AP 1.4 – Process simulation database

Model processes

Estelle van der Walt

Technical University of Munich – Straubing Campus

Professorship of Bioprocess engineering

Report date: 4.11.2024

Table of contents

1 Introduction

TransBIB aims to boost the German bio-economy to decrease the German environmental footprint and to become more economically independent from imports [1]. To build and strengthen the German bio-economy, commonly used products must be produced in Germany from German-produced biomass and wastes in a sustainable manner [2].

In this report, bio-processing plants are described for the production of three bio-compounds that are globally traded as fuel and/or chemicals in large scale. These are ethanol, isobutanol and lactic acid. All three of these compounds can be produced from fossil resources or from bio-based resources and for all three, the fermentation step in the bio-process requires sugars that can be sourced as a pure crystalline sugar or as a liquid that contains sugars, such as hydrolysate or molasses. Hydrolysate may also be produced on site from agricultural residues to further improve the environmental impact and costs.

For each model process, a process flow diagram (PFD), process description, mass balance and energy balance are given. The descriptions in this report do not include equipment sizing, auxiliary equipment, control philosophies, heat integration for process optimization, etc. The model processes are meant to enable users to draw analogies to their own processes or to adopt process steps for their own planning.

2 Bio-processing

Most bio-processing plants can be separated into a fermentation section and a downstream section. The fermentation section is where the production micro-organism is grown and then used for the conversion of a carbon source to a valuable product. In the downstream processing section, the product is isolated from the fermentation broth. [3] In some cases the two sections may overlap to a small degree and in most, optimized processing plants, many side-streams from the downstream processing side may be recycled to the fermentation side.

For all three processes described in this report, the description of the fermentation side is very similar, consuming the same carbon source and growing the production organism in a similar seed train. Therefore, the feedstock and seed train will be described in general before describing each of the three processes individually.

2.1 Feedstock

For the production processes described here, the feedstock is hydrolysate, which is a solution of fermentable sugars, produced through enzymatic hydrolysis of lignocellulosic biomass. It is therefore a second-generation feedstock [4]. This feedstock may be purchased from a producer of hydrolysate or it may be produced on site (recommended).

Depending on the composition of the hydrolysate, it may be necessary to provide nutritional supplements to the fermenter for the proper metabolic health of the production organism. This may include nitrogen, phosphorous and trace elements as well as vitamins.

2.2 Seed train/cell growth

Live cells such as *Saccharomyces cerevisiae* yeast (for ethanol), *Clostridium acetobutylicum* (for isobutanol) and *Lactobacillus* strains (for lactic acid) are responsible for turning sugars in the raw material into the final product. Therefore, the rate of conversion is dependent on (among others) the number of cells in the fermenter. A bio-production plant does not buy several tons of cells for this purpose. Instead, a starter culture of a few grams or kilograms is purchased and the production cultures are grown from the starter culture in seed fermenters of increasing volume.

Usually, the first culture is prepared using laboratory equipment such as shaking flasks and an incubator, followed by growth in aerobic bioreactors called seed fermenters of increasing volume until a suitable amount of cell mass have been generated. Most production organisms require oxygen to grow and it produces alcohols or organic acids when oxygen is not available. For this reason, the seed fermenters are aerated to supply the needed oxygen for growth. The same substrate is used in the seed fermenters as in the production fermenter in order to prepare the cell metabolism for consumption of the available sugars in the production fermenter. In the seed fermenters, more supplements are required than in the production fermenter because the supplements are consumed in the generation of new cells, but (usually) not in the production of alcohols or organic acids.

After fermentation, the cells are recovered from the broth using a disk stack centrifuge. These cells are normally viable for another two to four batches. Therefore, it is not necessary to operate the entire seed train for each new batch of product fermentation.

3 Bio-ethanol production process

Ethanol is most commonly produced by fermenting sugars using microbes such as yeast or bacteria under anaerobic conditions [5]. After fermentation, ethanol must be isolated from the fermentation broth. This is normally done in a two-step process of first distillation, followed by dehydration [5]. The process flow diagram of a standard second-generation ethanol production plant is shown in Figure 1. The feedstocks and seed train are as described in section 2.1 and 2.2.

