In situ extraction and recovery of volatile fatty acids from biogas-producing anaerobic digestion

by

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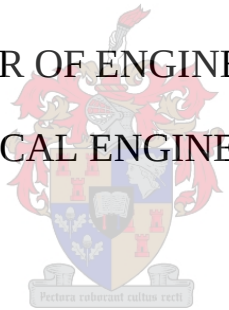

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DECLARATION

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ABSTRACT

An important set of compounds which are produced as intermediates in anaerobic digestion (AD) technologies, although they are not widely recovered as products in biogas plants, are volatile fatty acids (VFAs). Bio-based VFA production from AD using extractive fermentation is a promising approach to control against drastic pH reduction and unstable operational performance due to VFA accumulation in AD systems, while producing a second valuable product stream. This work explores the viability of integrating VFA extraction and recovery with AD using extractive fermentation without arresting the biogas productivity of the digester. Five extractants (tri-n-octylamine (TOA), tri-n-butyl phosphate (TBP), tri-n-octylphosphine oxide (TOPO), Aliquat 336 and trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl)phosphinate ([$P_{666,14}$][Phos])) in combination with oleyl alcohol, lamp oil and canola oil as diluents were investigated based on (i) extraction capacity at varying pH, (ii) biocompatibility with the microbial consortium and (iii) feasibility of VFA back-extraction.

Laboratory scale liquid-liquid extraction (LLE) experiments with synthetic VFA solutions revealed that the extractant Aliquat 336 had the highest capacity to extract VFAs at pH 3.9-6.8, attaining total VFA extractions of 50-70% using the diluents oleyl alcohol, lamp oil and canola oil. Extraction capacity decreased above the pKa of the acids with the rest of the extractants studied. However, TOA-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil extracted 10-25% total VFA (tVFA) at pH 5.6-6.8, which suggested that there are solvents with the capacity to extract acids within suitable pH ranges for biogas-producing AD, which are typically above the pKa of the extracted acids. Most solvent combinations, with the exception of [$P_{666,14}$][Phos], exhibited similar or even improved VFA extractions from wastewater systems, highlighting their potential for application in non-idealised systems.

Bench-scale biogas production experiments using industrial wastewater demonstrated that biocompatible extractant-solvent systems allow for co-production of biogas and VFAs, with enhanced biogas productivity in some cases. Systems containing TOA-oleyl alcohol, TBP-oleyl alcohol, TOPO-oleyl alcohol, TOPO-canola oil and [$P_{666,14}$][Phos]-oleyl alcohol produced two to five times more biogas than the control with average methane percentages of between 70-75% (compared to 55% achieved with the control) and analogous production was seen using TOPO-lamp oil and TOA-lamp oil relative to the control. The presence of Aliquat 336 resulted in minimal gas production regardless of the diluent used, and is therefore not recommended for application in biogas-producing AD.

Total back-extraction VFA recoveries of 80-100% were achieved from TOPO, TBP, TOA and [$P_{666,14}$][Phos] using $NaOH_{(aq)}$ to recover VFAs and regenerate the solvent. Aliquat 336 exhibited lower potential for back-extraction with recoveries between 40-50%. Back-extraction with solvents containing canola oil is not recommended due to observed emulsification in these systems.

The experiments outline that it is possible to select a biocompatible solvent combination that could be used in AD with the ability to co-produce biogas and VFAs, and even enhance productivity in biogas producing digester systems. This methodology could be integrated and used as a pH control strategy while promoting management and reduction of waste, resource recovery, and utilisation of renewable energy. TOPO-lamp oil, TOPO-oleyl alcohol, TOA-lamp oil, TOA-oleyl alcohol and TBP-oleyl alcohol would be recommended for further investigation as potential solvents for *in situ* VFA extraction from biogas-producing AD wastewater treatment systems.

OPSOMMING

’n Belangrike stel samestellings wat geproduseer word as intermediêre produkte in anaerobiese vertering (AD) -tegnologieë, al word hulle nie gewoonlik herwin as produkte in biogasaanlegte nie, is vlugtige vetsure (VFA’s). Bio-gebaseerde VFA-produksie vanuit AD deur ekstraktiewe fermentasie te gebruik, is ’n belowende benadering om te beheer teen drastiese pH-afname en onstabiele bedryfsdoeltreffendheid as gevolg van VFA-akkumulasie in AD-stelsels, terwyl ’n tweede waardevolle produkstroom geproduseer word. Hierdie werk ondersoek die lewensvatbaarheid van integrasie van VFA-ekstraksie en herwinning met AD deur ekstraktiewe fermentasie te gebruik sonder om die verteerder se biogas produktiwiteit te stuit. Vyf ekstraheermiddels (tri-n-oktielamien (TOA), tri-n-butielfosfaat (TBP), tri-n-oktielfosfienoksied (TOPO), Aliquat 336 en triheksiel(tetradektiel)fosfonium bis-2,4,4-(trimetielpentiel)fosfinaat([$P_{666,14}$][Phos])) in kombinasie met olielalkohol, lampolie en kanola-olie as verdunners is ondersoek gebaseer op (i) ekstraksiekapasiteit by variërende pH, (ii) biooverenigbaarheid met die mikrobiese konsortium en (iii) uitvoerbaarheid van VFA terug-ekstraksie.

Laboratoriumskaal vloeistof-vloeistof ekstraksie (LLE) -eksperimente met sintetiese VFA-oplossings het getoon dat die ekstraheermiddel Aliquat 336 die hoogste kapasiteit het om VFA’s by pH 3.9 — 6.8 te ekstraheer, wat ’n totaal van 50 — 70% VFA-ekstraksies bereik, deur die verdunners olielalkohol, lampolie en kanola-olie te gebruik. Ekstraksiekapasiteit het afgeneem bo die pKa van die sure vir die res van die ekstraheermiddels ondersoek. TOA-lampolie en TOPO-kanola-olie het 10 — 25% van totale VFA (tVFA) by pH 5.6 — 6.8 geëkstraheer, wat voorstel dat daar oplosmiddels is met die kapasiteit om sure te ekstraheer binne gepaste pH-bestekke vir biogas produserende AD, wat tipies bo die pKa van die geëkstraheerde sure is. Meeste oplosmiddelkombinasies, met die uitsondering van [$P_{666,14}$][Phos], het soortgelyke of selfs verbeterde VFA-ekstraksies van afvalwaterstelsels getoon, wat hul potensiaal vir toepassing in nie-ideale stelsels beklemtoon.

Biogasproduksie eksperimente op banktoetsskaal wat industriële afvalwater gebruik het gedemonstreer dat bioversoenbare ekstraksiemiddel-oplosmiddelstelsel koproduksie van biogas en VFA’s, met verbeterde biogasproduktiwiteit in sekere gevalle, toelaat. Stelsels wat TOA-olielalkohol, TBP-olielalkohol, TOPO-olielalkohol, TOPO-kanola-olie en [$P_{666,14}$][Phos]-olielalkohol bevat, het twee tot vyf keer meer biogas geproduseer as die kontrole met gemiddelde metaanpersentasies van tussen 70 en 75% (in vergelyking met 55% bereik met die kontrole) en analoë produksie is waargeneem toe TOPO-lampolie en TOA-lampolie gebruik is relatief tot die kontrole. Die teenwoordigheid van Aliquat 336 het minimale gasproduksie tot gevolg gehad ongeag die verdunner wat gebruik is, en word daarom nie voorgeskryf vir toepassing in biogasproduserende AD nie.

Totale terug-ekstraksie VFA-herwinning van 80 — 100% is bereik van TOPO, TBP, TOA en [$P_{666,14}$][Phos] deur $NaOH_{(aq)}$ te gebruik om VFA’s te herwin en die oplosmiddel te regenereer. Aliquat 336 het laer potensiaal

getoon vir terug-ekstraksie met herwinning tussen 40 en 50 %. Terug-ekstrahering met oplosmiddels wat kanola-olie bevat word nie voorgestel nie as gevolg van waargenome emulsifikasie in hierdie stelsels.

Die eksperimente dui breedweg aan dat dit moontlik is om ’n bioversoenbare oplosmiddelkombinasie te kies wat gebruik kan word in AD met die vermoë om biogas en VFA’s te koproduseer, en selfs produktiwiteit in biogasproduserende verteringstelsels te versterk. Hierdie metodologie kan geïntegreer en gebruik word as ’n pH-beheerstrategie terwyl bestuur en reduksie van afval, hulpbronherwinning, en gebruik van hernubare energie, bevorder word. TOA-olielalkohol, TOA-lampolie, TOPO-olielalkohol, TOPO-lampolie en TBP-olielalkohol word voorgestel vir verdere ondersoek as potensiële oplosmiddels vir *in situ* VFA-ekstraksie van biogasproduserende AD-afvalwaterbehandelingstelsels.

ARTICLES ARISING FROM THIS WORK

An article titled “Extraction of volatile fatty acids from wastewater anaerobic digestion using different extractant-diluent mixtures”, under revision in *Bioresource Technology*, delayed due to potential patenting.

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NOMENCLATURE

Acronyms and symbols	
AD	Anaerobic digestion
$[A^-]$	Concentration of dissociated acid
BMP	Biochemical methane potential
C/N	Carbon to nitrogen ratio
COD	Chemical Oxygen Demand
E%	Degree of extraction
[HA]	Concentration of undissociated acid
HRT	Hydraulic retention time
IL	Ionic Liquid
ISR	Inoculum to substrate ratio
K_D	Distribution coefficient
LLE	Liquid-liquid extraction
OLR	Organic loading rate
R%	Percentage Recovery
SRT	Solid retention time
S/F	Solvent to feed ratio
[TA]	Concentration of total acid
tVFA	Total volatile fatty acids
TS	Total solids
VFA	Volatile fatty acid
VS	Volatile solids
WWTF	Wastewater treatment facility

Subscripts and superscripts	
aq	Aqueous phase
b	Blank test
eq	Equilibrium
i	Initial
Ib	Inoculum present in the blank test
Is	Inoculum present in the sample test
o	Organic phase
i	Initial
s	Test sample
Sb	Substrate present in the blank test
Ss	Substrate present in the sample test

CHAPTER 1
INTRODUCTION

With continued growth in human populations and expansion of economies worldwide, global waste generation rates are rising. Sustainable waste management is necessary to minimize environmental degradation and to transition into a restorative and regenerative economy. Conventional waste management approaches are traditionally treatment orientated, with focus on meeting environmental regulatory classifications. This approach often neglects the potential of diverting waste streams and utilising components as feedstocks to produce added-value chemicals. Resource recovery from waste sources facilitates the simultaneous minimisation of waste and generation of valuable products [1,2]. Biomass and waste have been recognised as prominent future renewable energy sources due to their capability to generate energy and provide continuous power generation, with benefits of reduced dependence on fossil-orientated energy and a shift towards a circular economy [3–7]. Anaerobic processes can be used for the treatment of wastewater, as well as solid wastes, while presenting opportunities for the recovery of resources from waste streams [2,8].

During anaerobic digestion (AD), a series of microbial transformations take place, where organic material is converted to volatile fatty acid (VFA) intermediates and methane-bearing biogas through the action of a consortia of microorganisms in the absence of oxygen [2,9]. With the growing demand for alternative sustainable energy sources, AD has attracted increased attention as a process option for bio-based energy generation [4,10–12]. VFAs are valuable short chain monocarboxylic fatty acids with six or fewer carbon atoms, which are not widely recovered as products in biogas plants. These acids are considered platform chemicals that can be converted to a broad range of chemicals and materials [13,14], with applications in chemical fabrication fields, wastewater nutrient removal processes, bioenergy, pharmaceutical, and food and beverage industries [3,15–18]. Presently, commercial VFA production is primarily achieved using non-renewable petrochemical feedstocks. Therefore, it would be opportune to extract and recover VFAs formed during organic degradation processes, such as AD, as an alternative renewable option to fossil-derived carbon sources [2,19].

Bio-based VFA production from waste by acidogenic fermentation has recently drawn research interest as a promising option for resource recovery [1,2,8,16–18,20–26]. Most of these studies have considered waste derived VFA recovery through AD with the inhibition of methane production. Very little work on simultaneous co-production of biogas and VFAs has been presented in literature. This study, therefore, aimed to investigate the extraction and co-production of excess VFAs produced in AD without arresting biogas production.

In a typical AD process, fermentative bacteria metabolize organic molecules to produce VFAs during acidogenesis and acetogenesis, which serve as a carbon source for biogas-producing methanogenic bacteria [27]. The interactions between the organisms, the feedstock, and the intermediate compounds are complex, and from time to time there is an overabundance of VFAs produced [3,9,28–31], resulting in the pH of the

digester drastically decreasing. Acid accumulation and pH fluctuations adversely affect the microbial cultures, causing inhibition or death, and subsequent reduced digester performance [9].

The occurrence of "acid-crash", i.e. when VFAs accumulate in AD systems resulting in reduction of pH below the optimum range, which directly inhibits methanogens, is common. There is, therefore, a need to control VFA levels and the system pH within active AD systems. Industrial biogas producers go to great lengths to control their systems within the optimal pH range, often at a significant expense. A commonly applied method of pH control in commercial AD systems involves the addition of a base, which leads to the costly consumption of reagents. Additionally, feed streams are frequently halted or decreased to afford methanogenic bacteria time to consume the VFAs [30], which can result in waste treatment backlogs and decreased biogas production. Removing excess VFAs from the digester is a possible alternative option for pH adjustment, allowing tighter control of acid concentrations and aiding system stability.

Fermentation systems are complicated in both chemical composition and in fluid properties, and VFA concentrations attained in the fermentation broths are typically low due to inhibition caused by the acid products [15,32]. Various techniques have been applied for the recovery of organic acids from fermentation broths, among which, liquid–liquid extraction (LLE) has been recognised as an efficient, economical and environmentally friendly method for separation of carboxylic acids [33,34]. The LLE approach exhibits promising potential due to its success in the separation and removal of acids from dilute aqueous waste streams [2,23,31–33,35,36]. However, the majority of reported VFA extractions are conducted post-fermentation, which means they offer limited leverage in controlling the system pH. The simultaneous separation and *in situ* extraction of acids produced during fermentation processes has been proposed as a feasible solution to overcome the inhibitory effects of acid production [3,13].

Most studies which have investigated VFA production from AD systems have proposed the inhibition of methanogens to suppress biogas production and enhance acidification [2,17]. However, the accumulation of VFAs and subsequent lowering of the system pH in AD biogas plants could potentially be prevented through continuous *in situ* VFA extraction by removing excess VFAs as they are formed, which could enhance biogas production while providing benefits of increased digester loading capacity and the recovery of valuable VFAs. Increased productivity of bioreactors used for carboxylic acid production has been demonstrated using continuous *in situ* removal of acids as they are produced [2,15,37–41]. Despite this benefit, the *in situ* extraction and recovery of VFAs from fermentation systems is not common practice [2]. This study aimed to investigate the potential of integrating LLE in AD systems for the co-production of VFAs to enhance the overall performance of biogas plants.

To establish an appropriate *in situ* LLE system, an extractant that has a selectivity for VFAs needs to be identified to maximise extraction efficiency [35,42]. Organophosphates such as trioctylphosphine oxide (TOPO) and tri-n-butyl phosphate (TBP), and aliphatic amines including trioctylamine (TOA) have been found

to be more effective extractants for the extraction of organic acids in comparison to traditional solvents [3,33,35]. Ionic liquids, such as trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate ([$P_{666,14}$][Phos]) and Aliquat 336 have also been reported for extraction of VFAs, with superior extraction efficiency compared to conventional solvents [32,35,43].

The pH of the system plays an important role in carboxylic acid extraction, especially when simultaneous fermentation and extraction are to take place within the same system. To maintain consistently high methanogenic activity, fermentations at pH 6.5 to 7.2 are usually preferred [16,27], which are substantially higher than the pH levels ideal for LLE [44], where most solvents function best at a pH value much lower than the pKa value of the organic acid [15,34,45,46]. Therefore, for *in situ* VFA recovery that serves to simultaneously control the pH of the system, recover VFAs, and allow biogas production, it is necessary to select an extractant capable of extracting acids at pH values within the functional range for biogas-forming AD, even if at a pH value greater than the pKa of the acids being extracted, as well as lower pH values when AD systems may experience overproduction of VFAs.

Solvent toxicity to the microorganisms presents an additional challenge to *in situ* LLE. Reports suggested that solvents with high extraction capacities tend to also be toxic to consortia essential in fermentation processes [15,33,47]. Consequently, biocompatibility is a key factor for *in situ* removal of VFAs from AD systems and the selection of extractant and diluent for extractive fermentation should be done based on minimal toxicity and maximum capacity.

Finally, the success of extractive fermentation as an economical process lies in complete recovery of acids from the organic extract phase so that the solvent can be regenerated and recycled back to the LLE [15]. To regenerate the extraction solvent, the reversal of the reaction to recover the acids into the solvent phase needs to be possible. Back extraction, a low-energy solvent regeneration method, was considered in the present study, where acids are stripped out of the solvent into an alkaline product phase, and the acid-free solvent can then be recycled.

This investigation aimed to compare different extractants and diluents for their application in *in situ* VFA extraction and recovery from biogas-producing AD systems. Five extractants and three diluents were studied based on (i) extraction capacity at varying pH, (ii) biocompatibility with the methane-producing consortium and (iii) feasibility of VFA back-extraction. This was achieved through the use of (a) laboratory scale LLE experiments using aqueous solutions containing dilute VFA concentrations at varied pH ranges and wastewater from an AD plant (b) bench-scale biogas production tests to determine whether bacteria could continue to produce biogas in the presence of the solvents over a period of time, and (c) back-extraction of the solvents using sodium hydroxide to recover the extracted VFAs. These results were used as a basis for the selection of potential extraction solvents for use in continuous *in situ* LLE operation.

CHAPTER 2
LITERATURE REVIEW

Waste management is a crucial element of sustainable infrastructure which is regularly rated within the top three primary issues that need to be addressed by developing countries. With a direct impact on many aspects of society, the economy and the natural environment, handling of waste should be seen as a global concern and a political priority [48]. Rapidly increasing amounts of generated waste are becoming progressively more difficult to manage. This poses challenges in the disposal of municipal, agricultural and animal wastes, as well as the treatment of municipal and industrial wastewater. An estimated seven to ten billion tonnes of waste was generated worldwide in 2010, and it has been predicted that cities in developing nations (such as Africa and Asia) are expected to double their municipal waste generations within the next 20 years due to continuous population growth, urbanisation and economic development [48].

To achieve environmental sustainability, reductions in the consumption of raw materials and the generation of waste materials, are required. This can be achieved through the transition into a circular economy, with the development of resource recovery techniques, reuse and recycling [2]. Many waste disposal routes utilise landfills, which eliminates the potential for resource recovery [26] and presents several environmental challenges such as leachates, groundwater and soil contamination, and generation of greenhouse gases [20,49,50]. An alternative approach to landfilling involves the conversion waste materials into practical forms of energy using waste-to-energy techniques [51]. Various chemical and/or thermal waste-to-energy techniques such as incineration, gasification and pyrolysis have been employed to reduce and manage increasing amounts of biowaste, but are often energy intensive (particularly for high-moisture waste) and result in secondary impacts such as air pollution and subsequent environmental and health effects [29]. Great lengths have been taken to establish green technologies for converting wastewater treatment sludge into a renewable resource for bioenergy recovery, with difficulty due to the high moisture content of the biomass. Biological processes that utilise organic wastes as feedstock in aqueous environments could be used as an alternative to thermal techniques for the production of biofuels and bio-based products [26,52]. Anaerobic digestion (AD) is a biological process that can convert organic biomass to bioenergy while stabilizing waste [20,52,53], which has been proposed as an environmentally feasible and economical waste treatment alternative to landfilling and incineration [18].

2.1 Anaerobic digestion

Anaerobic digestion (AD) is a mature bioprocess technology where microbes metabolise and degrade biodegradable organic materials in an oxygen-poor environment. This disposal route has the ability to treat various types of waste with high biological pollution loads, including liquid and solid organic wastes, such as industrial wastewater, municipal solid waste (MSW), sewage sludge, agricultural and animal wastes [29,52]. The microbial decomposition of organic materials in AD breaks down waste matter, which results in the reduction of solids, stabilises suspended organic material, reduces pathogens and controls odours. AD can therefore reduce the operational cost of sludge disposal for sanitation services by using organic waste as a process input [27,54,55] and can play an important role in supporting modern Wastewater Treatment Facilities (WWTF) to meet water nutrient removal legislation standards and overcome eutrophication problems in receiving waters, by reducing pollution levels through the breakdown of organic material [3,55,56]. Additionally, in comparison to leaving the organic matter untreated or directly combusting biomass, AD reduces the emission of greenhouse gases [29,53,57], particularly methane and nitrous oxide which have 25 and 298 times more global warming potential than carbon dioxide respectively [52].

For decades AD has performed well primarily for waste treatment and stabilisation [7], however, the process yields additional valuable outputs. The main products of AD are biogas and digestate, shown in Figure 1. The effluent material that has been digested in AD processes is nutrient-rich, containing mineralised nitrogen and phosphorus, which can be used in agriculture as organic fertilizer and compost for agronomic benefits. Biogas is a mixture of gases composed mostly of methane (typically in the range of 50-75%), carbon dioxide and a small proportion (<1%) of hydrogen, which has a high calorific value and can be recovered as a source of renewable bioenergy [3,27,29,52]. The biogas produced is normally burned in a cogeneration unit to generate heat and power to maintain optimal operating conditions for the digestion process [54], and can be upgraded to be used as a fuel source for various other applications [52].

Biogas production from AD as a by-product of the treatment of various types of waste demonstrates immense potential for energy generation, with varying net energy capacities between 20 to 335 kWh per ton of waste reported [29], depending on the type of waste utilised. With the growing demand for substitute energy sources, interest in bio-based renewable energy technologies for bioenergy production as an alternative to fossil fuels has been steadily increasing over recent years [52,54]. As a result, there has been increased investment in AD for biogas production as a practical, energy efficient way of recycling organic bio-wastes and generating biofuel, bio-electricity and heat [7,54,58], and AD has been emerging in application for organic waste treatment, as well as continuous energy production, with an annual growth rate of 25% during recent years [7]. Although biogas technology has been predominantly deployed in Europe, with more than 10 000 active digesters and more than 500 biomethane installations in operation by the end of 2018, the use of AD to generate electricity and heat is expanding extensively to more countries across North and South America, Asia,

Philippines and the Middle East [51]. Within the South African context, it has been highlighted by the Department of Environmental Affairs that waste-to-energy treatment processes such as AD need to be further explored to promote diversion of organic wastes from landfills [59].

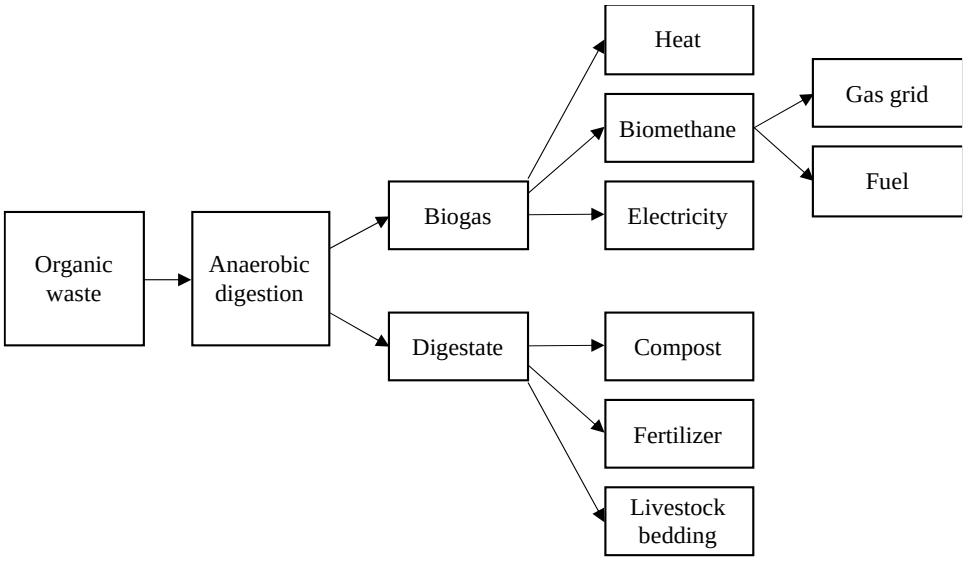


Figure 1: *Digestion of organic material for waste reduction and energy recovery (modified from Rabii et al. (2019)).*

As noted by Rabii et al. (2019), application and integration of anaerobic digestion for waste treatment can indeed lead to goals of waste reduction, integrated waste management and utilisation of renewable energy. However, a series of biological processes are involved during AD, which are influenced by various factors. These processes and their governing factors require consideration in the application of AD for waste treatment and biogas production.

2.1.1 Process overview

Anaerobic digestion is a multidimensional process that depends on the coordinated activity of communities of microorganisms to metabolize organic material through carboxylic acid intermediates to produce biogas [2,9,29]. Acting through a series of microbiological processes, diverse types of bacteria and archaea complete different tasks in four main successive phases. These four stages (illustrated in Figure 2) include hydrolysis, acidogenesis, acetogenesis and methanogenesis [17,27,28].

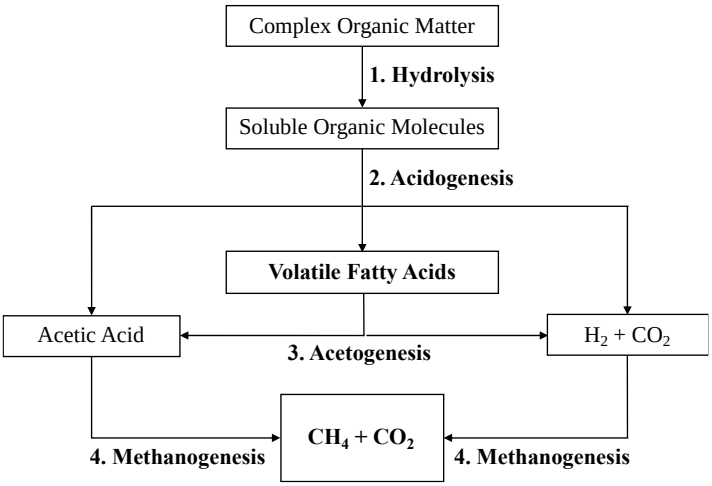


Figure 2: *Main phases of anaerobic digestion process (modified from Appels et al. (2008)).*

Hydrolysis is generally considered the rate limiting step of the AD process and involves the degradation of insoluble organic material and high molecular weight compounds into simpler soluble organic substances. Complex molecules such as lipids, proteins, polysaccharides and nucleic acids are hydrolysed into smaller organic compounds such as glucose, amino acids and long chain fatty acids (LCFAs) [27,29,50,52]. The components formed during hydrolysis are further broken down during the second phase, acidogenesis. Fermentative acidogenic bacteria facilitate the formation of VFAs (composed of mainly C2-C6 carboxylic acids, which may include acetic acid (C2), propionic acid (C3), butyric acid (C4), valeric acid (C5), caproic acid (C6) etc.) along with other by-products such as ammonia (NH_3), carbon dioxide (CO_2), hydrogen sulphide (H_2S) and alcohols. Acetogenesis is the third stage in AD, where the higher organic acids and alcohols produced by acidogenesis are further digested by acetogens to produce acetic acid and hydrogen (H_2). This conversion is controlled largely by the partial pressure of H_2 in the mixture [20,27,29,52]. Syntrophic bacteria oxidise higher chain fatty acids to acetic acid, H_2 and CO_2 [60] and homoacetogens utilise H_2 and CO_2 to produce acetic acid [16]. The final stage of the AD process is methanogenesis, where biogas is produced by two groups of methanogenic bacteria. The first group splits acetate into methane (CH_4) and CO_2 and the second group uses H_2 as an electron donor and CO_2 as an electron acceptor to produce CH_4 [4,20,27]. The stability of the AD process is contingent on the crucial balance between the symbiotic growth of these principal groups of acid forming bacteria, obligate hydrogen producing acetogens and methane producing methanogens [61].

2.1.2 Instability

Despite the numerous advantages of AD, there are inevitable limitations in the process. AD is an intricate sequential chemical and biochemical process with many factors that can affect its performance [30]. The microbiology is complex and delicate, involving several groups of bacteria and archaea. Each of the microbial groups has its own favoured growth conditions and can be highly sensitive to changes in process parameters

that could lead to an unsuitable environment, especially in the case of the methanogenic organisms. Factors that typically affect performance include pH, alkalinity, temperature and substrate characteristics, such as the amounts of volatile solids (VS), carbon to nitrogen (C/N) ratio, total solids (TS), concentration of other nutrients, organic loading rate (OLR), ammonia, and VFAs [27,29]. These parameters can be inhibiting to some or all bacterial groups [27] and it is, therefore, important to control and balance these factors in the AD process design to maximise productivity and ensure efficient operation [28].

A wide variety of inorganic and organic substances, which are either present in the digester substrate or are generated during digestion, have been reported to inhibit AD processes. Inhibition is usually evident from a decrease in the microbial population and methane production, the disappearance of hydrogen, the accumulation of VFAs and a lowered pH [30]. Monitoring the behaviour of the AD system is thus essential to control the process and optimise the breakdown of sludge. Biogas production and pH are traditionally monitored in most AD processes to facilitate process control because of the relative ease with which these parameters can be monitored on a routine basis. However, significant disturbances in pH and biogas production are generally only detected when the process has become severely unbalanced [62].

The microbial conversion of carbohydrates produces VFAs, which are important metabolic intermediates that govern the stability of the AD process. It has been recognized that levels of organic acid are important in digestion because VFAs (particularly acetic) are immediate precursors in the metabolic chain leading to methane formation [63]. However, at high concentrations, acids are known to cause stress in the microbial population. Under anaerobic conditions VFAs are degraded by proton-reducing acetogens in syntrophic association with hydrogen consuming methanogens [9]. A sufficient balance between the rates of hydrolysis, acetogenesis and methanogenesis is essential for continuous methane production, with rapid methanogenesis required to prevent accumulation of organic acids [29]. Fermentative bacteria tend to grow faster than methanogenic bacteria, resulting in the kinetic uncoupling between the acid producers and consumers, and subsequent greater relative VFA production rates [9,30,31]. Changes in VFA concentration can also be in response to variations in temperature, organic loading rates or the presence of toxicants [9].

When the VFA production rate exceeds the methanogenic VFA utilisation rate, methanogens are unable to remove the hydrogen and organic acid fast enough, and acids begin to accumulate in the system over time [28]. Subsequently, the methanogens are unable to counter the production of VFAs by making the environment more alkaline [27], causing the pH of the system to naturally decrease. Each group of micro-organisms has a different optimum pH range in the AD process. There is a strong pH limitation on methanogenesis [64] where methanogenic bacteria are inhibited at low pH values (with irreversible inhibition reported at around pH 3.3) resulting in little or no methane gas production [65]. The optimum pH range for the growth of methanogens lies between pH 6.5 and 7.2 [16,27,29], whereas the fermentative microorganisms are somewhat less sensitive and can function in wider pH ranges between pH 5.25 and 11 (most acidogens cannot survive in extremely

acidic environments below pH 3 or in alkaline environments above pH 12), depending on the type of waste used [1,29].

Excessive VFA concentrations in anaerobic systems are a leading cause of process failure due to a reduction in pH below the optimum range, which directly inhibits methanogens [63]. The system pH influences the reaction kinetics and impacts the enzymes and configurations of microorganisms. As the pH lowers, the methanogenic activity and VFA utilisation kinetics decrease, further advancing VFA accumulation and inhibiting methane production. This phenomenon is commonly known as “acid crash”. A narrow operating pH range of between pH 6.5 to 7.6 is, therefore, usualy recommended to avoid inhibition of digestion [28].

While pH fluctuations are known to inhibit methanogens, VFA accumulation and related inhibition of methanogens are not exclusively caused by decreased pH. In systems with high buffering capacity and minimal resultant pH fluctuations, the accumulation of VFAs has still resulted in methanogenic inhibition. One such case includes the partial inhibition and delayed methane production reported as a result of VFA accumulation with anaerobic co-digestion of swine manure and winery wastewater, where in spite of VFA accumulation, the pH was maintained within a range close to 7 due to the buffer capacity of the swine manure [66].

When inhibition of methanogenic bacteria persists, acetogens begin to predominate in digesters [63], which leads to another obstacle faced in carboxylic acid fermentation, namely end-product inhibition. Under these conditions acid-producing bacteria are inhibited by their acid products [13,37,39,67]. Accumulation of VFAs over time in AD systems can therefore be extremely detrimental to the microbial community and system performance, from inhibition of methanogenic as well as fermentative microorganisms, which in turn results in repressed biogas production and can ultimately lead to complete digester failure [27,50].

VFAs could therefore be more widely used as indicators of process imbalance and can be treated as a monitoring parameter, where the accumulation of VFAs illustrates an early warning sign to detect process disturbances and digester upset [68]. Mechichi and Sayadi (2005) confirmed VFA accumulation as a sign of anaerobic digester imbalance, observing a decrease in biogas production and methane yield with an accumulation of acetate, propionate, butyrate and valerate. While acetic acid is a key substrate for methanogenesis, propionic and butyric acids have been reported as inhibitory to methanogenic bacteria, and appropriate regulation of acids has been shown to stabilize the overall AD system [63]. A number of observations have been made regarding the level and ratio of organic acids and the correlation of these relationships with anaerobic digester performance, seen in Table 1.

Table 1: *Inhibitory VFA concentrations reported in literature for AD.*

Observation	Source
Acetic acid levels > 800 mg/L or a propionic to acetic acid ratio > 1.4 indicative of impending digester failure.	[69]
VFA concentrations between 6.7-9.0 mol/m^3 reported toxic to microorganisms, resulting in acid accumulation, pH reduction and inhibition.	[27,67]
Propionic acid concentration of 900 mg/L resulted in significant inhibition with reduced methanogenic activity and low methane yields.	[70]
Total VFA concentration < 500 mg/L as acetic acid in a well-designed and operated digester. VFA concentrations > 1500 to 2000 mg/L, could inhibit biogas production.	[71]
Maximum VFA concentrations for stable AD performance reported at 13 000 mg/L.	[63]
Acetic acid concentration of 2400 mg/L and butyric concentration of 1800 mg/L did not inhibit methanogens, but propionic acid concentration of 900 mg/L caused significant inhibition.	[72]

Regardless of the system-specific VFA concentration that onsets digester imbalance, there is certainly a correlation between VFA levels and digester performance, and a need to control VFA concentrations within active AD systems. In addition to pH and biogas production, which are traditionally measured, the continuous monitoring of VFAs could be used to regulate the digester performance and evaluate the system stability to provide a more accurate overview of the digester performance [62]. Through monitoring and controlling the VFA concentrations, the necessary operational changes can be made before the onset of digester failure. This study aimed to to address the viability of applying this strategy in biogas-producing AD.

2.1.3 Process control

Until recently, research has mainly been focused on the methane-production phase of the AD process. Fewer studies have been focused on the acid production phase of the process and less attention has been paid to the recovery and reuse of fermentation permeates such as VFAs, while still producing biogas. Most studies which have investigated VFA production from AD systems have proposed the inhibition of methanogens to suppress biogas production and enhance acidification [2,17]. However, there could be potential for integration of VFA extraction to enhance the overall performance of biogas plants while simultaneously co-producing VFAs.

Operational factors which influence biogas production and AD performance include inoculum to substrate ratio (ISR), pH, solid retention time (SRT), hydraulic retention time (HRT), temperature, pre-treatment, digester mixing and digester mode [29,50]. Many of these factors are taken into consideration in the digester design for treatment of specific types of waste and some are continuously controlled throughout the digestion process to ensure stable operation, particularly when there are fluctuations in environmental factors. Ammonia and VFA build-up in the digester are regulated by selecting a substrate with appropriate carbon/nitrogen (C/N) stoichiometry (the relative amount of organic carbon and nitrogen in the feedstock), which in turn influences

the system pH. An optimum C/N ratio in the range of 20-30 has been established to ensure adequate nitrogen and organic carbon for anaerobic microbes to grow, where lower C/N ratios can result in increased system pH and higher C/N ratios can result in rapid conversion of nitrogen and low biogas production [63]. However, when a biogas plant is required to treat a variety of substrates, controlling the C/N can be challenging. In addition to controlling the C/N ratio of the substrate, there are two main strategies commonly employed in industry for ensuring stable biogas production and correcting the low pH of AD systems to treat waste. These include allowing the methanogenic population time to reduce the concentration of VFAs by stopping the feed (increasing the retention time), and the addition of a base to raise the pH and provide additional buffering capacity to the system [30]. Stopping or reducing the influent feed rate results in decreased capacity for energy generation from the AD plant and prolongs the duration of waste treatment, which can lead to increased operational costs. Acid neutralisation to adjust the system pH has the drawback of large consumption of chemicals (such as sodium hydroxide or lime) and the formation of a waste salt sludge which requires disposal, both of which result in increased plant operating costs.

An alternative method of pH control and reduction of VFA accumulation in AD systems could be through the removal of excess VFAs from the digester. The prevention of acid accumulation through acid extraction could provide an alternative to the current practice of acid neutralisation, and enable the plant to handle larger loads without needing to reduce the frequency of influent pumping. The pH of the AD system and the VFA concentration could be maintained continuously throughout the AD process through the removal of excess VFA intermediates before they accumulate, while concurrently acquiring an economically valuable product and lowering plant operating costs. It was noted by Wu *et al.* (2016) that free pH control anaerobic fermentation may be an economically feasible method for preparing VFAs, with lower production costs and reduced operational complexity. This study, therefore, aimed to investigate the extraction of excess VFAs produced in AD to enhance the performance of biogas plants.

2.1.4 Biochemical methane potential tests

Although the application of AD technology is expanding, the high complexity of anaerobic degradation as a dynamic system (where biochemical, microbiological and physio-chemical aspects are interconnected) needs to be considered [61]. Biochemical methane potential (BMP) tests are a well-known technique to determine the methane potential and biodegradability of wastewater and waste biomass. These tests are used extensively for characterising a substrate's influence on the anaerobic digestion process and have become an important tool for the investigation of different digestion treatment options [28]. Methane productivity, a key output of BMP tests, has been widely used as a parameter in determining digester performance [31,62,69].

The conventional method involves the incubation of substrate material inoculated with anaerobic bacteria retrieved from an active digester for between 30 to 60 days, with regular monitoring of biogas production and its methane composition [73]. A higher fraction of inoculum than that of substrate in the test mixture is

recommended to ensure provision of nutrients, vitamins, trace elements, pH-buffering capacity and prevention of volatile fatty acid accumulation [61,73]. Consequently, the inoculum to substrate ratio (ISR), the ratio of volatile solids (VS) (or chemical oxygen demand (COD)) from the inoculum to VS (or COD) from the substrate, is a key parameter of BMP tests. For most applications, the recommended ratio is between two and four [61,73].

Mixing can be an important parameter for consideration in BMP determination and kinetic studies, as it facilitates contact between the microorganisms and the substrate, ensures distribution of nutrients, and prevents sedimentation of particulate materials and accumulation of intermediate materials [61]. It has been reported that manual mixing once a day is sufficient for BMP tests, especially when the digester content is easily degraded [28,73] where mixing can be facilitated by turning vessels up and down [61].

The methane content of the biogas produced is measured and used to determine the methane potential of the substrate. BMP is defined as the volume of methane produced per amount of organic substrate material added to the reactor (which is expressed per mass of volatile solids or COD added) [100], where the background methane production from the inoculum (determined from blank assays with medium and water, with no substrate) is subtracted from the methane production obtained in the substrate sample bottles [61].

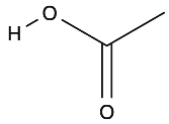
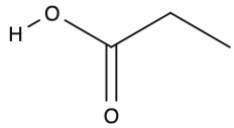
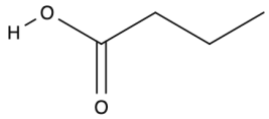
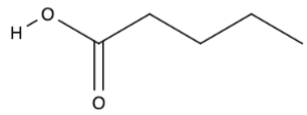
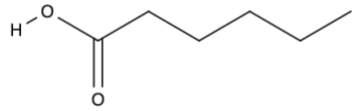
Determination of the BMP and organic load of the feedstock materials provides insight into the design parameters for anaerobic digesters [73] and is often necessary for the determination of various components (such as size and biogas output) for full-scale digestion plants [28]. The use of BMP tests is therefore important for both research and biogas plant management [74], and provides a useful tool for gaining insight into the dynamic, complex processes involved in anaerobic degradation.

2.2 Volatile fatty acids

Volatile fatty acids (VFAs), also referred to as short-chain fatty acids (SCFAs), are monocarboxylic fatty acids with six or less carbon atoms, illustrated in Table 2. VFAs are generally considered platform chemicals, which can be converted into a wide array of chemicals and materials for several manufacturing and bioenergy industries [13,14,17]. VFAs can be applied for the synthesis of complex polymers, additives and fertilizers, as well as serving as precursors to biofuels and chemical productions [18,22]. These versatile carboxylic acids are critical substrates for microorganisms involved in biological nutrient removal processes for wastewater treatment [17,18]. Waste-derived volatile fatty acids have been utilised for the production of biodegradable plastics, hydrogen, biodiesel and bioelectricity by way of microbial fuel cells and biogas production [1,2,17,19,23,32]. Through implementation of suitable methods, VFAs can be utilised as building blocks of various compounds such as alcohols, aldehydes, ketones, esters, alkanes, olefins, polyhydroxyalkanoates, (PHA), microalgal lipids and biohydrogen, and also find direct usage as additives in various products

[16,17,20]. Consequently, VFAs find diverse and extensive applications in chemical fabrication fields as well as the pharmaceutical, food and beverage, textile and leather industries [2,3,15,18,20]. Table 2 summarises the market size and potential applications of selected volatile fatty acids.

Table 2: *Market size, indicative prices and potential applications of individual volatile fatty acids.*

Volatile Fatty Acid	**Market Size (tonnes/year)**	**Market Price ($/ton)**	**Use/Application**
Acetic acid 	3,500,000 [3,20]	400 - 800 [3,20]	Food additives, plasticisers, dyes, polymers, adhesives, solvents, ester production [3,17]
Propionic acid 	180,000 [3,20]	1,500 - 1,700 [3,20]	Pharmaceuticals, resins, paints, food additives, chemical intermediates, solvents, flavouring agents [3,17]
Butyric acid 	30,000 [3,20]	2,000 - 2,500 [3,20]	Perfumes, textiles, varnishes, plastics, food additives, flavouring, pharmaceuticals, animal feed supplements [3,17]
Valeric acid 		2,500 - 3,000 [75]	Perfumes, plasticisers, lubricants [17]
Caproic acid 	25,000 [3,20]	2,250 - 2,500 [3,20]	Rubber, grease, tobacco flavour [17]

Atasoy *et al.* (2018) reported that the total global market demand for acetic, butyric and propionic acids will be approximately 18 500 kilotons in 2020, with growing compound annual growth rates for these acids. If harvested effectively, VFAs can enhance the environmental sustainability and economic viability of the AD process [16]. The provision of these value-added products from AD has the potential to enhance local and national economies through additional revenue generated through VFA and biogas sales, while reducing waste generation [56]. Various benefits of AD with VFA co-production are summarised in Figure 3 below.

