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Optimization of Bioethanol Production from Sawdust and Brewery Spent Grain

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Abstract
The choice of bioethanol as an alternative energy source is due to the high rate of depletion of the non-renewable energy source. Hence, this study investigated the production of bioethanol from Brewery Spent Grain (BSG) and Sawdust. Strains of Aspergillus spp and Saccharomyces cerevisiae were selected and characterized using morphological indices. The spores of Aspergillus flavus and colonies of Saccharomyces cerevisiae were cultured in a liquid fermentation medium containing brewery spent grain (BSG) and sawdust as substrates for bioethanol production. The process was optimized at pH range 4 - 8, temperature range 30 - 50 °C and substrate concentration 5-30 % for 96 h. Optimum bioethanol yield of 14 % was obtained from BSG at substrate concentration of 25 %, at temperature of 35 ^{o}C and pH 4.0. However, optimum bioethanol yield of 10 % was obtained with saw dust at substrate concentration of 25%, temperature of 35 ^{o}C and pH 6.0. Thus, there was an increased yield of bioethanol from BSG than sawdust. This study shows the potentials of Aspergillus flavus and Saccharomyces cerevisiae in the production of bioethanol from sawdust and BSG.

Keywords: Bioethanol, Brewery Spent Grain, Sawdust, *Aspergillus flavus*, *Saccharomyces cerevisiae*

Introduction
The recent attention being received by bioethanol as an alternative source of energy is due to the increasing depletion of the non-renewable energy sources (1). Bioethanol has been produced over the years from food sources such as corn and other starchy food sources (2). Attention has been shifted from the use of food based biomass to non-edible biomass for bioethanol production. This is due to the fact that the use of the food based biomass could lead to food insecurity (3). Current research is geared towards the search and development of alternative energy sources from non-edible food sources or biomass.
Nigeria is not fully producing bioethanol due to the problem of appropriate sourcing of raw material for bioethanol. However, sawdust is used as feedstock in the production of cellulosic ethanol (4). This lignocellulosic byproduct of agriculture is useful in the production of paper and paper products, cotton, linen, rayon for cloths, nitrocellulose for explosives, cellulose acetate for films, etc. (5).
Brewery Spent Grains (BSG) is another lignocellulosic biomass. BSG are the by-products of mashing process; which is one of the initial operations in beer production (6). In brewing, the quantity of BSG generated could be as high as 85% of the total by-products (7). The accumulation of these wastes in the environment can result into pollution (8). Thus, BSG is a readily available, high volume, low cost by-product of brewing and is a potentially valuable resource for industrial exploitation (9).
Industrial production of ethanol from lignocellulosic biomass is dependent on the availability of microorganisms that can utilize the sugars inherent in the starchy biomass (10, 11). The ability to grow and produce enzymes necessary for the bioconversion of sugars inherent in starchy biomass to ethanol is what distinguished the yeast cell as the most suitable group of microorganisms for bioethanol production. Some of these yeasts are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus* (12). Recently, the use of some food crops like pineapple, sugar cane, corn straw, millet, cassava have been reported as substrates for bioethanol production (13). However, report on the comparative production of bioethanol from brewery spent grain and sawdust is scanty. Moreover, BSG and sawdust are underutilized in their industrial applications (3). Hence this study investigated the potentials of BSG and sawdust in bioethanol production.

Materials and Methods
Collection and preparation of samples
Brewery spent grain was obtained from Sona Breweries, Ota, Ogun State. Sawdust was collected from a timber market in Benin City, Edo State, Nigeria. The samples were dried and milled to powdery form using a local milling machine.

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Isolation of Amylolytic and Cellulolytic moulds from the soil environment

Soil samples were collected around decomposing grasses and wood within Wellspring University, Benin City, Edo state. The soil samples were diluted separately and plated on sterilized potato dextrose agar (PDA). The prepared medium was sterilized for 15 minutes at 121 ^{o}C. After sterilization, the medium was allowed to cool and then dispensed into Petri dishes. The plates were incubated at 30^{o}C for 72 h (3).

Isolation and Characterization of yeast

Soil samples were collected from soil surrounding decomposing woods within Wellspring University, Benin City, Edo State. The soil samples were diluted and plated on potato dextrose agar (PDA) media. The plates were incubated at 30 ^{o}C for 72 h (3)

Screening and selection of amylolytic and cellulolytic moulds

Screening for amylolytic moulds

Screening of amylase producing moulds from the soil environment was carried out according to the method of Kareem *et al*. (14). The mould isolates were inoculated on starch agar plates and incubated at 30 ^{o}C for 48 h. The plates were flooded with Lugol's iodine solution after 48 h and zones of clearance observed around the moulds. The mould with the highest zone of clearance was then selected for further screening.

