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(Selective) Isolation of acetic acid and lactic acid from heterogeneous fermentation of xylose and glucose

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ABSTRACT

The present study focuses on the isolation of acetic and lactic acid from a fermentation broth produced by heterofermentative microorganisms using glucose and xylose as substrate. Especially xylose fermentation to lactic acid leads to unwanted by-product formation of acetic acid. Reactive liquid-liquid extraction is an energy-efficient downstream process, where the use of green solvents such as *d*-limonene opens the way to a more sustainable production. To find the optimum solvent/reactive extractant pairing, the reactive extractants trioctylamine, trioctylphosphine oxide, Aliquat 336, and tributyl phosphate were used diluted in 1-octanol, 1-decanol, *d*-limonene or a deep eutectic solvent. The phosphine-based extractants proved to be most effective for both acids. In the first extraction step, 19% of acetic acid and only 3% of lactic acid are extracted with trioctylphosphine oxide/1-octanol when the xylose feed is used without pH adjustment. The pKa value of the acids is responsible for the difference in extraction efficiency leading to the proposal of a two-step extraction separating first acetic acid and after pH adjustment, lactic acid can be extracted. The combination of trioctylphosphine oxide diluted in *d*-limonene leads to a surprisingly high selectivity for lactic acid isolation in the second extraction step. The present study shows for the first time that green solvents lead to similar extraction efficiencies compared to conventional solvents such as 1-octanol. For back extraction *n*-heptane and *p*-cymene are used as disintegration agents and water as stripping phase; *n*-heptane results in a lactic acid back extraction efficiency of up to 82% and *p*-cymene up to 70%. This shows that also for the back extraction *p*-cymene as a green disintegration agent leads to similar results as fossil-based *n*-heptane.

1. Introduction

Lactic acid is a naturally occurring organic acid that exists in two different isomers. It can be manufactured by chemical synthesis or microbial fermentation processes. In addition to the utilization of fossil fuels, the main disadvantage of chemical synthesis is that it produces only a racemic mixture of D(-) and L(+) lactic acid (D-LA; L-LA) which is not suitable for many applications [1,2]. Microbial fermentation can result either in optically pure D-LA or in optically pure L-LA, or a mixture of both depending on the microbe used [3].

The global market growth for lactic acid in the last 3 years was 8.2% and is also reported to increase by 8% per annum [4] with polylactic acid (PLA) accounting for a quarter of this as the main market driver. PLA has gained increased attention recently since it represents a bio-based and biodegradable thermoplastic polymer, which can already be produced on a close-to-cost competitiveness basis compared to conventional plastics [5].

It is well known that both the physical and mechanical properties of PLA can be influenced by variations of its isomeric composition [6–9], the production of optically pure L-LA and D-LA is thus essential for achieving cost-competitive production of PLA with tailored functional properties. Compared to L-LA production only little is currently known about D-LA production and its production is still limited to pilot scale only, due to unsolved cost-associated problems [10–12]. As is the case with L-LA the switch from cost-intensive sugar feedstock to industrial by-products can represent a method for cutting the production costs of D-LA [13]. As industrial by-products, for example from the pulping industry, often consist of mixtures of hexose and pentose sugars, utilization of both is preferable. Pentose sugars are metabolized by lactic acid bacteria via the phosphoketolase and the pentose phosphate/glycolytic

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pathway resulting in a homofermentative or heterofermentative production of LA [14]. Homofermentative production is preferable due to the simplified purification it offers, but homofermentation of pentose sugars is rare in nature [15–17] and has never been demonstrated for D-LA with naturally occurring isolates [10,18,19]. D-LA production from pentoses with natural isolates is generally limited. A screening of the integrated microbial genomes and microbiomes (IMG) database identified only 6 out of 5235 lactobacilli able to produce optically pure D-lactate from xylose. As acetic acid generation during fermentation cannot be avoided without genetic modification, efficient isolation technologies need to be developed for cost-efficient processing from xylose to lactic acid.

Reactive liquid-liquid extraction has shown to be effective for the isolation of carboxylic acids from different feed streams [20–22]. Also the in situ removal of lactic acid from a fermentation broth using a membrane-assisted reactive liquid-liquid extraction process was shown to be practicable [23]. The isolation of carboxylic acids from biobased process streams using reactive liquid-liquid extraction is generally accompanied by low selectivity for the targeted carboxylic acid and high crud formation. Crud describes the formation of a stable interfacial layer made from the two phases, proteins, cell debris, or alike are known to stabilize the formed crud layer [24]. Sufficient pre-treatment of the feed solution and the optimization of the solvent phase composition is necessary to overcome these topics [20]. The extraction efficiency depends on the solvent phase composition, such as the reactive extractant [25], the diluent, and the modifier [26], whereas the selectivity is influenced by the pKa value of the respective acid [27].

The present article shows the approach of using reactive liquid-liquid extraction for the separation of acetic acid and lactic acid from a glucose- and a xylose-based fermentation broth produced by heterogeneous fermenting species. In addition to commonly used solvents, the green solvents *d*-limonene and a deep eutectic solvent made from thymol and menthol [28] are now being tested. Xylose fermentation to lactic acid is accompanied by high acetic acid loads, selective separation of the two acids is targeted by varying the solvent phase and adjustment of the feed pH value. The back extraction of the acids in their protonated form is investigated with the disintegration agents *n*-heptane and *p*-cymene, and the stripping phase water. The use of *p*-cymene as disintegration agent is not described in the literature yet. Finally, a five-step extraction is performed to prove if the separation of acetic acid before lactic acid extraction is possible.

