Direct transesterification of microalgae to biodiesel using ionic liquid catalysts

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Increasing global energy demand coupled with the environmental effects of fossil fuels have, like global warming, reinvigorated the need for the commercialization and development of alternative renewable energy sources such as biodiesel. Microalgae are considered a sustainable feedstock for the commercial production of biofuels because they need not compete with food production, and they can use CO_2 and sunlight to produce the lipids needed for biodiesel production. Recently the idea to directly convert unbroken and wet microalgae to biodiesel via *in situ* transesterification has drawn attention. However, the high moisture content in microalgae biomass is still a main limiting factor for *in situ* transesterification processes.

The overall objective of this work was to develop and optimize the direct conversion of wet microalgae biomass into biodiesel using an ionic liquid catalyst. This process reduces the total operational steps through the simultaneous extraction and transesterification of intracellular lipids from algae biomass and eliminating the need for an energy-intensive drying step.

Four types of tetrabutylphosphonium carboxylate ionic liquids ($[P_{4444}][CA]$) were synthesized and were used to transesterify refined cooking oils (sunflower, canola, and corn oil) into biodiesel, and for the direct transesterification of wet microalgae biomass (*C. vulgaris*) into biodiesel. Phosphonium carboxylate ionic liquids were found to be good catalysts for transesterification in the presence of methanol and capable of both cell disruption and transesterification in a single step. The leading candidate ionic liquid ($[P_{4444}]$[Formate]) was selected for more in-depth characterization of the effect of process variables on fatty acid methyl ester (FAME) yield. The FAMEs composition and the major properties of synthesized biodiesel from both cooking oils and microalgae were calculated. All synthesized biodiesels fulfilled the biodiesel properties stipulated in the ASTM D6751 and EN 14214 biodiesel standards.

The effects of reaction parameters including ionic liquid anion size, reaction time, reaction temperature, the mass ratio of IL to microalgae biomass, and the water content of microalgae on FAME yield were investigated. This process was further optimized using response surface methodology (RSM). The optimal reaction conditions for the FAME yield was found to require a reaction time of 4.6 h, a reaction temperature of 102.4^{o}C, IL:microalgae mass ratio of 8:1, and water content of 40.6%. The FAME yield at these conditions was predicted to be 98.0 ± 2.48%. Finally, the reusability of the ionic liquid was verified. The major properties of the synthesized biodiesel from both cooking oils and microalgae were calculated using the FAME composition of the resulting biodiesel. Finally, the reusability of the ionic liquid was verified which will be necessary to reduce the environmental impact a direct transesterification process.

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Last but certainly not least, my wife Nazee. Nazee, your continuous love and support has allowed me to persevere to complete this project. Your patience throughout this long process cannot be overstated. I look forward to many more years of your love, support and friendship to help me achieve my future goals.

.

Dedication

To my wife, Nazee, for her continuous love, support and encouragement

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List of Abbreviations

Ace	Acetate
ANOVA	Analysis of variance
ASTM	American Society for Testing Materials
ASTM D6751	Specification for biodiesel (B100) blend stocks for distillate fuels
Buty	Butyrate
C11:0Me	Undecanoic acid methyl ester
C15:0Me	Pentadecanoic acid methyl ester
CCD	Central composite design
CFPP	Cold flow plugging point
CN	Cetane number
DCW	Dry cell weight
DT	Direct transesterification
EN14214	Fatty acid methyl esters (FAME) for diesel engines, requirements and test methods
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame ionization detector
For	Formate

GC	Gas chromatography
HHV	Higher heating value
IL	Ionic liquid
ISTD	Internal standard
IV	Iodine value
LAP	Laboratory analytical procedure
LCSF	Long chain saturation factor
MeOH	Methanol
NREL	National Renewable Energy Laboratory
Prop	Propionate
RSM	Response surface methodology
TAGs	Triacylglycerides
TG	Triglyceride

1 Introduction

1.1 Research Background

Increasing global energy demand coupled with the environmental effects of fossil fuels have, like global warming, reinvigorated the need for the commercialization and development of alternative renewable energy sources such as biodiesel. However, a competition of biofuel production with food production has led to a series of problems, such as increases in food prices and the overuse of agricultural land [1, 2]. Microalgae are considered a sustainable feedstock for the commercial production of biofuels because they do not need to compete with food production, and they can use CO_2 and sunlight to produce the lipids needed for biodiesel production [3, 4]. Compared with conventional sources of biofuels such as edible and non-edible fuel crops, microalgae have higher photosynthetic efficiency, higher growth rates than traditional land crops, and can be cultivated in brackish or wastewaters [5, 6]. Moreover, many microalgae species such as *Chlorella sp., Phaeodactylum sp., Nannochloropsis sp., and Chaetoceros sp.* can accumulate a substantial amount of their biomass as lipids (25 to 75% per dry algae weight) which are used for biodiesel production [7].

Despite these benefits, there are still some technical and economic bottlenecks that must be overcome to make the production of microalgae biofuels a cost-competitive industry. Microalgae biomass contains more than 60 wt.% water inside the cells after harvesting and dewatering. To use conventional methods of lipid extraction, microalgae biomass must be dried to a water content of less than 10% [8, 9]. This requires a significant energy investment increasing the processing costs [1]. The extraction process for microalgae starts with cell disruption using either mechanical, chemical or biological methods, followed by lipid and oil extraction using organic solvents [10, 11]. This can be challenging in microalgae as some species may have a complex and rigid cell wall, which significantly hinders the disruption step [12].

The extracted lipid oil is converted into biodiesel by transesterification in the presence of an alcohol (most commonly, methanol) and an acid, base, or heterogeneous catalyst [12-15]. However, recently the idea of direct use of unbroken and wet microalgae via *in situ* transesterification for biodiesel production in order to reduce the number of steps and decrease the processing costs has drawn attention [16-19]. Generally, *in situ* or direct transesterification refers to the simultaneous process of oil extraction and conversion into fatty acid methyl esters (FAMEs) directly from whole biomass. *In situ* transesterification streamlines and removes the expensive cell disruption and drying steps, potentially reducing the overall energy consumption for the process and decreasing the losses in yield that are intrinsically compounded by an increased number of processing steps [20-22]. However, high moisture content in microalgae biomass is still a main limiting factor for *in situ* transesterification processes as the synthesis of FAME is a reversible process, and the presence of water can hydrolyze the product converting it to free fatty acids (FFAs) and methanol again [9, 23, 24].

Furthermore, conventional *in situ* transesterification processes use expensive or unrecoverable catalysts and/or organic solvents, which emit volatile organic compounds (VOCs) that are harmful to the environment [25]. Pretreatments are also often necessary, although undesirable as they add additional cost and each additional step results in an additional loss in yield. Some of the pretreatments reported are expensive, have a high capital cost, or are not amenable to scale-up such as ultrasonication, microwave irradiation, or supercritical fluid extraction [1, 26-28]. Therefore, it is vital to explore alternative methods to address these challenges in order to advance the search for environmentally friendly processes for the production of renewable liquid fuels that are cost-competitive with unsustainable fossil-fuels.

Ionic liquids (ILs) have been shown to both facilitate lipid extraction [1, 7, 25, 29-31] and to facilitate the *in situ* transesterification process from wet microalgae using a homogenous base catalyst [19]. ILs, also known as “green designer solvents,” due to a large number of possible anion and cation combinations and their relatively high chemical and thermal stability,

non-flammability, low volatility, low melting point (below 100℃), and recyclability [8, 32-34]. Some ILs can dissolve recalcitrant biopolymers like lignin and cellulose by disrupting their hydrogen-bonding network leading researchers to explore their use in cell disruption technologies as many algae species possess cellulosic cell walls [1, 35, 36].

1.2 Research Objectives

The overall objective of this work was to develop and optimize the direct conversion of wet microalgae biomass into biodiesel using an ionic liquid as the catalyst in order to reduce the total operational steps by combining the extraction and transesterification steps and eliminating the need for an energy-intensive drying step. To the best of our knowledge, basic ionic liquid catalysts have not been tested for *in situ* transesterification for simultaneous lipid extraction and biodiesel production from microalgae. The research objectives can be divided into the following tasks:

1. Identify room temperature ionic liquids capable of transesterifying refined cooking oils (sunflower, canola, and corn oil) into biodiesel, as well as directly transesterifying wet microalgae biomass into biodiesel.

2. Study the effects of reaction parameters, including ionic liquid anion size, reaction time, reaction temperature, the mass ratio of IL to microalgae biomass, and the water content of microalgae on FAME yield.

3. Optimize the reaction conditions used for *in situ* transesterification of wet microalgae into FAME using response surface methodology (RSM).

4. Evaluate the reuse/recycling of the ionic liquid.

1.3 Outline of Thesis

This thesis is divided into five chapters. Chapter 1 outlines the research background and the specific objectives of this work. Chapter 2 presents a literature review of different processes using *in situ* transesterification of microalgae into biodiesel to give a fundamental understanding of this process. It also discusses the different generations of biodiesel and the direct transesterification mechanism. Chapter 3 provides the details of materials, methods, and experimental procedures followed during each research stage. The experimental results are presented in the fourth chapter, and the research findings are discussed and evaluated. Finally, in chapter 5, the research is summarized, conclusions are made, and possible future work avenues are outlined.

2 Literature Review

Biofuels have garnered growing interest globally as a result of the limited fossil resources and volatile fuel costs [22]. Conventional sources of energy like gas, oil, and coal are non-renewable. The usage of these traditional sources usually causes significant damage to the environment by raising the atmospheric load of CO_2 and greenhouse gases (GHGs) [37]. Regulation 2009/28/EC of the European Parliament and Council was adopted to encourage the use of biofuels and other renewable fuels for transportation objectives. It established a compulsory goal to raise the portion of biofuels used globally for transportation0% of all fuel consumption by 2020 [20]. Therefore, there is a global effort underway to increase the use of renewable energy sources and minimize the pollution triggered by fossil fuel consumption [37]. The use of renewable energy sources has been rising quickly in the last few years, and this trend is expected to continue in the future as it will be reinforced by a predicted increase in global energy demand of 30% by 2040 [37]. The benefits of biodiesel compared to conventional fuel include its low-toxicity and superior lubricity, which have been well recorded [22].

Based on the European Academies Science Advisory Council (EASAC) report in 2012, biodiesel is generally categorized as first (FGB), second (SGB), or third (TGB) generation. These generations are mainly based upon the origin of the feedstock material of the biodiesel. In contrast, fourth-generation biodiesel, now in the early stage of a primary investigation, is based on the genetically modified (GM) algae [2].

2.1 Generations of Biodiesel

First-generation biodiesel is produced from edible food crops such as palm oil, corn oil, canola oil, and other vegetable oils. Using edible feedstocks to produce biodiesel was popular at the start of the biodiesel era. The availability of crops and relatively simple conversion procedure are significant advantages of first-generation feedstocks [38]. These feedstocks' disadvantages

relate to food security issues as demand for food increases with the increasing global population and directing food crops towards energy production has already been shown to increaseood prices [39]. Furthermore, there is a limited amount of global arable land for food production and these crops are seasonal limiting the production depending on climate, prompting researchers to look for alternative feedstocks for biodiesel production [6].

Second-generation biodiesel (SGB) like other second-generation biofuels is produced from non-edible feedstocks such as non-edible plant oils like *Jatropha* oil, waste cooking oils, and animal fats like tallow [40] . It can also include oleaginous microorganisms grown on waste feedstocks for lipid production [41]. SGB alleviates many of the concerns about FGB by producing biofuels from waste products rather than food crops [5]. Moreover, many of the purpose grown biodiesel crops like *Jatropha curcus* require less land for farming and can use land unsuitable for current food crops [4]. Waste cooking oil in particular is very economical but highly heterogeneous raw material for the production of biodiesel. Using waste cooking oil for biodiesel production also decreases landfilling and water contamination by waste cooking oils [42]. Nevertheless, these oils tend to be less suitable for biodiesel production due to their high FFA content which generates soap during traditional transesterification processes [43].

Finally, the biodiesel generated from microalgae is described as third-generation biodiesel [43]. Using microalgae as a feedstock alleviates the concerns of all previous generations of biodiesel with respect to food security issues in addition to having an even smaller impact on the environment [44]. A summary of the three generations and their primary feedstocks, benefits and major limitations are shown in **Figure 2.1**.

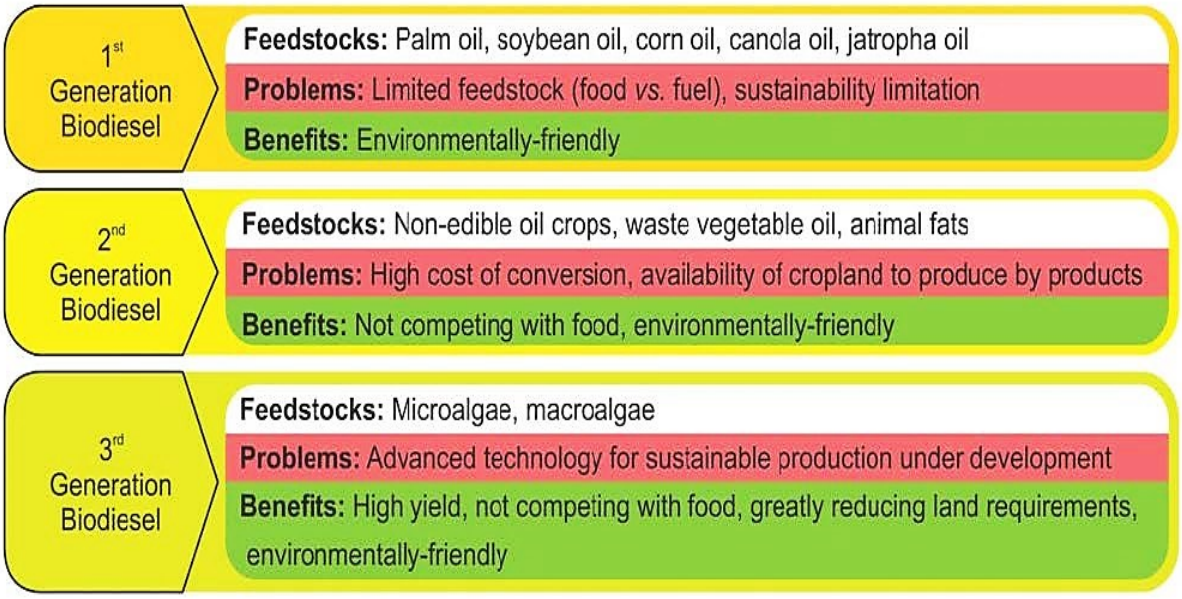

Figure 2.1: Biodiesel generations based on feedstock [45]

2.2 Algae as a Biomass

Research to obtain fuel from algae is not new. It was initially proposed in the 1950s, and with the oil crisis in the 1970s, several publicly funded research programs began, but when the crude oil price fell (around the '80s) the application of this research was halted. Today this topic is again at the forefront, with many governments and companies investing in research and development. Thus far, many algae species have been tested, and cultivation and oil extraction technologies have been improved, and several pilot plants have been constructed [46, 47]. Interested parties include the Bioenergy Technologies Office of the U.S. Department of Energy (US), the Algal Bioenergy Special Interest Group of Natural Environment Research Council (UK) and the Ministry of Science and Technology – MCT (Brazil) [48-50]. There have also been a number of private investments from companies such as Algenol, British Petroleum, Shell, Chevron, ExxonMobil, and others [51].

There are more than 30,000 species of algae described [12]. When classified by size, algae are divided into:

- **Macroalgae (‘‘seaweeds”):** multicellular plants (large algae, visible without a microscope) growing in salt or freshwater. They can be brown seaweed (*Phaeophyceae*), red seaweed (*Rhodophyceae*), and green seaweed (*Chlorophyceae*) based on their pigmentation [52].

- **Microalgae:** unicellular photosynthetic micro-organisms that are eukaryotic organisms containing chlorophyll A and a plastid. This excludes cyanobacteria, which are a type of bacteria, not algae, although several biofuels’ studies include this group because of their properties and potential [53].

Microalgae can grow autotrophically (when supplied with light, CO_2 and nutrients), mixotrophically or even heterotrophically (using organic substrates such as sugars). Heterotrophic growth may also enhance lipid production [53]. Microalgae are cultivated in aquatic media (seawater, freshwater, brackish water and domestic and industrial effluents) using nutrients and CO_2 as inputs. The nutrients, mostly nitrogen and phosphorus, can be provided from fertilizers or wastewater, while the CO_2 is usually provided by a pump system from power plants or industries that emit this gas. Production of 1 kg of algae biodiesel may require up to 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate (without recycling the wastewater), while recycling the wastewater can result in 84% less water usage and 55% less nutrients [54]. This is critical since the nutrients used for microalgae cultivation are also used for agricultural fertilizers and the overuse of fertilizers would lead to similar concerns to those of first and second-generation biodiesel. Researchers conclude that the only cost-effective strategy to produce microalgae on a large scale would be constructing the facility close to a source of nutrients and CO_2 [53]. However, microalgae can grow on non-arable land in many different environments (even under harsh conditions), so there are little to no negative impacts expected from land-use change [53].

The advantages of using microalgae as feedstock compared to traditional oil crops include [55-58]:

- Non-food based resource
- Non-productive land can be used for their cultivation
- Higher areal productivity
- Can accumulate a higher lipid content (more than 40% wt. of their dry biomass, compared to 25% of rapeseed)
- Higher photosynthesis efficiency (10% compared to 1% for typical crops)
- Faster growth rate
- Application of pesticides, herbicides or fungicides is not necessary
- Can use waste sources of CO_2 and 100 tonnes of microalgae biomass can fix 183 tonnes of CO_2
- Microalgae can produce other value-added products
- Microalgae biofuels have superior fuel characteristics

Microalgae lipids are composed of triacylglycerides (TG) containing fatty acids from 12 to 22 carbons in length, but predominantly C16:0, C18:1, and C18:2, which is a favourable fatty acid profile for biodiesel production [59]. After lipid extraction, the remaining biomass containing mainly starch and protein can be used to produce other biofuels, such as jet fuel, biogas, or ethanol [60]. Some value-added products including animal feed, antioxidants, colouring substances, fertilizers and soil conditioners, cosmetics, and pharmaceutical compounds can also be extracted from the lipid extracted algae (LEA) [55].

2.3 Transesterification of Lipids

After lipid extraction, biodiesel (fatty acid methyl or ethyl ester – FAME or FAEE) is produced by the transesterification and esterification of TGs and FFAs from biologically derived oils in the presence of an alcohol and catalyst [61]. The first patent for biodiesel production from vegetable oils was published in the 1940s [61]. Since its inception, the principal steps of the process have not changed significantly on a large scale, where biodiesel is produced using an alkaline catalyst in batch or flow reactors [62].