Screening for cellulolytic moulds

This was carried out by the methods of Mahasneh and Stewart (15) using Carboxyl methyl cellulose-Congo red (CMC-CR) medium. Pure mould isolates were inoculated on CMC-CR medium and incubated at 30 ^{o}C for 96 h. The mould with the highest zone of clearance was identified and selected for further studies.

Identification of cellulolytic and amylolytic moulds

Identification of the mould with amylolytic and cellulolytic property was carried out by modified needle mount preparation method (16). A small portion of the colony was removed with an inoculation sterile needle into a drop of 70 % ethanol. It was mixed gently to tease the colonies. A drop of Lactophenol Cotton Blue stain was then added and a clean cover slip was gently placed on the preparation. It was examined under X 40 objective power of the microscope.

Identification of the yeast

The Germ tube, Urea, Glucose and Cycloheximide tests were carried out to identify the yeast isolate according to the method described by Chessbrough (16).

Production and quantification of bioethanol from saw dust and brewery spent grain (BSG) by submerged fermentation under optimum conditions.

Spores of the mould were cultured on sterilized fermentation medium made up of 10 % saw dust and BSG powder in 250 ml Erlenmeyer flasks for each of the substrate. This was incubated at 30 ^{o}C for 96 h. The sugar produced was quantified at 12, 24, 36, 48, 60, 72, 84 and 96 h of fermentation using Abbe refractometer. The yeast strain was inoculated into the fermentation medium at 60 h and ethanol production monitored at 12, 24, 36, 48, 60, 72, 84 and 96 h of fermentation by fractional distillation using the method of James (17).

Optimization of the fermentation conditions for ethanol production from saw dust and BSG

Effects of substrate concentration on ethanol production

Ethanol production was carried out at 30° C and pH 5 using various substrate concentrations (5, 10, 15, 20, 25 and 30 %) of Saw dust and BSG. Ethanol production by yeast strains were determined as previously described.

Effects of temperature on ethanol production

The effect of temperature on ethanol production was investigated at 25 % substrate concentration and pH 5. The fermentation medium was incubated at different temperature (25, 30, 35, 40, 45 and 50 ^{o}C). Ethanol production was determined as previously described.

Effects of pH on ethanol production

The effect of pH on ethanol production was studied in the range 4.0-8.0 (4.0, 5.0, 6.0, 7.0 and 8.0) at 25 % substrate concentration and temperature of 35^{o} C. Ethanol production was determined as previously described

Effects of agitation on ethanol production

The effect of agitation on ethanol production was studied in the range 60 - 160 (60, 80, 100, 120, 140 and 1600) at 25 % substrate concentration, temperature of 35^{o} C and pH 6. Ethanol production was determined as previously described

Results and Discussion

Screening and identification of amylolytic and cellulolytic mould

Screening for cellulolytic moulds

In over 100 moulds screened from soil samples in Wellspring University, Benin city, one mould tested positive for amylase and Cellulase production by showing zone of clearance on the starch-agar plate (Plate 1) and the CMC-CR plate (Plate 2). The mould selected was identified as *Aspergillus flavus* using its morphological characteristics under X40 power of the objective lens of the microscope (Table 1). The clear zones around colony of the mould on Starch-Agar showed that it is an amylase producer able to degrade the starch in the medium around. This showed the mould to be an amylolytic microorganism. Furthermore, the same mould

demonstrated zones of clearance when cultured on CMC-CR plates. The mould is a cellulolytic microorganism because of its ability to degrade the cellulose in the surrounding medium. However, there were no zones of clearance around the microorganisms that are not amylase and Cellulase producers.