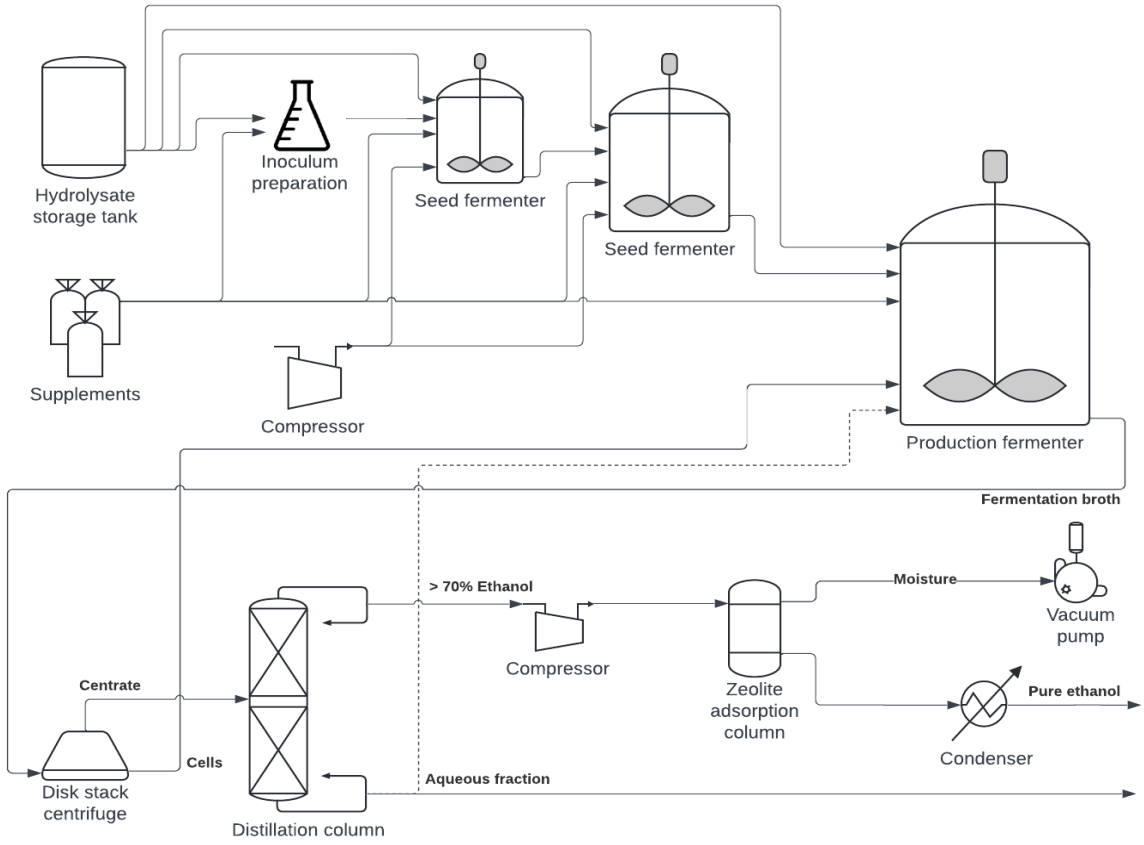

Figure 1: Process flow diagram for the production of Bio-ethanol

3.1 Production fermenter

In the production fermenter, cell growth is unwanted because it competes with the production of ethanol. The production fermenter is operated anaerobically to minimize the formation of new cells and to force the existing cells to generate ethanol to survive. [6]

Saccharomyces cerevisiae is the strain of yeast that has been used for brewing beer and baking breads since before the time that we knew of the existence of micro-organisms and it is still used for this purpose today. This yeast, as well as some engineered strains with higher production capacity, lower sensitivity to ethanol or organisms that are capable of consuming a wide range of different sugars for ethanol production are available for use in industrial bio-ethanol production. Depending on the strain and the operating conditions, an ethanol concentration of between 5% and 20% may be expected in the final broth. Yields in excess of 90% can be expected for glucose. For other sugars, the yields vary significantly between strains. [7]

The state-of-the-art fermentation method for ethanol production is in a batch process due to the simplicity and ease of sterilizing the equipment. Other configurations such as fed

batch or continuous operation or production with in-situ product removal may also be applied and result in higher productivity and yield, but with higher capital investment cost and an increased risk of contamination.

3.2 Downstream processing

After the sugars had been converted to ethanol, the cells must be harvested for re-use and the ethanol should be isolated from the rest of the broth as pure product. This is done using a centrifuge, a distillation column and a molecular sieve.

Firstly, a simple solid-liquid separation is conducted using a disk stack centrifuge. This is the state-of-the-art device used for cell recovery and is commercially available in different sizes from several suppliers. The cells are recovered in the centrifuge as an intermittently released slurry. The cell-free liquid fraction (centrate) is recovered from the top of the centrifuge and contains the ethanol product.

Distillation is the first step in isolating ethanol from the broth. By iterative steps of evaporation and condensation in a distillation column, ethanol of up to 96% purity can be obtained in the distillate. Ethanol and water form an azeotrope at 96%, therefore it is not possible to achieve a higher concentration of ethanol through conventional distillation [8]. The distillation column bottoms would contain mostly water, unconverted sugars and other broth components. This stream may be recycled or further processed depending on the stream composition and other processing facilities at or near the ethanol production plant.

In order to further purify the ethanol to >99% purity, the azeotropic distillate mixture is sent through a zeolite molecular sieve in gas phase. Water is trapped in the zeolite pores and ethanol passes through the molecular sieve, resulting in a pure ethanol product. The zeolite is regenerated by subjecting it to a vacuum.

Auxiliary equipment such as pumps, conveyors, instrumentation, sterilization equipment, vehicles, utilities, multiples of certain equipment types, laboratories, offices, storage and buildings are not shown in the process flow diagram for simplicity, but these cannot be omitted from the capital investment cost calculations.

3.3 Mass and energy balance

Table 1 shows the required flows of feed into and out of a bio-ethanol plant for the production of ethanol on a per-kilogram-ethanol basis. This is done on the assumption that one batch of cells from the seed train remains viable and productive for 4 production fermentation batches. For each kilogram of ethanol, 5.3 kg of hydrolysate (2.5 kg monosaccharides) and a few grams of supplements are required, 1.2 kg of CO_2 is emitted, 300 grams of yeast is generated and a side stream of 3.1 kg waste water is produced.

