2013 3rd International Conference on Energy and Environmental Science
IPCBEE vol.54 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V54. 13

Assessment of Lagenaria vulgaris Seeds Cake for Bioethanol and Biohydrogen Production

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Abstract. There is persistent energy supply problem globally, especially in the developing nations. This had resulted to periodic increase in fuel pump price which significantly affect socioeconomic well being of the populace of these countries. Therefore, diversification of energy sources especially by exploitation of biomass resources for biofuels can reduce the magnitude of dependency on the fossil fuel source; boost energy supply as well as mitigating emission of greenhouse gasses. The potential of biomass material for generation of biofuels varies from species and localities. However, production of biofuels results to formation of by-products which constitute waste into environment. Biodiesel production in many instances results to generation of cake which constitutes a waste in the environment. Thus, the potential of this type of cake was investigated using seeds cakes of *Lagenaria vulgaris*. Reducing sugar content of the cake was analyzed using dinitrosalicylic acid (DNS) reagent. The glucose content in the cake was fermented to bioethanol using *Saccharomyces* The results of the analyses indicate that the cake yielded 503 ppm reducing sugar and 0.4241 ±0.4865% bioethanol. Co-digestion of the cake at thermophilic condition also generated a biogas containing hydrogen gas as the major component. The results of this study indicate that indigenous seeds cakes of Lagenaria vulgaris have potentials of being a viable raw material for production of bio-based fuels. Thus, harnessing this form of biomass can positively improve the energy supply of the populace.

Keywords: Bioethanol, Reducing Sugar, Biohydrogen

1. Introduction

Diversification of energy sources especially by exploitation of biomass resources for biofuels can reduce the magnitude of dependency on fossil fuel source. Globally, utilization of biomass resource as potent energy resources has attracted interest for being derived from plants which are capable of being cultivated in many different environments as well as being carbon neutral [1].

Fermentation is one of the promising conversion techniques of biomass to fuels. Bioethanol and biogas are products resulted from this type of conversion. Hydrolysis of biomass is influenced by many factors [2, 3]. Consequent upon these, present study tend to investigate the influence of temperature, acid concentration and reaction time on reducing sugar yield on the hydrolysis of *Lagenaria vulgaris* seeds cake using response surface method (RSM). Furthermore, anaerobic digestion of biomass material was also established to be an avenue of generating a fuel in gaseous form. Compositional analysis of biogas yield different constituents in

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varied proportion depending on the nature of the substrate and experimental condition. Thus, study on the Batch digestion of *Lagenaria vulgaris* cake was investigated in the present study.

2. Materials and Methods

2.1. Sample Collection and Treatment

Defatted seeds cake of *Lagenaria vulgaris* cake obtained from the research laboratory of Energy Research Center Usmanu Danfodiyo University Sokoto, Nigeria was used in this study. The cake was dried in a hot air oven at 65^{o}C for 30 minutes to remove the residual n-hexane. The solvent free cake was sieve through a mesh and kept in a nylon bag until required for the next stages of the analyses.

2.2. Experimental Design

Response surface method (RSM) was used to design the experiment. The design consists of three levels and factors. Table 1 shows the levels and the parameters for the design.

Table 1: 3-level-3-Factor Experimental Design

Level	Acid concentration (%)	Temperature (^{o}C)	Time (min)
1	0.01	100	10
2	0.03	120	15
3	0.05	150	30

The factors were coded A, B and C to designate acid concentration,temperature and time respectively and values -1,0 and +1 represent low, middle and higher value of the factors respectively. Response yield (reducing sugar) was predicted from the response design using equation 1.

$$\text{Reducing sugar} = +279.32 + 144.98 * A + 41.56 * B + 18.77 * C + 64.48 * A^2 - 55.50 * B^2 - 6.83 * C^2 + 21.87 * A * B + 10.10 * A * C - 14.80 * B * C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

2.3. Estimation of Reducing Sugars and Concentration of Bioethanol

The hydrolysis of the cake and the fermentation of the hydrolysate were carried out according the method reported by Mohit *et al*., [4]. Bioethanol concentration was determined using procedure reported by Rabah *et al.* [5].

