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Concentrating second-generation lactic acid from sugarcane bagasse via hybrid short path evaporation: Operational challenges

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ABSTRACT

Production of lactic acid (LA) from lignocellulosic materials has become increasingly prominent in the market. However, for industrial scale to be reached, several challenges in second-generation lactic acid production must be overcome. Besides obstacles such as the hydrolysis process, one of the major challenges of the lactic acid industry is the separation and purification process. This study evaluates the separation of lactic acid produced from hemicellulose hydrolysate from sugarcane bagasse using a Hybrid Short Path Evaporation (HSPE) system. Results showed that a lactic acid concentration of 3.1 times the feed concentration (27.85 g/L) is achievable. Three operational parameters were studied: evaporator temperature, internal condenser temperature and feed flow rate. Maximum lactic acid concentration was 86.69 g/L with an evaporator temperature of 120 °C, condenser temperature of 13 °C, and feed flow rate of 8.27 mL/min. Separation of LA from hemicellulosic sugars using HSPE was a more difficult process than separation of LA from 6-carbon sugars.

1. Introduction

Lactic acid (LA) is a high added-value product that has been gaining market share every year [1]. It stands out in the environmental, ecological, and medical areas, especially as a building-block molecule. The use of LA has been common in the food industry for a long time and new uses become available every year [2].

LA production by fermentation has several advantages when compared to chemical synthesis, such as low cost of substrates, relatively low required temperatures, low energy consumption, better environmental traits, high purity [3] and easiness to achieve products with tailor-made characteristics [2]. About 90% of all industrially-produced LA worldwide comes from bacterial fermentation [4]. LA biorefineries can exploit various types of feedstock for obtaining second-generation (2G) LA, for example sugarcane bagasse [5], with many advantages such as employment of cheap and non-food competitive raw materials as substrates in the fermentation process. Brazil is the biggest sugarcane producer in the world. The production of sugarcane bagasse in Brazil (2015/2016) has reached 166.4 million tons [6], making this a substrate of great importance in the biofuel and biochemicals scenario.

Downstream processes are decisive for LA industry development. This is especially because in medical and pharmaceutical applications, for example, a high-purity product is often required [7]. It is also necessary to consider environmental issues, with increasingly stringent legislation governing the use of solvents and waste generation, in favor of a sustainable production chain [2]. The traditional LA production chain involves a series of downstream treatments like precipitation, conventional filtration, acidification, carbon adsorption, evaporation, crystallization and others. LA separation and purification require a simpler and cheaper alternative to conventional precipitation process, still achieving high purity and yield. In this sense, Short Path Evaporation (SPE) can be an attractive alternative. Economic feasibility analyses of HSPE have been carried out in a few studies, and in any case the proposed method had fewer separation steps in comparison to conventional LA downstream, allowing for a reduction in equipment and operational costs. Furthermore, industrial interests in the purification of LA via SPE is made abundantly clear by the many published patents [8].

Hybrid Short Path Evaporation (HSPE) is considered appropriate for the purification of thermally sensitive substances, such as liquids with

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low vapour pressure and high molecular weight [9–11]; it reduces the hazard of thermal decomposition and avoids abundant use of toxic solvents [10,12]. It is an environment-friendly and balanced technique with the scope and potential for large-scale application in pharmaceutical and cosmetic industries [13]. Besides, the process does not involve solvents, making further LA purification unnecessary [14]. In this sense, HSPE may be a suitable method for LA recovery and purification [10,11,15–18]. As a special form of SPE that operates at high vacuum, HSPE demands lower pressures, a desirable characteristic that decreases the boiling points of substances, reducing thermal decomposition [16]. Separation efficiency depends on operating conditions such as distilling temperature and pressure, feed flow rate, as well as their interactions [10,11,15–18]. An operating pressure of 1000 Pa, higher than usually employed in conventional SPE, as well as a single refining step, made the technique more suitable for LA purification than other approaches in the literature [18]. Based on these advantages, HSPE was chosen to study the LA purification process.

