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Bioethanol production from banana peels using different pretreatments

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Abstract

Bioethanol is an attractive biofuel having a higher potential for energy security and environmental safety over fossil fuels. In this study, to obtain high concentration of ethanol from cellulosic materials, banana peels was pretreated with H_2SO_4(0.5% v/v) and two types of ionic liquids (ILs) and cellulase enzyme. Fermentation process was conducted using *Saccharomyces cerevisiae* under anaerobic condition. All the pretreatment methods have been shown to effectively solubilize and hydrolyze cellulose and hemicellulose into monomeric sugars. It was proved that H_2SO_4gave the highest glucose concentration 34.2 g/L on the fourth day, while ethyl acetoacetate gave lower glucose concentration 27.3 g/L at fourth day. The concentration of the produced ethanol was measured every two days using ethanol sensor. The lowest mean concentration of ethanol produced from banana peels sample without pretreatment, H_2SO_4, ethyl acetoacetate, hexamethylphosphoramide and enzyme were 0.606, 3.936, 2.473, 6.286 and 2.553 g/L respectively, after 3 days fermentation. While the higher concentration of ethanol for the same samples (without pretreated and pretreated as above) were 8.143, 41.776, 24.853, 46.03 and 39.523 g/L respectively after 7 days of fermentation.

Key words : Bioethanol; Banana peels; Ionic liquid; Enzyme; Fermentation.

INTRODUCTION

Biofuels, generally defined as a fuels derived from biological materials, can be made from plants, vegetable oils, forest products, or waste materials. The raw materials can be grown specifically for fuel purposes, or can be the residues or wastes of existing supply and consumption chains, such as agricultural residues or municipal garbage [1]. Banana, a tropical fruit, is widely cultivated worldwide. Globally, its product (about 16%) is the second largest amount compared with other fruits [2]. Regarding ethanol production from banana peels, the search for processes able to efficiently perform the dissociation of the lignin-cellulose-hemicellulose complex and enable the attainment of high yields in fermentable sugars in hydrolyzed liquor and, most importantly, without generating elements that inhibit the fermentation process, is one of the great challenges for producing 2nd generation ethanol that is competitive with 1stgeneration ethanol from sugarcane, corn or sugar beet [3-4-5]. Routes most studied till now have been the use of chemical pretreatment with acids or bases, followed by enzymatic hydrolysis, of which the acid pretreatment is considered as one of the very important techniques and targets for high yields of sugars from lignocellulosics[6].In this study examined the ability of banana peels to produce bioethanol andinvestigate different pretreatment methods to enhance bio-digestibility for ethanol production.

MATERIALS AND METHODS

Collection banana peels

The gathered banana peels were chopped into small pieces approximately 2-4 cm in length using a knife and dried in an oven at 65°C for 24 h. The dried substrate was powdered with an electric grinder to a mesh size of 40, packed in polyethylene bags and stored at 4ºC in refrigerator for following experiment [7]. The experiments were done in two ways:

a. Without pretreatment banana peels :

One hundred grams of dried banana peels were weighted and prepared for fermentation process.

b. Pretreatment banana peels :

Acid hydrolysis: Fifty gram banana peels of dry powder was pretreat with H_2SO_4 (0.5%), 121°C for 60 min [8]Thereafter, the solid residues washing by deionized water to neutral pH generated. The pH was 4 and neutralized using NaOH (5% M) to adjust the pH to 5 [9].

Ionic liquid hydrolysis: The solubility of lignocellulosic materials in IL was performed at 130°C by mixing 50 gfrom banana peels with two kind of IL (Hexamethylphosphoramide $C_6H_{18}N_3OP$ and Ethyl acetoacetate $C_2H_{10}O_3$). After incubating the solution in the IL for 20 min at 130°C, the pretreated banana peels powder was precipitated with deionized water. The precipitationwas washed with additions of deionized water [10]. Washed solids were dried overnight in oven at 45°C and stored at 4°C until further use [11].

