



Review

Reactive extraction technologies for organic acids in industrial fermentation processes – A review

Sinah Tönjes^{a,b}, Evelien Uitterhaegen^b, Karel De Winter^{b,*}, Wim Soetaert^{a,b}^a Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium^b Bio Base Europe Pilot Plant (BBEPP), Rodenhuiszekaai 1, 9042 Ghent, Belgium

ARTICLE INFO

Editor: M. Freire

Keywords:

Reactive extraction

Fermentation

Organic acids

In situ product recovery

Scale-up

ABSTRACT

Reactive liquid–liquid extraction is an attractive, innovative technique to intensify the fermentative production and downstream processing of biobased organic acids through a selective partitioning to an organic phase. In an *in situ* product recovery (ISPR) approach, fermentation performance can be enhanced by alleviating product inhibition, yielding a relatively concentrated product stream. While reactive extraction has been widely studied for different organic acids in dilute aqueous solutions, its application to complex product streams from fermentation brings important added challenges. This review addresses these challenges and offers perspectives for future research and scale-up studies towards industrialisation. The complex interplay of solvent systems, extraction behaviour of different organic acids, and fermentation-dependent parameters is critically reviewed. Additionally, challenges faced upon implementation in industrial set-ups are discussed with a specific focus on ISPR technologies, discussing back extraction and resource recycling, as well as techno-economic considerations. To that end, potential process strategies and future research directions are proposed to overcome remaining limitations to bring this technology to an industrial level.

1. Introduction

The chemical industry has large potential to contribute to more sustainable and environmentally friendly processes. Yet, to date, 85 % of the feedstocks for organic chemicals are still derived from fossil resources [1–3]. Here, the production of chemicals by microorganisms in industrial biotechnology offers an alternative to the use of petrochemicals with a lower carbon footprint. The latter is achieved by using renewable carbon, hence avoiding the depletion of fossil resources, and through more environmentally friendly processes, typically with mild conditions [4,5].

In that respect, biobased organic acids can serve as platform chemicals to produce a variety of industrial building blocks and end-products [6]. Applications range from polymers, solvents, textiles, food and feed additives to detergents, cosmetics, and pharmaceuticals [7,8]. With the expected decrease of the use of petrochemicals, the demand for biobased organic acids is increasing and reached a market value of USD 26.5 billion in 2023 with the prediction to grow with a CAGR of 7.45 % from 2024 to 2030 [2,9]. Commonly known biobased organic acids produced

at commercial scale include citric acid, succinic acid, itaconic acid, and lactic acid, where the demand of the latter is for instance increasing due to the use of polylactic acid as biopolymer to replace traditional plastic [6,10–12]. Muconic acid is an example for an organic acid that is not yet commercially produced via fermentation but is gaining attention as a platform molecule to produce terephthalic acid, ϵ -caprolactam, and adipic acid [13]. The main hurdle to produce biobased organic acids to date is reaching cost-competitiveness to low-priced petrochemicals where especially the purification from dilute aqueous fermentation broths is challenging. The latter usually accounts for a large proportion of the process costs, reaching 30–40 % of the selling price for citric acid, and up to 50 % of the production costs for lactic acid. Additionally, during lactic acid production, up to one ton of gypsum (CaSO₄) per ton of product is generated, which is often landfilled and can generate additional disposal costs [14–19].

Reactive liquid–liquid extraction can intensify the purification of organic acids through the partitioning of the acids to an organic phase. In comparison to conventional liquid–liquid extraction, reactive extraction is based on the formation of complexes between organic acids and extractants that are soluble in the organic phase [20,21]. The

* Corresponding author.

E-mail addresses: sinah.toenjes@ugent.be (S. Tönjes), evelien.uitterhaegen@bheu.org (E. Uitterhaegen), karel.de.winter@bheu.org (K. De Winter), wim.soetaert@ugent.be (W. Soetaert).<https://doi.org/10.1016/j.seppur.2024.129881>

Received 23 August 2024; Received in revised form 15 September 2024; Accepted 24 September 2024

Available online 27 September 2024

1383-5866/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Nomenclature

Glossary

Compositions of commercial chemicals used in this study are listed below

Aliquat 336 methyltriocylammonium chloride

CYPHOS IL-101 trihexyltetradecylphosphonium chloride

CYPHOS IL-104 trihexyltetradecylphosphonium bis(2,4,4 trimethylpentyl)phosphinate

CYTOP 503 mixture of trialkyl phosphine oxides

CYANEX 923 mixture of trialkyl phosphine oxides

extraction can be performed subsequent to the fermentation in a traditional downstream processing (DSP, Fig. 1.1), or during the fermentation in an extractive fermentation process, also referred to as *in situ* product recovery (ISPR, Fig. 1.2). ISPR can be performed internally through the addition of an organic phase to the bioreactor (Fig. 1.2A), or externally by circulating the fermentation broth over an external extraction unit (Fig. 1.2B) [22–25].

DSP processes based on reactive extraction have been developed for industrial use as described in various patents for instance for lactic acid and propionic acid [25–29]. Here, an important advantage is the possibility to apply different extraction conditions as compared to the fermentation process such as a change in temperature, pH, and stirring intensity, or to remove contaminants that are present in the broth. On the other hand, applying ISPR for a continuous extraction of organic acids during fermentation can reduce product inhibition, which is especially pronounced for organic acids, and hence increase fermentation performance. Additionally, a partly purified product stream in the organic phase is obtained, which can reduce downstream purification efforts and costs [30,31]. In 2020, reactive extraction was reported as a technology under development with a technology readiness level (TRL) of 3–4, limiting the knowledge to laboratory and bench scale [32]. To date, ISPR studies emerge focussing on scalability and techno-economic considerations, however, industrial implementation is lacking.

This review addresses current challenges that are faced for industrial implementation of reactive extraction of organic acids. Firstly, the theoretical framework of reactive extraction is given, whereafter the impact of fermentation parameters such as pH and oxygen requirements, as well as fermentation broth composition, and solvent toxicity are discussed in-depth. Then, advanced processing set-ups are critically reviewed, especially focussing on ISPR technologies as well as their scalability and techno-economic considerations, including back

extraction and resource recycling. Finally, potential process strategies and future research directions are proposed to overcome remaining limitations and challenges to enable industrialization.

2. Theoretical framework of reactive extraction of organic acids

In reactive liquid–liquid extraction processes, a solute is transferred from an aqueous phase into an organic phase, where, in comparison to conventional solvent or liquid–liquid extraction, a reaction between the solute and an extractant is established [33,34]. This typically leads to the formation of complexes, in which the solute interacts with the extractant through chemical interactions, creating a species with high affinity for the organic phase (Fig. 2). To assess the performance of reactive extraction, key parameters include the distribution or partition coefficient (K_D), the extraction yield, and the selectivity. K_D is defined as the ratio of the concentration of the target product in the organic phase and in the aqueous phase (equation (1)), whereas the extraction yield is defined as the ratio of the total mass of the target compound in the organic phase and the initial mass (equation (2)).

$$K_D = \frac{[HA]_{org}}{[HA]_{aq}} \quad (1)$$

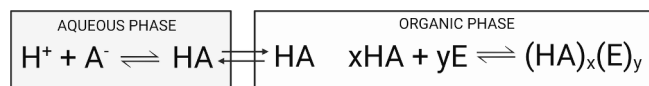


Fig. 2. Schematic overview of reactive extraction of organic acids. Created with BioRender.com.

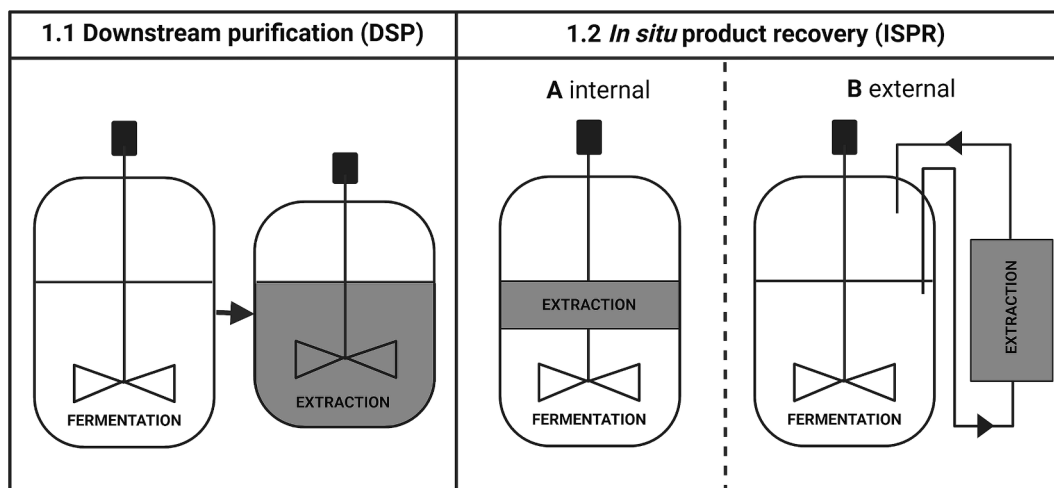


Fig. 1. Configurations of reactive extraction in fermentation processes. In downstream purification (DSP), the extraction takes place subsequent to the fermentation (1.1), whereas the extraction in *in situ* product recovery processes (ISPR) takes place during fermentation either internally (1.2A) or externally (1.2B). Created with BioRender.com.

$$\text{Extraction yield (\%)} = \frac{k_{\text{HA-org}}}{k_{\text{HA-aq}}} \cdot 100\% \quad (2)$$

If multiple extractable compounds are present, selectivity for one compound *a* can be calculated by the ratio of its K_D with the one of compound *b* (equation (3)).

$$\text{Selectivity} = \frac{K_{D,a}}{K_{D,b}} \quad (3)$$

For an efficient and selective reactive extraction process, multiple parameters should be considered, most importantly the organic phase composition, where properties such as hydrophilicity and viscosity are crucial. Moreover, this includes the characteristics and concentration of the target product, the pH, the volumetric ratio between organic and aqueous phase, as well as the temperature, as reviewed by multiple authors for organic acid extraction [20,21,34–38].

Selecting an effective organic phase is essential for the process performance, where extraction mechanisms vary depending on the extractant itself, as well as on so-called diluents or modifiers that are commonly mixed with the extractants. Reactive extractants can be classified into aminic and phosphorous-based extractants [20,36], where the most commonly used aminic extractant is trioctylamine (TOA) [39–49], and the most commonly used phosphorous-based reactive extractants are trioctylphosphine oxide (TOPO) and tributyl phosphate (TBP) [50–56]. Besides, ionic liquids (IL) have gained attention as extractants, where more specifically the aminic and phosphorous-based ones have been reported for organic acid extraction, including the quaternary ammonium salt Aliquat 336 as well as ILs based on tetraalkylphosphonium cations with varying anions. The latter are for instance commercially available as CYPHOS IL-104 or CYPHOS IL-101 [35,57,58]. The complex formation between organic acids and the extractants can occur through hydrogen bonding, ion pair formation, or a combination of both, depending on the type of extractant as well as the diluent [36,59]. In the case of TOA, many studies postulated the complex formation to be based on hydrogen bonding between the undissociated organic acid and the amine [45,60,61]. More recently, the complexes were analysed based on Fourier Transform Infrared (FTIR) spectroscopy suggesting that organic acids are deprotonated by the amine, leading to a negative charge of the acid and a positive charge of the amine, allowing ion pair formation (Fig. 3A). Aliquat 336 on the other hand has a positively charged cation, enabling ion pair formation directly with dissociated organic acids (Fig. 3B) [40,43].

ILs based on tetraalkylphosphonium cations have been reported to result in particularly high extraction yields for organic acids through the formation of clusters where multiple acids can be extracted per IL molecule [35,62–65]. The usage of diluents can limit the overall extractants costs and alter physical properties of the organic phase, for instance through a reduction of viscosity [20,36,38,49]. Diluents can

moreover improve the extraction depending on their nature, where active compounds such as hexanol are suggested to stabilize amine-acid complexes through hydrogen bonds. Inactive compounds such as hexene and dodecane on the other hand, do not participate in the complex formation. For more detailed information on the extraction mechanisms of the varying extractants in mixture with different diluents, the reader is referred to earlier published reviews [20,36].

3. Reactive extraction in fermentation processes

When applying reactive extraction to purify organic acids from fermentation, complexity increases, and additional challenges arise. The following sections are focused on the main fermentation parameters and composition of the fermentation broth, which are known to influence the performance of reactive extraction in both DSP and ISPR set-ups. For the latter, the toxicity of organic phases is of major importance due to the simultaneous fermentation and extraction, and is therefore discussed in-depth.

3.1. Influence of fermentation parameters

Multiple parameters are controlled during fermentation processes ensuring optimal performance, where the most important ones are pH, oxygen level, and temperature. Whereas the pH throughout fermentations is controlled at the optimum of the production organism, reactive extraction is also influenced by pH. Depending on the composition of the organic phase, extraction mechanisms vary, where the dissociation state of the target organic acid in aqueous solution is crucial. The latter is directly determined by the pH and the acid specific dissociation constant (pK_a). For extractants such as TOA and TBP, the necessity of a $pH < pK_a$ of the organic acids is reported to ensure that the majority of the acids are in their undissociated form [43,60,66,67]. On the other hand, Aliquat 336 for instance can directly form complexes with the dissociated form of organic acids at higher pH due to the positive charge of its cation [60,68]. Therefore, the organic phase for extraction needs to be chosen based on the pH of the fermentation. Although for DSP processes the pH could be adjusted before the extraction step, additional chemicals would be required impacting process economics.

For organic acid production, traditional fermentation processes have been performed at neutral pH, at the optimum of many producing organisms but also to reduce the pronounced product inhibition of organic acids at low pH. Indeed, undissociated acids are able to permeate the cell membrane of microorganisms and interfere with intracellular processes, thereby reducing the fermentation performance [69,70]. Strain engineering efforts have focussed on increasing acid tolerance and robustness of microorganisms to allow fermentation processes at reduced pH with the main goal to limit the use of neutralizers and hence reduce chemicals requirements and salt waste production. Moreover, organic acids can be produced in the undissociated form, potentially reducing

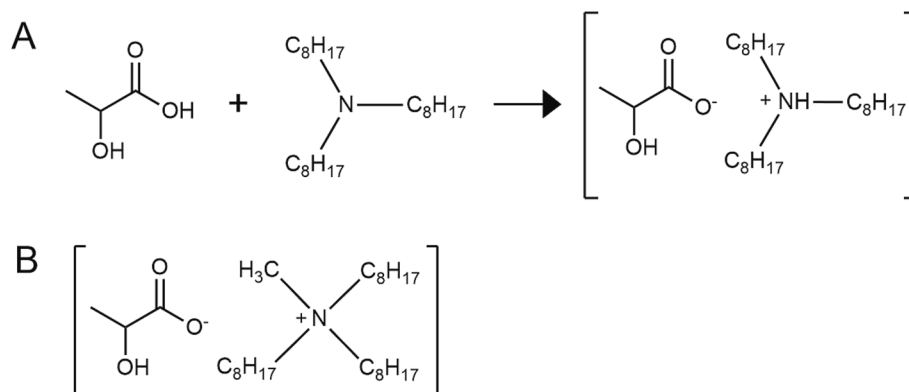


Fig. 3. Suggested complexation of lactic acid with TOA (A) or Aliquat 336 (B).

purification efforts. Great progress has for instance been made in the development of *Saccharomyces cerevisiae* strains, enabling the production of 233 g/L of malic acid and 119 g/L of L-lactic acid without base addition [71,72]. For other acids, product titers at low pH typically remain low. For muconic acid, the maximum titer using *Pichia occidentalis* at a pH of 3.5–4.0 was limited to 7 g/L, as compared to 39 g/L at a neutral pH [73]. It has to be noted that in ISPR processes, organic phases that are efficient at low pH are preferred. The target organic acid is continuously extracted from the fermentation broth resulting in an increase in pH. While the latter reduces product inhibition, the extraction yield is also negatively affected. Several studies report that during continuous ISPR processes, a balance is reached between extraction and production of organic acids, resulting in a constant, slightly acidic pH [74–76]. Hence ISPR can be used as a pH control without the need of neutralizing agents.