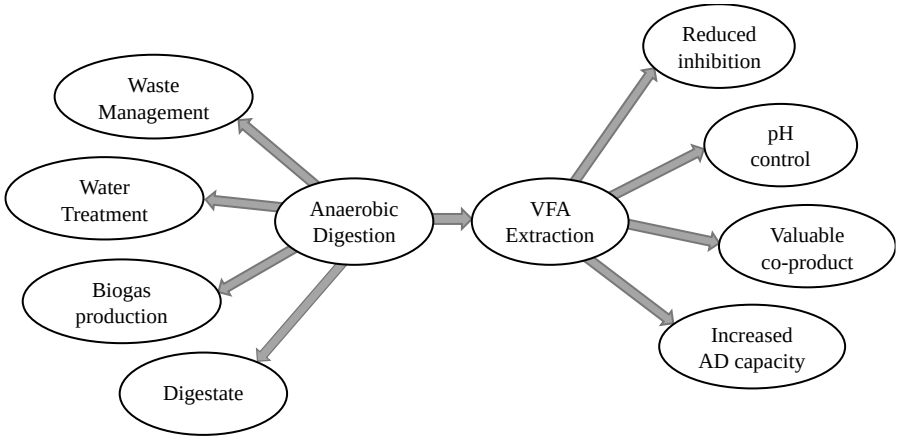


Figure 3: *Combined benefits of anaerobic digestion with VFA co-production.*

2.2.1 VFA production

As outlined in Section 2.1.1, VFAs are essential intermediates produced during acidogenesis and acetogenesis when organic materials are degraded by anaerobic digestion [18]. These valuable compounds which are produced in AD technologies are, however, not widely recovered as products in biogas plants. At present, commercial VFA production is accomplished predominantly by chemical routes based on non-renewable petrochemical feedstocks [2].

Preceding the development of petrochemical based VFA production, VFAs were customarily produced by fermentation methods, through either the microbial oxidation of ethanol or the anaerobic fermentation of hexose sugars or starch. These processes occur in aqueous solutions and traditionally require energy intensive separation techniques using distillation to recover the VFAs. Lower energy fermentation alternatives were investigated, but challenges were faced with more complex processing methods and lack of process robustness. Microbial fermentation processes were consequently superseded by oil-based VFA production processes, which are accomplished in the gaseous phase in the absence of water, thereby avoiding the significant energy costs related to removing water from the acid products [3]. Subsequently, VFAs have been predominantly produced from petrochemical feedstocks with significant greenhouse gas (GHG) emissions.

Environmentally benign biological recovery routes, such as AD, could provide a promising alternative to oil-based VFA production processes [2,19]. With a growing demand for VFAs, research has been refocused on developing alternative, more sustainable methods of VFA production due to the scarcity and rising costs of global petroleum resources, as well as the increasing awareness of the environmental impact of energy-inefficient processes [76]. There has been a renewed interest in fermentative processes, which can be restorative and regenerative by design, as a renewable alternative for VFA production [2]. Substrates derived from solid and liquid wastes from agricultural sources, as well as complex effluent streams from municipal and industrial wastewaters can be utilised for bio-based production of VFAs via AD, representing an alternative source of renewable carbon-based chemicals while valorising waste streams [2,3,15,19]. Effluent streams containing significant amounts of VFAs are currently treated to meet water quality standards and to reduce environmental pollution [27]. VFA recovery from AD systems could therefore have a positive impact on waste management through utilisation of organic carbon existing in waste products and providing relief to municipal treatment plants through the removal of excess VFAs.

The synthesis of these value-added chemicals using downstream recovery techniques as an economically viable process is, however, not straightforward due the challenges faced in the separation and purification of VFAs. Until now, the economic impact of fermentation chemicals remains limited largely due to the high cost and the difficulty of product recovery [77]. While bio-based VFA production is a budding way for resource recovery from waste streams, additional research and development is required to enable sustainable and economically feasible implementation of suitable recovery methods [2]. Despite the challenges faced, fermentative routes are still considered potentially viable alternatives to replace petroleum-based VFA productions due to the many advantages that biological processing routes offer [3,13,35].

2.2.2 VFA recovery

For VFA products produced via fermentative routes to penetrate the organic chemicals industry, substantial improvements in the existing recovery technology are needed [42]. AD streams are complicated in terms of chemical composition and fluid properties, which makes VFA recovery technically and economically challenging [15,32]. Various downstream processing methods have been investigated based on the physicochemical characteristics of streams to overcome these obstacles. Recovery methods, such as solvent extraction, distillation, absorption, adsorption, electrodialysis, nanofiltration, the use of membrane bioreactors, liquid surfactant membrane extraction, reverse osmosis, direct distillation, gas stripping, precipitation and ion-exchange have been explored for the recovery of organic acids from fermentation broths [2,3,33,35]. All of the processes have their own advantages, but also have significant shortcomings and limitations for feasible VFA recovery. An additional challenge faced in the commercialisation of VFA production using anaerobic bacteria is the low VFA concentration (<10%) attained in the fermentation broth due to inhibition caused by acid products and the high affinity of VFAs to water [13,42,44]. For this reason, energy efficiency is a critical

factor for the extraction of VFAs from complex streams and separation techniques that attempt to recover VFAs by directly removing the water fraction of fermented wastewater are not economical [15,32,35].

A conventional method of carboxylic acid recovery from fermentation broths is by calcium hydroxide precipitation. The precipitation involves the addition of calcium hydroxide to form calcium salt of carboxylic acid, whereafter sulfuric acid is added to liberate the free carboxylic acid. Both calcium hydroxide and sulphuric acid are thus consumed, and a waste sludge is formed which requires disposal. The environmental pollution and high costs associated with this recovery method have resulted in the need for alternative methods of VFA recovery from fermentation systems [77].

Affinity separation techniques to recover VFAs from fermentation broths have been suggested as a practical technique for VFA extraction. Liquid-liquid extraction (LLE) is a widely applied affinity separation technique, which could enable the effective separation of VFAs from dilute aqueous solutions in an energy efficient manner [32]. LLE does not affect the thermal stability of the bioproducts, is simple, clean, economic in operation [15,44,77], and exhibits promising potential over other separation methods due to its success in the removal of acids from dilute acid concentration waste streams [35].

2.3 Liquid-liquid extraction

Liquid-liquid extraction (LLE) is a process used for separating components dissolved in a liquid feed solution by contact with a second liquid phase known as the solvent. Components are transferred from one phase to the other by a deviation from the thermodynamic equilibrium, where the equilibrium state depends on the nature of the interactions between the feed components and the solvent phase. The process takes advantage of differences in chemical properties of the feed components, such as hydrophilic or hydrophobic character, or differences in polarity, to achieve separation. The potential for separating the feed components is determined based on the differences in these interactions [78].

The simplified LLE process is represented in Figure 4 below. The stream entering the LLE process is referred to as the feed stream and typically contains the solute components to be separated. The extraction solvent is the immiscible or partially miscible liquid that is added to the process to create a second liquid phase that serves the purpose of extracting one or more of the solutes from the feed [78]. The extraction solvent may be comprised of an extractant dissolved in a liquid diluent. In this case, the extractant species is primarily responsible for the extraction of the solute forming a reversible adduct, molecular complex or ion-pair. The extraction capacity is based on the strength of the interaction between solute and extractant. The diluent itself does not necessarily contribute significantly to the extraction of the solute. Diluents are generally used to improve the physical properties of viscous extractants, prevent third phase formation (due to association of

acid and extractant), and can affect the extraction power of the extractant by providing solvation or a stabilizing effect to the acid-extractant interaction [36,79].

After contacting the feed stream with the extraction solvent, the LLE process produces a stream that is referred to as the extract, which contains the extraction solvent with a portion of the feed stream containing the desired components. The liquid feed phase that remains after the feed has been contacted with the extraction solvent (i.e. that has been stripped of the solute) is referred to as the raffinate [78].

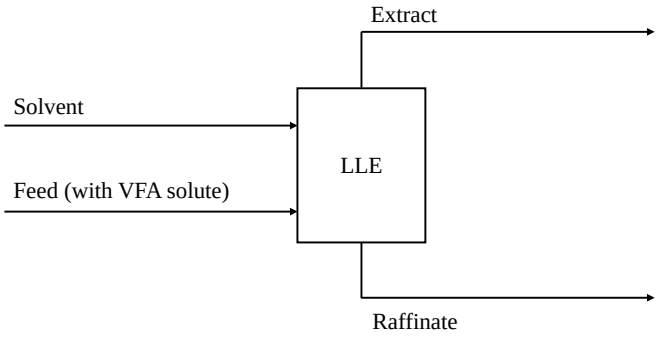


***Figure 4**: Graphical representation of components of LLE process.*

The mechanism for extraction of solutes is generally through physical interaction or chemical reaction with the extraction solvent and target molecules. Physical extractions typically involve the use of conventional solvents, such as inert non-reacting hydrocarbons and substituted hydrocarbons, whereas chemical extractions involve the extraction of a solute through reaction with the solvent. Traditionally, chemical reactions have been less widely applied for separations because of high consumption of expensive solvents [23], where the reaction product is usually less valuable than the original compound. However, this disadvantage can be overcome by using a solvent that reversibly complexes with the solute. In this case, the solvent complexes with the solute in one step and the reaction is reversed to regenerate the solvent in a second step, making chemical extraction a more promising method of separation [15].

2.4 Extractive fermentation

While LLE exhibits promising potential as an efficient method for carboxylic acid extractions, and effective separations of acids from dilute aqueous solutions have been achieved with various extraction solvents [2,3,23,32,35], the majority of these extractions have been conducted in the effluent, post-fermentation. This means they do not offer leverage in controlling the system pH and reducing acid accumulation.

Extractive fermentation is a technique used to segregate a desired product simultaneously in a fermentation process [80]. Continuous LLE of carboxylic acids has the potential to maintain low-level acid concentrations in fermentations, thereby reducing end-product inhibition by removing products *in situ* [3,13,37,38,81], and has shown to increase recovery efficiency compared to conventional downstream recovery techniques where acids are produced and then recovered from the effluent [2]. Furthermore, a system with integrated VFA recovery during fermentation may be able to provide a mechanism for controlling the pH of the AD through removal of excess VFAs (maintaining the pH within the optimal range for digestion, minimising the occurrence of acid-crash) and enhancing performance of biogas plants, while allowing for the recovery of VFAs as a second product stream.

The continuous *in-situ* removal of VFAs from AD systems using LLE could therefore yield an effective extraction strategy to overcome limitations in VFA production via fermentation [2,3,13,37,77]. However, research in characterising extractive fermentations for organic acid production has been relatively limited [38] and although *in situ* VFA recovery has been proposed to enhance bio-based VFA production and recovery from waste streams, the strategy is not currently used in common practice [2].

2.4.1 Improved productivity and yield

The application of extractive fermentation for continuous removal of carboxylic acids from systems while they are being formed has been shown to enhance the performance of bioreactors used to produce carboxylic acids. Most processes available in literature collectively have the following components: (1) fermentation system with extractive fermentation (2) regeneration and recycle of the extractant and (3) recovery of the extracted acids [42]. Table 3 illustrates various extractive fermentation methods with their corresponding improved process performances. It can be noted that fermentation coupled with acid extraction has been shown to exhibit higher productivities, as well as higher product purities and concentrations, compared to conventional fermentations. The improved performance for extractive fermentation can be attributed to reduced end-product inhibition from acid production on the acid-producing microorganisms, pH regulation due to acid removal by extraction [37,38,42], and an increased driving force for higher production rates through a metabolic pathway shift due to continuous removal of acid products from the fermentation while they are being formed [2,37]. Furthermore, with increased product concentration and purity, *in situ* product recovery techniques have the capacity to produce intermediate acids that can be directly used as chemicals and upgraded to fuels, resulting in decreased processing costs [82]. While the mechanisms and microbial kinetics of the biological processes in Table 3 may differ from those in anaerobic digestion processes, the strategies were evaluated with the possibility of applying the same approach of continuous acid removal for reduced microbial inhibition and pH control in AD technologies.

Table 3: *Extractive fermentation systems with corresponding improved process performances.*

Extractive fermentation system	Process performance	Source
Extractive fermentation in immobilised cell system with *Lactobacillus delbruekii* for continuous *in situ* lactic acid removal using 15% Alamine 336 in oleyl alcohol.	Higher bioreactor productivity (12g/L h) compared to the control fermentation (7g/Lh) due to decreased product inhibition.	[38]
Extractive fermentation for butyric acid production from glucose with immobilized cells of *Clostridium tyrobutyricum* using 10% Alamine 336 in oleyl alcohol contained in a hollow-fiber membrane extractor.	Higher product purity (91%), reactor productivity (7.37 g/Lh compared to 0.19 g/Lh), butyrate yield (0.45 g/g compared to 0.34 g/g) and final product concentration (301 g/L compared to 16.3g/L butyrate) relative to free-cell fermentation without extraction.	[37]
Extractive fermentation for acetic acid production by *Acetobacter aceti* with electrodialysis for continuous acetic acid removal.	Improved cell growth and higher productivity, 2.4 times greater acetic acid production and productivity 1.35 times higher than non-pH-controlled fermentation.	[39]
Pertractive extractive fermentation for butyric and hexanoic acid productions from glucose and lignocellulosic hydrolysate with *M. elsdenii* using 10% triocylamine with oleyl alcohol.	Butyric acid and hexanoic acid production up to 17 g/L demonstrated, productivities increased by 3-fold compared to batch for pertractive fermentation (0.26 g/L/h), glucose conversion rates also higher by ~ 3-fold.	[83]
Extractive lactic acid fermentation from glucose with *Lactobacillus delbrueckii* using 15% Alamine-336 with oleyl alcohol in sunflower oil immobilised cell system.	Maximum yield of 25.5 g/L and total lactic acid concentration ~ 2.5 times greater (25.59 gdm^{-3}) than that obtained from fermentation without organic solutions.	[84]
Extractive fermentation using ditridecylamine with oleyl alcohol in hollow-fiber membrane extractor for propionate production from lactose with *Propionibacterium acidipropionici.*	Increased productivity by 5-fold (~1 g/(Lh)), >20% increase in propionate yield (0.66 g/g), higher final product concentration (75 g/L), and increased product purity (~90%) compared to conventional batch fermentation.	[41]
Fermentation coupled with reactive extraction for lactic acid production.	Reactor productivities of up to 25 times greater than those achieved for plain fermentations.	[42]

2.4.2 Continuous pH control

During acid fermentation, the pH of the medium decreases as the acid is produced, which leads to the necessity of adding a neutralization agent to maintain the pH within optimal range for the microbial consortium. The continuous removal of acid products could be an alternative solution to control the pH, while also reducing product inhibition and thereby increasing the productivity and the performance of the bioreactor. Through the removal of acids from the fermentation broth, the accumulation of VFAs and subsequent lowering of the system pH can be prevented. If the product can be removed *in situ* of the reactor, a high pH can be maintained by removing excess acids as soon as they are formed [15]. The fermentation rate is usually higher at a higher

pH value, but the extraction rates tend to be higher at a lower pH value. The extractive fermentation can thus reach a pseudo-steady-state pH, at which the rate of acid production from fermentation equals the rate of acid removal by extraction [37]. The *in situ* LLE of acids is therefore potentially a self-regulating process, whereby a balance is established between acid production and acid removal by extraction [42,44]. Constant pH maintenance could therefore potentially be achieved without external control and the addition of a base to the fermentation system. This is a core aspect the present study aimed to address.

Table 4: *Extractive fermentation systems with corresponding continuous pH control.*

Extractive fermentation system	**Continuous pH control**	**Source**
Extractive fermentation in immobilised cell system with *Lactobacillus delbruekii* for continuous *in situ* lactic acid removal using 15% Alamine 336 in oleyl alcohol.	On/off pH controller monitored pH decrease in fermenter due to acid formation and accordingly activated solvent inlet and exit fluid pumps. Product concentration and pH were maintained constant through acid removal during fermentation.	[38]
Extractive fermentation using ditridecylamine with oleyl alcohol in hollow-fiber membrane extractor for propionate production from lactose with *Propionibacterium acidipropionici.*	Self-regulatory pH control in fermentation broth through balance established between fermentation rate and propionate extraction rate.	[41]
Extractive fermentation for butyric acid production from glucose with immobilized cells of *Clostridium tyrobutyricum* using 10% Alamine 336 in oleyl alcohol contained in a hollow-fiber membrane extractor.	No external pH control, self-regulation of fermentation pH by balance between acid production and acid removal by extraction, pH maintained at ~ 5.5.	[37]
Extractive fermentation for acetic acid production by *Acetobacter aceti* with electrodialysis for continuous acetic acid removal.	Computerized system used to supply direct current power as pH drops due to acid production. Acetate ions penetrate the anion exchange membrane, causing pH of fermentation broth to rise. Continuous pH maintenance through removal of produced acetic acid.	[39]
Pertractive extractive fermentation for butyric and hexanoic acid productions from glucose and lignocellulosic hydrolysate with *M. elsdenii* using 10% trioctylamine with oleyl alcohol.	Pertractive experiment run without external pH control. Initial pH of 6.5 dropped to 5.6 at 80 h before slowly rising to ~ 6.1 at 200 h.	[83]

2.4.3 Solvent regeneration

During the continuous *in situ* LLE process the acids in the feed solution are extracted by the solvent. With the continued production and extraction of acids, the extraction efficiency tends to decrease due to a loss in driving force as the solvent becomes increasingly loaded with acid [83]. The build-up of acid in the solvent phase can be mitigated through back-extraction of the acid-rich extractant, where the acids are stripped from the solvent

using an alkaline stripping solution. With a high recirculation rate all acids extracted by the organic solvent can be stripped almost simultaneously into the aqueous alkaline solution, allowing the solvent to continuously extract the acid to its depletion [37]. This allows for the recovery of acids from the solvent into a concentrated product phase, as well as regeneration of the extractant which can then be recycled back to the LLE process [37,38,42].

Table 5: *Methods of solvent regeneration applied in extractive fermentation system.*

Extractive fermentation system	Solvent regeneration	Source
Extractive fermentation in immobilised cell system with *Lactobacillus delbruekii* for continuous *in situ* lactic acid removal using 15% Alamine 336 in oleyl alcohol.	Lactic acid recovered from solvent through back extraction with 2 M $NaOH_{(aq)}$.	[38]
Extractive fermentation using ditridecylamine with oleyl alcohol in hollow-fiber membrane extractor for propionate production from lactose with *Propionibacterium acidipropionici.*	Solvent containing acetic acid and propionic acid simultaneously regenerated using back-extraction with 6 N $NaOH_{(aq)}$.	[41]
Extractive fermentation for butyric acid production from glucose with immobilized cells of *Clostridium tyrobutyricum* using 10% Alamine 336 in oleyl alcohol contained in a hollow-fiber membrane extractor.	Solvent simultaneously regenerated by stripping with 6 N $NaOH_{(aq)}$ in a second membrane back-extractor.	[37]
Pertractive extractive fermentation for butyric and hexanoic acid productions from glucose and lignocellulosic hydrolysate with *M. elsdenii* using 10% trioctylamine with oleyl alcohol.	Acids in organic phase extracted with 0.5 N $NaOH_{(aq)}$.	[83]

2.5 Process considerations

Extractive fermentation is indeed an emerging separation technique which exhibits considerable potential for VFA extraction. There is ample evidence that suggests *in situ* product recovery has a positive effect on microbial productivity as well as product purity [82], the self-adjusting process can eliminate the use of expensive pH control systems [42] and the solvent can be regenerated and recycled. With correct implementation, the development and optimization of an *in situ* product separation process can enhance the quality of the product produced while reducing the overall cost of production [40]. However, it is necessary to gain further knowledge on how best to integrate the extraction and recovery processes with anaerobic digestion.

While the generation and recovery of VFAs specifically from anaerobic digestion has been suggested as an alternative source of sustainable carbon based chemicals for industry, with bio-based VFA production from waste by acidogenic fermentation recently drawing increased research interest [1,2,16–18,20,21,23–26] and numerous proposed benefits of VFA recovery from complex effluent streams reported [3], there are still many

challenges that need to be resolved to make VFA recovery and production from waste streams a profitable option for carbon recovery [2]. In depth characterisation of extractive fermentations for VFA production from AD systems has been relatively limited, and *in situ* VFA recovery from waste streams using LLE is not widely applied in practice. Further, most studies have considered waste derived VFA recovery through AD with the inhibition of methane production. Very little work on simultaneous co-production of biogas and VFAs has been presented in the literature. This study, therefore, aimed to investigate the extraction and co-production of excess VFAs produced in AD without arresting biogas production.

The separation and recovery process should sufficiently and selectively extract the acids with minimal disruption of the digestion process itself [82]. Biochemical processes are complex in nature and AD systems are sensitive to various factors, therefore effective control of system parameters is essential to ensure process stability, maximise efficiency and prevent digester failure [71]. Additionally, LLE is governed by several physicochemical and operational parameters such as pH, temperature mass transfer characteristics, the type and properties of extraction solvent (extracts and diluents), composition and concentration of VFA-rich digestate, degree of extraction, loading ratio, rate of acid-extractant reaction, etc. [2,3].

The separation yield and efficacy of organic acid extraction depends highly on the nature of the acid extracted, concentration of the extractant in the diluent, the type of diluent and the pH of the system [3,33]. A primary fundamental step in the establishment of an appropriate system is the search for an efficient and selective extractant. It can be difficult to find a good extractant that can work well at a pH value close to the optimal pH, which is usually close to pH 6 or higher for fermentation (Wu et al, 2003). Accordingly, it is essential to understand the effects of pH on extraction as well as on fermentation before an extractive fermentation process can be designed. Additionally, solvents with high extraction capacities tend to also be toxic to bacterial cells which are essential in the anaerobic digestion process [15,47]. It is therefore important to find a biocompatible solvent that is minimally toxic to the microbial community in the AD that also has a high enough extraction capacity for the VFA products. Furthermore, the success of the reactive extraction process as an economical process lies in complete recovery of acid from the loaded organic phase so that the solvent can be regenerated and recycled back to the LLE [15]. Accordingly, the reversal of the reaction or interaction to recover the acid into the solvent phase needs to be possible in order to regenerate the extraction solvent.

The basis for the selection of an effective extraction solvent was therefore based on the fundamental criteria of (i) suitable extraction capacity at varying pH, (ii) biocompatibility, and (iii) feasibility of the solvent for back-extraction, which are essential considerations for the development of a functional continuous *in situ* LLE operation. The following sections will look at these criteria in detail, with an analysis of extraction solvents and their reported performance in terms of these fundamental process considerations.

2.5.1 Extractant types

An important starting point for the development of an effective LLE process for carboxylic acid recovery should be the identification of powerful extractants that have a high selectivity for the target acids to be extracted [35,42]. The study aimed to establish an appropriate extractant-diluent combination with a high enough extraction capacity to extract sufficient VFAs from the AD effluent into the extraction solvent phase, such that the pH of the AD system can be maintained within the desired range and product inhibition can be reduced.

The distribution coefficient and degree of extraction are commonly utilised measures to assess the extraction capacity of the extraction solvents. The distribution coefficient (K_D) can be described as the concentration of the desired product (VFA) in the organic phase over the concentration of the VFA remaining in the aqueous phase (raffinate), and gives an indication of the thermodynamic potential of a solvent for extracting a given solute [35,78]. The distribution coefficient can be calculated using Equation 1 below, where $[TA]_o$ and $[TA]_{aq}$ refer to the concentrations of acids in the organic solvent and aqueous phases respectively.

$$K_D = \frac{([TA]_o)_{extract}}{([TA]_{aq})_{raffinate}} \qquad \textit{[Equation 1]}$$

Most VFAs are relatively weak acids and thus partially ionise in aqueous solution [79].

$$HA \rightleftharpoons H^+ + A^- \qquad \textit{[Equation 2]}$$

The concentrations of undissociated acid [HA] and dissociated acid [A^-] are influenced by the system pH (or the concentration of the hydrogen ion [H^+], subject to equilibrium with the equilibrium constant.

$$K_a = \frac{[H^+]_{aq}[A^-]_{aq}}{[HA]_{aq}} \qquad \textit{[Equation 3]}$$

$$pH = pK_a + log\,\frac{[A^-]_{aq}}{[HA]_{aq}} \qquad \textit{[Equation 4]}$$

At extremely low pH (high [H^+]), the VFAs are present mostly in their undissociated form. At higher pH (lower [H^+]), the VFAs are present more in their dissociated form. Usually, acids are almost completely dissociated at pH = pKa + 1. In the case of VFAs, this is around pH ~ 5.8 - 5.9. In the intermediate region (pH ~ pKa), the VFAs are present as both dissociated and undissociated acids [79]. For consistency, the acid concentration in the study will be referred to as [TA] for inclusion of both dissociated and undissociated acids at varying pH conditions.

$$[TA] = [HA] + [A^-] \qquad \textit{[Equation 5]}$$

The weight percentage of acid transferred from the aqueous feed into the organic phase can be expressed as degree of extraction (E%), defined as the ratio of the VFAs in organic extract phase after extraction to the VFAs in the feed stream (or the sum of VFA in the organic and aqueous phases at equilibrium):

$$E\% = \frac{([TA]_o V_o)_{extract}}{([TA]_{aq} V_{aq})_i} \times 100 \qquad \textit{[Equation 6]}$$

The degree of extraction can be represented in terms of K_D as follows if the extraction proceeds to equilibrium:

$$E\% = \frac{K_D \times 100}{1 + K_D} \qquad \textit{[Equation 7]}$$

A wide range of organic solvents available for VFA recovery have been categorized into three major types, namely: (I) conventional oxygen-bearing hydrocarbon extractants such as octanol, decanol and methyl isobutyl ketone (MIBK), (II) phosphorus-bonded oxygen bearing extractants such as tri-n-butyl phosphate (TBP) and tri-n-octyl phosphine oxide (TOPO), and (III) high molecular weight aliphatic amines such as Aliquat 336, Alamine 336 and trioctylamine (TOA) [3,15,35]. In addition to the above-mentioned solvents, there has been an increasing interest in ionic liquids (ILs) as extractants for organic acids extractions from aqueous solutions using LLE. Room temperature ILs exist as molten salts at ambient temperature and usually consist of a charge–stabilized organic cation and an inorganic or organic anion [43].

It has been reported that physical extraction with conventional oxygen-bearing hydrocarbon solvents is not an efficient method for recovery of VFAs from aqueous feed solutions due to low distributions of VFAs into the extraction solvent [3,36]. In an attempt to increase selectivity and yield of acid, a combination of extractants and diluents have been investigated for chemical extractions of acids, with improved extraction results [3,15,35,36,77]. Table 6 summarises selected extractants that have been utilised for organic extraction and their reported mechanisms.

Table 6: *Summary of extractants and interaction types.*

Extractant Name	Type	Interaction Type
Tri-n-butyl phosphate (TBP)	Organophosphorus compound	Contains a phosphoryl group which has a marked tendency toward intermolecular hydrogen bonding. Undergoes specific interactions like self-association and molecular complex formation with diluents and VFA solutes [35]. Extractant interacts with the acid to form an acid-extractant complex, mainly through hydrogen bonding in the organic phase [15,77].
Tri-n-octyl phosphine oxide (TOPO)	Organophosphorus compound	Extractant interacts with the acid to form an acid-extractant complex [35]. Interaction between acid and extractant through solvation of the acid by donor bonds [15].
Trioctylamine (TOA)/ Alamine 336 (tri-octyl/decyl amine)	Tertiary amine	Interaction between tertiary amine and acid effected through hydrogen bonding of undissociated acid molecules [77]. Extractant serves as complexing agent, undergoing a specific, strong, yet reversible reaction with the acid due to strong interactions of basic nitrogen of the amine and the acidic portion of a carboxyl group [15].

Extractant Name	Type	Interaction Type
Aliquat 336	Quaternary amine, ionic liquid	Acid extraction via an anion exchange mechanism upon equilibration with an aqueous solution of an acid or its salt, extracts both dissociated and undissociated forms of acids [77] at both acidic and basic pH [85].
$[P_{666,14}][Phos]$	Ionic liquid	Extraction of undissociated acid molecules through hydrogen bonding [32,86]
$[P_{666,14}][Cl]$, $[P_{666,14}][Br]$	Ionic liquids	Acid extraction via ion exchange through which Cl^- or Br^- is replaced with the anion of the VFA [32].
$[P_{666,14}][N(CN)_2]$	Ionic liquid	Mechanism of hydrogen bonding with molecular acid [32].

2.5.2 Extraction capacity at varying pH

The pH of the system plays an important role in carboxylic acid extraction, especially when the fermentation and extraction are to take place in the same vessel. While simultaneous fermentation and extraction have been used to remove inhibiting products from bioprocesses and increase bioreactor productivity [33,38,42], many organic solvents do not have a high enough extraction capacity at the suitable fermentation pH range for efficient separation purposes [37,79]. Methanogenic bacteria function optimally at a pH between 6.5 and 7.2 [27], and pH adjustment in anaerobic processes is typically controlled by the interaction of the carbonic system and a net strong base in the pH range 6-7.5 [87]. Whereas fermentations require a higher pH (between pH 6–7.5) for the survival of methanogenic bacteria, LLE performance tends to be optimal at lower pH values (below pH 4.7) [44]. The concentration of undissociated acid in the system is a function of the pH of the aqueous phase. When the pH lies below the pKa of the acid to be extracted, it can be assumed that undissociated acids are predominantly involved in the extraction [35]. Since solvents are generally most effective when extracting VFAs in their undissociated form [3], most solvents function best at pH values lower than the pKa value of the organic acid to be extracted [15,34,45,46]. Hence, extraction efficiency tends to decrease drastically when the pH is higher than the acid pKa. Therefore, for *in situ* VFA recovery that serves to simultaneously control the pH of the system, recover VFAs, and allow biogas production, it is necessary to select an extractant capable of extracting acids at pH values within the functional range for biogas-forming AD at relatively high pH values between 5 and 7 [37], as well as lower pH values when AD systems may experience overproduction of VFAs.

While several extractants and diluents have been applied for carboxylic acid recovery and results have been promising in terms of the capacity for VFA separation from mixed streams, many studies which have investigated various solvents for VFA extraction have been performed under experimental conditions where the pH of the aqueous solution was lower than that of the acid be extracted, whereby only undissociated forms of the acid are extracted with negligible effects of acid dissociation [35,36,77,88]. The following sections will look at reported extractions using organophosphorus compounds, amines and ionic liquids.

2.5.2.1 Organophosphorus compounds

Organophosphorus compounds such as TBP and TOPO have been used as extractants to recover carboxylic acids through the solvation of the acids by donor bonds [15] and have provided higher acid distributions into the extraction solvent phase compared to conventional carbon-bonded oxygen donor and oxygen-bearing hydrocarbon extractants [35,42,88]. These compounds are chemically stable, therefore there is a high possibility of achieving a good separation effect with feed solutions containing chemically similar solutes [88]. Additionally, organophosphates exhibit low water coextraction and have a very low solubility in the aqueous phase. Increasing the TBP concentration in diluent increases the distribution coefficient obtained, however, due to the high viscosity of TBP, concentrations of 40% or lower are recommended [35]. Mostafa (1999) established an optimum concentration of 20% TOPO in kerosene at a solvent to feed ratio S:F of 1:1 for higher partitioning of VFA into the solvent phase.

Table 7 illustrates various VFA extractions reported in literature using organophosphorus extractants at varied concentrations with assorted diluents at differing initial aqueous phase pH values. Both TBP and TOPO exhibit potential for acid extraction, particularly at low pH values below the pKa of the acids to be extracted. Alkaya *et al.* (2009) assessed the influence of pH on VFA extractions using TOPO in kerosene. The highest recoveries of VFA from the fermentation broth were observed a pH of 2.5, while removal efficiencies decreased significantly at pH 5.5, as seen in Table 7. This trend was also noted by Wasewar, (2012), who reported that carboxylic acid extractions with phosphorous based extractants such as tri-n-octyl phosphoric acid tend to decrease with an increase in pH. Although TBP has been investigated for VFA extraction, there is limited literature data available for studies conducted at higher pH ranges.

Table 7: *Reported VFA extractions using organophosphorus extractants at various pH ranges.*

Extractant	Diluent	Acid extracted	K_D	E%	Initial pH	Source
TBP (20%)	sunflower oil	propionic	1.11	53%	pH 2.65-3.13	[77]
TBP (20%)	petroleum ether	propionic	1.35	58%	< pH 4.7	[90]
TBP (20%)	n-heptane	propionic	1.29	56%	< pH 4.7	[90]
TBP (20%)	toluene	propionic	1.78	64%	< pH 4.7	[90]
TBP (25-40%)	1-decanol, kerosene	propionic	5.89	85%	pH 2.4-3.48	[88]
TBP (40%)	1-octanol	propionic	2.79	74%	< pH 4.7	[35].
TOPO (20%)	kerosene	acetic, butyric, propionic, valeric	2.07	67%	pH 2.5	[33]
TOPO (20%)	kerosene	acetic, butyric, propionic, valeric	0.48	32%	pH 5.5	[33]
TOPO (20%)	kerosene	acetic	~3	~75%	< pH 4.7	[89]
TOPO (20%)	kerosene	propionic	~9	~90%	< pH 4.7	[89]

2.5.2.2 Amines

Aliphatic amines including Alamine 336, TOA and Aliquat 336 have been found to be slightly more effective and less expensive than phosphorus-bonded, oxygen-bearing extractants, and have been extensively employed for the extraction of carboxylic acids such as lactic, citric, nicotinic, butyric, valeric, glycolic and glyoxylic acids [15,91]. The nature of acid-amine interactions depends on the strength of acid and type of amines [35]. High-molecular weight amines have low solubilities in water and give rise to high distributions of VFAs into the extraction solvent [35]. Further, the high affinity of the organic amine base for the acid gives rise to selectivity for the acid over nonacidic components in the mixture [42], which is beneficial in preventing co-extraction of impurities in digester systems.

Maximum reported extractions have been achieved at amine concentrations between 20-40% [92], where the distribution coefficient tends to increase up to 40% amine extractant, after which it decreases [35,42]. Higher concentrations of amine increase the viscosity of the organic phase, which is not advantageous as high viscosity at the interface can hinder the reaction and transfer of the complex into the solvent phase. Furthermore, with concentrations of greater than 25% amine in diluent, a third emulsion phase has been observed at the interface between the aqueous and organic phases of the extraction system. The use of 10-20% amine in diluent has thus suggested [42,85]. Table 8 exhibits various VFA extractions using amine extractants at varied concentrations with assorted diluents at differing initial aqueous phase pH values. Tertiary amines have been reported as more suitable extractants for organic acid removal compared to primary and secondary amines [38], where the extractant reacts with undissociated acid solutes to form an acid-extractant complex, which facilitates a high distribution of acid from dilute aqueous phase into the organic phase [36]. It can be noted from Table 8 that tertiary amines TOA and Alamine 336 demonstrate high capacities for VFA extraction, however, the pH values of the systems are often maintained below the acid pKa in order to achieve maximum distribution coefficients. The extraction capacity is greater at acidic pH values when acids predominate in their undissociated form because tertiary amines are capable of primarily extracting undissociated acid molecules through hydrogen bonding [35,77]. The effect of pH on carboxylic acid extraction with tertiary amines is demonstrated in the results obtained by Shang-Tian Yang, Scott A. White (1991), where the distribution coefficients drastically decreased as the system pH increased, with no VFAs extracted above pH 7. Aliquat 336, on the other hand, exhibited significant capacity for VFA extraction even at pH values above the acid pKa, as seen in Table 8. This correlates with work by Wasewar (2012), who observed that carboxylic acid recoveries with tertiary amines tend to decrease with an increase in pH, while quaternary amines have a certain optimum pH for extraction. It has been proposed that the extraction power of amine solvents is dictated by the basicity of the amine and the basicity of the carboxylic acid anion, which is known from the pKa of the acid (pKa,A) [34,38] In cases where the amine is a much weaker base ($pK_{a,B} << pK_{a,A}$) the contribution of ion-pair formation is small, and extraction by the amine is mainly affected by hydrogen bonding or solvation of undissociated acid molecules. On the other hand, if the amine is a much stronger base than the anion of the extracted acid

(pKa,B>>pKa,A), the amine binds to the proton forming a positively charged protonated amine, which binds the anion of the extracted acid in a mechanism referred to as ion-pair formation [36]. Extraction is less influenced by the pH of the aqueous phase using Aliquat because the interaction between the quaternary ammonium salt is via anion exchange upon equilibration with an aqueous solution of an acid or its salt, which enables Aliquat to extract both the dissociated and undissociated forms of acids [77].

Table 8: *Reported VFA extractions using amine extractants at various pH ranges.*

Extractant	Diluent	Acid extracted	K_D	E%	Initial pH	Source
TOA (20%)	n-octanol	acetic	8.80	90%	pH 2.8	[32]
TOA (20%)	n-octanol	acetic	1.30	57%	pH 4.6	[32]
TOA (30%)	oleyl alcohol	propionic	10.11	91%	pH 2.65-3.14	[36]
TOA (30%)	ethyl acetate	propionic	2.83	74%	pH 2.65-3.14	[36]
TOA (30%)	petroleum ether	propionic	0.75	43%	pH 2.65-3.14	[36]
TOA (30%)	n-heptane	propionic	0.42	29%	pH 2.65-3.14	[36]
TOA (30%)	1-octanol	propionic	14.09	93%	< pH 4.7	[35]
TOA(20%)	sunflower oil	propionic	0.98	49%	pH 2.65-3.13	[77]
Alamine 336	-	acetic	0.55	35%	pH 2	[79]
Alamine 336	-	acetic	0.28	22%	pH 4.76	[79]
Alamine 336 (25%)	kerosene	propionic	2.09	68%	pH 2	[79]
Alamine 336 (25%)	kerosene	propionic	1.05	51%	pH 4.67	[79]
Alamine 336	-	butyric	3.30	77%	pH 2	[79]
Alamine 336	-	butyric	1.65	62%	pH 5.6	[79]
Aliquat 336 (20%)	oleyl alcohol	propionic	1.09	52%	< pH 4.7	[93]
Aliquat 336 (20%)	oleyl alcohol	butyric	9.12	90%	< pH 4.7	[93]
Aliquat 336 (50%)	1-octanol	propionic	1.66	62%	< pH 4.7	[35].
Aliquat 336 (20%)	sunflower oil	propionic	1.11	53%	pH 2.65-3.13	[77]
Aliquat 336 (25%)	kerosene	acetic	0.20	17%	pH 2	[79]
Aliquat 336 (25%)	kerosene	acetic	0.13	11%	pH 4.76	[79]
Aliquat 336 (25%)	kerosene	propionic	2.10	68%	pH 2	[79]
Aliquat 336 (25%)	kerosene	propionic	1.30	56%	pH 5.44	[79]
Aliquat 336 (25%)	kerosene	butyric	3.31	77%	pH 2	[79]
Aliquat 336 (25%)	kerosene	butyric	1.85	65%	pH 4.81	[79]

2.5.2.3 Ionic liquids

Through the combination of different ions, ILs have tuneable properties such density, viscosity, polarity and miscibility with other common solvents. Furthermore, ILs possess a range of unique properties such as negligible vapor pressure, high thermal stability and low chemical reactivity [43]. The combination of these properties presents promising opportunities for the application of ILs in extraction processes.

A range of mechanisms have been reported using ionic liquid extractants for organic acid extractions. Tetraalkylphosphonium ionic liquid (IL) with a bis 2,4,4-trimethylpentylphosphinic anion ([$P_{666,14}$][Phos]) was

suggested an effective extractant for lactic acid extraction, achieving distribution coefficients of above 40 through extraction of undissociated acid molecules via hydrogen bonding [86]. Reyhanitash *et al.* (2016) studied phosphonium-based ionic liquids (ILs) for extraction of VFAs from fermented wastewater. $[P_{666,14}]Cl$ and $[P_{666,14}]Br$ exhibited leaching into the aqueous phase, indicative that the acid extraction mechanisms used by these ILs is ion exchange through which Cl^- or Br^- are replaced with the anion of the VFA. Following extraction, the equilibrium pH of the raffinate decreased using these solvents. Both $[P_{666,14}][Phos]$ and $[P_{6,6,6,14}][N(CN)_2]$ were found to be hydrophobic and highly stable, with negligible leaching of ions into the aqueous phases and the pH of the raffinate increased following LLE. Here the extraction mechanism was confirmed to proceed through hydrogen bonding of molecular acid and not ion exchange of the acid anion, which corresponds with work by Marták and Schlosser (2007). Decreasing the pH of the aqueous solution resulted in increased distribution coefficients using $[P_{666,14}][Phos]$ and $[P_{6,6,6,14}][N(CN)_2]$. By lowering pH, acid dissociation takes place in the aqueous phase, with ongoing equilibria series of dissociation, partitioning and organic phase complexation, resulting in higher acid distributions in these extractants [32].

Table 9: *Reported VFA extractions using ionic liquid extractants at various pH ranges.*

Extractant	Diluent	Acid extracted	K_D	E%	Initial pH	Source
$[P_{666,14}][Phos]$	-	acetic	17.00	94%	pH 2.8	[32]
$[P_{666,14}][Phos]$	-	acetic	1.20	55%	pH 4.6	[32]
$[P_{666,14}][Br]$	-	acetic	2.20	69%	pH 2.8	[32]
$[P_{666,14}][Br]$	-	acetic	1.40	58%	pH 4.6	[32]
$[P_{666,14}][Cl]$	-	acetic	3.70	79%	pH 2.8	[32]
$[P_{666,14}][Cl]$	-	acetic	3.40	77%	pH 4.6	[32]
$[P_{666,14}][N(CN)_2]$	-	acetic	1.80	64%	pH 2.8	[32]
$[P_{666,14}][N(CN)_2]$	-	acetic	0.70	41%	pH 4.6	[32]

2.5.2.4 Diluents

Many extractants are solid or highly viscous at room temperature and are therefore often dissolved in low molecular weight and low viscosity diluents to allow easier handling [35]. Diluents are also used to prevent third phase formation that builds up due to the association of carboxylic acid and extractant [36], an important consideration as high viscosity at the interface can hinder the reaction and transfer of the complex into the solvent phase. Further, diluents are used to improve physical properties such as surface tension, density, water uptake and boiling point of the solvent phase [88]. In addition to providing solution to the extractant, the diluent can have an impact on the extraction equilibria of LLE by providing solvating capacity to the extractant for acid extractions [35,42,92]. The diluent can affect the basicity of the extractant, which in turn influences the stability of the ion pair formed and its solvation [38]. The stability of the ion-pair or the acid-extractant complex

determines the equilibrium conditions of the acid extraction, particularly with low aqueous acid concentrations [42].

a) Active diluents

Polar diluents have been reported to be more favourable than non-polar solvents for organic acid extractions [35,38,88]. There is increased extraction power with a diluent that stabilizes the acid-extractant complex effectively. Such diluents are referred to as 'active' diluents [94]. Various active polar and proton or electron donating diluents such as aromatic hydrocarbons, halogenated aliphatic hydrocarbons, nitrobenzenes ketones, and higher alcohols (such as octanol) tend to enhance the extraction of solutes [36]. These diluents provide general solvation to the system and affect the extraction power of the extractant by providing specific interaction or a stabilization effect between the acid and the extractant. The solvation of the extractant-acid complex is often based on dipole-dipole interactions and can play an important role in the neutralization reaction between acid and extractant. This interaction is promoted by increasing the polarity of the diluent [35,42]. The effect of diluent on extraction through hydrogen bonding to amines is governed by the diluent interaction with the undissociated acid and with the free amine. Extraction through ion-pair formation or complexation from aqueous solutions of low pH, and extraction of acids by relatively strong amines, are strongly dependent on solvation of the ion pair formed. Therefore, extraction is strongly enhanced by polar and particularly by protic diluents [34].

b) Inert diluents

Contrarily, nonpolar aprotic diluents which do not provide stabilization to the acid-extractant complex are referred to as 'inert' diluents [94]. Inert diluents, such as long chain paraffins, hexane, n-heptane and benzene tend to limit the solvent capacity, particularly when the interaction between the acid and the extractant is via hydrogen bonding or solvation [36]. As seen in Table 8, the lowest distribution coefficient was observed for TOA with *n*-heptane (compared to n-heptane, petroleum ether, ethyl acetate and oleyl alcohol) which is non-polar in nature, supporting the theory that non-polar diluents result in low distribution of acids into the extraction solvent. However, while the polarity rationale holds true for most diluents, it is not applicable for oleyl alcohol systems, illustrated in the high extraction achieved with TOA and oleyl alcohol where an almost 10-fold increase in distribution coefficient was achieved [36].

