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D(−)-Lactic Acid Production by *Leuconostoc mesenteroides* B512 Using Different Carbon and Nitrogen Sources

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Abstract Sugar concentration from sugarcane juice and yeast autolysate increased lactic acid production more than the other agro-industrial substrates tested. The concentrations of these two components were further optimized using the Plackett–Burman design and response surface method. A second-order polynomial regression model estimated that a maximal lactic acid production of 66.11 g/L would be obtained when the optimal values of sugar and yeast autolysate were 116.9 and 44.25 g/L, respectively. To validate the optimization of the medium composition, studies were carried out using the optimized conditions to confirm the result of the response surface analysis. After 48 h, lactic acid production using the shake-flask method was at 60.2 g/L.

Keywords Response surface method · Medium optimization · D(−)-Lactic acid · Residues

Introduction

Lactic acid has been used in biodegradable plastics, such as polylactic acid (PLA), and can be used to improve the physical properties in the production of garbage bags, agricultural plastic sheeting, and food packaging [1]. It can also be used in sutures and surgical implants due to its biocompatible and bioabsorbable characteristics [2]. Lactic acid is industrially produced either through chemical synthesis or microbial fermentation. The advantage of the biological method is that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a racemic mixture of lactic acid [3]. The optical purity of lactic acid is very important to the physical properties of PLA and obtaining a more stable crystalline polymer than that achieved with a racemic lactic acid [4, 5]. Polymers made with L(+)-lactic acid have a melting point of 175 °C. However, this melting point can be increased by adding D(−)-lactic acid, thereby producing a stereocomplex with a melting point of around 230 °C [6, 7].

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Leuconostoc mesenteroides B512F is a lactic bacteria that produces optically pure D(−)-lactic acid. This species as well as all lactic bacteria has complex nutritional requirements due to their limited ability to biosynthesize B vitamins and amino acids [8]. Refined sugars such as glicose, fructose, sucrose, and lactose are fermented by *Leuconostoc*. Furthermore, a considerable amount of an expensive complex nitrogen source, such as yeast extract, must be added to the medium to produce lactic acid within a reasonable timeframe. However, this is economically unfavorable, as pure sugars and pure complex nitrogen sources are expensive. Therefore, raw materials for the industrial production of lactic acid need to have characteristics such as low cost, low levels of contaminants, rapid fermentation, and year-round availability [3]. According to Tejayadi and Cheryan [9], the cost of raw materials represents 68% of the total cost of lactic acid production. A number of industrial bio-products or wastes have been evaluated as substrates for lactic acid production with the aim of decreasing the cost of the process, such as sugarcane [10], molasses [11], cassava wastewater [12], and whey [13] as carbon sources and corn steep liquor (CSL) [14] and yeast autolysate [15] as nitrogen sources. However, there are a few studies on D(−)-lactic acid fermentation using these substrates in comparison with L(+)-lactic acid.

Sugarcane juice, sugarcane molasses, and whey are renewable, abundant, and cheap sources of carbon. They were also proven to be an economically feasible raw material for industrial production of lactic acid once they have enough nutrients necessary for the growth of lactic acid bacteria. Furthermore, sugarcane molasses and sugarcane juice do not require treatment to convert starch to fermentable sugars as the sugar content is almost all in the form of sucrose [10] or in the form of lactose in the case of whey. This way, it is possible to reduce the cost of culture medium by using these substrates in order to increase economic viability. These substrates are renewable, abundant, and cheap sources of carbon and they have enough nutrients necessary for the growth of lactic acid bacteria.

The aim of the present study was to evaluate the potential use of different types of cheap agro-industrial substrates as well as investigate the effects of different medium components for the optimization of the production of D(−)-lactic acid by *L. mesenteroides* B512.

Materials and Methods

Microorganism

L. mesenteroides NRRL B 512 was provided by Department of Food Engineering, Unicamp, Brazil. The strain was stored in a Man, Rogosa, and Sharpe (MRS) medium with 20% (*v*/*v*) glycerol at −20 °C.