Another crucial parameter during fermentation is the dissolved oxygen, where an efficient transfer rate of oxygen from the sparged gas to the liquid phase is important for the process performance. Indeed, the oxygen mass transfer coefficient (k_La) has been described to be the rate-limiting step in aerobic processes, where limitations can negatively influence biomass and product formation as well as reduce yields. The k_La is dependent on varying parameters including the fermentation medium composition and process conditions such as temperature, aeration, and stirring rate. During scale-up, additional challenges are encountered for example through zones with varying oxygen concentrations in large reactors that potentially decrease performance [77,78]. While the dissolved oxygen is not affected by DSP set-ups, the organic phase in ISPR processes can influence the oxygen transfer through direct contact with the aqueous fermentation broth in internal ISPR set-ups, or through partial solubility of solvents in external ISPR set-ups. Whereas the effect of solvents has not been researched in the context of ISPR processes, researchers have evaluated the use of, for instance, alkanes and silicone oils to improve the k_La during fermentation because oxygen shows a higher solubility in these liquids. An improved oxygen transfer was indeed found when adding perfluorodecalin to a fermentation with *Yarrowia lipolytica* [79]. Oils such as olive oil, silicon oil, and soybean oil on the other hand, which are used as diluents in ISPR processes, were reported to decrease the k_La [79,80]. However, a maximum of 0.3 v% of oil was added to the medium, and the effect of higher amounts of hydrophobic compounds is not studied in detail to date. Therefore, further research is required to study the effect of an organic phase on oxygen levels and transfer rates in fermentations, especially when dealing with high cell densities or high oxygen-requiring fermentations and upon scale-up. Next to this, the addition of a second phase will change the volume and viscosity and potentially lead to emulsion formation and foaming. This will have a direct influence on the k_La and the mixing and sparging capacity of the bioreactor, and hence on the fermentation performance. As a consequence, the zoning effect during scale-up might be increased, where trials at pilot scale will be crucial to find an operating window in which the impact is limited, and to assess the feasibility of the approach on an industrial scale.

Next to the pH and oxygen transfer, the temperature during fermentation is important and is controlled at the optimum of the fermentation organisms. Although the temperature optimum is highly dependent on the specific organism, commonly used microbes such as *S. cerevisiae* and *Pichia pastoris* are fermented at 30 °C [71], and *Escherichia coli* at 37 °C [81]. Usually, organisms show little tolerance to changes in temperature, which limits the flexibility for internal ISPR processes [82]. Rewatkar et al. evaluated the extraction of gallic acid (2.55 g/L) with an organic phase containing either TBP, TOA, or Aliquat 336 in hexanol and temperatures ranging from 15 °C to 45 °C. Independent of the extractant, lower temperatures resulted in increased extraction yields most likely due to the decrease in solubility of the target organic acid in the aqueous solution. For TOA, the K_D decreased from 27 at 15 °C to 11 at 45 °C [83]. Similar results have been found by other authors, suggesting that the extraction should be executed at lower

temperatures [84,85]. While this is difficult in internal ISPR set-ups as the choice of temperature is limited by the organisms' tolerance, in external ISPR and DSP set-ups, another temperature could be applied during extraction. However, applying temperature changes at industrial scale significantly increases the energy requirements and hence the cost of the process due to cooling and heating cycles. Whether the cost increase is acceptable in relation to the increase in extraction yield needs to be addressed during techno-economic assessments (TEA) for specific processes.

3.2. Influence of fermentation broth compositions

The composition of fermentation broths is complex, where a multitude of different compounds is present to create an optimal environment for microorganisms. This includes nitrogen and carbon sources as well as trace elements and vitamins. The use of second-generation feedstocks, e. g., derived from lignocellulosic biomass, additionally adds compounds such as hydroxymethylfurfural or 4-hydroxybenzoic acid that are formed during pre-treatment of the biomass [86]. Moreover, acids and bases are used for pH control, adding salts to the reactor. Fermentation broths have been described to compromise reactive extraction for organic acid separation. As an example, the performance of an organic phase consisting of TOA in decanol to extract 3-hydroxypropionic acid (3-HP) was significantly reduced when the acid was present in complex fermentation broth as compared to a solution in water. Indeed, at a similar pH of 4.0 and a 3-HP concentration of 1 g/L, extraction yield and K_D were respectively reduced by 26 % and 71 % in the fermentation broth [87]. Multiple researchers have focused on evaluating the interference of the different compounds in fermentation broths on reactive extraction of organic acids as summarized in the table below (Table 1) and discussed in detail in the following sections.

3.2.1. Other organic acids

The presence of more than one organic acid in the fermentation broth can lead to competition between the acids, resulting in co-extraction and a reduced extraction yield of the target product. Moreover, the product purity after back extraction is most likely reduced. The competition was for instance studied by Prochaska et al. using reactive extraction to separate succinic acid from a fermentation broth containing 23.3 g/L of succinic acid as well as formic acid (9.9 g/L), acetic acid (8.4 g/L), and lactic acid (6.4 g/L). During reactive extraction with the extractant Cyanex 923 at pH 2.0, an extraction yield of 100 % for succinic acid was found, however, the extraction yield was also 100 % for formic acid, 49 % for lactic acid, and 92 % for acetic acid [91]. Similar results have been found by multiple other authors for varying organic acids and organic phase compositions (Table 1).

To increase selectivity, potential solutions could be the fine-tuning of solvent mixtures to balance extraction yields and selectivity or choosing a pH according to the varying pKa values of the acids. Different researchers have identified the polarity of the organic phase, the viscosity, and the extractant concentration as important factors for selectivity. For instance, in a study to extract fumaric acid from *Rhizopus oryzae*

Table 1
Compounds of fermentation broths and their interference with reactive extraction of organic acids.

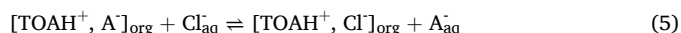
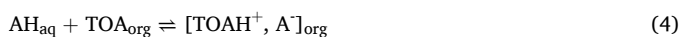
Fermentation broth compounds	Reason for interference	References
Other acids	Competition/co-extraction	[41,48,49,52,56,66,88–98]
Salts	Competition/co-extraction	[49,66,92,99–109]
Proteins, surfactants, cells	Mass transfer reduction	[110–112]
Compounds from second-generation feedstocks	Co-extraction	[113]

fermentation broth that additionally contained malic acid and succinic acid, selectivity was increased by using a stoichiometric amount of the tertiary amine Amberlite LA-2 for fumaric acid extraction mixed with the passive diluent dichloromethane. Indeed, selectivity factors of more than 9 were reported, which decreased to less than 1 when the extractant was present in excess [88]. As an example for fine-tuning the pH to increase selectivity, Omwene et al. evaluated the extraction of the target product succinic acid and the side product lactic acid using TOA at a pH range of 2.0–5.0. While at a pH of 5.0, the selectivity for succinic acid over lactic acid was 0.6, showing a preference for the side product, this was increased to 2.0 at pH 2.0 [90]. This demonstrated that pH has an important influence and can introduce or improve selectivity. Nevertheless, the selectivity is limited, and should therefore go alongside identification and optimization of a suitable extraction mixture to yield an overall performance level which allows efficient and selective extraction of the target organic acid.

One of the highest reported selectivities for organic acid mixtures was reported by Kloetzer et al., where the authors were able to design a membrane-based pertraction process for a selective recovery of fumaric acid from a mixture with malic acid and succinic acid. The target compound is transported from the feed phase mimicking fermentation broth to a back extraction phase through a liquid membrane, in this case Amberlite LA-2 dissolved in *n*-heptane. By tailoring the pH gradient between the two aqueous phases as well as the concentration of Amberlite LA-2, fumaric acid was extracted with a selectivity factor of 19 from a mixture with the other two organic acids [41]. This strategy could be applied in both ISPR and DSP set-ups, where an optimization of the exact membrane composition and the pH gradient between feed and back extraction phase will be needed depending on the target product. However, industrial usage of such systems needs further research to assess scalability and costs (see section 4). Although increased selectivity for a single acid can be reached with the described approaches, complete elimination of co-extraction is challenging. If fermentation streams with multiple organic acids are present, an additional separation step might be needed. Alternatively, the possibility of introducing selectivity in the back extraction step should be evaluated. In parallel, engineered strains with reduced side production of other organic acids as well as optimized fermentation conditions which favour selective production of the target organic acid are being developed to improve extraction and additionally increase fermentation yields for the target acid.

3.2.2. Salts

The presence of inorganic salts may also negatively affect the extraction of organic acids. More specifically, the anions of the salts compete with the organic acids for the extractant [49,103,107]. In the case of the extraction of an undissociated organic acid (AH) with TOA (equation (4)), the presence of chloride anions (Cl^-) causes the release of a dissociated weaker acid into the aqueous phase and the extraction of the Cl^- (originating from the stronger acid HCl) to the organic phase (Eq. (5)) [99]:



Chemarin et al. studied the competition with inorganic salts in detail in a reactive extraction process for 3-HP using TOA in octanol as organic phase. There, either KCl or KH_2PO_4 were added to the aqueous phase whereupon a reduction of the corresponding anions Cl^- and H_2PO_4^- as well as a release of the dissociated, charged form of 3-HP was reported [99]. For different types of salts, the influence on the extraction of the organic acid varies, where Kurzrock et al. showed that NH_4Cl , NaCl, and MgCl have a larger impact on succinic acid extraction using TOA in octanol compared to MgSO_4 , CaCl_2 , and KH_2PO_4 at an ionic strength of 1.0 mol/L [49]. While the cause for these differences was not further investigated, other researchers also found variations between different

salts, most likely caused by differences in size, acidity of the salt anion, and the affinity towards the extractant [99]. In difference to aminic extractants, the co-extraction of inorganic salts is lower when phosphorous-based extractants are used. The reason is most likely linked to the different mechanism to extract organic acids. As the extraction with phosphorous-based extractants is based on hydrogen bonds, an ion exchange with salts as presented for aminic extractants (equation (4), (5)), is not possible. Demmelmayer et al. extracted LA with various organic phases and found a co-extraction of H_3PO_4 of up to 51 % when using organic mixtures containing TOA, which was limited to 0.13 % using mixtures containing TOPO [109]. Hence, choosing an organic phase with phosphorous-based extractants can reduce the interference of salt co-extraction. Moreover, medium optimization can improve the extraction yields, for instance by evaluating whether chloride salts can be exchanged. Also, the inorganic salt concentration should be kept as low as possible, where Keshav et al. reported an increase of K_D for the extraction of propionic acid by 55 % when the ionic strength of NaCl was reduced from 1 mol/L to 0.1 mol/L [105]. If the influence however remains significant, and changes in the medium are not feasible, a removal of salts prior to the reactive extraction can be considered. However, this can only be applied if reactive extraction is performed downstream and is not feasible for ISPR processes with continuous extraction of acids during the fermentation.

3.2.3. Others fermentation broth compounds

Carbon sources, in difference to inorganic salts, do not compete with organic acids for the extractant, limiting their effect on reactive extraction. This has been shown by several authors for carbohydrates including glucose, lactose, and dextrose, as well as for polyols like glycerol. The extraction yield remained constant and no competition was reported [89,104–106,108]. However, when second-generation feedstocks are used, for instance lignocellulosic biomass, additional compounds can be found in the fermentation broth. In that respect, low molecular weight compounds and phenolics from lignocellulosic biomass such as furfural were shown to be co-extracted with acetic acid with the extractants TOA and Aliquat 336 in octanol [113]. When designing a process using second-generation feedstocks, the effect of such impurities and their co-extraction should be considered and evaluated for the specific process.

Other compounds present in fermentation broth such as proteins, phospholipids, and cells can influence reactive extraction through adsorption to fluid interfaces [110], where a reduction in mass transfer during liquid–liquid extraction in a water–octanol system of up to 70 % was reported earlier [112]. Microbial cells can stabilize oil-in-water emulsions by serving as Pickering-type colloidal particles, which can be problematic in internal ISPR processes as stable emulsions would hinder phase separation subsequent to the fermentation [114,115]. For external ISPR processes, the interference can be reduced by applying a cell recycling unit for instance using microfiltration to avoid cell contact with the organic phase, or by making use of membrane-based extraction. The latter has been tested by Chemarin et al. using TOA in decanol to extract 3-HP with a membrane contactor, where the addition of the protein albumin, which has previously been described to decrease the extraction yield, did not result in a negative effect on the extraction [99]. For DSP processes, cell removal and filtration are common practice after fermentation for instance using microfiltration, ultrafiltration, or nanofiltration steps, where interference with cells, remaining proteins, and lipids is hence unlikely.

3.3. Solvent toxicity

One of the most significant aspects to consider in the development of an ISPR process is the solvent toxicity. A majority of the extractants and solvents used for reactive extraction are toxic towards microbial production hosts, which is important to consider when aiming for an ISPR process. Many researchers have therefore focused on screening different

solvent classes for toxicity towards multiple organisms including yeasts [116,117], bacteria [75,117–123], and fungi [117,124], and have developed different strategies to counter this. The impact of toxicity in internal ISPR set-ups is generally higher compared to external set-ups due to the direct contact between microorganisms and the organic phase, also referred to as phase level toxicity [75,121].

In this context, De Brabander et al. screened the toxicity of over 60 different solvents and extractants towards *S. cerevisiae* for use in internal ISPR set-ups. There, the most promising extractants for adipic acid, namely TBP, CYTOP 503, CYPHOS IL-101, and Aliquat 336, were found toxic as demonstrated by a glucose consumption that was reduced by 44 %–100 %. However, mixing the extractants with biocompatible diluents could reduce toxicity while retaining the extraction yield, for example when mixing 12.5 v% of CYTOP 503 with canola oil [116]. This mixture has also shown potential in another study for the extraction of muconic acid during an internal ISPR process [125]. Other biocompatible mixtures for application in internal ISPR set-ups were found based on mixtures of TOA, mainly for *Lactobacillus* species [74,76]. In external ISPR set-ups, the impact of solvent toxicity is limited by the partial solubility of solvents in the aqueous phase, often referred to as molecular level toxicity. In a direct comparison between internal and external ISPR set-ups, Gössi et al. demonstrated the toxicity of common extractants and solvents towards the lactic acid producer *Lactobacillus plantarum*. By directly adding 17 v% of TOA to a batch fermentation, only 13 % of lactic acid was produced as compared to the control. In contrast, if the fermentation medium was contacted with TOA prior to the fermentation to mimic the partial solubility of the extractant in the medium as seen in external ISPR set-ups, lactic acid production amounted to 80 % as compared to the control. Full biocompatibility has been achieved by mixing 20 wt% of TOA with decanol [75]. An alternative strategy to

minimize direct contact between microorganisms and the organic phase is cell immobilisation, which is for instance described to reduce toxicity of inhibitors from lignocellulosic biomass [126]. To reduce solvent toxicity in ISPR processes, the use of alginate [127,128] or κ -carrageenan [129,130] has been described, where encapsulate cells can for instance be used in a packed-bed column over which broth and organic phase are cycled, representing a scalable option of the technique [127].

