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Evaluation of Cashew Apple Juice for the Production of Fuel Ethanol

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Abstract A commercial strain of *Saccharomyces cerevisiae* was used for the production of ethanol by fermentation of cashew apple juice. Growth kinetics and ethanol productivity were calculated for batch fermentation with different initial sugar (glucose + fructose) concentrations. Maximal ethanol, cell, and glycerol concentrations were obtained when 103.1 g L^{-1} of initial sugar concentration was used. Cell yield ($Y_{X/S}$) was calculated as 0.24 (g microorganism)/(g glucose + fructose) using cashew apple juice medium with 41.3 g L^{-1} of initial sugar concentration. Glucose was exhausted first, followed by fructose. Furthermore, the initial concentration of sugars did not influence ethanol selectivity. These results indicate that cashew apple juice is a suitable substrate for yeast growth and ethanol production.

Keywords Ethanol · Cashew apple juice · *Saccharomyces cerevisiae* · Batch cultivation · Kinetic parameters

Introduction

One of the greatest challenges for society in the twenty-first century is to meet the growing demand for energy for transportation, heating, and industrial processes and to provide raw material for the industry in a sustainable way. An increasing concern for the security of oil supply has been evidenced by increasing oil prices, which, during 2006, approached US$80 per barrel [1]. More importantly, the future energy supply must be fulfilled with a simultaneous substantial reduction of green house gas emissions [2]. Ethanol satisfies that

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requirement because its production and combustion do not contribute significantly to the total amount of carbon dioxide in the atmosphere [3].

Ethanol has already been introduced on a large scale in Brazil, USA, and some European countries, and it is expected to be one of the dominating renewable biofuels in the transport sector within the coming 20 years. Ethanol can be blended with petrol or used as neat alcohol in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization; furthermore, it is an excellent fuel for future advanced flexfuel hybrid vehicles [1]. Nearly all fuel ethanol is produced by fermentation of sucrose in Brazil or corn glucose in the USA; however, these raw material bases will not be sufficient to satisfy the international demand [4].

A very common argument against ethanol is its economic competitiveness against fossil fuels. Nevertheless, Goldemberg et al. [5] demonstrated, through the Brazilian experience with ethanol, that economy of scale and technological advances can lead to increased competitiveness of this renewable alternative, reducing the gap with conventional fossil fuels. Consequently, there is an intensified interest in the study of all the steps involved in ethanol production to reduce costs [6].

Impelled by the creation of the "Proálcool" project in the 1970s and 1980s, Brazil has become, in 2005–2006, the world's largest ethanol producer via fermentation, developing and improving many fermentative processes [5] using sugar cane as a source of sucrose. In the northeast of Brazil, however, the volume of ethanol produced does not represent an important amount compared to the national production. So, the optimization of alternative low-cost processes is imperative. In the state of Ceará, the cashew agroindustry has an outstanding role in the local economy. However, only 12% of the total peduncle, the part of the tree that connects it to the cashew nut, is processed and it does not play an important role in the economy of the state. Furthermore, the majority of the cashew apple production spoils in the soil. Those facts, together with its rich composition (reducing sugar, fibers, vitamins, and minerals salts), turns cashew apple juice (CAJ) into an interesting and inexpensive (R$1.00/kg) culture medium [7].

Cashew is produced in around 32 countries of the world, and the major cashew apple-producing countries and their production figures in the year of 2004, based on the Food and Agriculture Organization [8], are, approximately, Vietnam, 8.4 million tons; Nigeria, 5 million tons; India, 4 million tons; Brazil, 1.6 million tons; and Indonesia, 1 million tons. The official estimate for the Brazilian cashew crop for 2006/2007 is around 266 million tons [9], which accounts for 11% of the world production and corresponds to more than 2 billion tons of cashew apple. Considering that the use of agroindustrial residues can contribute for the reduction of production costs, cashew apple appears as an alternative raw material for ethanol production, due to its vast availability and high concentration of reducing sugars. Therefore, the aim of this work was to investigate the potential use of this alternative substrate (CAJ) as a carbon source for ethanol production by *Saccharomyces cerevisiae* (commercial strain). Experiments were conducted in a batch bioreactor and the process was monitored by measuring biomass, glucose, fructose, and ethanol concentrations.

