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Ethanol production from waste materials

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Abstract

Experiment was designed for ethanol production using corn and other organic waste material containing starch contents and cellulosic material while barely used for diastase and acidic digestion methods. The effect of temperature, yeast, barely diastase and various dilutions of acid (sulfuric acids) were investigated on ethanol production. The result showed that corn yielded high amount of ethanol (445ml) as compared to cellulosic material which produced 132ml of ethanol from one kg of weight. It was also noted that with the increase of barely and yeast amount in a proper manner can increase ethanol production from different starch sources. It was also noted that acid dilutions affected cellulose digestion where high yield of reducing sugar was noted at 0.75% of sulfuric acid dilution. It was concluded from the present experiment that economical sources of starch and various dilutions of acids should be tried on cellulose digestion for bio-fuel production to withstand in this energy crisis time.

Key words: Ethanol, Biomass, Cellulose, Fermentation, Municipal waste

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Introduction

Ethanol is also used as fuel in gasoline engine, and as preservative for biological specimens (Altınta et al. 2002). Ethanol is very old chemical and has been made since old times due to the sugar fermentation (Hughes et al. 2009). Majority of the beverage industry produce ethanol by fermentation process (Patle et al. 2008).

Ethanol is produced from any fodder crop which contains simple sugar in abundance or their polymers (Hughes et al. 2009). The polymers like starch and cellulose are broken down into simple sugars through chemical hydrolysis or enzymatic hydrolysis (saccharification), and then converted by fermentation process to ethanol *and carbon dioxide (Jamai et al., 2007), in saccharification,* process starch is converted into simple sugar(monosaccharide) using microorganism or enzymes such as glucoamylase and α-amylase (Shapouri et al. 2004).

Barley is first germinated, dried (baked on pan) and crushed for the whole process (Shapouri et al. 2002). The resulting sugars are then by help of yeast (Saccharomycescerevisiae.) converted into ethanol (Oner et al. 2005). Generally the yeast attack on hexose sugars, but carbohydrates containing pentose subunits could also be digested by specific yeast into ethanol (Ohgren et al., 2006).

Along with the production of industrial ethanol research studies are also focused upon the upgrading the bioconversion process of ethanol production. Ethanol as a fuel has promising prospects. For biofuel purpose 85-95% alcohol is needed; the distilled out product contains approximately 95% ethanol. However further treatment may lead to 99% pure ethanol. Dehydrating agents like CaCl2, MgCl2 etc can be used produce absolute ethanol (99%) (Shigechi et al. 2008).

Materials and methods

Ethanol is a basic ingredient of hot drinks but now-a-days its production is preferably doing as engine fuel. The present project was conducted keeping this objective in mind.

Carbon Raw Material

Corn grain: Corn was obtained from the local markets of Peshawar city. Waste vegetable (Municipal Waste): Waste vegetable was also collected from local vegetable shops and main sabzi mandi.

Germination of barley for diastase activation

Barley malt contains appreciable amount of diastase enzyme. For the hydrolysis starchy material during the experiment two kg of barley was malted as per the procedure of Davis et al. (2005). Two kg of barley was weighted and was taken in crates made of wood and that were watered so that excess amount of water would leach down and only desired moister retained for the germination of barley. This process was carried out in dark for 3 days and night and every day the moisture content were noted regularly. When the seedling of barley becomes 1cm long the process was stopped. After that barley seeds were dried and baked on using pan. The dried material was ground in home grinder and used as diastase source for ethanol production.

Isolation and selection of yeast strains

Yeasts samples were isolated in sterilized condition from corn (grains) samples collected from four regions of KPK, namely Swat, Peshawar, Hazara division and D I Khan, Pakistan. Yeast isolation was done at approximately 36 °C by using the technique which is called enrichment which contains sugar cane juice (five percent to eight percent total sugars) (Consuelo *et al*, 2010), 0.05% ammonium sulphate and 4% (volume by volume) ethanol and with about (pH 4.7) and without pH adjustment of about (pH 3.9). Cultures of pure yeast were isolated and were kept on agar slants (One percent yeast extract, two percent peptone, two percent glucose and two percent agar) and stored at about 10 °C.

Saccharification

Enzymatic hydrolysis

Saccharification of the raw material was conducted according to Guo et al. (2008) with some modifications. Two kg of corn grains were boiled in a steel pot of 8L size. The boiled material was cooled to 60oC and blended with home blender. (Generally the material in this condition is called mash. The dilution of this material is termed as wort). The pH of the material was made upto 4.85 with 0.05 M citrate buffer, and 20 g of diastase enzyme in the form of barley malt was added to 100 g of the raw material used for ethanol production (about 3%, dry basis) in a total working volume of 500 milliliter in one liter conical flask. These flasks were kept in incubation at 52-55oC on an orbital shaker operated at 140 rpm. The percentage of saccharification was calculated as follows:

$$\%\,Saccharification = \frac{Reducing\ sugars * 0.9}{Carbohydrates\ in\ substrate} * 100$$

Acid hydrolysis

Municipal waste was treated with different concentration (0.25%, 0.5%, 0.75%, 1%, 2%, 3%, 4%) of H2SO4. 2kg of the waste was boiled with 3 L of each concentration of the H2SO4 in glass pot of 8L size. The reducing sugar was calculated by Fehling test. Hydrolysis efficiency was calculated as follows. (Guo et al. 2008).

