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From second generation feed-stocks to innovative fermentation and downstream techniques for succinic acid production

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Succinic acid (SA) is one of the most important bio-building blocks in biorefinery. Its production from fermentation of renewable biomass sources is becoming a consolidated alternative that is more sustainable and potentially more economic than the traditional petroleum-based path for SA production. Fermentative production of SA has been successfully commercialized and a large and increasing number of SA-derivatives are promoting the economic stability of this production. However, the companies producing SA from fermentation are targeting specialized markets and the production is far from large-scale bulk chemical synthesis. In order to develop optimized and economic processes, the best candidates in every step of the SA production process must be identified. In this paper, the most promising biomass sources, pretreatment methods, fermentation conditions (i.e. host microorganism, fermenter design and operative mode) and separation techniques for industrial SA production are critically reviewed. Selection of the host microorganism is a key factor for SA production. However, the availability, potential and sustainability of feed-stocks, fermentation and separation process must also be carefully evaluated for a cost-effective and environmentally sustainable SA production.

Keywords: succinic acid; lignocellulose; biomass pretreatment; membrane separation; continuous and simultaneous saccharification and fermentation; *in situ* product recovery; large-scale production of succinic acid.

1.0. Introduction

Refining biomass (biorefinery) is a promising strategy to reduce dependency on petroleum, especially with respect to chemicals and fuel production. A biorefinery addresses several challenges at the same time, such as the depletion of fossil fuel resources (with the associated consequences), the requirement for increased human sustainability of production, waste management, and political concerns (Chandel, Garlapati, Singh, Antunes, & da Silva, 2018; Cherubini, 2010). Today, worldwide efforts are being made to develop efficient processes for bio-based production of chemicals, and succinic acid (SA) is widely recognized as a

66 fundamental building block in such efforts (Werpy & Petersen, 2004). Succinity, a company
67 producing biomass-based SA, reported a reduction of more than 60% in greenhouse gasses
68 (GHG) emissions compared to petroleum-based SA production (Succinity, 2019). Currently,
69 more than 30 commercially valuable products can be synthesized from SA (Figure 1) or
70 include a derivative of it, examples are: solvents and lubricants, synthetic resins, and
71 biodegradable polymers such as polybutylene succinate (PBS) and polyamides, as well as
72 cosmetics, food additives and pharmaceuticals intermediates (Arshadi et al., 2008; Beauprez,
73 De Mey, & Soetaert, 2010). Between 1999 to 2011 the global market for SA, which increased
74 at 10% per year, more than doubled (Pinazo, Domine, Parvulescu, & Petru, 2015) and this
75 market is expected to grow at a CAGR (compound annual growth rate) of around 24% by
76 2020 (Nghiem, Kleff, & Schwegmann, 2017). Until recently, petrochemical-based SA
77 dominated the market and up to 2011 biorefinery-based SA production was reported to be
78 less than 5% of the total SA production (IEA Bioenergy, 2012; Weastra, 2012). However,
79 biorefinery-based SA increased to 48.7% of the market in 2013 (EC-DGE, 2015) and was
80 forecasted to reach even 60% in 2015 (Pinazo et al., 2015). Pinazo et al. (2015) confirmed
81 this trend, reporting that petrochemical-based SA production has remained stable for years,
82 whereas SA from fermentation is responsible for the worldwide growth in SA production. In
83 2013 total SA production was around 38,000 t with a total market value of \$108 million
84 (approx. 2,860 \$/t), while petrochemical-based global SA production was approximately
85 40,000 t with a market value of \$100 million (approx. 2,500 \$/t). In 2015 the estimated
86 addressable market for SA-derived chemicals was between \$7 and \$10 billion, including 1.4
87 butandiol (BDO - up to \$4 billion), tetrahydrofurane (THF) and oxalan-2-one (GBL) (EC-
88 DGE, 2015). Because of the wealth of industrial activity focused around biorefinery-based
89 SA production, SA was reported as the fastest growing bio-based market in 2015. If SA from
90 fermentation is economically competitive, it could easily replace many fossil-based building

block alternatives. In a report entitled “From the sugar platform to biofuel and biochemicals”, the European Commission places SA production at a TRL between 7 and 8 today. This means that some processes are at commercial scale, while others still need further research and development to enter the market (EC-DGE, 2015). However, whilst significant advances have been made in the field, barriers remain for full exploitation of lignocellulose (EC-DGE, 2015) which is expected to be the future major feedstock for industrial SA production (Efe, van der Wielen, & Straathof, 2013; C. S. K. Lin et al., 2013).

Succinic acid has traditionally been a petrochemical by-product obtained from catalytic hydrogenation, paraffin oxidation and electrolytic reduction of maleic anhydride or maleic acid (Xu et al., 2018). The liquid-phase maleic anhydride hydrogenation to succinic anhydride is followed by the hydration to SA (Figure. 2) (Pinazo et al., 2015).

The petrochemical synthesis of SA occurs by means of Ni or Pd based catalysts at a temperature between 120 to 180 °C and moderate hydrogen pressure of 0.5 to 4.0 MPa, which saturates the double bonds to release heat ($\Delta H = -133.89 \text{ kJ mol}^{-1}$) (Fumagalli, 2006). The process efficiency reported in the literature is limited to the first step only (from maleic anhydride to succinic anhydride, see Figure 2) with yields close to the theoretical yield (Fumagalli, 2006; Pinazo et al., 2015). However, purification steps are still required to obtain a marketable product, and after removing the catalyst by filtration, the raw succinic anhydride is distilled under vacuum conditions and subsequently flaked (Fumagalli, 2006).

SA can also be chemically synthesized from levulinic acid, which is another renewable feedstock that can be easily obtained from lignocellulose treatment. The process is reported to be economically competitive compared to SA production from petroleum, and offers also advantages compared with SA from fermentation of lignocellulose (Cukalovic & Stevens, 2008). Nevertheless, SA synthesis from levulinic acid has only recently received attention and is currently still far from full-scale implementation (Kawasumi et al., 2017). In

contrast, several industrial actors such as: Biosuccinium (former Reverdia), Succinity, BioAmber and Myriant (Table 1), have already successfully commercialized SA based on microbial fermentation. To develop more economic and optimized bio-based processes, identification of the best candidates in every step must be performed.

This work comprehensively reviews the most recent advances in the development of cost-efficient second generation biorefinery processes for SA production with an emphasis on large-scale synthesis, and takes a look at the future. There are four main sections: the first investigates the characteristics and availability of biomass feedstock candidates with a focus on second generation biorefineries; the second provides an overview of the potential of SA production from different feedstock candidates and reviews the relevance of process configurations and operational modes that can be applied in the fermentation step; the third section reviews the major separation techniques applied for SA separation and purification; the last section identifies the best candidates for the process from a holistic point of view and the associated challenges, laying solid foundations for future work in process simulation.

2.0. Biomass-derived succinic acid: feedstock composition, distribution and availability

Biomass for SA production can originate from three main sources: agriculture and/or forestry sources, industrial by-products, and food waste (Vassilev & Vassileva, 2016). To date, large-scale production of SA has primarily focused on starch-based sugars, but for SA not to compete with food production, inexpensive lignocellulosic-derived sugars should ideally be extracted from non-food crops as feedstock for SA production (Salvachúa et al., 2016). First generation feed-stocks for SA production are typically rich in carbohydrates, for example wheat, corn, sugar beet, sugar cane or direct use of refined sugars, for example glucose (Salvachúa et al., 2016). For many of the plant sources of such carbohydrates,

however, only a small fraction of the aerial parts of the plant is utilized for SA production (Cherubini, 2010). Reduced chemical complexity and high concentration of degradable carbohydrates are the major advantages of the first generation feed-stocks. SA production has low dependence on a single feedstock since it can be chemically produced from basically any carbohydrate fraction (Table 4). This flexibility is useful in overcoming seasonal and geographical limitations that may be associated with producing biomass-based biorefinery products.

Lignocellulosic biomass has been proposed as the future feedstock for SA production (Efe et al., 2013; C. S. K. Lin et al., 2013). Unlike first generation feedstock, lignocellulosic biomass encompasses nearly the whole plant (Cherubini, 2010). The composition of such biomass ranges from 40-50% cellulose and 20-40% hemicellulose and lignin (Cherubini, 2010) and represents a cheap and abundant feedstock as well as a way to dispose of agricultural wastes (Mulvihill, Beach, Zimmerman, & Anastas, 2011). Fermentable sugars obtained from cellulose and hemicellulose, such as glucose, xylose, fructose, lactose are the sources for SA production (Werpy & Petersen, 2004). The annual production of lignocellulosic material from the agriculture industry and terrestrial plants is estimated to be about 180 million tons per year (Figure 3).

Food waste represents a rather diffuse unexploited (or not fully exploited) resource throughout the entire world. Nowadays, the vast majority is landfilled, burnt, or in the best-case scenario anaerobically digested for biogas production (C. S. K. Lin et al., 2013). Many studies have highlighted the great potential of food waste as potential feedstock for chemical synthesis (Brunklaus B, Rex E, Carlsson E, 2018; Erica, 2004; C. S. K. Lin et al., 2013). In 2012 the amount of dumped food worldwide was estimated to be around 1.3 billion t (1/3 of the food production) (Buchner et al., 2012), with FAO reporting as much as 50% of food wasted in the supply chain and after reaching the consumers. In the European Union, 89

million tons of food are wasted yearly, with 80% of this figure coming from manufacturing (38%) and household waste (42%) (C. S. K. Lin et al., 2013). In this respect, it is important to mention that SA has been produced successfully from selected food waste samples (Q. Li, J. A. Siles, I. P. Thompson, 2010; Zhang et al., 2013).

Bakery and bread wastes have been pointed out as particularly suitable for SA production because they are rich in easily fermentable carbohydrates (starch and simple sugars) and can provide the required nutrients for efficient SA biosynthesis (A. Y. Z. Zhang et al., 2013). Leung, Cheung, Zhang, Lam, & Lin (2012) used bread waste for solid state fermentation, and from the 59.8 wt% detected starch per gram of bread (dry weight), they obtained as much as 90.8% conversion to glucose, resulting in a sugar concentration of more than 100 g/L after hydrolysis. Similar amounts of carbohydrates were reported by Zhang et al. (2013) in pastry and cake residues, at 33.5 and 62.0% (g carbohydrate/g residue), respectively. Treatment of a 30% (w/v) solution residue, i.e. 10.05 g carbohydrate/L, with simultaneous hydrolysis and fungal autolysis released about 54.2 and 58.7 g/L glucose plus fructose, for pastry and cake residues, respectively.

