



Research Article

Production, Characterization and Optimization of
Bioethanol from *Microalgae* Obtained from Waste Water in
Maiduguri Metropolitan Council, Borno State- Nigeria

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ABSTRACT

Bioethanol is a renewable, colorless, less toxic, and readily biodegradable form of fuel from biological sources; that can be used for heat, electricity, and fuel. In this research study, an alternative feedstock known as microalgae was used. Bioethanol was produced from microalgae using enzymatic hydrolysis. The microalgae was pretreated with acid and alkaline. Hydrolysis was carried out by *Aspergillus niger* and *Saccharomyces cerevisiae* was used for fermentation. The concentration of the Bioethanol produced was determined using acid Potassium Dichromate. After hydrolysis, the reducing sugar concentration was determined using Dinitrosalicylic acid (DNS) colorimetric method. Results showed that there was significant difference at $p < 0.05$ in the yields of the reducing sugar produced between acid and alkaline pretreated samples after microbial hydrolysis. Microbial hydrolysis of alkaline pretreated samples was higher in yield. Also, the results showed that the highest bioethanol yield of 0.142ml/l was obtained after alkaline pretreated hydrolysates were fermented with *Saccharomyces cerevisiae* at 40°C and pH of 6.0. The highest bioethanol yield of 0.116ml/l was obtained from the acid pretreated hydrolysates at 35°C and pH of 5.5. The volatile compounds produced by alkaline pre-treated samples using Gas Chromatography Mass Spectroscopy (GCMS) shows that Ethanol and Di-n-decylsulfone were the highest volatile compounds with peak areas of 30.70% and 41.87% respectively while 2-hydroxy-1-(hydroxymethyl) ethyl ester (linolenic acid) was found to be least volatile compound with peak area of 3.34%. The volatile compounds produced by acid pre-treated samples shows that 2-ethyl-2-methyl-tridecanol was the highest volatile compound with peak area of 23.00% while Heptacosane was found to be the least volatile compound with peak area of 1.90%. Therefore bioethanol production using alkaline pretreated samples produced a large quantity of bioethanol and may be a preferred method. This study concluded that Microalgae is a suitable substrate for bioethanol production and hydrolyzing with *Aspergillus niger* and optimization of fermentation conditions at pH 6.0 and 40°C yielded higher bioethanol concentration.

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INTRODUCTION

The demand for energy continuously increases with the increase in population but the sources of fossil fuel are exhausting day by day which causes an increment in petroleum fuel prices (Bibi *et al.*, 2017). The increased use of fossil fuels from petroleum resources have concerns related to environment and energy security issues. Issues, including global climate change due to the emission of greenhouse gases make researchers find alternative ways for the production of fuels from sustainable bioresources (Bezerra and Ragauskas, 2016). Biofuel is used as an alternative for fossil-based fuel due to increased concern about environmental protection. Biomass energy can play an important role in reducing greenhouse gas emissions. Bioethanol production process only uses energy from renewable sources. Hence no net carbon dioxide is added to the atmosphere, making bioethanol an environmentally beneficial energy source (Bull *et al.*, 1992).

Algae which are the third generation feedstock for biofuels are an alternative for the first and second generation feedstocks due to their productivity, easy cultivation and convenient harvesting time (John *et al.*, 2011). Algae can grow phototrophically or heterotrophically. Phototrophic algae convert carbon dioxide in atmosphere to nutrients such as carbohydrate. Conversely, heterotrophic algae continue their development by utilizing organic carbon sources (Wen and Chen, 2003). Algae are classified as microalgae and macroalgae. Microalgae as their name implies, are prokaryotic or eukaryotic photosynthetic microorganisms. They can survive in hard conditions with their unicellular or simple colony structures (Mata *et al.*, 2010). High oil prices, competing demands between foods as a feedstock for biofuel production, and the world food crisis, have ignited interest in algae culture for making biodiesel, bioethanol, and other biofuels, using land that is not suitable

for agriculture. Among algal fuels' appealing characteristics is that, they can be grown with minimal impact on freshwater resources and can produce up to 300 times more oil than conventional crops (Yang *et al.*, 2010). Microalgae have a short harvesting cycle of 1 to 10 days; their cultivation permits several harvests in a very short time-frame, a strategy that differs it from yearly crops (Christi, 2007). Since bioethanol production from conventional feedstock is known for emitting more greenhouse gases than fossil fuels in consequence of the production steps and applications during the process, algal bioethanol production can overcome these problems. In comparison with conventional feedstocks, algal production areas don't occupy agricultural lands and they do not need any fertilizer for cultivation. With these advantages, higher ethanol yields are obtained from microalgae. Therefore, the use of algae as a renewable energy source for biofuel production may ensure self-sufficiency and energy security.

