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Production of Bioethanol Using Neem Tree Leaves

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The study was aimed to investigate the potential of Neem tree leaves (*Azadirachta indica*) for bioethanol production by using *Aspergillus niger* and *Saccharomyces cerevisiae as* fermenting organisms. Neem leaves were hydrolyzed using *Aspergillus niger* to determine reducing sugar content using UV spectrophotometer. The hydrolyzed samples were then fermented using *Saccharomyces Cerevisiae*. After the fermentation, the broths were distilled to obtain bioethanol. The result shows that neem leaves yield a high amount of reducing sugar content, density, viscosity, the quantitative and qualitative concentration at different temperatures (30, 35 and 40^{o}C). This study revealed that neem leaves possessed a significant amount reducing sugar which can be used for the production of bioethanol.

Keywords: *Azadirachta indica*, Bioethanol, UV Spectrophotometer, Density

1. Introduction

The microbial fermentation process results in the production of bioethanol which is a volatile and flammable liquid, which has a molecular formula of C_2H_5OH (Chandel *et al*., 2012). Lignocelluloses is a complex carbohydrate polymer of cellulose, hemicelluloses and lignin. Ethanol production from lignocellulosic materials has received extensive interest due to the availability, abundance and relatively low cost (Demirbas, 2008). The international energy Agency stated that cellulosic ethanol could allow ethanol fuels to play a much more significant role in the future than previously thought (IEA, 2006). The bioethanol is an oxygenated fuel consuming 35% oxygen which reduces particulates and nitrogen oxides (NOx) emissions from combustion. Also, is biodegradable and contributes to sustainability (Balat and Balat, 2009). However, this research is intended to come up with an alternative solution of converting wastes to wealth through the conversion process to bioethanol.

The Neem tree leaves were reported to have contained 60% of H_2O, 23% of carbohydrates,7% proteins and more than 3% minerals and 1% fat (Heinrich *et al*., 2005). It grows widely in Northern Nigerian region, its fruits and seeds are the sources of Neem oil. Neem tree is an incredible plant that has been declared the Tree of the 21st Century by the United Nations, 2012. Hence, the contribution of plants such as neem leaves that is not non-edible plant source raw material for bioethanol production. The study aims to pre-treat the substrate for bioethanol production using acidic pretreatment. To hydrolyze the pretreated substrate using *Aspergillus niger* and to carry out the fermentation process using *Saccharomyces cerevisiae* for the production of bioethanol.

2. Materials and Methods

2.1 Collection of Sample and Preparation

Neem tree leaves was collected at Achida area, Sokoto State in a sterilized polythene bags which were taken to the laboratory for analysis at Sokoto State University Sokoto, Microbiology Department.

2.2 Sample Pretreatment

The neem leaves were air-dried for three days to remove all the moisture present in it. The dried sample was ground using a pestle and a mortar.

2.3 Acidic Pretreatment

Thirty grams (30 g) of the powdered sample were poured into 500 ml conical flask, 300 ml of 1% H_2SO_4 was poured into the sample and were plug with cotton wool and covered with aluminum foil, it was allowed to stand for 24 hours, the suspension was filtered and raised with running tap water. The filtrate was poured

back into the conical flask. 300 ml of distilled water was added; it was covered, plug with cotton wool and covered with aluminum foil. The suspension was autoclaved at 121^{o}C for 15 minutes (Gabhane *et al*., 2014; Allison *et al*., 2016).

2.4 Spore Suspension

Ten (10 ml) of sterilized distilled water disposed into the plate of *Aspergillus niger* and it was then allowed to diffuse.

2.5 Enzymatic Hydrolysis

Two (2 ml) of the spore suspensions were added to the sample and incubated at 30, 35 and 40^{o}C, respectively for three days (Humprey and Caritas, 2007).

