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Ethanol Production by Fermentation from Waste Coffe Mucilage

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ABSTRACT *In order to take advantage of residues generated in coffee industries, Coffee mucilage was used as substrate to produce bioethanol. The microorganism used for the fermentation process was Sacharomyces cerevisiae. Batch fermentation experiments were carried out with 20 tests using central composite design with 5 levels leadings to evaluate the effect of pH, temperature and initial sugar, respect to yield in the carbohydrates conversion to bioethanol. The best condition in the tests was: pH 5.5, temperature 28 ºC, initial sugar 35 g, sugar consumed 98% and bioethanol produced 10.93 g, with yield 0.32 g ethanol per gram of sugar and 0.025 g ethanol per gram of coffee mucilage.*

1. Introduction

Coffee is a drink that is made from the roasted and ground fruit of the coffee plant seeds. Inside of different extraction methods, mechanical extraction of coffee grain reduces the amount of water used, in consequence allowed recovering the mucilage fraction (Belitz et al., 2009; González-Ríos et al., 2007; Joët et al., 2010).

Coffee mucilage is a viscous liquid residue generated by this process. Because of carbohydrate content (Avallone et al., 2000; Avallone et al., 1999) it can be used as substrate to produce biofuels: bioethanol and biogas. Bioethanol is an alternative to fossil fuels, it is made by fermentation with microorganism (Kataria and Ghosh, 2011), and it can be used as a blend in regular gasoline, as means of lowering the carbon dioxide emissions from the transport sector (Corro and Ayala, 2008).

2. Materials and methods

2.1 Samples Preparation

Coffee mucilage (CM) was extracted manually, the composition was 4 kg of coffee cherry per liter of water, initial pH was 4.5 and supplemented with 0.5 g/L ammonium sulfate as nitrogen source (Breisha, 2010). The CM was centrifuged at 7,000 rpm for 10 min and pasteurized (Chuck-Hernández et al., 2009).

2.2 Microorganism

The yeast used was *Saccharomyces cerevisiae* NRRL Y-2034. The strain was maintained in YPD agar (1% yeast extract, 2% peptone, 2% glucose, and 2% w/v agar) slants at 4°C and fresh cultures 48 h in YPD were used as inocula (Okuda et al., 2008). Strain was cultured in 250 mL shake erlenmeyer flask stirred at 200 rpm at 28°C (Boluda-Aguilar and López-Gómez, 2013). Growth proceeded overnight for 24 h to allow cell growth to exponential phase, after it was centrifuged at 10,000 rpm for 5 min and the cells were suspended in the fermentation medium.

2.3 Fermentation

Batch fermentation experiments were carried out according to the experimental design in serological bottles of 100 mL, stirring with a shaker with controlled temperature at 200 rpm during 48 h (Kwon et al., 2013). Cell density was adjusted to an optical density of 0.5 (600 nm) (Zheng, 2011). Were taken culture samples of 1 mL every 3 h and centrifuged at 10,000 rpm for 10 min. The fermentation yield was calculated assuming 0.51g ethanol/glucose in the culture medium.

2.4 Experimental design

Twenty (20) tests were realized using central compose design, full factorial with five levels leadings to evaluate the effect of pH (4.5-5.5) (Shanavas et al., 2011), temperature (28°-38 °C) (Li et al., 2009) and the initial sugar (35-65 g) as independent variables of the fermentation (Statistical Software Design expert, 7.00). The experimental design is shown in Table 1.

2.5 Analysis

The reducing sugar concentration was determined by dinitrosalicilic acid method (Miller, 1959) using glucose as standard. The sugars, bioethanol and other compounds were determined by the high-performance liquid chromatography (HPLC), using a Phenomenex column eluted at 60°C at a flow rate of 0.5 mL/min and having a RID. Minerals were determined by ICP-OES (Inductively coupled plasma–Optic emission spectroscopy) device.

3. Results and discussion

The composition in CM was reducing sugar 35.15 g, 37.67 g galactose, 35.65 g glucose, and 1.06 g lactose. According to the ICP-OES analysis, CM contains several minerals. Potassium was the most abundant element 239.9 mg/L, followed by phosphorus 41.55 mg/L, calcium 37.08 mg/L, sulfur 30.19 mg/L and magnesium 10.05 mg/L.

Table 1. Experimental design

Test	Factor X_1	Factor X_2 (ºC)	Factor X_3 (g/L)
1	5.05	32.5	50

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2	5.05	32.5	50
3	5.05	32.5	50
4	5.05	32.5	50
5	5.05	32.5	50
6	5.05	32.5	50
7	5.05	39.36	50
8	5.05	32.5	72.87
9	6.04	32.5	50
10	5.05	32.5	27.13
11	4.06	32.5	50
12	5.05	25.64	50
13	5.7	28	65
14	5.7	28	35
15	4.4	37	65
16	4.4	28	35
17	4.4	37	35
18	5.7	37	65
19	4.4	28	65
20	5.7	37	35

Factor X_1: pH, factor X_2: temperature, factor X_3: initial sugar.