Enzymatic hydrolysis: Enzymatic hydrolysis was carried out at 50°C, 0.01 M citrate buffer (pH 4.7) in 150 rpm shaking incubator for 72 h of saccharification. The activity of cellulase was 30 FPU/g of solid [12]. The suspensions were placed in a thermostatic bath at 80C for 30 min, to denature the enzyme. Next, the residual washed with deionized water, and the solid was separated by centrifuge (10,000 rpm, 15 min) and suspended in deionized water. At the end of these procedures, a suspension of cellulose was obtained and stored at 4°C ina closed container[13].

Measuring sugar content :

Glucose content was determined according to Miller method (1959). Three mililiter of 3-5-dinitrosalicylic acid (DNS) reagent solution was added to 3 ml of glucose sample in a lightly capped test tube. Then, the mixture was incubated in water bath for 5 to 15

min at 90°C until the red-brown color appeared. Then, 1 ml of Rochelle salt solution (potassium sodium tartrate) 40% was added to stabilize the color. The absorbance value of the reducing sugar was measured using spectrophotometer at 575 nm, after cooling to room temperature [15].

Preparation of yeast culture: The yeast *S. cerevisiae* were purchased from Baghdad local market. Before using in fermentation, the yeast was activated. About 1 g of dry yeast was added to 20 ml of 5% sterilized glucose solution, activated at 38ºC for 1h, cooled from 38ºC to 30ºC, and then used in the experiment. The yeast concentration was approximately 10^8 cells/ml[16].

Fermentation of raw materials

The activated yeast was added to banana peels sample for each experiment (with pretreatment and without pretreatment) in sterilized circumstances in order to prevent any contamination [16]. Added 1L of distilled water to each mixture, then autoclaved in 121ºC for 20 min. and left fermentation for7 days. It was kept in glass tank with pH 4.8 and air outlet to let the carbon dioxide formed to escape[17]. During that the ethanol concentration was determined every two days by using ethanol sensor device.

Distillation and Dehydration

This process is designed to increase the ethanol concentration up to maximum available purity. The columns use the differences in the boiling points of water and ethanol to will boil and separate the ethanol. The distillation process is a raise the concentration of alcohol from low purity to high purity [18].One liter of fermented raw materials was placed in flask and heated to preset evaporation temperature by a digital heating mantle at 80 ºC for 3hours. The cooling water supply was opened while the evaporating temperature was kept constant during the process. The vapor was condensed and collected in a flask. The concentration of ethanol was measured at 20 ºC. 250 ml of condensate was obtained from each liter of fermented raw materials. Toraise the ethanol concentration to 99.5%,the extractive distillation was used in the present work. The drying agent used was calcium oxide (CaO) [18]. So, 5 g of CaO were added to the ethanol and mixed well for 10 minute. The mixture was heated and evaporated again at 80°C. The output of this process was 99.5% ethanol.

Statistical analysis

In order to determine the impact of ethanol in banana peel, using analysis of variance, F-test, t-test in complete randomized design (CRD). To explain the differences between means, calculate least significant differences (LSD) values at $p \leq 0.05$, and expressed that as (Mean ± SEM) [19].

RESULTS

Chemical composition of banana peels: The compositions of banana peels are shown in table-1.

Table 1: Compositional analysis of corncobs on dry matter

Component	% w/w
Cellulose	21.89
Hemicellulose	17.96
Lignin	9.74
Other carbohydrates	19.83
Proteins	14.33
Lipids	5.30
Crude fibers	6.21
Ash	4.14

