

A Critical Review on Downstream Process to Recovery Succinic Acid from Fermentative Broth

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Succinic acid was a building block chemical of many industrial products, among these, one of the most interesting applications concerns the bioplastics market. This compound can be produced through the biotechnological routes, but several challenges still need to be addressed. One of the greatest challenges has been the downstream process to recover and purify succinic acid from the fermentative broth. Succinic acid must be taken from the fermentation broth, that was composed of various compounds, such as cellular residues, proteins, and other acids, to reach a recovery and a purity >90%, in order to be marketed. Generally, the downstream process can be divided into three phases: pretreatment, separation and purification. Several studies have been carried out on different technologies with the aim to improve recovery and purity. To date, all these methods were applied only on laboratory-scale, due to various critical issues that emerged, such as high costs, which prevented their application on an industrial scale. This review highlighted the best obtained results from the recent studies and discussed on critical aspects and future perspectives on the topic.

1. Introduction

Succinic acid (SA) was one of the most interesting building-block chemicals, a C4 carboxylic acid with four carbon atoms, widely used as precursor for various products such as surfactants, foaming agents, detergents, and biodegradable plastics (Nam et al., 2012). This acid could be produced through two processes: the petrochemical based on fossil sources and the fermentative based on biological processes. The production of bio-based SA through fermentation processes presented several advantages, including the use of abundant and low-cost renewable sources (glucose – rich feedstock) from agro-industrial sector. Succinic acid can be produced by many microorganisms (such as *Actinobacillus succinogenes*, *Basfia succiniciproducens*, *Escherichia coli*, *Saccharomyces cerevisiae*) as an intermediate of various biochemical pathways, including the Krebs cycle. The fermentation process generated a broth containing SA or his salt, the succinate, that needed to be separated from the fermentative broth and purified from other impurities and by-products. This downstream process was investigated in many studies however it still remained one of the critical topics for the production of bio-succinic acid for many aspects, such as the increase of recovery and purification of SA, the decrease of the costs of the process, that could accounted for more than 50% of the total production costs, and the reduction of the potential environmental impact due to chemical reagents and energy requirements (Dickson et al., 2021).

Nowadays, several studies have been conducted on different method, but choosing the correct strategy has not been easy because of several issues due to the fermentation process, the feedstock, the SA concentration, the presence of impurities, the scalability and cost of technologies.

2. The recovery of succinic acid from fermentation broth

In this review, the most recent downstreaming technologies were investigated based on a critical analysis from specific case studies to better understand the operational processes to optimize the recovery and purification of succinic acid from fermentation broth.

One of the most crucial challenges in the production of bio-based SA has been to effectively separate this acid (or its salt form, succinate) from a mixture of microbial cells and various compounds, such as water, residual sugars, byproducts (like ethanol, formate, lactate, acetate), macromolecules (especially proteins, nucleic acids and polysaccharides) and salts. The removal of all the impurities was fundamental, to obtain high purity SA, that could be used to produce biopolymers, such as those based on butylene succinate (Alexandri et al., 2019).

Due to the complexity of fermentation broth, typically, downstream process of bio-based succinic acid included three steps. In the first step, centrifugation and/or membrane filtration, and clarification could remove the microbial cells, the debris, macromolecules (proteins, residual of sugars and pigments) that made up more than 85% of the composition of the broth. These technologies could remove 97% of microbial cells and around 80% of proteins and macromolecules with no significant loss of succinic acid (Mancini et al., 2020). In second step, SA was separated from other by-products (organic acids, and ethanol) to recovery SA for the last step and the final purification through crystallization. Each of these steps consisted of several techniques that could be combined, in order to exploit the advantages of each, and to obtain SA of greater than 90% purity. In addition, the choice of the downstreaming process was also given to yield, operational costs, and impact on the environment.

2.1 Pretreatments

In studies conducted on the production of succinic acid through fermentation, many sources were used as growth medium, simulated fermentation broth or hydrolysates produced from agro-industrial resources (biomass-based hydrolysates). The use of synthetic growth media and simulated fermentative broths might not accurately show the issues faced in recovering SA from biomass-based hydrolysates as fermentative broth which were mainly addressed in the separation phase. Depending on the feedstock, several products can remain in fermentative broth. In the first step, the broth needed to be cleaned from microbial cells, macromolecules, such as proteins and polysaccharides and pigments. In most studies, as shown in table 1, the first step was the centrifugation of the fermentation broth to separate the microbial cells from the broth by means of centrifugal force at 8000 rpm for 10 min at laboratory scale. Dabkowska et al. (2019) tested centrifugation and ultrafiltration by using a polyacrylonitrile membrane 12%, with a pore size of 0.2 μm to obtain a clear broth with a slight loss of SA equal to $8.0 \pm 1.5\%$ respect to SA in concentration in the broth. In other works, only a filtration was employed (Omwene et al., 2021; Omwene et al., 2020; Lee et al., 2022) or salting-out extraction (Lutfhi et al., 2020) on the fermentation broth with the aim to develop new strategies for the recovery of succinic acid. After centrifugation, the decolorization process by activated carbon was performed to remove organic impurities and pigments in almost all the works analyzed, except for Garcia-Aguirre et al. (2020) and Lee et al. (2022).

