In situ extraction of volatile fatty acids from anaerobic digestion systems

by

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ABSTRACT

In recent decades anaerobic digestion (AD) technology has gained significant interest due to policymakers' intent to reduce non-renewable resources, and for the processing of organic wastes. AD is, however, faced with operational difficulties such as acid crash, and optimisation problems since feedstocks are variable and often intermittent. This thesis aimed at developing additional products from AD by investigating the co-production of volatile fatty acids (VFAs) and biogas, by the continuous removal of VFAs by *in* situ extraction. Gas stripping and liquid-liquid extraction (LLE) were identified as potential extraction methods. Gas stripping was investigated by an Aspen model. The model indicated that 100% recovery of VFAs could be achieved with a mass ratio of 230 for pure carbon dioxide and 150 for an equimolar mixture of carbon dioxide and methane. Gas-equilibrium experiments for both mixtures were compared to the model. The highest percentage of VFAs extracted was 0.91 ± 1.42% using carbon dioxide at pH 6.0 and 0.55% for the equimolar mixture at pH 3.5. A continuous gas stripping experiment showed that 4.48% of VFAs (0.013 g) were extracted out from a 20 mL of synthetic VFA solution (14.65 g/L) using 40.2 L of equimolar gas. The results indicated that the model significantly overestimated the viability of gas stripping as an *in situ* recovery method for VFAs. Gas stripping was concluded to be inefficient, and an alternative *in situ* method was proposed using LLE. From literature, trioctylamine (TOA) and tributyl phosphate (TBP) and three diluents (canola oil, lamp oil, and oleyl alcohol) were identified as suitable solvents. These solvents were investigated in liquid-liquid equilibrium experiments. These experiments showed that there was a strong dependence on pH for the extraction of VFAs. The highest degree of extraction at pH 5.0 was observed for TOA/oleyl alcohol (50.48 ± 0.13%) and the lowest for TOA/canola oil (25.64 ± 8.42%). Biochemical methane potential (BMP) tests were conducted, using the three best solvents, to test the biocompatibility of the solvents with AD bacteria. From these experiments, the samples containing TOA/canola oil and TBP/lamp oil performed better than the control in total gas production (168.00 mL ± 26.15 mL and 145.67 ± 5.03 mL) and methane percentage (12.62 ± 2.82% and 14.68 ± 6.73%). The control produced 114.50 ± 39.42 mL of gas (9.73 ± 1.33% of methane). Over a 28 day digestion period, 2.40 ± 0.30 g/L and 5.84 ± 0.36 g/L of VFAs in 10 mL of solvent were successfully recovered from TBP/lamp oil and TOA/ oleyl alcohol, respectively. A 17 L AD-bioreactor was modified by placing an *in situ* extraction tube inside the reactor, connected to a circulator and batch extraction unit. TOA/oleyl alcohol was selected for the 17 L scale-up, based on the equilibrium and biocompatibility tests.0.078 g of VFAs were extracted out and 6.71 L of biogas was produced with a methane percentage of 43% in the scale-up. To conclude, *in situ* LLE extraction may be industrially applicable as a potential co-production process for biogas and VFAs.

OPSOMMING

In onlangse dekades het anaerobiese vertering (AD) -tegnologie beduidende belangstelling verkry as gevolg van beleidmakers se voorneme om nie-herwinbare bronne te verminder, en vir die prosessering van organiese afval. AD staar egter bedryfsuitdagings soos suur-ineenstorting, en optimeringsprobleme in die gesig, omdat voermateriaal veranderlik is met gereeld onderbroke voorraad. Hierdie tesis het beoog om addisionele produkte uit AD te ontwikkel deur die koproduksie van vlugtige vetsure (VFAs) en biogas, deur die kontinue verwydering van VFAs deur *in situ* ekstrahering. Gasstroping en vloeistof-vloeistof ekstrahering (LLE) is geïdentifiseer as potensiële ekstraheringsmetodes. Gasstroping is deur ’n Aspen™-ekwilibriummodel ondersoek. Die model het aangedui dat 100% herwinning van VFAs bereik kan word met ’n massa ratio (gas na VFA) van 230 vir suiwer koolstofdioksied en 150 vir ’n ekwimolêre mengsel van koolstofdioksied en metaan. Gasekwilibriumeksperimente vir beide mengsels is met die model vergelyk. Die hoogste persentasie VFAs geëkstraheer was 0.91 ± 1.042% deur koolstofdioksied te gebruik by pH 6.0 en 0.55% vir die ekwimolêre mengsel by pH 3.5. ’n Kontinue gasstropingseksperiment het aangedui dat 4.48% VFAs (0.013 g) geëkstraheer is uit ’n 20 mL sintetiese VFA-oplossing (14.65 g/L) deur 40.2 L van ekwimolêre gas. Die resultate het aangedui dat die model die lewensvatbaarheid van gasstroping as ’n *in situ* herwinningmetode vir VFAs aansienlik oorskat het. Dis tot die gevolgtrekking gekom dat gasstroping oneffektief was, en ’n alternatiewe *in situ* metode was voorgestel deur LLE te gebruik. Uit literatuur is trioktielamien (TOA) en tributielfosfaat (TBP) en drie verdunners (kanola-olie, lampolie, en oleïelalkohol) geïdentifiseer as gepaste oplosmiddels. Hierdie oplosmiddels is ondersoek in vloeistof-vloeistof ekwilibriumeksperimente. Hierdie eksperimente het aangedui dat daar ’n sterk afhanklikheid op pH vir die ekstrahering van VFAs was. Die hoogste grade van ekstrahering by pH 5 was waargeneem vir TOA/oleïelalkohol (50.48 ± 0.13%) en die laagste vir TOA/kanola-olie (25.64 ± 8.42%). Biochemiese metaanpotensiaal (BMP)-toetse is uitgevoer, deur die drie beste oplosmiddels te gebruik, om die bio-verdraagbaarheid van die oplosmiddels met AD-bakterieë te toets. Uit hierdie eksperimente het die steekproewe wat TOA/kanola-olie en TBP/lampolie bevat beter gedoen as die kontrole in totale gasproduksie (168.00 mL ± 26.15 mL en 145.67 ± 5.03 mL) en metaanpersentasie (12.62 ± 2.82% en 14.68 ± 6.73%). Die kontrole het 114.50 ± 39.42 mL gas (9.73 ± 1.33% metaan) geproduseer. Oor ’n 28-dae verteringsperiode, was 2.40 ± 0.30 g/L en 5.84 ± 0.36 g/L VFAs in 10 mL oplosmiddel suksesvol herwin uit TBP/lampolie en TOA/oleïelalkohol, onderskeidelik. ’n 17 L AD-bioreaktoer is aangepas deur ’n *in situ* ekstraheringspyp binne die reaktor te plaas, wat aan ’n sirkuleerder en lotekstraheringseenheid gekonnekteer was. TOA/oleïelalkohol is gekies vir die 17 L opskaal, gebaseer op die toetse vir ekwilibrium en bio-verdraagbaarheid. 0.078 g VFAs is geëkstraheer en 6.71 L biogas is geproduseer met ’n metaanpersentasie van 43% in die opskaal. Om af te sluit, *in situ* LLE-ekstrahering kan industrieel toepaslik wees as ’n potensiële ko-vervaardigingsproses vir biogas en VFA

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Chapter 1: Introduction

Anaerobic digestion (AD) is said to be one of the oldest biotechnologies, dating back to the 10th century. In 1895, street lamps in Exeter, England, were fuelled by biogas from a sewage treatment facility (Monnet, 2003). The AD process began to improve as more advanced equipment and techniques became available. From this, a closed tank with heating and mixing were used for the optimisation of AD systems. Although this technology was used in small-scale for waste management and energy production, several failures were reported. A common problem associated with AD was the likely occurrence of acid crash due to the VFA accumulation (Edwiges, Frare, Alino, Triolo, Flotats & Silva de Mendonça Costa, 2018). In addition to the failures, the implementation of AD was affected severely by the use of coal and petroleum as well as the development of the aerobic process (Monnet, 2003). In recent decades, stringent environmental regulations and the increased cost of energy has led to further research and development into the use of sustainable energy and renewable resources (Volker, Gogerty, Bartholomay, Hennen-Bierwagen, Zhu & Bobik, 2014).

An issue with AD is that it has significant investment and operational costs, and is usually not used as a sole energy source. Due to this, AD is generally integrated into existing processes to reduce the energy demand from fossil fuels and to reduce the costs related to waste disposal (Verma, 2002). Apart from using AD as a waste management, AD provides two valuable products, which are biogas and digestate that can be used as a soil amendment, provided that the specific chemical loading, nutrient profile and pathogen loading is met (Makdi, Tomcsik & Orosz, 2012). However, there may be an opportunity for another valuable resource within AD, volatile fatty acids (VFAs).

VFAs are linear short-chained carboxylic acids and are valuable commodity chemicals and, with a wide range of applications in industry, ranging from bioplastics to additives in food (Lee, Chua, Yeoh & Ngoh, 2014). The majority of VFAs are currently produced commercially by petrochemical-based processes. However, VFAs are found as intermediates and are used as substrates for the production of biogas in AD systems (Mostafa, 1999). This implies that AD could potentially be used as a source for the production and recovery of VFAs.

Therefore, a potentially more sustainable and preferred approach to AD would be to co-produce VFAs and biogas, which might be best achieved by continuous *in situ* extraction of the VFAs from the AD process. There has been little to no work in the literature that focuses on co-producing VFAs and

biogas. However, there are studies that have reported the recovery of VFAs from the AD broth using adsorption, esterification, distillation, membrane separation, precipitation, electrodialysis and liquid-liquid extraction (Rebecchi, Pinelli, Bertin, Zama, Fava & Frascari, 2016; Weier, Glatz & Glatz, 1992; Zacharof & Lovitt, 2013). Many of the studies use these recovery methods for downstream processing (*ex situ*). Recently, studies have used these methods *in situ*, whereby the recovery method is used 'on-site' but in an external configuration. An *in situ* configuration is preferred as it improves productivity and increases yield, more importantly, it can be used to prevent product inhibition (Van Hecke, Kaur & De Wever, 2014). Alternatively, an *in situ*-internal configuration is possible whereby the extraction method is placed inside the reactor/digester. Although an *in situ* configuration internally could complicate the process, it can significantly reduce capital costs through the removal of additional equipment required for external *in situ*.

Hence, the aim of this study was to evaluate the possibility of co-producing biogas and VFAs by extracting VFAs *in situ*. The objectives for the thesis were: to identify the various processing methods that can be used for *in situ* extraction; to determine a process for the recovery of VFAs (after extraction of VFAs) and finally to design and construct a unit capable of continuously extracting VFAs *in situ*.

Chapter 2 will discuss the economic value and use of VFAs, the complex nature of AD and the different types of techniques that have been implemented to extract VFAs. This chapter will outline the advantages and disadvantages of downstream processing methods and whether these methods can be used for *in situ* extraction on an AD system. Two of these methods will be chosen for experimental evaluation for the recovery of VFAs in an anaerobic digestion system.

Chapter 2: Literature Review

2.1 Volatile Fatty Acids

VFAs are linear short-chain carboxylic acids, ranging from two to six carbon atoms. These include acetic, propionic, butyric, valeric and caproic acid (Scoma, Varela-Corredor, Bertin, Gostoli & Bandini, 2016). VFAs are valuable commodity chemicals and have a wide range of applications in industry due to their functional group, with uses ranging from the production of bioplastics to bioenergy (Strazzera, Battista, Garcia, Frison & Bolzonella, 2018). VFAs are usually commercially produced from fossil-oil-based chemical processing through the oxidation and carboxylation of precursors. The depleting oil reserves and market drivers away from fossil-fuel derived products has attracted the use of alternative energy that is renewable and sustainable because of the environmental impact of fossil fuel (Volker et al., 2014). Fossil fuels are non-renewable resources that are unstainable and contribute to global warming. AD has gained significant interest over time due to these factors. AD can not only produce biogas but could potentially be used as a VFA source, as VFAs are seen as intermediates in the AD process, while maintaining biogas production.

2.1.1 Applications

The high commodity value of VFAs, shown in Table 1, is due to the wide range of applications of VFAs in numerous industries (Tugtas, 2014) and the increased costs of raw materials derived from petroleum. In combination with these, tariff hikes and transport cost has also played a role in the increased value of VFAs (Zacharof & Lovitt, 2013). The global market for acetic acid was estimated to be worth $8.1 billion in 2018, with a compound annual growth rate (CAGR) of 2.68% between 2011 and 2018. By 2024, the estimated value of acetic acid is expected to rise to $11.4 billion with a CAGR of 5.83% between 2019 and 2024 (IMARC, 2019). Propionic acid was estimated to be worth $0.94 billion in 2012. In 2013, the market size was estimated to be 180 000 tons/year, with an estimated market value of 1500 – 1650 $/ton (Table 1). A 7.8% CAGR was estimated from 2013 to 2018 and an estimated global value of $1.6 billion in 2018 (MarketsandMarkets, 2019). The global market value of butyric acid is expected to have a CAGR of 6.8% from 2019 to 2026 (Acumen, 2019).

Table 1: VFA commodity value and market size.

Volatile fatty acid	**Chemical formula**	**Market size (tons/year) in 2013**	**Market value in 2013 ($/ton),** (Zacharof & Lovitt, 2013)	**Market value in 2019 ($/ton) based of Alibaba.com**
Acetic	CH_3COOH	3500000	400 – 800	400 – 950
Propionic	CH_3CH_2COOH	180000	1500 – 1650	1000 - 1500
Butyric	$CH_3(CH_2)_2COOH$	30000	2000 – 2500	1580 – 2950
Caproic	$CH_3(CH_2)_4COOH$	25000	2250 – 2500	1300 – 3000

Lee et al. (2014) discuss several uses of VFAs. One of the more recent applications is where VFAs are used by microorganisms to synthesis biodegradable polymers such as polhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB) and polylactic acid (PLA). Biodegradable polymers have various applications in industry and are environmentally friendly. Lee et al. (2014) mention that VFAs can be used to generate electricity directly with the use of a microbial fuel cell, with acetate, butyrate and propionate being the carbon sources. Although the power performance is not suitable for direct energy generation, VFAs can also be used as a precursor to biogas, hydrogen and to produce biodiesel. Some of the applications and uses of VFAs in industry are shown in Table 2 (Zacharof & Lovitt, 2013).

Table 2: Application of VFAs in numerous industries.

Volatile fatty acid	**Application**
Acetic acid	• Vinyl acetate monomer (polymers, dyes and adhesives) • Food additives • Ester production
Butyric acid	• Aroma additive • Food additive • Pharmaceuticals
Caproic acid	• Ester production • Food additive • Manufacture of hexyl derivatives
Valeric acid	• Ester production (lubricants) • Plasticisers • Vinyl stabiliser
Propionic acid	• Animal and food additive • Chemical intermediate • Solvent

2.1.2 Production methods

Petrochemical based processes are currently being used to produce VFAs (Katikaneni & Cheryan, 2002). Before the development of these processes, VFAs were produced by microbial fermentation (Mostafa, 1999). This was done by anaerobic fermentation of carbohydrates or microbial oxidation of ethanol (Table 3). The VFAs were extracted from these production methods through downstream processing techniques.

Zacharof and Lovitt (2013) state that distillation was employed to separate the VFAs in the fermentation processes. However, VFAs are present in a dilute aqueous solution, this results in distillation being an energy-intensive separation method for VFAs. The separation of VFAs at a low concentration from water proved to be a principal challenge and in order to do so made the process energetically unfeasible (Zacharof & Lovitt, 2013).

The article mentions that the development of petrochemical-based processes was preferred because the process avoided the significant purification energy costs of the bioprocesses. This petrochemical process is carried out in the gaseous phase without water. Therefore, avoiding the energy costs associated with the removal of water from VFAs (Joglekar, Rahman, Babu, Kulkarni & Joshi, 2006).

Table 3: Fermentation methods for the production of carboxylic acids (Zacharof & Lovitt, 2013).

Carboxylic acid	Chemical synthesis method	Bioprocess
Formic	Oxidation of alkanes Hydrogenation of carbon dioxide Methanol carbonylation	Oxidative fermentation Anaerobic fermentation
Acetic	Acetaldehyde oxidation Methanol carbonylation Ethylene oxidation	Oxidative fermentation Anaerobic fermentation
Propionic	Hydrocarboxylation of ethylene Aerobic oxidation of propionaldehyde	Anaerobic fermentation
Butyric	Chemical oxidation butyraldehyde	Fungal fermentation of glucose
Caproic	Ethylene oxidation	Anaerobic fermentation
Lactic	Chemical synthesis fermentation	Anaerobic fermentation

Alternative downstream processing solutions have been investigated for the separation of VFAs in fermentation processes (Omar et al., 2009 and Wan Omar et al., 2009). These processes make use of the physicochemical properties of the VFAs (Table 4) and will be discussed in the following sections to come. However, there are problems that are associated with downstream processing, these problems are usually that process is either energy-intensive, inefficient or use chemicals that may hinder the digestion process if these chemicals come into contact with the active broth. Although downstream processing techniques have several advantages and disadvantages, it is important to understand the AD process and the difficulties surrounding the process before discussing the downstream extraction processes. AD will be discussed in the next section and the problems associated with downstream processing is discussed in more detail in section 2.3 Downstream Processing Methods of VFAs.

Table 4: Physicochemical properties of VFAs (Perry & Green, 2007).

Carboxylic acid	Chemical formula	Molecular mass (g/gmol)	Density (kg/L)	Melting point (°C)	Boiling point (°C)	pKa
Caproic	$CH_3(CH2)_4COOH$	116.6	0.93	-34	205	4.88
Valeric	$CH_3(CH_2)_3COOH$	102.1	0.94	-34	186	4.84
Lactic	$CH_3CHOHCOOH$	90.08	1.20	53	122	3.86
Butyric	$CH_3(CH2)_2COOH$	88.11	0.96	-7.9	163	4.82
Propionic	CH_3CH_2COOH	74.08	0.99	-21	141	4.88
Acetic	CH_3COOH	60.05	1.04	16	118	4.79
Formic	HCOOH	46.03	1.22	8.4	101	3.77

2.2 Anaerobic Digestion

2.2.1 Process description

AD or anaerobic fermentation consists of an interconnected set of microbiological processes where, to simplify, digestible organic matter is converted to biogas in the absence of oxygen (Teghammar, 2013). The raw organic matter used is usually solid and liquid waste generated from industries, such as the agricultural, pulp and paper, dairy, food, and wastewater industries (Lee et al., 2014). There are several advantages associated with AD over non-renewable resources. However, the main attraction of AD is that it is used as a waste disposal method and produces two products that are commercially useful, namely: biogas and digestate.

A post-digestate that is rich in nitrogen can be used as fertiliser or it may be converted into biochar. The biochar is used to purify flue gas and wastewater or it can be used for nutrition for soil enhancement (Inyang, Gao, Pullammanappallil, Ding & Zimmerman, 2010). Biochar usually consists of an elemental composition of 50% carbon, 6% hydrogen, 43% oxygen, 0.7% nitrogen, and less than 1% of sulphur (Břendová, Tlustoš, Száková & Habart, 2012). The extraction of VFAs may not lead to a significant change in the biochar composition, as the amount of VFAs present in the broth would be relatively small compared to the organic matter. The end-product of the AD process is biogas. Biogas is a mixture of gases that mainly comprises of methane (50-75 vol%) and carbon dioxide (30-45 vol%) and trace amounts of hydrogen sulphide, hydrogen and siloxane (Borja, 2011: 786). Biogas has an energy potential of 6.0-6.5 kWh/m^3, which is equivalent to 0.6-0.65 L of crude oil per cubic meter (Tezel, Tandukar & Pavlostathis, 2011: 448). However, this is dependent on the composition of methane in the biogas. The lower heating value (LHV) and higher heating value (HHV) for pure methane is 13.9 and 15.4 kWh/kg, whereas biogas is between 7.21-8.65 kWh/kg for the HHV, which is significantly less than pure methane. Therefore, the composition of biogas is important and supplemental fuel would be required if the carbon dioxide composition is considerably large as the biogas will not produce a self-sustained burn (Gerardi, 2003: 73).

Although AD systems produce two valuable products, there is, however a possibility of another co-product in the microbiological process: VFAs. VFAs are present in the AD system as intermediate substrates and products, and are produced from organic matter during the acidogenesis phase. The acidogenesis phase is the second step in the four-step metabolic process (Figure 1). The other steps include: hydrolysis, acetogenesis and methanogenesis (Kashyap, Dadhich & Sharma, 2003).

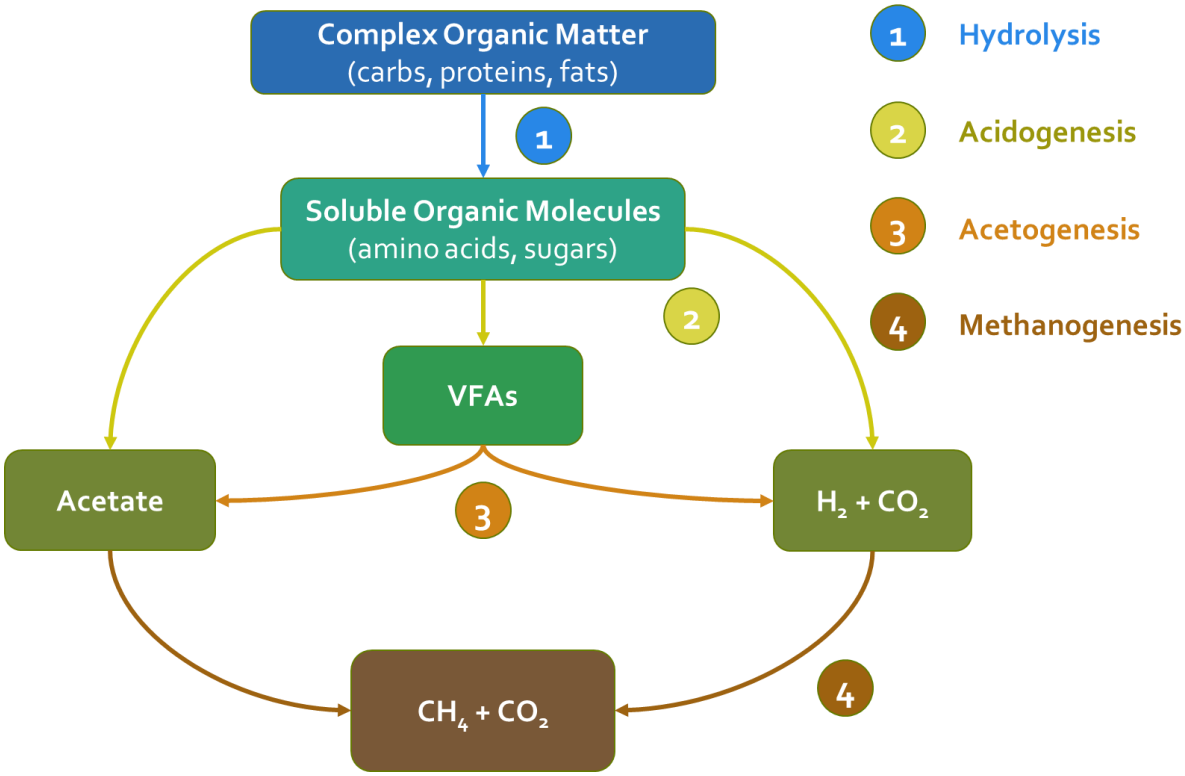

Figure 1: Metabolic pathways of microorganisms in the anaerobic digestion process.

Appels et al. (2008) described this series of steps as follows. In the hydrolysis step (reaction 1 in Figure 1), extracellular hydrolytic enzymes degrade polysaccharides, proteins and fats into soluble monomers such as amino acids, fatty acids and sugars (Teghammar, 2013). Teghammar (2013) explains that the hydrolysis step can be the rate-limiting step if there are complex structures (e.g. lignocellulose) present, as it could require weeks for complete hydrolysis of such structures. For easily degradable substrates, the methanogenesis step is the rate-limiting step. The hydrolysis step can be expressed as follows:

$$Biomass + H_2O \rightarrow Monomers + H_2 \quad (1)$$

In the acidogenesis step (reaction 2 in Figure 1), fermentative bacteria metabolise the monomers from the hydrolytic enzymes to produce VFAs (reaction 2), alcohols (reaction 3), ammonia, carbon dioxide and hydrogen (Gerardi, 2003: 54–55). An important factor in the acidogenesis step is the partial pressure of hydrogen. If the partial pressure of hydrogen is high in a stable process, more VFAs and alcohols are produced, whereas a low hydrogen partial pressure would result in acetate, carbon dioxide and hydrogen being formed (Schink, 1997).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (2)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (3)$$

From Figure 1, it can be seen that the products from the acidogenesis step can be either used directly by the methanogens or they can be degraded further into acetic acid, carbon dioxide and hydrogen. The products that can be degraded further are: VFAs, alcohols larger than one carbon atom, aromatic and branched chained fatty acids (Teghammar, 2013). The reactions below describe the main process that occurs during acetogenesis (Clifford, 2018).

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (4)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (5)$$

$$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \quad (6)$$

$$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2 \quad (7)$$

In the acetogenesis phase (reaction 3 in Figure 1), the acetogens involved are obligatory H_2 producers (Teghammar, 2013). Therefore, they require a low hydrogen partial pressure, due to this, these microorganisms are found in a symbiotic relationship with methanogens, which are H_2 consumers (Gerardi, 2003: 14). Apart from the acetic produced from the VFAs, homoacetogenic microorganisms convert carbon dioxide and hydrogen into acetic acid (Teghammar, 2013). Gerardi (2003) further mentions that the VFAs (beside acetic acid) and ethanol concentrations increase if the hydrogen concentration is excessively high. Increased concentrations these VFAs and ethanol cause a drop in the digester pH, which can cause a toxic environment for the methanogenic bacteria.

The last biological reaction in the four-step process is the methanogenesis step. In this phase, methanogenic archaea are highly sensitive towards oxygen and other environmental stressors such as unfavourable pH conditions and heavy metals (Chen, Cheng & Creamer, 2008). As mentioned earlier, methanogenesis is the rate-limiting step for easily hydrolysed materials. This is because the methanogenic archaea have the longest growth times (usually 2-25 days) amongst all the microorganisms in the digester (Teghammar, 2013). Methane is produced by three principal bacteria,

namely, acetotrophic methanogens, hydrogenotrophic methanogens and methyltrophic methanogens.

Acetotrophic methanogens, which account for approximately 70% of methane production, convert acetate to carbon dioxide and methane. About 30% is produced from hydrogenotrophic methanogens, which produce methane and water from hydrogen and carbon dioxide. Whereas, methyltrophic methanogens use methanol and methylamines to produce methane and have a small contribution to methane production (Gerardi, 2003: 26–27).

2.2.2 Co-production of VFAs and biogas from anaerobic digestion

There have been no studies to date, to the knowledge of the author, that have focussed on the co-production of biogas and VFA production, as the studies either focus solely on biogas or VFA production. However, there are useful studies where the amount of VFAs and methane yield were recorded. Bouallagui et al. (2009) investigated the effect of abattoir wastewater, activated sludge and fish waste as co-substrates with fruit and vegetable waste in the AD. A 70% water and 30% fruit and vegetable waste produced a maximum VFA yield of 2.8 g/L and 0.4 L/g VS of biogas. Whereas, the maximum biogas yield of 0.61 L/g VS was produced from digester with 70% fruit and vegetable waste and 30% abattoir wastewater, and only produced a maximum VFA yield of 2.0 g/L. Another study by Mata-Alvarez et al. (1993) was conducted using a two-phase AD system which consisted of a hydrolyser and methanisers. Shredded fruit and vegetables were used as the substrate for the hydrolyser with cow manure as inoculum. The methanisers were filled with water and inoculum from a pig-manure digester. In the study, the maximum total VFA concentration of 9.8 g/L and 5.1 g/L was obtained in the hydrolyser and the methanisers. This observation was seen around 8-10 days. At this time point, a maximum biogas production was observed in both the hydrolyser (1.2 L/day) and the methanisers (2.5 L/day). A mixed substrate interaction study performed by Kell (2019) achieved a VFA yield of 17.18 g/L using equal amounts of waste apples, retentate, food waste and cow manure. Due to large amount of VFAs present the sample only produced 102.93 mL/g VS of biogas with a methane yield of 13.08 mL/g VS_{fed}.

Therefore, from the gathered information there is potential for VFA extraction within the AD process. However, the drawback of producing excess amounts of VFAs was that there were low methane yields observed in the studies. Considering the long term value of this thesis, for co-production to be

economically viable, the process has to produce enough VFAs such that they can be sold as valuable products to increase the economic value of AD. Along with this, the process would have to produce biogas that is capable of supply energy to small utilities within the factories or plants. However, the thesis focused on the possibility of co-production of VFAs and biogas in an *in situ* manner using a suitable extraction process for VFAs. The extraction process is limited by the limitations of AD, there are several processing parameters that should be considered.

2.2.3 Process Parameters

There are numerous variables that influence AD. This is due to all three phases being present in the AD broth as well as a complex consortium of microorganisms. The two main phases that are of concern are the liquid and solid phase because these factors may have an influence on the recovery method for VFAs. These factors include: alkalinity, pH, temperature, organic loading rate, hydraulic retention time, different inhibitors and the type of substrates.

2.2.3.1 Alkalinity and pH

The vast majority of AD microorganisms are sensitive to pH changes, resulting in difficult unit operation exacerbated by them having different optimum activities at different pHs. Methanogens work optimally in a pH range of 6.5 - 8.0, whereas acetogens prefer a pH range of 5.0 - 8.5 (Boe, 2006). It is also reported that acceptable methane productivity does not occur when the pH drops below 6.2 (Gerardi, 2003: 99). Therefore, anaerobic digesters are usually operated optimally between a pH of 7.0 and 8.5. pHs outside this range can cause imbalances in the AD process (Gerardi, 2003: 99).

To ensure a stable pH is maintained, the alkalinity of the process is required to be high and stable. Alkalinity is defined as the number of basic compounds in the system and is directly associated with the buffering capacity of the AD system. This is based on the equilibrium of carbonate and dissolved carbon dioxide (Teghammar, 2013). However, there are some protein-rich substrates which release ammonia when degraded that can contribute to the alkalinity of the system.

2.2.3.2 Particle size and mixing

A study by Izumi et al. (2010) has demonstrated the significance of the particle size of food waste. Izumi et al. (2010) investigated the relationship between particle size and VFA. The reduction in particle size of the substrate improved biogas yield by 28%, as there was an increase in the solubilisation of the food waste and VFA production. Reducing particle size increases the surface area, resulting in more food being available to the microorganisms. However, VFA accumulation was observed when the substrate particle size was excessively reduced (particle size < 0.393 mm), which as a result decreased methane production. By reducing the particle size, the surface area of the substrate is significantly increased. By increasing the surface area of the substrate, the rate at which the microorganisms digest the substrate would be greater, implying that more VFAs are produced initially causing VFA accumulation.

Another method of increasing the contact area of the microorganism is by mixing the AD content. By mixing the AD content, the digestion process is enhanced as it helps distribute bacteria, nutrients and substrates throughout the reactor (Gerardi, 2003: 117). Slow and gentle mixing provides the necessary spatial contact area required by the acetogens and methanogens. This also allows for efficient hydrolysis of the substrates and production of VFAs. In addition, mixing prevents the settling of grit and reduces scum build-up. Solids accumulation reduced the hydraulics of the reactor, which can negatively impact the performance of the digester (Gerardi, 2003: 117). Another advantage of mixing is that the toxicity of toxic materials is minimised through dispersion.

2.2.3.3 Temperature

The AD process can be operated at various temperature ranges depending on the capabilities of the microbial community used in the process; different microorganisms have different temperature where their growth and metabolism is optimal (Duran & Speece, 1997). The following temperature ranges are described by Ward et al. (2008): the psychrophilic range have temperatures below 20°C, with a growth optima around 10°C. Mesophilic conditions are referred to a temperature range of 20-45°C and an operating temperature range of 45-60 °C is termed thermophilic. The thermophilic microorganisms exhibit a 25-50% higher activity than mesophilic microorganisms, which results in a higher methane yield (Levén, Eriksson & Schnürer, 2007). However, thermophilic microorganisms are more sensitive towards temperature disturbances and toxic compounds. Whereas, mesophilic microorganisms are

more stable and robust, which may be due to a more diverse microorganism community (Levén et al., 2007). Common industrial microbial communities have shown to operate best at 37 °C (Kundu, Sharma & Sreekrishnan, 2012). Therefore, pushing any community outside of its preferred operating temperatures would result in a loss of productivity. Temperature influences the metabolisms and growth rate of the microorganisms. An increase in temperature increases the solubility of the organic compounds as well as the biological and chemical reaction rates. If the extraction methods are operated above or below these temperature ranges, it may be necessary to extract the VFAs in an *ex situ* manner to avoid hindering the AD. If the methods are operated within these ranges, an *in situ* process scheme may be possible, provided that other factors affecting the AD are within the AD processing parameters. Depending on the rate of extraction, the operating temperature of the extraction method may have implications on the AD process. This is only a concern if (i) a high flow rate is required, which implies a short residence time in the VFAs extraction unit, and (ii) the temperature is much higher than in AD. If the temperature is higher than AD, another step is required to cool the process to normal AD operating temperatures, as the extraction process would increase the temperature of the digester if placed in an *in situ* manner

2.2.3.4 Organic loading rate and hydraulic retention time

Organic loading rate (OLR) is the amount of substrate added per time and volume of the digester. Mesophilic processes typically work between 2-3 kg volatile solids (VS)/m^3.day, while thermophilic processes work a higher OLR of 4-5 kg VS/m^3.day (Teghammar, 2013). The inhibition caused by VFA accumulation can occur if a high OLR is added for easily degradable substrates such as fruit waste (Fang, 2010). Hydraulic retention time (HRT) is defined as the time required to digest all the organic matter in the digester. The typical HRT for anaerobic digesters is usually about 10-25 days or longer (Schnürer & Jarvis, 2009). For slowly degrading substrates, a longer HRT is required compared to easily degradable substrates. Normally when the OLR is high, a longer HRT is required to prevent low degradation (Teghammar, 2013).

2.2.3.5 Substrates

Biogas yield and quantity is directly influenced by the type of substrate used. A higher biochemical methane potential was observed for organic matter rich in lipids/fats compared to carbohydrates or proteins, which is due to the extensive oxidation required to degrade fats than carbohydrates and

proteins (Neves, Oliveira & Alves, 2009). Typical digester substrates include: animal manure, sewage sludge, wastewater, energy crops and food wastes (Deublein & Steinhause, 2008). Proteins, fats and carbohydrates are used as an energy source for the microorganisms. The production of energy in the system is produced by the oxidation of the energy source, the electrons or protons are transferred through intermediates and finally accepted by CO_2 (Schnürer & Jarvis, 2009). Microorganisms also require macronutrients, such as carbon, iron, nitrogen, hydrogen, phosphorus, potassium and sulphur, and micronutrients (copper, cobalt, iron, selenium, tungsten and vitamins) for sustainable microbial growth (Kayhanian & Rich, 1995). Therefore, the feed that are used as substrate should contain both sets of nutrients.

In addition to the organic content, according to Liu and Whitman (2008) and Yadvika, Santosh, Sreekrishnan, Kohli & Rana (2004), the carbon to nitrogen (C/N) ratio is one of the most important factors for the biogas process. For the digester to be working optimally, it is said that the C/N ratio should be between 25 and 30. However, a C/N ratio of 10-30 can be used (Yadvika et al., 2004). Unfavourable conditions for the methanogens may occur if low C/N ratios are used, as there is a risk of ammonia inhibition. This results in VFA accumulation which decreases the pH of the system causing the digester to fail. Similarly, high C/N ratios are undesired as it may cause low methane yields due to the lack of nitrogen needed for cell growth (Alvarez & Lidén, 2008).

2.2.3.6 Toxic or inhibiting compounds

The methanogens in the AD system are the most sensitive microorganisms in the consortium and are the main concern regarding biogas production. There are many compounds that can cause inhibition in the anaerobic digester. Toxic compounds can originate internally or externally. Internally, the compounds can come from one of the metabolic pathways. There are some cases known where microorganisms tolerate toxic compounds and later phases dominate in the digester (Schnürer & Jarvis, 2009). Externally, the toxic compounds can originate from compounds or solvents used for the extraction of VFAs. However, this is usually not the case for general biodigesters.

Ammonia is the most common inhibitor in the anaerobic process and is produced from the degradation of urea or proteins, or it may originate from soluble ammonia in the feed. The most toxic form of ammonia is the non-ionised form because it can diffuse through the cell wall, which causes a proton imbalance and potassium deficiency (Chen et al., 2008). The toxicity of ammonia is influenced by the

solubility of ammonia which is directly influenced by pH and temperature and may depend on the buffer capacity of the AD system as well as the ability of the microorganisms to adapt to changes (Alvarez & Lidén, 2008; Kayhanian, 1999).

The inhibition of ammonia may lead to acidification as ammonia acts as a buffer in the process. Acidification is caused when there is an excessive accumulation of volatile fatty acids (VFAs) in the broth. This accumulation of VFAs reduces the pH of the broth, which can cause further methanogenic consortia decay and may cease the AD process (Hori, Akuzawa, Haruta, Ueno, Ogata, Ishii & Igarashi, 2014). VFAs are present in both their undissociated and dissociated form as this is pH-dependent, at lower pHs the undissociated VFA concentrations are higher. The undissociated form has an inhibitory effect as diffusion through the cell wall occurs (Deublein & Steinhause, 2008). Therefore, a good process indicator on the performance of the digester can be the accumulation of VFAs (Ahring, Sandberg & Angelidaki, 1995).

A study by Wang et al. (2009) investigated the extent to which individual VFA concentrations could cause inhibition of the methanogens. Acetic, propionic and butyric acid concentrations of 1.6, 0.3 g/L, 1.8 g/L produced the maximum amount of methane. It was found specifically that propionic acid caused inhibition in the methanogenic activity. Also, it has been reported that digester failure occurs at propionic concentrations over 3 g/L (Ward et al., 2008). However, a study by Franke-Whittle et al. (2014) showed that it was not possible to specifically determine the VFA concentrations for all systems at which inhibition occurs. Samples from two different anaerobic digesters in Austria were taken, it was reported that higher VFA concentrations were found than the concentrations known to cause instability in the digesters. Furthermore, methane production was found to be unaffected by the high VFA concentrations and digester failure did not occur. It was suggested that microbial consortia adapted to the high VFA concentrations. Therefore, it can be concluded that each AD process may have its own unique VFA concentration at which inhibition occurs which is dependent on the microbial community present in the broth.

