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Effects of Dilute Hydrochloric Acid Pretreatment Oneucalyptus Camaldulensis Sawdust for Bioethanol Production

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ABSTRACT

The problem of global warming has been undeniably accepted worldwide. One of the causes of this situation is the increase in the greenhouse gases. This study was carried out to produce bioethanol from sawdust of Eucalyptus camaldulensis. Sawdust of Eucalyptus camaldulensiswas pretreated with 3% HCl at 30oC, 40oC and 50oC for 20, 25 and 30 minutes prior to fermentation with Saccharomyces cerevisiaeand Saccharomyces carlbergensis. Result shows that the highest reducing sugar yield of 43.9% was obtained from Eucalyptus camaldulensissawdust pretreated at 40oC for 20 minutes while the lowest yield of 34.7% was obtained at 30oC for 30 minutes. Highest bioethanol concentration of 1.10% was obtained at 25 minutes when the hydrolysate was fermented by Saccharomyces cerevisiaeand Saccharomyces carlbergensisin synergy at 25 minutes. Saccharomyces cerevisiaeproduced the lowest bioethanol concentration of 0.63% at 30 minutes in 7 days of fermentation. The study concludes that synergy between Saccharomyces cerevisiaeand Saccharomyces carlbergensismay be a better combination for bioethanol production from sawdust of Eucalyptus camaldulensispretreated with 3% HCl at 40oC.

Keywords: *HCl, Bioethanol, Fermentation, Eucalyptus camaldulensis, Reducing sugar.*

INTRODUCTION

Worldwide high demand for energy, uncertainty of petroleum resources and concern about global climatic changes has led to the resurgence in the development of alternative liquid fuels. Ethanol has always been considered a better choice as it reduces the dependence on crude oil and promises cleaner combustion leading to a healthier environment. Developing ethanol as fuel beyond its current role of fuel oxygenate, would require lignocellulosics as a feedstock because of its renewable nature, abundance and low cost (Saha,2015). The chemical properties of lignocelluloses components make them of enormous biotechnological values for the production of affordable fuel ethanol. Also, it is less expensive than starch and sugar crops and is also renewable and available in large quantities (Aiyejagbara, 2015). Cellulose is the major polymeric component of plant material and is the most abundant polysaccharide on earth (Liming and Xueling, 2004).

Bioethanol is an alcohol produced by fermenting the sugar components of renewable plant biomass (Keith, 2009).Bioethanol is considered as an important renewable fuel to partly replace fossil-derived fuels. The world production of bioethanol increased from 50 million m3in 2007 to over 100 million m3in 2012 (Kang et al., 2014). Brazil and the United States represent approximately 80% of the world supply, mostly using corn or sugarcane. In developing economies, food-related feedstock is preferably replaced by nonfood raw materials, such as sweet sorghum or cassava. The use of common biomass could significantly increase the bioethanol production (Kang et al., 2014). Industrial ethanol is mainly produced petro chemically through the acid-catalyzed hydration of ethylene. Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as biofuel, is produced by fermentation where certain species of yeast (e.g., Saccharomyces cerevisiae) or bacteria (e.g., Zymomonasmobilis) metabolize sugars in oxygen-lean conditions to produce ethanol and carbon dioxide (Qian, 2014). The main reasons for the enhanced development of bioethanol are its use as a favorable and near carbon neutral renewable fuel, thus reducing CO2emissions and associated climate change; its use as octane

enhancer in unleaded gasoline; and its use as oxygenated fuel-mix for a cleaner combustion of gasoline. Hence reducing tailpipe pollutant emissions and improving the ambient air quality (Qian, 2014).

Owing to depleting reserves and competing industrial needs of petrochemical feedstocks, there is global emphasis in ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Ali et al., 2014). Therefore the present study was undertaken to utilize lignocellulosic biomass (sawdust of Eucalyptus camaldulensis) for bioethanol production.