MATERIALS AND METHODS

The samples used as raw materials for the study were obtained from Sanda Kyarimi park waste water in Maiduguri metropolis, Borno State. Maiduguri is located at longitude 11.50° North and latitude 13.15° East and elevation at about 364m above sea level in the North eastern part of the country, (Nigeria). Maiduguri temperature ranges between 30-47°C during the hot period (March to June) while the cold weather ranges between 19°C and 25°C. During the raining season, rainfall varies from 150-600mm with the same relative humidity.

Collection of samples

Fresh Microalgae was collected in a new clean sterilized plastic containers from waste water at Sanda Kyarimi Park in Maiduguri metropolis, Borno State where it was transported to the Department

of Microbiology, University of Maiduguri for analysis.

Preparation of the sample

The Microalgae sample was sun dried for seven (7) days for milling. The milled feedstock is called mash, this was done to increase the surface area and to make the cellulose extraction more effective.

Microorganisms used in the research

Aspergillus niger and Baker's Yeast (*Saccharomyces cerevisiae*) were used.

Identification of *Aspergillus niger*

Macro culture method was used to identify the organisms according to the method described by Gupta, (2006). Floor dust was collected using sterilized spatula and transferred to a sterilized sample container. The soil was serially diluted; a sample suspension was prepared by adding 1.0g of sample to 10ml of distilled water and mixed well for 10 minutes. The suspension was diluted serially 10^{-1} , 10^{-2} and 10^{-3} . From the third dilution factor, 1ml was measured using a syringe and inoculated into a Potato Dextrose Agar (PDA) plate and incubated at 37°C for five days. The initial white color of the colonies that later turns black at the top with pale yellow color at the bottom confirm the organism to be *Aspergillus niger*.

Reactivation of Baker's Yeast (*Saccharomyces cerevisiae*)

The method described by Alkasrawi (2006), was used to reactivate the baker's yeast. One (1) Gram of Baker's yeast was put in a flask containing a solution of warm distilled water, glucose broth media and yeast extract. The solution was then subjected to incubation for 24 hours. The reactivated yeast was then inoculated in to Potato Dextrose Agar (PDA) and subjected to incubation at 28°C for 5 days. After 5 days, the growth observed was used to form a pure culture. This was done to avoid inhibition of some important inocula such as proteins and fibers in the yeast.

Acid pretreatment of the Microalgae

Acid pretreatment was carried out using sulphuric acid. Exactly 10 g each of the ground microalgae was introduced into 3 conical flasks sized 250 ml containing 150ml of distilled water. The mixture was stirred thoroughly, covered with cotton wool and then wrapped with aluminum foil paper and subjected to autoclaving at 121°C for 15 minutes. After autoclaving, 1% H_2SO_4 was added to the mixture and boiled for 1 hour at 45°C in water bath. The samples were then removed and allowed to cool (Lamed and Zeikus, 1980).

Alkaline pretreatment of the Microalgae

The method of Lamed and Zeikus, (1980) was used for the alkaline pretreatment. In this method, 10 g each of the milled microalgae powder was dissolved in 150 ml of distilled water in 3 conical flasks of size 250mls. The mixture was shaken vigorously, covered with cotton wool and then wrapped with aluminum foil paper. The mixture was autoclaved at 121°C for 15 minutes. After autoclaving, 1ml of 2% prepared sodium hydroxide solution was added to the mixture and then subjected to water bath at 45°C for 1 hour. The samples were removed from the water bath and allowed to cool.

Microbial Hydrolysis of the Sample with *Aspergillus niger*

Microbial hydrolysis was carried out according to the method described by Gupta, (2006). In this method, the acidic and alkaline pre-treated samples were inoculated with 0.5 ml suspension of 96 hours culture of *Aspergillus niger*. Hydrolysis was carried out at room temperature for five days. Samples were taken daily for reducing sugar determination. The samples were then filtered using cheese cloth and the filtrates were used for fermentation.

