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Optimization of Biohydrogen Production (BHP) from Agro Waste Water (Cassava Waste Water): A Case of Box-Behnken Response Surface Methodology (RSM)

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Abstract: This work aims at optimization of biohydrogen production(BHP) from agro waste water. To determine the physicochemical properties of the cassava waste water the sample was subjected to analysis using standard methods. Broth medium was made and substrate preparation was carried out, inoculum pretreatment was carried out and the medium was cultivated following standard method. To optimize the process condition, three-factor-three-variables response surface methodology (RSM) was used; these gave 17 experimental runs and were carried out. Results showed that the physicochemical analysis of agro waste water had initial pH of 5.46 which indicated a low acidity, total diffuse solid of 3.93 mg/L, chemical oxygen demand of 0.25 mg/L and biochemical oxygen demand of 0.16 mg/L. The highest biohydrogen yield(BHY) obtained was 4.25 mlat a coded factors of X_1= 0, X_2=1 and X_3= 1, but the RSM statistical software predicted BHY of 4.009 ml at X_1= -1, X_2= -1.0 and X_3= -0.011 at desirability of 0.706. This was validated by carrying out three experiments which gave an average BHY of 4.00 ml. The coefficient of determination (R^2) of 0.9966% implies most variability can be explained by regression model. The experimental findings concluded that the use of RSM with appropriate experimental design can help in achieving the optimum yield of biohydrogen, which could serve as an alternative source of energy that could replace petroleum-based fuels.

Keywords: Optimization, Response Surface Methodology, Agro Waste Water, Physicochemical Properties, Biohydrogen, Coefficient of Determination

1. Introduction

In this 21st century, while the demand for energy for transportation, heating, and industrial processing is increasing day by day, environmental issues are a point of concern [1]. In our modern world of today where there is high demand for energy, Oil and its derivatives are the major sources of energy used to meet the societies need for energy. Fossil fuels are source of non-renewable energy and also have seriously negative impacts on the environment [2]. Hydrogen is an alternative source of energy that could replace petroleum-based fuels because water and energy are the only by products of its combustion with oxygen [3]. Hydrogen gas has energy content of 122kJ/g. It does not emit carbon dioxide, carbon monoxide and oxides of sulphur and nitrogen but water as its only by-product during combustion thereby reducing greenhouse effect. Thus, it is a clean source of energy. Hydrogen is the most abundant chemical element in the universe, containing almost three-quarters of the universe's entire mass [4]. It is often found naturally in a combined state with other elements. Recent reviews on hydrogen shows that the world's need for hydrogen is increasing with a growth rate of nearly 12% per year for the time being and contribution of hydrogen to total energy market will be 8-10% by 2025 [5]. Hydrogen can be produced through electrochemical, thermochemical, photochemical or photo electrochemical and biological processes. Most of the processes used for the production of hydrogen gas employ fossil fuels. Currently, 88 percent of hydrogen produced globally is heavily dependent on fossils fuels, 40 percent from steam reforming of methane and another 48 percent from crude oil and coal. However, production of hydrogen gas from fossil fuels by steam reforming, or from water by electrolysis and thermochemical decomposition, may not be environmentally friendly and can also be uneconomical [6]. In order to sustain the environment and also meet the high demand for energy, renewable energy sources have been employed as alternative sources of energy.

Biohydrogen production from renewable sources using microorganisms is a good alternative to the previously mentioned methods for hydrogen gas production. It appears to be the most attractive method compared to other hydrogen production processes because it has fewer environmental concerns. Biohydrogen production also known as green technology has received considerable attention in recent years [7]. Biological method mainly embraces photosynthetic hydrogen production (photo fermentation) and fermentative hydrogen production (dark fermentation) [8]. Broadly, biohydrogen production processes can be divided into biophotolysis (biophotolysis of water using algae and cyanobacteria), photo decomposition (photo decomposition of organic compounds by photosynthetic bacteria), and fermentation (fermentative hydrogen production from organic compounds) [9]. So far biohydrogen production by photosynthetic microorganisms has extensively been studied while biohydrogen production by fermentation was treated with little attention [9]. The production of biohydrogen through fermentation is of more advantage over photochemical process because various waste waters can be used as substrates. The use of agro-industrial residues in biological hydrogen production has also been investigated for the same reason [10]

