ISSN 1330-9862
(FTB-2363)

original scientific paper

The Use of Response Surface Methodology in Optimization of Lactic Acid Production: Focus on Medium Supplementation, Temperature and pH Control

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Received: October 20, 2009
Accepted: February 18, 2010

Summary

Two response surface methodologies involving central composite designs have been successfully applied to evaluate the effect of cheese whey, corn steep liquor, ammonium sulphate, temperature and pH control on lactic acid fermentation by *Lactobacillus* sp. LMI8 isolated from cassava flour wastewater. In the first central composite design, corn steep liquor and ammonium sulphate were investigated as low-cost nitrogen sources in combination with other components to substitute yeast extract for economical production. The best results were obtained with 55 g/L of lactose, 15 g/L of corn steep liquor and 5.625 g/L of ammonium sulphate. At the maximum point, the lactic acid concentration reached 18.68 g/L. After defining the optimal nutritional conditions for lactic acid production, a second central composite design was performed to determine the extent to which temperature and pH influence the lactic acid production with the aim of improving the fermentation process. The second-order polynomial regression model determined that the maximum lactic acid production of 52.37 g/L would be obtained when the optimum temperature and pH were 39.6 °C and 5.9, respectively. Comparing the lactic acid production in shake flask fermentation, there was an increase of 180 % after 30 h of processing, with a conversion efficiency of about 86.12 % of the initial lactose. In addition, lactic acid produced from whey lactose by *Lactobacillus* sp. LMI8 was optically almost pure D-lactic acid (over 98 % of total lactic acid produced).

Key words: lactic acid, whey cheese, nitrogen sources, response surface methodology, medium optimization

Introduction

Lactic acid has numerous applications in the food, chemical, textile, pharmaceutical, and other industries (*1*). Recently, there has been a great demand for lactic acid, as it can be used as a monomer for the production of the biodegradable polymer polylactic acid (PLA), which is an alternative to synthetic polymers derived from petroleum resources (*2*). While only racemic DL-lactic acid is produced through chemical synthesis, a desired stereoisomer (*i.e.* an optically pure L- or D-lactic acid) could be produced through a fermentative production of renewable resources if the proper microorganisms are chosen for lactic acid fermentation (*3*).

There has been increasing interest in solving the problem that PLA weakens under heat in order to expand the use of this renewable plastic. A polymer blend of poly-L-lactic acid and poly-D-lactic acid has been reported to yield a racemic crystal called stereocomplex. This stereocomplex polymer blend is characterized by its high melting temperature, which is approx. 50 °C higher than that of PLA (*4,5*). This finding has led to an increased importance of the production of D-lactic acid.

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Nutrient supplements, such as yeast extract and lactamine AA (casein hydrolysate), can improve the nutritional quality of the medium, as they contain growth-promoting compounds in addition to organic nitrogen and carbonaceous compounds. However, the use of these nutrient supplements in large quantities is very expensive and can reach as much as 32 % of the total cost of lactic acid production (*6*). Thus, there is a need to develop an industrially acceptable process that considers productivity, residual lactose and economical levels of nutrient supplements.

Whey is a major by-product of dairy industry and contains approx. 60 to 65 % (*m/V*) lactose as well as moieties of protein, fat and mineral salts. The increased demand for cheese on the market has led to a rise in whey production. Due to the low nutrient concentration, whey was discarded in the environment for a number of years without causing harm, but has become an environmental problem due to the volume produced and biochemical oxygen demand (BOD, 30 000 to 50 000 mg/L). Lactose is the main component responsible for high BOD values, as there is a BOD reduction of only 1000 mg/L when removing protein from cheese whey. The use of lactose in biotechnological processes reduces the BOD of cheese whey by as much as 75 % (*7*). In order to reduce the BOD level and acquire useful compounds, the whey can be used as a cheap carbohydrate source for the production of lactic acid by bacteria (*8,9*). However, lactic acid bacteria have complex nutrient requirements, as they have a limited capacity to synthesize vitamin B and amino acids (*1*).

