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Highly selective recovery of medium chain carboxylates from co-fermented organic wastes using anion exchange with carbon dioxide expanded methanol desorption

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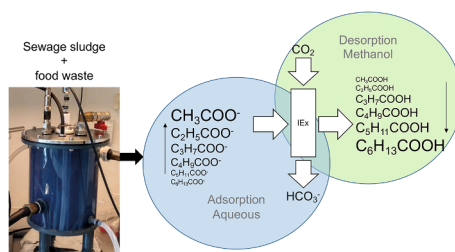
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HIGHLIGHTS

- C1-C7 carboxylates were successfully recovered from fermented organic waste.
- Strong anion exchange resin preferentially adsorbed hexanoate and heptanoate.
- Longer carboxylates were also easier desorbed, allowing their enrichment.
- Hexanoate and heptanoate were concentrated up to 0.29 mol/kg and 0.18 mol/kg, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this work was to recover a mixture of carboxylates ranging from 2 to 7 carbon atoms using a strong anion exchange resin, followed by desorption with CO₂-expanded methanol. Medium chain carboxylates hexanoate and heptanoate adsorbed better than acetate, and the corresponding medium chain carboxylic acids desorbed easier than acetic acid. Consequently, hexanoate and heptanoate were concentrated up to 14.6 and 20.7 times, respectively. These findings will enable effective separation and purification of the produced carboxylic acids. Notably, the presence of inorganic ions in the sample, such as chloride, decreased the adsorption affinity compared to a synthetic mixture only of carboxylates.

1. Introduction

The current depletion of natural resources due to their exacerbated consumption is requiring a transition from a linear to a circular economy. In such an economy, organic wastes play an important role since

they hold a great potential for different resources, such as nutrients and carbon. Finding a viable and efficient way to separate these valuable fractions still represents one of the main challenges.

Different technologies can be applied to organic wastes depending on the fraction to be recovered. In the past, incineration was a widely used

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technology in order to recover energy, but new techniques such as pyrolysis have been applied to convert the waste into other products, such as biofuels (Fahmy et al., 2020). Nowadays, the focus is switching to the production of biochemicals, which are more valuable than energy.

Some of the common technologies for resource recovery from organic wastes are mixed-culture fermentation for the production of carboxylates and hydrogen (Agler et al., 2011; Atasoy et al., 2018; Bastidas-Oyanedel et al., 2015; Hay et al., 2013; Marone et al., 2015) or the complete anaerobic digestion (AD) for the production of biogas (Maragkaki et al., 2017; Nghiem et al., 2014). Co-fermentation of different organic wastes has also been investigated as strategy to optimize resource recovery, in order to reduce possibly inhibitory compounds and exploit the synergistic effect (Eiteman et al., 2008; Mata-Alvarez et al., 2011). During anaerobic fermentation, hydrogen and carboxylic acids are produced by undefined mixed cultures. During acidogenic glucose fermentation, different reduction and oxidation reactions take place, in which glucose is converted to pyruvate and can be further transformed into carboxylic acids (Agler et al., 2011; Bastidas-Oyanedel et al., 2015; Hay et al., 2013). Production of carboxylic acids and hydrogen are correlated, since the partial pressure of hydrogen can influence the metabolic pathways and favor the production of certain carboxylic acids (Hay et al., 2013; van Lingen et al., 2016). One of the main challenges of mixed culture fermentation is the difficulty to focus on the production of an individual product, due to the metabolic activity of different microbial populations and interactions among them (Varrone et al., 2017). The use of mixed cultures complicates the long-term preservation of the inoculum and lowers the long-term stability of the process (Grimalt-Alemany et al., 2020; Varrone et al., 2018, 2017). In addition, the variability of microorganisms hinders the optimization of the process parameters and maximization of the yields, which may lead to more expensive downstream processing. The potential presence of pathogenic microorganisms in undefined mixed cultures can make their application unsuitable for the feed or pharma industry. When carboxylic acids are targeted, their conversion to methane by methanogenic bacteria has to be inhibited. Currently, the more valuable components (longer carboxylates) are in practice not separated from the rest.

Sewage sludge (SS) or animal manure are some of the most common substrates utilized for anaerobic digestion. In this process, energy is recovered in the form of biogas, while the nutrients remain in the digestate fraction that can be used as fertilizer. During anaerobic digestion, the produced biogas is separated from the liquid fraction due to the difference in density. Nevertheless, purification of the products from mixed-culture fermentation presents even a greater challenge, since it consists of a mixture of different carboxylates that have very similar properties. In addition, most of the compounds are in the dissociated form, since their pK_a is lower than the common operational pH (between 5 and 7) during mixed-culture fermentation. The methodologies applied for carboxylate recovery should then exploit this characteristic and focus on the properties of the dissociated compounds (López-Garzón and Straathof, 2014).

Some of the technologies studied for recovery of carboxylates from mixed-culture fermentation are electrodialysis (Scoma et al., 2016), nanofiltration (Zacharof et al., 2016), liquid-liquid extraction (Andersen et al., 2016; Reyhanitash et al., 2016) and ion exchange (Rebecchi et al., 2016). Compared to electrodialysis and nanofiltration, ion exchange avoids costs due to membrane fouling or high energy demand. Table 1 presents an overview of the applications, limitations and operational conditions of these technologies.

The recovery of carboxylates via anion exchange chromatography is a very promising technology since it targets the interaction between the dissociated compound and the active site of the resin, which forms an ionic bond. Anion exchange has already been successfully applied to some compounds such as acetate, propionate and butyrate (Cabrera-Rodríguez et al., 2017b). With this technology, the dissociated carboxylates are adsorbed to a strong anion exchange resin, followed by

Table 1
Overview of recovery technologies.