Cassava is a major economic sustenance cash crop produced in 24 states out of 36 states of the Nigeria thus making her the world's largest producer of cassava. Over 41 million metric tonnes is produced per annum [11]. Cassava (*Manihotesculenta*) is commonly used in the production of flour and starch in Nigeria. In Nigeria, cassava is cultivated in plantations by traditional farming means. Cassava is processed either by hand or by mechanized units. Cassava undergoes a pressing process during processing, and the wastewater derived from the pressing is known as *cassava waste water*. Cassava waste water has high energy content. It is rich in carbohydrate and is easily hydrolysable. Cassava waste water contains approximately 20-40 g of carbohydrate per litre. The wastewater contains high BOD, COD and Total Solids (TS) as organic substances are extracted from the cassava roots. Thus, the energy potential of cassava waste water makes it very attractive as a substrate for hydrogen production, and its use in this process would reduce the environmental impact of disposing it without prior treatment [12]. Of late, biohydrogen production through anaerobic fermentation using waste water as substrate has been attracting considerable attention [13].

Dark fermentation also known as anaerobic digestion is considered a viable process for biohydrogen production because it generates biohydrogen from carbohydrate substrates including biomass and organic waste materials. However, the yield of biohydrogen is relatively low, since hydrogen is produced as an intermediate and can be further reduced to methane, acetate and propionate by hydrogen-consuming bacteria during dark fermentation [9]. Inhibiting the activity of hydrogen-consuming bacteria and enriching hydrogen producing bacteria will increase the production rate of biohydrogen. Important factors to be considered in biohydrogen production are pH, temperature, feed concentration, bacterial population, retention period, etc.

The Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [14]. It is an important tool in designing, formulating, developing and analysing new products. According to Hill and Hunter, Response Surface Methodology (RSM) was introduced by G.E.P. Box and K.B. Wilson in 1951 [15]. They acknowledge that this model was only an approximation, but used it because such a model is easy to estimate and apply, even when little is known about the process [15].

By using of design of experiment for Response Surface Methodology, the input levels of each factor as well as the level of the selected response can be quantified. The central composite, Box-Behnken and Doehlert designs are among the common designs used for response surface methodology. In this study, the optimization of biohydrogen production from synthesized agro waste (cassava waste water) was studied.

The objective of this study was to optimise the production of biohydrogen from an agro waste using Box-Behnken Response Surface Methodology (RSM).

2. Materials and Methods

2.1. Microorganism

Hydrogen forming bacteria known as *Clostridium coliform* was collected from Microbiology Laboratory of the Akwa Ibom State University, Nigeria. The microorganism was cultured using standard methods

2.2. Broth Medium Preparations

1.1 g of nutrient broth powder was added to 1 litre of distilled water in equivalence of 5 litres in a medium. The medium was sterilized in an autoclave at 121˚C for 120 min and then placed in the refrigerator for 24 h.

2.3. Inoculum Preparation

The cultured *Clostridium coliform* was added to the nutrient broth medium to make an inoculum. The inoculum was sterilized in an autoclave at 121˚C for 20 min. The inoculum was pre-treated by raising its pH to 3.0 with 0.1M sulphuric acid. The inoculum was then incubated at 37˚C for 24 h.

2.4. Quality of Substrate

The substrate is the cassava waste water. The agro waste water (cassava waste water) was obtained from a cassava processing plant in Ikot Ekpene, Akwa Ibom State, Nigeria.

2.5. Physicochemical Properties

The physicochemical properties of cassava waste water such as total alkalinity, ammonia nitrogen, total phosphate, total solids, aluminum, potassium, copper, iron, magnesium, calcium, zinc, COD (Chemical Oxygen Demand) were analyzed using the ELE International Photometer while the analysis on organic carbon, total Kjedahl nitrogen, ash content, conductivity test were carried out using Walkley-black titration method, Kjeltex auto-distillation apparatus model, Muffle furnace, Electrochemical analyzer (Consort C6020), respectively. Meanwhile, volatile solids content was calculated from the ash content value.