A number of studies have shown that the lactic acid production of most lactobacilli is significantly improved by the addition of yeast extract, amino acids, protein concentrates, hydrolysates, vitamins and inorganic compounds such as $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ (*10–13*). Other studies have demonstrated the need to supplement cheese whey with commercially available growth supplements, such as corn steep liquor, yeast extract, casamino acids, peptone, neopeptone, molasses and trypticase (*13–15*). Corn steep liquor is a by-product of the corn milling industry and has been used as an inexpensive nutrient source for fermentation. It is an excellent source of nitrogen for most microorganisms, as it has high content of amino acids and polypeptides, with considerable amounts of B-complex vitamins (*16*).

Temperature and pH are the key environmental parameters that affect the lactic acid fermentation process. Lactic acid bacteria can grow at temperatures from 5 to 45 °C and, not surprisingly, are tolerant of acidic conditions, with most strains capable of growing at pH=4.4. It is therefore important to determine the temperature and pH at which optimal microbial growth is achieved. A better understanding of the effects of temperature and pH on lactose fermentation will facilitate the improvement of the process.

Response surface methodology has been successfully used to model and optimize biochemical and biotechnological processes related to food systems (*17–19*). This methodology could be employed to optimize media for lactic acid fermentation. Thus, the aim of the present study is to optimize the production of D-lactic acid from the isolated *Lactobacillus* sp. LMI8, controlling temperature and pH and using cheese whey as a low-cost medium, with corn steep liquor and ammonium sulphate as nitrogen sources.

Materials and Methods

Microorganism

Lactobacillus sp. LMI8 (D-lactic acid producer of highly optical purity) used in this study was isolated from cassava flour wastewater collected in the state of São Paulo (Brazil). The strain was stored in de Man, Rogosa and Sharpe (MRS) broth with 20 % (by volume) glycerol at –10 °C.

Cheese whey

Whey powder containing 72 % (*m/V*) lactose was obtained from Tavolaro Ltda, Brotas (São Paulo, Brazil). Deproteinization was carried out by heat treatment (100 °C for 10 min) of the acidified (pH=4.0) whey solution by adding 10 M HCl and cooled at room temperature (*20*). The resulting whey solution was centrifuged at 10 000×*g* to remove the solids and the supernatant (200 g/L of lactose) was diluted to reach the desired lactose concentration.

Media and growth conditions

The inoculum was prepared by transferring glycerol stock culture (1 mL) to an Erlenmeyer flask containing 100 mL of modified MRS medium and incubated at (37±1) °C for 18 h (time needed for the microorganism to reach the exponential growth phase) on a rotary shaker (New Brunswick Scientific Co, NJ, USA) at 200 rpm. Initial pH of the medium was adjusted to 6.5. Erlenmeyer flasks containing the production medium were inoculated with 10 % inoculum grown in the modified MRS medium. The composition of modified MRS medium was (in g/L): peptone 10, yeast extract 5, beef extract 10, glucose 20, sodium acetate 5, $Na_2HPO_4{\cdot}2H_2O$ 2, triammonium citrate 2, $MgSO_4{\cdot}7H_2O$ 0.1 and $MnSO_4{\cdot}4H_2O$ 0.05.

A central composite design (CCD) was used in the optimization of D-lactic acid production. The experiments were performed in 250-mL Erlenmeyer flasks containing 50 mL of production medium, kept on the rotary shaker at the agitation of 200 rpm for 48 h at (37±1) °C. The production medium consisted of the same salts used in the growth medium, with the addition of whey lactose (29.26 to 80.74 g/L), corn steep liquor, CSL (8.565 to 21.435 g/L) and $(NH_4)_2SO_4$ (0 to 11.625 g/L). Initial pH was 6.5 and it was not kept constant throughout the experiments. After choosing the best culture medium composition for lactic acid production, a second CCD was employed to optimize temperature (28.4 to 39.6 °C) and pH (5.29 to 6.7). The CCD was carried out in a 3.0-litre Biostat B. Fermentor (B. Braun Biotech International, Allentown, PA, USA) containing 1.5 L of production medium. Fermentations were run at the agitation of 200 rpm for 48 h and all media were sterilized at 121 °C for 15 min. The pH was controlled by the automatic addition of 5 M NaOH. All experiments were carried out in duplicate.