2.2 Succinic acid separation and purification

In Table 1 were reported succinic acid and other by-products produced after the fermentation of several feedstocks, and the most studied technologies to recovery SA. Several raw materials of different origin could be used for fermentation, from hydrolyzed lignocellulosic biomasses (Dabkowska et al., 2019; Law et al., 2019; Lee et al., 2022) to cheese/milk by-products. (Omwene et al. 2020, 2021)

Naturally, the variability of the composition of initial feedstock reflected on the fermentation performance and consequently in SA concentration. In fact, Omwene et al. (2020) obtained 11.9 g/L of SA from whey cheese while Jokodola et al. (2022) obtained 36.7 g/L of SA from pure xylose. The difference between the final concentration of SA was certainly influenced by the greater complexity of a biomass such as whey compared to a glucose-rich solution. The initial feedstock can also influence the production of by-products, as acetic acid (AA), formic acid (FA) or lactic acid (LA). In fact, it was possible to note a great concentration of lactic acid (14.1 g/L) compared to the other by-products, such as succinic acid (11.6 g/l), formic (2.83 g/L) and acetic acid (5.7 g/L) in the fermentation broth derived from cheese whey(Omwene et al., 2020). Lactic acid may also be present when other feedstocks were used (i.e. lignocellulosic biomass) and depending on the fermentation conditions and metabolic pathway used by the organic acid-producing microorganism. When lignocellulosic biomasses were used, acetic acid was the most abundant by-product 3.7 g/L respect to 29.2 g/l SA from hydrolyzed oil palm (Law et al., 2019), 5.0 g/L of AA and 24.0 g/L of SA from hydrolyzed miscanthus (Dabkowska et al., 2019). As known, acetic acid was a by-products of lignocellulosic biomass pre-treatment and fermentation process that can be considered one of the most relevant inhibitor compounds of fermentative

microorganisms at critical concentrations; indeed, AA had a negative effect on SA production. To minimize the presence of acetic acid and other unwanted compounds (i.e. formic, lactic acids), several strategies were tried before fermentation of lignocellulosic biomasses by improvement of pretreatments methodologies (Zhai et al., 2022) or by genetic engineering of microorganisms that has been tested to improve SA production and reduce the formation of other fermentation by-products (Jiang et al., 2017). In addition, their removal from the broth, during downstream processes was fundamental to enhance SA yield and purity.

Acetic acid and others organic acids can be removed after fermentation process through several technologies of separation that had been developed taking advantage of the chemical-physical properties of SA, that is hydrophilic and has a high boiling point (235 °C) respect to others organic acids and competitor compounds.

Table 1: Overview of SA and other by-products concentration at the end of fermentation, pre-treatments and separation techniques, yield (%) and purity (%) of SA obtained at the end of downstream process, NR= not reported; SA= succinic acid; LA= lactic acid; FA= formic acid; AA= acetic acid,

Pretreatments	Products concentration (g/L)	Separation techniques	Yield (%)	Purity (%)	Reference
Microfiltration (0.1 µm ceramic membrane)	11.9 SA , 0.1 LA, 3.8 FA, 4.4 AA	Chromatography (anionic resin) – evaporation	78.0	98.5	Omwene et al. (2021) ¹
		Chromatography (cationic resin) – NF/RO – vacuum distillation	65.0	96.7	Omwene et al. (2021) ²
Centrifugation (10 minutes; 8000 rpm)	36.7 SA 28.7 SA	Vacuum distillation – direct crystallization	79.1	NR	Jokodola et al. (2022) ¹
		Vacuum distillation – direct crystallization	76.5	NR	Jokodola et al. (2022) ²
		Vacuum distillation – direct crystallization	75.2	NR	Jokodola et al. (2022) ³
Centrifugation (NR)	36.8 SA , 4.2 LA, 1.4 FA, 7.9 AA	Forward osmosis	NR	NR	Garcia-Aguirre et al. (2020)
Ultrafiltration (NR)	11.6 SA , 14.1 LA, 2.8 FA, 5.7 AA	Vacuum distillation	57.3	NR	Omwene et al. (2020)
Salting-out extraction (30% w/w acetone and 20% w/w (NH ₄) ₂ SO ₄ ; pH 3.0; 8h) Centrifugation (20 min; 10000 rpm) – ultrafiltration (polyacrylonitrile membrane 0.2 µm)	36.3 SA , 3.1 FA, 7.8 AA	Distillation – salt dissolution	84.8	99.7	Luthfi et al. (2020) ¹
Ultrafiltration (hollow-fiber PVDF 0.05 µm)	24.0 SA , 2.5 FA, 5.0 AA	Chromatography – vacuum distillation	50.6	98.9	Dabkowska et al. (2019)
		Electrodialysis – distillation – precipitation – activated carbon	74.7	99.4	Lee et al. (2022)
Centrifugation (20 min; 8000 rpm)	17.5 SA 29.2 SA , 0.2 FA, 3.7 AA	Forward osmosis	NR	NR	Law et al. (2019) ¹
		Forward osmosis	67.1	90.51	Law et al. (2019) ²