2.4. Preparation of the Feed Materials for Biohydrogen Production

The slurry of de-fatted *Lagenaria vulgaris* cake was prepared by mixing 1kg of the cake with 3 liters of distilled water in a plastic bowl. The cattle dung slurry was prepared in ratio 1:1 with distilled water in a separate bowl. Four liters each of the prepared slurries were mixed and seeded with 500 ml of palm oil effluent (POME). The mixed slurry was used as the feedstock for the bio reactor.

2.5. Experimental Design for Biogas Production

Batch digestion process using eight liters of prepared mixed slurries was carried out in a 10 L Jacketed bioreactor (Sartorius Germany) at thermophilic temperature (52.8^{o}C). The retention period of seven days was chosen for both the sample and the control experiments

2.6. Determination of Total and Volatile Solids of the Slurry

Samples of raw and mixed slurries were analyzed for total solids (TS) and volatile solids (VS) using standard methods for examination of water and wastewater [6]. Likewise samples collected intermittently from the bioreactor were analyzed using the same method. Volatile fatty acids (VFAs) content of the slurries were determined using GC-MS with using polar column.

2.7. Determination of Biogas Composition

Biogas composition was determined by gas chromatograph (HP 6890N) equipped with a thermal conductivity detector (TCD) and HP Molesieve capillary column of 30m length x 0.5mm ID x 40µm film thickness (P/N 19095P).

3. Results and Discussion

Concentration of reducing sugar (ppm) obtained from the RSM design is presented in Table 3. The reducing sugar concentration varies with the experimental conditions. High concentration of glucose (503.684 ppm) was observed at run 15.

Table 2: Experimental Design Matrix and Reducing Sugar Content of *L.vulgaris* Seed Cake

Run	Acid conc., A (M)	Temp, B (^oC)	Time C (min)	Reducing sugar (ppm) *L..vulgaris*
1	0.03	125.00	30.00	305.334
2	0.03	125.00	20.00	286.713
3	0.05	125.00	20.00	508.09
4	0.03	125.00	20.00	276.018
5	0.00	125.00	20.00	199.56
6	0.03	125.00	20.00	281.78
7	0.00	150.00	30.00	138.068
8	0.05	150.00	10.00	461.208
9	0.00	100.00	30.00	149.88
10	0.05	100.00	10.00	326.335
11	0.00	150.00	10.00	168.70
12	0.03	150.00	20.00	285.89
13	0.00	100.00	10.00	88.59
14	0.03	125.00	20.00	282.65
15	0.05	150.00	30.00	503.684
16	0.03	125.00	20.00	253.600
17	0.03	100.00	20.00	181.799
18	0.03	125.00	20.00	255.100
19	0.03	125.00	10.00	259.690
20	0.05	100.00	30.00	395.30

The hydrolysis of the *Lagenaria vulgaris* seed cake was evaluated as a fuction of acid concentration, temperature and time (Table 2). The result revealed that reducing sugar yield increases with an increase in acid concentration, temperature and the residence time. The result indicates the likelihood of the cake to be a viable feedstock for bioethanol production. Research evidence has revealed that bioethanol yield has linear relation with reducing sugar conentration [3,4, 7). The concentration of reducing sugar obtained from the analysed seed cake is higher than the content reported in jatropha seed cake [4] and groundnut hulls [5].

However, the effect of variables and their possible interaction on the reducing sugar yield can be dipicted from the model analysis of variance (ANOVA) . The analyses indicate that the model is significant, and can be used to predict the response at 0.05 confidence level. The ANOVA also revealed that A, B, C, A^2, B^2 and BC are significant term that can be used to predict the response. The response yield increases linearly with increase in factors A, B, C. Similarly there is positive interaction between the factors AB and BC, with respect to the response yield and negative interaction between the factors AC. The extent of interaction is greater for AB (p-value 0.0053) than for BC (p-value 0.0375). The R^2 value 0.9880 signify that the 98.80% of the response is due to selected experimental parameter. Larger R^2 value showed the accuracy, applicability and adequacy of the model to describe the response [8, 9].

