JOBIMB, 2023, Vol 11, No 2, 51-56
https://doi.org/10.54987/jobimb.v11i2.860

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

Potency of Some Savanna Herbaceous Weeds in the Production of Bioethanol Using *Zymomonas mobilis* and *Saccharomyces cerevisiae*

Yusuf Ahmed[1]*, Mukhtar, M.D.[1], Sani Yahaya[1] and Abdullahi Adamu Faggo[2]

[1]Department of Microbiology, Faculty of Science, Bayero University Kano, PMB 3011 Gwarzo Road Kano, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, P.O. Box 1069, Nigeria.

*Corresponding author:
Yusuf Ahmed
Department of Microbiology,
Faculty of Science,
Bayero University Kano,
PMB 3011 Gwarzo Road Kano,
Nigeria.
Email: yusufahmed2852@gmail.com

HISTORY

Received: 27th Oct 2023
Received in revised form: 21st Dec 2023
Accepted: 29th Dec 2023

KEYWORDS

Lignocellulose biomass
Pretreatment
Zymomonas mobilis
Saccharomyces cerevisiae
Bioethanol

ABSTRACT

The uncertainty of the fossil fuel and global climate changes has invariably led to renewable energy demand. Thus, necessitating increasing biotechnological research efforts. Accordingly, the current work was designed to investigate the potency of some savanna herbaceous weeds in the production of bioethanol using *Zymomonas mobilis* and *Saccharomyces cerevisiae*. The biodiversity study and the proximate analysis of weed samples growing on Bayero University Kano old Campus, Nigeria were carried out. The substrates were pretreated with 2% of 0.233M sulfuric acid solution and hydrolysed using *Aspergillus niger*. The reducing sugars were estimated using the dinitrosalicylic acid method. Fermentation was carried out using local strains of *Zymomonas mobilis* and *Saccharomyces cerevisiae* isolated from fermented Zobo drink and palm wine under anaerobic conditions. The absorbance of the produced bioethanol was measured and compared with the known standard ethanol curve using Techme I Uv-vis spectrophotometry at 600nm. The dominant weeds include; *Cynodon dactylon* (30%), H*eteropogon contortus* (20%) and *panicum virgatum* (13.45%). The carbohydrate contents as hemicellulose ranged from (35.44 - 27.68%), cellulose (39.80 - 29.20%) and lignin contents (4.32 - 2.14%). The reducing sugar ranged from (24.67±0.01 - 94.79±0.01 mg/g) in all the substrates used. Maximum bioethanol yield was obtained with *Cynodon dactylon* (48.09±0.07ml/g) in fermentation with a cocktail of the microorganisms whereas, the minimum yield was observed with H*eteropogon contortus* (15.08±0.01 mL/g). *Zymomonas mobilis* had the maximum bioethanol yield (29.20±0.01ml/g) than *Saccharomyces cerevisiae* with (20.10±0.01 mL/g) bioethanol ($p>0.05$). The findings reveal the lignocellulosic weeds used in this study are good substrates which can be exploited in bioethanol production.

INTRODUCTION

Energy sustainability is becoming a global necessity and its consumption associated with the transportation sector has contributed to a world problem [1]. The world's energy demand is increasing every day and the problem of fossil fuel depletion is looming [2]. These challenges have led to new approaches that focus on energy consumption management and alternative sources of fuel so as to increase efficiency and reduce greenhouse gas emissions respectively [3]. Invasive herbaceous weed is an important lignocellulosic plant with great potential for bioethanol production. They are found in abundance, invading many important places such as roadsides, government agencies, educational institutions, etc. [4,5]. The issue of energy security resulting from the depletion of fossil fuel, increase of petroleum prices and environmental concerns has stimulated governments and researchers to look for alternative renewable energy sources that are technically feasible, economically competitive and environmentally acceptable [6].

There is a high abundance of herbaceous biomass weeds in Bayero University Kano, their growth invades every nook and cranny of the institution, secured are the buildings, roads and other paved surfaces. During the dry season, these important renewable resources are usually burned, putting biodiversity at high risk. It involves the production of air pollutants and other oxidants [7]. The use of herbaceous biomass weeds to generate energy in form of bioethanol will not only provide efficient and

inexpensive sources of energy but also help to solve environmental problems without competing with the food supply. It is suggested that the utilization of these plants weeds in the production of bioethanol with a view to addressing their mismanagement and other ecological, agricultural, economic and environmental challenges is imperative. So, the conversion of these weeds into bioethanol will helps to reduce environmental pollution and energy problem [6]. Finding an alternative method for managing herbaceous weeds is therefore crucial, thus justifying the current study.

