Optimization and Assessment of Different Parameters and Utilizing Food Waste from the College Canteen for Bioethanol Production

Sharif Neaz¹*, Sayeeda Monira Rahman², S. M. Rafiq Bapari³, Hasib Uddin Ahmed Chowdhury Rumi⁴, Abul Kaiser Bhuiyan⁵, and Sanjay BeLawar⁶

¹Dept. of Chemistry, Dhaka Commerce College, Dhaka, Bangladesh; ²Dept. of Biochemistry and Molecular Biology, Habibullah Bahar College, Dhaka, Bangladesh; ³Dept. of Biochemistry and Molecular Biology, Siddheswari College, Dhaka, Bangladesh; ⁴Dept. of Laboratory Medicine, Asgar Ali Hospital, 111/1/A Distillery Road, Gandaria, Dhaka, Bangladesh; ⁵Dept. of Biochemistry & Immunology, Popular Diagnostic Centre Ltd. Dhaka, Bangladesh; ⁶Dept. of Chemistry, Bangladesh University of Engineering and Technology (BUET), Dhaka, Bangladesh.

*Correspondence: sharifneaz@gmail.com (Sharif Neaz, Assistant Professor and Chairman, Dept. of Chemistry, Dhaka Commerce College, Dhaka, Bangladesh).

ABSTRACT

Bioethanol production from canteen food wastes not only resolves pollution issues by decreasing food waste management it also meets the requirement of bio-fuels. The development of alternatives to fossil fuels like bio-fuel is appropriate and increasingly urgent with the reduction of resources of fossil fuels and the progressively worsening situation of our atmosphere and natural surroundings. The usage of biofuels is one option to decrease the emission of greenhouse gases in the nearer future. Different promising raw materials have been considered for the production of bio-ethanol throughout the last few decades. Food waste from school and college canteens are increasing environmental problem. Food waste might be considered as an edible and non-toxic waste-derived during food production or consumption system. Food waste generated in canteens is rich in carbohydrate, which comprises 65% of total solids due to its high quantity of starch. Through the use of fermentation technology, this waste can be converted to useful by-products like bio-ethanol. Therefore, exploitation of hotel and restaurant food waste for bio-ethanol production can absolutely influence both the energy and environmental sustainability.

Keywords: Fossil fuels, Bioethanol, Canteen food waste, α-Amylase, Gluco-amylase, and S. cerevisiae.

INTRODUCTION:

For past few decades, the objectives to found national energy independence and to reduce the greenhouse gas emissions have led to the progress of renewable bio-fuel technologies based on agricultural materials. Ethanol is by far the most significant bio-fuel in the United States, accounting 94% of all bio-fuel production in 2012 (USDA, 2013). Ethanol is largely produced from corn in the U.S. and from sugar cane in Brazil (Walker et al., 2003). However, sugar cane and corn are used as food as well; excessive use of corn or sugar cane as feedstock for ethanol production results rivalry between food and fuel. In addition, rising prices of these two are the major drivers of the elevated cost of ethanol production. According to the earlier studies, cost of these sugar cane and corn feedstock contributed to 70-90% of the total ethanol production costs.
Researchers have investigated the production of ethanol from low-cost agricultural wastes (Lau and Dale, 2009; Md et al., 2014), wheat straw (Saha et al., 2005), sugar cane bagasse (Martín et al., 2012), etc.

Proficiently producing sugars from cellulose and hemicellulose is the main challenges of using cellulosic biomass amongst others (Matsakas et al., 2014). To rupture cellulose and hemicellulose into monosaccharides, the biomass materials have to be processed with a harsh pretreatment process, followed by hydrolysis with the addition of a high dosage of enzymes, which significantly increases the capital and processing costs of the ethanol production (Humbird et al., 2014). Food waste is a complex biomass from households, restaurants, cafeterias, and groceries and contributes a considerable portion of municipal solid waste (Yan et al., 2012). Food waste management raises noteworthy environmental concerns. Disposal of food waste in landfill is not only expensive but also causing potential environmental problems; with direct and indirect emissions of greenhouse gases (Moon et al., 2009). Incinerations is another approach to manage food waste but is prohibited in different countries because of environmental concerns. Moreover, energy recovery through incineration may not be practicable, because of the evaporation of large water content in food waste results energy loss (Lin et al., 2013).