MATERIALS AND METHODS

Sample Collection and Processing

One kilogram (1kg) of Eucalyptus camaldulensis was collected in clean polyethane bag from AfakaForest Reserve, Kaduna State and transported immediately to the Microbiology laboratory, Department of Biological Sciences, N.D.A Kaduna.The sample was washed several times to remove adhering dirt and oven dried at 150oC for 6 hours, then pulverized to powder using mortar and pestle (Galbe and Zacchi, 2007). The sample was then placed in capped wide mouthed plastic containers, labeled and stored at room temperature in the laboratory. Personal protective wears such as hand gloves and safety boots were used.

Pretreatment of Biomass

This was carried out according to the method described by Humphrey and Caritas (2007) as follows: One hundred grammes (100 g) of sawdust from Eucalyptus camaldulensiswas weighed into 2 litre capacity conical flasks. Then 1 litre of 3% dilute hydrochloric acid (3% HCl) was added into each of the conical flasks. The flasks were covered with cotton wool, wrapped in aluminium foil, heated in a water bath for 20, 25 and 30 minutes at 30oC, 40oC and 50oC and then autoclaved for 15 minutes at 121 oC. The flasks were allowed to cool, filtered through No1 Whatman filter paper and the pH was adjusted to 4.5 with 0.4M NaOH.

Determination of Reducing Sugar

The reducing sugar content following hydrolysis of the biomass was determined using the dinitrosalicylic acid (DNS) colorimetric method of Miller (1959). The reducing sugar content of the hydrolysates was assayed by adding 3ml 0f 3, 5 - dinitrosalicylic acid (DNS) to 3 ml of each hydrolysate sample. The mixture was heated inhot water bath for 10 minutes until red-brown color was observed. To the mixture, 1 ml of 40 % potassium sodium tartrate solution was then added to stabilize the color and the mixture cooled to room temperature under running tap. Absorbance of each sample was measured at 491 nm using UV-VIS spectrophotometer. The reducing sugar content wassubsequently determined by reference to a standard curve of known glucose concentration.

Inoculation of Yeast and Bioconversion of the Fermentable Sugars To Ethanol

This was carried out according to the method describe by (Brooks 2008).Two grammes of peptone water was added to the previously detoxified hydrolysate and the pH of the solution was adjusted to 5.6 by adding 10% sulphuric acid (H2SO4). The solution was autoclaved at 121oC for 15 minutes to sterilize the medium and 1.2g of both Saccharomyces cerevisiae and Saccharomyces carlsbergensiswere inoculated into the fermentation medium in ratio 1:1 in a sterilized environment. The fermentation set up was incubated for 3 weeks at 30oC. The fermented medium was aliquoted after 7 days, 14 days and 21 days interval and were distilled to assay ethanol content.

Determination of the Concentration of Bioethanol in the Distillate

This was carried out using UV-VIS quantitative analysis of alcohols using chromium (vi) reagent according to the methods described by Oyeleke and Jibrin (2009 : 1 ml of standard ethanol was diluted with 99 ml of distilled water to give a concentration of 1 %. Then each of 0, 2, 4, 6 and 8 mls of the 1% ethanol were diluted to 10 ml with distilled water to produce 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 and the readings were used to develop a standard ethanol curve. Then 4 ml of each bioethanol samples were put in test tubes and treated with 2 ml of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance

measured at 588 nm using the UV-VIS spectrophotometer.

STATISTICAL ANALYSIS

Data obtained were statistically analyzed using Duncan Multiple Range Test (Duncan, 1955).

RESULT

Reducing sugar

The result of reducing sugar yield obtained from the hydrolysates after hydrolysis with3% HClat 30oC, 40oC and 50oC for 20, 25 and 30 minutes are presented in Figure 3.1. In 3% HCl, the highest yield of 43.9% was obtained from Eucalyptus camaldulensisat 40oC for 20 minutes while the lowest yield of 34.7 % was obtained from Eucalyptus camaldulensissawdust at 30oC for 20 minutes (Fig. 3.1).