In earlier studies [10,16,18–20], our research group investigated HPSE purification of LA from fermented molasses broth. As previously reported [5], molasses from sugarcane and hemicellulose hydrolysate from sugarcane bagasse have different compositions. Molasses are basically composed of sucrose, glucose and fructose. On the other hand, hemicellulose hydrolysate is composed of xylose, glucose, arabinose and cellobiose. Since HSPE is affected by feed composition, new studies for LA recovery, employing variations in operational conditions and feed composition, are required.

Bearing all this in mind, the objective of this paper was to evaluate 2G-LA separation via HSPE in the presence of a high amount of hemicellulose hydrolysate total reducing sugars. The many patents reported in the literature point to the industrial interest and potential of molecular distillation for LA production [8]. However, to the best of our knowledge, this is the first study evaluating the challenges of using HSPE for 2G-LA separation. Once these challenges are elucidated, new studies may go further in order to optimize the process and make it industrially feasible.

2. Materials and methods

2.1. Propagation of microorganisms and inoculum preparation

The microorganism *Lactobacillus plantarum* CCT 3751 (Fundação André Tosello–Coleção de Culturas Tropical, Campinas, Brazil) was grown in MRS broth (de Man, Rogosa and Sharpe) [21] 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 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 6000 rpm. The supernatant was discarded, and the cell pellet was resuspended in 100 mL of sterile water, to be used as inoculum in the fermentation. The inoculum was added to the bioreactor in sterile mode, using a peristaltic pump [20].

2.2. Hemicellulosic liquor from sugarcane bagasse

Hemicellulose liquor from hydrolyzed sugarcane bagasse was kindly provided by the Brazilian Bioethanol Science and Technology Laboratory – CTBE (CNPEM, Campinas, Brazil). The sugarcane bagasse was collected from the mill and dried at room temperature. The material was pretreated in full form, without undergoing any washing process for removal of residual sugars or ash. Dilute acid pretreatment using 0.5% (v/v) sulfuric acid at 140 °C proceeded for 15 min, in order to hydrolyze the hemicellulose and obtain a liquor containing ≈80% xylose and a low concentration of inhibitory compounds [22]. At the end of the process, pH of the liquor was lower than 1.0. It was adjusted to 6.0 with the addition of solid NaOH. Subsequently, the liquor was centrifuged (Eppendorf, Hauppauge, USA) for 10 min, at 4 °C and 6000 rpm, in order to remove solids.

2.3. 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 125 g/L sugar (glucose and xylose) from sugarcane bagasse hemicellulose hydrolysate, 20 g/L yeast extract, and 5 g/L sodium acetate. It was then transferred to the bioreactor, to be sterilized in a vertical autoclave at 121 °C for 30 min. Bioreactor temperature was adjusted to 37 °C and an agitation speed of 200 rpm. pH was maintained at 6.0 ± 0.1 through automatic dosing of a sterile 4 M $Ca(OH)_2$ solution, via real-time monitoring of the fermentation process. Total fermentation time was 48 h.

2.4. Separation process

The fermented broth was first treated with H_2SO_4, to adjust the pH to 3.85 (LA pKa) and convert calcium lactate into LA. After pH adjustment, the broth was filtered and centrifuged to remove solids. The liquid stream was used as a feed stream in the separation process [20].

The concentration process was conducted in a Pope 2 Wiped Film Still short path evaporator (Pope Scientific Inc., Saukville, USA). In a modification of the equipment, an external condenser at −5 °C was attached to it, and the overall assembly was named “HSPE.” The evaporator is the main component of the system, with an evaporation area of 0.33 m^2. Liquid flows uniformly through the evaporator wall, leading some components of the mixture to evaporate. Water has higher vapour pressure values than LA, so the latter can be expected to preferentially volatilize [16]. Upon reaching the internal condenser, its low temperature causes molecules to condense. Thus, two main streams are generated, a distillate one and a residue one (formed from the non-evaporated portion of the liquid). In addition to the evaporator, the process requires other auxiliary components. Pressure control was done with a vacuum pump operating at 1 kPa, and a trap constantly fed with liquid nitrogen (−196 °C). Adjacent to this component, another external condenser generates the third stream, known as the light stream (mainly water from the evaporation process). Fig. 1 is a schematic representation of the equipment. System feeding (40 g of raw material) was conducted through a Cole Parmer Masterflex 77200-60 peristaltic pump (Cole Parmer, Chicago, USA) [20]. The agitation of the system was fixed at 250 rpm. Details of the equipment can be found in Komesu et al. [18].