Ethanol production via fermentation depends on the available sugars in the hydrolysate and the type of fermenting organism [10]. The result in Table 3 infers that *Lagenaria vulgaris* had a concentration of 0.4241 ± 0.4865% bioethanol. The ethanol concentration observed from the fermentation broth of *L. vulgaris* is low. It could be due presence of other monosaccharide and inhibitory compound which cannot be converted to ethanol by *Saccharomyces cerevisiae*. The bioethanol yield obtained is comparable to the values reported

for canola, sunflower, sesame and peanuts cakes [7] and higher than the ethanol yield obtained in the hydrolysate of millet husk [5].

Table 3: Bioethanol concentration(%) and Optimal Glucose Response for the Predicted and Experimental Tests at 95% Confidence Interval

Sample Cake	Variables			Glucose concentration (ppm)	
	A(M)	B(^{o}C)	C(min)	Predicted	Experimental
L. vulgaris	0.02	129.37	18.54	310.899	331.323
Bioethanol (%)	0.4241 ± 0.4865%				

Effectiveness of anaerobic biodegradation process depends on the amount of biogas released. Co-digestion gives a better performance in terms of biogas output [11, 12, 13, 14]. The higher the biogas output, the more likelihood of it being beneficial.

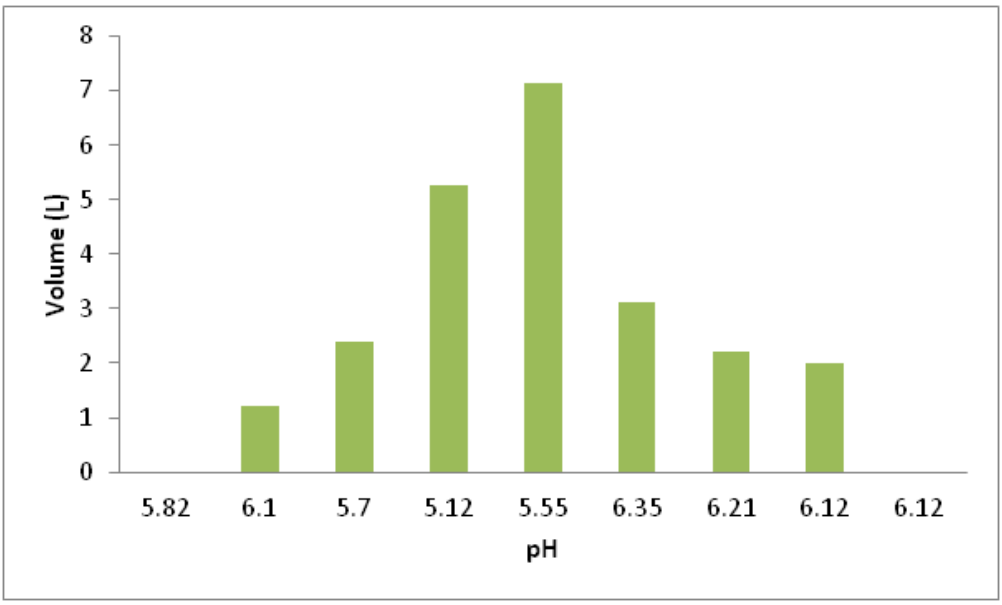

Fig 2: Daily Volume of Biogas Against pH for *Lagenaria vulgaris*

The results in Figures 2 show biogas generation as a function of pH. It was observed that the daily pH of the reactor increases with increase in the biogas volume. Figure 2 shows an irregular pH trends at the first 3 days of the experiment, and then exhibited linear relationship with the volume of produced biogas for the remaining hydraulic retention time. The highest volume of 7.15L was observed at pH 5.55 which could likely be the medium that triggered the activity of the fermenting organism.

Although the optimum pH for biogas production especially for hydrogen is debatable, substrates consisting of glucose were reported to yield optimal hydrogen gas at pH values of either 5.7 or 6.4 [15] and 5.5 [16]. The pH values observed in these experiments were compatible to the pH values (4-7) expected for the effective conversion of glucose to bio-hydrogen at mesophilic temperature [17].

