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ORIGINAL ARTICLE

Low-cost effective culture medium optimization for D-lactic acid production by *Lactobacillus coryniformis* subsp. *torquens* under oxygen-deprived condition

Lizeth Jaramillo[1] · Danielle Santos[2] · Elcio Borges[3] · Diogo Dias[1] · Nei Pereira[3]

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Abstract

Lactic acid is considered a commodity and its production is boosted by the synthesis of polylactic acid. D-lactic acid (DLA) isomer offers greater flexibility and biodegradability and it can only be obtained in its pure form through fermentation. The lactate dehydrogenase is stereospecific for homofermentative production of DLA isomer in the metabolic pathway of *Lactobacillus coryniformis* subsp. *torquens*, with optical purity of ≥ 99.9% under oxygen-deprived condition. A simple culture medium that increases DLA production and reduces fermentation costs is fundamental for industrial applicability. A central composite rotatable design was used to evaluate significant components influencing the DLA production. Concentrations were adjusted using the Design-Expert 7.0 optimization tool with a desirability coefficient of 0.693 and the best concentrations of each component were determined. Finally, an assay in the bioreactor with the modified culture medium resulted in a product yield of 0.95 g/g, volumetric productivity of 0.85 g/L.h and 95% of efficiency.

Keywords Fermentation · Optical purity · Central composite rotatable design · Optimization tool

Introduction

Lactic acid (LA) was discovered in 1780 by chemist Carl Wilhelm Scheele. It is an important hydroxycarboxylic acid with applications in the food, pharmaceutical, cosmetics, textile, and chemical industries (Abdel-Rahman et al. 2013). The world demand for lactic acid production is forecasted to reach over one million metric tons by the year 2020 with annual growth of 5–8% (Jem et al. 2010; Abdel-Rahman et al. 2013). The application of lactic acid in the production of biodegradable biopolymer poly-lactic acid (PLA) is growing due to its biodegradability characteristics (Nampoothiri et al. 2010).

Lactic acid has an asymmetric β-carbon as chiral center that produces two enantiomers: L(+) lactic acid (LLA) and D(−)lactic acid (DLA). While LLA and DLA are only obtained by microbial fermentation processes, racemic DL-lactic is always produced via chemical synthesis (Li and Cui 2010). Depending on the microorganism and the operating conditions employed, an optically pure product L(+) or D(−) and in some cases racemic mixtures can be produced (Narayanan et al. 2004).

The chemical, physical, and biological properties of PLA are determined by the isomeric composition, processing temperature, annealing time, and molecular weight. Polymerization of LLA results in poly-L(+) lactic acid (PLLA) and polymerization of DLA results in poly-D(−) lactic acid (PDLA). Due to the stereo regular chain microstructure, optical pure PLLA and PDLA are semi

✉ Lizeth Jaramillo
lizethacevedo@eq.ufrj.br

[1] Laboratory of Biocorrosion, Biodegradation and Biosynthesis, School of Chemistry, Federal University of Rio de Janeiro, Av. Horacio Macedo, 2030, Bloco E, Sala E-109, CT, Cidade Universitária - Ilha do Fundao, Rio de Janeiro, RJ CEP: 21949-900, Brazil

[2] Nucleus of Ecology and Socio-Environmental Development of Macaé, NUPEM, Federal University of Rio de Janeiro, Av. São José do Barreto, 764 -São José do Barreto, Macaé, RJ CEP: 27965-045, Brazil

[3] Department of Biochemical Engineering. School of Chemistry, Federal University of Rio de Janeiro, Av. Horacio Macedo, 2030, Bloco E, Sala E-203, CT, Cidade Universitária - Ilha do Fundao, Rio de Janeiro, RJ CEP: 21949-900, Brazil

crystalline. The majority of commercial PLA is poly (meso-lactide), a mixture of LLA (> 95%) and DLA (< 5%) (Nampoothiri et al. 2010).

Poly (meso-lactide) can be used in a wide range of applications. However, this type of PLA has no stereochemical structure and is highly amorphous. Also, the end products made from this PLA are not suitable for high temperatures applications (Shen et al. 2010). The physicochemical properties of optically active PDLA and PLLA are nearly the same, whereas the racemic PLA has very different characteristics. Properties of PLA biopolymers can be modified by varying the proportions of the enantiomers. For example, the melting temperature of PLLA can be increased to 40–50 °C and its heat deflection temperature can be increased from approximately 60–190 °C by physically blending the polymer with PDLA (Nampoothiri et al. 2010). The crystallization can be decreased with the presence of DLA isomer, resulting in amorphous polymers with controlled degradation, increasing biocompatibility and, therefore, better application in biomedical fields. Hence, the production of PDLA has gained importance and the optical purity of DLA plays a crucial role in determining the characteristics of the PLA biopolymer.

