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Reactive extraction for *in-situ* carboxylate recovery from mixed culture fermentation

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Abstract

Biorefinery wastewaters can be fermented to produce carboxylates which are high-value platform chemicals. However, the major challenges in this fermentation are limited product yields and productivities faced due to product inhibition and difficulty in carboxylate separation and recovery from fermentation broths. To mitigate the above problems, process optimization via integrated fermentation-separation i.e. *in-situ* product recovery (ISPR) systems can be considered. As a first step towards development of such coupled carboxylate bioprocesses, this study aimed to provide a detailed analysis of extraction behaviour of a wide array of extractants and diluents for C2-C6 carboxylates in synthetic solutions and real effluent from acidogenic fermentation. Compared to physical extraction without extractant, a 75-85% increase was achieved when using reactive extraction (RE) and the difference was more pronounced for short chain carboxylates, particularly at pH 4.5. Distribution coefficients and extraction efficiencies increased with increasing extractant concentration and reached an equilibrium at molar ratio of 1:2. Aliquat 336 and tri-octylphosphine oxide solvated in methyloctanoate emerged as the best RE systems and yielded high extraction efficiencies of 11.5 and 29.5 (acetic acid) to almost 100 (caproic acid) respectively. Testing with real fermentation effluent demonstrated similar high extraction yields as observed on synthetic solutions. Potential toxicity of RE on acidogenic fermentation was also investigated which suggested the application of an external ISPR configuration for these coupled bioprocesses.

Keywords: carboxylate, reactive extraction, resource recovery, acidogenic fermentation, waste stream, biorefinery.

Abbreviations: ISPR, *in-situ* product recovery; RE, reactive extraction; COD, chemical oxygen demand; AD, anaerobic digestion; VFA, volatile fatty acid; HRT, hydraulic retention time; TOPO, tri-octylphosphine oxide.

1. Introduction

Biorefinery concept is being increasingly emphasized in the recent times to drive the transition from linear (extract-process-consumption-disposal) to a closed loop economy, in which the value of products, materials and resources is maintained for as long as practically possible [1,2]. In this regard, the sourcing from renewable biological resources instead of fossil fuel resources for building block chemicals including solvents e.g. 1,3-propanediol, 2,3-butanediol, organic acids e.g. acetic acid, succinic acid, lactic acid etc. is imperative [3,4]. To fully realize the potential of biorefineries within the circular economy scheme, maximum recovery of resources not only from initial feedstock but also the waste streams arising from the production process is required.

A relevant example to illustrate this aspect is the bioethanol production process. Up to 20 L wastewater known as stillage is generated per liter of bioethanol produced by the wet milling process. Stillage constitutes the waste stream remaining after distillation of ethanol from the fermentation broth. It typically has a high chemical oxygen demand (COD), inorganic load and pH range between 4.0 and 5.5 [5,6]. Therefore, adequate treatment of stillage is needed for maximizing energy and resource recovery and to ensure the sustainability of the plant. For this, anaerobic digestion (AD) has traditionally been the starting point aiming to recover biogas from high organic content which is subsequently used in the form of electricity and heat [7]. However, both of these are low-value products, for example, electricity and heat are valued at € 0.10 kWh⁻¹ and € 0.05 kWh⁻¹ respectively. This implies that the economic value of biogas produced from 1 kg of COD amounts to only € 0.20-0.25, and is therefore not very lucrative [8]. Alternatively, the application of mixed culture fermentation of organic matter in

stillage under anaerobic conditions instead of digestion can be an attractive method to produce carboxylates which are high-value products. **In this case, specific treatments of mixed culture inoculum e.g. by use of heat are applied to avoid methanogenesis and produce only the carboxylates as the fermentation product [9].**

Crude acetate has a market price of € 600-800 per ton, which implies that the conversion of 1 kg of COD to acetate would earn € 0.55-0.75. This is more than double the economic value of biogas [10]. In fact, longer chain fatty acids such as butyrate and caproate can reach even higher market prices. Carboxylates are considered as platform chemicals for synthesis of a wide spectrum of currently fossil fuel-derived products such as polymers (plastics), alcohols, ketones and olefins; and therefore, their bio-based production via biorefinery scheme is particularly attractive [2].

The mixed culture fermentation process provides a green and renewable route to high-valued carboxylates, however the process has two main challenges. First is the inhibition (toxicity) caused by carboxylates (i.e. volatile fatty acids (VFA)) on fermentation. VFA can cause inhibition of fermentative organism functions and can stop the further production of acids [11]. In fact, the highest total carboxylate concentrations are around 20 g/L, even at increasing substrate concentrations, which point to product inhibition [12]. It is well known that undissociated acids can pass through the cell membrane and dissociate in the cell. **This affects the transmembrane pH and decreases the amount of energy available for cell growth, thereby reducing the acid production by cells [13,14].** Another challenge concerns the separation and recovery of carboxylates from dilute fermentation broths such as stillage. The separation method needs to target the carboxylates molecules while removing limited water [15].

An approach to overcome these limitations is to remove the VFAs while they are being formed in the fermenter [13]. Process intensification which integrates fermentation and

separation technology, i.e. *in situ* product recovery (ISPR) offers several benefits to the above bioprocess. It allows product enrichment, thereby leading to simpler downstream processing and reduced costs, increase in the volumetric productivity due to alleviation of product inhibition and reduction of process flows [16,17]. With this concept in mind, the objective of this work was to develop efficient reactive extraction (RE) systems for carboxylate recovery from mixed fermentations, mainly leading to the design of ISPR-coupled mixed fermentations for efficient VFA production in bioprocesses. RE involves a reversible reaction between acid in the aqueous phase and extractant in the organic (diluent) phase forming an acid-extractant complex. This results in a higher affinity of the acid for the organic phase and hence higher acid extraction yields than those achieved in physical extraction using diluents alone. The acid can be back-extracted from the RE complex, thereby regenerating the extractant to be used again [18]. Organophosphorous compounds, tertiary and quaternary amines are some of the extractants used in RE systems which give high extraction yields of organic acids from dilute aqueous solutions [18,19].

Studies on the extraction of carboxylates have been performed and reported in literature. Alkaya et al. (2009) tested only one extractant and diluent for recovery of mixed VFAs from real effluent but the VFA concentration in their study was very low (5 g/L) [20]. Reyhanitash et al. (2016) explored the use of ionic liquids and trioctylamine/octanol for VFA extraction using synthetic mixtures at pH lower and higher than pK_a of acids [21]. More recently, the extraction of only small chain acids C2-C4 from synthetic mixtures using two combinations of extractants and diluents was investigated [22]. Another recent study reported the potential of membrane based RE for separation of C2-C5 acids from thin stillage fermentation broth [23]. While high extraction efficiencies were obtained using synthetic carboxylate mixtures, the extraction rates decreased when real fermenter effluent was used. This was mainly caused due to the fouling of the membrane with acid-rich effluent. Thus, the above account shows

that a detailed investigation covering several aspects e.g. optimization of extraction parameters, testing with real fermentation broth and toxicity of extraction systems has been rarely performed. The same is therefore the focus of the present study. The present work is significant as it aims to provide a detailed analysis of extraction behaviour of a wide array of extractants and diluents for multiple VFAs (C2 to C6) from synthetic media at various pH, VFA:extractant ratios and varying VFA concentrations. Furthermore, the VFA extraction from real effluent of acidogenic fermentation containing high acid concentrations of up to 22 g/L is demonstrated. In addition, the potential toxicity of reactive extractant systems on acidogenic fermentation is also investigated to provide guidelines on the selection of an appropriate ISPR configuration for coupled VFA bioprocesses.

