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Production of Bioethanol from Agricultural Wastes Utilising Fermentation Potential of *Saccharomyces cerevisiae*

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ABSTRACT

The present study was designed to produce bioethanol from three lignocellulosic biomasses, such as sugarcane bagasse, rice straw and wheat straw utilising fermentation potential of *Saccharomyces cerevisiae*. After physical treatment, three substrates were pre-treated with 5% H_2SO_4 + 1% NaOH, followed by steaming at 121°C for 15 minutes. Then the substrates were hydrolysed by commercial alpha-amylase enzyme and fermented by *S. cerevisiae* under anaerobic and submerged conditions. Fermentation media were prepared at different pH (3.7, 3.9, 4 and 4.6) and the fermentation process was carried out at different temperature (37°C, 40°C and 45°C) and bioethanol yield was measured after different fermentation periods (72 hrs, 96 hrs, and 120 hrs) to investigate the effect of temperature, pH and fermentation period on bioethanol production. The highest bioethanol concentration (16.29%) was obtained from sugarcane bagasse when the pH of fermentation broth was 4.0 and the temperature was 40°C after 96 hours of the fermentation period. The lowest ethanol concentration (13.0%) was obtained from the wheat straw at pH 3.7 while the temperature was 37°C after 72 hours of the fermentation period. Among the tested substrates, sugarcane bagasse produced more bioethanol than that of rice straw and wheat straw by using the fermentation potential of *S. cerevisiae*. The result of the study suggested that agricultural wastes can be utilised as cost-effective substrates for bioethanol production.

Introduction

Bioethanol (C_2H_5OH) is a high-octane number, liquid, renewable, sustainable, and eco-friendly biofuel (Chin *et al.,* 2013; Di Nicola *et al.,* 2011). As it is bio-based and oxygenated (35% oxygen), hence providing a potential to reduce particulate and NO_2 emissions in compression-ignition engines (Panichelli *et al*., 2008). Bioethanol comes in a different number of forms and meets different energy needs. One such blend of bioethanol for light-duty vehicles is popularly known as E85 contains 85% Bioethanol and 15% Gasoline (Sundvor *et al.,* 2018). Bioethanol can be produced from plant material that contains glucose such as sugarcane, corn, sugar beet and other cereals such as maize and burley (Binod *et al*., 2010). Generally, bioethanol for fuel derives from sugarcane called the first-generation biofuel, and is used as pure or blended form with gasoline (24% bioethanol, 76% gasoline) (Busic *et al.,* 2018). Throughout the development, bioethanol has been produced from a variety of feedstocks such as sugarcane bagasse, miscanthus, sorghum, switchgrass, reed canary grass, cord grasses, hemp, kenaf, potatoes, sweet potatoes, cassava, sunflower, fruits, molasses, stover, wheat and Jerusalem artichoke (Binod *et al*., 2010).

However, the environmental and economic concerns about the first-generation bioethanol production process using sugar or starch from sugarcane, corn, and wheat have led to the development of a second-generation (or advanced) bioethanol process using waste feedstock, for example; municipal solid waste, crop residues, sludge and livestock manure (Lee *et al.,* 2013). Waste biomass in the form of lignocellulosic or starch-based origin is a potential source of free fermentable sugars for ethanol fermentation (Kim *et al*., 2008). Moreover, second-generation bioethanol is greener and sustainable because it is produced from the available feedstock or feedstock waste (Lennartsson *et*

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al., 2014). Many studies have been conducted extensively across the globe to develop sustainable technology from the available feedstock (Busic *et al.,* 2018). However, an industrial scale-up of the second-generation (advanced) bioethanol production process is still hampered by several critical technological issues and bottleneck steps (Lee *et al.,* 2013; Zhang *et al*., 2012). Biotechnological usefulness of *S. cerevisiae* resides in its unique biological characteristics, i.e., its fermentation capacity, accompanied by the production of alcohol and CO_2 and its resilience to adverse conditions of osmolarity and low pH (Maria Parapouli *et al*., 2020). Therefore, the present study was undertaken to optimise the protocol for bioethanol production from agricultural wastes such as rice straw, wheat straw and sugarcane bagasse utilising the fermentation potential of *S. cerevisiae*.