High-purity LLA or DLA monomers are necessary to achieve the desired polymer properties (Li and Cui 2010). Nowadays, most lactic acid is produced by microbial fermentation and many studies have reported optical pure LLA production at industrial scale. On the other hand, production of optically pure DLA is not well established (Cingadi et al. 2015). The fermentative production has the advantage of giving the ability to choose a strain of homofermentative lactic acid bacteria (LAB) that will produce only one optical isomer with high purity. Few wild type strains have been studied for the production of optical pure DLA (Cingadi et al. 2015).

LAB are Gram-positive, non-motile, non-spore forming bacteria, and most of the group are cocci. They are facultative anaerobes, catalase negative, with high acid tolerance and are generally recognized as safe (GRAS). LAB are unable to synthesize ATP by respiration and have complex nutrient requirements due to their limited ability to synthesize B vitamins and amino acids (Hofvendahl and Hahn-Hägerdal, 2000; Gao et al. 2011). The *Lactobacillus* strain needs complex nitrogen sources, vitamins, and minerals (Narayanan et al. 2004; Wee et al. 2006; Li and Cui 2010). Homo-fermentative *Lactobacillus* produces LA as the main product of the glucose metabolism. Through the Embden-Meyerhof-Parnas pathway, 1 mol of hexose is converted to 2 mol of lactic acid and 2 mol of ATP (Hofvendahl and Hahn-Hägerdal 2000; Li and Cui 2010).

Due to the nutritional requirements of *Lactobacillus* strains, the fermentation process may have high costs associated to the raw materials used in the process. The use of cheaper feedstock as carbon source along with higher productivity is a promising strategy to reduce the cost of DLA production. Various studies have reported the use of cheap raw material for DLA production (Tanaka et al. 2006; Nakano et al. 2012; Nguyen et al. 2013; Cingadi et al. 2015). However, few studies have showed the effect of medium components in the production of DLA. Such studies are important to establish a simple medium, with few components and low concentrations that offers a good balance between the nutritional needs of the microorganism and the economic viability of the process.

Response surface methodology (RSM) is a collection of statistical techniques and tools for constructing a functional relationship between a response variable and a set of design variables used in studies of developing, improving, and optimizing processes (Myers et al. 2009). This experimental methodology has been successfully used in optimization studies for different biotechnological processes (Dubey et al. 2011; Zhu et al. 2012; Kongruang and Kangsadan 2015; Dasgupta et al. 2013; Cingadi et al. 2015; Abdelwahed et al. 2017; Almeida et al. 2017, Imran et al. 2017; Ojha and Das 2018). There are different RSM experimental designs, and in this study, a statistical optimization of the producing medium was performed by employing a central composite rotatable design (CCRD) in order to obtain the maximum amount of reliable information with the fewest number of experiments as well as to achieve high production of optically pure DLA using low concentrations of components that influence the DLA production.

Materials and methods

Microorganism and inoculum preparation

Lactobacillus coryniformis subsp. *torquens* (ATCC 25600) was employed in this study, and it was acquired from the Collection of Institute Pasteur (CIP). This strain was selected among four *Lactobacillus* strains after preliminary tests where its homofermentative characteristic for production of DLA with high optical purity was verified by chiral chromatography (unpublished data).

Cells were activated in 100 mL sealed anaerobic bottles containing 50 mL of MRS medium. The anaerobic bottles were inoculated with 1 mL of the stock culture, which was preserved in glycerol and stored at − 80 °C. The inoculum was prepared in anaerobic bottles containing 100 mL of MRS medium and the inoculation volume was 10% of exponentially growing cells from the activation step.

The medium for activation and inoculum preparation contained the follow components: glucose, 10 g/L; peptone, 10 g/L; yeast extract, 5 g/L; meat extract, 1 g/L; ammonium citrate, 2 g/L; sodium acetate, 5 g/L; dipotassium phosphate, 2 g/L; magnesium sulfate, 0.1 g/L; manganese sulfate, 0.05 g/L; and Tween-80, 1 g/L. Glucose was aseptically added to the medium after autoclaving. All culture media were flushed

with nitrogen gas for 15 min to remove oxygen and autoclaved at 121 °C for 15 min. All liquid cultures were incubated in rotary shaker at 37 °C and 120 rpm.

Fermentation assays

Shake flask fermentation were performed for the development of experimental designs. All experiments were carried out in 100 mL anaerobic flasks containing 50 mL of producing medium with different concentrations according to the experimental design. The initial pH was adjusted to 6.8, and glucose was aseptically added to the medium after autoclaving. Anaerobic fermentations were performed at 37 °C and 120 rpm under uncontrolled pH condition for 12 h. All experiments were inoculated with cells at the exponential growing phase using inoculum size 10% (*v*/*v*) with initial cells concentration around 0.13 g/L.

