*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Scholars Research Library**

**Annals of Biological Research, 2014, 5 (1):106-111**
*(http://scholarsresearchlibrary.com/archive.html)*

Annals of Biological Research
Scholars Research Library
**ISSN 0976-1233**
**CODEN (USA): ABRNBW**

# Bioethanol production potential of Andropogon *gayanus*

**B. U. Bagudo, S. M. Dangoggo and J. Usman**

*Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto*

---

**ABSTRACT**

*Andropagon gayanus (Gamba grass) was assessed for Bio-ethanol production potentials. The grass was hydrolyzed using 5% and 10% sulphuric acid and fermentated using cultured bacteria (Zymomonas mobilis) and cultured yeast (Saccharomyces cerevisiae). It was found that the percentage yield of the ethanol in the distillate obtained from the process involving cultured bacteria (Zymomones mobilis) was 26.50% from 5% $H_2SO_4$ and 44.90 % from 10% $H_2SO_4$. For the process involving cultured yeast (Saccharomyces cerevisiae) the values were 35.80% from 5% $H_2SO_4$ and 35.30 from 10% $H_2SO_4$.*

**Key words:** Bioethanol, Renewable energy, hydrolysis, Distillation

---

## INTRODUCTION

The high demand for energy, uncertainty of petroleum resources and concern about climatic changes has led to the resurgence in the development of alternative liquid fuels [1]. The future fuel is presently focused on organic waste materials from both plants and animal sources Biodegradable output from industries, agricultural waste, forestry and household could also be potential sources of bio-energy, examples of these includes corn, straw, timbers, manure, sewages and dispersed seeds by animals even leftover. An inevitable depletion of the worldwide energy supply has prompted an increasing worldwide interest in these alternative sources of energy [16].

Bio-ethanol is one of the liquid fuels generated from carbonaceous material found to have an efficient calorific content capable of being used in automobiles [19]. In United States, it is the principal fuel used as a petrol substitute or as a blend for road transport vehicle.

This paper aims to determine the bioethanol production potentials of *Andropogon gayanus* (H: Gamba) a grass commonly found in almost all parts of northern Nigeria.

***Andropogon gayanus:*** Common names include gamba grass, bluestem (Africa, Australia); Rhodesian andropogon (southern Africa); Rhodesian blue grass (Zimbabwe); onga, tambuki grass (north-west Africa); sadabahar (India). It is a kind of tall annual or perennial, tussock grass, culms erect, up to 3m tall, more or less stout, about 0.6cm in diameter, glabrous, many-nodded, producing flowering branches from the third node upward; leaves glabrous or softly pubescent, rarely villous or tomentose; sheath tight, striate; ligule short, rounded or truncate, glabrous or somewhat hairy on back, rarely exceeding 0.2cm long; lamina linear to lanceolate-linear in the lower leaves, usually from a much attenuated base and there often forming a terete petiole, tapering to a fine point, over 30cm long, up to 1.6cm broad, glaucescent or reddish, margin scabrous [4]. The use of switch grass as substrate for bioethanol production started since in the early 1980s and now a more favored substrate for bioethanol production than corn which is also food crop. *Andropogon gayanus* has many similarities with switch grass i.e. being perennial, resistant to drought, low soil fertility requirement and availability in Nigeria this and many more reasons informed the used of this local grass in this piece of work.

**Bio-alcohols**

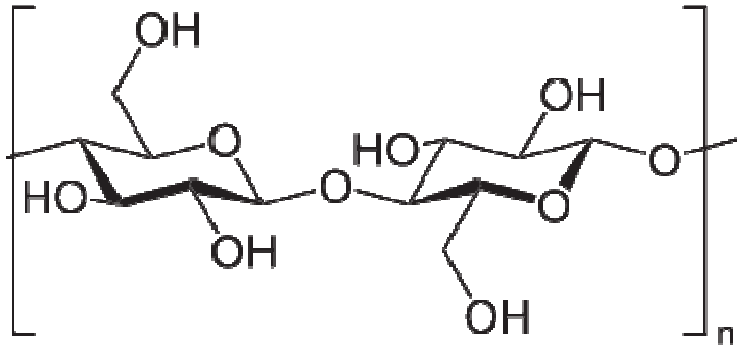
These are biologically produced alcohols. Common among these are ethanol and rare among these are propanol and butanol. Bio-butanol can be used directly in a gasoline engine and hence is considered as a direct replacement for gasoline. Butanol can be burnt straight in the existing gasoline engine without any alteration to the engine or carburetor. It is also claimed that this butanol produces more power and has less corrosive effect and is less soluble in water than ethanol [18].

