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Optimal fermentation conditions for maximizing the ethanol production by *Kluyveromyces fragilis* from cheese whey powder

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ABSTRACT

Cheese whey powder (CWP) is an attractive raw material for ethanol production since it is a dried and concentrated form of CW and contains lactose in addition to nitrogen, phosphate and other essential nutrients. In the present work, deproteinized CWP was utilized as fermentation medium for ethanol production by *Kluyveromyces fragilis*. The individual and combined effects of initial lactose concentration ($50–150$ kg m^{-3}), temperature ($25–35$ °C) and inoculum concentration ($1–3$ kg m^{-3}) were investigated through a 2^3 full-factorial central composite design, and the optimal conditions for maximizing the ethanol production were determined. According to the statistical analysis, in the studied range of values, only the initial lactose concentration had a significant effect on ethanol production, resulting in higher product formation as the initial substrate concentration was increased. Assays with initial lactose concentration varying from 150 to 250 kg m^{-3} were thus performed and revealed that the use of 200 kg m^{-3} initial lactose concentration, inoculum concentration of 1 kg m^{-3} and temperature of 35 °C were the best conditions for maximizing the ethanol production from CWP solution. Under these conditions, 80.95 kg m^{-3} of ethanol was obtained after 44 h of fermentation.

1. Introduction

The dairy industry represents an important part of the food processing industry and contributes significant liquid process residues that can be used for the production of ethanol [1]. Cheese whey (CW), a by-product of the cheese manufacturing process whose major components are lactose ($45–50$ kg m^{-3}), proteins ($6–8$ kg m^{-3}), lipids ($4–5$ kg m^{-3}), and mineral salts (8–10% of dried extract), constitutes an inexpensive and nutritionally rich raw material for the production of different compounds [2,3]. Ethanol production by bioconversion of whey is an alternative of great interest for reuse of this industrial by-product [4]. In addition, the development of ethanol production methods is stimulated by the possibility of using ethanol as a component in biofuels [5,6]. However, the production of ethanol from non-concentrated CW is not economically feasible because the levels of ethanol obtained at the end of fermentation reach only about $20–30$ dm^3 m^{-3}. Distillation costs for ethanol separation from dilute fermentation broths ($20–30$ dm^3 m^{-3} EtOH) is a major cost item in ethanol fermentation of CW [2]. Ultrafiltration (UF) processes have been used to concentrate lactose in CW before

fermentation [7]. UF improves the lactose concentration by a factor of 5–6, but is expensive (approx. 50 $ m^{-3} original dilute CW) [8].

Dry cheese whey powder (CWP) may be an attractive raw material for ethanol production. CWP is a dried and concentrated form of CW and contains lactose in addition to nitrogen, phosphate and other essential nutrients [9]. Utilization of CWP instead of CW for ethanol fermentations has significant advantages such as elimination of costly ultrafiltration processes to concentrate lactose before fermentation, compact volume, long-term stability and high concentrations of lactose and other nutrients yielding high ethanol concentrations by fermentation. Moreover, the cost of CWP production from CW by spray or drum drying varies between 0.2 and 0.4 $ per kg CWP (10–20 $ m^{-3} original dilute CW), which is much lower than distillation costs for pure ethanol production from dilute CW [8,10].

It is known that the fermentation process performance is affected by operational conditions such as temperature, stirring rate, initial inoculum and substrate concentrations, dissolved oxygen, among others. A suitable control of these variables is of great importance for a good process performance and obtainment of high-quality products. The present study aimed to optimize the conditions for ethanol production from CWP through RSM designed with central composite design. Three factors were selected as process (independent) variables: initial lactose concentration, temperature and inoculum concentration; while the ethanol concentration, substrate consumption and fermentative parameters (ethanol yield factor, $Y_{P/S}$; ethanol volumetric productivity, Q_P; ethanol yield per cell, $Y_{P/x}$; and bioconversion efficiency, η) were selected as responses (dependent) variables.