Materials and Methods

Microorganism

The microorganism used was a commercial yeast *S. cerevisiae* Saf-Instant (SAF Argentina, Buenos Aires, Argentina).

CAJ Preparation

CAJ was extracted by compressing the cashew apple (*Anarcardium occidentale* L.). After compressing, the juice was centrifuged at 3,500 rpm for 20 min (BIO ENG, BE-6000, Piracicaba, São Paulo, Brazil).

Media and Fermentation Assays

The medium for fermentation consisted of CAJ supplemented with the following components: $MgSO_4$ 0.65 g L^{-1}, KH_2PO_4 0.50 g L^{-1}, $(NH_4)_2SO_4$ 2.50 g L^{-1}, and $ZnSO_4$ (0.65 g L^{-1}). It was sterilized in autoclave (Phoenix, Araraquara, São Paulo, Brazil) at 110 °C for 10 min, and the initial pH was adjusted to 4.50 by using 1 N HCl. Batch fermentation was carried out in 500-ml Erlenmeyer flasks with 250 ml of medium in a rotary shaker TE240 (Tecnal, Piracicaba, São Paulo, Brazil) at 30 °C and 150 rpm. The initial yeast concentration inoculated into the fermentation medium was 10 g L^{-1} using dry baker yeast. Samples were collected at time-defined intervals and submitted to analysis.

Influence of CAJ (glucose + fructose) Concentration on Ethanol Production by *S. cerevisiae*

The effects of initial concentrations of glucose + fructose, present in CAJ, were examined. Different initial sugar concentrations (glucose + fructose) were evaluated, from 24.4 to 103.1 g L^{-1}. The initial sugar concentration was achieved by diluting or concentrating the CAJ, which has a natural concentration around 80 g L^{-1}. For each initial sugar concentration, specific microbial growth rates (μ_x), specific ethanol production rates (μ_p), maximum ethanol (P_{em}) or glycerol concentration (P_{gm}), maximum dry weight (X_m), ethanol ($Y_{P/So}$), and cell yields ($Y_{X/So}$) were calculated (variables are listed in the Appendix).

Analytical Methods

Biomass Content

Cell concentration was determined by dry weight [10]. Samples were taken from the fermentation media at certain time intervals and centrifuged at 3,000 rpm for 30 min in a BE-6000 centrifuge (BIO ENG). The pellet was dried at 80 °C on a Tecnal TE-397/4 stove (Tecnal) until constant weight was achieved. Supernatant was used for glucose, fructose, ethanol, and glycerol analysis.

pH Measurement

The pH of the fermentation medium was measured using a Tec-3MP model pH meter (Tecnal, Campinas, São Paulo, Brazil).

Glucose, Fructose, Ethanol, and Glycerol Concentrations

Substrate concentration (glucose and fructose) and product concentration (ethanol and glycerol) were measured by high-performance liquid chromatography (HPLC) using a Waters HPLC system (Waters, Milford, MA, USA) equipped with a refractive index Waters 2414 detector and a Shodex Sugar SC1011 column 8.0×300 mm (Shodex, Kawasaki,

Kanagawa, Japan). Water MiliQ (simplicity 185, Millipore, Billerica, MA, USA) was used as solvent with a flow rate of 0.6 mL min^{-1} at 80 °C. Samples were identified by comparing the retention times with those of carbohydrate, ethanol, and glycerol standards.

