Evaluation of Hybrid Short Path Evaporation to Concentrate Lactic Acid and Sugars from Fermentation

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Lactic acid is an important organic compound that finds various applications in the chemical, pharmaceutical, food, and medical industries. Many of these applications require lactic acid with high purity. Hybrid short path evaporation (HSPE) is a separation process well studied in the petrochemical sector that is mainly used to obtain compounds with high purity. It is also a process offering small residence time, low pressure, and environmentally friendliness. The concentration process of lactic acid was studied by using HSPE in the presence of high total reducing sugar content remaining from sugarcane molasses fermentation. In this work, the influence of operational conditions, such as evaporator temperature (86.4 °C to 153.6 °C), internal condenser temperature (7.95 °C to 18 °C), and feed flow rate (8.27 to 21.7 mL/min), on lactic acid concentration and mass percentages were evaluated. The results showed that all variables influenced the process. Mathematical models were developed for the mass percentage and concentration of the total reducing sugar in the distilled stream and for the mass percentage at residue stream. Under the best operational conditions, the concentration of lactic acid (≈ 247.7 g/L) was 2.5 times higher than the initial fermentation broth (≈ 100.1 g/L).

Keywords: Lactic acid; Downstream; Hybrid short path evaporation; Total reducing sugar

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INTRODUCTION

Lactic acid (LA) is an organic acid that can be produced either *via* fermentation or conventional organic synthesis. The production through fermentation is preferred because, among other advantages, the lactic acid can be produced from renewable substrates such as starchy materials (Juodeikiene *et al.* 2015), lignocellulosic biomass (Abdel-Rahman *et al.* 2011), food waste (Tang *et al.* 2016), glycerol (Murakami *et al*. 2016), microalgae (Vanthoor-Koopmans *et al*. 2013), and sugarcane molasses (Oliveira *et al*. 2016). Sugarcane molasses is a potential raw material for lactic acid production. It is an agro-industrial by-product generated from the sugar industry. Brazil is the largest sugarcane producer worldwide producing more than 600 million tons per year (MAPA 2015). During sugar production, approximately 17.9 million tons of molasses are generated as the by-product (Hauly *et al*. 2003). It contains approximately 40% to 60% of sucrose, which can be converted to lactic acid through the use of microorganisms (Dumbrepatil *et al*. 2008).

Lactic acid is the most important hydrocarboxylic acid (Wang *et al*. 2016), and it has a wide variety of applications in pharmaceutical products, cosmetics, food industry, package industry, chemistry, and medical areas (Komesu *et al*. 2014a). However, the versatility of applications can only be fully exploited if the compound has a cost-effective production (Komesu *et al*. 2016). The greatest challenge in its production is to obtain high purity lactic acid, which is related to downstream processes (Pal *et al*. 2009). Many processes have been developed for lactic acid separation and purification, such as electrodialysis, *via* ion-exchange membrane, adsorption, crystallization, and solvent extraction (Joglekar *et al*. 2006). However, there are still challenges to overcome *viz*., low efficiency, high cost, toxic solvent usage, and residual disposal, causing environmental problems (Yu *et al*. 2015). Some of these difficulties can be associated with some of the physical properties of lactic acid, such as its low volatility, association with water, and thermal decomposition at high temperature.

In this sense, hybrid short path evaporation (HSPE) may be a suitable process for lactic acid recovery and purification (Yu *et al*. 2015). It is an environmentally friendly and gentle technique with scope for large-scale application in pharmaceutical and cosmetic industries (Chen *et al*. 2012). It is also considered appropriate for the purification of other thermally sensitive substances like liquids with low vapor pressure and high molecular weight (Lin *et al*. 2014), besides reducing the hazard of thermal decomposition and avoiding the use of abundant toxic solvents (Wang *et al*. 2013). The HSPE is a special case of short path evaporation that works at high vacuum. Lower pressures are desirable because they decrease the boiling point of the substances, reducing the thermal decomposition (Komesu *et al.* 2015b). The separation efficiency depends on operation conditions like distilling temperature and pressure, feed flow rate, as well as their interactions (Yu *et al*. 2015).

In previous works (Komesu *et al.* 2013; 2014a; 2015b; Oliveira *et al.* 2017), our research group studied the purification of the lactic acid from fermentation broth using HSPE. In these works, the influence was evaluated of operational parameters that could affect the HSPE process using fermentation product containing about 5 % (w/w) of lactic acid. The results showed that LA with high purity (≈ 89.7 %) was obtained. By the fact that HSPE is affected by the feed composition, new studies for lactic acid recovery using different fermentation broth concentrations are required. Bearing all this in mind, the objective of this paper was to produce the lactic acid in high concentration (≈ 100 g/L) by fermentation route and evaluate the separation process using HSPE in presence of high amount of total reducing sugars. This will enable researchers to optimize fermentation broths to improve the performance of the separation process.

EXPERIMENTAL

Materials

Propagation of microorganisms and inoculum preparation

The microorganism *Lactobacillus plantarum* CCT 3751 (from Fundação André Tosello – Coleção de Culturas Tropical, Campinas, Brazil) was grown in MRS (de Man *et al.* 1960) broth (de Man *et al*. 1960) and incubated for 24 h at 37 °C in a vertical incubator. The inoculum was prepared in a 250-mL Erlenmeyer flask containing approximately 100 mL of MRS broth, and incubated for 18 h, at 37 °C, and 120 rpm in an orbital shaker. The inoculum media was centrifuged (Eppendorf, Hauppauge, USA) for 10 min, at 4 °C, and

6,000 rpm. The supernatant was discarded and the cell pellet was suspended again in 100 mL of sterile water to be used as an inoculum in the fermentation. The inoculum was added to the bioreactor in a sterile mode using a peristaltic pump of the bioreactor.

