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The potentiality of bioethanol production from corn (*Zea mays* L.) as a renewable source

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ABSTRACT

Corn (*Zea mays* L.) is one of the versatile crop which is used as food, feed, fodder and in recent past as a source of bio-fuel. The sub-tropical climate is very favorable for corn cultivation. Traditionally, corn was grown in South and Southeast Asia primarily as a subsistence food crop. Worldwide it is being cultivated in over 170 countries representing an area of 185 million ha with a productivity of 5.62 t ha⁻¹ (FAO, 2017). Out of world corn production of 1037 million MT, SAARC countries comprising of Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan and Sri Lanka represent 3.2 % with a productivity of 3.8 t ha⁻¹. Among SAARC countries, the highest productivity of 6.9 t ha⁻¹ is reported in Bangladesh. Corn can be an important renewable source for bioethanol production. This research was carried out to evaluate Bangladeshi Corn for optimum bioethanol production. A 100 g of corn flour was mixed with 300 ml distilled water was blended and sterilized. The experiment was conducted with a temperature of 30 °C, pH 6.0 and 20 % sugar concentration. For alcoholic fermentation, 200 ml yeast (*Saccharomyces cerevisiae* CCD) was added to make the total volume 500 ml. Addition of small amount of 1750 unit α -amylase enzyme to the substrate solution was found to enhance the fermentation process quicker. After 6-days of incubation time corn produces 63.57 ml of ethanol with 13.33 % (v/v) purity. The non-filtered solution produces comparatively more ethanol (63.57 ml with 13.33 % purity) than the filtered solution (53.66 ml with 10 % purity). The purity can be increased by re-distillation process.

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INTRODUCTION

Bioethanol is considered to be the most optimistic biofuel. With the inevitable depletion of the world's petroleum supply, there has been an increasing interest in alternative, non-petroleum based sources of energy. Additionally, ethanol from biomass-based waste materials is considered as bioethanol. Alcohol is an organic compound that has one or more hydroxyl (OH) groups attached to a carbon atom. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol fermented from renewable sources for fuel or fuel additives is known as bioethanol. Bioethanol, unlike petroleum, is a form of renewable energy source that can be produced from agricultural feedstocks. The first generation of ethanol production used corn as a substrate, later corn was considered as a feedstock lead to the second generation of production of ethanol which used microorganisms and different wastes as substrates [1]. Ethanol an important biofuel, having high calorific value has the added advantage of being less polluting than most sources of energy that are in use today. Reports available suggest that previous

natural substrates for ethanol production *via* saccharification have included sugarcane bagasse, wheat straw, corn, softwood etc. *Saccharomyces cerevisiae* is usually considered the typical yeast of wine and cider fermentations; among other species of the genus *Saccharomyces*, *S. bayanus*-characterized by high ethanol tolerance is used for the production of wine, sparkling wines, and cider, and it can also be used in industrial applications for bioethanol production [2]. The glucose can then be utilized by organisms of the genera *Saccharomyces* which can ferment the glucose into fuels such as ethanol [3]. The world ethanol production has reached about 51,000 million liters [4], being the USA and Brazil the first producers and India stands fourth among the top fuel ethanol producers. Since it is estimated that the fossil fuels will be running out by the next few decades, attention has currently been dedicated to the conversion of biomass into fuel ethanol. Main feedstocks for bioethanol production are sugarcane (in Brazil) and corn grains (in USA), while many other agricultural raw materials are also used worldwide. World ethanol production for transport fuel tripled between 2000 and 2007 from 17 billion to more than 52 billion liters. From 2007 to 2008, the share of ethanol in global gasoline

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type fuel use increased from 3.7% to 5.4%. In 2009 worldwide ethanol fuel production reached 19.5 billion gallons (73.9 billion liters). Ethanol is widely used in Brazil and in the United States, and together both countries were responsible for 89 percent of the world's ethanol fuel production in 2009. Most cars on the road today in the U.S. can run on blends of up to 10% ethanol, and the use of 10% ethanol gasoline is mandated in some U.S. states and cities. Since 1976 the Brazilian government has made it mandatory to blend ethanol with gasoline, and since 2007 the legal blend is around 25% ethanol and 75% gasoline (E25). In addition, by 2010 Brazil had a fleet of more than 10 million flexible-fuel vehicles regularly using neat ethanol fuel (Goettemoeller *et al.*, 2007). Each enzyme will have a different temperature range where it becomes inactive. Even if one essential enzyme stops working, the organism fails to grow. Hence, the first essential enzyme that gets deactivated defines the maximal temperature at which that organism can grow. At the lower end, it gets more complicated. Usually, the enzymes are not inactivated but rather just slow down [5]. The most well-known and commercially significant yeasts that been primarily used for bioethanol production are the related species and strains of *Saccharomyces cerevisiae* [6]. These organisms have long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages and in the baking industry [7]. Since there is no wild type microorganism that could efficiently accomplish this process. *Saccharomyces cerevisiae* is utilized as a part of the fermentation process since it changes over sugar with oxygen to give carbon dioxide. As indicated by the International Energy Agency, cellulosic ethanol could permit ethanol fuels to assume a much greater part later on than previously suspected [8]. However, the aim of this study was to evaluate Bangladeshi Corn as a good renewable source of bioethanol production by using the yeast *Saccharomyces cerevisiae* CCD.