The fermentation media resulted of the shake flask experiments were used for production of DLA at bioreactor scale. Batch fermentation was carried out in a 2 L bioreactor (New Brunswick Bio Flo®) with a working volume of 800 mL and the same conditions from the shake flask experiments. The pH was automatically controlled at 6.8 by the addition of 4 M NaOH and the fermentation time was 46 h considering that controlled pH condition maintained in the bioreactor may allow the reduction of cell inhibition by product formation, and it was possible to development a long-term fermentation with total substrate consumption.

Analytical methods

The concentrations of organic acid and glucose were determined by high-efficiency liquid chromatography (HPLC). Glucose was analyzed using an Aminex HPX-87P column (300 mm × 7.8 mm, 9 μm; Bio-Rad) and a refractive index detector (RI-410, Waters). The mobile phase was Milli-Q water at a flow rate of 1.0 mL/min, and the column was operated at 85 °C. Organic acid were analyzed using a column C18 (250 mm × 4.6 mm, 9 μm; StrodsIIPeek) and UV detector set at 210 nm. The mobile phase was phosphate buffer at a flow rate of 0.9 mL/min, and the column was operated at 50 °C. Enantiomer D(−) lactic acid was identified using a chiral column (150 mm × 4.6 mm; Chirex® 3126) with 1 mM $CuSO_4$ as the mobile phase, flow rate of 0.8 mL/min, temperature 50 °C, and UV detector set at 254 nm.

Experimental design

Response surface methodology was used to study the individual and interactive effects of the significant variables identified through the screening design on DLA production. The variables were studied at four levels of concentration and at the central point using central composite rotatable design (CCRD). The experimental data allowed describe the relationships the DLA production and the medium components by a mathematical model following second-order polynomial equation:

$$Y = \beta_o + \sum\beta_i X_i + \sum\beta_{ii} X_i^2 + \sum\beta_{ij} X_i X_j; \quad i \neq j, i, j = 1, 2, 3, \ldots k \tag{1}$$

where Y is the predicted response (DLA production, g/L), β_o is the interception coefficient, β_i is the linear term, β_{ii} is the quadratic term, β_{ij} is the interaction term, and X_i, X_j represent the independent variables. Experimental data from the CCRD were subjected to analysis of variance (ANOVA). Analysis of the model, calculation of the predicted responses, and the plotting of surface plots were done using Design-Expert® software version 7.0. The final adjustment of the model was done using the Design-Expert 7.0 optimization tool considering as main criteria reduction of component concentrations and maximized DLA production.

Calculation of kinetic parameters

The yield of DLA was calculated as the amount of DLA produced from 1 g of consumed glucose. Productivity was determined as the concentration of DLA per fermentation time. Efficiency was determined as the process yield divided by the theoretical yield, $Y_{p/s\ theoretical}$ is 1.0 g of LA produced per 1.0 g of glucose consumed (Abdel-Rahman et al. 2011).

Results and discussion

Preliminary screening of components

Plackett-Burman design was used for the screening of medium components that influence DLA production. Ten components, such as glucose, peptone, meat extract, yeast extract, ammonium citrate, sodium acetate, magnesium sulfate, manganese sulfate, dipotassium phosphate, and Tween-80 were used in the formulation of the fermentation medium. All factors were set up at two levels (− 1 or + 1), and the central point and 13 different medium conditions were evaluated in 15 trials (data not show). The results of these preliminary experiments showed concentrations of DLA from 6.65 to 13.23 g/L and productivity from 0.30 to 1.10 g/L · h. These results were similar to the data reported by Chauhan et al. (2007) when selecting components for *Lactobacillus* sp. KCP01, they obtained LA concentrations ranging from 5.775 to 16.860 g/L after 48 h of fermentation. The DLA concentration and productivity obtained in the present study were also compared to the results obtained by Slavica et al. (2015) with *L. coryniformis* subsp. *torquens*. These authors reported DLA production with concentration between 10.09 and 14.03 g/L

and productivity between 0.94 and 0.99 g/L · h using the MRS medium. Therefore, the results of the screening experiments were considered satisfactory.

The components were screened at the confidence level of 95%. As shown in the Pareto chart (see Fig. 1), the factors that positively affected DLA production were sodium acetate, meat extract, yeast extract, glucose, and dipotassium phosphate. Ammonium citrate had a negative effect. Manganese sulfate, tween-80, peptone, and magnesium sulfate were considered insignificant. In these experiments, pH was not maintained at a constant value and decreased during the fermentation process due to the growth-associated DLA production (Abdel-Rahman et al. 2011). Therefore, glucose was not completely depleted from the medium and had a moderate effect.

