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Modeling and kinetic study of bio-ethanol production from soy protein concentrate by-product

Lucas CALDEIRÃO^{1*}, Cyntia TANAKA¹, Elza IDA¹, Wilma SPINOSA¹

Abstract

The aim of this work was to evaluate a non-agitated process of bioethanol production from soybean molasses and the kinetic parameters of fermentation using a strain of *Saccharomyces cerevisiae* (ATCC[®] 2345). Kinetic experiment was conducted in medium with 30% (w v⁻¹) of soluble solids without supplementation or pH adjustment. The maximum ethanol concentration was in 44 hours, the ethanol productivity was 0.946 g L⁻¹ h⁻¹, the yield over total initial sugars (Y₁) was 47.87%, over consumed sugars (Y₂) was 88.08% and specific cells production rate was 0.006 h⁻¹. The mathematical polynomial was adjusted to the experimental data and provided very similar parameters of yield and productivity. Based in this study, for one ton of soybean molasses can be produced 103 kg of anhydrous bioethanol.

Keywords: alcoholic fermentation; bioethanol; soybean molasses; co-product valorization.

Practical Application: Static fermentation showed high yield to ethanol production from soybean molasses.

1 Introduction

Bioethanol is a viable alternative to petroleum-based fuels, since fossil fuels are limited and generate greater environmental impact. Bioethanol has low production costs, easy of operation and can be obtained from different raw materials materials (Cardona & Sánchez, 2007; Balat et al., 2008; Sage et al., 2009; Nikolic et al., 2010). Any sugar or starch raw material can be converted to ethanol by microorganisms. The substrate should allow growth of the microorganism without interfering with product recovery. The substrate must be economically viable and available in quantity for the industrialization of the fermentation process (Srichuwong et al., 2009; Oda & Nakamura, 2009; Behera et al., 2010).

In Brazil, after 1975, a program was implemented to encourage the use of sugarcane bioethanol to replace gasoline in addition to encouraging the mixture of ethanol and gasoline used in common cars. This measure increased production and consumption of ethanol in the country, and promoted research for various raw materials to obtain ethanol, in addition of sugar cane as well as the production of second generation ethanol (Gámez et al., 2005; Miller et al., 2012).

Soy is one of the crop most widely produced in Brazil and in the world due to its versatility in cultivation and processing, generating various products and co-products used by the food, animal feed and agrochemical industries (Silva et al., 2012). The soy molasses is a viscous liquid of brown color and sweet-sour taste obtained from the processing of the soy protein industry concentrate (70% dry basis). The defatted soy flour is washed with ethanol and water to the proteins concentration, so, the carbohydrates and other soluble compounds are drawn and subsequently concentrated by evaporation of the ethanol and part of the water, resulting in the molasses. The main components

of the molasses sugars are sucrose, raffinose and stachyose (Siqueira et al., 2008). The soybean molasses contains proteins, lipids, minerals and isoflavones in addition to carbohydrates (Hosny & Rosazza, 1999; Siqueira et al., 2008). Much of this co-product is used for animal feed or else discarded as waste. In Brazil already exist soybean processing industries that use the soy molasses for production of hydrated alcohol, however, the use of this ethanol is limited as an alternative energy for these industries (Machado, 1999; Siqueira et al., 2008, Romão et al., 2012). Besides fuel, the soybean molasses ethanol can be used as raw material for the food industry or even the development of a distilled beverages.

Industries conduce alcoholic fermentations without agitation due the lack of equipment and its high cost, so this study aimed to evaluate the static process of ethanol production from soy molasses and their kinetic parameters applying a mathematical modelling.

2 Materials and methods

2.1 Raw material

The soybean molasses (74.5% soluble solids) was donated by the Selecta[®] soybean factory, located in Araguari, Minas Gerais (Brazil). The samples were stored in plastic containers (5 kg) at 4 °C until they are ready to use.

2.2 Characterization of the soybean molasses

Dry extract and ashes were determined by gravimetric method after drying at 105 °C and calcination at 550 °C, respectively, until constant weight; proteins were determined by

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Kjeldahl method (Association of Official Analytical Chemists, 2005). Lipids were determined by Bligh-Dyer method (Bligh & Dyer 1959). Total sugars were determined by phenol-sulfuric acid method (DuBois et al., 1956). The results are presented as average of three analyses.

