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# Economic and environmental analysis of bio-succinic acid production: From established processes to a new continuous fermentation approach with *in-situ* electrolytic extraction

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## ABSTRACT

Many recent attempts to commercialize bio-succinic acid (bio-SA) ended to be unsuccessful after a start flourishing moment. Furthermore, the improved environmental performance of bio-SA production processes compared to petroleum-based SA is still uncertain. In this study a techno-economic analysis was conducted comparing four bio-SA manufacturing processes in terms of net present value and minimum selling price. Two of the simulated processes are based on patents released by bio-SA manufacturing companies (I) Roquette/DSM (Reverdia) and (II) DNP Green Technology/ARD (BioAmber). A third process is based on a Michigan State University patent (III) and a fourth process is conceptual (IV). The conceptual process IV was demonstrated to have <50% lower capital costs and ~40 to 55% lower manufacturing costs than the other processes. With a minimum selling price of 1.4 USD kg<sup>-1</sup>, process IV would be cheaper than petroleum based succinic acid (~2.0 USD kg<sup>-1</sup>). The Reverdia-based process can also be competitive, while process III and particularly the BioAmber-based process II are not profitable. Ion-exchange columns, nanofiltration and anion exchange membranes are shown to be key technologies for lowering bio-SA manufacturing costs. Continuous bio-SA fermentation with *in situ*-like extraction can change the bio-SA market, but the environmental sustainability assessment reveals only marginal differences compared with petroleum-based SA. Low pH “aerobic fermentation” is likely to be a more sustainable strategy compared to neutral pH “aerobic fermentation”.

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**Abbreviations:** AEM, Anion exchange membrane; Bio-SA, Bio-succinic acid (bio-based succinic acid); CAGR, Compound annual growth rate; GMO, Genetically modified microorganism; LCA, Life cycle assessment; NPV, Net present value; PA, Pyruvic acid or pyruvate; PEM, Proton exchange membrane; SA, Succinic acid; TRL, Technology Readiness Level; USD, United States dollar.

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## 1. Introduction

The current pace of utilizing products derived from fossil sources to meet growing demands for commodities is widely recognized as a precursor to global environmental changes. A report from the US Department of Energy (DOE) described bio-succinic acid (bio-SA) as a top 12 priority compound with the potential to become a platform chemical in a bio-based economy due to its strategic importance in the future chemical industry (Werpy and Petersen, 2004). Bio-SA is deemed important because it can be a building block in the production of over 30 valuable products and can offset climate change by saving 4.5–5 tons of CO<sub>2</sub> per ton of succinic acid produced compared to petrochemical-based succinic acid (SA) (Hermann et al., 2007). At the same time, low-value biomasses such as food, industrial and agricultural wastes can be used to produce bio-SA, which is then converted into value-added products (Werpy and Petersen, 2004; Cherubini, 2010; E4tech RE-CORD and WUR, 2015). The most notable bio-SA applications are in the food, pharmaceutical, cosmetic and chemical industries (Mancini et al., 2019). In addition, newer industrial applications such as 1,4-butanediol, poly-butylene succinate, alkyd resins and plasticizers are expected to boost the future growth of the bio-SA market (Clark, 2014). As a result of the far reaching applications of bio-SA, the global succinic acid market is expected to grow from USD 131.7 million in 2018 to USD 282.8 million by 2023, at a compound annual growth rate (CAGR) of 6.8% during the forecast period 2019–2023 (Newark, 2020).

In 2003, the competitiveness of bio-SA compared to its rivals was mainly due to very high oil prices (Pinazo et al., 2015). Thus, immediately after the peak oil prices in 2007, several industrial actors showed their interest in commercial-scale bio-SA production. As a consequence, four commercial-scale bio-SA plants were built in the period 2012–2015, i.e. Reverdia (10 kt y<sup>-1</sup> – a joint venture of DSM and Roquette), BioAmber (30–50 kt y<sup>-1</sup> – a joint venture of DNP Green Technology and ARD), Myriant (14 kt y<sup>-1</sup>) and Succinity (10 kt y<sup>-1</sup>), both from a joint venture between BASF and Corbion Purac (E4tech RE-CORD and WUR, 2015; Garg et al., 2019). Despite several efforts made in the past to produce bio-SA competitively, most of the commercial plants previously mentioned are no longer in operation (Barrett, 2018; De Guzman, 2019).

The catalytic instead of the fermentative way to produce bio-SA from biomasses is promising, offering also advantages compared with bio-SA from microbial fermentation (Verma et al., 2020; Cukalovic and Stevens, 2008). However, bio-SA from chemical synthesise is still far from full scale implementation (Kawasumi et al., 2017). One potential solution for increasing the competitiveness of bio-SA production from microbial fermentation is to lower the total production cost through a more sustainable production. Morales et al. (2016) described the distribution of the total production cost of bio-SA as follows: 10–15% goes to feedstock purchase, 20–25% to the fermentation process, and 60–80% to the purification process. This breakdown indicates that if technological breakthroughs can decrease the production cost associated with fermentation and purification, bio-SA can replace petroleum-based SA. The major challenges in bio-SA production are (1) intensive pretreatments, (2) low yields, productivity, and selectivity, (3) expensive recovery and separation of bio-SA acid from the rather diluted fermentation broth, rich of impurities and fermentation by-products (Mancini et al., 2019). Several bench-scale studies have been conducted to address the aforementioned challenges. For example, various pretreatment methods including acid thermal hydrolysis (Humbird et al., 2011), alkaline thermal hydrolysis (Kuhn et al., 2016), deacetylation (Salvachúa et al., 2016a), hot water wash (Alvarado-Morales et al., 2015) and enzymatic hydrolysis (Humbird et al., 2011) have been studied to prepare different biomass sources such as lignocellulosic material, glycerol and macroalgae for the fermentation. To increase yield, titre and productivity in fermentation, several parameters including host microorganism (Van de Graaf et al., 2015), fermenter configuration and operational technique have been studied (Mancini et al., 2019). For the downstream, since bio-SA purification is an energy-intensive process, a range of technologies have been investigated to address the challenge, such as electrodialysis, direct crystallization, reactive extraction,

ion-exchange column, reactive crystallization and membrane technology, including microfiltration, ultrafiltration and nanofiltration (Cheng et al., 2012). However, while huge advances have been made in developing GMO microbial hosts (Babaei et al., 2019; Chatterjee et al., 2001; McKinlay et al., 2007), the separation of bio-SA and in general the production approach with upstream unit operations (fermentation or aerobic bioprocesses) separated from those in the downstream has not changed for years.

Despite several bench-scale experimental studies on improving bio-SA production efficiency, little information is available on the economics and the environmental performance of commercial-scale bio-SA production. Regarding industrial-scale studies, Efe et al. (2013) conducted a techno-economic analysis of bio-SA production from cane sugar for a plant capacity of 30 kt y<sup>-1</sup> and calculated a selling price of 2.26 USD kg<sup>-1</sup> for bio-SA. Recently, Klein et al. (2017) found a production cost of 2.32 USD kg<sup>-1</sup> for bio-SA derived from bagasse and obtained from a sugarcane biorefinery. For our work, also considering the import-export data (ZAUBA, 2016; Alibaba, 2021), we assume a petroleum-based SA market price of 2.0 USD kg<sup>-1</sup>, suggesting that improvements are needed to reach economic competitiveness. Similarly, from the environmental perspective studies show that the impacts of bio-SA production vary considerably depending on many factors (e.g. type of feedstock, process conditions, modelling approach) (Morales et al., 2016; Cok et al., 2013; Gadkari et al., 2021), indicating that the environmental benefits of bio-SA production compared to conventional routes are still unclear (Ögmundarson et al., 2020). To the best of our knowledge, (1) no study has explicitly simulated commercial-scale biorefineries to investigate their economics and bottlenecks in the current market, and (2) assessment of the techno-economic profitability and environmental sustainability of commercial-scale bio-SA production using corn stover as a feedstock is until now limited to only one study (Dickson et al., 2021).

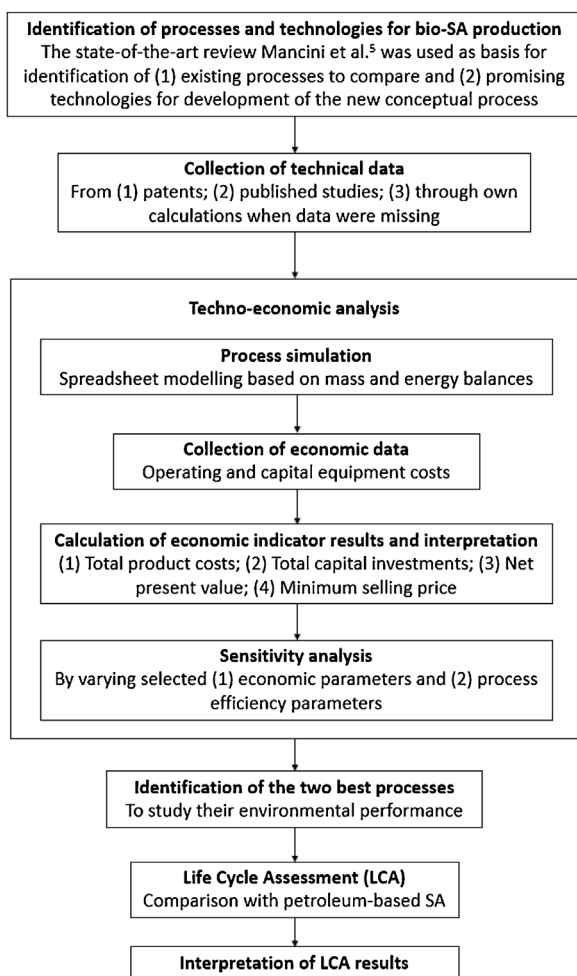
The aim of this study was therefore to assess the economic and environmental performance of four processes for commercial bio-SA production. Four process designs were analysed to investigate the economics of bio-SA production. Two of the designs are based on actual production processes of the companies Reverdia (Process I) and BioAmber (Process II), a third is based on a patent (Yedur et al., 2001) (Process III), and the fourth is a novel process consisting of immobilized cells with an *in situ*-like anion exchange electrolytic extraction of bio-SA (Process IV). Rigorous techno-economic models were used to determine the economic competitiveness of each process design, whereas life cycle assessment (LCA) was used for trade-off analysis of environmental impacts.

## 2. Methods

Fig. 1 provides an overview of the main methodological steps followed in this study to carry out the techno-economic and the environmental assessments. A detailed description of the processes considered for the comparison and the specific methodology adopted are presented in the following sections. Details on how the four processes have been modelled (i.e. including efficiencies and operating conditions) is provided in Table S4 of the Supplementary Material.