In conclusion, biocompatible mixtures typically consist of 10–40 v% of extractant in a diluent, in most cases vegetable oil, long-chain alcohols, alkanes, or fatty acid methyl esters. Solvent and extractant toxicity toward the microbial production host is a critical factor that must be carefully evaluated when designing an ISPR process, as it can significantly impact the efficiency and viability of the system. While diluents are traditionally used to reduce costs, improve extraction, as well as alter the extractants physical properties, they should also be selected based on their biocompatibility and the ability to decrease the overall toxicity of the organic phase. Hence, in most cases, a compromise between good extractability of the target organic acid and the biocompatibility has to be made.

4. From lab to industry

For industrialization of bioprocesses, the choice of equipment is crucial as well as understanding the main process parameters and scale-up challenges. For reactive extraction, various equipment is available and different set-ups can be used. An overview of possible ISPR configurations is given in Fig. 4 for both internal as well as external ISPR set-ups, and key studies on the performance of ISPR in fermentations targeting organic acid production at bioreactor scale are presented in Table 2.

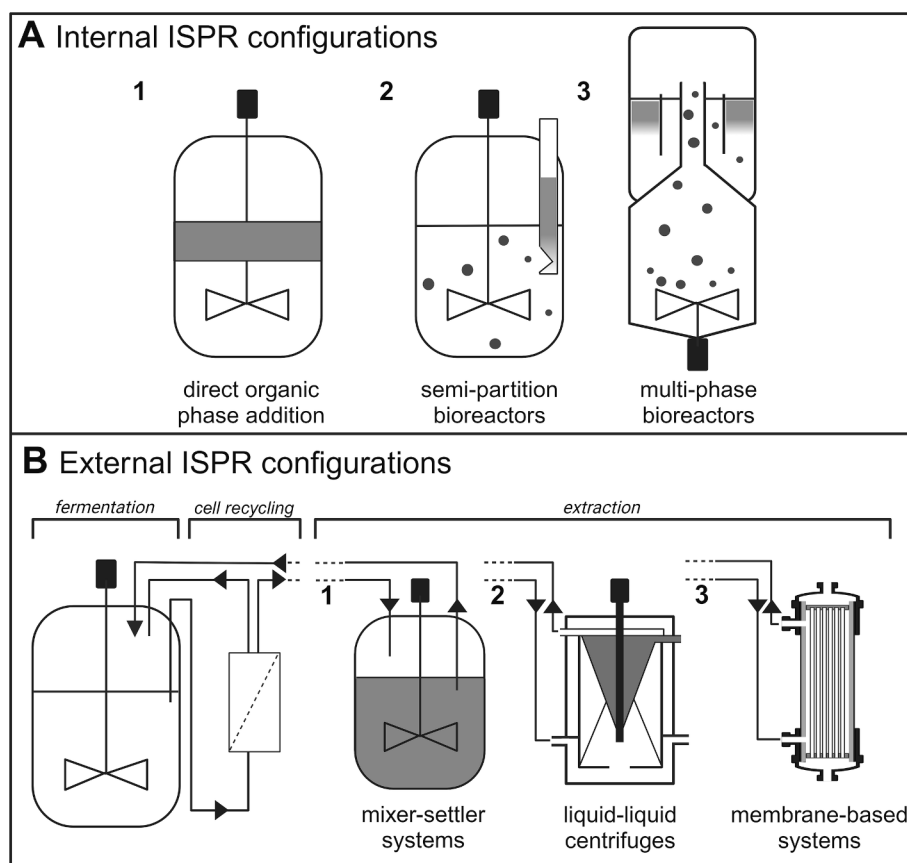


Fig. 4. Schematic overview of configurations of *in situ* product recovery (ISPR) based on reactive extraction. In internal ISPR configurations (A), the organic phase (dark grey) is in direct contact with the fermentation broth. In external ISPR processes (B), the broth is circulated over an external extraction unit. Created with BioRender.com.

Table 2

Key studies on the performance of ISPR in fermentations targeting organic acid production at bioreactor scale.

ISPR configuration	Reactive extraction equipment	Target product	Production organisms	Scale	Improvement to non-ISPR	Organic phase	Challenges/Remarks	Ref.
Internal	Direct organic phase addition	Lactic acid	<i>Lactobacillus delbrueckii</i>	1 L	36 % (titer ^a) 2 % (productivity ^b)	20 v% TOA in decanol, dodecane	– Ratio organic-to-aqueous phase 1:1 – Cell viability decreased over time	[76]
	Direct organic phase addition	Lactic acid	<i>Lactobacillus fructivorans</i>	0.35 L	26 % (productivity) 22 % (yield ^c)	22 v% TOA in decanol	– Ratio organic-to-aqueous phase 1:1 – Cells immobilized in alginate and CaCO ₃ capsules, operated in packed-bed reactor	[127]
	Direct organic phase addition	Muconic acid	<i>Saccharomyces cerevisiae</i>	10 L	44 % (biomass ^d) 18 % (titer)	12.5 v% CYTOP 503 in canola oil	– Ratio organic-to-aqueous phase 1:5	[125]
	Semi-partition reactor	Lactic acid	<i>Lactobacillus casei</i>	7.5 L	68 % (titer) 25 % (productivity) 75 % (yield)	35 v% TBP, 25 v% TOA in oleyl alcohol	– Ratio organic-to-aqueous phase 1:7 – Continuous extraction and back extraction – Cell growth decreased due to solvent toxicity	[74]
External	Mixer-settler system	Lactic acid	<i>Lactobacillus delbrueckii</i>	2 L	No information	40 v% TOA in oleyl alcohol	– Cell recycling before extraction – Sequential extraction with up to 4 stages	[143]
	Mixer-settler system	Lactic acid	<i>Lactobacillus delbrueckii</i>	2 L	71 % (productivity)	15 v% TOA in oleyl alcohol	– Cells immobilized in κ-carrageenan capsules and added to the fermenter	[130]
	Mixer-settler system	Lactic acid	<i>Lactobacillus delbrueckii</i>	1 L	25 % (biomass) 40 % (productivity)	40 v% TOA in oleyl alcohol	– Cell recycling before extraction – Sequential extraction with 2 stages	[144]
	MBSE	Lactic acid	<i>Lactobacillus plantarum</i>	2 L	430 % (yield)	20 v%TOA in decanol	– Membrane material: Teflon – Process stable for 7 days	[75]
	MBSE	Butyric acid	<i>Clostridium tyrobutyricum</i>	0.15 L	1389 % (titer) 44 % (productivity) 19 % (yield)	10 v% TOA in oleyl alcohol	– Membrane material: Polypropylene – Cells immobilized in a fibrous bed reactor – Process stable for 2 weeks	[155]
	MBSE	Itaconic acid	<i>Aspergillus terreus</i>	5 L	44 % (biomass)	2-methyl tetrahydrofuran	– Membrane material: Teflon – Cell recycling before extraction	[156]
	MBSE	Butyric acid	mixed cultures	5 L	No information	3 v% TOPO in light mineral oil	– Membrane fouling – Membrane material: Polypropylene – Stable process for 55 days	[158]

^a titer: product (organic and aqueous phase) formed per liter of aqueous phase (g_{org+aq}/L_{aq}).^b productivity: product formation rate per liter of aqueous phase ($g_{org+aq}/L_{aq}/h$).^c yield: product formed per substrate consumed (g_{org+aq}/g).^d growth: biomass formation in the aqueous phase based on cell dry weight (g/L_{aq}) or optical density at 600 nm (–).

4.1. Internal ISPR configurations

The direct addition of an organic phase to the fermenter is the least complex option to achieve internal ISPR based on reactive extraction (Fig. 4A.1). On lab scale, this approach can be used to evaluate the effectiveness of ISPR in a straight-forward way by adding the organic phase to a shake flask fermentation. This has been demonstrated to enhance the fermentation performance for the production of various organic acid including itaconic acid [124], malic acid [131], caproic acid [132], and lactic acid [128] using TOA as extractant in all cases mixed with varying diluents. Crucial parameters for this approach include the amount of extractant that is added to the fermentation, which is largely dependent on the solvent toxicity, as well as the stirring speed, which influences the extraction yield of the target organic acid.

Direct organic phase addition has also been investigated in lab scale bioreactors. Singhvi et al. added an organic phase containing 20 v% TOA, 10 v% decanol, and 70 v% dodecane in a ratio of 1:1 (v/v) with the aqueous phase to a 1 L bioreactor producing lactic acid with *Lactobacillus delbrueckii*. The extractant mixture was added after 48 h of fermentation. At the end of the process (96 h), the lactic acid titer was improved by 36 % with no impact on productivity as compared to the

fermentation without ISPR. Moreover, no neutralizing agent was added, and the initial pH value of 7.0 decreased to approximately 4.0, where it remained constant due to the acid extraction into the organic phase [76]. One limitation of this operation is the bioreactor vessel volume, as the organic phase should take up as little volume as possible. While current lab scale studies typically keep the volumetric ratio of the organic phase to the aqueous phase at 1:1 (v/v), this is not feasible on large scale. Therefore, researchers evaluated the addition of an organic phase containing 12.5 v% of CYTOP 503 in canola oil with a reduced volume ratio of 1:5 (v/v) to a fermentation process in a 10 L bioreactor to produce muconic acid using *S. cerevisiae*. This led to an enhancement of muconic acid production and growth by 44 % and 18 % as compared to the control fermentation without ISPR, respectively [114]. Nevertheless, in a batch set-up, the inhibitory effect of the target product is recurrent upon continued production with a constant equilibrium. To overcome this issue, continuous extraction systems should be targeted, requiring specialized equipment. Here, Teke et al. developed a semi-partition bioreactor at lab scale (Fig. 4A.2), inserting a hollow tube that functions as a settler in the fermenter with an opening for fluid exchange. The organic phase loaded with the target product settles in the tube, where it can be pumped to another vessel to perform back

extraction and recycled back to the reactor [133]. With this set-up, the titer of lactic acid in a 7.5 L bioreactor was increased by 68 % compared to the control fermentation without ISPR [74]. While this technique has proven effective on lab scale, recently, industrial-scale set-ups have been described and patented (Fig. 4A.3). In that respect, Delft Advanced Biofuels B.V. (DAB.bio) developed a reactor used at demonstration scale in which both production and separation take place simultaneously. The reactor consists of a separation compartment and a fermentation compartment with a continuous inflow of fresh organic phase and a continuous outflow of loaded organic phase. Production processes have for example been demonstrated for vanillic acid and butanol [134,135]. In another patent, Bednarz et al. developed a multiphase loop reactor, which is a modified airlift reactor that enables a counter-current, continuous liquid–liquid extraction [136] which has been described for instance to separate rhamnolipids during fermentation processes [137].

Another challenge of internal ISPR configurations is the formation of stable emulsions, for example caused by the usage of vegetable oils as non-toxic diluents. Besides, the ionic liquids CYPHOS IL-101 and CYPHOS IL-104 have been described to be prone to form emulsion [64]. Emulsion formation is especially favored due to the vigorous mixing and aeration in aerobic processes with high oxygen demand, as well as by microbial cells that can stabilize oil-in-water emulsions [114,115]. Stable emulsions in ISPR processes should be avoided as they are challenging to break and complicate further downstream purification [64]. If the formation cannot be averted, there are multiple options to destabilize emulsions, requiring extra processing steps for instance through the addition of chemicals or thermal treatments [138]. Besides the costs of these additional steps, added chemicals need to be removed from the product downstream, and thermal treatments are often energy-intensive and unsuitable for heat-sensitive products. Alternatively, the use of supercritical CO₂ can enable fast phase separation, however, extra costs for instance through the necessity for equipment that can withstand high pressure, needs to be taken into account [139].

4.2. External ISPR configurations

In external ISPR configurations, product recovery occurs simultaneously to the fermentation in external equipment. Typically, the cells are separated and recycled back to the fermenter which is achieved by technologies for continuous fermentations for instance through hollow fibre membranes [140,141], or cell immobilization [129]. The product-containing outflow is then subjected to reactive extraction, whereafter the nutrients can be recycled back to the fermenter. It has to be noted that although the focus of this section lays on ISPR configurations, the described equipment can also be applied to DSP subsequent to the fermentation. The equipment can generally be grouped into mixer-settler set-ups, column set-ups, centrifugal set-ups, and membrane-based solutions with numerous variations and manufacturers [142].

In mixer-settler systems (Fig. 4B.1), the fermentation output stream is mixed with the organic phase and subsequently left for settling, for example in separate tanks or chambers [142]. This technique is among the initial ones described for external ISPR and was already studied in the 1990s at bioreactor scale for lactic acid [130,143,144]. Yabannavar et al. for instance fermented *Lactobacillus delbrueckii* at pH 4 in a 2 L bioreactor and cycled the medium over separate mixer and settler units to continuously extract lactic acid using TOA in oleyl alcohol. This increased the productivity by 71 % compared to the control fermentation [130]. As an intensification approach at industrial scale, column contactors can be used, which are well-known in chemical processes. Various designs such as rotating disc contactors, packed columns, or sieve tray columns, are used for liquid–liquid extraction at industrial scale [142], but have not been applied for ISPR processes to produce organic acids to date.

As an alternative to mixer-settler approaches, liquid–liquid centrifugal contactors are gaining attention for reactive extraction (Fig. 4B.2).

With these devices, two phases are continuously brought into contact and separated based on density differences and applying high centrifugal forces. For information and details on various types, suppliers, and working principles of centrifugal contactors, the reader is referred to Hamamah et al. 2022 [145]. Main advantages include continuous operation, short residence times, good phase separation, high mass transfer efficiencies, and good scalability [40,145]. Researchers have successfully demonstrated the use of centrifugal contactors for reactive extraction of organic acids subsequent to the fermentation on lab scale. As an example, Notheisen et al. described the extraction of pyridinecarboxylic acid using liquid–liquid centrifuges at lab scale with a maximum flow rate of 2 L per minute. The authors were able to extract up to 95 % of the acid from a fermentation broth from *Pseudomonas putida* using an organic phase of TOA in octanol [40]. In another study, acetic acid extraction from pyrolysis oil was demonstrated with the same extractant in 2-ethylhexanol, where maximum recoveries of 71 % were reported in a cascade of two sequential centrifuges. The authors reported that the process was prone for emulsion formation in dependency of the applied flow rates and rotational frequencies, where an increase of both parameters led to reduced emulsion formation [95]. This indicates some drawbacks of centrifugal contactors. While increasing rotational frequency and decreasing flow rates might decrease emulsion formation, this also shortens the contact time between the organic phase and the aqueous phase, negatively affecting extraction yield. The latter can for instance be improved by extraction with multiple devices in sequence which will, however, increase the amount of process units. These challenges are most likely the reason for the scarcity of studies implementing the technique in ISPR settings for organic acids. Nevertheless, external ISPR processes with centrifugal contactors has been successfully implemented for other products such as L-phenylalanine at lab scale, suggesting that this is also possible for organic acids [146]. Moreover, suitable industrial-scale equipment for centrifugation is available, facilitating scale-up of the technique.

Alternatively, diverse types of membrane set-ups are described for reactive extraction (Fig. 4B.3), where the membrane acts as a barrier between the two immiscible phases. One main advantage is that mixing, dispersion, and emulsion formation are avoided yet large contact areas are present enabling efficient extraction [31]. Generally, a distinction can be made between membrane-based solvent extraction (MBSE) and the use of liquid membranes (LM). In MBSE, the membrane separates the feed and extractant phase, but has no active function (Fig. 5A). While MBSE processes require extra steps for back extraction of the organic acid and solvent regenerations, in LM processes, the extraction and back extraction of the target products take place in the same equipment. One realization of the latter are supported liquid membranes (SLM), in which the extractant is immobilized in a microporous support. The target product is simultaneously extracted from the feed solution and back-extracted with the same equipment (Fig. 5B) [147,148].