A summary of the most significant energy needs for the production of bio-ethanol is given in Table 2. The production of one kilogram of ethanol requires 1 kWh of electrical power,

1.3 kWh of steam and 1.5 kWh of cooling water. Most of the electrical power (98%) is for agitation of the production fermenter and most of the steam (98%) is consumed in the distillation column reboiler. Cooling water is used mostly for temperature control in the production fermenter (58%) and for condensation in the distillation column (41%). This total energy requirement is calculated before optimizing energy flows through heat integration and on the assumption that one batch of cells from the seed train remains viable and productive for four production fermentation batches. It also does not consider energy consumption for auxiliary equipment and administrative services. With that in mind, the calculated total energy consumption for the production of bio-ethanol is only 46% of the energy contained within ethanol as a fuel [9].

Table 1: Mass balance for bio-ethanol production

Description	Hydrolysate	Ammonium sulphate	CO_2 emissions from fermentations	Yeast cell recycle stream from centrifuge	Aqueous waste (Distillation bottoms)	Ethanol
Total Mass Flow (kg/kg EtOH)	5.3	0.004	1.2	0.3	3.1	1
Temperature (°C)	25	25	39	39	97.5	25
Pressure (bar)	1.013	1.013	1.013	1.013	1.013	1.013
Amm. Sulfate	-	100%	-	0.05%	0.1%	-
Cellulose	0.06%	-	-	1%	-	-
CO_2	-	-	100%	-	-	-
Ethyl Alcohol	-	-	-	13%	2%	100.00%
Glucose	28%	-	-	0.8%	2%	-
Lignin	0.2%	-	-	4%	-	-
Proteins	0.3%	-	-	0.2%	0.3%	-
Sodium Acetate	0.2%	-	-	0.1%	0.3%	-
Water	53%	-	-	49%	94%	-
Xylose	19%	-	-	0.5%	1%	-
Yeast	-	-	-	31%	-	-

Table 2: Energy requirements for bio-ethanol production

Energy flow per kg ethanol		1000L seed fermenter	25m^3 production fermenter	Centrifuge	Distillation	Molecular sieve	Ethanol condenser
Electrical power	kWh/kg	0.003	0.958	0.023	-	-	-
Heating	kWh/kg	-	-	-	1.283	0.022	-
Cooling	kWh/kg	0.002	0.878	-	0.622	-	0.010

4 Isobutanol production

Isobutanol can be produced by fermenting sugars using naturally occurring or engineered microbes such as yeast or bacteria under anaerobic conditions. Unlike ethanol, however, isobutanol negatively affects the metabolism of the cells from around 1,2% and kills most cells at a concentration of only 2%. It is therefore necessary to conduct in-situ product removal in order to achieve an economically viable titer without poisoning the production organism. There are several possibilities for in-situ product removal. [10] The process described here and depicted in Figure 2, makes use of liquid-liquid extraction to remove isobutanol from the fermentation broth during fermentation. The feedstocks and seed train are as described in section 2.1 and 2.2.

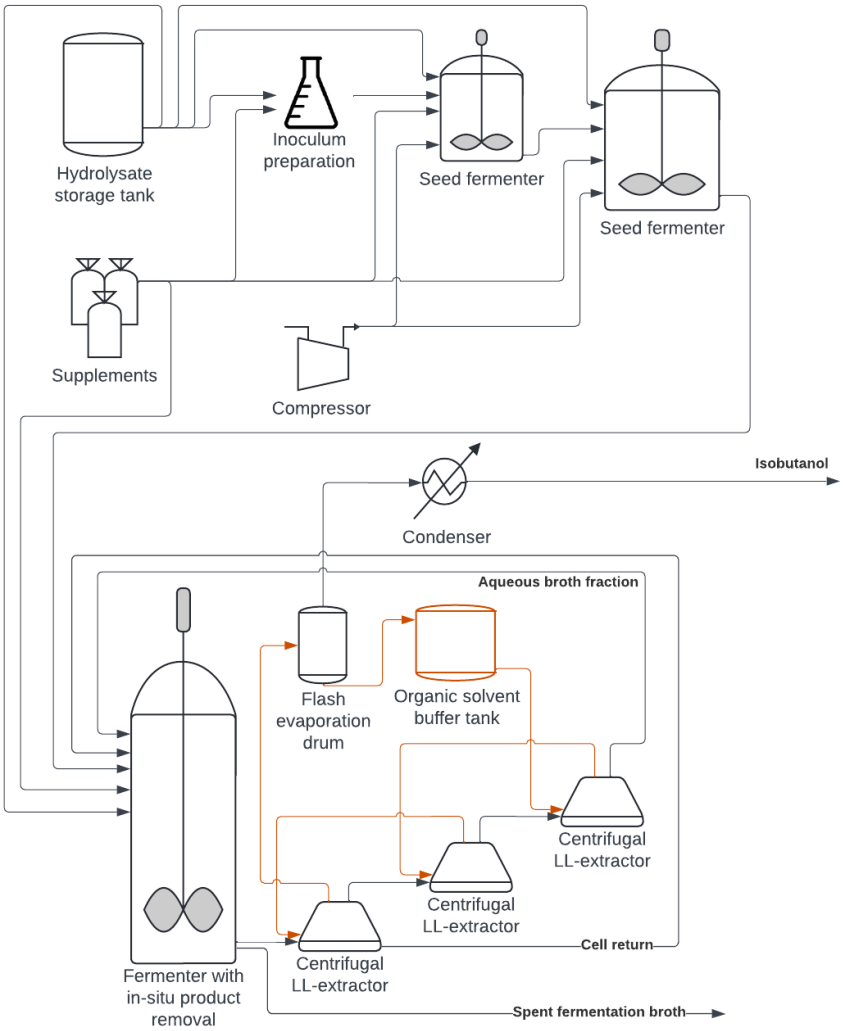

Figure 2: Process flow diagram for isobutanol production

4.1 Production fermenter

In the production fermenter, cell growth is unwanted because it competes with the production of isobutanol. The production fermenter is operated anaerobically to limit the formation of new cells and to force the existing cells to generate isobutanol to survive.