Analytical methods

The quantification of lactose and lactic acid concentrations were determined using a high performance liquid chromatograph (Thermo Separation Products, Rivera Beach FL, USA), equipped with a tunable UV detector set at 210 nm and a refraction index (RI) detector. An Aminex HPX-87H ion-exchange column (300 mm×7.8 mm, Bio-Rad, Hercules, CA, USA) was eluted with 0.005 M H_2SO_4 as a mobile phase at a flow rate of 0.6 mL/min. Column temperature was maintained at 60 °C. The optical purity of lactic acid was analyzed by an enzyme test kit (R-Biopharm AG, Darmstadt, Germany), as reported elsewhere (*3*). Cell growth was measured by a UV-160A spectrophotometer (Shimadzu Co, Tokyo, Japan) set at 650 nm. Dry cell mass was determined by a calibration curve measured at the absorbance of 650 nm for dry mass (g/L). The samples obtained at different time intervals were centrifuged at 15 000×*g*.

Experimental design

The response surface methodology was applied to understand the interaction of various variables and then used to find the optimum concentration of the main medium components that affect the response.

Central composite design

The first experimental CCD was carried out in order to identify and optimize the nutrient(s) of the production medium (whey lactose, CSL and ammonium sulphate) that have a significant effect on the D-lactic acid production. For the two factors, this design was made up of a full 2^3 factorial design with its eight cube points, augmented with three replications of the center points and the six star points, that is, points having an axial distance to the center of $\pm\alpha$ (1.287) for one factor, while the other factor is at level 0. Three or four center experiments should always be included in factorial designs, as the risk of missing non-linear relationships in the middle of the intervals has to be minimized and the repetition allows determining confidence intervals (*21*). To estimate the optimal point, a second order polynomial function was fitted to the experimental results. Thus, the influence of all experimental variables, factors and interaction effects on the response was investigated. The objective of the second experiment was to obtain a more precise estimate of the optimal operating conditions for the factors involved. Thus, a central composite circumscribed 2^2 experimental design was used, with two variables (temperature and pH), four star points ($\pm\alpha$=1.41) and three replicates at the center point, resulting in a total of 11 experiments. The independent variables, experimental range and levels investigated for both CCDs are given in Table 1. In performing the regression equation, the test variables were coded according to the following Eq. 1:

$$x_i = \frac{(X_i - X_{cp})}{\Delta X_i} \qquad /1/$$

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 change value.

Statistical data analysis

Statistica v. 7.0 software package (StatSoft, Tulsa, OK, USA) was used for the experimental design, data analysis and quadratic model building. Response surface and contour plots were generated to understand the interaction of different variables. The optimal points for the variables were obtained from Maple v. 9.5 (Waterloo Maple Inc, Ontario, Canada).

Results and Discussion

The first central composite design

Table 2 shows the results obtained from the central composite design regarding the studied variables: the concentrations of whey lactose (X_1), CSL (X_2) and $(NH_4)_2SO_4$ (X_3) using the isolated *Lactobacillus* sp. LMI8. The highest lactic acid production achieved in the verification experiment was 18.31 g/L (as seen in run 17) and it reached maximal biomass of 0.932 g/L. The optical purity of D-lactic acid produced at 30 h was 98 % (17.92 g/L).

Table 3 shows the results of the second-order response surface models for lactic acid production in the form of analysis of variance (ANOVA). The regression equation (Y_a) demonstrated that lactic acid production was an empirical function of test variables in coded units, as shown in Eq. 2:

$$Y_a = 18.532 + 0.813X_1 - 1.822X_1^2 - 1.592X_2^2 - 1.599X_3^2 \qquad /2/$$

Fisher's F-test ($F_{(9,17)} = S^2_m/S^2_S = 17.37 > Ft_{(9,17)} = 3.68$) with a very low probability value [$(p_{model} > F) < 0.0005$] indicated that the model was highly significant. The goodness-