METHODOLOGY

Sample collection &biodiversity studies

Multi-stage sampling technique was employed in this study and weed samples were collected using the 1x1 meter square quadrant method and the weed Plants in each quadrant were identified and their numbers were estimated in percentage for biodiversity analysis [8]. The identification of the plant species was carried out in plant science herbarium of Bayero University Kano, Kano State, Nigeria.

Biomass estimation

Weed biomass samples was dried in hot air oven at 105 °C overnight until the moisture completely evaporated [8].

$$\% \text{ of dry matter} = \frac{\text{W1 (g/m}^2\text{)} - \text{W2 (g)}}{\text{W3 (g/m}^2\text{)}} x100\%$$

Biomass preparation

The dried (herbaceous weeds sample) was grinded with mortar and pistil, after drying [9]. The samples were repackaged in clean plastic containers and stored at ambient temperature in storage cupboards for further analysis in the postgraduate laboratory department of microbiology, Bayero University before subsequent analysis.

Proximate analysis

Proximate compositional analyses of the powdered samples was carried out to determine the lignocellulose compositions (lignin, Hemicellulose, cellulose) and Nutritional composition moisture, crude Ash, crude fibre, crude fat, crude protein content, following the methods described by Association of Official Analytical Chemists [10].

Isolation and identification of microorganisms

Zymomonas mobilis was isolated from commercially sourced fermented zobo of *Hibiscus sabbdarifa* using nutrient agar medium and identified with reference to cultural, biochemical, morphological characteristics and using biotechnological method of molecular [11](Rabah *et al*., 2014).

Saccharomyces cerevisiae was isolated from palm wine using sabouraud dextrose agar and identified using cultural and morphological characteristics [12](Usman *et al*., 2019). *Aspergillus niger* was isolated from soil sample using Potato Dextrose Agar (PDA) and the plates were incubated at 28 ºC for 5 days as described by (Alhassan *et al*., 2020)[13].

Processes involved in the production of bioethanol
pretreatment

Dilute acid pre-treatment was carried out, in this method three sets of two conical flasks each were arranged (a-c). Set (a) contains 10 and 15g of powdered *Cynodon dactylon weed.* Set (b and c) contains 10 g and 15 g as above for *Panicum virgatum* and *H. contortus* respectively. These sets were duplicated accordingly. The sets were soaked with 5 mL and 10 mL of 2% (0.223 M) of dilute sulfuric acid and left for 30 min, after which were slurred with (100 mL and 150 mL) of sterile distilled water. The flask was covered with cotton wool, wrapped in aluminium foil, heated in a water bath at 60 °C for 1 hour and autoclaved at 121°C for 30 min at a pressure of 15 psi for delignification [14].

Hydrolysis

Aspergillus niger was used to hydrolyze the substrates. The acid-treated slurry samples in each of the conical flasks were inoculated with (1.0 mL and 1.5 mL) of *Aspergillus niger* suspension of according to McFarland standard ($3.0x10^8$ cells per mL). The pH was adjusted to 5.0 and the flasks were incubated for 5 days in an incubator shaker at 100 RPM at 30ºC for continuous shaking [15].

Determination of reducing sugar

The clear supernatant of the hydrolysates was taken on daily basis for estimation of reducing sugars using DNS method (3, 5-Dinitrosalicylic acid) For absorbance at 540nm in UV-VIS spectrophotometer Tichem I USA 20D and extrapolated the actual concentration using standard curve [15].

Fermentation

The sterilized hydrolysates were inoculated separately with 1.5 ml and 2.0 ml according to McFarland standard ($3.0x10^8$ cells per mL) each of the overnight incubated enriched medium of the *S. cerevisiae* and *Z. mobilis.* Also, the samples were inoculated with 1.5 ml each of the consortium of both microorganism (*Saccharomyces cerevisiae* and *Zymomonas mobilis*). The inoculated samples were incubated at 30 ℃ for five (5) days, the conical flasks were sealed completely to induce an anaerobic condition for efficient fermentation [16].