Ethanol is the most commonly used bio–fuel in the world and particularly in Brazil.. Ethanol can be used in petrol engine as a substitute for gasoline. Also it can be blended with gasoline in many different proportions. The contemporary automobile petrol engines can work on mixture of gasoline and ethanol that has 15% bio–ethanol. These mixtures of gasoline and ethanol have higher octane number which indicates that engine would burn hotter and more efficiently.

In higher altitude spots a mixture of gasoline and ethanol is used as a winter oxidizer and thereby decreasing atmospheric pollution. Ethanol fuel has less British thermal unit content, energy content, thus, to drive the same distance, more fuel is required. Also ethanol has a corrosive effect on combustion chamber, aluminum robber hoses and gasket and fuel system [6].

**Methods of Bioethanol production**

Ethanol can be produced from biomass by hydrolysis and sugar fermentation process. Biomass contains a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemicellulose and lignin [19].




**Cellulose**

In order to produce sugar from the biomass, it is usually pre – treated with acid or enzymes to reduce the size of the feedstock and to open up the plants structure. The cellulose and the hemicellulose portions are broken down (hydrolyzed) by enzymes or dilute acids to sugar that is then fermented into ethanol [19]. The stages involves in conversion of complex carbohydrate polymer to ethanol includes;

**Pretreatment**

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as it's submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieve more rapidly and with great yields [11] & [17]. Pretreatment affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. Pretreatment has been considered as one of the most expensive processing step in biomass to fermentation sugar conversion with as high as 30 cent/gallon ethanol produced.[11].

**Hydrolysis**

After pretreatment there are two types of process to hydrolysis the feedstock into monomeric sugar constituent required for fermentation into ethanol.

The hydrolysis method most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolysis efficiency, the lignin – hemicelluloses network has to be loosened for the better amenability of cellulose to residual carbohydrate fraction for sugar recovery.

Dilute acid treatment is employed for degradation of hemicellulose leaving lignin and cellulose network in substrate. Other treatments are alkaline hydrolysis or microbial pretreatment, with white – rot fungi, (*Phaenerc – chate chrysosporium).*

The dilute acid process is conducted under higher temperature and pressure and reaction in the range of seconds or minutes while the concentrated acid hydrolysis process uses relatively mild temperatures and a minimum. Reaction times are typically much longer than for dilute acid process [11]

**Dilute Acid Hydrolysis**

In dilute acid hydrolysis, the hemicellulose is depolymerized at relatively low temperatures. Dilute sulfuric acid is mixed with the biomass to hydrolyze the hemicellulose to xylose and other sugars. Dilute acid reacted with biomass at a temperature of 120-220 $^0$C where the hemicellulose fraction of plant cell is depolymerized leading to the enhancement of cellulose digestibility in the residual solids [13], [16] and [17]. Dilute acid hydrolysis however has some limitations, if higher temperatures (or longer residence time) are applied, the hemicellulose derived monoccharide will degrade and give rise to fermentation inhibitors like furan compound, weak carboxylic acids and phenolic compound [8], [9] & [14]. These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms. In order to remove the inhibitors and increase the bioethanol production, some biological methods have been used. These methods include over liming (Martinez *et al;* 2000), charcoal detoxification (Lopez *et al 2004), detoxification of acid.*

The lignocellulose material is first treated with dilute sulphuric acid and heated to approximately 50$^0$C followed by the addition of much stronger acid solution and the temperature raised to 190$^0$C. At this stage approximately 80% of the hemicellulose and 29% of cellulose are hydrolyzed. The hydrolysis is further incubated at lower temperature for a residence time of two hours to convert most of the oligosaccharides into monosaccharides followed by the separation of solid and liquid fractions. The solid materials again washed with plenty water to enhance sugar recovery [19].

