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Determination of Quantity of Bioethanol Produced from Microalgae (*Zygnema*)

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ABSTRACT

This research was aimed to determine the quantity of bioethanol produced from microalgal biomass (Zygnema) prior to proximate analysis and reducing sugar concentration of Zygnema (Maradun, 2023). The quantity of bioethanol was determined using UV-Visible Spectrophotometer, and also the percentage of ethanol was determined using the procedure adopted from Sirajo et al (2019). From the result obtained, bioethanol Produced for 3,6 and 9 of Retention time was higher at Day 9 Mean±SD (0.94±0.15g/dm³) with pH of 7.5 and 30°C and least at Day 3 (0.54±0.056g/dm³) with pH of 6.5 and 20°C as shown in table 1. Meanwhile, the quantity and viscosity determined were all found to be within the recommended range of ASTM standard. It was observed from this study that the bioethanol produced from microalgal biomass will not affect the engine cars since the density and viscosity were all found to be within the recommended range.

Keywords: Quantity, Viscosity, Density, *Zygnema,* Bioethanol.

Introduction

Rapid growth of the world population and improved developments over the past decade have increased the demand for energy which is mainly derived from fossil fuels. This has led to severe environmental impacts such as the release of greenhouse gases (GHG) emission to the atmosphere, which is one of the major contributors to global warming and ocean acidification (Chew *et al.,* 2018). To over-come these issues, an alternative source of energy is required to replace the depleting fossil fuels.

Bioethanol is one of the potential alternative biofuels that can reduce the dependence on fossil fuels in the near future. It can be classified into first-generation bioethanol derived from agricultural or food crops; second-generation bioethanol that are mainly produced from lignocellulosic (non-edible) materials and third-generation bioethanol which are derived from microalgae (Chew *et al.,* 2018; Phwan *et al.,* 2018). Bioethanol production from microalgae has many advantages over the first and second-generation biofuels due to its fast growth rate, ability to grow on wastelands for cultivation and does not pose food security issues (Chew *et al.,* 2018; Chia *et al.,* 2018). Moreover, microalgae cells are devoid of lignin content which makes them disrupt easily compared to lignocellulosic materials, in addition to the cheaper operation cost than second-generation biofuels (Chew, *et al.,* 2018).

Materials and Method

Determination of percentage ethanol concentration

Five centimeter cube (5 cm^3) of each sample was measured into different test tubes and then 2 cm^3 of the prepared potassium dichromate solution was added to each and shaken thoroughly, and allowed to stay for 20 minutes. The solution labeled in each test tube was poured into labeled cuticle in the UV/Visible spectrophotometer and analyzed to determine the percentage of ethanol concentration, which was extrapolated from the standard ethanol curve (Sirajo *et al*., 2019).

3.10 Reactivation of Baker's Yeast (*saccharomyces Cerevisae*)

Thebaker's yeast (*Saccharomyces. cerevisiae)* used in this study was purchased from Sokoto central market, Sokoto State prior to fermentation, the yeast was reactivated in the mycology laboratory of Usmanu Danfodiyo University, Sokoto (UDUS). The activation was done using warm water (36^0C) in accordance with method reported by Rabah *et al.,* 2011. The yeast dextrose agar was prepared according to the method described by the manufacturer and incubated at room temperature for 24 hours. Thereafter, a single colony of the yeast was inoculated into the yeast dextrose agar broth and incubated at room temperature for 24 hours.

Fermentations

For the fermentation, the hydrolysate was adjusted to a pH of 6.5, 7.0 and 7.5 which was done in triplicate using 2M NaOH and was supplemented with additional nutrients (per L): $MgSO_4.7H_2O$, 5 grams of *S.cerevisiae* extract, 5g $KH_2PO_4$and 2g $(NH_4)_2SO_4$. This was used as bioethanol production medium, a working volume of 100ml was transferred in to a 250ml flask and was sterilized in an autoclave at 121^0C, 15psi for 30 min. *Saccharomyces cerevisiae* was loaded into the sterilized medium (10%v/v). Subsequently, the fermentation process was commenced, the operating conditions of the temperature was maintained at 20^0C, 25^0C and 30^0C in triplicate and retention time of 3, 6 and 9 retention time also in triplicate, in which the broth was collected at 6 hours intervals. In order to fully analyze the varying effect of the parameters, all the parameters were presented in triplicates and all the means and standard deviations were determined at the end of the experiment. The experimental set up as outlined by Ojewumu *et al. (*2018) was used for the study

Fractional Distillation

The fermented broth was transferred into a round-bottom flask fixed to a distillation column with a running tap water through the column. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78.3 °C was used to heat the round-bottomed flask containing the fermented broth for each group. The distillate collected was measured using measuring cylinder (Oyeleke and Jibril 2009).

