BIOFILTRATION OF HEXANE: ENHANCEMENT OF PERFORMANCE BY NITROGEN SUPPLEMENTATION

by

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ABSTRACT

A study was conducted to investigate the improvement of hexane biofiltration through nutrient supplementation. Two supplementary nutrient addition protocols, low and high frequency additions, were applied to biofilters containing pearlite, peat and their 50:50% (v/v) mixture. In high frequency nutrient addition, 1 kg of nitrogen was added to 1 m^3 of biofilter medium every week. In low frequency nutrient supplementation, the addition was reduced to once every 30 days. The biofilters were inoculated with a mixed culture containing *Pseudomonas corrugata*, which was identified as the dominant species capable of utilizing hexane as the sole carbon source.

Biodegradation of hexane was found to strongly depend on nitrogen concentration in the solid media, such that media formulation and particle size had little effect on biofiltration rates. Hexane removal rate 50 $g/m^3.h$ at 400 ppm and a flow rate of 20 m/h during low nutrient supplementation for all media formulations. For high nutrient supplementation, the Hexane removal rate was up to 67 and 120 $g/m^3.h$ at hexane concentrations between 560–680 ppm and gas flow rates of 20 and 100 m/h, respectively. The elimination capacity for hexane was 75 $g/m^3.h$

Pressure drop ranges during high frequency addition of nutrients were 20 and 60 Pa/m to 230 and 2900 Pa/m for pearlite and the mixture, respectively at gas flow rate of 20 m/h. The pressure drop range during low frequency addition of nutrients was lower (10–250 Pa/m) for all media. In both cases, the pearlite exhibited the lowest pressure drop. The lime was effective in maintaining the pH value above 5 for more than 50 and 240 days of high and low nutrient addition, respectively. A long term biofiltration showed that the performance factor, a measure of the amount of hexane removed per unit volume, energy and time was higher for the pearlite averaging 5 $g/m^3.J.h$ than that of the peat and the mixture which was less than 1 $g/m^3.J.h$.

The hexane removal followed zero order kinetics. Mathematical models described by Ottengraf are effective in describing biofiltration kinetics. However, apparent reaction rate constants changed with nitrogen concentration in the solid media.

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DEDICATION

To my two beloved sons, Douglas Rugambwa and Brian Mujuni.

Contents

List of Figures

List of Tables

Chapter 1

INTRODUCTION

1.1 Air pollution from volatile compounds

Air pollution from Volatile Organic Compounds (VOCs) and Volatile Inorganic Compounds (VICs) is one of the major environmental pollution problems for our modern society. The amount of toxic VOCs released to the atmosphere in the USA alone in 1987 was more than 1.2 million tons [60]. In the late 80's, there were more than 85.000 leaking motor fuel underground storage tanks in USA with estimated clean up cost of more than 32 billion dollars [10]. A survey of 24 urban sites between 1990 and 1991 indicates that, Canadian ambient air contains hazardous VOCs which include aromatic and chlorinated compounds [22]. The VOCs with highest concentrations reported by the National Air Pollution Surveillance Network data and other sources are shown in Table 1.1. The VOCs have a great impact on our environment in many different ways. Some are toxic and hence hazardous to plants and animals. Others are responsible for the smog formation and ozone depletion which adversely affect our environment. Other VOCs may not be toxic but are a nuisance and cause discomfort due to their offensive odour.

The VOCs originate from either synthetic or natural sources. Synthetic VOCs

Table 1.1: VOC with high concentrations at urban sites in Canada

Compound	Ambient Canadian 24 sites (1990–1991) [$\mu g.m^{-3}$]		National Survey [$\mu g.m^{-3}$]	
	mean	maximum	mean	maximum
Toluene	9.02	389	40.8	5730
Decane	1.3	74.4	31.4	6450
m- and p-Xylene	5.02	389	20.7	1470
1,4-Dichlorobenzene	0.64	16.0	18.9	1390
Dichloromethane	1.55	32.3	16.1	1690
1,2,4-Trimethylbenzene	1.52	11.0	11.5	1920
Ethylbenzene	1.3	142	8.2	540
o-Xylene	1.59	143	5.6	320
Benzene	3.84	61.1	5.4	67.9
1,3,5-Trimethylbenzene	0.49	6.52	2.7	640
Tetrachloroethylene	0.98	45.7	2.7	313
Chloroform	0.21	3.78	1.5	98.6
Hexane	3.91	417	1.2	124
p-Cymene	0.15	9.57	1.2	115

source [22]

are produced by chemical industries dealing with synthetic flavouring, paint and dye, organic chemicals, pesticides, pharmaceuticals, printing and paper milling, and refineries. Most of the natural VOCs are those with offensive odours. They are mainly produced by food and food related industries which include: slaughterhouses, sugar factories, cocoa and chocolate factories, breweries and gin distilleries, cattle feeding and fish meal factories [101, 170]. Other sources are fermentation industries, refuse incineration, coffee roasting, tobacco factories, flour mills, yeast and alcohol factories, spices and aroma factories [114]. Other industries such as sewage treatment works, solid waste composting works, and textile industries tend to produce both natural and synthetic VOCs. Due to the increasing environmental awareness, industries are facing stringent government regulations which require the removal of VOCs from waste air and industrial effluent gases.

1.2 Air pollution abatement methods

There are several methods for treating air contaminated with VOCs. These methods are masking odorous VOCs, ozone oxidation, chemical scrubbing, adsorption, incineration, bio-scrubbing, trickling filtration and biofiltration. Masking of odorous compounds is done by introducing a new volatile compound with a pleasant smell which overrides the bad odour. The method neither removes the VOCs from the air nor eliminates the related hazards. Masking is common for indoor use for temporary odour problems. Ozone oxidation is done by oxidizing the VOCs using ozone gas to oxides such as carbon dioxide. The ozone oxidation method was discontinued due to its harmful health effect on bronchial tubes.

Chemical scrubbing is performed by transferring the VOCs from gas phase to liquid phase in which they are chemically oxidized using other chemicals. Bio-scrubbing is similar to chemical scrubbing except that the VOCs are biologically oxidized after being transferred to aqueous phase. In both chemical scrubbing and bio-scrubbing methods, the process of removing the VOCs from the gas phase into the continuous liquid phase and the oxidation take place in different units. The trickling filtration is similar to bio-scrubbing. However, both transfer of the VOCs to the aqueous phase and biological oxidation take place in the same unit. The flow of the aqueous phase in a trickling filter is much less than that in the bioscrubber.

Adsorption removes VOCs from a gas stream by means of adsorption onto a solid surface. Incineration employs a combustion process to oxidize VOCs. When the concentration of VOC under consideration is too low to support combustion, fuel is added to the waste gas to obtain a combustible fuel-air ratio. Biofiltration is a relatively new method for waste gas treatment. The VOCs are biologically oxidized in a packed bed containing microorganisms immobilized on the surfaces of solid supports without a continuous flow of aqueous phase.

1.3 Biofiltration: advantages and disadvantages

Biofiltration is becoming attractive to the waste gas treatment industry because of the following advantages over the other waste gas treatment methods:

- Biofiltration is the most environmentally friendly method. In most cases it results in total mineralization of VOCs to mainly carbon dioxide and water without creating a secondary stream which needs a subsequent disposal. Incineration on the other hand, produces nitrogen oxides which are secondary pollutants. Adsorption is usually followed by the disposal of the adsorbent containing the VOC as a solid waste, or VOC desorption and treatment by other methods. Chemical scrubbing and bio-scrubbing methods produce waste water which needs further treatment.

- Biofiltration uses less water than bio-scrubbers and trickling filters.

- Biofilters have the lowest investment and running cost as shown in Table 1.2.

- Biofilters are reliable and relatively easy to maintain. They do not have moving parts except fans for blowing air thorough the filter bed.

- Biofiltration has the ability to remove odorous compounds and other pollutants present in low concentrations from polluted air.

- Biofiltration is reported to be more efficient than wet scrubbing especially when the VOC concentration is relatively low.

- Biofiltration can achieve a satisfactory degradation of VOCs which are less soluble in water.

Despite all the advantages, biofiltration has some drawbacks. The method is only used for low VOC concentrations. Due to low reaction rates, biofiltration requires a

Table 1.2: Average costs of air treatment

Treatment method	Average total cost US $ per 1000 m^3 of air	
Incineration	5.35	4.59
Chemical Scrubbing	2.47	2.12
Ozone oxidation	2.47	2.12
Adsorption on Activated Carbon with regeneration	0.88	0.71
Biofiltration	0.35	0.28
References	Ottengraf [98]	Bohn [12]

high residence time, and hence huge filter volumes are required for waste air treatment. This becomes a limitation for less biodegradable and persistent VOCs, and/or when large volumes of gases are to be treated. Although biofiltration is relatively easy to maintain, long-term process control and stability or regulation of metabolism creates some challenges. Moreover, aging of the solid media is associated with solid media compaction, high pressure drop and air channelling problems [98, 142]. Nevertheless, biofiltration is still a promising technology, and continues to improve with time. For the last twenty years, there has been considerable improvement in methods of preventing an increase in pressure drop which is a major operating cost for biofiltration. By incorporating inert materials which have mechanical stability, such as polystyrene spheres in compost and peat biofilters, pressure drop and aging effects have been significantly reduced [98]. Inert material added to the filter medium to increase mechanical strength does not necessarily take part in the intrinsic biochemical reaction as a microbial support due to the lack of nutrients.

1.4 Biofiltration History

Biofiltration has been used for more than 50 years as a means of odour control on installations such as rendering and sewage treatment plants in Germany, UK, Netherlands, Japan and to a limited extent in the USA [98]. Soil biofilters are reported to have been used to treat odours from sewer exit gases in Long Beach California in 1953 [12, 80]. The first attempts to treat exhaust gas from a composting facility in Europe were made in Geneva-Villette [12]. Around 1959 a soil bed system was used at a municipal sewage treatment in Nuremberg, Germany [147]. In the early 1960s Carlson and Leiser [14, 80] conducted research on biofiltration in the USA to treat hydrogen sulfide from sewage. Since then there has been a steady progress in the application of biofiltration to treat waste gases. More details of the historical development are reported by Ottengraf [98, 99].

Having proven to be effective in treating odorous gases, biofiltration research in the past two decades has focussed on the removal of VOCs and air toxins from chemical and other industrial waste gases. In the 1980s, more emphasis was directed to the reduction of pressure drop [98] to improve air flow and increase operating life span. Current research activities are focussed on improving/optimizing operating parameters such as increasing reaction rates, understanding biomass dynamics and improving system modelling. Inoculation of the filter bed with pure or mixed cultures is reported to reduce biofilter acclimation time and increase biodegradation rates. There are reports of improvement of filter performance by addition of nutrients, especially nitrogen source [18, 82, 95].

1.5 Research Objectives

Although there has been a progressive improvement in biofiltration, the technology is not fully explored. There is still a lot to be learned about the mechanisms and factors influencing this technology for future improvements. Recent developments in biofiltration have concentrated on improving the mechanical structure of the solid media and reduction of pressure drop, which is the main operating cost in biofiltration [12, 98, 132]. There have been few reports on improving the reaction rates by providing favourable environments for microbial activity, and more needs to be done in this area.

The proposed research is intended to explore the influence of supplementary nutrients, particularly the nitrogen source, and other system variables on biofiltration performance through the following activities:

1. Selection and characterization of solid media: peat (as a reference), pearlite (as a potentially useful inert solid media); Peat-Pearlite mixtures (as a practical application).

2. Improvement and maintenance of high biodegradation rates of hexane (as a primary model VOC) by supplementary nutrient addition particularly nitrogen source. Assessment of overall biodegradation of hexane with respect to various operating conditions such as pressure drop, supplementary nutrient addition protocol, solid medium, microbial growth pattern, and long-term stability.

3. Evaluation and modification if necessary of available equations for modelling biodegradation kinetics of biofiltration performance for the above scenarios.

Chapter 2

LITERATURE REVIEW

2.1 Fundamental Concepts of Biofiltration

Biofiltration of polluted air is a biological process for removing volatile organic or inorganic compounds (VOCS or VICs) from air in a gas-solid phase packed bed. The process involves passing the contaminated air through a packed bed containing immobilized microorganisms which utilize the VOCs as a source of carbon, energy or sulfur. Common packing materials (solid media) are usually organic solids such as compost and peat. Biodegradation reactions take place inside a thin aqueous film known as the biofilm or biolayer adhering to the solid particles of the solid media. This biofilm supports a variety of different microorganisms, and because of its static nature and the low solubility of most VOCs, it is strongly believed that all bio-reactions take place on the surface of the particles in the biofilm and there is no intra-particle diffusion [98].

There are several mass transfer steps involved in biodegradation of VOCs. Figure 2.1 illustrates simplified mass transfer steps for biofiltration. First, VOC and oxygen molecules in the air must diffuse from the gas phase across the gas-liquid boundary to the biofilm. In the biofilm the VOC and oxygen molecules continue to diffuse

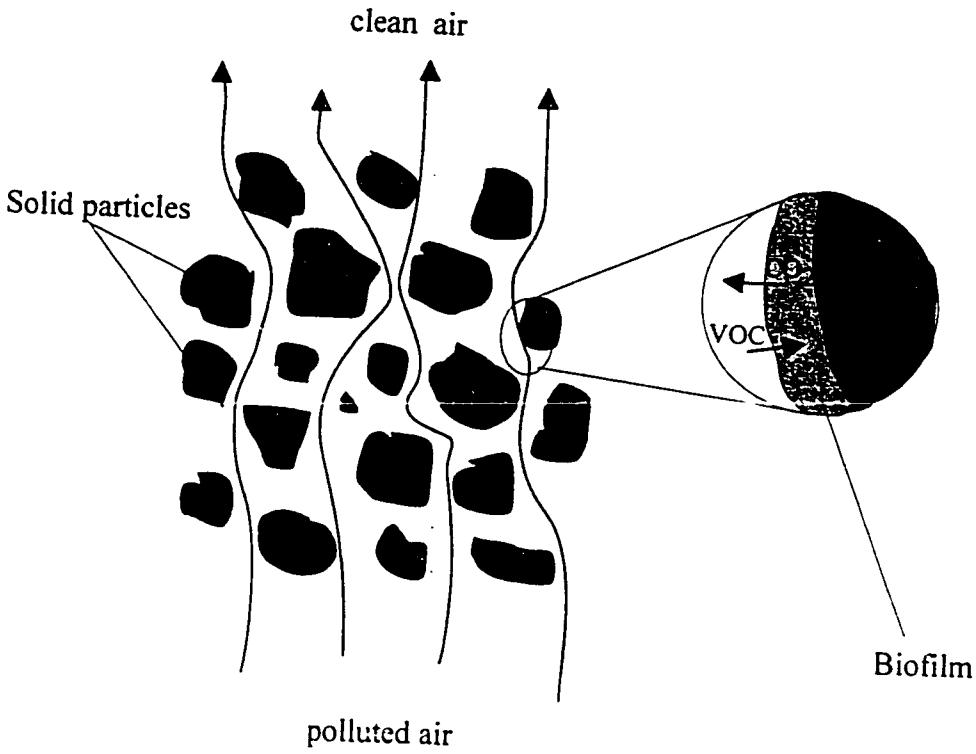


Figure 2.1: Mass transfer steps in biofiltration

further toward the surface of the solid particle until they come in contact with the microbial cell membrane. The molecules then penetrate into the microbial cell where they participate in biochemical reactions. Biochemical reactions always require other nutrients such as nitrogen, minerals and/or sulfur. In traditional biofilters the required nutrients are usually supplied by the solid medium. This is one of the major reasons why compost and peat are the common solid media for biofiltration. In recent studies, addition of supplementary nutrients is reported as being useful in enhancing biofiltration rates [18, 82]. Bio-reactions are followed by the transfer of products in the opposite direction. Gaseous products, mainly carbon dioxide, diffuse from the microbial cell to the aqueous phase in the biofilm and then to the gaseous phase. Other products that are highly soluble in water remain in the aqueous phase of the biofilm.

During biofiltration, there is a decrease in pH with time with consumption of the

VOC. Products, like hydrogen chloride from chlorinated VOCs and hydrogen sulphide from sulfur containing compounds, dissolve in the aqueous phase resulting in low pH. With the exception of sulfur utilizing bacteria, lower pH values decrease microbial activity and consequently lower VOC removal. The pH is controlled by addition of carbonate materials, such as lime to neutralize the acidic byproducts.

2.2 Biofilter Configuration

Biofilter beds come in different sizes and shapes. A simplified schematic diagram of the biofilter is shown in Figure 2.2. The biofilters are made of two main units; a packed bed unit and a humidification unit. Packed beds of rectangular cross section area are common for industrial applications [12, 99]. The packed height varies from 0.5 to 2 m and cross section area varies from 10 to more than 2,000 m^2 [80], depending on the flow volume of the waste gas to be treated, VOC concentration and the rate of degradation of the VOC to be removed. A medium biofilter has a capacity of treating 40,000 m^3/h waste gas and a large one has a capacity of treating 100,000 m^3/h of gas [33, 63, 99, 138, 141]. The packed bed is usually a single unit but where space is limited a stack of multiple units is a better option [122]. The filter bed is packed with natural organic materials such as compost, peat, heather, or soil. In most biofilters

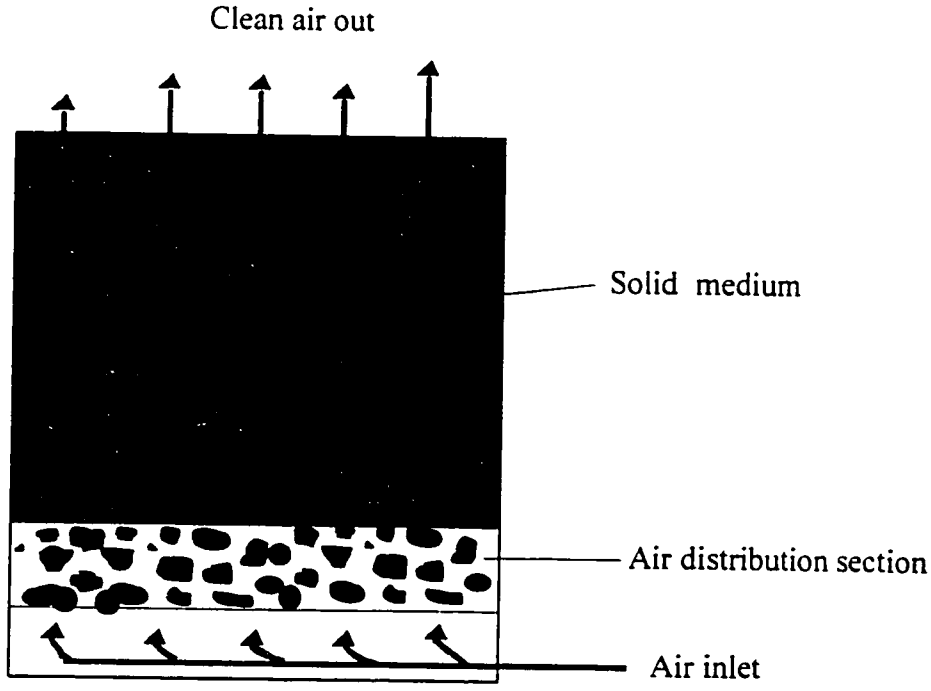


Figure 2.2: A simplified biofilter system

the polluted air flows upward through the packed bed [80, 98, 99, 147], but downward flow designs are also reported [131, 132, 142, 170]. Unlike upward flow operation, downward flow operation do not retain excess moisture which may create anoxic conditions and high pressure drop. However, high pressure drop can be experienced during downward flow operation if the packing material is compressible. For instance, pressure drop for downward flow operation observed by Togna and Skladany was 50% higher than that of upward flow operation due to compression of packing material [131].

The bottom of the packed bed has a water drainage structure for removing water resulting from rain and excess water added to restore moisture content. On top of the drainage system is an air distribution system followed by the solid medium. The air distribution system has the cavity through which air can flow freely at the bottom of the filter. The cavity can be made from perforated pipe surrounded by large solid particles such as gravel or open channels of concrete, metal or plastic material. The channels are covered with a perforated floor which supports the solid medium. Due to their large size, most biofilters are open to the atmosphere, but other biofilters are designed with a top cover.

Before the waste air is introduced into the packed bed, it passes through the humidification unit. The air must be as humid as possible in order to reduce moisture loss from the filter bed. The unit can either be a packed column which allows air to come into contact with water or a column equipped with a nozzle which emulsifies water droplets into the inlet air [12, 168].

2.3 Characteristics of solid media

The solid medium is the most important component of the biofiltration system. A good solid medium should have the following properties;

- High microbial immobilization capacity.
- High moisture retention capacity, for high microbial activity.
- High pneumatic conductivity for low pressure drop across the filter bed.
- Provide supplementary nutrients such as nitrogen and phosphorus required for microbial growth and maintenance energy.
- Ability to operate at constant pH.
- Adsorption capacity where shock loading operations are encountered.

In traditional biofilter systems, nutrients such as nitrogen and minerals required for growth are obtained from the solid media. In most cases the carbon source comes from the VOC. However, in situations where the air pollutant does not contain the carbon source, as is the case with VIC (e.g. hydrogen sulfide), or when microorganism can not utilize the VOC without an separate carbon source to support growth, the solid media acts as the source of carbon. Solid media of organic origin are the commonly used materials in biofiltration systems. Compost, peat, and humus are the most widely used. Others solid media used in experimental and industrial applications include soil, composted activated sludge, composted leaves, silt, bark compost, sand and clay. Compost and peat are superior to other solid media in providing the highest biofilter efficiency [32]. Soil is relatively effective in biofiltration and has an almost unlimited life time but has poor pneumatic conductivity.

The above solid media have proved useful despite variation within similar solid media due to differences of age or sources. There are limited number of reports

of thorough analysis of different solid media and their effects on microbial activity. Compost analysed by Martin and Loehr [83] contained a high content of heavy metals: cadmium, 6.6; copper, 144; nickel, 40; and lead, 86 (mg/kg on a dry basis). Such heavy metals could have an inhibitory effect on microbial activity. Certain types of soil such as clay and sandy soils do not have sufficient amounts of nutrients to support an adequate microbial population [12].

There are problems associated with the organic solid media mentioned above. They include clump formation, bed shrinkage, and crack formation which results in poor air distribution and high pressure drop. The problems have been effectively reduced by addition of inert material such as vermiculite, gypsum, pearlite or polystyrene spheres of 3–5 mm diameter [31, 98, 99]. In addition, the inert material stabilizes the structure of the solid medium. The use of pearlite as an inert material has been widely reported [80, 98, 142, 143].

Other materials added to biofilter media are activated carbon and calcium carbonate. Although the activated carbon is an optional component, its role is to reduce shock loading in systems where periodic VOC release or large fluctuations of VOC concentration is of major concern [74, 99, 142, 151]. The activated carbon has been reported to be integrated within the solid medium [74, 99, 142, 151], but Weber and Hartman [151] found the adsorption properties of the activated carbon to be reduced in the presence of water. Mohseni and Allen reported that granular activated carbon did not enhance the performance of the biofilter containing compost, wood-chips and activated carbon compared to that of the compost, wood-chips and pearlite biofilter when both biofilters were subjected to step changes in the loading rate of α-pinene. They further report that activated carbon was fully covered by a thick layer of biofilm and α-pinene could not diffuse through this layer to reach the activated carbon surface [93]. It is suggested that the activated carbon unit should be placed before the humidification unit [151]. Weber and Hartman report controlled fluctuations of

toluene concentration in the inlet polluted air from 0–1500 mg/m^3 to 200–800 mg/m^3 [151] by placing the activated carbon before the biofilter. Christina and Allen report that transient concentrations can be effectively treated by biofilters without activated carbon [30]. From the above literature information it reasonable to assume that if the VOC loading is close to the biofilter maximum elimination capacity, a VOC fluctuation that increases the concentration will likely result in a VOC breakthrough. But, if the fluctuations takes place when the VOC loading if far less than the biofilter elimination capacity the breakthrough is less likely.

The role of carbonate material in the biofilter is to stabilize the pH between optimum values of 6 and 8 by neutralizing acids formed from both decomposing medium and degraded VOCs. Common carbonate materials include lime, oyster shells, and marble [80, 99, 142].

In conventional compost biofilters, particle size ranges between 1–5 mm. [80, 98]. Medina *et al.* [99] used activated carbon of 4.75 mm size during biofiltration of gasoline vapours. Best results were obtained with bed porosity between 40 and 50%. It is reported that most biofiltration reactions take place on the exterior surface of the particles [98]. Therefore, it seems more likely that the main advantage of porous solid particles is to provide additional space for moisture [116]. Literature on the effect of porosity of the solid particles and how it affects the biofiltration process was not available.

Efforts to introduce new packing materials for biofiltration have been reported. Ziehr *et al.* [168] used micro-porous polyethylene tubes sintered with activated charcoal and obtained 75% degradation of alcohols at a residence time of 13.5 seconds. Cruse [20] patented a packing material made by curing 10–20 mm spheres of a mixture of leonardite, bentonite, calcium hydroxide, and nitrogen and phosphorus sources.

The solid medium used in this work consisted of peat and pearlite. The pH was controlled by addition of lime. The properties of these materials are detailed below.

2.3.1 Peat

Peat is formed as a result of the anaerobic decomposition of plants in a marsh land. The disintegration of plants in the marsh land starts as an aerobic process but stops before it is complete due to lack of oxygen at the bottom of the marsh, allowing the process to proceed anaerobically. Accumulation of 1 metre of peat takes approximately 3000 to 4000 years [44]. Mined peat contains 80–90% water and 10 20% solids. The composition of peat varies depending on the degree of decomposition. In slightly decomposed peat, plants can be identified from roots, leaves and pollen/spores. Highly decomposed peat looks like a thick mud soil [44].

There are two major types of peat depending on the habitat. They can be distinguished by their difference in acidity and ash content. Low-moor peat comes from peat deposits whose bog water is continuous with the mineral and underground water system. Low-moor peat contains a high mineral content (7–14% ash dry w/w) and woody materials and has a pH ranging from 4 to 7.5 [44]. High-moor peat comes from peat deposits whose bog water is high above and not continuous with the underground water. High-moor peat is characterized by plants such as sphagnum and cotton weeds. It contains a lower mineral content (less than 3% ash dry w/w) and has a pH ranging from 3 to 5 [44].

Table 2.1: Composition of peat (% dry weight) Source [44]

Components	Sphagnum high-moor peat	Sedge low-moor peat
Ash	1.5–3.0	7.7–14.5
Bitumen	3.1–9.1	3.2–3.9
Pectins	4.2–7.8	2.5–3.6
Polyoses (hydrolysates with 2% HCl)	9.8–40.4	12.3–21
Cellulose (hydrolysates with 80% H_2SO_4)	10.3–23.7	7.8–8.1
Residue (lignin and humic acids)	26–64	54–62
Protein	5.6–6.9	1–14
Total reducing sugars (calculated as glucose)	20–50	16–20

Apart from ash, peat consist of bitumen, cellulose, pectin, and other carbohydrates such as hemicellulose, helocellulose, glycosides and chitin [44]. The general composition of peat is shown in Table 2.1. Bitumen contains waxes which have aliphatic and cyclic hydrocarbons, alcohols and acids of C_{16} to C_{35}. Humic acids have high carboxylic acid content and a significant amount of nitrogen 1.5–10%. As much as 20 to 50% of the nitrogen is in a form of hydrolysed amino acids. Humic acids are insoluble in water but soluble in alkaline solutions and tend to form micellar aggregates. Reducing sugars include galactose, xylose and fructose.

The constituents of peat, especially carbohydrates, protein and amino acids, are beneficial to microorganism as nutrients and probably as growth factors. The presence of hydrocarbons, bitumen, alcohols and acids of C_{16} to C_{35} as well as wax in the peat might be used as substrates allowing microorganisms in the peat to develop hydrocarbon degrading enzymes.

2.3.2 Pearlite

Pearlite is a glassy volcanic rock. The definition includes the rock in its native or expanded form. Upon heating, the native rock expands 4 to 20 times. Pearlite is considered to be a chemically inert material. Chemically, it is essentially a metastable amorphous aluminum silicate. A typical average chemical analysis is 71–75% SiO_2, 12.5–18.0% Al_2O_3, 4–5% K_2O, 1–4% sodium and calcium oxides, and traces of metal oxides [89]. The bulk density of expanded pearlite varies from 32 to 321 kg/m^3 depending on the preparation and intended use. Pearlite for horticulture uses is reported to have a bulk density of 96–128 kg/m^3 [89].

2.3.3 Carbonate materials

Carbonate materials are added to the solid media to control pH which would otherwise decrease and reduce the microbial activity. The role of the carbonate materials is

to neutralize acids formed as a result of biodegradation of VOCs. The carbonate materials reported in the literature include oyster shells, lime, calcium hydroxide and marble [37, 58, 99, 119, 121, 142, 171]. The literature does not give any preference to a particular material and it appears oyster shells are more widely used.

2.4 Biodegradation Pathway of alkane VOC

Different biodegradation pathways exist depending on the molecular structure of the VOC. Since this study involves degradation of hexane, only the biodegradation pathway for alkanes will be presented. Aliphatic hydrocarbons pose many problems as substrates. They are insoluble in water and hence difficult to emulsify/dissolve at high concentrations in the aqueous phase. They are relatively chemically unreactive and hence require specialized enzymes. They also change the metabolism of microorganism from being glycolytic and lipogenic to being lypolytic and gluconeogenic [49, 113].

Aliphatic hydrocarbons are assimilated by a wide range of microorganisms. Saturated hydrocarbons are degraded more readily than unsaturated hydrocarbons. Also, straight chain hydrocarbons are degraded more readily than branched chain hydrocarbons [4, 49, 113]. Cell mass yield on hydrocarbon degraded increases with increasing chain length [4, 49, 113]. Utilization of C_1-C_4 is restricted to a few specialized species, while alkanes in the C_5-C_9 range are toxic to most microorganisms, being biodegraded by a few. Toxicity is due to their higher solubility in the aqueous phase: bacterial species reported to grow on short chain hydrocarbons increase as the hydrocarbon concentration is lowered [49]. Normal alkanes in the C_{10}–C_{22} range usually are readily metabolized. Higher molecular weight alkanes tends to be solid waxes and are not readily biodegradable. However, slow biodegradation of n-alkanes up to C_{44} has been reported [5, 49, 65, 81, 106, 113]. During biofiltration of gasoline vapours, C_6-C_9 are reported to be less biodegradable than other components [156]. Cycloalkanes have the lowest biodegradation rates compared to straight and branched chain alkanes, alkenes, and aromatic hydrocarbons [106].

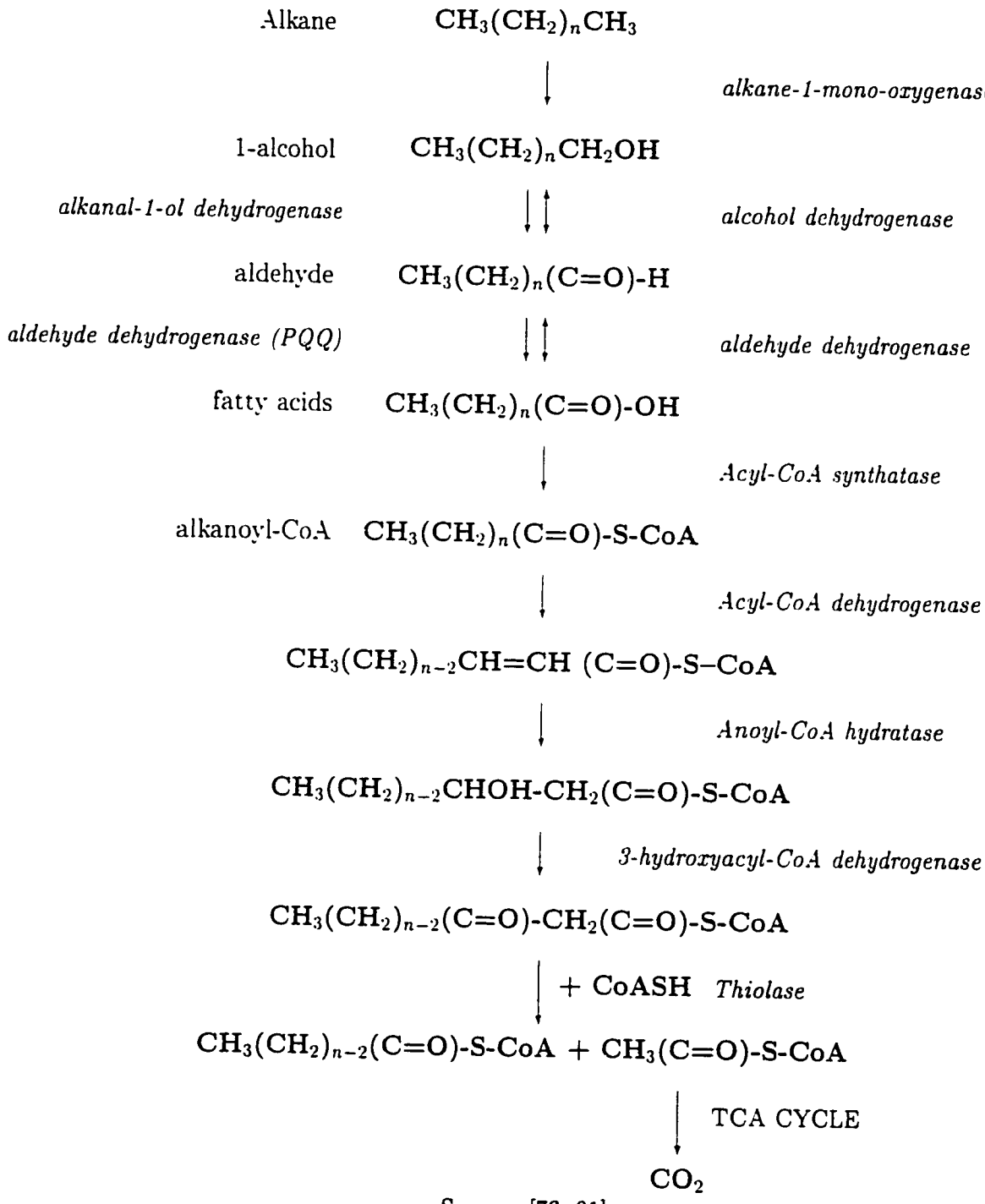
Unlike bacteria and yeast, filamentous fungi do not exhibit preferential degradation for a particular chain length [4]. Fungi in some cases were found to exhibit greater hydrocarbon degradation than bacteria in aquatic environments [4].

It is reported that in aqueous medium the uptake of hydrocarbons by microbial cells starts by the microorganism adhering to the submicron or larger hydrocarbon droplets. Internalization of the hydrocarbon is accomplished by direct partitioning in the cytoplasmic membrane and/or by solubilization with inducible surfactants produced by microorganisms. Transport is assumed to be passive diffusion, or if solubilizing agents are involved by facilitated diffusion [4, 49]. Surfactants are reported to increase the solubility of hydrocarbon resulting in higher biodegradation rates. Many microbial species have the capability of producing surface active agents resulting in higher hydrocarbon solubilization [4, 49, 55, 81]. These surfactants range from simple fatty acids to complex polymers [81]. The surfactants' emulsifying power has a pH range of 2–9 for bacteria as represented by *Pseudomonas* sp. and 0.2–11 for yeast as represented by *Candida* sp. [55]. The optimum emulsifying power of the surfactants is at pH 6.5 for both bacteria and yeast [55].

The biodegradation pathway for aliphatic hydrocarbons is shown in Figure 2.3. The most common pathway for alkane oxidation starts with monoterminal oxidation of the methyl group forming a primary alcohol. The enzyme catalysing this oxidation is designated as alkane-1-mono-oxygenase. The alcohol is oxidized to an aldehyde and then to a monocarboxylic acid. The corresponding enzymes for these biotransformations are, in respective order, alcohol dehydrogenase, and aldehyde dehydrogenase [40, 49, 88, 91, 113].

2.4.1 ω-oxidation

In some cases diterminal oxidation leading to the formation of dioic acids has been observed. The mechanism starts with the monoterminal oxidation described above until the monoic fatty acid is formed. Then the whole process starts all over on the other terminal leading to the formation of dioic acid [113, 150].



Source: [76, 91]

Figure 2.3: Biodegradation pathway for aliphatic hydrocarbons

2.4.2 β-oxidation

Once the fatty acid (monoic or dioic acid) has been synthesized, it is broken down by β-oxidation reactions. The reactions of the β-oxidation pathway starts with the formation of alkanoyl-CoA which is a long chain acyl CoA ester and ends with the synthesis of a two carbon atoms acyl CoA and a long chain acyl CoA esters. The long chain acyl CoA ester re-enters the β-oxidations while the short chain acyl CoA ester is broken down to carbon dioxide and water via the TCA cycle [4, 21, 49, 76, 91, 113].

2.4.3 Subterminal oxidation

Although alkane degradation occurs by terminal oxidation, there are reports on subterminal oxidation [49, 150]. The subterminal oxidation is a less common pathway, which is limited to only *Penicillium* sp. and *Pseudomonas aeruginosa* [49]. The oxidation proceeds in a similar manner to monoterminal oxidation but with oxidation taking place on a subterminal carbon atom. It starts with the formation of a secondary alcohol followed by methyl ketones, esters and finally fatty acids. The fatty acids may then be utilized for energy production or biosynthesis of cellular components via fatty acids β-oxidation and TCA cycle [21, 49, 113, 137].

2.4.4 Enzymology of biodegradation of aliphatic hydrocarbons

The genetics and enzymology of hydrocarbon degradation by *Pseudomonas* species have been reported in several reviews [4, 49, 113]. The genetic information for hydrocarbon degradation in these organisms generally has been found to be encoded on plasmids. The degradative plasmid for n-octane and other n-alkanes is designated as OCT [49, 113]. The plasmid is conjugate and non-specific, and therefore responsible for oxidation of all biodegradable aliphatic hydrocarbons. The induction of OTC

plasmid-coded alkane hydroxylase and alcohol dehydrogenase in *Pseudomonas* species is achieved by a wide range of compounds. Many straight chain and even those that cannot serve as growth substrates can induce activities provided that they have a chain length of C_6–C_{10} and that both ends do not have terminal or subterminal hydroxyl groups. A carbonyl, hydroxyl, or methyl group at the subterminal carbon of only one end of the hydrocarbon chain does not prevent induction [49, 113].

The complete enzyme system for the transformation of n-alkanes to their corresponding alcohols involves three proteins: rubredoxin; NADH: rubredoxin reductase, and monooxygenase. During the oxidative biotransformation of n-alkane to 1-alcohol, monooxygenase acts as a catalyst while rubredoxin, a red iron sulfur protein, is an electron carrier. Rubredoxin is then regenerated through NAD and NADH reactions as shown below. The reduced and oxidized form of this protein are designated as rubredoxin (red) and rubredoxin (ox) [38, 88, 91, 113].

$$CH_3(CH_2)_nCH_3 + O_2 \xrightarrow{\text{Alkane monooxygenase}} CH_3(CH_2)_nCH_2OH + H_2O$$

Rubredoxin (red) Rubredoxin (ox)

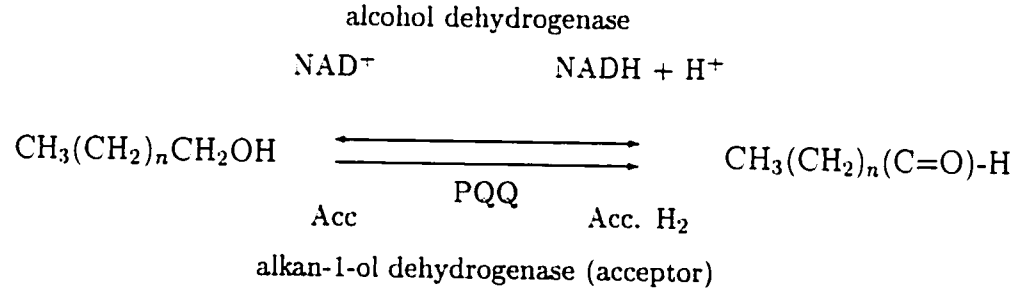
$$NAD^+ \xleftarrow[\text{Rubredoxin-NAD reductase}]{} NADH + H^+$$

Unlike in *Pseudomonas* sp., the oxidation of aliphatic hydrocarbons in yeast is achieved with a different enzyme. NADPH-cytochrome-P-450 reductase system is reported as the most common enzyme in yeast. This enzyme has also been isolated in some bacteria such as *Corynebacterium* and *Pseudomonas* [66, 113, 136, 150]

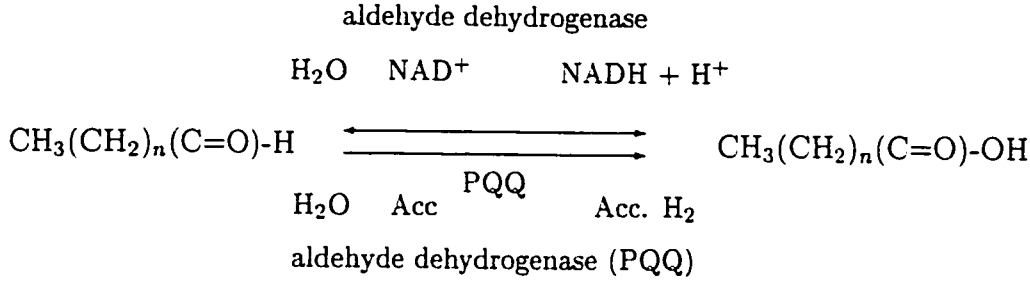
The oxidation of 1-alcohol to aldehyde is catalysed by either alcohol dehydrogenase or alkan-1-ol dehydrogenase [91]. The former is reversible and NAD^+ dependent [3, 91, 96]. It has an optimum pH of 10.5 for the forward reaction and 6.5 for the reverse

reaction [3]. The latter is irreversible and the reaction is regulated by a cofactor pyrroloquinoline quinone (PQQ) [54, 91]. The reaction is NAP(PH)$^+$ dependent and the nature of the cofactor is unknown [54]. Two quite different types of quinoprotein enzymes (enzyme with pyrroloquinoline quinone) appear to exist, quinoprotein and quinohemoprotein alcohol dehydrogenase. Quinoprotein alcohol dehydrogenase has optimum activity at a pH value of 9. The enzyme shows a broad substrate specificity: with primary and secondary alcohols as well as aldehydes as substrates [54].

Similarly, quinohemoprotein alcohol dehydrogenase shows a broad substrate specificity: with primary and secondary alcohols as well as aldehydes as substrates. Aldehydes are reported to be excellent substrates, and under appropriate conditions no free aldehyde is detected in the conversion of alcohol [54]. Quinohemoprotein alcohol dehydrogenase has an optimum pH of 7.5 [54].



The oxidation of aldehyde to fatty acids is also catalysed by the two enzymes, aldehyde dehydrogenase and aldehyde dehydrogenase (PQQ). The former is reversible and electron transfer is achieved by the NAD and NADH system. The latter is irreversible and the reaction is regulated by pyrroloquinoline quinone (PQQ) [39, 91]. The enzyme system has higher activity as the molecular weight of the aldehyde increases. For instance, aldehyde dehydrogenase derived from ethanol-grown *Acinetobacter* catalysed the oxidation of aldehydes at the following relative rates: acetaldehyde, 22; hexanal, 12; decanal, 98; dodecanal, 100 [39]. The above reaction is shown in the formula below:



Further degradation of the monoic or dioic fatty acid proceeds by β-oxidation and finally, carbon dioxide and water are liberated through oxidation of acetyl CoA via the TCA cycle [4, 49, 76, 91, 113].

2.5 Factors affecting Biofilter Performance

2.5.1 Solid media

The solid medium used as the packing material must be able provide conditions which support microbial activity as detailed in section 2.3. If conditions inside the biofilter are not favourable for microbial activity, the VOC degradation will be low.

2.5.2 Chemical nature of VOC

Compounds which are removed from air through biofiltration vary from organic to inorganic compounds. The physical, chemical and biochemical properties of these compounds can vary significantly. Properties of biofiltration importance include solubility, vapour pressure, microbial toxicity, and biodegradation rates. A simple classification puts them into two major groups; biogenic and anthropogenic compounds [99]. Most of the biogenic compounds are of natural origin, and are easily biodegraded. Anthropogenic compounds are man made compounds, and are generally less biodegradable. Anthropogenic compounds which resemble natural compounds are relatively more biodegradable. Compounds which do not resemble natural compounds are known as xenobiotics. Xenobiotic compounds are divided into two groups; slowly biodegradable compounds are known as recalcitrants, and non biodegradable ones are know as persistent compounds [99].

VOCs with excellent biodegradability contain oxygen, sulfur, and nitrogen functional groups. Similarly VICs with excellent biodegradability such as hydrogen sulfide, ammonia, and nitrogen oxides contain atoms of oxygen, sulfur, and nitrogen. Moderately biodegradable VOCs are hydrocarbons, phenols and carbon disulfide. Slowly biodegradable VOCs are halogenated and polyaromatic hydrocarbons. A detailed list of biodegradability of various compounds with different functional groups is shown in Table 2.2. The elimination capacity of biofilters can be as high as 80 to

200 g/m^3.h of organic carbon for easily biodegradable compounds and 5–100 g/m^3.h for moderately and slowly biodegradable compounds [98].

2.5.3 VOC Loading

Biodegradability of a particular VOC depends on its loading rate, provided other factors are favourable. The loading rate is controlled by the gas velocity through the bed and the concentration of VOC in the influent stream. At low loading the VOC can often be completely removed from the air stream.

VOC loading must consider the properties of the VOC. VOCs with low solubility and high Henry's constant in water, are likely to be affected by diffusion limitations due to their low solubility in aqueous phase and hence may require lower loading. Similarly, high concentrations of toxic VOC will inhibit biofiltration performance. For instance, hydrophobic compounds are less biodegradable and require lower loading rate than hydrophilic compounds [92]. The highest loading rate for complete removal of the VOC depends also on the other conditions such as pH, availability of nutrients, moisture content and microbial species.

2.5.3.1 VOC Concentration

The rate of degradation increases with increasing VOC concentration until the maximum removal capacity is reached. Further increases in loading results in break through. The best loading condition is obtained by the combination of both flow rate and concentration. Typical pollutant concentrations range from 0.3 to 100 g/m^3, which correspond to approximately 3 ppm to 1000 ppm [33]. Higher concentrations have been reported by Ebinger *et al.* [34] who obtained 92% removal of propane in a soil bed at a concentration of 10,000 ppm in a batch experiment.

Table 2.2: Biodegradability of various VOCs and VICs

Organic compounds	
Aliphatic hydrocarbons	
saturated aliphatic hydrocarbons	
- methane	+
- propane	+
- pentane	+
- hexane	++
cyclic aliphatic hydrocarbons	
- cyclohexane	+
Aromatic hydrocarbons	
Benzene	++
Toluene	+++
Xylene	+++
Styrene	+++
Oxygen containing compounds	
alcohols	+++
- methanol	+++
- butanol	+++
ethers	+
- tetrahydrofurane	+++
- diethylether	+
- dioxane	+
aldehydes	+++
- formaldehyde	+++
- acetaldehyde	+++
ketones	++
- acetone	++
carbonic acids	+++
- butyric acid	+++
esters of carbonic acids	++
- ethylacetate	++
phenols	++

Sulfur containing compounds	
sulfides (thioether)	++
- dimethyl sulfide	++
thiocyanates	++
sulfur heterocycles	++
- thiophene	++
mercaptans	++
- methyl mercaptan	++
carbon disulfide	++
Nitrogen containing compounds	
amides	++
amines	+++
- trimethylamine	+++
nitrogen heterocycles	++
- pyridine	++
nitro compounds	+
nitriles	++
- acetonitrile	++
isonitrile	++
Halogenated hydrocarbons	
dichloromethane	+
chlorophenols	++
1.1.1-trichloroethane	-
Inorganic Compounds	
hydrogen sulfide	+++
ammonia	+++
nitric oxide	++

Sources: [8, 11, 12, 13, 16, 18, 34, 42, 90, 95, 118, 119, 122, 158, 160]

KEY:

+++	Excellent biodegradability	+	low biodegradability
++	Good biodegradability	-	no biodegradability

2.5.3.2 Loading Velocity

Gas flow rates through the bed vary considerably. An average superficial velocity through the filter bed is about 10 to 100 m/h. Highly biodegradable VOC can be treated at much higher superficial velocity. Don [31], Zilli *et al.* [169] and Sabo [116], performed successful biofiltration at loading velocities ranging from 100 to 500 m/h.

2.5.4 Mixture of multiple VOCs

Biofiltration of air contaminated by several VOCs is reported in literature [18, 79, 92, 122, 129, 146, 158, 160]. There are different observations ranging from cometabolism to inhibition of biodegradation for certain hydrocarbons in the presence of other carbon sources or VOCs. Preferential biodegradation of aromatic hydrocarbons versus aliphatic hydrocarbons has been observed by Leson and Smith [79] during biofiltration of petroleum hydrocarbons. Wang *et al.* [146] and Stroo *et al.* [129] observed low biodegradation of phenol and phenanthrene respectively, in the presence of glucose. Mohseni and Allen [92] reported that the biodegradation rate of the hydrophobic compound α-pinene was reduced in the presence of a hydrophilic compound (methanol), but the methanol biodegradation rate was not affected by the presence of α-pinene at all concentrations. The removal rate of α-pinene was inversely proportional to the methanol loading rate [92]. Shoda *et al.* [122], observed a decrease in dimethyl sulfide removal in the presence of hydrogen sulfide and methanethiol during biofiltration.

If one of the VOCs in the air stream is toxic to the microorganisms, the microbial activity is reduced. Consequently, biodegradation rates of other VOCs in the presence of a toxic VOC are lower than biodegradation rates in the absence of the toxic VOC. Speiter Jr. and McLay [126], and Parvatiyar *et al.* [52] observed a decrease in the degradation of toluene from 80% to 63% at 600 ppm when tetrachloroethylene was introduced in the biofilter. When the toxicity of one VOC reduces the removal of others, a biofilter with multiple stages, each containing different types of microorganisms,

is reported as a viable solution [122]. The toxic compound is removed by a special culture in the first stages of the biofilter and the subsequent stages remove the less toxic compounds. When more than one compound is toxic to the microorganisms, the concentrations have to be reduced far below the toxic levels of the individual compounds because the toxicity effect is additive [102].

In other cases, multiple VOC components in an air stream are reported to have negligible effect on the biodegradability of an individual component [18, 158, 160]. Although literature on enhancement of biodegradation due to multiple components effect is scarce, one can not rule out such a possibility. Multiple component mixtures of VOCs and VICs might have a positive effect if one component acts as a supply of nutrients lacking in the biofilter. For instance, the removal of ammonia from a gas stream in a peat biofilter resulted in a pH increase from 4 to 8 and microbial growth, the carbon source was supplied by peat [84]. Deshusses reports that biodegradation of toluene increased from 10 to 80 $g/m^3.h$ a 100% removal, when ammonia gas was injected into the biofilter [25]. The carbon source could also be the VOC supplied through a gas phase, thus providing a possibility of removing both ammonia and the VOC during biofiltration.

2.5.5 Microorganisms

Biofiltration is achieved through microbial utilization of the VOC as a carbon, sulfur, nitrogen or energy source. Similar to the waste water treatment process, biofilters are inhabited by a mixed culture of different bacteria and fungi. The microbial species vary considerably and depend mainly on the chemical properties of the VOC. Different microorganisms differ in their ability to produce enzymes for digesting different compounds. For instance, *Pseudomonas* and *Candida* species are effective in the utilization of hydrocarbons [4, 49, 139, 140], the utilization of hydrogen sulfide is achieved by a *Thiobacillus* species [121]. Operating conditions such as pH and moisture content

also influence the microbial population and species as well. Low pH and low moisture content tend to favour fungi over bacteria. It is therefore necessary to have the right type of microorganisms and operating conditions for effective biofiltration. However for recalcitrant compounds, finding microorganisms with a high biodegradation ability might be difficult. In this case genetic improvement of microorganisms may offer a helping hand [15].

A list of microorganisms capable of degrading hydrocarbons is well documented by Atlas [4], Gibson [49] and Ratledge [113]. Atlas [4] in his review of biodegradation of petroleum hydrocarbons, reported an extensive list of different species of microorganisms rich in hydrocarbon assimilation strains. The genera of the most frequently isolated microorganisms capable of oxidizing aliphatic hydrocarbons is shown in Table 2.3. *Pseudomonas* sp. are the most reported microorganisms, and have been widely isolated from environments polluted with different types of hydrocarbon. For instance, *Pseudomonas* sp. have been isolated from soil contaminated with petroleum hydrocarbons [43], a trickle bed reactor treating aromatic hydrocarbons [128], and ground water degrading aromatic hydrocarbons [85]. *Pseudomonas* species have also been isolated from coastal sea water in which non-ionic surfactant was being biodegraded [97], and biofilters degrading benzene, toluene, ethyl benzene, and xylene [82]. Additional reports on isolation of *Pseudomonas* species may be found in the literature [35, 47, 54, 64, 88, 135, 145]. Among fungal species, *Candida* is the most commonly reported microorganism capable of degrading aliphatic and other hydrocarbons [4, 49, 21, 113, 136, 137].

The rate of biodegradation depends also on the biomass concentration in the biofilters. High biodegradation rates are achieved when the biomass concentration is high. The optimum cell concentration for maximum performance is not precisely known. However, high levels of VOC degradation have been reported in regions with cell density ranging from 10^6 to 10^8 CFU per gram of solid support [37]. Higher cell

densities have been reported in inoculated biofilter systems with nutrient addition. The number of colony forming units in a biofilter removing odour from a broiler chicken house is reported between $5x10^7$ to $3x10^{10}$ per gram of solids [103]. Kiared *et al.* [70] reported an average of 10^9 and maximum of 10^{13} CFU per gram of the solid support for biofiltration of ethanol and toluene in peat biofilters.

2.5.5.1 Effect of inoculum on biofiltration rates

Conventional biofilters depend on natural microorganisms found in compost, soil, peat or other solid media without microbial inoculation. Recent studies show that inoculation of the filter bed with an acclimatized culture, activated sludge or microorganisms isolated from contaminated environments improves biodegradation rates [70]. Shoda [119] enhanced biofiltration of sulfur-containing gases by using a mixed culture inoculum of *Thiobacillus thioparus*, *Thiobacillus*, *Xanthomonas*, and *Hyphomicrobium* species. Other reports on high biodegradation rates resulting from inoculation of the biofilters are available [18, 26, 70, 98, 112, 120]. The inoculum increases the biomass density, hence high biodegradation rates. For that reason, modern biofiltration research uses inocula to reduce biofilter acclimation time and improve overall biodegradation rates [112]. Acclimation time can now be achieved within one to two weeks if biofilters are inoculated with either activated sludge or acclimatized culture [26]. Typical sources of microorganisms for inoculum are the activated sludge and soil contaminated sites [26, 70, 82, 98, 120].