Yield Coefficients and Selectivity

The experimental data of products, substrates, and cells concentrations in a given period of time were used to calculate the yields in product ($Y_{P/S}$) and cells ($Y_{X/S}$) related to substrate and the specific rates for growth (μ_x), substrate consumption (μ_S), and product formation (μ_p).

Equations 1 and 2 represented a productivity (Pm) and ethanol selectivity (Se), respectively.

$$\mathrm{Pm} = \frac{C_{im} - C_{i0}}{t} \tag{1}$$

Where i stands for ethanol or glycerol, C_{i0} is the initial concentration, C_{im} is the highest i concentration, and t is the fermentation time when the highest i concentration is achieved.

$$\mathrm{Se} = \frac{C_{\mathrm{ETHANOL}}}{C_{\mathrm{GLICEROL}}} \tag{2}$$

Results and Discussion

In a previous work [11], CAJ was characterized in terms of physical–chemical parameters, and results showed it was rich in glucose, fructose, and several amino acids. However, some macro- and micronutrients were not present at the desired level for ethanol production and had to be supplemented. In this work, the effect of initial sugar concentrations (glucose + fructose − S_0) on ethanol production was investigated in the range of 24.4–103.1 g L^{-1}. Figure 1 shows the experimental results obtained for substrate consumption and ethanol and glycerol production and dry weights of the strain during fermentation time for each substrate concentration studied. It can be observed that, for all initial sugar concentrations evaluated, the biomass concentration with time is a typical curve of microbial growth. Furthermore, log phase growth occurred between 4 and 6 h for CAJ medium.

The yeast consumed both glucose and fructose, sugars that are present in CAJ, to produce ethanol and glycerol (Fig. 1), but glucose was exhausted first, followed by fructose. Maximum ethanol concentration, 44.4±4 g L^{-1}, was obtained when 103.1 g L^{-1} of the initial sugar concentration was used; however, higher productivity, 9.71±0.3 g L^{-1}, was achieved with 87.7 g L^{-1} (Fig. 1, Tables 1 and 2) of the initial concentration of substrate. Highest ethanol concentration was obtained after 4 h of fermentation in the medium with an initial sugar concentration of 87.7 g L^{-1} and after 6 h in the medium with an initial sugar concentration of 103.1 g L^{-1}. This result is probably due to yeast metabolism, which may be inhibited by the substrate or suffer from glucose repression. It can be observed that the rate of sugar consumption is very low when S_0=103.1 g $L^{-1}$was used; fructose and glucose concentrations remained almost constant for 2 h at the beginning of fermentation. Other authors [12] found that the growth of *S. cerevisiae* is inhibited equally by glucose and fructose. Another possibility is the presence of other chemicals that are partially inhibitory to the yeast fermentation, which had its concentration enhanced when concentrating the juice.

