CARPET GRASS AS A SUBSTRATE FOR BIOETHANOL PRODUCTION

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ABSTRACT

The production of bioethanol from carpet grass was examine by acid hydrolysis and fermentation process to hydrolyzed 100g of substrate at 35^0C for six days. The method used involved acid hydrolysis of carpet grass with varied acid molarities of 1M, 3M, and 5M. The hydrolyzate was further subjected to fermentation at varied yeast volume of $10cm^3$, $20cm^3$ and $30cm^3$ in order to obtain ethanol. The result obtain shows a gradual increase in the yield of ethanol with increasingly acid concentration. The final values of the bioethanol produced for different acid concentrations and volume of yeast are 20.31%, 23.61% and 26,27% from 1M, 24.29%, 25.61% and 29.61% from 3M, and 26.25%, 29,76% and 31,52% from 5M. A combination of acid concentration of 5M and $30cm^3$ of yeast gave high yield of ethanol at room temperature for acid hydrolysis of lignocellulosic content of carpet grass and fermentation of the resulting product. This result provide evidence that this method can be used to improve the production of ethanol from carpet grass.

Key words: Ethanol, Lignocelluloses , Fermentation, Pretreatment, Perennial grass

1. INTRODUCTION

The increase in demand for energy and the negative impacts of fossil fuels such as global warming, greenhouse gasses emissions, and the fast depletion of fossil resources have resulted in an increased interest in the research of alternative energy or sustainable energy such as biofuel (Muhammad and Saha, 2020). Combustion of fossil fuel has offered a numerous advantages to our life but it also lead to GHG which cause global warming and climate change (Zabed et al., 2017). The continuously increasing global demand for energy, fossil fuel resources on our planet are anticipated to become depleted within the several decades, endangering worldwide energy security. The production of biofuel has been proposed as a suitable alternative for energy generation in order to reduce the usage of fossil fuels which causes global warming, greenhouse effect, and environmentally friendliness (Dey et al., 2020). Among all, bioethanol is one of the most attractive as it can substitute gasoline (Efemwenkiekie et al., 2019). However, industrial-scale bioethanol production still faces a severe challenges of feedstock acquisition and economic viability (Robak et al., 2020). Lignocellulosic biomass is an abundant and renewable resources from plant mainly composed of polysaccharides (cellulose and hemicellulose) and an aromatic polymer (lignin). Lignocellulose biomass has a high potential as an alternative fossil resources to produce second generation biofuel.

Lignocellulose resources consist of virgin biomass comprises all naturally growing terrestrial plants, including herbaceous plants (annual, biennial, and perennial plants) and woody plants (trees bushes, and dwarf shrubs), as well as aquatic plants (e.g, water hyacinth, water fern, water lettuce, and duckweed). Energy crops include perennial grasses and other dedicated energy crops that produced a high yield of lignocellulosic biomass (e.g., switch grass, giant reed, elephant grass, and miscanthus). Waste biomass is a low-value by-product of different industrial sectors such as agriculture (bagasse, cereal, straws, stover, and husks), forestry (branches from dead trees, pruning, and thinning residues), and wood and paper production (bark, sawdust, and wood chips). It also includes an organic portion of municipal solid wastes (Arefin et al., 2021).

Lignocellulosic biomass is mainly composed of three polymers, cellulose ($C_6H_{10}O_5$)n, Hemicellulose ($C_5H_8O_4$)m, and lignin ($C_9H_{10}O_3(OCH_3)$0.9-17)x along with pectin . The cellulose, hemicelluloses and lignin content in a typical lignocellulosic biomass falls within the range of 30-60, 20-40 and 15-25% (Rezania et al., 2020). However, the composition of these major component varies depending on the source.

Cellulose is the main structural polysaccharides of the plant cell wall, providing it with high tensile strength and rigidity. Its amount usually ranges from 30% to 50% of the dry weight of lignocellulosic biomass. Regardless of its origin, cellulose is generally a highly crystalline and a high – molecular-weight polymer with a strong tendency to form high crystalline fibers. Hemicelluloses are the second most abundant polysaccharide of the plant cell wall (second primary polymer) which accounts for 15-30% of the lignocellulosic dry mass. They are complex polymers consisting of short linear and highly branched heteropolysaccharides. The lignin is the third principal component of lignocellulosic biomass, constituting about 15-30% of its dry mass. Present in all vascular plants, lignin is an amorphous, hyper-branched, and a cross linked three-dimensional network polymer with no regular repeating elements . .