Determination of Reducing Sugar

One (1) ml of each sample filtrate was taken into test tubes and 1ml of DNS reagent was added in the test tubes. A blank was prepared containing 1ml distilled water and 1ml DNS reagent. The

test tubes were boiled in a water bath for 10 minutes and allowed to cool. A colour developed fully after 20 minutes. One (1) ml of 40% sodium potassium tartrate was added to stabilize the colour and the absorbance was read at 540nm using ultraviolet (UV- VIS) spectrophotometer. A standard curve for glucose determination was prepared to calculate the percentage of the reducing sugar (Rabah *et al.* 2011).

pH Adjustment and sterilization of the hydrolyzed sample

Before addition of yeast to the above prepared samples, the pH of the samples were adjusted to 5.0, otherwise the yeast will be denatured in hyper acidic or basic state. A highly concentrated NaOH solution and concentrated HCl was prepared to adjust the pH and was regularly checked using a digital pH meter (Laopaiboon *et al.*, 2007). All the hydrolysed samples were sterilized by autoclaving at 121°C for 15 minutes prior to fermentation (Al-Hassan, 2008).

Fermentation of the Sample

The fermentation of the hydrolysate was carried out in triplicate in eighteen (18) different 250 ml conical flasks. The hydrolyzed samples were aseptically inoculated with 1ml suspension of the reactivated yeast. The flasks were then covered with cotton wool and wrapped with aluminum foil paper. The fermentation was carried out at room temperature for seven (7) days (Abu *et al.*, 2009).

Distillation of the Sample

Soxhlet apparatus was used for distillation of the fermented broth. All experiments were carried out at 78 °C (Wayne *et al.*, 2009). The fermented broth was dispensed into round-bottom conical flasks fixed to a distillation column enclosed under tap water. Another conical flask was attached to the other end of the distillation column to collect the distillate. Heating mantle with the temperature adjusted to 78°C was used

to heat the round buttoned flask containing the fermented broth.

Determination of the concentration of the Bio-ethanol Produced

The percentage of the bio-ethanol produced was carried out by quantitative analysis using acid potassium dichromate reagent according to the method of (Oyeleke and Jibrin, 2009). Exactly 1ml of standard ethanol was diluted with 99 ml of distilled water to give a concentration of 1 %. Each of 0,2,4,6 and 8ml of the 1% ethanol was diluted to 10ml of distilled water to produce 0, 0.2, 0.4, 0.6 and 0.8 concentrations. After that, 1ml each of the varying ethanol concentration was mixed up with 1 ml of the acid potassium dichromate and allowed to stand for an hour for colour development. The mixture was then developed fully to brown colour. The readings obtained were used to develop a standard curve of ethanol. Then, 1ml of each bioethanol samples was put into test tubes and treated with 2ml of the acid potassium dichromate. The mixture was allowed to stand for an hour and the absorbance was measured at 588nm using UV-VIS spectrophotometer and the reading was used to develop standard ethanol curve (Isah *et al.*, 2019).

Determination of the Volume (Quantity) of the Bio-ethanol Produced

The quantity of the bioethanol obtained was determined using the method of (Humphrey and Caritas, 2007). In this method, the distillate (Ethanol) collected over a slow heat at 78°C (for a period of 40min.), was measured using a measuring cylinder, and expressed as the quantity of ethanol produced in g/l by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033g/ml).

Density Measurements

The density of the bioethanol produced was carried out using density bottle. The bottle was placed on a level surface and filled with the bioethanol produced to ensure that the bioethanol level the density bottle. An empty

density bottle was weighed and the readings were taken. The empty density bottle was then filled with each bioethanol produced and weighed and the readings also taken. Another empty density bottle was filled with distilled water and weighed and readings also taken again. The specific density of the bioethanol was calculated according to the following formula given below (Amenaghawon *et al.*, 2014).

$$\text{Density} = (X_2 - X_1) / (X_3 - X_1)$$

Where: X_1 = weight (g) of empty density bottle

X_2 = weight (g) of empty density bottle + sample

X_3 = weight (g) of empty density bottle + water

Determination of Compounds Present in the Bioethanol Produced

Gas chromatography and mass spectroscopy (GS-MS) analysis was conducted in accordance with the procedure developed in the Department of Analytical Chemistry lab, Yobe State University Damaturu, Yobe State, Nigeria. Agilent Technologies 6890N Network GC system and agilent Technologies 5973 Network mass selective detector coupled with 7683B series injector was used. The carrier gas used was Helium at a flow rate 1.2 pml/ min. the injection volume was one Nanolitre (1nl). The inlet temperature was maintained at 230 °C. The oven temperature was programmed initially at 50°C for 5 min. it was programme again to increase to 300°C at a rate of 10 °C with 25 min and this temperature was held for 15 min. Total run time was 15 min. The Ms transfer line was maintained at a temperature of 250°C. The source temperature was also maintained at 230 °C and the MS Guaged at 150 °C. The ionization mode used is electron ionization mode at 70Ev. Total ion count (TIC) was used to evaluate for compounds identification and quantification. The spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NISTO2 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent software.