Different types of membrane contactors are commercially available, where hollow fibre membrane contactors are commonly used that are made from polyurethane, polypropylene, and polyethylene. Those contactors are available in different sizes with the possibility to run multiple units in parallel or in series allowing testing from lab to industrial scale [31,149,150]. Alternatively, hollow fibre membranes that are submerged in the fermentation broth have also been described [151]. Multiple authors have evaluated membrane contactors for organic acid extraction, where the studies focusing on ISPR implementation at bioreactor scale are summarized in Table 2. Often, the fermentation broth is directly circulated over the contactor without a separate cell recycling, which, however, resulted in shear stress to microorganisms [152,153]. Therefore, a cell recycling step is beneficial. Pérez et al. for instance retained the biomass from a lactic acid process using *L. casei* through a hollow fibre filter and subsequently subjected the supernatant to reactive extraction. The authors implemented a novel type of extraction based on a liquid membrane in Taylor flow which was described to enhance mass transfer and to have high stability. Overall,

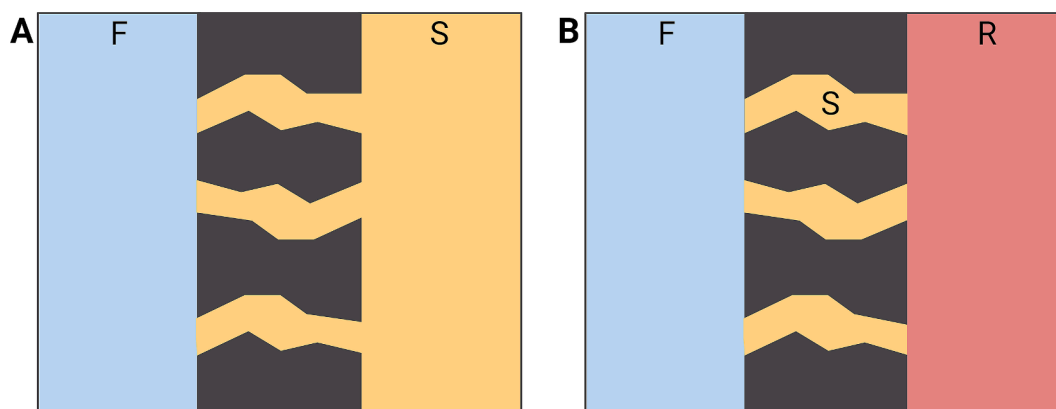


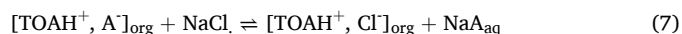
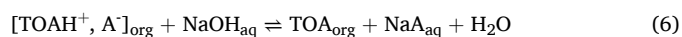
Fig. 5. Schematic illustration of membrane-based solvent extraction (A, MBSE), and extraction based on liquid membranes (LM) with the specific example of supported liquid membranes (B, SLM). The feed (F) is represented in blue, the organic phase (S) in yellow, and the solution for recovery (R) or back extraction in red. Created with [BioRender.com](https://www.biorender.com).

lactic acid production was increased by 42 % using this approach [154]. Other possibilities to avoid direct contact of the cells with the membrane contactor include cell immobilization techniques. A successful example is described by Wu et al., who immobilized the cells in a fibrous bed bioreactor and circulated the broth over a hollow fibre membrane contactor to extract butyric acid with an organic phase consisting of TOA and oleyl alcohol. With this set-up, the titer was increased 15-fold, and the productivity by 44 % in the ISPR process as compared to the control fermentation [155]. Other drawbacks using membrane contactors include poor resistance of the membrane module towards the organic phase as well as membrane fouling [152,156]. To reduce these issues, alternative types of membranes are being investigated, where polytetrafluoroethylene (Teflon, PTFE) or polyvinylidene fluoride (PVDF) for instance have proven more robust [157]. Pilot-scale studies are crucial to evaluate whether the process can run continuously over an extended period of time, and whether a cell removal step before the membrane-based reactive extraction is needed.

In conclusion, multiple set-ups for external ISPR processes have been described and proven effective on lab scale. Where mixer-settler units present more conventional equipment for reactive extraction, continuous processes using liquid–liquid centrifuges or membrane contactors have gained attention over the past years. To reach industrialization, further research is needed to align fermentation and extraction processes, where process optimization regarding flow rates, phase ratios, and material selection of the various equipment will be crucial. Moreover, the long term stability and robustness of the ISPR process over multiple cycles in an industrial setting needs to be assessed.

4.3. Back extraction and resource recycling

After extraction of the target organic acid to the organic phase, there are multiple options to recover the acid for further processing and solvent recycling; a prerequisite for economic viability. An overview of different back extraction strategies is given in Table 3. The most frequently described technique is the addition of an acid, base, or salt solution. In case of extraction of the undissociated acid ($\text{pH} < \text{pKa}$), the addition of base results in the neutralization of the acid groups and consequently a release of the dissociated acid in its salt form to the aqueous phase. Typically, NaOH is applied as exemplified below using TOA as extractant (equation 6) [89,159–163]. Alternatively, salts such as NaCl or mineral acids such as HCl can be added to replace the target organic acid via an anion exchange mechanism (equation 7) [130,164,165]. The latter is also used to back-extract organic acids from quaternary ammonium salts like Aliquat 336, where the extraction occurs via an ion exchange mechanism with the dissociated acid ($\text{pH} > \text{pKa}$) [40].



Multiple studies describe full recovery of the organic acid using this back extraction technique [89,90,162,166,167]. However, the main disadvantage is the high requirement of chemicals and the recovery of organic acids in their salt form in dilute aqueous streams. Hence, dependent on the application, subsequent processing is required to yield a concentrated stream of the organic acid, for example through acidification with the production of a salt by-product, separation of the latter, and most likely evaporation of water to yield high concentrations of the organic acid. Moreover, in case of the anion exchange mechanism using salts or mineral acids, the extractant is not recovered in its native form (equation 7) and needs to be regenerated, for instance using NaOH to enable recycling of the deprotonated extractant. The latter results in the need for larger processing plants, the production of waste streams, and high energy demands.

To circumvent re-acidification and the production of salt waste, the usage of volatile bases (e.g., trimethylamine, TMA) as alternative to NaOH has received attention owing to the possibility of evaporating the amine after back extraction and yielding the organic acid in its undissociated form. Moreover, the volatile base can directly be recovered by evaporation and recycled without the need for additional steps, allowing full resource recycling [168,169]. This approach can yield highly concentrated product streams, where, depending on the properties of the target organic acid, the latter can be recovered in its solid form (e.g., succinic acid) or liquid form (e.g., lactic acid). Kurzrock et al. used this approach to produce succinic acid, where reactive extraction was performed using diisooctylamine and dihexylamine in a mixture with hexanol and octanol. Back extraction was performed with TMA resulting in a recovery of 95 %, followed by an evaporation-based crystallization yielding succinic acid crystals with a purity of 99.5 %. Recycling of the organic phase was successfully demonstrated for four cycles with no decrease in extraction yield [161]. As mentioned above, the TMA could also be recovered by evaporation without the need for additional regeneration steps, whereas it is important to be mentioned that increased temperature and reduced pressure are required for a full recovery of both the organic acid and the TMA. An additional disadvantage of TMA is its strong, fishy odor, which could persist in the purified end product or other process streams and may require additional deodorization or other treatment steps. In the abovementioned example, temperatures of up to 160 °C and 4 mbar of pressure were applied to yield succinic acid crystals [161]. In other examples, lactic acid was recovered at temperatures of 100–120 °C with a pressure of 267 mbar [160], and acetic acid at 150 °C at atmospheric pressure [170].

Table 3

Comparison of techniques to back-extract organic acids from organic phases after reactive extraction.

Back extraction technique	Advantages	Disadvantages	References
Addition of acid, base, or salt (e.g., NaOH, NaCl, HCl)	- Reliable, complete recovery of organic acids for a wide range of organic acids and extraction phases	- Recovery of organic acids in salt form, additional processing required to yield undissociated acids - Recovery of organic acid in dilute aqueous streams - Additional processing required to enable recycling of organic phase and back extraction phase	[40,89,90,159,161–167]
Addition of volatile bases (e.g., TMA)	- Direct recovery of undissociated organic acids - Recovery of highly concentrated organic acid streams - Reliable, complete recovery of organic acids for a wide range of organic acids and extraction phases - Easy recovery of organic phase and back extraction phase	- High energy requirements for distillation - For volatile organic acids, extra separation required	[160,161,168–170]
Direct distillation	- Direct recovery of undissociated organic acids - Recovery of highly concentrated organic acid streams - No need for additional chemicals	- High energy requirements for distillation - Challenging for non-volatile organic acids - Contaminants/inhibitors might accumulate in the organic phase	[170–173]
Temperature swing	- Direct recovery of undissociated organic acids - No need for additional chemicals	- Recovery of organic acid in dilute aqueous streams - Varying performance for different organic acids and different extraction phases	[159,164,176,177]
Solvent swing	- Direct recovery of undissociated organic acids	- High energy requirements if distillation is needed - Recovery of organic acid in dilute aqueous streams - Additional processing required to enable recycling of organic phase and back extraction phase	[164,176,177]

Some authors suggested that direct distillation of volatile acids from the organic phase could also be considered to yield highly concentrated acid streams without the need for additional chemicals [170–172]. Nevertheless, the energy requirements for distillation are high and need to be critically assessed, especially if the initial acid concentration and the extraction yields are low. Moreover, if co-extraction of compounds from the fermentation broth occurs, these might accumulate in the organic phase, impeding the solvent capacity for the target product over time, or introducing inhibitory effects in the fermentation if applied in an ISPR set-up [173]. Saboe et al. provides an example of direct recovery of organic acids from the organic phase through distillation. The authors evaluated the distillation of short and medium chain organic acids, namely a mixture of acetic acid, butyric acid, and caproic acid, from various organic phases containing either aminic or phosphorous-based extractants. A complete acid recovery was achieved using a spinning band distillation column with 25 theoretical stages, and the recyclability of the organic phase was demonstrated over three cycles. In terms of energy requirements, the most crucial parameter is the acid concentration in the organic phase as well as the water content of the organic phase. In their model, a 50 g/L acetic acid stream from an ISPR process with membrane-based reactive extraction could be extracted and distilled with a total energy input of 2.6 MJ/kg acetic acid and a carbon footprint that was significantly reduced compared to the non-ISPR control process [171]. This marks a significant reduction in energy requirement compared to the direct distillation from aqueous broths for acetic acid, as the boiling points of water and acetic acid are close together, hampering efficient separation. In a direct comparison, energy requirements for the direct distillation from a 305 g/L aqueous solution of acetic acid were reported to be as high as 31.9 MJ/kg of acetic acid [174]. Although promising, other studies have reported that a full recovery of acetic acid was not possible if CYPHOS IL-104 was used as extractant due to the strong interactions of acetic acid and the ionic liquid [170]. Another important factor to consider when applying distillation is the tendency of some organic acids to copolymerize, where lactic acid for instance is described to form the lactide dimer [175].

Alternatively, a temperature increase can also be used to release the organic acid during back extraction into an aqueous phase. This process is referred to as temperature swing and makes use of the temperature-dependency of the extraction process. Here, Chemarin et al. reached a maximum recovery of 3-HP from an organic phase containing TOA in *n*-decanol of 78 % when heated to 140 °C [164]. However, lower recoveries of maximum 35 % were found for back extraction of propionic

acid from TOA with 1-decanol at 93 °C [159], most likely due to the higher hydrophobicity of propionic acid compared to 3-HP and hence a higher affinity to the organic phase. Whereas temperature swing is attractive because no extra chemicals are required and the acids can be recovered in their undissociated form, the technique is also less robust and flexible compared to the addition of acid, base, or salt solutions. The efficiency has to be assessed for the specific organic phase and target product, also considering the dilution effect when the acid is released into an aqueous phase, mostly into water.

Another back extraction technique is referred to as solvent swing, where a change in the composition of the organic phase leads to the release of the organic acid into an aqueous phase. This can be achieved either by adding another solvent, often referred to as an anti-solvent, to the organic phase, or by removing the diluent [176]. As an example, the addition of hexane to an organic phase containing 3-HP, TOA, and *n*-decanol led to a maximum recovery of 3-HP of 70 %. Hexane could afterwards be recovered through evaporation owing to its high volatility. However, similarly to the temperature swing technique, the target acids will be recovered in dilute streams, resulting most likely in the necessity of further processing steps to concentrate the product [164].

To assess the feasibility of both temperature swing and solvent swing, Sprakel et al. compared both back extraction techniques to recover succinic acid from fermentation broth in terms of energy inputs. The organic phase for extraction consisted of either TOPO or TOA in methyl isobutyl ketone (MIBK). Then, back extraction was achieved either by adding pentane or gaseous ethane as anti-solvent to the organic phase, by increasing the temperature to 60 °C, or by evaporating MIBK from the organic phase. Compared to a benchmark process in which water is directly evaporated from the fermentation broth to concentrate the acid, reactive extraction with either of all four back extraction techniques was advantageous in terms of energy requirements. The use of ethane as anti-solvent was most promising, reducing the energy input by 79 %, amounting to 13 MJ/kg of succinic acid as compared to 62 MJ/kg of succinic acid for the benchmark process [177].

Energy requirements and techno-economic considerations based on pilot-scale studies will be crucial to evaluate the feasibility of the different processes. Comparing all abovementioned back extraction strategies, there are clear benefits and limitations (Table 3). While the addition of a base solution is a robust and flexible technique capable of reaching full organic acid recovery, salt waste streams are produced, and further processing is most likely needed. Moreover, the acid will be recovered in a dilute aqueous stream, which is also the main

disadvantage of the alternative strategies of temperature swing and diluent swing. There are two process scenarios which can achieve full resource recycling, namely the direct evaporation of acids from an organic phase and the usage of volatile bases such as TMA with a subsequent distillation of the base (Fig. 6). In both cases, waste streams are avoided through resource recycling and the acid is recovered in a high concentration or in a crystalline form depending on the properties.

4.4. Techno-economic assessment

To assess the feasibility and economic viability of the different described approaches for reactive extraction of organic acids, TEAs are required. However, fully integrated pilot-scale studies for ISPR processes including fermentation, reactive extraction, and back extraction with resource recycling are scarce to date. As a result, the predictions for TEA analysis are more uncertain than for mature technologies and might change during further developments [32,178]. However, several studies have been published assessing reactive extraction as DSP method subsequent to the fermentation, where Prado-Rubio et al. compared different purification technologies to produce lactic acid from a

fermentation process. Using one fermentation process, three subsequent purification methods were assessed, where reactive distillation was used as benchmark technology and compared to electrodialysis and reactive extraction. The latter was modelled in a counter-current column using TOA and 1-dodecanol as organic phase, whereafter the loaded organic phase was sent to a distillation column to recover lactic acid. Comparing this purification method to the more mature technology of reactive distillation including steps of pre-concentration, esterification, hydrolysis, and alcohol recovery, the total annual costs (TAC) and the operational expenditures (OPEX) were reduced by 44 %. Moreover, energy requirements were reduced by 37 % [32]. In this study, the authors assumed a 79 % recovery of lactic acid with a purity of 86 % in a single stage with one extraction and one distillation column. As mentioned in section 4.3, a direct distillation of the organic acids or the solvent can be challenging and highly energy intensive, where pilot-scale studies in future research should substantiate the model.

In another approach, Magalhães et al. compared different purification technologies to recover itaconic acid from fermentation broth. This included adsorption, reactive extraction, and electrodialysis as compared to the conventional industrial process of crystallization.