Compositional analysis of the biogas (Figure 4) revealed the presence of hydrogen gas, methane, carbon monoxide and other residual gas. The result infers that hydrogen gas is the main component of biogas generated from the co-digestion of *L. vulgaris* seed cake and had a cumulative average % volume of 407.29 cm^3 (Table 7). The percentage volume of methane in the biogas was low which indicates that either the substrate is not a viable feedstock for methane production or the condition is not favourable for methanogenesis. Fakhru'l-razi *et al.* [18] opined that low or absence of methane in biogas could be due to deactivation of methogenes at high temperature and low pH.

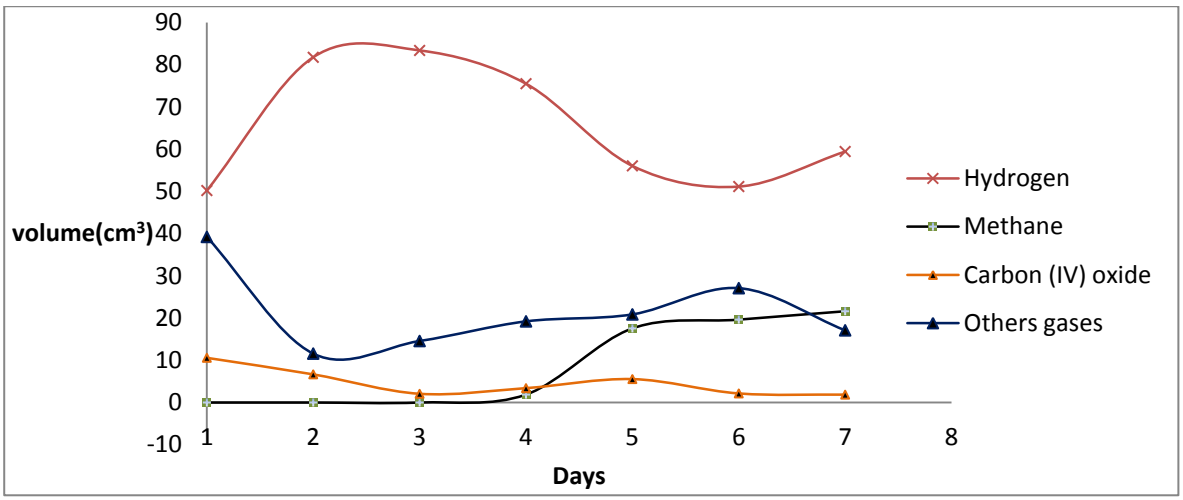

Fig 4: Volume of Hydrogen Generated from Co- Digested *L. vulgaris cake*

However, the composition of biogas obtained (Figure 4) agrees with the findings of Fakhru'l-razi *et al*., [18], who reported the presence of hydrogen, carbon monoxide and absence of methane in biogas generated from the anaerobic fermentation of waste water. Similarly the biogas composition shown in Figure 4 corresponds to the finding of Wu and Zhou [19] who reported hydrogen gas as the major constituent of biogas generated from anaerobic fermentation of municipal sludge. Therefore, the result obtained shows that biogas from the co-digestion of *L. vulgaris* cake could be a suitable source of hydrogen for possible application in hydrogen fuel cell.

The steady increase in percentage of hydrogen observed in the four consecutive days could be due to high decomposition of the substrate yielding acetic acid and propaonate (Table 4). Production of acetic acid during anaerobic digestion of substrate rich in glucose result in the increase in hydrogen content [20, 21]. High percentage of hydrogen obtained from the generated biogas could be due to absence of alcohols, low concentration of propanoate and lactic acid. The decrease in the % volume of hydrogen with increase in hydraulic retention time (Figure 4) could be due to the decrease in the activities of hydrogenic bacteria. Activities of acclimatised hydrogenic bacteria are high with short retention time (few days) [22]

Table 4: Percentage Volatile Fatty Acids Content of the Co-digested Slurry of the *L. vulgaris* Cake

Day	pH	Acetic acid (%)	Propanoic acid (%)	Butyric acid (%)	Other non-volatile acidic compounds
4	5.55	64.25	3.75	ND	32.00
6	6.21	43.89	9.36	3.20	43.55
8	6.12	41.16	8.72	3.7	46.42

nd = not detected

The VFAS contents obtained from the digested feed stocks indicate acetic acid as the major intermediary compound. Lower percentage concentration of butyric acid and propionaote were also detected. Presence of these compounds infers that hydrogen gas might be the major product of the digestion [18].This is attributed to the fact that hydrolytic decomposition of glucose yield ethanoic acid, CO_2 and volumes of hydrogen.