2.6. Experimental Design

The experiment was carried out under an anaerobic condition and a dark fermentation process. The response surface methodology was used in the experimental design with box-behnken, three-level-three-factors (for three variable) design was applied and 17 experimental runs were generated and were carried out. The variables considered were fermentation time (days), pH effect and substrate concentration (mg/L) (Table 1).

Table 1. *Variables factors considered for biohydrogen production.*

Variable	Symbol	Coded factor levels		
		-1	0	+1
Time (days)	X_1	1	2	3
pHeffect	X_2	5.80	6.0	6.20
S. conc. (mg/L)	X_3	3.70	4.0	4.30

The quadratic equation derived from ANOVA analysis was used in determining the optimum conditions of hydrogen yield.

$$Y_F = \rho_0 + \sum_{i=1}^{k} \rho_i X_i + \sum_{i=1}^{k} \rho_{ii} X_i^2 + \sum_{i<j}^{k} \rho_{ij} X_i X_j + \varepsilon \quad (1)$$

2.7. Biohydrogen Production

The experiment was conducted in an anaerobic condition using dark fermentation process to produce biohydrogen from cassava waste water. 80 ml of cassava waste water of different concentrations (samples) were measured into seventeen vacuum flasks with. The pH of cassava waste water was varied for each sample. The samples were sterilized in the autoclave at 121°C for 20 min and then cooled. 15 ml of the inoculum was added to each sample in the vacuum flasks and then closed with a stopper. The mixture was left for 1-3 days for biohydrogen yield to be monitored. Balloons were placed at the side arm (vacuum) of the flask to trap biohydrogen gas produced. The yield/volume of biohydrogen gas produced was determined by immersing the balloons filled gas in a container that held a known volume of water. The weight of volume displaced was taken as the volume of biohydrogen yield (BHY).

3. Results and Discussion

3.1. Optimization of BH Production

Shown in Table 2 are the coded experimental independent factors considered in this study with the response BHY, predicted and residual values. Design Expert dx10.0.3.1 was engaged to evaluate and determine the value of coefficients independent factor in a full regression model equation and their mathematical significance. The results of test of significance for all regression coefficient are presented in Table 3,observation shows that all p-values were significant ($p<0.0001$) except X_1 and X_2X_3 that were found non-significant at 95% confidence level (95% CI).

Table 2. *Experimental data for HBP, predicted and residue values.*

Std. run	X_1	X_2	X_3	BHY (ml)	Predicted	Residual
1	-1	-1	0	3.90	3.90	0.000
2	1	-1	0	3.90	3.90	0.000
3	-1	1	0	4.19	4.18	0.0075
4	1	1	0	3.80	3.81	-0.0050
5	-1	0	-1	4.08	4.07	-0.005
6	1	0	-1	4.00	4.01	0.013
7	-1	0	1	4.06	4.05	-0.012
8	1	0	1	3.90	3.90	0.013
9	0	-1	-1	3.73	3.74	0.000
10	0	1	-1	3.75	3.73	-0.0075
11	0	-1	1	3.94	3.95	0.020
12	0	1	1	3.90	3.90	-0.012
13	0	0	0	3.90	3.90	0.000
14	0	0	0	4.25	4.27	0.000
15	0	0	0	4.30	4.29	-0.020
16	0	0	0	3.80	3.81	0.0075
17	0	0	0	4.20	4.19	0.005

To minimize the error, the independent coefficient factors were taken into consideration. The model coefficients, residual, lack of fit, pure error and core total with their probability values are shown in Table 4 called ANOVA (analysis of variance of regression equation). The model F-value = 0.50 with $p<0.0001$, suggests significant model term and fittness of the chosen quadratic model. It revealed low standard deviation (0.016) and low mean values (3.98). The coefficient of determination (R^2) which plays an important role in the check of modeling also explained the proportion of variability that is explained by the model was obtained as 0.9966%, the value of R^2 being close to one (1) implies most variability can be explained by regression model. The low values of standard errors among the variables and their interactions observed shows that the regression model fitted the statistical data well with a good prediction (Table 5).