Citrus peel waste has also been studied for SA production. The major components of citrus waste are water (80 wt%), soluble sugars, cellulose up to 23.17 ± 0.64 wt% (dry weight) (Q. Li et al., 2010), hemicellulose, pectin and D-limonene. It is estimated that 31.2 million tons of citrus fruits are annually processed in the world, half of which is waste (calculated on a wet basis). This waste comes mainly from oranges, lemons, limes, grapefruits and tangerines, which are therefore potential substrates for SA production (Q. Li et al., 2010; C. S. K. Lin et al., 2013). Seventy percent of the world's supply in citrus fruits originates from Brazil, Italy, Spain, China, India, Egypt, South Africa, Morocco, Turkey, and USA (C. S. K. Lin et al., 2013).

Cheese whey is a by-product of the cheese-making industry, and different studies have reported on the potential of this low-cost substrate to produce SA in high concentration and yield by using different bacterial hosts (Lee, Lee, Hong, & Chang, 2003; Samuelov, Datta, Jain, & Zeikus, 1999; Wan, Li, Shahbazi, & Xiu, 2008). Cheese whey contains about 4.9% carbohydrate and 6 to 7% solids of which 70 to 80% is lactose and 10-15% consists of milk proteins, lactate and salts (Samuelov et al., 1999). After separation of lactose-rich and protein-rich fractions, the former could be used for SA production *via* fermentation (C. S. K. Lin et al., 2013). Whey production in the U.S., expressed as dry matter, is about 470,332 t (European Commission, 2018), whereas the global production was about 2.6 million tons in 2014 (FAO, 2018). Due to its high biological oxygen demand (BOD), whey cannot be released into the environment and most of it is disposed of (Lee, Lee, Hong, & Chang, 2003) or used in animal feed blends (Samuelov et al., 1999). The high organic carbon content makes cheese whey a good substrate for SA production but it lacks available nitrogen (Lee, Lee, Hong, & Chang, 2003). As a consequence, significant amounts of nitrogen could be necessary to produce SA from whey (Pateraki et al., 2016).

Glycerol is a by-product of the biodiesel and bioethanol industries, and about 100mL glycerol is produced with every liter of biodiesel (Borzani, 2006; Carvalho, Matos, Roca, & Reis, 2014). Glycerol is highly promising as a substrate for SA production due to its higher reduced chemical status as compared to C5 and C6 sugars (Pateraki et al., 2016). The cost of raw glycerol is low but its quality largely depends on the feedstock used and the quality of the produced biodiesel (Carvalho et al., 2014).

Lastly, algae are moderately rich in proteins although their organic composition can vary significantly depending on the species and/or the growth conditions. This variety makes this biomass very versatile for numerous commercial applications, such as production of biofuel, biochemicals, pharmaceuticals, food etc. The amount of algal carbohydrates reported

in the literature is on average 29.9 wt% with a maximum of 83.6 wt% and a minimum of 4.0 wt% (Vassilev & Vassileva, 2016).

To summarize, nearly all food crops production has been constantly increasing during the last 50 years – for example, fresh fruits and cereals production rose by around 4.5 fold (FAO, 2018) – and this growth has also generated a constant increase in lignocellulosic residues that can be utilized under the biorefinery concept. In this sense, the main SA production from lignocellulose could be supplemented with that from local organic solid waste (including food waste) or by exploiting regional resources. Important local resources for SA production are: algae from areas close to the sea, non-food crops such as grass, industrial wastes such as glycerol, cheese whey, spent sulfite liquor (from paper industry), citrus peel etc.

3.0. Manufacturing succinic acid

3.1. Feed-stocks potential

Waste biomass from cereal processing has been widely investigated as a potential feedstock for SA production. The overall potential yield of SA from corn stover under different conditions was 74 ± 2 wt% (J. Li et al., 2011; Salvachúa et al., 2016; Zheng et al., 2010) when the process was started from straw hydrolysates only. Zheng, Dong, Sun, Ni, & Fang (2009) reported a higher SA yield from corn straw (81 ± 2 wt%) compared with that from wheat straw (74 ± 2 wt%) and rice straw (63 ± 2 wt%). The same authors reported a yield as high as 89 ± 3 wt% from corncob only, which highlighted the potential of using specific parts of the corn stover for SA production. The potential of corn stalks for SA production is also relatively high in the lignocellulosic wastes group, and yields of about 83-87 wt% have been reported (Liang et al., 2013; D. Wang et al., 2011). Sugarcane bagasse and molasses are also

attractive biorefinery substrates due to their potential SA yield. Reported yields for the former are around 40 wt% (Borges & Pereira, 2011) up to 80 wt% (Chen, Tao, & Zheng, 2016) when multiple enzymatic pretreatment is applied. While for sugar cane molasses SA yields are between nearly 70 wt% (Cao et al., 2018b) to 80 wt% (Liu et al., 2008; Shen et al., 2015), depending on the pretreatment steps and nitrogen sources. For both sugarcane bagasse and molasses *A. succinogenes* was used as the microbial host.

Bread and bakery wastes are rich in fermentable carbohydrates and have a good potential for biochemical SA production, with values of 55% (g SA/g bread) from solid-state fermentation (Leung et al., 2012) and between 28 and 35% (g SA/g total bakery waste) (A. Y. Z. Zhang et al., 2013). In the UK alone, waste from bakeries and dried food amounted to 1 million t in 2009. The conclusion is that relevant quantities of succinic acid can be produced via fermentation of bakery products (C. S. K. Lin et al., 2013). For citrus waste, most of which is peels, about 15.6 million t (wet basis) of citrus waste is produced yearly worldwide (Q. Li et al., 2010; C. S. K. Lin et al., 2013). Q. Li et al., (2010), studied the potential SA production from different concentrations of pretreated orange peel through exposing the peel to the cellulolytic bacterium *F. succinogenes* S85 in an anaerobic batch reactor under a carbon dioxide atmosphere. After removing D-limonene (see section 3.2), fermentation of 10 g/L orange peel gave a maximum yield of more than 12% (g SA/g pretreated orange peel) with a production rate of 10 mg/L/h. Increasing the orange peel concentration significantly lowered the yield to about a third (< 4% - g SA/g pretreated orange peel at 60 g pretreated orange peel /L) but more than doubled the productivity (25 mg/L/h). Regarding cheese whey, SA yields are reported to be between 57 to 91 wt% depending on the microbe used and the fermentation conditions (K.-K. Cheng, Zhao, Zeng, & Zhang, 2012; Samuelov et al., 1999; Wan et al., 2008).

3.2. Pretreatment of biomass for SA production

Biomass pretreatment is essential to make the carbohydrates of the selected raw material available for fermentation. An efficient pretreatment aims to make as much as possible of the carbohydrate fraction of the biomass accessible while at the same time removing potentially inhibiting compounds in the mixture. On the other hand, feedstock production and grid intensity in biomass pretreatment for SA production is reported as a major source for GHG emissions (EC-DGE, 2015).

SA production from agricultural crops can exploit the already established treatment processes of food production. Du et al. (2008) suggested that the processing of the raw material fractions (e.g. flour separated from bran) and subsequent formation of a common feedstock for fermentation and SA production is more economic and sustainable. These authors reported that SA production from an integrated wheat biorefinery was twice that obtained from a biorefinery process not using fractionation of the raw material; SA yields of 40 wt% (Du et al., 2008) were obtained for the former process compared to 19 wt% for the latter (Du, Lin, Koutinas, Wang, & Webb, 2007).

With lignocellulosic material, pretreatment may involve harsh conditions to break down the robust lignocellulosic structure, and operations vary from simple drying and grinding (Q. Li, et al., 2010) to steam explosion at 215°C for 3 - 6 min (Kim et al., 2004; Lee, Lee, Hong, Chang, & Park, 2003). Nonetheless, enzymatic pretreatments (after thermochemical treatments) were reported as being less complex and more efficient and sustainable than non-enzymatic pretreatments and extracted up to 90% of the sugars (Chandel et al., 2018). Table 2 collects the advantages and disadvantages of the different pretreatment methods. However, the process itself may produce toxic compounds (see section 3.4). Salvachúa et al. (2016) significantly alleviated inhibition due to toxic compounds by applying

a deacetylation pretreatment before a diluted acid pretreatment to corn stover. When this deacetylated corn stover was compared to pure sugar as a substrate for SA production, the production rate only was lower while the final SA titer and yield were the same: a titer of 43 and 47 g/L and yield of 72 and 74 wt% for corn stover and pure sugar, respectively. The corn stover was knife-milled, sieved through a 19 mm mesh and deacetylated for 2h in a bath of 0.4 wt% NaOH and 80°C, for a corn stover with 8 wt% total solids (TS). The pretreatment was then run for 10 minutes at 160°C with addition of 8 g H₂SO₄ per kg of biomass. When deacetylation was performed before dilute acid pretreatment of corn stover, SA production yield was 42% higher (0.74 g SA/g sugars) with a 370% increase in production rate (1.27 g SA/L/h) than SA yield (0.52 g SA/g sugars) and production rate (0.27 g SA/L/h) without prior deacetylation. The gap between the theoretical yield and the obtained production in the experiment reported by Salvachúa et al. (2016) (1.12 and 0.74 g SA/g sugars, respectively) was explained by the generation of other co-products (i.e. formate, acetate) and biomass formation of *A. succinogenes*.

Food waste pretreatment might involve a simple blending followed by enzymatic hydrolysis and fungal autolysis, such as for bread and bakery waste (Leung et al., 2012; A. Y. Z. Zhang et al., 2013), or may require more complex steps, such as for citrus waste. For example, before conducting fermentation for producing SA, Q. Li, et al. (2010) minced citrus peel to a particle size of 2 mm, then dried the resulting particles for 120 h at 65 °C and finally applied steam to remove D-limonene which is a known antibacterial agent. According to the authors, concentrations of D-limonene of 0.06 vol% inhibit cell growth; 0.06 vol% corresponds to 27 g/L orange peel. Therefore limonene must be removed from orange peel in concentrations greater than 27 g/L prior to fermentation.