2. Materials and methods

2.1. Microorganism and inoculum preparation

Kluyveromyces fragilis (Kf1) from the culture collection of the Centre of Biological Engineering, University of Minho (Portugal), was the yeast strain employed in the experiments. This strain was supplied by University of Lavras (Department of Biology), Brazil, and was isolated from cocoa fermentation. Cells of this yeast were maintained at 4 °C on YPD agar plates. The inoculum was prepared by transferring a loopful of cells from a freshly grown culture (incubated at 30 °C for 30 h) to 500 ml Erlenmeyer flasks containing 100 ml sterile CWP solution (50 kg m^{-3} lactose). The flasks were incubated on a rotary shaker at 3.3 Hz, 30 °C for 24 h. After this time, the cells were recovered by centrifugation (4200 g, 15 min), washed with sterilized distilled water, and directly resuspended in the fermentation medium.

2.2. Medium and fermentation conditions

Cheese whey powder (CWP) was kindly supplied by Lactogal (Porto, Portugal). CWP composition (sample of January, 2008) included (w w^{-1}): >73% lactose, 12% proteins, 1.5% lipids and <5% moisture. To be used as fermentation medium, CWP solutions with different initial lactose concentrations were prepared, pH-adjusted to 5 by addition of 1 kmol m^{-3} citric acid, and deproteinized by heat treatment at 115 °C for 15 min.

The precipitates were removed by centrifugation at 5600 g and 10 °C for 15 min, and the supernatants were used as fermentation medium.

Batch fermentations were performed in 500 ml Erlenmeyer flasks containing 100 ml of medium. Flasks were maintained in an orbital shaker at 2.5 Hz for 44 h. Different values of initial lactose concentration, temperature and inoculum concentration were used in the experiments (Table 1).

In the second step, the assays for determination of the best initial lactose concentration (varying from 150 to 250 kg m^{-3}) were also performed in Erlenmeyer flasks as above described. However, in this case the temperature and initial inoculum concentration were fixed at 35 °C and 1 kg m^{-3}, respectively.

The fermentation runs were monitored through periodic sampling in order to determine the cell growth, lactose consumption and ethanol production. All the experiments were performed in duplicate and mean values are given. The kinetic parameters of fermentations were calculated at the end of the runs. The ethanol yield factor ($Y_{P/S}$, kg kg^{-1}) was defined as the ratio between the ethanol concentration (kg m^{-3}) and lactose consumed (kg m^{-3}). The ethanol yield per cell ($Y_{P/x}$, kg kg^{-1}) was defined as the ratio between ethanol and total cell concentrations (kg m^{-3}). The ethanol productivity (Q_P, kg m^{-3} h^{-1}) was defined as the ratio between ethanol concentration (kg m^{-3}) and fermentation time (h). The efficiency of ethanol production (η, %) was defined as the ratio between the ethanol concentration (kg m^{-3}) and the maximum theoretical ethanol concentration (kg m^{-3}) that could be achieved considering the theoretical value of 0.538 kg ethanol per kg consumed lactose [11].

2.3. Analytical methods

The fermented media samples were centrifuged at 2700 g for 10 min and the supernatant was used for lactose and ethanol quantification. The remaining solid was washed with distilled water, centrifuged and then, diluted with distilled water for analysis of biomass. The cell concentration was determined in a spectrophotometer at 600 nm, by means of a calibration curve (biomass dry weight vs. optical density (OD)) previously obtained. Samples were diluted to give an absorbance in the range of 0.05–0.7.

The lactose and ethanol concentrations in the supernatant were determined by high-performance liquid chromatography, in a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI) and a Chrompack (300 × 6.5 mm) column at 60 °C, using 5 mM sulfuric acid as the eluent at a flow rate of 0.5 ml min^{-1} and a sample volume of 20 μl.