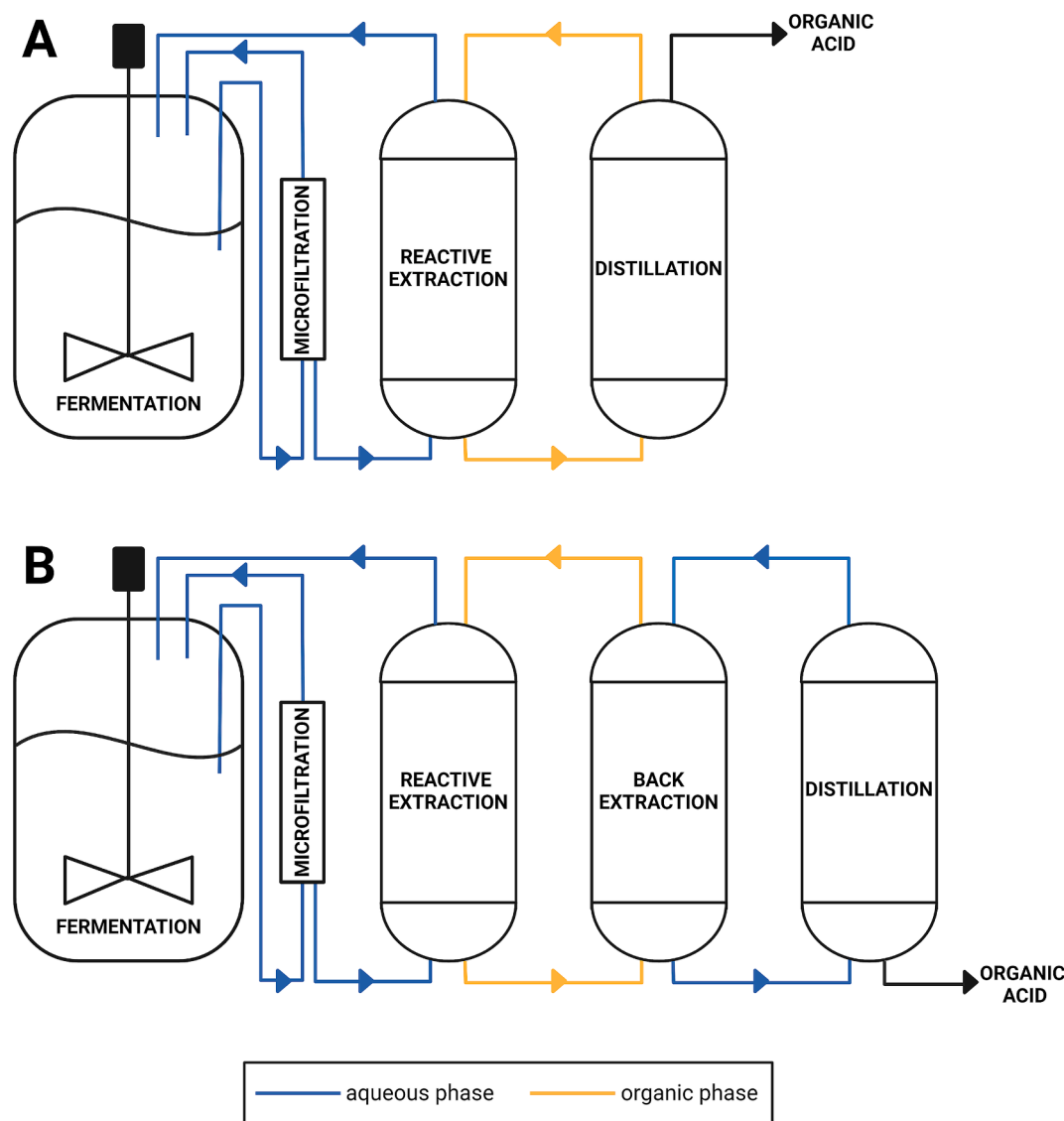


Fig. 6. Process scheme of integrated *in situ* reactive extraction including product purification and resource recycling. For volatile organic acids, direct distillation from the organic phase after extraction is applied (A), whereas non-volatile acids are back-extracted with a volatile base, which is subsequently distilled (B). Created with BioRender.com.

Reactive extraction was executed in rotating disc contactors using TOA in ethyl oleate as organic phase. Subsequent back extraction was performed using TMA whereafter itaconic acid was recovered via evaporation-based crystallization. The results indicate that although total investment costs of the reactive extraction process were approx. 75 % reduced compared to the crystallization process, the annual costs for raw materials, utility consumption, and energy costs are 55 % higher. The total processing costs of itaconic acid are ultimately comparable for both purification approaches, where a sensitivity analysis of varying parameters showed that the required amount and cost of the organic phase are crucial for the competitiveness of reactive extraction [179]. The authors assumed that the organic phase can be used for 1 year with minimum losses, which cannot be confirmed with experimental studies to date. In that respect, Oudshoorn et al. also described the highest operational expenses (OPEX) for ISPR processes during fermentation to be the solvent purchase and its regeneration, often performed by an acid or base wash [180], highlighting the importance of efficient back extraction with organic phase recycling.

In conclusion, initial TEA studies show that reactive extraction can present a promising technology to recover organic acids. However, comprehensive TEA studies on ISPR-based production pathways, including fermentation and the impact of improved fermentation performance, extraction, and back extraction, are needed to allow a true comparison to current industrial practices.

5. Conclusions and prospects

Reactive extraction is a promising technique to intensify the production of biobased organic acids. Its implementation in fermentation processes in an ISPR approach can improve the fermentation performance and reduce downstream purification costs. Internal ISPR processes, where the organic phase is directly added to the bioreactor, have recently been enabled on pilot scale, where the main challenges are overcoming solvent toxicity and emulsion formation. External ISPR processes require more complex processing set-ups, which have been limited to lab scale to date. The primary advantage of external ISPR is that the conditions of extraction and fermentation can be different, reducing solvent toxicity issues. However, fermentation parameters as well as the fermentation broth composition have a significant impact on the extraction during ISPR. Especially salts and other, non-targeted organic acids interfere through competition for the extractant and consequently co-extraction. Moreover, cells, proteins, and surfactants lead to reduced mass transfer and emulsion formation. To fully leverage the benefits of the technology and attain cost-competitive production processes, back extraction of the target organic acid from the organic phase is crucial. To this end, full resource recycling can be achieved via direct acid distillation or by using volatile bases such as TMA which are subsequently distilled and recovered. Initial TEA studies have shown promising results, however, future research is required to collect representative data on pilot and demonstration scale. These studies will allow a comprehensive assessment of the scalability of innovative ISPR set-ups based on reactive extraction and a precise modelling of costs, energy requirements, and environmental impact, which is scarce to date. Additionally, innovative liquid–liquid extraction techniques for further process intensification are being developed, such as liquid membrane extraction and integrated extraction-distillation, that can enhance separation efficiency by combining extraction with additional purification steps.

Overall, reactive extraction in an ISPR set-up is a high-potential, yet relatively complex and advanced processing technology, which will ask for a higher level of expertise for operation and specialised equipment with concomitant investment costs. By consequence, for certain organic acids like lactic acid and acetic acid, for which mature biobased processes are available to date, such extra investments might hinder short-term implementation. For novel biobased platform chemicals like muconic acid and acrylic acid, which currently lack biobased industrial

processes, intensified, integrated ISPR processes could enable viable biobased production routes and hence accelerate their introduction to the industrial landscape. In addition, these routes with the potential of full resource recycling could imply significant waste reduction and even prevention. As such, they could fit into the zero-waste biorefineries of the future. As pressure on industry to reduce carbon footprints and climate impact further increases, more sustainable technologies will become critical to enable a shift towards green manufacturing practices, where reactive extraction and ISPR could play a pivotal role.

Author contributions

Sinah Tönjes: Formal analysis, Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Evelien Uitterhaegen:** Conceptualization, Investigation, Writing – review & editing. **Karel De Winter:** Conceptualization, Writing – review & editing. **Wim Soetaert:** Conceptualization, Writing – review & editing.

Funding

We gratefully acknowledge the financial support of the Flemish Government and Flanders Innovation & Entrepreneurship (VLAIO) through the Moonshot project HYBRID (HBC.2023.0550).

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.

Data availability

No data was used for the research described in the article.

References

- [1] F. Kähler, M. Carus, O. Porc, C. Vom Berg, Turning off the tap for fossil carbon future prospects for a global chemical and derived material sector based on renewable carbon, *Ind. Biotechnol.* 17 (2021), <https://doi.org/10.1089/ind.2021.29261.fka>.
- [2] P. Gabrielli, L. Rosa, M. Gazzani, R. Meys, A. Bardow, M. Mazzotti, G. Sansavini, Net-zero emissions chemical industry in a world of limited resources, *One Earth* 6 (2023) 682–704, <https://doi.org/10.1016/j.oneear.2023.05.006>.
- [3] L. Cullen, F. Meng, R. Lupton, J.M. Cullen, Reducing uncertainties in greenhouse gas emissions from chemical production, *Nature, Chem. Eng.* 1 (2024) 311–322, <https://doi.org/10.1038/s44286-024-00047-z>.
- [4] M. Carus, L. Dammer, A. Raschka, P. Skoczinski, Renewable carbon: Key to a sustainable and future-oriented chemical and plastic industry: Definition, strategy, measures and potential, *Greenhouse Gases: Science and Technology* 10 (2020) 488–505, <https://doi.org/10.1002/ghg.1992>.
- [5] L.P. Yu, F.Q. Wu, G.Q. Chen, Next-generation industrial biotechnology-transforming the current industrial biotechnology into competitive processes, *Biotechnol J* 14 (2019) 1800437, <https://doi.org/10.1002/biot.201800437>.
- [6] M. Sauer, D. Porro, D. Mattanovich, P. Branduardi, Microbial production of organic acids: expanding the markets, *Trends Biotechnol* 26 (2008) 100–108, <https://doi.org/10.1016/j.tibtech.2007.11.006>.
- [7] L. Sun, M. Gong, X. Lv, Z. Huang, Y. Gu, J. Li, G. Du, L. Liu, Current advance in biological production of short-chain organic acid, *Appl Microbiol Biotechnol* 104 (2020) 9109–9124, <https://doi.org/10.1007/s00253-020-10917-0>.
- [8] Y.-S. Jang, B. Kim, J.H. Shin, Y.J. Choi, S. Choi, C.W. Song, J. Lee, H.G. Park, S. Y. Lee, Bio-Based Production of C2–C6 Platform Chemicals, *Biotechnol Bioeng* 109 (2012) 2437–2459, <https://doi.org/10.1002/bit.24599>.
- [9] Verified Market Research, Bio-Organic Acid Market Size And Forecast., <https://www.verifiedmarketresearch.com/Product/Bio-Organic-Acid-Market/>, (2024) accessed 22/03/2024.
- [10] M. Berovic, M. Legisa, Citric acid production, *Biotechnol Annu Rev* 13 (2007) 303–343, [https://doi.org/10.1016/S1387-2656\(07\)13011-8](https://doi.org/10.1016/S1387-2656(07)13011-8).
- [11] A. Carlson, B. Coggio, K. Lau, C. Mercogliano, J. Millis, Industrial Production of Succinic Acid, in: F. Cavani, S. Albonetti, F. Basile, A. Gandini (Eds.), *Chemicals and Fuels from Bio-Based Building Blocks*, Wiley-VCH Verlag GmbH & Co., KGaA, Weinheim, 2016, pp. 173–190, <https://doi.org/10.1002/9783527698202.ch7>.
- [12] L. Ranakoti, B. Gangil, S.K. Mishra, T. Singh, S. Sharma, R.A. Ilyas, S. El-Khatib, Critical review on polylactic acid: properties, structure, processing,