MATERIALS AND METHODS

Experimental Site and Duration

This experiment was conducted at Institute of Environmental Science Laboratory of Rajshahi University during from June to November 2017. The samples were collected as raw materials for ethanol production on July 2017 from the local market of Rajshahi city in Bangladesh. The steps for biofuel production are-

Raw Material Collection and Preparation

The corn and corn flour were collected from the local market of Rajshahi city, Bangladesh. It was boiled for 25-30 minutes. Then was blended with a blender machine and sterilized. This sterilized solution was used as substrate for further experiment.

Yeast Strain and Culture Media

The research was conducted in the IES laboratory. Yeast strain (*Saccharomyces cerevisiae* CCD) was collected from the Spirit Section of Carew and Co., Darsana, Bangladesh. For yeast culture, modified YMPD (Yeast-Malt-Peptone-Dextrose) broth culture media was used. The YMPD media was prepared with yeast extract (3.0 g), malt extract (3.0 g), peptone (5.0 g) and

dextrose (10.0 g). All of these ingredients were dissolved in 1000 ml of water and adjusted to pH 6.0.

Bioethanol Production from Corn

The Bangladeshi Corn was assessed for bioethanol production. It was boiled and blended with a blender machine. Then it was used as raw materials for bioethanol production. For optimum bioethanol production 200 ml of yeast culture was added in 300 ml solution (20 %) and incubated for 6 days. All the stated experiments were conducted at pH 6.0 and 30 °C.

Bioethanol Production from Filtering and Non-filtering Corn Solution

100 g samples of each were blended with 300 ml distilled water. Then one of them was filtered with a muslin cloth and the other was treated as non-filtered. About 1750 unit of α -amylase enzyme and 200 ml of yeast were added. All the stated experiments were conducted at pH 6.0 and 30 °C. After 6-days of fermentation the crude fermented solution was first centrifuged at 12,000 rpm for five minutes to remove the unused starch and yeast cell. Then, the clear solution was taken into rotary evaporator for separation of ethanol at 78.5 °C.

Estimation of Total Sugar Before and After Fermentation

Total sugar content of corn solution was determined by sugar measurement machine named "On Call Plus". Sugar concentration before and after fermentation was measured.

Distillation Process

Distillation was carried out at 78.5 °C. The condensation product was collected in a flask and used for estimation of ethanol concentration.

Measurement of Purity of Produced Alcohol

The percent of purity of produced bioethanol from corn was measured by using an alcohol meter (Jiujiingnongduji, China). This meter can measure the alcohol purity from 0-100%.

Data Analysis

The experimental design was completely randomized, with three replicates all data were expressed as mean values \pm SD (Standard Deviation).

RESULTS AND DISCUSSION

Bioethanol Production from Corn

Bioethanol production from corn of Bangladesh was assessed. It was shown that the sugar content was reduced to 5.33 m mol L⁻¹ from 8.23 m mol L⁻¹ after fermentation. 63.57 ml of ethanol was produced from corn of Bangladesh with purity of 13.33 % (Table 1 and Figure 1).