The transesterification reaction happens in three stages as shown in **Figure 2.2.** TG and FFA react with low molecular weight alcohol (usually methanol or ethanol) in the presence of a base or acid catalyst. The catalyst can be alkaline, acidic, or enzymatic with sodium hydroxide (NaOH), potassium hydroxide (KOH) and sulphuric acid being the most commonly used [63]. The reaction produces glycerol as a co-product. The stoichiometry requires 3 moles of alcohol for each mole of TG, but to achieve a higher yield a mole ratio of 6:1 is typically used [64]. Methanol is the most commonly used alcohol because of its low cost and higher reactivity [65]. Still, ethanol has been studied as an alternative because it can be produced from sugars using a renewable process [66]. Unfortunately, using ethanol results in lower yields and hampers glycerol separation processes [67].

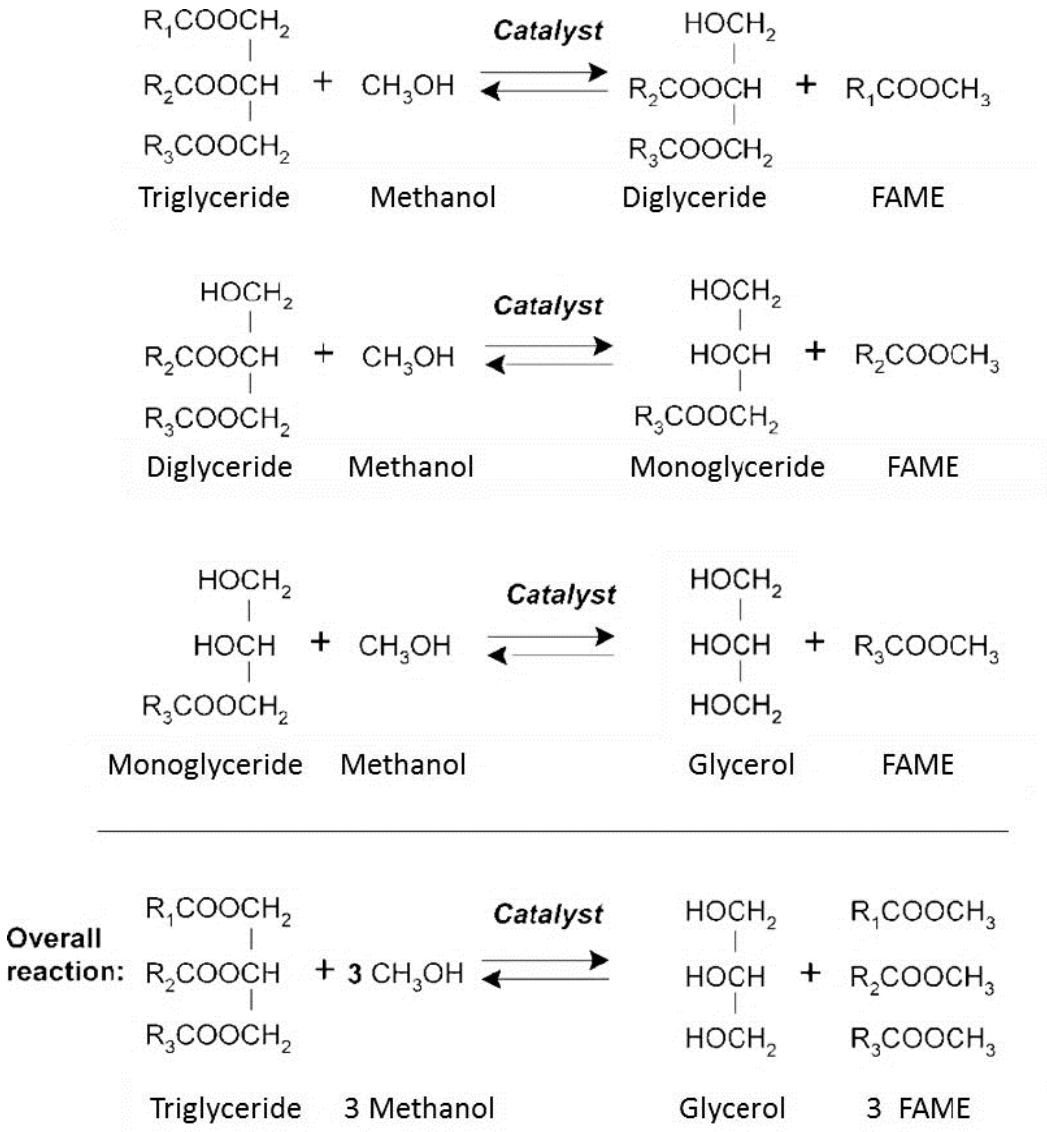

Figure 2.2: Transesterification of a TG with methanol [64]

2.4 *In situ* transesterification

In situ transesterification refers to the process of directly converting intracellular lipids in biomass to biodiesel in a single step without lipid extraction. It uses the same reagents as the traditional process described above but uses a much higher stoichiometric ratio (30:1) of

alcohol to lipid in order to achieve reasonable yields [64]. The combination of three steps, including lipid extraction, recovery of solvent, and transesterification into a single step, may provide a more cost-effective option for the production of biodiesel from microalgae since drying and solvent extraction steps account for about 90% of the process's required energy in the two-step transesterification of algae oil to biodiesel [68].

Over the last few years, lots of researchers have worked on the direct production of biodiesel from dry or wet microalgae, and several studies have found that high water content in microalgae biomass leads to reduced conversion of lipids to biodiesel [17]. In the *in situ* transesterifications of microalgae, alcohol has dual roles as both the extraction solvent and a transesterification reactant [69]. Using a co-solvent can help to enhance the process efficiency by improving extraction efficiency as well as creating a homogenous system between the oil, microalgae biomass, catalyst, and alcohol [5]. Direct biodiesel synthesis also minimizes the loss of lipids by reducing the number of process steps and depending on the catalyst used, can convert all types of lipids into biodiesel [70]. A comparison of conventional transesterification, conventional direct transesterification, and the proposed wet direct transesterification process is shown in **Figure 2.3**. *In situ* transesterification processes can be classified into catalytic and non-catalytic. In the following sections, the advantages and disadvantages of each method will be discussed.

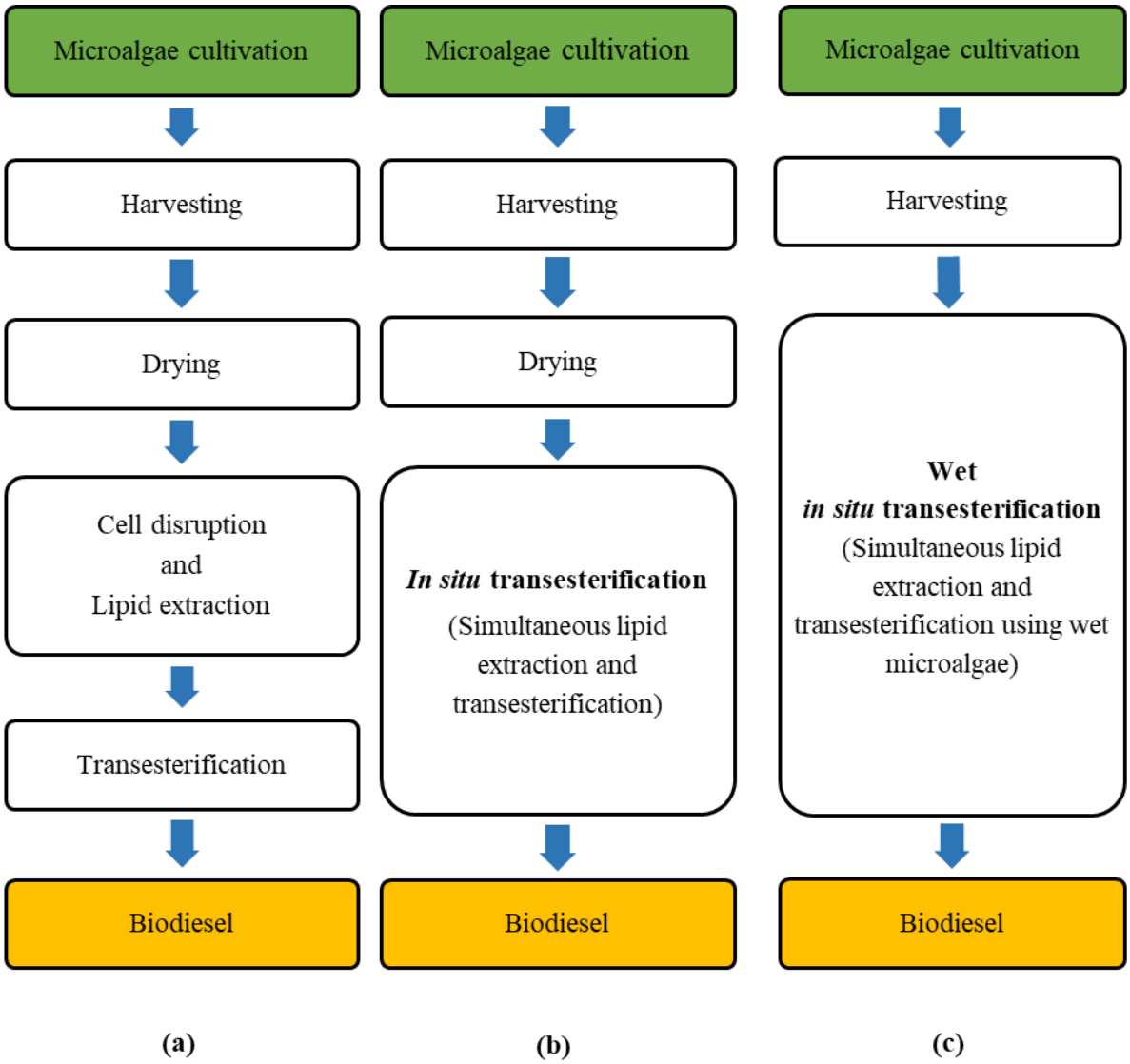

Figure 2.3: Flow diagram of the microalgae biodiesel production process. Depicted are (a) conventional transesterification; (b) conventional *in situ* transesterification [70]; (c) wet *in situ* transesterification [63]

2.4.1 Homogenous Catalytic *in situ* Transesterification

Homogenous catalytic systems use acid or base catalysts dissolved in the reaction solvent. The selection of catalysts is an important step to reaching a high yield in biodiesel production and depends on the content of FFAs in the oil. The lower yield of biodiesel in acid-catalyzed *in situ* transesterification compared to base-catalyzed is considered the main disadvantage of acid catalysts [21]. However, acid catalysts are able to convert both FFAs and TGs to biodiesel while alkaline catalysts cannot [71]. They are only useful and effective in the production of biodiesel from the lipids with lower FFA content (< 0.5%), but they exhibit much faster reaction rates than acid-catalyzed reactions [72]. Product yield using acid catalysts is less sensitive towards water and FFA content compared to base catalysts and therefore have been studied more extensively for *in situ* transesterification. In the acid-catalyzed reaction, the carbonyl group of a TG is protonated which is attacked by the alcohol forming a tetrahedral intermediate [73]. Among the acid catalysts, sulphuric acid is used extensively due to its selectivity in transesterification reaction, moisture tolerance, and relatively low price [64].

2.4.1.1 Acid-Catalyzed *in situ* Transesterification

Velasquez-Orta et al. [18] performed *in situ* transesterification process with *Chlorella sp.* and *Nannochloropsis oculata* biomass with moisture contents of 0, 1.5, and 10% using various acid and base catalysts (sodium methoxide, sodium hydroxide, and sulphuric acid). They found that sulfuric acid as a catalyst showed the highest FAME yield for both microalgae among the mentioned catalysts (73% for *Nannochloropsis oculate* and 92% for *Chlorella* sp.), and the FAME yield decreased with the increasing the moisture of algae.

Wahlen *et a*l. [73] produced biodiesel from mixed microalgae species biomass with sulphuric acid as the catalyst and obtained 77% of FAME yield at 0.3 h, molar ratio of methanol to lipid oil 1831:1 at 80°C. Moreover, they conducted the same experiment at a larger scale (100 g *vs.* 0.2 g) and reported a conversion yield of 84% using a higher amount of sulphuric acid (1.3 ml 1.8% (v/v) H_2SO_4 per CDW). They also found that the FAME yield was

proportional to the alcohol loading ratio and inversely proportional to the moisture content of microalgae.

2.4.1.2 Base-catalyzed *in situ* Transesterification

Few studies have been carried out using base catalyst for *in situ* transesterification of microalgae. In the base-catalyzed reaction, the base deprotonates the alcohol which attacks the carbonyl group of one of the fatty acids resulting in a fatty acid alkyl ester [19].

Salam et al. [19] used a 96% (w/w oil) base concentration and a high amount of methanol (molar ratio of methanol to oil of 925:1) at 60°C for the *in situ* transesterification of *C. vulgaris* biomass. A FAME yield of 96% was achieved in a reaction time of 10 min. Interestingly, a high FAME yield was achieved regardless of the high FFA level in the algae. This finding demonstrates the excessive amount of methanol and catalyst needed to achieve a short reaction time and can be utilized to prevent saponification and improve the production rate of biodiesel during base-catalyzed *in situ* transesterification of algae biomass.

2.4.2 Heterogeneous catalysts for *in situ* Transesterification

It is difficult to separate homogenous catalysts from the product which leads to extra cost for purification of the product as well as the production of extra waste [74]. As a result, using heterogeneous catalysts may be a better option for production of biodiesel from microalgae. This eliminates the need for recovery of the catalyst thereby reducing the process cost [15]. Solid acids and bases, including MgO, CaO, SrO, $SrCO_3$, BaO, and $MgCO_3$, are among the most popular heterogeneous catalysts using for production of biodiesel using refined oils [75].

Li et al. [76] studied the two-step lipid extraction and transesterification process of *Nannochloropsis* oil and the one-step *in situ* transesterification of *Nannochloropsis* with $Mg_2Zr_5O_{12}$ as a heterogeneous catalyst. The highest FAME yield for *in situ* transesterification was 60% which was obtained with reaction conditions of 65°C, for 4 h, and 10 wt.% catalyst . For the two-step transesterification process, the FAME yield was 47% using the same

transesterification conditions. They found that the biodiesel yield increased with increasing amount of catalyst, but very high catalyst amounts led to a decrease in the yield. Although heterogeneous catalysts have some benefits compared to homogeneous catalysts, the high cost of synthesizing a solid acid catalyst with high activity and specificity is one of the main disadvantages of these catalysts [77].

2.4.3 Solvent-assisted *in situ* Transesterification

Generally, a solvent is used to improve the mass transfer between the extracted oil from the microalgae and the reactant. The simplest option is utilizing a co-solvent such as chloroform or hexane along with the acid catalyst and alcohol during wet *in situ* transesterification [17].

Cao et al. [78] produced FAME directly from *Chlorella pyrenoidosa* containing 0-90 wt.% moisture content. Hexane was used as a co-solvent, and the maximum FAME yield was 91.8% with a 30 wt.% moisture content using 1 g of biomass, 6 mL hexane, 4 mL methanol, 0.5 M H_2SO_4/g DCW at 90℃ for 120 min. Additionally, reactions at 120 and 150℃ were conducted, and it was found that by increasing the reaction temperature, the negative effect of increasing moisture content can be minimized.

When using chloroform as a co-solvent for wet *in situ* transesterification (which has a higher density than water), it can facilitate separation of the biodiesel from the residual solids, water, and glycerol after transesterification (Figure 2.4).

Im et al. [17] reported *in situ* transesterification of wet *Nannochlropsis oceanica* (moisture content 65%) using sulphuric acid as a catalyst and chloroform as a co-solvent. They achieved high conversion yield of 91% using 0.3 g of sulphuric acid, 1 mL of methanol, and 2 mL of chloroform per 0.2 g of biomass at a reaction temperature of 95℃ for 90 min.

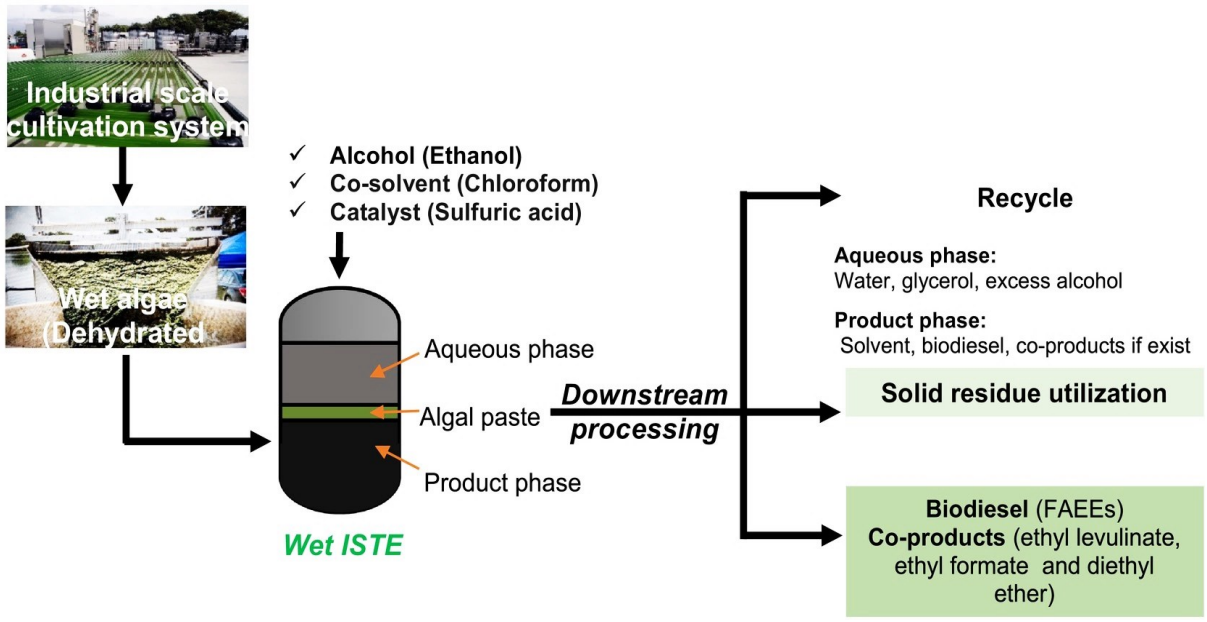

Figure 2.4: Characteristic diagram of solvent-assisted wet *in situ* transesterification [63]

2.4.4 Supercritical Solvent-assisted *in situ* Transesterification

Using supercritical alcohols for the production of biodiesel was presented by Saka et al. [79] in 2001 and its application to *in situ* transesterification was first discovered in 2010 by Lee et al. [80] using *Jatropha curcas* seed and was applied to microalgae (*Chlorella sp.)* in 2010 by Levine et al. [81].

Supercritical solvent assisted *in situ* transesterification does not require a catalyst or a co-solvent and decreases the reaction time significantly [82]. For supercritical transesterification, alcohols, such as ethanol or methanol, are usually used because they play a dual role of reactant and solvent. Tir supercritical conditions (critical temperature and pressure) are relatively easy to achieve compared to other alcohols [22]. Since short-chain alcohols have higher reactivity biodiesel can be obtained without any added catalyst [22].