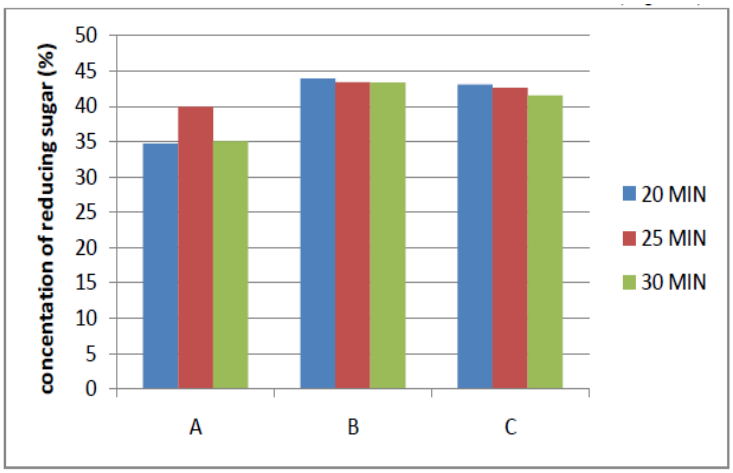

Figure 3.1. *Reducing sugar yield of hydrolysates after hydrolysis with 3 % HCl at 30 oC, 40 oC and 50 oC for 20, 25 and 30 minutes.*

Key: A: Sawdust of Eucalyptus camaldulensisat 30oC
B: Sawdust of Eucalyptus camaldulensisat 40oC
C: Sawdust of Eucalyptus camaldulensisat 50oC

Table 3.1. *Concentration of bioethanol (%) from sawdust of Eucalyptus camaldulensis pretreated with 3% HCl at 30^0C for 20,25 and 30 minutes using single and combined fermenting organism.*

		Fermentation days		
Fermenting organisms	pretreatment	7	14	21
S. cerevisiae	20	0.56^{a} ±0.01	0.78^{d}± 0.01	0.99^{g}± 0.01
S. carlsbergensis	20	0.95^{e}±0.01	0.97^{f} ± 0.02	1.21^{i} ±0.02
S. cerevisiae + S. carlsbergensis	20	0.98^{f} ± 0.01	0.80^{e}± 0.01	0.61^{b}± 0.02
S. cerevisiae	25	0.63^{b} ±0.03	0.67^{b} ±0.02	0.82^{d} ±0.02
S. carlsbergensis	25	1.05^{g}±0.01	1.10^{g}± 0.01	1.13^{h}±0.01
S. cerevisiae + S. carlsbergensis	25	1.12^{h} ± 0.01	0.97^{f}±0.01	0.94^{f}±0.01
S. cerevisiae	30	0.64^{b} ±0.01	0.69^{b} ±0.01	0.75^{c} ±0.01
S. carlsbergensis	30	0.68^{c}±0.02	0.73^{c}± 0.01	0.87^{e}±0.02
S. cerevisiae + S. carlsbergensis	30	0.81^{d} ± 0.01	0.56^{a}±0.01	0.51^{a}±0.02

Note: abcd means within a column with different superscripts are significantly different (p< 0.05). Values are means ± standard deviation of three replicates.

Concentration of Bioethanol (%) From Sawdust Of Eucalyptus Camaldulensispretreated With 3% Hcl At 300C For 20, 25 And 30 Minutes Using Either S. Cerevisiae,S. Carlsbergensis, Or Combinations Therefrom .

Table 3.1 shows the concentration of bioethanol over a three weeks period from sawdust of Eucalyptus camaldulensispretreated with 3 % HCl at 30oC for 20, 25 and 30 minutes using S. cerevisiaeand S. carlsbergensis. Highest concentration (1.21%) of bioethanol was achieved at 20 minutes when the hydrolysate was fermented by S. carlsbergensis at 21 days than other fermenting organisms. S. cerevisiae and S. carlsbergensis produced the lowest concentration of 0.51% at 30 minutes in 21 days of fermentation.

