Electronic Supplementary Information (ESI)

Sustainability Assessment of Succinic Acid Production Technologies from Biomass using Metabolic Engineering

Merten Morales,‡ Sara Badr, Sven Linster, Ioannis Kourlimpinis and Konrad Hungerbühler
Institute for Chemical and Bioengineering, Department of Chemistry and Applied Bioscience, ETH Zurich, CH-8093 Zurich, Switzerland

Meriç Ataman,‡ and Vassily Hatzimanikatis
Laboratory of Computational Systems Biotechnology, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Stavros Papadokonstantakis
Department for Energy and Environment, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

‡ These authors contributed equally to this work.
* Correspondence concerning this article should be addressed to S. Papadokonstantakis at stavros.papokonstantakis@chalmers.se; V. Hatzimanikatis at vassily.hatzimanikatis@epfl.ch.

1. Process modelling

1.1. Neutral fermentation with reactive extraction

The process flowsheet for the neutral fermentation with reactive extraction is shown in Figure 1 in the manuscript. The process comprises three main modelling steps: glucose (*GLU*) fermentation with *in situ* neutralisation of the as-formed bio-*SA* with calcium hydroxide to calcium succinate (Step-1), sulphuric acid-mediated hydrolysis of calcium succinate to bio-*SA* and gypsum (Step-2), reactive extraction for the recovery of bio-*SA* from fermentation medium through an extraction, a back extraction and washing of bio-*SA* crystals for the removing extraction solvents (Step-3). The process design for *SA-1* and *SA-6* is the same and can be only differentiated by the origin of the sugar feedstock (sugar in *SA-1* from lignocellulosic biomass, sugar in *SA-6* from sugar beet). The *LCI* for process design *SA-1/6* are presented in Table 3 in the manuscript. Details to these steps are provided in the following.

Step-1: *GLU* fermentation and in situ bio-*SA* neutralisation

The *GLU* solution consists of 10%wt *GLU* in water. Before the *GLU* solution enters the fermenter it is desterilized at 80°C to prevent bacteria deactivation through contamination. Besides the *GLU* solution a bacteria make up stream and the fermentation medium is added to the fermenter. The anaerobic fermentation is modelled in Aspen Plus® V8.6 with a RStoic model. The lumped reaction for the bio-*SA* production is expressed through the genetically engineered *E.coli* strain. The calcium succinate production is implemented in the model through a two-step reaction:

$$10\ C_6H_{12}O_6 + 0.001\ NH_3 + 0.0001\ H_3PO_4 + 2e^{-5}\ H_2SO_4 \rightarrow$$
$$1e^{-4}\ \textit{E.coli} + 14\ C_4H_6O_4 + 1.998\ C_4H_6O + 20.01\ H^+ + 2.0009\ H_2O$$

$$C_4H_6O_4 + Ca(OH)_2 \rightarrow C_4H_4O_4Ca + 2\ H_2O$$

The metabolically engineered *E.coli* strain is defined through the chemical formula:

$$C_{40.9}H_{63.0948}O_{15.75}N_{10.5092}P_{0.9274}S_{0.2448}$$

The batch fermentation takes place at 1bar and 32°C with a retention time of 72 hours. It is assumed that both reactions reach full conversion and therefore no *GLU* remains in the fermentation outlet. Furthermore a molar excess of 20% calcium hydroxide is added to the fermenter to control the pH level.[1] This high amount of excess calcium hydroxide presents a conservative control strategy to prevent acidity in the fermentation reactor. The decrease of the excess calcium hydroxide would lead to a slight improvement of environmental impact and operating costs. It is assumed that 90% of the *E.coli* can be recycled from the fermentation broth after a two stage centrifuge (SSplit model in Aspen Plus® V8.6). The anaerobic recovery of the *E.coli* takes place in another fermenter (RStoic model in Aspen Plus® V8.6) at 37°C, 1bar and with a retention time of 6 hours. Under these reactor conditions the *E.coli* strain uses the fermentation medium with a higher conversion for the reproduction of its bacterium and lowers at the same time the conversion of the fermentation medium to bio-*SA*. The *E.coli* recovery was lumped in a single-step reaction with the formula:

$$10\ C_6H_{12}O_6 + 3.15188\ NH_3 + 0.278\ H_3PO_4 + 0.0755949\ H_2SO_4 \rightarrow$$
$$0.3\ \textit{E.coli} + 9.88762\ C_4H_6O_4 + 8.1811\ CO_2 + 55.48\ H^+ + 0.775839\ H_2O$$

Step-2: Sulphuric acid-mediated hydrolysis of calcium succinate

The fermentation broth is directed into a crystallizer after the *E.coli* is recovered. It is assumed that calcium succinate is still dissolved at 32°C, namely the temperature of the fermentation broth. The solubility of calcium succinate, which decrease with increasing temperature, is approximated with the data from calcium

sulfate in Aspen Plus® due to data gaps in the literature. The crystallizer (Crystallizer model in Aspen Plus® V8.6) operates at 80°C and ambient pressure.[1] After the precipitation the solid calcium succinate is separated via filtration (SSplit model in Aspen Plus® V8.6) and rinsed with water to remove nutrients from the fermentation medium. In a sulphuric acid-mediated hydrolysis calcium succinate is converted to bio-*SA* and gypsum:

$$H_2SO_4 + C_4H_4O_4Ca \rightarrow C_4H_6O_4 + CaSO_4$$

The reactor (RStoic model in Aspen Plus® V8.6) operates at 130°C and 2.6 bar with a conversion of 90% of calcium succinate. The same conditions were assumed like in a similar production step for lactic acid, due to lack of literature data.[2] Sulfuric acid is added with a molar excess of 10%.[1] Gypsum is separated via filtration (SSplit model in Aspen Plus® V8.6) and landfilled after the hydrolysis. The aqueous solution has a bio-*SA* concentration of 16.6 %wt after this process step.

Step-3: Bio-*SA* recovery with reactive extraction

The recovery of bio-*SA* from water (aqueous phase) is done through three extraction columns (Sep model in Aspen Plus® V8.6) which have an individual separation efficiency of 0.86.[3] The extraction solvent (organic phase) consists of 87%wt 1-octanol and 13%wt trioctylamine. Each extraction column operates at 50°C and ambient pressure. According to Kurzrock et al.[3] the volumetric inlet flows of the organic and aqueous phase need to be equal for each extraction column to achieve the separation efficiency of 0.86. A solvent loss per extraction column of 0.21% 1-octanol into the aqueous phase is estimated with a decanter model (2-phase liquid-liquid decanter model in Aspen Plus® V8.6). Therefore, a constant make up of 1-octanol is required for the extraction. The remaining fermentation broth including also the 1-octanol lost in the aqueous phase leaves the third extraction column and is treated in a waste water treatment plant. In a back extraction column bio-*SA* is re-extracted from the organic phase. For this task, a solvent consisting of 25%wt trimethylamine and 75%wt water, extracts bio-*SA* with a separation efficiency of 1 from the organic phase into an aqueous phase (Sep model in Aspen Plus® V8.6). The minimum ratio between trimethylamine and bio-*SA*, which is required to achieve this separation efficiency, was experimental investigated by Kurzrock et al.[4] For the separation efficiency of 1 a minimum of 2 mol trimethylamine per mol bio-*SA* is required. Kurzrock et al.[4] suggested to use 9.3 mol of trimethylamine per mol bio-*SA* to secure the separation efficiency of 1, due to the high volatility of trimethylamine. A solvent loss of 0.46% 1-octanol in the back extraction column is again estimated with a 2-phase liquid-liquid decanter model in Aspen Plus® V8.6. The respective amount is constantly added as a make-up stream. Kurzrock et al.[3] stated that the back extraction operates at 100°C and ambient pressure. The aqueous bio-*SA* solution is treated in crystallizer after the back

extraction. The crystallizer (Crystallizer model in Aspen Plus® V8.6) operates at 20°C and ambient pressure with the solubility data for *SA* measured by Seidel.[5] In a filtration unit (Sep model in Aspen Plus® V8.6) the solid bio-*SA* is separated form liquid phase. The loss of 0.1% of the trimethylamine-water solvent through the filtration is constantly fed via a make-up stream into the system. The solid bio-*SA* is cleaned from the remaining extraction solvents (SWash model in Aspen Plus® V8.6) and dried with air (Dryer model in Aspen Plus® V8.6). The washing unit operates with a liquid-to-solid mass ratio of 2 and the dryer with preheated air at a temperature of 130°C.

1.2. Neutral fermentation with electrodialysis

The flowsheet for the neutral fermentation with electrodialysis purification (*SA-2*) is shown in Figure 2 in the manuscript and the relative *LCI* data is shown in Table 3. The process comprises four main modelling steps: *GLU* fermentation with *in situ* neutralisation of the as-formed bio-*SA* with sodium hydroxide to sodium succinate (Step-1), desalting electrodialysis to enrich the sodium succinate concentration (Step-2), water splitting electrodialysis to convert sodium succinate into an oversaturated bio-*SA* solution (Step-3) and further purification to high grade bio-*SA* (99.9%) through crystallization and washing (Step-4).

Step-1: *GLU* fermentation and in situ bio-*SA* neutralisation

The *GLU* solution consist of 10%wt *GLU* and water. The process step is identical to Step-1 for neutral fermentation with reactive extraction (*SA-1/6*) (presented in Figure 1 in the manuscript) with the only difference that sodium hydroxide is used to control the pH level during the fermentation. The reason for this is that the electrodialysis membranes cannot handle divalent ions. The same process modelling as for the process design *SA-1/6* was applied regarding to the 20% molar excess of sodium hydroxide in the fermentation, *E.coli* characteristics (e.g. chemical composition, lumped reactions, recovery via filtration) as well as the operating conditions (e.g. temperature, pressure, retention time) in both reactors.

