

# Hybrid Short Path Evaporation as an Option to Lactic Acid Recovery from Fermentation Broth

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Hybrid short path evaporation (HSPE) is proposed as an alternative separation process with potentiality for recovery and concentration of thermally unstable molecules such as lactic acid. This work aimed to analyze the influence of HSPE process variables on the concentration of lactic acid from sugarcane molasses. Through 22 factorial experimental design, the influence of evaporator temperature (from 115 to 135 °C) and internal condenser temperature (from 13 to 22 °C) on lactic acid concentration and mass percentage of residue and distillate stream were studied. The results showed that it is possible to increase the lactic acid concentration greater than twice from fermentation broth by HSPE. In addition, the effect of each variable studied and interactions between them were demonstrated.

## 1. Introduction

Lactic acid is an important chemical that has been gaining prominence in the market every year (Albuquerque, Borges and Fraga, 2015). The main way of producing lactic acid in the world is by fermentation. Once the fermentative process is complete, the fermented broth undergoes through processes of purification and concentration of the lactic acid produced. These processes can represent 20 to 50 % of the production costs (Ramaswamy, Huang and Ramarao, 2013). In this way, finding efficient downstream processes is the key step in lowering the cost of the end product. Many studies have been carried out using different techniques of extraction and purification of lactic acid. Among proposed technologies, hybrid short path evaporation (HSPE) is an alternative separation process that can be applied for the recovery and concentration of thermally unstable molecules such as lactic acid (Komesu, Patrícia Fazzio Martins, *et al.*, 2014). Recent studies have considered HSPE as a promising alternative for the downstream of lactic acid from fermentation (Komesu *et al.*, 2015) mainly due to its low evaporation temperature and short residence time, which minimizes problems of thermal decomposition (Komesu *et al.*, 2013; Komesu, Patrícia F Martins, *et al.*, 2014). In addition, as the process does not involve the use of a solvent as in extractive distillation, the product material is not polluted and no further purification is needed (Gorak and Schoenmakers, 2014). Bearing all this in mind, the purpose of this work was to evaluate the performance of a HSPE for the concentration of lactic acid from fermentation broth. The effect of evaporator temperature and condenser temperature were studied considering lactic acid concentration and mass percentages of residue and distillate streams.

## 2. Methodology

### 2.1 Sugarcane molasses fermentation

Fermentation process was performed in batch mode, using a 1.5 L New Brunswick Bioflo®/ Celligen® 115 bioreactor. The bioreactor was cleaned, assembled and equipped with probes of pH and temperature previously calibrated. The bioreactor with the fermentation broth was sterilized in a vertical autoclave, at 121 °C for 30 min. The fermentation medium was composed by  $\approx$  200 g/L of total reducing sugars (TRS), 20 g/L of

yeast extract, and 5 g/L of sodium acetate. The bioreactor temperature was adjusted at 37 °C, stirring of 200 rpm and bubbling nitrogen for 30 min before inoculation. The microorganism used in the process to convert TRA to lactic acid was *Lactobacillus plantarum* (Fundação André Tosello, Campinas, Brazil). The pH was kept at 6.00 through real-time addition of 4 mol/L Ca(OH)<sub>2</sub>. After 48 h of fermentation, broth was treated with H<sub>2</sub>SO<sub>4</sub> to reach the pH of 3.85 (lactic acid pKa), converting calcium lactate to lactic acid. The fermented broth with composition presented in Table 1 was used as feed stream in the HSPE.

Table 1: Composition of the fermented broth used in HSPE tests.

Lactic acid (g/L)	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)
86.54	4.13	6.74	86.02

## 2.2 High performance liquid chromatography analysis

Analyzes of the raw material and the product streams of the HSPE process were done using High Performance Liquid Chromatography (HPLC). For TRS, the column used was Bio-Rad Aminex ®HPX-87P (300 x 7,8 mm x 9 µm), at 55 °C. Milli-Q water was used as mobile phase at flow rate of 0.5 mL/min with automatic injection. For organic acids analyzes, it was used the column Bio-Rad Aminex ®HPX-87H (300 x 7,8 mm x 9 µm) at 35 °C, using sulfuric acid (5 mM) as mobile phase at flow rate of 0.6 mL/min with automatic injection.

