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ORIGINAL PAPER

Optimization of probiotic and lactic acid production by *Lactobacillus plantarum* in submerged bioreactor systems

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Abstract Biomass and lactic acid production by a *Lactobacillus plantarum* strain isolated from Serrano cheese, a microorganism traditionally used in foods and recognized as a potent probiotic, was optimized. Optimization procedures were carried out in submerged batch bioreactors using cheese whey as the main carbon source. Sequential experimental Plackett–Burman designs followed by central composite design (CCD) were used to assess the influence of temperature, pH, stirring, aeration rate, and concentrations of lactose, peptone, and yeast extract on biomass and lactic acid production. Results showed that temperature, pH, aeration rate, lactose, and peptone were the most influential variables for biomass formation. Under optimized conditions, the CCD for temperature and aeration rate showed that the model predicted maximal biomass production of 14.30 g l^{-1} (dw) of *L. plantarum*. At the central point of the CCD, a biomass of 10.2 g l^{-1} (dw), with conversion rates of 0.10 g of cell g^{-1} lactose and 1.08 g lactic acid g^{-1} lactose (w/w), was obtained. These results provide useful information about the optimal cultivation conditions for growing *L. plantarum* in batch bioreactors in order to boost biomass to be used as industrial probiotic and to obtain high yields of conversion of lactose to lactic acid.

Keywords *Lactobacillus plantarum* · Lactic acid production · Probiotic production · Plackett–Burman design · CCD experimental design

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Introduction

Probiotics are defined as active microorganisms that present health benefits for the host, whether humans or animals, by improving the properties of indigenous microflora when consumed in adequate amounts [19, 28]. Probiotics, among other characteristics, should have technological properties to allow their production on large scale and their incorporation into food products without losing viability and functionality, but without creating unpleasant flavors or textures [3]. Therefore, extensive research on metabolic kinetics of different lactobacilli is still necessary, especially for improvement of biomass production by fermentation.

Lactobacillus and *Bifidobacterium* are the main genera used as probiotics in commercial products. Lactobacilli have been in use in the food industry for a long time and are characterized by the production of lactic acid [3]. They comprise fastidious-growing bacteria with numerous requirements for growth. Therefore, lactobacilli need rich media containing expensive compounds such as amino acids, peptides, vitamins, and nucleic acids [1]. One of the most widespread *Lactobacillus* species used in food technologies is *L. plantarum*, which has a homofermentative metabolism and high acid tolerance, and is a

generally regarded as safe (GRAS) organism. Many *L. plantarum* strains are presently marketed as probiotics [7]. High acid tolerance is an important feature that prevents contamination during the fermentation process. *L. plantarum* is one of the best lactic acid producers [10, 18]. Lactic acid is a valuable industrial chemical used as an acidulant and preservative in food industries, as well as in other applications in the pharmaceutical industry. Recently, many companies have expressed interest in producing lactic acid for production of biodegradable plastics [10, 32].

In recent years, there has been growing interest in the use of *L. plantarum* or its products for many applications. Production of *L. plantarum* biomass and its metabolites is greatly dependent on the composition of the growth medium and culture conditions. For large-scale commercial applications, alternative media with low cost should be used. Cheese whey appears as an alternative medium to reduce costs of biomass production. Approximately 85% of the total milk used for manufacturing cheese is discarded as whey, which may contain as much as 55% of total milk nutrients [22]. The most abundant of these nutrients are lactose, soluble proteins, lipids, and mineral salts [22, 30]. Although mainly used as a food component, whey waste is the source of great economical losses for the dairy industry, since almost 50% of the total worldwide production is disposed of in wastewater treatment plants or on farm fields [25]. This organic waste could be used as a cheap, readily available substrate for microbial cell cultivation.

