the two reactors can be evaluated in two different ways. Average values indicate typical performance while probability plots are useful in determining the likelihood of exceeding a specified limit. Average values for the effluent quality during the pseudo-steady state periods of Sept. 21 - Oct. 27 and Apr. 5 - May 5 are presented in Table 5.11. A paired t test data found a significant difference between reactors for 20°C filtered effluent COD values at the 95% confidence level. At 14°C there was no significant difference in the effluent CODs of the two reactors. At 20°C the anoxic reactor produced more solids in the effluent than did the aerobic reactor; at 14°C the converse was true. Both temperature and the reactor has an impact on effluent quality.

Table 5.11 Average Effluent COD and TSS Concentrations

Parameter	Temperature (°C)	Aerobic	Anoxic	Degrees of Freedom	t-value	$t_{0.95}$
Filtered COD (mg/L)	20	32.7	39.6	16	-2.52	2.12
Filtered COD (mg/L)	14	48.4	46.9	13	+0.46	2.16
Effluent Solids (mg TSS/L)	20	8	22	30	-3.92	2.04
Effluent Solids (mg TSS/L)	14	37	20	34	+5.12	2.03

The effluent solids for the aerobic reactor increased from 8 to 37 mg TSS/L as the temperature was decreased from 20 to 14°C. Statistically, this is a significant difference as indicated by a t value of 12.1 on 37 df. The critical value, $t_{0.95}$, is 2.02. While this is a large increase in effluent solids, the quality of the effluent is comparable to other experimental systems. Sutton *et al.* (1977) found for a carbon-removal nitrification system with a clarifier that the mean effluent solids concentration was approximately 24 mg TSS/L. The effluent solids was less than 41 mg TSS/L 90% of the time. Hoepker and Schroeder (1979) examined the effect of loading rate on SBRs effluent quality. Effluent solids concentrations of 30 - 54 mg TSS/L were reported for systems

operating with feed strengths of 200 - 1600 mg/L measured as ultimate BOD. The aerobic reactor at 14°C had a loading of 325 - 500 mg COD/L. If it is assumed that all of the influent COD was biodegradable, then the loading of the 14°C reactor is within the range used by Hoepker and Schroeder (1979). The performance of the aerobic reactor at 14°C in terms of effluent solids was not as good as that at 20°C but it is comparable to values derived with other experimental systems.

vii) Consumptive Ratio

The organic substrate to nitrogen ratio required for complete denitrification is referred to as the consumptive ratio. In a post-denitrification system, the consumptive ratio indicates the minimum amount of exogenous carbon that must be supplied for total denitrification. Figure 5.17 shows the consumptive ratios for the SBR at 14 and 20°C as a function of time. The ratios have been calculated using composited influent and effluent samples. Organic consumption was calculated as the total COD of the influent less the filtered effluent COD. The non-filterable COD would be associated with effluent solids and is a reflection of the culture's settling characteristics. Nitrate consumption was calculated as the amount of nitrate added less the mass of nitrate lost in the effluent. Pseudo-steady state was assumed so neither organic nor nitrate consumption was corrected for changes in the reactor concentration.

A mean consumptive ratio and a standard deviation for each temperature during the pseudo-steady state period are listed in Table 5.12. A t-test was performed to determine if there was a significant difference in the consumptive ratios at 14 and 20°C. The test indicates that temperature does not impact this ratio (Table 5.12).

Table 5.12 Consumptive Ratios

Temperature (°C)	Mean (mg COD/mg NO_X-N)	Standard Deviation (mg COD/mg NO_X-N)	t value	$t_{0.95}$
20	5.46	0.38	-1.45	2.10
14	6.08	2.40		

Note: The t-value has 18 df associated with it.

Stensel (1981) as reported by U.S. EPA (1993) developed Equation 5.4 to define the required COD to nitrogen ratio for any organic substrate. Using the observed yield reported in Table 5.9, the consumptive ratios at 20°C and 14°C are 4.74 and 4.86 mg COD/mg NO_X-N, respectively. At 20°C there is a significant

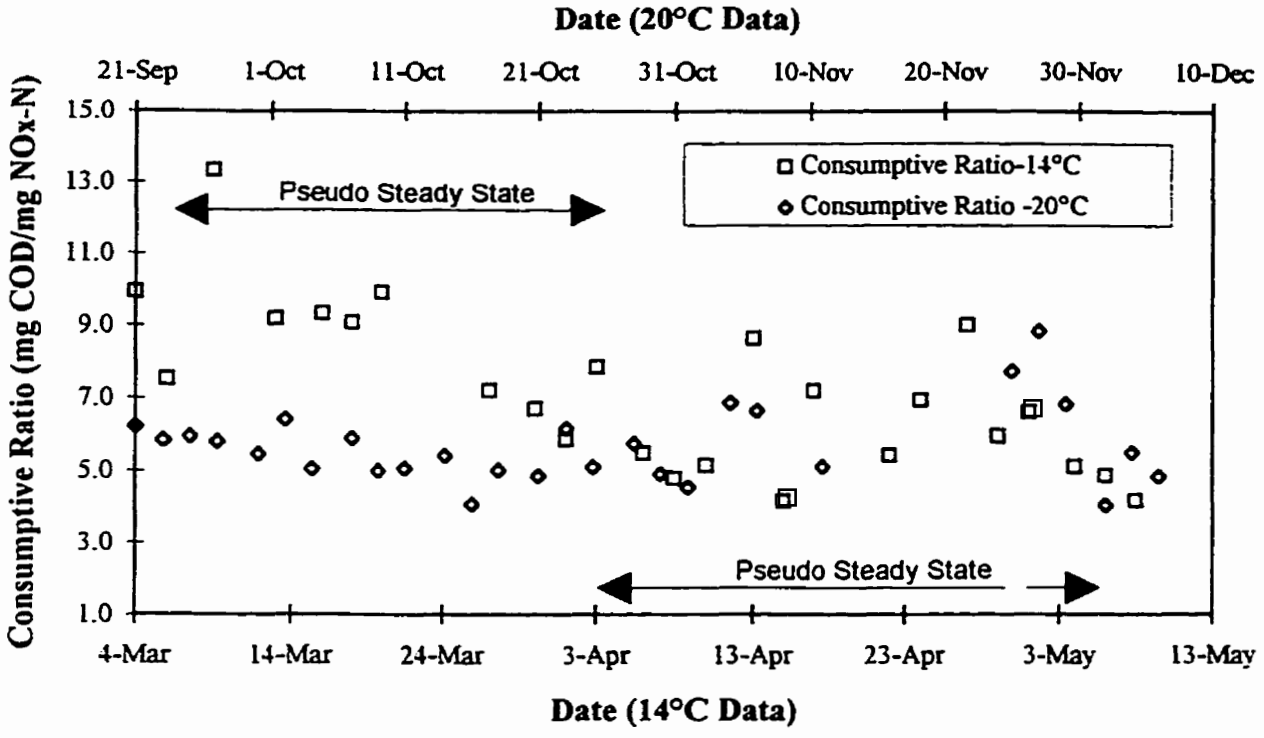
difference between the value calculated from Equation 5.4 and the experimental value. The t-value is 7.57 with 15 df; the critical value at the 95% confidence level is 2.13. At 14 °C, there is no significant difference between the two values as the t-value is 1.90 with 14 df. The critical value is 2.14 at the 95% confidence level.

$$\frac{COD}{N} = \frac{2.86}{1 - 1.134Y} \qquad \text{Eq. (5.4)}$$

where COD/N = required COD to nitrogen ratio

Y = biomass net yield based on COD, g VSS/g COD removed

Figure 5.17 Required Organic Substrate to Nitrogen Ratio



viii) Mass Balances

Barker and Dold (1995) stated that carbon and nitrogen mass balances are a fundamental requirement if conclusions drawn from the analysis of the experimental system are to be defended. With a SBR, COD balances are difficult to obtain because the OUR varies with time. It is easier to obtain mass balances on a stirred tank reactor with solids recycle, since the average OUR is a good approximation of the oxygen consumed. When using a SBR, this is not an option because of the dynamics of the process. The oxygen

consumed can be calculated if the following are known: amount of oxygen supplied, and oxygen concentration in the off-gas and liquid effluent. This was not possible with the current experimental system so COD balances were not performed.

Nitrogen balances were carried out for the aerobic reactors at 20 and 14°C. Total closure on nitrogen for the anoxic reactors requires the offgas to be analyzed for nitrogen. This type of analysis was not possible due to analytical limitations. With the anoxic reactors, a balance was done on Kjeldahl nitrogen. It was assumed that, in the presence of high concentrations of ammonia nitrogen, nitrate would not be used in cell biosynthesis. Under these conditions, it would be used only as a terminal electron acceptor. The balances assumed that the systems were in pseudo-steady state over the short time period considered and did not compensate for any changes in the reactor MLVSS occurring over this period. The procedure used in calculating the balances is outlined in Appendix D.

The balances for the reactors are presented in Tables 5.13 and 5.14. For the current experimental system, the average closure on the aerobic reactor was 99% with individual balances ranging from 86 - 116%. For the corresponding anoxic reactor, the average closure was 94% with individual balances ranging between 87 - 103%. There does not appear to be a trend between date and % closure for either the aerobic or anoxic systems. The closure for the anoxic reactor at 14°C is 95% which is comparable to the balance on this system at 20°C. Closure for balances on both the anoxic and aerobic reactors at both 20 and 14°C appears to be good.

Barker and Dold (1995) using the data of McClintock *et al.* (1988) found there was 96% closure on an aerobic system operating at a 10 d SRT. The anoxic system, operating at a 9.6 d sludge age, had a 95.9% closure. Both of these experiments were carried out at 20°C. These investigators also investigated the temperature dependency of mass balances in aerobic systems using the data of Schroeter *et al.* (1982). At a sludge age of 8 days, the closure on nitrogen was 100.2% and 97.5% for systems run at 14 and 20°C, respectively. With the current experimental system, nitrogen balances at 14°C resulted in average closure of 94% with a standard deviation of 10% whereas the closure at 20°C was 99% with a standard deviation of 10%. The balances do not show any temperature dependency and are comparable to literature values.

Table 5.13 Balances on Total Kjeldahl Nitrogen for Reactors at 20°C

Starting Date	Ending Date	Closure (%) on Aerobic Reactor	Closure (%) on Anoxic Reactor
Oct. 18	Oct. 20	116	93
Oct. 21	Oct. 22	101	95
Oct. 23	Oct. 24	102	99
Oct. 25	Oct. 27	86	88
Oct. 28	Oct. 29	95	99
Oct. 30	Oct. 31	90	101
Nov. 4	Nov. 5	86	90
Nov. 27	Nov. 28	99	87
Nov. 29	Dec. 1	115	88
Dec. 2	Dec. 3	110	103
Dec. 6	Dec. 8	92	93

Table 5.14 Balances on Total Kjeldahl Nitrogen for Reactors at 14°C

Starting Date	Ending Date	Closure (%) on Aerobic Reactor	Closure (%) on Anoxic Reactor
Mar. 30	Mar. 31	96	92
Apr. 1	Apr.2	98	90
Apr. 3	Apr. 5	117	106
Apr. 6	Apr. 7	83	89
Apr. 8	Apr. 9	87	101
Apr. 10	Apr. 12	94	93
Apr. 13	Apr. 14	96	109
Apr. 15	Apr. 16	107	93
Apr. 17	Apr. 19	92	95
Apr. 20	Apr. 21	80	81
Apr. 22	Apr.23	92	98
Apr. 24	Apr. 26	91	92
May 1	May 2	100	91
May 7	May 9		97

5.2.2. Phase 2 - Lactalbumin

a) Introduction

Lactalbumin was used in rate studies examining the effect of temperature, EAS and protein on the rate of hydrolysis. The details of these studies are found in Section 5.3.3.c. After initial rate studies, the need for a culture acclimated to lactalbumin became apparent. At this time, there was some questions regarding the biodegradability of lactalbumin.

The goals of this work were as follows:

a) Determine whether or not a continuous culture can be maintained using lactalbumin as a principal substrate.

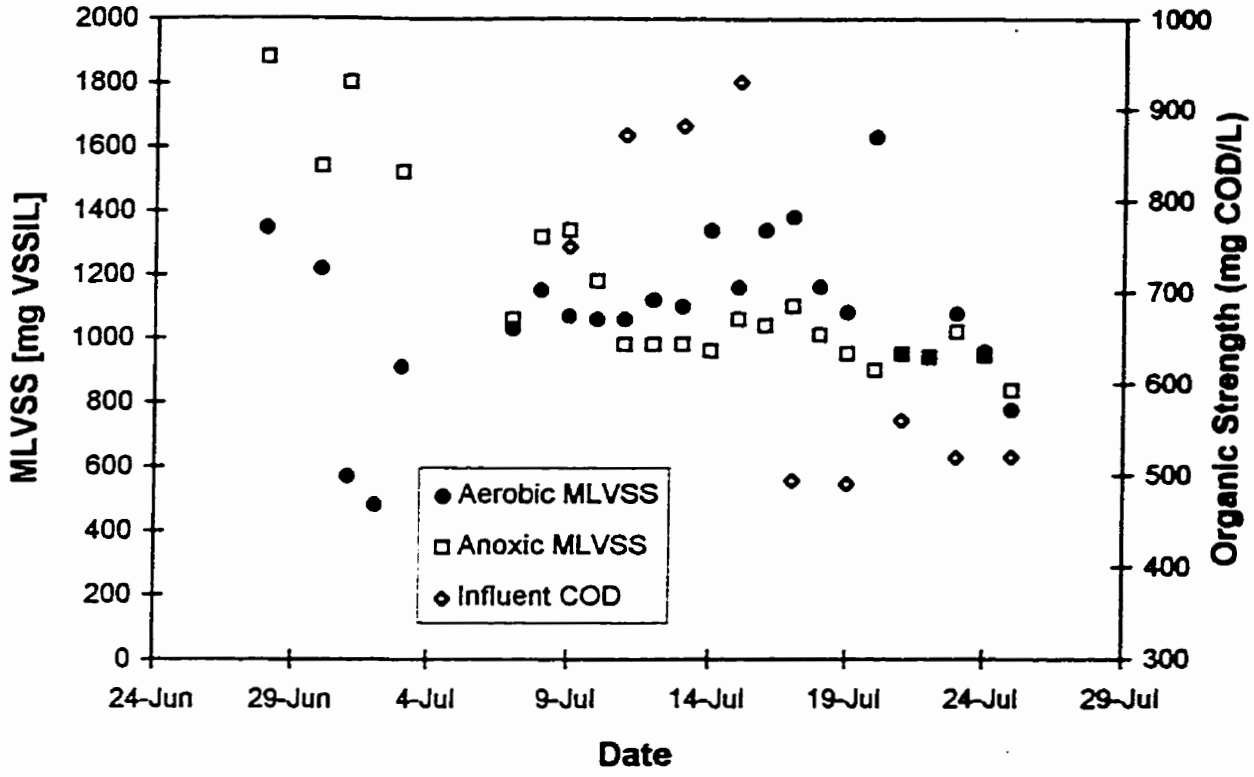
b) Provide biosolids acclimated to lactalbumin for use in batch rate study examining lactalbumin degradation. This culture would also be used as the starting culture for death and decay studies.

b) **Presentation of Experimental Results**

i) Influent Strength and MLVSS Profile

The feed was composed of sewage to lactalbumin in a 1:2 volume ratio. The total feed strength varied between 500 - 950 mg COD/L because different quantities of lactalbumin had been inadvertently added to the feed (Figure 5.18). The sewage strength remained relatively constant at 200 - 250 mg COD/L. The MLVSS profiles of the reactors reflect the variability in the feed strength. The reactors were not at pseudo-steady state because of the variation in reactor MLVSS but it was not required to prove biodegradability or generate the biosolids needed for rate studies.

Figure 5.18 Influent COD and Reactor MLVSS for Cultures using Lactalbumin at 20°C



ii) Ammonia Nitrogen Production

Figure 5.19 shows that the effluent ammonia nitrogen ranged between 20 - 60 mg N/L. In general, the effluent ammonia concentration of the aerobic reactor exceeded that of the anoxic reactor. During the period of July 11 - July 19, effluent ammonia nitrogen values declined for both the aerobic and anoxic reactor. After

July 19, the effluent ammonia nitrogen concentration continued to decline for the aerobic reactor while values for the anoxic reactor remained relatively constant. From July 9 to July 15 the organic strength of the influent is very high (750 - 925 mg COD/L) while from July 17 to July 25 the strength is much lower. Decreases in effluent strength would explain the declining effluent ammonia nitrogen concentrations. It appears the anoxic reactor reached an new equilibrium much faster than the aerobic reactor.

c) Discussion of Experimental Results

i) Biodegradability of Lactalbumin

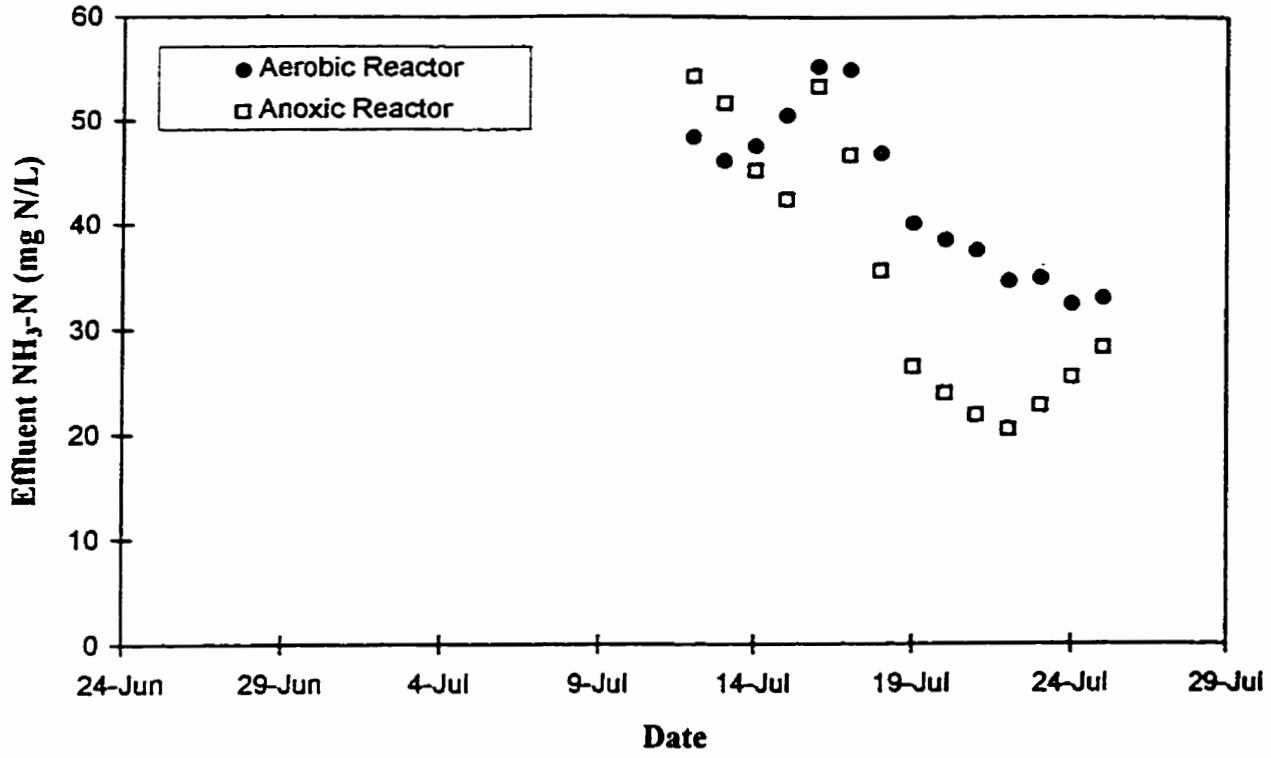
Lactalbumin is the whole protein of whey. The preferred treatment option for whey wastewaters is anaerobic treatment due to the high organic strength of the waste. Porges and Jasewicz (1959) examined the treatment of whey wastewaters by extended aeration at 30°C. A mass balance was done on the system over the 61 days of its operation. On average 77% of the influent whey COD was oxidized. The reactor effluent accounted for a further 19% of the influent COD. Increases in the reactor mixed liquor VSS accounted for the remaining COD. The investigators suggested with proper clarification 97% COD removal is possible. The results of this study suggest lactalbumin is degradable.

Orhon *et al.* (1993) examined the treatability of whey washwater. A batch rate study using an initial soluble COD of 1126 mg/L was used to define the degradation kinetics. Values for μ_m, K_s were 3.1 d^{-1} and 100 mg COD/L, respectively, were determined. After 240 h of degradation, a soluble COD concentration of 38 mg/L was measured. They attribute the residual COD to the formation of soluble residual products. A COD balance is lacking on the system so the fate of lactalbumin in unknown. As a particulate substrate, the lactalbumin component of whey may have accumulated in the MLVSS.

Proteins such as lactalbumin contain nitrogen in excess of growth requirements. Their deamination and utilization results in a net production of ammonia nitrogen. Monitoring the production of ammonia nitrogen was chosen as an easy way of determining the biodegradability of lactalbumin. Ammonia nitrogen production greater than the total Kjeldahl nitrogen content of sewage suggests lactalbumin degradation. Total Kjeldahl nitrogen was not done on the sewage itself but Tables 5.4 and 5.7 suggest a range of influent TKNs of 25.0 - 46.9 mg N/L. In the feed, sewage would contribute between 8.3 - 15.6 mg N/L. The lowest effluent ammonia

nitrogen reported in Figure 5.19 for the aerobic and anoxic reactor is 20.4 and 32.3 mg N/L, respectively. This would suggest that some ammonia nitrogen was originating from lactalbumin degradation.

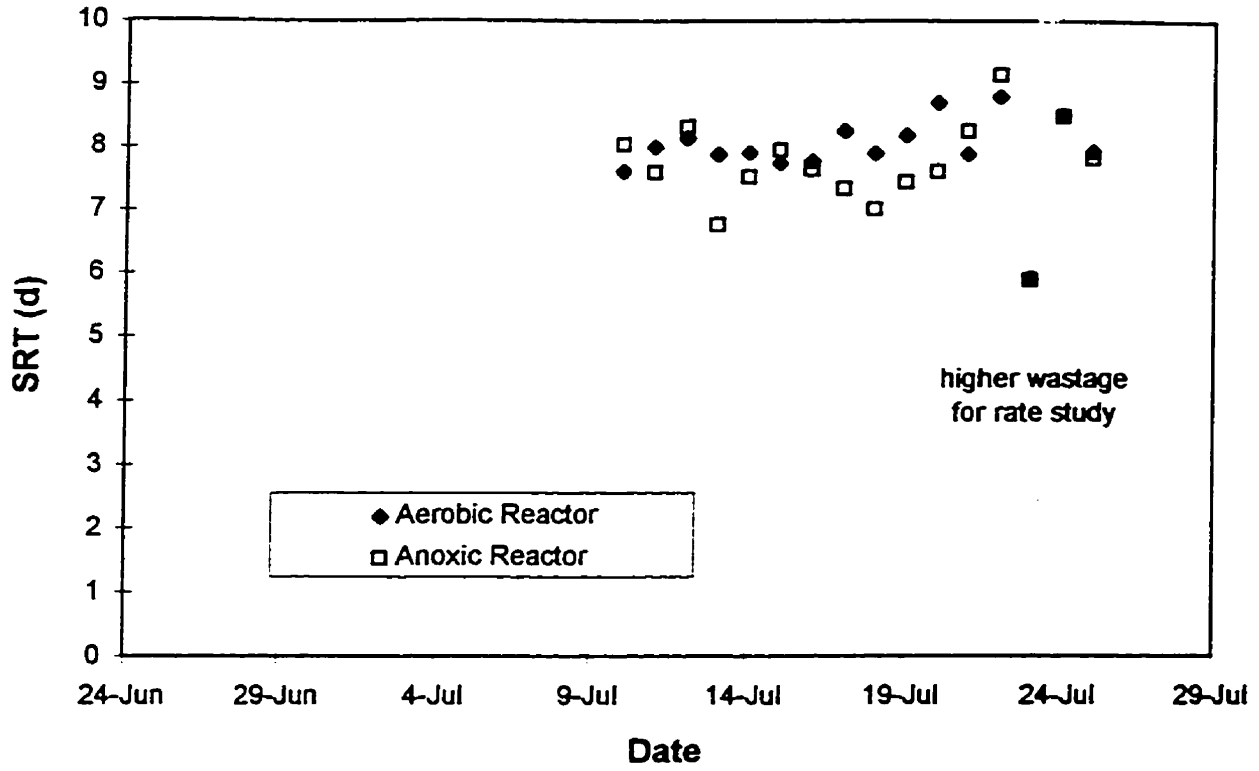
Figure 5.19 Effluent Ammonia Nitrogen for Cultures using Lactalbumin at 20°C



ii) Solids Retention Time

Using Equation 5.1, the solids retention time (SRT) for the reactor was calculated for the aerobic and anoxic reactors. Inspection of Figure 5.20 shows the SRT of the aerobic and anoxic reactors to be comparable. A lower SRT was seen on July 23 when more biosolids than normal were withdrawn from the reactor for a batch rate study. SRTs for the period before July 23 range between 7.5 and 9.5 days. Variation in the SRT was the result of daily differences in the unintentional losses in the effluent.

Figure 5.20 Solids Retention Time for Reactors Using Lactalbumin



d) Summary Statements

i) Phase 1

Biosolids Production

1. Carbon removal under anoxic conditions produced more biosolids that under aerobic conditions. The increase was 24 and 32% for temperatures of 20 and 14°C, respectively.
2. The observed yield follows this same trend with higher yields being associated with the anoxic reactors. The observed yield for the aerobic reactor decreased by 4% with decreasing temperature; whereas, the anoxic yield increased by 8% with a temperature decrease of 6°C.
3. If feed proteins had accumulated in the reactor, this may have been reflected in higher TKN/MLVSS ratios. The TKN/MLVSS ratio was compared for aerobic and anoxic reactors. There was no significant difference between the ratio of the aerobic and anoxic reactors at either temperature.

4. Use of AODC as an enumeration technique should allow an analysis of the bacterial component of the biomass. Analysis of the AODC data indicated that the reactor and temperature did not significantly impact the fraction of the biosolids composed of bacteria.
5. A second factorial analysis determined that on average there were significantly more cells in the anoxic reactor than there were in the aerobic reactor. Temperature was not identified as a significant factor. This may arise due to two factors: (1) predation may have contributed to the decreased number of bacteria in the aerobic reactor, (2) lack of oxygen in the anoxic reactor would have precluded the growth of most non-bacterial organisms.

Acridine Orange Direct Counts

1. AODC produced cell counts with narrow confidence limits which suggested a high level of precision for the procedure.
2. AODC measurements correlate well with MLVSS measurements for the anoxic reactor but do not correlate with MLVSS measurements for the aerobic reactors. The presence of a large non-bacterial component in the population may account for the lack of correlation with the aerobic MLVSS.

Performance of Carbon Removal under Aerobic and Anoxic Conditions

1. At 20°C, the aerobic reactor on average outperformed the anoxic reactor in both terms of effluent COD and effluent suspended solids concentrations.
2. At 14°C, the aerobic reactor had an average effluent COD that was not significantly different from the anoxic reactor. The aerobic reactor on average produced more effluent solids.
3. The aerobic reactor had a significantly higher effluent TKN than the anoxic reactor at both 14 and 20°C. The TKN was principally composed of ammonia nitrogen.

ii) Phase 2

1. Lactalbumin is biodegradable as indicated by the effluent ammonia nitrogen concentration.

5.3. Growth Kinetics and Stoichiometry

5.3.1. Introduction

It is important to understand why there was higher biosolids production in the anoxic SBR reactor. Batch studies were designed to provide information about the biotransformations involved in carbon removal under aerobic and anoxic conditions. Three specific areas were addressed; growth kinetics, stoichiometry, and substrate uptake and hydrolysis. The testing protocol proceeded with: one half of a stock culture being fed a protein hydrolysate (PH) while the remaining portion was fed a whole protein. Proteins are slowly biodegradable organics that require: uptake, storage and enzymatic degradation (i.e. hydrolysis) before being used as a growth substrate (Dold *et al.*, 1980). In contrast, PHs are simple substrates which can be directly used by the microorganism. The growth kinetics and stoichiometry derived using PH cultures are not confounded with the kinetics of hydrolysis. Differences between substrate utilization rates for cultures using proteins and those using PH would determine if hydrolysis was rate limiting.

A number of different proteins were used to determine if the rate hydrolysis is dependent on the molecular weight of the protein or its overall structure. Except for a few rate experiments, the PH remained the same, N-Z Amine A (CAA). The rate experiments using the PH formed a repeated two level factorial experiment with temperature (14 and 20°C) and EAS (aerobic and anoxic) as factors. Experiments done under using the same combination of EAS and temperature are not replicates in the strictest sense. The stock culture originated from the SBR on different days. The rate experiments were treated however as replicates so statistical analysis of the results could be carried out.

This particular section of the thesis examines growth kinetics and stoichiometry using data derived from batch rate studies using PH as substrates. These experiments also allowed acridine orange direct counts (AODC) to be further evaluated as an enumeration technique under conditions of active substrate metabolism. The specific objectives of these experiments are outlined below:

1. Determine if substrate uptake and hydrolysis is a rate limiting step under aerobic and anoxic conditions at temperatures of 14 and 20 °C when proteins are used as a model substrate. Determine if the rate of substrate

uptake and hydrolysis is dependent on the molecular weight of the protein or its overall structure and solubility.

2. Evaluate AODC as a method of enumerating the number of bacteria in a mixed microbial population.
3. Determine if there are any significant differences in the specific utilization rates for aerobic and anoxic cultures at 14 and 20°C based on electron equivalents. The impact of temperature on the utilization rate will be determined.
4. Determine if there are significant differences in the observed yield for aerobic and anoxic cultures under conditions of parallel active substrate removal.

Growth Kinetics and Stoichiometry follows the same general format as **Sequencing Batch Reactor Operation**. Initially, the oxygen utilization rate (OUR), ammonia nitrogen, filtered COD, MLVSS and bacteria counts will be presented for aerobic cultures. Similarly for the anoxic cultures, these data were presented with one exception. Nitrate utilization rates (NUR) were reported instead of the OUR. A discussion comparing the data to literature values follows along with further statistical analysis. To conclude the section, a number of summary statements are made.

5.3.2. Presentation of Experimental Data

a) Aerobic Cultures

i) Increases in Biomass and Bacteria Counts

This experimental program was unique because the bacterial population was enumerated in addition to the traditionally measured MLVSS. MLVSS quantifies the mass of all the organic components found in the activated sludge floc. Traditionally rate data (e.g. OUR, NUR, observed ammonia nitrogen production, soluble COD uptake) has been normalized on MLVSS concentration to facilitate comparisons between similar experiments.

In this experimental program, only the initial MLVSS concentration was considered for two reasons. MLVSS measurements are not very precise as indicated by a standard deviation of 5-50 mg/L for replicate analysis. For most PH cultures, the soluble COD concentration decreased by approximately 300 mg/L. Using a yield of 0.625 mg COD/mg COD derived by Hoover and Porges (1952) for growth on casein, the

maximum increase in bacteria would be 188 mg COD/L. Using the value of 1.42 g COD/g MLVSS suggested by Marais and Ekama (1976), cell growth would increase the MLVSS concentration by 140 mg VSS/L. With the given level of precision for MLVSS determinations, increases in MLVSS concentration may not have been accurately detected.

MLVSS measurements on the final culture would take into account increases in bacterial mass, the mass of substrate incorporated in floc as well as previously measured, partially degraded organics and non-biodegradable organics. PH addition to the stock culture resulted in an immediate uptake of substrate. As will be shown in Table 5.23, uptake varied from 16.3-73.3 mg COD/L. Differences in the MLVSS of the initial and final culture would overestimate the yield of cells by 12-51 mg VSS/L. Thus only initial MLVSS data were used and these are reported in Table 5.15.

Initial bacteria counts were adjusted counts of the stock culture. Final counts were done on the culture remaining at the end of the rate study. Bacteria counts with their 95% confidence intervals are presented in Table 5.15. Mean values and 95% confidence limits were presented in preference to a mean and standard deviation. Bacteria counts were treated as a log normal distribution. The standard deviation of a log normal distribution cannot be log transformed back to a normal distribution. The average of the initial and final bacteria count data that was used to normalize rate data unless otherwise noted. For 20°C cultures, three samples were taken independently through the dilution, staining and counting procedures; at 14°C two samples were taken through the process.

There were seven rate studies where data were available for both initial and final cultures. For five of those rate studies, there was a significant difference between the initial and final cultures. In four cultures, the final culture had a greater number of cells. For the remaining culture, the converse was true.

Increases in AODC reflects increases in the number of bacteria. Bacterial growth may be through multiplication or through increases in cell size. Cells smaller than 0.2 μm are not enumerated by AODC because it uses a polycarbonate membrane with 0.2 μm diameter pores. A small fraction of the bacterial population of activated sludge present in the initial culture may be smaller than 0.2 μm in the initial culture. They may become detectable by AODC in the final culture because of increases in size.

Whether bacteria increase in size or replicate will depend on the initial culture conditions. One of the factors to be considered is the initial substrate to microorganism ratios (S_0/X_0). For aerobic batch studies, this ratio was 1 or less (see Table 5.24). Chudoba (1992) states when S_0/X_0 is less than 2-4, depending on culture history, no cell multiplication takes place during exogenous substrate removal. Under these conditions, a biomass increase is mostly due to the synthesis of storage polymers.

The bacteria of the stock culture generated by the SBR were either chronically starved or starved. After the addition of PH or protein to the SBR stock culture, increases in cell size may have to occur before replication is initiated. For a starved *Vibrio* S14 it was found that DNA replication was initiated at a cell volume corresponding to approximately two times the theoretical minimal cell volume at all starvation times studied (Nystrom, 1990).

There were several sources of error present in AODC measurements. The samples were homogenized to breakdown the floc into its components. While this was achieved in principle, there always remained some very small flocs. Generally, these were only seen at the highest magnification 1250; the magnification at which the counting was done. If the field selected for counting contained a floc, then the number of bacteria would be too numerous to count. The number of bacteria present would be estimated. Estimates that were too high or too low would bias the value for the filter. A statistical comparison of this filter with repeated samples would then show a significant difference between filters. If this was the case, additional filters were done after further homogenization. Other errors may have been caused during serial dilution or counting. Acridine orange has a tendency to photo bleach making it difficult to count some samples.

ii) Oxygen Utilization Rate

The aerobic culture was not composed exclusively of bacteria as higher organisms would also be present. The presence of protozoa was confirmed by microscopic examination of the culture. The measured OUR would therefore include the oxygen demands of the higher organisms as well as those of the bacterial population. Prior to adding substrate, an OUR test was done on each half of the stock culture and averaged. This was defined as the basal OUR (OUR_B). It was the sum of the oxygen demands of the non-bacterial population and the endogenous respiration requirements of the bacteria.

Table 5.15 MLVSS and Bacteria Counts for Aerobic Cultures using Protein Hydrolysates

Temp (°C)	Protein	Date	Initial MLVSS (mg VSS/L)	Std. Dev. MLVSS (mg VSS/L)	Bacteria Counts for Initial Culture (bact/L) x10^{11} Mean	Lower 95% CI	Upper 95% CI	Bacteria Counts for Final Culture (bact/L) x10^{11} Mean	Lower 95% CI	Upper 95% CI	Increase in Mean
20	CAA	Nov. 27	455	11	5.63	4.62	6.86	7.04	5.78	8.58	25%
20	CAA	Nov. 29	490	18	18.6	17.2	20.1	10.9	12.2	9.68	-41%
20	CAA	Dec. 6	465	12	3.85	3.20	4.65	3.84	3.28	4.48	<1%
14	CAA	Mar. 27	500	38				10.6	9.2	12.3	
14	CAA	Apr. 4	450	5				9.6	8.6	10.8	
14	CAA	May 5	250	28	3.27	2.95	3.63	5.31	4.79	5.88	62%
14	CAA	May 7	420	35	4.06	3.47	4.75	7.66	6.71	8.74	89%
14	CAA	May 9	370	26	4.72	5.94	3.75	6.09	5.19	7.15	29%
14	LEH	Apr. 9	460	47	3.66	3.30	4.06				
14	LEH	Apr. 13	590	44	5.46	4.85	6.14	7.58	6.54	8.78	39%
14	LEH	Apr. 29	370	20				7.68	6.48	9.10	

The basal rate was measured at the end of a SBR cycle. The end of the cycle occurred eleven hours after feeding was complete. At this time, it is argued that the bacteria were respiring at an endogenous level with very little respiration due to substrate removal. In contrast, the respiration associated with higher life forms was assumed to remain relatively constant over the 4-6 h of the rate experiment. The OUR_B was subtracted from the OUR measured during active substrate removal (OUR_{sf}) resulting in a corrected OUR (OUR_C). The oxygen requirements of the bacteria during active substrate removal were better estimated using OUR_C.

In Tables 5.16-5.17, average basal respiration rates for the stock culture normalized on MLVSS or bacteria counts are presented. The test criterion for single outliers (Eq. 5.5) as given by (NUS Corporation, 1987) was used within data sets. In Table 5.17, the basal respiration rate for May 5 was identified as an outlier. With this value in the data set, the mean and relative standard deviation (RSD) were 8.2 mg O_2/(g VSS·h) and 47%, respectively. With the May 5 value excluded from the data set, the mean and RSD were 6.97 mg O_2/(g VSS·h) and 25%, respectively.

$$T_n = \frac{(X_n - \overline{X})}{S_x} \qquad \text{Eq. (5.5)}$$

where T_n = test criterion for single outliers
$\overline{X}$ = mean of all "n" values
S_X = standard deviation of all values in the sample population
X_n = the largest value in the sample population arranged in $X_1 \leq X_2 \leq X_3 \leq ... \leq X_n$

The numbers are highly variable regardless of the normalizing factor used as shown by large RSD. This is expected to be due in part to changing conditions within the stock culture as discussed previously in Section 5.2.1.b. The OUR of the stock culture would be 20% higher than the values listed in Table 5.16-5.17 because the stock culture was diluted by 20% by the addition of substrate. The upper and lower confidence limits of the basal respiration at 20°C and 14°C overlap suggesting that there was no significant difference between the rates.

Table 5.16 Basal Respiration Rates for 20°C Aerobic Culture

Date	MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	OUR_B (mg O_2/L h)	Normalized OUR_B (mg O_2/(g VSS·h))	Normalized OUR_B (mg O_2/bact h) x 10^{-12}
25-Oct	585		7.0	12.0	
2-Nov	330		3.7	11.2	
7-Nov	460		2.8	6.08	
27-Nov	455	5.63	3.5	7.69	6.2
29-Nov	490	18.6	3.54	7.22	1.90
6-Dec	465	3.85	4.3	9.25	12.6
23-Jul	860		11.3	13.1	
			Mean	9.51	6.90
			RSD	27.9%	78.0%
		Upper	95% CL	12.0	
		Lower	95% CL	7.06	

Table 5.17 Basal Respiration Rates for 14°C Aerobic Cultures

Date	MLVSS (mg VSS/L)	Cell Counts (bact/L) x10^{11}	OUR_B (mg O_2/L h)	Normalized OUR_B (mg O_2/(g VSS h))	Normalized OUR_B (mg O_2/bact h) x 10^{-12}
27-Mar	500		3.06	6.11	
4-Apr	450		2.68	5.96	
13-Apr	590	5.46	3.75	6.36	6.87
29-Apr	370		1.87	5.06	
5-May	250	3.27	4.22	**16.9**	12.9
7-May	420	4.06	4.31	10.3	10.6
9-May	370	4.72	2.56	6.91	5.42
9-Apr	460	3.66	3.75	8.15	10.2
			Mean	6.97	9.21
			RSD	25%	33%
		Upper	95% CL	8.58	
		Lower	95% CL	5.37	

Bolded values have been identified as outliers using the test criterion for single outliers (Eq. 5.5)

The OUR_C of PH cultures exhibited several different temporal patterns (Figures 5.21 - 5.24). A linear regression was fitted to all data sets but was found to be inappropriate for: Nov. 29 (CAA), Mar 27 (LEH), Apr. 4 (CAA) and May 9 (CAA). This is indicated by a low correlation coefficient. Since a linear regression was not applicable to all the data sets, a mean OUR_C was used. Listed in Tables 5.18 and 5.19 are the mean OUR_C values.

1. Within minutes of either CAA or LEH addition, the culture attained a new OUR_M. The OUR_M exhibited by the cultures was up to six times higher than OUR_B so it is concluded that CAA and LEH could be metabolized.

2. At 20 °C, the OURs were either relatively constant or increased slowly with time. In four of the five experiments, there was very little variability within a single rate experiment.

3. At 14°C, the data for some rate experiments showed a great deal of variation within the data set.

4. Cultures fed LEH at 14°C showed different responses depending on the culture. Some of the responses were seen previously with the CAA cultures. The variability in responses was due to the extent of acclimation. The cultures of Mar. 27 and Apr. 9 used the stock culture which was fed sewage, casein and albumin. Lactalbumin, the polymer analogue of LEH, was not part of the daily feed. The Apr. 13 and Apr. 29 cultures were allowed to acclimate to lactalbumin for a number of days prior to the rate study and as a result, the OURs were higher.

5. The OURs ranged from approximately 20 - 60 mg O_2/(g VSS·h) for both 14 and 20°C cultures.

Figure 5.21 Oxygen Utilization for Cultures using CAA at 20°C - Oct. 25 - Nov.7

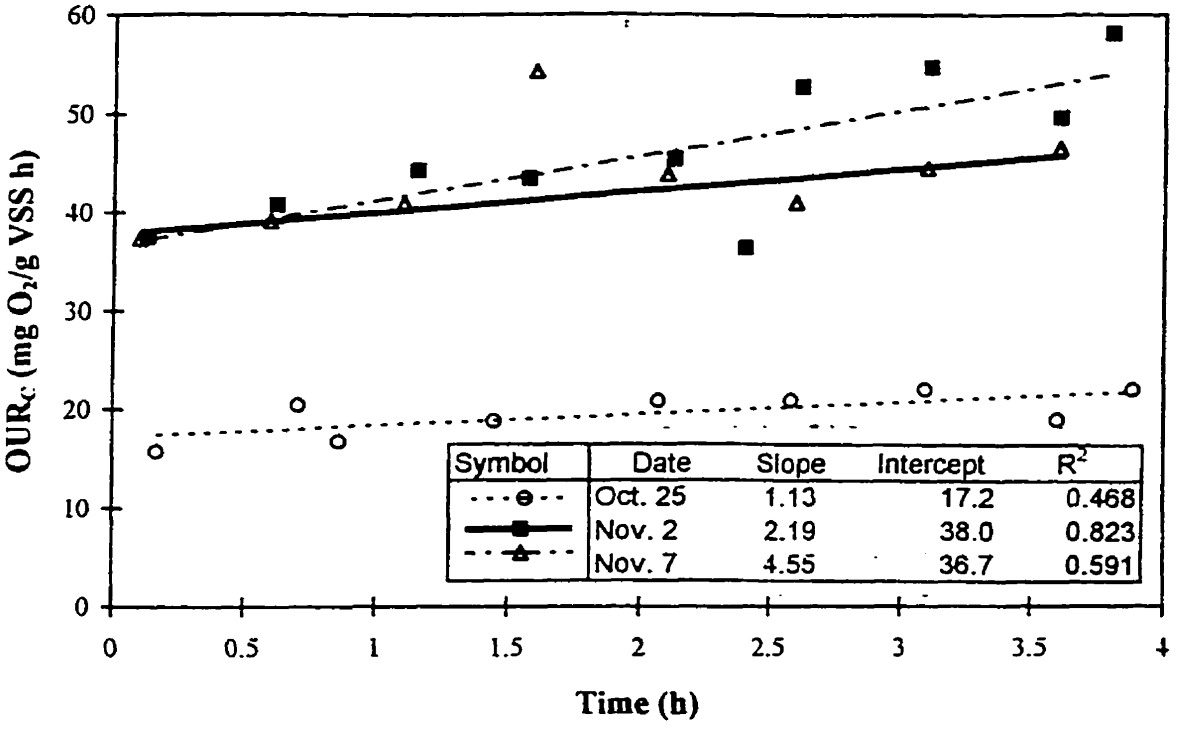
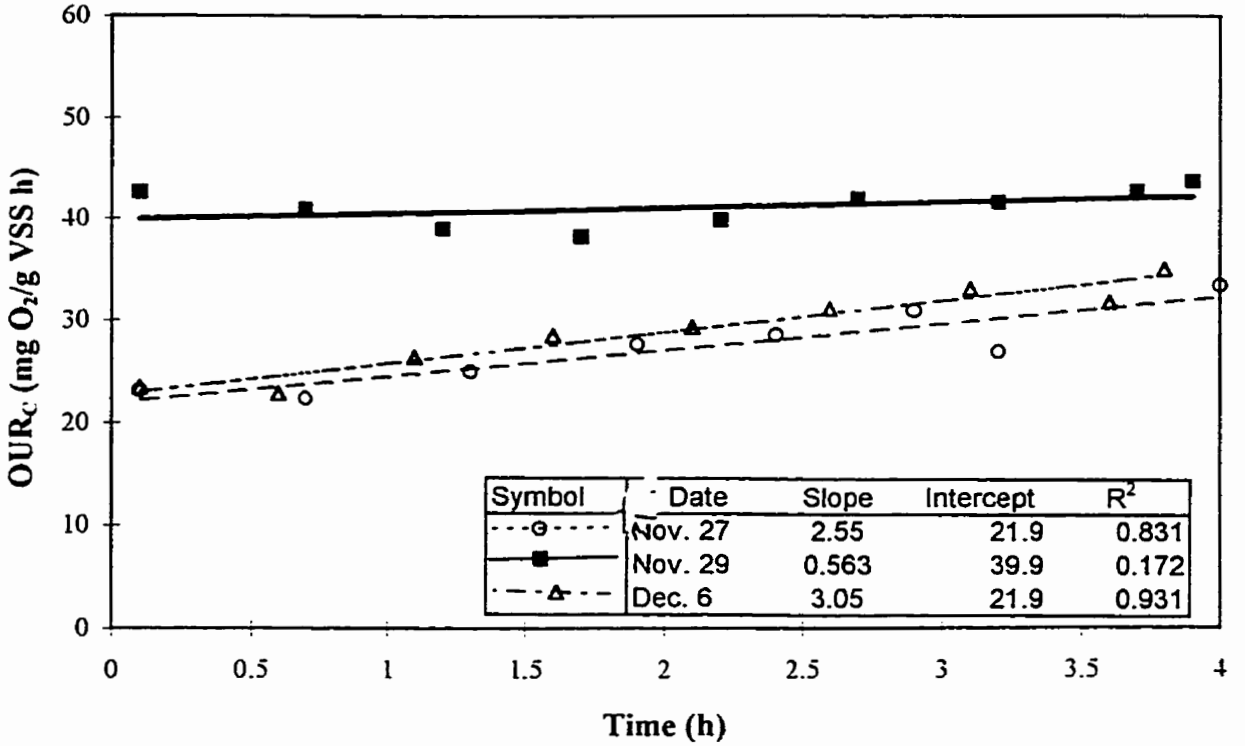
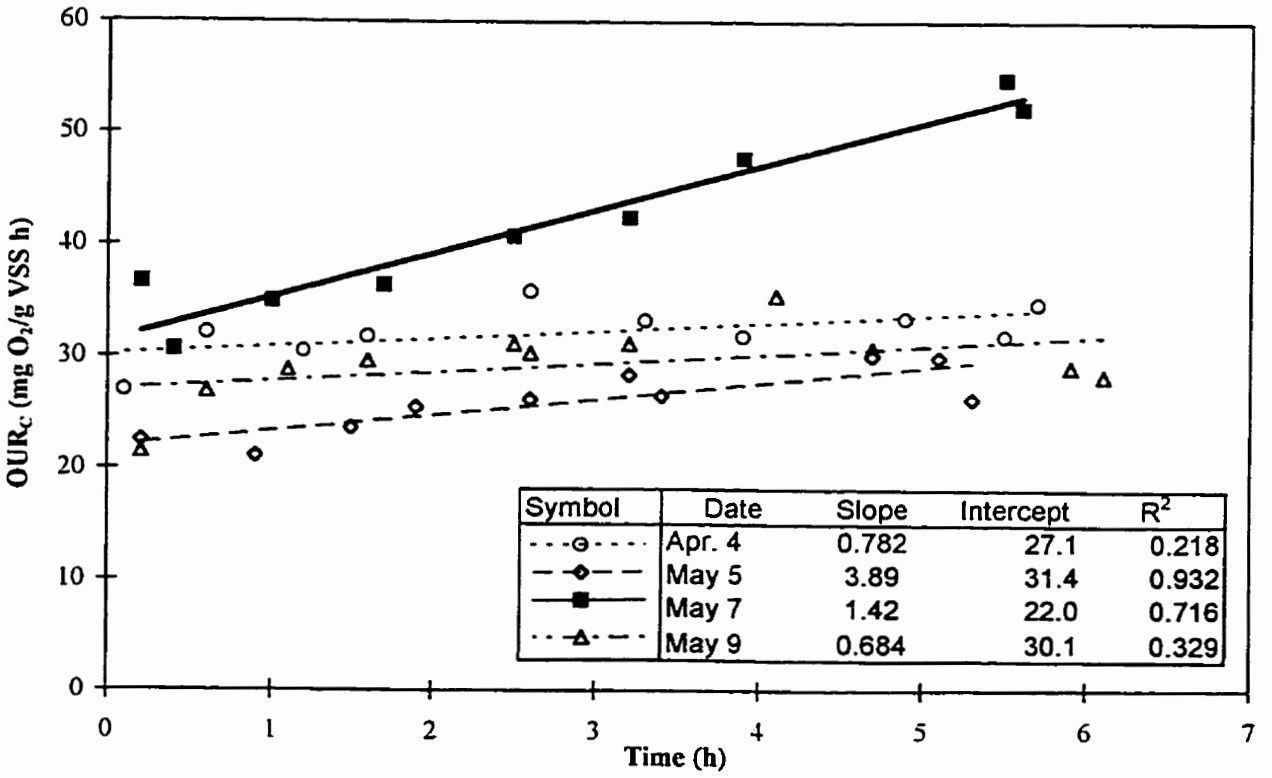


Figure 5.22 Oxygen Utilization for Cultures using CAA at 20°C - Nov. 27 - Dec. 6



Symbol	Date	Slope	Intercept	R^2
---o---	Nov. 27	2.55	21.9	0.831
——■——	Nov. 29	0.563	39.9	0.172
- - -△- - -	Dec. 6	3.05	21.9	0.931

Figure 5.23 Oxygen Utilization for Cultures using CAA at 14°C - Apr. 4 - May 9



Symbol	Date	Slope	Intercept	R^2
---o---	Apr. 4	0.782	27.1	0.218
- -◇- -	May 5	3.89	31.4	0.932
——■——	May 7	1.42	22.0	0.716
---△---	May 9	0.684	30.1	0.329

Table 5.18 Average OUR for CAA Cultures at 20°C - Normalized on MLVSS and Bacteria Counts

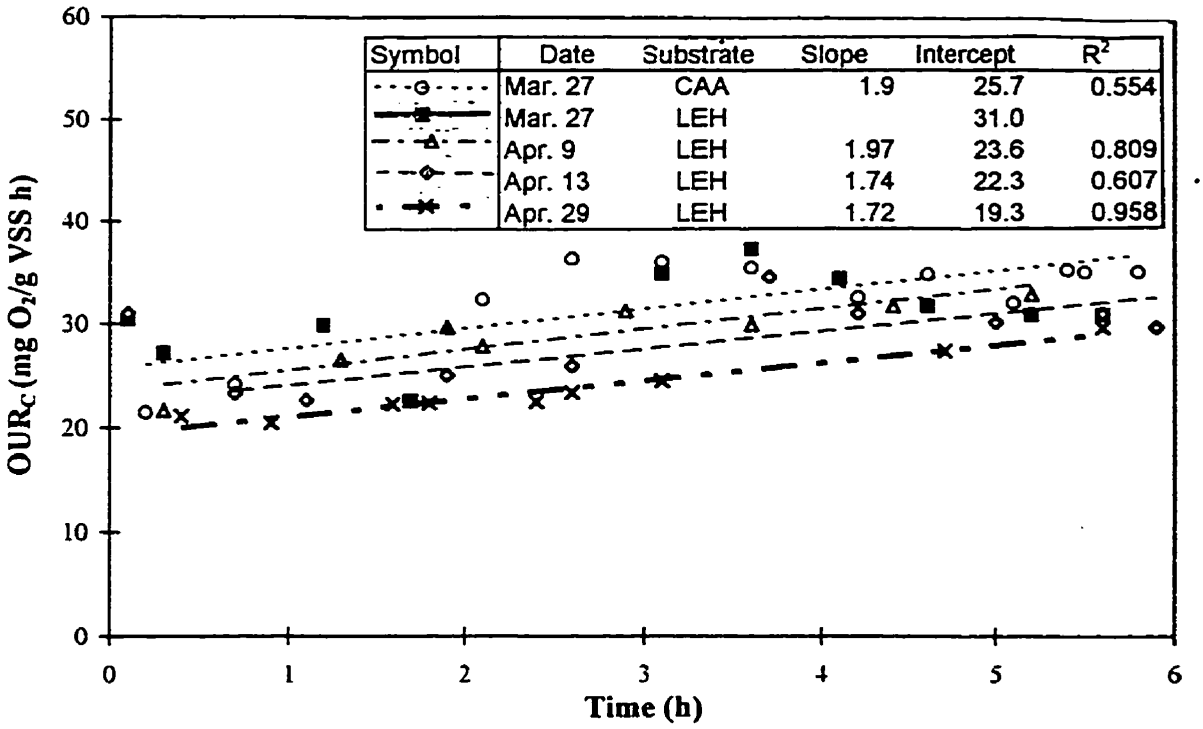
Date	MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	OUR_C (mg O_2/L h)	OUR_C (mg O_2/g VSS h)	OUR_C (mg O_2/bact h) x 10^{-12}
25-Oct	585		11.4	19.5	
2-Nov	330		14.2	43.0	
7-Nov	460		21.3	46.3	
27-Nov	455	6.34	12.4	27.3	19.6
29-Nov	490	14.8	20.2	41.4	13.6
6-Dec	465	3.85	13.5	29.0	35.2
			Mean	34.4	22.8
			RSD	31%	49%

Table 5.19 Average OUR for PH Cultures at 14°C - Normalized on MLVSS and Bacteria Counts

Substrate	Date	MLVSS (mg VSS/L)	Bacteria Counts (bact/L) x10^{11}	OUR_C (mg O_2/(L h))	OUR_C (mg O_2/(g VSS h))	OUR_C (mg O_2/bact h) x 10^{-12}
CAA	27-Mar	500	10.6	17.2	34.5	16.3
CAA	4-Apr	450	9.60	13.2	29.4	13.8
CAA	5-May	250	4.29	10.5	42.0	24.5
CAA	7-May	420	5.86	11.0	26.1	18.7
CAA	9-May	370	5.41	11.9	32.3	22.1
LEH	27-Mar	500		15.5	31.0	
LEH	9-Apr	460	*3.66*	13.3	28.9	36.3
LEH	13-Apr	590	6.52	13.1	22.2	20.1
LEH	29-Apr	370	7.68	9.52	25.7	12.4
			CAA	Mean	32.9	19.1
				RSD	18%	23%
			LEH	Mean	27.0	22.9
				RSD	14%	53%

Note: Bolded bacteria counts are based on final culture while those in italics are based on the initial culture.

Figure 5.24 Oxygen Utilization for Cultures using CAA and LEH at 14°C



iii) Enzyme Assays

The results of enzyme assays done on intact mixed liquor 75 minutes after the initiation of a rate study are listed in Table 5.20. Enzyme production was not chemically supressed so the enzyme levels in the table reflect the dynamic nature of the process. Enzymes consistently present in concentrations greater than 30 nanomoles in at least two of the three cultures were: phosphatase alkaline, esterase (C4), esterase (C8), phosphatase acids and phosphoamidase.

Phosphatase alkaline and phosphatase acids belong to a family of esterases that catalyze the hydrolysis of mono-phosphatase esters. ADP, ATP and AMP, examples of mono-phosphatase esters, are involved in energy transfer. The enzymes are classified on the basis of their optimum pH as either acid or alkaline phosphatase. Esterases are a large group of hydrolases acting on ester bonds. The acid portion of the ester may be a carboxylic, phosphoric or sulfuric acid.

In general, those enzymes present in high concentrations in the intact mixed liquor are involved in ester hydrolysis. The low levels of trypsin and chymotrypsin are surprising because both of these enzymes

hydrolyze protein and peptide bonds. Trypsin preferentially cleaves Arg and Lys bonds while chymotrypsin preferentially cleaves residues containing Tyr, Trp, Phe and Leu.