2.3 Activation and inoculum preparation

Lyophilized *Saccharomyces cerevisiae* strain (ATCC® 2345), obtained from tropical Research Foundation “André Tosello” culture collection, was activated in sterile yeast, malt broth (YM) (Himedia®) and incubated in a shaker (Cientec®, Brazil, CT-712 R) at 30 °C and agitated at 150 rpm for 24 h. After activation, 10% (v v⁻¹) of this broth was transferred to aqueous soybean molasses 10° Brix sterile (15 minutes at 121 °C; FABBE®, Brazil, Primar) and incubated in the same conditions of temperature and agitation of the activation step. After 24 h 10% (v v⁻¹) of this inoculum was transferred to sterile molasses diluted to 20 °Brix and incubated for 24 h at 30 °C. This last media was used as inoculum to conduction of the assays.

2.4 Effect of initial concentration of soybean molasses

The ethanol yield over total initial sugars (Y_1), yield over consumed sugars (Y_2) and productivity were evaluated from three initial soybean molasses concentrations (20, 25 and 30 °Brix). The fermentations were conducted in Erlenmeyer flasks and incubated (TECNAL®, Brazil, B.O.D. TE-391) at 30 °C with 10% (v v⁻¹) of inoculum (10^7 initial cells ml⁻¹) at a pH value of 5.5.

The fermentation was conducted until there was no liberation of CO₂ (visual analysis). Each assay was realized in duplicate.

2.5 Modeling, kinetic parameters and yield of fermentation

As showed the results of effect of initial concentration of soybean molasses over fermentation, the best condition (30 °Brix, 10% (v v⁻¹) initial inoculum, pH of 5.5 and 30 °C) was used to obtain the kinetic parameters. The essay was conducted in a bioreactor (Biofoco®, Brazil. Rubia basic). It was collected samples for counting cells, total sugars and ethanol content determinations until 140 h of fermentation.

Parameter of ethanol yields over total initial sugars (Y_1) (Equation 1), total consumed sugars (Y_2) (Equation 2), total sugars in ethanol conversion ($Y_{p/s}$) (Equation 3), ethanol productivity (Equation 4) and specific growth rate (μ_x) (Equation 8) were determined in maximum ethanol concentration. The parameters of growth rate (r_x) total sugars consumption rate (r_s) (and ethanol production rate (r_p) were determined from inclination (angle) of linear equation obtained from kinetic of cells and bioethanol formation and total sugars consumption data (Figure 1) represented by the Equations 5, 6 and 7, respectively. The cell generation time (t_g) was determined by division of the time of maximum number of cells by the number of yeast cell generations (n) (Equations 9 and 10). A polynomial equation of third order was adjusted to experimental data, representing cells, total sugars and ethanol concentrations as time dependent functions.

