

Chemical reaction as a method for the separation of biologically-derived carboxylic acids

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Abstract

The biotechnological production of organic compounds using renewable carbon sources is an approach consistent with sustainable development and green technologies. The development of these processes requires refinement of both the upstream stage, including the selection of microorganisms and the use of waste raw materials, and the downstream stage. The fermentation broth contains not only the main product but also unreacted substrates and by-products. The paper presents computer simulations that analyse the possibility of using esterification for the separation of lactic acid from acetic acid. The standard distillation approach does not allow for a high degree of separation, but a distillation step is possible for esters of both acids. As a result, high-purity ethyl lactate is obtained and, by introducing a hydrolysis step, pure lactic acid. The issue was analysed using Chemcad software with the UNIFAC thermodynamic model.

Keywords

fermentation broth, carboxylic acids, separation, esterification

1. INTRODUCTION

The biotechnological production of organic compounds is an important aspect in replacing processes based on non-renewable energy sources and is consistent with the sustainable development goals and the principles of green chemistry (Demmelmayer et al., 2024). The fermentation process uses a variety of microorganisms and different carbon sources depending on the final main product preferred. Technologies that use waste, renewable raw materials (including molasses, waste food raw materials) and those based on strains of bacteria that are safe for health are particularly desirable and allow to reduce production costs (Li et al., 2021b). Regardless of the parameters, the result is a fermentation broth that requires appropriately selected further processing operations after the upstream process (Vidal et al., 2024). The selection of the separation method depends on the physicochemical properties of the main product. In the case of carboxylic acids, the composition of the by-products and the pH of the fermentation are important. Most strains of bacteria that convert the carbon source into carboxylic acids (including lactic or succinic acid) require a neutral environment which is obtained by the addition of a neutralising agent (e.g. sodium hydroxide, calcium hydroxide). In this approach, the conversion efficiency and the content of the main product in the fermentation broth increase. Neutralisation of the fermentation broth during bioconversion results in the acquisition of a product in the form of a carboxylate (such as sodium lactate, calcium succinate), which requires acidification (Rübenach et al., 2024). Another important aspect is the presence of by-products. Biotechnological production of organic compounds does not require the

use of high temperatures or pressure, as in the case of many chemical reactions. This is very advantageous technologically, but as a result of fermentation a main product of limited concentration is obtained (Yin et al., 2023). Additionally, at the separation stage, unreacted substrates, auxiliary media, inorganic salts, biomass, and byproducts of the metabolic pathway should be taken into account. There are many separation methods described in the literature that can be used at the stage of downstream processing (Bui et al., 2024). At the initial stage of separation, filtration methods (including microfiltration and ultrafiltration) and centrifugation are used (Lee et al., 2017). In the next step, ion exchange, preparative chromatography, solvent extraction or reactive extraction (Mungma et al., 2019), esterification, and membrane methods (such as pervaporation, electrodialysis, and electrodeionization) can be used (Papadopoulou et al., 2023). Crystallisation and distillation are most often the final stage. Selecting the appropriate method requires knowledge of the composition of the fermentation broth and the amount of by-products. In the biotechnological production of lactic acid, parallel formation of other carboxylic acids, including acetic acid, is observed. Filtration and centrifugation are efficient methods of separating biomass and solid contaminants from raw fermentation broth. In a further stage, the use of extraction, ion exchange, or electrodialysis enables the separation of some impurities, but the purified solution is always a mixture of all carboxylic acids present in the raw broth. As a result of the biotechnological production of lactic acid fermentation broth, acetic acid is produced as a by-product (Lech and Trusek, 2019). The study analysed the possibility of separating both of these acids from aqueous solutions by distillation, as well



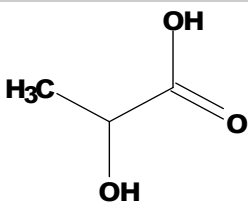
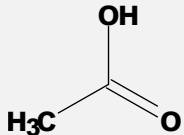
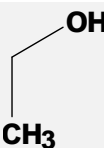
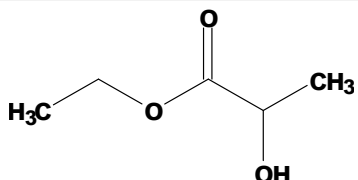
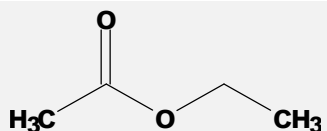
as by using reactive distillation. Chemcad software was used as the process simulation tool. UNIFAC was chosen as the thermodynamic model, which is based on functional group contributions, can be used for mixtures containing non-polar and polar organic compounds and one that does not require experimental data. Basic data on chemical compounds are presented in Table 1.