Experimental designs such as the Plackett–Burman (PB) and factorial designs are good methods for screening and optimization of media compositions and culture conditions in fermentation processes through a minimal number of experiments [12, 14, 33]. Concerning *L. plantarum*, only a few studies have reported on the production of biomass [2, 10, 13], while some works have focused on metabolic components such as lactic acid [10, 15], enzyme [21], bacteriocin [8, 17, 31], and exopolysaccharides [9]. The biomass production of *Lactobacillus* sp. reported in the literature, in works both with and without optimization tools, have shown low yields in batch bioreactors. The need to obtain high densities of cells is, therefore, a concern for research groups. In this work, the biomass and lactic acid production of a new strain of *L. plantarum*, isolated from Serrano cheese, was optimized in submerged bioreactors using cheese whey as medium. First, culture conditions and nutrients that could have the most influence on biomass production were investigated using PB methodology. Experimental factorial design was then used in order to optimize the process through the combination of the effects of temperature and aeration rate in bioreactors.

Materials and methods

Microorganism

A strain of *L. plantarum*, isolated from Serrano cheese [6], was used in this study. Stock cultures were maintained in frozen suspension in 20% glycerol.

Inocula preparation

Erlenmeyer flasks (1,000 ml) containing 200 ml MRS broth [5] were inoculated with 1.5 ml glycerol stock culture and incubated at 37°C in a rotatory shaker at 180 rpm and grown to optical density (OD) 1.0 at 600 nm. The cells were harvested by centrifugation at 5,000 × *g* for 10 min at 4°C. The cell pellet was washed and resuspended directly into the cultivation broth (200 ml), the composition of which was varied accordingly to the experimental design described in this work. This procedure was used as the standard inoculum preparation for all experiments.

Cultivation procedures

In the investigation of which variables had a significant effect on biomass production, cultivations were carried out with medium containing (g l^{-1}): $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.04. The other components of the medium were cheese whey powder, peptone, and raw yeast extracts (autolyzed unpurified yeast extract; Prodesa, Brazil), the concentrations of which were the independent variables in the statistical design (Table 1). To avoid protein precipitation during sterilization (121°C, 15 min), cheese whey proteins were hydrolyzed with a commercial protease (Alcalase 2.4 L FG, Novozymes, Brazil). Hydrolysis was performed using 1 ml enzyme over 140 g l^{-1} whey powder, for 1 h at 50°C. With this procedure, whey proteins remained soluble in the medium. In order to avoid the Maillard reaction, peptone and yeast extract were autoclaved separately from cheese whey and added after sterilization into the bioreactor.

An amount of 200 ml standard inoculum was transferred to a bioreactor filled with 1,800 ml medium composition according to the specific assay. These experiments were performed using a 2,000-ml Biostat B bioreactor (B. Braun Biotech International, Germany). pH was controlled by automatic addition of 10.0 M NaOH and 2.0 M H_3PO_4. Dissolved oxygen concentration of cultures was measured using a polarographic O_2 probe (Mettler-Toledo, Germany). Volumetric oxygen mass transfer rate (k_la) was calculated by the dynamic gassing out method [27]. Temperature, aeration rate, pH, and stirred agitation were set up according to the PB design. For the central composite design (CCD), temperature and aeration rate were the independent variables, and pH and stirred agitation were set up according to

Table 1 Plackett–Burman experimental design matrix for biomass production of *L. plantarum*

Trial no.	Random order	Variables[a]/levels[b]											Biomass in 48 h (g l^{-1})	$Y^{c}_{P/S}$
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	D_1	D_2	D_3	D_4		
1	6	+	−	+	−	−	−	+	+	+	−	+	5.00	1.26
2	1	+	+	−	+	−	−	−	+	+	+	−	3.43	1.26
3	10	−	+	+	−	+	−	−	−	+	+	+	8.47	0.68
4	13	+	−	+	+	−	+	−	−	−	+	+	4.21	1.07
5	15	+	+	−	+	+	−	+	−	−	−	+	1.53	–
6	2	+	+	+	−	+	+	−	+	−	−	−	3.05	1.50
7	9	−	+	+	+	−	+	+	−	+	−	−	10.98	0.99
8	5	−	−	+	+	+	−	+	+	−	+	−	14.97	1.04
9	12	−	−	−	+	+	+	−	+	+	−	+	8.61	1.29
10	3	+	−	−	−	+	+	+	−	+	+	−	2.98	1.23
11	8	−	+	−	−	−	+	+	+	−	+	+	7.01	1.03
12	14	−	−	−	−	−	−	−	−	−	−	−	7.57	0.96
13	4	0	0	0	0	0	0	0	0	0	0	0	9.52	0.80
14	11	0	0	0	0	0	0	0	0	0	0	0	8.20	1.31
15	7	0	0	0	0	0	0	0	0	0	0	0	9.94	1.11