2. Materials and methods

2.1. Acids and reactive extractant systems

An extensive range of extractants and diluents was screened in the present study for the selection of the best RE for VFA extraction from acidogenic broth. 5 extractants including amines, esters and organophosphorous compounds were investigated in the present study. Specifically, the extractants used were trioctylamine, tri-n-butyl phosphate, Aliquat 336, N-methyldioctylamine and tri-octylphosphine oxide (TOPO). These were used in combinations with 9 different types of diluents differing in their chemical nature i.e. presence or absence of functional groups and their type, polarity and chain length. These diluents included alkanes, sunflower oil, ketones, esters and alcohols and formed 45 different types of RE systems. Specifically, hexane, dichloromethane, o-xylene, methylisobutyl ketone, pentyl acetate, methyloctanoate, sunflower oil, 1-octanol and oleyl alcohol were used as diluents in the RE system. Trioctylamine, oleyl alcohol, methyloctanoate, hexane, dichloromethane, and C2-C4 carboxylic acids were procured from Merck, Germany. Extractants such as Aliquat 336, N-methyldioctylamine and TOPO, diluents such as o-xylene, methylisobutyl ketone, pentyl

acetate, and C5-C6 acids were supplied by Sigma Aldrich, Belgium. Tri-n-butyl phosphate was purchased from Alfa Aesar while n-octanol and sunflower oil were procured from Acros Organics, Belgium and S.A. Delhaize Group N.V., Belgium, respectively. All chemicals were used without further purification.

2.2. Experimental design and methodology

2.2.1. Screening of RE systems for VFA extraction from synthetic solutions

The first step in this study was the selection of the optimal RE system for VFA recovery. For this, RE (liquid-liquid) experiments were conducted with synthetic carboxylate-containing solutions (0.25 M) using 45 types of RE systems. Physical extraction using only diluents was performed to compare the extraction efficiency with RE. To prepare synthetic solutions, equimolar concentrations of acetic acid, propionic acid, butyric acid, valeric acid and caproic acid were prepared from commercial solutions by combining with distilled water. A final VFA concentration of 2 to 22 g/L (0.0625 to 0.25 M) was prepared. This concentration range was selected for RE studies since these VFA levels are usually obtained in mixed culture fermentations [24]. The initial pH was measured using a Multiline-Multi 340i pH meter (WTW, Belgium) and adjusted to 4.5 or 5.5 with 10 M NaOH. The electrical conductivity of VFA mixtures was measured using Multiline-P4 conductivity meter (Hach, Belgium) and was adjusted to the electrical conductivity of the real fermentation broth (15 mS/cm).

In the first set of RE experiments, 0.25 M VFA synthetic solution was mixed with a double concentration of extractant (0.5 M) to provide an excess of extractant in the RE mixture and ensure a complete extraction of acid in the organic (extractant) phase. Investigations were performed at both pH 4.5 and 5.5. The latter was performed to allow the analysis of the extraction behaviour of carboxylic acids at pH both lower and higher than their pK_a values, respectively. The methodology for extraction experiments was the same as detailed in our previous publications [18,25]. Briefly, equal volume of 0.5 mL each of synthetic solution

containing all C2-C6 acids (aqueous phase) and reactive extractants (or diluents only) were mixed for 16 h at ambient temperature. This was followed by phase separation by centrifugation and analysis of VFA concentration in the aqueous phase as described below. A comparison was performed among all extractant and diluent combinations to screen the most optimal combination for VFA extraction from synthetic solutions. All experiments were performed in duplicate. RE performance was analysed on the basis of two parameters as detailed in our previous publication [18]:

a) partition coefficient: $K = \frac{C_{a,org}}{C_{a,aq}}$

i.e., the ratio of VFA concentration in organic phase ($C_{a,org}$) and aqueous phase ($C_{a,aq}$)

b) extraction yield: $E\% = \frac{K(100)}{1+K}$

2.2.2. Influence of varying extractant and VFA concentrations on RE

In addition to the nature of extractant, its concentration can also influence the equilibrium extraction characteristics. Another important consideration is the VFA concentration in the aqueous solutions which provides the required driving force for extraction to the organic phase. Keeping these aspects in mind, the VFA:extractant ratios and VFA concentrations were varied and their effect on RE was determined. These experiments also allowed the selection of the optimal extractant concentration for maximum VFA extraction. For studying the effect of varying VFA:extractant ratios, the organic phase was prepared by dissolving the extractants in diluents according to total VFA:extractant molar ratios of 1:0, 2:1, 1:1, 1:2, 1:3 and 1:4. Meanwhile, the VFA concentrations were varied from 0.0625 M to 0.375 M. Experiments were performed with synthetic VFA solutions at pH 4.5 and 5.5. Furthermore, the effect of reaction time on extraction behaviour was also investigated. For this study, the reaction time was varied from 1 min, 2.5 min, 5 min, 30 min to 16 h.

2.2.3. Extraction of VFA from real effluent of acidogenic culture using RE systems

Based on the results of K and E% from the above experiments, the selected optimal extractant/diluent combination and their concentration was used for evaluation of VFA extraction efficiency using real effluent obtained from anaerobic fermentation of thin stillage from bioethanol plant. The thin stillage had a high organic load of 100 g COD/L and a pH of 5.0. Fermentation was performed in a 3-L double-jacketed glass reactor (Applikon, The Netherlands) and a working volume of 1 L was used. The inoculum used for acidogenic fermentation was granular sludge obtained from an anaerobic digester of the potato processing industry (Opure, Ede, The Netherlands). It was pre-treated by the method as described by Arslan et al. (2012) before adding to the reactor to avoid methanogenesis [24]. The sludge was washed with 20 mM potassium phosphate buffer (pH 5.0) and sieved with a mesh of 500 μm three times. After the last washing step, it was left in the same buffer overnight at room temperature. The next day, the sludge had a dry matter content of 9% and ash content of 11%, which were measured using analytical protocols as detailed in our previous publications [24,26]. The sludge was then treated by boiling in water for 15 min to avoid methanogenesis. To allow anaerobic conditions, N_2 sparging was performed in the reactor for 20 min. Temperature was maintained at 30°C by circulation of hot water and agitation was performed at 250 rpm. pH was maintained at 5.0 using 2 M NaOH. A semi-continuous feeding of undiluted thin stillage was performed wherein it was added four times a day to the reactor. An organic loading rate of 5.0 kg COD/ $\text{m}^3\cdot\text{d}$ and a hydraulic retention time (HRT) of 22 d during the start-up phase was used. When the volume in the reactor reached 2 L, the HRT was reduced to 15 d while keeping the same feeding regime as before. The outflow of the reactor was controlled by a level switch and occurred with 2.5 h of time delay compared to influent dosing. Samples were withdrawn from the reactor daily and stored at -20°C for subsequent use in RE experiments. Before usage for the extraction tests, the solid particles in samples were removed by centrifugation at 10,000 rpm for 5 min. Subsequently,

the supernatant was filtered through a 0.45µm syringe filter using a 25mm GD/X disposable filter device (GE Healthcare Life Sciences, Belgium).