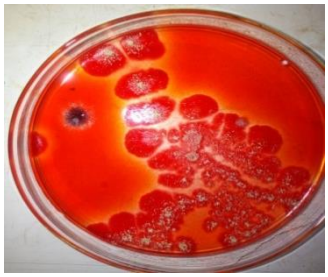

Plate 1: Cellulose producing mould on CMC-CR plates

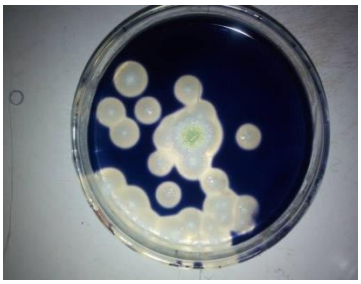

Plate 2: Amylase producing mould on Starch-Agar plate

Table 1: Identification of cellulolytic and amylolytic mould

LABEL	MACROSCOPY	MICROSCOPY	IDENTITY
1	Flat granular with radial grooves appearing yellowish green.	Conidia is radial in loose column, biserate from phliades on vesicle and globose. Conidiophores are coarsely rough close to vesicle.	*Aspergillus flavus*

Table 2: Identification of yeast

S/N	MACROSCOPY	MICROSCOPY	GGT	UREA	CYHEX	GROWTH@37	GLU	IDENTITY
1	Creamy colour, smooth glaborous and slightly raised	Large globose budding blastoconidia.	-	-	-	+	-	*Saccharomyces cerevisiae*

Production and quantification of bioethanol by fermentation under optimum conditions.

The quantity of sugar produced from BSG and Sawdust at an optimum fermentation time of 60 h was 10 % and 9 % respectively (Table 3). The yeast *Saccharomyces cerevisiae* was then inoculated into the fermentation medium at 60 h of fermentation. Similarly, the optimum fermentation time for bioethanol production was 60 h. The bioethanol yield of 6 % was produced from brewery spent grain (BSG) compared to 4 % bioethanol yield from sawdust (Table 4). The ethanol yield increased until 60 h after which it began to decrease. This increase may be due to the gradual breaking down of complex sugars to simple sugars during fermentation. The result is in agreement with the work of Rabah *et al*. (18) that reported *Saccharomyces cerevisiae* thrives on sweet medium thereby breaking down the sugars to ethanol.

Table 3: Sugar produced from brewery spent grain and sawdust using *Aspergillus flavus*

SUBSTRATE	TIME (HOURS)	SUGAR YIELD (%)							
		12	24	36	48	60	72	84	96
BSG		0.0	4.0	6.0	8.0	10.0	7.0	5.0	3.0
SAWDUST		0.0	2.0	5.0	7.0	9.0	5.0	4.0	2.0

Table 4: Production of ethanol from BSG and sawdust using *A. flavus* and *S. cerevisiae*

		BIOETHANOL YIELD (%)							
SUBSTRATE	TIME(HOURS)	12	24	36	48	60	72	84	96
BSG		0	1.0	2.0	3.5	6.0	4.0	2.5	1.5
SAWDUST		0	1.5	2.5	3.0	4.0	3.0	1.5	0.5

Optimization of the fermentation conditions for ethanol production

Effect of substrate concentration on ethanol production

Effect of substrate concentration on ethanol production is shown in figure 1. The optimum bioethanol yield of 8 % and 5 % were produced at substrate concentration of 25 % for both the BSG and Sawdust respectively. Increase in the substrate concentration beyond 25 % resulted in reduction of ethanol concentration in the medium. This could have been due to high concentration of complex sugars in the fermentation medium which could have inhibited the growth of the yeast and its ability to produce ethanol (19). Furthermore, decrease in ethanol production during fermentation could also be caused by the composition of the substrate, reduction of the enzyme's active sides, and the inefficiency of mass transfer (20). Moreover, ethanol production in this study was higher than that produced from corn straw which was reported as 3.4 % (21).

Effect of incubation temperature on ethanol production

Effect of different incubation temperature (25-50 ^{o}C) on bioethanol production by *A. flavus* grown in medium containing BSG and Sawdust was evaluated. The result shows optimum production of bioethanol of 9 % and 7 % at 35 ^{o}C for both BSG and Sawdust respectively (Fig. 2). The optimum temperature for bioethanol production from BSG and sawdust was 35 ^{o}C. At 35 ^{o}C, BSG had a yield of 9 % bioethanol, while sawdust produced 7 % bioethanol. Further increase in temperature resulted in decrease in bioethanol production. This could be due to the fact that higher temperature might disrupt membrane function and enzyme activity, thus resulting in decrease in ethanol production. In a related study, Ogbonda and Kiin-Kabari (22), reported that the optimum production of ethanol was achieved at 35°C. Furthermore, Slavikova and Nadketrova (23) reported that yeast generally, grow over an optimum temperature range of 30 to 37°C. This range of temperature agrees with the range at which the highest amounts of ethanol were produced in the present study

