

Full Length Research Paper

Bioethanol production from brewer's spent grain, bread wastes and corn fiber

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Accepted 22 January, 2011

The production of ethanol from bread wastes, brewer's spent grain (BSG) and corn fiber using dried active baker's yeast (*Saccaromyces cerevisiae*) was investigated. These items were considered industrial wastes and are readily disposed off as by-products thereby constituting environmental hazards and an economic waste. The milled brewer's waste and corn fiber were pre-treated with dilute acid prehydrolysis followed by delignification using NaOH. The pre-treated BSG was further fermented using the bakers' yeast. For the bread waste, it was fermented without pretreatment because it contains less lignocellulose. The reducing sugar concentration of the pretreated BSG was determined to assess the extent of the pretreatment process. The result showed that the ethanol content of the acid-treated BSG and bread wastes were 1.90 and 0.5% respectively while the proof spirit were 4.3 and 1.5% respectively. Bioethanol yield of corn fiber and base-treated BSG were negligible.

Key words: Delignification, bioethanol, wastes, lignocellulosics, biofuel.

INTRODUCTION

Bioethanol is an alcohol produced by fermenting the sugar components of plant materials (renewable biomass). It is made mostly from sugar and starch crops such as sugar cane and corn among others. In the "first-generation" technology bioethanol is produced by converting sugars directly ("first-generation" technology) from crops like sugarcane or sugar beets, indirectly through starch from corn, wheat, potatoes, or cassava into ethanol via fermentation followed by distillation (Wang, 2000). In the "second generation" technology ethanol is produced through cellulose from biomass ("second-generation" technology). The largely used substrate, however, are the food crops sugarcane and sugar beet. They contain large amounts of sucrose, which can be converted into its monomeric components and contribute to 60% of the world's bioethanol production (Zaldivar et al., 2001). Sugarcane and sugar beet by-products include wastes like bagasses and molasses, and fruit juice are also used for alcohol production. The expansion of biofuels production,

particularly in the United States, together with increased world-wide demand for grains and increased energy costs, has led to drastically higher grain prices (Hamelinck, 2003; Hahn-Hagerdal et al., 2006).

Agricultural wastes include corn (maize) stover, barley, wheat and rice straw, corncobs, sunflower stalks and heads, cotton waste, brewer's spent grain, grape pomace, tomato and orange peels etc., and wood constitute the source of cellulosic and lignocellulosic materials. Lignocellulosic biomass typically contains 50-80% (dry basis) carbohydrates that are polymers of 5C and 6C sugar units. Yield of ethanol from lignocellulose however is low, because of lack of suitable technology. Lignocellulosic raw materials minimize the potential conflict between land use for food (and feed) production and energy feedstock production. The raw material is less expensive than conventional agricultural feedstock and can be produced with lower input of fertilizers, pesticides, and energy. Biofuels from lignocellulose generate low net greenhouse gas emissions, reducing environmental impacts, particularly climate change. Brewer spent (BG) is a by-product of the brewing process, consisting of the solid residue remaining after mashing and lautering. It consists primarily of grain husks

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and other residual compounds not converted to fermentable sugars by the mashing process.

BG is the most abundant brewing by-product, corresponding to around 85% of total by-products generated (Xiros et al., 2008) and is mainly used as low-value cattle food. The chemical composition of BSG varies according to barley variety, harvest time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Huige, 1995; Santos et al., 2003). BSG according to Xiros et al. (2008) contains mainly hemicellulose in the form of arabinoxylans from the barley grain and cellulose. BSG has the potential to serve as a low-cost feedstock for the production of ethanol since hemicellulose and cellulose content corresponds to 52% w/w of dry BSG. Other substances such as proteins, lignin and fat are also present in BSG in significant quantities.

Corn fiber (CF), a waste from maize pap is a heterogeneous complex of carbohydrate polymers and lignin. It is primarily composed of the outer kernel covering or seed pericarp, along with 10 to 25% adherent starch. Carbohydrate analyses of corn fiber vary considerably according to the source of the material. Generally, corn fiber has been reported to include 30 to 50% arabinoxylan and 15 to 20% cellulose (Leathers, 1998; Gaspar et al., 2005). The other carbohydrate component in corn fiber is hemicellulose, a well-branched polymer of xylose substituted with arabinose, galactose, mannose and glucose (Sun et al., 2000). Some of the side chains may also contain acetyl groups of ferulate (Carpita and Gibeaut, 1993).