**Concentrated acid hydrolysis**

This method uses concentrated sulphuric acid followed by a dilution with water to dissolve and hydrolyses the substrate into sugars. This process provides complete and rapid conversion of cellulose to glucose and hermicellulose to xylose with a little degradation. The concentrated acid process used 70% sulphuric acid at a temperature of between 40 and 50$^0$ C for a period of between two to four hours in reactor. The low temperatures and pressure will minimize the sugar degradation. The hydrolyzed material is then washed to recover the sugar [19]. The solid resisure from first stage is de–watered and soaked in 30 – 40% sulfuric acid for 50 minutes at 100$^o$C for further cellulose hydrolysis. The primary advantage of the concentrated acid process is the potential for higher sugar recovery efficiency, about 90% of both hermicellulose and cellulose fractions get depolymerized into their monomeric units [18].

**Enzymatic Hydrolysis**

Instead of using acid to hydrolyze the biomass into sucrose, we can use enzymes to break down the biomass in a similar way.

[Condensation polymer of glucose starch + enzyme] $\longrightarrow$ $C_6O_6H_{12}$ Yeast $2C_2H_5OH + 2CO_2$
Glucose $\longrightarrow$ Ethanol

However this process is very expensive and is still in its early stages of development.[15].

**Fermentation Process**

The hydrolysis process breaks down the cellulose part of the biomass into sugar solution that can then be fermented into ethanol. Yeast is added to the solution which is then heated. The yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugar into glucose and fructose [5].

$C_{12}H_{22}O_{11}$ + $H_2O$ $\xrightarrow{\text{Invertase}}$ $C_6H_{12}O_6$ + $C_2H_{12}O_6$
Sucrose Water Catalyst Fructose Glucose

The fructose and glucose sugars then react with another enzyme called zymase, which is also contained in the yeast to produce ethanol and carbon dioxide.

$C_6H_{12}O_6$ $\xrightarrow{\text{Zymase}}$ $2C_2H_5OH$ + $2CO_2$
Fructose Catalyst Ethanol

**Fractional Distillation process:**

This is a process by which components in a chemical mixture are separated according to their different boiling points. Vapors from a boiling solution are passed along a column. The temperature of the column gradually

decreases along its length. Components with a higher boiling point condense on the column and return to the solution; components with a lower boiling point pass through the column and are collected.

The ethanol, which is produced from the fermentation process, still contains a significant quantity of water, which must be removed. This is achieved by the fractional distillation fermented mixture. Since ethanol has a lower boiling point of $78.3^0$C it will distill; first leaving water behind. Re distilling the distillate will give a product that is almost pure ethanol and which can directly be utilized as bio fuel. Ethanol ($C_2H_5OH$) is a biodegradable liquid with low toxicity and cause little environmental pollution, when split. It burns to produce carbon dioxide and water vapour.

$$C_2H_5OH \xrightarrow[\Delta]{} CO_2 + H_2O$$

Ethanol is high octane fuel and has been reported to replace tetra ethyl lead as octane enhancer in petrol. By blending ethanol with gasoline the fuel mixture can also oxygenated so that it burns more completely and produce less polluting emissions. Ethanol fuel blends are widely sold in the United States. The most common blends are 10% ethanol and 90% petrol (E10). Vehicle engines require no modifications to run on E10 and vehicle warranties are also unaffected. Only flexible fuel vehicles can run on up to 85% petrol blends (E85) [5].