Table 5.20 Enzyme Assays for Aerobic Cultures at 20°C

Enzyme	Oct. 25	Nov. 2	Nov. 7	Enzyme	Oct. 25	Nov. 2	Nov. 7
Phosphatase alkaline	5[1]	5	5	Phosphatase acids	5	3	5
Esterase (C4)	4	1	4	Phosphoamidase	5	1	5
Esterase (C8)	4	1	4	α-galactasidase	0	0	1
Lipase (C14)	0	0	1	β-galactasidase	1	1	1
Leucine arlymidase	1	2	2	β-glucuronidase	0	0	0
Valine arlymidase	3	1	2	α- glucosidase	1	1	1
Cystine arlymidase	1	1	1	β-glucosidase	1	1	1
Trypsin	2	1	2	N-acetyl-beta-glucosaminidase	3	1	3
Chymotrypsin	3	0	2	α-mannoisdase	0	0	1
				α-fucosidase	0	1	4

[1] Moles of test-strip substrate hydrolysed
1 - 5 nanomoles, 2 - 10 nanomoles, 3 - 20 nanomoles, 4 - 30 nanomoles, 5 - ≥40 nanomoles

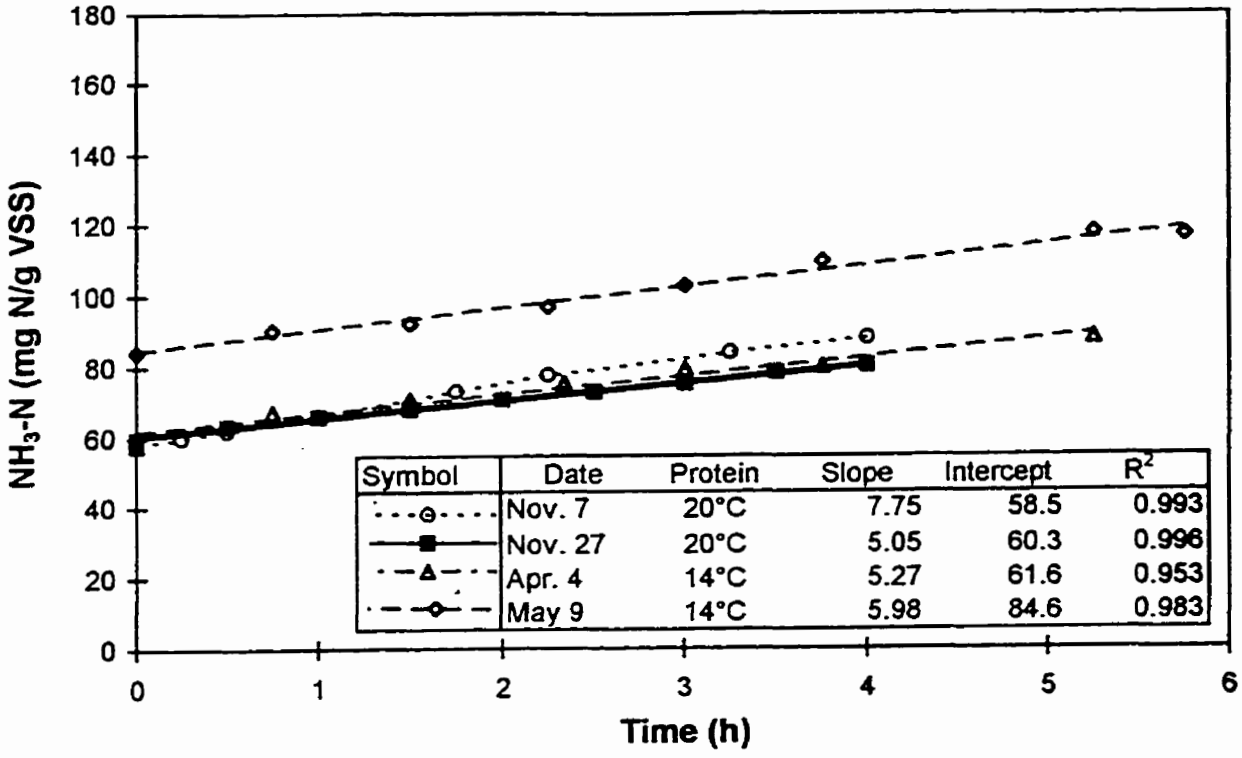
iv) Observed Ammonia Nitrogen Production

Typically, ammonia nitrogen is generated in wastewater by the degradation of urea, through the decay and lysis of cells and as a byproduct of protein degradation. Urea is commonly found in wastewater because it is the end-product of human nitrogen metabolism. This amide is broken down to carbon dioxide and ammonia by the enzyme urease (Payne, 1980). Lysis of intact cells will increase the concentration of ammonia nitrogen in the media because these bacteria are able to retain ammonia intracellularly at concentrations higher than in the culture media (Brown, 1980).

In the current experiments, nitrification was suppressed so it was possible to monitor the net ammonia nitrogen production. Low concentrations of suppressor could be used because *Nitrosomonas sp.* is very sensitive to chemical inhibition. These concentration levels were not expected to have any effect on other metabolic processes. The observed ammonia production may be an alternative way of comparing the hydrolysis and utilization of a protein to the rate of PH utilization. Figure 5.25 shows the observed ammonia nitrogen production for four different experiments with CAA. As the graph shows, the ammonia nitrogen concentration increases at a constant rate. The observed rate of production is the change in ammonia nitrogen concentration over time. This same pattern was seen for all of the aerobic cultures. Ammonia nitrogen data for the remaining cultures is found in Appendix B. The observed ammonia production data is summarized in Tables 5.21 and

5.22. The averages for ammonia production at 14 and 20°C are within one standard deviation of each other suggesting production is invariant with temperature. This conclusion arises from data normalized on MLVSS or bacteria counts.

Figure 5.25 Ammonia Nitrogen Production for Aerobic Cultures using CAA



v) Soluble COD Uptake

Figure 5.26 illustrates soluble COD uptake profiles for four experiments using CAA at 20 and 14°C. Shortly after the PH was added to the solution, some of it was taken up into the floc. The higher of the initial values shown in Figure 5.26 was based on a COD balance. The mass of PH added was known as well as its equivalent COD value. The filtered COD of the stock culture was the filtered effluent COD of the SBR composite adjusted for dilution. The lower of the initial points was the COD concentration measured after the stock culture was mixed with the PH. After the initial COD uptake occurred, COD disappearance was linear with respect to time for the remainder of the rate study. Table 5.23 lists the initial COD concentration derived from a mass balance and the initial COD calculated from a linear regression of COD concentration over time. The initial uptake of COD has been evaluated from this data.

Table 5.21 Ammonia Nitrogen Production for an Aerobic Culture at 20°C using CAA

Date	Initial MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	NH_3-N Production (mg NH_3-N/L h)	NH_3-N Production (mg NH_3-N/(g VSS h))	NH_3-N Production (mg NH_3-N/bact h) x 10^{-12}
Oct. 25	585		2.57	4.40	
Nov. 2	330		2.47	7.49	
Nov. 7	460		3.56	7.75	
Nov. 27	455	6.34	2.30	5.05	3.62
Nov. 29	490	14.8	2.89	5.89	1.95
Dec. 6	465	3.85	2.20	4.72	5.70
		Mean	2.67	5.88	3.76
		RSD	19%	24%	50%

Table 5.22 Ammonia Nitrogen Production for an Aerobic Culture at 14°C using CAA

Date	Initial MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	NH_3-N Production (mg NH_3-N/(L h))	NH_3-N Production (mg NH_3-N/(g VSS h))	NH_3-N Production (mg NH_3-N/bact h) x 10^{-12}
Mar. 27	500	**10.60**	2.02	4.05	1.91
Apr. 4	450	**9.60**	2.37	5.27	2.47
May 5	250	4.29	2.36	9.42	5.49
May 7	420	5.86	3.11	7.40	5.30
May 9	370	5.41	2.21	5.98	4.09
			Mean	6.42	3.85
			RSD	32%	42%

Note: Bolded bacteria counts are based solely on the final culture.

Uptake values in Table 5.23 range from -42 mg COD/L to 73 mg COD/L. In most cases, the immediate uptake of soluble substrate was indicated. This is consistent with the immediate increase in OUR noted previously in this section.

The calculated values of Oct. 25 and Nov. 7 were higher than the initial COD. The analytical protocol for determining COD has a standard deviation of 17 mg/L (Standard Methods, 1995). Differences of up to 34 mg COD/L would not be significant as they would fall within two standard deviations. This is the case for both Oct. 25 and Nov. 7. Negative values could occur if the value of the filtered COD of the stock solution was higher than the value indicated by the composited effluent of the SBR. The average uptake normalized on MLVSS was 0.052 and 0.138 mg COD/mg VSS for 20 and 14°C, respectively.

Figure 5.26 Soluble COD Uptake for Aerobic Cultures using CAA

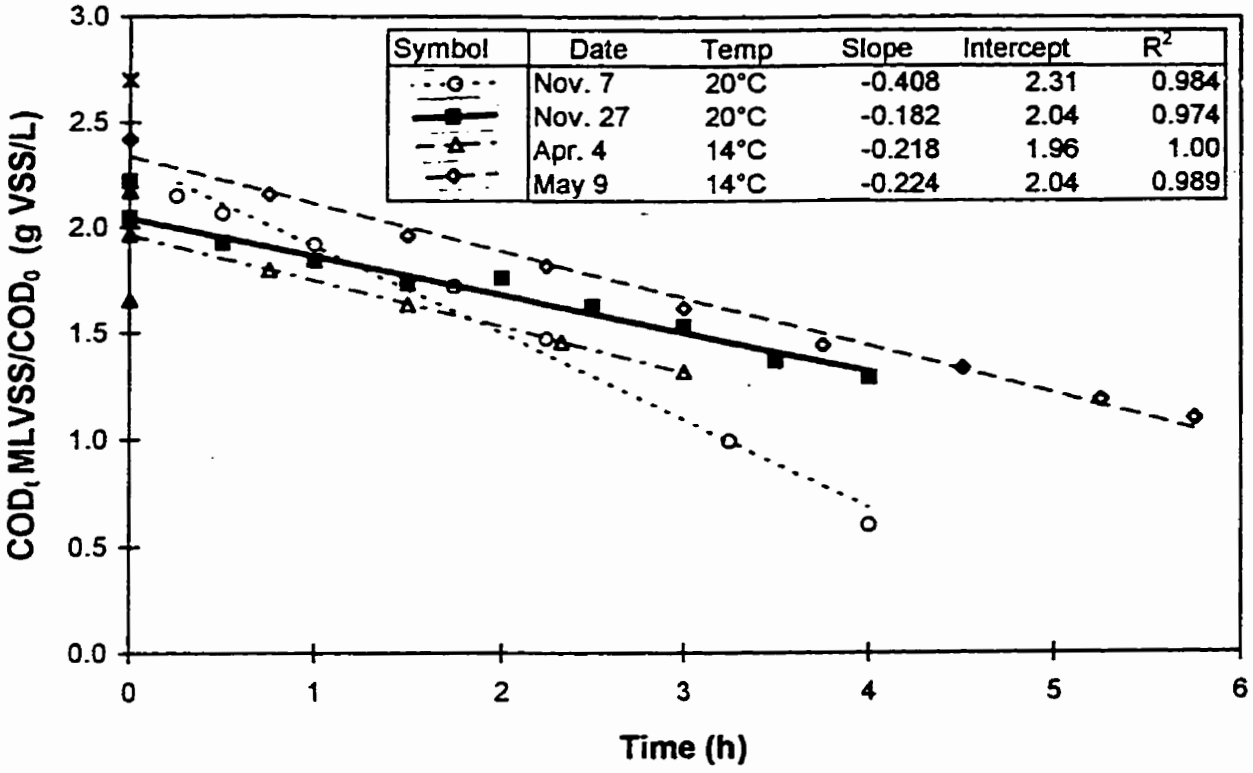


Table 5.23 COD Uptake and Storage for Aerobic Cultures using CAA

Temp. (°C)	Date	Initial COD (mg/L)	Calculated COD (mg/L)	Uptake in COD (mg COD/L)	Initial MLVSS (mg VSS/L)	COD Uptake (mg COD/mg VSS)
20	Oct. 25	458.1	500.1	-42	585	
20	Nov. 2	463.9	451.7	12.2	330	0.037
20	Nov. 7	479.8	511.7	-31.9	460	
20	Nov. 27	526.4	493.1	33.9	455	0.074
20	Nov. 29	538.9	522.9	16.0	490	0.033
20	Dec. 6	487.8	457.4	30.1	465	0.065
14	Mar. 27	502.0	432.3	69.7	500	0.139
14	Apr. 4	526.5	455.9	70.6	450	0.156
14	May 5	528.3[1]	512	16.3	250	0.065
14	May 7	544.0	489.5	54.5	420	0.130
14	May 9	531.0	457.7	73.3	370	0.198

[1] Effluent COD of stock culture has been estimated.

vi) Initial Substrate to Microorganism Ratio

The initial substrate to biomass ratio (S_0/X_0) as an important experimental variable because of its influence on kinetic parameters (Grady *et al.*, 1996). S_0/X_0 ratios were derived from the initial substrate concentrations, MLVSS concentrations and bacteria counts (Table 5.24). The filtered COD of the stock culture was not taken into consideration in these calculations because it was generated by the SBR eleven hours after the end of feeding. Most of the filtered COD consisted of non-biodegradable organics present initially in the sewage and refractory organics generated during bacterial growth.

Approximately the same initial concentration of CAA was used for all of the rate studies; however, the MLVSS concentration was variable. S_0/X_0 ratios range between 0.74 and 1.97 mg COD/mg VSS. For the majority of the cultures, the S_0/X_0 ratio is close to 1. S_0/X_0 ratios were not appropriate for LEH cultures because many of these cultures had lactalbumin present in addition to LEH. The presence of both substrates was due to the acclimation procedure. S_0/X_0 based on bacteria counts ranged from 3.28 to 12.5 x 10^{-10} mg COD/bacteria. This is a much wider range than is seen when the ratio is determined on the basis of MLVSS.

Table 5.24 Approximate S_0/X_0 Ratios for Aerobic Cultures using CAA

Temperature (°C)	Date	MLVSS $\frac{\text{mg VSS}}{\text{L}}$	Bacteria Count $\frac{\text{bact}}{\text{L}} \times 10^{11}$	COD (mg/L)	S_0/X_0 $\frac{\text{mg COD}}{\text{mg VSS}}$	S_0/X_0 $\frac{\text{mg COD}}{\text{Bact}} \times 10^{-10}$
20	Oct. 25	585		437.4	0.747	
20	Nov. 2	330		437.4	1.33	
20	Nov. 7	460		437.4	0.951	
20	Nov. 27	455	6.34	486.0	1.07	7.72
20	Nov. 29	490	14.8	486.4	0.992	3.28
20	Dec. 6	465	3.85	480.8	1.03	12.5
14	Mar. 27	500	**10.6**	480.1	0.960	4.52
14	Apr. 4	450	**9.6**	478.9	1.06	4.99
14	May 5	250	4.29	492.5	1.97	11.5
14	May 7	420	5.86	483.4	1.15	8.25
14	May 9	370	5.41	477.6	1.29	8.82

Bacteria counts in bold are based on numbers from final culture.

b) Anoxic Cultures

i) Increases in Biomass

One of the objectives of this portion of the experimental program was to evaluate acridine orange under conditions of active substrate metabolism. The limitations of AODC has been presented in Section 5.3.2.a.i-Increases in Biomass and Bacteria Counts. Initial and final bacteria counts are available for seven samples (Table 5.25). In five of the seven samples, there was a significant increase in the number of bacteria. In the remaining two samples, there is no significant difference between the initial and final samples. In the those samples where there is a significant difference, the mean increased by 38-94%. The results provided by AODC counts are reasonable. Cell replication may not have occurred in all cases because the cells have to obtain a minimum size before replication occurs.

ii) Nitrate Utilization Rate

The anoxic culture was assumed to be composed of bacteria only. The absence of non-bacterial species such as protozoa was confirmed by microscopic examination. The absence of higher order microorganisms is reasonable given the oxygen limitations of the reactor.

Table 5.25 MLVSS and Bacteria Counts for Anoxic Cultures using PH

Temp (°C)	PH	Date	Initial MLVSS (mg VSS/L)	Std. Dev. of MLVSS (mg VSS/L)	Bacteria Counts for Initial Culture (bact/L) x10^{11}			Bacteria Counts for Final Culture (bact/L) x10^{11}			Increase in Mean
					Mean	Lower 95% CI	Upper 95% CI	Mean	Lower 95% CI	Upper 95% CI	
20	CAA	Dec. 1	470	14	10.6		12.2	14.6	13.1	16.2	38%
20	CAA	Dec. 4	470	6	12.0	10.2	14.2	15.6	13.2	18.5	30%
20	CAA	Dec. 7	500	8				6.52	5.44	7.80	
14	CAA	Mar.29	465	21	16.0	14.1	18.2	17.5	15.4	19.8	9%
14	CAA	Apr. 1	495	32				22.7	20.6	25.0	
14	CAA	Apr. 4	570	5				21.2	20.3	24.1	
14	CAA	Apr. 24	590	16	13.4	12.0	15.0				
14	CAA	May 5	640	16	14.9	13.4	16.5	22.6	19.8	25.7	51%
14	CAA	May 9	585	49	9.28	7.36	11.7	18.0	14.7	22.0	94%
14	LEH	Apr. 1	495	32				14.5	12.5	17.0	
14	LEH	Apr. 9	640	28	8.64	7.84	9.52	26.3	18.3	24.2	67%
14	LEH	Apr. 13	740	55	8.88	7.79	10.2				
14	LEH	Apr. 29	645	23	10.2	8.40	12.6	17.6	15.5	20.0	73%

The endogenous respiration rate is more difficult to determine for anoxic cultures than for aerobic cultures because the rates are quite low. van Haandel *et al.* (1981) determined denitrification rates under two different reactor configurations at temperatures of 14-20°C; pre-denitrification and post-denitrification. The rate of pre-denitrification using an easily metabolizable substrate was 30 mg N/(g MLVSS·h). In contrast, the rate using a post denitrification configuration, where endogenous respiration supplied the energy needed, was 3 mg N/(g MLVSS·h). For a different system operating at 15°C and an anoxic sludge age of 6 d, the rate of endogenous nitrate respiration was 0.83 mg N/(g MLVSS·h). The denitrification rates cited were not corrected for the endogenous respiration rate because of measurement difficulties.

Nitrate and nitrite nitrogen were measured in the batch rate experiments of this research. The concentration of nitrite, an intermediate of nitrate reduction, was always less than 2 mg N/L. For convenience, nitrate will refer to the sum of nitrate nitrogen and nitrite nitrogen in the remainder of this chapter. Nitrate consumption for cultures using CAA and LEH at 20 and 14°C was constant over time (Figure 5.27); the rate of nitrate utilization is the slope of the regression line. The raw data and regression equations are found in Appendix B. Normalized NURs are summarized in Tables 5.26 and 5.27.

Figure 5.27 Nitrate Utilization for Anoxic Cultures using CAA at 20 and 14°C

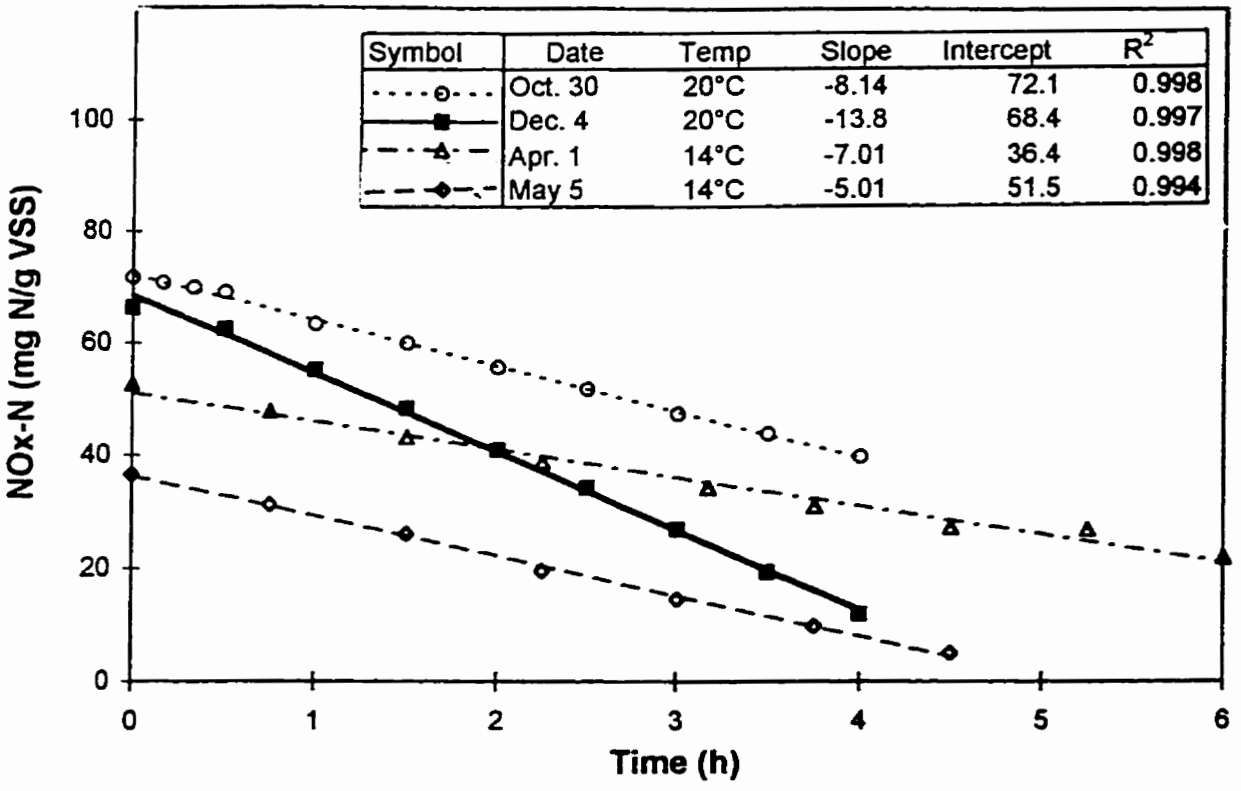


Table 5.26 NO_x Utilization for CAA Cultures at 20°C - Normalized on MLVSS and Bacteria Counts

Date	MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	NUR (mg NO_x-N/L h)	NUR (mg NO_x-N/g VSS h)	NUR (mg NO_x-N/bact h) x10^{-12}
20-Oct	650		5.39	8.29	
24-Oct	695		5.11	7.35	
30-Oct	550		4.47	8.13	
1-Dec	470	12.6	6.34	13.5	5.03
4-Dec	470	13.8	6.53	13.9	4.73
7-Dec	500	6.52	4.64	9.28	7.12
			Mean	10.1	5.63
			RSD	28.4%	23%

Table 5.27 NO_x Utilization for PH Culture at 14°C - Normalized on MLVSS and Bacteria Counts

Substrate	Date	MLVSS (mg VSS/L)	Bacteria Count (bact/L) x10^{11}	NUR (mg NO_x-N/(L h))	NUR (mg NO_x-N/(g VSS h))	NUR (mg NO_x-N/(bact h)) x10^{-12}
CAA	29-Mar	465	16.8	2.20	4.73	1.30
CAA	1-Apr	495	22.7	3.45	6.96	1.52
CAA	4-Apr	570	21.2	2.83	4.96	1.34
CAA	24-Apr	590	13.4	2.14	3.63	1.60
CAA	5-May	640	18.8	3.21	5.02	1.71
CAA	9-May	585	13.6	4.14	7.07	3.04
LEH	1-Apr	495	14.5	3.89	7.86	2.68
LEH	9-Apr	640	17.5	3.03	4.73	1.73
LEH	13-Apr	740	8.9	3.60	4.86	4.05
LEH	29-Apr	645	13.9	4.10	6.36	2.95
			CAA	Mean	5.39	1.49
				RSD	25.1%	12%
			LEH	Mean	5.89	2.46
				RSD	23.1%	26%

Note: Underlined datum has been found an outlier using Eq. 5.5

iii) Enzyme Assays

The results of enzyme assays done on intact mixed liquor 75 minutes after substrate addition are listed in Table 5.28. Enzymes present in concentrations in excess of 30 nanomoles or higher in at least two of the three cultures were: phosphatase alkaline, esterase (C4), leucine arlymidase, phosphatase acids, and phosphoamidase. With the exception of leucine arlymidase and esterase (C8), the predominant enzymes of the aerobic and anoxic cultures are the same. The expression of leucine arlymidase at these levels was specific to the anoxic culture. Leucine arlymidase belongs to a group of amino-peptidases which preferentially cleaves amino acid arlyamides. Leucine arlymidase would specifically hydrolyze leucine arylamides. Esterase (C8) was seen at high levels only in the aerobic cultures. In general, the enzyme profile of the aerobic and anoxic cultures are similar with enzymes promoting ester hydrolysis being predominant in both cultures.

Table 5.28 Enzyme Assays for Anoxic Cultures at 20°C

Enzyme	Oct. 20	Oct. 23	Oct. 30	Enzyme	Oct. 20	Oct. 23	Oct. 30
Phosphatase alkaline	5[1]	5	5	Phosphatase acids	5	5	1
Esterase (C4)	2	4	4	Phosphoamidase	4	5	0
Esterase (C8)	2	4	0	α-galactasidase	0	1	1
Lipase (C14)	0	0	5	β-galactasidase	1	2	0
Leucine arlymidase	5	5	1	β-glucuronidase	0	0	1
Valine arlymidase	1	1	0	α-glucosidase	0	2	0
Cystine arlymidase	0	0	1	β-glucosidase	0	1	3
Trypsin	1	1	1	N-acetyl-beta-glucosaminidase	3	2	0
Chymotrypsin	1	1	4	α-mannoisdase	0	0	2
				α-fucosidase	1	1	0

[1] Moles of test-strip substrate hydrolyzed

1 - 5 nanomoles, 2 - 10 nanomoles, 3 - 20 nanomoles, 4 - 30 nanomoles, 5 - ≥40 nanomoles

iv) Observed Ammonia Nitrogen Production

The anoxic culture was similar in its behaviour to the aerobic culture in the areas of ammonia nitrogen production (Fig. 5.28). The ammonia nitrogen concentration increased with time indicating ammonia was being generated as growth proceeded. The rate of production is the change in ammonia nitrogen concentration over time. The rate of production normalized on bacteria counts or MLVSS is presented in Tables 5.29 and 5.30. Bacteria counts of the initial and final cultures were used to normalize the production rate.

Figure 5.28 Ammonia Nitrogen Production for Anoxic Cultures using CAA at 20 and 14°C

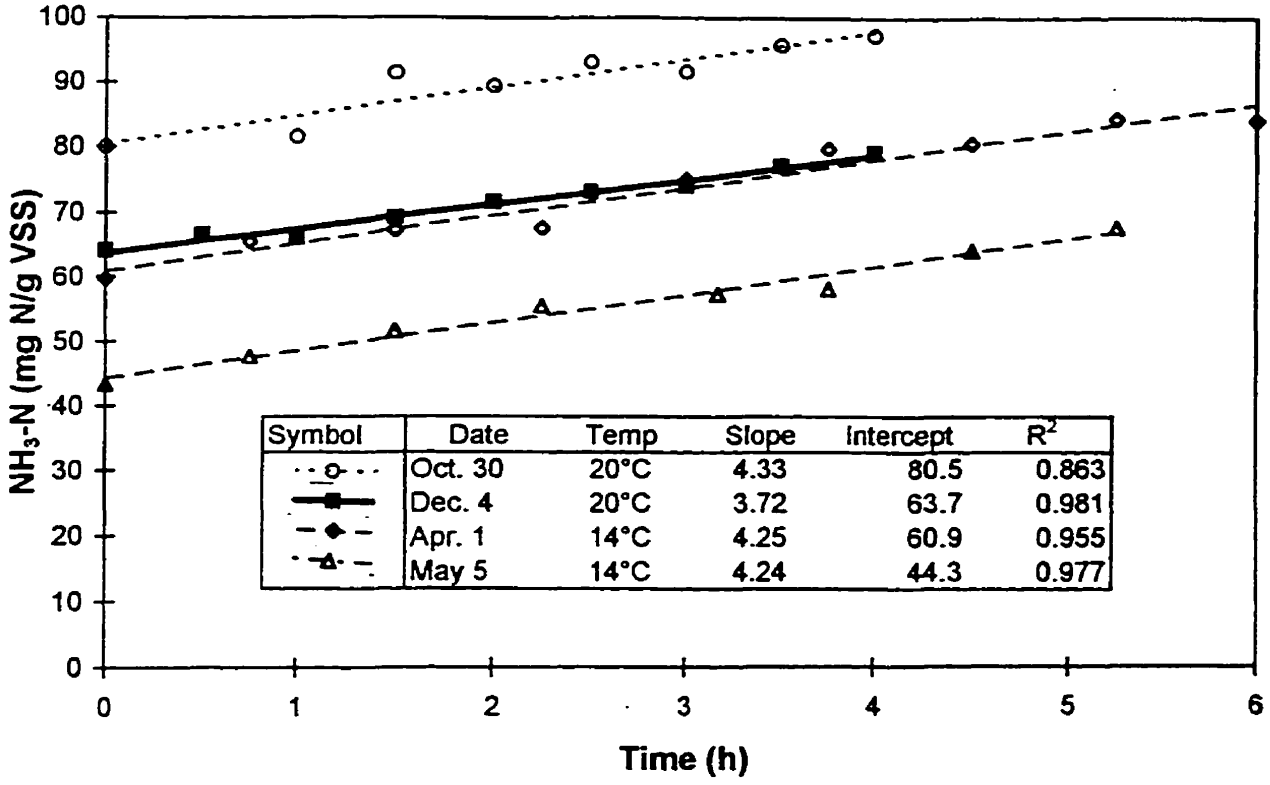


Table 5.29 Ammonia Nitrogen Production for an Anoxic Culture at 20°C using CAA

Date	NH_3-N Production (mg NH_3-N/(L h))	NH_3-N Production (mg NH_3-N/(g VSS h))	NH_3-N Production (mg NH_3-N/bact h) x 10^{-12}
Oct. 20	1.90	2.92	
Oct. 24	3.01	4.33	
Oct. 30	2.38	4.33	
Dec. 1	2.76	5.87	2.19
Dec. 4	1.75	3.72	1.27
Dec. 7	2.87	5.74	4.40
	Mean	4.49	1.73
	RSD	25.2%	38%

Note: Underlined datum has been found an outlier using Eq. 5.5

Table 5.30 Ammonia Nitrogen Production for an Anoxic Culture at 14°C using CAA

Date	NH_3-N Production (mg NH_3-N/(L h))	NH_3-N Production (mg NH_3-N/g VSS h)	NH_3-N Production (mg NH_3-N/bact h) x 10^{-12}
Mar. 29	1.62	3.48	0.97
Apr. 1	2.11	4.26	0.93
Apr. 4	2.14	3.75	1.01
May 5	2.72	4.25	1.45
May 9	3.22	5.50	2.37
	Mean	4.25	1.09
	RSD	18%	22%

Note: Samples of Apr. 24 have been excluded because the samples were preserved with formaldehyde which affected the results.

v) Soluble COD Uptake

Figure 5.29 illustrates soluble COD uptake profiles for four experiments using CAA at 20 and 14°C. Shortly after the PH was added to the solution, some of it was taken up into the floc. The higher of the initial values shown in Figure 5.29 was based on a COD balance. The material balance determination was carried out in the same fashion as was the determination for the aerobic culture. Table 5.31 lists the initial COD concentration derived from a mass balance and the initial COD calculated from a linear regression of COD concentration over time. The initial uptake of COD has been evaluated from this data. In general, the COD uptake of the anoxic culture was much higher than it was for the aerobic culture. In ten of the eleven rate studies, the COD uptake exceeds the lower detection limit of 20 mg/L. For anoxic cultures values of 0.146 and 0.108 mg COD/mg VSS were obtained for 20 and 14°C, respectively.

Table 5.31 COD Uptake and Storage for Anoxic Cultures using CAA

Temp (°C)	Date	COD (mg/L)	Expected COD (mg/L)	Uptake in COD (mg/L)	Initial MLVSS (mg VSS/L)	COD Uptake Normalized on MLVSS (mg COD/mg VSS)
20	Oct. 20	506.4	389.9	116.6	650	0.179
20	Oct. 24	506.5	412.4	94.1	695	0.135
20	Oct. 30	532.9	489.9	43	550	0.078
20	Dec. 1	501.7	398.3	103.4	470	0.220
20	Dec. 4	514.1[1]	456.9	57.2	470	0.122
20	Dec. 7	544.6	473.5	71.5	500	0.143
14	Mar. 29	494.2	480.1	14.1	465	0.030
14	Apr. 1	521.5	466.1	55.4	495	0.112
14	Apr. 4	520.0	455.9	64.1	570	0.112
14	Apr. 24	533.4	430.9	102.6	590	0.174
14	May 5	526.9	455.7	71.2	640	0.111
14	May 9	520.7	314.3	206.4	585	0.353[2]

1. Effluent COD of stock culture has been estimated
2. Excluded from average after being identified as a outlier using the test criterion for single outliers (Eq. 5.5)

vi) Initial Substrate to Microorganism Ratios

Table 5.32 lists S_0/X_0 ratios for anoxic cultures at 20 and 14°C. These ratios have been calculated in the same way as they were for aerobic cultures. The S_0/X_0 ratios are lower for the anoxic cultures than they were for the aerobic cultures due to higher initial biomass concentrations. Values range between 0.69 and 1.04

mg COD/mg VSS. Later analysis will use the S_0/X_0 ratio to determine if a correlation exists between it and the normalized OURs and NURs.

Figure 5.29 COD Uptake for Anoxic Cultures using CAA at 20 and 14°C

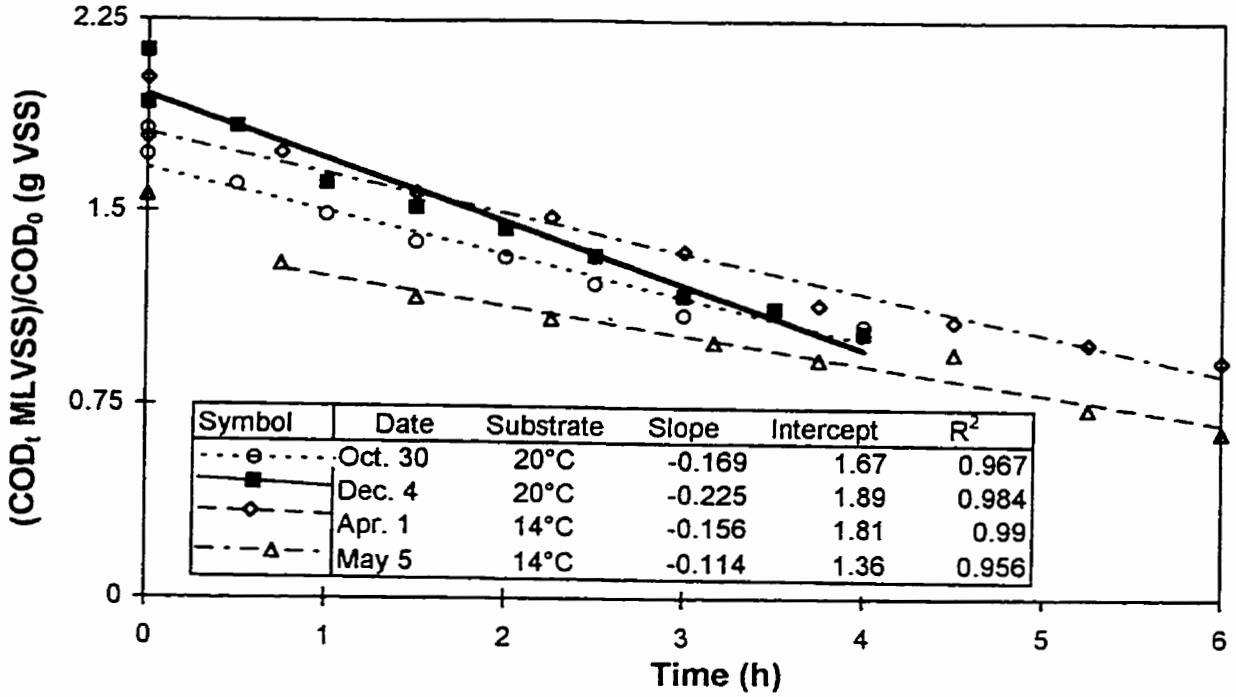


Table 5.32 Approximate S_0/X_0 Ratios for Anoxic Cultures using CAA

Temp. (°C)	Date	Initial MLVSS $\frac{\text{mg VSS}}{\text{L}}$	Bacteria Count $\frac{\text{cell}}{\text{L}} \times 10^{11}$	COD (mg/L)	S_0/X_0 $\frac{\text{mg COD}}{\text{mg VSS}}$	S_0/X_0 $\frac{\text{mg COD}}{\text{Cell}} \times 10^{-10}$
20	Oct. 20	650		479.3	0.737	
20	Oct. 24	695		479.3	0.689	
20	Oct. 30	550		489.0	0.889	
20	Dec. 1	470	10.6	487.6	1.04	4.6
20	Dec. 4	470	12.0	487.2	1.04	4.06
20	Dec. 7	500	**6.5**	482.9	0.966	7.43
14	Mar. 29	465	16.0	478.2	1.03	2.99
14	Apr. 1	495	**22.7**	479.5	0.970	2.11
14	Apr. 4	570	**21.2**	480.3	0.842	2.27
14	Apr. 24	590	13.4	478.6	0.811	3.57
14	May 5	640	14.9	526.9	0.823	3.53
14	May 9	585	9.3	477.7	0.817	5.13

1. Bacteria counts in bold were based on the final culture.

5.3.3. Analysis and Discussion of Experimental Data

a) Immediate increases beyond the basal respiration rate of the aerobic cultures

Within minutes of either CAA or LEH addition, the aerobic cultures attained a higher OUR. The increase in OUR exhibited by the aerobic culture was up to six times higher than the corresponding basal respiration rate. The almost immediate increase in OUR upon substrate addition indicated that substrate uptake and utilization happened very quickly. Low basal respiration rates indicated the stock culture had a very low initial metabolic rate. The speed at which the metabolic rate changed was surprising but this phenomena has been noted by other investigators.

In general, the degree to which a culture can immediately increase its growth and substrate removal has been referred to as the available reduction potential (McLellan and Busch, 1969). This phenomena has been exhibited by both pure cultures and mixed populations. Daigger and Grady (1982) suggested that the new rate will be greater than the one exhibited before the transition but it will be less than the maximum obtainable. This may explain in part the linear increase in OUR seen with some of the PH cultures. Selna and Schroeder (1978) saw a rapid increase in the OUR of an activated sludge system in response to pulse loadings of organics.

The rapid response to a nutrient shift-up has been noted in systems existing under starvation conditions by several investigators. After starvation for 7 weeks, *Ant-300* cells responded to the addition of glucose immediately and multiplication of the cells occurred (Novitsky and Morita, 1977). Morita (1993) suggested that this response is necessary for the cell to obtain energy during starvation-survival. Non-differentiating bacteria lack the genetic security of spore formation during growth arrest.

b) Comparison of Nitrate Utilization rates with Literature

Most of the literature nitrate utilization data has been derived for combined sludge systems where the biomass is exposed to alternating aerobic and anoxic conditions (Sutton *et al.*, 1977; Halmø and Eimhjellen, 1981; Oleszkiewicz and Berquist, 1988). Only a fraction of the active heterotrophic biomass in such cases will be capable of denitrification. In the anoxic experimental system of this research, there was little dissolved oxygen, no alternating aerobic/anoxic operation and excess nitrate available. Some non-denitrifiers will continually enter the reactors with the sewage but unfavorable conditions will limit their growth. Thus it was

assumed that most of the active heterotrophs were denitrifiers. The current experimental system most closely parallels a separate sludge system in its operation.

Murphy and Sutton (1975) reported denitrification rates for a separate sludge system using methanol as a supplementary carbon source and operating at an approximately 9 day sludge age. Rates of 6.8-9.3 mg NO_x-N/(g VSS·h) were reported for operation at 20°C. Using the data of Table 5.26, the lower and upper 95% confidence limit are 7.09 -13.1 mg NO_x-N/(g VSS·h), respectively at 20°C. There is no significant difference between the rates reported for this study and those reported by Murphy and Sutton. This comparison is appropriate because for both systems, the mixed liquor was expected to have a high proportion of denitrifiers. The single sludge system of Murphy and Sutton received a clarified effluent from an upstream aeration basin supplemented with methanol and nitrate. Under these conditions there was very little opportunity for the growth of obligate aerobes.

van Haandel *et al.* (1981) reported the occurrence of three distinct phases with characteristic rates when activated sludge was mixed with influent wastewater under batch conditions. The initial phase proceeds until the rapidly biodegradable COD present in the influent is depleted. During this phase, the rate of denitrification was fast. This situation would be comparable to growth on CAA. van Haandel *et al.* (1981) calculated the rate kinetics from numerous nitrate profiles over a range of sludge ages (10-20 d), temperatures (14-20°C) and influent CODs (300-800 mg COD/L). The rate of denitrification when the culture was using rapidly biodegradable organics was 30 mg NO_x-N/(g VSS·h). The value presented by the investigators is based on active biomass concentration only and thus would be expected to be higher than the values presented in this work and by Murphy and Sutton. The investigators commented that even though the standard deviation associated with the mean was small there was a wide variation (±25%) in the rate constants. They attributed this variation to changes in the sewage composition.

Henze *et al.* (1993) examined the denitrification rate as it was influenced by the wastewater fraction used as substrate. Rapidly biodegradable substrates as defined in the context of ASM1 were further partitioned into directly and easily degradable substrates (Ekama *et al.*, 1986). Table 5.33 gives examples of substrates belonging to the directly and easily degradable fractions, and the rate denitrification realized when these fractions are used at 20°C. The CAA used in the current experiments would belong to the easily degradable

category. The rate of 7.09 -13.1 mg NO_x-N/(g VSS·h) is much higher than the value cited by Henze *et al.* (1993).

Table 5.33 Classification of Rapidly Biodegradable Substrates as proposed by Henze *et al.* (1993)

Fraction	Substrates	Rate of Denitrification mg N/(g VSS·h)
Directly degradable	Acetic acid, lower volatile fatty acids	10
Easily degradable	Higher volatile fatty acids, lower amino acids and simple carbohydrates	3

c) **Temperature Dependency of Denitrification**

Changes in temperature can cause changes in the MLVSS composition, ecology and cellular growth kinetics. Consequently, the relative proportions of slowly biodegradable substrate, bacteria, and non-bacterial species in the mixed liquor may change. The ratio of respiring denitrifiers to MLVSS is a function of temperature. For this reason, expressing changes in kinetics on the basis of AODC may give a better representation of the observed effects.

A modified Arrhenius coefficient (θ_{DN}) is frequently used to express the temperature dependency of rate coefficients (Eq. 2.1). Table 5.34 summarizes modified Arrhenius coefficients for a number of different experimental systems reported in the literature; the value of θ_{DN} ranges between 1.05-1.30. A value for θ_{DN} was derived for rate data presented in Tables 5.26 - 5.27 with the use of a program determining non-linear least squares. For rate data normalized on MLVSS, θ_{DN} was 1.09. It is comparable to the value of 1.10-1.13 reported by Sutton (1973). Sutton's work employed a separate sludge culture operating at a sludge age of 7-9 days using methanol as a carbon source.

A value of 1.25 was determined for θ_{DN} using data normalized on bacteria counts. This is much higher than any of the literature values reported in Table 5.34 but these values were based on rates normalized on MLVSS. Sutton *et al.* (1977) reported an increasing temperature sensitivity with shorter SRTs for separate sludge systems. For a separate sludge system at a SRT of 4 d, θ_{DN} was 1.17. With shorter SRTs, a greater proportion of the MLVSS will be active denitrifiers. The increased sensitivity of θ_{DN} could reflect changes in the underlying population as well as changes in the growth kinetics. θ_{DN} of 1.25 is not unreasonable given the

value was calculated on a the number of bacteria and the culture conditions precluded the growth of many non-denitrifying organisms.

d) **Temperature Dependency for Aerobic Cultures**

As with the anoxic cultures, changes in temperature can cause changes in the MLVSS composition, ecology and cellular growth kinetics of the bacteria present in the aerobic reactor. Temperature effects may be more or less pronounced under aerobic conditions because it is expected that the non-bacterial population would make up a larger part of the MLSS. A modified Arrhenius coefficient was calculated using UWHAUS (University of Wisconsin, 1964) for respiration rates normalized on MLVSS and bacteria counts. Values of 1.01 and 1.03 were found for data normalized on MLVSS and bacteria counts, respectively.

Freidman and Schroeder (1972) quantified the temperature effects on growth in activated sludge systems using a continuous-flow stirred tank reactor. Reactor and effluent biosolids concentrations and the effluent flow were monitored frequently over approximately 6 hours. A unit growth rate was calculated as the ratio of the mass of solids in the effluent to the mass of solids in the reactor. The unit growth rate was corrected to account for different starting MLVSS concentrations. Over the temperature range of 3.7 to 20°C, the modified Arrhenius coefficient for the unit growth rate was 1.046.

Table 5.34 Modified Arrhenius Coefficients θ_{DN} for Denitrifying Systems

Culture	Temperature Range (°C)	Culture Conditions	Growth Substrate	θ values	Reference
Activated Sludge	14-20	Batch, SRT = 8 d	Sewage/Protein	1.09	This Study
Activated Sludge, Separate Sludge	5-25	SRT=4 d	Methanol	1.17	Sutton *et al.* (1977)
Activated Sludge, Separate Sludge	5-25	SRT=7 d	Methanol	1.13	Sutton *et al.* (1977)
Activated Sludge, Separate Sludge	5-25	SRT=9 d	Methanol	1.10	Sutton *et al.* (1977)
Activated Sludge. Separate Sludge	5-25	SRT=10 d	Methanol	1.08	Sutton *et al.* (1977)
Activated Sludge	2.5-25	Non-Acclimated	Methanol	1.07	Lewandoswki (1982)
Activated Sludge	2.5-25	Non-Acclimated	Acetic Acid	1.07	Lewandoswki (1982)
Activated Sludge	2.5-25	Non-Acclimated	Acetone	1.07	Lewandoswki (1982)
Activated Sludge	7-15	SBR	Sewage	1.06	Oleszkiewicz and Berquist (1988)
Activated Sludge	2-7	SBR	Sewage	1.30	Oleszkiewicz and Berquist (1988)
Pseudomonas denitrificans	3-27	Batch	Sodium Citrate	1.12	Dawson and Murphy (1973)
Pseudomonas denitrificans	10-20	Batch	Sodium Citrate	1.10	Dawson and Murphy (1973)
Pseudomonas denitrificans	30-38	Batch	Methanol	1.05	Wang *et al.* (1995)
Activated Sludge	ng	Batch	ng.	1.06	Nakajima *et al.* (1984)
Denitrifying Culture Isolated from Lake Water	ng	Batch	ng	1.08	Nakajima *et al.* (1984)

ng - not given

The unit growth rate is proportional to the oxygen utilization rate reported for this study. The temperature sensitivity of these two variables should be equivalent as long as the true yield is invariant. The value of 1.046 is only slightly higher than that of 1.03 found for respiration rates normalized on bacteria counts.

e) **Variability in OUR or NUR data for Repeated Experiments**

OUR and NUR data obtained under similar conditions of PH loading, EAS and temperature was variable (Tables 5.18, 5.19, 5.26 and 5.27). The variance in the OUR data presented in Tables 5.18 and 5.19 was exaggerated because the OUR_C was used in preference to OUR_M. The correction reduced the mean value while the standard deviation of the data set remained unchanged.

Due to time limitations and the amount of stock culture produced, it was impossible to do several experiments at once. Both the 14 and 20 °C rate studies were done over a 7 week period. Changes in the stock culture were evident from changes in the observable characteristics of the SBR: MLVSS concentrations, effluent COD and ammonia nitrogen concentration and nitrate consumption ratios. With these observable changes, it is reasonable to expect differences in the underlying microbial population. Sewage was added on a daily basis specifically to maintain a diverse population.

Grady *et al.* (1996) suggested that culture history (the type of environmental conditions imposed and the duration of their imposition) was the major factor contributing to measured differences in kinetic parameters describing biodegradation. With mixed cultures, some members of the community are selected over others because of their growth kinetics. Even within certain species of bacteria, a bacterium can selectively express enzyme systems with differing substrate affinities. When grown under relatively steady ambient conditions, bacteria may alter their macromolecular composition to optimize growth. The authors concluded that the initial S_0/X_0 ratio used in the experiment is particularly important because it influences the kinetics.

In this research, repeated experiments were carried out at differing S_0/X_0 ratios. This was the consequence of the stock culture having varying MLVSS concentrations while the amount of substrate remained relatively constant. In theory, S_0/X_0 ratios calculated on bacteria counts should be more precise because AODC enumerates the total bacterial population whereas MLVSS is a surrogate measurement.

Sufficient data were available to determine if the variation in normalized OURs and NURs was the result of varying S_0/X_0 ratios. The analysis was done using S_0/X_0 defined on MLVSS concentration and bacteria counts. The S_0/X_0 ratios calculated in Tables 5.24 and 5.32 and the rate data summarized in Tables 5.18, 5.19, 5.26 and 5.27 were used in the calculations. For these calculations, only data for cultures using CAA were considered.

The data normalized on either MLVSS or bacteria counts were correlated to S_0/X_0. Only for those data sets having a correlation coefficient (r) greater than the critical value was the correlation considered significant. The values of r and the corresponding critical values at the 95% and 90% confidence level are listed in Table 5.35.

Table 5.35 Correlation Coefficients for Oxygen and Nitrate Utilization

Normalizing Factor	Temperature (°C)	EAS	Degrees of Freedom	r	$r_{0.95}$	$r_{0.90}$
MLVSS	20	Aerobic	4	0.527	0.811	0.729
	20	Anoxic	4	**0.849**	0.811	0.729
	14	Aerobic	3	0.769	0.878	0.805
	14	Anoxic	4	0.151	0.811	0.729
Bacteria	20	Aerobic	1	0.974	0.997	0.988
Counts	20	Anoxic	1	**0.999**	0.997	0.988
	14	Aerobic	3	**0.974**	0.878	0.805
	14	Anoxic	4	**0.881**	0.811	0.729

Significant r values are bolded.

For the data sets where a significant correlation did exist, the variance as measured by the relative standard deviation is overstated (Tables 5.18, 5.19, 5.26 and 5.27). Some of the variance is due to the dependency of the rate on the initial S_0/X_0. The residual error remaining after the regression is a better estimate of the unexplained variation in kinetics.

The original hypothesis was that the variation in the kinetics would be greater for data normalized on MLVSS than on bacteria counts. This seemed reasonable given MLVSS is a measure of organic mass that evaluates all constituents of the activated sludge floc. In theory, there should be greater reproducibility with data normalized on bacteria counts.

Revised estimates of the variation in the data sets are presented in Table 5.36. For three of the four culture types, rates expressed on bacteria counts show better reproducibility. The exception to this is the aerobic culture at 20°C. The results of this data set may be less reproducible than the others because there were only

three samples in the data set and the presence of filaments was noted in the SBR during the early part of December. The morphology of filaments makes it difficult to count the number of individual bacteria. Filaments were noted in the Dec. 6 sample.

Table 5.36 Revised Estimates of Relative Standard Deviations for Repeated Experiments

EAS	Temperature (°C)	Normalized on MLVSS	Normalized on Bacteria Counts
Aerobic	20	31%	49%
Aerobic	14	18%	9%
Anoxic	20	17%	1%
Anoxic	14	25%	18%

f) **Relative Rates of Oxygen and Nitrate Utilization**

The rate of nitrate and oxygen utilization can be related to each other using the half reactions presented (Eq. 5.6 and 5.7). Based on these equations 1 mg NO_3-N is equal to 2.86 mg O_2. The reader is referred to Barker and Dold (1995) for a complete derivation.

$$e^- + \frac{1}{5}NO_3^- + \frac{6}{5}H^+ \rightarrow \frac{1}{10}N_2 + \frac{3}{5}H_2O \qquad \text{Eq. (5.6)}$$

$$e^- + \frac{1}{4}O_2 + H^+ \rightarrow \frac{1}{2}H_2O \qquad \text{Eq. (5.7)}$$

Using a common basis allows for easier comparison of oxygen and nitrate respiration rates. The utilization rate of either oxygen or nitrate expressed in terms of oxygen equivalents is (1-Y) times the rate of substrate utilization. The true yield, Y, is the observed yield in the absence of maintenance energy requirements. It will be shown later in section that there is no significant difference at the 95% CL in the observed yield under conditions of active substrate removal and aerobic and anoxic conditions. In this case, comparing respiration rates is equivalent to comparing substrate utilization rates.

In Tables 5.37 - 5.38, the rates of CAA utilization are summarized in terms of oxygen equivalents. The data indicates that at 20 °C, substrate utilization proceeds at a comparable rate for both aerobic and anoxic cultures when normalized on MLVSS concentration. At 14°C, nitrate respiration is much slower than the aerobic respiration. When the rates of PH utilization at 20°C are normalized on bacteria counts, there is no difference in substrate utilization at the 95% confidence level. At the 14°C, substrate utilization occurs more slowly under anoxic conditions.

Table 5.37 PH Utilization in Oxygen Equivalents-Normalized on MLVSS

EAS	Temperature (°C)	Utilization of Oxygen or Nitrate (mg O_2/g VSS h) Mean	Approximate Upper 95% CL	Approximate Lower 95% CL
Aerobic	20	34.4	45.6	23.1
Aerobic	14	32.9	40.3	25.6
Anoxic	20	28.9	37.5	20.3
Anoxic	14	15.4	19.5	11.4

Raw data are found in Table 5.18, 5.19, 5.26 and 5.27

Table 5.38 PH Utilization in Oxygen Equivalents -Normalized on Bacteria Counts

EAS	Temperature (°C)	Utilization of Oxygen or Nitrate (mg O_2/cell h) x 10^{-12} Mean	Approximate Upper 95% Confidence Limit	Approximate Lower 95% Confidence Limit
Aerobic	20	22.8	50.6	-4.9
Aerobic	14	19.1	24.6	13.7
Anoxic	20	16.1	25.4	6.81
Anoxic	14	4.26	4.53	3.98

Raw data are found in Table 5.18, 5.19, 5.26 and 5.27

A statistical analysis of the replicated 2^2 factorial examined the effects of EAS and temperature or any interaction between them on normalized respiration rates. A standard deviation for the effects is estimated from the variance between repeated runs. The ratio of the effect to the estimated standard deviation, the t-value, follows a normal distribution. If the t-value is greater than the critical value at the 95% confidence level, then the effect is significant.

For data normalized on MLVSS, none of the factors considered were found to be significant (Table 5.39 with supporting data in Appendix C). When the same problem was examined using data normalized on cell counts, the EAS was significant. On average, respiration rates were highest under aerobic conditions.

Table 5.39 Analysis of Respiration Rates Normalized on MLVSS

Effect		Normalized on MLVSS (mg e^-/g VSS h) Estimated Effect	Calculated t
Main Effects	EAS	-5.75	1.83
	Temperature	3.75	1.19
Interaction	EAS x Temperature	3.00	0.955

Effects have associated with them 19 df and a critical value, $t_{0.95}$, of 2.09.
Bolded effects are significant at the 95% CL.

Table 5.40 Analysis of Respiration Rates Normalized on Bacteria Counts

		Normalized on Bacteria Counts (mg e^-/cell h) $\times 10^{-12}$	
Effect		Estimated Effect	Calculated t
Main Effects	EAS	**-5.39**	2.67
	Temperature	3.89	1.93
Interaction	EAS x Temperature	2.03	1.00

Effects have associated with them 13 df and a critical value, $t_{0.95}$, of 2.16.
Bolded effects are significant at the 95% CL.

g) **Ammonia Nitrogen Production**

Ammonia production data was analysed to see if the rate correlated with the rate of oxygen or nitrate utilization expressed in terms of oxygen equivalents (Table 5.41). Critical values are presented for the 90% and 95% confidence levels. A significant correlation exists if the calculated correlation coefficient, r, exceeds the critical value. A significant correlation existed for the aerobic culture at 20°C and the anoxic culture at 14°C.

Table 5.41 Correlation between NH_3-N Production and Consumption of Oxygen or Nitrate

EAS	Temp. (°C)	Degrees of Freedom	Correlation Coefficient (r)	$r_{0.95}$	$r_{0.90}$
Aerobic	20	4	**0.853**	0.811	0.729
Aerobic	14	3	0.607	0.878	0.805
Anoxic	20	4	-0.414	0.811	0.729
Anoxic	14	3	**0.882**	0.878	0.805

Bolded r values are significant.

The lack of consistency is not surprising when the complexity of ammonia production is considered. The first step involves the hydrolysis of amide bonds present in peptides to form ammonia nitrogen. This may be accomplished by several different classes of enzymes. Many proteolytic enzymes have been found to have esterase and amidase activities for low molecular proteinaceous weight substrates (Clarke, 1980). Chymotrypsin, papain and pepsin are all enzymes noted for their ability to hydrolyze amides. For example, chymotrypsin hydrolyses peptide bonds adjacent to the carbonyl group of aromatic amino acids but also hydrolyses esters of N-acetlphenylalanine and N-acetyltryptophan and amides such as benzoylglycineamide (Clarke, 1980).

Ammonia nitrogen produced by these mechanisms is used to produce glutamate, an essential amino acid. Glutamate is a key building block in organic synthesis. With proteins, often the amount of ammonia

nitrogen needed for bacteria synthesis is less than that produced by peptide deamination resulting in a net production of ammonia nitrogen.

In some instances, there was a lack of correlation between the observed rate of ammonia nitrogen production and the rate of respiration. Some possible reasons are as follows:

1. The nitrogen requirements of the cell may vary with the growth regime. The cell makes a transition from a chronically starved state at the experiment's onset to growing at a much higher rate at its completion.
2. With a solution of amino acids and peptides, selective adsorption on the cell surface may occur. Amino acids such as threonine, valine, and leucine do not contain as much nitrogen as other amino acids. Their generation from dipeptides and olgiopeptides will not add as much ammonia nitrogen to the pool.
3. There is evidence that ammonia nitrogen can accumulate within the cell. Stevenson and Silver (1977) reported that three strains of *Escherichia coli* concentrated methylammonia, an ammonium analogue, some 100-fold intracellularly. Depending on the bacteria present and the starting conditions of the culture, this could be a sink for some of the produced ammonia nitrogen.

With the current experimental system, it is not possible to suggest any one reason to explain the behaviour described above.

Ammonia nitrogen production data formed a replicated 2^2 factorial design with EAS (aerobic/anoxic) and temperature (14 and 20°C) as factors. The analysis was done in the manner described in the previous section using data in Tables 5.21, 5.22, 5.29, and 5.30. Results are summarized in Table 5.42. Regardless of the normalizing factor, the EAS had a significant effect on the rate of production. Ammonia nitrogen production was highest under aerobic conditions. Temperature and the EAS-temperature interaction were not significant factors.