2.3 Downstream Processing Methods of VFAs

Although there are several difficulties that are related to recovering VFAs from AD, numerous studies have reported to have effectively recovered VFAs from this system (Katikaneni and Cheryan, 2002; Rebecchi et al., 2016 and Scoma et al., 2016). However, the techniques applied were downstream

processing techniques, after separation of the supernatant and solids present in the AD digestate. These include: liquid-liquid extraction (LLE), adsorption, precipitation, membrane processing, distillation and esterification. Downstream processing methods are also known as *ex situ* extraction methods. The understanding of the fundamentals of these techniques would show insight on how VFAs are extracted and give an indication of a possible *in situ* extraction method. An *in situ* extraction method is whereby the method is completed within the process cycle loop, usually by having a recycle stream back to the process. This differs from *ex situ* whereby the extraction method is completed after the process cycle. An *in situ* configuration is preferable as it was reported to improve the recovery efficiency for the recovery of VFAs as compared to the *ex situ* way (Ataei & Vasheghani-Farahani, 2008; Roume, Arends, Ameril, Patil & Rabaey, 2016). Additionally, *in situ* extraction has shown to prevent inhibitory effects of VFAs, resulting in higher VFA production rates (Ge, Usack, Spirito & Angenent, 2015; Trad, Akimbomi, Vial, Larroche, Taherzadeh & Fontaine, 2015). The methods discussed below are for the recovery of carboxylic acids, as mentioned before, VFAs are low molecular weight carboxylic acids.

2.3.1 Adsorption

Adsorption is a surface phenomenon whereby a substance is separated from one phase (either liquid or gas) followed by the accumulation or concentration of the substance at the surface of a solid (Ramaswamy, Ramarao & Huang, 2013: 103–104). In adsorption, the solid phase is known as the absorbent, the component or product in the absorbed state on the solid phase (absorbent) as the adsorbate and the product in the bulk fluid phase as the adsorptive (Ramaswamy et al., 2013: 103–104).

Adsorption can be classified into two types based on the type of forces of attraction between the adsorbate and adsorbent: physical adsorption or chemical adsorption (Ramaswamy et al., 2013: 104). Perry and Green (2007) describe these two types in the following manner. Physical adsorption or physisorption involves weak van der Waals forces and electrostatic forces. The physisorption process is generally exothermic and is easily reversible. This is done by heating or decreasing the pressure of the adsorbate. This makes physisorption well suited for a regeneration process (a process of reusing the adsorbents for a new cycle). Chemical adsorption or chemisorption is governed by chemical bonding which are strong forces of attraction and are usually irreversible or may not be fully reversible due to the sharing of electrons between the adsorbate and the surface of the adsorbent (Kwon, Fan,

DaCosta, Russell, Berchtold & Dubey, 2011). Thus, the regeneration of chemisorption can be energy-intensive. Chemisorption destroys the capacity of the adsorbent and is not usually utilised widely in industry (Perry and Green, 2007, p. 16–4).

Another form of physisorption is ion exchange, which is based on electrostatic forces and is due to the Coulomb-attractive forces between charged functional groups and ions. This form of adsorption uses ion exchangers, which are polymeric solids that have the ability to take up charge ions (cations or anions) from a solution and replacing dissimilar ions (of the same charge) in the solid (Ramaswamy et al., 2013: 149–153).

Figure 2 depicts a simplistic cyclic process for carboxylic acid recovery in a packed column and is explained by López-Garzón and Straathof (2014). However, the combination of adsorption with AD can be a complex process. The red text corresponds to a strong anion and black to a weak anion exchange operation. In stage 1, interaction occurs between the solute and the functional group on the resin. The impurities flow through the column. During stage 2, desorption of the acid occurs due to the change in resin characteristics. Stage 3 is the regeneration process whereby the resin is reused for a new cycle. The intermediate steps for washing and repacking of the resin contribute to a discontinuous process (López-Garzón & Straathof, 2014).

For the ion exchange process to be industrially applicable, a minimum capacity of 0.05 g/g for carboxylic acids is required (Davison, Nghiem & Richardson, 2004). Some of the advantages and disadvantages have been reported for ion exchange and have been listed below in Table 5 (Ramaswamy et al., 2013: 151). The consideration of these pros and cons, along with a required minimum carboxylic acid capacity of 0.05 g/g to be industrially applicable (Davison et al., 2004), proves to be a difficult task. This is due to the economic feasibility of ion exchange.

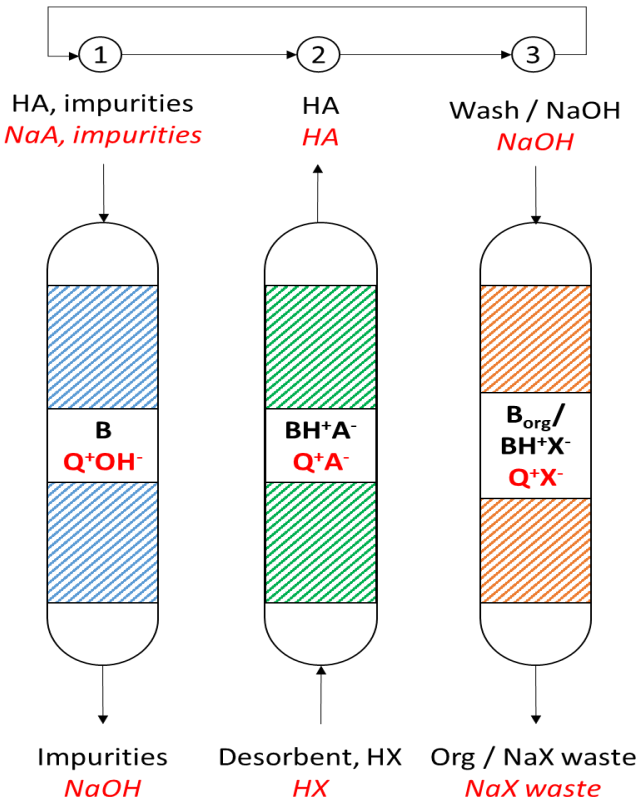

Figure 2: An anion exchange-based process diagram for the recovery of carboxylic acids or carboxylates using adsorption, adapted from López-Garzón and Straathof (2014).

Table 5: Advantages and disadvantages of adsorption.

Advantages	Disadvantages
All ions can be removed from aqueous liquids	Prefiltration is required if suspended particles > 50 mg/L
Recovery of valuable substances are possible	Low-temperature resistance of organic ion exchangers
Large variety of specific resins available	Interference of competing cations in wastewater
High efficiency	

2.3.2 Distillation and esterification

Other separation techniques include distillation and esterification. Distillation is the most common separation technique in the chemical industry (Wankat, 2012). The driving force behind distillation is the difference in the relative volatility of the components. The volatile component will be more concentrated in the vapour stream and the least volatile components in the liquid stream (Wankat,

2013: 79). Although distillation is the simplest separation technique, it requires significant amounts of energy to separate acetic acid and water (Zhou, 2005). This is due to the relative volatilities of these two components being close to unity (Zhou, 2005). Therefore, the recovery of VFAs by distillation is likely unsuitable for the recovery from AD broth, especially for dilute VFA streams.

The recovery of acetic acid can be processed by esterification. Esterification involves the conversion of acetic acid to an ester and takes place at temperatures exceeding 55 °C (Tolvanen, Kilpiö, Mäki-Arvela, Murzin & Salmi, 2014). This is done by reacting the acid with an alcohol, using an acid catalyst. To form the final acid product, the ester is hydrolysed (Katikaneni & Cheryan, 2002). Esterification can be severely affected by large amounts of water in the fermentation broth. The presence of large amounts of water reduced the efficiency of the process and gave yields of 5-20% (Horiuchi, Shimizu, Tada, Kanno & Kobayashi, 2002). The addition of the alcohol and reaction temperatures of esterification may cause harm or hinder the AD-biogas process. As a result of this, esterification may not be a viable *in situ* recovery method.

2.3.3 Membrane processes

Membranes allow for selective separation to occur by using semi-permeable barriers. This implies that different solvents and solutes flow through the membrane at different rates (Perry and Green, 2007, p. 20–36). Combined with relatively low capital (compared to adsorption) and energy costs, membrane technology can be suitable for the extraction of VFAs. There are two main driving forces for membrane separation. These include a pressure-driven or an electrical field-driven force. Membranes that are driven by an electrical field is briefly discussed later on.

Pressure driven processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) and pervaporation have been used for the treatment of wastewater (Jones et al., 2015 and Longo et al., 2015). MF and UF membranes work according to the principle of pores. This, however, is partially true for NF and RO membranes, separation also occurs by diffusion of substances through the membrane (Schaep, Van der Bruggen, Vandecasteele & Wilms, 1998).

MF and UF are not commonly applied to the recovery of VFAs because these separation processes do not only allow VFAs through but some of the digestate as well due to the pore size of the filters.

Therefore, the use of these processes can be excluded from primary recovery processes of VFAs. MF and UF, however, can be used to pretreat the process stream before the extraction process to separate the larger particles in the broth (Zacharof & Lovitt, 2013). NF and RO membranes can separate ions from relatively small molecules (Schaep et al., 1998), this suggests that NF and RO can be used as primary recovery techniques for recovering VFAs from AD.

Timmer, Kromkamp and Robbertsen (1994) tested NF and RO membranes for the dewatering of filtered lactic acid fermentation broth. NF membranes reported to be better than RO membranes. However, it was recommended that UF should be used as a pretreatment as protein fouling occurred. Cho, Lee and Park (2012) passed ultrafiltered butyric acid from a fermentation broth through NF and RO membranes. It was reported that both membranes separated butyric acid and water from ions and larger molecules. NF was also reported to have a good recovery but low purity. NF and RO membranes may not be suitable for primary recovery of VFAs due to both methods not leading to a concentrated permeate (López-Garzón & Straathof, 2014).

Other membrane methods such as pervaporation have also been used to remove VFAs from fermentation broths. Pervaporation is a process whereby a liquid feed stream is heated up and brought into contact with the active site of the membrane (Pervatech, 2014). The better permeating component passes through the membrane and is removed from the permeate side of the membrane in the vapour form.

This technique was used by Choudhari et al. (2015) to separate butyric acid using a polyether block amide based composite membrane. A butyric acid concentration of 5.95 g/L was used in a 2 L anaerobic broth. The study reported that 120 g/m^{2}h of butyric acid was removed. Although this technique was effective for the recovery of butyric acid, this technique was impacted negatively when a model solution of VFAs (comprising of acetic, butyric, propionic and valeric acid) was used. It was suggested that high solubility of acetic and propionic acid in water contributed to the high fluxes of water seen in the study. Therefore, pervaporation may not be suitable for recovering VFAs from AD due to the complex nature of AD.

There are several advantages of using membrane process such as: ability to recover acids in a relatively concentrated form, low pressure operation, ease of up-scaling, *in situ* regeneration of the polymeric liquid, relatively low operation cost and low energy consumption. However, the major disadvantage

of any membrane separation process is fouling (Zamani, Ullah, Akhondi, Tanudjaja, Cornelissen, Honciuc, Fane & Chew, 2016) and is a result of solid particles present in the fermentation broth and by concentration polarisation (Field, 2010). Fouling leads to unscheduled downtime as membrane cleaning is required (Field, 2010). AD involves the use of waste resources which can cause severe fouling in membranes. This can have a drastic effect on the process of the system, especially considering the sensitivity of the microbial consortia with changes in the environment. Another disadvantage of using NF, in this case, is that the rejection ratio of VFAs is strongly dependent on the pH of the feed solution to the membrane (Cho et al., 2012). Cho, Lee and Park (2012) explain that at low pH, the surface charge of the membrane becomes positive. This allows VFAs to penetrate the positively charged membrane while retaining large neutral molecules. This implies that an external *in situ* configuration would be required to decrease the pH to the desired removal rate of VFAs. This may be beneficial in the long run as the configuration would be the simplest configuration to incorporate downtime caused by fouling and maintenance of the process

This thesis deals with the possibility of a continuous separation process, because of the unscheduled downtimes due to membrane fouling, an integrated system that takes into account solid removal may be required. By considering this, as well as, the effect of pH on NF, membrane technology may not be a suitable solution.

2.3.4 Electrodialysis

Electrodialysis (ED) is a form of membrane separation process where the driving force behind the process is an electric field. For the separation of VFAs from a fermentation broth, there are two types of ED that have been investigated in literature (Wankat, 2013, p. 723). The two types are conventional electrodialysis (CED), seen in Figure 3, and a bipolar membrane electrodialysis (BMED).

CED uses cation and anion exchange membranes that are stacked between the anode and the cathode (Ramaswamy et al., 2013: 425). The ions can only pass their respectable membranes. The BMED process uses bipolar membranes to split water into H^+ and OH^- ions (Wang, Wang, Zhang, Feng & Xu, 2013). However, due to the cost of the bipolar membranes, this separation method can be expensive (Jones et al., 2015). Both CED and BMED have been used as an *in situ* separation method of carboxylic acids during fermentation (Murali, Srinivas & Ahring, 2017)

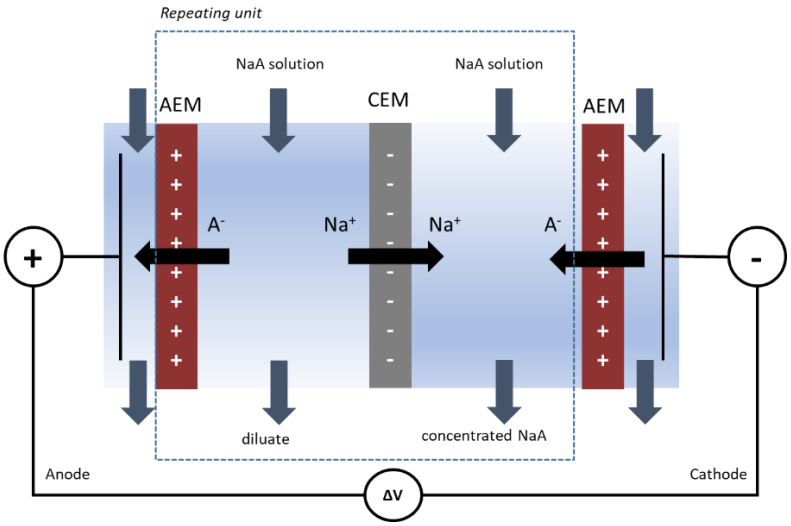

Figure 3: A bipolar membrane cell with cation exchange membranes (CEM) and anion exchange membranes (AEM) used for electrodialysis, adapted from López-Garzón and Straathof (2014).

ED is a form of membrane separation and one of the major problems of membranes is that they are susceptible to membrane fouling (Zamani et al., 2016). Therefore, preventative measures such as clarifying the fermentation broth may be required to prevent membrane fouling. Another issue of using ED for separating VFAs is the high energy costs. This is due to the low electrical conductivity of carboxylic acids, which results in a low current efficiency (Nagarale, Gohil, Shani, Trivedi, Thampy & Rangarajan, 2014). For the concentration of sodium lactate and sodium propionate, the energy consumed for the ED process was roughly 0.22 kWh/kg under optimised conditions (Hábová, Melzoch, Rychtera & Sekavová, 2004). More than 1 kWh/kg would be required for organic acids (Ramaswamy et al., 2013: 425). However, in South Africa, electricity is currently around R1/kWh (Eskom, 2019) and the retail of value of acetic acid on Alibaba.com is roughly R7.5/kg (when purchasing in bulk). Therefore, the energy demand can be overlooked depending on the effectiveness of the method.

Jones et al. (2015) reported to reduce acetic and butyric acid concentrations by 97% and 96%, using 20 cell pairs with an effective membrane area of 0.128 m^2, the CED stack contained 250 mL of concentrate, 1000 mL of electrolyte and 250 mL of a model VFA solution. Tao et al. (2016) used a chain of processes to extract VFAs for the production of polyhydroxyalkanoates. The effluent stream was microfiltered before it passed through the CED cell. The study reported to have removed 92% of VFAs to the concentrated stream. However, in both studies, fouling was not discussed and may be due to the small scale set-up used in the studies. Another study by Scoma et al. (2016) reported to have only

removed 30-35% VFAs from pre-treated Olive Mill Wastewaters. In the study, it was reported that there was no damage done to the membranes by fouling. This may be a result of the vigorous washing of the membranes after every experimental run.

Although ED is a promising technique for extraction, it can be energy-intensive on an industrial scale (1 kWh/kg). In addition to membrane clogging, membranes are placed in an external configuration for maintenance because the internal configuration is practically infeasible (Van Hecke et al., 2014). Although this does not affect the *in situ* requirement, it adds to the capital cost for extra equipment, along with extra equipment costs for an integrated system for particle removal.

2.3.5 Precipitation

Precipitation involves the conversion of a soluble carboxylic acid into an insoluble carboxylate. The precipitate is separated from the impurities and water by means of filtration. Calcium hydroxide and calcium carbonate can be used for precipitation (Heding & Gupta, 1975). The recovery of the acid is based on the solubility of the carboxylate. This method of recovery could prove to be effective, especially if the VFAs are in the gaseous phase.

This concept was used in combination with gas stripping in Li et al. (2015) where VFAs were recovered as VFA calcium salts after they were stripped from the fermentation broth. In this study, 200 mL of 2 M calcium carbonate slurry was used to capture the VFAs from the gaseous phase. 3.11 g of total VFAs was reported to have been recovered. The recovered composition of the VFA calcium salts were reported as 82.9% butyrate and 11.4% acetate, with small amounts of propionate (2.3%) and valerate (3.5%). The duration of gas stripping in which resulted in the 3.11 g of VFAs is unclear.

Another principle was applied for the recovery of butyric acid, this principle was based on the higher solubility of the carboxylate compared to the solubility of the carboxylic acid. An example of this is the solubility of sodium fumarate and fumaric acid. Sodium fumarate has a high solubility of 220 g/L at 25°C and fumaric acid has a low solubility of 6.3 g/L (PubChem, 2017). This implied that fumarate could be recovered as solid fumaric acid. Sulphuric acid was added to an aqueous sodium butyrate solution. This resulted in an oily layer of butyric acid on the aqueous phase (López-Garzón & Straathof, 2014).

2.3.6 Gas stripping

Gas stripping or desorption is a process whereby a separating agent is added (gas stream) to remove one or more components from a liquid stream. The components are vaporised into the insoluble gas stream (Wankat, 2013, p. 453). Separation by gas stripping can be implemented in two ways, a separate gas-stripper column or an integrated method with the bioreactor. In the separate stripping column scenario, the fermented broth and the stripping gas is sent to the stripper via two different streams. The stripping gas captures the solvents, and the feed stream is then recycled back to the reactor. The gas stream passes through a condenser to condense the solvents. The desired volatile solvents are enriched in the condensate stream. In the integrated method, the bioreactor acts as the stripping column itself. The stripping gas is passed through the bioreactor, capturing the volatile solvents. The gas stream is then sent to a condenser. This method can be seen in Figure 4.

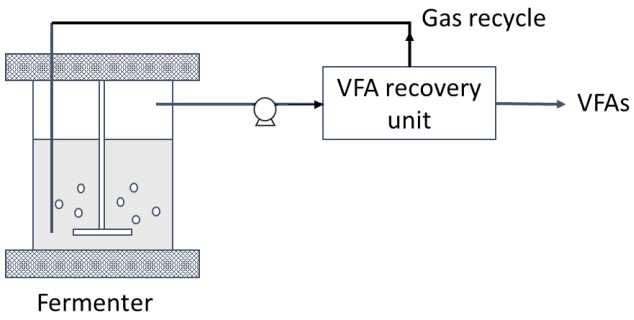

Figure 4: An *in situ* extraction gas stripping method.

The stripping gas is usually non-condensable gases, such as nitrogen. Fermentation gases have also been known to be used in acetone, butanol and ethanol (ABE) fermentation (Ezeji, Qureshi & Blaschek, 2004). Such gases include hydrogen and carbon dioxide. There are also, two ways of operating the gas flow. The first, the gas is released to the atmosphere, which is called the single-pass mode. Solvent loss may occur with the single-pass depending on the efficiency of the condenser. The second is the recycle mode. In this mode, after the gas passes through the condenser, it is sent back to the stripping column to be reused. Solvent loss is prevented as the process is a closed-loop.

There are several advantages of using gas stripping as an integrated processing method for product recovery. These include (Qureshi & Blaschek, 2001; Vane, 2008): operating at fermentation

temperature, the use of fermentation gas as a stripping agent in the ABE fermentation and processing of broth with or without the presence of solids

The vapour pressure of the components can be described by Raoult's law (Equation 8). From the Raoult's law, P_i^{sat} is dependent on temperature. Therefore, increasing the temperature would favour a high vapour pressure and stripping rate. However, both the volatile solvent and water follow this principle. An optimal temperature would depend on the selectivity of the solvent and water (Ramaswamy, Ramarao and Huang, 2013, p. 414).

$$P_i = x_i P_i^{sat} = y_i P_{total} \quad (8)$$

where,

P_{total} = total pressure of the gas phase

P_i = partial pressure of component *i*

P_i^{sat} = saturated vapour pressure

Y_i = mole fraction of component in the gas phase

X_i = mole fraction of component in the mixture

Gas stripping has been implemented to remove butanol from (ABE fermentation broth (Cai et al., 2016 and Xue et al., 2016). However, there are only a few studies on the recovery of VFAs from anaerobic digesters (this study is discussed later on). Li et al. (2015) reason that this may be due to the high Henry's law constants of VFAs compared to the strippable components. Gas stripping is normally used when the Henry's law constants of the volatile solvents are less than 10 M/atm (Li et al., 2015). The Henry's law constants of VFAs are significantly higher and are between 2.2-5.7 × 10^3 M/atm (Sander, 1999).

Gas stripping in industry is usually fast when Henry's law constants are small. Although anaerobic digestion is a slow process, gas stripping can still be effective if the VFAs are present in their undissociated form (Li et al., 2015). This is because the undissociated form is more volatile and are able

to evaporate at atmospheric pressure. The pH of the broth determines the form of the VFAs and therefore should be maintained at levels that favour the undissociated forms (Li et al., 2015).

Another factor that can indirectly affect gas stripping is the composition of gas used. This can be explained by Henry's law (Equation 9), where the partial pressure of the gas (P_i) is proportional to the concentration of the solute in the solvent (C_i). This implies that a high Henry's law constant (K_H) would result in a low concentration of solute in the solvent.

$$P_i = C_i K_H \tag{9}$$

Other factors that can affect stripping include: bubble size, contact time, gas flow rate, mass transfer coefficient and cooling temperature (Ramaswamy et al., 2013: 413). The effects of the bubble size and the gas flow rate were studied by Ezeji et al. (2005) on butanol recovery. The study reported that no effect was observed for bubble sizes in between 0.5 and 5 mm. However, the gas flow rate had an effect on the study. They observed a 2.5-fold increase in the stripping rate when increasing from a flow rate of 43 cm^3/s to 80 cm^3/s. It was also reported that the contact time of the gas bubbles in a 2 L reactor was 0.14 s. They concluded that smaller bubbles of < 0.5 mm reduced the productivity of the reactor. It should be noted that the study by Ezeji et al. (2005) used an *in situ* configuration that was internal as seen in Figure 4. An external configuration where a separate gas stripping unit is used may be influenced by the flow rate and bubble size.

Li et al. (2015) used *in situ* gas stripping to extract VFAs from an anaerobic bioreactor that produces methane. The stripping gas used in the study was nitrogen and a calcium carbonate recovery solution (as discussed earlier in section 2.3.5 Precipitation) was used to recover the VFAs from the nitrogen stream. The study reported the pH of the broth increased as the VFAs were stripped and that stripping only began once the broth reached a pH below 4.8 (average pKa of the VFAs). It should be noted that at a pH of 4.8, no methane production was observed. Therefore, the co-production of VFAs and biogas was not observed in this study.

The study above indicates that gas stripping can be a promising technique for the *in situ* removal of VFAs on an AD-biogas system. However, a major drawback from this study was that the methanogenesis phase was purposely inhibited to produce more VFAs. Therefore, there is a possibility

of gas stripping as an *in situ* recovery process for VFAs in an AD system that co-produces biogas and VFAs. The studies above used either nitrogen, carbon dioxide or hydrogen gas as the stripping gas. However, there have been no reports that studied biogas from an AD-biogas process as stripping gas.

Biogas could be potentially an alternative stripping gas because it mostly comprising of non-condensable gases (methane and carbon dioxide). Although, the composition of biogas could affect the stripping process.

2.3.7 Liquid-Liquid Extraction (LLE): Solvent Extraction and Reactive Extraction

LLE has been a well-established extraction technique for the primary recovery of VFAs. LLE of VFAs can be categorised into three groups: (1) carbon-bonded oxygen-bearing and hydrocarbon extractants (2) phosphorous-bonded oxygen-bearing extractants and (3) aliphatic amines (Kertes & King, 1986). Groups 1 and 2 are nonreactive extractants and extract VFAs by the solvation of the VFAs by donor bonds, this extraction is termed as solvent extraction. Whereas, group 3 involves a chemical reaction that produces an acid-amine complex and is termed reactive extraction. Group 3 usually extract more due to the acid-amine complex (Wasewar, Yawalkar, Moulijn & Pangarkar, 2004).

Solvent extraction is a separation process used to separate components from a liquid feed to another, immiscible, solution. The process is based on the difference of solubility of the component in the two solvents. The carrier solvent or feed solvent is the major liquid in the feed. Solutes are the minor components in the solution. The extraction solvent or extractant is an immiscible or partially immiscible liquid and the liquid phase remaining after the extract phase is known as the raffinate (Perry and Green, 2007, p. 15–10).

Figure 5 represents a general concept of solvent extraction and is explained by Koch and Shiveler (2015), where A is the carrier solvent, B is the extractant and C is the solutes in the feed. The first step of the process involves the addition of the extractant. A dispersion is formed due to the two immiscible liquids (carrier and extract solvent). One liquid is dispersed in the other as droplets. The mass transfer takes place between the dispersed phase and the continuous phase (surrounding liquid). The two liquids must have different densities in order to be separated. Depending on the relative densities, droplets accumulate either above or below the continuous phase. The interface is regarded as the

boundary between the dispersed phase and the continuous phase. The next step involves mixing the two solvents as mixing improves the interface between the extractant and the diluent (Koch & Shiveler, 2015). The process is generally performed in a counter-current flow pattern (Wankat, 2012). However, for an *in situ* extraction process in AD, it would be preferable for the effluent to remain in the digester rather than using an external unit for the extraction, as this would reduce equipment costs.

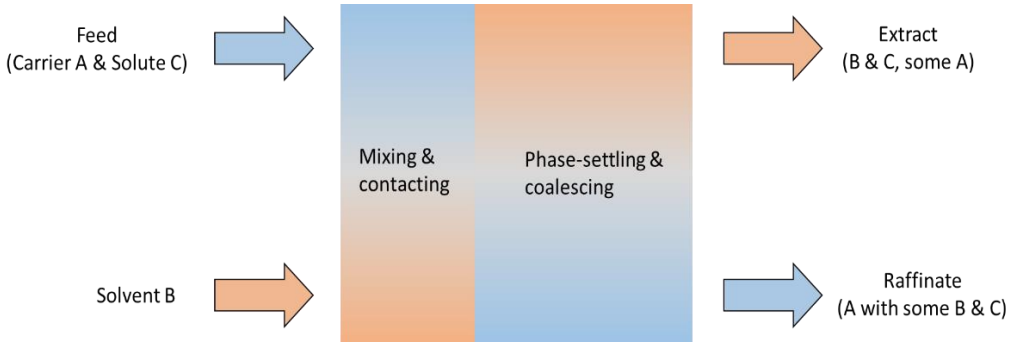

Figure 5: An extraction unit for liquid-liquid extraction, adapted from Koch and Shiveler (2015).

In reactive extraction, primary, secondary and tertiary amines are used to form an association complex or chemical compound in between the extractant and solute via interactions through hydrogen bonding and ion pairing (Kertes & King, 1986). The complexation in the organic phase can be described below for tertiary amines, it should be noted that the exact chemistry is still unknown and is simplified.

$$HA_{(aq)} + R_3N_{(org)} \rightleftharpoons R_3NH^+A^-_{(org)} \quad (10)$$

$$nHA_{(aq)} + R_3N_{(org)} \rightleftharpoons R_3N(H^+A^-)_{n(org)} \quad (11)$$

There are two basic requirements for the selection of a suitable extractant in LLE: a high distribution coefficient and a high selectivity for the product (Wasewar et al., 2004). Although literature states a high distribution coefficient is required, low distribution coefficients can still work because the system is continuous. The distribution coefficient (k_d) is determined by the concentration of acid in the organic phase over the concentration of acid in the aqueous phase, which can be seen in the equation below, where HA represents the acid. In addition to these basic requirements, the extractant should have (i) a low viscosity, (ii) no reaction between the extractant and raffinate, (iii) higher density than the raffinate, (iv) a low solubility in the raffinate and (v) low toxicity (Wasewar et al., 2004).

$$k_d = \frac{[HA]_{org}}{[HA]_{aq}} \quad (12)$$

Solvent extraction of VFAs with conventional extractants, such as alcohols, aliphatic hydrocarbons, ethers and ketones, were determined to have low distribution coefficients due to their low aqueous activity when applied to dilute VFA solutions such as fermentation broths and wastewater. This implies that conventional extractants are not efficient enough to be selected as a suitable extractant. On the other hand, phosphorous-bonded oxygen-bearing extractants and high molecular weight amines have higher distribution coefficients (compared to conventional extractants under similar conditions) and low solubility in water (Zacharof & Lovitt, 2013). Solvents may have toxic effects on the anaerobic microorganism, a low solubility in water may reduce the toxic effects. Among the phosphorous-bonded oxygenated donor extractants, trioctlyphosphine oxide (TOPO) and tri-n-butyl phosphate (TBP) have been used extensively in literature (Alkaya, Kaptan, Ozkan, Uludag-Demirer & Demirer, 2009; Keshav, Wasewar & Chand, 2009; Wasewar et al., 2004).

Aliphatic amines are said to be more effective than the phosphorous-bonded oxygenated donor extractants (Wasewar et al., 2004). This is due to the formation of acid-amine complexes through strong amine interactions result in high distribution coefficients. In addition, aliphatic amines have a high selectivity for VFAs. However, the solvation power of the amine is dependent on the type of diluent used and the amount of amine present in the diluent (Ricker, Michaels & King, 1979). The basicity of the amine is affected by diluent, which in turn affects the stability of the ion pair (formation of the acid-amine complex) and its ability to solvate the acid-amine complex. The distribution coefficient is directly influenced by this, as a diluent can prevent a separate phase from forming and prevent precipitation of the acid-amine complex (Senol, 2004). The ability of the amine to solvate the acid-amine complex depends on the dipole-dipole interactions. It was found that polar diluents can significantly improve the solvation power of the amine when compared to nonpolar diluents (Kertes & King, 1986). A trend was observed in a study by Hong and Hong (2000), the trend showed that as the chain length of a tertiary amine increase, so does the extraction power of the amine in a polar diluent. However, when a nonpolar diluent was used (n-heptane), the opposite was seen. From the aliphatic amines, tertiary amines are preferred over primary and secondary amines because primary amines have a high mutual solubility with water and secondary amines have a tendency to form amides when heated. Also, the extractive power of the primary and secondary amines are significantly lower than

tertiary amines (Wasewar et al., 2004). The distribution coefficient does not only depend on the extractant and the type of diluent but also with temperature and has a strong dependence on pH.

A study by Tamada and King (1990) showed the effect of temperature for the extraction of lactic acid using TOA. The study showed that as the temperature increases, the extraction efficiency decreases. This was said to be due to the exothermic reaction between the acid and the amine, which involves a hydrogen-bond formation or a proton-transfer-reaction. This temperature effect was found to be significantly small for TBP when compared to TOA (Wasewar et al., 2004). However, between 20 °C and 90 °C, the temperature only had a slight effect for physical solvents (which can be used as diluents) such as alcohols, diethyl carbinol, ether and ketones (Kertes & King, 1986). AD is generally operated below 40°C (depending on the consortia). Therefore, in an *in situ* configuration, LLE is limited by temperature.

Another limitation of extracting VFAs by LLE is the significance pH on the distribution coefficient. Most extractants are mainly effective at acidic conditions, where the pH is below the pKa value of the VFA. Below the pKa value, the undissociated form of the VFA is dominant and the complexation reaction between the acid and amine takes places with the undissociated form (Yang, White & Hsu, 1991). AD was identified as a potential source for co-producing VFAs and biogas. However, the normal operating pH conditions (usually between 6 and 8) could be problematic as the pH conditions are considerably higher than the average pKa value of the VFAs. Although, Aliquat 336 has been reported to extract VFAs in both forms, the undissociated and dissociated form (Yang et al., 1991). However, overloading the quaternary amine could lead to the formation of a third phase. Furthermore, because the quaternary amine has the ability to extract both VFA forms, the regeneration of the solvent or stripping of the solvent can be a difficult task achieve (Yang et al., 1991).

One of the most crucial factors that should be considered is the toxicity of the solvent or extractant on the microbial community, especially if the extraction method will be an *in situ* method. If the method is applied *in situ*, the solvent would be in contact with the active broth. This implies that a toxic solvent can hinder the AD process causing major downtime. The solvent can be toxic to the microorganisms on a molecular level and at a phase level. On a molecular level, the dissolved solvent can inhibit enzymes or change the permeability of the cell membrane. Phase level, the solvent can prevent diffusion of nutrients from the medium to the cell (caused by solvent coating) when the solvent comes into direct contact with the cells (Keshav, Wasewar, et al., 2009). Cell immobilisation and cell membranes have successfully employed to decrease phase toxicity of the solvent by preventing the microbes from making direct contact with the solvent. However, membranes are susceptible to

membrane fouling. Membranes are also expensive and could rupture during the operation process (Keshav, Wasewar, et al., 2009). An alternative solution would be to use a diluent that is capable of reducing the toxicity of the extractant. This can be solved by introducing a toxic extractant in a nontoxic diluent.

A study by Datta (1981) showed the effects of solvents on anaerobic acid producing bacteria. TOPO and TOA were observed to have no toxic effects in amyl acetate, diesel and toluene at saturation conditions. Playne and Smith (1983) investigated the effects of several organic solvents on anaerobic acid producing bacteria from commercial inoculum. From the thirty solvents investigated, thirteen were found to be nontoxic, fifteen were toxic and two were partially toxic at saturation conditions. The thirteen nontoxic solvents and the two partially toxic solvents can be seen in Table 6. Most the solvents listed below are relatively inexpensive, generally ranging between R400 and R2000 for 1 L (based off Sigma-Aldrich). These solvents may not be toxic to anaerobic acid-producing bacteria but most of them are toxic to aquatic life and are have acute toxicity on humans. These solvents should be handled with care and be disposed of in an orderly manner.

In contrast, Marták et al. (1995) also studied the toxicity effects of organic solvents on specific fermentation bacteria. The study reported that 100% TBP showed a significant decrease in growth for the anaerobic bacteria and was deemed to be toxic. 20% and 30% TOA were reported to inhibit acid production and biomass growth. With this in mind, if LLE is chosen, the solvent should be investigated to verify its biocompatibility within the digestive broth before implementation.

Yabannavar and Wang (1990) successfully implemented an extractive fermentation process using 15% TOA and oleyl alcohol to continuously remove lactic acid. The two-step process, whereby the medium was mixed with the solvent in the mixer and allowed to settle in the settler. The aqueous phase was recycled. The study incorporated both methods to reduce toxicity, cell immobilisation with the use of cell beads and the use of a diluent. The study reported a 71% increase in productivity (from 7 g/L to 12 g/L).

Table 6: Toxicity of extraction solvents on anaerobic acid producing bacteria from inoculum, adapted from Playne and Smith (1983).

Nontoxic solvents	**Partially toxic solvents**
Aliquat 336	Amberlite LA-2
Dibutyl phthalate	Primene JMT
di-isoamyl ether	
di-isoamyl phthalate	
Freon 113	
iso-octane	
Kerosene	
n-decane	
n-hexane	
Tributyl phosphate (TBP)	
Trioctylamine (TOA)	
Trioctyl phosphine oxide (TOPO)	
Tritolyl phosphate	

Oleyl alcohol not only lowers the toxicity of the extractant but also considerably increases the solvation power of TOA. Keshav, Wasewar and Chand (2008) investigated the effects of TOA in different diluents for the extraction of propionic acid. From the diluents used, 30% TOA in oleyl alcohol proved to have the highest distribution coefficient. This was due to the polarity of oleyl alcohol, TOA is a nonpolar extractant, this implies that more polar diluents would significantly improve the solvation power of TOA (Keshav et al., 2008). Other diluents that could enhance the biocompatibility of extractants is lamp oil and sunflower oil. Lamp oil and sunflower oil are nonpolar diluents and are insoluble in water. These diluents were investigated by Yang, White and Hsu (1991) and Keshav, Wasewar and Chand (2009). Although these diluents do not significantly increase the extractive power of TOA and TBP compared to 1-decanol, they increase the biocompatibility and increase the loading factor of the extractants. This allows the extractant to extract more VFAs from the aqueous solution.

LLE has proved to be one of the most efficient and simplest techniques for extracting VFAs from an AD system. When considering LLE for *in situ* extraction, the most important factor is the selection of the solvent (extractant and diluent, if any). To extract VFAs from the AD system, the solvent should not only be able to extract at high a pH but also have nontoxic effects on the inoculum. However, inhibitory compounds may not affect all systems as these systems may have microorganisms that may have adapted over time to inhibitory compounds. Therefore, compounds that are toxic to a certain reactor or system may not have the same effects on another (Franke-Whittle et al., 2014). Based on the

literature, TOA and TBP have been used extensively in several diluents to extract VFAs and can be easily back-extracted with a sodium hydroxide solution (Wasewar et al., 2004).

2.3.8 Summary

A summary of the process methods that have been discussed is summarised in Table 7.

Table 7: A summary of the advantages and disadvantages of the separation methods for VFAs, adapted from (Zacharof & Lovitt, 2013).