2.5.3 Toxicity

The following section investigates toxicity of extractant-diluent systems and why this is an important factor for consideration in *in situ* LLE of VFAs from biological systems. In order to integrate LLE *in situ* without disrupting the AD process itself, the extractant and diluent should ideally be biocompatible with the anaerobic microorganisms (particularly if solvents are to come into direct contact with microbes continuously during process) to ensure continued production of VFAs and methane in the presence of the solvent. Atasoy *et al.*

(2018) noted a considerable drawback of solvent extraction for *in situ* extraction of VFAs being that some solvents may be toxic or inhibitory to microorganisms. Solvent toxicity can be differentiated into two groups; molecular level toxicity which refers to the toxicity of the solvent due to the soluble portion of the solvent, and phase level toxicity which arises due to the presence of two phases [15]. The problem of toxicity becomes increasingly pertinent when recovery is carried out *in situ* of the bioreactor where extractant and diluent can exert toxicity both at the molecular level, where the dissolved organic extractant and diluent can inhibit enzymes or modify cell membrane permeability, and at the phase level by direct contact of the solvent phase with cells, where extractant or diluent coating of the cells may block nutrient diffusion and may also disrupt the cell wall due to increased surface tension [44,77].

Most organic solvents are toxic to anaerobic bacteria, giving rise to a series of physical microbial and biochemical effects on the catalytic activity of the microorganisms. This ultimately either inhibits cell growth or results in the death of microorganisms [37,42] which negatively impacts product formation. Consequently the toxicity level of solvents to microbes limits their compatibility with fermentation broths [43], making biocompatibility a key factor for *in situ* removal of VFAs from AD systems. Various approaches have been adopted to reduce the toxic effects of solvent systems. Avoidance of direct contact of the organism with the extractants can substantially reduce toxic effects. Several investigators have used membranes to prevent direct contact of the solvent with the microorganisms present in the broth [3,37,41]. Cell immobilization is another method that has been employed to protect the cells by reducing the contact of the immiscible solvent with the microbes [42]. Cell immobilization and the use of membranes can minimize phase toxicity but they can also pose further problems, such as membrane fouling, high membrane cost, rupture during operation and low distribution coefficients with the use of insoluble solvents [77]. Alternatively, it may be possible to use a non-toxic diluent in combination with a toxic extractant to yield a biocompatible mixture [15], this concept is further explored in Section 2.5.3.2.

2.5.3.1 Extractants

Organic solvents such as hexane, chloroform and ethers which have been extensively used in the extraction phase of various LLE systems tend to be volatile, highly combustible and are often toxic to acid-producing microorganisms. New extraction media are thus required to replace conventional solvents to facilitate the development of more environmentally friendly extraction approaches [95]. Toxicity of organophosphorus compounds, amines and ILs has been considered in numerous studies, which will be discussed in more detail. However, toxicity is often not a key component that is taken into consideration in VFA extraction studies using different extractant-diluent combinations and biocompatibility is seldomly quantitively investigated in combination with evaluation of the extraction capacity of solvent systems. There are far fewer studies that make direct comparison of different solvents and their relative toxicities in fermentation systems compared to

studies which have drawn comparison to the extraction ability of various extractant-diluent systems, yet toxicity is a critical factor which requires consideration for application of LLE in biological systems.

It was reported by Zacharof and Lovitt (2013) that the use TOPO as a solvent yielded relatively low VFA extractions with no enhancement of acid production over the 120-hour extractive fermentation for propionic and acetic acid extractive fermentations, however, the cell growth and production of acids were not hindered in the presence of the extractant. This may imply that the organophosphorus compound was non-toxic to the consortia. Keshav, Wasewar and Chand (2008c) reported that TBP and Aliquat are toxic to bacteria for propionic acid production, whereas TOA was considered non-toxic. In a study of itaconic acid extraction using TBP and Aliquat 336 in sunflower oil, Aliquat 336 was shown to be less toxic to microbes than TBP [44], indicating Aliquat 336 may be the more suitable extractant for acid extraction.

Studies using pertractive fermentation to recover VFAs suggested that TOA with oleyl alcohol was less inhibitory to microbial cultures when compared to trihexylamine-octanol [82]. However Wu and Yang (2003), stated that although TOA was reported minimally toxic to some bacteria, the use of 10% TOA in oleyl alcohol was toxic to free cells of *C. tyro-butyricum* in suspension for butyric acid production, but not harmful to cells immobilized in the fibrous bed. In an investigation of solvents for lactic acid extraction, no bacterial cell growth was observed in extractions utilising tertiary amine concentrations above 40% and no cell growth was observed in all cases with dioctyl adipate (DOA) and di-n-decyl-amine in mediums tested with greater than 10 % solvent [3]. Yabannavar and Wang (1991) found that TOA exhibited slight toxicity in lactic acid fermentations while Tik, Bayraktar and Ü. Mehmetoglu (2001) noted a toxic effect with 15-50% TOA in oleyl alcohol for extractive lactic acid fermentation. Wasewar *et al.* (2004) concluded that TOA in oleyl alcohol would serve as an ideal extraction system for lactic acid, however, TOA exhibited symptoms of molecular level toxicity at 5% saturation, and high phase level toxicity was observed even at a low aqueous to organic ratios of 100:1. These comparisons of published data demonstrate that toxicity depends largely on the combination of microorganism and solvent used, where some strains of bacteria may be more sensitive or more resilient to the solvent than others. It is therefore difficult to characterize the biocompatibility of solvents and extractants based off results obtained from studies and reviews reported in literature due to the variability of microorganisms, systems and subsequent results obtained.

Ionic liquids have been suggested as a sustainable alternative to classical organic solvents, however, Tonova (2017) highlighted that some of the most commonly employed ILs ($[P_{6,6,6,14}]Cl$, for example) exhibit much higher levels of ecotoxicity in aquatic environments compared to conventional organic solvents. Therefore, not all ILs can be labelled as green, biodegradable, nontoxic solvents and the environmental impact, biodegradability and biocompatibility of ILs requires further investigation.

It can be noted from these various studies that although toxicity of solvents has been recognised and mentioned as a matter of concern in several extractive fermentation investigations, and some extractant-diluent systems

have been compared in terms of their biocompatibility in certain systems, the comparisons are largely qualitative in nature and it is difficult to draw a conclusion on which extractants would be most suitable for application in AD systems. When assessing extractants and diluents for application in biological systems, biocompatibility is a key factor which needs to be taken into consideration, and further research on the impact of solvents in AD systems, with comparison of their relative effects on AD productivity, would provide more clarity on this subject. This study therefore aimed to compare a number of extractants and diluents based on their biocompatibility with the biogas-producing AD consortia.

2.5.3.2 Diluents

Diluents are often used to enhance extraction performance and improve physical properties of the extractant. However, diluents can also have an effect on the toxicity of the solvent. Octanol, which has been widely applied as a diluent in LLE with high partition coefficients for acid extractions when used with suitable extractants [32,35,97], has been shown to exhibit toxicity to propionic acid producing bacteria [97]. When investigating lactic acid extractions, Wasewar *et al.* (2004) noted that both octanol and MIBK were highly toxic to acid-producing microorganisms. Therefore, despite their good extraction performance, these diluents would not be suitable for VFA extraction from AD systems.

An alternative approach to mitigating solvent toxicity could be limiting the concentration of the toxic extractant with a non-toxic diluent. Two strategies suggested by Zhong, Glatz and Glatz (1998) to eliminate solvent toxicity included the replacement of the toxic diluent with a nontoxic diluent or entrapment of the dissolved toxic solvent in the culture growth medium with vegetable oils such as corn, olive, or soybean oils. Paraffinic liquid, which displays low levels of toxicity was suggested as more biocompatible alternative to toxic diluents for simultaneous extraction during fermentation [42]. Oleyl alcohol has also been suggested as an alternate suitable diluent for biological extraction systems due to its non-toxic characteristic and insignificant inhibitory effect on bacterial groups [38,42,83,93,96,97]. Non-toxic, natural diluents such as sunflower oil, castor oil, rice bran oil and soybean oil have been shown to substantially reduce toxic effects of extractants by avoiding direct contact of microorganisms with the organic solvent phase, and have even aided in the extraction of target molecules [15,42,44,77,96]. Keshav, Wasewar and Chand (2008c) investigated the extraction of propionic acid using sunflower oil diluent and concluded that toxicity can be minimised through the use of a combination of less toxic extractants with non-toxic diluents. Reactive extraction of itaconic acid was also carried out using sunflower oil diluent, with enhancement of extraction using Aliquat 336 in sunflower oil, illustrating that natural non-toxic diluents can be successfully employed in extractive fermentation [44]. However, the extent to which the toxicity was minimized through the use of the oil was not clearly demonstrated in these studies. Additionally, fats and oils can be used as carbon sources for bacterial groups, being broken down into soluble organic substances and fatty acids during AD process. This is an important factor that would need to be

considered in the long-term usage and regeneration of solvents, as the consumed diluent would need to be replenished.

While using a non-toxic diluent to improve biocompatibility of solvents may not completely eliminate toxicity, the use of low concentrations (10–30%) of extractant in diluent has been reported to provide a biocompatible solution for the recovery of acids [44,85], with a suitable concession between high extractability and biocompatibility [38]. The use of 20% extractant in diluent was therefore selected for the current study. Due to their reported non-toxic characteristics, oleyl alcohol, lamp oil (paraffinic liquid of C_{14}-C_{20}) and canola oil were for chosen for investigation as potentially suitable, selective, biocompatible diluents for VFA extraction from AD systems.

2.5.4 Feasibility for back-extraction

With continued production and extraction of VFAs in AD systems, the extraction efficiency decreases as the solvent becomes increasingly loaded with acid. An alternative to adding more solvent to the system would be to remove VFAs from the loaded solvent to alleviate accumulation and build-up of acids in the extract phase [83]. Ultimately, the *in situ* extraction of VFAs from AD systems needs to be sustainable, with minimal waste stream production and low reagent consumption. The success of extractive fermentation as an economical process therefore depends largely on the complete recovery of acid from the organic extract phase [15]. The acid can be back extracted from the solvent phase using various regeneration methods. The regeneration step follows the extraction step and involves the reversal of the reaction to extract the acid to remove the acid from the loaded solvent, allowing for product recovery and recycling of the solvent back to the LLE.

Regeneration methods should ideally require low energy inputs, generate minimal by-products and contain non-toxic components, as the organic phase which is recycled to the *in situ* LLE unit may contain residual dissolved components of the back-extractant [42]. Two well-established regeneration methods are distillation and back extraction [3], however, distillation is an energy intensive separation process which requires complex design and optimisation to ensure efficient recoveries. Back extraction is a relatively simple technique that involves the recovery of the extractant through contacting the acid-rich solvent phase with alkaline stripping solution. The acids are stripped out of the solvent into an alkaline product phase and thereafter the acid free solvent can be recycled back into the LLE system to obtain a closed-loop recovery process. Complete recovery of VFAs renders the regenerated acid-free solvent functional for recycle and recovery of more VFAs, thereby reducing the consumption of extra reagents, reducing extraction costs and maintaining a sustainable extractive fermentation setup. Alkaline back extraction using sodium hydroxide solution was investigated in the present study for the recovery of extracted VFAs and regeneration of the solvent phase as a starting point to demonstrate the reversibility of extraction mechanisms with different solvent combinations.

2.5.4.1 Extractants

For recovery of VFAs from the loaded solvent into an alkaline product phase, the reversal of the reaction to extract the acid needs to be possible. Therefore, for alkaline back-extraction be feasible it is important to ensure that an extractant with a reversible mechanism for VFA extraction is selected for *in situ* LLE. This section will explore reports of alkaline back-extraction for VFA recovery with various extractants.

As outlined in section 2.4.3, back extraction of carboxylic acids from loaded organic phases has been used to successfully recover acids by contacting the solvent phase with $NaOH_{(aq)}$ solution in extractive fermentation systems. With use of $NaOH_{(aq)}$ in excess stoichiometric amount, complete recovery of VFA can be obtained, provided the extraction reaction is reversible [15]. During extraction with tertiary amines, the amine extractant recovers the acid by reacting with it to form an acid-amine complex that is solubilized into the extractant phase. Regeneration methods have been used to reverse the complexation reaction, enabling recovery of the acids into a product phase [94]. Following acid extractions using oleyl alcohol with extractants Alamine 336 [37,38] and TOA [83], $NaOH_{(aq)}$ has been used to completely strip acids from the solvents into the alkaline phase, highlighting the potential for back-extraction of solvents containing tertiary amines. Extractants TBP and TOPO also undergo specific molecular complex formation with solutes [15,35], therefore if the complexation reaction is reversible it should be possible to regenerate and recover these organophosphorus extractants.

When the reaction between the acid and extractant takes place via an ion exchange mechanism in an irreversible interaction, it may not be possible to regenerate and recycle the solvent using alkaline back-extraction. Reyhanitash *et al.* (2016) found that the extraction interaction using the ionic liquids $[P_{666,14}][Cl]$ and $[P_{666,14}][Br]$ to extract VFAs involved the replacement of the Cl^- and Br^- in an anion exchange mechanism with the anion of the VFA, while the reaction mechanism using $[P_{666,14}][Phos]$ was confirmed to proceed through hydrogen bonding of molecular VFAs and not ion exchange. Extraction with $[P_{666,14}][Phos]$ exhibited the advantage of easy stripping of acid from the solvent, which was not the case for IL with a chloride anion $[P_{666,14}][Cl]$ [98].

Further investigation of selected solvents for back-extraction is required to determine the ease with which they can be stripped of VFAs. In addition to the reversibility of the extraction mechanism, the phase behaviour and interactions between the solvents and sodium hydroxide solution would also influence the practicality for alkaline back-extraction. The study therefore aimed to explore each of the extractant-diluent combinations for VFA recovery and solvent regeneration to provide clearer insights on the feasibility for alkaline back extraction with the selected solvents for study.

2.5.5 Additional considerations

2.5.5.1 Temperature

Temperature has a significant influence on the AD process, with a direct effect on physical and biochemical reactions [99]. Many industrial scale anaerobic digesters are operated in the mesophilic range, with a temperature between 30°C and 38°C. An increasing temperature has numerous benefits in AD such as increased death rate of pathogens, higher solubility of organic compounds, and enhanced chemical and biological reaction rates. However, the application of high thermophilic temperature between 50°C and 57°C has counteractive adverse effects such as increased proportions of free ammonia [27] increased VFA concentrations and decreased biogas yields [99]. Methanogens are the most sensitive AD microorganisms to temperature fluctuations, where variations in temperature often result in process instabilities [27,99]. It is therefore important to maintain a stable operating temperature in the digester to avoid process failure, which has been reported at temperature fluctuations in excess of 1°C/day [27].

On the other hand, LLE is generally an exothermic process. Extraction is thus expected to decrease with an increase in temperature [42]. Wasewar (2012) noted up to 50% reductions in carboxylic acid extraction capacity at increased operating temperatures. It is therefore important to ensure that the extractant can operate efficiently in the operating temperature range of the digester.

Through the application of *in situ* VFA extraction, AD systems may become more stable and less sensitive to fluctuations in temperature within the thermophilic range, which could facilitate operation at higher temperatures with increased AD throughputs in the future. However, for the current study the AD systems were operated in the mesophilic temperature region with continuous temperature control to minimise fluctuations, to evaluate the efficiency of the extractants within the widely applied mesophilic temperature range.

2.5.5.2 Impurities

Impurities such as salts (including NaCl, Na_2SO_4, K_2HPO_4), biosurfactants, ions, fats and proteins are inherently present in AD systems. These impurities are likely to have a significant effect on mass transfer, kinetics, equilibrium and may influence the stability of two-phase systems. A knowledge of the effect of impurities on the VFA extraction capacity of solvents is therefore necessary for the design and scale-up of an *in situ* LLE system.

It has been reported that the presence of salts may cause the salting out of VFAs, resulting in an increase in distribution coefficient, which would be beneficial for the removal of VFAs from the system. However decreased distribution coefficients in the presence of dissolved salts have also been reported which may be due to interactions between salt ions (Cl^-, SO_4^{2-}, HPO_4^{2-}) and the hydrogen ion of the acid to be extracted, resulting

in the formation of HCl, H_2SO_4 and H_3PO_4. If salting out of HCl, H_2SO_4 and H_3PO_4 occurs more than that of acids, the VFA extraction capacity of the solvent would be reduced [15]. Reyhanitash *et al.* (2016) conducted experiments with dissolved salts and ions (Cl^-, SO_4^2, HPO_4^{2-} , Na^+ and K^+) present in the aqueous VFA feed streams and it was observed that extractions using $[P_{666,14}]$[Phos] and TOA were strongly affected by salt ions present in the feed, while $[P_{6,6,6,14}]$Cl and $[P_{6,6,6,14}][N(CN)_2]$ maintained constant extraction capacities. Extraction of VFAs using $[P_{666,14}]$[Phos] and TOA was significantly reduced in solutions with impurities compared to extractions using model solutions without dissolved salts at the same initial pH. This was attributed to the co-extraction of significant amounts of anions present in the solution which resulted in reduced extraction capacity with these solvents [32].

It is evident from published data that the presence of impurities in AD systems could contribute to enhanced extraction performance or could lead to decreased VFA extraction, depending on the system and the solvent investigated. This study aimed to establish how each of the selected extractant-diluent systems were influenced by impurities present in AD fermented wastewater to determine which solvents show potentiality for application *in situ.*

2.6 Conclusions from literature

Although AD is growing in application and has been widely used for the treatment of organic waste, the digestion mechanism is highly complex and is not yet completely understood. The process is subject to instability and digester imbalance, as outlined in Section 2.1.2. There is a correlation between VFA levels and digester performance, and a need to control VFA concentrations and system pH within active digesters. Removing excess VFAs from the digester is a possible alternative process control strategy for pH adjustment, which could aid system stability. Effective control of pH and VFA concentrations through removal of excess VFAs could provide an alternative to the current practice of acid neutralisation, and would allow AD systems increased capacity to handle larger loads without stopping the feed. While it has been identified that simultaneous separation and *in situ* extraction of acids produced during fermentation processes could be a feasible solution to overcome the inhibitory effects of acid production, this strategy has not been extensively investigated or applied in industry. Further, there is limited published data available regarding the feasibility of integrating LLE with the AD process without arresting the biogas productivity of the digester.

Section 2.5 highlights studies that have investigated LLE of carboxylic acids from aqueous solutions using different extractant-diluent mixtures, with consideration of extraction capacity, pH dependency and toxicity. Nonetheless, seldom have these factors been collectively considered within a specific study, making it difficult to draw a reasonable conclusion from the literature regarding a suitable solvent for VFA extraction from AD systems. While the extraction of carboxylic acids from aqueous streams has been explored, studies involving

the comparison of the extractants are sparse, particularly for the simultaneous extraction of a range of different acids with different carbon chain lengths. Furthermore, most studies have focused on extraction at the optimal extraction pH, below the pKa of the acids to be extracted, with limited emphasis on the feasibility of extraction at higher pH setpoints, as seen in Section 2.5.2. Extraction solvents have been applied in fermentation systems for acid extraction with consideration of toxicity, as discussed in Section 2.5.3, however, there is no published data available regarding the methanogenic biocompatibility of solvents for application in AD systems. Various methods for solvent regeneration have been suggested, however there is minimal information available regarding practical applications of back-extraction methods using a range of solvents for complete product recovery.

Most studies that considered the production of VFAs from AD systems have proposed the inhibition of methanogenesis to optimise VFA production. The approach is generally to maximise VFA production by blocking methanogenesis or maximisation of methane yield by controlling various operational factors discussed in Section 2.1.3. Another strategy is to minimise VFA build-up through extraction of excess VFAs in AD systems to avoid process instability and subsequent process failure. However, to the knowledge of the author, there is no published data that explores this strategy. In this context, *in situ* LLE was investigated for the extraction of VFAs from biogas-producing AD systems as a valuable co-product. The approach is partial removal of VFAs to reduce the inhibitory effects of VFAs and prevent acid crash. In other words, the aim is not complete removal of VFAs, as this would remove the carbon source for methane production, but rather use of a pH-dependent, self-regulating system mediated by VFA extractants. The design of an extractive fermentation process requires an understanding of the dependence of different parameters on the overall extraction. The study aimed to investigate solvents for potential to extract and recover VFAs from AD systems based on the parameters of extraction capacity, biocompatibility and feasibility of VFA back extraction. These criteria were independently investigated to characterise the solvent performance.

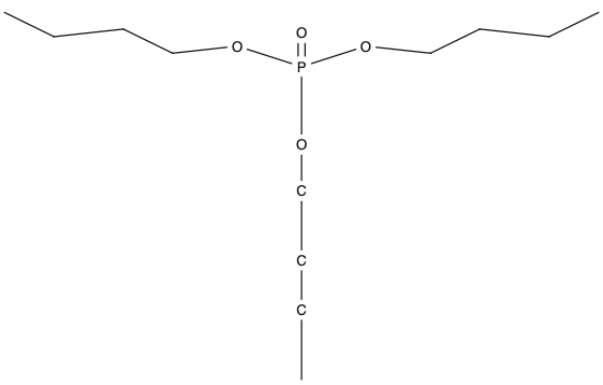
Numerous extractants such as organophosphorus bonded compounds, aliphatic amines and ionic liquids have been reported in literature with promising potential for the extraction of VFAs from dilute aqueous streams, but there is limited data available regarding the ability of the extractants in simultaneously collectively meeting the criteria of sufficient extraction capacity at different pH ranges, biocompatibility of solvents with microbial communities and feasibility for back extraction of the solvent. There is literature available regarding extractants and their performance with respect to one or two of these parameters, but extractants have rarely been considered with concurrent consideration of all the criteria. While an extractant may yield a high degree of extraction at an optimal pH, it may not extract VFAs at higher pH values, it may prove to be toxic to the microorganisms, or the extraction mechanism may be irreversible presenting difficulty for solvent regeneration, for example.

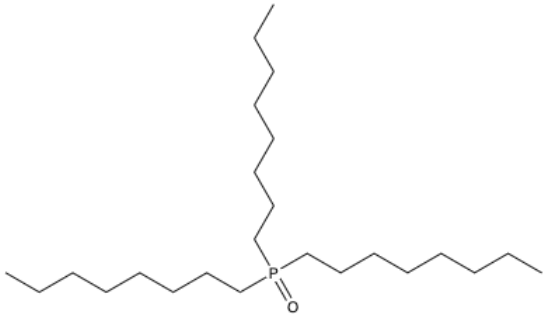
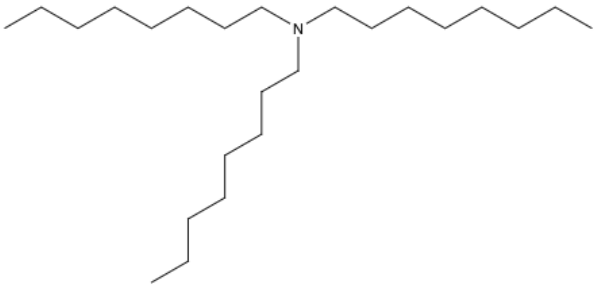
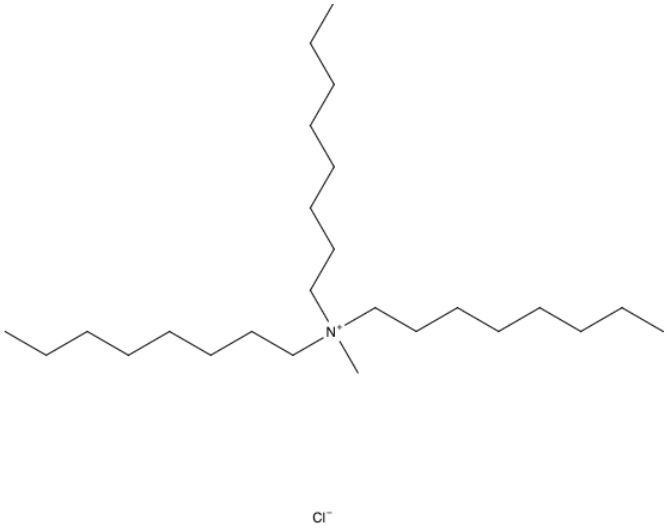
Investigation into how to integrate VFA extraction and product recovery with the AD process, without disrupting the AD process itself, is still required. Shen Lee *et al.* (2014) noted the necessity of investigating synergistic or antagonistic interactions amongst variables, and how different operating conditions affect microbial dynamics, in order to devise functional operating strategies that can be used in anaerobic technologies. This project aimed to investigate the potential for extraction of VFAs using LLE from AD systems with alkaline back extraction of the extract to recover the acids and regenerate the solvent, thus enabling recycling of the solvent back to the LLE. Extractants and diluents were investigated and selected based on their VFA extraction capacity at varying pH, biocompatibility, and feasibility for back extraction. This would allow for evaluation of the proposed process as a solution to inhibitory effects of acid production, with the benefits of valorised waste effluents.

2.7 Solvents selected for study

Extractants TOA, TBP, TOPO, $[P_{666,14}][Phos]$ and Aliquat 336, with reported affinity for VFAs [3,32,33,35,43], were selected for study (depicted in Table 10) in combination with three diluents. Sunflower oil has been proposed as a natural diluent to reduce toxic effects of extractants in fermentation systems [15,42,44,77], oleyl alcohol was suggested as a non-toxic diluent for solvent extractions [97] and kerosene has been used successfully with TOPO for the recovery of acids from anaerobic acidification broth by LLE [33]. Oleyl alcohol, canola oil and lamp oil (in the same chemical family as kerosene) were therefore investigated as potentially non-toxic diluents for application in AD systems (summarised in Table 11).

***Table 10**: Chemical structure of extractants selected for study.*

Name	Formula	Structural Diagram
Tri-n-butyl phosphate (TBP)	$C_{12}H_{27}O_4P$	

Name	Formula	Structural Diagram
Tri-n-octylphosphine oxide (TOPO)	$C_{24}H_{51}OP$	
Tri-n-octylamine (TOA)	$C_{24}H_{51}N$	
Methyltrioctylammonium chloride (Aliquat 336)	$C_{25}H_{54}ClN$	

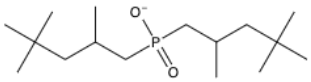
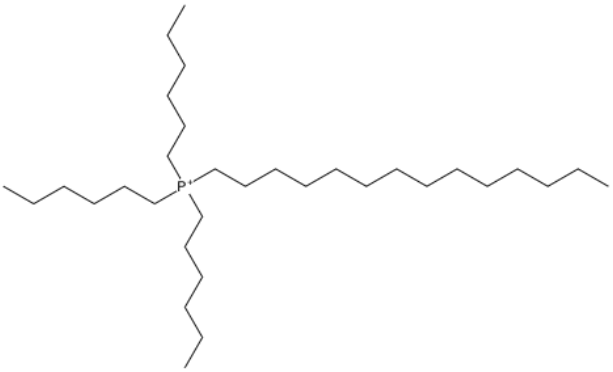
Name	Formula	Structural Diagram
Trihexyltetradecylphosphonium bis(2,4,4-trimethylpentyl)phosphinate ([$P_{666,14}$][Phos])	$C_{48}H_{102}O_2P_2$	 

***Table 11**: Summary of diluents selected for study.*

Diluent	Description
Oleyl alcohol ($C_{18}H_{36}O$)	Active, protic, low polarity, biodegradable
Lamp oil (C_{14}-C_{20})	Inert, aprotic, non-polar, non-biodegradable
Canola oil	Inert, aprotic, non-polar, biodegradable

CHAPTER 3
PROJECT SCOPE

It is clear from the literature that the AD process presents promising potential for the valorisation of waste and wastewater streams through the production of biogas, as well as the co-production of VFAs. There is potential for the extraction of VFAs using *in situ* LLE as a mechanism for continuous pH control, while VFA co-production exhibits process possibilities for resource recovery, as well as increased productivity of both VFAs and biogas production due to reduced inhibition. This project aimed to investigate the integration of an *in situ* extraction process to extract VFAs from AD systems using LLE, with back-extraction of the VFA products to regenerate the solvent and recover the VFAs into a product phase. However, in order to achieve this, a suitable extraction solvent needs to be selected. If the objective of the extraction is to control the VFA concentration to prevent acid accumulation, such that the pH of the system is maintained and the methanogenic archaea are not inhibited, then extractant selection may not necessarily be based primarily on the pursuit of the highest degree of VFA extraction. In the present study solvents were investigated in terms of extraction capacity at varying pH, biocompatibility with the methane-producing consortia and feasibility for VFA back-extraction, to establish whether a LLE system is potentially able to extract sufficient VFAs to reduce inhibition (thereby optimising methane production and recovering a valuable VFA co-product) within suitable pH ranges for AD, without negatively impacting the AD microbial population.

3.1 Aim

The aim of this project was to investigate the integration of an *in situ* separation technology to remove excess VFAs as they are produced from biogas-producing anaerobic digestion, for the co-production of VFAs without arresting methanogenesis. Extraction solvents were investigated based on (i) extraction capacity at varying pH, (ii) biocompatibility with the methane-producing microbial consortium and (iii) feasibility of VFA recovery from the extractant-diluent mixture using back-extraction, to establish whether a biocompatible solvent combination could be used in AD with the ability to co-produce biogas and VFAs in biogas producing digester systems.

3.2 Objectives

- Establish fundamental criteria for the development of a continuous *in situ* LLE operation in AD systems.
- Identify potential extractants and diluents for extraction of VFAs from AD systems.
- Determine the VFA extraction capacity of selected extractants and diluents at varied system pH.

- Establish whether biogas production is possible when solvents are introduced in an *in situ* manner in the AD system.
- Determine whether recovery of VFAs using alkaline back-extraction is possible.

3.3 Relevance

- Minimisation of VFA build-up in AD systems through partial removal of VFAs could result in reduction of inhibitory effect of VFAs and prevention of acid crash.
- Diversification of the slate of renewable chemicals and fuels that can be produced from an AD unit: VFA reclamation benefits from AD systems include the formulation of a valorised waste, further supporting the scope for the application of AD (for waste treatment, production of biogas as well as co-production of VFAs).
- Utilisation of cost advantage waste feedstock directly addresses a key cost barrier for energy generation and renewable chemicals.

3.4 Project hypotheses

- Simultaneous co-production of VFAs and biogas is possible in AD systems.
- *In Situ* LLE is an effective method for the extraction of VFAs from AD systems.
- Biocompatible solvents can be utilised for partial removal of VFAs from AD to reduce the inhibitory effects of VFAs.

3.5 Research questions

The main research question that this study addresses is whether an *in situ* LLE process can be applied to a biogas-producing AD system for the co-production of VFAs without arresting biogas production. To provide further insight, the main research question was coupled with further sub-questions:

1. What possible extractants and diluents can be used for the extraction of VFAs from dilute aqueous streams?
2. How is the extraction capacity of solvents influenced by the system pH and do solvents have extraction capacity to extract VFAs under a range of pH conditions?
3. How would the presence of impurities in AD systems influence the LLE capacity of solvents and which solvents could suitable for application in non-idealised systems?
4. Can these solvents be applied in an *in situ* manner in AD systems for the extraction of VFAs without inhibiting methanogens or arresting biogas production, and if so, how would the biogas productivity be influenced by the presence of the solvents in the system?
5. Is it possible to regenerate solvents by recovering VFAs using alkaline back-extraction and what would the recovery of VFAs be?

CHAPTER 4
MATERIALS AND METHODS

4.1 Experimental Plan

Extraction solvents were investigated on the basis of (i) extraction capacity varying pH, (ii) biocompatibility with the AD consortia and (iii) feasibility for back-extraction through the use of (a) laboratory scale LLE experiments using aqueous solutions containing dilute VFA concentrations at varied pH ranges and wastewater from an AD plant (b) bench-scale biogas production tests, and (c) back-extraction of solvents using sodium hydroxide to recover the extracted VFAs.

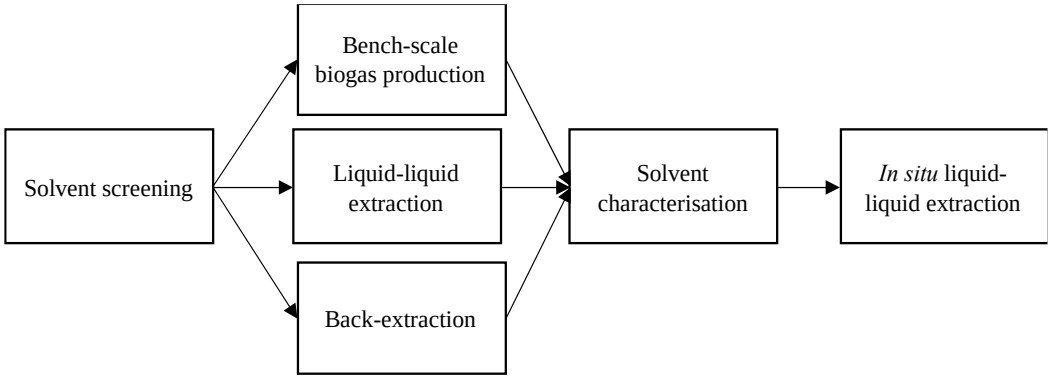


Figure 5: *Experimental approach utilised in the study.*

The following five extractants and three diluents were assessed using LLE experiments, bench-scale biogas production tests and back-extraction experiments.

Table 12: *Extractants and diluents selected for solvent screening.*

Extractants	Name	Extractant Type
TOA	Tri-*n*-octylamine	Tertiary amine
TBP	Tri-*n*-butylphosphate	Organophosphorus compound
TOPO	Tri-*n*-octylphosphine oxide	Organophosphorus compound
Aliquat 336	Methyltrioctylammonium chloride	Quaternary amine/ ionic liquid
$[P_{666,14}][Phos]$	trihexyl(tetradecyl)phosphonium bis-2,4,4- (trimethylpentyl) phosphinate	Ionic liquid

4.2 Materials

Acetic acid (HAc, >99.7%), propionic acid (HPr, >99.5%), butyric acid (HBu, >99%), valeric acid (HVa>99.7%), caproic acid (HCa, >99.7%), trihexyl(tetradecyl)phosphoniumbis-2,4,4-(trimethylpentyl)phosphinate (Phos >99.7%), tri-n-octylamine (TOA, 98%), tri-n-butylphosphate (TBP, 98%), Tri-n-octylphosphine oxide (TOPO, 98%), oleyl alcohol (98%), sodium hydroxide (NaOH >99%), hydrochloric acid (>99%), and perchloric acid (>99%) were purchased from Merck (Pty) Ltd, Johannesburg, South Africa. Methyltrioctylammonium chloride (Aliquat >90%) was purchased from KIMIX Chemical & Lab Supplies, Cape Town, South Africa. Lamp oil (C_{14}-C_{20}) was sourced from Sasol, Secunda, South Africa. Canola oil (99%) was purchased from the local supermarket.

4.3 Case study wastewater treatment facility

Industry wastewater substrate and inoculum were obtained from a wastewater treatment facility (WWTF) in Stellenbosch (Western Cape, South Africa) which experiences digester upset due to VFA accumulation. The facility combines and treats wastewater from three operational plants in Stellenbosch including effluent streams from grape processing, marula fruit processing, cider processing and occasionally small volumes of sewage waste at optimal temperature between 36-37°C. The plant biogas production varies due to seasonal fluctuations in the feed and effluent quality. The biogas (50-65% CH_4) is used by a steam boiler for the production of steam which is used for heating of the plant feed and surplus energy is available for use at the facility. The pH of the system is critical and is strictly maintained between 6.5-7.5 by dosing with either lime or $NaOH_{(aq)}$. Acid crash is typically experienced during overloading of the system, a sudden increase in influent COD or during temperature fluctuations

Fresh batches of substrate and inoculum were sourced and stored in a 50 L active CSTR anaerobic digester at 35-37°C at Stellenbosch University for further usage. The inoculum was manually fed with substrate on a weekly basis to ensure survival of the bacteria and archaea and sustained biogas production. The fermentation broth was subjected to subsequent bench-scale biogas production tests.

4.4 Solvent combinations

The five extractants selected for study were investigated in combination with three diluents, namely oleyl alcohol, lamp oil and canola oil to yield 16 resultant solvent combinations are summarised in Table 13.

Table 13: Extractant-diluent solvent combinations investigated in this study.

Solvent	Extractant	Diluent
1	Tri-*n*-octylamine (TOA)	Oleyl alcohol
2	Tri-*n*-octylamine (TOA)	Lamp oil
3	Tri-*n*-octylamine (TOA)	Canola oil
4	Tri-*n*-butylphosphate (TBP)	Oleyl alcohol
5	Tri-*n*-butylphosphate (TBP)	Lamp oil
6	Tri-*n*-butylphosphate (TBP)	Canola oil
7	Tri-*n*-octylphosphine oxide (TOPO)	Oleyl alcohol
8	Tri-*n*-octylphosphine oxide (TOPO)	Lamp oil
9	Tri-*n*-octylphosphine oxide (TOPO)	Canola oil
10	Aliquat 336	Oleyl alcohol
11	Aliquat 336	Lamp oil
12	Aliquat 336	Canola oil
13	$[P_{666,14}]$[Phos]	Oleyl alcohol
14	$[P_{666,14}]$[Phos]	Lamp oil
15	$[P_{666,14}]$[Phos]	Canola oil
16	$[P_{666,14}]$[Phos]	No diluent

The use of 10-20% extractant in diluent has been suggested to avoid solvent toxicity as well as increased organic phase viscosity and third emulsion phase formation at the interface between aqueous and organic phases in LLE systems [36,42,44]**.** Extractant concentrations of 20% v/v in diluent (80% v/v) were used in the present study.

4.5 Model aqueous solutions

LLE experiments were performed using synthetic model aqueous solutions containing only VFAs and water. Acid concentrations were selected to resemble similar total VFA concentrations exhibited in AD systems. The growth rate of anaerobic microorganisms in AD systems depends highly on the composition of the feedstock [29]. Therefore, the concentration and proportion of dominant VFAs differs across systems, substrates and bacterial populations, depending on substrate loading, retention times and pH conditions, as seen in various studies with varying concentrations of acetic, propionic, butyric, valeric and caproic acids produced [18,20,32,33,63,72]. Table 14 illustrates a few reported VFA concentrations observed in literature.

Table 14: *Summary of various reported VFA concentrations in AD systems.*

Reported VFA Concentrations	Source
Typical VFA concentrations of acetic, propionic and butyric acid between 2.5-10 g/L in fermented wastewater systems.	[32]
Maximum VFA concentrations for stable AD performance 13 g/L.	[63]
Acetate concentrations between 2-5 g/L, n-butyrate between 1-10 g/L, and propionate between 2-6 g/L.	[72]

The VFA concentrations used in the present study to create a simplified system are summarised in Table 15.

Table 15: *Composition of model aqueous solutions used in LLE experiments.*

Component	Chemical formula	Concentration (g/L)	pKa [78]
Acetic acid	CH_3COOH	2.5	4.76
Propionic acid	CH_3CH_2COOH	2.4	4.88
Butyric acid	$CH_3(CH_2)_2COOH$	2.4	4.82
Valeric acid	$CH_3(CH2)_3COOH$	0.5	4.84
Caproic acid	$CH_3(CH_2)_4COOH$	0.5	4.88

4.6 Liquid-liquid extraction

Laboratory scale LLE experiments were conducted with the solvent combinations listed in Table 13 using model aqueous solutions (Table15) and wastewater from the WWTF AD plant to note the effect of impurities on extraction efficiency. Extractions using synthetic VFA solutions were carried out at three pH intervals (pH 3.9, pH 5.6 and pH 6.8), with pH adjustments done using sodium hydroxide NaOH (2M). Extractions using wastewater were not pH adjusted. The pH of the aqueous solutions was measured before LLE and after the extraction, where the effect of VFA removal on the pH of the solution was noted.

Extractant (1 mL) was combined with diluent (4 mL) in a 15 mL centrifuge tube for all extractant-diluent solvent combinations. For extraction using extractant without diluent, 1 mL of extractant was used as the solvent. Mixtures were vortexed for 5 minutes to ensure a homogenous solvent. Thereafter 5 mL of aqueous VFA solution was added to the solvent and the system was vortexed for 5 minutes to promote mixing. The centrifuge tubes were placed horizontally in a temperature controlled orbital shaking incubator (model LM-575D) shaking at 150 rpm, at temperature 35±1°C for 24 hours to reach equilibrium. After mixing, phases

were separated using a Hermle Z366 centrifuge at 8000 rpm for 5 minutes to ensure complete separation of the aqueous phase and the organic phase. After the two phases were separated, the aqueous phase was withdrawn and prepared for HPLC analysis. Experiments were carried out in triplicate for statistical significance.

The organic extract VFA concentration at equilibrium was determined by mass balance using the initial aqueous phase VFA concentration and equilibrium aqueous phase VFA concentration following LLE:

$$([TA]_o)_{extract} = \frac{([TA]_{aq}V_{aq})_i - ([TA]_{aq}V_{aq})_{eq}}{(V_o)_{extract}} \qquad \textit{[Equation 8]}$$

where V_{aq} and V_o refer to the volume of aqueous and organic phases (mL) respectively. The extent to which the VFAs were extracted was expressed as the degree of extraction (E%), the weight percentage of acid transferred from the aqueous phase into organic phase, illustrated in Equation 6. The degree of extraction can be expressed by Equation 9 by substituting Equation 8 into Equation 6 (details in Appendix C). Summation of VFA concentrations were expressed as total VFA concentration (tVFA) as well as separate VFAs (acetic, propionic, butyric, and valeric) for extraction calculations.

$$E\% = 1 - \frac{([TA]_{aq}V_{aq})_{eq}}{([TA]_{aq}V_{aq})_i} \qquad \textit{[Equation 9]}$$

The initial and equilibrium VFA concentrations of the organic and aqueous phases are reported in Appendix E. Uncertainty was expressed in terms of the uncertainty parameter (Δ), with significance level (α) = 0.05 and sample size (n) = 3. Details of error propagation can be found in Appendix C.

4.7 Bench-scale biogas production

Standard biochemical methane potential (BMP) test protocols outlined in literature [28,61,73] were utilised for the design of bench-scale biogas production tests to assess the biocompatibility of a series of extractants and diluents. Gas production of less than 5 L/day was confirmed before obtaining inoculum for the bench-scale biogas production tests. As suggested by Angelidaki *et al.* (2009) and Holliger *et al.* (2016), coarse inert materials were removed from the inoculum and substrate samples with minimal additional preparation to avoid alteration of the properties and digestibility of the materials. The inoculum was degassed (pre-incubated in order to deplete the residual biodegradable organic material present) until no significant methane production was observed.

