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Pretreatment of Agro Residues for Bioethanol Production

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ABSTRACT: Crop residue left in the field after grain harvests have a large potential as a bioenergy feedstock. Crop residues of interest for bioenergy include; wheat straw, soybean straw, and rice straw, paddy straw etc. Paddy straw and wheat straw are potential substrate which can be exploited in industries in future for bioethanol (biofuel) production as they are cheap, abundant and high cellulose content. In this study rice (paddy) and wheat straw had been given both acid (1%-2.5%) and heat treatment (100ºc for 1 hour) for better sugar extraction in media and different parameters were optimized for bioethanol production. Conc. of reducing sugar in the sample after heat treatment were 0.30µg/ml (wheat straw), 15µg/ml (paddy straw), 18µg/ml (mixed sample). After acid treatment total reducing sugar was 56µg/ml (wheat straw), 70 µg/ml (paddy straw), and 170µg/ml (mixed). The optimized parameters for bioethanol production were pH =5.5, Temp=37ºc, conc. of $(NH4)_2SO_4$ = 0.6mg/ml and yield of ethanol was 5.4%. The concentration of bioethanol for acid treatment of 1%, 2%, 2.5% were 1.3%, 3.4%,5.4%.

KEYWORDS: Paddy straw, Wheat straw, Optimization, Acid treatment, Heat treatment, Bioethanol.

1 INTRODUCTION

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used for the production of fuel, electricity and other goods [1]. Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the earth's atmosphere has drastically increased [2]. With the expansion of human population and increase of industrial prosperity, global energy consumption also has increased gradually. Import of transport fuel is affected by limited reserves of fossil fuel. Annual global oil production will begin to decline within the near future [3]. In this scenario, renewable sources might serve as an alternative. Wind, water, sun, biomass, geothermal heat can be the renewable sources for the energy industry whereas fuel production and the chemical industry may depend on biomass as an alternative source in the near future [4]. All petroleum-based fuels can be replaced by renewable biomass fuels such as bioethanol, bio-diesel, bio-hydrogen, etc., derived from sugarcane, corn, switchgrass, algae, etc. Requirements of electricity may be supplied by solar- and wind-farms. The energy consumption rate includes each person's share of electricity and fuel used in making foods and goods and their transport. Biogas has also been identified as a possible motor fuel on organic farms in the short and medium terms. Biogas is produced by anaerobic digestion of organic material. When used as biofuel, CO2 is removed from the gas to increase the energy content and the gaseous fuel can be stored at high pressure. Biogas can be substituted for natural gas or propane as fuel for boilers and for electricity generation in rural areas. Approximately 1281 megawatt biogas is potentially produced from agrowastes in India [5]. Annual methane production in Sweden from organic waste is about 38 PJ, catering to 11% of the domestic energy requirement for transportation in 2007 and projected to be sufficient for fulfilling the EU target for 2020 [6]. Countries across the globe have considered and directed state policies toward the increased and economic utilization of biomass for meeting their future energy demands in order to meet carbon dioxide reduction targets as specified in the Kyoto Protocol as well as to decrease reliance and dependence on the supply of fossil fuels. Although biomass can be a huge source of transport fuels such as bioethanol, biomass is commonly used to generate both power and heat, generally through combustion. Ethanol is at present the most widely used liquid biofuel for motor vehicles [7,8]. The importance of ethanol is increasing due to a number of reasons such as global warming and climate change. Bioethanol has been receiving