Food waste can be diverted from landfills and incinerators by turning it into compost to improve the soil fertility, but it may cause harsh pollution to surface and underground water (Uncu and Cekmecelioglu, 2011). Additionally, food waste contains abundant nourishment (starch, glucose, protein, etc.), making it a good raw material for bio-fuel production. At this time, most of the research has been focusing on the utilization of food waste to produce biogas through anaerobic digestion (Kim et al., 2013; Lee et al., 2013). Food waste can also be used as a low cost feedstock for producing ethanol (Lee et al., 2013), which is a more valuable fuel compared to biogas. It is noteworthy that higher solids contents of fermenting material can decrease the costs of ethanol production, since higher solid content results reduction of energy and water consumptions with the volumes of the processing equipment as well (Kim et al., 2013). Alternatively, fermentation of higher solids causes increased ethanol concentration, which in turn inhibits yeast activity, thus causing decreased ethanol production and lesser fermentation efficiency. In the vacuum fermentation system, a low ethanol concentration can be maintained in the fermenter during fermentation, thereby eliminating or minimizing yeast ethanol inhibition (Shihadeh et al., 2013; Mariano et al., 2011)

Uses of enzymes like α-Amylase and Gluco-amylase appreciably lessen the huge energy requirement and thus making the manufacturing process of starch-based products much straight-forward since there would be no call for liquefaction and saccharification (Wang et al., 2007; Yan et al., 2011). The energy input for starch liquefaction and saccharification represents about 30-40 % of the total energy used during starch-based ethanol production (Sun et al., 2010). An additional research showed that competent application of raw starch-digesting enzyme during ethanol production results in significant reduction of about 10-20 % fuel value in total ethanol product (Lee et al., 2012). The objective of this study was to fermentative production and separation of ethanol from food waste generated from a college canteen with high solids content. Food waste from canteens and hostels is an increasing environmental problem, particularly in hostel areas of school, colleges and universities. Canteen waste consists of canteen discards, waste from food preparation, large amounts of oils and fats. Food waste can be defined as any edible waste generated from food production, transportation, distribution and consumption.

MATERIALS AND METHODS:

Raw Material - Food waste used in this study was collecting from college canteens. First plastic, straws and toothpicks and paper were removed and then the samples. Since majority of food supplied from canteen are fast food, food wastes are mainly white bread buns of burgers, hot dogs etc. Breads were separated and chopped into small pieces (about 5 × 5 mm). The bread was dried in ambient conditions and partly powdered by a mortar and pestle. The crushed bread was afterward classified according to size by a mesh sieve,
and mixed so the size distribution matches to that of flour. Then this coarse bread powder are stored for further use. Raw materials were analyzed for moisture, ash, crude fat, crude fiber, crude protein, and crude carbohydrate according to AOAC, (2000). The crude fiber, crude protein, and the fat content were subtracted from organic matter; the remainder accounted for carbohydrates: % carbohydrate = 100-protein (%) + fat (%) + ash (%)

**Enzymes** - Enzymes used in this research are α-Amylase and Gluco-amylase. Alpha-amylase (EC 3.2.1.1) is an enzyme which catalyzes the hydrolysis of α-1, 4-glycosidic bonds and α-1, 6-glycosidic bonds of the inner branched chains of starch. This mainly causes the production of maltose, minor oligosaccharides, and dextrin (Sharma and Satyanarayana, 2013). On the other hand, Gluco-amylase (EC 3.2.1.3) enzyme mainly hydrolyzes α-1, 4-glycosidic bonds from the non-reducing ends of starch chains, results the production of glucose molecules (Marín-Navarro and Polaina, 2011). Usually, conversion of starch to smaller oligosaccharides and monosaccharide glucose in industry comprises two steps which are energy demanding liquefaction step and saccharification step (Uddin et al., 2017). This saccharification step involves hydrolysis by α-amylase and Gluco-amylase under high temperature conditions. During primary liquefaction step, starch is gelatinized first and then converted to dextrin and other minor oligosaccharide molecules by α-amylase enzyme at high temperature (95-105°C) and pH is maintained within 6.0 to 6.5. Then in the saccharification step, the liquid starch slurry is cooled first and the pH is maintained from 4.0 to 4.5. Gluco-amylase is added further in the medium to hydrolyze the oligosaccharides to glucose at temperatures of 60-65°C.