2.5. Analytical procedures

All samples were analyzed using high-performance liquid chromatography (HPLC) (Agilent, Santa Clara, USA). Sugar analysis employed a Bio-Rad Aminex ®HPX-87P column (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 and automatic injection. Organic acids analysis employed the Bio-Rad Aminex ®HPX-87H column (300 mm × 7.8 mm × 9 µm) at 35 °C, with sulfuric acid (5 mM) as the mobile phase at a flow rate of 0.6 mL/min and automatic injection [20].

2.6. Experimental design

A central composite experimental design—with 3 replicates at central point, resulting in 17 experiments—was used to study the influence of the following three factors on the HSPE process: evaporator temperature (T_{evap} °C), internal condenser temperature (T_{cond} °C) and

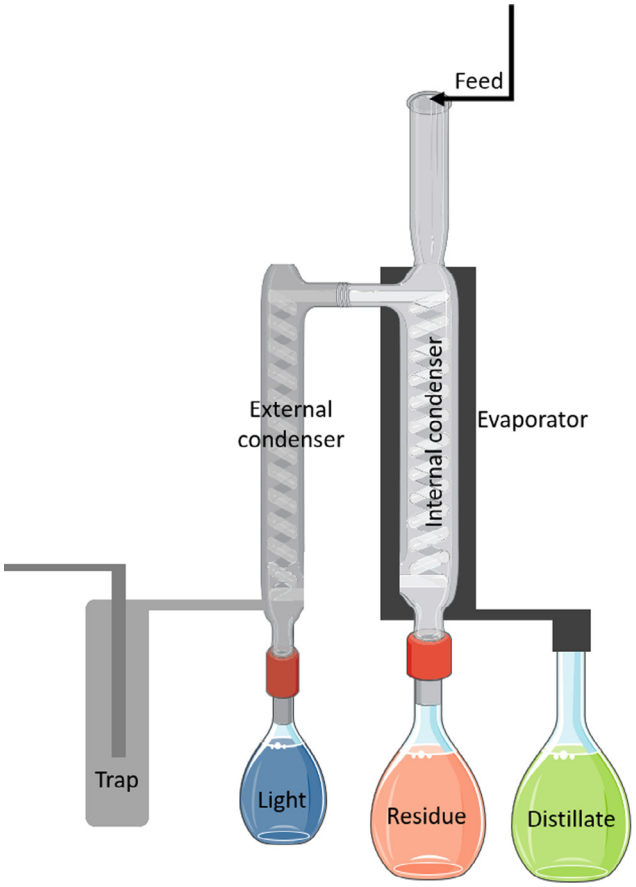

Fig. 1. Schematic representation of the hybrid short path evaporator used in this study.

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	−1	−1	100	10	11
2	-1	−1	1	100	10	19
3	−1	1	−1	100	16	11
4	−1	1	1	100	16	19
5	1	−1	−1	140	10	11
6	1	−1	1	140	10	19
7	1	1	−1	140	16	11
8	1	1	1	140	16	19
9	−1.68	0	0	86.4	13	15
10	1.68	0	0	153.6	13	15
11	0	−1.68	0	120	7.8	15
12	0	1.68	0	120	18	15
13	0	0	−1.68	120	13	8.3
14	0	0	1.68	120	13	21.7
15	0	0	0	120	13	15
16	0	0	0	120	13	15
17	0	0	0	120	13	15

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

feed flow rate (FFR mL/min). Response variables were LA concentration, TRS concentration, and mass percentage of residue and distillate streams. Real variables were described in coded form and their experimental ranges are shown in Table 1.

Statistica 7.0 (Statsoft Inc., Palo Alto, USA) was used to calculate the effect of each variable and their interactions. The relationship between factors and their response was modeled using polynomial 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.

$$Y = \beta_0 + \beta_1 X_1 + \beta_1 X_1^2 + \beta_2 X_2 + \beta_2 X_2^2 + \beta_3 X_3 + \beta_3 X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Table 2
Concentration of each component of the feed stream of Hybrid Short Path Evaporation process.