	Operational conditions	Applications	Limitations
Electrodialysis	Ambient conditions, conducting aqueous solution, electric field	Water desalination Wastewater treatment	Less economical for low ion concentration Scaling Membrane fouling High energy demand
Nanofiltration	Ambient temperature, pressure 5 - 70 bar, aqueous solution	Water desalination Micropollutant removal Wastewater treatment	Membrane fouling High energy demand Limited particle size retention
Liquid-liquid extraction	Ambient conditions, aqueous-nonaqueous biphasic mixture	Capture of compounds in chemical, food and pharmaceutical industry	High consumption of chemicals Scaling Extractant regeneration to avoid waste production
Ion exchange	Ambient conditions, aqueous solution	Deminerallization Water softening Separation of organic acids, peptides and proteins	Resin lifetime Chemicals consumption for resin regeneration
Anion exchange + CO ₂ -expanded alcohol regeneration	Pressure 10 - 20 bar, aqueous solution	Single step recovery Protonation of organic acids	Solubility of the acid Desorption efficiency Resin lifetime

recovery through desorption with CO₂ and methanol, which results in the desired protonated form of the carboxylate. In addition, esterification of these compounds has been proven successful for a mixture of carboxylates from paper mill wastewater (Cabrera-Rodríguez et al., 2017a). Furthermore, the use of CO₂-expanded methanol results in no stoichiometric waste salt production, while the high solubility of CO₂ in methanol decreases the CO₂ pressure needed and therefore the cost of this technology.

However, the recovery of longer compounds such as hexanoate and heptanoate has been less studied. These medium chain carboxylates, generated during chain elongation of shorter carboxylates (usually using ethanol as electron donor), have a higher market value than volatile fatty acids (VFA) (Zacharof and Lovitt, 2013). It represents a typical example of secondary fermentation (*sensu* Agler et al., 2011) and has been already described in detail (de Cavalcante et al., 2017). The chain elongation process has been studied under different operating conditions, achieving titers up to 1.7 g/l of hexanoate without external ethanol addition, 2.7 g/l with ethanol addition in a single reactor, and 12.6 g/l with ethanol addition in a two-stage system (Grootscholten et al., 2014, 2013). However, higher concentrations are limited by the solubility of hexanoate and the high product inhibition. Therefore, to favor process intensification, carboxylate removal can be coupled to fermentation, in order to reduce product inhibition and increase the final productivity, as successfully proved with anion-exchange resin (Yu et al., 2019). Besides anion exchange, other technologies such as membrane electrolysis (Andersen et al., 2014; Xu et al., 2015) and liquid-liquid extraction (Agler et al., 2012; Saboe et al., 2018) have also been coupled to carboxylate removal from fermentation processes. The liquid-liquid extraction system (pertraction), for instance, consists of a pH gradient (5.5–9) acting as driving force to extract the carboxylic acids by diffusion through membranes (Agler et al., 2012). Furthermore, a membrane electrolysis cell can be added to the pertraction system, achieving a final effluent with up to 90% of hexanoic and octanoic acids

(Xu et al., 2015). This system was based on a pH gradient in a membrane electrolysis cell and the application of a constant current, thus avoiding the addition of chemicals.

The objective of the current paper was to study the recovery of carboxylic acids from a mixture of carboxylates obtained from complex co-fermented organic wastes. After anion exchange, carboxylates were desorbed as carboxylic acids using a CO₂-expanded alcohol technology, which has already been proven successful for short carboxylates. The focus was on obtaining enrichment towards the more valuable medium chain carboxylic acids hexanoic and heptanoic acid, investigating the effect of carbon chain length on the affinity during adsorption and desorption.

2. Materials and methods

2.1. Mixture of carboxylates for adsorption and desorption experiments

Two different samples were used for the experiments: a co-fermented organic waste and a synthetic mixture with a similar composition of carboxylates. The organic wastes utilized were municipal sewage sludge (SS) and the organic fraction of municipal solid waste (OFMSW). SS was obtained in Copenhagen from BIOFOS, the largest wastewater treatment plant in Denmark. Samples of OFMSW were obtained from HCS Environment & Waste, a Danish company that pretreats this organic waste. It consists of the organic fraction collected from households, restaurants and supermarkets. OFMSW is pretreated by the company with a hammer mill and mixed with rain water to produce a pulp.

Both substrates were mixed in a SS/OFMSW ratio of 40/60, based on volatile solids (VS) content, and co-fermented in a 3 L custom-made reactor with hydraulic retention time (HRT) of 4 days. The effluent was centrifuged at 10,000 rpm and 4 °C for 20 min (Thermo Scientific Sorvall RC 6 Plus, USA), and residual solid particles were removed by microfiltration (pore size 0.2 µm).

A synthetic mixture with similar composition to the real fermented sample was prepared by dissolving acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, hexanoic acid and heptanoic acid in deionized water (Milli-Q). pH was adjusted to 5.18 with sodium hydroxide. These mixtures were compared with a pure solution of hexanoic acid, which was prepared by dissolving hexanoic acid in deionized water and the pH was adjusted to 5.17 with sodium hydroxide.

2.2. Other materials

All the chemicals were analytical grade: Dowex Marathon MSA resin (type I; macroporous, chloride form), anhydrous methanol (≥99.8%), sodium hydroxide (98%), acetic acid (≥99%), propionic acid (≥99%), isobutyric acid (≥99%), butyric acid (≥99%), isovaleric acid (≥99%), valeric acid (≥99%), hexanoic acid (≥99%) and heptanoic acid (≥99%) were obtained from Sigma Aldrich. Sodium bicarbonate (≥97%) was obtained from J. T. Baker. Carbon dioxide (≥99.8%) was supplied by Linde as compressed gas.

2.3. Resin preparation

Dowex Marathon MSA, a strong basic resin, was supplied in the chloride form (Sigma Aldrich). A weighed amount of resin (5 g of wet resin chloride form (65–68% w/w water)) was placed in an adjustable height Omnifit glass column (1 cm of internal diameter × 15 cm height), leading to a bed volume (BV) of 7.3 ml. The resin was converted from the chloride form to the bicarbonate form by column elution technique, as described in (Cabrera-Rodríguez et al., 2017a). In this preparation, the resin was washed with constant flow (1.5 ml/min) of sodium bicarbonate solution (20 g/l), until the absorbance at 210 nm at the outlet of the column was constant. The resin was then washed with a constant flow (1.5 ml/min) of deionized water until the absorbance at 210 nm at

the outlet of the column remained constant.

2.4. Adsorption and desorption experiments

Breakthrough experiments were performed in a Thermo Scientific Dionex Ultimate 3000 system. Carboxylate solutions were pumped through the column with a flowrate of 1.5 ml/min and room temperature. Fractions of 0.75 ml were collected during the experiment. Feed and collected samples were analyzed for carboxylates. All the experiments were performed until $C_i/C_{i,feed}$ reached 1 for most of the compounds (C_i , effluent concentration; $C_{i,feed}$, feed concentration).

