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Effect of Pretreatment on the Production of Bioethanol from Kitchen Waste

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Abstract: ***Production of Bioethanol from fermentation of kitchen wastes mainly vegetable peels as raw materials was carried out using saccharomyces cerevasia. The major objective of this research work is to find out the role of pretreatment for higher alcohol production. The process optimization was done by different pretreatment methods. Acid pretreatment was carried out using HCl before fermentation. The reducing sugar content with and without pretreatment is determined. The effect of fermentation temperature and pH on the yield of ethanol was studied. The results indicate that the alcohol concentration increased with increase in temperature and maximum concentration was obtained in acid treated sample at 31^{o}C and at pH 5.0. The concentration of reducing sugar was also found to be maximum in acid pretreatment compared to the one without any pretreatment.***

Keywords: Pretreatment; Bioethanol; Saccharification; Fermentation; Distillation

I. INTRODUCTION

Bioethanol is a source of energy that can be obtained from renewable sources of plant origin. It can be produced from fermentation of sugars from microorganisms. Bioethanol can be used as an alternative fuel due to its high octane number and high flammability. Bio fuels like Bioethanol and biodiesel that are produced from biomasses can be blended with petrol or diesel at 5-10% [1, 2] and used in engines, which significantly reduce pollution. These biofuels are sustainable and can contribute in abating global warming to remarkable extent [3]. Bioethanol is the first biofuel produced from food based feedstock's such as sugarcane, corn, millet, wheat, etc.[3,4]. Biofuel production from food stuffs is not an appropriate idea as there is food scarcity [5]. Alternatively, agricultural wastes viz rice straw, wheat straw, baggase are abundant and are available throughout the year [5, 6, 7]. Theses agricultural wastes can be effectively sued as source of biofuel. Researches have reported the synthesis of biofuels from non-edible bio wastes and lignocellulosic biomasses such as fruit and vegetable wastes [8]. These biomasses mainly consists of heterogeneous cellulose and hemicellulose which require physical, chemical or enzymatic pretreatment [1, 9, 10].

The pretreatment of biomass is one of the most important step in the production of biofuels. Pretreatment is done to breakdown cellulose and hemicellulose into monomeric sugars such as glucose and xylose [11, 12, 13, 14]. The pretreatment can be achieved by hot water, acid, alkali, steam or by enzymes [14, 15, 16, 17]. Fermentation converts monomeric sugars into ethanol in an anaerobic environment.

In this research work, the role of acid hydrolysis on the yield of bioethanol is studied. The concentration of acid, temperature and pH was varied to optimize the conditions for higher yield of bioethanol using saccharomyces cerevsea.

II. MATERIALS AND METHODS

The chemicals used in this research work are Sucrose, peptone, urea, yeast, Potassium dichromate, DNSA reagent, HCl and NaOH. LR grade chemicals and distilled water is used for the process.

The equipment used are heating mantle, autoclave, BOD incubator, distillation unit, photoelectric calorimeter and hydrometer.

The Raw materials like peels of carrot and beetroot were obtained from local hostel.

1) *Substrate Preparation:* The raw materials were washed thoroughly with distilled water to remove the dust and other impurities and air dried. The dried materials were crushed and pulverized to get fine powder.
2) *Pretreatment:* the pulvarised raw materials were treated with HCl for acid pretreatment. The percentage of HCl was varied from 2 to 8%. The samples were autoclaved at 120^{o}C for 30 minutes. After the pretreatment the samples were cooled and filtered with cloth to remove the solid residue. The pH of the samples was adjusted to desired value by using Sodium carbonate solutions. The filtrate was used for further studies and fermentation process.
3) *Estimation of reducing sugar:* The acid and base pretreated samples were tested for reducing sugars. Estimation of reducing sugar was carried out by DNSA method with and without pretreatment.