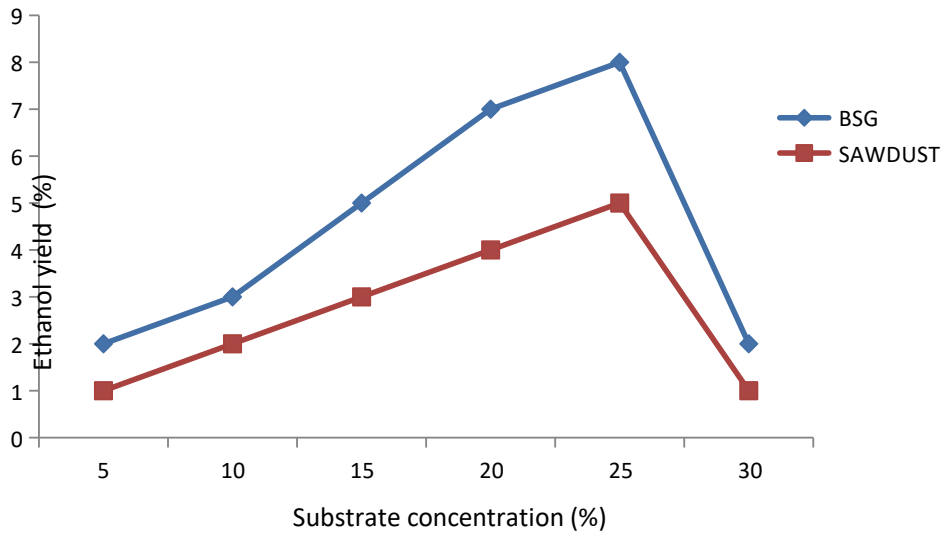

Fig.1: Effect of substrate concentration on ethanol production from BSG and sawdust by Aspergillus flavus and Saccharomyces cerevisiae.

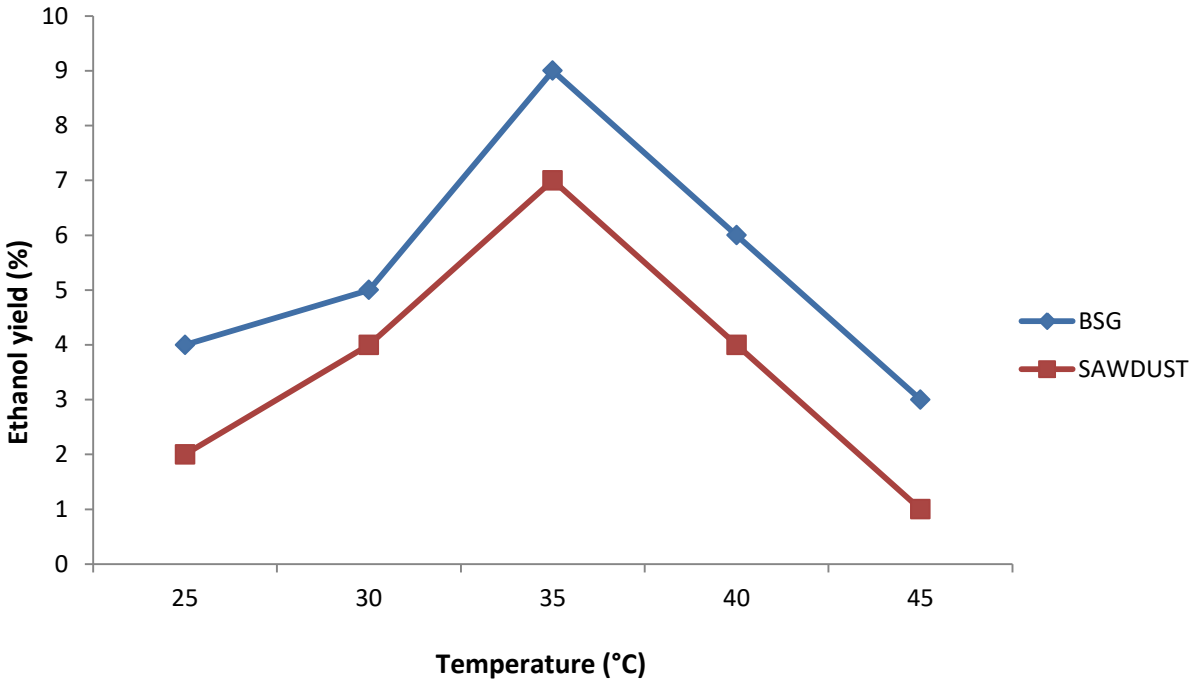

Fig.2: Effect of temperature on ethanol production from BSG and sawdust peels by Aspergillus flavus and Saccharomyces cerevisiae