2. Materials and methods

2.1. Feed preparation and characterization

To verify how the used microorganism *Weissella oryzae* (DSMZ 25,784) converts glucose and xylose, the two feed streams were investigated separately. stream. *Weissella oryzae* (DSMZ 25,784) was cultivated using MRS media. The fermentation was performed as an anaerobic fed-batch process in a 2 l glass vessels with 1500 ml working volume. The process was initiated by a batch phase with 20 g l^{-1} sugar concentration. Feeding with 600 g l^{-1} sugar solution and a feeding rate of 3.75 ml h^{-1} started after 8 h. Agitation at 400 rpm was maintained using an integrated stirrer (DasGip, Julich, Germany), and the temperature was maintained at 30 °C. The pH of the medium was maintained at 5.0 by the automatic addition of 8 M NaOH. Temperature, agitation, and pH were monitored and controlled using the DasGip monitoring and control system. The biomass was removed from the fermentation broth by centrifugation at 7100 relative centrifugal force (Sorvall Evolution RC) and further filtered using a 0.2 μm PES membrane bottle top filter (Thermo Scientific, Nalgene). The sterile fermentation broth was stored at 4 °C until further processing. Both fermentation broths appeared as clear yellow-colored liquid. Optical purity for both feeds was determined to be 100% D-LA. Table 1 summarizes the concentration of lactic acid and acetic acid, and the pH for the two investigated fermentation broths.

Table 1
Summary of the two investigated feed streams, glucose feed (GF) and xylose feed (XF). c_{LA} is the lactic acid concentration and c_{AA} is the acetic acid concentration.

Feed	c_{LA} / g l^{-1}	c_{AA} / g l^{-1}	pH
Glucose feed (*GF*)	15.92 ± 0.77	0.78 ± 0.06	4.84–4.85
Xylose feed (*XF*)	12.14 ± 0.59	9.45 ± 0.65	4.87–4.88

The comparably low concentration of acetic acid when glucose is used as feeding material is a result of using the heterofermentative species *Weissella oryzae* during fermentation.

2.2. Materials

Trioctylamine (TOA, 98%, CAS 214-242-1, Sigma-Aldrich), Aliquat 336 (AQ, 88.2-93%, CAS 63,393-96-4, Alfa Aesar), tributyl phosphate (TBP, 97%, CAS 126-73-8, Sigma Aldrich) and trioctylphosphine oxide (TOPO, 91%, CAS 78-50-2, Cytec) were used as extractants. 1-octanol (≥99%, CAS 111-87-5, Carl Roth), 1-decanol (>98%, CAS 112-30-1, Alfa Aesar), *d*-limonene (100%, CAS 5989-27-5, Carl Roth), and a deep eutectic solvent produced from 33% thymol (≥99%, CAS 89-83-8, Carl Roth) and 67% menthol (≥99%, CAS 1490-04-6, Sigma Aldrich) were used as solvents in reactive liquid-liquid extraction. Preparation of the DES was performed as described in [28]. For the back extraction *n*-heptane (≥99%, CAS 110-54-3, Carl Roth) and *p*-cymene (99%, CAS 99-87-6, Sigma Aldrich) were used as disintegration agents. Distilled water was used as the stripping phase. Sulfuric acid was used for pH adjustment (98%, 7664-93-9, Sigma Aldrich). The mobile phase was prepared using a 1 N sulfuric acid solution (CAS 7664-93-9, Carl Roth) and ultrapure water. The internal standard solution was prepared using dimethyl sulphoxide (DMSO, >99.91%, CAS 67-68-5, ThermoFisher Scientific) in ultrapure water for HPLC measurement.

2.3. Analytics

2.3.1. Carboxylic acids

The carboxylic acid concentrations in the aqueous samples were measured using an HPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific) with a REZEX-ROA column (Rezex™ ROA-Organic Acid *H* + 8%, LC Column 300 × 7.8 mm, Ea from Phenomenex), an UV-Vis detector (operated at 210 nm), and an external column oven (Dionex STH 585 from Thermo Fisher Scientific); the column oven was set to 30 °C. As the mobile phase, a 0.0025 M H_2SO_4 solution was used at a flow rate of 0.5 ml min^{-1} [20]. Sample preparation was done by diluting 0.8 ml of glucose feed, 1.0 ml of xylose feed, 1.2 ml of glucose raffinate, 1.4 ml of xylose raffinate, or 1.6 ml of loaded stripping phase with 1.0 ml 0.0025 M H_2SO_4 and 1 ml DMSO solution (0.001 g ml^{-1}), that was used as internal standard. Subsequently, the samples were filtered using a 0.45 μm syringe filter (AAFPES4525-100, Altmann Analytik).

2.3.2. Proteins

The total protein concentration was determined using the Bradford method [29]. The assay was performed in microplate wells. Bovine serum albumin was used as protein standard solution with concentrations between 1 and 0.025 mg ml^{-1}. 10 μl of each standard/sample/blank was mixed with 200 μl of diluted Bradford reagent (ROTI®Quant 5x Carl Roth) and incubated at room temperature for 5 min while slow shaking. The absorbance values were measured at 595 nm and a standard curve was generated using Microsoft Office Excel software. The protein concentration of the respective sample was calculated using the equation of the standard curve.