STATISTICAL ANALYSIS OF DATA

The data obtained was statistically analyzed using T-test with MINITAB software to test the concentration of the reducing sugar produced. The results are expressed as Mean \pm Standard deviation and $p \leq 0.05$ was considered as the level of significance.

RESULTS

Morphological Identification of *Aspergillus niger*

Aspergillus niger isolated from soil revealed that it has a black mycelium on the agar medium, it had septed hyphae, long and smooth conidiospores, and long unbranched sporangiospores with a large and round head. The initial white color of the colonies that later turned black at the top with pale yellow color at the bottom confirmed the organism to be *Aspergillus niger*.

Concentration of reducing sugar produced from acid and alkaline pretreatment overtime

Concentration of reducing sugar produced from acid and alkaline pre-treated samples for 120 hours revealed that the highest yield of reducing sugar of 0.519 g/l was obtained from Alkaline pretreatment after 72 hours of microbial hydrolysis, whereas the highest yield of 0.504 g/l was obtained from acidic pretreatment after 120 hours of hydrolysis. A low reducing sugar yield of 0.328 g/l was obtained from acidic pre-treatment after 24 hours of hydrolysis and a low reducing sugar yield of 0.331 g/l was obtained from alkaline pretreatment after 24 hours of hydrolysis (Table 2).

Effect of temperature and pH on the yield of Bioethanol produced from Acid and Alkaline pretreatment

The result revealed that fermentation of the alkaline pre-treated samples produced the highest bioethanol yield of 0.142ml/l at 40°C and pH of 6.0 after 120 hours and its lowest bioethanol yield of 0.109ml/l at 35°C and pH of 5.5. Microbial hydrolysis of the acidic pre-treated samples produced its bioethanol yield of

0.116ml/l at 35°C and pH 5.5 and its yield of of fermentation (Table 3)
0.074ml/l at 30°C and pH of 5.0 after 120 hours

Table 1: Morphological Characteristics of *Aspergillus niger* Isolated from the Soil

Isolate	Colony characterization	Cell shape	Organism
1	Black dotted surface as conidia	Filamentous with septed hyphae	<i>Aspergillus niger</i>

Table 2: Concentration of reducing sugar produced from acid and alkaline pretreatment overtime (Concentration of Reducing Sugar Produced (g/l))

Hour's	Acidic	Alkaline	p-Value
24	0.328 ± 0.016 ^c	0.331 ± 0.095 ^c	0.239
48	0.384 ± 0.108 ^{bc}	0.353 ± 0.019 ^c	0.507
72	0.398 ± 0.047 ^{bc}	0.519 ± 0.239 ^a	0.161
96	0.436 ± 0.058 ^b	0.471 ± 0.154 ^b	0.251
120	0.504 ± 0.088 ^a	0.432 ± 0.132 ^{bc}	0.450

The Values are Mean ± SD, T-Test of difference < 0.05, a, b, c means within a column with different superscripts, there is significant difference using t-test ($p < 0.05$)

Table 3: Effect of temperature and pH on the yield of bioethanol produced from acid and alkaline pretreatment (Concentration of Bioethanol Produced (ml/l))

Temp(°C)	Sample	pH	Bioethanol yield (ml/l)	Sample	pH	Bioethanol yield (ml/l)	p-value
30	Acid 1	5.0	0.074 ± 0.016 ^c	Alkaline 1	5.0	0.114 ± 0.017 ^b	0.035
35	Acid 2	5.5	0.116 ± 0.103 ^a	Alkaline 2	5.5	0.109 ± 0.004 ^c	0.010
40	Acid 3	6.0	0.098 ± 0.08 ^b	Alkaline 3	6.0	0.142 ± 0.048 ^a	0.054

The Values are Mean ± SD, T-Test of difference = 0.05, a, b, c means within a column with different superscripts, there is significant difference using t-test ($p < 0.05$)

Volume of the Bioethanol produced from Acid and Alkaline pre-treatment.