[a] X_1 temperature at highest level of 42°C, central level of 38°C, and lowest level of 34°C; X_2 pH at highest level of 6.4, central level of 5.8, and lowest level of 5.2; X_3 lactose at highest concentration of 140.0 g l^{-1}, central concentration of 110.0 g l^{-1}, and lowest concentration of 80.0 g l^{-1}; X_4 peptone at highest concentration of 25.0 g l^{-1}, central concentration of 15.0 g l^{-1}, and lowest concentration of 5.0 g l^{-1}; X_5 yeast extract at highest concentration of 15.0 g l^{-1}, central concentration of 10.0 g l^{-1}, and lowest concentration of 5.0 g l^{-1}; X_6 stirred agitation at highest level of 400 rpm, central level of 300 rpm, and lowest level of 200 rpm; X_7 aeration rate at highest level of 2 vvm, central level of 1 vvm, and lowest level of 0 vvm; D_1, D_2, D_3, and D_4 are dummy variables

[b] (+) Highest concentration of variable; (−) lower concentration of variable; (0) central level of variable

[c] $Y_{P/S}$ yields, as lactose conversion to lactic acid

the results of the PB experiments; the medium contained (g l^{-1}): $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.04; peptone, 15; yeast extract, 5; lactose, 140.

Experimental design

Plackett–Burman statistical design

To determine what nutrients and conditions had a significant effect on *L. plantarum* biomass production, a PB design was used [24]. Seven variables and four dummy variables were screened in 15 trials, with triplicates at the central point. The minimal and maximal ranges selected for the seven parameters are presented in Table 1, in which each column represents an independent variable and each row represents a trial. Variables with confidence levels >90% were considered to have significant influence on biomass production.

Central composite design

The optimization of key culture conditions in order to maximize biomass and lactic acid production was determined by applying the central composite design methodology. The variables and the coded and uncoded values of the variables at various levels are given in Table 2, which shows 11 trials of the two variables, each at five levels. The design was a central composite design ($k = 2$) with triplicates at the central point (all factors at level 0) and the four axial points, which have, for one factor, an axial distance to the centre of $\pm\alpha$, whereas the other factor is at level 0. The axial distance α was chosen to be 1.41 to make this design orthogonal. In each case, the productions of biomass and lactic acid (measured as yields, $Y_{P/S}$ g acid g lactose^{-1}) were determined. The quadratic equation for the variables was as follows:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2, \quad (1)$$

where Y is the response variable, β_0 the constant, β_i the coefficient for the linear effect, β_{ii} the coefficient for the quadratic effect, β_{ij} the coefficient for the interaction effect, and x_i and x_j the coded levels of variables X_i and X_j. The above quadratic equation was used to plot surfaces for the variables.