Preparation of the bioreactor and fermentation broth

Fermentations were carried out in a New Brunswick Bioflo®/Celligen® 115 bioreactor (New Brunswick Scientific, New Jersey, USA) with a working volume of 1 L. The bioreactor was cleaned, assembled, and equipped with previously calibrated probes. The fermentation broth was prepared with 200 g/L of total reducing sugar (TRS) content from sugarcane molasses (Brazilian Bioethanol Science and Technology Laboratory-CTBE, Campinas, Brazil) without pretreatment, 20 g/L of yeast extract, and 5 g/L of sodium acetate. Then, the fermentation broth was transferred to the bioreactor to be sterilized in a vertical autoclave, at 121 °C for 30 min. The bioreactor temperature was adjusted to 37 °C and an agitation speed of 200 rpm. The pH was maintained at 6.0 ± 0.1 through automatic dosing of a sterile 4 M $Ca(OH)_2$ solution, through real-time monitoring during the fermentation process.

Methods

Samples from fermentation were utilized for measuring LA production, TRS consumption, cell growth, and by-product formations. The temperature and pH were continuously monitored. Cell dry weight was used to characterize the microorganism growth with time. All of the samples from fermentation and after separation processes were analyzed using high-performance liquid chromatography (HPLC) (Agilent, Santa Clara, USA). For sugar analysis, the column used was Bio-Rad Aminex ®HPX-87P (Bio-Rad, Hercules, USA) (300 mm × 7.8 mm × 9 μm) at 55 °C. Milli-Q water was used as the mobile phase at a flow rate of 0.5 mL/min with automatic injection. For organic acids analysis, the column Bio-Rad Aminex ®HPX-87H (300 mm × 7.8 mm × 9 μm) was used at 35 °C, using sulfuric acid (5 mM) as the mobile phase at a flow rate of 0.6 mL/min with automatic injection.

Separation process

The fermented broth was first treated with H_2SO_4 to adjust the pH to 3.85 (lactic acid pKa) to convert calcium lactate to lactic acid. After the pH adjustment, the broth was filtrated and centrifuged to remove the solids. The liquid stream was used as a feed stream in the separation process.

The concentration process was conducted in a short path evaporator (model: Pope 2 Wiped Film Still; Pope Scientific Inc., Saukville, WI, USA). An external condenser at -5 °C was connected to the evaporator, which was named as HSPE. The main component of the system is the evaporator, with an evaporation area of 0.33 m^2. The liquid flows uniformly through the evaporator wall, causing some components of the mixture to evaporate. Water has higher vapor pressure values than lactic acid, which can be expected to volatilize preferentially (Komesu *et al.* 2015b). Upon reaching the internal condenser, its low temperature causes the molecules to condense to the liquid state. Thus, two main streams are generated, one of distillate and another one of residue (formed from the non-evaporated portion of the liquid). In addition to the evaporator, the process requires other auxiliary systems. Control of the pressure was done with a vacuum pump operating at 1 kPa and a trap constantly fed with liquid nitrogen (-196 °C). Next to this system, another external condenser generates the third stream, called light. This stream was mainly

composed of water from the evaporation process. The system feeding (40 g of raw material) was conducted through a peristaltic pump Cole Parmer Masterflex 77200-60 (Cole Parmer, Chicago, USA). The agitation of the system was fixed at 250 rpm. Details of the equipment can be found in Komesu *et al*. (2014a).

Experimental design

A central composite experimental design with three replicates in central point was used to study the influence of the following three factors in the HSPE process: evaporator temperature (T_{evap}, °C), internal condenser temperature (T_{cond}, °C), and feed flow rate (FFR, mL/min). The results used from this analysis were on LA concentration, TRS concentration, and mass percentage from residue and distillate streams. Real variables were described in coded form and their experimental ranges are shown in Table 1. The software used to calculate the effect of each variable and their interactions was Statistica 7.0 from Statsoft Inc. (Palo Alto, USA).

The relationship between the factors and their response was modeled using the polynomial equation given by Eq. 1, in which: X_1, X_2, and X_3 denote the independent coded variables; β_0, β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} represent the regression coefficients; and Y indicates the response function:

$$Y = \beta_0+\beta_1X_1+\beta_1X_1^2+\beta_2X_2+\beta_2X_2^2+\beta_3X_3+\beta_3X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad (1)$$

Table 1. Central Composite Experimental Design Matrix with Experimental Range

Runs	Coded Variables			Real Variables		
	X_1	X_2	X_3	T_{evap} (°C)	T_{cond} (°C)	*FFR* (mL/min)
1	-1.00	-1.00	-1.00	100.00	10.00	11.00
2	-1.00	-1.00	1.00	100.00	10.00	19.00
3	-1.00	1.00	-1.00	100.00	16.00	11.00
4	-1.00	1.00	1.00	100.00	16.00	19.00
5	1.00	-1.00	-1.00	140.00	10.00	11.00
6	1.00	-1.00	1.00	140.00	10.00	19.00
7	1.00	1.00	-1.00	140.00	16.00	11.00
8	1.00	1.00	1.00	140.00	16.00	19.00
9	-1.68	0.00	0.00	86.40	13.00	15.00
10	1.68	0.00	0.00	153.60	13.00	15.00
11	0.00	-1.68	0.00	120.00	7.95	15.00
12	0.00	1.68	0.00	120.00	18.00	15.00
13	0.00	0.00	-1.68	120.00	13.00	8.27
14	0.00	0.00	1.68	120.00	13.00	21.70
15	0.00	0.00	0.00	120.00	13.00	15.00
16	0.00	0.00	0.00	120.00	13.00	15.00
17	0.00	0.00	0.00	120.00	13.00	15.00