4) Saccharification and fermentation: Fermentation is a process in which the sugar is converted to ethanol under anaerobic conditions by microorganisms.1.5 grams of peptone was added to the filtrate obtained after pretreatment and autoclaved at 120°C for 20 minutes. After cooling, the solution was added with yeast solution which is previously prepared by adding 8 grams of Baker's yeast in luke warm water. The samples were kept for fermentation for three days and at different temperatures. The fermented samples were subjected to distillation to get bioethanol. The ethanol yield was estimated by potassium dichromate method.

III. RESULTS AND DISCUSSION

Finely grinded sample was washed with distilled water. The Slurry was prepared from the cleaned sample by adding 500mL distilled water. The slurry was autoclaved at 120°C for 30minutes. Another set of samples were subjected to acid pretreatment with 2%, 4%, 6% and 8% HCl. The samples were autoclaved at 120°C for 30minutes. The samples were cooled and pH was adjusted to 6.0 by using dilute Sodium carbonate. The samples were filtered and small quantity of filtrate was used to estimate the reducing sugar by DNSA method. The results are tabulated in Table 1 and 2. Figure 1 & 2 shows the effect of pretreatment on the concentration of reducing sugar. The reducing sugar concentration was found to be 11.2mg/mL without pretreatment. The sugar content was found to be more in samples subjected for pretreatment. Maximum sugar content 15.8mg/mL was obtained when 4%HCl was used for pretreatment.

Table 1
Reducing sugar content with and without pretreatment

Method	Concentration of reducing Sugar in mg/mL
Without Pretreatment	11.2
With acid pretreatment	15.8

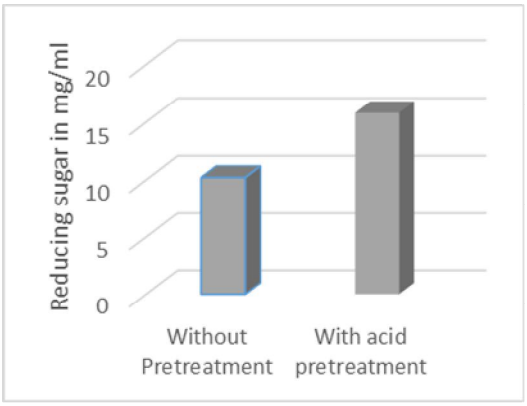

Fig 1: Effect of Pretreatment on concentration of reducing sugar

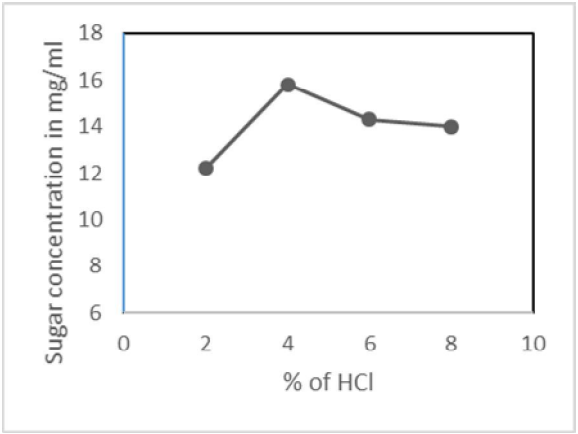

Fig 2: Variation of sugar content with acid concentration during pretreatment

Table 2
Effect of acid concentration on the sugar content

% HCl used	Sugar concentration mg/mL
2	12.2
4	15.8
6	14.3
8	14.0

From the above data it is understood that the reducing sugar is maximum when the samples were treated with 4% HCl, hence the same was optimized and used for further studies.

The effect of fermentation temperature on the yield of ethanol was studied by treating the samples with 4% HCl and the fermentation temperature was varied from 20°C to 35°C. Fermentation was carried out for three days. After three days of fermentation the samples were subjected to double distillation and the alcohol content in the samples were estimated by titration method using Potassium dichromate. The results are shown in Figure 3 and Table 3. The yield of ethanol was increased with increase in temperature and was found to be maximum at 30°C. Low temperature inhibits the activity of yeast and higher temperature of kills the microorganism.

Table 3
Effect of temperature on the fermentation process.