2.5.6 Nutrients

Nutrients are needed for biodegradation of hydrocarbons. The petroleum reserves have existed for millions of years without substantial biodegradation due exclusively to nutrient limitations. The limiting nutrients in the petroleum reserves include oxygen, nitrogen, phosphorus and trace elements [41]. Addition of nutrients is reported

Table 2.3: Genera of common microorganisms containing aliphatic hydrocarbons-oxidizing species

Bacteria	Yeast and Fungi
Anthrobacter	*Aspergillus*
Achromobacter	*Candida*
Acinetobacter	*Cryptococcus*
Actinomyces	*Debaryomyces*
Aeromonas	*Endomyces*
Alcaligenes	*Hansenula*
Arthrobacter	*Mycotolura*
Bacillus	*Penicillium*
Beneckea	*Phichia*
Brevibacterium	*Rhodotorula*
Corynebacterium	*Saccharomyces*
Flavobacterium	*Selenotila*
Micrococcus	*Sporidiobolus*
Micromonospora	*Sporobolomyces*
Mycobacterium	*Torulopsis*
Nocardia	*Trichosporon*
Pseudomonas	*Tricoderma*
Rhodococcus	
Spirillum	
Vibrio	

Source: [3, 4, 21, 49, 113, 150]

to increase the bioremediation of soil contaminated with petroleum fuels [9, 41]. Similarly in biofiltration biodegradation is limited by availability of nutrients. Earlier generation biofilters were operated without nutrient addition. Basic nutrients such as nitrogen, phosphorus and sulfur were supplied by the solid support. Nitrogen is reported to be a limiting factor in biofiltration. In recent years, there have been reports on addition of supplementary nutrients to improve degradation rates in biofilters [18, 53, 82, 95, 115, 154, 156, 171]. nutrient addition is also reported to improve bioremediation of petroleum hydrocarbon contaminants in a marine environment [56]. Corsi and Seed [18] reported that available nitrogen decreased from 347 to 68 mg N/kg of composted sewage sludge medium within 16 days during biofiltration of toluene.

Don [32] observed a dramatic increase in toluene degradation at a loading rate of 50 $g/m^3.h$ when nitrogen source and other supplementary nutrients were added to a compost biofilter. Morgenroth *et al.* [95], also reported improved biofiltration of hexane by addition of a nitrogen source to the biofilter. In biodegradation of petroleum hydrocarbons, addition of both nitrogen and phosphorus was found to improve biodegradation as long as the phosphorus concentration was not too high to inhibit bacterial growth [4].

It is assumed that nutrients are recycled and reused by young cells when old ones die and lyse, but the process is not 100% effective [80, 98, 99, 142]. Nitrogen is removed from the solid media by the following mechanisms: uptake of soluble nitrogen to make new biomass as the VOC is degraded; uptake of soluble nitrogen to make new biomass as the solid media is biodegraded; loss of soluble nitrogen through leaching when water is added to restore moisture; loss of nitrogen through stripping of ammonia from the biofilter; loss of nitrogen through denitrification in the case of high VOC loading in compact solid media with anoxic zones [53]. As a result, recycle efficiency cannot be 100%. Nutrients must therefore be added from time to time to restore high microbial activity.

2.5.7 Moisture Content

Moisture is an important requirement for microbial activity. Media moisture content is one the operating parameters most relevant to the biofiltration performance [143]. Optimum water content for compost and peat ranges from 45 to 60% by weight, while moisture contents of the sand and loam soil are much less for the same water activity. A moisture content below 33% (w/w) for compost and peat results in poor microbial activity while values above 60% reduce mass transfer for hydrophobic substances, clogs available pore spaces thereby increasing pressure drops, contributes to slime formation, may result in the formation of anoxic regions, and causes irreversible

structural damage to the media through washing out of small particles [98, 143, 156]. Highly porous solid media treating hydrophilic VOC may have an optimum moisture content above 60% [143]. To avoid bed drying, influent air must be humidified in a separate unit. However, the heat of reaction and air flow through the filter bed removes moisture and occasional addition of water is necessary. If the bed is allowed to dry completely, wetting of the solid medium is difficult because of the hydrophobic surface nature of compost and other natural organic materials [11, 13, 51, 116]. Large quantities of water might be added to remove non-gaseous products which can accumulate in the bed and affect microbial activity.

2.5.8 Temperature

Biofiltration, like chemical and other biological reactions is affected by temperature. It is expected that optimum biodegradation rates are achieved at the optimum temperature of the active microorganisms in biofilters. However, for economic reasons, biofilters operate within a temperature range of 5°C to 40°C depending mainly on the inlet temperature of the gas. For most biofilter processes, the optimum temperature is 30–37°C [13, 31, 99]. Wright *et al.* [156], Ebinger *et al.* [34], and Zurlinden *et al.* [171] obtained higher biodegradation rates of gasoline vapours when the biofilter temperature was 29–32°C compared to 18–22°C. When the temperature is greater than 40°C a cooling device is often necessary. Hot gases such as those from foundries and bakeries must be cooled to a lower temperature range which allows microbial activity [13]. Cooling is achieved by diluting the contaminated waste gas with ambient air, in many cases a pre-humidification system provides enough cooling [142].

Biofiltration at high temperature of up to 65°C is possible with thermophilic bacteria. Matteau and Ramsay [86, 87] performed thermophilic biofiltration of toluene at 50–60°C and obtained a very high toluene removal of up to 289 g carbon/m^3.h in a compost biofilter. However, thermophilic reactions need the right microbial strain

for a biotransformation of particular organic compound. Tripathi and Allen reported that removal of a chemical oxygen demand of bleached craft pulp mill effluent was lower at 55 and 60°C than at 35°C although long chain fatty acids removal was better [134].

Biotransformation reactions produce heat during biofiltration. A large amount of the heat of reaction is removed by the flow of air through the filter bed, allowing a temperature rise of only 2 to 4°C, and in few cases up to 10°C [142, 143]. With such a small temperature range, the biofilter can be considered to operate at the inlet gas temperature.

Reports on biofiltration at low temperatures is scarce. Although hydrocarbons degrading microorganisms have been isolated at temperatures as low as 2.5°C [133], biofiltration rates are likely to be very slow at such low temperatures. Understanding biofilter behaviour at low temperatures is important during shut down periods. When a biofilter is shut down, the temperature may decrease especially during winter seasons. It is reported that start up problems are not encountered as long as the temperature is kept above the freezing point. A feasibility study done by Lehtomäki *et al.* [78] indicates that biofilters operated at 30°C had no start up problems after shut down when the temperature dropped to 4°C. However, when the temperature drops to -4°C, a longer start up period was required [78].

2.5.9 Effect of pH

The type of microorganisms degrading the VOC are mainly bacteria. Hence, most biofilters operate between pH of 6 and 8, which favours the bacterial activity [80, 98, 99, 142]. The pH value affects the enzymatic reactions in all biochemical reactions. A study of enzymatic biodegradation of long chain aliphatic hydrocarbons (C_{12}–C_{16}) to their corresponding alcohol by Finnerty [38] indicated that, the monooxygenase enzyme system has an optimum pH value of 7.8–8.5. The optimum values for enzymes

responsible for conversion of alcohol to aldehydes and aldehydes to fatty acids varies depending on the type of enzyme. One enzyme, alcohol and aldehyde dehydrogenase with cofactor pyrroloquinoline quinone, which catalyses both reactions has an optimum pH value of 7.5 [54]. In addition to bacterial activity, biofiltration under acidic conditions is discouraged to avoid fungal growth. Filamentous fungal growth leads to biofilter clogging and high pressure drop.

Like most biological systems, pH values in biofilters, unless controlled, will decrease due to formation of acidic compounds such as acetic and humic acids from the solid medium, and biodegradation of VOCs. VOCs and VICs with high potential for decreasing pH include chlorinated, nitrogen and sulfur containing compounds such as chloroethane, dichloromethane, ammonia, amines, mercaptans and sulfur dioxide gas [36, 71, 124]. Their degradation produces hydrogen chloride, nitrates, sulfates and their acids. For instance, Smet *et al.* [124] observed decreasing pH accompanied by low biofiltration rates of methyl sulfides without addition of a neutralizing agent. Ergas *et al.* [36] reported similar observations during biodegradation of dichloromethane in compost biofilters.

Acidic conditions are not limited to the biodegradation of the above mentioned compounds. During biodegradation of hydrocarbons, fatty acids have been found to accumulate causing lower pH values [4]. Devinny [29] observed accumulation of acidic products in biofilters overloaded with ethanol. The control of pH is achieved by addition of about 10% insoluble carbonates in the form of oyster shells, lime, and marble to the solid medium.

There are cases where low pH is encouraged in biofilter systems. For instance, low pH had little effect on biofiltration of hydrogen sulfide [152]. Sulfur oxidising bacteria used to treat sulfur dioxide and hydrogen sulphide have an optimum pH way below 4. In such cases, the biofilter is operated under acidic conditions [17, 118, 119, 120, 121, 122, 123].

2.5.10 Oxygen

Oxygen is required as one of the reactants in the biological oxidation of the VOC. Oxygen is always supplied to the biofilter in excess of the stochiometric amount due to the high concentration of oxygen in the gas phase (approximately 21%) compared to the concentration of VOC which is usually up to a few thousands ppm. Under such high oxygen supply, biofiltration is an aerobic process and oxygen is a non-limiting reactant. It is reported that even when the partial pressure of oxygen is half that of oxygen in the air, degradation capacity remains the same [31]. Mohseni *et al.* [92] did not observe any change in biodegradation of α-pinene when air was replaced by pure oxygen.

However, oxygen has been reported to be a limiting reactant in biofiltration. Zarook *et al.* [165] suggests that oxygen was a limiting reactant during biofiltration of methanol under nutrient supplementation conditions in biofilters whose solid medium consisted of individual or mixtures of polyurethane foam, milled peat moss, vermiculite, and pearlite. Also, Cox *et al* [19] observed an increase in biodegradation of styrene from 63 to 91 $g/m^3.h$ when the oxygen concentration in the gas phase was increased from 20 to 40%. The biofilter was inoculated with the fungus *Exophiala jeanselmei* and operated under supplementary nutrient addition once every 2–3 weeks. The oxygen limitation reported by Zarook might have been caused by the milled solid media (milled peat moss) which is very likely to develop anoxic conditions. In addition, biodegradation of the milled peat moss under nutrient supplementation is likely to be fast, hence increasing oxygen consumption. The fungus which is highly oxidative might be the cause of the oxygen limitation in the results reported by Cox. Moreover, the growth of fungus might have reduced the pneumatic conductivity of the medium leading to anoxic zones.

2.6 Biofilter adaptation time

Biofilter adaptation time is considered to be the period required to attain a steady state performance. The adaptation time depends mainly on three factors: characteristics and complexity of the gas stream, microbial type and population, and VOC loading rate. Adaptation time can be attained in a week for easily biodegradable VOCs while it can take three weeks to two months for poorly biodegradable VOCs [98, 165]. When the bed is inoculated with microorganisms the adaptation time is considerably reduced [18, 26, 28, 32, 82, 119, 120, 121]. Sometimes the initial loading of the VOC must be reduced to allow microbes to adapt to a new substrate. The loading can then be increased to the operating value. If the starting VOC loading is as high as the operating loading, the adaptation time increases and there is a possibility that VOC removal may be completely inhibited [32]. Biofilters do not have start up problems after being shut down for several weeks. They quickly recover to the original efficiency especially if humid air is continuously supplied during shut down. Long shut down periods increase re-adaptation time [78, 83].

2.7 Biofiltration Kinetics

Few theoretical models have been developed to describe the kinetics for biofiltration of polluted air. Unlike aqueous biological reactions which can be performed at more or less the same conditions, biofiltration is a heterogeneous and more complicated system. Even solid media of the same kind such as peat or compost varies significantly in their moisture retaining capacity, microbial content and rate of decomposition depending on their age and origin. In addition to degradation of VOC, other reactions such as decomposition of solid media, and neutralization of acid products, take place in the biofilters simultaneously.

Despite the complexity of the system, biofiltration kinetic models have been developed based on the following simplifying assumptions:

1. Nutrients in the biofilm are transported by diffusion described by effective diffusivity D_e [98, 164].

2. The biofilm thickness is very small relative to the particle size and planar geometry can be assumed [98].

3. Micro-kinetics of substrate elimination is described by Monod equation [98, 159].

4. The gas flow through the filter bed is plug flow [98, 164].

5. Mass transfer resistance in the gas phase is negligible. Ottengraf [98, 100] proved this assumption by showing that the number of transfer units in the gas phase is at least 10 fold more than the number of reaction units in the biofilm.

6. At steady state the biomass population can be considered constant [98, 159, 164].

7. The biofilm forms on the exterior surface of the particle and there is no internal particle diffusion [164].

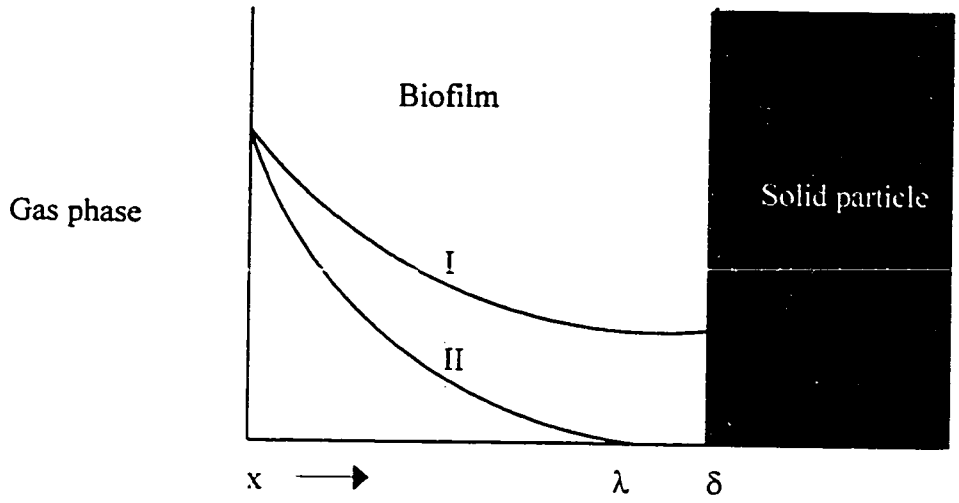


Figure 2.4: Mass transfer of VOC in the biofilter

Ottengraf [98], developed the biofilter model by modifying the catalytic bed reactor model. According to this model, mass transfer of the VOC from the gas phase to the liquid film surrounding the solid particle is illustrated in Figure 2.4. The mass balance of substrate utilization is given by:

$$D_e \frac{d^2 C_{li}}{dx^2} - r_i = 0 \tag{2.1}$$

where C_{li} is concentration of VOC inside the biofilm. C_{li} at the interface is in equilibrium with the concentration of the VOC in bulk gas, hence the first boundary conditions are:

$$x = 0, \qquad C_{li} = \frac{C_{gi}}{m_i} \tag{2.2}$$

The rate of substrate (VOC) utilization in the biofilm at a constant cell concentration X, follows the Monod equation given below.

$$r_i = \frac{dC_{li}}{dt} = \frac{\mu_m}{Y_i} \frac{C_{li}}{C_{li} + K_{si}} X \tag{2.3}$$

2.7.1 First order Kinetics

For first order kinetics, the Monod constant $K_{si} \gg C_{li}$, and Equation 2.3 approaches first order with respect to the substrate concentration and becomes:

$$r_i = \frac{dC_{li}}{dt} = \frac{\mu_m}{Y_i}\frac{C_{li}}{K_{si}}X \tag{2.4}$$

Substituting Equation 2.4 into the 2.1, the following differential equation is obtained.

$$D_e\frac{d^2C_{li}}{dx^2} - \frac{\mu_m}{Y_i}\frac{C_{li}}{K_{si}}X = 0 \tag{2.5}$$

In order for the reaction to follow first order kinetics, the biofilm must be fully active (curve I) hence, the second boundary condition is:

$$x = \delta, \qquad \frac{C_{li}}{dx} = 0 \tag{2.6}$$

At a constant biomass concentration, the solution for the Differential Equation 2.5 is:

$$\frac{C_{li}}{\frac{C_{gi}}{m_i}} = \frac{\cosh\{\phi_1(1-\sigma)\}}{\cosh\phi_1} \tag{2.7}$$

where $\phi_1 = \delta\sqrt{\frac{k_1}{D_e}}$ is the Thiele modulus and $\sigma = x/\delta$ is the dimensionless length coordinate in the biolayer. The first order reaction constant k_1 is given by:

$$k_1 = \frac{\mu_m}{Y_i K_{si}}X \tag{2.8}$$

From a differential mass balance, the gas phase concentration C_{gi} can be expressed as a function of biofilter height h according to:

$$-U_g\frac{dC_{gi}}{dh} = N.a \tag{2.9}$$

where N (in mol/m^2.s) is the substrate flux into the biofilm. From this equation it follows for N:

$$N = -D_e \left(\frac{dC_{li}}{dx} \right)_{x=0} = \frac{D_e C_{gi}}{\delta m_i} \phi_1 \tanh \phi_1 \tag{2.10}$$

The VOC exit concentration C_{ge} in the gas phase is given by:

$$\frac{C_{ge}}{C_{go}} = \exp \left(-\frac{HaD_e}{m_i U_g \delta} \phi_1 \tanh \phi_1 \right) = \exp \left(-\frac{HK_1}{m_i U_g} \right) \tag{2.11}$$

where the apparent first order constant is given by:

$$K_1 = \frac{a D_e}{\delta} \phi_1 \tanh \phi_1 \tag{2.12}$$

2.7.2 Zero order Kinetics

For zero order kinetics, the Monod constant $K_{si} \ll C_{li}$ and the differential Equation 2.3 approaches zero order with respect to the substrate concentration and becomes:

$$r_i = \frac{dC_{li}}{dt} = \frac{\mu_m}{Y_i} X \tag{2.13}$$

Substituting Equation 2.13 into 2.1, the following differential equation is obtained:

$$D_e \frac{d^2 C_{li}}{dx^2} - \frac{\mu_m}{Y_i} X = 0 \tag{2.14}$$

Zero order kinetics may be limited/controlled by either the reaction or diffusion rate. The kinetics are said to be under reaction limited conditions when the biofilm is fully active and there is no diffusion limitation. When there is a diffusion limitation and the biofilm is not fully active, then the kinetics are said to be under diffusion limited conditions.

2.7.2.1 Reaction limitation

When reaction is limiting and the biomass density is assumed to be constant, Equation 2.14 gives the following final solution:

$$\frac{C_{ge}}{C_{go}} = 1 - \frac{HK_0}{U_g C_{go}} \tag{2.15}$$

2.7.2.2 Diffusion limitation

When diffusion is limiting and the biomass density is assumed to be constant, the solution to Equation 2.14 becomes:

$$\frac{C_{ge}}{C_{go}} = \left(1 - \frac{H}{U_g}\sqrt{\frac{K_0 D_e a}{2m_i C_{go}\delta}}\right)^2 \tag{2.16}$$

where K_0 is the apparent zero order reaction rate constant expressed as:

$$K_0 = k_0 \cdot a \cdot \delta \tag{2.17}$$

and

$$k_0 = \frac{\mu_m}{Y_i} X \tag{2.18}$$

The approach of Ottengraf [98] to kinetic modelling of biofilters was supported by Lith [142], Zilli [169] and Deshusses [24]. Ottengraf [98] indicated that most VOCs are removed through zero-order kinetics. Bohn [13] and Hodge *et al.* [57] suggested a first-order kinetics removal. Kordono and Allen [75] reported that biofiltration of n-butane followed first order kinetics at low concentrations (below 20 ppm) and zero order, diffusion-limited kinetics at higher concentration (20–80 ppm). Yang and Allen [158, 159] developed a biofilter model from another approach, similar to the homogeneous plug flow system. They developed zero, first and fractional order reaction kinetic models. The final solutions of the differential equations for the zero

and first order kinetics are similar to those developed by Ottengraf. By varying the concentration of the substrate (hydrogen sulfide) Yang and Allen [158, 159] were able to run the biofilter under all three scenarios.

Other models include transient behaviour of biofilters developed by Deshusses *et al.* [27, 28], Zarook *et al.* [167, 166] and Baltiz *et al.* [164, 165]. In these models interactive microbial kinetics, which are a modified Monod kinetics for two or more substrates were used and oxygen was considered as a limiting reactant in addition to the VOC. Zarook and Shaikh [166] reported that the results calculated using the interactive model were comparable to those calculated using zero and first order models developed by Ottengraf [98]. The use of a kinetic expression resulting from shake flask experiments by Zarook and Shaikh [166] in the comparison of different models may not represent the actual situation in the biofilter to justify that oxygen is a limiting reactant.

2.8 Pressure Drop

Pressure drop is the main cause of energy consumption in biofilters. It depends on flow properties of air through the bed, moisture content and physical properties of the solid medium. For a fixed bed containing uniform spherical particles, pressure drop can be estimated by the following Ergun Equation:

$$\frac{dP}{dh} = 150\frac{(1-\varepsilon)^2\mu^* U_g}{\varepsilon^3 d_p^2} + 1.75\frac{(1-\varepsilon)\rho U_g^2}{\varepsilon^3 d_p} \tag{2.19}$$

Particles with an average size above 3 mm are recommended for relatively low pressure drop [80, 98, 99]. For conventional biofilters containing materials such as compost and peat, pressure drop changes with time due to aging. Aging is accompanied by bed shrinkage, cracking and cluster formation. Problems associated with aging have been delayed by addition of inert materials such as polystyrene spheres to improve mechanical structure of the solid medium. High moisture content reduces porosity causing an increase in pressure drop [98]. For compost filters, pressure drop ranges between 100 and 700 Pa [37, 98]. Typical power consumption in biofilter ranges from 1.8 - 2.8 kWh/1000 m^3 [12, 80, 98].

2.9 Concluding remarks

Biofiltration is a reliable and cost effective method for controlling the VOCs. Good biofiltration media must allow microorganisms responsible for biodegradation to flourish. Moisture retention capacity, good immobilization capacity, good pneumatic conductivity, and stable pH are necessary properties of a good medium. The peat and compost, with addition of inert and carbonate materials, have remained the major biofiltration media reported in literature. An efficient biofiltration process requires the right type of microbial species, which according to recent studies come from inoculum of acclimatized culture or activated sludge. Good microbial density of more that 10^8 CFU per gram of solid medium is required for high biofiltration rates. Average operating conditions, especially pH value near 7.0 and moisture content of approximately 60% by weight, must be maintained by integrating carbonate materials into the solid media and periodic addition of water, respectively. Operating temperature depends on the inlet temperature of the polluted air. Cooling is achieved by mixing the inlet air with ambient cooler air. Addition of supplementary nutrients to biofilters have been shown to improve the biodegradation rates.

Pressure drop is a major component associated to the biofilter operating costs. High pressure drop across the biofilter has been reduced by using media with particle size of 3–5 mm and adding inert material, especially pearlite and styrene spheres. The biofiltration kinetics can be modelled in a similar manner to the packed bed reactors. The kinetics may follow zero or first order depending on the VOC concentration and VOC diffusion properties.

Biofiltration technology suffers from low biodegradation rates of VOCs leading to high biofilter volumes and poor removal of persistent compounds at high concentrations. In addition the organic solid media used as packings have a disadvantage of aging, potentially leading to channelling, poor biofiltration rates and high pressure drop. Periodic replacement of the aging media raises the running costs.

The technology needs improvements to increase biofilter performances. More studies are needed to look into ways to increase the biodegradation rates, and to delay or completely eliminate the aging problem by using inorganic or synthetic media and addition of supplementary nutrients, respectively. It is the purpose of this research to look into the improvement and optimization of reaction rates by nutrient supplementation. Given the small amount of literature available on biofiltration of aliphatic hydrocarbon, hexane was chosen as the model compound to represent moderately biodegradable compounds.

Chapter 3

Aqueous culture studies

An aqueous suspension mixed culture study was conducted in shake flasks containing mineral medium and hexane as the sole carbon source. The main objectives of this study were to acclimatize the mixed culture for inoculating the biofilters and to determine the growth characteristics of the microorganisms involved in the biodegradation of hexane. The microbial growth rate at different temperatures and changes of pH with time were studied to understand the conditions affecting the activity of the microorganisms during biodegradation of hexane as the sole carbon source.

3.1 Materials and methods

3.1.1 Acclimatization of microorganisms

According to the recent literature, inoculation of biofilters with acclimatized culture reduces biofilter adaptation time and improves overall biodegradation rates as detailed in section 2.5.5. Microorganisms used in this study were therefore acclimatized to hexane in liquid culture before being used to inoculate the biofilters. The source of the microorganisms was an activated sludge obtained from the City of Waterloo Municipal Waste Water Treatment Plant. The activated sludge was examined under

a light microscope for evidence of viable microorganisms. The hexane biodegradation potential of the microorganisms was tested by adding 5 mL of hexane to 150 mL of the activated sludge in a 500 mL Erlenmeyer flask. The flask was incubated at room temperature ($\approx$ 23°C) on a shaker at 150 rpm. Due to the low solubility of hexane (0.014% w/w at 15°C [105]), the added amount appeared as a thin layer on top of the activated sludge surface. Added hexane was visually monitored until it disappeared, at which point more hexane was added in 5 mL volumes.

The growth of microorganisms was monitored with a light microscope by counting bacteria in a Petroff-Hauser Counting Chamber. After 72 hours, the activated sludge broth was subcultured into an mineral medium containing hexane as the only source of carbon. The mineral medium was made from the Basal Inorganic Medium with composition shown in Table 3.1. Each 1 L of the basal medium was complemented with 2 mL of trace elements solution, and its pH was adjusted to 7 using sodium hydroxide solution. This medium was similar to the media used by Weckhuysen *et al.* [154], Ergas *et al.* [37], Apel *et al.* [2], and Mallakin [82] and reported by Holt [59].

The subculturing was performed by inoculating 150 mL of the fresh mineral medium with 15 mL of the culture that had been growing for two days, followed by addition of 5 mL of hexane, in a 500 mL Erlenmeyer flask. The Erlenmeyer flask was incubated at room temperature on a shaker at 150 rpm. More hexane was added after 24 hours and the broth was subcultured every other day until the suspended solids from the activated sludge were diluted out. Once the suspended solids had been eliminated, the microbial growth was monitored by optical density at 600 nm in addition to the microscopic examination. The acclimatized culture was stored as 20 mL aliquots in 25 mL bottles at 4 °C for later use as an inoculum. The stock was subcultured every six months as recommended by Gherna [48] for bacterial cultures which had been stored at 5–8°C.

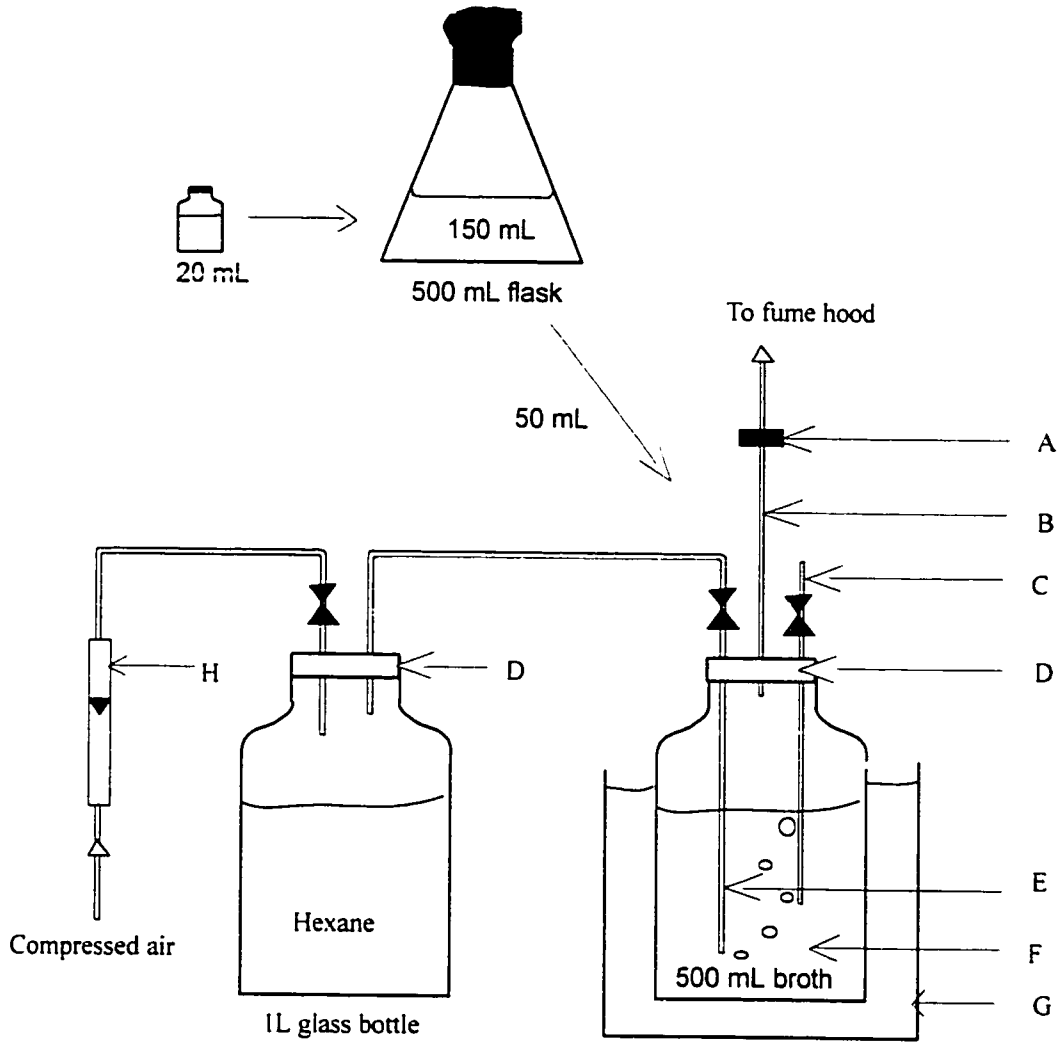
Table 3.1: Composition of the mineral medium

Component	Quantity
Basal Medium	
K_2HPO_4	1.00 g
KH_2PO_4	0.10 g
$(NH_4)_2SO_4$	1.00 g
$MgCl_2.6H_2O$	0.50 g
$FeSO_4.7H_2O$	0.02 g
Deionized water	1.00 L
Trace element solution	2 mL
Trace Elements	
Trace element solution was prepared by dissolving into 1L, 200 mg of each of the following $CaCl_2$, $MnSO_4.5H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, and $(NH_4)_6Mo_7O_{24}.4H_2O$	

3.1.2 Growth characteristics in free suspension mixed culture

The stock culture was used for studying growth characteristics in a free suspension culture. The 20 mL stock culture was allowed to come to room temperature and then used to inoculate 150 mL mineral medium containing 5 mL of hexane in a 500 mL Erlenmeyer flask. After 24 hours of incubation at room temperature on a shaker (150 rpm), 50 mL of the growing culture was used to inoculate 500 mL of the mineral medium in a 1L flask. In this flask, hexane was added in a vapour form as shown in Figure 3.1. The growth of the mixed culture was conducted at controlled temperatures of 25, 30 and 35°C using a water bath.

Compressed air was passed through a vapour head space in a 1L bottle containing hexane. On leaving the bottle, the air carried hexane vapour with it to the fermentation broth. The air-hexane vapour mixture was bubbled through the fermentation broth throughout the experiment. The flow of air-hexane vapour mixture through the fermentation broth was 0.2 v/v.min (40 mL/min). This setup had the following



KEY:
A: Air filter
B: Air outlet
C: Broth sampling tube
D: Rubber stop
E: Air and hexane vapor inlet
F: Fermentation broth
G: Water bath
H: Flow meter

Units: mm Not to scale	Project: Biofiltration Designed and Drawn by: O. Kibazohi

Figure 3.1: Aqueous fermentation: experimental setup

advantages;

- The system had a close similarity to a biofiltration system where the VOC (hexane) is transferred from a gas phase to the liquid phase.
- It was possible to continuously supply hexane into the fermentation broth to keep the hexane concentration as high as possible (relative to it maximum solubility of 0.014% (w/w) at 15°C [105]) in order to reduce the possibility of mass transfer limitation arising from a hexane concentration gradient.
- It eliminated the use of liquid hexane in the fermentation broth which made biomass estimation difficult. In the presence of liquid hexane, micelles containing most of the microbial cells were formed on the surface of the fermentation broth, leaving the lower broth layer with reduced microbial density.
- The setup ensured a continuous supply of oxygen in order to avoid oxygen limitation if shake flask experiment was to be used [81].

3.1.3 Analysis

3.1.3.1 Cell dry weight

A 5 mL sample of fermentation broth was withdrawn with a 10 mL plastic syringe. The sample was filtered through a dried tared 0.2 μm filter paper. The filter paper containing cells was placed in a tared aluminum dish and dried to constant weight for 48 h in an oven at 100°C. The sample was stored in a desiccator for at least 20 minutes, after which dry weight was recorded. The cell dry weight concentration was calculated from the following equation:

$$X\,(g/L) = \frac{w_T - (w_D + w_{FP})}{5(mL)} \cdot 1000(mL) \tag{3.1}$$

where X is the cell concentration (g/ L), w_T is total weight of filter paper, dry cells and weighing dish, w_D is the weight of the empty dish and w_{FP} is the weight of the dry filter paper.

3.1.3.2 Optical density

Approximately 5 mL of fermentation broth was withdrawn with a 10 mL plastic syringe and placed in a cuvette. The sample was gently mixed with a vortex for 30–40 seconds and optical density measured with a spectrophotometer (PYE UNICAM SP6-550 UV/VIS) at 600 nm. The cell density was evaluated from the calibration curve between the optical density and dry cell weight (Figure D.1). The spectrophotometer was set to zero absorbance using the mineral medium (as a blank) each time before taking the measurement.

3.1.3.3 pH

Evaluation of pH was done using pH meter (ORION research digital pH/mV meter 611). To reduce over sampling of the fermentation broth, the 5 mL of a sample used for optical density was transferred into a larger 20 mL test tube and the pH measured. The pH electrode was calibrated to the required slope between pH 4 and 7 with standards once a day before taking the measurements [155].

3.2 Results and discussion

3.2.1 Acclimatization of mixed culture

Examination of the activated sludge under a light microscope revealed the presence of bacteria, yeast and algae. When hexane was added, microscopic examination after 6, 12, 24, and 48 hours showed a significant increase in the bacterial population. After 48 hours, visual observation confirmed that a thin layer of hexane on top of the activated sludge broth had completely disappeared. The next 5 mL of hexane disappeared within 24 hours. Since the microorganism population was increasing, the disappearance of the hexane layer may be attributed in part to microbial degradation.

After subculturing microorganisms in the mineral medium, microorganisms continued to exhibit growth. Four subsequent subculturings diluted out most of the suspended solid originating from the activated sludge. Growth characteristics of this culture are detailed in the next section 3.2.2. It can be concluded that microorganisms utilized hexane in both activated sludge and mineral medium such that within a week a fully acclimatized culture was available. In addition, the microorganisms were able to utilize hexane as the sole carbon source in a mineral medium.

3.2.2 Growth characteristics in a free suspension mixed culture

The growth pattern of the mixed culture under a continuous supply of hexane vapour is shown in Figure 3.2. At both 25°C and 30°C the culture showed a lag phase of approximately 10 hours followed by 30 hours of an exponential growth phase. The growth entered a stationary phase after approximately 40 hours of fermentation. The maximum dry weight of the biomass attained was 1.52 gL^{-1}. The value was obtained as a difference of total biomass (1.6 g/L) and the average initial biomass concentration

(0.08 g/L). A simple mass balance indicates that the nitrogen source added to the medium was 1 g/L as $(NH_4)_2SO_4$, which is equivalent to 0.212 g/L of nitrogen. If all nitrogen was assimilated, dry cell weight would have contained 13.9% nitrogen which corresponds to an apparent cell yield of 7.17 (w/w). The total nitrogen content of the dry cells measured at the end of fermentation by the Kjeldahl method was 12.6% (w/w). This amount is equivalent to biomass yield of 7.93 (w/w) based on nitrogen. The difference was likely caused by ammonia lost due to bubbling of air through the medium. According to the literature, bacterial biomass contains 12% (w/w) nitrogen [108]. Some bacteria such as *E. coli* are reported to contain 14% nitrogen [7]. The percentage nitrogen in a dry cell is 12.4% based on a common molecular formula for microbial cell of $C_5H_7O_2N$. The value observed in this work is therefore in good agreement with the reported literature values. Since oxygen, carbon, phosphorus and sulfur were supplied in excess amounts, one might suggest that nitrogen was a limiting reactant, at least towards the end of growth phase.

The exponential phase was accompanied by a pH decrease from 7 to 4. The trend indicates a formation of acidic end products. Literature reports an accumulation of organic acids such as acetic acid during biodegradation of petroleum hydrocarbons [4, 29]. More details on acid formation during biodegradation of hydrocarbons is available in section 2.3.

The microbial growth was observed at 25°C and 30°C, but there was no measurable growth at 35°C by either optical density or cell dry weight method (Figure 3.2). The maximum growth rates (μ_{max}) and Monod constants (K_S) at 25°C and 30°C were evaluated using the following modified Monod equation:

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_s}{\mu_{max}} \cdot \frac{1}{S_N} \tag{3.2}$$

where μ is given by:

$$\mu = \frac{ln\left(\frac{X}{X_0}\right)}{t} \tag{3.3}$$

and

$$S_N = S_{N0} - \frac{1}{Y} \cdot (X - X_0) \tag{3.4}$$

where X_0 and S_{N0} are the cell density (dry weight) and nitrogen concentration at time $t = 0$, X and S_N are the cell density (dry weight) and nitrogen concentration at time t. Y is a cell yield coefficient based on nitrogen and μ is a specific growth rate.

Table 3.2: Maximum growth rate μ_{max}

Temperature (°C)	25	30	35
μ_{max} [h^{-1}]	0.15	0.17	0
K_S [g/L]	0.038	0.04	-

The maximum growth rates and Monod constants were obtained from Figure 3.3 plotted using equation 3.2. Table 3.2 show that maximum growth rates were 0.181 h^{-1} at 25°C and 0.177 h^{-1} at 30°C and 0.0 h^{-1} at 35°C. The corresponding Monod constants were 0.038 and 0.04 g/L. At 95% confidence interval there was no significant difference between the values at 25°C and 30°C as shown in Figure 3.3. The temperature range is in agreement with the optimum temperature (25–30°C) for *Pseudomonas* sp. [111] which was identified (Chapter 4) as the only species of bacteria capable of growing on hexane as the sole carbon source. The maximum growth rates were slightly higher than those obtained by other researchers. Pol *et al.* [109] obtained a maximum growth rate of 0.14 h^{-1} when *Hyphomicrobium* sp. was grown on methanol as the only carbon source at 30°C. Ottengraf [98] reports maximum growth rates with values between 0.025–0.1 h^{-1} (temperature not mentioned) when the biomass was grown on at least one of the following; toluene, ethyl acetate butyl acetate or butanol in shake flasks. The differences in maximum growth rates may

be accounted for by the difference in carbon source, toxicity of carbon source to microorganisms, variation in mixed culture as well as the experimental conditions.

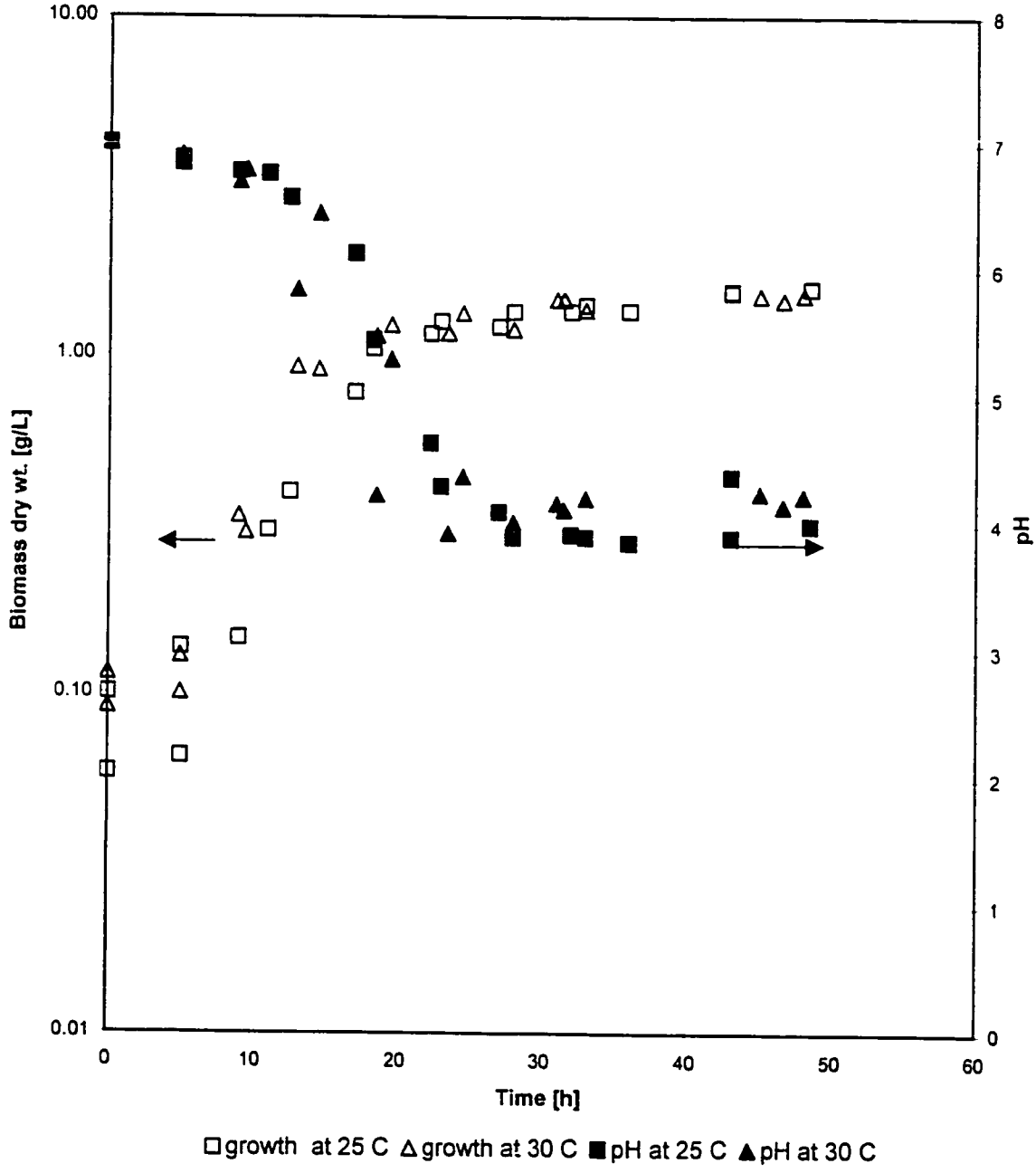


Figure 3.2: Growth curves in aqueous suspension (hexane as the only carbon source)

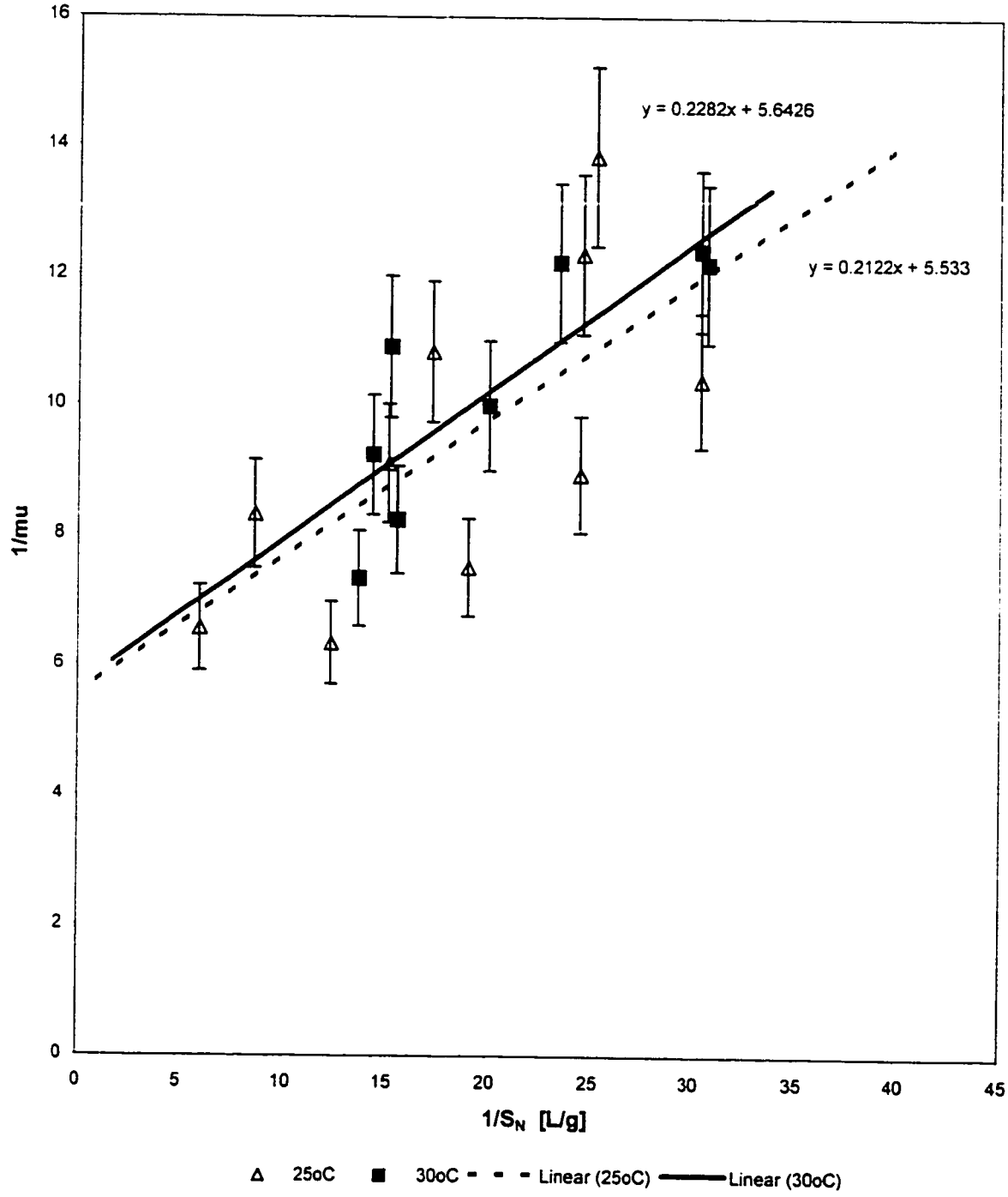


Figure 3.3: Evaluation of growth rate parameters

Chapter 4

Isolation and Identification of Microorganisms

The microorganisms involved in the biodegradation of hexane in both biofilters and the acclimatized culture were isolated and identified to determine their genera. The isolates were also grown in a special medium containing hexane as the only source of carbon to determine which microorganism could utilize hexane as the sole carbon source.

4.1 Materials and methods

4.1.1 Isolation of microorganisms

Microbial identification started with isolation of microbes into pure cultures. Samples of solid media were withdrawn from the columns through solid sampling ports. Approximately 1g of each sample was added into a test tube containing 9 mL of sterile saline solution. The biomass was released into the saline, by grinding the solids on the test tube wall with a sterile spatula until a fine slurry was formed. The suspension

was thoroughly mixed with a vortex mixer to detach cells from the solids and then serially diluted with fresh saline solution to give a final concentration of 10^{-9} of the original slurry. In a similar manner, 1 mL samples from the aqueous fermentation broth of acclimatized culture were diluted with saline.

Samples that had been diluted to 10^{-9} to 10^{-7} of initial slurry/broth were cultured on agar plates. A volume of 150 μL from each sample was spread on an agar plate. Nutrient Agar (NA), and Malt Extract Agar (MEA) were used as non-enrichment media to allow for the growth of different types of bacteria and fungi, respectively. The inoculated plates were incubated for 48 h at 30°C. Single colonies with different morphology and pigmentation were isolated and cultured on new agar plates and incubated at 30°C for 48 hours. The procedure was repeated until each isolate was pure with the same physical morphology and pigmentation. Visual observation was complemented by examining the cells under the light microscope to ensure the culture was comprised of the same type of cells with the same shape and size.

4.1.2 Identification of microorganisms

4.1.2.1 Bacteria

bacterial species were identified using the Biolog automated microbial identification system supplied by Biolog Inc. (Hayward, California). The major component of the Biolog method is a 96-microwells panel. All the microwells (except one used as a control) contain different substrates (carbon sources) which are utilized by bacteria and tetrazolium dyes which change in colour as they are reduced in presence of respiring bacteria. The Biolog system is fast and easy to use, and its accuracy in identifying bacteria is satisfactory (greater than 86%) [45, 73, 144].

Each pure culture of bacteria from nutrient agar was gram stained to identify its gram reaction [104, 111, 117]. The cultures were then grown on two special agars, R2A and BUGM for 24 h, then harvested, diluted to the required concentration, and

inoculated into microwells of the Biolog panel. The R2A is a low-nutrient growth medium and one of the solid media used in Biolog test and the BUGM is a Biolog Universal Growth Medium for both gram negative and gram positive bacteria [6]. Better results are obtained when the bacteria are grown on the above mentioned agars prior to inoculation of the Biolog panel. The Biolog plates were incubated at 30°C and the colour change patterns of the Biolog panels were entered into a computer after 12 and 24 hours for positive identification of bacteria. A database of environmental bacterial isolates was used in the identification process. Biolog identifications were accepted as correct if the similarity index of the genus and species name was 0.500 or greater at 24 h. [45, 73, 94, 104, 144]. Although the Biolog method can be used to identify both bacteria and yeast, the yeast database was unavailable. The detailed procedure of the Biolog method is described in Appendix C.

4.1.2.2 Fungi

Each pure culture of fungus from potato dextrose agar was examined under a light microscope to determine the physical characteristics. The physiological characteristics of interest were the hyphae morphology, mycelium, fruiting body, spores and colony colour. Care was taken to avoid damaging physical features during morphological examination. Observed physiological characteristics were compared to pictures and sketches in an atlas of known fungi [149] to determine fungal family genera.

4.1.3 Aqueous fermentation of isolates

Individual bacterial and fungal isolates were used to inoculate an artificial aqueous medium containing hexane as the only source of carbon to check their growth characteristics. The isolates were first grown on agar plates for 48 h, then removed from agar plates using wet sterile cotton swabs. The biomass was then shaken off the swab into 20 mL of artificial media until the solution was milky. The resulting mixture was

used to inoculate 200 mL of the artificial medium in 500 mL flasks. The composition of the artificial medium is described on Table 3.1 on page 51. The flasks were incubated at 30°C and the biomass growth was monitored by both dry weight and optical density methods, described in sections 3.1.3.1 and 3.1.3.2.

4.2 Results and discussion

4.2.1 Microbial isolation and identification

4.2.1.1 Bacteria

A total of eight different genera of bacteria were isolated from the biofiltration columns. Five of them were positively identified using the computer aided Biolog method. A bacterium is said to be positively identified when the percentage of identification certainty is above 50%. The identified bacteria are *Pseudomonas corrugata*, *Bacillus brevis*, *Micrococcus diversus*, *Acinetobacter genospicies* 15 and *Corynebacterium jeikeium A*. *Pseudomonas corrugata* was the most abundant bacteria in all samples. A sample of Biolog identification is shown in Figure C.1, Appendix C.

4.2.1.2 Yeast and Fungi

A total of nine types of yeast and fungi were isolated from the biofiltration columns. Two of them were filamentous fungi and when their physiological properties were compared to the sketches of the known fungi [149], their genera were identified as *Aspergillus* sp. and *Penicillium* sp.

A different study was conducted to identify bacteria, yeast and fungi as well as their distribution in the same packed columns using physiological tests [162]. The generic names of bacteria and yeast and fungi identified by both Biolog and physiological tests are listed in Table 4.1.

Most of the microorganisms isolated from the medium and biofilters have been reported elsewhere in the literature. Atlas [4] reported an extensive number of different petroleum-oil degrading microorganisms that had been isolated from aquatic and contaminated soil environments. Britton [49] compiled a list of microorganism capable of oxidizing hydrocarbons. The most frequently isolated microorganisms capable of oxidizing aliphatic hydrocarbons reported in literature are listed in Table 2.3. Among

Table 4.1: Genera of microorganisms isolates from the biofilters degrading hexane

Bacteria	Yeast and Fungi
Pseudomonas sp.	*Candida* sp.
Bacillus sp.	*Phichia* sp.
Clostridium sp.	*Rhodotorula* sp.
Corynebacterium sp.	*Fusarium* sp.
Rhodococcus sp.	*Penicillium* sp.
Staphylococcus sp.	*Aspergillus* sp.
Acinetobacter sp.	*Mucor* sp.
Micrococcus sp.	*Tricoderma* sp.
	Polypaecium sp.

16 microorganisms isolated in this study, 11 are listed in Table 2.3. Our isolates therefore, agree well with the general population of known isolated microorganisms capable of oxidizing aliphatic hydrocarbons.

Pseudomonas sp. is the most reported microorganism in the biodegradation literature and most widely isolated from hydrocarbon polluted environments. In yeast and fungi, *Candida* sp. is the most reported yeast in biodegradation of aliphatic hydrocarbons. The isolation of the two microorganisms in this study is in agreement with a wide number of published reports which indicate that the two microorganisms are capable of biodegrading a wide range of compounds including aliphatic and aromatic hydrocarbons in different environments [2, 4, 35, 46, 47, 49, 54, 64, 72, 88, 113, 135, 136, 137, 145, 153, 163].

The distribution of microorganisms is illustrated in Figure 4.1. The microorganisms were found throughout the biofiltration columns. All types of bacteria were found in the bottom, middle and top sections of all columns and the nature of the solid medium did not seem to affect the distribution of bacteria. Yeast and fungi were also evenly distributed throughout individual columns. There was a difference in distribution between columns of different solid media. Yeast and fungal species of *Mucor, Tricoderma, Fusarium* and *Polypaecilum* were absent in a column containing pearlite but present in a column containing a mixture of 50% peat and 50% pearlite

(v/v). It is assumed that the variation in micro-environment due to the presence of peat might be a contributing factor. For instance, the pH in columns where peat was a component of the solid media was always lower than that in the column containing pearlite alone. Also peat, which contains lignin, chitin and cellulose [44], is a substrate for some of the microorganisms capable of hydrocarbon degradation. Such microorganisms include *Tricoderma, Aspergillus, Rhodotorula, Bacillus, Micrococcus, Candida, Mucor, Clostridium, and Penicillium* species [50, 62].