Table 2 shows a summary of the results of SC, BP, gBEtOH/gS, gS/gCM and gBEtOH/gCM. BP values varied in the range 8.28 to 14.93 g, the highest predicted BP of 14.93 g was attained when the pH, temperature and initial sugar were 5.05, 32.5 °C and 50 g, respectively. gBEtOH/gS values varied in the range 0.19 to 0.32 g for the tests corresponding to 8 and 14, the maximum predicted gBEtOH/gS of 0.32 g was attained when the pH, the temperature and the initial sugar were 5.7, 28°C and 35 g, respectively. gS/gCM values varied in the range 0.042 to 0.112 g, the maximum predicted was attained when the initial sugar was the highest. gBEtOH/gCM values varied in the range 0.021 to 0.036 g for the tests corresponding to 10 and 8 respectively.

In Figure 1 all tests, the sugar consumed was higher than 97 %, while bioethanol produced was higher than 36 %. Test 8 has the highest concentration of sugar, although it is mostly consumed, it has the least amount of bioethanol produced relative to the theoretical value. For other hand, the test 14 has a low concentration of sugars and a high production of bioethanol.

Table 2. Summary of results analysis

Test	SC (g)	SC (%)	BP (g)	BP (%)	gBEtOH/gS	gS/gCM	gBEtOH/gCM
1	49.02	98.20	13.60	53.31	0.28	0.08	0.03
2	49.05	98.25	14.16	55.51	0.29	0.078	0.032
3	49.08	98.32	14.10	55.26	0.29	0.078	0.032
4	49.09	98.34	14.93	58.52	0.30	0.078	0.034
5	49.09	98.33	14.35	56.25	0.29	0.078	0.033
6	49.41	98.98	13.77	53.96	0.28	0.078	0.031
7	48.98	98.10	14.00	54.86	0.29	0.078	0.032
8	73.06	98.77	13.68	36.18	0.19	0.116	0.021
9	48.99	98.12	13.47	52.82	0.28	0.078	0.031
10	26.55	98.23	8.28	59.97	0.31	0.042	0.035
11	49.02	98.19	13.06	51.19	0.27	0.078	0.030
12	49.21	98.57	13.06	51.19	0.27	0.078	0.030
13	63.64	98.08	14.38	43.37	0.23	0.101	0.025
14	34.17	98.30	10.93	61.53	0.32	0.054	0.036
15	63.48	97.83	14.43	43.51	0.23	0.101	0.025
16	34.21	98.43	9.01	50.74	0.26	0.054	0.030
17	33.90	97.53	9.06	51.01	0.27	0.054	0.030
18	63.64	98.08	14.20	42.82	0.22	0.101	0.025
19	63.96	98.58	14.92	45.00	0.23	0.101	0.026
20	34.22	98.45	8.82	49.68	0.26	0.054	0.029

SC: sugar consumed, BP: bioethanol produced, gBEtOH/gS: gram of bioethanol per gram of sugar, gS/gCM: gram of sugar per gram of CM, gBEtOH/gCM: gram of bioethanol per gram of CM.

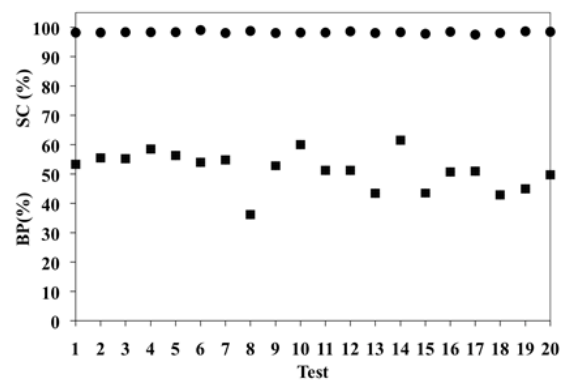

Figure 1. Bioethanol production and sugar consumption in percent for each test.

3.1 Statistical analysis

The statistical significance of the corresponding model equation was checked by *F* test analysis of variance (Table 3). The adequacy of the models was expressed by the coefficient of determination R^2, which proved to be 0.88 for the production of bioethanol in grams per gram of coffee mucilage. These values indicate 88% of the variability in the responses for the studied region, with the remaining that 12 % corresponding to the residue. Values of *P*-value less than 0.0500 indicate model terms are significant. In this case X_3 and X_3^2 are significant model terms.

3.2 Response surface

Figure 2 shows 3D graphics for response surface plotting the regression equation. In chart a), b) and c), it shows the interaction between pH, temperature, initial sugar and their optimal level, a) fixed initial sugar at optimum point of 35 g/L; (b) fixed temperature at optimum point of 28 °C; and (c) fixed pH level at optimum point of 5.5.