The results revealed that 40 ml/l was the highest volume produced by microbial hydrolysis of the

acid pretreated samples whereas microbial hydrolysis of the alkaline pre-treated samples produced 45 ml/l as the highest volume. However, the lowest volume of 30 ml/l was

produced from microbial hydrolysis of the alkaline pre-treated samples whereas microbial hydrolysis of the acid pre-treated samples produced 24 ml/l as its lowest volume (Table 4).

Densities of the Bioethanol produced through Acid and Alkaline pre-treatment.

The results revealed that 0.984g/cm³ was the highest density produced by the alkaline pre-treated samples whereas acid pre-treated samples produced 0.988g/cm³ as the highest density. The results revealed that samples with lowest densities have the highest percentage of bioethanol (Table 5).

Volatile compounds of the Produced Bioethanol by Alkaline and Acid Pretreatment

Volatile profile of the compounds produced by alkaline pretreatment showed that Ethanol and Di-n-decylsulfone were the highest volatile compounds with peak area of 30.70% and 41.87% respectively while 2-hydroxy-1-(hydroxymethyl) ethyl ester (linolenic acid) was found to be least volatile compound with peak area of 3.34%. (Table 6) while that of acid pretreatment shows that 2-ethyl-2-methyl-Tridecanol was the highest volatile compound with peak area of 23.00% while Heptacosane was found to be the least volatile compound with peak area of 1.90%. (Table 7)

Table 4: Volume (quantity) of the Bioethanol Produced from Acid and Alkaline Pre-treatment

Volume of Bioethanol produced (ml)			
pH	Acid	Alkaline	p-Value
5.0	38.00± 1.0 ^b	36.00 ± 2.0 ^b	0.078
5.5	40.00± 4.0 ^a	45.00 ± 1.0 ^a	0.231
6.0	33.00± 3.0 ^c	30.00 ± 3.0 ^c	0.021

The Values are Mean ± SD, T-Test of difference = 0.05, a, b, c means within a column with different superscripts, there is significant difference using t-test ($p > 0.05$)

Table 5: Densities of the Bioethanol Produced through Acid and Alkaline pretreatment with varying pH

Densities of Bioethanol Produced (g/cm ³)			
pH	Acid	Alkaline	p-Value
5.0	0.965± 2.0 ^c	0.980 ± 1.0 ^c	0.058
5.5	0.976± 3.0 ^b	0.982 ± 2.0 ^b	0.341
6.0	0.988± 2.0 ^a	0.984 ± 2.0 ^a	0.134

The Values are Mean ± SD, T-Test of difference = 0.05, a, b, c means within a column with different superscripts, there is significant difference using t-test ($p < 0.05$)

Table 6: Volatile Compounds of the Produced Bioethanol by Alkaline Pretreatment

S/N	Retention Time (min)	Peak Area (kwp)	Volatile Compounds	Formula	Peak Area (%)
1	9.479	163309.32	Ethanol	C ₂ H ₆ O	30.70
2	12.123	17784.54	2-hydroxy-1-ethyl ester	C ₂₁ H ₃₆ O ₄	3.34
3	14.732	222748.21	Di-n-decylsulfone	C ₂₀ H ₄₂ O ₂ S	41.87
4	17.564	60476.73	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	C ₁₄ H ₂₄ Si ₂	11.37
5	18.005	67620.64	1-decylsulfonyl-d-Mannitol	C ₁₆ H ₃₄ O ₇ S	12.71

Table 7: Volatile Compounds of the Produced Bioethanol by Acid Pretreatment

S/N	Retention Time (min)	Peak Area (kwp)	Volatile Compounds	Formula	Peak Area (%)
1	8.894	12018.31	1-Propanol	C ₃ H ₈ O	2.34
2	9.306	117982.78	2-ethyl-2-methyl-Tridecanol	C ₁₆ H ₃₄ O	23.00
3	10.044	47304.79	1-iodo-Octadecane	C ₁₈ H ₃₇ I	9.22
4	10.382	18683.29	Pentadecyl 2-propyl ester	C ₁₈ H ₃₈ O ₃ S	3.64
5	10.846	13910.22	2-ethyl-2-methyl-Tridecanol	C ₁₆ H ₃₄ O	2.71
6	11.601	19691.58	2-ethyl-2-methyl-Tridecanol	C ₁₆ H ₃₄ O	3.84
7	14.07	21508.8	2-ethyl-2-methyl-Tridecanol	C ₁₆ H ₃₄ O	4.19
8	17.031	42327.77	2-ethyl-2-methyl-Tridecanol	C ₁₆ H ₃₄ O	8.25
9	18.055	81680.95	2-methyl-Eicosane	C ₂₁ H ₄₄	15.92
10	18.198	59643.22	2,3,5,8-tetramethyl-Decane	C ₁₄ H ₃₀	11.63
11	18.37	21772.36	Heptacosane	C ₂₇ H ₅₆	4.25
12	18.524	17271.83	1-Bromoeicosane	C ₂₀ H ₄₁ Br	3.37
13	18.73	29254.1	1-Bromoeicosane	C ₂₀ H ₄₁ Br	5.70