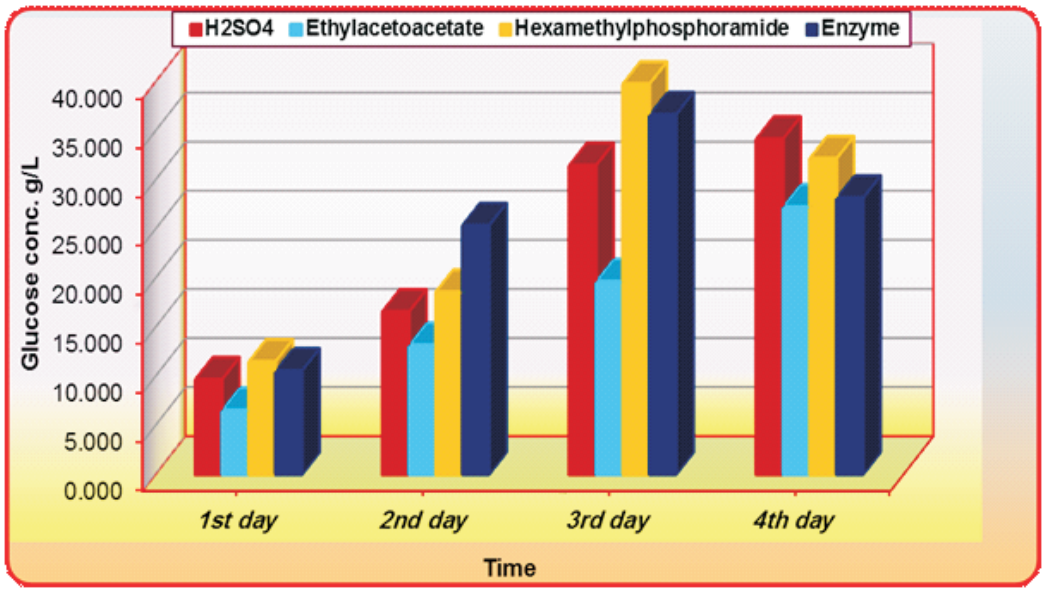

Figure 1: Concentration of reducing sugar from banana peels samples after pretreatment with diluted H2SO4, ILs and enzyme

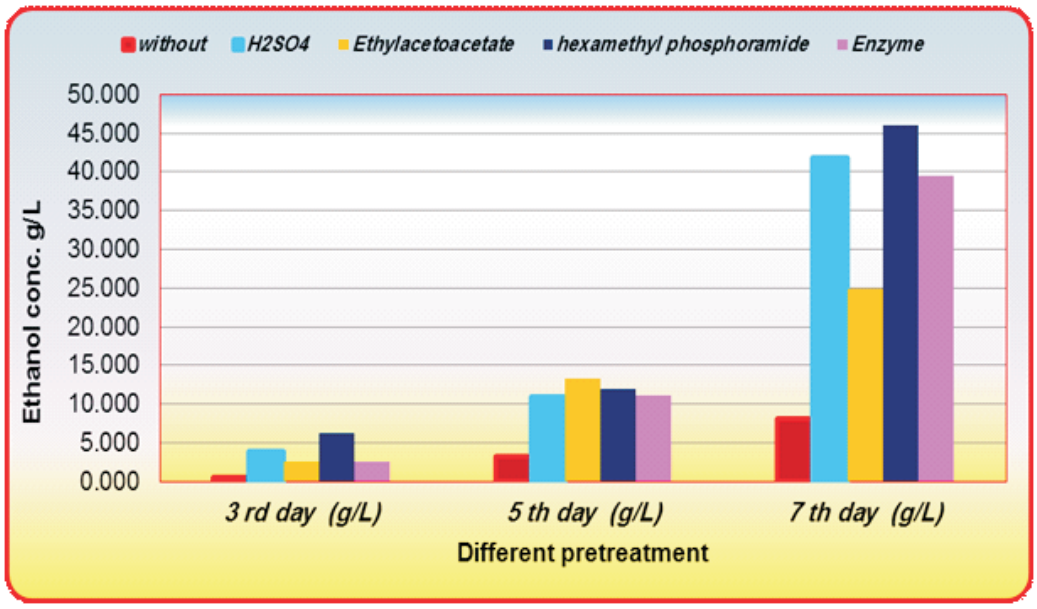

Figure 2: The mean concentration of ethanol from banana peels samples (pretreatment and without pretreatment) after 3, 5 and 7 days of fermentation

The parts of banana peels were mainly composed of cellulose (glucan), hemicellulose (xylan and arabinan) and lignin, other polysaccharides and a few content of proteins, lipids, crude fibers and ash were determined before pretreatment. Themain goal of pretreatment is to increase the enzyme accessibility to cellulose during the hydrolysis step, stressing the importance of the enzyme-dependent step. Cellulase enzymes are quite expensive and contribute significantly to the final cost of the biofuel[20].

Influence of different pretreatment methods: The effect of different pretreatment methods on glucose and ethanol returns for banana peels samples as shown in figure-1 was studied to determine the most effective material for the production of bioethanol.

