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Bio-ethanol Obtained by Fermentation Process with Continuous Feeding of Yeast

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Abstract. In our ongoing search for renewable energy, a study on the fermentation of starch contained in sorghum and cassava was developed with the addition of commercial yeast for bio-ethanol production. The optimal reaction conditions for starch hydrolysis were determined to obtain the maximum amount of fermentable sugars. In addition, the optimal conditions of sugar concentration and fermentation time for bio-ethanol production were found. Under the applied reaction conditions, the efficiency of sorghum fermentation is higher than that of cassava fermentation. On the other hand, the effect of phosphate inorganic salts added to both fermentation processes increase ethanol production in the sorghum hydrolyzed solution. The conversion yield was higher than 84% at 72 h of fermentation (sorghum 84.10% and cassava 55.80%).
Key words: Bio-ethanol, fermentation process, starch, yeast.

Resumen. En la búsqueda de energía renovable, en el presente trabajo se desarrolló un estudio de la fermentación de almidones de sorgo y yuca adicionando levadura comercial para obtener bio-etanol. Se determinaron las condiciones de reacción óptimas para llevar a cabo la hidrólisis de los almidones y obtener así la máxima cantidad de azúcar fermentable. De igual manera se encontraron las condiciones óptimas de concentración de azúcar y tiempo de fermentación fueron para producir la mayor cantidad de bio-etanol. Bajo las condiciones de reacción aplicadas la eficiencia de fermentación del sorgo es mayor a la de la yuca, permitiendo obtener rendimientos mayores al 84% de conversión (sorgo 84.10% y yuca 55.80%).
Palabras clave: Bio-etanol, procesos de fermentación, almidón, levadura.

Introduction

From ancient times the humankind has used the fermentation process to produce alcoholic beverages as the main product [1]. In the last century, since the 70's, the fermentation process was applied to produce bio-ethanol as an alternative fuel derived from agricultural wastes [1]. Current efforts are focused on the improvement of the reactor conditions to increase bio-ethanol production and batch reactor efficiencies. For example, when the fermentation activity cannot be maintained one must supply yeast cells for every batch resulting in an increment on energy consumption, which adds to the costs of yeast cell preparation [2]. It is known that bio-ethanol production shows economical and environmental advantages over the traditional extraction of fossil fuels such as non-expensive production, high energetic content and low emission of pollutants.

Bio-ethanol can be obtained from sugar or starch fermentation; however, these processes consume more energy than the energy released from its combustion. There are now engines that consume the blend of ethanol and gasoline mixtures as follows: E5 (5% ethanol), E10 (10% ethanol) and E85 (85% ethanol) [1].

Several countries such as Brazil, USA, China, India, and Pakistan have started using bio-ethanol as an alternative fuel. Brazil is the main producer and consumer of bio-ethanol fuel in the world; it presently replaces approximately 40% of gasoline in Brazil representing 3% of gasoline substitution in the world [3-4]. However, the use of agricultural harvests as the source of bio-ethanol production could eventually increase and drive the prices of these sources [5].

At present, bio-ethanol is mainly produced from corn and sugar cane fermentation because its production technologies are cheaper than other fermentation processes and bio-fuels made from vegetable oil. On the other hand, bio-ethanol production from cellulose of stem or leaf is about 33% of all plants matter, following this idea the aim of bio-fuel production projects should be an increase on the use of raw materials harvested in less fertile lands.

Forage sorghum has attracted interest as a potential energy crop. It can be grown under conditions that are unfavorable for corn production and offers high biomass yields. Sorghum plants can grow 6-15 ft tall [6-7]. On the other hand, Cassava (also called yucca or manioc) is mainly used as animal feeding, particularly as an additive to reduce ammonia and fecal odors in animal excreta [8]. Cassava is an important alternative source of starch producing from it the glucose syrups that can be used for ethanol production from either the whole cassava tuber or only from the starch extracted from it [9].

