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Purification of Lactic Acid Produced from Sugarcane Molasses

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In this work, fermentation process was carried out using sugarcane molasses without pretreatment and *Lactobacillus plantarum inoculum*. The purification of the fermentation product was conducted by using the hybrid short path evaporation. The system allowed to employ lower temperatures of operation due to the use of vacuum and short residence time was achieved, avoiding the tendency of lactic acid decomposition. For tests were used: feed flow rate of 8 mL/min, stirring of 750 rpm, pressure of 1 kPa and it was varied the evaporator temperature from 30 to 170 °C. The experimental results showed 5 times higher the lactic acid concentration than the initial content in the raw material.

1. Introduction

In recent years, concerns over the microbial production of commercially valuable products have been growing. This is mainly attributed to escalating global energy and environmental problems, which have stimulated researchers worldwide to develop methods for producing almost everything through green solvents (Abdel-Rahman et al., 2013). Of these, lactic acid has attracted a great deal of attention due to its widespread variety of applications in synthesizing products such as bio-plastics, pharmaceuticals, food, preservatives, cosmetics and fertilizers (Krishna et al., 2012). The polylactic acid polymers have several applications in the textile, medical and pharmaceutical industries (Martinez et al., 2013). The Global Industry Analyst Inc. announced in January 2011 that the global market for lactic acid is forecast to reach approximately 329.000 t (metric) by the year 2015 (Martinez et al., 2013).
Lactic acid is an organic acid that can be produced by fermentative or chemical synthesis. The production by fermentation process has attracted interest due to its advantages compared with chemical synthesis such as production of pure isomers (L(+) or D(-) lactic acid), use of renewable and low cost raw materials, low energy consumption and mild operation conditions. There are many cheap and renewable raw materials used for lactic acid production, such as starch, wheat, corn, potato, rice, lignocellulose, whey, sugar beet, sugarcane molasses and others. Renewable resources do not give any net contribution of carbon dioxide to the atmosphere, as do the limited oil- and fossil-fuel-based sources (Hofvendahl and Hahn-Hägerdal, 2000). Lactic acid can be biosynthesized by some bacteria and filamentous fungi (Lunelli et al., 2011). The lactic acid bacteria (LAB) can be classified in two groups: homofermentative and heterofermentative (Obligatory and Facultative). *Lactobacillus plantarum* is an example of microorganism Facultative Heterofermentative that ferments sugar by 6-phosphogluconate/phosphoketolase and by Embden-Meyerhof-Parnas pathways.
The development of an efficient method of separation and purification of the lactic acid from fermentation broth is very important, because, actually, these steps are responsible by 50 % of the production costs (Wasewar et al., 2002) and it is still difficult to recover it with high purity. A considerable amount of researches have done a great deal of work on the purification procedures (Chen et al., 2012), such as solvent extraction, separation with membranes, reactive distillation and others.

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Hybrid short path evaporation (Martins et al., 2012) is an alternative separation process with potential for the recovery and concentration of thermally unstable molecules such as lactic acid. The unit operation use gravity force to promote a thin film on the evaporating cylinder (evaporator), usually with a wiping element that mixes and distributes the liquid over the whole evaporator surface (Fregolente et al., 2007). It has been recognized as a promising technology mainly because its low evaporation temperature and short residence time, which minimize problems with thermal decomposition (Komesu et al., 2013).
In previous work (Komesu et al., 2013), our research group studied the influence of evaporator temperature on residue, distillate and light percentages, lactic acid purity and recovery using synthetic mixture of water:lactic acid. The experimental results showed that it is possible to concentrate the lactic acid using the evaporation system. Lactic acid was concentrated in residue stream in about 2.4 times with a recovery of 94 % using low temperature (50 °C).
The objective of this paper was to produce lactic acid from sugarcane molasses and to evaluate the lactic acid purification by hybrid short path evaporation system. In the studied process higher operating pressure (1000 Pa), compared to that usually employed in conventional molecular distillation, and one step of refining were used, which make this technique more suitable for lactic acid purification than the literature published works.