After adsorption, the solvent in the column was exchanged with methanol with a flowrate of 1.5 ml/min during 15 min. The pressure setup was prepared for desorption experiments by mixture of methanol and CO₂ in a mixing vessel. CO₂ was sparged from the bottom until it reached equilibrium with methanol (when the pressure stabilized). The pressure was maintained constant at 10 bar. Desorption experiments were performed with a flowrate of 1 ml/min and fractions of 0.75 ml were collected from the outlet of the column.

After carboxylates were desorbed, the column was washed with deionized water with constant flow (1.5 ml/min) until the absorbance at 210 nm at the outlet of the column was constant. A wavelength of 210 nm was used to ensure the complete absence of organic acids in the resin after column regeneration. Subsequently, the column was regenerated with constant flow (1.5 ml/min) of sodium bicarbonate solution (20 g/l) until the absorbance at 210 nm at the outlet of the column was constant. This led to reproducible adsorption profiles.

2.5. Analytical methods

Acetic, propionic, isobutyric, butyric, isovaleric, valeric, hexanoic and heptanoic acid concentrations were analyzed on a Waters HPLC system using a Bio-Rad Aminex HPX-87H column (78 × 300 mm) at 60 °C and a Refractive Index Detector (RID). Phosphoric acid (1.5 mmol/l at 0.6 ml/min) was used as an eluent. Phosphate (Hach-Lange LCK 350), nitrate (Hach-Lange LCK 339), calcium (Hach-Lange LCK327), magnesium (Hach-Lange LCK327), chloride (Sigma-Aldrich MAK023) and chemical oxygen demand (COD) (Hach-Lange LCK114) were determined spectrophotometrically using the specific test kits.

2.6. Calculation of adsorbed amount and concentration factor

The adsorbed amount Q_i (in mmol/g resin) of the different compounds was determined according to Equation (1), where $C_{i, out}$ represents the measured concentration of compound i leaving the column (in mmol/kg) and $C_{i, feed}$ the concentration in the feed sample (in mmol/kg). F is the volume flow rate (in ml/min) and M is the wet resin mass (in grams).

$$Q_i = \frac{F(C_{i,feed}t - \int_0^t C_{i,out} dt)}{M} \quad (1)$$

The concentration factor (CF) was calculated according to Equation (2), where $C_{i, des}$ represents the maximum desorption concentration (in mmol/kg).

$$CF = \frac{C_{i,des}}{C_{i,feed}} \quad (2)$$

3. Results and discussion

3.1. Characterization of co-fermentation products

The two organic wastes (municipal sewage sludge and OFMSW) were co-fermented in order to produce an effluent rich in carboxylates. In addition to the carboxylates, the co-fermented sample also contained several inorganic ions, as presented in Table 2. The high level of calcium

Table 2
Characterization of the co-fermented sludge and food waste.

Compound	Co-fermented sample		Synthetic mixture	
	mg/l	mmol/l	mg/l	mmol/l
Acetate	1826 ± 9	30.4 ± 0.2	1900 ± 100	31.6 ± 1.7
Propionate	706 ± 8	9.5 ± 0.1	875 ± 102	11.8 ± 1.4
Isobutyrate	248 ± 32	2.8 ± 0.4	214 ± 69	2.4 ± 0.8
Butyrate	1228 ± 1	13.9 ± 0.0	1244 ± 18	14.1 ± 0.2
Isovalerate	1355 ± 7	13.3 ± 0.1	954 ± 24	9.3 ± 0.2
Valerate	1013 ± 10	9.9 ± 0.1	1056 ± 8	10.3 ± 0.1
Hexanoate	1870 ± 41	16.1 ± 0.4	2268 ± 24	19.5 ± 0.2
Heptanoate	823 ± 116	6.3 ± 0.9	1149 ± 143	8.8 ± 1.1
Total	9069 ± 56	102.3 ± 0.6	9661 ± 65	108 ± 0.1
Ethanol	153.5 ± 76.7	3.3 ± 1.7	–	–
Chloride	388.7 ± 10.3	11.0 ± 0.3	–	–
Nitrate	11.0 ± 0.3	0.2 ± 0.0	–	–
Phosphate	457.8 ± 0.4	4.8 ± 0.0	–	–
Calcium	236.5 ± 1.4	5.9 ± 0.0	–	–
Magnesium	58.3 ± 0.4	2.4 ± 0.0	–	–

in the co-fermented sample was due to the water hardness, typical in the Copenhagen area, where the municipal sludge was collected. The co-fermented sample was composed of a mixture of carboxylates, mainly acetate, butyrate, valerate and hexanoate. The total concentration of carboxylates was 9,069 mg/l, which represented 94% of the total soluble COD of the sample (17,633 mg/l). The pH during fermentation was around 5.5, therefore most of the compounds were dissociated, since their pKa is below this pH value.

The characterization of the co-fermented samples was used to prepare a synthetic mixture with similar composition. This mixture was used to analyze the effect of inorganic impurities during adsorption and desorption and to compare the affinities of the different components.

3.2. Adsorption of carboxylates during anion exchange chromatography

Two different solutions were used for the adsorption experiments: the synthetic mixture and the co-fermented organic waste.

During adsorption, the anion exchange resin, originally loaded with bicarbonate ($Q^+HCO_3^-$), was loaded with the dissociated carboxylates (R_iCOO^-), as presented in Equation (3):

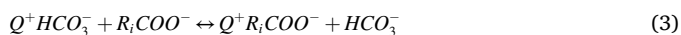


Fig. 1 presents the elution profiles of the carboxylates as breakthrough curves for the synthetic mixture and co-fermented sample. Breakthrough started at 2 BV in both the synthetic mixture (Fig. 1A) and in the co-fermented sample (Fig. 1B). Propionate was the only compound that presented a shorter breakthrough, 0.6 BV, in the co-fermented sample, which may be due to a measurement problem. On the other hand, a similar behavior for propionate was already observed (during the first 20 BV) in a previous study using acidified paper mill wastewater (Cabrera-Rodríguez et al., 2017a).