2.6 Determination of Reducing Sugar Content

The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations. Then, the Hydrolysates filtrate samples were treated with 3, 5-dinitrosalicylic acid (DNS) reagent (Miller, 1959) and the mixture was boiled in a water bath for 10 minutes to develop red-brown colour, and 1cm^{3} of 40% Potassium sodium titrate solution was added to stabilize the colour. The absorbance of the portion of the mixture from each test tube was measured using UV-visible spectrophotometer at 540nm (Akpinar, 2009).

2.7 Fermentation of the Hydrolysate

The hydrolysates samples were filtered using Whatman's No 1 filter paper. The flasks containing the filtrate were covered with cotton wool wrapped in aluminum foil, then autoclaved at 121^{o}C for 15 minutes. The sterile hydrolysates were allowed to cool, and 2ml of *Saccharomyces cerevisiae* suspension was added to the sample and incubated at 30, 35 and 40^{o}C for 5 days (Oyeleke *et al*., 2012).

2.8 Distillation

The fermented broths were filtered using filter paper. Each sample weighed into Kjedhal flasks and heated at 78^{o}C (boiling point of ethanol) on the microkjedhal apparatus until the solution turns colourless (Oyeleke *et al*., 2012).

2.9 Determination of the Quantity of Bioethanol Produced

The distillate collected was measured using a measuring cylinder and expressed as the quantity of ethanol produced in g/ml by multiplying the volume of distillate by the density of ethanol 0.0725 g/ml (Akpinar, 2009).

2.10 Determination of Concentration of Bioethanol Produced

This was carried out using UV-VIS quantitative analysis of alcohols using chromium VI reagent according to the methods described by (Patel *et al.,* 2007). A quantity (1 ml) of standard ethanol was diluted with 100 ml of distilled water to give a concentration of 1 %. Then, each of 0, 2, 4, 6 and 8 ml of the 1 % ethanol was diluted to 10 ml with distilled water to produced 0, 0.2, 0.4, 0.6 and 0.8 % of the ethanol. To each of the varying ethanol concentrations 2 ml of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 588 nm using UV-VIS spectrophotometer (UV-1650pc, Shimadzu) and the readings used to developed standard ethanol curve. Then 5 ml of each bioethanol samples were put in test tubes and treated with 2 mls of the chromium reagent. The mixture was allowed to standard for an hour and the absorbance measured at 588 nm using the UV-VIS spectrophotometer (UV-1650pc, Shimadzu).

2.11 Determination of Viscosity of Bioethanol

A cleaned, dried viscometer with a flow time above 200 seconds for the fluid to be tested was selected. The sample was filtered through a sintered glass to eliminate dust and other solid materials in the liquid sample. The viscometer was charged with the sample by inverting the tube's thinner arm into the liquid sample, and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 40^{o}C and allowed approximately 10 minutes for the thinner arm to draw the samples slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flows freely from the upper timing mark to the lower timing mark was recorded (Akpinar, 2009).

2.12 Determination of the Density of Bioethanol

To determine the density of the sample, an empty graduated cylinder was placed on a scale, and then it was tarred. The tare button on the scale was pressed to cancel out the mass of the graduated cylinder so that only the mass of its content will be displayed. A sample of ethanol was poured into the graduated cylinder, and the volume was recorded. The reading was taken at eye level with emphasis at the lower meniscus. The graduated cylinder with the ethanol was placed on the tarred scale, and the mass was read and recorded. The group obtained from the scale were divided by the volume read from the graduated cylinder. This was recorded as the density of ethanol (Akpinar, 2009).