Pretreatment process, disrupt the structure of lignocellulosic biomass and the cellulose fibre is exposed. This is carried out to overcome recalcitrance through the combination of chemical and structural changes to the lignin and carbohydrates (Singh et al., 2017). The hydrolysis of lignocellulosic biomass results in the release of various reducing sugars which are highly valued in the production of biofuel such as bioethanol, biogas, various organic acids, phenols and aldehydes. This pretreatment has lead to the development of various pretreatment techniques utilizing various physical, chemical, physico-chemical and biological approach.

Physical pretreatment is a pre-requisite prior to any other pretreatment methods. It is primarily carried out to reduce the particle size that result in the increase surface area, and decrease in degree of polymerization and crystallinity. Consequently, the process become more effective and easier (Chen et al., 2017). The methods are eco-friendly and seldom produce any toxic material .

Chemical pretreatment employs various chemicals, including alkalis, acids, gases, salts, ionic liquids, oxidizing agents, or organic solvents, to release polysaccharides from lignocellulosic complex and make them more susceptible to enzymatic hydrolysis (Cardona et al., 2018). Chemical pretreatment aims at removing hemicellulose and lignin in order to improve the biodegradability of cellulose.

Physico-chemical methods utilize both physical (high temperature and pressure) and chemical processes to effectively pretreatment of lignocellulosic biomass. Among such are steam explosion, carbon dioxide explosion, ammonia method, wet oxidation and liquid hot water.

Biological treatment of lignocellulosic materials is conducted to degrade the materials, the process may involve fungal pretreatment, enzymatic hydrolysis and aeration (Sharma et al., 2002). The fungi secrete the enzymes which are involved in the hydrolysis of cellulosic compounds to simple fermentable sugars. Enzymes such as xylanase, hemicellulase, α-amylase, and arabinose can be used to achieve hydrolysis. The biological process is safe and environmental friendly.

There are various combinations and several other techniques that are being developed to overcome the main drawbacks of the existing techniques to improve the utilization of the liginocellulosic complex, making the bioethanol production process more economical, efficient and environmentally friends (Rezania et al., 2020). One of them is alkaline hydrogen peroxide (AHP) pretreatment that combines the application of NaOH and H_2O_2. The main advantages are high efficiency of enzymatic hydrolysis, high lignin and hemicelluloses solubilisation values for the liquid fraction, low energy consumption, availability of chemicals needed, no furan derievatives produced, no need for special reactors.

Detoxification aims to remove or reduce the concentration of all the toxic compounds from pretreated biomass or hydrolysates, including fermentation inhibitors (such as furan, aldehydes, aliphatic acids, and phenoloc compounds) that could minimize the enzymes' efficiency and restrict microbial growth and activity during fermentation. The most common methods to discard inhibitors from biomass and ensure higher bioethanol yield and productivity are various in-situ strategies, including membrane extraction, solvent extraction, ion exchange, membrane bioreactors, adsorption, microbial adaption, using microbial consortium or engineered microorganism.

2. MATERIALS AND METHODS

a. Raw Materials

The renewable lignocellulosic biomass, carpet grass was collected around Aliero, kebbi state to be utilized as substrate for bioethanol production. The feedstock was washed with water to remove the impurities present and then sundried for 2weeks to remove the moisture in the substrate. The dried carpet grass was then subjected to powder form using pestle and mortal and in a blender to reduce the particle size. Finally, the powder substrate was sieved to have an homogeneous size.

b. Biomass Pretreatment

The pretreatment of the biomass was done by acid hydrolysis method. The 100g of the carpet grass was soaked in dilute tetraoxosulphate vi acid and heated at a temperature of 90^0C for 2hours. The slurry was autoclave at 121^0C for 2hours, and the solid separated from the liquid by filtration using a muslin cloth.