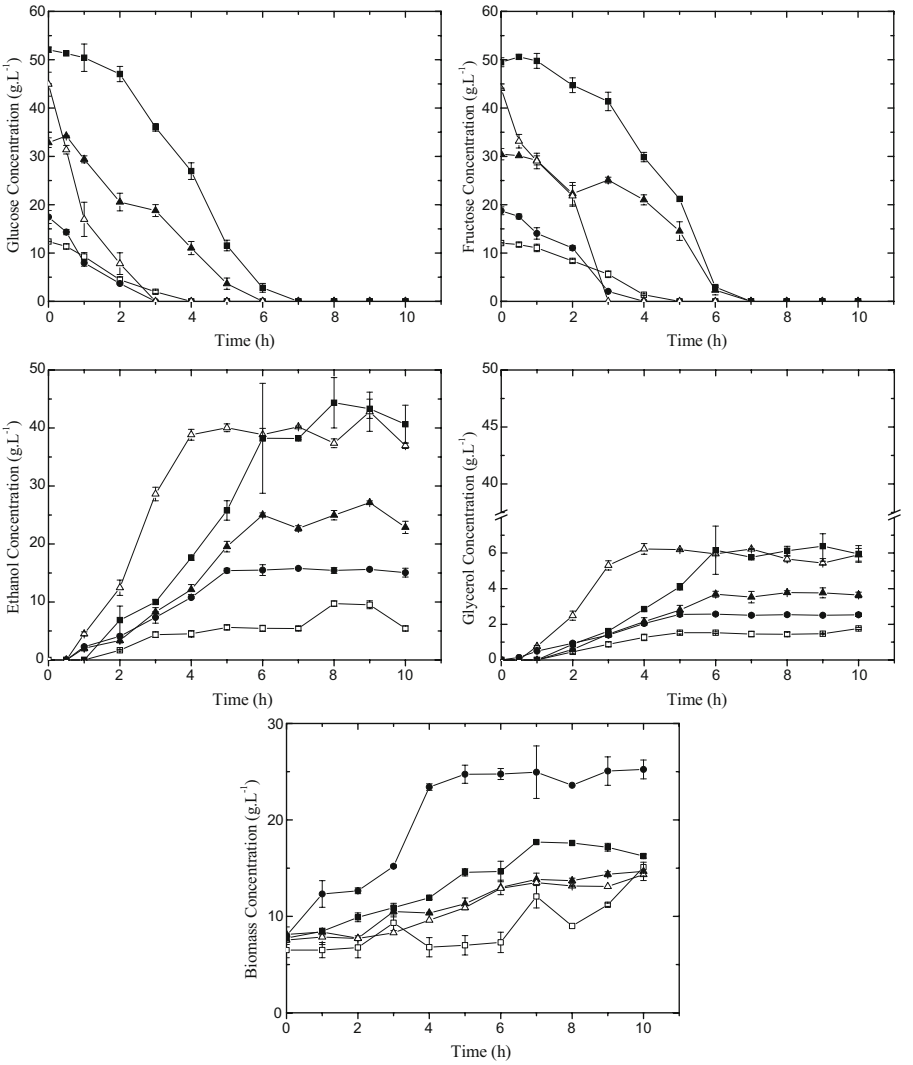

Fig. 1 Effect of sugar (glucose + fructose) on ethanol production by *S. cerevisiae* at 30 °C and 150 rpm. Initial sugar (glucose + fructose) concentration: *open squares*, 24.4; *closed circles*, 41.3; *closed triangles*, 62.9; *open triangles*, 87.7; and *closed squares*, 103.1 g L^{-1}. Data points represent the mean and standard deviation from at least three separate experiments

It can be observed that glycerol was produced in all assays (Fig. 1 and Table 2), and maximum glycerol concentration was obtained (5.8±0.1 g L^{-1}) when 103.1 g L^{-1} of initial sugar concentration was used. Glycerol is produced and accumulated in the yeast cell as a response to osmotic stress. In addition to osmotic regulation, glycerol also has a role in the redox balance of the yeast cell. Under anaerobic conditions, glycerol is formed to reoxidize the NADH formed in anabolism and in the synthesis of organic acids [13, 14]. Yalçin and Özbas [15] evaluated grape juice as a medium for glycerol production; their obtained maximum glycerol concentration and dry weight were 14.1 and 8.0 g L^{-1}, respectively. Although the objective of this work is to produce ethanol, glycerol has been an important

Table 1 Effect of initial sugar (glucose + fructose) concentration (S_0) on ethanol and glycerol productivity (Pm) and ethanol selectivity (Se) during fermentation of CAJ by *S. cerevisiae* at 30 °C and 150 rpm.