Commercial yeast cells for bakery purpose, *Saccharomyces cerevisiae*, are in non-proliferating state mainly due to nutrients lack [10]. Such cells are physiologically differentiated from those exponentially-divided cells, especially regarding stress resistance [11]. They are able to sustain high viability for extended periods of time in the absence of nutrients; when the starving cells are supplied with nutrients the fermentation takes place under anaerobic conditions.

In this work, bio-ethanol was obtained from sorghum and cassava fermentation studying the effect of three main parameters: addition of two kind of commercial yeast available in México as bakery baking powder during the fermentation process of every raw material, the continuous and regular addition of the same yeast to every fermentation process and the effect of simultaneous addition of inorganic salts during the fermentation with yeast.

Results and discussion

Fermentation of cassava and sorghum

In order to obtain the highest amount of sugars from both raw materials to input the fermentation process for ethanol production, data presented in a previous report [12] on the solubility of each unprocessed material was considered. This report determined that 170 g/L was the optimal concentration of hydrolyzed cassava solution and 200 g/L was the corresponding concentration of hydrolyzed sorghum solution. From these concentrations several dilutions were studied and the ethanol yields are presented in Table 1. It can be observed that the highest yield of ethanol was obtained with 170 g/L of hydrolyzed sugars of cassava and 200 g/L of hydrolyzed sugars of sorghum, respectively. From this data it is possible to calculate the amount of cassava and sorghum needed: only 30% of cassava content is starch while sorghum contains 70% of starch and 700 mL of reaction volume was used in every case. We have:

170 g of cassava starch/0.3 = 566.7 g of cassava in 1 L

But in 0.7 L of solution we used 396.7 g of cassava.

Applying the same calculation for sorghum we found the optimal amount of 200 g.

Two kinds of bakery baking powder trade marks were added in the fermentation of cassava and sorghum for comparison purpose: LEVADA and TRADIPAN, as explained in the experimental section.

Table 1. Concentration of hydrolyzed solutions of cassava and sorghum and ethanol yield.

C_i (g/L)	V_{hs} (mL)	V_f (mL)	C_f (g/L)	% ethanol
170	45	545	14.03	1.034
170	90	590	25.93	4.23
170	135	635	36.14	5.71
170	500	500	170	8.47
200	45	545	16.51	1.07
200	90	590	30.50	6.10
200	135	635	42.51	8.34
200	500	500	200	10.99

C_i = initial concentration, C_f = final concentration
V_{hs} = volume of hydrolyzed solution, V_f = final volume.

Table 2 shows the amount of ethanol obtained from cassava fermentation using these two types of commercial baking powder available in México. These results show that fermentation with LEVADA produces higher contents of ethanol than that with TRADIPAN.

Effect of continuous yeast feeding

To observe the influence on ethanol production of continuous yeast addition during the fermentation processes, 250 mL of every hydrolyzed solution were studied at a constant temperature of 40°C, pH = 6.0 and 2.5 g of yeast was initially added to the solutions, adding the same quantity every 24 h for 72 h. Table 2 shows a marked increase in ethanol yield using LEVADA yeast from 24 to 48 h; which is a two-fold the effect on TRADIPAN under the same time. Considering these results we worked with LEVADA baking powder for the rest of the experiments.

Inorganic salts effect

Table 3 shows the yield of ethanol produced from the fermentation of cassava and sorghum with inorganic salts added: ammonium sulfate, potassium phosphate acid and peptone. We can see that in the case of sorghum, with regular addition of commercial yeast and salts, the ethanol yield increased more than the double from 48 to 72 h while without these additions it is 4.73% lower at 72 h.

The addition of phosphate salt allowed the efficiency of the fermentation process of sorghum as it increases the formation of adenosine triphosphate (ATP). However, this effect is not observed in the cassava fermentation. This could be explained by the displacement of the reaction equilibrium differentiated by the concentration of fermentable sugars in both solutions. In the cassava solution, phosphate ions would be consumed due to the low concentration of fermentable sugars but the opposite case occurs in the sorghum solution where fermentable sugars are found in a higher concentration. The final step of fermentation is exponentially affected by the addition of salts, after that, the salt effect is no longer appreciated and the concentration of fermentable sugar in cassava solution remains constant being lower than the one in sorghum. When the fermentation continues the appearance of the corresponding aldehyde group takes place at the expense of ethanol.