X_1- T_{evap}: Evaporator temperature; X_2- T_{cond}: Internal condenser temperature; and X_3- *FFR*: Feed flow rate

RESULTS AND DISCUSSION

Fermentation Processes

The fermentation process was carried out four times to obtain a sufficient volume of medium for the HSPE process. For each process, the yield (*Y*), productivity (*P*), and remaining total reducing sugar content (R_{TRS}) were calculated to evaluate the processes using Eqs. 2, 3, and 4, respectively:

$$Y_{(g/g)} = \frac{Lactic\ acid\ produced_{(g)}}{TRS_{\text{consumed (g)}}} \quad (2)$$

$$P_{(gL^{-1}h^{-1})} = \frac{Lactic\ acid_{(g/L)}}{Time_{(h)}} \quad (3)$$

$$R_{TRS\ (\%)} = \left(\frac{TRS\ final_{\ (g)}}{TRS\ initial_{\ (g)}}\right) x\ 100 \quad (4)$$

The results of *Y*, *P*, and R_{TRS} for the fermentation process are shown in Table 2, which indicates a high LA yield (Y = 0.98 g/g) and productivity (P = 2.84 $gL^{-1}h^{-1}$). The microorganism presented a homofermentative process with the total amount of monosaccharides being consumed first to produce LA, and without any by-product formations. The remaining sugar after the fermentation process was mainly sucrose in a concentration of 82.16 g/L. In all the cases, the fermentative process was interrupted before the TRS consumption, once it was aimed to analyze the sucrose effect in the HSPE process. Even though, the final concentration of LA was high (100.11 g/L), it was similar to many previous studies with concentrations around 100 g/L (Hu *et al*. 2016; Nair *et al*. 2016; Ou *et al*. 2016; Wang *et al*. 2016; Zhou *et al*. 2016).

Table 2. Fermentation Broth Composition for Lactic Acid Production before and after the Treatment with H_2SO_4.

	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	Lactic acid (g/L)	*Y* (g/g)	*P* (g L^{-1} h^{-1})	R_{TRS} (%)
Fermented broth *	82.16	0.00	1.35	100.11	0.98	2.84	42.38
Standard deviation	21.18	0.00	1.79	22.98	0.01	0.46	8.66
Treated broth H_2SO_4	76.01	6.46	3.90	96.70	-	-	-

* The values for the Fermented broth are average of the results of four fermentation batches.
Y: Yield; *P*: Productivity; and R_{TRS}: Remaining total reducing sugar

Hybrid Short Path Evaporation Process

Table 2 shows that the feed stream used in HSPE process had high concentrations of TRS and LA. The results of the experimental design for the distillate and residue streams are presented in Table 3. The light stream was not subjected to statistical analysis because it did not have any of the analyzed components (lactic acid, sucrose, glucose, and fructose). Mass percentages of distilled and residue streams were defined as shown in Eqs. 5 and 6.

$$D\ (\%) = \left(\frac{Distilled\ mass_{(g)}}{Distilled\ mass_{(g)} + Residue\ mass_{(g)} + Light\ mass_{(g)}}\right) x\ 100 \quad (5)$$

$$R\ (\%) = \left(\frac{Residue\ mass_{(g)}}{Distilled\ mass_{(g)} + Residue\ mass_{(g)} + Light\ mass_{(g)}}\right) x\ 100 \quad (6)$$

In Table 3, the runs identified by the numbers 15, 16, and 17 correspond to central points, performed in triplicates at the same operating conditions to determine the experimental error.

Table 3. Lactic Acid and Total Reducing Sugar Concentrations, and Mass Percentages Produced by Hybrid Short Path Evaporation

Runs	Distilled Stream			Residue Stream		
	Lactic Acid (g/L)	*TRS* (g/L)	*D* (%)	Lactic Acid (g/L)	*TRS* (g/L)	*R* (%)
1	59.43	28.73	12.53	247.69	141.64	35.67
2	47.74	44.14	7.02	139.71	108.55	54.98
3	0.00	0.00	0.00	154.25	132.31	50.68
4	87.72	76.48	1.89	143.06	126.21	49.66
5	103.66	89.05	9.76	175.40	154.59	19.59
6	85.91	77.95	9.95	180.26	143.64	44.59
7	109.13	92.97	2.27	166.93	140.42	32.41
8	108.33	92.74	2.98	148.28	122.57	47.20
9	32.97	27.88	1.44	148.25	123.21	51.01
10	111.44	91.75	5.08	164.36	137.03	36.61
11	64.14	55.97	14.52	156.03	128.83	31.43
12	0.00	0.00	4.90	242.41	119.83	21.95
13	56.15	45.46	1.38	164.83	133.85	28.96
14	92.89	75.99	8.13	145.80	120.49	53.45
15	91.36	70.35	2.84	166.87	133.61	44.82
16	89.38	74.23	5.71	143.33	118.47	52.77
17	93.69	62.95	4.51	172.28	118.12	51.36

TRS: Total reducing sugar; *D*: Mass percentage in the distilled stream; and *R*: Mass percentage in the residue stream

In the distilled stream, central points presented are 91.48 g/L ± 2.16 g/L of LA, 69.18 g/L ± 5.73 g/L of TRS, and 4.35% ± 1.44% of D. For the residue stream, LA was 160.83 g/L ± 15.39 g/L, TRS was 123.40 g/L ± 8.84 g/L and R was 49.65% ± 4.24%. The error of each central point was less than 10%. The only response with a higher variation was D, which corresponded to 33% and was highly associated with the small amount of mass in this stream that unfortunately maximized the error. In addition, the increased temperature allowed the evaporation of higher portions of water to the light stream, and consequently, the distillate stream became more viscous, hindering the material flow inside the equipment, which made collecting the distillate stream more difficult and caused higher variations in the mass percentage value. This problem could be solved in different ways, such as:

- Removal of TRS *a prior* to the HSP

- Optimizations of the HSPE operational parameters in order to find a temperature that no cause crystallization of the sugars;
- Fermentation of all the sugars before goes to the HSPE process.