Culturing

The inoculum was prepared through the transference of 1 mL of stock culture to Erlenmeyer flasks containing 100 mL of growth medium (MRS). The following was the composition of the medium (g/L): peptone (10.0), yeast extract (5.0), meat extract (10.0), glucose (20.0), sodium acetate (5.0), ammonium citrate (2.0), K_2HPO_4 (5.0), $MgSO_4 \cdot 7H_2O$ (0.1), and $MnSO_4 \cdot 4H_2O$ (0.05). All experiments and inoculum preparations were carried out in flask cultures in orbital shakers at 200 rpm and 30 °C. The initial pH was adjusted to 6.2. 10% (*v*/*v*) of the inoculum and 100 g/L of calcium carbonate was added to 250-mL Erlenmeyer flasks with 20 mL of the experimental media.

Effect of Different Carbon Sources

Fermentations were carried out with MRS medium without glucose and supplemented with different carbon sources at an initial concentration of 120 g/L of reducing sugar. Three carbon sources were studied: cheese whey, sugarcane juice, and sugarcane molasses. The sugarcane molasses and sugarcane juice were supplied by the Santa Lucia sugar processing plant and the cheese whey powder was provided by Tavolaro Dairy. Both factories are located in the state of São Paulo, Brazil.

The cheese whey powder containing 72% lactose was dissolved in water and the resulting solution was heated to boiling point for 5 min in order to coagulate the proteins. The solution was then cooled naturally to room temperature and filtered through a coffee filter to remove the proteins; the supernatant contained 200 g/L of lactose. The sugarcane molasses and sugarcane juice contained 1,000 and 240 g/L of reducing sugar, respectively. These three carbon sources (whey, sugarcane molasses, and sugarcane juice) were diluted to reach the desired sugar concentration (120 g/L).

Effect of Different Nitrogen Sources

The fermentation medium was composed of sugarcane juice (120 g/L of sugar), 5 g/L of sodium acetate, 0.1 g/L of magnesium sulfate, 0.05 g/L of sulfate manganese, 2 g/L of ammonium citrate, and 1 mL/L of Tween 80, complemented with four nitrogen sources: yeast autolysate, yeast extract, corn steep liquor, and pro-flo (cottonseed protein).

The corn steep liquor and pro-flo were supplied by Corn Products and the US company Traders Protein, respectively. Both factories are located in the state of São Paulo, Brazil. Table 1 displays the concentrations of nitrogen sources.

Plackett–Burman Experimental Design

The purpose of this first optimization step was to identify the medium components that have a significant effect on lactic acid production. Twelve experiments were generated using eight factors: sugar from sugarcane juice, sodium acetate, ammonium citrate, magnesium sulfate ($MgSO_4$), manganese sulfate ($MnSO_4$), potassium phosphate (K_2HPO_4), Tween 80, and yeast autolysate. Variables with a confidence level greater than 95% were considered to have a significant influence over lactic acid production. The Plackett–Burman experimental design was based on the first-order model with no interaction among the factors. Table 2 displays the concentrations used for each variable. A central composite design (CCD) was performed with the variables that significantly increased the production of lactic acid.

Table 1 Concentration of each nitrogen source

Nitrogen source[a]	Amount (g/L)
Yeast extract	30
Yeast autolysate	25.56
CSL	73.5
Pró-floo	30.94

[a] Concentration of each nitrogen source determined based on the concentration of nitrogen contained in 30 g/L of yeast extract

Table 2 Variables and levels used in design

Variables	Codes	Range and levels	
		−1	+1
Sugar from sugarcane juice (g/L)	X_1	50	150
Sodium acetate (g/L)	X_2	0	10
Amonium citrate (g/L)	X_3	0	4
$MgSO_4$ (g/L)	X_4	0	0.4
$MnSO_4$ (g/L)	X_5	0	0.2
K_2HPO_4 (g/L)	X_6	0	4
Tween 80 (mL/L)	X_7	0	2
Yeast autolysate (g/L)	X_8	0	40

Central Composite Design

A CCD for two independent variables, each at five levels with four star points (α=1.41) and four replicates at the center points, was used to develop a second-order polynomial model, which determined the optimal values of variables for lactic acid production. Screened through the previous work, sugar from sugarcane juice and yeast autolysate was taken as the variable for investigation.