Clostridium acetobutylicum is the organism used for acetone–butanol–ethanol (ABE) fermentations during the first world war in order to manufacture explosives. This *Clostridium* strain, as well as some other engineered strains of yeasts and bacteria with higher production capacity and specifity, lower sensitivity to isobutanol or organisms that are capable of consuming a wide range of different sugars for isobutanol production exist and are being developed for use in industrial isobutanol production. [11]

Depending on the strain and the operating conditions, an isobutanol concentration of up to 2% can be achieved before the cells die off. This is a very low titer, which is unlikely to result in an economically viable production process unless the product is constantly removed from the fermentation broth during fermentation in order to maintain a low concentration in the fermenter.

The in-situ product removal method shown in Figure 2 is a liquid-liquid extraction system, using an organic solvent to continuously extract isobutanol from the aqueous broth.

4.2 Downstream processing

Due to the toxicity of isobutanol to the production organism, it must be continually removed from the fermenter as it is being produced. In the process described here, liquid-liquid extraction (LLE) is used for this purpose. An immiscible and non-toxic organic solvent, such as oleyl alcohol, is brought into close contact with the aqueous broth, which causes the isobutanol to diffuse out of the aqueous broth into the organic solvent, from where it can be recovered by flash evaporation.

Many different configurations of equipment are available for LLE. In this case, a 3-stage counter-current configuration with centrifugal extractors is used. Counter-current operation allows recovery of more product from the broth than a single stage LLE setup and does so using less solvent than a cross-current configuration. Oleyl alcohol is used because it is immiscible in water, non-toxic, relatively inexpensive and has a high partition coefficient for recovering butanol from an aqueous solution [12]. It is therefore assumed to have a similar partition coefficient for isobutanol.

In the first stage of centrifugation, the cells are also centrifuged out of the broth and returned to the fermenter along with the broth from the 3rd stage that has been stripped of isobutanol.

After passing through the three stages of LLE, the organic solvent is loaded with isobutanol. Isobutanol is recovered from the loaded organic solvent by means of flash evapora-

tion. The vapour fraction from the flash drum contains pure isobutanol that must be condensed and stored or packaged for sale. The liquid fraction from the flash drum contains organic solvent with no- or insignificant amounts of isobutanol and can therefore be re-used for further cycles of LLE.

Auxiliary equipment such as pumps, conveyors, instrumentation, sterilization equipment, vehicles, utilities, multiples of certain equipment types, laboratories, offices, storage and buildings are not shown in the process flow diagram for simplicity, but these cannot be omitted from the capital investment cost calculations.

4.3 Mass and energy balance

Table 3 and Table 4 show the material flows in and out of the seed train and production sections of the isobutanol production process depicted in Figure 2 on a per-kilogram-isobutanol basis. In this case, it was assumed that each new batch of product requires a new batch of cells. For each kilogram of isobutanol, 9.2 kg hydrolysate (4.3 kg monosaccharides) and a few grams of supplements are required, 1.4 kg CO_2 is emitted, 60 grams of cells is generated and a side stream of 7 kg (including the cells) is produced. For in-situ LLE product removal, 8.2 kg of oleyl alcohol is circulated per kg of isobutanol per hour.

Table 3: Mass balance for the seed train in the isobutanol production process

Description	**Seed train hydrolysate**	**Seed train supplements**	**Seed train emissions**	**Pre-culture**
Total Mass Flow (kg/kg)	0.44	0.0004	0.22	0.44
Temperature (°C)	25	25	37	37
Pressure (bar)	1.013	1.013	1.013	1.013
Amm. Sulphate	-	100%	-	0.1%
Cellulose	0.1%	-	-	0.1%
CO_2	-	-	100%	-
Glucose	28%	-	-	1%
Lignin	0.2%	-	-	0.2%
Phenol	0.2%	-	0.4%	-
Proteins	0.3%	-	-	0.3%
Sodium Acetate	0.2%	-	-	0.2%
Water	53%	-	-	73%
Xylose	19%	-	-	12%
Cells	-	-	-	13%

A summary of the most significant energy needs for the production of isobutanol is given in Table 5. The production of one kilogram of isobutanol requires 0.3 kWh of electrical power, 0.85 kWh of steam and 0.84 kWh of cooling water. Most of the electrical power

(93%) is for agitation of the production fermenter and all of the steam is consumed in separation of isobutanol from the oleyl alcohol solvent. Cooling water is used mostly for cooling the oleyl alcohol down for recycle (77%).

The total energy requirement reported is calculated before optimizing energy flows through heat integration. It also does not consider energy consumption for auxiliary equipment and administrative services. With that in mind, the calculated total energy consumption for the production of isobutanol is only 2% of the energy contained within isobutanol as a fuel [13].