### 2.1. Process descriptions

Two of the four company production processes, i.e. Reverdia (Van de Graaf et al., 2015; Boit et al., 2019) and BioAmber (Fruchey et al., 2011, 2012; Rush and Fosmer, 2014) were evaluated using a mass balance model and specified by split-fractions according to the patents released by the companies themselves combined with information from other related publications (Ahn et al., 2016; Nghiem et al., 2017). A third promising downstream process was modelled from the patent of Yedur et al. (2001) while process IV is a novel process,



**Fig. 1 – Overview of the major methodological steps in this study.**

developed based on process intensification principles. Therefore, the processes were modelled in spreadsheets based on patent and literature data. Patents include conditions, compositions and reactions and when information was not available in the literature, empirical equations were used, as reported in the Supplementary Material. Process IV is conceptual and was devised based on: (1) bioprocessing trends such as perfusion and continuous fermentation (Lindskog, 2018), (2) an extensive literature review that considers both industrial and bench-scale processes (Mancini et al., 2019) and (3) other industrial-scale simulation studies (Garg et al., 2019). The separation of bio-SA is typically a rather intense process with numerous steps and unit operations. Therefore, the principles based on which process IV was developed are the reduction of unit operations and the integration of functions. The key idea is to separate bio-SA while simultaneously improving the fermentation performance.

Information about the actual feedstocks used by the companies is not fully available and therefore corn stover was used as the feedstock for all considered processes. The primary reason for selecting corn stover as feedstock is due to its non-competition with food supplies, high potential for bio-SA production (Mancini et al., 2019; Salvachúa et al., 2016a), and constantly increasing global production, which was 1.1 billion tons in 2019 (Salvilla et al., 2020). Considering the yearly worldwide volume production of bio-SA of about 38 kt  $y^{-1}$  (E4tech RE-CORD and WUR, 2015) and other studies in the field (Efe

et al., 2013; Dickson et al., 2021), all bio-SA processes studied in this work were set to produce 30 kt  $y^{-1}$ .

### 2.1.1. Process I: reverdia

Fig. 2 is a schematic of the process flow diagram used by Reverdia. The process starts with pretreatment of corn stover according to a method developed by Salvachúa et al (2016a) and Shekiro et al. (2014) at pilot-scale. In this method, corn stover was first mixed with water to reach 8 wt% total solid concentration. The slurry is heated to 80 °C and sent to the deacetylation reactor (R-101) for 2 h, where 0.4 wt% NaOH is used to remove acetate and lignin. The de-acetylated slurry is then washed with water to remove alkali and re-suspended to achieve 10% total solid loadings. The slurry is heated to 160 °C and sent to the pretreatment reactor (R-102), where hydrolysis for 10 min with a 0.8 wt% sulphuric acid solution as catalyst takes place to result in conversion of 77% of the corn stover into fermentable sugars, as reported by Shekiro et al. (2014). The slurry from the pretreatment reactor (R-102) is pumped into a continuous Archimedean screw press (D-103) to separate unreacted biomass from the hydrolysate and cooled at 30 °C prior to entering the fermenter (F-104), where an engineered strain of the yeast *S. cerevisiae* converts sugar monomers to bio-SA. The “aerobic fermentation” lasts for 95 h at a pH of 3.1 to achieve a titre of 43 g  $L^{-1}$  with a yield of 0.69 g-SA  $g^{-1}$ -sugar (Van de Graaf et al., 2015). KOH is used as a buffering agent (Ahn et al., 2016; Nghiem et al., 2017), to control the pH of the “aerobic fermentation” broth, and the required amount was estimated using the BATE pH calculator - acid/base equilibrium, titration curves from ChemBuddy (see Supplementary Material). Due to the low pH of the “aerobic fermentation”, succinic acid is dominant in the broth (Supplementary Material, Figure S1); however, some succinate, and thus some potassium succinate (KAS) salt, can also be generated.

After “aerobic fermentation”, the broth is sent for centrifugation (CE-105) for cell removal, and clean broth free from cells is concentrated to supersaturation (420 g-SA  $kg^{-1}$ -solution) by evaporating (E-106) the water at 80 °C (Boit et al., 2019). Bio-SA in the concentrated slurry is then crystallized (C-107) at pH 1.5 and a cooling rate of 5 °C  $h^{-1}$  to reach 20 °C to recover 91% of the SA present in the “aerobic fermentation” broth. The impure crystals are separated by centrifugation (CE-108) and then purified with a hydrogen exchange column (H-109). The purified crystals are sent for crystallization (C-110) followed by centrifugation (CE-111) to obtain high purity SA, while the bio-SA solution from H-109 is sent to a series of membranes (M-112; N-113; N-114) to remove impurities before recycling. The percentage of the stream to be recirculated is a trade-off between SA recovery and energy consumption.

### 2.1.2. Process II: BioAmber

The BioAmber process was mainly based on patents released by the company itself and starts with feed pretreatment (R-201; R-202; D-203) as described in process I (Fig. 3). The fermentable liquor free from microbial cells is then fermented (F-204) for 72 h at 30 °C and pH 3 to achieve a bio-SA yield of 0.45 g  $g^{-1}$  sugars and a titre of 48.2 g  $L^{-1}$ . The “aerobic fermentation” uses an engineered strain of *Issaichenkia orientalis* and  $NH_4OH$  as microbial host and buffering agent, respectively (Table 1). After the “aerobic fermentation”, the broth is clarified and concentrated by microfiltration (M-205) followed by ultrafiltration (U-206) to remove the microbial cells and the proteins. The clarified broth is then sent to a reac-

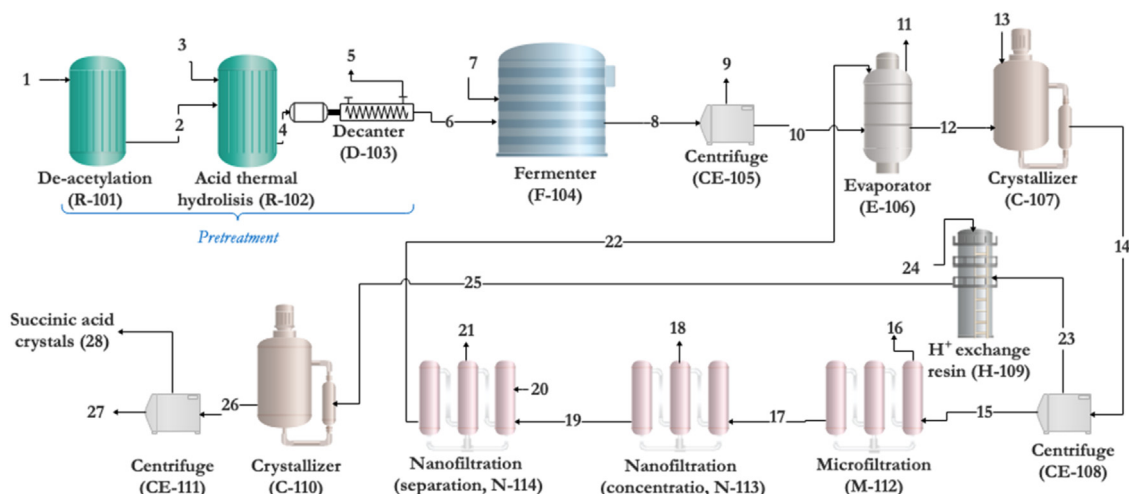


Fig. 2 – Process I diagram with biomass pretreatment and the Reverdia process for 30 kt y<sup>-1</sup> bio-SA production. The mass balance and energy consumption of each unit operation is represented by the numbers on the streams and the values can be found in Table S10 (Van de Graaf et al., 2015; Boit et al., 2019; Ahn et al., 2016).

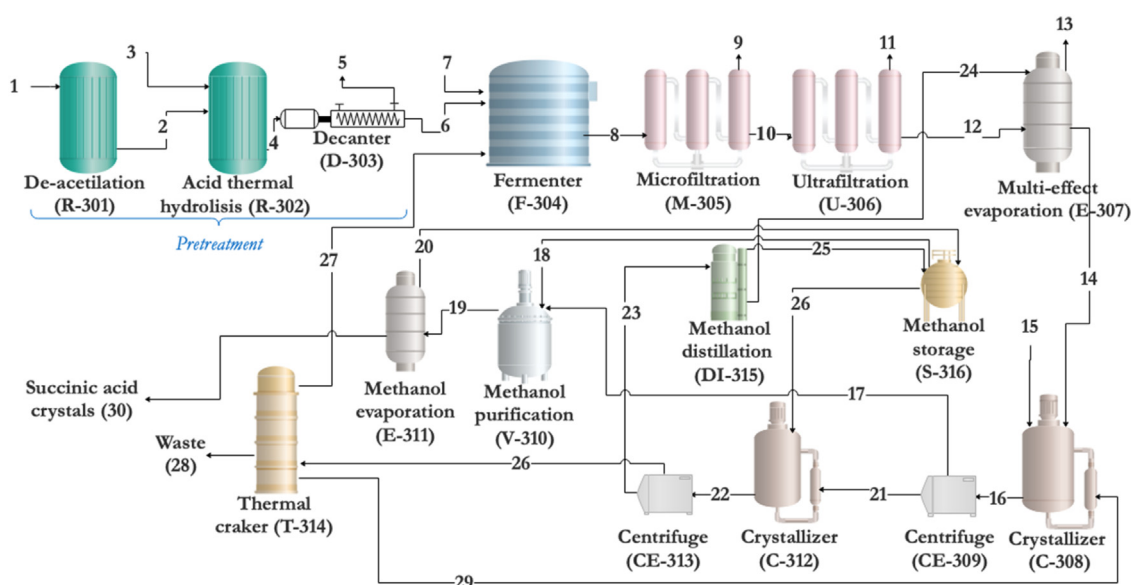


Fig. 3 – Process II diagram of biomass pretreatment and the BioAmber process for producing 30 kt y<sup>-1</sup> of bio-SA. The mass balance and energy consumption of each unit operation is represented by the numbers on the streams and the values can be found in Table S10 (Fruchey et al., 2012; Rush and Fosmer, 2014; Ahn et al., 2016; Nghiem et al., 2017; Finley et al., 2013).

Table 1 – Fermentative conditions, yield, SA concentration and productivity values used to simulate each process.

Process	Bioprocess: operative conditions	Yield (wt%)	Concentration (g L <sup>-1</sup> )	Productivity (g L <sup>-1</sup> h <sup>-1</sup> )	Reference
Process I (Reverdia)	<i>S. cerevisiae</i> ; batch; O <sub>2</sub> ; CO <sub>2</sub> ; 95 h; 30 °C; pH 3.1	0.69	43	0.45	(Van de Graaf et al., 2015)
Process II (BioAmber)	<i>I. orientalis</i> ; batch; O <sub>2</sub> ; CO <sub>2</sub> ; 72 h; 30 °C; pH 3.0	0.45	48.2	0.97	(Fruchey et al., 2011; Rush and Fosmer, 2014; Ahn et al., 2016)
Process III	<i>A. succinogenes</i> ; batch; CO <sub>2</sub> ; 72 h; 37 °C; pH 6.8	0.74	42.8	0.43	(Salvachúa et al., 2016a; Yedur et al., 2001)
Process IV	<i>A. succinogenes</i> ; cont.; CO <sub>2</sub> ; 0.04 h <sup>-1</sup> ; 37 °C; pH 6.8	0.78	39.6 (45.7) <sup>a</sup>	1.77 (2.16) <sup>a</sup>	(Bradfield et al., 2015; Pateraki et al., 2019)

<sup>a</sup> Continuous fermentation with a 15.49% improvement over the base value as a result of the anion exchange membrane (AEM) electrolytic cell effect on fermentation (Pateraki et al., 2019).

tive evaporator (E-207) where triglyme at 8 wt% concentration is used to strip ammonia at 110 °C. The bottom product of the reactive evaporator is then concentrated by evaporation (E-208) and crystallized in an evaporative crystallizer (C-209).