Table 5.42 Analysis of the Factorial Design - Ammonia Nitrogen Production

	Normalized on MLVSS x 10^{-3}		Normalized on Bacteria Counts x 10^{-12}	
	Estimated Effect	T value	Estimated Effect	T value
EAS	**-1.78**	2.68	**2.41**	3.76
Temperature.	-0.015	0.226	-0.265	0.414
EAS x Temp.	0.390	0.588	0.355	0.554

MLVSS-Effects have associated with them 18 df and a critical value, $T_{0.95}$, of 2.10.
Bacteria Counts -Effects have associated with them 12 df and a critical value, $T_{0.95}$, of 2.18.
Bolded effects are significant at the 95% CL.

h) **Substrate Uptake**

Uptake mechanisms and kinetics are difficult to determine because once the protein is incorporated into the floc it is not easily differentiated from the floc itself. Monitoring the disappearance of the protein from the bulk liquid is one way of approaching the problem; however, by measuring lumped parameters (e.g. COD or TCA soluble protein) there are no assurances that byproducts from substrate degradation or bacteria lysis are not included in these measurements. Information gained following the disappearance of a specified protein from the bulk solution should be interpreted with caution. More sophisticated techniques must be employed if information on the rate and mechanism of protein uptake is desired.

There are a number of processes that must occur before a biodegradable substrate can be used as a substrate. These may include: transport to the floc surface and adsorption onto or enmeshment into the floc. Protein adsorption is affected by the properties of the surface, the nature of the protein, and the solution conditions (Wahlgren and Arnebrant, 1991). If the protein is enmeshed into the floc then the size, geometry and physical state of the substrate will influence the rate of uptake differently than it will if adsorption is the prevailing mechanism.

The initial substrate uptakes were summarized in Tables 5.23 and 5.31 for the aerobic and anoxic cultures, respectively. Although the same amount of CAA was added in each case, the estimated initial value is quite variable. In the case of the aerobic culture, initial substrate values vary from 314.3 to 502 mg COD/L. There is less variation in the values of the anoxic culture where values range from 432.3 to 511.7 mg COD/L.

The initial uptake normalized on the initial MLVSS concentration show a great deal of scatter. The average uptake normalized on MLVSS for the aerobic cultures was 0.051 and 0.138 mg COD/mg VSS for temperatures of 20 and 14°C, respectively. For anoxic cultures values of 0.145 and 0.108 mg COD/mg VSS were obtained for 20 and 14°C, respectively. There is no clear trend with respect to either EAS or temperature. If substrate was adsorbed to the surface of the floc, then surface characteristics such as charge, area and porosity will affect of adsorption. These characteristics most likely will not be a function of the MLVSS concentration.

Torrijos *et al.* (1994) examined the uptake and utilization of a soluble fraction of wastewater (i.e. size less <0.1 μm). At the beginning of the culture, the rate of disappearance of substrate was higher than the

rate of oxygen consumption. Physical adsorption on the flocs or absorption by the bacteria was cited by the researchers as the reason for the initial rapid uptake of substrate.

McLoughlin and Crombie-Quilty (1983) examined the kinetics of bovine serum albumin (BSA) utilization by activated sludge. Upon addition of the BSA to the culture, there was immediate and substantial uptake. They suggest these kinetics are consistent with those of adsorption. After this occurred, the rate of uptake was much slower and linear with respect to time.

Potentially there could be three distinct phases in the substrate removal curve. In the first phase, substrate uptake is immediate. This phase would correspond to the emeshment of the substrate into the floc or physical adsorption of the substrate onto the floc. The rate of substrate disappearance would be greater than the OUR or NUR. The second phase begins once the all the surface sites of floc are saturated with substrate. The rate of substrate disappearance would be linear and the rate of substrate uptake is equal to the rate of utilization. During the third phase, there is no longer substrate present in the culture media in excess. Only a fraction of the sites on the floc's surface are saturated. Growth is now substrate limited.

For both aerobic and anoxic cultures studied in this research, soluble substrate uptake was initially immediate and then linear with respect to time (Fig. 5.26 and 5.9). A constant rate of substrate uptake and high final organic concentrations in the culture media supports the hypothesis that the floc remains saturated for the duration of the rate study. Under these conditions the rate of substrate uptake would be equal to the rate of utilization. This behaviour is consistent with the behaviour described by McLoughlin and Crombie-Quilty and would correspond to phases one and two. The experiments were not carried out long enough to reach phase three.

i) **Observed Yield**

The observed yield measured under conditions of active substrate metabolism differs from the observed yield calculated for the SBR. The observed yield for the SBR, a chronically starved system, is the ratio of biosolids production to the mass of substrate consumed. In this system, predation, cell lysis and regeneration, and the accumulation of non-biodegradable and biodegradable material is accounted for in the calculated observed yield. In this instance, the observed yield quantifies the effect of all these processes. Under conditions

of active substrate metabolism, the observed yield quantifies the stoichiometry of growth. It is calculated using the ratio of the respiration rate to the rate of substrate utilization.

The observed yield is always less than the true yield. For every unit of substrate COD consumed, Y units of cells are produced but some fraction (f) of the Y units is lost for maintenance energy requirements. The amount of substrate oxidized will be 1-Y(1+f). In terms of oxygen consumption this can be thought as:

$$OUR_{Total} = OUR_T + OUR_E \quad \text{Eq. (5.8)}$$

$$= (1-Y)\frac{dS}{dt} - fY\frac{dS}{dt} \quad \text{Eq. (5.9)}$$

$$= [1-Y(1+f)]\frac{dS}{dt} \quad \text{Eq. (5.10)}$$

where OUR_{Total} = total oxygen utilization rate (mg O_2/L·h)
OUR_T = oxygen utilization rate associated with cell growth (mg O_2/L·h)
OUR_E = oxygen utilization rate associated with endogenous respiration (mg O_2/L·h)
Y = true yield (mg COD/mg COD)
$\frac{dS}{dt}$ = rate of substrate consumption (mg COD/L·h)
f = fraction of the cell mass directed towards endogenous respiration

The measured OUR prior to substrate addition was defined is the basal respiration rate, OUR_B. When growth proceeds under substrate limitations, OUR_B is the sum of the oxygen demands of the non-bacterial population and endogenous respiration requirements of the bacterial population (OUR_E). Very little of the oxygen demand is due to substrate degradation.

The basal respiration rate (OUR_B) was subtracted from the OUR measured during active substrate removal (OUR_M) to produce a corrected OUR(OUR_C). It was assumed that addition of substrate did not affect the oxygen demand of the non-bacterial population over the 4-6 h of the rate study. Maintenance energy requirements and hence endogenous respiration can be affected by the growth regime so OUR_E may be under-estimated during active substrate removal.

OUR_C was used in Equation 5.11. Oxygen consumption was not constant over the duration of the rate experiment (Fig 5.23 and 5.24). Arithmetically averaging OUR_C gives each measurement equal weight; however, the actual measurements were made over different lengths of time. Time-weighted averages of OUR_C and substituting the average into Equation 5.11 was deemed appropriate.

In rate studies done under aerobic conditions, some soluble substrate was taken up into the floc shortly after substrate was added. Continued substrate uptake followed at a constant rate over the remainder of the rate study. Examples of this behaviour are seen in Figure 5.28.

$$\frac{dO_2/dt}{dS/dt} = -(1 - Y_{obs}) \qquad \text{Eq. (5.11)}$$

where dO_2/dt = the rate of oxygen consumption (mg $O_2 \cdot L^{-1} \cdot h^{-1}$)
dS/dt = the rate of biodegradable substrate disappearance (mg $COD \cdot L^{-1} \cdot h^{-1}$)
Y_{obs} = observed yield (mg COD/mg COD)

With the anoxic cultures, the rate of nitrate consumption was constant over time for PH and protein utilizing cultures (Figure 5.29 and 5.39). After the initial rapid uptake of substrate, disappearance of substrate from the bulk liquid was linear with respect to time. The constant utilization rates of substrate and nitrate are illustrated in Figure 5.29 and 5.31 for PH cultures. Nitrate consumption rates were not corrected for endogenous requirements because of the difficulties outlined in Section 5.3.2.b.ii - Nitrate Utilization. The rate of substrate uptake and nitrate consumption were substituted into Equation 5.12 to calculate the observed yield.

$$\frac{dS_{NO_3}/dt}{dS/dt} = \frac{1 - Y_{obs}}{2.86} \qquad \text{Eq. (5.12)}$$

where dS_{NO3}/dt = the rate of nitrate consumption (mg $NO_3\text{-}N \cdot L^{-1} \cdot h^{-1}$)
dS/dt = the rate of biodegradable substrate disappearance (mg $COD \cdot L^{-1} \cdot h^{-1}$)
Y_{obs} = observed yield (mg COD/mg COD)

Yield data derived from CAA cultures were grouped according to temperature (Tables 5.43 and 5.44) and are summarized in Table 5.45. When the rate of growth is a maximum, the observed yield is thought to be closest to the true yield because maintenance energy demands are much less than the energy used for synthesis (Grady and Lim, 1977). For these reasons, the average observed yield for the culture utilizing PH may approximate the true yield. The observed yield is not equal to the true yield because the estimates of maintenance energy made while the bacteria were chronically starved may underestimate the requirements under active growth. No estimate of maintenance energy was made for the anoxic culture.

Table 5.43 Yield Data from Aerobic Cultures using CAA

Date	dS/dt mg COD/L h	dO_2/dt mg O/L h	Yield Coefficient mg COD/mg COD
25-Oct	33.6	11.0	0.67
2-Nov	39.5	12.9	0.67
7-Nov	74.1	45.4	0.39
27-Nov	43.9	16.5	0.62
29-Nov	96.1	18.9	0.80
6-Dec	67.0	12.3	0.82
27-Mar	43.3	14.8	0.66
4-Apr	57.6	13.0	0.77
5-May	34.8	10.4	0.70
7-May	40.9	10.5	0.74
9-May	44.0	11.7	0.73

Table 5.44 Yield Data from Anoxic Cultures using CAA

Date	dS/dt mg COD/L h	dNO_3-N/dt mg NO_x-N/L h	Yield Coefficient mg COD/mg COD
7-Dec	66.5	4.64	0.82
20-Oct	59.7	5.39	0.77
24-Oct	35.7	5.11	0.63
30-Oct	49.4	4.47	0.77
1-Dec	59.7	6.34	0.73
4-Dec	56.7	6.54	0.70
29-Mar	37.3	2.2	0.85
1-Apr	40.3	3.44	0.78
4-Apr	44.6	2.83	0.84
5-May	38.5	3.21	0.79

Summaries of literature values for true yield under anoxic and aerobic conditions are presented in Tables 5.46 and 5.47, for general comparisons of various bacterial species, substrates and growth conditions.

Table 5.45 Yield on CAA under Different Culture Conditions

Culture	Starting Date	Final Date	Average Yield mg COD/mg COD	Median Y	Std. Dev
Aerobic 20°C	Oct. 25	Dec. 6	0.67	0.67	0.16
Aerobic 14°C	Mar. 27	May 9	0.73	0.72	0.04
Anoxic 20°C	Oct. 20	Dec. 7	0.72	0.71	0.072
Anoxic 14°C	Mar. 29	May 5	0.79	0.79	0.039

Table 5.46 True Yield of Aerobic Growth of Various Organisms on Glucose in Minimal Media (after Grady and Lim (1980)

Organism	Y_g (g COD/g COD removed)
Aerobacter aerogenes	0.54
Aerobacter cloacae	0.58
Arthrobacter globiformis	0.70
Bacterium HR	0.54
Escherichia coli	0.68
Pseudomonas aeruginosa	0.53
Pseudomonas fluorscens	0.51

Note. g of cells have been converted to g cell COD by multiplying by 1.42 (Marais and Ekama, 1976)

Table 5.47 Literature Values for Yield under Anoxic Conditions

Pure Culture	Substrate	Yield (g COD/g COD)	Study
Pseudomonas denitrificans	Ethanol	0.68	Wang *et al.* (1995)
Pseudomonas denitrificans	Glutamate	1.01	Koike and Hattori (1975a)
Pseudomonas denitrificans	Glutamate	0.64	Koike and Hattori (1975b)

A true yield value of 0.67 mg COD/mg COD has been used as the default value in ASM1 and ASM2 for both aerobic and anoxic growth (IAWQ Task Group, 1995; Henze *et al.*, 1986). The default value is a true yields obtained when mixed populations were grown on domestic sewage. The growth environment, including media complexity, pH, and temperature can all affect the true yield (Grady and Lim, 1977). The average observed yield for aerobic cultures derived from the current research (Table 5.45) was compared to the default value using a Student's t test. As indicated in Table 5.48, the observed value determined under aerobic conditions was not significantly different from 0.67 mg COD/mg COD. The same held true for the values (Table 5.44) derived using the anoxic culture.

Table 5.48 Summary of Statistics Tests Comparing Observed Yields

Hypothesis	df	t	$t_{0.95}$
Aerobic yield equal to 0.67 g COD/g COD	10	0.553	2.23
Anoxic yield equal to 0.67 g COD/g COD	9	0.973	2.26
Anoxic yield equal to aerobic yield	19	1.18	2.09
Yield at 20°C equal to yield at 14°C for CAA (aerobic)	8	-0.893	2.45
Yield at 20°C equal to yield at 14°C for CAA (anoxic)	8	2.13	2.31

From these tests the following can be concluded at the 95% CL.

1. The observed yield was invariant with temperature under both aerobic and anoxic conditions.

2. The observed yield for CAA cultures under aerobic conditions was not statistically different from that realized under anoxic conditions.

5.3.4. Summary Statements

a) Kinetics and Stoichiometry

1. Addition of PH to the stock culture resulted in an immediate increase in the OUR by 205-612%. It was concluded that CAA and LEH could be rapidly metabolized. This behaviour is consistent with the response of wastewater treatment plants to shock loadings of organics. The increased OUR either remained constant or increased slightly with time.
2. Respiration rates under aerobic and anoxic conditions were expressed in terms of oxygen equivalents. The confidence limits of the respiration rates under aerobic conditions at 14 and 20°C and the anoxic culture at 20°C all overlapped. It was concluded that the rate of respiration under these conditions were not significantly different. The rate of respiration under anoxic conditions at 14°C was significantly lower than under the other conditions. These trends were seen regardless if the rate data was normalized on MLVSS or bacteria counts.
3. Nitrate utilization rates normalized on cell counts showed a greater temperature sensitivity with a θ_{DN} of 1.25 as a opposed to 1.09 for data normalized on MLVSS. The modified Arrhenius coefficient for data normalized on MLVSS was consistent with literature values.
4. Oxygen utilization rates were less temperature sensitive than nitrate utilization rates. Values of 1.01 and 1.03 were found for the modified Arrhenius coefficient for data normalized on MLVSS and bacteria counts. respectively.
5. The initial substrate to microorganisms ratio, S_0/X_0, calculated on the basis of bacterial counts was correlated with respiration rates under the following conditions: aerobic 14°C; anoxic 20°C and anoxic 14°C. When the ratio was calculated using MLVSS a correlation only existed for the anoxic 20°C culture.
6. Average observed ammonia production rates normalized on cell counts was highest under aerobic conditions.

7. Significant correlations between observed ammonia nitrogen production and either OUR or NUR existed under some conditions. As correlations did not exist under all experimental conditions, ammonia production can not be directly correlated to respiration.
8. The observed yield under conditions of active substrate removal is not significantly different from 0.67 mg COD/mg COD under either aerobic or anoxic conditions.
9. The observed yield under conditions of active substrate removal under both aerobic and anoxic conditions is invariant with temperature.

b) **Acridine Orange Direct Counts**

1. In the majority of cases, there was either an increase or no significant difference in the counts of the final culture in comparison to the initial culture. Cell growth can occur as an increase in size or by replication. Growth of the second type will not be detected by increases in AODC.
2. In three out of four culture types there was a significant correlation between S_0/X_0 expressed on the basis of bacteria counts and the rate of respiration. Revised standard deviations for repeated experiments show data normalized on AODCs to have better reproducibility.

c) **Enzyme Assays**

1. Enzyme assays for aerobic cultures showed high levels of phosphatase alkaline, phosphatase acids, phosphoamidase, esterase (C4), esterase (C8).
2. Similar enzyme titers were observed for the aerobic and anoxic cultures. Leucine arylmidase was detected for the anoxic cultures but not for the aerobic cultures. Esterase (C8) was found at lower levels by the anoxic culture.

5.4. Substrate Uptake and Hydrolysis

5.4.1. Introduction

The primary objective of this portion of the experimental program was to determine the impact of substrate, temperature and electron acceptor system (EAS) on the rate of hydrolysis. The impact of overall protein structure and molecular weight were to be evaluated. A better understanding of substrate uptake and hydrolysis would be helpful in rationalizing biosolids production.

In this section, various attributes of protein cultures are compared to cultures using protein hydrolysates. Protein hydrolysates are simple substrates not requiring hydrolysis. Cultures using α-casein or ovalbumin behaved much differently than did lactabumin utilizing cultures. For this reason the behaviour of cultures using α-casein and ovalbumin will be discussed below while a presentation on the lactalbumin cultures appears later.

5.4.2. Presentation of Results for α-casein and Ovalbumin Rate Studies

a) Aerobic Cultures

i) Biomass Increases

The rate data (e.g. OUR, NH_3-N production and soluble COD uptake) were normalized on either MLVSS or bacteria counts to facilitate comparisons between similar experiments. When MLVSS was used as a normalizing factor, only the initial (adjusted concentration of the stock culture) was used. The MLVSS of the final culture was not considered for two reasons. First, immediately after substrate addition, substrate was enmeshed into the floc. Increases in MLVSS values would reflect the accumulation of substrate as well as increases in the mass of microorganisms. Second, the calculations of Section 5.3.2.a.i - Increases in Biomass show a possible increase of 188 mg VSS/L. Given precision of the MLVSS measurement technique, increases of this magnitude could be masked by large errors in analysis.

Rate data normalized on bacteria counts used an average of the bacteria counts of the initial and final cultures. Mean bacteria counts and their 95% confidence limits are summarized in Table 5.49. In three of the five cultures, there was a significant difference between the initial and final counts. In two cases, the mean

Table 5.49 Bacteria Count Numbers for Aerobic Cultures using α-casein and Ovalbumin

Temp (°C)	Protein	Date	Initial MLVSS (mg VSS/L)	Bacteria Counts for Initial Culture (bact/L) $x10^{11}$ Mean	Lower 95% CI	Upper 95% CI	Bacteria Counts for Final Culture (bact/L) $x10^{11}$ Mean	Lower 95% CI	Upper 95% CI	Increase in Mean
20	Ovalbumin	Nov. 27	455	5.63	4.62	6.86	9.99	8.05	12.4	77%
20	α-casein	Dec. 6	465	3.85	3.20	4.65	2.56	2.22	2.96	-34%
14	Ovalbumin	Apr. 4	450				3.04	2.66	3.48	
14	Ovalbumin	May 5	250	3.27	2.95	3.63	6.62	5.73	7.66	102%
14	α-casein	May 7	420	4.06	3.47	4.75	3.57	3.34	3.81	-14%
14	α-casein	May 9	370	4.72	5.94	3.75	5.17	4.45	6.01	10%

of the final culture is higher than the initial. In the third case, the mean is significantly lower. The limitations of the enumeration technique and possible errors have been previously discussed in Section 5.33.a.i-Increases in Biomass and Bacteria Counts.

ii) Oxygen Utilization Rates

As mentioned previously, the aerobic culture was not composed exclusively of bacteria as protozoa and possibly rotifiers and amoebaes would be present. The respiration rate during active substrate utilization, OUR_C, was calculated as the measured value (OUR_M) less the basal respiration rate (OUR_B) for the stock cultures (Table 5.21 and 5.22).

Figures 5.30 - 5.33 illustrate the OUR_c of split cultures receiving a protein and a PH in parallel experiments. The PH and protein cultures were derived from the same stock cultures. The responses of the PH cultures were previously discussed and were included in the figures below for ease of comparison. The following observations are applicable to the protein utilizing cultures.

1. Addition of proteins to the cultures at either 14 or 20°C led to an immediate increase in the OUR. The OUR_C increased by 220-610% above basal levels. The intercept is the value of OUR_C immediately after substrate addition.
2. Similar behaviour was seen for the cultures receiving protein of Nov. 27, Dec. 6, May 7 and May 9. The OUR_C was relatively constant for 2-3 h and then increased substantially for a very short time. It leveled off at this time. This type of behaviour was seen with both proteins and at the two temperature levels. Experiments repeated under the same environmental conditions and using the same protein did not always produce this response. This suggests that the characteristics of the stock culture influence the response. In parallel cultures receiving CAA on these same dates, the OURs increased linearly. The delay in metabolizing the whole protein may be caused by a slower rate of protein uptake and hydrolysis. For a more complete discussion see Section 5.4.3.a
3. The Nov. 7 and May 5 ovalbumin cultures had OURs that increased linearly with time. The May 5 culture, a 14°C culture, had an OUR_C that increased by 150% from its initial OUR_C over the first 4.5 h of the rate study. After this time, the OUR_C decreased so at the end of the rate study the OUR_C was only 25% greater

than the initial value. None of the other cultures experienced such a high demand or such a pattern of OUR utilization. For this reason, the May 5th experiment should be considered an anomalous experiment.

4. The Apr. 4 culture, a culture using ovalbumin, had an OUR_C that remained relatively constant with time.

5. The OUR_C of the protein culture at 20 °C bore no correlation to the PH utilizing culture derived from the same stock solution. In three of the four rate studies at 14°C, the OUR_C of the protein culture was substantially greater than that of the PH culture of the pair. In the fourth instance, the protein and PH utilizing cultures had similar oxygen demands.

Figure 5.30 OUR_C for α-casein and CAA at 20°C Normalized on MLVSS

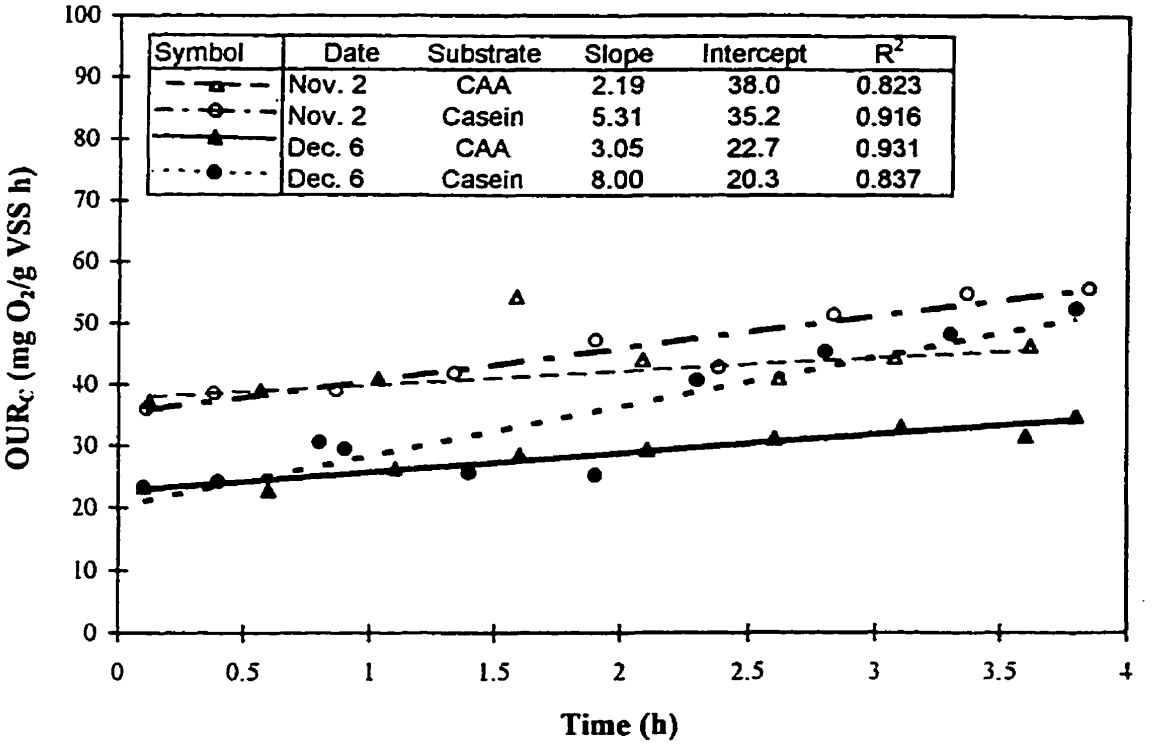


Figure 5.31 OUR_C for Ovalbumin and CAA at 20°C Normalized on MLVSS

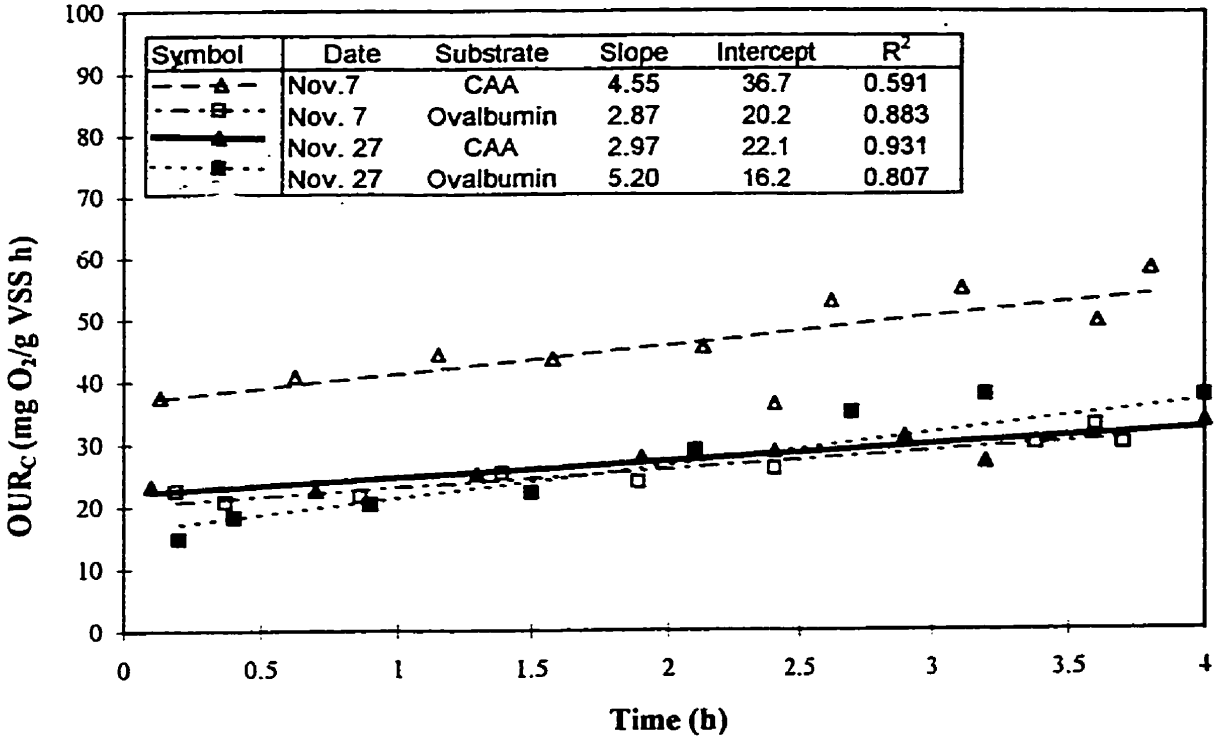


Figure 5.32 OUR_C for α-casein and CAA at 14°C Normalized on MLVSS

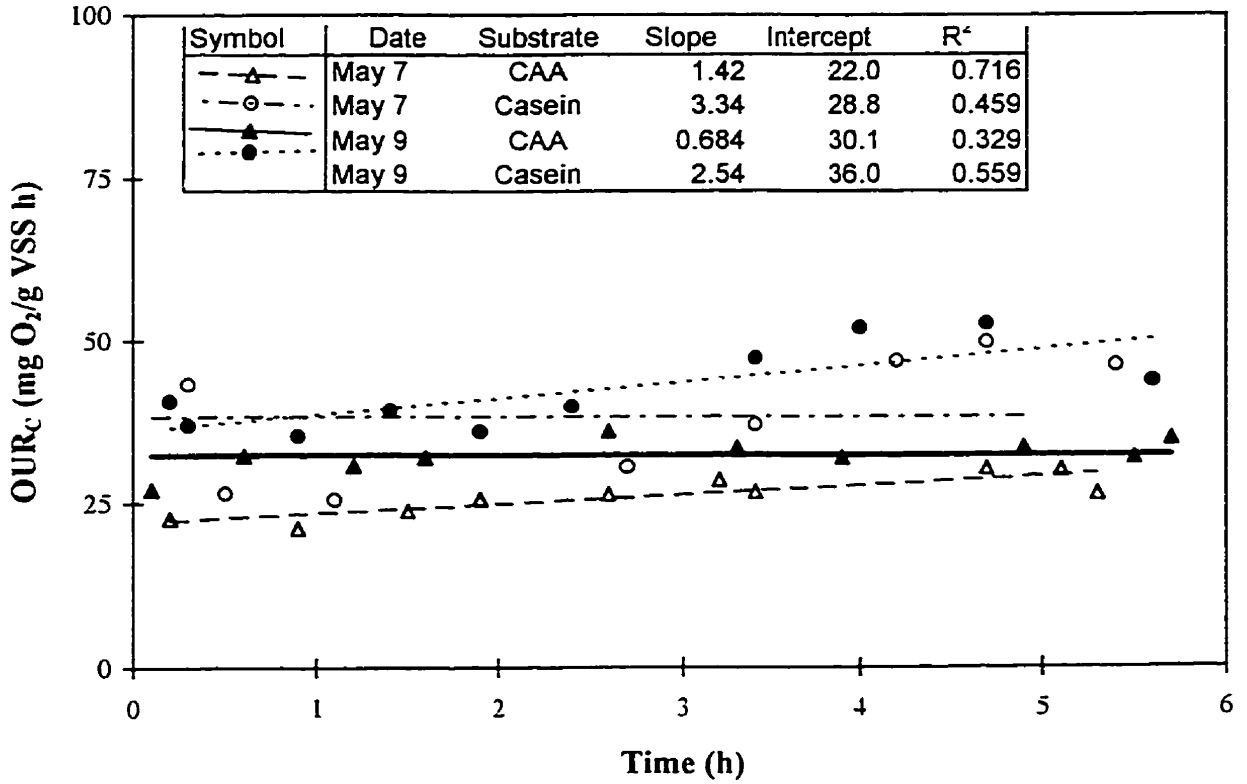
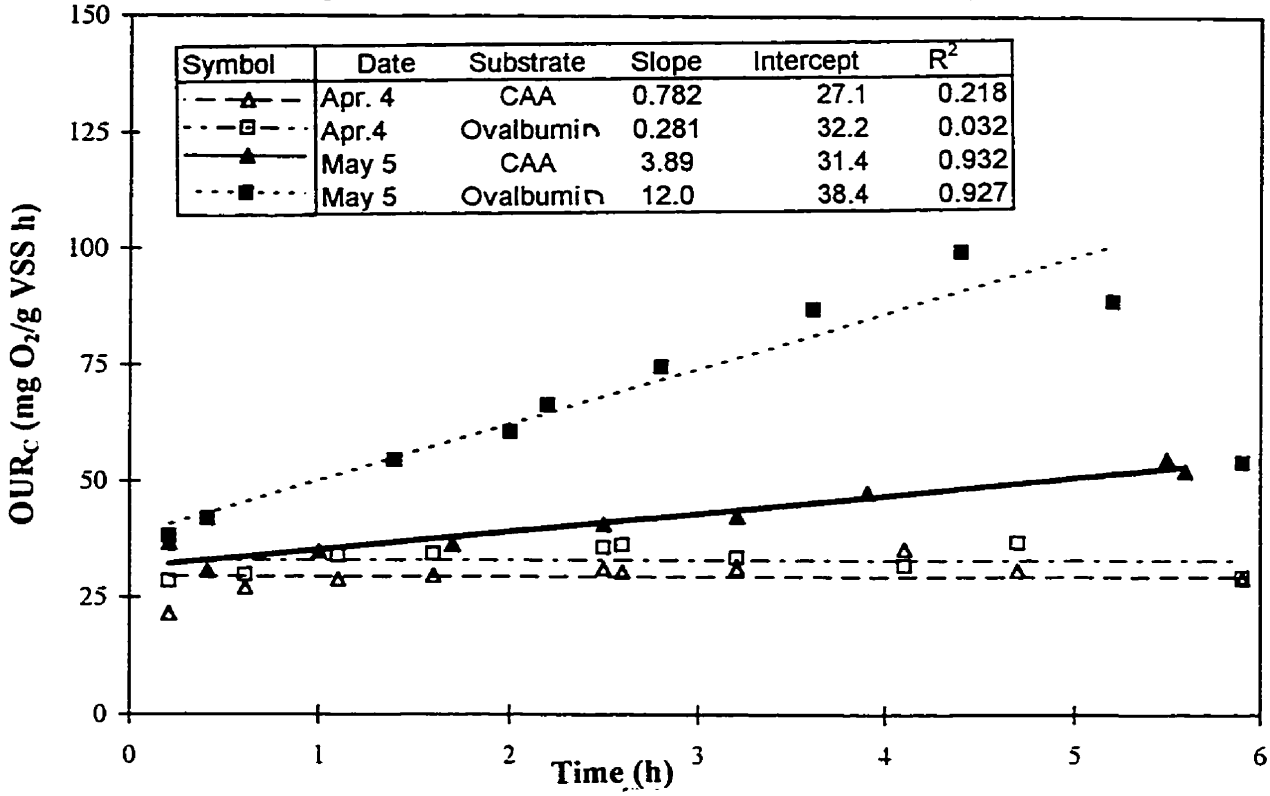


Figure 5.33 OUR_C for Ovalbumin and CAA at 14°C Normalized on MLVSS



OUR_C varied with time in several different ways. The marked increase in OUR_C after protein addition was seen with PH cultures. This behaviour has been exhibited by both pure cultures and natural populations after a nutrient upshift and is called available reduction potential (see Section 5.3.3.a.). Consistent with the treatment of OUR data for PH cultures, a mean OUR_C was calculated for protein cultures (Tables 5.50 and 5.51).

For repeated ovalbumin rate studies at 20°C, OUR_C normalized on MLVSS rate differed by less than 5%. There was a 20% difference in OUR_C for repeated rate experiments using α-casein under similar conditions. At 14°C, the ovalbumin cultures show more than a 50% difference in their normalized OUR_C. This was in contrast to the excellent reproducibility realized for α-casein cultures at 14°C.

Table 5.50 Average OUR_C for Protein Cultures at 20°C-Normalized on MLVSS and Bacteria Counts

Protein	Date	OUR_c mg O_2/L-h	Initial MLVSS (mg VSS/L)	Bacteria (bact/L) x10^{11}	OUR_C (mg O_2/gVSS-h)	OUR_C x 10^{-12} (mg O_2/bact-h)
Ovalbumin	Nov. 7	11.9	460		25.8	
Ovalbumin	Nov. 27	12.3	455	7.81	27.0	15.8
α-casein	Nov. 2	14.9	330		45.2	
α-casein	Dec. 6	16.9	465	3.20	36.3	52.8

Table 5.51 Average OUR_C for Protein Cultures at 14°C-Normalized on MLVSS and Bacteria Counts

Protein	Date	OUR_c mg O_2/L·h	Initial MLVSS (mg VSS/L)	Bacteria (bact/L) x10^{11}	OUR_C (mg O_2/gVSS·h)	OUR_C x 10^{-12} (mg O_2/bact·h)
Ovalbumin	Apr. 4	14.8	450	3.04	32.9	48.6
Ovalbumin	May 5	16.6	250	4.94	66.4	33.6
α-casein	May 7	16.0	420	3.81	38.1	42.0
a-casein	May 9	15.6	370	4.94	42.1	31.5

iii) Enzyme Assays

Enzyme assays for two protein utilizing cultures at 20°C were done on intact mixed liquor 75 minutes after substrate addition and are outlined in Table 5.52. The enzymes predominant in CAA cultures were also present at high levels in protein cultures. The stock culture was grown on the crude proteins containing substantial amounts of ovalbumin and α-casein and thus large changes in the enzyme complement were not expected.

Enzyme levels were based on the colour produced on a test strip. In some instances, a difference of only one colour unit was noted between ovalbumin and α-casein cultures; this was not considered to be significant. There is a substantial difference in the phosphamidase, n-acetyl-beta-glucosaminidase and α-fucosidase levels for the two cultures. These same differences were noted for the stock cultures used in these rate experiments.

Table 5.52 Enzyme Assays for Aerobic Protein Utilizing Cultures at 20°C

Enzyme	Ovalbumin Nov. 27	α-casein Nov. 2	Enzyme	Ovalbumin Nov. 27	α-casein Nov. 2
Phosphatase alkaline	4[1]	5	Phosphoamidase	5	3
Esterase (C4)	4	4	α-galactasidase	1	0
Esterase (C8)	4	4	β-galactasidase	1	2
Lipase (C14)	1	1	β-glucuronidase	0	0
Leucine arlymidase	2	2	α-glucosidase	1	0
Valine arlymidase	2	2	β-glucosidase	1	1
Cystine arlymidase	1	0	N-acetyl-beta-glucosaminidase	3	1
Trypsin	2	0	α-mannoisdase	1	0
Chymotrypsin	2	0	α-fucosidase	4	0
Phosphatase acids	5	5			

[1]Moles of test-strip substrate hydrolysed
1 - 5 nanomoles, 2 - 10 nanomoles, 3 - 20 nanomoles, 4 - 30 nanomoles, 5 - ≥ 40 nanomoles

iv) Observed Ammonia Nitrogen Production

Figure 5.34 illustrates observed ammonia nitrogen production for four different cultures using ovalbumin. The observed production rate is the rate of ammonia nitrogen production less requirements for bacteria synthesis. Nitrification was not occurring concurrently because a nitrification inhibitor was added to the stock culture on a daily basis. The observed ammonia nitrogen production was linear with respect to time for all cultures regardless of the protein source or temperature. This is indicated by very high correlation coefficients. This same type of behaviour was seen with the PH cultures.

Ammonia nitrogen rates normalized on MLVSS and bacteria counts are presented in Tables 5.53 and 5.54. The mean observed ammonia nitrogen production rate normalized on MLVSS is lower at 20°C than it is at 14°C. These average values are not significantly different than the comparable values for aerobic cultures using PH (Tables 5.26 and 5.27) as they fall with one standard deviation of each other. A more detailed statistical analysis of the results is presented in Section 5.4.3.c.

Figure 5.34 Observed Ammonia Nitrogen Production for Aerobic Cultures using Ovalbumin

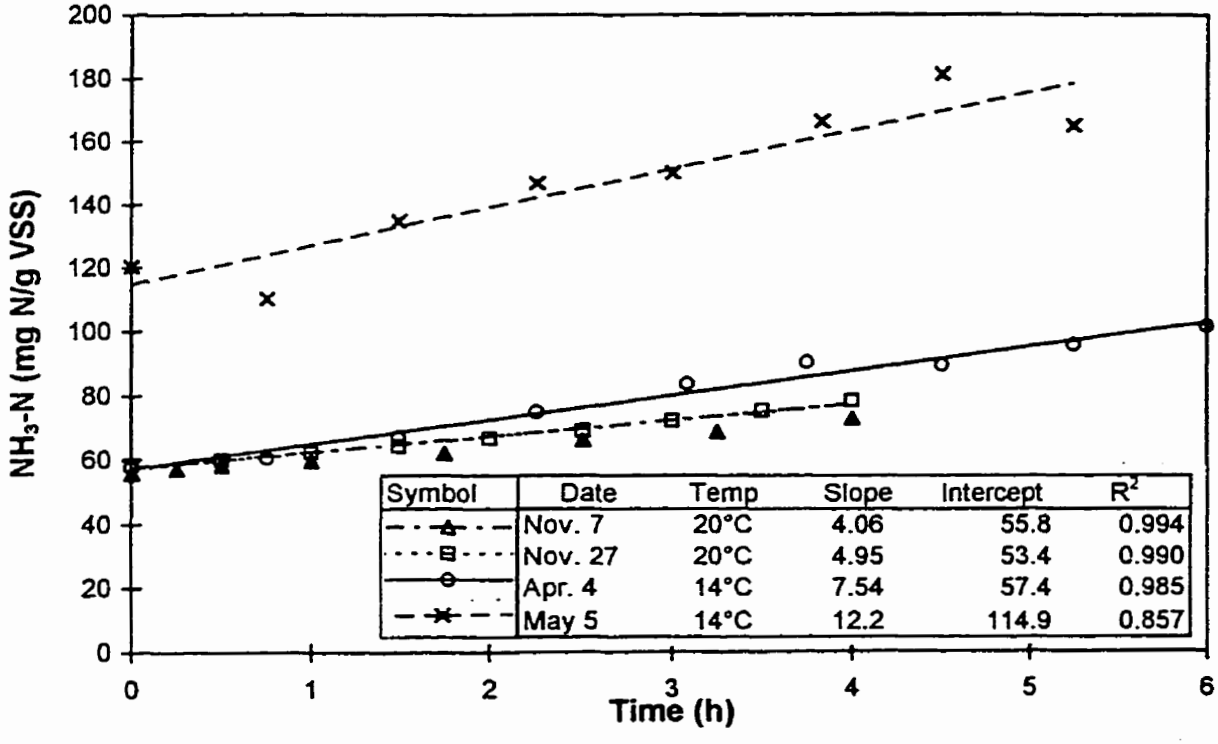


Table 5.53 Observed Ammonia Nitrogen Production Rates for Aerobic Cultures using Proteins at 20°C

Protein	Date	Production Rate mg NH_3-N/L·h	Production Rate mg NH_3-N/(gVSS·h)	Production Rate (mg NH_3-N/bact·h) x 10^{-12}
Ovalbumin	Nov. 7	1.87	4.06	
Ovalbumin	Nov. 27	2.25	4.94	2.88
α-casein	Nov. 2	2.36	7.15	
α-casein	Dec. 6	2.76	5.93	8.62

Table 5.54 Observed Ammonia Nitrogen Production for an Aerobic Culture using Proteins at 14°C

Protein	Date	Production Rate mg NH_3-N/(L·h)	Production Rate mg NH_3-N/(gVSS·h)	Production Rate (mg NH_3-N/bact·h) x 10^{-12}
Ovalbumin	Apr. 4	3.39	7.54	11.2
Ovalbumin	May 5	3.03	12.1	6.13
α-casein	May 7	3.64	8.67	9.56
α-casein	May 9	3.59	9.70	7.26

v) Soluble COD

Within the context of ASM1 and ASM2, α-casein and ovalbumin are examples of slowly biodegradable substrates. After filtering these proteins through a 0.45 μm filter there was no measurable difference between the organic strength of the original protein solution and its filtrate. The size of the protein colloids may influence the mechanism of substrate uptake.

When substrate was added to the MLVSS, there was an almost immediate uptake of substrate into or onto the floc. Figure 5.35 illustrates that the uptake of ovalbumin occurred at a constant rate after the initial uptake. Similar behaviour was seen was for α-casein cultures. The Nov. 7 culture was a notable exception because COD did not disappear from the bulk liquid. COD disappearance for the Nov. 7 culture is best described by a mean value because a linear regression is inappropriate for this culture. A constant rate of soluble substrate uptake was also observed for aerobic cultures using PH (see Section 5.3.2.a.v.). For a more detailed discussion regarding the mechanism of COD uptake see Section 5.3.3.h.

Figure 5.35 Soluble COD Uptake for Aerobic Cultures using Ovalbumin

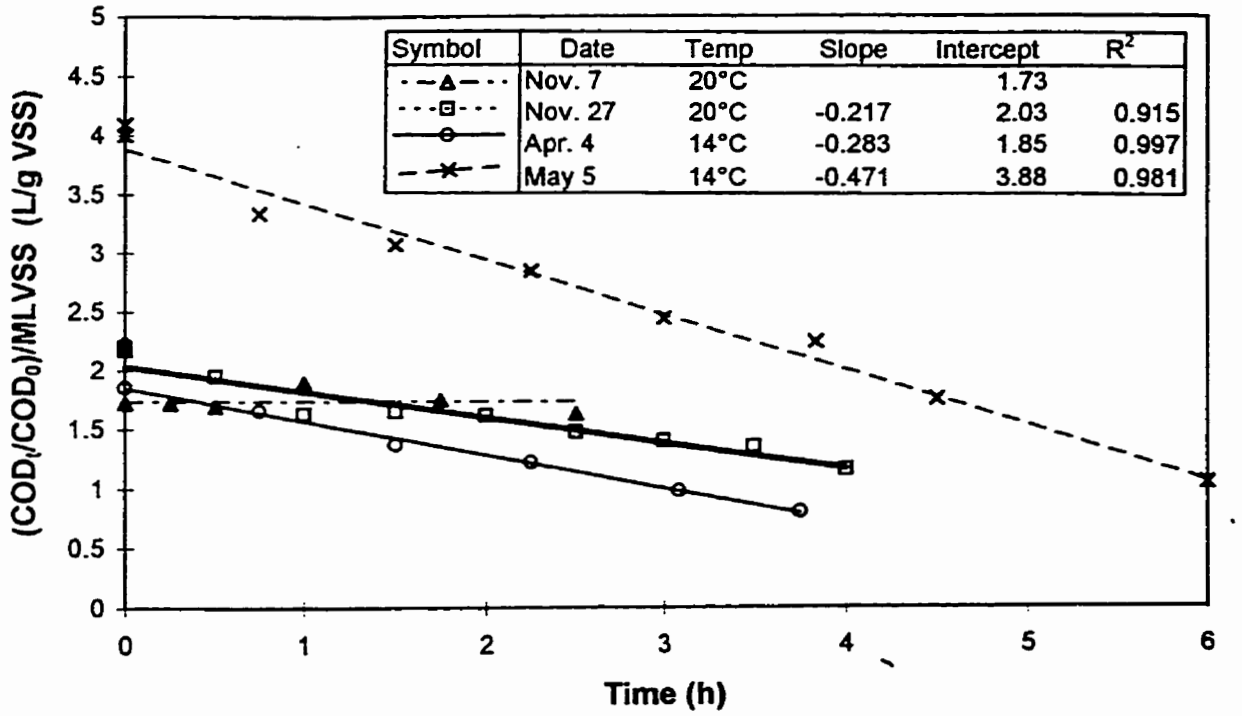


Table 5.55 quantifies the amount of substrate COD that was taken up by the floc. The higher initial COD was calculated knowing the organic strength and quantity of the protein added and having an estimate of the soluble COD present in the stock culture. A second initial value was obtained from a linear regression of the COD concentrations over time. COD uptake was normalized on the initial MLVSS and bacteria counts for the initial culture. Since uptake occurred at the very beginning of the rate experiment, it was appropriate to use only the bacteria counts of the initial culture. As with the aerobic PH cultures, the COD uptake normalized on MLVSS and bacteria counts appeared to be independent of the initial MLVSS or bacteria. The average COD uptake normalized on MLVSS was 0.181 mg COD/mg VSS with a RSD of 47.8%.

vi) Initial Substrate to Microorganism Ratio

As mentioned previously, the S_0/X_0 is important because it influences both parameter identifiability and the expression of culture history. The S_0/X_0 will be used later to determine if there was any correlation between this ratio and the normalized OURs. S_0/X_0 ratios were derived from the initial substrate concentration,

Table 5.55 COD Uptake and Storage for Aerobic Cultures using α-casein and Ovalbumin

Temp. (°C)	Date	Protein	Initial COD (mg COD/L)	Expected COD (mg COD/L)	Uptake in COD (mg COD/L)	MLVSS $\frac{\text{mg VSS}}{\text{L}}$	COD Uptake (mg COD/mg VSS)	Bacteria bact/L x 10^{11}	COD Uptake COD/bactx 10^{-11}
20	Nov. 27	Ovalbumin	539.5	498.5	41	455	0.090	5.63	7.28
20	Nov. 2	α-casein	519.4	424.6	94.8	330	0.287		
20	Dec. 6	α-casein	500.7	432.0	68.7	465	0.148	3.85	17.8
14	Apr. 4	Ovalbumin	549.5	452.8	96.7	450	0.214	**3.04**	31.8
14	May 5	Ovalbumin	540.0	523.9	16.1	250	0.064	3.27	4.92
14	May 7	α-casein	551.5	471.1	80.4	420	0.191	4.06	19.8
14	May 9	α-casein	549.3	448.8	100.5	370	0.272	4.72	21.3

Bacteria counts in bold are based on numbers from final culture

vii) Variation in pH

The PH cultures were maintained at a pH of 7.2 by acid addition while equal amounts of acid were added to the protein cultures. The pH of the protein cultures did not remain at pH 7.2. In Tables 5.56-5.57 the maximum, minimum and average pH experienced by the protein cultures from the time gas sparging began to the end of the rate study are listed. pH measurements were recorded every minute. Figure 5.36 illustrates the fluctuation in pH over time. Similar data exists for other cultures in Appendix B. In general, pH fluctuations measured for the 20°C cultures were less than those of the 14°C cultures. The pH of the aerobic cultures at 20°C ranged between 6.9-7.5. pHs of the 14°C cultures were more variable with pHs varying between 6.6 and 7.8.

Cultures may have experienced these pH extremes for a very short time. A low pH was a temporary situation since alkalinity and ammonia production and gas sparging tend to increase the pH. The pH was measured in the bulk liquid while the majority of the bacteria are associated with the extracellular polysaccharide matrix of the activated sludge floc. Short term fluctuations in pH may not be seen by the bacteria within the floc. The pH fluctuations at 20°C are 0.2 - 0.3 units away from the setpoint of 7.2. It is expected that small fluctuations will have minimal impact on the culture. At 14°C, fluctuations away from the setpoint are as large as 0.6 pH units. The impact of pH on kinetics will be discussed in Section 5.4.3.d.

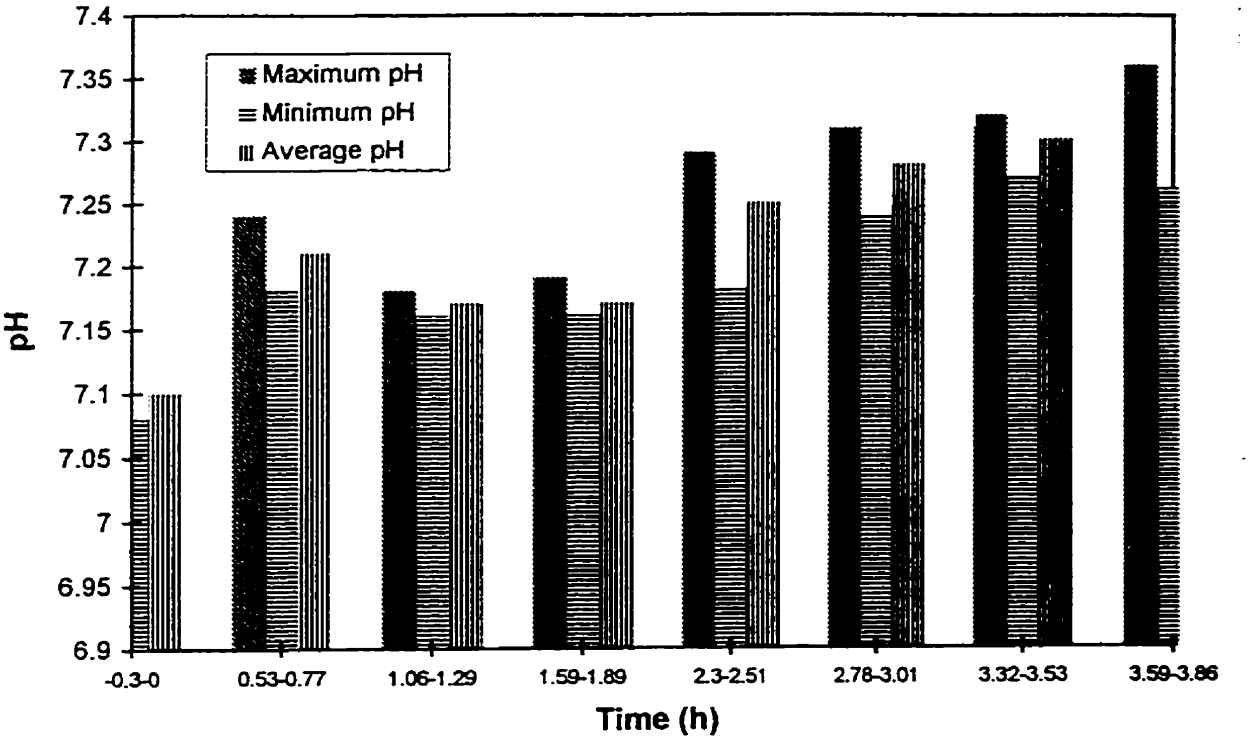
Table 5.56 Maximum and Minimum pH of Aerobic Protein Cultures at 20°C

Date	Protein	Maximum pH	Minimum pH	Average pH
Nov. 27	Ovalbumin	7.4	7.1	7.2
Nov. 7	Ovalbumin	7.5	7.0	7.2
Nov. 2	α-casein	7.4	7.0	7.3
Dec. 6	α-casein	7.2	6.9	7.1

Table 5.57 Maximum and Minimum pH of Aerobic Protein Cultures at 14°C

Date	Protein	Maximum pH	Minimum pH	Average pH
Apr. 4	Ovalbumin	7.3	7.1	7.0
May 5	Ovalbumin	7.8	7.0	7.3
May 7	α-casein	7.7	7.0	7.3
May 9	α-casein	7.4	6.7	7.2

Figure 5.36 pH profile of Nov. 27 Aerobic Culture



b) Anoxic Cultures

i) Increases in Biomass

Consistent with previous data treatment, rate data (e.g. NUR, ammonia nitrogen production and soluble COD uptake) was normalized on either MLVSS or bacteria counts. When MLVSS was used as a normalizing factor, only the initial concentration was used. The reasons discussed in Section 5.3.2.a.i are applicable in this situation as well.

Rate data normalized on bacteria counts used an averaged value based on the counts of the initial and final culture. Both initial and final bacterial counts are available for five rate studies (Table 5.58). For four of the five rate studies, there was no significant difference in the number of cells. In these instances, cell growth may have resulted in larger bacteria rather than more bacteria. For the fifth rate study, there is a significant increase in the number of cells. The mean of the final culture was 96% greater than the initial culture.

Table 5.58 Bacteria Counts for Anoxic Cultures using Proteins

Temp (°C)	Protein	Date	Initial MLVSS (mg VSS/L)	Bacteria Counts for Initial Culture (bact/L) x10^{11}			Bacteria Counts for Final Culture (bact/L) x10^{11}			Increase in Mean
				Mean	Lower 95% CI	Lower 95% CI	Mean	Lower 95% CI	Upper 95% CI	
20	Ovalbumin	Oct. 20	650							
20	Ovalbumin	Dec. 4	470	12	10.2	14.2	10.8	6.9	16.8	-10%
20	α-casein	Oct. 30	550							
20	α-casein	Dec. 1	470	10.6		12.2	8.53	6.94	10.5	-20%
14	Ovalbumin	Apr. 4	570				26.8	24.0	29.6	
14	Ovalbumin	May 5	640	14.9	13.4	16.5	15.8	14.7	17.10	-6%
14	α-casein	Mar. 29	465	16.0	14.1	18.2				
14	α-casein	Apr. 24	590	13.4	12.0	15.0	13.0	13.0	13.8	-3%
14	α-casein	May 9	585	9.28	7.36	11.7	18.2	16.4	20.4	96%

In contrast, five of the seven anoxic cultures using PH cultures showed significant increases in the bacteria counts of (See Table 5.30). Increases in the counts of the final cultures were 9-94% higher than the initial culture. Increases of 38% or higher were considered significant.

ii) NO_X Consumption

In these experiments, the nitrate and nitrate nitrogen was measured and is reported as the NO_X concentration. Typically, the nitrite concentration was less than 2 mg N/L suggesting that the nitrate and nitrite reduction was occurring at comparable rates. The NO_x decay was linear over the 4-6 hours of a rate experiment and thus the rate of denitrification is constant (Fig. 5.37). Similar behaviour was seen with cultures using either α-casein and PHs.

The denitrification rates cited are raw values that have not been corrected for the endogenous respiration rate. The endogenous rate is difficult to determine and the error incurred in neglecting it is small (see Section 5.3.2.a.ii for more details). Data normalized on MLVSS and bacteria counts are summarized in Tables 5.59 and 5.60 with raw data and regression equations located in Appendix B. The MLVSS concentration varied from 465 to 650 mg VSS/L.