The food waste is collected from canteens. Initially 250 gram of food waste was taken which was crushed with blend in a blender and distilled water was added into the mash. After the pretreatment process enzymatic hydrolysis is done to convert the Starch to fermentable sugars. It was treated with α-Amylase at 90° C, P^H 6.5 for 2 hours and then hydrolysed by gluco-amylase at 40 °C, P^H 4.8 for 2 hours. This hydrolysate is then subjected to autoclave for 15 min at 121° C and 15 psi. After the enzymatic digestion, the samples were inoculated with Saccharomyces cerevisiae (Baker yeast) inoculums collected from local store. The inoculums broth was prepared by introducing 0.8 g dry yeast in 10 ml distilled water. Fermentation was conducted for 72 h at 32 °C with continuous agitation at 30 rpm. Hydrolysis gradually reduced the slurry viscosity, thus causing an increase in revolutions per minute (rpm) of the agitator higher than 30. Therefore, the agitation rate was checked and manually reset to 30 rpm after each sampling.

![Fig 1: Schematic diagram of production of bio-ethanol from canteen food waste.](image-url)
Fermentation was monitored by taking 10 mL of slurry sample at 24, 48, and 72 h and centrifuged at 5000 rpm for 30 min at 10 °C and the supernatant was used to estimate the glucose and ethanol concentration. Glucose content was determined according to the method of Miller. The reducing sugars produced from these reactions were evaluated by using conventional 3, 5-dinitrosalicylic acid (DNS) method, using glucose as standard. In this method 3 milliliters of DNS reagent is added to 3 ml of glucose sample in a test tube and mixed thoroughly. The absorbance of resulting mixture was measured by an UV-vis spectrophotometer at 575 nm. Ethanol concentration is determined according to the method of Williams and Darwin (Vamadevan and Bertoft, 2015). At first 1 g of potassium dichromate in concentrated (6N) sulfuric acid is dissolved properly to prepare 100 ml of potassium dichromate reagent solution. Then by dissolving 1 g of S- Diphenylcarbazide to 1 ml of 95% ethanol saturated S- Diphenylcarbazide solution is prepared. The combination is then added with 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution to stabilize the color. The absorbance of colored solution was evaluated at 575 nm by an UV-vis spectrophotometer. The absorbance values were compared to the ethanol standard graph and the percentage of ethanol had been calculated. Then fractional distillation was done to separate ethanol from the broth. Samples given feasible results in the colorimetric test were distilled and the percentages of ethanol in the distillates were determined.

RESULT:

In this present work, it has been showed that ethanol can be produced from college canteen bread waste. Therefore other studies should be done to check the efficiency of ethanol production from these products of the canteen bread waste by taking different parameters in consideration. Waste bread is very nutritious food waste which is rich in organic carbon and nitrogen and can be used for fermentation. Proximate analysis of waste bread from college canteen is given in Fig 2.

| Water | Carbohydrate | Protein | Fat | Total fibre | Ash |
|-------|--------------|---------|-----|-------------|-----|
| 30.21 | 53.29        | 6.98    | 7.24| 2.9         | 1.7 |

The effect of different parameters on the production of ethanol from college canteen bread waste are presented below -

Effect of time on production of reducing sugar -
Effect of fermentation time on the production of reducing sugar was studied. The isolate was grown on waste bread medium, at different incubation period (0, 24, 48 and 72) hours at pH 5.0 and 30°C in shaker incubator at 30 rpm (Fig 3).

Effect of fermentation time on Ethanol production
- Effects of time on production of ethanol was studied by inoculating waste bread medium with *Saccharomyces cerevisiae* (Baker yeast) inoculums, at
different incubation period (0, 24, 48, and 72) hr at \(P^H\) 5.0 and 30°C in shaker incubator at 30 rpm. Ethanol was monitored by taking 10 mL of slurry sample at 24, 48, and 72 h and subjected to centrifuge at 5000 rpm for 30 min at 10°C and the supernatant was used to estimate the concentration (Fig 4).

