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Ethanol production from okra (*Hibiscus esculentus*) stalk using acid and enzymatic hydrolysis.

Omoniyi, T. E[1] and Oloruntola, S. K[2].

[1]Wood Engineering Department, University of Ibadan, Ibadan – Nigeria

[2]Agricultural and Environmental Engineering Department, University of Ibadan, Ibadan – Nigeria

*temidayoomoniyi@gmail.com, Stephen.oloruntola@gmail.com

08037881122, 07031988315

ABSTRACT

Bioethanol was produced from okra *(Hibiscus esculentus)* stalk, a waste agricultural residue using both acid and enzymatic hydrolysis. Acid hydrolysis was carried out on okra stalk using microwave as a medium of heat. The microwave power, heating time and acid concentration were 300Watts, 13.2min and 2M, respectively. After heating the pH was adjusted to 4.5 and yeast (Saccharomyces cerevisiae) was added to induce fermentation at room temperature. Enzymatic hydrolysis was carried out using the acid hydrolysis as a pre-treatment method, the mixture was detoxified using calcium chloride. Alpha amylase and gluco-amylase enzymes were added for liquefaction and saccharification at optimum condition (6.5, 60ºC), (5.0, 55ºC) pH and temperature respectively. Yeast (Saccharomyces cerevisiae) was introduced at pH 4.5 at room temperature. The resultant mixture of the acid and enzymatic hydrolysis processes was filtered and distilled at 78ºC and the properties of the ethanol determined. The ethanol yields from acid and enzymatic hydrolysis were 8.5ml and 12.9ml, respectively. The flash points (20.5ºC, 22.5ºC), pour points (5.77, 5.12), cloud points (20.26ºC, 21.76ºC), specific gravities at 29ºC, were (0.892, 0.920), kinematic viscosities (1.25mm2/s, 1.29mm2/s), moisture contents (0.52, 2.00) and densities (0.970g/cm^3, 0.982g/cm^3) from acid and enzymatic hydrolysis process respectively were in-line with the ASTM specification for Bioethanol. Okra stalk was successfully used to produce bioethanol using acid and enzymatic methods at 300Watts, 13.2min and 2M of acid.

Keywords: *Acid and enzymatic hydrolysis, Okra stalk, Bioethanol production, yeast (Saccharomyces cerevisiae), Agricultural residue*

INTRODUCTION

The natural energy resources such as fossil fuels (petroleum and coal) are being utilized at a rapid rate and these fossil fuel resources are gradually depleting and will be exhausted in the nearest future.

The production and consumption of fossil fuels have given off high levels of contaminants such as carbon dioxide (CO_2), sulfur oxides (SO_2, SO_3), nitrogen oxides (NOx), and methane (CH_4) which have caused environmental damage and, as a consequence, directly or indirectly, contaminating natural environments, water and food sources, causing immense damage to humanity (Cinthia *et al*., 2015).

The concern about environmental pollution and diminishing supply of petroleum- derived fuels are the key factors leading to search for the alternative sources to petroleum-based fuels. Biofuels have been promoted as one possible and promising solution to the declining reserves of fossil fuels, and the environmental unfriendliness resulting from the combustion of fossil fuels and the most feasible options for reducing carbon emissions in the transport sector. The term biofuel is used here to mean any liquid fuel made from plant material that can be used as a substitute for petroleum-derived fuel.

Bio-ethanol is the most promising resource because of its biological and renewable origins, normally derived from energy crops such as maize, sugarcane, cassava, sweet potato, mahula flower and by-products of agriculture and forestry (Ward and Singh, 2002).

Ethanol made biologically from a variety of cellulosic biomass sources such as agricultural and forestry residues, grasses, and fast growing wood is widely recognized as a unique sustainable liquid transportation fuel with high economic, environmental, and strategic attributes (Simone and Charles, 2009). Ethanol is used as an automotive fuel; it can be used alone in specially designed engines, or blended with gasoline and used without any engine modifications.

According to Simone and Charles, (2009) first generation ethanol made from starch-rich materials such as corn and wheat or from sugar feedstock is well proven. However, these raw materials are insufficient to meet the increasing demand for fuels, and there have been upsets that competition between the use of agricultural commodities for fuel production is driving up food costs because of the relatively high production costs, also the quest for high profit may result in environmentally detrimental indirect land use changes, e.g. the deforestation of tropical rainforest to gain more farmland. Ethanol can be produced from lignocellulosic materials such as agricultural residues, wood, paper and yard waste in municipal solid waste, and dedicated energy crops, which constitute the most abundant renewable organic component in the biosphere (Aiduan li., 2008). Moreover, utilization of a cheaper substrate such as lignocellulose could make bioethanol more competitive with fossil fuel, without the ethical concerns associated with the use of potential food resources. It is therefore imperative for a country such as Nigeria to begin research on production of ethanol from available and economically feasible feedstock to back up her dependency on fossil fuel. A good example of this lignocellulose feedstock is the Okra (*Hibiscus esculentus*) stalk.