RE tests with real effluent from acidogenic fermentation followed the same experimental conditions as described in Section 2.2.1 for synthetic solutions. All experiments were performed in duplicate.

2.2.4. Toxicity of RE systems on acidogenic fermentation

The influence of RE systems on acidogenic fermentation was investigated in serum vials.

Inoculum and wastewater were the same as those used for obtaining real effluent from acidogenic fermentation (Section 2.2.3). 12.5 g of feed and 2.5 g of heat pretreated wet granular sludge were added to 160 mL serum vials. To this, extractant saturated growth medium was added and volume was made up to 50 mL. For preparation of extractant saturated growth medium, the extractant was dissolved up to its solubility limit in distilled water. Methyl octanoate containing 0.125 M Aliquat 336 and 0.125 M TOPO were prepared in distilled water. The solutions in distilled water were mixed for 1 h at 20°C under constant agitation of 180 rpm to saturate the medium and then poured in a funnel to allow phase separation. After 1 h, the aqueous (bottom) phase was separated from the solvent and collected in a bottle which was stored at room temperature. These solutions were added to serum vials and pH of the resulting media in vials was re-adjusted to 5.0 using 2 M NaOH and HCl. The bottles were closed with rubber caps and sealed with aluminium crimp caps. The headspace was flushed with N₂ gas at atmospheric pressure for 10 min to ensure anaerobic conditions in the vial. Finally, the vials were placed on a rotating shaker and incubated at 30°C for 1 month under constant shaking at 170 rpm. Samples were withdrawn after 1 week and 4 weeks of fermentation, and analysed for VFA as per the protocols discussed below in analytical methods. Calculations were performed taking into account the volume changes due to sampling.

2.3. Analytical methods

Acetic acid, propionic acid, butyric acid, valeric acid and caproic acid were determined in the aqueous phase by High Performance Liquid Chromatography (Agilent, Belgium), as per the analytical protocols detailed in our previous publication [23]. VFA concentrations in the organic phase were calculated by using the mass balance as in our previous studies [18].

2.4. Statistical analyses

All statistical analyses were performed using SPSS program by one-way analysis of variance (ANOVA). Changes were considered to be significant with p -value < 0.05.

3. Results and discussion

3.1. Selection of RE systems for VFA extraction

The extraction capacity of an RE system is influenced by the nature of extractant and solvent used. When selecting the extractants, the objective is to obtain a high partition coefficient (K) of the acid for the organic extractant phase. During acid extraction from aqueous phase, these extractants operate by forming either an ionic bond e.g. quaternary ammonium salt Aliquat 336 or non-ionic hydrogen bond with acids e.g. trioctylamine. The extractants are often diluted with organic diluents to decrease the viscosity of the organic phase. Therefore, the chemical characteristics exhibited by diluent(s) are another important factor for RE systems [18]. The RE performance is influenced by the ability of diluents to solvate and stabilize the polar ion-pair acid-amine complex by means of dipole interactions or hydrogen bonding. Consequently, two broad categories of diluents are recognized i.e. hydrocarbon diluents and functional diluents [19]. Hydrocarbon diluents e.g. hexane, xylene etc. contain no functional group and operate by partitioning of acids into them due to hydrophobic interactions. Functional diluents on the other hand, contain an alcohol or ketone functional group, allowing them to form a hydrogen bond with the acid. Properties of diluents such as high K to facilitate high organic loading, and low volatility to avoid co-distillation with acid from

extractant are desirable. In general, using amines as extractant phase, alcohols are the best diluents while esters and alkanes are the next best ones, based on K_{dil} , as indicated by our previous extraction studies [18]. When selecting extractants and diluents for ISPR system, an additional concern is the potential toxicity resulting from their residues. This mostly depends on the nature and solubility of extractants.

Synthetic carboxylate mixtures containing equimolar amounts of VFA from C2 to C6 acids were extracted with 45 extractant/diluent combinations (RE systems) at pH 4.5 and 5.5.

Figure 1 shows the results of extraction efficiency ($E\%$) of acetic acid (C2), propionic acid (C3), butyric acid (C4), valeric acid (C5) and caproic acid (C6) using diluents alone (i.e. no extractant) and diluent/extractant combinations (i.e. RE system). The results of K values for selected RE systems based on one diluent and one extractant from each category of diluents and extractants investigated in this study are shown in Table 1. Among the diluents-based extraction at pH 4.5, hexane could not extract any of the C2-C6 acids **while**, a very low $E\%_{dil}$ and partition coefficient K_{dil} were obtained at high pH of 5.5. **At pH 5.5**, the $E\%_{dil}$ were particularly low for short chain acids (C2-C3) at 1.35-3% and it increased with increasing length of acids, giving the highest $E\%_{dil}$ for caproic acid (35.35%) (Figure 1). Generally, the extraction capacity varied with increasing polarity of diluents. For example, between o-xylene and dichloromethane, slightly higher $E\%_{dil}$ and K_{dil} values (*p-value*<0.05) were exhibited by dichloromethane for all acids (especially at pH 5.5) which could be attributed to its higher polarity than o-xylene and thus better ability to solvate the acid. Comparatively, extraction with esters alone provided higher extraction yields for both short and long-chain acids. Pentylacetate being more polar than methyloctanoate resulted in a higher extractability. The most favourable results among diluents were obtained for 1-octanol with an $E\%_{dil}$ of ~20% (C2), 56% (C3), 80% (C4), 93% (C5) and 98% (C6) acids at pH 4.5. The K_{dil} values also increased with the increasing carbon chain length of acids. For example, the K_{dil} values

with 1-octanol increased from 0.24 (C2), 1.29 (C3), 3.92 (C4), 13.58 (C5) to 49.52 for C6 acid at pH 4.5 (*p-value*<0.05) (Table 1).

Compared to physical extraction, an increase by 75-85% was achieved when using RE.

However, the difference between physical extraction and RE was more pronounced for short chain VFAs, particularly for pH 4.5. For example, the E% of acetic acid was higher by 81.9%

(*p-value*<0.005) when Aliquat 336 was used with methyloctanoate as compared to when methyloctanoate was used alone for extraction. The result is significant since it shows a clear superior effect of RE over physical extraction for enhanced VFA extraction, particularly for short-chain VFA which are usually difficult to extract by liquid-liquid extraction. Among all

the extractants, Aliquat 336 emerged as the best extractant for all VFAs at both pH 4.5 and 5.5 (Figure 1). Aliquat 336 is a quaternary amine which can also react with the charged form of acids unlike the tertiary amines investigated in this study which lack this ability. Thus, the highest extraction efficiency obtained with Aliquat 336 could be attributed to the specific chemical interactions between amine and the acid molecules to form acid-amine complexes in the organic phase, thereby allowing more VFAs to be extracted from the aqueous phase.