L. coryniformis subsp. *torquens* has complex nutrient requirements due to their limited ability to synthesize B vitamins and amino acids (Abdel-Rahman et al. 2013). Complex nitrogen sources such as meat extract and yeast extract contain vitamins, mineral salts, trace elements (magnesium, manganese, zinc and selenium), B vitamins (B_1, B_2, B_6), and amino acids that can fulfill the microbial nutritional requirements and reduce the production time. Meat extract and yeast extract were considered significant factors and selected as nitrogen sources. In this study, peptone was not found to be a significant factor. This result is different from the results reported by Chauhan et al. (2007) using a similar design for *Lactobacillus* sp. KCP01 and Naveena et al. (2005) for *Lactobacillus amylophilis* GV6. In both studies, peptone had a significant impact on the acid production. Considering the high cost of peptone and the desire to improve the economic parameters of DLA production, the fact that peptone was not significant to the process is a positive result.

Sodium acetate was found to be significant. According to Hertzberger et al. (2013), acetate is used in the formation of acetyl-CoA and enhances cell growth which influences DLA production. Dipotassium phosphate has also been reported as a significant component to the process (Chauhan et al. 2007). This could be due to the buffer effect of this substance. In these experiments, which were conducted without pH control, the buffer effect reduced the metabolism inhibition by the acidification of the medium.

Ammonium citrate had a negative effect on the fermentation process. The citrate ion might have had an inhibitor effect on pyruvate formation at PDH complex. This negative effect was also reported by Chauhan et al. (2007). Magnesium sulfate and manganese sulfate did not show a significant impact in the process and were excluded. Magnesium and manganese act as co-factors and were probably already present in the needed concentrations in the complex nitrogen sources. Tween-80 was also insignificant to the process, according to the results. These five factors were not further studied.

Study of main components using the response surface methodology

The response surface methodology was used to model and optimize the biotechnology process. The variables meat extract, yeast extract, sodium acetate, and dipotassium phosphate were studied using a central composite rotatable design keeping a fixed concentration of glucose. The factors were set up at four levels (− 1, + 1, − 2, + 2) and the central point (see Table 1). Table 2 represents the experimental design, and the results that were obtained. Different medium conditions were evaluated in 30 trials, and the results showed DLA concentrations ranging from 7.06 to 12.29 g/L (see Table 2). The variation of DLA concentration in this study was higher than the one reported by Bustos et al. (2004) using the response surface methodology for evaluate different organic nitrogen sources (corn steep liquor, peptone, and yeast extract) in complex composition medium, which lactic acid ranged from 9.8 to

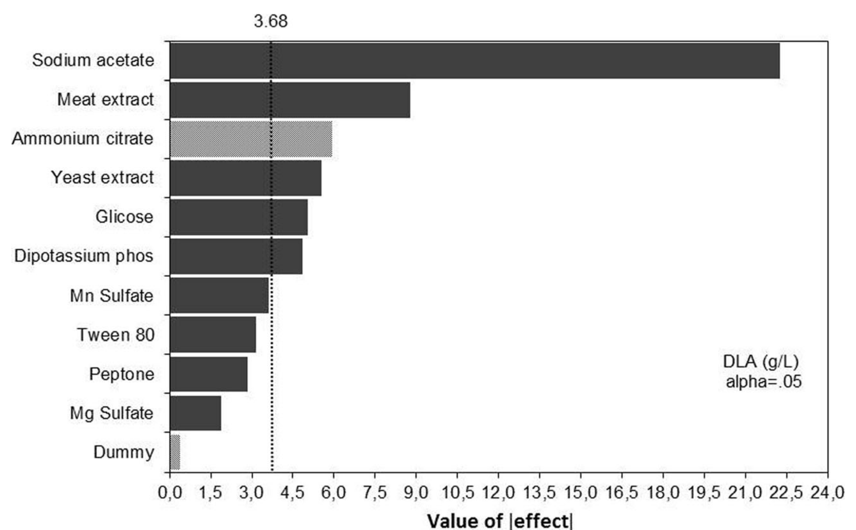

Fig. 1 Pareto chart of standardized effects for ten-factor on DLA production by *L. coryniformis* subsp. *torquens* at 12 h fermentation time. Positive effect ■, negative effect ■ for alpha = 0.05

Table 1 Coded and real values of factors in the central composite rotatable experimental design

Factor	Level of factor				
	−2	−1	0	1	2
Sodium acetate (A, g/L)	0.05	1.70	3.35	5.00	6.65
Meat extract (B, g/L)	0.25	3.50	6.75	10.00	13.25
Dipotassium phosphate (C, g/L)	0.05	0.70	1.35	2.00	2.65
Yeast extract (D, g/L)	0.05	1.70	3.35	5.00	6.65

9.9 g/L after 20 h of shake flask fermentations using calcium carbonate (100 g/L) to neutralize the acid production.