Table 1. Experimental range and levels of the independent variables (X_i and Z_i, i=1, 2 and 3) used in the central composite design

Independent variables		Range and levels				
		$-\alpha$	–1	0	+1	$+\alpha$
Production medium optimization, γ/(g/L)						
Whey lactose	X_1	29.26	35	55	75	80.74
Corn steep liquor (CSL)	X_2	8.565	10	15	20	21.435
Ammonium sulphate ($(NH_4)_2SO_4$)	X_3	0	1.25	5.625	10	11.625
Optimum operating conditions						
Temperature/°C	Z_1	28.4	30	34	38	39.6
pH	Z_2	5.29	5.5	6.0	6.5	6.7

Table 2. Central composite design for optimization of three variables (each on five levels) for the production of D-lactic acid by isolated *Lactobacillus* sp. LMI8

Runs	X_1	X_2	X_3	γ(lactic acid)/(g/L) Experimental values*
1	–1.00	–1.00	–1.00	12.44
2	–1.00	–1.00	1.00	11.64
3	–1.00	1.00	–1.00	11.92
4	–1.00	1.00	1.00	13.04
5	1.00	–1.00	–1.00	15.68
6	1.00	–1.00	1.00	14.44
7	1.00	1.00	–1.00	14.02
8	1.00	1.00	1.00	14.22
9	–1.287	0.00	0.00	12.98
10	1.287	0.00	0.00	14.04
11	0.00	–1.287	0.00	14.70
12	0.00	1.287	0.00	13.62
13	0.00	0.00	–1.287	13.68
14	0.00	0.00	1.287	14.60
15	0.00	0.00	0.00	18.16
16	0.00	0.00	0.00	18.18
17	0.00	0.00	0.00	18.31

X_1 – lactose concentration, X_2 – CSL concentration, X_3 – $(NH_4)_2SO_4$ concentration
*Values indicate the mean of triplicate observations

Table 3. Analysis of variance (ANOVA) for full quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F-value	Probe>F
Model	72.174	9	8.019	17.369	0.0005
Residual	3.232	7	0.461		
Lack of fit	3.162	5	0.632	17.746	0.0542
Pure error	0.071	2	0.035		
Total	75.406				

R^2=0.957, adj. R^2=0.902

-of-fit of the model was checked by the determination coefficient (R^2) of 95.7 %. In this case, the R^2 value (0.957) for Eq. 2 indicates that the sample variation for lactic acid of 95.7 % was attributed to the independent variables and only 4.3 % of the total variation cannot be explained by the model. The adjusted determination coefficient (adj. R^2=0.902) was also satisfactory for confirming the significance of the model.

Table 4 displays the Student's *t*-distribution and the corresponding values, along with the estimated parameters. The probability (p) values were used as a tool to check the significance of each of the coefficients. A larger magnitude of the *t*-test and smaller p-value denote greater significance of the corresponding coefficient (*22*).

The results show that the independent variable (X_1) had a significant effect, as it had a positive coefficient (Table 4), according to which an increase in its concentration led to an increased yield. The same is observed with the X_2X_3 interaction. The negative signs of the X_1X_2 interaction and squared variables X_1^2, X_2^2 and X_3^2 revealed a reduction in lactic acid production when their concentration was increased in the system. The independent variables X_2 and X_3 were not significant within the range of this study.

Table 4. Model coefficient estimated by linear regression

Factor	Coefficient	Standard error	Computed *t*-value	p-value
Intercept	18.532	0.109	170.372	0.000
X_1	0.813	0.051	15.916	0.004
X_2	–0.206	0.051	–4.037	0.056
X_3	0.061	0.051	1.186	0.357
X_1^2	–1.822	0.056	–32.408	0.001
X_2^2	–1.592	0.056	–28.321	0.001
X_3^2	–1.599	0.056	–28.447	0.001
X_1X_2	–0.345	0.067	–5.169	0.035
X_1X_3	–0.170	0.067	–2.547	0.126
X_2X_3	0.420	0.067	6.293	0.024