2.3.3. Sugars

The sugar concentrations in fermentation, as well as extraction samples, were determined using an HPLC system with RID (Shimadzu Prominence), equipped with an Aminex™ HPX87H (300·x 7.8 mm)

column (Bio-Rad) operated at an oven temperature of 40 °C using 5 mmol l^{-1} H_2SO_4 as eluent at a flow rate of 0.6 ml min^{-1} [30]. All samples were filtered using a 0.2 μm syringe filter and diluted with mobile phase if necessary to reach a sugar concentration below 5 g L^{-1}.

2.4. Extraction experiments

The extraction experiments were performed in double-walled separation funnels on a mechanical shaker (Edmund Bühler SM 25); the funnels were connected to a thermostat (Lauda M3-MS). The phase ratio between the fermentation broth and the solvent phase was 1:1 (v/v), the solvent phase was in each case composed of 30 wt% reactive extractant and 70 wt% solvent. 10 ml of the two phases were transferred into the funnel and mixed for 60 min at 170 rpm and 25 °C (±1 °C) [28]. Morales et al. [31] stated, that the extraction equilibrium for monocarboxylic acids is reached in 5-10 min. After mixing, the phases settled for 20 h under gravity. The mixing and settling time was chosen to ensure phase equilibrium and good phase separation. After phase separation, the equilibrium pH value ($pH_{equ,feed}$) was measured (electrode: SI Analytics A 164 1M-DIN-ID; pH meter: WTW Inolab pH level) and the acid concentrations in the aqueous phase were determined with HPLC. The water content of the solvent phase was measured using an auto titrator (SI Analytics TitroLine® 7500 KF). To ensure reproducibility, each experiment was performed twice.

For the five-step extraction experiments, the raffinate from the previous step was contacted with fresh solvent resulting in a cross-current operation.

2.5. Back extraction experiments

The back extraction was performed using 5 ml of extract and 10 ml of water as the stripping phase, further to this 15 ml of either *n*-heptane or *p*-cymene was added as disintegration agent. The solvent, the disintegration agent, and the water were mixed at 70 °C; speed, mixing time, and settling time were the same as for the extraction. After mixing and phase separation, the loaded stripping phase was analyzed for equilibrium pH value ($pH_{equ,strip}$) and acid content. The procedure was adapted as suggested by Gössi et al. [23].

2.6. Extraction efficiencies and extractant loading

The experimental data were evaluated using the efficiency for extraction and back extraction. The acid concentrations were measured in the aqueous phase, and the corresponding concentrations in the solvent phase were calculated based on the mass balance. The water content in the solvent phase changed during the extraction from 0.032-0.83 wt% to 0.32-8.14 wt%, which influences the phase ratio and, hence, needs to be taken into account in the calculations. The carboxylic acid equilibrium concentration in the solvent phase ($c_{ca,\ solvent,equ}$) was calculated according to Eq. (1), where $V_{aqu,in}$ and $V_{solvent,in}$ is the volume of the aqueous and solvent phase at the beginning of the experiment, $c_{ca,\ in}$ is the initial acid concentration in the fermentation broth, $c_{ca,aqu,equ}$ is the acid concentration in equilibrium, and $\Delta V_{H20,equ}$ is the change in volume of the solvent phase.

$$c_{ca,solvent,equ} = \frac{\left(\left(V_{aqu,in}\cdot c_{ca,aqu,in}\right) - \left(V_{aqu,in} - \Delta V_{H2O,equ}\right)\cdot c_{ca,aqu,equ}\right)}{\left(V_{solvent,\ in} + \Delta V_{H2O,equ}\right)} \quad (1)$$

The extraction efficiency (E_{extr}) and the back extraction efficiency (E_{back}), as well as the overall extraction efficiency (E_{tot}), were calculated using Eqs. (2), (3), and (4). Where V_{strip} is the volume of the stripping phase $c_{ca,strip}$ represents the carboxylic acid concentration in the stripping phase and $V_{solvent}$ is the volume of the loaden solvent phase.

$$E_{extr} = \frac{\left(V_{solvent,in} + \Delta V_{H2O,equ}\right)\cdot c_{ca,solvent}}{V_{aqu}\cdot c_{ca,aqu,in}}\cdot 100 \quad (2)$$

$$E_{back} = \frac{V_{strip}\cdot c_{ca,strip}}{V_{solvent}\cdot c_{ca,solvent,equ}}\cdot 100 \quad (3)$$

$$E_{tot} = \frac{E_{extr}}{100}\frac{E_{back}}{100}\cdot 100 \quad (4)$$

The composition of the solvent phase is regularly stated in weight percent. Due to the different molar weight of the respective extractant, the loading of the solvent phase (*z*) varies and was calculated using Eq. (5) [20].

$$z = n_{acid}/n_{extractant} \quad (5)$$

3. Results and discussion

3.1. Extractant screening

The feed streams had a pH of 4.8-4.9, which is unfavorable for carboxylic acid extraction because the generally used extraction agents extract the undissociated acid. Fig. 1 shows the Haegg diagram for lactic acid and acetic acid. It can be seen that at the feed pH value of 4.8 only 11% of the lactic acid is present in its protonated form, whereas at a pH of 2.8, 96% is present in the protonated form. For acetic acid at a pH of 4.8, 48%, and at a pH of 2.8, 99% are present as protonated acid molecules. The mechanism for the most commonly used extractants TOA or TOPO is based on anion exchange and on hydrogen bonding. Hence, the extraction works best at low pH values [32].