The biomass was characterized with regard to total (TS) and volatiles solids (VS). The dry inorganic and organic compounds are expressed as TS, and are measured according to standard protocol where the biomass is heated up to 105 °C in order to remove all water content.

$$TS\% = \frac{m_{dried}}{m_{wet}} \quad \textit{[Equation 10]}$$

VS content provides an estimation of the organic compounds in the sample and is determined by heating the sample up to 550 °C for 2 hours to burn off the organic matter. The weight difference between the sample after heating at 105 °C and 550 °C reflects the VS content of the biomass. VS is expressed as the ratio between the difference in the amount of sample after drying and burning, and the initial amount of sample.

$$VS\% = \frac{m_{dried} - m_{burned}}{m_{wet}} \quad \textit{[Equation 11]}$$

For wastewater samples, chemical oxygen demand (COD) is often used instead of VS due to the low masses obtained in the samples [100]. The COD of samples was determined using Merck test kits (details in Section 4.10). Bench-scale biogas production tests were conducted in triplicates using 100 mL serum bottles (small volumes are suitable for homogenous substrates [73]). Three vessels were used as blanks containing only inoculum, three vessels as reference controls with substrate and inoculum, and the rest of the vessels contained samples of inoculum, substrate and solvent. A low headspace volume of 30% and an inoculum to substrate ratio (ISR) of three was selected in order to generate high gas volumes. With a 30% headspace, the serum bottles had a total working volume of 70 mL available for the substrate, inoculum and solvent. A volume of 5 mL solvent (20% v/v extractant) was added to the substrate and inoculum to yield a total liquid volume of 70 mL. Volumes of the blank tests and control tests were topped up to a volume of 70 mL with deionized water. The mass of the inoculum and substrates for the tests were calculated using the ISR and the constraint of a total volume of 65 mL for the inoculum and substrate. The ISR, defined by the AMPTS II method [100] is described in Equation 12, where m_{Is} and m_{Ss} are the total masses of inoculum and substrate in the sample. The ISR was based on the chemical oxygen demand (COD) rather than volatile solids (VS) due to the low VS of the wastewater.

$$ISR = \frac{m_{Is}COD_{Is}}{m_{Ss}COD_{Ss}} = 3 \quad \textit{[Equation 12]}$$

$$\frac{m_{Ss}}{\rho_S} + \frac{m_{Is}}{\rho_I} = 65\ mL \quad \textit{[Equation 13]}$$

The test batches were prepared such that there was minimal contact with air. The headspaces of the serum bottles were flushed with nitrogen gas for 60 s and thereafter the vessels were sealed with gastight rubber septas to create an anaerobic environment. The bench-scale biogas production tests were conducted for a duration of four to five weeks at a temperature of 35±2°C, stored in a temperature-controlled environment, with shaking of the bottles daily to promote mixing. The once a day mixing strategy for the bench-scale biogas production tests was adopted according to methodology used for the execution of BMP syringe tests, where it has been reported that manual mixing once a day is sufficient [28,73].

Triplicate biogas samples were collected using a gas tight syringe and subjected to gas chromatography (GC) analysis once per week. The volume of methane produced was obtained by multiplying the volume of biogas collected by the percentage of CH_4 in the headspace, as determined by GC analysis.

$$V_{CH_4} = V_{biogas}(\%CH_4) \qquad \textit{[Equation 14]}$$

The methane yield (or methane potential) was calculated as the volume of biomethane produced per amount of organic substrate material added (expressed per mass of COD added) to each of the reactors, with subtraction of methane produced from the blank. This was determined as the difference between the accumulated volume of biomethane from the reactor containing the sample with inoculum and substrate (V_S) and the volume of biomethane coming from only the inoculum present in the sample bottle is (V_I), divided by the mass of COD of the substrate in the sample bottle ($m_{COD,Ss}$).

$$Methane\ Yield\ \left(\frac{mL}{gCOD}\right) = \frac{V_S - V_I}{m_{COD,Ss}} \qquad \textit{[Equation 15]}$$

The volume of biomethane coming from the inoculum present in the sample bottle is (V_I) was determined as the ratio between the total amount of the inoculum in the sample (m_{Is}) and the one in the blank (m_{Ib}).

$$Methane\ Yield\ \left(\frac{mL}{gCOD}\right) = \frac{V_S - V_I}{m_{COD,Ss}} = \frac{V_S - V_B\left(\frac{m_{Is}}{m_{Ib}}\right)}{m_{COD,Ss}} \qquad \textit{[Equation 16]}$$

For the calculation of the biomethane yields of tests containing solvent and the control, the biomethane production standard deviation (SD) of blanks and sample tests were taken into account by using the formula:

$$Yield_{s/control} = Yield_{avg,test/control} \pm \sqrt{(SD_b)^2 + \left(SD_{s/control}\right)^2} \qquad \textit{[Equation 17]}$$

The theoretical COD of methane is 64 gram of oxygen per mole of methane [101]. 1 mole of methane can therefore be generated per 64g of COD. For every gram of COD, 350ml of methane can theoretically be produced.

$1\ mole\ of\ CH_4 = 22.4\ L\ at\ STP, per\ 64\ g\ COD$

$\therefore 1\ g\ COD = \frac{22.4\ L\ CH_4}{64} = 350\ mL\ CH_4$

The volume of generated biogas can therefore be predicted using a COD to methane conversion ratio for conditions at STP illustrated below [28].

$$1\ g\ COD = 0.35\ L\ CH_4 \qquad \textit{[Equation 18]}$$

The bench-scale biogas production tests were terminated when CH_4 production in the inoculum-substrate control (without solvent) tests was negligible (<5 ml/day) and daily methane production during three consecutive days was less than 1% of the accumulated volume of methane as suggested in literature [73,102].

4.8 Back-extraction

VFAs were recovered out of the solvent extract phase using back-extraction with 1M $NaOH_{(aq)}$. Separated loaded organic solvents from LLE experiments (Section 2.5) were contacted with $NaOH_{(aq)}$ in 1:2 volumetric ratio by combining 5 mL of solvent with 10 mL of 1M NaOH in a centrifuge tube. The solutions vortexed for 5 minutes to promote mixing. Centrifuge tubes were placed horizontally in a temperature controlled shaking incubator at 30 ± 1°C at 200 rpm for 24 hours to reach equilibrium. Thereafter phases were separated using a centrifuge at 8000 rpm for 5 minutes. The aqueous phase was removed from the system and prepared for HPLC analysis. Experiments were carried out in triplicate for statistical significance.

The weight percentage of acid transferred from the organic extract phase into the alkaline aqueous phase was expressed as the percentage recovery (R%) according to Equation 19. Summation of VFA concentrations were expressed as total VFA concentration (tVFA) as well as separate VFAs (acetic, propionic, butyric, and valeric) for recovery calculations.

$$R\% = \frac{([HA]_{aq}V_{aq})_{equilibrium}}{([HA]_{o}V_{o})_{extract}} \qquad \textit{[Equation 19]}$$

Details of error propagation can be found in Appendix C.

4.9 *In situ* liquid-liquid extraction

A modified semi-partitioned reactor setup [103] for *in situ* LLE of VFAs, with recovery of VFAs using $NaOH_{(aq)}$ in an external back-extraction unit was used to remove VFAs continuously from a synthetic VFA aqueous solution. A volume of 1 L of 20% TOA in oleyl alcohol was used as the extraction solvent and 250 mL of 6M $NaOH_{(aq)}$ was used as the alkaline stripping medium. The contents of the reactor were stirred using an impeller at 150-200 rpm at room temperature. The extract phase was continuously removed from the system at 19 mL/min and pumped into the back-extraction unit containing $NaOH_{(aq)}$, where the solvent could bubble up through the alkaline medium. The stripped solvent was simultaneously continuously pumped from the back extraction into the dispersed phases at 19 mL/min. The VFA concentration and pH of the system were recorded in order to evaluate the efficacy of the modified reactor for *in situ* VFA extraction and to elucidate the pH control mechanism through monitoring of the gradual pH increase (preliminary results presented in Appendix A).

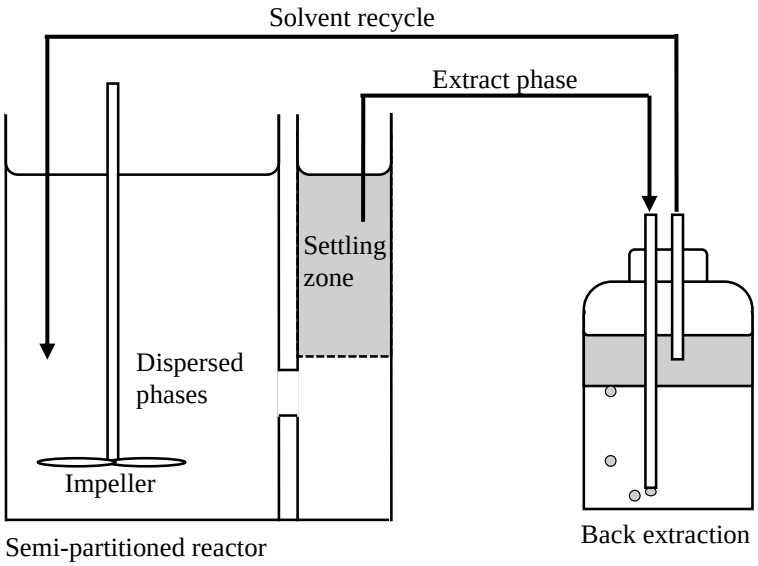


Figure 6: *Semi-partitioned reactor* [103] *schematic used for in situ LLE of VFAs from aqueous solution.*

4.10 Analysis

The biogas methane concentration was analysed by gas chromatography (GC) analysis using a GAS Compact GC 4.0 (Global Analyser Solutions), using a TCD detector with TCD temperature 110°C and helium carrier gas, to determine the methane content of the biogas produced. VFA analysis of the aqueous phase was done using high performance liquid chromatography (HPLC) analysis with a Dionex Ultimate 3000 HPLC, Biorad Aminex HPX-87H column. Column temperature of 65°C was used, eluent of 0.6 mL/min 5 mM. Sulphuric Acid UV Detection 210 nm. Standards were purchased from Sigma Aldrich. The pH of samples was measured using a HANNA (HI 5522) pH meter. The total COD content was determined using Supelco Spectroquant COD Cell Test (photometric 500-10 000 mg/L COD), total nitrogen content was determined using Supelco Spectroquant Nitogen (total) Cell Test (photometric 0.5-15 mg/L N) and alkalinity of samples was quantified using Supelco Spectroquant Acid Capacity (total alkalinity) Cell Test to pH 4.3 (photometric 0.40-8.00 mmol/L 20 - 400 mg/L $CaCO_3$). The moisture content of the substrate and inoculum were determined using a Kern DBS sample analyser (DBS 60-3).

CHAPTER 5
RESULTS AND DISCUSSION

5.1 Liquid-liquid extraction from synthetic solutions containing only water and acids

To demonstrate *in situ* VFA extraction during biogas producing AD, first one must demonstrate LLE of VFAs within appropriate pH ranges where both fermentation and extraction are possible. It is therefore essential to identify appropriate solvents with the capacity to extract carboxylic acids at varied pH conditions. The extractants TOA, TBP, TOPO, [$P_{666,14}$][Phos] and Aliquat 336, with reported affinity for VFAs [3,32,33,35,43], were selected for the investigation in combination with three diluents, namely oleyl alcohol, lamp oil and canola oil. There is limited published information regarding the performance of these extractants at higher pH ranges, and their ability to simultaneously extract VFAs that differ in length. Extraction efficiency is highly dependent on the number of carbons in the acid to be extracted, where longer acids tend to result in higher extractions (this is further explored in Section 5.1.3), which makes it difficult to compare solvent extraction capacities across different studies. Furthermore, these extractants have not previously been directly compared under similar conditions with the same sets of diluents for the extraction of multiple acids. These experiments aimed to extend VFA extraction knowledge by giving a controlled, repeated comparison between extractants with different diluents and their ability in extracting a range VFAs from aqueous streams.

Liquid-liquid extraction experiments were performed at three pH setpoints: below the lowest pKa of the acids to be extracted (< pH 4.8), above the pKa of the acids (> pH 5.5) and substantially higher than the pKa at optimal methanogenic pH (between pH 6.5 and 7.2 [16,27]). This was to determine whether extractants have the capacity to extract VFAs within suitable pH ranges for AD, to potentially minimise VFA build-up in the AD system, noting that the aim is to not completely remove VFAs but rather control the VFA concentration to prevent accumulation and hence, to avoid negative inhibitory effects on the AD consortia. While sustained biogas production in AD systems at pH 3.9 is likely not possible, the low pH was investigated to validate experimental methods and results with values reported previously in the literature, where most studies have carried out LLE experiments below the pKa of the acids to ensure predominance of undissociated forms of VFAs [32,33,35,36]. Additionally, when there is overproduction of VFAs, the digester pH decreases, which in turn inhibits the methanogens. If the partial removal of VFAs is to be used as a pH control mechanism, it would be necessary for solvents to be capable of extracting VFAs at low pH values in cases of severe acid crash (i.e. when the pH drops well below the optimal pH range for methanogen growth, resulting in inhibition of methanogenesis), when acids are in excess. A range of pH values were, therefore, investigated to establish the VFA extraction capacity of the solvents for a range of potential conditions.

Section 5.1 investigates LLE using model aqueous solutions (details in Section 4.5), which do not contain impurities, to enable the evaluation of the extraction capacity of the solvents without additional contaminants and microorganisms which may influence the extraction equilibria. The mode of contact in the LLE experiments was through continuous mixing at 200 rpm for 24 hours, placing the centrifuge tubes horizontally in a temperature-controlled shaking incubator at 35 ± 1°C. It has been reported that the time to reach equilibrium was less than 1 h for VFA extractions with continuous stirring of feed-solvent systems [32,33]. It was therefore assumed that equilibrium was attained after 24 h for all solvents, enabling direct comparison of the VFA extraction capacity of the solvents under the same conditions. Extractions were carried out using the extractant-diluent combinations listed in Table 13. Results are presented in terms of degree of VFA extraction (E%) in the following sections. Section 5.1.1 aimed to evaluate the performance of the selected extractants with each of the diluents under the same experimental conditions, while Section 5.1.2 presents the consolidated extraction results so as to compare and evaluate the relative extraction performance across solvents containing different extractants at varied levels of acid dissociation.

5.1.1 Extractant performance with different diluents

The use of conventional diluents alone to extract acids from dilute aqueous streams has been reported unsuitable due to low degree of acid extraction, which would result in increased processing costs due to a requirement for high solvent flow rates and significant dilution of acids. Most extractants are viscous and are, therefore, widely used together with diluents to ease handling and improve the physical properties of the extractant. The diluent generally functions as a medium to dilute the extractant to a desired concentration in a solvent mixture and to control the density and viscosity of the solvent phase [36]. However, diluents can also have an impact on the extraction equilibria of the LLE. It is, therefore, necessary to select a suitable diluent that does not interfere with the capacity of the extractant to extract VFAs from aqueous solutions.

It has been noted in literature that higher proportions of extractant improve extraction efficiency, however, increasing extractant concentration also tends to intensify solvent toxicity and a trade-off between high extractability and biocompatibility was thus suggested [38]. Higher extractant concentrations (>25%) also pose the problem of increased organic phase viscosity and third emulsion phase formation at the interface between aqueous and organic phases when using viscous extractants, which is not favourable for extractions. To overcome these challenges the use of 10-20% extractant in diluent has been suggested in literature [42,44,77], and 20% v/v extractant in diluent was utilised in the current study for LLE experiments with extractant-diluent mixtures.

This section will evaluate each one of the extractants (TOA, TBP, TOPO, [$P_{666,14}$][Phos] and Aliquat 336) separately in combination with diluents oleyl alcohol, lamp oil and canola oil to assess how the diluents influence the extraction capacity. Extractants paired with diluents provided varying levels of total VFA (tVFA) extraction (total acetic, propionic, butyric, valeric and caproic acid) at different pH ranges, where the degree

of extraction was significantly impacted by the diluent with some extractant-diluent combinations and remained fairly consistent with others. Figure 7 illustrates the degree of tVFA with TBP and diluents oleyl alcohol, lamp oil and canola oil. TBP alone has been reported to yield favourable acid extractions [35]. However, due to the high viscosity and density of the extractant, it is preferable to combine TBP with a lower density, lower viscosity diluent to facilitate phase separation for continuous extraction of VFAs. The extraction capacity of TBP was consistent with all three diluents, achieving extractions of 56.8 ± 0.1%, 56.7 ± 1.6% and 53.8 ± 0.7% with TBP-oleyl alcohol, TBP-lamp oil and TBP-canola oil respectively at pH 3.9. While tVFA extraction decreased at pH 5.6 using TBP-oleyl alcohol, TBP-lamp oil and TBP-canola oil with respective tVFA extractions of 13.3 ± 0.5%, 14.7 ± 0.6% and 15.9 ± 0.8%, the extraction capacity was consistent at the higher pH with the use of all three diluents. The use of aprotic and protic diluents had a similar effect, with comparable extractions achieved with all TBP-diluent combinations.

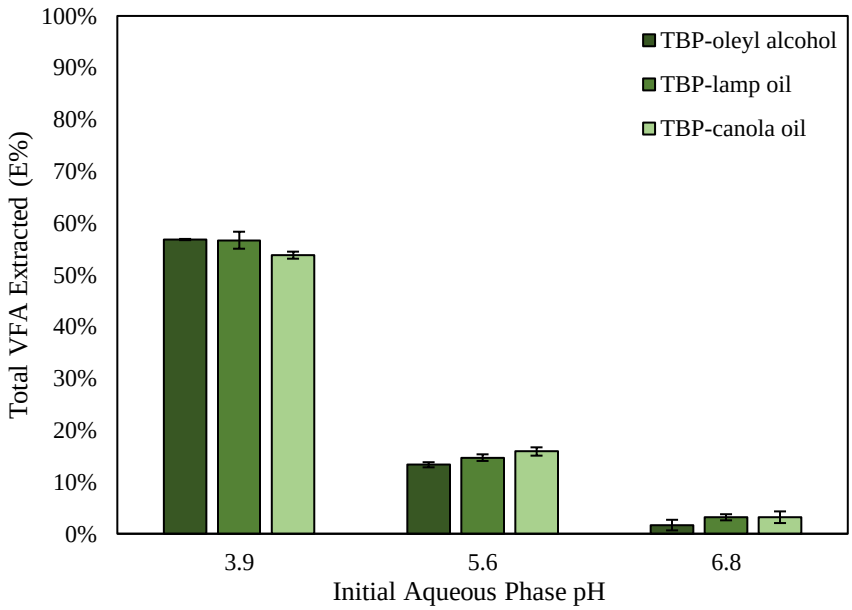


Figure 7: *Percentage total VFA extracted from aqueous phase using extractant TBP with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to tVFA extracted.*

TOPO is advantageous as an extractant due to its high boiling point and chemical stability. However, the extractant is solid at room temperature, with a melting point between 50-54 °C. It is therefore necessary to dissolve TOPO in a liquid diluent for use in LLE. Extractions of 60.2 ± 3.7%, 80.4 ± 4.9% and 75.7 ± 0.8% were achieved at pH 3.9 with TOPO-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil respectively. Similar reported results were obtained using 20% TOPO in kerosene at pH 2.5 [33]. The use of diluents lamp oil and canola oil appeared to provide enhanced extraction performance with TOPO relative to oleyl alcohol at pH of

3.9, as seen in Figure 8. The diluent effect was less apparent at higher pH values with respective tVFA extractions of 19.8 ± 3.2%, 22.6 ± 1.1% and 20.8 ± 6.6% attained at pH 5.6 and 7.8 ± 1.0%, 13.0 ± 0.8% and 9.9 ± 1.4% at pH 6.8 for TOPO-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil.

According to Keshav, Wasewar and Chand (2008a), organophosphorus compounds are chemically stable, with a marked tendency toward intermolecular hydrogen bonding. Due to the presence of both electron donor and electron acceptor groups within the extractants, specific interactions such as self-association and molecular complex formation take place with diluents and solutes [35]. The presence of protic and aprotic diluents did not appear to limit the solvent capacity considerably, with efficient extractions achieved using both TBP and TOPO with diluents oleyl alcohol, lamp oil and canola oil.

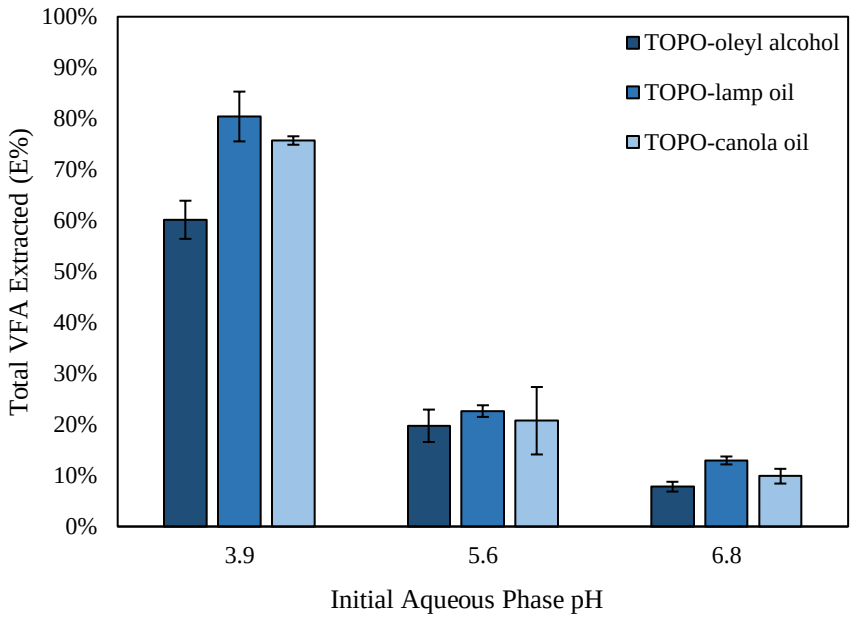


Figure 8: *Percentage total VFA extracted from aqueous phase using extractant TOPO with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

Extraction using TOA was more dependent on the nature of the diluent than other extraction systems. This behaviour concurs with what has been reported in literature, where polar and nonpolar diluents were less active in improving extractions with Aliquat 336 and TBP compared to TOA [35,42]. The use of active and inert diluents had a marked impact on the extraction capacity of TOA, illustrated in Figure 9. The use of TOA with oleyl alcohol resulted in more than double the degree of extraction compared to lamp oil, with results obtained using TOA-oleyl alcohol comparable to published results using 20% TOA in n-octanol for acetic acid extraction [32] and propionic extraction using 30% TOA in oleyl alcohol [36]. Extraction achieved using TOA-canola oil was also similar to published results for propionic extraction with 20% TOA in sunflower oil [77].

Extractions of 81.6 ± 0.7%, 33.1 ± 0.5% and 48 ± 0.5% tVFA were attained for TOA-oleyl alcohol, TOA-lamp oil and TOA-canola oil respectively at pH 3.9. These results indicate that the use of inert diluents limited the capacity of TOA to extract VFAs. It has been noted that TOA is a relatively poor solvating medium and the ability of the extractant to load more acid increases with increasing diluent activity [35]. It may be expected that diluents which are non-polar in nature would yield low extractions, as it has been reported that non-polar diluents do not provide stabilization to the acid-extractant complex [94]. However, oleyl alcohol is generally considered non-polar in nature [104], with particularly small polar head groups [105]. The high extraction achieved using low polarity diluent oleyl alcohol can therefore not be explained by the polarity rationale alone. Keshav, Wasewar and Chand (2008b) interpreted oleyl alcohol's high degree of extraction as a result of the ability of the diluent to solvate complexes and to prevent the complexes from interacting with one another. High extractions of acid using TOA-oleyl could therefore be attributed to the provision of enhanced solvation of TOA-acid complexes with the use of oleyl alcohol [36].

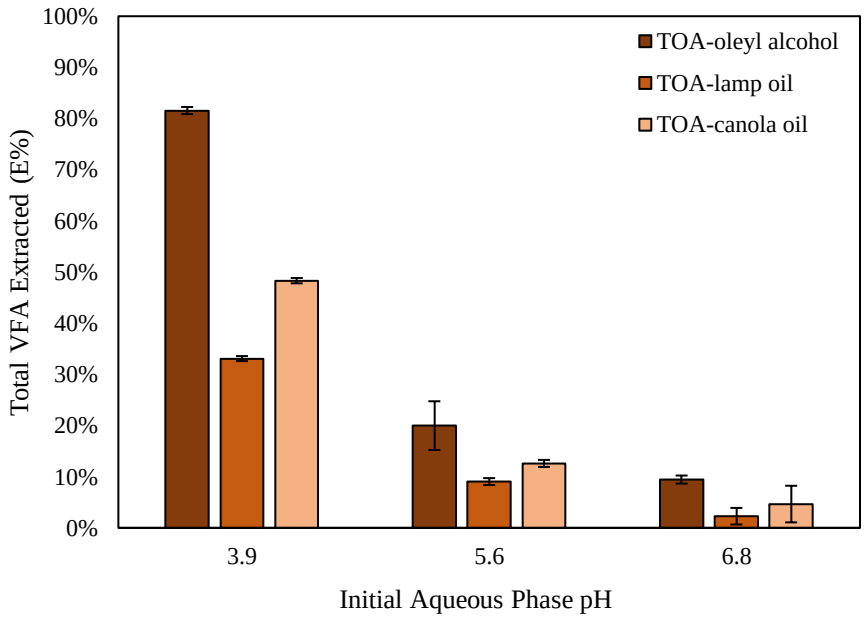


Figure 9: *Percentage total VFA extracted from aqueous phase using extractant TOA with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

Aliquat is highly viscous, therefore lower viscosity diluents are required for easier solvent handling. Lower solvent viscosity decreases the surface tension at the interface and facilitates good phase separation. Additionally, high viscosity at the interface has been reported to hinder the reaction between extractant and acid, hampering the transfer of the complex. The diluent can thus enhance solvation and penetration of the complex into the organic phase from the interface [35]. It can be seen from Figure 10 that use of protic and

aprotic diluents yielded similar degrees of tVFA extraction with Aliquat at all three pH ranges with all three diluents, contrary to the extraction trends seen using the other extractants. Aliquat-oleyl alcohol attained extractions of 65.3 ± 0.6%, 66.9 ± 0.8% and 63.8 ± 1.3% at pH 3.9, pH 5.6 and pH 6.8 respectively while Aliquat-lamp oil attained extractions of 66.9 ± 0.8%, 60.3 ± 1.5% and 55.6 ± 1.0% and Aliquat-canola oil achieved 67.4 ± 0.6%, 60.7 ± 2.9% and 53.8 ± 0.8% at pH 3.9, pH 5.6 and pH 6.8 respectively. These extractions are comparable to published results using 20% Aliquat 336 with sunflower oil for propionic acid extraction [77]. All three solvent combinations were capable of extracting significant tVFA at pH below the pKa, as well as pH values above the pKa of the acids to be extracted. This would imply that the quaternary ammonium chloride solvent extracted both dissociated and undissociated forms of VFAs, and the use of protic as well as aprotic non-polar diluents are suitable for use with Aliquat for LLE of VFAs. This corresponds with literature, where more Aliquat-acid complexes were formed when more diluent was present to solvate the complexes, which could be penetrated into the organic phase from the interface [35,42].

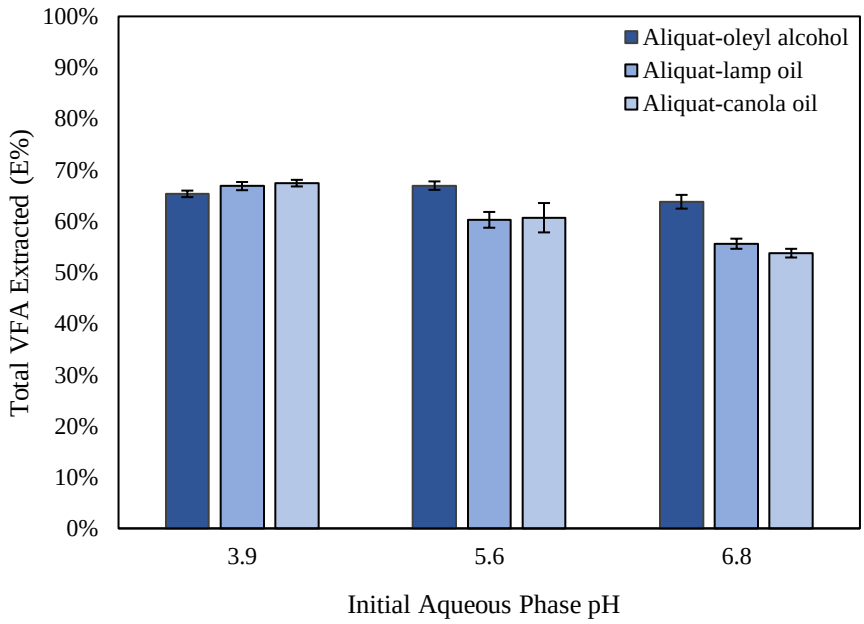


Figure 10: *Percentage total VFA extracted from aqueous phase using extractant Aliquat with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

Reyhanitash *et al.* (2016) identified [$P_{666,14}$][Phos] as a promising VFA-extracting solvent with the ability to deliver a concentrated VFA stream at a low solvent to feed ratio, where the extractant was used without a diluent in the LLE study. Due to the high viscosity of the IL, [$P_{666,14}$][Phos] was studied in combination with diluents oleyl alcohol, lamp oil and canola oil to determine its efficiency as an extractant in combination with various diluents. Extractions were also carried out using [$P_{666,14}$][Phos] alone as a solvent without diluent, to

enable comparison of the extraction performance using the same volume of extractant with and without diluents. The various degrees of extraction are depicted in Figure 11, where it can be seen that the extraction performance of $[P_{666,14}][Phos]$ was consistent with all three diluents, achieving extractions of 62.6 ± 0.9%, 70.5 ± 1.0% and 68.0 ± 5.4% tVFA with $[P_{666,14}][Phos]$-oleyl alcohol, $[P_{666,14}][Phos]$-lamp oil and $[P_{666,14}][Phos]$-canola oil respectively at pH 3.9. These extractions were in line with those achieved using $[P_{666,14}][Phos]$ without a diluent, which attained tVFA extraction of 68.9 ± 2.72% at pH 3.9. The dilution of $[P_{666,14}][Phos]$ in the extractant-diluent mixtures did not appear to enhance or impede the performance of $[P_{666,14}][Phos]$ as an extractant, and it would therefore be recommended to use $[P_{666,14}][Phos]$ in combination with diluents for LLE. The extraction capacity of $[P_{666,14}][Phos]$ drastically decreased above the pKa of the acids with negligible tVFA extractions at pH 5.6 and pH 6.8, regardless of the diluent (or absence of diluent) used. This information would be useful in the design of an extraction system for the recovery of VFAs using $[P_{666,14}][Phos]$, particularly when necessary to control the density or viscosity of the solvent.

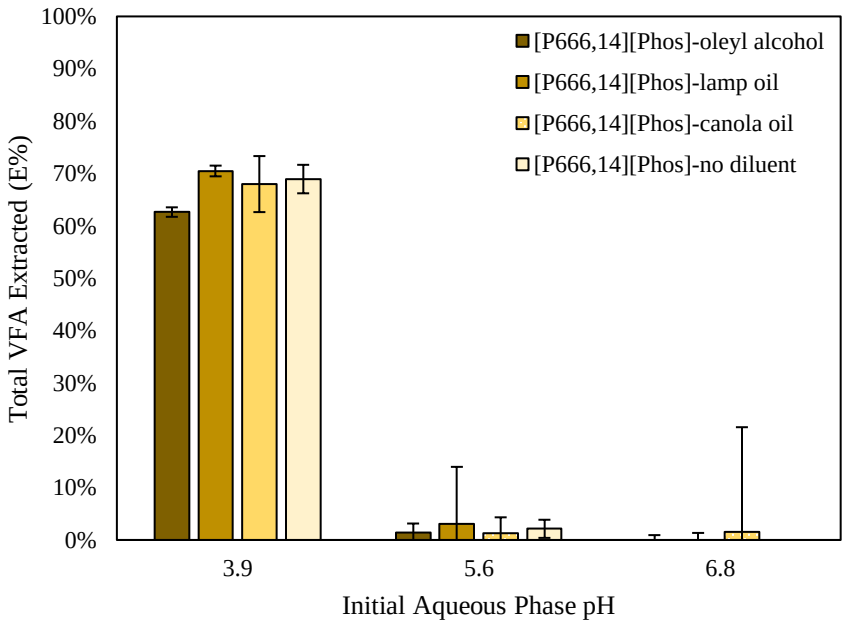


Figure 11: *Percentage total VFA extracted from aqueous phase using extractant $[P_{666,14}][Phos]$ with oleyl alcohol, lamp oil, canola oil and no diluent at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

It can be concluded that in addition to providing solution for extractants, diluents can also affect the extraction process and improve the equilibrium through stabilisation of the acid-extractant complexes. Although polar diluents have been reported more favourable than non-polar solvents for extraction of organic acids [35,38], the current study indicated that this is not always the case. As pointed out by Keshav, Wasewar and Chand (2008a), active polar diluents can be favourable for the solvation extractant-acid complexes and can enhance

neutralization reactions between acid and extractant. However, as seen with extractions using TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos] in combination with diluents oleyl alcohol, lamp oil and canola oil, enhanced extraction power can also be achieved with the use of low polarity diluents, with increased stabilisation of the acid-extractant complexes.

5.1.2 Comparison solvent extraction capacity at varying pH

The LLE results discussed in Figures 7-11 are consolidated and summarised for extractions using organophosphates and tertiary amines in Figure 12, and ionic liquid extractions in Figure 13, to enable direct comparison of extraction performance between different extractants and to evaluate their relative performance at varied levels of acid dissociation. As expected, VFA extraction capacity with TOA, TBP and TOPO was strongly pH dependent, evident from increased VFA extraction at low pH values. This result corresponds with literature, where several extractants and solvents have been applied for carboxylic acid recovery with the best results for VFA separation at pH values below the pKa of the acids that were extracted [32,33,35,36,77]. When the pH is lower than the pKa of the acids to be extracted, the acids are expected to exist mainly in their undissociated form in the aqueous phase, with negligible effect of acid dissociation. Generally, TOA-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil resulted in the highest VFA recoveries at the lowest pH value of 3.9 with respective recoveries of between 75-80%. Increasing the pH to pH 5.6 and above resulted in the extraction efficiency decreasing by more than 50% for all combinations containing TOA, TBP and TOPO. The pH dependency of VFA extraction demonstrated in Figure 12 could be attributed to the fact that when the pH is lowered, the dissociation equilibrium shifts towards the presence of protonated, molecular acids and extractions that are controlled by solvation or hydrogen bonding are largely determined by the concentration of the undissociated acid, which strongly depends on the pH of the system lying close to or below the pKa of the acid (listed in Table 15) [79]. It may be that these solvents are predominantly capable of extracting undissociated acid molecules at acidic pH values, through the mechanism of complexation or hydrogen bonding of the protonated molecular acid with the solvent, as suggested in literature [32].

Despite the fact that extraction capacity was reduced at the higher pH set points, tVFA extractions of 20-25% were achieved at pH 5.6 and 10-15% at the highest pH of 6.8 using TOA-oleyl alcohol, TOPO-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil. TBP-oleyl alcohol, TBP-lamp oil, TBP-canola oil, TOA-lamp oil and TOA-canola oil achieved extractions of 10-15% at pH 5.6. These solvents could potentially be applied *in situ* for excess acid removal, where the aim is to control the concentration of excess VFAs while not completely removing all the acids from the system. Although sufficient extraction efficiency is necessary to recover VFAs from AD systems, it should be noted that VFAs are important precursors for methane production in the AD process [63], and biogas production would be arrested if all produced VFAs were extracted using LLE. The experimental approach was therefore not complete removal of VFAs at all pH setpoints, as this would remove the carbon source for methane production, but rather partial removal of VFAs (which could potentially be

achieved at 10-25% VFA removal) to reduce the inhibitory effects of VFA accumulation and prevent acid crash. Fermentation rates and yields tend to be higher at a higher pH, contrary to the acid extraction rate, which is typically higher at lower pH values [37]. Extractive fermentation could, therefore, reach a regulating balance between excess acid production and acid extraction. When VFAs are produced in excess, the pH of the system decreases, which could lead to acid crash. On the other hand, the LLE efficiency increases as the pH of the system decreases. Therefore, as the pH drops, more acid is extracted, which would result in an increase in pH, which implies that the system would be hypothetically self-regulating. The increase in pH in turn reduces the VFA extraction via LLE. As the system approaches acid crash, excess VFAs could potentially be extracted to maintain a higher system pH, and when the pH increases, the rate of VFA removal decreases, which would eliminate the potential of complete VFA removal. The net result is possibly a pH control mechanism that benefits methanogenesis, enabling simultaneous VFA extraction and biogas production.

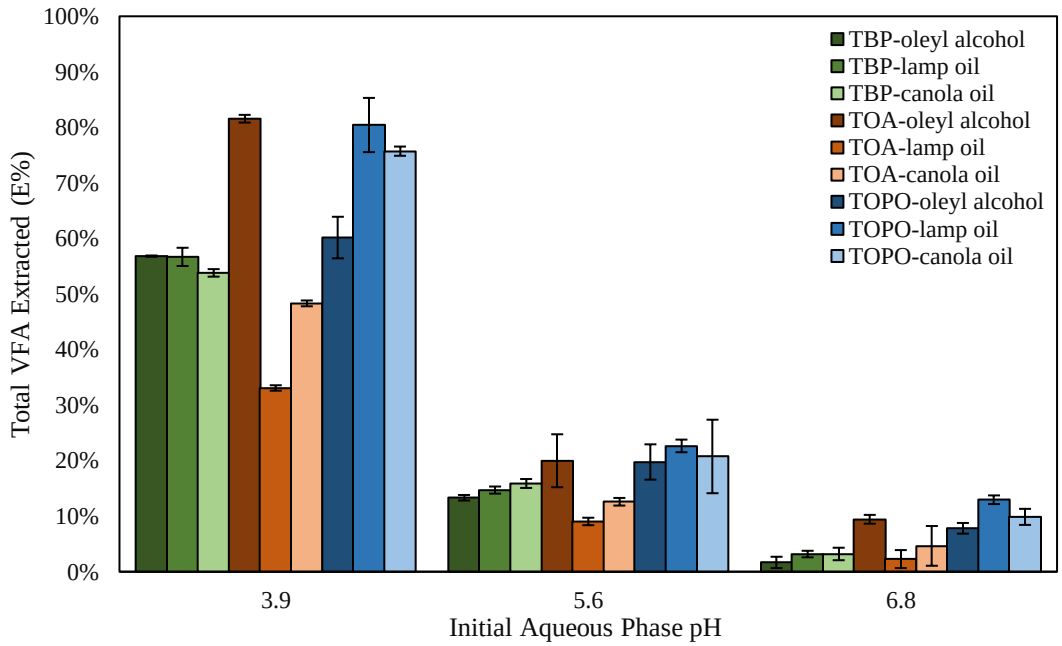


Figure 12: *Percentage total VFA extracted from aqueous phase using extractants TBP, TOA and TOPO with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

Figure 13 illustrates tVFA extractions using ionic liquids $[P_{666,14}]$[Phos] and Aliquat 336. Despite the ionic nature of $[P_{666,14}]$[Phos], an aqueous solution at a low pH value still resulted in increased VFA extractions with all diluents, where an increase in the reaction pH also resulted in an increase in error for some of the mixtures. Total VFA extractions using the ionic liquid demonstrated reasonable extraction of 65-70% at pH 3.9, but drastically decreased to negligible extractions at pH 5.6 and pH 6.8, indicating that $[P_{666,14}]$[Phos] would not be suitable for *in situ* LLE at pH above the pKa. This finding suggested the nature of the extraction mechanism

is based on hydrogen bonding of molecular VFAs and not ion exchange of the dissociated acids, which corresponds with what has been reported in literature using [$P_{666,14}$][Phos] for VFA extraction [32]. Whereas [$P_{666,14}$][Phos] showed minimal VFA extraction capacity at increased pH, the VFA extraction capacity using Aliquat 336 was not notably impacted by the increase in pH of the aqueous phase, evident from tVFA extraction that remained relatively consistent across the three pH setpoints with each of the diluents. Extractions between 50-70 % tVFA were achieved between pH 3.9 and pH 6.8 using Aliquat 336, and Aliquat 336-oleyl alcohol extracted greater than 60% tVFA at all three pH setpoints. The extractant is composed of an organic cation associated with a chloride ion and can therefore function as an anion-exchange reagent in an aqueous solution consisting of an acid or its salt under both acidic and basic conditions. This property enables Aliquat 336 to extract both the dissociated and undissociated forms of acids [79]. When ion-pair formation is the dominating mechanism and extraction is determined by protonation at the given pH, strongly basic extractants are efficient in the extraction of acids through protonation, even when the pH values are much higher than pKa value of the acid to be extracted [34]. Extraction of VFAs was, therefore, less influenced by the pH of the aqueous phase when using Aliquat 336 as an extractant.

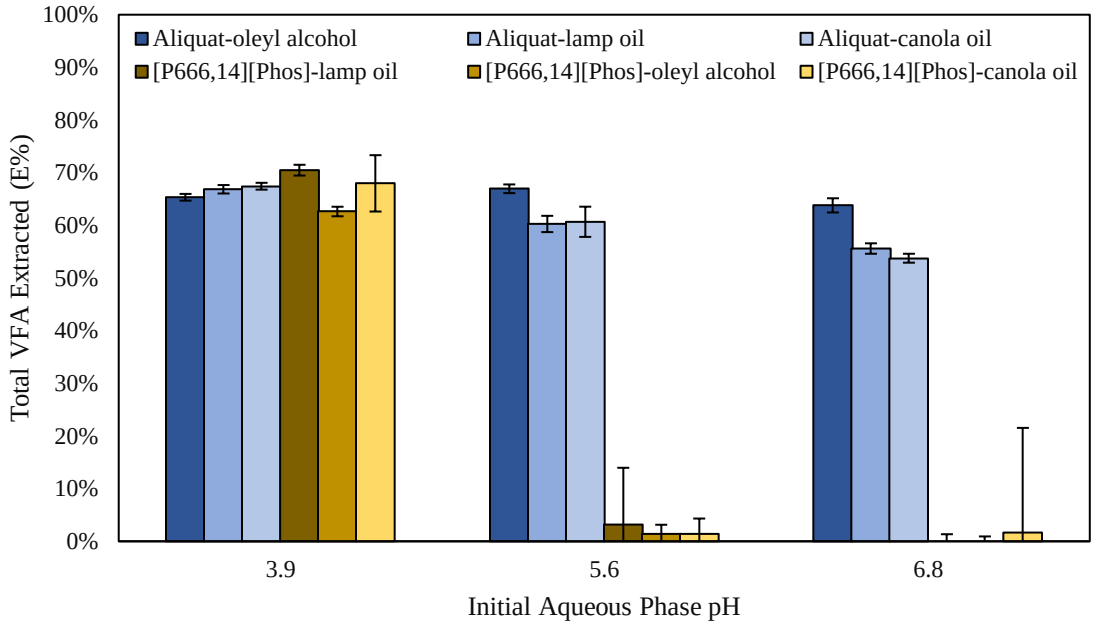


Figure 13: *Percentage total VFA extracted using extractants Aliquat 336 and [$P_{666,14}$][Phos] (ionic liquids) with diluents oleyl alcohol, lamp oil and canola oil at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats. Error bars represent standard deviations of concentration measurement that were propagated to total VFA extracted.*

From the LLE data it was evident that the nature of the extractant and the system pH were indeed key parameters which controlled the equilibrium VFA concentration between the aqueous and organic phases. Solvents differ in their extraction abilities at varying pH ranges and it is, therefore, crucial to select an

appropriate extractant for application in AD systems. Aliquat 336 showed the greatest potential to extract VFAs at higher pH setpoints, attaining extractions of greater than 50% at pH 6.8, and greater than 60% at pH 5.6 with all diluents. TOA-oleyl alcohol, TOPO-lamp oil and TOPO-canola oil performed reasonably well, extracting 10-15% tVFA at pH 6.8 and 20-25% tVFA at pH 5.6. The LLE results suggested that although extraction capacity generally decreased above the pKa of the acids, there are solvents with the capacity to extract acids at the pH values required for AD, which supported a key premise upon which this study was based.