An algorithm carried out using Maple v. 9.5 program was applied to calculate the stationary point (P_0) for lactic acid production. The λ values (λ_1=–1.917, λ_2=–1.777 and λ_3=–1.318) indicate that these responses have a maximal point, as they have equal and negative signs. Lactic acid production was 18.64 g/L at the optimization point from the codified variables x_1 (0.231), x_2 (–0.090), and x_3 (–0.005). As expected, this value was very close to that obtained in experiments 15, 16 and 17, as the variable of the maximization (lactic acid production) point was close to the central point. In this case, the optimal medium composition for lactic acid production by the isolated *Lactobacillus* sp. LMI8 consists of (in g/L): lactose 59.64, CSL 14.55 and $(NH_4)_2SO_4$ 5.65.

The 3D response surface plot is a graphical representation of the regression equation. It is plotted to understand the interaction of the variables and locate the optimal level of each variable for maximal response. Each response surface plotted for lactic acid production represents the different combinations of two test variables at one time while maintaining the other variable at the zero level. The convex response surfaces suggest that there are well-defined optimal variables. If the surfaces are rather symmetrical and flat near the optimum, the optimized values may not vary widely from the single variable conditions (*23*). The graphic representation of response surface shown in Fig. 1 helps to visualize the effects of lactose and CSL.

Lactose and CSL are the dominant nutrients that control the biosynthesis of lactic acid. Hence, a strong interaction between them for lactic acid fermentation is inevitable. Maximal lactic acid production (18.31 g/L) was obtained for values of lactose (55 g/L) and CSL (15 g/L) in the central point region (Fig. 1). Since lactic acid bacteria are nutritionally fastidious, requiring various amino acids and vitamins for growth, choosing the right type of the nitrogen source appears to be very important. CSL has long been proved to be an inexpensive alternative to much more expensive materials, such as yeast extract and peptone (*24*). However, to our knowledge, CSL is only

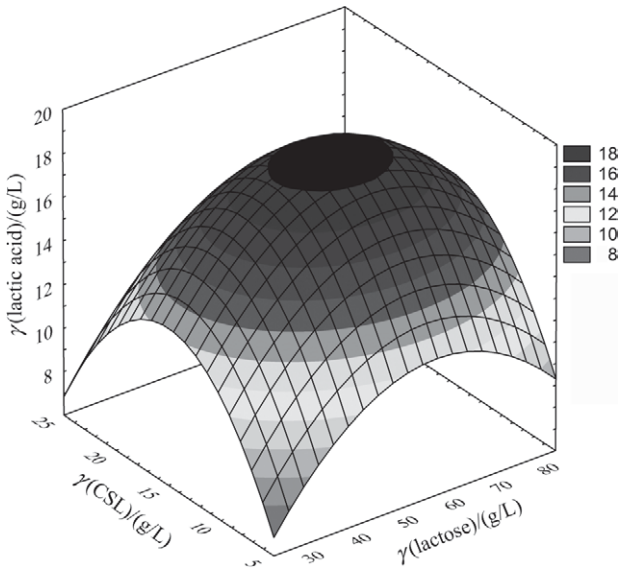

Fig. 1. Response surface plot showing the effect of lactose and CSL concentrations on lactic acid production. The value of the variable $(NH_4)_2SO_4$ was fixed at the central point

used as a partial nitrogen source in the culture for lactic acid production (*25*). In $(NH_4)_2SO_4$-supplemented medium, ammonium ions influence the metabolism of certain amino acids in lactobacilli by their incorporation with either α-ketoglutarate or glutamate (*26,27*). Thus, when $(NH_4)_2SO_4$ is used, the inorganic nitrogen should first be converted to amino acids and then used for the synthesis of proteins needed for growth and lactic acid production. Arasaratnam *et al*. (*28*) proposed that whey (total sugar 30 g/L) supplemented with 10 g/L of $(NH_4)_2SO_4$ resulted in 22 g/L of lactic acid at 48 h. Wee *et al*. (*29*) investigated the fermentative production of lactic acid from cheese whey and corn steep liquor as cheap raw materials using *Lactobacillus* sp. RKY1. When the cheese whey containing 100 g/L of lactose and 15–60 g/L of corn steep liquor was used as carbon and nitrogen sources, respectively, lactic acid was produced up to 91 g/L. Plessas *et al*. (*30*) used cheese whey (initial lactose concentration of 36 g/L) and sourdough (1 %), which resulted in maximum production of 6.9 g/L of lactic acid (by single culture) and 8.8 g/L of lactic acid (by mixed cultures).