Using the fermentation broth without pH adjustment is targeted. In the first step, an extractant screening was performed using 1-octanol as a modifier, and TOA, AQ, TOPO, and TBP as reactive extractants. AQ is a quaternary amine and can also extract the dissociated acid. Fig. 2 summarizes the data for both feed streams, *GF* and *XF*, and all the extraction agents tested. As expected, a higher extraction efficiency is obtained for acetic acid compared to lactic acid, which is related to the higher pKa of acetic acid. The two phosphorous-based extractants TOPO and TBP in particular show a high efficiency for acetic acid combined with a low efficiency for lactic acid. With these two extractants, a selective separation of acetic and lactic acid at the feed starting pH of 4.8 may be possible.

The results will further be discussed using the *XF*, as the acetic acid

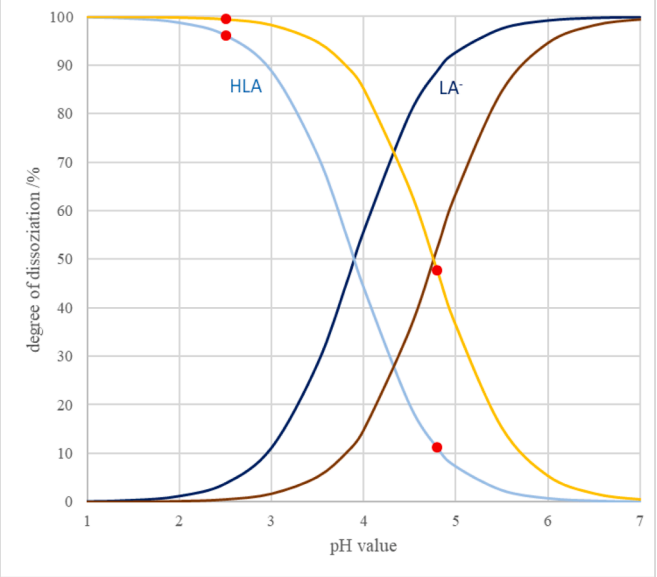


Fig. 1. Haegg diagram for lactic acid, pKa = 3.86 [30], in light blue HLA and in dark blue LA^-, and acetic acid pKa = 4.75, orange HAA, brown AA^-; red dots for the two pH values investigated; 4.8 = fermentation broth; 2.57 ± 0.04 adjusted pH value.

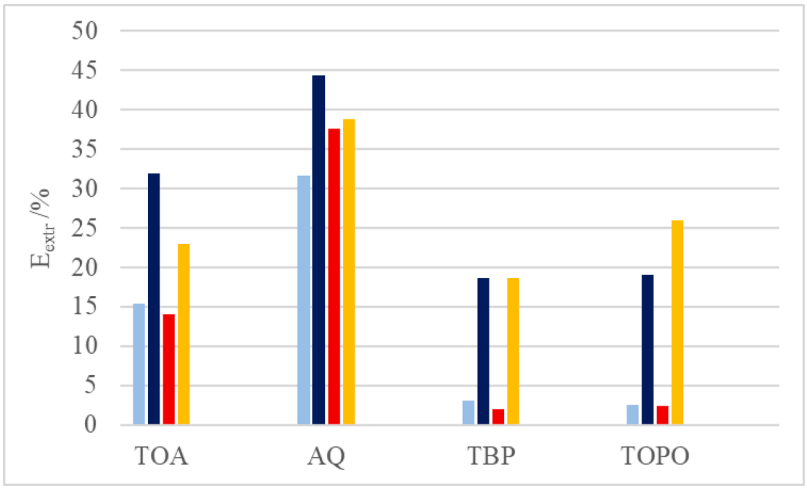


Fig. 2. Extraction efficiency for the tested extractants in 1-octanol, $pH_{initial} = 4.88$, $T = 25$ °C, red lactic acid in *GF*, yellow acetic acid in *GF*, light blue lactic acid in *XF*, dark blue acetic acid in *XF*.

concentration is considerably higher with 9.45 ± 0.65 g l^{-1} than in the *GF* with 0.78 ± 0.06 g·l^{-1}; however, all the explanations are also applicable for the *GF*.

The extraction efficiency at a pH of 4.8 is with 37.6 ± 0.0% for acetic acid and 31.7 ± 0.1% for lactic acid highest with AQ in 1-octanol. The high extraction efficiency can be attributed to the extraction mechanism, where lactate or acetate ions are preferably extracted by anion exchange, however, back extraction with AQ is challenging [33,34].

The phosphorous-based extractants TBP and TOPO exhibit an acetic acid extraction efficiency of 18.6 ± 0.7% and 19.1 ± 0.1%, whereas for lactic acid the extraction efficiency is comparatively low with 3.1 ± 0.9% and 2.6 ± 0.3%. The extraction ability for carboxylic acids is for both phosphorous-based extractants a result of the solvating character of the oxygen donor; the phosphoryl group is a stronger Lewis base than the carbonyl group at the acid resulting in high partitioning coefficients. Hano et al. found out that for TOPO the solvation number is controlled by the hydrophobicity and, hence, the number of carboxyl groups on each acid and not by the pKa value [35]. In our case, acetic acid offers a lower polarity than lactic acid, which results in a higher extraction efficiency thereof.