Table 1 – Experimental ranges and levels of the independent process variables according to the 2^3 full-factorial central composite design.

Independent variable	Symbol	Range and levels		
		−1	0	+1
Initial lactose concentration (kg m^{-3})	X_1	50	100	150
Temperature (°C)	X_2	25	30	35
Inoculum concentration (kg m^{-3})	X_3	1	2	3

2.4. Experimental design and optimization by response surface methodology

A 2^3 full-factorial central composite design with three coded levels, leading to 17 sets of experiments was made to establish the effects of the variables (initial lactose concentration, temperature, and inoculum concentration) on ethanol production from CWP solution. For statistical analysis, the independent variables were coded according to the Eq. (1), where each independent variable is represented by x_i (coded value), X_i (real value), X_0 (real value at the center point), and ΔX_i (step change value). The range and the levels of the variables are given in Table 1. The ethanol concentration, substrate consumption, ethanol yield factor, ethanol volumetric productivity, ethanol yield per cell, and bioconversion efficiency were taken as dependent variables or responses of the experimental design.

$$x_i = (X_i - X_0)/\Delta X_i \tag{1}$$

The experimental results were fitted with a second-order polynomial equation by multiple regression analysis. The quadratic mode for predicting the optimal point was expressed according to eq. (2), where $\widehat{y}_i$ represents the response variable, b_0 is the interception coefficient, b_i, b_{ii} and b_{ij} are the regression coefficients, n is the number of studied variables, and X_i and X_j represent the independent variables. Where possible, the model was simplified by elimination of statistically insignificant terms.

$$\widehat{y}_i = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j \tag{2}$$

The quality of the fitted polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked by the F-test. The significance of the regression coefficients was tested by t-value. Results were analyzed by the Experimental Design Module of the Statistica 5.0 software (Statsoft, USA). The model permitted evaluation of the effects of linear, quadratic and interactive terms of the independent variables on the chosen dependent variables.

3. Results and discussion

The yeast strain used in the present work was a K. fragilis selected among 8 Kluyveromyces strains (unpublished results). The experimental results obtained by cultivation of this yeast in deproteinized CWP solution, under different operational conditions according to a 2^3 central composite design, are shown in Table 2. It can be noted that K. fragilis was able to growth and produce ethanol under all the evaluated fermentation conditions, however, the production strongly varied according to the levels employed for the independent variables. The highest ethanol concentration (55.9 kg m^{-3}) was obtained when using an initial lactose concentration of 150 kg m^{-3}, 30 °C, and 2 kg m^{-3} inoculum concentration (conditions of run 10). Under these same conditions, the ethanol yield factor and volumetric productivity also achieved the highest values ($Y_{P/S} = 0.37$ kg kg^{-1}; $Q_P = 1.27$ kg m^{-3} h^{-1}).

Due to the large difference observed in the ethanol production, a statistical analysis was carried out to identify the variables that had the greatest influence on this bioconversion process. Table 3 shows the Student's t-test and p-values used to determine the statistical significance of the independent variables (initial lactose concentration, temperature, and inoculum concentration) on the response variables (ethanol concentration, $Y_{P/S}$, Q_P, η, $Y_{P/x}$, and substrate consumption). According to this analysis the initial lactose concentration was the variable that affected all the analyzed responses. In addition, it was the unique variable with