The variables of the experiments were coded based on the following equation:

$$x_i = \frac{\left(X_i - X_{\mathrm{cp}}\right)}{\Delta X_{\mathrm{i}}} \quad i = 1, 2, \ldots\ldots, K \tag{1}$$

in which x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_{cp} is the real value of an independent variable at the center point, and ΔX_i is the step chance value.

The behavior of the system was explained by the following quadratic equation:

$$Y = \mathrm{b0} + \sum \mathrm{bixi} + \sum \mathrm{biixi2} + \sum \mathrm{bijxixj} \tag{2}$$

in which Y is the predicted response, i.e., lactic acid concentration; b0 is the offset term; bi is the linear effect; bii is the squared effect; bij is the interaction effect; and xi is the independent variable.

The Statistica 7.0 software package (Stat Soft, USA) was used for the experimental design and regression analysis of the experimental data. The response surface was generated to determine interactions among the variables. The optimal points for the variables were obtained from Maple 9.5 (Waterloo Maple Inc., Ontario, Canada).

Using the CCD method, a total of 12 experiments was conducted with various combinations of sugar from sugarcane juice and yeast autolysate. Table 3 displays the range and concentrations of the variables investigated in these experiments.

To validate the optimization of the medium composition, tests were carried out using the optimized conditions in order to confirm the results of the response surface analysis.

Analysis

Lactic acid concentrations were determined using a high-performance liquid chromatography system (HPLC) equipped with a UV detector at 210 nm. A Rezex ROA (300×7.8 mm,

Table 3 Real values of variables used in central composite design

Variables (g/L)	Codes	Range and levels				
		−1.41	−1	0	+1	+1.41
Sugar from sugarcane juice	X_1	29.5	50	100	150	170.5
Yeast autolysate	X_2	8.85	15	30	45	51.15

The independent variables used in the central composite design were identified as significant to increasing lactic acid production using a Plackett–Burman two-level factorial design

phenomenex) column was eluted with 5 mM H_2SO_4 as a mobile phase at a flow rate of 0.4 mL/min and the column temperature was maintained at 60 °C. Optical purity of D(−)-lactic acid was determined with HPLC using a chirex 3126 phenomenex (150×4.6 mm) column with 1 mM of $CuSO_4$ as the mobile phase at 1 mL/min (30 °C). Reducing sugars were measured using the 3,5-dinitro salicylic acid method [16].

Results and Discussion

Effect of Different Carbon Sources

The results of the influence of three carbon sources (cheese whey, sugar cane juice, and molasses) over the production and productivity of D (−)-lactic acid are displayed in Table 4.

Among the three carbon sources investigated, *L. mesenteroides* achieved the highest production and productivity of D(−)-lactic acid with sugarcane juice. Sugarcane juice contains 13% to 16% (*w*/*v*) of sucrose and has the advantage of being a renewable, abundant, cheap carbon source. Lower production was observed with molasses, probably due to the presence of inhibitory compounds. Furthermore, when molasses is used in fermentation, it may cause serious problems in the purification and treatment of the substrate [17].

Effect of Different Nitrogen Sources

The results of the influence of four nitrogen sources added to sugarcane juice, yeast autolysate, yeast extract, corn steep liquor, and pro-flo (cottonseed protein) over the production and productivity of D(−)-lactic acid after 48 h of fermentation are displayed in Table 5.

Among the four nitrogen sources evaluated, yeast extract and yeast autolysate achieved the highest D(−)-lactic acid production (59.2 and 44.48 g/L, respectively) and productivity (2.3 and 1.61 g/L h, respectively). Vahvaselka and Linko [18] investigated the effect of yeast extract, hydrolyzed casein, and hydrolyzed protein from whey cheese, corn steep liquor, and

Table 4 Effect of different carbon sources on the production and productivity of D(−)-lactic acid after 48 h of fermentation with 120 g/L of initial sugar

Carbon source	Production (g/L)	Productivity (g/L h)
Cheese whey	41.16	0.85
Molasses	38.4	0.80
Sugarcane juice	43.8	0.91