As is well known, HSPE is a process widely used in the petrochemical sector to separate components of economic interest. The result is a set of compounds with very high purity and high concentration, ready to be used. In the case of lactic acid, this approach would be very interesting, especially for using this molecule in the medical sector. The removal of sugars *a priori* or after HSPE implies the addition of one more step in the overall process. However, the changes in the operational parameters for the optimization of the lactic acid separation may result in a downstream process that uses fewer steps as possible, and resulting in a product of very high purity and concentration, as needed for the medical industry.

Distilled stream analysis

Statistical analyses were developed for the distilled stream for the responses: LA, TRS, and D. The variable effects are shown in Table 4, considering a confidence level of 95%.

For LA, the statistically significant variables were: T_{evap} (linear and quadratic), T_{cond} (linear and quadratic), *FFR* (linear), and the interactions between: T_{evap} and T_{cond}, T_{evap} and *FFR*, and T_{cond} and *FFR* (Table 4). The T_{evap} was the variable of highest effect. Table 3 shows that the higher LA concentrations were obtained in the residue stream, which means that the best operation condition for LA concentration was to minimize the amount of LA in the distillate stream.

A regression model for LA concentration in the distillate stream is given in Eq. 7:

$$LA = 90.3445+50.3935X_1-5.8251X_1^2-14.5609X_2-34.2027X_2^2+17.4655X_3+11.8390X_1X_2-23.6454X_1X_3+29.0857X_2X_3 \quad (7)$$

In Eq. 7, the variables X_1, X_2, and X_3 represent the coded values of T_{evap}, T_{cond}, and *FFR*, respectively. The ANOVA for LA concentration is given in Table 5. For Eq. 7, the $F_{9,7}$ calculated (4.04) was higher than $F_{9,7}$ tabulated (3.68) at the 95% confidence level, which showed that the model adequately explained the experimental data variation. However, $F_{5,2}$ calculated (139.59) was higher than $F_{5,2}$ tabulated (19.30) indicating that the model cannot be used to make predictions. Observing Eq. 7, the T_{evap} should be the absolute minimum value to minimize the LA concentration on the distilled stream, and the same should happen with the FFR. However, the T_{cond} is a harder behaviour to predict and could be minimized or elevated to the extreme of the studied values, to obtain a lower LA concentration.

Variable effects for the TRS concentration are presented in Table 4. It is possible to see that: T_{evap} (linear), T_{cond} (quadratic), FFR (linear), and the interactions between: T_{evap} and *FFR*, and T_{cond} and *FFR*, are statistically significant variables of the process. The T_{evap} was the variable of highest effect, as well as the LA concentration.

The mathematical model for TRS concentration as a function of operating conditions is given by Eq. 8, in which the variables X_1, X_2, and X_3 represent coded values of T_{evap}, T_{cond}, and FFR, respectively.

$$TRS = 68.2454+45.5120X_1-22.7135X_2^2+19.3158X_3-25.8063X_1X_3+17.9875X_2X_3 \quad (8)$$

Table 4. Estimated Effects on the Distilled and Residue Streams at 95% Confidence Level