All the pretreatment methods (dilute acid, ILs, enzymes) have been shown to effectively solubilize and hydrolyze cellulose and hemicellulose into monomeric sugars, removing it from the cellulose fibers.

The contents of released sugars in the hydrolysates varied among various pretreatment and pretreatment conditions. Among all pretreatment, hexamethyl phosphoramide for 72h showed the greatest release of soluble reducing sugars 39.8 g/L, while the best reducing sugar was after 72 h when enzymatic pretreatment was 36.7 g/L, and a greater release sugar at dilute acid pretreatment after 96 h was 34.2 g/L.

Production of ethanol from banana peels: The means of ethanol values produced from banana peels samples without

Table 2: Static comparison for ethanol production among the days and among the different pretreatment for banana peels samples

Days after treatment	Ethanol concentration (g/L)					LSD P = 0.05
	Without pretreatment	H_2SO_4	Ethylacetoacetate	Hexamethylph-osphoramide	Enzyme	
3 rd day	0.607 Dc ± 0.075	3.937 Bc ± 0.325	2.473 Cc ± 0.393	6.287 Ac ± 0.604	2.553 Cc ± 0.161	1.142
5 th day	3.180 Cb ± 0.555	10.967 Bb ± 0.127	13.330 Ab ± 1.067	11.920 Abb ± 0.584	11.040 Bb ± 0.906	2.282
7 th day	8.143 Da ± 0.304	41.777 Aba ± 1.250	24.853 Ca ± 2.709	46.030 Aa ± 2.096	39.523 Ba ± 0.607	5.227
LSD P ≤ 0.05	1.272	2.593	5.870	4.512	2.202	

*Small letters indicate to comparison in column, while capital letters indicate to comparison in row. Similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test).

pretreatment were 0.607, 3.180 and 8.143 g/L after 3,5 and 7 days, respectively, while it was 3.937, 10.967 and 41.777 g/L, respectively for the same days after fermentation for the samples pretreatment with H_2SO_4 (0.5%). The highest concentration of ethanol was through the pretreatment of banana peels with diluted sulfuric acid.

The means of ethanol values produced from banana peels samples pretreated with ILs were 2.473, 13.330 and 24.853g/L for ethyl acetoacetate after 3,5 and 7 days, respectively, while it was 6.287, 11.920 and 46.030 g/L, respectively for the same days to sample pretreated with hexamethylphosphoramide after fermentation (figure-2).

The means of ethanol values produced from banana peels samples pretreated with enzyme was made between the days showed that there was a significant difference between them at were 2.553, 11.040 and 39.523 g/L after 3,5 and 7 days, respectively. A statistical comparison was made between the days showed that there was a significant difference between them at ($p \leq 0.05$) as it appears in table-2.

The results correspondent with Arumugam and Manikandan (2011) showed the fermentation of enzymatic hydrolyzed of diluted acid pretreated mixed fruit pulps (banana and mango) by yeast (*S. cerevisiae*) showed for maximum ethanol of 35.86 % corresponding to a fermentation efficiency of 70.33%. In peels of the same samples, the maximum yield of ethanol was 13.84 % in banana and 9.68 % in mango.

Also agreed with Abdulhay and Aljoborey, (2015) mentioned this rise in ethanol concentration can be returned to the contact time between substrate and yeast. Our results were disagreed with Singh *et al.,* (1984) has found that the fermentation of enzymatic hydrolysates showed better fermentation efficiencies in comparison to acid hydrolysates of agricultural residues.

The liquid of fermentation stage was heated and condensed then the ethanol purity were measured. Ethanol purity was 63% when banana peels without pretreatment, while 87% when banana peels with pretreatment. After dehydration stage, purity of ethanol was 99.5% . this result agreed with Dohetry and Malone (2001) studied the effect of different drying agents on alcohol concentration and found that ethylene glycol and CaO give highest concentration, also its agrees with Ismael *et al.,*(2008) used calcium carbide as a dehydration agent and compare it with calcium oxide and carboxymethyl cellulose.

CONCLUSION

From the obtained results of this study, banana peels have high contents of cellulose and hemicellulose. The different pretreatments gave different efficiency to turn the basic material into monosaccharides. The maximum amount of ethanol from banana peels was 46.03 g/L after 7 days fermentation.

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