Step-2: Desalting electrodialysis to recover bio-*SA* salt

The fermentation broth with disodium succinate is further treated in two desalting electrodialysis (*DED*) (Sep model in Aspen Plus® V8.6) based on the experimental results from Datta et al.[6, 7] The target of both units is to separate the disodium succinate salt from the non-ionic species of the fermentation broth. The driving force of the electrodialysis is the electrical potential difference across an alternating series of positive and negative charged polymer membranes. The fermentation broth enters the first *DED* and 80% of the sodium succinate are recovered based on the experimental results of Datta et al.[7] Half of the enriched electrolyte is recycled back to the first *DED* to decrease raw material costs for the electrolyte (i.e., fresh

sodium succinate). The rest of the electrolyte is further concentrated in a second *DED*, which recovers around 75% of sodium succinate from the diluting stream. The concentrating stream, which exists the second *DED*, has a concentration of 22.8 %wt sodium succinate. The energy consumption for both *DED* units is approximated based on the work of Garde[8] with 3.5kWh electricity consumed per kg of sodium succinate in the entering diluting stream.

Step-3: Recovery of bio-*SA* by water splitting electrodialysis

The concentrating stream (electrolyte), which contains the bio-*SA* salt is further treated in water splitting electrodialysis (*WSED*) to convert sodium succinate to supersaturated bio-*SA* solution.[7] The *WSED* is modelled with RStoic and Sep unit in Aspen Plus® V8.6 which are arranged in series. Glassner et al.[6] stated that the feed stream of the *WSED* needs to be undersaturated succinate solution (< 25 %wt), which is then converted into a supersaturated bio-*SA* solution by passing through the *WSED* unit. Sodium succinate is converted to bio-*SA* with a fractional conversion of 1 in the RStoic model according to the following reaction.

$$C_4H_4O_4Na_2 + 2\ H_2O \rightarrow C_4H_6O_4 + 2\ NaOH$$

This means that the complete sodium succinate is converted into bio-*SA* in the *WSED*. In the Sep unit the sodium hydroxide is separated from the supersaturated bio-*SA* solution and recycled back to the fermentation. The bio-*SA* stream leaving the *WSED* contains, besides bio-*SA* (19%wt), residual sodium cations, amino acids and sulfate ions. The electricity consumption for the *WSED* was approximated with 2.5kWh per kg of sodium succinate entering the *WSED* based on the experimental work of Garde et al.[8]

Step-4: Crystallization and washing of bio-*SA*

The supersaturated bio-*SA* solution is treated in a crystallizer at 20°C (Crystallizer model in Aspen Plus® V8.6) to separate bio-*SA* from the solution without any ion exchange polishing needed in prior.[6] Bio-*SA* solubility in water measurements were provided by Seidel.[5] Bio-*SA* crystals with 99.9% purity were obtained through subsequent filtration and washing steps.[6]

1.3. Neutral fermentation with ion exchange

The flowsheet for the fermentation with ion exchange purification is shown in Figure 3 in the manuscript and the relative *LCI* data are shown in Table 3. The process comprises four main modelling steps: *GLU* fermentation with *in situ* neutralisation of the as-formed bio-*SA* with calcium hydroxide to calcium succinate (Step-1), sulphuric acid-mediated hydrolysis of calcium succinate to bio-*SA* and gypsum (Step-2), ion

exchange treatment for the removal of ionic impurities (Step-3) and crystallization and washing of the product bio-*SA* (Step-4).

Step-1: *GLU* fermentation and in situ bio-*SA* neutralisation

The *GLU* solution consist of 10%wt *GLU* and water. The process step is identical to Step-1 for neutral fermentation with reactive extraction (*SA-1/6*) (presented in Figure 1 in the manuscript). The same process modelling as for the process design *SA-1/6* was applied regarding to the 20% molar excess of calcium hydroxide in the fermentation, *E.coli* characteristics (e.g., chemical composition, lumped reactions, recovery via filtration) as well as the operating conditions (e.g., temperature, pressure, retention time) in both reactors.

Step-2: Sulphuric acid-mediated hydrolysis of calcium succinate

This process step is identical with the process Step-2 for the process design neutral fermentation with reactive extraction (*SA-1/6*).

Step-3: Ion exchange treatment

The purification technology is developed based on the experimental work of Datta et al.[1] Ion exchangers are applied for the removal of the residual cations and anions impurities for the product stream. The treatment is separated into the purification step with a strong cation exchanger to remove all positive charge ions and a weak anion exchanger to separate all negative charged ions without the removal of bio-*SA*. Each of the exchangers need to be regenerated every 48 hours. The main advantage of ion exchanger is the low energy consumption. The main disadvantage is the regeneration of the resin after a certain period caused by fouling and contamination of the bed material. Therefore, an identical unit for both ion exchangers exists in parallel to operate during the regeneration process of the other unit. The strong cation exchanger (Sep model in Aspen Plus® V8.6) contains Dowex 50 WX8 resin. It was assumed that the cation exchanger adsorbs all calcium- and ammonium cations. For the regeneration of the resin an 8%wt hydrogen chloride solution is used. The weak anion exchanger (Sep model in Aspen Plus® V8.6) contains the Rohm and Haas Amberlite IRA-96 resin. The anion exchanger is able to adsorb for instance sulfate- , phosphate and chloride anions. It was assumed that all anionic impurities are removed, without the adsorption of bio-*SA*. For the regeneration of the resin an 8%wt sodium hydroxide solution is applied. Datta et al.[1] experimentally proved that the product stream contains 80-99% bio-*SA*, on a dry basis, less than 1% nitrogenous impurities and less than 10 ppm of sulfate ions or other contaminating ions. The bed volume for each exchanger was approximated based on design guideline of Dow[9] with the following equation:

$$Bedvolume\ [\mathrm{kg}] = 7.5\ [\mathrm{hr}] \cdot m_{Feed}\ [\mathrm{kg\ hr^{-1}}]$$

The regeneration procedure for each exchanger consists of one bed volume process water, four bed volumes of hydrogen chloride solution (cation exchanger) or 3 bed volumes of sodium hydroxide solution (anion exchanger), and one bed volume process water at the end of the procedure. The rising solution is discharged in a waste water treatment plant. The maintenance cost for the replacement of the resin was not considered in this study.

Step-4: Crystallization and washing of bio-*SA*

The recovered bio-*SA* from the fermentation broth is further purified via crystallization and washing step with the same models, which were presented in the process design *SA-1*.

1.4 Acidic pH fermentation with reactive extraction

In this process design, the *E.coli* bacteria are able to resist an acidic pH level in the fermentation. This process is under current investigation by industry, but no detailed information about the bacteria or fermentation conditions are published in literature.[10] The main goal of this process design is to reduce the downstream cost through eliminating the generation step of succinate salt. The flowsheet in Figure 4 comprises three main steps: Acidic *GLU* fermentation to bio-*SA* (Step-1), recovery of bio-*SA* from the fermentation broth via reactive extraction (Step-2) and further purification of bio-*SA* by crystallization and washing to 99% bio-*SA* purity (Step-3).

Step-1: Acidic *GLU* fermentation with reactive extraction

The *GLU* solution consist of 10%wt *GLU* and water from lignocellulosic feedstock. The fermentation is not controlled by a buffer solution, because the *E.coli* strain is able to resist low pH levels by achieving the same yield as in neutral pH environment. The lumped reaction and elemental composition of *E.coli* for the bio-*SA* production and recovery are the same as presented in process design *SA-1*. The fermentation broth contains bio-*SA* instead of calcium succinate due to the missing buffer solution. The aqueous solution, which contains besides bio-*SA* the byproduct ethanol, is further treated in reactive extraction.

Step-2: Succinic acid recovery by reactive extraction

The reactive extraction was modelled identically like in process design *SA-1*. The main difference is that through the missing reconversion of calcium succinate, a higher volume stream of aqueous solution is treated in the three extraction columns compare to process design *SA-1*. Therefore, for the recovery of the bio-*SA* from the fermentation broth, a higher solvent volume is required based on the experimental work of Kurzrock.[4] This leads automatically to a higher make up stream of octanol and trimethylamine in the process.

Step-3: Crystallization and washing of bio-*SA*

The recovered bio-*SA* from the fermentation broth is further purified via crystallization and washing step with the same models, which were presented in the process design *SA-1*.

1.5 Glucose production from lignocellulosic biomass

The production of *GLU* solution from wood was simulated via concentrated sulfuric acid solution in Aspen Plus® V8.6 based on the work of Farone et al.[11], which presents the most mature technology for the *GLU* solution production from lignocellulosic feedstock so far. It should to be underlined that a *GLU* solution is produced, which can be directly used as an educt in a fermentation. The process design is displayed in Figure S1 and the assessment results are shown in Table S1. The process comprises four main modelling steps: wood is separated from solid impurities and reduced in particle size (Step-1), then it is decrystallized with concentrated acid and cellulose as well as hemicellulose are hydrolysed to their corresponding sugars (Step-2), acid is recovered from the sugar solution (Step-3) and sugar is purified to 10%wt solution in water for being further processed to bio-*SA* (Step-4).