5.1.3 Distribution of acids extracted

Although some work has been done on the recovery of short-chain carboxylic acids using extractants TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos], studies involving comparison of the extractants are sparse, particularly for the simultaneous extraction of a range of acids that differ in length at varied pH ranges. Extraction capacity of solvents is highly dependent on the number of carbons in the acid to be extracted, which makes it difficult to compare solvent extraction efficacy across different studies. This section therefore aimed to investigate and evaluate the extraction performance of each of the solvent combinations for the extraction of each VFA (acetic, propionic, butyric, valeric and caproic acid) in aqueous solution at varying pH.

Interestingly, extractants paired with different diluents provided varying levels of individual VFA extraction, where VFAs were not extracted in equal proportions. In general, longer chain-length VFAs were more susceptible to extraction at all pH ranges for all solvent combinations. This behaviour is supported by findings in literature of increased extraction affinity of organic acids parallel to increasing chain length when using tertiary and quaternary amines [79], TOPO [33] and [$P_{666,14}$][Phos] [32], which could be due to the growing hydrophobic domain of the acid as the carboxylic acid chain length increases [32,33,79].

Preferential extraction of longer chain VFAs could be promising for application in AD systems as the extraction of longer chain VFAs can be used to facilitate pH control even at higher fermentation pH ranges. Additionally, it has been reported that longer-chain VFAs such as valerate are most toxic in anaerobic acidification, followed by propionate, butyrate and acetate, respectively. The COD contribution of longer chain-length VFAs is also higher than shorter-length VFAs [33]. The higher susceptibility of longer chain acids for removal could, therefore, minimize toxicity in acidification processes and enhance waste stabilisation. Furthermore, the market price of VFAs tends to increase with increasing chain length [2,3], making longer-chain acid recovery economically beneficial.

It is worth noting that while sufficient extraction efficiency is required for the recovery of VFAs from the AD system, if too many VFAs (particularly acetic acid, being an immediate precursor) are extracted, methanogenesis would be inhibited, which would negatively impact the biogas productivity of the plant. Therefore, extractant selection may not necessarily be based on the pursuit of the highest degree of extraction,

but rather on the ability of the extractant to extract excess VFAs when need be (ideally an extractant with selectivity for the specific VFAs that tend to be produced in excess would be selected) to avoid VFA accumulation in the system and in doing so, prevent the occurrence of acid-crash. Knowledge of these individual acid extractions could therefore be useful for application in various AD systems where specific VFAs tend to predominate, in addition to understanding how the preferential extraction of particular acids may influence the performance of AD plants, where the individual acid extractions can potentially be correlated to the biogas performance.

Figures 14-16 illustrate the distribution of VFAs extracted by chain length using TBP with oleyl alcohol, lamp oil and canola oil with synthetic VFA solutions at pH 3.9, pH 5.6 and pH 6.8. TBP provided similar relative levels of individual VFA extraction with each of the different diluents. Acetic acid extractions of 15-20% and propionic acid extractions of 50-55% were achieved at pH 3.9, which drastically decreased below 10% at the higher pH setpoints. Butyric acid extractions of between 75-80% at pH 3.9 decreased to 15-20% at pH 5.6 and were negligible at pH 6.8. While the extraction capacity decreased above the pKa, significant valeric acid extractions of 45-50% and caproic acid extractions in the range of 80% were obtained above the pKa of the acids at pH 5.6 and extractions of valeric and caproic acids between 5-20% were attained at pH 6.8.

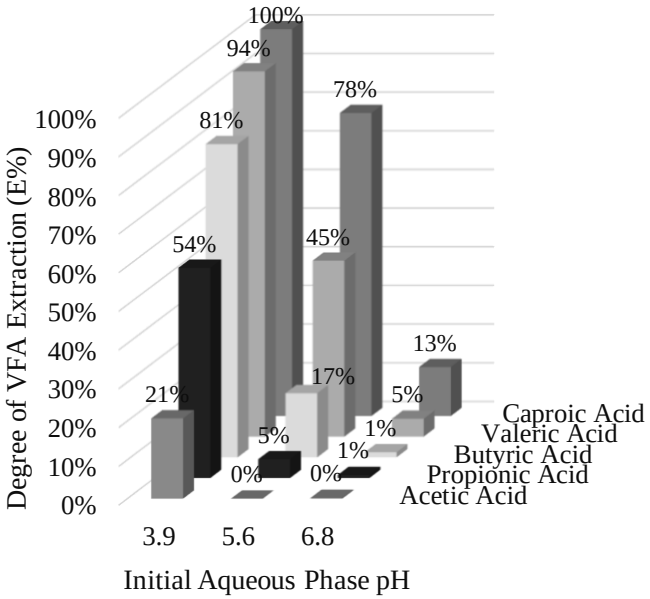


Figure 14: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TBP-oleyl alcohol with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

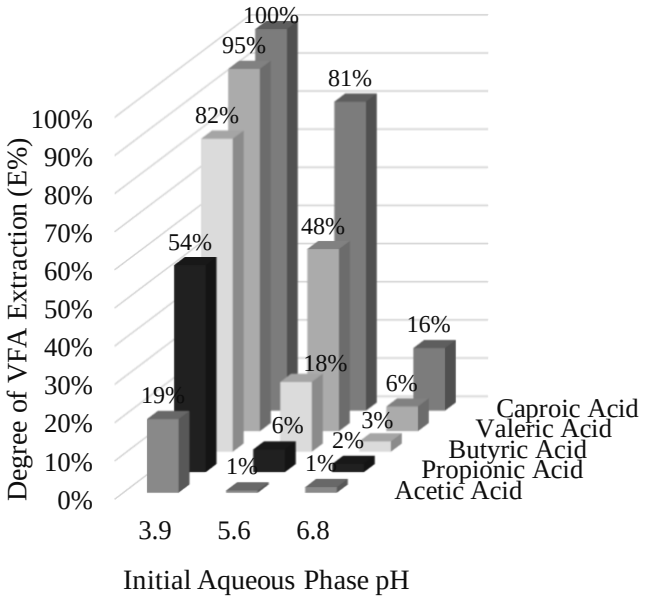


Figure 15: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TBP-lamp oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

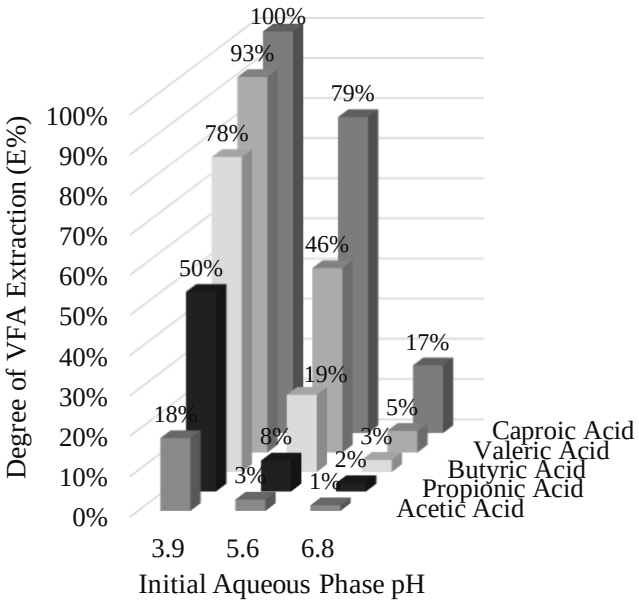


***Figure* 16:** *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TBP-canola oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

Figures 17-19 illustrate the distribution of acetic, propionic, butyric, valeric and caproic acid extracted using TOPO with oleyl alcohol, lamp oil and canola oil. Compared to TBP, TOPO achieved improved VFA extractions at all three pH setpoints, with the capability to extract more acids above the pKa. Acetic acid extraction was significantly lower using oleyl alcohol (23±5%) compared to TOPO-lamp oil and TOPO-canola oil at the lowest pH, which achieved extractions of greater than 40% at pH 3.9. Acetic acid extractions decreased to negligible values at pH above the pKa for all three solvent combinations. Propionic acid extractions between 60-85% decreased to 10% at pH 5.6, with minimal extraction at pH 6.8. TOPO extracted significant butyric (25-35%), valeric (55-70%) and caproic (85-90%) acid at pH 5.6. Extractions of 10-15% butyric acid, 30-45% valeric acid and 60-80% caproic acid were achieved at pH 6.8, demonstrating potential for TOPO to extract these VFAs above the pKa.

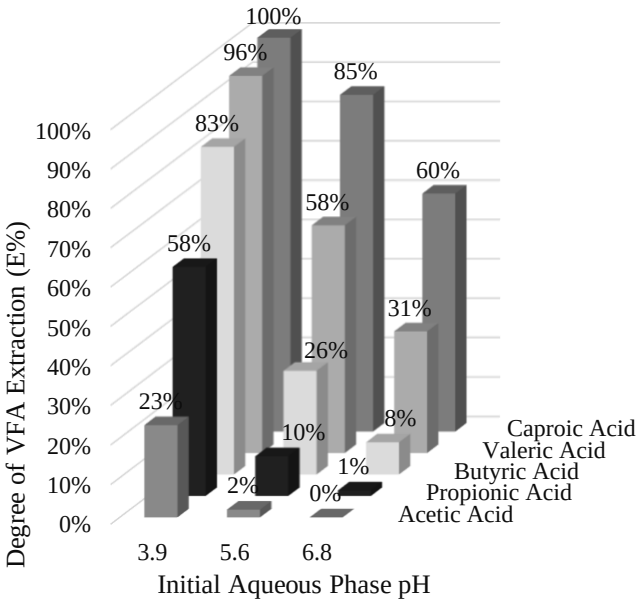


Figure 17: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOPO-oleyl alcohol with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

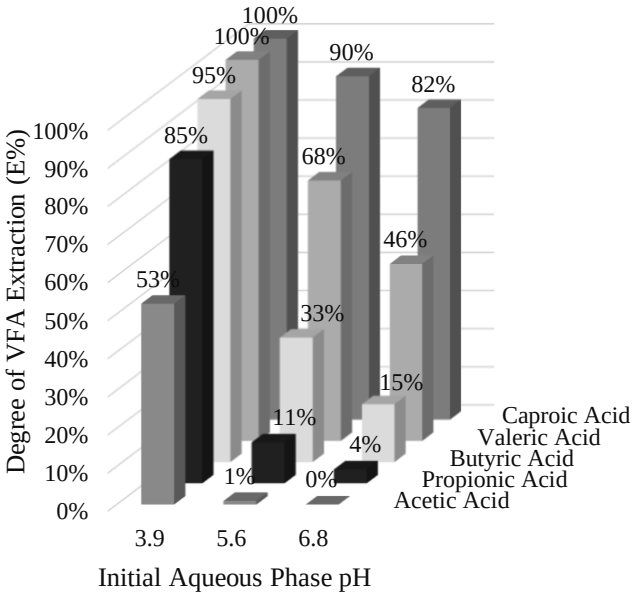


Figure 18: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOPO-lamp oil with synthetic VFA solution at* pH 3.9, pH 5.6 and pH 6.8 *for triplicate repeats.*

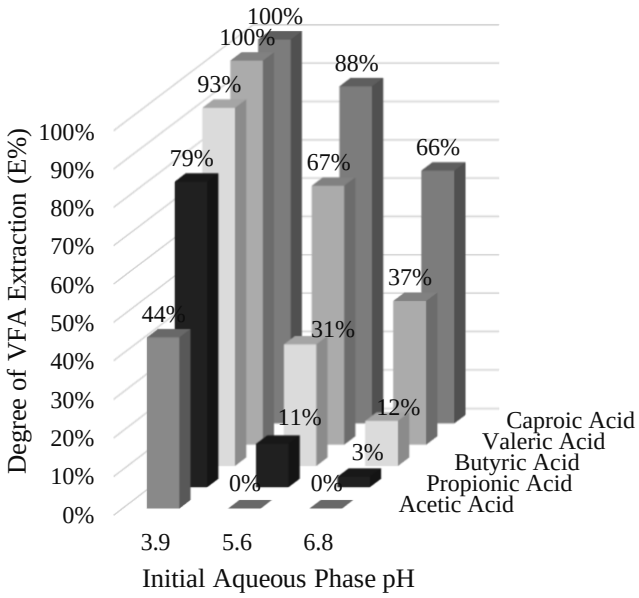


Figure 19: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOPO-canola oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

The distributions of VFAs extracted using TOA with oleyl alcohol, lamp oil and canola oil at varying pH are exhibited in Figures 20-22. The extraction capacity of TOA-oleyl alcohol was significantly higher for the extraction of all acids compared to TOA-lamp oil and TOA-canola oil. Acetic acid extraction of 61 ± 1% was achieved using TOA-oleyl alcohol at pH 3.9 while extractions of 5-15% were attained using lamp oil and canola oil. All acetic acid extractions were negligible at pH above the pKa. TOA-oleyl alcohol extracted 83 ± 1% propionic acid at pH 3.9 and 9 ± 3% at pH 5.6 while TOA-lamp oil and TOA-canola oil extracted 20-40% propionic acid at pH 3.9, with negligible extraction above the pKa. Butyric acid extractions of 94 ± 0% , 26 ± 8% and 10 ± 1%, valeric acid extractions of 100 ± 0%, 61 ± 13% and 30 ± 3%, and caproic acid extractions of 100 ± 0%, 90 ± 9% and 63 ± 5% were obtained with TOA-oleyl alcohol at pH 3.9, pH 5.6 and pH 6.8 respectively. TOA-lamp oil and TOA-canola oil achieved valeric acid extractions of 30-40% at pH 5.6 and 5-15% at pH 6.8, and caproic acid extractions of 70-80% at pH 5.6 and 20-40% at pH 6.8 respectively, suggesting that the solvent combinations could be applied for the extraction of these acids at varying pH.

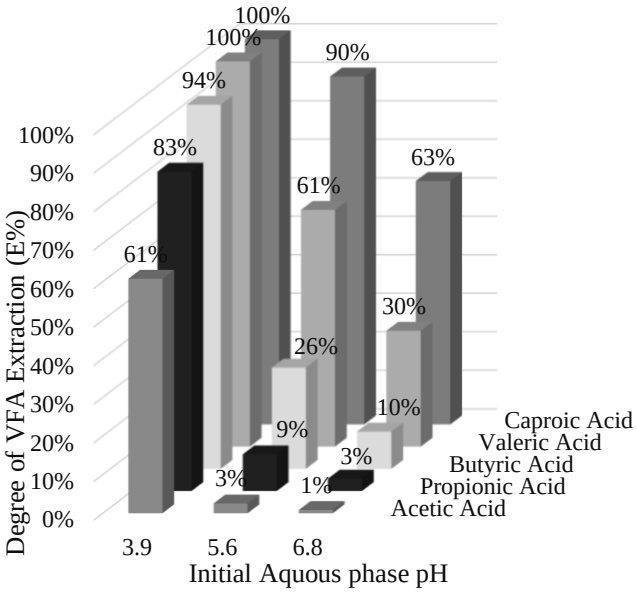


Figure 20: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOA-oleyl alcohol with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

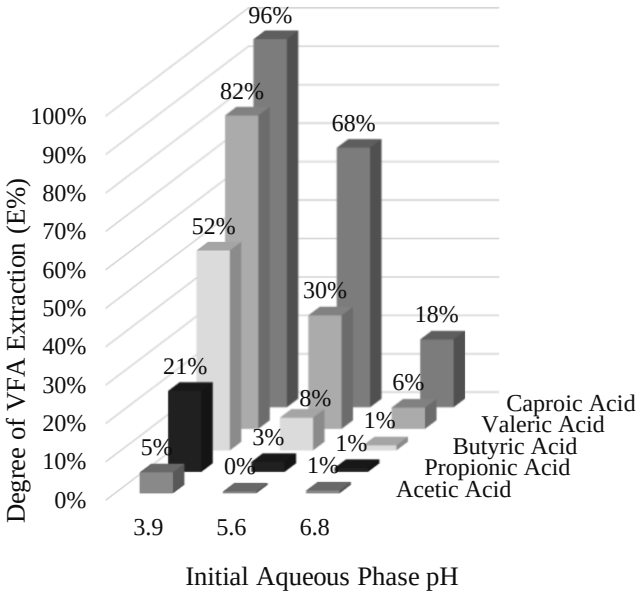


Figure 21: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOA-lamp oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

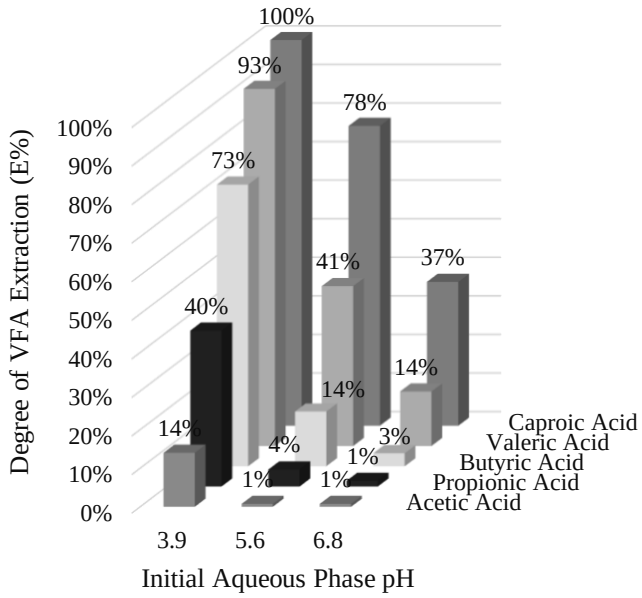


Figure 22: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using TOA-canola oil with synthetic VFA solution at* pH 3.9, pH 5.6 and pH 6.8 *for triplicate repeats.*

The distributions of acids extracted using Aliquat 336 are depicted in Figures 23-25, showing minimal pH dependency of acid extraction for each of the acids extracted. Higher extractions of shorter-chain VFAs were achieved using Aliquat 336 at higher pH values compared to other solvents studied, with extractions of 30-40% acetic acid achieved at pH 5.6-6.8. Extractions of 50-65 % propionic acid, 70-85 % valeric acid and 95-100 % were accomplished at all three pH setpoints, highlighting the potentiality Aliquat possesses to extract acetic, propionic, butyric and valeric acids at pH both below and above the pKa of the acids to be extracted.

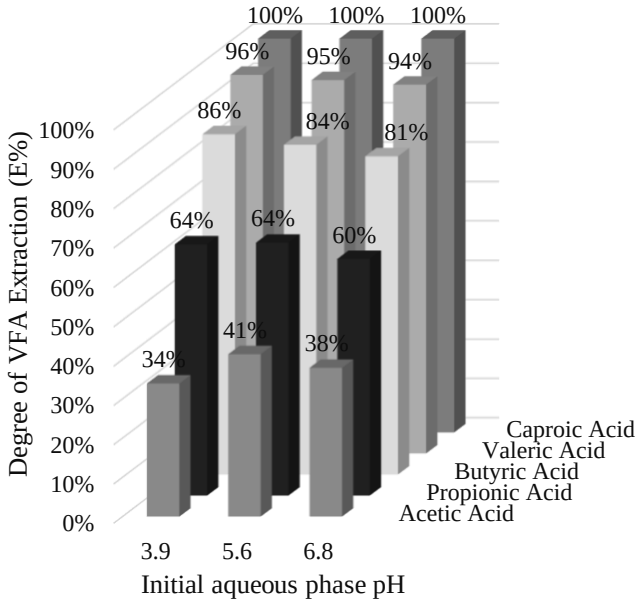


Figure 23: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using Aliquat-oleyl alcohol with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

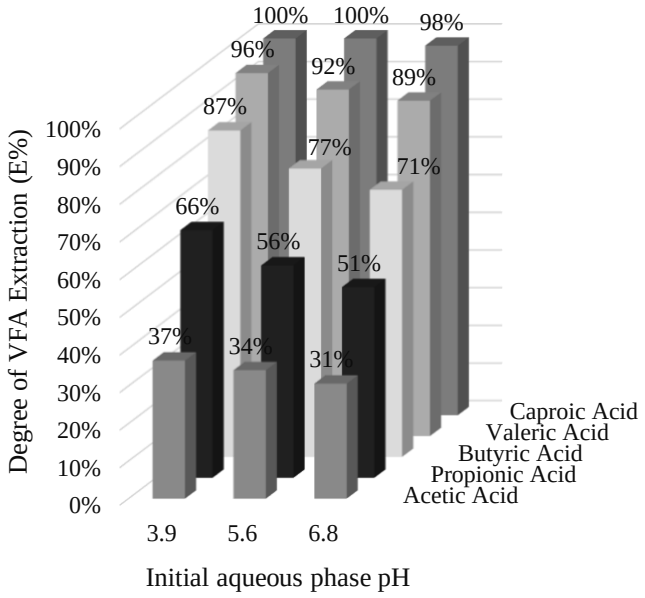


Figure 24: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using Aliquat-lamp oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

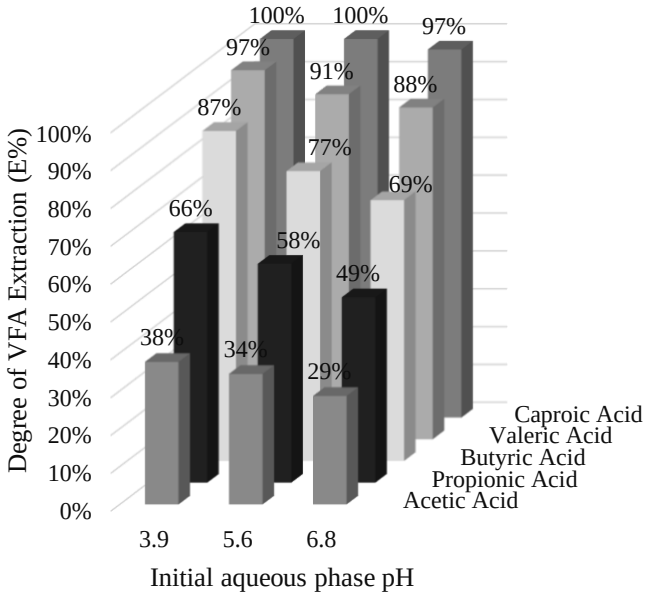


Figure 25: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using Aliquat-canola oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

Figures 26-28 illustrate the distribution of acetic, propionic, butyric, valeric and caproic acid extracted using [$P_{666,14}$][Phos] with oleyl alcohol, lamp oil and canola oil. At pH 3.9 extractions of 30-40% acetic acid, 60-70% propionic acid and 80-90% butyric acid were attained using [$P_{666,14}$][Phos] with all three diluents, however negligible extractions were achieved at pH 5.6 and 6.8 for these VFAs. Valeric acid extractions decreased from greater than 95% at pH 3.9 to 5-10 % at pH 5.6. Caproic acid extractions of 95-100 % were achieved below the pKa, which decreased to below 40% at pH 5.6. With minimal extractions observed at higher pH ranges, [$P_{666,14}$][Phos] would not be recommended for VFA extraction at pH ranges above the pKa of the acids.

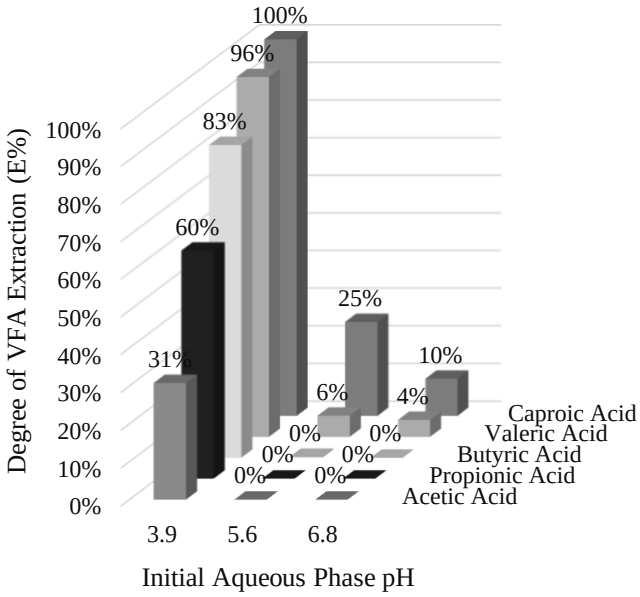


Figure 26: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using [$P_{666,14}$][Phos]-oleyl alcohol with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

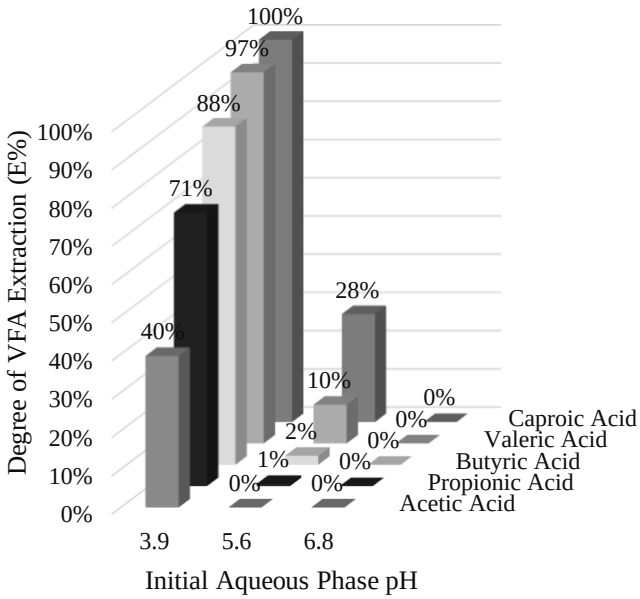


Figure 27: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using [$P_{666,14}$][Phos]-lamp oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

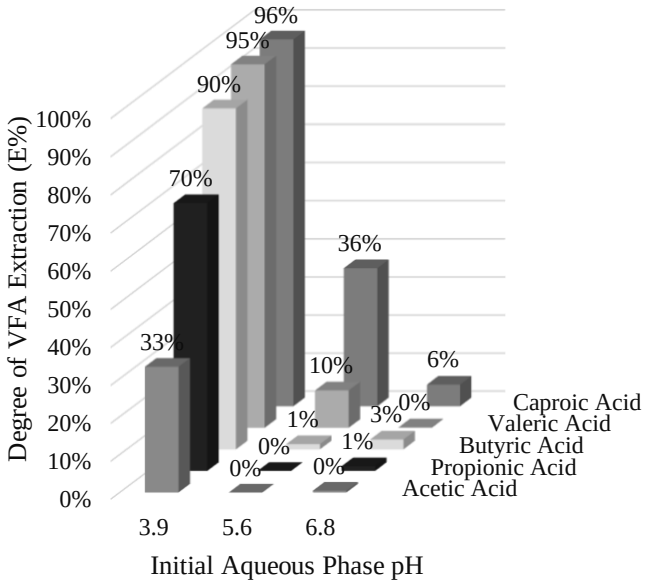


Figure 28: *Distribution of acetic (pKa 4.76), propionic (pKa 4.88), butyric (pKa 4.82), valeric (pKa 4.84) and caproic acid (pKa 4.88) extracted by chain length using [$P_{666,14}$][Phos]-canola oil with synthetic VFA solution at pH 3.9, pH 5.6 and pH 6.8 for triplicate repeats.*

Comparable trends were exhibited for extractants TOA, TBP, TOPO and [$P_{666,14}$][Phos] for the extraction of acetic, propionic, butyric, valeric and caproic acids. The pH dependence of extraction was evident for each of the individual acids, with highest extractions at the lowest pH for all acids using these extractants. At the highest pH (optimal for methanogenesis), almost no acetic acid was extracted - a benefit to the co-production of VFAs and biogas since acetic acid is an immediate precursor for biogas production [69], therefore complete immediate removal of acetic acid from the system would reduce the rate of methane production. The impact of pH was less apparent for extraction of the longer chain acids due to their increased extraction affinity. This could have a positive implication for the prospect of VFA extraction as a process control strategy, where even at pH above the pKa, significant longer chain acids (which tend to be most inhibitory to the AD process) can still be extracted. Aliquat did not always achieve the maximum relative degree of VFA extraction at pH 3.9 compared to extractants TOA, TBP, TOPO and [$P_{666,14}$][Phos], but provided consistent VFA extractions across all three pH ranges, highlighting the potential of this extractant for VFA extraction at varying pH, particularly at pH above the pKa of the acids to be extracted.

5.1.4 Effect of extraction on aqueous phase pH

In order to investigate whether VFA extraction could in principle be applied as a pH control strategy, the effect of the acid extraction on the system pH was observed by measuring the pH of the aqueous phase before and after the LLE experiments. One may expect that the system pH would increase following the extraction of

VFAs. However, this was not the case when using Aliquat 336 in LLE. In fact, the pH of the aqueous raffinate phase decreased after the extraction of the acids at all three pH setpoints, illustrated in Figures 29-31. It has been reported that when the mechanism for VFA extraction is anion exchange between the dissociated acid and the ionic solvent, the pH decreases due to the increased concentration of H+ ions as a result of the exchange mechanism [32]. This effect could counteract the pH increase due to the removal of acid anions. Therefore, in AD systems where the aim of VFA removal from the reactor would be to maintain a high pH for the survival microorganisms, LLE using Aliquat 336 would not be suitable. Furthermore, despite increased pH of the aqueous phase at pH 3.9 and pH 5.6, the pH of the aqueous raffinate was slightly lower than the initial aqueous feed for extraction using TOPO-oleyl alcohol at pH 6.8 (Figure 31), corroborating that the extraction of VFAs does not always guarantee corresponding proportionate increase in system pH at equilibrium.

It should be mentioned that in AD systems with high buffering capacity, the accumulation of VFAs tends to result in microbial inhibition, even if the pH of the system is maintained relatively constant. Partial inhibition of biogas production in a highly buffered AD system for co-digestion using winery waste and swine manure was reported due to VFA accumulation, even though the system pH was maintained at pH 7 [66]. Despite the maintenance of the system pH, the biogas production was negatively impacted by the accumulation of VFAs. This section investigated the extraction of VFAs as a pH control mechanism (as well as a strategy to prevent VFA accumulation), however, it is worth noting that there is value in the extraction of VFAs simply as an acid concentration control strategy to stabilise AD systems. In systems with high buffering capacity there may be less focus on VFA extraction as a pH control mechanism, where the application of these solvents may be suitable primarily for the prevention of VFA accumulation.

The pH of the aqueous raffinate phase was higher than the initial aqueous feed when TOA, TBP and $[P_{666,14}][Phos]$ were used in combination with diluents oleyl alcohol, lamp oil and canola oil at all three pH setpoints, displayed in Figures 29-31. This behaviour supports the idea that VFAs are extracted via interfacial protonation of the extractant followed by extraction of their anions to maintain charge balance [32]. The pH of the aqueous phase increased after extraction as a result of the selective removal of protonated acids, thus the transferral of acid to the solvent phase can facilitate control of the pH of the aqueous phase. Solvents containing TOA, TBP and $[P_{666,14}][Phos]$ therefore show considerable potential for use in pH control mechanisms to aid stability in AD systems at varying system pH.

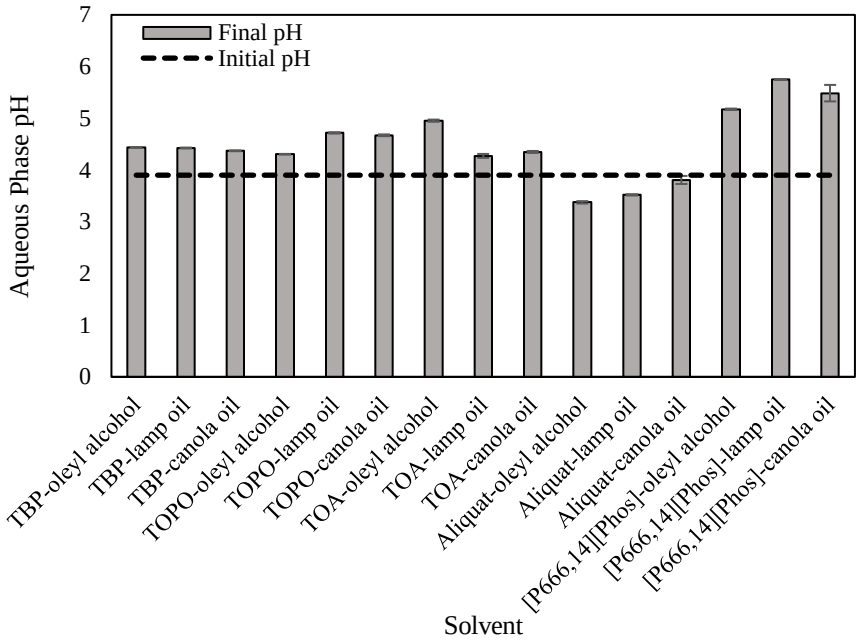


Figure 29: *Aqueous phase pH before and after LLE using TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos] with oleyl alcohol, lamp oil and canola oil using synthetic VFA solution at starting pH 3.9 for triplicate repeats, error bars given as sample standard deviation*

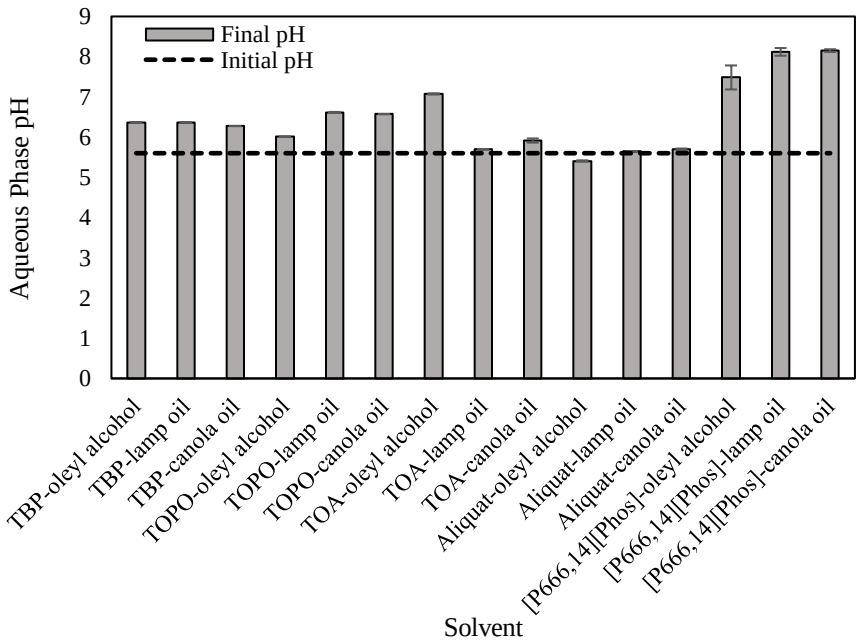


Figure 30: *Aqueous phase pH before and after LLE using TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos] with oleyl alcohol, lamp oil and canola oil using synthetic VFA solution at starting pH 5.6 for triplicate repeats, error bars given as sample standard deviation*

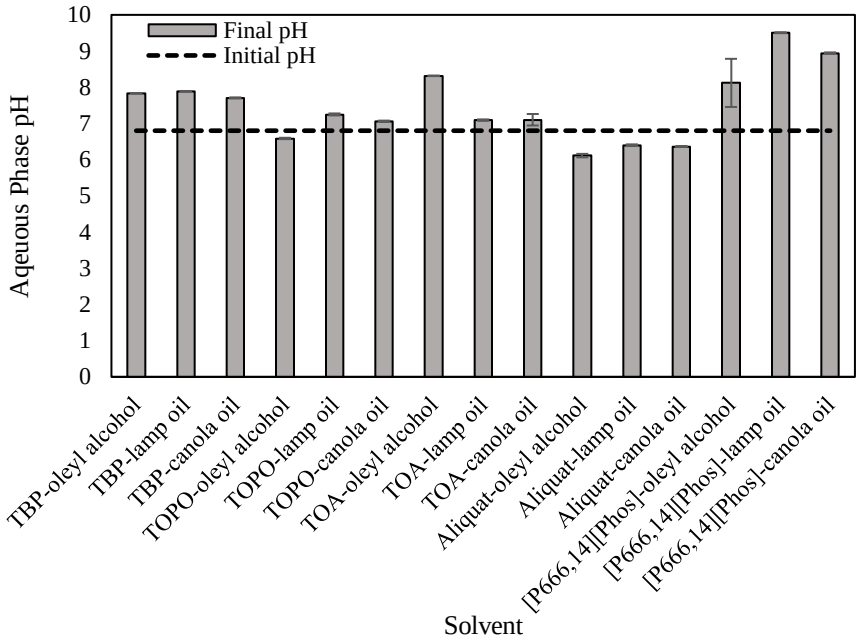


Figure 31: *Aqueous phase pH before and after LLE using TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos] with oleyl alcohol, lamp oil and canola oil using synthetic VFA solution at starting pH 6.8 for triplicate repeats, error bars given as sample standard deviation*

As illustrated in Figures 29-31, pH measurements indicated that the pH increased by between 0.2-2.5 units following extraction at various initial aqueous phase pH ranges with solvents TOA, TOPO, TBP and [$P_{666,14}$][Phos]. It is likely that even low tVFA extractions (less than 50%) from aqueous systems can be used to adjust the system pH and recover a VFA side stream through effective control and optimisation of extractant flow rate volume ratios with these extractants. By increasing (or decreasing) the solvent flow rate in the AD system, the mass transfer between solvents and VFAs can be controlled (as mass transfer is directly proportion to the solvent area), thereby influencing the degree of VFA extraction from the aqueous phase. Based on these equilibrium results, LLE with Aliquat 336 would not be a suitable solution in AD systems where the aim of VFA removal from the reactor would be to maintain a high pH for optimal digester performance.

It is important to highlight that equilibrium experiments do not provide comprehensive insight into the dynamic pH effect (or potential pH control) due to VFA extraction, and only elucidate part of the mechanism once the system has stabilised, where equilibrium pH does not necessarily represent the total extent of pH change due to VFA extraction. These results are, however, useful for provision of insight into further understanding the mechanisms of VFA extraction with various solvents and obtaining an indication of the potential pH effect these solvents could have on the aqueous systems.

5.2 LLE with fermented wastewater

There are significant amounts of impurities, dissolved salts, ions, microorganisms and various other components in fermented wastewater which accompany VFAs, making wastewater a complex aqueous solution containing a variety of different constituents. Dissolved salts present in fermented wastewater could negatively impact VFA extraction efficiency due to co-extraction of salt anions [32]. The LLE experiments discussed in Section 5.1 were conducted using synthetic model solutions with VFAs and water. These LLE systems did not contain impurities, salts and ions that are inherently present in AD systems. Impurities such as salts, ions, fats, proteins and biosurfactants are likely to play a large role in mass transfer and affect the kinetics, stability and equilibrium of two-phase systems. Atasoy *et al.* (2018) noted the need to devise strategies to overcome negative effects caused by co-existing anionic species, which are inevitably present in AD systems. This study therefore aimed to evaluate the performance of the solvent combinations for VFA extraction from industrial wastewater to establish whether the solvents are significantly limited in capacity due to impurities present in the AD system.

Extraction experiments were conducted using industrial wastewater from an AD plant. The measured pH of the aqueous feed was pH 4.3, which is lower than the pKa of the acids to be extracted. The LLE results obtained using fermented wastewater were therefore compared to LLE results obtained below the pKa at pH 3.9 using model aqueous solutions to contrast the extraction performance of the different systems. Figure 32 illustrates the percentage tVFA extracted from fermented wastewater and synthetic aqueous solutions. From figure 32 it is evident that most solvent combinations exhibited similar or even improved relative potential for extraction of VFAs from non-idealised solutions containing impurities compared to idealised aqueous systems, except for TOA-oleyl alcohol and [$P_{666,14}$][Phos]. The similar and even improved extraction performance of extractants from AD wastewater suggests that the co-existing impurities and anionic species present in the AD system did not have a negative impact on the extraction capacity of these solvents, a promising result for the application of these solvents in non-idealised systems. This behaviour has been reported in literature [15], where the enhanced extraction performance could be attributed to salting out of acid due to the presence of various salts in the system, resulting in an increase in degree of VFA extraction.

The degree of tVFA extraction decreased by more than 10% in the fermented wastewater extraction using TOA-oleyl alcohol and decreased by around 50% for all extractions using [$P_{666,14}$][Phos] with each of the three diluents. A similar effect was noted by Reyhanitash et al. (2016) using TOA and [$P_{666,14}$][Phos], with decreased extraction capacity for extraction of VFAs from fermented wastewater compared to model VFA solutions. Dissolved salt ions (such as Cl^-,HPO_4^{2-}, SO_4^{2-}) potentially interact with the hydrogen ion of acid in the aqueous phase, resulting in the salting out of salt-liquid phase complexes (such as HCl, H_2SO_4 and H_3PO_4) which then react with extractants to form complexes in the organic phase [15]. The reduced extraction performance could therefore possibly be due to co-extraction of ions, which would result in reduced VFA extraction capacity of

the solvents [32]. Although the pH was below the pKa of the acids to be extracted, extraction by [$P_{666,14}$][Phos] was drastically affected by impurities present in the feed, with tVFA extractions of only 10-15% achieved. These results suggest [$P_{666,14}$][Phos] would not be a suitable extractant for *in situ* LLE in active AD systems.

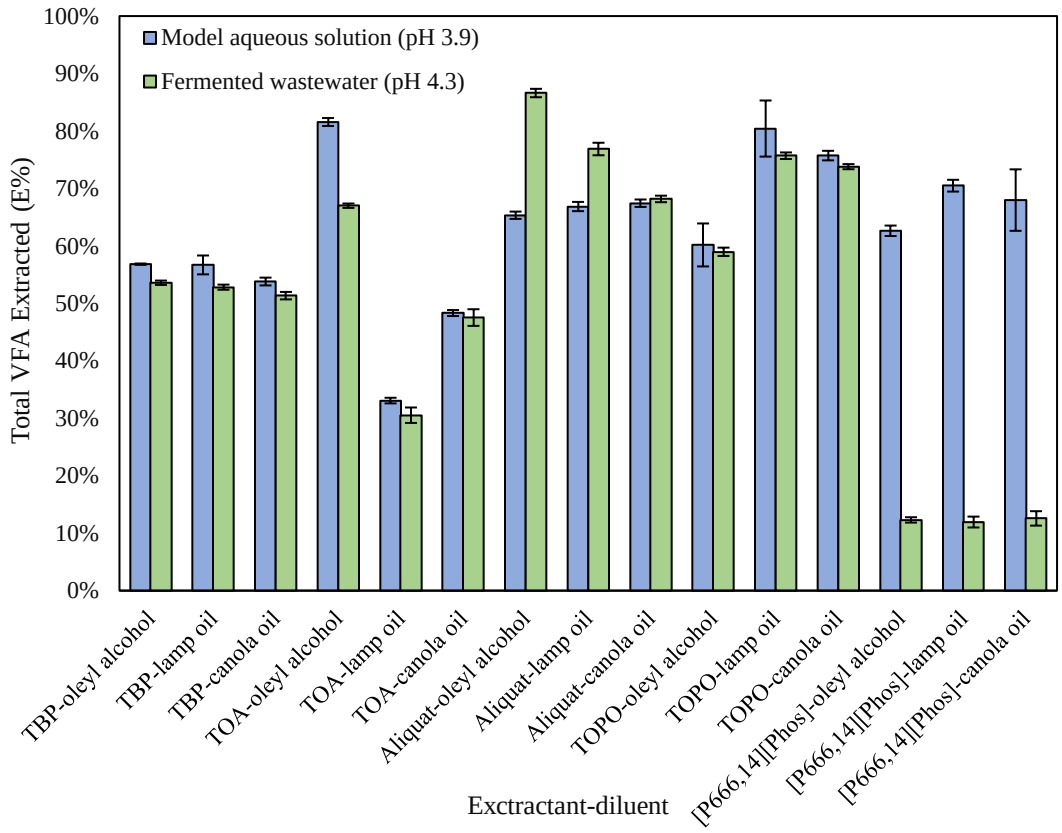


Figure 32: *Percentage total VFA extracted from model aqueous phase at pH 3.9 and fermented wastewater at pH 4.3 using extractants TBP, TOA, Aliquat, TOPO and [Phos] with diluents oleyl alcohol, lamp oil and canola oil. Error bars represent standard deviations of concentration measurements that were propagated to total VFA extracted.*

The pH of the systems following extraction were measured (illustrated in Figure 33), with similar results observed to those discussed in Section 5.1.4 for VFA extraction from idealised aqueous systems. The decreased pH of the raffinates obtained after extraction with Aliquat-oleyl alcohol and Aliquat-lamp oil confirm the predominance of ion exchange as the mechanism for extraction, where the dissociated acids in solution are possibly replaced with Cl^-, resulting in a lower relative pH compared to the aqueous feed solution. In addition to decreased pH, the leaching of Cl^- into the AD system is undesirable. Therefore, despite the significantly enhanced extraction performance with solvents Aliquat-oleyl alcohol and Aliquat-lamp oil in the AD system, these two solvents would not be recommended for application in biogas-producing AD.