Based on the results, acetic acid may be removed in the first step, after that the pH needs to be adjusted to a value below the pKa of lactic acid to isolate lactic acid from the fermentation broth.

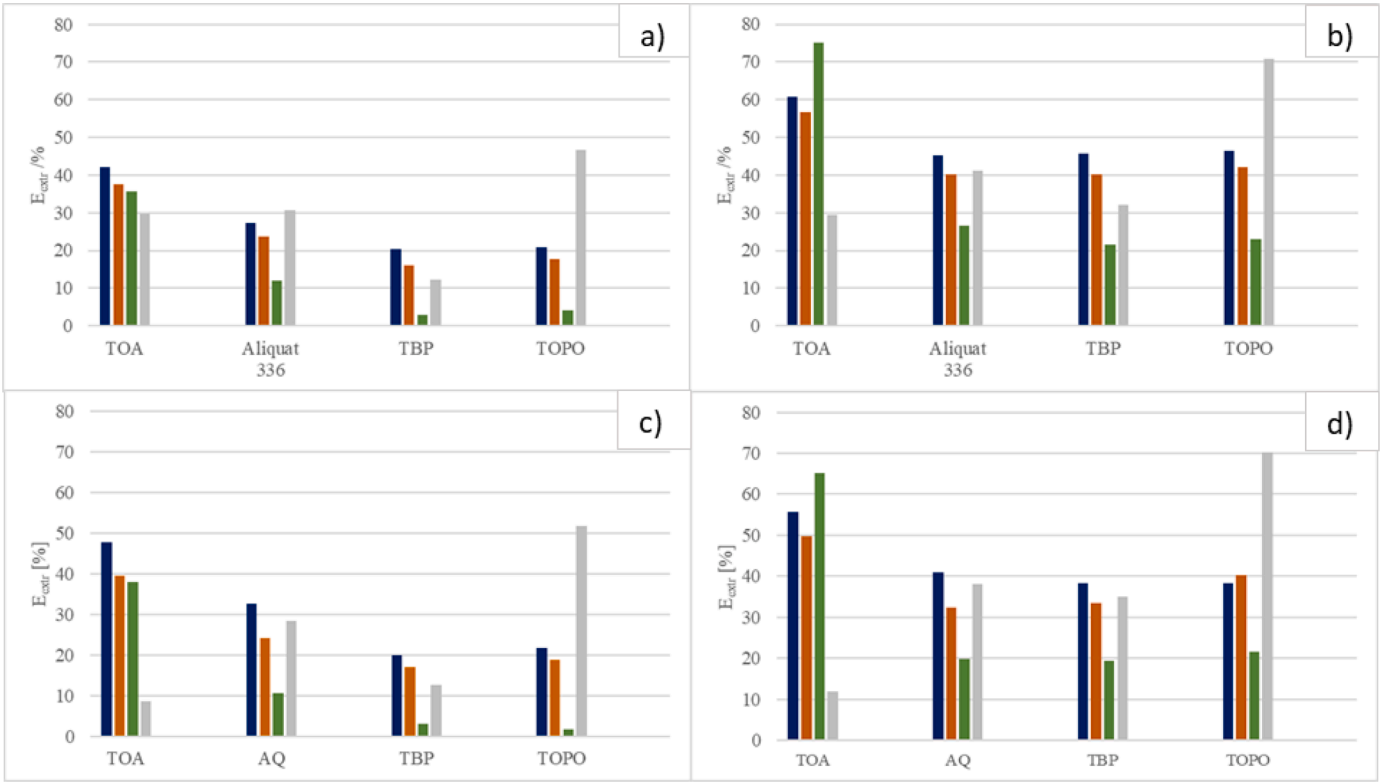


Fig. 3. Extraction efficiency for the different modifiers in the *XF*, a) lactic acid and b) acetic acid, and in the *GF* c) lactic acid and d) acetic acid. Dark blue = 1-octanol; orange = 1-decanol; green = DES (33% thymol, 65% menthol); grey = *d*-limonene. $pH_{initial,XF} = 2.57 \pm 0.04$, $pH_{initial,GF} = 2.51 \pm 1.18$ $T = 25$ °C.

3.2. Modifier screening with adjusted pH value

Besides the reactive extractant, the modifier plays a major role in the extraction in terms of crud formation and efficiency. Commonly used modifiers are 1-octanol and due to its lower water solubility more often 1-decanol. Besides the commonly used modifiers, *d*-limonene and a deep eutectic solvent made from thymol and menthol were tested [28]. To ensure that lactic acid is also removed from the fermentation broth, the pH value was adjusted to 2.57 ± 0.04, which is well below the pKa value of lactic acid. Fig. 3 summarizes the results for the *XF* (a and b) and the *GF* (c and d).