The purified crystals are then separated from the suspended solution by centrifugation (CE-210) to obtain high-purity bio-SA crystals. The solution from CE-210 contains a significant amount of unrecovered bio-SA (about 20% of the total bio-SA

from the fermenter), which can dramatically impact the process economics. The solution from CE-210 is therefore further processed using two crystallizers followed by crystal recycling as shown in Fig. 3. As a result, centrifugation of the broth from unit CE-212 generates impure crystals, which are re-suspended (V-213) and re-crystallized (C-209). The liquid fraction from unit CE-212 is instead crystallized in unit C-214 and the resulting impure crystals are separated (unit CE-215 Fig. 3), dissolved and re-crystallized in unit C-211, while the liquid fraction from CE-215 is disposed of.

### 2.1.3. Process III: Yedur et al

This process was designed based on the fermentation developed by Salvachúa et al. (2016b) and the purification process developed by Yedur et al. (2001). As shown in Fig. 4, the process starts with the pretreatment of corn stover, which follows the same strategy as described in process I. The pretreated slurry is then separated from the unreacted biomass and sent to the fermenter (F-303) for 72 h at 37 °C using *A. succinogenes* and NH<sub>4</sub>OH as a buffer to maintain the pH of the broth at 6.8. The final bio-SA concentration and yield are 42.8 g L<sup>-1</sup> and 0.74 g g<sup>-1</sup>, respectively (Table 1). The fermentation broth is then clarified, removing microbial cells and proteins via microfiltration (M-304) and ultrafiltration (U-305), and subsequently concentrated using a multi-effect evaporator (E-306). The bio-SA in the concentrated slurry is then crystallized (C-307) in the presence of ammonia sulphate at a pH below 1.8. The impure bio-SA crystals are subsequently separated by centrifugation (CE-308) and purified with methanol (V-309), which is then evaporated (unit E-310), condensed, and stored in the storage tank (S-315). The sulphates in the liquid stream from CE-308 are crystallized in unit C-311 and sent to a centrifuge (CE-312). The methanol-rich solution from the centrifuge CE-312 is sent to a distillation column (DI-314) to recover methanol in the distillate, which is stored in S-315. The recovered crystals from centrifugation (CE-312), containing mostly ammonium sulphate and some ammonium bisulphate are sent to a thermal cracker (T-313) for recovery of ammonia and ammonia bisulphate, which are recycled to the fermenter (F-303) and the crystallizer (C-307), respectively.

### 2.1.4. Process IV: continuous process

On the basis of an extensive literature review and some model-based analyses, a conceptual process was designed (Mancini et al., 2019; Garg et al., 2019). Corn stover is first pretreated as described in process I, then the fermentable liquor of sugars is fed to *A. succinogenes* in the fermenter. Compared to the other considered processes in this study, the fermentation and extraction in process IV are continuous. Immobilization of the microbial cells allows the concentration of slow growing bacteria, such as *A. succinogenes*, to increase, and biofilm then forms naturally. Even though, continuous operation is more complex to design and operate compared to batch and fed-batch cultivation, and despite the higher sensitivity to microbial contamination and risks of genetic drifting (Lindskog, 2018), these challenges are not insurmountable (Ozturk, 2015) and large-scale continuous operation is likely to become more common in the future (Lindskog, 2018). This is because a continuous production system with immobilized microbial cells clearly offers several advantages compared to suspended batch cultivation. For example, there is an increase in bio-SA productivity and yield (Ferone et al., 2018), particularly for *A. succinogenes*, while simultaneously reducing the fermenter's size (Ferone et al., 2019). A further advan-

tage of the microbial immobilization is stability and protection against environmental stresses (pH, temperature, inhibiting substrates and products etc.) (Bradfield et al., 2015). This is particularly important when fermenting thermochemically pretreated lignocellulosic biomasses (as simulated in this work) which typically contain inhibitory substances (Mancini et al., 2019). *A. succinogenes*, is known to suffer from lack of reducing power in its metabolic pathway. However, when *A. succinogenes* is immobilized, it has been found to be able to activate additional redox power compared to the suspended culture. This increment in the reducing power enhances the metabolism and the bio-SA yield (Bradfield and Nicol, 2016).

The separation of bio-SA starts with the direct and continuous product removal system, which is composed of an electrochemical cell equipped with an anion exchange membrane (AEM). The AEM allows the passage of many carboxylic acids, including succinate, as well as inorganic anions but not microbial cells, proteins, non-fermented substrates, and some dyes. Therefore, in terms of the downstream process, the electrochemical separation system performs an important degree of separation of bio-SA from the other components in the fermentation broth, simultaneously protonating succinate back to succinic acid. This prevents the addition of high acid loads that bring the pH from neutral to below 3 (Supplementary material, Figure S1) (Pateraki et al., 2019). The application of the electrochemical system does not only separate bio-SA, but it also improves the fermentation performance and reduces the amount of buffer required. Specifically, (1) the continuous removal of fermentation products eliminates the product inhibition effect of the fermentation, (2) the electrolysis generates protons that can be used as an extra proton motive force (Pateraki et al., 2019) and (3) the generation of hydroxyl groups during the electrolysis reduces the amount of required buffering agent (Pateraki et al., 2019). A similar technology to the AEM electrolytic cell used in process IV is electrodialysis, which has already been explored for manufacturing bio-SA (Van de Graaf et al., 2015). However, electrodialysis has higher operating and capital costs than the AEM electrolytic cell. Specifically, the AEM electrolytic cell does not require a proton exchange membrane (PEM) and thus does not require costly precious metals as catalysts in proton PEM electrolysis (Lichner, 2020).

After the direct bio-SA electrochemical extraction, the fermentation broth is concentrated via evaporation to facilitate crystallization (C-407, Fig. 5). The impure crystals are then dissolved and re-crystallized, while the bio-SA still in solution from the first crystallization step (C-407) is recirculated for further extraction of the bio-SA. The solution with non-crystallized bio-SA from unit C-410 is also recirculated in the evaporator for further extraction to reach a final purity above 99%.

## 2.2. Techno-economic analysis

All the equations to calculate the discount cash flow rate, to estimate the minimum bio-SA selling price, the total capital investment, and the total manufacturing cost of all simulated processes are made according to Peters and Timmerhaus (1994). All the assumptions made in the techno-economic model are shown in the Supplementary Material (Table S1).

The total capital investment comprises total direct costs, total indirect costs, working capital costs, and land costs. The total direct costs are further subdivided and consist of installed equipment costs, site development, and addi-

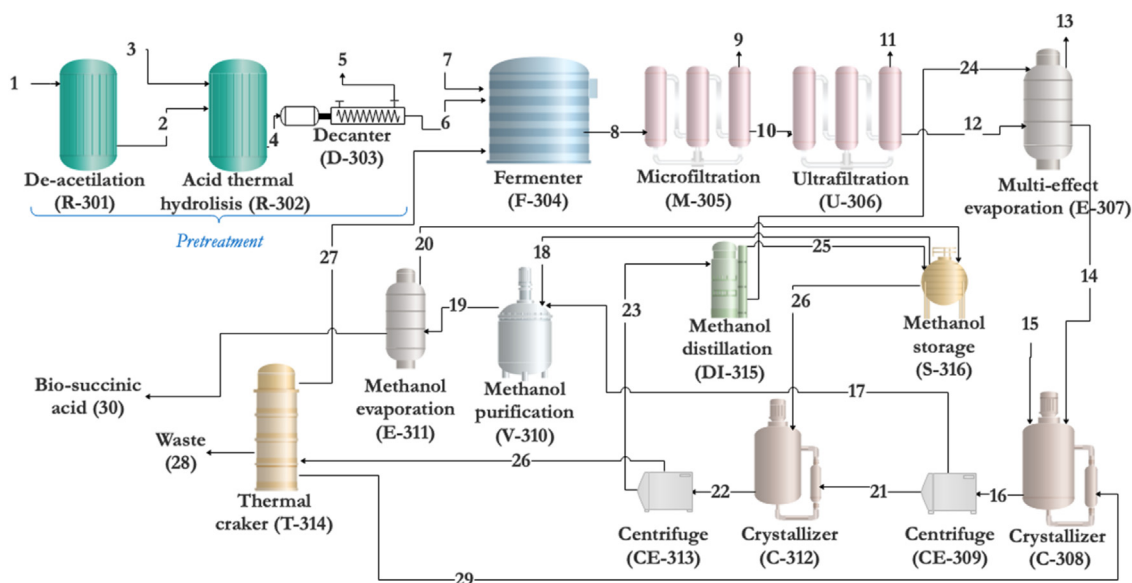


Fig. 4 – Process III diagram with mass and energy balances for 30 kt y<sup>-1</sup> bio-SA production. The diagram includes (I) biomass pretreatment: de-acetylation, acid thermal hydrolysis and fermentable liquor separation; (II) fermentation of Salvachúa et al. (2016a); (III) the downstream process patented by Yedur et al. (2001). The mass balance and energy consumption of each unit operation is represented by the numbers on the streams and the values can be found in Table S10.

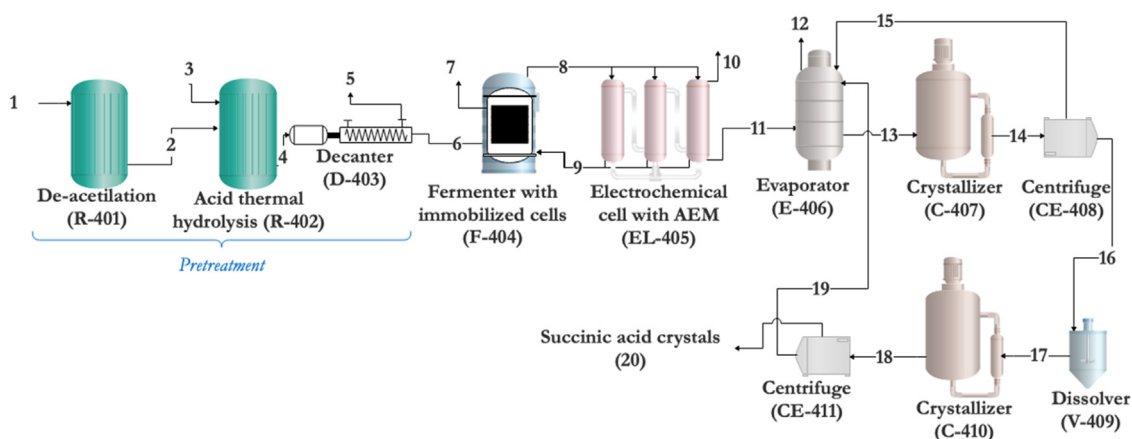


Fig. 5 – Process IV diagram of the conceptual plant for producing 30 kt y<sup>-1</sup> of bio-SA. The mass balance and energy consumption of each unit operation is represented by the numbers on the streams and the values can be found in Table S10.

tional piping and instrumentation. Likewise, total indirect costs include portable expenses, field expenses, home office and construction fees, project contingency, and other costs (start-up, permits, etc.). The equipment costs were estimated using the economic data (Supplementary Material, Table S2) reported in the literature.

