ABSTRACT

Second generation ethanol is produced from non-food based including waste from food crops, wood chips and agricultural residue. Lignocellulosic and starchy materials in them are converted to fermentable sugars which are further processed to produce bioethanol. Rice bran is an agricultural residue with abundant carbohydrate for bioconversion into ethanol. This study was designed to evaluate the potential of two varieties of rice bran (Sipi and Wita) to produce bioethanol. Compositional analysis of Wita rice bran showed 40% cellulose, 23% hemicellulose and 16% lignin content. Sipi variety contains 35% cellulose, 27% hemicellulose and 13% lignin content. Sodium hydroxide pretreatment was carried out at different concentrations (0.5%, 1%, 2% and 3%) and residence time of (15, 30, 60, and 90min). It was observed from the present study, pretreatment of rice bran with 2% NaOH for 90min is considered as effective pretreatment condition for bioethanol production from rice bran. Simultaneous saccharification and fermentation of cellulosic biomass was carried out for 72h with Saccharomyces cerevisiae and Mucor indicus. Fermentation of Wita variety with S.cerevisiae produced highest bioethanol yield of 1.36% while Mucor indicus produced 0.75% bioethanol yield. From the result of these findings, it can be concluded that rice bran could be considered as a promising substrate for the fermentation of second generation ethanol.
1. INTRODUCTION

Worldwide interest is increasing towards alternative sources of energy due to inevitable depletion of energy supply. The increased in the prices of fossil-based fuels, strict government regulations on exhaust emissions and future depletion of worldwide petroleum reserves, encourage studies searching for alternative fuels. Fogarty and Mccally [1] reported that the major greenhouse gas is Carbon dioxide (CO$_2$), it traps the earth’s heat and contributes to climate change. Efforts for the production of ethanol from starch and sugar-based crops have been intensified worldwide to lessen the dependency on fossil based fuel and provide environmental security Tiwari et al. [2].

The secondary biofuels can be categorized into three generations: first, second and third generation biofuels on the basis of different parameters, such as the type of feedstock, processing technology or their level of development Nigam and Singh [3]. First generation biofuels mainly constitute the ethanol produced from food-based crops Antizar-Ladislao and Turrion-Gomez, [4]. Biofuels produced from lignocellulosic materials are known as Second-generation biofuels. lignocellulosic materials include cereal straw, forest residues, bagasse, and purpose-grown energy crops such as vegetative grasses and short rotation forests Demirbas [5]. Cellulose, hemicellulose, and lignin are the main component of lignocellulosic biomass.

Third generation biofuels are derived from microalgae and are considered to be the possible and reliable alternative energy resource that avoid the major drawbacks associated with first and second generation biofuels Nigam and Singh [3].

Jorgensen et al., [6] reported that Lignocelluloses residues was regarded as the largest known renewable carbohydrate that account for almost all the total biomass present in the world, this has led to it utilization as substrate for biofuel production.

Harismah et al., [7] observed that rice bran is one of the most interesting lignocellulosic biomass that can be used as source of sugar and substrate for bioethanol production. It was reported by Tiwari et al. [2] that rice bran contains high amount of carbohydrate, therefore, it can be regarded as the best choice of substrate for fermentation technology to produce bioethanol. Two varieties of rice bran were used in this study, namely; Wita and Sipi. The technology of ethanol production from biomass feedstocks involved several steps and varies based on the type of feedstock used. For the production of bioethanol from cellulosic biomass, four major unit operations are required: pretreatment, hydrolysis, fermentation and separation (Taherzadeh and karimi [8]).

Pretreatment is needed to alter the biomass structure and size as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate in to monomeric sugars can be achieved more rapidly and with greater yields Sun and Cheng [9]. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, decreases the degree of polymerization decreases crystallinity, degradation of the lignin structure and separation of structural linkages between carbohydrate and lignin Fan et al., [10].

In SSF processed, both saccharification and fermentation are achieved simultaneously in a single vessel at optimized enzyme activity. This process assures less contamination by microorganism during ethanol production Montesinos and Navarro [11].

In the present study, NaOH was employed for the pretreatment of rice bran. After pretreatment, Substrates were subjected to 72h fermentation at temperature of 35°C using Saccharomyces cerevisae and Mucor indicus to produced ethanol. This study was aimed to investigate the potential of rice bran to produce bioethanol.