	Distilled Stream				Residue Stream			
	RC	SE	t(2)	p	RC	SE	t(2)	p
	Lactic Acid Concentration				Lactic Acid Concentration			
Mean	90.3445	1.2447	72.5824	0.0002	160.9173	8.8704	18.1409	0.0030
(1) T_{evap} (L)	50.3935	1.1691	43.1060	0.0005	1.9397	8.3313	0.2328	0.8376
T_{evap} (Q)	-5.8251	1.2867	-4.5271	0.0455	-3.8227	9.1698	-0.4169	0.7173
(2) T_{cond} (L)	-14.5609	1.1691	-12.4552	0.0064	2.1550	8.3313	0.2587	0.8201
T_{cond} (Q)	-34.2027	1.2867	-26.5813	0.0014	26.5218	9.1698	2.8923	0.1016
(3) FFR (L)	17.4655	1.1691	14.9398	0.0045	-24.1603	8.3313	-2.9000	0.1012
FFR (Q)	-4.1877	1.2867	-3.2546	0.0828	-4.5206	9.1698	-0.4930	0.6708
(1) * (2)	11.8390	1.5274	7.7508	0.0162	12.4078	10.8853	1.1399	0.3725
(1) * (3)	-23.6454	1.5274	-15.4803	0.0041	26.3473	10.8853	2.4204	0.1366
(2) * (3)	29.0857	1.5274	19.0420	0.0027	18.3221	10.8853	1.6832	0.2344
	TRS Concentration				TRS Concentration			
Mean	68.2454	3.3015	20.6713	0.0023	122.9943	5.0939	24.1452	0.0017
(1) T_{evap} (L)	45.5120	3.1008	14.6776	0.0046	11.0927	4.7843	2.3185	0.1463
T_{evap} (Q)	-0.2067	3.4129	-0.0606	0.9572	7.5483	5.2658	1.4335	0.2881
(2) T_{cond} (L)	-10.5181	3.1008	-3.3921	0.0770	-6.1592	4.7843	-1.2874	0.3268
T_{cond} (Q)	-22.7135	3.4129	-6.6553	0.0218	3.4571	5.2658	0.6565	0.5789
(3) FFR (L)	19.3158	3.1008	6.2293	0.0248	-13.2477	4.7843	-2.7690	0.1094
FFR (Q)	0.4369	3.4129	0.1280	0.9098	5.4636	5.2658	1.0375	0.4085
(1) * (2)	3.7717	4.0514	0.9310	0.4501	-10.8922	6.2510	-1.7425	0.2236
(1) * (3)	-25.8063	4.0514	-6.3698	0.0238	2.5952	6.2510	0.4152	0.7183
(2) * (3)	17.9875	4.0514	4.4399	0.0472	5.0200	6.2510	0.8031	0.5062
	Mass Percentage				Mass Percentage			
Mean	0.0437	0.0083	5.2629	0.0343	0.4930	0.0244	20.1778	0.0024
(1) T_{evap} (L)	0.0141	0.0078	1.8142	0.2113	-0.1046	0.0229	-4.5578	0.0449
T_{evap} (Q)	-0.0086	0.0086	-1.0058	0.4204	-0.0175	0.0253	-0.6910	0.5610
(2) T_{cond} (L)	-0.0707	0.0078	-9.0729	0.0119	0.0135	0.0229	0.5869	0.6167
T_{cond} (Q)	0.0370	0.0086	4.3114	0.0498	-0.1385	0.0253	-5.4837	0.0317
(3) FFR (L)	0.0126	0.0078	1.6222	0.2462	0.1454	0.0229	6.3342	0.0240
FFR (Q)	0.0020	0.0086	0.2277	0.8410	-0.0359	0.0253	-1.4200	0.2915
(1) * (2)	0.0080	0.0102	0.7849	0.5147	0.0143	0.0300	0.4783	0.6796
(1) * (3)	0.0113	0.0102	1.1065	0.3838	0.0537	0.0300	1.7918	0.2150
(2) * (3)	0.0198	0.0102	1.9441	0.1913	-0.0763	0.0300	-2.5453	0.1259

RC: Regression coefficient; SE: standard error; L: Linear constant; and Q: Quadratic constant

Table 5. ANOVA of Distilled and Residue Streams at 95% Confidence Level

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	$F_{calculated}$	$F_{tabulated}$
Distilled					
Lactic Acid Concentration					
Regression	16959.260697	9	1884.362300	4.04	$F_{9,7} = 3.68$
Residues	3265.993440	7	466.570491	139.59	$F_{5,2} = 19.30$
Lack of fit	3256.661047	5	651.332209	*Non-predictive Model*	
Pure error	9.332393	2	4.666197		
Total	20225.254137	16			
TRS Concentration					
Regression	12420.101311	9	1380.011257	3.96	$F_{9,7} = 3.68$
Residues	2436.700696	7	348.100099	14.45	$F_{5,2} = 19.30$
Lack of fit	2371.046444	5	474.209289	*Predictive Model*	
Pure error	65.654251	2	32.827126		
Total	14856.802007	16			
Mass Percentage					
Regression	0.024553	9	0.002728	5.57	$F_{9,7} = 3.68$
Residues	0.003427	7	0.000490	2.91	$F_{5,2} = 19.30$
Lack of fit	0.003013	5	0.000603	*Predictive Model*	
Pure error	0.000415	2	0.000207		
Total	0.027980	16			
Residue					
Mass Percentage					
Regression	0.183709	9	0.020412	5.32	$F_{9,7} = 3.68$
Residues	0.026692	7	0.003813	2.57	$F_{5,2} = 19.30$
Lack of fit	0.023096	5	0.004619	*Predictive Model*	
Pure error	0.003596	2	0.001798		
Total	0.210401	16			

The model adequacy was analyzed by an ANOVA, obtaining a correlation coefficient of 0.9956. The ANOVA data for TRS concentration are presented in Table 5. The model constructed was considered predictive according to the F-test (Table 5). The $F_{9,7}$ calculated (3.96) was higher than $F_{9,7}$ tabulated (3.68) at a 95% confidence level, which shows that the model adequately explains the experimental data variation. The $F_{5,2}$ calculated (14.95) was lower than $F_{5,2}$ tabulated (19.30), indicating that the model can be used to make predictions. Using the model (Eq. 8), it was possible to obtain the response surface in the distilled stream in functions of T_{cond} and T_{evap} (Fig. 1a), *FFR* and T_{evap} (Fig. 1b), and *FFR* and T_{cond} (Fig. 1c).