S_0 (g L^{-1})	Productivity (g L^{-1} h^{-1})[a]		Selectivity (Se)
	Ethanol	Glycerol	
24.4	1.455±0.06	0.255±0.01	5.86±0.3
41.3	3.082±0.02	0.429±0.01	6.18±0.1
62.9	4.168±0.22	0.418±0.03	6.70±0.5
87.7	9.708±0.25	0.889±0.00	6.64±0.2
103.1	6.370±0.38	0.747±0.02	6.64±0.6

[a] Values were calculated at maximum ethanol and glycerol concentrations.

industrial material with different uses in many industries, such as food, pharmaceutical, cosmetics, drug, toothpaste, leather, textile, tobacco, and other industries [15]. Many growth factors affect glycerol metabolism of yeast cells, such as the type of substrate and the initial substrate concentration, temperature, pH, inoculation rate, aeration rate, nitrogen source, etc. [15]. It is reported that an increase in the initial sugar concentration enhances glycerol production because of osmotic stress. In this study, however, initial sugar concentration had no important effect on ethanol selectivity (Table 1).

Specific growth rates (μ_x), specific substrate rates (μ_S), and ethanol production rates (μ_p) were calculated, and results are shown in Fig. 2 for S_0=103.1 g L^{-1}. Similar profiles were obtained for the other initial substrate concentration studied (data not shown). As can be observed, specific growth rates, substrate consumption, and product formation followed a typical pattern for ethanol fermentation [16]. The specific rate of substrate consumption (μ_S) and ethanol production (μ_p) present similar profiles, thus correlating it very well. The specific growth rate (μ_x) presents, approximately, the same course of the others two curves. Then, ethanol formation is associated with growth, consumption of substrate, and catabolism reaction, typical of a primary metabolite (Fig. 2).

Table 2 shows the effect of initial sugar (glucose + fructose) concentration on maximum ethanol concentration (C_{em}), together with dry weight (X_m) and glycerol concentration (C_g) when C_{em} was achieved. Table 3 shows the results on cell ($Y_{X/S}$), ethanol ($Y_{Pe/S}$), and glycerol ($Y_{Pg/S}$) yield coefficients, as well as those on ethanol yield that were calculated considering theoretical yield ($Y_{Pe/S}$=0.511) as reference [17]. The specific growth rate (Table 3) increased almost twofold when sugar concentration was increased from 24.4 to 87.7 or 103.1 g L^{-1}. However, specific growth rate remained almost constant when sugar concentration was increased from 87.7 to 103.1 g L^{-1}. The biomass yield ($Y_{X/S}$) in the media decreased from 0.228 to 0.091 g per gram of sugar concentration. Other authors [17]

Table 2 CAJ fermentation by *S. cerevisiae* at 30 °C and 150 rpm: maximum ethanol (C_{em}), glycerol (C_{gm}), and biomass concentrations (dry weight) for media with different initial concentrations of sugar (glucose + fructose).

S_0 (g L^{-1})	X_0 (g L^{-1})	X_m (g L^{-1})	C_{em} (g L^{-1})	C_g (g L^{-1})	ΔX (g L^{-1})	ΔS (g L^{-1})
24.4	6.50±0.8	12.07±1.2	9.7±1	1.4±0.1	5.57	24.4
41.3	8.00±0.9	16.85±4.9	15.6±0	2.5±0.1	8.85	41.3
62.9	8.13±0.1	14.67±1.0	27.1±0	3.8±0.1	6.54	62.9
87.7	7.55±0.1	14.33±0.1	42.8±3	5.4±0.1	6.78	87.7
103.1	7.75±0.1	17.17±0.4	44.4±4	5.8±0.1	9.42	103.1