## MATERIALS AND METHODS

**Samples:** The samples of fresh *Andropogon gayanus* were obtained from Dange and Tureta local Govt areas of Sokoto state and at the back of Sokoto Energy Research Center in the Main Campus of Usmanu Danfodiyo University Sokoto. The grass samples were first chopped into small pieces and then mashed mechanically using pestle and mortar. The mashed samples were sun dried for three days and then in a hot air oven. The oven dried samples were later grounded into powder using wooden mortar and pestle and then into a fine powder using an electric blender. The powdered material was stored at room temperature in a polythene bag prior to commencement of the analysis.

**Bio- ethanol Production:** Bio-ethanol production involves acids hydrolysis, fermentation and fractional distillation process. 5% and 10% dilute acid hydrolysis process was used in this conversion. Two different fermentation agents were used i.e. bacterial *(Zymomonas mobilis)* and culture yeast *(Saccharomyces cerevisiae)* in order to see which one gives high yield of bio – ethanol after distillation.

**Acid hydrolysis:**
Dilute acid hydrolysis was employed in this investigation and the following procedure was used. Fifty grams (50g) of dried powdered samples of *Andropogon gayanus* were taken into twelve different 500ml beakers, so that sample samples from Dange were contained in four Beakers labeled D1, D2, D3 and D4 the sample from Tureta were labeled T1, T2, T3 and T4, While the samples from the Back of Energy Research Center were labeled E1, E2, E3 and E4 respectively. From each group two of the powdered samples were treated with 400 ml of 5% sulphuric acid and the remaining two with 10% sulphuric acid. The beakers are then covered with cotton wool and aluminum foil gently heated to $50^o$C for 30mins. The beakers were allowed to cool and then filtered through Whatman filter paper and the pH adjusted to 4.0 with 10M NaOH solution so as to neutralize the acidity.

**Fermentation Process**
The fermentation was carried out with saccharification (simultaneous saccharification and fermentation [SSF]) as described by[8] and [14]. The beakers containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminum foil and then autoclaved for 30minutes at $121^o$C in order to arrest any microorganisms present in the filtrate before proceeding to fermentation process. They were afterward allowed to cool at room temperature then, 7.5ml of cultured yeast *(Saccharomyces cerevisiae)* was added into $1^{st}$ beaker containing 5% $H_2SO_4$ hydrolyzed sample and $2^{nd}$ containing 10% $H_2SO_4$ hydrolyzed sample respectively and stirred thoroughly. 7.5ml of cultured bacterial (*Zymomonas mobilis)* was added into $3^{rd}$ containing 5% $H_2SO_4$ and $4^{th}$ beakers containing 10% $H_2SO_4$ hydrolyzed samples respectively and stirred thoroughly. The 12 beakers were covered with cotton wool and wrapped in aluminum foil paper, then were stored for a week with constant string everyday.

**Distillation Process**
Each beaker containing the fermented liquor was dispensed into round – bottom flask, which was fitted to distillation column. A heating mantle with the temperature adjusted to $80^o$C was used to heat the round bottom flask containing the fermented liquor. A clean conical flask was fixed to the other end of the condenser for collection of the distillate as the bio – ethanol produced. The distillate which may still contain a significant amount of water was redistilled for the second time using the same procedure so as to obtain the pure ethanol.

**Quantification of Bio – ethanol Yield**

The volume of the ethanol yield was measured using a measuring cylinder, which was expressed in g/I by multiplying the volume of the distilled (bio – ethanol) by the density of ethanol (0.8033g/ml).

## RESULTS

The results of the various experiments conducted in this research work are presented in the Tables given below:

**Table 1: Percentage of Bioethanol yield (v/v) using *Saccharomyces cerevisaie* as fermenting agent**

| Samples | 5% $H_2SO_4$ | 10% $H_2SO_4$ |
|---|---|---|
| D | 35.00 | 38.50 |
| T | 37.50 | 37.80 |
| E | 37.00 | 38.20 |

**Table 2: percentage Bio ethanol yield (v/v) using *Zymomonas mobilis* as fermenting agent**

| Samples | 5% $H_2SO_4$ | 10% $H_2SO_4$ |
|---|---|---|
| D | 35.70 | 44.90 |
| T | 36.50 | 42.80 |
| E | 36.60 | 43.00 |
| | | |