The equipment costs were rescaled and updated to the year of analysis using a power law (Eq. 1) and a chemical engineering plant cost index (Eq. 2) to determine the installed cost of specific equipment, which were then summed to yield the total installed cost of equipment. Total direct and indirect costs were determined based on the factor approach. The complete methodology to determine total capital investment is reported in the Supplementary Material (Table S3).

$$C_n = I_n C_{n,o} \left( \frac{Q_n}{Q_{n,o}} \right)^{a_n}, \forall n \in N, \quad (1)$$

In Eq. 1,  $C_n$  is installation cost of equipment  $n$ ,  $C_{n,o}$  is the cost of the baseline equipment  $n$ ,  $Q_n$  and  $Q_{n,o}$  are rescaled and base-

line capacities of the equipment  $n$ , and  $a_n$  is a scaling exponent which varies depending on the equipment  $n$ .

$$UC_n = C_n \left( \frac{CEPCI_{2019}}{CEPCI_{ref}} \right), \forall n \in N, \quad (2)$$

In Eq. 2,  $UC_n$  is the updated equipment cost in the year of interest, and  $CEPCI_{2019}$  and  $CEPCI_{ref}$  are the chemical engineering plant cost index in the year 2020 and the baseline, respectively.

The total production costs comprise manufacturing costs and general expenses. These costs are further sub-divided; for example, manufacturing costs consist of direct production costs, fixed charges, and plant overhead costs. Direct production costs consist of expenditures associated with the production process and include the following: operating labour and supervision, utilities, maintenance and repairs, operating supplies and laboratory charges. Fixed charges are expenditure that remains constant throughout the year regardless of production rate, and involves expenditures such as depreciation, property taxes, insurance, and rent. Plant

overhead costs are like fixed charges in that they are not dependent on the production rate. General plant maintenance and overhead, safety, payroll overhead, etc. can be classified as plant overhead. By contrast, general costs include administrative expenses, distribution and marketing expenses, research and development expenses, financing expenses, and gross-earning expenses. To determine all aforementioned costs, we used the methodology proposed by Peters and Timmerhaus (1994).

The net present value (NPV) was calculated as in Eq. 3 according to Udugama et al. (2018):

$$NPV = \sum_{t=1}^n \frac{Net\ Profit_{p,a}}{(1 + Discount\ rate)^t} - Total\ Capital\ Costs \quad (3)$$

where,  $Net\ Profit_{p,a}$  stands for the revenues from succinic acid sales (per annum) minus the cost of operations;  $n$  stands for the payback period, which is set to 15 years; and the discount rate is calculated according to Fernando and Khartit (2020).

Further details of the methodology for the techno-economic assessment of the aforementioned processes can be found in the Supplementary Material.

### 2.3. Life cycle assessment

The goal of the LCA was to assess and compare the environmental impacts of different production routes to produce succinic acid at industrial scale. The two best economically viable processes for bio-SA production identified in the techno-economic assessment, namely process I and IV, were selected and compared with conventional petroleum-based SA obtained from catalytic hydrogenation of maleic anhydride (Pinazo et al., 2015). For a fair comparison of the three analysed production routes, the functional unit was defined as the 'production of 1 kg of high purity succinic acid ( $\geq 99$  wt%) at the factory gate, in Europe'. It was assumed that, in the mid-term, bio-SA will not replace entirely conventional production from fossil sources but will represent only a share of the total world production. This was considered realistic given the relative immaturity (process IV, being just a conceptual process) and lack of competitiveness (process I) of the analysed bio-SA production routes. Hence, according to the EU Commission's ILCD guidelines (EC-JRC, 2010), the study is defined a microlevel decision context (type A), where production of bio-SA is not expected to cause structural large-scale changes in other product systems (such as installation of new power plants). Following ILCD recommendations, system expansion was systematically applied to handle multifunctional processes using the consequential version of the ecoinvent database. This database, however, uses marginal rather than average data as would be recommended for microlevel decision context. The implications of this modelling choice were analysed in a sensitivity analysis presented in the Supplementary Material (Section S2.3).

The system boundaries consider all processes from *cradle to gate*, i.e. including extraction of raw materials, transportation (including feedstock collection) generation of energy and chemical supplies, production of SA and disposal of waste streams. For the bio-SA processes, the avoided conventional use of corn stover feedstock was also included, consistent with the system expansion approach. Construction and disposal of infrastructure and equipment in the foreground system were

instead excluded from the assessment, due to lack of specific data enabling the comparison between processes.

Data for background processes (such as electricity generation or wastewater treatment) was based on the ecoinvent database, version 3.3 (Wernet et al., 2016), whereas specific data for the foreground processes (e.g. unit operations) are based on the mass and energy balances calculated for the techno-economic assessment. The software SimaPro version 9.1.0.11 (PRé Consultants bv, the Netherlands) was used to model the product systems. Conventional SA production from maleic anhydride was modelled using the inventory in Pinazo et al. (2015). Details about input parameters and type of process used for the three compared scenarios are presented in the Supplementary Material (Section S2.1). Environmental impacts were calculated using ReCiPe 2016 (Huijbregts et al., 2017) (using midpoint indicators and hierarchist perspective), which includes methods for assessing 17 impact categories.

## 3. Results and discussion

The four described processes are rather different from each other, not only in regard to pretreatment but also in the type of fermentation and in the downstream operations. The type of fermentation and microbial host directly affects the downstream process and, indirectly, the pretreatment. For example, the lower bio-SA yield from *I. orientalis* in process II compared with the hosts in the other processes demands a higher amount of biomass, which means more pretreatment. On the other hand, *I. orientalis* in process II can tolerate the highest bio-SA concentration among the four investigated processes (Table 1), which means lower total working volume of the fermenter. This size difference emerges when comparing the three processes based on batch fermentation (process I, II and III), where process II has the smallest fermenter total working volume (11633, 7189, 8421 m<sup>3</sup> for process I, II, III respectively). The fermentation in process I and II is aerobic and with engineered microorganisms, while in process III and IV it is anaerobic and employs a naturally occurring bacterium. Anaerobic fermentation in general has lower capital and operating costs than aerobic fermentation (Crater et al., 2017), but the type of host microorganism dictates the performance of the fermentation and may cause burden-shifting in bio-SA production. For example, even though natural microorganisms, such as *A. succinogenes*, can yield more bio-SA than some engineered hosts, natural microbial hosts typically produce more by-products (Jiang et al., 2017). The presence of more by-products in the fermentation broth shifts the burden of bio-SA production from the fermentation to the downstream operations, which therefore need to be more sophisticated to separate bio-SA.

### 3.1. Economic implications

From the techno-economic analysis, process I (Reverdia) and IV (conceptual) emerge as less costly than processes II (BioAmber) and III, both in terms of operational and capital costs (Tables 2 and 3 respectively). Specifically, process I and IV have operational costs between 25 to less than 35 MM\$ p.a.<sup>-1</sup>, while process II and III are above 35 MM\$ p.a.<sup>-1</sup> (Table 2). The higher complexity of processes II and III was estimated to increase the bio-SA recovery efficiency compared to process I (82%, 78% and 71% for process II, III and I, respectively) but at a higher operational cost in basically every aspect – raw material, cost of labour, maintenance etc. (Table 2).

**Table 2 – Operational costs distribution in millions of USD per annum (MM\$ p.a.<sup>-1</sup>) for the four processes calculated based on Peters and Timmerhaus (1994).**

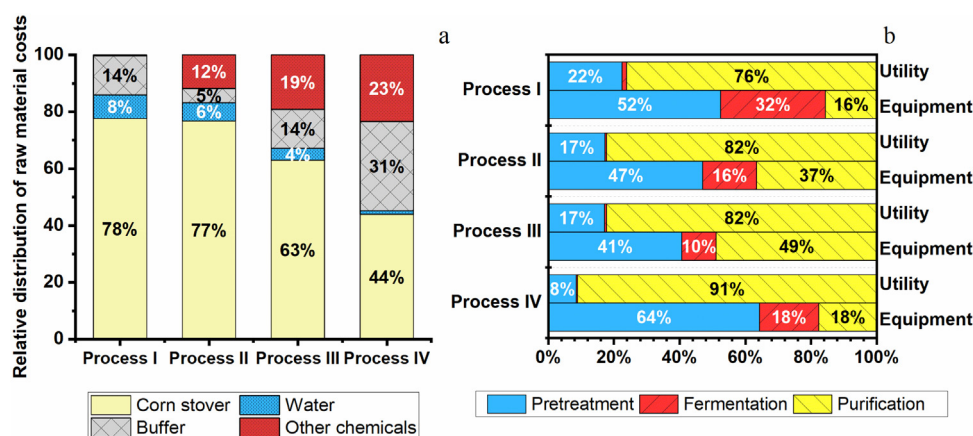
	Process I	Process II	Process III	Process IV
Raw material (MM\$ p.a. <sup>-1</sup> )	7.80	11.10	8.18	9.84
Utilities (MM\$ p.a. <sup>-1</sup> ) <sup>a</sup>	2.72	8.26	2.96	5.09
Cost of labour <sup>b</sup> (MM\$ p.a. <sup>-1</sup> )	1.69	2.31	1.98	0.77
Maintenance and repairs (MM\$ p.a. <sup>-1</sup> )	5.29	7.25	6.21	2.42
Direct production costs (MM\$ p.a. <sup>-1</sup> ) <sup>c</sup>	10.71	30.31	20.51	18.58
Fixed charges <sup>d</sup> (MM\$ p.a. <sup>-1</sup> )	5.29	7.25	6.21	2.42
General expenses <sup>e</sup> (MM\$ p.a. <sup>-1</sup> )	5.42	8.04	6.21	3.73
<b>Total cost of production (MM\$ p.a.<sup>-1</sup>)</b>	<b>33.40</b>	<b>51.34</b>	<b>37.83</b>	<b>26.63</b>

<sup>a</sup> Includes operational cost of utilities and waste disposal.  
<sup>b</sup> Includes operating labour and operating supervision.  
<sup>c</sup> Includes operating costs of labour and supervision, utilities, maintenance and repairs, operating supplies and laboratory charges.  
<sup>d</sup> Includes property taxes, financing and insurance.  
<sup>e</sup> Includes: (I) cost of administration, (II) sales & distribution and (III) research & development.

