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Bioethanol Production By Zymomonas mobilis MTCC No. 2427 Using Orange Peels As Low Cost Substrates

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Abstract: The paper aims to investigate best possible method for hydrolysis of orange peels, which was suspected to contain a high amount of sugar and complex carbohydrate, so that later it can be fermented to ethanol. Different methods of hydrolysis were carried out and the hydrolysate was later fermented to ethanol using *Z mobilis* obtained from IMTECH, Chandigarh. Amount of reducing sugar and total sugar was estimated using standard DNS method for reducing sugar and phenol-sulphuric acid. All the tests were done on the non-edible part of the orange peels which is considered as waste of the agriculture. The present work is carried out with a motive of producing fuel with cheap and cost effective manner.
Keywords: Orange peels, pretreatment, ethanol, fermentation.

Introduction

In India tons of waste is generated on daily basis, but of no use, it is either burnt or disposed by different techniques such as landfilling, burning and direct disposal to river and water bodies, very rarely it's been converted into some useful products like biogas, fuel and electricity. If we add some amount of value to these waste products we get essential value added products. This paper is an attempt to convert zero value products to a high value product. The waste is mostly organic in nature, which accumulates at different stages of agricultural production such as storage, transportation, mishandling leading to damage and microbial spoilage. If this waste is converted to fuel and electricity then the whole process will become economical. The conversion of waste to fuel is a laborious process which involves pretreatment, the first step in fuel production. The purpose of pre-treatment is to convert the complex molecules into simpler one. Here the complex molecule of starch and complex carbohydrate is broken down into simple sugar, so that the conversion of the waste to ethanol and fuel takes lesser time, thereby making the process efficient, and also it become easier for the microbe to carry out the process. Later steps involves fermentation and distillation of the fermented broth. Recently, there has been growing interest in applying microwave heating to rapid thermal digestion of the complex molecule to simple utilizable molecules (1). Degradation rates of the complex molecule are significantly enhanced by the presence

of alkali and hence the sugar conversion to ethanol is increased in the process, thereby making the process more efficient (2-3). The process of ethanol production from fruit waste involves pre-treatment of waste, inoculum preparation, fermentation media preparation and inoculation followed by fermentation and distillation of ethanol (4-6). Different fruit waste peels i.e. Orange, Pineapple and Watermelon peels (7-12) can be used, in this paper we have focused on Orange peels for our study. The fermentation can be operated in different mode (13, 15). The microbe used in the fermentation is mostly yeast (9, 13, 14), bacteria such as *Zymomonas mobilis* (5) and mutant strains are sometimes (12) used, we have done batch study using *Zymomonas mobilis*. This work is carried out in order to produce fuel using a low cost substrate.

Materials And Method

Microorganisms and cultivation

The culture of the *Zymomonas mobilis* MTCC No. 2427 was obtained from IMTECH, Chandigarh and was maintained on RM agar Medium containing Glucose 20g/l, Yeast extract 10.0g/l, KH_2PO_4 2.0g/l, Agar 15.0g, dH2O 1.0l and pH was Adjusted to 6.0. Microbial culture was sub-cultured at every 2 months and stored at 4° C.

Collection and Dry weight analysis of Peels

The orange peels were collected from fruit juice shop in a sterile plastic bag. The peels were washed under tap water and dried in air for 2 hours. Wet weight of the peels was taken. The orange peels were dried in the oven for 2 days at 40^0 C to completely remove the water content. Dry weight of the peels was determined and the water content of the peels was calculated.

Pre-treatment of Peels

Pre-treatment of orange peels was carried out by different methods followed by filtration using muslin cloth and estimation reducing sugar and total sugar by DNS method and phenol-sulphuric acid respectively. Physical pre-treatment was done by different methods i.e. heating, soaking and steaming. In heating, dried Peels (10% w/v) were mixed with distilled water and heated on hot plate at 60° C for different period of time. In soaking, dried Peels (10% w/v) were soaked in distilled water for different time intervals and in steaming also dried Peels (10% w/v) were steamed in an autoclave with one-fourth lid open for different intervals of time at 121^0C. Similarly chemical method of pre-treatment was also performed in which hydrolysis was carried out using H_2SO_4 and HCl individually. In Acid Hydrolysis, dried peels (10% w/v) were added to 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 (% v/v) % H_2SO_4 and HCl acid solution respectively in distilled water, followed by autoclaving at 121°C at 15 psi pressure for a time period of 15 minutes. The solution was cooled down to room temperature and pH was adjusted to 7. The peels were squeezed using muslin cloth to get the hydrolysate.

Inoculum Preparation and fermentation

Lyophilized culture of *Zymomonas mobilis* was subculture in 100 ml RM Broth containing Glucose 20g/l, Yeast extract 10.0g/l, KH_2PO_4 2.0g/l, and pH was Adjusted to 6.0. 1 ml of the exponential phase culture was inculcated into 250 ml of hydrolysate containing Yeast extract 10.0g/l, KH_2PO_4 2.0g/l at incubated for 120 hours at 37°C for fermentation. Fruit peel hydrolysate was used as Carbon source in the media and hence no external carbon source was added. Fermented broth was distilled and 50 ml of the distillate was collected for analysing the ethanol concentrated. Ethanol assay was done by standard dichromate test.