The test variables were coded according to the following regression equation:

Table 2 Experimental design and results of CCD

Trial no.	Actual levels [X_1 = T (°C); X_2 = aeration rate (vvm)]		Variables/levels		Biomass in 48 h (g l^{-1})	$Y_{P/S}$
	X_1	X_2	x_1	x_2		
1	31	2.5	−1	−1	11.55	0.90
2	31	4.5	−1	+1	13.05	0.88
3	37	2.5	+1	−1	9.12	0.81
4	37	4.5	+1	+1	9.65	1.08
5	30	3.5	−1.41	0	12.27	0.97
6	38	3.5	1.41	0	8.93	1.02
7	34	2	0	−1.41	10.35	1.00
8	34	5	0	1.41	11.33	0.97
9	34	3.5	0	0	10.77	0.93
10	34	3.5	0	0	11.87	0.96
11	34	3.5	0	0	11.19	1.00

Table 3 Effect estimates for biomass production from the result of PB design

Variables	Parameters	Effect	*P*-value
X_1	Temperature*	−6.74	0.0060
X_2	pH*	−1.99	0.0632
X_3	Lactose concentration*	2.08	0.0580
X_4	Peptone concentration¯	2.12	0.0563
X_5	Yeast extract concentration	−0.27	0.6550
X_6	Stirring	−1.20	0.1500
X_7	Aeration rate*	1.70	0.0830

Standard error = 0.52; *P*-values $\leq$0.10; R^2: 0.89

* Statistically significant at 90% confidence level

$$x_i = \left(\frac{X_i - X_0}{\Delta X_i}\right), \quad (2)$$

where x_i is the coded value, X_i is the actual value of the independent variable, X_0 is the actual value at the center point, and ΔX_i is the step change value.

Method of steepest ascent

The method of maximal ascending path (steepest ascent) is a procedure for moving sequentially along the path of ascent in the direction of maximal increase in response [20]. In this work, this method was used to check whether we were prospecting the best range of temperature and aeration rates. To achieve this, we divided the linear coefficient value of temperature by the linear coefficient value of aeration rate, both obtained from the CCD, in order to design a series of five experiments starting from the CCD central point. Table 3 presents the maximal ascending path experimental design for five trials, with their respective values of temperature and aeration rate.

Analytical methods

Samples of 10 ml culture broth were centrifuged at 3,500 × *g* for 20 min at 4°C. The cell-free supernatant was used for the estimation of lactose and lactic acid concentrations.

Biomass was quantified gravimetrically as dry weight of cells. Samples were centrifuged, twice washed with cold distilled water, and dried in preweighed plastic tubes at 80°C to constant weight in vacuum ovens. Lactose concentration was determined by dinitrosalicylic method (DNS) using lactose as standard [4]. Lactic acid concentrations were determined by high-performance liquid chromatography (HPLC; Shimadzu LC-10A, Japan) equipped with reverse-phase column Supelcosil C18, 5 μm, 250 mm × 4.6 mm (Supelco, USA) and detected at 210 nm (detector UV–VIS SPD-10A Shimadzu, Japan). A 0.01 M solution of H_2SO_4 was used as eluent at flow rate of 1 ml min^{-1}.

Data analysis

All experimental designs and results analyses were carried out using Statistica 7.0 (Statsoft, Tulsa, USA). Statistical verification of the model was performed by analysis of variance (ANOVA). Significance of the regression coefficients and the associated probabilities, *P*(*t*), were determined by Student's *t*-test; the second-order model equation significance was determined by Fisher's *F*-test. The variance explained by the model is given by the multiple determination coefficients, R^2.