High water content does not interfere in the reaction compared to the standard acid-catalyzed *in situ* transesterification. The main disadvantage is the higher reaction pressure and temperature (20 MPa and 300°C) to perform the reaction, compare to <100°C and standard pressure for traditional transesterification processes [82]. There is also a high start-up cost for the equipment needed and the need for a more skilled operator with knowledge of supercritical operations [83].

Jazzar et al. [84] reported that supercritical methanol was used for *in situ* transesterification of microalgae. During the transesterification reaction of wet *Chlorella* sp. and *Nannochloropsis gaditana*, a maximum biodiesel yield of 45.62% and 21.79% was obtained, respectively; using the following reaction conditions: 265°C for 50 min, a mass ratio of methanol to dry microalgae of 10:1, and a moisture content of 75 wt% for both microalgae. Patil et al. [85] studied the production of biodiesel using wet microalgae having approximately 90 wt% water using supercritical methanol without a catalyst. They used response surface methodology (RSM) to optimize the reaction conditions and reached a maximum FAME yield of 85.7% using a wet microalgae/methanol ratio of 1:9 at 255°C for 25 min. Levine et al. [81] used a supercritical method for direct transesterification to produce biodiesel from wet *C. vulgaris* (80 wt% moisture content). First, microalgae were hydrolyzed with water at a reaction temperature of 250°C, then supercritical ethanol was used without a catalyst to perform direct supercritical transesterification at 325°C to produce FAEE. A yield of approximately 66% was achieved using an ethanol/wet algae ratio of 8 and a reaction time of 3 h.

2.5 Novel Approaches in the *in situ* Transesterification of Microalgae

Recently, in order to enhance the *in situ* transesterification process, different physical assistance processes have been used to enhance cell lysis including ultrasound and microwave technology. In addition, the use of green solvents such as ionic liquids instead of conventional

organic solvents is also an active area of research. In these studies, the main purpose of these additional treatments has been to improve cell lysis and increase transesterification yield.

2.5.1 Microwave or Ultrasound-assisted *in situ* Transesterification

Ultrasonic or microwave assistance during *in situ* transesterification has been shown to enhance lipids' conversion into biodiesel. Using these techniques can decrease reaction time by improving mass transfer [86]. The major differences between ultrasonic and microwave technologies are shown in **Table 2.1**.

Table 2.1: Difference between microwave and ultrasound assistance processes [86-90].

Microwave assistance	**Ultrasound assistance**
Mode of operation: Microwaves generate electromagnetic fields that create heat and align polar molecules because of friction from the slower molecules orientation as well as changing the time rate of the fields. Methanol and ethanol are active microwave absorption media due to their strong polarity. **Advantages:** • Microwave technology permits safe, rapid, and economical production of microalgae biodiesel without the need for drying. • Microwave heating simplifies manipulation, decreases analysis time and produces products with higher purity. **Disadvantages:** • Low reaction volume • Not easy to use in continuous conditions • Overheating and generation of hotspots • Expensive to use for large-scale pretreatment of biomass	**Mode of operation:** High temperature and pressure, turbulence, high shear forces, and acoustic microstreaming create better emulsions in between immiscible fluids that improve transesterification reaction rates and mass transfer. **Advantages:** • High pressure and temperature conditions make free radicals that trigger the reaction to occur immediately. • Approximately 5000 K and 100 MPa are produced throughout the collapse of ultrasonic bubbles. • Ultrasonic can be useful in the extraction of valuable co-products such as carotenoids and pigments. **Disadvantages:** • Usually use in batch reactors • The high start-up cost for the equipment needed

2.5.1.1 Microwave-assisted *in situ* Transesterification Process

Cheng et al. [91] used microwave irradiation to speed up the disruption of the microalgae cell wall as well as to heat the reactants to the reaction temperature. Microwave irradiation resulted in the disruption of approximately 78% of the algae cells. In comparison with the conventional two-step transesterification (lipid extraction followed by a separate transesterification), microwave-assisted *in situ* transesterification improved the yield of biodiesel from 8.3 to 10.5% of dried algae, respectively. Furthermore, the kinetic rate of biodiesel production from wet algae was increased six-fold. By using the same microwave irradiation method, Cheng et al. [92] investigated the role of the solvent using 20 mL of methanol and chloroform and 1 mL of sulfuric acid per gram of CDW. The addition of chloroform improved yield to almost 100% using wet *Nannochlropsis oceanica* biomass (moisture content 80 wt.%).

Loong et al. [93] used sodium hydroxide catalyzed *in situ* transesterification with simultaneous microwave heating for the transesterification of wet *Nannochloropsis sp*. biomass (20%-wt of moisture content), which resulted in a yield of 75% after only 10 min. The simultaneous cooling and microwave heating (SCMH) process counteracts the drawbacks of traditional microwave methods, including overheating and the formation of hot spots. SCMH maintains the temperature of the reaction at the designated temperature without overheating, which results in even penetration of microwave irradiation over the reaction medium. The use of SCMH increased the *in situ* transesterification yield from 15.4% (microwave-assisted *in situ* transesterification without simultaneous cooling) to 75%.

In another study, Chen et al. [94] use microwave irradiation to facilitate oil extraction from wet *C. vulgaris* (moisture content 40 wt.%). They performed *in situ* transesterification using 0.5 wt% NaOH in methanol. Almost 100% yield was obtained in 15 min at 45°C. Interestingly, in comparison with the other acid-catalyzed *in situ* transesterification processes with or without physical assistance, the reaction temperature was relatively low.

2.5.1.2 Ultrasound-assisted *in situ* Transesterification Process

Sonication can be performed directly using an ultrasound horn or probe and indirectly using an ultrasonic bath. Ultrasonication is an appealing technique as it promotes the mixing of solutions, does not need a high temperature for disrupting the cell walls, reduces reaction time, and possibly reduces material consumption. This technique uses sound waves to circulate pressure fluctuations which causes cavitation [82]. Microalgae cells are fragmented by the ultrasounds, improving the interaction between the reagents and the oil [22, 82]. Ehinem et al. [95] reported that with a combination of a cosolvent and sonication, the molar ratio of methanol to oil was significantly reduced in the transesterification of *Chlorella* sp.. Using the traditional process, a FAME conversion of 55.6% was reached after 0.5 h of mechanical stirring with diethyl ether and methanol:oil molar ratio of 315:1. With the combination of mechanical stirring and ultrasonication, the conversion yield increased to 91% using the same amount of time and a lower methanol/oil molar ratio of 105:1. X. Zhang et al. [96] used ultrasonication for the production of biodiesel from *Trichosporon oleaginosus* biomass. They found that without ultrasonication, transesterification from yeast biomass resulted in 90.4% FAME after 12 h, whereas *in situ* transesterification using ultrasonication reached a FAME yield of 94.1% in 20 min using a 6 times lower methanol/oil molar ratio.

2.5.2 Ionic Liquids (ILs)

As mentioned in previous sections, almost all conventional *in situ* transesterification processes use expensive or unrecoverable catalysts such as acid or base catalysts and/or organic solvents, which emit volatile organic compounds (VOCs) and are harmful to the environment. Furthermore, other pretreatments methods such as microwaves, ultrasonication, or supercritical extraction are expensive, have a high capital cost, or are not amenable to scale-up. Therefore, it is vital to explore alternative methods to address these challenges in

order to advance the search for environmentally friendly processes for the production of fossil fuels that is cost-competitive with unsustainably fossil-fuels.

Recently, the possibility of utilizing ILs as an alternative solvent in lipid extraction and as a co-solvent fir *in situ* transesterification processes from wet microalgae has been studied. ILs, also known as "green designer solvents", include a large number of possible anion and cation combinations and they generally have a relatively high chemical and thermal stability, are non-flammable, have low volatility, have a low melting point (below 100℃), and are easily recovered for reuse [8, 32-34]. Some ILs can dissolve recalcitrant biopolymers like lignin and cellulose by disrupting their hydrogen-bonding network leading researchers to explore their use in cell disruption as many algae species possess cellulosic cell walls [1, 35, 36].

Orr et al. [1] reported that the IL, 1-ethyl-3-methylimidazolium ethyl sulphate [C_2mim][$EtSO_4$] could disrupt the cell wall of the difficult to lyse species, *Chlorella vulgaris* at room temperature. High lipid extraction yields (~100%) were obtained with a reaction time of 75 min at ambient temperature and were compatible with a wide range of water contents from 0-82 wt.%. Combined with methanol and KOH, this IL could also be used for the direct transesterification of intracellular lipids in the wet biomass of the oleaginous yeast *Rhodosporidium diobovatum* [97]. This process used low temperature (65^{o}C) and short reaction time (2.5 h) to recover over 97% of the TGs as fatty acid methyl esters (FAME) in fresh yeast biomass containing up to 80 wt.% water. However, while the IL was readily recovered, the homogenous catalyst used in this study (KOH) was not.

Wahidin et al. [33] utilized a simultaneous microwave-irradiation using an IL cosolvent for *in situ* transesterification of wet microalgae, *Nannochloropsis* sp., with 80% moisture content. Three ionic liquids; 1-ethyl-3-methylimmidazolium methyl sulphate [C_2mim][$MeSO_4$], 1-butyl-3-metyhlimidazolium chloride [C_4mim][Cl], and 1-butyl-3-methylimidazolium trifluoromethane sulfonate; [C_4mim][CF_3SO_3] were evaluated. Among tested ionic liquids, [EMIM][$MeSO_4$] showed the highest cell disruption efficiency of 99.7%. The highest biodiesel yield was 36.79% which was not much better than chloroform alone (28.82%).

Lee et al. [98] performed *in situ* transesterification assisted by 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([C_4mim][CF_3SO_3]) using wet *N. oceanic* (water content of 65%) with acetyl chloride as a catalyst and methanol. A FAME yield of 54% was obtained at a temperature between 55 and 75°C; However, there was a high methanol usage (50 mL/g CDW).

Sun et al. [4] used 1-butyl-3-methylimidazolium hydrogen sulphate ([C_4mim][HSO_4]) as an acid catalyst and solvent for *in situ* transesterification with methanol. They explored the effect of reaction time and temperature as well as the mass ratio of wet microalgae to ionic liquid ([C_4mim] [HSO_4]) on wet *Nannochloropsis* sp. (water content 62%). The biodiesel yield of 95.3% was achieved at the reaction condition of 200°C in 30 min with the mass ratio of IL: wet *Nannochloropsis* sp. of 0.9:1. Moreover, [C_4mim][HSO_4] could be recycled up to four times with a biodiesel yield of 81.2%.

In previous studies, ILs have been mainly used to facilitate cell disruption when combined with other pretreatment methods such as microwave-irradiation or facilitated direct transesterification when combined with other homogeneous catalysts such as KOH and acetyl chloride. The work of Sun et al. [4], which used an acidic IL as a solvent for lipid extraction and as a catalyst for *in situ* transesterification of microalgae lipids used a high reaction temperature zone (200°C) and is the only study thus far using an IL catalyst for direct transesterification. To the best of our knowledge, IL catalysts have not been tested for *in situ* transesterification for simultaneous lipid extraction and biodiesel production from microalgae in the low (under 100°C) and mid (100°C to 200°C) reaction temperature zone. Moreover, basic IL catalysts have not been tested as a solvent for lipid extraction and a base catalyst for *in situ* transesterification process for direct biodiesel production from microalgae.

3 Materials and Methods

3.1 Materials

All materials were purchased from Thermo Fisher Scientific or VWR or Sigma-Aldrich except where otherwise stated. Tetrabutylphosphonium bromide [P_{4444}][Br] (25% aqueous solution) was donated by SOLVAY, Niagara Falls, Canada. Cooking oils, including corn, sunflower and canola oil were edible grade oil purchased from a retail grocery store.

3.2 Strain and Culture Conditions

C. vulgaris strain UTEX 2714 was purchased from The Culture Collection of Algae at the University of Texas Austin. The culture was maintained in liquid media utilizing an aseptic technique in 150 mL Tris-acetate-phosphate (TAP) media in 500 mL shaker flasks. All cultures were grown at pH 6.5 and the temperature of 25℃ at 150 rpm under a light intensity of 100 μmol/m^2 s with a 16 h: 8 h light/dark cyclic illumination. The TAP medium contained Tris base (20 mM), KH_2PO_4 (2.4 mM), K_2HPO_4 (1.58 mM), $MgSO_4$ (0.83 mM), NH_4Cl (7.0 mM), $CaCl_2$ (0.34 mM), glacial acetic acid (1 mL/L), and Hutner's trace element solution (1 mL/L) [99]. After 48 h, a growing seed culture was harvested aseptically by centrifugation in the Eppendorf 5810 RT centrifuge. The centrifuged seed culture was inoculated into 75 mL of modified media (Tris base (20 mM), $CaCl_2 . 2H_2O$ (0.04 g/L), KH_2PO_4 (1.74 g/L), Hutner's trace element solution (1 mL/L), $NaNO_3$ (1.11 g/L), $MgSO_4 . 7H_2O$ (2.5 g/L), and glucose (18.8 g/L)) in 250 mL shaker flasks at 1% v/v; and cultured for six days (144 h) at 25 ℃ and 150 rpm and pH 6.8 which adjusted using NaOH (5 M) in order to induce lipid production [100].

3.3 Harvesting and Freeze-Drying

Microalgae cells were harvested by centrifugation at 3500 rpm for 15 min. The cell pellets were resuspended in DI water and washed 3 times using resuspension and centrifugation in order to eliminate residual salts. The washed cells were frozen at -20℃ for at least 12 h and lyophilized utilizing a 4.5 L benchtop freeze-dryer (Labconco) for 36 h or till the weight no longer fluctuated, and then stored in a desiccator until further use. For wet *in situ* transesterification process, microalgae were harvested with the mentioned method and resuspended in different amounts of DI water.

3.4 Synthesis of Ionic Liquids

Four ILs, tetrabutylphosphonium formate ($[P_{4444}][For]$), tetrabutylphosphonium acetate ($[P_{4444}][Ace]$), tetrabutylphosphonium propionate ($[P_{4444}][Prop]$), and tetrabutylphosphonium butyrate ($[P_{4444}][Buty]$), were synthesized using the following procedure. Tetrabutylphosphonium hydroxide ($[P_{4444}][OH]$) (60% aqueous solution) was neutralized with a slight excess of carboxylic acid (formic, acetic, propionic, or butyric acid) by stirring at 25℃ for 12 h. After neutralization, the mixture was dried under vacuum using a rotary evaporator at 80℃ for 8 h. Then, the synthesized IL was placed in a vacuum oven, including P_2O_5 at 85℃ for 48 h in order to remove the remaining water and the excess acid prior to use. The structure of synthesized ILs were characterized by ^1H NMR spectra using a Bruker-300 Ultrashield NMR. All the characterization data are available in the Appendix.

3.5 Determination of Total Lipid content in Biomass

The total lipid content in the microalgae biomass was determined as a FAME by the *in situ* transesterification standard laboratory procedure developed by the National Renewable Energy Laboratory (NREL) [101]. In brief, approximately 10 mg of freeze-dried microalgae was mixed with 300 μL of 0.6 M HCl in methanol, 25 μL of methyl pentadecanoate (C15:0Me) at 10 mg/mL as the recovery standard, and 200 μL of chloroform: methanol (2:1, v/v), and

consequently incubated at 85°C for 60 min in a water bath with stirring at 1000 rpm using a magnetic stirrer. After cooling to room temperature, 1 mL of n-hexane was added to the sample and vortexed the mixture. The sample was allowed to stand undisturbed for 1 h, and then the mixture was centrifuged and 450 µL of the clear top hexane phase was withdrawn and mixed with 50 µL of the internal standard (ISTD), methyl undecanoate (C11:0Me) at a final concentration of 100 µg/mL. The FAME was analyzed using GC according to the procedure will be described in Section 3.6. The total amount of FAME by weight was determined according to the process described by the NREL LAP procedure by adjusting the calculated FAME mass using the extraction recovery standard (C15:0Me) and dividing by the total mass of microalgae used in the analysis.

3.6 Gas Chromatography (GC)

FAME samples were mixed with the ISTD and separated and analyzed using an Agilent 6890 GC equipped with a flame ionizing detector (FID) and Agilent DB-Wax capillary column (30 m, 0.25 mm, 0.25 µm) by the standard laboratory procedure developed by the National Renewable Energy Laboratory (NREL) [101]. Helium was employed as the carrier gas with a constant flow rate of 1 mL/min. The injection was performed in split mode with 10:1 split ratio, and the injection volume was 1 µL. The FID detector was operated at 280°C, and FAMEs were eluted using the following program: 100°C for 1 min, 25°C/min up to 200°C and hold for 1 min, 5°C /min up to 250°C, hold for 7 min. Each of FAMEs was quantified by calibrating the method to an analytical standard mixture (Supelco 37, Sigma Aldrich) and using the ISTD, C11:0Me. Unidentified FAMEs were quantified by applying the RF factor of the closest known peak. The schematic diagram of GC-FID is shown in **Figure 3.1**.