1 or 2% of bread baked in large bakeries is unsuitable for sale as it does not satisfy the required specifications with the bread ending up as cattle feed or waste. The bread residues can be fermented to get the ethanol yield around 0.35 g/g substrate (Ebrahimi et al., 2007). Bread has always been a valuable item - either home baked or purchased from the baker - although much cheaper in comparison to other foodstuffs. Every year, in Austria, an estimated amount of 60,000 to 65,000 tons of bakery goods are thrown away - not including private waste! Empirical studies being carried out by the Institute of Waste Management, BOKU - University of Natural Resources and Applied Life Sciences in accordance with internal extrapolation, branch experts agree that this figure is probably reliable (Laura, 2009). Cellulosic biomass including forestry residue, agricultural residues, pulp mill refuse, switch grass and lawn, garden wastes and municipal solid wastes (MSW), is a potential feedstock for the synthesis of biofuels. Lignocellulosic biomass is a renewable resource and has great potentials for the production of fuel ethanol because it is less expensive than starch (for example, corn) and sucrose (for example sugarcane) producing crops and available in large quantities.

Agricultural lignocellulosic residues are abundant renewable resources for bioconversion to sugars, which

can then be fermented to fuel ethanol. The most important benefit of fuel ethanol production from biomass is reduced CO₂ emissions, thus reducing the greenhouse effect. Conventional production of ethanol from cellulose via fermentation involves a complex process of pretreatment in attempt to recover a maximum amount of sugars from the hydrolysis of cellulose and hemicellulose, and to ferment them into ethanol. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition and to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. The pretreatment aims to increase pore size and reduce cellulose crystallinity. In acid-catalyzed pretreatment, the hemicellulose layer is hydrolyzed, whereas in alkali-catalyzed pretreatment, mainly, a part of the lignin is removed and hemicellulose has to be hydrolysed by the use of hemicellulases. Hence, pretreatment is necessary to expose the cellulose fibers to the enzymes or to at least make the cellulose more accessible to the enzymes. An efficient pretreatment can substantially reduce the enzyme requirements, which make up a large part of the production cost. Pretreatment techniques have generally been divided into three distinct categories, including physical, chemical, and biological pretreatment.

Fermentation is an ATP-generating process in which organic compounds act as both donors and acceptors of electrons; it could either be aerobic or anaerobic, by which oxidation of a substrate occurs, with an inorganic substance acting as the final electron acceptor (Verhagen, 1981; Lange et al., 2000).

Two groups of microorganisms - enteric bacteria and some yeasts - are able to ferment pentoses, but with low ethanol yields. Furthermore, in the case of xylose fermenting yeasts (*Pachysolen tannophilus*, *Candida shehatae*, and *Pichia stipitis*), large-scale utilization is hampered by their sensitivity to high concentrations of ethanol (≥ 40 g/l), the requirement for carefully monitored microaerophilic conditions, high sensitivity to inhibitors, and the inability to ferment xylose at low pH. Some filamentous fungi have been shown to ferment most of the sugars found in pretreated biomass hydrolysates, such as glucose, mannose, galactose, xylose, and arabinose. Some fungi, such as *Monilia*, *Fusarium*, *Rhizopus*, *Aspergillus*, *Neocallimastix* and *Trichoderma* have been reported to possess the ability to convert cellulose to ethanol.

Recent genetic improvements focused on the transformation of *Saccharomyces cerevisiae* and *Zymomonas mobilis* could result in good fermentative performances on pentoses (Hahn-Hagerdal et al., 2006). Three approaches have been attempted to enable the xylose to be utilized:

- (i) to clone genes from pentose utilizing species into *S. cerevisiae*;
- (ii) to co-culture two different strains of genetically modified *Z. mobilis*;
- and (iii) to clone pentose-

utilizing genes into ethanol-resistant strains of *Escherichia coli*.