2. BIOPRODUCTION OF LACTIC ACID

Efficient and affordable technology for the production of platform chemicals is a necessary condition for the development of the bio-based economy. The upstream and downstream stages are important in the balance of production costs of biocompounds, including carboxylic acids. Additionally, the conditions of the upstream stage, the media used, and the degree of substrate contamination determine the complexity of the downstream stage. The possibility of using industrial side streams (e.g. from the pulp and paper industry) as a bioconversion substrate is a sustainable approach (Olszewska-Widdrat et al., 2023). Microbial fermentation of lactic acid can be carried out using various common strains of microorganisms,

including *Lactobacillus*, *Bacillus*, *Escherichia coli*, *Rhizopus oryzae*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Lactobacillus cruzi* (Huang et al., 2023). The bioproduction of lactic acid requires the selection of microorganisms, biomass as a substrate, auxiliary media such as a neutralising agent (calcium hydroxide, sodium hydroxide, ammonia) and inorganic salts. These ingredients are found in the fermentation broth and, together with by-products (acetic acid, ethanol), require separation (Huang et al., 2021). The use of lactic acid increases every year, hence the development of new production paths, including new strains with excellent lactic acid production properties and other strategies that effectively improve lactic acid production is a current issue. Strain modification technology is one of the main areas of microbiology research and includes mutagenesis, adaptive evolution, and metabolic engineering. As a result, the use of safe and effective breeding technology can effectively improve productivity (Liu et al., 2023; Tian et al., 2021). Lactic acid is produced in various fermentation modes including batch, fed-batch, and continuous bioreactors (Ojo and de Smidt, 2023). Regardless of the reactor operating mode, the result is a fermentation broth that requires purification and downstream processing toward a final product of high purity and quality.

Table 1. Chemical properties of chemical compounds used in computer simulation.

	Structure	Molecular Formula	SMILES Notation	Formula Weight
Lactic Acid		C ₃ H ₆ O ₃	O=C(O)C(O)C	90.078
Acetic Acid		C ₂ H ₄ O ₂	O=C(O)C	60.052
Water	H—OH	H ₂ O	O	18.015
Ethanol		C ₂ H ₆ O	OCC	46.069
Ethyl Lactate		C ₅ H ₁₀ O ₃	O=C(OCC)C(O)C	118.132
Ethyl Acetate		C ₄ H ₈ O ₂	O=C(C)OCC	88.106

3. DISTILLATION OF CARBOXYLIC ACIDS

Lactic acid has a wide range of applications in various industries, and its required quality and purity need effective downstream methods (Swetha et al., 2023; Zhang et al., 2022). Lactic acid has two optically active isomeric forms: L(+) lactic acid and D(-) lactic acid (Augustiniene et al., 2021). This carboxylic acid is important for the production of polylactic acid (PLA), where the isomeric composition has a significant impact (Kienberger et al., 2023). The production of lactic acid by chemical synthesis always leads to a mixture of both isomers. Additionally, impurities in lactic acid can significantly affect the PLA production process, as well as the physical and chemical properties of PLA (Ahmad et al., 2024). Therefore, the biotechnological route is of great importance in production and is gaining more attention due to the high optical purity of the final product (González-Navarrete et al., 2022).

The biotechnologically obtained lactic acid solution is purified and then usually concentrated. Most often, the process is carried out to obtain 80–88% of the product due to the polycondensation reaction observed at a higher degree of dehydration. The polycondensation reaction is an undesirable process, therefore this aspect should be taken into account in the technological process.

In the simulations used to design the process, calculations were made for various concentrations of the final product. Simulations were performed using Chemcad software (Chemstations). In the first stage, a computer simulation of the distillation of an aqueous solution of carboxylic acids was performed. Figure 1 shows a distillation scheme in which the liquid and gas phase vapor liquid equilibrium was calculated based on the UNIFAC model.

The column was fed with raw material (a composition taking into account the content of fermentation broths): 10 kg/h lactic acid, 1 kg/h acetic acid and 89 kg/h water (weight percentage of lactic acid: 10%). The process was carried out at a pressure of 101325 Pa. Depending on the required hydration of the final product (bottom stream), partial contamination of lactic acid with acetic acid was observed. The results obtained are summarized in Table 2 and Table 3.