Table 5.59 NO_X Utilization for Protein Cultures at 20°C-Normalized on MLVSS and Bacteria Counts

Substrate	Date	NUR $\frac{\text{mg NOx-N}}{\text{L h}}$	Initial MLVSS $\frac{\text{mg VSS}}{\text{L}}$	Bacteria Counts $\frac{\text{mg cell}}{\text{L}} \times 10^{11}$	NUR $\frac{\text{mg NOx-N}}{\text{g VSS h}}$	NUR x 10^{-12} $\frac{\text{mg NOx-N}}{\text{cell h}}$
Ovalbumin	Oct. 20	4.51	650		6.94	
Ovalbumin	Dec. 4	3.26	470	11.4	6.94	2.86
α-casein	Oct. 30	4.71	550		8.56	
α-casein	Dec. 1	6.79	470	9.57	14.5	7.10

Figure 5.37 NO_X Utilization for Anoxic Cultures using Ovalbumin at 20 and 14°C

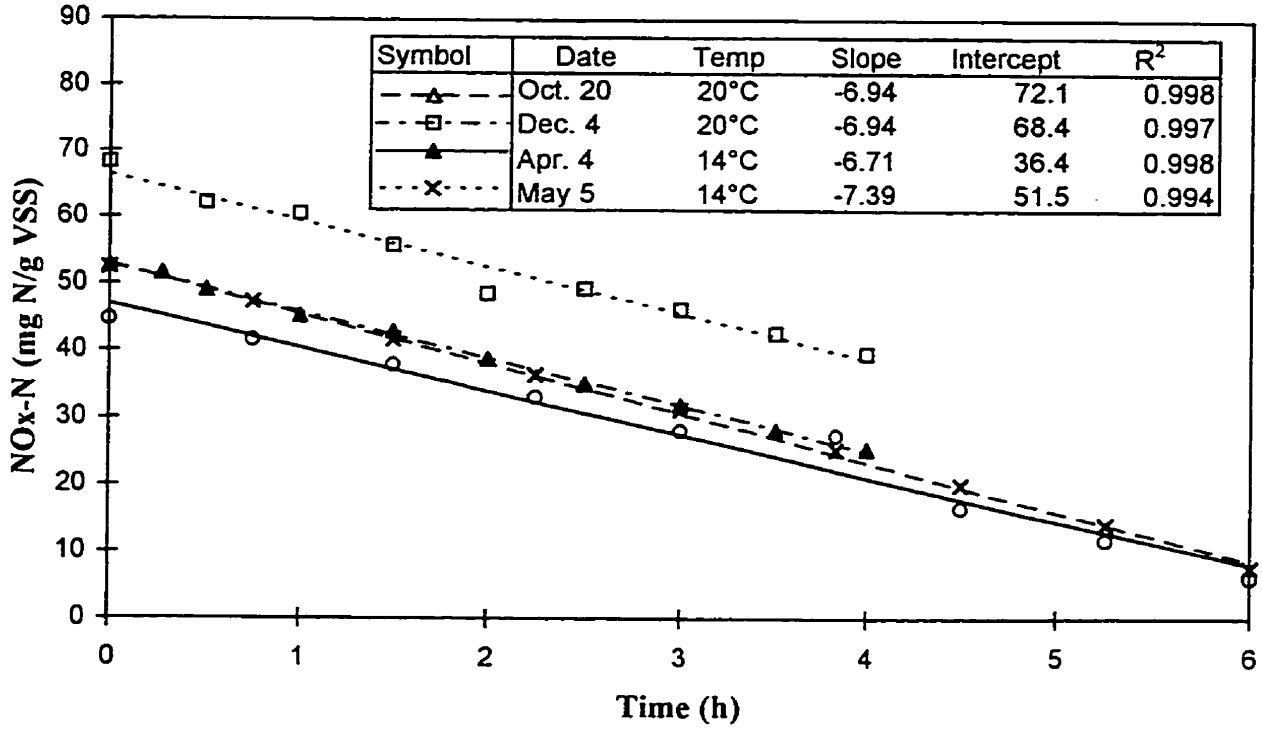


Table 5.60 NO_X Utilization for Protein Cultures at 14°C-Normalized on MLVSS and Bacteria Counts

Substrate	Date	NUR $\frac{\text{mg NOx-N}}{\text{L h}}$	Initial MLVSS $\frac{\text{mg VSS}}{\text{L}}$	Bacteria Counts $\frac{\text{mg cell}}{\text{L}} \times 10^{11}$	NUR $\frac{\text{mg NOx-N}}{\text{g VSS h}}$	NUR x 10^{-12} $\frac{\text{mg NOx-N}}{\text{cell h}}$
Ovalbumin	Apr. 4	3.69	570	**26.8**	6.47	1.38
Ovalbumin	May 5	4.73	640	15.4	7.39	3.07
α-casein	Mar. 29	1.82	465	**16.0**	3.91	1.14
α-casein	Apr. 24	5.20	590	13.2	8.81	3.94
α-casein	May 9	3.88	585	13.7	6.63	2.83

Note: **Bolded** number was a final bacteria count

Very good reproducibility in NUR normalized on MLVSS was seen for ovalbumin cultures. This was not the case for casein where the rates differed by as much as 50%. With the aerobic cultures, the OUR was either constant or increased linearly over time; however, for all of the anoxic cultures the NUR was constant with time. This was shown by high linearity in the NO_x decay curves. At 20°C, the highest rate of OUR increase was 4.20 mg O_2/(gVSS h^2) which corresponds to a change in the denitrification rate of

1.57 mg NO_X-N/(gVSS h^2). A change of this magnitude could have been detected in the rate of NO_X-N utilization but was not.

iii) Enzyme Assays

The results of enzyme assays for two protein-utilizing cultures at 20°C are outlined in Table 5.61. Assays were initiated 75 minutes after substrate addition. Enzyme levels are comparable to CAA cultures with the exception of esterase (C4). It was present at lower levels than in the CAA cultures. Both cultures were derived from the same stock culture and therefore large changes in the enzyme profile was not expected.

Chymotrypsin is a protease that preferentially attacks phenylalanyl, tyrosyl, and tryptophanyl peptide and ester bonds. Higher activities of chymotrypsin in the ovalbumin culture than in the casein culture would suggest more active protein hydrolysis, but as shown in the following sections this was not borne out by either OURs or ammonia nitrogen production rates.

Table 5.61 Enzyme Assays for Anoxic Protein Utilizing Cultures at 20°C

Enzyme	α-casein Oct. 30	Ovalbumin Oct. 20	Enzyme	α-casein Oct. 30	Ovalbumin Oct. 20
Phosphatase alkaline	5[1]	5	Phosphatase acids	4	5
Esterase (C4)	3	3	Phosphoamidase	1	1
Esterase (C8)	4	3	α-galactasidase	0	0
Lipase (C14)	0	0	β-galactasidase	1	0
Leucine arlymidase	5	5	β-glucuronidase	0	0
Valine arlymidase	1	2	α-glucosidase	1	1
Cystine arlymidase	0	1	β-glucosidase	0	0
Trypsin	1	2	N-acetyl-beta-glucosaminidase	3	2
Chymotrypsin	1	3	α-mannoisdase	0	0
			α-fucosidase	2	1

[1]Moles of test-strip substrate hydrolysed
1 - 5 nanomoles, 2 - 10 nanomoles, 3 - 20 nanomoles, 4 - 30 nanomoles, 5 - ≥ 40 nanomoles

iv) Observed Ammonia Nitrogen Production

The concentration of ammonia nitrogen increased linearly with time for anoxic cultures at 14 and 20°C using either α-casein or ovalbumin (Fig. 5.38). This same behaviour was seen with aerobic cultures using either proteins or PH and with anoxic cultures utilizing PH. The slope of the graph of ammonia nitrogen concentration versus time was the observed ammonia nitrogen production rate which takes into account both generation of ammonia nitrogen and its use in cell synthesis. The observed production rate normalized on either

MLVSS or bacteria counts is presented in Tables 5.62 and 5.63. The data for the Apr. 24 is not presented in the tables because these samples were preserved with formaldehyde which affected the ammonia analysis.

Production rates normalized on the MLVSS or bacteria counts shows a great deal of scatter for repeated rate experiments. The average rate of production normalized on MLVSS was 158% higher at 20°C for ovalbumin than it was at 14°C. For α-casein, the average production normalized on MLVSS was 233% higher at 20°C, than it was at the lower temperature.

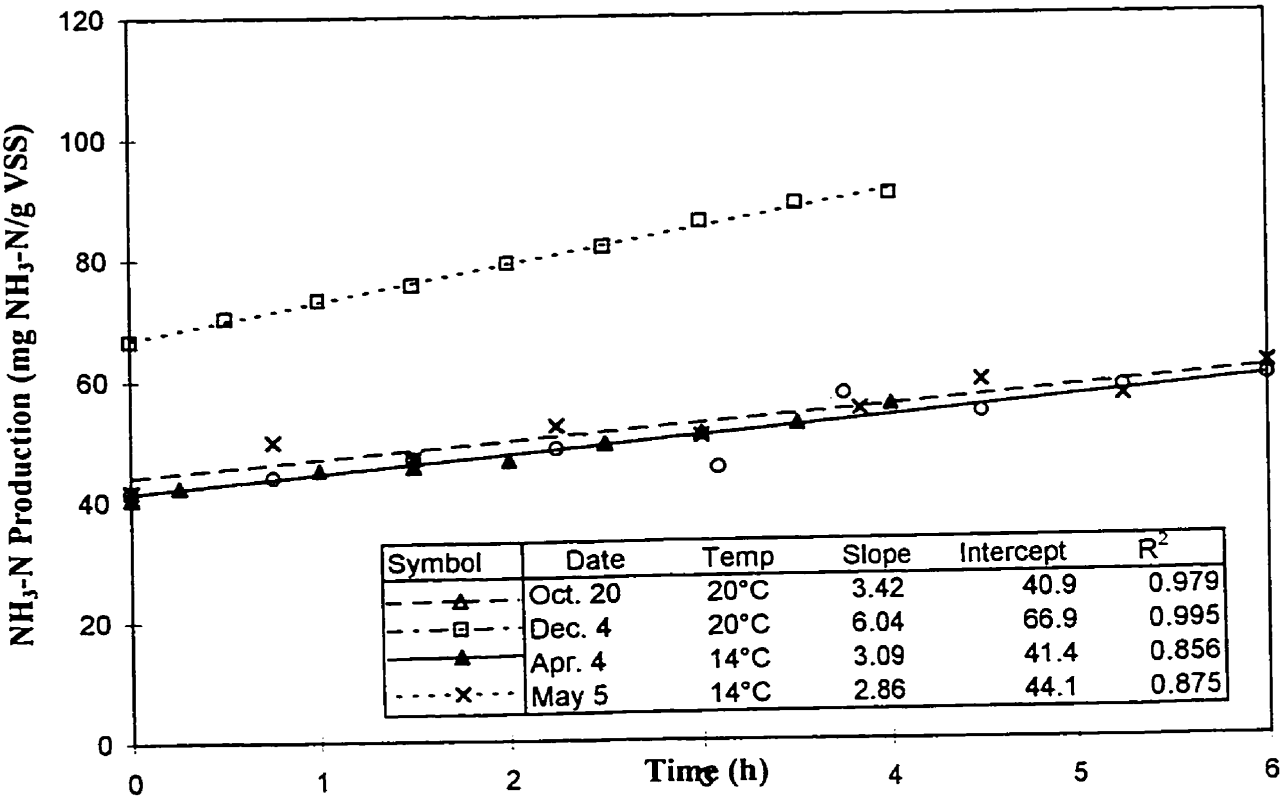


Table 5.62 Observed NH_3-N Production for an Anoxic Culture at 20°C using Proteins

Substrate	Date	Production Rate $\frac{mg\ NH_3\text{-}N}{L\ h}$	Production Rate $\frac{mg\ NH_3\text{-}N}{g\ VSS\ h}$	Production Rate $\frac{mg\ NH_3\text{-}N}{cell\ h} \times 10^{-13}$
Ovalbumin	Oct. 20	2.22	3.42	
Ovalbumin	Dec. 4	2.84	6.04	24.9
α-casein	Oct. 30	3.84	6.98	
α-casein	Dec. 1	2.21	4.70	23.1

Table 5.63 Observed NH_3-N Production for an Anoxic Culture at 14°C using Proteins

Substrate	Date	Production Rate $\frac{mg\ NH_3\text{-}N}{L\ h}$	Production Rate $\frac{mg\ NH_3\text{-}N}{g\ VSS\ h}$	Production Rate $\frac{mg\ NH_3\text{-}N}{cell\ h} \times 10^{-13}$
Ovalbumin	Apr. 4	1.76	3.09	6.57
Ovalbumin	May 5	1.83	2.86	11.9
α-casein	Mar 29	0.721	1.55	4.51
α-casein	May 9	2.02	3.45	14.7

v) Soluble COD

Figure 5.39 illustrates the uptake of soluble COD from solution over time when ovalbumin was used. There are two values for the initial time. Shortly after the protein was added to the solution, a portion was taken up into the floc. The higher initial COD was calculated knowing the organic strength and quantity of the protein added and having an estimate of the soluble COD present in the stock culture. A graph of soluble COD concentration over time has a slope equal to the rate of soluble substrate uptake. A constant rate of soluble substrate uptake was noted for aerobic cultures using either α-casein or a PH. The samples for May 9 were lost during freezing so uptake data for this date are not available. For a more detailed discussion of soluble COD uptake see Section 5.3.3.h.

Table 5.65 reports the amount of soluble COD taken up into the floc. The amount taken up ranges between 35.2 and 85.9 mg COD/L with the value for Apr. 24 excluded. The value for Apr. 24 of 235.1 mg COD/L was much higher than values for other cultures. By visual inspection there does not appear to any relationship between COD uptake and temperature, initial MLVSS or protein type. The average value derived from data of both 14 and 20°C cultures was 0.157 mg COD/L higher than the value of 0.122 mg COD/mg VSS derived for anoxic cultures using CAA.

vi) Initial Substrate to Microorganism Ratio

Table 5.64 lists S_0/X_0 ratios for anoxic cultures at 20 and 14°C. These ratios have been calculated for anoxic cultures in the same way the were for aerobic cultures. The S_0/X_0 ratios were lower for the anoxic cultures than they were for the aerobic cultures due to higher initial biomass concentrations. Values range

between 0.76 and 1.06 mg COD/mg VSS. Later analysis will use the S_0/X_0 ratio to determine if a correlation exists between it and the normalized NURs.

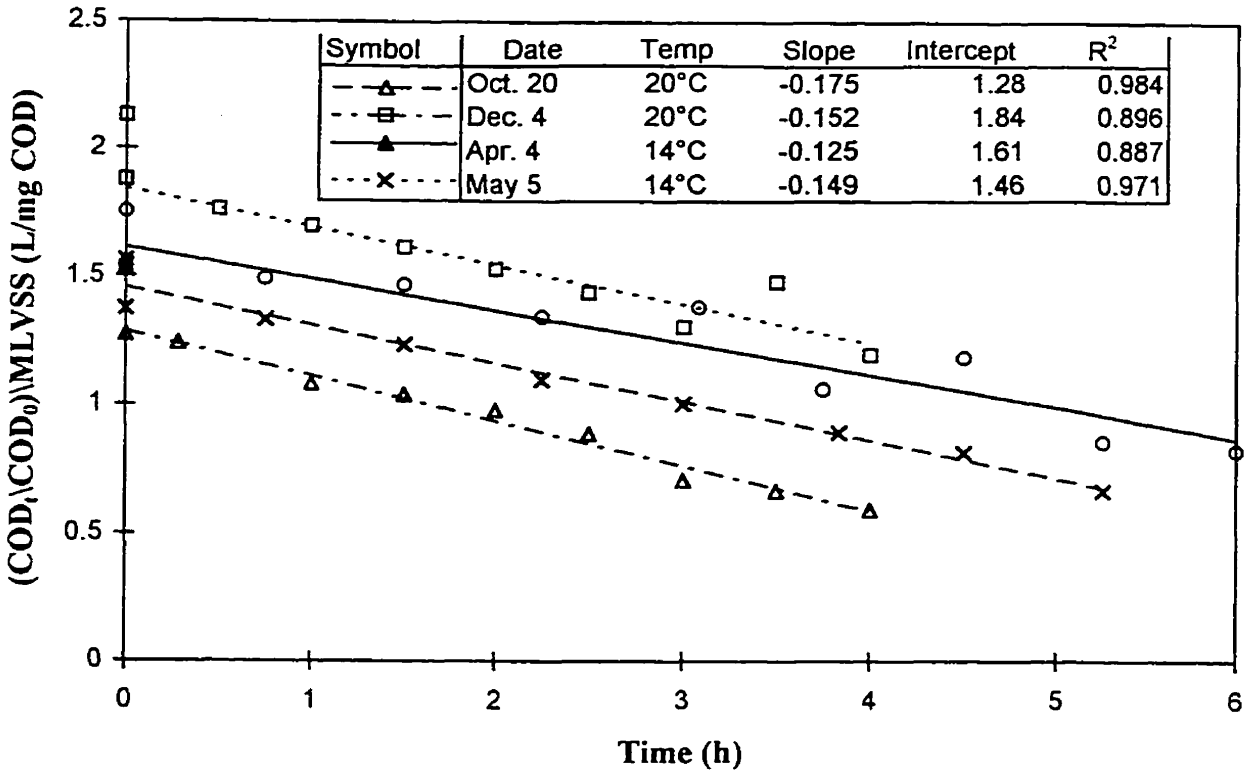


Table 5.64 Approximate S_0/X_0 Ratios for Anoxic Cultures using α-casein and Ovalbumin

Temp. (°C)	Date	Protein	MLVSS $\frac{mg\ VSS}{L}$	Bacteria Count $\frac{cell}{L} \times 10^{11}$	Initial COD $\frac{mg\ COD}{L}$	S_0/X_0 $\frac{mg\ COD}{mg\ VSS}$	S_0/X_0 $\frac{mg\ COD}{Cell} \times 10^{-10}$
20	Oct. 20	Ovalbumin	650		494.2	0.760	
20	Dec. 4	Ovalbumin	470	12	500.3	1.06	4.17
20	Oct. 30	α-casein	550		493.2	0.897	
20	Dec. 1	α-casein	470	10.6	488.0	1.04	4.60
14	Apr. 4	Ovalbumin	570	**26.8**	493.2	0.865	1.84
14	May 5	Ovalbumin	640	14.9	493.0	0.770	3.31
14	Mar. 29	α-casein	465	16.0	475.0	1.02	2.97
14	Apr. 24	α-casein	590	13.4	494.0	0.837	3.69
14	May 9	α-casein	585	9.28	493.0	0.843	5.31

Bacteria counts in bold are based on numbers from final culture.

Table 5.65 COD Uptake and Storage for Anoxic Cultures using α-casein and Ovalbumin

Temperature (°C)	Date	Protein	MLVSS $\frac{mg\ VSS}{L}$	Bacteria Count $\frac{cell}{L} \times 10^{11}$	Initial COD $\frac{mg\ COD}{L}$	Expected COD $\frac{mg\ COD}{L}$	COD Uptake $\frac{mg\ COD}{L}$	COD Uptake $\frac{mg\ COD}{mg\ VSS}$	COD Uptake $\frac{mg\ COD}{Cell} \times 10^{-11}$
20	Oct. 20	Ovalbumin	650		521.4	435.5	85.9	0.132	
20	Dec. 4	Ovalbumin	470	12	561.3	486.8	74.5	0.159	6.21
20	Oct. 30	α-casein	550		528.3	493.1	35.2	0.064	
20	Dec. 1	α-casein	470	10.6	512.6	419.7	92.9	0.198	8.76
14	Apr. 4	Ovalbumin	570	**26.8**	532.9	471.0	61.9	0.107	2.31
14	May 5	Ovalbumin	640	14.9	540.5	490.5	50.0	0.078	3.36
14	Mar. 29	α-casein	465	16.0	491.0	434.7	56.3	0.121	3.52
14	Apr. 24	α-casein	590	13.4	549.4	314.3	235.1	0.398	17.5

Bacteria counts in bold are based on numbers from final culture

vii) Variation in pH

As mentioned previously, addition of equal amounts of acid to the PH and protein culture might have resulted in fluctuations in the pH of the protein culture. pH measurements were taken every minute from the time gas sparging began to the end of the rate study. The maximum, minimum and average pHs are listed in Tables 5.66 and 5.67. Figure 5.40 shows the pH of the culture media over time for the Oct. 20 culture. pH data for additional cultures are found in Appendix C. Decreases in the pH would be temporary in nature because ammonia production, denitrification and gas sparging all tend to drive the pH up.

For 20°C cultures, the pH varied by less than 0.7 pH units away from the target pH of 7.2. In general, the difference between the maximum pH and the setpoint was greater than the setpoint and the minimum pH. This was a reflection of the sources cited above driving the pH up. The pH data for 14°C cultures shows the pH to be as much as 1 pH unit away from the set point. The minimum pHs for the 14°C cultures tend to be lower than for the 20°C cultures. The graphs of nitrate and nitrite consumption with time (see Figure 5.37 as an example) were highly linear suggesting that fluctuations in pH did not have an observable impact on nitrate and nitrite consumption. For example for the cultures of Oct. 20 and Dec. 4 pH ranges during the time of monitoring were 7.0-7.6 and 7.05-7.69, respectively. The impact of pH on growth will be discussed later in this section.

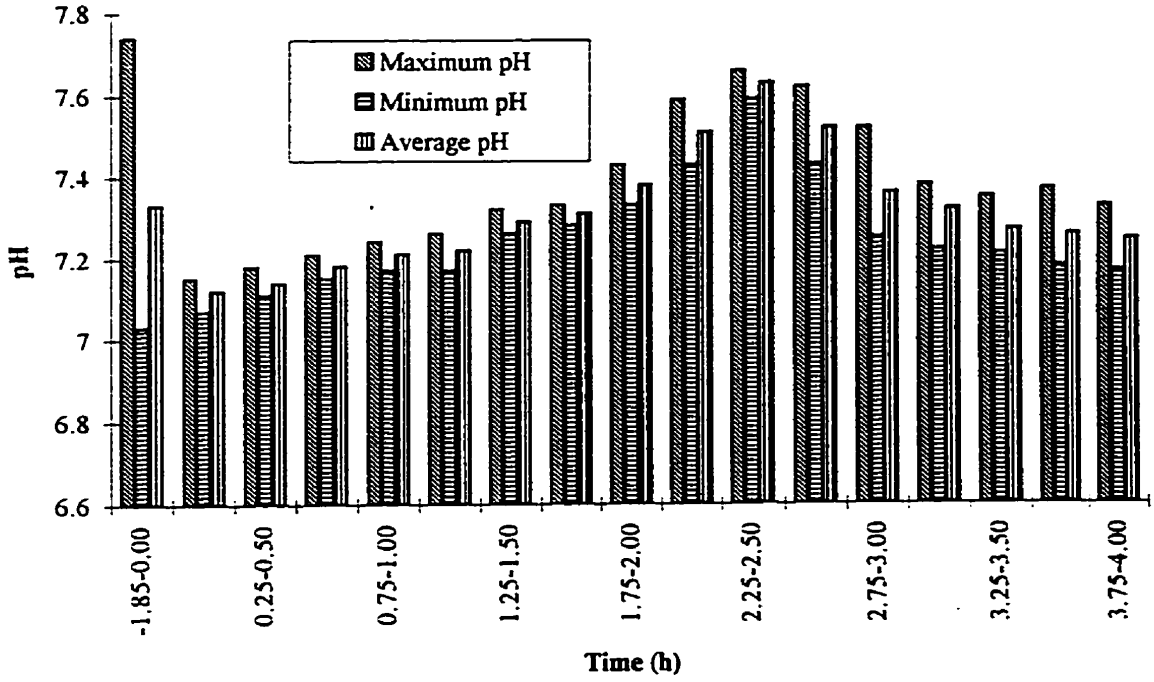
Table 5.66 Maximum and minimum pH of Anoxic Protein Cultures at 20°C

Date	Protein	Maximum pH	Minimum pH	Average pH
Oct. 20	Ovalbumin	7.7	7.0	7.3
Dec. 4	Ovalbumin	7.9	7.0	7.4
Oct. 30	α-casein	6.7	6.9	6.8
Dec. 1	α-casein	7.7	7.0	7.3

Table 5.67 Maximum and minimum pH of Anoxic Protein Cultures at 14°C

Date	Protein	Maximum pH	Minimum pH	Average pH
Apr. 4	Ovalbumin	7.7	6.1	7.06
May 5	Ovalbumin	7.8	6.7	7.00
Mar. 29	α-casein	7.1	6.4	6.77
Apr. 24	α-casein	8.0	6.8	7.04
May 9	α-casein	7.3	6.6	6.78

Figure 5.40 pH profile of Oct. 20 Anoxic Culture



5.4.3. Discussion of Ovalbumin and α-casein Experimental Data

a) Transitional Behaviour with Aerobic Cultures

With the cultures of Nov. 27 (ovalbumin), Dec. 6 (α-casein), May 7 (α-casein) and May 9 (α-casein), similar behaviour was seen. The OUR was relatively constant for 2-3 h depending on the culture. After this time, the OUR increased substantially over a very short time and then leveled off. This behaviour was not specific to a particular protein or temperature. The response was not seen in the following experiments: Nov. 2 (α-casein), Nov. 7 (ovalbumin), Apr. 4 (ovalbumin) and May 5 (ovalbumin). It was noted in both the Mar. 27 PH cultures.

As part of the experimental protocol, the stock culture was taken from conditions where there were low levels of usable substrate and placed in a rich media. It was suggested that the behaviour outlined above may be related to the change in culture media. If this was the reason, then the same type of behaviour should be seen in the companion PH utilizing culture. The two cultures were derived from the same stock culture and differ only in the substrate added. The PH utilizing cultures for these dates have OURs that increase linearly

with time or they remain constant. With PH utilizing cultures behaving in this manner it is unlikely that the transitional behaviour was caused by a change in media.

The changes in respiration rates reflects changes in the cell's metabolism. Possible reasons for an increased rate of metabolism could include increased substrate availability and accessibility of a preferred substrate. Certain portions of the protein may take longer for exoenzymes to degrade but yield a combination of amino acids and peptides favoured by the bacteria. By-products of protein degradation could act as surfactants changing the surface tension of the media and promoting increased mass transfer. For the PH utilizing cultures, the same arguments may apply as all dipeptides and olgiopeptides will not have the same surface characteristics. Based on the observations of Boczar *et al.* (1992), Dold *et al.* (1992), and Frolund *et al.* (1995) hydrolysis occurs within the floc structure. If this is accepted then the substrate must be enmeshed or adsorbed onto the floc.

b) **Hydrolysis Rate Limiting**

For parallel experiments, differences in the OUR of the protein and PH culture could be attributed to substrate uptake and hydrolysis. It was not necessary to correct for the basal OUR because both cultures were derived from the same stock culture. The results of a t-test done to detect differences in the means are listed in Tables 5.68 and 5.69. A requirement of the t-test was a pooled variance. The method of calculating the pooled variance and the resulting degrees of freedom depended on whether the variances associated with the two means were derived from the same distribution or not. The details of the analysis are summarized in Appendix C.

At 20°C , there was no significant difference in the rate of oxygen utilization for the protein and PH culture in three of the four rate studies. In the rate study of Nov. 7, ovalbumin was used more slowly than CAA. The second rate study done on Nov. 27, under comparable conditions does not show this. The discrepancy between repeated rate studies does not reflect inadequacies in the way in which the experiment was carried out or sample analysis. It was expected that more reproducible results would have arisen if the rate studies were undertaken on the same day. This was impossible because of time and equipment limitations. Repeated experiments were carried out at the same temperature using the sample protein but a different stock culture was used in each case. The ecology, physiological state of the bacteria present and enzyme levels will all be

different. Enzyme assays shown in Table 5.52 in part illustrate the dynamic nature of the system. Differences in repeated rate studies illustrates the difficulty in determining the kinetics of a mixed microbial population.

At 14°C, the results consistently show that proteins were used more quickly than PH based on a comparison of OURs. Proteins require hydrolysis prior to being used in cell synthesis. Even if hydrolysis was not rate limiting, the rate of cell synthesis using peptides and amino acids derived from the protein should be comparable to that of the PHs. It is possible that some factor was missing from the PH but was present in the purified proteins. Alternatively, even though the purified proteins were of relatively high purity it is possible contaminants present in them provided a more readily useable source than the PH.

Table 5.68 Comparison of OUR for Protein and CAA Utilizing Cultures at 20°C

Substrate	Date	Mean OUR (mg O_2/L h)	Standard Deviation	F	d.f.	Sig. Difference in Variance	Value of t	df	$t_{0.95}$
Ovalbumin	Nov. 27	15.8	3.90	5.25	(8,7)	Y	0.04	11	2.20
CAA		15.8	1.70						
Ovalbumin	Nov. 7	14.7	3.37	4.1	(8,7)	Y	8.04	14	2.15
CAA		24.1	1.67						
α–casein	Nov. 2	18.6	1.74	1.91	(8,7)	N	0.59	15	2.13
CAA		18	2.40						
α–casein	Dec. 6	20.5	5.11	6.96	(9,8)	Y	1.50	12	2.18
CAA		17.8	1.94						

Table 5.69 Comparison of OURs for Protein and CAA Utilizing Cultures at 14°C

Substrate	Date	Mean OUR (mg O_2/Lh)	Standard Deviation	F	d.f.	Sig. Difference in Variance	Value of t	d.f	$t_{0.95}$
Ovalbumin	Apr. 4	17.5	1.53	1.28	(10,9)	N	2.54	19	2.09
CAA		15.9	1.36						
Ovalbumin	May 5	20.8	2.06	6.23	(9,8)	Y	3.49	13	2.16
CAA		14.7	5.14						
α–casein	May 7	20.3	1.25	10	(7,9)	Y	3.38	9	2.26
CAA		15.3	4.08						
α–casein	May 9	18.2	0.9	6.84	(10,10)	Y	4.66	12	2.18
CAA		14.5	2.35						

Using the same reasoning as discussed before the NUR of the protein and PH utilization were compared for the anoxic cultures. Tables 5.70 and 5.71 summarize the NUR for the protein and PH cultures. A comparison of slopes was completed to determine if there was any significant difference between the NUR of the two cultures. At 20°C, the NUR of the ovalbumin culture was significantly slower than for the CAA culture

at the 95% confidence limit. This trend was confirmed by data from a second experiment done under similar conditions. This suggested that substrate uptake and hydrolysis was limiting when ovalbumin was used at 20°C with anoxic cultures. There was no significant difference between the NUR of the α-casein and CAA cultures at the 95% confidence level and thus substrate uptake and hydrolysis were not important factors.

At 14°C, there was a very different response to ovalbumin. At this temperature, ovalbumin was used at a significantly higher rate than the PH in both rate studies. In two of three rate studies using α-casein, there was no significant difference in the NUR for α-casein and CAA cultures. In a third rate study, the α-casein culture used NO_X at a significantly greater rate than did the CAA culture. As discussed above, differences in repeated experiments may be the result of differences in the stock cultures.

A second strategy for examining the role hydrolysis plays is through a comparison of ammonia production rates. The observed rate of production is the ammonia nitrogen released through protein degradation less the amount required for growth. Earlier it was shown that ammonia nitrogen production did not correlate with either the rate of oxygen or NO_X consumption for all culture types. The ammonia production rates realized for PH and protein utilizing cultures in parallel experiments are summarized in Tables 5.72-5.74. A comparison of slopes was completed to determine if there was any significant difference between the ammonia production rate of the two cultures.

Table 5.70 Comparison of NUR for Protein and CAA utilizing cultures at 20°C

Substrate	Date	Mean NUR $\frac{mg\ NOx\text{-}N}{L\ h}$	F Value	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Oct. 20	4.51	42.7	(1,16)	4.49
CAA		5.39			
Ovalbumin	Dec. 4	3.26	163.8	(1,14)	4.6
CAA		6.52			
α-casein	Oct. 30	4.71	3.3	(1,18)	4.41
CAA		4.47			
α-casein	Dec. 1	6.79	3.4	(1,14)	4.6
CAA		6.34			

Table 5.71 Comparison of NUR for Protein and CAA utilizing cultures at 14°C

Substrate	Date	Mean NUR $\frac{\text{mg NOx-N}}{\text{L h}}$	F Value	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Apr. 4	3.69	11.3	(1,14)	4.6
CAA		2.83			
Ovalbumin	May 5	4.73	86.3	(1,14)	4.6
CAA		3.21			
α–casein	Mar. 29	1.82	3.7	(1,14)	4.6
CAA		2.2			
α–casein	Apr. 24	5.2	226.4	(1,14)	4.6
CAA		2.12			
α–casein	May 9	3.88	1.1	(1,14)	4.6
CAA		4.14			

The ovalbumin culture of Nov. 7 shows significantly less ammonia nitrogen production than does its' PH utilizing counterpart. As shown previously, the Nov. 7 PH culture had a significantly higher OUR than did the parallel protein culture. The Dec. 6 shows the casein culture to produce significantly more ammonia nitrogen than does the PH culture. The mean OUR for the casein culture was higher than the PH culture but this difference was statistically insignificant. The pooled variance for the Dec. 6 experiment was large because the OUR data for the casein culture had a large variance. The large variance arises from the casein culture showing transitional behaviour. At 14°C, only the Apr. 4 cultures show a significant difference in the ammonia nitrogen production rates. Production was higher for the ovalbumin culture. At 14°C, all of the protein cultures had a significantly higher OUR than did the PH cultures.

For anoxic cultures, one of the four cultures had a significant difference in the ammonia production rate. The cultures of Dec. 4 showed a significant difference with the CAA culture producing more ammonia. This was one of two rate studies where the CAA culture had a higher OUR. Two of the four rate studies at 14°C had significant differences in the production rates. One pair of rate studies used ovalbumin/CAA while the other used α-casein /CAA.

An analysis of ammonia nitrogen production rates finds that a few of the rate studies having significant difference in ammonia production also had significant differences in either the OUR or NUR, as appropriate. There is no consistent pattern. This was also the case with comparisons of either OUR or NUR.

Table 5.72 Comparison of NH_3-N Production for Aerobic Protein and CAA cultures at 20°C

Substrate	Date	Production Rate (mg NH_3-N/L h)	F Value	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Nov. 7	1.86	155.9	(1,12)	4.74
CAA		3.56			
Ovalbumin	Nov. 27	2.25	0.205	(1,14)	4.60
CAA		2.3			
α-casein	Nov. 2	2.36	1.36	(1,12)	4.74
CAA		2.47			
α-casein	Dec. 6	2.76	91.7	(1,14)	4.60
CAA		2.19			

Table 5.73 Comparison of NH_3-N Production for Aerobic Protein and CAA cultures at 14°C

Substrate	Date	Production Rate (mg NH_4-N/L h)	F	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Apr. 4	3.39	11.3	(1,12)	4.74
CAA		2.37			
Ovalbumin	May 5	3.03	1.84	(1,12)	4.74
CAA		2.35			
α-casein	May 7	3.64	4.29	(1,12)	4.74
CAA		3.11			
α-casein	May 9	3.59	35.1	(1,12)	4.74
CAA		2.21			

Table 5.74 Comparison of NH_3-N Production for Anoxic Protein and CAA cultures at 20°C

Substrate	Date	Production Rate (mg NH_3-N/L h)	F	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Oct. 20	2.22	0.34	(1,12)	4.74
CAA		1.90			
Ovalbumin	Dec. 4	2.83	88.9	(1,13)	4.67
CAA		1.74			
α-casein	Oct. 30	3.84	4.59	(1,12)	4.74
CAA		2.38			
α-casein	Dec. 1	2.21	0.49	(1,12)	4.74
CAA		2.74			

Table 5.75 Comparison of NH_3-N Production for Anoxic Protein and CAA cultures at 14°C

Substrate	Date	Production. Rate (mg NH_3-N/L h)	F	Degrees of Freedom	$F_{0.95}$
Ovalbumin	Apr. 4	1.76	0.85	(1,13)	4.67
CAA		2.14			
Ovalbumin	May 5	1.83	6.86	(1,13)	4.67
CAA		2.72			
α-casein	Mar. 29	0.721	33.5	(1,14)	4.60
CAA		1.62			
α-casein	May 9	2.02	3.59	(1,14)	4.60
CAA		3.21			

In summary, no blanket statement can be made regarding hydrolysis. It may be that the role it plays depends on the temperature, EAS (aerobic or anoxic), protein (α-casein or ovalbumin) and stock culture. In some instances, different overall results were seen depending on the stock culture used. At 14°C under either aerobic or anoxic conditions, there were a number of rate studies where the protein was utilized more quickly than the PH. In many rate studies, the protein was used as quickly as the PH. Given these results, the molecular weight of the protein does not appear to have a significant impact on the rate at which they are used. The overall structure of α-casein and ovalbumin did not limit the rate at which the proteins were used.

c) **Ammonia Nitrogen Production**

It was shown for PH cultures that the observed ammonia nitrogen production correlates with NUR or OUR for only some cultures. Analysis of the data resulted in r values outlined in Table 5.76. There was no correlation between ammonia nitrogen production and NO_X utilization at the 95% confidence level; however, there was one between the average OUR and the rate of ammonia nitrogen production for aerobic cultures at 14 and 20°C. Possible reasons for the lack of correlation have been previously discussed in Section 5.3.3.f.

Table 5.76 Correlation between Ammonia Nitrogen Production and OUR and NUR

EAS	Temperature (°C)	Degrees of Freedom	r	$r_{0.95}$	$r_{0.90}$
Aerobic	20	2	0.997	0.950	0.900
Aerobic	14	2	0.976	0.950	0.900
Anoxic	20	2	-0.140	0.950	0.900
Anoxic	14	2	0.874	0.950	0.900

Ammonia nitrogen production normalized on MLVSS and bacteria counts was summarized in Tables 5.53, 5.54, 5.62 and 5.63. The data form a 2^3 factorial design with protein type, temperature and EAS as

factors. The analysis shows no significant effects at the 95% CL for data normalized on MLVSS (Table 5.77 with data in Appendix C). EAS and EAS-temperature were significant at the 90% CL.

The effect of EAS is confounded with a EAS-temperature interaction. Ammonia production was approximately 50% higher at the lower temperature for both electron acceptor systems. Ammonia production at 20°C was 5.52 and 2.74 mg NH_3-N/g h, respectively, under aerobic and anoxic conditions. The absolute increase in production rates is much different. This is the reason for a significant EAS-temperature interaction.

When the data were normalized on bacteria counts, only the EAS had a significant bearing at the 90% CL. Ammonia nitrogen production was highest under aerobic conditions when all other factors were equal. This same observation holds true for PH cultures (Table 5.47). Consistently higher ammonia nitrogen production values under aerobic conditions may reflect differences in the metabolic pathway of the bacteria or ecological differences.

Table 5.77 Analysis of the Factorial Design for Ammonia Production

Effect or Interaction	Normalized on MLVSS x 10^{-5}		Normalized on Bacteria Counts x 10^{-13}	
	Estimated Effect	T-value	Estimated Effect	T-value
Protein	-0.255	0.323	-6.67	0.575
Temperature	-0.360	0.456	-3.33	0.287
EAS	-1.75	*2.22*	-27.4	*2.36*
Protein x Temperature	-0.533	0.676	-7.23	-0.623
Protein x EAS	0.195	0.247	7.03	0.606
Temperature x EAS	1.63	*2.06*	10.6	0.916
Protein x Temperature x EAS	0.135	0.171	7.77	0.670

MLVSS results have 8 df. The t critical value at the 95% CL and 90% CL is 2.31 and 1.86, respectively. Similarly bacteria counts have 4 df. $t_{0.95}$ and $t_{0.90}$ have values of 2.78 and 2.13, respectively.
Bolded effects are significant at the 95% CL and those in italics are significant at the 90% CL.

d) **Effect of pH on Kinetics**

The pH of protein cultures deviated from 7.2 because pH control was not implemented. pH fluctuations can change the activity of exoenzymes as well as influencing bacterial growth kinetics. Enzyme systems are complex. Each participating enzyme possesses a pH range over which its performance is optimal. pH values outside of the optimum range can result in: competitive inhibition, non-competitive inhibition or irreversible denaturation.

The pH of the aerobic cultures at 20°C ranged between 6.9 and 7.5 while those of the 14°C cultures were more variable with pHs ranging between 6.6 and 7.8. The data were analyzed to see if the average OUR normalized on VSS correlated with the minimum, maximum or average pH. Correlation coefficients arising from this analysis are summarized in Table 5.78. There was no significant correlation between pH and OUR for the aerobic cultures. It is concluded that deviations away from a pH of 7.2 had no effect on aerobic metabolizing cultures.

Table 5.78 Correlation between pH and OUR

	Correlation Coefficient (r)		
Temperature (°C)	Maximum pH	Minimum pH	Average pH
14	0.713	-0.049	0.576
20	-0.069	-0.397	0.216

Critical value of r, $r_{0.95}$, is 0.950 with 2 df.

A review of the literature indicates that the reported optimal pH for denitrification is between 6.5 and 8.3 (Table 5.79). For the experimental system, pHs ranged from 6.2-8.2 for anoxic protein utilizing cultures. The minimum value is less than the lower value of the optimal range so further examination of the effect of pH on the cultures is warranted.

Beaubien *et al.* (1995) examined the impact of long term changes in the pH setpoint versus fluctuations in the culture pH. Using a denitrifying culture isolated from a drinking water plant, metabolic activity was monitored using gas production measurements. Metabolic activity was unchanged at pH setpoints varying between 6.5-8.5. Short term fluctuations in pH had a significant impact on gas production when they were greater than 1 unit from the optimum. Fluctuations of this magnitude decreased the denitrification activity by 20%.

All of the rate studies carried out under anoxic conditions had pH deviations of less than 1 unit from the set point of 7.2. The Apr. 4 rate study was an exception because the pH deviated by 1.1 units. The nitrite and nitrate consumption profiles are highly linear (see Fig. 5.37 as an example) suggesting that the rate of denitrification did not change over the course of the experiment. This was different than what was expected based on the results of Beaubien *et al.* (1995).

Table 5.79 Optimum pH Range for Denitrifying Cultures

Culture	Substrate	Temp (°C)	Optimum pH Range	Investigator
Pseudomonas Denitrificans	Ethanol/Nitrate	30	7.45-7.60	Wang *et al.* (1995)
Pseudomonas Denitrificans	Ethanol/Nitrite	30	7.20-7.30	Wang *et al.* (1995)
Pseudomonas Denitrificans	Sodium Citrate/ Nitrate		7.0	Dawson and Murphy (1973)
Hyphomicrobium Sp.	Methanol/Nitrate	25	8.3	Timmermans and Van Haute (1983)
Paracoccus Denitrificans	Asparate/Nitrate Yeast Extract/Nitrate Peptone/Nitrate		6.5	Kokufuta *et al.* (1986)
Denitrifying Culture	Methanol/Nitrate	20	6.5-7.5	Beccarri *et al.* (1983)
Denitrifying Culture	Methanol/Nitrite	20	7.5	Beccarri *et al.* (1983)
Denitrifying Culture	Citric Acid/Nitrate	20	6.5-8.5	Beaubien *et al.* (1995)

e) **Effect of S_0/X_0 on NUR and OUR**

The S_0/X_0 was used to determine if there was a significant correlation between this ratio and the normalized OURs and NURs. Table 5.80 outlines the correlation coefficients derived for the aerobic and anoxic cultures at different temperatures using either MLVSS or bacteria counts as a normalizing factor. Correlation coefficients were not calculated for cultures operating under aerobic and anoxic conditions at 20°C using bacteria counts because there was insufficient data. There were significant correlations for aerobic data normalized on MLVSS at 14 and 20°C at the 90% confidence level. A significant correlation was also seen for the data normalized on MLVSS at 14°C when PH was used as a substrate. The lack of correlation between S_0/X_0 and either OUR or NUR in many of the cases suggests that S_0/X_0 does not have a major impact on NUR or OUR in this particular instance.

Table 5.80 Correlation between S_0/X_0 and OUR and NUR

EAS	Temperature (°C)	Basis	r	df	$r_{0.95}$	$r_{0.90}$
Aerobic	20	MLVSS	0.819	2	0.950	0.900
Aerobic	14	MLVSS	0.997	2	0.950	0.900
Aerobic	14	Bacteria	0.599	2	0.950	0.900
Anoxic	20	MLVSS	0.460	2	0.950	0.900
Anoxic	14	MLVSS	-0.839	3	0.878	0.805
Anoxic	14	Bacteria	0.551	3	0.878	0.805

f) **Observed Yield using Proteins**

Observed yields under conditions of active substrate utilization were calculated in a similar manner to that described in Section 5.3.3.i. The same procedure as outlined previously produced the observed yield of

aerobic and anoxic cultures presented in Tables 5.81 and 5.82. Visual inspection of the tables suggests that there is no temperature dependency. The 95% CL are presented Table 5.83 along with a summary of the data. It can be seen from the table that all the 95% CL overlap so the protein or the culture does not significantly affect the yield.

Table 5.81 Yield Data for Aerobic Cultures using Proteins

Date	Substrate	dS/dt mg COD/L h	dO_2/dt mg O/L h	Yield Coefficient mg COD/mg COD	Median Y	Average Y	Std. Dev
7-May	α-casein	52.3	15.0	0.71	0.71	0.71	0.006
9-May	α-casein	54.4	15.8	0.71			
2-Nov	α-casein	47.8	14.2	0.70			
6-Dec	α-casein	53.8	16.1	0.70			
4-Apr	Ovalbumin	69.3	14.6	0.79	0.75	0.77	0.021
5-May	Ovalbumin	63.5	15.9	0.75			
27-Nov	Ovalbumin	53.3	12.0	0.78			

Table 5.82 Yield Data for Anoxic Cultures using Proteins

Date	Substrate	dS/dt mg COD/L h	dNO_3-N/dt mg NO_x-N/L h	Yield Coefficient mg COD/mg COD	Median Y	Average Y	Std. Dev
4-Apr	Ovalbumin	36.5	3.69	0.74	0.75	0.77	0.031
5-May	Ovalbumin	48.6	4.73	0.75			
20-Oct	Ovalbumin	59.2	4.51	0.80			
4-Dec	Ovalbumin	40	3.26	0.79			
30-Oct	α-casein	67	4.48	0.83	0.74	0.76	0.056
29-Mar	α-casein	16.8	1.819	0.72			
1-Dec	α-casein	68.1	6.79	0.74			

Table 5.83 95% CL for Yield Data Derived using Proteins

Protein	EAS	Upper 95% CL	Lower 95% CL
α-casein	aerobic	0.72	0.70
ovalbumin	aerobic	0.80	0.70
α-casein	anoxic	0.90	0.62
ovalbumin	anoxic	0.82	0.72

5.4.4. Presentation of Results for Lactalbumin Rate Studies

a) Aerobic

i) Acclimated and Unacclimated Cultures

Using the protocol detailed in Section 4.2.2.c.ii, lactalbumin was added to one half of a stock culture sample while a PH was added to the remaining half of the sample. Rate studies carried out in the Fall of

1995 at 20°C used a stock culture acclimated to casein and albumin, but not lactalbumin. Further rate studies using lactalbumin at 20°C were carried in July 1996 using a stock culture acclimated to lactalbumin. The stock culture was produced by a SBR fed lactalbumin and sewage on a continuous basis for four weeks (approximately 3 sludge ages). It was expected that this system would produce a culture acclimated to lactalbumin. High effluent ammonia nitrogen levels for the SBR confirmed that the reactor was degrading lactalbumin (See Section 5.2.3 for details).

For 14°C rate studies, a different approach was taken in developing a culture capable of using lactalbumin. An unacclimated culture was fed lactalbumin and the degradation of the protein was followed by periodically monitoring nitrate levels. If nitrate levels decreased markedly, then the culture was actively respiring and hence using lactalbumin. It was assumed that a culture degrading lactalbumin would possess a enzyme complement and ecology favourable for lactalbumin degradation. Rate studies used this exposed mixed microbial culture.

Anoxic and aerobic rate studies using exposed cultures were carried out in parallel as a time saving measure. An unacclimated anoxic culture was monitored frequently after lactalbumin addition to determine when lactalbumin degradation and subsequent nitrate utilization began. Addition of lactalbumin to the unacclimated aerobic culture occurred at the same time, but minimal sampling occurred. The anoxic culture used PH more slowly than its aerobic counterpart at 14°C (see Table 5.45). Based on the slower growth rate, it was anticipated that the anoxic culture would take longer to use the lactalbumin. Rate studies using the exposed cultures were carried out on April 13, 96 h after the cultures were fed lactalbumin. At this time the anoxic culture was actively denitrifying. The degradation of lactalbumin by the aerobic culture was confirmed by ammonia nitrogen measurements. On April 9 and 13, the ammonia nitrogen concentration was 34.3 and 57.3 mg NH_3-N/L, respectively. The increase in the ammonia nitrogen concentration is consistent with lactalbumin degradation.

ii) Oxygen Utilization Rates

Figure 5.41 shows the OURs of split stock cultures fed lactalbumin and PH in parallel experiments at 20°C. To permit comparisons between the cultures, the OUR_M has been normalized on the adjusted mixed liquor VSS of the stock culture (see Section 5.3.2.a.i for details on normalizing procedure).

As indicated by Figure 5.41, PHs were quickly degraded by cultures acclimated to the whole protein analogue. Table 5.84 summarizes the rates of PH utilization for acclimated and unacclimated cultures and using CAA and LEH. The average OUR_C varies by 117% for the Oct. 25 and Nov. 29 rate studies using CAA. Both of these rate studies used a stock culture grown on casein and albumin. As seen in Table 5.23, the average OUR_C of 34.4 mg O_2/g VSS·h was calculated using data from six repeat experiments. The average OUR_C for the Oct. 25 and Nov. 29 cultures was 30.5 mg O_2/gVSS·h which was comparable to the overall average.

Table 5.84 Average OUR_C for Lactalbumin and Companion PH Cultures at 20°C (mg O_2/g VSS·h)

Date	Temperature (°C)	Previous Exposure to Lactalbumin	Lactalbumin Culture	CAA	LEH
Oct. 25	20	No	6.06	19.5	
Nov. 29	20	No	6.53	41.4	
July 23	20	Yes	2.1		28.8

The same general trend was seen when lactalbumin was added to either acclimated or unacclimated cultures. Addition of lactalbumin to the stock culture caused an immediate increase in the OUR_M above the OUR_B (Table 5.85). Generally, the initial OUR_M of the lactalbumin culture was comparable to that of its companion culture. The Nov. 29 culture is an exception because the CAA culture exhibited an exceptionally high OUR of 41.4 mg O_2/(g VSS·h). Typically, the OUR_M of the lactalbumin culture remained at this higher level for a short time before it decreased. The decline continued until OUR_M was equal to OUR_B. It remained at the OUR_B for the remainder of the rate study.

Figure 5.41 Aerobic Degradation of Lactalbumin in Comparison to CAA and LEH at 20°C

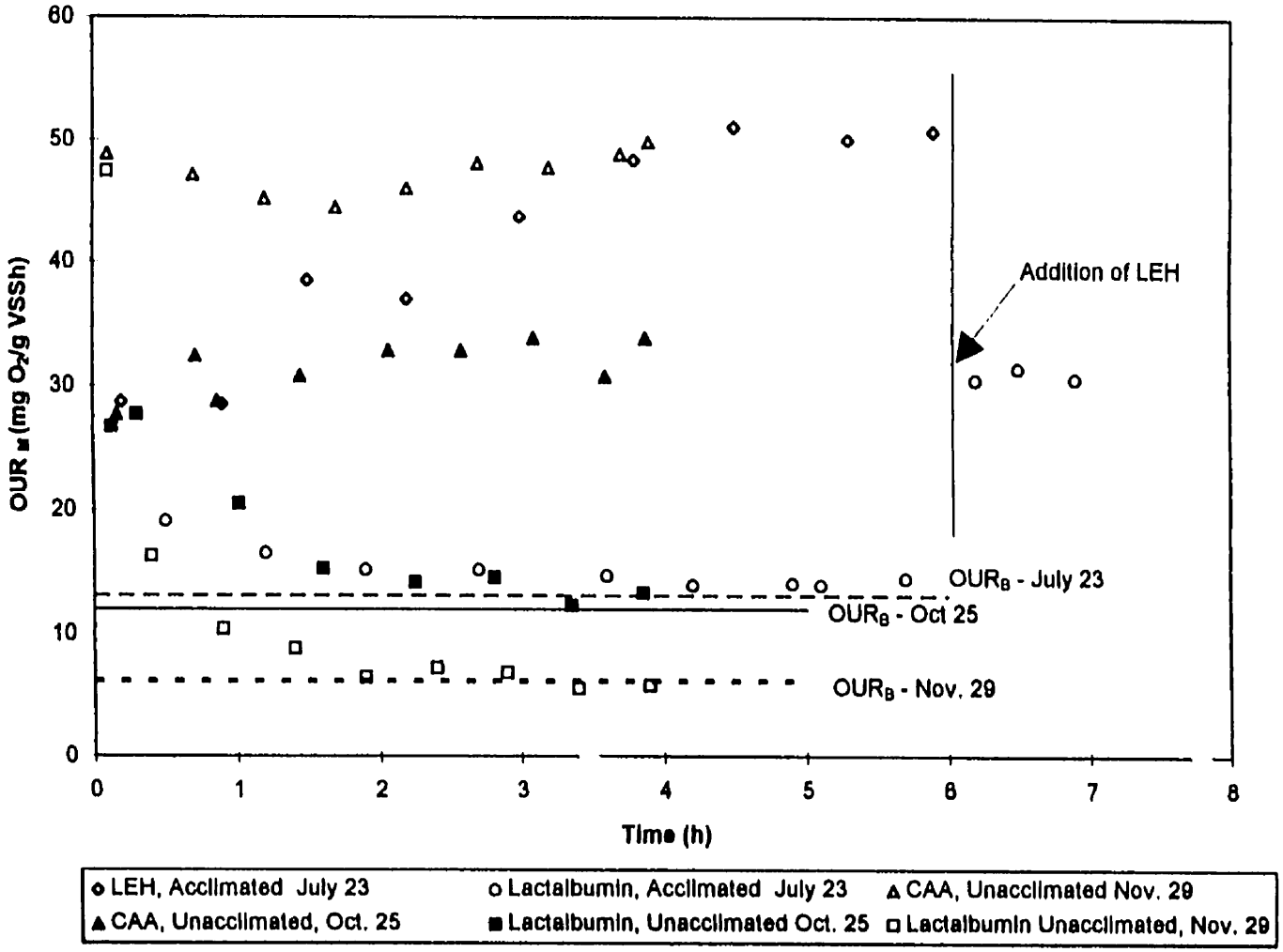


Table 5.85 Initial OUR_M for 20°C Lactalbumin and PH Cultures

Date	Previously Exposed to Lactalbumin	Substrate	Initial OUR_M mg O_2/(g VSS·h)	OUR_B mg O_2/(g VSS·h)
Oct. 25	No	CAA	25.0	12.0
Oct. 25	No	Lactalbumin	24.0	12.0
Nov. 29	No	CAA	52.0	6.20
Nov. 29	No	Lactalbumin	23.2	6.20
July 23	Yes	LEH	41.9	13.1
July 23	Yes	Lactalbumin	15.2	13.1

Lactalbumin was poorly degraded by both the unacclimated (Oct. 25 and Nov. 29) and acclimated cultures (July 23). These cultures had an average OUR_C that was 7-60% of the value of the companion culture (Table 5.84). At the end of the July 23 rate study, LEH was added to the acclimated lactalbumin culture. The mass of LEH added was equal to the mass of lactalbumin added to the culture initially. Shortly after LEH addition there was a substantial increase in the OUR. The response of the culture to LEH shows the culture was capable of responding in the presence of a suitable substrate.

A comparison of OURs for split cultures fed lactalbumin and LEH in parallel experiments at 14°C show the same general trends as seen previously at 20°C (Figure 5.42). The trends are summarized below.

1. At 20°C, OUR_M for the lactalbumin culture returned to OUR_B whereas at 14°C OUR_M for the exposed cultures (Apr. 13 and Apr. 29) approached but did not reach OUR_B. OUR_M for the unacclimated culture (Apr. 9) did return to the basal respiration rate. The difference in behaviour suggests that the exposed cultures were using some of the lactalbumin.
2. The unacclimated culture (Apr. 9) fed LEH exerted a higher OUR than the exposed cultures (Apr. 13 and Apr. 29). The OURs of the exposed cultures were comparable to each other (See Table 5.86).
3. With the two exposed lactalbumin cultures (Apr. 13 and Apr. 29), LEH was spiked into the culture at the end of the rate study. The OUR of the culture increased substantially and quickly thereby confirming the ability of the culture to exert a high OUR in the presence of a suitable substrate. The acclimated culture using lactalbumin at 20°C exhibited this same response upon LEH addition.

Figure 5.42 Aerobic Growth of Lactalbumin in Comparison to LEH at 14°C- Normalized on MLVSS

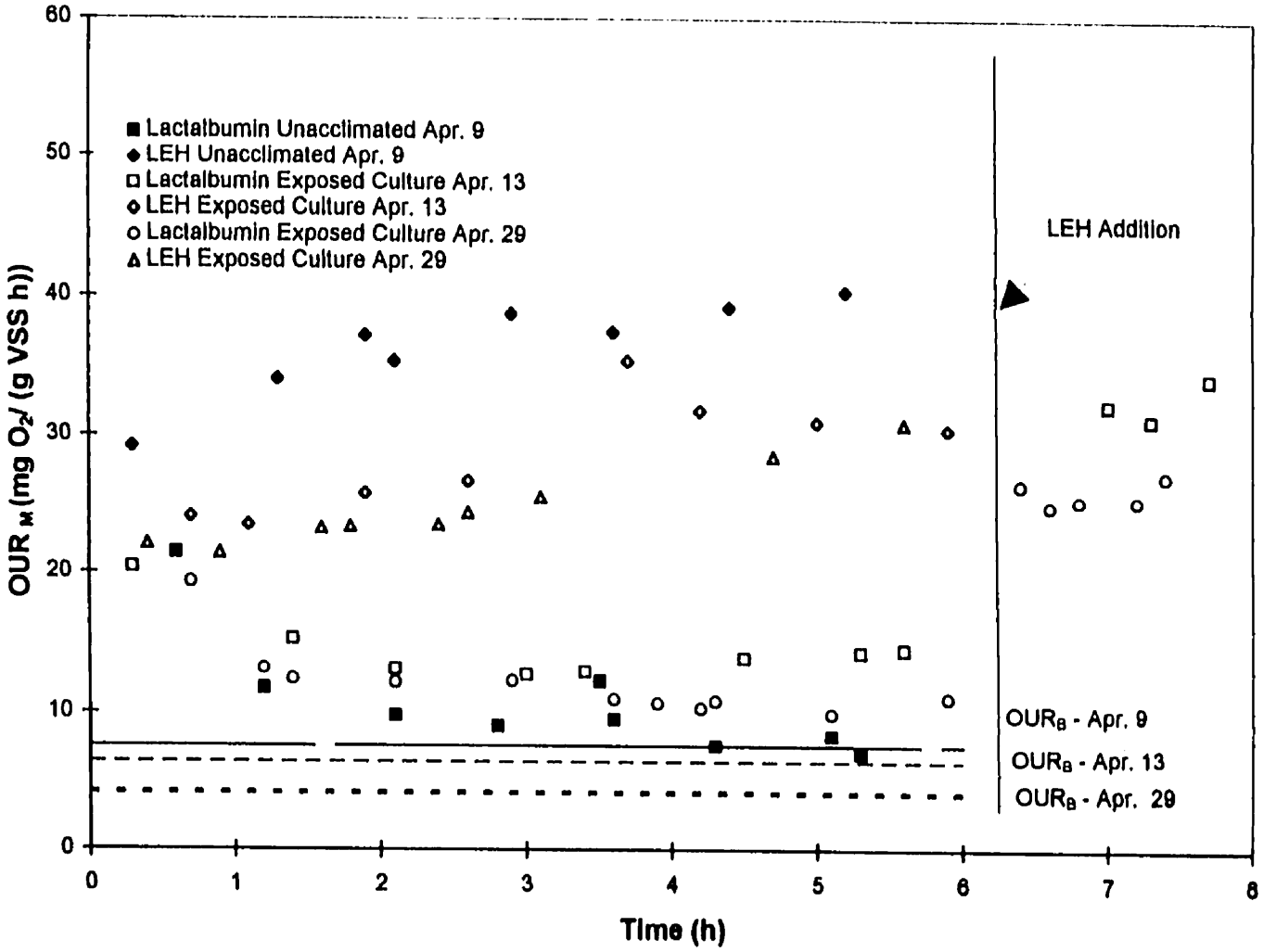


Table 5.86 Average OUR_C for Lactalbumin and Companion PH Cultures at 14°C (mg O_2/gVSS·h)

Date	Temperature (°C)	Previous Exposure to Lactalbumin	Lactalbumin Culture	CAA	LEH
Apr. 9	14	No	3.14		28.9
Apr. 13	14	Yes	8.29		22.2
Apr. 29	14	Yes	9.97		25.7

The existence of bacteria counts allowed the data of the 14°C rate studies to be analyzed in a different manner (Figure 5.43). The basal rates were normalized on the number of cells present in the initial culture. As discussed previously in Section 5.3.2.i - Oxygen Utilization Rates, the basal respiration rate does not correlate with the number of cells. All other respiration rates were normalized on cell counts of the initial and final culture.

iii) COD Uptake

Lactalbumin at a strength of approximately 580 mg total COD/L was used for rate studies. Monitoring the uptake of lactalbumin was of limited value because only 70 mg COD/L was filterable. The remaining 510 mg COD/L exists as a particulate and can not be differentiated from the floc.

iv) Observed Ammonia Nitrogen Production

Ammonia nitrogen production for the three rate studies done at 20°C is depicted in Figure 5.44. The observed levels of ammonia nitrogen increased linearly with time for PH cultures showing a constant rate of production. Fluctuations in the OUR were not reflected by changes in the rate of ammonia nitrogen production.