Table 2 – Experimental matrix and results of ethanol concentration (Et), ethanol per biomass yield factor ($Y_{P/x}$), and ethanol yield factor ($Y_{P/S}$), with coded levels of the variables according to a 2^3 full-factorial central composite design.									
Runs	Independent variables[a]			Responses						
	X_1	X_2	X_3	Cell (kg m^{-3})	S cons. (%)	Et (kg m^{-3})	$Y_{P/x}$ (kg kg^{-1})	$Y_{P/S}$ (kg kg^{-1})	Q_P (kg m^{-3} h^{-1})	η (%)
1	−1	−1	−1	4.9	100	12.7	2.59	0.25	0.29	45.67
2	−1	−1	+1	6.8	100	13.4	1.97	0.26	0.30	48.83
3	−1	+1	−1	5.5	100	12.0	2.18	0.27	0.27	54.86
4	−1	+1	+1	7.2	100	13.4	1.86	0.35	0.30	66.07
5	+1	−1	−1	7.4	94.6	48.2	6.51	0.34	1.10	60.13
6	+1	−1	+1	9.1	95.3	47.0	5.16	0.36	1.07	64.29
7	+1	+1	−1	7.4	100	48.5	6.55	0.35	1.10	64.76
8	+1	+1	+1	7.5	100	41.5	5.53	0.28	0.94	52.44
9	−1	0	0	6.6	100	10.3	1.56	0.22	0.23	40.65
10	+1	0	0	8.7	100	55.9	6.43	0.37	1.27	69.04
11	0	−1	0	7.8	100	28.8	3.69	0.33	0.66	62.03
12	0	+1	0	7.3	100	24.5	3.36	0.28	0.56	51.81
13	0	0	−1	7.5	100	32.4	4.32	0.33	0.74	60.58
14	0	0	+1	9.0	100	28.8	3.20	0.29	0.66	53.26
15	0	0	0	8.4	100	26.1	3.11	0.30	0.59	55.64
16	0	0	0	8.3	100	25.6	3.08	0.30	0.58	55.00
17	0	0	0	8.3	100	23.8	2.87	0.29	0.54	53.88

a X_1 = coded values of lactose; X_2 = coded values of temperature; X_3 = coded values of inoculum concentration.

Table 3 – Effect estimates, standard errors and ethanol concentration (Et), substrate consumption (Sc), bioconversion efficiency (η), ethanol yield per cell ($Y_{P/x}$), ethanol yield factor ($Y_{P/S}$), and ethanol volumetric productivity (Q_P) during the bioconversion of cheese whey power solution by *Kluyveromyces fragilis*, according to the 2^3 full-factorial central composite design.

	Estimated effects	Standard errors	t_{value}	Estimated effects	Standard errors	t_{value}	Estimated effects	Standard errors	t_{value}
Variables and interactions	Et			Sc			η		
X_1	35.860	±2.498	14.357[a]	−2.016	±0.570	−3.538[a]	10.916	±4.724	2.310[b]
X_1^2	7.641	±4.826	1.583	−0.994	±1.101	−0.903	−1.468	±9.127	−0.161
X_2	−2.040	±2.498	−0.817	2.016	±0.570	3.538[a]	1.798	±4.724	0.381
X_2^2	−5.259	±4.826	−1.090	−0.994	±1.101	−0.903	2.682	±9.127	0.294
X_3	−1.940	±2.498	−0.777	0.132	±0.570	0.232	−0.222	±4.724	−0.047
X_3^2	2.641	±4.826	0.547	−0.994	±1.101	−0.903	2.682	±9.127	0.294
X_1X_2	−1.125	±2.793	−0.403	2.520	±0.637	3.956[a]	−8.413	±5.282	−1.593
X_1X_3	−2.575	±2.793	−0.922	0.165	±0.637	0.259	−5.633	±5.282	−1.066
X_2X_3	−1.275	±2.793	−0.457	−0.165	±0.637	−0.259	−2.108	±5.282	−0.399
Variables and interactions	$Y_{P/x}$			$Y_{P/S}$			Q_P		
X_1	4.004	±0.246	16.288[a]	0.070	±0.024	2.968[b]	0.818	±0.058	14.182[a]
X_1^2	1.006	±0.475	2.119	−0.011	±0.046	−0.232	0.164	±0.111	1.470
X_2	−0.088	±0.246	−0.358	−0.002	±0.024	−0.085	−0.050	±0.058	−0.867
X_2^2	0.066	±0.475	0.140	0.009	±0.046	0.207	−0.116	±0.111	−1.043
X_3	−0.886	±0.246	−3.604[a]	0.000	±0.024	0.000	−0.046	±0.058	−0.798
X_3^2	0.536	±0.475	1.130	0.019	±0.046	0.427	0.064	±0.111	0.573
X_1X_2	0.233	±0.275	0.846	−0.045	±0.026	−1.707	−0.028	±0.064	−0.426
X_1X_3	−0.358	±0.275	−1.301	−0.035	±0.026	−1.327	−0.058	±0.064	−0.892
X_2X_3	0.158	±0.275	0.573	−0.005	±0.026	−0.190	−0.028	±0.064	−0.426