On analysis of the results obtained, it can be indicated that it is not possible to obtain complete separation for an aqueous concentrated solution of lactic acid. Acetic acid can be separated at a lactic acid concentration above 98%, which is not advantageous due to the ongoing polymerisation process.

4. REACTIVE DISTILLATION OF CARBOXYLIC ACIDS

In the next stage of computer simulations, the distillation process was analysed for an aqueous mixture of acids and their ethyl esters.

The study included a reactive distillation process in which a hybrid esterification-distillation process was used. Esterification is indicated as a method that enables the production of high-purity biolactic acid (Li et al., 2021a). Carboxylic acids in the environment of alcohol and an acid catalyst easily form esters of this alcohol. Sulphuric acid can be used as a catalyst, but a more preferred solution is heterogeneous catalysis on an acidic ion exchanger, which can be easily separated after the process and used in the next reaction step. Esterification is a reversible reaction, so simultaneous distillation is preferred as a method of shifting the reaction equilibrium. At the same time, the hydrolysis reaction is the easiest way to obtain the

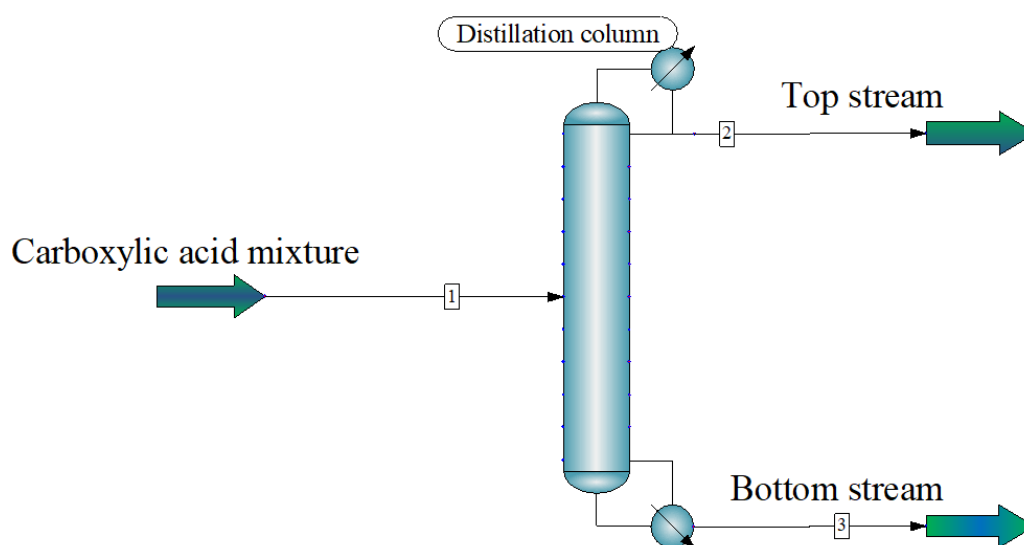


Figure 1. Distillation scheme: a multi-stage vapor-liquid equilibrium module with one feed stream and two product streams (feed: 10 kg/h lactic acid, 1 kg/h acetic acid, 89 kg/h water).

Table 2. Results of the distillation simulation for lactic acid and acetic acid with a variable amount of the received bottom stream.

Lp	No of stages	Feed stage for stream 1	Reflux ratio	Bottom component mass flow rate	Top stream [kg/h]			Bottom stream [kg/h]			Weight % for LA	Weight % for AA	
					LA	AA	W	LA	AA	W			
1	10	5	1	9	kg/h of LA	1	1	89	9	0	0	100	0
2	10	5	1	9.5	kg/h of LA	0.5	1	89	9.5	0	0	100	0
3	10	5	1	9.8	kg/h of LA	0.2	1	89	9.8	0	0	100	0
4	10	5	1	10	kg/h of LA	0	0.12	25.54	10	0.88	63.46	13.45	1.18
5	10	5	1	5	kg/h of W	0	0.5	84	10	0.5	5	64.52	3.22
6	10	5	1	3	kg/h of W	0	0.57	86	10	0.43	3	74.44	3.23
7	10	5	1	2	kg/h of W	0	0.63	87	10	0.37	2	80.81	3.02
8	10	5	1	1	kg/h of W	0	0.73	88	10	0.27	1	88.72	2.41
9	10	5	1	0.5	kg/h of W	0	0.81	88.5	10	0.19	0.5	93.56	1.76
10	10	5	1	0.1	kg/h of W	0	0.91	88.9	10	0.09	0.1	98.1	0.89