**Table 3 – Comparison of the capital costs of the four processes in millions of USD.**

	Process I	Process II	Process III	Process IV
Pretreatment	44.51	54.53	40.40	22.60
Fermentation	27.07	19.33	10.48	6.31
Purification	13.31	42.56	48.72	6.25
Total installed costs <sup>a</sup>	93.38	128.06	109.56	42.67
Total indirect costs	66.11	90.67	77.57	30.21
Total direct costs	110.19	151.11	129.28	50.35
Fixed capital investment	176.30	241.78	206.84	80.56
Working capital	8.81	12.09	10.34	4.03
Total capital investment	190.71	261.55	223.76	87.15

<sup>a</sup> Includes the costs for pumps and heat exchanger.

**Fig. 6 – Relative cost distribution of raw materials, i.e. corn stover, water, buffering agent and other chemicals (a) and total utility and equipment costs distribution between pretreatment, fermentation and downstream activities (b).**

In other words, a higher bio-SA recovery efficiency process could cost more than the income from selling the residual bio-SA obtained. As widely reported (Mancini et al., 2019; Dickson et al., 2021; Rosengart et al., 2017), the downstream of bio-SA production represents the vast majority of the operating costs: 76, 82, 82 and 91% for process I, II, III and IV respectively (Fig. 6b). Analysis of the relative utility cost distribution shows that pretreatment accounts for 17–20% of the costs in process I, II and III and 8% of costs in process IV. The major relative utility cost for process IV is the electrochemical system (85%). For process II and III the major relative costs are for evaporation and distillation units (54 and 37% respectively).

Although bio-SA recovery from process I is the lowest (75%) compared to the other processes, process I has the lowest direct production cost (Table 2). This is mainly due to use of

an ion-exchange column in process I, which results in 72.3%, 7.07%, and 46.5% less utility use than consumed in process II, III, and IV, respectively. When the four processes are examined for fixed capital investment, process IV requires the lowest capital investments compared to all the other processes. Lower fixed capital investment also means lower labour costs, lower fixed charges and lower general expenses. Overall, process IV shows the lowest operating costs at half of those estimated for process II and ~20% lower than those for process I (Table 2). Raw material and utilities costs constitute a major operating cost burden for process IV. Regarding raw material, the microbial host requires significant amounts of a nitrogen source to grow and a significant amount of NaOH to buffer the fermentation at the optimal pH of 6.8. Therefore, nitrogen source and buffering agent represent 23 and 31% of the



total raw material costs, respectively (Fig. 6a). The high utility costs for process IV come from the electrochemical system, which demands about 60 GW h  $y^{-1}$  (Fig. 5). The higher relative impact of other chemicals and buffer in process IV is also due to the lower amount of corn stover required. *A. succinogenes* offers a higher bio-SA yield compared to *S. cerevisiae* and *I. orientalis* (Table 1); this factor, together with the even better performance offered by *A. succinogenes* in continuous immobilized fermentation, and the direct bio-SA extraction, further improves the bio-SA yield.

For process II, the high utility costs for the reactive evaporator unit (E-207 – Fig. 3) derive from the required energy for distillation but, most importantly, from the non-hazardous wastewater (with ammonia) disposal (3% of the total operating costs). The management of ammonia in process II is important, and in this work 90% of ammonia wastewater is assumed to be recycled, even though there is no information in the patents about ammonia recycling. A full disposal of wastewater from unit E-207 is expected to increase the minimum selling price of bio-SA to about 3.7 USD  $kg^{-1}$ , i.e. 15% more than simulated in this work. A further significant cost in process II is the use of tryglyme to facilitate the stripping of ammonia from the fermentation broth through the reactive evaporator (E-207); the cost of tryglyme for process II is 12% of the total raw material costs (Fig. 6a).

In process III, the high operating costs come mostly from methanol evaporation (E-310) which accounts for 21% of the total operating costs. However, process III also uses ammonia like process II, but 95% of the ammonia is recycled. Process III is the most complex process investigated in this study, which inevitably increases uncertainty about the most optimal simulation regarding operating conditions and recycling streams. With this respect, a limitation of this study is that mass and heat integration has not been performed, which may affect the result of this very integrated process. For process III, a neutral fermentation process was simulated upstream of process III; the costs of buffering the fermentation and the acidification to convert diammonium succinate into succinic acid during the crystallization step (C-307) represent 10 and 21% of the total raw material costs, respectively. However, according to Cok et al. (2013) low-pH fermentation is economically advantageous end environmentally more sustainable than neutral pH fermentation.

The cost distribution analysis for pretreatment, fermentation and purification of bio-SA production shows that purification dominates the utility costs, while pretreatment is the most relevant stage for equipment costs, with process III as an exception (Fig. 6b). The high number of unit operations together with intense downstream operational conditions of process III impact both the utility and equipment costs.

Looking at the capital investments, the plants with a lower number of unit operations tend to be cheaper; capital costs for process IV  $< I < III$  and II (Table 3). Even though process III has more unit operations than process II, the former is 14% cheaper and the reason is directly and indirectly connected with the fermentation. Aerobic fermenters (process II) are more expensive than anaerobic fermenters (process III) (Crater et al., 2017) and the lower bio-SA yield offered by *I. orientalis* in process II versus *A. succinogenes* in process III requires provision of a larger amount of corn stover for pretreatment (Table 2). The lower downstream costs of process II versus process III (13% less), are not sufficient to lower the total installed costs, and therefore the other costs for pro-

cess II are also higher than for process III: total direct and indirect costs, fixed capital investments and working capital (Table 3).

Process I has the second lowest capital costs among the four processes,  $\sim 30$  and 15% lower than process II and III, respectively. Even though the fermentation of process I is the most expensive, the downstream capital investment is only  $\sim 30\%$  of that needed for processes II and III, respectively. Process IV is the most economical to build (Table 3) and the bio-SA productivity of the continuous fermentation in process IV is higher than that from the batch fermentation setup that is incorporated in the other processes. This higher productivity together with the high bio-SA yield of process IV significantly lowers the size and costs of pretreatment and fermentation equipment. The equipment costs for the pretreatment in process IV are about half those for the other processes, while for the fermentation the difference in capital costs ranges between 40 to more than 75% compared to process III and I, respectively (Table 3). The electrochemical system in process IV is considered downstream, but in reality, the direct bio-SA removal improves the performance of the fermentation as well. Therefore, the AEM electrolytic cell and the fermenter are not isolated from each other, as it is typically the case. Consequently, the distribution of the costs between the fermentation and the downstream of bio-SA should be seen as an approximation for process IV.

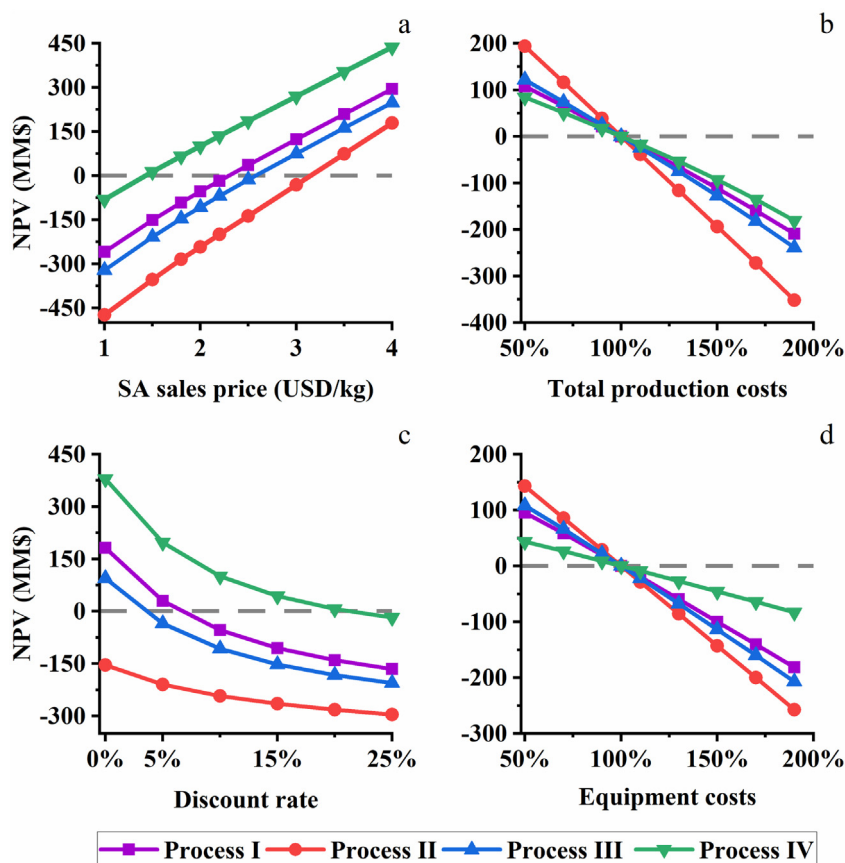
Looking at the resulting minimum selling prices of the four processes, the values are 2.3, 3.2, 2.6 and 1.4 USD  $kg^{-1}$  for process I, II, III and IV respectively. This means that the minimum selling price of the novel process IV is 38% lower than process I, and even up to 55% lower than the most expensive process II. Process IV is also the only process with a predicted bio-SA cost of production below 2 USD  $kg^{-1}$ . This is of importance because the current sales price of SA produced from petroleum refinery operations is thought to have a sales price of around 2 USD  $kg^{-1}$  (ZAUBA, 2016; Alibaba, 2021).

### 3.2. Sensitivity analysis

Univariate sensitivity analysis was conducted to analyse the impact of key economic parameters on the overall economics to assess the feasibility of the considered processes (Fig. 7).

The sales price of bio-SA was the first key economic variable analysed. As illustrated in Fig. 7a, bio-SA sales price that ranged from USD 1.5–4.0 per kg was considered. Fig. 7a shows that process IV is the only process with a positive net present value (NPV) at USD 2.0 per kg for a 15 years project life, with a 7.5 years payback period at a 10% discount rate (Dickson et al., 2018) and 30% taxation rate. Process I and III show a positive NPV between 2 and 2.5 USD  $kg^{-1}$ , while process II has a positive NPV only at a sales price that is above USD 3.0 per kg of bio-SA sold. As expected, a clear, linear correlation is seen between NPV and sales price. It can also be concluded that at a price of  $\sim 2$  USD per kg, only process IV provides a positive return on investments, indicating its economic competitiveness compared to the other processes.

The operating cost is the next key economic parameter analysed in Fig. 7b. This parameter changes the overall cost of operations, which accounts for market variations such as cost of utilities, raw materials, labour etc., but also technical variations such as the overall efficiency of the production process. If the operating cost is reduced to 75% of the calculated value, all the processes have a positive NPV.