4.2.2 Growth of microorganisms on hexane as the sole carbon source

When the individual isolates were grown in suspension culture shake flasks containing hexane as the sole carbon source, only one species of bacteria, *Pseudomonas corrugata* exhibited growth characteristics. Significant growth was not detected for the other bacteria, yeast and fungi. From this observation, *Pseudomonas corrugata* was identified as the only species capable of degrading hexane at the saturation concentration (in water) in the absence of other carbon sources. Other microorganisms were not able to utilize hexane as the only carbon source for two main reasons. First, the hexane and other aliphatic hydrocarbons with chain length below C_9 are considered toxic to microorganisms when present at high concentrations [4, 5, 49]. The second reason is the lack of cometabolite. All microorganisms listed in Table 4.1 are not necessarily capable of assimilating hexane as the sole carbon source. Some of them can only assimilate hexane in the presence of another carbon source. Yet others may not oxidize hexane at all but are capable of oxidizing intermediates of hexane biodegradation such as alcohol, ketones and organic acids. Cometabolism is reported by Britton [49] in a review of biodegradation of aliphatic hydrocarbons. For example, *Pseudomonas methanica* growing on methane could oxidize ethane, propane, and butane to corresponding alcohols, aldehydes and acids. Yet none of them could serve as

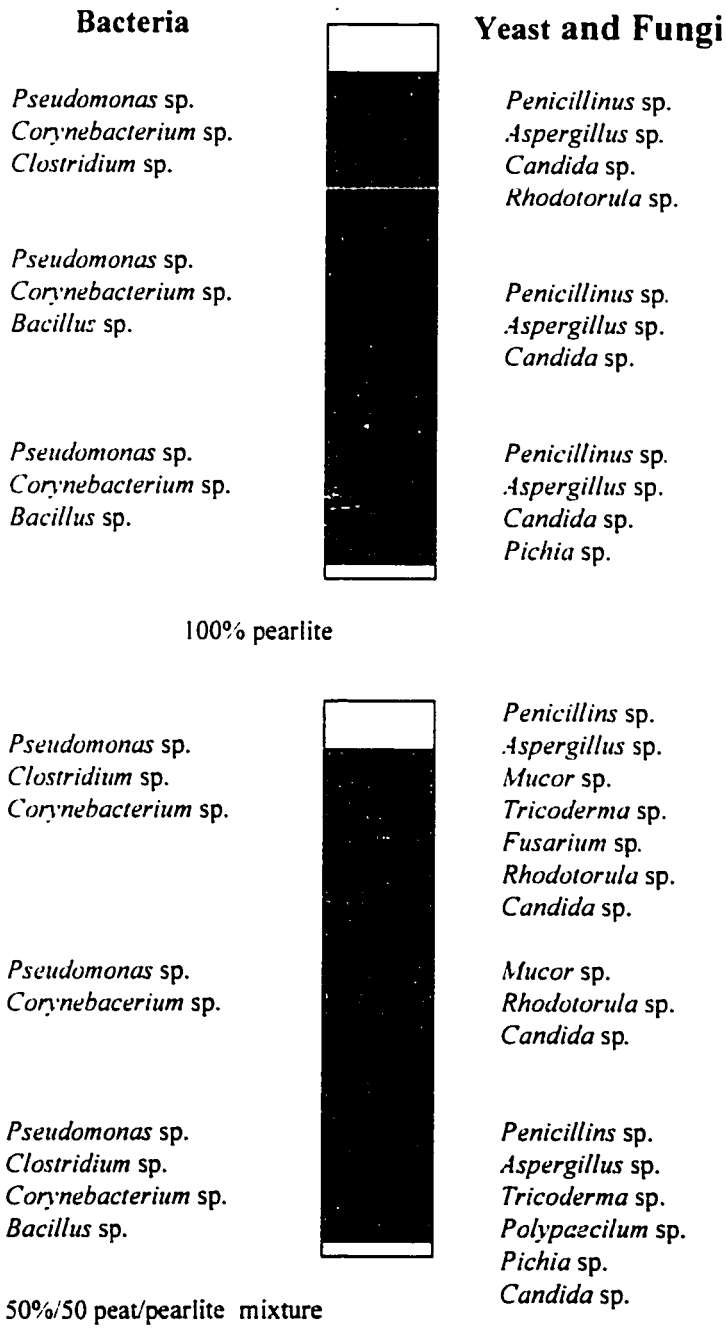


Figure 4.1: Distribution of microorganisms in the packed columns

a growth substrate. *Bacillus* species could not grow on C_{14} to C_{18} but a few of them oxidized alkanes in the presence of other growth substrates [49]. It can therefore be hypothesized that toxicity and a lack of cometabolite were two major known reasons for the inhibition of cell growth other than those of *Pseudomonas corrugata*. The growth of *Pseudomonas corrugata* under saturated concentration of hexane indicates that, biofiltration of hexane should be performed without experiencing hexane toxicity in the presence of *Pseudomonas* sp. considering the fact that hexane will be introduced into the biofilters at a concentration far below a saturation point.

Chapter 5

Biofiltration of hexane

Biofiltration of hexane in the vapour phase was conducted to study the effects of the solid media (peat and pearlite) on hexane (as a model VOC) removal rates under nutrient supplementation conditions. The effect of nitrogen content of the solid medium on hexane removal rates was investigated to determine its importance in biofiltration processes. The pH and pressure drop changes with time were monitored and correlated to the changes in biofiltration removal rates. The experimental procedure and equipment specifications are detailed below.

5.1 Material and methods

5.1.1 Biofiltration procedure

The biofiltration process started by evaporating and mixing hexane with compressed air to obtained the desired hexane-air concentration, followed by biological removal of hexane in the biofilters (Figure 5.1). Compressed air was passed through a humidification column and a hexane evaporation unit connected in parallel. The air-hexane vapour mixture from the evaporation unit was mixed with humid air from the humidification column. The hexane concentration in the final gas mixture was controlled

by regulating the quantity of air flow through the evaporation unit.

The final air-hexane mixture was passed through packed columns in which hexane was biologically removed by immobilized microorganisms. The quantities of air flowing through the columns were regulated by flow meters placed before the inlets of the biofiltration columns. Treated air was sent to the fume hood and the water draining from the columns was stored in 1 L bottles connected to the columns and later recycled to their respective columns. At the end of experimental run, waste water was pumped to a waste water storage container where it was later autoclaved and disposed of. The biofiltration process was performed at room temperature which varied between 22–24°C.

The study investigated the performance of three solid media formulations, 100% peat, 100% pearlite and their 50% (v/v) mixture to determine which formulation offers the highest removal, low pressure drop across the column and long-term operational stability. Further, the study was conducted at two levels of nutrient supplementation to assess the benefits and determine the quantity and frequency of nutrient addition. In the first scenario (high nitrogen supplementation), nutrient solution was added to the columns weekly. In the second scenario, (low nitrogen supplementation) nutrient solution was added only when biofiltration removal efficiencies fell below 50%.

At high nitrogen supplementation, 1 kg nitrogen per m^3 of solid media was added to the biofilters in the first and second weeks. In subsequent weeks nitrogen addition was decreased to 289 g/m^3 to reduce excessive biomass growth. Two solid media formulations were used: pearlite and an even 50:50% (v/v) mixture of both peat and pearlite. The experiment was conducted at gas flow rates of 20 $m^3/m^2.h$ for the first 14 days, then changed to 100, 30 and then 20 $m^3/m^2.h$ after 14, 24 and 46 days, respectively. Hexane inlet concentrations during this experimental run were between 100 and 800 ppm. Important parameters investigated were the performance of biofilters at high nitrogen content supplementation, and the effect of air flow rates

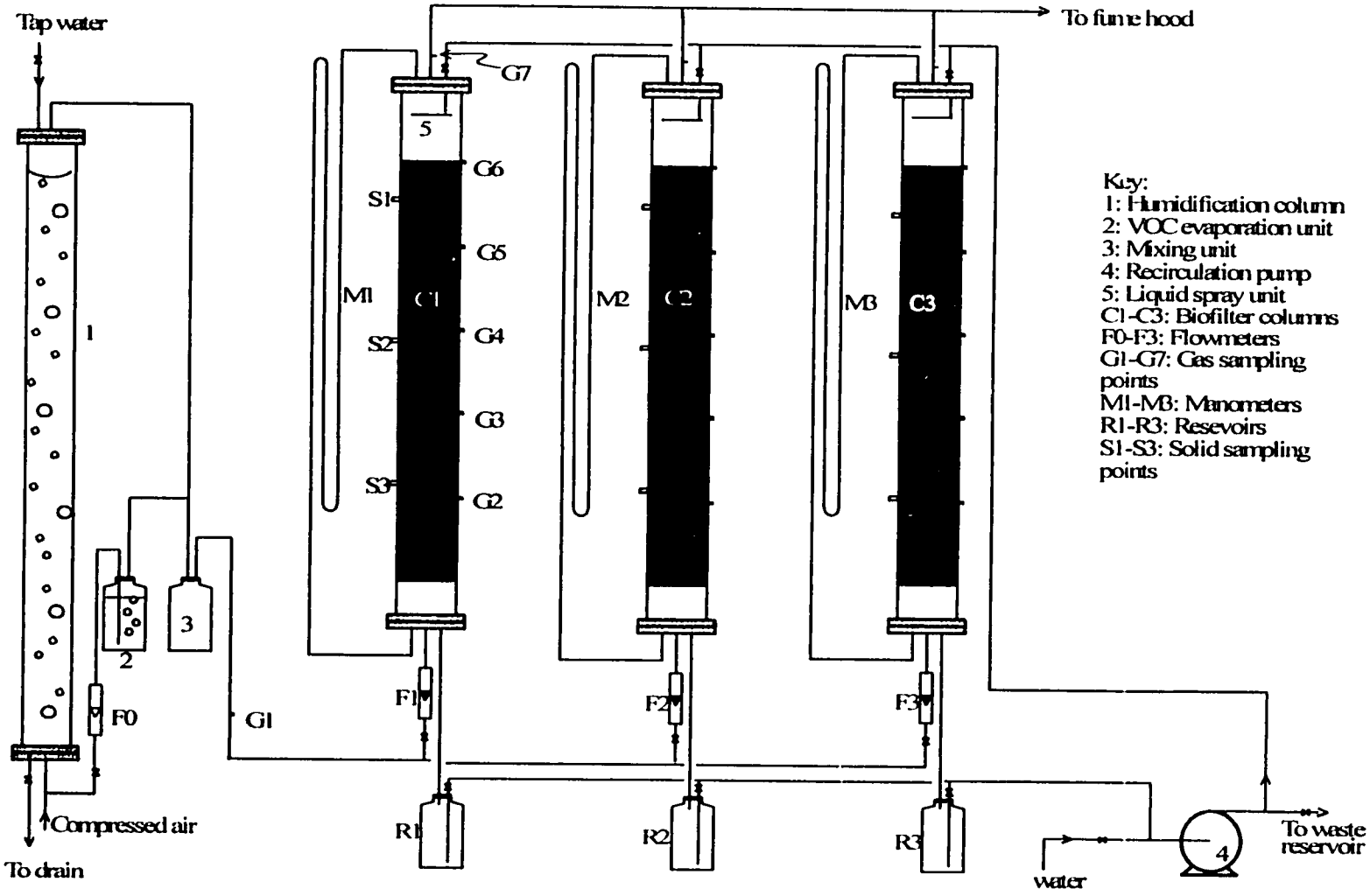


Figure 5.1: Experimental se up

on biofiltration kinetics.

At low nitrogen supplementation, 0.5 kg nitrogen per m^3 of solid media was periodically added to the biofilters. A total of four columns were used, one for each solid medium formulation. The fourth column contained the mixture packed in five stages to assess the effect of gas mixing between sections on biofiltration. Each section was packed with 12 cm high solid medium and spaced at 4 cm from the next one. The nutrient solution was mixed with the solid media before packing, and more nutrient solution was added after 30 days. In this experimental run, air flow rate through the column was maintained at 20 $m^3/m^2.h$. Important parameters investigated were the performance of the biofilters and the degradation kinetics at decreasing nitrogen content. In both cases of high and low nitrogen supplementations, changes of pH, pressure drop, and their correlation to biofilter performances, effect of moisture content, and biofilter life spans were investigated.

An additional experimental run was conducted at the low nutrient supplementation to assess the long-term stability of the biofilters. Fertilizer solution containing 0.5 kg of nitrogen was added to m^3 of solid media at the beginning of the experiment. Subsequent additions of the same amount were performed whenever hexane removal fell below 50%. Media formulation used for long-term stability analysis were peat, pearlite and their 50:50% (v/v) mixture. The air flow rates through the biofilters were set at 20 $m^3/m^2.h$.

5.1.2 Biofiltration Equipment

The Biofiltration equipment was made up of three main pieces; the packed columns, air humidification column, and hexane evaporation unit as illustrated in Figure 5.1. There were additional auxiliary units which included flow meters, vapour and air mixing unit, manometers, water re-circulation pump, column drain containment units and a waste water containment unit. With the exception of the pump and flow meters,

all units were designed and fabricated in-house in our glass blowing and mechanical workshops. The following are descriptions of individual units and their role in the biofiltration process.

5.1.2.1 Packed columns

The four columns used in this study were constructed from glass tubes. A detailed sketch of the complete assembled column is illustrated in Figure 5.2. All columns were 95 cm high. The internal diameter of two of the columns was 11.5 cm, and it was 10.5 cm for the other two. A fully packed column had three sections; a 6 cm high empty space at the bottom (gas distribution section), followed by a 75 cm height of solid medium and a 14 cm height of empty space at the top. The corresponding solid media volumes were 7.8 L and 6.5 L for the 11.5 and 10.5 cm column diameters, respectively. Each column had five gas sampling ports and three solid sampling ports spaced 15 cm and 20 cm apart, respectively. The gas sampling ports had a 6 mm internal diameter and were sealed with a Teflon lined septum supported by plastic screw caps. A centre hole in the screw cap allowed the insertion of a syringe needle through the Teflon seal to collect gas samples. The solid sampling ports had 18 mm internal diameter openings and were sealed with screw-on plastic caps lined with Teflon.

Both column ends were closed with Plexiglas flanges and an air tight seal was achieved by using an o-ring between the column and the flanges (Figures 5.3 and 5.4). The bolts connecting the flanges and clamping ring (Figure 5.5) provided the necessary pressure to the o-ring for an effective air tight seal. Each flange had three 1/4 inch pipe thread holes. Two holes were fitted with through tube Swagelock connectors and the other with a barbed tube connector. On both top and bottom flanges, the centre holes were used for air inlet and outlet. A second pair of holes was used for the manometer connection. The last pair was used for draining excess water

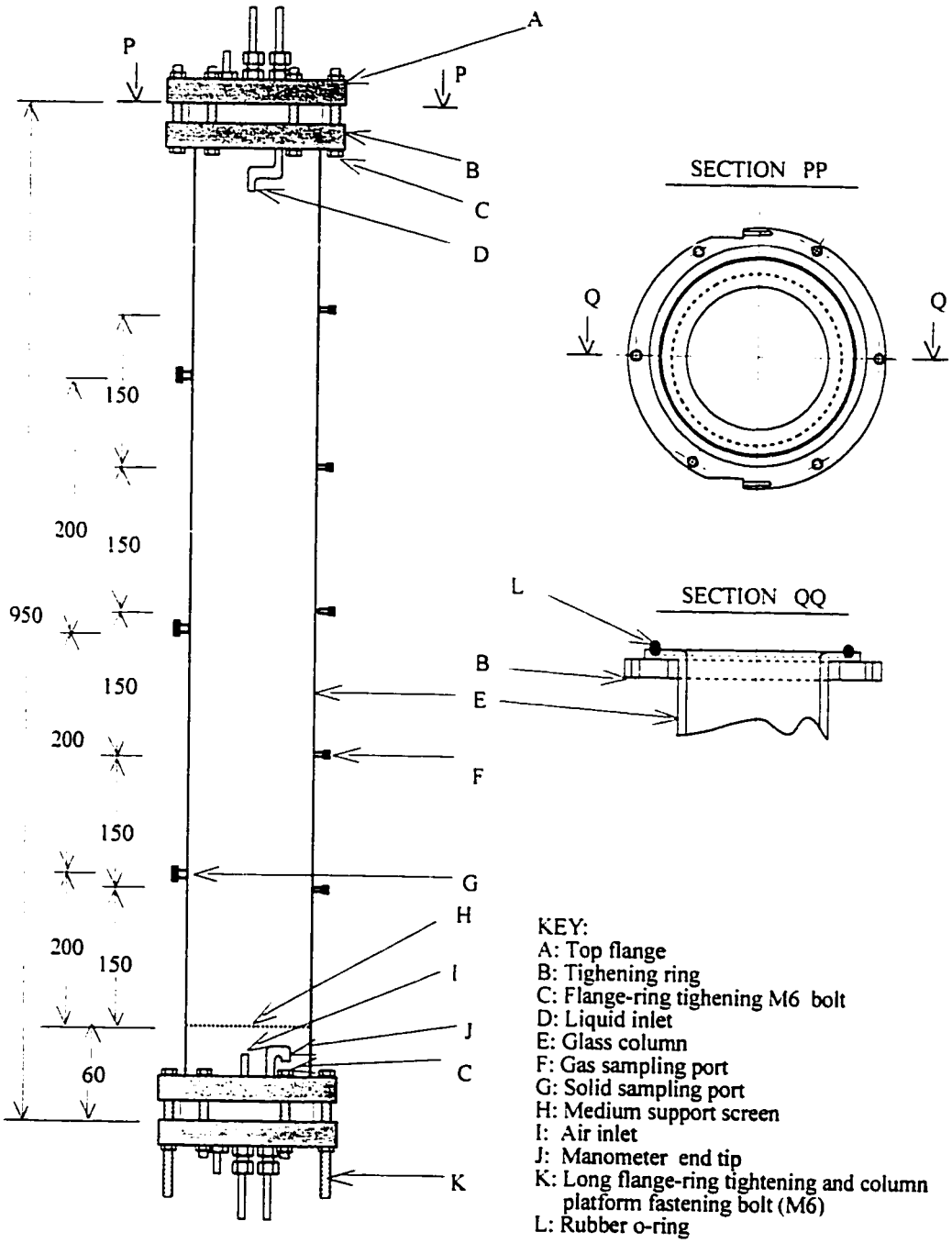


Figure 5.2: A biofiltration packed column

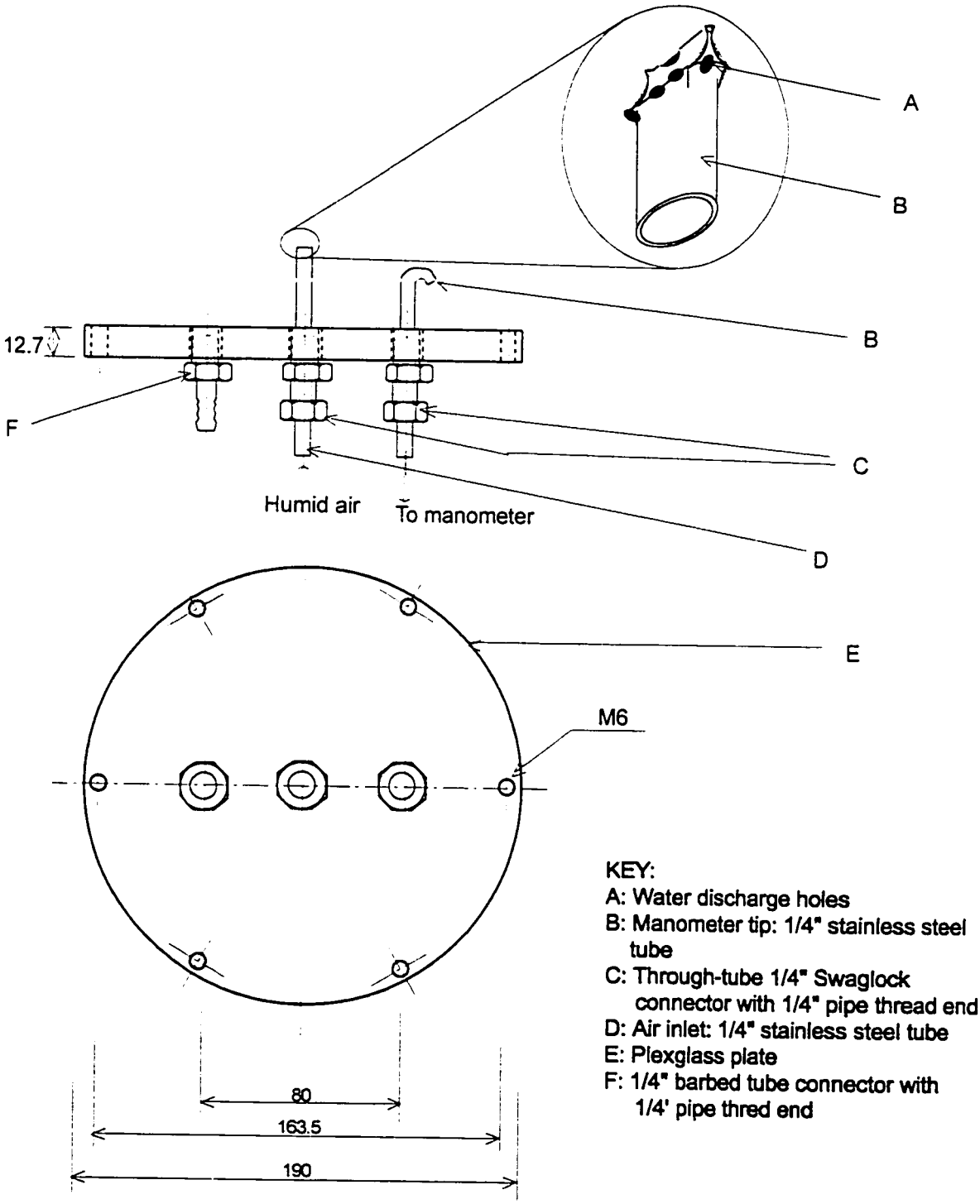
from the column and supplying water and nutrients into the column. The air inlet tube opening was raised 3 cm above the bottom flange to prevent water from entering the inlet tube. Similarly, a manometer tube opening was 3 cm high above the bottom flange and bent downwards to avoid water from dripping into the manometer system.

Stainless steel screens of 14 mesh (opening size 1.4 mm) were used to support the solid media in the columns (Figure 5.6). The screens were reinforced with 15 mm diameter stainless steel rods to prevent them from bending. The screens were supported to the required height of 6 cm above the bottom flange by three L-shaped, 15 mm diameter stainless steel rods. Several screens could be stacked on each to make multiple staged packing by adjusting the length of the support rods and adding a ring at the other end of the support rods to prevent them from penetrating another screen.

The columns were mounted on a 26 cm wide, 150 cm long and 60 cm high platform made from a steel frame and wooden top. Circular holes of 11 cm diameter were cut through the wooden top to allow tubing connections to the bottom flanges. Each bottom flange was fixed to the platform by two bolts, hence anchoring the column in position. The columns were vertically supported at the middle with metal clamps.

5.1.2.2 Humidification column

The humidification column was made of an 8 cm internal diameter and 1.3 m long glass column. Like the packed columns, the seal between the flange and the column was achieved with o-rings. The entire column was packed with 16 mm plastic Pall rings. The column had two openings on both bottom and upper flanges for air and water inlets and outlets. The humidification column was also mounted on the platform in the same way as the packed columns. Tap water was added daily through the top flange connection so that the column was filled with water during operation.



KEY:
A: Water discharge holes
B: Manometer tip: 1/4" stainless steel tube
C: Through-tube 1/4" Swaglock connector with 1/4" pipe thread end
D: Air inlet: 1/4" stainless steel tube
E: Plexglass plate
F: 1/4" barbed tube connector with 1/4' pipe thred end

Units: mm Not to scale	Project: Biofiltration Designed and Drawn by: O. Kibazohi

Figure 5.3: Bottom flange

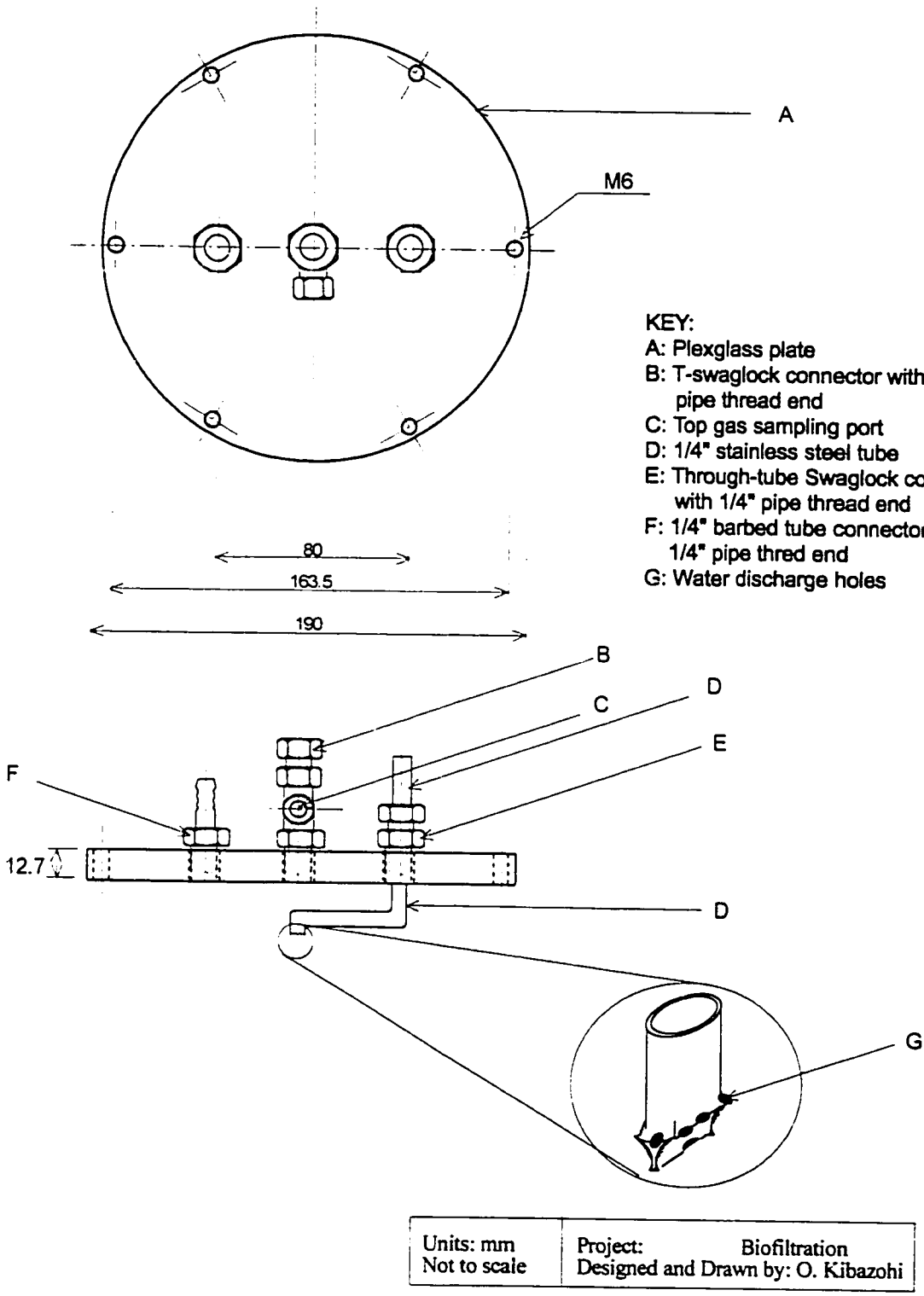


Figure 5.4: Top flange

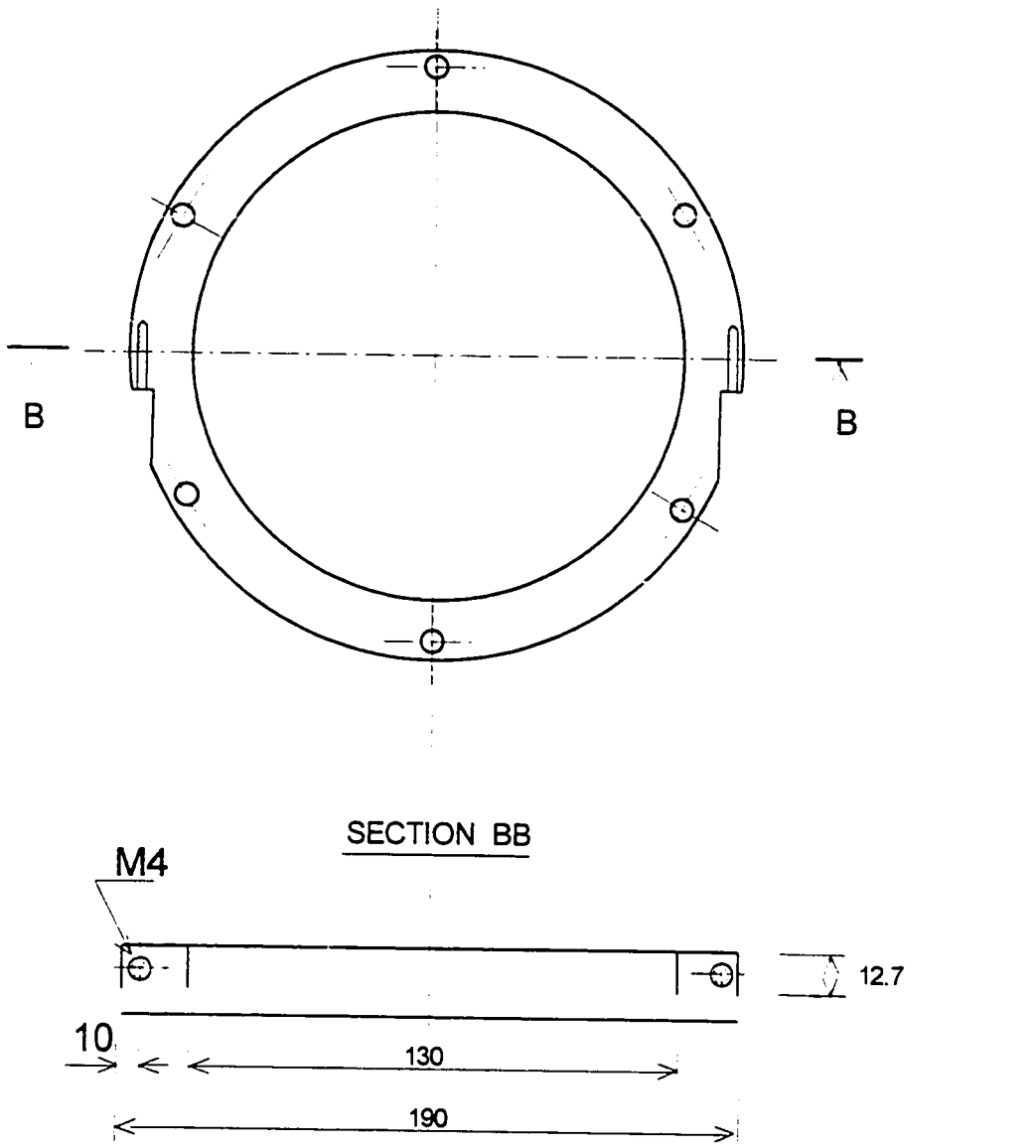
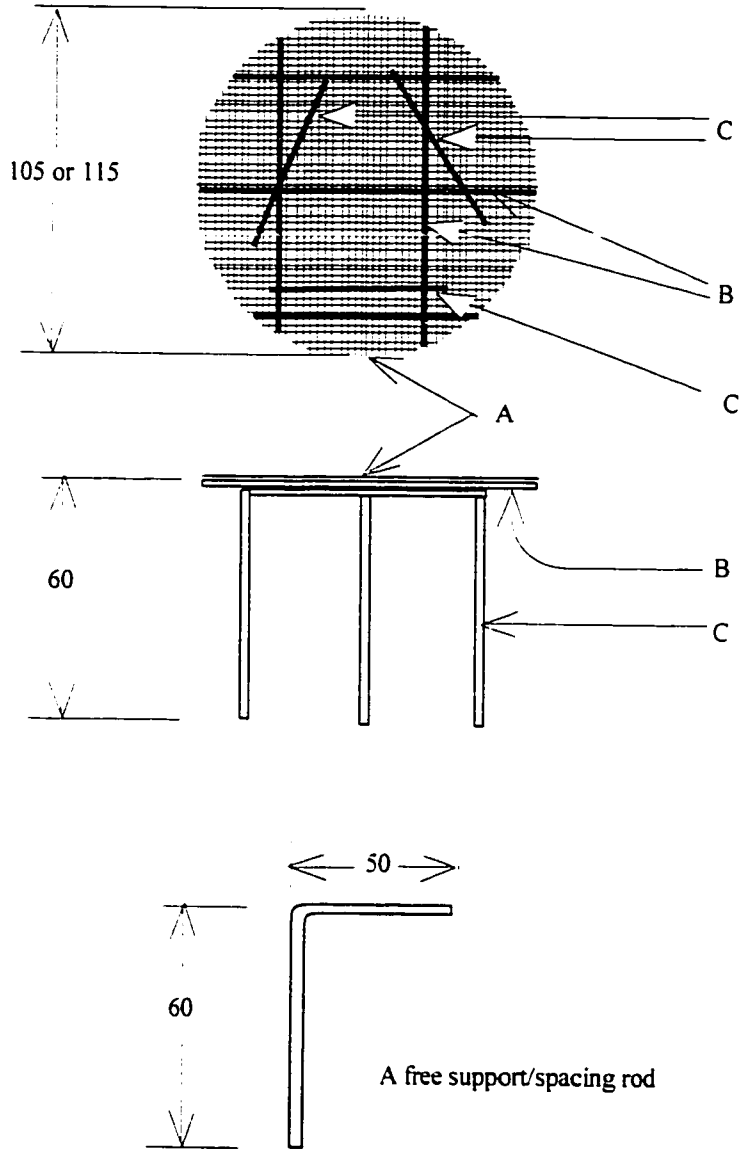


Figure 5.5: Flange clamping ring



KEY:

A: Screen
B: Reinforcement rods
C: Support and spacing rods

Units: mm Not to scale	Project: Biofiltration Designed and Drawn by: O. Kibazohi

Figure 5.6: Medium support

5.1.2.3 Hexane evaporation unit

The hexane evaporation unit was designed and constructed from two 500 mL glass bottles as illustrated in Figure 5.7. Both bottles contained hexane but evaporation took place only in the lower bottle. The upper bottle served as a hexane reservoir for maintaining a constant level in the lower bottle. The lid of the lower bottle was connected to the air inlet and outlet via 1/8 inch copper tubes. Compressed air was bubbled through the hexane near the bottom, and the air-hexane vapour mixture was collected from the top of the lower bottle. Due to different pressure drop across the evaporation unit and the humidification column, a pressure regulator was installed before the humidification unit so that higher pressure was directed towards the evaporation unit and its flow meter. The lower and upper bottles were connected by 1/4 inch Teflon and 1/8 inch copper concentric tubes. It is through this connection that air and hexane exchanged positions between the bottles. Whenever liquid hexane in the lower bottle fell below the end of the concentric tube, air would flow to the upper bottle, while liquid hexane from upper bottle would flow down to the lower bottle. By this design, hexane was maintained at a constant level in the lower bottle, and a constant evaporation rate of hexane at the same air flow rate was achieved.

The air flow through the evaporation unit for the a desired hexane concentration was estimated using the ideal gas law. The exact hexane concentration was determined by gas chromatography. Assuming the air from the evaporation bottle was saturated with hexane then;

$$P_{hex} = P^*_{hex}$$

From the ideal gas law, the molar flow rates in the final mixed stream is given by

$$n_{air} = \frac{P_{air1}V_1 + P_{air2}V_2}{RT}$$

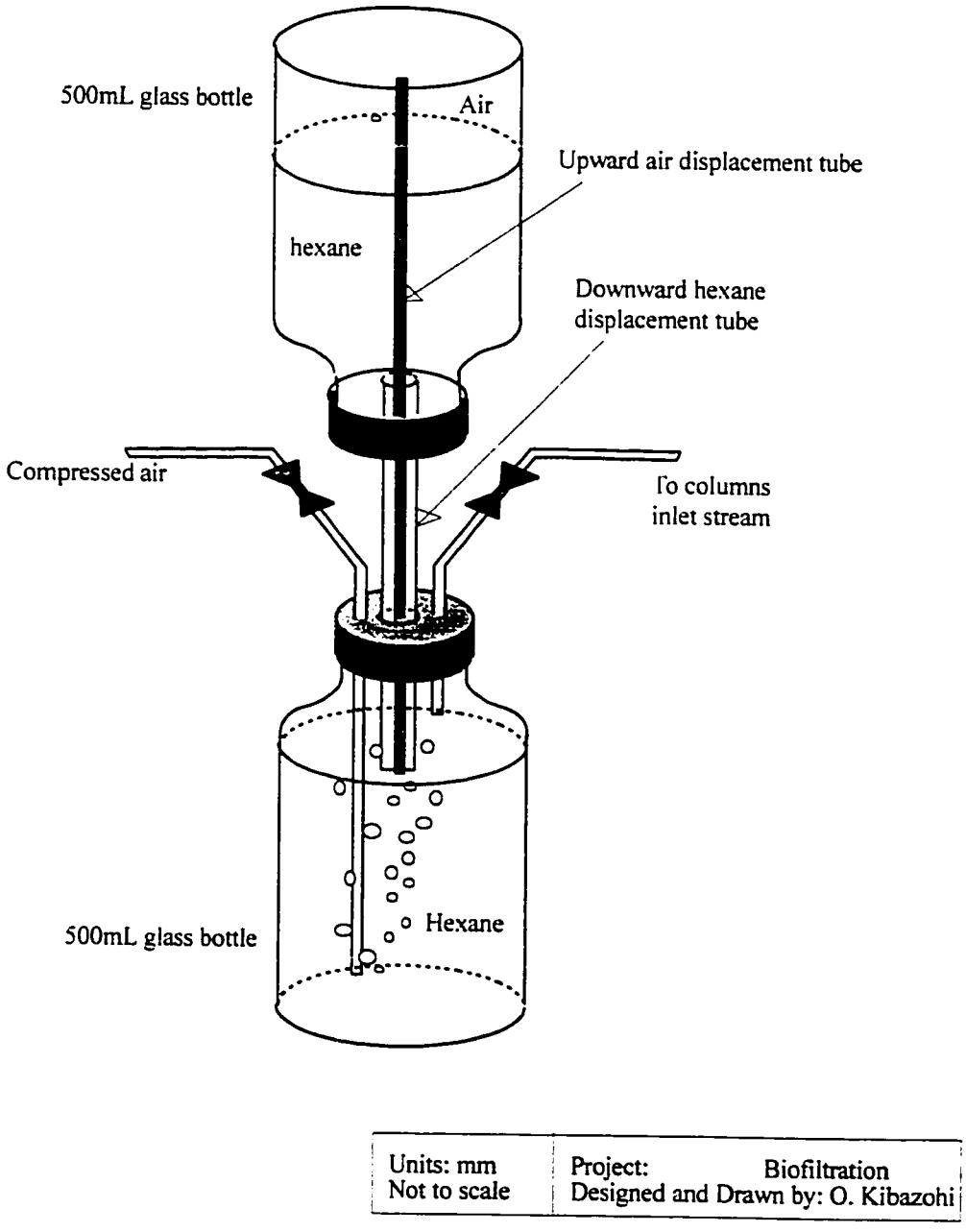
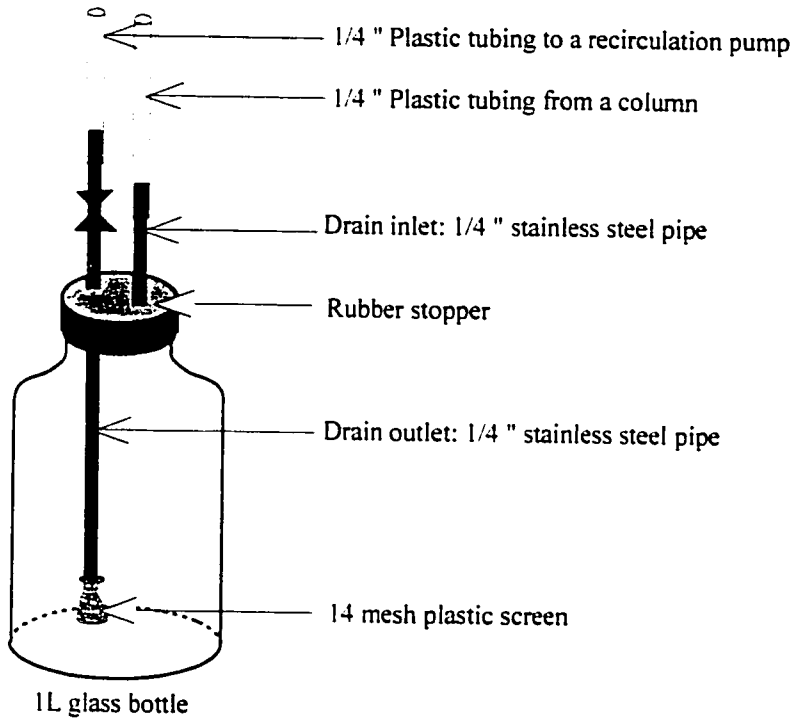


Figure 5.7: Hexane evaporation unit



Units: mm Not to scale	Project: Biofiltration Designed and Drawn by: O. Kibazohi

Figure 5.8: Column drain bottle

and

$$n_{hex} = \frac{P^*_{hex} V_2}{RT}$$

For $n_{air} \gg n_{hex}$, and $V_1 \gg V_2$, hexane concentration is given by

$$C_{hex}\,(ppm) = \frac{n_{hex}}{n_{air}} = \frac{P^*_{hex} V_2}{P_{air1} V_1 + P_{air2} V_2} \cdot 10^6$$

$$= \frac{P^*_{hex} V_2}{P_{air2} V_1} \cdot 10^6$$

where n is the molar flow rate, P is the partial pressure, P^*_{hex} is vapour pressure of hexane, R is the universal gas constant, T is the absolute temperature and V is the volumetric flow rate. The subscripts *air* refers to air, *hex* refers to hexane, and addition of 1 and 2 to the subscripts refer to air through the humidification column and air through the evaporation unit, respectively.

5.1.2.4 Auxiliary units

Air-hexane vapour mixing chamber

The final air-hexane vapour mixture was passed in the following order through the mixing chamber, flow meters, and the packed columns. The mixing chamber was made from a 1 L plastic bottle. The air inlet and outlet were connected to the bottle cap with Swagelock connectors. The mixing chamber was necessary to ensure all the packed columns received the same hexane concentration. The mixing unit was also used to prevent water overflowing from the humidification column from going to the flow meters.

Drainage collection and waste water containment unit

Water draining from the columns was collected in 1 L glass bottles fitted with rubber stoppers as illustrated in Figure 5.8. Each stopper had two holes through which 1/4

inch stainless steel pipes connected to rubber tubing were used as inlet and outlet for excess water from the packed columns. The lower end of the inlet tube coincided with the lower end of the rubber stopper while the outlet extended to the bottom of the bottle. The bottom of the outlet pipe was covered with 14 mesh (1.4 mm opening) plastic screen to prevent large particles from entering the tubing and clogging the pumping system.

All outlet pipes were connected to the plastic tubing which went through a peristaltic pump to the water inlets at the top of the packed columns. This design allowed a recirculation of drainage or introduction of fresh water or nutrient solution to the columns using the peristaltic pump without further connections. A fibreglass reinforced plastic adhesive tape was wrapped around the bottles for safety purposes to prevent flying glass in case of breakage. The tape was also used to hold down rubber stoppers to ensure air tight conditions. At the end of the experimental run, the liquid drained from the column was pumped into a 50 L glass waste water containment unit and later autoclaved and disposed of.

Flow meters

Flow meters with a capacity up to 50 L per minute were installed between the mixing chamber and the packed columns to control air flow rates. Another 50 L/min flow meter was used for carbon dioxide flow through an infrared analyser. A flow meter of up to 4.4 L per minute capacity was used for air flowing through the hexane evaporation unit. All flow meters were produced by Gilmont Instruments Inc.

Manometers

Water manometers for measuring pressure drop across the packed columns were constructed from 1/4 inch plastic tubing. The U-shape section was vertically mounted on a graph paper graduated in mm for reading pressure differences. A plastic T-joint connected at the bottom of the manometer allowed fast and easy addition of water

to the manometer.

Recirculation pump

A peristaltic pump was used for addition of fresh water and nutrient solution as well as recycling the drained liquid to the packed columns. The pump had an adjustable speed of 0 to 3000 rpm and 1/4 inch silicon tubing was used for transferring liquid.

5.1.3 Solid media

The solid media used in this research was composed of peat and pearlite in three different combinations 100% peat, 100% pearlite and their (50% by 50% v/v) mixture. The peat used in this research was sphagnum peat (high-moor peat) purchased from a local flower garden store know as White Rose. Since plants could not be recognized, it can be stated here that it was a highly decomposed peat. Laboratory evaluation of this peat showed that it had a bulk density of 166 $kg.m^{-3}$ (dry weight) and pH value of 3.6. Pearlite used in this study was of horticultural grade and had a bulk density of 100 kg/m^3 (dry weight). To allow for good pneumatic conductivity, the media materials were sieved to sizes between 1.7 and 4.75 mm.

Natural lime was added to all media formulations to control pH . According to the specifications, the lime contained 55% calcium, 5% magnesium and had a neutralizing value of 43.6% (w/w). The lime particles had diameters less than 10 mesh (2 mm screen opening) and 15% passed through 100 mesh (150 μm screen opening). When 1 g of lime was mixed thoroughly with 5 mL of deionized water, the pH of the resulting slurry was 8.4 indicating that the lime was in a form of calcium carbonate. In this research, approximately 8 kg lime per m^3 of the packed volume was mixed with the solid medium prior to packing the columns.

5.1.4 Supplementary Nutrients

Commercial organic based fertilizer was used as a source of supplementary nutrients. This fertilizer manufactured by Nitrite of Elmira, Ontario, had a NPK ratio of 14:7:14. The actual composition is shown on Table 5.1 below.

Table 5.1: Composition of fertilizer

Component	*Amount % (w/w)*
Total Nitrogen (N)	14.00
Available Phosphoric acid (P_2O_5)	7.00
Water soluble potash (K_2O)	14.00
Water soluble magnesium (Mg)	1.00
Water soluble sulfur (S)	5.00
Iron (Fe) actual	0.10
Zinc (Zn) actual	0.05
Manganese (Mn) actual	0.05
Copper (Cu) actual	0.05
Boron (B) actual	0.02
Molybdenum (Mo) actual	0.0005
Organic matter	15.00

The fertilizer which was added in the form of liquid, was prepared as follows. The required amount of fertilizer was dissolved in deionized water in a 200 mL flask by agitating with a magnetic stirrer for 4 h. The stirring was stopped, undissolved solids allowed to settle, and the supernatant decanted and used as a solution of nutrients, while the undissolved solids were discarded. At the beginning of an experiment the solution of nutrients was mixed with the solid media and inoculum before packing the columns. Subsequent nutrient solution was introduced through the top liquid inlet by pumping with the recirculation pump. The drained solution was continuously recirculated for 10 to 15 minutes for even distribution.

5.1.5 Hexane

Technical grade hexane was used as a model VOC. It was composed of approximately 88% n-hexane, 9% methyl cyclopentane and 3% 3-methyl pentane by weight. In this context, hexane refers to the mixture of the three components in the ratio given above. Solubility of these components in water is quite low. All components are reported to be practically insoluble in water [127]. On the other hand, n-hexane is reported to have a solubility of 0.014 g in 100 g of water at 15°C [105]. Physical properties of individual components are given in Table 5.2. Hexane was evaporated and introduced into a gas stream prior to biofiltration as described in section 5.1.2.4.

Table 5.2: Physical properties of hexane components

Physical properties	n-hexane	methyl cyclopentane	3-methyl pentane	reference
Boiling point at 760 mmHg (°C)	68.744	71.812	63.282	[110, 172]
Vapour pressure at 25°C (mmHg)	150	140	163	[161]
Solubility in water (g/100g)	0.014 (15°C)	insoluble	insoluble	[105, 127]
Liquid density at 15°C (g/mL)	0.66364	0.75340	0.66880	[110]

5.1.6 Inoculum preparation and immobilization

Before inoculation of the solid media, 1 L of inoculum was prepared for each column. 20 mL of the stock culture was allowed to come to room temperature and then used to inoculate into a 150 mL mineral medium containing 5 mL of hexane in a 500 mL flask. After 24 hours incubation at room temperature on a shaker, 100 mL of the growing culture was used to inoculate 1 L of the mineral medium in a 2 L flask containing 20 mL hexane. The culture broth was incubated at room temperature for 24 h while being aerated by bubbling air through it. The cells were immobilized by mixing the

resulting inoculum with the solid medium and the supplementary nutrients. The whole mixture was then introduced into the column. The total liquid volume used during start up was restricted to less than 2 L per column to minimize nutrient leaching.

The inoculation procedure described above initiated the immobilization process. The microorganisms from the inoculum became attached after being introduced to the solid media. Adsorption is considered to be the mechanism involved in microbial immobilization in this work.

5.1.7 Analysis

5.1.7.1 Gas analysis

Hexane concentration

The hexane concentration in the gas phase was analysed using a Hewlett Packard (Avondale, PA) 5890 Series II gas chromatograph (GC) equipped with a flame ionization detector (FID). The column was a RTX-502.2 fused silica megabore column with 30 m x 0.53 mm ID and 3.00 μm film thickness (Chromatographic Specialties Inc.). Helium was used as the carrier gas and the auxiliary gas was nitrogen. The flow of carrier in the column was set to 15 mL per min. A split/splitless technique and a temperature program were used for analysis. A 1700 series, 500 μL Hamilton gas tight syringe was used to withdraw a 200 μL sample from the column and inject it in the GC. A run was started with splitless flow mode and after nine seconds the purge valve was automatically turned on to provide a split flow mode. The initial oven temperature was 50°C for three minutes, followed by a ramp of 20°C per min until a final temperature of 80°C was reached and held for 1.5 minutes.

Data was automatically acquired and integrated by the software package Peaksimple II (Peak Simple Chromatography Data System, SRI Instruments, Torrance, CA) installed on a personal computer. The concentration of hexane in gas samples

was obtained from calibration curves prepared prior to the experiments.

Due to the low hexane solubility in water, a gas phase calibration curve was prepared. Approximately 5 mL each of pure n-hexane and methyl cyclopentane were introduced into 25 mL sample bottles. The bottles were closed with Teflon lined septums and vapour-liquid allowed to come to equilibrium for 5 hours. A sample of saturated vapour was withdrawn with a gas tight syringe and diluted to lower concentrations. The diluted vapour mixture was allowed to come to equilibrium for at least 30 minutes, then 50–500 μL samples volumes were injected into the GC. The concentration of the diluted samples were calculated from ideal gas law. For the gas sample volume v_s injected into the GC, the number of moles of hexane and air are given by:

$$n_{hex} = \frac{P^*_{hex} v_s}{RT \cdot \psi} \tag{5.1}$$

$$n_{air} = \frac{P_{atm} v_s}{RT} \tag{5.2}$$

and for $n_{air} \gg n_{hex}$

$$C\,[\text{ppm}] \approx \frac{n_{hex}}{n_{air}} \cdot 10^6 = \frac{P^*_{hex}}{P_{atm}\psi} \tag{5.3}$$

where n_{hex} are moles of hexane, n_{air} are moles of air, P_{air} absolute pressure of the gas stream, P^*_{hex} is vapour pressure of hexane, P_{atm} is atmospheric pressure, R is the universal gas constant, T is the absolute temperature, v_s is the sample volume injected into the GC, and ψ is the dilution factor (for calibration samples) The amount of hexane removed from the gas phase was calculated from the following relationship:

$$\text{Hexane removal } (\text{g/m}^3\text{.h}) = \frac{(C_{in} - C_{out})}{10^6} \frac{P_{air} V}{RT} M \tag{5.4}$$

where C_{in} and C_{out} are inlet and outlet concentrations of hexane in ppm, M is molar weight of hexane, and V is the volumetric flow rates of gas through the column.

Carbon dioxide

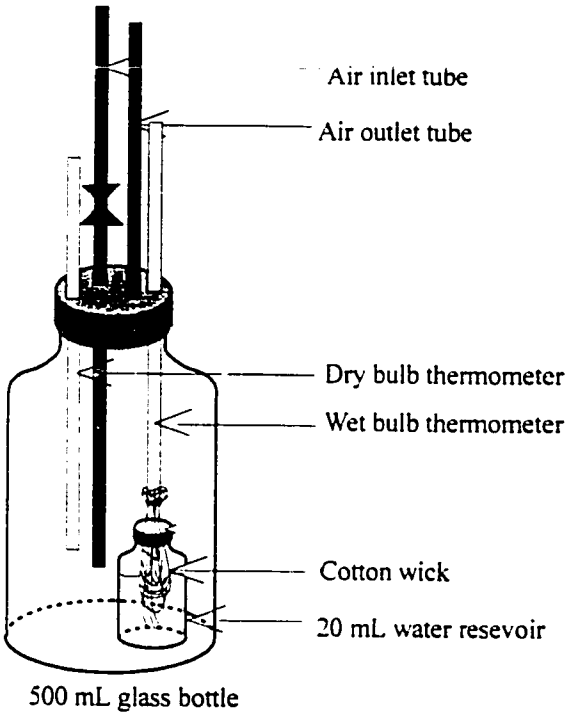
Carbon dioxide in the inlet air and packed columns exit gas streams was measured using an Infrared Gas Analyser (IR)(Beckman model 864). Before each measurement, the analyser was calibrated using pure nitrogen as a zero reference and a standard mixture of nitrogen and known concentrations of CO_2 for non-zero reference points. The measured stream was passed through the IR at the same flow rates as the calibration samples and the values of CO_2 was obtained from calibration curves. The amount of CO_2 generated in the biofilter was calculated as the difference between the CO_2 of the column exit stream and the CO_2 of the inlet stream.

Humidity

Air humidity was measured using a dry and wet bulb temperature hygrometer illustrated in Figure 5.9. The meter was designed and constructed in-house using a 500 mL glass bottle and two mercury thermometers with a sensitivity of 0.1°C. A cotton cloth wick and 20 mL container were used for the wet bulb temperature. The gas stream was passed through the hydrometer for 15 min, then wet and dry bulb temperatures were recorded. The relative humidity was evaluated from the psychrometric chart.

5.1.7.2 Ammonia analysis

A method capable of distinguishing ammonia nitrogen at low concentrations from other nitrogen forms was used. Ammonia content in the solid phase was monitored using an ammonia electrode (Ammonia electrode meter, Orion 9512 BN). Three solid samples of 1 g each were withdrawn from each column at the lower, middle and top sections. Each sample was homogenized in a test tube using a clean spatula and mixed with 100 mL of deionized water. The mixture was filtered into a 150 mL beaker using medium Whatman filter paper. Two mL of sodium hydroxide solution (concentration 450 g/L) were added to the filtrate. The mixture was stirred with a magnetic stirrer



Units: mm Not to scale	Project: Biofiltration Designed and Drawn by: O. Kibazohi

Figure 5.9: Hygrometer

and the ammonium content was directly measured using the ammonia electrode. The electrode was pre-calibrated with standard ammonia solutions which had been treated in the same manner each time before measurement.

5.1.7.3 pH measurement

Monitoring of pH was done using two methods, pH probe and litmus paper. The pH probe method was more precise than the litmus paper method. The pH probe method was performed by withdrawing 1 g solid media sample from the packed column via solid sampling ports. The sample was thoroughly mixed with 5 mL of deionized water in a test tube followed by measurement of the pH of the mixture with the pH probe.