Production of SA from macroalgae such as *Laminaria digitata* and *Laminaria japonica* requires pretreating by drying, chopping and milling followed by enzymatic

hydrolysis to release the intermediate sugars, such as glucose and mannose (Alvarado-Morales et al., 2015; Vassilev & Vassileva, 2016). Micro and macroalgae have huge potential in a biorefinery either for fuel or for chemicals production. However, up to the present there have been only few studies on using algae for SA production. In the context of the biorefinery as a cluster of bio-based facilities, algae hold a key role since they have few geographical limitations, do not suffer from competition with arable land, and have wide natural variety in their composition. For example, *Laminaria digitata* and *Saccharina latissimi* are macroalgae consisting of about 60% carbohydrates, and some other species can contain more than 80% carbohydrate (Vassilev & Vassileva, 2016), which also makes them suitable as a substrate source for SA synthesis (Holdt & Kraan, 2011). Alvarado-Morales et al. (2015) obtained a sugar solubilization of more than 78% from *L. digitata*, from which as much as 86.5 wt% of the total sugars were converted to SA. Similarly, Bai et al. (2015) obtained about 81 wt% yield of SA from total sugars from the macroalgae *Laminaria japonica*, which was about 73% of the maximum theoretical potential.

3.3. Biological synthesis

3.3.1. Theoretical production

One part of the process utilized for SA production, the purification step, is considered as a major cost driver. Therefore organisms capable of producing SA at near-maximum theoretical yields would contribute considerably to the cost-efficiency of the SA production process. Thus, the potential SA yield of the different feed-stocks and particularly the theoretical SA yields are benchmarks for evaluating the effective bacterial performance (McKinlay, Vieille, & Zeikus, 2007). Theoretically, a mole of glucose can lead to about 1.71 moles of SA, as illustrated below:

333 $C_6H_{12}O_6 + 0.86 HCO_3^- \rightarrow 1.71 C_4H_4O_4^{2-} + 1.74 H_2O + 2.58 H^+$ (McKinlay et al., 2007)

334 $\Delta GH^{0'} = -173 \text{ Kj/mol}$

335 In the presence of CO_2 and additional reducing power (e.g. H_2), two moles of succinate per
336 mole glucose can theoretically be obtained:

337 $C_6H_{12}O_6 + 2HCO_3^- + 2H_2 \rightarrow 2 C_4H_4O_4^{2-} + 2 H_2O + 2 H^+$ (McKinlay et al., 2007)

338 $\Delta GH^{0'} = -317 \text{ Kj/mol}$

339 Fermentation of xylose, fructose and glycerol can theoretically generate 1.43, 1.20 and 1 mol
340 SA/mol substrate, respectively (Andersson, Hodge, Berglund, & Rova, 2007).

341 *3.3.2. Fermentation: process configuration and operational techniques*

342 After pretreatment, the biomass can be fermented through four main process configurations
343 and three main operational techniques. The configurations are SHF (Separate Hydrolysis and
344 Fermentation), SSF (Simultaneous Saccharification and Fermentation), SSCF (Simultaneous
345 Saccharification and Co-Fermentation), and CBP (Consolidated By-Processing), while the
346 operational modes can be batch, fed-batch and continuous. The two most relevant
347 configurations for SA production are SHF and SSF.

348 SHF is a configuration in which hydrolysis and fermentation occur in two separate
349 steps and it has been largely studied for SA production as the review from Akhtar, Idris, &
350 Abd. Aziz, (2014) shows. In SSF instead, hydrolysis and fermentation occur in the same
351 reactor simultaneously. Temperatures used in SSF are between 37 °C to 39 °C and pH is kept
352 neutral when the host microorganism is a bacterium and low pH when the host is yeast
353 (pH~3) (Chandel et al., 2018). The optimal configuration depends on the microbial host, from

the starting feedstock (Akhtar et al., 2014). Table 3 collects the advantages and disadvantages of SHF and SSF. However, one of the main conclusions from the review of Akhtar, Idris, & Abd. Aziz, (2014) on SA production from SHF and SSF is that SFF has a promising future for SA production from lignocellulosic biomass. A recent study on organic acid production (including SA) from various lignocellulosic biomasses and through SHF and SSF confirmed the higher performance of SSF (Maslova, Stepanov, Senko, & Efremenko, 2019).

Regarding the operational techniques, companies producing SA at commercial scale use batch or fed-batch (Table 1), which are simple and efficient in terms of production yield. However, continuous production systems offer higher production rate (Table 4) and require less sterilization times (Ferone, Raganati, Olivieri, & Marzocchella, 2019). The review of Ferone et al., (2019) on bioreactors for SA production offers a clear view of the advantages of continuous production systems, particularly for the possibility to operate the continuous with immobilized cultures (biofilm), which significantly increase the productivity. The increasing SA production yield observed when using immobilized cell bioreactors is particularly interesting for *A. succinogenes*. The biofilm, naturally created by this bacteria, activates and additional redox power, which permits to overcome one of the biggest limits of *A. succinogenes* in SA synthesis, which is the lack of reducing power (see section 3.3.3.) (Bradfield & Nicol, 2016; Maharaj, Bradfield, & Nicol, 2014). Table 3 shows major advantages and disadvantages of the main reactor's configuration and operational modes.

To conclude, SSF in a continuous bioreactor system with immobilized cells emerges as a very promising option for large-scale production of succinic acid.

3.3.3. Succinic acid producers

3.3.3.1. Wild-type microorganisms. SA is biologically synthesized as an intermediate in the normal metabolic pathway of several anaerobic and facultative aerobic microorganisms

(Kawasumi et al., 2017). Three major pathways can be identified: (1) the TCA cycle (oxidative pathway) also called the Krebs cycle, (2) the glyoxylate cycle, and (3) the reductive TCA cycle. However, for wild-type microorganisms, the first two pathways cannot be exploited for SA production because SA itself is an intermediate in the pathways, whereas the last pathway allows the accumulation of SA in the cell (Nghiem et al., 2017). Furthermore, metabolic pathways to SA by either the TCA or the glyoxylate cycle release CO₂ and therefore only four of the six carbons in the glycolysis pathway are preserved. In contrast, the reductive TCA pathway can produce two four-carbon SA molecules from one six-carbon glucose molecule by incorporating CO₂. Therefore the anaerobic pathway is preferred for SA production (Saxena, Saran, Isar, & Kaushik, 2016). Most anaerobic and facultative anaerobe microorganisms ferment carbohydrates to a mixture of acids containing mainly acetic, lactate, formate and succinate as the final products of the metabolism (Van Der Werf, Guettler, Jain, & Zeikus, 1997). Phosphoenol pyruvate (PEP) is the key intermediate in the TCA cycle, i.e. it can be converted to pyruvate and consequently to acetate, formate etc., or to oxaloacetate (OAA) then malate, fumarate and succinate (Figure 4) (Agarwal, Isar, Meghwanshi, & Saxena, 2007; Macy, Ljungdahl, & Gottschalk, 1978). The reductive TCA cycle, also identified as the fermentative pathway, occurs under anaerobic conditions where the enzyme phosphoenolpyruvate carboxylase (PEPC) fixes CO₂ into a molecule of phosphoenolpyruvate (PEP), converting the PEP to oxaloacetate (OAA). Subsequently, the fermentative pathway converts OAA into malate, fumarate and finally succinate. Therefore 2 moles of NADH and a mole of CO₂ are needed for every mole of SA produced from PEP (Figure 5).

Even though the reductive TCA cycle can potentially generate two moles of SA from a mole of glucose - instead of one as in the oxidative TCA cycle (where 2 moles of CO₂ are fixed in the reductive pathway) - the maximum theoretical production is limited by the lack

of a reductant e.g. H₂ or NADH (see Figure 5) (K.-K. Cheng et al., 2012; McKinlay et al., 2007; Vemuri, Eiteman, & Altman, 2002). Whilst engineered *Escherichia coli* is currently used for commercial SA production (Nghiem et al., 2017), naturally occurring wild-type *E. coli* produces SA as a minor fermentation product at an average of only 0.12 mol/mol (Van Der Werf et al., 1997) and up to no more than 0.2 mol of succinate per mol of glucose consumed (Chatterjee, Millard, Champion, Clark, & Donnelly, 2001).

The major wild-type SA producers are bacteria (*Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Ruminococcus flavefaciens*, *Anaerobiospirillum succiniciproducens*, *Corynebacterium crenatum*), fungi (*Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium viniferum*, *Byssoschlamys nivea*, *Lentinus degener*, and *Paecilomyces varioti*) and the yeast *Saccharomyces cerevisiae* (Beauprez et al., 2010; Jiang et al., 2017; Nghiem et al., 2017) (Table 4). Fungi and yeasts produce SA as a by-product which they can synthesize under both aerobic and anaerobic conditions. However, production of SA seems more favorable with bacteria than with fungi because succinate has to cross two membranes (mitochondrial and cytoplasmic) in fungi rather than only one in bacteria in order to be excreted (Roa Engel, Straathof, Zijlmans, Van Gulik, & Van Der Wielen, 2008). *Actinobacillus succinogenes* and *Anaerobiospirillum succiniciproducens* are known to be the highest SA producers, with the former recognized as the most promising for industrial scale SA production (Carvalho, Roca, & Reis, 2016). *M. succiniciproducens*, *B. fragilis* (very recently screened wild-type microorganisms) and *A. succinogenes* can utilize various carbon sources, including carbon dioxide, to produce SA (Beauprez et al., 2010). Specifically, *A. succinogenes*, among other carbon sources can use glycerol, maltose, lactose, fructose, xylose, arabinose etc. (Bechthold, Bretz, Kabasci, Kopitzky, & Springer, 2008). *A. succinogenes* is a highly versatile host since (I) it can efficiently ferment various cheap feedstocks (even mixed) while fixating CO₂ (Guettler, Rumler, & Jainf, 1999), (II) it can resist to

high glucose (S. K. C. Lin, Du, Koutinas, Wang, & Webb, 2008) and SA (Guettler et al., 1999) concentrations, (III) it is non-pathogenic, (IV) it has the ability to form biofilms and (V) can tolerate inhibitors from pretreatment e.g. furfural and HMF (Dessie et al., 2018; Diaz, Blandino, & Caro, 2018; Van Der Werf et al., 1997).

3.3.3.2. Engineered microorganisms. Natural SA producing microorganisms are limited by a series of auxotrophies (cofactors and/or nutrients) which inevitably increase the number of required substrates and the production cost (Beauprez et al., 2010). Several metabolic engineering strategies have therefore been explored to take account of the need to channel microbial pathways to SA and divert fluxes away from alternative products (McKinlay et al., 2007). However, genetic tools to modify the host must be developed (Beauprez et al., 2010) and the currently applied strategies can be grouped in four categories: (1) deletion of pathways involved in accumulation of by-product, (2) amelioration of pathways that lead to SA synthesis, (3) enhancement of substrate transport, and (4) optimization of cofactor metabolism. Recombinant *Saccharomyces cerevisiae* and *Escherichia coli* are model engineered microbes both used for commercial SA production (Table 1).