Materials and Methods

Collection and processing of agricultural wastes

Agricultural wastes such as rice straw, wheat straw and sugarcane bagasse were collected from different areas of Mymensingh division. Rice straw and wheat straw were collected from Bangladesh Institute of Nuclear Agriculture (BINA), and sugarcane bagasse was collected from Ganginar par, Mymensingh using sterile plastic bags and brought to the laboratory for further processing. After collection, the raw samples were cleaned, oven-dried, and pH of the raw materials was recorded. Here, original gravity was measured by hydrometer.

Pre-treatment of the substrates for fermentation

To reduce the biomass size and cellulose content, the raw samples were chipped, ground, milled, and finally pre-treated with acid hydrolysis following the protocol of Braide *et al.* (2016). Acid pre-treatment was done by dissolving 25gm of each substrate into 250ml of 5% H_2SO_4 using a 500ml conical flask to achieve delignification. The mixtures were hydrolysed by autoclaving at 121°C for 15minutes. Then the pre-treated samples were filtered using a 24cm pleated filter paper into a 500 ml conical flask. The filtrates were incubated in a water bath at 50°C for 30 minutes. The residue was washed with 1% NaOH to neutralise the acid and then with distilled water and finally dried in an oven at 70°C for 24h.

Enzymatic hydrolysis

Enzymatic hydrolysis was done following the protocol of Braide *et al.* (2016). The cellulosic substrate was autoclaved for 15 minutes, followed by hydrolysis with the commercial enzyme Termamyl (Alpha-amylase) at 50^{0}C. This enzyme helps to break down the cellulose into simple-sugar (glucose) for yeast action. Then the mixtures of the cellulosic substrates and termamyl enzyme were placed into a programmed thermostatic mashing bath at 70^{0}C with stirring condition. After that, the mixture was allowed to boil for 30 minutes.

Wort production

The volume of the samples in each beaker was made up to 250ml by the addition of distilled water. It was then boiled at 90^{0}C for one hour to halt enzymatic activity. The resultant sample (also called mash) was then cooled to 45^{0}C, and the addition of distilled water made up the volume of each mash. The mash was then filtered into a measuring cylinder by the use of 24cm pleated filter paper placed in a funnel. 250 ml of the resultant liquid called wort was then added into 500 ml sterile conical flask.

Inoculation of S. cerevisiae source

The yeast *S. cerevisiae* was used from the collection of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh. Before measuring bioethanol production from different agricultural wastes, six *S. cerevisiae* strains such as SC-O, SC-B, SC-P, SC-G, SC-Pp, and SC-L were sub-cultured and checked for their viability and growth at a higher temperature. The strain, which showed fast and active growth at 37°C was selected for further study.

Inoculum development for fermentation process

A loop-full of the yeast colony was transferred from the agar plate into 100ml of the 5% yeast extract peptone dextrose (YEPD) broth and incubated at 30°C on a shaker at 120 rpm for 24 hrs. 7ml of the broth was centrifuged at 4500 rpm for 5min. The supernatant was decanted, and the pellet was re-suspended in 10ml of sterile distilled water, centrifuged and the supernatant decanted. The pellet was re-suspended in 1/10th of 50ml citrate buffer of working solution for each flask and was used as its inoculums. This process was performed in a centrifuge tube to obtain pure *S. cerevisiae* yeast.

Determination of the effect of pH, temperature and incubation time on bioethanol production

To find out the optimum pH for bioethanol production, substrates were fermented in the fermentation broth having different pH values from 3.7, 3.9, 4.0, and 4.6 while the temperature and incubation period were kept constant at 40^{0}C and 96 hours, respectively. Samples were fermented at different temperatures condition, i.e., 37^{0}C, 40^{0}C and 45^{0}C having constant pH and incubation time (pH 4 and 96 hours) to determine the optimum temperature. Furthermore, samples were incubated for the different incubation periods of 72, 96, and 120 hours at pH 4.0 and temperature 40^{0}C to find the optimum incubation time for bioethanol production.

Calculation for bioethanol concentration

The following formula was used for calculation of bioethanol yield:

Bioethanol conc. (%) = [(Original gravity – Final gravity)/100] * 131

Here, 131= A mandated specific factor to calculate bioethanol concentration (Spedding *et al*., 2016).

Statistical analysis

All analyses were carried out in three replicates, and statistical analysis was performed by one-way ANOVA followed by the post-hoc test using the Statistical Package for Social Sciences (SPSS, 2007, version 16.0). All results were presented as mean ± SD, and P-values <0.05 were considered significant.