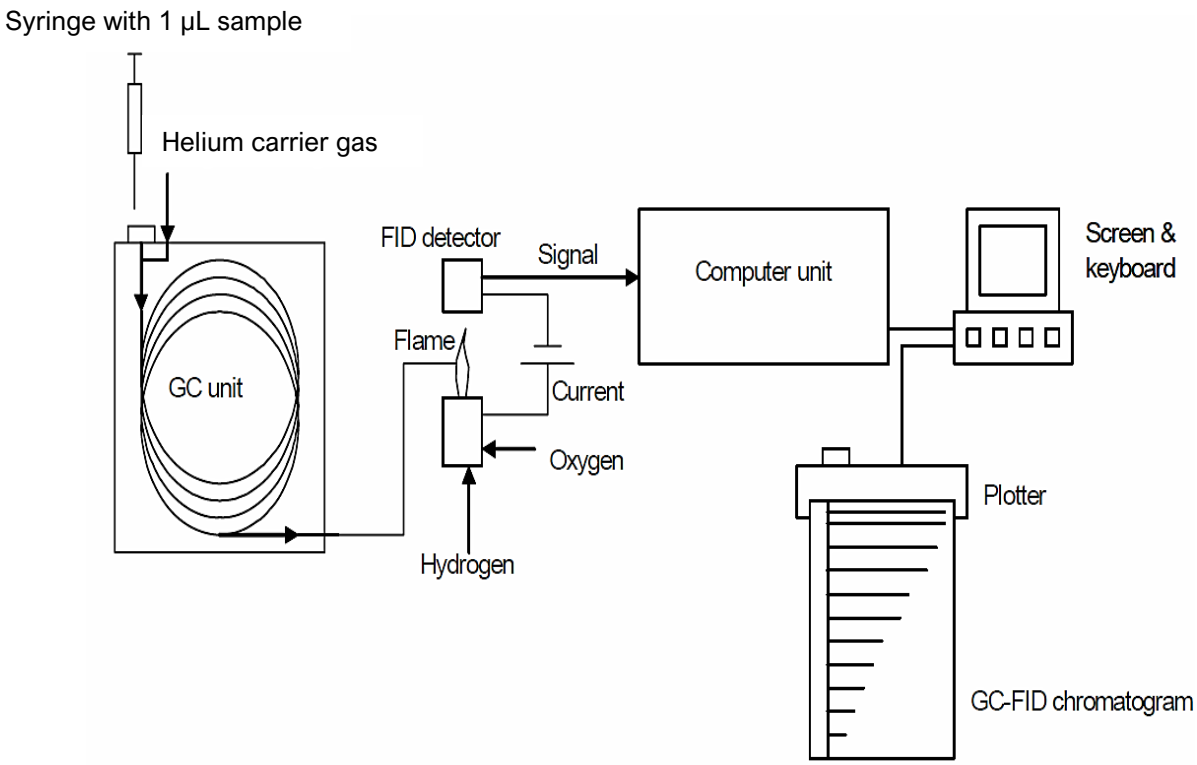

Figure 3.1: A Schematic diagram of the GC-FID set-up used in the present work (originally from Pedersen (2002)). [102]

An example calculation for FAME concentration (conc.) normalized by FAME extraction efficiency using the C15:0 recovery standard is shown below:

$$FAME\ conc\ _{Norm}\left(\frac{\mu g}{mL}\right) = \frac{\sum_{C4}^{C24} Conc\ FAME_i}{Conc\ FAME_{C15:0}\ measured} * Conc\ FAME_{C15:0}\ added * DF$$

$$FAME\ content\ _{Norm}(\%\ wt.) = \frac{FAME\ conc\ _{Norm}\left(\frac{mg}{mL}\right) * V\ (mL)}{DCW\ sample\ (mg)} * 100\%$$

3.7 Transesterification of Cooking Oils

The transesterification of cooking oils (corn, canola, and sunflower oil) with methanol and ionic liquids ([P_{4444}][For], [P_{4444}][Ace], [P_{4444}][Prop], [P_{4444}][Buty]) were performed as follows: 15 mg of oil, ionic liquid, and methanol were mixed at the designed ratio in a 5 mL vial. The mixture was heated to the indicated temperatures in an oil bath for the specified incubation time with stirring on a magnetic hot plate at 600 rpm. After cooling, the upper phase which contained FAME (visible as a thin layer) could not be easily recovered; thus, 4 mL of hexane was added to facilitate the extraction of FAME by vortexing for 1 min followed by 10 min stirring at 1000 rpm. The sample was settled for 5 min, then the top hexane phase was withdrawn the FAME content was determined by GC according to the procedure previously described. The FAME yield was calculated by the following equation:

$$Yield\ (\%) = \frac{FAME\ (mg)}{mass\ of\ oil\ (mg)\ \times\ FAME\ content\ (\%wt)} \times 100\% \qquad 3\text{-}1$$

The maximum FAME content from oil was calculated following the method described by EU regulation 2568/91 [103]. 100 mg of oil was dissolved in 10 mL hexane, and then 100 µL of 2 N KOH in methanol was added to the sample. Samples were vortexed for 30 s, followed by centrifugation, and the supernatant was spiked with the ISTD and separated on a GC.

3.8 *In situ* Transesterification of *C. vulgaris* with Methanol

The *in situ* transesterification reactions were performed as follows: 50 mg of microalgae was combined with ionic liquid and methanol were mixed at the designed ratio in a 5 mL vial. The mixture was heated to the indicated temperature in an oil bath for the specified incubation time with stirring on a magnetic hot plate at 600 rpm. After cooling, 4 mL of hexane was added to the mixture to facilitate the extraction of FAME by vortexing for 1 min followed by 10 min stirring at 1000 rpm. The sample was settled for 5 min, then 450 µL of the clear top hexane

phase was spiked with 50 μL of ISTD. The prepared sample was used to quantify the FAME yield using GC according to the procedure previously described. The *in situ* transesterification of wet microalgae was carried out using freeze-dried *C. vulgaris,* which was resuspended in different amounts of DI water to simulate the wet microalgae with different water contents. The FAME yield was calculated by the equation given below, as the FAME recovered during the *in situ* transesterification divided by total available FAME in the *C. vulgaris* biomass:

$$Yield\ (\%) = \frac{FAME\ (mg)}{mass\ of\ algae\ (mg)\ \times\ FAME\ content\ (\%wt)} \times 100\% \qquad 3\text{-}2$$

3.9 Calculation of Biodiesel Properties Using the FAME Composition

Several important biodiesel properties including cetane number (CN), iodine value (IV), cold filter plugging point (CFPP), higher heating value (HHV), and kinematic viscosity (υ) of FAME produced from refined oils and microalgae with $[P_{4444}][For]$ were calculated using the FAME composition. The cetane number of biodiesel was calculated by the following equation [104]:

$$CN = \sum_i -7.8 + 0.302 \times M_i - 20 \times N \qquad 3\text{-}3$$

where CN is the cetane number, M_i represents the molecular weight of the i^{th} FAME, and N is the number of double bonds in the FAME. The iodine value (IV) in gI_2100g^{-1} and the cold filter plugging point (CFPP) in ºC are predicted by the following equations [105, 106]:

$$IV = \sum_i \left(\frac{560\ \times\ N_i}{M_i}\right) \qquad 3\text{-}4$$

$$CFPP = (3.1417 \times LCSF) - 16.477 \quad 3\text{-}5$$

$$LCSF = (0.1 \times C16{:}0) + (0.5 \times C18{:}0) + (1 \times C20{:}0) + (2 \times C24{:}0) \quad 3\text{-}6$$

where N_i and M_i represent the percentage and the molecular weight of the i^{th} FAME, respectively. LCFS is the long chain saturation factor. The higher heating value (HHV) and kinematic viscosity (υ) of the were calculated by Equations (3-2) and (3-3), respectively [107].

$$\ln(\upsilon) = \sum_i -12.503 + 2.496 \times \ln(M_i) - 0.178 \times N \quad 3\text{-}7$$

$$HHV = 46.19 - \frac{4.9}{M_i} + 0.0118 \times N \quad 3\text{-}8$$

where N is the number of double bonds in the i^{th} FAME. The HHV is in MJ/Kg and υ is the kinematic viscosity of produced FAME at 40°C in mm^2/s.

3.10 Response Surface Design and Polynomials

Response surface methodology (RSM) is a collection of statistical and mathematical methods that is beneficial for analyzing and modelling problems in which multiple variables influence the response of interest. The optimization of this response is the main purpose of these methods [108]. Recently, RSM has been applied to optimize different biodiesel production processes [25, 85, 98, 109].

In this study, the *in situ* transesterification reaction was optimized by response surface methodology (RSM) using central composite design (CCD) with Design-Expert software 10

(State-Ease, USA). The CCD is a standard RSM design which is ideal for fitting a quadratic response surface polynomial design. Optimization of the significant variables with a minimum number of experiments and analyzing the interactions between variables are the main characteristics of this method [108]. The CCD was used to study the following variables: reaction time (x_1), reaction temperature (x_2), the mass ratio of IL: *C. vulgaris* (x_3), and the water content of microalgae biomass (x_4). The levels and ranges of four studied factors, including actual and coded levels, are summarized in **Table 3.1**. A five-level CCD with four factors ($k = 4$), $\alpha = 2$ was carried out to fit a quadratic response surface polynomial design. The design was fully replicated three times, and six replicates at the center points were used to determine the pure error of the experiment, creating a total of 90 runs. The general form of the regression model is as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad 3\text{-}9$$

where y is the predicted response (FAME yield (%)), β_0, β_i, β_{ii}, and β_{ij} represent the regression coefficients, k represents the number of factors, x_i and x_j represent the coded factors, and ε is the random error. Analysis of variance (ANOVA) and lack of fit of tests were performed to evaluate the significance of the model.

Table 3.1: Coded and uncoded variables used in CCD

		Coded Level and Actual Values				
Factor	**Label**	-2	-1	0	1	2
Time (h)	x_1	2	4	6	8	10
Temperature	x_2	55	75	95	115	135
Mass ratio IL:microalgae	x_3	2	4	6	8	10
Water content (% wt.)	x_4	0	21	42	63	84

3.11 Ionic Liquid Recycling

The reusability of $[P_{4444}][For]$ was investigated after the *in situ* transesterification reaction was carried out in triplicate under the optimal conditions obtained by RSM: 8 g IL/g algae, 9 g MeOH/g algae, at 102.4°C for 4 h and 36 min using wet *C. vulgaris* (water content of 40.62%). After FAME extraction, the microalgae residue was separated from the IL/methanol mixture by adding an extra 10 mL of methanol as an anti-solvent in order to precipitate dissolved solids. The resulting IL/methanol mixture was vacuum filtered using a fine porosity Buchner funnel. The IL was recovered by evaporation of residual water and methanol using a rotary vacuum evaporator at 80°C until the weight no longer fluctuated. The recycled IL was reused for *in situ* transesterification process described above in order to determine the effect of recycling IL on the performance of the wet *in situ* transesterification process.

4 Results and Discussion

4.1 Screening of Ionic Liquids for Transesterification

The total lipid content in the microalgae biomass (*C. vulgaris*) was determined as a FAME to be 31.17 ± 1.05 wt% using standard laboratory procedure developed by NREL.

First, the catalytic performance of the phosphonium carboxylate ([P_{4444}][CA]) ILs was investigated for their ability to convert refined cooking oils (corn, canola, and sunflower oil) into FAME. All experiments were conducted with a mass ratio of IL to oil/microalgae of 6:1, mass ratio of methanol to oil/microalgae of 9:1, at reaction temperature of 85ºC for 6 h. The initial reaction conditions were chosen based on our previous work [1]. The results of FAME yield for different oils using [P_{4444}][CA] ILs were presented in **Figure 4.1**.

Phosphonium ILs have relatively high thermal and chemical stabilities compared to the corresponding imidazolium and ammonium ILs. Moreover, unlike the imidazolium cation, the absence of acidic protons in the phosphonium cation indicates that phosphonium ILs can be utilized in strong basic environments [110]. Thus, it is possible to use them for the *in situ* transesterification process to produce biodiesel as the only catalyst of the process or as the catalyst and co-solvent, particularly in the basic environment. Furthermore, unlike halide anions resulting in chemical corrosion and are harmful to the environment, the carboxylates anions are environmentally friendly [111]. Consequently, [P_{4444}][CA] ILs could be an excellent catalysts for the production of biodiesel in order to have a green process.

It can be seen that using the formate anion resulted in a higher FAME yield than the longer chain carboxylates like acetate, propionate, and butyrate. This is not entirely unexpected since formate is a much smaller ion ,which reduces steric hindrance making it a stronger nucleophile for deprotonating the methanol [4, 112]. A similar trend was seen when the IL catalysts were reacted with *C. vulgaris* biomass with [P_{4444}][For] yielding the highest amount of FAME (**Figure 4.2**). In order to confirm the role of the IL plays in *in situ* transesterification, the

carboxylic acids, their sodium salts, and the original tetrabutylphosphonium hydroxide or bromide IL were tested for their ability to directly transesterify FAME on their own. All the experiments were performed in triplicate.

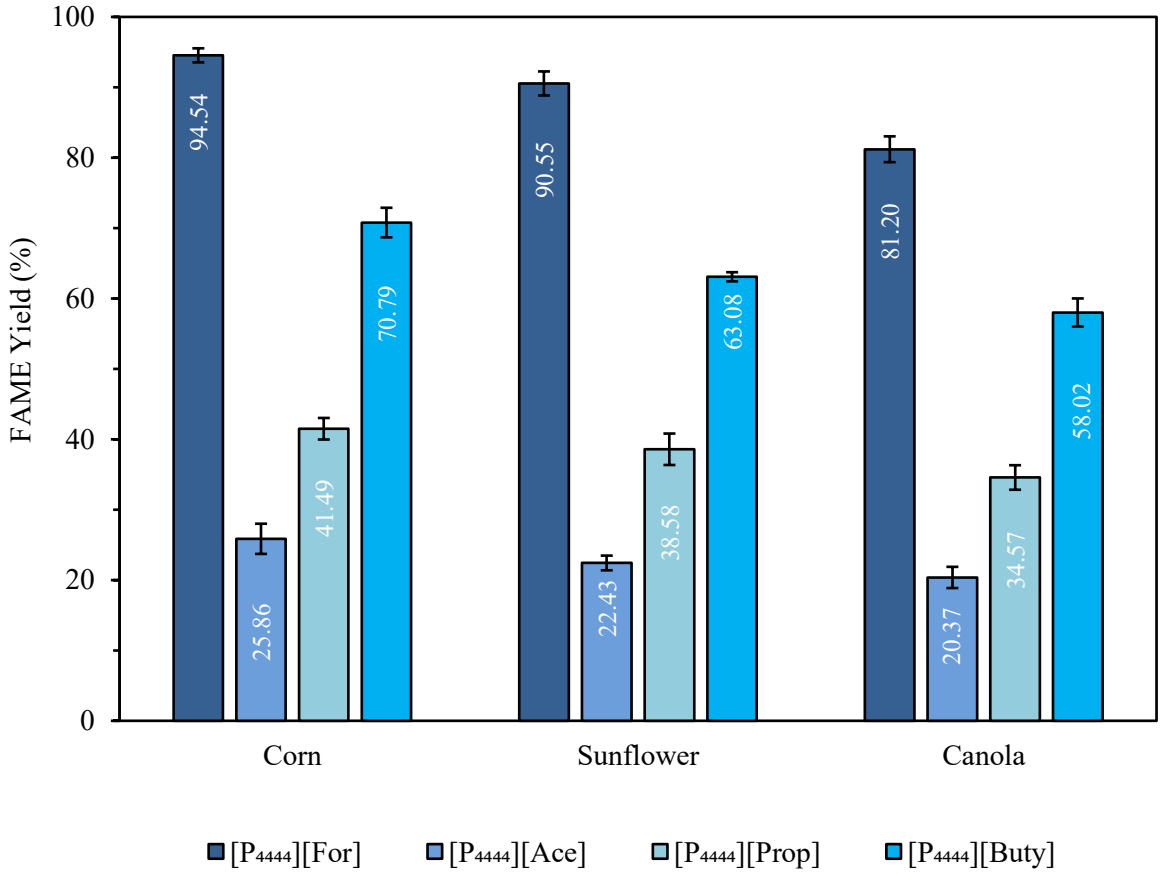

Figure 4.1: Direct transesterification of refined corn, sunflower, and canola oil using phosphonium carboxylate ILs.

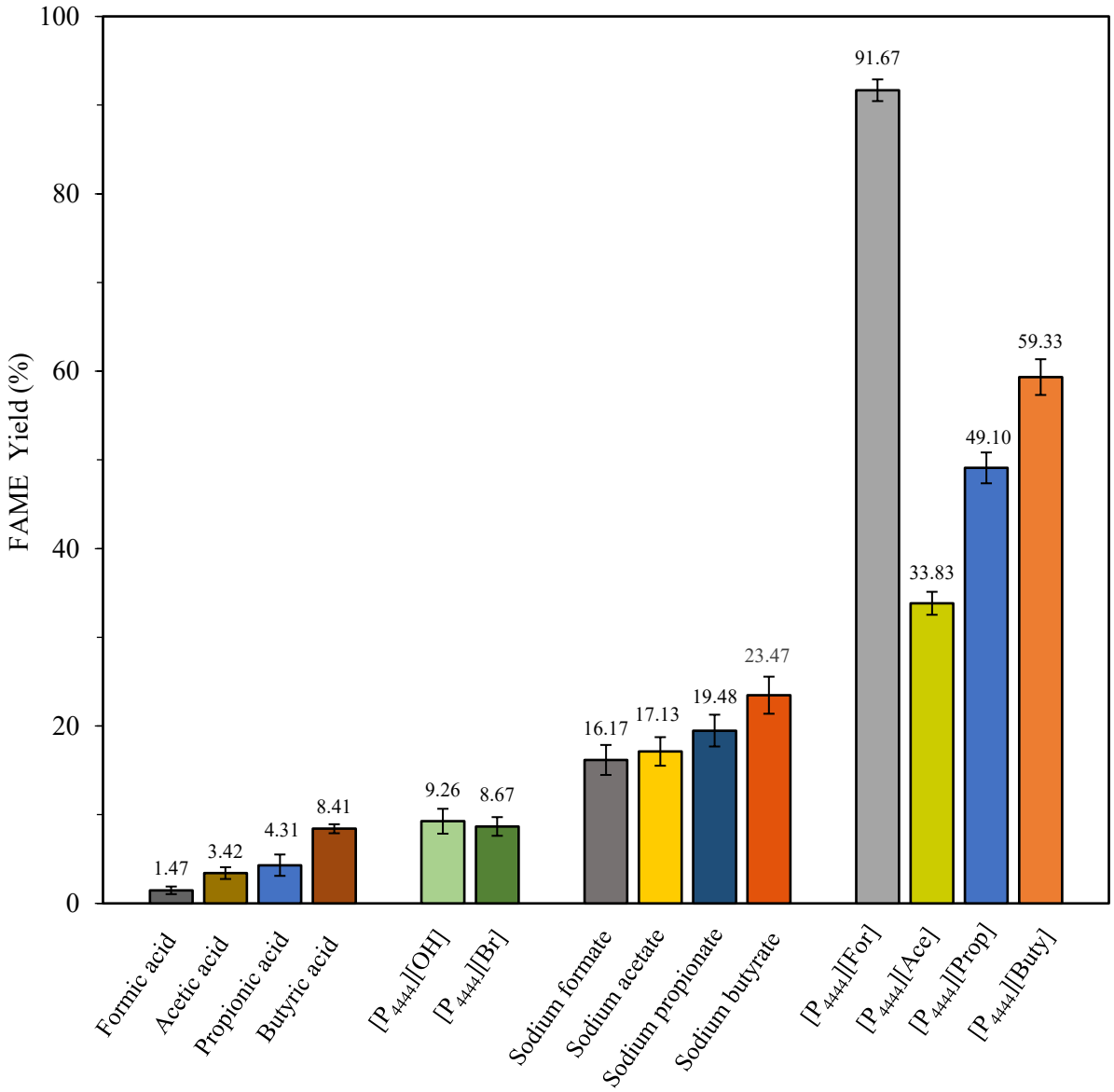

Figure 4.2: Direct transesterification of microalgae biomass using phosphonium ILs, sodium carboxylate salts, and carboxylic acids.

[P_{4444}][For] showed better performance compared to [P_{4444}][Ace], even though acetate anion is more basic than formate anion according to pK_a data from aqueous chemistry [113]. Based on previous literature, the basicity of the IL could be one of the main factors affecting the yield of the transesterification reaction [114]. In our system, the IL plays the role of both solvent for lipid extraction and catalyst for the transesterification reaction. Therefore, it can be concluded that the basicity of IL may be confounded with other factors during the *in situ* transesterification of microalgae.