Table 3 shows the analysis of variance (ANOVA) for the model and its statistics. Values of *Prob > Fisher* inferior to 0.0500 indicate that the model terms are significant. The analysis of the coefficients in Table 3 showed that the main factors were meat extract, yeast extract, and sodium acetate. Dipotassium phosphate did not show a significant effect as an individual factor, which could be related to the absence of the buffer effect for such nutrient. On the other hand, dipotassium phosphate was kept in the model because it had a small effect in the interaction with sodium acetate. Contrary to this study, Bustos et al. (2004) reported that yeast extract did not show a significant effect as an individual factor although it presented significant interactions with other two organic nitrogen sources considered the main factors. For this case, yeast extract was of less importance for the metabolic activity, since in the conditions studied by the authors the requirements of B vitamins and proteins were provided by corn steep liquor and peptone, which were more easily assimilable. The authors maintained the three nitrogen sources in the model for fermentation of 20 h, and in this way, we could consider that the characteristics of that medium are more complex than those presented in this study.

Table 2 Central composite rotatable experimental design matrix with experimental values of DLA produced by *L. coryniformis* subsp. *torquens*

Run	A	B	C	D	DLA (g/l)
1	−1.00	−1.00	−1.00	−1.00	8.42 ± 0.08
2	−1.00	−1.00	1.00	1.00	10.45 ± 0.06
3	1.00	−1.00	−1.00	1.00	9.62 ± 0.07
4	0.00	−2.00	0.00	0.00	8.69 ± 0.01
5	−2.00	0.00	0.00	0.00	9.26 ± 0.06
6	0.00	2.00	0.00	0.00	12.29 ± 0.06
7	0.00	0.00	0.00	0.00	9.84 ± 0.121
8	1.00	1.00	1.00	−1.00	10.92 ± 0.03
9	0.00	0.00	0.00	0.00	10.59 ± 0.05
10	0.00	0.00	−2.00	0.00	11.30 ± 0.08
11	−1.00	1.00	1.00	−1.00	10.12 ± 0.07
12	0.00	0.00	2.00	0.00	10.85 ± 0.09
13	0.00	0.00	0.00	0.00	10.99 ± 0.03
14	2.00	0.00	0.00	0.00	11.42 ± 0.07
15	1.00	−1.00	−1.00	−1.00	9.27 ± 0.09
16	0.00	0.00	0.00	0.00	10.82 ± 0.06
17	−1.00	1.00	−1.00	1.00	11.92 ± 0.07
18	−1.00	1.00	1.00	1.00	10.56 ± 0.04
19	1.00	−1.00	1.00	−1.00	9.16 ± 0.03
20	0.00	0.00	0.00	2.00	10.90 ± 0.08
21	1.00	1.00	−1.00	1.00	11.92 ± 0.07
22	0.00	0.00	0.00	−2.00	8.67 ± 0.04
23	−1.00	−1.00	−1.00	1.00	10.35 ± 0.03
24	0.00	0.00	0.00	0.00	10.88 ± 0.06
25	1.00	1.00	−1.00	−1.00	11.19 ± 0.04
26	1.00	−1.00	1.00	1.00	11.42 ± 0.04
27	−1.00	−1.00	1.00	−1.00	7.06 ± 0.06
28	0.00	0.00	0.00	0.00	10.28 ± 0.05
29	−1.00	1.00	−1.00	−1.00	10.34 ± 0.03
30	1.00	1.00	1.00	1.00	12.17 ± 0.07

The regression equation was obtained through the analysis of variance, which gave the response (DLA concentration g/L) as a function of four variables. A second-order polynomial (Eq. 2) was obtained using the terms that were considered significant to the process. The equation in terms of coded factors can be used to make predictions about the response for a given level of each factor. Even though the model coefficients were obtained experimentally, the model can be useful to predict results of untested conditions.

$$\mathrm{DLA}\ (\mathrm{g/L}) = 10.56 + 0.45\mathrm{A} + 0.86\ \mathrm{B} - 0.09\ \mathrm{C} + 0.68\ \mathrm{D} + 0.28\ \mathrm{AC} - 0.21\ \mathrm{D}^2$$

$$(\mathrm{A, sodium\ acetate; B, meat\ extract; C, dipotassium\ phosphate; D, yeast\ extract}) \quad (2)$$

The F-value of 23.11 implies that the model is significant and that the model was accurate in describing the experimental data. The Lack of Fit F-value was 1.49. A non-significant Lack of Fit value means that the model fits. The R^2 coefficient indicates good agreement between experimental and predicted data for a microbiological process and suggests that the model is reliable for depicting DLA production by *L. coryniformis* subsp. *torquens.*

Figure 2 shows the surface plots obtained using the model equation. The main factors were meat extract and yeast extract concentrations (Fig. 2a–c) while sodium acetate and dipotassium phosphate had little effect (Fig. 2b–d). The results showed that increasing concentrations of meat extract and yeast extract resulted in increased DLA concentration. However, high concentrations of these products represent higher production cost. Therefore, in order to make the process economically feasible, a lower concentration of these products must be used without, however, affecting the performance of the microorganism.