## 2.3 Hybrid short path evaporation system for lactic acid concentration

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). An external condenser was associated to the evaporation system which was named hybrid short path evaporation. The surface area of the evaporator is 0.033 m<sup>2</sup>. During the experiments, the external condenser was fixed at -5 °C. A trap was coupled to an external condenser which was continuously fed with liquid nitrogen (-196 °C). The transfer of raw material (about 40 g at room temperature) at flow rate of 14 mL/min to the equipment was conducted by using a peristaltic metering pump Cole Palmer Masterflex 77200-60. Operating pressure was fixed at 1 kPa and agitation of 750 rpm. In this system, it was possible to collect 03 streams: light, residue and distillate streams which were collected in glass flasks and analyzed by HPLC to determine lactic acid and TRS concentrations. Details of the equipment can be seen in Komesu *et al.* (2014).

## 2.4 Experimental design

A 2<sup>2</sup> factorial experimental design was used to study the influence of evaporator temperature ( $T_{evap}$ ) and condenser temperature ( $T_{cond}$ ) on lactic acid concentration, TRS concentration, % mass (mass of residue or distillate / sum of residue, distillate and light mass after evaporation) obtained in residue and distillate streams. Real variables values were described in coded form and their experimental ranges were shown in Table 2. The experiments were performed in random order using 3 replicates at the central point to estimate the pure error. The software used to calculate the effect of each variable and their interactions was Statistica 7.0 from Statsoft Inc. The relationship between factors and response was modeled using polynomial equation given by Eq. (1), in which  $X_1$  and  $X_2$  are the independent coded variables,  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_{12}$  are the regression coefficients, and  $Y$  is the response function.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \quad (1)$$

## 3. Results and discussion

### 3.1 Separation process results

Table 2 shows the experimental results obtained for residue and distilled streams in mass percentages (% mass), lactic acid concentrations (LA), and total reducing sugars concentrations (TRS). Mass percentages were defined as the ratio between the mass of the stream (residue or distilled) and the sum of residue, distilled and light streams after the process. Runs identified by the numbers 5, 6 and 7 correspond to central points. Central points are runs performed in triplicates at the same operating conditions to determine experimental error. It was verified that, while residue mass percentage ( $47.7 \pm 4.5$  %), LA concentration ( $181.22 \pm 5.37$ ) and TRS concentration ( $171.99 \pm 12.78$ ) at residue stream presented low standard deviations, distilled mass percentage ( $7.4 \pm 0.8$  %), LA concentration ( $49.73 \pm 3.68$ ) and TRS concentration ( $44.58 \pm 3.03$ ) at distilled stream presented a higher variation. This variation can be explained due the temperature increasing allowed

the evaporation of higher portions of water to light stream, and consequently the distillate stream was becoming more viscous, hindering the material flow inside the equipment and its collection at distillate stream. Besides, the interest of the process is on obtaining richer lactic acid fractions. Residue stream produced is composed mainly by lactic acid but TRS are present in high concentrations. The highest concentration of lactic acid was obtained on the residue stream in run 1 (199.17 g/L), in which it was possible to concentrate the lactic acid in 2.3 times compared to the input current (86.54 g/L). In this case, the TRS was concentrated in 1.8 times, representing 91 % of the concentration of lactic acid. For many lactic acid applications, such as medical prostheses, the presences of TRS are undesirable. In this case, the use of lactic acid in distillate stream is more advantageous because of the lower amount of sugars.

Table 2: Two level factorial design matrix and experimental results at residue and distilled streams.

Runs	Coded variables		Real Variables		Residue			Distilled		
	X1	X2	T <sub>evap</sub> (°C)	T <sub>cond</sub> (°C)	% mass	LA (g/L)	TRS (g/L)	% mass	LA (g/L)	TRS (g/L)
1	-1	-1	115	13	27.4	199.17	182.22	9.5	49.89	45.63
2	1	-1	135	13	38.4	189.77	194.94	9.3	60.70	56.10
3	-1	1	115	22	60.6	162.12	151.46	4.2	62.85	58.96
4	1	1	135	22	40.9	197.16	209.01	5.1	80.11	75.29
5 (C)	0	0	125	17.5	50.0	185.42	161.93	8.2	47.10	43.06
6 (C)	0	0	125	17.5	50.7	175.17	186.37	6.6	48.16	42.61
7 (C)	0	0	125	15.7	42.5	183.06	167.68	7.3	53.94	48.07

T<sub>evap</sub> = evaporator temperature; T<sub>cond</sub> = condenser temperature; % mass= mass of residue or distillate / sum of residue, distillate and light mass after evaporation; LA: lactic acid concentration; TRS: total reducing sugars concentration.