Three different concentrations of 1M, 3M, and 5M of tetraoxosulphate vi acid were prepared and 10g of the powdered sample was added into each concentration. The mixture was soaked for two hours. The PH of each hydrolysate was adjusted to 6-7 using sodium hydroxide. 10ml 20ml 30ml of prepared yeast was added to each of the mixture respectively and then shaken for 5mins to activate the yeast. This whole mixture was left for six days to ferment.

c. Hydrolysis

Preparation of NaOH (8.5M)

The preparation of 48.58g of sodium hydroxide pellets were dissolved in 500cm^{3}conical flask of prepared peptone water and stirred to dissolve.

Preparation of 1M H$_2$SO$_4$

94.6ml of distilled water was measured into 100ml volumetric flask and 5.4ml of concentrated sulphuric acid was added to make it up to the mark.

Preparation of 3M H$_2$SO$_4$

83.6ml of distilled water was measured into 100ml volumetric flask and 15.2ml of concentrated sulphuric acid was added to make it up to the mark.

Preparation of 5M H$_2$SO4

73ml of distilled water was measured into 100ml of volumetric flask and 27ml of concentrated sulphuric acid was added to make it up to the mark.

3. CHEMICAL ANALYSIS OF THE CARPET GRASS

The chemical analysis of the biomass was subjected to estimation of the chemical composition, and the reducing sugars.

a. Microorganisms

Peptone water preparation

The preparation of 7.5g of peptone water was dissolved in 100ml distilled water in a 250ml conical flask, the dissolution was done using Magnetic stirrer. The conical flask was then placed on the hot plate for about eight minutes. After dissolution, it was then autoclaved until the temperature rose to 121°c, the solution was then sterilized, and allowed to cool.

Preparation of yeast

The preparation of 1.5g of baking yeast was poured into a 100ml sterilized peptone water in a 250ml conical flask and covered with cotton wool and aluminum foil was kept in an incubator

b. Production of Bioethanol

The production of bioethanol was carried out using sacharification and fermentation process.

After four days, the mixtures were removed and filtered. The black chunk that was left in the filter paper was the lignin which could be dried and used for fuel. The filtrate contains ethanol and some other impurities. 200cm^3 of the mixture was then distilled using simple distillation at 80°C for 35 minutes. The distillate was collected in a conical flask placed at the end of the distillation column and the test for the presence of ethanol was carried out afterward.

The Bioethanol which was produced from the fermentation process contains significantly amount of water which has to be removed. This is achieved using distillation set up at 78°C. The product ethanol turns vapour at its boiling point before the water.

The fermentation was done by adding the activated yeast into different conical flask containing the samples. The conical flask were covered with cotton wool and wrapped with foil sheet to avoid oxygen penetrating into the fermentation medium. The samples were taken into the incubator and incubated at room temperature for 6days.

Enzymatic hydrolysis and Fermentation

The carpet grass which had undergone pretreatment are used for enzymatic hydrolysis process to convert the cellulose to glucose. The substrates was autoclaved and the yeast beaker resulting to increasing of high yield of reducing sugars for fermentation process (Ali et al., 2014). The hydrolysis lasted for 24hours at a temperature of 50^{0}C , stirring continuously and placed in the incubator. The vacuum filtration to separate the solid from the liquid part was used for fermentation. 1.0g of the yeast was added to the 200ml of the slurry to commence the fermentation process. This was carried out at room temperature for 21days in a conical flask sealed with an aluminum foil (Ali et al., 2014).

4. RESULTS AND DISCUSSION

Bioethanol was produced by saccharification and fermentation of the carpet grass. Table 1 shows the chemical composition of feedstock. The production of bioethanol by yeast is essential for the conversion of the carpet grass to reducing sugars as shown in table 2.

Table 1. Chemical composition of carpet grass

Composition	Cellulose	Hemicellulose	Lignin	Ash
Percentage	36.5	24.9	17.5	6.4

a. Saccharification

During this process, the reducing sugars was released by using DNS method, which indicates released of highest amount of sugars at 3M, and 3g of yeast after 24hours. Results shown in table 4.