The demand for bioethanol is expected to increase dramatically until 2020. In 1999, the US signed an executive order specifying a tripling in the production of biobased products and bioenergy by the year 2010. As a consequence, US oil imports will be reduced by nearly 4 billion barrels over that time. Efforts to decrease greenhouse gas (GHG) emissions are expected to spur the production of renewable energy sources by 6% within the European Union by 2010 (Zaldivar et al., 2001). In France, the approval of a clean air act could increase ethanol production to 500 million liters. Similar projects in Spain, Sweden and the Netherlands are expected to increase the utilization of ethanol to account for 15% of transportation fuels by 2010 (Mansson and Foo, 1998). The EU market for fuel ethanol will grow considerably in the coming years, as a result of the EU policy to substitute 8% of fossil transport fuels by renewable biofuels by the year 2020.

The aim of this work was hydrolysis and bioconversion of lignocellulosic byproducts from breweries, fermented corn pomace ('Ogi') and bakery (bread) waste to ethanol. Economically acceptable production of ethanol from lignocellulosic material could be also solution to dilemma: Food, feed or biofuel, which has appeared in last years as a result of growing demand for bioethanol on world's market. Industrial production of fuel ethanol is predominantly from agricultural crops, which also serve as food or animal feed. In order to meet the increasing demand for alternative biofuels, biomass sources other than those used as food need to be explored. This study has identified spent grains from breweries, 'Ogi waste' and waste bread as a potential biomass source for bioethanol.

MATERIALS AND METHODS

Materials

Materials include fermenter, a fractional distillation column, and an attrition mill among others.

Feedstocks

Raw materials used include spoilt bread (2 to 3 weeks old), obtained from a bakery and stored below 5°C. Corn fiber was obtained from maize slurry made according to the method of Akingbala et al. (1981), and brewer's spent grain was obtained from International Brewery, Ilesha, Nigeria.

Chemicals and reagents

Chemicals and reagents were of analytical grade and products of SIGMA chemical Company Limited, USA and British Drug Houses (BDH) Limited, Poole, England. Dried baker's yeast (*S. cerevisiae*) is the product of GYMA ZI. Le Terradou 84200 CARPENTRAS, TEWEX 431184, France.

Methods

The wastes materials collected were sundried and milled using an attrition mill, the milled raw materials were passed through 1.7 mm screen according to Badal et al. (2006) prior to pretreatment and 500 gm powder of each raw material was used as carbon source.

Pretreatment

A two-stage process which combines the Dilute Acid Prehydrolysis (DAPH-100-121) and alkaline delignification using NaOH as described by Dehnavi (2009) was used. In this step, dry materials were submitted to a reaction with dilute sulfuric acid which consist the use of 1.25% (w/v) H₂SO₄ solution in a 1:8 g: g solid: liquid ratio. One step Dilute Acid Prehydrolysis (DAPH-100) was performed in water bath at 100°C for 1 h. One step Dilute Acid Prehydrolysis (DAPH-121) was performed in autoclave at 121°C for 17 min. The solids were treated with 5% (w/v) sodium hydroxide solution in a solid: liquid ratio of 1:20 g: g, 120°C for 90 min. After that, the residual solid material (cellulose pulp) separated by filtration was washed with water to remove the residual alkali, and was dried at 50 ± 5°C for 24 h.

Preparation of yeast culture

A modified method of kirimi et al. (2006) was used. The inoculums were prepared using media containing (g/l): glucose, 100; yeast extract, 10; (NH₄)₂SO₄, 15; K₂HPO₄, 7; MgSO₄·7H₂O, 1.5; CaCl₂·2H₂O, 2 and 0.05 M buffer citrate at pH 5.5 ± 0.1. Volumes of 100 ml media were autoclaved at a pressure of 103.4KNm⁻² temperature 121°C for 15 min to allow for sterilization of the flask content without degrading the essential nutrients for yeast growth. The sterilized content was cool and inoculated in 500 ml cotton-plugged Erlenmeyer flasks, and then incubated for 30 h at 35 ± 0.5°C and shake at 150 rpm. Urea 5 g, KOH, phosphate 5 g, Yeast 20 g were added to 100 ml of H₂O using magnetic stirrer to stir. Adaptation was allowed for 4 h and it was later poured into the fermenter which contains total sample.