In the current study, most carboxylates ranging from 2 to 5 carbon atoms (acetate to isovalerate), showed a similar trend during adsorption in the synthetic mixture. Valerate and especially hexanoate and heptanoate showed a stronger adsorption than shorter compounds, according to the faster breakthrough of the shorter compounds. The reason for the stronger adsorption might be higher hydrophobicity, since hexanoic and heptanoic acid have a partition coefficient (Log P) of 1.92 and 2.42, respectively, compared to -0.17 of acetate, 0.33 of propionate, 0.79 of butyrate and 1.39 of valerate (PubChem, 2020). Furthermore, previous studies suggested higher adsorption affinity for hexanoate compared to acetate and butyrate, based on the different partition coefficients of the compounds as well (Rebecchi et al., 2016; Yu et al., 2019). These studies compared different anion exchange resins and concluded that hexanoate had a higher affinity for all of them, compared to shorter compounds. However, these experiments were performed with a synthetic mixture of carboxylates in batch vials, until equilibrium was reached. In the current

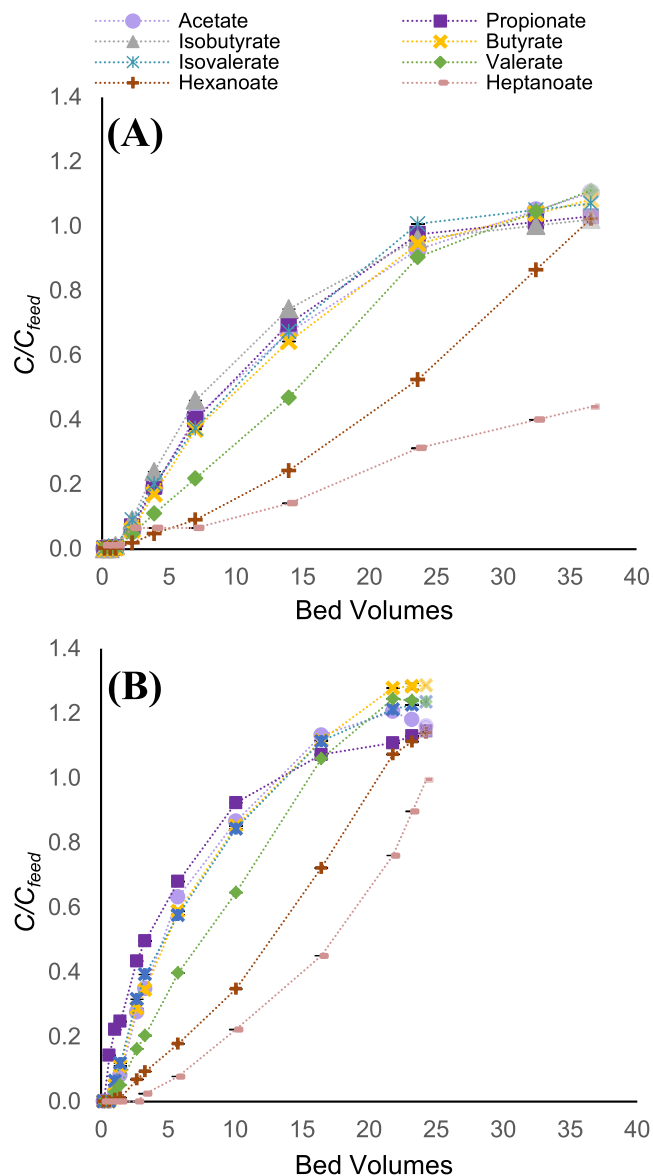


Fig. 1. Breakthrough curves for A) synthetic mixture of carboxylates and B) co-fermented sewage sludge and OFMSW (C , effluent concentration; C_{feed} , feed concentration). Average values of duplicate experiments. Last bed volume as an estimation. Isobutyrate results are not presented in the co-fermented sample due to difficulties during quantification of this compound.

study, on the other hand, the mixture of carboxylates was pumped through the column at a constant flowrate until the carboxylate concentration in the effluent was similar to the concentration in the feed sample. Nonetheless, a higher adsorption affinity for longer compounds was also found with this mode of operation.

In comparison, preliminary experiments with a solution of pure hexanoate presented the start of breakthrough of this compound at 2 BV, similar to hexanoate in the synthetic mixture and co-fermented sample. Hexanoate saturation of the resin took place at 33.5 BV for pure hexanoate, 37 BV for the synthetic mixture and around 20 BV for the co-fermented sample. In both experiments, saturation of the shorter compounds took place almost at the same time, followed firstly by hexanoate and then heptanoate. In the synthetic mixture, carboxylates ranging from 2 to 5 carbon atoms reached a C/C_{feed} ratio of 1 after approximately 24 BV, while in the co-fermented sample the same ratio was achieved in less than 16 BV. Moreover, this sample presented overshooting of the shorter carboxylates in the last 10 BV of adsorption. These compounds

started desorbing and, consequently, the amount of hexanoate and heptanoate in the column increased, reaching a C/C_{feed} ratio of 1.1 and 0.90 at 23 BV, respectively. Hexanoate also started desorbing after 20 BV.

As can be seen in Fig. 2, the solution of hexanoate saturated the column around 33 BV, while the synthetic mixture did not reach complete saturation after 36 BV. However, the co-fermented sample reached clear column saturation around 16 BV, when the adsorbed amount started decreasing. All the single compounds followed the same trend except hexanoate and heptanoate, which started decreasing around 24 BV. Therefore, the adsorption of the co-fermented sample should be stopped around 16 BV.

The difference between synthetic and co-fermented sample was probably mainly caused by the presence of inorganic ions such as chloride in the co-fermented sample, as presented in Table 2. Overshooting of the shorter carboxylates (C2 to C4) during experiments with the co-fermented sample was caused by the higher affinity of inorganics and medium chain carboxylates, which displaced the C2 to C4 carboxylates. In addition, the possible presence of other impurities may have enhanced this adsorption difference between the synthetic and co-fermented sample. The presence of biomass in the sample may affect the long-term regeneration of the resin. Therefore, a study of multiple cycles with different pretreatment methods to remove biomass should be performed. The total adsorbed amounts in all the experiments are presented in Table 3, including 16.4 BV of the co-fermented sample. As expected, the adsorbed amount of all the compounds was higher using the synthetic mixture while it decreased with the co-fermented sample, due to displacement by other (undetected) anions with higher affinity. Moreover, the feed volume using the co-fermented sample had an impact on the type of compound that was loaded on the column. After a feed volume of 24.3 BV, the total adsorbed amounts per gram of resin for all the short carboxylates were lower than after the feed volume of 16.4 BV. On the other hand, the amount of hexanoate on the resin was almost the same, while heptanoate was 20% higher. This difference between shorter and longer compounds can be explained by overshooting of the smaller compounds at $BV > \sim 16.4$. Similar displacement of shorter carboxylates was already observed during adsorption of acidified paper mill wastewater (Cabrera-Rodríguez et al., 2017a). For the desorption, an additional experiment with co-fermented sample was performed for 15 BV in order to maximize the carboxylates load in the column and avoid overshooting, as presented in the following Section 3.3.