Table 5 Effect of different nitrogen sources added to sugarcane juice (120 g/L of initial sugar) on lactic acid production and productivity

Nitrogen source[a] (g/L or mL/L)	Production[b] (g/L)	Productivity[c] (g/L h)
Yeast extract	59.2	2.3
Yeast autolysate	44.48	1.61
CSL	36	1.25
Pro-flo	26.4	1.31

[a] Initial amount of each nitrogen source determined based on the concentration of nitrogen contained in 30 g/L of yeast extract, i.e., 2.94 g/L of nitrogen

[b] Lactic acid production in 48 h of fermentation

[c] Lactic acid productivity in 24 h of fermentation

malt extract on lactic acid production by *Lactobacillus helveticus* in ultra-filtered milk. Yeast extract (5 g/L) achieved the best result, followed by the hydrolysates, whereas corn steep liquor and malt extract had little effect on production. Cox and MacBean [19] report a significantly better effect with yeast extract in comparison to corn steep liquor for a strain of *Lactobacillus bulgaricus*. However, the high cost of yeast extract is unfavorable to industrial production [20]. Thus, autolyzed yeast may be a viable alternative.

Plackett–Burman Experimental Design

The Plackett–Burman design matrix (real and coded values) of 12 experiments with eight variables (X_1=sugar from sugarcane juice, X_2=acetate, X_3=citrate, X_4=$MgSO_4$, X_5=$MnSO_4$, X_6=K_2HPO_4, X_7=Tween80, and X_8=yeast autolysate) and respective results (D-lactic acid

Table 6 Plackett–Burman design (real and coded values) with respective results regarding lactic acid production

Run	Independent variables (g/L or mL/L)								Result
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Lactic acid (g/L)
1	150 (1)	0 (−1)	4 (1)	0 (−1)	0 (−1)	0 (−1)	2 (1)	40 (1)	48
2	150 (1)	10 (1)	0 (−1)	0.4(1)	0 (−1)	0 (−1)	0 (−1)	40 (1)	45.2
3	50 (−1)	10 (1)	4 (1)	0 (−1)	0.2(1)	0 (−1)	0 (−1)	0 (−1)	12
4	150 (1)	0 (−1)	4 (1)	0.4(1)	0 (−1)	4 (1)	0 (−1)	0 (−1)	8
5	150 (1)	10 (1)	0 (−1)	0.4(1)	0.2(1)	0 (−1)	2 (1)	0 (−1)	10.2
6	150 (1)	10 (1)	4 (1)	0 (−1)	0.2(1)	4 (1)	0 (−1)	40 (1)	17.5
7	50 (−1)	10 (1)	4 (1)	0.4(1)	0 (−1)	4 (1)	2 (1)	0 (−1)	2.1
8	50 (−1)	0 (−1)	4 (1)	0.4(1)	0.2(1)	0 (−1)	2 (1)	40 (1)	27
9	50 (−1)	0 (−1)	0 (−1)	0.4(1)	0.2(1)	4 (1)	0 (−1)	40 (1)	16
10	150 (1)	0 (−1)	0 (−1)	0 (−1)	0.2(1)	4 (1)	2 (1)	0 (−1)	1.15
11	50 (−1)	10 (1)	0 (−1)	0 (−1)	0 (−1)	4 (1)	2 (1)	40 (1)	24
12	50 (−1)	0 (−1)	0 (−1)	0 (−1)	0 (−1)	0 (−1)	0 (−1)	0 (−1)	26

(−1) and (1) are coded levels

X_1 sugar from sugarcane juice, X_2 acetate, X_3 citrate, X_4 $MgSO_4$, X_5 $MnSO_4$, X_6 K_2HPO_4, X_7 Tween80, X_8 yeast autolysate

production) are displayed in Table 6. It was also observed in Table 6 that the decrease in nitrogen source results in a decreased production of lactic acid, indicating nutrient limitation.