a $p < 0.01$.
b $p < 0.05$; X_1 = coded values of initial lactose concentration; X_2 = coded values of temperature; X_3 = coded values of inoculum concentration.

significant influence on ethanol production, $Y_{P/S}$, Q_P, and η values. For all of these responses, only the linear effect of the initial lactose concentration was significant at 95% confidence level. Such effect had a positive signal, indicating that the ethanol concentration, $Y_{P/S}$, Q_P, and η values increased by increasing the initial lactose concentration. Temperature and inoculum concentration did not present main significant effect for these responses, suggesting that temperature between 25 and 35 °C and inoculum concentration varying from 1 to 3 kg m^{-3} did not affect the $Y_{P/S}$, Q_P, η, and ethanol production by *K. fragilis* from CWP solution. Interaction effects among the studied variables were also not significant at 95% confidence level.

The ethanol yield per cell ($Y_{P/x}$) was also mainly affected by the initial lactose concentration, resulting in higher ethanol production by cell as the initial substrate concentration was increased (positive effect) (Table 3). Moreover, the inoculum concentration had also a significant effect at 95% confidence level, which had a negative signal, indicating that the lower the inoculum concentration, the higher the ethanol amount produced by cell. In addition, the statistical analysis carried out for substrate consumption revealed that the inoculum variation from 1 to 3 kg m^{-3} did not have influence in the lactose consumption by yeast. Inoculum variation from 1 to 3 kg m^{-3} was also not significant for ethanol production. By considering these facts it can be concluded that when using 1 kg m^{-3} inoculum, the cells were able to consume the same lactose amount and produce the same final ethanol concentration that when using 3 kg m^{-3} inoculum. Consequently, the product formation by cell was higher. As can be seen in Table 3, when the inoculum concentration was decreased from 3 to

1 kg m^{-3}, an average increase of 3.604 kg kg^{-1} was observed in $Y_{P/x}$.

Regarding the substrate consumption, although the lactose was completely consumed in almost all the fermentations, the statistical analysis pointed out that this response was influenced by the initial lactose concentration and temperature (Table 3). The initial lactose concentration had a main and negative effect, indicating that the substrate consumption increased as the initial substrate concentration used in the experiments decreased. On the other hand, the temperature had a main and positive effect, revealing that the temperature increase favored the substrate consumption by the microorganism. Increasing the fermentation temperature from 23 °C to 42 °C also enhanced the lactose utilization by *Lactobacillus helveticus* [12]. According to the authors, the rate of reaction for microorganisms really increases with increasing temperature, until a limiting maximum value is reached.

After identification of the main variables affecting the ethanol production, a multiple regression analysis was performed to fit the experimental data to polynomial equations, obtaining the coefficients given in Table 4. The models were simplified by elimination of statistically insignificant terms. The quality of the fitted polynomial models was expressed by the coefficient of determination R^2. As can be observed, models explaining more than 90% of the variations observed in the responses ($R^2 > 0.9$) could be adjusted for the responses ethanol concentration, Q_P, and $Y_{P/x}$. The high R^2 values mean that the models accurately represent the data in the experimental region studied, explaining more than 90% of the variability in the responses.