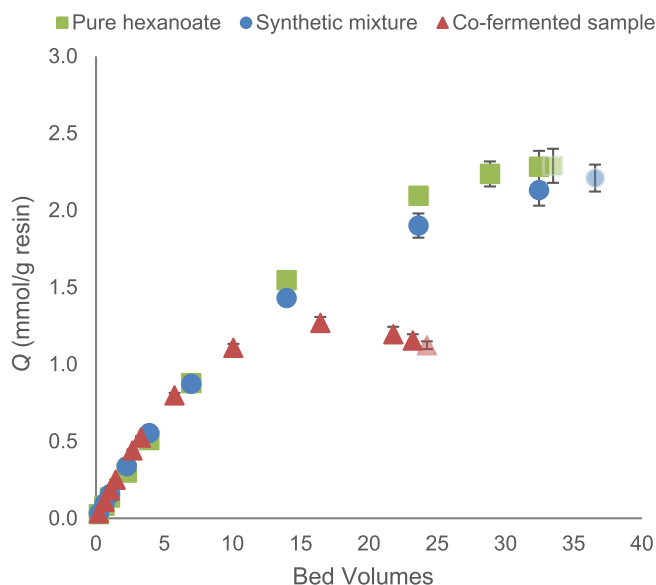


Fig. 2. Saturation of the column using the pure hexanoate, synthetic mixture and the co-fermented sample.

Table 3

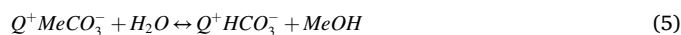
Adsorbed amounts (Q) of different carboxylates at the end of the two experiments.

Compound	Synthetic mixture	Co-fermented sample	
		16.4 BV	24.3 BV
Adsorbed amount (mmol/g resin)			
Acetate	0.48 ± 0.06	0.30 ± 0.00	0.23 ± 0.00
Propionate	0.18 ± 0.04	0.14 ± 0.00	0.12 ± 0.00
Isobutyrate	0.03 ± 0.01	–	–
Butyrate	0.22 ± 0.00	0.15 ± 0.07	0.10 ± 0.07
Isovalerate	0.13 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Valerate	0.20 ± 0.02	0.15 ± 0.00	0.13 ± 0.00
Hexanoate	0.59 ± 0.04	0.31 ± 0.01	0.32 ± 0.02
Heptanoate	0.36 ± 0.02	0.15 ± 0.01	0.18 ± 0.02
TOTAL	2.21 ± 0.10	1.24 ± 0.04	1.09 ± 0.03

3.3. Desorption of carboxylates during anion exchange chromatography

Three desorption experiments were performed after the adsorption tests (presented in Section 3.2.): i) synthetic mixture, ii) co-fermented sample for 24.3 BV and iii) co-fermented sample for 15 BV. The adsorption experiment with co-fermented sample at 15 BV was performed in order to start the desorption experiment from the capacity point reached at this number of bed volumes. This third (desorption) experiment, the co-fermented sample with 15 BV, had a very similar adsorption profile compared to 16.4 BV of the second experiment presented in Table 3. Small differences were due to slight variations in the initial concentration and sampling time.

It is worth noting that during desorption, the anion exchange resin, previously loaded with the dissociated carboxylates, was converted to the bicarbonate form, as presented in Equations (4) and (5). At the same time, the recovered carboxylates were protonated, resulting in no stoichiometric waste salt production (Cabrera-Rodríguez et al., 2017b).



Preliminary desorption experiments with pure hexanoate showed a maximum desorption concentration of 0.97 mol/kg solvent around 2 $BV_{desorption}$. Similar profiles are presented in Fig. 3 for the synthetic mixture and for co-fermented sample from 24.3 $BV_{adsorption}$ and 15 $BV_{adsorption}$. Here, the desorption concentration reached 0.29, 0.11 and 0.14 mol/kg solvent, respectively. All the compounds achieved their maximum concentration around 2 BV as well, followed by a pronounced tailing, especially for acetate. The final concentrations were lower for the experiments with co-fermented sample, since the total amount loaded in the column was generally lower, as presented in Table 3. Concentration of the carboxylates after desorption, compared to the initial feed concentration, increased according to the number of carbons. More specifically, the heptanoate concentration was 20.7 times higher after desorption (compared to the initial feed in the synthetic mixture), 13.6 times higher for the co-fermented sample from 24.3 $BV_{adsorption}$ and 7.9 times higher for the co-fermented sample from 15 $BV_{adsorption}$. In the case of hexanoate, these concentrations increased 14.6, 5.9 and 7 times, respectively.

Hexanoate and acetate were the compounds with the highest adsorbed amount in all the experiments (Table 3), however while hexanoate reached the highest concentration of all during desorption, this was not the case for acetate. Acetate only reached a maximum concentration of 0.09, 0.04 and 0.07 mol/kg solvent, respectively.

The total desorbed amount for each experiment, on the other hand, was lower than the adsorbed amount. After adsorption of the synthetic mixture, 2.21 mmol were loaded per gram of resin, but 2.00 mmol/g resin were desorbed during the desorption experiment. The co-fermented sample with 24.3 BV and 15 BV (the third experiment) loaded 1.09 and 1.24 mmol/g resin, while the desorption yielded 0.61