Table 4: Material flows for the isobutanol fermenter with in-situ product removal

Description	Pre-culture	Production hydrolysate	Production supplements	Circulating oleyl alcohol	Isobutanol product	Production fermentation emissions	Spent aqueous fermentation broth
Total Mass Flow (kg/kg)	0.437	8.785	0.007	8.195	1.016	1.151	7.026
Temperature (°C)	37	25	25	37	40	37	37
Pressure (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Amm. Sulphate	0.1%	-	100%	-	-	-	0.1%
Cellulose	0.1%	0.1%	-	-	-	-	0.1%
CO_2	-	-	-	-	-	99.96%	-
Glucose	1%	28%	-	-	-	-	1%
Isobutanol	-	-	-	0.01%	98%	-	0.05%
Lignin	0.2%	0.2%	-	-	-	-	0.3%
Oleyl alcohol	-	-	-	99.99%	-	-	-
Phenol	-	0.2%	-	-	2%	0.04%	-
Proteins	0.3%	0.3%	-	-	-	-	0.3%
Sodium Acetate	0.2%	0.2%	-	-	-	-	0.3%
Water	73%	53%	-	-	-	-	74%
Xylose	12%	19%	-	-	-	-	24%
Cells	13%	-	-	-	-	-	1%

Table 5: Energy requirements for the isobutanol process per kg product

Energy flux per kg isobutanol		**1000L seed fermenter**	**25m^3 production fermenter**	**Cell separation centrifuge**	**Flash drum**	**solvent cooler**	**Isobutanol condenser**
Electrical power	kWh/kg	0.036	0.255	0.015	-	-	-
Heating	kWh/kg	-	-	-	0.851	-	-
Cooling	kWh/kg	0.027	0.120	-	-	0.646	0.050

5 Lactic acid production

Lactic acid is produced from grass by naturally occurring lactic acid bacteria (LAB) in silage. We, as humans, also produce lactic acid in our muscles when we work so hard that we consume energy faster than our blood stream can carry oxygen to burn sugar [14]. Lactic acid producing microbes can be sorted into two groups based on the products that they produce under anaerobic conditions. Homofermentative LAB produce only lactic acid and heterofermentative LAB produce lactic acid as well as other products such as acetic acid and ethanol. [15]

The production of lactic acid in the fermenter inevitably causes a decrease in pH, which must be counteracted by the addition of an alkaline component or removal of the acid or both. The state-of-the-art method makes use of lime (CaO) to neutralize and precipitate the acid as calcium lactate. Sulphuric acid is then used downstream to recover lactic acid from the calcium lactate, thereby producing large quanities of solid gypsum waste. After recovery from the solid calcium lactate form, the lactic acid is purified through reactive distillation with an alcohol. In the process presented here and depicted in Figure3, lime is replaced with ammonia, which can be recycled, does not create a precipitate and allows purification through reactive distillation without producing any solid waste [15]. The feedstocks and seed train are as described in section 2.1 and 2.2.

5.1 Production fermenter

In the production fermenter, cell growth is unwanted because it competes with the production of lactic acid. Homofermentative organisms are also preferred above heterofermentative organisms both for lactic acid yield as well as simplifying downstream processes. The production fermenter is operated anaerobically to inhibit the formation of new cells and to force the existing cells to generate lactic acid to survive.

Bacteria from the genera *Lactobacillus, Lactococcus, Enterococcus, Pediococcus, Leuconostoc, Streptococcus, Carnobacterium, Fructobacillus, Oenococcus,* and *Weissella* naturally produce lactic acid efficiently. They are normally also resistant to low pH and high salt concentrations [16]. Some engineered strains of homofermentative yeasts and bacteria with high productivity, lower sensitivity to changes in pH or organisms that are capable of consuming a wide range of different sugars for lactic acid production exist and are being developed for improving industrial lactic acid production [17].

Most LAB ferment well in a pH range between 5 and 6 [18]. Since the production of lactic acid changes the acidity in the broth, pH control is important for this process. In the process described here and depicted in Figure3, pH control and in-situ product removal are both achieved by passing the broth through an ion exchanger with anionic exchange resin. The resin captures the lactate anion from the broth and replaces it with hydroxide anions, thereby increasing the pH.