Fermentation

Fermentation of the carbon raw material is carried out with the help of yeast according to the method of Voca et al. (2009). First of all the sample was prepared and filtered, meanwhile the yeast was dissolved make its solution, about 10 ml of the yeast solution was added in the sample and was kept for fermentation in dark up to seven days. The process of ethanol fermentation was done by using glass fermenter and mud pitcher as fermenter tank containing 650 milliliter of 0.05 M sodium citrate buffer (pH 4.9), this process was done by following the two-step of the first pre-saccharification and then the simultaneous saccharification and fermentation (SSF).

Distillation

The mixture after fermentation was distilled out with the reflux column and reflux condenser for the maximum percentage of alcohol, the method of Vander Griend et al. (2007) with some modification was followed for research work to get good results.

Results and Discussion

The ethanol production from maize, the effect of barley and amylase enzyme, time effect on saccharification, temperature effect on ethanol production and also the effect of various yeast strains isolated from grain belonging to different ecological regions were studied and results were presented in the form of tables and figures
The minimum saccharification %age was noted at 48 hours intervalswhile analyzing reducing sugar at this interval time. However the similar research was also conducted by Park et al. (2009) strongly supported the present work. The present work is also in line with Hoyong Sohn et al. (2008) which worked on the saccharification process.

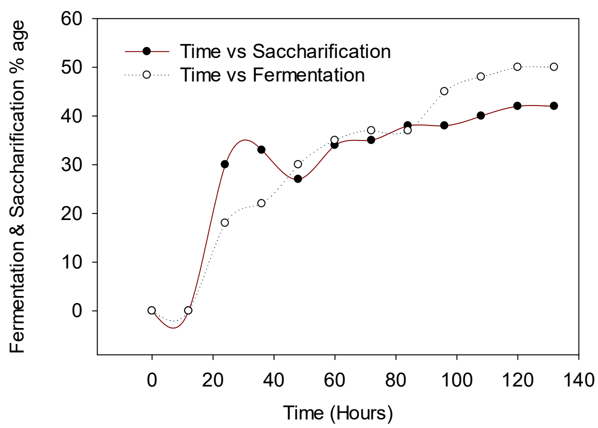

Figure 1:Effects of time interval on saccharification and fermentation of maize through amylase enzyme at 60 °C

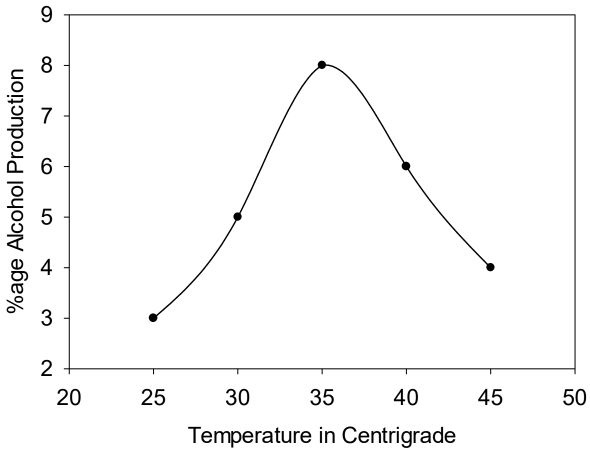

Figure 2: Ethanol Production on the basis of temperature

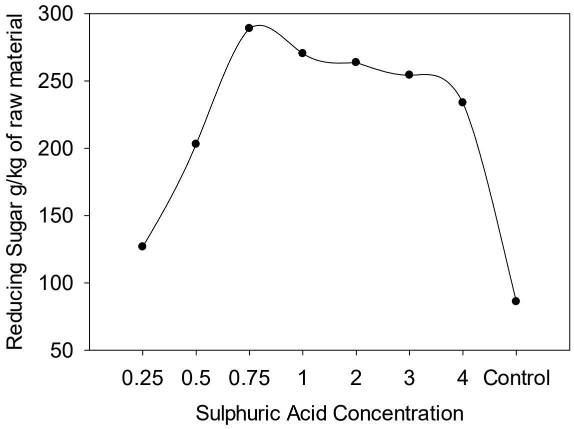

Figure 3: Hydrolysis of Organic Material with different concentrations of sulphuric acid