Table 1: Bioethanol production from corn

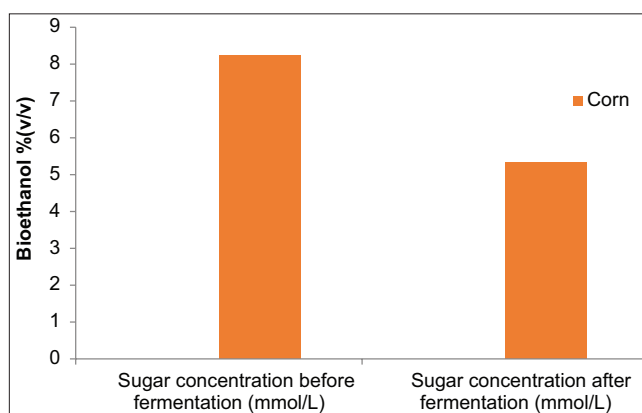
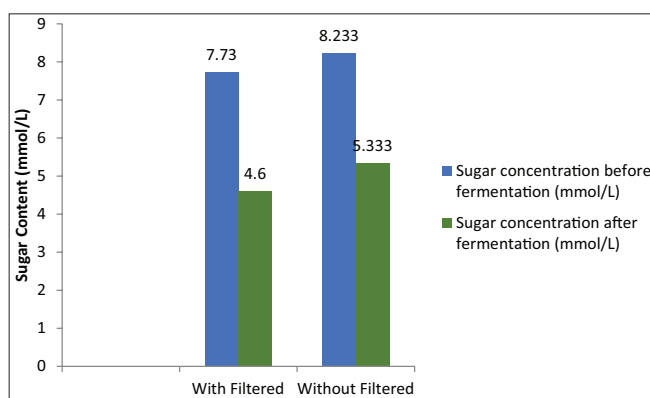
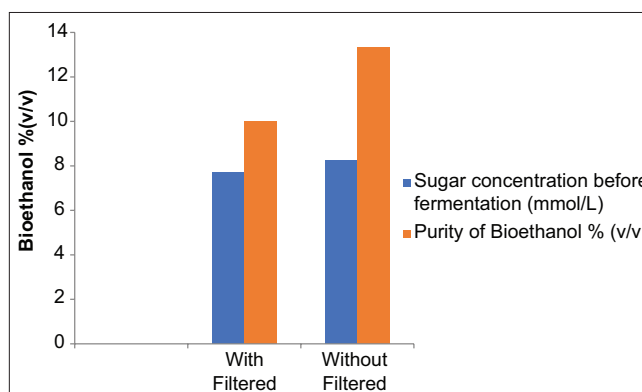
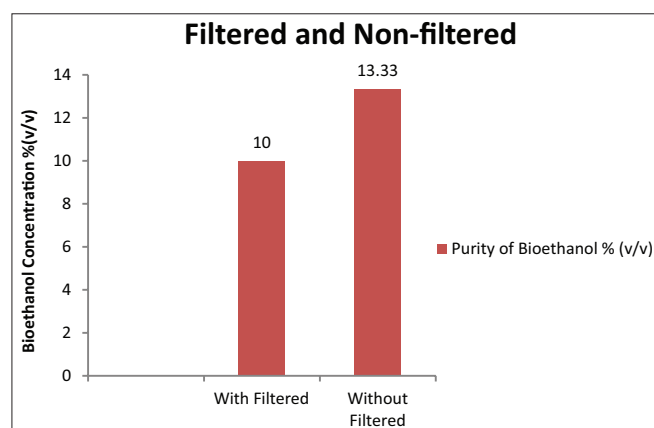
Substrate name	Sugar concentration before fermentation (m mol L ⁻¹)	Sugar concentration after fermentation (m mol L ⁻¹)	OD at 610 nm	Volume after fermentation (ml)	Volume after distillation (ml)	Purity of Bioethanol % (v/v)
Corn	8.23±0.43	5.33±0.22	0.44±0.02	462±1.15	63.57±0.58	13.33±0.88

Values are the means±SD of three replicates each.

Table 2: Bioethanol production from filtered and non-filtered Corn solution

Parameter	Sugar concentration before fermentation (mmol/L)	Sugar concentration after fermentation (mmol/L)	OD at 610 nm	Volume after fermentation (ml)	Volume after distillation (ml)	Purity of Bioethanol % (v/v)
With Filtered	7.73±0.60	4.60±0.52	0.36±0.03	454.30±3.38	53.66±4.63	10±0.58
Without Filtered	8.23±0.43	5.33±0.22	0.44±0.02	462±1.15	63.57±0.58	13.33±0.88

Values are the means±SD of three replicates each.

**Figure 1: Sugar content of corn before and after fermentation****Figure 2: Bioethanol from filtered and non-filtered Corn solution**

The result is quite similar with the report of Janani et al. (2013) [9] the concentration of ethanol present in the grape sample was 6.21 % and the ethanol in the papaya sample was 4.19 %, whereas the concentration of ethanol present in the apple sample was 4.72 % and ethanol present in banana was 5.40 %. With the increase in sugar concentration, the ethanol production increased significantly.

Bioethanol Production from Filtering and Non-filtering Corn

About each 100 g substrate with 300 ml distilled water was blended and prepared 20 % concentration. Then two experiments were done, one was filtered with a muslin cloth and the other was kept without filtered. In non-filtered solution the sugar

concentration (8.23 m mol L⁻¹) and yield (63.57 ml) of bioethanol is comparatively high than the filtered solution (7.73 m mol L⁻¹ and 53.66 ml) as shown in Figure 2 and Table 2.

This result is similar with Sandesh Babu et al. (2014), [10] reported that after two weeks they obtained 520 µg/ml and 470 µg/ml of ethanol from 100 mL of fruit juices of Sugarcane, Grape and then followed by Watermelon and least being the Mousambi after distillation and maintaining a pH of 4 and temperature of 35°C. Starch from starchy crops, such as cereals, to become fermentable needs a pre-treatment composed of three steps: gelatinization, to allow the starch to lose its crystallinity and become an amorphous gel; liquefaction, where

starch is hydrolyzed to dextrans by an alfa-amylase and viscosity is reduced; and saccharification, where a gluco-amylase is added to convert dextrans to glucose [11,12]. Saccharification can be managed to be simultaneous to fermentation: this makes the glucose gradually available to microorganisms and reduces contamination risks, process duration, and costs [13,14,15].

CONCLUSION

We can conclude that more concentrated form of ethanol could be obtained from corn by re-distillation the product ethanol obtained initially using a higher grade of distillation setup and if the ethanol tolerance capability of yeast species is improvised by mutating the yeast species. This more concentrated form of ethanol could be used as a biofuel, which releases no toxic gases out in the environment. This process is environment friendly and the left over residues after fermentation can be disposed in the soil acting as a fertilizer for the soil. So even a common man may develop this process and produce it on commercial basis.

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