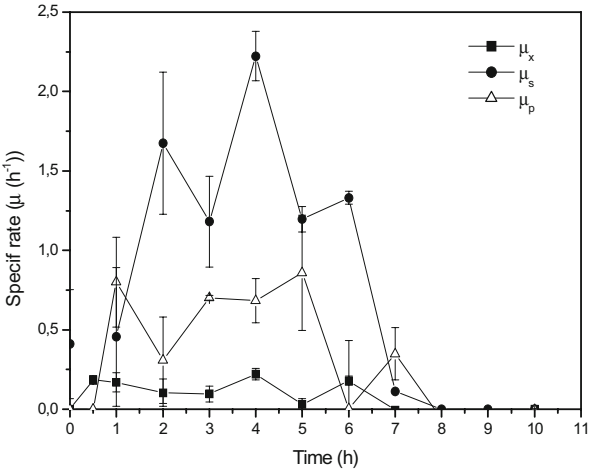

Fig. 2 Specific rates of growth (μ_x), substrate consumption (μ_S), and ethanol production (μ_p) for the fermentation of CAJ medium by *S. cerevisiae* at 30 °C, 150 rpm, and initial sugar (glucose + fructose) concentration of 101.05 g L^{-1}

observed the same behavior for biomass yield, which decreased from 0.217 to 0.075 g per gram of sugar concentration when sucrose concentration was increased from 34.6 to 257.4 g L^{-1}, and they attributed the decrease on $Y_{X/S}$ to growth inhibition due to high substrate concentrations. Ethanol yield varied from 74 to 95.5% of the theoretical values, the highest value being achieved when 87.7 g L^{-1} of initial sugar concentration was used.

Conclusion

Maximum ethanol concentration was obtained (≅44 g L^{-1}) when 87.7 and 103.1 g L^{-1} of initial sugar concentration was used in the fermentation medium. Specific rates of growth, substrate consumption, and product formation followed a typical pattern for ethanol fermentation. Maximum ethanol yield (0.488 g g^{-1}) and productivity (9.71 g L^{-1} h^{-1}) were obtained when 87.7 g L^{-1} of initial sugar concentration was used. Finally, initial sugar concentration had no important effect on selectivity. The results obtained indicate that CAJ is a suitable substrate for ethanol production. Moreover, the use of the CAJ as a medium will not only reduce the cost of the resulting ethanol production, but it will also make use of an agricultural waste that is otherwise discarded in the field.

Table 3 Effect of initial sugar (glucose + fructose) concentration on specific growth rates, yield coefficients and ethanol yield during CAJ fermentation by *S. cerevisiae* at 30 °C and 150 rpm.

S_0 (g L^{-1})	X_0 (g L^{-1})	$Y_{X/S}$ (g g^{-1})	$Y_{X/Pe}$ (g g^{-1})	$Y_{Pe/S}$ (g g^{-1})	$Y_{Pg/S}$ (g g^{-1})	Specific growth rate, μ_x (h^{-1})	Ethanol yield[a] (%)
24.4	6.50±0.8	0.228	0.574	0.398	0.059	0.061±0.02	77.8
41.3	8.00±0.9	0.214	0.567	0.378	0.061	0.071±0.01	73.9
62.9	8.13±0.1	0.104	0.241	0.431	0.060	0.078±0.01	84.3
87.7	7.55±0.1	0.077	0.158	0.488	0.062	0.115±0.01	95.5
103.1	7.75±0.1	0.091	0.212	0.431	0.056	0.120±0.01	84.3

[a] Values were calculated as a comparison between experimental and theoretical values of ethanol yield coefficients ($Y_{Pe/S}$).

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Appendix

Ks	substrate saturation parameter (g L^{-1})
P_{max}	product concentration when cell growth ceases (g L^{-1})
S	substrate concentration (g L^{-1})
X	biomass concentration (g L^{-1})
X_{max}	biomass concentration when cell growth ceases (g L^{-1})
$Y_{P/X}$	yield of product based on cell growth (g/g)
$Y_{X/S}$	yield of cell growth based on substrate consumptions (g/g)
$Y_{P/S}$	yield of product based on substrate consumptions (g/g)
C_{em}	maximum ethanol concentration (g L^{-1})

Greek letter

μ_{max}	maximum specific growth rate (h^{-1})
μ_p	specific rate of product formation (g/[L.h])
μ_S	specific rate of substrate consumption (g/[L.h])
μ_x	specific rate of growth (g/[L.h])

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