	Xylose (g/L)	Glucose (g/L)	Arabinose (g/L)	Cellobiose (g/L)	Lactic acid (g/L)
Feed	36.77	0.31	4.42	2.35	27.85

Table 3
Lactic acid and C5 concentrations, and mass percentages produced by Hybrid Short Path Evaporation.

Runs	Distillate stream			Residue stream		
	Lactic acid (g/L)	C5 (g/L)	D (%)	Lactic acid (g/L)	C5 (g/L)	R (%)
1	64.59	83.41	33.0	46.78	61.80	18.9
2	40.36	52.62	27.7	53.56	66.35	32.4
3	55.25	75.93	24.0	71.31	93.11	17.8
4	45.30	58.91	28.2	51.36	69.69	27.6
5	47.69	63.61	30.8	77.42	105.06	9.0
6	51.64	70.89	34.4	37.88	49.65	22.0
7	54.51	74.08	27.1	70.69	99.36	14.5
8	43.54	54.19	30.8	55.34	75.10	20.3
9	41.98	54.94	32.1	55.07	75.94	25.4
10	48.94	61.52	29.4	64.37	86.83	7.5
11	46.25	59.27	40.7	71.48	101.51	5.8
12	52.51	71.11	28.1	71.66	99.15	11.8
13	58.79	80.56	29.3	86.69	119.95	6.0
14	40.39	53.31	32.8	50.73	69.90	26.5
15	40.44	53.20	34.6	56.74	78.37	21.9
16	42.96	56.60	29.4	54.42	74.99	23.7
17	43.66	55.80	32.8	58.84	81.31	22.3

C5: xylose + arabinose + cellobiose; D: Mass percentage in the distillate stream; and R: Mass percentage in the residue stream.

3. Results and discussion

Table 2 shows feed stream concentrations of sugars and LA used in the HSPE process. Results of the experimental design for the distilled and residue streams are shown in Table 3. The light stream was not subjected to statistical analysis because it did not have any of the analyzed components (xylose, glucose, arabinose, cellobiose and LA). Mass percentages of distilled and residue streams were defined as shown in Eqs. (2) and (3).

$$D\ (\%) = \left(\frac{\text{Distilled mass}_{(g)}}{\text{Distilled mass}_{(g)} + \text{Residue mass}_{(g)} + \text{Light mass}_{(g)}} \right) \times 100 \quad (2)$$

$$R\ (\%) = \left(\frac{\text{Residue mass}_{(g)}}{\text{Distilled mass}_{(g)} + \text{Residue mass}_{(g)} + \text{Light mass}_{(g)}} \right) \times 100 \quad (3)$$

In Table 3, runs identified by the numbers 15, 16, and 17 correspond to central points, performed in triplicates under uniform operational conditions to determine experimental error. In the distillate stream, central points had LA = 42.80 ± 1.65 g/L; C5 = 55.96 ± 2.10 g/L; D (%) = 32.4 ± 2.2%. In the residue stream, LA = 56.67 ± 2.21 g/L; C5 = 78.22 ± 3.16 g/L; R (%) = 22.6 ± 0.9%. The error of each central point was less than 10%.

Table 4
Estimated effects on the distillate and residue streams at 90% confidence level.