Results and Discussion

Bioethanol is a type of biofuel made through the fermentation of plant sugars from crops and biomass resources. Lignocellulosic substances, such as agricultural wastes, are attractive feedstock for bioethanol production. In this study, three different substrates, for example, sugarcane bagasse, rice straw, and wheat straw, were used for bioethanol production utilising the fermentation potential of *S. cerevisiae.*

Selection of S. cerevisiae strain for bioethanol production

Six previously collected strains of *S. cerevisiae* were cultured in YEPD medium at 37°C for 24 hours. The strain SC-O showed active and fast growth at 37°C. This strain was used for further study (Figure 1).

Assessment of raw materials for bioethanol production

To determine the optimum temperature, the physical parameters of the substrates sugarcane bagasse, rice straw, and wheat straw were measured before processing (Table 1). A hydrometer was used for measurement of original gravity, and the pH of the substrate was measured by pH meter.

Table 1. Physical parameters of the substrates before fermentation

Raw materials	Original Gravity	pH
Sugarcane bagasse	12.50	4.87
Rice straw	11.20	4.75
Wheat straw	11.50	4.70

Effect of different factors on bioethanol production

Effect of pH on bioethanol concentration

The study was carried out to determine the influence of pH on bioethanol concentration. The sample was fermented at different pH values (3.7, 3.9, 4.0 and 4.6) while the temperature was kept constant at 40^0C to obtain the maximum bioethanol yield. Different pH values of fermentation broth significantly affected the bioethanol concentration. The highest concentration (15.04%) of bioethanol was obtained from sugarcane bagasse at pH 4, while the lowest amount (13.01%) was calculated in rice straw at pH 3.7 (Figure 2). The maximum ethanol concentration at pH 4 reflects that the enzymes involved in the fermentation process worked better at pH 4 while the lower ethanol concentration at other pH indicates lesser enzyme activity of *S. cerevisiae.* These findings are in line with the experimental results of Wong *et al.,* 2014, who utilised the fermentation potential of *S. cerevisiae* for bioethanol production.

Effects of temperature on bioethanol concentration

Temperature is one of the major factors that significantly affect bioethanol production. In this study, fermentation was conducted at three different temperature conditions such as 37°C, 40°C, and 45°C. A significantly higher concentration of ethanol (15.21%) was obtained from sugarcane bagasse at 40°C. However, the lowest concentration of bioethanol (12.8%) was obtained from the wheat straw at 37°C (Figure 3). The fermentation process requires an optimum temperature for yeast growth, and enzyme activities essential for ethanol production and comparatively higher temperature inhibits the growth of the *S. cerevisiae* cells which significantly decreases the rate of the fermentation process (Wong *et al.,* 2014). In this study, ethanol concentration declined considerably after 40^0C, which indicated the inhibition effect of higher temperature on *S. cerevisiae* activities.

The rate of an enzyme-catalysed reaction increases with the increase in temperature up to a certain level, and then the enzyme begins to denature. At lower temperature, the catalysis of enzyme become slower compared to a higher temperature (Yah *et al.,* 2010). Therefore, an optimum temperature condition is very important for the effective fermentation process. The findings of this study revealed that 37°C to 40°C is the optimum temperature range for bioethanol production using agricultural waste materials.

Effect of fermentation time on bioethanol concentration

Production of bioethanol from three agricultural waste samples was affected by the length of the fermentation period (72, 96, 120 hours). Based on the result, the significantly highest concentration (16.29%) of bioethanol was obtained from sugarcane bagasse after 96 hours of fermentation, while the lowest amount (13.20%) was found in wheat straw after 72 hours of fermentation. Here, 96 hours fermentation period was found optimum for the production of bioethanol. These findings explain that the conversion rates of sugars into ethanol from sugarcane bagasse worked rapidly than rice and wheat straw (Irfan *et al.,* 2014).