**Fig. 7 – Sensitivity analysis of net present value (NPV) variation with sales price, cost of operations, discount rate and capital costs.**

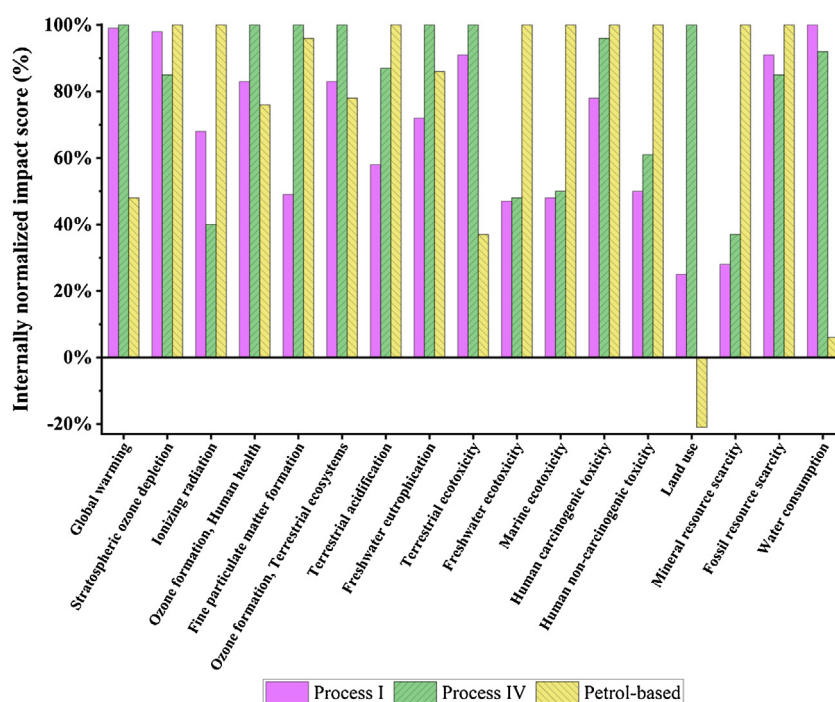
Two important financial parameters that can influence the NPV and an investment decision are the discount rate and the equipment costs (Fig. 7c and d, respectively). The discount rate in the context of this manuscript represents the return on capital investment an investor would like to have. A positive NPV, at a given discount rate, means that the project has the ability to generate this return and still have cash reserves. From NPV (Eq. 3) and from the Fig. 7c it can be inferred that with an increase in the discount rate, the present value of net profits will be reduced. In general, due to the risk appetite of investors, a higher discount rate is warranted for more technologically risky projects (Silk et al., 2020). From a practical point of view and for these projects, a discount rate of 0%, i.e. accepting no return on the investment, will only happen if a Government or a Charitable Foundation funds the projects. According to Skok and Reiss (2015), the discount rate of 15% for established processes such as process I and II is considered safer than the 10% applied in this study, while for process III and IV a discount rate of 20% is recommended because of the higher risk of investing in a non-established process. From Fig. 7c, it can be seen that process I and III would have a positive NPV at a discount rate below 5%, while process II is never profitable. Therefore, it is not convenient to invest in either process I, II and III. For process IV, on the contrary, investment would still be profitable at a discount rate of 20% (NPV = 1.4 MM USD for bio-SA 2.0 USD kg<sup>-1</sup> – Fig. 7c), but profitability would change to zero for a discount rate of ~21%.

The sensitivity of the variation of the total capital cost factor on the NPV was investigated from 50% to 200% of the total costs, which means that at 100% of the equipment costs, the values in MM USD are those reported in Table 3 and so on for

the other percentages. Analysis of the sensitivity of variation of capital costs on the NPV gives the following order: process II » III > I » IV. Thus, the influence of the capital costs on NPV variation is highest for process II and lowest for process IV (Fig. 7d), and a 10% reduction in the capital costs would generate a positive NPV of about 28.6 MM USD for process II and nearly half of that for process IV (15.3 MM USD).

Different microbial hosts can produce and tolerate significantly different bio-SA yields and titre (Mancini et al., 2019; Jiang et al., 2017; Kuenz et al., 2020). Analysing the variation of minimum selling price along with bio-SA yields and titres in the fermenter, we can see that both process I and III can become profitable (Figure S2 in Supplementary Material). Specifically, for process I, bio-SA yields above 100 wt% (CO<sub>2</sub> is fed alongside) as reported from some studies (Sánchez et al., 2005; Li et al., 2010; Yu et al., 2016) or titre above 90 g L<sup>-1</sup> (Mancini et al., 2019; Kuenz et al., 2020), can lower the minimum selling price below 2.0 USD kg<sup>-1</sup>. Process III efficiency is not significantly affected by the bio-SA, but with acceptably high titre (80 g L<sup>-1</sup>) the process is expected to become competitive (Figure S2).

In process IV, important sources of uncertainty are the capital and operating costs of the AEM electrolytic cell, for which no information was found. The capital costs for the AEM electrolytic cell adopted in this work are those from electrodialysis. However, the AEM electrolytic cell is cheaper in both capital and life cycle costs. Another uncertainty concerns the power required from this unit, which was estimated starting from a laboratory cell<sup>43</sup>; however, the efficiency and the required power of a real commercial AEM electrolytic cell would probably differ significantly. The estimated power required based on



**Fig. 8 – Comparison between process I, IV and petrol-based SA, where impact scores are normalized with respect to the worst performing process per each impact category. Characterized impact scores can be found in Table S8 in the Supplementary Material.**

the laboratory AEM electrolytic cell and reported by Pateraki et al. (2019) is  $86.5 \text{ kW h m}^{-3}$ , while for electro dialysis applied for desalination values between  $0.8\text{--}11 \text{ kW h m}^{-3}$  are reported by Thomas (1997). Other investigated scenarios on the capital and life cycle costs over 15 years of this unit showed a minimum selling price between  $1.4\text{--}1.5 \text{ USD kg}^{-1}$  at a 10% discount rate.  $1.4 \text{ USD kg}^{-1}$  is the minimum selling price obtained when the capital cost of the electrolytic cell is that resulting from a linear scaling up of laboratory equipment ( $\sim 800,000 \text{ USD}$  total capital costs), while a minimum selling price of  $1.5 \text{ USD kg}^{-1}$  results from the capital costs of electro dialysis of  $\sim 5,350,000 \text{ USD}$  ( $0.5 \text{ \$ m}^3 \text{ y}^{-1}$ ,  $280 \text{ \$ m}^{-3} \text{ d}^{-1}$ ). An extreme scenario was also explored, considering 20% discount rate, capital costs of  $280 \text{ \$ m}^{-3} \text{ d}^{-1}$  and  $1 \text{ \$ m}^{-3} \text{ y}^{-1}$  of lifecycle costs (15-year project life), resulting in total electrolytic cell cost of  $\sim 11,640,000 \text{ USD}$  and a final minimum selling price of  $2.20 \text{ USD kg}^{-1}$  of bio-SA. However, this is very unlikely, considering the lower costs of the AEM electrolytic cell compared to electro dialysis (Lichner, 2020).

Overall, the following recommendations can be made based on the observed trends:

a) Technologies like ion-exchange columns and membrane systems, such as nanofiltration and anion exchange, appear to be key technologies to lower bio-SA manufacturing costs. However, a technological advance is needed to overcome the strong dependence of bio-SA manufacturing success on fluctuations of the petroleum prices and thus on petroleum-based SA. This study shows the potential of continuous bio-SA production and of *in situ*-like product extraction to lower manufacturing costs. Thus, the key technology for obtaining a successful bio-SA manufacturing cost could be the establishment of a hybrid between the fermentation stage and the downstream stage, where the downstream component also improves the fermentation performance.

- b) Process IV is the only process with a positive NPV at a bio-SA sales price of USD 2 per kg under the expected cost of operations, at a discount rate up to 20%, and a payback period of 15 years and 30% taxation rate. The NPV of process IV becomes negative for a bio-SA sales price below  $1.92 \text{ USD kg}^{-1}$ .
- c) Process IV is only conceptual even though the advantages of continuous fermentation of immobilized culture emerge from the literature (Ferone et al., 2019) and the potential of the direct (or even better *in situ*) extraction is increasingly supported by experimental proof (Pateraki et al., 2019). However, to our knowledge, continuous fermentation of immobilized microorganisms does not have commercial application (Kumar et al., 2020). The AEM electrolytic cell is a technology that is closely related to electro dialysis which has TRL 9, and therefore a rather high TRL can be assumed for the AEM electrolytic cell. On the contrary, the other processes are all based on fully developed technologies (TRL 9) and process I and II are real operating processes.
- d) It is estimated that, from a purely economic investment point of view, process I (Reverdia), II (BioAmber) and III (Yedur et al., 2001) are not convenient under current market conditions. Process IV would easily become non-profitable at a discount rate above 20%.
- e) With bio-SA sales price increases, a reduction in operations costs due to government subsidies or cheaper raw materials, process I would likely be able to demonstrate a positive NPV, while significant changes are needed before process II and III become profitable.

### 3.3. Environmental assessment

Results from the life cycle impact assessment show that none of the three analysed processes performs as best in all environmental impact categories, but the ranking varies depending on the type of impact considered (Fig. 8 and Table S8 in the

Supplementary Material). Process I seems to be the slightly preferable option as it shows the lowest impacts in 8 categories (particulate matter formation, terrestrial acidification, freshwater eutrophication, mineral resource scarcity, freshwater, marine and human toxicity) and the worst performance only in water consumption. Process IV ranks better than the petroleum-based process in 9 impact categories, however the two processes show both burdens and benefits in similar proportions, making it less straightforward which process is preferable. In addition, differences between the three processes are relatively small, with scores differing by less than a factor 2 in most of the cases. Therefore, no process can be considered as markedly more environmentally sustainable than the others. Nevertheless, if considering only the total numbers of impact categories in which a process scores better than another, process I is slightly preferable, followed by process IV and finally petroleum-based SA.

The process contribution analysis revealed that, for process I, the fermentation and, to a lower extent, the pretreatment steps are the main drivers of impacts depending on the impact category. On average, they account for 57% and 27% of the total impacts, respectively (see Supplementary Material, Section S2.4). This is mainly caused by power consumed during the production of the oxygen supply needed in the fermenter and by emissions from production of steam used in the pretreatment. Larger impacts on water consumption reflect the fact that the total water input (including cooling water) is 42% higher compared to process IV.

In process IV, the fermentation and the purification steps have the major contribution to total impacts, with fermentation ranging between 17 and 86% and purification between 8–77% of total impacts depending on the category (see Supplementary Material, Section S2.4). The somewhat worse performance of process IV compared to process I in several categories is mostly caused by the larger amount of NaOH required for pH control in the fermentation and from the high electricity consumption of the AEM electrolytic cell (purification). This result is in agreement with the economic analysis, where process IV showed higher costs in terms of raw materials and utilities compared to process I. As infrastructure and equipment materials were not modelled in the LCA, the environmental analysis is not able to capture any benefits that might be associated with the smaller plant size of process IV, which was the main reason of its overall better economic performance compared to process I (offsetting the larger raw materials and utility costs). Infrastructure and equipment materials are generally found to have only a marginal influence on the overall results (Smidt et al., 2016). However, given the considerable difference in plant size (e.g. the fermenter is around 6 times smaller for process IV) and the lower number of unit operations in the purification phase, substantial reductions of some impacts (such as mineral resource scarcity) cannot be excluded if this aspect is accounted for.