Fermentation

A modified method of Akin-Osanaiye et al. (2008) was used. 400 g portion of the pretreated sample was weighed into conical flask and 400 cm³ distilled water was added. This was pasteurized by boiling in a water bath for 15 min. It was allowed to cool and the inoculums were added. The fermentation was then monitored from day 1 (24 h) to day 7. The pH of the sample was adjusted with 0.5 M NaOH, from 4.2 to 5.0. At the end of the fermentation, the fermented sample was poured into a cheese cloth to drain out the fermented broth. This procedure was carried out in triplicates.

Analytical methods

Determination of total carbohydrate content

The carbohydrate content of untreated and pretreated raw materials for fermentation were measured by phenol sulphuric acid method with glucose as standard.

Determination of crude fiber

The crude fiber content was determined as described by Pearson (1976). 3 g of sample of sample was weighed into a Soxhlet

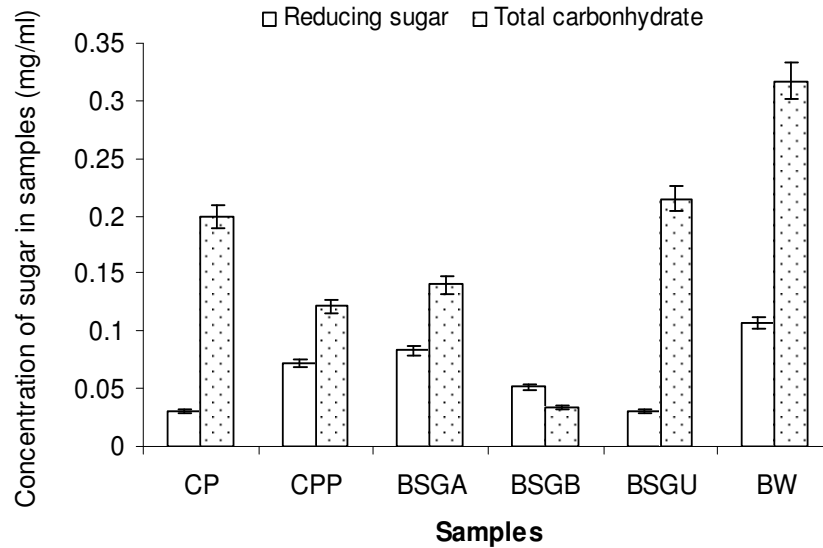


Figure 1. Reducing sugar and total carbohydrate of samples. CP, corn fiber; CPP, pretreated corn fiber; BSGA, acid treated brewer's waste; BSGB, base treated brewer's waste; BSGU, untreated brewer's waste; BW, bread waste.

apparatus and extracted with petroleum ether. The extracted sample was air dried and transferred to a dry 100 ml conical flask and 200 ml of 0.128 M H_2SO_4 was added. The mixture was later boiled for exactly 30 min with the constant rotation of the flask every few minutes so as to mix the contents and remove particles from the sides. After boiling for 30 min, the mixture was allowed to cool for a minute and gently poured into an already prepared Buchner funnel. The suction was adjusted so that the filtration of the bulk of 200 ml was completed within 10 min. The insoluble matter was washed with boiling water until the washing were free from acid, then washed back into the original flask by means of a wash bottle containing 200 ml of 0.313 M NaOH solution measured at ordinary temperature and brought to boiling point. The mixture was further boiled for another 30 min and allowed to stand for 1 min and then filtered immediately through a Whatman filter paper.

The insoluble matter was later transferred to a weight ashless filter paper and dried at 100°C to a constant weight. The filter paper and its content were incinerated to an ash at a dull red heat. The weight of the ash was subtracted from the increase of the weight on the filter paper due to the insoluble material and the difference reported as fiber content.

Determination of ash content

The ash content was determined as described by AOAC (1990).