Temperature	20°C	25°C	30°C	35°C
Ethanol concentration	2.4	2.8	5.2	4.3

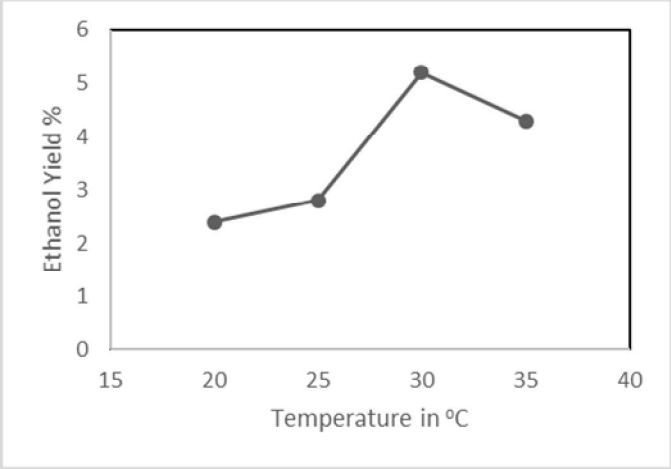

Fig 3: Effect of temperature on ethanol production

The effect of pH on the fermentation process was studied by varying the pH of the solutions from 4 to 7 keeping the acid pretreatment at 4%HCl and fermentation temperature 30°C. The maximum ethanol yield was obtained at pH 5.0 which is 5.4%. The same is also reported by [18]. With the increase in pH the yield of ethanol decreased to 3.3% at pH 7.0. It is found that the activity of saccharomyces cervasia is maximum in acidic pH and decreased with increase in fermentation pH. Figure 4, shows the effect of pH on fermentation process.

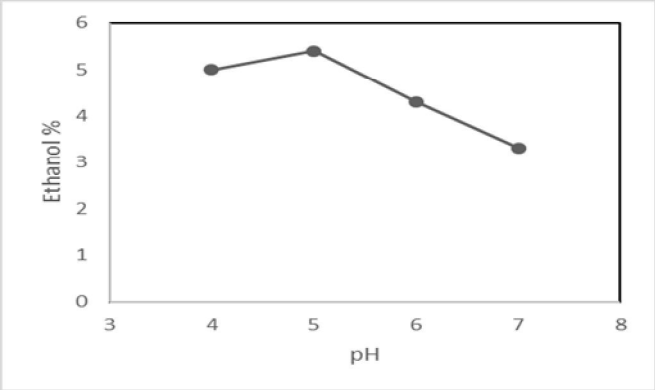

Fig 4: Effect of pH on ethanol yield

The yield of ethanol at different HCl Concentration during Pretreatment is studied and is shown in Figure 5. The ethanol yield was more when 4% HCl was used for fermentation and decreased with increase in acid concentration.

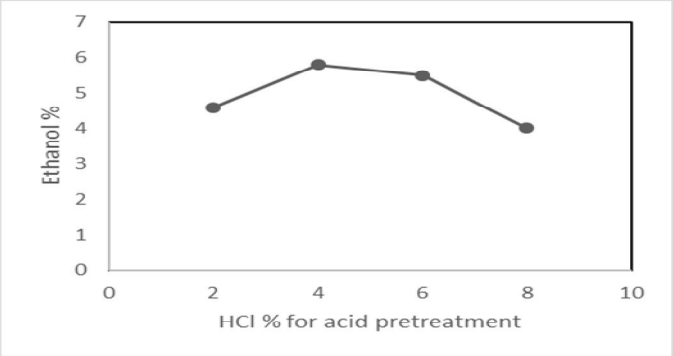

Fig 5: Effect of acid concentration during pretreatment on ethanol yield

IV. CONCLUSIONS

A simple ecofriendly method was optimized for the synthesis of bioethanol where in Kitchen wastes can be converted as source of energy. Optimization of the process for the synthesis of bioethanol was done by varying the concentration of HCl for pretreatment. Effect of temperature and pH on fermentation process was also studied. The sugar content was found to be more with acid pretreatment compared to that without pretreatment. The reducing sugar was found to increase with increase in HCl concentration and was maximum when 4% HCl was used for Pretreatment. Ethanol yield was found to be maximum when the fermentation was carried out at 30^{O}c and at pH 5.0.