Distillation

Distillation of the fermented hydrolyzed sample was carried out using simple fractionating distillation apparatus. The temperature of thermo- regulated heating mantle was maintained at 78°C which was used to heat the round bottomed flask containing the fermented wort-water-mixture. As the distillation proceeded, the lighter low boiling constituents notably aldehydes gradually evolved and was collected [17].

Dehydration of the distillate

Calcium oxide was used for dehydrating the resulting distillate. Ten 10 g of CaO_2 was added to the distillate, stirred thoroughly and kept overnight for the slaking to take place. After 24 h, the upper layer which was thought to be bioethanol on top was carefully decanted, leaving the water layer below [17].

Determination of quantity of bioethanol produced

The distillate collected from substrates were measured using a measuring cylinder and expressed as quantity of ethanol produced in mL/g. The percentage bioethanol yield was calculated according to the expression proposed by Gunasekaran and Kamini [6].

$$\text{Bioethanol Yield (\%)} = \frac{\text{Vol. of Bioethanol Produced}}{\text{Vol. of Fermented Sample Used}} x100\%$$

Confirmatory test for ethanol produced
potassium dichromate test

Two drops of acetone were introduced into a test tube already containing four drops of fractionated bioethanol. Subsequently, two drops of chromic acid were carefully added. The test tube was then sealed tightly with a cork and vigorously shaken to facilitate observation for any discernible color change [18].

Statistical analysis of the data

The data obtained were analyzed statistically using SPSS 21.0. A paired t-test was used to ascertain the difference between the bioethanol yield by the individual microorganisms as well as that by the combined microorganisms at 95% probability level. The results were expressed as Mean± Standard deviation.

RESULTS

Biodiversity study of the herbaceous weeds

The biodiversity analysis of herbaceous weed samples exhibited a range from 2.75% to 30%. Notably, *Cynodon dactylon* emerged with the highest percentage of occurrence (30%), followed by *Heteropogon contortus* (20.75%), (13.25%) *Parnicum virgatum*, (12.25) *Imperata cylindrica*, (7.25%) *Crepsis foetida*, (4.35), *Syndrella nodiflora*, (3.75) *Polypogon monspeciensis*, (3.15%) *Combopogon citratus*, (2.75%), *Gomphrena serrata* L. while *Heterotheca grandiflora* had the least percentage of occurrence of (2.5%) respectively (**Fig. 1**).

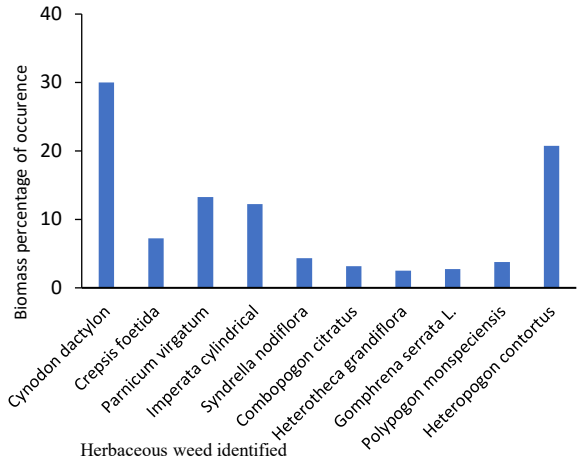

Fig. 1. Biodiversity distribution of savanna herbaceous weeds sample identified in Buk, Old Campus.

Determination of biomass concentration in g/m^2.

The relative percentage concentrations of biomass harvested from each sampling point indicate that the results ranged from 41.48% to 16.19%. The highest concentration of biomass was harvested from the hostel area, at 41.48%, followed by the sports complex at 22.80%, and the staff quarters at 19.53%. The academic complex had the lowest percentage concentration of biomass, at 16.19% (**Fig. 2**).

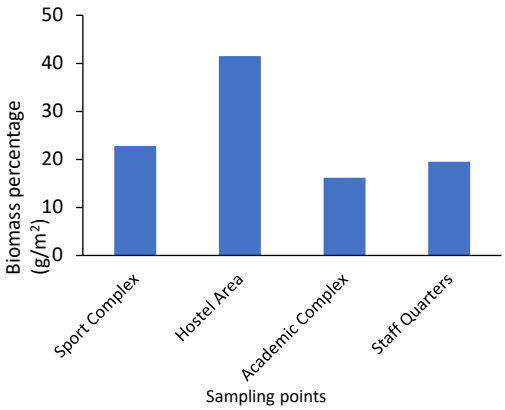

Fig. 2. Relative percentage of biomass estimated per sampling sites in gram/m^2.