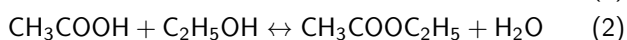
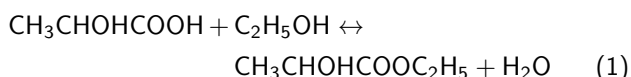
LA – Lactic Acid; AA – Acetic Acid; W – Water

Table 3. Distillation simulation results for lactic acid and acetic acid with a constant amount of received bottom stream.

Lp	No of stages	Feed stage for stream 1	Reflux ratio	Bottom component mass flow rate	Top stream [kg/h]			Bottom stream [kg/h]			Weight % for LA	Weight % for AA	
					LA	AA	W	LA	AA	W			
7	10	5	1	2	kg/h of W	0	0.63	87	10	0.37	2	80.81	3.02
11	5	3	1	2	kg/h of W	0.01	0.8	87	9.99	0.2	2	81.99	1.61
12	10	5	2	2	kg/h of W	0	0.59	87	10	0.41	2	80.56	3.33
13	10	5	0.5	2	kg/h of W	0	0.66	87	10	0.34	2	81.02	2.78

LA – Lactic Acid; AA – Acetic Acid; W – Water

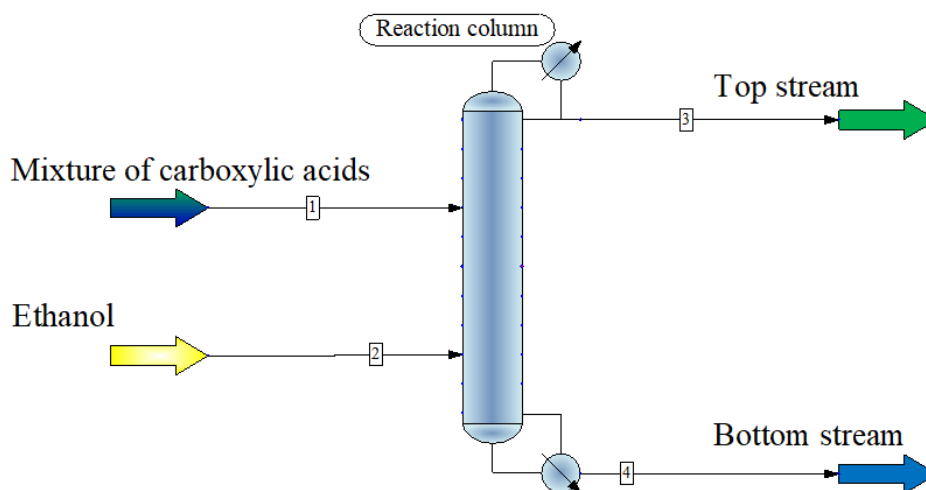
acidic form from the ester (lactic acid), after prior separation of the lactate from the impurities. Other carboxylic acids present in the fermentation broth also undergo esterification – Equations (1) and (2) present the esterification reaction of lactic and acetic acid in ethanol:



The selection of alcohol significantly affects the cost of the process – both due to the price of the substrate and the boiling point of the obtained esters. At the same time, the boiling points of the esters affect the possibility of separating both esters and subsequently obtaining high-purity lactic acid by hydrolysis. For ethyl acetate and ethyl lactate, the boiling points at atmospheric pressure are: 78 °C and 154 °C, respectively. The difference in these values allows the esters to be separated during distillation. If other alcohols are used,

a higher boiling point of the esters will be obtained, which involves a greater energy input at the distillation stage. For example, in a butanol environment, butyl lactate with a boiling point of 188 °C will be obtained. In this case, the process may be more efficient if the partial immiscibility of butanol with water and the additional coupling with the extraction method are taken into account.