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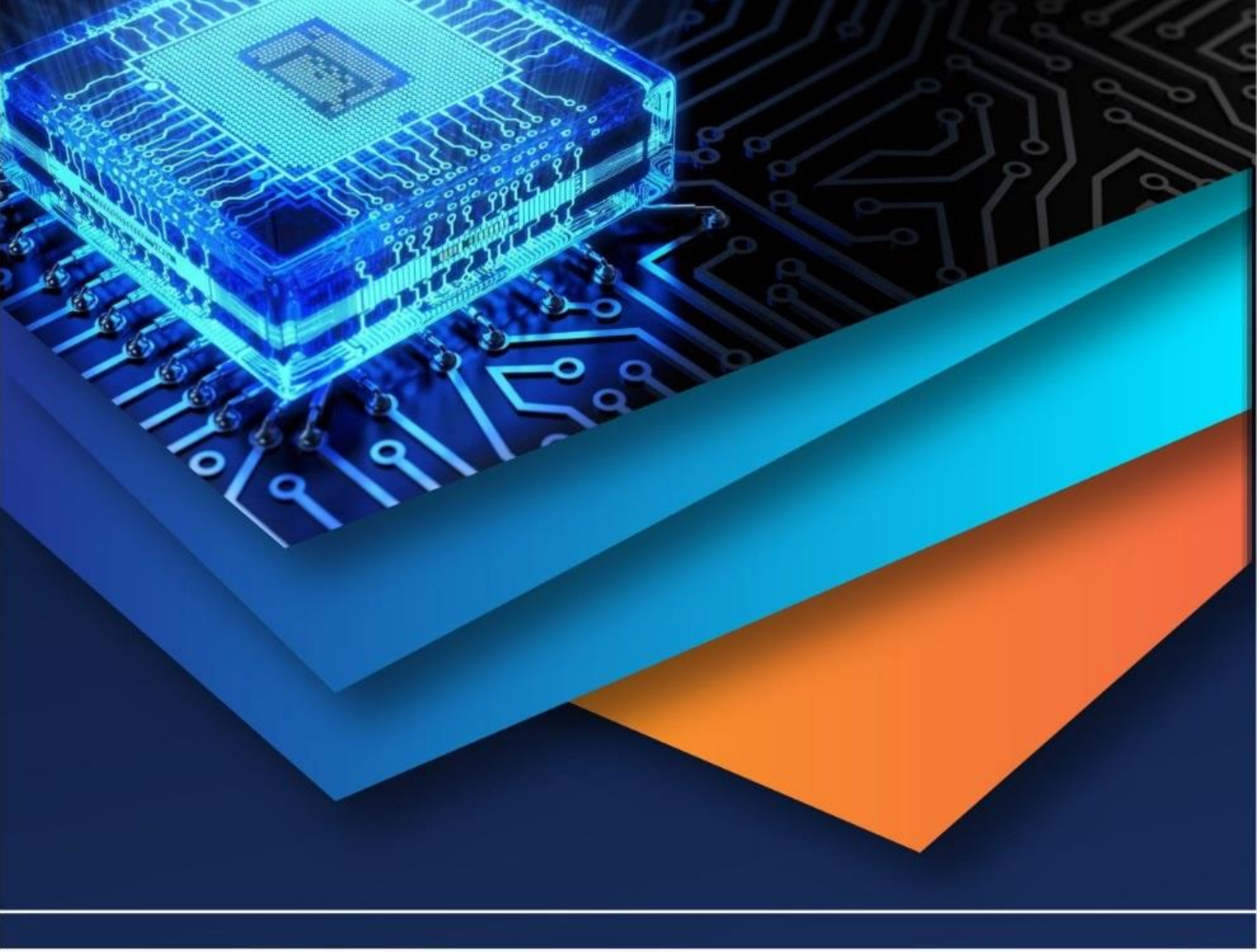

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