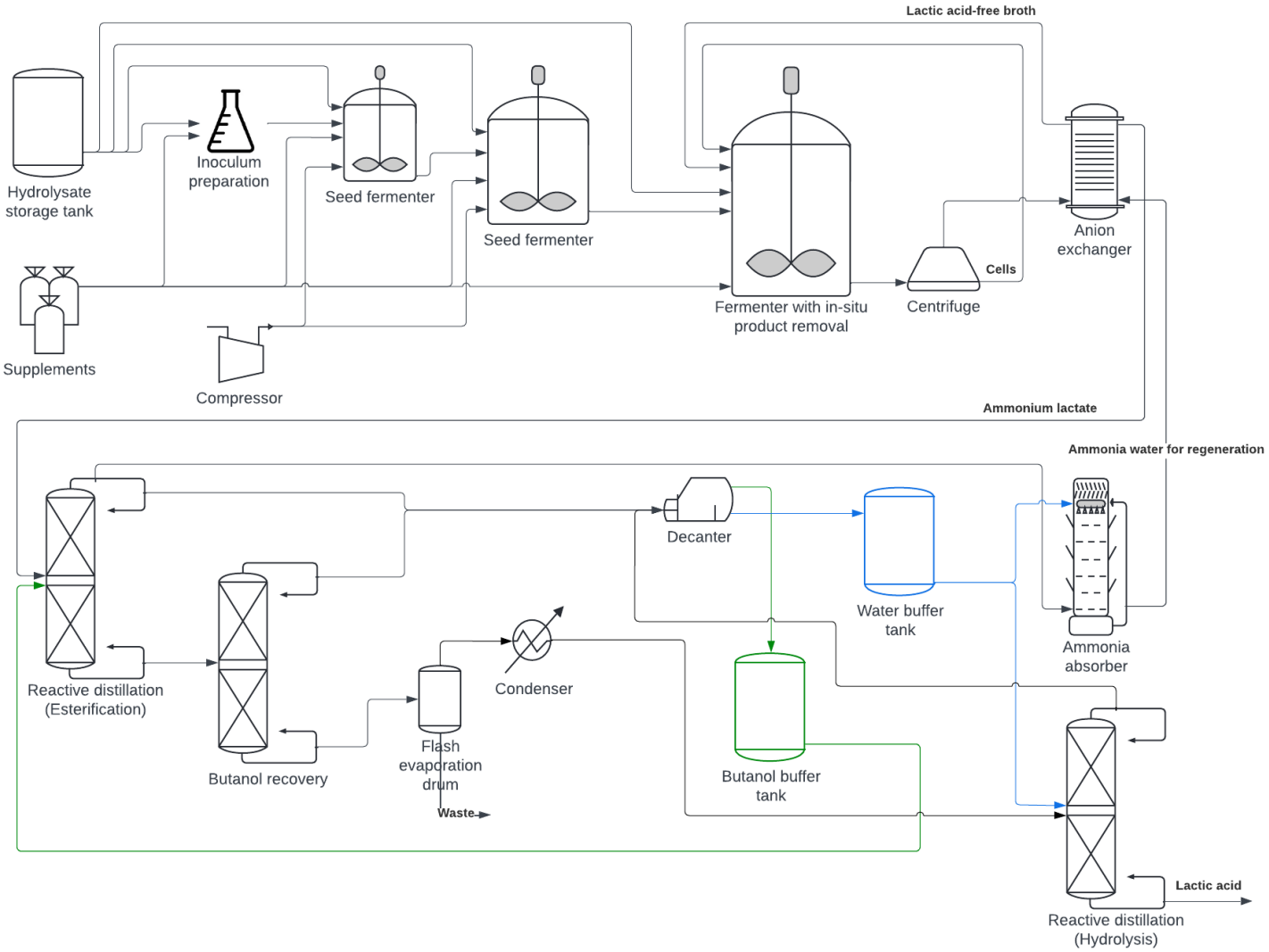

Figure3: Process flow diagram for lactic acid production

5.2 Downstream processing

Lactic acid is removed from the broth using ion exchange and then purified through reactive distillation.

5.2.1 Product recovery and pH control

Ion exchange resin easily becomes blocked with solids, therefore cells and any other solid particles must be removed from the broth before passing it through the ion exchanger. A disk stack centrifuge is used for this step. The solid fraction is returned to the fermenter while the liquid fraction is passed through the ion exchanger.

Ion exchangers are operated in cycles of two to three operations. These are loading, eluting and regenerating. In some cases, such as for the process described here, elution and regeneration can be achieved in one combined operation. During the loading operation,

the lactate anion is bound to the resin, which carries a positive charge. The lactate ions replace hydroxide ions that were bound to the resin before. The released hydroxide ions along with the rest of the broth is returned to the fermenter. Once the resin is saturated with lactate and has no more hydroxide to give, the lactate ions are and the resin is regenerated with hydroxide ions by running an ammonium hydroxide solution through it. In this operation lactate ions are released into the solution and hydroxide ions bind to the resin. Ammonium lactate in solution exits the ion exchanger. This operation is run until all the lactate ions on the resin have been replaced by hydroxides. Thereafter the cycle starts anew by feeding centrate through the ion exchanger to recover lactate.

5.2.2 Purification through reactive distillation

The ammonium lactate stream emerging from the ion exchanger during the elution operation is converted (esterified) to butyl lactate in a reactive distillation column with a catalyst. Sun *et.al.* successfully tested a catalyst for this purpose. The catalyst is a cationic exchange resin modified with stannous chloride [19]. In the esterification reactive distillation column, ammonium lactate is reacted with butanol in a 1:3 molar ratio in contact with the catalyst to produce butyl lactate. Ammonia is evolved from this reaction and collected from the top of the reactive distillation column [19]. This stream absorbed in water in an absorption column and re-used as the regeneration liquid for the ion exchanger. Distillate from the esterification reactive distillation column contains water and butanol, which are immiscible to a great extent and can therefore be separated by decanting. The two fractions, water and butanol, are returned to their respective holding tanks for re-use. The bottoms from the esterification reactive distillation column contains the produced butyl lactate as well as some unreacted ammonium lactate, butanol and water. Butanol is removed from this mixture through vacuum evaporation and returned to the butanol buffer tank. To isolate butyl lactate from the other components and potential contaminants in the stream, it is evaporated in a flash drum and recondensed as purified butyl lactate.

A second reactive distillation column is used for hydrolysis of the butyl lactate to yield pure lactic acid. The catalyst used here is a cation exchange resin in the H+ form. To hydrolyze the butyl lactate, it is brought into contact with the catalyst and an excess of water (1:15 molar ratio). [19] Hydrolysis of butyl lactate yields butanol and lactic acid. Butanol and water are recovered from the distillate of the hydrolysis reactive distillation column, decanted and returned to the butanol- and water buffer tanks for re-use. The hydrolysis reactive distillation column bottoms contain >90% pure lactic acid product.

Auxiliary equipment such as pumps, conveyors, instrumentation, sterilization equipment, vehicles, utilities, multiples of certain equipment types, laboratories, offices, storage and buildings are not shown in the process flow diagram for simplicity, but these cannot be omitted from the capital investment cost calculations.