Table 7 shows the total percentage volume of hydrogen gas generated from the mixed slurry of the respective cakes with cattle dung. The results show an increase in percentage of hydrogen gas in the cake mixed with cattle dung than the hydrogen content in the raw cattle dung. *L. vulgaris* had a volume increase of 86.44 % over the control sample.

Table 7: Total and Volatile Solid Contents and Average Volume of H_2 gas for *L. vulgaris and* Cattle Dung Slurries

Parameter	*L. vulgaris*	Cattle Dung
Total Solid (%)	25.658±0.001	10.886±0.230
Total Solid (g/L)	129.212±0.011	96.354±0.054
Volatile Solids (%)	57.508±0.321	86.545±0.005

Volatile Solids(g/L)	83.613±0.026	83.416±0.044
Average Volume H_2 gas (cm^3)	407.29	320.56
Increased volume H_2 gas (cm^3)	86.44	-

Total solids and volatile solids are principal components that contribute to the organic strength of biogas feedstock [23]. Substrate with high TS content makes mixing to be problematic and hinders homogeneity of the slurry as well as inadequate biodegradation of the substrate by fermenting organism. The TS concentrations (Table 7) content is within the limit that will not hamper efficient mixing [23]. The VS content is an indicator for suitability of substrate to generate substantial volume of gas. Slurry with high VS content could generate more gas than the one with low VS content under favourable fermentation conditions.

4. Conclusion

Residual waste generated from the production of biodiesel can be harness to generate bioethanol and biohydrogen via fermentation and anaerobic digestion respectively. Hydrolysis is upon influenced by acid concentration, temperature and resident time. Co-digestion of *Lagenaria vulgaris* gave significant amount of biogas with hydrogen as the major component.

5. Acknowledgements

The authors wish to acknowledge the financial support received from the Tertiary Education Trust fund of Nigeria (TERTFUND) for the conduct of this research. Access to laboratories granted by Institute of Advanced Technology (ITMA) Universiti Putra, Malaysia is also acknowledged.

6. Reference

[1] P. V. Amish, N. .Subrahmanyam, and A. P. Payal.. Production of Biodiesel through Trans-esterification of Jatropha oil Using KNO_3/Al_2O_3. *Fuel*, 2009, 88: 625 – 628

[2] J.D McMillan. Pretreatment of lignocellosic Biomass In:Himmel , M.E., Baker,J.O., Overend, R.P. Enzymatic Conversion of Biomass for fuels production . American Chemical Society, Washington, D.C. . 1994 : 292 – 324

[3] S.Ye, and C. Jiayang, . Hydrolysis of Lignocellulosic Materials for Ethanol Production: A Review. *Journ.. of Biores. Tech.*, 2002: 83:1-11.

[4] S. M. Mohit,., , B. Chandrashekhar; C. Tanusheree,, and S. Kanwal,. Acid Hydrolysis and Fermentation by *Saccharomyces Cervisiae*. *International Journal of Biotechnology Applications*, 2001: **3(1)**: 41-47

[5] A. B, Rabah,., S. B. Oyeleke,, S. B. Manga,. and L. G. Hassan. Utilization of Millet and Guinea corn Husks for Bioethanol Production. *African Journal of Microbiology Research,2011* **5(31)**: 5721- 5724.

[6] D.E Andrew., A.D Eaton., L.S. Clercer and A.E. Greenberg. Standard Methods for Examination of Water and Wastewater. American Public Health Association, Washington DC, 2005.