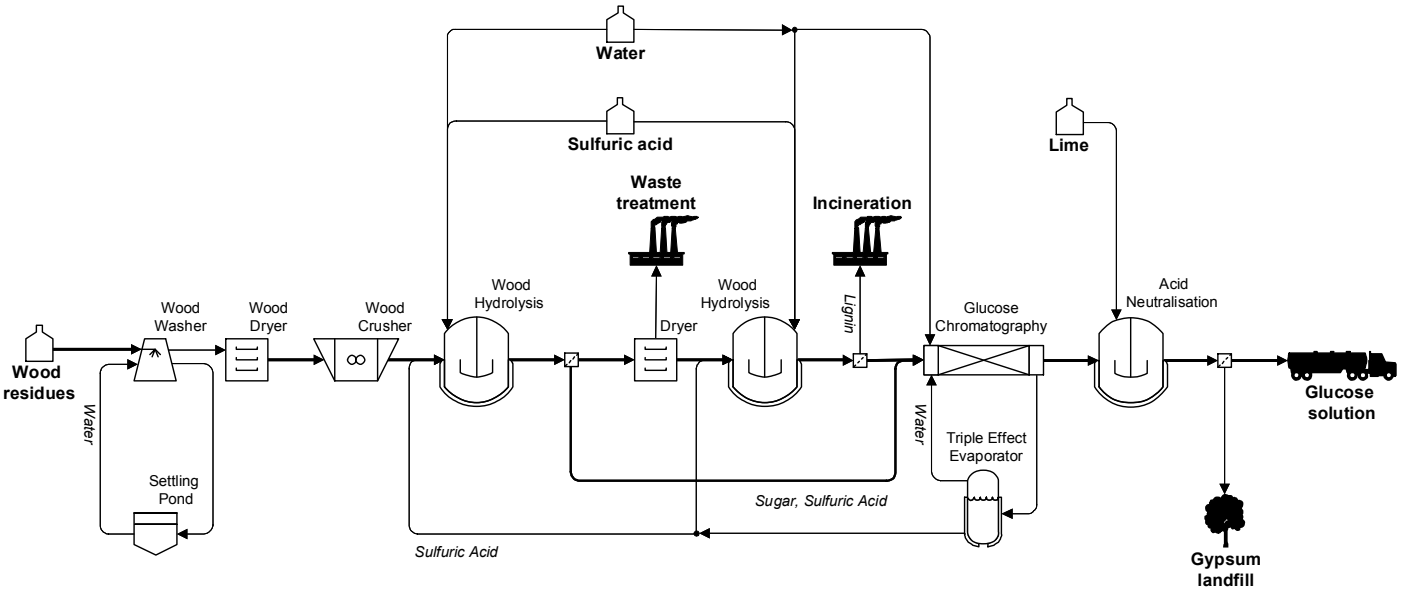

Fig. S1 Flowsheet for the production of *GLU* solution from wood residues.

The advantage of this method is the absence of degradation products and the performance of the hydrolysis at low temperatures of around 100°C. Furthermore, the process flexibility to the feedstock input, such as rice straw or waste from the pulp and paper industry, makes the process attractive.[11] Details to these steps are provided in the following.

Step-1: Wood treatment

The lignocellulosic biomass is washed (Swash model in Aspen Plus® V8.6) in a first step with water to separated solid impurities, which could influence the hydrolysis. The water used for the washing is then transferred to a settling pond (Mixer model in Aspen Plus® V8.6) where the solid particles sediment and water is recycled. Afterwards the wood is dried to a moisture content of 10%wt and is crushed to 3-7 mm in size (Crusher model in Aspen Plus® V8.6), with the mean diameter of 5 mm.[11] The electricity required for the size reduction was calculated using Bond`s law with a Bond index of 413 kWh per metric ton of wood.[12] The following equations were applied:

$$P_{el}\,[kW] = W \cdot \dot{m}$$

$$W\,[kWh\ t^{-1}] = 10\ W_i\ (P_{80}^{-0.5} - F_{80}^{-0.5})$$

P_{el} is the electrical power requirement [kW], $\dot{m}$ is the feed flow rate into the crusher [t h^{-1}], W is the work required to form the particles [kWh t^{-1}], W_i is the Bond index [kWh t^{-1}], and F_{80} and P_{80} are respectively the sieve sizes at which 80% of the product and feed pass [µm]. Here, a product size of 5 mm and a feed size of 25 cm was assumed. The conservative assumption of large feed size leads to higher P_{el} for crushing the wood. The work needed to produce crushed wood chips is 50 kWh ton^{-1}.

Step-2: Decrystallization and hydrolysis

The pretreated wood is mixed with 77%wt sulfuric acid solution for 50 minutes at 35°C and a pressure of 0.37 bar.[11] The acid solution is mixed with wood in a mass ratio of 1.25:1. During this step most of the hemicellulose (90%) is converted to its corresponding sugars (Rstoic model in Aspen Plus® V8.6). After the decrystallization, the reactor temperature is increased to 100°C and process water is added to dilute the acid concentration to 30%wt. Under these conditions, cellulose is hydrolysed (RStoic model in Aspen Plus® V8.6) for 60 minutes to *GLU* with a conversion of 70%. The sugar concentration was estimated to be 17%wt after the first hydrolysis step based on the experimental work of Farone et al.[11] After the first hydrolysis the liquid and solid phase are separated from each other (SSplit model in Aspen Plus® V8.6). The remaining

solids, which is mainly lignocellulosic biomass, are dried to 10%wt moisture content and then directed into a second hydrolysis step. The reason for this is that the sugar degrades in the presence of sulfuric acid to hydroxymethylfurfural and furfural. Therefore, the residence time is limited in each hydrolysis step. The remaining cellulose in this second hydrolysis step (Rstoic model in Aspen Plus® V8.6) is converted to its corresponding sugars with a conversion rate of 65%. The mass ratio of sulfuric acid to cellulose is also 1.25:1 during the second hydrolysis. The mixture is heated to 100°C and diluted to 30%wt acid concentration. The reaction time is 50 min and afterwards the liquid phase is separated from the solid phase, which is mainly lignin.[11] The liquid phases obtained in this two-stage hydrolysis are mixed (i.e., resulting in a concentration of 42%wt water, 38%wt sulfuric acid, 12%wt glucose and 8%wt other sugars) and directed to the downstream section.

Step-3: Acid sugar separation

The acid sugar solution is separated by chromatographic separation (Sep model in Aspen Plus® V8.6) using a cation exchange resin in pseudo moving bed column with water as an eluent. The column temperature is 60°C and 95% of the sugar and 98% of the acid is recovered.[11] The produced sugar stream contains 10%wt *GLU* and 5%wt of other sugars in water. The acid stream is reconverted with a triple effect evaporator (Flash2 model in Aspen Plus® V8.6) to a concentration of 77%wt and it recycled back to the decrystallization steps. The triple effect evaporator consists of three flash units in series. The vapour stream from each flash serves to provide the heat to the following flash. Hence, only the first flash requires external heat.

Step-4: Acid neutralisation

The sugar stream might still contain acid, therefore it is neutralised with lime. The following reaction was implemented:

$$H_2SO_4 + Ca(OH)_2 \rightarrow CaSO_4 + 2\ H_2O$$

The conversion was assumed to be 1 (Rstoic model in Aspen Plus® V8.6). Afterwards the gypsum is separated from the sugar stream via filtration (SSplit model in Aspen Plus® V8.6) and landfilled. The final sugar stream contains 10%wt *GLU* and 5%wt other sugars (e.g., mannose, arabinose, xylose, dextrose) in water. The higher *GLU* concentration of 30%wt for process design *SA-5* is reached with an additional evaporation step (Flash2 model in Aspen Plus® V8.6), which is not shown in the flowsheet. The assessment

results for the production of 10%wt and 30%wt *GLU* concentration with and without heat integration is shown in the following Table S1. For the bio-*SA* production the designs including heat integration of the *GLU* production were used. It was assumed that in the bio-*SA* production other sugars than *GLU* behave like inert chemicals and are not converted to bio-*SA* by the genetically engineered *E.coli* strains.

Table S1 Results of the environmental and economic assessment of the 10%wt and 30%wt *GLU* solution production from wood via concentrated acid technology with and without heat integration.

	10%wt *GLU* without heat integration	10%wt *GLU* with heat integration	30%wt *GLU* without heat integration	30%wt *GLU* with heat integration	Units
CED	1.24	0.51	4.04	3.18	$MJ_{eq}\ kg_{product}^{-1}$
EI-99	0.0071	0.0044	0.018	0.014	Points $kg_{product}^{-1}$
GWP	0.28	0.23	0.46	0.40	$CO_{2\text{-}eq}\ kg_{product}^{-1}$
Operating Cost	0.059	0.055	0.077	0.072	USD $kg_{product}^{-1}$

1.6 Conventional Production of *SA* from non-renewable resources

Pinazo et al.[13] has developed an inventory of utilizes raw materials, energy and produces waste based on experimental data for the production of *SA* from catalytic hydrogenation of maleic anhydride. The inventory, which was used based on work of Pinazo et al.[13] is shown in Table S2.