In process optimisation, the distillation process of a mixture of acids and their ethyl esters was analysed. The simulation assumed a mixture after esterification for partially concentrated acids (30% LA by weight and 3% AA by weight) and a two-fold excess of ethanol as the reagent. Figure 2 shows the esterification reactions of lactic acid and acetic acid with ethanol assuming 96% conversion (the degree of conversion). The results obtained from this process (Figure 2) were used to analyse the distillation process of the post-reaction mixture. Figure 3 shows the diagram and composition of the feed dosed to the rectification column. The purity of ethyl lactate in the



Stream No.	1	2	3	4
Flow rates in kg/h				
Lactic Acid	30	0	0.0045	1.3062
Acetic Acid	3	0	0.0566	0.0549
Water	67	0	42.0938	31.5104
Ethanol	0	262	235.3703	9.7415
Ethyl Lactate	0	0	0.2381	37.386
Ethyl Acetate	0	0	4.2369	0.0009

Figure 2. Simulation of esterification of an aqueous solution of lactic acid and acetic acid with ethanol.

bottom stream after esterification (Figure 2) was 46.73% by weight (content of other chemicals: 39.39% water, 12.18% ethanol, 1.63% lactic acid, 0.07% acetic acid).

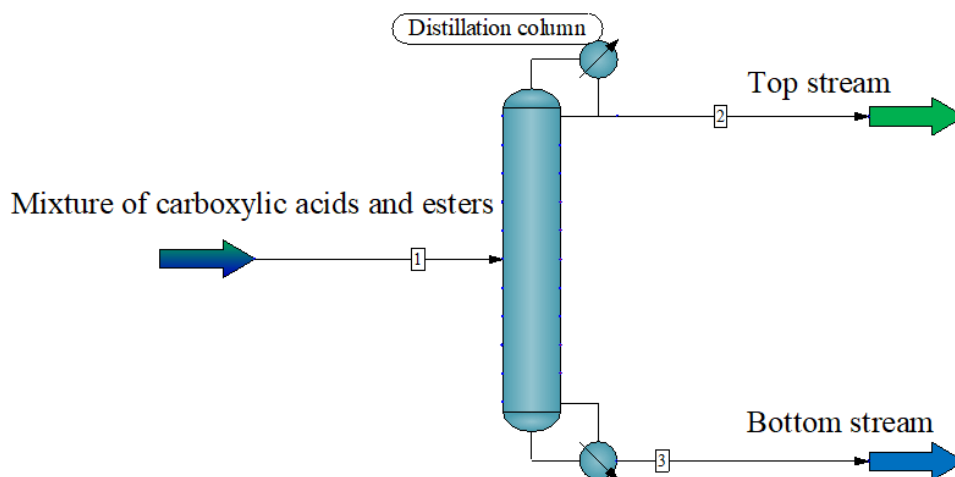
After distillation of the post-reaction mixture, a bottom aqueous stream was obtained (Fig. 3, stream no. 3) rich in ethyl lactate (80.67%) also containing lactic acid (3.51%) and acetic acid (0.13%). In this solution, a partial loss of the product was assumed (ethyl lactate content in the upper stream: 7.39 kg/h). If 37 kg/h of ethyl lactate is assumed to be collected in the bottom stream, the degree of product dilution increases (mass concentration 49.35%), while the acetic acid content is only slightly lower (0.07%). Therefore, it is worth collecting the entire stream of ethyl lactate and hydrolysing it in the next stage (water content is not an obstacle). The results obtained indicate that acetic acid is difficult to separate, regardless of the difference in boiling points. The best solution is to convert carboxylic acids into the appropriate esters as much as possible and then separate them by distillation. In such a case (Table 4), a pure aqueous solution of ethyl lactate is obtained, which can be used as the final product or hydrolysed to produce high-purity lactic acid.

The separation of acetic acid by distillation, despite the difference in the boiling points of the solution components, is a difficult issue to implement. During experimental tests, other values of retention time in relation to the boiling points of

Table 4. Ester distillation simulation results (ethyl acetate and ethyl lactate) in a water-ethanol mixture.

Stream No.	1	2	3
	Mixture	Top stream	Bottom stream
Molar flow [kmol/h]	2.4	0.8	1.6
Mass flow [kg/h]	100.0	28.9	71.1
Temp. [K]	293.0	346.2	373.5
Pressure [Pa]	101325.0	101325.0	101325.0
Flow rates in kg/h			
Lactic Acid	0.0	0.0	0.0
Acetic Acid	0.0	0.0	0.0
Water	30.0	7.9	22.1
Ethanol	10.0	10.0	0.0
Ethyl Lactate	50.0	1.0	49.0
Ethyl Acetate	10.0	10.0	0.0

the analysed components were also observed. Figure 4 shows example chromatograms obtained from qualitative analysis using gas chromatography. A capillary column PERMABOND CW 20M (30 m × 0.53 mm) was used as the stationary phase. Analyses were carried out using the same parameters for com-