The second central composite design

Table 5 shows the design matrix of the variables in coded units with the experiment results. The highest lactic acid production was 52.14 g/L as seen in run 6.

Multiple regression analysis yielded regression Eq. 3 for the experimental data:

$$Y_b=48.933+3.674Z_1-0.641Z_2+ +1.11Z_1Z_2-0.403Z_1^2-1.6761Z_2^2 \quad /3/$$

where Y_b is the predicted response, that is, lactic acid concentration, and Z_1 and Z_2 are the coded values of the test variables temperature and pH, respectively.

Table 6 shows the results of the second-order response surface models for lactic acid production in the form of analysis of variance (ANOVA).

Table 5. Central composite design for optimization of two variables in experimental values for production of D-lactic acid by the isolated *Lactobacillus* sp. LMI8

Runs	Z_1	Z_2	γ(lactic acid)/(g/L)
1	–1.00	–1.00	43.74
2	–1.00	1.00	41.00
3	1.00	–1.00	46.30
4	1.00	1.00	48.00
5	–1.41	0.00	40.07
6	1.41	0.00	52.14
7	0.00	–1.41	45.12
8	0.00	1.41	42.23
9	0.00	0.00	47.40
10	0.00	0.00	46.40
11	0.00	0.00	47.00

Z_1 – temperature, Z_2 – pH

Table 6. Analysis of variance (ANOVA) for the quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F-value	Probe>F
Model	114.95	5	22.989	12.215	0.0079
Residual	9.41	5	1.882		
Lack of fit	107.86	5	21.573	85.159	0.0116
Pure error	0.5067	2	0.253		
Total	124.36				

R^2=0.924, adj. R^2=0.848

The ANOVA of the quadratic regression model demonstrates that the model is very significant, as is evident from the Fisher's F-test ($F_{(5.5)}=S^2_m/S^2_S=12.21>Ft_{(5.5)}=4.46$) with a low probability value [$(p_{model}>F)<0.0079$], which indicated that the model was significant. The coefficient of determination (R^2) was 0.924, indicating that 92.4 % of the variability in the response could be explained by the model. The adjusted determination coefficient (adj. R^2=0.848) was also satisfactory for confirming the significance of the model. Fig. 2 displays the surface response plot of the model equation. The determination of the significant parameters was performed through a hypothesis test (Student's *t*-test) with a 5 % level of significance. Parameters with a level of significance higher than this value were dismissed. The empirically adjusted equation (Y_c), which represents lactic acid production, is described in Eq. 4:

$$Y_c=48.553+3.369Z_1-1.557Z_2^2 \quad /4/$$

In Eq. 4, only the isolated variable Z_1 and quadratic variable Z_2^2 significantly influence the process. A positive Z_1 and negative Z_2^2 demonstrate that a rise in temperature and reduction in pH lead to an increase in lactic acid production. The determination coefficient was 0.851, indicating that 85.1 % of the variability in the response could be explained by the model. The coordinates of the stationary points for lactic acid production were calculated from the complete Eq. 3 and using Maple v. 9.5 program: z_1=1.41 and z_2=–0.28. At the stationary point,