Though more accurate, the pH probe method could not be used frequently, since a lot of solid media would have been removed from the packed columns. The pH probe method was therefore used when solid samples were withdrawn from the columns for determining nitrogen content and microbial density. For frequent pH measurement litmus paper with a pH sensitivity of 0.5 units was used. The method involved stopping the air flow through the columns and inserting a piece of litmus paper to the columns through the solid sampling ports. The paper was removed within 5 minutes and colour change matched to the standard colour chart.

5.1.7.4 Microbial density

Approximately 1 g solid media sample were withdrawn from the columns through solid sampling ports and added into test tubes containing 10 mL of sterile saline solution. The biomass was released into the saline by grinding the solids on the test tube wall with a sterile spatula until a fine slurry was formed. The suspension was thoroughly mixed with a vortex mixer to detach cells from the solids and then serially diluted with fresh saline to give a final concentration of 10^{-9} of the original slurry.

A volume of 150 μL from each sample that had been diluted to 10^{-9} to 10^{-7}

of initial slurry/broth was cultured on agar plates. Nutrient Agar (NA), and Malt Extract Agar (MEA) were used for bacteria and fungi respectively. Chloramphenicol at a concentration of 25 mg/L of MEA was used for inhibition of bacterial growth on MEA. The agar plates were incubated for 48 h at 30°C and CFU counted with a Quebec Colony Counter after 24, 36 and 48 hours of incubation to evaluate the number of viable cells in the sample [117].

5.1.7.5 Solid media moisture content

The moisture content of the solid media was determined by the dry weight method. Solid samples (0.5–1 g) were withdrawn from the columns and placed in tared dried aluminum dishes. The weights of wet samples were recorded and samples were dried to constant weights for 48 h at 100 °C. The samples were placed in a desiccator for at least 20 minutes and dry weights recorded. The percentage moisture content (w/w) was then determined using the following equation.

$$MS = \frac{w_{Td} - w_D}{w_{Tw} - w_D} 100 \tag{5.5}$$

where w_{Td} is total dry weight of solids and weighing dish, w_D is the weight of the empty dish and w_{Tw} is total wet weight of solids and weighing dish.

5.1.7.6 Pressure drop

The pressure drop was monitored using water manometers connected to each column end. The manometer scale had a sensitivity of 1 mm H_2O. Before recording the readings, the flow meters regulating air flow through the packed columns were checked and adjusted to the required flow rate. The pressure drop per meter of packed bed was evaluated using the following equation:

$$\Delta P\,(\text{Pa/m}) = \frac{\rho g \Delta L}{0.75} \tag{5.6}$$

where ΔL is the differential water manometer reading, g is the gravitational acceleration, 0.75 refers to the height of the packed volume and ρ is the density of water.

5.2 Results and Discussion

5.2.1 Biodegradation

The study has led to an improved understanding of the fundamentals of biofiltration technology. Biodegradation of VOC during biofiltration depends on many factors including the availability of nutrients, the type and composition of solid medium, microbial density, VOC concentration, and environmental conditions, such as pH and moisture content. Figures 5.10 through 5.41 and 5.33 illustrate the biodegradation patterns of hexane in the biofilters containing the different solid media (peat, pearlite, and their mixtures) at different time courses and levels of supplementary nutrients. Under supplementary nutrient conditions, the biodegradation of hexane was accompanied by nitrogen utilization and carbon dioxide generation which are illustrated in Figures 5.19, and 5.20. The carbon balance was investigated. Figure 5.21 illustrates the ratio of measured carbon dioxide to the equivalent hexane removed by biofiltration. Time-courses of pressure drop and pH during the biofiltration are illustrated in Figures 5.24 through 5.28. The effects of pH and solid media particle size on the biofiltration are illustrated in Figures 5.31 and 5.38. The comparisons of the biofilters in terms of the solid media using their rates of biodegradation and performance indicator are presented in Figures 5.33 and 5.35. Details of the results are presented below.

5.2.1.1 Effect of supplementary nutrients

The biodegradation of hexane has been found to strongly depend on the availability of nutrients in the solid medium, particularly nitrogen. Preliminary experimental work (Figure 5.10) showed that without addition of supplementary nutrients, hexane biodegradation rates were negligible. Hexane removal remained below 10% for more than a week when inlet concentrations were between 100 to 200 ppm. However, hexane

removal increased gradually to more than 40% when a solution of basal medium was added to the biofilter containing 100% peat. Other researchers reported similar observations of low or negligible VOC removal in peat biofilters without inoculation or addition of supplementary nutrients or both [18, 53, 82, 95]. High biodegradation rates were further observed when supplementary nutrient solution was added at high and low supplementation levels as detailed below. This observation was an indication that nutrient availability in the solid media improves the biodegradation rates. The quantity and frequency of the nutrient supplementation and its industrial applicability was the subject of further investigation which is addressed in the next sections.

5.2.1.2 Effect of High (weekly) nutrient supplementation

The biofiltration of hexane during addition of 1 kg of nitrogen per m^3 of solid medium per week is illustrated in Figures 5.11, and 5.12. Availability of nutrients resulted in a high removal of hexane. Within the first 5 days, the percent removal had reached 75% for the mixture and 66% for the pearlite. The percent removal strongly depended on gas flow rate and inlet concentrations of hexane. The values of percentage removal for both pearlite and the mixture media decreased as the flow rates were increased from 20, to 30 and 100 $m^3/m^2.h$ at the same range of hexane inlet concentrations (Figure 5.11 and Table 5.3). A similar trend was observed by Tang *et al.* [130] during biofiltration of triethylamine in compost biofilters. The flow rate affects the residence time of hexane in the biofilter, hence this trend of the lower percentage removal at higher gas flow rates was expected.

Similarly, the overall amount of hexane removed depended on the flow rates and concentration. Unlike the percentage removal, the amount of hexane removed in $g/m^3.h$ increased with both the flow rate and the inlet concentration until an average elimination capacity of 70 $g/m^3.h$ was reached (Figures 5.12, 5.13 and Table 5.3). For the same range of hexane inlet concentration, the amount removed was highest up

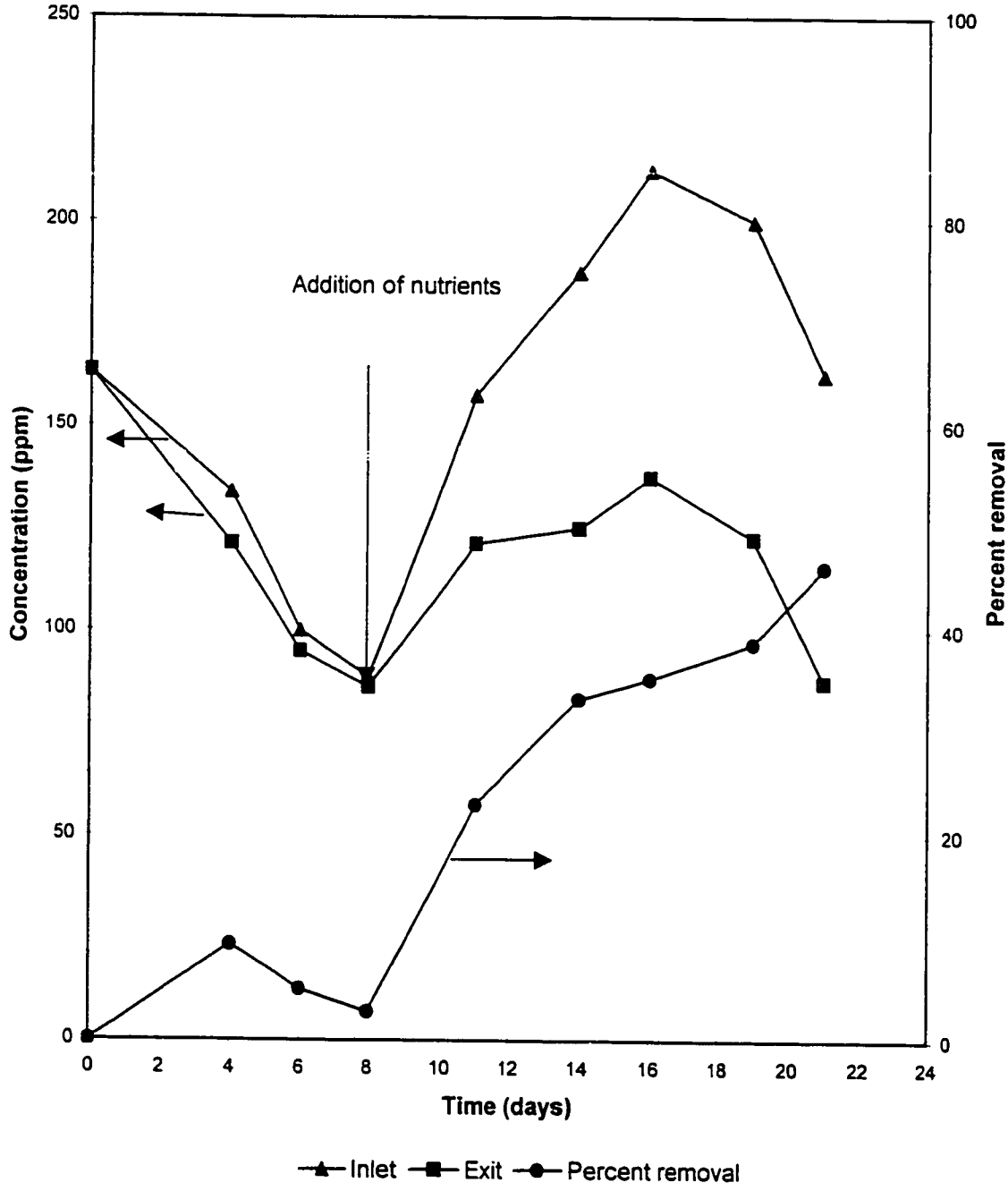


Figure 5.10: Effect of nutrient addition on biodegradation of hexane (100% peat)

Table 5.3: Hexane removal at different air flow rates (high nutrient supplementation)

Time scale	Flow rate	Inlet conc.	Pearlite		Mixture	
(day)	($m^3/m^2.h$)	(ppm)	(%)	($g/m^3.h$)	(%)	($g/m^3.h$)
5–13	20	560–680	40–67	34–56	73–80	50–67
14-27	100	500–720	10–24	36–96	11–24	34-120
28-46	30	320–980	27–54	20–80	36–75	28 -71
47-75	20	280–640	30–50	16–40	40–50	16-40

to 100 $m^3/m^2.h$, and lowest for 20 $m^3/m^2.h$. For instance, when the flow rate was changed from 20 to 100 $m^3/m^2.h$, the amount of hexane removed increased from 56 to more than 95 $g/m^3.h$. Literature reports similar observations whereby the amount of acetone removed was dependent on the inlet concentration [77]. Although the percentage removal showed the inverse relationship to the gas flow rate, the fact that the amount of the hexane removed increased with the gas flow rate suggests that a waste gas flow rate can be increased during biofilter operation to increase overall removal rate, as long as the exit concentration of VOC from the biofilter is below the required level. The above biofiltration characteristics are further elaborated in the modelling (Chapter 6).

In both pearlite and the mixed solid media, biomass growth was observed. After 45 days the biofiltration efficiency decreased to 45% for the mixture and 37% for pearlite. The decrease in efficiency was accompanied by a sharp rise in pressure drop and a decrease in pH for the column containing the mixture (Sections 5.2.5 and 5.2.6). Beyond 60 days of operation, high biomass overgrowth was observed in both solid media (pearlite and the mixture) such that addition of supplementary nutrient solution did not improve the hexane removal. More biomass overgrowth was observed in the top section at which the nutrient solution was introduced to the columns. Consequently, supplementary nutrient solution could not easily pass down

through the columns. Due to high pressure drop and the difficulty in adding nutrient solution, the experimental run was stopped after 70 days.

Statistical analysis for the biodegradation of hexane for the two solid media (pearlite and the mixture) was performed using fractional conversion of hexane data at different concentrations and flow rates. The comparison of the least squares means (Figure 5.14) shows that although pearlite had a lower overall fractional conversion of hexane, the two solid media did not significantly differ in biodegradation of hexane at a 95% confidence level.

From the above observation, it can be concluded that addition of supplementary nutrients increased the biodegradation rates of hexane. However, a weekly addition of 1 kg (N)/m^3 was too high and resulted in high biomass overgrowth, which in turn reduced the pneumatic conductivity of the solid media. Deshusses [25] suggests that continuous supply of nutrients is not desirable because rapid biofilter clogging by excessive biomass growth would occur. It is therefore necessary to properly manage the amount of nitrogen added to the biofilter such that reasonably high biofiltration rates are achieved while the biomass growth is controlled and a relatively low pressure drop is maintained for long-term operation. It can also be concluded that under high nutrient supplementation, the type of the solid medium did not significantly affect the biodegradation efficiency. It is therefore the nutrient supplementation which controls the biofiltration rates. The observation is more interesting in the sense that if the method to remove excess biomass from biofilters is developed, biofilters can be operated under the high nutrient supplementation protocol with a periodical removal of the excess biomass. Such operation will increase overall biofiltration rates and reduce biofilter volumes on a long-term basis.

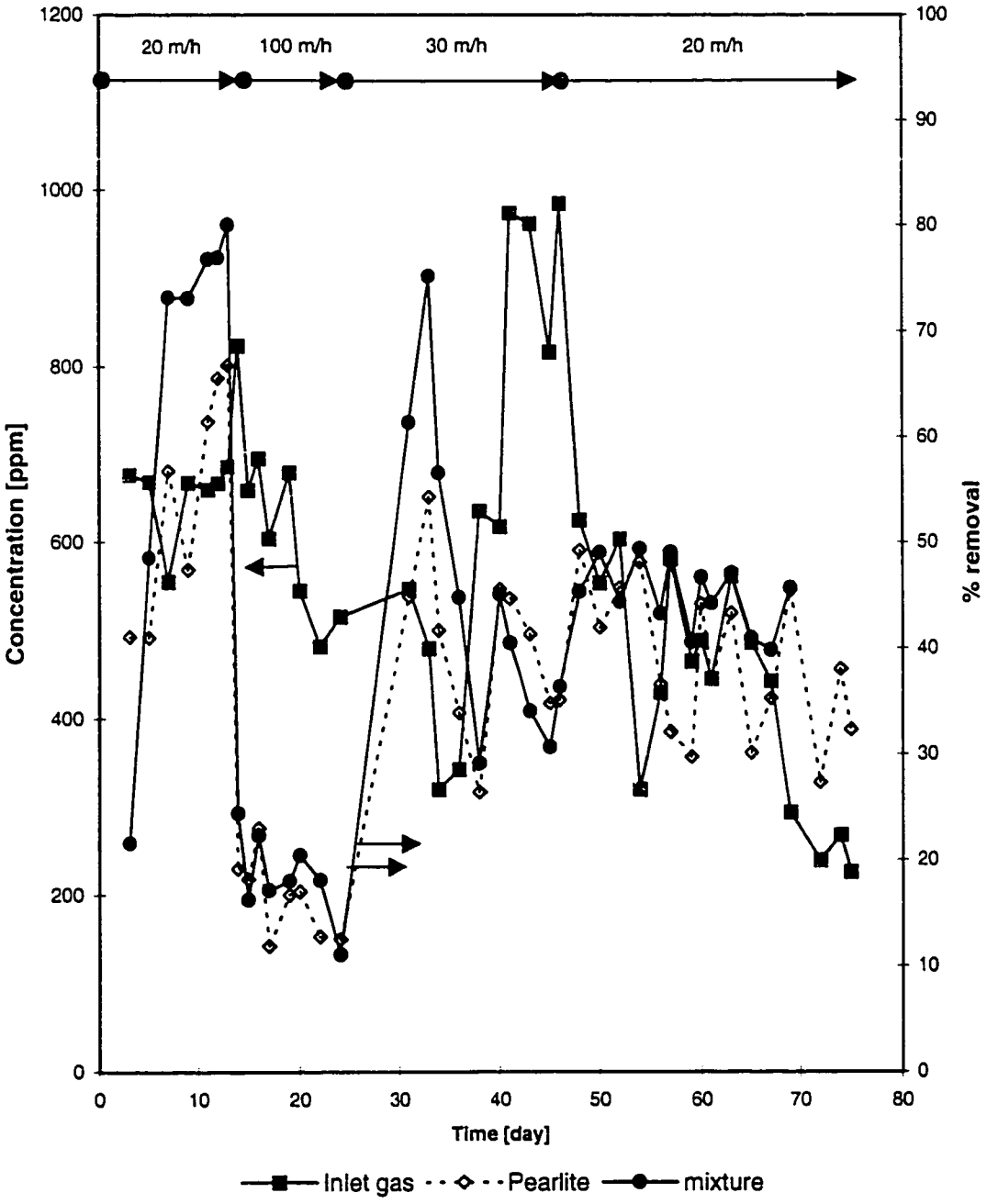


Figure 5.11: Hexane percent removal (high nutrient supplementation)

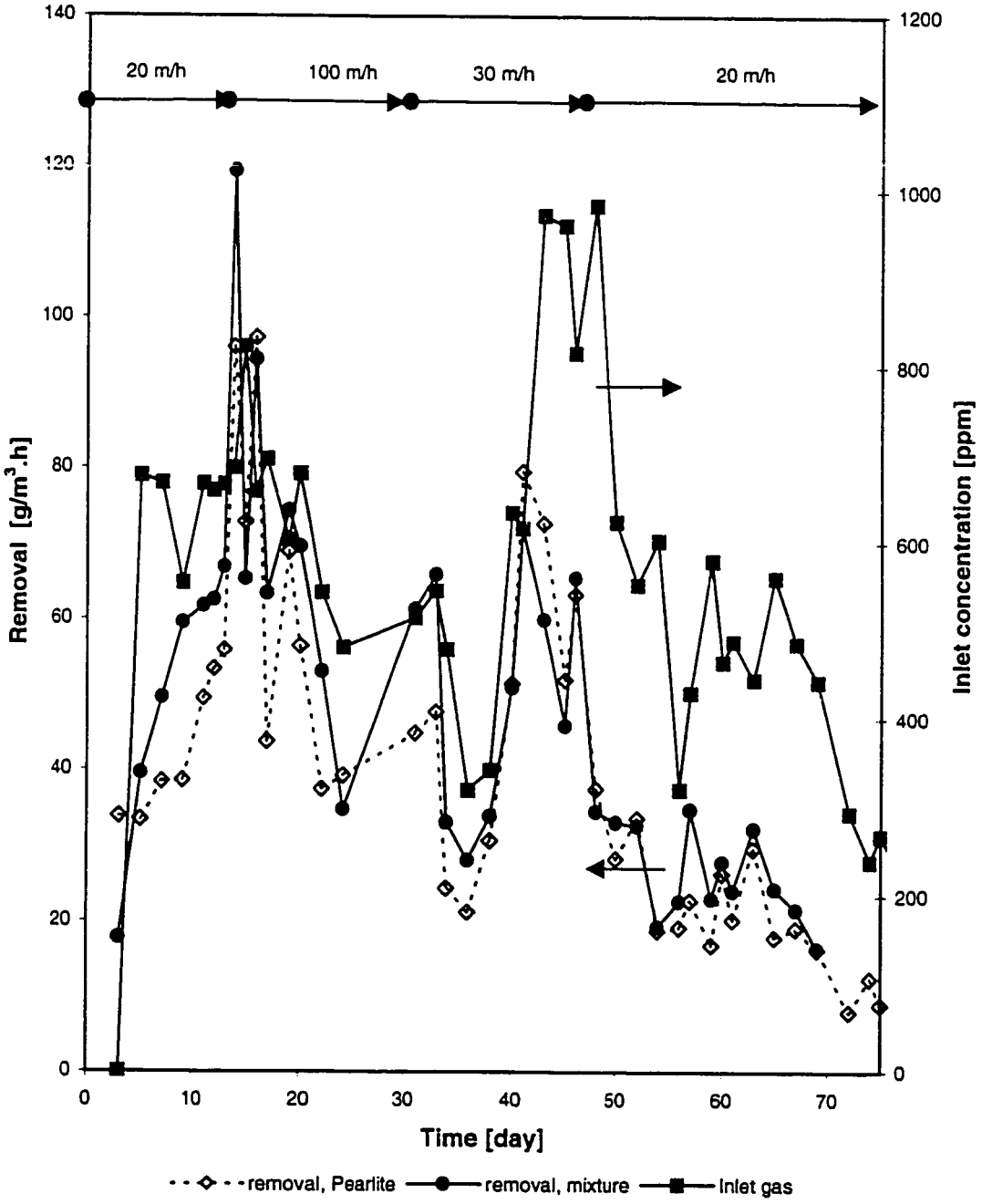


Figure 5.12: Hexane removal rates in $g/m^3.h$ (high nutrient supplementation)

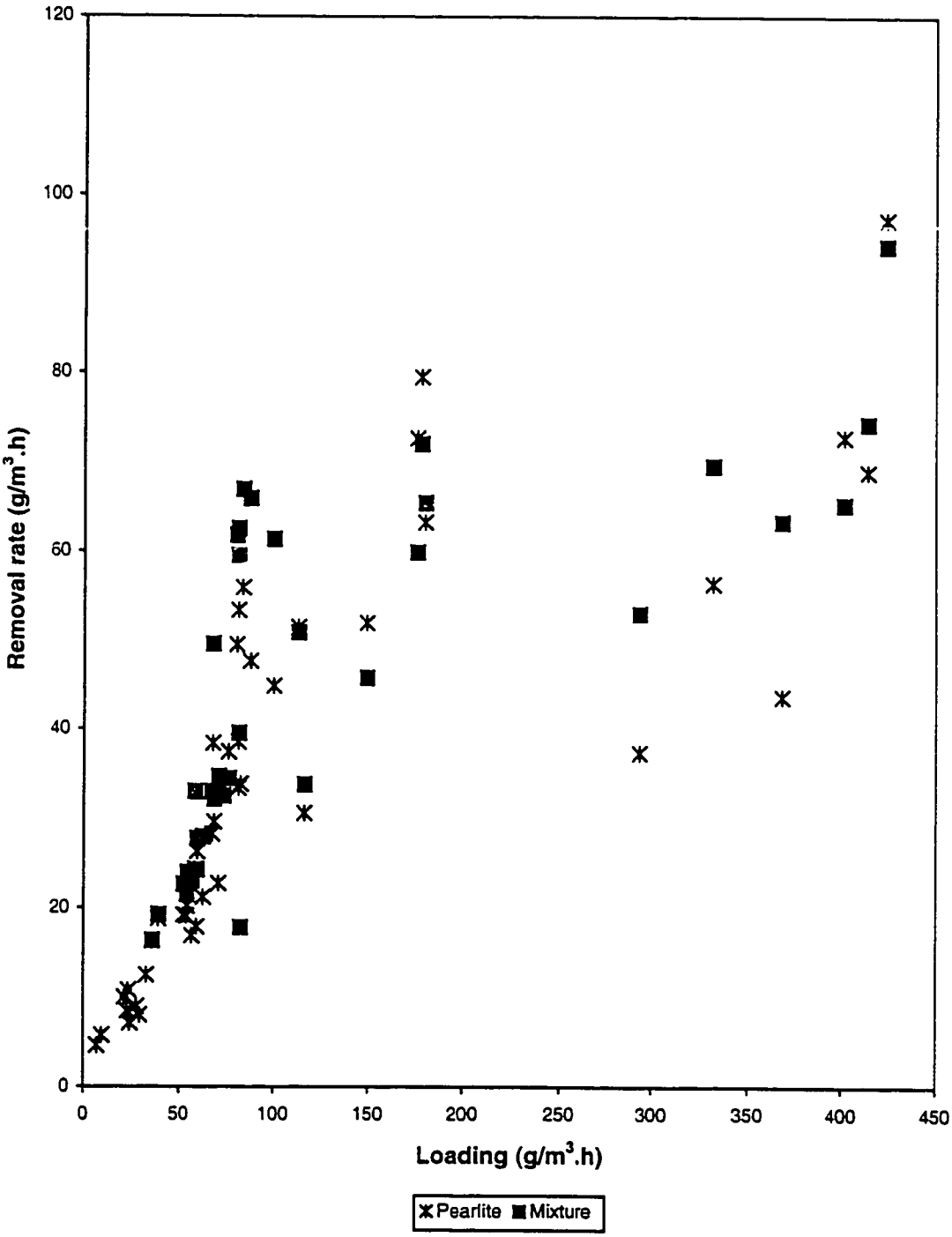


Figure 5.13: Variation of hexane removal rates with hexane loading

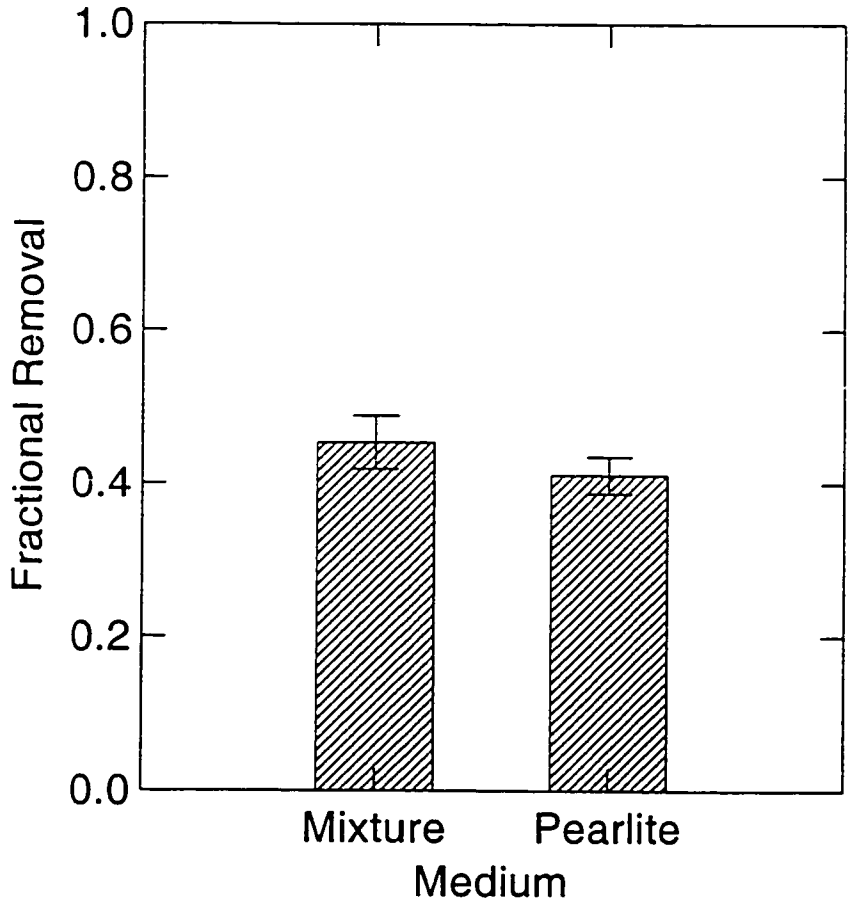


Figure 5.14: Means comparison for hexane fractional removal (high nutrient supplementation)

Table 5.4: Hexane removal at 20 $m^3/m^2.h$, and average concentration of 400 ppm (Low nutrient supplementation)

Time scale	Pearlite		Peat		Mixture		Mixture (stagewise)	
(day)	(%)	($g/m^3.h$)	(%)	($g/m^3.h$)	(%)	($g/m^3.h$)	(%)	($g/m^3.h$)
4-30	16–76	6–50	30–84	12–50	60–99	24–54	38–88	16–50
30–60	20–72	6–40	44–92	14–48	38–100	15-58	38–88	17–50

5.2.1.3 Effect of low nutrient supplementation

In this experimental run, supplementary nutrient solution was added whenever hexane removal dropped below 50%. The drained liquid which contained unused nitrogen was recycled back to the columns twice a week. This approach was used to avoid the excessive biomass growth observed during the high nutrient supplementation, and at the same time maintain relatively high biodegradation rates and long term biofilter operation. Figures 5.15 and 5.16 show the performance of biofilters containing solid media made of 100% peat, 100% pearlite and the mixtures.

The results indicate that the biodegradation of hexane increased from zero soon after the start of the experimental run in all biofilters due to the availability of the supplementary nutrients in the solid media. The biodegradation of hexane decreased gradually with time until another addition of the supplementary nutrients. The hexane removal (Figures 5.15 and 5.16) increased during the first 6 days of biofiltration, reaching the maximum values. With the exception of the column containing pearlite, the hexane removal remained at a maximum until the 14^{th} day then decreased gradually until the 30^{th} day when the second nutrient solution was added. From the 30^{th} to 60^{th} day the hexane removal pattern was similar to the first 30 days such that the ranges of percentage removal and amount of hexane removed were the same (Table 5.4). A similar rebounding property of biofilter operation was observed when nutrients were re-added to the solid medium (Wittorf *et al.* [168]) during biofiltration of a mixture of alcohols.

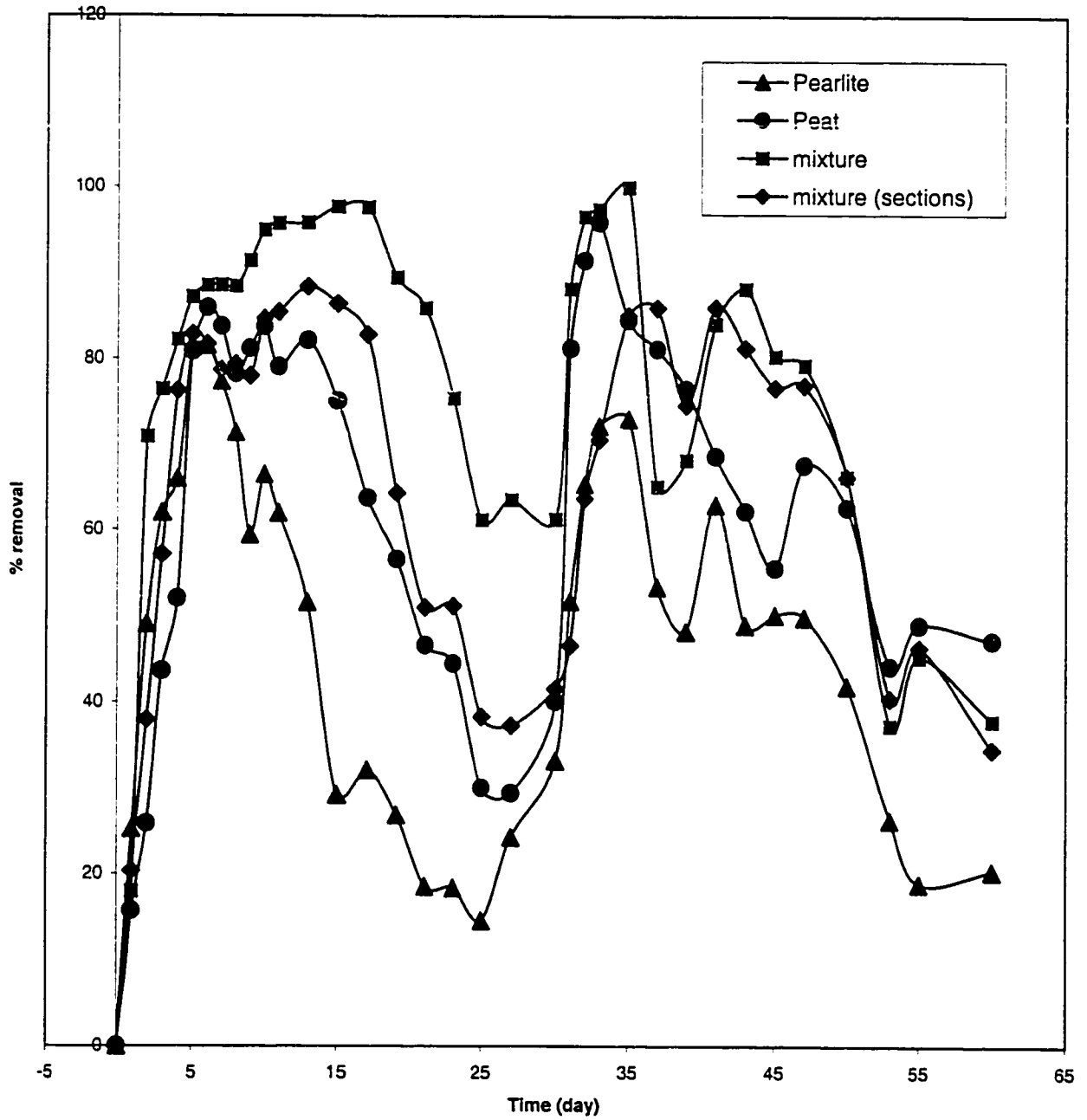


Figure 5.15: Hexane percent removal (low nutrient supplementation)

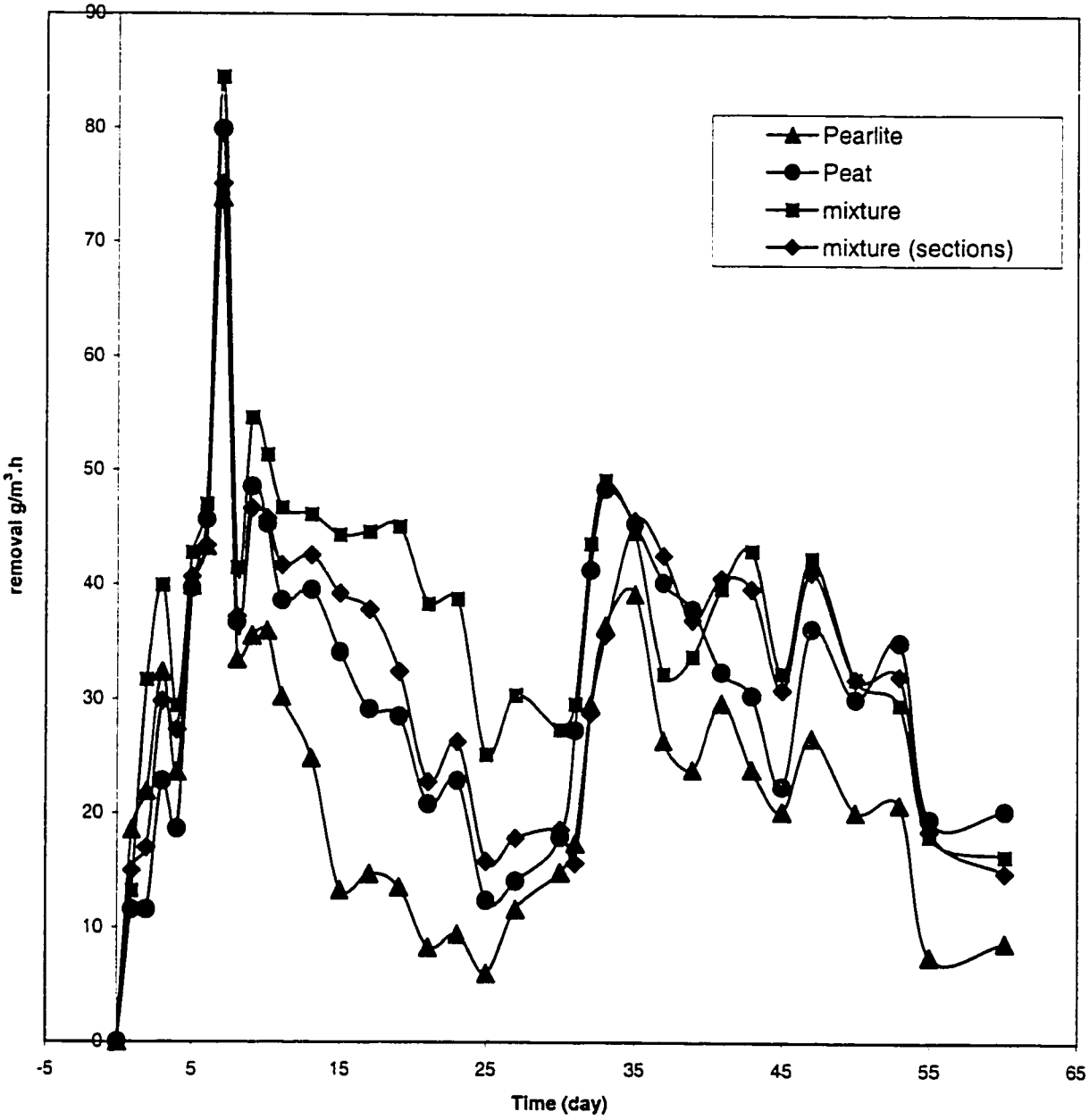


Figure 5.16: Hexane removal rates in $g/m^3.h$ (low nutrient supplementation)

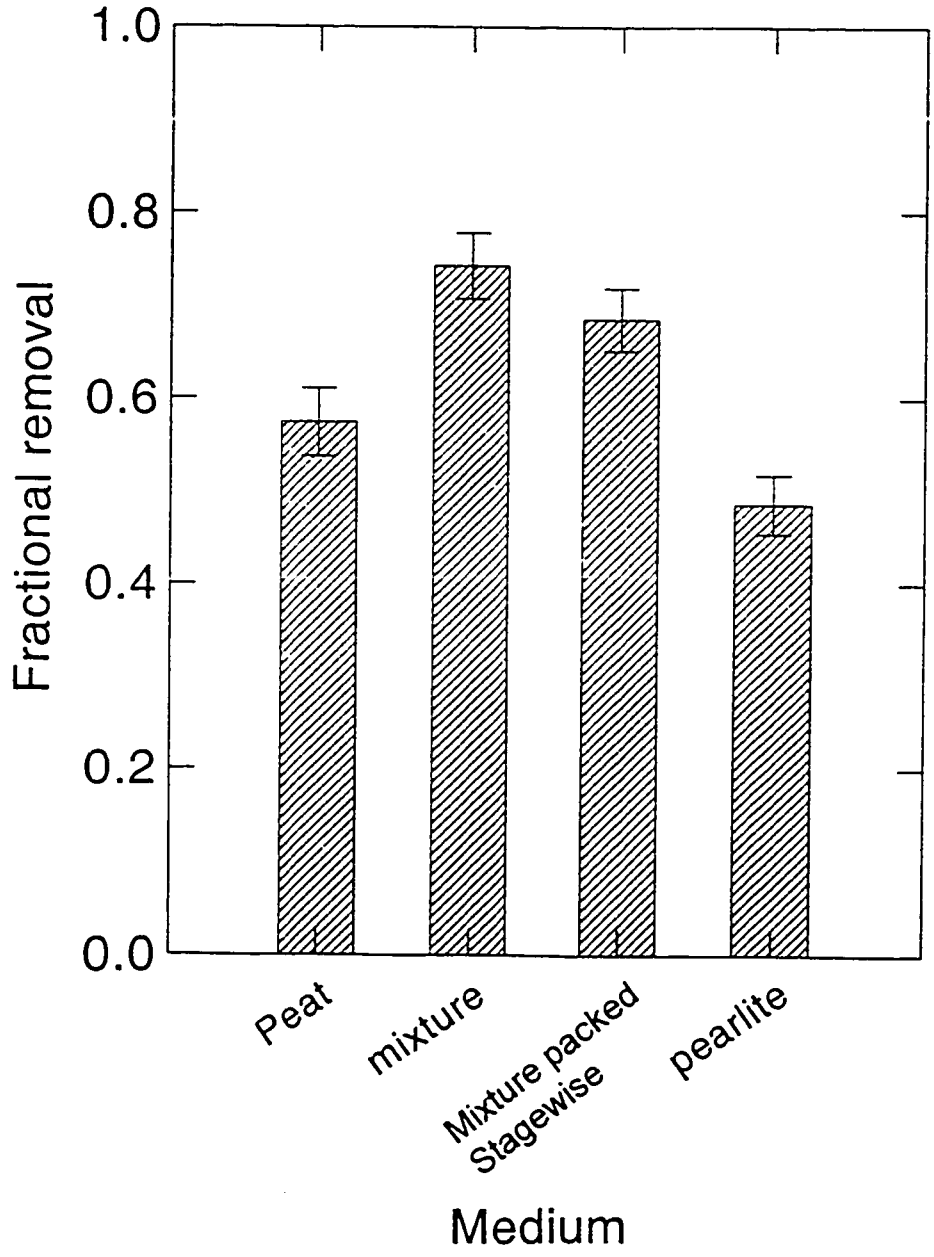


Figure 5.17: Mean comparison for hexane fractional removal (low nutrient supplementation)

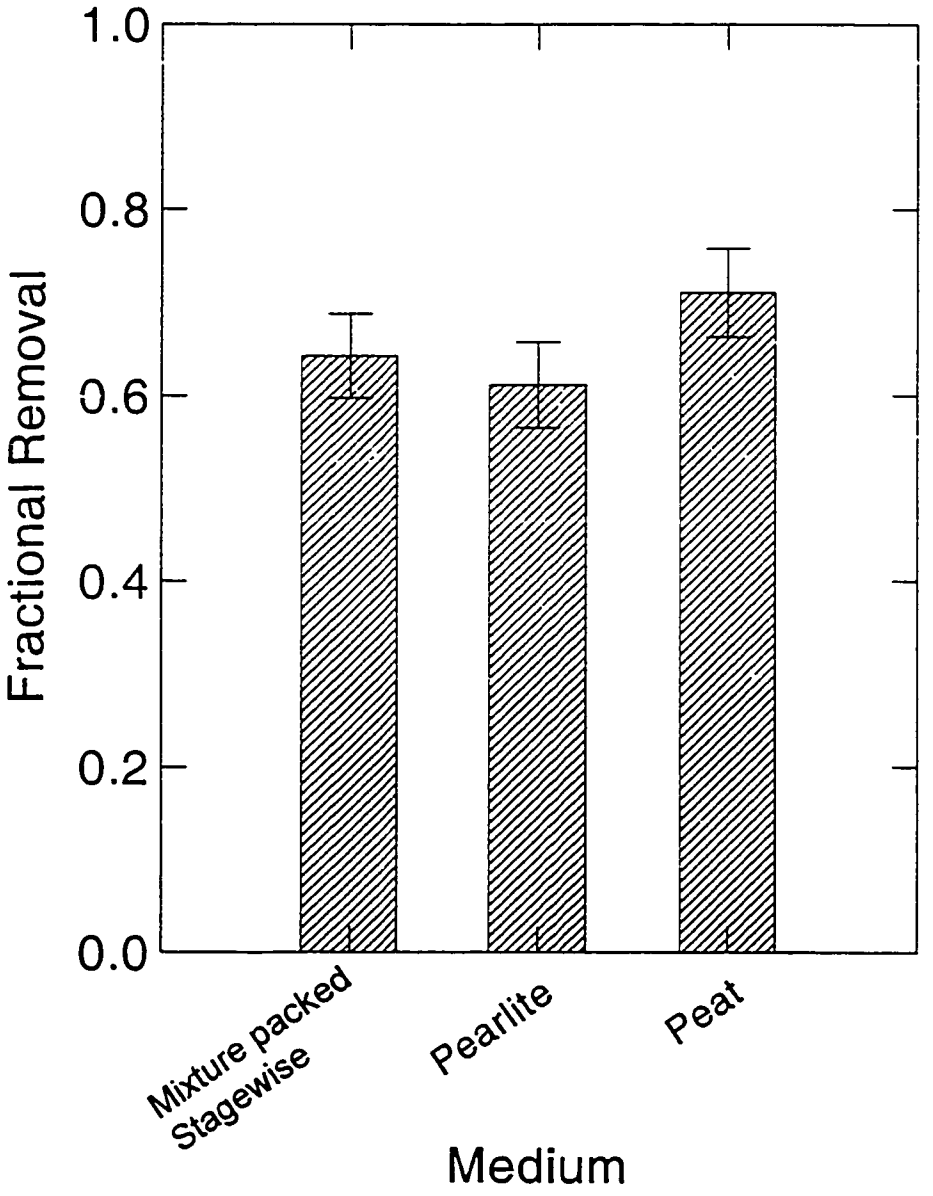


Figure 5.18: Mean comparison for hexane fractional removal, long term biofiltration (low nutrient supplementation)

The biofiltration operation period was extended beyond 11 months during low nutrient supplementation operation compared to 2 months for high nutrient supplementation. Long term biofiltration is illustrated in Figures 5.40 and 5.41. The biofilter containing peat was able to operate for 11 months before failure. Similarly, the columns containing pearlite and the mixture (packed in stages) were operated for more than 11 months, as shown in Figures D.2 through D.5 in appendix D. Throughout the long term biofiltration, the hexane removal rates were maintained between 10 and 50 $g/m^3.h$ depending on the hexane inlet concentration and the time course following addition of supplementary nutrients. From the above mentioned Figures, it was observed that each time the new supplementary nutrients were added, the hexane percent removal increased from 50% or less to more than 90%. The observation confirmed the strong dependency of VOCs' removal rates on availability of nitrogen in the solid media. Biofiltration rates can therefore be increased by periodic addition of supplementary nutrients. Unlike the high nutrient supplementation protocol which failed within 60 days, biofilters under reduced nutrient addition protocol can be operated without failure for at least 11 months. This opportunity of long-term biofilter operation comes with one technical difficulty. The Periodic oscillation of biofiltration rate due to changes in nitrogen concentration does not allow biofilters to be operated at constant removal rates. It is therefore suggested that more work is needed to reduce the oscillation of biofiltration rates. Addition of low concentration of ammonia gas or adding less nutrients more frequently are suggested methods for reducing oscillating conditions.

Figures 5.15 and 5.16 show that the removal was highest for the column containing the mixture, followed by the column containing mixture in section, then peat and pearlite. Statistical analysis for fractional conversion of hexane data show that at a 95% confidence level the least squares means (Figure 5.17) of both media composed of the mixture were not significantly different. Hence, the presence of stages

in one column did not significantly influence the biodegradation of hexane. However, fractional conversion for the mixtures were significantly higher than that of pearlite, and only the mixture (without stages) showed a significantly higher fractional conversion than that of the peat medium. The fractional conversion for both peat and pearlite media were not significantly different. The observation indicates that within a short time of 60 days, the highest biodegradation is achieved by a combination of both pearlite and peat into a single medium. The results were not surprising since the mixture provides better properties than peat or pearlite alone. The mixture had more nitrogen content (from the peat) than pearlite (Section 5.2.2) and better pneumatic conductivity than the peat alone. Peat provides a high water retention and immobilization capacity while pearlite provides mechanical stability and improves pneumatic conductivity. Statistical analysis of long term biofiltration suggested that at low nutrient supplementation (Figure 5.18), there were no significant differences among the fractional conversions of hexane for all three media (pearlite, peat and the mixture) at a 90% confidence level. At 95% confidence level, the average removal rate of hexane in the column containing peat medium was higher than average removal rates in the columns containing pearlite and the mixture. This observation suggests that once there is sufficient biomass accumulation from growth, the type of the biofilter medium does not have a significant effect on the biofiltration rate. Since the medium has little influence on the biofiltration rates it suggested here that a mechanically stable solid medium be used. This does not only offer the opportunity of reducing the aging process and better pneumatic conductivity of the solid medium, but also the possibility of reducing the oscillation properties of biofiltration rates. The mechanically strong solid medium can withstand a mechanical treatment such as scouring to remove excess biomass, hence allowing biofiltration process to be conducted at a nutrient addition protocol higher than once a month in order to reduce the oscillation of biodegradation rates.

5.2.2 Nitrogen utilization in the biofilters

The biodegradation of hexane was accompanied by utilization of nitrogen. Analysis of ammonia nitrogen concentration in the solid media (Figure 5.19) showed that increased removal rates occurred when a significant amount of ammonia was available in the solid medium and decreased as the ammonia content fell. The dependency of biodegradation rates on nitrogen suggests that hexane removal was mainly due to microbial growth; i.e. through assimilation into biomass and oxidation to provide energy for both growth and maintenance. In the absence of a nitrogen source, hexane was removed through oxidation to provide maintenance energy, and limited microbial growth depending on how fast the nitrogen was recycled from dead cells. VOC removal can therefore be described by the following mechanism:

$$\text{VOC} + O_2 + \text{N, P} \longrightarrow \text{Biomass} + \text{Energy} + CO_2 \qquad (5.7)$$

From Equation 5.7, VOC utilization must be higher when there is a significant amount of nitrogen to support the growth than when nitrogen concentration in the biofilter is low. The ratio of carbon removal to nitrogen utilization (C/N ratio), which is variable depending on the frequency and amount of nitrogen added, supports the utilization of the nitrogen for growth and maintenance. Table 5.5 shows that during high nutrient supplementation, the C/N ratio was low (14-15), and high during low nutrient supplementation ranging from 52 to 66. The C/N results indicate that the fraction of total carbon used for maintenance energy during low nutrient supplementation was higher than during high nutrient supplementation due to overall low nitrogen concentration in the solid media.

This explains why high removal rates (up to 50 $g/m^3.h$) were observed when average values of ammonia nitrogen in the three sections (top, middle and bottom) of the solid media were between 100-500 g per m^3. Hexane removal decreased considerably

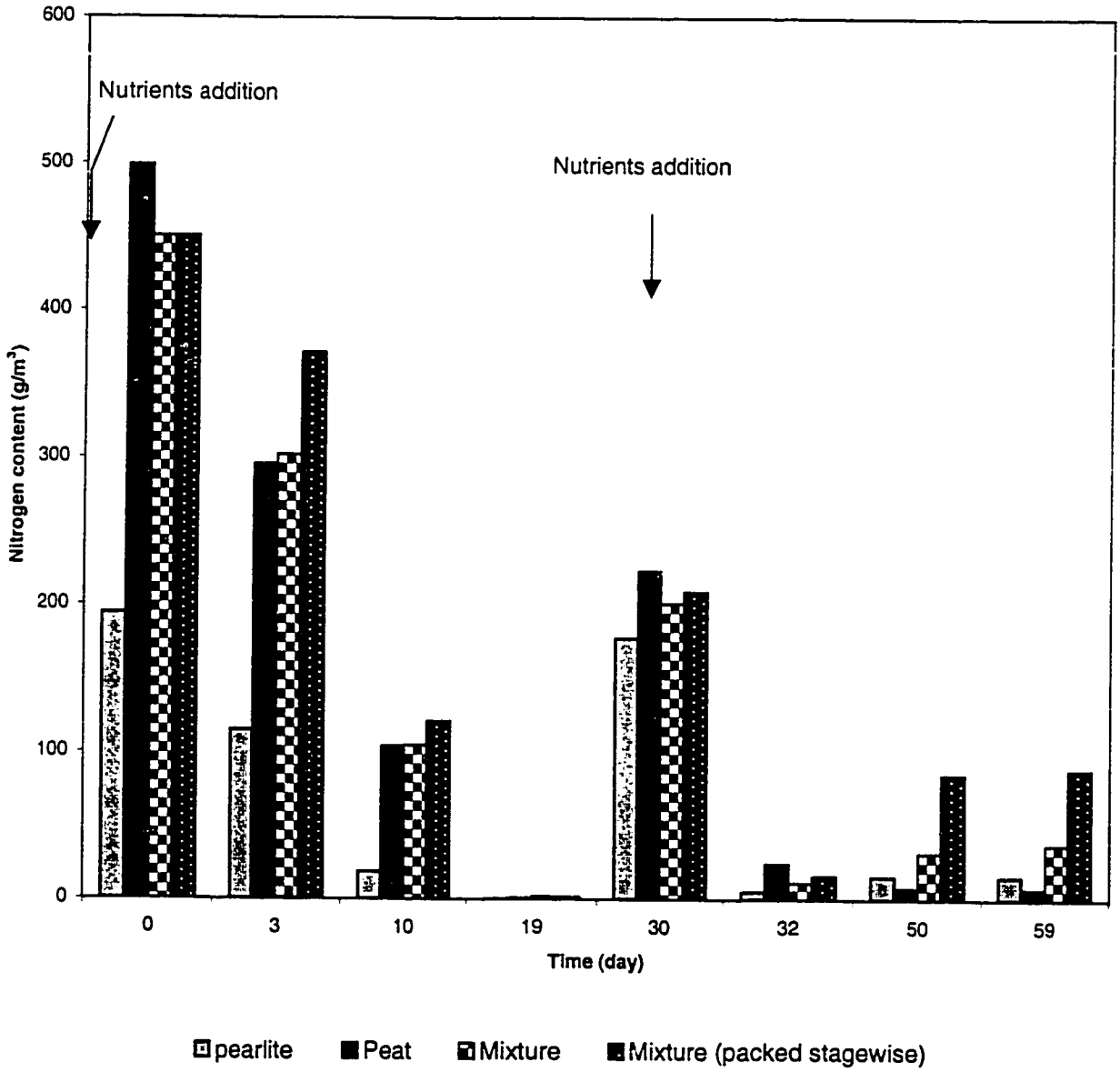


Figure 5.19: Nitrogen utilization in biofilters (low nutrient supplementation)

(to below 20 $g/m^3.h$) after average values of ammonia nitrogen content in the solid media fell below 1 g/m^3 (Figures 5.15, 5.16 and 5.19).

At the beginning of an experimental run, all columns received the same amount of ammonia nitrogen (from the same solution) per unit volume, but the columns containing peat and mixtures registered higher ammonia nitrogen (500 and 450 g/m^3 respectively) than the column containing pearlite (200 g/m^3) despite the fact that negligible amounts of liquid drained from the columns (Figure 5.19). The extra ammonia nitrogen can only come from the peat. The peat used in this research contained 4% (w/w) total nitrogen (Kjeldahl method), which is equivalent to 6640 g/m^3 of dry peat (density 166 kg/m^3). A fraction of this nitrogen is assumed to be available for microbial growth. This explains why ammonia nitrogen was higher in the columns containing peat and mixtures than the column containing pearlite. When nutrient solution was added to the columns for the second time (day 30, Figure 5.19), variation in ammonia adsorption capacity was observed. The amount of immobilized nitrogen was calculated as the difference in ammonia nitrogen contents of the added nutrient solution and the liquid drained from the columns. Peat and the mixtures showed higher ammonia immobilization capacity (240 and 200 g/m^3 respectively) than pearlite (180 g/m^3). This indicated that the peat adsorption capacity for ammonia is 30% more than pearlite.

The concentration of ammonia nitrogen within the columns decreased faster in the bottom section than in the middle and top sections in all media formulations except 100% pearlite (Figures D.6 through D.9, in appendix D). The nitrogen concentration gradient along the biofilter columns was due to high removal rates of nutrients in the bottom sections where the polluted gas entered the column. As explained in the previous section, the hexane removal rate was dependent on hexane concentration. Therefore the hexane removal rate was higher at the bottom where the hexane concentration was high, resulting in higher nitrogen utilization than in

the top sections. Secondly, the drained water was recycled through the top of the columns resulting in a high accumulation of nitrogen in the top sections.

From the above observations, supplementary nutrients, particularly nitrogen, play an important role in biodegradation process and should be supplied to biofilters to improve biodegradation rates. Managing the excess biomass produced by microbial growth remains a challenge, hence further work is needed in this area.