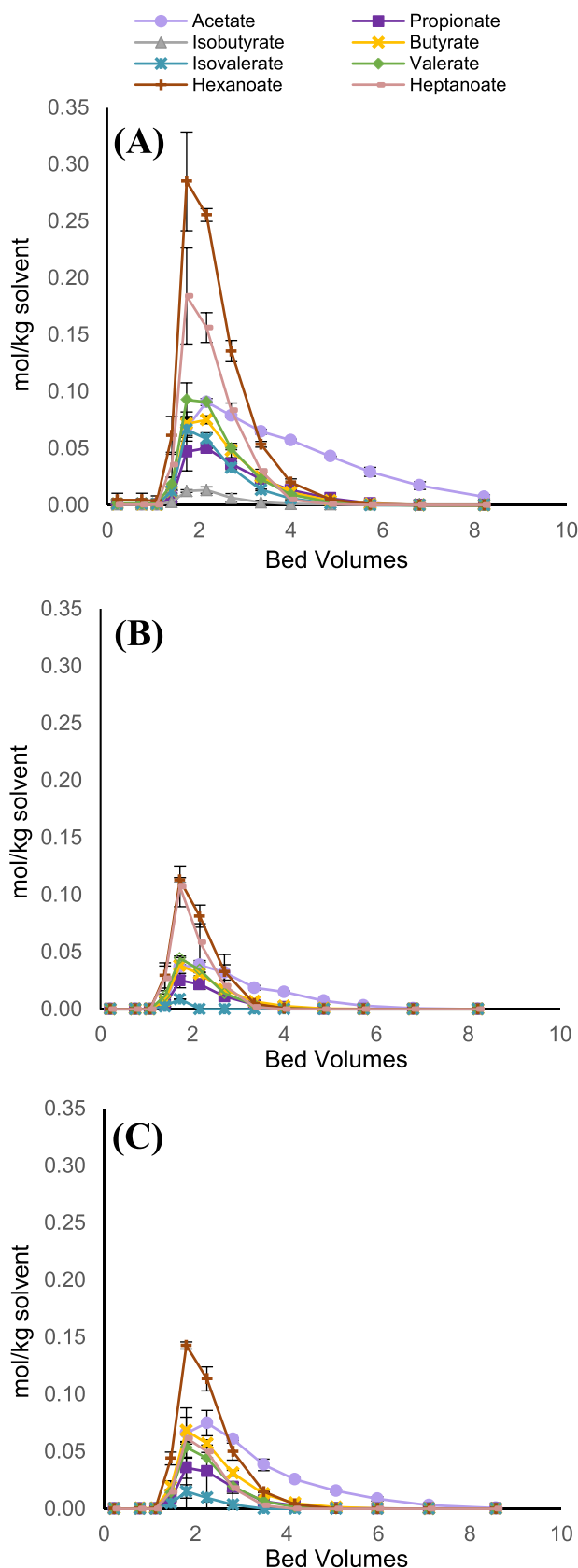


Fig. 3. Desorption concentration (in mol/kg solvent) for A) synthetic mixture of carboxylates, B) co-fermented sample 24.3 BV and C) co-fermented sample 15 BV. Average values of duplicate experiments.

and 0.89 mmol/g resin, respectively. None of the three experiments reached a desorption percentage of 100%, but this was due to the steps of washing with water and solvent exchange, which were performed between adsorption and desorption experiments, as well as column regeneration with sodium bicarbonate at the end of the experiment. During these steps, part of the loaded compounds was lost in the different solvents. The synthetic mixture had the highest desorption percentage of 90.5%, while this number decreased to 73.6% with the co-fermented sample at 15 BV and even lower to almost 60% with the co-fermented sample at 24.3 BV. These variations between the total adsorbed amount and the maximum concentration during desorption, for the different carboxylates, indicate higher affinity for longer compounds. The positive impact of carboxylate length was previously suggested for the desorption with only ethanol and a mixture of ethanol and NaOH, which was applied in batch mode to different resins loaded with VFA in a vial (Rebecchi et al., 2016). In that experiment, the authors showed positive increase in the desorption yield along with the number of carbons. Another study used different temperatures and concentrations of NaOH during desorption of resins loaded from a fermentation broth (Yu et al., 2019). However, no significant differences in the desorption efficiency were found, comparing hexanoate, butyrate and acetate, besides a slightly better efficiency for acetate. In the current study, desorption experiments were performed at a constant flowrate, using a column loaded with the resin and carboxylates after the adsorption (Section 3.2.). The use of CO₂-expanded methanol enabled the recovery of carboxylates in the protonated form, avoiding the generation of salt waste and regenerating the column to the bicarbonate form (Cabrera-Rodríguez et al., 2017b).

3.4. Higher concentration factor for medium chain carboxylates

In order to study the influence of the number of carbons during the experiments, the concentration factor for the different compounds was calculated (Fig. 4) as described in Section 2.6. Fig. 4 clearly presents an increase in concentration factor, along with the number of carbons. The shorter carboxylates, ranging from 2 to 4 carbon atoms, present a similar CF, which increases with longer compounds. With acetate as reference, the CF ranged between 4 and 5 times higher for hexanoate and up to 13 times for heptanoate, in the co-fermented sample 24.3 BV.

In comparison, the application of membrane electrodialysis (with a single electrochemical step) for carboxylate recovery did not present

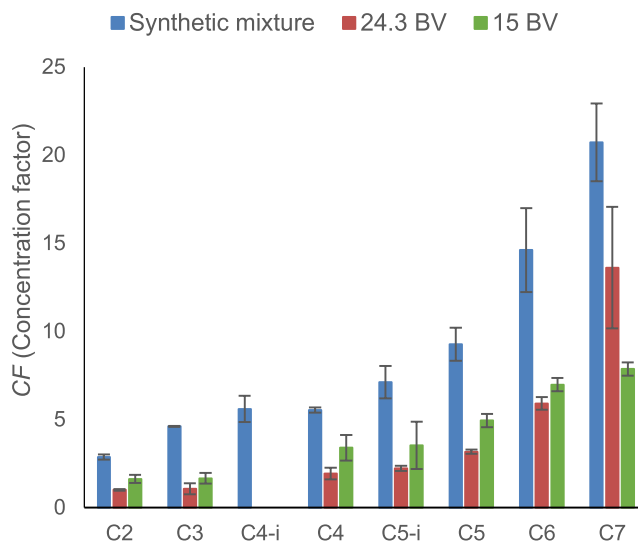


Fig. 4. Concentration factor for the different carboxylates in the synthetic mixture and co-fermented samples (C2, acetate; C3, propionate; C4-i, isobutyrate; C4, butyrate; C5-i, isovalerate; C5, valerate; C6, hexanoate; C7, heptanoate).

different selectivities between compounds ranging from acetate to hexanoate (Vertova et al., 2009). However, a membrane electrolysis cell coupled to carboxylate fermentation and working with a pH gradient achieved a selective medium-chain carboxylate separation (Xu et al., 2015). The accumulation of carboxylates after the anion exchange membrane lead to concentrations slightly above the solubility of longer compounds (C6 and C8), which enabled phase separation. However, the concentration of shorter compounds (C2 to C5) was below their solubility. Therefore, the new medium chain carboxylate molecules transferred through the membrane were separated from the solution, while the shorter carboxylates did not.