Okra (*Hibiscus esculentus*) is a member of the Malvaceae family and is widely distributed in the tropical to subtropical regions from Africa to Asia, Southern European and Mediterranean countries, and America (Oyelade *et al.,* 2003).

Okra cultivation and production has been widely practiced because of its importance to the economy development and can be found in almost every market in Africa.

According to Ngbede, (2014) Okra production worldwide is estimated at six million tons per year. In West Africa, it is estimated at 500 to 600 thousand tons per year (Burkil, 1997). The total area under cultivation has increased over the years. India is the world largest producer followed by Nigeria and Sudan (Varmudy, 2011). According to CBN (1996), the average growth rate of vegetable crop including okra produced in Nigeria between 1989 and 1993 was 14.0% compared to 6.4% of cassava, 18% for palm oil and 3.8% for maize.

Okra is grown for its tender fresh pods which are rich in vitamins, minerals and protein (Mbah *et al.,* 2009). In view of all the nutritional and economic importance of okra pods, leaves and dried seeds, the stalk of the okra is left to constitute nuisance on the farm after the farmer has harvested the desired part of the crop. Therefore, the okra stalk which is readily available and abundant due to its low economic importance was used in this research.

Materials and method

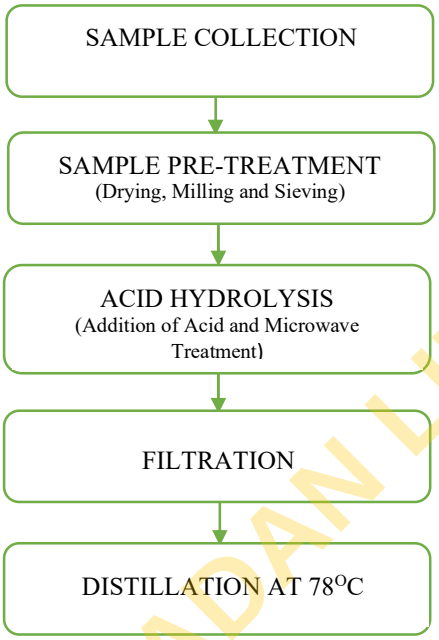

Fig.1: Experimental Procedure

Sample Collection

The okra (Hibiscus esculentus) stalk was obtained from, Ayetoro Gbede, Kogi State. The sample was obtained after harvest.

Sample pre-treatment

Dirt's lodging within the stalk were removed and then air dried for 2 days in order to reduce the moisture content of the sample from 35 to 18%. The okra stalk was then chopped into smaller bits of 4 – 6 inches as shown in plate 1 using a knife and a chop board. A milling machine (electronic ignition, GX200 japan model) was used to mill the sample to fine particle size of about 0.8 – 1mm, this is because particle size has effect on the success of hydrolysis (Aiduan li., 2008). The milled sample is shown in plate 2. After which a sieve was used to select a sieve size of 1mm

Plate 1: Chopped Okra stalk

Plate 2: Milled Sample of Okra stalk

Ethanol production using acid hydrolysis

Acid hydrolysis of okra stalk as a pre-treatment method and ethanol production method was used in this experiment.

100g of the okra stalk, was mixed with 2 litres of 2 M tetraoxosulphateIV acid, then heated in a microwave at 300watt power for 13.2min. the pH value was adjusted to 4.5 which is the optimum pH for the fermentation process.

Production of ethanol using enzymatic hydrolysis

100g of the sample (okra stalk) was hydrolyzed using 2 litres of 2 M tetraoxosulphateIV, in a microwave at 300W of power for 13.2Min, then the hydrolyzed mixture was thoroughly washed. The hydrolyzed solid was then mixed with 2 litres of water and the pH value was adjusted to 6.5, Calcium chloride was added for activation of the Enzyme assay before 200ml of alpha amylase was added for liquefaction of biomass. The mixture was then kept in a water bath for 3 hours at a constant temperature of 60^{o}C.

The pH value of the mixture was readjusted to 5.0 after which 200ml of gluco-amylase enzyme was added for the scarification. and the mixture was placed in a water bath at constant temperature of 55^{o}C for an hour.