5.2.3 Carbon dioxide from biofilters

The amount of carbon dioxide generated from the biofilters differed depending on the type of the solid media. In order of magnitude from highest to lowest, CO_2 was generated from columns containing the mixture, mixture (packed stagewise), peat and pearlite (Figure 5.20). The pattern of CO_2 generation is similar to the pattern of hexane removal rates (Figures 5.15 and 5.16). As expected, the more hexane removal the more CO_2 was generated. During the first 5 days of biofiltration and after a second addition of nutrient solution, carbon dioxide released from the biofilters increased rapidly from zero to more than 1400 ppm, then decreased to values between 1000 and 200 ppm after 30 days of biofiltration. Upon the second addition of nutrient solution, the CO_2 generation pattern was repeated. The carbon dioxide released was high (1200–1600 ppm) for the first three days after addition of supplementary nutrients, it gradually decreased to 400–800 ppm within the next 30 days as nitrogen concentration was depleted. The results show a strong correlation between the hexane removal rates and CO_2 generation in the biofilters, they both had the same pattern which strongly depended on nitrogen content.

The amount of carbon in the carbon dioxide released from the biofilters was compared to the carbon calculated from the biodegraded hexane. The ratio of carbon in the CO_2 to the carbon of the biodegraded hexane was less than 0.5 in the first week when the nitrogen content was relatively high (Figure 5.21). The ratio increased

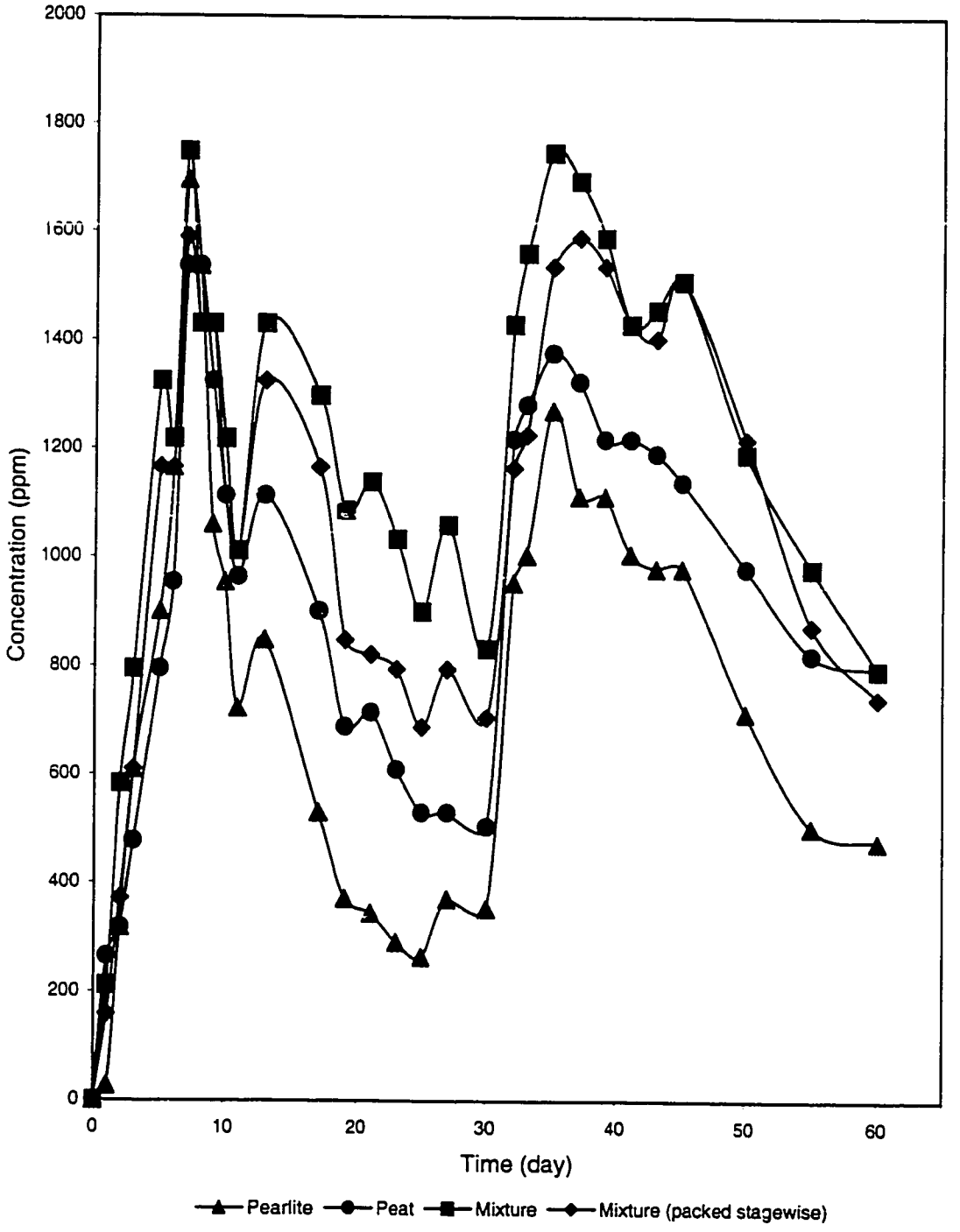


Figure 5.20: Measured CO_2 from the biofilters (low nutrient supplementation)

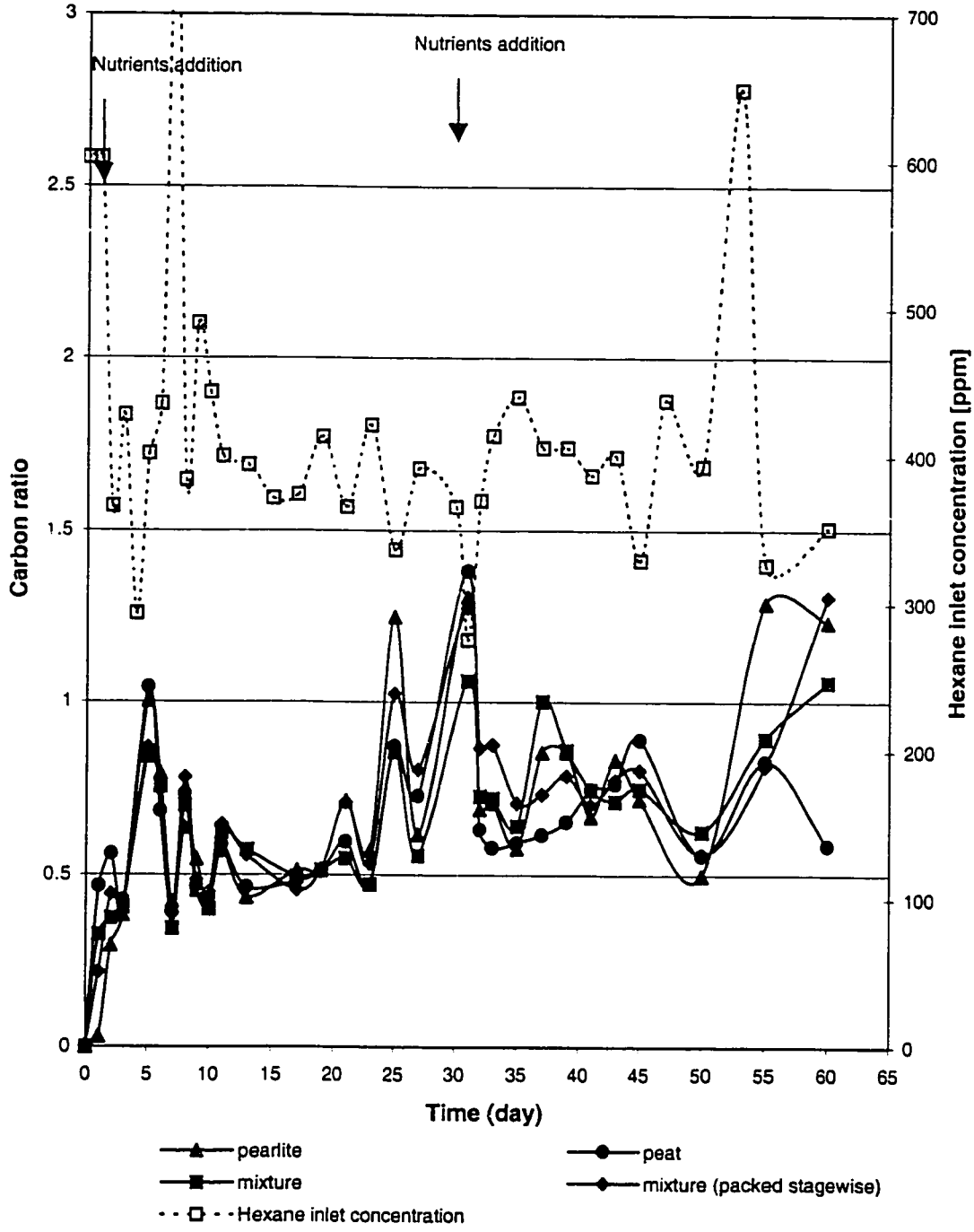


Figure 5.21: Ratio of carbon in CO_2 to the carbon of the biodegraded hexane

gradually to values between 0.5 and 1 after three weeks of biofiltration and remained within the same range after the second addition of nutrients. The data suggests that, during the first week, approximately half of the biodegraded hexane was used for microbial growth and the other half was used for maintenance energy. As the nitrogen concentration decreases the ratio of the biodegraded hexane used for maintenance increased to approximately 0.7 (Figure 5.21). The ratio of carbon in CO_2 to carbon of the biodegraded hexane did not decrease significantly after the second addition of supplementary nutrients. Since the quantity of the nitrogen immobilized was lower during the second addition of nutrients (Figure 5.19) it might have been insufficient to lower the carbon ratio to 0.5. Moreover, the carbon dioxide in the biofilters was not produced as a result of microbial growth and maintenance metabolism alone, it also came from a decomposition of dying cells and neutralization of acid products by calcium carbonate. In addition, the maintenance energy depends on many factors such as pH, ionic strength and the source of nutrients [108] which might have changed between the first and second addition of nutrients.

From the above observation, carbon dioxide release from biofilters can be used as a measure of biofiltration performance. Low concentration of carbon dioxide indicated low biodegradation rates. The ratio of carbon in the CO_2 to carbon of the biodegraded hexane can give an estimations of carbon, hence biomass accumulating in biofilters. In the absence of other measurements, CO_2 cannot give an overall biodegradation performance especially when decaying solid media such as peat and compost are used or when there is high microbial death which releases more CO_2. Biofiltration conditions such as high ionic strength, and nitrogen source which requires more energy to utilize, might enhances high carbon ratio (i.e. more VOC used for maintenance energy than for growth) might reduce the biomass yield from the VOC leading to low biomass accumulation in biofilters. However, this might come with the price of lower average biodegradation rates.

5.2.4 Microbial density in biofilters

The microbial density in the biofilters is illustrated in Figures 5.22 and 5.23. In all solid media formulations, the average bacterial density was more than 10^{13} CFU per gram of wet solid medium after 10 days of biofiltration. The bacterial cell density remained approximately constant between 10^{13} and $10^{12.5}$ by day 30. Addition of the second nutrient solution did not significantly change the cell density values except for the column containing pearlite alone.

The microbial density for fungi was lower than that for bacteria (Figure 5.23). The average fungal density reached the maximum values of 10^{10} CFU/g by the 3rd day of biofiltration and thereafter decreased and remained at 10^{9}. The fact that the microbial density for bacteria was greater than that of fungi was mainly due to environmental conditions. The pH values for all columns were above 5 favouring growth of bacteria. Secondly, the substrate (hexane) is toxic to most microorganisms except *Pseudomonas* sp. (Chapter 4).

The microbial density indicates there was a significant biological activity. Other researchers have reported a varying number of microbial density in the biofilters. Microbial density without addition of nutrients ranges between 10^{6} to 10^{8} CFU per gram of solid medium [37, 103]. With supplementary nutrients added to biofilters, microbial density is reported to be higher. Kiared *et al.* [68] reports maximum bacterial density of 10^{13} which stabilized at 10^{9} per gram (wet basis). Francis *et al.* [125] reports microbial density 5 x10^{9} and 5.5x10^{8} during biofiltration of toluene using ammonia and nitrate supplements, respectively. High bacterial density 10^{9}–10^{11} is also reported in the removal of ammonia and toluene in biofilters [1, 84]. The microbial density observed in this study was slightly higher but comparable to the literature values.

The results and other information from the literature show that, high biodegradation rates are achieved when viable bacterial density is 10^{9} or more per gram of

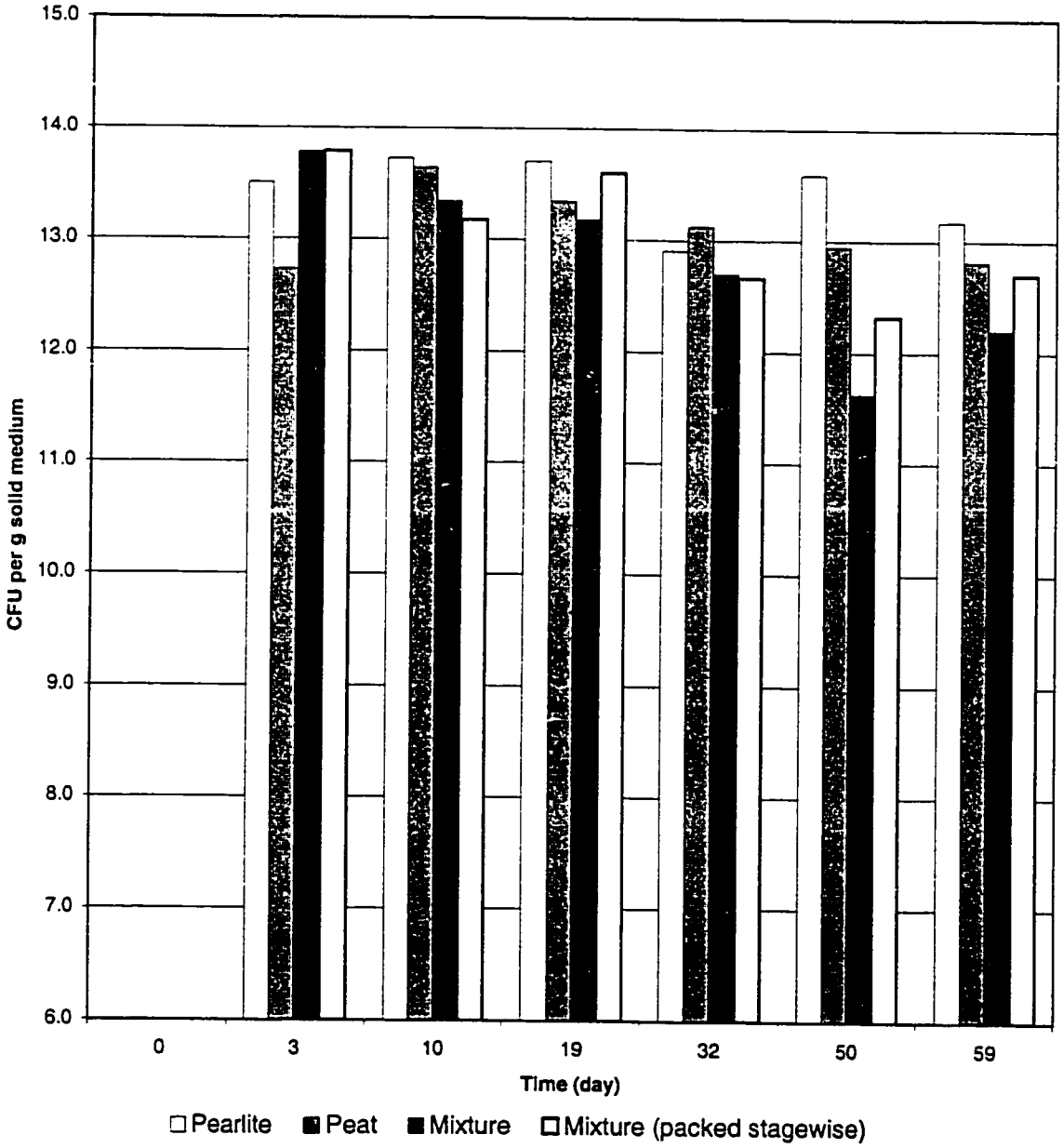


Figure 5.22: Microbial density for bacteria in the biofilters

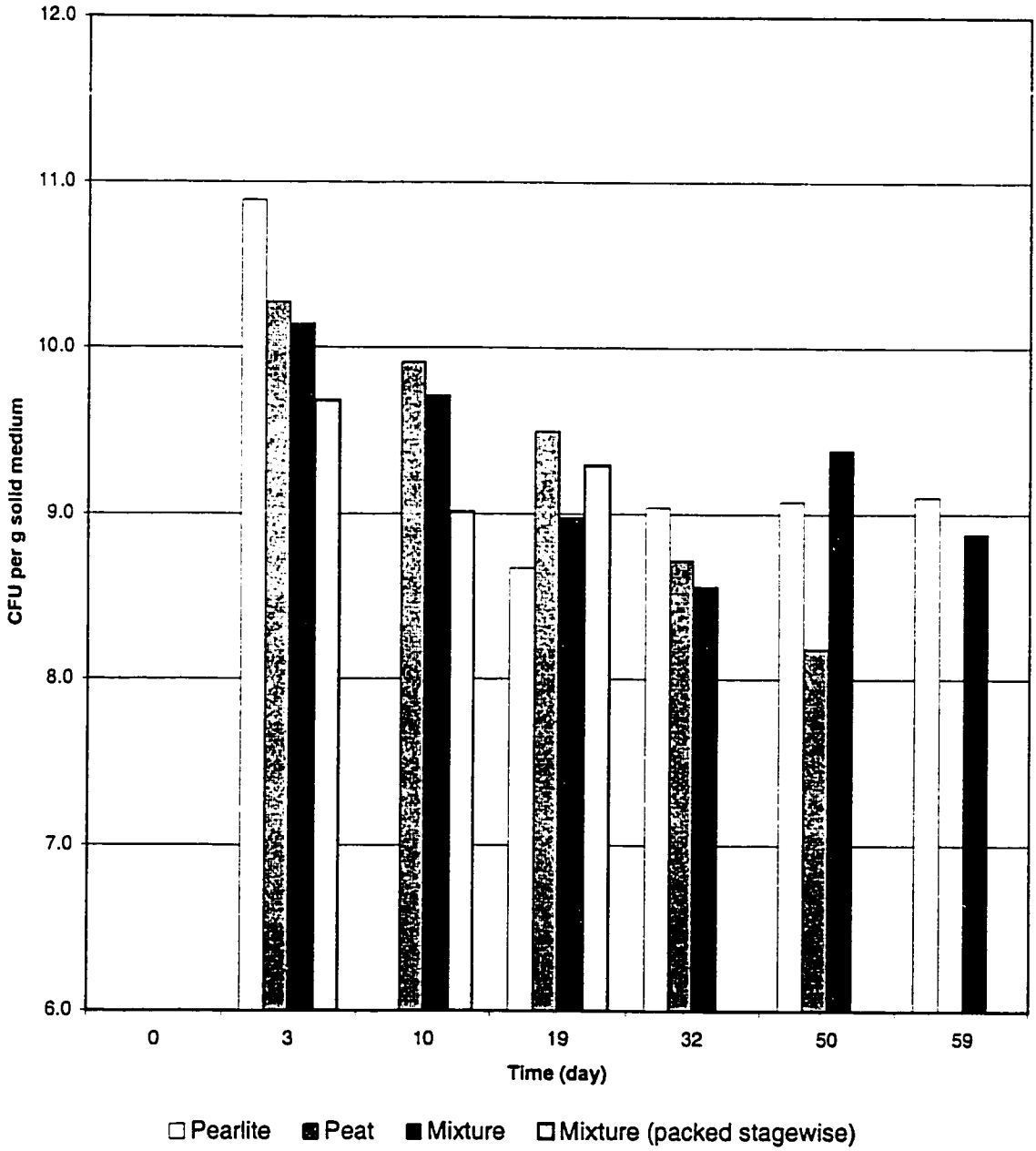


Figure 5.23: Microbial density for fungi in the biofilters

wet solid medium. Therefore, factors favouring high bacterial density such as pH and availability of nutrients must be maintained. Since the microbial density was satisfactory in the inert medium (the pearlite), and the biofiltration rate in the column containing pearlite was comparable to the biofiltration rates in the columns containing other media formulations (peat and the mixture), pearlite can be used as the only component of the solid medium for biofiltration. The inoculum provides the source of microorganisms which under nutrient addition conditions are able to survive and multiply. It is therefore important that this area of using inert medium for biodegradation process be explored further in order to eliminate the aging problem caused by organic solid media.

5.2.5 Pressure drop

Pressure drop values across the biofilters columns for the different media formulations were measured without biofiltration and during biofiltration under high and low nutrient supplementation conditions. Results indicate an increase of pressure drop with time during biofiltration and sensitivity of pressure drop to moisture content before and during biofiltration.

5.2.5.1 Pressure drop of fresh solid media

Results of pressure drop across the columns containing peat and the mixture before biofiltration are shown in Figures 5.24 and D.10, appendix D. Neither hexane was flowing through the columns nor inoculation had been performed when the measurements were taken. For a particular moisture content, all solid media showed an increase in pressure drop with increasing superficial velocity between 0 and 40 $m^3/m^2.h$. For a particular gas flow rate, the pressure drop for peat and the mixture showed peak values with increasing solid moisture contents as shown in Figure 5.25.

The pressure drop values for solid media composed of 100% peat and the mixture of peat and pearlite exhibited maximum values at moisture contents of 47% and 30% (w/w), respectively. A similar pressure drop pattern for a mixture of compost and polystyrene spheres was observed by Ottengraf [98]. The pressure drop value of the solid medium composed of 100% pearlite at a given gas flow rate did not vary with solid moisture content.

It is clear from the composition of the solid media that the peak values of pressure drop when the moisture content is between 30 and 50% is caused by the presence of peat. This phenomena can be explained from the characteristics of peat in the presence of water. It was visually observed that when dry peat was placed in a beaker of water: it absorbed water and swelled. The swelling effect reduces the inter-particle spaces and results in higher pressure drop. At much higher moisture content beyond the maximum pressure drop values, it is assumed the peat particles agglomerate, resulting in a system which behaves as if the solid particles were large. The swelling properties of the peat may negatively affect the biofiltration process by reducing pneumatic conductivity of a solid medium and increasing pressure drop.

5.2.5.2 Pressure drop during high nutrient supplementation

Figure 5.26 shows pressure drop trends across the columns during high (weekly) nutrient supplementation. The column containing the mixture showed higher pressure drop values than the column containing pearlite. The pressure drop for pearlite increased as the gas flow rate increased. During the first 10 days, at 20 $m^3/m^2.h$, the pressure drop was between 20–30 Pa/m. It increased to 130–230 Pa/m at 100 $m^3/m^2.h$, then decreased to 50–100 Pa/m at 30 $m^3/m^2.h$. At the time of failure, reducing flow rate from 30 to 20 $m^3/m^2.h$ did not reduce the pressure drop. Instead it increased to 80–120 Pa/m. Similarly for the mixture, the pressure drop was 58–94 Pa/m at 20 $m^3/m^2.h$. It increased to 890–1730 Pa/m at 100 $m^3/m^2.h$, then decreased

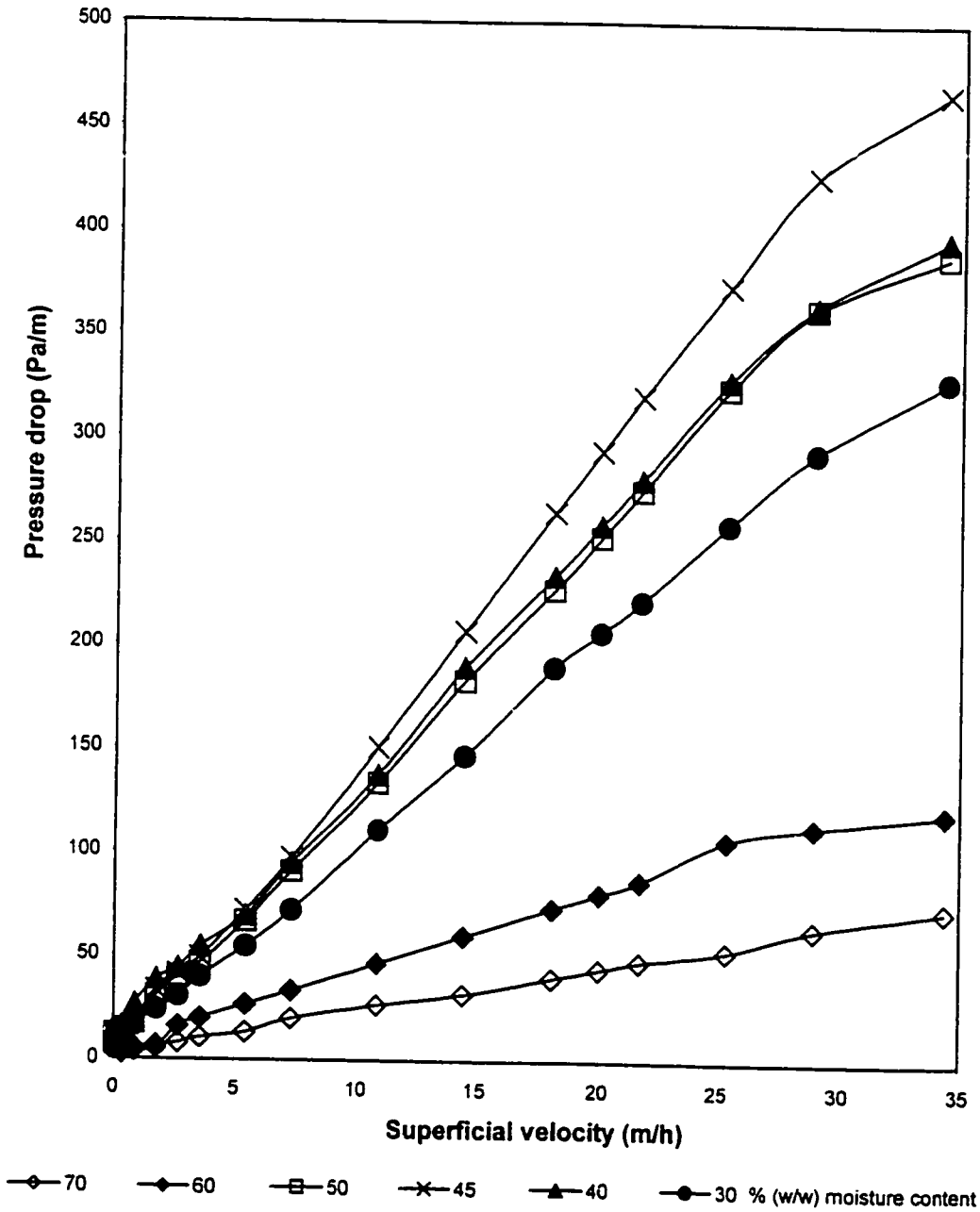


Figure 5.24: Variation of pressure drop of fresh peat with moisture content and flow rate

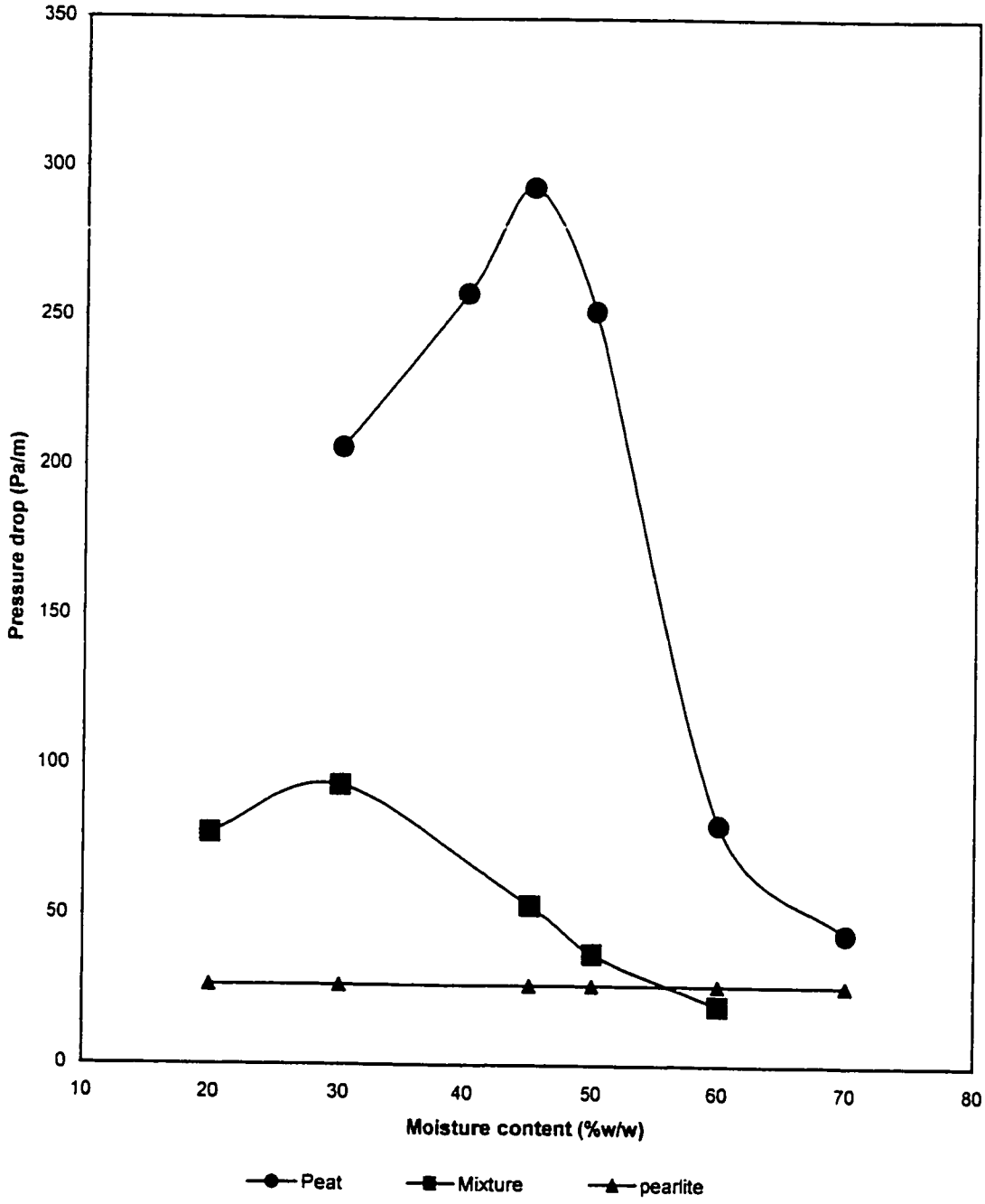


Figure 5.25: Variation of pressure drop with moisture content at 20 $m^3/m^2.h$ superficial velocity

to 320–1770 Pa/m at 30 $m^3/m^2.h$. At the time of failure, reducing the flow rate from 30 to 20 $m^3/m^2.h$ did not reduce the pressure drop. Instead it increased to 2400–2930 Pa/m.

Due to the addition of supplementary nutrients, biomass overgrowth was visually observed. The biomass overgrowth was so high that even with relatively low pressure drop, supplementary nutrient solution could not pass down the pearlite medium during the last week of the experiment. At the point of closing the experiment, disintegration of peat particles into much smaller ones was visually observed. It was noted that high pressure drop and low pH values (Section 5.2.6) were observed prior to the biofiltration failure.

High pressure drop is not desired in biofilter operations due to a high running cost associated with it. It is therefore necessary that the pressure drop remain as low as possible. Under high nutrient supplementation, the column containing the pearlite showed a lower pressure drop than the column containing the mixture. Since the biofiltration rates for both columns containing the pearlite and the mixture were not significantly different, the pearlite offers an opportunity of running a biofiltration process at a lower and stable pressure drop. However, due to excessive biomass growth in the top section as a result of introducing the nutrient solution at the top of the column, the nutrient solution did not pass down the column containing pearlite, hence long-term operation is uncertain. A better method of introducing the nutrient solution into the column, or more important, controlling the pH to reduce fungal growth and biofilter failure might make the pearlite more attractive as the solid medium for a long-term biofiltration process.

5.2.5.3 Pressure drop during low nutrient supplementation

Pressure drop profiles under reduced supplementary nutrient addition are shown in Figure 5.27. Throughout 60 days of the experiment, the column containing 100%

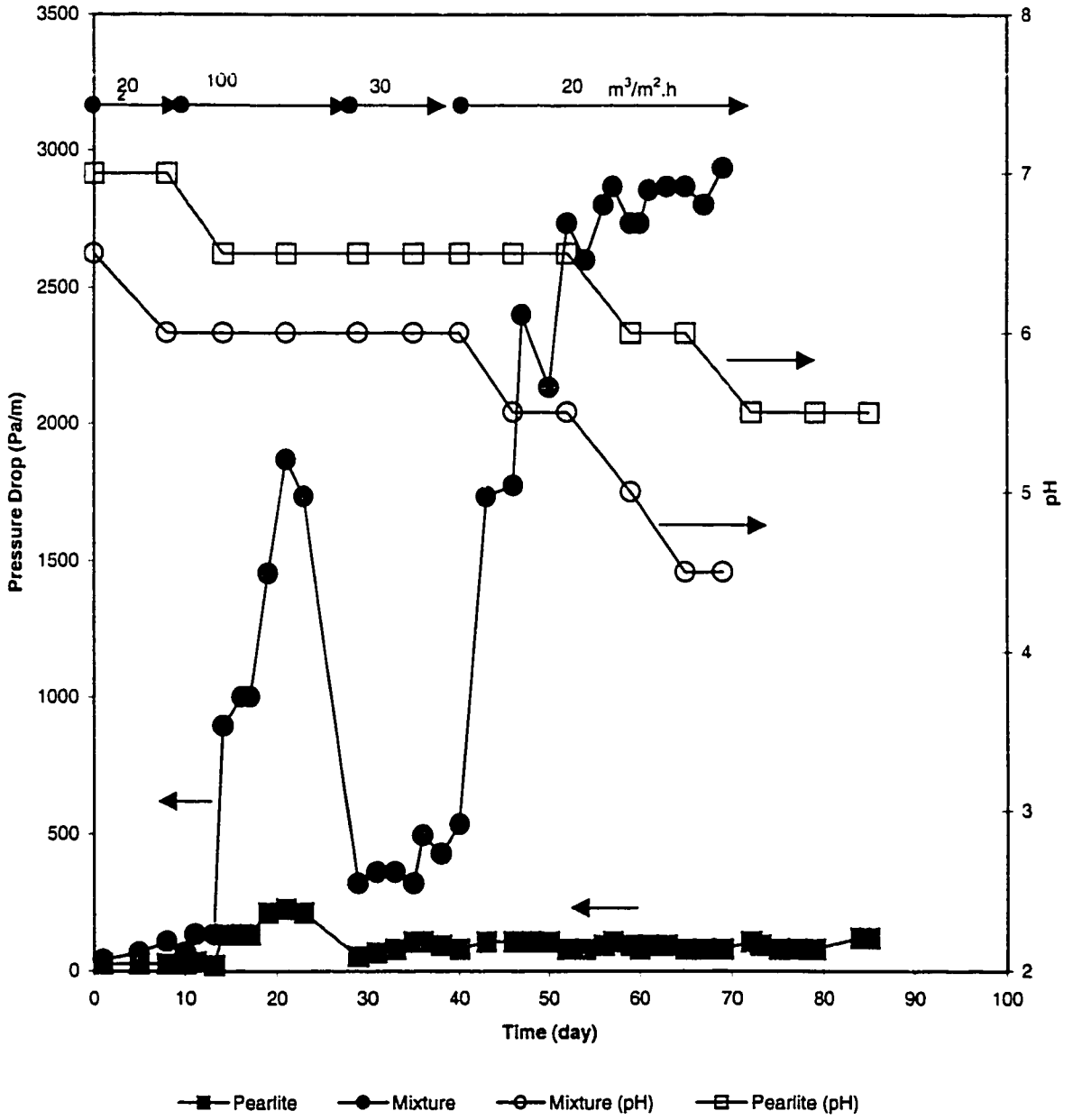


Figure 5.26: Variation of pressure drop and pH during high nutrient supplementation

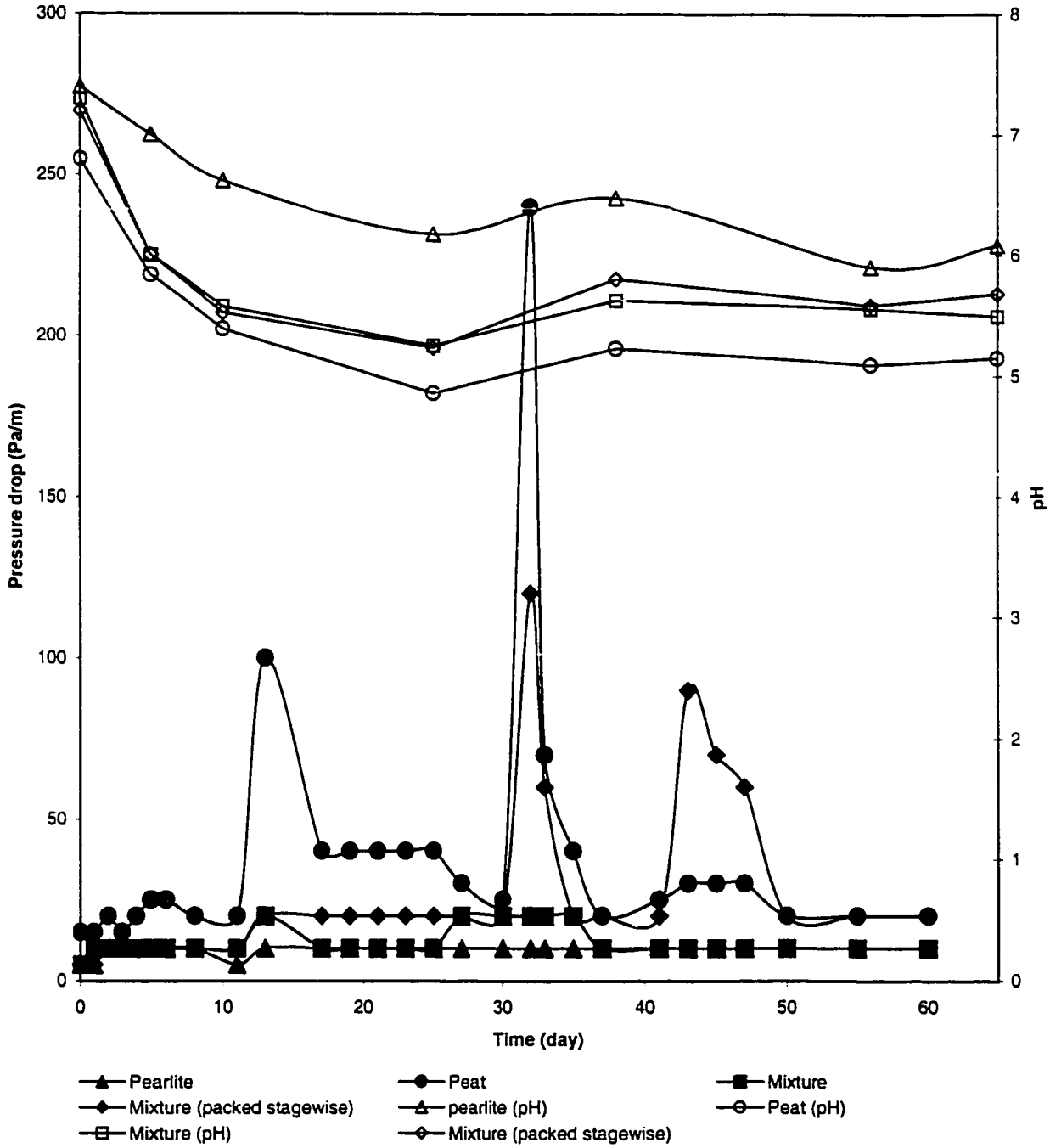


Figure 5.27: Variation of pressure drop and pH during low nutrient supplementation

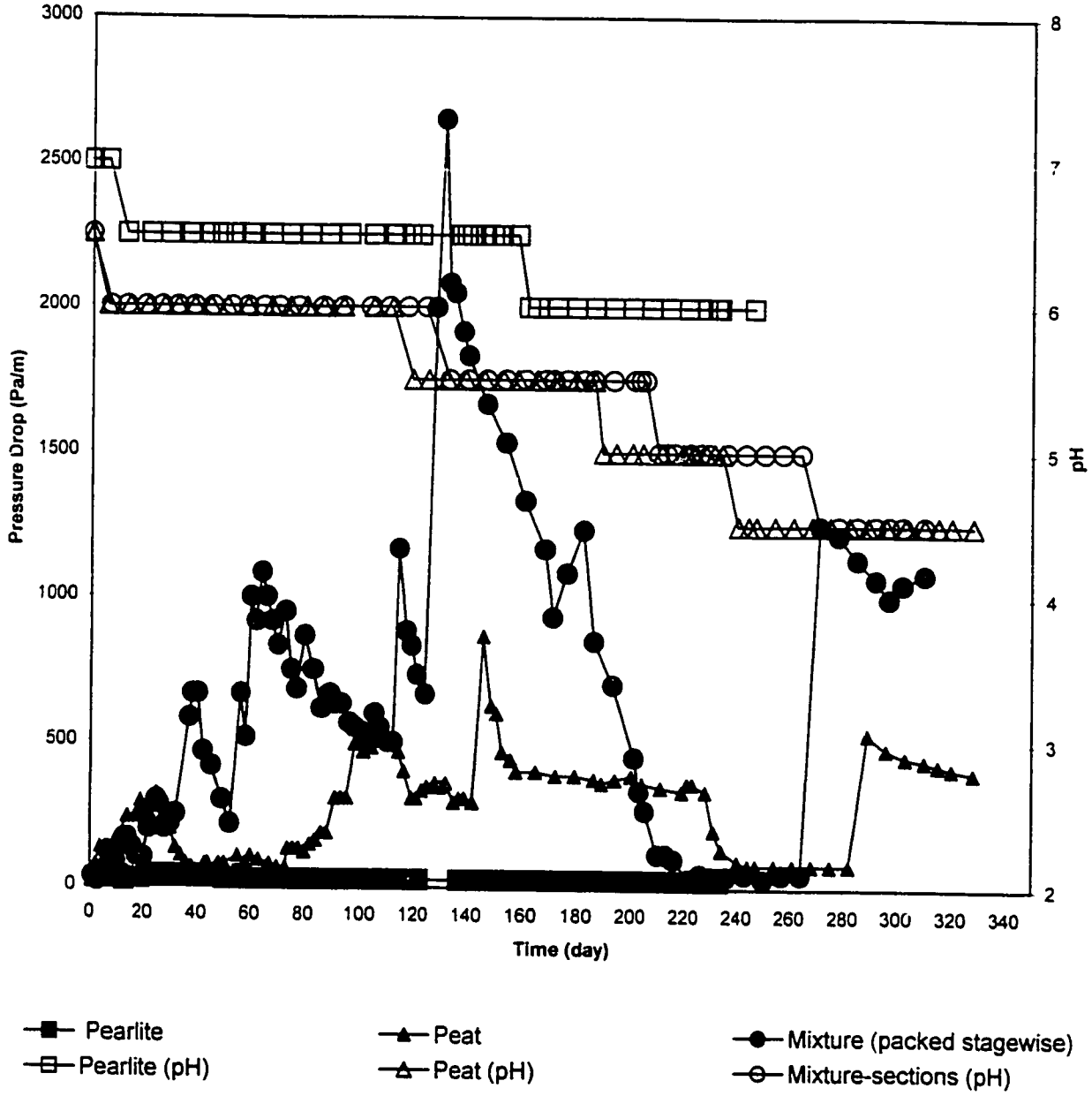


Figure 5.28: Variation of pressure drop and pH during low nutrient supplementation; long term biofiltration

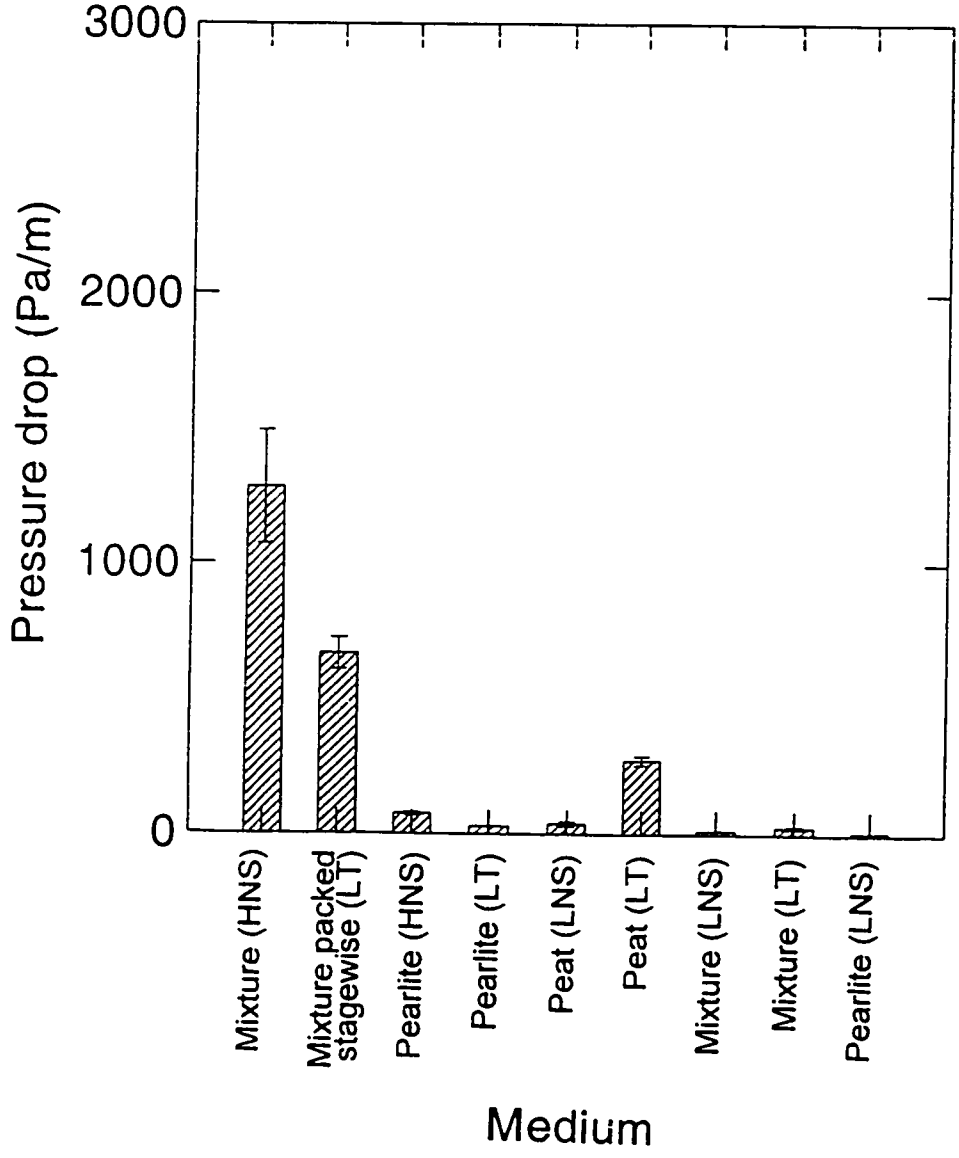


Figure 5.29: Mean comparison for the pressure drops of different media and different supplementary nutrient addition protocol

pearlite showed the lowest pressure drop with an average value of 10 Pa/m while the peat-containing column showed an average pressure drop of 30–50 Pa/m and a maximum peak value of 250 Pa/m. Pressure drop for the mixture-containing column ranged between 10–20 Pa/m while that of the column with the mixture packed stagewise averaged 30 Pa/m with maximum peaks of 120 Pa/m. Pressure drop for the columns containing peat and the mixture packed stagewise were sensitive to moisture content. The peak values were observed after addition of supplementary nutrients or water to the columns. The pressure drop values for the column containing peat was related to the swelling properties of peat when water was added. The high pressure drop values for the column containing the mixture packed stagewise was due to the large number of screens used to support the solid medium. The medium was supported by five screens compared to one screen in the other mixture-containing column that was not sensitive to moisture addition. The biomass growth had a tendency to obstruct the screen openings, hence increasing the pressure drop. Addition experiment confirmed that pressure drop for both pearlite and the mixture was higher when the solid media packed stagewise than when the solid media was packed without sections.

Long term operation of three columns containing pearlite, peat and the mixture packed in stages was conducted for more than 11 months. Results showed that the maximum pressure drop for pearlite was 40 Pa/m (Figure 5.28). The column containing the mixture packed in stages showed the highest pressure drop ranging between 80 Pa/m to 1000 Pa/m and had a peak value of 2500 Pa/m. The pressure drop for peat had an average value of 250 Pa/m and a peak value of 800 Pa/m. Kiared *et al.*, reports a pressure drop of 600 Pa/m at a flow rate of 70 $m^3/m^2.h$ for biofiltration of toluene under nutrient supplementation [68]. Tang *et al.* reports an initial pressure drop of 500 Pa/m which increased to 850 at 80 $m^3/m^2.h$ during biofiltration of acetone [130]. With the exception of a few peaks, the values of the pressure drop

were within the range of recommended pressure drop values 100 and 700 Pa/m for practical application [37, 98]. The sieving of the solid media to size between 1.7–4.75 mm is considered as one of the factors responsible for maintaining the pressure drop in this work within the values suggested by the literature.

The long-term pressure drop trends are of particular interest when it comes to practical applications. The long-term study showed that the biodegradation rates in the column containing peat was significantly higher than the mixture and pearlite, both of which were not significantly different from each other (Figure 5.18). As reported earlier, the peat-containing column showed a significantly higher pressure drop (Figure 5.29) on long-term basis. Moreover, columns containing the peat and the mixture media were sensitive to the addition of the water to restore moisture. Therefore, pearlite appears to be attractive as a solid medium for long-term biofiltration processes under low nutrient supplementation conditions. The mixture is good if its pressure drop sensitivity to the addition of water is controlled (Section 5.2.5.4 below). The mixture still suffers from the aging process of the peat.

5.2.5.4 Effect of moisture content on pressure drop during biofiltration

As mentioned earlier, the columns containing peat showed sensitivity to pressure drop after addition of moisture due to its swelling characteristics. The columns containing the mixture packed in stages showed sensitivity to pressure drop due to five screen supports which were found to be clogged with biomass at the end of experiment. The screen supports added additional resistance to air flow compared to only one screen in other columns. Similar results were obtained when additional experiments were conducted using columns containing pearlite. While pressure drop for the pearlite packed in stages increased to 460 Pa/m in 60 days, the pressure drop of the same medium pearlite without stages remained less than 30 Pa/m. Under nutrient supplementation conditions, the medium support must be properly designed to reduce

unnecessary pressure drop.

With both the peat and the mixture, the peaks occurred within a week after addition of supplementary nutrients. The addition of water (without nutrients) to restore the moisture content in biofilters caused a slight increase in pressure drop which was far less compared to the changes caused by the addition of supplementary nutrients. This association of high values of pressure drop one week after addition of supplementary nutrients suggests that the pressure drop is related to biomass growth.

As the pressure drop increased in columns containing peat and the mixture, an investigation was made on the possibility of restoring it by reducing the moisture content. The addition of water to restore moisture content in the columns was reduced in the long term experimental run with low nutrient supplementation (Figure 5.28). With the exception of the column containing pearlite, a decreasing pressure drop for both peat (between day 245 to 280) and the mixture (between day 220 to 255) was observed. The Pressure drop in the column containing peat decreased from 300 Pa/m to 80 Pa/m while that of the mixture packed stagewise decreased from 1200 Pa/m to 33 Pa/m. The moisture content in the solid media varied from 28% in the driest bottom section to 35% in the top section. The columns lost moisture from bottom upward due to upflow operation. The solid media became bound together by the biomass and the solid media shrank. The shrinkage left an approximate 3 mm gap between the solid matrix and the wall of the column which is approximately 10% of the volume in the most dry sections. The observed low pressure drops were therefore caused by air channelling between the medium and column wall. Shrinking was negligible for the solid medium composed of 100% pearlite. When moisture was restored, swelling of the peat and the mixture were visually observed and the pressure drop values jumped back to high values of 300 Pa/m and 1300 Pa/m, respectively.

The increase in pressure drop due to addition of water to restore moisture was also caused by water being held up in the solid medium by upward flowing air. The

effect could be reduced by having an efficient humidification system and operating the biofilter with air flowing downward. In this work the relative humidity of the inlet air varied between 83–95% depending on atmospheric conditions. The lowest values were recorded during winter months such that water to restore moisture was added three times a week and only twice during summer months. It is therefore important that moisture content be maintained within optimum values 45–60% (w/w) [99, 143] to avoid channelling due to solid media shrinkage and consequently lower biodegradation rates.

5.2.5.5 Concluding remarks

Figure 5.29) shows the analysis of variance of the pressure drop values under different nutrient supplementation conditions. Starting with the highest, the pressure drop of the following three media were significantly high compared to others:

- mixture under high nutrient supplementation,
- mixture (packed in stages) under low nutrient supplementation, long term and
- peat under low nutrient supplementation, long term

The pressure drop for the other media listed below were low and did not differ significantly from each other:

- mixture under low nutrient supplementation, short term
- mixture (packed in stages) under low nutrient supplementation, short term
- peat under low nutrient supplementation, short term
- pearlite under high nutrient supplementation
- pearlite under low nutrient supplementation, long term

- pearlite under low nutrient supplementation, short term

From the above observation, it is evident that for a short term (60 days) biofiltration under low nutrient supplementation, the pressure drop for all solids was not significantly different and packing the solid medium in stages did not show a significant effect on pressure drop. On a short-term basis, there was not much biomass accumulation to cause high pressure drop in the biofilters. For a long term biofiltration under low nutrient supplementation, the pressure drop for the peat and the mixture packed in stages was significantly higher than that of pearlite. Under high nutrient supplementation, the pressure drop for the mixture was significantly higher than that of pearlite. It can be concluded that although the supplementary nutrients are necessary to increase biodegradation and maintain it at high rates, high amounts of nutrients causes high pressure drop. For practical application on a long-term basis, lower pressure drop is preferred. The pearlite medium offers the lowest pressure drop values, hence it appears to be a good candidate for long-term applications. Besides, pressure drop the pearlite is not sensitive to the addition of water nor does the pearlite deteriorate with time. The only drawback for the pearlite is that low nutrient supplementation is accompanied by oscillating biofiltration rates. Managing biomass accumulation is needed to reduce the oscillation of biofiltration rates by adding nutrients more frequently.

5.2.6 Variations of pH during biofiltration

Changes in pH were observed during the biofiltration of hexane. There was a correlation between pH and pressure drop changes. The pressure drop values were very high whenever pH values decreased to 5 or less. The pH changes during biofiltration are depicted in Figures 5.26 and 5.28. Literature indicated that organic acids accumulate during biodegradation of aliphatic hydrocarbons [4]. Devinny [29] also observed accumulation of organic acids during biofiltration of ethanol at high loading. The

organic acids decrease pH, favouring fungal growth which clogs biofilters resulting in high pressure drop. Despite using calcium carbonate which has an equilibrium pH

$$(NH_4)_2\,SO_4 \longrightarrow \text{Biomass} + 2H^+ + SO_4{}^{2-} \tag{5.8}$$

$$CaCO_3 + SO_4{}^{2-} \longrightarrow CaSO_4 + CO_3{}^{2-} \tag{5.9}$$

value of 8–9, the pH in all biofilters decreased quickly to values between 6–6.5 within the first 10 days. The effect was caused by ammonia sulphate used as the nitrogen source. The sulphate reacts with calcium forming gypsum leaving behind hydrogen and carbonate ions. The system becomes bicarbonate ($H^+/HCO_3{}^-$) buffered around pH 6.0–6.5 (Equations 5.8 and 5.9).