Additionally, the use of ionic liquids for carboxylates recovery presented higher selectivity for butyrate and propionate, compared to acetate (Reyhantash et al., 2016). On the other hand, the application of nanofiltration to a mixture of acetate and butyrate did not show clear selectivity differences (Zacharof et al., 2016). Nevertheless, the addition of sodium bicarbonate presented encouraging results, showing the potential of salt addition as selectivity agent that could favor the retention of one acid over the other.

In the current study, the synthetic mixture presented a higher CF for all the compounds, compared to the co-fermented samples, while 24.3 BV and 15 BV were very similar, with the exceptions of isovalerate and heptanoate. The C7 compound presented almost 2 times higher CF in the co-fermented sample 24.3 BV compared to the 15 BV. The difference between synthetic mixture and co-fermented samples may be due to the content of inorganic ions in the resin, but the concentration factor still raises as the number of carbons increases. This difference between compounds can be exploited for developing an industrial chromatographic separation process (Nicoud, 2015).

3.5. Perspectives on the applications of this technology

In this study, the production and recovery of carboxylates from co-fermentation of organic wastes was studied. The effluent after fermentation was centrifuged and filtered in order to remove impurities. The remaining fractions, after this pretreatment, could be used for anaerobic digestion, thus coupling the production of biogas. Duplicate experiments were performed using the same resin in order to evaluate a possible decrease in adsorption capacity due to impurities in the sample. In future studies, the lifetime of the resin should be investigated more in detail, but the use of CO₂-expanded methanol as desorption technology allows to regenerate the column to the original bicarbonate form. In addition, the high solubility of CO₂ in methanol reduces the pressure needed and therefore the operational costs (Cabrera-Rodríguez et al., 2017b). A techno-economic design of a conceptual full scale process should be performed to assess its practical applicability.

4. Conclusions

This study confirmed that the use of anion-exchange chromatography and CO₂-expanded methanol can recover carboxylic acids from a complex carboxylate mixture obtained by fermentation of organic wastes. The method proved to be selective for longer chain carboxylates, leading to up to about 20-fold higher concentration of desorbed heptanoic acid compared to heptanoate in the feed. Notably, the inorganic ions in the feed resin took up resin capacity and negatively affected the process.

CRedit authorship contribution statement

Clara Fernando-Foncillas: Investigation, Methodology, Writing - original draft. **Carlos I. Cabrera-Rodríguez:** Conceptualization, Methodology, Writing - review & editing. **Francisco Caparrós-Salvador:** Methodology, Writing - review & editing. **Cristiano Varrone:** Supervision, Writing - review & editing. **Adrie J.J. Straathof:** Supervision, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2020.124178>.

References

- Agler, M.T., Spirito, C.M., Usack, J.G., Werner, J.J., Angenent, L.T., 2012. Chain elongation with reactor microbiomes: upgrading dilute ethanol to medium-chain carboxylates. *Energy Environ. Sci.* 5, 8189. <https://doi.org/10.1039/c2ee22101b>.
- Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: The carboxylate platform. *Trends Biotechnol.* 29, 70–78. <https://doi.org/10.1016/j.tibtech.2010.11.006>.
- Andersen, S.J., Berton, J.K.E.T., Naert, P., Gildemyn, S., Rabaey, K., Stevens, C.V., 2016. Extraction and esterification of low-titer short-chain volatile fatty acids from anaerobic fermentation with ionic liquids. *ChemSusChem* 9, 2059–2063. <https://doi.org/10.1002/cssc.201600473>.
- Andersen, S.J., Hennebel, T., Gildemyn, S., Coma, M., Desloover, J., Berton, J., Tsukamoto, J., Stevens, C., Rabaey, K., 2014. Electrolytic membrane extraction enables production of fine chemicals from biorefinery sidestreams. *Environ. Sci. Technol.* 48, 7135–7142. <https://doi.org/10.1021/es500483w>.
- Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2018. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresour. Technol.* 268, 773–786. <https://doi.org/10.1016/j.biortech.2018.07.042>.
- Bastidas-Oyanedel, J.-R., Bonk, F., Thomsen, M.H., Schmidt, J.E., 2015. Dark fermentation biorefinery in the present and future (bio)chemical industry. *Rev. Environ. Sci. Bio/Technol.* 14, 473–498. <https://doi.org/10.1007/s11557-015-9369-3>.
- Cabrera-Rodríguez, C.I., Moreno-González, M., de Weerd, F.A., Viswanathan, V., van der Wielen, L.A.M., Straathof, A.J.J., 2017a. Esters production via carboxylates from anaerobic paper mill wastewater treatment. *Bioresour. Technol.* 237, 186–192. <https://doi.org/10.1016/j.biortech.2017.02.030>.
- Cabrera-Rodríguez, C.I., Paltrinieri, L., de Smet, L.C.P.M., van der Wielen, L.A.M., Straathof, A.J.J., 2017b. Recovery and esterification of aqueous carboxylates by using CO₂-expanded alcohols with anion exchange. *Green Chem.* 19, 729–738. <https://doi.org/10.1039/C6GC01391K>.
- Cavalcante, W. de A., Leitão, R.C., Gehring, T.A., Angenent, L.T., Santaella, S.T., 2017. Anaerobic fermentation for n-caproic acid production: a review. *Process Biochem.* 54, 106–119. <https://doi.org/10.1016/j.procbio.2016.12.024>.
- Eiteman, M.A., Lee, S.A., Altman, E., 2008. A co-fermentation strategy to consume sugar mixtures effectively. *J. Biol. Eng.* 2, 3. <https://doi.org/10.1186/1754-1611-2-3>.
- Fahmy, T.Y.A., Fahmy, Y., Mobarak, F., El-Sakhawy, M., Abou-Zeid, R.E., 2020. Biomass pyrolysis: past, present, and future. *Environ. Dev. Sustain.* 22, 17–32. <https://doi.org/10.1007/s10668-018-0200-5>.
- Grimalt-Alemany, A., Łężyk, M., Asimakopoulos, K., Skiadas, I.V., Gavala, H.N., 2020. Cryopreservation and fast recovery of enriched syngas-converting microbial communities. *Water Res.* 177, 115747. <https://doi.org/10.1016/j.watres.2020.115747>.
- Grootscholten, T.I.M., Kinsky dal Borgo, F., Hamelers, H.V.M., Buisman, C.J.N., 2013. Promoting chain elongation in mixed culture acidification reactors by addition of ethanol. *Biomass and Bioenergy* 48, 10–16. <https://doi.org/10.1016/j.biombioe.2012.11.019>.
- Grootscholten, T.I.M., Strik, D.P.B.T.B., Steinbusch, K.J.J., Buisman, C.J.N., Hamelers, H.V.M., 2014. Two-stage medium chain fatty acid (MCFA) production from municipal solid waste and ethanol. *Appl. Energy* 116, 223–229. <https://doi.org/10.1016/j.apenergy.2013.11.061>.
- Hay, J.X.W., Wu, T.Y., Juan, J.C., Jahim, J.M., 2013. Biohydrogen production through photo fermentation or dark fermentation using waste as a substrate: overview, economics, and future prospects of hydrogen usage. *Biofuels Bioprod. Biorefining* 7, 334–352. <https://doi.org/10.1002/bbb.1403>.
- López-Garzón, C.S., Straathof, A.J.J., 2014. Recovery of carboxylic acids produced by fermentation. *Biotechnol. Adv.* 32, 873–904. <https://doi.org/10.1016/j.biotechadv.2014.04.002>.
- Maragkaki, A.E., Fountoulakis, M., Gypakis, A., Kyriakou, A., Lasaridi, K., Manios, T., 2017. Pilot-scale anaerobic co-digestion of sewage sludge with agro-industrial by-products for increased biogas production of existing digesters at wastewater