Results and discussion

Plackett–Burman experimental design

Plackett–Burman design was used to evaluate the effects of concentrations of lactose, peptone, and yeast extract, temperature, pH, stirred agitation, and aeration rate on *L. plantarum* biomass production. Table 1 represents the PB experimental design for 15 trials with two study levels for each variable and the corresponding biomass production in 48 h of bioreactor cultivation. Biomass production varied markedly in a range between 0 and 14.97 g l^{-1} for the different trials, which reflected the importance of optimization to obtain higher production. Temperature seems to be the most important variable. There was low biomass production at higher levels of temperature, with maximal biomass value of 5.00 g l^{-1}. However, in all trials with the temperature at the lower level, the minimal value of biomass was 7.01 g l^{-1}, 40% higher than the maximal value obtained with the high level of temperature. The biomass production was much higher than that obtained by Kask et al. [15] on A-stat cultures of *L. plantarum*, where maximal cell density was 2.5 g l^{-1} (dw). Fu and Mathews [10] reported biomass production of 11 g l^{-1} of *L. plantarum* in experiments conducted with pH control varying from 5 to 6. Lactose conversion was high, with high lactic acid production, even in those runs with high biomass production (Table 1). The maximal theoretical conversion yield of lactose to lactic acid is 1.05. In this work, $Y_{P/S}$ of 1.50 was obtained, showing that *L. plantarum* can not only efficiently convert lactose from whey, but can also use other culture ingredients such as peptone and yeast extract for its growth and acid production [10]. This observation indicates the great potential of *L. plantarum* as a highly productive strain for lactic acid production from whey when fermentation to biomass production is performed.

Table 3 presents the statistical analysis of the studied variables for biomass production. The temperature (X_1), pH (X_2), lactose concentration (X_3), peptone concentration (X_4), and aeration rate (X_7) were found to be significant at the 90% level for biomass production. Temperature and pH negatively influenced biomass production. The maximal viable cell count of *L. plantarum* was obtained with MSE-extra medium in bioreactor fermentation when pH was controlled at 5.0 [16], which is near the lowest level used in the PB design (5.2).

Lactose, peptone concentration, and aeration rate all showed positive influence on biomass production. Whey and peptone are complex ingredients which contain several essential amino acids and important minerals and vitamins [22, 26, 30]. Bevilacqua et al. [2] have shown that biomass production increased in a nonlinear way with increasing lactose up to a threshold value (20 g l^{-1}) and for pH (6.0), but found that the effect of the interaction of pH and lactose in the model on biomass was small. The positive effect of aeration on biomass production was also observed by other researchers. Fu and Mathews [10] have shown that cell yields in anaerobiosis were about 80% of those obtained under aerobic cultivation. Stevens et al. [29] obtained approximately 25% higher cell densities in aerobiosis when compared with anaerobic cultures. A similar influence of aeration on biomass was observed by Pintado et al. [23], who obtained higher cell densities under aerobiosis compared with anaerobic culture when different polysaccharides were used to supplement a basal culture medium. *L. plantarum* is a facultative bacterium and can use oxygen as an electron acceptor for cell growth and product metabolism [7, 10, 23]. The positive effect of aeration rate on biomass production can be attributed to differences in metabolic pathways under aerobic and anaerobic conditions; in particular, the generation of additional adenosine triphosphate (ATP) benefits cell growth in the presence of oxygen [1, 10, 23].

Lactose concentration was set at the highest value of the PB experiments (140.0 g l^{-1}) for the CCD experiments. In spite of the effect of peptone concentration, as indicated by its highest level from the PB results, in the CCD experiments its concentration was chosen at the central level because of its high cost. The lower level of pH (5.2) was selected as the set point for the CCD experiments. When pH is low, cells of *Lactobacillus* remained resistant, and it avoids possible contamination [10]. Yeast extract concentration and stirred agitation, which showed no significant influence on cell production, were set at 5 g l^{-1} and 200 rpm, respectively. Laitila et al. [16] also observed that increased concentration of yeast extract from 4 to 8 g l^{-1} did not improve cell growth.

Central composite designs (CCD)

Based on the results of the PB design, for the next step of optimization the influences of temperature and aeration rate were tested using the CCD methodology. Temperature was varied in the range between 30°C and 38°C, while aeration rate was tested between 2 and 5 vvm. Table 2 presents the experimental design with the obtained results. The biomass production varied from 8.93 to 13.06 g l^{-1} according to the different levels of the tested variables. The highest productions of biomass, 13.06 and 12.27 g l^{-1}, were obtained at low temperature levels (30°C and 31°C), while the lowest productions of biomass, 8.93, 9.12, and 9.65 g l^{-1}, were obtained with temperature at high levels (37°C and 38°C), showing the strong negative influence of this variable on the process. The biomass concentrations obtained in this research are the highest reported in the literature [10, 11, 13].