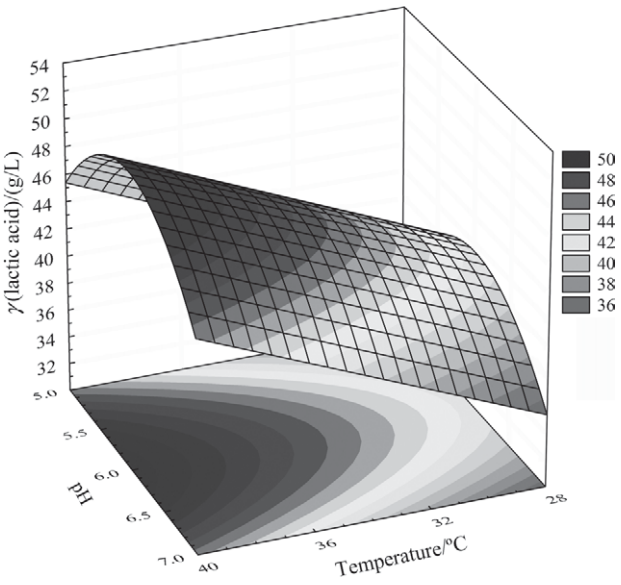

Fig. 2. Response surface plot in relation to temperature and pH for lactic acid production

coordinates z_1 and z_2 are inside the experimental region. The stationary points and respective λ values (λ_1=–1.562 and λ_2=–0.513) relative to lactic acid production suggest that the response is the maximal point. The maximal point of the model was at 39.6 °C and pH=5.9. The model predicted a maximal response for lactic acid concentration of 52.492 g/L for this point. In order to confirm the predicted results of the model, experiments using the medium representing this maximal point were performed and a value of 52.37 g/L was obtained (triplicate experiments were carried out). This value represents an increase of 180 % of lactic acid production when the pH of the production medium was controlled, with a conversion efficiency of about 86.12 % of the initial lactose. The optical purity of D-lactic acid that was produced at 30 h and reached maximal biomass of 1.64 g/L was 51.45 g/L. Although the L-lactic acid was produced in small amounts (approx. 0.5 g/L) during fermentation by *Lactobacillus* sp. LMI8, D-lactic acid content in total lactic acid produced were more than 98 % in all experiments using whey lactose as a source of carbon. The pH control did not affect the optical purity, however, when the pH was not controlled, the microorganism metabolism was affected and the lactose consumption and lactic acid production practically ceased after 16 h of process, when the medium pH reached approx. 4.2 (results not shown). According to some authors (*31*,*32*), weak acids, *e.g.* lactic acid, inhibit bacterial growth because as the external pH decreases, the acid is protonized as soon as it is exported out of the bacteria. Uncharged, it diffuses back into the cell and dissociates due to the higher intracellular pH. The cell then has to use ATP to pump out protons, and eventually the energy is depleted, causing the stop of the growth and the death of bacteria.

Mussatto *et al.* (*33*) reported that a glucose-rich hydrolysate (50 g/L) obtained from brewer's spent grain was used as substrate for lactic acid production. The effects of pH control and nutrient supplementation of the hydrolysate were investigated. The lactic acid production in the MRS broth with controlled pH at 6.0 was 35.54 g/L, while in the medium without pH control, it was only 13.02 g/L. These values represent an increase of 170 % of lactic acid production when the pH of the supplemented hydrolysate was controlled.

Fig. 2 illustrates that an increase in temperature leads to increased production of lactic acid. Maximal lactic acid production was obtained at pH=6.0. The optimal range for lactic acid production was from 34 to 39.6 °C and pH=5.5 to 6.5.

Conclusion

The central composite design and response surface methodology enabled the determination of optimal operating conditions for obtaining greater lactic acid production. The validity of the model was proven by fitting the values of the variables to the model equation and by carrying out experiments using these values. The optimization of the analyzed responses demonstrated that the best results for lactic acid production (52.37 g/L) were obtained with 59.64 g/L of lactose, 14.55 g/L of CSL and 5.65 g/L of $(NH_4)_2SO_4$, at 39.6 °C and pH=5.9. All points were located near the central point of the design. Lactic acid produced from lactose was mostly D-lactic acid with the content of more than 98 % based on total lactic acid produced. Thus, the isolated *Lactobacillus* sp. LMI8 proves to have great potential for lactic acid production when grown on whey cheese and corn steep liquor.

Acknowledgements

We thank the São Paulo Research Foundation (FAPESP) for fellowships and financial support.

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