**Fig 3:** Glucose concentration at different time intervals.

| Time (hours) | Glucose concentration (v/v %) |
|-------------|-------------------------------|
| 0           | 1st run 13.8  2nd run 12.5  3rd run 12.9  4th run 13.5 |
| 24          | 7.2  6.9  6.2  6.8 |
| 48          | 2.9  3.1  2.9  3 |
| 72          | 0.8  0.6  0.5  0.5 |

**Fig 4:** Ethanol concentration at different time intervals.

| Time (hours) | Ethanol concentration (w/v%) |
|-------------|------------------------------|
| 0           | 1st run 0  2nd run 0  3rd run 0  4th run 0 |
| 24          | 4.1  3.8  4  4.5 |
| 48          | 6.8  7.1  7.9  7.8 |
| 72          | 6.2  6.8  6.9  6.4 |

**Effect of temperature** - Temperature plays an important part in ethanol production, since increase of temperature causes the increase of the rate of alcoholic fermentation. The optimum temperature of ethanol ranges between 30°C to 40°C which depends on room temperature.

**Effect of \(P^H\) -** \(P^H\) value plays significant contribution on alcoholic fermentation. The pH of ethanol ranges from 4 to 6. Yeast generally tolerates slightly acidic environment from \(P^H\) 4 to 6. In this research ethanol produced from bread wastes had elevated alcoholic content when the \(P^H\) is 5.0.
DISCUSSION:

Starch is one of the most plentiful carbohydrate reserves among a variety of plants, such as tuberous plants, cereals, and legumes etc. Starches are a polymer of glucose, which is mostly, consists of amylose and amylopectin. Amylose is a typically linear molecule containing α-D glucosyl residues which are linked by α-1, 4-glycosidic linkages, whereas amylopectin is a extremely branched structure composed of long polymers of α-1, 4-glycosidic bonds linked α-D glucosyl units with α-1, 6-linked side chains (Waterschoot et al., 2015). These amyloses can be used to produce many valuable food products in the food processing industry, such as maltose, glucose, fructose, glucose–fructose syrups, organic acids, amino acids, etc (Favarot et al., 2015).

Additionally, starch is also considered as feedstock in the fermentation industry for production of ethanol, which can be used as beverages or as biofuel (Favarot et al., 2015). Although alcohol making is a potential choice for food waste utilization, there are several research gaps and constraints that must be addressed to further extension of the technology. There is a huge demand to develop energy efficient recovery techniques to get improved alcohol production from food and food derived wastes now a day. There is also a requirement to optimize a cheaper and efficient pretreatment method to recover highest amount of fermentable carbohydrates from food or food derived wastes. It would be helpful to build up a co-fermentation process to employ food wastes while there is a growing demand for emerging technologies and cheaper raw materials which are essential to link this gap. Furthermore, it is not easy to meet a noteworthy portion of fuel demand with existing alcohol production potential of food waste. In this regard, use of food wastes in manufacture of alcohols for industrial, educational and medical applications may be a more constructive option to think about.

CONCLUSION:

The result of this study has shown that effect of different parameters on the production of bio-ethanol. From this study, it is clear that the
maximum yield of ethanol was obtained at temperature 34°C; pH 5.0. From this study we conclude that the process is cheaper than the others because the raw materials used in this procedure comes from waste and does not produce any toxic residues during the process. This bio-ethanol production process can be used for small and large scale plant because carbohydrate based food waste can be obtained from school, college and university canteens continuously. It is also noteworthy that, results were derived from limited parameters and only a single isolate of microorganism was employed in this study. More studies should be done to elaborate the characteristics of fermentation and compare those parameters to develop a procedure to get more bio-ethanol from canteen waste fermentation. This study was intended towards fermentation and optimization only. An inexpensive, effective downstream processing scheme of the ethanol generated in this procedure also requires further development.

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CONFLICT OF INTERESTS:
The authors declared no potential conflicts of the interest with respect to the research, authorship of this article.

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