Table S2 *LCI* data for conventional *SA* production route

Materials and Energy	**Conv-SA**	Units
Bio-*SA*	1000	$kg\ hr^{-1}$
Maleic anhydride	0.89	$kg\ kg_{SA}^{-1}$
Hydrogen	0.25	$kg\ kg_{SA}^{-1}$
Process water	0.30	$kg\ kg_{SA}^{-1}$
Nitrogen	0.073	$kg\ kg_{SA}^{-1}$
Palladium catalyst	1e-3	$kg\ kg_{SA}^{-1}$
Natural gas	0.10	$kg\ kg_{SA}^{-1}$
Electricity	0.36	$kg\ kg_{SA}^{-1}$
Waste Treatment	0.32	$kg\ kg_{SA}^{-1}$

[a] Electricity production mix of Europe
[b] Waste Treatment by incineration

2. Sustainability assessment

2.1 Global warming potential

The global warming potential 100a (*GWP*) values for the different process designs with and without heat integration are shown in Figure S2 and Figure S3. It can be seen that the results for the *GWP* follow a similar trend with the *CED* results presented and discussed in the main manuscript. For the purification technologies (*SA- 1 – SA-6*), the neutral fermentation with reactive extraction (*SA-1*) has the lowest *GWP* value of 12.0 $CO_{2\text{- eq}}$ kg_{SA}^{-1} (Figure S2), whereas the purification technology with electrodialysis (*SA-2*) has the biggest impact with 29.6 $CO_{2\text{-eq}}$ kg_{SA}^{-1} due to the high consumption of electricity. The results for the upstream design (*SA-4 – SA-6*) show that all technologies lead to an environmental improvement of at least 12% compared to process design *SA-1*. The main difference between the results of the *GWP* compared to the *CED* is that no bio-based technology has a smaller environmental impact than conventional technology, even after the heat integration. For the *GWP* indicator the material impact, mainly allocated to the *GLU* solution, contributes more to the overall impact compared to the *CED* indicator.

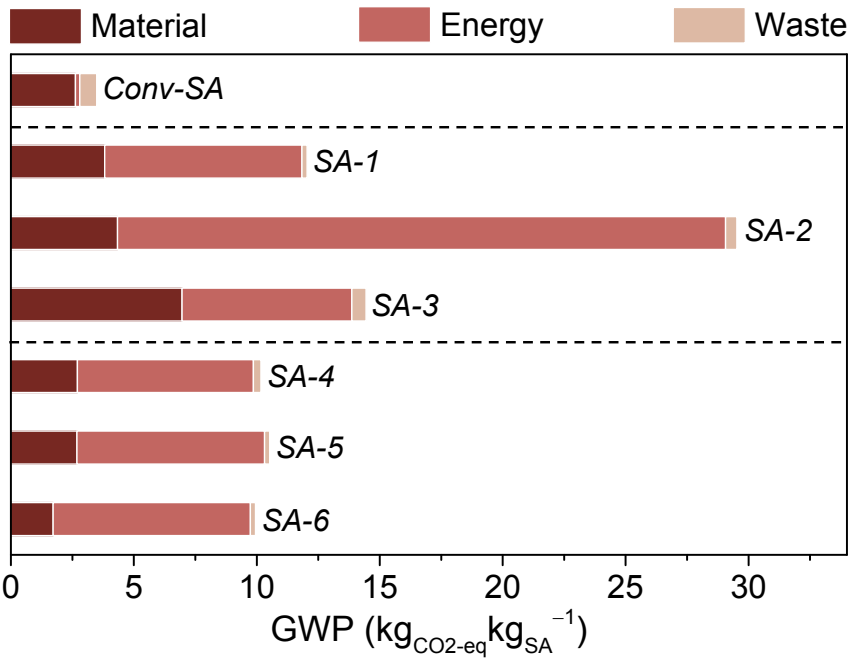

Fig. S2 Cradle-to-gate *LCA* according to the *GWP* metric for various process scenarios without heat integration.

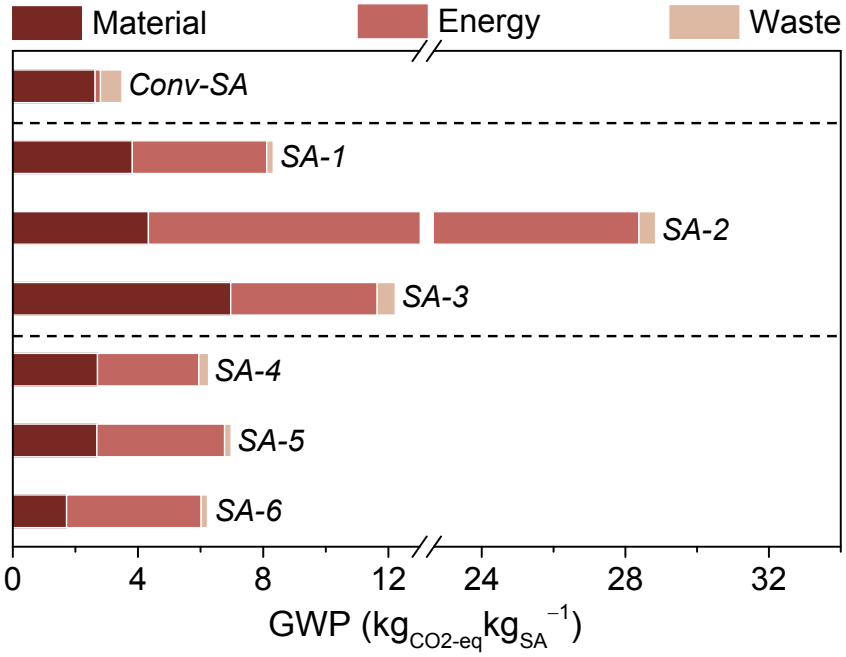

Fig. S3 Cradle-to-gate *LCA* according to the *GWP* metric for various process scenarios with heat integration.

2.2 Eco-Indicator 99

The trends of the *EI-99* values for the process design without heat integration and with heat integration are shown in Figure S4 and Figure S5. The results for the *EI-99* follow more strongly the trend of the *CED* indicator presented in the manuscript. The reactive extraction process design *SA-1* has the lowest value (0.66 Points kg_{SA}^{-1}) among the downstream technologies (*SA-1* – *SA-3*). Due to the high electricity requirements process design *SA-2* has the biggest impact with 1.56 Points kg_{SA}^{-1}. For the upstream technologies (*SA-4* – *SA-6*), *SA-4* has the lowest environmental impact (0.51 Points kg_{SA}^{-1}) for the same reasons mentioned in the case of the *CED* (i.e., lower material consumption because of lacking the process steps for pH neutralisation and hydrolysis for reconverting bio-*SA*, as well as the lower volume of generated waste). It is demonstrated that the process design *SA-5* and *SA-6* does not lead to an improvement of the *EI-99* compared to *SA-1* because the change to a higher *GLU* concentration (*SA-5*) or sugar source (*SA-6*) leads to a stronger decrease of the *EI-99* indicator compared to the *GWP*. Furthermore, none of the technologies has a lower impact than the conventional process without heat integration. When heat integration is included, all process designs show a significant improvement of the *EI-99* score, except *SA-2*. The reason for this is the high demand of electricity in process design *SA-2,* which is not part of the heat integration. The only technology achieving a lower *EI-99* score than the conventional production is process design *SA-4* (0.29 Points kg_{prod}^{-1} *versus* 0.34 Points kg_{prod}^{-1}).

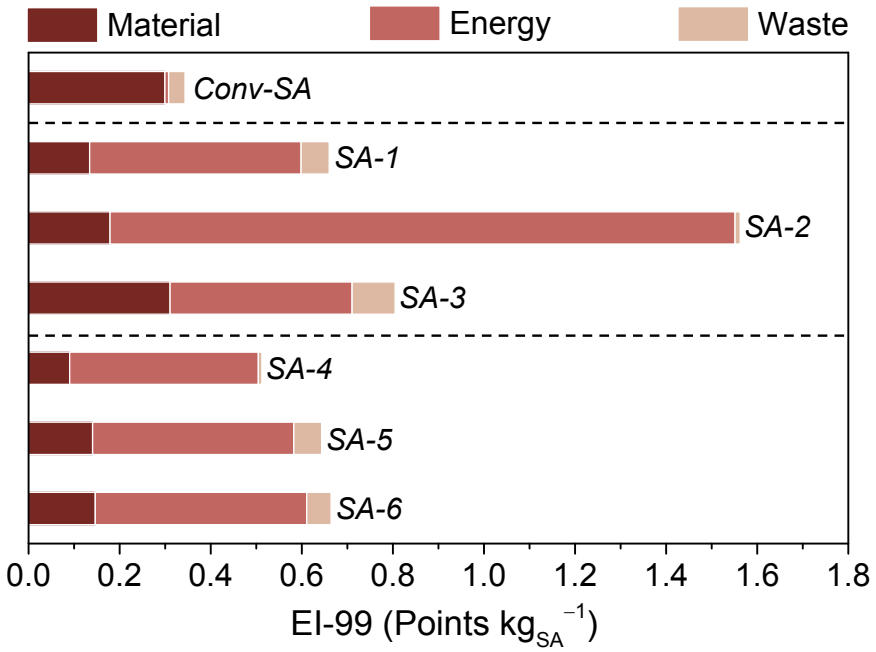

Fig. S4 Cradle-to-gate *LCA* according to the *EI-99* metric for various process scenarios without heat integration.