Analytical Methods

Reducing sugar was estimated by mixing 1 ml of the sample with 3 ml of DNS reagent and checking absorption at 540 nm. Total sugar estimation involves mixing of the 1mlof hydrolysate sample, 1 ml of phenol, 5 ml of sulphuric acid and measurement of absorption at 490 nm. Absorption was plotted on standard graph to obtain the correct sugar concentration and multiplied with their dilution factor. Glucose is commonly used to create the standard curve.

Ethanol assay was done by dichromate test. The reaction mixture with 1 ml each of the sample, potassium dichromate 50 g/L and saturated di-phenylcarbazide was heated at 90°C for 10 minutes. After the formation of brown colour, 1 ml of sodium potassium tartrate (40%) was added. Formation of brown colour was indication of ethanol and it was estimated by extrapolating on ethanol standard curve. The absorbance was measured at 575 nm.

Results

Dry weight analysis of the peels showed a constant decrease in weight of the sample over a period of 48 hours. After 48 hours the weight was constant and decrease in weight was plotted against time as shown in the graph. Figure 1 show dry weight analysis of peels against time, the water content in the orange peels after calculation was 74.5%.

During chemical pre-treatment it was observed that the amount of sugar concentration increased with initial increase in acid concentration and then after reaching a maximum value it was constant with further increase in acid concentration. In chemical pre-treatment maximum sugar leaching was obtained by 2.5% HCL hydrolysis, 17.6% reducing sugar and 21.01% total sugar was obtained whereas 18.5% reducing sugar and 21.54% total sugar was obtained by 3.5% H_2SO_4 hydrolysis. Figure 2 and figure 3 shows amount of reducing sugar and total sugar obtained after acid pre-treatment.

During Physical pre-treatment it was observed that the amount of sugar concentration increased with time. In heating method 19.85% reducing sugar and 23.45% total sugar was obtained after 60 minutes. In Soaking method 13.1% reducing sugar and 17.41% total sugar was obtained after 24 hours. In Steaming method 9.2% reducing sugar and 13.56% total sugar was obtained after 90 minutes. Figure 4, 5, 6 shows amount of sugar obtained after soaking, heating and steaming method of pre-treatment. 2.5% of ethanol was present in the fermented broth was calculated by extrapolating the absorption of the sample on the standard curve of Ethanol using dichromate test.

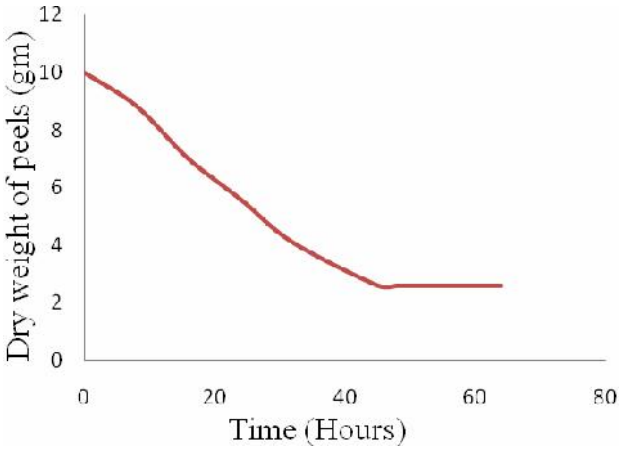

Figure 1: Shows dry weight analysis of peels against time

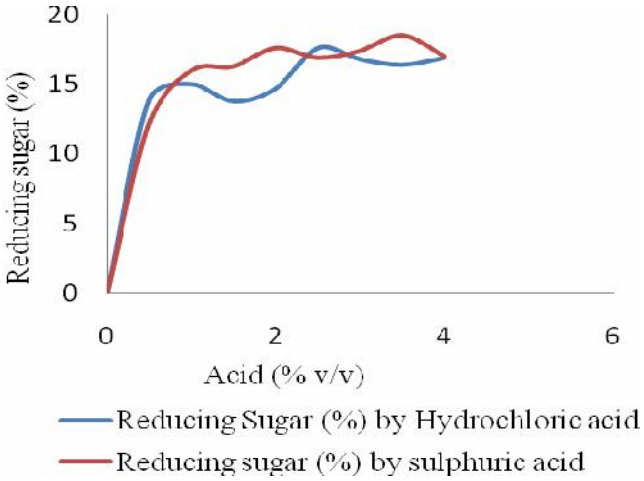

Figure 2: Shows amount of reducing sugar obtained after acid pre-treatment.