Table 4 Coefficient estimates by the regression model in CCD

Independent variables (parameter)	Effect	Coefficient (β)	Standard error	*t*-Value	*P*-value
Intercept	11.28	11.28	0.32	35.14	0.0008
X_1*	−2.64	−1.32	0.20	−6.72	0.0214*
X_1X_1	−0.62	−0.31	0.23	−1.32	0.3172
X_7	0.86	0.43	0.20	2.18	0.1615
X_7X_7	−0.38	−0.19	0.24	−0.80	0.5062
X_1X_7	−0.49	−0.24	0.28	−0.89	0.4730

* Statistically significant at 95% confidence level

Table 4 shows the significance of coefficients determined by Student's *t*-test and *P*-values. The first-order temperature coefficient of the model was the only significant variable (95% confidence level). This effect was negative, indicating that the biomass decreases inversely with temperature. No work evaluating the influence of temperature and aeration rate on biomass production of *L. plantarum* could be found in the literature, confirming the importance of this study. Leal-Sánchez et al. [17], when optimizing bacteriocin production by *L. plantarum* LPCO10, obtained the following best conditions: NaCl concentration of 2.5%, temperature ranging from 22°C to 27°C, and inoculum size of $10^{7.4}$ colony-forming units (CFU) ml^{-1}.

In spite of the fact that only the first-order temperature coefficient was significant, ANOVA analysis showed that the model using all coefficients was very reliable, with R^2 of 0.95, meaning that 95% of the total variation is explained by the model. This suggests a satisfactory representation of the process model and a good correlation between the experimental and predicted values. We therefore propose the following second-order polynomial equation:

$$Y = 11.27833 - 1.32069x_1 - 0.30919x_1^2 + 0.42769x_7 - 0.18784x_7^2 - 0.24375x_1x_7, \quad (3)$$

where Y is the predicted response, and x_1 and x_7 are the coded values of temperature and aeration rate, respectively. The computed *F*-value (19.02) was greater than the tabulated *F*-value (3.14), reflecting the statistical significance of the model equation. This shows that the model, as expressed in Eq. 3, is suitable to describe the response of *L. plantarum* biomass production.

Figure 1 shows the planned series as a contour plot generated from the predicted model presented in Eq. 3. The predicted model indicates for maximal biomass production (14.30 g l^{-1}) a point outside the studied region ($x_{1c} = 3.47255$, $x_{7c} = 3.39147$). This response could indicate that the strong negative effect of temperature presented by the PB shows that the temperature could be even lower than that used in CCD, the same happening with the aeration rates used in CCD. For this reason, we used the method of steepest ascent [20], which allows searching for a better interval of study. Table 5 represents the maximal ascendant path experimental design. The maximum attained with this design was very close to that obtained by the CCD. As can be seen, no statistically different results were obtained compared with the central point of the CCD. This indicates the importance of proper evaluation of experimental design applied to biological processes in order to avoid planning unsound experiments. The model was validated at the CCD central point conditions. Figure 2 presents the biomass and lactic acid productions, lactose consumption, and pO_2 evolution. The biomass production was 10.2 g l^{-1}, very close to the predicted value of 11.3 g l^{-1} at the central point. This value of biomass was similar to [10] or higher [11, 13] those reported in the literature for other tested culture conditions and variables. The cell yield coefficient and the product yield coefficient, measured at 48 h of cultivation, were 0.10 g of cell g^{-1} lactose and 1.08 g lactic