**Table3: Qualitative test of all the distillates (Bio – ethanol**

| Test | Observation | Inference |
|---|---|---|
| Distilled + Acidified $KMnO_4$ | The purple coloration of $KMnO_4$, decolorized immediately | Presence of OH |
| Distillates + Sodium metal | A vigorous effervescence with evolution of colourless gas, which does not have any effect on litmus paper. | Presence of OH group |
| Distillates + Red litmus paper followed by blue litmus paper. | No effects on both litmus paper | Neutral to litmus paper |
| Distillates + $CH_3COOH$ + few drops of conc. $H_2SO_4$ | A pleasant fruity character was observed | Presence of OH |
| Distillate + $K_2Cr_2O_7$ solution ($5cm^3$; 0.1M) of conc. $H_2SO_4$ then warmed | The orange colour of $K_2Cr_2O_7$ changes to green immediately. | Presence of OH |

## DISCUSSION

The Results presented in Tables 1and 2 revealed that the percentage yield of bioethanol with cultured yeast (*Saccharomyces cerevisiae)* from 5% sulphuric acid hydrolyzed was 36.70% while that from 10% sulphuric with cultured yeast (*Saccharomyces cerevisiae)* yielded 38.50 % (v/v) for samples from Dange (D). For the samples from Tureta (T) and Energy Research center the yield from 5% sulphuric acid using cultured yeast (*Saccharomyces cerevisiae)* were 36.50 and 36.60 respectively While that from 10% dilute Sulphuric acid hydrolyzed with the same fermenting agent are 37.80 and 38.20 respectively. This result indicates that the strength of the hydrolyzing acid can influence the bioethanol yield due to the greater hydrolyzing power leading to high sugar recovery. Fermentation with cultured bacterial (*Zymomonas mobilis)* on the other hand yielded the followings; with 5 % sulphuric D = 35.70, T= 36.50 and E had 36.60 indicating a very similar result in the entire sample. But hydrolysis with 10% Sulphuric acid shows that D = 44.90, T =42.80 and E = 43.00. This result also indicated that hydrolysis with 10% sulphuric acid is more beneficial than using 5% sulphuric acid.

The results presented in Table 3 shows the result of qualitative analysis on the sample distillates. The distillates obtained was tested with few drops of acidified $KMnO_4$, the purple coloration of the $KMnO_4$ was tested decolorized immediately which inferences the presence of OH group. A piece of sodium metal was dropped into the distillates, a vigorous effervescence with evolution of colourless gas, which does not have any effect on litmus paper, was also observed indicating presence of OH groups. The sample where further tested with litmus paper (Both blue and Red), which have no any effects on both paper, indicating neutral to litmus paper.

A pleasant fruity scent character was observed when few drops of acetic acid ($CH_3OOH$) were added followed by few drops of concentrated $H_2SO_4$, which signified the presence of OH. Few drops of acidified $K_2Cr_2O_7$ were added to the distillates which changed the orange coloration of $K_2Cr_2O_7$ to greenish coloration, indicating the presence of OH groups. The samples generally yielded bioethanol at relatively high percentages i.e 35 – 44.90 this shows a great

potential for bioethanol production from *Androapogo gayanus* which is local perennial grass that can grow even during dry season throughout northern Nigeria.

## CONCLUSION

From the results presented in this research work, bio – ethanol can be produced from *Andropogon gayanus* (Gamba grass) by using cultured bacterial (*Zymomonas mobilis)* and cultured yeast (*Saccharomyces cerevisiae)*. There is great potential yield of bio – ethanol from *Andropagon gayanus* (Gamba grass) using cultured bacteria (*Zymomonas mobilis)*.

*Recommendation*
From what was observed it is recommended that, further research should be conducted on this using higher variables dilute acid and enzymatic hydrolysis in order to examine the structure of the produced ethanol, kinetics studies of the production process should be conducted. All these should be with a view to explore means of properly harnessing these agricultural products towards the production of ethanol for uses in energy sector as bio – fuel as against its production as an alcoholic drink.