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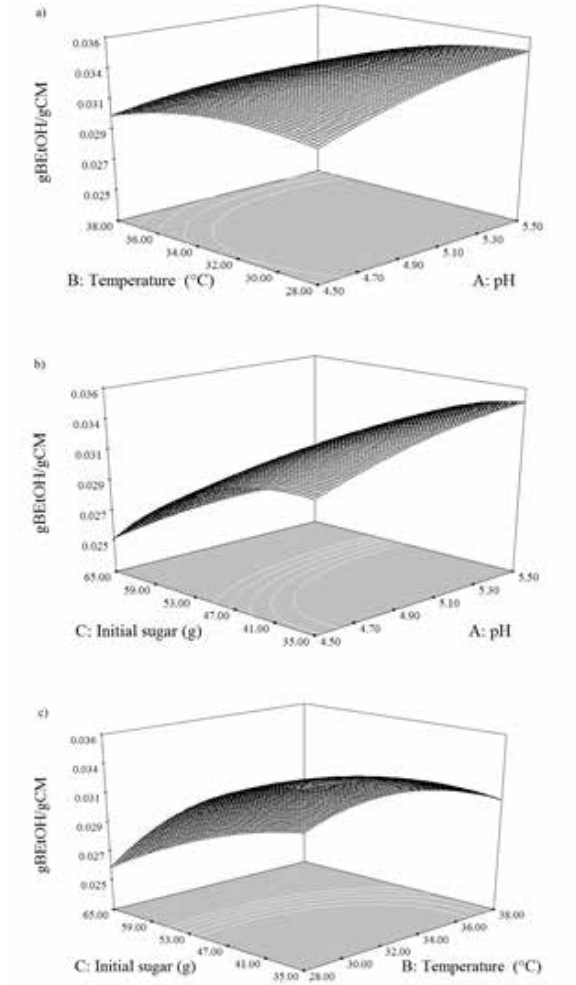

Figure 2. Three-dimensional graphs of the quadratic model for gBEtOH/gCM within a full factorial central composition design.

Figure 3 shows kinetic of sugar consumption and kinetic of bioethanol production, to the best condition between tests of experimental design. Sugar consumed was 98.3 % and bioethanol produced 10.93 g corresponding at 61.53 % conversion sugar to bioethanol.

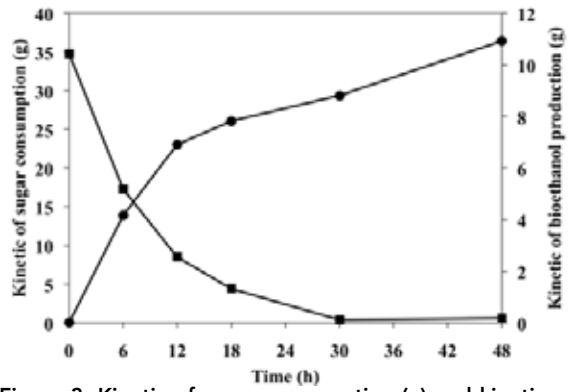

Figure 3. Kinetic of sugar consumption (n) and kinetic of bioethanol production (l), to the best condition.

Table 3 Variance analysis of bioethanol production in grams per gram of coffee mucilage.

Source	Polynomial coefficient	Sum of squares	Degree freedom	Mean square	*F*-Ratio	*P*-Value
Coefficient	0.032300					
X_1	0.000399	0.000003	1	0.000003	1.02	0.3370
X_2	-0.000543	0.000003	1	0.000003	0.89	0.3690
X_3	-0.003290	0.000133	1	0.000133	41.60	0.0001
X_1^2	-0.000695	0.000013	1	0.000004	3.89	0.0767
$X_1 X_2$	-0.000660	0.000005	1	0.000005	1.65	0.2270
$X_1 X_3$	0.000773	0.000006	1	0.000006	1.84	0.2050
X_2^2	-0.000636	0.000007	1	0.000013	2.25	0.1650
$X_2 X_3$	-0.001010	0.000004	1	0.000004	1.21	0.2970
X_3^2	-0.002060	0.000046	1	0.000007	14.30	0.0036
Total error		0.002	10	0.000001		
Total (corr.)		0.050	19			

X_1: pH, X_2: temperature, X_3: initial sugar, *F*: Fisher test, *P*-value: probability distribution. The correlation coefficient (R^2) was 0.88, adjusted correlation coefficient was 0.78.

4. Conclusion

We can say that CM is a good raw material of the coffee industry composed by sugars for getting bioethanol. A central composite design was employed to analyze CM fermentation for the efficient production of bioethanol.

According to the bioethanol conversion we conclude that the yield in gram of ethanol by gram of sugar, the best condition in the tests was pH 5.5, temperature 28 °C, initial sugar 35 g, sugar consumed 98% and bioethanol produced 10.93 g, with yield 0.32 gBEtOH/gSugar and 0.025 gBEtOH/gCM.

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