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widespread interest at the international, national and regional levels. The global market for bioethanol has entered a phase of rapid, transitional growth. Many countries around the world are shifting their focus toward renewable sources for power production because of depleting crude oil reserves. The trend is extending to transport fuel as well. Ethanol has potential as a valuable replacement of gasoline in the transport fuel market. However, the cost of bioethanol production is more compared to fossil fuels. The world bioethanol production in 2001was 31 billion liters [9].It has grown to 39 billion liters in 2006 and is expected to reach 100 billion liters in 2015 [10]. Brazil and the USA are the two major ethanol producers accounting for 62% of the world production [11]. Large scale production of fuel ethanol is mainly based on sucrose from sugarcane in Brazil or starch, mainly from corn, in the USA. Current ethanol production based on corn, starch and sugar substances may not be desirable due to their food and feed value. Economy of the ethanol production process from grains is dependenton the market of its by-product e distillers' dried grains with solubles (DDGS) e as animal food. The market of DDGS may not expand like that of ethanol in the future [10]. Cost is an important factor for large scale expansion of bioethanol production. The green gold fuel from lignocellulosic wastes avoids the existing competition of food versus fuel caused by grain based bioethanol production [12]. It has been estimated that 442 billion liters of bioethanol can be produced from lignocellulosic biomass and that total crop residues and wasted crops can produce 491 billion liters of bioethanol per year, about 16 times higher than the actual world bioethanol production [11]. Lignocellulosic materials are renewable, low cost and are abundantly available. It includes crop residues, grasses, sawdust, wood chips, etc. Extensive research has been carried out on ethanol production from lignocellulosics in the past two decades [13-15]. Hence bioethanol production could be the route to the effective utilization of agricultural wastes. Rice straw, wheat straw, corn straw, and sugarcane bagasse are the major agricultural wastes in terms of quantity of biomass available [11].

2 MATERIAL AND METHODS

2.1 MATERIAL

Crop residues are the most promising non-conventional source for energy generation. Paddy and wheat are the widely cultivated crops in India. With the increase in production, the amount of crop-residues generated each year has also increased. These residues are generally burnt in the field as a means of disposal. But these organics are rich source of lignocelluloses. Generally they are used as animal feed. But they can also be used as alternate source for fuel generation. The substrate for paddy straw and wheat straw were pasiala, sahbad, sidora.

2.2 CULTURE USED

Yeast (*Saccharomyces cerevisiae)* was used. The yeast culture was maintained on yeast extract broth. The composition of yeast extract broth are KH_2PO_4 (0.2mg/100ml), $MgSO_4.7H_2O$ (0.05mg/100ml), Yeast extract (0.2mg/100ml), $(NH_4)_2SO_4$ (0.6mg/100ml).

2.3 PRETREATMENT

The most important processing challenge in the production of biofuel is pretreatment of the biomass. Lignocellulosic biomass is composed of three main constituents namely hemicellulose, lignin and cellulose. Pretreatment methods refer to the solubilization and separation of one or more of these components of biomass. It makes the remaining solid biomass more accessible to further chemical or biological treatment [7]. The lignocellulosic complex is made up of a matrix of cellulose and lignin bound by hemicelluloses chains. The pretreatment is done to break the matrix in order to reduce the degree of crystallinity of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack [16]. Pretreatment is undertaken to bring about a change in the macroscopic and microscopic size and structure of biomass as well as submicroscopic structure and chemical composition. It makes the lignocellulosic biomass susceptible to quick hydrolysis with increased yields of monomeric sugars [17]. Goals of an effective pretreatment process are (i) formation of sugars directly or subsequently by hydrolysis (ii) to avoid loss and/ or degradation of sugars formed (iii) to limit formation of inhibitory products (iv) to reduce energy demands and (v) to minimize costs.

2.3.1 PRETREATMENT OF SUBSTRATE BY HOT AIR OVEN TREATMENT

The substrates *viz.,* paddy straw, wheat straw were dried at 45 ºC in a hot air oven and powdered in a grinder (dry milling) and sieved to obtain particle sizes of 500µ and 1cm in each substrate.

2.3.2 PRETREATMENT OF SUBSTRATE BY HEAT TREATMENT

The following substrate goes for heat treatment are Paddy straw (20g/100ml, 20g/150ml, 20g/200ml), Wheat straw (20g/100ml, 20g/150ml, 20g/200ml), Mixed, 20g/200ml. These substrates were autoclaved at 121ºC, 15 lb pressure for 1 h. The heat treatment temperture was 121°Cfor 15 min which breakdown the cellulose lignin, hemicellulose present in the sample.