2. MATERIALS AND METHODS

2.1 Material

Rice bran was obtained from Kura local Government area, Kano state. Two local varieties were obtained, known as Wita and Sipi. Rice bran were air dried for a week, and then milled to reduce its size. Samples were authenticated by a Taxonomist at Bayero University Kano, Department of Plant Biology with accession number BUKHAN 0289 (Wita) and BUKHAN 0288 (Sipi).
2.2 Microorganism

Saccharomyces cerevisae (active dry yeast) was reactivated as described by Almodares et al., [12] with some modifications. Yeast powder was suspended in sterile distilled water; it was then incubated for 25min at temperature of 30°C.

The fungal spore was prepared based on the method described by Abo-State, et al., [13] with some modifications. Fungal isolates were inoculated onto 100 ml Potato Dextrose Agar medium in 250 ml Erlenmeyer flasks. The inoculated media was incubated at room temperature for 5days, and then the spores were collected by adding 30 ml sterile saline containing 1.0% Tween-80. The spore suspension was collected in new sterile flask as stock for inoculation.

2.3 Complex Carbohydrate Composition of Rice Bran

The lignin, cellulose and hemicelluloses content of both rice bran were analyzed using Chessson method as described by Hernawan et al. [14]. To one g (a) of dry sample, 150 ml water was added and refluxed at 100 °C in water bath for 1 hour. It was then filtered, and the residue was washed with 300 ml hot water. The residue was then dried in an oven then weighed (b). To the dried Residue, 150 ml of 1N H2SO4 was added and then refluxed in a water bath at 100 °C for 1 hour. The residue was filtered and washed with 300 ml of hot water; it was dried and weight (c). 10 ml of 72% H2SO4 was added to the dried residue and soaked at room temperature for 4 hours and then filtered. 150 ml of 1 N H2SO4 was added again to the residue and refluxed in a water bath for 1 hour. It was filtered, washed with 400 ml H2O, then dried in an oven at temperature of 105°C and weighed (d). The residue was ashed in the furnace and weighed (e).

Lignin content = [(d-e)/a] x 100%;
Cellulose content = [(c-d)/a] x 100%;
Hemicellulose content = [(b-c)/a] x 100%.

2.4 Pretreatment of Biomass

Biomass was pretreated with NaOH according to the method described by Xu et al., [15] with slight modification. Biomass were subjected to NaOH delignification in an auto clave. Pretreatment was carried out with different concentration of NaOH (0.5, 1.0, 2.0, and 3.0 w/v) and residence time of (15, 30, 60 and 90 min). 4g of biomass sample and 40ml NaOH solution of desired concentration was mixed in a conical flask. The pretreated biomass was then recovered by filtration (vacuum filtration) and washed with 400ml deionized water to remove excess alkali.

2.5 Simultaneous Saccharification and Fermentation with S. cerevisae

Simultaneous saccharification and fermentation that is, hydrolysis and fermentation was carried out in same vessel according to the method described by Omidvar et al., [16] with slight modifications. 1g of pre-treated rice bran was mixed to nutrient suspension containing containing the following compounds: g/L (yeast extract, 5.0; (NH4)2SO4, 7.5; MgSO4·7H2O, 0.75; KH2PO4, 3.5; CaCl2·2H2O, 1.0) and 0.05 M sodium citrate buffer (pH 4.8) was added to a 100 ml flask. The suspension was then autoclaved for 20 min at 121°C. After cooling to room temperature, the solutions were inoculated with S.cerevisiae and then supplemented with 25FPU cellulase per gram of dried substrate. Finally, the flask was sealed and incubated at 35°C and 120 rpm for 72h. Liquid samples were taken and analyzed by UV visible spectrophotometer after 72h fermentation.

2.6 Simultaneous Saccharification and Fermentation with Mucor indicus

Simultaneous saccarification and fermentation for ethanol production with fungi was conducted as described by Omidvar et al., [16] with slight modifications. 1g of pre-treated rice bran was mixed to nutrient suspension containing the following compounds: g/L (yeast extract, 5.0; (NH4)2SO4, 7.5; MgSO4·7H2O, 0.75; KH2PO4, 3.5; CaCl2·2H2O, 1.0) and 0.05 M sodium citrate buffer (pH 4.8) was added to a 100 ml flask. The suspension was then autoclaved for 20 min at 121°C. After cooling to room temperature, the solutions were inoculated with 100µL Mucor indicus spore suspension, and then supplemented with 25FPU cellulase per gram of dried substrate. Finally, the flask was sealed and incubated at 35°C and 120 rpm for 72h. Liquid samples were taken and analyzed by UV visible spectrophotometer after 72h fermentation.