- biocomposites, and nanocomposites, *Materials* 15 (2022) 4312, <https://doi.org/10.3390/ma15124312>.
- [13] I. Khalil, G. Quintens, T. Junkers, M. Dusselier, Muconic acid isomers as platform chemicals and monomers in the biobased economy, *Green Chem.* 22 (2020) 1517–1541, <https://doi.org/10.1039/c9gc04161c>.
 - [14] A. Kumar, A. Thakur, P.S. Panesar, Lactic acid and its separation and purification techniques: A review, *Rev Environ Sci Biotechnol* 18 (2019) 823–853, <https://doi.org/10.1007/s11157-019-09517-w>.
 - [15] A.G. Dafu, J.F. Görgens, Techno-economic analysis and environmental impact assessment of lignocellulosic lactic acid production, *Chem Eng Sci* 162 (2017) 53–65, <https://doi.org/10.1016/j.ces.2016.12.054>.
 - [16] A. Komesu, R.M. Wolf Maciel, R.M. Filho, Separation and purification technologies for lactic acid – a brief review, *BioResources* 12 (2017) 6885–6901, <https://doi.org/10.15376/biores.12.3.6885-6901>.
 - [17] J.-F. Bobier, T. Cerisy, A.-D. Coulin, C. Blecher, V. Sassoon, B. Alexander, Breaking the Cost Barrier in Biomanufacturing, Boston Consulting Group, 2024.
 - [18] A.J.J. Straathof, The proportion of downstream costs in fermentative production processes, in: *Comprehensive biotechnology*, Elsevier, 2011, pp. 811–814, <https://doi.org/10.1016/B978-0-08-088504-9.00492-X>.
 - [19] A. Manandhar, A. Shah, Techno-economic analysis of bio-based lactic acid production utilizing corn grain as feedstock, *Processes* 8 (2020) 199, <https://doi.org/10.3390/pr8020199>.
 - [20] S. Kumar, S. Pandey, K.L. Wasewar, N. Ak, H. Uslu, Reactive Extraction as an Intensifying Approach for the Recovery of Organic Acids from Aqueous Solution: A Comprehensive Review on Experimental and Theoretical Studies, *J Chem Eng Data* 66 (2021) 1557–1573, <https://doi.org/10.1021/acs.jced.0c00405>.
 - [21] M. Djas, M. Henczka, Reactive extraction of carboxylic acids using organic solvents and supercritical fluids: A review, *Sep Purif Technol* 201 (2018) 106–119, <https://doi.org/10.1016/j.seppur.2018.02.010>.
 - [22] E.M. Buque-Taboada, A.J.J. Straathof, J.J. Heijnen, L.A.M. Van Der Wielen, In situ product recovery (ISPR) by crystallization: Basic principles, design, and potential applications in whole-cell biocatalysis, *Appl Microbiol Biotechnol* 71 (2006) 1–12, <https://doi.org/10.1007/s00253-006-0378-6>.
 - [23] A.G. Santos, T.L. de Albuquerque, B.D. Ribeiro, M.A.Z. Coelho, In situ product recovery techniques aiming to obtain biotechnological products: A glance to current knowledge, *Biotechnol Appl Biochem* 68 (2021) 1044–1057, <https://doi.org/10.1002/bab.2024>.
 - [24] U.A. Salas-Villalobos, R.V. Gómez-Acata, J. Castillo-Reyna, O. Aguilar, In situ product recovery as a strategy for bioprocess integration and depletion of inhibitory products, *J. Chem. Technol. Biotechnol.* 96 (2021) 2735–2743, <https://doi.org/10.1002/jctb.6797>.
 - [25] P. Chaichol, N. Weeranoppanant, Advances in in situ and in-line liquid-liquid extraction for bioprocess intensification, *React Chem Eng* 8 (2023) 2387–2402, <https://doi.org/10.1039/d3re00266g>.
 - [26] A.B. De Haan, J. Van Krieken, T. Dekic Zivkovic, Lactic acid extraction, *US10017451 B2*, 2018.
 - [27] A.B. De Haan, A. Krzyzaniak, B. Schuur, Extraction of carboxylic acids from a dilute aqueous stream, *EP2998285 B1*, 2013.
 - [28] T. Granstrom, J. Ahola, P. Hietala, Method for recovering and purifying propionic acid, *UA20140330039 A1*, 2014.
 - [29] A. Eggert, M. Gausmann, T. Massmann, Transferring a target substance between two liquid phases, *US11471787 B2*, 2020.
 - [30] F.M. Antony, K.L. Wasewar, The Sustainable Approach of Process Intensification in Biorefinery Through Reactive Extraction Coupled with Regeneration for Recovery of Protocatechuic Acid, *Appl Biochem Biotechnol* (2023) 1570–1591, <https://doi.org/10.1007/s12010-023-04659-8>.
 - [31] M. Chadni, M. Moussa, V. Athès, F. Allais, I. Ioannou, Membrane contactors-assisted liquid-liquid extraction of biomolecules from biorefinery liquid streams: A case study on organic acids, *Sep Purif Technol* 317 (2023) 123927, <https://doi.org/10.1016/j.seppur.2023.123927>.
 - [32] O.A. Prado-Rubio, R. Gasca-González, J. Fontalvo, F.I. Gómez-Castro, E.S. Pérez-Cisneros, R. Morales-Rodríguez, Design and evaluation of intensified downstream technologies towards feasible lactic acid bioproduction, *Chem. Eng. Process. - Process Intensif.* 158 (2020) 108174, <https://doi.org/10.1016/j.cep.2020.108174>.
 - [33] J.A. Tamada, A.S. Kertes, C.J. King, Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, *Industrial & engineering chemistry research* 29 (1990) 1319–1326, <https://doi.org/10.1021/ie00103a035>.
 - [34] D. Datta, S. Kumar, H. Uslu, Status of the Reactive Extraction as a Method of Separation, *J Chem* (2015) 853789, <https://doi.org/10.1155/2015/853789>.
 - [35] A.C. Blaga, A. Tucaliuc, L. Klotzter, Applications of Ionic Liquids in Carboxylic Acids Separation, *Membranes* (Basel) 12 (2022) 771, <https://doi.org/10.3390/membranes12080771>.
 - [36] L.M.J. Sprakel, B. Schuur, Solvent developments for liquid-liquid extraction of carboxylic acids in perspective, *Sep Purif Technol* 211 (2019) 935–957, <https://doi.org/10.1016/j.seppur.2018.10.023>.
 - [37] K.L. Wasewar, Reactive Extraction: An Intensifying Approach for Carboxylic Acid Separation, *International Journal of Chemical Engineering and Applications* (2012) 249–255, <https://doi.org/10.7763/ijcea.2012.v3.195>.
 - [38] A.S. Kertes, C.J. King, Extraction chemistry of fermentation product carboxylic acids, *Biotechnol Bioeng* 28 (1986) 269–282, <https://doi.org/10.1002/bit.260280217>.
 - [39] A.C. Blaga, L. Klotzter, D. Cascaval, A.-I. Galaction, A. Tucaliuc, Studies on Reactive Extraction of Itaconic Acid from Fermentation Broths, *Processes* 12 (2024) 725, <https://doi.org/10.3390/pr12040725>.
 - [40] J. Notheisen, R. Takors, Reactive extraction of pyridinedicarboxylic acid (PDCA) with tri-n-octylamine and Aliquat 336 in 1-octanol – A promising approach to access microbially produced PDCA, *Sep Purif Technol* 333 (2024) 125826, <https://doi.org/10.1016/j.seppur.2023.125826>.
 - [41] L. Klotzter, A.C. Blaga, D. Cascaval, A.I. Galaction, Selective pertraction of dicarboxylic acids from simulated *Rhizopus oryzae* fermentation broths, *Sci Rep* 13 (2023) 7170, <https://doi.org/10.1038/s41598-023-34100-3>.
 - [42] B. Kiriş, Y.S. Aşçı, M. Zahoor, S.S. ul Hassan, S. Bungau, Separation of mandelic acid by a reactive extraction method using tertiary amine in different organic diluents, *Molecules* 27 (2022) 5986, <https://doi.org/10.3390/molecules27185986>.
 - [43] L. Nolte, M. Nowaczyk, C. Brandenbusch, Monitoring and investigating reactive extraction of (di-)carboxylic acids using online FTIR – Part I: Characterization of the complex formed between itaconic acid and tri-n-octylamine, *J Mol Liq* 352 (2022) 118721, <https://doi.org/10.1016/j.molliq.2022.118721>.
 - [44] F.M. Antony, K. Wasewar, Effect of temperature on equilibria for physical and reactive extraction of protocatechuic acid, *Heliyon* 6 (2020) e03664, <https://doi.org/10.1016/j.heliyon.2020.e03664>.
 - [45] V. Inyang, D. Lokhat, Reactive Extraction of Malic Acid using Trioctylamine in 1-Decanol: Equilibrium Studies by Response Surface Methodology Using Box Behnken Optimization Technique, *Sci Rep* 10 (2020) 2400, <https://doi.org/10.1038/s41598-020-59273-z>.
 - [46] F. Chemarin, M. Moussa, M. Chadni, B. Pollet, P. Lieben, F. Allais, I.C. Trelea, V. Athès, New insights in reactive extraction mechanisms of organic acids: An experimental approach for 3-hydroxypropionic acid extraction with tri-n-octylamine, *Sep Purif Technol* 179 (2017) 523–532, <https://doi.org/10.1016/j.seppur.2017.02.018>.
 - [47] S.-T. Yang, S.A. White, S.-T. Hsu, Extraction of Carboxylic Acids with Tertiary and Quaternary Amines: Effect of pH, *Ind Eng Chem Res* 30 (1991) 1335–1342, <https://doi.org/10.1021/ie00054a040>.
 - [48] B. Choudhury, A. Basha, T. Swaminathan, Study of Lactic Acid Extraction with Higher Molecular Weight Aliphatic Amines, *J. Chem. Technol. Biotechnol.* 72 (1998) 111–116, [https://doi.org/10.1002/\(SICI\)1097-4660\(199806\)72:2<111::AID-JCTB878>3.0.CO;2-O](https://doi.org/10.1002/(SICI)1097-4660(199806)72:2<111::AID-JCTB878>3.0.CO;2-O).
 - [49] T. Kurzrock, D. Weuster-Botz, New reactive extraction systems for separation of bio-succinic acid, *Bioprocess Biosyst Eng* 34 (2011) 779–787, <https://doi.org/10.1007/s00449-011-0526-y>.
 - [50] V.R. Dhongde, B.S. De, K.L. Wasewar, Experimental Study on Reactive Extraction of Malonic Acid with Validation by Fourier Transform Infrared Spectroscopy, *J Chem Eng Data* 64 (2019) 1072–1084, <https://doi.org/10.1021/acs.jced.8b00972>.
 - [51] S.S. Raghuvanshi, K.L. Wasewar, K.K. Athankar, Appraisal of the capacity of natural oils for partitioning of itaconic acid via reactive extraction, *J. Chem. Technol. Biotechnol.* (2023) 270–278, <https://doi.org/10.1002/jctb.7535>.
 - [52] D. Chakraborty, S.G. Palani, M.M. Ghangrekar, J.W.C. Wong, Reactive extraction of lactic and acetic acids from leached bed reactor leachate and process optimization by response surface methodology, *Environ Technol* (2023) 1–16, <https://doi.org/10.1080/09593330.2023.2186272>.
 - [53] V. Agnihotri, A. Kumar, D. Shende, K. Wasewar, Separation of Itaconic Acid from the Aqueous Phase using Phosphoric Extractant, *Chemical Review and Letters* 6 (2023) 256–261, <https://doi.org/10.22034/crl.2023.414245.1242>.
 - [54] V.R. Dhongde, B.S. De, K.L. Wasewar, P. Gupta, S. Kumar, Experimental perspective for reactive separation of malonic acid using TBP in natural non-toxic solvents, *J. Ind. Eng. Chem.* 91 (2020) 273–284, <https://doi.org/10.1016/j.jiec.2020.08.011>.
 - [55] N. Joshi, A. Keshav, A.K. Poonia, Reactive Extraction of Gallic Acid Using Tributyl Phosphate in Different Classes of Diluents, *J Chem Eng Data* 64 (2019) 2826–2835, <https://doi.org/10.1021/acs.jced.9b00192>.
 - [56] T. Brouwer, M. Blahusiak, K. Babic, B. Schuur, Reactive extraction and recovery of levulinic acid, formic acid and furfural from aqueous solutions containing sulphuric acid, *Sep Purif Technol* 185 (2017) 186–195, <https://doi.org/10.1016/j.seppur.2017.05.036>.
 - [57] J. Marták, T. Liptaj, Š. Schlosser, Extraction of Butyric Acid by Phosphonium Decanoate Ionic Liquid, *J Chem Eng Data* 64 (2019) 2973–2984, <https://doi.org/10.1021/acs.jced.9b00057>.
 - [58] E.N. Dragoi, A.C. Blaga, D. Cascaval, A.I. Galaction, Experimental, modeling and optimisation of adipic acid reactive extraction using ionic liquids, *J Mol Liq* 410 (2024) 125564, <https://doi.org/10.1016/j.molliq.2024.125564>.
 - [59] C. Scheler, M. Popovic, E. Iannotti, R. Mueller, R. Bajpa, Chemical Interactions between Aqueous and Organic Phases in a Reactive Extraction Process, in: C. E. Wyman, B.H. Davison (Eds.), *Seventeenth Symposium on Biotechnology for Fuels and Chemicals*. ABAB Symposium, vol 57/58, Humana Press, Totowa, NJ, 1996, pp. 29–38, https://doi.org/10.1007/978-1-4612-0223-3_3.
 - [60] M.E. Marti, H. Zeidan, Using eco-friendly alternatives for the recovery of pyruvic acid by reactive extraction, *Sep Purif Technol* 312 (2023) 123309, <https://doi.org/10.1016/j.seppur.2023.123309>.
 - [61] D. Yankov, J. Molinier, J. Albet, G. Malmay, G. Kyuchoukov, Lactic acid extraction from aqueous solutions with tri- n -octylamine dissolved in decanol and dodecane, *Biochem Eng J* 21 (2004) 63–71, <https://doi.org/10.1016/j.bej.2004.03.006>.
 - [62] Š. Schlosser, J. Marták, M. Blahusiak, Specific phenomena in carboxylic acids extraction by selected types of hydrophobic ionic liquids, *Chem. Pap.* 72 (2018) 567–584, <https://doi.org/10.1007/s11696-017-0365-7>.
 - [63] J. Marták, Š. Schlosser, Influence of anion and cation structure of ionic liquids on carboxylic acids extraction, *Front Chem* 7 (2019) 117, <https://doi.org/10.3389/fchem.2019.00117>.