Chromatographic separation, distillation, membrane technologies and crystallization have been the most tested technologies for the separation and purification of SA. Given the mixture of the organic acids produced during the fermentation process and which were not eliminated by pretreatments as the centrifugation and/or filtration phases, more separation technologies have been combined to improve the downstream process.

Omwene et al. (2021) tested two chromatographic separations: the first by Amberlite IRA900 Cl anionic resin followed by an evaporation performed at 80 °C and the second by cationic resin followed by membrane filtration (nanofiltration/reverse osmosis NF/RO) and vacuum distillation at 80°C 350 mbar). At the end of the

two processes, a crystallization at 4°C and pH 2 were performed to purify and recover the succinic acid after a washing at 4°C and a drying at 60°C for 24h. The chromatographic separation by anionic resin, first eluted lactic, acetic, and formic acid; this suggested that sorption of SA was stronger compared to the other organic acids. The best separation efficiencies till to 69% was obtained at a low flow rate (0.42 BV/h) than 1.8 BV/h that decreased the efficiency till to 39% (Omwene et al. (2021)). In the second process, the cationic exchanger Lewatit®MonoPlus S100 removed the cations from the fermentation broth, such as Ca²⁺, Co²⁺, K⁺, and Na⁺, and converted the organic acids into their acid form by the acidification of the broth to pH 2.7. A cation-exchange resin (Amberlite IR120H) was also tested by Dabkowska et al. (2019) at a flow rate of 10 ml/min, that reached a recovery of succinate around 75% after vacuum distillation (65 °C 30 mmHG), and the crystallization (4°C, 12 h). The use of vacuum distillation after the chromatographic separation can be efficiently performed to separate organic acids since the boiling point of acetic acid (118 °C) and formic acid (101 °C) were lower than the one of SA, so these compounds evaporated sooner (Vlysidis et al., 2011) without no significant loss of SA compared to other techniques. Membrane filtration can also be applied to recovery SA by nano-filtration (NF), ultra-filtration (UF) and reverse osmosis (RO) and forward osmosis (FO). Omwene et al. (2021) experimented NF and RO by obtaining the best SA retention (97%) at pH 6.8 for NF and a double pass mode for RO was sufficient to reach a retention of 96% of SA. Law et al., 2019 performed the forward osmosis on fermentative broth by concentrating SA 3.9-fold from an initial concentration of 28.9 g/L to 111.3 g/L. After centrifugation, adsorption and distillation, a direct crystallization process was tested by Jokodola et al. (2020) on three different acidified broths, such as pure xylose, hydrolyzed olive pits and sugarcane bagasse. The authors reached a recovery yield of SA higher than 70% in all the tested broths without the adding of further steps as precipitation. Multiple crystallization was tested after pretreatments and distillation of the broth by Luthfi et al. (2020) that observed an improvement of 17% in the recovery of SA crystals after the increase of the crystallization time from 6 to 9 hours. Afterwards, the authors tried the crystallization in three consecutive stages, and found an improvement on the recovery percentage of SA from 55% to 84.8% and a high purity (99.7%).

An alternative process to recover and purify SA was employed by Lee et al. (2022) on the clarified broth that was electrodialyzed to separate succinate from other contaminated ions by using two anionic and cationic membranes positioned between anode and cathode. After electrodialysis, the solution was heated at 100 °C (distillation) and a series of cooling-heating cycles was performed for the purification of SA. After this approach, a yield and a purity equal to 74.7% and 99.4% was respectively obtained (Lee et al. (2022)).