***Table 3.** Test of Significance for Every Regression Coefficient.*

Source	Sum of squares	df	Mean Square	F-value	p-value
X_1	8.000E-004	1	8.000E-004	3.29	0.1124
X_2	0.011	1	0.011	46.32	0.0003
X_3	0.43	1	0.43	1780.68	< 0.0001
X_1X_2	1.600E-003	1	1.600E-003	6.59	0.0372
X_1X_3	8.100E-003	1	8.100E-003	33.35	0.0007
X_2X_3	0.000	1	0.000	0.000	1.0000
X_1^2	0.016	1	0.016	67.72	< 0.0001
X_2^2	0.014	1	0.014	57.32	0.0001
X_3^2	7.605E-003	1	7.605E-003	31.32	0.0008

***Table 4.** Analysis of Variance (ANOVA) of Regression Equation.*

Source	Sum of squares	df	Mean Square	F-value	p-value
Model	0.50	9	0.0550000	227.15	< 0.0001
Residual	0.0017	7	0.0002429		
Lack of Fit	0.0017	3	0.0005667		
Pure Error	0.000	4	0.0000000		
Cor. Total	0.500	16			
RSM: S.D =0.016; Mean = 3.98; R-Sq. = 99.66%, R-Sq.(adj.) = 99.22%					

The variance inflation factor (VIF) of 1.00 to 1.01 obtained indicated that the center points were orthogonal to the axes points. The final equation that takes into account the linear factors, the interactions, the quadratic and the error terms is expressed in Eqn. (2).

***Table 5.** Regression Coefficients and Significance of Response Surface Quadratic.*

Factor	Coefficient Estimate	df	Standard Error.	95%CI Low	95%CI High	VIF
Intercept	3.90	1	6.969E-003	3.88	3.92	-
X_1	-1.000E-002	1	5.510E-003	-0.023	0.003028	1.00
X_2	0.038	1	5.510E-003	0.024	0.051	1.00
X_3	0.23	1	5.510E-003	0.22	0.25	1.00
X_1X_2	0.020	1	7.792E-003	0.001575	0.038	1.00
X_1X_3	-0.045	1	7.792E-003	-0.063	-0.027	1.00
X_2X_3	0.000	1	7.792E-003	-0.018	0.018	1.00
X_1^2	0.062	1	7.595E-003	0.045	0.080	1.01
X_2^2	0.057	1	7.595E-003	0.040	0.075	1.01
X_3^2	0.043	1	7.595E-003	0.025	0.060	1.01

$$BHY(ml) = 3.90 - 0.01X_1 + 0.038X_2 + 0.23X_3 + 0.020X_1X_2 - 0.045X_1X_3 + 0.00X_2X_3 + 0.062X_1^2 + 0.057X_2^2 + 0.043X_3^2 \quad (2)$$

The contour and the 3-D diagram can be used to provide a pictorial method of interaction between the response (HBY) and the independent factors considered. Shown in Fig. 1 is the plot of predicted against the actual. It was noted that the predicted and actual values were in agreement with each other. Fig. 2 shows the contours and the3-D plots representing the effect of fermentation time, pH and the substrate concentration. Observation from the graph showed that there was a high mutual interaction among the fermentation time and the pH (X_1X_3) than the interaction among the fermentation time and substrate concentration (X_1X_2). However, there was no significant interaction among the fermentation time and pH (X_2X_3).