S. cerevisiae can produce SA either anaerobically or aerobically but the natural fermentative pathway does not efficiently produce SA (Nghiem et al., 2017). The most important advantage offered by engineered *S. cerevisiae* is the ability to produce SA under low pH fermentative conditions. Such tolerance reduces the costs and efforts to neutralize pH during fermentation (Raab, Gebhardt, Bolotina, Weuster-Botz, & Lang, 2010). In fact, low pH fermentation has been reported to be a key factor for an economic and sustainable SA production (Cok, Ioannis, Alexander L., & Martin K., 2013). However, the metabolic flux of *S. cerevisiae* is different and therefore, for an efficient SA production, aeration during fermentation must be applied (Mazière, Pepijn, García, Luque, & Len, 2017).

E. coli is a very well-known engineered bacterium that can efficiently grow on a restricted medium and thus reduce the number of required nutrients compared with naturally occurring microbes (Beauprez et al., 2010). Nonetheless, *E. coli* is sensitive to high acetate concentrations, which is typically found in cellulosic streams (Nghiem et al., 2017), lowering therefore the potential application of this host for second generation biomasses. Furthermore, major SA productivity of *E. coli* takes place through a dual-phase strategy where the produced CO₂ is released and wasted (Vemuri et al., 2002). This factor also influences capital and operating costs since oxygen must be supplied for *E. coli* to grow (Pateraki et al., 2016). Metabolic engineering manipulation of *A. succinogenes* where recently performed to overcome the limits of the natural strain in by-product formation, auxotrophy, pH tolerance and product inhibition (Dessie et al., 2018). Even though manipulations of the *A. succinogenes*' metabolism is possible (Joshi, Schindler, McPherson, Tiwari, & Vieille, 2014), the results are not effective as those obtained for other metabolically modified SA-producing strains (Dessie et al., 2018). However, metabolic engineering strategies of *A. succinogenes* are still at its infancy (Dessie et al., 2018; Pateraki et al., 2016). To conclude, *S. cerevisiae*, *E. coli*, and *A. succinogenes* amongst the best candidates for large-scale SA production. A summary of the advantages and disadvantages of their use can be found in the supplementary material Table S1.

3.4. Separation of succinic acid

Depending on the feedstock, pretreatment and fermentation processes, non-desired by-products such as lactic acid, acetic acid and ethanol may be generated together with SA. These by-products must be separated from SA since they not only reduce the purity (and thus the value) of the SA stream but they also may act as inhibitors of SA production (McKinlay et al., 2007). For example, pretreating lignocellulosic material could release acids (acetic,

formic, levulinic), furan derivatives (furfural, 5- hydroxymethylfurfural (HMF)) and phenolic compounds, such as vanillin, phenol, and p-hydroxybenzoic acid (Palmqvist & Hahn-Hagerdal, 2000). Separation of SA from the fermentation broth is estimated to account for more than 60% to 80% of the total costs and represents the most important source of expenses in SA production (Bechthold et al., 2008). No single specific method has been identified as the best for SA separation and purification, however, the review of K. K. Cheng et al. (2012) on the subject, reported direct crystallization, precipitation, membrane separation, extraction, chromatography and *in situ* separation as major techniques for SA separation. SA is hydrophilic and has a high boiling point. After fermentation, the next step is usually the separation of microbial cells from the liquid phase by using membrane technologies or centrifugation. Subsequently, SA is separated from the other compounds in the fermentation broth and finally purified. Therefore several techniques are typically integrated to separate SA from the fermentation broth. A high purity of the SA stream is required to produce biopolymers, such as those based on butylene succinate (Alexandri et al., 2019), and the polymerization process is inhibited by fermentation by-products such as acetic and formic acid (López-Garzón & Straathof, 2014).

3.4.1. Membrane separation

Membranes play a fundamental role in purifying fermentation products, not only downstream but also during product formation itself (i.e. membrane bioreactor), and potentially lower the total number of unit operations needed to manufacture SA (Alexandri et al., 2019). Cao et al. (2018) investigated the synthesis and separation of SA from glucose and CO₂ with a membrane bioreactor while applying *A. succinogenes* as a production host. Up to 97% separation and recycling of *A. succinogenes* was obtained with a ceramic membrane of 300 KDa pore size and 0.16-m² surface area. This pore size was found to be the best option of the

range studied, i.e. 0.2 μm , and 300, 150 and 50KDa. Cao et al. (2018) used NaOH to buffer the pH during fermentation and consequent organic acid formation instead of the traditional MgCO_3 . The latter is reported to be unattractive for large-scale SA production due to its cost (J. Li et al., 2011), difficult solubilization and the need to handle the large amounts of CaSO_4 that accumulate in the SA extraction process. The use of NaOH simultaneously enables exogenous CO_2 capture instead of the (by microorganisms) preferred intrinsic CO_3^{2-} from MgCO_3 (Cao et al., 2018a). On the other hand, high Na^+ concentrations are toxic and therefore the applied membrane in the bioreactor also separates Na^+ along with SA. Under the studied conditions of 0.4 bar CO_2 and NaOH as buffer, the SA production from repeated batch membrane bioreactors ranged from a product concentration of 27.8 to 30.4 g/L and a productivity of up to 1.39 g/L/h, which identified a concentration limit for SA accumulation at which *A. succinogenes* was inhibited. Only partial SA purification was performed after lowering the pH to 2.0 and recovering unconsumed nitrogen with a spiral wound NF270 membrane. The final SA yield and purity were not investigated, but with this membrane bioreactor and *in situ* separation of salts, SA productivity and CO_2 fixation were 1.39 g/L/h and 0.52 g/L/h, respectively, which was an increase of 39.2% compared to batch culture. Lubsungneon et al. (2014) exploited nanofiltration (NF) coupled with vapor permeation (VP)-assisted esterification to purify SA from glucose-based fermentation broth. After pH adjustment to 2.0 with H_2SO_4 (to obtain organic acids in non-dissociated form – Figure 6), the *A. succinogenes* ATTC 55618 microorganisms were removed by centrifugation and a subsequent cross-flow microfiltration unit (MF), which achieved up to 80% protein removal (to 0.48 g/L). The authors reported membrane fouling by macromolecules and protein adsorption as one of the main issues during the process. The final step was SA recovery carried out through NF and subsequent VP-assisted esterification. Diananofiltration with a tubular membrane module (membrane surface area of 55 cm^2 made of a selective layer of

TiO₂ coated on the supportive α -Al₂O₃ layer) was used to separate organic acids from the fermentation broth. The subsequent SA recovery yield (in the retentate) was up to 98% of the original concentration detected in the fermentation broth before separation. The filtration was carried out over 205h and under a pressure of 400 KPa, at a pH equal to 2.0 and temperature of 30.5 °C. To separate SA from the other organic acids, Lubsungneon et al. (2014) applied a VP-assisted esterification. Permeate was concentrated with a rotary evaporator and then SA was esterified with ethanol to produce diethyl succinate (highest reaction rate 11.13 g/L/h at 80-95°C, equilibrium time reached in 60 to 90 min). The reaction also generated water and highly pure diethyl succinate was obtained through water removal (dehydration) which consequently shifts the equilibrium towards product formation. Afterwards vacuum distillation was applied, followed by ethanol dehydration (in VP with a NaA zeolite membrane) and recirculation. The diethyl succinate was then hydrolyzed to obtain highly pure SA as the final product.

Electrodialysis is a technology based on altering the concentration of electrolytes in a solution and transporting them to another solution that is separated from the first solution by an ion-exchange membrane. The driving force is the applied electrical potential. A key study on SA recovery from a fermentation broth through electrodialysis was done in US Patent No. 5,143,834 (1992). In this study, *A. succiniciproducens* was grown on corn steep liquor and CO₂ and SA purification was performed as follows: (1) the cells and succinate (as well as the other ions) were separated from uncharged compounds e.g. proteins and from the water by electrodialysis (viable cells were recycled). Subsequently, (2) the obtained sodium succinate was converted through a water-splitting electrodialysis to NaOH and SA, and finally, (3) the aqueous SA solution was subjected to an ion exchange purification process to obtain 60 and 80 wt% SA yield and purity, respectively. In contrast, Prochaska et al. (2018) explored reactive extraction associated with bipolar membrane electrodialysis (EDBM) and obtained

up to 90 wt% SA extraction from a glycerol fermentation broth. The actual post-fermentation broth (pH=8.5) was centrifuged for biomass removal, then filtrated with ultrafiltration, and finally subjected to EDBM. The two major advantages of EDBM are: (1) the simultaneous separation of cells (that can be recycled) and SA, with no need to incorporate a cell separation step; and (2) NaOH is economically and theoretically completely recyclable (Yedur, Berglund, & Dunuwila, 2001). Major disadvantages are the potential inhibition by Na^+ in the fermentation step (Cao et al., 2018a), potential membrane fouling (Szczygiełda, Antczak, & Prochaska, 2017), the robustness and lifetime of EDBM (Jansen & van Gulik, 2014), and the high capital and operative costs (K. K. Cheng et al., 2012). However, some recent studies have claimed that electrodialysis is cost-effective and can be used as a process step for SA recovery in a large-scale fermentation plant (Fu et al., 2014; Szczygiełda et al., 2017).

Overall, membrane technologies are key components in the preliminary downstream steps (such as for cell and macromolecule separation) of fermentation-based SA production (Jansen & van Gulik, 2014). Moreover, the toxic Na^+ can be separated *in situ* when using cheaply available NaOH to buffer the fermentation broth. The major problem associated with membrane application is that filtration of post fermentation broths is based on pressure-driven membrane techniques, which may lead to membrane fouling phenomena (Prochaska et al., 2018). However, the physicochemical processes that occur in membrane fouling are rather well-known (C. Wang et al., 2012) and several cleaning techniques have been established at industrial scale (Shi, Tal, Hankins, & Gitis, 2014). Due to the relevance of membrane technologies in SA separation, methods to control permeate flux decline and therefore also membrane fouling (one of the biggest problems in membrane technology) are worth to be mentioned. Actions made to reduce membrane fouling are related to (I) the selection of appropriate membrane and modulus with specific characteristics, (II) selection of the

operating parameters, such as shear stress, permeate flux, pressure and temperature and finally, (III) adjustment of the feed-water composition with respect of foulant components, pH and ionic strength. In SA production membranes can be used in different steps, consequently requiring different sets of modules, membranes, and operating conditions. For removal of large-molecules when using UF a factor to control fouling is to ensure an operating pressure below the so-called threshold pressure, while in SA separation with NF, the isoelectric point of the membrane and the pH of the solution are key factors for an effective separation (W. Zhang, Luo, Ding, & Ja, 2015).