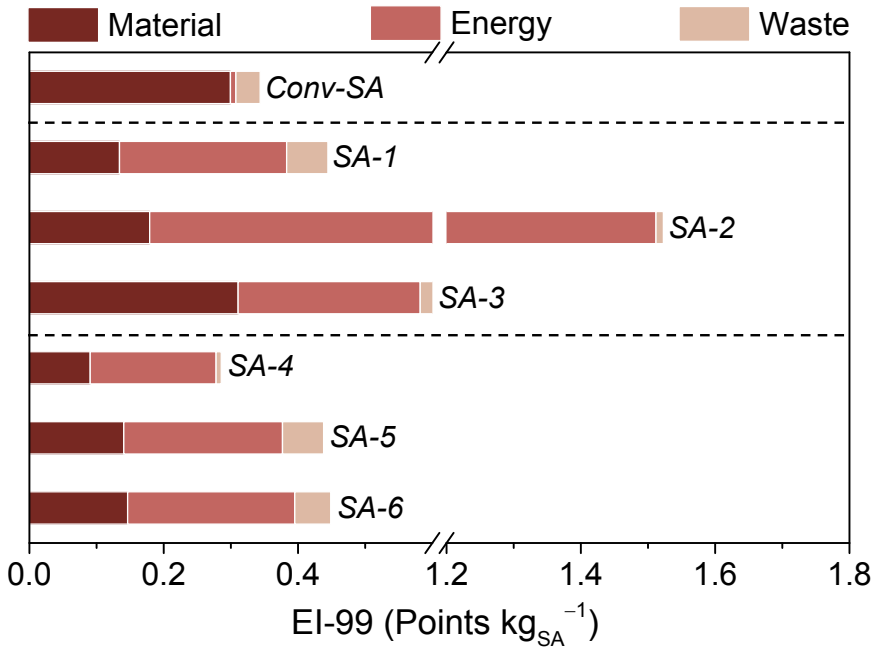

Fig. S5 Cradle-to-gate *LCA* according to the *EI-99* metric for various process scenarios with heat integration.

2.3 Profit

The results of the economic assessment without heat integration are shown in Figure S6.

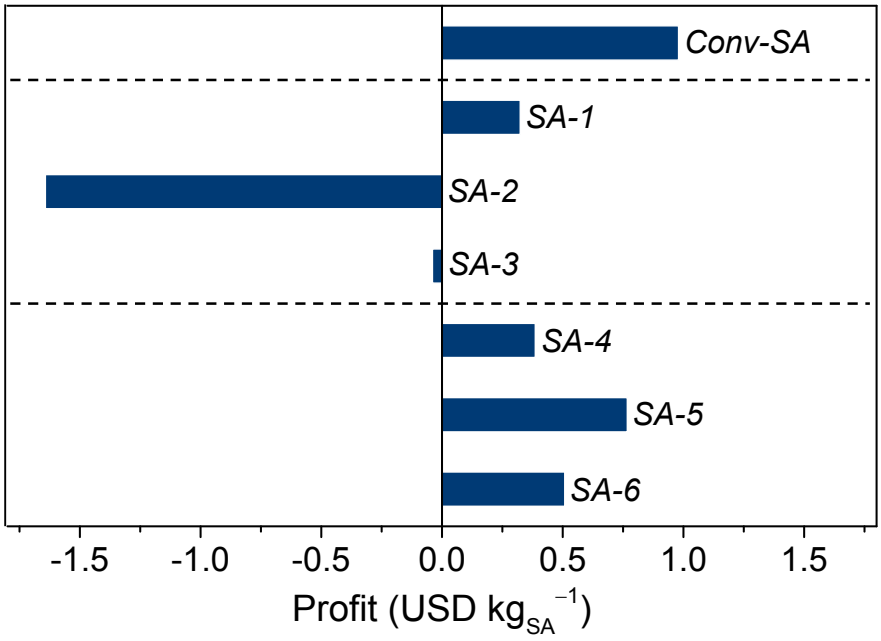

Fig. S6 Profit for various process scenarios without heat integration.

The selling price of *SA* was approximated with 2.9 USD kg_{SA}^{-1} for west Europe.[14] The operating cost were calculated based on the inventory shown in Table S2 with 1.92 USD kg_{SA}^{-1} for the conventional production of *SA*. Thus, the profit for *SA* in the conventional production is 0.98 USD kg_{SA}^{-1}. The most profitable purification technology is process design *SA-1* with a profit of 0.32 USD kg_{SA}^{-1} for the non-integrated designs. A high *GLU* concentration in the fermenter (*SA-5*) leads to a further improvement of around 141% compared to *SA-1*. The expected profit for the non-integrated designs are below the conventional production route (*Conv-SA*).

An improvement of the profitability can be reached when heat integration is included in the process designs. Figure S7 shows that the process design *SA-5* has an improved profitability of around 76% by reducing the heat requirements through heat integration. Therefore a 39% higher profit than the conventional production of *SA* from maleic anhydride (*Conv-SA*) is reached (1.36 *versus* 0.98 USD kg_{SA}^{-1}). All profit calculations mentioned above did not include the respective investment expenditure.

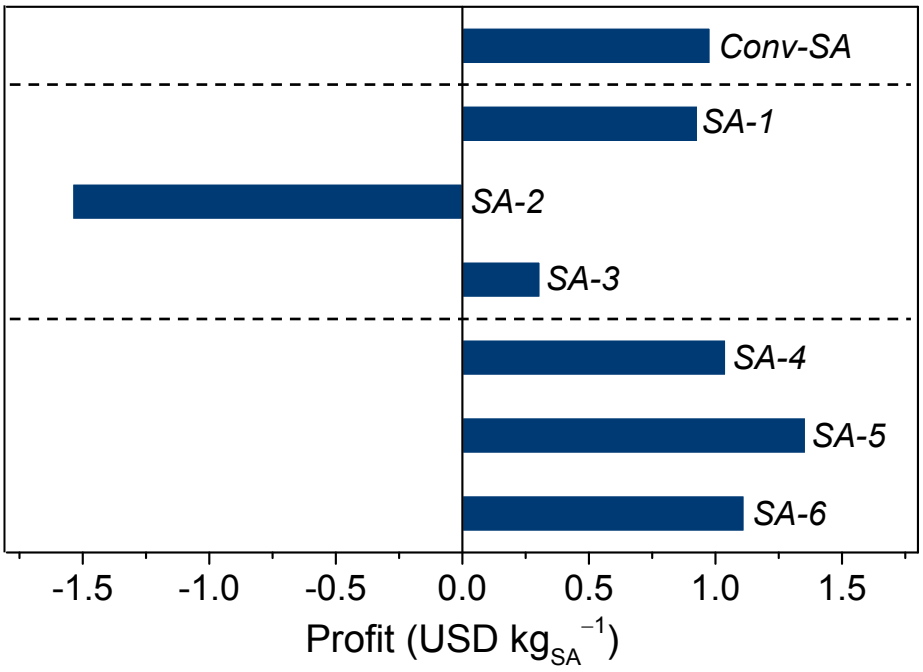

Fig. S7 Profit for various process scenarios with heat integration.

2.4 Hazard assessment

The results of the hazard assessment in Figure 7 were calculated for the weighting factors of 0.4 for safety, 0.2 for health and 0.4 for environment. For this combination, the process design with the lowest score is the neutral fermentation with electrodialysis *SA-2*. Figure S8 shows the process with the lowest score for all different combinations of the weighting factors. It can been seen that *SA-2* has the lowest hazard score for almost all of the combinations, only in the extreme case where the weighting factor for safety and environment is set to 0 and the one for health to 1 all process designs have the same total score due to the fact of the presence of the same inorganic acids.

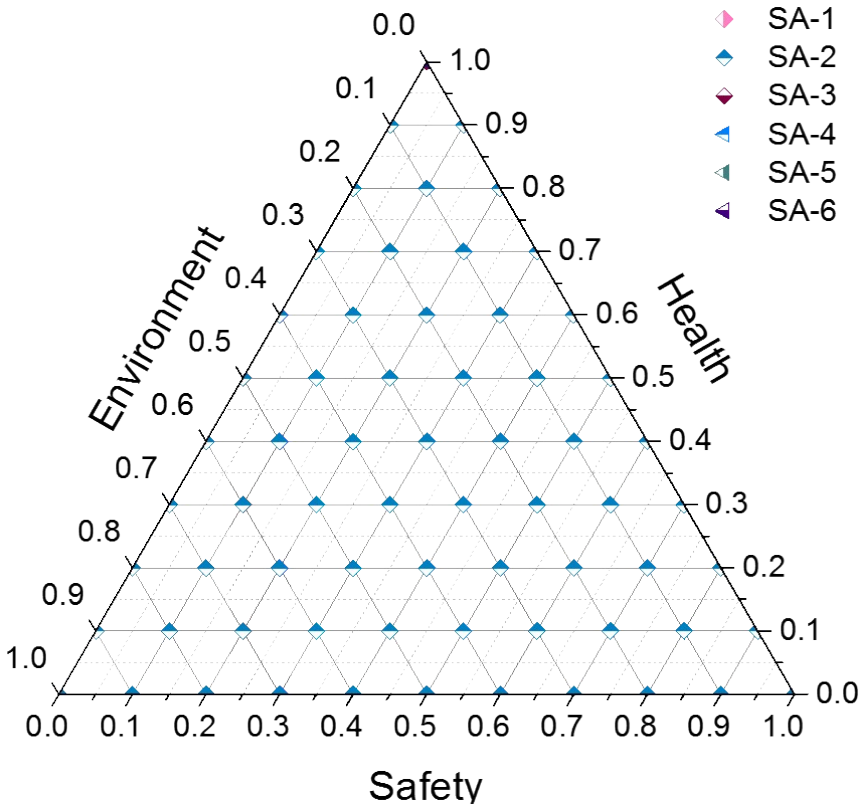

Fig. S8 Total Score for the *EHS* assessment with Sugiyama method for different weighting factors