The relation between variables and ethanol concentration can be best visualized by examining the surface plots given in

Table 4 – Model equations for the response surfaces fitted to the experimental data points, and the respective R^2.

Response	Model equations	R^2
Ethanol concentration (Et, in kg m^{-3})	$Et = 28.99 + 17.93X_1$	0.94
Ethanol volumetric productivity (Q_P, in kg m^{-3} h^{-1})	$Q_P = 0.659 + 0.409X_1$	0.94
Ethanol yield by cell ($Y_{P/x}$, in kg kg^{-1})	$Y_{P/x} = 3.376 + 2.002X_1 + 0.658X_1^2 - 0.443X_3$	0.96

X_1 = initial lactose concentration; X_3 = inoculum concentration.

Fig. 1, which were plotted as a function of two variables at a time and holding the other variable at a fixed level. Fig. 1 clearly shows that increasing initial lactose concentration resulted in higher ethanol production, with maxima values ($\geq$41.5 kg m^{-3}) being achieved under the maximum tested concentration (150 kg m^{-3}). Similar plot surfaces were

obtained for the Q_P response (not shown). A comparable behavior was also verified for *Kluyveromyces marxianus* DSMZ-7239 yeast using initial lactose concentrations up to 75 kg m^{-3} [13]. However, maxima ethanol amounts produced in the present study were higher than those obtained by direct fermentation of crude (non-concentrated) cheese whey [14] or cheese whey powder [13].

Based on the statistical analysis results, assays were performed in a following step to evaluate the possibility of increasing the ethanol concentration by increasing the initial lactose concentration to values above 150 kg m^{-3} (up to 250 kg m^{-3}). The values of temperature and inoculum used in these experiments were fixed at 35 °C and 1 kg m^{-3}, respectively. This temperature was chosen because the solubility of lactose solutions increase with the temperature increase and this is important as higher the concentration of the cheese whey solution used. On the other hand, the inoculum concentration was fixed at 1 kg m^{-3} due to economical and practical reasons.

Fig. 2 shows the experimental results obtained in these experiments. As can be seen, initial lactose concentrations up to 200 kg m^{-3} favored the bioconversion to ethanol, but higher lactose concentration values drastically affected all the fermentative parameters. Cell growth was slightly increased by increasing the lactose concentration between 150 and 200 kg m^{-3} (Fig. 2A). In this same range of values, the substrate
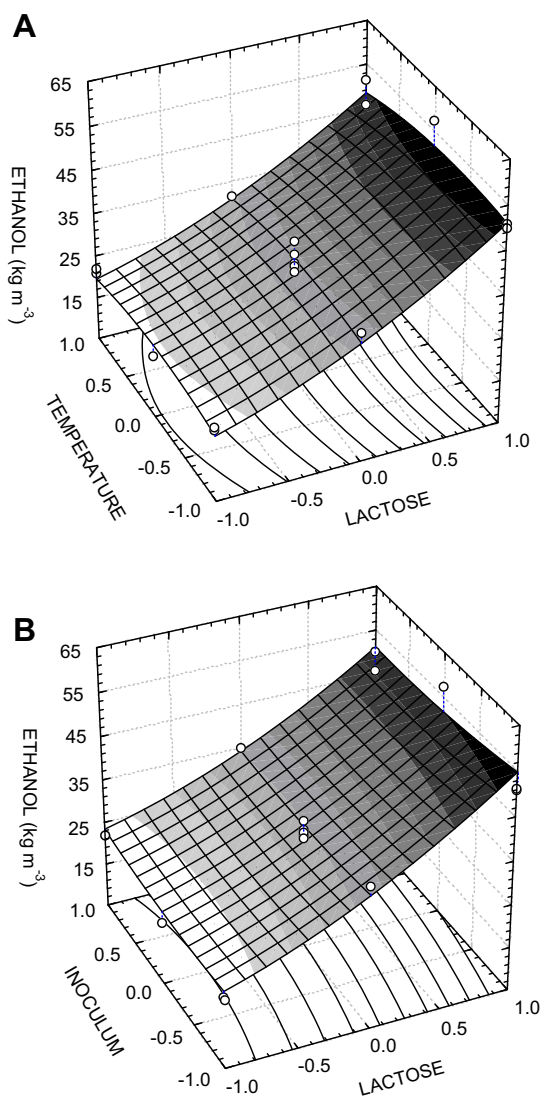