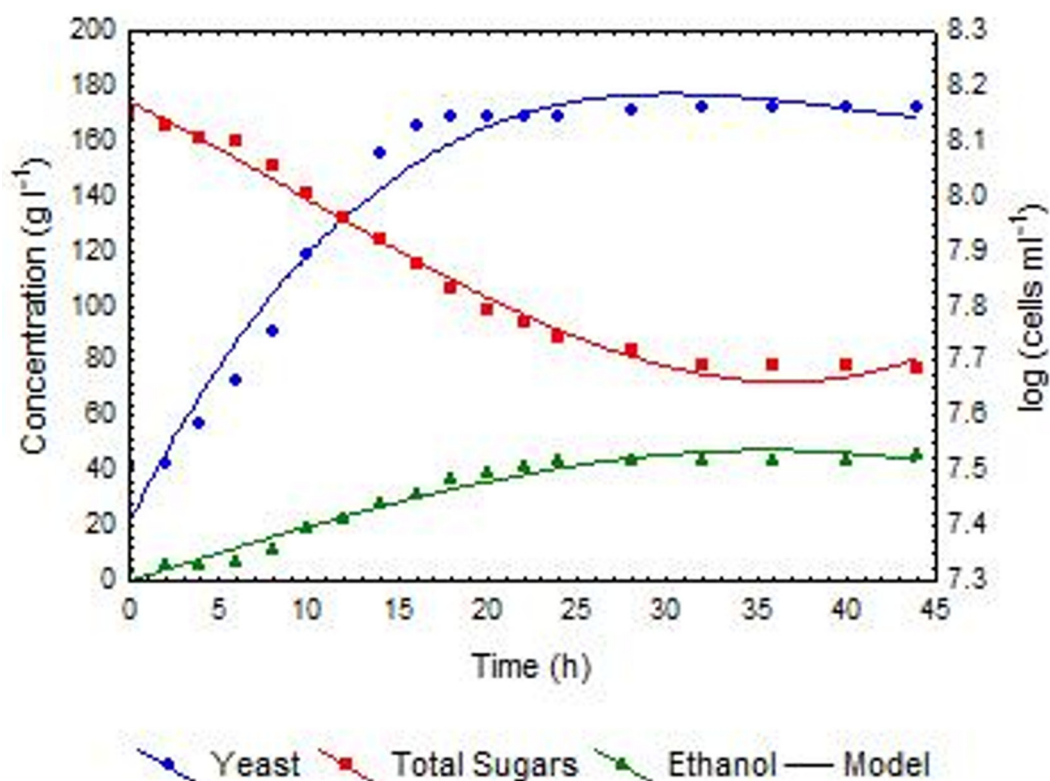


Figure 1. Evolution of ethanol concentration, total sugars and yeast cells as a function of the fermentation time from practical and theoretical data.

$$Y_1(\%) = \frac{(Et_f - Et_i) * 100}{(TS_i) * 0.511} \quad (1)$$

$$Y_2(\%) = \frac{(Et_f - Et_i) * 100}{(TS_i - TS_f) * 0.511} \quad (2)$$

$$Y_{P/S}(\%) = \frac{(Et_f - Et_i) * 100}{(TS_i - TS_f)} \quad (3)$$

$$Productivity(g L^{-1} h^{-1}) = \frac{Et_f}{t_f} \quad (4)$$

$$rx(\log cel mL^{-1} h^{-1}) = \frac{X_f - X_i}{t_f - t_i} \quad (5)$$

$$rs(g L^{-1} h^{-1}) = \frac{TS_i - TS_f}{t_f - t_i} \quad (6)$$

$$rp(g L^{-1} h^{-1}) = \frac{Et_f - Et_i}{t_f - t_i} \quad (7)$$

$$\mu x(h) = rx * \frac{1}{X} \quad (8)$$

$$tg(h) = \frac{t}{n} \quad (9)$$

$$n = \frac{X_f - X_i}{\log 2} \quad (10)$$

Where, Et_f is the final ethanol content ($g L^{-1}$); Et_i is the initial ethanol content ($g L^{-1}$); TS_i is the final total sugar content ($g L^{-1}$); TS_f is the final total sugar content ($g L^{-1}$); 0.511 is the maximum theoretical conversion (stoichiometric) of hexose to ethanol; t is time (h); X_f is the final cell concentration ($\log cells mL^{-1}$); X_i is the initial cell concentration ($\log cells mL^{-1}$); t_f is the final fermentation time (h); t_i is the initial fermentation time (h); t_t is the time at the maximum counting cell value (h); n is the number of cell generations.

2.6 Determinations of total sugars, ethanol and cells count

Total sugars were determined by phenol-sulfuric acid reaction, using glucose as standard. The analysis consists in addition of 2.5 mL of sulfuric acid and 0.5 mL 5% (w v⁻¹) phenol solution over 0.5 mL of diluted sample and keep the reaction at room temperature for 20 minutes. After the reaction time, the optical density is determined at 480 nm in spectrophotometer (DuBois et al., 1956).

Alcoholic fermented samples were previously distilled in micro-distiller of alcohol (TECNAL®, Brazil, TE-012) and alcohol concentration was determined by digital densitometry (Rudolph Research Analytical®, EUA, DDM 2909) (Spinosa et al., 2015).

Cell concentration was quantified in Neubauer counting. The viable cells were differentiated by the methylene-blue staining method (1% w v⁻¹) and the concentration expressed as the logarithm of cells per mL ($\log cells mL^{-1}$) (Silva et al., 2012).

2.7 Statistical analysis

When required, Tukey test ($p = 0.05$) was used to determine statistical differences between means. The polynomial regression was obtained from Statistica 7.0 (Statsoft, 2004).

3 Results and discussion

3.1 Characterization of soybean molasses

Table 1 shows the main components in soybean molasses. Carbohydrates representing 64.05 g 100 g⁻¹ in dry basis. Molasses contains also significant amounts of proteins (8.91 g 100 g⁻¹) and ashes (7.52 g 100 g⁻¹) which represent, respectively, nitrogen and minerals sources, these components are important for yeast cell growth (Nahvi et al., 2002; Siqueira et al., 2008).