The excellent catalytic activity of [P_{4444}][For] compared to other ILs with the same cation might be due to the nucleophilicity of the formate anion. The formate anion is less bulky than other carboxylate anions; thus, it is the strongest nucleophile [112]. On the other hand, the viscosity of ILs increases with increasing the chain length of the anion, which negatively impacts the transesterification process by increasing the mass transfer resistance [115]. Thus, the lower viscosity of [P_{4444}][For] compared to other ILs could be another reason for the highest FAME yield of this IL. Among the other ILs, [P_{4444}][Buty] shows better catalytic activity with a FAME yield of 59.33%. There were two possible reasons to explain the higher FAME yield of [P_{4444}][Buty] compared to [P_{4444}][Ace] and [P_{4444}][Prop]. Firstly, the higher solubility of extracted oil from microalgae in the more hydrophobic IL, [P_{4444}]Buty], considering that the hydrophobicity of the ILs comprising carboxylate anions increases with the chain length of the anion [116, 117]. Secondly, among the carboxylate anions, butyrate has relatively high β value (Kamlet–Taft parameter), which describes the ability of an IL's anion to accept the hydrogen-bond as well as the hydrogen-bond basicity and correlates with the ability of anion to dissolve cellulose and biomass disruption. This means that butyrate anion may have an improved ability to interact with the cell wall of microalgae and disrupt it compared to the acetate and propionate anions [118-120]. However, it needs to be mentioned that although the hydrogen-bond basicity of anion is one of the important factors in cell disruption of microalgae, it is not the sole mechanism that comes into play during the microalgae cell wall lysis.

As shown in **Figure 4.2**, for all sodium salts and reaction intermediaries ([P_{4444}][Br] and [P_{4444}][OH]) low FAME yields (less than 30%) were obtained. As can be seen in **Figure 4.2**, FAME production for the ILs neared 92% with [P_{4444}][For] confirming that both carboxylate and cation are required for direct transesterification.

4.2 FAME composition

The composition of FAME produced from the refined oils (corn, canola, and sunflower oil) using direct IL transesterification using phosphonium carboxylate catalysts is shown in **Figure 4.3.** In addition, the composition of microalgae FAME using the same IL catalysts is presented in **Figure 4.4**. All experiments were conducted with a mass ratio of IL to oil/microalgae of 6:1, mass ratio of methanol to oil of 9:1, at reaction temperature of 85ºC for 6 h.

Interestingly, transesterification of both microalgae and refined oils using [P_{4444}][Ace] consistently resulted in a higher proportion of C18:1 and a reduction in C18:2 FAME. This could suggest that this IL acts more readily on unsaturated TGs FAs, or it may result in the saturation of these bonds during the reaction. Another possibility is that the IL catalysts are also able to convert the FFAs in these oils to FAME, since methanolic KOH was used to determine the amount of saponifiable lipids which would not convert FFAs. This is in contrast to the microalgae biomass which was converted to FAME using a strong acid catalyst and methanol as is recommended by NREL. In this case, acid catalysts can convert all available lipids including FFAs and phospholipids into FAME for analysis and the composition did not vary significantly when the IL catalysts were used.

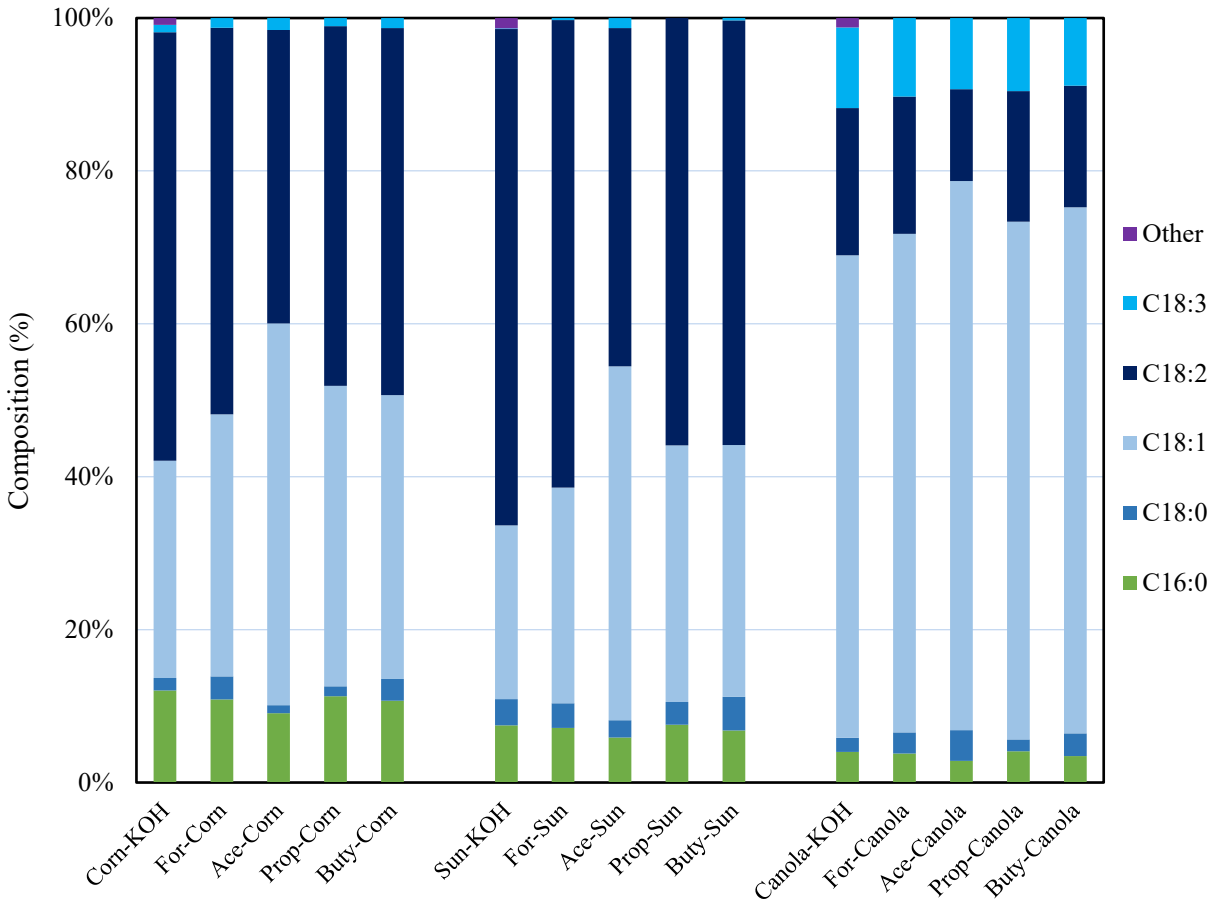

Figure 4.3: The composition of FAME produced from refined oils with direct transesterification using phosphonium carboxylate ionic liquid catalysts.

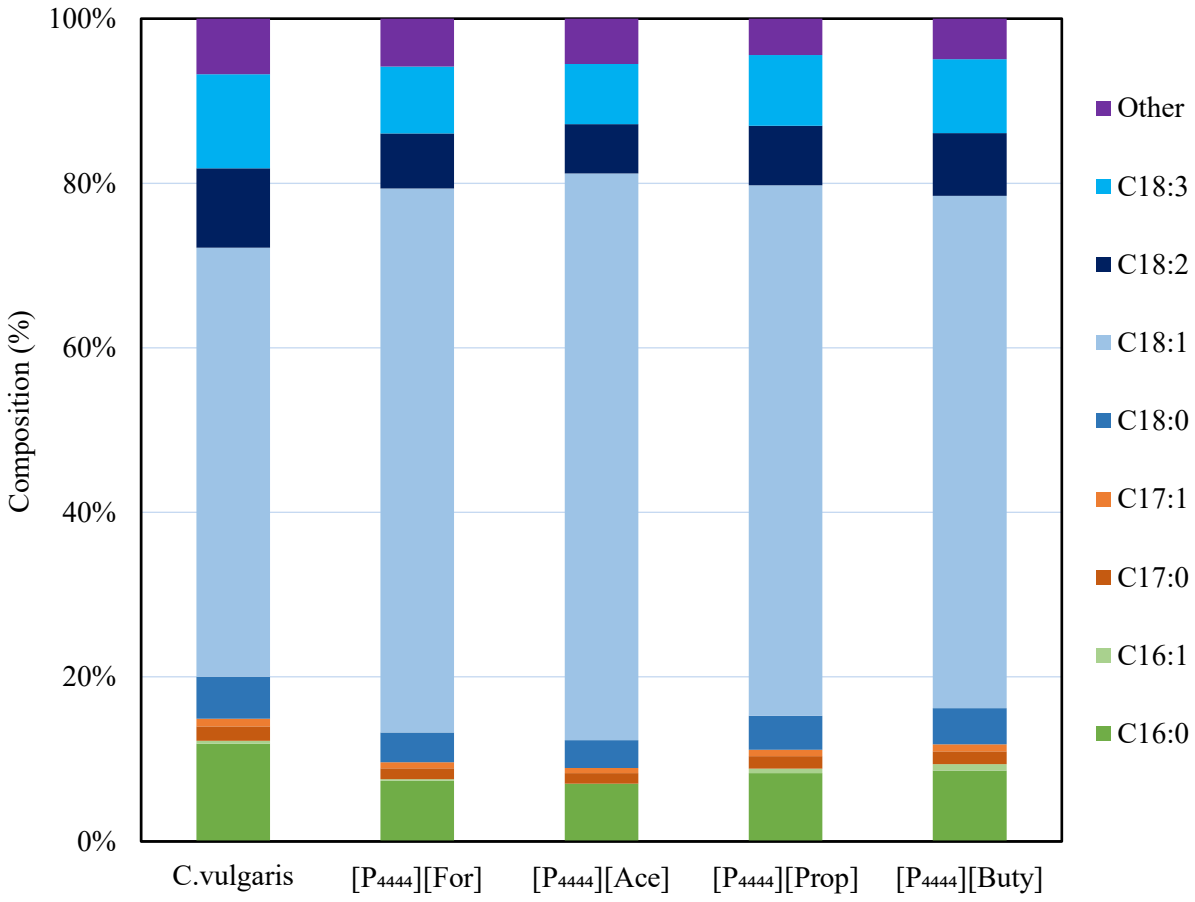

Figure 4.4: The composition of microalgae FAME produced by direct transesterification using phosphonium carboxylate ionic liquid catalysts.

4.3 Calculation of Biodiesel Properties Using the FAME Composition

The major biodiesel properties including cetane number (CN), iodine value (IV), cold filter plugging point (CFPP), higher heating value (HHV), and kinematic viscosity (υ) of FAME produced from refined oils and microalgae with [P_{4444}][For] were calculated using the FAME composition. All calculated biodiesel properties as well as standards amounts according to ASTM D6751 and EN 14214 are presented in **Table 4.1**.

The cetane number (CN) is among the most important indicators for identifying diesel combustion behaviour and indicative of delay time in the fuel ignition. The shorter the ignition time, the higher the CN [121]. Furthermore, the cold filter plugging point (CFPP) is another significant biodiesel property, which is generally utilized to predict biodiesel's flow performance at low-temperature levels [122]. The crystallization of biodiesel molecules increases and agglomerates at the lower temperatures, which leads to clogging fuel pipes and filters; thus, the appropriate amount of CFPP for any synthesized biodiesel according to the climate condition is vital [123].

A higher heating value (HHV) is the heat amount generated by the complete combustion of a unit quantity of fuel [107]. Moreover, biodiesel should have a suitable kinematic viscosity (υ) in order to guarantee that a sufficient fuel supply gets to injectors at different temperatures [124]. Furthermore, the possibility of oxidation is one of the main properties of biodiesel. The Iodine value (IV) describes the tendency of biodiesel to react with oxygen at ambient temperature. The higher the IV, the higher the deposits formation and the oxidation possibility of biodiesel [121].

As can be seen, all synthesized biodiesels fulfill the ASTM D6751and EN 14214 biodiesel standards for almost all properties, indicating direct transesterification of both microalgae and refined oils using [P_{4444}][For] resulted in high-quality biodiesel.

Table 4.1: Biodiesel of FAME produced using [P_{4444}][For]

	Standard ASTM D6751	Standard EN 14214	Corn	Sunflower	Canola	*C. vulgaris*
CN	≥ 47	≥ 51	52.2	50.5	54.0	53.9
IV (g I_2/100 g)	NA	≤ 120	118.9	130.44	112.49	88.5
CFPP (°C)	19 (max.)	0 (max.)	-8.4	-9.2	-11.0	-8.6
Kinematic viscosity (υ) (mm^2/s)	1.9-6.0	3.5-5.0	4.09	4.08	4.19	3.90
HHV (MJ/Kg)	NA	NA	39.6	39.9	39.5	36.9

4.4 Effect of Process Conditions on FAME Production from *C. vulgaris*

It was evident from the first experiments that [P_{4444}][For] outperformed the other [P_{4444}][CA] ILs. Therefore, [P_{4444}][For] was selected for a more in-depth characterization of the effect of process variables on FAME yield. All the experiments were performed in triplicate.

4.4.1 Effect of Methanol Ratio

The amount of methanol is one of the most important factors that affects the FAME yield during the *in situ* transesterification process. The typical stoichiometric molar ratio of transesterification of refined oils using methanol to oil is 3:1; however, during *in situ* transesterification methanol plays a role in acting as a solvent for lipid extraction, reducing the viscosity of the dissolved biomass, in addition to its role as a reactant in the transesterification reaction [25]. Therefore the effect of mass ratio of methanol to freeze-dried *C. vulgaris* on FAME yield was further investigated in the range of 0.15:1 (equal to methanol to oil molar ratio of 3:1 in our system) to 18:1. Five experiments were conducted under fixed operational conditions of mass ratio of [P_{4444}][For] to algae was 6:1, reaction temperature of 85ºC for 6 h, and results are presented in **Figure 4.5**.

When the mass ratio of methanol to microalgae increased from 0.15:1 to 9:1, the FAME yield was significantly increased from 51.54% to 91.67%. Since the samples with low methanol ratios were viscose and poorly covered by this small volume of liquid, it is possible that the low yields are related to the poor contact and mixing in these samples [109]. Further increases in mass ratio of methanol to *C. vulgaris* from 9:1 to 18:1, resulted in only a minor increase in the FAME yield. Thus, a ratio of 9:1 was selected for the remaining experiments.

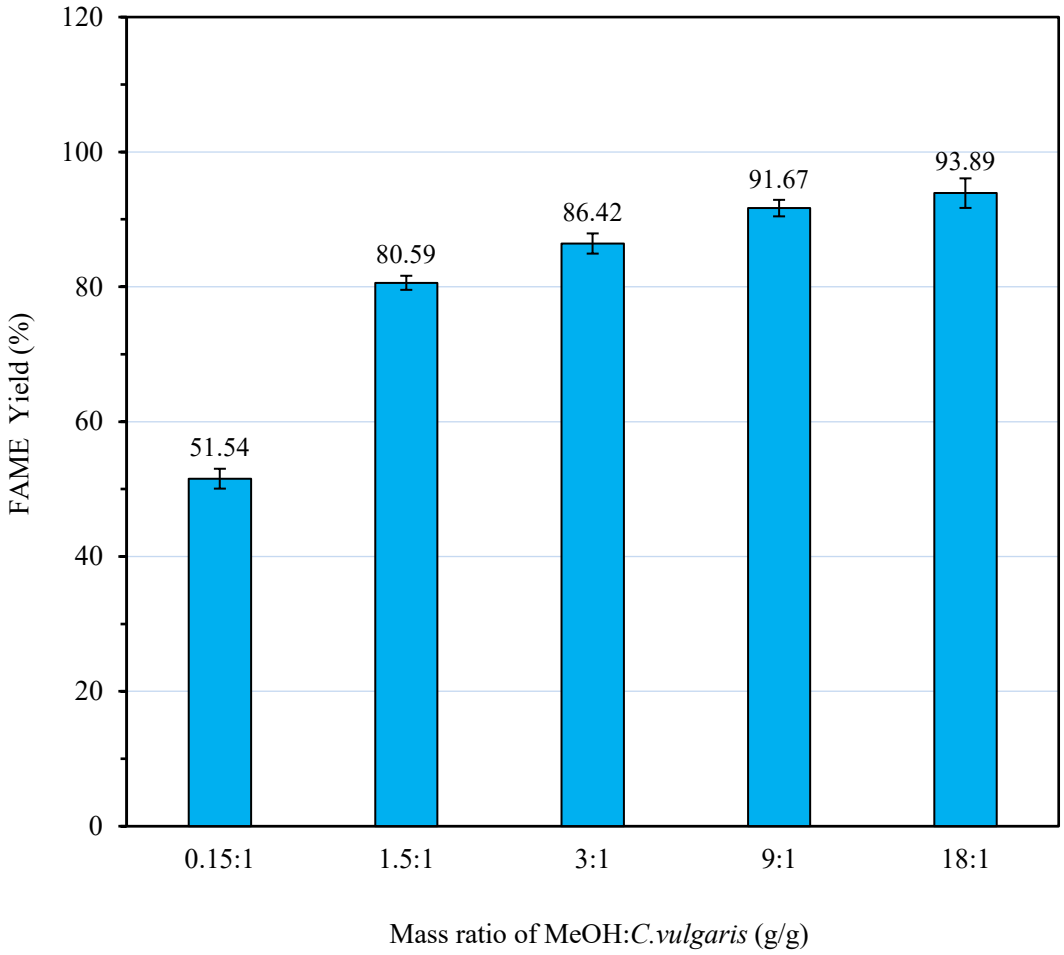

Figure 4.5: Effect of methanol ratio on FAME yield during transesterification using [P_{4444}][For]

4.4.2 Effect of Mass Ratio of IL to Microalgae

The effect of the mass ratio of [P_{4444}][For] to *C. vulgaris* biomass on FAME yield is shown in **Figure 4.6**. All experiments were performed at a constant reaction temperature of 85ºC with a reaction time of 6 h, the mass ratio of methanol to microalgae of 9:1, when the mass ratio of [P_{4444}][For] to *C. vulgaris* was varied from 3:1, 6:1, and 9:1.

The results show that increasing the amount of IL has a positive effect on FAME yield, which increased from 60.5± 0.92% to 102.9 ± 2.15% when the mass ratio was increased from 3:1 to 9:1. Since transesterification reactions are reversible, increasing the concentration of the reactant, methoxide by increasing the amount of [P_{4444}][For] could play a role in the effect of mass ratio of IL:microalgae [4].