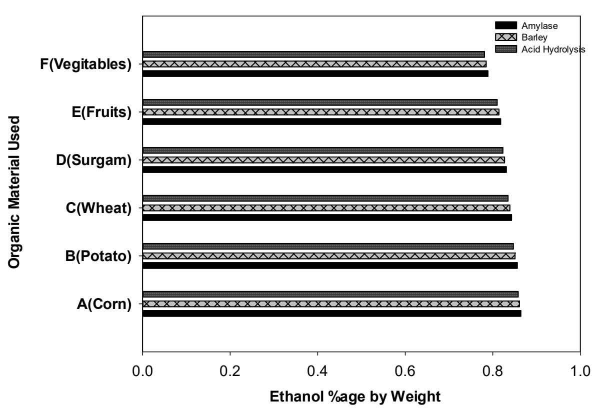

Figure 4: Effect of various agents (Amaylase, Barley, Acid Hydrolysis) on different Carbohydrate sources

The saccharified maize sample, subjected to fermentation process by yeast (micro-organism) at 370C was presented in Fig.2. Here the maximum alcohol content was achieved at 37 0C on further rise of temperature will reduce the alcoholic content up to great extent in Fig. 2. The minimum concentration of alcohol was achieved at 250C than at 450C.

The maximum saccharified sample is subjected to the further process of fermentation as shown in Fig.1. The minimum fermentation in terms of sample alcohol content was observed at 24th hour, where before 24th hour no alcohol was detected in the sample which indicates that fermentation was not yet started. While the fermentation reached to its maximum at 120th hour after that no appreciable change was observed. This means that fermentation required a great span of time, which should be up to 120th hour at 50 0C in Fig. 1. Such type of experiment was also conducted by Kyunghee et al. (2010).

Municipal waste was treated with different dilution of sulphuric acid (H2SO4). The hydrolysis of the sample measured in the form of reducing sugars showed that dilution had profound effect on hydrolysis. The minimum hydrolysis of the waste was obtained at 0.25% of the acid dilution while maximum was got on 0.75% in Fig. 3. Although the higher dilution was efficiently hydrolyzed the waste but that decreased with higher concentration of the acids (Zhao and Bai, 2009). For different waste the acid dilution should be adjusted. The present work was in line with Patel et al. (2008). Although the study of Joseph Di Pardo (2000), was on the same subject,so from the above graph we can say that for hydrolysis of municipal waste 0.75% dilution of acid is quite well to get maximum results in Fig 3.

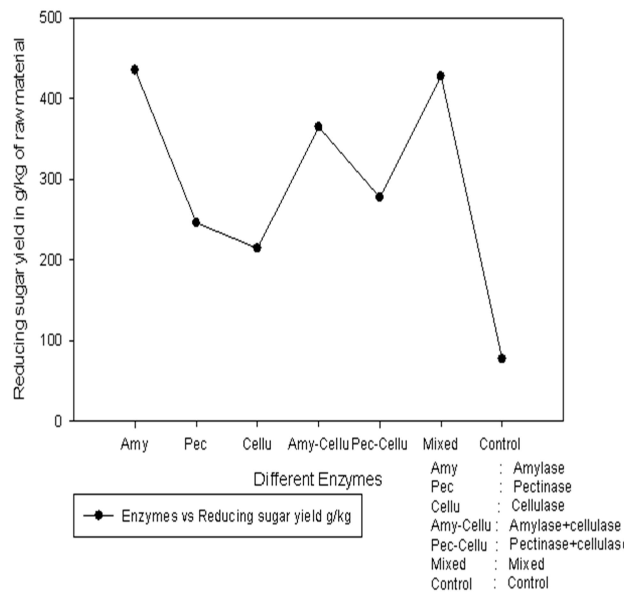

Figure 5: Hydrolysis of municipal waste with different enzymes.

Various carbohydrates sources (corn, potato, wheat, fruits and vegetables (mixed waste)) treated with three different hydrolyzing agents, been taken according to their equivalency action as in Fig 4. The result showed that the best hydrolyzing agent was amylase showed in Fig 4, which gave maximum hydrolyzing percentage in case of all sources followed by barley malt and acids. Among the different sources corn, wheat and potato showed best hydrolyzing percentage while the minimum percentage was noted in vegetables which might be due to some hindering substances (e.g. corn) (Hashem and Darwish, 2010). This work is in line with Patel et al. (2008) which support our study.

Similarly on various carbohydrate sources to get maximum reducing sugar content g/kg of the raw material we use different enzymes alone and in combination. With amalyase enzyme we got maximum reducing sugar content of 435 g/kg followed by the combination of enzymes 427.6 g/kg and minimum of 214.4 g/kg of reducing sugar by using cellulase enzyme as in Fig 5. This work is in line with Hashem and Darwish 2010.

Conclusion

Enzymatic hydrolysis of cellulosic material was best for obtaining maximum amount of reducing sugar which further used for alcohol fermentation. Acid hydrolysis had posed various hindrances. Best fermentation temperature was found at 37-50^{o}C depending upon the conditions and best fermentation time was 120hrs. Yeast strains had variability in their ethanol production.

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