Among the discussed recent studies, the best results in terms of yield and purity were obtained by Luthfi et al. 2020, which achieved up to 84.4% yield and 99.7% of purity when a three steps of crystallization were performed after salting-out extraction of fermentative broths, adsorption by active carbon and evaporation at 100 °C. A yield of 50.6% and a purity of 98.9% was reached after vacuum distillation combined with crystallization (Dabkowska et al. (2019)). After chromatographic separation by the use of anionic resin, and evaporation, Omwene et al. (2021)² reached a yield of SA equal to 78.0% and a purity of 98.5%. Interesting results on yield (74.7%) and purity (99.4%) was also obtained by Lee et al., 2022 that performed active carbon decolorization, precipitation, distillation and electrodialysis. The results obtained from these studies demonstrated once again how the strategy of using several technologies can be successful on a laboratory or computational scale, but further problems need to be addressed for their development on an industrial scale. The main advantages and disadvantages of the major technologies used in downstreaming processes are reported in Table 2 to discuss the most important issues towards their scalability.

Table 2: advantages and disadvantages of the various techniques adopted to separate and purify succinic acid from fermentation broth.

Separation techniques	Advantages	Disadvantages
Chromatography	High selectivity Easy scalability	Regeneration of chromatographic matrix, large amount of acids and/or alkali
Direct crystallization	Few unit operations, easy to apply	Low yield and purity of the product
Distillation	Efficient remove of water, acetic and formic acid	High energy consumption
Membrane technologies	High yield and purity obtained	High equipment costs and fouling
Precipitation	Viable process for commercial production	Large dosage of chemicals, difficult reuse of chemicals and disposal of by-products, low-value byproducts

Chromatography can be a good candidate technology for industrial scale-up but for the disadvantages on the regeneration of the matrix and the use of many chemicals it is not an ideal solution. Crystallization needed few operation units and can be scale-up but this technology did not allow high yield and purity. Distillation can be efficient for the removal of other acids but it is an energy-intensive techniques for industrial scale. Membrane-based techniques can be considered very promising, but energy consumption was found to be limited by about 90% compared to distillation (Sholl & Lively, 2016). However, fouling problems can reduce the permeability of membranes due to the accumulation of microbial cells, macromolecules, and other impurities on their surfaces (Kumar et al., 2020). The need for continuous cleaning and/or replacement of these membranes, to date, is a limitation for the scale-up of these techniques. Precipitation was used at industrial scale but due to the use of excessive amounts of chemicals should be replaced with less environmentally impactful technologies.

To overcome some of the outlined problems, an integration of fermentation and downstream process was explored by Pateraki et al. (2019) that performed a fermentative production of SA by an electrochemical membrane reactor for an integrated production and *in-situ* extraction of SA, with the production of base in the cathodic process, that regulated the pH of fermentation and avoided cell toxicity derived from product inhibition. The developed integrated process demonstrated a great result for the obtained yield of 79% and the purity of 99% (less than 0.09 mol of acetic acid content). In addition, a preliminary technical-economic evaluation showed that the designed integrated system could reduce production costs for a plant of 40,000 tonnes/year by 20 % due to the reduction of downstreaming operations (Pateraki et al. 2019). This study was conducted on laboratory scale, but it can be considered a promising strategy, for the results obtained.

In addition, the techno-economic and environmental comparative analysis of four different simulated processes for the production of bio-SA from corn stover, two industrial processes and two conceptual processes highlighted, that a continuous process of fermentation by immobilized cells and extraction by electrochemical cell equipped with an anionic exchange membrane followed by evaporation, crystallization and centrifugation could be an interesting solution to be developed at industrial scale (Mancini et al. 2022). Indeed, it has been shown that this process had a lower total production cost than the other processes. And although 91% of the utility cost items were due to the downstream process, of which the largest contribution was made by the energy and maintenance requirements of the electrochemical system, it was observed a decrease in operation costs of between 20-50% compared to conventional industrial processes. Furthermore, in particular market situations and with a 15-year projection, the developed system could decrease the price of AS by up to about USD 1.4 if the developed system also came to industrial scale while maintaining the same performance. In addition to the economic and environmental factors that led the production of bio-succinic acid to be uncompetitive in the market, other key aspects to be considered in the production of bio-succinic acid are the yield and purity of the final product that must be at least of 90 and 99.5%, respectively for industrial-scale application (Dessie et al., 2023).

3. Conclusion

Downstreaming remained a critical step for the production of bio-succinic acid for many aspects, such as yield, purity, and production costs. Several studies have been carried out on various technologies and for each it has been demonstrated that the strategy of integrated use of technologies was a promising way to optimize yield and purity, but many aspects need to be optimized. In particular, the most important aspect was the scalability of technologies on an industrial scale and their contribution to the total cost of production. Hence, further studies must be carried out regarding the optimization of upstream and downstream processes, to reduce the current critical disadvantages.

Acknowledgments

This work was supported by The National Recovery and Resilience Plan (NRRP) - Next Generation EU – Mission 4, Component 2, Investment 1.4, under the “National Research Centre for Agricultural Technologies”, Agritech, [grant number I63C22000350007].

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