Concentration of Bioethanol (%) From Sawdust Of Eucalyptus

Camaldulensispretreated With 3% Hcl At 400C For 20, 25 And 30 Minutes Using Either S. Cerevisiae,S. Carlsbergensis, Or Combinations Therefrom .

Table 3.2 shows the concentration of bioethanol over a three weeks period from sawdust ofEucalyptus camaldulensispretreated with 3 % HCl at 40oC for 20,25 and 30 minutes using S. cerevisiaeand S. carlsbergensis.Highest concentration (1.10%) of bioethanol was achieved at 25 minutes when the hydrolysate was fermented by S. cerevisiaeand S. carlsbergensis at 7 days than other fermenting organisms. S. cerevisiaeproduced the lowest concentration of 0.63 % at 30 minutes in 7 days of fermentation.

Table 3.2. *Concentration of bioethanol (%) from sawdust of Eucalyptus camaldulensis pretreated with 3% HCl at 40^0C for 20, 25 and 30 minutes using single and combined fermenting organism.*

		Fermentation days		
Fermenting organisms	pretreatment	7	14	21
S. cerevisiae	20	0.67^{a}±0.01	0.71^{b}± 0.02	0.93^{d}± 0.01
S. carlsbergensis	20	0.69^{c}±0.00	0.88^{d} ± 0.01	0.97^{e} ±0.01
S. cerevisiae + S. carlsbergensis	20	1.05^{f} ± 0.01	0.94^{f}± 0.01	0.88^{c}± 0.02
S. cerevisiae	25	0.77^{d} ±0.02	0.89^{d} 0.01	1.09^{f} ±0.01
S. carlsbergensis	25	0.76^{d}±0.01	0.82^{c}± 0.02	0.91^{d}±0.02
S. cerevisiae + S. carlsbergensis	25	1.10^{g} ± 0.01	0.91^{e}±0.02	0.84^{b}±0.01
S. cerevisiae	30	0.63^{a} ±0.01	0.69^{a} ±0.00	0.91^{d} ±0.02
S. carlsbergensis	30	0.64^{a}±0.01	0.92^{ef}± 0.02	0.95^{e}±0.01
S. cerevisiae + S. carlsbergensis	30	1.01^{e} ± 0.01	0.98^{g}±0.01	0.71^{a}±0.02

Note: abcd means within a column with different superscripts are significantly different (p< 0.05). Values are means ± standard deviation of three replicates.

Concentration of Bioethanol (%) From Sawdust Of Eucalyptus Camaldulensispretreated With 3% Hcl At 500C For 20, 25 And 30 Minutes Using Either S. Cerevisiae,S. Carlsbergensis, Or Combinations Therefrom .

Table 3.3 shows the concentration of bioethanol over a three weeks period from sawdust of Eucalyptus camaldulensispre treated with 3 % HCl at 50oC for 20,25 and 30 minutes using S. cerevisiae and S. carlsbergensis. Highest concentration (1.01%) of bioethanol was achieved at 20 minutes when the hydrolysate was fermented by S. cerevisiae and S. carlsbergensis at 7 days than other fermenting organisms. S. cerevisiae produced the lowest concentration of 0.55 % at 30 minutes in 7 days of fermentation.