Glucose standard

As fermentation commenced, 10 g of glucose was placed in a 100 ml volumetric flask and was dissolved with distilled water. The solution was made up to 100 ml to give a concentration of 100 mg/ml of glucose solution. Serial dilutions of the solution were made to obtain glucose solution of various concentrations. 1ml of each of the solution was placed in a test tube and two drops of alkaline 3, 5-dinitrosalicylic acid (DNS) were added and shaken. The test tube was then placed in boiling water for 5 min. The solution was allowed to cool to room temperature and the extinction

was measured at 540 nm. The glucose concentration (mg/ml) of the various glucose solutions versus their extinction values at 540 nm were used to plot a glucose standard curve (Figure 4).

Determination of reducing sugar in the fermenting sample

2 ml of the fermenting sample was placed in a test-tube and 1gm of activated charcoal was added. The mixture was shaken thoroughly. The mixture was filtered with filter paper until a colourless filtrate was obtained. 1 ml of filtrate was placed in a test-tube and two drops of alkaline DNSA were added and the tube was placed in boiling water for 5 min. The mixture was allowed to cool and the extinction was measured at 540 nm. This measurement was taken as the absorbance value at zero hour. At 24 h of the commencement of the fermentation, 2 ml of the fermenting sample was withdrawn and decolorized as earlier described. This procedure was repeated at 48, 72, 96, 120, 144 and 168 h respectively.

Analysis of the percentage alcohol produced

The alcohol content was determined as described by AOAC (1990).

RESULTS AND DISCUSSION

The production of ethanol from food and industrial waste by the efficient lignocellulolytic enzyme system of *S. cerevisiae* was essential for the bioconversion of corn fiber, brewer's spent grain and spoilt bread to useful and economic important product (ethanol) (Muria et al., 1998). The optimization of the bioconversion of these wastes took into consideration the pH, temperature and considering the waste as the carbon growth substrate and other important factors. Figure 1 shows the

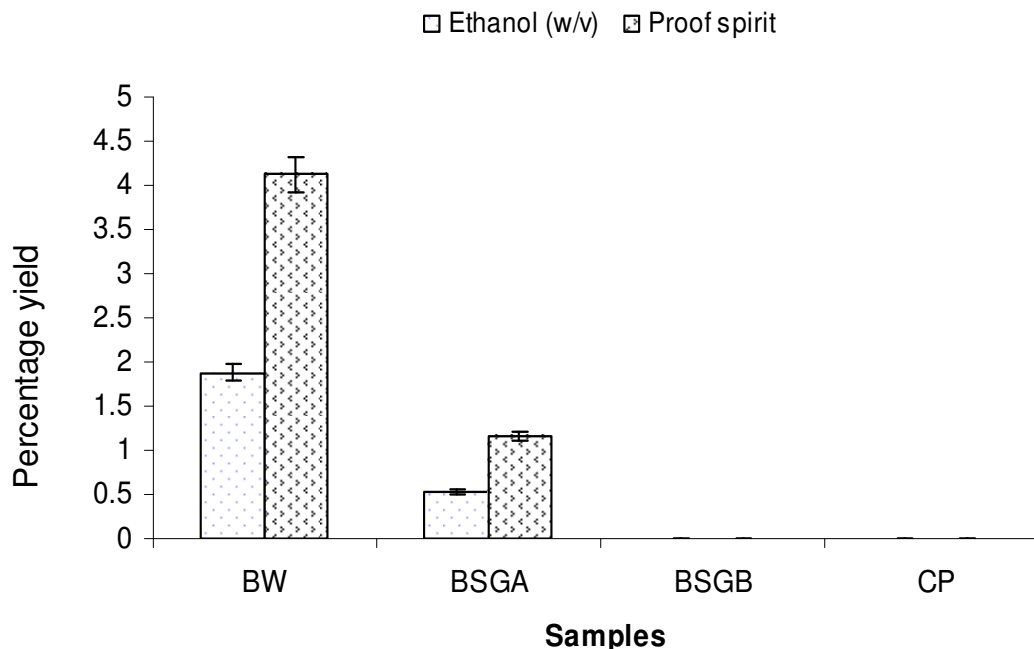


Figure 2. The percentage yield of ethanol and proof spirit of the distillate. CP, corn fiber; CPP, pretreated corn fiber; BSGA, acid treated brewer's waste; BSGB, base treated brewer's waste; BSGU, untreated brewer's waste; BW, bread waste.