Considering the extraction performance, the E% and K were found to decrease with increasing equilibrium pH of the aqueous phase for Aliquat 336 (Figure 1 and Table 1).

Figure 1 (left column) shows that extraction efficiencies of ~45% (C2), ~75% (C3) and 86% to 97-99% (higher VFAs) were obtained using Aliquat 336 at pH 4.5. These values

drastically decreased by 1.5-2 fold for all extracted acids at pH > pK_a. For example, a 2.14-fold higher (*p-value*<0.005) E% of acetic acid was obtained using Aliquat

336/methyloctanoate at pH 4.5 as compared to the E% obtained when the extraction was performed at pH 5.5. An understanding on the observed differences in extraction behaviour at

different pH can be obtained from the extractive mechanism of Aliquat 336. At pH > pK_a of the acid, Aliquat 336 performs ion exchange due to the presence of quaternary ammonium

group. However, at low pH, such mechanism has been proven to be thermodynamically unfeasible and therefore the RE of carboxylic acids possibly occurs by other mechanism such as hydrogen bonding [27]. Another important point is that although Aliquat 336 can extract both undissociated and dissociated forms of carboxylic acids, a larger proportion of undissociated acid molecules are removed by it particularly at low pH conditions and low acid concentrations. The ratio of undissociated and dissociated molecules extracted changes with the increasing pH. Kyuchoukov et al. (2004) also reported similar findings as above for the effect of pH on extraction of lactic acid using Aliquat 336 solved in 1-decanol. The authors reported that at $\text{pH} > \text{pK}_a$ of the acid, more than 50% of the acid molecules were dissociated which favoured the extraction via ion exchange mechanism [28]. This points out to the fact that the concentration of undissociated acid extracted is a function of pH of the aqueous phase and consequently the best results for RE using Aliquat 336 were obtained at pH 4.5 in the present study.

TOPO was the second best extractant. Similar to Aliquat 336, the E% and K were found to decrease with increasing equilibrium pH of the aqueous phase for TOPO. The extraction efficiency of TOPO was lower than Aliquat 336 at higher pH values ($p\text{-value} < 0.05$) (Figure 1, right column). The E%_s for TOPO at pH 5.5 were similar to the ones obtained with physical extraction ($p\text{-value} > 0.05$). The different pH-behavior for Aliquat 336 and TOPO can be explained on the basis of their extraction mechanism. TOPO is a phosphorous-bonded oxygen donor extractant containing a phosphoryl group. It is a Lewis base and forms non-stoichiometric compound with neutral solutes. Their extraction efficiencies are lower than those of aliphatic amines at $\text{pH} < \text{pK}_a$ and thus low distribution coefficients are obtained [29]. The latter explains the inefficient RE with TOPO at $\text{pH} > \text{pK}_a$ in the present study.

It is important to note that for all the RE systems under investigation in the present work, the E% and K increased with increase in the length of the carboxylic acid chain (Figure 1). Thus,

the highest E% and K values were obtained for C6 (caproic acid), in line with the physical extraction with diluents alone. As reported by Qin et al. (2003) [30], the degree of extraction is mainly influenced by the nature of the acid being extracted i.e. its hydrophobicity and acidity. Thus, a higher extraction efficiency is expected with increasing hydrophobicity and acidity. The longer chain carboxylic acids are more hydrophobic, i.e. caproic acid ($pK_a = 4.88$, $\log P = 1.95$) > butyric acid ($pK_a = 4.78$, $\log P = 0.751$) > propionic acid ($pK_a = 4.67$, $\log P = 0.29$) > acetic acid ($pK_a = 4.76$, $\log P = -0.30$). This clearly shows that higher the pK_a and $\log P$ values of the acid, higher the equilibrium complexation constant and hence the E% (Figure 1). Plácido and Zhang (2017) obtained similar results as above for short-chain VFA recovery using different ratios of octanol and trioctylamine [31].

Overall, the best extractants from first phase of the present work were Aliquat 336 and TOPO. Among all the diluents tested with Aliquat 336 and TOPO, methyloctanoate was the most suitable to work with both the selected extractants for all C2-C6 acids. E% of 38-40% (C2), 70-76% (C3), 87-92% (C4), and >96% (C5 and C6) acids were obtained using methyloctanoate with these extractants at pH 4.5 (see Figure 1, left column). The K values for methyloctanoate-based RE systems using both Aliquat 336 and TOPO corresponded to the higher E% achieved for these systems (Table 1). For example, the K values obtained using Aliquat 336 were 0.66 (C2), 2.4 (C3), 7.2 (C4), 26.5 (C5) and 179.4 (C6) at pH 4.5. As detailed by Saboe et al. (2018) [19] regarding the characteristics of diluents for RE of acids, the low volatility of the diluent is another important consideration in addition to high partitioning of acids in the RE system. High volatility would result in co-distillation with the acid from the heavy organic extractant phase and therefore, low volatility diluents are required to allow the acid to be recovered as a distillate. Methyloctanoate as a diluent fulfils this requirement while also providing high acid extraction efficiencies. Thus, following the

results from the first phase of the present work, Aliquat 336 and TOPO as extractants and methyloctanoate as diluent were selected for subsequent studies.

3.2. Effect of varying extractant and VFA concentration on RE performance

Both the concentration of extractant and acid being extracted influence the extraction behaviour in liquid-liquid extraction [32]. The selected combinations of extractants and diluents from experiments above were used in this phase of study to investigate the effect of varying VFA:extractant ratios on RE at pH 4.5 and 5.5. The results for extraction efficiencies obtained for C2 to C6 at different VFA:extractant molar ratios of 2:1, 1:1, 1:2, 1:3 and 1:4 are shown in Figure 2. For Aliquat 336, the E% for C2 to C4 acids increased with increasing the VFA:extractant ratios at both pH 4.5 and 5.5 for all VFA concentrations under study. However, for long-chain VFAs (C5 and C6), the E% first increased with increasing Aliquat 336 concentration and thereafter reached an equilibrium when VFA:extractant molar ratio was higher than 1:2. For example, for valeric acid, the increase in E% with increase from 1:2 to 1:3 ratio at 0.25 M acid concentration and pH 4.5 was insignificant ($p\text{-value}>0.05$). Similar result was seen at pH 5.5 for valeric acid. Results of K values (not shown) followed the same trend as E%. Similar trends for variation of E% with increase in extractant concentration have been reported in literature. Although these reports dealt with a single acid extraction only and not mixed VFA, a comparison with results of this study is meaningful to show the application of RE to mixed acids. Keshav et al. (2009) reported that 30% Aliquat 336 in the organic phase, which corresponds to a ratio of 1:3 for the solution 0.250 M in the present study, was the optimum concentration for achieving high extraction efficiencies of propionic acid [32]. At this concentration, problems of three-phase formation, difficulty in phase separation and high turbidity were not observed [32,33]. On the contrary, it was reported that increase in Aliquat 336 concentration above 40% decreased the equilibrium concentration of long chain fatty acid caproic acid in the organic phase and hence the K values [34].