Table 2. Ethanol produced from cassava fermentation process adding two types of yeast.

	Tradipan*	Levada*
time (h)	% v/v ethanol	% v/v ethanol
24	4.62	3.26
48	6.10	8.55
72	9.22	9.57

*2.5 g added every 24 h.

Table 3. Effect of inorganic salts addition during fermentation process on ethanol yield

Exp.	time (h)	Peptone (g)	$(NH_4)_2SO_4$ (g)	KH_2PO_4 (g)	% v/v ethanol Cassava	Sorghum
1	24				3.98	1.50
	48	0.0	0.0	0.0	5.96	3.28
	72				7.32	6.25
2	24				2.38	2.15
	48	7.5	0.5	1.5	4.11	4.60
	72				6.15	10.98

Additionally, the amount of fermentable sugar found in the sorghum hydrolyzed solution is 200 g/L higher than that found in the cassava hydrolyzed solution, it is in agreement with the results of Montaldo [12]. This could also support the observation of higher production of ethanol from the sorghum hydrolyzed solutions.

Reaction efficiency

The stoichiometry of sugar fermentation reaction is

$$C_6H_{12}O_6 + 2P_i + 2ADP \rightarrow 2CH_3\text{-}CH_2OH + 2CO_2 + 2ATP + 25.5\ \text{Kcal}$$

Table 4 shows the theoretical and experimental amount of ethanol produced by both fermentation processes up to 72 hours. At this maximum time the efficiency of sorghum fermentation is 84.10% when LEVADA yeast and salts are added while the efficiency of cassava fermentation is 55.80% at the same conditions. The efficiency of ethanol yield is higher in sorghum fermentation than in cassava fermentation.

Conclusions

Concentrations of 170 g/L and 200 g/L of hydrolyzed sugar solutions of cassava and sorghum are respectively required to obtain the highest yield of bio-ethanol through a fermentation process under the present study reaction conditions.

The best effect on the fermentation of the raw materials obtained with baking powder addition came with LEVADA, so the fermentation processes were carried out using this yeast. The regular addition of yeast every 24 h is important for increasing the yield of bio-ethanol.

The addition of inorganic salts and peptone to the fermentation medium improves the production of ethanol in the case of sorghum source but the cassava fermentation process shows its maximum volume of ethanol without inorganic salts added.

The best ratio of bio-ethanol yield based on the raw materials weighted was found in sorghum since just 200 g delivered almost the double yield of ethanol than the 396.7 g of cassava yield at 72 h of fermentation in salted medium.

Experimental

Hydrolysis of sorghum and cassava

200 g of sorghum and almost the double of cassava, 396.7 g, were milled and hydrolyzed to obtain 140 and 119 g. of starch, respectively. The hydrolysis process was carried out in 700 mL of water adding several drops of concentrated HCl to fix the pH at 1.0 giving the initial concentrations of 170 g/L and 200 g/L of hydrolyzed solutions of cassava and sorghum, respectively. The process was carried out for 6 hours with constant agitation at 90-95°C. From this mixture, the solids were separated; the solution of hydrolysis was neutralized with NaOH at 30% and refrigerated at 8°C until the fermentation process started. This solution obtained from the hydrolysis was used to prepare the diluted solutions shown in Table 1.

Table 4. Yield conversion of ethanol from cassava and sorghum in salted medium.