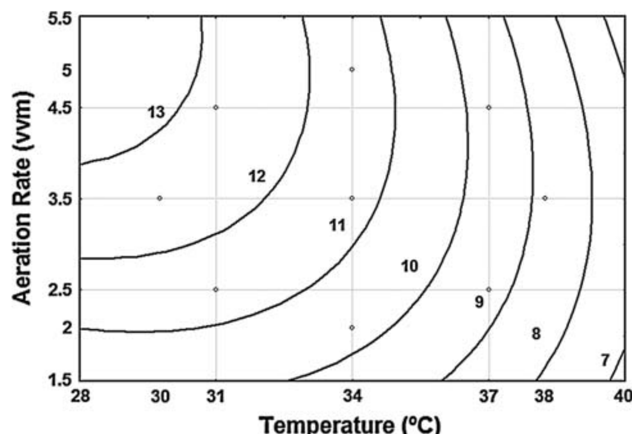

Fig. 1 Contour plot of biomass production of *Lactobacillus plantarum*. The *numbers* inside the contour plots indicate biomass concentration (g l^{-1})

Table 5 Maximal ascendant path experimental design

Trial no.	Temperature (°C)	Aeration rate (vvm)	Biomass in 48 h (g l^{-1})
1	34	3.5	11.75 ± 0.07[a]
2	31	4.5	9.83 ± 0.85[a,b]
3	28	5.5	12.41 ± 0.25[a,c]
4	25	6.5	10.80 ± 0.30[a]
5	22	7.5	11.71 ± 0.05[a]
Central point CCD	34	3.5	11.26 ± 0.56[a]

[a, b, c] The same letters indicate no statistical difference at 95% confidence level

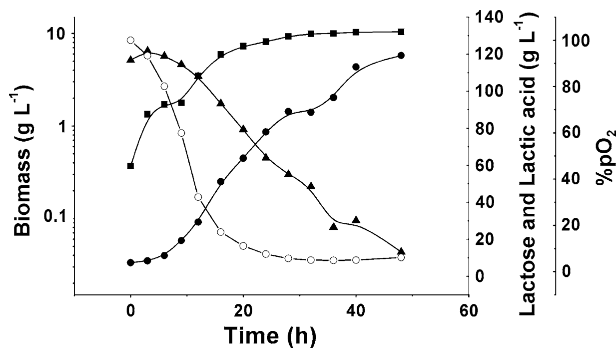

Fig. 2 Time course of batch fermentations of *Lactobacillus plantarum* on whey medium containing (g l^{-1}): $MgSO_4{\cdot}7H_2O$, 0.2; $MnSO_4{\cdot}H_2O$, 0.04; peptone, 15; yeast extract, 5; lactose, 140. Culture conditions: 34°C; 3.5 vvm; 200 rpm, pH 5.2. *FIlled square* dry cell weight; *open circle* % pO_2; *Filled circle* lactic acid concentration; *filled triagle* lactose concentration

acid g^{-1} lactose (w/w), respectively. The lactic acid yield coefficient was higher than the theoretical value of 1.05 which is obtained considering lactose as the only carbon source in lactic acid production, indicating that the use of cheese whey (a complex ingredient) supplemented with raw peptone and yeast extract is an extremely interesting culture medium, not only for biomass production but also to boost lactic acid formation. The volumetric oxygen transfer rate, k_la, measured for these optimal culture conditions, was 16.3 h^{-1} at mid-exponential growth phase, indicating that, in future work, this aeration condition can be kept while testing other variables.

Conclusions

Plackett–Burman design provided an efficient and rapid method for screening and selecting culture components and conditions with a minimal number of experiments. This method showed that the variables that would have more influence on the production of biomass and lactic acid by *L. plantarum* were temperature, pH, lactose and peptone concentrations, and aeration rate. Temperature and aeration rate tested in a CCD produced a model to optimize the biomass production of *L. plantarum*. The validation of this model at its central point showed that the obtained conditions would allow for the highest biomass production so far reported for batch cultures of this microorganism, which is around 14 g l^{-1} at maximal conditions. The combination of optimization and the use of very cheap medium formulation, as presented in the results of this research, might help in reducing the costs of production of biomass of *L. plantarum*, an important microorganism recognized as a probiotic, as well as for the production of lactic acid, its main commercial metabolite.

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