2.3.3 PRETREATMENT OF SUBSTRATE BY ACID TREATMENT

For the acid treatment of the substrates, used the sulphuric acid (H_2SO_4) at different concentration (1%-2.5%). After acid treatment sample (wheat straw) and mixed were placed at 60ºc in water bath for 1h.

3 TREATMENT OF SUBSTRATES WITH COMMERCIAL CELLULASE ENZYME:

Five grams of the delignified samples (all two substrates of 500 µ size) were taken in flask of 250ml capacity separately. The cellulase were used to treat substrates at substrate to enzyme ratio 1:14 (5 g substrate : 70 ml enzyme). The flasks were incubated at 50ºC in a water bath for 48h (11).

4 CHEMICAL ANALYSIS:

4.1 ESTIMATION THE CONCENTRATION OF REDUCING SUGAR BY DNSA METHOD (18)

The reducing sugar present reduce the nitro groups present in dinitro salicylic acid to amino groups and itself get oxidized to sugar acids.The orange colour solution formed shows absorption maximum at 540nm.

4.2 ESTIMATION THE CONCENTRATION OF CARBOHYDRATE BY ANTHRONE METHOD (19)

The anthrone reaction is a rapid and convenient method for determination of hexoses and aldopentose ,hexuronic acid either free present in polysaccharide. The blue green solution formed shows absorption maxima at 620 nm.The reaction is not suitable when proteins containing a large amount of tryptophan are present.

5 FERMENTATION

Filter the sample (wheat straw & mixed) and take liquid parts. Add the media in proper way

6 ETHANOL ESTIMATION BY SPECTROPHOTOMETRIC METHOD (20)

The yeast (*Saccharomyces cerevisiae)* convert the fermentable sugar (glucose, fructose, & sucrose) into ethanol and CO_2. Molasses a byproduct in sugar industry contain 45-55% w/v fermentable by yeast through temp and alcohol and CO_2 are the end product of the fermentation**.**

7 PURIFICATION

The purification of the sample was done by distillation process.

8 RESULTS AND DISCUSSION

Concentration of reducing glucose content in the sample after heat treatment is shown in figure 1.