- treatment plants. *Waste Manag.* 59, 362–370. <https://doi.org/10.1016/j.wasman.2016.10.043>.
- Marone, A., Varrone, C., Fiocchetti, F., Giussani, B., Izzo, G., Mentuccia, L., Rosa, S., Signorini, A., 2015. Optimization of substrate composition for biohydrogen production from buffalo slurry co-fermented with cheese whey and crude glycerol, using microbial mixed culture. *Int. J. Hydrogen Energy* 40, 209–218. <https://doi.org/10.1016/j.ijhydene.2014.11.008>.
- Mata-Alvarez, J., Dosta, J., Macé, S., Astals, S., 2011. Codigestion of solid wastes: A review of its uses and perspectives including modeling. *Crit. Rev. Biotechnol.* 31, 99–111. <https://doi.org/10.3109/07388551.2010.525496>.
- Nghiem, L.D., Nguyen, T.T., Manassa, P., Fitzgerald, S.K., Dawson, M., Vierboom, S., 2014. Co-digestion of sewage sludge and crude glycerol for on-demand biogas production. *Int. Biodeterior. Biodegradation* 95, 160–166. <https://doi.org/10.1016/j.ibiod.2014.04.023>.
- Nicoud, R.-M., 2015. *Chromatographic Processes - Modeling, Simulation, and Design*. Cambridge University Press.
- PubChem, 2020. National Center for Biotechnology Information. URL <https://pubchem.ncbi.nlm.nih.gov/> (accessed 4.30.20).
- Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., Frascari, D., 2016. Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. *Chem. Eng. J.* 306, 629–639. <https://doi.org/10.1016/j.cej.2016.07.101>.
- Reyhanitash, E., Zaalberg, B., Kersten, S.R.A., Schuur, B., 2016. Extraction of volatile fatty acids from fermented wastewater. *Sep. Purif. Technol.* 161, 61–68. <https://doi.org/10.1016/j.seppur.2016.01.037>.
- Saboe, P.O., Manker, L.P., Michener, W.E., Peterson, D.J., Brandner, D.G., Deutch, S.P., Kumar, M., Cywar, R.M., Beckham, G.T., Karp, E.M., 2018. In situ recovery of bio-based carboxylic acids. *Green Chem.* 20, 1791–1804. <https://doi.org/10.1039/C7GC03747C>.
- Scoma, A., Varela-Corredor, F., Bertin, L., Gostoli, C., Bandini, S., 2016. Recovery of VFAs from anaerobic digestion of dephenolized olive mill wastewaters by electro dialysis. *Sep. Purif. Technol.* 159, 81–91. <https://doi.org/10.1016/j.seppur.2015.12.029>.
- van Lingen, H.J., Plugge, C.M., Fadel, J.G., Kebreab, E., Bannink, A., Dijkstra, J., 2016. Thermodynamic driving force of hydrogen on rumen microbial metabolism: a theoretical investigation. *PLOS One* 11. <https://doi.org/10.1371/journal.pone.0161362>.
- Varrone, C., Floriotis, G., Heggset, T.M.B., Le, S.B., Markussen, S., Skiadas, I.V., Gavala, H.N., 2017. Continuous fermentation and kinetic experiments for the conversion of crude glycerol derived from second-generation biodiesel into 1,3 propanediol and butyric acid. *Biochem. Eng. J.* 128, 149–161. <https://doi.org/10.1016/j.bej.2017.09.012>.
- Varrone, C., Skiadas, I.V., Gavala, H.N., 2018. Effect of hydraulic retention time on the modelling and optimization of joint 1,3 PDO and BuA production from 2G glycerol in a chemostat process. *Chem. Eng. J.* 347, 525–534. <https://doi.org/10.1016/j.cej.2018.04.071>.
- Vertova, A., Aricci, G., Rondinini, S., Miglio, R., Carnelli, L., D'Olimpio, P., 2009. Electrolytic recovery of light carboxylic acids from industrial aqueous wastes. *J. Appl. Electrochem.* 39, 2051–2059. <https://doi.org/10.1007/s10800-009-9871-9>.
- Xu, J., Guzman, J.J.L., Andersen, S.J., Rabaey, K., Angenent, L.T., 2015. In-line and selective phase separation of medium-chain carboxylic acids using membrane electrolysis. *Chem. Commun.* 51, 6847–6850. <https://doi.org/10.1039/C5CC01897H>.
- Yu, J., Liao, J., Huang, Z., Wu, P., Zhao, M., Liu, C., Ruan, W., 2019. Enhanced anaerobic mixed culture fermentation with anion-exchange resin for caproate production. *Processes* 7, 404. <https://doi.org/10.3390/pr7070404>.
- Zacharof, M.-P., Lovitt, R.W., 2013. Complex effluent streams as a potential source of volatile fatty acids. *Waste Biomass Valorization* 4, 557–581. <https://doi.org/10.1007/s12649-013-9202-6>.
- Zacharof, M.P., Mandale, S.J., Williams, P.M., Lovitt, R.W., 2016. Nanofiltration of treated digested agricultural wastewater for recovery of carboxylic acids. *J. Clean. Prod.* 112, 4749–4761. <https://doi.org/10.1016/j.jclepro.2015.07.004>.