Stream No.	1	2	3
Temperature, K	293	360.5822	378.166
Pressure, Pa	101325	101325	101325
Flow rates in kg/h			
Lactic Acid	1.3062	0.0018	1.3044
Acetic Acid	0.0549	0.0072	0.0477
Water	31.5104	25.6738	5.8366
Ethanol	9.7415	9.7415	0
Ethyl Lactate	37.386	7.386	30
Ethyl Acetate	0.0009	0.0009	0

Figure 3. Distillation scheme: a multi-stage vapor-liquid equilibrium module with one feed stream and two product streams (composition of the feed in relation to the main stream product – ethyl lactate; esterification simulation in Figure 2).

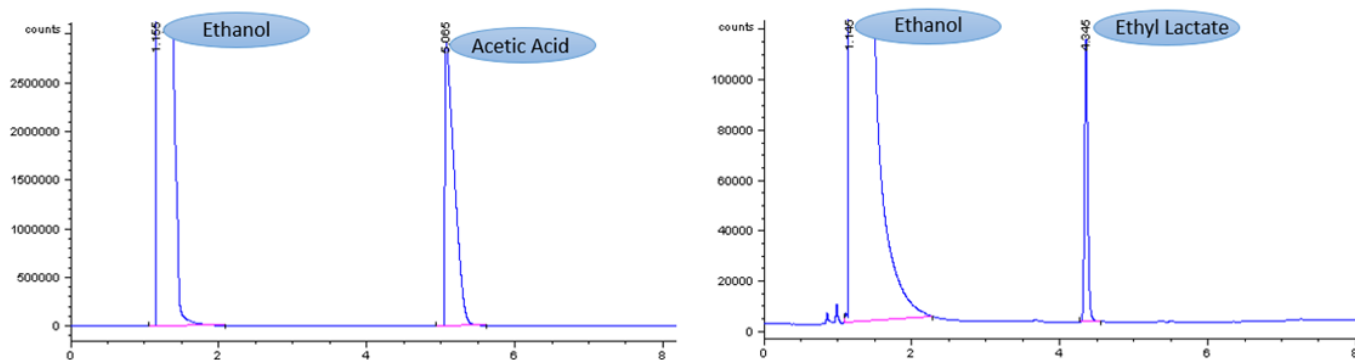


Figure 4. Chromatograms for ethanol, acetic acid and ethyl lactate (gas chromatography, FID detector).

ponents whose boiling points at atmospheric pressure are: 78 °C ethanol, 118 °C acetic acid, 154 °C ethyl lactate. The retention times for these components were, respectively: 1.2 min. of ethanol, 4.4 min. of ethyl lactate and 5.1 min. acetic acid.

The presented results indicate the considerable difficulty of acetic acid separation and the need to use complex separation techniques. The approach based on esterification, which is a reaction that does not require stringent conditions, allows for the separation of lactic and acetic acid.

5. CONCLUSIONS

The downstream processing of the fermentation broth has a significant impact on the costs of biotechnological production. At the same time, the possibility of using techniques that do not interfere with the optimisation of the upstream stage is the best approach due to the sensitivity of microorganisms and their environmental and culture medium requirements. In the case of lactic acid, fermentation broths are most often

obtained in which acetic acid is a by-product. Adjusting the downstream stage in which the separation of these two carboxylic acids is possible is an important aspect. The results showed that the esterification reaction in an acidic environment leading to esters (lactates and acetates) is a solution that enables the separation of both esters by distillation. The selection of alcohol as an esterification substrate depends on the required form of the product – if the final product is lactic acid obtained by hydrolysis, methanol or ethanol are suitable due to their low boiling points. For the production of lactic acid esters, esterification as a separation step is an even more suitable approach. Solid contaminants (such as biomass) may precipitate in the alcohol environment, which limits the implementation of a multistage purification process. The final lactic acid ester is purified in the distillation step and the reversible hydrolysis reaction can be omitted. In this approach, other alcohols can be used for esterification (e.g. butanol). The partial immiscibility of the solvent with the aqueous solution may have a beneficial effect on the separation and purification process and is an issue worth further analysis. The results indicate that esterification is a preferred method that allows the separation of impurities and the obtaining of a pure product in the form of an ester or carboxylic acid. Reaction conditions and the possibility of regenerating heterogeneous catalysts are advantages that may allow the use of the presented method on an industrial scale.

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