Separation methods	Description	Advantages	Disadvantages
Precipitation	Calcium salts are used to neutralise the acids, resulting in a calcium carboxylate solution. The solution is concentrated by evaporation and crystallised	Low capital cost, high product yield, high purity products	Sulphuric acid is used to recover the VFAs from the calcium carboxylates, which generates solids
Distillation	Acids are neutralised with ammonia to form ammonia carboxylate. Esters are formed when ammonia carboxylate is mixed with alcohol. These esters are separated by distillation	Fertiliser as a byproduct and high purity of the product	Energy-intensive as well as high capital costs to separate the alcohol from the VFAs
Adsorption	The exchanging of ions are carried out by the use of ion-exchange resins	Ease of use	Cost of resin is relatively high, resin regeneration is energy-intensive, low selectivity
Electrodialysis	An electric current is used to pass the carboxylate ions through the membrane	Concentrated carboxylate is found in an aqueous solution, which does not require pH adjustment	High impurities, difficulty in scaling-up, energy-intensive
Solvent extraction	The extraction of VFAs through the use of organic acids	Low costs of carboxylate salt production	Requires regeneration of extractants and

			acidified feed for efficiency
Membrane separation	Use of membrane can act as filters to treat the mixed effluents for solids removal and fractionate the desired product for recovery	Ease of scalability, relatively low costs, yields of products are high	Membrane fouling, clogging
Gas Stripping	A stripping gas is used to strip the volatile solvents from an aqueous solution	Fermentation gas can be utilised as the separating agent, operated at fermentation temperatures, does not harm the cells and can be used with or without separating the solids	The use of fermentation gases, such as methane, can be potentially released into the atmosphere. This causes foul odours and can contribute to the greenhouse effect

2.4 Conclusions

VFAs are short-chained fatty acids with a considerable number of uses in industry. As a result, they have significant commodity value. VFAs are currently produced via the petrochemical industry. This method of producing VFAs may not be viable in the future as the world turns to alternative and sustainable energy and renewable resources, due to associated environmental benefits of bio-based products. Therefore, alternative methods have been investigated to produce VFAs in a sustainable manner. One of these methods is the process by AD. AD comprises of complex metabolic pathways that convert organic waste into beneficial byproducts. These byproducts include biogas, hydrogen and VFAs. Biogas is a renewable fuel, which consists of methane and carbon dioxide. This renewable fuel can be used to provide heat and transport. Biogas can be easily extracted by means of a valve. VFAs, on the other hand, need more complicated processes for extraction.

Downstream extraction methods have been employed to extract profitable VFAs from the AD process. These processes include liquid-liquid extraction, membrane processing (such as NF, UF and electrodialysis), adsorption, gas stripping, precipitation, esterification and distillation. All these extraction methods have their pros and cons, however, there are certain extraction methods that are limited to downstream processing due to the nature of the process. Distillation and adsorption can separate VFAs from the broth but they are high energy processes. As a result, the distillation and adsorption systems are inefficient and infeasible to industry. A regeneration step can be used to recover and reuse the resins to reduce production costs. Although membrane processing is a viable alternative, it is coupled with unscheduled downtime due to membrane fouling. Membranes also have poor recoveries of VFAs. However, membranes can be used to purify the VFA stream due to the selective property of the membrane. This could potentially be used to recover the VFAs from the gaseous phase but would result in more equipment costs. The AD process consists mainly of water, which implies that esterification can be used to recover the VFAs but would have a poor recovery ratio, as the esterification is inhibited by large amounts of water. Precipitation produces unwanted solid waste. Therefore, filtration equipment is required, which would increase the capital costs of the extraction scheme.

Gas stripping and LLE are two promising techniques that were investigated further. Gas stripping was chosen because it can be used as an *in situ* extraction method with minimal cons to the AD system. This is because gas stripping can utilise the fermentation gas, already produced by the AD process, to

strip the VFAs from the fermentation broth. The employment of this technique can be advantageous as it can process the broth with or without solids. This reduces the costs as there is no need for solid-liquid equipment. Gas stripping was investigated using two mixtures of gas, namely, 100% carbon dioxide and a mixture of carbon dioxide and methane gas to simulate biogas within the bioreactor.

The second promising technique, LLE, is cost-effective, efficient and relatively simple to implement in an *in situ* configuration. However, LLE is highly dependent on the type of solvent used as the solvent is dependent on pH, temperature and the type of diluent. The solvent should also be able to extract VFAs at considerably higher pH conditions and should not have any or minimal toxic effects on the bacteria that is present in the AD broth. Diluents are normally used with extractants to minimalise the toxic effects of the extractant. Diluents can also provide better solvation power depending on the polarity of the diluent. Two extractants, TOA and TBP, have been used extensively in literature and were investigated. Three diluents, two nonpolar diluents (canola oil and lamp oil) and one polar diluent (oleyl alcohol) have been chosen from literature. Canola oil was selected over sunflower oil as there have been studies for the use of sunflower oil as diluents. Canola oil has similar properties and should have similar effects seen in the study by Keshav, Chand and Wasewar (2009).

AD can be used as a source to harvest VFAs. The problem with extracting VFAs from this system is that the extraction method used is often a downstream processing method. There are few reports that have used an *in situ* extraction process to extract VFAs from this system. The proposed *in situ* process scheme is likely to feature more than one extraction method. The extraction methods were investigated initially in synthetic solution before actual AD effluent to verify the possibility of the extraction process. The use of solvents (extractant and diluent) for the extraction of VFAs should be investigated for their biocompatibility as well as their ability to extract VFAs at high pH ranges which are usually seen within the AD process.

Chapter 3: Project Scope

3.1 Aim and Objectives

The aim of this research project was to develop an *in situ* extraction for the continuous recovery of VFAs in an anaerobic digester that co-produces VFAs and biogas.

The objectives are outlined as follows:

1) Identify the separation processes that can be used for an *in situ* VFA extraction process scheme
2) Determine the extraction parameters that would affect AD
3) Determine a suitable recovery method (if any) for the VFAs
4) Design and construct a unit capable of continuously extracting VFAs from an AD reactor and test the functionality of the unit in terms of VFA yield and possible impacts on the AD bioprocess

3.2 Research Questions

The main research question that this study addresses was: can an *in situ* extraction process be applied to an AD system that co-produces VFAs and biogas for the continuous recovery of VFAs? This question was coupled with sub-questions to provide insight in order to answer the main question. These sub-questions are:

1) What are the possible extraction methods for the recovery VFAs in an AD system?
2) Can these methods be applied in an *in situ* manner in the AD system for VFA recovery?
3) Would the preliminary experiments justify models predictions under a set range of conditions?
4) How would the VFAs be recovered and what would be the recovery of VFAs be?
5) Would the implementation of such a process increase the productivity of both VFAs and biogas?

6) Would the process generally be energy-efficient and capable of extracting VFAs continuously without obstructing the digestion process?

3.3 Project Hypothesis

The hypothesis for the research project is outlined below:

1) LLE is an effective method for the *in situ* extraction of VFAs in an AD system that co-produces VFAs and biogas.

2) The solvent used in the LLE will be biocompatible with the microorganisms in the active digester.

3.4 Limitations

The different types of bacteria that are present in the inoculum have different living conditions. It is important for the extraction method to work within these conditions to have minimal negative effects on the system. Conditions or process parameters were found from literature where the digestion process runs optimally, from these process parameters, temperature and pH are of main concern as these parameters would have an effect on the extraction method. Optimal temperature conditions were found to be at 37°C and optimal pH conditions of 7.0 – 8.5. It should be noted that low methane production would be observed at pHs less than 6.2.

VFAs have an average pKa value of 4.7, below this value, the undissociated form of the VFAs are dominant. The undissociated form is more volatile than the dissociated form, this implies that VFAs are easier to extract in their undissociated form. Therefore, extraction processes that are reliant on the undissociated form can be severely affected by higher pHs. If this is the case, an external configuration of the extraction method may be required. The same is applied for temperature. Temperature increases the solubility of the VFAs and may increase or decrease if the temperature is changed. Preliminary experiments should be investigated to see the effect of pH. On the other hand, the temperature was kept at 37°C to prevent further complexity of the extraction process.

Chapter 4: Materials and Methods

In preparation of the methods below: acetic acid (98% purity), propionic acid (99.5% purity), butyric acid (98% purity), valeric acid (99% purity) were purchased from Sigma Aldrich. Tributyl phosphate (>97% purity), an organophosphate compound, and trioctylamine, a tertiary amine (>98% purity) were used as extractants (supplied by Sigma Aldrich). Oleyl alcohol (technical grade, 85% purity) was procured from Sigma Aldrich. 100% Canola seed oil was purchased from a local supermarket and lamp oil (C_{14}-C_{20} hydrocarbons) was supplied by Sasol.

4.1 Gas Stripping Modelling

Aspen Plus v8.8 was used for the modelling of a simple gas stripping process. The Aspen model was done by a postdoctoral researcher, Dr. Abdul Muhaymin Petersen. The goal of the study was to design an *in situ* process, this implied that there would be no stages in the gas stripper as the stripping process would most likely occur inside the bioreactor. Therefore, a simple flash drum was used as the gas stripper which can be seen in Figure 6. The conditions of the flash were set at atmospheric pressure and zero duty. The feed stream (W3) contained a model solution of VFAs in an aqueous solution with a typical concentration seen in the fermented broth, 400.2 tons/hour water and 15.9 tons/hour acetic acid. The second stream (GAS) to the stripper is the gas stream which is used as the stripping agent. The temperature of the feed stream was set to 37°C and the gas stream was set to 25°C. The gas stream was set to 25°C as the gas would come from an external source that would not be preheated to the operating temperature of the digester. The separating agent would be the fermented gas that is found in AD. These gases are methane and carbon dioxide. Two gases were used as the stripping agent for comparison purposes, namely: pure carbon dioxide and a mixture of 50 mol% carbon dioxide and 50 mol% methane. The NRTL-HOC method was used as this method takes into account non-condensable gases. A sensitivity analysis was completed using the model analysis tools (sensitivity tool). The amount of gas fed was varied with the target variable set to the flowrate of VFAs in the outlet gas stream.

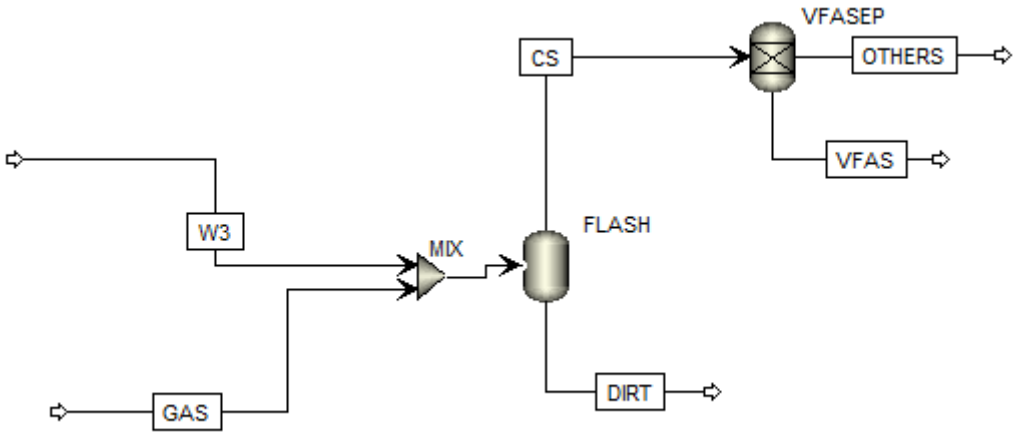

Figure 6: Aspen flow diagram of the model for gas stripping as an extraction method for VFAs from an AD digester.

4.2 Gas Equilibrium Studies

Gas equilibrium experiments were performed as an initial extraction method. These experiments were done using two potential stripping gases, namely, carbon dioxide and a mixture containing 50 mol% methane and 50 mol% carbon dioxide gas (to simulate biogas produced from the anaerobic digester). The gases were supplied by an AFROX local supplier. The experiments were conducted in 100 mL Schott bottles. The bottles were initially sparged either with 1 L of carbon dioxide or the biogas mixture (measured using a measuring cylinder in a water bath) and clamped immediately afterwards as shown in Figure 7. The setup was placed in a fume hood to disperse the flammable gas once it has passed through the inverted measuring cylinder. The rate at which gas was used was well below the flammability limits of the mixture. The bottles were then unclamped and filled with 20 mL of 14.56 ± 0.21 g/L of synthetic VFA solution to ensure the pressure of the bottle was at atmospheric pressure. The bottles were clamped immediately after and placed overnight (24 hours) in a low-temperature orbital shaker incubator (model LM-575D) at 40°C and 150 rpm. The synthetic VFA solution consisted of 50% acetic acid, 30% propionic acid and 20% valeric acid. The gas equilibrium experiments using carbon dioxide gas were done in duplicate. The experiments with the mixture of carbon dioxide and methane were not repeated as this mixture of gas was used for the continuous gas stripping experiment which is described in the next section.

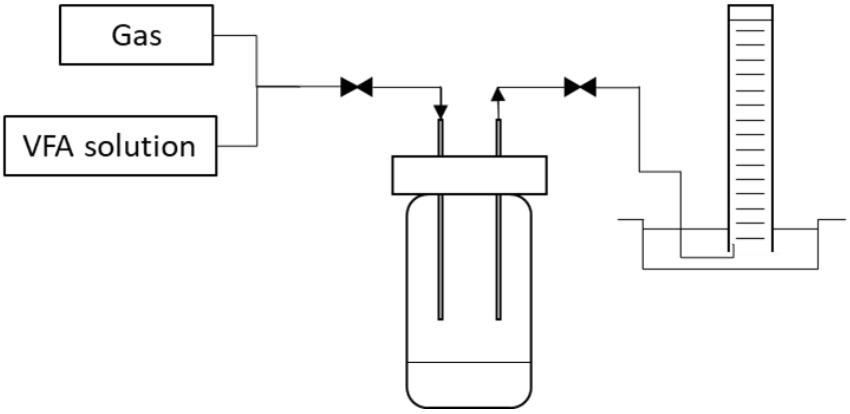

Figure 7: Experimental procedure for the preparation of the gas equilibrium experiments using a 100 mL Schott bottle and an inverted measuring cylinder to measure the gas flow. 1 L of gas mixture was passed into the bottle first before the VFA solution. The bottle was sealed immediately with crocodile clips after the 20 mL of VFA solution was added.

The percentage of VFAs extracted by gas stripping was calculated by the following equation:

$$\%\, Total\ VFAs\ extracted = \frac{Initial\ VFAs - VFAs\ after\ stripping}{Initial\ VFAs} \times 100 \qquad (13)$$

4.3 Continuous Gas Stripping Experiment

The continuous gas stripping was used to simulate a scenario where the gas would be continuously flowing through the AD digester. This was done using a 20 mL of 14.65 g/L synthetic VFA solution at a pH of 3.3 in a 250 mL Schott bottle. The synthetic VFA mixture contained 64.93% acetic acid, 14.60% propionic acid, 13.52% butyric acid and 6.95% valeric acid. Butyric acid was added to see if this would influence the result. A mixture of equimolar carbon dioxide and methane gas was used as the stripping agent. The gas was first bubbled through distilled water to saturate the carbon dioxide in the gas mixture with water. This was done to prevent the loss of the volume of the VFA solution. No change in volume of the VFA solution was observed. The gas mixture was then continuously passed through the solution using a metal sparger that was placed in contact with the VFA solution for 134 minutes at 1.2 vvm (Appendix C). The percentage of total VFAs extracted was calculated using the above equation. The process is described in Figure 8.

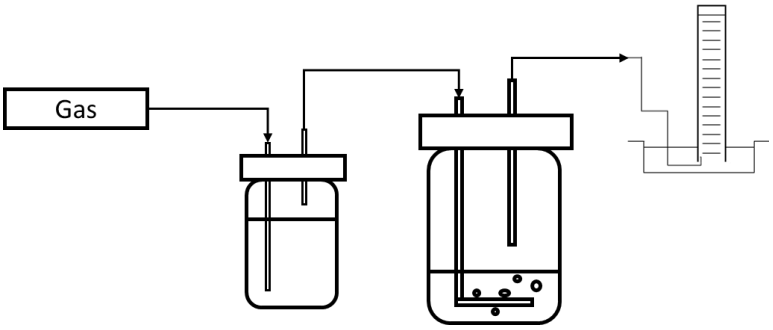

Figure 8: Continuous gas stripping experimental setup using an equimolar carbon dioxide and methane gas mixture. The gas was saturated in a Schott bottle containing 1 L of water before continuously passing through a metal sparger that was in contact with the 20 mL of VFA solution. The gas flow rate of 1.2 vvm was used for 134 minutes (calculation in Appendix C) in 250 mL Schott bottle.

4.4 Laboratory-scale LLE Experiments

Initial LLE experiments were completed for both synthetic and AD effluent solutions using two extractants, TBP and TOA, and three diluents (canola oil, lamp oil and oleyl alcohol). To investigate the effects of pH and extractant concentration for LLE, an overall central composite design (CCD), executed on Statistica™ v13.3, was used and applied to each possible scenario of solvent, i.e. TBP and TOA in the three different diluents. The CCD included two factors (pH and extractant concentration) and three levels with centre point triplicates. An input range of 3.8 – 6.2 (midpoint at 5.0) and 10 – 30 vol% (midpoint at 20 vol%) was used for pH and extractant concentration. An output of eleven experiments per scenario (twelve scenarios) was calculated using this software, which can be seen in Table 8 and Table 9. The pH range was chosen to see the implications of pH on the extraction process and the concentration of the extractant was chosen from typical extractant concentrations used within literature.

Table 8: CCD output using two factors and three levels, including two repeats at the centre point (C).

Sample	pH	Extractant in solvent (vol%)
1	3.8	10
2	3.8	30
3	6.2	10
4	6.2	30
5	3.3	20
6	6.7	20
7	5	3.8
8	5	34
9 (C)	5	20
10 (C)	5	20
11 (C)	5	20

Table 9: Scenarios for different extractants and diluents.

Scenario	Extractant	Diluent	Type of aqueous solution
1	TOA	Canola oil	Synthetic
2	TOA	Oleyl alcohol	Synthetic
3	TOA	Lamp oil	Synthetic
4	TBP	Canola oil	Synthetic
5	TBP	Oleyl alcohol	Synthetic
6	TBP	Lamp oil	Synthetic
7	TOA	Canola oil	AD effluent
8	TOA	Oleyl alcohol	AD effluent
9	TOA	Lamp oil	AD effluent
10	TBP	Canola oil	AD effluent
11	TBP	Oleyl alcohol	AD effluent
12	TBP	Lamp oil	AD effluent

LLE experiments were conducted in batch using 15 mL Falcon tubes, an equal volume of solvent (organic phase) was added to the aqueous phase and vortexed for 30 seconds. The samples were kept in an orbital shaker incubator (model LM-575D) at 37°C and 150 rpm, and left for 24 hours to ensure equilibrium was reached. Optimum digester temperature of 37°C as opposed to the 40°C used in the gas stripping experiments. The samples were centrifuged at 8000 rpm for 10 minutes in a Hermle Z366 centrifuge and allowed to settle for 2 hours before determining the VFA concentration in the aqueous phase using HPLC. This was done to ensure complete separation of the aqueous phase and the organic phase. The synthetic solution consisted of approximately 14.4 ± 0.3 g/L of VFAs (65% acetic acid, 14.5%

propionic acid, 13.5% butyric acid and 7% valeric acid) in distilled water. The AD effluent was obtained from a 50 L digester containing 50 w/w% cow manure, 20 w/w% lignocellulosic biomass (LCB) and 30 w/w% waste apples Kell (2019). The digester was buffered with 1 w/v% of calcium carbonate. The solvent comprised of extractant and diluent. The extractant was mixed in the diluent and vortexed for 2 minutes to ensure homogeneity. An additional set of LLE experiments were done at pHs 3.8 and 6.2 using 20% extractant concentration to verify the trend of pH. The pH of the samples was adjusted accordingly using 7 M NaOH or 7 M H_2SO_4. VFA concentrations of the aqueous phase were measured before and after equilibrium was reached. The VFA concentration of the solvent phase was determined by mass balance. The extent to which the VFAs are extracted is measured by the degree of extraction which is measured by the distribution coefficient k_d. The equations are given below.

$$k_d = \frac{VFAs\ in\ organic\ phase\ (\frac{g}{L})}{VFAs\ in\ aqueous\ phase\ (\frac{g}{L})} \tag{14}$$

$$Degree\ of\ extraction\ (\%\ E) = \frac{k_d}{k_d+1} \times 100 \tag{15}$$

4.5 Biochemical Methane Potential Tests (BMPs)

An important factor of using LLE as an *in situ* method is to examine the biocompatibility of the solvent on the microbial consortia. To test the biocompatibility of the solvents, a series of BMPs were conducted with the solvent present in the broth. The protocol by Angelidaki et al. (2009) was followed for the BMPs.

The BMPs were conducted in triplicate using 100 mL serum bottles. A total working volume of 70 mL was used, which took into account 30 mL of headspace for gas production and 10 mL solvent. Therefore, 60 mL was used for the total mass of the substrates and inoculum. The mass of the inoculum and substrates were calculated using the equations below. These calculations are defined by the AMPTS II method (Bioprocess Control Sweden AB, 2016) using an inoculum to substrate ratio (ISR) of 2:1. Equation 16 took into account two substrates, namely, cow manure and apple pomace, which was supplied Welgevallen Experimental Farm and Elgin Fruit Juices.

$$\frac{m_I VS_I}{m_{ap} VS_{ap} + m_{cm} VS_{cm}} = 2 \quad (16)$$

$$m_I + m_{ap} + m_{cm} = 60 \quad (17)$$

$$\frac{m_{cm}}{m_{ap}} = \frac{3}{7} \quad (18)$$

where, m_I, m_{cm}, m_{ap}, represents the mass of inoculum, cow manure and apple pomace, respectively. VS_I, VS_{cm} and VS_{ap} represent the volatile solids fraction of the inoculum, cow manure and apple pomace, respectively.

Due to the high solids percentage present in the apple pomace and cow manure, at 16% and 21% respectively, the individual substrates were reduced to 6% solids using distilled water and homogenised using a 600 W NutriBullet blender for 10 seconds. A master mix of 70% of apple pomace and 30% cow manure was mixed and blended further to provide homogeneity. The pH of the master mix was measured and adjusted to a pH between 7.0 and 8.0 using 7 M KOH. The substrate master mix was added to the serum bottles, followed by 10 mL of solvent and immediately after with the inoculum to reduce oxygen exposure. The serum bottles were sealed with a butyl stopper and crimped with an aluminium crimp. The bottles were then sparged for 5 minutes using nitrogen gas to established anaerobic conditions. The bottles were then placed in a shaker incubator at 37°C for 28 days. No buffer solution was added to the BMPs. No buffer was added to see the effects of the solvent on a high C/N ratio substrate.

Pre-digestion and post-digestion samples were taken for VFA analysis. The samples were centrifuged at 8000 rpm for 10 minutes in Hermle Z366 centrifuge and the supernatant of the aqueous phase was used for the VFA analysis. The solvent was separated using a pipette and back-extracted using an equal volume of 1 M NaOH.

4.6 17 L Scale-up

4.6.1 Modification for LLE

A 17 L bioreactor was modified for *in situ* LLE by inserting a 40 mm diameter Perspex tubing into the bioreactor (height of 280 mm). The Perspex tube was attached to the top of the digester lid and positioned such that the tubing was in contact with the AD broth. The solvent was added into the tube. Due to the immiscibility of the solvent, the solvent remained above the AD broth, provided that the mixing did not create a vortex in which the solvent would be pulled outside the tube. The modified bioreactor can be seen in Figure 9. The solvent was regenerated using a pH-swing back-extraction unit that uses 400 mL of 1 M NaOH solution at a volume ratio of 1:1 with the solvent. The solvent was pumped using a peristaltic pump with a doubleheader at a rate of 4 mL/min. The pump calibration curve is given in Appendix A.

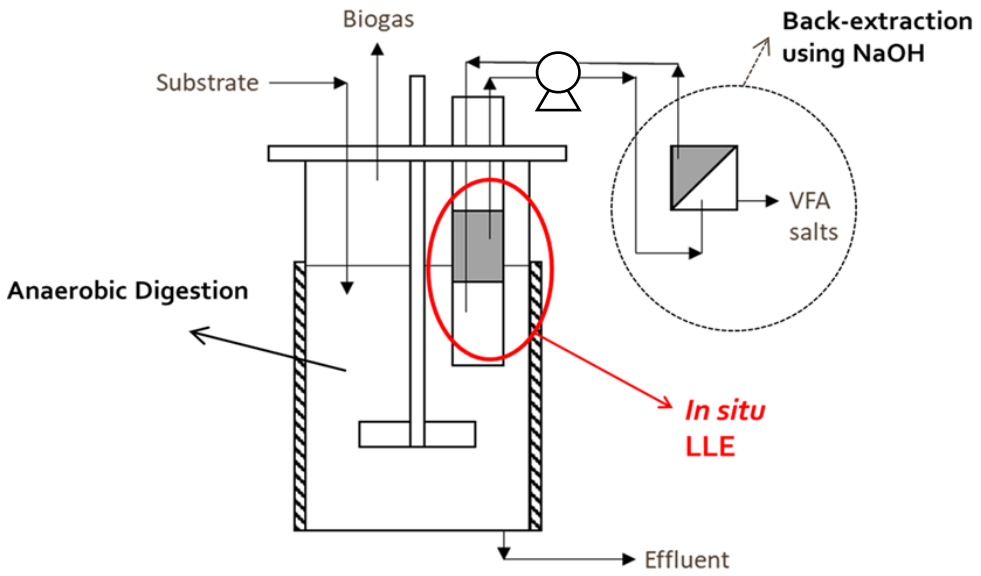

Figure 9: Modified 17 L bioreactor that incorporates an *in situ* LLE for VFAs using a pH-swing back-extraction unit with 1 M NaOH for solvent regeneration.

4.6.2 Substrate

A 70% working volume of the 17 L bioreactor was used for the scale-up experiment to allow for gas production and to ensure the mixer was not overloaded. For the initial 17 L experiment, an 80:20 ratio was used for cow manure and apple pomace. Additional calcium carbonate buffer of 1% [w/v] (i.e. 120

g calcium carbonate added to 12 L) was added to the system to ensure the stability of the system in terms of pH. For this experiment, an increased ISR of 2.5 was used to compensate for the high solids loading of 10.1%.

Therefore, 4040 g of cow manure was mixed with 1010 g of apple pomace in a 10 L bucket. 120 g of calcium carbonate was added to the substrate. 6950 g of degassed inoculum was obtained from a 30 L digester from Stellenbosch University and added initially into the bioreactor. The mixture of cow manure, apple pomace and buffer was then added into the reactor, the reactor was then closed and sealed. For the LLE, 70 mL of a solvent made up of 20 vol% TBP and 80 vol% lamp oil was added into the clear Perspex tubing. The back-extraction unit comprised of 400 mL of solvent and 400 mL of 1 M NaOH in a 1 L Scott bottle. A slow flowrate of 4 mL/min with a doubleheader peristaltic pump was used to allow for mass transfer of the VFAs into the organic phase. The LLE was only switched on day 5 of the digestion period. Table 10 below shows the detailed summary of the 17 L run.

Table 10: Summary of materials used in the initial 17 L digester run.

Bioreactor working volume	12 L
ISR	2.5
Cow manure to apple pomace	4:1
Mass of inoculum	6950 g
Mass of cow manure	4040 g
Mass of apple pomace	1010 g
Total solids	10.1% (1214.3 g)
Volatile solids	7.8% (937.1 g)
Back-extraction unit	1 L Schott bottle
Solvent in bioreactor	70 mL of 20 vol% TBP in lamp oil
Solvent in back-extraction unit	400 mL of 20 vol% TBP in lamp oil
Aqueous phase in back-extraction unit	400 mL of 1 M NaOH
Temperature	37°C
Initial pH	7.37
Buffer	120 g of calcium carbonate

For the second run, a 60:40 apple pomace to cow manure ratio was used with an inoculum to substrate ratio (ISR) of 2:1. This implied that 8385 g of inoculum, 2169 g of apple pomace and 1446 g of cow manure was added to the reactor. The substrate was not diluted in the run, which implied the solids loading was 10.3% (total volatile solids 611 g). The pH of the substrate was increased to 7.3 using KOH. 70 mL of 20 vol% TOA in oleyl alcohol was used in the tube of the LLE tube with an additional 400 mL of solvent in a 1 L Schott bottle, which was used for the back-extraction. The Schott bottled also

contained 400 mL of 1 M NaOH (1:1 ratio of solvent to NaOH). The solvent was pumped using a peristaltic pump with a flowrate of 52 mL/min.

4.7 Analytical Methods

4.7.1 VFA analysis

All VFA analyses were measured using high performance liquid chromatography (HPLC). The total VFA concentrations were obtained by the sum of the individual VFAs present in the sample. The parameters and specification for HPLC are shown in Table 11 below.

Table 11: HPLC specifications and parameters for VFA analysis

Column description:	Biorad HPX-87H column, 250 x 7.8 mm with guard cartridge
Column temperature:	65°C
Flow rate:	0.6 mL/min
Instrument 1:	Thermo Separations Product (TSP) HPLC UV detector
Instrument 2:	Dionex UltiMate 3000 HPLC UV detector
Mobile phase:	0.005 M sulphuric acid
UV detector wavelength:	210 nm

4.7.2 Moisture content and volatile solids

The moisture content and volatile solids (VS) of the substrate and inoculum were determined using the standardised method ("2540 SOLIDS", 2017). The wet samples were dried in a convection oven at 105°C until a constant weight was attained. The mass of the samples before and after drying was recorded and used to calculate the moisture content in the sample.

$$\%\ Moisture = \frac{mass\ of\ wet\ sample - mass\ of\ dried\ sample}{mass\ of\ wet\ sample} \times 100 \qquad (19)$$

The dried sample was then used to determine the VS and ash content. The dried sample was burnt in a muffle furnace at 550°C for approximately 2 – 3 hours until a constant mass was achieved. The ash was weighed and used to calculate the VS content using the formula below:

$$\%\, Volatile\ Solids = \frac{mass\ of\ ash}{mass\ of\ wet\ sample} \times 100 \quad (20)$$

The moisture and VS content were done in triplicates for each substrate and inoculum. The average of the triplicates was used for further calculations.

4.7.3 Gas composition and volume

The volume of the gas produced was measured using a needle attached to a syringe. The needle was injected through the butyl rubber stopper, the gas volume was recorded (in mL) once plunger of the syringe halted. The volume of the gas was recorded every 2 – 3 days, depending on the amount of gas produced in the bottles. The total gas production was calculated by the sum of all the recordings. Gas composition was measured using a CompactGC 4.0 Gas Chromatograph analyser (from Global Analyser Solutions). The specifications of the gas chromatograph are given below in Table 12.

Table 12: Specifications for CompactGC 4.0.

Carrier gas flow rate:	5.0 mL/min
Carrier gases:	Argon and helium
Detector 1 oven temperature:	50°C
Detector 2 oven temperature:	65°C
Filament temperature:	210°C
Injector temperature:	60°C
Reference gas flow rate:	1.0 mL/min
Thermal conductivity detector temperature:	110°C

The GC consisted of one flame ionisation detector for channel 1 and two Thermal Conductivity Detectors (TCD) for channel 2 and 3. Channel 2 was used to analyse the composition of carbon dioxide. Channel 3 was used to identify the composition of hydrogen, methane, oxygen and nitrogen. Channel 1 was not used for the gas sample analysis. An error occurred when the sum of the gas compositions

did not add up to 100%, this was assumed to be due to a variation in the sample injection pressure or to other compounds present in the sample.

The maintenance and calibration of the GC were completed every six months. Maintenance was done by increasing the temperature 50°C higher than the normal operating temperature and left overnight, by doing so, any residual compounds are purged out of the column. The GC was calibrated by injecting known compositions of gas samples at normal operating temperature settings and the calibration curves were adjusted accordingly. The calibration curves for carbon dioxide, hydrogen, methane, nitrogen and oxygen were used and can be seen in Appendix B.

Chapter 5: Results and Discussion

The complexity of AD systems means that most separation methods for VFAs would be excluded as the operability of such methods interfere with either the reactions or the microorganisms itself. Moreover, these methods are either expensive, inefficient, energy-intensive or cause downtime due to high maintenance. The methodology chosen must balance VFA recovery with operability in complex AD reactors. For this thesis, two methods for *in situ* VFA recovery were investigated: gas stripping and LLE.

5.1 Gas Stripping

Gas stripping has several advantages such as: easy implementation, low maintenance and the fermentation gas itself could be used as the stripping agent. The use of gas stripping has been implemented in ABE fermentation for the extraction of ethanol. However, to the author's knowledge, there were no studies that have indicated the use of gas stripping for VFA extraction using AD gas. The use of AD gas as a stripping agent could potentially be beneficial to the extraction process, as AD gas can serve as two functions. Firstly, the biogas can be used as a source of energy. Secondly, the biogas can be used as the stripping agent for the extraction of VFAs, which could bring additional income to AD. Therefore, carbon dioxide and biogas were chosen as potential gas agents. The feasibility of gas stripping was investigated initially with a steady-state model in Aspen (developed in collaboration with Dr. Abdul Petersen). The viability of the model was determined by gas-liquid equilibrium and a continuous gas stripping experiment.

5.1.1 Modelling

It is important to bear in mind that the AD process is a complicated process to model, which can be time-consuming due to the vast consortia of bacteria present in the process, as well as the complex substrate used as feed. Therefore, a simple steady-state simulation in Aspen was opted for and executed in collaboration with a postdoctoral researcher, Dr. Abdul Petersen. The model was completed by Dr. Petersen, whereas the input data for the gas stripping was completed by the author. This simple simulation was used as a scoping investigation for the use of gas stripping as an option for the extraction of VFAs. A sensitivity analysis was conducted to gain more insight into the amount of

gas that would be required to extract a certain amount of VFAs. In the sensitivity analysis, the mass fraction of gas was varied and the output variable was the amount of VFAs extracted in the gaseous phase of the flash drum. The results for the Aspen simulation are given below in Table 13 and Table 14 for the relevant stripping agents. In Table 13, 100% of the VFAs are extracted when the ratio of CO_2 to VFAs is 230 (by mass). When methane was used as the stripping agent, in combination with carbon dioxide (equimolar), the ratio of gas to VFAs significantly reduced to 150 for 100% recovery. This could be attributed to the difference of the solubility of the VFAs and the affinity of the VFAs towards the different gases. In the case of an AD process, the gases produced are carbon dioxide and methane with small amounts of hydrogen. Therefore, the simulation using equimolar amounts of carbon dioxide and methane would be a more accurate representation of the AD process as compared to the simulation with just carbon dioxide. Based on the results from Aspen, the potential of gas stripping looked promising and was selected for further experimental work.

Table 13: The model examined the required amount of carbon dioxide (in terms of mass ratio) needed to strip x% of VFAs from a synthetic aqueous solution of VFAs using the sensitivity analysis tool in Aspen.

Mass ratio of CO_2 to VFAs [CO_2:VFAs]	**VFAs extracted [%]**
90	19.7
110	25.5
130	32.6
150	41.3
170	52.4
190	66.5
210	84.9
230	100

Table 14: The model examined the required amount of equimolar carbon dioxide and methane (in terms of mass ratio) needed to strip x% of VFAs from a synthetic aqueous solution of VFAs using the sensitivity analysis tool in Aspen.

Mass fraction of equimolar CO_2 and CH_4 to VFAs [CO_2 + CH_4:VFAs]	**VFAs extracted [%]**
10	3.6
30	8.6
50	14.6
70	22.4
90	33.4
110	49.3
130	73.8
150	100

5.1.2 Experimental Gas Equilibrium Results

The gas equilibrium results are seen in Table 15 and Table 16. Gas equilibrium experiments were used to verify the model predictions for gas stripping as a suitable method. From the tables, it was clearly evident that neither gas stripping (CO_2 or CH_4) agent was able to strip a reasonable amount of VFAs. The results show less than 2.5% recovery of VFAs from 20 mL of 14.56 ± 0.21 g/L of synthetic VFA solution at 40°c. This implies that it would require a tremendous amount of gas in order to strip even a small amount of VFAs. The negative values seen in the tables can be attributed to the accuracy of the HPLC. The low recovery of VFAs can be explained by the high Henry constants of the VFAs. As mentioned in section 2.3.6 Gas stripping, the Henry constants of VFAs are at least 220 times larger than the volatile solvents which are normally extracted by gas stripping (Li et al., 2015). Even at low pHs, where VFAs are dominate in the undissociated form, low extraction percentages were observed.

Table 15: Percentage of total VFAs extracted using carbon dioxide (gas to VFAs mass ratio of 0.17) as a stripping agent at 40°C from a Schott bottle containing 20 mL of 14.56 ± 0.21 g/L of synthetic VFA solution.

Sample	pH	% Extracted
1	2.0	0.18 ± 0.95
2	3.5	-0.12 ± 0.70
3	4.5	0.30 ± 0.13
4	5.0	-0.03 ± 0.55
5	6.0	0.91 ± 1.42
6	7.0	-0.08 ± 0.29

The results obtained from the experimental work did not correspond to the Aspen model. This could be due to the feasibility of the thermodynamic model used in the model. An alternative explanation could be that the model did not take into account the saturation of the carbon dioxide, as compared to the experimental set-up, where the gas was purged through water before it entered the VFA solution. If the model did not take this into account, the gas would be saturated with the VFA solution in the model. Therefore, increasing the amount of VFAs stripped. Although the mass ratios were relatively small compared to the Aspen Model, 0.17 and 0.32 for the pure carbon dioxide gas and the equimolar gas, respectively, the experimental results suggested that significantly more gas would be required compared to the model. To substantiate this, a continuous gas stripping experiment was conducted using the equimolar gas mixture, whereby the gas to VFA mass ratio was increased to 168.25. The continuous gas stripping only resulted in 4.48% of VFAs (0.013 g of VFAs) extracted from 20 mL of a 14.56 ± 0.21 g/L synthetic VFA solution. The volume of gas used was calculated to be 40.20 L of equimolar gas, as compared to the 80 mL of gas in the equilibrium experiments. The calculations for the continuous gas stripping which was based on the results obtained from the model can be found in Appendix C. This result showed that the more gas used, the more VFAs are stripped out. Even though an improved extraction was seen by increasing the amount of gas, the continuous stripping did not show the same amount of VFAs extracted as opposed to the model. Although AD can be used as waste management, AD is limited by the amount of biogas the system produced. Therefore, based on the experimental work, the effectiveness of implementing gas stripping in an active system is unlikely, as most of the biogas produced would be required to extract a reasonable amount of VFAs for the system to be economically viable. This implies that there would be small amounts of biogas remaining, if any, for energy usage.