Table 3 Analysis of variance (ANOVA) for the selected model

Source	Sum of squares	df	Mean square	F value	P value Prob > F
Model	36.46	6	6.08	23.11	< 0.0001
A–sodium acetate	4.83	1	4.83	18.38	0.0003
B–meat extract	17.66	1	17.66	67.19	< 0.0001
C–dipotassium phosphate	0.18	1	0.18	0.68	0.4184
D–yeast extract	11.19	1	11.19	42.58	< 0.0001
AC	1.27	1	1.27	4.84	0.0382
D^2	1.32	1	1.32	5.02	0.0350
Residual	6.05	23	0.26		
Lack of fit	5.09	18	0.28	1.49	0.3508
Pure error	0.95	5	0.19		< 0.0001
Cor total	42.51	29			0.0003

[R^2 = 0.858; Adj R^2 = 0.821]

From a technical point of view, the highest DLA concentration should be considered the main objective of this optimization. However, the economic aspect must also be considered. In order to optimize the medium (considering

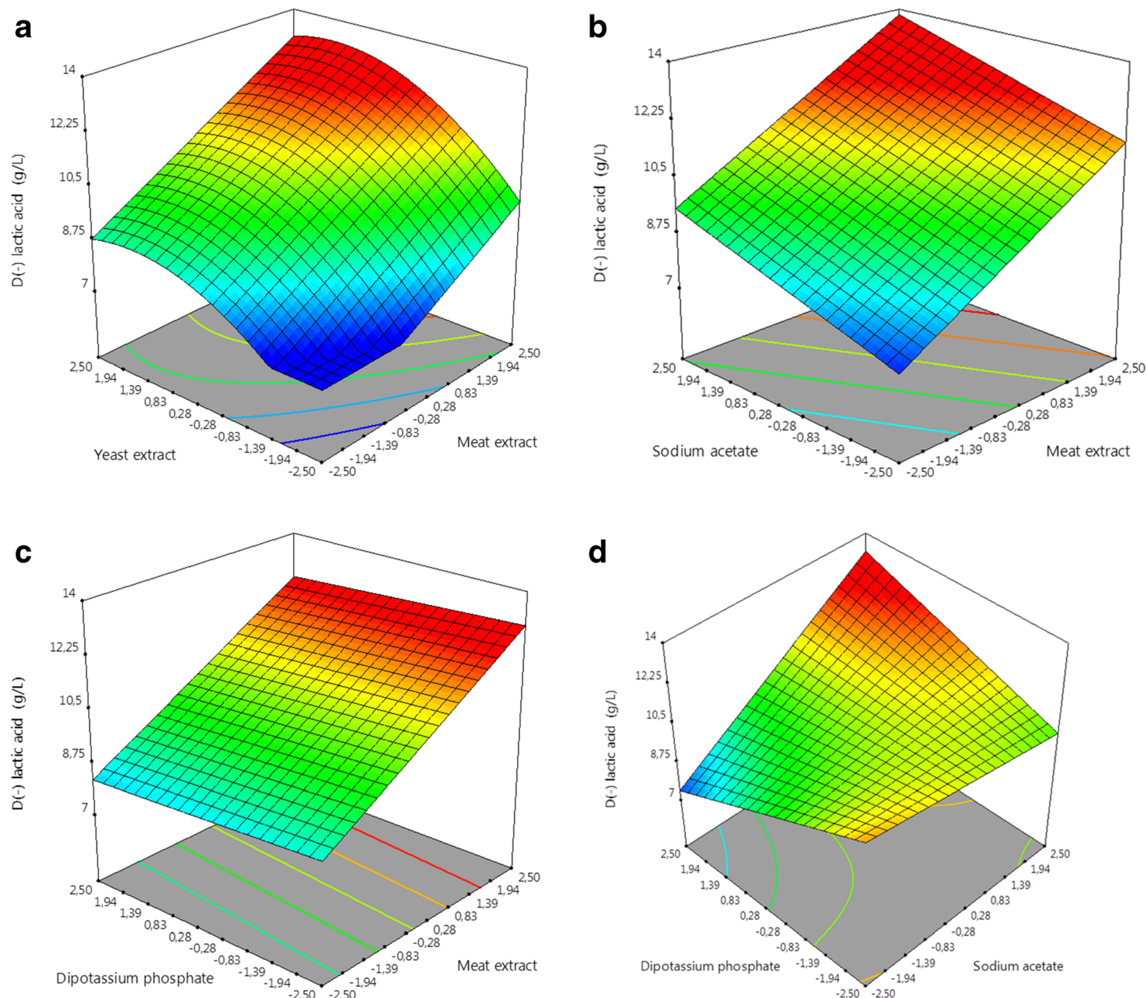

Fig. 2 Surface plots for interactions between the different components of the media that were optimized to increase the DLA production by *L. coryniformis* subsp. *torquens* at 12h fermentation time where (**a**) represents the effect of yeast extract and meat extract, (**b**) sodium acetate and meat extract, (**c**) dipotassium phosphate and meat extract, and (**d**) dipotassium phosphate and sodium acetate. The effect of two variables with the other two variables maintained at their respective zero