It should be emphasised that while these equilibrium experiments are useful for investigating the extraction mechanism of various solvents and can provide some insight into the potential pH effect using these solvents,

these results only demonstrate part of the story. Whereas higher final pH is encouraging for the potential application of VFA extraction as a mechanism for positive pH control, the equilibrium pH does not necessarily represent the total extent of pH change due to VFA extraction, nor does it imply anything about the kinetics, and although the magnitude of final pH increase is not always high, it does not necessarily mean it is not as promising for application in continuous *in situ* LLE. Continuous pH measurement throughout the extraction mechanism, measuring the real-time pH throughout the duration of the LLE, would provide more insight for dynamic pH control, which is recommended for further study.

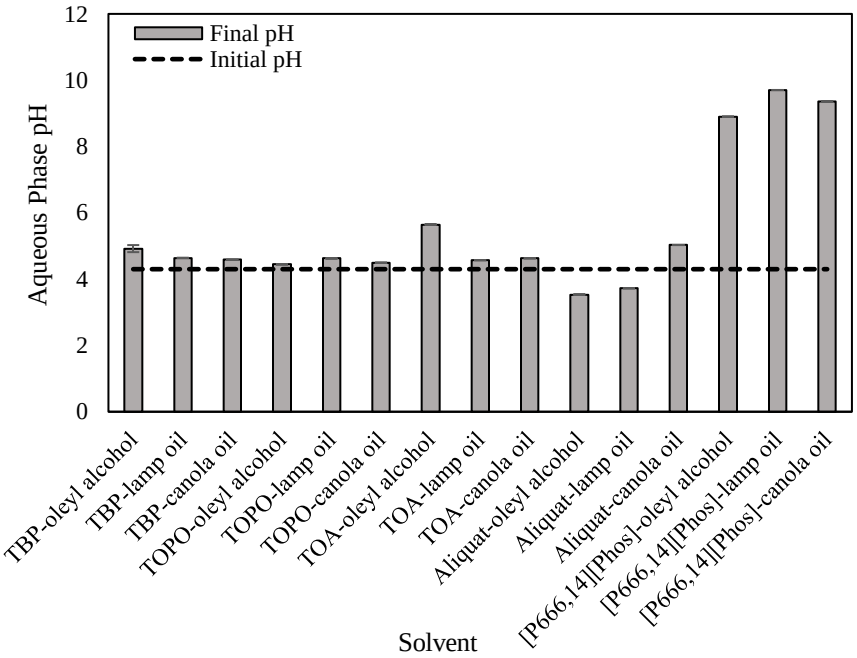


Figure 33: *Aqueous phase pH before and after LLE using TBP, TOPO, TOA, Aliquat and [$P_{666,14}$][Phos] with oleyl alcohol, lamp oil and canola oil using fermented wastewater at starting pH 4.3 for triplicate repeats, error bars given as sample standard error.*

5.3 Bench-scale biogas production tests

Given their potential toxicity to one or more members of the microbial consortium, the nature of the solvent(s) used in AD fermentation for *in-situ* VFA extraction should be carefully considered. The selection of extractant and diluent for *in situ* LLE of VFAs should therefore be done on the basis of minimal toxicity and maximum capacity. The degree of solvent toxicity depends on the combination of microorganism and the solvent used, with some strains of bacteria more resistant or more sensitive to the solvent than others [42,44,77]. The characterization and implementation of a system with a robust, biocompatible extractant-diluent combination is thus challenging due to variations in microbial consortiums. The bench-scale biogas tests were used to

establish whether microbes were able to continue producing methane in the presence of the solvents over a period of time, as a proxy measure for evaluating the consortium health. It was assumed that methane production was indicative of the survival of fermentative as well as the methanogenic bacteria due to the fact that VFAs are produced as precursors and utilised for methane production.

Methane productivity has been widely used as a parameter in determining digester performance [28,31,62,69,73]. The approach utilised in BMP tests to characterise a substrate's influence on the AD process was adopted in the bench-scale biogas production tests to investigate the influence of the extractant-diluent combinations on AD systems. As suggested by Angelidaki *et al.* (2009) inoculum was sampled at steady state from an active AD plant digesting complex organic matter to ensure provision of a highly diverse microbial community. The substrate and inoculum were characterised according to chemical oxygen demand (COD), total nitrogen, pH, tVFA, alkalinity, total solids (TS%), volatile solids (VS%), the details of which can be found in Appendix B. Substrates were inoculated with anaerobic bacteria and solvent combinations listed in Table 13 and thereafter incubated for a period of four to five weeks, where the production of biogas together with its methane composition were monitored throughout the test. Solvent-free inoculum-substrate tests served as the control.

5.3.1 Analysis of extractants with diluents

A considerable drawback of solvent extraction for *in situ* extraction of VFAs is that extractants may be toxic or inhibitory to microorganisms, therefore when solvents come in direct contact with AD consortia, they can negatively impact microbial growth and product formation. There is limited published data regarding the methanogenic biocompatibility of solvents and the usage of solvents in biogas-producing AD systems for VFA extraction. While chemical pre-treatment using organic solvents and ionic liquids such as N-methylmorpholine N-oxide (NMMO) [106,107], and triethanolamine (TEA) [108] have enhanced biomethane yields, there is no available published data regarding the methanogenic biocompatibility of TOA, Aliquat 336, [$P_{666,14}$][Phos] TBP and TOPO. The ability of AD consortia to continue producing biogas and methane while in contact with solvents over a period of time was used to identify potentially biocompatible solvents that could be applied in AD without inhibiting process performance.

Sunflower oil has been proposed as a natural diluent to reduce toxic effects of extractants in fermentation systems [15,42,44,77], oleyl alcohol has been suggested as a non-toxic diluent for solvent extractions [97] and kerosene has been used successfully with TOPO for the recovery of acids from anaerobic acidification broth using LLE [33]. Oleyl alcohol, canola oil and lamp oil were therefore investigated as potentially non-toxic diluents, to ascertain whether these diluents can indeed reduce the toxic effects of extractants in biological systems, and even enhance biogas production in AD systems. This section aimed to evaluate the impact of each of the diluents with each extractant on the biogas production.

Figure 34 illustrates the accumulated biomethane production of sample tests containing TOA with oleyl alcohol, lamp oil and canola oil. Systems containing TOA-oleyl alcohol produced significantly higher volumes of biomethane with a production of 148 ± 8 mL by week four of the experiment compared to TOA-lamp oil and TOA-canola oil, with total productions of 29 mL and 3 mL respectively. The biomethane production of TOA-lamp oil was not significantly enhanced nor inhibited, with comparable results to the biomethane production seen in the inoculum-substrate control, indicating that the consortia were able to survive in the presence of the solvent. There was, however, severe inhibition observed with the use of TOA-canola oil, which resulted in negligible biogas production over the four-week test duration.

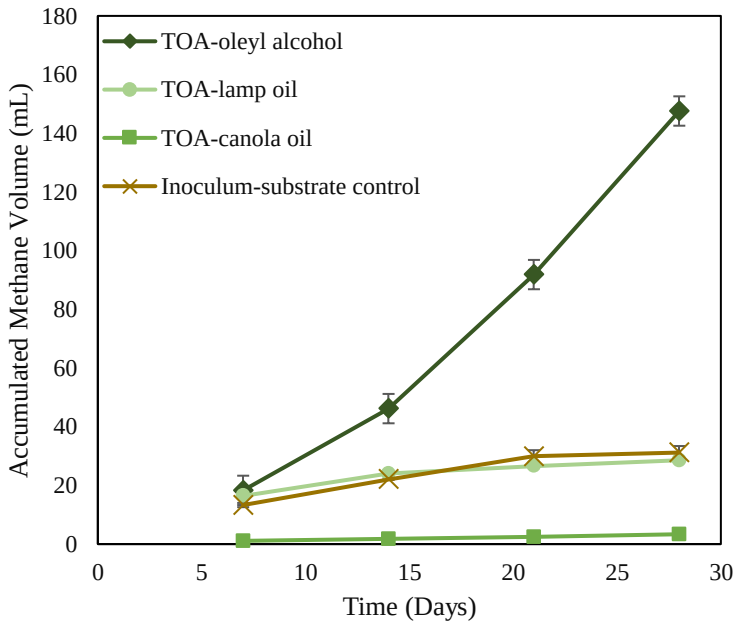


Figure 34: *Accumulated biomethane volume over four-week duration using TOA with diluents oleyl alcohol, lamp oil and canola oil relative to inoculum substrate control tests for triplicate repeats, error bars given as sample standard error.*

Figure 35 depicts the biomethane production in systems containing TBP-oleyl alcohol, TBP-lamp oil and TBP-canola oil. The use of TBP-oleyl alcohol resulted in enhanced biomethane production relative to the control, with an accumulated methane volume of 95 ± 5 mL on day 28 of the experiment. The use of TBP-lamp oil and TBP-canola oil resulted in negligible biogas production over the duration of the experiment, indicating that these solvents were not biocompatible with the AD consortia.

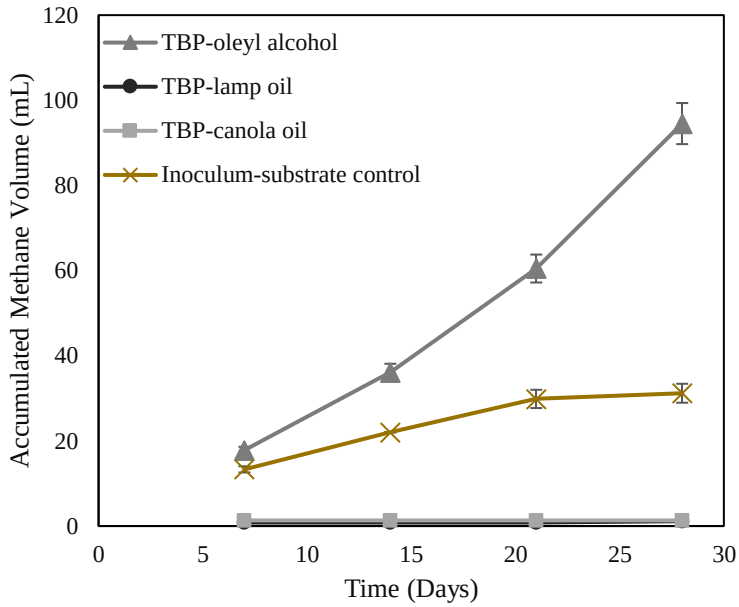


Figure 35: *Accumulated biomethane volume over four-week duration using TBP with diluents oleyl alcohol, lamp oil and canola oil relative to inoculum substrate control tests for triplicate repeats, error bars given as sample standard error.*

Promising results were obtained in systems containing TOPO with oleyl alcohol, lamp oil and canola oil, with significant biomethane productions seen in the presence of all three solvents, presented in Figure 36. TOPO-canola oil yielded the highest methane productivity on week two of the experiment, with an accumulated methane volume of 65 ± 3 mL compared to TOPO-oleyl alcohol, TOPO-lamp oil and the inoculum-substrate control which produced 41 ± 1 mL, 28 ± 2 mL and 22 mL respectively. By week four of the experiment, TOPO-oleyl alcohol had produced the highest volume of methane with an accumulated volume of 136 ± 7 mL, followed by TOPO-canola oil, TOPO-lamp oil and the inoculum-substrate control with respective accumulated methane productions of 97 ± 11 mL, 32 ± 2 mL and 31 ± 2 mL. These results would suggest that TOPO exhibited compatibility with the AD consortia when used in combination with all three diluents oleyl alcohol, lamp oil and canola oil.

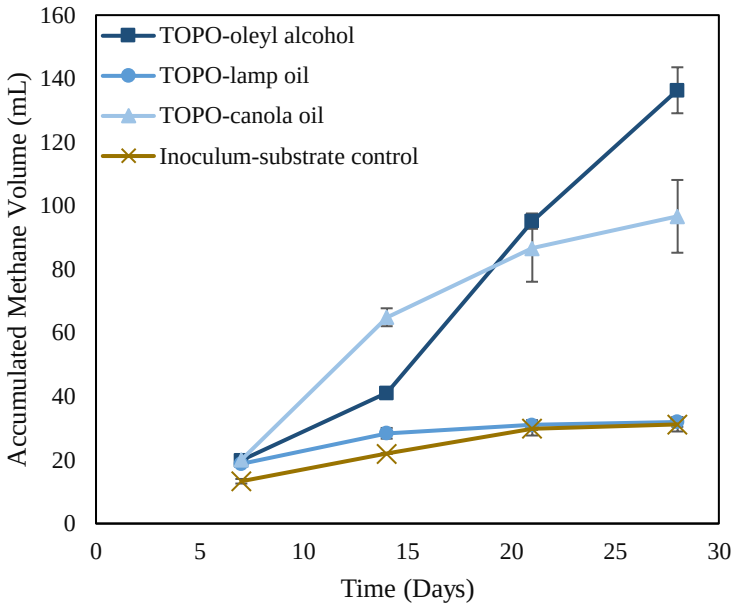


Figure 36: *Accumulated biomethane volume over four-week duration using TOPO with diluents oleyl alcohol, lamp oil and canola oil relative to inoculum substrate control tests for triplicate repeats, error bars given as sample standard error.*

The accumulated biomethane volumes produced from AD systems in the presence of $[P_{666,14}][Phos]$ are depicted in Figure 37. It can be seen that $[P_{666,14}][Phos]$-lamp oil and $[P_{666,14}][Phos]$-canola oil severely inhibited the production of methane in AD systems, with negligible volumes of biomethane produced in the presence of these solvents. However, significantly improved methane production relative to the inoculum-substrate control was observed in systems containing $[P_{666,14}][Phos]$-oleyl alcohol, which produced 211 ± 3 mL by week four of the experiment, indicating that $[P_{666,14}][Phos]$-oleyl alcohol did not have a toxic effect on the methane-producing consortia.

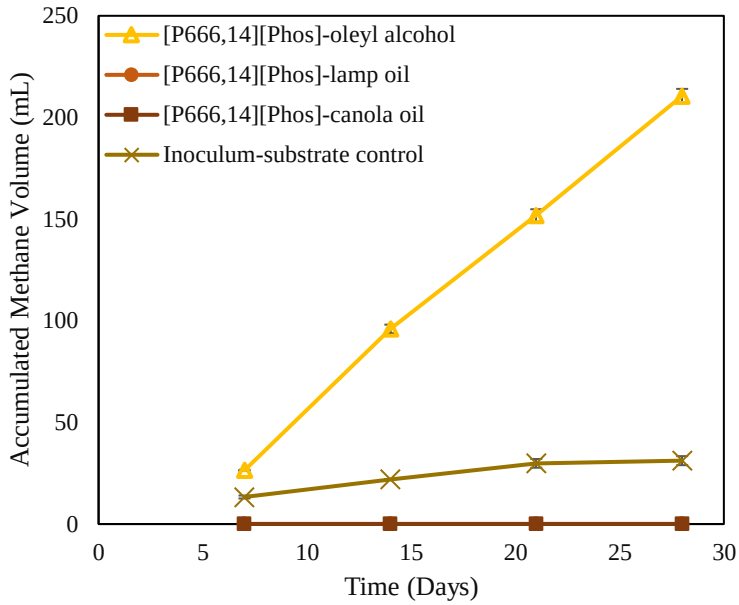


Figure 37: *Accumulated biomethane volume over four-week duration using [$P_{666,14}$][Phos] with diluents oleyl alcohol, lamp oil and canola oil relative to inoculum substrate control tests for triplicate repeats, error bars given as sample standard error.*

The combination of the extractant with diluent appeared to have a major influence on the biocompatibility of the solvent combinations. The results suggested that the use of oleyl alcohol as a non-toxic diluent mitigated extractant toxicity in most AD systems. This idea has been discussed in literature, where it has been proposed that toxicity in reactive extraction can be reduced through use of non-toxic diluents, which can assist in avoiding direct contact of the bacteria with toxic extractants, or entrapment of the dissolved toxic solvent with vegetable oils [42,44,77,92]. In this work, the use of canola oil generally resulted in reduced biogas production relative to the control, with the exception of increased biogas production seen with TOPO-canola. While canola oil may have reduced direct contact with the extractant, oil can interfere with the biological activity of inoculum and cover the carbohydrate content of organic waste, making it unavailable for further digestion [16]. The use of lamp oil with most extractants generally resulted inhibited biogas production, except for TOPO-lamp oil and TOA-lamp oil where biogas productions analogous to the control were seen.

5.3.2 Comparison of solvent combinations

The current section aimed to compare the performance of the AD systems in the presence of the different extractants studied. Screening of different extractants in AD cultivations revealed marked differences and improvements in terms of biomethane yield, which allowed selection of at least two solvents for future study. The total cumulative biogas and biomethane productions with each of the solvent combinations is summarised in Figure 38 to enable direct comparison of the solvent combinations. Increased biogas production was seen for all extractants with oleyl alcohol diluent, except for Aliquat 336. [$P_{666,14}$][Phos]-oleyl alcohol produced almost five times the amount of biogas relative to the control, while TOA-oleyl alcohol, TBP-oleyl alcohol

and TOPO-oleyl alcohol produced two to three times more total biogas compared to the inoculum-substrate control over the four-week test period. There was also increased biogas production with TOPO-canola oil, producing three times the total biogas production of the control, and comparable levels of biogas production using TOPO-lamp oil and TOA-lamp oil with similar relative total biogas productions to the control.

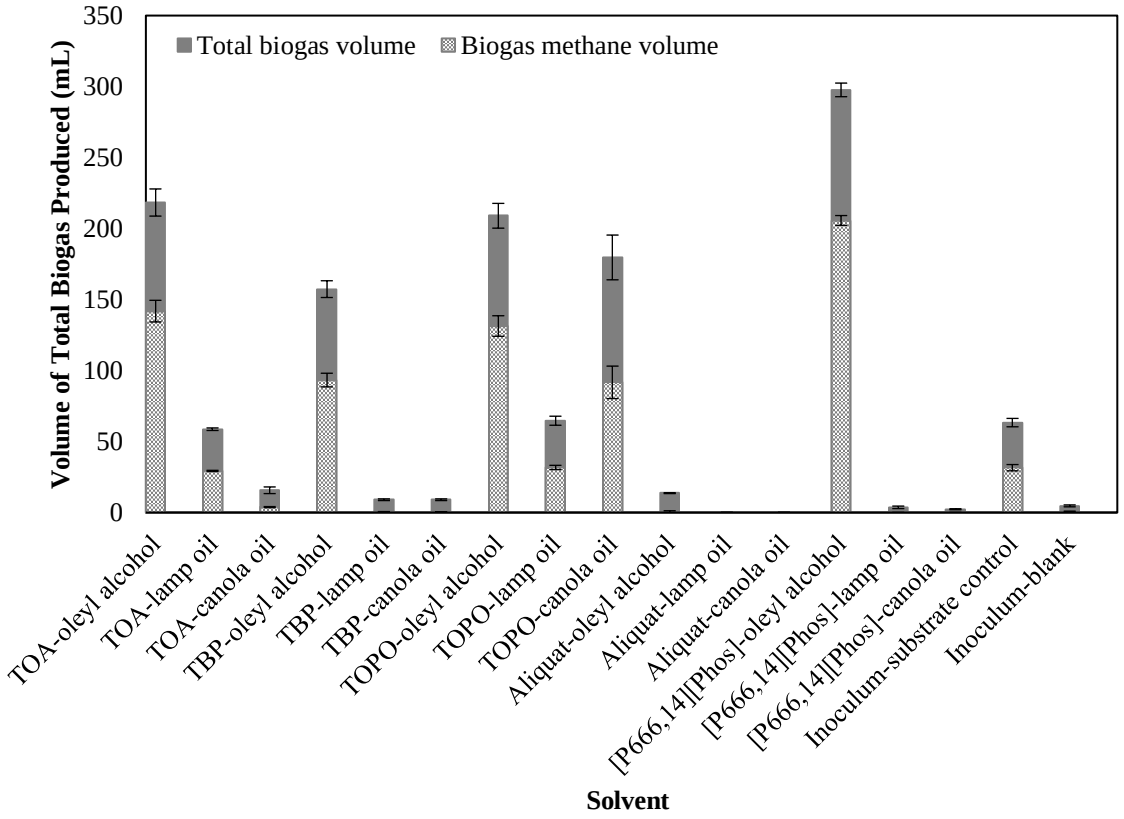


Figure 38: *Total biogas production with methane proportion after four weeks of bench-scale AD tests using the different extractant-diluent combinations, relative to inoculum-substrate control and inoculum-blank tests. Error bars represent the standard error of triplicate experiments.*

The biogas production was severely inhibited in the presence of several solvent combinations, evident from the decreased total biogas production relative to the inoculum-substrate control. The reduced biogas production is likely due to a toxic effect of the solvents on the methanogenic microbial population. The presence of Aliquat 336 in all biogas production tests resulted in minimal gas production, regardless of the diluent used. One might infer that Aliquat 336 had a toxic effect on the microbial population, which could not be mitigated with any of the diluents. It has been suggested in literature that although Aliquat 336 was the best extractant for reactive extraction of propionic acid, its toxicity is of concern [77] which is borne out of this data. Additionally, the pH of the aqueous raffinate decreased following extraction with Aliquat 336, which may have negatively impacted the consortium. Considering the LLE results presented in Figures 23-25, the significant acetic acid extractions

at a higher system pH and/or the decrease in system pH following contact with the aqueous phase could also have contributed to the repressed biogas production.

The cumulative biogas production in systems with similar or enhanced biogas production relative to the inoculum-substrate control are illustrated in the Figure 39 to evaluate the biogas productivity over the duration of the experiment. Compared to the inoculum-substrate control, the volume of biogas produced in systems the containing of TOA, TBP, TOPO and [$P_{666,14}$][Phos] with oleyl alcohol and TOPO-canola oil was consistently higher over the test duration, with sustained biogas production for a longer period of time. The methane proportion with extractant-diluent combinations that yielded similar or enhanced productivity relative to inoculum-substrate control are illustrated in Figure 40, which correspond with expected biogas methane percentages of 50-75 % reported in literature [29]. The biogas of the systems containing TOA, TBP, TOPO and [$P_{666,14}$][Phos] with oleyl alcohol achieved maximum methane concentrations of 70-75% of gas produced, while TOPO-canola oil, TOPO-lamp oil and TOA lamp oil achieved maximum concentrations of 50-60%, and the control reached a maximum methane percentage of 55 ± 3% on day 21 of the experiment. These results suggested sustained survival of the microbial population without severe inhibition or impending digester failure due to the presence of the solvents.

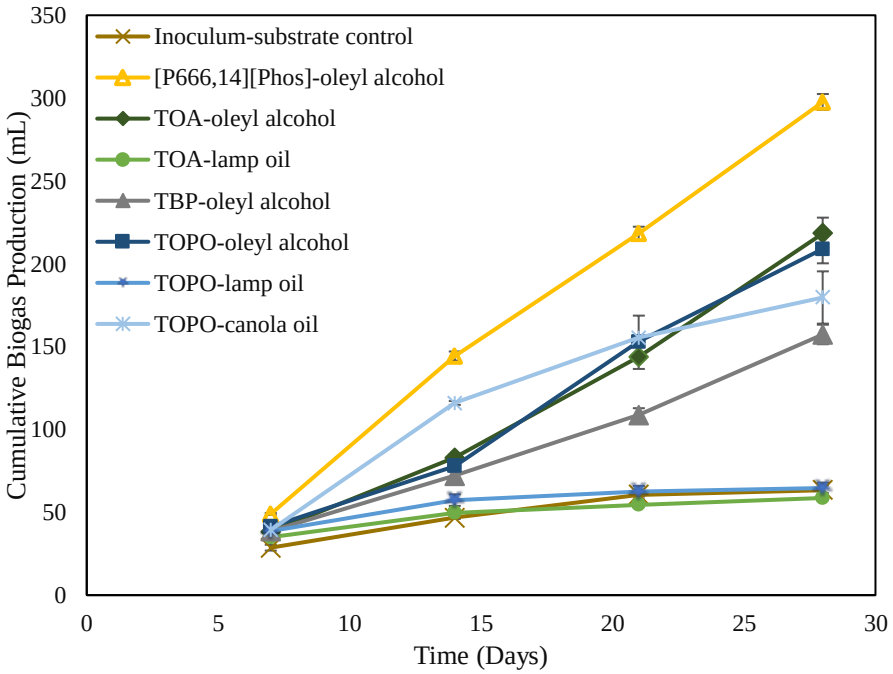


Figure 39: *Cumulative biogas production with extractant-diluent combinations that yielded enhanced or similar production relative to inoculum-substrate control. Error bars represent the standard error of triplicate experiments.*

If acetate is being removed (even in small amounts) from the AD system, a shift may occur towards hydrogenotrophic methanogenesis due to the acetate sink. Higher contributions of hydrogen and subsequent increased utilisation of carbon dioxide in AD could result biogas production with an enhanced methane concentration. The enhanced biogas quality seen in the bench scale biogas production tests could potentially be attributed to this phenomenon. Intuitively it would seem that the methanogenesis is shifting towards hydrogen conversion to methane through adjustment of the VFA composition, however at this stage, the discussion remains speculative as we do not know what is happening mechanistically. In-line hydrogen measurement, genome analyses or a metagenomic approach may be useful to characterise the methanogenic communities and provide further insights into the overall microbial consortia.

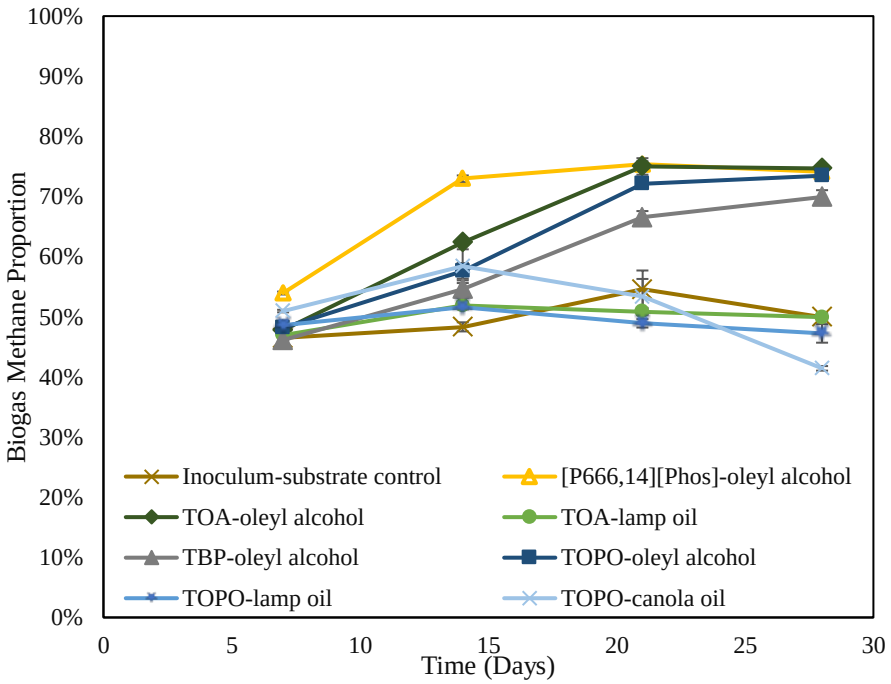


Figure 40: *Biogas methane proportion with extractant-diluent combinations that yielded similar or enhanced productivity relative to inoculum-substrate control. Error bars represent the standard error of triplicate experiments.*

The methane yield (or theoretical methane potential) was calculated with the methane generated from the substrate in terms of COD contributed by the substrate, the results are presented in Figure 41. The theoretical COD of methane is 64 g oxygen per mole of methane, which is in theory, the maximum amount of methane that you can get if all COD is converted into methane [101]. Therefore, in an optimally performing digester where all COD is converted to methane at standard temperature and pressure conditions (STP), for every gram of COD present in the substrate, 350ml of methane can theoretically be produced. Adjusting the ratio to conditions at 35 °C, one gram of COD should theoretically produce a maximum 395 mL CH4 [28].

It can be seen from Figure 41 that the methane yields of [P666,14][Phos]-oleyl alcohol, TOA-oleyl alcohol, TOPO-oleyl alcohol, TBP-oleyl alcohol and TOPO-canola oil far exceed the theoretical maximum of 395 mL CH_4/g COD. This would imply that the solvents may have provided an additional carbon source for the methane-producing consortia. It was noted by Wasewar *et al.* (2004), that fats and oils in fermentation processes can be used as carbon sources for acid-producing bacteria, where co-immobilisation with sunflower oil appeared to affect the metabolism of microbes for the production of lactic acid. There is limited literature available regarding the digestibility of oleyl alcohol in fermentations for VFA production, however, it has been reported that fatty alcohols up to carbon chain length C_{18} are biodegradable and field studies at wastewater treatment plants have shown up to 99% removal of fatty alcohols between C_{12}-C_{18} [109]. In these cases where the biomethane production exceeds the expected theoretical maximum, it is likely that that canola oil and oleyl alcohol were metabolised by the AD consortia, being broken down into soluble organic substances and fatty acids during the fermentation process. While the mitigation of solvent toxicity is a positive result for application of *in situ* LLE, it would be necessary to find a diluent that is not readily digested by the AD consortia to maximise the long-term reusability of the solvents. That being said, it is likely that the diluent degradation rate would be very small considering the volume of diluent used and the limited surface area of the solvent that makes contact with the AD system. Further investigation of the diluent degradability to quantify the fraction of diluent digested is therefore recommended for future research.

The methane yields of TOPO-lamp oil and TOA-lamp oil were comparable with that of the inoculum-substrate control, with respective yields of 171 ± 3 mL CH_4/gCOD, 153 ± 1 mL CH_4/gCOD, and 169 ± 4 mL CH_4/gCOD. TOPO-lamp oil and TOA-lamp oil were therefore unlikely partially consumed by the AD consortia. The presence of these solvents did not result in repressed biogas production, which demonstrates they could potentially be applied in for *in situ* LLE without impeding biogas productivity.

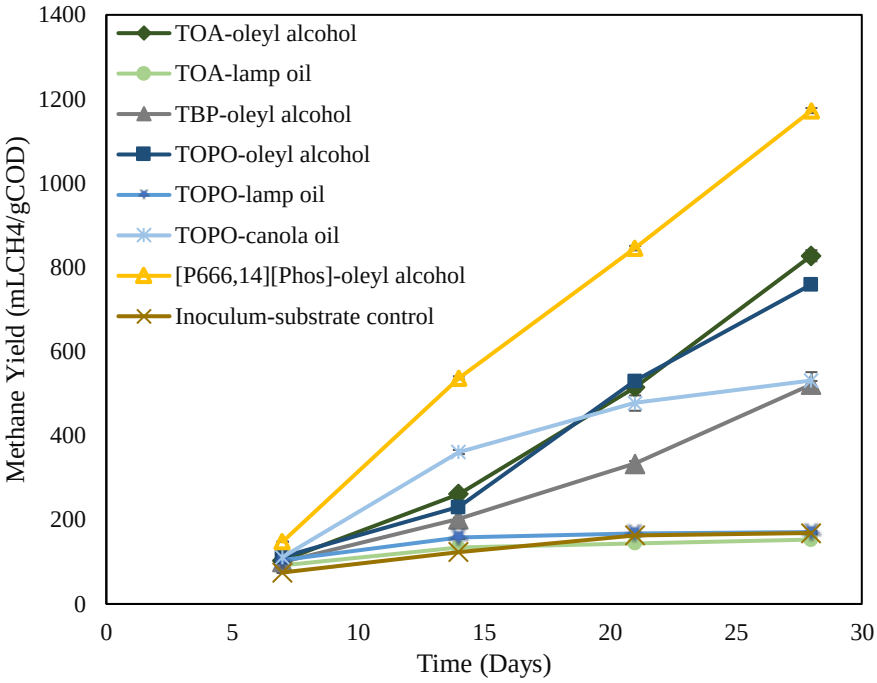


Figure 41: *Methane yield with extractant-diluent combinations that yielded similar or enhanced productivity relative to inoculum-substrate control. Error bars represent the error resulting from standard deviation of triplicate blanks and test samples.*

VFAs are known to cause stress in microbial populations at high concentrations, which can lead to process failure [69] and there is ample published evidence that suggests *in situ* product recovery has a positive effect on microbial productivity through selective removal of inhibiting products from bioprocesses [33,37,38,40,42]. Increased biogas and methane production seen in bench-scale AD tests with the solvents could, therefore, potentially be attributed to solvent extraction of excess acid produced in the AD systems. Reduction of acid accumulation could minimise stress on the microbial populations and enhance methanogenic activity. When there is a surplus of acid in a system, the pH drops, which would enable the solvents to extract more VFAs. In principle, the solvents possibly act as a store for the acids, releasing them slowly as the aqueous VFA concentration drops, resulting in consistent, improved productivity. However, enhanced biomethane yields, particularly in the presence of oleyl alcohol, could also be attributed to partial consumption of solvents. It is likely that the solvents served as a nutrient source for the microbes where biomethane yields exceeding 395 mL methane per gram of COD were seen. Careful solvent choice is therefore important, and degradability of the solvents should be further investigated.

A repeat set of biogas production tests were carried out using a different batch of freshly sourced wastewater treatment facility (WWTF) substrate and inoculum, results are presented in Figure 42. Growth rates of anaerobic microorganisms and subsequent biogas production in digesters is highly dependent on the composition of the organic matter in the feedstock [29]. Constituents of the feed are selectively digested by various microbes, therefore the overall digestion performance in terms of biogas productivity, organic

reduction, inhibition characteristics, and process stability, is dependent on the characteristics of the feed stream [20]. Due to fluctuating waste streams entering active digesters, feed composition varies over time in AD plants, resulting in changes in the microbial consortium and their growth rates. The repeat experiments therefore served to verify the biocompatibility of solvents containing TOA, TBP, TOPO, [$P_{666,14}$][Phos] and diluent oleyl alcohol. Due to the comparatively high biogas production seen with [$P_{666,14}$][Phos]-oleyl alcohol, [$P_{666,14}$][Phos] was also tested without the oleyl alcohol to observe the effect of the diluent in this system. Results were consistent with the initial bench-scale biogas production tests, with systems containing TOA-oleyl alcohol, TBP-oleyl alcohol, TOPO-oleyl alcohol and [$P_{666,14}$][Phos]-oleyl alcohol producing four to five times more biogas compared to the control tests over the experimental duration. The biogas production was, however, repressed in the presence of [$P_{666,14}$][Phos] without oleyl alcohol. This result confirmed that the diluent had a significant impact in mitigating the toxicity of the extractant in this solvent combination within biogas-producing AD systems, possibly reducing contact of the toxic extractant with the consortia to yield a more biocompatible solution, as suggested in literature [92].

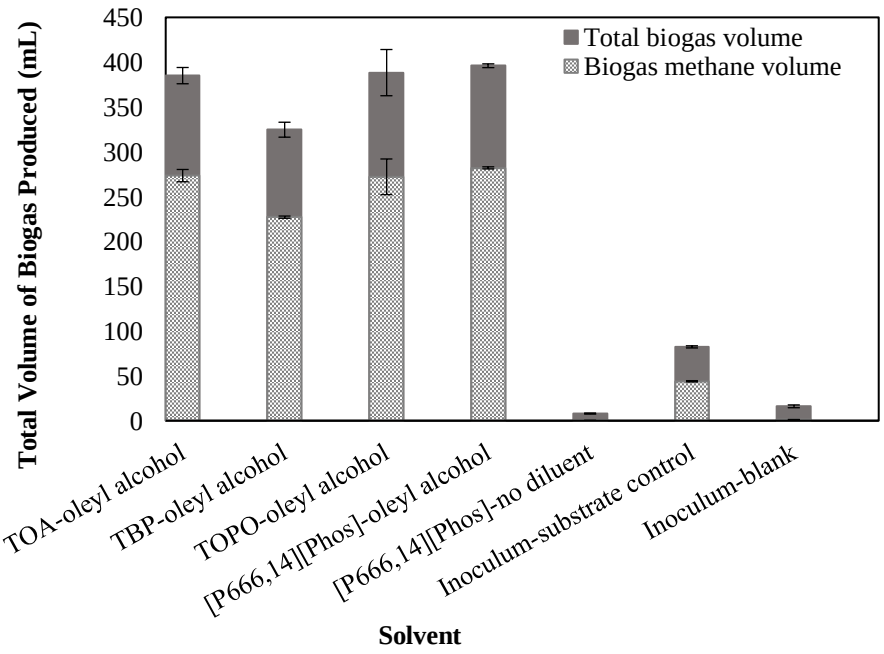


Figure 42: *Total biogas production with methane proportion over five-week duration bench-scale AD tests using extractants TOA, TBP, TOPO, [$P_{666,14}$][Phos] and diluent oleyl alcohol, relative to inoculum substrate control and inoculum blank tests for triplicate repeats, error bars given as sample standard error.*

The accumulated biomethane productions seen in the repeat bench-scale biogas production tests are illustrated in Figure 43. In both sets of experiments the biogas production was sustained for a longer time period in systems containing the solvents with oleyl alcohol, with continuous production up to week four of the

experiment, whereas biogas production in the inoculum-substrate control systems diminished by week three. This result could suggest that the solvents not only enhanced but promoted prolonged biogas production in the AD systems. While some of the solvents may have served as a carbon source for the bacteria, the microbial population were able to survive in the presence of the solvents, illustrating that diluents can be used to attenuate solvent toxicity.

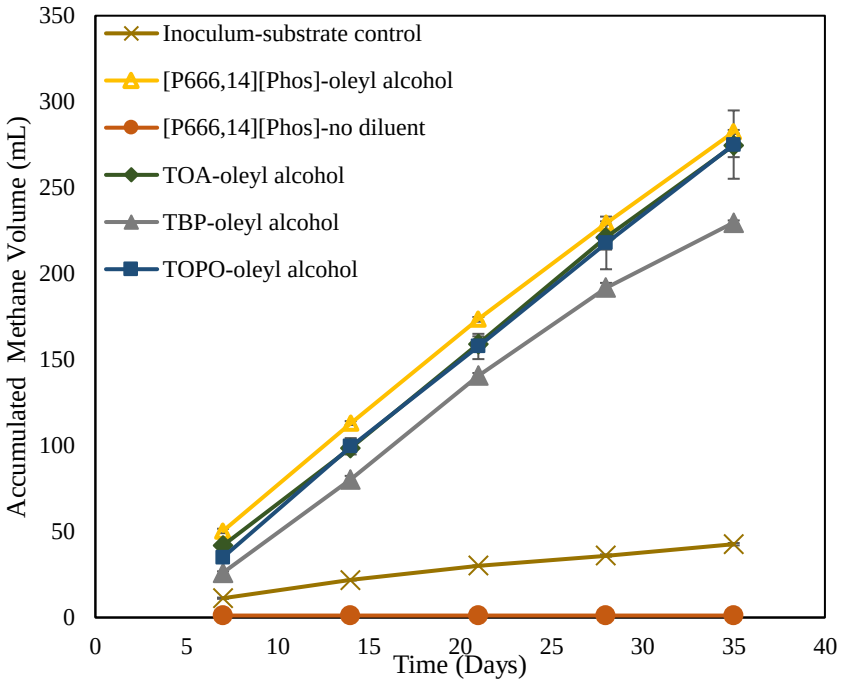


***Figure 43**: Accumulated methane production over five-week duration bench-scale AD tests using extractants TOA, TBP, TOPO, [$P_{666,14}$][Phos] and diluent oleyl alcohol, relative to inoculum substrate control and inoculum blank tests for triplicate repeats, error bars given as sample standard error.*

The biocompatibility of the solvents was inferred from the biogas production and methane content of the biogas produced. The toxicity of solvents can be mitigated through the use of effective diluents; however, it is vital to ensure that the solvents are not consumed by the microorganisms. An in depth understanding of the microbial activities in the AD system would provide insights to support a robust, effective control strategy [29] and investigation of the composition and mechanisms of the microbial community is recommended to further elucidate the effect of the solvents and VFA extraction on the AD process. Undoubtedly, the effect of VFA extraction would influence population balances and the operation of the AD consortium. However, for the purpose of this discussion, it is sufficient to note that the sustained increased biogas production in the presence of the solvents seen in the bench-scale biogas production tests is a promising result for potential *in situ* extraction of VFAs from active AD systems. Moreover, the results confirm that there are biocompatible solvent combinations that could be used in biological systems with ability to co-produce biogas and VFAs, and indeed even improve biogas productivity.

5.4 Back-Extraction of loaded solvent phase

Back-extraction experiments were performed to determine whether VFAs could be stripped out of the solvent into an alkaline aqueous phase, to enable regeneration and recycling of the acid-free solvent. Loaded organic phases from LLE experiments (Section 5.1) were stripped with double the solvent volume of 1 M sodium hydroxide solution. Back-extractions using solvents from LLE at pH 3.9 were analysed to identify which solvents show potential for stripping and regeneration. The total acids extracted out of the solvent phase (R%) are summarised in Table 16.

Table 16: *Total VFAs recovered from solvents using back-extraction for triplicate repeats, Δ represents standard deviations of concentration measurements that were propagated to tVFA recovered.*

Solvent	R%	ΔR%
TBP-Oleyl alcohol	92.5%	9.0%
TBP-Kerosene	96.0%	2.9%
TBP-Canola oil	89.7%	5.7%
TOA-oleyl alcohol	83.6%	16.0%
TOA-lamp oil	100.0%	9.5%
TOA-canola oil	100.0%	16.0%
Aliquat-oleyl alcohol	53.5%	6.5%
Aliquat-lamp oil	44.0%	3.7%
TOPO-Oleyl alcohol	92.3%	5.7%
TOPO-Kerosene	90.7%	9.3%
TOPO-Canola oil	94.5%	20.3%
$P_{666,14}$][Phos]-Oleyl alcohol	89.2%	4.2%
$P_{666,14}$][Phos]-Kerosene	82.9%	18.2%
[$P_{666,14}$][Phos]-no diluent	78.9%	3.7%

Extractants TOA, TBP, TOPO and [$P_{666,14}$][Phos] with oleyl alcohol and lamp oil exhibited considerable potential for back-extraction, recovering between 80-100% tVFA from the solvent phase. Aliquat 336-oleyl alcohol and Aliquat 336-lamp oil showed lower potential for back-extraction, only achieving tVFA back-extraction recoveries between 40-50%. This corresponds with literature, where it has been suggested that because Aliquat 336 functions as an anion-exchange reagent under both acidic and basic conditions, extractant regeneration by stripping may be difficult [79]. Recovery of VFAs and regeneration of these solvents would therefore be less feasible using back-extraction with an alkaline solution.