Yeast autolysate proved as the most influential variable in the production of lactic acid, followed by K_2HPO_4, $MnSO_4$, and sugar from sugarcane juice. Among these variables, only sugar and yeast autolysate had a significant positive effect on lactic acid production with a 95% confidence level and were therefore used to optimize the production of lactic acid. The effects of the variables are illustrated in Fig. 1 (Pareto chart).

Using the Plackett–Burman design to study solid-state fermentation with *Lactobacillus amylophilus* GV6, Naveena et al. [21] report that $MnSO_4 \cdot H_2O$ achieved a negative coefficient, whereas $MgSO_4$, sodium acetate, and corn steep liquor were found to be insignificant. However, ammonium citrate and Tween 80 improved the production of lactic acid.

According to Honorato et al. [22], the addition of phosphate to the culture medium increases microorganism growth and lactic acid production as this component maintains the pH near the optimal value for growth and allows the conduction of fermentation for a longer period of time. However, K_2HPO_4 had a negative effect on lactic acid production (Fig. 1). This may be explained by the excess of this component in the medium. Thus, the addition of K_2HPO_4 and $MnSO_4$ to sugarcane juice is not necessary and should be avoided.

Response Surface Method

Sugar and yeast autolysate were further optimized using response surface optimization. The design matrix of the variables in coded units and real values and the respective experimental results are displayed in Table 7.

By comparing tests 9, 10, 11, and 12 with test 7 where the concentration of X_2 was reduced by threefold (maintaining X_1 constant), the lactic acid production was reduced,

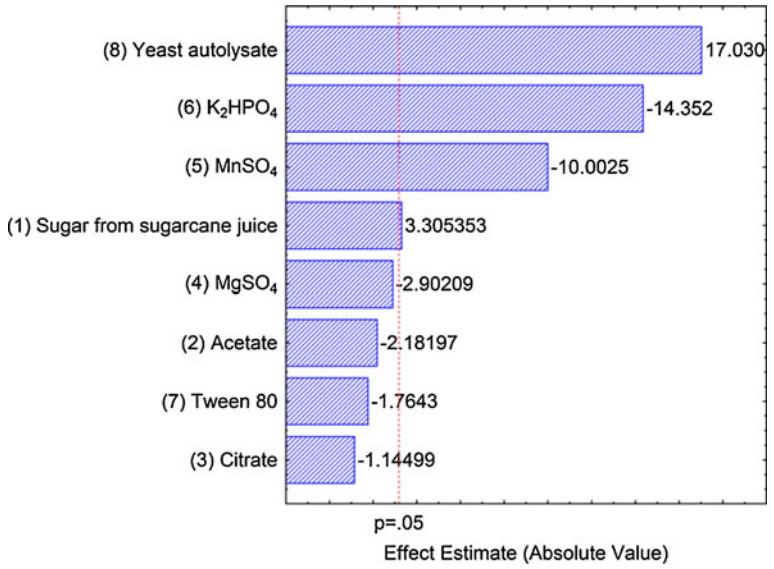

Fig. 1 Pareto chart for lactic acid production

Table 7 Central composite design for optimization of two variables (each at five levels) and experimental values for production of lactic acid

Run	Independent variables (g/L)		Results (g/L)
	Sugar from sugarcane juice (X_1)	Yeast autolysate (X_2)	Lactic acid
1	50 (−1)	15 (−1)	16
2	50 (−1)	45 (1)	28.56
3	150 (1)	15 (−1)	6.4
4	150 (1)	45 (1)	58.8
5	29.5 (−1.41)	30 (0)	6.8
6	170.5 (1.41)	30 (0)	18.4
7	100 (0)	8.85 (−1.41)	4.72
8	100 (0)	51.15 (1.41)	59.4
9	100 (0)	30 (0)	56.8
10	100 (0)	30 (0)	56.5
11	100 (0)	30 (0)	57.12
12	100 (0)	30 (0)	57

(−1.41), (−1), (0), (1), and (1.41) are coded levels

indicating nutrient limitation. In the same way, by reducing X_1 by half (test 2), lactic acid decreases by the same amount.