5.3 Mass and energy balance

on a per-kilogram-lactic acid basis. In this case, it was assumed that a batch of fresh cells from the seed train is sufficient for three production runs in the fermenter. For each kilogram of lactic acid, 4.7 kg hydrolysate (1.3 kg monosaccharides) and a few grams of supplements are required, 0.4 kg ammonium hydroxide (recycled) is used for pH control, 30 grams of cells is generated, a side stream of 3.9 kg (including the cells) is produced and an intermediate ammonium lactate stream of 2.6 kg is produced in the fermentation section. In the purification section, the intermediate ammonium lactate stream from the fermentation section is esterified with butanol (2.6 kg/kg lactic acid recycled with 70 g/kg lactic acid topped up) and hydrolyzed with water (4.4 kg/kg lactic acid recycled). Ammonia from the esterification reaction is captured in water and returned to the fermentation section for pH control, and an aqueous waste stream of 160 g/kg lactic acid is produced.

A summary of the most significant energy needs for the production of lactic acid is given in Table 8. The production of one kilogram of lactic acid requires 1.42 kWh of electrical power, 8.73 kWh of steam and 9.11 kWh of cooling water. Most of the electrical power (51%) is for agitation of the production fermenter. The remaining electrical power is mostly used for continually separating cells from the broth in the centrifuge. 97% of the steam is consumed in the reactive distillation columns for esterification and hydrolysis. 92% of the cooling water is used in the reactive distillation columns' condensers.

The total energy requirement reported is calculated before optimizing energy flows through heat integration. It also does not consider energy consumption for auxiliary equipment and administrative services. With that in mind, the calculated total energy cost for the production of lactic acid equates to around 80% of the selling price for a racemic mix of lactic acid in Germany [20].

Table 6: Mass balance for the fermentation section with integrated pH control and product removal

Stream flows (kg/kg lactic acid)	**Hydrolysate (incl. 1/3 seed train)**	**Supplements (1/3 seed train)**	**Ammonia water eluent recycled**	**Cells generated and recycled**	**Ammonium lactate eluate**	**Spent broth**
Temperature (°C)	25	25	25	37	25	37
Pressure (bar)	1.013	1.013	1.013	1.013	1.013	1.013
Total Contents (kg/kg)	4.74	0.00002	1.71	0.03	2.59	3.94
Amm. Acetate	-	-	-	-	0.01	-
Amm. Hydroxide	-	-	0.42	-	-	-
Amm. Lactate	-	-	-	-	1.29	-
Amm. Sulphate	-	0.00002	-	-	0.00007	-
Cellulose	0.003	-	-	-	-	0.003
Glucose	1.32	-	-	-	-	0.27
Lactic Acid	-	-	-	-	-	0.03
Lignin	0.010	-	-	-	-	0.01
Phenol	0.009	-	-	-	-	0.009
Proteins	0.013	-	-	-	0.01	0.001
Sodium Acetate	0.011	-	-	-	-	-
Water	2.50	-	1.29	-	1.29	2.78
Xylose	0.88	-	-	-	-	0.84
Cells	-	-	-	0.03	-	0.03

Table 7: Mass balance stream flow for the downstream

Stream flows (kg/kg lactic acid)	Fresh butanol feed	Ammonium lactate eluate	Butanol recycle	Water recycle	Ammonia water eluent recycled	Lactic acid	Aqueous waste
Temperature (°C)	25	25	56.5	56.5	25	142	56.5
Pressure (bar)	1.013	1.013	0.067	0.067	1.013	0.067	0.067
Total Contents (kg/kg)	0.07	2.59	2.60	4.36	1.71	1.05	0.16
Amm. Acetate	-	0.01	-	-	-	-	-
Amm. Hydroxide	-	-	-	-	0.42	-	-
Amm. Lactate	-	1.29	-	-	-	-	-
Amm. Sulphate	-	0.00007	-	-	-	-	-
Butanol	0.07	-	2.60	0.04	-	-	0.001
Butyl lactate	-	-	0.001	0.08	-	0.05	0.004
Lactic Acid	-	-	-	-	-	1.00	-
Proteins	-	0.01	-	-	-	-	-
Water	-	1.29	-	4.24	1.29	-	0.15

Table 8: Energy requirements for the production of lactic acid per kg product

Energy	Production fermenter 25m^3	Centrifuge	Reactive distillation for esterification	Butanol recovery column	Butyl lactate flash rectifier	Butanol condenser	Reactive distillation for hydrolysis
Electrical power (kWh/kg)	0.73	0.68	0.00	-	-	-	0.00
Heating (kWh/kg)	-	-	2.72	0.13	0.09	-	5.80
Cooling (kWh/kg)	0.52	-	2.47	0.14	-	0.05	5.92

6 Recommendations

Any person or entity wishing to use the information in this report for their own business plans free to do so and is advised to consult the One-stop-shop database on the TransBIB website to find additional information, expert consultants in the field, piloting facilities and equipment manufacturers that may assist in the process.

Any person or entity wishing to contribute to the German bio-economy is also advised to contact TransBIB through any of the platforms mentioned on the website so that they may become integrated into the TransBIB network.