Table 4 Medium modified using the Design Expert 7.0 optimization tool for DLA production by *L. coryniformis* subsp. *torquens*

Constraints				
Factor	Goal	Lower limit	Upper limit	Importance
Sodium acetate	Minimize	− 2	2	3
Meat extract	Minimize	− 2	2	5
Dipotassium phosphate	Minimize	− 2	2	3
Yeast extract	Minimize	− 2	2	4
D(−) lactic acid	Maximize	7.06	12.29	5
Solution-optimization				
Factor	Coded values	Real values (g/L)	Desirability	
Sodium acetate	− 2.00	0.05	0.715	
Meat extract	− 1.08	3.23		
Dipotassium phosphate	− 2.00	0.05		
Yeast extract	− 0.43	2.63		
Response	Prediction	SE Pred	95% PI low	95% PI high
D-lactic acid (DLA, g/L)	9.70	0.80	8.04	11.35

both the technical and economic point) two aspects were targeted: increasing DLA concentration and reducing the concentration of components, mainly meat extract. Under these conditions, the concentrations were optimized using the quadratic model of the Design Expert 7.0 optimization tool. The parameters and results were summarized in Table 4. The final modified medium contained 3.23 g/L of meat extract, 2.63 g/L of yeast extract, 0.05 g/L of sodium acetate, and 0.05 g/L of dipotassium phosphate. The differences are the reductions in concentrations of meat extract from 10 to 3.23 g/L, yeast extract from 5.0 to 2.63 g/L, sodium acetate from 5.0 to 0.05 g/L, and dipotassium phosphate from 2.0 to 0.05 g/L. The desirability coefficient of 0.715 was obtained, indicating that approximately 72% of the production and cost reduction requirements were achieved.

In order to validate the model, DLA was produced by *L. coryniformis* subsp. *torquens* using MRS medium and the modified medium under the same conditions of the shake flask experiments. The concentration of DLA using the modified medium was 10.72 g/L with 0.89 g/L.h of productivity. This result corroborates the model prediction (Table 4) and shows the adequacy of the model. The concentration of DLA using MRS medium was 12.69 g/L. This concentration was 1.19-fold higher than the concentration obtained when the modified medium was used (10.72 g/L). This difference is a result of the reduction in the number and concentrations of medium components. From the technical-economic point of view, the modified medium showed a satisfactory result since the decrease in the DLA concentration was only 20%, especially when considering the higher cost of the MRS medium.

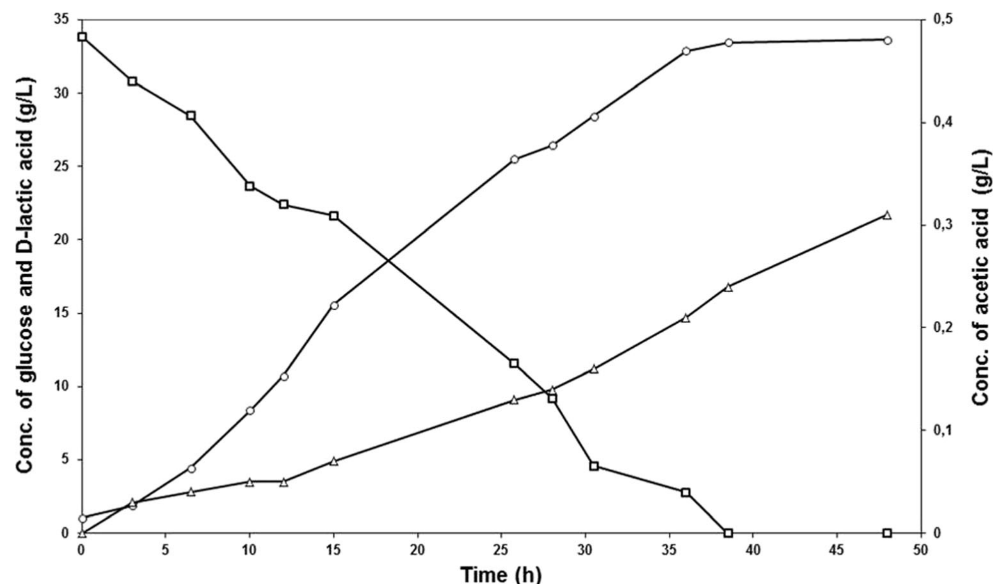

Fig. 3 Profile of glucose consumption (□), DLA production (○) and acetic acid production (Δ) in batch fermentation by *L. coryniformis* subsp. *torquens* using the modified medium at 37 °C, 120 rpm and pH 6.8 controlled with NaOH (4 M)