These results indicate that the increased availability of nitrogen assimilated by *L. mesenteroides* B512 probably influences the increased production of lactic acid during the fermentation process. Since lactic acid bacteria are nutritionally fastidious, amino acids and vitamins are required for growth. Choosing the right type of nitrogen source appears to be very important because of the rise in biomass concentration.

The application of multiple regression analysis methods yielded the following regression equation (Eq. 3) for the experimental data:

$$Y = 56.85 + 4.63X_1 + 17.78X_2 - 20.85X_1X_1 + 9.96X_1X_2 - 11.12X_2X_2 \qquad (3)$$

in which Y is the predicted response (lactic acid production) and X_1 and X_2 are, respectively, the coded values of the test variables sugar from sugarcane juice and yeast autolysate.

The highest production of lactic acid was 59.4 g/L, obtained from 100 mL/L of sugarcane juice and 51.15 g/L of yeast autolysate (Table 7). The response surface quadratic model was performed in the form of analysis of variance (ANOVA) and the results are summarized in Table 8. Fisher's F-test was used to check the statistical significance of Eq. 3.

ANOVA of the quadratic regression model demonstrates that the model is highly significant [as evident from Fisher's test (F_{calc} (5,6)=100.8525>Ft (5,6)=4.39)] and has a very low probability value [(P_{model}>F)=0.000011]. The fit of the model was checked by the coefficient of determination (R^2) and multiple correlation coefficient (R). The R^2 value (0.988) for Eq. 3 indicates that the sample variation of 98.8% for lactic acid was attributed to the independent variables and only 1.2% of the total variation cannot be explained by the model. The adjusted R^2 value (0.978) is also high, which indicates the high significance of the model. The high R value (0.994) demonstrates a strong agreement between observed and predicted values.

Table 8 Analysis of variance for the quadratic model

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*P*>*F*
Model	6,203.364	5	1,240.673	100.8525	0.000011
Error	73.811	6	12.302		
Lack of fit	73.591	3	24.530	334.05	
Pure error	0.220	3	0.073		
Total	6,277.176				

R^2 =0.988; adjusted R^2 =0.978; R=0.994

Table 9 displays the Student's *t*-distribution and probability (*P*) values. These values serve as a tool to check the significance of each coefficient, which represents the interaction pattern between the test variables. A smaller *P*-value denotes a more significant corresponding coefficient. All independent variables had a significant effect (small *P*-values). The parameter estimate indicate that X_2 (yeast autolysate) is the parameter that contributes more strongly to the response (lactic acid). Moreover the variables X_1 and X_2 and their interaction (X_1X_2) had a positive effect, with an increase in their concentration leading to an increase in the response (lactic acid production).

Figure 2 displays the 3D response surface and contour plots, a graphical representation of the regression equation plotted for one to understand the interaction of the variables and locate the optimal level of each variable for maximal response.

There was a strong interaction between sugar and yeast autolysate in the production of lactic acid (Fig. 2). The area of greatest lactic acid production is located between 102 and 132 g/L of sugar from sugarcane sugar and between 37 and 52 g/L of yeast autolysate. However, both variables may inhibit lactic acid production at higher concentrations due to significant carbon and nitrogen repression. As lactic acid bacteria are nutritionally fastidious and require various amino acids and vitamins for growth, choosing the right nitrogen and carbon sources is very important. Nitrogen is necessary to the synthesis of amino acids, lipids, enzyme cofactors, some carbohydrates, and other substances. The nitrogen source is a major factor of influence over the growth of *Lactobacillus* [23]. As the synthesis of lactic acid by fermentation is associated with cell growth, there is no product formation if the medium does not have an adequate concentration of nitrogen [24]. However, high concentrations of nitrogen can lead to cell death [15].