	Distillate stream				Residue stream			
	RC	SE	t(2)	p	RC	SE	t(2)	p
	Lactic acid concentration				*Lactic acid concentration*			
Mean	42.2323	0.9755	43.2936	0.0005	57.2613	1.2732	44.9736	0.0005
(1) T_{evap} (L)	0.5254	0.9162	0.5734	0.6242	4.9720	1.1958	4.1578	0.0533
T_{evap} (Q)	3.0252	1.0084	3.0000	0.0955	−1.9383	1.3162	−1.4727	0.2787
(2) T_{cond} (L)	0.7116	0.9162	0.7766	0.5186	4.8854	1.1958	4.0853	0.0550
T_{cond} (Q)	5.7997	1.0084	5.7514	0.0289	6.4393	1.3162	4.8924	0.0393
(3) FFR (L)	−10.5654	0.9162	−11.5318	0.0074	−18.8227	1.1958	−15.7403	0.0040
FFR (Q)	5.9466	1.0084	5.8970	0.0276	4.4157	1.3162	3.3549	0.0785
(1) * (2)	0.7788	1.1971	0.6506	0.5821	−2.9014	1.5624	−1.8570	0.2044
(1) * (3)	6.7904	1.1971	5.6725	0.0297	−10.4296	1.5624	−6.6753	0.0217
(2) * (3)	−0.1647	1.1971	−0.1376	0.9032	−0.6364	1.5624	−0.4073	0.7232
	C5 concentration				*C5 concentration*			
Mean	55.0233	1.0230	53.7888	0.0003	79.2247	1.8236	43.4447	0.0005
(1) T_{evap} (L)	0.4352	0.9608	0.4529	0.6950	8.2790	1.7127	4.8338	0.0402
T_{evap} (Q)	3.3644	1.0575	3.1815	0.0862	−4.6649	1.8851	−2.4746	0.1318
(2) T_{cond} (L)	1.8271	0.9608	1.9017	0.1976	7.3867	1.7127	4.3128	0.0498
T_{cond} (Q)	8.2850	1.0575	7.8347	0.0159	8.7314	1.8851	4.6318	0.0436
(3) FFR (L)	−15.5574	0.9608	−16.1926	0.0038	−26.7610	1.7127	−15.6248	0.0041
FFR (Q)	9.5213	1.0575	9.0038	0.0121	4.9051	1.8851	2.6020	0.1214
(1) * (2)	−1.2624	1.2553	−1.0056	0.4205	−3.7277	2.2378	−1.6658	0.2377
(1) * (3)	8.8001	1.2553	7.0103	0.0197	−15.2022	2.2378	−6.7934	0.0210
(2) * (3)	−3.3499	1.2553	−2.6686	0.1164	0.7943	2.2378	0.3549	0.7566
	Mass percentage				*Mass percentage*			
Mean	0.3247	0.0152	21.3568	0.0022	0.2216	0.0055	40.6052	0.0006
(1) T_{evap} (L)	0.0083	0.0143	0.5791	0.6211	−0.0895	0.0051	−17.4661	0.0033
T_{evap} (Q)	−0.0221	0.0157	−1.4050	0.2952	−0.0123	0.0056	−2.1823	0.1608
(2) T_{cond} (L)	−0.0542	0.0143	−3.7980	0.0629	0.0115	0.0051	2.2425	0.1542
T_{cond} (Q)	0.0035	0.0157	0.2226	0.8445	−0.0665	0.0056	−11.7774	0.0071
(3) FFR (L)	0.0175	0.0143	1.2228	0.3459	0.1123	0.0051	21.9064	0.0021
FFR (Q)	−0.0203	0.0157	−1.2942	0.3249	−0.0138	0.0056	−2.4370	0.1351
(1) * (2)	0.0029	0.0187	0.1554	0.8908	0.0240	0.0067	3.5876	0.0697
(1) * (3)	0.0212	0.0187	1.1339	0.3744	−0.0110	0.0067	−1.6477	0.2412
(2) * (3)	0.0239	0.0187	1.2827	0.3282	−0.0271	0.0067	−4.0473	0.0560

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

3.1. Distillate stream analysis

Distillate stream statistical analyses were carried out for the following responses: LA, C5 (xylose + arabinose + cellobiose), and D (%). Variable effects are shown in Table 4, with a confidence level of 90%. For LA, statistically significant variables were T_{evap} (quadratic), T_{cond} (quadratic) and FFR (linear and quadratic), as well as the interactions between T_{evap} and FFR (Table 4).

Table 3 shows the highest LA concentration in the distillate stream was obtained during run 1 (64.59 g/L), with the following conditions: FFR = 11 mL/min, T_{evap} = 100 °C, and T_{cond} = 10 °C.