With an initial pH value of 2.57 ± 0.04 in the *XF*, a higher extraction efficiency is obtained for both acids. Also, the extraction efficiency decreases with increasing chain length of the modifiers 1-octanol and 1-decanol. The equilibrium pH is in line with the highest extraction efficiency of 75.1 ± 0.6% obtained for acetic acid when TOA diluted with 1-octanol is used; TOA diluted with the *d*-limonene leads with 70.8 ± 0.2% to the second highest extraction efficiency. The same behavior as for the *XF* is observed for the *GF*, also for the *GF*, the highest extraction efficiencies are achieved with TOA/DES and TOPO/*d*-limonene. Data for the equilibrium pH value and the water content are summarized in Table 2.

The deep eutectic solvent is not as effective for lactic acid as for acetic acid. The highest lactic acid extraction efficiency is obtained using the modifier *d*-limonene with the phosphorous-based extractant TOPO. Matsumoto et al. investigated the effect of combining TOA and TBP; the synergistic effect was more prominent for hydroxyl carboxylic acids [36]. Hence, this would be a possibility to increase the extraction efficiency for lactic acid.

TOPO diluted in *d*-limonene leads to the highest extraction efficiency for lactic acid, hence, this combination shall be used for further investigations.

3.3. Two-step extraction and back extraction

Based on the results discussed in 3.1. and 3.2., the concept of removing first acetic acid using TOPO diluted in 1-octanol, and second lactic acid, here either TOPO diluted in *d*-limonene or TOA diluted in 1-octanol were tested.

Common back extraction methods use a pH swing extraction with an alkaline solution, hence, a subsequent acidification step is needed to obtain the free acid, that is generally needed for industrial applications [20]. Gössi et al. suggested disintegrating lactic acid by using *n*-heptane as a disintegration agent and water as stripping phase [23]. To ensure greener production, *p*-cymene was tested as an alternative to *n*-heptane. The applied disintegration agents weaken the hydrogen bonding between the reactive extractant and the lactic and acetic acid, which leads to a shift of the phase equilibrium to the stripping phase [37]. The back extraction method that is performed, benefits from the fact that protonated lactic acid is liberated in the aqueous phase. After the back extraction, the *n*-heptane or *p*-cymene can be removed from the solvent phase by distillation [23].

Scheme 1 shows a schematic of the process tested for lactic and acetic acid separation.

In the *XF*, lactic acid had a concentration of 12.14 ± 0.59 g l^{-1} and acetic acid 9.45±0.65 g l^{-1}. With 13.41 g l^{-1} the lactic acid concentration in raffinate 1 is slightly higher than in the fermentation broth, which can be attributed to the water transfer from the feed phase into the solvent phase. Acetic acid was extracted into the extract, but 8.69 g l^{-1} remained in the raffinate. The extraction efficiency for lactic and acetic acid is 2.45% and 19.44% respectively. Raffinate 1 was then adjusted to a pH value of 2.51 ± 0.1.

When the solvent phase for the second extraction step consisted of TOA diluted in 1-octanol, raffinate 2 had a lactic acid concentration of 7.83 g l^{-1} and 3.37 g l^{-1} of acetic acid leading to an extraction efficiency of 43.32% and 62.39% respectively. The solvent phase consisting of TOPO/*d*-limonene leads to an extraction efficiency for lactic acid of 48.95%, and for acetic acid 73.99% are obtained. Both reactive extractants show high extraction efficiencies for acetic acid; as an efficient separation of the two acids is targeted, the first extraction step needs to be performed with more than one theoretical stages. However, a minor quantity of lactic acid is lost to the solvent phase.

Extract 2 was used for the back extraction. Table 3 summarizes the back extraction efficiencies for the *XF*, the two acids, and the two investigated solvent phases. Data for the *GF* are summarized in the supplementary information (STable 1).