concentration of the reducing sugar and total carbohydrate of waste bread, corn fiber and brewer's waste (before and after treatment). The result showed that the BW (bread waste) has the highest value of total carbohydrate (0.317 mg/ml) with a corresponding reducing sugar (0.107 mg/ml). The table also revealed that all other waste that was pretreated lost some of their total carbohydrate and gained more of the reducing sugar. The initial total carbohydrate values of unpretreated corn fiber (CP) and brewers spent (BSGU) was 0.20 and 0.215 mg/ml, respectively and reducing sugar 0.03 and 0.032 mg/ml respectively. For the pretreated sample of brewers spent; acid pretreated (BSGA) and alkaline pretreated (BSGB) has values of 0.14 and 0.033 for total carbohydrate respectively and 0.084 and 0.051 mg/ml respectively for the reducing sugar.

This result indicated that pretreatment with either acid (H_2SO_4) or alkaline (NaOH) generally reduce the availability of total carbohydrate and reducing sugar for fermentation (Kumar et al., 2009). The levels of reducing sugar in the sample determine to some extent the percentage of ethanol that will be produced from the fermenting medium. Figure 2 shows the percentage yield of ethanol and proof spirit. There was indication of high percentage of proof spirit in the BW (bread waste) with value of 4.12%, with corresponding 1.8% in ethanol yield. The results for acid treated brewers spent (BSGA) for ethanol and proof spirit were 1.16 and 0.53% respectively, while the value of ethanol for alkaline treated brewers spent brewers waste (BSGB) and corn

fiber were not significant. This indicated that using some wastes containing between 0.1 to 0.5% reducing sugar a maximum ethanol yield of about 4.5% and a minimum yield of 0.5% can be achieved. The specific gravity of the fermented waste bread (0.99) corn fiber (1.0) and brewer's waste pretreated with acid, (BSGA) (0.99), brewer's waste pretreated with alkaline (BSGB), (1.0) were shown in Figure 3. The specific gravity is defined as the ratio of the mass of that product to the mass of an equal volume of water at 4°C. It can also be used to determine the ethanol content in the fermented sample using hygrometer (Akpan et al., 2005).

Table 1 shows the crude fiber of the waste bread, corn fiber and brewer's waste (before and after treatment). The crude fiber of the untreated brewers spent (BSGU); $34.23 \pm 0.16\%$ was higher than the rest of the fermented waste, this was followed by acid treated brewer's spent grain (BSGA); $31.43 \pm 0.33\%$, alkaline treated brewer's spent grain (BSGB); $29.41 \pm 0.78\%$, treated corn fiber (CPP); $0.05 \pm 0.01\%$ and untreated corn fiber (CP); $7.25 \pm 0.14\%$ and bread waste (BW); $0.05 \pm 0.01\%$. After pretreatment, the level of the crude fiber was found to have significantly reduced for all the studied samples. The crude fiber quantity will affect the production of bioethanol from the samples. As expected, bread waste with the least amount of crude fiber produces the highest quantity of bioethanol. The acid treated brewer's spent grain though with higher concentration of crude fiber than corn fiber-treated and untreated, produces more bioethanol. The only explanation that could be adduced