2.7 Filtration and Distillation

Samples were filtered with filter paper to separate the solid substrate from liquid and then distillation was done at temperature range between 78-80°C to get the ethanol sample for UV visible analysis.
2.8 Determination of Bioethanol Concentration

Determination of concentration of bioethanol produced was carried out using the method described by Rabah et al., [17]. 1ml of standard ethanol was diluted with 100 ml of distilled water to give a concentration of 1 %. From this stock solution 0, 0.2, 0.4, 0.6 and 0.8 % of the ethanol was prepared by diluting it with distilled water. To each of the varying ethanol concentrations 2mls of chromium reagent was added and allowed to stand for an hour for color development. The absorbance of each concentration was measured at 588nm using UV-VIS spectrophotometer and the readings used to developed standard ethanol curve. Then 5mls of each bioethanol samples were put in test tubes and treated with 2mls of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance was measured at 588nm. The absorbance values were compared to the ethanol standard graph and percentage of ethanol was calculated.

3. RESULTS

The composition of both samples are presented in Table 2. Wita rice bran has cellulose content of 40%, 23% hemicelluloses and 16% lignin. As well Sipi rice bran contains 35% cellulose, 27% hemicelluloses and 13% lignin. Cellulose and lignin content were observed to be higher in Wita variety than Sipi variety. Higher hemicellulose content was recorded in Sipi rice bran.

3.1 Bioethanol Produced from Wita and Sipi Rice Brans by Simultaneous Saccharification and Fermentation Process with S. cerevisiae

Fig. 1 shows ethanol produced by Simultaneous Saccharification and fermentation process using S. cerevisiae. The result show that (Wita rice bran) pretreated with 2% NaOH for 90min produced highest ethanol yield (1.36%) after 72h fermentation. Lowest ethanol concentration (yield) of 0.15% was recorded after fermentation of substrate pretreated with 1% NaOH.

Fig. 2 shows substrate pretreated with 1% NaOH for 30 minutes produced 0.76% maximum ethanol concentration after fermentation with Saccharomyces cerevisiae. S. cerevisiae was able to utilize sugars and convert it to ethanol. Lowest bioethanol concentration recorded was 0.21% after fermentation of substrate pretreated with 0.5% NaOH for 15 minutes.

3.2 Bioethanol Produced From Wita and Sipi Rice Brans by Simultaneous Saccharification and Fermentation Process With Mucor Indicus

Result presented in Fig. 3 shows maximum bioethanol concentration (0.69%) was obtained after SSF of pretreated substrate with 2% NaOH for 60 min. lowest ethanol yield recorded was 0.23% after SSF of substrate pretreated with 1% NaOH for 30 minutes.

From the result presented in Fig. 4, highest concentration of bioethanol (0.75%) was recorded after fermentation of substrate pretreated with 2% NaOH for 60 minutes. Lowest concentration of bioethanol was recorded after SSF of biomass (sipi rice bran) pretreated with 0.5% NaOH for 30 minutes.

4. DISCUSSION

Lignocellulosic biomass is composed of 40-50% cellulose, 25-30% hemicellulose and 15-20% lignin Tayyab et al., [18] but, due to their complex nature differences were observed in their composition Sluiter et al., [19]. Pratap and Kumar [20] recorded cellulose, hemicellulose and lignin composition of rice bran closely similar to the result obtained in the present study. High cellulose content in lignocellulosic material is a promising condition for bioethanol production. According to Chang and Holtzapple [21] high content of lignin might result to low digestibility of biomass therefore it removal by pretreatment increases the digestibility.

The mechanism of alkaline pretreatment is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components. Lignocellulosic materials porosity increases with the removal of crosslinks Tarkow and Feist, [22]. Dilute NaOH treatment of lignocellulosic materials caused swelling, which lead to an increase in the internal surface area, a decrease in crystallinity, separation of structural linkage between lignin and carbohydrates, and disruption of the structure of lignin Fan et al., [10]. Alkali pretreatment as compared to acid pretreatment is less harsh and can be carried out effectively at an ambient condition Tayyab et al., [18]. Also in acid pretreatment method inhibitory compounds are formed and it requires longer pretreatment duration.
According to Alvarez et al., [23] for chemical pretreatment, higher yield are achieved when higher pretreatment time and alkaline concentration were used. From this study, 90mins pretreatment time with 2% NaOH was found to be more effective for pretreatment of rice bran and maximum ethanol yield was obtained. Omidvar et al., [16] reported 2.6M NaOH at 67°C for 150 min as optimum pretreatment condition of rice husk in their study of enhanced ethanol and glucosamine production from rice husk by NaOH pretreatment and fermentation by Mucor hiemalis. However the effect of alkaline pretreatment method depends on lignin content of lignocellulosic biomass McMillan [24]. Extended pretreatment time is required to remove lignin Bradeur et al., [25]. Also kataria and Ghosh [26] from their study of NaOH Pretreatment and Enzymatic Hydrolysis of Saccharum spontaneum for Reducing Sugars Production reported that best pretreatment condition for reducing sugar release with sodium hydroxide is at higher residence time. Kaur and Singh [27] obtained maximum ethanol yield (5.89%) with 3% NaOH pretreated sample, they studied bioethanol production from rice husk using SSF with optimization of pretreatment method.