3. Results and Discussion

3.1. Results

The figure shows the mean of the reducing sugar produced from neem tree leaves at a range of temperatures from 30, 35 and 40°C which had the reducing sugar value of 10.71, 63.49 and 87.37 mg/ml respectively. Figure 2 shows the mean concentration of bioethanol produced from Neem tree leaves from neem tree leaves at a temperature range from 30, 35 and 40°C with a profound concentration value of 75.30, 69.37 and 64.19 mg/l, respectively. The mean quantity of bioethanol produced from Neem tree leaves at different temperature presented in Figure 3. The result measured at a temperature of 30, 35 and 40°C, was 153, 175 and 175m/s respectively. As depicted in Figure 4 the mean viscosity which was measured at a temperature of 30, 35 and 40°C, the value of the viscosity produced are 4.7, 6.6 and 5.00g/ml respectively. The density was measured at a range of temperature from 30,35 and 40°C, and the mean value of the density was found to be 310.3g, 318.0g and 323.7g, respectively (Figure 5).

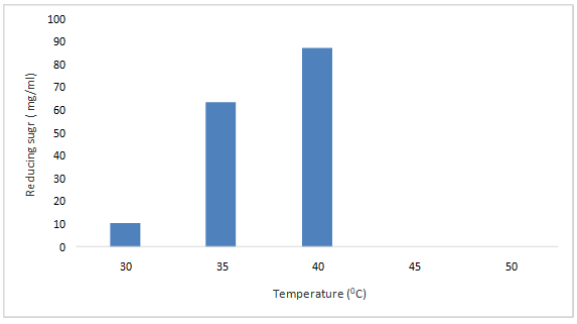

Figure 1. Mean Reducing Sugar Concentration Produced from Neem tree leaves at 30, 35 & 40°C.

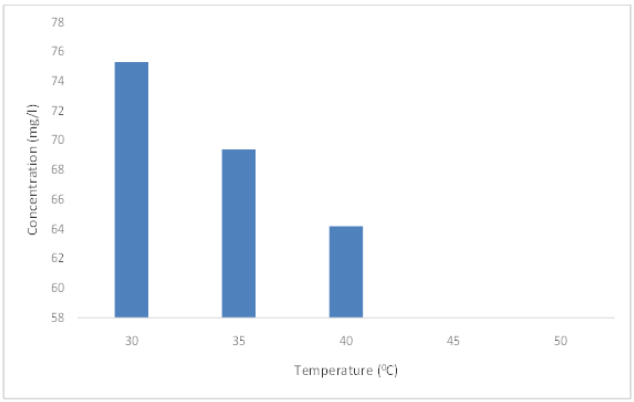

Figure 2. Mean Concentration of bioethanol produced from Neem tree leaves at 30, 35 & 40°C.

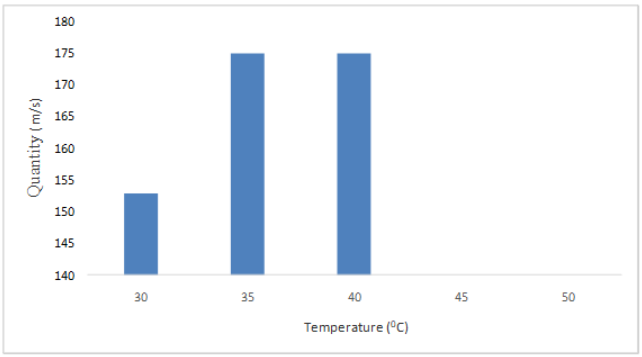

Figure 3. Mean Quantity of bioethanol produced from neem tree leaves at 30, 35 & 40°C.

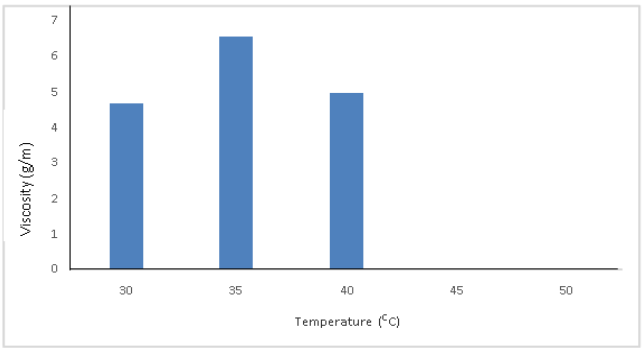

Figure 4. Mean Viscosity of Bioethanol Produced from Neem tree leaves at 30, 35 & 40°C.