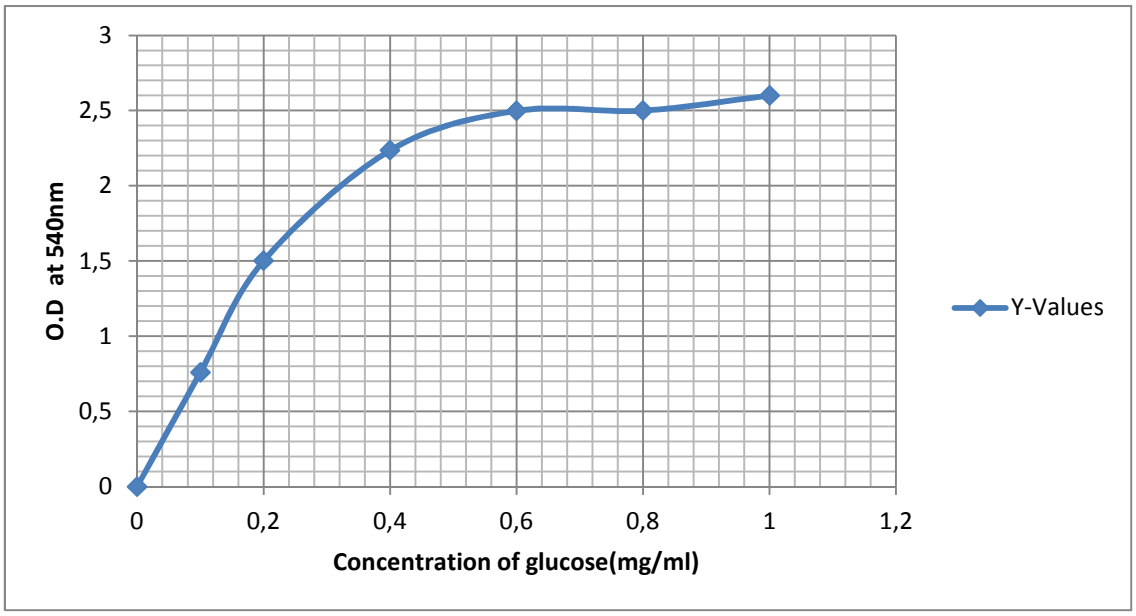

Fig. 1. The concentration of the reducing sugar in the sample after heat treatment were 30µg/ml(wheat straw),15µg/ml(paddy straw),18µg/ml(mixed).

Concentration of total sugar content in the sample after heat treatment is shown in figure 2.

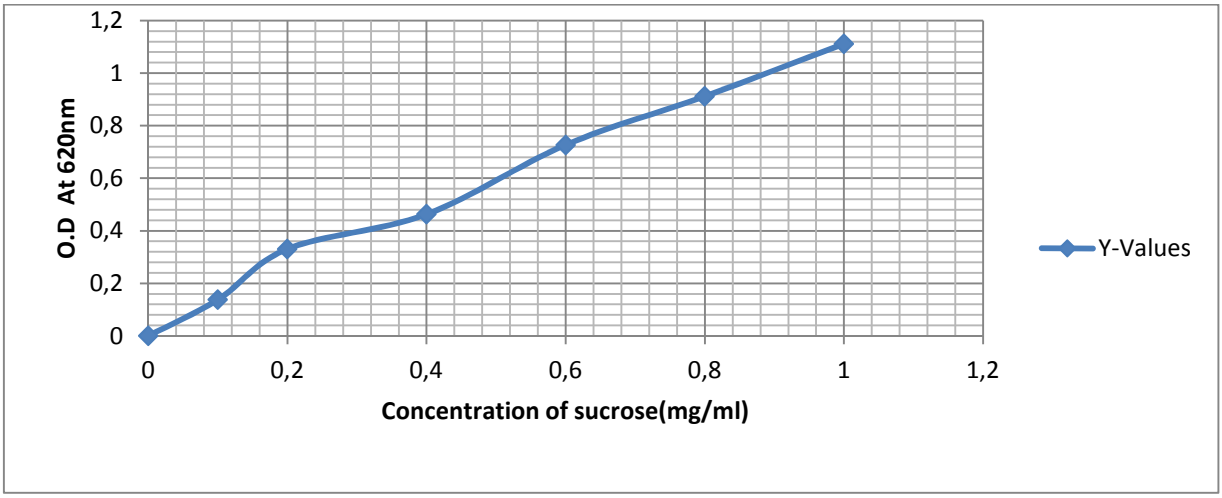

Fig. 2. The concentration of total sugar in sample after heat treatment were 50µg/ml(wheat straw),33µg/ml(paddy straw),45µg/ml(mixed).

Concentration of reducing sugar content in the sample after acid treatment is shown in figure 3.