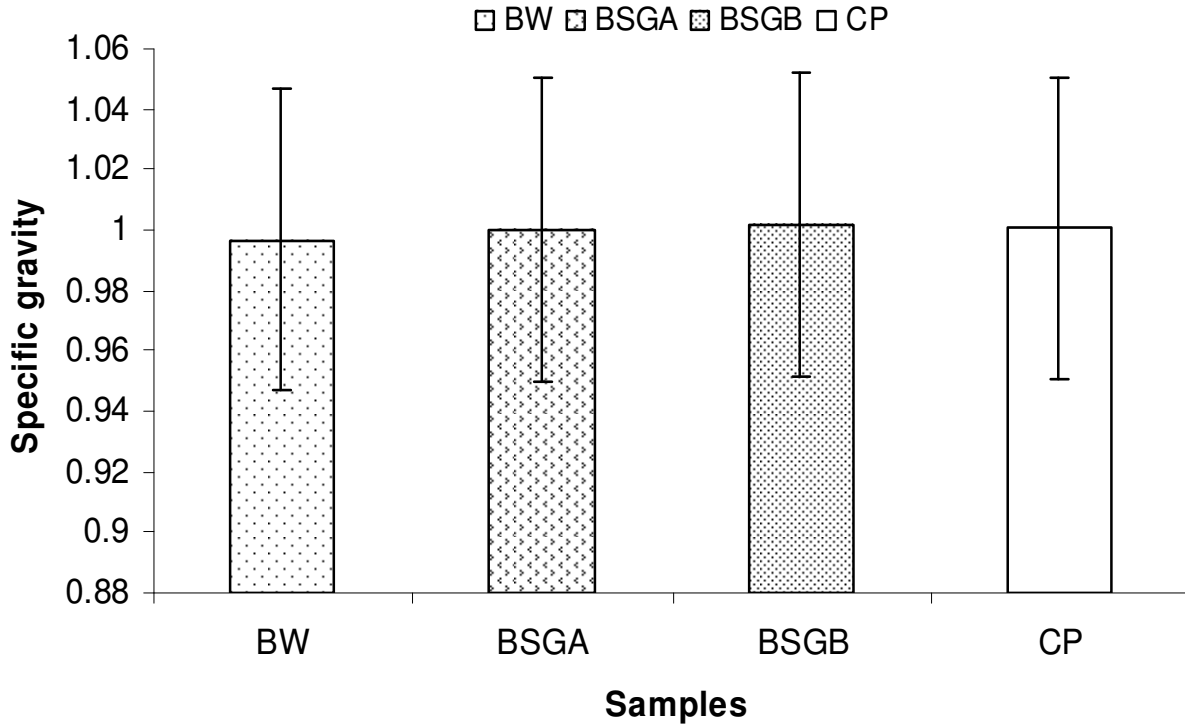


Figure 3. The specific gravity of the distillate. CP, corn fiber; CPP, pretreated corn fiber; BSGA, acid treated brewer's waste; BSGB, base treated brewer's waste; BSGU, untreated brewer's waste; BW, bread waste.