3.4.2. Precipitation

Precipitation with $\text{Ca}(\text{OH})_2$ or CaO is a traditional and commercialized method for isolation of organic acids from fermentation broths. The process consists of precipitating calcium succinate by adding calcium ion sources directly into the fermentation broth. However, most specialty and commodity-based SA commercial products require free SA (Bechthold et al., 2008). Therefore, after calcium succinate recovery by filtration, SA is released by adding H_2SO_4 and subsequently purified with active carbon absorption or ion exchange. SA concentration is finally achieved by evaporation and then crystallization (US Patent No. 5,168,055, 1992). In the patented method (US Patent No. 5,168,055, 1992), the authors separated SA from an *A. succiniciproducens* fermentation broth and obtained 94.2 % purity. More recently, Alexandri et al. (2019) compared different methods for SA separation, including calcium precipitation. The broths were from (1) a fermented synthetic media exposed to *A. succinogenes* and (2) from a filtered spent sulfite liquor as feedstock (a by-product of the paper industry) exposed to *Basfia succiniciproducens*. The SA yields from calcium precipitation were 8.1% and 13.1% (g dry weight of recovered SA/g dry weight of SA in the initial liquid medium) and the purities were 87.2 and 81% (g dry weight of

recovered SA per/ g total dry weight of recovered sample) for the two fermentation broths, respectively. The SA purity from calcium precipitation was slightly lower than that reported in the aforementioned patent (US Patent No. 5,168,055, 1992) (81 and 94%, respectively), but the former was from an industrial waste which is a more complex feedstock than that used in the patented work, i.e. glucose. Note that the yield is the same as that reported by Luque et al. (2009) who achieved a yield of 13% (g dry weight SA recovered crystals per/g initial dry weight of SA in the fermentation broth) by applying calcium precipitation in a fermentation broth of a wheat flour hydrolysate medium exposed to *A. succinogenes*. Even though the application of this well-known precipitation method with $\text{Ca}(\text{OH})_2$ or CaO would reduce the potential risks of establishing a different technology for large-scale production of SA, a large number of reagents (not repeatedly usable) is needed, which consequently produce large quantities of solids and slurry e.g. calcium sulfate (produced in equal amounts to SA) (Zeikus, Jain, & Elankovan, 1999). These solids and slurries must be treated and disposed of, which inevitably contributes to an increase in the operational costs. Furthermore, the process is reported as being neither rapid nor energy efficient (Hestekin, Snyder, & Davison, 2002).

Separation based on precipitation can also be achieved by using ammonia which reacts with SA to produce di-ammonium succinate. The following addition of sulfuric acid in the fermentation broth leads to SA precipitation and ammonium sulfate formation. Subsequent purification of SA is achieved by addition of methanol and recrystallization. The reagents can be recovered by pyrolyzing the by-product, ammonium sulfate, then regenerating ammonia and ammonium bisulfate. Yedur et al. (2001) patented a method based on di-ammonium succinate in which by-products are nearly completely regenerated. In this process, pH is kept neutral at 8 with ammonium cations, and the di-ammonium succinate formed is then reacted with ammonium bisulfate or with sulfuric acid at very low pH ranges

(1.5-1.8). The reaction leads to succinic acid and ammonium sulfate formation. Reagent regeneration was carried out at about 300°C by cracking the ammonium sulfate. The maximum final reported SA yield was 93.3 wt%. The advantage of using ammonia precipitation is reduced waste formation and the fact that the reagents are to a large extent reusable. The main drawbacks are the high energy consumption for reagent regeneration and corrosion of equipment due to the low pH (K. K. Cheng et al., 2012). It is worth highlighting that this technology is currently used by Myriant in a 14kt/y SA plant in the United States (Table 1).

3.4.3. Crystallization

Direct crystallization either from acidification or using ion exchange resins has provided better performances than traditional calcium precipitation (Alexandri et al., 2019; Luque et al., 2009). Luque et al. (2009) separated SA by vacuum distillation-crystallization from two synthetic broths and one real fermentation broth from which 35.7 g/L of SA were produced from a wheat flour hydrolysate medium exposed to *A. succinogenes*. After removal of biomass and impurities from the fermentation broth by centrifugation, membrane filtration and activated carbon, separation was applied using vacuum distillation (at 60°C) and subsequent crystallization (at 4°C) under controlled pH conditions (kept at 4.2) with hydrochloric acid. Selective SA crystallization from the fermentation broth was achieved by exploiting the different solubility of organic acids, which resulted in a purity of 45% (g SA crystals per/ g total acid crystals) and yield of 28% (g dry weight SA recovered crystals per/g initial dry weight of SA in the fermentation broth). This result represented a 50% and 87% improvement in purity and yield, respectively, compared to a calcium precipitation process. Much better results were reported from mock hydrolysates used, to obtain up to 97 and 75 wt% purity and yield, respectively. Similar purity but much higher yield was obtained with

648 direct crystallization (60-75 wt%) compared to calcium precipitation (20-27 wt%) of mock
649 hydrolysates (Luque et al., 2009). Currently, the highest SA recovery purity and yield values
650 from direct crystallization were reported by S. K. C. Lin et al. (2010). These authors exposed
651 a wheat hydrolysate medium to *A. succinogenes* and reported up to 99 and 89.5 wt% purity
652 and yield, respectively, as a result of applying a resin-based vacuum distillation-
653 crystallization method. Interestingly, Alexandri et al. (2019) in their comparative study of
654 different downstream separation processes, identified vacuum evaporation, cooling rate and
655 the previously reported pH (S. K. C. Lin et al., 2010) as the key factors for a successful
656 crystallization process. Vacuum evaporation enabled acetic and formic acid removal (which
657 prevent SA crystallization), while pH and cooling rate affected the form in which SA was
658 obtained (dissociated or non-dissociated – Figure 6) and the crystal formation process,
659 respectively. Optimal pH for direct crystallization of SA was reported at pH 2.0, where SA is
660 non-dissociated and can be selectively crystallized with higher yields (S. K. C. Lin et al.,
661 2010). Under this pH condition, only 3 to 4% of SA is solubilized, while the other organic
662 acids e.g. acetic acid and lactic acid are fully water miscible (S. K. C. Lin et al., 2010).
663 However, Alexandri et al. (2019) reported higher purity and yield by means of ion-exchange
664 resins compared to just lowering the pH to 2.0 (with H₂SO₄). Specifically, after vacuum
665 distillation and crystallization, the SA yield and purity from a real fermentation broth were,
666 respectively, 38.6% and 6.7% higher from cation-exchange than from pH decrease (79%
667 yield and 96% purity from cation-exchange and 57% yield and 90% purity from lowering the
668 pH). The lower values in the work of Alexandri et al. (2019) compared to the values reported
669 in the work of S. K. C. Lin et al. (2010), i.e. 99% yield and 89.5% purity, were attributed to
670 the higher complexity of the spent sulfite liquor used by the former authors instead of the
671 wheat hydrolysates used by S. K. C. Lin et al. (2010). High SA purity with less than 0.09
672 mol% of impurities is required for polymer synthesis (Alexandri et al., 2019). Even though

direct crystallization enables a rather good yield of SA crystals to be obtained without many unit operations (Q. Li, Wang, et al., 2010), the purity is low since other compounds in the fermentation broth can crystallize with SA (Q. Li, Wang, et al., 2010; Thuy, Kongkaew, Flood, & Boontawan, 2017). Therefore crystallization is used and recommended as the final step to purify SA (K. K. Cheng et al., 2012).

3.4.4. Extraction

Salting out is a potential SA separation method which simultaneously removes cells and proteins from the fermentation broth and thus centrifugation and filtration steps can be omitted (Sun, Yan, Fu, & Xiu, 2014). The process is based on the interaction between electrolyte and non-electrolyte compounds, where (the non-electrolyte) would become less soluble under high salt concentration conditions and as a consequence precipitates out. The method allows the extraction of hydrophilic compounds, such as some organic solvents, from an aqueous solution. For example, Sun et al. (2014) investigated SA separation from a real (glucose-based fed-batch fermentation) and a synthetic fermentation broth by means of salting out and subsequent crystallization. The salting out mechanism for SA separation is governed by factors such as salt and solvent concentrations and SA dissociation form. In their study, Sun et al. (2014) first lowered the fermentation broth pH (from *A. succinogenes* on spent sulfite liquor feedstock) to 3.0 with H₂SO₄, then added acetone (30%) and (NH₄)₂SO₄ (20%) to induce SA partitioning. The SA-acetone phase was purified with activated carbon which was then removed by filtration under vacuum evaporation to enable acetone recovery. Subsequently, crystallization was carried out at pH 2.0 and 4°C for 24h. Finally, SA crystals were washed and dried at 70°C for 12h. SA yield and purity were 65% and 97%, respectively, from the synthetic fermentation broth, whereas the values for yield and purity were 65% and 91%, respectively, from the actual fermentation broth, and 99.03% of the cells

and 90.82% of the proteins were removed by direct salting out (without any preceding filtration steps). The same process was investigated by Alexandri et al. (2019) in their comparative separation and purification study (previously mentioned) which achieved 50% and 86% yield and purity, respectively. Even though extraction can lead to high SA purity through simultaneously separating cells and proteins from the fermentation broth and thus replacing for centrifugation and/or filtration steps, the yield is limited. Furthermore, if xylose is present in the fermentation broth, it will crystalize with SA and lower the final product purity. Therefore, since lignocellulosic material (which is rich in xylose) has been identified as the future most important feedstock for SA production, a combination of salting out and crystallization for product recovery would potentially not be a successful strategy to separate and purify the SA if the fermentation process is not highly controlled to avoid the presence of residual xylose.

To summarize, membrane separation and crystallization emerge as promising techniques for SA production from biomass fermentation. However, several combinations of the mentioned separation techniques could be potentially more efficient for SA production.

4.0. Perspective on process alternatives

Every process and unit operation candidate potentially used for SA production has its own merits and limits. Different feedstock sources and host microorganisms will (I) require different pre-treatments, (II) have different sensitivity to formation of fermentation process inhibitors, (III) require a specific set of fermentation conditions, (IV) have specific by-product formation patterns and (V), require a different downstream technique or combination of techniques.