Table 16: Percentage of total VFAs extracted using an equimolar mixture of carbon dioxide and methane (gas to VFAs mass ratio of 0.32) at 40°C from a Schott bottle containing 20 mL of 14.56 ± 0.21 g/L of synthetic VFA solution.

Sample	pH	% Extracted
1	2.0	0.18
2	3.5	0.55
3	4.5	0.09
4	5.0	-0.19
5	6.0	-0.76
6	7.0	-0.92

The recovery of the VFAs via gas stripping could be increased by increasing the pressure and temperature. However, increasing pressure would require additional operational and equipment expenses, and an increase in pressure could have negative effects on the microorganism as AD is usually operated at atmospheric pressure. The temperature would be dependent on the type of microorganisms that are used. Increasing the temperature beyond the bounds of the microorganism would result in AD crash, as the organisms die at a temperature above their operating temperature. In all, gas stripping is capable of extracting a very small amount of VFAs, over a long period. However, for the purpose of this investigation, the gas stripping process was found to be inefficient due to the small amount of VFAs extracted. If the VFAs are sold for additional revenue, the additional revenue would not be able to cover the costs involved in implementing the method.

5.2 Laboratory-scale LLE Experiments

After the completion of the gas stripping experimental work, an alternative method based on extraction to a second liquid phase was proposed. LLE was decided upon as several extractants, like TOA and TBP, are capable of extracting of VFAs. From literature, the diluents commonly associated with these extractants were kerosene and oleyl alcohol. The difference between the extractant and the diluents are that extractants have strong interactions with the solute (VFAs in this case), whereas, diluents are used to decrease the solubility of the solvent in the aqueous phase and can provide additional solvation power. The use of canola oil was decided upon based on an article by Wasewar, Shende and Keshav (2011). In the article, sunflower oil was used as a natural diluent which may be considerably cheaper than other diluents such as lamp oil and oleyl alcohol.

The solvents selected for experimentation were TOA and TBP, while oleyl alcohol, lamp oil and canola oil were used as diluents. These were chosen because of their low toxicity on anaerobic bacteria (Playne & Smith, 1983). In these experiments, it was important to test the ability of the solvents to extract VFAs at various pHs (3.0 to 7.0), as this would enable valuable insight into the type of configuration used for the LLE (*in situ* or *ex situ*). The solvents were tested on a synthetic solution of VFAs, followed by LLE experiments with AD effluent. This was done to verify the use of LLE in the AD bioreactor. The substrate used in AD systems often contains an assortment of complex organic matter and chemicals. For example, food waste can contain a variety of compounds due to food processing. These compounds could have an effect on the extraction process. Due to the complexity of the system, and potential interactions between variables, a CCD was designed which took into account two main variables: pH (a variable important for AD operation) and the volume of the extractant (extractant is expensive, so minimising this component renders the system more viable) in the solvent, with the measured response variable being VFAs extracted. Even though the temperature does influence the LLE, it was not selected as a variable as digesters are commonly kept constant at 37°C. Although thermophilic conditions may result in higher amounts of biogas, the microbial consortia in this temperature range (45 - 60°C, usually 50°C) are more sensitive to toxic compounds or changes in the operating conditions (Duran & Speece, 1997). Therefore, the implementation of these solvents in thermophilic digesters may be unlikely. The volume of the extractant was varied from 10 to 30 vol%. Even though these solvents have low toxicity, the extractant percentage was limited to 30% to limit potential toxic effects, and since higher concentrations would likely affect cost significantly. This was done as a precautionary measure, as the inoculum that would be used in the bioreactor may or may not contain the same bacteria used by Playne and Smith (1983). Bacteria can be conditioned or acclimate to certain living conditions, such as high VFA concentrations (Teghammar, 2013). AD bioreactors that use fruit waste could exhibit higher tolerances to VFA inhibition than AD bioreactors that use lower C/N (carbon to nitrogen) ratios. Therefore, the bacteria used by Playne and Smith (1983) may not have the same tolerances as the one used in this thesis.

BMPs were also conducted to investigate whether the use of these solvents inhibits the growth and biogas production of the microbial community within the broth. The type of configuration, *in situ* (where extraction takes place 'on-site') or *ex situ* (extraction process takes place away from the process, usually downstream processing), was based on the laboratory-scale experiments and the biocompatibility test with the BMPs. This section is subdivided into two subheadings, namely: effects of pH and the volume of extractant (used in the solvent) and the analysis of the degree of extraction from the laboratory scale experiments.

5.2.1 Effects of pH and volume of extractant

In this section, the parameters that influence LLE is discussed. The two main variables that were taken into account were the pH and the amount of extractant added in the solvent. The difference in the degree of extraction between the different solvents is discussed in detail in the next section to come, as well as the difference observed between the synthetic VFA solution and the AD effluent.

The response surface curve for TOA in canola oil from a synthetic VFA solution was plotted in Figure 10. The response surface showed the trend of the degree of extraction as pH range and the vol% of the extractant changed. The total volume of the solvent (diluent and extractant) was kept at 5 mL. A higher degree of extraction was observed at pHs below the pKa value when the extractant vol% was greater than 10%. This was because of the stoichiometry of the acid-amine complexes. The more solvent used, the more acid-amine complexes can be formed. As pH increases, the degree of extraction decreases. The figure also showed that higher vol% of extractant used results in a higher degree of extraction except at pHs greater than 5.5, where the colour bands tend to be more vertical. This implies at higher pHs, increasing the extractant vol% does not significantly improve the degree of extraction. In other words, the degree of extraction was relatively insensitive to changes in the extractant concentration. This does correspond with literature which can be seen in Wasewar et al. (2004). Wasewar et al. found that the distribution coefficient increased when pH decreased except at extremely low and high pHs. At these extremes, the distribution coefficient did not change significantly. There were three terms that had an effect of the degree of extraction using TOA in canola oil and a synthetic VFA solution. These terms were pH (linear), extractant volume in the diluent (linear) and the linear interaction between pH and the extractant volume which can be seen in Figure 11. The curve seen in Figure 10 was due to this linear interaction term.

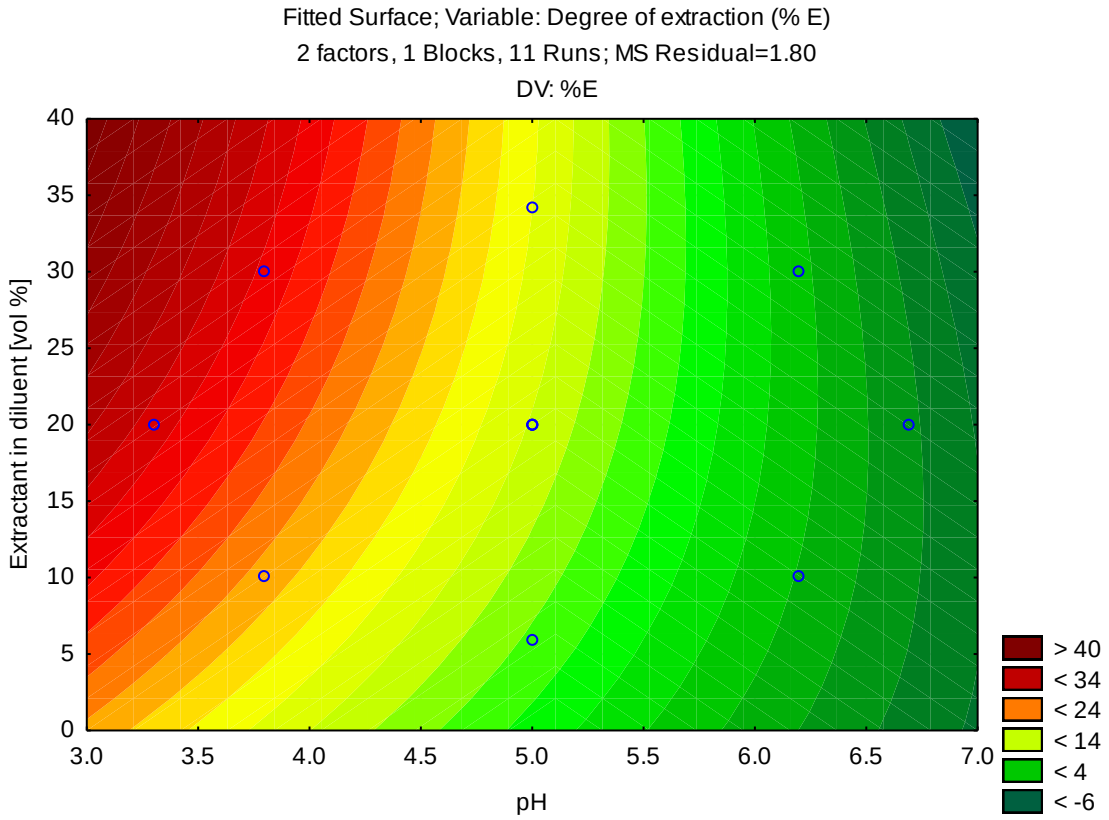

Figure 10: Surface response curve plotted for the degree of extraction (% E) of total VFAs using LLE from a synthetic VFA solution with varying amounts of TOA in canola oil at different pHs and at 37°C. The error is given as MS residual.

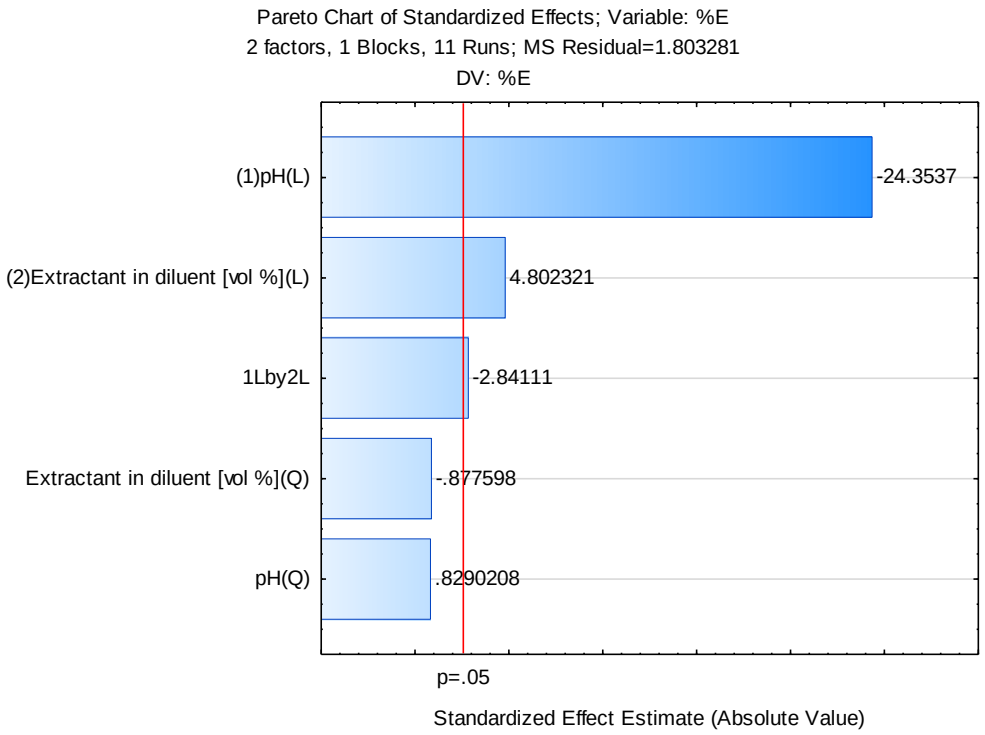

Figure 11: Pareto chart showing the effects that contribute to the degree of extraction (% E) for TOA in canola oil using a synthetic solution.

However, when the AD effluent was used as the VFA source, the extractant concentration did not have a significant effect; the curvature in Figure 12 was less pronounced. As a result, pH was found to be the only significant variable that had an effect for the LLE, which can be seen in the Pareto chart (Figure 13). Similar results were significant effects were due to obtained for all AD effluent samples except for TBP in lamp oil, where the significant effects were resultant of pH (linear), extractant volume (linear) and the linear interaction between those two terms (seen in Figure 14). The same was observed for the synthetic solution (TBP in lamp oil). For visual references, Figure 15 shows the difference between the synthetic solution and the AD effluent. It should be noted that the variables that influence the degree of extraction were the same variable seen in the AD effluent. However, the linear interaction term between pH and extractant volume as well as the linear term of the extractant volume were more pronounced in the synthetic solution. This means that there is some interference between the AD components and the solvent. This implies that low concentrations of extractant can be used for AD systems. This was a favourable result as third phase formations were found when the extractant concentration was increased to greater than 30 vol% (Eda, Kumari, Thella, Satyavathi & Rajarathinam, 2017). The response curves and Pareto charts for all possible scenarios of extractant and diluents can be found in Appendix D.

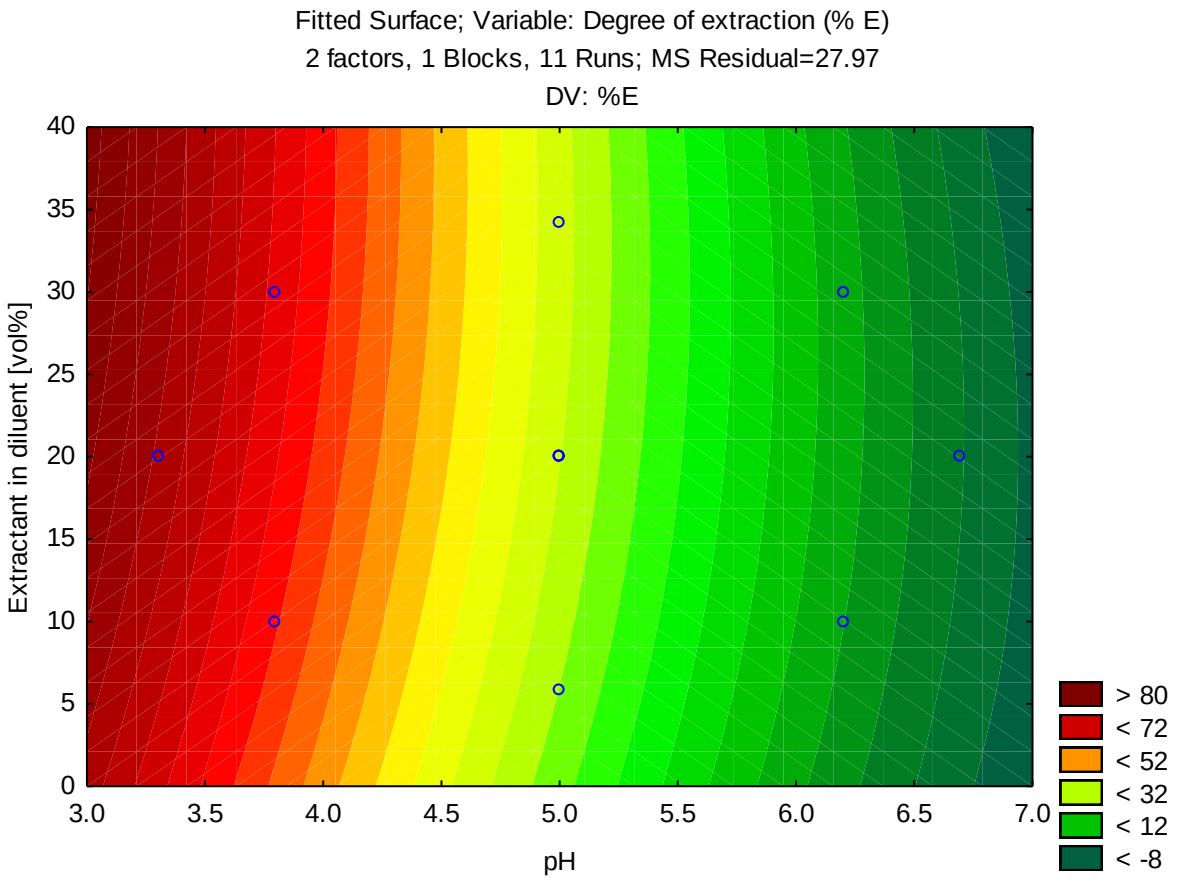

Figure 12: Response curves for degree of extraction (% E) of total VFAs for LLE on AD effluent using TOA in canola oil with varying amounts of extractant at different pHs and at 37°C.

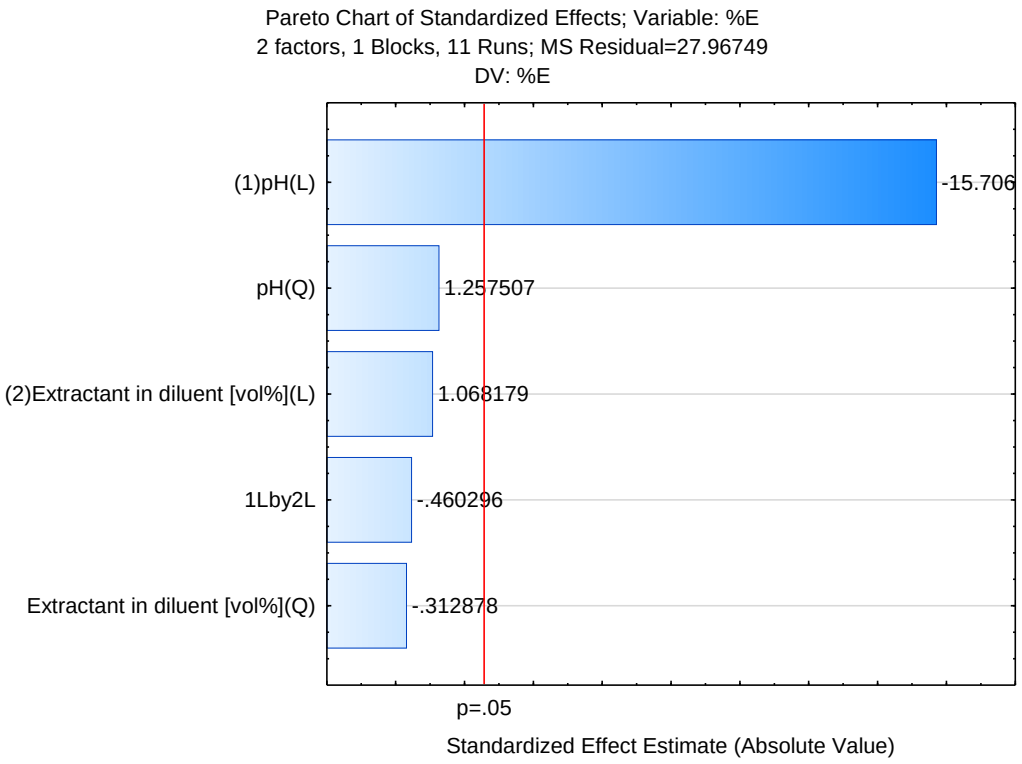

Figure 13: Pareto chart showing the effects that contribute to the degree of extraction (% E) for TOA in canola oil using AD effluent.

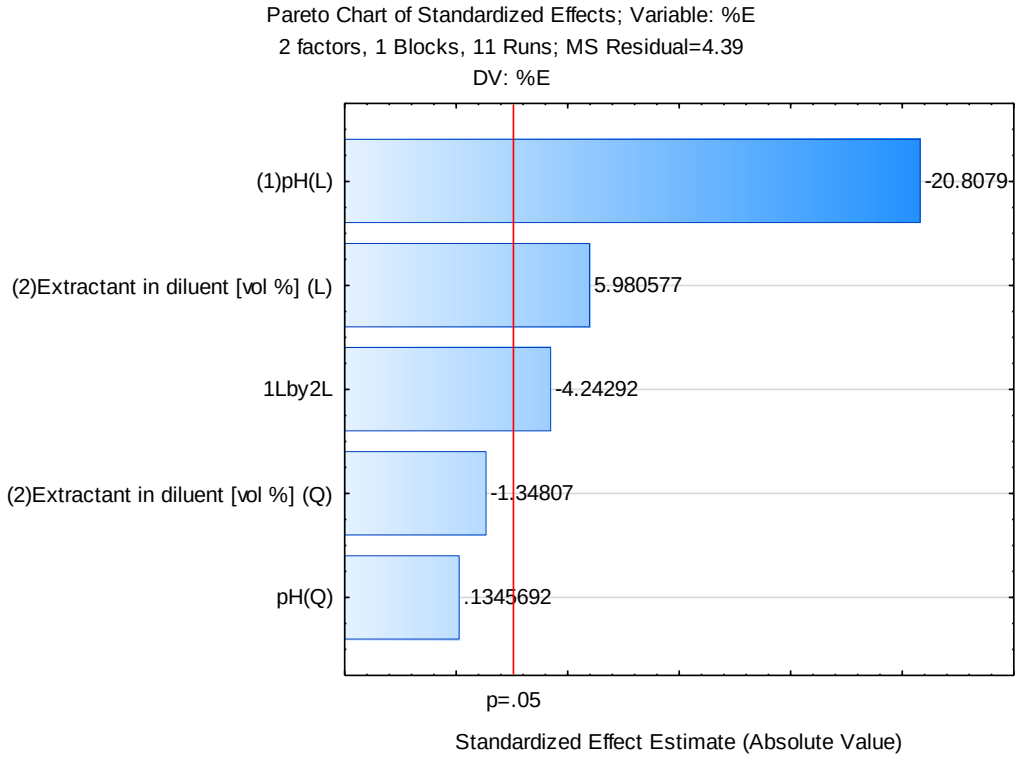

Figure 14: Pareto chart showing the effects that contribute to the degree of extraction (% E) for TBP in lamp oil using AD effluent.

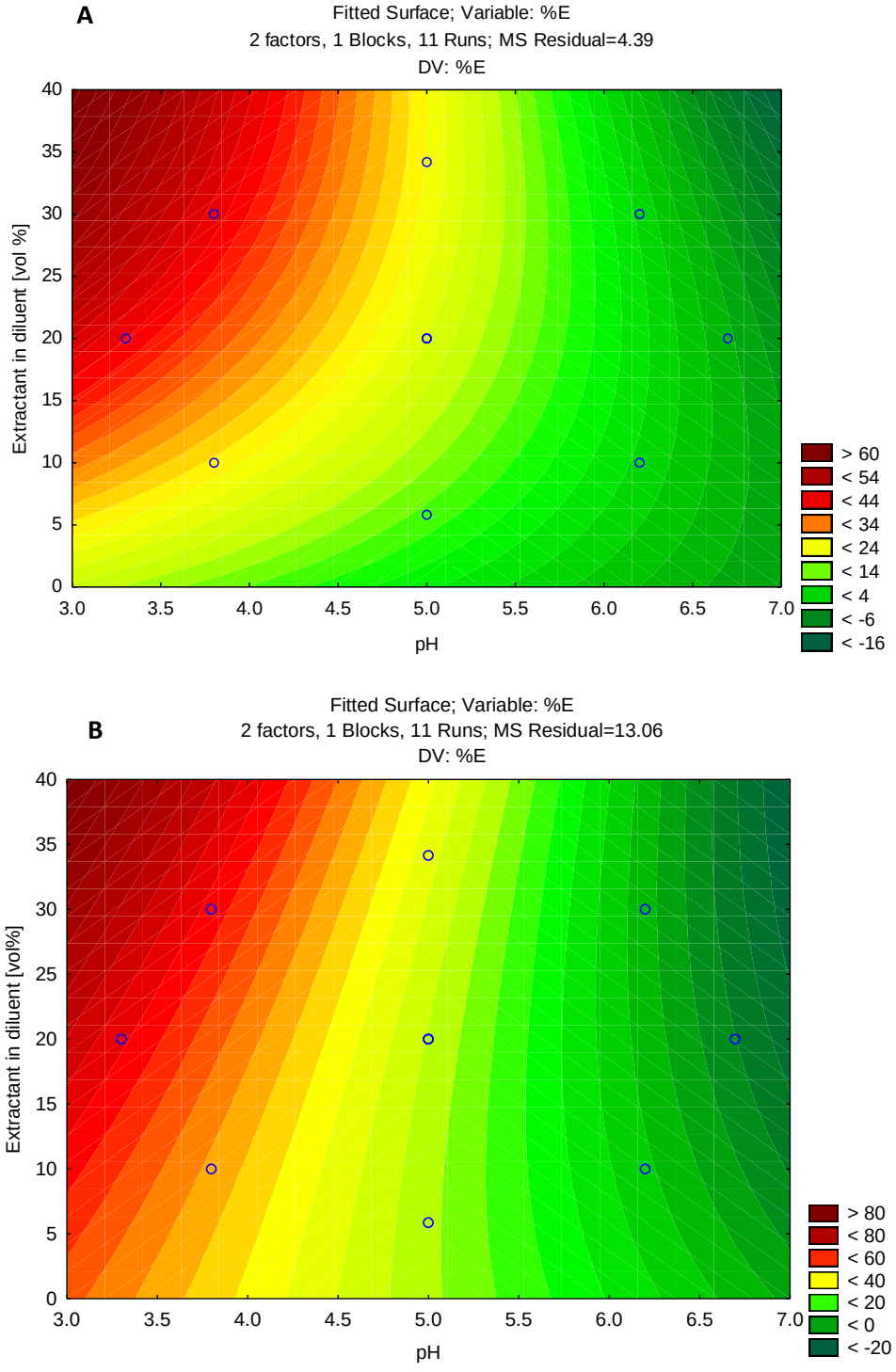

Figure 15: Response curves for degree of extraction (% E) of total VFAs for LLE using TBP in lamp oil with varying amounts of extractant at different pHs and at 37°C for A) a synthetic solution and B) AD effluent.

A summary of the effects using a synthetic VFA solution and AD effluent are given in *Table 17* and *Table 18*. The significance of the effects was determined from ANOVA analysis and can be seen from the Pareto charts in Appendix D. From these tables, it was evident that the main effect in the degree of extraction is pH. This is consistent for solvents that are only able to extract the undissociated form of the VFAs, as pH dictates the dissociation of the acid. When comparing the AD effluent with the synthetic solution, the only effect in the AD effluent is the effect from the pH of the solution, with the exception of TBP in lamp oil. However, pH is still the dominating variable that influences the extraction. In the synthetic solutions, the effect of pH is seen for all the solvents. However, both extractants in canola oil and lamp oil were affected by pH, extractant concentration and a linear interaction between the extractant concentration and pH. This could be due to the presence of ions such as sulphates, phosphates and other compounds in the AD effluent which may affect the extraction efficiency of the solvents. The difference is discussed in more detail in the next section. From this, it can be concluded that the main variable that influences the extraction efficiency is pH when using AD effluent. This means that the amount of extractant used in the solvent can be limited to 20 vol%. This is an ideal situation, as the use of a high vol% of extractant can hinder bacterial growth, which can lead to a digester that is inefficient in biogas production or it can lead to digester failure. In the case of digester acidification caused by VFA accumulation, 20 vol% extractant was preferred over 10 vol% to prevent potential overloading of the solvent with acid.

Table 17: Summary of effects based on the Pareto charts on solvents with AD effluent at 37°C

	pH (linear)	**Extractant concentration (linear)**	**Linear interaction between pH and extractant concentration**
TOA / Canola oil	✓	✗	✗
TOA / Oleyl alcohol	✓	✗	✗
TOA / Lamp oil	✓	✗	✗
TBP / Canola oil	✓	✗	✗
TBP / Oleyl alcohol	✓	✗	✗
TBP / Lamp oil	✓	✗	✓

Table 18: Summary of effects based on the Pareto charts on solvents with a synthetic VFA solution at 37°C

	pH (linear)	Extractant concentration (linear)	Linear interaction between pH and extractant concentration
TOA / Canola oil	✓	✓	✓
TOA / Oleyl alcohol	✓	✗	✗
TOA / Lamp oil	✓	✓	✓
TBP / Canola oil	✓	✓	✓
TBP / Oleyl alcohol	✓	✗	✗
TBP / Lamp oil	✓	✓	✓

5.2.2 Analyses of the degree of extraction

The extraction equilibria of VFAs in an anaerobic digestion broth by 20 vol% extractants in different diluents was studied over a pH range. The highest degree of extraction (81.7 ± 0.95%) was observed at a pH of 3.3 with TOA in oleyl alcohol. TOA in oleyl alcohol (Figure 16B) had significantly the best degree of extraction throughout the pH range. Overall, TOA in lamp oil had the lowest degree of extraction. The difference between the extraction efficiencies of the extractants in the diluents can be explained by the solvation capabilities of the diluent with the extractants (Keshav et al., 2008). The effects of diluents were studied by Tamada and King (1990). It was suggested that the extracting power of nonpolar amines is increased by a polar diluent (such as oleyl alcohol) which provides additional solvation power. This allows higher amounts of polar acid-amine complexes to remain in the organic phase. Whereas, a nonpolar diluent (lamp oil or canola oil) will not affect nonpolar amines (Kertes & King, 1986). Although this may significantly improve the extraction power of the solvent at low pHs, the effect is minimal at high pHs (Figure 16). This implies that at high pHs where AD operates, the solvents would extract smaller amounts of VFAs. This is not necessarily a bad thing, as VFAs are used as substrates for the methanogenic bacteria, excessive VFA extraction could lead to substrate limitation whereas extraction of smaller amounts of VFAs could improve biogas yield by preventing VFA accumulation, while still allowing for significant concentrations of VFAs available for digestion (Teghammar, 2013). The negative values seen in Figure 16 can be attributed to the accuracy of the HPLC. However, an outlier occurred for TBP in lamp oil with AD effluent, with a degree of extraction of -10.38. This was possibly due to the microorganisms still being active in the effluent and is explained in more detail in the coming sections.

An interesting trend can be seen in Figure 16, where all digester effluent had a significantly higher degree of extraction in both extractants and all diluents, as opposed to the synthetic solution. The significant difference between the synthetic solution and the digester effluent could be a result of salt ions present in the broth (Reyhanitash, Zaalberg, Kersten & Schuur, 2016). A study by Reyhanitash et al. (2016) showed that the distribution coefficient can be affected when Cl^-, SO_4^{2-} or HPO_4^{2-} ions were present for 20 wt% TOA in n-octanol. Although the study only used a synthetic solution and not actual AD effluent, the presence of such ions in the synthetic solution influenced the extraction process. Ions and other components found in the substrate, such as proteins, fats and biosurfactants could affect the extraction of the solvent significantly. This could be the case as the AD effluent was obtained from a digester that used food waste as a substrate. An exception was observed for TOA in oleyl alcohol in a synthetic solution, which had similar extraction efficiencies below the pKa value of the VFAs. This exception can be explained by the additional solvation power of oleyl alcohol when combined with TOA.

Upon further investigation, an alternate explanation could be due to the degree of extraction of the individual acids. The solubility of the individual VFAs are all different. This implies that more carbon atoms the VFA has, the higher affinity for the organic phase. Therefore, the individual VFAs would have different distribution coefficients or degree of extractions (the function of distribution coefficient). The difference in the overall VFA extractant efficiency can be mainly attributed to the caproic acid present in the anaerobic digester effluent, which the synthetic solution did not contain. Caproic acid has six carbon atoms and has a higher affinity towards the organic phase. This implies that caproic acid would have a large degree of extraction, as compared to acetic or propionic acid. Thus, the total VFA degree of extraction would be larger when higher carbon-chained VFAs are present. A study by Yang et al. (1991) showed that the distribution coefficient increased as the carbon length of the VFA increased. Although the study only took into account acetic, propionic, lactic and butyric acid, a similar trend can be seen in Figure 17.

This trend can not only be seen from using canola oil and TBP but for all combinations given for the two extractants (TOA and TBP) and the three diluents (canola oil, oleyl alcohol and lamp oil), which are given from Figure 17 to Figure 22. The degree of extraction increases as the carbon chain length increases. The significant negative degrees of extractions, observed only at pHs above the pKa value of the VFAs, are speculated to be due to the microbial community of acidogens still active in the effluent. If the acidogens are still producing VFAs, this could cause a negative net change in VFAs as the concentration of VFAs in the organic phase was determined by mass balance. These values could

be a sign that the microbial community are still active despite being in contact with the solvents and that the extractions are underestimated due to this.

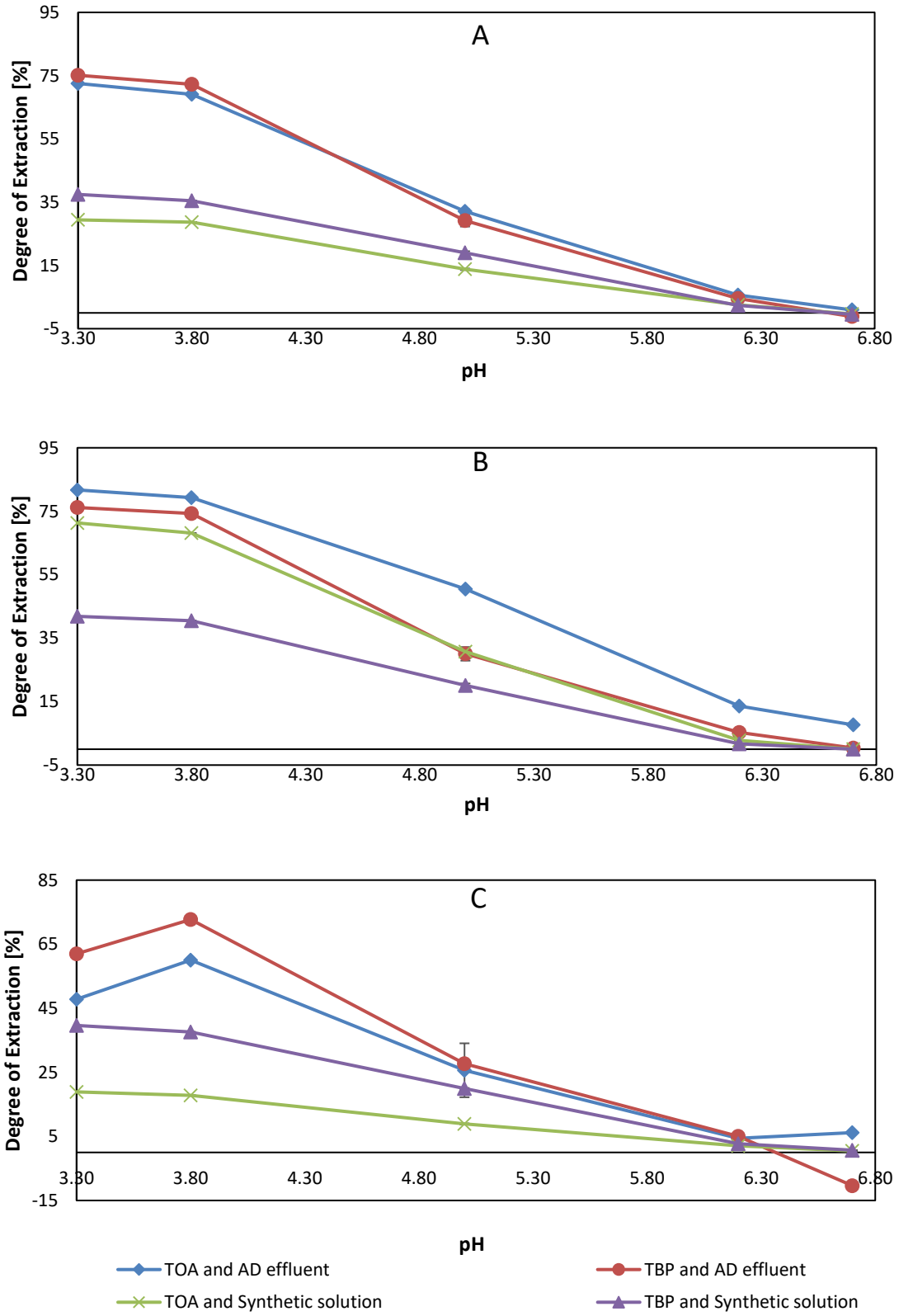

Figure 16: Degree of extraction (% E) of total VFAs from 20 vol% TOA or 20 vol% TBP in canola oil (A), oleyl alcohol (B) and lamp oil (C). LLE extraction at 37°C was performed using AD effluent and a

synthetic solution of VFAs in a range of pHs, error bars are calculated using the error propagation method in Appendix E.

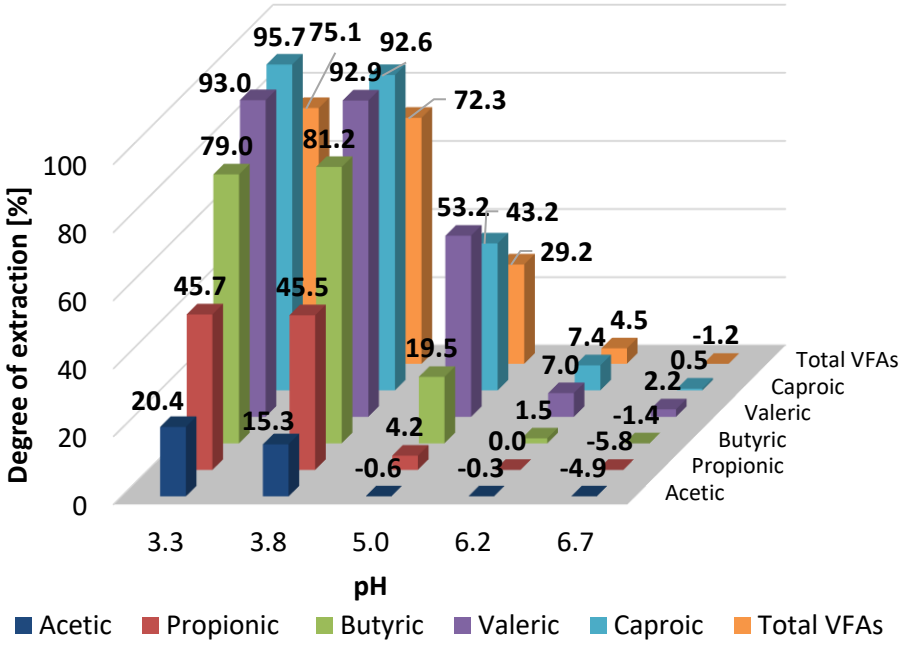

Figure 17: Degree of extraction of individual VFAs from LLE using 20 vol% TBP in canola oil on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

There was also a strong correlation between the degree of extraction of the solvent and the pH of the solution. A high degree of extraction can be seen at pHs below the pKa value of the VFAs and decreases as pH increases. This was due to the extractants' ability to extract VFAs predominately in their undissociated form (Yang et al., 1991). This implies that at high pHs where anaerobic digestion operates optimally, lower extraction of VFAs is possible, although non-zero.