2. Materials and Methods

2.1 Fermentation process

Fermentation process was carried out in a 7 L New Brunswick Scientific BioFlo 415 bioreactor. Sugarcane molasses (48 % sucrose w/w) without pretreatment, typical of large scale industrial mills (from Costa Pinto Mill, Piracicaba, Brazil) was diluted with distilled water in order to obtain an initial sucrose concentration of 32 g/L approximately. The fermentation medium was enriched with 4 g/L of yeast extract to attend the nutritional requirements of the bacterium. The *Lactobacillus plantarum inoculum*, from Fundação Tropical de Pesquisa e Tecnologia André Tosello (Campinas, Brazil), with adequate preparation (Lunelli et al., 2010) was added to the fermenter. The temperature was maintained at 37 °C, pH at 5.0 by adding 4M NaOH and agitation speed at 200 rpm. A pulse of diluted molasses (32 g/L) was carried out after the sucrose had been completely consumed in order to avoid the inhibition of the cell growth by high sucrose concentration as well as to increase of lactic acid end concentration (Lunelli et al., 2010). The total time of fermentation was approximately 30 h. The fermentation product containing about 5 % (w/w) lactic acid was vacuum filtered, centrifuged (5000 rpm for 15 min at room temperature), added sulfuric acid to convert sodium lactate to lactic acid and used as raw material for the investigation of the evaporation system.

2.2 Apparatus

Lactic acid was concentrated in an evaporation system composed by a short path evaporator, Model Pope 2 Wiped Film Still, manufactured by Pope Scientific Inc. (Saukville, WI, USA). The surface area of the evaporator is 0.033 m^2 and the distance between evaporator-internal condenser is 17 mm. Details of the evaporation system was described in Komesu et al. (2013).
Short path principle of separation is based on the difference of components' vapour pressures (Martins et al., 2013). In this system, it was possible to collect 03 streams: light, residue and distillate. The substances of higher vapour pressure were collected predominantly in the light stream, while substances with intermediate vapour pressure in the distillate and substances with lower vapour pressure in the residue. The streams (light, residue and distillate) were collected in glass flasks and analysed by liquid chromatography to determine lactic acid concentration.

2.3 Procedure

The evaporator temperature was varied from 30 to 170 °C in order to study the effect of this operational condition on percentages, lactic acid purities and lactic acid recoveries at residue, distillate and light streams.
The fixed operational conditions were used: feed flow rate of 8 mL/min, stirring of 750 rpm, external condenser temperature at -5 °C, internal condenser temperature at 10 °C and pressure of 1 kPa.

2.4 Chromatographic analysis

The concentration of lactic acid was determined in an equipment of high performance liquid chromatography (HPLC), Agilent model 1260, equipped with UV detector (UV/vis) connected in series with

the chromatography column Bio-Rad Aminex, model HPX-87H (300 x 7,8 mm). The equipment was controlled through OpenLab software. Sulfuric acid solution with 5 mM was used as mobile phase at flow rate of 0.6 mL/min. The column temperature was kept constant at 37 °C. In each run, an injection volume of 25 μL was used. For lactic acid detection and quantification, the wavelength of 215 nm was used in the UV detection system. The lactic acid concentrations were determined using the calibration curve obtained with standard solutions of DL-Lactic acid 90 %.

3. Results and discussions

3.1 Fermentation product analysis

After the fermentation, the resulting product was analyzed by HPLC. This step is important to determine the concentration of the substances obtained in each stream (light stream, residue and distillate) and thus, it will be used to evaluate operating conditions and separation process performance. The analysis by HPLC allowed the identification of 04 substances, to know: sucrose, glucose, fructose and lactic acid. By calibration curve, the lactic acid mass concentration in the fermentation product was about 5 % (w/w).

3.2 Stream percentage analyses

Stream percentage was defined as the ratio between mass of distillate, residue or light stream and the sum of residue, distillate and light mass after evaporation.