As shown in Figure 5.44, the Oct. 25 and July 23 cultures produced 1.61 and 1.38 mg NH_3-N/g VSS·h, respectively. The Nov. 29 culture produced negligible amounts of ammonia nitrogen; the ammonia nitrogen concentration increased by 1.0 mg NH_3-N/L over the 4 hours of the rate study. The limitations of monitoring ammonia nitrogen production in the bulk liquid were outlined in Section 5.3.2.b.vi.

v) Variation in pH

For a discussion on the effects pH could have on substrate utilization see Section 5.3.2.c.iv.

Figure 5.43 Aerobic Growth on Lactalbumin in Comparison to LEH at 14°C-Normalized on Bacteria Counts

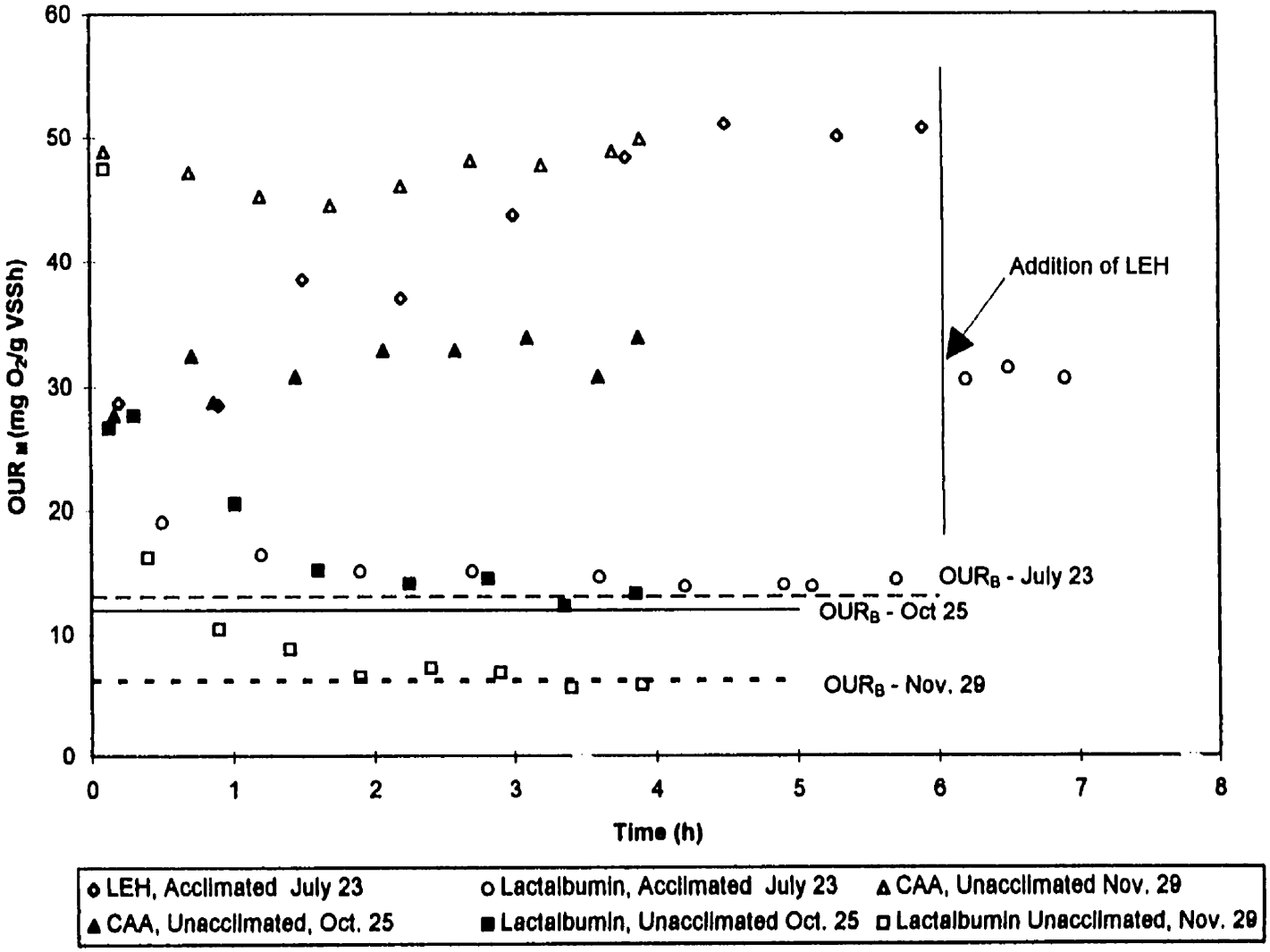


Table 5.87 Maximum and Minimum pH of Aerobic Lactalbumin Cultures

Date	Temperature (°C)	Maximum pH	Minimum pH
Oct. 25	20	7.4	7.1
Nov. 29	20	7.4	7.1
July 23	20	7.4	6.6
Apr. 9	14	7.6	7.1
Apr. 13	14	6.77	7.53

Data for Apr. 29 is not available

b) Anoxic

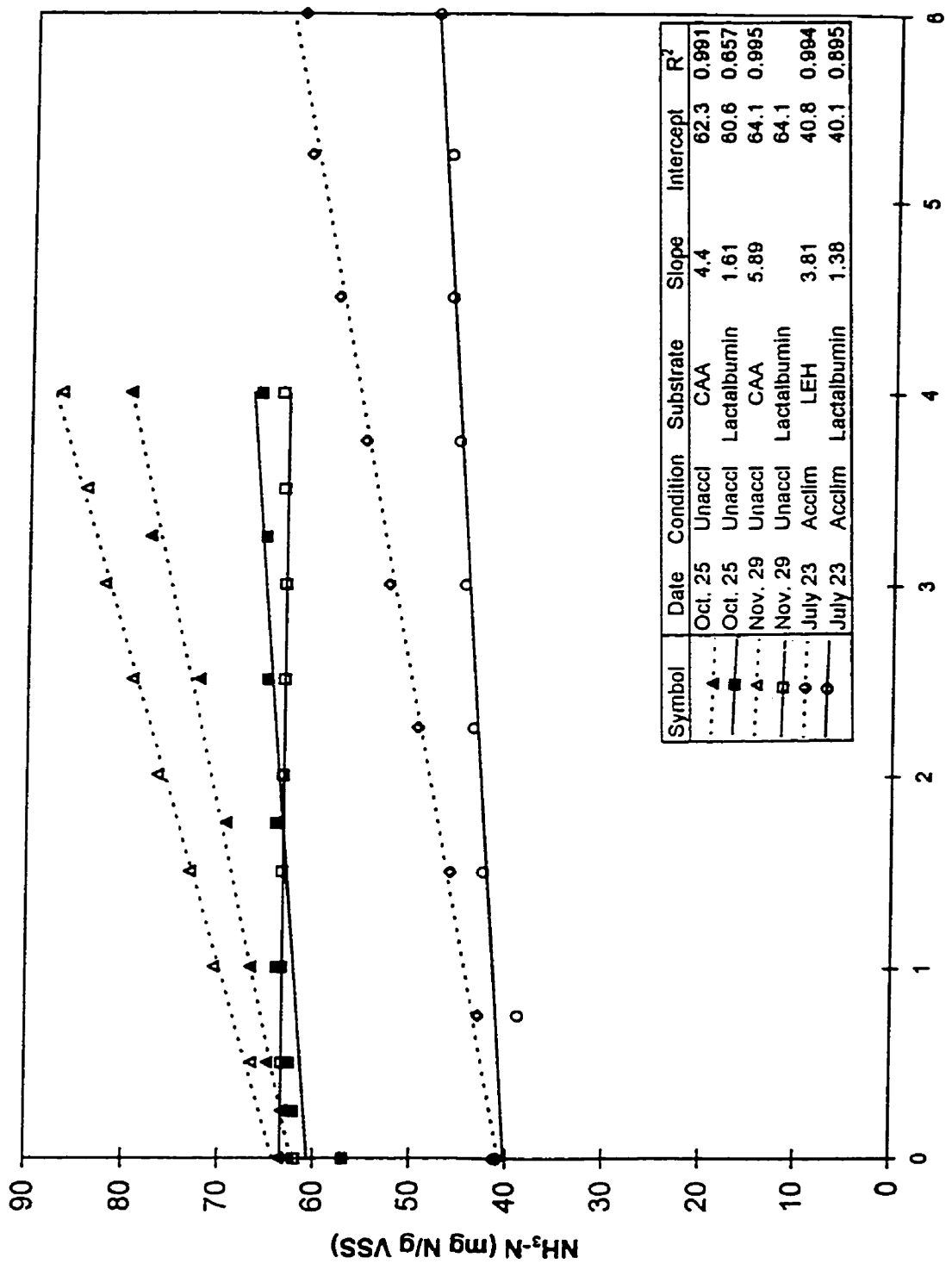
i) Acclimated and Unacclimated Cultures

The terminology used to describe aerobic cultures is also applicable to anoxic cultures. As previously discussed, rate studies examining lactalbumin degradation in the Fall of 1995 used a stock culture acclimated to casein and albumin but not lactalbumin. Subsequent 20°C rate studies were done using a stock culture grown on lactalbumin. The SBR reactor generating the anoxic stock culture for these rates was operated in parallel to the aerobic SBR. The reactor was considered acclimated because of the length of time the reactor had operated and the high effluent ammonia nitrogen levels suggesting lactalbumin degradation (see Section 5.2.3).

For 14°C rate studies, a different approach was taken to developing a culture capable of using lactalbumin. An unacclimated stock culture was fed lactalbumin and the degradation of the protein was followed by periodically monitoring nitrate reduction. The consumption of more than 10 mg NO_3-N/L would indicate active substrate removal. At this point, the exposed culture was capable of metabolizing lactalbumin. Nitrate reduction using this exposed mixed microbial culture may proceed more slowly than with a fully acclimated culture, but the rate would give some indication of how easily lactalbumin was degraded.

After the initial rate study of April 9, the sparging with nitrogen gas was continued and the pH of the culture was maintained at 7.2. During the first 31 h of the study, there was no detectable decrease in the concentration of nitrate. After a further 16.5 h of exposure, a decrease of 8 mg NO_3-N/L was observed. The culture began to consume nitrate 31-47.5 h after it was fed lactalbumin. Over the next eight hours, the nitrate was monitored hourly and the rate of denitrification was 0.5 mg NO_3-N/(L·h). The rate study with the exposed

Figure 5.44. Ammonia-N Production for Lactalbumin and LE11 Cultures at 20°C - Aerobic



culture began on April 13, 96 h after the culture was fed lactalbumin. The details of the April 9 and April 13 rate study follow.

The second rate study, used a stock culture produced by the SBR on April 26. This stock culture had been exposed to casein and albumin. As in previous rate studies lactalbumin was added to the culture to a concentration of approximately 580 mg COD/L. The culture was sparged with nitrogen gas and the pH was maintained at 7.2 by acid addition. After 21.5 h, there was no detectable decrease in nitrate. When the nitrate level was measured 44.75 h after the lactalbumin had been added to the culture there was a decrease of 4 mg NO_3-N/L. Measurements were taken periodically over the next 26 h. The rate of denitrification was 0.23 mg NO_3-N/(L·h). The rate study of April 29th, used a culture exposed to lactalbumin for a period of 72 h. The data collected for the April 29 rate study is presented below.

ii) Nitrate Utilization Rate

Nitrate utilization in the presence of lactalbumin, CAA or LEH at 20°C is shown in Figure 5.45. The nitrate concentration has been normalized on the adjusted mixed liquor VSS of the appropriate unacclimated, acclimated or exposed culture. See Section 5.3.2.a.i for details. Addition of CAA to the unacclimated stock cultures of Oct. 24 and Dec. 7 resulted in denitrification rates of 7.36 and 9.28 mg NOx-N/g VSS·h. A third unacclimated culture (Dec. 9) was used to determine if lactalbumin could be degraded over a longer period of time. Lactalbumin was added to the culture and the pH was maintained at 7.2. Figure 5.46 shows that under these conditions only 1.8 mg NO_3-N/L was consumed over 27 h. This low rate of nitrate consumption suggests that little or no lactalbumin was used.

In the fourth study at 20°C (July 23), lactalbumin and LEH were added to a stock culture that had been acclimated to lactalbumin. LEH was used and consumed nitrate at a rate of 9.79 mg NO_3-N/g VSS·h. This rate was slightly higher than the denitrification rate realized with other cultures. In the companion culture, lactalbumin was degraded and nitrate consumed at a rate of 2.7 mg NO_3-N/(g·h). Over the 6 h of the rate period, 17.7 mg NO_3-N/L had been consumed. At the end of the rate study, the lactalbumin culture was spiked with LEH. This produced no detectable change in the rate of denitrification. The results of the four rate studies have been summarized in Table 5.88.

Figure 5.45 Anoxic Degradation of Lactalbumin in Comparison to CAA and LEH at 20°C

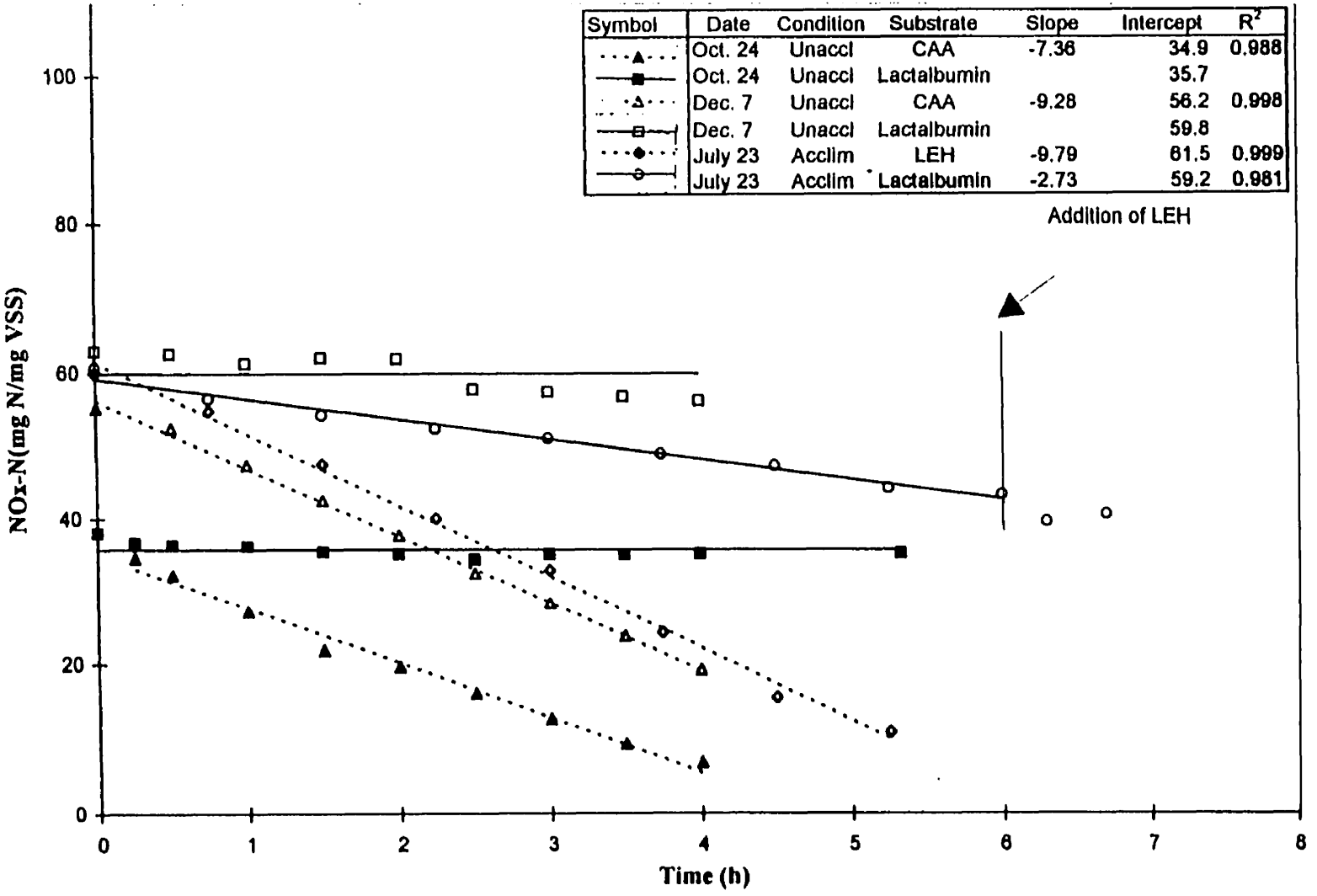


Figure 5.46 Anoxic Degradation of Lactalbumin pH=7.2

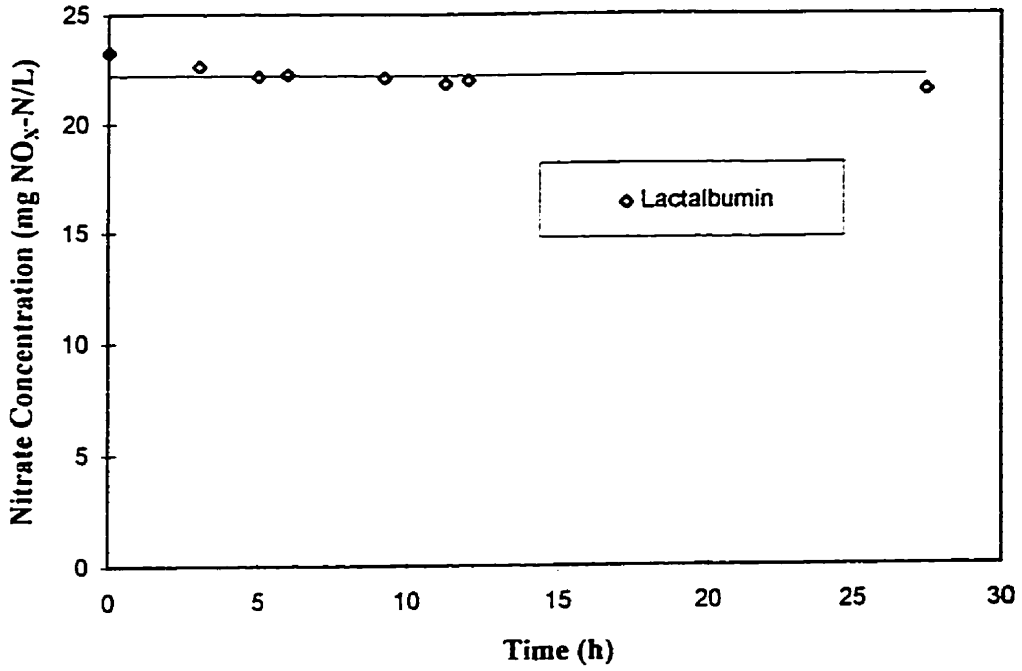


Table 5.88 Denitrification Rates for Lactalbumin/PH Cultures (mg NO_3-N/gVSS·h) at 20°C

Date	Previous Exposure to Lactalbumin	Lactalbumin Culture Lactalbumin	LEH	Protein Hydrolysate Culture CAA	LEH
Oct. 24	No	Non-det.		7.36	
Dec. 7	No	Non-det		9.28	
Dec. 9	No	Non-det			
July 23	Yes	2.73	2.73		9.79

Non-det. denotes a non-detectable reduction in nitrate nitrogen

At 14°C, anoxic rate studies were carried out in parallel with aerobic rate studies at this temperature. LEH was used as the PH in three rate studies shown in Figure 5.47. The rate of denitrification normalized on the adjusted stock culture VSS was significantly different for the three cultures. In order of the fastest to the slowest culture, the rates were: Apr. 29 (6.35 mg NO_3-N/g VSS h), Apr. 13 (4.87 mg NO_3-N/g VSS h) and Apr. 9 (4.21 mg NO_3-N/g VSS h). The two cultures exposed to lactalbumin for three days had the greater rates of denitrification.

Figure 5.47 shows the response of lactalbumin cultures both unacclimated and acclimated to be the similar. Lactalbumin was not metabolized over the 6 h of the rate study as indicated by NUR. LEH was added to the acclimated lactalbumin cultures (Apr. 13 and Apr. 29) at the end of the rate study. Both cultures began to

consume nitrate shortly after LEH addition. LEH was consumed at a rate of 6.03 and 4.60 mg NOx-N/g VSS h for the Apr. 13 and Apr. 29 cultures, respectively. As seen with the aerobic cultures, the lactalbumin cultures were capable of responding if given a favourable substrate.

A different perspective on the data was gained by plotting nitrate decay normalized on the basis of bacteria counts (Figure 5.48). Bacteria counts averaged on the initial and the final cultures were used where possible. The rate of LEH utilization was significantly different for each of the rate studies. The rate of utilization was faster for the acclimated cultures than it was for the non-acclimated culture. This was expected as the cultures would have been growing on lactalbumin, the polymer analogue of LEH. Addition of LEH to the Apr. 13 and Apr. 29 cultures resulted in a nitrate consumption rate of 4.11×10^{-13} and 3.59×10^{-13} mg NO_3-N/bact ·h, respectively. It appears that the observed rates were comparable upon addition of LEH.

iii) Observed Ammonia Nitrogen Production

Figure 5.49 shows that the concentration of ammonia nitrogen increased over time for anoxic 20°C cultures using PH. The change in ammonia nitrogen concentration over time is referred to as the observed ammonia nitrogen production rate. For the two CAA cultures, the observed production rate was 4.32 and 5.52 mg NH_3-N/(g VSS h). This was higher than the 3.2 mg NH_3-N/(gVSS h) realized by the LEH culture.

The unacclimated lactalbumin cultures did not produce ammonia nitrogen. These same cultures did not denitrify (see Figure 5.47). In contrast, the acclimated lactalbumin culture did produce ammonia nitrogen at a rate of 1.3 mgNH_3-N/gVSS·h. This culture did denitrify at a rate of 2.7 mg NO_3-N/L·h. In general, denitrifying cultures also produced ammonia nitrogen.

iv) Variation in pH

The maximum and minimum pHs for the cultures are listed in Table 5.89. For a discussion on the effects pH could have on substrate utilization see Section 5.3.3.c.iv.

Table 5.89 Maximum and minimum pH of Anoxic Lactalbumin Cultures

Date	Temperature (°C)	Maximum pH	Minimum pH
Dec. 7	20	7.9	6.1
July 23	20	7.6	6.1
Apr. 9	14	7.5	7.0
Apr. 13	14	8.0	7.0
Apr. 29	14	7.6	7.0

Figure 5.47 Anoxic Growth on Lactalbumin/LEH at 14°C-Normalized on MLVSS

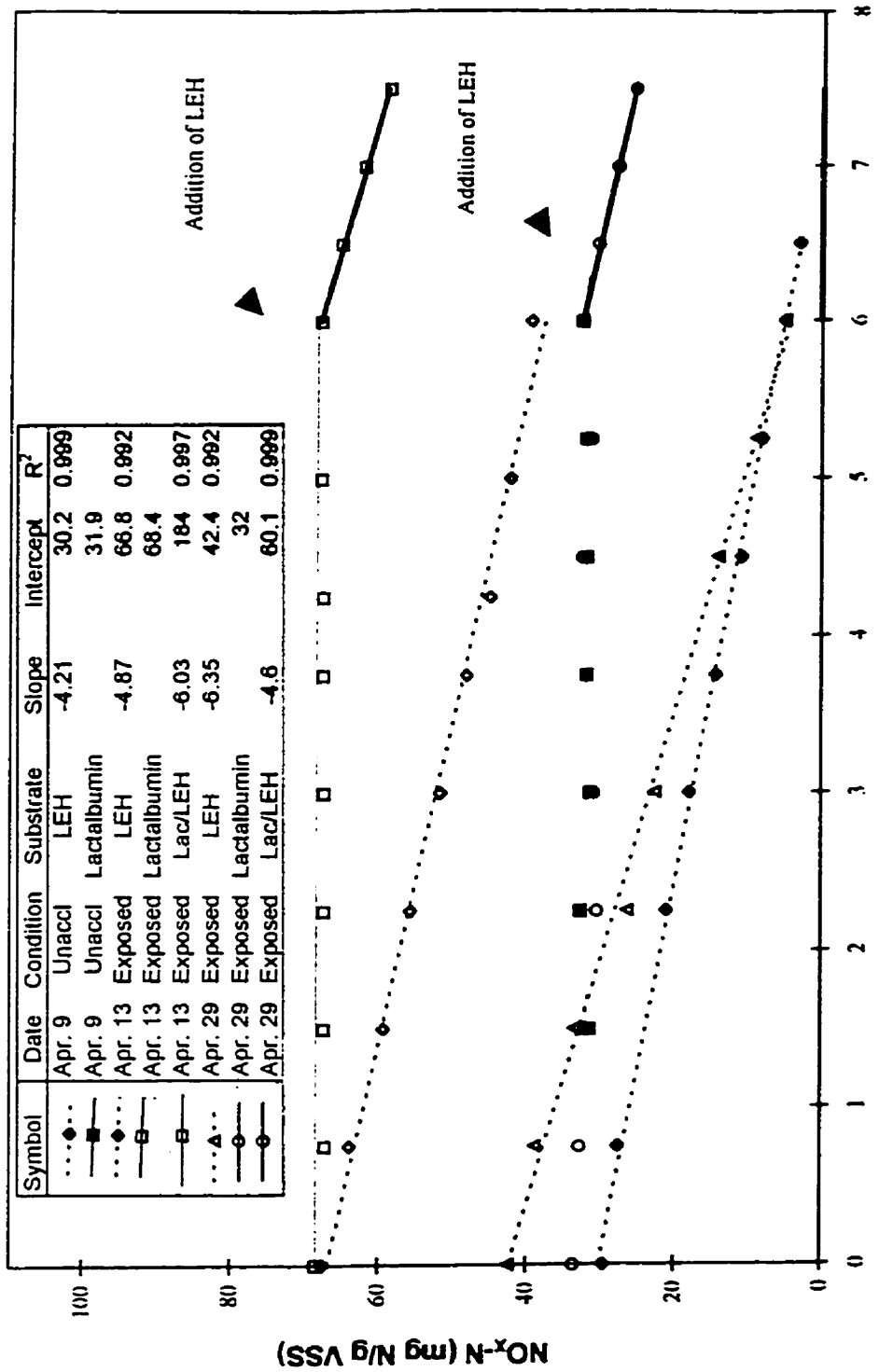
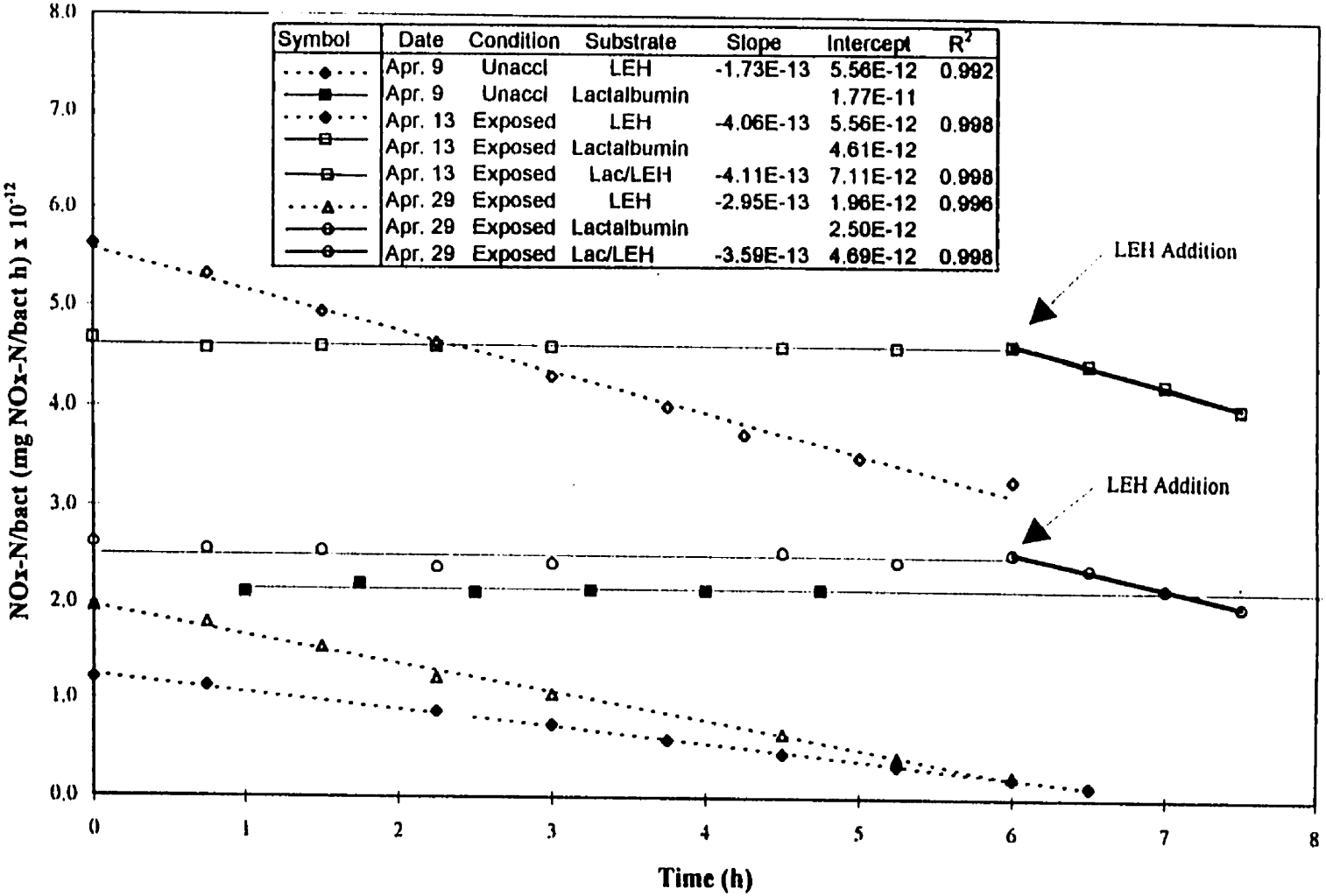


Figure 5.48 Anoxic Growth on Lactalbumin/LEH at 14°C-Normalized on Bacteria Counts



5.4.5. Discussion of Experimental Data for Lactalbumin

i) Initial Response of Cultures Fed Lactalbumin as Substrate

Cultures fed lactalbumin exhibited a high OUR initially. This behaviour would seem to contradict the hypothesis that lactalbumin is a protein resistant to degradation. The lactalbumin is a crude protein that may be contaminated with other milk proteins and sugars. Lactose is one possible contaminant. Lactalbumin dissolved in the salts media did form visible particulates. Approximately 12% of organic strength was attributable to the fraction filterable through a 0.45μm filter. The filterable fraction may contain impurities or serum albumin, a constituent protein of lactalbumin. It was assumed that these contaminants caused the initial increased OUR.

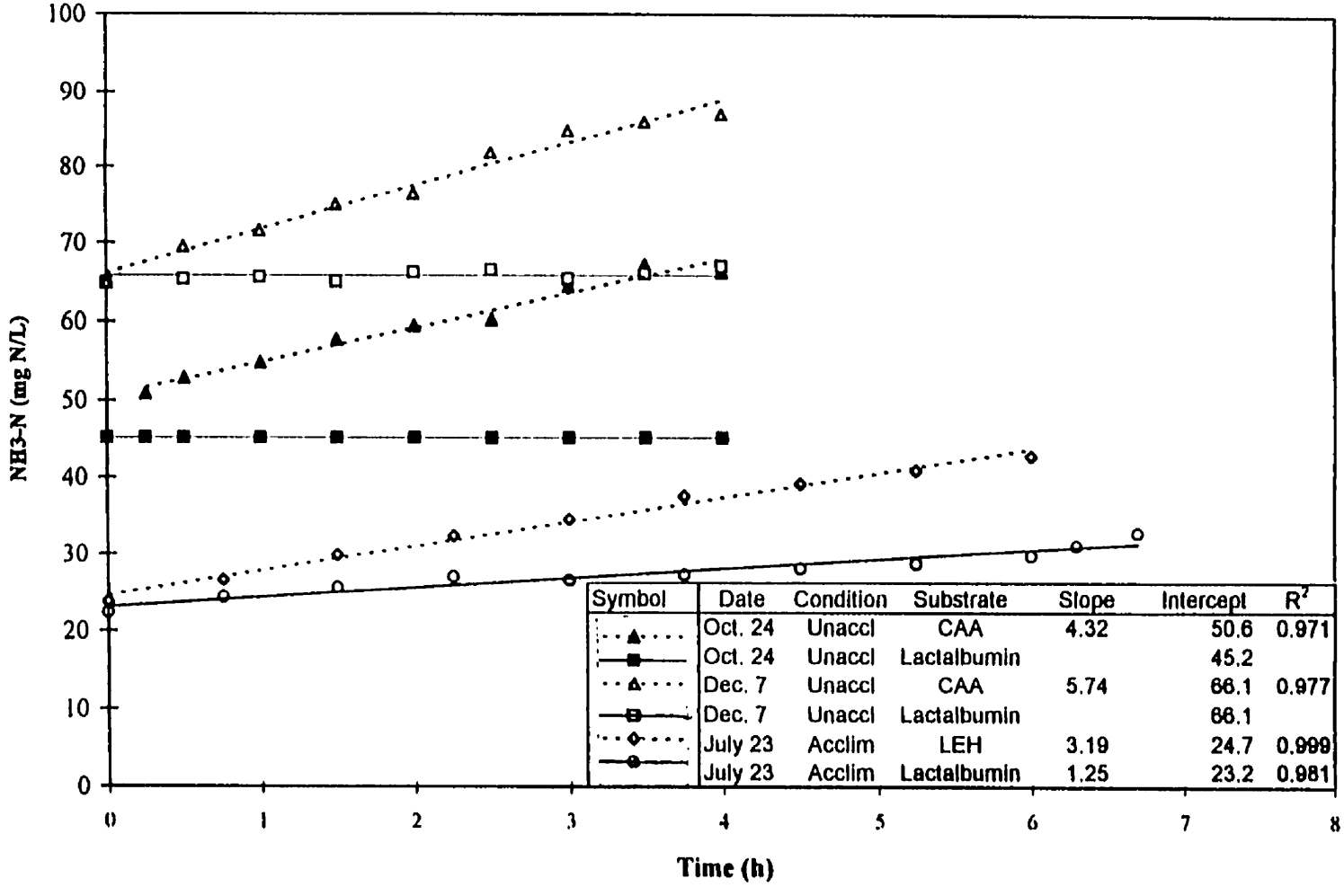
Estimates of the amount of substrate consumed were calculated from the amount of oxygen consumed. The OUR_M was corrected for the basal respiration rate because some of the oxygen demand would be associated with non-bacterial consumption to yield a corrected OUR (OUR_C). (See Section 5.3.2.i - Oxygen Utilization Rates for more of a discussion). Plots of OUR_C versus time were integrated. The amount of oxygen consumed prior to the respiration rate returning to OUR_B was between 8-16 mg O_2/L. An observed yield of 0.71 (see Table 5.43) was used to calculate the equivalent amount of substrate consumed. For every unit of substrate COD consumed, 0.71 units was used for cell synthesis and 0.29 units was used for respiration. The oxygen consumed was therefore equivalent to a substrate COD of 31-57 mg/L (Table 5.90).

Table 5.90 Oxygen/Nitrate Utilization for Lactalbumin Cultures

Date	Temperature °C	Available Substrate mg COD/L	Nitrate Equivalents mg NO_3-N/L	Prior Exposure to Lactalbumin
Oct. 25	20	44	3.39	No
Nov. 29	20	31.2	2.40	No
July 23	20	56.7	4.37	Yes
Apr. 9	14	120	9.28	No
Apr. 13	14	76.6	5.91	Yes
Apr. 29	14	91.0	7.03	Yes

Initial lactalbumin concentration was 464 mg COD/L.

Figure 5.49. Ammonia-N Production for Lactalbumin/LEH Cultures at 20°C - Anoxic



Using the calculation procedure outlined above for the 20°C cultures, 77 - 120 mg COD/L of substrate was consumed over 6 h (Table 5.90) by the 14°C cultures. These calculated values were higher than those at 20°C because the OUR_M did not return to the basal level for acclimated cultures. This would suggest that the exposed cultures were using the protein. If the portion of the curve prior to the OUR_M reaching a plateau is considered, the COD consumed was between 44 - 78 mg COD/L which is comparable to the consumption at 20°C. Consumption of 44 - 78 mg COD/L is approximately 10-15% of the 460 mg COD/L of lactalbumin. It is reasonable to expect impurities at this level in a crude protein.

Anoxic cultures did not use detectable amounts of nitrate in the presence of lactalbumin and its impurities. In this respect, the anoxic cultures were dissimilar to the aerobic cultures using lactalbumin. Using estimates of impurities levels gained from the response of the aerobic cultures, a theoretical nitrate consumption was calculated using Equation 5.13 and calculated values of available substrate listed in Table 5.90. At 20°C, conversion of the impurities present requires 2.72 - 4.95 mg NO_3-N/L. At 14°C, 6.69-10.5 mg NO_3-N/L would be required if all the impurities present were consumed. In Table 5.91 shows the amount of nitrate consumed for each of the lactalbumin cultures. Decreases of this magnitude would have been detectable suggesting that the anoxic cultures did not use the contaminants present.

$$\frac{\Delta N}{\Delta S} = \frac{1 - Y_{obs}}{2.86} \qquad \text{Eq. (5.13)}$$

where ΔN = nitrate consumed (mg NO_3-N/L)
ΔS = biodegradable substrate consumed (mg COD/L)
Y_{obs} = observed yield (mg COD/mg COD)
= 0.77 (mg COD/mg COD) (Table 5.44)

Table 5.91 Nitrate Consumption for Lactalbumin Cultures

Date	Temperature (°C)	Nitrate Consumption (mg NO_3-N/L)
Oct. 24	20	1.8
Dec. 7	20	3.3
Dec. 9	20	1.8
July 23	20	20.4
Apr. 9	14	1.8
Apr. 13	14	0.4
Apr. 29	14	0.9

5.4.6. Closure

a) Relevance of Protein Structure to Hydrolysis

In this research, the uptake and hydrolysis of three model proteins was examined under anoxic and aerobic conditions. These proteins, by virtue of their high molecular weight would have to be degraded by exoenzymes before they would be available as growth substrates and thus are defined as slowly biodegradable substrates. From this study, it appears protein structure is important in determining the rate of uptake and hydrolysis.

α-Casein and ovalbumin are considered soft proteins because they have minimal cross-linking between segments of the protein molecule. α-Casein has a number of physiochemical properties that indicate that the tertiary structure of this protein is more flexible than typical globular proteins (Swaisgood, 1993). The protein contains domains of rather unstable structure that may result in an appreciable fraction of the molecular backbone and side chains being exposed to water (Swaisgood, 1982). Ovalbumin is not highly cross-linked containing only one disulfide bond per molecule (Osuga and Feeney, 1977). As shown previously, uptake and hydrolysis of these proteins may or may not be rate limiting depending on the experimental conditions. For example at 14°C under either aerobic or anoxic conditions, there were occasions when the protein was utilized more quickly than the hydrolysate.

Lactalbumin is a protein mixture consisting of β-lactoglobulin, α-lactalbumin and serum albumin. Lactalbumin can be thought of as a hard protein because it contains β-lactoglobulin that is cross-linked by two disulfide bonds. Swasigood (1982) suggested that the presence of a thiol group in β-lactoglobulin limits the accessibility of a portion of this protein to chemical reagents. Barurquio and Van de Voort (1988) suggest as a consequence of the manufacturing process, the proteins present in lactalbumin denature resulting in the loss of solubility.

The cultures receiving lactalbumin exhibited low OUR_C and NUR in comparison to the companion PH culture indicating that uptake and hydrolysis of this protein was very slow. This behaviour was seen with aerobic and anoxic cultures at both 20 and 14°C. Acclimated and non-acclimated cultures at 20°C produced comparable results suggesting that the observed behaviour was not the result of culture conditions.

Slowly biodegradable organics require: uptake, storage, and enzymatic degradation before being used as a growth substrate. Uptake and storage occurring prior to hydrolysis is consistent with enzymes being located within the floc (Boczar *et al.* (1992), Dold *et al.* (1991) and Frolund *et al.* (1995)). Uptake and storage of substrate are considered to be essentially immediate (Dold *et al.*, 1980) and therefore were excluded from the list of significant biotransformations described in ASM2. With lactalbumin having limited solubility, uptake and storage may not be immediate. Lactalbumin may be too large for enmeshment in the floc. If this was the case, the bacteria would have to colonize the protein or the protein has to come into contact with the floc before hydrolysis could proceed.

Even if uptake and storage were not rate limiting events, there is experimental evidence suggesting that hydrolysis of lactalbumin will be proceed more slowly than α-casein and ovalbumin because of enzyme accessibility dictated by the overall protein structure. Mitchell and Marshall (1989) examined the hydrolysis of caseins and whey proteins by the heat stable proteases of *Pseudomonas fluorescens*. Specific activities of the proteases were significantly higher on caseins (e.g. α, β and κ-casein) than on the three whey proteins (e.g. β-lactoglobulin, α-lactalbumin and bovine serum albumin). The investigators concluded that whey proteins are generally more resistant to proteolysis by heat stable proteases from *Pseudomonas sp.* than are caseins. They further reasoned that the resistance of whey proteins to proteolysis is probably due to their compact tertiary structure which protects most of the peptide bonds. This study is particularly applicable because *Pseudomonas fluorescens* could represent from 13-96.8% of a denitrifying culture depending on the carbon source (See Table 2.4).

In summary, hydrolysis does not limit the rate of utilization for all substrates defined as slowly biodegradable. For the model proteins used in this experimental work, molecular weight does not seem to be the exclusive governing principal determining the rate of hydrolysis. Uptake and hydrolysis did not limit ovalbumin utilization but it did lactalbumin. The overall structure seems to be important in defining its rate of hydrolysis.

b) Hydrolysis as Affected by the Presence of Oxygen or Nitrate

Henze *et al.* (1986) hypothesized that hydrolysis under anoxic conditions proceeds at a much slower rate than it does under aerobic conditions. The task-group developing ASM2 accepted this hypothesis. When

α-casein and ovalbumin were used as substrates, hydrolysis was not rate limiting in most cases. In these particular instances, the rate of protein utilization was limited by the rate of synthesis occurring on peptides and amino acids derived from the protein. Respiration rates expressed on the basis of oxygen equivalents derived from approximate repeat runs using PH (Table 5.23 and Table 5.24) indicates that there was no significant difference in the rate of respiration under aerobic and anoxic conditions at 20°C. However, at 14°C the respiration rates normalized on either MLVSS or bacteria counts were significantly different. The rate of respiration under aerobic conditions was approximately 100-500% higher than that under anoxic conditions. This would suggest that for some conditions synthesis under anoxic conditions proceeds at a much slower rate than it does under aerobic conditions and not hydrolysis.

5.5. Discussion of Assumptions used in Batch Protocol

Three assumptions were inherent to the batch protocol:

1. Monod kinetics are applicable; therefore the rate of nitrate or oxygen utilization is proportional to the amount of substrate oxidized.
2. Substrates having equal observed yields would consume the same amount of oxygen or nitrate for every unit of substrate COD consumed. Comparing the rate of oxygen utilization for two substrates having equal yields under conditions where Monod kinetics are applicable is the same as comparing their rates of substrate consumption.
3. The protein requires hydrolysis prior to becoming available as a growth substrate while the protein hydrolysate does not.

These assumptions make it possible to compare the OURs/NURs of the protein/PH pairs and attribute differences in the respiration rates to substrate uptake and hydrolysis.

5.5.1. Monod Kinetics

In assuming that Monod kinetics were applicable, the rate of nitrate or oxygen utilization was proportional to the amount of substrate oxidized. That is, if the NUR or OUR was high, then large amounts of substrate COD were being oxidized. A cell may accumulate substrate in excess of growth requirements within

its interior. Examples of such microbial storage products are glycogen and poly-β-hydroxybutyric acid (PHB). There would be an increase in the amount of cell mass as measured by MLVSS or COD due to the presence of the storage products. If this was to occur, the rate of measured substrate disappearance would exceed the rate of oxygen utilization. In the context of the current experimental program, if a significant amount of storage did occur, the OUR_M would underestimate the rate of substrate uptake and hydrolysis.

5.5.2. Equal Observed Yields

There is no significant difference between the observed yield for the protein and PH utilizing cultures under either aerobic or anoxic conditions. The 95% CL for the protein utilizing cultures were presented in Table 5.83. The 95% CL encompass the mean values of the PH cultures outlined in Table 5.45 so there is no significant difference in the observed yield .

5.5.3. Protein Hydrolysis

When considering substrate transport, it is important to recognize that the bacteria of activated sludge are predominantly Gram-negative (Hawkes, 1963). The mechanism of substrate transport differs significantly in Gram-negative and Gram-positive bacteria. Many Gram-negative bacteria exclude hydrophobic, amphiphilic and hydrophillic molecules above a given size limit although there are exceptions (Hancock, 1991). A better approach to differentiating between rapidly and slowly biodegradable organics is based on a size exclusion limit. Porins are water filled protein channels, that allow small hydrophilic molecules to diffuse through the phospholipid and lipopolysaccharide layers of the cell wall of Gram-negative bacteria. Diffusion through non-selective porins is generally limited to compounds with sizes less than the porin exclusion limit of 600-1400 Da.

As mentioned previously, slowly biodegradable substrates require hydrolysis before becoming available growth substrates whereas readily biodegradable substrates can be used directly by the cell (Ekama *et al.*, 1986). The three proteins used in the rate study are slowly biodegradable substrates because of their large size and high molecular weight (18-45 kd). The protein hydrolysates, CAA and LEH, are a mixture of dipeptides and oligopeptides. CAA and LEH have MW of 247 and 302 Da, respectively. It was assumed that hydrolysis of these small protein fragments was not needed before they diffused through the porins of the outer

membrane of the cell. Use of protein hydrolysates or hydrolysized proteins once inside the cytoplasmic region of the cell would be described by Monod growth kinetics.

6. Conclusions and Comments

Conclusions drawn from this research are listed below:

1. For the SBR, biosolids production under anoxic conditions exceeded that under parallel aerobic conditions at both 20°C and 14°C. Similarly, the observed yield realized under anoxic conditions was higher than those under aerobic conditions.
    - The economic evaluations of McClintock *et al.* (1988) and Ip *et al.* (1987) found carbon removal under anoxic conditions to be more cost effective than under aerobic conditions. Cost reductions were recognized principally through decreased biosolids production. The results of this research are therefore in direct conflict with these earlier findings.
2. A factorial analysis revealed that there were on average, more bacteria in the anoxic reactor than in the aerobic reactor. A hypothesis that predation is a causative factor was put forth since stalked protozoan were observed in the aerobic system but not the anoxic system.
    - The impact of predation on the bacterial population was not acknowledged in the development of ASM2. As well, the death and lysis coefficient of ASM2 is invariant with temperature and SRT. However, the type and number of predators is known to be a function of temperature and SRT.
    - Under conditions of equal specific utilization rates and stoichiometry, a predenitrification reactor will have fewer cells than a post-denitrification reactor due to the presence of predators. With a higher number of bacteria in the post-denitrification reactor, there will be a higher overall rate of removal.
3. The rates of hydrolysis of proteins with molecular weights ranging between 18,000 and 45,000 Da were not significantly different.
    - The rate of hydrolysis of slowly biodegradable proteins is independent of the length of the protein molecule over this size range. A large molecule does not take longer to degrade than a small molecule.

4. The overall protein structure is important in defining the rate of uptake and hydrolysis. Proteins containing a high degree of cross-linking undergo uptake and hydrolysis very slowly. For proteins having minimal cross-linking, uptake and hydrolysis may not be the rate limiting step.
    - The uptake and hydrolysis of the soluble proteins α-casein and ovalbumin were not rate limiting at 14°C or under aerobic conditions. Under anoxic conditions at 20°C, uptake and hydrolysis of α-casein were rate limiting.
        - The characterization protocol of Mamais *et al.* (1993) is based on solubility. It would have incorrectly characterized the soluble proteins of α-casein and ovalbumin as rapidly biodegradable proteins. Given their high molecular weight, these proteins require enzymatic degradation before use as a growth substrate and thus are slowly biodegradable.
        - Ekama *et al.* (1976) suggested a characterization protocol based on metabolic response. It would have characterized α-casein and ovalbumin as rapidly biodegradable proteins at 14°C or under aerobic conditions. Under anoxic conditions at 20°C, α-casein would have been characterized as slowly biodegradable. Ovalbumin and α-casein is a slowly biodegradable substrate regardless of the temperature or terminal electron acceptor. The kinetics of hydrolysis change in response to these factors. In using Ekama's protocol the characterization is confounded with the kinetics of hydrolysis.
    - Slow uptake and hydrolysis of lactalbumin, a cross-linked protein forming settleable particles, were observed regardless of culture conditions. This may be a consequence of the limited solubility of the protein or the poor accessibility of regions of the protein to hydrolytic enzymes. If the protein is too large to become enmeshed in the floc, hydrolysis will proceed only when the protein has become colonized or comes into contact with the floc.
5. An operational definition of slowly biodegradable substrates having a molecular weight of approximately 1000 Da. For proteins with a molecular weight much greater than 1000 Da, the overall

protein structure has no impact on whether or not extracellular enzymes are required. For proteins with a molecular weight near 1000 Da, overall protein structure is important.

6. AODC is a promising method for enumerating the bacterial populations under both aerobic and anoxic, mixed culture conditions. Under all conditions tested, it was possible to differentiate between bacteria and other components of the activated sludge floc.
    - Direct counts are not likely to be useful as a measurement for full scale, daily process control. However, they will be useful over larger time scales and for research on process mechanisms.
7. There was no significant difference in the normalized respiration rates for aerobic and anoxic conditions expressed on the basis of oxygen equivalents at 20°C. However at 14°C, the anoxic respiration rate was much lower than the aerobic rate. Denitrification kinetics were highly temperature sensitive as indicated by a θ_{DN} of 1.25 for kinetics determined on the basis of bacteria counts. Carbon removal under aerobic conditions appeared to be less temperature sensitive as indicated by a value of θ of 1.03 on the basis of bacterial counts.
    - To maintain the same level of denitrification under both winter and summer conditions, higher SRTs have to be maintained during the winter.
    - Henze *et al.* (1986) hypothesized that hydrolysis under anoxic conditions proceeds at a much slower rate than it does under aerobic conditions. The task-group developing ASM2 accepted the hypothesis. At 14°C, α-casein and ovalbumin were used as quickly as the protein hydrolysate. The experimental data shows that hydrolysis does not proceed more slowly under anoxic conditions; however, respiration does.
8. The observed yield under conditions of active substrate utilization for the aerobic and anoxic reactors was not significantly different from the 0.67 g COD/g COD value used in ASM2. The observed yield was invariant with temperature for both aerobic and anoxic reactors.
9. Performance of the aerobic reactor was found to be better than the anoxic reactor at 20°C in terms of effluent COD and solids. At 14°C equivalent performance was seen with respect to the effluent COD but the aerobic reactor produced an effluent with higher solids. At either 14 or 20°C, the average performance of these reactors was acceptable with respect to typical effluent discharge standards.

10. There was no significant difference at the 95% confidence level between the consumptive ratios at 14°C and 20°C. The consumptive ratios derived at 14°C and 20°C were compared to theoretical values calculated from a equation proposed by Stensel (1981). There was a significant difference between the experimental and calculated value at 20°C but not at 14°C.

7. Recommendations for Future Work

1. AODC should be further pursued as a enumeration technique for activated sludge. A more complete data base is required for the technique to be fully evaluated. Automated image analysis should be pursued as this technique is time intensive.
2. Enumeration techniques differentiating the respiring population from the total population should be evaluated. This may be accomplished with improved current techniques or by using fluorophores.
3. The mechanism for uptake and hydrolysis of large proteins is an important area warranting further study because proteins account for 28% of the influent strength of raw sewage as measured in terms of COD (Raunkjer *et al.*, 1994). Using the data of Hunter and Heuklekian (1965) settleable and supracolloidal material account for 23.9% and 35.8% of influent COD, respectively. In a typical activated sludge plant 60% of the solids are removed in the primary clarifier. A primary clarifier maybe excluded from the treatment train when pre-denitrification is implemented. Approximately 15% of the input would then be settleable and supracolloidal material.
4. Ectoenzymes are in constant communication with the originating bacterium and thus are subject to feedback. Under conditions of high concentrations of rapidly biodegradable substrate, hydrolysis as carried out by these enzymes may cease. If these enzymes are responsible for hydrolysis then their dynamic nature should be reflected by mathematical models such as ASM2.
5. Further study on the uptake mechanism for large particles is required as it is unclear how these particles come in contact with hydrolytic enzymes and how they are subsequently degraded. ASM2 assumes that substrate uptake and storage is immediate and thus is not an important biotransformation. This may not be the case. Substrate uptake and storage will be important for nitrogen removal in the pre-denitrification configuration. When this configuration is implemented, influent sewage is often added directly to the anoxic basin. Large particles typically removed during primary clarification are present in the influent sewage .
6. The current experimental program should be carried out at lower temperatures to quantify the impact of temperature on hydrolysis and denitrification kinetics.

7. The role of the non-bacterial population warrants further attention especially since most mathematical models such as ASM2, describe bacterial growth. The loss of cells due to predation and decay is mathematically described by the lysis coefficient and is proportional to the active bacterial mass.