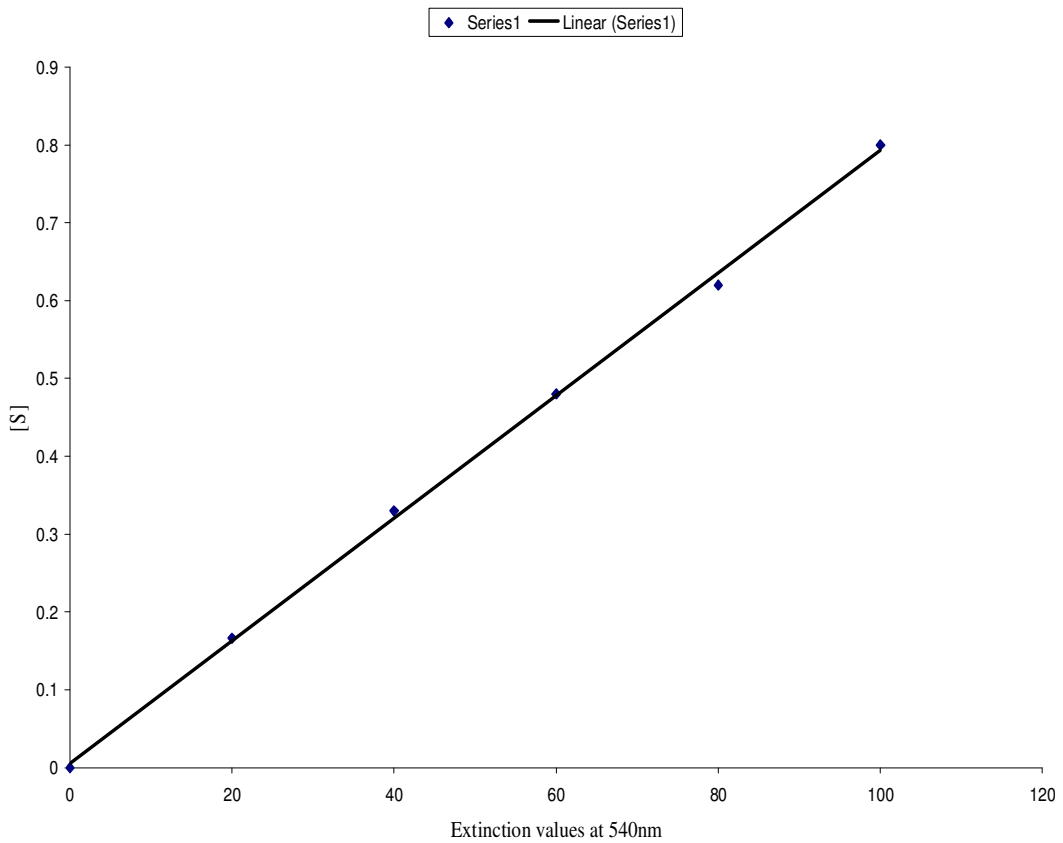


Figure 4. Graph of standard glucose concentration (mg/ml) vs. the extinction values at 540 nm.

Table 1. The crude fiber content of feedstock for ethanol production.

Sample	Percentage fiber
Bread waste	0.05 ± 0.01 ^f
Untreated corn fiber	7.25 ± 0.14 ^e
Treated corn fiber	5.86 ± 0.25 ^f
Untreated brewer's spent grain	34.23 ± 0.16 ^a
Acid treated brewer's spent grain	31.43 ± 0.33 ^b
Base treated brewer's spent grain	29.41 ± 0.78 ^c

Table 2. Ash of distillate.

Sample	% Ash
Bread waste	2.08 ± 1.96 ^a
Corn fiber	0.12 ± 0.00 ^b
Acid treated brewer's waste	0.27 ± 0.00 ^b
Base treated brewer's waste	0.21 ± 0.00 ^b

Table 3. The pH of the feedstock for ethanol production.

Day	Bread waste	Brewer's spent grain (acid treated)	Brewer's spent grain (treated)	Corn fiber
1	6.36	7.00	7.11	6.29
2	6.32	6.35	7.07	6.24
3	6.26	6.30	6.73	6.19
4	6.20	6.27	6.73	6.19
5	6.08	6.27	6.73	6.19
6	6.08	6.27	6.73	6.19
7	6.08	6.27	6.73	6.19

to this is that the higher concentration of total carbohydrate and reducing sugar in the acid treated brewer's spent grain are responsible for the more bioethanol production as explained in Figures 1 and 2.

Table 2 shows the ash content of the distillate from the waste bread, corn fiber and brewer's waste. The result indicated that there was a significant higher level of ash in the ethanol distillate from the bread waste (2.08 ± 1.96) than all other waste distillate for ethanol. The ash content, which is a measure of the mineral content of food (Nnamani et al., 2009), was found to be significantly high in the distillate of waste bread and least in distillate of corn fiber (0.12 ± 0.00).

Table 3 shows the pH of the waste feedstock for the production of ethanol, from the result, the pH of the feedstock generally decreased during the period of fermentation. This is an indication that the fermentation process becomes more acidic as a result of the production of other secondary metabolites and activities of other lactobacteria in the fermentation medium. In addition, the decrease in pH remain constant on the 5th day for the bread waste, on the 4th day for the acid

treated brewer's spent grain, and on the 3rd day for both the base treated brewer's spent grain and corn fiber. This also is in consonance with the ethanol production as bread waste with the longest fermentation as deduced from the time the pH become constant has highest ethanol production rate, followed by acid treated brewer's spent grain, base treated brewer's spent grain and corn fiber in that order.

Conclusion

The result of this study show that the rate of alcohol production through fermentation of industrial and food waste by baker's yeast (*S. cerevisiae*) increases with fermentation time. The finding of this work suggests that bioethanol can be produced from bread waste and brewer's spent grain that has been pretreated with acid. In addition, the quantity of bioethanol production is directly proportion to the amount of total carbohydrate and reducing sugar in the samples and inversely proportion to the fiber content of the sample.

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