- [64] J. Marták, Š. Schlosser, Phosphonium ionic liquids as new, reactive extractants of lactic acid, *Chem. Pap.* 60 (2006) 395–398, <https://doi.org/10.2478/s11696-006-0072-2>.
- [65] J. Marták, Š. Schlosser, Extraction of lactic acid by phosphonium ionic liquids, *Sep Purif Technol* 57 (2007) 483–494, <https://doi.org/10.1016/j.seppur.2006.09.013>.
- [66] Y.S. Jun, E.Z. Lee, Y.S. Huh, Y.K. Hong, W.H. Hong, S.Y. Lee, Kinetic study for the extraction of succinic acid with TOA in fermentation broth; effects of pH, salt and contaminated acid, *Biochem Eng J* 36 (2007) 8–13, <https://doi.org/10.1016/j.bej.2006.06.011>.
- [67] R. Canari, A.M. Eyal, Effect of pH on Dicarboxylic Acids Extraction by Amine-Based Extractants, *Ind Eng Chem Res* (2003) 1293–1300, <https://doi.org/10.1021/ie010579p>.
- [68] G. Kyuchoukov, M. Marinova, J. Molinier, J. Albet, G. Malmay, Extraction of lactic acid by means of a mixed extractant, *Ind Eng Chem Res* 40 (2001) 5635–5639, <https://doi.org/10.1021/ie010137d>.
- [69] A. Peetermans, M.R. Foulquié-Moreno, J.M. Thevelein, Mechanisms underlying lactic acid tolerance and its influence on lactic acid production in *Saccharomyces cerevisiae*, *Microbial, Cell* 8 (2021) 111–130, <https://doi.org/10.15698/mic2021.06.751>.
- [70] B.E. Bauer, D. Rossington, M. Mollapour, Y. Mamnun, K. Kuchler, P.W. Piper, Weak organic acid stress inhibits aromatic amino acid uptake by yeast, causing a strong influence of amino acid auxotrophies on the phenotypes of membrane transporter mutants, *Eur J Biochem* 270 (2003) 3189–3195, <https://doi.org/10.1046/j.1432-1033.2003.03701.x>.
- [71] L. Sun, Q. Zhang, X. Kong, Y. Liu, J. Li, G. Du, X. Lv, R. Ledesma-Amaro, J. Chen, L. Liu, Highly efficient neutralizer-free L-malic acid production using engineered *Saccharomyces cerevisiae*, *Bioresour Technol* 370 (2023) 128580, <https://doi.org/10.1016/j.biortech.2023.128580>.
- [72] B. Jang, Y. Ju, D. Jeong, S. Jung, C. Kim, Y. Chung, S. Kim, L-Lactic Acid Production Using Engineered *Saccharomyces cerevisiae* with Improved Organic Acid Tolerance, *Journal of Fungi* 7 (2021) 928, <https://doi.org/10.3390/jof7110928>.
- [73] M.E. Pyne, J.A. Bagley, L. Narcross, K. Kevvai, K. Exley, M. Davies, Q. Wang, M. Whiteway, V.J.J. Martin, Screening non-conventional yeasts for acid tolerance and engineering *Pichia occidentalis* for production of muconic acid, *Nat Commun* 14 (2023) 5294, <https://doi.org/10.1038/s41467-023-41064-5>.
- [74] G.M. Teke, G.K. Gakingo, R.W.M. Pott, The liquid-liquid extractive fermentation of L-lactic acid in a novel semi-partition bioreactor (SPB), *J Biotechnol* 360 (2022) 55–61, <https://doi.org/10.1016/j.jbiotec.2022.10.017>.
- [75] A. Gössi, F. Burgener, D. Kohler, A. Urso, B.A. Kolvenbach, W. Riedl, B. Schuur, In-situ recovery of carboxylic acids from fermentation broths through membrane supported reactive extraction using membrane modules with improved stability, *Sep Purif Technol* 241 (2020) 116694, <https://doi.org/10.1016/j.seppur.2020.116694>.
- [76] M. Singhvi, T. Zendo, D. Gokhale, K. Sonomoto, Greener L-lactic acid production through in situ extractive fermentation by an acid-tolerant *Lactobacillus* strain, *Appl Microbiol Biotechnol* 102 (2018) 6425–6435, <https://doi.org/10.1007/s00253-018-9084-4>.
- [77] P. De Brabander, E. Uitterhaegen, T. Delmulle, K. De Winter, W. Soetaert, Challenges and progress towards industrial recombinant protein production in yeasts: A review, *Biotechnol Adv* 64 (2023) 108121, <https://doi.org/10.1016/j.biotechadv.2023.108121>.
- [78] F. García-Ochoa, E. Gomez, Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview, *Biotechnol Adv* 27 (2009) 153–176, <https://doi.org/10.1016/j.biotechadv.2008.10.006>.
- [79] P.F.F. Amaral, M.G. Freire, M.H.M. Rocha-Leão, I.M. Marrucho, J.A.P. Coutinho, M.A.Z. Coelho, Optimization of oxygen mass transfer in a multiphase bioreactor with perfluorodecalin as a second liquid phase, *Biotechnol Bioeng* 99 (2008) 588–598, <https://doi.org/10.1002/bit.21640>.
- [80] A. Morão, C.I. Maia, M.M.R. Fonseca, J.M.T. Vasconcelos, S.S. Alves, Effect of antifoam addition on gas-liquid mass transfer in stirred fermenters, *Bioprocess Eng.* 20 (1999) 165–172, <https://doi.org/10.1007/s004490050576>.
- [81] I. Shahzadi, M.A. Al-Ghamdi, M.S. Nadeem, M. Sajjad, A. Ali, J.A. Khan, I. Kazmi, Scale-up fermentation of *Escherichia coli* for the production of recombinant endoglucanase from *Clostridium thermocellum*, *Sci Rep* 11 (2021), <https://doi.org/10.1038/s41598-021-86000-z>.
- [82] M. Jesús Torija, N. Rozé, M. Poblet, J.M. Guzmán, A. Mas, Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*, n.d. www.elsevier.com/locate/ijfoodmicro.
- [83] K. Rewatkar, D.Z. Shende, K.L. Wasewar, Effect of Temperature on Reactive Extraction of Gallic Acid Using Tri-n-butyl Phosphate, Tri-n-octylamine and Aliquat 336, *J Chem Eng Data* 61 (2016) 3217–3224, <https://doi.org/10.1021/acs.jced.6b00310>.
- [84] S. Pandey, D. Pal, S. Kumar, Reactive extraction of gallic acid using tri-n-octylamine in a biocompatible mixture of diluents, *Chem. Data Collect.* 41 (2022) 100908, <https://doi.org/10.1016/j.cdc.2022.100908>.
- [85] S. Mukherjee, B. Munshi, Experimental and theoretical analysis of reactive extraction of caproic acid by using TBP in green diluents, *Chem. Eng. Process. - Process Intensif.* 153 (2020) 107926, <https://doi.org/10.1016/j.cep.2020.107926>.
- [86] E. Palmqvist, B. Arbel, H.-H. Agerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, *Bioresour Technol* 74 (2000) 25–33, [https://doi.org/10.1016/S0960-8524\(99\)00161-3](https://doi.org/10.1016/S0960-8524(99)00161-3).
- [87] G. Burgé, F. Chemarin, M. Moussa, C. Saulou-Bérion, F. Allais, H.E. Spinnler, V. Athès, Reactive extraction of bio-based 3-hydroxypropionic acid assisted by hollow-fiber membrane contactor using TOA and Aliquat 336 in n-decanol, *J. Chem. Technol. Biotechnol.* 91 (2016) 2705–2712, <https://doi.org/10.1002/jctb.4878>.
- [88] L. Kloetzer, A. Tucaliuc, A.I. Galaction, D. Cașcaval, Fractionation of dicarboxylic acids produced by *Rhizopus oryzae* using reactive extraction, *Sci Rep* 12 (2022) 2020, <https://doi.org/10.1038/s41598-022-06069-y>.
- [89] A. Krzyżkowska, M. Regel-Rosocka, The effect of fermentation broth composition on removal of carboxylic acids by reactive extraction with Cyanex 923, *Sep Purif Technol* 236 (2020) 116289, <https://doi.org/10.1016/j.seppur.2019.116289>.
- [90] P.I. Omwene, M. Yagcioglu, Z.B. Ocal Sarihan, A. Karagunduz, B. Keskinler, Recovery of succinic acid from whey fermentation broth by reactive extraction coupled with multistage processes, *J Environ Chem Eng* 8 (2020) 104216, <https://doi.org/10.1016/j.jece.2020.104216>.
- [91] K. Prochaska, J. Antczak, M. Regel-Rosocka, M. Szczygielda, Removal of succinic acid from fermentation broth by multistage process (membrane separation and reactive extraction), *Sep Purif Technol* 192 (2018) 360–368, <https://doi.org/10.1016/j.seppur.2017.10.043>.
- [92] J. Gorden, E. Geiser, N. Wierckx, L.M. Blank, T. Zeiner, C. Brandenbusch, Integrated process development of a reactive extraction concept for itaconic acid and application to a real fermentation broth, *Eng Life Sci* 17 (2017) 809–816, <https://doi.org/10.1002/elsc.201600253>.
- [93] Y. Ki Hong, W. Hi Hong, H. Nam Chang, Selective extraction of succinic acid from binary mixture of succinic acid and acetic acid, *Biotechnol Lett.* 22 (2000) 871–874, <https://doi.org/10.1023/A:1005627816323>.
- [94] P. Von Frieling, K. Schügerl, Recovery of lactic acid from aqueous model solutions and fermentation broths, *Process Biochem.* 34 (1999) 685–696, [https://doi.org/10.1016/S0032-9592\(98\)00143-5](https://doi.org/10.1016/S0032-9592(98)00143-5).
- [95] C.B. Rasrendra, B. Girisuta, H.H. Van de Bovenkamp, J.G.M. Winkelman, E. J. Leijenhof, R.H. Venderbosch, M. Windt, D. Meier, H.J. Heeres, Recovery of acetic acid from an aqueous pyrolysis oil phase by reactive extraction using tri-n-octylamine, *Chem. Eng. J.* 176–177 (2011) 244–252, <https://doi.org/10.1016/j.cej.2011.08.082>.
- [96] M.E. Marti, T. Gurkan, Selective recovery of pyruvic acid from two and three acid aqueous solutions by reactive extraction, *Sep Purif Technol* 156 (2015) 148–157, <https://doi.org/10.1016/j.seppur.2015.09.067>.
- [97] Y. Suk Huh, Y. Ki Hong, W. Hi Hong, H. Nam Chang, Selective extraction of acetic acid from the fermentation broth produced by *Mannheimia succiniciproducens*, *Biotechnol Lett* 26 (2004) 1581–1584, <https://doi.org/10.1023/B:BILE.0000045656.00138.93>.
- [98] E. Bressler, S. Braun, Conversion of citric acid to itaconic acid in a novel liquid membrane bioreactor, *J. Chem. Technol. Biotechnol.* 75 (2000) 66–72, [https://doi.org/10.1002/\(SICI\)1097-4660\(200001\)75:1<66::AID-JCTB176>3.0.CO;2-U](https://doi.org/10.1002/(SICI)1097-4660(200001)75:1<66::AID-JCTB176>3.0.CO;2-U).
- [99] F. Chemarin, V. Athès, M. Bedu, T. Loty, F. Allais, I.C. Trelea, M. Moussa, Towards an in situ product recovery of bio-based 3-hydroxypropionic acid: influence of bioconversion broth components on membrane-assisted reactive extraction, *J. Chem. Technol. Biotechnol.* 94 (2019) 964–972, <https://doi.org/10.1002/jctb.5845>.
- [100] K. Lan, S. Xu, J. Li, C. Hu, Recovery of Lactic Acid from Corn Stover Hemicellulose-Derived Liquor, *ACS Omega* 4 (2019) 10571–10579, <https://doi.org/10.1021/acsomega.9b00794>.
- [101] E. Riveiro, L. Villar, O.G. Sas, B. González, J. Canosa, A. Domínguez, Extraction of Carboxylic Acids from Aqueous Solutions by Using [BMim][NTf2] and Salting-out Agents, *J Chem Eng Data* 64 (2019) 4717–4723, <https://doi.org/10.1021/acs.jced.9b00041>.
- [102] M. Matsumoto, M. Shibasaki, R. Yuasa, K. Kondo, Salting-out Extraction of 3-Hydroxypropionic Acid with Reactive Extraction and Aqueous Two-phase Systems, *Solvent Extr. Res. Dev., Jpn.* 24 (2017) 141–147, <https://doi.org/10.15261/serdj.24.141>.
- [103] 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, <https://doi.org/10.1016/j.seppur.2016.01.037>.
- [104] 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) 45029–45039, <https://doi.org/10.1039/c4ra06612j>.
- [105] A. Keshav, K.L. Wasewar, S. Chand, Extraction of propionic acid from model solutions: Effect of pH, salts, substrate, and temperature, *AIChE J* 55 (2009) 1705–1711, <https://doi.org/10.1002/aic.11780>.
- [106] M.M. Hossain, J.L. Maisuria, Effects of organic phase, fermentation media, and operating conditions on lactic acid extraction, *Biotechnol Prog* 24 (2008) 757–765, <https://doi.org/10.1021/bp702267>.
- [107] A. Schunk, G. Maurer, On the influence of some strong electrolytes on the partitioning of acetic acid to aqueous/organic two-phase systems in the presence of tri-n-octylamine: Part I: Methyl isobutyl ketone as organic solvent, *Fluid Phase Equilib* 239 (2006) 223–239, <https://doi.org/10.1016/j.fluid.2005.11.022>.
- [108] M. San-Martin, C. Pazos, J. Coca, Reactive Extraction of Lactic Acid with Alamine 336 in the Presence of Salts and Lactose, *J. Chem. Technol. Biotechnol.* 54 (1992) 1–6, <https://doi.org/10.1002/jctb.280540102>.
- [109] P. Demmelmayer, M. Čosić, M. Kienberger, Mineral Acid Co-Extraction in Reactive Extraction of Lactic Acid Using a Thymol-Menthol Deep Eutectic Solvent as a Green Modifier, *Molecules* 29 (2024) 1722, <https://doi.org/10.3390/molecules29081722>.
- [110] V.B. Fainerman, E.H. Lucassen-Reynders, R. Miller, Adsorption of surfactants and proteins at fluid interfaces, *Colloids Surf A Physicochem Eng Asp* 143 (1998) 141–165, [https://doi.org/10.1016/S0927-7757\(98\)00585-8](https://doi.org/10.1016/S0927-7757(98)00585-8).
- [111] M. Moussa, G. Burgé, F. Chemarin, R. Bounader, C. Saulou-Bérion, F. Allais, H. E. Spinnler, V. Athès, Reactive extraction of 3-hydroxypropionic acid from model aqueous solutions and real bioconversion media. Comparison with its isomer 2-