Bustos et al. (2004) reported a model with higher production using 5 g/L of CSL, 3.6 g/L of yeast extract, and 10 g/L of peptone for LA production by *L. coryniformis* subsp. *torquens*. The authors obtained a concentration of 58.9 g/L and 0.61 g/L.h of productivity using 100 g/L of glucose after 96 h. The productivity reported by these authors are lower than the one obtained in this study, where lower concentrations of expensive nitrogen sources were employed. The DLA concentration and productivity (10.72 g/L and 0.89 g/L) obtained using the modified medium was also comparable to the results obtained by Slavica et al. (2015). These authors obtained 12.95 g/L of DLA and productivity of 0.99 g/L.h by *L. coryniformis* subsp. *torquens* using MRS medium after 13 h of fermentation. Özcelik et al. (2016) found LA concentrations ranging from 0.27 to 0.56 g/L for eight lactic acid bacteria (LAB) strains in MRM medium after 4 days of fermentation at 37 °C. Cingadi et al. (2015) reported that homo-fermentative LAB strains studied for DLA production by batch culture in MRS medium produced between 0.22 and 11.32 g/L by *L. coryniformis* bacteria (NCDC367, NCDC368, NCDC369) produced DLA acid ranging from 9.76 to 11.32 g/L in shake flask experiments.

DLA production in bioreactor

Batch fermentation was carried out in a bioreactor to study the DLA production using the modified medium under controlled operating conditions. Figure 3 shows the profiles of glucose utilization and organic acid production during the fermentation at pH 6.8 and initial glucose of 33.85 g/L. Under controlled pH conditions, glucose was completely consumed by *L. coryniformis* subsp. *torquens* after 38 h—and DLA production was increased due to the reducing of inhibition by acid products formation. DLA with an optical purity of ≥ 99.0% was produced with concentration of 33.6 g/L, yield factor of 0.95 g/g, productivity of 0.88 g/L · h, and 95% of fermentation efficiency. A small quantity of acetic acid was also produced but other products were not identified. The acetic acid production was 0.31 g/L at the end of the fermentation. Acetic acid was still being produced while DLA concentration remained constant in the stationary phase.

These results of yield factor (0.95 g/g) and productivity (0.88 g/L · h) were observed to be high in contrast to DLA production by *Lactobacillus delbrueckii* IFO3202 reported for Tanaka et al. (2006) with an estimated yield factor of 0.9 g/g and productivity of 0.75 g/L · h using the MRS medium and after 36 h from 30 g/L of glucose at pH 6.0. In our present study, yield factor was similar to that obtained by Zhao et al. (2010) who reported conversion of glucose to DLA of 0.94–0.99 g/g for repeated production in one-reactor system at pH 5.6–5.8 by *Sporolactobacillus* sp. CASD. Others studies for production and optimization of lactic acid in batch fermentation have presented lower yield factor values, for example Bernardo et al. (2016) used *Lactobacillus rhamnosus* B103 and lactose (approximately 90 g/L of reducing sugar) as the carbon source and obtained a lactic acid production of 57 g/L and yield factor of 0.63 g/g of reducing sugar after 48 h at pH 6.2 and Wang et al. (2016) reported lactic acid concentration of 36 g/L and yield factor of 0.69 g/g at pH 6.25 by *L. rhamnosus* LA-04-1.

The results of this study showed that the modified medium, which has a lower concentration of nutrients, lead to the total consumption of the glucose by *L. coryniformis* subsp. *torquens*. The DLA production showed no inhibitory effects by nutrient limitation or by-products formation, and a 0.95 yield factor was obtained.

Conclusions

In this study, the preliminary screening showed the significant effect sodium acetate, meat extract, yeast extract, glucose, and dipotassium phosphate had on the DLA production. These components were studied using a central composite rotatable design, and a quadratic model was obtained to describe the relationship between the DLA production and the medium components. The optimization of model employing the Design-Expert 7.0 tool resulted in the modified medium with the following composition: 2.3 g/L of meat extract, 3.58 g/L of yeast extract, 0.05 g/L of sodium acetate, and 0.05 g/L of dipotassium phosphate. The scale-up from flask to bioreactor under controlled pH condition using the modified medium showed the total consumption of glucose by *L. coryniformis* subsp. *torquens* without inhibitory effects, little formation of by-product acetic acid, and yield factor of 0.95 g/g. The modified medium obtained in this work could contribute to the development of processes economically viable for the optically pure DLA production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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