Determination of Density of Bioethanol Produced

The procedure described by Emtron (2015) was employed for density determination. The volume of the bioethanol distillate from each fermentative organism with reference to the original mass of the sample utilized was employed to determine the density of the produced bioethanol produced according to the expression using equation below

$$= \quad \underline{\qquad}$$

Determination of quantity of bioethanol produced

The distillates collected was measured using a measuring cylinder, and expressed as the quantity of bioethanol produced in g/l by multiplying the volume of the distillates collected by the density of bioethanol (0.8033g/ml). It was noted that g/l is equivalent to the yield of 100g of dried substrate (Humphrey *et al.,* 2007). -

Determination of viscosity of Bioethanol Produced

The viscometer was charged with the sample for 30 minutes to adapt with the test temperature. The sample was placed in the capillarity tube of the viscometer and the level of the test sample was adjusted with the aid of suction pump to the mark position on the capillary arm of the instrument about 5 mm ahead of the first timing mark. With the sample flowing freely, the time required for the meniscus to pass from the first to the second timing mark was measured in seconds. The process was repeated three times and average value was taken. Kinematic viscosity was calculated from measured flow time and instrument constant C (ASTM D445, 1984) using equation below

$$\text{Kinematic viscosity} = C \times t$$

Results and Discussion

Table 1: Bioethanol concentration at day 3, 6 and 9 Retention time using algae

Retention time (Days)	pH	Temp (°C)	Bioethanol concentration(g/dmt)
3	6.5	20	0.11±0.21
6	7	25	0.078±0.13
9	7.5	30	0.048±0.14

Results are expressed as Mean ± Standard deviation

The bioethanol concentration day 6 had the highest bioethanol concentration of 0.078±0.13 at 25^OC, followed by day 9 with the concentration of 0.048±0.14 at 30^OC pH of 7.5 and day 3 have the least bioethanol concentration of 0.11±0.21 at 20^OC pH of 6.5 respectively. The variation of bioethanol concentration could be as a result of most yeasts can convert a range of hexose sugars to bioethanol via glycolysis. However, *Saccharomyces cerevisiae* is by far the most used yeast organism for alcoholic fermentation due to its robustness and tolerances. *S. cerevisiae* has several advantages over other yeasts as it is a facultative anaerobe capable of growing under both aerobic and anaerobic conditions in the presence of glucose (Krantz *et al.,* 2004) and is tolerant of elevated bioethanol concentrations (Claassen *et al.,* 1999). Under anaerobic conditions, *S. cerevisiae* will produce acetaldehyde, which is further reduced to bioethanol (Walker and Basso 2020). During inoculation and fermentation, yeast cells are subjected to several stressors that can affect bioethanol yields, including biological (e.g., cellular ageing, microbial competition), chemical (e.g., toxicity from bioethanol and its metabolites, pH), and physical stressors (e.g., temperature shock, osmotic pressure) (Walker and Basso2020). Stress can result in increased mutations, microbial contamination, altered yeast flocculation, increased glycerol production, decreased bioethanol production, and

production of undesired compounds (e.g., flavor and aromatic compounds in fermented beverages) (Cray *et al.,* 2015). Poor activity and declines in yeast viability from stress can also cause stuck or sluggish fermentations. Fortunately, several methods have been developed to reduce these stresses including increasing fermentation temperature and pitching rate (Deparis *et al.,* 2017), nutritional supplementation (Pham *et al.,* 2010; Dragone *et al.,* 2003), using mutant yeast strains (Blieck *et al.,* 2007), immobilizing yeast (Norton *et al.,* 1995), and enhancing aeration efficiency (Debourg, 2010). Fermentation success is also influenced by various additional factors, including nutrition imbalances (e.g., nitrogen, vitamins, mineral deficiencies), medium composition (e.g., sugar concentration), and inoculum size. Biotic stress factors (e.g., microbial contamination) can also affect fermentation efficiency. These factors primarily involve the presence of contaminating microorganisms, such as lactic acid bacteria (LAB) (Erten *et al.,* 2007).