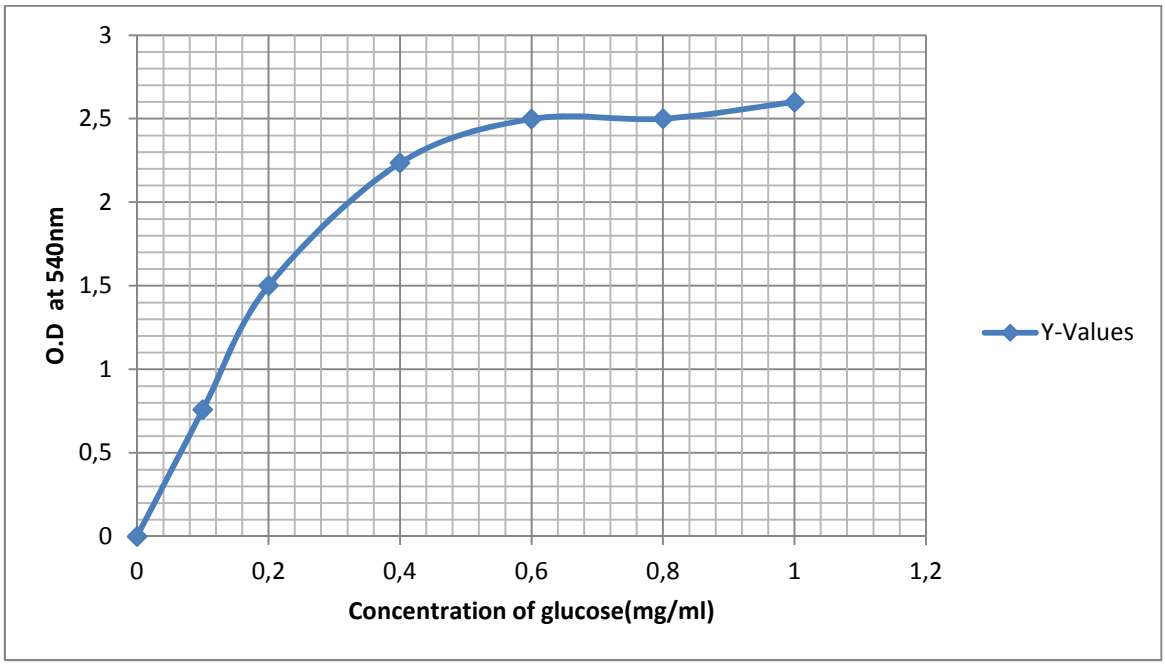

Fig. 3. The concentration of reducing sugar in sample after acid treatment was 56µg/ml (wheat straw) and 170µg/ml (for mixed sample).

Concentration of total sugar content in the sample after acid treatment is shown in figure 4.

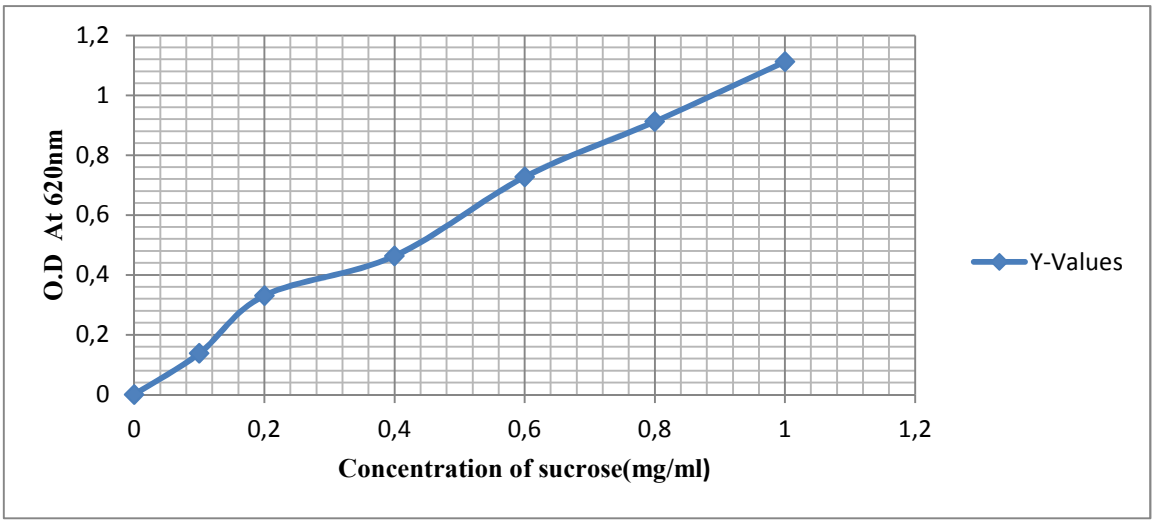

Fig. 4. The concentrations of total sugar in sample after acid treatment were 134µg/ml in wheat straw sample and 70µg/ml in paddy straw sample.