Similar results as obtained above for Aliquat 336 were observed for TOPO at both pH 4.5 and 5.5. Both E% and K increased with increasing TOPO concentration at a given VFA concentration for C2-C6 acids while reaching an equilibrium at VFA:TOPO ratio higher than 1:2 ($p\text{-value}>0.05$) (Figure 2). An exception was however seen for the most concentrated solution i.e. 0.375 M at pH 5.5, where both parameters decreased with increasing TOPO concentrations for reasons unknown. For example, comparing the E% across ratios 2:1 through 1:2 for butyric acid at pH 5.5, there was a significant decrease in E% at 0.375 M acid concentration ($p\text{-value}<0.05$). Furthermore, at pH 5.5, increasing the ratio between 1:2 to 1:4 demonstrated only a marginal increase in E% and K for all tested VFA concentrations. An important consideration for process flow design is to determine the extractant concentration required for achieving maximum VFA extraction. For e.g., a high extractant concentration would result in higher E% and K of product (VFA) in one stage only. On the other hand, a similar separation efficiency might be obtained in 2-3 stages by using a lower extractant concentration which would also be more cost-efficient [18]. Thus, from the above results, a VFA:extractant molar ratio of 1:2 was considered to be the most suitable for VFA extraction using RE systems.

In addition to the extractant concentrations, the VFA concentration of the VFA-containing aqueous broth is another critical parameter. The concentration of VFA should be maximized in order to ensure enough driving force for the extraction while on the other hand, it should be low enough to avoid significant product inhibition. Therefore, the understanding of variation in extraction performance in response to VFA concentration is essential. In the present work, this information was obtained from the equilibrium curves for the selected RE systems. The extraction efficiency of acids from C2 to C6 with Aliquat 336 was usually found to increase with increase in the VFA concentration for a given VFA:extractant ratio (Figure 2). Similar trends as the E% were obtained for K values (data not shown). The results

are in good agreement with the studies on propionic acid extraction by Keshav et al. (2009) [32]. They reported that higher loading ratios i.e. the ratio of total acid concentration in organic phase to total concentration of amine in the organic phase were obtained at higher acid concentrations at both Aliquat 336 concentrations of 20% and 30% tested in their study. However, high initial acid concentrations and high Aliquat 336 content caused the problem of third phase formation.

For TOPO-based extractions at $\text{pH} < \text{pK}_a$, the trends were similar to those obtained with Aliquat 336 and the extraction efficiency increased with increasing VFA concentration.

Similar results have been reported using tri-butyl phosphate which is also a phosphorous-bonded oxygen donor extractant similar to TOPO [35]. At pH 4.5, the effect of acid dissociation on extraction is immaterial since this pH condition is below the pK_a of all acids under investigation as explained above. Thus, the increase in extraction efficiency observed under this condition is mainly influenced by the (increasing) acid concentration for both Aliquat 336 and TOPO. On the contrary, the variation of E% and K with increasing initial acid concentration showed a different trend at pH 5.5. At this pH ($\text{pH} > \text{pK}_a$), both parameters were found to decrease with increasing acid concentrations. The decrease occurred sharply at high extractant concentrations. This trend is consistent with the results obtained in the study by Kertes and King using tri-butyl phosphate as extractant [36]. These authors reported that the decrease in K values for tartaric acid extraction with tri-butyl phosphate was much more pronounced at high extractant concentrations.

3.3. Effect of reaction time on extraction performance

During the reaction between VFA and extractants, the VFAs are transferred from aqueous solution to the organic phase. Experiments were performed to investigate the effect of reaction time on RE performance. It is usually expected that E% increases with increasing incubation time i.e. increasing the contact time between acid and extractant, until an

equilibrium is reached which exhibits the highest level of extraction [37,38]. The results using Aliquat 336 as a function of time showed that the percentage of VFAs extracted after 1 min of reaction did not increase with increasing the incubation time at pH 4.5 and 5.5 for all VFAs under study. This indicated that the acids were rapidly transferred to the organic phase once the acid-extractant complex was formed. At pH 5.5, however, a small significant increase in E% was observed for C2-C4 acids with increase in the incubation time from 1 min to 5 min. For e.g., E% for acetic acid increased by 9.5% ($p\text{-value}<0.05$), propionic acid by 6.9% ($p\text{-value}<0.02$) and butyric acid by 3.5% ($p\text{-value}<0.05$). For TOPO, the E% remained the same at all incubation times for all VFAs at pH 4.5 and 5.5.

The reaction rate for the complexation between organic acids and different types of complexants has been studied for several types of RE systems. The reaction rate has been found to be first order in acid and zero or first order in complexant depending on the organic acid and complexation agent studied [37,38,39]. Some examples of reported reaction rates are $0.94\text{ m}^3\text{ kmol}^{-1}\text{s}^{-1}$ [37], $0.013\text{ m}^3\text{ kmol}^{-1}\text{s}^{-1}$ [38], $0.035\text{ m}^3\text{ kmol}^{-1}\text{s}^{-1}$ [39], which are all indicative of fast processes under well mixed conditions. Such similar well mixed conditions were used in the present study with a focus to determine the effect of contact time on K and E% under equilibrium conditions. While specific tests to determine the precise reaction order and rate were outside of the scope of the present study, nevertheless, under most of the conditions studied, equilibrium was reached quickly i.e. within a minute of contact time which was coherent with the expected fast reaction rate, as reported in literature.

3.4. Performance of extractants on VFA recovery from real effluent of acidogenic fermentation

The selected RE systems resulted in high C2-C6 E% when used for VFA extraction from synthetic solutions. The real suitability of RE should be tested with real acidogenic fermentation broth, however this is usually lacking in literature reports. Therefore, after the

extraction optimization experiments with synthetic solutions, the RE systems were used for VFA extraction from real effluents obtained from fermentation. The selection of the best extractant/diluent i.e. methyloctanoate in Aliquat 336 and TOPO and their concentration for optimal RE of carboxylic acids was obtained from results as shown in Section 3.1 and 3.2. The selected extractant concentration i.e. a VFA/extractant ratio of 1:2 was used to perform RE on samples obtained from acidogenic fermentation. Three samples were withdrawn from acidogenic culture at different periods of fermentation and VFAs were extracted from these sample solutions. The characteristics of the acid-rich thin stillage collected during different time periods is given in Table 2. Two samples (solution 1 and 2) were taken for RE experiments and these solutions differed in their total as well as individual VFA concentrations. For example, solution 1 had a higher total VFA concentration (0.291 M) as compared to 0.133 M VFA concentration in solution 2. Furthermore, solution 1 had a higher fraction of C2 and C3 acids while a lower fraction of C5 acids as compared to solution 2. The results of RE using real effluents are shown in Figure 3. The results show that RE has a higher extraction performance as compared to physical extraction using methyloctanoate alone. It further confirmed that Aliquat 336/methyloctanoate was the best extraction system. The E% increased with increase in the chain length of VFAs which is due to the acid hydrophobicity, as also previously observed for tests on synthetic solutions above and reported in studies [35,36].