Back-extraction of Aliquat 336-canola oil and [$P_{666,14}$][Phos]-canola oil with sodium hydroxide resulted in the formation of emulsified, viscous systems with indistinct phases (images provided in Appendix D), which is not practical for solvent regeneration or VFA recovery due to difficulty in separating the two phases. Back

extractions using TOPO, TOA and TBP with canola oil achieved VFA recoveries of 80-100%. However, emulsification in the systems was observed, which required centrifugation to separate the phases. Centrifugation is unlikely to be feasible on a larger scale for a continuous process. Back extraction of solvents containing canola oil is therefore not recommended.

These results suggest there is potential for back-extraction using solvents with TOPO, TBP, TOA and [$P_{666,14}$][Phos] with diluents oleyl alcohol and lamp oil to recover VFAs and regenerate the solvents. Similar results have been reported in literature, where back-extraction from loaded organic phases with sodium hydroxide has been used to successfully recover acids and regenerate solvents such as tertiary amines and organophosphates, which predominantly extract undissociated acids under acidic conditions [15,37,38,79]

5.5 Summary of experimental results

The LLE, bench-scale biogas production and back-extraction experiments were used as a basis for the selection of potential extraction solvents for use in continuous *in situ* LLE operation. Table 17 was employed to highlight and summarise which solvents show capacity for application in biogas-producing AD systems to extract and co-produce VFAs without disrupting the AD process itself. Extraction results (LLE) were categorised according to the degree of extraction at varying pH and the performance of solvents in non-idealised systems. LLE results with degree of tVFA extractions (E%) of 10% or greater at pH above the pKa of the acids (pH 5.6) were indicated with a tick mark, provided the solvent was not drastically affected by impurities present in the feed (>15% reduction in E%). It should be noted that this simplified categorisation was used merely to summarise results, and further investigation of *in situ* LLE of acids as a pH control strategy for enhanced biogas production is still required. Bench-scale biogas production experiments where biogas production was not supressed in the presence of the solvent, with biogas volumes and methane percentages greater than, or analogous to, those produced by the inoculum-substrate control were indicated with a tick for ‘Biogas’. Solvents were designated a tick for ‘BE’ for total VFA back-extraction recoveries (R%) of greater than or equal to 80% from the organic phase, where emulsification was not observed.

Based on the experimental results, TOA-lamp oil, TOA-oleyl alcohol, TOPO-lamp oil, TOPO-oleyl alcohol and TBP-oleyl alcohol would be recommended for further study and implementation as potential solvents for *in situ* VFA extraction from biogas-producing AD wastewater treatment facility systems.

***Table 17**: Summary of solvent screening results based on experimental results from liquid-liquid extraction (LLE), bench-scale biogas production tests (Biogas) and back-extraction (BE) experiments*

Extractant	**Diluent**	**LLE**	**Biogas**	**BE**
TOA	Oleyl alcohol	✓	✓	✓
TOA	Lamp oil	✓	✓	✓
TOA	Canola oil	✓	✓	
TBP	Oleyl alcohol	✓	✓	✓
TBP	Lamp oil	✓		✓
TBP	Canola oil	✓		
TOPO	Oleyl alcohol	✓	✓	✓
TOPO	Lamp oil	✓	✓	✓
TOPO	Canola oil	✓		
Aliquat 336	Oleyl alcohol	✓		
Aliquat 336	Lamp oil	✓		
Aliquat 336	Canola oil	✓		
[$P_{666,14}$][Phos]	Oleyl alcohol		✓	✓
[$P_{666,14}$][Phos]	Lamp oil			✓
[$P_{666,14}$][Phos]	Canola oil			

CHAPTER 6
CONCLUSIONS

This study aimed to investigate potential solvents for co-production and extraction of VFAs from biogas-producing AD systems using *in situ* LLE based on (i) VFA extraction capacity at the desired pH, (ii) biocompatibility with the microbial community and (iii) feasibility for back extraction to regenerate the solvent. The experimental results demonstrated that there is potential for integrating the product recovery process with the fermentation process, with scope to extract and recover VFAs from AD systems at the desired pH range using biocompatible solvents.

LLE experiments conducted at varying pH levels revealed that there are several solvents with the capacity to extract acids (at varying amounts, but most sufficient for *in situ* continuous extraction) above the pKa of the acids, within suitable pH ranges for biogas-producing AD. Specifically, Aliquat 336, TOA and TOPO performed well at high pH values. Most solvent combinations exhibited similar or even improved VFA extractions from AD wastewater systems containing impurities compared to idealised aqueous systems, a promising result for the application of these solvents in non-idealised systems. However, extraction using [$P_{666,14}$][Phos] drastically decreased by around 50%, indicating it is not suitable for application in active AD systems.

Given that optimal AD operation occurs between pH 6.5 to 7.6 and VFA extraction is usually better at lower pH values, the obvious route would be to resort to solvents which are less influenced by the pH of the aqueous phase. However, it is crucial to test such a hypothesis using live cultures, as was proven in this study, a good solvent system in terms of VFA extraction is not necessarily good for the AD system and the organisms it harbours. Rather, there is a play-off between biocompatible solvents, their extraction efficiency and maintaining a suitable pH for the methanogens. Bench-scale biogas production tests indicated that microbes could survive and continue producing methane-bearing biogas in the presence of selected solvents under standard operation of the digester, with substrate and inoculum sourced from an operational AD plant. This elucidates potential for integration of *in situ* LLE for VFA extraction in an operational digester on a larger scale. The study has shown that solvents can be used to control the VFA content of an AD system with minimal apparent disruption of the microbial consortium, evident from similar and even increased volumes of biogas with elevated methane percentages seen in bench-scale AD systems containing TOA, TOPO, TBP and [$P_{666,14}$][Phos] compared to the inoculum-substrate control tests. The diluent had a significant impact on the biocompatibility of solvent combinations in biogas-producing AD systems, mitigating the toxic effect of the extractants. This was demonstrated in particular with oleyl alcohol, where up to five times the biogas production was seen using this diluent compared to the control. Solvents not only enhanced the biogas

production but promoted prolonged production in the AD system. This could be attributed to the stabilising effect of the solvents and/or to partial consumption of the solvents (containing oleyl alcohol and canola oil) by the microbes. Further investigation of the microbial activities and solvent degradability is recommended to ensure the solvents are not partially consumed by the consortia. Nevertheless, sustained and increased biogas production in the presence of these solvents exemplifies that there is potential for integrating LLE in AD systems to enhance the overall performance of biogas plants.

Finally, good VFA back-extraction recoveries were attained from TOA, TOPO, TBP and $[P_{666,14}][Phos]$, illustrating that these extractants can be effectively back-extracted to recover extracted VFAs and regenerate the solvents by stripping with sodium hydroxide. This will allow recycling of the solvent, thereby reducing extraction costs and maintaining a sustainable *in situ* LLE setup. Alkaline back-extraction was found to be unsuitable for solvents containing diluent canola oil and extractant Aliquat 336.

While most extractant-diluent combinations demonstrated good performance in at least one of the three areas of interest, TOPO-lamp oil, TOPO-oleyl alcohol, TOA-lamp oil, TOA-oleyl alcohol and TBP-oleyl alcohol would be recommended for further study and implementation in *in situ* VFA extraction from biogas-producing AD systems. Bio-based VFA production from AD using extractive fermentation could indeed be a promising way for resource recovery from AD systems and can lead to integrated management and reduction of waste, resource recovery, and utilisation of renewable energy. The data obtained in the study will be useful in the design of an *in situ* LLE unit for recovery of VFAs from biogas-producing AD systems. However, further investigation is required to develop and integrate the extraction and recovery process for sustainable operation of a system for the continuous co-production of VFAs from biogas-producing AD.

This study successfully achieved its aims through the completion of the objectives. Fundamental criteria were established for the development of a continuous *in situ* LLE operation in AD systems. Potential extractants and diluents for extraction of VFAs from AD systems were identified and the VFA extraction capacities of these solvents were determined at varied system pH. It was established that biogas production is indeed possible when solvents are introduced in an *in situ* manner in AD systems, and recovery and purification of VFAs using alkaline back-extraction is feasible with selected solvents. The results indicate that there is potential to control the VFA composition in AD and improve methane production through partial removal of VFAs to reduce the inhibitory effects of acid accumulation in active digesters.

CHAPTER 7
RECCOMMENDATIONS

Additional research is still required to design a robust, effective and self-regulating control strategy for *in situ* VFA extraction as a means for pH control and valuable side stream generation. The methodology applied in this study could be integrated and used towards developing an *in situ* LLE setup for the extraction and co-production of VFAs from biogas-producing AD systems.

A prototype modified semi-partitioned reactor system in which the concept for *in situ* LLE might be accomplished [103] was used in a preliminary study, which aimed to evaluate the efficacy of the modified reactor for *in situ* VFA extraction and to observe the effect of continuous VFA extraction on the system pH. The details of this study and the preliminary results obtained can be found in Appendix A. The reactor was devised to allow for integration of *in situ* LLE within an operational AD system by inserting a partition to the existing digester for minimal disruption of the established digestion process. An inlet from the partition to the reactor allows for contacting of the extracting phase with the digester media to provide a well-mixed system and the partition or settling zone provides an area for mechanical disengagement, to allow for removal of the solvent phase which can then be pumped to the back extraction unit (recovering the product from the solvent phase). In this way, a continuous process for simultaneous extraction and back extraction of VFAs could be developed. If successful, this concept could be applied to a bioprocess unit for *in situ* product extraction to reduce the effect of product inhibition in AD operation.

The experiments illustrated that the semi-partitioned reactor setup was successful in continuously extracting VFAs, with a direct correlation between VFA extraction and system pH, provided that the solvent is sufficiently stripped. However, the system requires further design adjustments and optimisation. The mode of contact and mixing to facilitate mass transfer are important considerations for the scale-up of the LLE system. Mass transfer due to the contact mode is a key parameter which controls the equilibrium of the system, which is suggested for further investigation in the *in situ* LLE reactor design. Reaction kinetics of the acid extraction also require further research, with determination of kinetic parameters in order to calculate optimal solvent flow rates and ensure sufficient residence times in the reactor and back extraction unit. Furthermore, the design of the back-extraction unit requires further consideration, with improved mass transfer through more efficient mixing of the solvent with the alkaline phase, as the solvent was limited in capacity to continue extracting VFAs. During LLE the solvent became progressively more loaded with VFAs, resulting in decreased extraction efficiency. If the solvent is sufficiently back extracted, with complete removal of the acids from solvent phase, the accumulation of VFAs in the solvent can be alleviated. Once the modes of contact for sufficient mass transfer between the aqueous and organic phases (for both extraction and back-extraction) have

been established and reaction kinetics have been determined, a more comprehensive scale-up of the *in situ* LLE using synthetic VFA solutions for continuous VFA extraction could be attempted, with evaluation of the resultant effect on the pH of the aqueous phase to elucidate the feasibility of the prototype reactor for *in situ* LLE as a self-regulating pH control mechanism.

Thereafter the potential for *in situ* LLE could be investigated using a variety of inoculum and substrate sources to test the robustness of the extraction unit and to allow for investigation of the solvent effect on a range of different microorganisms. The methodology based on (i) VFA extraction capacity at the desired pH, (ii) biocompatibility with the microbial community and (iii) feasibility for back extraction to regenerate the solvent could be applied for the screening of solvents for various AD systems. For future bench-scale biogas production tests, an additional control test sample with pH adjustment prior to the start of the tests, as well as continuous pH measurement and VFA sampling of all samples throughout duration of the experiments are recommended to clarify how different operating conditions affect microbial dynamics and to correlate the pH of the systems with the biogas productivity. While mode of contact and mixing have a significant impact on extraction efficiency, they will likely also increase the effect of solvent toxicity on the AD consortia due to increased contact and prolonged exposure to the extractants. An in-depth investigation of the microbial activities in the AD system in the presence of solvents is recommended using selected solvents in an anaerobic process with continuous *in situ* VFA extraction, with continual monitoring of the pH, VFA concentration and biogas productivity. Further investigation of the composition and mechanisms of the microbial community are also recommended to further elucidate the effect of the solvents and VFA extraction on the AD process.

Additional factors such as co-extraction and solvent degradability are important elements that would have a significant impact on the success of extractive fermentation in AD. Surfactant effects are also suggested for future research. Biosurfactants will likely affect the interaction of the two phases in AD systems, and there may be losses due to surfactants in a continuous *in situ* LLE setup. Re-usability of the extractant could be a limiting factor in the overall process and non-VFA build up in the solvents is an important consideration, both of which are recommended for further investigation.

Finally, techniques for downstream VFA purification are recommended for future research, as this was not the core focus of the present study. Using alkaline back extraction, it may be possible to increase the VFA concentration in the $NaOH_{(aq)}$ until the VFAs begin to precipitate out of solution. Using smaller volumes of solvent and stripping solution at higher recirculation rates in continuous LLE could yield a more concentrated solvent and stripping solution following LLE. This concept was seen for LLE and back-extraction of $[P_{666,14}][Phos]$ with 1 mL of solvent and 2 mL of $NaOH_{(aq)}$, achieving ~31 g/L of VFA in the solvent and ~12 g/L of VFA in the stripping solution. Additional back extraction techniques, such as distillation, could also be investigated to compare downstream purification techniques.

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APPENDIX A

A.1 Preliminary *in situ* LLE investigation

A simulated bioprocess using VFA addition to simulate VFA production was used. The idealised system containing only VFAs and water was utilised to assess of the effect of VFA extraction on the pH of the aqueous phase without interferences and interactions due to organisms and impurities inherent in AD systems. The experimental setup is illustrated in Section 4.9.

The solvent selected for *in situ* operation was 20% (v/v) TOA in oleyl alcohol, due to promising results obtained in the LLE, bench-scale biogas production and back extraction experiments. It should be noted that a proof of concept approach was taken for this experiment, and further investigation is required to select the optimal extractant-diluent combination as outlined in Section 5.5. The contents of the reactor were stirred using an impeller which allowed for mixing of the VFA medium with the solvent. The extract phase was continuously removed from the system and pumped into the back-extraction unit containing 6 M $NaOH_{(aq)}$, where the solvent could bubble up through the alkaline medium. The stripped solvent which had settled to the top phase of the back-extraction unit was simultaneously continuously pumped from the back extraction into the dispersed phases. The pH and the tVFA concentration were monitored at sampling, where triplicate samples were extracted from the aqueous phase at regular time intervals of 15-30 mins and pH measurements were recorded for the duration of sampling (10-15 seconds). An initial tVFA concentration of 2.5g/L at pH 5 (pH adjusted using 2 M $NaOH_{(aq)}$) was utilised in the reactor setup. The VFA content of the reactor was then increased by adding acetic, propionic, butyric, valeric and caproic acid to the digester until the pH of the system decreased to pH 4.4, to simulate VFA accumulation (acid crash). As evident from Figures 44 and 45, as the tVFA concentration increased from 2.5 g/L to 4.6 g/L, the pH drastically decreased. With continuous VFA extraction, the pH stabilised at around pH 4.7, with a corresponding tVFA concentration of 3.1 g/L.

During LLE the solvent becomes progressively more loaded with VFA, causing the extraction efficiency to decrease due to a decline in driving force. If the solvent is sufficiently back-extracted to strip the acids from solvent phase, the accumulation of VFAs in the solvent can be alleviated [83]. It was suspected that the solvent capacity to continuously extract VFAs was limited by overloading of the solvent with VFAs and inefficient back extraction due to inadequate mixing in the back extraction unit. An additional volume of 100 mL of solvent was added to the system to test this theory. The tVFA concentration thereafter decreased to approximately 2.6 g/L and stabilised at pH 4.9, as seen in Figures 44 and 45 by the decreased in tVFA concentration and jump in pH. These results indicate that the design of the back extraction unit requires optimisation with more efficient mixing, as the solvent was indeed limited in capacity to continue extracting VFAs since introducing more solvent resulted in improved extraction capacity. HPLC analysis of the alkaline

back extraction solution revealed tVFA concentration of 2.3 g/L. Considering the VFA medium volume of 1000 mL and the $NaOH_{(aq)}$ volume of 200 mL, only 23 % of the extracted VFAs were recovered into the alkaline product, confirming that only a small proportion of the extracted VFAs were recovered into the alkaline product phase.

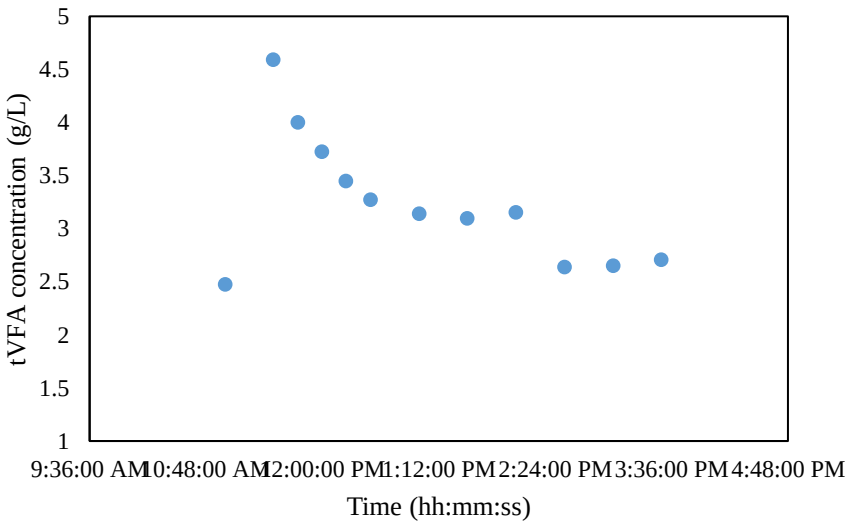

Figure 44: *Total VFA concentration of aqueous medium over experimental duration. Error bars represent standard error of triplicate measurements.*

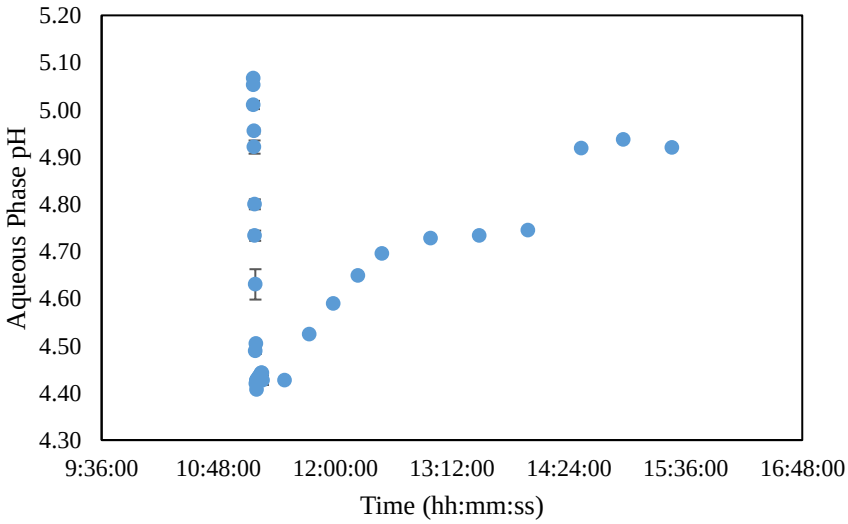

Figure 45: *pH measurements of the aqueous phase over experimental duration, error bars represent standard error of pH measurements during sampling time.*

An additional *in situ* experimental run was conducted at a starting pH above the pKa of the acids at pH 5.7 and tVFA concentration of 2.5 g/L. Over the course of the experimental run the pH stabilised at pH 6 and tVFA concentration of 2.35 g/L, illustrated in Figures 46 and 47.

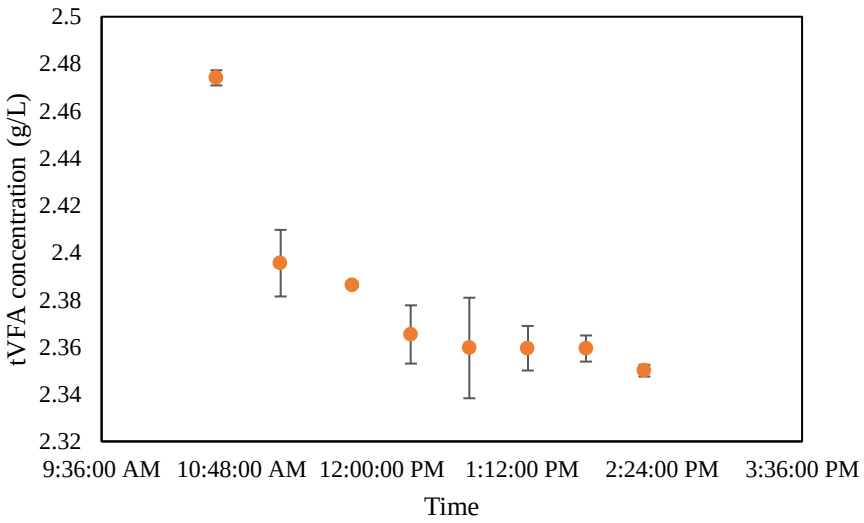

Figure 46: *Total VFA concentration of aqueous medium over experimental duration. Error bars represent standard error of triplicate measurements*

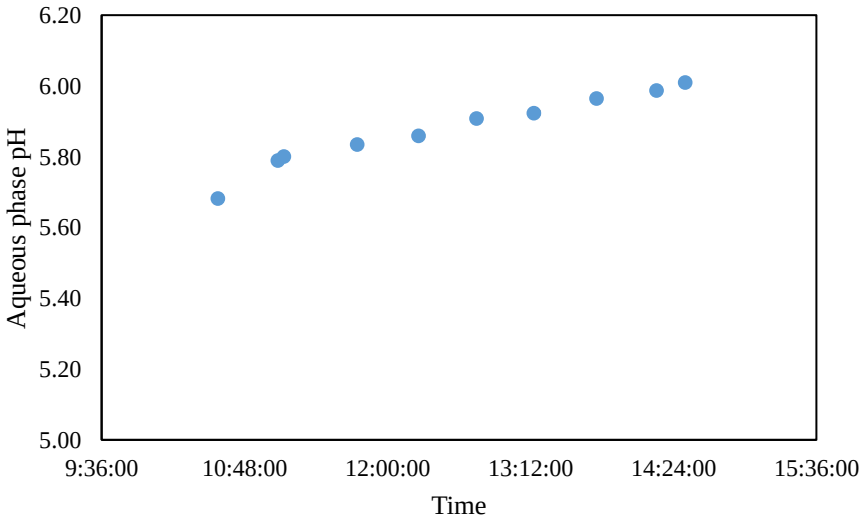

Figure 47: *pH measurements of the aqueous phase over experimental duration, error bars represent standard error of pH measurements during sampling time*

Although the system requires further design adjustments and optimisation, the experiments illustrate that the semi-partitioned reactor setup was successful in continuously extracting VFAs, with a direct correlation between VFA extraction and system pH, provided that the solvent is sufficiently stripped. From the back

extraction experiments conducted (details of which are discussed in Section 5.4) it is evident that complete recovery can be achieved with sufficient mixing of the solvent with alkaline solution, which needs to be considered in the future design of the back extraction unit. With sufficient stripping of the solvent and adjustment of the solvent flow rate, VFA extraction could indeed be used to control the system pH, even at pH above the pKa of the acids to be extracted. With improved design of the back extraction, this concept could be applied to a bioprocess unit for AD operation for *in situ* product extraction to reduce the effect of product inhibition.

APPENDIX B

B.1 Supplementary data for bench-scale biogas production tests

Table 18: *Characteristics of inoculum and substrate for bench-scale biogas production tests.*

Parameter	Inoculum (Test 1)	Substrate (Test 1)	Inoculum (Test 2)	Substrate (Test 2)
pH	6.95	5.69	7.17	4.34
COD (g/L)	11.30	13.90	7.95	11.15
Total Nitrogen (mg/L)	0.22	0.04	0.28	0.03
Alkalinity (mg $CaCO_3$/L)	2.46	-	1.17	-
tVFA (g/L)	0.10	1.38	0.03	2.23
TS %	2.38	0.55	1.13	0.26
VS %	1.75	0.53	1.03	0.24
Moisture content (%)	98.74	98.78	98.85	98.89

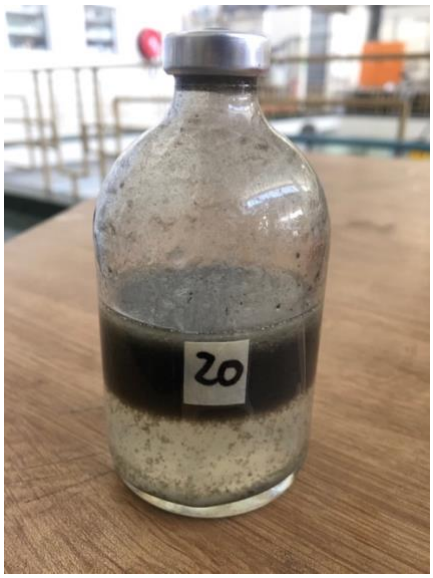

Figure 48: *Bench-scale biogas production sample test with inoculum, substrate and solvent.*

APPENDIX C

C.1 Error propagation

Uncertainty was expressed in terms of the uncertainty parameter (Δ), with significance level (α) = 0.05 and sample size (n) = 3.

$$\Delta = t(\alpha, n-1)S_n$$

$$S_n = \frac{s}{\sqrt{n}}$$

Uncertainty propagation of a given function f(x, y) was determined using the following equation:

$$\Delta_f = \sqrt{\left[\left(\frac{\partial f}{\partial x}\right)_{\bar{x}\,\bar{y}} \Delta_x\right]^2 + \left[\left(\frac{\partial f}{\partial y}\right)_{\bar{x}\,\bar{y}} \Delta_y\right]^2}$$

Where $\left(\frac{\partial f}{\partial x}\right)_{\bar{x}\,\bar{y}}$ refers to the partial derivative of the function with respect to x and $\left(\frac{\partial f}{\partial y}\right)_{\bar{x}\,\bar{y}}$ refers to the partial derivative of the function with respect to variable y.

The uncertainty parameters Δ_x and Δ_y were calculated using the sample standard error and the student's t-statistic. Table 19 below summarises the MS Excel commands used.

Table 19: *Summary of functions used for error propagation.*

	Symbol	Function
Number of samples	n	$count(x); count(y)$
Sample mean	$\bar{x}; \bar{y}$	$average(x); average(y)$
Sample standard deviation	s	$stdev(x); stdev(y)$
Sample standard error	s_n	$s/sqrt(n)$
Significant level	α	0.05
Student's t-statistic	$t(\alpha, n-1)$	$t.inv.2t(\alpha, n-1)$
Uncertainty parameter	$\Delta_x; \Delta_y$	$t(\alpha, n-1) * S_n$

Uncertainty from initial and equilibrium aqueous concentration measurements was considered using the standard deviations of triplicate measurements which were propagated in the $\%E$ function with the following assumptions; errors were small, measurements were normally distributed, and errors were independent. The uncertainty propagation was completed using partial derivatives of the function with respect to $\left([TA]_{aq}\right)_i$ and $\left([TA]_{aq}\right)_{eq}$:

$$\frac{\partial \%E}{\partial \left([TA]_{aq}\right)_{eq}} = -\frac{\left(V_{aq}\right)_{eq}}{\left([TA]_{aq} V_{aq}\right)_i}$$

$$\frac{\partial \%E}{\partial([TA]_{aq})_i} = \frac{([TA]_{aq}V_{aq})_{eq}}{(V_{aq}[TA]_{aq}^2)_i}$$

Error bars were used to represent uncertainty propagation, calculated using equation:

$$\Delta_{\%E} = \sqrt{\left[\left(\frac{\partial \%E}{\partial([TA]_{aq})_{eq}}\right)_{\overline{[TA]}_{aq,i}\overline{[TA]}_{aq,eq}} \Delta_{([TA]_{aq})_{eq}}\right]^2 + \left[\left(\frac{\partial \%E}{\partial([TA]_{aq})_i}\right)_{\overline{[TA]}_{aq,i}\overline{[TA]}_{aq,eq}} \Delta_{([TA]_{aq})_i}\right]^2}$$

Uncertainty from aqueous and organic concentration measurements was considered using the standard deviations of triplicate measurements which were propagated in the $\%R$.

$$\frac{\partial \%R}{\partial([TA]_{aq})_{eq}} = \frac{(V_{aq})_{eq}}{([TA]_o V_o)_i}$$

$$\frac{\partial \%R}{\partial([TA]_o)_i} = -\frac{([TA]_{aq}V_{aq})_{eq}}{(V_o[TA]_o^2)_i}$$

$$\Delta_{\%R} = \sqrt{\left[\left(\frac{\partial \%R}{\partial([TA]_{aq})_{eq}}\right)_{\overline{[TA]}_{aq}\overline{[TA]}_o} \Delta_{([TA]_{aq})_{eq}}\right]^2 + \left[\left(\frac{\partial \%R}{\partial([TA]_o)_i}\right)_{\overline{[TA]}_{aq}\overline{[TA]}_o} \Delta_{([TA]_o)_i}\right]^2}$$

C.2 Liquid-liquid extraction

Expression of degree of extraction in terms of initial and final aqueous phase VFA concentrations:

$$[TA]_{o,extract} = \frac{([TA]_{aq}V_{aq})_i - ([TA]_{aq}V_{aq})_{eq}}{V_{o,extract}}$$

$$\%E = \frac{([TA]_o V_o)_{extract}}{([TA]_{aq}V_{aq})_i} \times 100$$

$$\%E = \frac{\frac{([TA]_{aq}V_{aq})_i - ([TA]_{aq}V_{aq})_{eq}}{V_{o,extract}} V_{o,extract}}{([TA]_{aq}V_{aq})_i} \times 100$$

$$\%E = 1 - \frac{([TA]_{aq}V_{aq})_{eq}}{([TA]_{aq}V_{aq})_i} \times 100$$

Example

Total VFA (tVFA) extraction using TBP-oleyl alcohol at pH 3.9

$[TA]_{aq,i}$ (g/L)	$[TA]_{aq,eq}$ (g/L)
8.1435	3.5196
8.1595	3.5203
8.1474	3.5178

$$\overline{[TA]}_{aq,i} = \frac{8.1435 + 8.1595 + 8.1474}{3} = 8.1501\ g/L$$

$$\overline{[TA]}_{aq,eq} = \frac{3.5196 + 3.5203 + 3.5178}{3} = 3.5192\ g/L$$

$$\%E = 1 - \frac{([TA]_{aq}V_{aq})_{eq}}{([TA]_{aq}V_{aq})_{i}} = 1 - \frac{\left(3.5192\frac{g}{L}\right)(0.005\ L)}{\left(8.1501\frac{g}{L}\right)(0.005\ L)} \times 100 = 56.82\ \%$$

Example

Total VFA (tVFA) extraction using TBP-oleyl alcohol at pH 3.9

$$\frac{\partial \%E}{\partial([TA]_{aq})_{eq}} = -\frac{(V_{aq})_{eq}}{([TA]_{aq}V_{aq})_{i}} = -\frac{0.005}{(8.1501)\,(0.005)} = -0.1227$$

$$\frac{\partial \%E}{\partial([TA]_{aq})_{i}} = \frac{([TA]_{aq}V_{aq})_{eq}}{(V_{aq}[TA]_{aq}^{2})_{i}} = \frac{(3.5192)(0.005)}{(0.005)(8.1501)^2} = 0.0530$$

$$\Delta_{([TA]_{aq})_{eq}} = t(\alpha, n-1)S_n = (4.3027)(0.0007) = 0.0032$$

$$\Delta_{([TA]_{aq})_{i}} = t(\alpha, n-1)S_n = (4.3027)(0.0048) = 0.0207$$

$$\Delta_{\%E} = \sqrt{\left[\left(\frac{\partial \%E}{\partial([TA]_{aq})_{eq}}\right)_{\overline{[TA]}_{aq,i}\overline{[TA]}_{aq,eq}} \Delta_{([TA]_{aq})_{eq}}\right]^2 + \left[\left(\frac{\partial \%E}{\partial([TA]_{aq})_{i}}\right)_{\overline{[TA]}_{aq,i}\overline{[TA]}_{aq,eq}} \Delta_{([TA]_{aq})_{i}}\right]^2}$$

$$\Delta_{\%E} = \sqrt{[(-0.1227)(0.0207)]^2 + [(0.0530)(0.0032)]^2} = 0.0012 = 0.12\%$$

C.3 Bench-scale biogas production tests

The methane yield was calculated as the volume of biomethane produced per amount of organic substrate material added to each of the reactors. This was determined as the difference between the accumulated volume of biomethane from the reactor containing the sample with inoculum and substrate (V_S) and the volume of biomethane coming from the inoculum present in the sample bottle is (V_I), divided by the mass of COD of the substrate in the sample bottle ($m_{COD,Ss}$). The volume of biomethane coming from the inoculum present in the sample bottle is (V_I) was determined as the ratio between the total amount of the inoculum in the sample (m_{Is}) and the one in the blank (m_{Ib}).

m_{Ss}: mass of substrate in sample bottle
m_{Is}: mass of inoculum in sample bottle
m_{Ib}: mass of inoculum in blank bottle
V_{Ss}: accumulated biomethane volume from sample bottle
V_b: accumulated biomethane volume from blank bottle

$$Methane\ Yield\ \left(\frac{mL}{gCOD}\right) = \frac{V_S - V_I}{m_{COD,Ss}} = \frac{V_S - V_B\left(\frac{m_{Is}}{m_{Ib}}\right)}{m_{COD,Ss}}$$

Example

Day 21 for TOA-oleyl alcohol:

Sample cumulative biomethane volume (mL)	Blank cumulative biomethane volume (mL)
95.41	0.33
100.09	1.65
80.06	0.31

$$COD_{Ss} = 13900\,\frac{mg}{L}$$

$$m_{Ss} = 13.310\ g, m_{IS} = 49.253\ g, m_{Ib} = 49.313\ g$$

$$V_{Ss} = 13.310\ g\ \div\ 1.046\,\frac{g}{mL} =\ 12.725\ mL$$

$$m_{COD,Ss} = m_{COD,Ss} \times\ V_{Ss} = 13.310\ g\ \times\ 12.725\ mL = 0.177g$$

$$Methane\ Yield\ \left(\frac{mL}{gCOD}\right) = \frac{V_S - V_B\left(\frac{m_{Is}}{m_{Ib}}\right)}{m_{COD,Ss}} = \frac{91.85\ mL - 0.76mL\left(\frac{49.253\ g}{49.313g}\right)}{0.177g} = 515\,\frac{mL}{gCOD}$$

$$Uncertainty_{test/control} = \sqrt{(SD_b)^2 + \left(SD_{s/control}\right)^2} = \sqrt{(0.768)^2 + (10.481)^2} = 10.51$$

C.4 Back-extraction

The weight percentage of acid transferred from the organic extract phase ($[TA]_{o,extract}$) into the alkaline aqueous phase $\left([TA]_{aq}V_{aq}\right)_{equilibrium}$ was expressed as the percentage recovery ($\%R$) according to the corresponding acid.

$$\%R = \frac{\left([TA]_{aq}V_{aq}\right)_{equilibrium}}{([TA]_oV_o)_{extract}}$$

Example

Total VFA (tVFA) recovery from TBP-oleyl alcohol at pH 3.9

$[\boldsymbol{TA}]_{\boldsymbol{aq,eq}}$ (g/L)	$[\boldsymbol{TA}]_{\boldsymbol{o,extract}}$ (g/L)
2.0471	4.6239
2.2069	4.6392
2.1729	4.6296

$$[\overline{TA}]_{o,extract} = \frac{4.6239 + 4.6392 + 4.6296}{3} = 4.6309\ g/L$$

$$\%R = \frac{\left([TA]_{aq}V_{aq}\right)_{eq}}{([TA]_oV_o)_{extract}} = \frac{\left(2.1423\frac{g}{L}\right)(0.01\ L)}{\left(4.6309\frac{g}{L}\right)(0.005\ L)} = 92.52\ \%$$

$$\frac{\partial \%R}{\partial\left([TA]_{aq}\right)_{eq}} = \frac{\left(V_{aq}\right)_{eq}}{([TA]_oV_o)_i} = \frac{0.01}{(4.6309)(0.005)} = 0.4319$$

$$\frac{\partial \%R}{\partial([TA]_o)_i} = -\frac{\left([TA]_{aq}V_{aq}\right)_{eq}}{\left(V_o[TA]_o^2\right)_i} = -\frac{(2.1423)(0.01)}{(0.005)(4.6309)^2} = -0.1998$$

$$\Delta_{\%R} = \sqrt{\left[\left(\frac{\partial \%R}{\partial\left([TA]_{aq}\right)_{eq}}\right)_{\overline{[TA]}_{aq}\overline{[TA]}_o} \Delta_{\left([TA]_{aq}\right)_{eq}}\right]^2 + \left[\left(\frac{\partial \%R}{\partial([TA]_o)_i}\right)_{\overline{[TA]}_{aq}\overline{[TA]}_o} \Delta_{([TA]_o)_i}\right]^2}$$

$$\Delta_{\left([TA]_{aq}\right)_{eq}} = t(\alpha, n-1)S_n = 0.2091,\ \Delta_{([TA]_o)_i} = t(\alpha, n-1)S_n = 0.0192$$

$$\Delta_{\%R} = \sqrt{[(0.4319)(0.2091)]^2 + [(-0.1998)(0.0192)]^2} = 0.9039 = 9.04\%$$

APPENDIX D

D.1 Back-extraction supplementary data

Figure 49: *Emulsification with back-extraction of Aliquat-canola oil*

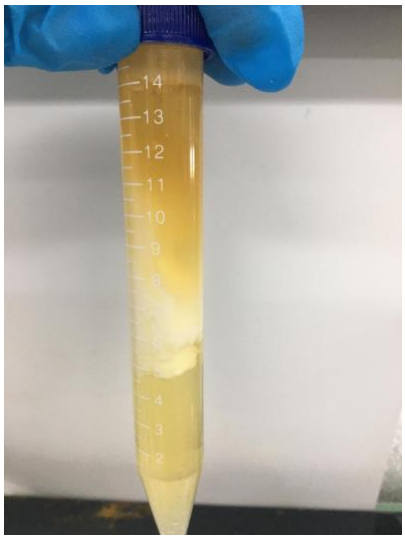

Figure 50: *Emulsification with back-extraction of $[P_{666,14}][Phos]$-canola oil*

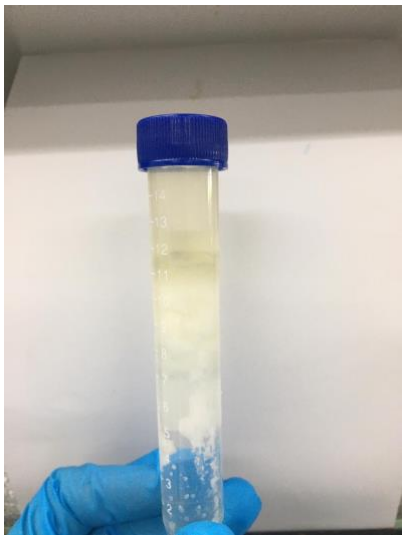

Figure 51: *Emulsification with back-extraction of TOPO-canola oil*

Figure 52: *Emulsification with back-extraction of TOA-canola oil*

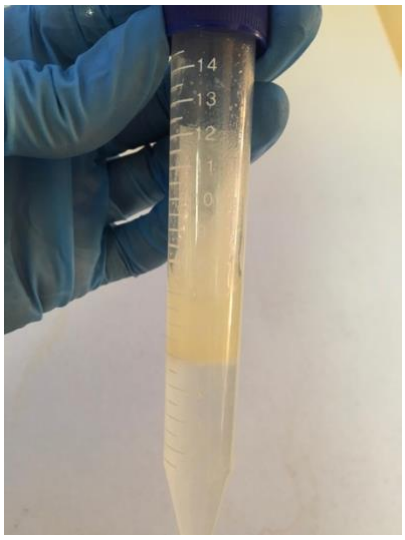

Figure 53: *Emulsification with back-extraction of TBP-canola oil*

APPENDIX E

E.1 Liquid-liquid extraction raw data with model aqueous solutions

Table 20: *Initial and final aqueous phase VFA concentrations from LLE experiments with TBP and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)						Initial aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TBP	oleyl alcohol	3.9	1.943	1.103	0.448	0.025	0.000	3.520	2.442	2.419	2.356	0.463	0.463	8.144
TBP	oleyl alcohol	3.9	1.933	1.110	0.452	0.026	0.000	3.520	2.440	2.421	2.356	0.466	0.476	8.160
TBP	oleyl alcohol	3.9	1.933	1.108	0.450	0.027	0.000	3.518	2.441	2.420	2.371	0.464	0.453	8.147
TBP	oleyl alcohol	5.6	2.349	2.211	1.894	0.244	0.100	6.798	2.352	2.328	2.274	0.448	0.465	7.866
TBP	oleyl alcohol	5.6	2.342	2.214	1.897	0.246	0.102	6.801	2.332	2.322	2.269	0.446	0.463	7.833
TBP	oleyl alcohol	5.6	2.355	2.216	1.897	0.242	0.100	6.809	2.335	2.326	2.271	0.447	0.465	7.845
TBP	oleyl alcohol	6.8	2.330	2.301	2.234	0.428	0.400	7.693	2.337	2.319	2.264	0.447	0.460	7.827
TBP	oleyl alcohol	6.8	2.328	2.302	2.236	0.429	0.410	7.706	2.325	2.312	2.258	0.451	0.461	7.808
TBP	oleyl alcohol	6.8	2.312	2.286	2.221	0.427	0.403	7.649	2.322	2.312	2.256	0.448	0.467	7.806
TBP	lamp oil	3.9	1.953	1.083	0.410	0.022	0.000	3.468	2.442	2.419	2.356	0.463	0.463	8.144
TBP	lamp oil	3.9	1.978	1.121	0.432	0.024	0.000	3.555	2.440	2.421	2.356	0.466	0.476	8.160
TBP	lamp oil	3.9	1.984	1.124	0.435	0.024	0.000	3.567	2.441	2.420	2.371	0.464	0.453	8.147
TBP	lamp oil	5.6	2.326	2.181	1.850	0.235	0.087	6.679	2.352	2.328	2.274	0.448	0.465	7.866
TBP	lamp oil	5.6	2.327	2.192	1.864	0.235	0.089	6.707	2.332	2.322	2.269	0.446	0.463	7.833
TBP	lamp oil	5.6	2.326	2.190	1.859	0.232	0.089	6.696	2.335	2.326	2.271	0.447	0.465	7.845
TBP	lamp oil	6.8	2.291	2.258	2.193	0.418	0.388	7.548	2.337	2.319	2.264	0.447	0.460	7.827
TBP	lamp oil	6.8	2.295	2.269	2.202	0.420	0.384	7.570	2.325	2.312	2.258	0.451	0.461	7.808
TBP	lamp oil	6.8	2.296	2.268	2.201	0.421	0.390	7.576	2.322	2.312	2.256	0.448	0.467	7.806
TBP	canola oil	3.9	2.001	1.220	0.511	0.031	0.000	3.763	2.442	2.419	2.356	0.463	0.463	8.144
TBP	canola oil	3.9	1.992	1.214	0.509	0.030	0.000	3.744	2.440	2.421	2.356	0.466	0.476	8.160
TBP	canola oil	3.9	2.011	1.231	0.516	0.031	0.000	3.788	2.441	2.420	2.371	0.464	0.453	8.147
TBP	canola oil	5.6	2.279	2.155	1.843	0.242	0.101	6.621	2.352	2.328	2.274	0.448	0.465	7.866
TBP	canola oil	5.6	2.276	2.149	1.838	0.243	0.099	6.604	2.332	2.322	2.269	0.446	0.463	7.833
TBP	canola oil	5.6	2.267	2.140	1.832	0.243	0.097	6.579	2.335	2.326	2.271	0.447	0.465	7.845
TBP	canola oil	6.8	2.289	2.259	2.186	0.424	0.383	7.539	2.337	2.319	2.264	0.447	0.460	7.827
TBP	canola oil	6.8	2.306	2.279	2.202	0.426	0.390	7.602	2.325	2.312	2.258	0.451	0.461	7.808
TBP	canola oil	6.8	2.292	2.263	2.187	0.425	0.384	7.550	2.322	2.312	2.256	0.448	0.467	7.806