Table3.3. *Concentration of bioethanol (%) from sawdust of Eucalyptus camaldulensispretreated with 3% HCl at 50^0C for 20, 25 and 30 minutes using single and combined fermenting organism.*

		Fermentation days		
Fermenting organisms	pretreatment	7	14	21
S. cerevisiae	20	0.58^{ab} ±0.02	0.69^{b}± 0.01	0.90^{d}±0.01
S. carlsbergensis	20	0.61^{b}±0.02	0.84^{e} ± 0.01	0.95^{f} ±0.02
S. cerevisiae + S. carlsbergensis	20	1.01^{e} ± 0.00	0.90^{f}± 0.01	0.84^{b}±0.01
S. cerevisiae	25	0.70^{c} ±0.01	0.81^{d} ±0.02	0.93^{ef}±0.02
S. carlsbergensis	25	0.69^{c}±0.03	0.77^{c}± 0.02	0.89^{cd}±0.02
S. cerevisiae + S. carlsbergensis	25	0.98^{de} ±0.01	0.95^{g}±0.01	0.80^{a}±0.01
S. cerevisiae	30	0.55^{a} ±0.01	0.66^{a} ±0.02	0.87^{c} ±0.01
S. carlsbergensis	30	0.61^{b}±0.08	0.89^{f} ± 0.01	0.91^{de}±0.02
S. cerevisiae + S. carlsbergensis	30	0.94^{d} ± 0.02	0.91^{f}±0.02	0.83^{b}±0.02

Note: abcd means within a column with different superscripts are significantly different (p< 0.05). Values are means ± standard deviation of three replicates.

DISCUSSION

With increased population growth there is a corresponding demand for energy resources especially for non-renewable forms. This over dependence has result in the depletion of the resource base and gross degradation of the environment. This has led to the search for alternative and renewable energy sources (Agarwal, 2005). In the present investigation, we explored the production of bio-ethanol from saw dust of Eucalyptus camaldulensis waste. The results on the hydrolysis of the sawdust

from Eucalyptus camaldulensis pretreated with 3% HCl at 30oC, 40oC and 50oC for 20, 25 and 30 minutes revealed high yield of reducing sugars which is similar to the findings of Yahaya et al 2017. This results revealed a high presence of reducing sugar in Eucalyptus camaldulensis sawdust when treatment time were considered in all the reducing sugar yield, on the average the highest yield of 43.9 % was obtained at 40oC for 20 minutes treatment period. To ensure successful biological conversion of lignocellulosic materials, the interaction between lignin and the polysaccharide components of the cell wall must be reduced through pretreatment, a process that is considered to be one of the most important steps in the process (Wyman et al., 2005). Dilute acid treatment is one of the most effective pretreatment methods for lignocellulosic biomass. The treatment offers good performance in terms of recovering hemicellulose sugars (Kootstraet al., 2009).

Highest bioethanol concentration of 1.10% was obtained at 25 minutes when the hydrolysate was fermented by Saccharomyces cerevisiaeand Saccharomyces carlbergensisin synergy at 25 minutes. Saccharomyces cerevisiaeproduced the lowest bioethanol concentration of 0.63% at 30 minutes in 7 days of fermentation whichare higher than that of millet husk (0.11%) and bark of E. tereticornis(0.22%) as reperted by Rabahet al. 2011 and Usmanet al.2015 respectively. An integral part of the fermentation process is the choice of organism. The ability to ferment pentoses along with hexoses is not wide spread among organisms. Saccharomyces cerevisiaeis the most favored organism for bioethanol production from hexoses due to their high bioethanol tolerance, being able to out-compete other yeasts and greater resistance to contamination and inhibitors generated from biomass (Jeffries, 2006).

CONCLUSION

The purpose of this study was to determine the ethanol yielding capacity of sawdust of Eucalyptus camaldulensis. The results show that dilute acid pretreatment could serve as a pretreatment option in freeing reducing sugar content of lignocellulosic biomass such as sawdust of Eucalyptus. Camaldulensis which is in abundance, does not compete with food materials and the plants are renewable. Bioconversion offers a cheap and safe method of not only disposing the agricultural and forestry residues, but also it has the potential to convert lignocellulosic waste into usable forms such as reducing sugars that could be used for ethanol production. Hence the conversion of lignocellulosic "wastes" into biofuel such as ethanol will help reduce environmental pollution, contribute toward the mitigation of greenhouse gases emissions and serve as a sustainable solid waste management strategy.

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