Fig. 1 – Response surface of ethanol production from cheese whey powder by *K. fragilis* as a function of: (A) initial lactose concentration and temperature, (B) initial lactose and inoculum concentrations.
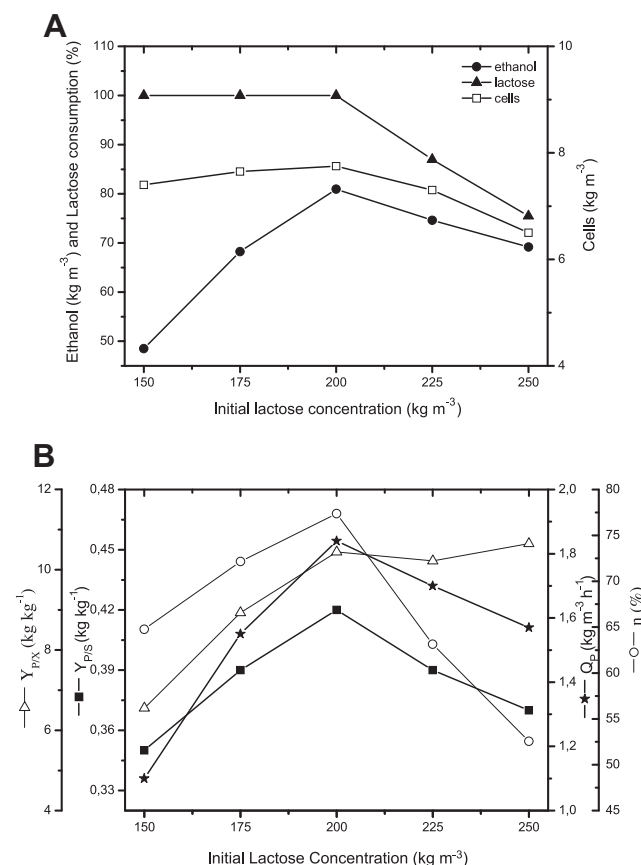

Fig. 2 – Effect of the initial lactose concentration on (A) cell growth, lactose consumption, ethanol production, and (B) in the fermentative parameters for ethanol production by *K. fragilis* in cheese whey powder solution. Fermentation temperature: 35 °C; inoculum concentration: 1 kg m^{-3}.

consumption by the microorganism was not affected, and the ethanol production increased with the initial lactose concentration increase, achieving a maximum value of 80.95 kg m^{-3} (77.4% of the theoretical value) when a deproteinized CWP solution containing 200 kg m^{-3} lactose was fermented. Lactose concentration values higher than 200 kg m^{-3} affected the ethanol production by the yeast, as can be seen in the profiles given in Fig. 2A and B. The observed ethanol concentration reduction by elevated initial sugar concentration is probably related with a substrate inhibition, which might have inactivated the cells due to high osmotic pressure encountered at high sugar content, causing high maintenance requirements [8].

The results here attained can be favorably compared with others reported in the literature. For example, fermentation of whey by *K. marxianus* MTCC 1288 under different initial lactose concentrations yielded maximum ethanol production (3.98 kg m^{-3}) when using 50 kg m^{-3} lactose. Higher lactose concentrations led to a drastic decrease in product formation and substrate utilization [15]. Lactose concentrations higher than 100 kg m^{-3} had an inhibitory effect on the specific growth rate, lactose utilization rate, and ethanol production rate by *Candida pseudotropicalis* [16]. Ethanol bioconversion by a recombinant *Saccharomyces cerevisiae* was also affected by initial lactose concentrations higher than 100 kg m^{-3}. The highest value (59%) decreased to 53% when using initial lactose concentrations higher than 100 kg m^{-3} [17]. Ethanol production by *K. fragilis* (present work) was only affected by lactose concentrations higher than 200 kg m^{-3}. This finding represents an advantage because the costs of the process can be significantly reduced with the lactose concentration increase [2].