Table 2. Chemical composition of pretreated carpet grass

Composition	Total sugars	Reducing sugars	Cellulose
Percentage	18.56	6.8	22.56

b. Estimation of Bioethanol

The estimation of ethanol was observed from the results as shown in table 3 - 6.

Table 3.1.1; 3.1.2; and 3.1.3; shows the results of the percentage yield of ethanol using 1M Acid hydrolysis; 3M Acid hydrolysis; and 5M Acid hydrolysis at different volume of yeast.

Table 3 Percentage yield of ethanol at 1M concentration and different volume of yeast

S/N	Volume of yeast Cm^3	Volume of hydrolysate cm^3	Volume of 4 distillate cm^3	Percentage Yield %
1	10	64	13	20.31
2	20	72	17	23.61
3	30	82	22	26.27

Table 4 Percentage yield of ethanol at 3M concentration and different volume of yeast

S/N	Volume of Yeast cm^3	Volume of Hydrolysate cm^3	Volume of distillate Cm^3	Percentage Yield %
1	10	70	17	24.29
2	20	80	21	25.61
3	30	87	24	27.61

Table 5: Percentage yield of ethanol at 5M concentration and different volume of yeast

S/N	Volume of Yeast cm^3	Volume of Hydrolysate Cm^3	Volume of Distillate Cm^3	Percentage Yield %
1	10	80	21	26.25
2	20	84	25	29.76
3	30	92	29	31.52

Table 6. Characterization of produced Bioethanol

Properties (S.I Units)	Carpet grass	ASTM Standard
Boiling point (^{0}C)	78	78
Cloud point (^{0}C)	20	23
Density (g/cm^3)	1.03	0.79
Flash point (^{0}C)	19.50	18.60
Pour point (^{0}C)	5.0	5.2
Viscosity (Cst)	0	0.98

The effects of acid concentration and variation of yeast concentration was carried out using 1M, 3M, 5M concentration of tetraoxosulphate vi acid.

Table 3.1.1, shows the variation of ethanol yield, derived from the fermentation of hydrolyzed lignocellulosic content of carpet grass with 1M concentration of tetraoxosulphate vi acid and different volume of yeast. It was observed that the percentage of ethanol increases with increase volume of yeast. As shown in Table 3.1.2, it can be deduced that the percentage yield of ethanol increases with increase in the volume of yeast. Table 3.1.3 showed that ethanol yield also increase with increase in acid concentration which also confirmed that the fermentable sugar present in hydrolyzed lignocellulosic content of carpet increases with increase in the concentration of the acid. At 1M concentration, and highest volume of yeast of $30cm^3$, the maximum yield of ethanol was $26.27cm$. At 3M concentration and highest volume of yeast of $30cm^3$, the maximum yield of ethanol was $27.61cm^3$. At 5M concentration and highest volume of yeast at $30cm^3$, the maximum yield of ethanol was $31.52cm^3$.

It can be deduced that increase in the yield of ethanol is directly proportional to the increase in the concentration of the acid.

5. CONCLUSION

By analyzing the results above, it can be concluded that acid hydrolysis can be used to improve the production of ethanol from the lignocellulosic content of carpet grass. Ethanol with moderate percentage yields was produced from carpet grass using three different acid concentrations in the hydrolysis processes. The concentration of yeast also affect the yield of ethanol obtained from the fermentation of the fermentable sugar derived from the hydrolyzed lignocellulosic content of carpet grass.

Conclusively, this present work has clearly shows that acid hydrolysis at 5M tetraoxosulphate vi acid with moderate yeast concentration can be used to improve the production of bioethanol production.

6. RECOMMENDATIONS

This research has successfully proved that production of bioethanol from sawdust is a good venture that could reduce unemployment in Nigeria. As a result, l will recommend that:

i . Further research should be done using non-acid hydrolysis as an alternative to chemical methods to reduce toxicity.

ii . Bioethanol should be produced on industrial scale so as to provide a lasting solution to the gradual depleting ozone layer as a result of green house gasses emission.

Iii . More research should be conducted on the production of bio energy which are renewable.

iv . Various industries should utilize huge volume of cellulosic wastes which provides a low-cost and sustainable resources for production of ethanol in order to reduce the emission of green house gas cause by fossil fuel.