Arasi and Sashi [28] reported maximum ethanol yield (0.28%) by 72h SSF of rice bran pretreated with 1N NaOH for 60min, saccharified with Bacillus pumilus and Psuedomonas aueruginosa. Harismah et al. [7] achieved high amount of ethanol concentration from rice bran after 3 days fermentation at temperature of 60°C with Saccharomyces cerevisae using enzyme pretreatment method. Enzymes are quite expensive, the choice of alkali pretreatment in the present study is affordable and considered economical. Maximum ethanol concentration recorded for sipi rice bran after 72h SSF was 0.76% which is lower compared to wita, it may be due to high amount of carbohydrate in wita rice bran. Yamba et al., [29] observed that sugar content was directly proportional to the bioethanol produced.

Simultaneous saccharification and fermentation of sipi rice bran with Mucor indicus produces maximum ethanol concentration of 0.75% which is lower compared to the ethanol produced by Saccharomyces cerevisae. Ethanol and glucosamine yield produced by Mucor highly depends on the medium composition Sues et al., [30]. It was observed by Teresa [31] low sugar consumption by Mucor indicus may be as a result of short fermentation duration.
Table 1. Pretreatment of biomass at different concentration of sodium hydroxide and time

<table>
<thead>
<tr>
<th>Pretreatment condition time (min)</th>
<th>NaOH concentration (%)</th>
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<tbody>
<tr>
<td>15</td>
<td>0.5</td>
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<td></td>
<td>1.0</td>
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<td></td>
<td>2.0</td>
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<td></td>
<td>3.0</td>
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<tr>
<td>30</td>
<td>0.5</td>
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<td></td>
<td>1.0</td>
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<td>2.0</td>
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<td></td>
<td>3.0</td>
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<tr>
<td>60</td>
<td>0.5</td>
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<td></td>
<td>1.0</td>
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<td>2.0</td>
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<td>90</td>
<td>0.5</td>
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<tr>
<td></td>
<td>1.0</td>
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<td>2.0</td>
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Table 2. Composition of sipi and wita rice bran

<table>
<thead>
<tr>
<th>Lignocellulosic</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wita</td>
<td>40</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>Sipi</td>
<td>35</td>
<td>27</td>
<td>13</td>
</tr>
</tbody>
</table>

Fig. 2. Bioethanol produced from simultaneous Saccharification and fermentation of Sipi rice bran pretreated with NaOH at different concentrations (0.5%, 1%, 2% and 3%) and pretreatment time (15 min, 30 min, 60 min and 90 min)
Fig. 3. Bioethanol produced from simultaneous saccharification and fermentation of *wita* rice bran pretreated with NaOH at different concentrations (0.5%, 1%, 2% and 3%) and time (15 min, 30 min, 60 min and 90 min)

Fig. 4. Bioethanol produced from simultaneous saccharification and fermentation of *Sipi* rice bran pretreated with NaOH at different concentrations (0.5%, 1%, 2% and 3%) and varied pretreatment time (15 min, 30 min, 60 min and 90 min)

5. CONCLUSION

Once our result showed that, the fermentation of rice bran pretreated with 2% NaOH for 90min with *Saccharomyces cerevisae* showed a production of 1.36% ethanol yield. Therefore, it can be concluded that rice bran could be considered as a promising agro-based residue for the fermentation of second generation ethanol. Alkaline pretreatment with 2% NaOH for 90min can be considered as optimum pretreatment condition for ethanol production from rice bran. Further studies should explore various pretreatment processes in order to
choose the one that best fit the structure of rice bran. Also, genetically engineered organism that is capable of hydrolyzing cellulose and fermenting sugars should be employed to achieve greater fermentation performance of rice bran and maximum bioethanol yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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