5.2.6.1 Variations of pH during high nutrient supplementation

pH values remained above 6 and 6.5 for both solid media composed of the mixture and 100% pearlite. After 40 days of biofiltration, pH for the mixture started decreasing gradually and reached 4.5 within 30 days (Figure 5.26). The change corresponded to a sharp increase in pressure drop. For the solid medium composed of 100% pearlite the pH decreased at a slower rate. It remained at an average of 6.5 and decreased to 5.5 after 60 days of biofiltration. It was observed that low pH was accompanied by high fungal overgrowth which resulted in high pressure drop. From the results, it is very likely that high pressure drop can be delayed by keeping the pH above 5.

5.2.6.2 Variations of pH during low nutrient supplementation

For a short-term, there was no significant decrease in pH for all media formulations. In all cases the pH value remained above 5. However, a decrease in pH was observed on the long-term biofiltration experiments. The pH values started decreasing as the biofilter aged. After 120 days of operation the pH in both solid media composed of

peat and the mixture started decreasing from a value of 6. By the 260^{th} day the pH had dropped to 4.5. In the solid medium composed of pearlite, the pH started decreasing from a value of 6.6 to 5.5 after 160 days. It remained at this value until the end of the experiment. Fungal growth was also observed at low pH. After 300 days of operation, particles in the column containing peat had disintegrated to much smaller particles and nutrient addition was difficult.

From the above results, it is clear that the pH values in solid media composed of 100% pearlite decreased at a slower rate than pH for peat and the mixture. This difference was due to the presence of extra acidic products from decomposition of peat. Since the amount of calcium carbonate added to columns in both cases of low and high nutrient supplementation were the same (8 kg/m^3), it is suggested that the right amount of calcium carbonate added to a biofilter should be based on the potential of production of acids by the system which in turn depends among other things, on the nutrient availability. To avoid the failure caused by high pressure drop, which happens at low pH (when calcium carbonate has been exhausted), optimization of calcium carbonate is needed. So far there is no clear guideline for the quantity of calcium carbonate to be added to biofilters. Suggested amounts of calcium carbonate added to biofilters range from 0.5 kg/m^3 [87] to 25 kg/m^3 [147]. It is suggested that unless biodegradation rates are reduced, the maximum possible amount of calcium carbonate should be added.

5.2.6.3 Effect of calcium carbonate on pH

Experiments were performed with three columns containing 100% pearlite as the only solid medium. Calcium carbonate was added to two columns (one packed in stages) and the other column was operated without addition of calcium carbonate. The column without calcium carbonate (uncontrolled) started showing lower biodegradation rates than the other two after 5 days of the experiment (Figures 5.30 and 5.31). In

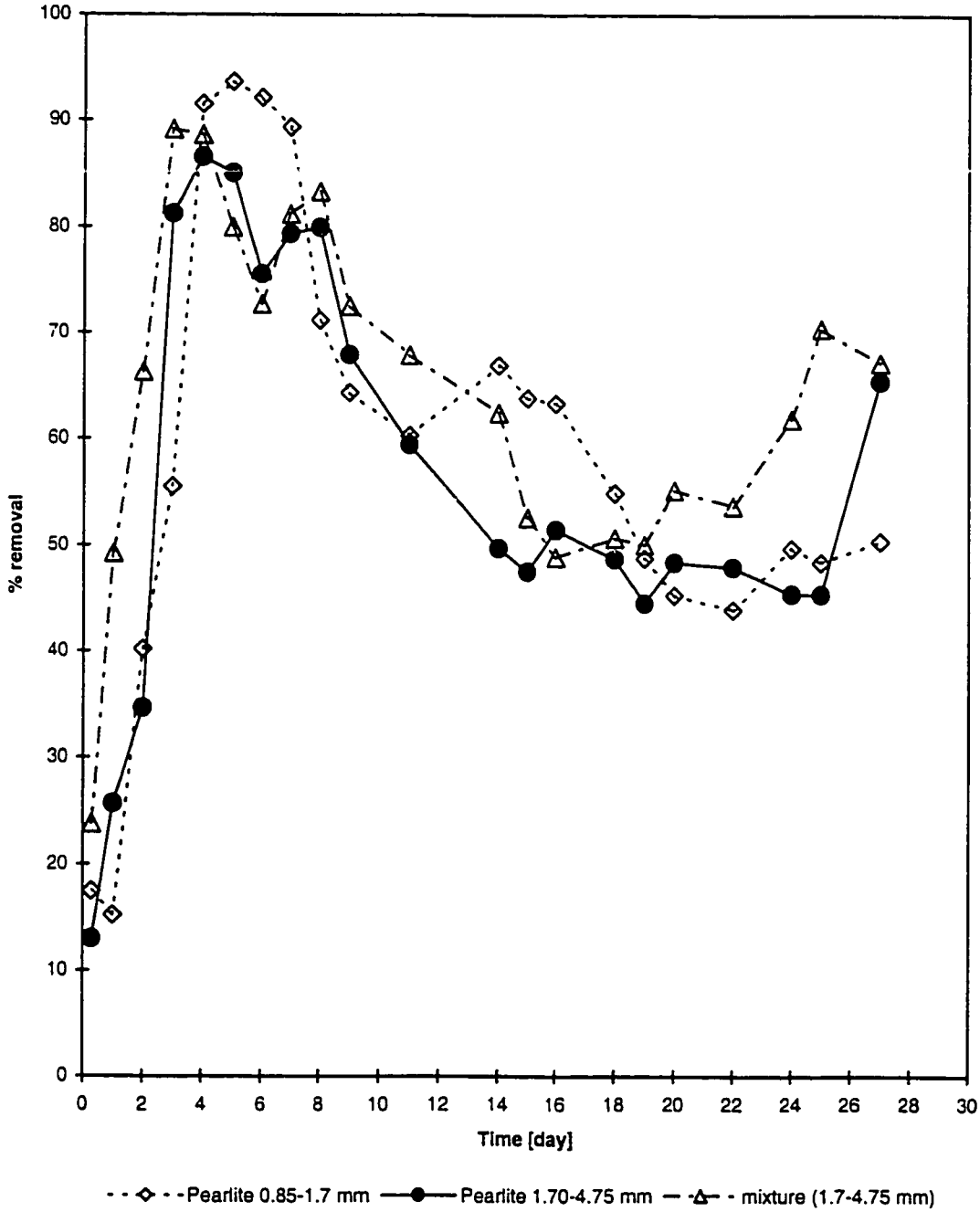


Figure 5.30: Effect of $CaCO_3$ and stagewise packing of the pearlite on the hexane removal

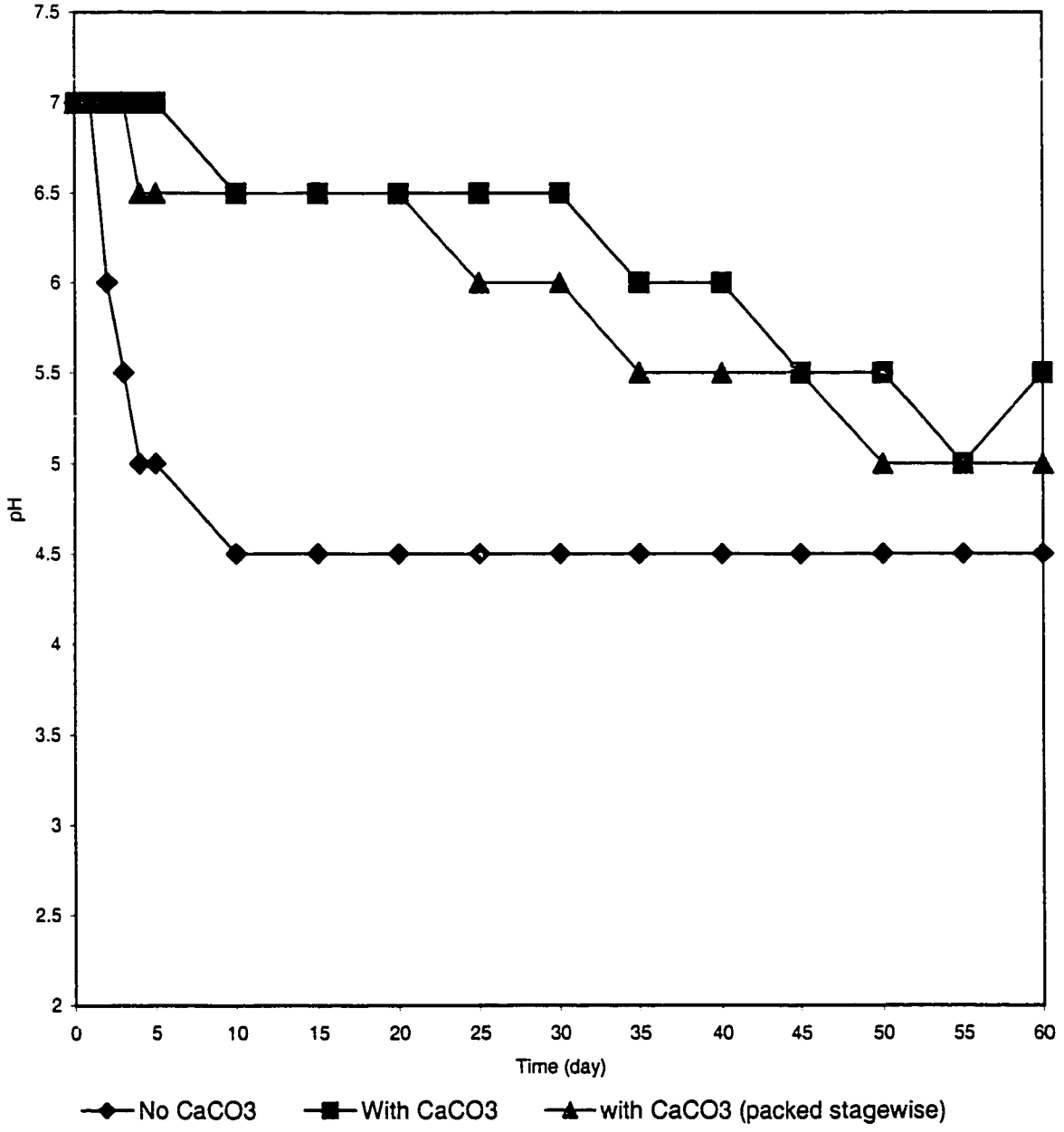


Figure 5.31: Effect of $CaCO_3$ on pH

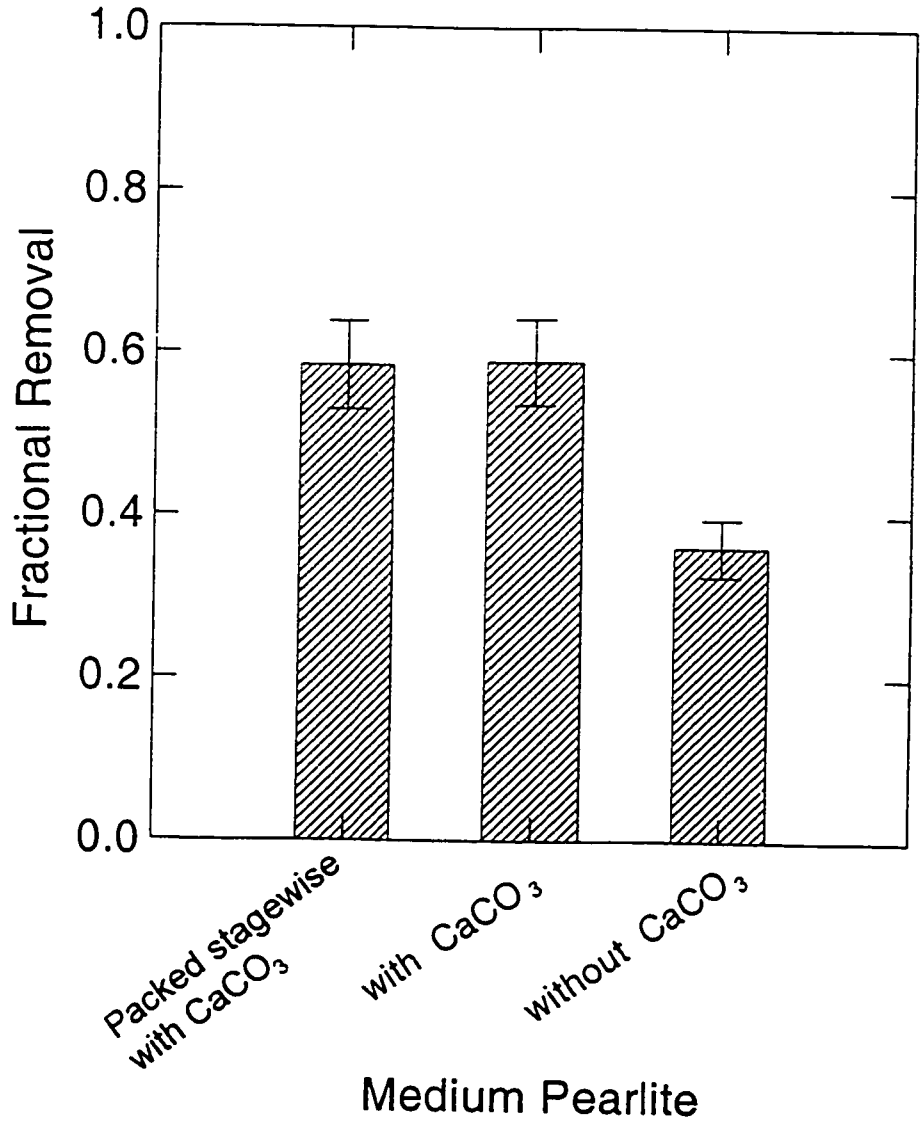


Figure 5.32: Mean comparison for fractional removal of hexane in pearlite with and without calcium carbonate

the second and third week of experiment, percent removal for the column containing $CaCO_3$ was about 40%, the percent removal for the uncontrolled column was about 30%. A second addition of supplementary nutrients resulted in the average percentage removals of 80% and 40% for the controlled and the uncontrolled pH, respectively. Analysis of variance shows that the hexane conversion was lower in the absence of calcium carbonate material (Figure 5.32). The observation supports the need to control the pH during biofiltration.

From Figure 5.31 the pH value of the solid medium without calcium carbonate had dropped to 4.5 within a week while pH values of the other two remained above 5.5. Weckhuysen *et al.* [154] observed a similar trend during biofiltration of butanol in compost biofilters. Without addition of calcium carbonate, the biodegradation rate was low and the biofilter became unstable after 25 days due to accumulation of acidic products. It is therefore important to maintain pH above 5.5 when a near neutral pH environment is required by bacteria (such as *Pseudomonas*) which perform the primary role in the biodegradation sequence [111]. As suggested earlier, optimization of calcium carbonate is needed so that biofiltration rates can be maintained at a high levels under nutrient supplementation on a long-term basis.

5.2.7 Comparison of biofilter performance

5.2.7.1 Removal rates

The comparison of biofilters in terms of amount of hexane removed in percentage and $g/m^3.h$, must take into consideration operating conditions, especially flow rate and concentrations. A good biofilter comparison is the one which represents the information in terms of kinetic parameters such as reaction rate constants. Since most literature reports the removal in terms of percentage and $g/m^3.h$, the following comparison will be in $g/m^3.h$. Operating conditions will be addressed to clarify the comparison.

Hexane biodegradation rates observed in this study are comparable to available literature values as shown in Table 5.5. Morgenroth *et al.* [95] reported hexane biodegradation of up to 85–99% while working with solid medium composed of 50% pearlite and 50% compost mixture at a hexane loading of 21 $g/m^3.h$ (200 ppm) and residence time of 1–2 min. In this study, the maximum hexane removal rates obtained while operating at an inlet concentration of 400 ppm and residence time of 2.25 min was (56 $g/m^3.h$), more than twice the amount obtained by Morgenroth. Since the removal was found to depend on hexane concentration, the high removal rates observed in this study were due to a high inlet concentration (400 ppm) compared to the lower concentration (200 ppm) used by Morgenroth *et al.* [95]. Wright *et al.* [156] achieved a removal rate of 8–5 $g/m^3.h$ of petroleum hydrocarbons in a gasoline vapour mixture using a mixture of compost and pearlite. Medina *et al.* [90], reports gasoline removal at a rate of 4–11 $g/m^3.h$, using granular activated carbon as a solid medium at a superficial residence time of 15 min. Hodge *et al.* [58] reports a much lower biofiltration rate (6 $g/m^3.h$) of hydrocarbon fuel vapours in activated carbon and soil biofilters despite addition of nutrients and inoculum. Although other research groups mentioned above used inoculated biofilters and added supplementary nutrients, the overall hydrocarbon removal achieved was low mainly due to low VOC loading [90, 95, 156]. Others used different VOC components in the gas mixture some of which (e.g. branched alkane and cyclic hydrocarbons) are less biodegradable [58, 90, 156]. In addition, this work was performed by a periodic addition of the supplementary nutrients, while most other researchers added the supplementary nutrients only once at the beginning of biofiltration [90].

From the above results, it has been shown that in the presence of nutrients high biodegradation rates can be achieved. Recent studies on biofiltration of other non aliphatic hydrocarbons indicates that there has been a considerable improvement in biofiltration due to nutrients. High removal rates are achieved when supplementary

nutrients are added to the solid medium. Kiared *et al.* achieved a removal rate of 165 $g/m^3.h$ for toluene in a 2 m high peat biofilter when nutrients were added from time to time [69]. Toluene removal of more that 135 $g/m^3.h$ was observed at a gas flow rate of 24–120 $m^3/m^2.h$ [154, 157]. A high removal rate of 289 g carbon per $m^3.h$ was achieved during thermophilic biofiltration of toluene [87]. Up to 120 $g/m^3.h$ hydrogen sulfide was eliminated in a compost-pearlite biofilter [148].

Biofilters of different solid media formulations performed in this study are compared to each other in Figures 5.33 and Table 5.5. Removal rates as a function of inlet concentration was higher for biofilters conducted under low nutrient supplementation than those conducted under high nutrient supplementation. Solid media composed of the mixtures showed the highest removal rates, followed by peat then pearlite. It was interesting to note that media composed of pearlite in both cases of low and high nutrient supplementation, had the same average removal rates (Figure 5.33). Furthermore, the linear regression slopes for all solid media formulations except the mixture under high nutrient supplementation were almost the same. It is not clear at this point why the solid medium composed of the mixture under high nutrient supplementation had a linear regression line with a slope different from others. Observations in Figure 5.33 are supported by a statistical analysis (Figure 5.34). At a 95% confidence level fractional removal for both mixture and pearlite media under high nutrient supplementation were not significantly different from each other but were significantly lower than all other media formulations under low nutrient supplementation, for short and long term operation except pearlite for short term. Peat and the mixture under low nutrient supplementation showed the highest average fractional removal.

In addition, the columns with weekly addition of supplementary nutrients had a higher utilization of nitrogen source. The ratio of the amount of hexane-carbon removed to the nitrogen utilization (C/N ratio) was 52-66 g/g for biofilters con-

Table 5.5: Comparison of the hexane removal, C/N ratio and pressure drop at different residence time

Packing material	VOC	RT[1] (min)	removal (g/m^3.h)	removal (%)	C/N ratio	ΔP (Pa/m)	Ref.
High nutrient supplementation							
100% pearlite	hexane	2.25	16–56	30–67	15	20–120	⋆
100% pearlite	hexane	0.45	36–96	10–24	15	130–230	⋆
50:50 mixture	hexane	2.25	10–67	40–80	14	60–2933⊗	⋆
50:50 mixture	hexane	0.45	34–120	11–24	14	890–1730⊗	⋆
Low nutrient supplementation							
100% pearlite	hexane	2.25	6–50	16–76	56	10–20	⋆
100% peat	hexane	2.25	12-50	30–92	54	30–250	⋆
50:50 mixture	hexane	2.25	15-58	38–100	66	20–30	⋆
50:50 mixture (stagewise)	hexane	2.25	16-50	38–88	52	30–120	⋆
Low nutrient supplementation, long term biofiltration							
100% pearlite	hexane	2.25	3–38	12–94		10–40	⋆
100% peat	hexane	2.25	2-81	2–100		30–800	⋆
50:50 mixture (stagewise)	hexane	2.25	2-76	7–100		30–2500	⋆
Literature							
COMP & pearlite + nutrients	hexane	1–2	18–21	> 99	10		[95]
COMP & pearlite + nutrients	gasoline	2.2	8–15	40–100		1500	[156]
GAC + nutrients	gasoline	15	4–11	>80			[90]
Envirogen[2] + nutrients	gasoline	3–6	3–34	75–85			[132]
COMP + nutrients	n-butane 200 ppm	2		> 90			[75]
COMP + nutrients	kerosine 100–500 ppm	3		70–90			[107]

KEY:
COMP = compost, Envirogen[2] = Trade name, packing material type not mentioned,
GAC = granular activated carbon, RT[1] = superficial residence time ⋆ = this study
⊗ maximum pressure drop was lower at low residence time than at high residence time because it was conducted before biomass accumulation period only.

ducted under low nutrient supplementation after considering all nitrogen (including nitrogen from the peat). The carbon-nitrogen ratio was 14–15 g/g for biofilters conducted under high nutrient supplementation without considering nitrogen from the peat. Morgenroth *et al.* [95] reports C/N ratio of 10. The difference in nitrogen ratio between high and low nutrient supplementation can be explained by the two biodegradation mechanisms. At low nutrient concentration, VOC is removed mainly by maintenance mechanisms with little utilization of nitrogen. At high nutrient concentration, microbial growth, which utilizes a lot of nitrogen, is predominant.

5.2.7.2 Performance-Indicator

Combining both biodegradation rates and pressure drop into a single parameter brings another way of looking at biofilter performance, particularly in terms of biofiltration economics. For that reason, a biofiltration **Performance-Indicator** was introduced. The **Performance-Indicator** is the ratio of the amount of VOC removed per unit biofilter volume to the amount of energy required to blow the air through the biofilter. The **Performance-Indicator** is intended to address the biofiltration economics, it gives a direct and straight forward comparison of biofilters. The higher the value of the Performance-indicator the better the biofiltration economics. The Indicator can be used a primary screening parameter for selection of biofilters. A precautionary note on using this parameter is that if pressure drop is too low, the Performance-Indicator might be high even when the biodegradation rate is low. This can transform into a high biofilter volume, hence high capital investment cost. The Performance-Indicator should be used in conjunction with other information especially biofiltration rates. Performance-Indicator will work well where biodegradation rates of different biofilters more or less the same.

Results in Figures 5.35 through 5.37 show that all columns conducted under low nutrient supplementation for a short period of time (60 days) had higher performance-

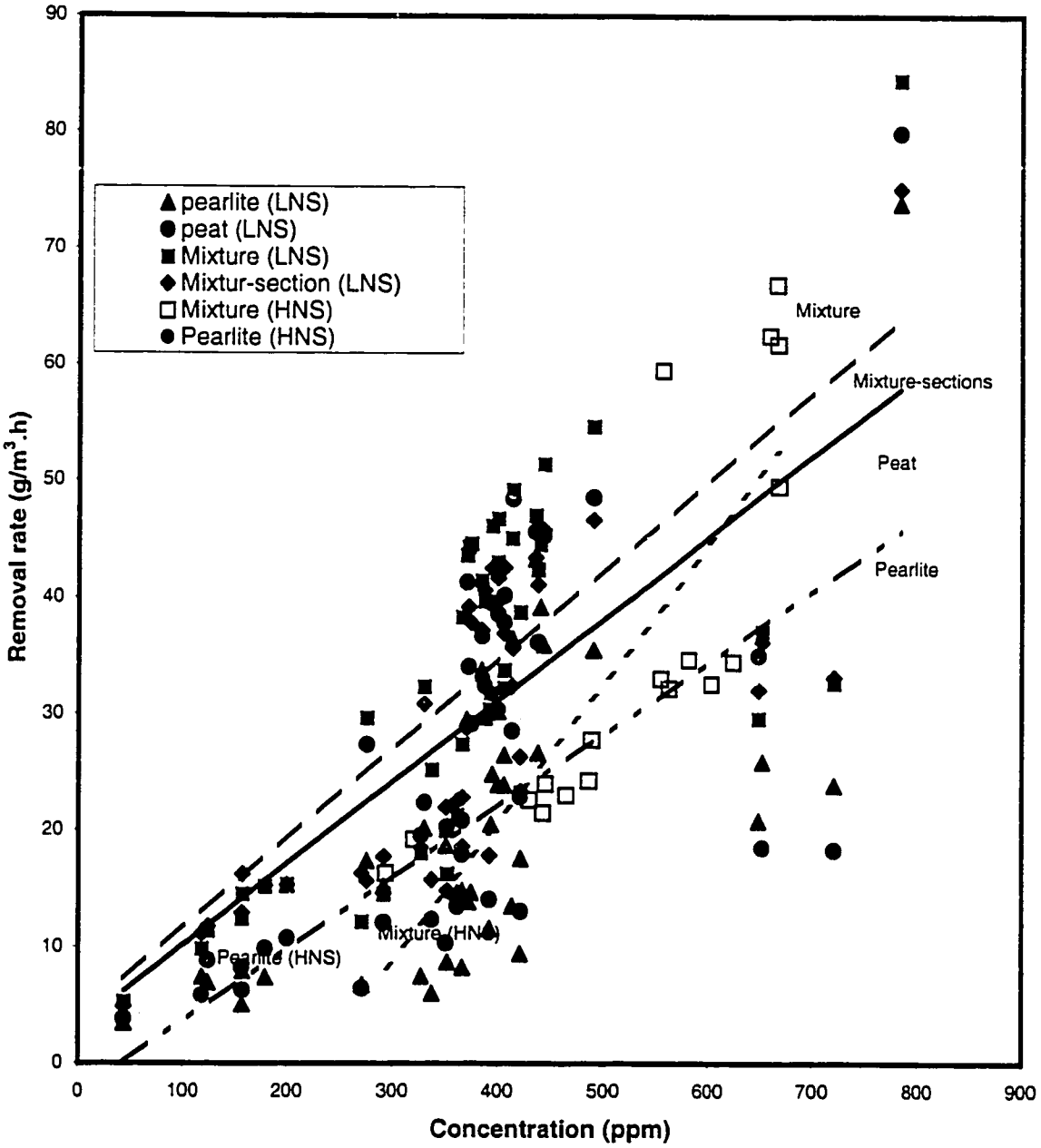


Figure 5.33: Comparison of the biofilters in term of biodegradation rates

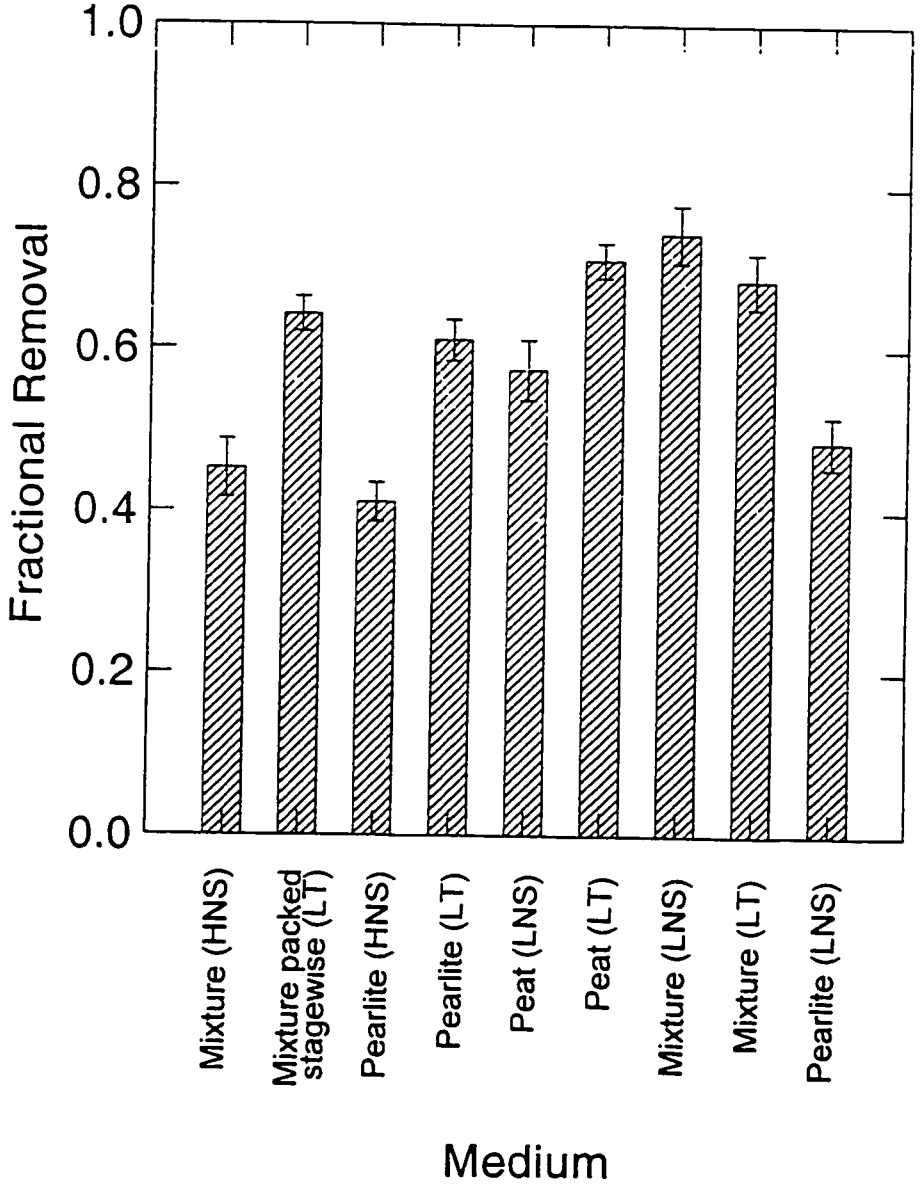


Figure 5.34: Mean comparison for fractional removal for different media and nutrient addition protocol

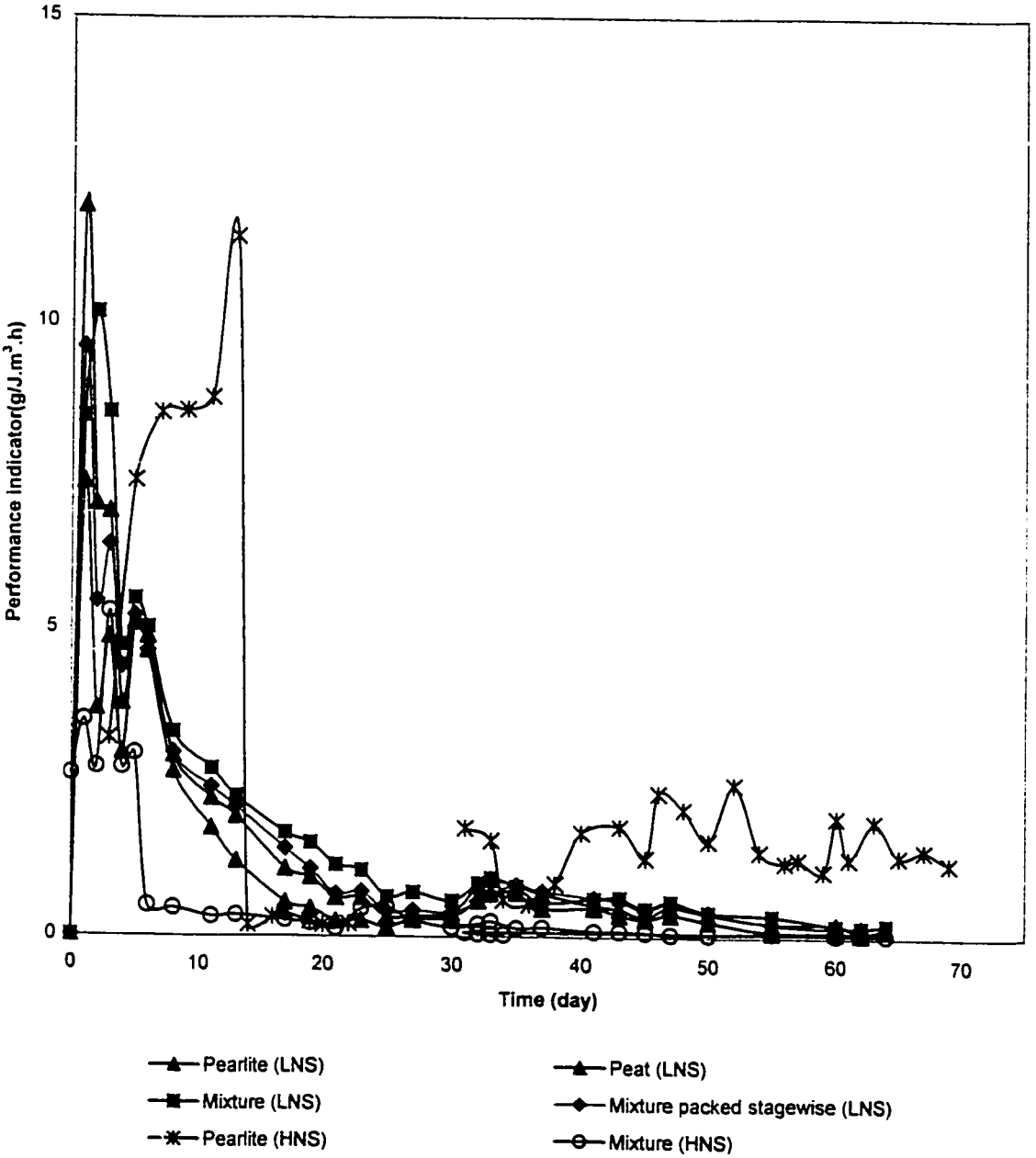


Figure 5.35: Performance-indicator

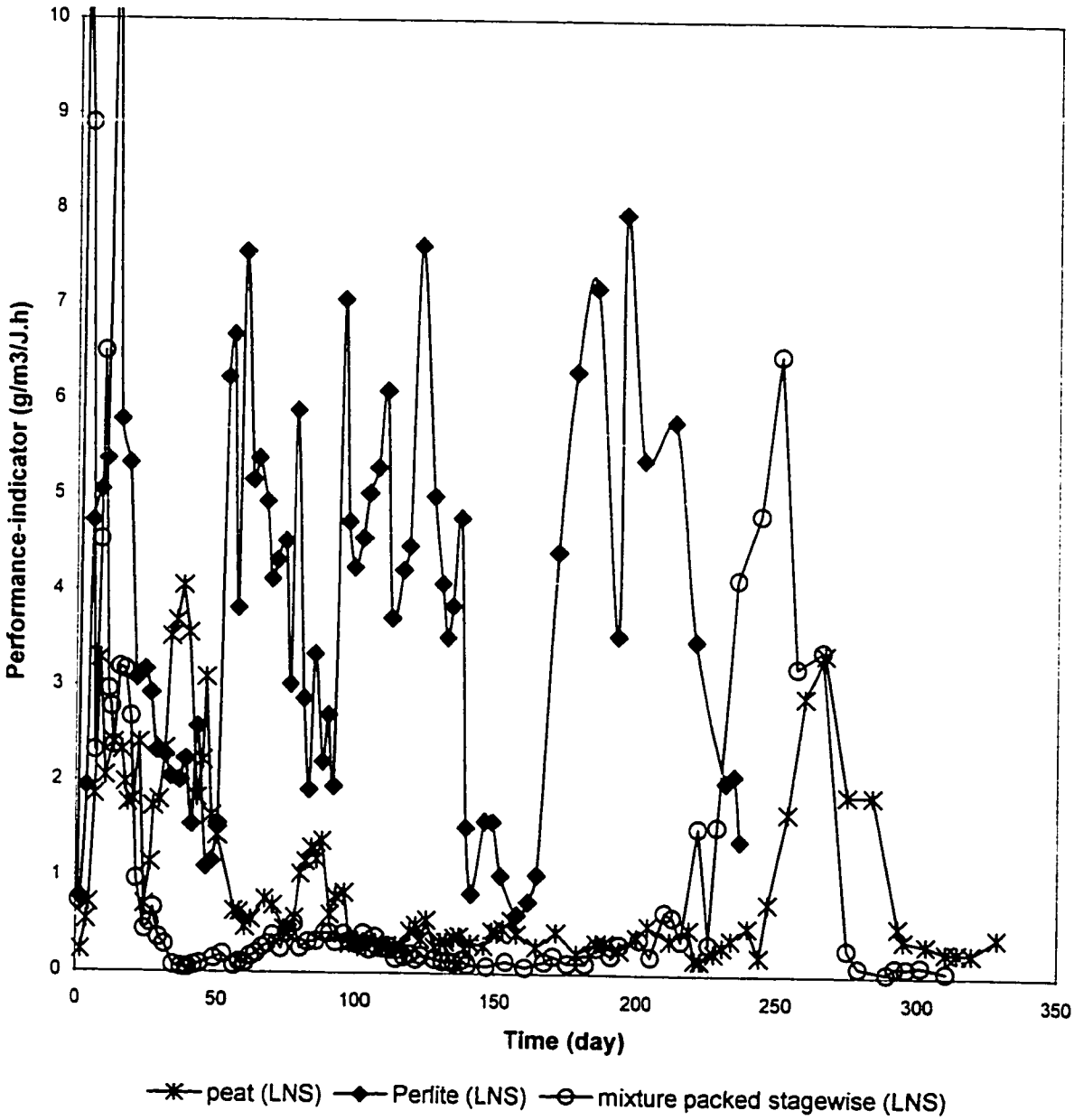


Figure 5.36: Performance-indicator, long term biofiltration

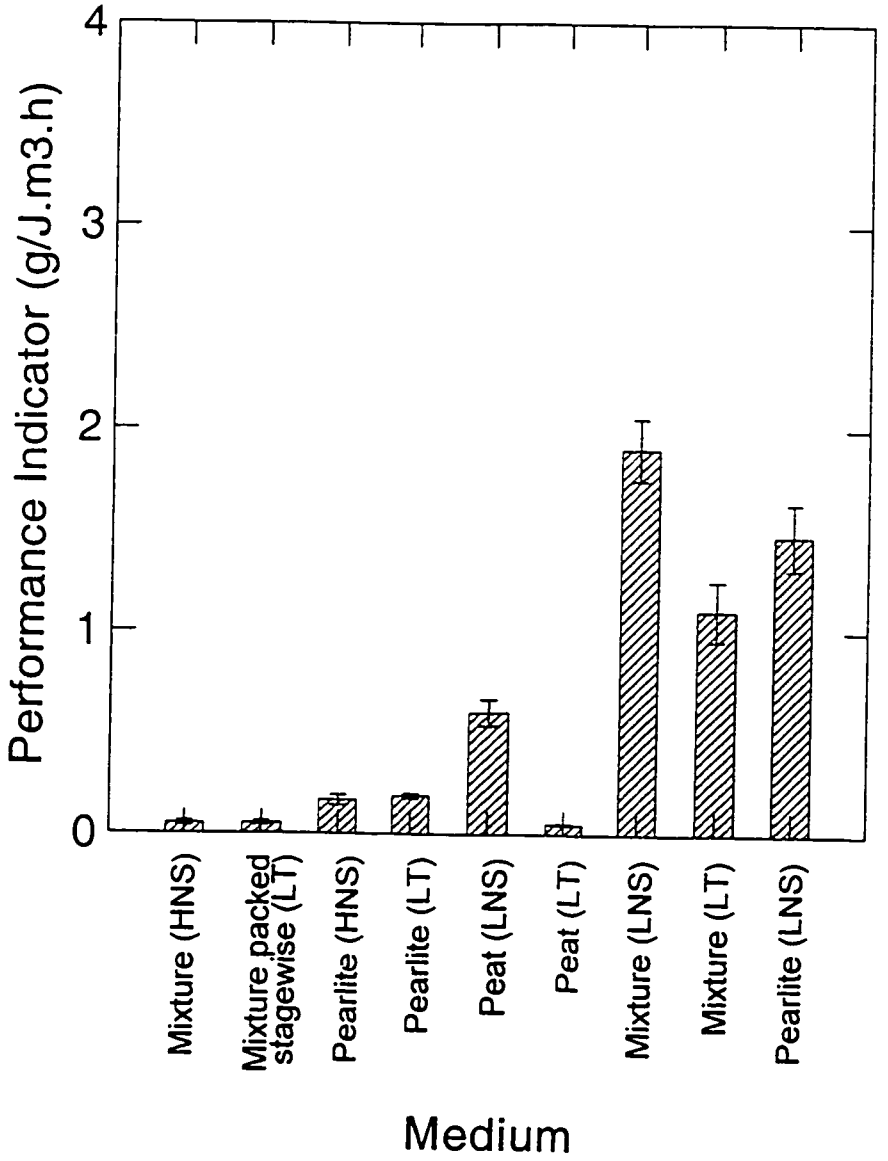


Figure 5.37: Mean comparison for performance-indicator for different media and nutrient addition protocol

indicators than those with high nutrient supplementation and low nutrient supplementation operated for a long term. Under low nutrient supplementation (short term), the performance-indicator was higher than 4 g/m^3.J.h. within the first week of the biofiltration. This value decreased to below 0.5 g/m^3.J.h. due to nutrient depletion and increased to more than 8 after the second addition of nutrients. After 30 days of operation all solid media with low nutrient supplementation had almost the same performance-indicator ranging from 0.1–0.8 g/m^3.J.h. Under low nutrient supplementation and short term operation, there were no high pressure drops and hence all media had high performance indicators. Among all media formulations under high nutrient supplementation and low nutrient supplementation (long term), only pearlite showed a performance-indicator significantly higher than others. In terms of biofiltration economics, Pearlite is therefore a cost effective medium for long term biofiltration.

Table 5.6 compares the performance indicators obtained in this work under low nutrient supplementation on long term basis with Performance indicators calculated from literature values. The Performance indicator achieved in this work was the highest (0.02–4 g/J.m^3.h) compared to values achieved by other researcher (0.006–0.211 g/J.m^3.h) for hexane and gasoline. This achievement was due to high biodegradation rates attained through nutrients supplementation and low pressure drop. High performance indicator are easily attained when the VOC is highly biodegradable. Mohseni and Allen achieved a performance indicator ranging from 0.06–3.038 during biofiltration of methanol [92]. A performance indicator of 0.338–0.696 was achieved by Kiared *et al* during biofiltration of toluene [69].

5.2.8 Effect of particle size on biofiltration

Biofiltration was conducted with a column containing pearlite sieved to sizes between 0.85–1.70 mm and two columns containing pearlite and the mixture both with par-

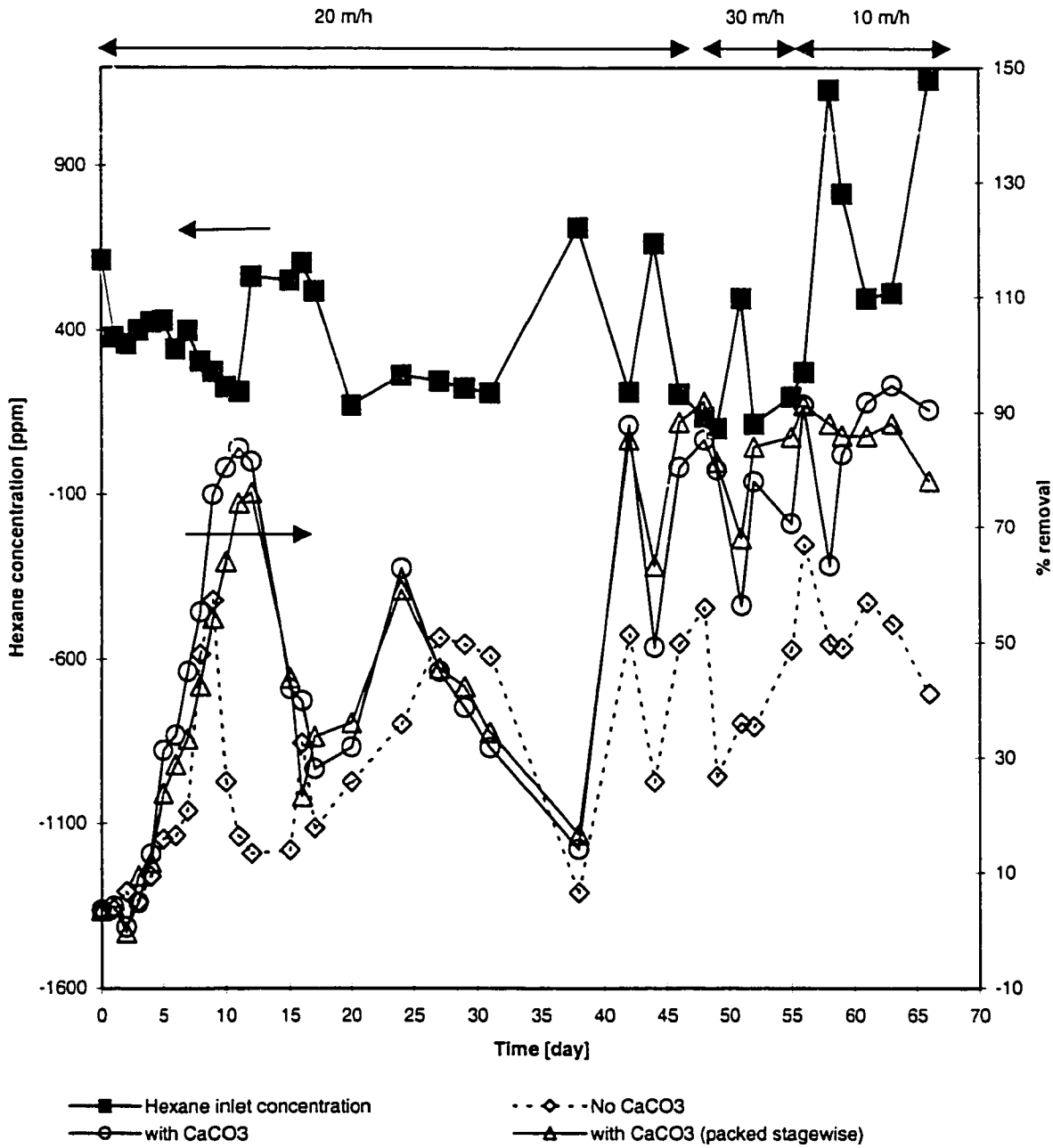


Figure 5.38: Effect of particle size on the biofiltration of hexane under nutrient supplementation condition

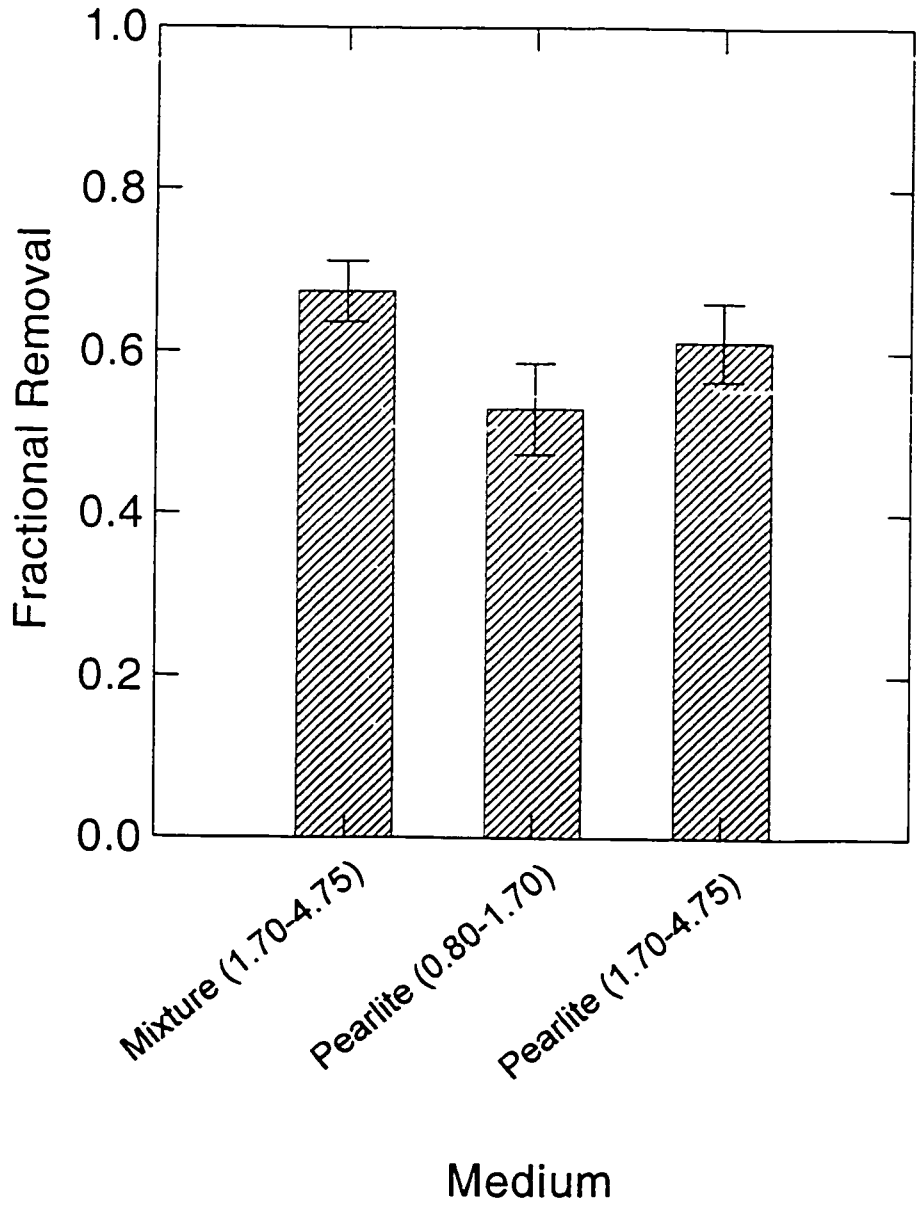


Figure 5.39: Mean comparison for fractional conversion for different particle sizes

Table 5.6: Comparison of Performance indicators

Packing material	VOC	Pressure drop (Pa)	Removal rate ($g/m^3.h$)	Performance Indicator ($g/J.m^3.h$)	Reference
Pearlite	hexane	10–40	12–94	0.6–4	This study
Peat	hexane	30–800	2–100	0.26–1.31	This study
Mixture	hexane	30–2500	7–100	0.02–0.42	This study
Compost + Pearlite	hexane	889–1143	8.4–21	0.006–0.02	Morgenroth *et al* 1996
GAC	gasoline	100	5–25	0.04–0.211	Wright *et al* 1997
Compost + clay	Acetone	500–850	82–105	0.08–0.177	Tang *et al* 1997
Synthetic Agent	Toluene	200	80–165	o.338–0.696	Kiared *et al* 1997
Peat or PU foam	Methanol	554–680	40–113	0.05–0.172	Shareefdeen & Baltiz 1993
Wood chips Compost + pearlite	Methanol	50–250	180	0.606–3.038	Mohseni and Allen

Key: GAC= Granular activated carbon PU = Polyurethane

ticles sieved to sizes 1.7–4.75 mm. There was no significant difference in percentage removal among the three columns at a 95% confidence level (Figures 5.38 and 5.39). Since the columns were operated simultaneously, it can be concluded that smaller particle size did not increase biofiltration rates despite their higher specific surface area. Wu *et al.* obtained similar results during biofiltration of toluene [157]. The results suggests that particle size has a negligible effect on biodegradation in the presence of supplementary nutrients. Based on this information, the size of the solid media for biofilters can be increased to minimize pressure drop up to a size which will have an influence on reaction rates. Solid medium of large particle size (10–20 mm) supplemented with nutrients is reported to successively provide good biodegradation rates of VOCs during biofiltration [23]. At this juncture, it appears that the

advantages of surface area provided by the media particle size on reaction rate are much less than the advantages of nutrient supplementation. Further work on the combined effects of media particle size and nutrient supplementation is needed. As the particle size is increased, there will be an minimum particle size whereby the biofiltration rates will start decreasing. This size may likely depend on the quantity and frequency of supplementary nutrient addition. What ever the case, one advantage is eminent, the pressure drop will be reduced further due to large particle size, hence high performance indicator.

5.2.9 Biofilter acclimation period

The acclimation of the biofilters for maximum hexane removal was achieved within five days in both cases of high and low nutrient supplementation. During low nutrient supplementation, biofilters regained high performance within three days after subsequent additions of nutrient solution. Literature indicates that acclimation may take a few weeks to a month [98, 142]. Acclimation of a compost biofilter removing hexane is reported to be 16 days when nutrients are added to the solid medium and activated sludge is used as an inoculum [95]. A one week acclimation period was also observed by Peters *et al.* during biofiltration of fuel-derived VOCs [107] in a compost biofilter and Shareefdeen *et al.* [165] during biofiltration of methanol in a peat-pearlite mixture biofilter. Easily biodegradable compound may have acclimation periods of less than a week. Deshusses reports an acclimation of 3–5 days for methyl ethyl ketone in compost biofilters when nutrients were added to biofilters and a specific enrichment culture was used [26]. Results here agree with the literature and suggest that the acclimation period can be shortened to 5 days by the use of acclimatized culture and supplementary nutrients.

5.2.10 Biofilter operating life and long term stability

The long term stability of biofilters supplemented with nutrients depended on the nutrient addition protocol. For the weekly addition of nutrients, the biofilters failed within 70 days. Such a short period of time is not economical for industrial application. When the frequency of nutrient addition was reduced, biofilter life spans were extended beyond 11 months.

In all cases, biofiltration failure was accompanied by low biofiltration rates, low pH values and high pressure drop, as well as visually observed biomass overgrowth (Sections 5.2.5 and 5.2.6). Low biodegradation rates and failure of biofilters observed as the pH decreases below 4.5 is attributed to the following factors:

- Low pH is known to be unfavourable to bacteria which in this case were responsible for hexane biodegradation, and favour fungi whose growth clogs the biofilters resulting in poor air flow. Poor pneumatic conductivity reduces the effective biofilter residence time, and solid media surface area in contact with the flowing gas while increasing channelling effects. Such physical changes reduce biodegradation rates. Kennes *et at* [67] reports similar observations, at a pH lower than 4.5, the biofilters were dominated by fungi and the biodegradation of alkylbenzene vapours in pearlite biofilters was lower than that at higher pH values.

- At pH below 5.0 the enzymic activities responsible for biodegradation of hydrocarbons are reduced [54].

- It is reported that a pH below 5.0 reduces the emulsifying power of the surfactants produced by microorganisms to increase the solubility of hydrocarbons [55]. Assuming the VOC (hexane) is not toxic to the microorganisms, reducing its solubility results in a lower amount of hexane accessible to the microorganism; hence, low biodegradation rates.