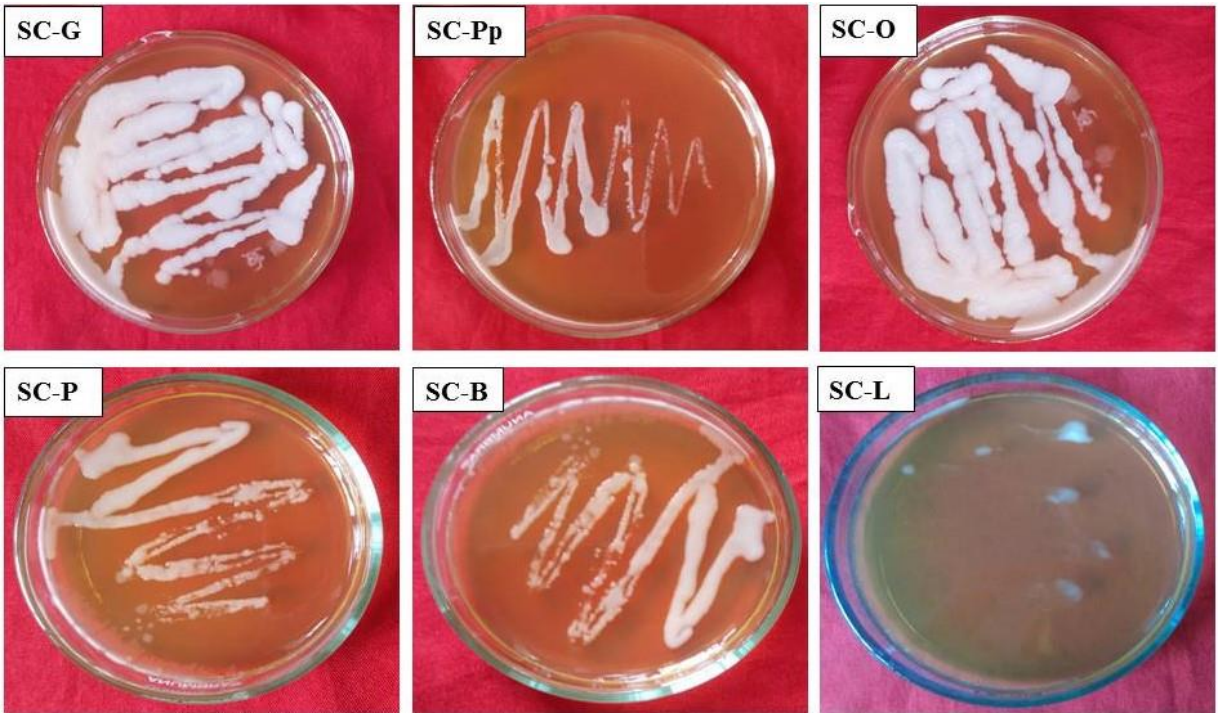

Figure 1. Culture of different *S. cerevisiae* isolates in YEPD medium at 37°C for 24 hours. (Here, SC-G= *S. cerevisiae* isolate collected from Guava, SC-Pp= *S. cerevisiae* isolate collected from Papaya, SC-O= *S. cerevisiae* isolate collected from Orange, SC-P= *S. cerevisiae* isolate collected from Pineapple, SC-B= *S. cerevisiae* isolate collected from Banana, SC-L= *S. cerevisiae* isolate collected from Lemon).

Figure 2. Effect of pH on bioethanol concentration. Here, the fermentation temperature was 40°C, and the fermentation period was 96 hours

Figure 3. Effect of temperature on bioethanol concentration. Here, the pH of fermentation broth was 4.0 and the fermentation period was 96 hours