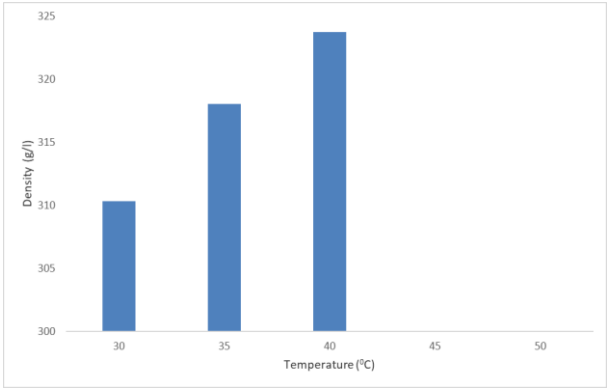

Figure 5. Mean density of Bioethanol Produced from Neem tree leaves at 30, 35 & 40°C.

3.2. Discussion

The results of the present study revealed that the neem tree leaves had a high sugar content, which is an essential source for bioethanol production. High sugar content (87.37 mg/ml) was recorded at a temperature of 40^{o}C followed by 63.49 mg/ml, which was recorded at 35^{o}C. Lastly, the lowest sugar content (10.71 mg/ml) was recorded at a temperature 30^{o}C as showed in figure 1. When the levels of temperature increase hydrolysis resulted in a higher yield of reducing sugar content because the cellulosic and hemicellulosic substances in the substrates had been broken down into more straightforward sugar which the fermenting organisms can utilize. The result of this study is in agreement with the report of Sabba *et al*. (2018) who study the production of parameters of bioethanol from fruit pulp (*Azadirachta indica*). The mean concentration produced was also recorded at a range of temperature from 30-40^{o}C, the highest quantity produced (75.30 mg/l) was observed at 30^{o}C followed by the concentration value of 69.4 mg/l at 35^{o}C, and lowest concentration amount was 64.2mg/l at a temperature of 40^{o}C of which was presented in Figure 2. The concentration of the quality and the quantity recorded appear to increase with the decrease in temperature. This is supported by the work of Shabib (2018) who studied the comparative study on bioethanol production neem leaves using *Saccharomyces cerevisiae* and *Bacillus* spp.

Viscosity increases at the beginning of temperature 4.7 g/ml at 30^{o}C, while the optimum temperature was recorded 6.6 g/ml at 35^{o}C and viscosities decreases at high temperature 5.0 g/ml at 40^{o}C which was presented in Figure 4. The range of values recorded may be due to the afflux time when timing the flow of the sample with their respective temperature, which is in line with the report of previous work on the production of parameters of bioethanol from the fruit pulp of *Azadirachta indica* (Sabba *et al*., 2018). The densities increase from 310.3g, 318g, 323g at temperature 35,35 & 40 ^{o}C respectively as presented in Figure 5. A similar finding was also reported by Itelima *et al*. (2013) that gradual increase in cell densities from day one to seven day of the fermentation periods suggested that substantially more carbon was utilized for ethanol production instead of cell production and this due to the ability of yeast *S. cerevisiae* to ferment the sugar to ethanol.

4. Conclusion

In conclusion, the study has revealed that neem tree leaves show high amount concentration of reducing sugar, density, viscosity, quantity and the quality of the concentration. Hence neem leaves can be used for the production of bioethanol. Based on recommendations; Lignocelluloses biomass such as neem leaves should be utilized in the production of bio-ethanol, biofuels and reduce environmental pollution. Further research is needed for proper pretreatment and optimizing the conditions for maximum production of bioethanol from neem leaves.

Conflict of interest

The authors declare no conflict of interest.

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