The manufacturing sectors should established a joint venture with energy institution, Universities, Polytechnics and other private sector to come up with simpler and more ways of producing ethanol I n large marketable quantities to serve as substitute for other fuels, thereby creating room for job empowerment in the society.

## REFERENCES

[1] Anuj K.C. Ravinder R, Lakshmi M.N, Rao V, Ravindra P. (**2007**): *Bioethanol. Mole. Biol. Rev*. 2(1): 14 – 32.
[2] Berg, C. (2004): World Ethanol Production and Trade – A qualitative analyses, World Ethanol Conference, 5 – 7, November **2003**, London, U.K
[3] Burgaje, I.M and Mohammad, I.A (**2008**): "Handbook of Biofuels Production Technology" ISBN: 978 – 057 – 061 – 8, PP.56
[4] Bowden, B.N. (2003): Studies on Andropogon gayanus Kunth. I. The use of Andropogan gayanus in agriculture. Empire J. Exper. Agric. 31(123):267 – 273
[5] Biofuels, (**2011**) [online] [Accessed on 5th January, 2011].
Available from: http://www.Esru,strath.ac.uk/EandE/websites/02-03/biofuels/whatbioethanol.htm
[6] Clines, T. (**2006**). "Brew Better Ethanol" [online] [accessed on 29/12/2010].
Available from: http://www.pop.sci.com/popsci/energy/67562260360abolovgnvcm1000004eecbccdrcrdhtml.
[7] Fuelethanol, (**2010**) [online]Accessed 5th January, 2011].
Available from: http://www.comalc.com/fuelethanol.htm.whatisethanol
[8] Klinke, H.B, Thomson, A.B and Abung, B.K (**2004**): *Appl.Microbiol.Bioethanol*.66:10-26.
[9] Larson,S., Manchanda, M and Sigh, A. (**1999**): *Bioproc.Eng*.30:47-125.
[10] Lopez, M.J, Nichols, N.N. Dien, B.S, Moreno, J and Bothast R.J (**2004**): *Appl.Microb.Biotechnol* 64:125-131.
[11] Moiser, N, Wyman C, Dale B, Elander R, Lee YY, Hottzapple, M and Ladisch, M. (**2005**): *Biores. Technol.* 96:173-686.
[12] Martinez, A, Rodriguez, M.E, York S.W, Petron and J.E, Ingram L.O (**2001**): Detoxification of acid hydrolysates of lignocellulose with Time.Prog.17:287-293.
[13] Nigam, J.L, (**2001**)*: J.Biotechnol*.87:27.
[14] Olsson, L. and Hahn-Hagardal, B. (**1996**): *Enzyme Microbe. Technol*.18:312-331.
[15] Palmqvist, E and HahnHagerdal, B (**2000**): *Bioresour.Technol*.74:17-33
[16] Saha BC., Iten Cotta MA and Wu, YY (**2005**): *Pro. Biochm*,40:3693-3700
[17] Sun, Y and Cheng, J (**2002**): *Biores Technol* 83:1-11
[18] Wikipedia, (**2007**) [online] [Accessed on 29-12-2010@2:57pm]
Available from: http://www.un.org/news/Briefing/docs/2007/070302.Bio-fuelsdoc.htm.
[19] Wooley, R, Ruth, M, Sheehan, J. Ibesen, K, Majdeski, H and galvoez A (**1999**): Lingnocellulosic Biomass to Ethanol Process Design and economics Utilizing Co-current Dilute Acid Prehydrolysis and Enzymatic hydrolysis: current and Futuristic Scenarios Nrel/Tp-580-26157, 1999 National Renewable energy Laboratory, Goden, Co.
Wyman, C.E and Hinman, N.D (**1990**): Ethanol Fundamentals of Production from renewable feed stocks and Used as Transport Fuel. Appl. Biochem. Biotechnol.24/25:735-775.