In acid pretreatment, dilute sulphuric acid is added to the feedstock to hydrolyze hemicellulose (1%-2.5%, temperature was 37°C). Sometimes, concentrated sulphuric acid is also utilized for feedstock pretreatment but care should be taken as

this might lead to the formation of fermentation inhibit phenolic compounds. Moreover, the acid must be removed or neutralized before fermentation. Dilute acid pretreatment is the most preferred method for feedstock pretreatment. Acid treatment gives more amount of reducing sugar and total sugar as compare to the heat treatment, so the author prefer the acid treatment for the bioethanol production.

After acid treatment of 1% the concentration of ethanol is shown in figure 5.

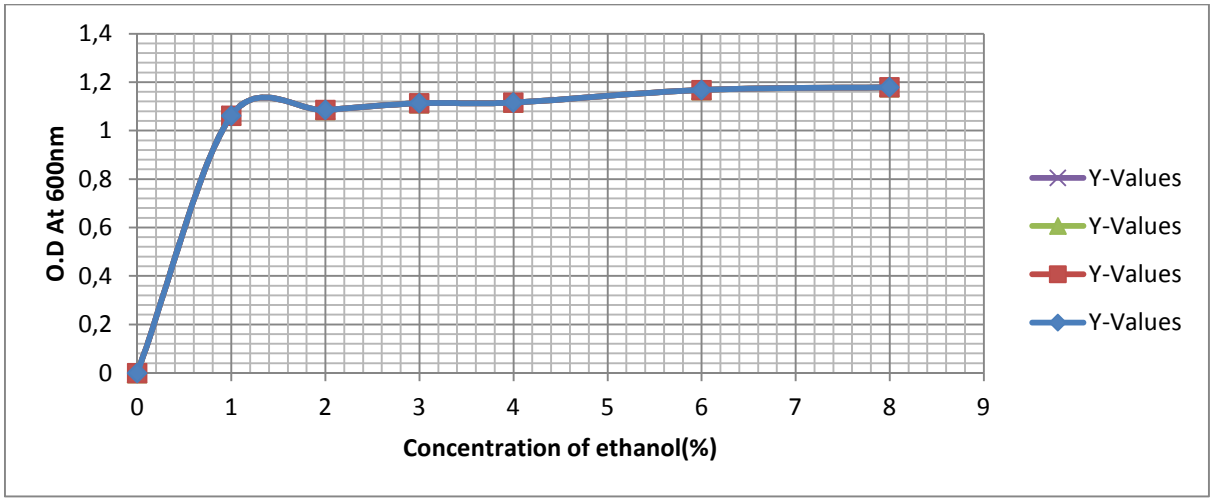

Fig. 5. The concentration of ethanol for acid treatment of 1% was 1.3%.

After acid treatment of 2% the concentration of ethanol is shown in figure 6.