3.2 Effect of initial concentration of soybean molasses

The fermentation time (Table 2) increased proportionally to the initial total sugar concentration. Despite the increase of the fermentation time, the ethanol productivity was not affected by elevation of initial concentration of molasses, it kept statistically equal for the three assays. It is possible to observe that initial concentration of soybean molasses does not influenced in ethanol yield over total sugar (Y_1), however, the ethanol yield over consumed sugar (Y_2) was higher in 30 °Brix initial concentration than in 20 and 25 °Brix. The experiments conducted in 20 and 25 °Brix were not significantly different. The ethanol concentration increased with increasing of initial concentration of molasses, this occurred due the higher concentration of available total sugar to be converted. Remaining sugars are the non-fermentable fractions of total sugar, raffinose and stachyose. This carbohydrates present α -1,6 bounds, which are not broken by *S. cerevisiae* enzymes (Lan et al., 2007).

In a similar study, Siqueira et al. (2008) using agitation, observed a yield (Y_1) of 40.97% and 56 g L⁻¹ of final ethanol concentration, starting from 30 °Brix soybean molasses, after 30 hours of fermentation.

The assay with 30 °Brix initial concentration was chosen for the kinetic studies due its higher yield (Y_2) and ethanol production, when compared to the other assays.

3.3 Modeling, kinetic parameters and yield of fermentation

The fermentation time was 44 hours (Table 3), after this, the ethanol concentration was maximum and constant. From the experimental data it was proposed a third order polynomial mathematical model (Equations 11, 12 and 13), in which, it was used to determine the theoretical data of yeast cell concentration, total sugars and ethanol content.

Table 1. Composition of soybean molasses in dry basis.

Component	g 100 g ⁻¹
Ashes	7.52 ± 0.05
Lipids	9.46 ± 0.26
Proteins	8.91 ± 0.08
Total sugars	64.05 ± 0.24
Total dry extract	72.61 ± 0.27

Table 2. Effect of soybean molasses concentration (Brix) on ethanol yield and productivity*.

Brix	Time (h)	TS _i (g L ⁻¹)	TS _f (g L ⁻¹)	Et (g L ⁻¹)	Y ₁ (%)	Y ₂ (%)	Productivity (g L ⁻¹ h ⁻¹)
20	48	126.93	56.68	22.83	35.64 ± 0.63 ^a	65.10 ± 2.12 ^b	0.474 ± 0.00 ^a
	48	123.38	56.52	22.75			
25	72	143.88	54.26	30.82	43.09 ± 1.63 ^a	68.65 ± 1.92 ^b	0.439 ± 0.01 ^a
	72	143.72	52.81	32.53			
30	96	190.23	95.28	40.79	39.51 ± 3.46 ^a	86.03 ± 2.79 ^a	0.401 ± 0.03 ^a
	96	191.36	110.78	36.24			

*TS_i, total initial sugars; TS_f, total final sugars; Et, ethanol concentration; Y₁, yield over total initial sugars, Y₂, yield over consumed sugars. Means followed by different letters on the same line indicates significant difference (p < 0.05).

Table 3. Experimental data obtained from kinetic study and theoretical data determined from mathematical models*.

Time (h)	Experimental data			Theoretical data		
	Log (cells mL ⁻¹)	TS (g L ⁻¹)	Et (g L ⁻¹)	Log (cells mL ⁻¹)	TS (g L ⁻¹)	Et (g L ⁻¹)
0	7.51	170.20	4.74	7.41	174.31	0.00
2	7.51	165.62	5.21	7.53	167.65	4.06
4	7.59	161.15	6.16	7.64	160.66	8.13
6	7.66	159.55	7.27	7.74	153.42	12.13
8	7.75	150.67	11.14	7.82	146.01	16.02
10	7.89	140.66	18.88	7.90	138.52	19.79
12	7.96	131.77	22.67	7.96	131.02	23.40
14	8.08	123.93	27.73	8.02	123.59	26.84
16	8.13	115.30	31.05	8.06	116.32	30.08
18	8.14	105.97	37.13	8.10	109.29	33.10
20	8.14	98.95	39.58	8.13	102.59	35.87
22	8.15	93.40	41.63	8.16	96.28	38.36
24	8.15	88.01	43.13	8.17	90.46	40.57
28	8.15	83.33	43.14	8.19	80.60	43.99
32	8.16	78.48	43.21	8.20	73.65	45.95
36	8.16	78.24	43.61	8.19	70.27	46.24
40	8.16	77.95	43.87	8.18	71.11	44.68
44	8.16	77.71	46.37	8.17	76.82	41.08

*TS, total sugars; Et, ethanol concentration.