### 3.2 Residue stream analysis

Table 3 shows variable effects on percentage mass, LA and TRS concentration. Considering a confidence level of 90 %, a factor is considering statistically significant if its p value was lower than 0.1. According to Table 3, internal condenser temperature (T<sub>cond</sub>) and interaction between evaporator temperature (T<sub>evap</sub>) and internal condenser temperature were statistically significant variables for the mass percentage in the residue stream. The mass variation in this case was from ≈ 27 to 60 %. Experimental data of mass percentage of residue were modeled by fitting the polynomial equation given by Eq. 2 in which %R corresponds to residue mass percentage, and the variables X<sub>1</sub> and X<sub>2</sub> represent coded values of evaporator temperature and internal condenser temperature, respectively.

$$\%R = 0.4437 - 0.0432X_1 + 0.1783X_2 - 0.1533X_1X_2 \quad (2)$$

Table 4 shows the ANOVA for the residue percentage. In order to evaluate if the models are statistically significant with a confidence level of 90 %, one criterion is to attend the F-test. According to Table 4, the linear model to the residue mass percentage is statistically significant and can be used to make predictions in the range studied. So, 85.000 % of variation is explained by the model of Eq. 2, showing the linear model for mass percentage is statistically significant in a confidence level of 90 %.

Considering the lactic acid concentration, the interaction of T<sub>cond</sub> and T<sub>evap</sub> is significant. The highest value was obtained in the run 1 (199.17 g/L). Lactic acid concentration model is represented by Eq. 3, in which LA<sub>R</sub> corresponds to lactic acid concentration at residue stream, and the variables X<sub>1</sub> and X<sub>2</sub> represent coded values of evaporator temperature and internal condenser temperature, respectively. According to the ANOVA (Table 4), lactic acid concentration presented a correlation coefficient of 0.8832. Therefore, Eq. 3 adjust well the experimental data.

$$LA_R = 184.5534 + 12.8198X_1 - 14.8293X_2 + 22.2189X_1X_2 \quad (3)$$

For TRS concentrations it was no possible to generate a model. Therefore, the linear model is not satisfactory to fit the experimental data of lactic acid concentration at residue stream.

Figure 1 shows in fact that the mass percentage (Figure 1a) suffers a greater influence of the temperature of the internal condenser, while lactic acid concentration (Figure 1b) is strongly determined by the interaction between evaporator and internal condenser temperatures.

Table 3: Estimated effects on residue and distilled streams with a confidence level of 90 %. Significant effects are shown in bold (\*RC: Regression coefficient; \*\*SE: Standard error).