4. Conclusions

The initial lactose concentration in deproteinized cheese whey powder solution exerted great influence on ethanol production by *K. fragilis*, being the maximum product formation (80.95 kg m^{-3}) obtained when using an initial lactose concentration of 200 kg m^{-3}. This value is about 4 times higher than that achieved during the fermentation of non-concentrated cheese whey, and represents thus an interesting alternative to decrease the distillation costs for ethanol production from cheese whey.

Acknowledgments

REFERENCES

[1] Ghaly AE, El-Taweel AA. Kinetic modelling of continuous production of ethanol from cheese whey. Biomass Bioenerg 1997;6:461–72.

[2] González Siso MI. The biotechnological utilization of cheese whey: a review. Bioresour Technol 1996;57:1–11.

[3] Panesar PS, Kennedy JF, Gandhi DN, Bunko K. Bioutilisation of whey for lactic acid production. Food Chem 2007;105:1–14.

[4] Dragone G, Mussatto SI, Oliveira JM, Teixeira JA. Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation. Food Chem 2009; 112:929–35.

[5] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenerg 2004;26: 361–75.

[6] Staniszewski M, Kujawski W, Lewandowska M. Semi-continuous ethanol production in bioreactor from whey with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product − Kinetic model predictions considering glucose repression. J Food Eng 2009; 91:240–9.

[7] Guadix A, Sorenson E, Papageorgiou LG, Guadix EM. Optimal design and operation of ultrafiltration plants. J Membr Sci 2004;235:131–8.

[8] Ozmihci S, Kargi F. Fermentation of cheese whey powder solution to ethanol in a packed-column bioreactor: effects of feed sugar concentration. J Chem Technol Biotechnol 2009; 84:106–11.

[9] Kargi F, Ozmihci S. Utilization of cheese whey powder (CWP) for ethanol fermentations: effects of operating parameters. Enzym Microb Technol 2006;38:711–8.

[10] Ozmihci S, Kargi F. Continuous ethanol fermentation of cheese whey powder solution: effects of hydraulic residence time. Bioproc Biosyst Eng 2007;30:79–86.

[11] Mawson AJ. Bioconversions for whey utilization and waste abatement. Bioresour Technol 1994;47:195–203.

[12] Tango MSA, Ghaly AE. Effect of temperature on lactic acid production from cheese whey using *Lactobacillus helveticus* under batch conditions. Biomass Bioenerg 1999;16:61–78.

[13] Ozmihci S, Kargi F. Kinetics of batch ethanol fermentation of cheese-whey powder (CWP) solution as function of substrate and yeast concentrations. Bioresour Technol 2007;98: 2978–84.

[14] Zafar S, Owais M. Ethanol production from crude whey by *Kluyveromyces marxianus*. Biochem Eng J 2006;27:295–8.

[15] Zafar S, Owais M, Saleemuddin M, Husain S. Batch kinetics and modelling of ethanolic fermentation of whey. Int J Food Sci Tech 2005;40:597–604.

[16] Ghaly AE, El-Taweel AA. Effect of lactose concentration on batch production of ethanol from cheese whey using *Candida pseudotropicalis*. Trans ASAE 1995;38:1113–20.

[17] Guimarães P, Klein J, Domingues L, Teixeira JA. Fermentation performance of a recombinant lactose-consuming flocculating *Saccharomyces cerevisiae* strain. Braz J Food Technol 2005;3:34–9.