[7] B. Venkatesh, , A. Chad,., P. Shishir, , C. Leonardo, and E..Bruce, Conversion of Extracted Oil Cake Fibers into Bioethanol including DDGs, Canola, Sunflower, Sesame, Soy and Peanut for Integrated Biodiesel Processing. *Journal of American Chemical Society*, 2009: 86:157- 165.

[8] A. A. Shatalov, and H. Pereira.. Biorefinery of Energy crop (cynaracardunculus. L)- hydrolytic Xylose production as Entry to complex Fractionation Scheme. *Journal of Chemical Engineering Process Technology*, 2011: **2(5):**118

[9] Y Bao,., , Z. Yan, H. Wang,and L. Chen,. Optimization of Bio-ethanol Production during simultaneous Saccharification and Fermentation in very high-gravity cassava mash. *Antonie van Leeuwenhoek*, 2011: 99:329-339.

[10] K.C., Anuj, E..S Chan, R. Ravinder, , M. N. Lakshmi, L.R. Venkateswar and R. Pogaku.. Economics and Environmental Impact of Bioethanol Production Technologies: An Appraisal. *Biotechnology and Molecular Biology Review*. 2007 : **2 (1**), 014-032.

[11] J.B. Van-lier. A. Tilche,. B. K.. Ahring, , H. Matarie,., R . Moletta.,. M. Dohanyos,. L.. Pol. , w. H., , P Levis. and W .Verstraete,. New Perspective in Anearobic Digestion . *Journal of Water Science and Technology*, 2001: 43: 1-18.

[12] B.. Ahring, .Perspectives for Anaerobic Digestion. *Advances in Biochemical Engineering and Biotechnology*, 2003*:*

81: 1-30.

[13] S. Yadvika,., T. Sreekrishnan, S. Kohli, and V. Rana., Enhancement of biogas production from solid substrates using different techniques-a review. *Bioresource Technology*, 2004: 95(1): 1-10.

[14] R. Alvaraz, and G. Linden. Semi-continuous Co-digestion of Solid Slaughter House Waste, Manure and Fruits and Vegetable waste. *Journal of Renewable Energy*, 2008: 33: 726 – 734.

[15] Y. Ueno, S. Otsuka.,M. Morimoto. Hydrogen production from Industrial Wastewater by Aneorobic Microflora in Chemostat culture. Fermentation and Bioenergy,1996: **82(2)** : 194 - 197

[16] H. Fang, and H. Liu,. Effect of pH on Hydrogen Production from Glucose by a Mixed Culture. *Journal of Bioresource Technology*, 2002: 82:87 – 93

[17] K. Vijayaraghvan,, and M.M. Soom.Trends in Biohydrogen Generation- A review. *Journal of Environmental Sciences*, 2006**: 3**(4):255-271.

[18] A. Fakhru'l-Razi, A.A. Yassin,, S.E. Lyuke,, M.A. Ngan, and M. Morimoto. ,Bio-Hydrogen Synthesis from Wastewater by Anaerobic Fermentation Using Microflora. *International Journal of Green Energy*, 2005: 2(4):287-396.

[19] F. Wu,. And S. Zhou. Continuous Pro-Hydrogen by Anaerobic Fermentation of Municipal Sludge. *International Journal of Green Energy*, 2011: **8**(3):130-145

[20] R. Zoetemeyer, J. Van den Heuvel, and A. Cohen. pH Influence on Acidogenic Dissimilation of Glucose in an Anaerobic Digester. *Water Research*, 1982 :**16(**3): 303-311.

[21] C.G. Chen, C.Y. Lin, andJ.S. Chang. Kinetics of Hydrogen Production with Continuous Anaerobic Cultures Utilizing Sucrose as the Limiting Substrate. *Journal of Applied Microbiology and Biotechnology*,

[22] C.Y. Lin, and R.C. Chang,. Hydrogen Production during the Anaerobic Acidogenic Conversion of Glucose. *Journal of Chemical Technology and Biotechnoology*, 1999: 74:498 - 500.

[23] A.C. Wilkie. Biogas Feedstock accessed at ***biogas**.ifas.ufl.edu/feedstocks.asp.* Retrieved on 21, October, 2012