Figure 1 shows the experimental mass percentage of distillate (% D), residue (% R) and light (% L) streams varying the evaporator temperature (T evaporator).

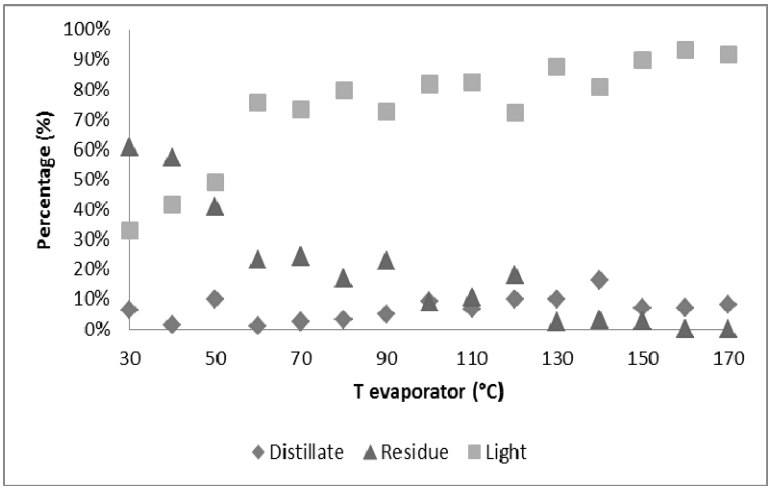

Figure 1: Percentage of distillate, residue and light as function of evaporator temperature (T evaporator)

Observing Figure 1, the amount of residues decreased with increase in temperature because the temperature increasing promotes the evaporation of a larger amount of material, increasing therefore the percentage of light and distillate. Similar behavior was obtained with synthetic solution lactic acid: water in previous work (Komesu et al., 2013).

3.3 Lactic acid purity analyses

Figure 2 shows the lactic acid mass purities on distillate, light and residues streams varying the evaporator temperature.

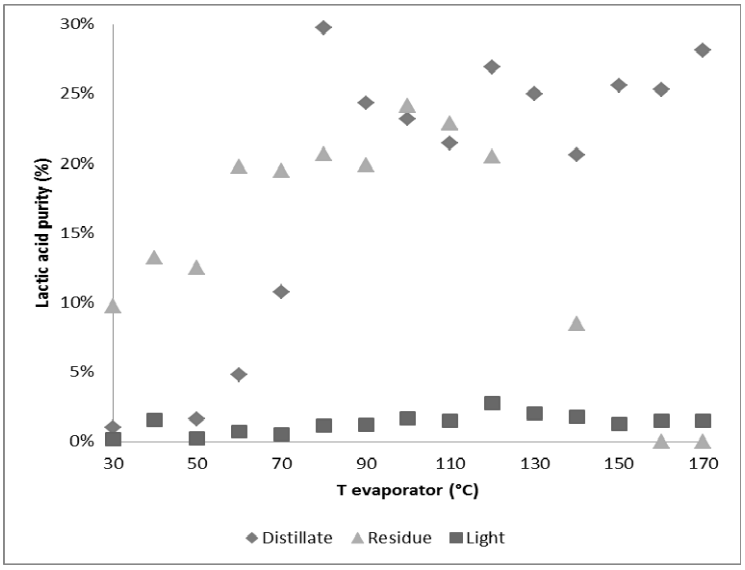

Figure 2: Lactic acid purities on distillate, residue and light as function of evaporator temperature (T evaporator)

The behavior of the curves obtained with synthetic solution lactic acid: water in previous work (Komesu et al., 2013) was different when compared with this study. Fermentation broth is a more complex mixture than synthetic mixture, by the presence of residual sugars and other organic acids, and this affects the performance of the separation process.

Lactic acid purity showed a sharp increase with the temperature in the distillate, which was expected primarily because the increment of temperature enlarged the evaporation rate (Chen et al., 2012). However, at 80°C or higher temperature, lactic acid purity in the distillate stream showed a decline, since the more volatile component but also the heavier components were evaporated and carried into the distillate. The maximum lactic acid purity obtained in distillate was 29.76 % at 80 °C, concentrating the solution in 6.32 times.