It is indeed important to note that the results on real fermentation broth were comparable to those obtained on synthetic medium pH 4.5, although the pH of real broth solutions 1 and 2 lied in the middle of tested pH values of 4.5 and 5.5 for the synthetic media (Table 3). The highest E% was obtained for solution 1 which contained the highest total VFA concentration. This supported the finding from equilibrium studies above which showed the increase in E% with an increase in VFA concentration in the solution. A total acid concentration of 0.140 M

was extracted using Aliquat 336/methyloctanoate RE system from solution 1. This demonstrated that the chosen RE system and its optimal concentration were suitable for VFA recovery for real acidogenic mixed cultures.

3.5. Toxicity of selected RE systems on acidogenic fermentation

One of the main features in designing of an appropriate ISPR configuration for a bioprocess is the biocompatibility of extractants with the microbial culture [17]. ISPR-coupled fermentation facilitates alleviation of product inhibition, however the presence of extractant and/or diluent can result in physical, microbial and biochemical effects on the metabolic activity of the fermenting microorganism(s). Yabannavar and Wang (1991) reported the toxic effects of commonly used extractant systems comprising tri-octylamine and oleyl alcohol on lactic acid fermentation [40]. The authors reported that the cell growth was completely inhibited in the presence of 30% tri-octylamine, however a detailed analysis of fermentation behaviour e.g. acid production, in the presence of extractants was not performed. Similar results for tri-octylamine have also been reported for itaconic acid fermentation [17]. This implies that in addition to the requirement of a high extraction performance for an RE system, the investigation of impact of their presence on the fermenting microorganism is also essential to design the right ISPR configuration i.e. with or without cell contact, reduced cell contact e.g. immobilized cell systems etc. [40] for a coupled bioprocess. While developing the optimal system for *in-situ* product extraction, the focus has been largely on E%, while biocompatibility of the extractants has often been ignored in the literature reports. Therefore, both diluent alone and extractant/diluent combinations in RE systems were investigated for their potential toxicity to the acidogenic fermentation in the present study. Only methyloctanoate dissolved in distilled water was used to assess the effect of diluent. Aliquat 336 and TOPO concentrations were decided on the basis of total VFA concentration in the feed and double molarity for extractants was used. The results of total VFA production and

individual VFA distribution as a function of time are shown in Figure 4. The results clearly show the toxic effect of Aliquat 336 and TOPO when solved in methyloctanoate and used as a medium for acidogenic fermentation. After one week of fermentation, the culture grown on distilled water produced the highest concentration of total VFAs (29 mM). **The total VFA in both diluent and the two extractant/diluent mixtures grown cultures was reduced by 80% (p -value<0.005), and 71% (p -value<0.05) and 51% (p -value<0.05), respectively.** In all the treatments, acetic acid was the main VFA produced, which was followed by butyric acid. After four weeks of fermentation, the total VFA in distilled water grown culture changed very little (26 mM) (p -value>0.05) while it increased in other treatments and propionic acid was also produced. However, the overall toxic effects of the reactive extractants were still evident and the highest VFA concentrations were seen for distilled water grown culture. Only methyloctanoate-grown culture was a better medium. It produced a comparable total VFA concentration (25 mM) as the control with distilled water and higher production than Aliquat 336/methyloctanoate (19 mM) (p -value<0.05) and TOPO/methyloctanoate (18 mM) (p -value<0.05) grown cultures. **Overall, after four weeks of acidogenic fermentation, acid production with the addition of Aliquat 336/methyloctanoate and TOPO/methyloctanoate was reduced by 26.9% and 30.7% respectively as compared to the control fermentation.** The toxic effects of Aliquat 336 and TOPO have indeed been previously reported [41,42]. **Aliquat 336 in combination with di-isopropyl ether was reported to be toxic to the fatty acid forming bacteria [41]. The acid production was reduced to less than 25% of the control, which is consistent with the findings of the present study.**

Considering the toxicity of RE systems on acidogenic fermentation and inhibition of VFA production in the presence of extractants, this suggests the selection of an external ISPR configuration involving no contact with the fermenting microbial population for VFA

recovery. The conceptual design for such a coupled acidogenic fermentation-ISPR process is shown in Figure 5.

4. Conclusions

Mixed culture VFA fermentations based on wastewater biorefinery concept provide an efficient method to produce VFA as high-valued platform chemicals. Product inhibition and difficult recovery from fermentation broth however remain as two important challenges facing these bioprocesses. The present study aimed at debottlenecking these problems and leading towards the development of ISPR-coupled VFA bioprocesses to allow their sustainable production. For fulfilment of this objective, a comprehensive investigation on the applicability of RE systems for *in-situ* recovery of multiple VFAs was performed and the effect of process parameters such as pH, VFA:extractant ratios, VFA concentration and contact time was determined. First, 45 types of RE systems for VFA extraction were evaluated. It was found that the extraction efficiencies for C2-C6 VFAs were higher by 75-85% using RE as compared to those achieved using pure diluents alone. Among the 45 RE systems tested in this study, Aliquat 336 and TOPO solved in methyloctanoate emerged as the most favourable RE systems for VFA extraction from synthetic solutions at both high and low pH values. These RE systems were then used further to investigate the effect of varying VFA:extractant molar ratios which demonstrated an increase in K and E% values with increasing extractant concentration and reaching an equilibrium at a molar ratio of 1:2. Additionally, the extraction efficiency of C2-C6 acids greatly increased with increasing VFA concentrations at pH 4.5 and 5.5 when using Aliquat 336/methyloctanoate. The effect of contact time on extraction performance was also evaluated. It was found that the reaction proceeded very quickly and equilibrium was reached within a minute under most conditions with an exception to Aliquat 336/methyloctanoate at pH 5.5 in which the equilibrium was

reached in 5 min. This indicated that RE was very efficient in extracting C2-C6 acids from solutions.

While optimization of extraction parameters is often performed in liquid-liquid extraction studies, these are usually carried out using synthetic (mock) solutions while real fermentation broth has been rarely used in the literature. Therefore, following the tests with synthetic solutions, the present study aimed at overcoming this knowledge gap by testing the selected RE systems with real effluent from acidogenic fermentation. The performance of two selected RE systems was tested on real effluent and Aliquat 336/methyloctanoate emerged as the best system yielding comparable results as those obtained for synthetic solutions. This strongly supports the applicability of RE systems for recovery of VFA from real acidogenic mixed cultures. Regarding the use of RE for real acidogenic culture, the purity of acid product in the extractant stream is an important consideration. This is because the other organic molecules present in the fermentation broth might also be extracted along with the carboxylates. For organic molecules such as glucose, our previous reports on separation of itaconic acid from fermentation broth have shown that glucose was not fractionated into the extractant rich organic layer [18]. A similar result was also obtained for the salts present in the fermentation medium. However, depending on the substrate used in acidogenic fermentation e.g. woody or grassy biomass, sewage sludge, biorefinery wastewater etc., various organic molecules other than carboxylates in the fermentation broth might co-extract with the latter. Therefore, it would be worthwhile to perform a detailed investigation involving carbon purity calculations for the extracted stream. These would be the subject of attention in our future research.