Companies producing SA from biomass fermentation at commercial scale targets specialized markets and the production is far from large-scale bulk SA synthesis. In addition,

every company producing SA uses its own specific process which is different from the others (supplementary material Figure S1). Other options and potential processes have been also proposed (Klein et al., 2017; J. Li et al., 2011; Posada, Rincón, & Cardona, 2012).

Recently, Garg, Woodley, Gani, & Kontogeorgis, (2019) carried out an extensive study which proposes a systematic methodology that integrates process synthesis-intensification and it is capable of providing tools to evaluate a large search space of process alternatives. Such methodology has been applied to produce SA from a co-fermentation with CO₂, obtaining a base case process alternative from a superstructure optimization approach, which was applied for process intensification. Thus, three more economic and sustainable intensified options for SA production, compared with the current processes, were developed (Figure 7). The optimized processes highlight the key role of membranes used both for the synthesis (membrane bioreactor) and in the downstream, and also put emphasis on the use of activated carbon and crystallization. However, the study of Garg et al. (2019) is based on first generation biomasses and thus, it does not include biomass pretreatment.

Therefore, more studies need to be done to find an optimal processing pathway for sustainable production of SA using a systematic approach. The lack of systematic studies on how operation conditions and equipment design affect the operating cost, with regard to fixed productivity, production and purity of SA, prevents the establishment of a standard technology for large-scale production in an economically feasible way (Figure 8). In order to carry out systematic studies, a clear view of the best candidates in every step of the succinic acid production process is needed. In terms of availability, cost, potential, efficiency and technological development, some major candidates can be identified:

(1) Feedstock. Valuable feed-stocks are glycerol, cheese whey, corn stover and other cereal crop residues, sugarcane molasses and bread and bakery wastes. Glycerol and

cheese whey are waste streams and no pretreatment is required before fermentation, consequently reducing greenhouse gasses (GHG) emissions (EC-DGE, 2015). Furthermore, both cheese whey and glycerol could be part of an integrated biorefinery system; valuable proteins could be extracted from the former prior to fermentation to SA (C. S. K. Lin et al., 2013), while glycerol could be combined with biodiesel production (Loureiro da Costa lira Gargalo, 2017). However, depending on the host microorganism, a nutrient supply may be required to optimize the fermentation of both cheese whey and glycerol (Carvalho et al., 2014; Mansouri et al., 2013) inevitably rising the operative costs. Co-substrate fermentation, such as glycerol with Kraft paper by-product (Carvalho et al., 2014) and cheese whey with corn steep liquor (Lee, Lee, Hong, & Chang, 2003) could lower the costs of nutrient supply. High SA yields were also reported from corn stover and other crop residues. These feed-stocks are abundant and have less geographical limitations. However, harsh pretreating condition are needed to be efficiently fermented. Bread and bakery waste were also found to be optimal for SA production and provide all the required nutrients after blending and hydrolysis and fungal autolysis as pretreatment (Leung et al., 2012; A. Y. Z. Zhang et al., 2013).

(2) **Pretreatment.** Efficient and economic pretreatment methods allow extraction of carbon and nourishment from the feedstock while simultaneously avoiding the presence of fermentation inhibitors. While glycerol and cheese whey do not need pretreatments, and bakery and molasses only demand simple pretreatments, lignocellulose feed-stocks (corn stover, sugarcane, wheat flour by-products) pose additional challenges due to energy consuming and wastewater production pretreatment methods and the formation of fermentation inhibitors. However, some promising methods can efficiently solubilize up to 90% sugars (Chandel et al., 2018)

and successfully remove fermentation inhibitors (Salvachúa et al., 2016), leading to high SA yields (Table 4). Valuable pretreatment methods include a thermochemical step with H₂SO₄ or H₂O₂ and especially an enzymatic step (Table 2). Deacetylation with NaOH can also be done to limit the formation of inhibitory compounds (Salvachúa et al., 2016).

- (3) **Fermentation.** *A. succinogenes*, *S. cerevisiae* and *E. coli* are the most promising and investigated SA producers. Engineered *S. cerevisiae* can efficiently produce SA at low pH saving energy and cost in the downstream, while *E. coli* offers high conversion efficiency and requires limited nutrient supply, however, both *S. cerevisiae* and *E. coli* require aeration for efficiently produce SA. *A. succinogenes* captures CO₂ to produce SA, can use various carbon sources rather efficiently, even those derived from crude renewable sources, and can adequately tolerate inhibitors. However, *A. succinogenes* may need nutrient supplies such as nitrogen (Pateraki et al., 2016), and its biochemistry still needs to be fully understood (Beauprez et al., 2010), which limits its potential for engineering manipulation. Another advantage of *A. succinogenes* is the natural ability to create biofilms, which enables chemical reactions capable of compensating the lack of cofactors in the feedstock (Bradfield & Nicol, 2016). Biofilm shows also potential to detoxify inhibitory compounds in fermentation (Bradfield et al., 2015). Continuous systems, different from batch, can be operated with immobilized cells. Continuous operation typically has lower yields compared to batch and fed-batch but higher productivity, less sterilization times and lower contamination risks. SSF in a continuous bioreactor system with immobilized cells emerges as very promising for large-scale production of succinic acid.
- (4) **Downstream.** The downstream of SA production can be divided into some major steps for which different technologies can be efficiently applied.

- 795 • *Cell separation.* Centrifugation and/or microfiltration are typically used to separate

796 cells from the fermentation broth (Alexandri et al., 2019). Membrane bioreactor in a

797 continuous fermentation system and with *in situ* cell recycle and inhibitors removal

798 (Na^+) (Cao et al., 2018a) is highly potential (Ferone et al., 2019).
- 799 • *Concentration, clarification and impurity removal.* This step is done to concentrate

800 SA and remove colors and impurities. Processes typically adopted are: evaporation for

801 removal of water or acetic acid, solvent extraction, adsorption with activated carbon,

802 centrifugation or ultrafiltration (K. K. Cheng et al., 2012). Adsorption through

803 activated carbon comes out as a key step to remove colorants (Garg et al., 2019) while

804 for protein removal, ultrafiltration has been reported to be more efficient than

805 centrifugation (C. Wang et al., 2013) and has been widely reported as economic, low

806 energy consuming and easily scalable (Chaiklahan, Chirasuwan, Loha, Tia, &

807 Bunnag, 2011; Shao, Hou, & Song, 2010; C. Wang et al., 2012). However, membrane

808 fouling can be severe in membrane separation (Lubsungneon et al., 2014) and

809 inexpensive membrane fouling removal techniques need to be developed.
- 810 • *Succinic acid separation.* Several technologies are used to separate SA, for example:

811 precipitation, absorption (e.g. ion exchange resin, zeolite), reactive extraction, bipolar

812 membrane electrodialysis, direct crystallization and nanofiltration. All these

813 technologies have different potentials. Direct crystallization is reported to be a better

814 solution than traditional precipitation (Alexandri et al., 2019; Luque et al., 2009), but

815 the yield is low and impurities could crystalize with SA (K.-K. Cheng et al., 2012).

816 Bipolar membrane electrodialysis has great potential to separate not only SA but also

817 proteins and to recycle cell and titrant (US Patent No. 5,143,834, 1992; Yedur et al.,

818 2001). Even though recent studies suggested bipolar membrane electrodialysis as an

819 efficient and economical solution for large-scale SA production (Fu et al., 2014;

Szczygiełda et al., 2017), doubts about its robustness and lifetime remain (Jansen & van Gulik, 2014; Szczygiełda et al., 2017). Nanofiltration is a rather new technology with unexplored potential for SA separation. High SA yields have been reported for use of NF, but fouling can be severe if macromolecules are not removed beforehand (Lubsungneon et al., 2014), and to date SA separation from other impurities has only been partially achieved (Choi, Fukushi, & Yamamoto, 2008). Therefore further studies on nanofiltration selectivity to SA need to be conducted.

- *Succinic acid purification and dried crystal production.* The final step is product isolation and dried crystals formation. Crystallization is a major technology to produce pure SA crystals. High purity is necessary for polymers synthesis (Alexandri et al., 2019).

The arduous task of identifying an optimal route to cost-effective and sustainable production of SA could be partially tackled by an integrated biorefinery system that combines production of SA and other building block chemicals of significant value. For example, Loureiro da Costa et al. (2017) investigated the potential of integrating SA and biodiesel production, and reported that SA production is among the top three solutions for potentially valorizing glycerol: adding SA production from glycerol carries less economic risk and improves the environmental sustainability of the biodiesel production process. In this sense, economic risk assessment of process alternatives from different feed-stocks would be essential as a decision-support tool towards process implementations for SA production (Mansouri et al., 2019).

5.0. Conclusions

Succinic acid is currently an established platform chemical that forms the basis for producing

several commercially valuable products and chemicals. Industrially produced SA, including that derived from second generation biomasses, is entering the market. However, environmentally sustainable bulk SA production requires major integration between different feed-stocks and separation technologies and also requires production of other products in an integrated biorefinery system; thus, systematic studies are needed in this direction. Some key factors for a competitive SA production from biomass fermentation are identified in this review:

- Many studies and the SA-producing companies themselves are focusing on first generation biomasses for SA production. However, various second generation biomasses show great potential and superior sustainability indicators compared to first generation biomasses. Important feed-stocks are: corn stover, wheat flour by-products, sugarcane molasses, glycerol, cheese whey and bread/bakery wastes. However, important second generation feed-stocks, such as the lignocellulosic one, may require harsh pretreatments to be used. On the other hand, co-fermentation of strategically mixed feed-stocks can compensate auxotrophies. In each case, CO₂ should be fed alongside.
- While glycerol and cheese whey do not need elaborated pretreatment and bread/bakery wastes require only simple operation, lignocellulosic feed-stocks must undergo more complex pretreating conditions. Among the various pretreatments used for the lignocellulosic matter, thermochemical steps with H₂SO₄ or H₂O₂ followed by an enzymatic pretreatment step seem to offer better performances for SA production. In addition, deacetylation during pretreatment can remove inhibitory compounds from lignocellulosic biomasses, consequently improving the SA yields and potentially reducing the separation steps in the downstream.