The maximum lactic acid purity obtained in residue was 24.16 % at 100 °C. The initial concentration of lactic acid in fermentation broth was 4.71 %, therefore, the concentration process was responsible by producing a solution 5.13 times more concentrated. The lactic acid purity at light stream was practically constant.

The results obtained from these experiments are promising because with one step of evaporation and low temperature it was possible to concentrate the lactic acid from fermentation broth. In addition, technical grade lactic acid (22-44 %) achieved in this work can be used as an acidulant in vegetable and leather tanning industries (Narayanan et al., 2004).

3.4 Lactic acid recovery analyses

Besides concentration, another important process parameter to evaluate the separation process is the lactic acid recovery. Lactic acid recovery in residue, distillate and light streams varying the evaporator temperatures is defined by Eq. 1:

$$\mathrm{Re}\,c(\%) = \frac{m_i \times \%LA_i}{\sum_{i=1}^{3} m_i \times \%LA_i} \times 100 \qquad (1)$$

In which i=residue or distillate or light, m is the residue, distillate or light mass (g) and %LA is the fraction of lactic acid in residue, distillate and light.

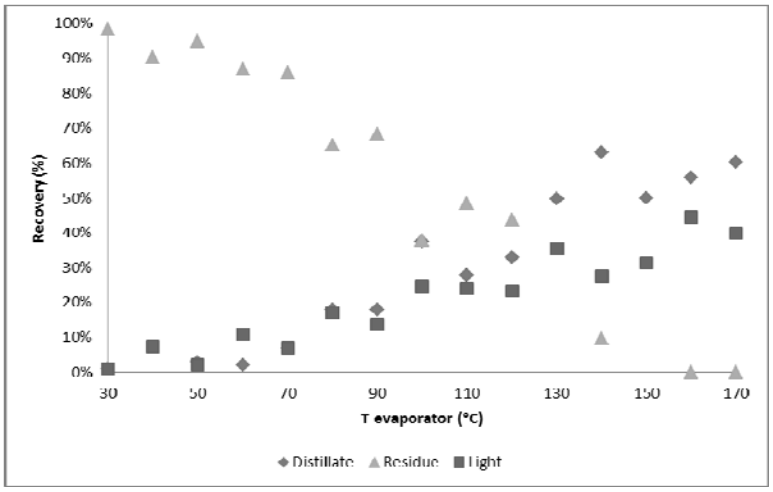

Figure 3: Lactic acid recoveries in distillate, light and residue as function of evaporator temperature (T evaporator)

Lactic acid recovery at residue decreased with the increase of evaporator temperature as can be seen in Figure 3. This is in accordance with preliminary results with synthetic mixture of water: lactic acid (Komesu et al., 2013).

Although lactic acid purity obtained in distillate stream was higher than in residue stream, lactic acid recovery in residue was approximately 37.85 % (at 100 °C) and, in the case of distillate, it was about 17.85 % (at 80 °C), testifying that the best option to concentrate lactic acid under the operating conditions of this study is to concentrate lactic acid in the residue stream. In future works, the interaction of the other operation conditions as feed flow rate, agitation, condenser temperature on residue and distillate percentages, lactic acid purity and recovery will be studied

One strategy for allying high recovery and increase the lactic acid concentration would carry out multiple evaporations in the residue stream.

4. Conclusions

The influence of evaporator temperature on the purification process of lactic acid from fermentation broth was studied in this work. Lactic acid was concentrated in residue stream in about 5 times higher lactic acid concentration than the initial content in the raw material.

Based on the results, it can be concluded that carrying out the lactic acid concentration by using hybrid short path evaporation system is technically feasible and advantageous because the use of high operating pressure (1000 Pa), compared to that usually employed in conventional molecular distillation, and one step of refining, significantly reduces the process costs.

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