This may or may not be an important factor for an *in situ* extraction method. On the one hand, anaerobic digestion has long digestion periods, often spanning between a few days or weeks, depending on the substrate (Teghammar, 2013). The low extractions of VFAs could increase the productivity of both VFAs and methane by the removal of VFAs. This is especially useful as VFA accumulation often leads to digester failure due to methanogenic and acidogenic inhibition (Hori et al., 2014). On the other hand, insufficient VFA extraction may not be economically viable and would increase the complexity of the anaerobic process, increasing capital costs. However, for *in* situ LLE, the implementation of solvents with lower degrees of extraction could be increased by increasing the

solvent flowrate into the broth. By increasing the flowrate, the mass transfer between the solvents and the VFAs is increased as the area is directly proportional to the mass transfer. This is provided that the flowrate to the LLE is in direct contact with the broth.

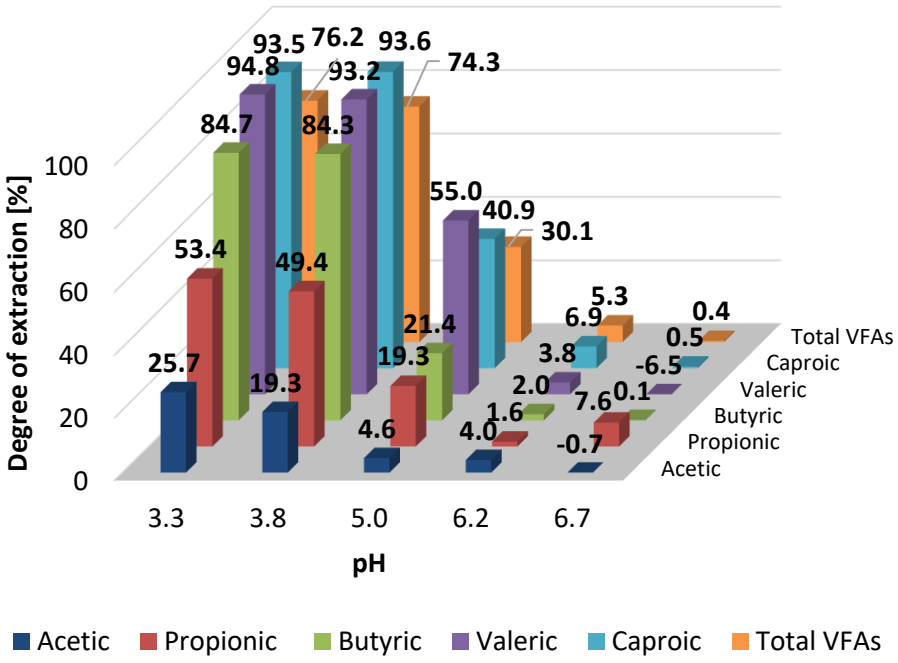

Figure 18: Degree of extraction of individual VFAs from LLE using 20 vol% TBP in oleyl alcohol on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

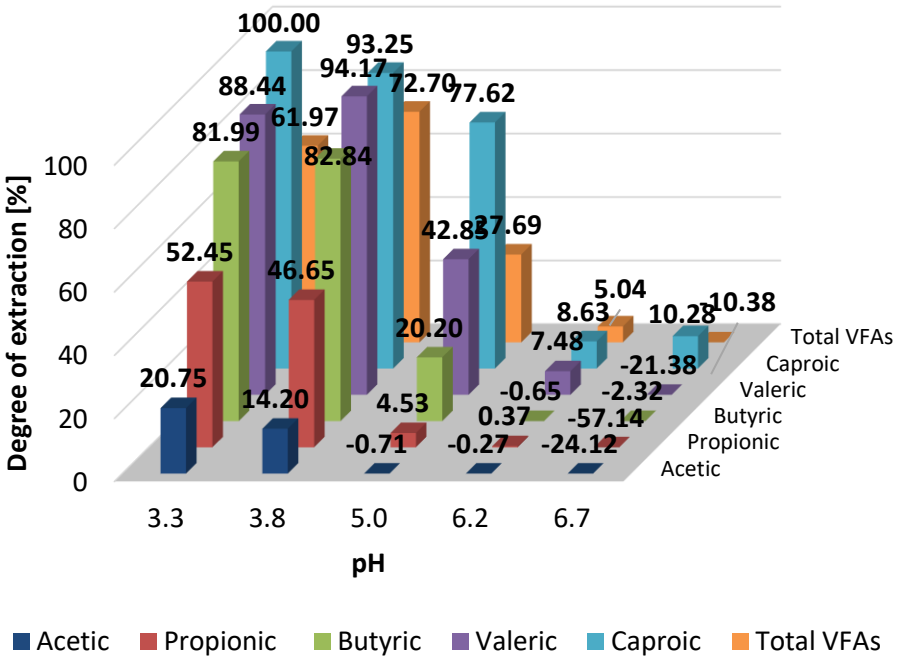

Figure 19: Degree of extraction of individual VFAs from LLE using 20 vol% TBP in lamp oil on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

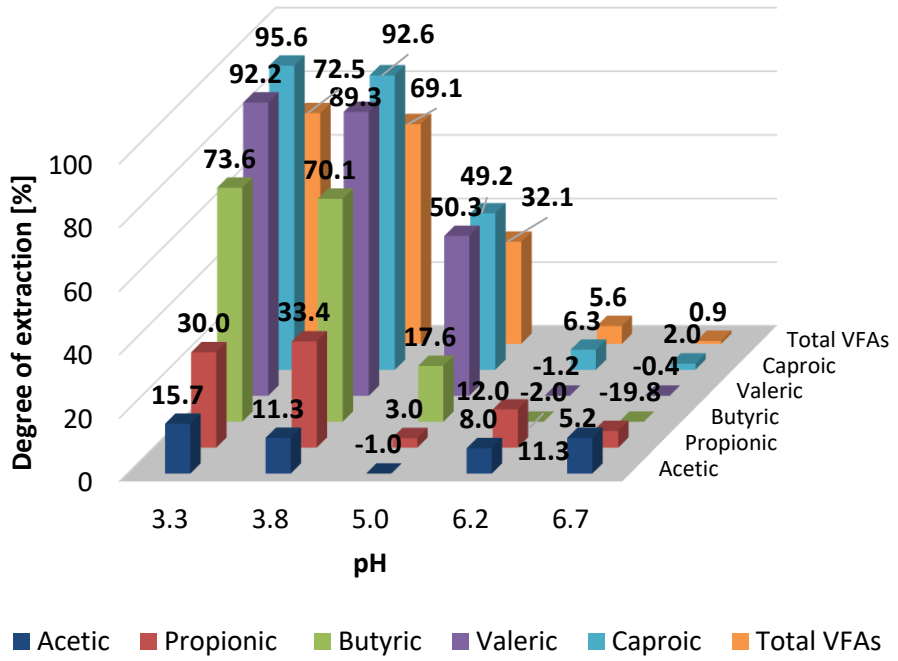

Figure 20: Degree of extraction of individual VFAs from LLE using 20 vol% TOA in canola oil on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

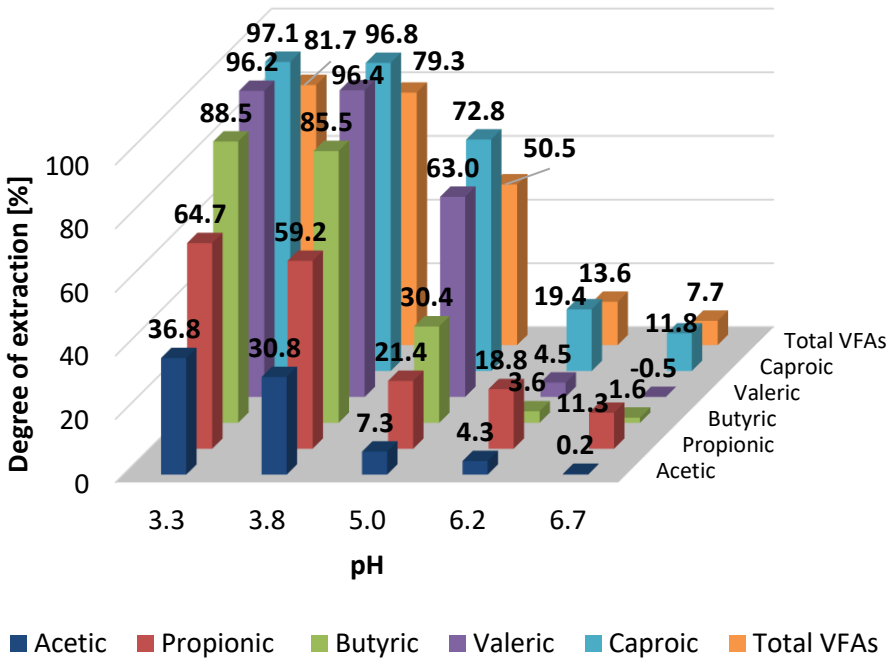

Figure 21: Degree of extraction of individual VFAs from LLE using 20 vol% TOA in oleyl alcohol on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

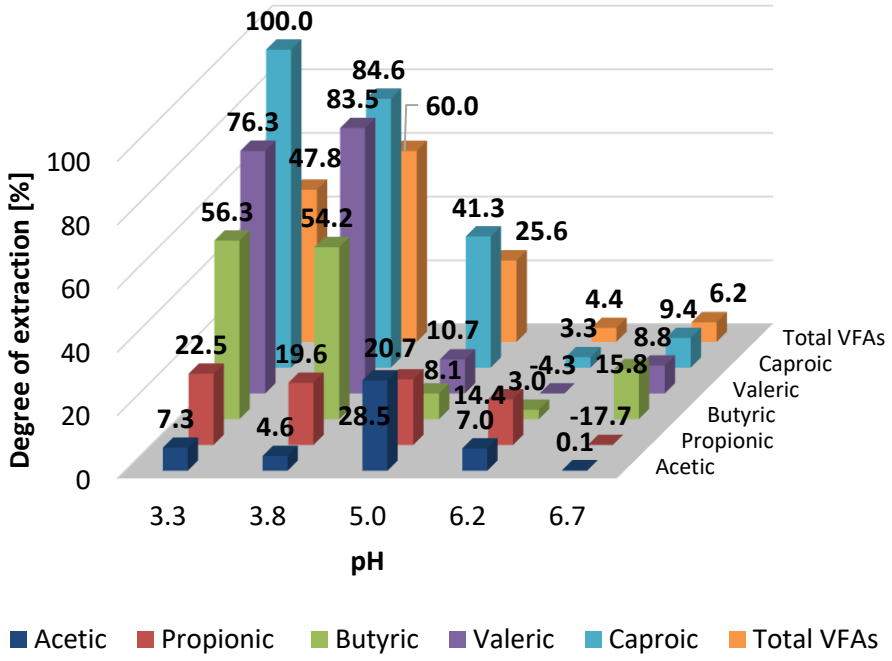

Figure 22: Degree of extraction of individual VFAs from LLE using 20 vol% TOA in lamp oil on AD effluent for a range of pHs at 37°C. The standard deviation was calculated to be less than 0.95 at pH 5.0 in triplicate.

For the co-production of VFAs and methane, one should consider the pH of the system as pH is one of the most important factors in AD. Suitable enzymatic activity for methane-forming bacteria occurs above pH 6.2. Optimal pH conditions are observed between 6.8 and 7.2 (Gerardi, 2003: 99). Therefore, considering the different combinations of extractant and diluent (Figure 17 to Figure 22). The highest extraction efficiencies at a pH of 6.7 were observed for TOA and oleyl alcohol (total VFA extraction of 7.65 ± 0.13%) followed closely by TOA and lamp oil (total VFA degree extraction of 6.21 ± 8.42%). TOA and oleyl alcohol performed slightly better as an active diluent (polar diluent) was used. However, the degree of extraction for propionic acid for TOA and lamp oil was observed to be -17.69 ± 11.65%. This suggests that the active microbial consortia produced more propionic acid. The concentration of the organic phase was determined by mass balance by measuring the aqueous phase. Therefore, the true amount of the VFAs or concentration of VFAs in the organic phase can be largely underestimated due to the production of VFAs within the time period of the experiment. Thus, the VFA extractions observed at higher pHs (active broth) throughout the solvents could be significantly higher.

TBP in lamp oil showed interesting results, which can be seen in Figure 19. At pH 6.7, the lowest degree of extraction was observed for propionic acid, -57.14 ± 0.10 % (the error propagation parameter for the corresponding individual acids at pH 5.0 was used as the uncertainty parameter). This was followed by -24.12 ± 0.47%, -21.38 ± 1.00%, -2.32 ± 0.56% and 10.28 ± 0.95% for acetic, valeric, butyric and caproic acid, respectively. The total degree of extraction was -10.38 ± 0.33%. As mentioned above, extreme negative values cannot be attributed to HPLC error alone and are hypothesised to be due to the active bacteria. Therefore, these negative values may be an indication of the possibility of TBP and lamp oil to be a biocompatible solvent. This implies that if TBP and lamp oil are used as a solvent, toxicity may not be an issue. However, this results in low VFAs extracted out of the system, which can still be potentially useful in continuous *in situ* extraction.

5.3 BMP Studies

BMP experiments were conducted to test the biocompatibility of the solvents on active microbial consortia – since, even if excellent VFA extraction is seen with a particular extractant, if it is inhibitory or toxic to the organisms, it is not a viable option for *in situ* extraction. The function of BMPs is to see the methane potential from a certain substrate. These tests were modified by adding the solvent into the AD medium (inoculum and substrate). This was done to see the performance or the influence the solvent would have on the microorganisms. The measured variables of the BMPs were the total gas

production and the methane percentage of the gas. The results of the BMPs with the solvent were compared to a control, which only included the substrate. To check for the biocompatibility of the solvent, the results from the BMPs with the solvent should show similar or improved results, as this would suggest that the organisms in the BMPs are able to survive and grow in such conditions. An inoculum control was performed as well to see the effect of the substrate. This was done as the methane potential is dependent on the composition of the substrate (C/N ratio and amount of VS). Therefore, the substrate control and the inoculum control were performed as a baseline experiment.

Marták et al. (1995) reported that TBP was toxic to anaerobic bacteria, whereas TOA was non-toxic. To overcome the toxicity of TBP, a non-toxic diluent was used and TBP vol% was kept relatively low, typically 20 vol% (Keshav, Wasewar, et al., 2009). It should be noted that a high sugar composition (high apple pomace content) was used to increase the initial amount of VFAs produced in the BMPs to test the solvents capabilities of extracting the VFAs effectively. The best extractant and diluent combinations were used for the BMP studies, i.e. TOA in canola oil, TBP in lamp oil and TOA in oleyl alcohol.

From Figure 23, the control produced 114.5 mL ± 39.42 mL (error given as standard deviation) of biogas, with maximum methane of 9.73% ± 1.33%. The inoculum control only produced 66 mL ± 7.21 mL but had the highest methane composition of 16.94% ± 6.87%. TOA in canola oil and TBP in lamp oil significantly affected the activity of the microorganisms. Higher methane percentages were observed for these solvents, 12.62% ± 2.82% and 14.68% ± 6.73% respectively, as well as higher amounts of biogas produced (168.0 mL ± 26.15 mL and 145.7 ± 5.03 mL). TOA in oleyl alcohol performed slightly worse compared to the control, with lower gas production of 90.7 mL ± 30.37 mL, but still maintaining a slightly higher methane percentage of 10.44% ± 0.13%. To confirm the difference statistically, an ANOVA analysis was performed with an alpha value of 0.05 or a 95% confidence and can be seen in Table 19. From these results, the null hypothesis (all samples are the same) can be rejected as the p-value was less than 0.05.

These results did not only show that all the selected solvents were biocompatible with the AD microorganisms but, more interesting, showed improved methane production when the solvent was used. This suggests that the microorganisms are able to survive and grow under these conditions. Additionally, the use of TOA in canola oil and TBP in lamp oil showed both improved methane production and total gas production. This could be due to the high sugar substrate used, which

increases the likelihood of the system to produce excess amounts of VFAs, leading to acid crash. This would suggest that the solvent aids or could possibly prevent acid crash from happening within the system. This could be justified by measuring the amount of VFAs in the solvent and the broth.

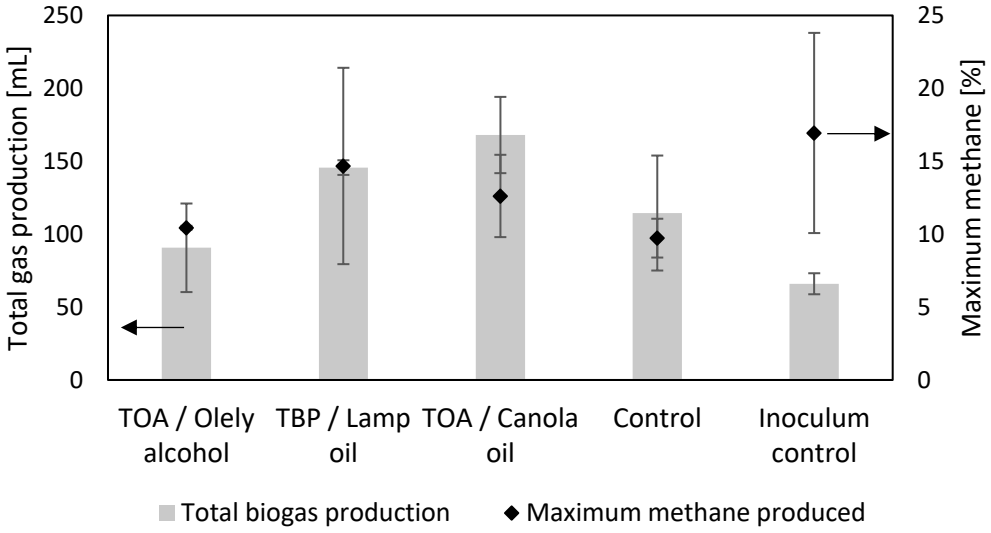

Figure 23: Biocompatibility test of solvents in BMPs by measuring the total biogas production and maximum methane percentage observed in a 28 day digestion period. Solvents consisted of 20 vol% extractant and diluent.

Table 19: ANOVA analysis of the three solvents, the control and the inoculum control used in the BMPs.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	9848.68	4	2462.17	7.37	0.000809	2.87
Columns	81254.22	1	81254.22	243.27	1.17E-12	4.35
Interaction	10428.75	4	2607.19	7.81	0.000584	2.87
Within	6680.26	20	334.01			
Total	108211.9	29				

The VFAs in the solvent and before and after digestion are given in Table 20. In the table, 5.84 ± 0.36 g/L of total VFAs were extracted using TOA and oleyl alcohol, and 2.40 ± 0.30 g/L of total VFAs using TBP and lamp oil. The net VFA production was determined by the difference between the post- and pre-digestion. Surprisingly, the net VFA production of 5.10 ± 0.33, 1.71 ± 0.58, 1.03 ± 0.50, 3.27 ± 0.33 and -0.10 ± 0.01 g/L was observed for TOA/canola, TBP/lamp oil, TOA/oleyl alcohol, substrate control and inoculum control, respectively. These results clearly indicate the potential use of solvents to

prevent acidification of the AD system. The amount of VFAs in the post-digestion was measured to be 11.07 ± 0.29 g/L. Whereas, TOA in canola oil showed improved total gas production and had 12.90 ± 0.29 g/L of VFAs. Furthermore, 9.51 ± 0.56 g/L of VFAs were measured for the TBP in lamp oil BMP test, which had the best methane percentage and improved total gas production (compared to the control). These results substantiate the idea that the solvent can help prevent severe VFA accumulation (which causes acidification). It should be noted that the VFAs extracted using TOA and canola oil could not be determined as an emulsion formed. The emulsion prevented the use of back-extraction to regenerate the solvent. Therefore, the use of TOA and canola oil was concluded to be not a suitable solvent due to the severe implications the emulsion would have on the AD system and the LLE system.

The difference in total gas production for TOA in oleyl alcohol could be a result of the solvent extracting excessive amounts of VFAs initially, which can hinder the methanogenic microorganisms as there may not be enough food for the microorganisms to survive. These results support the possibility that the methanogens may not have enough food due to the amount of VFAs extracted. This implies that extracting excessive amounts of VFAs initially may disrupt the anaerobic process by preventing the growth and sustenance of the methanogens.

These results indicate that the solvents are not affecting the microorganisms in the anaerobic process and that an *in situ* extraction method of VFAs in an anaerobic digester could be possible. Low methane percentages were expected in this study due to the substrate ratio. A substrate ratio of 70% apple pomace and 30% of cow manure was used in this study. The high apple pomace loading meant that a high C/N ratio was expected. A high C/N ratio was said to result in low methane yields due to the lack of nitrogen for cell growth (Alvarez & Lidén, 2008).

Although this thesis does not focus on the optimisation of methane, there are other contributing factors that may influence the gas composition. These other contributing factors could be due to the total VS and the small particle size of the substrate after blending, which was done to ensure a homogenous mixture. By decreasing the total solids to 6%, the amount of VS in the substrate was significantly decreased. The reduction in total solids was equivalent to a 59.37% reduction from the original calculation of VS. VS is directly related to the amount of VFAs formed, a higher VS loading usually results in higher amounts of VFAs being formed in the digester (Gerardi, 2003: 72). Therefore, by reducing the total solids to 6%, VS was reduced, which reduces the amount of VFAs formed in the

digester. This implies that there was less substrate or food for the sustainable growth of the methanogens, which can decrease the amount of biogas produced. The reduction of particle size would result in a faster hydrolytic rate, which increases the rate of the acidogenic rate. The increased rate would result in more VFAs produced over a span time, if this rate is considerably large, this can lead to VFA accumulation, which as a result can cause acidification as the pH of the bioreactor would drop to undesirable conditions for the methanogenic bacteria. Methanogens would be unable to grow under these conditions, implying a low methane yield.

Table 20: Total VFAs before and after digestion from the BMP test and VFAs in the solvent (where applicable).

	Pre-digestion [g/L]* **	**Post-digestion [g/L]*** **	**Net VFAs produced [g/L]**	**VFAs in 10 mL of solvent [g/L]***
TOA and canola oil	7.80 ± 0.15	12.90 ± 0.29	5.10 ± 0.33	n/a **
TBP and lamp oil	7.80 ± 0.15	9.51 ± 0.56	1.71 ± 0.58	2.40 ± 0.30
TOA and oleyl alcohol	7.80 ± 0.15	8.83 ± 0.48	1.03 ± 0.50	5.84 ± 0.36
Control	7.80 ± 0.15	11.07 ± 0.29	3.27 ± 0.33	n/a
Inoculum control	0.10 ± 0.01	0	-0.10 ± 0.01	n/a

* VFAs measured after the digestion period through back-extraction using 1 M NaOH solution
** Could not be determined due to the formation of an emulsion with the solvent
*** The volume of the pre-digestion and post-digestion broth was 60 mL

The results from the BMPs tests not only showed the biocompatibility of the solvents but an interesting conclusion can be drawn. From the analysis of the degree of extraction results, higher degrees of extractions were achieved at a lower pH. However, the BMP results show the ability of the solvent to extract VFAs from an active broth. If VFA accumulation occurs, this would lead to a decrease in the pH. As pH decreases, the degree of extraction of the solvent increases, which implies that more VFAs are extracted. If VFAs are extracted at a greater rate than the VFA production rate inside the digester, this would increase the pH. Therefore, there is an opportunity for *in situ* LLE to not only co-produce VFAs and biogas but could potentially be used as a pH control mechanism. Although pH control using LLE has been investigated previously by Yabannavar and Wang (1990), for lactic acid production from a fermentation system using 15% TOA in oleyl alcohol in external configuration, in the study the fermenter was conditioned to produce only lactic acid and not biogas. The introduction of an *in situ*

extraction can increase the economic value of AD by co-producing VFAs and can reduce costs relating to buffer solutions that are added to stabilise the pH in AD.

The BMP results are promising for *in situ* removal of VFAs. However, a scale-up run was investigated as it was known that differences in biogas yields can occur between BMP results and scale-up results, which were observed in Kell (2019), where the BMP results are usually better than the scale-up.

5.4 17 Litre Bioreactor Run with Continuous Extraction

The same concept of the BMPs was applied for this experiment. However, the implementation of the LLE was applied differently to the bioreactor than the batch tests. A tube was inserted into the bioreactor, in which the LLE would take place. The solvent was pumped back and forth from the bioreactor to the back-extraction unit at 4 mL/min, where regeneration of the solvent would occur. This concept can be seen in Figure 24. To increase the mass transfer of the solvent, the solvent inlet pipe into the bioreactor was placed inside the broth.

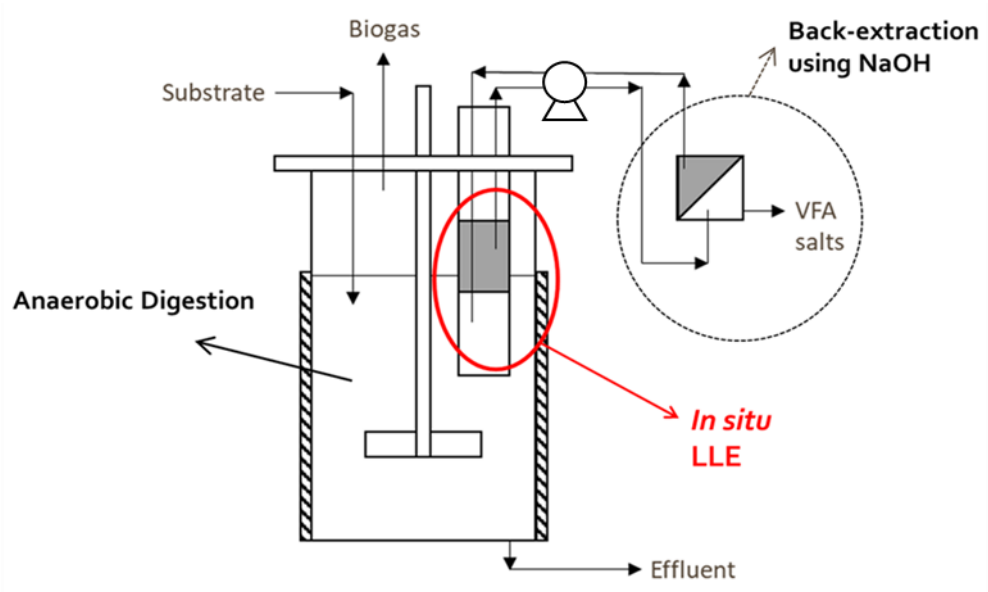

Figure 24: Modified 17 L bioreactor that incorporates an *in situ* LLE for VFAs using a pH-swing back-extraction unit with 1 M NaOH for solvent regeneration.

For this experiment, 20 vol% TBP in lamp oil was chosen as a suitable solvent. This choice was based on the ability of the solvent to have minimal toxic effects on the AD broth and the ability to regenerate the solvent through a pH-swing back-extraction. TOA in oleyl alcohol was not chosen initially for this experiment due to the lower gas production (from the BMPs) and the potential of the solvent of extracting large amounts VFAs, causing inefficient growth activity of the methanogens. The aim of this experiment was to show the concept of continuous *in situ* LLE in an AD system and that such a system can be used to co-produce VFAs and methane. Therefore, methane percentage was measured instead of total gas production.

Due to the stability of the system as well as low methane yields observed in the BMPs, the substrate ratio was changed to an increased buffering capacity by increasing the cow manure to 80% and reducing the apple pomace to 20%. This reduces the C/N ratio from a high ratio to a low ratio due to the nitrogen content in cow manure (Kell, 2019). Additional buffer was added to increase the pH stability of the system. *In situ* VFA extraction may have potential to be used as a pH control mechanisms. However, for the basis of this thesis, a proof of concept approach was taken. This meant that due to two complex systems of AD and LLE, as well as trying to incorporate the one into the other, a simplified system was used. This system was simplified to have no acid crash by having a stable pH system such that continuous extraction of VFAs may occur.

The experiment was kept at a constant temperature of 37°C and ran for 15 days. The LLE unit was switched on day 11. This was done to allow for the initial growth of the methanogens before exposing the methanogens to the solvent. On day 8, a sample was taken and analysed. A methane percentage of 38% was observed. To measure the amount of VFAs extracted from the solvent, the NaOH solution was analysed for VFAs. However, no VFAs were found. This was an interesting result as this experiment was expected to have VFAs, if not small amounts of VFAs. This could be due to increased stability of the system as pH was relatively constant at 7.20 to 7.35 throughout the incubation period. This was a result of the increased cow manure percentage and the 1 w/v% calcium carbonate buffer. From Section 5.2.2 Analyses of the degree of extraction, low extractions were observed for pH 6.7. An alternative explanation could be due to the inefficient mass transfer in the LLE unit. The diameter of the tube was relatively small (40 mm) as compared to the bioreactor. Therefore, the surface area of the interface area where the mass transfer occurs, combined with the slow flowrate (4 mL/min) was relatively small compared to the BMPs, where the serum bottles were shaken periodically (increasing the contact area of the solvent).

However, the experiment was repeated with 20 vol% TOA and oleyl alcohol, as TOA in oleyl alcohol (active diluent) had the ability to extract higher amounts of VFAs compared to TBP in lamp oil. An increased solvent flowrate of 52 mL/min was used instead of 4 mL/min to increase the surface area of the solvent in the broth. No buffer was added to this experiment to allow for fluctuations in pH such that better extraction can occur in the early stages of the digestion period, as a significant decrease in pH will occur during the hydrolysis and acidogenic steps, where the organic matter is converted to VFAs.

The LLE unit was switched on initially for 8 days in which the solvent (TOA and oleyl alcohol) extracted 0.141 g/L of total VFAs (45.9% acetic acid, 29.5% propionic acid and 24.6% butyric acid). The solvent that settled inside the reactor over the duration of the experiment (22 days) due to vigorous mixing was back-extracted and analysed. An additional 0.045 g/L of total VFAs (92.1% propionic acid and 7.9% acetic acid) were extracted out. Therefore, a combined total of 0.186 g/L of total VFAs were extracted out using TOA and oleyl alcohol. Gas production was measured to be 6.71 L of biogas over 22 days with a methane content of 43%. The production of VFAs in the early stages of AD decreases the pH of the system. The drop in pH allows for better extraction of the VFAs using a suitable solvent and prevents acidification of the bioreactor from occurring. Therefore, satisfying the growth conditions of the methanogenic microorganisms, which allows for methane production. These results show the possibility of co-production of biogas and VFAs using continuous *in situ* extraction of VFAs by LLE.

Chapter 6: Conclusion

To summarise the work done in this thesis, **the aim of this thesis was to develop a method that was capable of continuously extracting VFAs *in situ* in AD systems to co-produce VFAs and biogas.** This would be achieved by: 1) Identifying possible methods for *in situ* extraction, 2) Determining the extraction parameters that would influence AD, 3) Determining the recovery method for VFAs once they were extracted 4) To design and evaluate the extraction unit.

Objective 1 was achieved by identifying several downstream processing methods, such as: adsorption, distillation and esterification, membrane technology, electrodialysis, precipitation, gas stripping and LLE. Based on the literature, gas stripping and LLE was identified as a potential *in situ* VFA extraction method in AD. For the second objective, the amount of gas required for gas stripping was influenced by the stripping gas composition. A 35% reduction of gas was observed for an equimolar gas mixture of carbon dioxide and methane when compared to pure carbon dioxide. For LLE, TBP and TOA were chosen as extractants and canola oil, lamp oil and oleyl alcohol were chosen as suitable diluents. An experimental CCD was designed to test the ability of the solvents to extract VFAs from various pHs. LLE was found to have a strong pH dependence, whereby higher degrees of extractions were observed below the pKa value of the VFAs. Additionally, a major concern was the biocompatibility of the solvents with the AD bacteria. BMPs were conducted to test the biocompatibility of the solvent. BMP experiments with the solvent resulted in improved gas production and methane percentage when compared to a substrate control. The third objective was met by selecting a pH-swing to back-extract the solvent (regenerate the solvent) using a 1 M NaOH solution. Gas stripping was concluded to be inefficient for the purpose of this thesis due to the large volumes of gas required to strip VFAs. The final objective was achieved by designing a reactor prototype, whereby the LLE would occur inside the bioreactor. This was achieved by placing a tube into the bioreactor. The solvent was regenerated in a separate back-extraction unit outside the reactor. However, no VFA recovery was observed in the NaOH solution.

For the hypothesis: LLE is an effective method for *in situ* extraction, the scale-up experiment did not show any VFA recovery and was concluded to be due to the large buffering capacity of the substrate and low mass transfer between the solvent and AD broth. However, excellent VFA recovery was observed for TOA/oleyl alcohol (5.84 ± 0.36 g/L) and TBP/lamp oil (2.40 ± 0.30 g/L) in the BMP tests. Therefore, this hypothesis can be partially accepted due to the BMP results, whereby improved methane percentage and improved biogas production was observed. Due to this as well, the second

hypothesis that the solvent would be biocompatible with the AD bacteria can be accepted. Therefore, it can be concluded that the overall aim of the thesis was achieved.

Successful implementation in the scale-up using TOA and oleyl alcohol, in combination with the BMP results shows that there is tremendous potential for *in situ* extraction in AD. The difference between the BMPs and the scale-up was the composition of the substrate, high C/N ratio for the BMPs and low C/N ratio for the scale-up. High C/N ratios usually produce significantly more VFAs than lower C/N ratios (Kell, 2019). The low C/N ratio in this experiment could cause lower degrees of extractions due to the small amount of VFAs produced. After 15 days, the VFAs in the bioreactor was measured to be 5.01 g/L. The post-digestate VFAs were almost doubled the amount observed in the bioreactor. Therefore, further experimentation using optimised conditions for LLE is required to increase the recovery of VFAs in a larger scale.

Such conditions include: increasing the mass transfer of the VFAs to the solvent, the composition of the substrate and a more suitable solvent. The mass transfer of the LLE can be increased by increasing the flowrate of the solvent. By increasing the solvent flowrate into the bioreactor, the contact area of the solvent would be increased resulting in better mass transfer. Additionally, the diameter of the tube can be increased to increase the contact surface area between the solvent and the broth. For the substrate composition, the substrate with high amounts of sugar should be used to increase the amount of VFAs produced. This should be coupled with the addition of no buffer to allow the pH of the system to decrease rapidly due to extreme VFA production. Further research should be investigated as well into solvents that are capable of extracting VFAs in both their dissociated and undissociated form. A suitable solvent is necessary for *in situ* extraction and must be economically feasible.

Chapter 7: Recommendations and Future Research

7.1 Limitations and Proposed Solutions

Recommendation for problems regarding BMP tests:

- To accurately measure gas production and methane potential, the use of Automatic Methane Potential Test System (AMPTS) II is proposed. The device accurately measures and analyses low biogas samples. Conditions such as pressure build-up and sparge gas overestimation are mitigated by calculations, which increases the accuracy of measurements.
- BMPs should be conducted in larger volumes to minimise variations in biogas production. Tedlar gas sampling bags should be attached to BMPs to prevent pressure-build up and to prevent gas contaminations when using the GC.

Recommendations concerning LLE:

- Due to time constraints, scale-up experiments with solvents used in the BMP tests should be tests and compared with another to see the effects on biogas production
- Due to low degrees of extractions, it is recommended that more solvents should be investigated in BMPs and tested on the modified reactor to see the effects these solvents would have on VFA extraction and biogas production.

Recommendations to problems concerning the scale-up experiment:

- Due to the design of the reactor, it was difficult to determine the level of the solvent in the reactor. The LLE unit should be placed against the sidewall of the reactor such that the level of the solvent can be clearly visible.
- Due to reasons beyond our control, the inoculum and substrate used in the scale-up should be preferably used from the same source to minimise any additional error between scale-up and BMPs.
- Scale-up experiments should be conducted in triplicate to mitigate errors in VFA production and methane potential. However, due to time constraints, this was not possible.

7.2 Future Work

Based on the work completed in this thesis, future research such as investigating the possibility of pH control could be useful and industrially applicable due to the acidification of AD systems associated with high carbon feedstock. This can be achieved by investigating an AD system that is prone to acid crash and applying the LLE unit to such a system. In addition, *in situ* LLE extraction should be tested on different inoculum sources to test the robustness of the extraction unit.

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Appendix A: Pump Calibration Curve

A pump calibration curve was used to determine the flowrate settings for the solvent. The pump was calibrated using the data in Table 21. The pump setting was changed and the volume of the solvent was measured every minute. The pump curve is shown in Figure 25.

Table 21: Measurements taken to determine the pump curve.

Pump setting	Flowrate [mL/min]
1	4
5	22
7	30
10	45
20	85

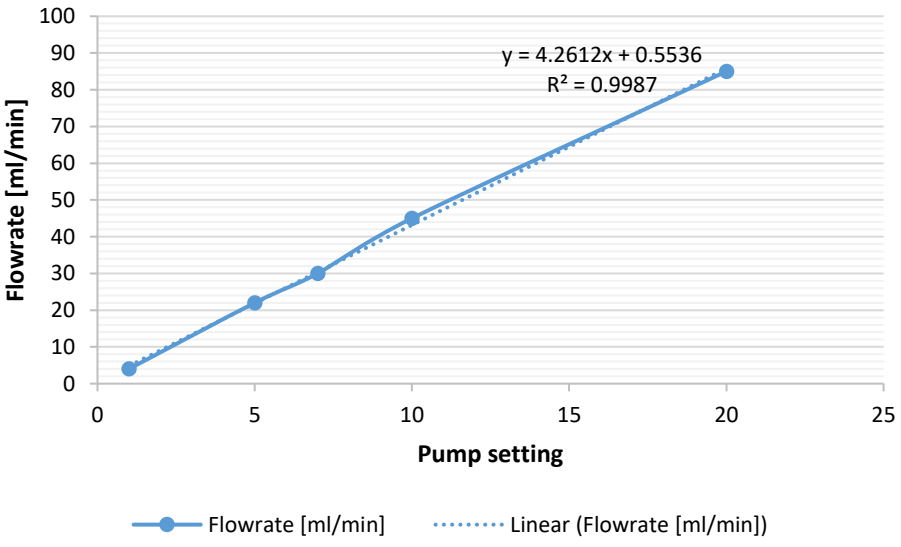

Figure 25: Pump calibration curve using lamp oil and 20 vol% TBP

Appendix B: GC Calibration Curves

Calibration Details	N2		
Calibration Type	Lin	*Offset (C0)*	0.0000
Evaluation Type	Area	*Slope (C1)*	0.0031
Number of Calibration Points	8	*Curve (C2)*	0.0000
Number of disabled Calibration Points	0	*R-Square*	0.9998

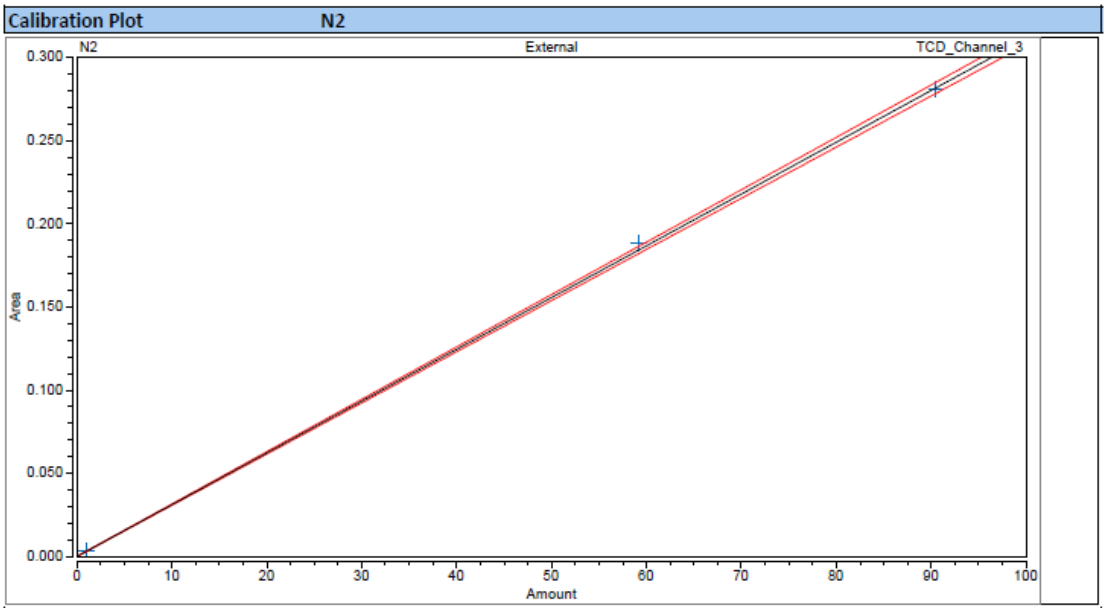

Figure 26: Nitrogen calibration curve for GC analysis.