Table 2: Quantity of Bioethanol Produced for 3,6 and 9 of Retention time using algae

Retention time (Days)	pH	Temp (˚C)	Quantity (g/ml)
3	6.5	20	0.54±0.056
6	7.0	25	0.85±0.078
9	7.5	30	0.94±0.15

Results are expressed as Mean ± Standard deviation

The quantity of bioethanol produced was found to be within the range of at 0.94±0.15 at day 9, 30^{0}C pH of 7.5, followed by day 6 with 0.849±0.078 at 25°C pH of 7 and day 3 with the least bioethanol concentration of 0.537±0.056 at 20^{O}C with pH of 6.5. The result is also in line with the finding of Kemka *et al*. (2013) where ethanol of 10.50% was achieved at temperature above 30°C. The result is similarly compatible with the findings of Asmanaw *et al.* (2021) with highest ethanol quantity of 7.97g/L beyond 72 hrs. Ajay ha the same fin dings, having maximum bioethanol of 25% in 3, 4, 5 days respectively at 30°C. From the compared literature and the result obtained, the quantity of bioethanol was seemed to be achieved higher when the pH os 7 or 7.5, and retention time above 6 days.

Table 3: Density of Bioethanol Produced for 3, 6 and 9 of Retention time using algae

Retention time (Days)	pH	Temp (^{0}C)	Density (g/cmt)
3	6.5	20	0.54±0.58
6	7.0	25	0.85±0.08
9	7.5	30	0.94±0.15

Results are expressed as Mean ± Standard deviation

The results on density of Bioethanol Produced for 3,6 and 9 of Retention time is higher at Day 9Mean±SD **(**0.94±0.15g/dm³) with pH of 7.5 and 30°C while at Day 3 (0.54±0.056g/dm³) with pH of 6.5 and 20°C as shown in table3.

The density of bioethanol produced from table 3, the level of density was found to be within the range of 0.54±0.58-0.94±0.15 for 3, 6 and 9 days respectively. Since the standard range of bioethanol is around 0.789g/cm3. Density is the ratio fuel mass to the volume of fuel at a temperature of 150^{0}C. The found range above the standard shows the likely possibility of engine to be knocked at high temperature and pressure. This is in line with the findings of Anonymous, 2006 and Raj 2019 respectively.

Table 4: Viscosity of Bioethanol at 3, 6 and 9 Retention time using algae

Retention time (Days)	pH	Temp (^{0}C)	Viscosity
3	6.5	20	1.89±0.35
6	7	25	2.23±0.65
9	7.5	30	1.83±0.22

Results are expressed as Mean ± Standard deviation

The results on Viscosity of Bioethanol Produced for 3,6 and 9 of Retention timeis higher at Day 6Mean±SD **(**2.23±0.65g/dm³) with pH of 7.0 and 25°C while at Day 9 (1.83±0.22g/dm³) with pH of 7.5 and 30°C as shown in table 4.

The viscosity of bioethanol produced Table 4 illustrated the level of viscosity found within 3, 6 and 9 days of retention time after the production of bioethanol. The highest viscosity of 2.23±0.64 was achieved at 25^{0}C pH of 7, followed by day 3 having 1.89±0.35 at 20^{0}C, and day 9 with the least viscosity of 1.83±0.22Cst at 30^{0}C. The standard of viscosity in bioethanol is 1.525 cst, while the table shows that the lowest viscosity of green algae was found to be 1.5. Fluid viscosity measure material resistance to a steam. Viscosity depends on temperature; it decreases as the temperature increases. Viscosity is essential property in the storage and use of fuel. If the viscosity is too thick, it will make the engine difficult in pumping, igniting the burner and flowing. In addition, the high level viscosity in the fuel worsens the atomization, triggering the formation carbon deposition on the cylinder wall of the engine (IOP Conference, 2019).

Conclusion

The result on Bioethanol concentration at day 3, 6 and 9 of Retention time was higher at Day 3 Mean±SD **(**0.11±0.21g/dm³) with pH of 6.5 and 20°C The results on Quantity of Bioethanol Produced for 3,6 and 9 of Retention time is higher at Day 9Mean±SD **(**0.94±0.15g/dm³) with pH of 7.5 and 30°CThe results on Density of Bioethanol Produced for 3,6 and 9 of Retention time is higher at Day 9 Mean±SD **(**0.94±0.15g/dm³) with pH of 7.5 and 30°C. The results on Viscosity of Bioethanol Produced for 3,6 and 9 of Retention time is higher at Day 6Mean±SD **(**2.23±0.65g/dm³) with pH of 7.0 and 25°C. This showed that the microalgal biomass could be the best substitute feedstock over second generation and first generation for the production of bioethanol.

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