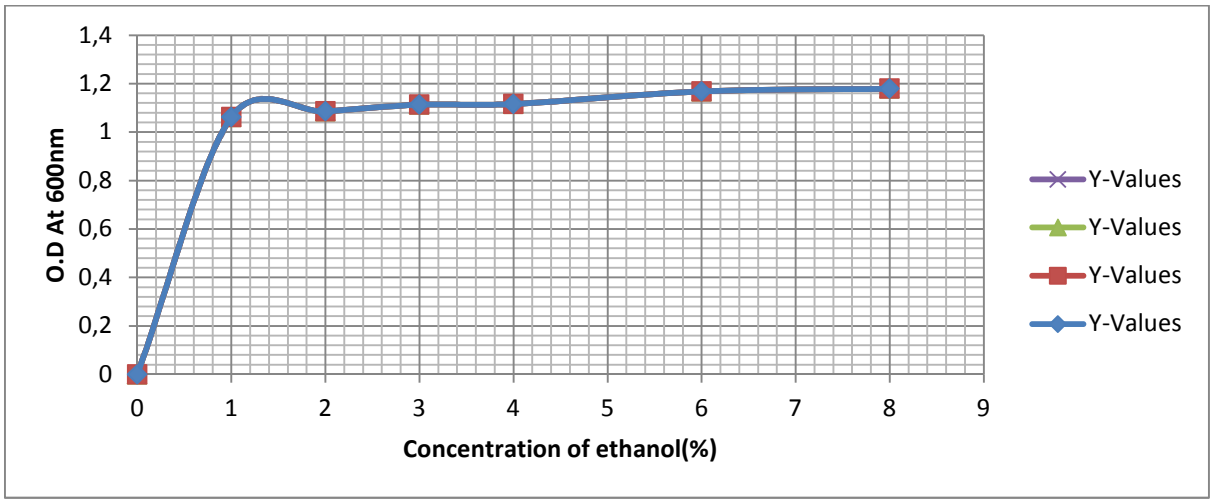

Figure6. The concentration of ethanol for acid treatment of 2% was 3.4%.

After acid treatment of 2.5% the concentration of ethanol is shown in figure 7.

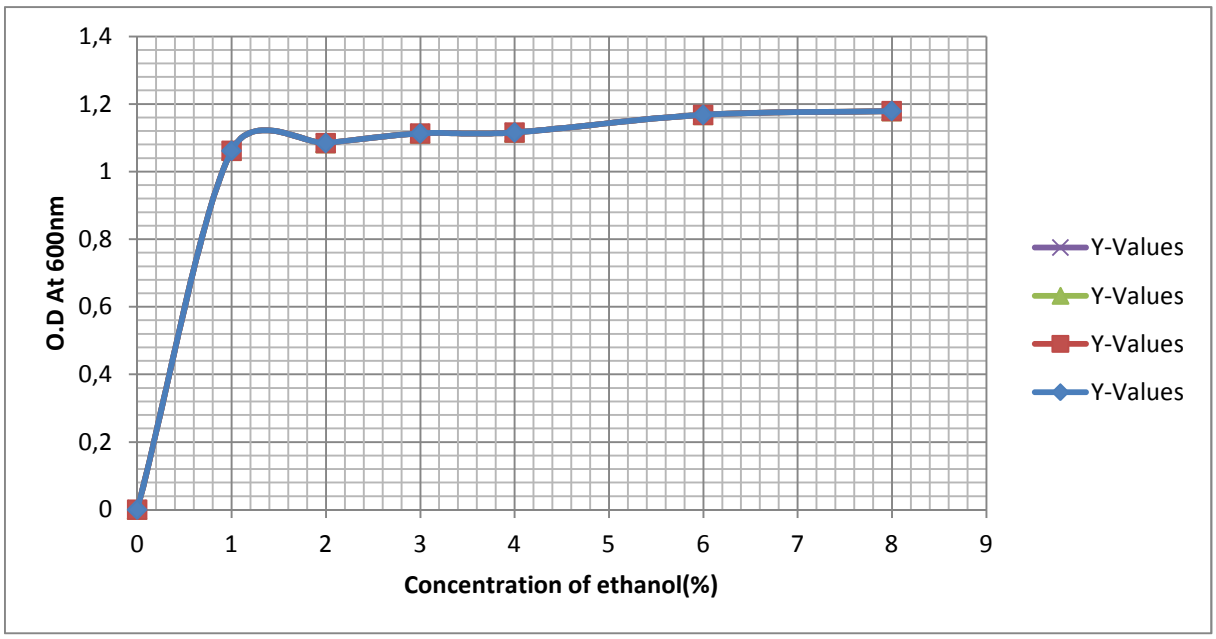

Fig. 7. The concentration of ethanol for acid treatment of 2.5% was 5.4%.