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9. Glossary of Terms

A	-	wetted area of 25 mm filter (μm^2)
a	-	the specific maintenance rate (d^{-1})
a_{net}	-	area of net micrometer field (μm^2)
AODC	-	acridine orange direct counts
Ala	-	alanine
ANOVA	-	Analysis of Variance
AS	-	activated sludge
Asn	-	asparagine
ASM1	-	IAWPRC Activated Sludge Model No. 1
ASM2	-	IAWQ Activated Sludge Model No 2
ASTM	-	American Society for Testing and Materials
Asp	-	aspartic acid
APase	-	alkaline phospatase
ATP	-	adenosine triphosphate
b	-	net endogenous respiration coefficient (d^{-1})
b'	-	slope of the OUR curve when plotted against time (d^{-1})
b_H	-	death and decay rate (d^{-1})
BOD_5	-	5 day biological oxygen demand ($mg \cdot L^{-1}$)
BSA	-	bovine serum albumin
CAA	-	N-Z Amine A
CAmp	-	cyclic adenosine monophosphate
CL	-	confidence limit
COD	-	chemical oxygen demand ($mg \cdot L^{-1}$)
CTC	-	5-cyano-2,3-ditolyl tetrazolium chloride
Cys	-	cysteine
Da	-	Daltons
D	-	dilution factor
DAPI	-	4',6-diamidino-2-phenyl indole
Decr	-	decrease
df	-	degrees of freedom
DNA	-	deoxyribonucleic acid
DO	-	dissolved oxygen concentration ($mg \cdot L^{-1}$)
Dorm	-	dormant bacteria

DSA	-	dynamic solids age (d)
EAS	-	electron acceptor system
EPA	-	Environmental Protection Agency
EPS	-	extracellular polymeric substance
ETS	-	electron transport system
F:M	-	food to microorganism ratio
Glu	-	glutamine
His	-	histidine
HPA	-	hide powder azure
HRT	-	hydraulic residence time (d)
IAWPRC	-	International Association on Water Pollution Research and Control (now named International Association for Water Quality)
IAWQ	-	International Association for Water Quality
Ile	-	isoleucine
Incr	-	increased
INT	-	2-(p-iodophenyl-3-(p-nitrophenyll)-5-phenyltetrazolium chloride
k	-	temperature dependent rate coefficient
K_M	-	Michaelis-Menten half saturation coefficient
K_S	-	half velocity coefficient for growth (mg COD·L^{-1})
K_H	-	maximum specific rate of hydrolysis (d^{-1})
K_X	-	half saturation coefficient for hydrolysis (mg COD·L^{-1})
K_N	-	half saturation coefficient for nitrate-nitrogen (mg N·L^{-1})
LEH	-	lactalbumin enzymatic hydrolysate
Leu	-	leucine
Lys	-	lysine
MED	-	maintenance energy demand
Met	-	methionine
MLVSS	-	mixed liquor volatile suspended solids (mg·L^{-1})
MTT	-	methylthiazolydiphenyltetrazolium
MW	-	molecular weight
n	-	average number of bacteria per net micrometer field
N	-	concentration of nitrate-nitrogen (mg N·L^{-1})
N_b	-	number of bacteria per net micrometer field
NC	-	no change
ng	-	not given
N-Serve		2-chloro-6(trichloromethyl)-pyridine

NBT	-	nitroblue
Non-Rep	-	non-replicating bacteria
NUR	-	nitrate and nitrite utilization rate (mg NOx-N·L^{-1}·h^{-1})
NR	-	nitrogen removal
OUR	-	oxygen utilization rate (mg·L^{-1}·h^{-1})
OUR_B	-	basal oxygen utilization rate (mg·L^{-1}·h^{-1})
OUR_C	-	corrected oxygen utilization rate (mg·L^{-1}·h^{-1})
OUR_M	-	measured oxygen utilization rate (mg·L^{-1}·h^{-1})
PH	-	protein hydrolysate
PHB	-	poly-β-hyroxybutyric acid polymers
Q_e	-	effluent volume (L)
Q_W	-	wastage volume (L)
r	-	correlation coefficient
Rep	-	replicating bacteria
RSD	-	relative standard deviation
RNA		ribonucleic acid
S	-	concentration of biodegradable carbonaceous material (mg·L^{-1})
S_0	-	initial carbanceous substrate concentration (mg·L^{-1})
S_x	-	standard deviation of all values in the sample population
SBCOD	-	slowly biodegradable organics
SBR	-	sequencing batch reactor
Ser	-	serine
SRT	-	solids retention time (d)
Std Dev	-	standard deviation
STP	-	sewage treatment plant
T	-	temperature (°C)
T_n	-	test criterion for single outliers
TCA	-	trichloroacetic acid soluble protein
TKN	-	total Kjeldahl nitrogen (mg N·L^{-1})
Tr	-	thrice weekly
TSS	-	total suspended solids (mg ·L^{-1})
TTC	-	2,3,5,-triphenyltetrazolium chloride
Typ	-	tryptophan
Tyr	-	tyrosine
Tween 80	-	Polyoxyethylene 20 Sorbitan Monoleate
V	-	volume

Val	-	valine
VFA	-	volatile fatty acid
VSS	-	volatile suspended solids ($mg \cdot L^{-1}$)
VBNC	-	viable but non-culturable bacteria
W	-	weekly
X	-	cell concentration ($mg \cdot L^{-1}$)
$\bar{X}$	-	mean of the sample population
X_D	-	concentration of denitrifiers (mg $COD \cdot L^{-1}$)
$X_{D,V}$	-	concentration of viable denitrifiers (mg $COD \cdot L^{-1}$)
X_H	-	concentration of heterotrophes (mg $COD \cdot L^{-1}$)
$X_{H,V}$	-	concentration of viable heterotrophes (mg $COD \cdot L^{-1}$)
X_0	-	initial microorganism concentration
X_n	-	largest value in the sample population
X_S	-	concentration of slowly biodegradable substrate (mg $COD \cdot L^{-1}$)
Y	-	true cell yield (g COD ·g COD^{-1})
Y_{Obs}	-	biomass net yield (g COD ·g COD^{-1})
α	-	the growing fraction
μ	-	specific rate of organism growth (d^{-1})
μ_m	-	maximum specific rate of growth (d^{-1})
μ_M	-	Michaelis Menten maximum velocity (d^{-1})
θ	-	modified Arrhenius coefficient
θ_{DN}	-	modified Arrhenius coefficient for denitrification

Appendix A

A.1 Analytical Protocols

Ammonia Nitrogen

Ammonia nitrogen measurements were done using ion chromatography on a Dionex Ion Chromatograph equipped with an CS12 column (Dionex, Sunnyvale, CA) and a conductivity cell. A gradient technique using 23 mM methansulfonic acid and water was designed specifically for this matrix. The loop size for this analysis was 100 µl with a partial loop injection of 50 µl.

Oxygen Utilization Rate (OUR)

Direct measurement of oxygen is possible using a YSI Model 54 A Membrane Electrode , Oxygen (421F) or an alternative (see equipment list). Prior to starting the procedure the dissolved oxygen meter is calibrated. The mixed liquor is placed in a BOD bottle with a stir bottle and placed on a Styrofoam insulated stir plate. A dissolved oxygen meter in conjunction with a personal computer is used to log the dissolved oxygen every 15 s.

Nitrate and Nitrite Nitrogen

Nitrate measurements were done using ion chromatography on a Dionex Ion Chromatograph equipped with an AS4A column (Dionex, Sunnyvale, CA) with UV detection at 214 nm. For the 20°C work (excluding the Death and Decay study) a 18 mM bicarbonate/carbonate solution was used at 2 mls/min. When the temperature was decreased to 14 °C, the matrix changed and a unidentified species coeluded with the nitrate. This necessitated changing the eluent to 15 mM sodium chloride (NaCl) solution. When NaCl was used as an eluant, the column was regenerated with NaOH at the end of each run.

A calibration curve was done with six standards ranging in concentration from 1 to 40 mg NO_3-N/l and 1-10 mg NO_2-N/l. The linear correlation was excellent with correlation coefficients typically exceeding 0.99. The loop size for this analysis was 100 µl with a partial loop injection of 50 µl.

pH

Direct measurement with a pH meter assembly

Suspended Solids

Total Suspended Solids Dried at 103-105°C as per Standard Methods (1995) (SM209C)

Volatile Suspended Solids

Fixed and Volatile Suspended Solids Ignited at 550°C as per Stamdard Methods (SM2540D)

TKN

Semi-micro Kjeldahl Method (SM4500C) followed by Ammonia Nitrogen determination as described above.

Enzyme Assay

API-Zym - Bio Merieux SA

1. The mixed liquor sample was homogenized using a Poter tube. In view of the absence of growth during the test, no paticular precaution concerning sterility or incubation need be taken.
2. An incubation tray and lid were setup. Approximately 5 mL of tap water was added to the incubation tray to provide humid atmosphere during incubation. Remove the API Zym strip from the sealed envelope and place it in the incubation tray.
3. With a micropipettor, 65 µL was added to culture was added to each cupule of the strip.
4. After inoculation, the plastic lid was placed on the tray and incubated for 4 h at 37°C.
5. After incubation, one drom of ZYM A and one drop of ZYM B reagents were added to the strip.
6. The strip was exposed to UV light for approximately 5 minutes.
7. The colours of the strip were compared to a colour strip supplied by the manufacturer

A.2 Sample Preservation

Table A.1 Sample Preservation

Parameter	Presevation	Preservation Time
COD	Refrigerate add H_2SO_4 to pH<2	28 d
Ammonia	Refrigerate add H_2SO_4 to pH<2	28 d
NOx-N	Refrigerate add H_2SO_4 to pH<2	28 d
TKN	Refrigerate add H_2SO_4 to pH<2	28 d
Cell Counts	Addition of Formaldehyde	

Samples for COD, Ammonia, NOx-N and TKN were sometimes frozen to extend the preservation time.

A.3 Equipment

Table A.2 Pumps Required for SBR Operation

Use	Pump Manufacturer & Location	Model	Description	Pump Head Manufacturer & Location	Model
Acid Delivery	Cole-Parmer (Chicago, IL)	7543-12	12 rpm Acid Additon	Heads Purchased with Pumps	
Effluent	Cole-Parmer (Chicago, IL)	7553-80	Masterflex Standard Drive	Barnant Co. (Barrington, IL)	Masterflex Size 18 MR-07018-20
Feed	Cole-Parmer (Chicago, IL)	7520-35	Standard Console Drive	Barnant Co. (Barrington, IL.)	Easy Load Masterflex Model 7518-00
Nitrate	Cole-Parmer (Chicago, IL)	7013-52	Master Servodyne 0-300 rpm	Barnant Co. (Barrington, IL)	Masterflex Size 13 Model 4445-30
Waste	Cole-Parmer (Chicago, IL)	7553-80	Masterflex Standard Drive 1-100 RPM	Barnant Co. (Barrington, IL)	Masterflex Size 17 MR-07017-20

Table A.3 Equipment Required for SBR Operation

	Manufacturer & Location	Description
pH Controller	Cole-Parmer (Chicago, IL)	pH/ORP Controller Model 5656-00
	Cole-Parmer (Chicago, IL)	pH/ORP Controller PHCN-36
pH Electrodes	VWR Scientific	Baxter/Canlab sealed pH electrodes 34105-023
Sequence Timer	Mastercraft (Canadian Tire)	Digital Lamp and Appliance Timer Model 52-8851-2
Interval Timer	Potter-Brumfield	
Aeration	Rolf C Hagen Inc. (Montreal, Que.)	Optima Air Pump Air output 500 cc/min-5500 cc/min
Reactor Mixers	Caframo (Wiarton, Ont.)	Model R2RT-64, Setting 2.5
Mixer for Feed Tank	Heldolph (Canlab)	Model 50115

Table A.4 Equipment Required for SBR Characterization and Batch Rate Studies

Type	Manufacturer
Acid Delivery Pumps	Cole Parmer Standard Servodyne 0-300 rpm Cat 4440-30 2 Pump Heads Model 7013-52
Centrifuge	Sorvall RC5B with SS-34 Rotor (Refrigerated) Sorvall MC12V Microcentrifuge
Ion Chromatograph	Dionex (Sunnydale California)
Microscope	Nikon Labophot 2Awith Episcopic-Fluorescence Attachment EF-D
Dissolved Oxygen Meters	Orion Model 810 Dissolved Oxygen Meter
pH Control	New Brunswick Scientific Automatic pH Controller Model pH410 Ωmega pH/ORP Controller pHCN-36 YSI Model 57 Oxygen Meter pH/ORP Controller Model pH410
pH Meters	Corning Model 810 Ion Analyzer 250 Radiometer PHM82 Standard Meter

A.4 Sequence of Rate Experiments

Table A.1 Sequence of 20 °C Batch Rate Studies

Electron Acceptor	Protein	Hydrolysate	Date
Aerobic	Ovalbumin	CAA	Nov. 7/95
			Nov. 27/95
Aerobic	α-casein	CAA	Nov. 2/95
			Dec. 6/95
Aerobic	Lactalbumin	CAA	Oct. 25/95
			Nov. 29/95
		LEH	July 23/96
Anoxic	Ovalbumin	CAA	Oct. 20/95
			Dec. 4/95
Anoxic	α-casein	CAA	Oct. 30/95
			Dec. 1/95
Anoxic	Lactalbumin	CAA	Oct. 24/95
			Dec. 7/95
			Dec. 9/95
		LEH	July 23/96

Table A.2 Sequence of 14°C Batch Rate Studies

Electron Acceptor	Protein	Hydrolysate	Date
Aerobic	Ovalbumin	CAA	Apr. 4/96
			May 5/96
Aerobic	α-casein	CAA	May 7/96
			May 9/96
Aerobic	Lactalbumin	LEH	Apr. 9/96
			Apr. 29/96
Aerobic		LEH	Mar. 27/96
		CAA	
Anoxic	Ovalbumin	CAA	Apr. 4/96
			May 5/96
Anoxic	α-casein	CAA	Mar. 29/96
			Apr. 24/96
			May 9/96
Anoxic	Lactalbumin	LEH	Apr. 9/96
			Apr. 29/96
Anoxic		LEH	Apr. 1/96
		CAA	

Appendix B

Oxygen Utilization Data Rate Studies

Nov. 2 /95

Temperature 20 C Protein: α-Casein
PH: CAA
Basal Respiration 0.061 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.1	0.26	0.1	0.267
0.4	0.274	0.6	0.277
0.9	0.277	1.0	0.287
1.3	0.292	1.6	0.36
1.9	0.321	2.1	0.303
2.4	0.297	2.6	0.287
2.8	0.344	3.1	0.306
3.4	0.363	3.6	0.317
3.9	0.368		

Nov. 27 /95

Temperature 20 C Protein: Ovalbumin
PH: CAA
Basal Respiration 0.059 (mg.L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg.(L min))	Time (h)	OUR (mg.(L min))
0.1	0.17	0.0	0.234
0.3	0.196	0.6	0.228
0.8	0.212	1.2	0.248
1.4	0.226	1.9	0.268
2.0	0.276	2.3	0.275
2.6	0.322	2.8	0.293
3.1	0.343	3.1	0.262
3.9	0.341	4.0	0.311
4.1	0.289		

Nov. 7/95

Temperature 20 C Protein: Ovalbumin
PH: CAA
Basal Respiration 0.047 (mg/L.min)

rotein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.2	0.22	0.1	0.335
0.4	0.206	0.6	0.36
0.9	0.213	1.2	0.387
1.4	0.242	1.6	0.381
1.9	0.23	2.1	0.396
2.4	0.246	2.4	0.326
3.4	0.277	2.6	0.452
3.6	0.299	3.1	0.467
3.7	0.277	3.6	0.427
		3.8	0.493

Dec. 6 /95

Temperature 20 C Protein: α-Casein
PH: CAA
Basal Respiration 0.059 (mg.L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg.(L min))	Time (h)	OUR (mg.(L min))
0.1	0.253	0.1	0.253
0.4	0.265	0.6	0.249
0.8	0.309	1.1	0.276
0.9	0.301	1.6	0.293
1.4	0.271	2.1	0.299
1.9	0.268	2.6	0.313
2.3	0.387	3.1	0.328
2.8	0.423	3.6	0.317
3.3	0.446	3.8	0.342
3.8	0.479		

Oct. 25/95

Temperature 20 C Protein: Lactalbumin
PH: CAA
Basal Respiration 0.117 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.1	0.26	0.2	0.27
0.3	0.27	0.7	0.316
1.0	0.2	0.9	0.28
1.6	0.149	1.4	0.3
2.3	0.138	2.1	0.32
2.8	0.142	2.6	0.32
3.4	0.12	3.1	0.33
3.9	0.13	3.6	0.3
		3.9	0.33

Nov. 29 /95

Temperature 20 C Protein: -Lactalbumin
PH: CAA
Basal Respiration 0.051 (mg.L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg.(L min))	Time (h)	OUR (mg.(L min))
0.1	0.387	0.1	0.399
0.4	0.133	0.7	0.385
0.9	0.085	1.2	0.369
1.4	0.072	1.7	0.363
1.9	0.053	2.2	0.376
2.4	0.059	2.7	0.393
2.9	0.056	3.2	0.39
3.4	0.046	3.7	0.399
3.9	0.048	3.9	0.407

Mar. 27/96

Temperature 14 C PH: LEH
PH: CAA

Basal Respiration 0.051 (mg/L.min)

PH Culture-LEH		PH Culture-CAA	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.1	0.304	0.2	0.23
0.3	0.277	0.7	0.252
1.2	0.299	2.1	0.32
1.7	0.239	2.6	0.353
3.1	0.341	3.1	0.351
3.6	0.361	3.6	0.346
4.1	0.337	4.2	0.322
4.6	0.315	4.6	0.341
5.2	0.308	5.1	0.318
5.6	0.308	5.4	0.345
		5.5	0.343
		5.8	0.343

Apr. 13/96

Temperature 14 C Protein: Lactalbumin
PH: LEH

Basal Respiration 0.063 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.3	0.2	0.7	0.237
1.4	0.15	1.1	0.231
2.1	0.129	1.9	0.254
3	0.125	2.6	0.263
3.4	0.127	3.7	0.348
4.5	0.137	4.2	0.313
5.3	0.141	5.0	0.305
5.6	0.143	5.9	0.3
7	0.317		
7.3	0.307		
7.7	0.336		

July 23/96

Temperature 20 C Protein: Lactalbumin
PH: LEH

Basal Respiration 0.188 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.5	0.274	0.2	0.412
1.2	0.237	0.9	0.409
1.9	0.218	1.5	0.553
2.7	0.218	2.2	0.531
3.6	0.211	3.0	0.628
4.2	0.2	3.8	0.695
4.9	0.202	4.5	0.734
5.1	0.2	5.3	0.719
5.7	0.208	5.9	0.729
6.2	0.438		
6.5	0.451		
6.9	0.439		

Apr. 9/96

Temperature 14 C Protein: Lactalbumin
PH: LEH

Basal Respiration 0.058 (mg L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.6	0.164	0.3	0.224
1.2	0.09	1.3	0.261
2.1	0.075	1.9	0.285
2.8	0.069	2.1	0.271
3.5	0.094	2.9	0.297
3.6	0.073	3.6	0.287
4.3	0.058	4.4	0.301
5.1	0.064	5.2	0.31
5.3	0.054		

Apr. 29/96

Temperature 14 C Protein: Lactalbumin
PH: LEH

Basal Respiration 0.031 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.7	0.148	0.4	0.169
1.2	0.101	0.9	0.164
1.4	0.095	1.6	0.178
2.1	0.093	1.8	0.179
2.9	0.094	2.4	0.18
3.6	0.084	2.6	0.187
3.9	0.082	3.1	0.196
4.2	0.079	4.7	0.219
4.3	0.083	5.6	0.237
5.1	0.076		
5.9	0.085		
6.2	0.221		
6.4	0.203		
6.6	0.191		
6.8	0.194		
7.2	0.194		
7.4	0.208		

Apr. 4/96

Temperature 14 C Protein: Ovalbumin
PH: CAA
Basal Respiration 0.045 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.4	0.258	0.2	0.206
0.6	0.268	0.6	0.247
1.2	0.299	1.1	0.261
1.6	0.303	1.6	0.267
2.1	0.313	2.5	0.279
2.7	0.317	2.6	0.273
3.7	0.296	3.2	0.279
4.3	0.284	4.1	0.311
5.2	0.321	4.7	0.276
5.9	0.265	5.9	0.263
		6.1	0.257

May 5/96

Temperature 14 C Protein: Ovalbumin
PH: CAA
Basal Respiration 0.070 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.2	0.229	0.2	0.223
0.4	0.245	0.4	0.198
1.4	0.298	1.0	0.216
2.0	0.323	1.7	0.222
2.2	0.347	2.5	0.24
2.8	0.382	3.2	0.247
3.6	0.433	3.9	0.269
4.4	0.485	5.5	0.299
5.2	0.44	5.6	0.288
5.9	0.296		

May 7/96

Temperature 14 C Protein: β-Casein
PH: CAA
Basal Respiration 0.072 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.3	0.375	0.2	0.23
0.5	0.257	0.9	0.22
1.1	0.25	1.5	0.238
2.7	0.285	1.9	0.251
3.4	0.33	2.6	0.256
4.2	0.4	3.2	0.271
4.7	0.42	3.4	0.258
5.4	0.395	4.7	0.283
		5.1	0.282
		5.3	0.256

May 9/96

Temperature 14 C Protein: Ovalbumin
PH:
Basal Respiration 0.043 (mg/L.min)

Protein Culture		PH Culture	
Time (h)	OUR (mg/(L min))	Time (h)	OUR (mg/(L min))
0.2	0.293	0.1	0.209
0.3	0.27	0.6	0.241
0.9	0.26	1.2	0.231
1.4	0.284	1.6	0.239
1.9	0.264	2.6	0.264
2.4	0.288	3.3	0.248
3.4	0.334	3.9	0.239
4.0	0.363	4.9	0.249
4.7	0.367	5.5	0.239
5.6	0.312	5.7	0.257

Nitrate Utilization Data Rate Studies

Oct. 20/95

Temperature 20 C Protein: Ovalbumin PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	34.2	Slope	4.51	0	34.1	Slope	5.39
0.267	33.5	Intercept	34.3	0.283	31.6	Intercept	33.2
0.5	31.9	R^2	0.998	0.5	30.3	R^2	0.996
1	29.4	F	4792	1	27.8	F	2096
1.5	27.9	df	8	1.5	24.4	df	8
2	25.2			2	22.3		
2.5	22.8			2.5	19.1		
3	20.8			3	17.0		
3.5	18.3			3.5	14.6		
4	16.5			4	12.1		

Oct. 30/95

Temperature 20 C Protein: Casein PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L.)	Linear Regression		Time (h)	NOx-N (mg N/L.)	Linear Regression	
0.0	39.4	Slope	-4.48	0.0	39.7	Slope	-4.71
0.2	38.9	Intercept	39.7	0.2	38.2	Intercept	39.7
0.3	38.4	R^2	0.998	0.3	38.4	R^2	0.995
0.5	38.0	F	5638.9	0.5	38.2	F	1727.1
1.0	34.8	df	9	1.0	35.0	df	9
1.5	32.9			1.5	32.3		
2.0	30.6			2.0	30.1		
2.5	28.5			2.5	28.0		
3.0	26.1			3.0	26.4		
3.5	24.1			3.5	23.0		
4.0	21.9			4.0	20.6		

Dec. 4/95

Temperature 20 C Protein: Ovalbumin PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	32.1	Slope	-3.26	0	31.1	Slope	-6.53
0.5	29.2	Intercept	31.2	0.5	29.3	Intercept	32.1
1	28.4	R^2	0.969	1	25.9	R^2	0.997
1.5	26.2	F	222.2	1.5	22.7	F	2467
2	22.8	df	7	2	19.2	df	7
2.5	23.2			2.5	16.1		
3	21.7			3	12.6		
3.5	20.0			3.5	9.1		
4	18.6			4	5.6		

Dec. 1/95

Temperature 20 C Protein: Casein PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	32.0	Slope	-6.79	0.0	30.3	Slope	-6.34
0.5	30.0	Intercept	32.3	0.5	27.9	Intercept	31.1
1	25.1	R^2	0.994	1.0	25.7	R^2	0.996
1.50	22.0	F	1263	1.5	22.0	F	603
2	19.0	df	7	2.0	18.2	df	7
2.5	14.1			2.5	15.6		
3	12.6			3.0	12.3		
3.5	9.0			3.5	8.1		
4	5.0			4.0	5.8		

May 5/96

Temperature 14 C Protein: Ovalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0.0	33.5	Slope	-4.73	0.0	33.7	Slope	-3.21
0.8	30.3	Intercept	33.9	0.8	30.6	Intercept	32.7
1.5	26.7	R^2	0.998	1.5	27.6	R^2	0.984
2.3	23.3	F	6169.0	2.3	24.8	F	442.7
3.0	20.0	df	7	3.2	21.9	df	7
3.8	16.2			3.8	19.8		
4.5	12.8			4.5	17.5		
5.3	9.0			5.3	17.2		
6.0	4.9			6.0	14		

Apr. 24/96

Temperature 14 C Protein:α-casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0.00	37.6	Slope	-5.20	0	37.5	Slope	-2.14
0.50	35.1	Intercept	37.0	0.75	35.2	Intercept	36.6
1.25	30.6	R^2	0.993	1.5	33	R^2	0.978
2.00	26.2	F	875.0	2.25	31.2	F	315.3
2.75	21.7	df	7	3	29.4	df	7
3.50	17.6			3.75	28		
4.25	14.3			4.5	26.9		
5.00	11.1			5.25	25.3		
5.75	8.6			5.75	25.3		

Mar. 29/96

Temperature 14 C Protein:α-casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	27.6	Slope	-1.819	0.00	27.4	Slope	-2.2
0.75	25.8	Intercept	26.6	0.75	23.8	Intercept	26.6
1.5	23.3	R^2	0.962	1.50	24.1	R^2	0.971
2.25	21.7	F	177.8	2.25	20.5	F	235.1
3	20.3	df	7	3.00	20.6	df	7
3.75	19.2			3.75	18.8		
4.5	18.3			4.50	16.8		
5.25	17.2			5.25	15.2		
6	16.7			6.00	13.2		

May 9/96

Temperature 14 C Protein:α-casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	40.2	Slope	-3.88	0	40	Slope	-4.14
0.5	39.1	Intercept	40.3	0.75	34.9	Intercept	38
1.25	35.9	R^2	0.99	1.5	31.3	R^2	0.979
2	32.5	F	660.9	2.25	27.6	F	325.4
2.75	28.6	df	7	3	23.8	df	7
3.5	25.9			3.75	21.6		
4.25	23.0			4.5	19.2		
5	20.8			5.25	16.8		
5.75	19.4			5.75	15.7		

Oct. 24/95

Temperature 20 C Protein: Lactalbumin

PH: CAA

Protein Culture			PH Culture			
Time (h)	NOx-N (mg N/L.)	Linear Regression	Time (h)	NOx-N (mg N/L.)	Linear Regression	
0.0	26.5	Slope	0.3	24.0	Slope	-5.11
0.3	25.5	Intercept	0.5	22.4	Intercept	24.3
0.5	25.3	R^2	1.0	18.8	R^2	0.987
1.0	25.2	F	1.5	15.2	F	367.6
1.5	24.6	df	2.0	13.7	df	7
2.0	24.5		2.5	11.2		
2.5	23.9		3.0	8.8		
3.0	24.4		3.5	6.5		
3.5	24.4		4.0	4.7		
4.0	24.5					
5.3	24.5					

Dec. 9/95

Temperature 20 C Protein: Lactalbumin

Protein Culture		
Time (h)	NOx-N (mg N/L)	Linear Regression
0.0	26.5	Slope
0.3	25.5	Intercept
0.5	25.3	R^2
1.0	25.2	F
1.5	24.6	df
2.0	24.5	
2.5	23.9	
3.0	24.4	
3.5	24.4	
4.0	24.5	
5.3	24.5	

Dec. 7/95

Temperature 20 C Protein: Lactalbumin

PH: CAA

Protein Culture			PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression	Time (h)	NOx-N (mg N/L)	Linear Regression	
0	31.4	Slope	0	27.6	Slope	-4.64
0.5	31.3	Intercept	0.5	26.21	Intercept	28.1
1	30.6	R^2	1	23.72	R^2	0.998
1.50	31.0	F	1.5	21.3	F	3726
2	30.9	df	2	18.88	df	7
2.5	28.8		2.5	16.23		
3	28.7		3	14.15		
3.5	28.4		3.5	11.87		
4	28.1		4	9.61		

Apr. 4/96

Temperature 14 C Protein: Ovalbumin

PH: CAA

Protein Culture			PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression	Time (h)	NOx-N (mg N/L)	Linear Regression	
0.0	25.5	Slope -3.69	0.0	25.6	Slope	-2.83
0.8	23.7	Intercept 26.7	0.8	23.1	Intercept	25
1.5	21.6	R^2 0.972	1.5	20.7	R^2	0.992
2.3	18.9	F 242.6	2.3	18.3	F	938.7
3.0	16.0	df 7	3.2	15.4	df	7
3.8	15.6		3.8	14		
4.5	9.4		4.5	12.5		
5.3	6.7		5.3	9.8		
6.0	3.5		6.0	8.9		

Apr. 1/96

Temperature 14 C PH: LEH

PH: CAA

LEH Culture				CAA Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0.0	33.5	Slope	-3.89	0.0	18.1	Slope	-3.45
0.8	30.3	Intercept	32.7	0.8	15.4	Intercept	17.9
1.5	26.5	R^2	0.993	1.5	12.9	R^2	0.998
2.3	23.7	F	933.9	2.3	9.7	F	2947.2
3.0	20.2	df	7	3.0	7.2	df	6
3.8	17.4			3.8	4.9		
4.5	15.0			4.5	2.5		
5.3	12.6			5.3	0.1		
6.0	10.5						

Apr. 13/96

Temperature 14 C Protein: Lactalbumin

PH: LEH

Protein Culture				CAA Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression		Time (h)	NOx-N (mg N/L)	Linear Regression	
0	68.4	Slope		0	50.0	Slope	-3.60
0.75	67.0	Intercept		0.75	47.2	Intercept	49.5
1.5	67.3	R^2		1.5	43.9	R^2	0.991
2.25	67.3	F		2.25	41.2	F	812.1
3	67.3	df		3	38.2	df	7
3.75	67.4			3.75	35.6		
4.25	67.4			4.25	33.2		
5	67.7			5	31.2		
6	67.8			6	[illegible]		
6.5	65.1						
7	62.2						
7.5	58.8						

APR. 9/96

Temperature 14 C Protein: Lactalbumin

PH: LEH

Protein Culture			PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression	Time (h)	NOx-N (mg N/L)	Linear Regression	
1	1		0	29.6	Slope	-4.22
1.75	1.8		0.75	27.6	Intercept	30.3
2.5	2.5		2.25	21.1	R^2	0.997
3.25	3.3		3	17.9	F	2816
4	4		3.75	14.3	df	6
4.75	4.8		4.5	10.8		
21.5	21.5		5.25	8.1		
			6	5.0		
			6.5	3.1		
			6.5	39.7		
			22	12.6		
			23.5	10.7		
			24.75	10.6		

Apr. 29/96

Temperature 14 C Protein: Lactalbumin

PH: LEH

Protein Culture			PH Culture			
Time (h)	NOx-N (mg N/L)	Linear Regression	Time (h)	NOx-N (mg N/L)	Linear Regression	
0	28		0.0	27.3	Slope	-4.09
0.75	27.3		0.8	25	Intercept	27.3
1.5	27.2		1.5	21.5	R^2	0.996
2.25	25.4		2.3	17	F	1443
3.00	25.8		3.0	14.6	df	6
4.50	27.1		4.5	9		
5.25	26.1		5.3	5.7		
6	27.1		6.0	3.2		
6.5	25.4					
7	23.3					
7.5	21.4					

Ammonia Production Data Rate Studies

Nov. 7/95

Temperature 20 C — Protein: Ovalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	31.2	Slope	1.87	0	26.7	Slope	3.56
0.25	31.8	Intercept	31.2	0.25	27.6	Intercept	32.4
0.5	32.3	R^2	0.995	0.5	28.5	R^2	0.993
1	32.9	F	1103.4	1	30.4	F	820.9
1.75	34.1	df	6	1.8	33.6	df	6
2.5	35.9			2.25	35.7		
3.25	37.1			3.25	38.6		
4	38.9			4	40.5		

Nov. 2/95

Temperature 20 C — Protein: Casein

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	32.5	Slope	2.36	0	36.4	Slope	2.47
0.25	32.8	Intercept	32.4	0.25	36.9	Intercept	36.5
0.5	33.8	R^2	0.998	0.5	38.2	R^2	0.993
1	34.6	F	2573.5	1.00	39.4	F	901.6
1.75	36.4	df	6	1.75	40.6	df	6
2.5	38.1			2.5	42.4		
3.25	40			3.25	44.6		
4	42			4	46.6		

Nov. 27/95

Temperature 20 C — Protein: Ovalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	26.6	Slope	2.25	0	27.1	Slope	2.30
0.5	27.3	Intercept	26.1	0.5	28.8	Intercept	27.4
1.0	28.4	R^2	0.990	1	29.9	R^2	0.996
1.5	29.3	F	691.1	1.50	30.9	F	1927.6
2.0	30.4	df	9	2	32.1	df	7
2.5	31.4			2.5	33.1		
3.0	32.8			3	34.2		
3.5	34.2			3.5	35.6		
4.0	35.6			4.0	36.5		

Dec. 6/95

Temperature 20 C — Protein: Casein

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	25.6	Slope	2.76	0	32.0	Slope	2.2
0.5	27.0	Intercept	25.3	0.5	33.0	Intercept	27.3
1	27.9	R^2	0.996	1	34.1	R^2	0.995
1.50	29.2	F	1990.5	1.50	35.5	F	1347.5
2	30.8	df	7	2	36.0	df	7
2.5	32.1			2.5	37.8		
3	33.5			3	38.7		
3.5	35.0			.5	39.5		
4	36.7			4.0	40.7		

Nov. 29/95

Temperature 20 C — Protein: Lactalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	30.3	Slope		0.0	31.1	Slope	2.89
0.5	31.0	Intercept		0.5	32.6	Intercept	31.4
1.0	31.1	R^2		1.0	34.6	R^2	0.994
1.5	31.1	F		1.5	35.9	F	1344.8
2.0	31.1	df		2.0	37.6	df	7
2.5	31.1			2.5	38.9		
3.0	31.1			3.0	40.3		
3.5	31.2			3.5	41.3		
4.0	31.3			4.0	42.6		

Oct. 25/95

Temperature 20 C — Protein: Lactalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	33.3	Slope		0.0	36.6	Slope	2.89
0.25	36.3	Intercept		0.3	37.0	Intercept	31.4
0.5	36.6	R^2		0.5	37.9	R^2	0.994
1.00	37.4	F		1.0	39.0	F	1344.8
1.75	37.5	df		1.8	40.6	df	7
2.5	38.1			2.5	42.3		
3.25	38.3			3.3	45.4		
4	38.7			4.0	46.7		

Apr. 4/96

Temperature 14 C — Protein: Ovalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	26.0	Slope	3.39	0.0	26.1	Slope	2.37
0.8	27.3	Intercept	25.8	0.8	30.3	Intercept	27.7
1.5	30.2	R^2	0.977	1.5	31.9	R^2	0.953
2.3	33.8	F	296.0	2.3	33.9	F	101.3
3.1	37.6	df	7	3.0	35.7	df	5
3.8	40.6			3.8	36.0		
4.5	40.2			5.3	39.6		
5.3	43.0						
6.0	45.7						

May 5/96

Temperature 14 C — Protein: Ovalbumin

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	30.1	Slope	3.03	0.0	31.0	Slope	2.36
0.8	27.6	Intercept	28.7	0.8	32.6	Intercept	30.7
1.5	33.7	R^2	0.857	1.5	34.1	R^2	0.994
2.3	36.7	F	36.1	2.3	35.5	F	97.4
3.0	37.5	df	6	3.2	37.6	df	6
3.8	41.6			3.8	39.7		
4.5	45.3			4.5	41.8		
5.3	41.3			6.0	44.8		

Apr. 1/96

Temperature 14 C PH: LEH

PH: CAA

LEH Culture				CAA Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	28.1	Slope	2.25	0	29.6	Slope	2.11
0.8	29.1	Intercept	28.4	0.75	32.4	Intercept	30.1
1.5	32.9	R^2	0.979	1.5	33.3	R^2	0.954
2.3	34.2	F	334.1	2.25	33.4	F	148.3
3.0	35.2	df	7	3	37.2	df	7
3.8	36.5			3.75	39.4		
4.5	39.0			4.5	39.8		
5.3	40.6			5.25	41.6		
6.0	41.3			6	41.6		

Mar. 29/96

Temperature 14 C Protein:α-casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	20.7	Slope	0.721	0.00	21.9	Slope	1.62
0.8	21.4	Intercept	20.9	0.75	23.5	Intercept	21.9
1.5	22.3	R^2	0.982	1.50	24.7	R^2	0.942
2.3	22.9	F	499	2.25	23.7	F	114.5
3.0	23.2	df	7	3.00	27.3	df	7
3.8	23.6			3.75	28.2		
4.5	24.1			4.50	30.3		
5.3	24.5			5.25	30.1		
6.0	25.4			6.00	31.3		

July 23/96

Temperature 20 C PH: LEH

Protein: Lactalbumin

LEH Culture			CAA Culture		
Time (h)	NH3-N (mg N/L)	Linear Regression	Time (h)	NH3-N (mg N/L)	Linear Regression
0	24.3	Slope	0	22.9	Slope
0.75	27.2	Intercept	0.75	24.9	Intercept
1.5	30.5	R^2	1.5	26.2	R^2
2.25	33.1	F	2.25	27.6	F
3.00	35.3	df	3.00	27.2	df
3.75	38.4		3.75	27.9	
4.5	40.1		4.5	28.8	
5.25	41.9		5.25	29.4	
6	43.8		6	30.5	
			6.3	31.9	
			6.7	33.6	

May 9/96

Temperature 14 C Protein:α-casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	38.7	Slope	2.02	0	40.4	Slope	3.21
0.5	39.7	Intercept	39.9	0.75	43.3	Intercept	40.7
1.25	43.0	R^2	0.909	1.5	44.4	R^2	0.928
2	45.0	F	70.2	2.25	47.4	F	89.5
2.75	47.5	df	7	3	53.3	df	7
3.5	47.3			3.75	54.8		
4.25	49.7			4.5	53.4		
5	48.6			5.25	55.1		
5.75	50.5			5.8	60.7		

Oct. 20/95

Temperature 20 C Protein: Ovalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	26.28	Slope	2.22	0	32.3	Slope	1.9
0.25	27.58	Intercept	26.7	0.3	37.5	Intercept	34
1	29.36	R^2	0.979	0.55	37.2	R^2	0.641
1.50	29.66	F	285	1	31.8	F	1249
2	30.3	df	6	2.0	38.1	df	7
2.5	32.0			2.5	39.6		
3	33.1			3	39.5		
3.5	34.1			3.5	41.5		
4.0	36.1			4.0	41.1		

Oct. 30/95

Temperature 20 C Protein: Casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	39.0	Slope	3.84	0	44.0	Slope	2.38
0.5	44.0	Intercept	41.5	1	44.9	Intercept	44.3
1.0	46.7	R^2	0.883	1.5	50.3	R^2	0.862
1.5	47.9	F	45.4	2.00	49.2	F	37.7
2.0	51.7	df	6	2.5	51.2	df	6
2.5	49.4			3	50.4		
3.0	53.6			3.5	52.7		
3.5	53.5			4	53.5		

Dec. 4/95

Temperature 20 C Protein: Ovalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	31.28	Slope	2.84	0	30.1	Slope	1.75
0.5	33.05	Intercept	31.5	0.5	31.3	Intercept	29.9
1	34.42	R^2	0.997	1	31.0	R^2	0.981
1.5	35.5	F	1585	1.50	32.5	F	364
2	37.2	df	7	2	33.6	df	7
2.5	38.42			2.5	34.3		
3	40.36			3	34.8		
3.5	41.69			3.5	36.2		
4	42.42			4.0	37.1		

Dec. 1/95

Temperature 20 C Protein: Casein

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
1	27.2	Slope	2.21	0	25.8	Slope	2.76
1.5	25.7	Intercept	23.4	0.5	28.1	Intercept	24.5
2	26.7	R^2	0.871	1	26.0	R^2	26.6
2.50	28.7	F	34	1.50	26.9	F	114.6
3	30.2	df	5	2	28.6	df	7
3.5	31.2			2.5	30.1		
4	32.7			3	31.4		
				3.5	37.8		
				4.0	35.8		

Oct. 24/95

Temperature 20 C Protein: Lactalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	31.4	Slope	0.51	0.3	35.4	Slope	3.01
0.3	32.4	Intercept	32.1	0.5	36.8	Intercept	35.2
0.5	32.8	R^2	0.778	1.0	38.1	R^2	0.971
1.0	33.0	F	24.6	1.5	40.1	F	233.4
1.5	33.0	df	7	2.0	41.4	df	7
2.0	33.1			2.5	41.9		
2.5	33.3			3.0	44.8		
3.0	33.5			3.5	46.7		
3.5	33.9			4.0	46.0		
4.0	33.9						

Dec. 7/95

Temperature 20 C Protein: Lactalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	32.5	Slope	0.189	0.0	32.5	Slope	2.87
0.5	32.7	Intercept	32.5	0.5	34.8	Intercept	33.02
1	32.8	R^2	0.536	1.0	35.8	R^2	0.976
1.50	32.5	F	8.07	1.5	37.5	F	295.5
2	33.1	df	7	2.0	38.2	df	7
2.5	33.3			2.5	41.0		
3	32.7			3.0	42.5		
3.5	33.0			3.5	43.1		
4	33.5			4.0	43.6		

May 5/96

Temperature 14 C Protein: Ovalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	26.7	Slope	1.83	0.0	27.8	Slope	2.71
0.8	31.9	Intercept	28.2	0.8	30.5	Intercept	28.3
1.5	30.1	R^2	0.875	1.5	33.0	R^2	0.976
2.3	33.5	F	49.1	2.3	35.4	F	251.2
3.0	32.3	df	7	3.2	36.6	df	6
3.8	35.1			3.8	37.0		
4.5	38.0			4.5	40.7		
5.3	36.4			5.3	43.1		
6.0	39.6						

Apr. 4/96

Temperature 14 C Protein: Ovalbumin

PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	23.7	Slope	1.75	0.0	25.6	Slope	2.14
0.8	25.1	Intercept	23.6	0.8	28.8	Intercept	26.8
1.5	26.7	R^2	0.856	1.5	28.8	R^2	0.889
2.3	27.7	F	41.9	2.3	33.1	F	47.9
3.1	25.8	df	7	3.0	35.8	df	6
3.8	32.7			3.8	34.9		
4.5	30.9			4.5	35.7		
5.3	33.2			5.3	36.8		
6.0	34.1						

Mar. 27/96

Temperature 14 C — PH: LEH

Aerobic — PH: CAA

LEH Culture				CAA Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	25.3	Slope	1.85	0	24.5	Slope	2.02
0.8	26.6	Intercept	26.1	0.75	26.3	Intercept	24.7
1.5	29.1	R^2	0.873	1.5	28.2	R^2	0.982
2.3	33.9	F	48.1	2.25	29.1	F	387.4
3.0	31.2	df	7	3	30.7	df	7
3.8	32.4			3.75	31.6		
4.5	33.6			4.25	34.6		
5.0	35.2			5.25	35.4		
6.0	37.4			6	36.5		

July 23/96

Temperature 20 C — PH: LEH

Aerobic — Protein: Lactalbumin

LEH Culture				CAA Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0	35.5	Slope		0	35.2	Slope	
0.75	33.5	Intercept		0.75	37.1	Intercept	
1.5	36.7	R^2		1.5	39.7	R^2	
2.25	37.7	F		2.25	42.7	F	
3.00	38.6	df		3.00	45.4	df	
3.75	39.3			3.75	47.7		
4.5	40.1			4.5	50.3		
5.25	40.3			5.25	52.9		
6	41.7			6	53.7		
6.3	43.2						
6.7	49.8						

May 7/96

Temperature 14 C — Protein: -casein

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.0	30.6	Slope	3.64	0.00	30.6	Slope	3.11
0.8	33.6	Intercept	30.98	1.00	35.8	Intercept	31.6
1.5	36.7	R^2	0.981	1.75	36.9	R^2	0.99
2.3	39.3	F	358.3	2.50	39.8	F	476.1
3.0	40.9	df	7	4.00	43.8	df	5
3.8	46.0			4.75	46.1		
4.5	47.3			5.25	47.9		
5.3	51.7						
6.0	51.1						

May 9/96

Temperature 14 C — Protein: -casein

Aerobic — PH: CAA

Protein Culture				PH Culture			
Time (h)	NH3-N (mg N/L)	Linear Regression		Time (h)	NH3-N (mg N/L)	Linear Regression	
0.5	31.7	Slope	3.59	0	31.2	Slope	2.21
1.25	33.55	Intercept	29.3	0.75	33.4	Intercept	31.3
2	36.2	R^2	0.981	1.5	34.1	R^2	0.983
2.75	38.9	F	309.1	2.25	35.9	F	340.8
3.5	40.3	df	6	3	38.0	df	6
4.25	45.9			3.75	40.5		
5	48.2			5.25	43.5		
5.75	49.3			5.75	43.2		

pH Data Rate Studies

Nov. 2 /95

Temperature 20 C Protein: β-Casein

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.9	0.0	7.4	7.13	7.48
0.5	0.7	7.1	6.99	7.07
1.0	1.2	7.2	7.05	7.13
1.5	1.8	7.3	7.13	7.21
2.0	2.2	7.3	7.15	7.22
2.5	2.7	7.3	7.13	7.2
3.0	3.2	7.3	7.11	7.17
3.5	3.7	7.2	7.07	7.12

Oct. 25 /95

Temperature 20 C Protein: Lactalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.3	-1.0	7.2	7.37	7.27
-0.3	0.0	7.3	7.29	7.28
0.5	0.8	7.2	7.26	7.25
1.2	1.3	7.2	7.25	7.24
1.3	1.4	7.2	7.26	7.24
1.8	2.1	7.1	7.15	7.14
2.5	2.7	7.1	7.16	7.15
3.0	3.2	7.2	7.17	7.16
3.5	3.7	7.2	7.16	7.16

Nov. 27 /95

Temperature 20 C Protein: Ovalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.3	0.0	7.16	7.08	7.1
0.53	0.8	7.24	7.18	7.21
1.06	1.3	7.18	7.16	7.17
1.59	1.9	7.19	7.16	7.17
2.3	2.5	7.29	7.18	7.25
2.78	3.0	7.31	7.24	7.28
3.32	3.5	7.32	7.27	7.3
3.59	3.9	7.36	7.26	7.33

Nov. 29/95

Temperature 20 C Protein: Lactalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.0	-0.2	7.16	7.08	7.10
0.6	0.8	7.24	7.18	7.21
1.1	1.3	7.18	7.16	7.17
1.5	1.7	7.19	7.16	7.17
2.1	2.2	7.29	7.18	7.25
2.6	2.8	7.31	7.24	7.28
3.1	3.3	7.32	7.27	7.30
3.6	3.8	7.36	7.26	7.33

Nov. 7/95

Temperature 20 C Protein: Ovalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.9	-0.7	7.11	7.26	7.2
-0.2	0.0	7.07	7.15	7.12
0.6	0.7	7.34	7.4	7.37
1.0	1.2	7.37	7.46	7.41
1.6	1.7	7.28	7.34	7.31
2.0	2.3	6.99	7.36	7.33
2.5	2.7	7.06	7.16	7.1
3.0	3.2	7.08	7.21	7.17
3.5	3.7	7.12	7.24	7.2

Dec. 6/95

Temperature 20 C Protein: -casein

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.2	-0.25	7.22	6.87	7.08
0.25	0.54	7.13	7.09	7.11
0.54	0.84	7.09	7.08	7.08
1.1	1.22	7.12	7.1	7.1
1.54	1.73	7.18	7.16	7.17
2.03	2.23	7.22	7.19	7.21
2.52	2.73	7.24	7.21	7.23
3.5	3.67	7.19	7.11	7.15

Dec. 7/95

Temperature 20 C Protein: lactalbumin

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.4	0.0	7.89	7.13	7.48
0.0	0.3	7.12	6.99	7.07
0.3	0.5	7.21	7.05	7.13
0.5	0.8	7.28	7.13	7.21
0.8	1.0	7.28	7.15	7.22
1.0	1.3	7.26	7.13	7.2
1.3	1.5	7.27	7.11	7.17
1.5	1.8	7.21	7.07	7.12
1.8	2.0	7.16	6.99	7.06
2.0	2.3	7.05	6.87	6.96
2.3	2.5	6.95	6.76	6.88
2.5	2.8	6.81	6.6	6.76
2.8	3.0	6.64	6.61	6.62
3.0	3.3	6.64	6.4	6.43
3.3	3.5	6.43	6.1	6.23
3.5	3.8	6.13	6.12	6.12
3.8	4.0	6.15	6.13	6.14

Dec. 4/95

Temperature 20 C Protein: Ovalbumin

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.55	0	7.85	7.19	7.36
0	0.25	7.26	7.19	7.22
0.25	0.5	7.24	7.16	7.19
0.5	0.75	7.4	7.19	7.3
0.75	1	7.53	7.4	7.47
1	1.25	7.61	7.41	7.51
1.25	1.5	7.57	7.47	7.53
1.5	1.75	7.68	7.58	7.63
1.75	2	7.69	7.43	7.5
2	2.25	7 58	7.21	7.37
2.25	2.5	7.41	7.29	7.35
2.5	2.75	7.48	7.41	7.44
2.75	3	7.53	7.18	7.42
3	3.25	7.29	7.2	7.25
3.25	3.5	7.34	7.05	7.25
3.5	3.75	7.14	7.1	7.12
3.75	4	7.16	7.03	7.11

Apr. 8/96

Temperature 14 C Protein: Lactalbumin
Reactor: Anoxic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.0	-0.5	6.96	6.96	6.96
-0.5	0.0	7.55	1.68	6.96
0.5	1.0	7.13	7.01	7.07
1.0	1.5	7.18	7.06	7.13
1.5	2.0	7.20	7.12	7.16
2.0	2.5	7.27	7.16	7.21
2.5	3.0	7.36	7.26	7.30
3.0	3.5	7.46	7.36	7.40
3.5	4.0	7.38	7.18	7.28
4.0	4.5	7.29	7.18	7.28
4.5	5.0	7.37	7.14	7.21
5.0	5.5	7.28	7.17	7.22
5.5	6.0	7.29	7.13	7.23

April 29/96

Temperature 14 C Protein: Lactalbumin
Reactor: Anoxic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
0.0	0.5	7.23	6.95	7.09
0.5	1.0	7.11	7.09	7.10
1.5	2.0	7.19	7.17	7.18
2.0	2.5	7.18	7.17	7.17
2.5	3.0	7.19	7.18	7.18
3.0	3.5	7.21	7.17	7.20
3.5	4.0	7.22	7.21	7.22
4.0	4.5	7.23	7.22	7.23
4.5	5.0	7.24	7.23	7.24
5.0	5.5	7.25	7.24	7.24
5.5	6.0	7.25	7.25	7.25
6.0	6.5	7.27	7.24	7.25
6.5	7.0	7.30	7.27	7.28
7.0	7.5	7.57	7.30	7.39

Apr. 13/96

Temperature 14 C Protein: Lactalbumin
Reactor: Anoxic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.1	-0.6	7.00	7.00	7.01
-0.5	0.0	7.01	7.01	7.13
0.0	0.5	7.25	7.22	7.23
0.5	1.0	7.29	7.25	7.27
1.0	1.5	7.34	7.29	7.32
1.5	2.0	7.36	7.34	7.35
2.0	2.5	7.39	7.36	7.38
2.5	3.0	7.41	7.39	7.40
3.0	3.5	7.44	7.41	7.42
3.5	4.0	7.51	7.44	7.48
4.0	4.5	7.55	7.51	7.53
4.5	5.0	7.57	7.55	7.56
5.0	5.5	7.58	7.57	7.58
5.5	6.0	7.59	7.58	7.58
6.0	6.5	7.63	7.53	7.58
6.5	7.0	7.84	7.64	7.74
7.0	7.5	8.04	7.85	7.95

Apr. 1/96

Temperature 14 C PH: CAA
Reactor: Anoxic PH: LEH pH Measurements on this C

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.1	-0.6	7.71	6.94	7.39
-0.6	-0.2	7.03	6.83	6.93
-0.1	0.4	7.07	6.89	6.98
0.4	0.9	7.08	6.95	7.00
0.9	1.4	7.22	7.01	7.09
1.4	1.9	7.28	7.07	7.18
1.9	2.4	7.29	7.05	7.13
2.4	2.9	7.24	7.04	7.11
2.9	3.4	7.20	7.03	7.09
3.4	3.9	7.21	7.08	7.17
3.9	4.4	7.24	6.99	7.05
4.4	4.9	7.25	7.09	7.17
4.9	5.4	7.14	7.08	7.11
5.4	5.9	7.24	6.99	7.16
5.9	6.0	7.03	7.00	7.02

May 7/96

Temperature 14 C Protein: -casein

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.9	0.0	7.74	7.03	7.33
0.0	0.2	7.15	7.07	7.12
0.3	0.5	7.18	7.11	7.14
0.5	0.7	7.21	7.15	7.18
0.8	1.0	7.24	7.17	7.21
1.0	1.2	7.26	7.17	7.22
1.3	1.5	7.32	7.26	7.29
1.5	1.7	7.33	7.28	7.31
1.8	2.0	7.43	7.33	7.38
2.0	2.2	7.59	7.43	7.51
2.3	2.5	7.66	7.59	7.63
2.5	2.7	7.62	7.43	7.52
2.8	3.0	7.52	7.25	7.36
3.0	3.2	7.38	7.22	7.32
3.3	3.5	7.35	7.21	7.27
3.5	3.7	7.37	7.18	7.26
3.8	4.0	7.33	7.17	7.25

May 9/96

Temperature 14 C Protein: -casein

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.5	-1.0	7.27	6.85	7.09
-1.0	-0.5	7.14	6.69	6.99
0.0	0.1	7.20	7.18	7.19
0.4	0.7	7.18	7.11	7.14
1.0	1.3	7.15	7.08	7.11
1.5	1.8	7.16	7.08	7.13
1.9	2.3	7.17	7.08	7.13
2.5	3.3	7.26	7.14	7.21
3.5	3.9	7.28	7.14	7.17
4.2	4.6	7.40	7.21	7.26
4.8	5.5	7.30	7.22	7.33

Apr. 8/96

Temperature 14 C Protein: Lactalbumin

Reactor: Aerobic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.2	0.0	7.61	7.08	7.34
0.0	0.5	7.27	7.21	7.23
1.3	1.9	7.33	7.24	7.29
2.2	2.7	7.29	7.18	7.26
2.9	3.4	7.34	7.29	7.31
3.7	3.8	7.40	7.39	7.40
3.7	4.2	7.40	7.21	7.36
4.4	4.9	7.41	7.25	7.32

Mar. 26/96

Temperature 14 C PH: LEH culture- pH is measured

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.7	-1.3	7.15	6.87	7.07
-1.2	-0.8	7.19	7.07	7.16
-0.3	0.0	7.31	7.22	7.28
0.0	0.1	7.28	7.24	7.27
0.5	1.0	7.37	7.28	7.32
1.0	1.3	7.37	7.31	7.34
1.5	2.0	7.42	7.37	7.39
2.3	2.6	7.49	7.41	7.45
2.9	3.5	7.58	7.41	7.47
3.8	3.9	6.98	7.44	7.49
4.5	4.6	7.54	6.87	6.92
5.3	5.4	7.02	7.00	7.01

Oct. 30/95

Temperature 20 C Protein: -casein

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.08	0.00	6.72	6.78	6.75
0.00	0.25	6.75	6.78	6.77
0.25	0.50	6.78	6.80	6.79
0.50	0.75	6.77	6.80	6.79
0.75	1.00	6.79	6.81	6.80
1.00	1.25	6.79	6.81	6.80
1.25	1.50	6.79	6.80	6.80
1.50	1.75	6.79	6.81	6.80
1.75	2.00	6.80	6.82	6.81
2.00	2.25	6.82	6.84	6.83
2.25	2.50	6.83	6.85	6.84
2.50	2.75	6.84	6.85	6.85
2.75	3.00	6.85	6.88	6.86
3.00	3.25	6.79	6.82	6.81
3.25	3.50	6.80	6.83	6.82
3.50	3.75	6.79	6.84	6.82
3.75	4.00	6.80	6.82	6.80

Dec. 1/95

Temperature 20 C Protein: -casein

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.9	0.0	7.74	7.03	7.33
0.0	0.2	7.15	7.07	7.12
0.3	0.5	7.18	7.11	7.14
0.5	0.7	7.21	7.15	7.18
0.8	1.0	7.24	7.17	7.21
1.0	1.2	7.26	7.17	7.22
1.3	1.5	7.32	7.26	7.29
1.5	1.7	7.33	7.28	7.31
1.8	2.0	7.43	7.33	7.38
2.0	2.2	7.59	7.43	7.51
2.3	2.5	7.66	7.59	7.63
2.5	2.7	7.62	7.43	7.52
2.8	3.0	7.52	7.25	7.36
3.0	3.2	7.38	7.22	7.32
3.3	3.5	7.35	7.21	7.27
3.5	3.7	7.37	7.18	7.26
3.8	4.0	7.33	7.17	7.25

Apr. 4/96

Temperature 14 C Protein: Ovalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.88	-0.68	7.20	6.58	7.02
-0.50	-0.22	6.64	6.47	6.57
0.62	1.08	6.92	6.74	6.89
1.25	1.[illegible]	6.98	6.90	6.95
1.67	2.04	7.04	6.91	7.00
2.21	2.53	7.09	7.02	7.05
2.88	3.33	7.17	7.04	7.12
3.33	3.52	7.18	7.16	7.17
3.83	4.22	7.25	7.12	7.19
4.42	5.08	7.20	6.93	7.10
5.28	5.76	7.29	7.10	7.14

May 5/96

Temperature 14 C Protein: Ovalbumin

Reactor: Aerobic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.75	-0.34	6.98	6.82	6.93
-0.30	0.00	7.28	7.15	7.20
0.49	1.00	7.35	7.13	7.21
1.00	1.35	7.24	7.15	7.20
1.50	1.97	7.28	7.17	7.22
2.27	2.59	7.65	7.33	7.52
2.91	3.33	7.81	7.52	7.68
3.34	3.52	7.57	7.20	7.48
3.77	4.28	7.43	7.20	7.32
4.48	5.04	7.26	7.07	7.24
5.28	5.77	7.37	6.74	6.96

Apr. 4/96

Temperature 14 C Protein: Ovalbumin

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.00	-0.52	7.38	6.81	6.99
-0.50	-0.02	6.91	6.74	6.82
0.50	0.98	6.91	6.79	6.85
1.00	1.48	6.99	6.84	6.90
1.50	1.98	7.20	6.99	7.11
2.00	2.48	7.39	7.21	7.30
2.50	2.98	7.50	7.39	7.44
3.00	3.48	7.61	7.51	7.57
3.50	3.98	7.73	7.39	7.66
4.00	4.48	6.58	6.08	7.27
4.50	4.98	7.54	6.15	6.41
5.00	5.48	6.76	6.59	6.68
5.50	5.98	6.91	6.76	6.84

May 5/96

Temperature 14 C Protein: Ovalbumin

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
0.0	0.5	7.48	6.66	7.18
0.5	1.0	7.83	7.49	7.68
1.5	2.0	7.15	6.89	6.99
2.0	2.5	6.93	6.82	6.87
2.5	3.0	6.88	6.80	6.84
3.1	3.5	6.88	6.79	6.83
3.6	4.1	6.90	6.81	6.86
4.1	4.6	6.95	6.85	6.90
4.6	5.1	6.99	6.86	6.92
5.1	5.6	7.05	6.91	6.95
5.6	6.1	6.99	6.93	6.97

Mar. 29/96

Temperature 14 C Protein: Casein

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.0	-0.5	7.12	6.77	6.91
-0.5	-0.1	6.87	6.64	6.75
0.0	0.5	6.84	6.68	6.76
0.5	1.0	6.98	6.82	6.89
1.0	1.5	6.96	6.81	6.89
1.5	2.0	6.99	6.84	6.89
2.0	2.5	6.99	6.81	6.92
2.5	3.0	6.90	6.80	6.85
3.0	3.5	6.96	6.70	6.88
3.5	4.0	6.84	6.70	6.74
4.0	4.5	6.78	6.50	6.73
4.5	5.0	6.58	6.51	6.55
5.0	5.5	6.63	6.58	6.61
5.5	6.0	6.64	6.37	6.42