In terms of climate change impacts, it was found that the assessed bio-SA processes do not yet seem competitive with respect to the conventional petrol-based route. Similar results were found in other studies (Morales et al., 2016; Dickson et al., 2021) for bio-SA obtained from different feedstocks and technologies. For process IV, the burdens derived from the use of NaOH for pH control represent 22% of the total climate change impacts and, overall, constitute between 10–66% of all environmental impacts. Therefore, it is expected that reducing the need for buffering agent by conducting a low pH fermentation

has a great potential to substantially reduce several impacts, making the process more environmentally favourable. This corroborates earlier findings suggesting that fermentation at low pH is preferable to a neutral pH process when considering environmental sustainability (Cok et al., 2013; Dickson et al., 2021). On the contrary, changing to a different biomass type is not seen as the main priority to enhance the environmental performance of the process, given that biomass pretreatment is not found as a major contributor to total impacts (Figure S4).

## 4. Conclusions

The techno-economic analysis presented here highlights the strengths and weaknesses of two commercial processes and a patented technology, and potentially identifies the reasons for the commercial failure. Low pH fermentation allows to lower the downstream costs, but in process I and II, the low bio-SA yield from *S. cerevisiae* and *I. orientalis* demands a higher fermentation volume and a higher amount of corn stover to be processed, shifting the costs from downstream to upstream. A better performing microbial host in process I (100 g-bioSA g-sugar<sup>-1</sup>) would make the process commercially viable, while process II is never found competitive when varying technical parameters within potential ranges. Regeneration of pH buffering, as adopted in process III, can significantly lower the production costs, but higher titres (>80 g L<sup>-1</sup>) in fermentation are needed for the process to become profitable. Process I showed the second lowest minimum selling price of 2.3 USD kg<sup>-1</sup>. The key technologies of process I in the intensive separation step are ion-exchange columns and nanofiltration, which result in lower operating costs than in process II and III. The conceptual process IV combines high bio-SA volumetric productivity with a limited number of unit operations in the downstream. The energy demand is high and this impacts the environmental sustainability, but process IV can potentially produce bio-SA at minimum selling price of 1.4 USD kg<sup>-1</sup>, process IV would be cheaper than petroleum based succinic acid (~2.0 USD kg<sup>-1</sup>). The Reverdia-based process I and process III could also be competitive, while BioAmber-based process II is not profitable. Nonetheless, continuous fermentation with immobilized culture, combined with an *in situ* anion exchange membrane electrolytic extraction, as investigated here, could completely change the bio-SA acid market. However, the estimated high electricity and NaOH consumptions limit its environmental sustainability.

## Declaration of Competing Interest

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

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.cherd.2022.01.040>.

## References

- Ahn, J.H., Jang, Y.S., Lee, S.Y., 2016. Production of succinic acid by metabolically engineered microorganisms. *Curr. Opin. Biotechnol.* 42, 54–66.
- Alibaba, 2021. High Quality Succinic Acid 99.5% Industrial Grade White Crystal or Powder With CAS No. 110-15-6 S, Available at: <https://www.alibaba.com/product-detail/High-quality-Succinic-acid-99-5-60808319140.html>. (Accessed: 21st November 2021).
- Alvarado-Morales, M., et al., 2015. *Laminaria digitata* as a potential carbon source for succinic acid and bioenergy production in a biorefinery perspective. *Algal Res.* 9, 126–132.
- Babaei, M., et al., 2019. Engineering oleaginous yeast as the host for fermentative succinic acid production from glucose. *Front. Bioeng. Biotechnol.* 1, 361 [www.frontiersin.org](http://www.frontiersin.org).
- Barrett, A., 2018. BioAmber Is Bankrupt, Available at: <https://bioplasticsnews.com/2018/05/07/bioamber-is-bankrupt/>. (Accessed: 14th November 2021).
- Boit, B., Fiey, G. & Van De Graaf, M. J. Process for manufacturing succinic acid from a fermentation broth using nanofiltration to purify recycled mother liquor. Patent no. US 10,189,767 B2. 2, (2019).
- Bradfield, M.F.A., Nicol, W., 2016. The pentose phosphate pathway leads to enhanced succinic acid flux in biofilms of wild-type *Actinobacillus succinogenes*. *Appl. Microbiol. Biotechnol.* 100, 9641–9652.
- Bradfield, M.F.A., et al., 2015. Continuous succinic acid production by *Actinobacillus succinogenes* on xylose-enriched hydrolysate. *Biotechnol. Biofuels* 8, 181.
- Chatterjee, R., Millard, C.S., Champion, K., Clark, D.P., Donnelly, M.I., 2001. Mutation of the *ptsG* gene results in increased production of succinate in fermentation of glucose by *Escherichia coli*. *Appl. Environ. Microbiol.* 67, 148–154.
- Cheng, K.K., et al., 2012. Downstream processing of biotechnological produced succinic acid. *Appl. Microbiol. Biotechnol.* 95, 841–850.
- Cherubini, F., 2010. The biorefinery concept: using biomass instead of oil for producing energy and chemicals. *Energy Convers. Manage.*, <http://dx.doi.org/10.1016/j.enconman.2010.01.015>.
- Clark, S., 2014. *Allied Marke Research: Bio Succinic Acid Market by Application*.
- Cok, B., Ioannis, T., Roes, A.L., Patel, M.K., 2013. Succinic acid production derived from carbohydrates: An energy and greenhouse gas assessment of a platform chemical toward a bio-based economy. *Biofuels, Bioprod. Biorefining*, 16–29, <http://dx.doi.org/10.1002/bbb.1427>.
- Crater, J., Galleher, C., Lievense, J., 2017. *Consultancy on Large-Scale Submerged Aerobic Cultivation Process Design - Final Technical Report*.
- Cukalovic, A., Stevens, C.V., 2008. Feasibility of production methods for succinic acid derivatives: a marriage of renewable resources and chemical technology. *Biofuels Bioprod. Biorefining*, 505–529, <http://dx.doi.org/10.1002/bbb.105>.
- De Guzman, D., 2019. The Changing Landscape of Bio-Based Succinic Acid, Available at: <https://greenchemicalsblog.com/2019/02/24/the-changing-landscape-of-bio-based-succinic-acid/comment-page-1/>. (Accessed: 14th November 2021).
- Dickson, R., Ryu, J.H., Liu, J.J., 2018. Optimal plant design for integrated biorefinery producing bioethanol and protein from *Saccharina japonica*: a superstructure-based approach. *Energy* 164, 1257–1270.
- Dickson, R., et al., 2021. Sustainable bio-succinic acid production: superstructure optimization, techno-economic, and lifecycle assessment. *Energy Environ. Sci.*, <http://dx.doi.org/10.1039/d0ee03545a>.
- E4tech RE-CORD and WUR, 2015. *From the Sugar Platform to biofuels and biochemicals*. E4Tech, RE - CORD (Consorzio per la Ricerca e la Dimostrazione sulle Energie Rinnovabili), Wageningen University and Research Center, doi:contract No. ENER/C2/423-2012/SI2.673791.
- EC-JRC, 2010. *International Reference Life Cycle Data System (ILCD) Handbook – General guide for Life Cycle Assessment – Detailed guidance*. Luxembourg. Publications Office of the European Union.
- Efe, Ç., van der Wielen, L.A.M., Straathof, A.J.J., 2013. Techno-economic analysis of succinic acid production using adsorption from fermentation medium. *Biomass Bioenergy* 56, 479–492.
- Fernando, J., Khartit, K., 2020. Discounted Cash Flow (DCF), Available at: <https://www.investopedia.com/terms/d/DCF.asp>. (Accessed: 21st February 2021).
- Ferone, M., et al., 2018. Continuous succinic acid fermentation by *Actinobacillus succinogenes* in a packed-bed biofilm reactor. *Biotechnol. Biofuels* 11, 1–11.
- Ferone, M., Raganati, F., Olivieri, G., Marzocchella, A., 2019. Bioreactors for succinic acid production processes. *Crit. Rev. Biotechnol.* 39, 571–586.
- Finley, K. R. et al. Compositions and methods for succinate production. Patent no. US 2013/0302866 A1. (2013).
- Fruchey, O. S. et al. Processes for producing monoammonium succinate from fermentation broths containing diammonium succinate, monoammonium succinate and/or succinic acid, and conversion of monoammonium succinate to succinic acid. Patent no. US 2011 / 0104761 A1. 12–15 (2011).
- Fruchey, O. S. et al. Processes for producing succinic acid from fermentation broths containing diammonium succinate. Patent no. US 2011/O297.527 A1. (2012).
- Gadkari, S., Kumar, D., Qin, Zhao, Ki Lin, C.S., Kumar, V., 2021. Life cycle analysis of fermentative production of succinic acid from bread waste. *Waste Manag.* 126, 861–871.
- Garg, N., Woodley, J.M., Gani, R., Kontogeorgis, G.M., 2019. Sustainable solutions by integrating process synthesis-intensification. *Comput. Chem. Eng.* 126, 499–519.
- Hermann, B.G., Blok, K., Patel, M.K., 2007. Producing bio-based bulk chemicals using industrial biotechnology saves energy and combats climate change. *Environ. Sci. Technol.* 41, 7915–7921.
- Huijbregts, M.A.J., et al., 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *Int. J. Life Cycle Assess.* 22, 138–147.
- Humbird, D., et al., 2011. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. National Renewable Energy Laboratory, <http://dx.doi.org/10.2172/1107470>.
- Jiang, M., et al., 2017. Progress of succinic acid production from renewable resources: metabolic and fermentative strategies. *Bioresour. Technol.* 245, 1710–1717.
- Kawasumi, R., et al., 2017. One-step conversion of levulinic acid to succinic acid using I2/t-BuOK system: the Iodoform reaction revisited. *Sci. Rep.* 7, 4–11.
- Klein, B.C., et al., 2017. Process development and techno-economic analysis of bio-based succinic acid derived from pentoses integrated to a sugarcane biorefinery. *Biofuels Bioprod. Bioref.* 11, 1051–1064.
- Kuenz, A., Hoffmann, L., Goy, K., Bromann, S., Prüße, U., 2020. High-level production of succinic acid from crude glycerol by a wild type organism. *Catalysts* 10.
- Kuhn, E.M., O'Brien, M.H., Ciesielski, P.N., Schell, D.J., 2016. Pilot-scale batch alkaline pretreatment of corn stover. *ACS Sustain. Chem. Eng.* 4, 944–956.
- Kumar, A., Udugama, I.A., Gargalo, C.L., Germaey, K.V., 2020. Why is batch processing still dominating the biologics landscape? Towards an integrated continuous bioprocessing alternative. *Processes* 8, 1–19.
- Li, J., et al., 2010. Effect of redox potential regulation on succinic acid production by *Actinobacillus succinogenes*. *Bioprocess Biosyst. Eng.* 33, 911–920.
- Lichner, C., 2020. Electrolyzer Overview: Lowering the Cost of Hydrogen and Distributing Its Production, Available at: <https://pv-magazine-usa.com/2020/03/26/electrolyzer/>