Another important consideration for ISPR-coupled bioprocesses is the toxicity of the extractant system to microbial cells. As found from the toxicity studies, the presence of reactive extractants in the fermentation medium negatively affected the VFA production. This

suggested that an external ISPR configuration which separated the fermenting cells from extractants would be more appropriate for VFA recovery in the coupled bioprocess. Our future studies would focus on the impact of integration of RE-based ISPR unit with a running acidogenic fermenter i.e. an *in-line* system on fermentation performance in terms of enhanced VFA yields and productivity. Furthermore, back extraction methods for the recovery of VFAs from VFA-extractant complex need to be established. Additionally, strategies might also need to be developed to separate the components of VFA recovered from waste streams and make them more valuable. Research efforts to investigate such methods for high purity VFA separation and recovery are recommended. This would allow to cater to more specific applications requiring individual VFAs and therefore their separation would be the topic for our future investigations.

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Competing interests

Authors declare no competing interests.

References

- [1] G. Kaur, K. Uisan, K. L. Ong, C.S.K. Lin, Recent Trends in Green and Sustainable Chemistry & Waste Valorisation: Rethinking Plastics in a circular economy, *Curr. Opinion Green Sustain. Chem.* 9 (2018) 30-39.
- [2] D. Pleissner, Q. Qi, C. Gao, C.P. Rivero, C. Webb, C.S.K. Lin, J. Venus, Valorization of organic residues for the production of added value chemicals: A contribution to the bio-based economy, *Biochem. Eng. J.* 116 (2016) 3-16.
- [3] T.H. Kwan, A. Vlysidis, Z. Wu, Y. Hu, A. Koutinas, C.S.K. Lin, Lactic acid fermentation modelling of *Streptococcus thermophilus* YI-B1 and *Lactobacillus casei* Shirota using food waste derived media, *Biochem. Eng. J.* 127 (2017) 97-109.
- [4] K.L. Ong, C. Li, X. Li, Y. Zhang, J. Xu, C.S.K. Lin, Co-fermentation of glucose and xylose from sugarcane bagasse into succinic acid by *Yarrowia lipolytica*, *Biochem. Eng. J.* 148 (2019) 108-115.
- [5] A.C. Wilkie, K.J. Riedesel, J.M. Owens, Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks, *Biomass Bioenerg.* 19 (2000) 63-102.
- [6] J.D. Vrieze, M. Coma, M. Debeuckelaere, P.V. der Meeren, K. Rabaey, High salinity in molasses wastewaters shifts anaerobic digestion to carboxylate production, *Water Res.* 98 (2016) 293-301.
- [7] K. Syutsubo, T. Onodera, P. Choeisai, J. Khodphuvieng, P. Prammanee, W. Yoochatchaval, W. Kaewpradit, K. Kubota, Development of appropriate technology for treatment of molasses-based wastewater, *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* 48 (2013) 1114-1121.
- [8] W. Verstraete, S.E. Vlaeminck, ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future, *Int. J. Sustain. Dev. World Ecol.* 18 (2011) 253-264.
- [9] M.M.H. Coelho, N.W.S. Morais, E.L. Pereira, R.C. Leitão, A.B. dos Santos, Potential assessment and kinetic modeling of carboxylic acids production using dairy wastewater as substrate, *Biochem. Eng. J.* 156 (2020) 107502.
- [10] R. Kleerebezem, B. Joosse, R. Rozendal, M.C.M. Van Loosdrecht, Anaerobic digestion without biogas? *Rev. Environ. Sci. Biotechnol.* 14 (2015) 787-801.
- [11] S. Pratt, D. Liewa, D.J. Batstone, A. Werker, G.F. Morgan-Sagastume, P.A. Lanta, Inhibition by fatty acids during fermentation of pre-treated waste activated sludge, *J. Biotechnol.* 159 (2012) 38-43.
- [12] I. Siegert, C. Banks, The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors, *Process Biochem.* 40 (2005) 3412-3418.
- [13] M.T. Agler, B.A. Wrenn, S.H. Zinder, L.T. Angenent, Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform, *Trends Biotechnol.* 29 (2011) 70-78.
- [14] W. Van Hecke, G. Kaur, H. De Wever, Advances in in-situ product recovery (ISPR) in whole cell biotechnology during the last decade, *Biotechnol. Adv.* 32 (2014) 1245-1255.
- [15] S. Wainaina, M. Parchami, A. Mahboubi, I.S. Horváth, M.J. Taherzadeh, Food waste-derived volatile fatty acids platform using an immersed membrane bioreactor, *Bioresour. Technol.* 274 (2019) 329-334.
- [16] G. Kaur, A.K. Srivastava, S. Chand, Debottlenecking product inhibition in 1,3-propanediol fermentation by In-Situ Product Recovery, *Bioresour. Technol.* 197 (2015) 451-457.