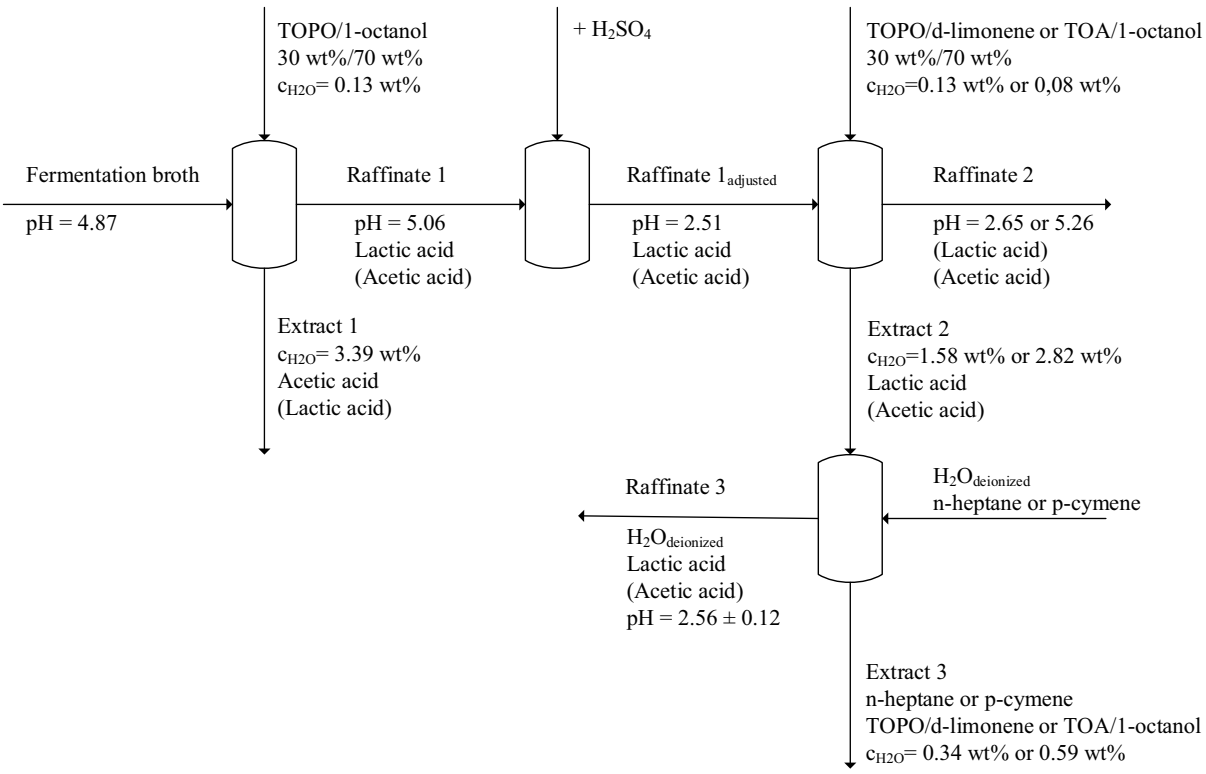
3.4. Five step cross current extraction

A five-step cross-current extraction without pH adjustment was performed to prove whether a selective separation of acetic and lactic acid is possible. The XF was thus used at the pH value resulting from the fermentation of 4.88. The results as shown previously suggest the use of the phosphine-based extractant TOPO diluted in 1-octanol for this purpose. Scheme 2 shows the schematic of the five-step extraction.

In each extraction step, fresh solvent was added to the raffinate stream from the previous extraction step. It can be seen that the solvent phase takes up 3.58 ± 0.16 wt% of water, a recycle of the solvent phase was not investigated. The water uptake of the solvent phase results in a concentration increase of lactic acid in the raffinate stream, which can

Table 2
Equilibrium pH and water content in the solvent phase for the reactive extractants TOA, AQ, TBP, and TOPO in combination with the modifier 1-octanol, 1-decanol, deep eutectic solvent (33 mol% thymol, 65 mol% menthol), and *d*-limonene. All extractants were used in a ratio of 30 wt% reactive extractant to 70 wt% modifier.

		XF				*GF*			
Extractant	Modifier	$pH_{initial}$	pH_{equ}	$H_2O_{solvent}$ [wt%]	$H_2O_{extract}$ [wt%]	$pH_{initial}$	pH_{equ}	$H_2O_{solvent}$ [wt%]	$H_2O_{extract}$ [wt%]
TOA	1-octanol	2.54	5.21	0.08	3.81	2.45	5.36	0.08	3.69
AQ		2.54	2.63	0.63	6.97	2.45	2.54	0.63	7.16
TBP		2.54	2.56	0.08	3.68	2.51	2.51	0.08	4.27
TOPO		2.54	2.57	0.13	3.40	2.58	2.54	0.13	3.25
TOA	1-decanol	2.54	5.05	0.14	3.13	2.58	5.20	0.14	3.08
AQ		2.57	2.64	0.62	6.44	2.50	2.57	0.62	6.57
TBP		2.57	2.58	0.28	3.40	2.50	2.51	0.28	3.45
TOPO		2.57	2.56	0.11	2.89	2.49	2.47	0.11	2.89
TOA	DES	2.58	6.49	0.09	1.52	2.49	6.63	0.09	1.47
AQ		2.58	2.49	0.57	2.26	2.49	2.41	0.57	2.43
TBP		2.58	2.55	0.07	1.42	2.52	2.50	0.07	1.44
TOPO		2.58	2.53	0.10	1.27	2.52	2.46	0.10	1.27
TOA	*d*-limonene	2.57	3.24	0.03	0.32	2.52	3.13	0.03	0.38
AQ		2.57	2.69	0.83	7.95	2.52	2.60	0.83	8.06
TBP		2.57	2.60	0.08	0.73	2.52	2.51	0.08	0.79
TOPO		2.57	2.65	0.12	1.23	2.52	2.56	0.12	1.56



Scheme 1. Schematic of the two-step extraction and back extraction. Compounds given in brackets mean that small quantities thereof are expected, e.g. extract 1 consists mainly of acetic acid, but small amounts of lactic acid are expected, the same applies to the other streams.