Since the biofilters with high nutrient supplementation failed much faster than those with low nutrient supplementation, it can be concluded that overall acid formation is high during high nitrogen content. Consequently the carbonate material is neutralized faster leading to a faster decrease in pH. The decrease in pH observed during the aqueous fermentation of hexane (section 3.2) supports acid formation during the growth phase. Accumulation of acid intermediates in the overloaded ethanol biofilters is reported by Devinny [29]. Atlas [4] reports that there is accumulation of organic acids during biodegradation of aliphatic hydrocarbons. It can also be concluded that while nutrient supplementation improved biofiltration performance, it is very important to manage the quantity and frequency of addition of the nutrients to achieve both long term stability and improved biodegradation. Increasing the amount of the carbonate materials as suggested in section 5.2.6, will increase the biofilter life span.

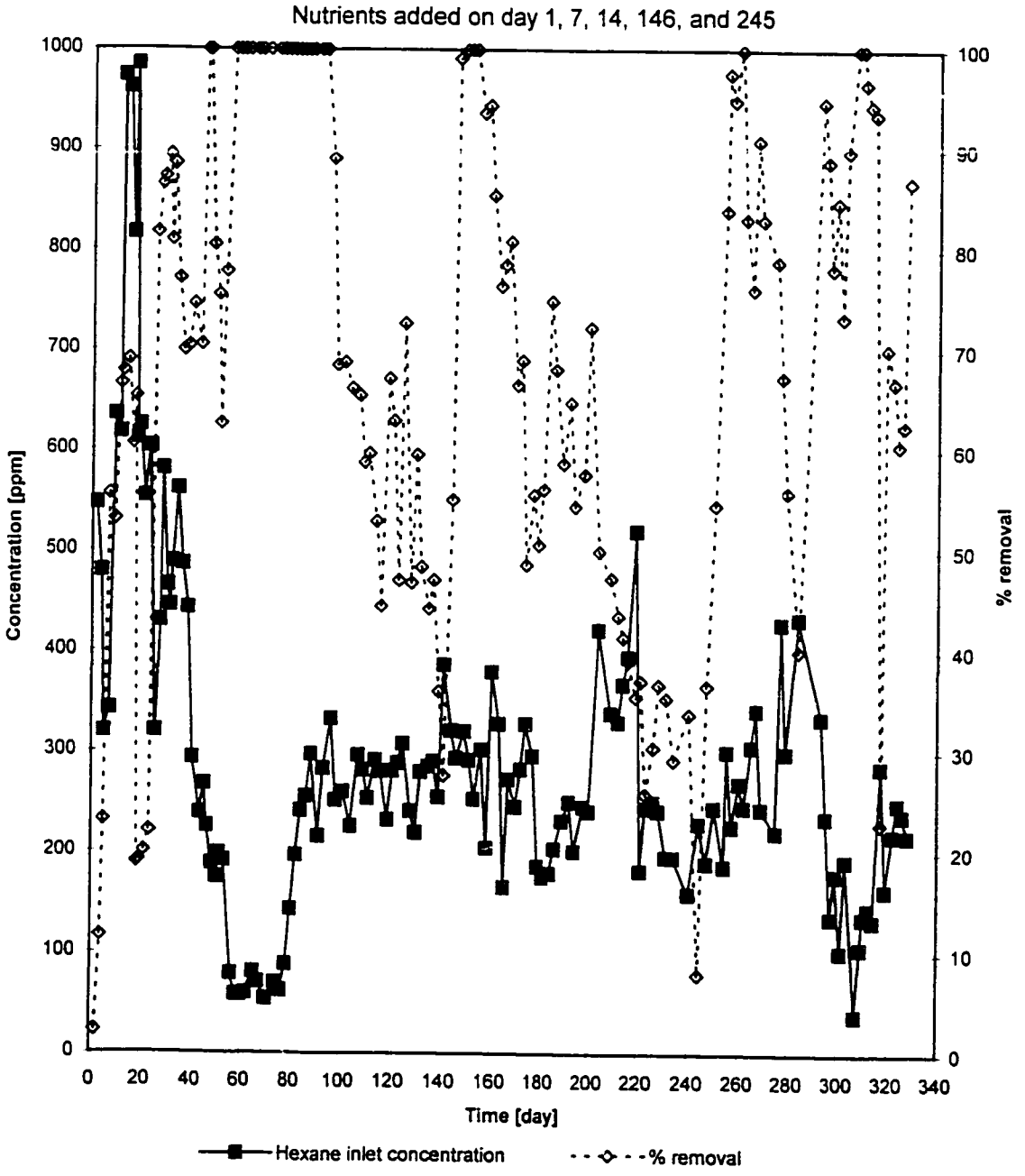


Figure 5.40: Hexane percent removal in the column containing 100% peat, at 20 m/h; Long term biofiltration

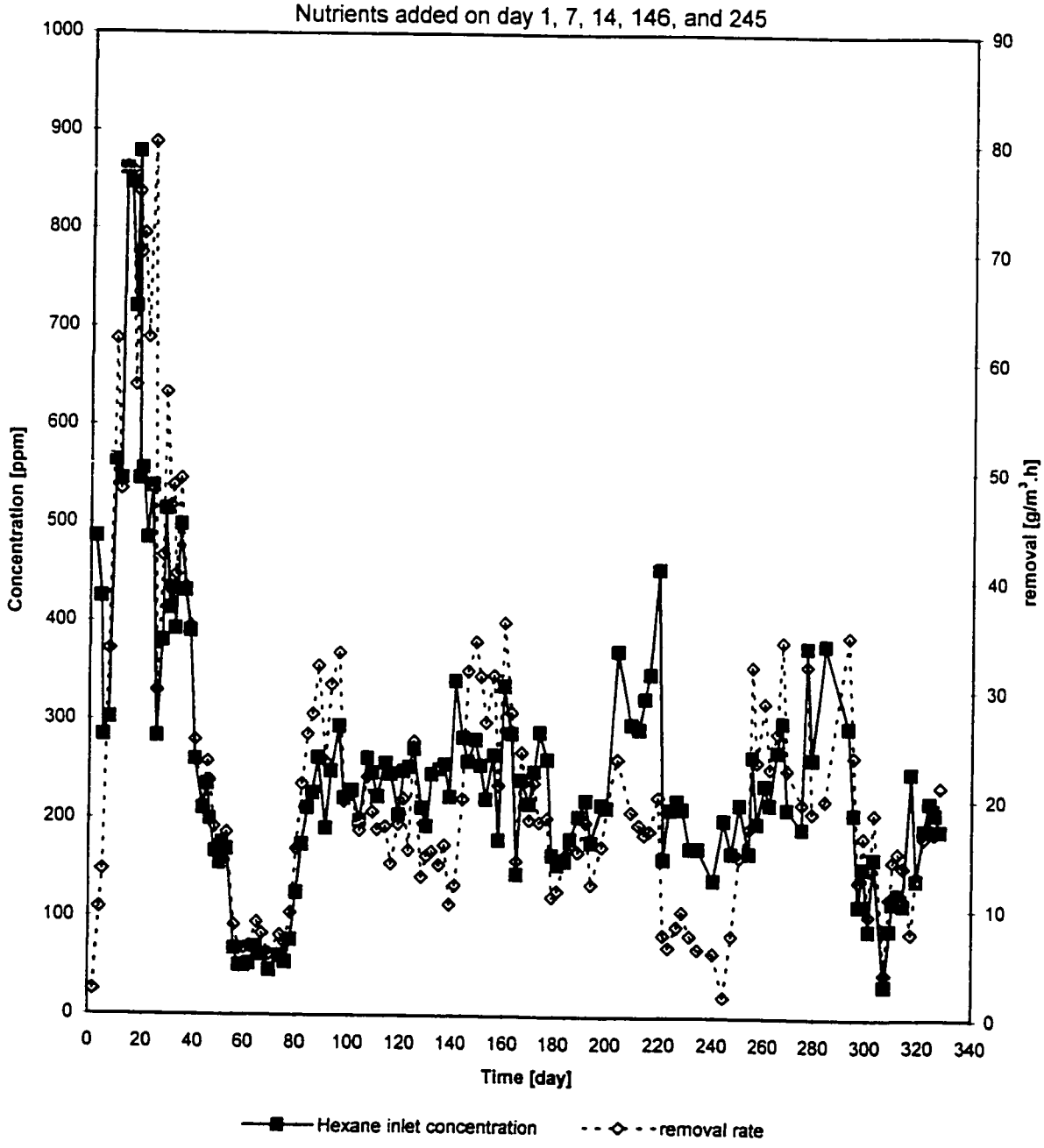


Figure 5.41: Hexane removal rates in the column containing 100% peat at 20 m/h; Long term biofiltration

Chapter 6

Modeling

6.1 Model development

Biofiltration systems are complex and difficult to model. Although biofilters are similar to packed bed reactors, there are a lot of differences arising from biomass growth, interactions of microbial species and solid medium decomposition which cause the kinetics, and the hydrodynamics to change with time. Factors contributing to the complexity of the system include:

- The physical characteristics of the VOC, such as solubility and partition coefficient, affect the mass transfer properties of the VOC from the gas phase to the biofilm. It is reported that microorganisms produce surfactants which increase the solubility of hydrophobic VOCs in the aqueous phase during biodegradation [4, 49, 55, 81]. The quantitative and qualitative parameters of the surfactants produced by microorganisms at different conditions and their variation with time are not well understood.

- The biodegradation rates of different VOCs are affected by their chemical properties. Certain VOCs are easily biodegradable while others are less biodegrad-

able. Other recalcitrant VOCs are difficult to biodegrade and may have inhibitory effects on the microorganism.

- Chemical composition of organic solid media may differ significantly depending on source and age. Solid media differences, such as nitrogen and phosphorus content influence microbial growth, which directly affects biodegradation rates. Also, differences in solid media particle size influence pneumatic conductivity of the media and may indirectly affect the biodegradation rates. In addition the physical structure of the solid media is continuously changing due to aging. Organic solid media undergo biodegradation during biofiltration, resulting in a more compact bed and poor pneumatic conductivity.

- The microbial population in biofilters consists of a mixed culture which changes with time due to changes in environmental factors, particularly pH, moisture content, and availability of supplementary nutrients. Such changes are accompanied by changes in VOC biodegradation rates, which decrease with time as the concentrations of supplementary nutrients and/or pH decrease.

- Growth of microorganisms under nutrient supplementation conditions influence the pneumatic conductivity of the biofilter due to accumulation of biomass with time.

- When supplementary nutrients are added in a batch mode, the biofilter system becomes more active. The microbial cells go through a growth phase cycle. The microbial density increases during high concentration of supplementary nutrients and decreases as the nutrients are depleted.

Despite all the complex factors, it is important to have an adequate working model describing the biofiltration process. The biofiltration process under periodic nutrient supplementation has not been addressed extensively. All models reported in the

literature including the models proposed by Ottengraf [98] assume a constant microbial density. The models do not address the long-term changes in microbial activity resulting from addition of supplementary nutrients. From the results, it has been shown that biofiltration rates are dependent on the concentration of supplementary nutrients. Addition of nitrogen and other nutrients to the solid media, as is the case in this research, increases the microbial activity. When supplementary nutrients are depleted, the microbial activity decreases. Therefore microbial activity is a time dependent variable and cannot be assumed constant on a long term basis if biofilters are operated under periodic addition of supplementary nutrients. It is therefore very important to address the time course of biofiltration rates under nutrient supplementation. Assumptions required to describe the nutrient supplementation process include:

1. Assumptions 1–5 listed in section 2.7 page 40, which assume nutrient diffusion transportation within the biofilm, planar biofilm geometry, Monod kinetics, gas plug flow through the biofilter and negligible gas film mass transfer.

2. Under nutrient supplementation conditions, the growth of microorganisms is controlled by the availability of nutrients particularly nitrogen and carbon source. On a long-term basis, the nitrogen is depleted and becomes a limiting reactant reducing an effective elimination capacity.

3. The VOC is biodegraded through two scenarios:

   (a) biodegradation through microbial growth which depends on the availability of a limiting reactant (nitrogen) and

   (b) biodegradation through maintenance which depends only on the microbial density.

6.1.1 Nitrogen utilization

The microbial growth in both aqueous suspension cultures and biofilters can be expressed by multiple substrate kinetics as follows [61]:

$$\frac{dX}{dt} = (\mu - \kappa) Y \left(\frac{C}{C + K_C}\right) \left(\frac{S_N}{S_N + K_{sN}}\right) X \tag{6.1}$$

where Y is the cell yield based on the limiting reactant; C, and S_N are the concentrations of carbon and nitrogen sources respectively; X is the cell density; K_C, and K_{sN} are Monod constants for carbon and nitrogen sources respectively. μ is the specific growth rate constant and κ is the specific death rate constant. When nitrogen is the sole limiting substrate, Equation 6.1 becomes:

$$\frac{dX}{dt} = (\mu - \kappa) Y \left(\frac{S_N}{S_N + K_{sN}}\right) X \tag{6.2}$$

Where Y is the cell yield based on the limiting substrate nitrogen. Assuming $\kappa \ll \mu$ then Equation 6.2 becomes:

$$\frac{dX}{dt} = \mu_{max} Y \left(\frac{S_N}{S_N + K_{sN}}\right) X \tag{6.3}$$

Where μ_{max} is the apparent maximum growth rate. The VOC (hexane) might be the limiting reactant when its concentration is low and the nitrogen is available in large quantities. In that case Equation 6.1 should be used.

Nitrogen mass balance:

Assuming a constant biomass yield on nitrogen, the amount of nitrogen in the aqueous phase or biofilter can be calculated from the following relationship:

$$Y = \frac{X - X_0}{S_{N0} - S_N} \tag{6.4}$$

6.1.2 Hexane Removal in biofilters

The kinetics of VOC removal is discussed in section 2.7. Despite the fact that nitrogen concentration has a significant influence on the biofiltration kinetics, such as changing biomass density and the elimination capacity of the VOC in g/m^3.h, the instantaneous VOC removal can be modelled by assuming the constant biomass density. This assumption is supported by the fact that the residence time of VOC (hexane) is too small (less than 3 minutes) compared to the rate of biomass doubling time (in liquid phase) which is more than 4 hours at the maximum growth rate parameter μ_{max} of 0.17 h^{-1} (Section 3.2.2). The biomass growth in the solid phase (in biofilters) is much smaller as indicated by the rate of nitrogen utilization (Figure 5.19). Depending on several factors such as hexane gas concentration, hexane diffusion in the liquid phase (biofilm) and the biodegradation rate in the absence of mass transfer limitations, hexane removal may follow first order, zero order reaction limited or zero order diffusion limited kinetics [98].

The solution for the first order kinetics (Equation 2.11) presented by Ottengraf [98] is repeated here below as Equation 6.5. Sample curves of hexane concentration along the column for the first order reaction kinetics are shown in Figure 6.1.

$$\frac{C_{ge}}{C_{go}} = \exp\left(-\frac{HaD_e}{m_i U_g \delta}\phi_1 \tanh\phi_1\right) = \exp\left(-\frac{HK_1}{m_i U_g}\right) \tag{6.5}$$

If the hexane removal follows zero order reaction limited kinetics, the solution (Equation 2.15) presented by Ottengraf [98] is repeated here below as Equation 6.6. Sample curves of hexane concentration along the column for zero order reaction limited kinetics are shown in Figure 6.2.

$$\frac{C_{ge}}{C_{go}} = 1 - \frac{HK_0}{U_g C_{go}} \tag{6.6}$$

If the hexane removal follows zero order diffusion limited kinetics, the solution (Equation 2.14) presented by Ottengraf [98] is repeated here below as Equation 6.7. Sample curves of hexane concentration along the column for zero order diffusion limited kinetics are shown in Figure 6.3.

$$\frac{C_{ge}}{C_{go}} = \left(1 - \frac{H}{U_g}\sqrt{\frac{K_0 D_e a}{2m_i C_{yo}\delta}}\right)^2 \tag{6.7}$$

In all cases, the first and zero order reaction kinetics, the VOC fractional concentration along the column decreases faster with increasing reaction rate constants. The curves for reaction limited zero order reaction kinetics are straight lines. The curves for diffusion limited zero order reaction kinetics tend to be straight lines when the VOC concentration is high. However, when biodegradation is high or inlet VOC inlet concentration is low such that almost all the VOC is eliminated, the diffusion limited, zero order kinetics reaction shifts towards curved lines (Figures 6.3).

6.1.3 Long-term effect of nitrogen depletion on hexane removal kinetics

As the nitrogen concentration changes with time, the kinetics of VOC removal also changes. This arises as nitrogen, which is a limiting factor, decreases to low levels and the microorganisms stop growing and utilizes the VOC for maintenance energy only. In that case the rate of hexane removal from the gas phase can be described by modifying Equation 6.1 to the following relationship:

$$-r_C = \frac{dC_{li}}{dt} = \mu_{max} Y_c \left(\frac{S_N}{S_N + K_{sN}}\right)\left(\frac{C_{li}}{C_{li} + K_{C1}}\right) X + \Psi \left(\frac{C_{li}}{C_{li} + K_{C2}}\right) X \tag{6.8}$$

Where K_{C1} and K_{C2} are Monod constants for hexane removal during growth and maintenance respectively. The Biodegradation of the VOC during growth is affected by the availability of nitrogen due to microbial growth. Similarly the VOC removal

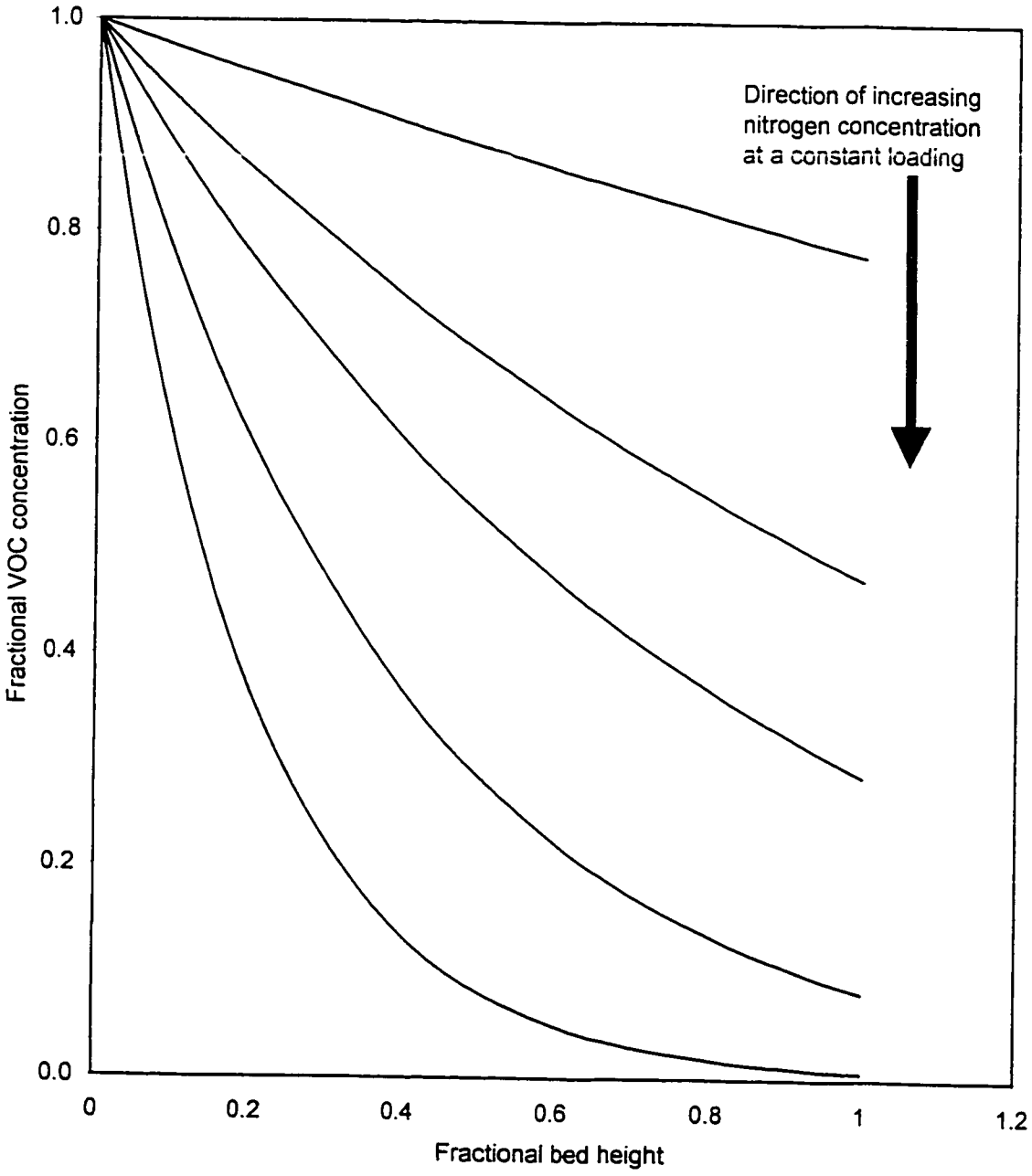


Figure 6.1: First order reaction kinetics curves

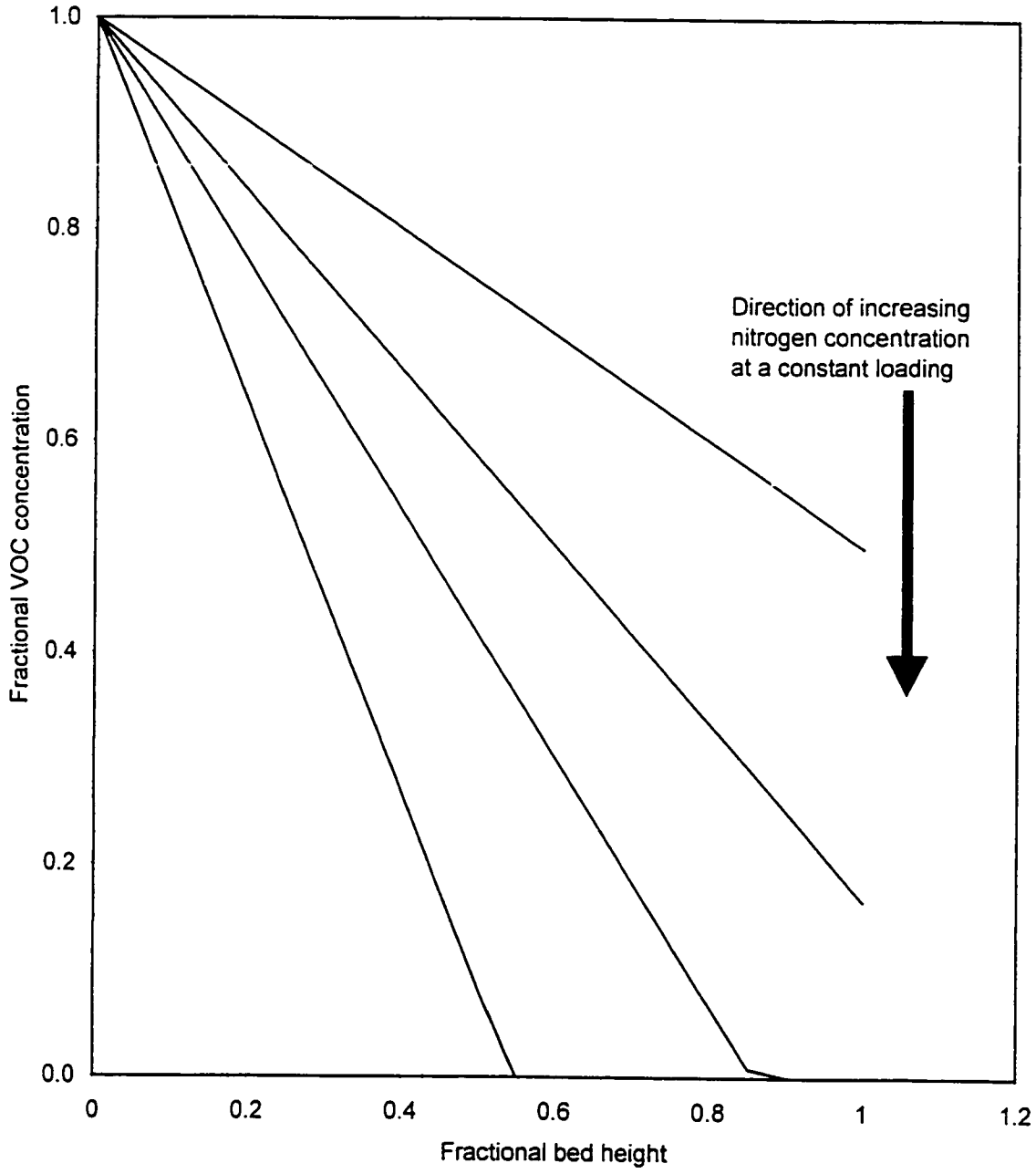


Figure 6.2: Zero order reaction limited kinetics curves

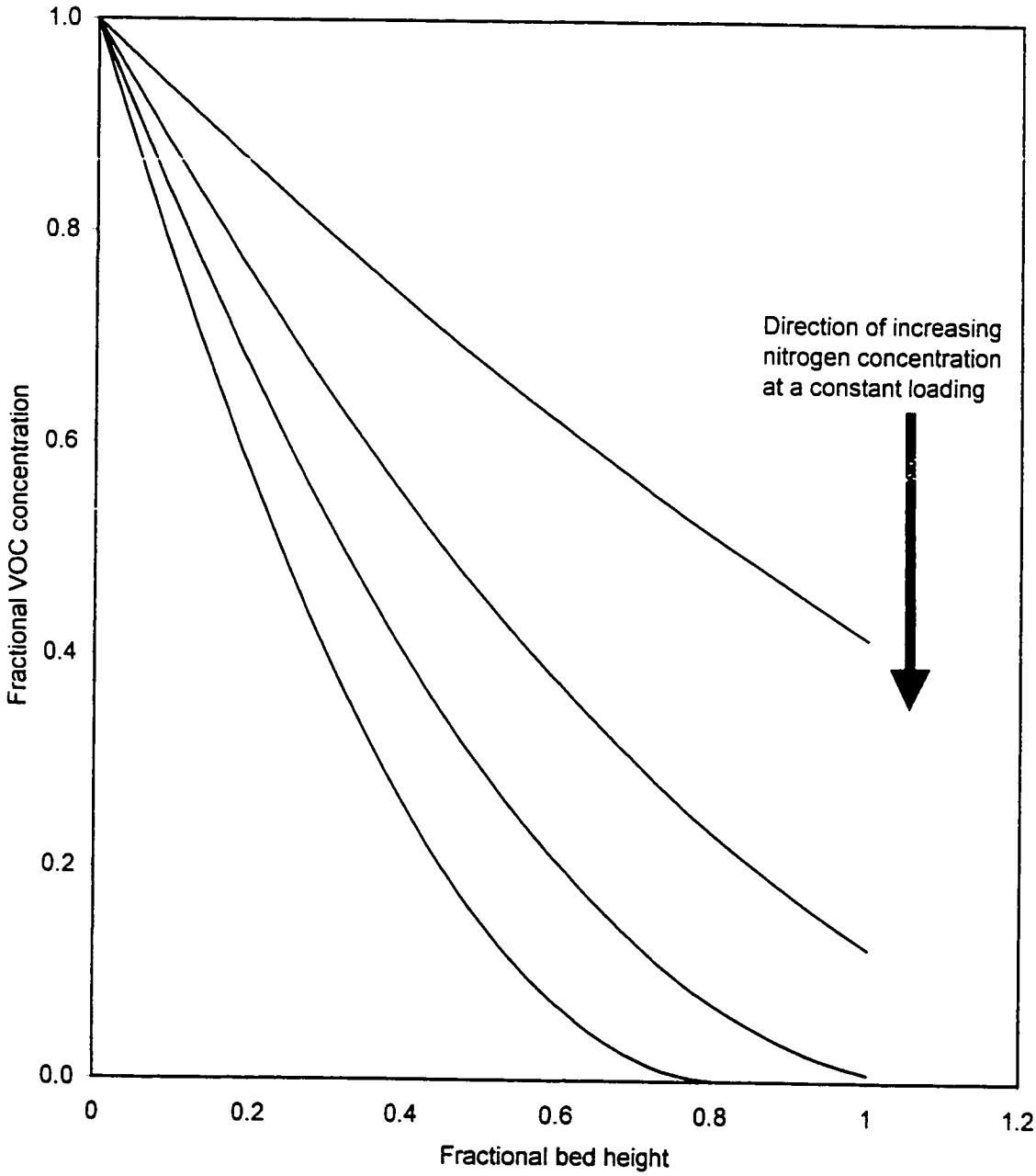


Figure 6.3: Zero order diffusion limited kinetics curves

during maintenance is affected by the change of microbial density (X) arising from the consumption of the nitrogen source and may shift/increase each time nutrients solution is added to the biofilter.

When the assimilable nitrogen concentration is too low, the hexane removal in biofilters is predominantly through the maintenance mechanism. At zero assimilable nitrogen concentration Equation 6.8 becomes:

$$-r_C = \frac{dC_{li}}{\mathrm{d}t} = \Psi \left(\frac{C}{C + K_{C2}} \right) \mathrm{X} \tag{6.9}$$

The values of Ψ and the order of reaction based on carbon source under zero nitrogen concentration condition can be evaluated from Equation 6.9, when elimination capacity has reached a steady state and nitrogen concentration in the biofilter is zero. The information obtained during zero nitrogen concentration can be substituted in equation 6.8 to evaluate the μ_{max} and order of reaction for the first part of the right hand side of Equation 6.8.

Hexane mass balance:

The hexane mass balance is described by effective diffusion:

$$D_e \frac{d^2 C_{li}}{dx^2} - r_i = 0 \tag{6.10}$$

where C_{li} is the concentration of hexane inside the biolayer. C_{li} at the interface is in equilibrium with the concentration of hexane in the bulk gas, hence the first boundary conditions are;

$$x = 0, \qquad C_{li} = \frac{C_{gi}}{m_i} \tag{6.11}$$

The hexane mass balance in the gas phase along the column is given by:

$$-U_g\left(\frac{dC_g}{dh}\right) = N_T \cdot a \tag{6.12}$$

where N_T is the number of transfer units and a is the specific particle surface area per unit volume(m^2/m^3).

The differential Equations 6.8, 6.10 and 6.12 describe the biofiltration process performed under supplementary nutrient conditions. Equation 6.8 addresses biodegradation rates while accounting for changes arising from the nitrogen concentration. Equation 6.10 addresses the mass transfer in the biofilter and 6.12 describes the hexane concentration change along the column. Under the experimental conditions used in this work, the kinetic parameters could not be determined accurately. This modeling approach is therefore recommended for future work.

6.2 Results

6.2.1 Aqueous fermentation

The aqueous fermentation experimental data and model curves are shown in Figure 6.4. The parameters $K_{s,N} = 0.04$ g/L, and $\mu_{max} = 0.17$ h^{-1} as estimated in section 3.2 were used in the differential Equation 6.3 which was solved using the fourth order Runge-Kutta numerical tools in Mathcad to generate the model curve D.13. The model curve fits the experimental data showing that growth of microorganisms on hexane as the sole carbon source followed Monod kinetics and nitrogen is a limiting reactant. This implies that microorganisms in the biofilters will also grow according to the Monod kinetics if there are no mass transfer limitation due to solid state limitations and low hexane VOC concentrations.

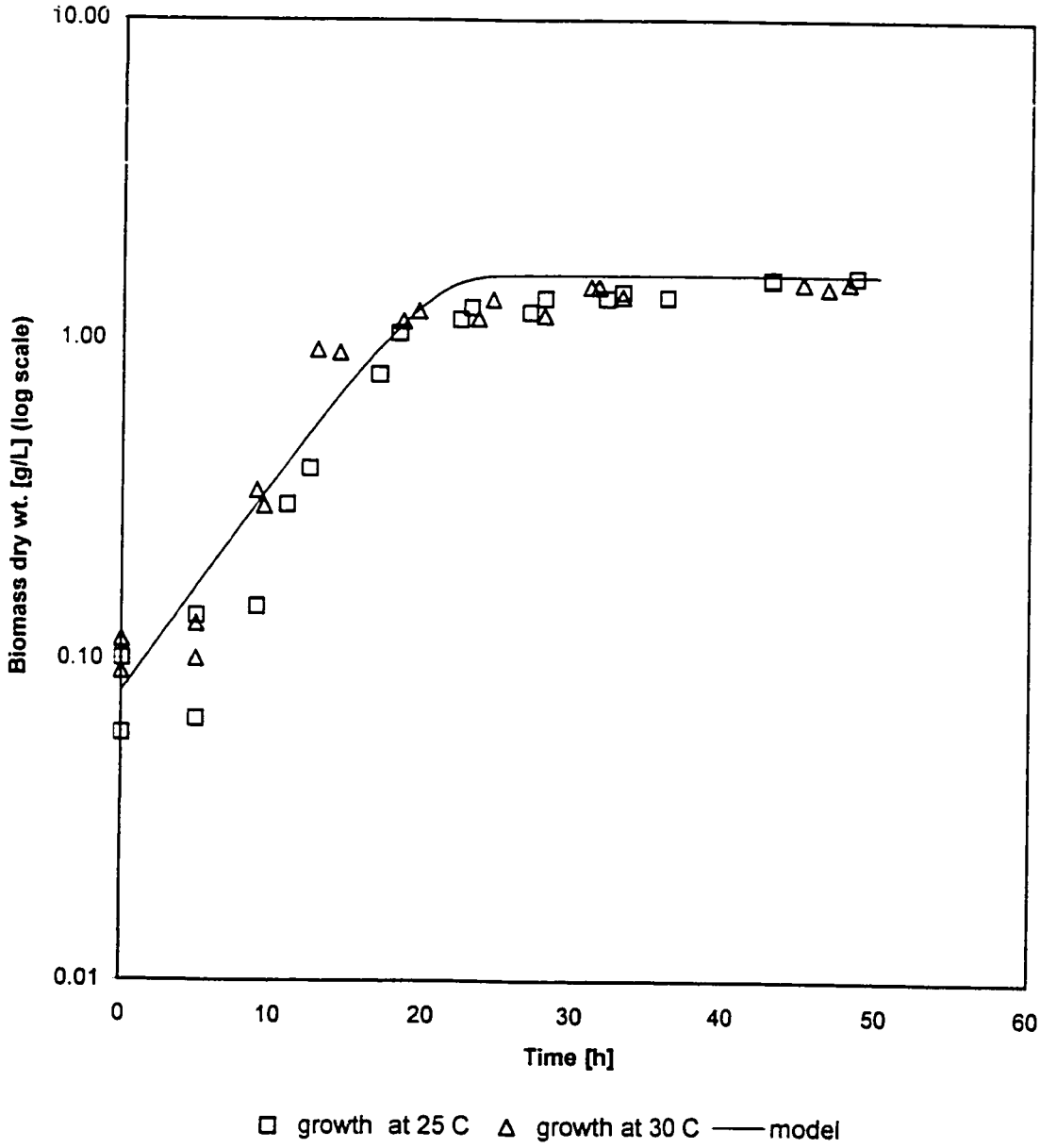


Figure 6.4: Aqueous fermentation experimental and model curves ($K_{sN} = 0.04$ g/L, and $\mu_{max} = 0.17$ h^{-1})

6.2.2 Hexane removal

Figures 6.5 through 6.7 show that the hexane removal followed zero order kinetics. The fractional concentration of hexane along the columns gave a more or less straight line trend confirming the order. Zero order kinetics was exhibited in all experiments of high and low nutrient supplementation as well as long term biofiltration experiments. Additional figures for the long-term biofiltration are shown on Figures D.11 and D.12.

The concentration of hexane did not affect the order of the reaction; it only changed the rate of decrease in concentration along the column. When the zero order kinetics was exhibited throughout the range of hexane concentration (50–400 ppm) covered in this experimental work. Even at low hexane concentrations resulting in complete removal (days 67 and 78 Figure 6.7), zero order kinetics were exhibited.

The time after addition of supplementary nutrients did not affect the order of the reaction. The influence of time after addition of supplementary nutrients was the slope of the curves. The longer the time after adding a batch of supplementary nutrients the lower the rate of removal. The trend was expected since a decrease in nitrogen concentration in the solid media was accompanied by lower orders of reaction, other conditions such gas flow rate being the same.

The type of the solid media has no influence on the order of the reaction. All three media formulations pearlite, peat and the mixture showed the same zero order kinetics trend. The difference among the media is the slope of the curves depending on how active the biofilter was. The more active the biofilter was the less the slope of the curve, hence the higher the reaction rate constant.

The above results indicates that the zero order mathematical model Equations presented by Ottengraf ([98])adequately describes the biofiltration process under the nutrient supplementary conditions at any single moment in time but not as a long term model. The model however cannot describe a periodic nutrient supplemented biofiltration process on a long-term basis. The process is dynamic due to changes of

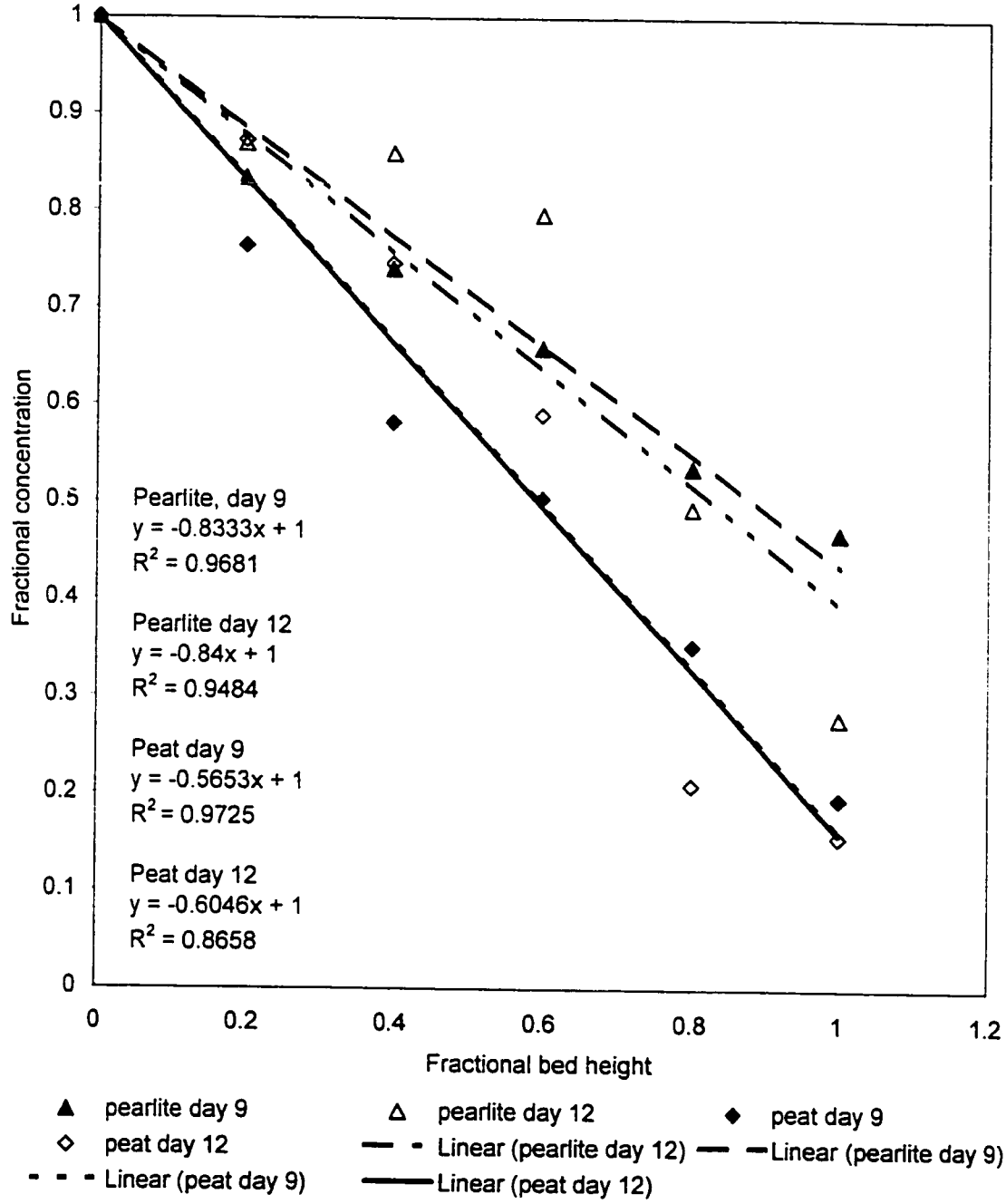


Figure 6.5: Zero order reaction kinetics for n-hexane (high nutrient supplementation)

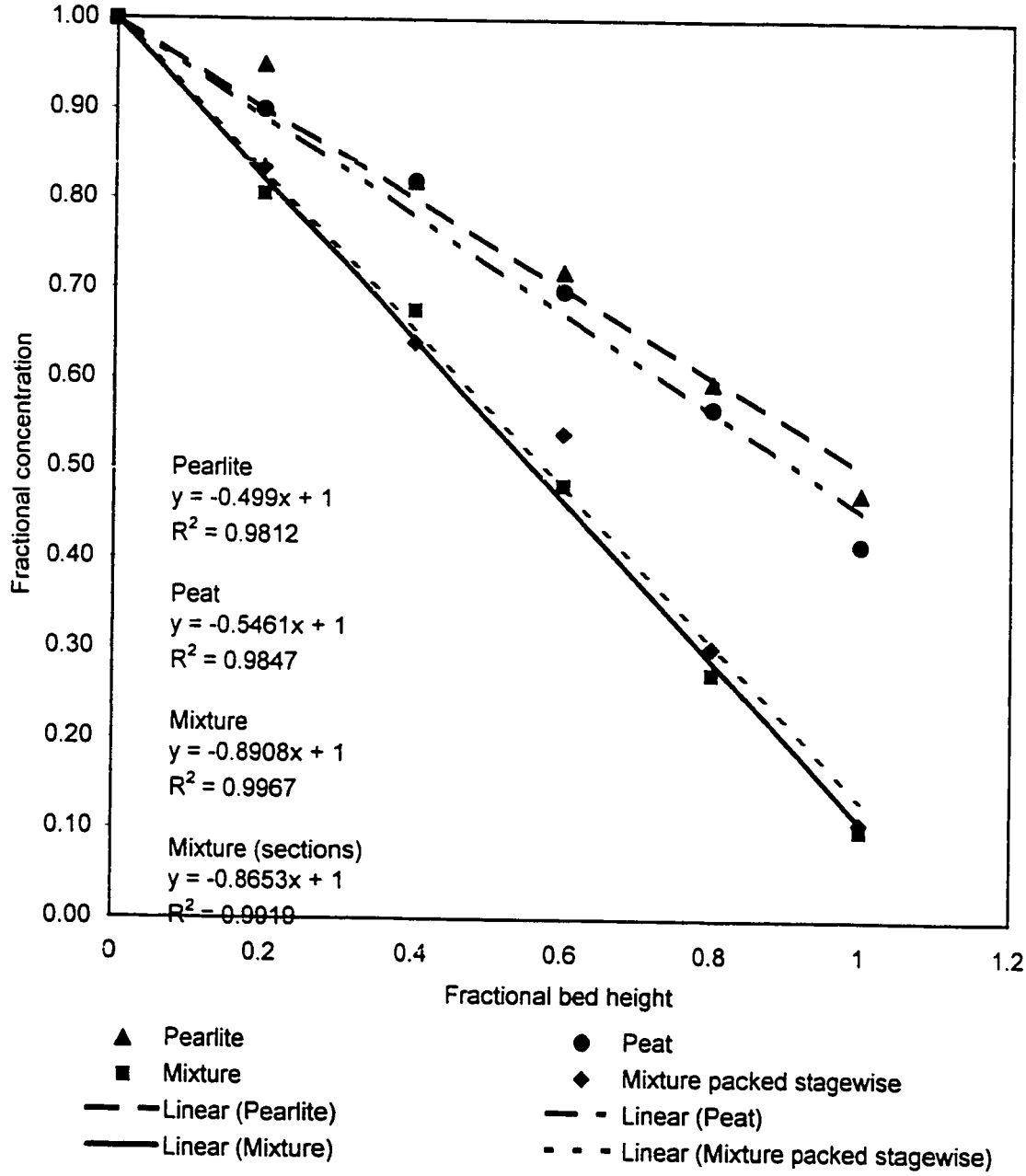


Figure 6.6: Zero order reaction kinetics for n-hexane (low nutrient supplementation)

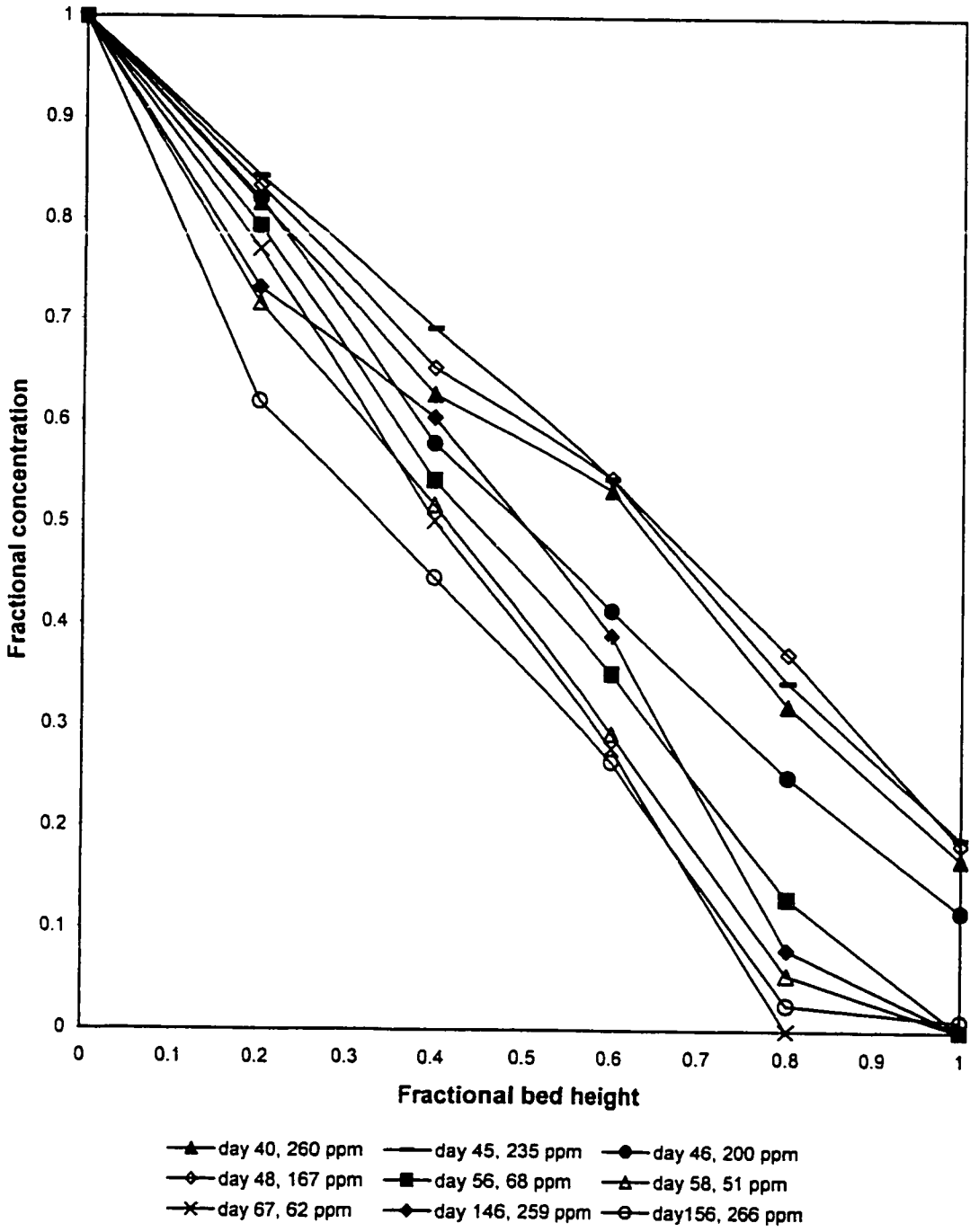


Figure 6.7: Zero order reaction kinetics for n-hexane in the column containing peat (long term biofiltration)

reaction rate constant arising from changes of the nutrient concentrations and biomass density. A more rigorous model which address the time dependent changes is needed.

6.2.3 Concluding remarks

It can be concluded here that zero order kinetic model with diffusion is adequate for the biofiltration of hexane. The nature of the media did not affect the order of the reaction. The hexane concentration in the gas phase and flow rates also had little influence on the order of the reaction. Due to the decrease in nitrogen content with time the model need to use different values of reaction rate constant for different days. A better model which addresses time dependent changes is therefore needed, unless the physical problem of oscillating reaction rate constant is eliminated by modifying the nutrient addition protocol.

Chapter 7

Conclusions

The following can be concluded from this research:

1. Activated sludge is a good source for the microorganisms in the biofiltration process. The microorganisms from the activated sludge were easily acclimatized to utilize hexane as a sole carbon source. *Pseudomonas corrugata* was identified as the most abundant species in the biofilters as well as the only bacteria capable of utilizing hexane at saturation conditions as the sole carbon source.

2. Nitrogen is a limiting nutrient in both aqueous suspension mixed culture, and the biofilters. The biofiltration rates were strongly dependent on the supplementary nutrients such that the type of the solid media, and the particle size had insignificant influence on the biofiltration. This observation is a contribution to biofiltration technology which should reduce the use of organic materials such as peat and compost whose operating life is limited due to aging. The inorganic solid media such as pearlite under nutrient supplementation offer a alternative solid media which has no aging problems.

3. Under high supplementary nutrient addition conditions, both pearlite and the mixture media removed hexane from the air at approximately equal rates. Un-

der low supplementary nutrient addition conditions, the mixture medium was significantly better than either pearlite or peat alone in removing hexane from the air on short time basis. The low biodegradation rates in pearlite containing columns was due to lower nitrogen concentrations since peat supplied additional nitrogen in other columns containing peat and the mixture. However, on a long term basis, pearlite and the mixture were not significantly different. Given the aging problem or organic media pearlite or other inert solid media offers an attractive solution to biofiltration.

4. The pH is an important factor during the biofiltration. The pH values below 5 reduced the biodegradation rates. Calcium carbonate was found to be effective in controlling pH above 5.5, keeping the biodegradation rates relatively high and prolonging the biofilter life. Biofilter failure occurred immediately after the pH value dropped to 4.5 or lower. All effort must be made to keep the pH above 5.5 in order to prolong the biofilter life.

5. Under our experimental conditions, there was no observed difference in biodegradation of hexane between biofilters containing pearlite with particle sizes 1.70–4.75 mm and 0.85–1.70mm. Under supplementary nutrient conditions, the difference in surface area due to different particle size has negligible effects on reaction rate. This observation offers a change to biofiltration technology to increase particle size, hence reducing pressure drop when operating under nutrient supplementary conditions.

6. Pearlite was confirmed as a novel solid medium for biofilters. It is non biodegradable and yet offers an acceptable biodegradation rates for the VOC. It has an excellent hydrodynamic conductivity and stable lower pressure drop values than peat and the mixture. The increase in pressure drop when water or supplementary nutrient solution are added to the biofilters containing pearlite is negligible

compared to the increase in pressure drop for the biofilters containing either the mixture or peat. Given the above mentioned facts, the pearlite offers the highest performance-indicator, hence lowest operating cost at more or less the same biodegradation rates. Controlling the quantity and frequency of supplementation nutrient addition is essential in order to improve biodegradation rates while limiting biomass overgrowth which results in higher pressure drops and shorter biofilter life. This work has demonstrated a potential of using non organic medium for biofiltration through nutrient supplementation strategy to improve biodegradation rates on both short long term basis. This work contributes towards revolutionasing biofiltration industry from using organic to inorganic medium such a pearlite to avoid aging rated problems.

7. Microbial growth in the suspension aqueous culture followed Monod kinetics where nitrogen was a limiting reactant and hexane was the sole carbon source.

8. Hexane was removed from the biofilters according to zero order reaction kinetics. The mathematical model equations presented by Ottengraf are effective in describing the biofiltration process at any given point in time. However, the reaction rate constant decreases with nitrogen concentration in the solid media.

From this study, biofiltration process under nutrient supplementation conditions has been expanded. It is now clear that the nutrient supplementation improved biofiltration rates. But quantity and frequency of nutrient addition is very important in maintaining high biodegradation rates and low pressure drop. More important, the inert solid medium is +as equally good as the organic media in terms of biodegradation and has an advantage of low pressure drop. However, the process has an oscillating biofiltration rates and hence more improvement is needed.

Chapter 8

Recommendations

1. While high nutrient concentrations limit the life span of the biofilter due to biomass overgrowth, the choice of nitrogen source such as nitrate to ammonia might reduce microbial growth [125] and still provide the benefit of improved biodegradation rate. Alternatively, developing an effective method to remove the biomass from the solid medium could be a key solution to the problem. Scouring using air through a column filled with water removed a significant amount of cells from the column containing pearlite. Negligible microbial removal was observed for columns containing peat and the mixture (this study). Solid media must be mechanically strong enough to resist scouring. Microbial removal from biofilters will allow supplementary nutrients to be added at a higher rate and achieve high biofiltration rates, as long as biomass disposal does not outweigh the benefits of high biodegradation rates.

2. The biofilter life span can be extended beyond the observed values by adding more calcium carbonate to neutralize organic acids. It is the exhaustion of calcium carbonate which leads to the biofilter failure in this study. The maximum amount of calcium carbonate to be added needs optimization.

3. The biofilters moisture loss starts at the air entrance section and progresses towards the remaining sections. It seems more appropriate to operate biofilters in a down-flow mode than up-flow mode. Down-flow operation will reduce moisture hold-up caused by up-flow movement of air and consequently resulting in high pressure drop. In addition, water added to restore moisture will be added directly to where drying rate is fast and excess moisture will be forced from the media by air flowing downward.

4. The particle size tested did not have any influence on the biodegradation rates. Further work on the combined effects of media particle size and nutrient supplementation is needed to evaluate the optimum media particle size. Increasing the particle size until it has a significant effect on biodegradation rate will significantly reduce the pressure drop, and lower operating costs of biofiltration.

5. Further work is needed to study the distribution and thickness of biofilm on the solid particles and its change with time. This will increase the understanding of biomass accumulation in the biofilter as well as its long-term effect on the residence time distribution and channelling.

6. The study of microbial community and its changes (succession) is needed to understanding of intrinsic kinetics, yield coefficients and overall changes in biofiltration rates.

7. The effect of nitrogen concentration on reaction kinetics needs further study so that the rate of VOC removal can be predicted several days ahead, after addition of supplementary nutrients for a given biofilter loading.