- [17] D. Kreyenschulte, B. Heyman, A. Eggert, T. Maßmann, C. Kalvelage, R. Kossack, L. Regestein, A. Jupke, J. Büchs, In situ reactive extraction of itaconic acid during fermentation of *Aspergillus terreus*, *Biochem. Eng. J.* 135 (2018) 133-141.
- [18] G. Kaur, K. Elst, Development of reactive extraction systems for itaconic acid: a step towards in situ product recovery for itaconic acid fermentation, *RSC Adv.* 4 (2014) 45029.
- [19] P.O. Saboe, L. Manker, W.E. Michener, D.J. Peterson, D. Brandner, S. Deutch, M. Kumar, R.M. Cywar, G.T. Beckham, E.M. Karp, In situ recovery of bio-based carboxylic acids, *Green Chem.* 20 (2018) 1791-1804.
- [20] E. Alkaya, S. Kaptan, L. Ozkan, S. Uludag-Demirer, G.N. Demirer, Recovery of acids from anaerobic acidification broth by liquid-liquid extraction, *Chemosphere* 77 (2009) 1137-1142.
- [21] E. Reyhanitash, B. Zaalberg, S.R.A. Kersten, B. Schuur, Extraction of volatile fatty acids from fermented wastewater, *Sep. Purif. Technol.* 161 (2016) 61-68.
- [22] S. Eda, A. Kumari, P.K. Thella, B. Satyavathi, P. Rajarathinam, Recovery of volatile fatty acids by reactive extraction using tri-n-octylamine and tri-butyl phosphate in different solvents: Equilibrium studies, pH and temperature effect, and optimization using multivariate taguchi approach, *Can. J. Chem. Eng.* 95 (2017) 1373-1387.
- [23] K. De Sitter, L. Garcia-Gonzalez, C. Matassa, L. Bertin, H. De Wever, The use of membrane based reactive extraction for the recovery of carboxylic acids from thin stillage, *Sep. Purif. Technol.* 206 (2018) 177-185.
- [24] D. Arslan, K.J.J. Steinbusch, L. Diels, H. De Wever, C.J. Buisman, H.V. Hamelers, Effect of hydrogen and carbon dioxide on carboxylic acids patterns in mixed culture fermentation, *Bioresour. Technol.* 118 (2012) 227-234.
- [25] G. Kaur, M. Maesen, L. Garcia-Gonzalez, H. De Wever, K. Elst, Novel Intensified Back Extraction Process for Itaconic Acid: Toward in Situ Product Recovery for Itaconic Acid Fermentation, *ACS Sustainable Chem. Eng.* 6 (2018) 7403-7411.
- [26] G. Kaur, L. Luo, G. Chen, J.W.C. Wong, Integrated food waste and sewage treatment – A better approach than conventional food waste-sludge co-digestion for higher energy recovery via anaerobic digestion, *Bioresour. Technol.* 289 (2019) 121698.
- [27] A.M. Eyal, R. Canari, pH Dependence of Carboxylic and Mineral Acid Extraction by Amine-Based Extractants: Effects of pKa, Amine Basicity, and Diluent Properties, *Ind. Eng. Chem. Res.* 34 (1995) 1789-98.
- [28] G. Kyuchoukov, M. Marinova, J. Albet, J. Molinier, New Method for the Extraction of Lactic Acid by Means of a Modified Extractant (Aliquat 336), *Ind. Eng. Chem. Res.* 43 (2004) 1179-84.
- [29] K.Y. Hong, W.H. Hong, Y.K. Chang, Effect of pH on the extraction characteristics of succinic and formic acids with Tri-n-octylamine dissolved in 1-octanol, *Biotechnol. Bioprocess Eng.* 6 (2001) 347-351.
- [30] W. Qin, Z. Li, Y. Dai, Extraction of Monocarboxylic Acids with Trioctylamine: Equilibria and Correlation of Apparent Reactive Equilibrium Constant, *Ind. Eng. Chem. Res.* 42 (2003) 6196-6204.
- [31] J. Plácido, Y. Zhang, Evaluation of esterification and membrane based solvent extraction as methods for the recovery of short chain volatile fatty acids from slaughterhouse blood anaerobic mixed fermentation, *Waste Biomass Valorization* (2017) 1-11.
- [32] A. Keshav, K. L. Wasewar, S. Chand, Reactive extraction of propionic acid using tri-n-octylamine, tri-n-butyl phosphate and aliquat 336 in sunflower oil as diluent, *J. Chem. Technol. Biotechnol.* 84 (2009) 484-489.

- [33] A. Keshav, K. L. Wasewar, S. Chand, Extraction of Acrylic, Propionic, and Butyric Acid Using Aliquat 336 in Oleyl Alcohol: Equilibria and Effect of Temperature, *Ind. Eng. Chem. Res.* 48 (2009) 888-893.
- [34] K.L. Wasewar, D.Z. Shende, Equilibrium Study for Reactive Extraction of Caproic Acid in Mibk and Xylene, *Engineering* 3 (2011) 829-835.
- [35] A. Keshav, S. Chand, K.L. Wasewar, Equilibrium Studies for Extraction of Propionic Acid Using Tri-n-Butyl Phosphate in Different Solvents, *J. Chem. Eng. Data* 53 (2008) 1424-1430.
- [36] A.S. Kertes, C.J. King, Extraction chemistry of fermentation product carboxylic acids, *Biotechnol. Bioeng.* 28 (1986) 269-282.
- [37] M.E. Marti, T. Gurkan, L.K. Doraiswamy, Equilibrium and Kinetic Studies on Reactive Extraction of Pyruvic Acid with Trioctylamine in 1-Octanol, *Ind. Eng. Chem. Res.* 50 (2011) 13518-13525.
- [38] B.P. Nikhade, J.A. Moulijn, V.G. Pangarkar, Extraction of citric acid from aqueous solutions with Alamine 336: equilibrium and kinetics, *J. Chem. Technol. Biotechnol.* 79 (2004) 1155-1161.
- [39] N. Thakre, D. Datta, A.K. Prajapati, P.K. Chaudhari, D. Pal, Reactive Extraction of Citric Acid Using Different Extractants: Equilibrium, Kinetics and Modelling, *Chem. Biochem. Eng. Q.* 31 (2017) 437-446.
- [40] V.M. Yabannavar, D.I.C. Wong, Strategies for Reducing Solvent Toxicity in Extractive Fermentations, *Biotechnol. Bioeng.* 37 (1991) 716-722.
- [41] G. Hayward, I. Lau, Toxicity of Organic Solvents to Fatty Acid Forming Bacteria, *Can. J. Chem. Eng.* 67 (1989) 157-161.
- [42] C.S. Lopez-Garzon, A.J.J. Straathof, Recovery of carboxylic acids produced by fermentation, *Biotechnol. Adv.* 32 (2014) 873-904.

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Fig. 1. Total VFA and individual C2-C6 VFA extraction yields for diluents alone and reactive extraction systems comprising amines, esters and organophosphorous compounds at pH below (pH 4.5, left column) and above (pH 5.5, right column) the pK_a of carboxylic acids. (A) shows the total VFA extraction yields and (B)-(F) refers to extraction yields for individual acids i.e. C2 (acetic acid), C3 (propionic acid), C4 (butyric acid), C5 (valeric acid) and C6 (caproic acid), respectively.

Fig. 2. Effect of varying VFA and extractant (Aliquat 336 and TOPO) concentration on extraction yield of C2-C6 VFAs at pH 4.5 (right column) and pH 5.5 (left column). VFA:extractant molar ratio is varied from 2:1 to 1:4 and VFA concentration is increased from 0.0625 M to 0.375 M. E% for all VFA:extractant ratios at all VFA concentrations is shown. (A)-(E) refers to extraction yields for C2 (acetic acid), C3 (propionic acid), C4 (butyric acid), C5 (valeric acid) and C6 (caproic acid), respectively.

Fig. 3. Extraction yields of C2-C6 acids obtained from real effluent of acidogenic fermentation using (a) Aliquat/methyloctanoate and (b) TOPO/methyloctanoate RE systems. Effluent 1 (left column) and Effluent 2 (right column) indicate solution 1 and 2 respectively obtained from acid-rich thin stillage. (E% values for C6 extraction using Aliquat/methyloctanoate for Effluent 2 are close to 100% and not shown in the figure).

Fig. 4. Toxicity effect of diluent alone (methyloctanoate) and RE systems (Aliquat/methyloctanoate, TOPO/methyloctanoate) on VFA production in acidogenic fermentation.

Fig. 5. Schematic of process flow design for coupled VFA fermentation-ISPR system. Note that the exit (aqueous) stream from reactive extraction step can be recycled back to the fermenter for chain elongation of residual (un-extracted) short-chain VFA.