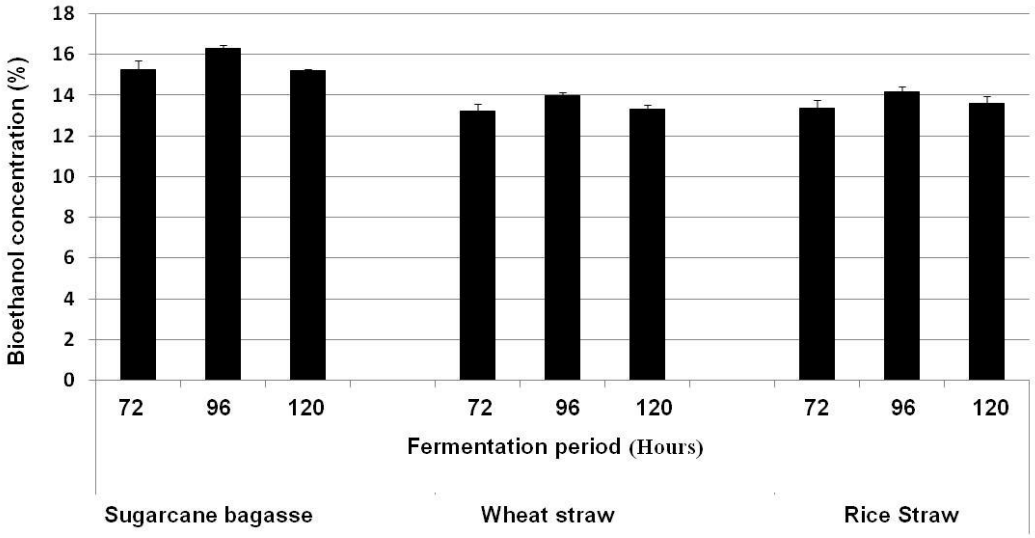

Figure 4. Effect of fermentation period on bioethanol concentration. Here, fermentation temperature was 40°C and pH of fermentation broth was 4.0

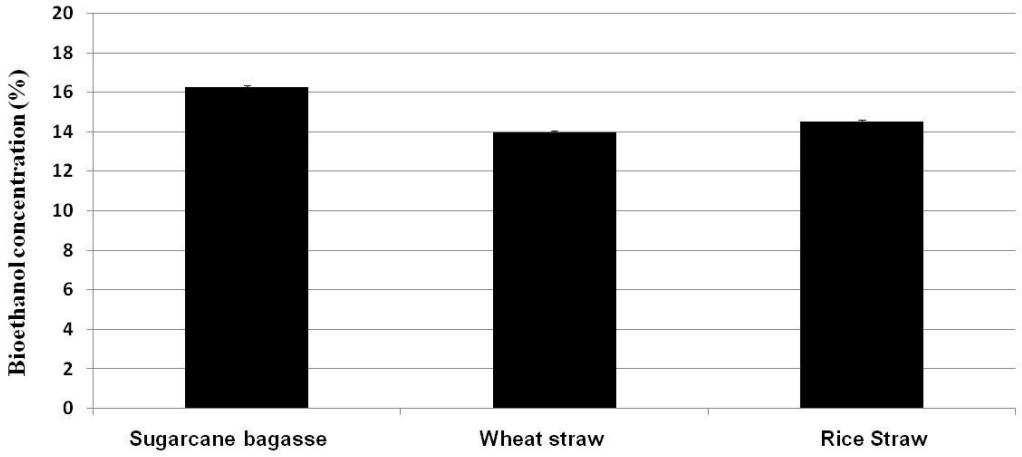

Figure 5. Comparative analysis of substrate on bioethanol production. Here, fermentation temperature was 40°C; pH of fermentation broth was 4.0 and fermentation period was 96 hours

Comparative analysis of substrate on bioethanol production

A significant difference was observed in bioethanol concentration depending on substrate variation. To compare the ethanol production from different agricultural wastes, i.e., sugarcane bagasse, rice straw, and wheat straw, a constant pH (4.0) and temperature (40°C) were maintained. After 96 hours of the fermentation period, the significantly highest amount of bioethanol concentration (16.21%) was obtained from sugarcane bagasse. Bioethanol concentration was 13.59% and 13.42% for the rice straw and wheat straw, respectively. This result explains that the sugars from sugarcane bagasse produced by enzymatic hydrolysis are rapidly converted into bioethanol than wheat straw, and rice straw due to more cellulose and saccharification rate also vary with the presence of cellulosic content in biomasses (Akhtar *et al.,* 2001). Among all the substrates tested in this study, sugarcane bagasse produced significantly more bioethanol than rice straw and wheat straw by using *S. cerevisiae* (Figure 5).

Conclusion

Bioethanol production from agricultural wastes has excellent economic and environmental significance. In this study, three different agricultural wastes such as sugarcane bagasse, rice straw, and wheat straw were compared for bioethanol production by *S. cerevisiae* at different temperatures, pH, and fermentation period. This study revealed that the significantly highest bioethanol concentration (16.29%) was obtained from sugarcane bagasse while the pH of fermentation broth was 4.0 and temperature was 40^0C. Although lesser than sugarcane bagasse, rice, and wheat straw also produced a significant amount of bioethanol. Therefore, it can be concluded that agricultural wastes such as sugarcane bagasse, wheat straw, and rice straw can be utilised as cost-effective substrates for bioethanol production and can serve as an alternative energy source.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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