2.4 Investment Costs

The investment costs for the alternative production routes *SA-1 – SA-6* were calculated based on the cost functions for chemical equipment published by Peters et al..[15] The cost of the ion exchangers and the electrodialysis was approximated with the capital costs by Sexton et al.[16] The results of the calculation are shown in Figure S9. *SA-2* has for the purification technologies the highest investment costs expressed as bare module costs with $27.1 \cdot 10^6$ USD. The high investment costs are a result of the equipment cost for the electrodialysis, which was estimated of around $21.9 \cdot 10^6$ USD. *SA-3* has the second highest investment costs of $10.2 \cdot 10^6$ USD. Around 40% of the bare module costs are allocated to equipment costs of the ion exchangers. The batch reactors contribute 40% to the overall investment costs for *SA-3*. The flowsheet *SA-1* has the smallest investment costs with $7.1 \cdot 10^6$ USD. Here the extraction columns contribute minor to the investment costs. The biggest cost contribution of 60% is allocated to the batch reactors, such as the fermentation and recovery of the *E.coli*. The improvement of the upstream technology (*SA-4 – SA-6*) leads only for *SA-4* to a decrease of the investment costs. In *SA-4* no succinic salt is produced during the fermentation. The missing processing steps (e.g. crystallization, filtration and hydrolysis) leads to 19% decrease of the investment costs compared to *SA-1*. The higher *GLU* concentration in *SA-5* leads to higher production rate, which consequently leads to bigger equipment size in the upstream and downstream section.

Especially the bigger batch reactors and filters lead to investment costs of $9.0 \cdot 10^6$ USD, which is a 26% increase compared to *SA-1*. *SA-6* has the same investment costs than *SA-1*, due to the fact that only the origin of the sugar source is different.

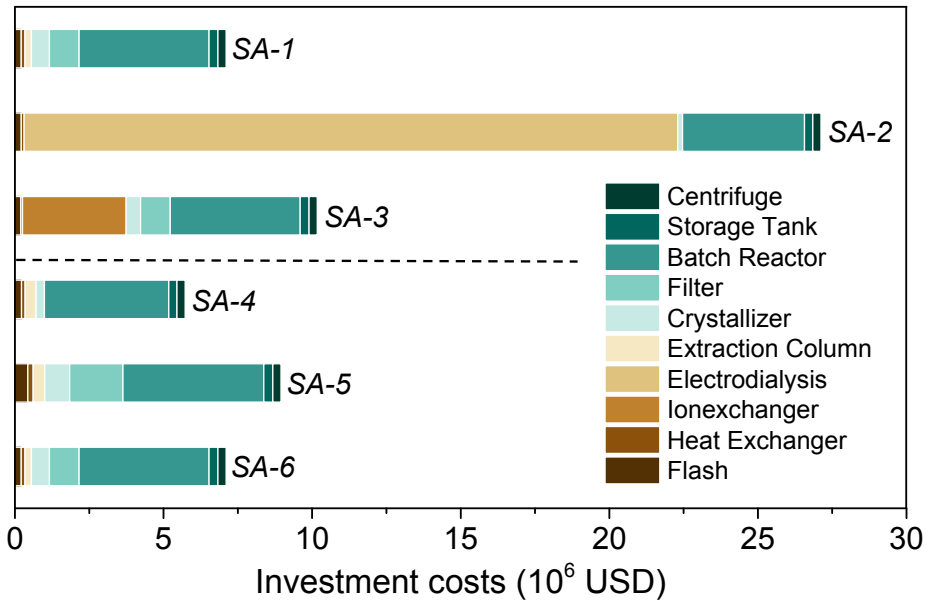

Fig. S9 Investment costs of the alternative technologies

2.5 Maintenance Costs

The maintenance cost of the alternative production routes *SA-1* – *SA-6* were calculated based on Peters et al.[15] for an average processes with normal operating conditions. Therefore the maintenance cost were estimated with 6% of the investment cost on an annual basis. The results are shown in Figure S10. It can be seen that *SA-2* has the highest maintenance costs with $1.6 \cdot 10^6$ USD a^{-1}, whereas the lowest maintenance cost has the acidic fermentation with reactive extraction (*SA-4*) with 0.4 USD a^{-1}.

2.6 Labour Costs

The labour cost were approximated with the correlation by Alkhayat and Gerrard.[17] Based on Turton et al.[18] a number of approximately 4.5 operators needs to be hired for each operator needed in the plant at any time. A yearly brutto salary of a plant and system operator in Europe was estimated with 75000 USD. The results of the calculations are shown in Figure S11 and illustrate that the labour costs for all plants are expected to be around $1.1 \cdot 10^6$ – $1.2 \cdot 10^6$ USD a^{-1}.

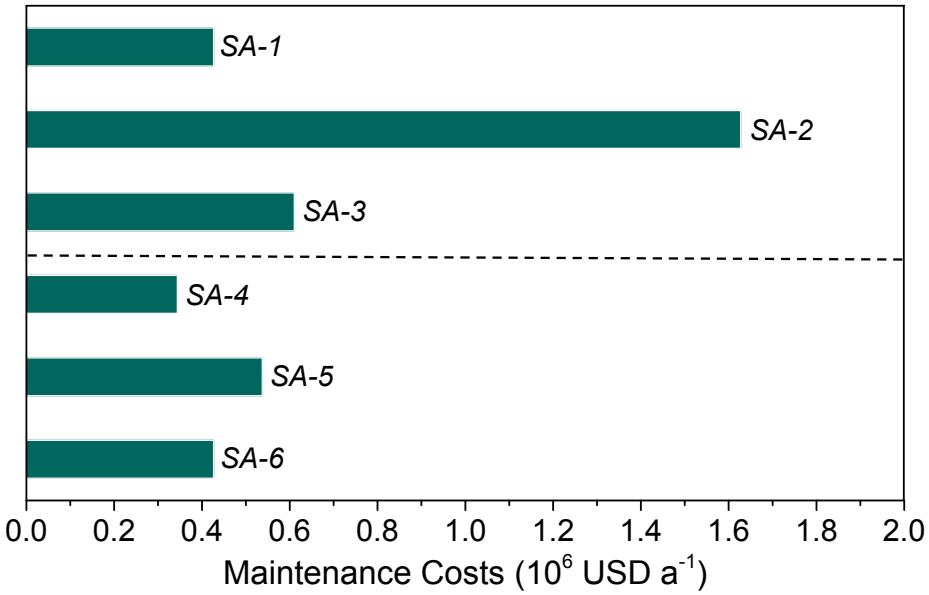

Fig. S10 Maintenance costs of the alternative technologies

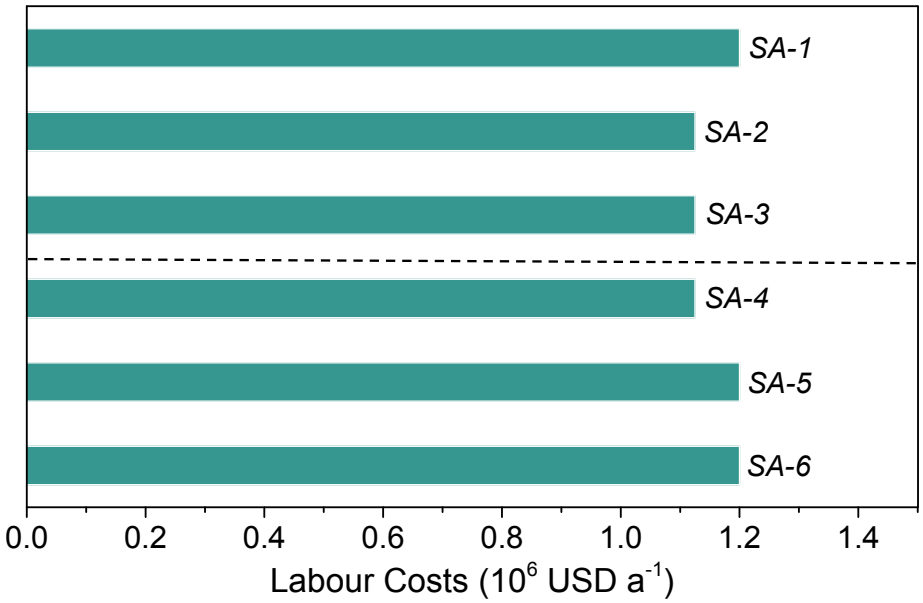

Fig. S11 Labour costs of the alternative technologies

3. Heat integration

Figures S12 to S16 present the results for the heat integration of the *SA-1* to *SA-5* process designs in the form of grand composite curves (*GCC*) assuming a minimum temperature approach (ΔTmin) of 10 Kelvin. An overview of the heat integration results in terms of pinch temperatures, heat recovery and hot and cold utlility consumption is given in Table S3. Energy requirements for the fermentation of *GLU* ($Q_{Fermentation}$) and the recovery of the *E.coli* bacteria (Q_{Ecoli}) were not included in the heat integration because of their operation in batch mode. Q_{hot} and Q_{cold} refer to the utility consumption for the continuous part of the processes Q_{hot_total} and Q_{cold_total} are the sum of all hot- and cold utilities in a process, whereas the reduction of the integrated compared to the non-integrated process design is presented as $\Delta Q_{hot\_Reduction}$ and $\Delta Q_{cold\_Reduction}$.