Factor	Residue				Distilled			
	RC*	SE**	t(2)	p	RC*	SE**	t(2)	p
	% mass				% mass			
Mean	0.4437	0.0171	25.9310	0.0015	0.0717	0.0030	24.0775	0.0017
(1)Tevap	-0.0432	0.0453	-0.9534	0.4410	0.0041	0.0079	0.5192	0.6554
(2)Tcond	<b>0.1783</b>	<b>0.0453</b>	<b>3.9377</b>	<b>0.0589</b>	<b>-0.0476</b>	<b>0.0079</b>	<b>-6.0413</b>	<b>0.0263</b>
(1) * (2)	<b>-0.1533</b>	<b>0.0453</b>	<b>-3.3859</b>	<b>0.0773</b>	0.0055	0.0079	0.6985	0.5572
	Lactic acid concentration				Lactic acid concentration			
Mean	184.5534	2.0279	91.0065	0.0001	57.5345	1.3916	41.3439	0.0006
(1)Tevap	12.8198	5.3654	2.3894	0.1394	<b>14.0345</b>	<b>3.6819</b>	<b>3.8118</b>	<b>0.0624</b>
(2)Tcond	-14.8293	5.3654	-2.7639	0.1098	<b>16.1811</b>	<b>3.6819</b>	<b>4.3948</b>	<b>0.0481</b>
(1) * (2)	<b>22.2189</b>	<b>5.3654</b>	<b>4.1412</b>	<b>0.0537</b>	3.2294	3.6819	0.8771	0.4729
	TRS concentration				TRS concentration			
Mean	179.0862	4.8300	37.0781	0.0007	52.8178	1.1463	46.0759	0.0005
(1)Tevap	35.1321	12.7789	2.7492	0.1108	<b>13.4014</b>	<b>3.0329</b>	<b>4.4187</b>	<b>0.0476</b>
(2)Tcond	-8.3488	12.7789	-0.6533	0.5806	<b>16.2547</b>	<b>3.0329</b>	<b>5.3595</b>	<b>0.0331</b>
(1) * (2)	<b>22.4161</b>	<b>12.7789</b>	<b>1.7542</b>	<b>0.2215</b>	2.9302	3.0329	0.9661	0.4359

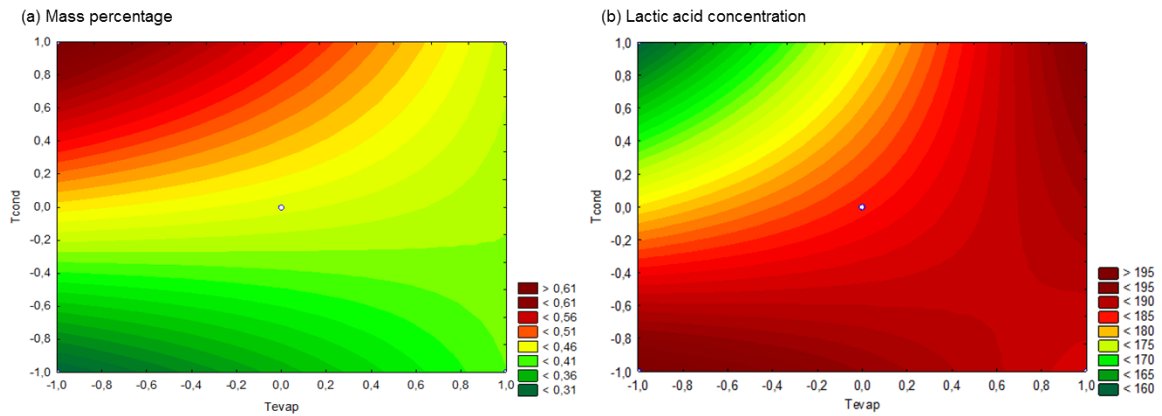


Figure 1: Response of (a) mass percentage and (b) lactic concentration in function of internal condenser temperature and evaporator temperature on the residue stream.

### 3.3 Distilled stream analysis

In the distilled stream, the internal condenser temperature was the mean factor affecting the results. For lactic acid concentration and TRS concentration, both variables ( $T_{\text{evap}}$  and  $T_{\text{cond}}$ ) were important, but not the interaction between them. The mass variation in this stream was from  $\approx 4$  to 9 %, considerable lower than variation in the residue stream. This means that more material remained in the residue stream. The regressed model for distilled percentage is given by Eq. 4 in which %D corresponds to distilled mass percentage and the variables  $X_1$  and  $X_2$  represent coded values of evaporator temperature and internal condenser temperature, respectively. The model on the Eq. 4 explains 93.90 % of data variation, so the linear model is statistically significant in a confidence level of 90 %.

$$\%D = 0.0717 + 0.0041X_1 - 0.0476X_2 + 0.0055X_1X_2 \quad (4)$$

When analyzing the results of Table 4 for the distilled, it is noted that according to the F-test, only the model developed for the mass percentage (Eq. 4) is predictive. In this case, only the internal condenser temperature influences the process, so the variation of the evaporator temperature did not influence the resulting amount of distillate mass (Figure 2).