May 9/96

Temperature 14 C Protein: Casein

Reactor: Anoxic PH: CAA

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.4	-0.9	7.32	6.82	7.01
-0.9	-0.4	6.88	6.73	6.81
0.0	0.5	6.89	6.66	6.74
0.5	1.0	6.81	6.66	6.74
1.0	1.5	6.93	6.78	6.85
1.5	2.0	6.95	6.78	6.83
2.0	2.5	6.86	6.78	6.81
2.5	3.0	6.82	6.71	6.77
3.0	3.5	6.79	6.68	6.73
3.5	4.0	6.83	6.68	6.72
4.0	4.5	6.78	6.68	6.78
4.5	5.0	6.7	6.6	6.7
5.0	5.5	6.7	6.6	6.7
5.5	5.9	6.8	6.7	6.7

July 23/96

Temperature 14 C Protein: Lactalbumin

Reactor: Anoxic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-0.6	-0.1	7.4	6.8	7.0
2.0	2.4	7.2	7.0	7.1
2.4	2.9	7.4	7.2	7.3
2.9	3.4	7.5	7.4	7.4
3.4	3.9	7.6	7.5	7.6
3.9	4.4	7.5	7.6	7.7
4.4	4.9	7.7	6.1	7.3
4.9	5.4	6.6	6.2	6.4
5.4	5.9	6.8	6.6	6.7
5.9	6.4	6.9	6.8	6.8
6.9	7.4	6.9	6.7	6.9
7.4	7.9	7.0	6.9	6.9

July 23/96

Temperature 14 C Protein: Lactalbumin

Reactor: Aerobic PH: LEH

Time		pH		
Minimum	Maximum	Maximum	Minimum	Average
-1.6	0.0	7.35	6.59	7.17
0.0	0.3	7.14	7.02	7.12
1.4	1.8	7.19	7.10	7.15
2.0	2.5	7.13	7.02	7.09
2.9	3.4	7.21	7.12	7.18
3.7	4.0	7.20	7.11	7.17
4.4	4.8	7.24	7.03	7.21
5.2	5.5	7.18	7.10	7.20
5.8	6.1	7.24	7.17	7.21
6.4	6.4	7.23	7.20	7.20
6.5	6.7	7.20	7.18	7.21

SBR Data - Sept. 21 - Dec. 10/95

Sewage

Sample	Starting Date	Ending Date	Total COD (mg COD/L)				Filtered COD (mg COD/L)				Total TKN (mg N/L)			Filtered TKN (mg N/L)	NH_3-N (mg N/L)	Nitrate (mg N/L)	
			Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Avg				
1	21-Sep	22-Sep	236.4	219.1	265.9	240.5	86.1	83.7	98.5	89.4						ND	
2	23-Sep	24-Sep	236.4	219.1	265.9	240.5	86.1	83.7	98.5	89.4						ND	
3	25-Sep	26-Sep	139.8	144.8	103.3	129.3	90	102.9	100.1	97.7						ND	
4	27-Sep	29-Sep	233.5	236.4	239.4	236.4	95.9	94.9	96.1	95.6						ND	
5	30-Sep	1-Oct	71.4	81.3	81.3	78.0	56.3	49.2	49.2	51.6						ND	
6	2-Oct	3-Oct	71.4	81.3	81.3	78.0	56.3	49.2	49.2	51.6						ND	
7	4-Oct	6-Oct	170	165.6	196.4	177.3	89.4	91.5	85.2	88.7					35.3	ND	
8	7-Oct	8-Oct	199.4	225.9	236.3	220.5	64	66.5	61.6	64.0					33.9	ND	
9	9-Oct	10-Oct	108.3	110.8	120.6	113.2	54.2	64	66.5	61.6					21.5	ND	
10	11-Oct	13-Oct	169.9	174.8	169.9	171.5	116.5	94.6	94.6	101.9							
11	14-Oct	15-Oct	159.1	161.6	174.1	164.9	77.1	77.1	77.1	77.1					40.1	ND	
12	16-Oct	17-Oct	72.1	114.3	96.9	94.4	69.6	62.2	64.5	65.4							
13	18-Oct	20-Oct	154.2	171.6	164.1	163.3	106.9	109.4	111.9	109.4	36.2		36.2	33.8	34.3	ND	
14	21-Oct	22-Oct	96.8	97	101.9	98.6	109.4	106.9	119.3	111.9	39		39	31.2	35.4	ND	
15	23-Oct	24-Oct	99.5	109.4	144.2	117.7	92	94.4	94.5	93.6	24.5		24.5	31.4	30.9	ND	
16	25-Oct	27-Oct	248.7	238.7	241.2	242.9	79.6	87	94.5	87.0	46.9		46.9	33.1	29.1	ND	
17	28-Oct	29-Oct	241.2	251.1	263.6	252.0	104.4	121.9	99.5	108.6	45.3		45.3	33.1	32.2	ND	
18	30-Oct	31-Oct	111.9	124.3	116.9	117.7	116.9	124.3		120.6	48.2		48.2	50.9	35.6	ND	
19	1-Nov	3-Nov	285.9	293.4	290.9	290.1	121.8	131.8	126.8	126.8				32.1	28.2	ND	
20	4-Nov	5-Nov	256.1	196.5	208.9	220.5	99.5	198.9	141.7	146.7	38.8	36.3	37.55	28.3	27.8	ND	
21	6-Nov	7-Nov	256.1	196.5	208.9	220.5	99.5	198.9	141.7	146.7	38.8	36.3	37.55	28.3	27.8	ND	
22	8-Nov	10-Nov	256.1	226.3	241.2	241.2	151.7	151.3	119.3	140.8					30.2	ND	
23	11-Nov	12-Nov	169.1	141.7	136.7	149.2	87	79.6	121.8	96.1					30	ND	
24	25-Nov	26-Nov	178.2	172.9	164.1	171.7	74.5	82.5	98.4	85.1					20.7		0.18
25	27-Nov	28-Nov	162.2	175.5	236.7	191.5	69.1	103.7	101.1	91.3	29.9	33.1	31.5	21.9	23.15	ND	
26	29-Nov	1-Dec	276.6	172.9	207.5	219.0	114.4	125.8	103.7	114.6	28		28.0	16.6	19.5		0.18
27	2-Dec	3-Dec	109.1	132.9	135.6	125.9	31.9	21.2	37.2	30.1	24.6	25.3	25.0	23.1	19.1	ND	
28	4-Dec	5-Dec	66.5	55.8	58.5	60.3	87.7	63.8	50.5	67.3					16.2	ND	
29	6-Dec	8-Dec	212.8	218.1	223.4	218.1	82.4	93.1	103.7	93.1	37.7	37.4	37.6		25.7	ND	
30	9-Dec	10-Dec	154.3	156.9	146.2	152.5	58.6	69.1	71.8	66.5							

Synthetic Feed

Sample	Starting Date	Ending Date	Total COD (mg COD/L.) Vial 1	Vial 2	Vial 3	Average	Filtered COD (mg COD/L.) Vial 1	Vial 2	Vial 3	Average	Total TKN (mg N/L.) Sampl	Sampl	Averag	Filtered TKN (mg N/L.)	NH_3-N (mg N/L.)
1	21-Sep	22-Sep	465.5	495	495	485.2	381.6	384.1	386.6	384.1					
2	23-Sep	24-Sep	465.5	495	495	485.2	381.6	384.1	386.6	384.1					
3	25-Sep	26-Sep	465.4	460.5	467.9	464.6	381.7	386.6	391.5	386.6					24.7
4	27-Sep	29-Sep	628	632.9	632.9	458.3	364.1	389.1	374.3	375.8					
5	30-Sep	1-Oct	522	546.6	568.8	545.8	433.4	433.4	462.9	443.2					26.1
6	2-Oct	3-Oct	522	546.6	568.8	545.8	433.4	433.4	462.9	443.2					26.1
7	4-Oct	6-Oct	497.4	539.3	526.9	521.2	391.5	389.1	386.6	389.1					23.4
8	7-Oct	8-Oct	539.4	541.8	544.3	541.8	458.1	458.1	463	459.7					21.4
9	9-Oct	10-Oct	539.4	541.8	544.3	541.8	458.1	458.1	463	459.7					21.4
10	11-Oct	13-Oct	517.1	497.4	462.9	492.5	441.6	422.3	429.6	431.2					
11	14-Oct	15-Oct	559.5	586.8	594.3	580.2	465	477.5	455	465.8				76.2	
12	16-Oct	17-Oct	559.5	586.8	594.3	580.2	465	477.5	455	465.8				76.2	
13	18-Oct	20-Oct	455.1	470	467.5	464.2	370.5	382.9		376.7	57.9	63.5	60.7	55	
14	21-Oct	22-Oct	420.3	415.3	422.7	419.4	348.1	335.7	340.7	341.5	58.9	58.4	58.7	52.4	
15	23-Oct	24-Oct	460	467.5	474.9	467.5	323.2	333.2	353.2	336.5	65.8		65.8	64.8	
16	25-Oct	27-Oct	422.7	422.7	435.2	426.9	385.4	380.5	363.1	376.3	68.75		68.8	61.5	
17	28-Oct	29-Oct	484.9	482.4	472.5	479.9	350.6	355.6	358.1	354.8	66.9	64.5	65.7	54.2	
18	30-Oct	31-Oct	410.3	435.2	422.7	422.7	365.6	358.1	375.5	366.4	65.5	58.2	61.9	60.2	
19	1-Nov	3-Nov	420.3	430.2	447.6	432.7	375.5	400.4	397.9	391.3	58.5		58.5		
20	4-Nov	5-Nov	487.4	497.4	502.4	495.7					70.2	69.8	70.0		
21	6-Nov	7-Nov	487.4	497.4	502.4	495.7					70.2	69.8	70.0		
22	8-Nov	10-Nov					460	462.5	494.8	472.4					
23	11-Nov	12-Nov	539.6	512.3	532.2	528.0	397.9	380.4	385.5	387.9					
24	25-Nov	26-Nov	518.7	521.3	577.2	539.1	436.2	430.9	449.5	438.9	67.8		67.8		
25	27-Nov	28-Nov	441.5	441.5	409.6	430.9	353.7	369.7	396.3	373.2	41.1	62.4	51.8	56.1	
26	29-Nov	1-Dec	462.8	510.7	468.1	480.5	412.8	409.6	406.9	409.8	62.6	65	63.8	69.9	
27	2-Dec	3-Dec	457.4	460.1	489.4	469.0	380.3	401.6	396.3	392.7	53.7	56.2	55.0	45.8	
28	4-Dec	5-Dec	473.4	521.3	484.1	492.9	375	385.7	367	375.9				55.7	
29	6-Dec	8-Dec	478.7	478.7	470.8	476.1	449.5	449.5	492	463.7	91.6	58.8	75.2	68.8	
30	9-Dec	10-Dec	497.4	481.4	470.8	483.2				0.0					

1. Based on filtered value.

Aerobic Reactor

Sample	Starting Date	Ending Date	Total COD (mg COD/L.) Vial 1	Vial 2	Vial 3	Average	Filtered COD (mg COD/L.) Vial 1	Vial 2	Vial 3	Average	Total TKN (mg N/L.) Sampl	Sampl	Averag	Filtered TKN (mg N/L.)	NH_3-N (mg N/L.)	Nitrate (mg N/L.)
1	21-Sep	22-Sep	78.1	97.5	115.3	97.0	29.4	24.7	21.8	25.3						ND
2	23-Sep	24-Sep	34.7	31.4	32.7	32.9	46.8	29	37	37.6						ND
3	25-Sep	26-Sep	36.4	37.2	33.8	35.8	40.3	33	22.1	31.8					40.8	ND
4	27-Sep	29-Sep	32	40	36.3	36.1	31.5	29.4	29.6	30.2						ND
5	30-Sep	1-Oct	30.6	42.6	37.5	36.9	43.5	22.8	34.4	33.6						ND
6	2-Oct	3-Oct	29.6	34.5	29.6	31.2	22.1	27.1	27.1	25.4					43.4	ND
7	4-Oct	6-Oct	39.4	34.4	32	35.3	19.7	22.1	17.2	19.7					43.6	ND
8	7-Oct	8-Oct	34.5	40.8	43	39.4	38.5	29.6	38.5	35.5					50	ND
9	9-Oct	10-Oct	91.1	91.1	105.9	96.0	19.7	17.3	15.8	17.6					42.6	ND
10	11-Oct	13-Oct	39.3	36.9	41.9	39.4	45.6	30.4	21.2	32.4						
11	14-Oct	15-Oct	67.1	27.3	32.3	42.2	32.3	19.9	34.8	29.0					53.5	ND
12	16-Oct	17-Oct	47.2	37.3	42.3	42.3	27.3	37.3	39.8	34.8					57.9	
13	18-Oct	20-Oct	49.7	89.5	96.9	78.7	39.8	39.7		39.8	49.2			44.3	40.2	ND
14	21-Oct	22-Oct	57.1	44.8	42.3	48.1	47.2	34.8	34.8	38.9	42.3				40.2	ND
15	23-Oct	24-Oct	34.8	44.7	59.6	46.4	49.7	44.7	47.2	47.2	41.4			43.8	43.5	ND
16	25-Oct	27-Oct					19.9	29.8	27.4	25.7	43			41.2	44.1	13.1
17	28-Oct	29-Oct	57.2	57.2	49.7	54.7	57.2	54.7	42.3	51.4	46.1			45.2	42.6	ND
18	30-Oct	31-Oct	37.3	37.3	37.3	37.3	22.4	22.4	22.4	22.4	45.8			44.6	41.9	ND
19	1-Nov	3-Nov	29.8	29.8		29.8	32.3	32.3	34.8	33.1					38.4	ND
20	4-Nov	5-Nov	47.2			47.2	49.7	47.2	44.8	47.2	41	39.2		37.8	47	ND
21	6-Nov	7-Nov	62.2	62.2	49.7	58.0	47.2	52.2	59.6	53.0					39	ND
22	8-Nov	10-Nov	64.6	69.7	57.1	63.8	64.6	89.5	82	78.7					42.8	ND
23	11-Nov	12-Nov	228.7	221.3	208.9	219.6	159.1	151.7	159.1	156.6					32	ND
24	25-Nov	26-Nov					19.9	19.9	19.9	19.9					35.7	ND
25	27-Nov	28-Nov	52.2	52.2	59.7	54.7	23.9	53.1	74.5	50.5	42.2	42		39.2	36.4	ND
26	29-Nov	1-Dec	236.7	204.8	188.9	210.1	77.1	58.5	61.1	65.6	48.6	49.7		41.6	37.9	ND
27	2-Dec	3-Dec	167.6	170.2	178.2	172.0	24.5	27.1		25.8					44.4	ND
28	4-Dec	5-Dec					58.5	21.3	26.6	35.5					37.8	ND
29	6-Dec	8-Dec	45.8	79.8	61.1	62.2	10.6	10.6	5.3	8.8	44.2	44.3		41.5	39.4	ND
30	9-Dec	10-Dec	140.9	82.4	82.4	101.9	87.8	69.1	82.5	79.8					34.1	ND

Anoxic Reactor

Sample	Starting Date	Ending Date	Total COD (mg COD/L)				Filtered COD (mg COD/L)				Total TKN (mg N/L)			Filtered TKN	NH_3-N	Nitrate
			Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Vial 3	Average	Sampl	Sampl	Averag	(mg N/L)	(mg N/L)	(mg N/L)
1	21-Sep	22-Sep	46.6	39.1	72.6	52.8	43.2	38.9	40.2	40.8						29.1
2	23-Sep	24-Sep	36.9	48.6	41.3	42.3	36	30.4	33	33.1						25.5
3	25-Sep	26-Sep	45.9	48.4	40.4	44.9	49.9	42.9	81.7	58.2						14.8
4	27-Sep	29-Sep	61.6	59.1	64	61.6	34.7	36.4	47.1	39.4						35.8
5	30-Sep	1-Oct	81.1	73.7	85.2	80.0	31.7	31.4	38.1	33.7						14.6
6	2-Oct	3-Oct	54.1	54.2	56.6	55.0	24.6	29.5	34.5	29.5						15.8
7	4-Oct	6-Oct	158.9	166.8	154	159.9	39.4	27.4	34.4	33.7						5.7
8	7-Oct	8-Oct	46.8	54.2	49.2	50.1	45.3	36.9	29.6	37.3					43.9	8.9
9	9-Oct	10-Oct	27.8	29.5	32	29.8	32	24.6	19.7	25.4					49.2	13
10	11-Oct	13-Oct	54.7	54.7	57.7	55.7	48.6	42.6	48.6	46.6						16.7
11	14-Oct	15-Oct	74.6	84.6	96.9	85.4	29.8	42.3	34.8	35.6						0.7
12	16-Oct	17-Oct	49.7	52.2	52.2	51.4	54.7	49.7	42.3	48.9						0.9
13	18-Oct	20-Oct	49.7	52.2	52.2	51.4	34.8	34.8	32.3	34.0	34.9		34.9	42.5	40.2	8.9
14	21-Oct	22-Oct	34.8	52.2	39.7	42.2	37.3	37.3	37.3	37.3				37	38.3	18.3
15	23-Oct	24-Oct	32.3	34.8	34.8	34.0	32.3	44.7	34.8	37.3				36.8	39.5	27.9
16	25-Oct	27-Oct	24.9	14.9	24.8	21.5	52.2	89.9	24.9	55.7	40.3		40.3	43.9	43.5	8.6
17	28-Oct	29-Oct	54.7	39.7	42.3	45.6	62.1	37.3	39.8	46.4	44.9		44.9	42.4	41.9	8.6
18	30-Oct	31-Oct	37.3	37.3	49.7	41.4	54.7	37.3	39.7	43.9	47.2		47.2	46.3	43.4	13.1
19	1-Nov	3-Nov	29.8	29.8		29.8	32.3	32.3	34.8	33.1						0.2
20	4-Nov	5-Nov	74.6	72.1		73.4	42.3	44.8	42.3	43.1	42.8	45.5	44.15	40	37.9	18.4
21	6-Nov	7-Nov	111.9	114.4	116.9	114.4	67.1	72.1	72.2	70.5					38.7	23
22	8-Nov	10-Nov	104.5	101.9	111.9	106.1									38.5	14.7
23	11-Nov	12-Nov	104.5	99.5	106.9	103.6	69.6	59.6	49.7	59.6					34.6	0.2
24	25-Nov	26-Nov	39.7	39.7	39.7	39.7	29.8	32.3	29.8	30.6					34.7	23
25	27-Nov	28-Nov	18.6	39.8		29.2	21.3	21.3		21.3	36	34.9	35.45	35.4	33.4	38.6
26	29-Nov	1-Dec	42.5	34.5	34.5	37.2	21.3	18.6	13.2	17.7	35.8	36.5	36.15	39.5	36.5	27.2
2.	2-Dec	3-Dec	26.5	21.3		23.9		23.9	29.5	26.7	27.7	39.6	33.65	35.9	3..6	0.4
28	4-Dec	5-Dec	71.8	93.1	63.8	76.2									39.1	12
29	6-Dec	8-Dec	66.4	63.8	42.5	57.6	71.8	95.7	63.8	77.1	29.3	44.4	36.85	43.1	40	17.8
30	9-Dec	10-Dec	98.4	79.8	87.7	88.6	21.3	31.9	21.3	24.8					36.9	27.8

Aerobic Reactor

Sample	Starting Date	Ending Date	Effluent (mL) Day 1	Day 2	Day 3	Total	Wastage (mL) Day 1	Day 2	Day 3	Total	Acid Addition (mL)	Volume of Feed (mL)	Organic Removal Based on Tot. Effluent (mg/d)	Based on Filt. Effluent (mg/d)
1	21-Sep	22-Sep	7020	8390		15410	950	925		1875	661	16624	2502	3121
2	23-Sep	24-Sep	7590	7590		15180	917	917		1834	604	16411	3017	2977
3	25-Sep	26-Sep	7300	7300[1]		14600	1125	875		2000	554	16046	2559	2592
4	27-Sep	29-Sep	7525	7380	7230	22135	955	875	1035	2865	608	24392	2813	2862
5	30-Sep	1-Oct	7525	7525		15050	988	988		1976	361	16665	2913	2941
6	2-Oct	3-Oct	7280	7280		14560	865	815		1680	393	15847	2815	2862
7	4-Oct	6-Oct	7240	7240	7100	21580	933	933	875	2741	480	23841	2923	3049
8	7-Oct	8-Oct	6858	6858		13716	938	938		1876	252	15340	2970	3000
9	9-Oct	10-Oct	7000	6870		13870	935	910		1845	300	15415	2305	2921
10	11-Oct	13-Oct	7017	7017	6980	21014	790	790	795	2375	289	23100	2654	2709
11	14-Oct	15-Oct	7335	7200		14535	800	795		1595	289	15841	3140	3246
12	16-Oct	17-Oct	6735	6280		13015	860	835		1695	212	14498	2496	2551
13	18-Oct	20-Oct	7330	7270	7210	21810	990	990	1000	2980	608	24182	2256	2577
14	21-Oct	22-Oct	6920	6920		13840	992.5	992.5		1985	79	15746	2073	2145
15	23-Oct	24-Oct	6600	6600		13200	977.5	977.5		1955	238	14917	2218	2212
16	25-Oct	27-Oct	6900	6980	6770	20650	920	925	1000	2845	355	23140	2832	2630
17	28-Oct	29-Oct	6790	6950		13740	1000	1000		2000	149	15591	2687	2713
18	30-Oct	31-Oct	6915	6915		13830	1050	1000		2050	232	15648	2198	2316
19	1-Nov	3-Nov	6900	7030	5635	19565	930	975	985	2890	365	22090	2600	2575
20	4-Nov	5-Nov	6995	6995		13990	1025	1025		2050	367	15673	2877	2877
21	6-Nov	7-Nov	6695	7200		13895	1075	1085		2160	320	15736	2803	2843
22	8-Nov	10-Nov	6928	6928	6920	20776	950	925	1100	2975	492	23259	2596	2478
23	11-Nov	12-Nov	6633	6632		13265	989	976		1965	345	14885	1364	1844
24	25-Nov	26-Nov	7120	7120		14240	1028	1027.5		2055		16295	3409	3246
25	27-Nov	28-Nov	7070	7070		14140	1030	1025		2055		16195	2318	2352
26	29-Nov	1-Dec	7170	7170	6330	20670	980	980	1075	3035	477	23228	1331	2474
27	2-Dec	3-Dec	7600	7600		15200	780	780		1560	90	16671	1465	2690
28	4-Dec	5-Dec	6970	6970		13940	975	975		1950	359	15531	2721	2439
29	6-Dec	8-Dec	6500	6500	6530	19530	1025	1075	1075	3175	352	22353	2419	2824
30	9-Dec	10-Dec	7095	7095		14190	1950			1950	352	15788	2124	2302

1. Estimated value.

Anoxic Reactor

Sample	Starting Date	Ending Date	Effluent (mL.) Day 1	Day 2	Day 3	Total	Wastage (mL.) Day 1	Day 2	Day 3	Total	Acid Addition (mL.)	Nitrate Addition (mL.)	Concentration of Nitrate Stock (mg NO_3-N/mL)	Total Feed Volume (mL.)
1	21-Sep	22-Sep	7360	6965		14325	1000	875		1875	132	106.4	13.000	15961
2	23-Sep	24-Sep	14550			14550	2000			2000	363	108	13.000	16079
3	25-Sep	26-Sep	7460	7460		14920	1250	1000		2250	679	78.9	13.000	16412
4	27-Sep	29-Sep	6900	7140	6920	20960	1000	1025	875	2900	224	162.2	13.900	23474
5	30-Sep	1-Oct	14100			14100	2100			2100	103	88.1	13.900	16009
6	2-Oct	3-Oct	7000	7535		14535	1000	935		1935	123	83.7	13.900	16264
7	4-Oct	6-Oct	14210	7030		21240	2025	1000		3025	481	132.1	13.900	23652
8	7-Oct	8-Oct	14085			14085	1965			1965	200	85.4	13.900	15764
9	9-Oct	10-Oct	7070	7080		14150	1000	910		1910	249	98.8	13.900	15712
10	11-Oct	13-Oct	13760	7095		20855	1940	960		2900	335	152.7	12.650	23267
11	14-Oct	15-Oct	7040	6880		13920	950	960		1910		91.1	12.650	15739
12	16-Oct	17-Oct	6895	6345		13240	1000	775		1775	239	97.3	12.650	14679
13	18-Oct	20-Oct	7180	7150	7140	21470	980	980	975	2935	122	135.9	13.250	24147
14	21-Oct	22-Oct	14080			14080	1970			1970	48	90.9	13.250	15911
15	23-Oct	24-Oct	12775			12775	1000	1125		2125	99	87.4	13.250	14714
16	25-Oct	27-Oct	6850	6895	6870	20615	1150	1035	1000	3185	128	127.5	12.495	23544
17	28-Oct	29-Oct	6770	6430		13200	1000	975		1975	121	83.8	12.495	14970
18	30-Oct	31-Oct	13830			13830	1050	1000		2050	117	87.1	12.495	15676
19	1-Nov	3-Nov	6950	7010	6660	20620	930	910	1025	2865	117	137.9	12.830	23230
20	4-Nov	5-Nov	13685			13685	2000			2000	109	90.2	12.830	15486
21	6-Nov	7-Nov	6610	6610		13220	1075	940		2015	119	87.9	12.830	15029
22	8-Nov	10-Nov	14340	6920		21260	875	475	1050	2400	184	132.6	12.550	23343
23	11-Nov	12-Nov	13160			13160	1257	1257		2514	91	82	12.550	15501
24	25-Nov	26-Nov	6915	6915		13830	1033	1000		2033		97.2	12.305	15766
25	27-Nov	28-Nov	6825	6825		13650	1000	1085		2085		97.2	12.305	15638
26	29-Nov	1-Dec	7040	7040	5400	19480	1008	1007	870	2885	108	149.3	11.830	22108
27	2-Dec	3-Dec	6830	6830		13660	1013	1012		2025	68	100.5	11.830	15516
28	4-Dec	5-Dec	6555	6555		13110	1075	1015		2090	89	97.9	11.830	15013
29	6-Dec	8-Dec	6400	6400	6795	19595	1015	1025	1050	3090	130	149.5	12.305	22406
30	9-Dec	10-Dec	6855	6855		13710	895	1025		1920	149.7	102.2	12.305	15378

Anoxic Reactor

Sample	Starting Date	Ending Date	Organic Removal Based on Tot. Effluent (mg/d)	Organic Removal Based on Filt. Effluent (mg/d)	Nitrate Consumption (mg NO_3-N/d)	Consumptive Ratio Total	Consumptive Ratio Filtered
1	21-Sep	22-Sep	2780	2877	463	6.0	6.2
2	23-Sep	24-Sep	2881	2956	507	5.7	5.8
3	25-Sep	26-Sep	2536	2422	408	6.2	5.9
4	27-Sep	29-Sep	2507	2683	462	5.4	5.8
5	30-Sep	1-Oct	2452	2827	518	4.7	5.5
6	2-Oct	3-Oct	2697	2906	454	5.9	6.4
7	4-Oct	6-Oct	1890	2910	575	3.3	5.1
8	7-Oct	8-Oct	2966	3069	521	5.7	5.9
9	9-Oct	10-Oct	2879	2914	583	4.9	5.0
10	11-Oct	13-Oct	2542	2614	515	4.9	5.1
11	14-Oct	15-Oct	2782	3176	587	4.7	5.4
12	16-Oct	17-Oct	2456	2475	609	4.0	4.1
13	18-Oct	20-Oct	2484	2625	524	4.7	5.0
14	21-Oct	22-Oct	2140	2179	448	4.8	4.9
15	23-Oct	24-Oct	2281	2257	367	6.2	6.2
16	25-Oct	27-Oct	2710	2439	477	5.7	5.1
17	28-Oct	29-Oct	2648	2642	460	5.8	5.7
18	30-Oct	31-Oct	2170	2150	438	5.0	4.9
19	1-Nov	3-Nov	2735	2709	597	4.6	4.5
20	4-Nov	5-Nov	2642	2879	420	6.3	6.9
21	6-Nov	7-Nov	2250	2585	389	5.8	6.6
22	8-Nov	10-Nov	2275	3112	447	5.1	
23	11-Nov	12-Nov	2350	2695	528	4.5	5.1
24	25-Nov	26-Nov	2983	3055	395	7.6	7.7
25	27-Nov	28-Nov	2436	2498	282	8.6	8.8
26	29-Nov	1-Dec	2570	2716	398	6.5	6.8
27	2-Dec	3-Dec	2518	2496	618	4.1	4.0
28	4-Dec	5-Dec	2051	2631	479	4.3	5.5
29	6-Dec	8-Dec	2462	2314	478	5.2	4.8
30	9-Dec	10-Dec	2177	2675	409	5.3	

20 C Nitrogen Balances

Aerobic Reactor

Starting Date	Ending Date	Feed Volume (L.)	Sewage TKN (mg N/L.)	Protein TKN (mg N/L.)	Total Mass of N IN (mg N)	Effluent Volume (mL.)	Effluent TKN (mg N/L.)	Waste Volume (mL.)	Mixed Liquor TKN (mg N/L.)	Total Mass of N OUT (mg N)	Closure OUT/IN %
6-Dec	8-Dec	22.35	37.6	73.7	1376.7	19.53	44.4	3.18	125.9	1266.9	92%
2-Dec	3-Dec	16.67	25.0	55.0	749.4	15.20	44.4	1.56	96.7	825.7	110%
29-Nov	1-Dec	23.23	28.0	63.8	1203.6	20.67	49.2	3.04	119.9	1380.9	115%
27-Nov	28-Nov	16.20	31.5	62.4	842.9	14.14	42.1	2.06	117.9	837.6	99%
4-Nov	5-Nov	15.67	37.6	70.0	926.9	13.99	40.1	2.05	116.1	799.0	86%
30-Oct	31-Oct	15.65	48.2	61.9	896.3	13.83	45.8	2.05	83.7	805.0	90%
28-Oct	29-Oct	15.59	45.3	65.7	917.4	13.74	46.1	2.00	118.0	869.4	95%
25-Oct	27-Oct	23.14	46.9	68.8	1421.7	20.65	43.0	2.85	119.1	1226.8	86%
23-Oct	24-Oct	14.92	24.5	65.8	775.4	13.20	41.4	1.96	123.3	787.5	102%
21-Oct	22-Oct	15.75	39.0	58.7	820.1	13.84	42.3	1.99	123.0	829.6	101%
18-Oct	20-Oct	24.18	36.2	57.7	1220.8	21.81	49.2	2.98	114.3	1413.7	116%
										Average	99%
										Range	86%
										Range	116%
										Deviation	11%

Anoxic Reactor

Starting Date	Ending Date	Feed Volume (L.)	Sewage TKN (mg N/L.)	Protein TKN (mg N/L.)	Total Mass of N IN (mg N)	Effluent Volume (mL.)	Effluent TKN (mg N/L.)	Waste Volume (mL.)	Mixed Liquor TKN (mg N/L.)	Total Mass of N OUT (mg N)	Closure OUT/IN %
6-Dec	8-Dec	22.40	37.6	73.7	1379.6	19.60	44.4	3.09	134.3	1285.0	93%
2-Dec	3-Dec	15.52	25.0	55.0	697.5	13.66	33.7	2.03	127.4	718.3	103%
29-Nov	1-Dec	22.11	28.0	63.8	1145.5	19.48	36.2	2.89	104.1	1005.5	88%
27-Nov	28-Nov	15.64	31.5	62.4	813.9	13.65	35.5	2.09	108.7	711.2	87%
4-Nov	5-Nov	15.48	37.6	70.0	915.7	13.69	44.2	2.00	111.7	828.3	90%
30-Oct	31-Oct	15.69	48.2	61.9	898.9	13.83	47.2	2.05	123.9	906.8	101%
28-Oct	29-Oct	14.97	45.3	65.7	880.7	13.20	44.9	1.98	142.1	873.3	99%
25-Oct	27-Oct	23.59	46.9	68.8	1449.2	20.62	40.3	3.19	138.5	1271.9	88%
23-Oct	24-Oct	14.71	24.5	65.8	764.9	12.78	36.8	2.13	134.8	756.6	99%
21-Oct	22-Oct	15.91	39.0	58.7	828.7	14.08	37.0	1.97	136.1	789.1	95%
18-Oct	20-Oct	24.15	36.2	57.7	1219.0	21.47	34.9	2.94	132.0	1136.7	93%
										Average	94%
									Lower	Range	87%
									Upper	Range	103%
									Standard	Deviation	6%

SBR Data - Mar. 4 -May 9/96

Sewage

Sample	Starting Date	Ending Date	Total COD (mg COD/L) Vial 1	Vial 2	Vial 3	Average	Filtered COD (mg COD/L) Vial 1	Vial 2	Vial 3	Average	Total TKN (mg N/L) Sample 1	Sample Average	Filtered TKN (mg N/L)	NH_3-N (mg N/L)	Nitrate (mg N/L)
1	4-Mar	5-Mar	153	144.8	131.1	143.0	51.9	60.1	71	61.0					
2	6-Mar	8-Mar	243.1	254	248.6	248.6	98.3	92.8	103.7	98.3					
3	9-Mar	10-Mar	232.2	226.7	218.5	225.8	101.1	90.1	92.9	94.7					
4	11-Mar	12-Mar	232.2	226.7	218.5	225.8	101.1	90.1	92.9	94.7					
5	13-Mar	15-Mar	423.4	385.1	377	395.2	286.8	259.5	264.9	270.4					
6	16-Mar	17-Mar	259.5	245.9	243.1	249.5	101.1	106.5	98.3	102.0					
7	18-Mar	19-Mar	259.5	245.9	243.1	249.5	101.1	106.5	98.3	102.0					
8	20-Mar	22-Mar	152.8	166.5	161.9	160.4	141.4	150.5	132.8	141.6					
9	23-Mar	24-Mar	152.8	166.5	161.9	160.4	141.4	150.5	132.8	141.6					
10	25-Mar	26-Mar	152.8	166.5	161.9	160.4	141.4	150.5	132.8	141.6					
11	27-Mar	29-Mar	127.8	130	145.6	134.5	73	73	68.4	71.5			28.7	26.7	1.5
12	30-Mar	31-Mar	234.9	232.6	234.9	234.1	193.9	198.4	198.4	196.9					1.5
13	1-Apr	2-Apr	234.9	232.6	234.9	234.1	193.9	198.4	198.4	196.9					ND
14	3-Apr	5-Apr	255.4	256	257.7	256.4	205.3	200.7	207.5	204.5	28.2	28.2	25.2	18.5	
15	6-Apr	7-Apr	77.5	116.5	77.5	90.5	50.2	61.5		55.9	*28.5*	*28.5*	*24.6*	24.1	0.18
16	8-Apr	9-Apr	77.5	116.5	77.5	90.5	50.2	61.5		55.9	*28.5*	*28.5*	*24.6*	24.1	0.18
17	10-Apr	12-Apr	248.6	253.1	255.4	252.4	143.7	150.5	152.8	149.0	29.9	29.9	20	16.7	ND
18	13-Apr	14-Apr	216.7	193.8	184.7	198.4	91.2	86.7	95.8	91.2	25.5	25.5	17.1	17.8	ND
19	15-Apr	16-Apr	216.7	193.8	184.7	198.4	91.2	86.7	95.8	91.2	25.5	25.5	17.1	17.8	ND
20	17-Apr	19-Apr	119.6	104.9		112.3	107.4	85.5	122.1	105.0	25.3	25.3	18.8		2.28
21	20-Apr	21-Apr	97.7	83	100.1	93.6	63.5	63.5	58.6	61.9	21.5	21.5	18.8	20.1	ND
22	22-Apr	23-Apr	97.7	83	100.1	93.6	63.5	63.5	58.6	61.9	21.5	21.5	18.8	20.1	ND
23	24-Apr	26-Apr	322.3	354	371.1	349.1	107.4	97.6	104.9	103.3	28.7	28.7	22.7	16.7	0.9
24	27-Apr	28-Apr	322.3	354	371.1	349.1	107.4	97.6	104.9	103.3					
25	29-Apr	30-Apr	322.3	354	371.1	349.1	107.4	97.6	104.9	103.3					
26	1-May	3-May	234.4	207.5	239.2	227.0	100.1	112.3	114.7	109.0	31.1	31.1	25.4		ND
27	4-May	5-May	95.2	92.7	92.8	93.6	61	58.6	70.9	63.5				21.3	
28	6-May	7-May	95.2	92.7	92.8	93.6	61	58.6	70.9	63.5				21.3	
29	8-May	9-May	156.3	153.8	156.3	155.5	109.9	114.7	97.7	107.4	25.7	25.7	23.7		ND

Synthetic Feed

Sample	Starting Date	Ending Date	Total COD (mg COD/L)				Filtered COD (mg COD/L)				Total TKN (mg N/L)			Filtered TKN (mg N/L)
			Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Avg.	
1	4-Mar	5-Mar	707.4	734.8	691	711.1	573.6	557.2	581.8	570.9				
2	6-Mar	8-Mar	671.9	682.9	666.5	673.8	453.4	483.5	502.6	479.8				
3	9-Mar	10-Mar	494.4	491.6	505.3	497.1	437	404.3	423.4	421.6				
4	11-Mar	12-Mar	494.4	491.6	505.3	497.1	437	404.3	423.4	421.6				
5	13-Mar	15-Mar	423.4	385.1	377	395.2	286.8	259.5	264.9	270.4				
6	16-Mar	17-Mar	453.4	519	472.5	481.6	374.2	379.7	371.5	375.1				
7	18-Mar	19-Mar	453.4	519	472.5	481.6	374.2	379.7	371.5	375.1				
8	20-Mar	22-Mar	546.3	542.4	538.1	542.3	417.9	431.6	428.8	426.1				
9	23-Mar	24-Mar												
10	25-Mar	26-Mar												
11	27-Mar	29-Mar	394.5	408.2	399.1	400.6	317	298.8	314.8	310.2	48.6	47.6	48.1	26.2
12	30-Mar	31-Mar	572.4	554.2	558.8	561.8	456.1	453.9	440.2	450.1	63.1		63.1	47.4
13	1-Apr	2-Apr	483.5	478.9	481.2	481.2	332.9	330.7	351.2	338.3	63.1		63.1	47.4
14	3-Apr	5-Apr	492.6	490.3	488.1	490.3	355.8	355.8	351.2	354.3	53.2	52.5	52.9	36.9
15	6-Apr	7-Apr	458.4	449.3	449.3	452.3	385.4	380.9	380.9	382.4	74.3	72.3	73.3	61.9
16	8-Apr	9-Apr	458.4	449.3	449.3	452.3	385.4	380.9	380.9	382.4	74.3	72.3	73.3	61.9
17	10-Apr	12-Apr	460.7	478.9	492.6	477.4	351.2	351.2	353.5	352.0	71.3	60.4	65.9	41.1
18	13-Apr	14-Apr	449.3	437.9	449.2	445.5	328.4	342.1	342.1	337.5	58.7	56.5	57.6	41.9
19	15-Apr	16-Apr	405.9	408.2	396.8	403.6	328.4	323.8	314.7	322.3	50.2	44.1	47.2	45.5
20	17-Apr	19-Apr	495.6	473.7	481	483.4	424.8	427.3	429.7	427.3	62.9	54	58.5	53.4
21	20-Apr	21-Apr	441.9	439.5	439.5	440.3	368.7	380.9	380.9	376.8	55.2	54.7	55.0	46.2
22	22-Apr	23-Apr	441.9	439.5	439.5	440.3	368.7	380.9	380.9	376.8	55.2	54.7	55.0	46.2
23	24-Apr	26-Apr	549.4	544.5	507.8	533.9	410.2	429.7	451.7	430.5	66.8	74.4	70.6	52.6
24	27-Apr	28-Apr				433.0				353.0				
25	29-Apr	30-Apr				433.0				353.0				
26	1-May	3-May	456.6	459	451.7	455.8	419.9	410.2	402.8	411.0	62.4	60.2	61.3	
27	4-May	5-May	454.1	463.9	415.1	444.4	405.3	378.5	378.5	387.4				
28	6-May	7-May	454.1	463.9	415.1	444.4	405.3	378.5	378.5	387.4				
29	8-May	9-May	485.8	488.3	537.1	503.7	344.2	349.1	263.8	319.0	72.4	69.5	71.0	

Aerobic Reactor

Sample	Starting Date	Ending Date	Total COD (mg COD/L)				Filtered COD (mg COD/L)				Total TKN (mg N/L)			Filtered TKN (mg N/L)	NH_3-N (mg N/L)	Nitrate (mg N/L)
			Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Vial 3	Average	Sample 1	Sample	Average			
1	4-Mar	5-Mar	32.8	27.3	27.3	29.1	20	25	20	21.7						ND
2	6-Mar	8-Mar	43.7	46.4	41	43.7	35.5	35.5	32.8	34.6						ND
3	9-Mar	10-Mar	20	20	20	20.0	20	20	20	20.0						ND
4	11-Mar	12-Mar	20	20	20	15.0	20	20	20	20.0						ND
5	13-Mar	15-Mar	20	20	20	20.0	20	20	20	20.0						
6	16-Mar	17-Mar	21.9	20	20	20.6	20	20	20	20.0						
7	18-Mar	19-Mar	34.7	34	39.2	36.0	23.3	36.7	26.1	28.7						ND
8	20-Mar	22-Mar	57	50.2	55.8	54.3	31.1	38	36.8	35.3						ND
9	23-Mar	24-Mar	70.7	82.1	70.7	74.5	45.3	57	41.1	47.8						ND
10	25-Mar	26-Mar	111.7	111.7	111.7	111.7	47.9	45.6	47.9	47.1						ND
11	27-Mar	29-Mar	91.2	89	98.1	92.8	25.1	29.6	27.4	27.4	39.6		39.6	33.9	30	
12	30-Mar	31-Mar	104.9	100.4	104.9	103.4	34.2	34.2	29.7	32.7	42	43.4	42.7	52.8	34	0.42
13	1-Apr	2-Apr	134.6	120.9	120.9	125.5	45.6	47.8	38.8	44.1	45.9	47.2	46.6	41.5	39.1	
14	3-Apr	5-Apr	155	148	145	149.3	57	61.5	60	59.5	44.9		44.9	33.1	31.7	0.19
15	6-Apr	7-Apr	127.7	84.4	114.1	108.7	36.5	31.9	36.4	34.9	39.6	37	38.3		29.1	0.15
16	8-Apr	9-Apr	84.4	61.5	72.9	72.9	34.2	34.2	29.6	32.7	40.6	40	40.3	31.4	29.2	
17	10-Apr	12-Apr	84.4	84.3	88.9	85.9	29.6	36.4		33.0	42.8		42.8	38.4	34.3	
18	13-Apr	14-Apr	100.3	100.3	91.2	97.3	36.5	38.7		37.6	38.7		38.7		33.1	
19	15-Apr	16-Apr	54.7	50.1	66.1	57.0	22.8	38.8	20.5	27.4	36.5		36.5	31.1	35.6	ND
20	17-Apr	19-Apr	95.2	95.2	97.7	96.0	46.4	51.2	48.8	48.8	40.8		40.8	34.3		0.4
21	20-Apr	21-Apr	90.3	100.1	95.2	95.2	48.8	46.3	58.6	51.2	34.2		34.2	33.7	31.9	ND
22	22-Apr	23-Apr	107.4	90.3		98.9	58.6	53.7	63.5	58.6	34.3		34.3	38	36.5	ND
23	24-Apr	26-Apr	109.9			109.9	83	63.5	65.9	70.8	45.9		45.9	41.2	35.9	ND
24	27-Apr	28-Apr	85.5	75.7		80.6	41.5	39.1	65.9	48.8					36.9	0.2
25	29-Apr	30-Apr	104.9	107.4	107.4	106.6	41.5	43.9	53.7	46.4					36.2	ND
26	1-May	3-May	61	63.5	65.9	63.5	39.1	43.9	51.3	44.8	37.2		37.2	47.7		0.2
27	4-May	5-May	107.4	100.1	104.9	104.1									36.1	0.3
28	6-May	7-May	129.4	11 .3	119.6	120.4	83	75.6	68.4	75.7					36.5	
29	8-May	9-May	109.9	107.4	87.9	101.7	68.4	70.8	61	66.7	39.1		39.1	38.1	35.7	0.3

Anoxic Reactor

Sample	Starting Date	Ending Date	Total COD (mg COD/L)				Filtered COD (mg COD/L)				Total TKN (mg N/L)			Filtered TKN (mg N/L)	NH_3-N (mg N/L)	Nitrate (mg N/L)
			Vial 1	Vial 2	Vial 3	Average	Vial 1	Vial 2	Vial 3	Average	Sample 1	Sample	Average			
1	4-Mar	5-Mar	131.1	128.4	142	133.8	27.3	43.7	30.1	33.7						16.7
2	6-Mar	8-Mar	90.1	106.5	112	102.9	60.1	57.4	46.4	54.6						31.3
3	9-Mar	10-Mar	46.4	57.4	54.6	52.8	20	20	20	20.0						40.9
4	11-Mar	12-Mar	60.1	57.4	65.5	61.0	30	20	20	23.3						54.8
5	13-Mar	15-Mar	90.1	76.5	87.4	84.7	30	20	20	23.3						32.4
6	16-Mar	17-Mar	79.2	81.9	92.9	84.7	27.3	20	20	22.4						7.5
7	18-Mar	19-Mar	115.1	122.1	125.8	121.0	48.1	44.9	48.8	47.3						12.9
8	20-Mar	22-Mar	98.8	107.2	104.9	103.6	57	68.9	58.6	61.5						4.4
9	23-Mar	24-Mar	63.9	68.4	68.4	66.9	54.7	57	59.3	57.0						23
10	25-Mar	26-Mar	68.4	75.3	70.7	71.5	54.7	38.8	54.7	49.4						29.2
11	27-Mar	29-Mar	59.3	59.3	57	58.5	20	20	20	20.0						32.4
12	30-Mar	31-Mar	77.5	82.1	63.9	74.5	50.2	34.2	36.5	40.3	39		39	35.9	32.4	22.4
13	1-Apr	2-Apr	91.2	86.6	77.5	85.1	41.1	45.6	70.7	52.5				36.6	39.4	21.9
14	3-Apr	5-Apr	70.6	68.4	63.8	67.6	43.3	45.6	60	49.6	36.9		36.9		32.5	21.7
15	6-Apr	7-Apr	52.4	47.9	66.1	55.5	41.1	52.4	43.3	45.6	38.2	34.6	36.4	33.8	32	13.4
16	8-Apr	9-Apr	59.3	59.3	57	58.5	61.5	31.9	34.2	42.5	42.3		42.3	43.1	40.2	24.1
17	10-Apr	12-Apr	66.1	61.5	63.9	63.8	59.3	47.8	47.8	51.6	39.6		39.6	37.5	33.3	11.2
18	13-Apr	14-Apr	72.9	70.7	75.5	73.0	31.9	34.2	31.9	32.7	37.6		37.6	32	29.6	19.8
19	15-Apr	16-Apr	59.3	70.7	47.8	59.3	38.7	34.2	34.2	35.7	30.1		30.1	27.3	31.5	79.6
20	17-Apr	19-Apr	68.4	68.3	65.9	67.5	61	58.6	51.3	57.0				34.2		14.3
21	20-Apr	21-Apr	58.6	65.9	51.2	58.6	31.7	34.2	75.6	47.2	27.6		27.6	27.6	32.3	
22	22-Apr	23-Apr	107.4	90.3		98.9	58.6	53.7	63.5	58.6	33.3		33.3			33.7
23	24-Apr	26-Apr	112.3	102.5	83	99.3	90.6	61	56.2	69.3	38.8		38.8		32.9	23.8
24	27-Apr	28-Apr	53.7	51.3	48.8	51.3	36.6	39.1	34.2	36.6					35.9	49.4
25	29-Apr	30-Apr	43.9	39.1	58.6	47.2	31.7	39.1	46.4	39.1					31.4	32.4
26	1-May	3-May	68.4	63.5	61	64.3	46.4	39	34.2	39.9	38.3		38.3	35.4		21.6
27	4-May	5-May	85.4	95.2	85.4	88.7	56.1	58.6	63.5	59.4					33.6	31.3
28	6-May	7-May	124.5	122.	102.5	116.4	53.7	43.9	43.9	47.2					39.7	17.3
29	8-May	9-May	119.6	97.6	87.9	101.7	46.3	56.1	58.6	53.7	42.4		42.4	38.5	35.7	0.288

Aerobic Reactor

Sample	Starting Date	Ending Date	Effluent (mL) Day 1	Day 2	Day 3	Total	Wastage (mL) Day 1	Day 2	Day 3	Total	Acid Addition (mL)	Volume of Feed (mL)	Organic Removal Based on Tot. Effluent (mg/d)	Based on Filt. Effluent (mg/d)
1	4-Mar	5-Mar	6440	6440		12880	187	855		1042		13922	3166	3218
2	6-Mar	8-Mar	6572	6572	6430	19573	929	947	971	2847		22420	3399	3467
3	9-Mar	10-Mar	6540	6540		13080	968.5	968.5		1937		15017	2773	2773
4	11-Mar	12-Mar	6361	6361		12721	975	977		1952		14673	2746	2709
5	13-Mar	15-Mar	6582	6582	6693	19857	893	957	955	2805		22662	2921	2921
6	16-Mar	17-Mar	6484	6484		12967	775	775		1550		14517	2690	2695
7	18-Mar	19-Mar	6183	6782		12965	935	931		1866		14831	2634	2688
8	20-Mar	22-Mar	6729	6490	7000	20219	923	985		1908		22127	2430	2570
9	23-Mar	24-Mar				0	600	600		1200		1200		
10	25-Mar	26-Mar	6489	6489		12978	927	975		1902		14880		
11	27-Mar	29-Mar	6850	6850	6850	20550	955	885	1021	2861		23411	1690	2201
12	30-Mar	31-Mar	6825	6825		13650	875	875		1750		15400	2687	3232
13	1-Apr	2-Apr	6772	6772		13544	875	875		1750		15294	2089	2712
14	3-Apr	5-Apr	6693	6693	6690	20075	891	1010	1040	2941		23016	2012	2701
15	6-Apr	7-Apr	6060	6060		12120	1058	1058		2116.67		14237	1844	2369
16	8-Apr	9-Apr	5630	5630		11260	1058	1190		2248		13508	1716	1988
17	10-Apr	12-Apr	6625	6625	6886	20136	1000	1038	1059	3097		23233	2438	2848
18	13-Apr	14-Apr	6595	6595		13190	1068	1069		2137		15327	2156	2614
19	15-Apr	16-Apr	6580	6580		13160	870	1024		1894		15054	2138	2361
20	17-Apr	19-Apr	7395	7395	7220	22010	700	727	727	2154		24164	2141	2521
21	20-Apr	21-Apr	7250	7250		14500	1027	1028		2055		16555	1909	2273
22	22-Apr	23-Apr	7090	7090		14180	1081	650		1731		15911	1805	2126
23	24-Apr	26-Apr	7003	7003	6250	20255	1031	1103	1050	3184		23439	2758	3064
24	27-Apr	28-Apr	6900	6900		13800	1026.5	1026.5		2053		15853	2498	2749
25	29-Apr	30-Apr	6365	6365		12730		972		972		13702	2067	2480
26	1-May	3-May	7270	7270	7270	21810	1045	800	923	2768		24578	2577	2731
27	4-May	5-May	7270	7190		14460	1053	870		1923		16383	1831	2684
28	6-May	7-May	7090	7090		14180	988	1030		2018		16198	1678	2041
29	8-May	9-May	6975	6975		13950	1027	1090		2117		16067	2296	2577

Anoxic Reactor

Sample	Starting Date	Ending Date	Effluent (mL) Day 1	Day 2	Day 3	Total	Wastage (mL) Day 1	Day 2	Day 3	Total	Nitrate Addition (mL)	Concentration of Nitrate Stock (mg NO_3-N/mL)
1	4-Mar	5-Mar	6440	6440		12880	187	855		1042	66.4	12.948
2	6-Mar	8-Mar	6571.5	6571.5	6430	19573	929	947	971	2847	148.1	12.990
3	9-Mar	10-Mar	6740	6740		13480	887.5	887.5		1775	77.2	12.990
4	11-Mar	12-Mar	6360.5	6360.5		12721	975	977		1952	77.2	12.990
5	13-Mar	15-Mar	6736.5	6736.5	6693	20166	893	907	935	2735	140.3	12.990
6	16-Mar	17-Mar	6541	6541		13082	758.5	758.5		1517	60.3	12.990
7	18-Mar	19-Mar	6230	6810		13040	857	921		1778	56.1	12.990
8	20-Mar	22-Mar	6729	6490	7000	20219	923	985	985	2893	69.4	12.990
9	23-Mar	24-Mar				0	600	600		1200	86.1	12.990
10	25-Mar	26-Mar	6586	6586		13172	935	875		1810	86.1	12.990
11	27-Mar	29-Mar	6850	6850	6850	20550	874.5	874.5	970	2719	129.7	12.809
12	30-Mar	31-Mar	6850	6850		13700	875	875		1750	103.6	12.809
13	1-Apr	2-Apr	6767	6767		13534	970	875		1845	96.7	12.809
14	3-Apr	5-Apr	6833.5	6833.5	6890	20557	782	980	835	2597	120	13.038
15	6-Apr	7-Apr	6347.5	6347.5		12695	1225	1225		2450	85.6	13.065
16	8-Apr	9-Apr	5850	5850		11700	1225	1240		2465	86.7	13.065
17	10-Apr	12-Apr	6845	6845	6847	20537	1000	1026	1049	3075	147.7	13.065
18	13-Apr	14-Apr	6660	6660		13320	1166.5	1166.5		2333	68.8	13.065
19	15-Apr	16-Apr	6640	6640		13280	870	1024		1894	157.8	13.071
20	17-Apr	19-Apr	6995	6995	7020	21010	800	1035	1035	2870	133.1	13.072
21	20-Apr	21-Apr	7240	7240		14480	1017	1017		2034	93.4	13.072
22	22-Apr	23-Apr	7250	7250		14500	990	926		1916	93.4	13.072
23	24-Apr	26-Apr	7075	7075	6550	20700	1000	1000	960	2960	149.3	13.072
24	27-Apr	28-Apr	7025	7025		14050	1026.5	1026.5		2053	101.4	13.072
25	29-Apr	30-Apr	6550	6550		13100	0	972		972	106.2	13.072
26	1-May	3-May	7390	7390	7260	22040	1045	835	923	2803	142.4	13.072
27	4-May	5-May	7260	7190		14450	923	1000		1923	108.5	12.201
28	6-May	7-May	7050	7050		14100	992	960		1952	102.7	12.201
29	8-May	9-May	6745	6745		13490	1092	990		2082	107.6	12.201

Sample	Starting Date	Ending Date	Total Feed Volume (mL)	Organic Removal Based on Tot. Effluent (mg/d)	Organic Removal Based on Filt. Effluent (mg/d)	Nitrate Consumption (mg NO_3-N/d)	Consumptive Ratio Total	Consumptive Ratio Filtered
1	4-Mar	5-Mar	13856	2421	3118	314	7.7	9.9
2	6-Mar	8-Mar	22272	2932	3292	438	6.7	7.5
3	9-Mar	10-Mar	15178	2552	2802	210	12.1	13.3
4	11-Mar	12-Mar	14596	2393	2670	129	18.5	20.6
5	13-Mar	15-Mar	22761	2439	2907	317	7.7	9.2
6	16-Mar	17-Mar	14539	2226	2680	287	7.7	9.3
7	18-Mar	19-Mar	14762	1991	2537	280	7.1	9.1
8	20-Mar	22-Mar	23043	2149	2474	250	8.6	9.9
9	23-Mar	24-Mar	1114			584		
10	25-Mar	26-Mar	14896			354		
11	27-Mar	29-Mar	23139	1932	2231	310	6.2	7.2
12	30-Mar	31-Mar	15346	2896	3160	472	6.1	6.7
13	1-Apr	2-Apr	15282	2392	2643	451	5.3	5.9
14	3-Apr	5-Apr	23034	2639	2777	355	7.4	7.8
15	6-Apr	7-Apr	15059	2349	2424	442	5.3	5.5
16	8-Apr	9-Apr	14078	1888	2001	418	4.5	4.8
17	10-Apr	12-Apr	23464	2632	2728	530	5.0	5.1
18	13-Apr	14-Apr	15584	2379	2695	312	7.6	8.6
19	15-Apr	16-Apr	15016	2110	2289	553	3.8	4.1
20	17-Apr	19-Apr	23747	2326	2410	336	6.9	7.2
21	20-Apr	21-Apr	16421	2191	2285			
22	22-Apr	23-Apr	16323	1847	2178	403	4.6	5.4
23	24-Apr	26-Apr	23511	2845	3081	444	6.4	6.9
24	27-Apr	28-Apr	16002	2753	2871	319	8.6	9.0
25	29-Apr	30-Apr	13966	2519	2576	434	5.8	5.9
26	1-May	3-May	24701	2580	2783	421	6.1	6.6
27	4-May	5-May	16265	1939	2178	427	4.5	5.1
28	6-May	7-May	15949	1679	2234	461	3.6	4.9
29	8-May	9-May	15464	2205	2579	620	3.6	4.2

14 C Nitrogen Balance

Aerobic Reactors

Sample	Starting Date	Ending Date	Starting Date	Ending Date	Feed Volume (L.)	Sewage TKN (mg N/L.)	Protein TKN (mg N/L.)	Total Mass of N IN (mg N)	Effluent Volume (mL.)	Effluent TKN (mg N/L.)	Waste Volume (mL.)	Mixed Liquor TKN (mg N/L.)	Total Mass of N OUT (mg N)	Closure OUT/IN %
12	30-Mar	31-Mar	26	27	15.4	28.7	63.1	794.4	13.65	42.7	1.75	102.6	762.4	96%
13	1-Apr	2-Apr	28	29	15.294	28.7	63.1	788.9	13.544	45.9	1.75	87.9	775.5	98%
14	3-Apr	5-Apr	30	32	23.016	28.2	52.9	1027.0	20.075	44.9	2.94	103.1	1204.5	117%
15	6-Apr	7-Apr	33	34	14.234	28.5	73.3	830.0	12.12	39.6	2.116	100.5	692.6	83%
16	8-Apr	9-Apr	35	36	13.508	28.5	73.3	787.6	11.26	40.3	2.248	103.9	687.3	87%
17	10-Apr	12-Apr	37	39	23.233	30	65.9	1251.8	20.136	42.8	3.097	101.4	1175.9	94%
18	13-Apr	14-Apr	40	41	15.327	25.5	57.6	718.1	13.19	38.7	2.137	84.5	691.0	96%
19	15-Apr	16-Apr	42	43	15.054	25.5	50.2	646.4	13.16	36.5	1.992	107.6	694.7	107%
20	17-Apr	19-Apr	44	46	24.164	25.4	58.5	1145.8	22.01	40.8	2.154	70.8	1050.5	92%
21	20-Apr	21-Apr	47	48	16.555	25.4	58.5	785.0	14.5	34.2	2.055	63.1	625.6	80%
22	22-Apr	23-Apr	49	50	15.91	21.5	55	696.7	14.18	38	1.731	57.3	638.0	92%
23	24-Apr	26-Apr	51	53	23.439	28.7	70.6	1326.1	20.255	45.9	3.184	86	1203.5	91%
26	1-May	2-May	58	59	24.578	31.1	61.3	1258.0	21.81	47.7	2.768	78.8	1258.5	100%

Notes

Mar 30-Mar 31 Used the ammonia-nitrogen value because Total TKN is too low.