- hydroxypropionic (lactic) acid, *J. Chem. Technol. Biotechnol.* 91 (2016) 2276–2285, <https://doi.org/10.1002/jctb.4813>.
- [112] M.R. Pursell, M.A. Mendes-Tatis, D.C. Stuckey, Effect of Fermentation Broth and Biosurfactants on Mass Transfer during Liquid-Liquid Extraction, *Biotechnol. Bioeng.* 85 (2004) 155–165, <https://doi.org/10.1002/bit.10840>.
- [113] D.L. Grzenia, D.J. Schell, S.R. Wickramasinghe, Membrane extraction for removal of acetic acid from biomass hydrolysates, *J. Memb. Sci.* 322 (2008) 189–195, <https://doi.org/10.1016/j.memsci.2008.05.030>.
- [114] H. Firoozmand, D. Rousseau, Microbial cells as colloidal particles: Pickering oil-in-water emulsions stabilized by bacteria and yeast, *Food Res. Int.* 81 (2016) 66–73, <https://doi.org/10.1016/j.foodres.2015.10.018>.
- [115] L.S. Dorobantu, A.K.C. Yeung, J.M. Foght, M.R. Gray, Stabilization of oil-water emulsions by hydrophobic bacteria, *Appl. Environ. Microbiol.* 70 (2004) 6333–6336, <https://doi.org/10.1128/AEM.70.10.6333-6336.2004>.
- [116] P. De Brabander, E. Uitterhaegen, E. Verhoeven, C. Vander Cruysen, K. De Winter, W. Soetaert, In Situ Product Recovery of Bio-Based Industrial Platform Chemicals: A Guideline to Solvent Selection, *Fermentation* 7 (2021) 26, <https://doi.org/10.3390/fermentation7010026>.
- [117] J. Marták, M. Rosenberg, S. Schlosser, L. Krstifiková, Toxicity of organic solvents used in situ in microbial fermentation, *Biotechnol. Tech.* 9 (1995) 247–252, <https://doi.org/10.1007/BF00151570>.
- [118] A.K. Sánchez-Castaneda, M. Moussa, L. Ngansop, I.C. Trelea, V. Athès, Organic phase screening for in-stream reactive extraction of bio-based 3-hydroxypropionic acid: biocompatibility and extraction performances, *J. Chem. Technol. Biotechnol.* 95 (2020) 1046–1056, <https://doi.org/10.1002/jctb.6284>.
- [119] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Molecular toxicity of potential liquid membranes for lactic acid removal from fermentation broths using *Lactobacillus casei* ATCC 393, *Dyna* 85 (2018) 360–366, <https://doi.org/10.15446/dyna.v85n207.72374>.
- [120] M. Matsumoto, K. Mochiduki, K. Fukunishi, K. Kondo, Extraction of organic acids using imidazolium-based ionic liquids and their toxicity to *Lactobacillus rhamnosus*, *Sep. Purif. Technol.* 40 (2004) 97–101, <https://doi.org/10.1016/j.seppur.2004.01.009>.
- [121] N.A. Marinova, D.S. Yankov, Toxicity of some solvents and extractants towards *Lactobacillus casei* cells, *Bul. Chem. Commun.* 41 (2009) 368–373.
- [122] Y. Tong, M. Hirata, H. Takashashi, T. Hano, M. Matsumoto, S. Miura, Solvent screening for production of lactic acid by extractive fermentation, *Sep. Sci. Technol.* 33 (1998) 1439–1453, <https://doi.org/10.1080/01496399808545059>.
- [123] M.S. Solichien, D. O'Brien, E.G. Hammond, C.E. Glatz, Membrane-based extractive fermentation to produce propionic and acetic acids: Toxicity and mass transfer considerations, *Enzyme Microb. Technol.* (1995) 23–31, [https://doi.org/10.1016/0141-0229\(94\)00086-7](https://doi.org/10.1016/0141-0229(94)00086-7).
- [124] 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, <https://doi.org/10.1016/j.bej.2018.04.014>.
- [125] S. Tönjes, E. Uitterhaegen, P. De Brabander, E. Verhoeven, T. Delmulle, K. De Winter, W. Soetaert, In situ product recovery as a powerful tool to improve the fermentative production of muconic acid in *Saccharomyces cerevisiae*, *Biochem. Eng. J.* 190 (2023) 108746, <https://doi.org/10.1016/j.bej.2022.108746>.
- [126] L.B. Soares, J.M. da Silveira, L.E. Biazzi, L. Longo, D. de Oliveira, A. Furigo Júnior, J.L. Ienczak, An overview on fermentation strategies to overcome lignocellulosic inhibitors in second-generation ethanol production using cell immobilization, *Crit. Rev. Biotechnol.* 43 (2023) 1150–1171, <https://doi.org/10.1080/07388551.2022.2109452>.
- [127] M. Matsumoto, M. Nishimura, Extractive Fermentation of Lactic Acid with *Hiocli* Bacteria in a Two-Liquid Phase System, *Ferment. Technol.* 5 (2016) 1000129, <https://doi.org/10.4172/2167-7972.1000129>.
- [128] N. Tik, E. Bayraktar, U. Mehmetoglu, In situ reactive extraction of lactic acid from fermentation media, *J. Chem. Technol. Biotechnol.* 76 (2001) 764–768, <https://doi.org/10.1002/jctb.449>.
- [129] V.M. Yabannavar, D.I.C. Wang, Strategies for reducing solvent toxicity in extractive ethanol fermentation, *Applied Biochemistry and Biotechnology - Part A: Enzyme Engineering and Biotechnology* 37 (1991) 716–722, <https://doi.org/10.1007/BF02787775>.
- [130] V.M. Yabannavar, D.I.C. Wang, Extractive Fermentation for Lactic Acid Production, *Biotechnol. Adv.* 37 (1991) 1095–1100, <https://doi.org/10.1002/bit.260371115>.
- [131] J. Iyyappan, G. Baskar, B. Bharathiraja, M. Gopinath, Enhanced malic acid production using *Aspergillus niger* coupled with in situ product recovery, *Bioresour. Technol.* 308 (2020) 123259, <https://doi.org/10.1016/j.biortech.2020.123259>.
- [132] H. Kim, O. Choi, B.S. Jeon, W.S. Choe, B.I. Sang, Impact of feedstocks and downstream processing technologies on the economics of caproic acid production in fermentation by *Megasphaera elsdenii* T81, *Bioresour. Technol.* 301 (2020) 122794, <https://doi.org/10.1016/j.biortech.2020.122794>.
- [133] G.M. Teke, R.W.M. Pott, Design and evaluation of a continuous semipartition bioreactor for in situ liquid-liquid extractive fermentation, *Biotechnol. Bioeng.* 118 (2020) 58–71, <https://doi.org/10.1002/bit.27550>.
- [134] A. Oudshoorn, K.J.J. Steinbusch, R.W.H. Kerste, D.J.R. Woolner, Integrated system for biocatalytically producing, WO202101822 A1, 2021.
- [135] dab.bio, FAST™ Fermentation Accelerated by Separation Technology, <https://dab.bio/technology>, (2024) accessed 30.05.2024.
- [136] A. Bednarz, A. Jupke, M. Schmidt, B. Weber, Multi-phase loop reactor and method of operation, WO2017149099 A1, 2017.
- [137] M. von Campenhausen, P. Demling, P. Bongartz, A. Scheele, T. Tiso, M. Wessling, L.M. Blank, A. Jupke, Novel multiphase loop reactor with improved aeration prevents excessive foaming in Rhamnolipid production by *Pseudomonas putida*, *Discover. Chemical Engineering* 3 (2023) 2, <https://doi.org/10.1007/s43938-023-00018-5>.
- [138] R. Grace, Commercial Emulsion Breaking, in: L.L. Schramm (Ed.), *Advances in Chemistry*, American Chemical Society, Washington, DC, 1992, pp. 313–339, <https://doi.org/10.1021/ba-1992-0231.ch009>.
- [139] C. Brandenbusch, B. Bühler, P. Hoffmann, G. Sadowski, A. Schmid, Efficient phase separation and product recovery in organic-aqueous bioprocessing using supercritical carbon dioxide, *Biotechnol. Bioeng.* 107 (2010) 642–651, <https://doi.org/10.1002/bit.22846>.
- [140] J. Zheng, Y. Tashiro, T. Yoshida, M. Gao, Q. Wang, K. Sonomoto, Continuous butanol fermentation from xylose with high cell density by cell recycling system, *Bioresour. Technol.* 129 (2013) 360–365, <https://doi.org/10.1016/j.biortech.2012.11.066>.
- [141] R.K. Lee, H.W. Ryu, H. Oh, M. Kim, Y.J. Wee, Cell-recycle continuous fermentation of *Enterococcus faecalis* RKY1 for economical production of lactic acid by reduction of yeast extract supplementation, *J. Microbiol. Biotechnol.* 24 (2014) 661–666, <https://doi.org/10.4014/jmb.1402.02017>.
- [142] L.R. Weatherley, *Intensification of Liquid-Liquid Processes*, Cambridge University Press, Cambridge, 2020, <https://doi.org/10.1017/9781108355865>.
- [143] K. Ye, S. Jin, K. Shimizu, Performance Improvement of Lactic Acid Fermentation by Multistage Extractive Fermentation, *J. Ferment. Bioeng.* 81 (1996) 240–246, [https://doi.org/10.1016/0922-338X\(96\)82215-X](https://doi.org/10.1016/0922-338X(96)82215-X).
- [144] H. Honda, Y. Toyama, H. Takahashi, T. Nakazeko, T. Kobayashi, Effective Lactic Acid Production by Two-Stage Extractive Fermentation, *J. Ferment. Bioeng.* 79 (1995) 589–593, [https://doi.org/10.1016/0922-338X\(95\)94753-E](https://doi.org/10.1016/0922-338X(95)94753-E).
- [145] Z.A. Hamamah, T. Grützner, Liquid-Liquid Centrifugal Extractors: Types and Recent Applications – a Review, *ChemBioEng. Rev.* 9 (2022) 286–318, <https://doi.org/10.1002/cben.202100035>.
- [146] N. Rüffer, U. Heidersdorf, I. Kretzers, G.A. Sprenger, L. Raeven, R. Takors, Fully integrated L-phenylalanine separation and concentration using reactive-extraction with liquid-liquid centrifuges in a fed-batch process with *E. coli*, *Bioprocess Biosyst. Eng.* 26 (2004) 239–248, <https://doi.org/10.1007/s00449-004-0354-4>.
- [147] G. Van Eygen, B. Van der Bruggen, A. Buekenhoudt, P. Luis Alconero, Efficient membrane-based affinity separations for chemical applications: A review, *Chemical Engineering and Processing - Process Intensification* 169 (2021) 108613, <https://doi.org/10.1016/j.ccep.2021.108613>.
- [148] S. Schlosser, R. Kertész, J. Marták, Recovery and separation of organic acids by membrane-based solvent extraction and pertraction: An overview with a case study on recovery of MPCA, *Sep. Purif. Technol.* 41 (2005) 237–266, <https://doi.org/10.1016/j.seppur.2004.07.019>.
- [149] R. Kertész, S. Schlosser, Design and simulation of two phase hollow fiber contactors for simultaneous membrane based solvent extraction and stripping of organic acids and bases, *Sep. Purif. Technol.* 41 (2005) 275–287, <https://doi.org/10.1016/j.seppur.2004.09.007>.
- [150] D. Maass, M.R. Gerigk, A. Kreutzer, D. Weuster-Botz, M. Wubbolts, R. Takors, Integrated L-phenylalanine separation in an *E. coli* fed-batch process: From laboratory to pilot scale, *Bioprocess Biosyst. Eng.* 25 (2002) 85–96, <https://doi.org/10.1007/s00449-002-0279-8>.
- [151] J. Xu, B. Bian, L.T. Angenent, P.E. Saikaly, Long-Term Continuous Extraction of Medium-Chain Carboxylates by Pertraction With Submerged Hollow-Fiber Membranes, *Front. Bioeng. Biotechnol.* 9 (2021) 726946, <https://doi.org/10.3389/fbioe.2021.726946>.
- [152] 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, <https://doi.org/10.1016/j.seppur.2018.06.001>.
- [153] J. Combes, N. Imatoukene, M. Moussa, N. Coquart, F. Chemarin, V. Athès, C. Fojcik, M. Chadni, I. Ioannou, M. Lopez, F. Allais, In-stream product recovery of p-coumaric acid heterologously produced: Implementation of a continuous liquid-liquid extraction assisted by hollow fiber membrane contactor, *Sep. Purif. Technol.* 293 (2022) 121083, <https://doi.org/10.1016/j.seppur.2022.121083>.
- [154] A.D. Pérez, S. Rodríguez-Barona, J. Fontalvo, Integration of a liquid membrane in Taylor flow regime with a fermentation by *Lactobacillus casei* ATCC 393 for in-situ lactic acid removal, *Chem. Eng. Process. - Process Intensif.* 140 (2019) 85–90, <https://doi.org/10.1016/j.ccep.2019.05.002>.
- [155] Z. Wu, S.T. Yang, Extractive fermentation for butyric acid production from glucose by *Clostridium tyrobutyricum*, *Biotechnol. Bioeng.* 82 (2003) 93–102, <https://doi.org/10.1002/bit.10542>.
- [156] A. Li, S. Sachdeva, J.H. Urbanus, P.J. Punt, In-stream itaconic acid recovery from *aspergillus terreus* fedbatch fermentation, *Ind. Biotechnol.* 9 (2013) 139–145, <https://doi.org/10.1089/ind.2013.0009>.
- [157] H. Sawai, T. Mimitsuka, S.I. Minegishi, M. Henmi, K. Yamada, S. Shimizu, T. Yonehara, A novel membrane-integrated fermentation reactor system: Application to pyruvic acid production in continuous culture by *Torulopsis glabrata*, *Bioprocess Biosyst. Eng.* 34 (2011) 721–725, <https://doi.org/10.1007/s00449-011-0521-3>.
- [158] S. Ge, J.G. Usack, C.M. Spirito, L.T. Angenent, Long-Term n-Caproic Acid Production from Yeast-Fermentation Beer in an Anaerobic Bioreactor with Continuous Product Extraction, *Environ. Sci. Technol.* 49 (2015) 8012–8021, <https://doi.org/10.1021/acs.est.5b00238>.

- [159] A. Keshav, K.L. Wasewar, Back extraction of propionic acid from loaded organic phase, *Chem Eng Sci* 65 (2010) 2751–2757, <https://doi.org/10.1016/j.ces.2010.01.010>.
- [160] K.L. Wasewar, A.B.M. Heesink, G.F. Versteeg, V.G. Pangarkar, Intensification of conversion of glucose to lactic acid: Equilibria and kinetics for back extraction of lactic acid using trimethylamine, *Chem Eng Sci* 59 (2004) 2315–2320, <https://doi.org/10.1016/j.ces.2003.11.023>.
- [161] T. Kurzrock, S. Schallinger, D. Weuster-Botz, Integrated separation process for isolation and purification of biosuccinic acid, *Biotechnol Prog* 27 (2011) 1623–1628, <https://doi.org/10.1002/btpr.673>.
- [162] P. Demmelmayer, M. Kienberger, Reactive extraction of lactic acid from sweet sorghum silage press juice, *Sep Purif Technol* 282 (2022) 120090, <https://doi.org/10.1016/j.seppur.2021.120090>.
- [163] L. Roxana Georgiana, B. Alexandra Cristina, D. Elena Niculina, G. Anca Irina, C. Dan, Mechanism, influencing factors exploration and modelling on the reactive extraction of 2-ketogluconic acid in presence of a phase modifier, *Sep Purif Technol* 255 (2021) 117740, <https://doi.org/10.1016/j.seppur.2020.117740>.
- [164] F. Chemarin, M. Moussa, F. Allais, I.C. Trelea, V. Athès, Recovery of 3-hydroxy-propionic acid from organic phases after reactive extraction with amines in an alcohol-type solvent, *Sep Purif Technol* 219 (2019) 260–267, <https://doi.org/10.1016/j.seppur.2019.02.026>.
- [165] M. Puttemans, L. Dryon, D.L. Massart, Extraction of Organic Acids by Ion-Pair Formation with Tri-n-octylamine. Part 2. Back-extraction, *Anal Chim Acta* (1984) 161, [https://doi.org/10.1016/S0003-2670\(00\)85811-0](https://doi.org/10.1016/S0003-2670(00)85811-0).
- [166] F. Chemarin, V. Athès, W. Buquet, M. Bedu, F. Allais, A.F. Teixeira, M. Moussa, I. C. Trelea, Predictive modeling and experimental implementation of organic acids in stream recovery by reactive extraction in membrane contactors, *Chem. Eng. J.* 431 (2022) 134067, <https://doi.org/10.1016/j.cej.2021.134067>.
- [167] G. Kyuchoukov, D. Yankov, Theoretical and experimental study of lactic acid stripping from loaded organic phase, *Ind Eng Chem Res* 49 (2010) 8238–8243, <https://doi.org/10.1021/ie100914n>.
- [168] J. Gorden, T. Zeiner, G. Sadowski, C. Brandenbusch, Recovery of cis, cis-muconic acid from organic phase after reactive extraction, *Sep Purif Technol* 169 (2016) 1–8, <https://doi.org/10.1016/j.seppur.2016.05.032>.
- [169] L.J. Poole, C.J. King, Regeneration of Carboxylic Acid-Amine Extracts by Back-Extraction with an Aqueous Solution of a Volatile Amine, *Ind Eng Chem Res* 30 (1991) 923–929, <https://doi.org/10.1021/ie00053a015>.
- [170] E. Reyhanitash, E. Fufachev, K.D. Van Munster, M.B.M. Van Beek, L.M.J. Sprakel, C.N. Edelijn, B.M. Weckhuysen, S.R.A. Kersten, P.C.A. Bruijninx, B. Schuur, Recovery and conversion of acetic acid from a phosphonium phosphinate ionic liquid to enable valorization of fermented wastewater, *Green Chem.* 21 (2019) 2023–2034, <https://doi.org/10.1039/c9gc00725c>.
- [171] P.O. Saboe, L.P. Manker, W.E. Michener, D.J. Peterson, D.G. Brandner, S. P. 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, <https://doi.org/10.1039/C7GC03747C>.
- [172] M. Blahusiak, S. Schlosser, J. Cvengros, J. Marták, New approach to regeneration of an ionic liquid containing solvent by molecular distillation, *Chem. Pap.* 65 (2011) 603–607, <https://doi.org/10.2478/s11696-011-0053-y>.
- [173] E. Reyhanitash, T. Brouwer, S.R.A. Kersten, A.G.J. van der Ham, B. Schuur, Liquid-liquid extraction-based process concepts for recovery of carboxylic acids from aqueous streams evaluated for dilute streams, *Chem. Eng. Res. Des.* 137 (2018) 510–533, <https://doi.org/10.1016/j.cherd.2018.07.038>.
- [174] K.-L. Li, I.-L. Chien, C. Cheng-Liang, Design and Optimization of Acetic Acid Dehydration Processes, *Proceedings of the International Conference on Control of Industrial Processes (ADCONIP 2014)* (2014) 126–131. https://folk.ntnu.no/sko/ge/prost/proceedings/adconip-2014/pdf/SUBS21TO40/0032/0032_FI.pdf.
- [175] S. Xu, K. Lan, J. Li, T. He, C. Hu, Separation of lactic acid from synthetic solutions and the mixture directly derived from corn stover by aqueous two phase extraction, *Sep Purif Technol* 204 (2018) 281–289, <https://doi.org/10.1016/j.seppur.2018.04.086>.
- [176] J.A. Tamada, C.J. King, Extraction of Carboxylic Acids with Amine Extractants. 3. Effect of Temperature, Water Coextraction, and Process Considerations, 336 (1990) 1333–1338. <https://doi.org/10.1021/ie00103a037>.
- [177] L.M.J. Sprakel, A.F.M. Holtkamp, R. Bassa, B. Schuur, Swing processes for solvent regeneration in liquid-liquid extraction of succinic acid, *Chem. Eng. Process. - Process Intensif.* 143 (2019) 107600, <https://doi.org/10.1016/j.cep.2019.107600>.
- [178] R. Mahmud, S.M. Moni, K. High, M. Carbajales-Dale, Integration of techno-economic analysis and life cycle assessment for sustainable process design – A review, *J Clean Prod* 317 (2021) 128247, <https://doi.org/10.1016/j.jclepro.2021.128247>.
- [179] A.I. Magalhães, J.C. de Carvalho, J.F. Thoms, J.D.C. Medina, C.R. Soccol, Techno-economic analysis of downstream processes in itaconic acid production from fermentation broth, *J Clean Prod* 206 (2019) 336–348, <https://doi.org/10.1016/j.jclepro.2018.09.204>.
- [180] A. Oudshoorn, C. van den Berg, C.P.M. Roelands, A.J.J. Straathof, L.A.M. van der Wielen, Short-cut calculations for integrated product recovery options in fermentative production of bio-bulk chemicals, *Process Biochem.* 45 (2010) 1605–1615, <https://doi.org/10.1016/j.procbio.2010.07.003>.