Table 21: *Initial and final aqueous phase VFA concentrations from LLE experiments with TOPO and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	**Diluent**	**Initial pH**	**Final aqueous phase acid concentration (g/L)**						**Initial aqueous phase acid concentration (g/L)**					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOPO	oleyl alcohol	3.9	1.999	1.108	0.447	0.024	0.000	3.578	2.537	2.494	2.474	0.520	0.659	8.684
TOPO	oleyl alcohol	3.9	1.905	1.013	0.403	0.022	0.000	3.343	2.542	2.500	2.479	0.519	0.664	8.704
TOPO	oleyl alcohol	3.9	1.943	1.026	0.406	0.021	0.000	3.396	2.543	2.495	2.468	0.517	0.492	8.515
TOPO	oleyl alcohol	5.6	2.400	2.168	1.765	0.212	0.073	6.617	2.423	2.381	2.361	0.497	0.495	8.157
TOPO	oleyl alcohol	5.6	2.336	2.105	1.710	0.206	0.068	6.424	2.426	2.388	2.367	0.498	0.487	8.165
TOPO	oleyl alcohol	5.6	2.387	2.157	1.758	0.213	0.073	6.588	2.415	2.376	2.358	0.496	0.496	8.141
TOPO	oleyl alcohol	6.8	2.436	2.405	2.224	0.355	0.195	7.616	2.386	2.432	2.415	0.519	0.483	8.235
TOPO	oleyl alcohol	6.8	2.444	2.407	2.221	0.356	0.196	7.623	2.395	2.434	2.418	0.509	0.490	8.246
TOPO	oleyl alcohol	6.8	2.423	2.387	2.214	0.353	0.193	7.569	2.405	2.433	2.414	0.509	0.502	8.263
TOPO	lamp oil	3.9	1.286	0.402	0.126	0.000	0.000	1.814	2.537	2.494	2.474	0.520	0.659	8.684
TOPO	lamp oil	3.9	1.070	0.328	0.101	0.000	0.000	1.498	2.542	2.500	2.479	0.519	0.664	8.704
TOPO	lamp oil	3.9	1.249	0.388	0.121	0.000	0.000	1.758	2.543	2.495	2.468	0.517	0.492	8.515
TOPO	lamp oil	5.6	2.414	2.184	1.638	0.162	0.050	6.448	2.444	2.464	2.457	0.516	0.509	8.390
TOPO	lamp oil	5.6	2.414	2.189	1.645	0.164	0.049	6.462	2.437	2.455	2.430	0.506	0.527	8.354
TOPO	lamp oil	5.6	2.416	2.189	1.642	0.160	0.051	6.458	2.432	2.435	2.427	0.515	0.485	8.294
TOPO	lamp oil	6.8	2.425	2.341	2.047	0.281	0.091	7.184	2.386	2.432	2.415	0.519	0.483	8.235
TOPO	lamp oil	6.8	2.429	2.349	2.052	0.275	0.093	7.199	2.395	2.434	2.418	0.509	0.490	8.246
TOPO	lamp oil	6.8	2.413	2.343	2.046	0.267	0.085	7.154	2.405	2.433	2.414	0.509	0.502	8.263
TOPO	canola oil	3.9	1.421	0.510	0.173	0.000	0.000	2.103	2.537	2.494	2.474	0.520	0.659	8.684
TOPO	canola oil	3.9	1.419	0.519	0.167	0.000	0.000	2.106	2.542	2.500	2.479	0.519	0.664	8.704
TOPO	canola oil	3.9	1.405	0.512	0.164	0.000	0.000	2.080	2.543	2.495	2.468	0.517	0.492	8.515
TOPO	canola oil	5.6	2.411	2.187	1.670	0.165	0.054	6.485	2.444	2.464	2.457	0.516	0.509	8.390
TOPO	canola oil	5.6	2.404	2.188	1.676	0.166	0.055	6.490	2.437	2.455	2.430	0.506	0.527	8.354
TOPO	canola oil	5.6	2.799	2.153	1.666	0.171	0.077	6.867	2.432	2.435	2.427	0.515	0.485	8.294
TOPO	canola oil	6.8	2.472	2.377	2.124	0.326	0.178	7.477	2.386	2.432	2.415	0.519	0.483	8.235
TOPO	canola oil	6.8	2.420	2.351	2.131	0.318	0.165	7.385	2.395	2.434	2.418	0.509	0.490	8.246
TOPO	canola oil	6.8	2.433	2.377	2.148	0.320	0.161	7.438	2.405	2.433	2.414	0.509	0.502	8.263

Table 22: *Initial and final aqueous phase VFA concentrations from LLE experiments with TOA and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)						Initial aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOA	oleyl alcohol	3.9	0.972	0.423	0.133	0.000	0.000	1.528	2.442	2.419	2.356	0.463	0.463	8.144
TOA	oleyl alcohol	3.9	0.954	0.413	0.131	0.000	0.000	1.498	2.440	2.421	2.356	0.466	0.476	8.160
TOA	oleyl alcohol	3.9	0.947	0.409	0.127	0.000	0.000	1.483	2.441	2.420	2.371	0.464	0.453	8.147
TOA	oleyl alcohol	5.6	2.280	2.077	1.615	0.152	0.033	6.157	2.352	2.328	2.274	0.448	0.465	7.866
TOA	oleyl alcohol	5.6	2.275	2.099	1.658	0.166	0.038	6.237	2.332	2.322	2.269	0.446	0.463	7.833
TOA	oleyl alcohol	5.6	2.288	2.140	1.756	0.199	0.064	6.447	2.335	2.326	2.271	0.447	0.465	7.845
TOA	oleyl alcohol	6.8	2.316	2.239	2.041	0.318	0.179	7.093	2.337	2.319	2.264	0.447	0.460	7.827
TOA	oleyl alcohol	6.8	2.304	2.240	2.037	0.308	0.161	7.050	2.325	2.312	2.258	0.451	0.461	7.808
TOA	oleyl alcohol	6.8	2.306	2.242	2.048	0.316	0.171	7.084	2.322	2.312	2.256	0.448	0.467	7.806
TOA	lamp oil	3.9	2.313	1.912	1.140	0.086	0.021	5.471	2.442	2.419	2.356	0.463	0.463	8.144
TOA	lamp oil	3.9	2.306	1.909	1.127	0.085	0.018	5.446	2.440	2.421	2.356	0.466	0.476	8.160
TOA	lamp oil	3.9	2.302	1.906	1.130	0.086	0.020	5.444	2.441	2.420	2.371	0.464	0.453	8.147
TOA	lamp oil	5.6	2.333	2.266	2.079	0.313	0.151	7.142	2.352	2.328	2.274	0.448	0.465	7.866
TOA	lamp oil	5.6	2.327	2.267	2.082	0.320	0.154	7.151	2.332	2.322	2.269	0.446	0.463	7.833
TOA	lamp oil	5.6	2.325	2.263	2.075	0.312	0.147	7.122	2.335	2.326	2.271	0.447	0.465	7.845
TOA	lamp oil	6.8	2.317	2.293	2.239	0.428	0.393	7.671	2.337	2.319	2.264	0.447	0.460	7.827
TOA	lamp oil	6.8	2.307	2.285	2.216	0.413	0.358	7.579	2.325	2.312	2.258	0.451	0.461	7.808
TOA	lamp oil	6.8	2.307	2.294	2.234	0.430	0.393	7.657	2.322	2.312	2.256	0.448	0.467	7.806
TOA	canola oil	3.9	2.110	1.448	0.636	0.035	0.000	4.229	2.442	2.419	2.356	0.463	0.463	8.144
TOA	canola oil	3.9	2.093	1.438	0.633	0.033	0.000	4.196	2.440	2.421	2.356	0.466	0.476	8.160
TOA	canola oil	3.9	2.095	1.440	0.641	0.036	0.000	4.212	2.441	2.420	2.371	0.464	0.453	8.147
TOA	canola oil	5.6	2.333	2.225	1.949	0.265	0.106	6.877	2.352	2.328	2.274	0.448	0.465	7.866
TOA	canola oil	5.6	2.320	2.223	1.953	0.261	0.099	6.855	2.332	2.322	2.269	0.446	0.463	7.833
TOA	canola oil	5.6	2.314	2.220	1.946	0.260	0.105	6.846	2.335	2.326	2.271	0.447	0.465	7.845
TOA	canola oil	6.8	2.327	2.289	2.196	0.401	0.309	7.521	2.337	2.319	2.264	0.447	0.460	7.827
TOA	canola oil	6.8	2.310	2.288	2.203	0.397	0.311	7.508	2.325	2.312	2.258	0.451	0.461	7.808
TOA	canola oil	6.8	2.296	2.265	2.151	0.358	0.251	7.321	2.322	2.312	2.256	0.448	0.467	7.806

Table 23: *Initial and final aqueous phase VFA concentrations from LLE experiments with Aliquat 336].and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	**Diluent**	**Initial pH**	**Final aqueous phase acid concentration (g/L)**						**Initial aqueous phase acid concentration (g/L)**					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
Aliquat	oleyl alcohol	3.9	1.608	0.863	0.313	0.018	0.000	2.801	2.442	2.419	2.356	0.463	0.463	8.144
Aliquat	oleyl alcohol	3.9	1.616	0.881	0.323	0.018	0.000	2.837	2.440	2.421	2.356	0.466	0.476	8.160
Aliquat	oleyl alcohol	3.9	1.617	0.880	0.323	0.018	0.000	2.838	2.441	2.420	2.371	0.464	0.453	8.147
Aliquat	oleyl alcohol	5.6	1.390	0.841	0.368	0.024	0.000	2.623	2.352	2.328	2.274	0.448	0.465	7.866
Aliquat	oleyl alcohol	5.6	1.363	0.828	0.372	0.023	0.000	2.586	2.332	2.322	2.269	0.446	0.463	7.833
Aliquat	oleyl alcohol	5.6	1.363	0.823	0.365	0.023	0.000	2.574	2.335	2.326	2.271	0.447	0.465	7.845
Aliquat	oleyl alcohol	6.8	1.452	0.921	0.431	0.029	0.000	2.833	2.337	2.319	2.264	0.447	0.460	7.827
Aliquat	oleyl alcohol	6.8	1.427	0.906	0.424	0.028	0.000	2.785	2.325	2.312	2.258	0.451	0.461	7.808
Aliquat	oleyl alcohol	6.8	1.459	0.939	0.442	0.029	0.000	2.869	2.322	2.312	2.256	0.448	0.467	7.806
Aliquat	lamp oil	3.9	1.552	0.827	0.314	0.017	0.000	2.710	2.442	2.419	2.356	0.463	0.463	8.144
Aliquat	lamp oil	3.9	1.549	0.839	0.318	0.017	0.000	2.722	2.440	2.421	2.356	0.466	0.476	8.160
Aliquat	lamp oil	3.9	1.530	0.815	0.311	0.017	0.000	2.672	2.441	2.420	2.371	0.464	0.453	8.147
Aliquat	lamp oil	5.6	1.528	0.991	0.524	0.035	0.000	3.077	2.352	2.328	2.274	0.448	0.465	7.866
Aliquat	lamp oil	5.6	1.556	1.034	0.545	0.037	0.000	3.172	2.332	2.322	2.269	0.446	0.463	7.833
Aliquat	lamp oil	5.6	1.534	1.012	0.525	0.036	0.000	3.107	2.335	2.326	2.271	0.447	0.465	7.845
Aliquat	lamp oil	6.8	1.627	1.146	0.659	0.050	0.013	3.495	2.337	2.319	2.264	0.447	0.460	7.827
Aliquat	lamp oil	6.8	1.613	1.142	0.660	0.050	0.013	3.478	2.325	2.312	2.258	0.451	0.461	7.808
Aliquat	lamp oil	6.8	1.606	1.134	0.648	0.048	0.000	3.435	2.322	2.312	2.256	0.448	0.467	7.806
Aliquat	canola oil	3.9	1.529	0.817	0.302	0.000	0.000	2.648	2.442	2.419	2.356	0.463	0.463	8.144
Aliquat	canola oil	3.9	1.511	0.809	0.300	0.017	0.000	2.637	2.440	2.421	2.356	0.466	0.476	8.160
Aliquat	canola oil	3.9	1.528	0.826	0.306	0.018	0.000	2.678	2.441	2.420	2.371	0.464	0.453	8.147
Aliquat	canola oil	5.6	1.504	0.937	0.504	0.038	0.000	2.983	2.352	2.328	2.274	0.448	0.465	7.866
Aliquat	canola oil	5.6	1.543	0.994	0.547	0.040	0.000	3.124	2.332	2.322	2.269	0.446	0.463	7.833
Aliquat	canola oil	5.6	1.553	1.012	0.546	0.041	0.000	3.152	2.335	2.326	2.271	0.447	0.465	7.845
Aliquat	canola oil	6.8	1.673	1.196	0.703	0.056	0.013	3.640	2.337	2.319	2.264	0.447	0.460	7.827
Aliquat	canola oil	6.8	1.651	1.171	0.698	0.055	0.013	3.588	2.325	2.312	2.258	0.451	0.461	7.808
Aliquat	canola oil	6.8	1.660	1.176	0.707	0.055	0.013	3.610	2.322	2.312	2.256	0.448	0.467	7.806

Table 24: *Initial and final aqueous phase VFA concentrations from LLE experiments with [P666,14][Phos].and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)						Initial aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
$[P_{666,14}][Phos]$	oleyl alcohol	3.9	1.767	1.009	0.424	0.026	0.000	3.226	2.538	2.562	2.544	0.532	0.532	8.708
$[P_{666,14}][Phos]$	oleyl alcohol	3.9	1.755	1.002	0.439	0.022	0.000	3.218	2.541	2.528	2.500	0.520	0.458	8.547
$[P_{666,14}][Phos]$	oleyl alcohol	3.9	1.756	1.011	0.426	0.022	0.000	3.215	2.543	2.533	2.514	0.523	0.479	8.593
$[P_{666,14}][Phos]$	oleyl alcohol	5.6	2.463	2.474	2.436	0.482	0.379	8.233	2.444	2.464	2.457	0.516	0.509	8.390
$[P_{666,14}][Phos]$	oleyl alcohol	5.6	2.452	2.466	2.415	0.483	0.376	8.194	2.437	2.455	2.430	0.506	0.527	8.354
$[P_{666,14}][Phos]$	oleyl alcohol	5.6	2.466	2.476	2.445	0.487	0.388	8.262	2.432	2.435	2.427	0.515	0.485	8.294
$[P_{666,14}][Phos]$	oleyl alcohol	6.8	2.444	2.464	2.451	0.487	0.444	8.290	2.386	2.432	2.415	0.519	0.483	8.235
$[P_{666,14}][Phos]$	oleyl alcohol	6.8	2.442	2.457	2.439	0.489	0.436	8.263	2.395	2.434	2.418	0.509	0.490	8.246
$[P_{666,14}][Phos]$	oleyl alcohol	6.8	2.364	2.459	2.470	0.493	0.450	8.235	2.405	2.433	2.414	0.509	0.502	8.263
$[P_{666,14}][Phos]$	lamp oil	3.9	1.540	0.715	0.288	0.015	0.000	2.558	2.537	2.494	2.474	0.520	0.659	8.684
$[P_{666,14}][Phos]$	lamp oil	3.9	1.543	0.715	0.288	0.014	0.000	2.560	2.542	2.500	2.479	0.519	0.664	8.704
$[P_{666,14}][Phos]$	lamp oil	3.9	1.522	0.706	0.284	0.017	0.000	2.528	2.543	2.495	2.468	0.517	0.492	8.515
$[P_{666,14}][Phos]$	lamp oil	5.6	2.311	2.256	2.196	0.424	0.309	7.496	2.423	2.381	2.361	0.497	0.495	8.157
$[P_{666,14}][Phos]$	lamp oil	5.6	2.473	2.413	2.352	0.455	0.339	8.032	2.426	2.388	2.367	0.498	0.487	8.165
$[P_{666,14}][Phos]$	lamp oil	5.6	2.491	2.434	2.372	0.460	0.412	8.169	2.415	2.376	2.358	0.496	0.496	8.141
$[P_{666,14}][Phos]$	lamp oil	6.8	2.496	2.453	2.414	0.494	0.536	8.394	2.389	2.349	2.330	0.489	0.488	8.045
$[P_{666,14}][Phos]$	lamp oil	6.8	2.483	2.443	2.405	0.492	0.534	8.357	2.414	2.373	2.349	0.495	0.490	8.121
$[P_{666,14}][Phos]$	lamp oil	6.8	2.489	2.446	2.410	0.493	0.545	8.382	2.400	2.358	2.341	0.492	0.483	8.074
$[P_{666,14}][Phos]$	canola oil	3.9	1.614	0.721	0.264	0.023	0.000	2.622	2.538	2.562	2.544	0.532	0.532	8.708
$[P_{666,14}][Phos]$	canola oil	3.9	1.673	0.697	0.265	0.025	0.028	2.688	2.541	2.528	2.500	0.520	0.458	8.547
$[P_{666,14}][Phos]$	canola oil	3.9	1.810	0.841	0.265	0.025	0.027	2.968	2.543	2.533	2.514	0.523	0.479	8.593
$[P_{666,14}][Phos]$	canola oil	5.6	2.512	2.417	2.322	0.446	0.291	7.988	2.423	2.381	2.361	0.497	0.495	8.157
$[P_{666,14}][Phos]$	canola oil	5.6	2.524	2.418	2.316	0.448	0.282	7.988	2.426	2.388	2.367	0.498	0.487	8.165
$[P_{666,14}][Phos]$	canola oil	5.6	2.547	2.441	2.351	0.450	0.368	8.156	2.415	2.376	2.358	0.496	0.496	8.141
$[P_{666,14}][Phos]$	canola oil	6.8	2.511	2.451	2.400	0.518	0.465	8.344	2.389	2.349	2.330	0.489	0.488	8.045
$[P_{666,14}][Phos]$	canola oil	6.8	2.155	2.107	2.063	0.444	0.434	7.204	2.414	2.373	2.349	0.495	0.490	8.121
$[P_{666,14}][Phos]$	canola oil	6.8	2.504	2.435	2.375	0.513	0.479	8.305	2.400	2.358	2.341	0.492	0.483	8.074

E.2 Back-extraction extraction raw data

Table 25: *Initial and final aqueous phase VFA concentrations from LLE experiments with [$P_{666,14}$][Phos].*

Extractant	Diluent	Initial aqueous pH	Final aqueous phase acid concentration (g/L)						Initial aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
[$P_{666,14}$][Phos]	-	3.9	1.526	0.753	0.486	0.015	0.000	2.781	2.538	2.562	2.544	0.532	0.532	8.708
[$P_{666,14}$][Phos]	-	3.9	1.525	0.757	0.334	0.000	0.000	2.616	2.541	2.528	2.500	0.520	0.458	8.547
[$P_{666,14}$][Phos]	-	3.9	1.543	0.768	0.322	0.000	0.000	2.633	2.543	2.533	2.514	0.523	0.479	8.593
[$P_{666,14}$][Phos]	-	5.6	2.505	2.503	2.412	0.466	0.317	8.204	2.444	2.464	2.457	0.516	0.509	8.390
[$P_{666,14}$][Phos]	-	5.6	2.504	2.495	2.398	0.458	0.308	8.163	2.437	2.455	2.430	0.506	0.527	8.354
[$P_{666,14}$][Phos]	-	5.6	2.496	2.477	2.403	0.457	0.305	8.137	2.432	2.435	2.427	0.515	0.485	8.294
[$P_{666,14}$][Phos]	-	6.8	2.485	2.472	2.447	0.492	0.404	8.300	2.395	2.434	2.418	0.509	0.490	8.246
[$P_{666,14}$][Phos]	-	6.8	2.490	2.485	2.455	0.496	0.405	8.330	2.405	2.433	2.414	0.509	0.502	8.263

*Organic phase concentration determined by mass balance

Table 26: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with TBP and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TBP	oleyl alcohol	3.9	0.246	0.582	0.839	0.186	0.195	2.047	0.499	1.316	1.908	0.438	0.463	4.624
TBP	oleyl alcohol	3.9	0.254	0.637	0.891	0.205	0.219	2.207	0.507	1.312	1.904	0.440	0.476	4.639
TBP	oleyl alcohol	3.9	0.251	0.631	0.876	0.202	0.212	2.173	0.508	1.312	1.920	0.438	0.453	4.630
TBP	lamp oil	3.9	0.246	0.646	0.912	0.208	0.218	2.230	0.489	1.336	1.946	0.441	0.463	4.675
TBP	lamp oil	3.9	0.236	0.647	0.915	0.206	0.201	2.204	0.462	1.301	1.925	0.442	0.476	4.605
TBP	lamp oil	3.9	0.233	0.642	0.913	0.210	0.220	2.218	0.457	1.296	1.936	0.440	0.453	4.581
TBP	canola oil	3.9	0.260	0.600	0.859	0.164	0.109	1.991	0.441	1.198	1.845	0.432	0.463	4.380
TBP	canola oil	3.9	0.239	0.595	0.859	0.174	0.133	2.000	0.448	1.207	1.848	0.436	0.476	4.415
TBP	canola oil	3.9	0.226	0.585	0.838	0.161	0.103	1.912	0.430	1.189	1.855	0.433	0.453	4.359

*Organic phase concentration determined by mass balance

Table 27: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with TOPO and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOPO	oleyl alcohol	3.9	0.689	0.689	0.989	0.213	0.204	2.360	0.556	1.511	2.169	0.532	0.532	5.300
TOPO	oleyl alcohol	3.9	0.685	0.685	0.986	0.225	0.228	2.388	0.577	1.438	2.085	0.520	0.458	5.078
TOPO	oleyl alcohol	3.9	0.676	0.676	0.981	0.221	0.266	2.405	0.578	1.443	2.092	0.523	0.479	5.116
TOPO	lamp oil	3.9	0.542	0.984	1.096	0.224	0.218	3.063	1.251	2.092	2.348	0.520	0.659	6.870
TOPO	lamp oil	3.9	0.592	1.004	1.126	0.228	0.226	3.175	1.473	2.172	2.378	0.519	0.664	7.206
TOPO	lamp oil	3.9	0.590	1.018	1.141	0.234	0.226	3.209	1.294	2.107	2.347	0.517	0.492	6.757
TOPO	canola oil	3.9	0.546	0.920	0.996	0.215	0.165	2.841	1.116	1.984	2.302	0.520	0.659	6.581
TOPO	canola oil	3.9	0.587	0.980	1.086	0.228	0.179	3.060	1.123	1.981	2.311	0.519	0.664	6.598
TOPO	canola oil	3.9	0.636	1.067	1.195	0.256	0.210	3.366	1.138	1.983	2.304	0.517	0.492	6.434

*Organic phase concentration determined by mass balance

Table 28: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with TOAand diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOA	oleyl alcohol	3.9	0.691	0.923	1.026	0.197	0.186	3.022	1.471	1.996	2.223	0.463	0.463	6.616
TOA	oleyl alcohol	3.9	0.633	0.842	0.903	0.148	0.093	2.619	1.486	2.008	2.225	0.466	0.476	6.661
TOA	oleyl alcohol	3.9	0.684	0.899	0.919	0.137	0.059	2.698	1.493	2.011	2.243	0.464	0.453	6.665
TOA	lamp oil	3.9	0.084	0.248	0.612	0.223	0.264	1.431	0.125	0.479	1.235	0.418	0.504	2.761
TOA	lamp oil	3.9	0.075	0.244	0.608	0.219	0.245	1.391	0.108	0.448	1.192	0.406	0.431	2.585
TOA	lamp oil	3.9	0.059	0.252	0.636	0.204	0.246	1.397	0.116	0.458	1.219	0.417	0.453	2.662
TOA	canola oil	3.9	0.248	0.579	0.984	0.293	0.301	2.406	0.340	1.054	1.845	0.475	0.532	4.246
TOA	canola oil	3.9	0.248	0.571	0.957	0.301	0.261	2.338	0.336	1.005	1.782	0.463	0.458	4.044
TOA	canola oil	3.9	0.250	0.618	1.039	0.313	0.335	2.555	0.427	1.079	1.839	0.469	0.479	4.293

*Organic phase concentration determined by mass balance

Table 29: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with Aliquat 336 and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
Aliquat	oleyl alcohol	3.9	0.350	0.535	0.460	0.043	0.000	1.389	0.834	1.556	2.043	0.445	0.463	5.342
Aliquat	oleyl alcohol	3.9	0.377	0.573	0.504	0.051	0.000	1.506	0.824	1.541	2.034	0.448	0.476	5.323
Aliquat	oleyl alcohol	3.9	0.341	0.529	0.464	0.048	0.000	1.382	0.824	1.540	2.048	0.446	0.453	5.310
Aliquat	lamp oil	3.9	0.299	0.427	0.438	0.036	0.000	1.199	0.891	1.591	2.042	0.446	0.463	5.434
Aliquat	lamp oil	3.9	0.272	0.412	0.438	0.036	0.000	1.157	0.891	1.583	2.039	0.449	0.476	5.437
Aliquat	lamp oil	3.9	0.300	0.443	0.457	0.038	0.000	1.238	0.911	1.605	2.060	0.448	0.453	5.475

*Organic phase concentration determined by mass balance

Table 30: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with [P666,14][Phos] and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
[$P_{666,14}$][Phos]	oleyl alcohol	3.9	0.354	0.686	0.928	0.204	0.228	2.400	0.771	1.554	2.119	0.506	0.532	5.482
[$P_{666,14}$][Phos]	oleyl alcohol	3.9	0.357	0.700	0.948	0.206	0.229	2.440	0.786	1.526	2.061	0.498	0.458	5.329
[$P_{666,14}$][Phos]	oleyl alcohol	3.9	0.347	0.683	0.944	0.202	0.205	2.381	0.788	1.523	2.088	0.501	0.479	5.379
[$P_{666,14}$][Phos]	lamp oil	3.9	0.376	0.683	0.867	0.180	0.182	2.287	0.997	1.779	2.186	0.505	0.659	6.126
[$P_{666,14}$][Phos]	lamp oil	3.9	0.423	0.784	0.952	0.202	0.192	2.552	0.999	1.785	2.191	0.505	0.664	6.144
[$P_{666,14}$][Phos]	lamp oil	3.9	0.453	0.819	1.040	0.210	0.202	2.724	1.021	1.789	2.184	0.500	0.492	5.986
[$P_{666,14}$][Phos]	canola oil	3.9	0.584	1.055	1.200	0.334	0.233	3.405	0.924	1.841	2.279	0.509	0.532	6.085
[$P_{666,14}$][Phos]	canola oil	3.9	0.588	1.061	1.146	0.335	0.249	3.379	0.868	1.831	2.236	0.495	0.430	5.859
[$P_{666,14}$][Phos]	canola oil	3.9	0.614	1.104	1.190	0.357	0.269	3.534	0.734	1.692	2.250	0.498	0.452	5.625

*Organic phase concentration determined by mass balance

Table 31: *Final queous phase VFA concentrations and initial organic phase VFA concentraions for back-extraction experiments with [P666,14][Phos].*

Extractant	Diluent	LLE pH	Final aqueous phase acid concentration (g/L)						Initial organic phase acid concentration* (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA	Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
[$P_{666,14}$][Phos]	-	3.9	2.396	4.091	4.564	0.743	0.315	12.109	5.057	9.046	12.231	2.583	2.661	31.578
[$P_{666,14}$][Phos]	-	3.9	2.386	4.072	4.722	0.865	0.449	12.495	5.079	8.857	12.166	2.600	2.291	30.993
[$P_{666,14}$][Phos]	-	3.9	2.361	4.051	4.672	0.867	0.395	12.346	4.999	8.827	12.251	2.615	2.397	31.088

*Organic phase concentration determined by mass balance

E.3 Bench-scale biogas production test raw data

Table 32: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing TOA with diluents oleyl alcohol, lamp oil and canola oil over four week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	**14**	**21**	**28**	**7**	**14**	**21**	**28**
TOA	oleyl alcohol	47.62%	62.80%	76.46%	75.06%	39	42	66	76
TOA	oleyl alcohol	48.32%	62.20%	76.29%	73.83%	38	48	68	78
TOA	oleyl alcohol	47.42%	62.17%	72.26%	75.10%	38	44	48	70
TOA	lamp oil	45.26%	52.03%	50.73%	49.95%	36	16	5	2
TOA	lamp oil	47.93%	52.01%	50.10%	50.96%	35	14	4	4
TOA	lamp oil	47.67%	51.62%	51.81%	48.77%	34	14	6	6
TOA	canola oil	15.74%	20.01%	52.52%	17.63%	6	2	2	2
TOA	canola oil	15.61%	20.47%	34.63%	23.97%	10	2	2	6
TOA	canola oil	16.37%	22.88%	25.49%	25.11%	5	4	2	4

Table 33: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing TBP with diluents oleyl alcohol, lamp oil and canola oil over four week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	14	21	28	7	14	21	28
TBP	oleyl alcohol	44.17%	51.81%	64.41%	67.75%	39	30	36	48
TBP	oleyl alcohol	47.29%	56.65%	67.70%	71.55%	41	36	40	52
TBP	oleyl alcohol	46.93%	55.36%	67.46%	70.47%	36	34	34	46
TBP	lamp oil	12.98%	0.00%	0.00%	15.63%	6	0	0	2
TBP	lamp oil	11.59%	0.00%	0.00%	16.60%	7	0	0	2
TBP	lamp oil	11.56%	0.00%	0.00%	15.53%	8	0	0	2
TBP	canola oil	14.72%	0.00%	0.00%	0.00%	10	0	0	0
TBP	canola oil	14.78%	0.00%	0.00%	0.00%	8	0	0	0
TBP	canola oil	14.82%	0.00%	0.00%	0.00%	9	0	0	0

Table 34: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing TOPO with diluents oleyl alcohol, lamp oil and canola oil over four week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	14	21	28	7	14	21	28
TOPO	oleyl alcohol	48.46%	59.70%	72.89%	74.25%	41	38	76	70
TOPO	oleyl alcohol	47.98%	58.00%	71.44%	73.54%	42	38	77	50
TOPO	oleyl alcohol	48.11%	55.21%	72.04%	72.59%	41	34	72	48
TOPO	lamp oil	48.78%	51.23%	50.27%	45.69%	38	20	5	2
TOPO	lamp oil	48.33%	51.23%	47.92%	45.70%	40	23	5	2
TOPO	lamp oil	48.72%	52.31%	48.55%	50.32%	38	13	6	2
TOPO	canola oil	50.86%	55.04%	47.95%	42.19%	39	75	20	23
TOPO	canola oil	50.65%	63.99%	58.11%	41.04%	38	80	62	30
TOPO	canola oil	51.36%	56.23%	53.98%	41.06%	41	75	36	20

Table 35: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing Aliquat 336 with diluents oleyl alcohol, lamp oil and canola oil over four week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	14	21	28	7	14	21	28
Aliquat	oleyl alcohol	0.54%	0.00%	1.64%	0.00%	12	0	2	0
Aliquat	oleyl alcohol	0.00%	8.03%	41.01%	0.00%	5	5	4	0
Aliquat	oleyl alcohol	0.63%	0.00%	2.37%	0.00%	10	0	3	0
Aliquat	lamp oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0
Aliquat	lamp oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0
Aliquat	lamp oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0
Aliquat	canola oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0
Aliquat	canola oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0
Aliquat	canola oil	0.00%	0.00%	0.00%	0.00%	0	0	0	0

Table 36: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing [P666,14][Phos] with diluents oleyl alcohol, lamp oil and canola oil over four week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	14	21	28	7	14	21	28
[P666,14][Phos]	oleyl alcohol	53.52%	74.06%	74.40%	74.38%	50	96	65	80
[P666,14][Phos]	oleyl alcohol	53.96%	72.42%	75.83%	73.88%	48	100	77	82
[P666,14][Phos]	oleyl alcohol	54.37%	72.36%	75.90%	74.14%	49	90	80	76
[P666,14][Phos]	lamp oil	2.57%	0.00%	0.00%	0.00%	5	0	0	0
[P666,14][Phos]	lamp oil	2.48%	0.00%	0.00%	0.00%	2	0	0	0
[P666,14][Phos]	lamp oil	2.31%	0.00%	0.00%	0.00%	4	0	0	0
[P666,14][Phos]	canola oil	2.55%	0.00%	0.00%	0.00%	3	0	0	0
[P666,14][Phos]	canola oil	2.58%	0.00%	0.00%	0.00%	2	0	0	0
[P666,14][Phos]	canola oil	0.00%	0.00%	0.00%	0.00%	2	0	0	0

Table 37: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for inoculum-substrate control and inoculum-blank tests over four week test duration.*

Control tests		Biogas methane proportion measured at 7 day intervals (CH4 %)				Biogas volume measured at 7 day intervals (mL)			
		7	**14**	**21**	**28**	**7**	**14**	**21**	**28**
Inoculum	substrate	46.55%	49.26%	60.78%	50.67%	26	20	20	3
Inoculum	substrate	45.63%	46.83%	51.95%	48.65%	32	16	12	2
Inoculum	substrate	47.31%	48.89%	51.05%	50.52%	28	18	10	3
Inoculum	blank	0.00%	0.00%	16.62%	23.52%	0	0	2	2
Inoculum	blank	0.00%	0.00%	41.28%	20.79%	0	0	4	2
Inoculum	blank	0.00%	0.00%	15.50%	20.79%	0	0	2	2

Table 38: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for systems containing TOA, TBP, TOPO and [P666,14][Phos] with diluent oleyl alcohol over five week test duration.*

Extractant	Diluent	Biogas methane proportion measured at 7 day intervals (CH4%)					Biogas volume measured at 7 day intervals (mL)				
		7	**14**	**21**	**28**	**35**	**7**	**14**	**21**	**28**	**35**
TOA	oleyl alcohol	61.48%	74.73%	73.68%	73.13%	72.95%	68	75	82	90	81
TOA	oleyl alcohol	61.85%	74.24%	73.45%	72.87%	72.42%	68	75	74	76	74
TOA	oleyl alcohol	60.76%	73.44%	74.65%	73.83%	71.87%	69	79	90	88	66
TBP	oleyl alcohol	53.45%	68.54%	73.42%	73.13%	70.81%	46	76	85	68	58
TBP	oleyl alcohol	55.37%	71.12%	72.75%	72.83%	71.18%	48	79	78	68	60
TBP	oleyl alcohol	58.94%	71.21%	77.12%	80.31%	79.04%	46	77	80	67	38
TOPO	oleyl alcohol	56.17%	73.04%	72.17%	72.82%	72.46%	60	78	73	66	69
TOPO	oleyl alcohol	62.21%	73.67%	72.24%	73.55%	72.54%	63	84	84	76	77
TOPO	oleyl alcohol	55.48%	74.07%	73.27%	74.42%	73.03%	58	101	83	103	90
[P666,14][Phos]	oleyl alcohol	65.42%	74.33%	72.18%	72.70%	71.71%	74	84	83	79	73
[P666,14][Phos]	oleyl alcohol	66.45%	73.65%	73.13%	71.43%	71.85%	75	87	84	75	74
[P666,14][Phos]	oleyl alcohol	65.16%	73.50%	72.68%	72.39%	71.95%	81	84	82	79	74
[P666,14][Phos]	-	16.31%	0.00%	0.00%	0.00%	0.00%	8	1	0	0	0
[P666,14][Phos]	-	16.10%	0.00%	0.00%	0.00%	0.00%	7	1	0	0	0
[P666,14][Phos]	-	14.39%	0.00%	0.00%	0.00%	0.00%	7	0	0	0	0

Table 39: *Biogas methane proportions and total biogas volumes measured at seven day sampling intervals for inoculum-substrate control and inoculum-blank tests over five week test duration.*

Control tests		**Biogas methane proportion measured at 7 day intervals (CH4%)**					**Biogas volume measured at 7 day intervals (mL)**				
		7	**14**	**21**	**28**	**35**	**7**	**14**	**21**	**28**	**35**
Inoculum	substrate	46.22%	48.63%	55.06%	58.53%	55.78%	23	22	16	10	12
Inoculum	substrate	46.67%	48.47%	55.96%	58.59%	58.65%	24	22	14	9	11
Inoculum	substrate	47.49%	48.22%	56.28%	56.42%	59.97%	25	21	15	12	11
Inoculum	blank	18.41%	18.22%	0.00%	0.00%	0.00%	6	6	2	3	2
Inoculum	blank	11.89%	18.12%	0.00%	0.00%	0.00%	4	4	2	2	2
Inoculum	blank	11.90%	17.81%	0.00%	0.00%	0.00%	5	4	2	2	2

E.4 Liquid-liquid extraction with fermented wastewater raw data

Table 40: *Initial aqueous VFA concentration of AD wastewater used in LLE experiment.*

Initial aqueous phase acid concentration (g/L)					
Acetic	**Propionic**	**Butyric**	**Valeric**	**Caproic**	**tVFA**
0.655	0.437	0.481	0.209	0.000	1.782
0.646	0.437	0.492	0.203	0.000	1.779
0.602	0.400	0.449	0.193	0.000	1.644

Table 41: *Final aqueous phase VFA concentration of AD wastewater from LLE experiment with TOA and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOA	oleyl alcohol	4.3	0.388	0.144	0.058	0.000	0.000	0.590
TOA	oleyl alcohol	4.3	0.389	0.153	0.043	0.000	0.000	0.585
TOA	oleyl alcohol	4.3	0.393	0.152	0.043	0.000	0.000	0.587
TOA	lamp oil	4.3	0.580	0.351	0.227	0.064	0.000	1.222
TOA	lamp oil	4.3	0.581	0.345	0.252	0.063	0.000	1.241
TOA	lamp oil	4.3	0.582	0.347	0.256	0.062	0.000	1.248
TOA	canola oil	4.3	0.539	0.281	0.097	0.027	0.000	0.944
TOA	canola oil	4.3	0.519	0.265	0.097	0.028	0.000	0.909
TOA	canola oil	4.3	0.443	0.248	0.000	0.024	0.000	0.714

Table 42: *Final aqueous phase VFA concentration of AD wastewater from LLE experiment with Aliquat and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
Aliquat	oleyl alcohol	4.3	0.160	0.067	0.023	0.000	0.000	0.251
Aliquat	oleyl alcohol	4.3	0.142	0.058	0.000	0.000	0.000	0.200
Aliquat	oleyl alcohol	4.3	0.178	0.068	0.019	0.000	0.000	0.264
Aliquat	lamp oil	4.3	0.290	0.112	0.037	0.000	0.000	0.439
Aliquat	lamp oil	4.3	0.278	0.111	0.032	0.000	0.000	0.420
Aliquat	lamp oil	4.3	0.248	0.100	0.029	0.000	0.000	0.377
Aliquat	canola oil	4.3	0.376	0.147	0.037	0.000	0.000	0.560
Aliquat	canola oil	4.3	0.376	0.148	0.039	0.000	0.000	0.562
Aliquat	canola oil	4.3	0.379	0.160	0.040	0.000	0.000	0.578

Table 43: *Final aqueous phase VFA concentration of AD wastewater from LLE experiment with TBP and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TBP	oleyl alcohol	4.3	0.515	0.222	0.089	0.000	0.000	0.826
TBP	oleyl alcohol	4.3	0.514	0.220	0.091	0.000	0.000	0.825
TBP	oleyl alcohol	4.3	0.514	0.223	0.091	0.000	0.000	0.828
TBP	lamp oil	4.3	0.519	0.222	0.103	0.000	0.000	0.844
TBP	lamp oil	4.3	0.519	0.218	0.099	0.000	0.000	0.837
TBP	lamp oil	4.3	0.518	0.224	0.098	0.000	0.000	0.840
TBP	canola oil	4.3	0.528	0.245	0.101	0.000	0.000	0.874
TBP	canola oil	4.3	0.526	0.241	0.100	0.000	0.000	0.867
TBP	canola oil	4.3	0.528	0.242	0.088	0.000	0.000	0.858

Table 44: *Final aqueous phase VFA concentration of AD wastewater from LLE experiment with TOPO and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
TOPO	oleyl alcohol	4.3	0.486	0.179	0.073	0.000	0.000	0.738
TOPO	oleyl alcohol	4.3	0.489	0.174	0.072	0.000	0.000	0.735
TOPO	oleyl alcohol	4.3	0.477	0.171	0.070	0.000	0.000	0.718
TOPO	lamp oil	4.3	0.339	0.083	0.021	0.000	0.000	0.443
TOPO	lamp oil	4.3	0.336	0.082	0.020	0.000	0.000	0.438
TOPO	lamp oil	4.3	0.322	0.078	0.019	0.000	0.000	0.418
TOPO	canola oil	4.3	0.357	0.086	0.023	0.000	0.000	0.467
TOPO	canola oil	4.3	0.353	0.081	0.026	0.000	0.000	0.460
TOPO	canola oil	4.3	0.353	0.093	0.027	0.000	0.000	0.473

Table 45: *Final aqueous phase VFA concentration of AD wastewater from LLE experiment with [P666,14][Phos] and diluents oleyl alcohol, lamp oil and canola oil.*

Extractant	Diluent	Initial pH	Final aqueous phase acid concentration (g/L)					
			Acetic	Propionic	Butyric	Valeric	Caproic	tVFA
[P666,14][Phos]	oleyl alcohol	4.3	0.624	0.415	0.332	0.192	0.000	1.563
[P666,14][Phos]	oleyl alcohol	4.3	0.625	0.418	0.331	0.185	0.000	1.559
[P666,14][Phos]	oleyl alcohol	4.3	0.625	0.418	0.328	0.192	0.000	1.562
[P666,14][Phos]	lamp oil	4.3	0.630	0.416	0.327	0.187	0.000	1.560
[P666,14][Phos]	lamp oil	4.3	0.624	0.417	0.332	0.199	0.000	1.573
[P666,14][Phos]	lamp oil	4.3	0.630	0.420	0.329	0.193	0.000	1.571
[P666,14][Phos]	canola oil	4.3	0.643	0.433	0.318	0.173	0.000	1.567
[P666,14][Phos]	canola oil	4.3	0.641	0.430	0.308	0.168	0.000	1.547
[P666,14][Phos]	canola oil	4.3	0.644	0.429	0.314	0.170	0.000	1.557