	Average	94%
Lower	Range	80%
Upper	Range	117%
Standard	Deviation	10%

Anoxic Reactors

Sample	Starting Date	Ending Date	Starting Date	Ending Date	Feed Volume (L.)	Sewage TKN (mg N/L.)	Protein TKN (mg N/L.)	Total Mass of N IN (mg N)	Effluent Volume (mL.)	Effluent TKN (mg N/L.)	Waste Volume (mL.)	Mixed Liquor TKN (mg N/L.)	Total Mass of N OUT (mg N)	Closure OUT/IN %
12	30-Mar	31-Mar	26	27	15.45	28.7	63.1	796.9	13.7	39	1.75	111.8	730.0	92%
13	1-Apr	2-Apr	28	29	15.379	28.7	63.1	793.3	13.534	36.6	1.845	118.8	714.5	90%
14	3-Apr	5-Apr	30	32	23.154	28.2	52.9	1033.2	20.557	36.9	2.597	131.1	1099.0	106%
15	6-Apr	7-Apr	33	34	15.145	28.5	73.3	883.1	12.695	36.4	2.45	130.9	782.8	89%
16	8-Apr	9-Apr	35	36	14.15	28.5	73.3	825.1	11.7	42.3	2.45	136.5	829.3	101%
17	10-Apr	12-Apr	37	39	23.634	30	65.9	1273.4	20.537	39.6	3.097	118.9	1181.5	93%
18	13-Apr	14-Apr	40	41	15.653	25.5	57.6	733.4	13.32	37.6	2.333	127.4	798.1	109%
19	15-Apr	16-Apr	42	43	15.272	25.5	50.2	655.7	13.28	30.1	1.992	104.9	608.7	93%
20	17-Apr	19-Apr	44	46	23.88	25.4	58.5	1132.4	21.01	34.2	2.87	123.3	1072.4	95%
21	20-Apr	21-Apr	47	48	16.514	25.4	58.5	783.1	14.48	27.6	2.034	114.6	632.7	81%
22	22-Apr	23-Apr	49	50	16.416	21.5	55	718.8	14.5	33.3	1.916	117.4	707.8	98%
23	24-Apr	26-Apr	51	53	23.66	28.7	70.6	1338.6	20.7	38.8	2.96	144.7	1231.5	92%
26	1-May	3-May	58	60	24.727	31.1	61.3	1265.6	21.92	38.3	2.807	110.8	1150.6	91%
29	7-May	9-May	64	66	15.572	25.7	71	869.6	13.49	42.4	2.082	131.2	845.1	97%

Notes

Apr 15-Apr 16 Used Filtered TKN because Total TKN is unavailable

Apr 17-Apr 19 Used Filtered TKN because Total TKN is unavailable

Aerobic Reactor - 20 C

		Mixed Liquor									Effluent				
Day	Date	Wastage (mL)	TSS 1	TSS 2	TSS 3	Average TSS (mg/L)	VSS1	VSS2	VSS3	Average VSS (mg/L)	Effluent (mL)	TSS1	TSS2	TSS3	Averag TSS (mg/L)
0	21-Sep	950	670	730	740	710	620	620	610	620	7020	6	5	6	6
1	22-Sep	925	820	890	870	860	720	800	790	770	8390	15	19	17	17
2	23-Sep	917	780	730	700	740	670	640	620	640	7590	1	6	4	4
3	24-Sep	917	780	730	700	740	670	640	620	640	7590	1	6	4	4
4	25-Sep	1125	750	760	700	740	710	720	660	700	7300	6	8	5	6
5	26-Sep	875	760	700	720	730	720	660	700	690	7300	5	5	6	5
6	27-Sep	955	690	720	710	710	640	650	650	650	7525	6	13		10
7	28-Sep	875	760	750	630	710	650	650	590	630	7380				10
8	29-Sep	1035	770	770	720	750	650	690	630	660	7230	11	12	6	10
9	30-Sep	988	780	820	810	800	710	720	730	720	7525	11	0	12	8
10	1-Oct	988	780	820	810	800	710	720	730	720	7525	11	0	12	8
11	2-Oct	865	790	840	740	790	720	790	740	750	7280	4	7	3	5
12	3-Oct	815	750	710	700	720	610	550	600	590	7280	2	15	8	8
13	4-Oct	933	830	810		820	730	760	750	750	7240	4	9	6	6
14	5-Oct	933	830	810		820	730	760	750	750	7240	4	9	6	6
15	6-Oct	875	800	860	780	810	710	770	690	720	7100	8	12	13	11
16	7-Oct	938	930	930	900	920	820	830	790	810	6858	12	15	12	13
17	8-Oct	938	930	930	900	920	820	830	790	810	6858	12	15	12	13
18	9-Oct	935	890	910	840	880	830	790	720	780	7000	8	15	7	10
19	10-Oct	910			830	830			750	750	6870	4	2	4	3
20	11-Oct	790	880	910	850	880	810	860	790	820	7017	15	13	9	12
21	12-Oct	790	880	910	850	880	810	860	790	820	7017	15	13	9	12
22	13-Oct	795	860	830	850	850	780	750	780	770	6980	12	8	7	9
23	14-Oct	800	850	890	890	880	770	780	760	770	7335	12	12	9	11
24	15-Oct	795	870	860		870	780	800		790	7200	12	8	7	9
25	16-Oct	860	1010	950	1010	990	920	890	920	910	6735	7	13	12	11
26	17-Oct	835	1080	1050	1100	1080	1020	980	1000	1000	6280	8	4	9	7
27	18-Oct	990	880	830	860	860	810	710	780	770	7330	5	4	3	4
28	19-Oct	980	790	810	780	790	690	720	660	690	7270	5	9	6	7
29	20-Oct	1000	910	850	830	860	790	780	770	780	7210	5	15	8	9
30	21-Oct	992.5	720	730	750	730	650	680	700	680	6920	1	6	4	4
31	22-Oct	992.5	720	730	750	730	650	680	700	680	6920	1	6	4	4
32	23-Oct	977.5	770	800	730	770	700	730	700	710	6600	15	3	3	7
33	24-Oct	977.5	770	800		790	700	730		720	6600	15	3	3	7
34	25-Oct	920	810	820	790	810	750	730	710	730	6900	4	6	4	5
35	26-Oct	925	780	790	760	780	730	730	730	730	6980	5	5	9	6
36	27-Oct	1000	720	650	620	660	680	580	580	610	6770	5	5	13	8

		Mixed Liquor									Effluent				
Day	Date	Wastage (mL)	TSS 1	TSS 2	TSS 3	Average TSS (mg/L)	VSS1	VSS2	VSS3	Average VSS (mg/L)	Effluent (mL)	TSS1	TSS2	TSS3	Averag TSS (mg/L)
37	28-Oct	1000	700	680	690	690	620	650	630	630	6790	5	6	1	4
38	29-Oct	1000	600	700	680	660	580	600	640	610	6950	1	5	5	4
39	30-Oct	1050	770	750	780	770	690	680	700	690	6915	3	11	11	8
40	31-Oct	1000	720	710		720	650	710		680	6915	3	11	11	8
41	1-Nov	930	700	710	710	710	680	640	670	660	6900	1	1	1	1
42	2-Nov	975	460	500		480	380	440		410	7030	1	1	1	1
43	3-Nov	985	550	600	560	570	500	540	520	520	5635	1	1	1	1
44	4-Nov	1025	730	780	740	750	640	670	660	660	6995	1	7	8	5
45	5-Nov	1025	730	780	740	750	640	670	660	660	6995	1	7	8	5
46	6-Nov	1075	620	655	620	630	570	570	560	570	6695	19	1	8	9
47	7-Nov	1085	600	720	660	660	520	610	600	580	7200	11	8	12	10
48	8-Nov	950	660	680	680	670	550	610	600	590	6928	16	8	6	10
49	9-Nov	925	580	600	670	620	540	560	590	560	6928	16	8	6	10
50	10-Nov	1100	650	600		630	590	580		590	6920	15	13	16	15
51	11-Nov	0				**630**				**600**	6633	20	28	20	23
52	12-Nov	0				**630**				**600**	6633	20	28	20	23
65	25-Nov	1027.5	730	560	670	650	600	620		610	7120	20	13		17
66	26-Nov	1027.5	730	560	670	650	600	620		610	7120	20	13		17
67	27-Nov	1030	600	610	610	610	570	560		570	7070	24	22	24	23
68	28-Nov	1025	600	620	600	610	620	580	600	600	7070	24	22	24	23
69	29-Nov	980	700	580		640	620	600		610	7170	94	90	90	91
70	30-Nov	980	640	880	700	740	580	580	620	590	7170	94	90	90	91
71	1-Dec	1075	760	740	880	790	640	620	560	610	6330	212	208	212	211
72	2-Dec	780	540	530	500	520	470	480	480	480	7600	42			42
73	3-Dec	780	540	530	500	520	470	480	480	480	7600	42			42
74	4-Dec	975	460	530	450	480	390	430		410	6970	26	26		26
75	5-Dec	975	460	530	450	480	390	430		410	6970	26	26		26
76	6-Dec	1025	620	660	650	640	580	570	600	580	6500	34	36	36	35
77	7-Dec	1075	680	700	640	670	630	600	590	610	6500	27	35	32	31
78	8-Dec	1075	680	700	640	670	630	600	590	610	6530	27	35	32	31
79	9-Dec	975	580	550	500	540	530	450		490	7095	20	34	28	27
80	10-Dec	975	580	550	500	540	530	450		490	7095	20	34	28	27

Bolded Values are Estimates

Anoxic Reactor -20 C

		Mixed Liquor									Effluent				
Day	Date	Wastag (ml)	TSS 1	TSS 2	TSS 3	Average TSS (mg/L)	VSS1	VSS2	VSS3	Average VSS (mg/L)	Effluen (mL)	TSS1	TSS2	TSS3	Average TSS (mg/L)
0	21-Sep	1000	930	810	820	850	810	750	780	780	7360	21	20	15	19
1	22-Sep	875	770	760	800	780	710	700	690	700	6965	16	10	9	12
2	23-Sep	1000	850	850	810	840	790	810	710	770	7275	7	10	10	9
3	24-Sep	1000	850	850	810	840	790	810	710	770	7275	7	10	10	9
4	25-Sep	1250	850			850	770	790	720	760	7460	13	17	19	16
5	26-Sep	1000	860	970	910	910	770	790	550	700	**7460**	70	101	94	88
6	27-Sep	1000	860	830	830	840	790	750	780	770	6900	15	20	16	17
7	28-Sep	1025		790	790	790	590	710	750	680	7140	19	26	22	22
8	29-Sep	875	840	950	840	880	730	870	820	810	6920	21	24		23
9	30-Sep	1050	870	830	880	860	800	780	770	780	7050	18	14	16	16
10	1-Oct	1050	870	830	880	860	800	780	770	780	7050	18	14	16	16
11	2-Oct	1000	890	890	870	880	770	730	750	750	7000	26	39	26	30
12	3-Oct	935	890	780	920	860	820	710	820	780	7535	21	15	15	17
13	4-Oct	1012	960	980		970	850	860		860	7105	54	44	58	52
14	5-Oct	1012	960	980		970	850	860		860	7105	54	44	58	52
15	6-Oct	1000	700	820	890	800	690	740	710	710	7030	21	28	25	25
16	7-Oct	938	950	930	920	930	780	820	810	800	7043	19	23	21	21
17	8-Oct	938	950	930	920	930	780	820	810	800	7043	19	23	21	21
18	9-Oct	1000	900	790	880	860	720	710	800	740	7070	24	27	24	25
19	10-Oct	910	820	790	830	810	700	670	750	710	7080	35	37	69	47
20	11-Oct	970	880	910	850	880	810	860	790	820	6880	4	5	9	6
21	12-Oct	970	880	910	850	880	810	860	790	820	6880	4	5	9	6
22	13-Oct	960	800	850	850	830	760	810	790	790	7095	4	8	7	6
23	14-Oct	950	880	890	860	880	790	810	760	790	7040	21	30	29	27
24	15-Oct	960	930	960	940	940	830	800	820	820	68[illegible]	6	8		7
25	16-Oct	1000	990	830	960	930	870	830	840	850	6895	16	19	4	13
26	17-Oct	775	1020	1010	980	1000	1020	980	1000	1000	6345	11	11	11	11
27	18-Oct	980	870	890	920	890	750	800	820	790	7180	17	17	12	15
28	19-Oct	980	720	840	910	820	760	850	840	820	7150	11	15	15	14
29	20-Oct	975	920	980	870	920	850	840	750	810	7140	21	19	11	17
30	21-Oct	985	950		920	940	800		760	780	7040	10	6	1	6
31	22-Oct	985	950		920	940	800		760	780	7040	10	6	1	6

		Mixed Liquor									Effluent				
Day	Date	Wastag (ml)	TSS 1	TSS 2	TSS 3	Average TSS (mg/L)	VSS1	VSS2	VSS3	Average VSS (mg/L)	Effluen (mL)	TSS1	TSS2	TSS3	Average TSS (mg/L)
32	23-Oct	1000	1030	970	1030	1010	850	790	890	840	6387.5	9	8	1	6
33	24-Oct	1125	960	920	920	930	870	870	870	870	6387.5	9	8	1	6
34	25-Oct	1150	950	940	940	940	820	810	820	820	6850	1	4	1	2
35	26-Oct	1035	830	890	800	840	770	790	710	760	6895	27	1	6	11
36	27-Oct	1000	870	850	830	850	810	760	750	770	6870	7	4	8	6
37	28-Oct	1000	840	810	910	850	780	760	810	780	6770	1	5	3	3
38	29-Oct	975	890	920	850	890	830	860	790	830	6430	1	5	5	4
39	30-Oct	1050	770	750	780	770	690	680	700	690	6915	3	11	11	8
40	31-Oct	1000	720	710		720	650	710		680	6915	3	11	11	8
41	1-Nov	930	770	760	780	770	720	720	660	700	6900	1	1	1	1
42	2-Nov	910	660	660	740	690	660	570	690	640	7010	45	31	24	33
43	3-Nov	1025	690	710	730	710	680	660	650	660	6660	96	98	110	101
44	4-Nov	1000	640	680	700	670	600	580	640	610	6843	32	23	24	26
45	5-Nov	1000	640	680	700	670	600	580	640	610	6843	32	23	24	26
46	6-Nov	1075	700	580	580	620	560	480	520	520	6610	82	96	76	85
47	7-Nov	940	800	790	770	790	740	780	730	750	**7200**	38	48	39	42
48	8-Nov	875	760	710	770	750	700	640	680	670	7170	32	31	28	30
49	9-Nov	475	760	720	710	730	690	660	660	670	7170	32	31	28	30
50	10-Nov	1050	740	790	860	800	740	780	670	730	6920	62	64	64	63
65	25-Nov	1032.5	560	500		530	560	560		560	6915	3	12	12	9
66	26-Nov	1000	**640**	**660**	**480**	**590**	**540**	**600**	**440**	**530**	6915	3	12	12	9
67	27-Nov	1000	**640**	**660**	**480**	**590**	**540**	**600**	**440**	**530**	6825	9	15	15	13
68	28-Nov	1085	640	660	480	590	540	600	440	530	6825	9	15	15	13
69	29-Nov	1007.5	800	580		690	500	720		610	7040	12	12	34	19
70	30-Nov	1007.5	800	580		690	500	720		610	7040	12	12	34	19
71	1-Dec	870	700	700	660	690	600	580	580	590	5400	16	11	15	14
72	2-Dec	1012.5	800	750	680	740	720	660	580	650	6830	1	9	7	6
73	3-Dec	1012.5	800	750	680	740	720	660	580	650	6830	1	9	7	6
74	4-Dec	1075	680	630		660	590	580		590	6555	37			37
75	5-Dec	1015	630	680	670	660	570	600	630	600	6555	37			37
76	6-Dec	1015	690	700	740	710	600	590	680	620	6400	42	32	33	36

Bolded Values are estimated

Aerobic Reactor - 14 C

Day	Date	Mixed Liquor Wastage (mL)	TSS 1	TSS 2	TSS 3	Averag (mg/L)	VSS 1	VSS 2	VSS 3	Averag (mg/L)	Effluent Effluen (mL)	TSS1	TSS	TSS3	Average (mg/L)
0	4-Mar	187	1070	1090	1120	1090	980	980	980	980	6440	26	26	26	26
1	5-Mar	855	1130	1150	1150	1140	1090	1110	1090	1100	6440	26	26	26	26
2	6-Mar	929	1020	990	1080	1030	960	880	950	930	6572	24	14	32	23
3	7-Mar	947	1030	960	960	980	920	860	910	900	6572	24	14	32	23
4	8-Mar	971	950	950	910	940	850	870	810	840	6430	16	24	30	23
5	9-Mar	968.5	770	810	760	780	710	700	770	730	6540	26	22	18	22
6	10-Mar	968.5	770	810	760	780	710	700	770	730	6540	26	22	18	22
7	11-Mar	975	780	750	730	750	700	690	680	690	6361	16	6	10	11
8	12-Mar	977	630	690	710	680	630	640	640	640	6361	16	6	10	11
9	13-Mar	893	780	810	830	810	770	770	800	780	6582	26	16	6	16
10	14-Mar	957	780	760	800	780	720	670	710	700	6582	26	16	6	16
11	15-Mar	955	750	730	780	750	700	710	670	690	6693	20	24	14	19
12	16-Mar	775	740	670	750	720	660	600	660	640	6484	12	8		10
13	17-Mar	775	740	670	750	720	660	600	660	640	6484	12	8		10
14	18-Mar	935	760	720	800	760	680	650	700	680	6183	30	10	14	18
15	19-Mar	931	640	640	660	650	590	560	590	580	6782	16	16	16	16
16	20-Mar	923	780	720	790	760	720	730	720	720	6729	36	20	18	25
17	21-Mar	985	650	660	670	660	570	600	600	590	6490	38	34	40	37
18	22-Mar	**900**	850	850	850	850	650	650	650	820	7000	68	46	56	57
19	23-Mar	600	840	820	850	840	780	770	770	770	**6800**	54	44	48	49
20	24-Mar	600	840	820	850	840	780	770	770	770	**6800**	54	44	48	49
21	25-Mar	927	860	860	860	860	810	740	770	770	6489	64	64	68	65
22	26-Mar	975	720	770	830	770	710	700	740	720	6489	64	64	68	65
23	27-Mar	955	780	700	650	710	680	610	590	630	**6850**	**34**	**32**	**36**	34
24	28-Mar	885	750	720	740	740	650	620	650	640	**6850**	34	32	36	34
25	29-Mar	1021	720	700	740	720	600	620	650	620	**6850**	34	42	40	39
26	30-Mar	875	710	720	680	700	620	680	660	650	6825	82	82	82	82
27	31-Mar	875	710	720	680	700	620	680	660	650	6825	82	82	82	82
28	1-Apr	875	700	760	750	740	670	680	677	680	6772	80	72	74	75
29	2-Apr	875	670	720	710	700	650	650	690	660	6772	80	72	74	75
30	3-Apr	891	830	810	830	820	730	730	730	730	6693	82	84	72	79
31	4-Apr	1010	570	580	610	590	560	560	570	560	6693	82	84	72	79
32	5-Apr	1040	620	630	630	630	580	580	580	580	6690	166	166	152	161
33	6-Apr	1058	560	620	600	590	510	570	560	550	6060	54	50	52	52
34	7-Apr	1058	560	620	600	590	510	570	560	550	6060	54	50	52	52

		Mixed Liquor									Effluent				
Day	Date	Wastage (mL)	TSS 1	TSS 2	TSS 3	Averag (mg/L)	VSS 1	VSS 2	VSS 3	Averag (mg/L)	Effluen (mL)	TSS1	TSS	TSS3	Average (mg/L)
35	8-Apr	1058	560	620	600	590	510	570	560	550	5630	42	60	52	51
36	9-Apr	1190	610	650	670	640	580	600	600	590	5630	42	60	52	51
37	10-Apr	1000	530	550	520	530	450	520	490	490	6625	46	38	42	42
38	11-Apr	1038	510	570	550	540	460	500	500	490	6625	46	38	42	42
39	12-Apr	1059	490	480	470	480	430	390	410	410	6886	48	54	50	51
40	13-Apr	1069	460	410		440	420	350	420	400	6595	27	34	26	29
41	14-Apr	1069	460	410		440	420	350	420	400	6595	27	34	26	29
42	15-Apr	870	430	430	420	430	380	370	360	370	6580	25	30	15	23
43	16-Apr	1024	410	370		390	370	330	290	330	6580	25	30	15	23
44	17-Apr	700	410	400	350	390	360	360	340	350	7395	30	36	28	31
45	18-Apr	727	390	400	390	390	340	350	370	350	7395	30	36	28	31
46	19-Apr	727	390	400	390	390	340	350	370	350	7220	38	34	40	37
47	20-Apr	1028	410	450	420	430	390	400	370	390	7250	32	37	37	35
48	21-Apr	1028	410	450	420	430	390	400	370	390	7250	32	37	37	35
49	22-Apr	1081	370	340	340	350	300	310	290	300	7090	34	32	23	30
50	23-Apr	650	280	290	200	260	270	290	240	270	7090	34	32	23	30
51	24-Apr	1031	510	500		510	470	490	480	480	7003	33	36	40	36
52	25-Apr	1103	470	430	450	450	440	390	430	420	7003	33	36	40	36
53	26-Apr	1050	480	440	430	450	440	420	390	420	6250	50	47	50	49
54	27-Apr	1026.5	450	410	380	410	450	370	320	380	6900	58	52	60	57
55	28-Apr	1026.5	450	410	380	410	450	370	320	380	6900	58	52	60	57
56	29-Apr	0	410	380		400	370	320		350	6365	52	60	58	57
57	30-Apr	972	410	380		400	370	320		350	6365	52	60	58	57
58	1-May	1045	600	620	690	640	580	590	650	610	7270	24	44	45	38
59	2-May	800	510	540	540	530	470			470	7270	24	44	45	38
60	3-May	923	440	460	410	440	360			360	7270				21
61	4-May	1053	440	460	410	440	360			360	7270				21
62	5-May	870	280	400	400	360	270	330	330	310	7190	8	2	48	19
63	6-May	988	520	490	470	490	480	440	410	440	7090	9	32	30	24
64	7-May	1030	550	560	580	560	460	530	540	510	7090	9	32	30	24
65	8-May	1027	560	560	730	620	510	560	540	540	6975	12	12	16	13
66	9-May	1090	500	500	530	510	440	450	500	460	6975	12	12	16	13

Bolded Values are Estimates

Anoxic Reactor - 14 C

Day	Date	Mixed Liquor Wastage (mL)	TSS 1	TSS 2	TSS 3	Averag (mg/L)	VSS 1	VSS 2	VSS 3	Average (mg/L)	Effluent (mL)	TSS1	TSS2	TSS3	Averag (mg/L)
0	4-Mar	995	630	610	670	640	560	560	590	570	6440	26	26	26	26
1	5-Mar	845	850	720	690	750	830	650	650	710	6440	26	26	26	26
2	6-Mar	885	620	630	640	630	560	540	580	560	6701.5	32	32	32	32
3	7-Mar	895	650	600	620	620	570	520	530	540	6701.5	32	32	32	32
4	8-Mar	901	680	640	620	650	630	590	570	600	6612	16	24	30	23
5	9-Mar	887.5	590	610	600	600	560	590	580	580	6640	34	34	30	33
6	10-Mar	887.5	590	610	600	600	560	590	580	580	6640	34	34	30	33
7	11-Mar	903	630	570	660	620	580	560	610	580	6360.5	42	38	40	40
8	12-Mar	903	630	570	660	620	580	560	610	580	6360.5	42	38	40	40
9	13-Mar	891	610	630	650	630	590	560	600	580	6582	26	16	6	16
10	14-Mar	907	620	600	600	610	520	540	540	530	6582	26	16	6	16
11	15-Mar	935	600	570	570	580	500	540	500	510	6794	64	68	62	65
12	16-Mar	758.5	540	500	600	550	460	480	520	490	6541	24	30	30	28
13	17-Mar	758.5	540	500	600	550	460	480	520	490	6541	24	30	30	28
14	18-Mar	857	560	560	560	560	500	500	490	500	6230	48	50	50	49
15	19-Mar	921	630	590	600	610	540	520	520	530	6810	64	52	58	58
16	20-Mar	907	590	630	550	590	540	600	540	560	6718	64	56	60	60
17	21-Mar	960	510	490	450	480	460	420	400	430	6685	48	48	52	49
18	22-Mar	900				660				620	6605	42	42	44	43
19	23-Mar	930	640	690	650	660	610	580	560	580	**6850**	16	20	18	18
20	24-Mar	930	640	690	650	660	610	580	560	580	**6850**	16	20	18	18
21	25-Mar	935	620	610	640	620	550	540	600	560	6586	32	28	48	36
22	26-Mar	875	600	610	750	650	540	530	570	550	6586	32	28	48	36
23	27-Mar	875	570	620	630	610	530	530	560	540	**6850**	74	80	68	74
24	28-Mar	875	570	620	630	610	530	530	560	540	**6850**	74	80	68	74
25	29-Mar	970	590	640	610	610	550	600	590	580	**6850**	88	76	78	81
26	30-Mar	875	620	640	640	630	600	630	620	620	6850	40	30	26	32
27	31-Mar	875	620	640	640	630	60	630	620	620	6850	40	30	26	32
28	1-Apr	970	800	670	720	730	660	610	580	620	6772	80	72	74	75
29	2-Apr	875	660	670	690	670	630	620	650	630	6767	32	32	28	31
30	3-Apr	782	790	840	770	800	750	760	740	750	6834	30	32	34	32
31	4-Apr	980	750	760	720	740	690	700	690	690	6834	30	32	34	32
32	5-Apr	835	720	740	720	730	680	680	700	690	6890	40	64	44	49
33	6-Apr	1225	720	740	740	730	680	720	700	700	6348	30	28	22	27
34	7-Apr	1225	720	740	740	730	680	720	700	700	6348	30	28	22	27

Day	Date	Mixed Liquor									Effluent				
		Wastage (mL)	TSS 1	TSS 2	TSS 3	Averag (mg/L)	VSS 1	VSS 2	VSS 3	Average (mg/L)	(mL)	TSS1	TSS2	TSS3	Averag (mg/L)
35	8-Apr	1225	860	860	880	870	820	820	760	800	5850	20	34	16	23
36	9-Apr	1240	860	860	880	870	820	820	760	800	5850	20	34	16	23
37	10-Apr	1000	800	880	900	860	780	760	860	800	6845	16	20	16	17
38	11-Apr	1026	840	820	880	850	740	740	740	740	6845	16	20	16	17
39	12-Apr	1049	880	900	880	890	800	840	810	820	6847	38	32	30	33
40	13-Apr	1166.5	800	790		800	640	580		610	6660	15	17	20	17
41	14-Apr	1166.5	800	790		800	640	580		610	6660	15	17	20	17
42	15-Apr	887	740	810	720	760	720	700	680	700	6640	19	25	26	23
43	16-Apr	1105	680	740	840	750	640	680	700	670	6640	19	25	26	23
44	17-Apr	800	860	660	790	770	790	720	700	740	6995	4	18	10	11
45	18-Apr	1035	860	780	820	820	780	720	720	740	6995	4	18	10	11
46	19-Apr	1035	860	780	820	820	780	720	720	740	7020	12	24	12	16
47	20-Apr	1017	780	720	840	780	700	700	780	730	7240	5	5	5	5
48	21-Apr	1017	780	720	840	780	700	700	780	730	7240	5	5	5	5
49	22-Apr	990	700	780	740	740	660	680	680	670	7250	16	24	13	18
50	23-Apr	930	840	880	760	830	760	740	720	740	7250	16	24	13	18
51	24-Apr	1000	760	700	800	750	720	740	760	740	7075	14	18	15	16
52	25-Apr	1000	760	760	740	750	700	780	680	720	7075	14	18	15	16
53	26-Apr	960	900	900	960	920	780	840	880	830	6550	3	18	17	13
54	27-Apr	1026.5	680	820	800	770	600	780	700	690	7025	4	10	14	9
55	28-Apr	1026.5	680	820	800	770	600	780	700	690	7025	4	10	14	9
56	29-Apr	0				**910**				760	6550	8	4	12	8
57	30-Apr	972	940	900	900	910	840	840	800	830	6550	8	4	12	8
58	1-May	1050	800	820	840	820	760	720		740	7390	20	22	16	19
59	2-May	835	800	860	800	820	660	700		680	7390	20	22	16	19
60	3-May	923	1100	830	800	910				**830**	7140				20
61	4-May	923	1100	830	800	910				**830**	7140				20
62	5-May	1000	800	820	860	830	800	780	820	800	7190	24	20		22
63	6-May	392	760	940		850	760	940		30	7050	36	45	32	38
64	7-May	960	900	880	880	890	860	920	920	900	7050	36	45	32	38
65	8-May	1092	660	940	1060	890	900	940	700	850	6745	26	20		23
66	9-May	990	700	760	800	750	680	720	800	730	6745	26	20		23

Cell Counts 14C - Example Data

Calculations

Filtration Area

		1000x Magnification	
Filter Diameter	20 15 mm	Micrometer Area	Grids per
Filter Area	318 89 mm2	6 40E+03 um2	Filter
	3 19E+08 um2		49826 457

Date	Electron Acceptor	Reactor	Replicate	Correction Factor	Dilution Factor	Number of Dilutions	Final Vol of Dilution	Squares Counted	Field 1	Field 2	Field 3	Field 4	Field 5	Field 6	Field 7	Field 8	Field 9	Field 10
27-Mar	Aerobic	RB	1	1	10	3	10	20	52	63	51	52	33	49	34			
27-Mar	Aerobic	RB	2	1	10	3	10	20	35	38	52	26	50	64	38	53	28	34
4-Apr	Aerobic	RB	3	1	10	4	10	100	18	24	25	14	15	32	24	20	25	10
4-Apr	Aerobic	RB	4	1	10	3	10	30	65	61	62	97	73	87	43	51	46	73
4-Apr	Aerobic	SB	1	1	10	3	9	30	22	21	13	18	21	19	9	14	15	19
4-Apr	Aerobic	SB	2	1	10	3	9	30	18	10	21	15	27	11	18	19	15	16
9-Apr	Aerobic	R1	1	0 8	10	3	10	40	47	50	46	35	53	48	50	60	42	51
9-Apr	Aerobic	R1	1	0 8	10	3	10	40	49	30	41	26	51	62	48	40	57	53
9-Apr	Aerobic	RB	1	1	10	3	9	30	65	49	51	61	46	57	69	44	63	57
9-Apr	Aerobic	RB	2	1	10	3	9	30	21	26	21	43	28	41	65	45	39	25
9-Apr	Anoxic	RB	3	1	10	3	10	30	46	26	31	29	34	36	54	49	54	41
9-Apr	Anoxic	RB	4	1	10	3	10	30	46	20	39	49	33	43	53	52	34	42
10-Apr	Aerobic	RB	1	1	10	3	10	20	43	31	35	41	48	49	46	39	56	41
10-Apr	Aerobic	RB	1	1	10	3	10	20	45	35	50	40	38	44	45	34	31	42
13-Apr	Aerobic	R1	1	0 8	10	3	9	30	30	45	60	50	51	58	53	58	29	57
13-Apr	Aerobic	R1	1	0 8	10	3	9	30	47	51	49	25	42	42	60	49	49	43
13-Apr	Aerobic	RB	1	1	10	4	10	100	20	13	24	14	10	20	25	16	11	19
13-Apr	Aerobic	RB	1	1	10	3	9	20	25	25	19	27	46	21	24	18	40	20
13-Apr	Aerobic	SB	1	1	10	3	9	40	37	48	44	41	50	49	50	44	61	
13-Apr	Aerobic	SB	1	1	10	3	9	40	57	61	52	50	50	48	23	58	66	43
24-Apr	Aerobic	SB	1	1	10	4	10	40	23	35	26	18	35	17	11	21	41	25
24-Apr	Aerobic	SB	2	1	10	4	10	40	20	21	12	17	17	29	28	49	32	26
26-Apr	Aerobic	R1	1	0 8	10	3	10	20	30	23	28	24	23	29	28	41	31	43
26-Apr	Aerobic	R1	2	0 8	10	4	10	100	41	45	40	36	30	60	52	45	47	26
26-Apr	Aerobic	R1	3	0 8	10	4	10	100	24	35	24	29	28	31	39	32	39	31
26-Apr	Aerobic	R1	4	0 8	10	4	10	100	36	32	41	32	27	29	47	47	12	19
29-Apr	Aerobic	RB	1	1	10	3	9	100	28	74	66	55	40	45	43	49	34	50
29-Apr	Aerobic	RB	2	1	10	3	10	20	59	47	14	38	26	26	18	23	29	38
29-Apr	Aerobic	RB	3	1	10	3	10	20	44	37	45	20	29	26	30	44	31	29
29-Apr	Aerobic	SB	1	1	10	3	9	50	18	23	21	18	27	22	49	38	24	22
29-Apr	Aerobic	SB	2	1	10	3	10	30	26	29	27	26	16	33	23	26	29	60
29-Apr	Aerobic	SB	3	1	10	3	10	30	43	33	39	53	23	33	35	53	34	22
29-Apr	Aerobic	SB	4	1	10	3	10	30	20	57	24	35	49	40	35	25	29	45
5-May	Aerobic	R1	1	0 8	10	3	9	30	25	29	30	25	35	31	37	34	21	28
5-May	Aerobic	R1	2	0 8	10	3	9	30	28	26	27	21	34	15	30	28	31	30
5-May	Aerobic	RB	2	1	10	3	9	30	27	28	27	21	36	28	26	29	32	24
5-May	Aerobic	RB	2	1	10	3	9	30	43	24	32	25	37	37	35	17	33	29
5-May	Aerobic	SB	1	1	10	3	9	30	42	36	31	46	36	34	21	40	45	32
5-May	Aerobic	SB	1	1	10	3	9	30	21	27	42	24	47	53	53	24	42	52
7-May	Aerobic	R1	1	0 8	10	3	9	30	52	46	31	46	44	21	58	55	32	28
7-May	Aerobic	R1	1	0 8	10	3	9	30	48	25	21	19	24	36	40	34	31	34

Date	Electron Acceptor	Reactor	Replicate	Correction Factor	Dilution Factor	Number of Dilutions	Final Vol of Dilution	Squares Counted	Field 1	Field 2	Field 3	Field 4	Field 5	Field 6	Field 7	Field 8	Field 9	Field 10
7-May	Aerobic	RB	1	1	10	3	10	30	44	81	60	60	33	31	42	43	47	69
7-May	Aerobic	RB	1	1	10	3	10	30	40	44	45	43	38	31	59	49		
7-May	Aerobic	SB	1	1	10	3	9	40	26	24	23	31	25	32	24	28	23	26
9-May	Aerobic	R1	1	0 8	10	3	9	30	58	54	45	59	53	61	17	45	22	55
9-May	Aerobic	R1	2	0 8	10	3	9	30	28	14	44	56	42	15	56	47	49	55
9-May	Aerobic	RB	1	1	10	3	9	30	35	26	53	27	18	21	38	30	18	21
9-May	Aerobic	RB	2	1	10	3	9	30	41	39	54	53	29	46	19	38	57	44
9-May	Aerobic	SB	1	1	10	3	9	30	24	38	18	42	41	41	44	15	30	27
9-May	Aerobic	SB	2	1	10	3	9	30	22	29	23	25	29	30	21	27	21	24

Appendix C

Statistical Analysis of Biosolids Production Rates at 20°C

	Deviations from Regression			F	$F_{0.95}$
	df	Sum of Squares	Regression Mean Square Error		
Within					
Aerobic	34	8.738E4			
Anoxic	34	1.795E6			
Sum	68	2.608E6	38363		
Pooled	69	6.200E7			
Difference	1	2.570E6	2.570E6	1620	4.00

For more details of the analysis see Snedcor and Cochran (1967)

Statistical Analysis of Biosolids Production Rates at 14°C

	Deviations from Regression			F	$F_{0.95}$
	df	Sum of Squares	Regression Mean Square Error		
Within					
Aerobic	33	3.879E6			
Anoxic	33	6.127E6			
Sum	66	1.00E7	1.5160E5		
Pooled	67	8.99E7			
Difference	1	7.99E7		527.1	4.00

Factorial Analysis of Biosoids Composition

For Details of the Analysis see Box *et al.* (1978)
Data Table

Reactor	Temp (°C)	Mean	Std. Deviation	n
Aerobic	20	19.6	16.1	3
Anoxic	20	18.5	7.94	3
Aerobic	14	14.8	7.47	6
Anoxic	14	24.9	8.64	8

Pooled Variance =90.4
Variance (Effect) = 18.1
Std. Dev. (Effect) = 3.45

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.13

Factorial Analysis Number of Cells Present

Reactor	Temp (°C)	Mean	Std. Deviation	n
Aerobic	20	5.93	1.57	2
Anoxic	20	11.0	4.60	3
Aerobic	14	6.43	2.94	6
Anoxic	14	18.3	4.47	8

Pooled Variance =15.2
Variance (Effect) =3.19
Std. Dev. (Effect) =1.78

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.13

Factorial Analysis of Respiration Rates Normalized on MLVSS

Reactor	Temp (°C)	Mean	Std. Deviation	n
Aerobic	20	34.4	10.7	6
Anoxic	20	32.9	5.92	5
Aerobic	14	28.9	8.20	6
Anoxic	14	15.4	3.87	6

Pooled Variance =59.1
Variance (Effect) =9.86
Std. Dev. (Effect) =3.14

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.44

Factorial Analysis of Respiration Rates Normalized on Cell Counts

Reactor	Temp (°C)	Mean	Std. Deviation	n
Aerobic	20	22.8	11.2	6
Anoxic	20	19.1	4.39	5
Aerobic	14	16.1	3.70	6
Anoxic	14	4.26	0.61	6

Pooled Variance =27.5
Variance (Effect) =4.06
Std. Dev. (Effect) =2.02

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.45

Factorial Analysis of Ammonia Production Normalized on MLVSS

Reactor	Temp (°C)	Mean	Std. Deviation	n
Aerobic	20	5.88	1.41	6
Anoxic	20	4.49	2.05	5
Aerobic	14	6.42	1.13	6
Anoxic	14	4.25	0.765	5

Pooled Variance = 2.42
Variance (Effect) = 0.439
Std. Dev. (Effect) = 0.663

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.47

Factorial Analysis of Ammonia Production Normalized on Cell Counts

Reactor	Temp (°C)	Mean $x10^{-12}$	Std. Deviation $x\ 10^{-12}$	n
Aerobic	20	3.76	1.88	2
Anoxic	20	1.71	0.67	2
Aerobic	14	3.85	1.62	5
Anoxic	14	1.09	0.24	5

Pooled Variance =1.46 $x10^{-24}$
Variance (Effect) =0.41 $x10^{-24}$
Std. Dev. (Effect) =0.64 $x10^{-12}$

Experimental Levels: Reactor Aerobic (-) Anoxic (+)
Temperature 14°C(-) 20°C (+)

ANOVA Table - See Table 5.47

Statistical Analysis of Nitrate Utilization Data

ANOVA Table - Oct. 20

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	10	-4.514	8	0.602	0.075
Rapidly Biodegradable	10	-5.390	8	1.958	0.245
			16	2.560	0.160
Pooled	19	-4.951	17	9.355	0.550
Difference between Slopes			1	6.795	
Comparison of Slopes		F	42.4 (1,16)	Sig	
		$F_{0.05}$	4.49		

ANOVA Table - Dec. 4

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-3.261	7	5.02	0.718
Rapidly Biodegradable	9	-6.529	7	1.81	0.259
			14	6.83	0.488
Pooled	17	-4.895	15	86.9	5.79
Difference between Slopes			1	80.1	
Comparison of Slopes		F	164 (1,14)	Sig	
		$F_{0.05}$	4.60		

ANOVA Table - Oct. 30

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Rapidly Biodegradable	11	-4.48	9	0.640	0.071
Slowly Biodegradable	11	-4.71	9	2.31	0.257
			18	2.95	0.164
Pooled	21	-4.59	19	3.50	0.184
Difference between Slopes			1	0.542	
Comparison of Slopes		F	3.30 (1,18)	N. Sig	
		$F_{0.05}$	4.41		

ANOVA Table - Dec. 1

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-6.79	7	3.83	0.548
Rapidly Biodegradable	9	-6.34	7	2.47	0.353
			14	6.30	0.450
Pooled	17	-6.57	15	7.81	0.521
Difference between Slopes			1	1.51	
Comparison of Slopes		F	3.35 (1,14)	N. Sig	
		$F_{0.05}$	4.60		

ANOVA Table - Apr. 4

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-3.690	7	13.3	1.90
Rapidly Biodegradable	9	-2.834	7	2.02	0.289
			14	15.3	1.10
Pooled	17	-3.263	15	27.7	1.84
Difference between Slopes			1	12.4	
Comparison of Slopes		F	11.3 (1,14)	Sig	
		$F_{0.05}$	4.60		

ANOVA Table - May. 5

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-4.73	7	0.859	
Rapidly Biodegradable	9	-3.21	7	5.49	
			14	6.35	0.454
Pooled		-3.96	15	45.4	
Difference between Slopes			1	39.1	
Comparison of Slopes		F	(1,14)	86.1	Sig
		$F_{0.05}$	4.60		

ANOVA Table - Mar. 29

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-1.82	7	4.40	0.628
Rapidly Biodegradable	9	-2.20	7	4.87	0.695
			14	9.26	0.662
Pooled	17	-2.01	15	11.73	0.782
Difference between Slopes			1	2.46	
Comparison of Slopes		F	3.72 (1,14)	N. Sig.	
		$F_{0.05}$	4.60		

ANOVA - Apr. 24

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-5.20	7	6.08	0.868
Rapidly Biodegradable	9	-2.14	7	3.29	0.470
			14	9.37	0.669
Pooled	17	-3.67	15	161	10.7
Difference between Slopes			1	152	
Comparison of Slopes		F	226 (1,14)	Sig	
		$F_{0.05}$	4.60		

ANOVA - May 9

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	-3.875	7	5.14	0.734
Rapidly Biodegradable	9	-4.141	7	11.9	1.70
			14	17.0	1.22
Pooled	17	-4.008	15	18.2	1.21
Difference between Slopes			1	1.14	
Comparison of Slopes		F	0.94 (1,14)	N.Sig	
		$F_{0.05}$	4.60		

Statistical Analysis of Ammonia Production Data - Anoxic Cultures

ANOVA - Oct. 20

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	2.22	7	1.66	0.238
Rapidly Biodegradable	9	1.90	7	34.95	4.99
			14	36.6	2.62
Pooled	17	2.05	15	37.5	2.50
Difference between Slopes			1	0.878	
Comparison of Slopes		F	0.34 (1,12)	N.Sig	
		$F_{0.05}$	4.75		

ANOVA Table - Dec. 4

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	1.75	7	0.881	0.126
Rapidly Biodegradable	9	2.84	7	0.535	0.076
			14	1.42	0.101
Pooled	17	2.30	15	10.4	0.694
Difference between Slopes			1	8.993	
Comparison of Slopes		F	88.9 (1,13)	Sig	
		$F_{0.05}$	4.67		

ANOVA Table - Oct. 30

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	8	3.843	6	20.4	3.42
Rapidly Biodegradable	8	2.383	6	11.2	1.88
			12	31.8	2.65
Pooled	15	3.050	13	43.9	3.38
Difference between Slopes			1	12.1	
Comparison of Slopes		F	4.59 (1,12)	N.Sig	
		$F_{0.05}$	4.75		

ANOVA Table - Dec. 1

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	7	2.22	5	5.05	1.01
Rapidly Biodegradable	9	2.77	7	30.1	4.31
	16		12	35.2	2.94
Pooled	15	2.59	13	36.7	2.82
Difference between Slopes			1	1.44	
Comparison of Slopes		F	0.49 (1,12)	N. Sig	
		$F_{0.05}$	4.75		

ANOVA Table - Apr. 4

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	1.76	7	17.4	2.49
Rapidly Biodegradable	8	2.14	6	13.50	2.25
			13	30.9	2.38
Pooled	17	1.91	14	32.9	2.35
Difference between Slopes			1	2.03	
Comparison of Slopes		F	0.85 (1,13)	N.Sig.	
		$F_{0.05}$	4.66		

ANOVA Table - May 5

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	1.83	7	16.2	2.32
Rapidly Biodegradable	8	2.71	6	4.17	0.695
	17		13	20.38	1.57
Pooled			14	31.1	2.22
Difference between Slopes			1	10.8	
Comparison of Slopes		F	6.86 (1,13)	Sig	
		$F_{0.05}$	4.67		

ANOVA Table - Mar. 29

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	0.721	7	0.307	
Rapidly Biodegradable	9	1.623	7	5.43	
			14	5.74	0.410
Pooled	18	1.171	15	19.5	1.30
Difference between Slopes			1	13.7	
Comparison of Slopes		F	33.5 (1,14)	Sig	
		$F_{0.05}$	4.60		

ANOVA Table - May 9

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	2.027	7	13.2	1.89
Rapidly Biodegradable	9	3.218	7	26.1	3.73
			14	39.4	2.81
Pooled	15	2.468	13	49.5	3.81
Difference between Slopes			1	10.1	
Comparison of Slopes		F	3.59 (1,14)	N. Sig	
		$F_{0.05}$	4.60		

Statistical Analysis of Ammonia Production Data - Anoxic Cultures

ANOVA - Nov. 7

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	8	1.87	6	0.288	0.048
Rapidly Biodegradable	8	3.56	6	1.38	0.230
			12	1.67	0.139
Pooled	16	2.71	13	23.4	1.80
Difference between Slopes			1	21.7	
Comparison of Slopes		F	155.9 (1,12)	Sig	
		$F_{0.05}$	4.74		

ANOVA - Nov. 27

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	2.25	7	0.770	0.110
Rapidly Biodegradable	9	2.30	7	0.287	0.0410
			14	1.06	0.0755
Pooled	18	2.27	15	1.07	0.0715
Difference between Slopes			1	0.0155	
Comparison of Slopes		F	0.205 (1,14)	N.S.	
		$F_{0.05}$	4.60		

ANOVA - Nov. 2

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	8	2.36	6	0.198	
Rapidly Biodegradable	8	2.47	6	0.619	
			12	0.817	0.0680
Pooled					
Difference between Slopes			1	0.0928	
Comparison of Slopes		F	1.36	(1,14)	N.S.
		$F_{0.05}$	4.60		

ANOVA - Dec. 6

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	2.76	7	0.041	0.006
Rapidly Biodegradable	9	2.20	7	0.376	0.054
			14	0.417	0.030
Pooled	18	2.48	15	3.15	0.210
Difference between Slopes			1	2.73	
Comparison of Slopes		F	91.7 (1,14)	Sig	
		$F_{0.05}$	4.60		

ANOVA - Apr. 4

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	3.39	7	9.19	1.31
Rapidly Biodegradable	7	2.37	5	5.44	1.09
			12	14.6	1.22
Pooled	15	3.026	13	28.4	2.18
Difference between Slopes			1	13.7	
Comparison of Slopes		F	11.3 (1,12)	Sig	
		$F_{0.05}$	4.75		

ANOVA - May 5

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	8	3.03	6	36.4	6.07
Rapidly Biodegradable	8	2.36	6	0.965	0.161
			12	37.4	3.11
Pooled			13	43.2	3.32
Difference between Slopes			1	5.86	
Comparison of Slopes		F	1.88 (1,12)	N. Sig.	
		$F_{0.05}$	4.75		

ANOVA - May 7

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	3.64	7	8.75	1.25
Rapidly Biodegradable	7	3.11	5	2.38	0.477
			12	11.1	0.928
Pooled	16	3.42	13	15.1	1.163
Difference between Slopes			1	3.98	
Comparison of Slopes		F	4.29 (1,12)	N.S.	
		$F_{0.05}$	4.75		

ANOVA - May 9

	No. of Obs	Reg. Coef.	Deviations from Regression		
Within			d.f.	S.S.	M.S
Slowly Biodegradable	9	2.03	7	13.2	1.89
Rapidly Biodegradable	9	3.22	7	26.1	3.73
			14	39.4	2.81
Pooled	15	2.47	13	49.5	3.81
Difference between Slopes			1	10.1	
Comparison of Slopes		F	3.59 (1,14)	N. Sig	
		$F_{0.05}$	4.60		

Factorial Analysis of Ammonia Production using Proteins - Normalized on MLVSS

Reactor	Temp (°C)	Protein	Mean	Variance	n
Anoxic	20	ovalbumin	4.73	3.43	2
Anoxic	20	α-casein	5.84	2.59	2
Anoxic	14	ovalbumin	2.98	0.026	2
Anoxic	14	α-casein	2.50	1.805	2
Aerobic	20	ovalbumin	4.50	0.387	2
Aerobic	20	α-casein	6.54	0.744	2
Aerobic	14	ovalbumin	9.82	10.39	2
Aerobic	14	α-casein	9.19	0.530	2

Pooled Variance = 2.49
Variance (Effect) = 0.622
Std. Dev. (Effect) = 0.789

Experimental Levels: Reactor: Aerobic (-) Anoxic (+)
Temperature: 14°C(-) 20°C (+)
Protein: α-casein (-) ovalbumin (+)

ANOVA Table - See Table 5.77

Factorial Analysis of Ammonia Production using Proteins - Normalized on Bacterial Counts

Reactor	Temp (°C)	Protein	Mean x 10^{-13}	Variance x 10^{-26}	n
Anoxic	20	ovalbumin	24.9		1
Anoxic	20	α-casein	23.1		1
Anoxic	14	ovalbumin	9.24	14.2	2
Anoxic	14	α-casein	9.60	51.9	2
Aerobic	20	ovalbumin	28.8		1
Aerobic	20	α-casein	86.2		1
Aerobic	14	ovalbumin	86.7	1285.2	2
Aerobic	14	α-casein	84.1	26.5	2

Pooled Variance = 404 x 10^{-26}
Variance (Effect) = 134.6 x 10^{-26}
Std. Dev. (Effect) = 11.6 x 10^{-13}

Experimental Levels: Reactor: Aerobic (-) Anoxic (+)
Temperature: 14°C(-) 20°C (+)
Protein: α-casein (-) ovalbumin (+)

ANOVA Table - See Table 5.77

Appendix D

D.1 Dynamic Sludge Age

Presented in this section is the method of computing the DSA. For a complete derivation and further instructions the reader is referred to Vaccari *et al.* (1985). To compute the DSA, W, the average wastage rate since the last time the DSA was calculated is required. In addition, you need to know the DSA the last time you computed it DSA_0. Also required is the previous solids concentration (M_0), and the time in days (t) since the DSA was lasted computed. To use the equations, first compute the net growth rate, K, and the production rate, P, averaged over the period of calculation

$$K = (M-M_0) / t$$

$$P = (K+W)$$

One of four possibilities may arise, and one question is selected based on the case which occurs to compute the new DSA:

Case 1) W = 0; No sludge wasted or lost over the period t.

$$DSA = (DSA_0 + t) M_0 / M + (1 - M_0 / M) t / 2 \qquad \text{Eqn. (D.1)}$$

Case 2) K = 0; There was no net sludge production (W = P).

$$DSA = (DSA_0 + M_0 / W) e^{(-Wt/M_0)} \qquad \text{Eqn. (D.2)}$$

Case 3) W = 2P; Twice as much sludge is wasted as produced.

$$DSA = DSA_0 (M / M_0) - (T + M_0 / K) \ln(M / M_0) \qquad \text{Eqn. (D.3)}$$

Case 4) If none of the above conditions hold use this equation:

$$DSA = (DSA_0 - M_0 / (P + K))(M / M_0)^{(-P/K)} + M / (P + K) \qquad \text{Eqn. (D.4)}$$

D.2 Mass Balance Calculation Procedure

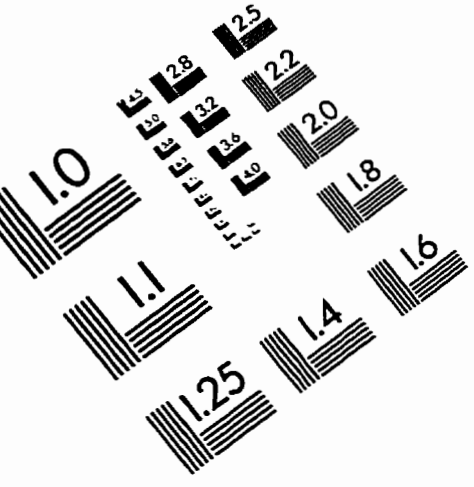
Total Kjedahl Nitrogen In = Total Kjedahl Nitrogen Out

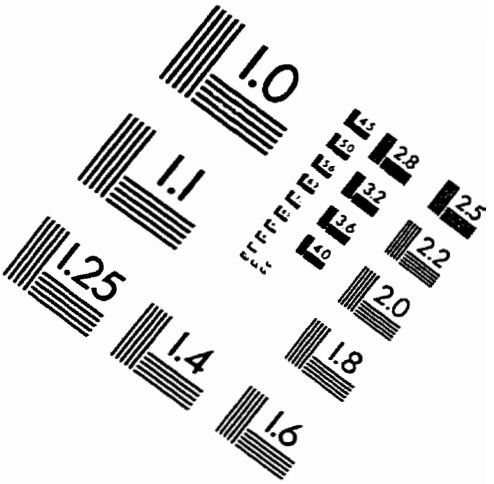
$$V_S T_S + V_P T_P = V_E T_E + V_W T_W \qquad \text{Eqn. (D.5)}$$

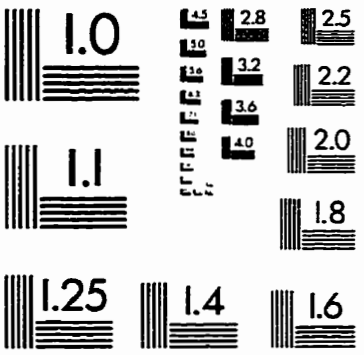
where:

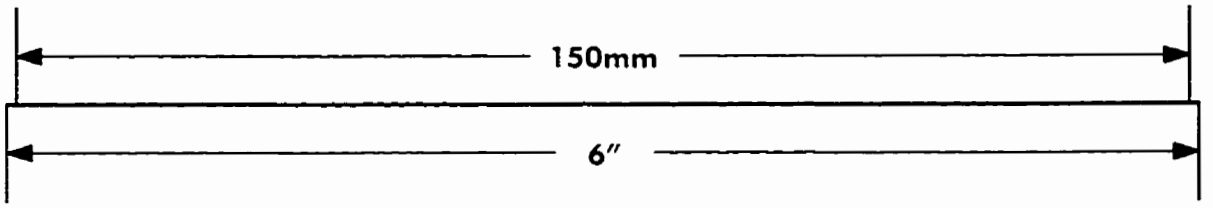
VS	=	Volume of sewage added to the reactor (L)
TS	=	Total Kjedahl Nitrogen of sewage (mg N/L)
VP	=	Volume of protein feed added to the reactor (L)
TP	=	Total Kjedahl Nitrogen of Protein Feed (mg N/L)
VE	=	Effluent Volume (L)
TE	=	Total Kjedahl Nitrogen of Effluent (mg N/L)
VW	=	Volume of Waste Mixed Liquor (L)
TW	=	Total Kjedahl Nitrogen of Waste Mixed Liquor (mg N/L)

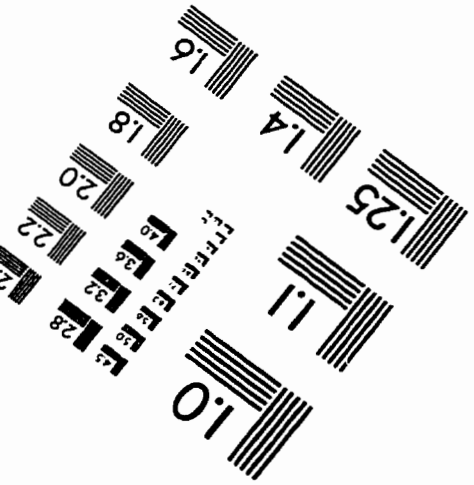
IMAGE EVALUATION
TEST TARGET (QA-3)

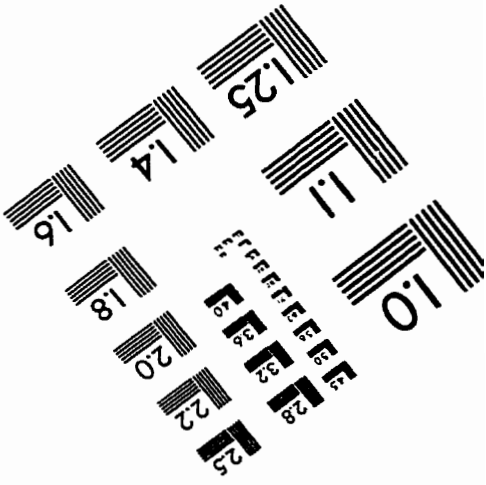












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