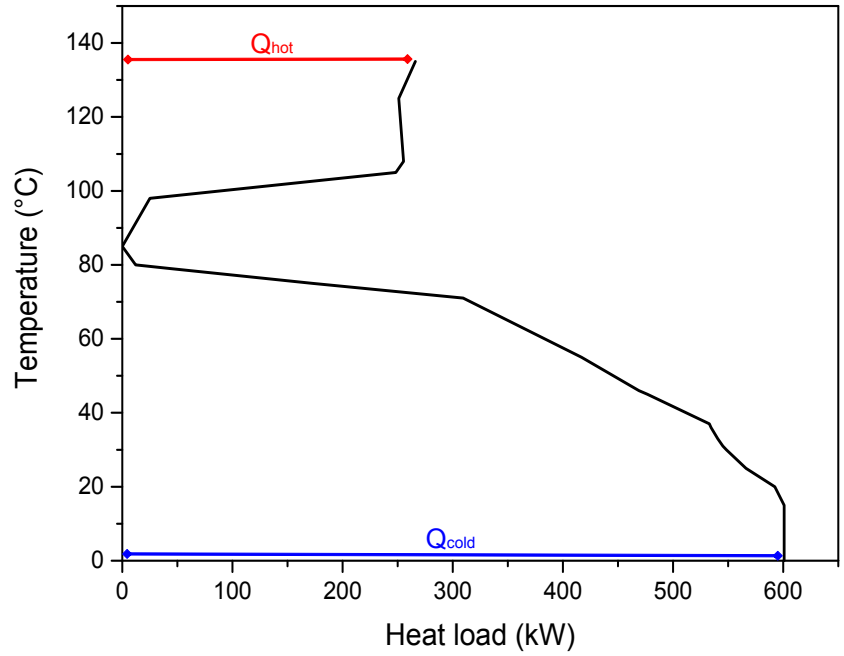

Fig. S12 *GCC* for the process designs *SA-1* and *SA-6*.

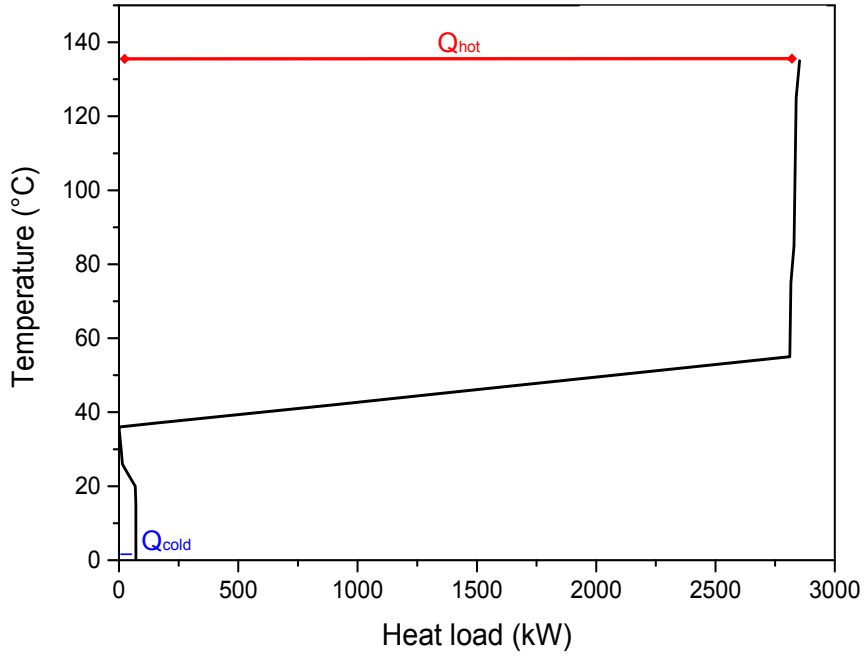

Fig. S13 *GCC* for the process design *SA-2*.

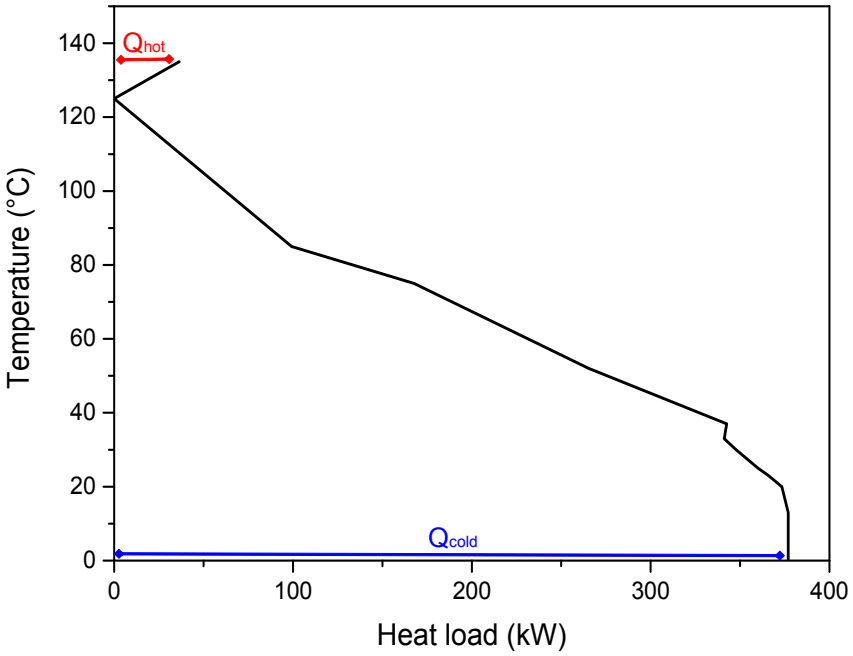

Fig. S14 *GCC* for the process design *SA-3*.

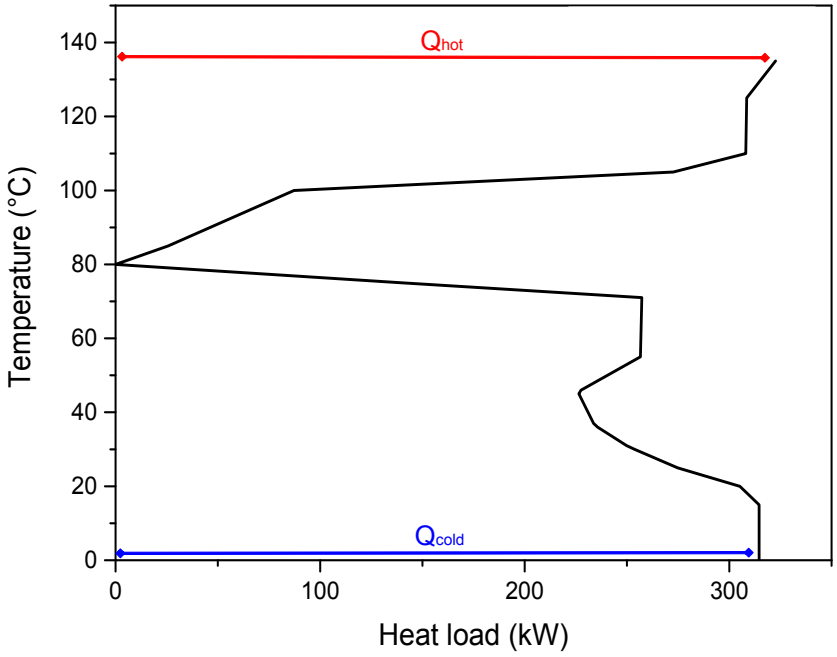

Fig. S15 *GCC* for the process design *SA-4*.

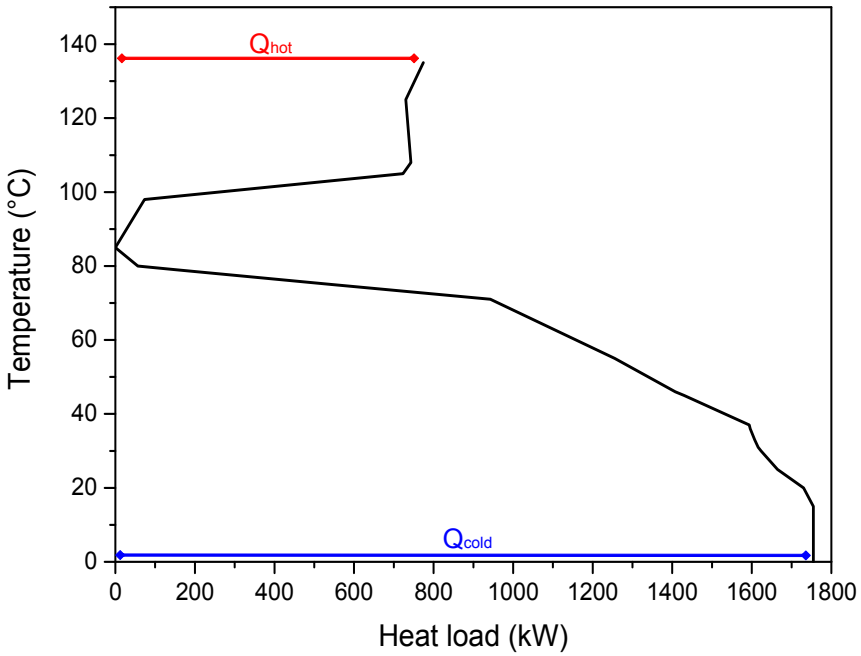

Fig. S16 *GCC* for the process design *SA-5*.

Table S3 Heat integration results for bio-*SA* production from biomass.

