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Bioethanol production from coffee mucilage

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Abstract

Chiapas is one of the largest coffee-producing states in Mexico. In this industry, there are large quantities of waste, which are toxic and harmful to the environment. During the extraction process of coffee bean the waste generated are: pulp, mucilage and parchment. Recently, investigations have been done to utilize these residues for bioenergy generation. This paper provides an overview of coffee and one of its major industrial wastes. The objective of this research was the production of bioethanol from coffee mucilage at laboratory scale and extract and characterize the substrate used as raw material and establish a fermentation process for the production of bioethanol. Our results show the kinetics of fermentation of coffee mucilage as a substrate to yeast *Saccharomyces cerevisiae*. The cell density, concentration of ethanol, reducing sugar consumption, physico-chemical variables such as pH and temperature were analyzed. The Fermentation parameters such as growth rate, saturation constant and yields were estimated.

Keywords: Coffee mucilage; fermentation; bioethanol.

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1. Introduction

The law of promotion and development of bioenergy passed on February 2008 indicates mandatory use of ethanol as a gasoline oxygenator with a minimum value of 2% of the volume produced [3]. Ethanol demand could be around 39.2 million liters a year. Mexico covers only 20% of the demand for PEMEX obtaining ethanol from sugarcane. Sugar cane as raw material is very costly and is restricted to areas with special soil for it.

Coffee is one of the most consumed beverages in the world and is the second most traded commodity after oil [1]. Due to the demand for this product in the coffee industry, large amounts of waste are generated, which are toxic and harmful to the environment. During the coffee processing we obtain the following wastes: pulp, mucilage, coffee husk etc [2].

In some countries, mucilage of coffee has been used for extracting pectin. However, this residue is not used in Mexico, but directly discharges to the environment. When released to the environments it causes water pollution due to its toxic nature.

Recently, attempts have been made to utilize these wastes to produce energy. Fermentation is one of the most important processes for agro-waste reuse producing yeast and clean fuels [2]. This process does not require the use of toxic substances; this makes it an environmentally friendly process.

Bioethanol is produced from biological way by alcoholic fermentation of simple sugars, which is a strong candidate to replace fossil fuels. The advantages over fossil fuels are: they are clean, renewable, have a more complete combustion and less waste in general [4]. The development of biofuel technology also seeks to solve the current energy problem, substantially reducing economic dependence on fossil fuels.

2. Materials and methods

2.1. Substrate

The coffee mucilage was extracted using a pulper located in the municipality of Yajalón, Chiapas. The substrate had a reducing sugar concentration of 60 g/L.

This was supplemented with the following: salt, 0.02 g/L of magnesium sulfate ($MgSO_47H_2O$), 0.2 g/L of ammonium phosphate ($(NH_4)_2SO_4$) and 2 g/L of yeast extract [5].

2.2. Microorganism

The yeast *Saccharomyces cerevisiae* Y2034 [6] was graciously donated by the ceparium of the Agroindustrial engineering laboratory of the Universidad Politécnica de Chiapas.

The strain was grown in YPD medium (Yeast extract 1%, peptone 2%, dextrose 2%). The cells were grown to 28º C during 48 h [5,7].

2.3. Fermentation process

The fermentation process was carried out by triplicate in a flask of 250 mL with constant stirring at 150 rpm, pH of 5 and temperature of 30º [8]. The final volume of the medium used for the fermentations was 150 mL which was stirred during 16 h, with initial cell concentration of 1E+6 ufc/mL [9].

2.4. Analytic methods

During the fermentation, the pH and temperature were measured. The sugar concentration was determined by Miller method [10]. Viable cell counts were done with Neubauer chamber adapted with optical microscopy [11]. Trypan blue dye was used as the vital cells [12]. To determine the bioethanol concentration the sample was taken in the aqueous phase. It was centrifuged at 3000 x g during 15 minutes at 5º C. The supernatant solution was changed to another tube and the precipitate was removed.

2.5. Experimental design

The experimental design was the response surfaces with a factor of an input variable concentration of reducing sugars and an output variable, the kinetic growth (μ). 60 g/L was the maximum concentration of reducing sugar diluted in coffee mucilage. Two series of 3 experiments were carried out. The Design Expert software version 8.0.7.1 was used for the experimental design [13].

2.6. FTIR analysis

The samples were taken from the exhaust and distillate medium. Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the samples. This technique is used to obtain infrared spectrum of adsorption, emission, photoconductivity or Raman spectrum of solid, liquid or gas. Samples EtOH 20, 40, 60 were distilled. Samples EtOH 20, 40, 60 medium exhaust were taken after fermentation.

3. Results

The series of experiments designed are shown in the following table.

Table 1. Layout design

	Factor 1	Response 1
Treatments	Reduction sugar concentration (g/L)	Kinetic growth (μ)
1	20	
2	40	Experimental variable
3	60	

Kinetics growth of *Saccharomyces cerevisiae* Y2034 yeast is displayed in figure 1. It was observed that higher concentration of sugars increased cell concentration. Lag phase is observed in the time interval from 0 to 4 hours, the exponential phase lasted for 8 hours (4 to 12 hours) and the death phase 4 hours (12 to 16 hours). 60 g / L of reducing sugar initial cell concentration was higher, reaching 4.0E+8 cfu/mL.