Proximate compositional analysis of herbaceous weed samples dry matter basis

The result of the proximate composition of dry matter presented the nutritional and lignocellulose content as follows: For the nutritional contents: The moisture content values ranged from 3.29 to 5.22%. Substrate A (*C. dactylon*) had the highest percentage of (5.22%), followed by substrate C (*Heteropogon contortus*) (4.21%), while substrate B (*Panicum virgatum*) had the lowest count of (3.29%). Crude protein ranged from 6.64% to 12.77%. Substrate B had the highest count of 12.77, followed by substrate C (9.66), and substrate A recorded the lowest count of 6.64%. The crude fibre ranged from 9.6 to 22.46%. Substrate A recorded the highest count of 22.46%, followed by substrate C (19.4) respectively, and substrate B recorded the lowest count of 9.6%. Ash content ranged from 9.63 to 4.43%.

Substrate A had the highest ash content (9.63), followed by substrate C (6.02), while the lowest count was recorded at substrate B (4.43%). The results of the fat content ranged from 7.55 to 6.5%. Substrate C recorded the highest percent (7.55%), followed by substrate A (6.6%) and substrate B had the lowest percentage (6.5%). For the lignocellulose components: The hemicellulose contents ranged from 35.44 to 27.68%. The highest content was recorded in substrate A (*C. dactylon*) constituting (35.44%), followed by substrate B (34.01%) and substrate C had the lowest content of (27.68%). The cellulose content was ranged from 39.80 to 29.20 %. Substrate A constituted the highest content (39.80%), followed by substrate B with (35.78%) and substrate C constituting the least content of (29.20%). The lignin contents ranged from 4.32 to 2.14. Substrate A, haven the highest content of (4.32%), followed by substrate B with (2.31%), while substrate C had the least percent of (2.14%) respectively (**Fig. 3**).

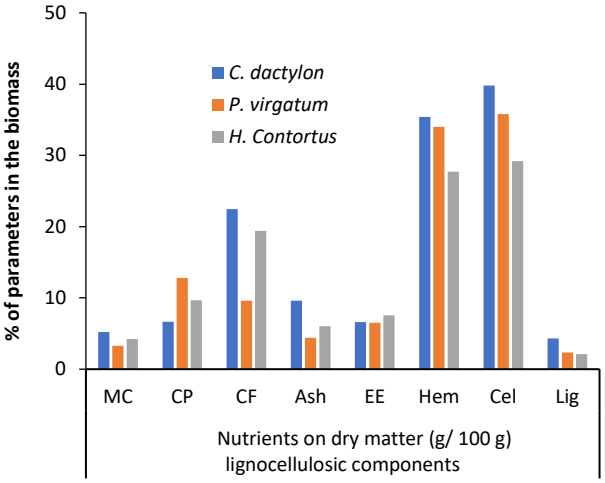

Fig. 3. Proximate composition of savanna herbaceous feedstock showing nutritional and lignocellulose content. MC=Moisture content, CP=Crude Protein, CF=Crude fiber, EE=Ether extract (fat), Hem=Hemicellulose, Cel=Cellulose, L=Lignin.

Morphological characterization of *Aspergillus niger* isolated from soil

The organism shows a black mycelium on the agar medium, it had septate hyphae, long and smooth conidiospores, and long unbranched sporangiospores with a large and round head (**Table 1**).

Colonial and morphological characterization of *Saccharomyces cerevisiae*

Macroscopically, the organism shows a fluffy cream colony, with purple and oval shapes. Microscopically, the vegetative cells isolated view under 100 objective lenses of the compound microscope are oval, circular in clusters and chains with budding (**Table 2**).

Colonial, morphological characterization and biochemical characteristics *Zymomonas mobilis.*

The isolates were Gram–negative, short rods, motile, catalase-positive, oxidase negative and with the evolution of gas (**Table 3**).

Reducing sugar

After hydrolysis of pretreated samples for five (5) days using *Aspergillus niger* is presented in **Fig. 4**. The reducing sugar values ranged from 25.34±0.01, 25.26±0.01, 24.67±0.001 for day one (1) to 94.62±0.02, 94.79±0.01, 94.47±0.03 (mg/g) for day five (5) in sample A, B and C.