Bibliography

[1] “Startseite - TransBIB.” Accessed: Nov. 04, 2024. [Online]. Available: https://transbib.de/de/

[2] “Bioeconomy - BMBF,” Federal Ministry of Education and Research - BMBF. Accessed: Nov. 04, 2024. [Online]. Available: https://www.bmbf.de/bmbf/en/research/energy-and-economy/bioeconomy/bioeconomy_node.html

[3] Venair, “Upstream vs. Downstream Processing in Biomanufacturing | Venair.” Accessed: Nov. 04, 2024. [Online]. Available: https://venair.com/en/blog/articles/upstream-downstream

[4] E. Palmqvist and B. Hahn-Hägerdal, “Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition,” *Bioresour. Technol.*, vol. 74, no. 1, pp. 25–33, Aug. 2000, doi: 10.1016/S0960-8524(99)00161-3.

[5] Y. N. Guragain, K. V. Probst, and P. V. Vadlani, “Fuel Alcohol Production,” in *Reference Module in Food Science*, Elsevier, 2016. doi: 10.1016/B978-0-08-100596-5.00137-2.

[6] T. L. Nissen, C. W. Hamann, M. C. Kielland-Brandt, J. Nielsen, and J. Villadsen, “Anaerobic and aerobic batch cultivations of Saccharomyces cerevisiae mutants impaired in glycerol synthesis,” *Yeast*, vol. 16, no. 5, pp. 463–474, 2000, doi: 10.1002/(SICI)1097-0061(20000330)16:5<463::AID-YEA535>3.0.CO;2-3.

[7] D. G. Olson, R. Sparling, and L. R. Lynd, “Ethanol production by engineered thermophiles,” *Curr. Opin. Biotechnol.*, vol. 33, pp. 130–141, Jun. 2015, doi: 10.1016/j.copbio.2015.02.006.

[8] V. Carravetta *et al.*, “An atomistic explanation of the ethanol–water azeotrope,” *Phys. Chem. Chem. Phys.*, vol. 24, no. 42, pp. 26037–26045, Nov. 2022, doi: 10.1039/D2CP03145K.

[9] N. O. of D. and Informatics, “NIST Chemistry WebBook.” Accessed: Nov. 04, 2024. [Online]. Available: https://webbook.nist.gov/chemistry/

[10] J. Borger, “Solvent characterization for in-situ liquid/liquid extraction of isobutanol from fermentation broth,” presented at the Himmelfahrtstagung on Bioprocess Engineering 2024 - Novel Strategies and Technologies for Sustainable Bioprocesses and Bioproducts, 2024. Accessed: Oct. 24, 2024. [Online]. Available: https://dechema.converia.de/frontend/index.php?page_id=16391&additions_conferenceschedule_action=detail&additions_conferenceschedule_controller=paperList&pid=49997&hash=f948e36161bb53d8859875c8e623181321f92edd335148b7c5f8190f976937fb

[11] H. G. Moon, Y.-S. Jang, C. Cho, J. Lee, R. Binkley, and S. Y. Lee, “One hundred years of clostridial butanol fermentation,” *FEMS Microbiol. Lett.*, vol. 363, no. 3, p. fnw001, Feb. 2016, doi: 10.1093/femsle/fnw001.

[12] K. A. Motghare, K. L. Wasewar, and D. Z. Shende, “Separation of Butanol Using Tetradecyl(trihexyl)phosphonium Bis(2,4,4-trimethylpentyl)phosphinate, Oleyl Alcohol, and Castor Oil,” *J. Chem. Eng. Data*, vol. 64, no. 12, pp. 5079–5088, Dec. 2019, doi: 10.1021/acs.jced.9b00211.

[13] “ISOBUTANOL,” MIDSIS-TROCS. Accessed: Nov. 04, 2024. [Online]. Available: https://midsis.rempec.org/en/find-chemical/isobutanol

[14] “What is lactic acid? Respiration and gas exchange - KS3 Biology,” BBC Bitesize. Accessed: Nov. 04, 2024. [Online]. Available: https://www.bbc.co.uk/bitesize/articles/ztdmrwx

[15] A. O. Ojo and O. de Smidt, “Lactic Acid: A Comprehensive Review of Production to Purification,” *Processes*, vol. 11, no. 3, Art. no. 3, Mar. 2023, doi: 10.3390/pr11030688.

[16]V. M. Dillon, “NATURAL ANTI-MICROBIAL SYSTEMS | Preservative Effects During Storage,” in *Encyclopedia of Food Microbiology (Second Edition)*, C. A. Batt and M. L. Tortorello, Eds., Oxford: Academic Press, 2014, pp. 941–947. doi: 10.1016/B978-0-12-384730-0.00238-X.

[17]M. Sauer, D. Porro, D. Mattanovich, and P. Branduardi, “16 years research on lactic acid production with yeast – ready for the market?,” *Biotechnol. Genet. Eng. Rev.*, vol. 27, no. 1, pp. 229–256, Jan. 2010, doi: 10.1080/02648725.2010.10648152.

[18]P. Pal, J. Sikder, S. Roy, and L. Giorno, “Process intensification in lactic acid production: A review of membrane based processes,” *Chem. Eng. Process. Process Intensif.*, vol. 48, no. 11, pp. 1549–1559, Nov. 2009, doi: 10.1016/j.cep.2009.09.003.

[19]X. Sun, Q. Wang, W. Zhao, H. Ma, and K. Sakata, “Extraction and purification of lactic acid from fermentation broth by esterification and hydrolysis method,” *Sep. Purif. Technol.*, vol. 49, no. 1, pp. 43–48, Apr. 2006, doi: 10.1016/j.seppur.2005.08.005.

[20]“Milchsäure, 80 % (E 270),” chemiekontor.de. Accessed: Nov. 04, 2024. [Online]. Available: https://www.chemiekontor.de/organische-saeuren/milchsaeure-80-e-270/