7. REFERENCES

[1] Ali, N., and Ahire, M., Ubhrani, P., Tagotra, M (2014). A step towards Environmental wastes Management and Biofuel Production from waste banana peelings. Ame. J. Eng. Res. 3 (5): 110-116.

[2] Arefin, M.A.; Rashid, F.; and Islam, A. A (2021). Review of Biofuel Production from Floating Aquatic Plants: An Emerging Source of Bio-Renewable Energy. *Biofuels Bioprod. Biorefin. 15*, 574–591.

[3] Cardona, E., Liano B., Penuela M., Pena J. and Rios L. A., (2018). Liquid-hot water pretreatment of palm-oil residues for ethanol production: An economic approach to the selection of the processing conditions, Energy. 160 (C), 441-51.

[4] Chen, J.; Zhang, B.; Luo, L.; Zhang, F.; Yi, Y.; Shan, Y.; Liu, B.; Zhou, Y.; Wang, X.; and Lü, X. A (2018). Mixing Alkali pretreatment and Acid Pretreated Biomass for Cellulosic Ethanol Production Featuring Reduced Chemical Use and Decreased lnhibitory Effect. Lnd. Crops Prod. 124, 719-725.

[5] Das, N.; Jena, P.K.; Padhi, D.; Kumar Mohanty, M.; and Sahoo, G. A (2021). Comprehensive Review of Characterization, Pretreatment and Its Applications on Different Lignocellulosic Biomass for Bioethanol Production. *Biomass Conv. Bioref. 82*, 1–25.

[6] Dey, P.; Pal, P.; Kevin, J.D.; and Das, D.B (2020). Lignocellulosic Bioethanol Production: Prospects of Emerging Membrane Technologies to Improve the Process—A Critical Review. *Rev. Chem. Eng. 36*, 333–367.

[7] Efemwenkiekie, U.K.; Oyedepo, S.O.; Idiku, U.D.; Uguru-Okorie, and D.C.; Kuhek (2019). A. Comparative Analysis of a Four Stroke Spark Ignition Engine Performance Using Local Ethanol and Gasoline Blends. *Procedia Manuf. 35*, 1079–1086.

[8] Fan, M., Zhao, C., Huang, X., Zhang, H., and Xie, J.(2021). Enhanced Digestibility and Fermentability of sugarcane Bagasse in Biofuel production by Surfactant-Assisted Dilute Acid Pretreatment. lnd. Crops prod. 172, 114006.

[9] Hoang, T., and Nghiem, N.P (2021). Recent Development ND Current Status of Commercial Production of Fuel Ethanol, Fermentation, 7(4), 314.

[10] Rezania, S., Oryani, B., Cho, J., Talaiekhozani, A., Sabbagh, F., Hashemi, B., Rupani, P.F., Muhammadi, A.A. (2020). Different pretreatment Technologies of Lignocellulosic Biomass for Bioethanol Production. An Overview. Energy, 199, 117457.

[11] Robak, K; and Balverek, M (2019). Current State-of-the-Art in ethanol production from lignocellulosic S., Eds; Academic press: Cambridge, MA, USA, pp. 45-59. ISBN 978-0-1

[12] Roberto, I.C., Castro, R.C.A., Silva, J.P.A., and Mussato, S.I (2020). Ethanol production from High Solid Loading of Rice Straw by Simulteneous Sacharification and Fermentation in a Non-Conventional Reactor. Energies. 13, 2090.

[13] Sharma S.K., Kaira k.L., Grewal, H.S. (2002). Enzymatic saccharification ofr preteated sunflower stalks. Biomass Bioenergy. 23 (3): 237-241.

[14] Singh, N., Gupta, A., Mathur A.S., Barrow, C., and Puri, M. (2020). Integrated consolidated bioprocessing for simultaneous production of Omega-3 fatty acids and bioethanol, Biomass Bioenergy. 137:10555.

[15] Zabed, H.; Sahu, J.N.; Suely, A.; Boyce, A.N.; Faruq, G (2017). Bioethanol Production from Renewable Sources: Current Perspectives and Technological Progress. *Renew. Sustain. Energy Rev. 71*, 475–501.