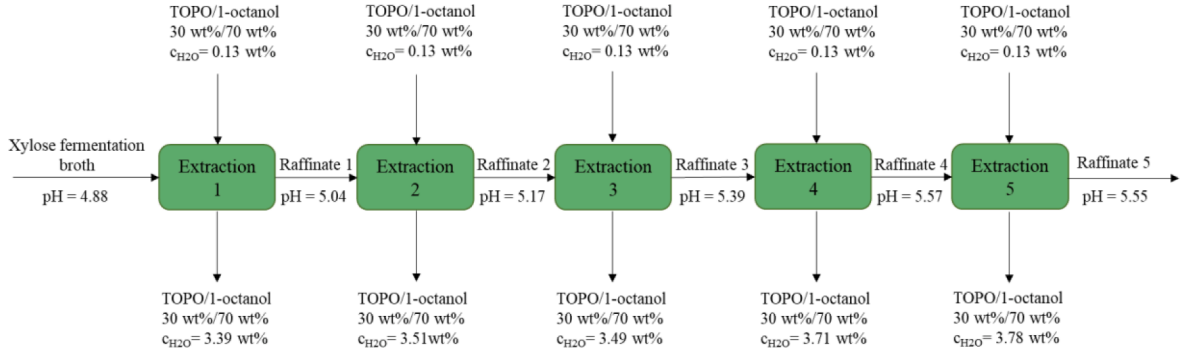
Table 3
Back extraction efficiencies for *n*-heptane and *p*-cymene into water for lactic acid and acetic acid for raffinate 3. Equilibrium pH = 2.4 ± 0.1, T = 70 °C.

Feed	Solvent phase	Stripping agent	Lactic acid E_{back} [%]	Acetic acid E_{back} [%]
Xylose	TOA:1-octanol	*p*-Cymene	54.4 ± 0.1	54.7 ± 0.5
		n-heptane	62.3 ± 0.7	61.5 ± 0.4
	TOPO:*d*-limonene	*p*-Cymene	65.0 ± 0.3	43.1 ± 0.2
		n-heptane	68.9 ± 2.5	47.6 ± 3.3

be seen in Fig. 4; the lactic acid slightly increases from step to step, although approximately 2% of the lactic acid is co-extracted in each step with acetic acid. The equilibrium pH value increases from extraction step one to four from 5.04 to 5.57 as a result of the acid extraction. After that, the pH remained stable, at this pH value 2% of lactic acid and 13% of acetic acid molecules are protonated, which results in a decreased extraction efficiency.

The results show that with the applied parameters a selective separation is not possible within five steps. Nevertheless, the results are promising, since neither the solvent phase nor the phase ratio or loading of the solvent phase were optimized. Saboe et al. showed the potential for increasing the efficiency of the process when varying the modifier, solvent, and phase ratio. They stated that with decreasing phase ratio (V_{org}/V_{aqu}) the extraction efficiency increases; decreasing the phase ratio from one to 8.5 resulted in an increased loading factor of the solvent phase, also from one to 8.5 [38].

If the pH value is adjusted to 4.90 ± 0.10 in each stage, 10 theoretical stages would be required to reduce the acetic acid concentration to 1 g l^{-1}, which equals 10% of the initial acetic acid concentration. At the same time, roughly 20% or 2 g 1^{-1} of lactic acid are co-extracted. Depending on the needed purity, the acetic acid can be removed, however, the data show that an integrated process design is necessary to ensure product quality and also cost efficiency.



Scheme 2. Schematic of the five-step extraction.

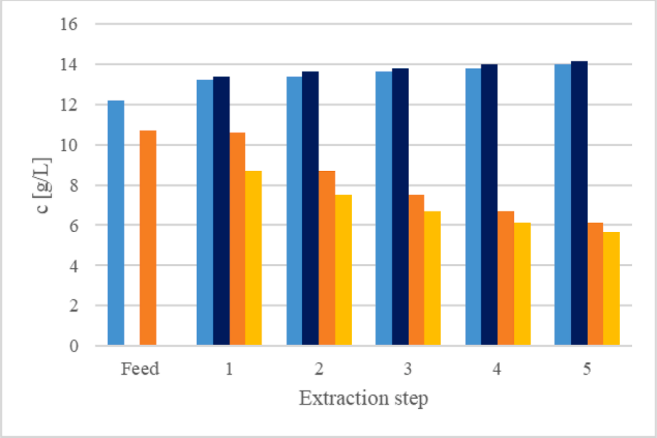


Fig. 4. Acid concentrations in the feed and raffinate of the five extraction steps. Light blue = lactic acid in the respective input stream; dark blue = lactic acid in the respective raffinate stream; orange = acetic acid in the respective input stream; yellow = acetic acid in the respective raffinate stream; phase ratio = 1, T = 25 °C, solvent phase: TOPO/1-octanol.

4. Conclusion

Xylose as raw material for D-lactic acid production is accompanied by a high load of acetic acid when natural LA producers are used. Reactive liquid-liquid extraction using the phosphine-based extractant TOPO diluted in 1-octanol proved to be efficient for acetic acid removal at a pH of 4.88. After this the pH value was adjusted to 2.5 and lactic acid was extracted using TOPO diluted in *d*-limonene. A concept of a first extraction step at pH 4.8, which is the native pH value of the fermentation broth, to remove acetic acid, followed by a second step at pH 2.5 for lactic acid isolation proved to be promising. In the back extraction the disintegration agents *n*-heptane and *p*-cymene result in a back extraction efficiency of up to 70%.

CRediT authorship contribution statement

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

Marlene Kienberger: supervision, Writing - original draft, Conceptualization, Supervision, Methodology. **Paul Demmelmayer:** Validation and Visualization of liquid-liquid extraction experiments, Writing - review & editing, Methodology. **Christoph Weinzettel:** data curation of liquid-liquid extraction, Investigation. **Viktoria Leitner:** Data curation of fermentation experiments, Writing - review & editing. **Michael Egermeier:** Data curation of fermentation experiments

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Viktoria Leitner reports financial support was provided by Wood K plus.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ceja.2023.100552.

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