Comparing the theoretical and experimental data, it was possible to observe that, for practical cell concentration, the maximum value was 8.16 log cells mL⁻¹ after 32 hours of fermentation, while the model presented 8.20 log cells mL⁻¹ at the same time. For the final time, the theoretical data presented 8.17 log cells mL⁻¹ versus 8.16 log cells mL⁻¹ of experimental data. The values determined by sugars consumption equation was very similar to the experimental, however, the model proposes the minimum total sugar concentration after 36 hours of fermentation, whilst practical data showed the minimum after 44 hours (Figure 1).

$$Yeast (\log \text{ cells mL}^{-1}) = 7.4078 + 0.0645t - 0.0017t^2 + 1.42E^{-5}t^3 \quad (11)$$

$$Total \text{ Sugars } (g \text{ L}^{-1}) = 174.3064 - 3.232t - 0.0517t^2 + 0.0017t^3 \quad (12)$$

$$Ethanol (g \text{ L}^{-1}) = 0.00 + 2.074t - 0.0039t^2 - 0.0005t^3 \quad (13)$$

Regarding the ethanol concentration, the maximum experimental value was 46.47 g L⁻¹ after 44 hours, at this time, the mathematical model showed 41.08 g L⁻¹, but the real produced

ethanol (Et_f – Et_i) was 41.63 g L⁻¹, very close of the predicted value. The maximum ethanol concentration determined from the model was 46.24 g L⁻¹ after 36 hours of fermentation, what did not occur.

For 44 hours of fermentation (Table 4), it was observed the maximum consumption of total sugars and production of ethanol. Consumption of total sugars (dTS) and ethanol production (dEt) were, respectively, 92.49 g L⁻¹ and 41.63 g L⁻¹. The yield from sugar (Y_{p/s}) was 45.01%, this value represents 80.08% of maximum theoretical (Y₂). The yield over total initial sugars (Y₁) indicates the amount of ethanol was generated from total initial sugar in the fermentative process, the apparent low value (45.01%) in this study is related the amount of fermentative sugars in soybean molasses. Remaining sugars represents, mainly, the amount of stachyose and raffinose, non-fermentable by *S. cerevisiae* as it is showed by other industries (Machado, 1999; Siqueira et al., 2008, Romão et al., 2012). However, the low cost of soybean molasses and high fermentable sugar to ethanol conversion (88.08%) are indicatives that this study is relevant.

The mathematical model indicated 97.49 g L⁻¹ for sugars consumption and 41.08 g L⁻¹ for ethanol production, which represented the yield ($Y_{p/s}$) of 42.14% and 82.46% for yield over consumed sugars (Y_2). The yield over total initial sugars (Y_1) proposed by the model was 46.12%. The practical and theoretical productivity was 0.946 g L⁻¹ h⁻¹ and 0.934 g L⁻¹ h⁻¹, respectively.

The similarity of yields and productivity indicates that the models are well-adjusted to the experimental data, generating values closed to the practical, and can be used for prediction of yield, productivity, concentrations of yeast cells, sugars and ethanol.

Siqueira et al. (2008) obtained yields of 45.4% ($Y_{p/s}$) and 45.8% (Y_1) at laboratory scale using agitation, similar to this study. Romão et al. (2012) studied the alcoholic fermentation of acid hydrolyzed soybean molasses and obtained yields of 60% (Y_1) and 78% (Y_2) at optimum conditions of hydrolysis. Silva et al. (2012) obtained yields of 67.5% (Y_1) and 80.8% (Y_2) in optimum conditions of enzymatic hydrolysis and fermentation. The higher values of Y_1 in Romão et al. (2012) and Silva et al. (2012) studies is due the hydrolysis of non-fermentable to fermentable sugars, increasing the ethanol production and total sugar consumption.

The kinetic parameters were determinate from inclination of linear regression of experimental and mathematical data at the same range. Table 5 shows the kinetic parameters and time of cell generation. The experimental cell production (rx) was 0.046 log cells mL⁻¹ h⁻¹, while theoretical determined from model was 0.037 log cells mL⁻¹ h⁻¹, this value is 80.5% of the experimental data, which is considered a good estimative for a biological process.