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Appendix A

Nomenclature

Symbol	*Units*	*Description*
a	m^2/m^3	specific surface area of a fixed bed
C_{in}	mol/m^3	gas phase concentration of the exit stream
C_{out}	mol/m^3	gas phase concentration of the inlet stream
C_l	mol/m^3	liquid phase concentration
D_e	m^2/s	diffusion coefficient
D_f		dilution factor
d_p	m	particle diameter
K_o	$mol/m^3.s$	apparent zero order reaction rate constant
K_L	m/s	overall liquid mass transfer coefficient
K_s	mol/m^3	Monod constant for substrate S
k_o	$mol/m^3.s$	zero order reaction rate constant
k_1	1/s	fist order reaction rate constant
K_N	mol/m^3	Monod constant for nitrogen
H	m	total bed height
h	m	height coordinate
m		partition coefficient
MS		fraction moisture content
N	mol/s	mass transfer rate
N_T		number of transfer units
CFU_p		agar plate colony forming units
r	$mol/m^3.s$	reaction rate
s	g/m^3	substrate concentration
t	s	time
S_N	mol/m^3	substrate nitrogen
U_g	m/s	superficial gas velocity

Symbol	*Units*	*Description*
V_m	mol/kg.s	maximum substrate utilization rate
w	g	Sample dry weight
X	kg/m^3	cell concentration
x	m	length coordinate
Greek		
ΔC	g/m^3	change in concentration
ΔP	Pa	pressure drop
δ	m	biofilm thickness
ε		bed void fraction
λ	m	biofilm penetration depth
ϕ		Thiele modulus
μ	h^{-1}	specific growth rate conatant
μ_{max}	h^{-1}	maximum growth rate conatant
μ^*	Pa.s	dynamic viscosity
ρ	kg/m^3	density
ρ_{ds}	kg/m^3	bulk density of the solid medium
Ψ		reaction rate constant without microbial growth
Subscripts		
C		Carbon
D		Weighing dish
FP	g	Filter paper
N		Nitrogen
NO		Nitrogen at time t= 0
Td	g	Total dry weight

Appendix B

Abbreviations

Abbreviation	*Description*
B	Bottom Column section
CFU	Colony forming units
Co.	Company
FID	Flame ionization detector
GC	Gas chromatograph
HNS	High frequency nutrients supplementation condition
ID	Internal diameter
IR	Infrared gas analyzer
LNS	Low frequency nutrients supplementation condition
LT	long term biofiltration
M	Middle column section
Mfg.	Manufacturing
NA	Nutrient agar
NPK	Nitrogen, phosphorus, potassium
ox	oxidized
PDA	Potato dextrose agar
PQQ	pyrroloquinoline quinone
red	reduced
sp.	specie
sps.	species
T	top column section
TCA	Tricarboxylic acid cycle
v/v	Volume by volume
VIC	Volatile inorganic compound
VICs	Volatile inorganic compounds
VOC	Volatile organic compound

Abbreviation	*Description*
VOCs	Volatile organic compounds
w/w	weight by weight

Appendix C

Identification of bacteria by Biolog

Procedure:
A) **Smear preparation** [104, 117]

- Add a drop of sterile water on a clean slide
- With a wire loop transfer a small amount of a pure bacterial culture to the water drop and mix.
- Spread the mixture on a slide to form a thin film
- Dry the slide by holding it above a Bunsen burner flame
- Pass the slide, film side up, three times through the Bunsen burner flame to fix and kill the bacteria.

B) **Gram-staining** [104, 117]

- Stain the smear with crystal violet for about 30 seconds.
- Rinse with water.
- Cover the film with Gram-iodine for 30 seconds
- Rinse with water.
- Decolorize the film with a mixture of 75% ethanol and 25% acetone for 10 to 20 seconds.
- Rinse with water.
- Counter stain with safranin for 20 to 30 seconds

- Rinse with water.
- Examine the film using light microscope under oil immersion objective lens (magnification 1000) and determine if the bacteria are gram negative or positive as well as shape and relative size of bacteria.

C) Biolog technique [45, 73, 94, 111, 144]
Biolog identification method is a computer aided system used to identify bacteria and yeast through the following procedure.

- Prepare a young bacterial culture (24 hours) on an agar plate.
- Make a standard culture by transferring biomass from the agar plate using a wet sterile cotton swab stick into a test tube containing 20 mL of normal saline. Gently collect bacteria with the swab stick from the agar surface. Dispense the biomass into the saline by gently rolling the swab stick against a tube wall.
- Measure the light absorbance of the resulting broth culture. The light absorbance should be within the the lower and upper limits of Biolog standard for Gram-positive or Gram-negative bacteria respectively.
- Using a micro pipette, transfer 150 μL volumes into 96 wells of Biolog panel. Incubate for 4–72 hours. The wells contain special nutrient/carbon sources and indicator for detecting the metabolic bioproducts. A purple color change of a well indicates a positive reaction.
- Enter results of color patterns of the wells for into a computer for definitive identification of the microorganism.

```
MICROLOG (TM) 1 RELEASE 3.50

Date         : 26/03/98
Hour         : 24
Plate Type   : GN
Media Type   : TSA/BUGM
Plate #      : 8
Strain Name  : ?
Strain #     : C
Other Info   : 48 HOURS
Input Mode   : Well-By-Well
Data Base    : MicroLog GN

POSITIVE/NEGATIVE DATA
"XX" = positive, "B" = borderline, ".." = negative
 ..+ = negative, "=>" ID choice positive > 90% of time
 XX- = positive, "=>" ID choice positive < 10% of time

    1     2     3     4     5     6     7     8     9     10    11    12
  +-----------------------------------------------------------------------
A |  ..    ..    ..    ..    XX    XX    ..    ..    ..    XX    XX    ..
B |  ..     B    ..    XX    ..    XX    XX    ..    ..    ..    XX    XX
C |  ..    ..    ..    ..    ..    ..    ..+   ..-   ..    ..    XX    XX
D |   B    XX    XX    ..    XX    XX    XX    XX    XX    ..    XX    ..
E |  ..+   ..    ..    XX    ..    XX    XX    XX    XX    XX    ..    XX
F |  XX    XX    ..    ..    XX    XX    XX    XX    XX    XX     B-   XX
G |   B-   XX    XX     B    ..    XX    ..+   ..    XX     B    XX    XX
H |  XX    XX     B    ..    ..    XX    ..    ..    XX    ..    ..    ..

 BIO-NUMBER : 0306-2543-0003-7372-0575-6377-7517-7110

  SPECIES IDENTIFICATION     : PSEUDOMONAS CORRUGATA

   CLOSEST SPECIES :.............................SIM....DIST...AVG....MAX
=> 1)   PSEUDOMONAS CORRUGATA                     0.652  5.334  0.211  2.747
   2)   PSEUDOMONAS VIRIDIFLAVA B                 0.000  8.071  0.406  2.202
   3)   PSEUDOMONAS SYRINGAE PV LACHRYMANS        0.000  8.116  0.531  1.275
   4)   PSEUDOMONAS SYRINGAE PV SYRINGAE A        0.000  8.840  0.656  5.181
   5)   PSEUDOMONAS SYRINGAE PV HIBISCI           0.000  9.159  1.188  2.219
   6)   PSEUDOMONAS TOLAASII                      0.000  9.203  0.313  1.606
   7)   PSEUDOMONAS FUSCOVAGINAE                  0.000  9.207  0.438  2.162
   8)   PSEUDOMONAS SYRINGAE PV MORSPRUNO         0.000  9.492  0.083  1.225
   9)   PSEUDOMONAS SYRINGAE PV APTATA            0.000 10.476  0.490  2.553
  10)   PSEUDOMONAS CICHORII                      0.000 10.597  0.738  4.172
   other :                                        -----  -----  -----  -----
```

Figure C.1: A sample of Biolog identification pattern

Appendix D

Additional Results

The following are additional graphical results of the biodegradation of hexane, pressure drop across the biofilters, nutrient concentrations in the columns and modelling referred in the literature.

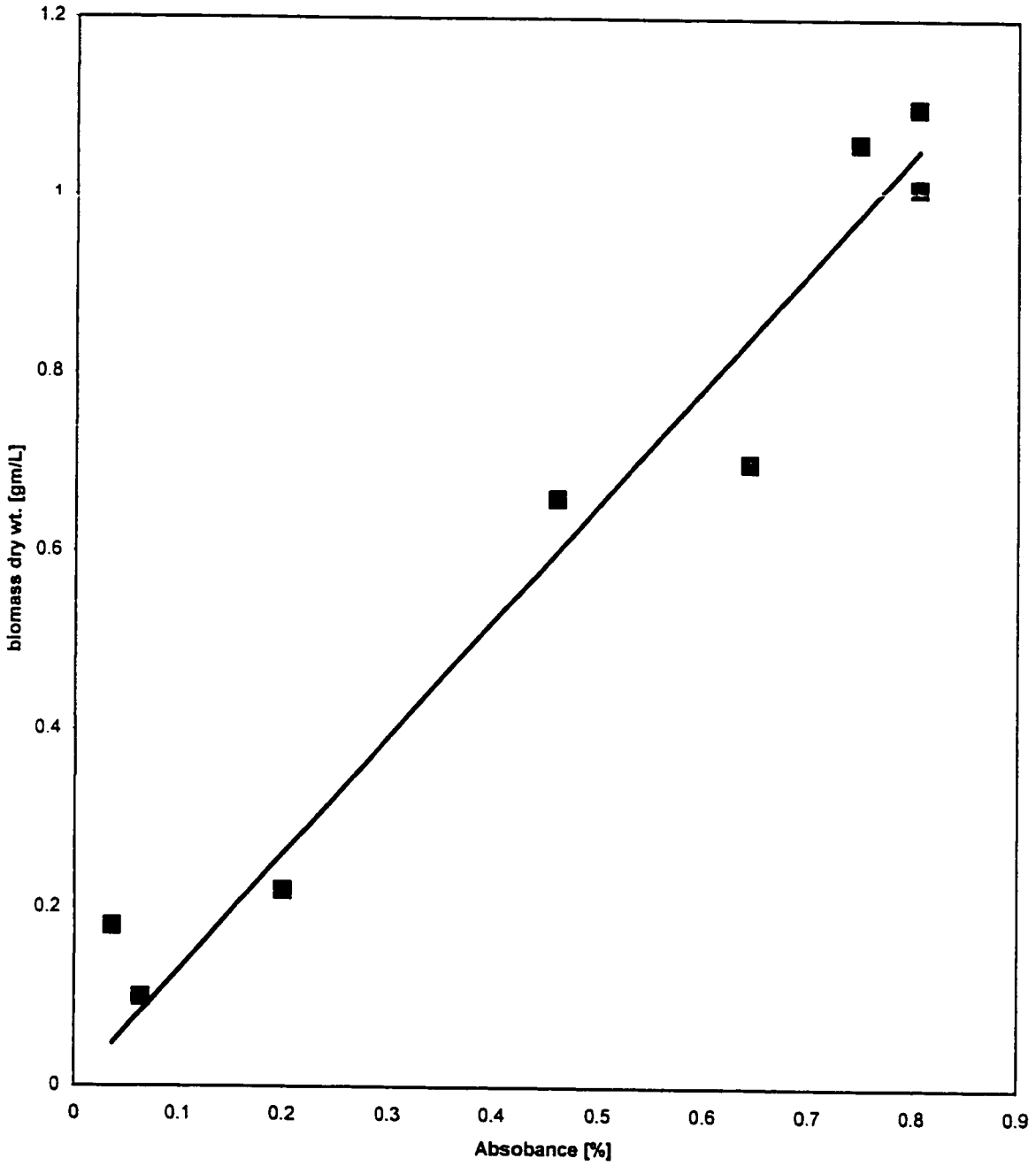

Figure D.1: Optical density-dry weight calibration curve

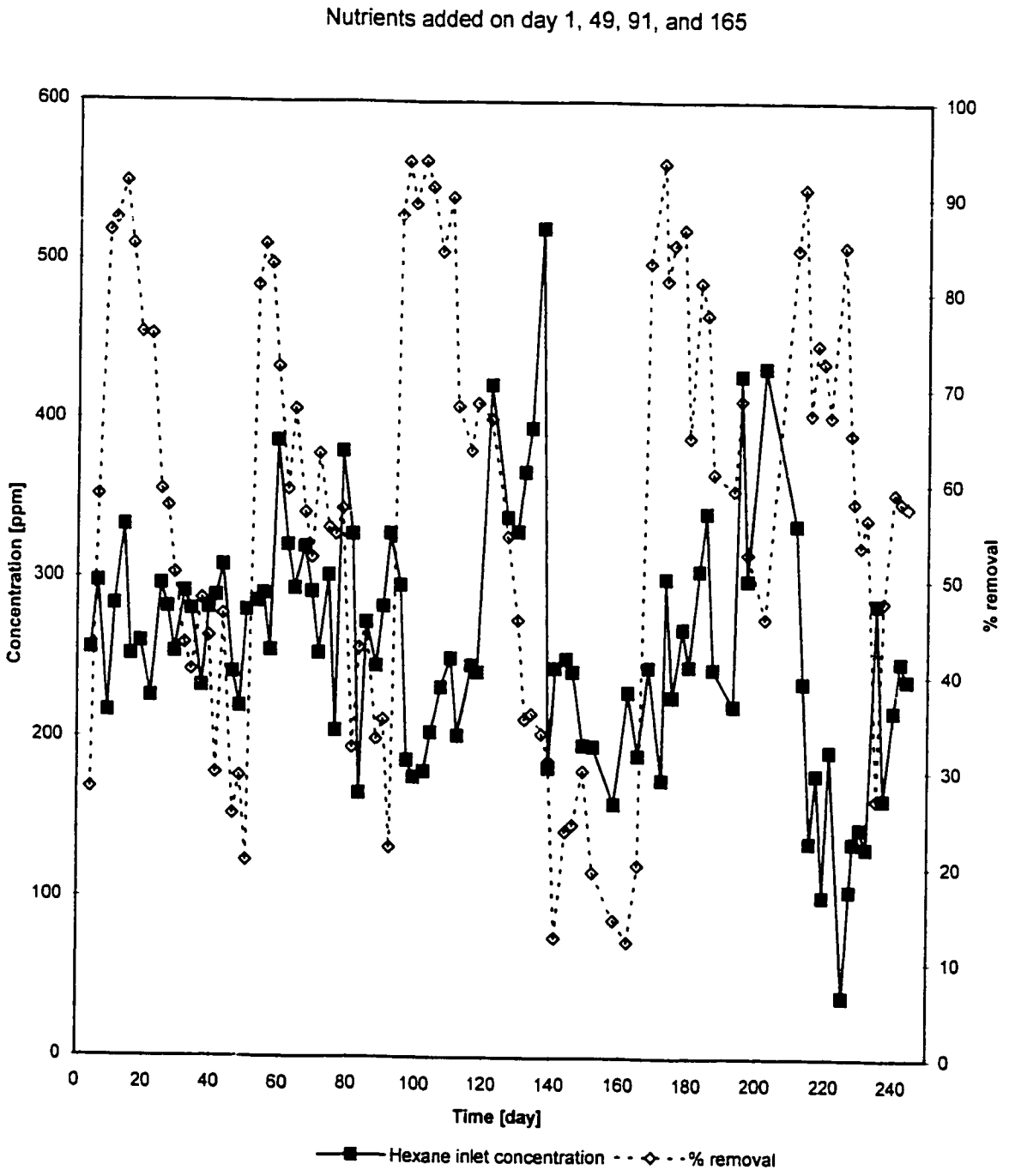

Figure D.2: Hexane percent removal in the column containing 100% pearlite under low nutrient supplementation, long term

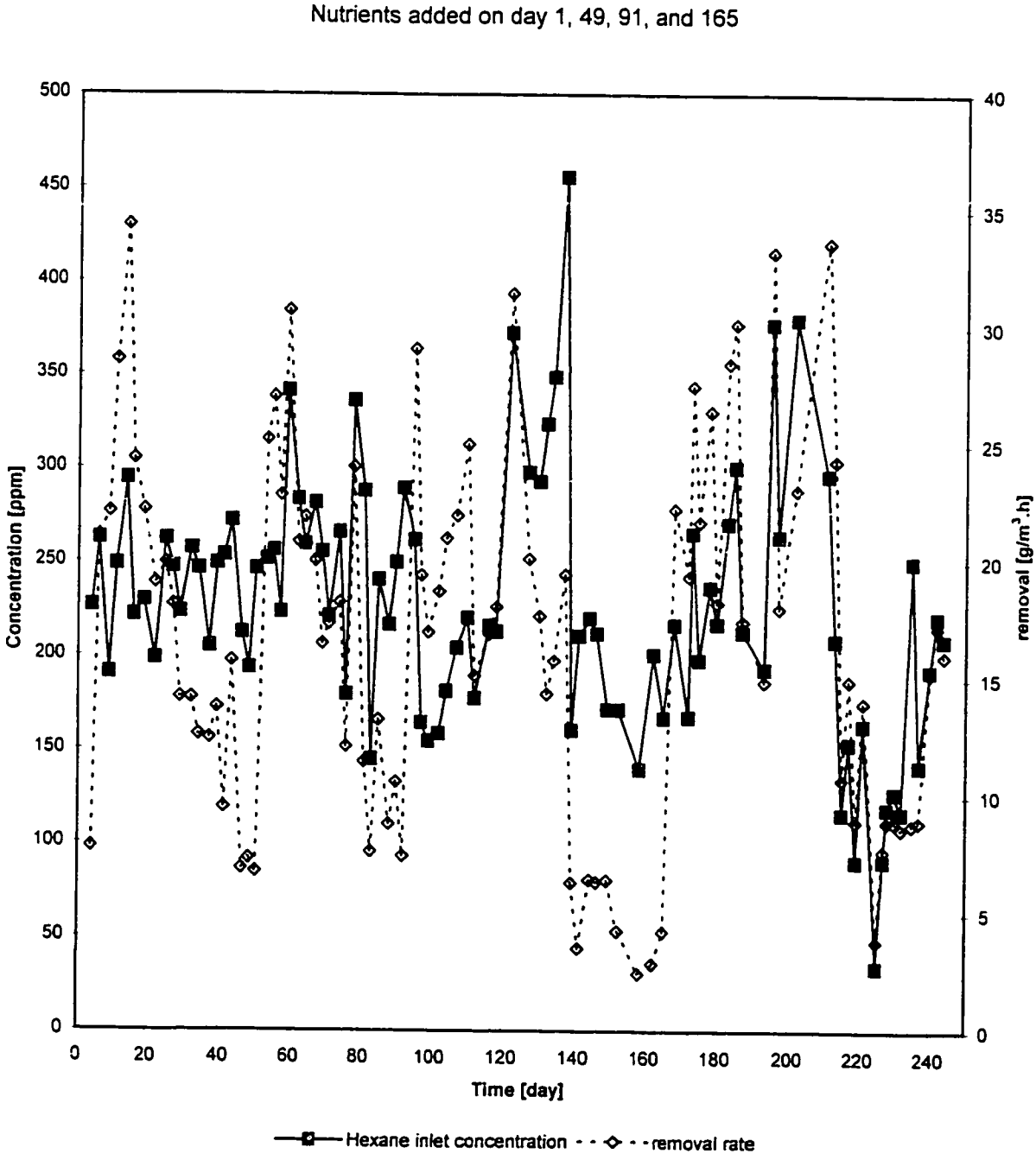

Figure D.3: Hexane removal rates in the column containing 100% pearlite under low nutrient supplementation, long term

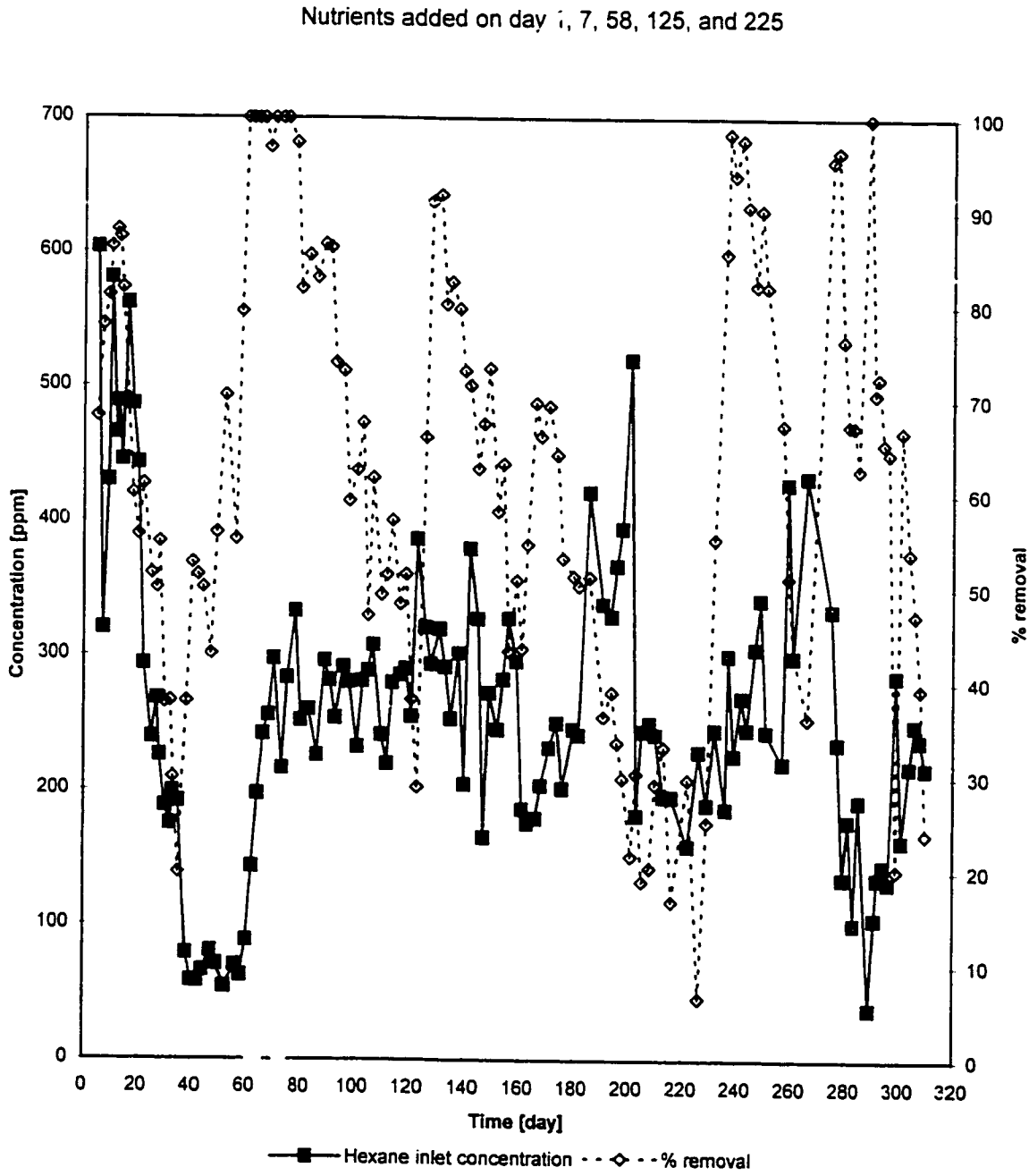

Figure D.4: Hexane percent removal in the column containing the mixture under low nutrient supplementation, long term

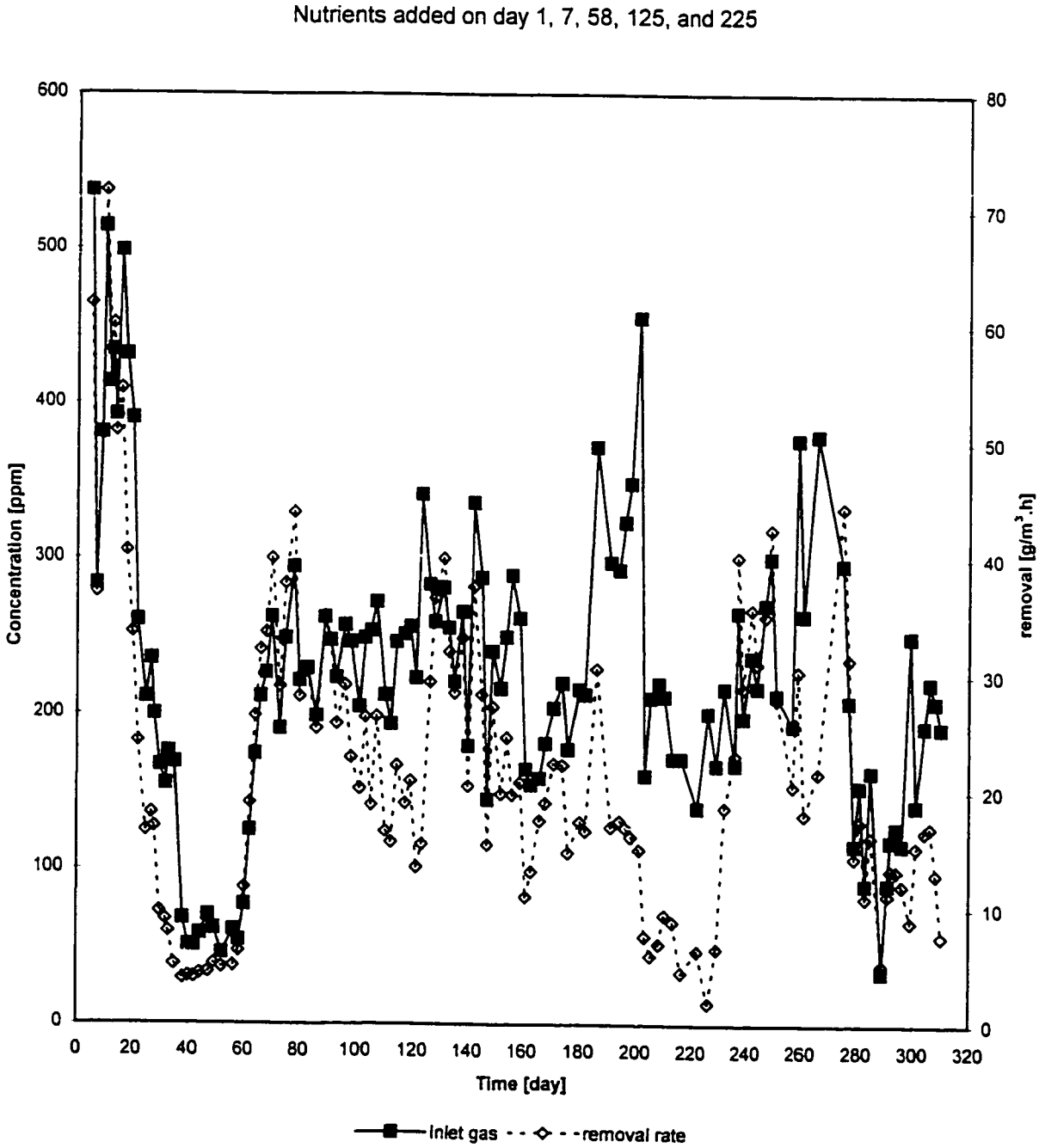

Figure D.5: Hexane removal rates in the column containing the mixture under low nutrient supplementation, long term

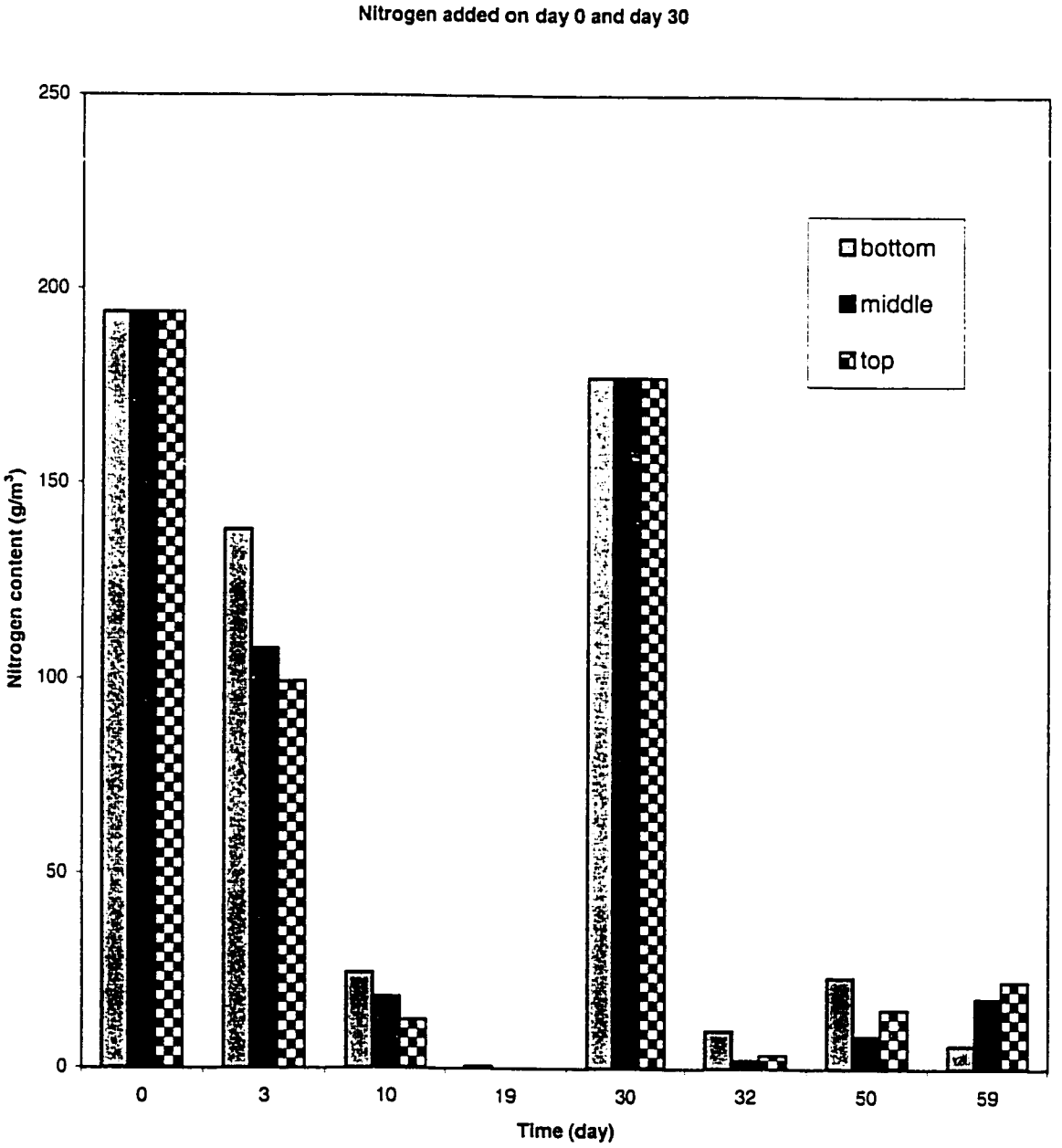

Figure D.6: Nitrogen content in the top, middle and bottom sections of the column containing the pearlite

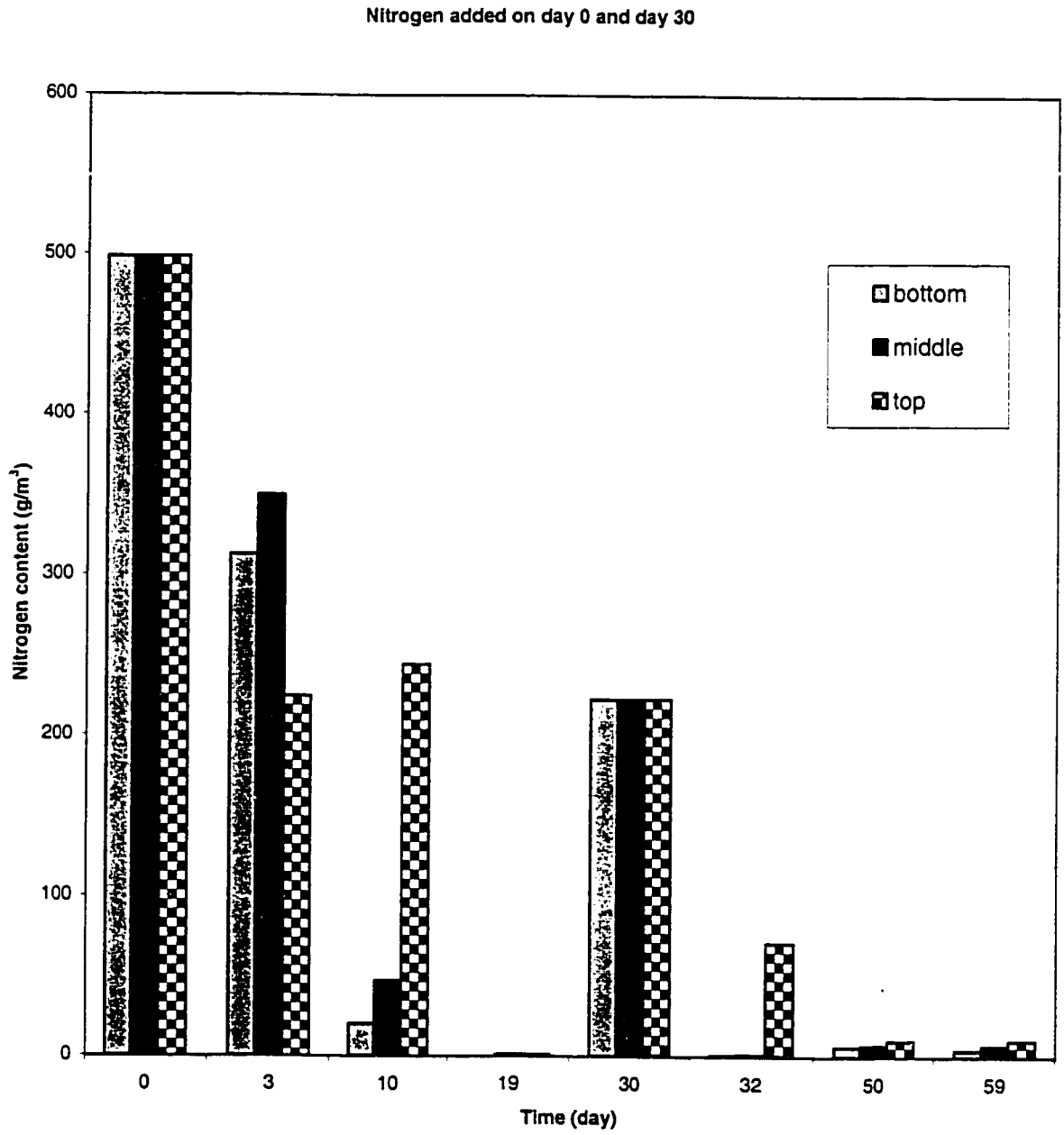

Figure D.7: Nitrogen content in the top, middle and bottom sections of the column containing the peat

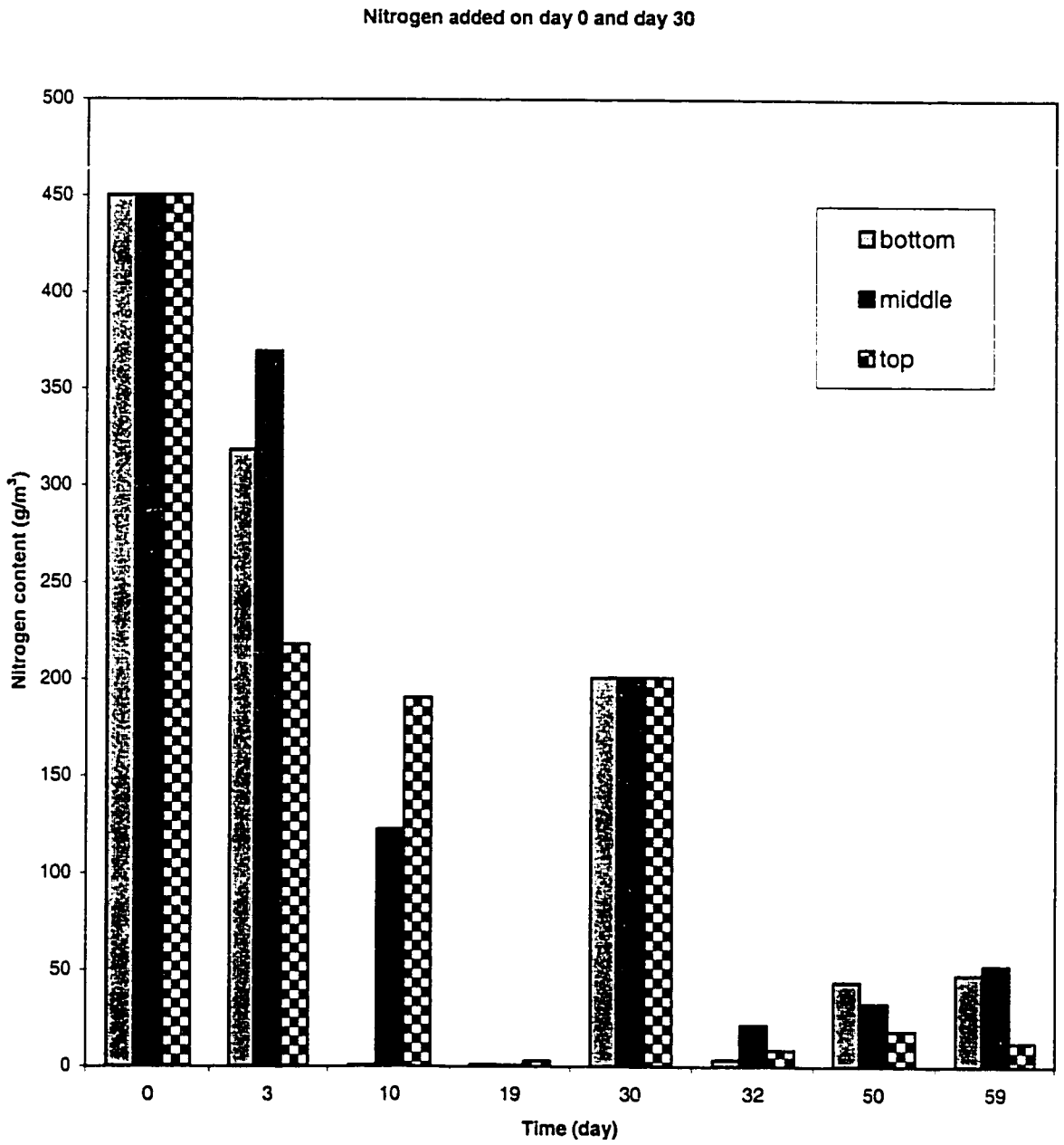

Figure D.8: Nitrogen content in the top, middle and bottom sections of the column containing the mixture

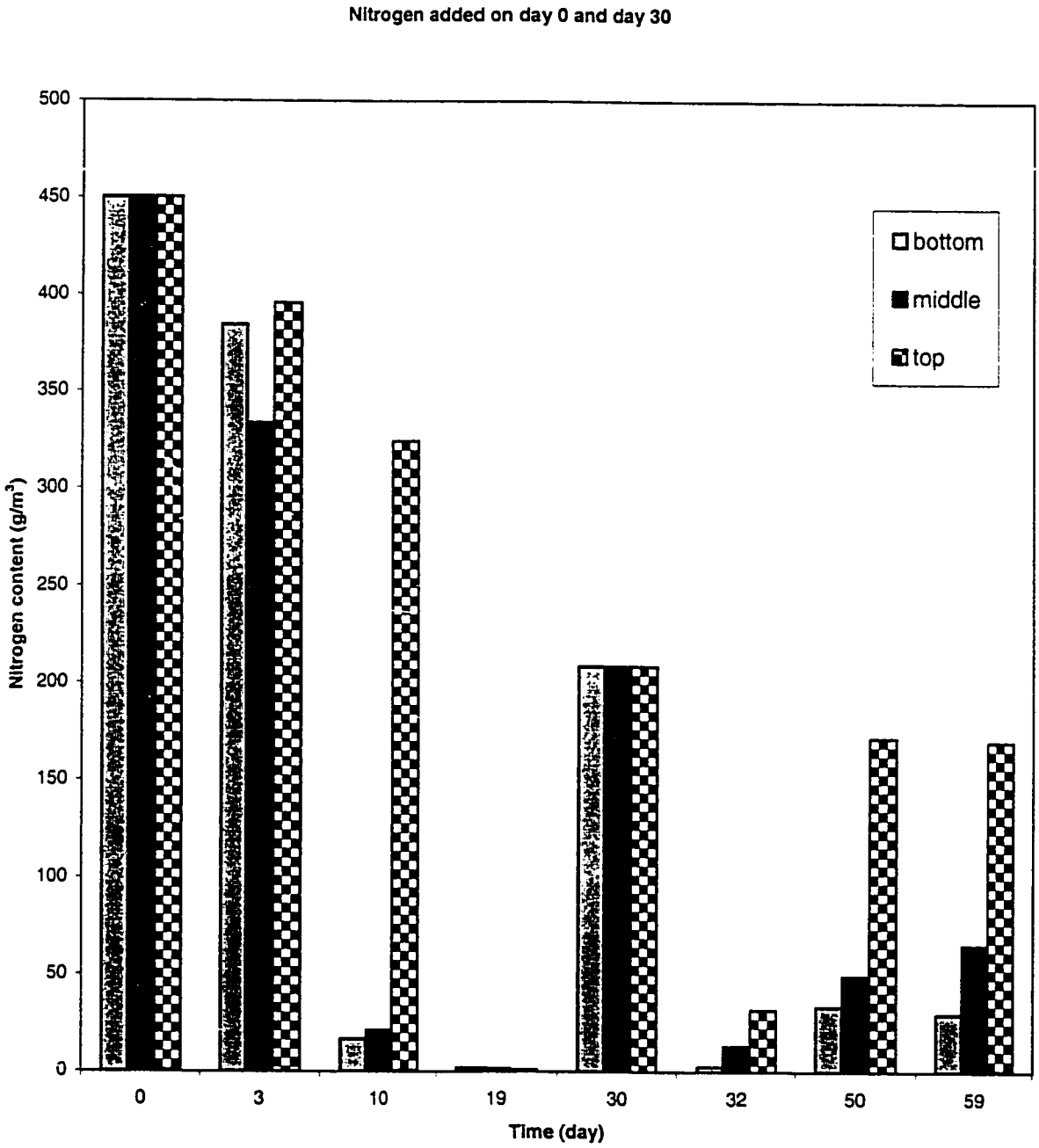

Figure D.9: Nitrogen content in the top, middle and bottom sections of the column containing the mixture packed stagewise

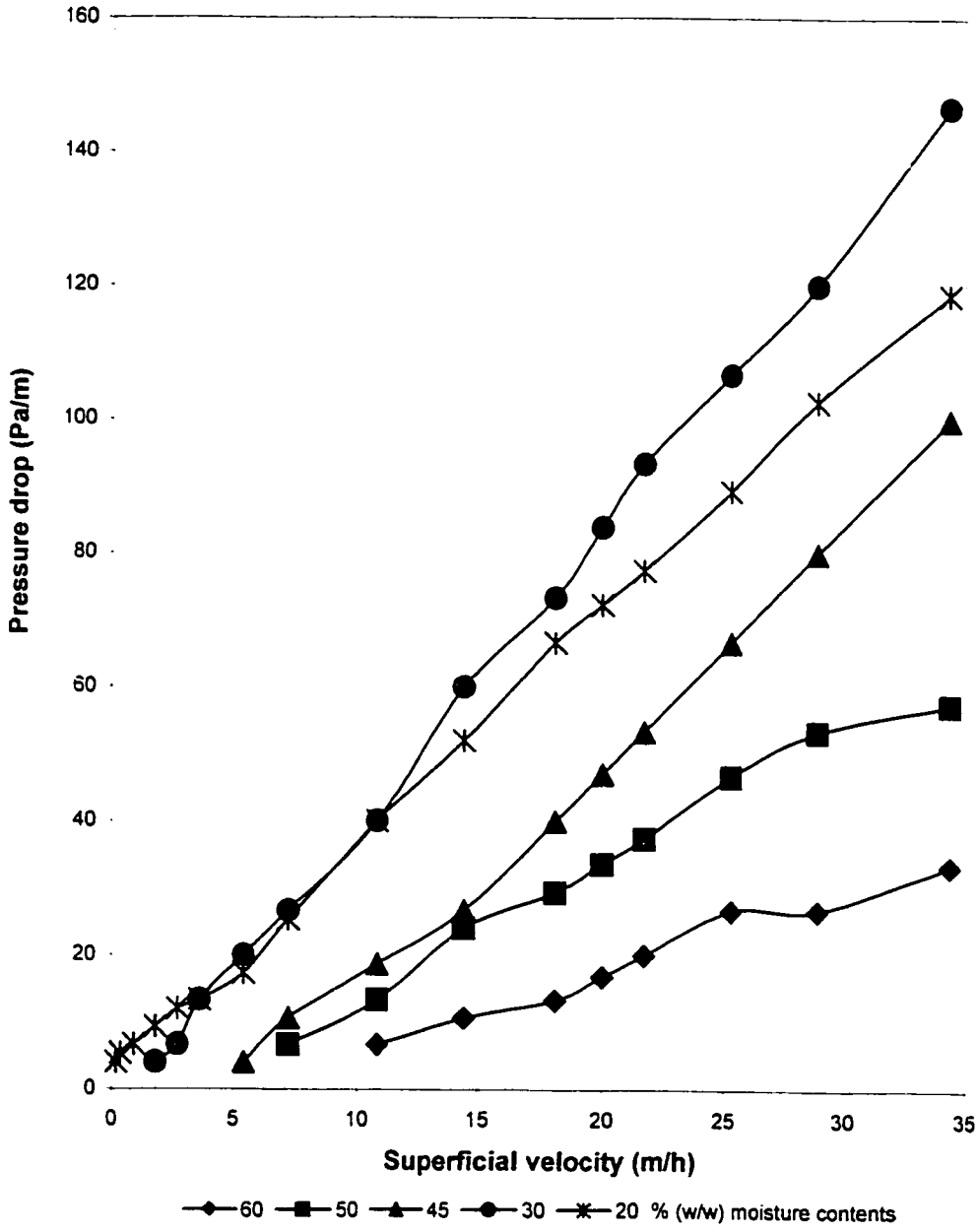

Figure D.10: Pressure drop the column containing the mixture before biofiltration

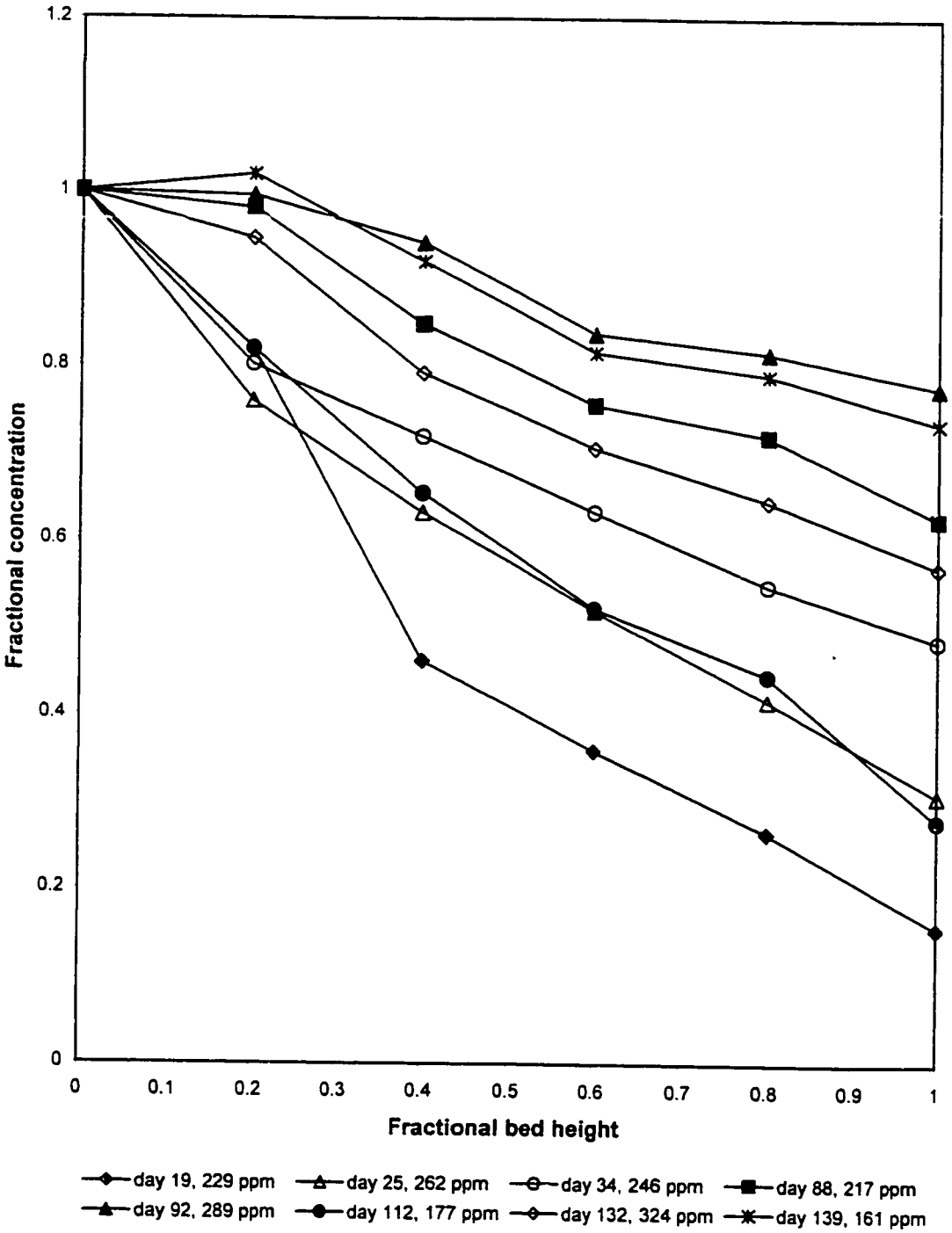

Figure D.11: Zero order reaction kinetics for n-hexane in the column containing the pearlite (long term biofiltration)

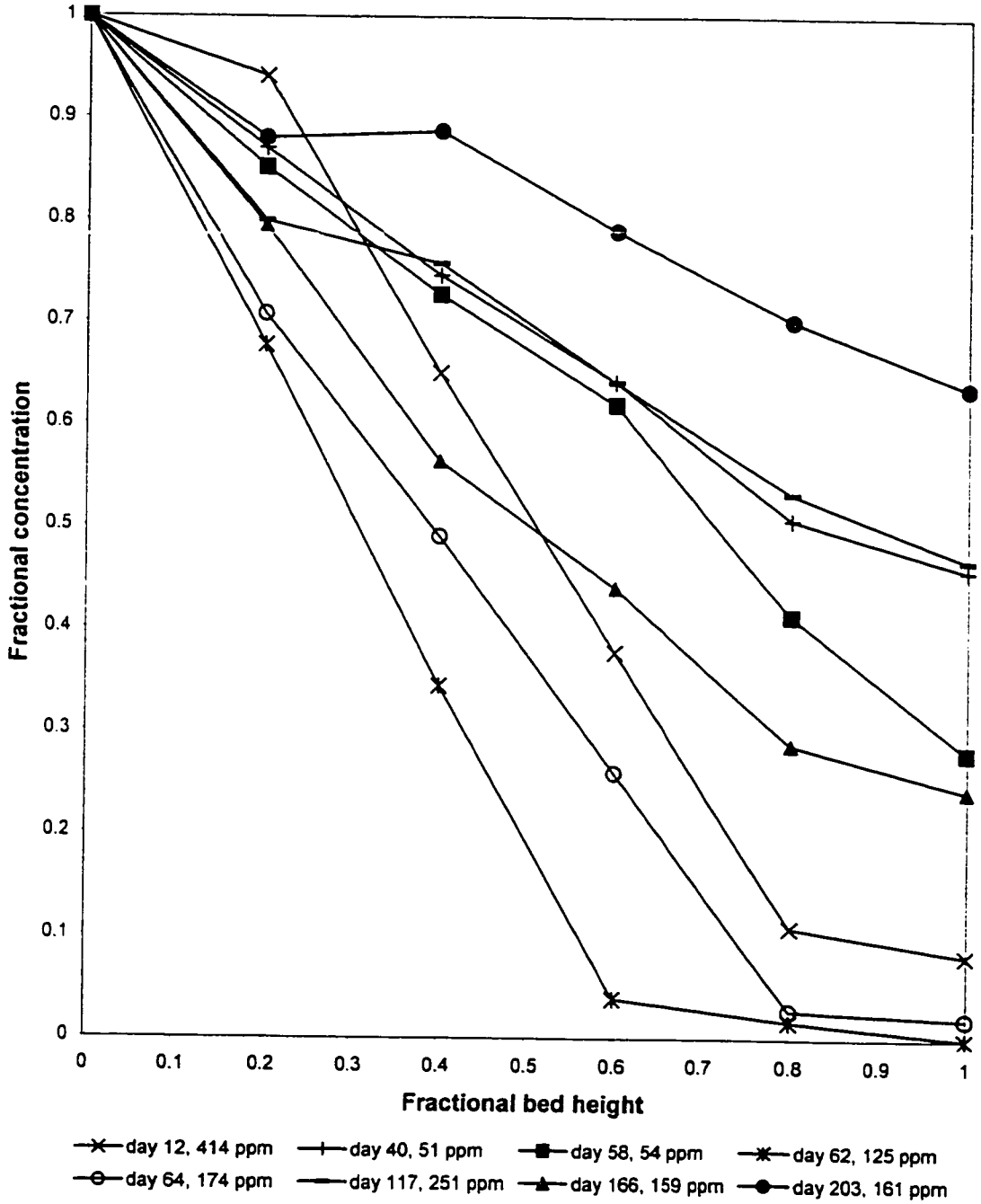

Figure D.12: Zero order reaction kinetics for n-hexane in the column containing the mixture packed stagewise (long term biofiltration)

BIOMASS GROWTH AND Ammonia/Nitrogen utilization in aqueous suspension culture

$K_s := 0.04$ $\mu_{max} := 0.17$ $Y := 7.17$ $S_0 := 0.212$ $X_0 := 0.08$

BIOMASS GROWTH

$$D(t,F) := \begin{bmatrix} \mu_{max} \cdot F_0 \cdot \dfrac{\left(S_0 - \dfrac{F_0 - X_0}{Y}\right)}{K_s + S_0 - \dfrac{F_0 - X_0}{Y}} \\ \dfrac{-\mu_{max}}{Y} \cdot \dfrac{F_1}{F_1 + K_s} \cdot \left[(S_0 - F_1) \cdot Y + X_0\right] \end{bmatrix}$$

$$init\_val := \begin{bmatrix} 0.08 \\ 0.212 \end{bmatrix}$$

sol := rkfixed(init_val, 0, 50, 100, D)

Time $t := sol^{<0>}$

Cell concentration, $X := sol^{<1>}$

Nitrogen concentration, $S := sol^{<2>}$

Sample data

sol =

	0	1	2
0	0	0.08	0.212
1	0.5	0.086	0.211
2	1	0.092	0.21
3	1.5	0.099	0.209
4	2	0.106	0.208
5	2.5	0.114	0.207
6	3	0.123	0.206
7	3.5	0.132	0.205
8	4	0.142	0.203
9	4.5	0.152	0.202
10	5	0.163	0.2
11	5.5	0.175	0.199
12	6	0.188	0.197

Figure D.13: Fourth order Rungekutta numerical simulation of aqueous fermentation of the hexane