- overview-lowering-the-cost-of-hydrogen-and-distributing-its-productionhydrogen-industry-overview-lowering-the-cost-and-distributing-production/. (Accessed: 20th November 2021).
- Lindskog, E.K., 2018. The Upstream Process: Principal Modes of Operation. Biopharmaceutical Processing: Development, Design, and Implementation of Manufacturing Processes. Elsevier Ltd., <http://dx.doi.org/10.1016/B978-0-08-100623-8.00031-1>.
- Mancini, E., Mansouri, S.S., Gernaey, K.V., Pinelo, M., Luo, J., 2019. From second generation feed-stocks to innovative fermentation and downstream techniques for succinic acid production. *Crit. Rev. Environ. Sci. Technol.* 0, 1–45.
- McKinlay, J.B., Vieille, C., Zeikus, J.G., 2007. Prospects for a bio-based succinate industry. *Appl. Microbiol. Biotechnol.* 76, 727–740.
- Morales, M., et al., 2016. Sustainability assessment of succinic acid production technologies from biomass using metabolic engineering. *Energy Environ. Sci.* 9, 2794–2805.
- Newark, N., 2020. Global Succinic Acid Market, Available at: <https://www.globenewswire.com/news-release/2020/01/30/1977366/0/en/Global-Succinic-Acid-Market-is-Expected-to-Reach-USD-218-14-Million-by-2025-Fior-Markets.html>. (Accessed: 20th November 2021).
- Nghiem, N.P., Kleff, S., Schwegmann, S., 2017. Succinic acid: technology development and commercialization. *Fermentation*, 601–630, <http://dx.doi.org/10.3390/fermentation3020026>.
- Ögmundarson, Ó., Herrgård, M.J., Forster, J., Hauschild, M.Z., Fantke, P., 2020. Addressing environmental sustainability of biochemicals. *Nat. Sustain.* 3, 167–174.
- Ozturk, S.S., 2015. Opportunities and challenges for the implementation of continuous processing in biomanufacturing. In: *Continuous Processing in Pharmaceutical Manufacturing*, pp. 457–478, <http://dx.doi.org/10.1002/9783527673681.ch18>.
- Pateraki, C., Andersen, S.J., Ladakis, D., Koutinas, A., Rabaey, K., 2019. Direct electrochemical extraction increases microbial succinic acid production from spent sulphite liquor. *Green Chem.* 21, 2401–2411.
- Peters, M., Timmerhaus, K., 1994. *Plant Design and Economic for Chemical Engineers. Seminars for Nurse Managers*, 2.
- Pinazo, J.M., Domine, M.E., Parvulescu, V., Petru, F., 2015. Sustainability metrics for succinic acid production: a comparison between biomass-based and petrochemical routes. *Catal. Today* 239, 17–24.
- Rosengart, A., Vizzi, M., Manenti, F., Citterio, A., 2017. Development of an ultrafiltration predictive model to estimate the cost of downstream in biorefineries: effects of epistemic experimental uncertainties. *Energy Convers. Manage.* 149, 875–884.
- Rush, B. J. & Fosmer, A. M. Methods for Succinate Production. Patent no. US2014/0363862A1. 1, (2014).
- Salvachúa, D., et al., 2016a. Succinic acid production on xylose-enriched biorefinery streams by *Actinobacillus succinogenes* in batch fermentation. *Biotechnol. Biofuels* 9, 1–15.
- Salvachúa, D., et al., 2016b. Succinic acid production from lignocellulosic hydrolysate by *Basfia succiniciproducens*. *Bioresour. Technol.* 214, 558–566.
- Salvilla, J.N.V., et al., 2020. Synergistic co-pyrolysis of polyolefin plastics with wood and agricultural wastes for biofuel production. *Appl. Energy* 279, 115668.
- Sánchez, A.M., Bennett, G.N., San, K.Y., 2005. Novel pathway engineering design of the anaerobic central metabolic pathway in *Escherichia coli* to increase succinate yield and productivity. *Metab. Eng.* 7, 229–239.
- Shekro, J., et al., 2014. Characterization of pilot-scale dilute acid pretreatment performance using deacetylated corn stover. *Biotechnol. Biofuels* 7, 1–10.
- Silk, D., et al., 2020. A decision-support framework for techno-economic-sustainability assessment of resource recovery alternatives. *J. Clean. Prod.* 266, 121854.
- Skok, D., Reiss, S., 2015. The Discount Rate Should Be the Company's WACC, Available at: [https://www.forentrepreneurs.com/discount-rate-for-dcf/?fbclid=IwAR3V80eweBDdOGAu0LA0LbtZHqXP\\_A\\_r6E1De5f53CDOMG7zimq9OYJbB.k](https://www.forentrepreneurs.com/discount-rate-for-dcf/?fbclid=IwAR3V80eweBDdOGAu0LA0LbtZHqXP_A_r6E1De5f53CDOMG7zimq9OYJbB.k). (Accessed: 20th February 2021).
- Smidt, M., Hollander, J.D., Bosch, H., Xiang, Y., 2016. Life cycle assessment of biobased and fossil-based succinic acid. In: *Sustainability Assessment of Renewables-Based Products: Methods and Case Studies*. John Wiley & Sons, Ltd.
- Thomas, K.E., 1997. Overview of Village Scale, renewable energy powered desalination. *Renew. Energy*, NREL617p-440-22083 UC Category: 1210 DE97000240.
- Udugama, I.A., et al., 2018. A systematic methodology for comprehensive economic assessment of process control structures. *Ind. Eng. Chem. Res.* 57, 13116–13130.
- Van de Graaf, M. J., Valianpoer, F., Fiey, G., Delattre, L. & Schulten, E. A. M. Process for the crystallization of succinic acid. Patent no. US 2015/0057425 A1. 1, (2015).
- Verma, M., Mandyal, P., Singh, D., Gupta, N., 2020. Recent developments in heterogeneous catalytic routes for the sustainable production of succinic acid from biomass resources. *ChemSusChem* 13, 4026–4034.
- Wernet, G., et al., 2016. The ecoinvent database version 3 (part I): overview and methodology. *Int. J. Life Cycle Assess.* 21, 1218–1230.
- Werpy, T., Petersen, G., 2004. Top Value Added Chemicals From Biomass. Volume I: Results of Screening for Potential Candidates From Sugars and Synthesis Gas. Produced by the Staff at the Pacific Northwest National Laboratory (PNNL) and the National Renewable Energy Laboratory (NREL)., <http://dx.doi.org/10.2172/15008859>.
- Yedur, S., Berglund, K. A. & Dunuwila, D. D. Succinic acid production and purification. Patent no. US patent No. US 6,265,190 B1. 11 (2001).
- Yu, J.H., et al., 2016. Combinatorial optimization of CO<sub>2</sub> transport and fixation to improve succinate production by promoter engineering. *Biotechnol. Bioeng.* 113, 1531–1541.
- ZAUBA, 2016. Search Import Export Data of India, Available at: <https://www.zauba.com/import-succinic+acid+99-hs-code.html>. (Accessed: 20th July 2021).

**Update**

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## Corrigendum

## Corrigendum to ‘Economic and environmental analysis of bio-succinic acid production: From established processes to a new continuous fermentation approach with in-situ electrolytic extraction’ [Chemical Engineering Research and Design Volume 179, March 2022, Pages 401–414]

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The authors regret that an incorrect image is shown as Fig. 3 in the published article. The correct Fig. 3 is shown below:

The authors apologize for any inconvenience caused by this mistake.

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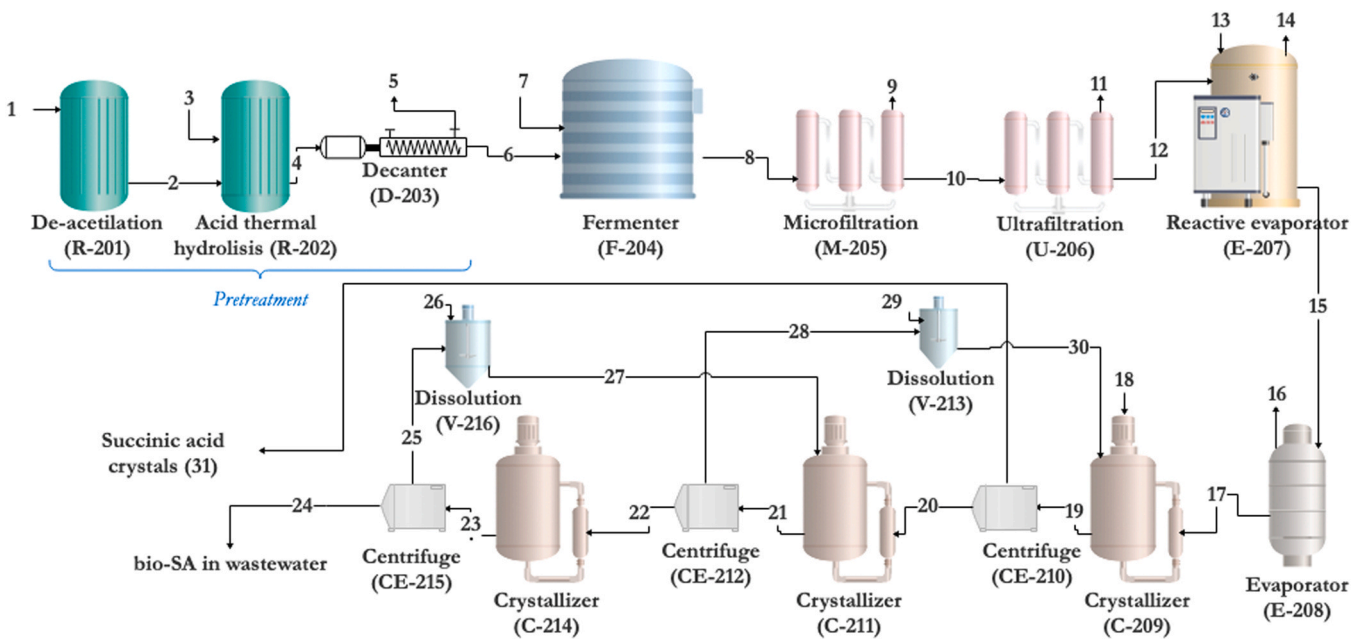
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**Fig. 3 – Process II diagram of biomass pretreatment and the BioAmber process for producing 30 kt y<sup>-1</sup> of bio-SA. The mass balance and energy consumption of each unit operation is represented by the numbers on the streams and the values can be found in Table S10. (Ahn et al., 2016; Nghiem et al., 2017; Rush and Fosmer, 2014; Finley, 2013; Fruchey, 2012).**

## References

- Ahn, J.H., Jang, Y.S., Lee, S.Y., 2016. Production of succinic acid by metabolically engineered microorganisms. *Curr. Opin. Biotechnol.* 42, 54–66.
- Nghiem, N.P., Kleff, S., Schwegmann, S., 2017. Succinic acid: technology development and commercialization. *Fermentation* 601–630. <https://doi.org/10.3390/fermentation3020026>

- Rush, B.J., Fosmer, A.M., 2014. Methods for Succinate Production. Patent no. US2014/0363862A1 1.
- Finley, K. R. et al. Compositions and methods for succinate production. Patent no. US 2013/0302866 A1. (2013).
- O. S. Fruchey et al. Processes for producing succinic acid from fermentation broths containing diammonium succinate. Patent no. US 2011/0297.527 A1. (2012).