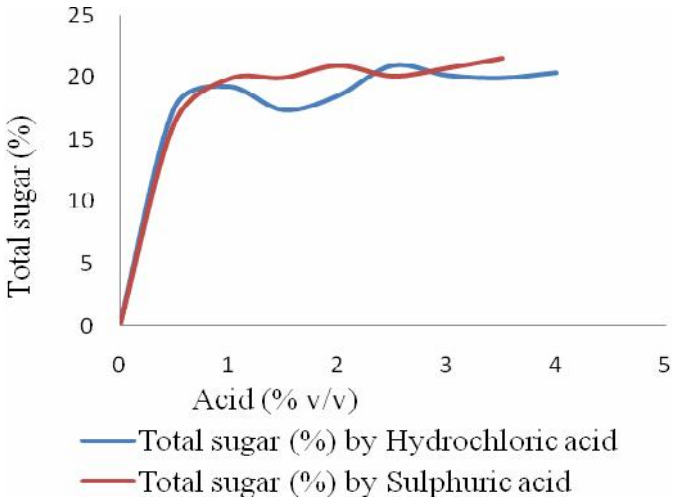

Figure 3: Shows amount of Total sugar obtained after acid pre-treatment

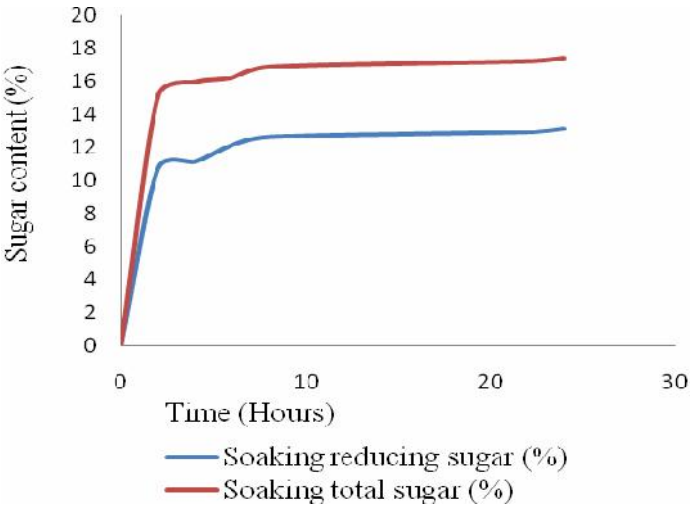

Figure 4: Shows amount of sugar obtained soaking in distilled water.

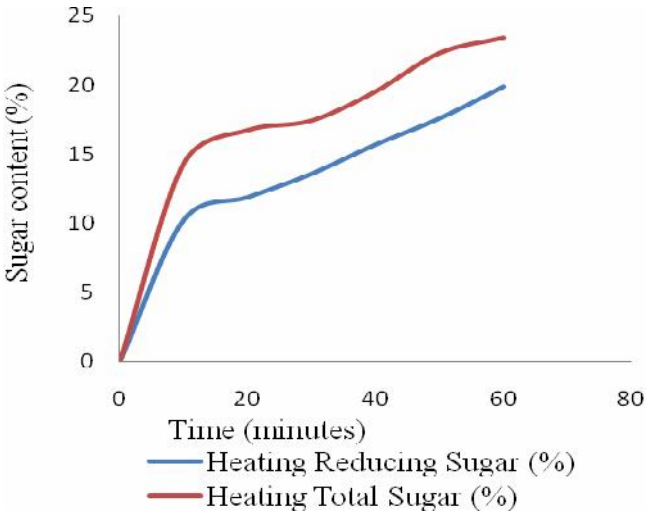

Figure 5: Shows amount of sugar obtained after heating method of pre-treatment.

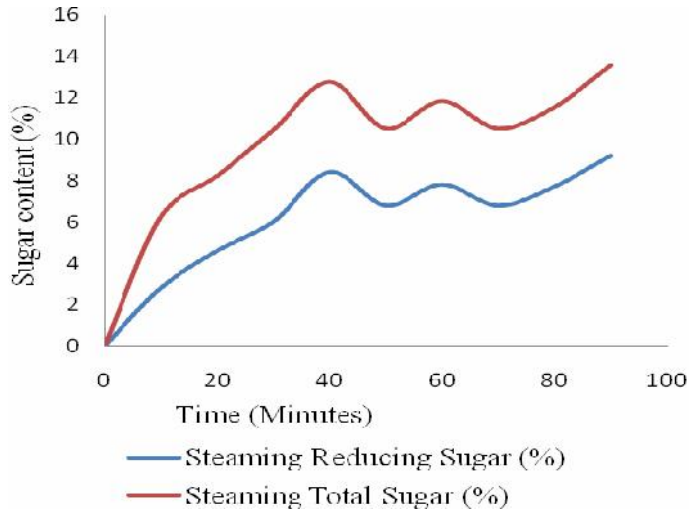

Figure 6: Shows amount of sugar obtained after steaming method of pre-treatment.

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