	SA-1/6	*SA-2*	*SA-3*	*SA-4*	*SA-5*	Units
Q_{hot}	266	2852	36	323	774	kW
Q_{cold}	601	71	377	315	1755	kW
$Q_{Fermentation}$	663	1467	663	448	1973	kW
Q_{Ecoli}	101	101	101	101	101	kW
T_{Pinch}	85	36	125	80	85	°C
Q_{hot_total}	1030	4420	800	871	2848	kW
Q_{cold_total}	601	71	377	315	1755	kW
$\Delta Q_{hot\_Reduction}$	46	3.5	32	55	47	%
$\Delta Q_{cold\_Reduction}$	58	63	46	77	58	%

4. Background data for environmental and economic assessment

The background data used in this study for the environmental and economic assessment with respect to the consumption of resources are presented in Table S4, while those related to the environmental impact of the process emissions (i.e. those generated by the incineration units or waste water treatment plants, as no other direct process emissions are considered in this study) are presented in Table S5. The respective impacts for producing *GLU* from lignocellulosic biomass have already been presented in Table S1.

Table S4 Background data for the environmental (without renewable resources) and economic assessment with respect to resources consumption (Source: Ecoinvent database V2.2, www.alibaba.com and refs.)[19, 20]

Substance	$CED_{non\text{-}renewable}$ (MJ_{eq} kg^{-1})	*EI-99* (Points kg^{-1})	GWP_{100a} ($kg_{CO2\text{-}eq}$ kg^{-1})	Price (USD ton^{-1})
Process water	2.79e-04	1.83e-06	2.45e-05	1
Ammonia	41.67	0.18	2.10	625
Phosphoric acid	20.00	0.23	1.42	900
Glucose from sugar beet	6.49	0.05	0.51	390
Sulphuric acid	2.02	0.04	0.12	150
Trioctylamine[a]	113.39	0.82	6.58	5000
Octanol[a]	79.92	0.34	2.03	5250
Trimethylamine[a]	84.81	0.31	2.47	2000
Calcium hydroxide	5.50	0.03	0.99	110
Sodium hydroxide	21.40	0.6	1.10	500
E.coli	18.60	0.08	1.04	1000[e]
Sodiumsuccinate[a]	81.02	0.36	4.50	2100
Hydrogen chloride[a]	68.54	0.26	2.56	190
Steam (6bar)[b]	1.56	0.01	0.10	20
Electricity[b]	9.87	0.02	0.49	0.10[c]
Cooling water from river	0.00	0.00	0.00	0.15[c]
Natural gas[b]	1.24	4.03e-03	1.22e-02	600[c]
Sodium hydroxide 30 wt.%	22.8	6.26e-02	1.09	570
Hydrochloric acid 32 wt.%	17.5	0.060	0.853	190
Polydimethylsiloxane	62.7	0.22	2.71	1000
Iron(III) chloride 40 wt.%	16.3	6.35e-02	0.803	360
Calcium chloride	11.0	5.18e-02	0.854	440

Gypsum landfill	0.65	4.22e-02	1.34e-02	4.81e-05
Maleic anhydride	68.05	0.24	2.37	1500
Hydrogen	69.70	0.23	1.67	1800
Nitrogen	8.73	0.02	0.43	75
Palladium catalyst	90.88	0.32	4.13	1700
Natural gas	63.19	0.21	0.612	100
Waste Treatment [d]	27.31	0.11	2.17	2.45e-4

[a] Impact calculated via the Finechem Tool

[b] Functional unit for steam as well as natural gas is MJ and for electricity kWh

[c] Values based on the work of Rerat et al.[20]

[d] Waste treatment of conventional refinery sludge by incineration

[e] Metabolic engineered *E.coli* is assumed to have the same price as commercially available *E.coli*

Table S5 Background data for the environmental impact for the emissions (Source: Ecoinvent database V2.2 and ref.)[20]

Substance	*EI-99* (Points kg^{-1})	GWP_{100a} ($kg_{CO2\text{-}eq}$ kg^{-1})
Carbon dioxide	5.46e-02	1
Carbon monoxide	8.36e-02	-
Nitrogen dioxide	2.75	1.57
Particles	9.74	-
Ammonia	3.42	-

4.2 Calculation of *CED*, *GWP*, *EI-99*

The *LCA* calculations for the flowsheet were performed based on the background data collected in Tables S4 and S5. Tables S6 and S7, for example, show the calculations for the most promising bio-*SA* process designs.

Table S6 *LCA* calculations for bio-*SA* production process design *SA-4* including heat integration

	Material									Energy			Waste	
	Water	Octanol	Trimethylamine	10%wt *GLU*[f]	*GLU*[g]	Phosphate	Sulphate	Ammonia	*E.coli*	Steam (6bar)	Cooling water	Electricity	WWTP	Gypsum landfill
Flow (kg h^{-1})	3.47	12.39	0.045	1000	7.61	0.12	0.03	0.23	0.088	3136	54115	0	921	0
CED (MJ_{eq} h^{-1})[b]	9.67e-04	9.90e+02	3.82	5.11e+02	4.93e+01	2.30	6.27e-02	9.46	1.64	4905	0	0	331	0
EI-99 (Points h^{-1})[b]	6.33e-06	4.25	1.40e-02	4.00	3.84e-01	2.62e-02	1.2e-03	4.10e-02	7.38e-03	18.09	0	0	0.73	0
GWP (kg_{CO2eq} h^{-1})[b]	8.48e-05	2.51e+01	1.11e-01	2.31e+02	3.85	1.63e-01	3.76e-03	4.76e-01	9.15e-02	3.12e+02	0	0	30.05	0
∑*CED* (MJ_{eq} h^{-1})[c]	1568									4905			331	
∑*EI-99* (Points h^{-1})[c]	8.72									18.09			0.73	
∑*GWP* (kg_{CO2eq} h^{-1})[c]	261									3.12e+02			30.05	
CED (MJ_{eq} kg_{SA}^{-1})[d]	16.26									50.87			3.43	
EI-99 (Points kg_{SA}^{-1})[d]	0.09									0.19			7.61e-03	
GWP (kg_{CO2eq} kg_{SA}^{-1})[d]	2.71									3.24			0.312	
$CED_{Process}$ (MJ_{eq} kg_{SA}^{-1})[e]	70.56													
$EI\text{-}99_{Process}$ (Points kg_{SA}^{-1})[e]	0.29													
$GWP_{Process}$ ($kg_{CO2\text{-}eq}$ kg_{SA}^{-1})[e]	6.26													

[a] Flow (kW)
[b] Obtained by multiplying the flow with the background data in Tables S4 and S5
[c] Summation of *CED*/*EI-99*/*GWP* values for material, energy and waste
[d] Summation of *CED*/*EI-99*/*GWP* values for material, energy and waste divided by the bio-*SA* productivity calculated for this process (96.4 kg h^{-1})
[e] Summation of *CED*/*EI-99*/*GWP* values of material, energy and waste
[f] *GLU* solution with 10%wt concentration in water from wood via concentrated acid technology
[g] Pure *GLU* from sugar beet

Table S7 *LCA* calculations for bio-*SA* production process design *SA-5* including heat integration

	Material										Energy			Waste	
	Water	Octanol	Trimethyl amine	30%wt *GLU*[f]	*GLU*[g]	Phosphate	Sulphate	Ammonia	Calcium hydroxide	*E.coli*	Steam (6bar)	Cooling water	Electricity	WWTP	Gypsum landfill
Flow (kg h^{-1})	1297	14.90	0.12	1000	7.61	0.12	256	0.23	210	0.088	2849	1755	0	2187	336
CED (MJ_{eq} h^{-1})[b]	0.36	1.19e+03	9.89	3.15e+03	49.4	2.34	516	9.59	1.15e+03	1.64	16040	0	0	529	219
EI-99 (Points h^{-1})[b]	2.37e-03	5.10	3.62e-02	14.20	0.38	2.66e-02	9.90	4.15e-02	5.48	7.38e-03	59.16	0	0	1.17	14.17
GWP (kg_{CO2eq} h^{-1})[b]	3.17e-02	30.20	0.288	399	3.85	0.16	31	0.48	207	9.15e-02	1020	0	0	48	4.51
∑*CED* (MJ_{eq} h^{-1})[c]	6079										16040			748	
∑*EI-99* (Points h^{-1})[c]	35.19										59.16			15.35	
∑*GWP* (kg_{CO2eq} h^{-1})[c]	672										1020			53	
CED (MJ_{eq} kg_{SA}^{-1})[d]	24.28										64.06			2.99	
EI-99 (Points kg_{SA}^{-1})[d]	0.14										0.24			0.06	
GWP (kg_{CO2eq} kg_{SA}^{-1})[d]	2.69										4.08			0.21	
$CED_{Process}$ (MJ_{eq} kg_{SA}^{-1})[e]	91														
$EI\text{-}99_{Process}$ (Points kg_{SA}^{-1})[e]	0.44														
$GWP_{Process}$ ($kg_{CO2\text{-}eq}$ kg_{SA}^{-1})[e]	6.98														

[a] Flow (kW)
[b] Obtained by multiplying the flow with the background data in Tables S4 and S5
[c] Summation of *CED*/*EI-99*/*GWP* values for material, energy and waste
[d] Summation of *CED*/*EI-99*/*GWP* values for material, energy and waste divided by the bio-*SA* productivity calculated for this process (250.4 kg h^{-1})
[e] Summation of *CED*/*EI-99*/*GWP* values of material, energy and waste
[f] *GLU* solution with 30%wt concentration in water from wood via concentrated acid technology
[g] Pure *GLU* from sugar beet

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