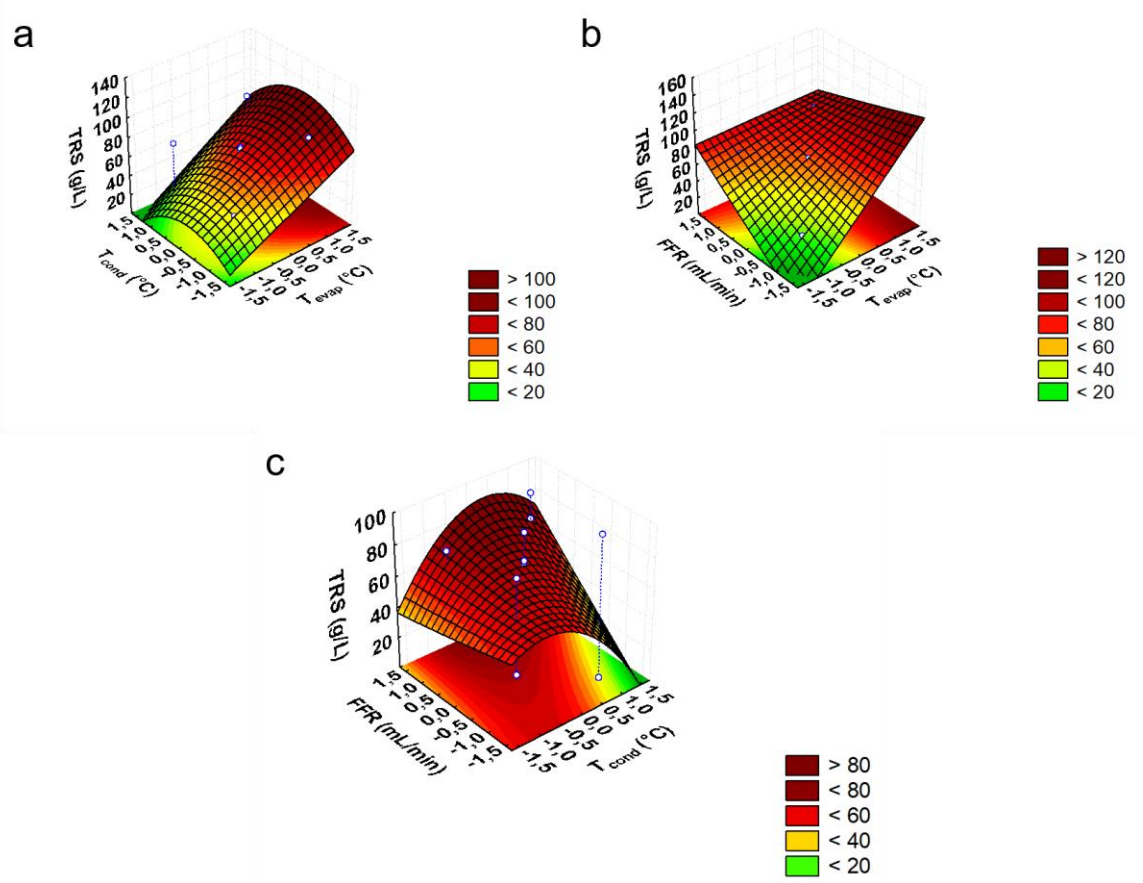

Fig. 1. Response surface for TRS in the distilled stream as function of: a) T_{cond} and T_{evap}; b) *FFR* and T_{evap}; and c) *FFR* and T_{cond}

To keep the TRS in the highest possible concentration in the distilled stream, Fig. 1 shows that T_{evap} and *FFR* should be high (153.6 °C and 21.7 mL/min), and the T_{cond} should be an intermediate temperature (13.0 °C). This combination would allow the distilled stream to have the highest possible TRS concentration, which is quite desirable to separate the LA in the residue stream. In this case, it is important to highlight that the feed stream used here had a high sugar concentration to allow the analysis of the behaviour of these compounds during the process. As demonstrated by Komesu *et al*. (2014b), the composition of a real fermented broth has an influence on the process, when compared to the results of a synthetic LA solution (Komesu *et al*. 2013). Using the synthetic media, the authors achieved only a lower lactic acid concentration than that using a fermented broth from molasses. This was attributed to the main difference between the solutions of the presence of sugars in the fermented broth. But in general, this fact can be also be associated to the synergistic effects of the components from the fermentation, such as sugars, salts, proteins, and other organic compounds. In fact, the residual sugars in the feed stream represent an important challenge in the separation process. Depending on the evaporator temperature, the sugars can be caramelized and, in this case, accumulating them inside the equipment. It causes a loss of mass from the feed flow and thus creates an operational problem.

Upon analyzing the mass percentage response in Table 4, only the T_{cond} (linear and quadratic) was statistically significant ($p < 0.05$) for the process. The model for D is shown in Eq. 9, in which the variable X_2 represents the coded value of T_{cond}.

$$D\ (\%) = 0.0437 - 0.0707X_2 + 0.3070X_2^2 \quad (9)$$

The ANOVA test showed that the model was predictive (Table 5). The $F_{9,7}$ calculated (5.57) was higher than $F_{9,7}$ tabulated (3.68) at a 95% confidence level, which shows that the model adequately explains the experimental data variation. Furthermore, the $F_{5,2}$ calculated (2.91) was lower than $F_{5,2}$ tabulated (19.30) indicating that the model can be used to make predictions. The graphical response as a two-dimensional contour plot is shown in Fig. 2.