Starch content	ethanol_{Theo} (mL)	time (h)	% v/v ethanol_{Exp}	ethanol $_{Exp}$ (mL)	% Yield conversion
119 g		24	2.38	16.66	21.60
Cassava	77.13	48	4.11	28.77	37.30
		72	6.15	43.05	55.80
140 g		24	2.15	15.05	16.58
Sorghum	90.74	48	4.60	31.99	35.25
		72	10.98	76.30	84.10

Fermentation process

Several pieces of Tradipan and Levada baking powder were added in the fermentation process. It is well-known that baking powder contains mainly *Saccharomyce cerevisiae* cells as yeast, in some cases contains up to 30% added sucrose or glucose/fructose syrup as a sweetener agent, often combined with fat, powder milk and salt [13]. The baking powder used in this work contains no sweetener agents. 2.5 g of yeast were added to 250 mL of the hydrolyzed cassava solution at 40°C and some amount of NaOH solution at 30% was added to fix the pH at 6. Every 24 h, 2.5 g of yeast was continuously added to the reaction solution, before that an aliquot was collected to analyze the ethanol content in order to follow the conversion reaction.

Additionally, the effect of inorganic salts over the fermentation process was studied. 250 mL of hydrolyzed solution was treated with 2.5 g of yeast, 7.5 g of peptone (Aldrich), 0.5 g of $(NH_4)_2SO_4$ (Aldrich) and 1.5 g of KH_2PO_4 (Aldrich) at pH = 6 imposed by a buffer solution at 40°C. The samples were collected when the fermentation was stopped and were analyzed by gas chromatography without distillation process. Ethanol quantification was carried out by gas chromatography, Agilent 6890 with thermal conductivity detector at 200°C, ATWax Column 30 m × 0.25 mm × 0.25μm was used, injector temperature at 150°C, 1mL/min, Split 200. Ethanol (Aldrich) was used as standard [14].

References

1. Sánchez, O. J.; Cardona, C. A. *Interciencia* **2005**, *30*, 671- 678.
2. Moukamnerd, C.; Kino-oka, M.; Sugiyama, M.; Kaneko, Y.; Boonchird, C.; Harashima, S.; Noda, H.; Ninomiya, K.; Shioya, S.; Katakura, Y. *Appl. Microbiol. Biothecnol.* **2010**, *88*, 87-94.
3. Goldemberg, J. *Biothecnol. Biofuels* **2008**, *6*, 1-7.
4. Lora, E. S.; Andrade, R. V. *Renew. Sust. Energ. Rev.* **2009**, *13*, 777-788.
5. Ferreira-Leitão, V.; Fortes Gottschalk, L. M.; Ferrara, M. A.; Lima Nepomuceno, A.; Correa Molinari, H. B.; Bon, E. P. S. *Waste Biomass Valor* **2010**, *1*, 65-76.
6. Sarath, G.; Mitchell, R. B.; Sattler, S. E.; Funnell, D.; Pedersen, J. F.; Graybosch, R. A. *J. Ind. Microbiol. Biothecnol* **2008**, *35*, 343-354.
7. Vermerris, W.; Saballos, A.; Ejeta, G.; Mosier, N. S.; Ladisch, M. R.; Carpita, N. C. *Crop. Sci.* **2007**, *47*, S-142-153.
8. Cheeke, P. R. *Proc. Phytochem. Soc. Eur.* **2000**, *45* 241-254.
9. Sánchez, O. J.; Cordona, C. A. *Bioresour. Technol.* **2008**, *99*, 5270-5295.
10. Fuge, E. K.; Werner-Washburne, M. *Stationary phase in the yeast Saccharomyces cerevisiae* In *Yeast Stress Respons*. Eds S. Hohmann, W. H. Mager, Chapman & Hall, New York.
11. Werner-Washburne, M.; Braun, E.; Johnston, G. C., Singer, R. A. *Mircrobiol. Rev.* **1993**, *57*, 383-401.
12. Montaldo, A. *La yuca frente al hambre del mundo tropical* Ed. Universidad Central de Venezuela, 1996.
13. Sultan W. J. *Sweet yeast dough products*. In *Practical baking*, 5th eds., van Nostrand Reinhold, New York, 1990, pp. 217-327.
14. Vivanco Loyo, P; Juárez Gutiérrez, M. Bases de diseño para planta productora de bioetanol a partir de sorgo y yuca; Universidad Nacional Autónoma de México, Facultad de Química, 2009.