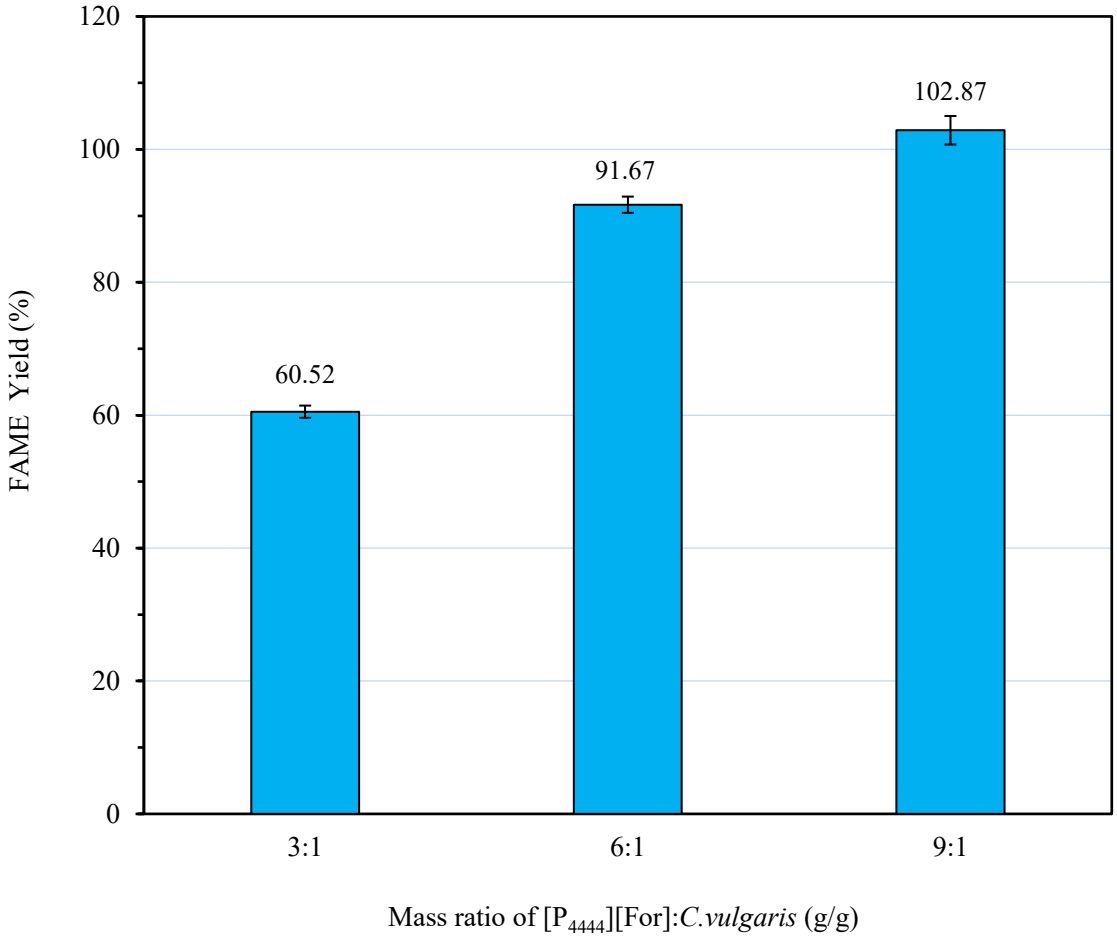

Figure 4.6: Effect of mass ratio of IL to microalgae on FAME yield during transesterification using [P_{4444}][For]

4.4.3 Effect of Reaction Time

The effect of reaction time on *in situ* transesterification process was studied next. When the reaction time was increased, the yield was also increased as is seen in **Figure 4.7**. This figure shows the plot of FAME yield vs. reaction time at the constant reaction temperature of 85ºC, mass ratio of $[P_{4444}][For]$ to *C. vulgaris* of 6:1, and mass ratio of methanol to microalgae of 9:1.

As can be seen, the FAME yield significantly increased from 60.27% at a reaction time of 1 h to 91.67% at a reaction time of 6 h. This is likely because the direct transesterification process is a simultaneous two step process where first the biomass must be disrupted, then the transesterification reaction can occur. However, no considerable change in the FAME yield was seen when the reaction time increased to 9 h. Therefore, considering the reaction efficiency and energy consumption, the reaction time of 6 h was selected as the optimum reaction time for further experiments.

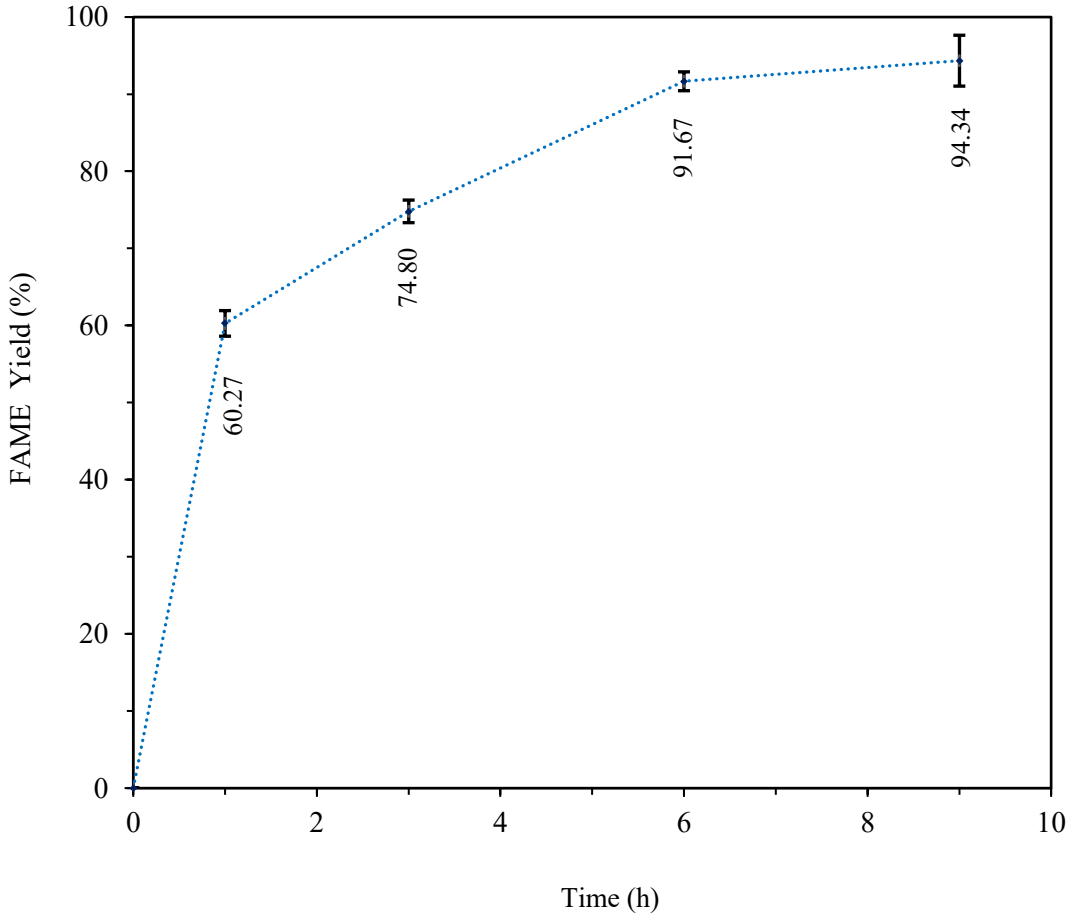

Figure 4.7: Effect of reaction time on FAME yield during transesterification using [P_{4444}][For]

4.4.4 Effect of Water Content and Temperature

Recently, some studies have demonstrated the increased compatibility of lipid extraction and transesterification with water by using IL cosolvents [1, 4, 97, 125]. However, very high moisture contents in microalgae biomass is still a main limiting factor for *in situ* transesterification processed as the synthesis of FAME is a reversible process and the presence of water can hydrolyze the product converting it to FFAs and methanol again (**Figure 4.8**).

One of the main goals of this study was also to enhance the water compatibility of *in situ* transesterification of wet microalgae. Thus, the effects of temperature and water content on the production of FAME were investigated. The different water contents were simulated by pre-wetting a fixed amount of freeze-dried *C. vulgaris* using deionized water and allowing the microalgae to rehydrate for 30 min. The experiments were carried out at reaction temperatures of 85ºC and 115ºC, and water contents ranging from 50 to 90 wt.%. The other variables were fixed as a mass ratio of $[P_{4444}][For]$ to algae of 6:1, a mass ratio of methanol to dried algae of 9:1 for 6 h. The results are presented in **Figure 4.9**.

It was observed that the FAME yield decreased considerably with increasing water content. This was not surprising as the presence of water can cause hydrolyze TAGs forming FFAs, decreasing the FAME yield [97, 125]. The negative impact of water on direct transesterification can be somewhat mitigated by increasing the reaction temperature. There are multiple possible mechanisms as play. First, transesterification is an endothermic process requiring some heat and therefore, higher temperatures result in higher yields [125, 126]. Secondly, higher temperatures can also lead to a decrease in the viscosity of $[P_{4444}][For]$ and enhance the contact between reactants and mass transfer between the phases [127].

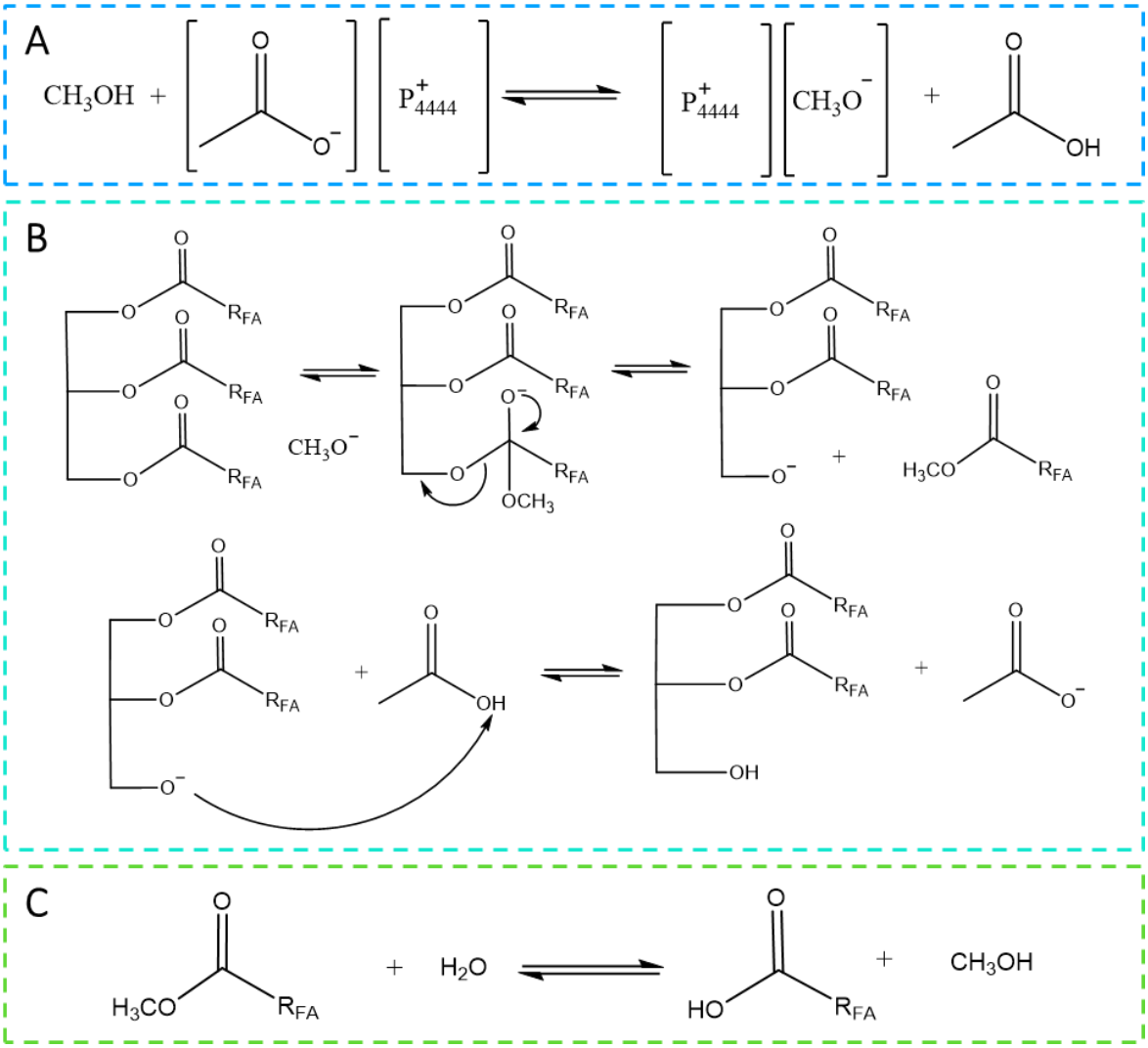

Figure 4.8: Schematic of biodiesel transesterification reaction. (A) The equilibrium between the methoxide ion, formate anion which comes from [P_{4444}][For] IL, and methanol. (**B**) The mechanism of formation of fatty acid alkyl esters from glycerol lipids using an alkyl oxide catalyst [4] and (**C**) the hydrolysis of FAME to alcohol and FFAs in the presence of water.

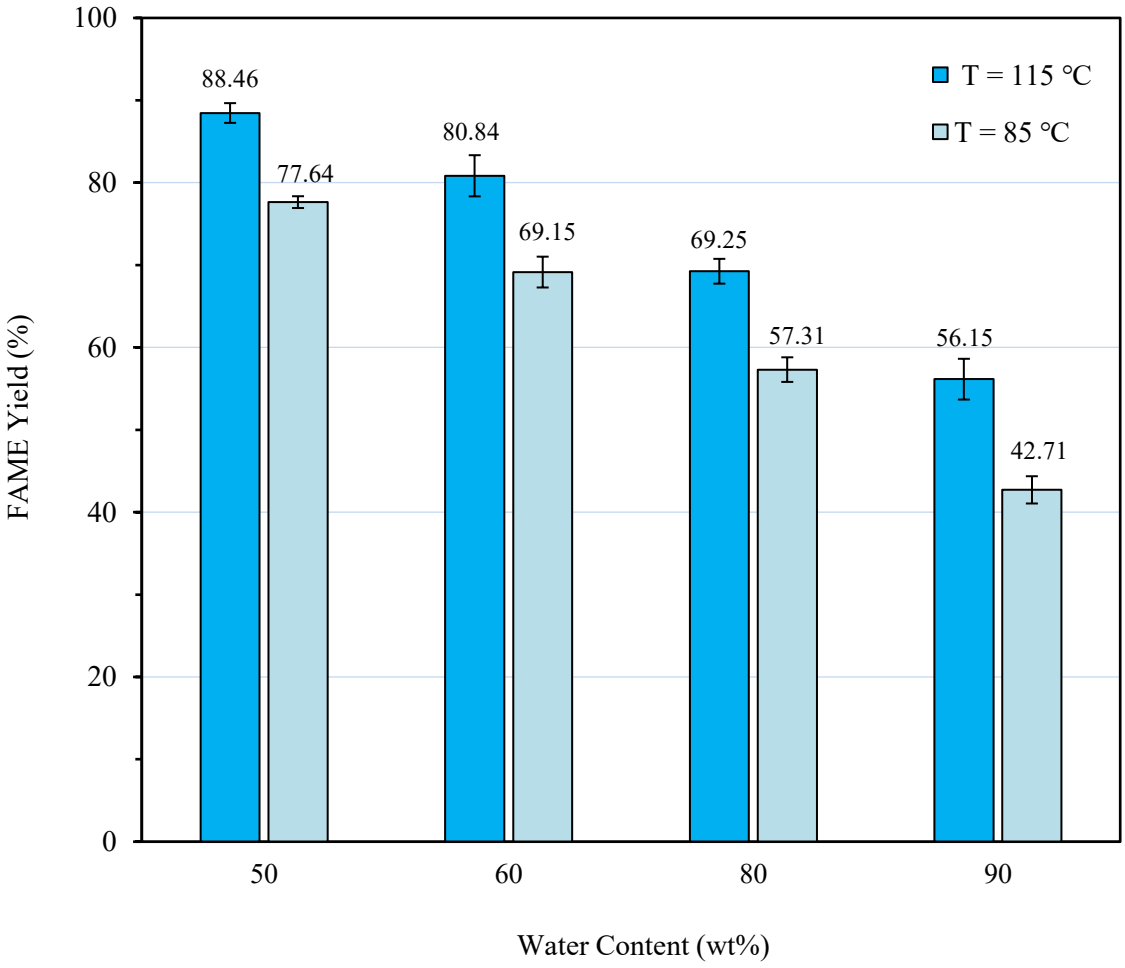

Figure 4.9: Effect of water content and temperature on FAME yield during transesterification using [P_{4444}][For]

4.5 Optimization of Wet *in situ* Transesterification Reaction Using Response Surface Methodology (RSM)

In order to confirm the optimal conditions identified in the previous experiments, minimize the consumption of catalyst and methanol, maximize the FAME yield, and to understand the possible synergistic effects of multiple factors, response surface methodology (RSM) was applied to the reaction conditions.

Based on the previous experimental results, reaction time (x_1), reaction temperature (x_2), mass ratio of [P_{4444}][For]:*C. vulgaris* (x_3), and the water content of *C. vulgaris* (x_4), were selected as the main variables for the further optimization of the response variable, FAME yield. A four-factor, five-level central composite design (CCD) was used. The experimental results are presented in **Table A1**. A quadratic model was chosen as the best fit according to analysis of variance and a lack of fit tests. The externally studentized normal probability plots and the residual plots did not reveal any issues with the model. The resulting model equation is given as:

$$y = 92.38 - 5.28x_1 + 10.91x_2 + 7.77x_3 - 11.97x_4 - 2.25x_1^2 - 8.43x_2^2 - 1.05 - 5.32x_4^2 - 2.67x_{12} - 2.21x_{13} + 1.09x_{14} - 6.43x_{23} + 1.42x_{24} - 2.86x_{34} \quad \text{4-1}$$

Moreover, the ANOVA results are presented in **Table 4.2**.

Table 4.2: ANOVA results for the CCD using [P_{4444}][For]

Source	SS	df	MS	*F*-value	*p*-value	
Block	28.72	2	14.36			
Model	35677.33	14	2548.38	414.95	< 0.0001	significant
x_1(Time)	2005.83	1	2005.83	326.61	< 0.0001	
x_2 (Temp.)	8564.55	1	8564.55	1394.56	< 0.0001	
x_3 (IL/algae)	4351.25	1	4351.25	708.51	< 0.0001	
x_4 (Water cont.)	10309.93	1	10309.93	1678.76	< 0.0001	
x_1x_2	341.64	1	341.64	55.63	< 0.0001	
x_1x_3	235.39	1	235.39	38.33	< 0.0001	
x_1x_4	56.53	1	56.53	9.20	0.0033	
x_2x_3	1986.14	1	1986.14	323.40	< 0.0001	
x_2x_4	97.12	1	97.12	15.81	0.0002	
x_3x_4	393.77	1	393.77	64.12	< 0.0001	
x_1^2	417.16	1	417.16	67.93	< 0.0001	
x_2^2	5843.23	1	5843.23	951.45	< 0.0001	
x_3^2	91.21	1	91.21	14.85	0.0002	
x_4^2	2327.76	1	2327.76	379.03	< 0.0001	
Residual	448.32	73	6.14			
Lack of Fit	43.15	10	4.32	0.6710	0.7468	not significant
Pure Error	405.17	63	6.43			
Corrected Total	36154.37	89				

The lack of fit was not significant indicating the data was well represented by the model and that the residuals (SS = 448.32) were well represented by the pure error (SS=405.17). The model had an R^2 of 0.9876, which was very close to the adjusted R^2 value (0.9852) indicating that the model was well fit by the quadratic model and has a prediction R^2 value of 0.9809 indicating it has good predictive ability in the design space. In addition, the low value of CV (3.15%) indicating a high level of precision and good reliability in the experimental data.