Calibration Details	CH4		
Calibration Type	Lin	*Offset (C0)*	0.0000
Evaluation Type	Area	*Slope (C1)*	0.0079
Number of Calibration Points	5	*Curve (C2)*	0.0000
Number of disabled Calibration Points	0	*R-Square*	0.9999

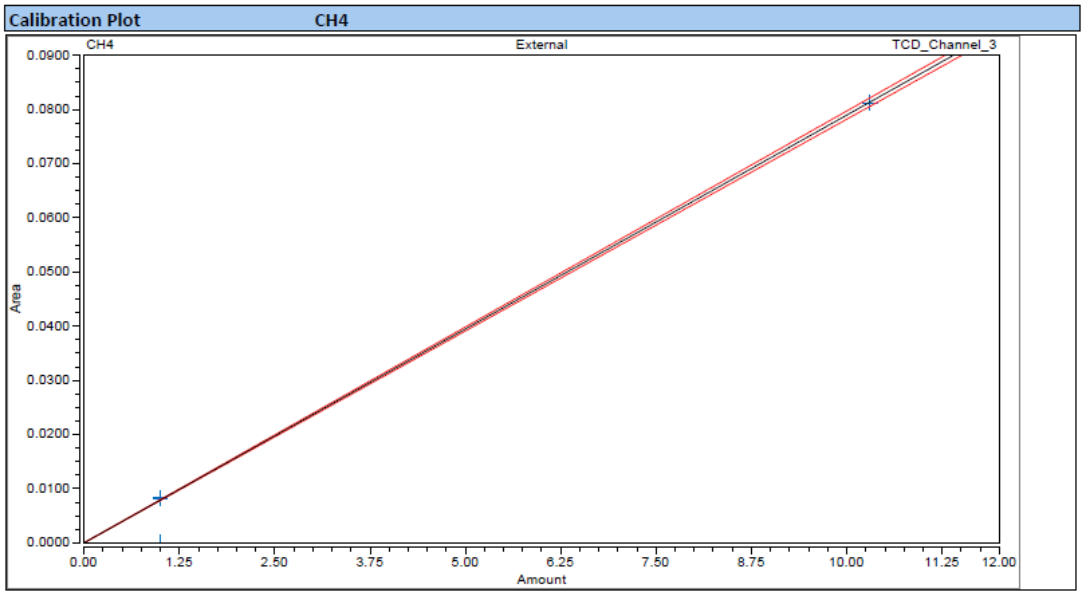

Figure 27: Methane calibration curve for GC analysis

Calibration Details	CO2		
Calibration Type	Lin	*Offset (C0)*	0.0000
Evaluation Type	Area	*Slope (C1)*	0.0293
Number of Calibration Points	5	*Curve (C2)*	0.0000
Number of disabled Calibration Points	0	*R-Square*	0.9997

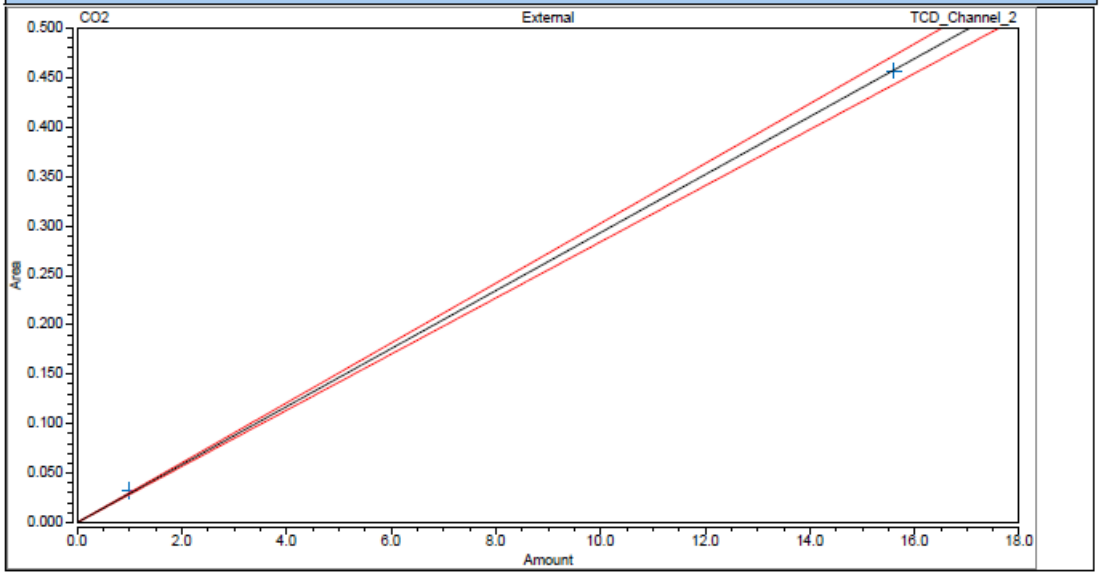

Figure 28: Carbon dioxide calibration curve for GC analysis.

Calibration Details	Oxygen		
Calibration Type	Lin	*Offset (C0)*	0.0000
Evaluation Type	Area	*Slope (C1)*	0.0037
Number of Calibration Points	3	*Curve (C2)*	0.0000
Number of disabled Calibration Points	0	*R-Square*	0.0000

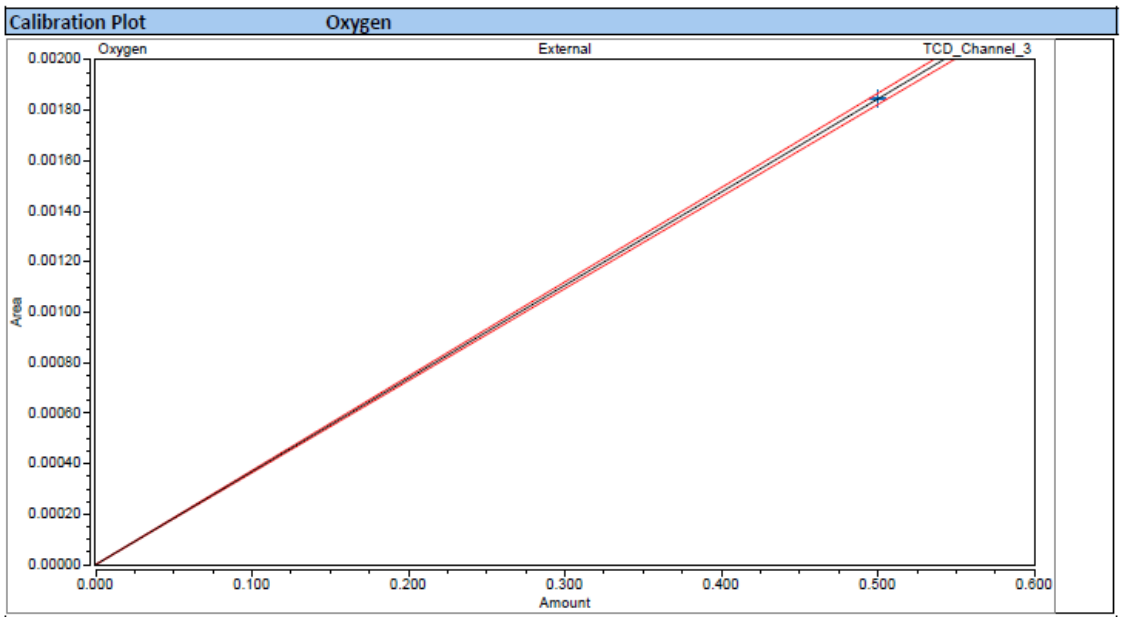

Figure 29: Oxygen calibration curve for GC analysis.

Appendix C: Continuous Gas Stripping Calculations

For 100% recovery of VFAs using an equimolar carbon dioxide and methane gas:

From Table 14, mass fraction of gas to VFAs = 150

Volume of VFAs used = 20 mL of 14.65 g/L

⟶ 0.29 grams of VFAs

Therefore, 0.29 x 150 = 43.94 grams of gas required

Since equimolar gas ⟶ molar mass of gas = 30 g/gmol

Therefore, 43.94/30 = 1.47 mol of gas

Assuming ideal gas behaviour, PV = nRT using (T = 273.15 + 37 and P = 101325 Pa)

⟶ V = 0.03728 m^3 ⟶ **V = 37.28 L of gas required**

Volume of gas used in continuous experimental run:

Volume of bottle = 250 mL = 0.25 L

The flowrate of the gas was set to 1.2 vvm

Stripping time = 134 mins

Therefore, 1.2 x 134 x 0.25 = 40.2 L of gas used

Appendix D: Response Surface and Pareto Charts

D.1 TOA in Diluents with a Synthetic VFA solution

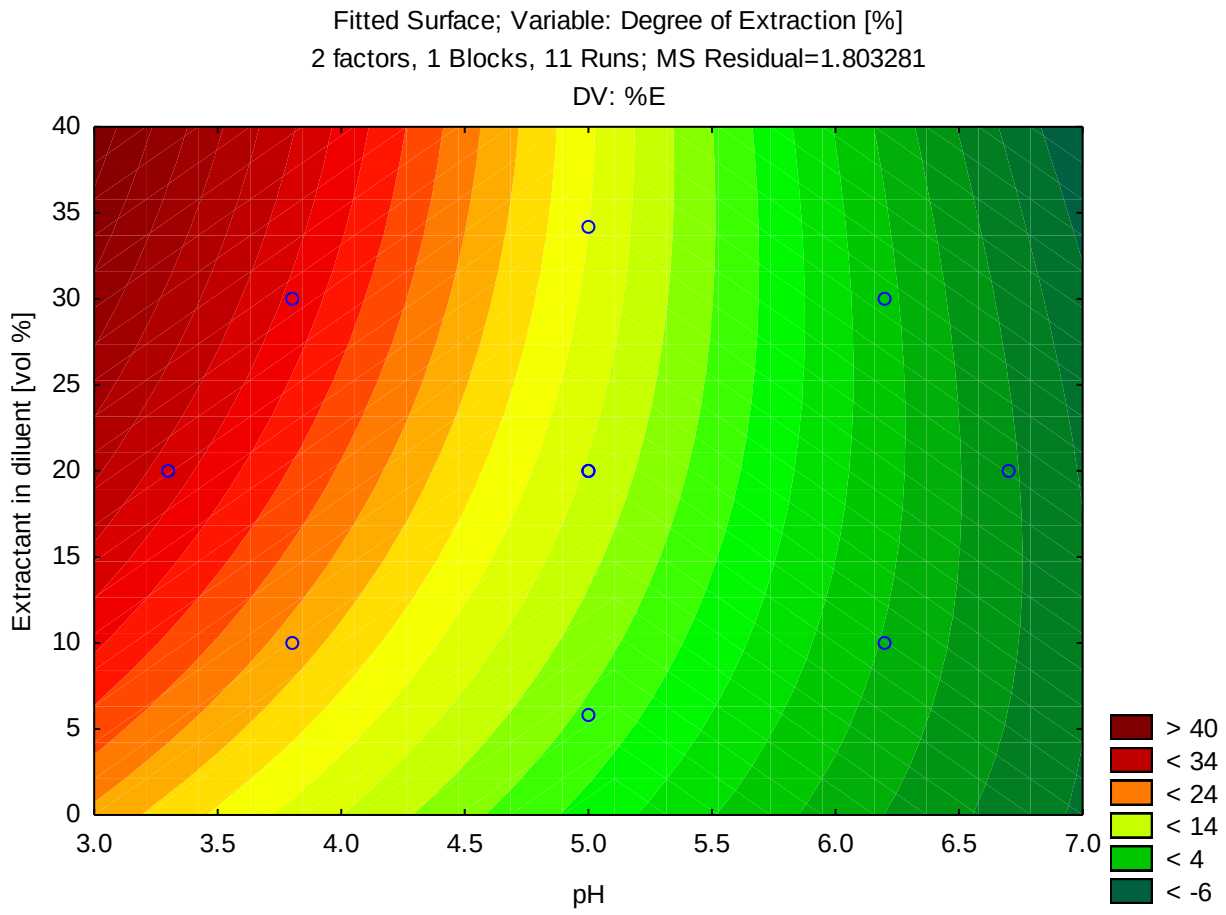

Figure 30: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and canola oil in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

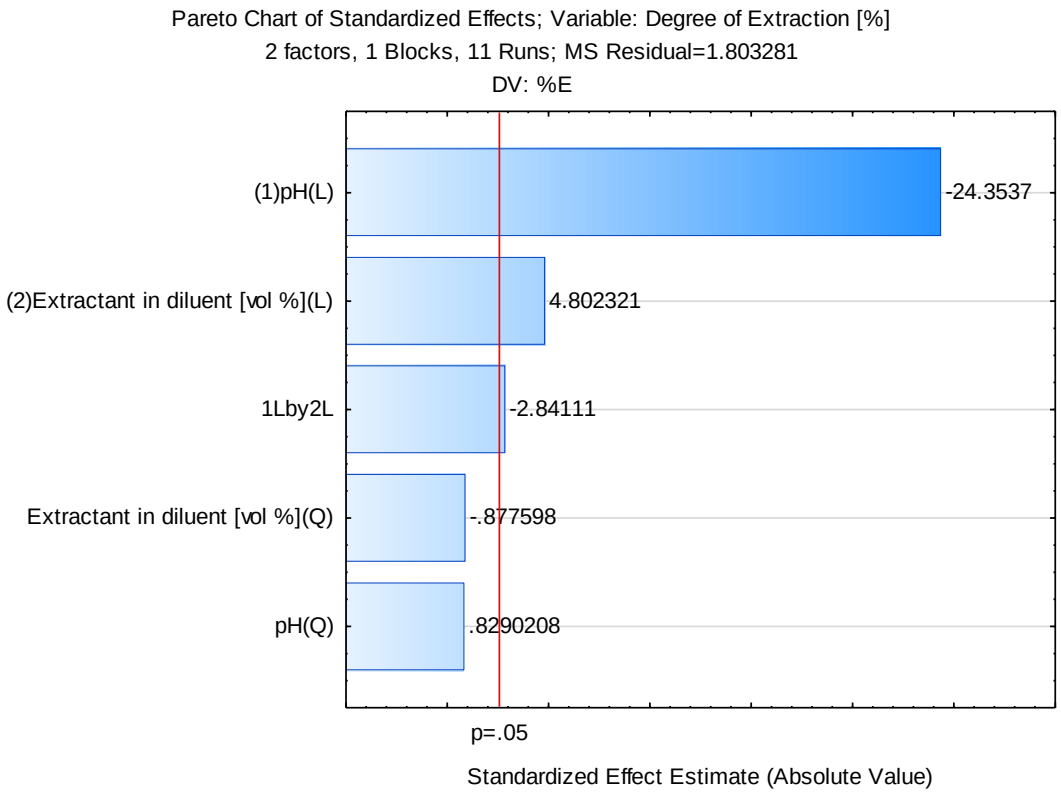

Figure 31: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TOA and canola oil at 37°C.

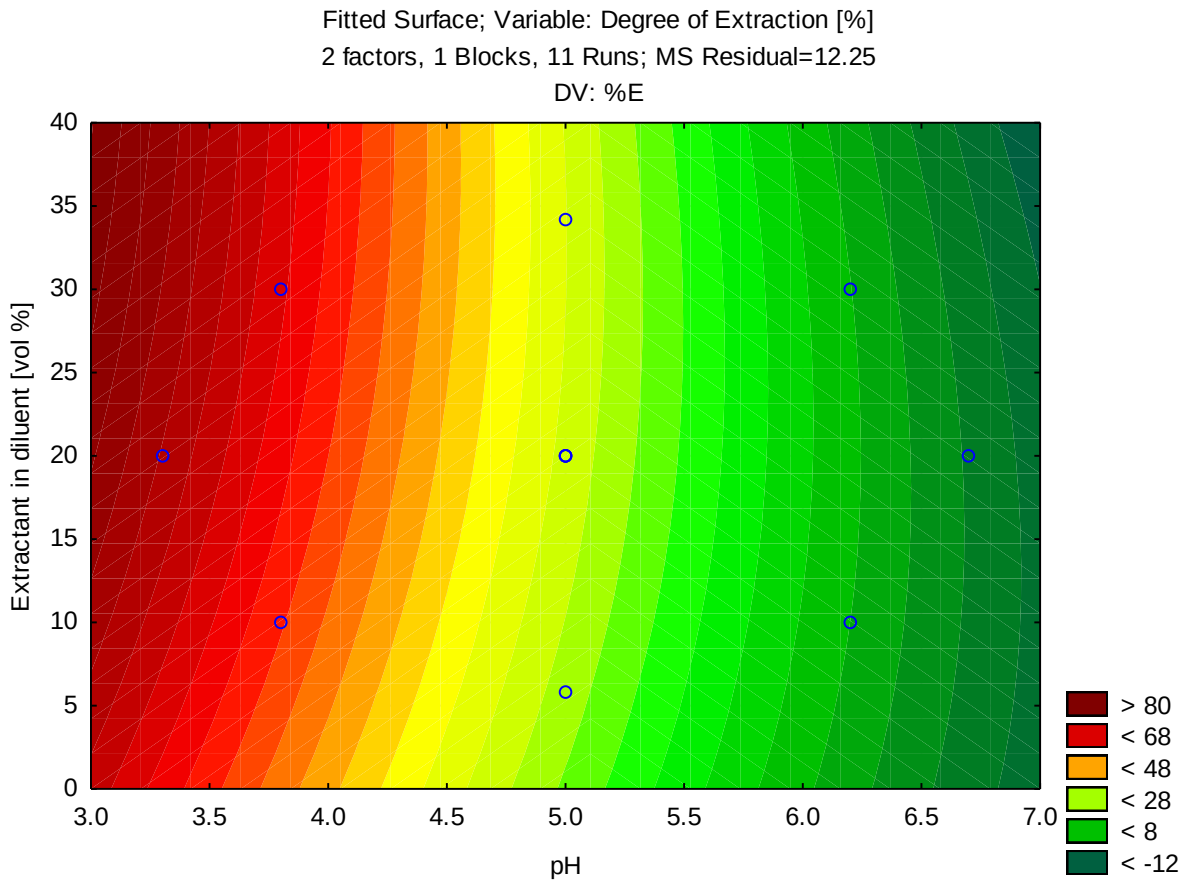

Figure 32: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and oleyl alcohol in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

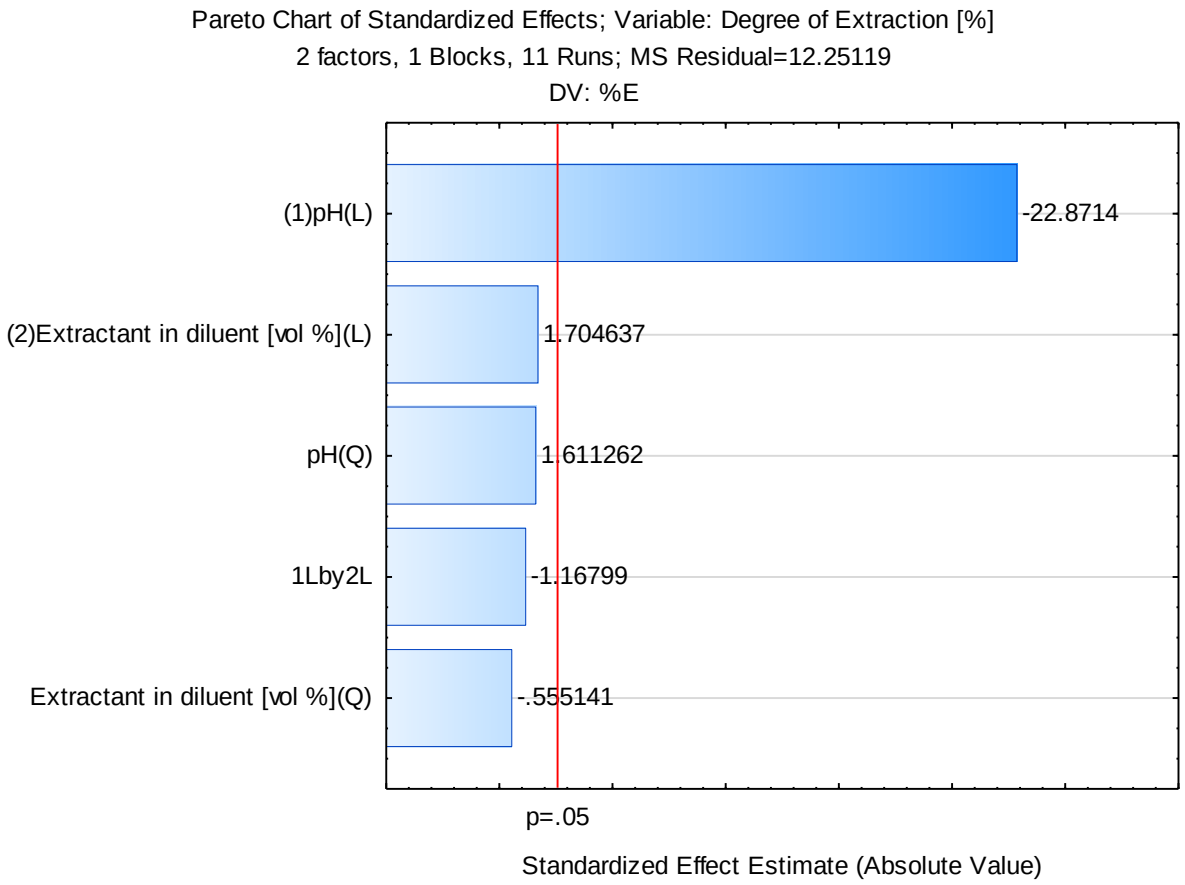

Figure 33: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TOA and oleyl alcohol at 37°C.

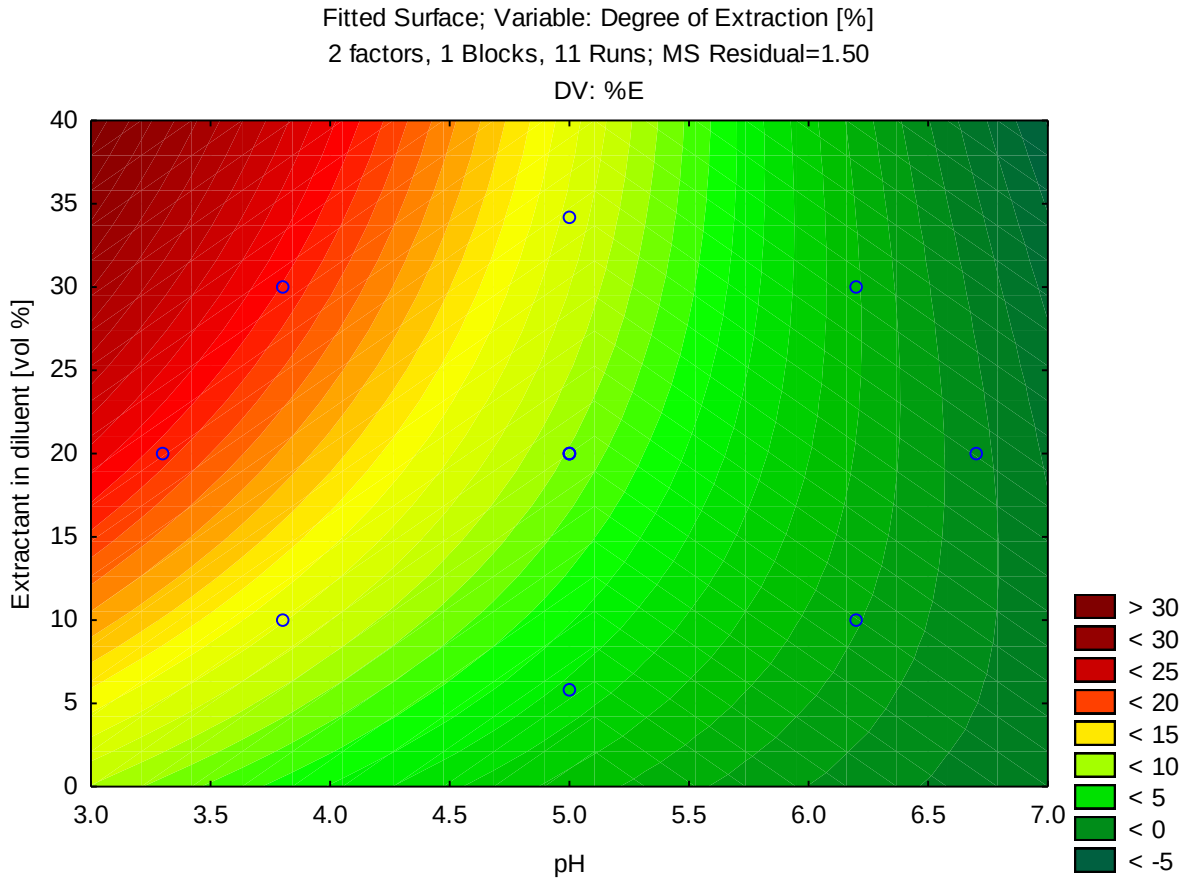

Figure 34: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and lamp oil in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

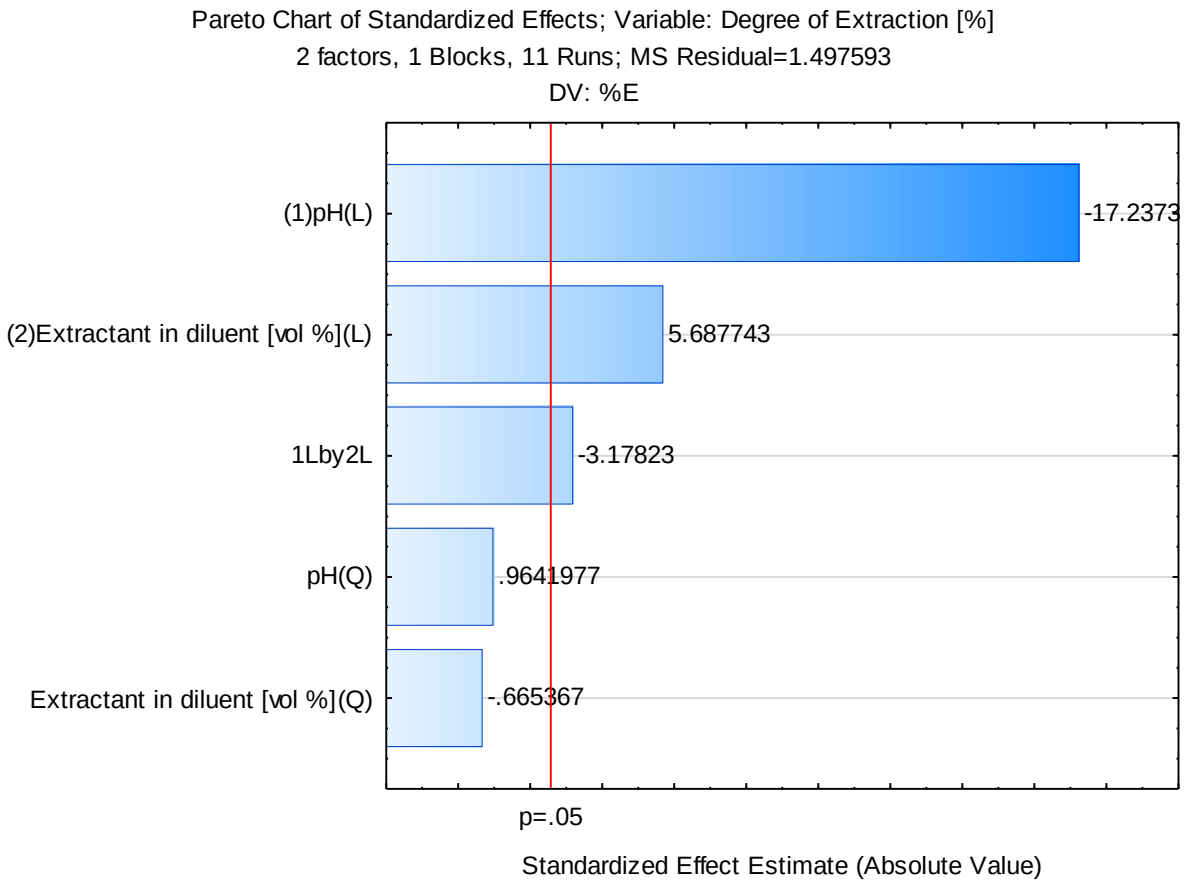

Figure 35: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TOA and lamp oil at 37°C.

D.2 TBP in Diluents with Synthetic VFA Solution

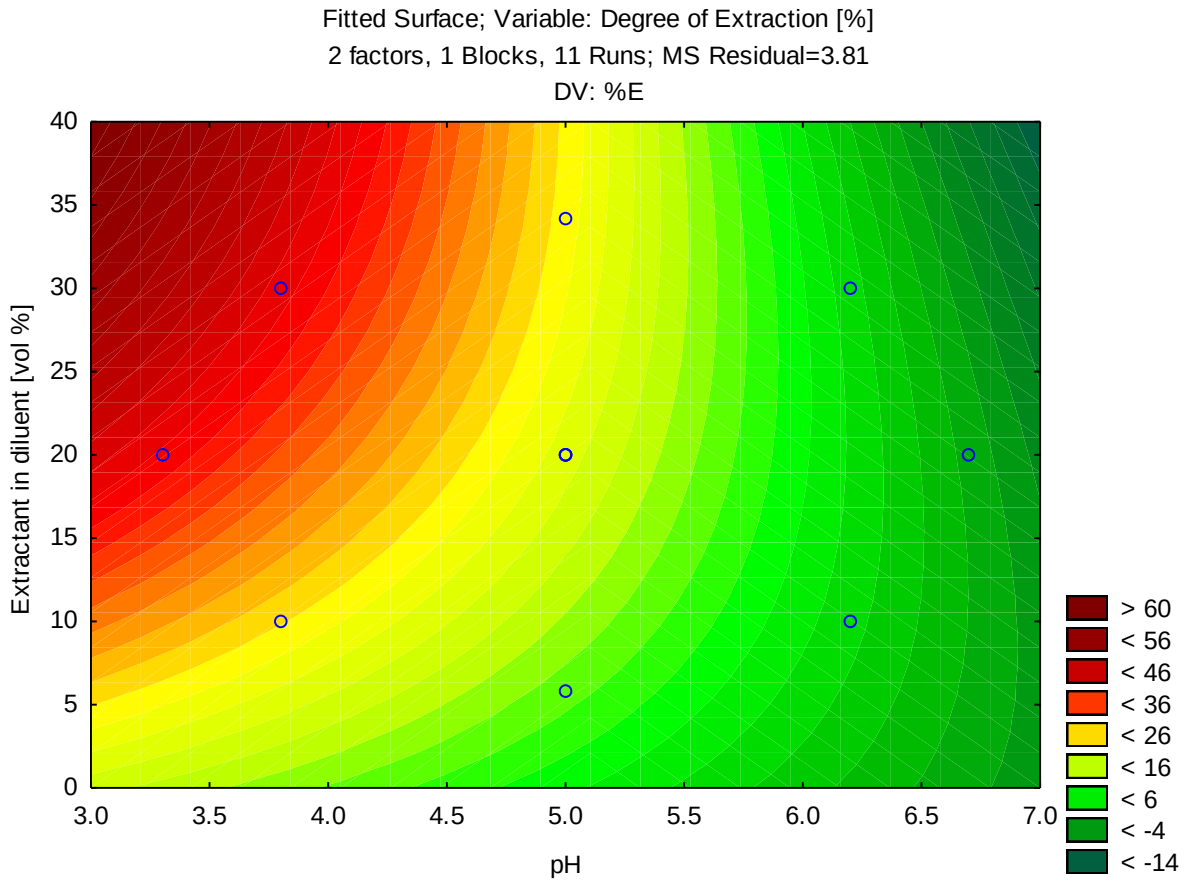

Figure 36: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and canola oil in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

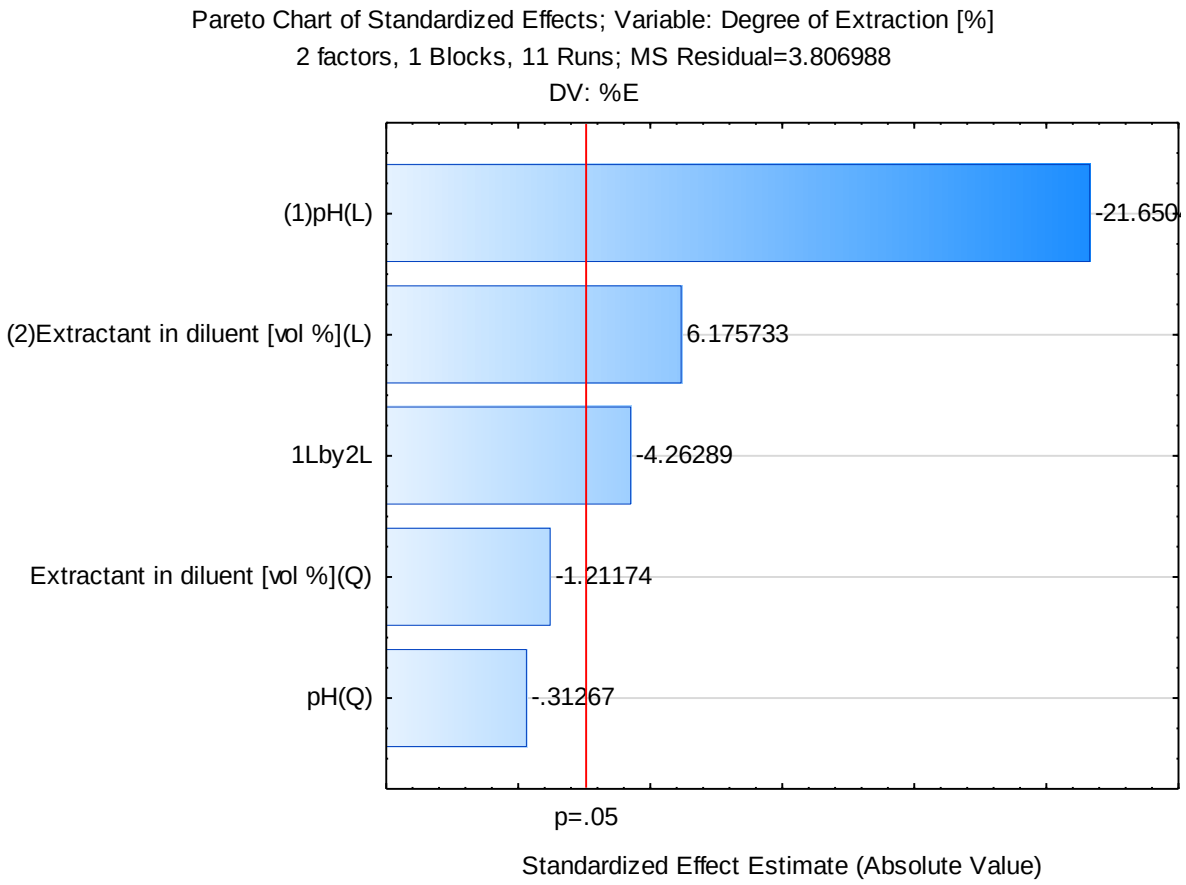

Figure 37: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TBP and canola oil at 37°C.