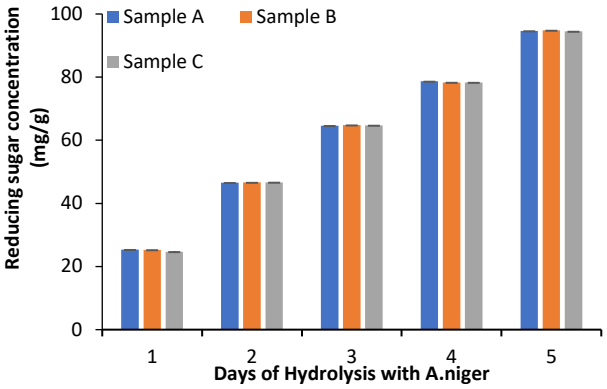

Fig. 4. Reducing sugar yield from hydrolysis of treated samples using *Aspergillus niger.*

Confirmatory test for bioethanol produced

The confirmatory test indicates that all the produced distillate from fermented substrates (A, B and C) turns from orange colour (dichromate colour) to blue-green colour within a few seconds of adding two drops of dichromate acid solution. This change in distillate colouration to blue-green indicates strong evidence for the existence of ethanol (**Table 4**).

Bioethanol yield

The distillate collected from substrates A, B, and C were measured using a measuring cylinder and expressed as a quantity of ethanol produced in g/mL by dividing with the volume of the fermented distillate by multiplying with 100%. The highest ethanol yield was absorbed in Substrate A3 (48.09 g/mL) with a substrate concentration of 15 g/mL and the lowest ethanol yield was observed in substrate C1 (15.08 g/mL) with a substrate concentration of 10 g/mL. The result proved that the quantity of ethanol increased with an increase in substrate concentration. The samples with maximum ethanol yield were those fermented with a mixed culture of *Z. mobilis* and *S. cerevisiae*. When comparing single organisms *Zymomonas mobilis* yield more ethanol than *Saccharomyces cerevisiae* (**Fig. 5**).

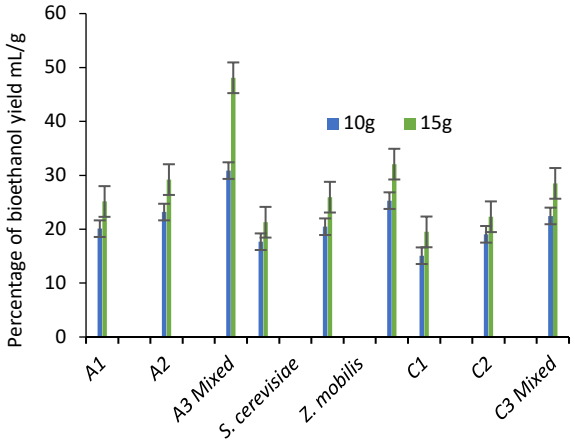

Fig. 5. Quantity of bioethanol yield % (mL/g). Keys: A1, A2 and A3= *Cynodon dactylon* + *S. cerevisiae*, *Cynodon dactylon* + *Z. Mobilis, Cynodon dactylon* + Mixed culture. B1, B2 and B3= *Panicum virgatum* + *S. cerevisiae*, *Panicum virgatum* + *Z. Mobilis*, *Panicum virgatum* + Mixed culture. C1, C2 and C3= *Heteropogon contortus* + *S. cerevisiae*, *Heteropogon contortus* + *Z. Mobilis*, *H. contortus* + Mixed culture.

Table 1. Morphological characterization of *Aspergillus niger* isolated from soil.

Isolate	Results
Colony shape	Black dotted surface as Conidia
Cell Shape	Filamentous, with septate hyphae
Suspected organism	*Aspergillus niger*

Table 2. Morphological characterization of *Saccharomyces cerevisiae* isolated from fresh palm wine.

Isolate	Results
Colony shape	Fluffy cream colonies
Cell Shape	Purple oval shape
Gram's reaction	+
Suspected organism	*Saccharomyces cerevisiae*

Table 3. Morphological and biochemical characterization of organism isolated from fresh Zobo drink.

Isolated organism/Tests	Results
Colony shape	Brilliant white to cream color
Cell shape	Plump with rounded ends
Gram reaction	– rod
Motility	+
Catalase	+
Urease	–
Mannitol	+
Oxidase	–
Organism	*Zymomonas mobilis*

Key: +: positive; –negative

Table 4. Confirmatory (qualitative) test for bioethanol produced.