- 867 • Simultaneous saccharification and fermentation (SSF) reactors have shown several
868 advantages compared to other reactor configurations, including better performance
869 when fermenting lignocellulosic biomasses. Most of the studies and the companies
870 themselves use batch and fed-batch to produce SA substantially focusing on
871 maximizing the yield from (among others) simple feed-stocks. However, continuous
872 fermentation offers several important advantages such as cell immobilization.
873 Simultaneous saccharification and fermentation in a continuous immobilized cell
874 bioreactor, with *in situ* cell recycle has been reported to increase the biomass
875 concentration and thus increase the overall SA productivity. At the same time the
876 capital and operative costs would be reduced since a reduced dilution is required,
877 consequently reducing the needed reactor size.
- 878 • Engineered *E. coli* and *S. cerevisiae* are well established and efficient hosts for SA
879 production, however, pathogenicity, required aeration, emission of CO₂ during
880 production and low tolerance to some inhibitors are important limitations to their
881 utilization. *A. succinogenes* is a promising host and the development of engineering
882 tools for metabolic pathway manipulations, together with the development of
883 integrated biorefinery strategies, could open the door to the large-scale utilization of
884 *A. succinogenes* for SA production.
- 885 • Succinic acid recovery should be carried out at low pH, since lower environmental
886 impacts have been reported under those conditions. However, only yeasts, such as *S.*
887 *cerevisiae*, can tolerate low pH conditions. Membranes, activated carbon and
888 crystallization appear as key technologies for downstream processing of SA.

889 Further process optimization studies based on the data collected in this review are
890 needed to identify optimal processes. The conclusions of this work can be used to elaborate a

891 superstructure optimization that may suggest viable processes and sequences of processes for
892 feasibly large-scale production of SA.

893

894

Figure 1. Overview of some selected specialty and commodity chemicals that can be synthesized from succinic acid (Arshadi et al., 2008; McKinlay et al., 2007).

Figure 2. Production of succinic acid from petrochemical derived maleic anhydride.

Figure 3. Distribution of world food waste that would be suitable for succinic acid production. With the exception for data on rice waste in Asia, which are from the work of Gunarathne et al. (2019), all the other data are based on the work of C. S. K. Lin et al. (2013).

Phosphoenol pyruvate

Succinic acid

Figure 5. Reductive process in the tricarboxylic acid cycle (Saxena et al., 2016).

912

913 Figure 4. General TCA cycle found in many natural fermentative microorganisms, including
 914 *E. coli*, *A. succinogenes*, *A. succiniciproducens* and *M. succiniciproducens*. Lactate is not
 915 produced by *A. succinogenes*, ethanol is not produced by *M. succiniciproducens* when grown
 916 on glucose, and *A. succiniciproducens* does not synthesize formate (McKinlay et al., 2007).
 917 The reductive pathway of the TCA cycle is shown in red, while the pathway that specifically
 918 occurs in *A. succinogenes* for xylose and glycerol is shown in red burgundy. The glyoxylate
 919 shunt and the oxidative branch of the TCA cycle represented in blue (Carvalho et al., 2014;
 920 McKinlay et al., 2007; Pateraki et al., 2016; Xu et al., 2018). These metabolic pathways are
 921 exploited in anaerobic succinate engineered *E. coli* (McKinlay et al., 2007). G6P: glucose-6-
 922 phosphate; F6P: fructose-6-phosphate; F1,6P: fructose-1,6-biphosphate; G3P: glycerate-3-
 923 phosphate; GA3P: glyceraldehyde-3-phosphate; G2P: glycerate-2-phosphate; PEP:
 924 phosphoenolpyruvate; OAA: oxaloacetate.

925

926 Figure 6. Effect of pH on succinic acid dissociation to form HAS- ($\text{C}_4\text{H}_5\text{O}_4^-$) and SA2-
 927 ($\text{C}_4\text{H}_4\text{O}_4^{2-}$); the $\text{pK}_{\text{a}1} = 4.16$, $\text{pK}_{\text{a}2} = 5.6$ (Jansen & van Gulik, 2014).

928 .

Figure 8. Generic process for succinic acid production listing the most relevant second generation feed-stocks, the proposed pretreatments and fermentation conditions and the optimal range under which major separation techniques can operate.

a) Flowsheet alternative 1

b) Flowsheet alternative 2

c) Flowsheet alternative 3

933

934 Figure 7. Generated alternative processes for the production of bio-based SA (with permission
935 from Garg et al. (2019).

936 Table 1. Overview of the major industrial actors producing succinic acid (SA) from fermentation today, their presumed technologies
 937 and resultant challenges.

Company	Capacity (kt/year)	Operative	Raw material	Fermentation/ Microorganism	Downstream recovery	Potential problems/Challenges	Location	Ref.
BioAmber ¹	3 t/y demo plant	2010	Wheat glucose	<i>E. coli</i>	Electrodialysis	- Electricity costs for EDBM - Effect of sodium in fermentation	Pomacle, France	(EC-DGE, 2015)
BioAmber ¹ Mitsui & Co	30-50	2015	Corn glucose	<i>Candida krusei</i> /pH 3, aerobic batch	DAS ² + reactive evaporation		Sarnia, Canada	(Cavani, Albonetti, Basile, & Gandini, 2016; EC-DGE, 2015; Finley et al., 2013)
Biosuccinium (Roquette)	10	2012	Starch/Sugar	pH 3, dual phase fed-batch/ Recombinant <i>S. cerevisiae</i> (by DSM) ³ .	Direct separation of SA	- Effect of low pH on fermentation performance.	Cassano, Spinola, Italy	(EC-DGE, 2015; Ferone et al., 2019; Jansen & van Gulik, 2014; Nghiem et al., 2017)
Myriant	14	2013	Glucose/ Sugars ⁴	<i>E. coli</i> ⁵	Ammonia precipitation	- SA recovery in di-ammonium - Ammonia effect in fermentation	Lake providence, Luisiana, USA	(EC-DGE, 2015; Myriant, 2019)
Succinity (joint venture BASF & Corbion-Purac)	10	2014	Glycerol/ Sugar/CO ₂	Anaerobic fed-batch/ <i>B. succiniciproducens</i>	MgOH as neutralizer followed by recycling	- Dependency on two recycles in process - Cost and performance of MgCl ₂ cracking - SA recovery in MgCl ₂ -stream	Montmelo, Spain	(BASF, 2014; EC-DGE, 2015; Pateraki et al., 2016)

938 1. BioAmber is currently in CCAA proceedings (Companies' Creditor Arrangement Act)(Blain, 2019)

939 2. DAS: diammonium succinate.

- 940 3. The company has developed a recombined *S. cerevisiae* for co-production of ethanol and SA. It is not clear if this is the strain used in the plant.
- 941 4. The glucose is obtained from sorghum, while sugars are extracted from lignocellulosic biomasses.
- 942 5. The *E. coli* strain was specifically developed to produce succinic acid from lignocellulose-derived sugars.

943 Table 2. Summary of some pretreatment methods used in biorefinery with their advantages and disadvantages and their use for SA

944 production (Modified from Kumar et al., (2009)).

Pretreatment method	Advantages	Disadvantages and limits	Examples in SA production	Specific details on used pretreatments/Notes	References
Steam explosion	Degradation of hemicellulose and lignin transformation; cost-effective	Partial destruction of xylan and of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms.	Oak and wood chips Crop stalks (including corn and cotton)	215 °C for 3 min in an 8 l exploder followed by enzymatic hydrolysis at 50°C for 3 d. 10 min at 1.5 MPa then filtration, dehydration, explosion to 1 % (w/v) NaOH and 4% (v/v) H ₂ O ₂ for 24 h at the room temperature, followed by enzymatic pretreatment.	(Kim et al., 2004; Lee, Lee, Hong, Chang, et al., 2003) (Q. Li, Yang, et al., 2010)
Ammonia Fiber explosion	Increase accessible surface area, partial removal of lignin and hemicellulose, does not produce inhibitors for downstream processes.	Not efficient for lignin-rich biomass.	-	-	

CO ₂ explosion	Increase accessible surface area; no fermentation of inhibitory compounds; cost-effective	Does not modify lignin or hemicelluloses	-	-	
Alkaline hydrolysis	Increase accessible surface area; removal of hemicellulose and lignin.	Long residence times required; irrecoverable salts formed and incorporated into biomass.	Corn stover	Soaked in 2% (v/v) H ₂ O ₂ solution (solid–liquid ratio of 1:15), then 4 M NaOH to pH 11.5 at 30 °C for 16 h	(Zheng et al., 2010)
Acid hydrolysis	Hydrolyze hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances.	Corn stover	Hydrothermal pretreatment of 200°C, 0.75% H ₂ SO ₄	(T. Zhang, Kumar, Tsai, Elander, & Wyman, 2015)
			Sugarcane bagasse	H ₂ SO ₄ , 1% (v/v); solid : liquid ratio, 1:2; 121°C; 40-min	(Borges & Pereira, 2011)
			Sugarcane molasses	Soaked in 5 M H ₂ SO ₄ and heated at 60°C for 2h	
Oxidative agents	High conversion efficiency; no toxic compounds released	Incomplete lignin solubilization.	Hemp	Chopped and then cutting milled to <1 mm particle size; 2 M NaOH to pH 11.5, then autoclaved with H ₂ O ₂ at 121°C for 1h.	(Gunnarsson, Kuglarz, Karakashev, & Angelidaki, 2015)
Mechanical comminution	Reduce cellulose crystallinity	Usually requires more energy than the inherent biomass	Various lignocellulosic biomasses	This step is largely used in pretreatments of	

		energy; high greenhouse gas emissions ^a .		lignocellulosic biomasses also for SA production.	
Organosolv	Hydrolyze lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost	-	-	
Biological	Degrade lignin and hemicelluloses; low energy requirements; less corrosion issues	Hydrolysis rate is very low. Cellulosic enzymes are expensive.	Various lignocellulosic biomasses	Many studies on SA production from lignocellulosic matter use an enzymatic step in the pretreatment process.	(Gunnarsson et al., 2015; Kim et al., 2004; Lee, Lee, Hong, Chang, et al., 2003; Q. Li, Yang, et al., 2010; Salvachúa et al., 2016)

945

946 Table 3. Major advantages and disadvantages of the two most relevant configurations for SA production (SHF and SSF) and the
947 operational techniques (batch, fed-batch and continuous).

Advantages		Disadvantages
<i>Reactor's configuration</i>		
SHF	Optimization of hydrolysis and fermentation processes. Higher control of fermentation inhibitors and potential reduction of downstream processes.	High capital and operative costs. Low yield with <i>E. coli</i> on glucose, galactose and sucrose (Akhtar et al., 2014).
SSF	Simple; cost-effective since low capital cost and low energy consumption; reduced substrate toxicity (Zheng et al., 2010).	Softwood lignocellulosic biomass contains 10% silicon, which is toxic for enzymes in SSF (Akhtar et al., 2014).