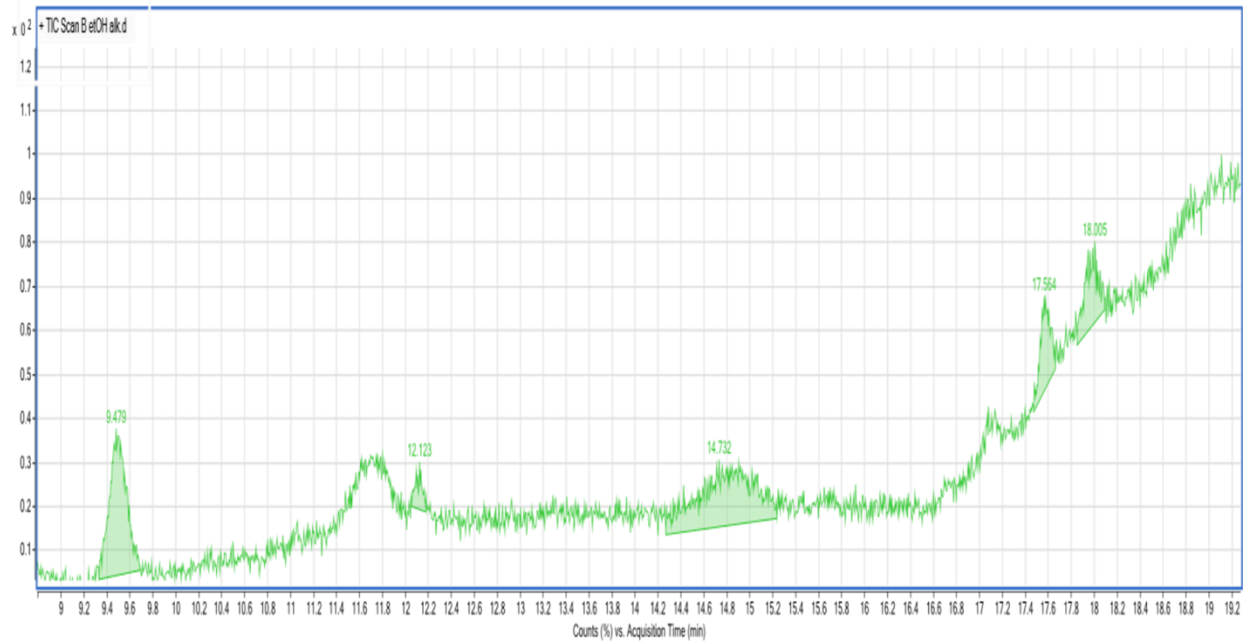


Figure 1: Chromatogram of the Bioethanol produced by Alkaline Pretreatment

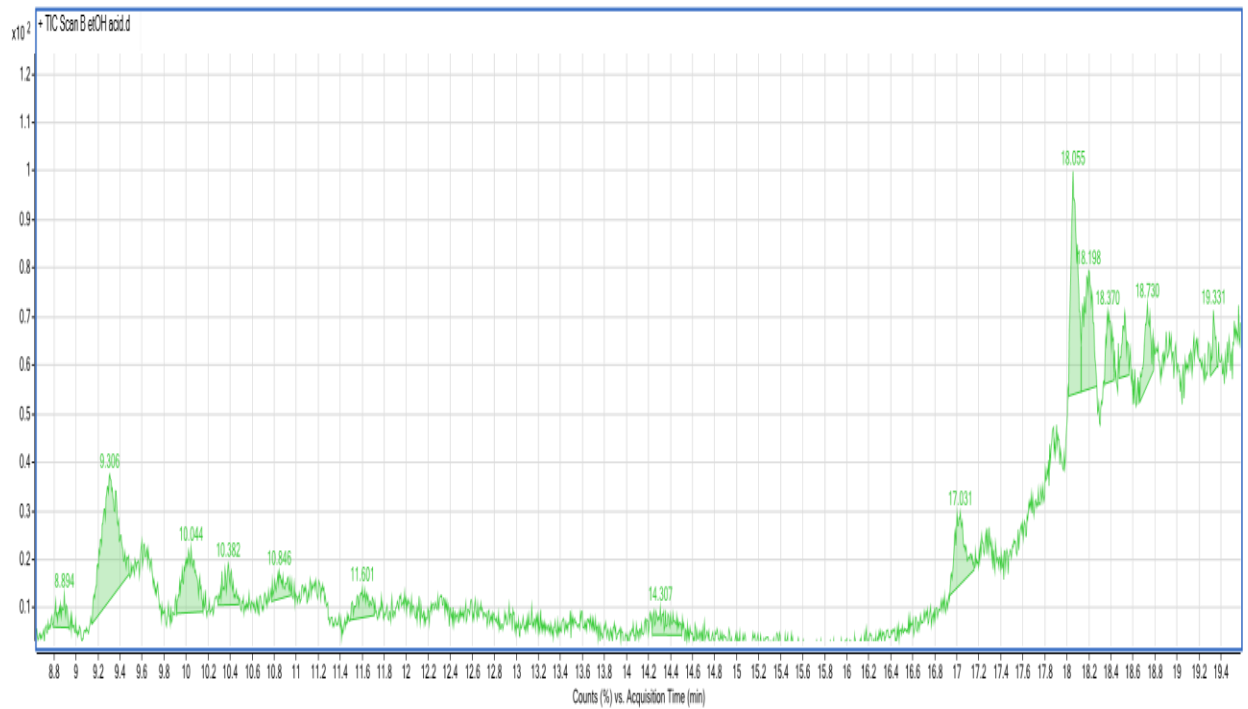


Figure 2: Chromatogram of the Bioethanol produced by Acid Pretreatment

DISCUSSION

The organisms (*Aspergillus nige* and *Saccharomyces cerevisiae*) used proved very promising in this study. The yeast was found

to be fermentative in the breakdown of hexose and pentose sugars for the production of ethanol and carbon dioxide. Previously, several workers such as Oyeleke and Jibrin

(2009), Mohd *et al.* (2011) and Schneider *et al.* (1981) have reported the activities of yeast in bioethanol production. The experimental set-ups in this study were allowed to ferment for 24-120 hours and then distillation was carried out under controlled temperature of 78°C.

Ethanol acts as a solvent for a wide range of industrial products, including paints, oils and dyes. Moreover, some are used as raw material in chemical synthesis and in the form of fuel (Morrison and Boyd, 1972; Goldemberg *et al.*, 1985). Ethanol ranks second after water as the most used solvent widely in chemical industry and as these industries have expanded, the demand for industrial ethanol has increased.