The regression model for LA concentration in the distillate stream is given by Eq. (4). X_1, X_2 and X_3 are the independent variables T_{evap}, T_{cond} and FFR, respectively.

$$LA = 42.2323 + 0.5254X_1 + 3.0252X_1^2 + 0.7116X_2 + 5.7997X_2^2 - 10.5654X_3 + 5.9466X_3^2 + 0.7788X_1X_2 + 6.7904X_1X_3 - 0.1647X_2X_3 \quad (4)$$

According to Eq. (4), higher LA concentrations in the distillate stream can be obtained by increasing T_{evap} and T_{cond} and decreasing FFR. ANOVA analysis for LA concentration is given in Table 5. $F_{9,7}$ calculated (2.76) was higher than $F_{9,7}$ tabulated (2.72) at a 90% confidence level, demonstrating the model adequately explained experimental data variation. However, $F_{5,2}$ calculated (11.96) was higher than $F_{5,2}$ tabulated (9.29), indicating that the model cannot be used to make predictions.

Variable effects of C5 concentration are shown in Table 4. It is possible to verify that T_{evap} (quadratic), T_{cond} (quadratic), and FFR (linear and quadratic), as well as the interactions between T_{evap} and FFR, are statistically significant variables. The mathematical model for C5 concentration as a function of operating conditions is given by Eq. (5). X_1, X_2 and X_3 are the independent variables T_{evap}, T_{cond} and FFR, respectively.

$$C5 = 55.0233 + 0.4352X_1 + 3.3644X_1^2 + 1.8271X_2 + 8.2850X_2^2 - 15.5574X_3 + 9.5213X_3^2 - 1.2624X_1X_2 + 8.8001X_1X_3 - 3.3499X_2X_3 \quad (5)$$

According to Eq. (5), higher C5 concentrations in the distillate stream can be obtained by increasing T_{evap} and T_{cond}, and decreasing FFR. ANOVA data for C5 concentration are presented in Table 5. Similar to LA, the model was considered non-predictive according to F-test with a 90% confidence level.

Upon analyzing the distilled mass percentage response (D) in Table 4, it becomes clear that only T_{cond} (linear) was statistically significant ($p < 0.10$) to the process. The model for D is shown in Eq. (6), in which X_1, X_2 and X_3 are the independent variables T_{evap}, T_{cond} and FFR, respectively.

$$D(\%) = 0.3247 + 0.0083X_1 - -0.0221X_1^2 - 0.0542X_2 + 0.0035X_2^2 + 0.0175X_3 - 0.0203X_3^2 + 0.0029X_1X_2 + 0.0212X_1X_3 + 0.0239X_2X_3 \quad (6)$$

According to Eq. (6), higher mass percentage in the distillate stream can be obtained by decreasing T_{cond}. ANOVA data analysis (Table 5) leads to the conclusion that this model is also non-predictive.

3.2. Residue stream analysis

Residue stream analysis was done by evaluating the effects of FFR, T_{evap} and T_{cond}, with a 90% confidence level for LA, C5, and R responses (Table 4). Table 3 shows that the highest LA concentration in the residue stream was obtained during run 13 (86.69 g/L), with the

Table 5
ANOVA of distillate and residue streams at 90% of confidence level.

Source of variation	Sum of squares	Degrees of Freedom	Mean square	$F_{calculated}$	$F_{tabulated}$
Distillate					
Lactic acid concentration					
Regression	627.80	9	69.76	2.76	$F_{9,7} = 2.72$
Residues	177.17	7	25.31	11.96	$F_{5,2} = 9.29$
Lack of fit	171.44	5	34.29	*Non-predictive Model*	
Pure error	5.73	2	2.87		
Total	804.98	16			
C5 concentration					
Regression	1368.46	9	152.05	3.17	$F_{9,7} = 2.72$
Residues	335.85	7	47.98	20.91	$F_{5,2} = 9.29$
Lack of fit	329.54	5	65.91	*Non-predictive Model*	
Pure error	6.30	2	3.15		
Total	1704.31	16			
Mass percentage					
Regression	0.0159	9	0.0018	1.77	$F_{9,7} = 2.72$
Residues	0.0070	7	0.0010	1.61	$F_{5,2} = 9.29$
Lack of fit	0.0056	5	0.0011	*Non-predictive Model*	
Pure error	0.0014	2	0.0007		
Total	0.0229	16			
Residue					
Lactic acid concentration					
Regression	1810.55	9	201.17	2.08	$F_{9,7} = 2.72$
Residues	677.18	7	96.74	27.34	$F_{5,2} = 9.29$
Lack of fit	667.42	5	133.48	*Non-predictive Model*	
Pure error	9.76	2	4.88		
Total	2487.73	16			
C5 concentration					
Regression	3785.12	9	420.57	2.04	$F_{9,7} = 2.72$
Residues	1443.23	7	206.18	28.42	$F_{5,2} = 9.29$
Lack of fit	1423.20	5	284.64	*Non-predictive Model*	
Pure error	20.03	2	10.02		
Total	5228.35	16			
Mass percentage					
Regression	0.0864	9	0.0096	3.66	$F_{9,7} = 2.72$
Residues	0.0184	7	0.0026	40.56	$F_{5,2} = 9.29$
Lack of fit	0.0182	5	0.0036	*Non-predictive Model*	
Pure error	0.0002	2	0.0001		
Total	0.1048	16			