Test	Substrate A	Substrate B	Substrate C	Inference
	Observation			
4 drops of distillate + 2 drops of potassium dichromate acid solution	Blue-green	Blue-green	Blue-green	Ethanol presence

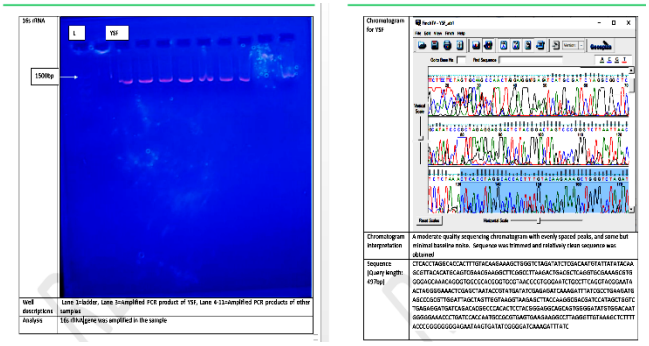

Fig. 6. Biotechnological method of molecular confirmation of *Zymomonas mobilis.*

DISCUSSION

The dominant identified weeds were *Cynodon dactylon* with 30% occurrence, H*eteropogon contortus* with 20% and *panicum virgatum* (13.45%). The proximate composition of the dry biomass matter for the *Cynodon dactylon* (5.22%) moisture content, crude protein (6.64%), fibre (22.45%), ash (9.63%) and ether extract had (6.60%). The *panicum virgatum* had (3.29%) moisture content, crude protein (12.77%), fibre (9.62%), ash content (4.42%) and ether extract of (6.48%) whereas *heteropogon contortus* had 4.21 % moisture content, 9.65% crude protein, 19.39% crude fibre, 6.01% ash content and 7.54% fat content. The proximate result from this research is nearly similar to the result obtained by [19] on proximate analysis of lignocellulose dry biomass matter where they obtained 3.9% moisture content, 9.11% ash content, 13.13% crude protein and 3.31% fat content.

The 94.79±0.01mg/g reducing sugar obtained in this study is almost in agreement with the 98% reducing sugar for methanol-treated substrates reported by Olanbinwoninu and Odunfa [20] and higher than 88% from alkali treatment reported by the same work. Maximum bioethanol yield was obtained with *Cynodon dactylon* (48.09 mL/g) in combined fermentation, followed by substrate B3 *Panicum virgatum* (32.07 mL/g) also in combined fermentation and the minimum yield was observed with *Heteropogon contortus* (15.08 mL/g). When comparing single organisms, *Zymomonas mobilis* was found to be most productive, had the maximum bioethanol yield of 29.20±0.01 mL/g, whereas bioethanol produced through fermentation with *Saccharomyces cerevisiae* had the maximum yield of 25.14±0.01 mL/g bioethanol ($p>0.05$). The findings from this study on bioethanol yield are lower than the one reported by [21] where *Zymomonas mobilis* produces 70 mL/g and *Saccharomyces cerevisiae* yields 40 mL/g using 20 g/200 mL powdered substrate.

CONCLUSION

The following weeds namely, *Cynodon dactylon*, *Panicum virgatum* and *Heteropogon contortus* were dominant weed species in Bayero University Kano, old campus and *Cynodon dactylon* had the highest percentage of occurrence. *Zymomonas mobilis* and *Saccharomyces cerevisiae* demonstrated high activity in the generation of bioethanol individually and in combination with these plant weeds. *Zymomonas mobilis* had the maximum bioethanol yield of 29.20±0.0/mL/g than *Saccharomyces cerevisiae* with 20.10±0.01 mL/g bioethanol ($P>0.05$). From the results obtained from this research analysis, the ethanol yield increased with an increase in substrate and inoculum concentration. Sodium potassium dichromate test, specific gravity curve test, qualitative test, physical colour and flammability test were carried out to confirm the produced ethanol respectively.

RECOMMENDATION

The result of the experiment shows lignocellulosic materials are good substrates which can be exploited in industries and on a commercial basis as they are cheap and more importantly renewable. It was recommended that the use of these lignocellulosic materials for bioethanol production could serve as a means of controlling the recalcitrant menace of weeds and environmental pollution. Other pretreatment methods such as biological, Steam expulsion and hot water method should be carried out to determine the efficacy and quality of the bioethanol yield. Further research has to be carried out at a large scale in order to identify the potential of other identified herbaceous weeds.