Effect of pH on ethanol production

The effect of varying pH on bioethanol production is presented in Figure 3. Optimum ethanol production of 11 % and pH 8 % was obtained at pH 6.0 for BSG and sawdust respectively.). However, bioethanol yield reduced drastically to 3 % and 1 % with BSG and Sawdust respectively as the pH of the medium was increased to pH 8, indicating a decrease in ascorbic acid production beyond the optimum pH of 6. This study correlates with the findings of Oiwoh *et al*. (24) who reported that the optimum pH for bioethanol production from pineapple peels using *Saccharomyces cerevisiae* was pH value of 6. The acidity or alkalinity of the fermentation medium is one of the important factors that affect the performance of Saccharification and Fermentation.

Effect of agitation on ethanol production

A proper agitation speed is important for appropriate air supply and proper mixing of media components, hence the effect of agitation speed on ethanol production was studied. The study as shown in figure 4 revealed that optimum ethanol yields of 14 % and 10 % were produced at an agitation speed of 100 revolutions per minute from BSG and sawdust respectively. Further increase in agitation speed resulted in reduction in ethanol yield. The decrease in ethanol production at higher agitation speeds might be due to the harmful effect of the shear forces on the fungal mycelium as a result of agitation speed (25). The reduction in ethanol production at lower agitation speeds might be due to improper mixing of the fermentation medium (26). The distribution and transportation of air and nutrients to the microbial cells is dependent on different agitation speeds (27).

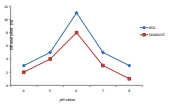

Fig.3: Effect of pH on ethanol production from BSG and sawdust by Aspergillus flavus and Saccharomyces cerevisiae

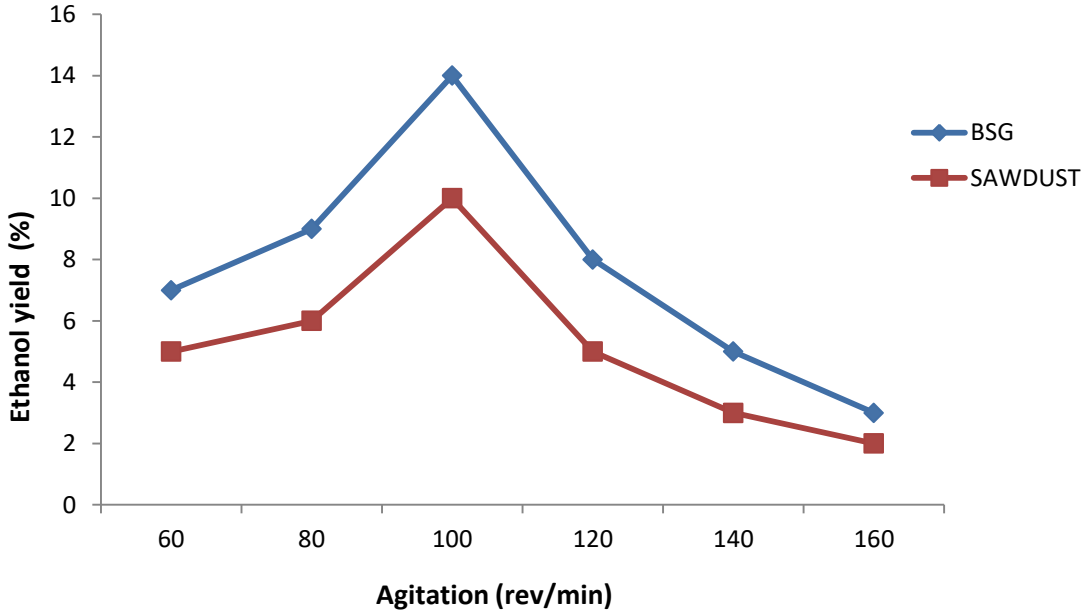

Fig.4: Effect of agitation on ethanol production from BSG and sawdust by Aspergillus flavus and Saccharomyces cerevisiae

Conclusion

In conclusion, this study has shown the potentials of *Aspergillus flavus* and *Saccharomyces cerevisiae* in the production of bioethanol from brewery spent grain and sawdust. Optimum ethanol production of 14 % and 10 % was achieved with BSG and Sawdust respectively at substrate concentration of 25 % (w/v), temperature of 35 ^{o}C, pH 6.0 and agitation speed of 100 rev/min. This study has been able to establish in a comparative manner the potentials of these industrial wastes (BSG and Sawdust) as substrates for the production of bioethanol.

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