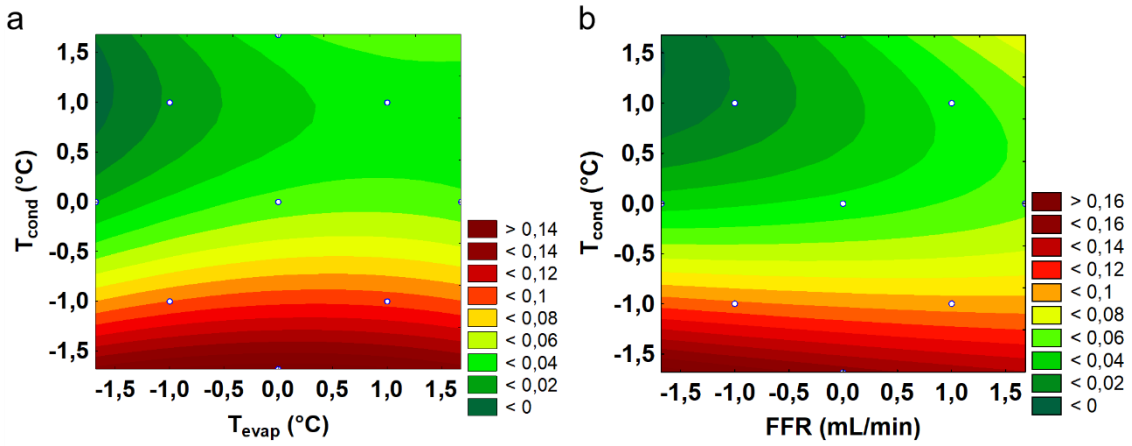

Fig. 2. Response of D in function of a) T_{cond} and T_{evap}, and b) T_{cond} and *FFR*

As was expected, Figs. 2a and 2b clearly show that the T_{evap} and *FFR* do not significantly affect *D*. In fact, to obtain a lower *D* value, the T_{cond} should be kept high (18 °C).

Residue stream analysis

The residue stream analysis was done by evaluating the effects of T_{evap}, T_{cond}, and *FFR*, with a confidence level of 95% for the responses LA, TRS, and *R* (Table 4). However, the LA and TRS concentrations were not affected by any of the studied variables. Even reducing the confidence level to 85%, the parameters still did not affect the responses. Thus far, it was not possible to develop a statistical model for these responses. This result was not expected, once in the previous works it was observed a high influence of all these parameters in the residue stream responses (Komesu *et al*. 2013, 2014b; a, 2015a; Oliveira *et al*. 2017).

However, *R* was influenced by all of the variables, such as T_{evap} (linear), T_{cond} (quadratic), and *FFR* (linear), as indicated by Table 4. Using the statistically significant variables, the regression model to R in the function of operational conditions is shown by Eq. 10, in which the variables X_1, X_2, and X_3 represent the coded values of T_{evap}, T_{cond}, and *FFR*, respectively.

$$R\ (\%) = 0.4930 - 0.1046X_1 - 0.1385X_2^2 + 0.1454X_3 \quad (10)$$

The $F_{9,7}$ calculated (5.35) was higher than $F_{9,7}$ tabulated (3.68) at a 95% confidence level, which shows that the model adequately explained the experimental data variation. The $F_{5,2}$ calculated (2.57) was lower than $F_{5,2}$ tabulated (19.30) indicating that the model can be used to make predictions. Based on the model, Fig. 3 represents the relationship between the dependent and independent variables in three-dimensional response surfaces. The model explains 98.28% of the data variation. In this case, to obtain a higher percentage of mass in the residue stream, the T_{cond} should in the middle (13.0 °C), T_{evap} should be low (86.4 °C), and *FFR* should be high (21.7 mL/min).

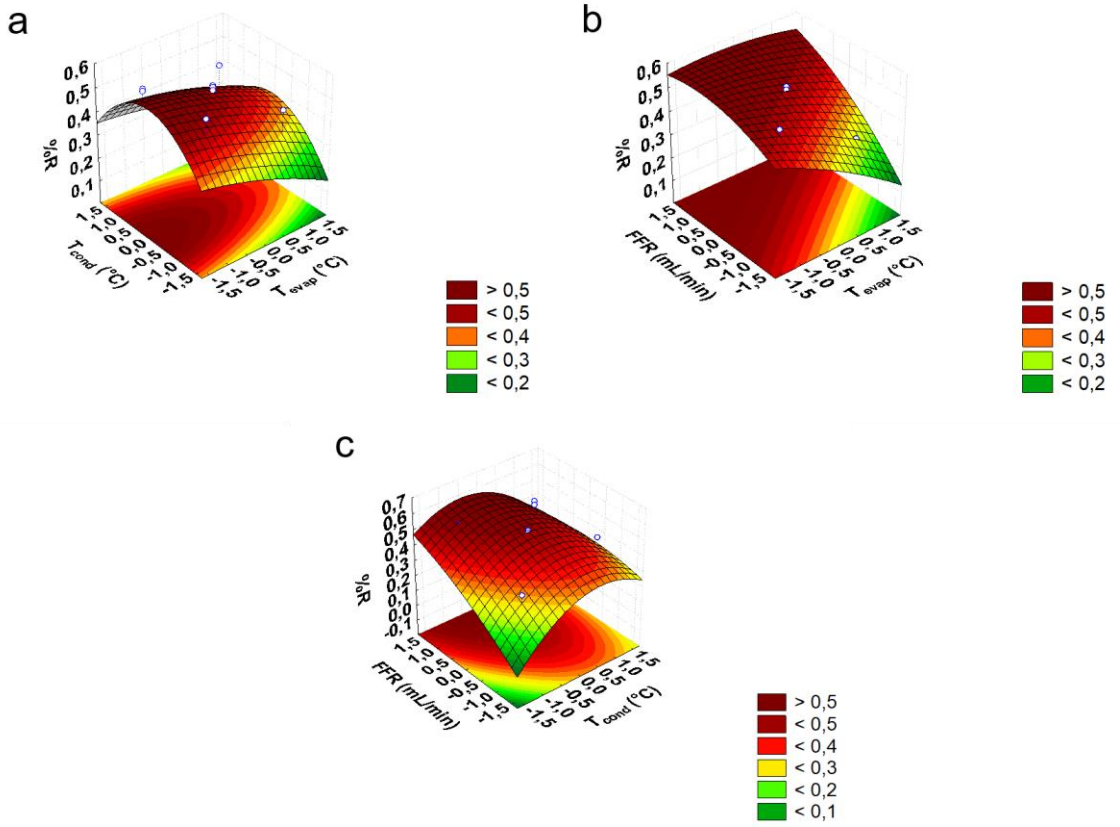

Fig. 3. Response surface for R as a function of: a) T_{cond} and T_{evap}, b) *FFR* and T_{evap}, and c) *FFR* and T_{cond}

General Analysis

The main goal of the process studied was to obtain richer LA fractions. In this study, it was achieved in the residue stream. The residue and distilled streams contained mainly LA, but TRS were present in high concentrations in both streams. For the distilled stream, the lowest LA concentration obtained was 32.9 g/L in run 9. In this case, the TRS content was 27.9 g/L, which represents almost 85% of the LA concentration.