following conditions: FFR = 8.27 mL/min, T_{evap} = 120 °C, and T_{cond} = 13 °C. This run also had the highest C5 concentration (119.95 g/L).

Mathematical models for LA, C5 and R are presented in Eqs. (7)–(9), respectively. X_1, X_2 and X_3 are the independent variables T_{evap}, T_{cond} and FFR, respectively.

$$LA = 57.2613 + 4.9720X_1 - 1.9383X_1^2 + 4.8854X_2 + 6.4393X_2^2 - 18.8227X_3 + 4.4157X_3^2 - 2.9014X_1X_2 - 10.4296X_1X_3 - 0.6364X_2X_3 \quad (7)$$

$$C5 = 79.2247 + 8.2790X_1 - 4.6649X_1^2 + 7.3867X_2 + 8.7314X_2^2 - 26.7610X_3 + 4.9051X_3^2 - 3.7277X_1X_2 - 15.2022X_1X_3 + 0.7943X_2X_3 \quad (8)$$

$$R(\%) = 0.2216 - 0.0895X_1 - 0.0123X_1^2 + 0.0115X_2 - 0.0665X_2^2 + 0.1123X_3 - 0.0138X_3^2 + 0.0240X_1X_2 - 0.0110X_1X_3 - 0.0271X_2X_3 \quad (9)$$

For LA concentration (Eq. (7)), statistically significant variables were T_{evap} (linear), T_{cond} (linear and quadratic), and FFR (linear and quadratic), as well as the interactions between T_{evap} and FFR (Table 4). Therefore, increasing T_{evap} and T_{cond} and decreasing FFR should result in a higher LA concentration of in residue stream.

For C5 concentration (Eq. (8)), statistically significant variables were T_{evap} (linear), T_{cond} (quadratic), and FFR (linear), as well as the interactions between T_{evap} and FFR (Table 4). Higher C5 concentrations can be obtained by increasing T_{evap} and T_{cond} and decreasing FFR.

For R (Eq. (9)), statistically significant variables were T_{evap} (linear), T_{cond} (quadratic), and FFR (linear), as well as the interactions between T_{evap} and FFR, and T_{cond} and FFR (Table 4). Higher mass percentages in the residue stream can be obtained by decreasing T_{evap} and increasing T_{cond} and FFR.

ANOVA analysis was performed considering Eqs. (7)–(9). Results are shown in Table 5. Similar to the distillate stream, F-test showed that none of the mathematical models of the residue stream were predictive.

3.3. General analysis

Selection of parameters for this study was in part based on previous studies [10,16,18–20], in which the most important factors in the concentration of 1G-LA were evaluated. However, it was found that studying 2G-LA separation required evaluation of other parameters, such as working pressure. Thus, this study presents results heretofore not demonstrated in the literature, regarding fundamental differences in the downstream processing of 1G and 2G products, which have different compositions due to fermentation.

The main goal of the studied process is to obtain higher LA concentrations. In this study, the highest LA concentration, 86.69 g/L, was obtained in the residue stream, making it the most effective stream in concentrating LA and minimizing residual sugar. In comparison to the feed stream, this value represents a 3.1 times increase in LA concentration, a result corroborated by previous works [10,16,18–20]. As such, optimization of the process requires concentrating LA in the residue stream, and upcoming studies should further investigate relevant parameters, in order to maximize the concentration of sugars in the distillate stream.