Table 4: ANOVA of residue and distilled streams with a confidence level of 90 %.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F <sub>calculated</sub>	F <sub>tabulated</sub>
<b>Residue</b>					
% mass					
Regression	0.05713	3	0.01904	5.67	F <sub>3,3</sub> = 5.39
Residues	0.01008	3	0.00336	2.92	F <sub>1,2</sub> = 8.53
Lack of fit	0.00598	1	0.00598		
Pure error	0.00410	2	0.00205		<i>Predictive model</i>
Total	0.06721	6			
Lactic acid concentration					
Regression	877.936	3	292.645	7.56	F <sub>3,3</sub> = 5.39
Residues	116.078	3	38.693	2.03	F <sub>1,2</sub> = 8.53
Lack of fit	58.504	1	58.504		
Pure error	57.574	2	28.787		<i>Predictive model</i>
Total	994.014	6			
<b>Distilled</b>					
% mass					
Regression	0.00231	3	0.00077	15.70	F <sub>3,3</sub> = 5.39
Residues	0.00015	3	0.00005	0.37	F <sub>1,2</sub> = 8.53
Lack of fit	0.00002	1	0.00002		
Pure error	0.00012	2	0.00006		<i>Predictive model</i>
Total	0.00246	6			
Lactic acid concentration					
Regression	469.224	3	156.408	1.35	F <sub>3,3</sub> = 5.39
Residues	346.760	3	115.587	23.58	F <sub>1,2</sub> = 8.53
Lack of fit	319.648	1	319.648		
Pure error	27.112	2	13.556		<i>Non-predictive model</i>
Total	815.984	6			
TRS concentration					
Regression	452.400	3	150.800	1.21	F <sub>3,3</sub> = 5.39
Residues	374.631	3	124.877	38.73	F <sub>1,2</sub> = 8.53
Lack of fit	356.234	1	356.234		
Pure error	18.397	2	9.198		<i>Non-predictive model</i>
Total	827.031	6			

For lactic acid concentration and TRS concentration was not possible to generate a predictive model. However, in a qualitative analysis it is possible to note that the ratio of lactic acid and TRS in this stream is kept more or less constant in all runs, once the TRS represented 92 % of the lactic acid concentration, with a variation of only 2 %. It indicates that on the analyzed conditions, there were TRS at relatively high concentration in the distilled stream. This fact shows that in future studies it is important to analyze the influence of TRS in this separation process, in order to understand how residual sugars in different concentrations can influence the performance of the HSPE process.

In any case, the concentration of lactic acid is always higher than the concentration of TRS in the distilled stream, which is expected since the lactic acid molecule has a lower molecular weight than the sugars that make up the TRS. Considering the lactic acid concentration, the highest value on the distilled stream was 80.11 g/L at the run 4. In this case, the distilled stream presented 94 % of TRS relative to lactic acid concentration. Considering this, it is possible to recommend to analyze a *multiple stage system of HSPE process* in which the interest response stream goes again to the process as an input stream as many times it is necessary. It can make possible to obtain a final stream with highest lactic acid concentration and a lower TRS content.

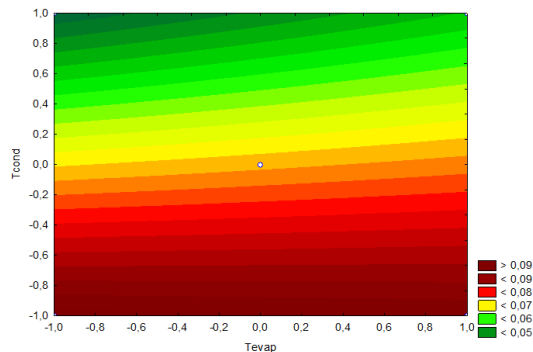


Figure 2: Response of mass percentage in function of internal condenser temperature and evaporator temperature on the distilled stream.

#### 4. Conclusions

Finally, it is concluded that the hybrid short path evaporation process is indicated for the recovery of lactic acid obtained by fermentation, allowing a concentration of lactic acid in the outflow stream greater than twice. The process still leads to a reduction in the relative concentration of TRS in all cases studied, so that the serial process of the outflow currents could lead to a higher concentration of the product of interest with a lower concentration of TRS. In addition, the evaporator and internal condenser temperatures are important variables in the process, both to obtain high concentrations of lactic acid and to reduce the concentration of TRS.

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