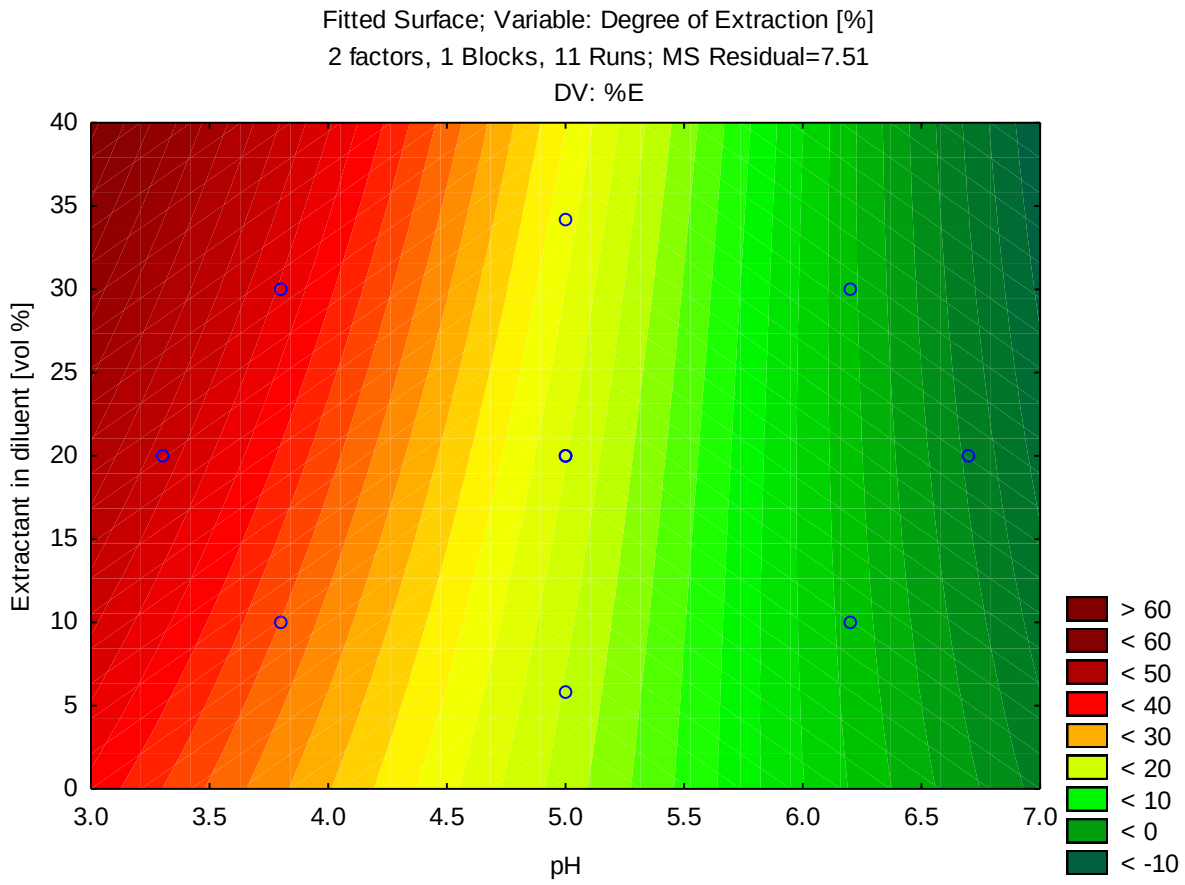

Figure 38: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and oleyl alcohol in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

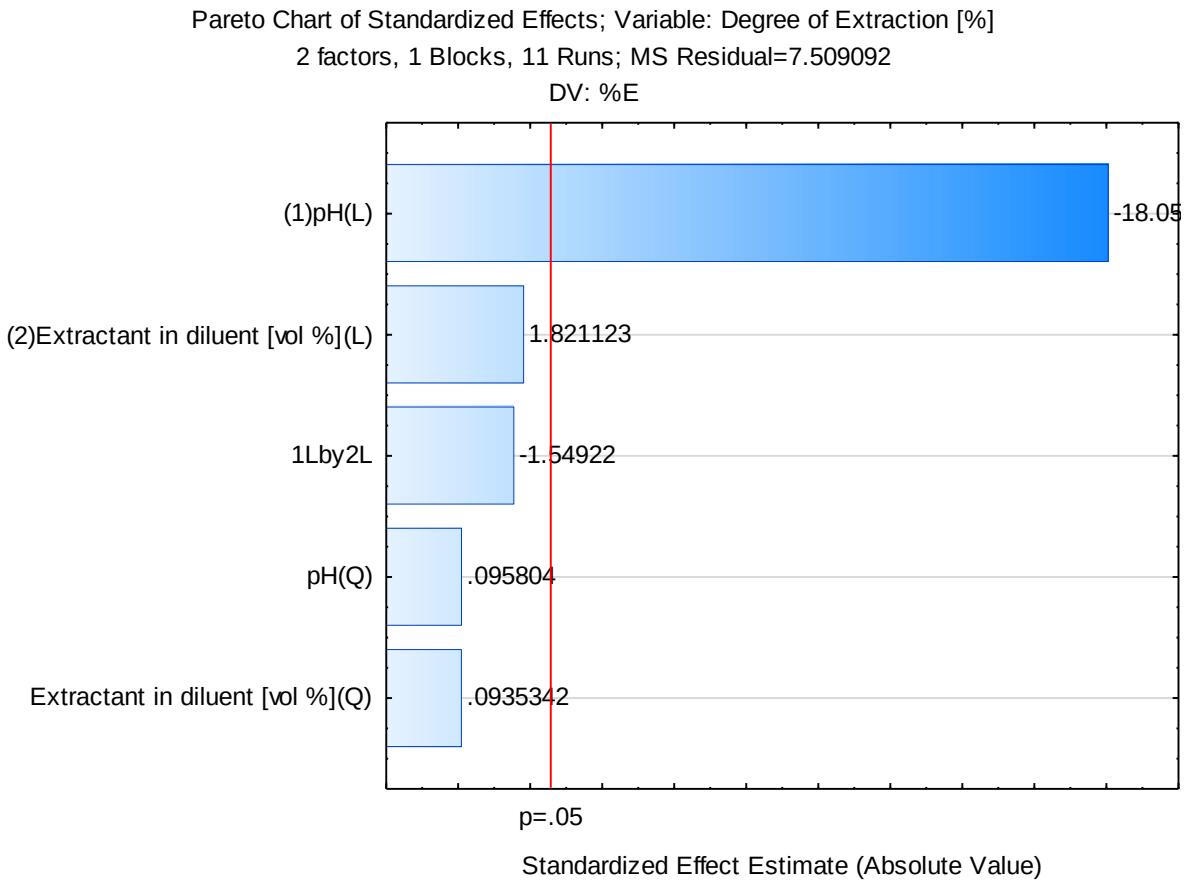

Figure 39: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TBP and oleyl alcohol at 37°C.

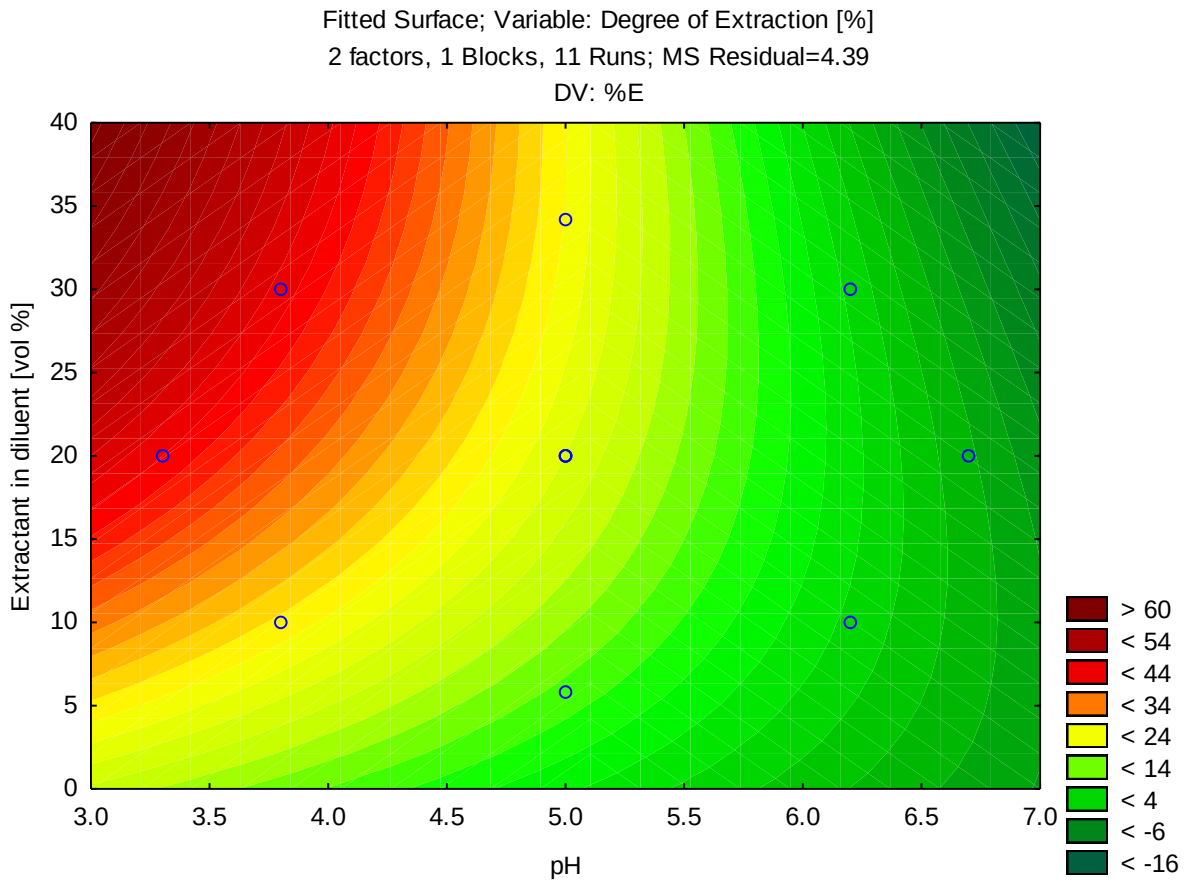

Figure 40: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and lamp oil in a synthetic VFA solution by varying amount of extractant in the solvent and pH at 37°C.

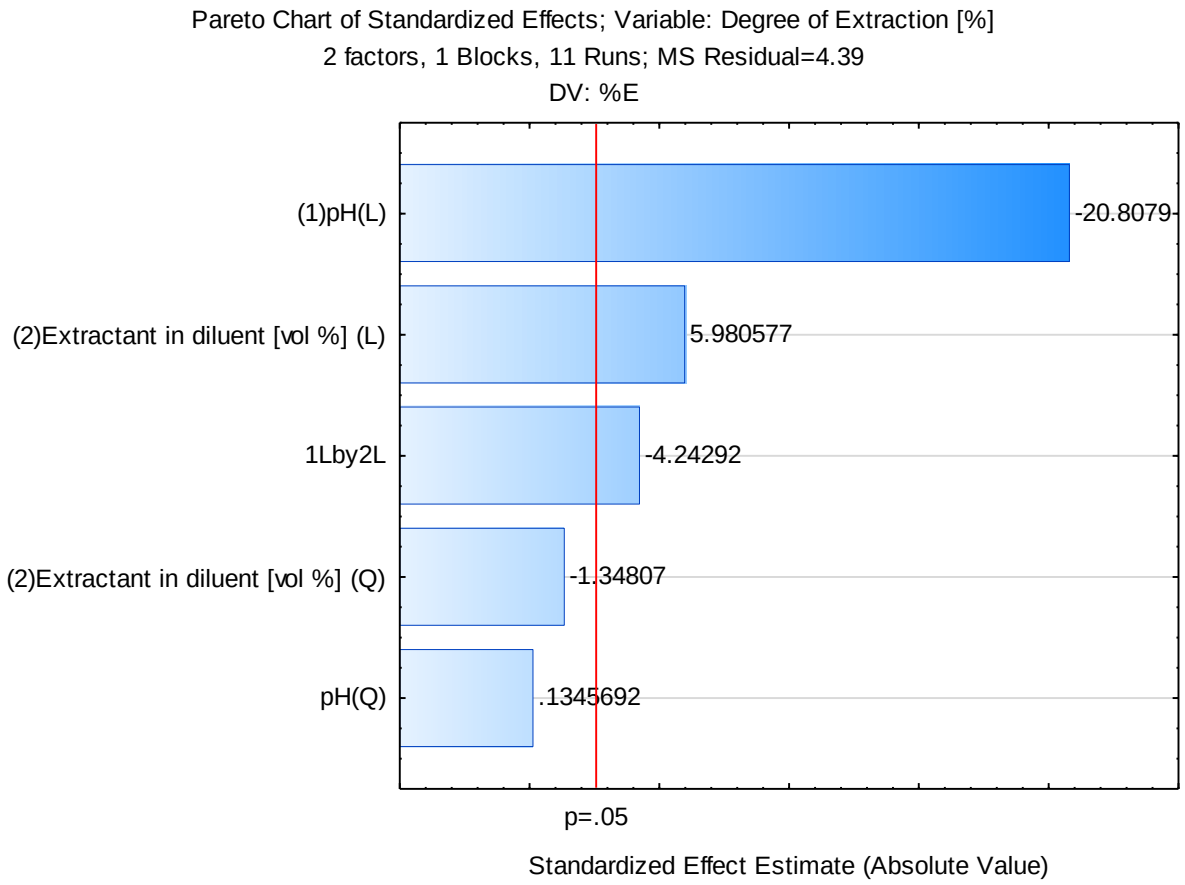

Figure 41: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using a synthetic VFA solution with TBP and lamp oil at 37°C.

D.3 TOA in Diluents with AD effluent

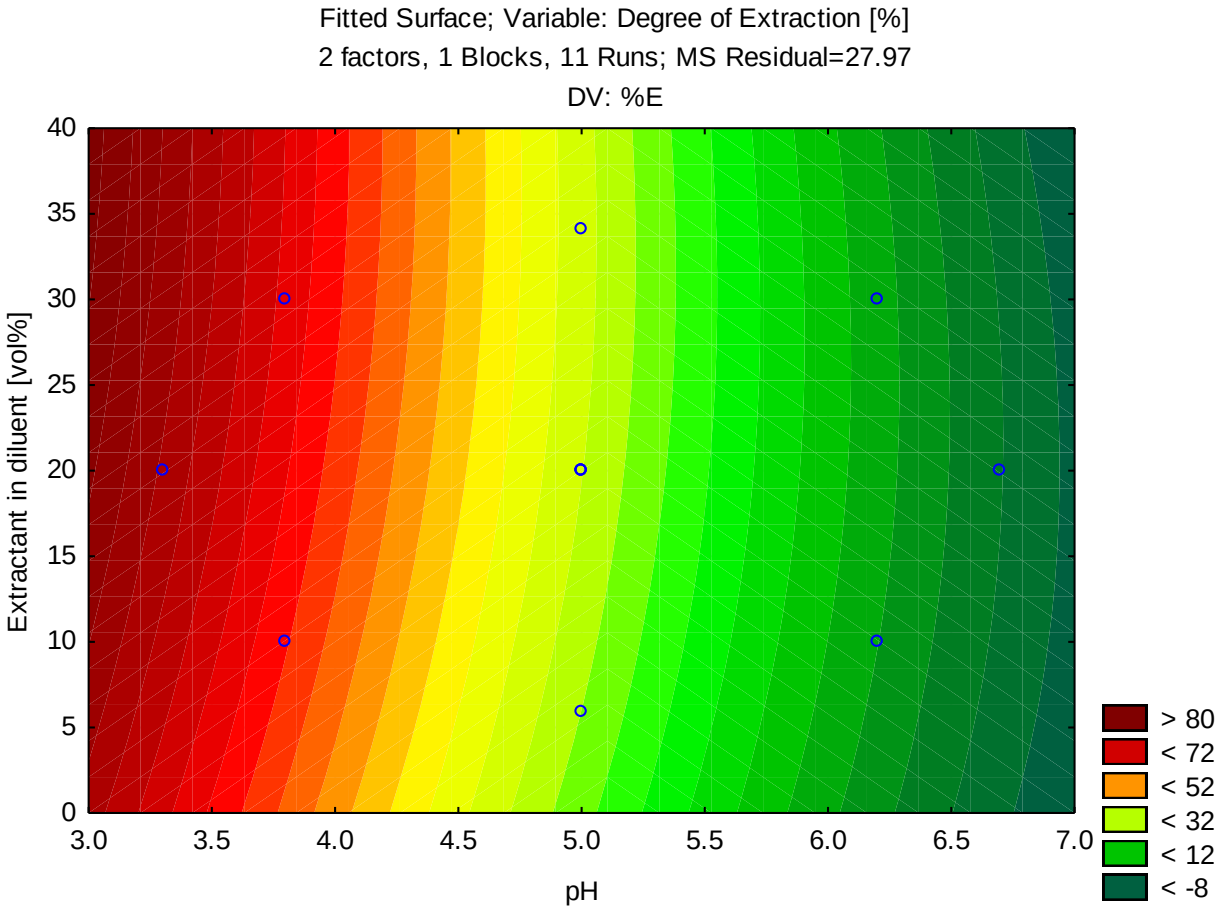

Figure 42: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and canola oil with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

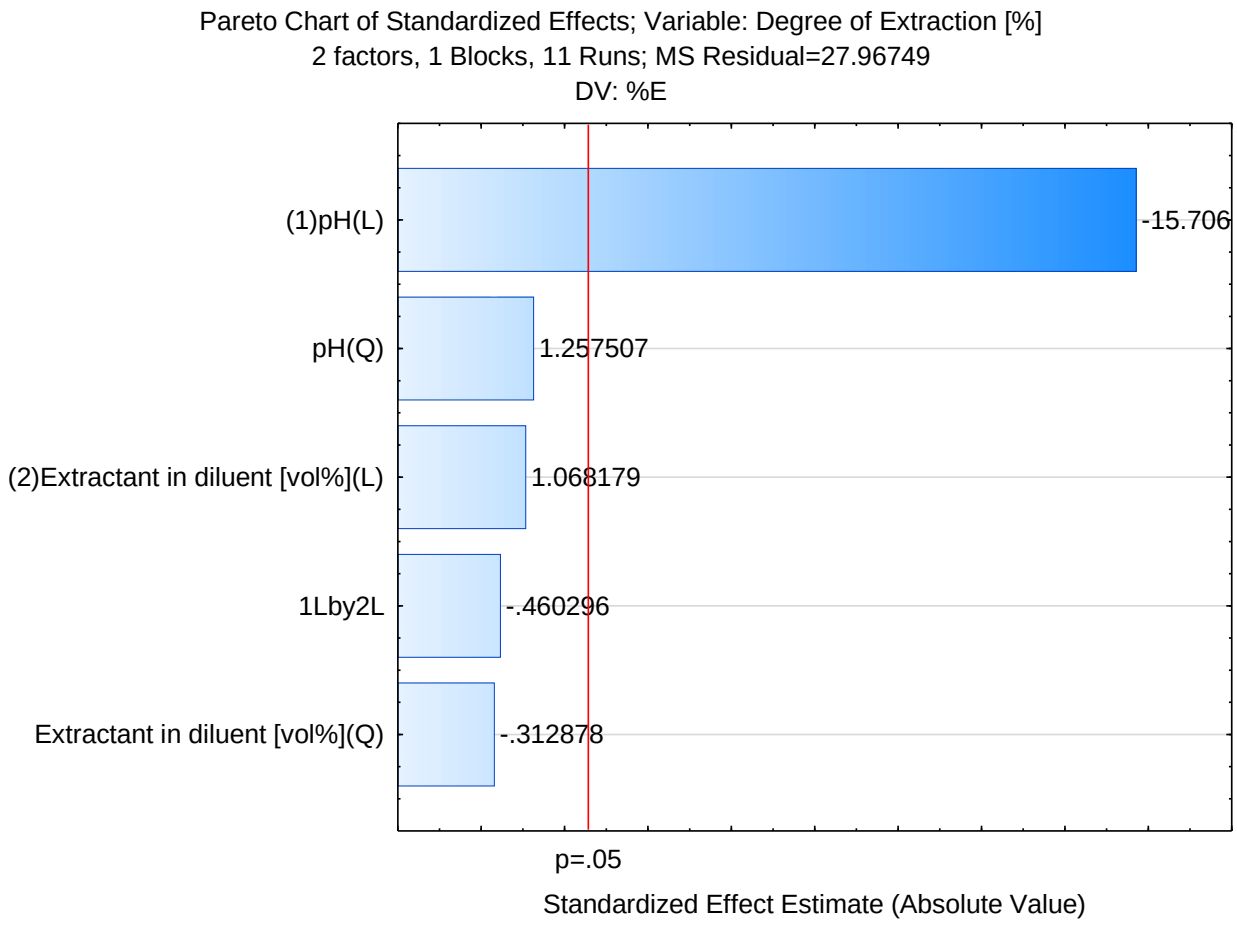

Figure 43: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TOA and canola oil at 37°C.

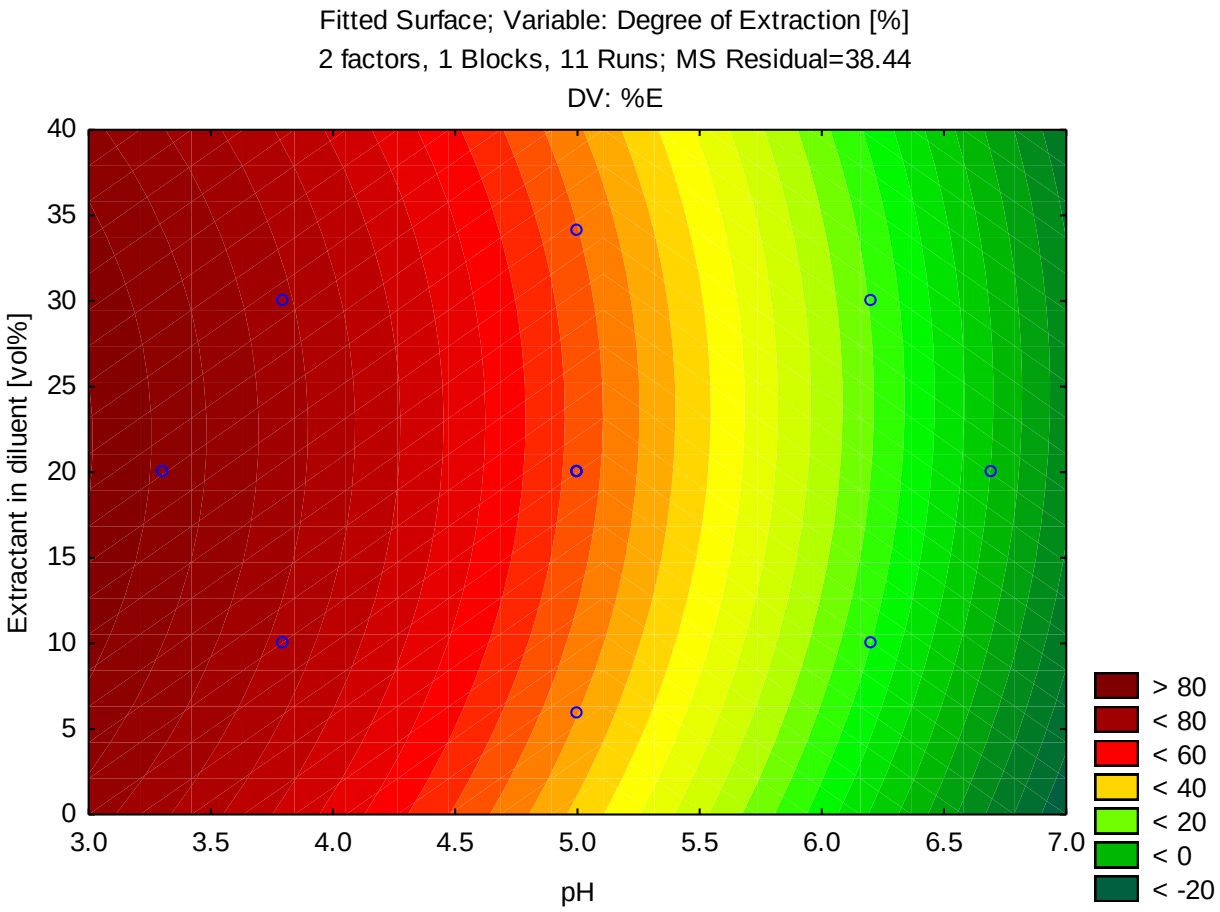

Figure 44: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and oleyl alcohol with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

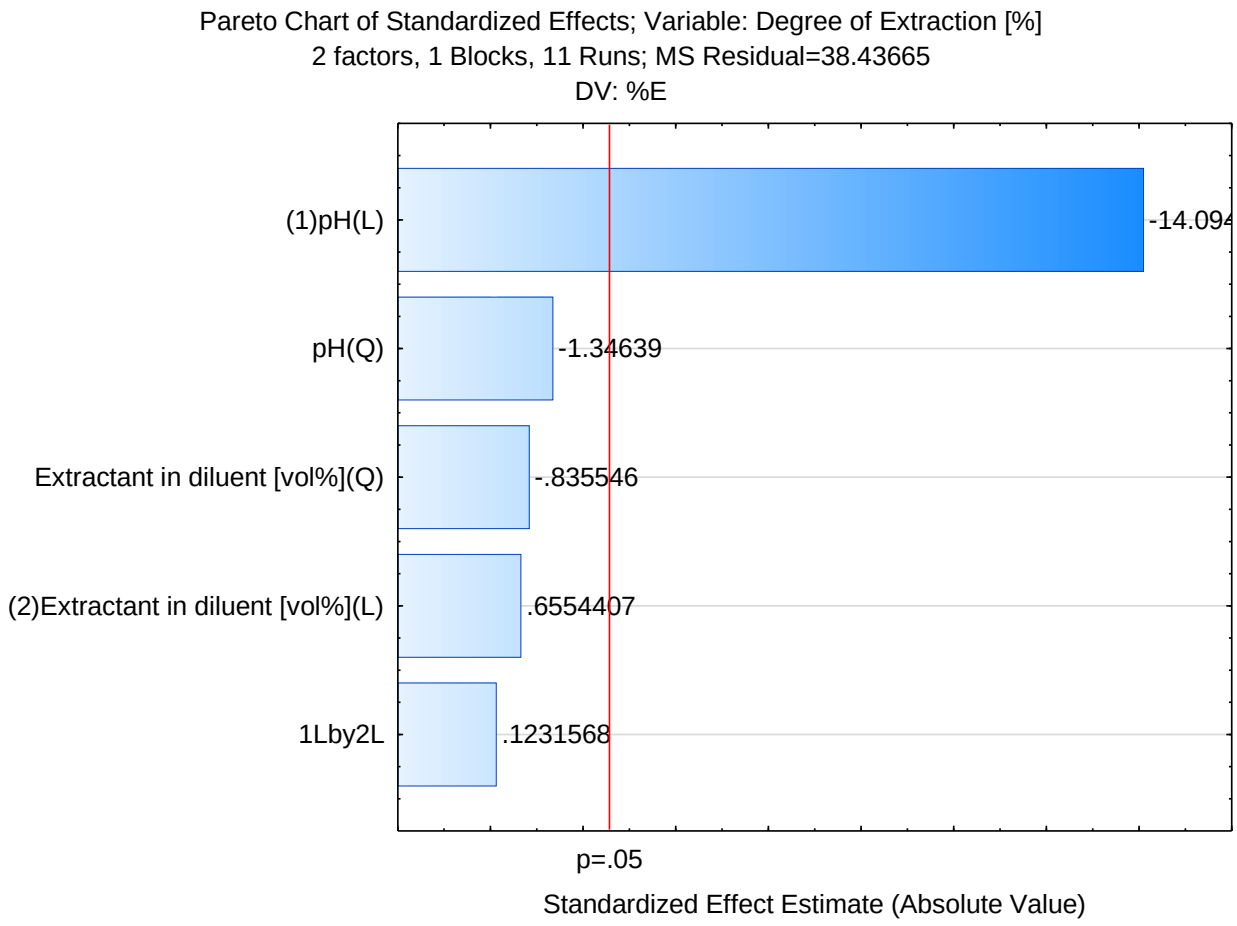

Figure 45: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TOA and oleyl alcohol at 37°C.

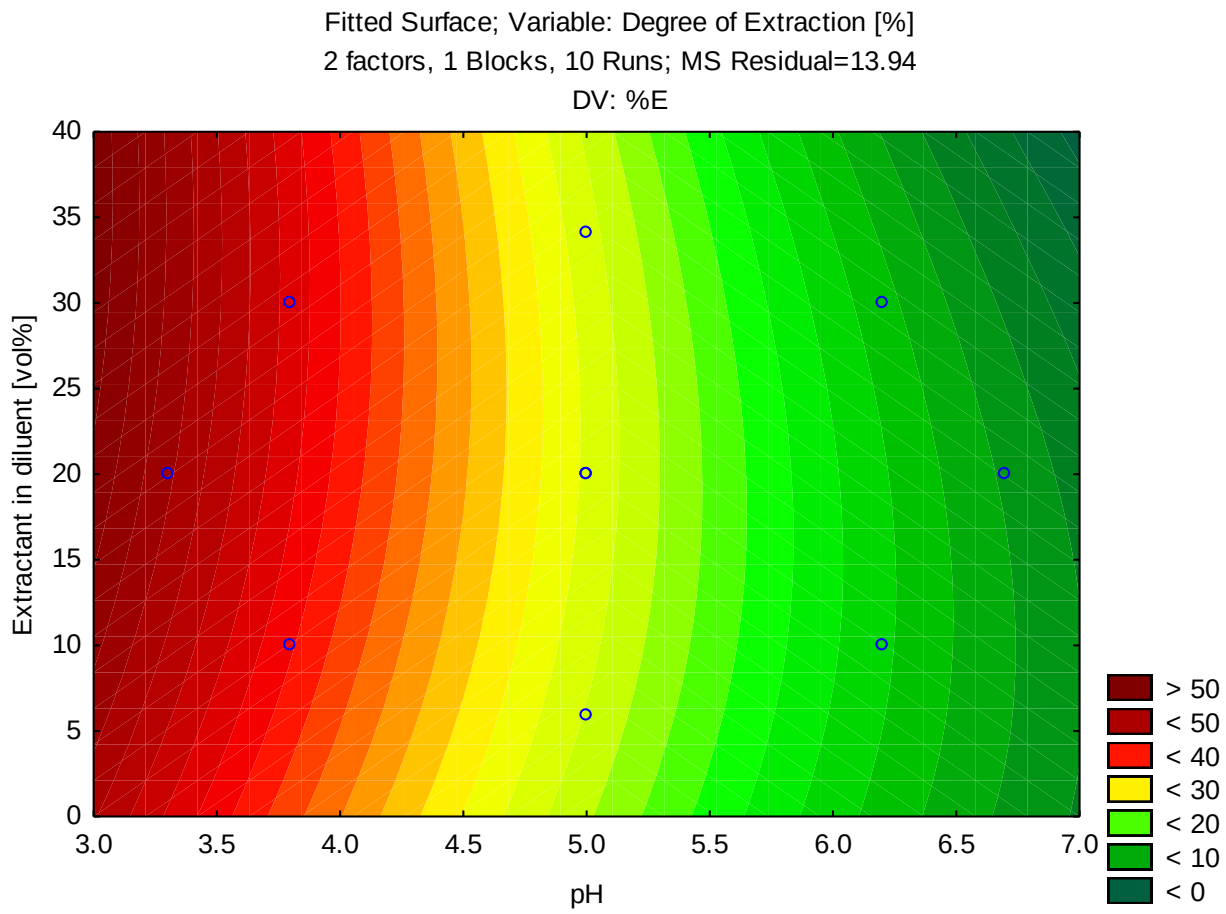

Figure 46: Response curves for degree of extraction (% E) of total VFAs for LLE with TOA and lamp oil with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

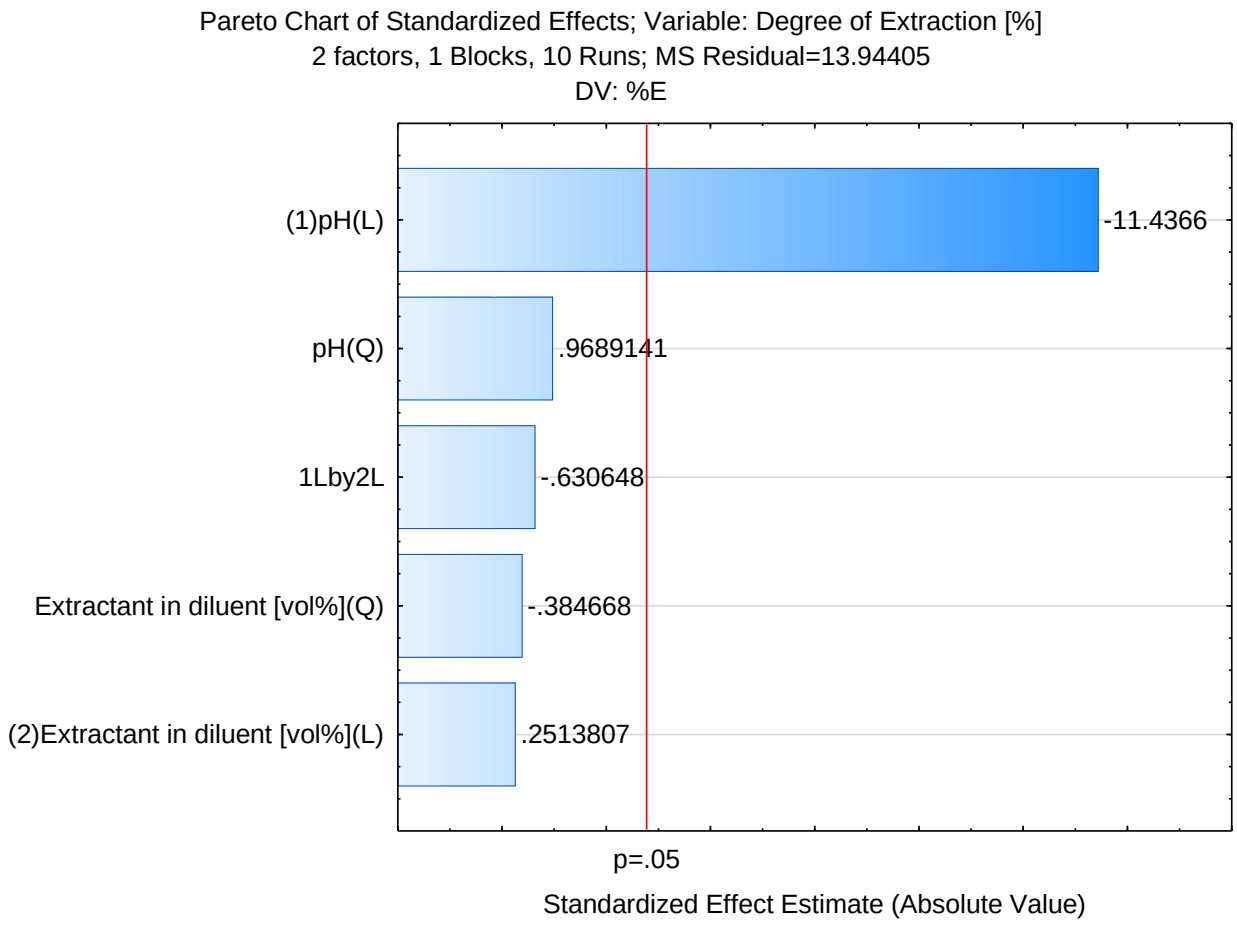

Figure 47: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TOA and lamp oil at 37°C.

D.4 TBP in Diluents with AD effluent

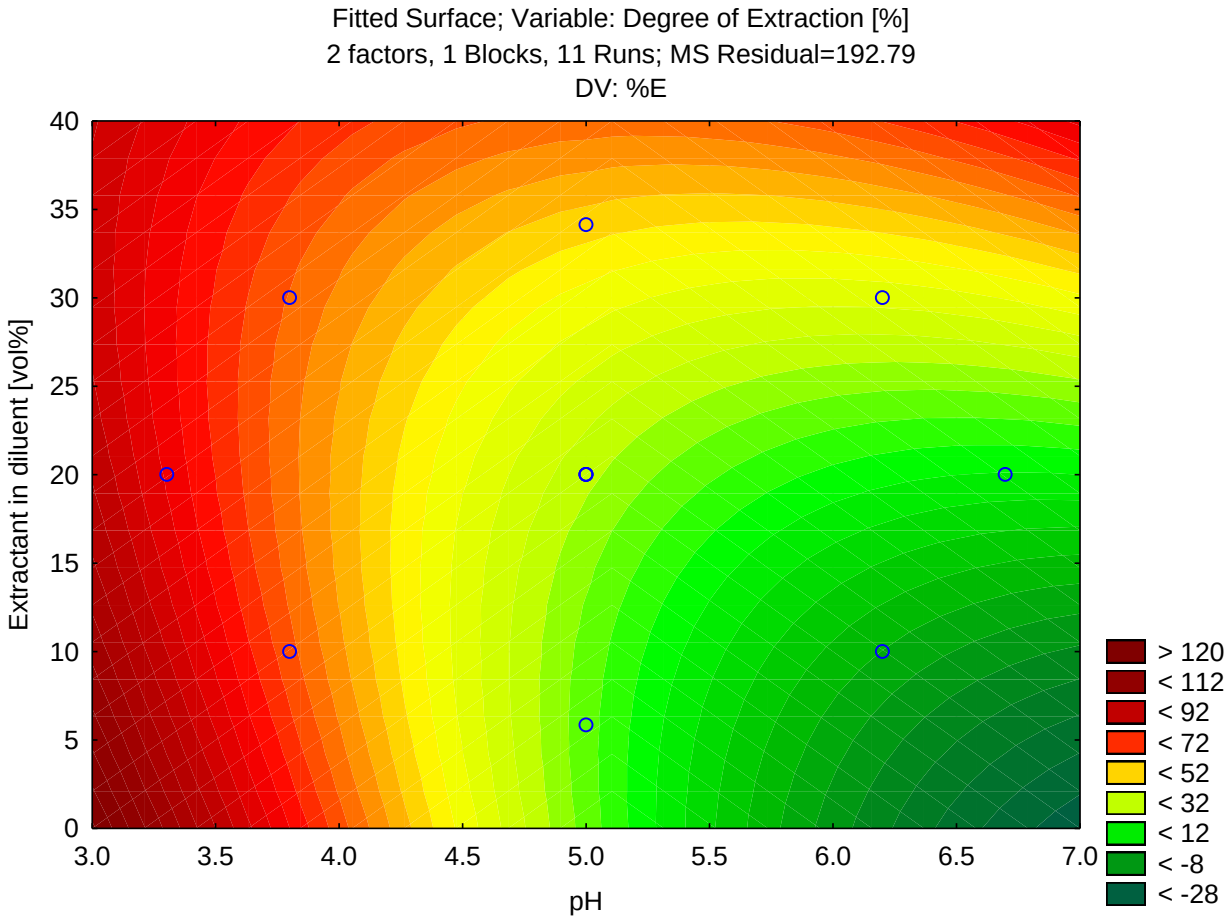

Figure 48: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and canola oil with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

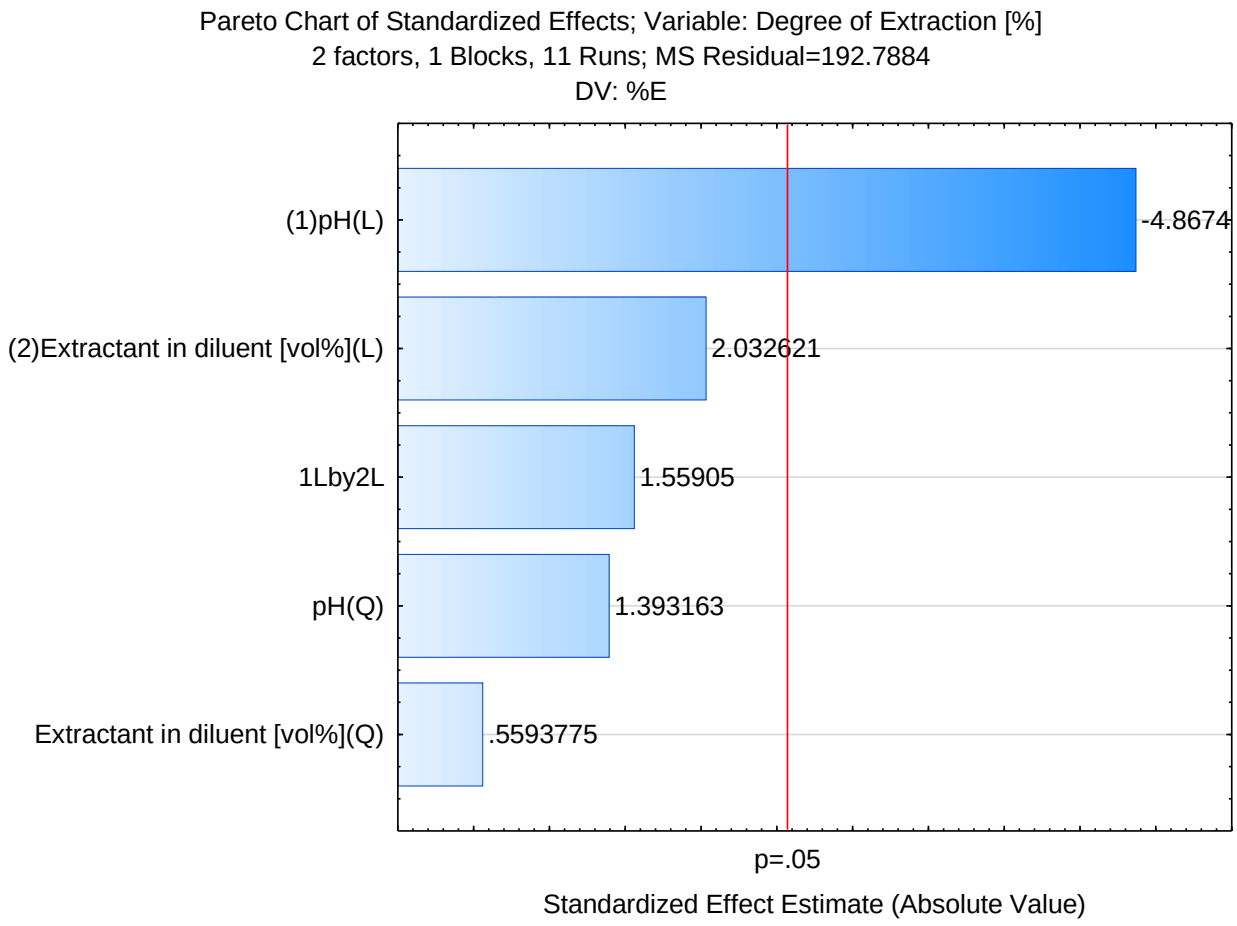

Figure 49: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TBP and canola oil at 37°C.