Henning Jørgensen reported that Wheat straw hydrolysate produced by enzymatic hydrolysis of hydrothermal pretreated wheat straw at a very high solids concentration of 30% dry matter (w/w) was used for testing the effect of nutrients on their ability to improve fermentation performance of Saccharomyces cerevisiae. The highest volumetric ethanol productivity was 1.16 g kg-1 h-1 and with an ethanol yield close to maximum theoretical. The use of urea or (NH4)2SO4 separately, together or in combination with MgSO4 or vitamins did not improve fermentation rate and resulted in increased glycerol formation compared to the use of yeast extract. Yeast extract was the single best component in improving fermentation performance and a concentration of 3.5 g kg-1 resulted in high ethanol yield and a volumetric productivity of 0.6 g kg-1 h-1[21]. Héctor A. Ruiz et al. reported that hydrothermal pretreated wheat straw with high cellulose content (>60%) at 180°C for 30 min was used as substrate in simultaneous saccharification and fermentation (SSF) process for bioethanol production using a thermotolerant flocculating strain of Saccharomyces cerevisiae CA11. In order to evaluate the effects of temperature, substrate concentration (as effective cellulose) and enzyme loading on: (1) ethanol conversion yield, (2) ethanol concentration, and (3) CO2 concentration a central composite design (CCD) was used. Results showed that the ethanol conversion yield was mainly affected by enzyme loading, whereas for ethanol and CO2 concentration, enzyme loading and substrate concentration were found to be the most significant parameters. The highest ethanol conversion yield of 85.71% was obtained at 30 _C, 2% substrate and 30 FPU of enzyme loading, whereas the maximum ethanol and CO2 concentrations (14.84 and 14.27 g/L, respectively) were obtained at 45 _C, 3% substrate and 30 FPU of enzyme loading, corresponding to an ethanol yield of 82.4%, demonstrating a low enzyme inhibition and a good yeast performance during SSF process. The high cellulose content obtained in hydrothermal pretreatment and the use of a thermotolerant flocculating strain of S. cerevisiae in SSF suggest as a very promising process for bioethanol production[22]. Wi et al. reported that the optimal doses of cellulase and xylanase enzymes were 23 FPU and 62 IU/g biomass, respectively. Using the optimized enzyme condition and popping pretreatment of rice straw (15% substrate loading, w/v), a sugar recovery of 0.567 g/g biomass (glucose; 0.394 g/g) was obtained in 48 h, which was significantly higher than that from untreated rice straw (total sugar recovery; 0.270 g/g biomass). Fermentation of the hydrolyzates by Saccharomyces cerevisiae resulted in 0.172 g ethanol/g biomass after 24 h, equivalent to 80.9% of the maximum theoretical yield (based on the amount of glucose in raw material) [23].

9 CONCLUSION

Finally the author conclude that acid treatment is better then heat treatment because after acid treatment he got more amount of reducing sugar, which is further used for bioethanol production. 2.5% sulphuric acid gives more amount of reducing sugar. The pH of media was 5.5 with 0.6mg/100ml $(NH4)_2SO_4$ with 37ºc temperature .The amount of ethanol

produced was 5.4 %(v/v). So the autthor can say that reducing sugar and carbohydrates of agro wastes, particularly paddy straw and wheat straw are potential substrates which can be exploited in industries for bioethanol (biofuel) production in future. These agro residues (wheat straw and paddy straw) are cheap, abundant and more importantly renewable source for bioethanol production. Based on the results obtained, it can be conclude that reducing sugar and carbohydrates of agro residues can be used as raw materials for bio-ethanol production. Acid treatment is more effective method for extraction of reducing sugar from these cellulosic agro residues.

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