The highest concentration of LA was found on the residue stream from run 1 (247.7 g/L), which also contained approximately 57% of TRS compared to the LA concentration. However, in run 12 (242.4 g/L), LA was concentrated 2.5 times compared to the input stream (96.7 g/L). The TRS was concentrated at 1.38 times compared to the input stream, representing less than 50% of the LA concentration. This denotes the lowest amount of TRS related to the LA concentration for the residue stream. As it is well known, for most of LA applications, the presence of TRS are undesirable, which makes the concentration

of LA in run 12 of the residue stream more advantageous, when compared with the other runs. Run 12 also obtained one of the lowest mass percentages. In the distilled stream, the same run presented no LA and TRS, with approximately 5% of the residue mass. This observation makes the conditions used in this run the best for achieving a high LA concentration with lower TRS content. The optimum conditions for this run were: T_{evap} and *FFR* in the middle range (120 °C and 15 mL/min), and a high T_{cond} (18 °C).

The results obtained in this work were quite different from those obtained in previous studies. The main difference between these studies was the feed composition stream used in the separation process. In previous studies (Komesu *et al*. 2013, 2014a,b, 2015a,b), the authors' research group has studied the LA concentration using a fermentation broth composed by 5% (w/w) of LA containing a minimal amount of residual sugar in the HSPE. In this work, the fermentation broth used had high LA (approximately 100 g/L) and TRS (approximately 85 g/L) concentrations. The results obtained in this work showed that high sugar concentration had a crucial role in terms of separation behaviour and operational difficulties. Komesu *et al*. (2014b) found the maximum LA concentration in the residue stream, as well as in this work, keeping the same operational parameters. However, the feed stream was composed mainly of 5% (w/w) of LA, focusing on a study in which the sucrose from molasses had already been completely depleted. However, as it is well known, just by varying the equipment parameters, it is possible to obtain completely different results. This is clear from comparing another work from Komesu *et al*. (2015b), which shows that just by changing the T_{evap} conditions, it was possible to attain a higher lactic acid amount in the distilled stream, even using a similar feed stream from molasses fermentation composed of 5% (w/w) LA.

The majority of previous studies used synthetic solutions for developing the models when the fermentation broth was a more complex mixture. In fact, the presence of residual sugar hardly affects the performance of the separation process and every single parameter has to be re-adjusted in accordance with the sugar content.

As for each of the models developed, the parameters should be studied according to different aims, and always with very specific feed streams. For example, to obtain:

• Higher TRS in the distilled stream: The evaporator temperature should be high (153.6 °C), the internal condenser temperature should be an intermediate temperature (13 °C), and the feed flow rate should be high (21.7 mL/min);

• Lower mass percentage in the distilled stream: Internal condenser temperature should be high (18 °C);

• Higher mass percentage in the distilled stream: Evaporator temperature should be low (86.4 °C), internal condenser temperature should be an intermediate temperature (13 °C), and the feed flow rate should be high (21.7 mL/min).

• Lower lactic acid concentration in the distilled stream, to concentrate it in the residue stream: Evaporator temperature and feed flow rate should be low (86.4 °C and 8.27 mL/min, respectively), and internal condenser temperature could be in the minimum or maximum level (7.95 °C or 18 °C, respectively).

Based on the results obtained in this work, it can be concluded that carrying out the lactic acid concentration by HSPE is advantageous because of the high operating pressure (1000 Pa) compared to the literature which uses conventional molecular distillation. In addition, the operating pressure is usually below 0.1 Pa and two or more steps of refining are required (Komesu *et al.* 2014a). Moreover, many patents reported in the literature show the industrial interest and potential of molecular distillation for lactic acid production (Komesu *et al.* 2017).

CONCLUSIONS

1. The methodology used allowed an estimate of the parameters that matter for obtaining a higher total reducing sugar (TRS) concentration in the distilled stream, which means it may be possible to separate the sugars from organic acids using the hybrid short path evaporation (HSPE) process.
2. Working with three operational variables, such as evaporator temperature, internal condenser temperature, and feed flow rate, it was possible to obtain models for the mass percentage, concentrations of the TRS and lactic acid in the distilled stream, and the mass percentage in the residue stream. It was observed that the HSPE operational parameters could change for different feed compositions.
3. It was observed that lactic concentration was influenced by internal condenser temperature, evaporator temperature, and feed flow rate in the distilled stream.
4. The HSPE process is suitable and has advantages for lactic acid separation and purification, such as being environmentally friendly, reduces the hazard of thermal decomposition, and avoids the use of toxic solvents.
5. *L. plantarum* was effective in producing a high concentration of lactic acid (100.11 g/L) with a high conversion rate using molasses as a substrate (98%). In addition, the productivity was also satisfactory (2.84 g $L^{-1}h^{-1}$).

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