Using an MRS medium containing 20 g/L of yeast extract and 120 g/L of sugarcane juice, Calabia and Tokiwa [10] obtained 107 g/L of lactic acid. The authors attribute this high production to the high nutritional value of sugarcane juice, which contains natural

Table 9 Least-squares fit and parameter estimates

Term	Estimate	Standard error	*t*	Pr>−*t*|
Intercept	56.855	1.75	32.42	0.000
X_1	4.63	1.24	3.73	0.0096
X_2	17.786	1.24	14.34	0.000007
X_1X_2	9.96	1.753	5.678	0.001284
X_{12}	−20.85	1.386	−15.038	0.000005
X_{22}	−11.12	1.386	−8.02	0.000201

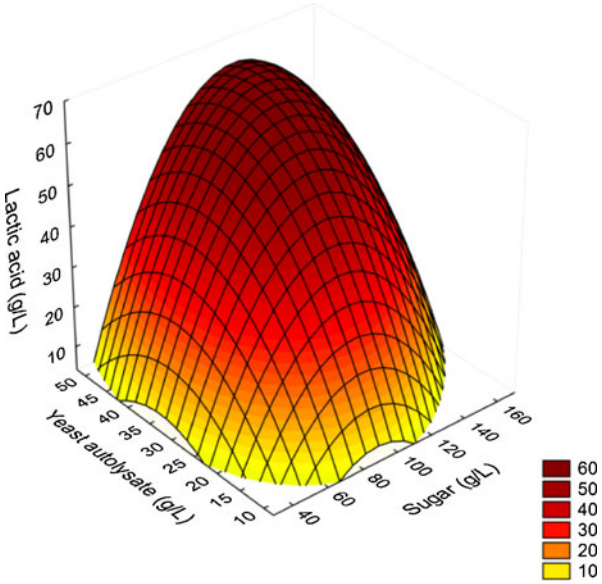

Fig. 2 Response surface and contour plots of D(−)-lactic acid production by *L. mesenteroides* showing the interaction between sugar from sugarcane juice and yeast autolysate

sugars, proteins, amino acids, and vitamins essential to the growth of lactic acid bacteria. Sule et al. [25] tested different concentrations of sucrose in a culture medium (5, 30, 50, and 100 g/L) and achieved maximal lactic acid production (21 g/L) using 50 g/L of sucrose, whereas the use of 100 g/L of sucrose resulted in a decrease in the production of lactic acid. The authors attribute this to the increased viscosity of the medium due to the high concentration of sucrose.

The point of maximal lactic acid production was determined through a canonical analysis of the adjusted model. A study was carried out to identify the nature of the stationary point (maximal point, low response, or even a saddle point). An algorithm carried out on the Maple 9.5 program (Waterloo Maple, Inc., Canada) was used to calculate the stationary point (P0) for the synthesis of lactic acid. These values are displayed in Table 10.

The λ values for sugar and yeast autolysate indicate that these responses have a maximal point, as they have equal and negative signs (Table 10). A second-order polynomial regression model estimated that a maximal lactic acid production of 66.11 g/L would be obtained when optimal sugar concentration from sugar and yeast autolysate values were 116.9 and 44.25 g/L, respectively. All optimal points were located within the experimental region and varied around their center points to different extents. Three additional experiments in a shaker were performed with this optimal medium composition in which mean lactic acid production was 60.2 g/L, which was a bit smaller than the predicted value (66.11 g/L).

Table 10 Stationary point for lactic acid production and codified values of variables X_1 and X_2 on the optimization point

P0	Lactic acid	Coordinates	Lactic acid
λ_1	−22.947	X_1	0.338
λ_2	−9.023	X_2	0.951

Sugarcane juice is a renewable, abundant, and cheap source of carbon. Furthermore, it is an economically feasible raw material for the industrial production of lactic acid once it has enough nutrients necessary for the growth of lactic acid bacteria. Therefore, optically pure D-lactic acid can be obtained using *L. mesenteroides* B512F, and the cost of culture medium could be reduced by using this cheap substrate.

Conclusions

Based on the optimization of the responses, the best result for D(−)-lactic acid production (60.2 g/L) was obtained with 116.9 g/L of sugar and 44.25 g/L of yeast autolysate. Thus, the use of these substances with *L. mesenteroides* is feasible as there was considerable production of D(−)-lactic acid, requiring only the completion of a cheap source of nitrogen (yeast autolysate). The Plackett–Burman design, central composite design, and response surface method, including regression analysis and model generation, were effective methods regarding medium optimization for the production of D(−)-lactic acid.

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