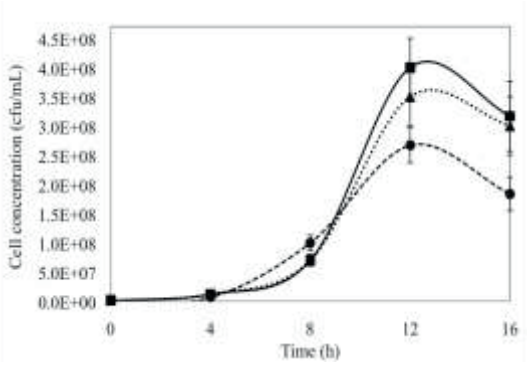

Fig. 1. Yeast growth kinetics of *Saccharomyces cerevisiae* Y2034 for three different initial concentrations of reducing sugars (■ 60 g/L, ▲ 40 g/L, ● 20 g/L).

Figure 2 shows the consumption of reducing sugars with different concentrations of yeast. This consumption was 19,263, 39,263 and 59,053 g/L respectively. This means the yeast consumed 96.315, 98.157 and 98.421% and was converted into alcohol.

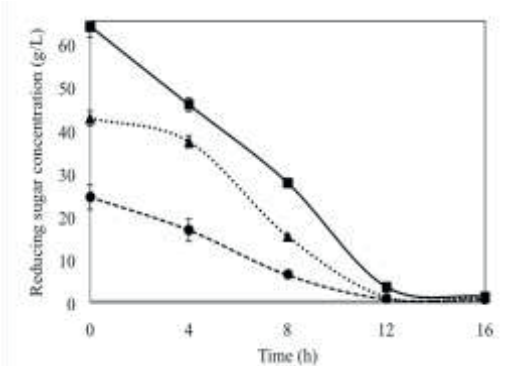

Fig. 2. Kinetics of consumption of reducing sugar with three different initial concentrations. (■ 60 g/L, ▲ 40 g/L, ● 20 g/L)

According to the analysis of sugar concentration and growth rate of the yeast, the behaviour of the graph in figure 3 shows that for 20 to 40 g/L sugar concentration the growth speed increased from 0.344 to 0.361 h^{-1} and for 40 to 60 g/L sugar concentration the growth speed increased from 0.361 to 0.367 h^{-1}, the maximum growth rate calculated by the Monod equation being 0.380 h^{-1} with a saturation constant (K) equal to 5,615 g/L.The saturation constant gives us an idea of the affinity of yeast by the substrate. The lower K_s the higher affinity. Ilona et al 2001 [14] report glucose K_s 2.31 g/L which mean that the yeast *Sacchoromyces cerevisiae* Y2034 is affined to coffee mucilage.

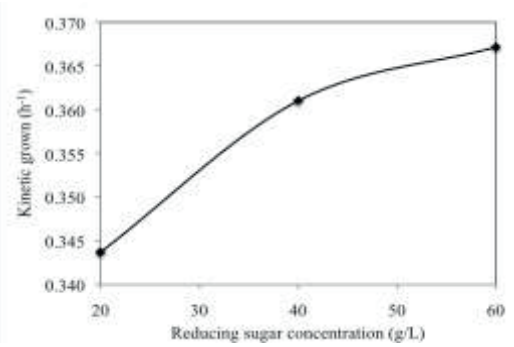

Fig. 3. Effect of the concentration of sugars on the net growth rate of the yeast *S. cerevisiae* Y2034 (1.0E+6 cfu/mL initial).

The following table summarizes the results obtained.

Table 2. Kinetic parameters [15, 16]

X (cfu/mL)	S (g/L)	μ (h^{-1})	td (h^{-1})	K_s (g/L)	$^{1}/_{\mu} = \mu_m$ (h^{-1})
1.0E+06	60	0.3672	1.8879		
1.0E+06	40	0.3615	1.9176	2.136	0.3805
1.0E+06	20	0.3437	2.0169		

X - cell concentration

S - sugar concentration

μ - Kinetic growth

Td - doubling time

K_s - saturation constant (substrate mass/ volume unit)

μ_m - maximum kinetic growth

According to the infrared spectrum it can be seen the absorption bands around 3400 cm^{-1}, which are associated with the vibration modes of the hydroxyl group (OH) characteristic of alcohols. At 2900 m^{-1} is the absorption of alkanes (CH), near 2100 cm^{-1} is the alkyne group (C ≡ C), around 1700 cm^{-1} is the ester absorption and at 1650 cm^{-1} is the group belonging to the alkene (C = C).

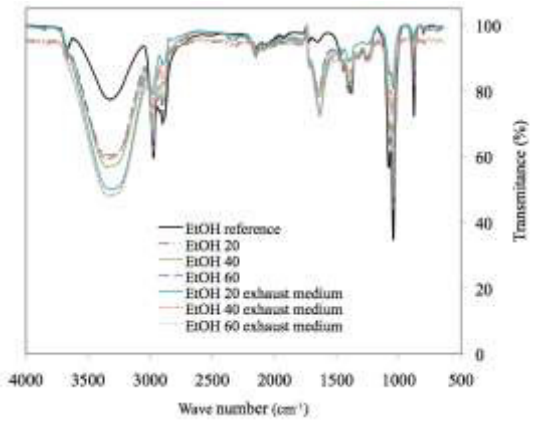

Fig. 4. Bioethanol infrared spectrum

4. Conclusion

This study established and developed a methodology for the production of bioethanol from coffee mucilage. The consumption of sugars in the different concentrations is approximately 98%, which means the yeast converted reducing sugar in ethyl alcohol. Kinetic analysis under yeast K_s has a 2.136 g/L, means coffee mucilage is akin to the substrate. FTIR analysis shows the infrared spectrum with absorption bands corresponding to ethyl alcohol. The mucilage of coffee has potential to produce bioethanol.

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