All of the quadratic model terms were found to be significant ($p < 0.01$) and the coded coefficients were plotted in **Figure 4.10** in order to visualize the factors with the greatest effects. In terms of factors which positively influenced the FAME yield, the main factors, temperature, time, and the IL mass ratio to microalgae were amongst the greatest (**Figure 4.10**). Water content had the greatest negative effect on FAME yield as was expected; however, both time and temperature interacted with water content in a positive manner. The most significant interaction was the mass ratio of IL to microalgae and temperature. As a result, less IL can be used if the reaction temperature is increased without sacrificing yield.

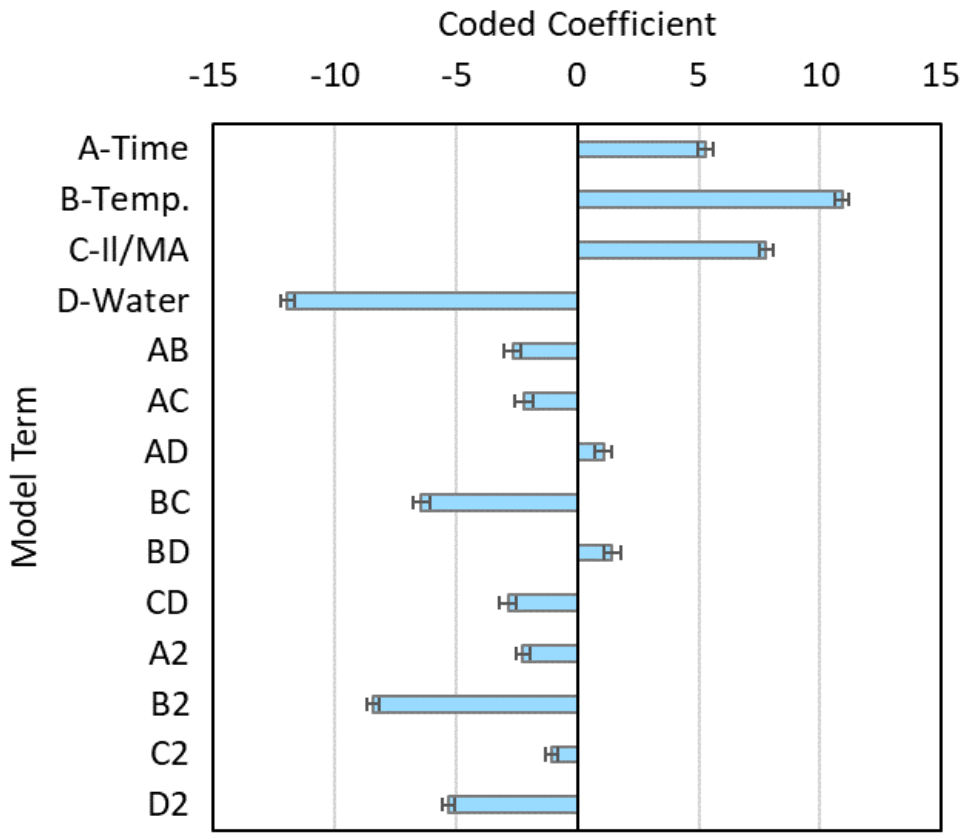

Figure 4.10: Comparison of coded coefficients

A number of three-dimensional (3D) surfaces were plotted to analyze the four greatest interactions between factors further and are presented in **Figures 4.11-4.14**.

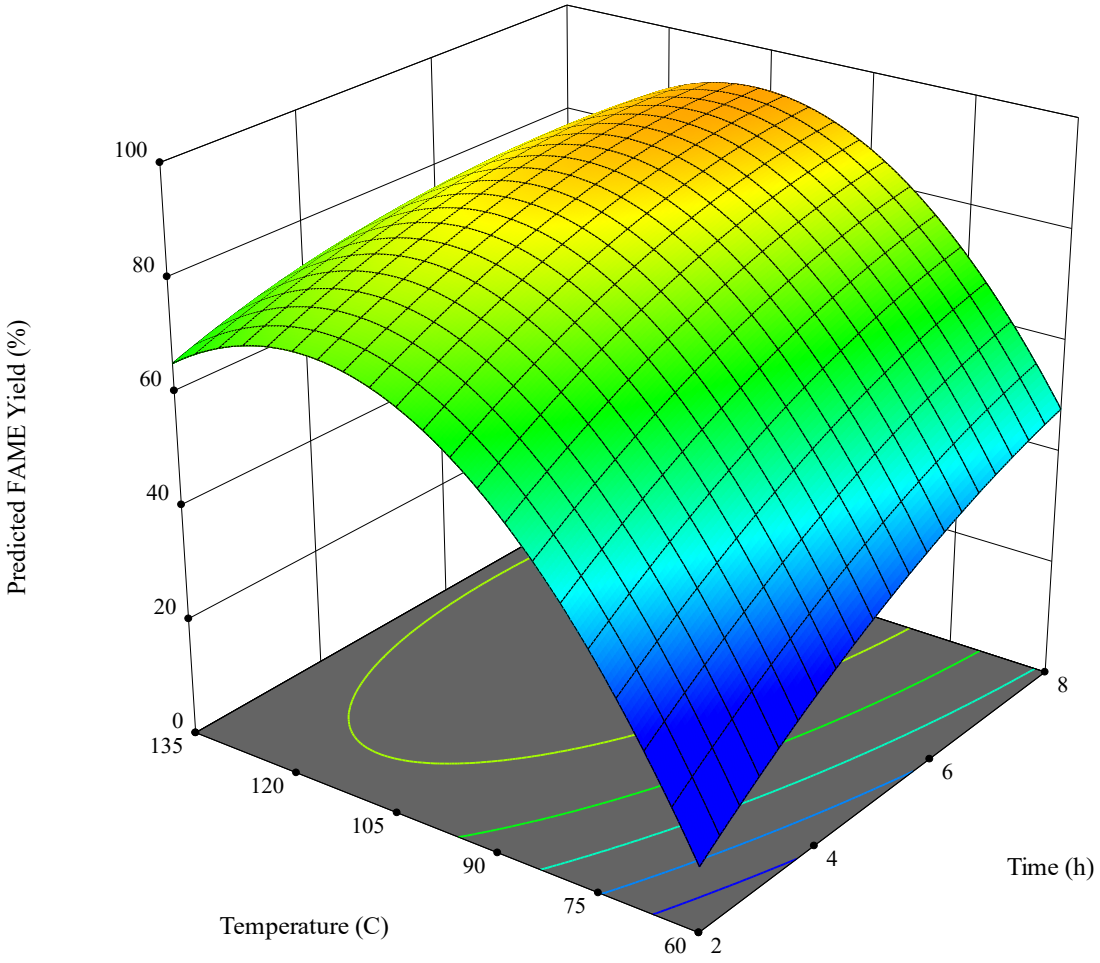

Figure 4.11: Surface plot of the interaction of reaction time and temperature (AB) and their effect on FAME yield. IL:microalgae mass ratio (C) and water content (D) were held constant at 6:1 and 50%, respectively.

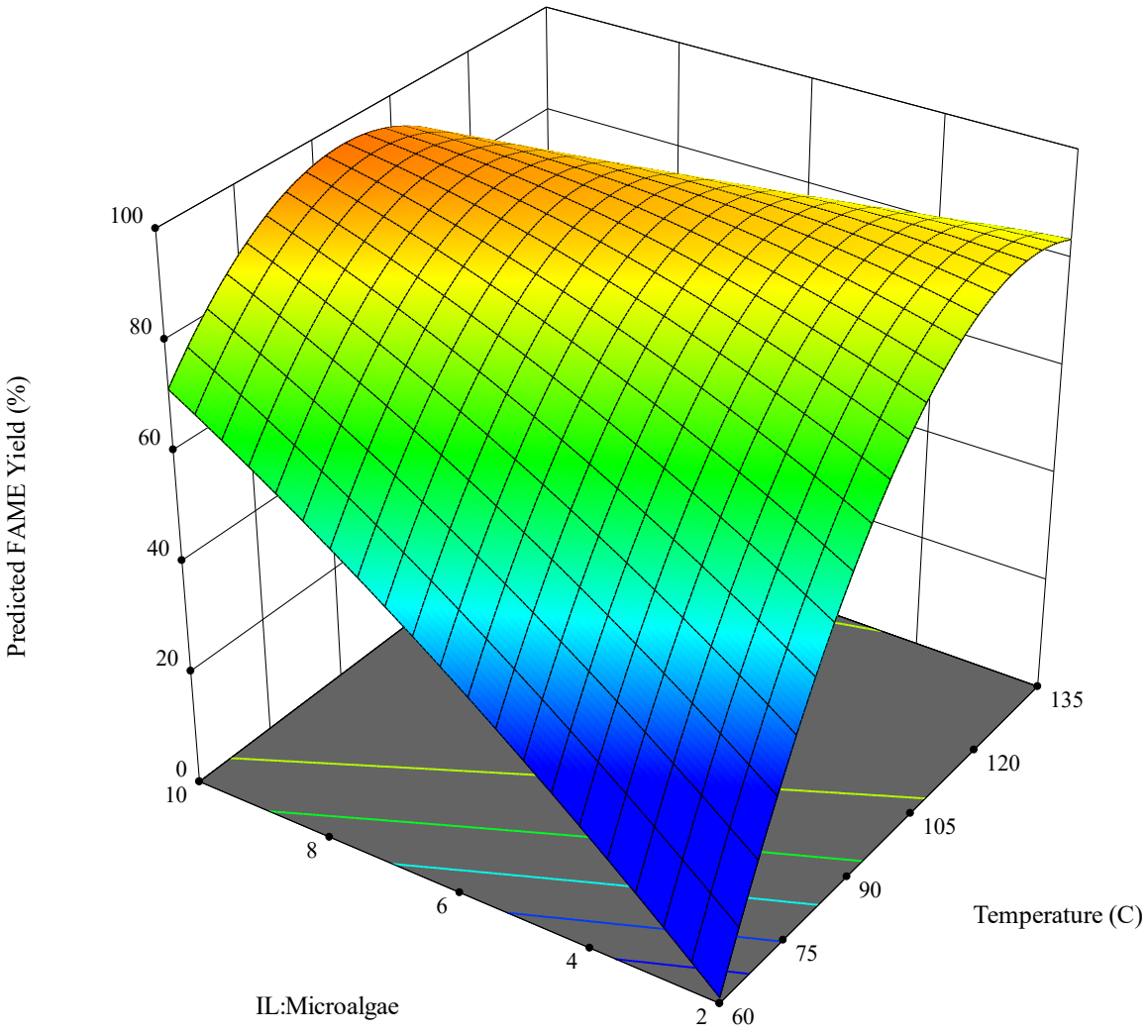

Figure 4.12: Surface plot of the interaction of temperature and IL:microalgae mass ratio (BC) and their effect on FAME yield. Reaction time (A) and water content (D) were held constant at 6 h and 50%, respectively.

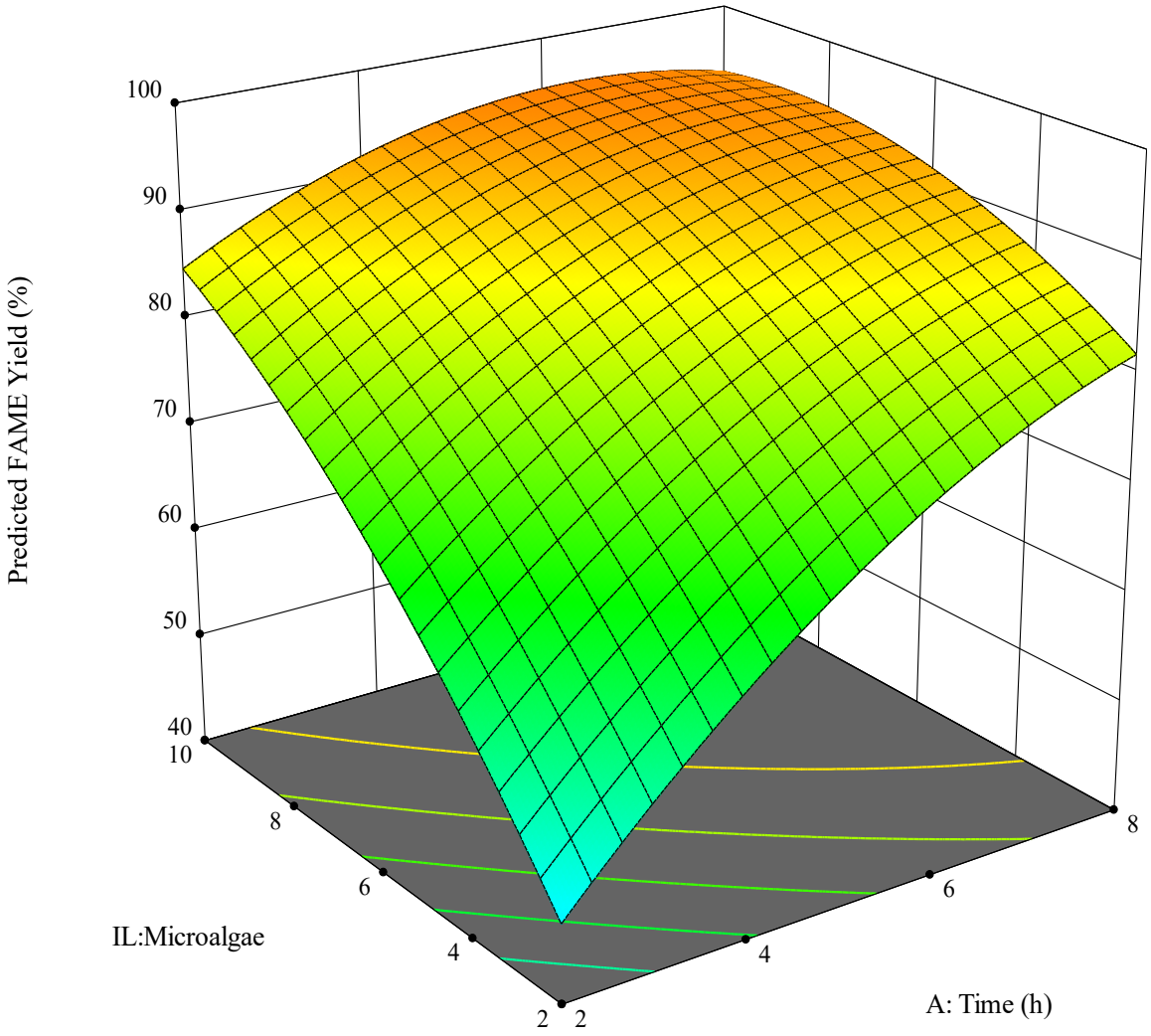

Figure 4.13: Surface plot of the interaction of reaction time and IL;microalgae mass ratio (AC), and their effect on FAME yield. Temperature (B) and water content (D) were held constant at 100°C and 50%, respectively.

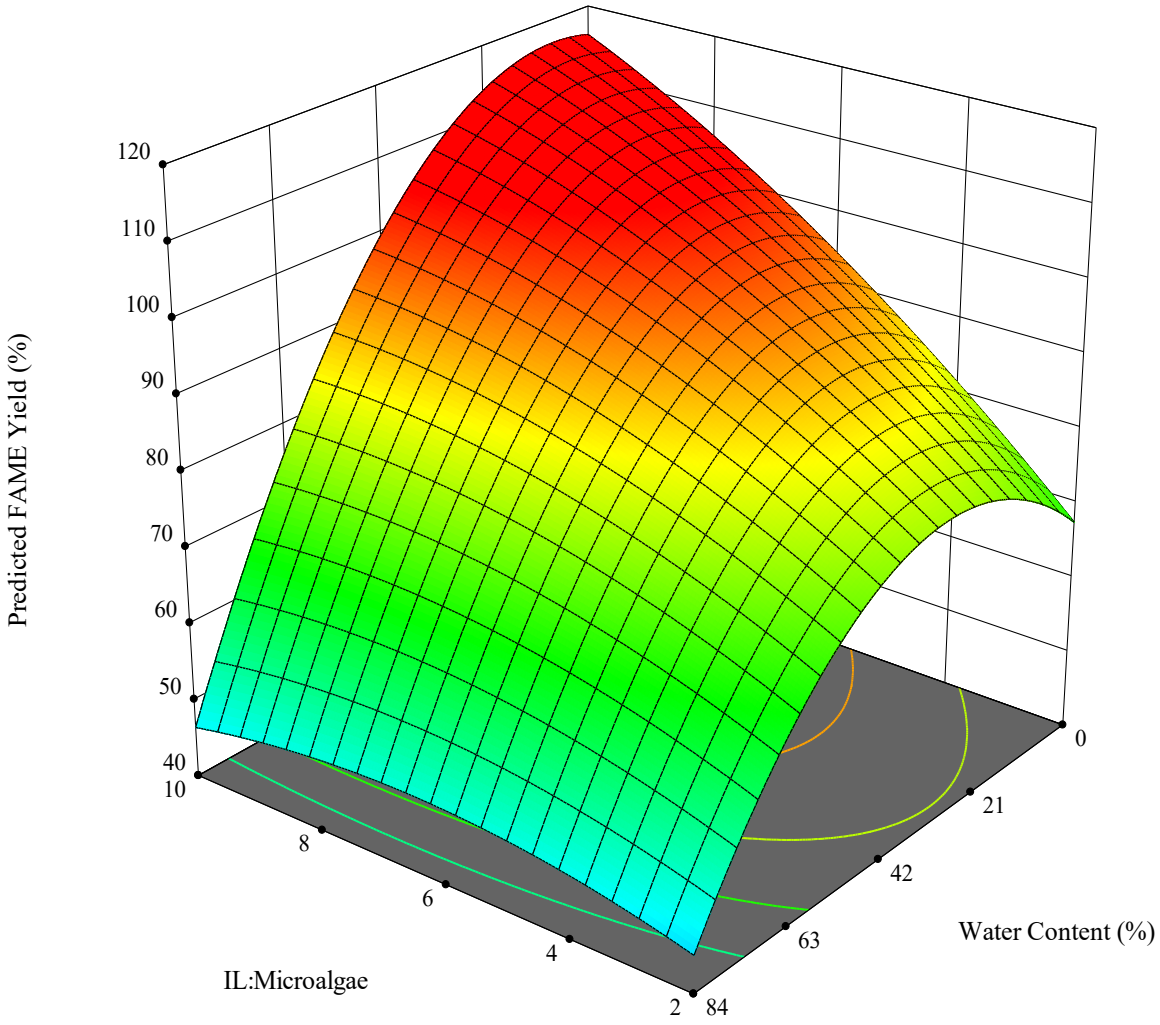

Figure 4.14: Surface plot of the interaction of IL:microalgae mass ratio and water content (CD), and their effect on FAME yield. Reaction time (A) and temperature (B) were held constant at 6 h and 100°C, respectively.