Fermentation was induced using 20 gram of yeast (*Saccharomyces cerevisiae*) per 100gram of sample at 30^{o}C and 4.5 temperature and pH, respectively. and was allowed to ferment for 48 hours, after which the mixture was filtered and distilled to collect ethanol at 78^{o}C

Plate 3: Remnant of fermentation and filtration process of ethanol production using enzymatic hydrolysis

Plate 4: Fermentation process for Acid and Enzymatic hydrolyses

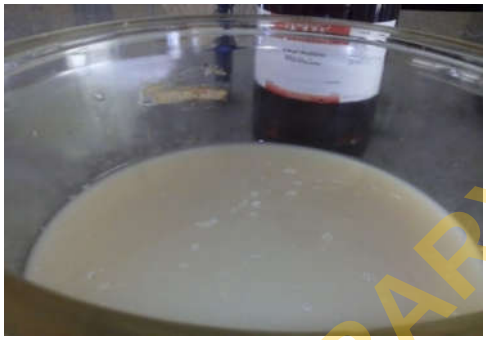

Plate 5: The filtrate from the fermented enzymatic hydrolyses process

Results and Discussion

The results obtained in this work are percentages Bio-ethanol yield from Acid hydrolysis and Enzymatic hydrolysis using acid hydrolysis as a pre-treatment method.

Acid hydrolysis

The acid hydrolysis process produced 8.5ml of ethanol which is approximately 43.6%v/v, as compared to the National renewable energy laboratory benchmark of 75%v/v of ethanol, this may be due to presence of inhibitors and low level of liquefaction and saccharification achieved during the process.

Enzymatic hydrolysis

The enzymatic hydrolysis process produced 12.9ml of ethanol which is approximately 65.8%v/v, as compared to the National renewable energy laboratory (NREL) benchmark of 75%v/v of ethanol.

Table 1: Fuel properties of bioethanol from okra stalk.

Properties	**Units**	**Experimental values**		**ASTM Standards**
		Acid hydrolysis	**Enzymatic Hydrolysis**	
Moisture content	%	0.52	2.00	< 20.00
Density	g/cm^3	0.970	0.982	0.99
Specific gravity		0.892	0.920	0.87
Flash point	^oC	20.5	22.1	18.60
Viscosity	mm^2/s	1.25	1.29	1.20
Cloud point	^oC	20.26	21.76	23.00
Pour point	^oC	5.77	5.12	5.20

Conclusion

Bioethanol was successfully produced from the okra stalk using acid and enzymatic hydrolysis, with yields of (8.5ml and 12.9ml)/100g sample, approximately (85litres and 129litres)/tonnes. The yields obtained are relatively low when compared to NREL standard of 196litres/tonnes.

The properties of the biofuel produced were within the ASTM standard values indicating its suitability as fuel. It may be concluded that the okra stalk is a potential raw material for bioethanol production.

Reference

Aiduan L. (1998) bioconversion of biodegradable municipal solid waste (BMSW) to glucose for bio-ethanol production. Department of Civil, Environmental and Geomantic Engineering University College London, London, pg. 79

Burkil, H.M. (1997): The useful plant of West African 2nd edition. Vol. 4, Families M-R, Royal Botanic Garden. Kew United Kingdom pp. 969pp

Central Bank of Nigeria (CBN) (1996): Annual Report and Statement of Account, Nigeria.
Cinthia D.S, Carreiro D.L, Fernando B. Mainier and Luciane P. (2015). Evaluation of Oilseeds for Biodiesel Production. American Journal of Environmental Engineering 2015, 5(2): 47-51.

Claassen, P.A.M., van Lier, J.B., Contreras, A.M.L., van Niel, E.W.J., Sijtsma, L., Stams, A.J.M., de Vries, S.S., Weusthuis, R.A., (1999). Utilisation of biomass for the supply of energy carriers. Appl. Microbiology Biotechnology. 52 (6), 741–755.

Mbah, E. U., Nottindge, D. O. and Keke, C. I. (2009): Growth and Yield of Cassava and Okra as influenced by Cassava Varieties, in Cassava / Okra intercrop on an Acid Ultisol. Proceedings of the 43rd Annual Conference of Agricultural Society of Nigeria (ASN), Abuja, 20th – 23rd October, Pp 19 – 23.

Ngbede S. O, H.N. Ibekwe, S.C OKpara, U. N Onyegbule and L. Adejumo. (2014) Greener journal of Agricultural Sciences Vol. 4, pp. 136 – 143.

Oyelade, O. J., Ade-Omowaye, B. I. O., Adeomi, V. F. (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. J. food Eng., 57: 111-114

Simone K.W, Charles O. P, (2009) Ethanol from biomass by enzymatic hydrolysis. Hem. Eng. Prog. 84.

Varmudy, V. (2011): Marketing Survey needed to boost okra exports. Department of economic Vive Kananda College, Puttur Karnataka.

Ward OP, Singh A. (2002) Bioethanol technology: development and perspectives. Adv Appl Microbiol; 51:53–8