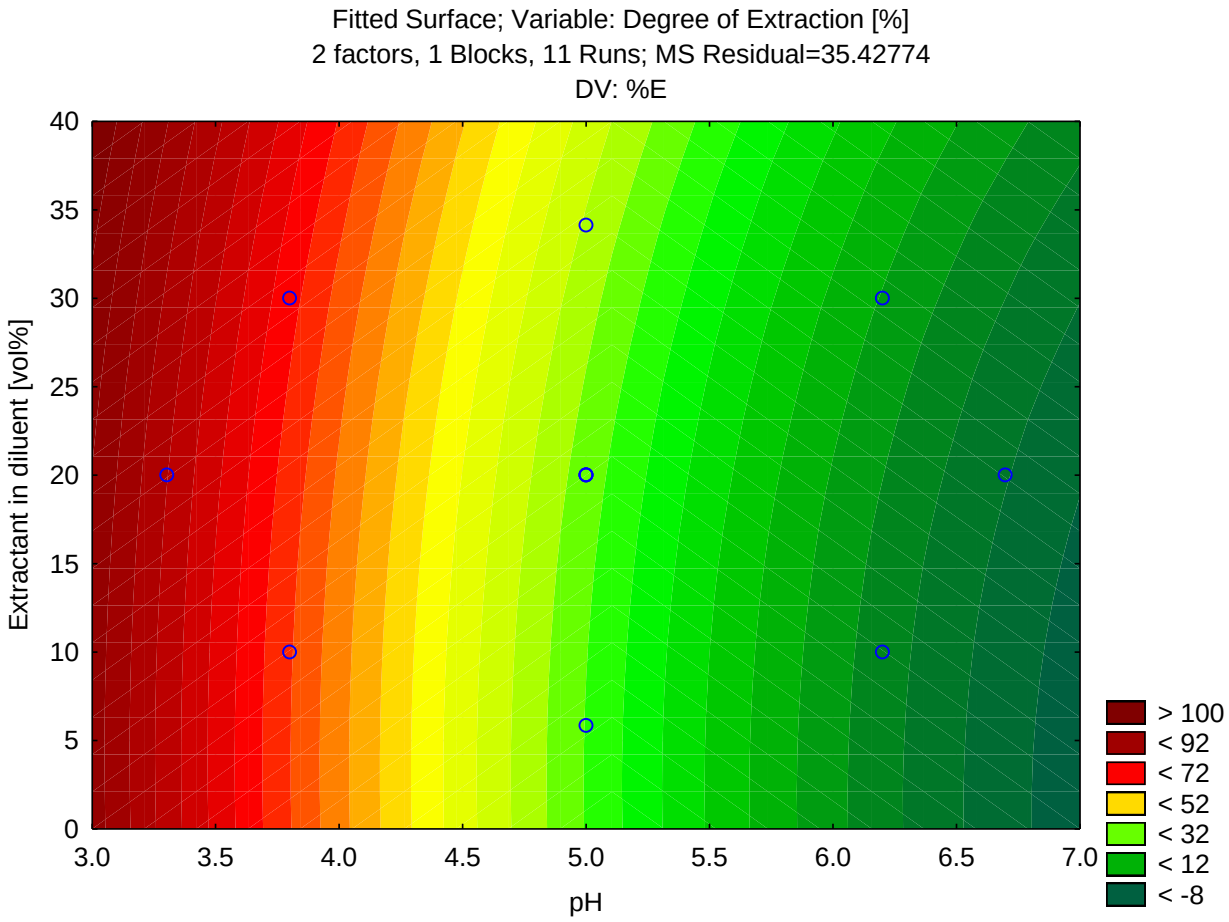

Figure 50: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and oleyl alcohol with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

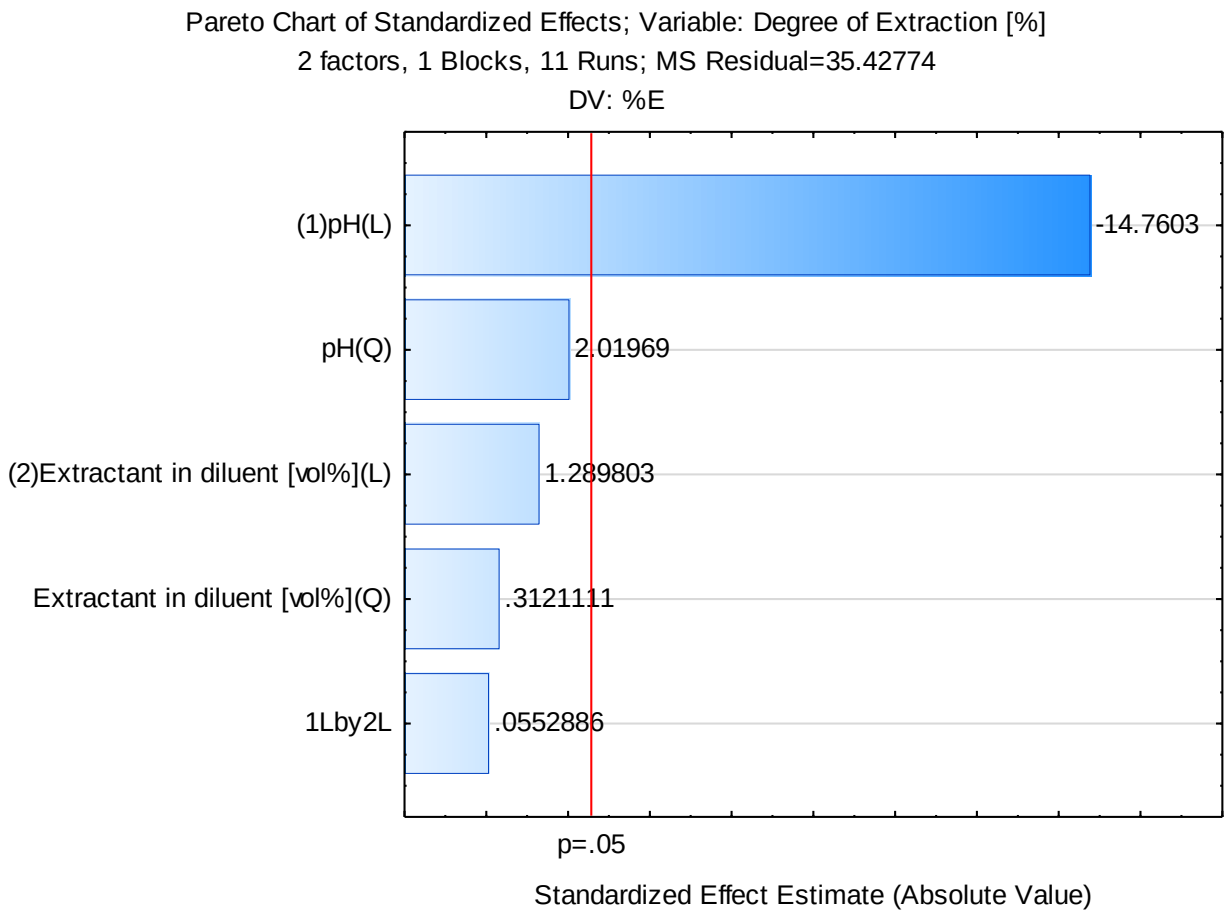

Figure 51: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TBP and oleyl alcohol at 37°C.

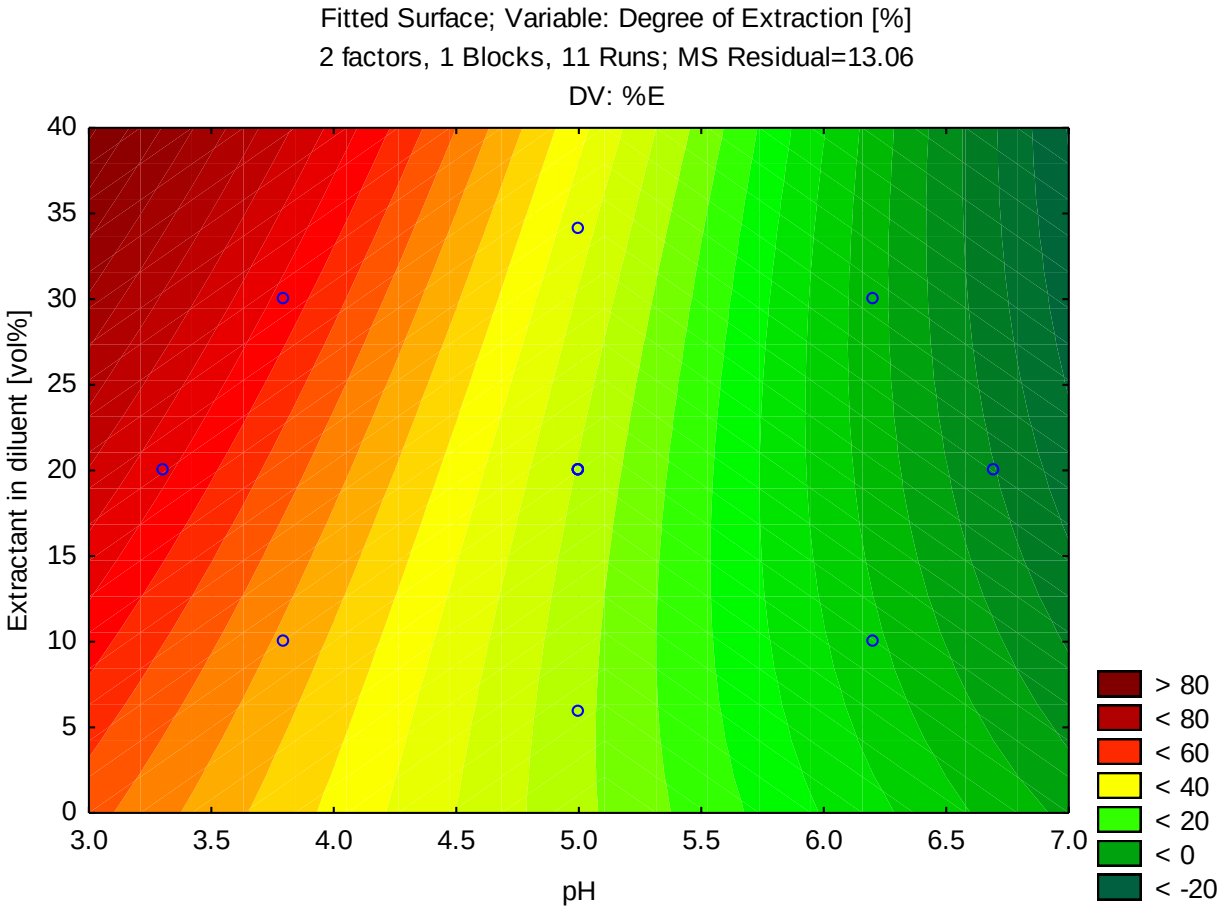

Figure 52: Response curves for degree of extraction (% E) of total VFAs for LLE with TBP and lamp oil with real AD effluent by varying amount of extractant in the solvent and pH at 37°C.

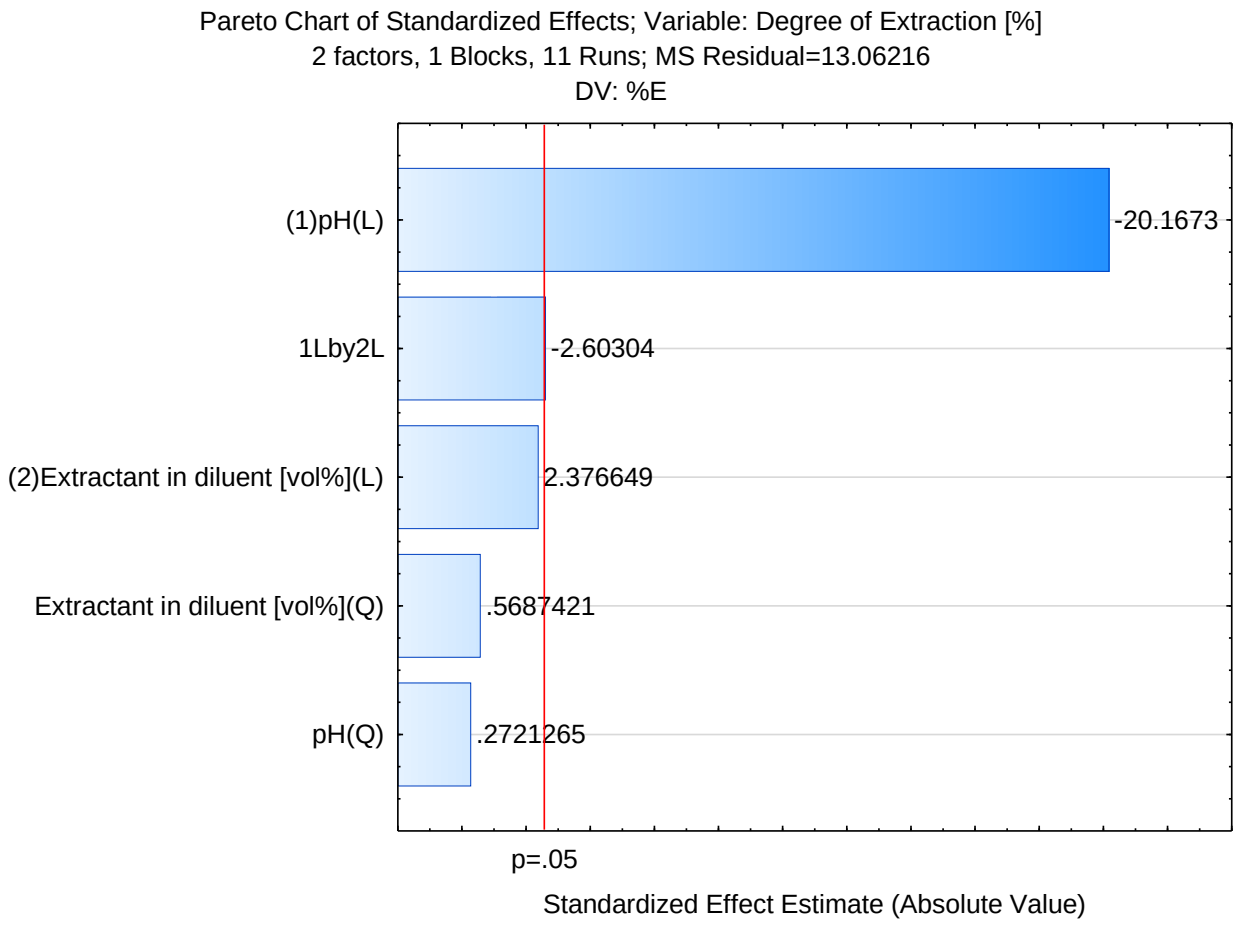

Figure 53: Pareto chart to show the effects of pH and extractant concentration in the solvent on LLE using real AD effluent with TBP and lamp oil at 37°

Appendix E: Error Propagation Calculations

Assumption:

- Errors are small
- Measurements are normally distributed
- Errors are independent

Given a function *f*(x,y), the uncertainty or error propagation for the function is given by the following equation:

$$\Delta e = \sqrt{\left[\left(\frac{\partial f}{\partial x}\right)_{\bar{x},\bar{y}} \Delta_x\right]^2 + \left[\left(\frac{\partial f}{\partial y}\right)_{\bar{x},\bar{y}} \Delta_y\right]^2} \quad (21)$$

Where,

$\left(\frac{\partial f}{\partial x}\right)_{\bar{x},\bar{y}}$ = the partial derivative with respect to x and $\left(\frac{\partial f}{\partial y}\right)_{\bar{x},\bar{y}}$ = the partial derivative to y.

Δ_x and Δ_y are the uncertainty parameter for x and y measurements and calculated using the student's t-statistic and the sample standard error.

Example:

Measurement of VFAs [g/L]	
VFA in aqueous phase	VFA in organic acid
4.780	0.358
4.770	0.368
4.741	0.398

Let the degree of extraction = f(z), VFA in organic phase = x and VFA in aqueous phase = y.

⟶ Degree of extraction (% E) = $\frac{K_d}{K_d+1} \times 100$. However, since $K_d = \frac{x}{y}$.

Therefore, $\% E = \frac{\frac{x}{y}}{\frac{x}{y}+1} \times 100$.

⟶ $\left(\frac{\partial f}{\partial x}\right)_{\bar{x},\bar{y}} = \frac{y}{(x+y)^2}$ and $\left(\frac{\partial f}{\partial y}\right)_{\bar{x},\bar{y}} = -\frac{x}{(x+y)^2}$

MS Excel was used to compute the rest of the calculations. The table below shows the generated data from the given example.

Table 22: Calculations for error propagation using excel

		VFA_{aq} (y)	VFA_{org} (x)	Excel command
Number of samples	n	3	3	count
Sample mean	ybar; xbar	4.764	0.375	average
Sample standard deviation	s	0.021	0.021	stdev
Sample standard error	sn	0.012	0.012	s/sqrt(n)
Significance level	alpha	0.05	0.05	
Student's t-statistic	t(alpha,n-1)	4.303	4.303	t.inv.2t(alpha,n-1)
Uncertainty parameter	Delta_y; Delta_x	0.051	0.051	t(alpha,n-1)*sn
Derivative	df/dy; df/dx	-0.017	0.210	given above
Calculated degree of extraction	% E	7.290		given above
Uncertainty parameter (% E)	Delta_E	0.930		given above

⟶ **% E = 7.29 ± 0.93**

Appendix F: Raw Data

Table 23: VFA concentrations of the aqueous phase and organic phase

					Concentration of acid in the aqueous phase[g/L]					Concentration of acid in the organic phase[g/L]*				
Extractant	Diluent	Type of solution	pH	Vol% extractant	Acetic	Propionic	Butyric	Valeric	Caproic	Acetic	Propionic	Butyric	Valeric	Caproic
TOA	Lamp oil	AD effluent	3.30	20.00	5.01	1.19	3.10	0.78	3.54	0.36	0.27	1.75	0.60	3.54
			3.80	10.00	4.97	1.18	3.08	0.79	3.39	0.16	0.15	1.26	0.53	3.39
			3.80	30.00	4.97	1.18	3.08	0.79	3.39	0.38	0.27	1.82	0.47	3.39
			3.80	20.00	5.06	1.26	3.36	0.72	13.59	0.23	0.25	1.82	0.60	11.49
			5.00	34.14	5.16	1.22	3.20	0.78	3.75	0.51	0.07	0.38	0.20	2.25
			5.00	5.86	5.16	1.22	3.20	0.78	3.75	0.92	0.15	0.25	0.07	1.50
			5.00	20.00	5.16	1.22	3.20	0.78	3.75	1.29	0.20	0.25	0.10	1.66
			5.00	20.00	5.16	1.22	3.20	0.78	3.75	1.66	0.31	0.27	0.06	1.43
			6.20	30.00	5.13	1.23	3.29	0.79	3.86	0.68	0.15	0.31	0.08	0.55
			6.20	10.00	5.13	1.23	3.29	0.79	3.86	0.84	0.19	0.39	0.11	0.47
			6.20	20.00	5.32	1.33	3.52	0.73	14.64	0.37	0.19	0.10	-0.03	0.49
			6.70	20.00	4.26	0.80	3.24	0.77	3.80	0.01	-0.14	0.51	0.07	0.36
		Synthetic	3.30	20.00	9.51	2.14	1.98	1.02	0.00	0.50	0.44	1.00	0.83	0.00
			3.80	10.00	9.47	2.13	1.98	1.01	0.00	0.28	0.26	0.69	0.71	0.00
			3.80	30.00	9.47	2.13	1.98	1.01	0.00	0.63	0.55	1.16	0.88	0.00
			3.80	20.00	9.53	2.13	1.98	1.02	0.00	0.43	0.41	0.96	0.82	0.00
			5.00	5.86	9.27	2.09	1.93	0.99	0.00	0.05	0.06	0.20	0.32	0.00
			5.00	34.14	9.27	2.09	1.93	0.99	0.00	0.17	0.22	0.60	0.66	0.00
			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.14	0.15	0.44	0.55	0.00

			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.14	0.15	0.43	0.54	0.00
			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.12	0.15	0.44	0.55	0.00
			6.20	10.00	9.13	2.05	1.90	0.98	0.00	-0.02	0.00	0.02	0.05	0.00
			6.20	30.00	9.13	2.05	1.90	0.98	0.00	0.00	0.01	0.06	0.12	0.00
			6.20	20.00	9.21	2.06	1.91	0.99	0.00	0.06	0.03	0.05	0.15	0.00
			6.70	20.00	9.15	2.06	1.90	0.99	0.00	0.00	0.01	0.01	0.04	0.00
			3.30	20.00	5.25	1.29	3.47	0.72	14.53	1.93	0.84	3.07	0.69	14.11
			3.80	10.00	5.04	1.26	3.33	0.71	13.46	1.38	0.69	2.85	0.68	13.12
			3.80	30.00	5.04	1.26	3.33	0.71	13.46	1.52	0.74	2.90	0.68	13.11
			3.80	20.00	5.06	1.26	3.36	0.72	13.59	1.56	0.75	2.87	0.69	13.16
			5.00	5.90	5.14	1.28	3.42	0.75	14.30	0.42	0.28	0.91	0.43	9.05
			5.00	34.10	5.14	1.28	3.42	0.75	14.30	0.41	0.30	1.08	0.50	10.21
		AD effluent	5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.36	0.27	1.02	0.47	10.43
			5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.37	0.27	1.06	0.43	10.43
			5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.40	0.28	1.05	0.50	10.36
			6.20	10.00	4.90	1.24	3.35	0.80	14.34	-0.22	0.16	-0.06	0.07	1.69
	Oleyl alcohol		6.20	30.00	4.90	1.24	3.35	0.80	14.34	-0.21	0.12	-0.02	0.10	2.28
			6.20	20.00	5.32	1.33	3.52	0.73	14.64	0.23	0.25	0.13	0.03	2.84
			6.70	20.00	5.12	1.28	3.48	0.74	14.33	0.01	0.14	0.06	0.00	1.70
			3.30	20.00	9.58	2.14	1.99	1.03	0.00	5.85	1.77	1.87	1.01	0.00
			3.80	10.00	9.53	2.13	1.98	1.02	0.00	4.36	1.55	1.79	1.00	0.00
			3.80	30.00	9.53	2.13	1.98	1.02	0.00	5.40	1.73	1.86	1.01	0.00
			3.80	20.00	9.53	2.13	1.98	1.02	0.00	5.40	1.72	1.85	1.00	0.00
		Synthetic	5.00	5.86	9.33	2.09	1.94	1.01	0.00	0.96	0.69	1.22	0.88	0.00
			5.00	34.14	9.33	2.09	1.94	1.01	0.00	1.37	0.85	1.37	0.91	0.00
			5.00	20.00	9.33	2.09	1.94	1.01	0.00	1.34	0.83	1.35	0.91	0.00
			5.00	20.00	9.33	2.09	1.94	1.01	0.00	1.34	0.83	1.35	0.91	0.00
			5.00	20.00	9.33	2.09	1.94	1.01	0.00	1.33	0.83	1.35	0.91	0.00

		6.20	10.00	9.21	2.06	1.91	0.99	0.00	-0.12	0.00	0.10	0.22	0.00
		6.20	30.00	9.21	2.06	1.91	0.99	0.00	-0.08	0.01	0.12	0.23	0.00
		6.20	20.00	9.21	2.06	1.91	0.99	0.00	-0.05	0.02	0.13	0.29	0.00
		6.70	20.00	9.12	2.06	1.91	0.99	0.00	-0.20	-0.03	0.01	0.08	0.00
Canola oil	AD effluent	3.30	20.00	5.25	1.29	3.47	0.72	14.53	0.82	0.39	2.56	0.66	13.89
		3.80	10.00	5.04	1.26	3.33	0.71	13.46	0.38	0.33	2.07	0.62	12.25
		3.80	30.00	5.04	1.26	3.33	0.71	13.46	0.66	0.49	2.47	0.65	12.77
		3.80	20.00	5.06	1.26	3.36	0.72	13.59	0.57	0.42	2.35	0.64	12.58
		5.00	5.90	5.14	1.28	3.42	0.75	14.30	-0.06	0.03	0.48	0.30	5.86
		5.00	34.10	5.14	1.28	3.42	0.75	14.30	-0.05	0.04	0.66	0.40	7.15
		5.00	20.00	5.14	1.28	3.42	0.75	14.30	-0.02	0.05	0.63	0.38	7.06
		5.00	20.00	5.14	1.28	3.42	0.75	14.30	-0.07	0.03	0.59	0.38	7.03
		5.00	20.00	5.14	1.28	3.42	0.75	14.30	-0.07	0.03	0.58	0.37	7.03
		6.20	10.00	4.90	1.24	3.35	0.80	14.34	0.50	0.02	-0.99	0.03	0.75
		6.20	30.00	4.90	1.24	3.35	0.80	14.34	1.12	0.06	-1.82	0.06	1.16
		6.20	20.00	5.32	1.33	3.52	0.73	14.64	0.43	0.16	-0.07	-0.01	0.93
		6.70	20.00	5.12	1.28	3.48	0.74	14.33	0.58	0.07	-0.69	0.00	0.28
	Synthetic	3.30	20.00	9.58	2.14	1.99	1.03	0.00	1.23	0.80	1.38	0.93	0.00
		3.80	10.00	9.53	2.13	1.98	1.02	0.00	0.73	0.56	1.14	0.85	0.00
		3.80	30.00	9.53	2.13	1.98	1.02	0.00	1.29	0.86	1.43	0.93	0.00
		3.80	20.00	9.53	2.13	1.98	1.02	0.00	1.15	0.78	1.36	0.92	0.00
		5.00	5.86	9.33	2.09	1.94	1.01	0.00	0.13	0.17	0.48	0.57	0.00
		5.00	34.14	9.33	2.09	1.94	1.01	0.00	0.32	0.36	0.84	0.76	0.00
		5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.24	0.29	0.72	0.71	0.00
		5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.25	0.29	0.73	0.71	0.00
		5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.25	0.29	0.74	0.71	0.00
		6.20	10.00	9.21	2.06	1.91	0.99	0.00	-0.01	0.01	0.04	0.09	0.00
		6.20	30.00	9.21	2.06	1.91	0.99	0.00	0.00	0.02	0.09	0.14	0.00

			6.20	20.00	9.21	2.06	1.91	0.99	0.00	0.06	0.03	0.08	0.20	0.00
			6.70	20.00	9.12	2.06	1.91	0.99	0.00	-0.09	-0.01	0.02	0.03	0.00
TBP	Lamp oil	AD effluent	3.30	20.00	5.01	1.19	3.10	0.78	3.54	1.04	0.63	2.55	0.69	3.54
			3.80	10.00	4.97	1.18	3.08	0.79	3.39	0.41	0.36	1.96	0.61	3.24
			3.80	30.00	4.97	1.18	3.08	0.79	3.39	1.41	0.72	2.65	0.71	3.39
			3.80	20.00	5.06	1.26	3.36	0.72	13.59	0.72	0.59	2.78	0.68	12.67
			5.00	5.86	5.16	1.22	3.20	0.78	3.75	0.87	0.07	0.30	0.16	1.93
			5.00	34.14	5.16	1.22	3.20	0.78	3.75	-0.06	0.07	0.73	0.37	3.11
			5.00	20.00	5.16	1.22	3.20	0.78	3.75	-0.04	0.05	0.64	0.33	2.89
			5.00	20.00	5.16	1.22	3.20	0.78	3.75	-0.05	0.07	0.66	0.34	2.91
			5.00	20.00	5.16	1.22	3.20	0.78	3.75	-0.03	0.04	0.64	0.33	2.93
			6.20	10.00	5.13	1.23	3.29	0.79	3.86	0.50	-0.03	0.01	-0.01	0.47
			6.20	30.00	5.13	1.23	3.29	0.79	3.86	-0.17	-0.02	0.01	0.05	0.83
			6.20	20.00	5.32	1.33	3.52	0.73	14.64	-0.01	0.00	-0.02	0.05	1.26
			6.70	20.00	4.26	0.80	3.24	0.77	3.80	-1.03	-0.46	-0.08	-0.16	0.39
		Synthetic	3.30	20.00	9.51	2.14	1.98	1.02	0.00	2.02	1.18	1.63	0.97	0.00
			3.80	10.00	9.47	2.13	1.98	1.01	0.00	0.95	0.76	1.34	0.90	0.00
			3.80	30.00	9.47	2.13	1.98	1.01	0.00	2.53	1.35	1.71	0.98	0.00
			3.80	20.00	9.53	2.13	1.98	1.02	0.00	1.82	1.13	1.60	0.97	0.00
			5.00	5.86	9.27	2.09	1.93	0.99	0.00	0.19	0.22	0.59	0.64	0.00
			5.00	34.14	9.27	2.09	1.93	0.99	0.00	0.65	0.66	1.20	0.87	0.00
			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.47	0.50	1.04	0.83	0.00
			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.48	0.51	1.04	0.84	0.00
			5.00	20.00	9.27	2.09	1.93	0.99	0.00	0.47	0.51	1.04	0.83	0.00
			6.20	10.00	9.13	2.05	1.90	0.98	0.00	-0.02	0.02	0.08	0.17	0.00
			6.20	30.00	9.13	2.05	1.90	0.98	0.00	-0.08	0.02	0.11	0.23	0.00
			6.20	20.00	9.21	2.06	1.91	0.99	0.00	0.00	0.03	0.11	0.25	0.00
			6.70	20.00	9.15	2.06	1.90	0.99	0.00	-0.03	0.01	0.04	0.08	0.00

		3.30	20.00	5.25	1.29	3.47	0.72	14.53	1.35	0.69	2.94	0.68	13.58
		3.80	10.00	5.04	1.26	3.33	0.71	13.46	0.68	0.55	2.58	0.65	12.27
		3.80	30.00	5.04	1.26	3.33	0.71	13.46	1.13	0.71	2.81	0.67	12.76
		3.80	20.00	5.06	1.26	3.36	0.72	13.59	0.98	0.62	2.83	0.67	12.72
		5.00	5.90	5.14	1.28	3.42	0.75	14.30	0.22	0.22	0.71	0.38	5.01
		5.00	34.10	5.14	1.28	3.42	0.75	14.30	0.33	0.22	0.86	0.44	6.40
	AD effluent	5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.24	0.25	0.74	0.41	6.17
		5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.26	0.24	0.73	0.41	5.64
		5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.22	0.26	0.73	0.41	5.75
		6.20	10.00	4.90	1.24	3.35	0.80	14.34	-0.22	0.08	-0.12	-0.01	0.10
		6.20	30.00	4.90	1.24	3.35	0.80	14.34	-0.25	0.04	-0.06	0.01	1.64
		6.20	20.00	5.32	1.33	3.52	0.73	14.64	0.21	0.02	0.07	0.03	1.02
Oleyl		6.70	20.00	5.12	1.28	3.48	0.74	14.33	-0.04	0.10	0.00	-0.05	0.07
alcohol		3.30	20.00	9.58	2.14	1.99	1.03	0.00	2.31	1.23	1.65	0.98	0.00
		3.80	10.00	9.53	2.13	1.98	1.02	0.00	1.71	1.07	1.55	0.95	0.00
		3.80	30.00	9.53	2.13	1.98	1.02	0.00	2.52	1.31	1.68	0.98	0.00
		3.80	20.00	9.53	2.13	1.98	1.02	0.00	2.13	1.21	1.63	0.97	0.00
		5.00	5.86	9.33	2.09	1.94	1.01	0.00	0.37	0.45	0.96	0.80	0.00
		5.00	34.14	9.33	2.09	1.94	1.01	0.00	0.56	0.63	1.16	0.86	0.00
	Synthetic	5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.49	0.53	1.06	0.83	0.00
		5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.42	0.53	1.06	0.84	0.00
		5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.46	0.54	1.07	0.84	0.00
		6.20	10.00	9.21	2.06	1.91	0.99	0.00	-0.10	0.00	0.07	0.18	0.00
		6.20	30.00	9.21	2.06	1.91	0.99	0.00	-0.15	0.00	0.07	0.19	0.00
		6.20	20.00	9.21	2.06	1.91	0.99	0.00	-0.10	0.01	0.09	0.24	0.00
		6.70	20.00	9.12	2.06	1.91	0.99	0.00	-0.20	-0.03	-0.02	0.05	0.00
Canola	AD	3.30	20.00	5.25	1.29	3.47	0.72	14.53	1.07	0.59	2.74	0.67	13.90
oil	effluent	3.80	10.00	5.04	1.26	3.33	0.71	13.46	0.37	0.35	2.09	0.61	11.93

	3.80	30.00	5.04	1.26	3.33	0.71	13.46	1.03	0.66	2.76	0.66	12.82
	3.80	20.00	5.06	1.26	3.36	0.72	13.59	0.77	0.57	2.72	0.67	12.59
	5.00	5.90	5.14	1.28	3.42	0.75	14.30	0.28	0.05	0.45	0.29	4.40
	5.00	34.10	5.14	1.28	3.42	0.75	14.30	-0.03	0.08	0.78	0.43	6.85
	5.00	20.00	5.14	1.28	3.42	0.75	14.30	-0.08	0.05	0.64	0.40	6.23
	5.00	20.00	5.14	1.28	3.42	0.75	14.30	-0.04	0.04	0.64	0.39	5.99
	5.00	20.00	5.14	1.28	3.42	0.75	14.30	0.02	0.07	0.73	0.40	6.30
	6.20	10.00	4.90	1.24	3.35	0.80	14.34	-0.15	0.07	-0.08	0.08	0.37
	6.20	30.00	4.90	1.24	3.35	0.80	14.34	-0.48	-0.11	-0.14	0.10	14.24
	6.20	20.00	5.32	1.33	3.52	0.73	14.64	-0.02	0.00	0.05	0.05	1.08
	6.70	20.00	5.12	1.28	3.48	0.74	14.33	-0.25	-0.07	-0.05	0.02	0.07
Synthetic	3.30	20.00	9.58	2.14	1.99	1.03	0.00	1.89	1.09	1.57	0.96	0.00
	3.80	10.00	9.53	2.13	1.98	1.02	0.00	0.90	0.70	1.26	0.90	0.00
	3.80	30.00	9.53	2.13	1.98	1.02	0.00	2.37	1.26	1.65	0.97	0.00
	3.80	20.00	9.53	2.13	1.98	1.02	0.00	1.68	1.04	1.53	0.95	0.00
	5.00	5.86	9.33	2.09	1.94	1.01	0.00	0.17	0.22	0.58	0.63	0.00
	5.00	34.14	9.33	2.09	1.94	1.01	0.00	0.63	0.63	1.16	0.86	0.00
	5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.45	0.47	0.97	0.81	0.00
	5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.45	0.48	0.97	0.81	0.00
	5.00	20.00	9.33	2.09	1.94	1.01	0.00	0.47	0.49	0.99	0.82	0.00
	6.20	10.00	9.21	2.06	1.91	0.99	0.00	-0.06	0.01	0.06	0.13	0.00
	6.20	30.00	9.21	2.06	1.91	0.99	0.00	-0.10	0.01	0.10	0.19	0.00
	6.20	20.00	9.21	2.06	1.91	0.99	0.00	0.02	0.03	0.10	0.18	0.00
	6.70	20.00	9.12	2.06	1.91	0.99	0.00	-0.11	-0.01	0.00	0.05	0.00

*Determined by mass balance