The total reducing sugar released, increased gradually with increase in time of incubation in all the flasks containing acid and alkaline treated biomass where more quantity of sugar was released on 3rd day but drops on the 4th and 5th day leading to more production of bioethanol by the alkaline pretreated biomass as shown in (Table 2) for the sugar released and (Table 3) for the bioethanol produced respectively.

However, the reducing sugar concentration of the microbial hydrolysis increased gradually from 24 hours to 120 hours. This means that hydrolysis for 120 hours is highly recommended for microbial hydrolysis as reported by Oyeleke *et al.* (2012). The sugar yield of the samples after microbial hydrolysis indicates that the highest value of 0.519g/l was recorded in alkaline pretreated biomass using 2% NaOH which was then followed by the value of 0.504g/l recorded in the acid pretreated biomass using 1% H₂SO₄. This is due to the acid medium which attacks the polysaccharides especially the cellulose.

This study agrees with the report of Subashini *et al.* (2011), in which NaOH was a better hydrolyzing agent than the H₂SO₄ when sago wastes were used as substrates. It can be observed that NaOH was a good hydrolyzing

agent as compared to H₂SO₄ (as can be seen in Table 4.2) as H₂SO₄ recorded low yield as seen in the NaOH treatment of the sample biomass; a reason that, this obstructs the degree of polymerization of the cellulose and its association with other protective polymeric structures such as pectin, hemicelluloses etc. (Taherzadeh and Karimi, 2007).