Operational parameters affected all the studied responses. Furthermore, it was possible to remove a considerable amount of water collected in the light stream. In this sense, the modification made in the equipment [18] was essential to separate LA from water, considering these two molecules have a strong affinity and separating them by simple evaporation or distillation proves difficult. The best operational conditions were obtained during run 13: T_{evap} = 120 °C, T_{cond} = 13 °C and FFR = 8.3 mL/min. These values represent T_{evap} and T_{cond} in the central point, and the FFR value was the lowest studied (Table 1).

This result is in agreement with a previous study [20]. In comparison to this study, it had the same operational parameters (T_{evap}, T_{cond} and FFR), with values in the same range. The main difference concerned the feed stream used in the separation process: the fermented broth used as a feed stream had residual sugars from the molasses fermentation (mainly sucrose). Sucrose has 12 carbons, while LA has 3. This makes the separation of sucrose and LA easier than the separation of xylose (5 carbons) and LA (3 carbons). This is probably because in the latter case the molecules have very similar sizes, making it harder to find the ideal operating range for separation. As a matter of fact, both studies showed that high sugar concentration has an important role in separation behaviour and operational difficulties.

The majority of HSPE studies in the literature use synthetic solutions to develop their models. As shown by our previous study [20], fermentation broth is a much more complex mixture, making it harder to deal with. In fact, the presence of residual sugar affects the performance of the separation process, and every single parameter has to be re-adjusted in accordance to sugar contents [20].

In a similar fashion, Komesu et al. [11] obtained the highest LA concentration from the residue stream. In that case, however, sucrose was completely depleted and no residual sugars were present in the feed stream. Another work by Komesu et al. [16] shows that by simply adjusting equipment parameters it is possible to obtain entirely different results. In this study, even when using a similar molasses fermentation feed stream with 5% (w/w) LA, merely changing the T_{evap} conditions resulted in a higher LA amount in the distillate stream.

In another experiment, Komesu et al. [14] evaluated the influence of glucose, xylose and sucrose in a synthetic solution on LA separation via HSPE. Xylose concentration had a strong influence on the LA concentration process. The same result was found here, considering high LA concentrations were always concomitant with high C5 sugar concentrations. This implies in

important differences between alternative downstream techniques for first (1G) and second (2G) generation LA, as the different molecules present in each one may have high influence on the efficiency of the process.

It is evident that operational parameters should correspond to different production goals and feed streams. When using LA produced from hemicellulose hydrolysate, downstream operational parameters should be adjusted in order to:

- Obtain higher C5 concentration in the distillate stream: increase T_{evap} and T_{cond} and decrease FFR;
- Obtain higher mass percentage in the residue stream: decrease T_{evap} and increase T_{cond} and FFR;
- Obtain higher LA concentration in the residue stream: increase T_{evap} and T_{cond} and decrease FFR.

HSPE is a complex process, involving many variables. The methodology presented here may be feasible for the concentration of 2G-LA. However, this process is even more complex than that of concentrating 1G-LA, due to the strong interaction between xylose and water. Variables studied here were based on previous studies for concentration of 1G-LA and other parameters, such as operation pressure, need to be evaluated for the process to be effective. To the best of our knowledge, this is the first study to demonstrate that downstream processing for 1G and 2G-LA may present fundamental operational differences, making it an important source of information for researchers in the area.

In order to avoid the accumulation of sugars and LA in the same stream, some options would be: consumption of all sugars during the fermentation process, optimization of HSPE operational parameters in order to find better separation conditions, or removal of sugars prior to the HSPE process.

4. Conclusions

- Lactic acid (LA) concentration by Hybrid Short Path Evaporation (HSPE) was influenced by internal condenser temperature, evaporator temperature, and feed flow rate.
- LA was more concentrated in the residue stream.
- HSPE downstream processing of LA from hemicellulose sugars was harder than from 6-carbon sugars.
- Other operational parameters need to be studied in order to optimize the HSPE process for 2G-LA purification.

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Declarations of interest

None.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.seppur.2018.07.012.

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