Operational techniques

Batch	Simple to operate; high yield	Low production rate; repeated inoculation and sterilization times; low biomass concentration which leads to big reactor's volume required.
Fed-batch	Simple; efficient for toxic feed-stocks; biomass can be concentrated, thus reduced reactor's volume are needed.	Reduced production rate; repeated inoculation and sterilization times.
Continuous	High production rate; high yield with cell immobilization; biomass concentration and thus reduced reactors volume.	Complex to operate; low yield if no cell immobilization applied.

948

949 Table 4. Fermentation-based succinic acid (SA) production from different carbon sources: the microorganisms, the substrates, the final
950 SA titer, production rate and SA yield are presented.

Raw material	Intermediate platform	Type of fermentation	Microorganism	Titer (g/L)	Productivity (g/l/h)	Yield	Ref.
<i>Pure carbon sources and first generation biomasses</i>							
	Glucose	Dual-phase batch	<i>E. coli</i> (Tang1528)	89.4	1.24	83.0 wt%	(Yu et al., 2016)
	Glucose	Micro-aerobic, fed-batch with membrane for cell recycling	<i>C. glutamicum</i> (Δ ldhA-pCRA717)	146	3.2	92.0 wt%	(Okino et al., 2008)
	Glucose	Anaerobic batch	<i>A. succinogenes</i>	39.4 $\pm$ 0.7	-	79.3 $\pm$ 1.5 wt%	(Liu et al., 2008)
	Glucose	Continuous with immobilized cells	<i>A. succinogenes</i>	12.0 at D = 0.56 h ⁻¹	6.35	69 $\pm$ 2 wt%	(van Heerden & Nicol, 2013)
	Glucose	Continuous with immobilized cells	<i>A. succinogenes</i>	18.0 at D = 0.5 h ⁻¹	9.2	70 wt%	(Brink & Nicol, 2014)
	Sucrose	Anaerobic batch	<i>E. flavescens</i>	2.82 $\pm$ 0.12	-	-	(Agarwal et al., 2007)

Sucrose	Fed-batch	<i>A. succinogenes</i> (NJ113)	60.4	2.16	83.0 wt%	(Jiang et al., 2014)
Sucrose	Anaerobic batch	<i>A. succinogenes</i>	40.3 ± 0.8	-	81.4 ± 1.6 wt%	(Liu et al., 2008)
Fructose	Anaerobic batch	<i>E. flavescens</i>	0.93 ± 0.04	-	-	(Agarwal et al., 2007)
Fructose	Anaerobic batch	<i>A. succinogenes</i>	1.2 ± 0.4	-	78.6 ± 1.8 wt%	(Liu et al., 2008)
Maltose	Anaerobic batch	<i>E. flavescens</i>	1.3 ± 0.07	-	-	(Agarwal et al., 2007)
Xylose	Anaerobic batch	<i>E. flavescens</i>	0.52 ± 0.02	-	-	(Agarwal et al., 2007)
Xylose	Anaerobic batch	<i>A. succinogenes</i>	32.6 ± 1.2	-	76.9 ± 2.7 wt%	(Liu et al., 2008)
Lactose	Anaerobic batch	<i>E. flavescens</i>	2.1 ± 0.09	-	-	(Agarwal et al., 2007)
Galactose	Anaerobic batch	<i>E. flavescens</i>	0.66 ± 0.03	-	-	(Agarwal et al., 2007)
Sorbitol	Anaerobic batch	<i>E. flavescens</i>	0.61 – 14.8	-	-	(Agarwal et al., 2007)
Mannitol	Anaerobic batch	<i>E. flavescens</i>	0.21±0.03	-	-	(Agarwal et al., 2007)
Rhamnose	Anaerobic batch	<i>E. flavescens</i>	0.24±0.04	-	-	(Agarwal et al., 2007)
Arabinose	Anaerobic batch	<i>E. flavescens</i>	0.13±0.04	-	-	(Agarwal et al., 2007)
Glycerol	Anaerobic batch	<i>A. succinogenes</i> (ATCC 55618)	24.39 ± 4.5	2.13 ± 0.56	95±20 wt%	(Carvalho et al., 2014)
Glycerol	Anaerobic fed-batch	<i>A. succinogenes</i> (ATCC 55618)	49.62	0.96	64 wt%	(Carvalho et al., 2014)
Glycerol	Anaerobic batch	<i>E. flavescens</i>	1.3±0.07	-	-	(Agarwal et al., 2007)
GAX (Glucose, Arabinose, Xylose)	Continuous with immobilized cells	<i>A. succinogenes</i>	20.5 at D = 0.7 h ⁻¹	15.0	0.56	(Ferone et al., 2018)

Starch		Anaerobic batch	<i>E. flavescens</i>	0.13±0.006	-	-	(Agarwal et al., 2007)
Wheat		SmF-based ¹	<i>A. succinogenes</i> (ATCC 55618)	16	0.31	19 wt%	(Du et al., 2007)
Wheat		Solid state fermentation	<i>A. succinogenes</i> (ATCC 55618)	64.2 ± 1.0	1.19 ± 0.05	40 wt%	(Du et al., 2008)
Second generation biomass							
<i>Arundo donax</i>	Glucose Xylose	Anaerobic batch	<i>B. succiniciproducens</i> BPP7	17	0.35	54% (g SA/g glucose+xylose)	(Cimini et al., 2016)
Cane molasses		Anaerobic batch	<i>A. succinogenes</i>	46.4	0.97	79.5% (g SA/g glucose)	(Liu et al., 2008)
Cane molasses		Anaerobic fed-batch	<i>A. succinogenes</i>	55.2	1.15	94% (g SA/g glucose)	(Liu et al., 2008)
Cane molasses		Anaerobic batch	<i>E. flavescens</i>	0.5 ± 0.02	-	-	(Agarwal et al., 2007)
Cane bagasse	Hemicellulose	Anaerobic batch	<i>A. succinogenes</i> (CIP 106512)	22.5	1.01	43 wt%	(Borges & Pereira, 2011)
Cane bagasse		Anaerobic batch	<i>A. succinogenes</i> (CCTCCM2012036)	120	1.65	80.5 wt%	(Chen et al., 2016)
Cane bagasse		Anaerobic batch	<i>E. coli</i> (BA305)	83	-	87.0 wt%	(Liang et al., 2013)
Wheat milling by-products		Solid state fermentation	<i>A. succinogenes</i> (ATCC55618)	62.1	0.91	8.7 wt%	(Dorado et al., 2009)
Wheat straw ²		Anaerobic batch	<i>F. succinogenes</i> S85 (ATCC 19169)	2.02	≈ 22.5	≈ 3 wt%	(Q. Li et al., 2010)
Corn straw hydrolysate	Glucose, Xylose	Anaerobic fed-batch	<i>A. succinogenes</i> (CGMCC1593)	53.2	1.21	82.5 wt%	(Zheng et al., 2009)
Corn straw hydrolysate	Glucose, Xylose	Anaerobic batch	<i>A. succinogenes</i> (CGMCC1593)	45.5	0.95	80.7 wt%	(Zheng et al., 2009)
Corn stalk		Anaerobic batch	<i>A. succinogenes</i> (BE-1)	15.8	0.56	66.0% (g SA/g total sugars)	(Q. Li, Yang, et al., 2010)
Corn stover		Anaerobic batch	<i>A. succinogenes</i> 130Z (ATCC 55618)	42.8	1.51	0.74% (g SA/g total sugars)	(Salvachúa et al., 2016)

Whey	Anaerobic fed-batch	<i>A. succiniciproducens</i>	24.0	2.1	72.0 wt%	(Samuelov et al., 1999)	
Bread waste	Anaerobic batch	<i>A. succinogenes</i> (ATCC 55618)	47.3	1.12	55 wt%	(Leung et al., 2012)	
Bakery waste	Solid state fermentation	<i>A. succinogenes</i>	24.8 ⁽³⁾	0.79 ⁽³⁾	28 wt% ⁽⁴⁾	(A. Y. Z. Zhang et al., 2013)	
			31.7 ⁽⁵⁾	0.87 ⁽⁵⁾	35 wt% ⁽⁵⁾		
Third generation biomass							
Macroalgae <i>L. japonica</i>	Mannitol	Dual-phase batch	<i>E. coli</i> (BS002)	14.32 ± 0.09	-	1.39 ± 0.01 (mol SA/mol total sugars)	(Bai et al., 2015)
	Glucose	Dual-phase batch	<i>E. coli</i> (BS002)	9.86 ± 0.48	-	1.01 ± 0.05 (mol SA/mol total sugars)	(Bai et al., 2015)
Macroalgae <i>L. digitata</i>	Anaerobic batch	<i>A. succinogenes</i> 130Z (DSM 22257)	-	0.50	86.49% (g SA/g total sugars)	(Alvarado-Morales et al., 2015)	

951

952 1. Submerged Fermentation

953 2. Not pretreated

954 3. Pretreated

955 4. From cake waste

956 5. From pastry waste

957

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1392

1393 **Supplementary material**

1394 Table S1. Summary of advantages and disadvantages of three of the most relevant microorganisms for SA production.

Microorganism	Advantages	Disadvantages
<i>S. cerevisiae</i>	1. Can work at low pH being then cost effective and “green”.	1. Complex gene editing required;
	2. Among the best known engineered microbes;	2. Oxygen required for the best performance;
<i>E. coli</i>	1. Among the best known engineered microbes;	3. Complex gene editing
	2. High yield and high efficiency;	1. Gene editing required;
	3. Restricted amount of nutrients required	2. Limited application for second generation biomasses;
		3. High capital and operative costs (Dual-phase reactor);
		4. Pathogenic
		5. CO ₂ emission and oxygen provision required for best performance.
<i>A. succinogenes</i>	1. High natural SA producer (no gene editing required);	1. Requires auxotrophies, especially nitrogen;
	2. Versatile to many substrates;	2. By-products formation;
	3. Tolerant towards pollutants from pretreatment of lignocellulose biomass:	3. Relatively new microbe with limited engineering tools and knowledge;
	4. Natural biofilm producer;	
	5. Low capital costs;	
	6. CO ₂ uptake;	

1395

BioAmber

Reverdia

Succinity

Myriant

Figure S1. Presumed processes used by the companies producing SA from fermentation at commercial scale. While the Myriant flow process was released by the company itself (Shmorhum, 2015), the other processes were draw based on the review of Nghiem et al., (2017).