The effect of temperature and pH on bioethanol production by the *Saccharomyces cerevisiae* from acid and alkaline pretreated samples is presented in (Table 4.3). Slavikova and Vadketrova (2003), Reported that yeast generally, will grow over an optimum temperature range of 30 to 38°C. This range of temperature corroborate with the range at which the highest amounts of ethanol were produced in this study. This clearly showed that each parameter (pH or temperature) has a modulating effect on the other. It would, therefore, be necessary to adjust the pH and temperature of the fermentation medium appropriately in order to maximize ethanol production by the organism. This corroborates with the findings reported by Ogbonda *et al.* (2007) who determined the influence of temperature and pH on biomass production and protein biosynthesis in putative *Spirulina* species.

Highest bioethanol yield of 0.142ml/l at 40°C and pH of 6.0 was observed in the microalgae biomass treated with 2% NaOH followed by the microalgae biomass treated with 1% H₂SO₄ which recorded the yield of 0.116ml/l at 35°C and pH of 5.5. The least yield was therefore, at the acid treated biomass at p<0.05 significant level. This was due to a problem encountered when treating the sample with acid as it forms the furan derivatives and other non-identified toxic products which is particularly true in the case of xylans, that easily leads to furfural production and these products inhibits the fermentation of the substrates using *Saccharomyces cerevisiae* (Cardona *et al.*, 2010). Hence, the hydrolysates are used as

fermentation media as the concentration of inhibitors is assumed to be low.

Neutralization of the substrate before inoculation of the microorganism brings changes and detoxifies the alcohol producing inhibitors such as the pentoses, aliphatic acids, furan derivatives etc. that are formed during pretreatment of the lignocelluloses (Cardona *et al.*, 2010).

These results revealed a higher production by microbial hydrolysis of the alkaline pretreated samples which may be because *Aspergillus niger* possess pyruvate decarboxylase as reported by Oyeleke *et al.* (2009) which tends to facilitate the breaking down of the microalgae. All these might be responsible for high bioethanol produced from the hydrolysates. Harun *et al.* (2009) reported that fermentation of microalgae at pH 5.0 after microbial hydrolysis produced the highest yield.

The volatile profile (GCMS analysis) of the bioethanol produced revealed that Ethanol and Di-n-decylsulfone were the highest compounds found in alkaline pretreated sample, with peak area of 30.70% and 41.87% respectively, which corroborate with the findings reported by Juliarnita *et al.* (2018) who obtained peak area of 58.00% and 37.25% for ethanol and linolenic acid respectively from mixed culture of microalgae biomass.

Finally, in all the experiments conducted and the results obtained for both the acid and alkaline pretreated biomass, it was observed and proved that conversion efficiency of cellulosic materials

into sugars was dependent on the suitability of cellulose for saccharification and the activity of enzymes and enhancement in total sugars. Bioethanol production is dependent on the availability of sugars and the activity of enzymes produced by the *Aspergillus niger*. Hence, it is noticed that saccharification and fermentation are moving hand in hand with each other. Therefore, increased production of ethanol was observed when sugars are more in the suspension and the activity of enzymes decreased gradually when sugars were decreased gradually (Fuad *et al.*, 2010) as presented and justified in the results of this study.

CONCLUSION

From this study, it was found that pretreatment of Microalgae by acid and alkaline pretreatment methods enable the accessibility and ease of hydrolysis of the Microalgae. This study also showed that microbial hydrolysis after alkaline pretreatment yielded more reducing sugars compared to acid pretreatment. Also, Bioethanol produced by alkaline pretreatment yielded highest percentage concentration than Bioethanol produced by acid pretreatment which yielded highest percentage concentration of 0.116ml/l. This study concluded that Microalgae may be a suitable substrate for bioethanol production and hydrolyzing with *Aspergillus niger* and optimization of fermentation conditions at pH 6.0 and 40°C yielded higher bioethanol concentration.

Declarations

We hereby declare that we carried out the work presented in this research at the Centre for Biotechnology, University of Maiduguri, Borno State. We solemnly declare that this is entirely a product of our own research and that to the best of our knowledge it has not been written and presented elsewhere. All references made were duly acknowledged.

Conflict of Interest: (None)

There is no conflict of interest from the authors.

Contributions

The authors contributed equally based on what the group assigned for each other.

Conflict of interest

The author(s) declares no conflict of interest.

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