REFERENCE

1. Adetunji OR, Youdeowei PK, Kolawole OO. Production of Bio-ethanol from Cassava Peels. In: International Conference on Renewable Energy and Power. Atlanta, Georgia, U.S.A; 2015.
2. Iodice P, Senatore A. Atmospheric pollution from point and diffuse sources in a National Interest Priority Site located in Italy. Energy Environ. 2016;27(5):586–96.
3. Yusuf AA, Inambao FL. Progress in alcohol-gasoline blends and their effects on the performance and emissions in SI engines under different operating conditions. Int J Ambient Energy. 2018;1–17.
4. Yan Q, Wang Y, Rodiahwati W, Spiess A, Modigell M. Alkaline-assisted screw press pretreatment affecting enzymatic hydrolysis of wheat straw. Bioprocess Biosyst Eng. 2017;40(2):221–9.
5. Borah AJ, Singh S, Goyal A, Moholkar VS. An assessment of the potential of invasive weeds as multiple feedstocks for biofuel production. RSC Adv. 2015;6(52):47151–63.
6. Okoh E, Valentine C. Bioethanol production from Jatropha seed cake via dilute acid hydrolysis and fermentation by Saccharomyces cerevisiae. GSC Biol Pharm Sci. 2021;15(01):049–54.
7. Hamid AA, Usman LA, Elaigwu SE, Zubair MF. Environmental and health risk of bush burning. Adv Environ Biol. 2010;4(2):241–9.
8. Adney B, Baker J. Measurement/Estimation of cellulose biomass activities. Laboratory Analytical Procedure (LAP. Golden, CO: National Renewable Energy Laboratory; 2008 p. 510-42628.
9. Venkatraman U, Uihlein A, Schbek L. Environmental impacts of a lignocellulosic feedstock biorefinery system: an assessment. Biomass Bioenergy. 2009;33:793–802.
10. Helrich K. Official Methods of Analysis of the Association of Official Analytical Chemists. Fifteenth Edition. Two Volumes. 15th edition. Arlington, Va: Aoac Intl; 1990. 1298 p.
11. Rabah AB, Oyeleke SB, Manga SB, Gusau LH. Utilization of Rice Husks and Groundnut Shells for Bioethanol Production. Int J Environ Sci Dev. 2014;5(4):5721–4.
12. Usman Y, Umar YA, Denwe SD, Muhammad MN, Mohammed S. Evaluation of bioethanol production potential from Eucalyptus Tereticornis biowaste using Bacillus subtilis, Escherichia coli, and Saccharomyces cerevisiae. Acad J Sci Eng. 2015;9(1):31–40.
13. Alhassan SA, Sani Y, Haruna M, Kasim M, Mukhtar MD. Utilization of rumen content by indigenous microorganisms in a modified anaerobic digester. Energy Res J. 2020;11:12–21.
14. Saranraj P. Production of Ethanol by Zymomonas mobilis and Saccharomyces cerevisiae Using Sunflower Head Wastes - A Comparative Study. Microbiol Res. 2014 Jan 1;5:208–16.
15. Ballesteros I, Negro MJ, Oliva JM, Cabañas A, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pretreated wheat straw. Appl Biochem Biotechnol. 2014;130:496–508.
16. Kumar N, Singh JP, Ranjan R, Devi CS, Srinivasan MV. Bioethanol production from weed plant. Adv Appl Sci Res. 2013;4(4):299–302.

17. Ofoefule AU, Mathewson SW. The Manual for the Home and Farm Production of Alcohol Fuel. Mathewson SW, editor. Chapter 12 Dry Alcohol. 2013;
18. Friedemann TE, Dubowski KM. Chemical testing procedures for the determination of ethyl alcohol. J Am Med Assoc. 1959 May 2;170(1):47–71.
19. Azeke CI, Eze UI, Kuroshi L. The potential of elephant grass (Pennisetum purpureum Schum. In: a Nigerian indigenous grass, in bioethanol production: A decarbonization alternative for the maritime industry. 2015.
20. Olanbiwoninu AA, Odunfa SA. Enhancing the Production of Reducing Sugars from Cassava Peels by Pretreatment Methods. Int J Sci Technol. 2012;2(9).
21. Bello S, Galan-Martin A, Feijoo G, Moreira MT. BECCS based on bioethanol from wood residues: Potential towards a carbon-negative transport and side-effects. Appl Energy. 2020;279:115884.