The theoretical total sugars rate (rs) was 3.559 g L⁻¹ h⁻¹, very similar to the practical rate (3.960 g L⁻¹ h⁻¹). The rate of ethanol production was 1.701 g L⁻¹ h⁻¹ and 2.367 g L⁻¹ h⁻¹ determined from experimental and theoretical data, respectively. The predicted value represents 72% of the practical. The specific growth rate (μ_x) and time of cell generation were 0.006 h⁻¹ and 6.850 h for experimental data and 0.005 h⁻¹ and 7.908 h for data determined by mathematical model, respectively.

Siqueira et al. (2008) reported the maximum ethanol productivity of 8.08 g L⁻¹ h⁻¹ conducting alcoholic fermentation of

soybean molasses under agitation and initial cell concentration of 10⁸ cells mL⁻¹ of *S. cerevisiae*. The low productivity and long cell generation time were expected due the low initial population (10⁷ cells mL⁻¹) and absence of agitation, because of this, the yeast cells remain decanted, lowering the contact area between cells and substrate (sugars). Other factor that affects the yeast development is the high concentration of sugars in the media (30 °Brix), which promotes high osmotic pressure in the cells, delaying its growth (Ochoa-Estopier et al., 2011). The low specific cell production rate (μ_x) can be explained by the low rs value in relation of high yeast population at the end of exponential phase of growth. A low μ_x of *S. cerevisiae* (0.0189 h⁻¹) in soybean molasses fermentation was also founded by Siqueira et al. (2008).

3.4 Mass balance

Based in ethanol produced in this work, was determined the mass balance, and carbonic gas was estimated by stoichiometry (Equation 14) of ethanol production, where, for each 100 kg of ethanol, 96.65 kg of carbonic gas is also produced. This way, 1000 kg of soybean molasses can produce 103 kg of bioethanol and 98 kg of carbonic gas (Figure 2).

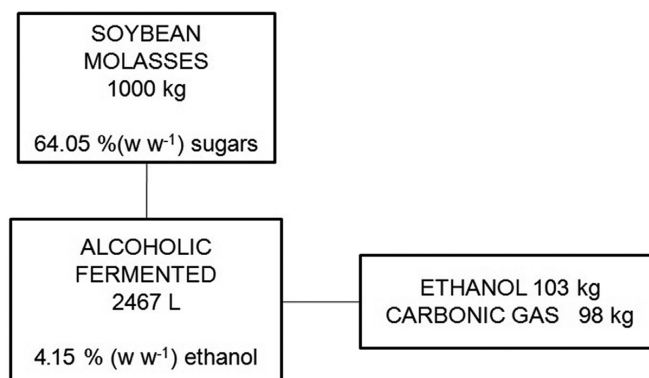
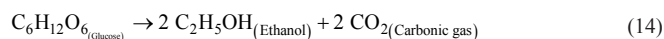


Figure 2. Mass balance from soybean molasses to ethanol.

Table 4. Fermentation yield and productivity for 44 hours of fermentation*.

	Time (h)	dTS (g L ⁻¹)	dEt (g L ⁻¹)	$Y_{p/s}$ (%)	Y_1 (%)	Y_2 (%)	Productivity (g L ⁻¹ h ⁻¹)
Experimental data	44	92.49	41.63	45.01	47.87	88.08	0.946
Mathematical model	44	97.49	41.08	42.14	46.12	82.46	0.934

*dTS, total consumed sugars; dEt, produced ethanol; $Y_{p/s}$, yield from sugar; Y_1 , yield over total initial sugars, Y_2 , yield over consumed sugars.

Table 5. Fermentation kinetic parameters determined by using experimental data and mathematical model*.

	rx (log cells mL ⁻¹ h ⁻¹)	rs (g L ⁻¹ h ⁻¹)	rp (g L ⁻¹ h ⁻¹)	μ_x (h ⁻¹)	tg (h)
Experimental data	0.046	3.960	2.367	0.006	6.850
Mathematical model	0.037	3.559	1.701	0.005	7.908

*rx, cells production rate; rs, sugar consumption rate; rp, ethanol production rate; μ_x , specific growth rate; tg, cells generation time.

4 Conclusion

Increasing soybean molasses concentration presented a positive effect in ethanol production and, despite the high concentration of soluble solids in the fermentative process (30 °Brix), the yeast had a good growing, which favored the yield and production of ethanol from soybean molasses.

The proposed mathematical models showed similar data to practical, mainly, yield and productivity parameters, then, the models can be used for bioethanol production at industrial scale.

Static fermentation is a cheap and simple process so the soybean molasses showed a potential raw material to production of bioethanol.

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