It can be seen from these surface plots that at high temperatures, FAME yield decreases with increasing temperature (**Figure 4.11**). Whereas the IL:microalgae mass ratio can be minimized at high temperatures reducing the amount of IL needed. This is particularly important as the cost of the IL starting material and the make-up of IL is anticipated to contribute a significant portion of the cost of IL based bioprocesses. Increasing the reaction time generally improved yields particularly at low IL:microalgae mass ratios which was expected since this process involves two distinct steps of cell lysis by denaturation or dissolution which is likely depend on the amount of IL present followed by the faster transesterification step. Finally, increasing the amount of IL used also helps to overcome the negative effect of increasing water content in the microalgae. This was in line with our previous results [97]. However, even at low IL mass ratios, yields of greater than 80% are possible with less than 35% water content.

The optimal reaction conditions for maximizing the FAME yield were obtained using numerical optimization with the following conditions from highest importance to lowest: FAME yield was maximized with the highest importance, water content was maximized, reaction time was minimized with the least importance, the mass ratio of IL:microalgae and the temperature were maintained in the range of 4-8 and 2-10 h, respectively. The optimal reaction conditions for the FAME yield was found to require a reaction time of 4.6 h, a reaction temperature of 102.4^{o}C, IL:microalgae mass ratio of 8:1, and water content of 40.6%. The FAME yield at these conditions was predicted to be 98.0 ± 2.48%. In order to verify the reliability of the predicted model, this point was validated in triplicate. The average of experimental FAME yield was 98.6 ± 1.82%, which aligned with the predicted value indicating that the developed model has the ability to accurately predict the response in the design space.

In the only comparable study using an IL catalyst for direct transesterification, Sun et al. [4] reported approximately the same FAME yield using their acidic IL catalyst ([C_4mim] [HSO_4]) however we could achieve the same results at a much lower reaction temperature (102.4^{o}C compared to 200^{o}C), potentially reducing the overall energy consumption for the process. They

used a comparable mass loading ratio of 0.9:1 IL:wet microalgae biomass and a lower methanol to wet algae ratio (3:1).

4.6 Reusability of [P_{4444}][For]

The reusability of catalysts can significantly decrease the cost of biodiesel production. Considering the relatively high cost of ILs and their low volatility, they are often claimed to be readily recycled; however, it is important to verify the possibility of recycling and reusing [P_{4444}][For] before making any claims towards the sustainability or cost of a direct transesterification process. Five cycles of direct transesterification were carried out in triplicate under the optimum reaction conditions. After each cycle, FAME was recovered and analyzed by GC, and [P_{4444}][For] was recovered from the residual solids by anti-solvent precipitation using methanol followed by filtration and drying. After each cycle, the recovered [P_{4444}][For] was reused with fresh wet microalgae in order to evaluate the stability and efficiency of the recycled IL.

As shown in **Figure 4.15**, the FAME yield slightly decreased from 98.63% to 93.76% after five consecutive cycles, possibly due to the decrease in the purity of [P_{4444}][For]. Moreover, the difference in the FAME yield between each cycle is shown in **Figure 4.16**. Overall, [P_{4444}][For] was readily recycled; and maybe a promising catalyst for direct transesterification of microalgae biomass to biodiesel. This has important implications on the solvent and catalyst environmental impact parameter, f, as methanol and the IL are both recycled.

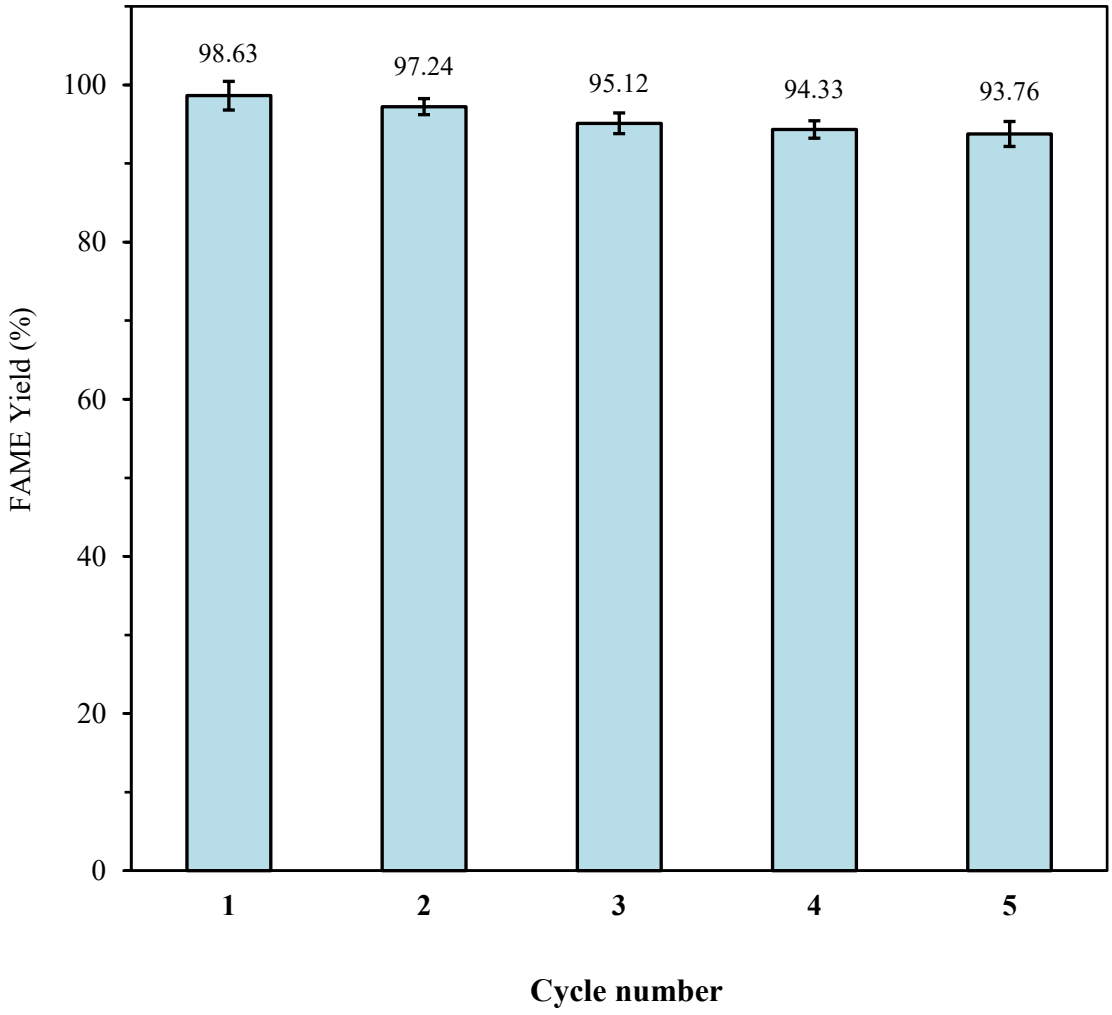

Figure 4.15: Reusability of [P_{4444}][For] under the optimal reaction conditions.

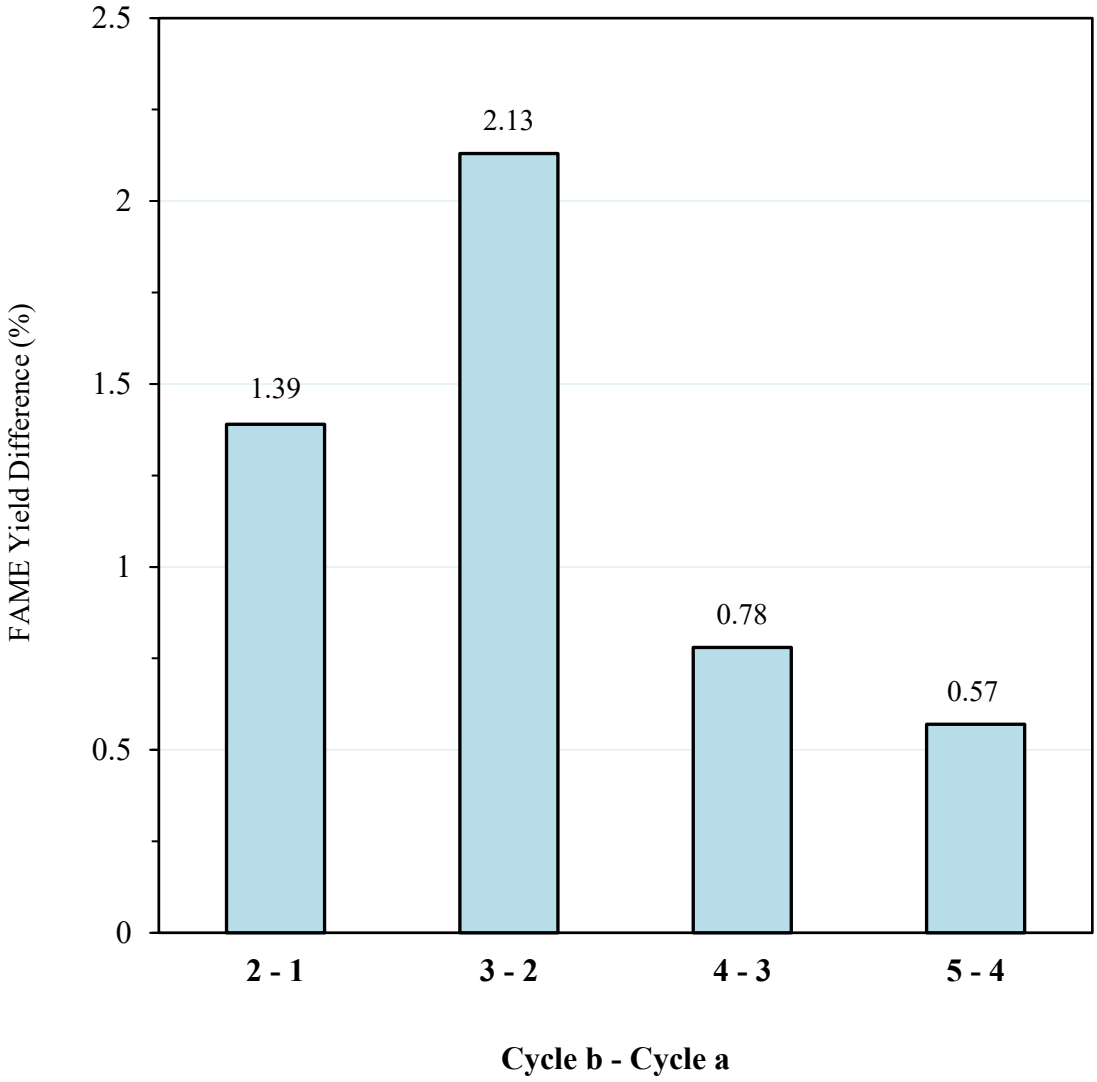

Figure 4.16: The difference in the FAME yield between each cycle.

5 Conclusions and Recommendations

5.1 Conclusions

The overall objective of this work was to develop and optimize the direct conversion of wet microalgae biomass into biodiesel using ionic liquid as a catalyst. Four types of tetrabutylphosphonium carboxylate ionic liquids ([P_{4444}][CA]) were synthesized and were used to transesterify refined cooking oils (sunflower, canola, and corn oil) into biodiesel, and for the direct transesterification of wet microalgae biomass (*C. vulgaris*) into biodiesel. Among the studied ionic liquids, [P_{4444}][For] yielded the highest amount of FAME for both refined cooking oil and microalgae. Therefore, it was chosen for a more in-depth characterization of the effect of process variables on FAME yield. The FAMEs composition and the major properties of synthesized biodiesel from both cooking oils and microalgae were calculated. The effects of reaction parameters including ionic liquid anion size, reaction time, reaction temperature, the mass ratio of IL to microalgae biomass, and the water content of microalgae on FAME yield were investigated. This process was further optimized using response surface methodology (RSM). Finally, the reusability of the ionic liquid was verified.

The following major conclusion can be drawn from this study.

1. Phosphonium carboxylate ionic liquids are good catalysts for transesterification in the presence of methanol and capable of both cell disruption and transesterification in a single step. This work was the first to investigate the use of a basic ionic liquid catalyst for *in situ* transesterification for simultaneous lipid extraction and biodiesel production from microalgae. Furthermore, the use of any ionic liquid catalysts for *in situ* transesterification process for direct biodiesel production in the low (under 100°C) and mid (100°C to 200°C) reaction temperature zone had not been reported prior to this work.

2. [P_{4444}][For] yielded the highest amount of FAME for both refined cooking oil and microalgae. The FAME yields of 94.54% and 91.67% were achieved for corn oil and *C. vulgaris* microalgae, respectively. This was likely due to the smaller size of formate ion compare to other carboxylate ions, which reduces steric hindrance making it a stronger nucleophile for deprotonating the methanol.

3. All synthesized biodiesels are predicted to fulfill the biodiesel properties stipulated in the ASTM D6751 and EN 14214 biodiesel standards, indicating direct transesterification of both microalgae and refined oils using [P_{4444}][For] resulted in high-quality biodiesel.

4. One of the major limitations to reducing the volume of methanol needed for direct transesterification was the need to fully wet the biomass in order to have sufficient mixing. As a result, the methanol was always in excess to the stoichiometric needs of the reaction.

5. Increasing the amount of IL had a positive effect on FAME yield, which increased from 60.5 ± 0.92% to 102.9 ± 2.15% when the mass ratio was increased from 3:1 to 9:1. Since transesterification reactions are reversible, increasing the concentration of the reactant, methoxide by increasing the amount of [P_{4444}][For] could play a role in the effect of mass ratio of IL:microalgae.

6. Increase reaction time was favourable to FAME yield. The FAME yield significantly increased from 60.27% at a reaction time of 1 h to 91.67% at 6 h. This is likely because the direct transesterification process is a two-step process where first the biomass must be disrupted, then the transesterification reaction can occur.

7. The water content of microalgae had a negative effect on biodiesel production. The FAME yield decreased considerably with increasing water content. This was not surprising as the presence of water can cause hydrolysis of TAGs forming FFAs, decreasing the FAME yield.

8. The negative impact of water on direct transesterification can be somewhat mitigated by increasing the reaction temperature. There are possibly multiple mechanisms as play. First, transesterification is an endothermic process requiring some heat and therefore, higher temperatures result in higher yields. Secondly, higher temperatures can also lead to a decrease in the viscosity of $[P_{4444}][For]$ and enhance the contact between reactants and mass transfer between the phases.

9. The optimal reaction conditions for the FAME yield was found to require a reaction time of 4.6 h, a reaction temperature of 102.4°C, IL:microalgae mass ratio of 8:1, and water content of 40.6%. The FAME yield at these conditions was predicted to be 98.0 ± 2.48%. Furthermore, it can be concluded that the effect of water content on FAME yield could be somewhat mitigated by increased processing temperatures, reaction time, and the amount of IL and methanol used which was an important finding. This would allow some flexibility in processing biomass with variable water content.

10. Reusability of the IL was confirmed which will be necessary to reduce the environmental impact of the direct IL transesterification process. The FAME yield slightly decreased from 98.63% to 93.76% after five consecutive cycles, possibly due to the decrease in the purity of $[P_{4444}][For]$. Overall, $[P_{4444}][For]$ was readily recycled; and it is a promising catalyst for direct transesterification of microalgae biomass to biodiesel.

5.2 Recommendations

The following recommendations are based on the results of this thesis:

- Study the transesterification of other microalgae species such as *Phaeodactylum,* and *Nannochloropsis*

- Combine IL transesterification with microwave irradiation or ultrasonication

- Investigate different kinds of alcohols such as ethanol for the transesterification process

- Study the ability of other phosphonium carboxylate ionic liquids to direct conversion of microalgae into FAME

- Develop a process model and evaluate kinetics for the direct transesterification process

- Perform the economic assessment for the established process in order to investigate the feasibility of process and the environmental impact

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Appendix A

Table A 1. Complete data set for all reactions performed in the response surface study

Factor A: Time (h)	Factor B: Temp (°C)	Factor C: IL/algae	Factor D: water (wt.%)	FAME Yield (%)
4	75	4	21	48.8
4	75	4	21	49.6
4	75	4	21	52.4
8	75	4	21	68
8	75	4	21	71
8	75	4	21	73.3
4	115	4	21	92.9
4	115	4	21	88.7
4	115	4	21	88.4
8	115	4	21	97.4
8	115	4	21	93.6
8	115	4	21	94.5
4	75	8	21	90.1
4	75	8	21	93.7
4	75	8	21	91.8
8	75	8	21	99.8
8	75	8	21	102.6

8	75	8	21	96.8
4	115	8	21	100
4	115	8	21	103.2
4	115	8	21	99.1
8	115	8	21	98.6
8	115	8	21	100
8	115	8	21	103.1
4	75	4	63	30.4
4	75	4	63	28.5
4	75	4	63	31.6
8	75	4	63	51.3
8	75	4	63	48.3
8	75	4	63	53.4
4	115	4	63	73.8
4	115	4	63	68.3
4	115	4	63	68.7
8	115	4	63	86.1
8	115	4	63	82
8	115	4	63	82.5
4	75	8	63	58.7
4	75	8	63	55.7
4	75	8	63	53.1

8	75	8	63	72.9
8	75	8	63	70.2
8	75	8	63	67.1
4	115	8	63	70.2
4	115	8	63	76.2
4	115	8	63	74.9
8	115	8	63	78.3
8	115	8	63	73.7
8	115	8	63	76.6
6	95	6	42	95
6	95	6	42	89.1
6	95	6	42	94.5
6	95	6	42	93.9
6	95	6	42	91.5
6	95	6	42	90.1
6	95	6	42	90.4
6	95	6	42	90.1
6	95	6	42	94.7
6	95	6	42	94.2
6	95	6	42	89.7
6	95	6	42	91.9
2	95	6	42	72.3

2	95	6	42	75.4
2	95	6	42	68.7
10	95	6	42	89
10	95	6	42	95.2
10	95	6	42	96
6	55	6	42	37.1
6	55	6	42	32.3
6	55	6	42	36.5
6	135	6	42	80.6
6	135	6	42	83.7
6	135	6	42	78.3
6	95	2	42	73.3
6	95	2	42	74.7
6	95	2	42	70.5
6	95	10	42	104.9
6	95	10	42	102.4
6	95	10	42	99.7
6	95	6	0	92.8
6	95	6	0	97.6
6	95	6	0	95.3
6	95	6	84	48.7
6	95	6	84	43.6

6	95	6	84	45.1
6	95	6	42	91.6
6	95	6	42	88.8
6	95	6	42	94.7
6	95	6	42	93.8
6	95	6	42	93.9
6	95	6	42	94.9