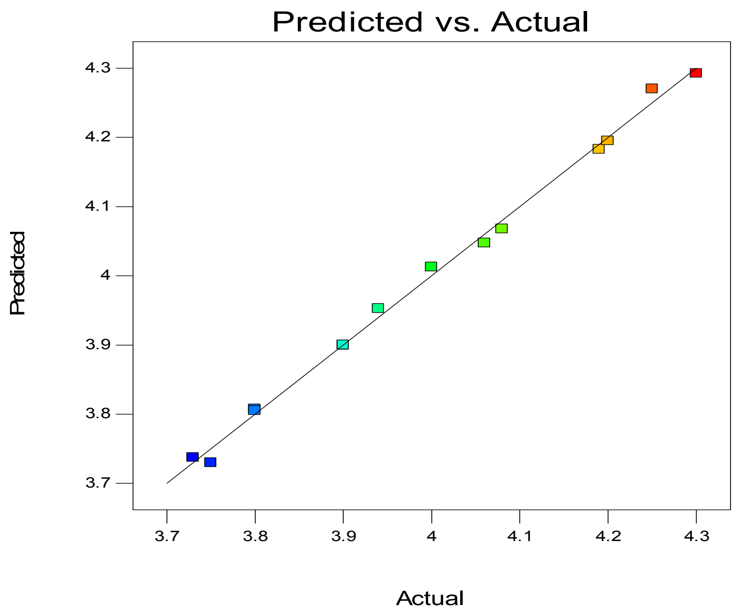
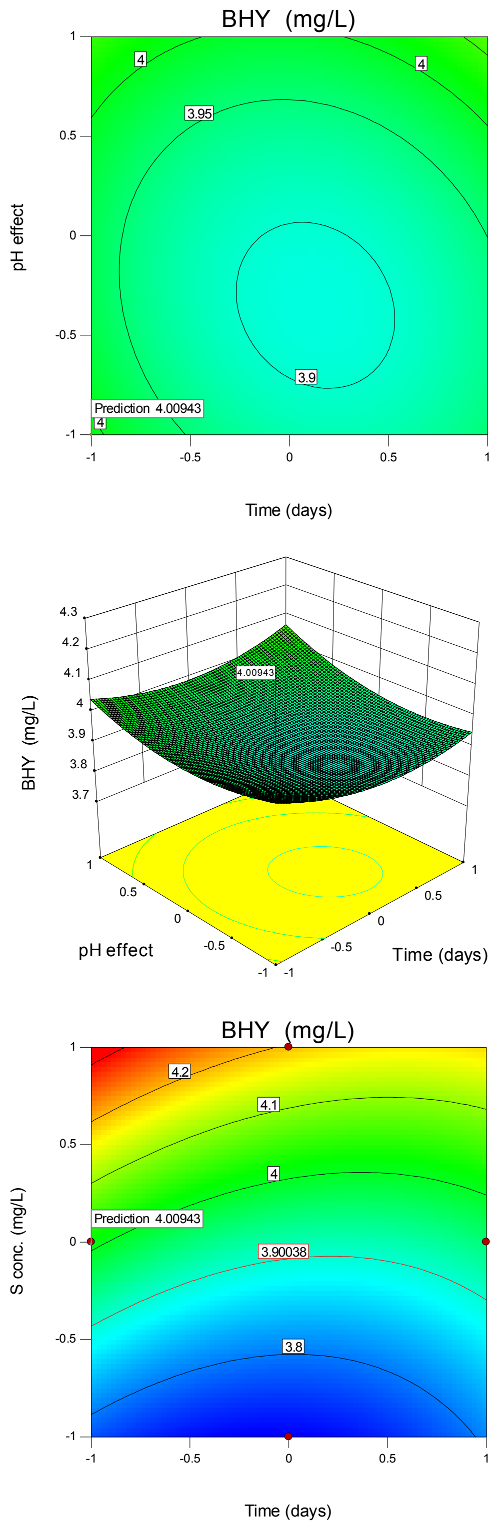


***Fig. 1.** RSM Plots of predicted against the actual.*

The highest BHY obtained was 4.25 ml at a coded factors of X_1= 0, X_2 =1 and X_3= 1, but the RSM statistical software predicted BHY of 4.009 ml at X_1= -1, X_2 = -1.0 and X_3 = -0.011 at desirability of 0.706. This was validated by carrying out three experiments, and the average BHY was 4.00 ml. The results of this study demonstrated that RSM with appropriate experimental design can be successfully useful for the optimization of biohydrogen production.



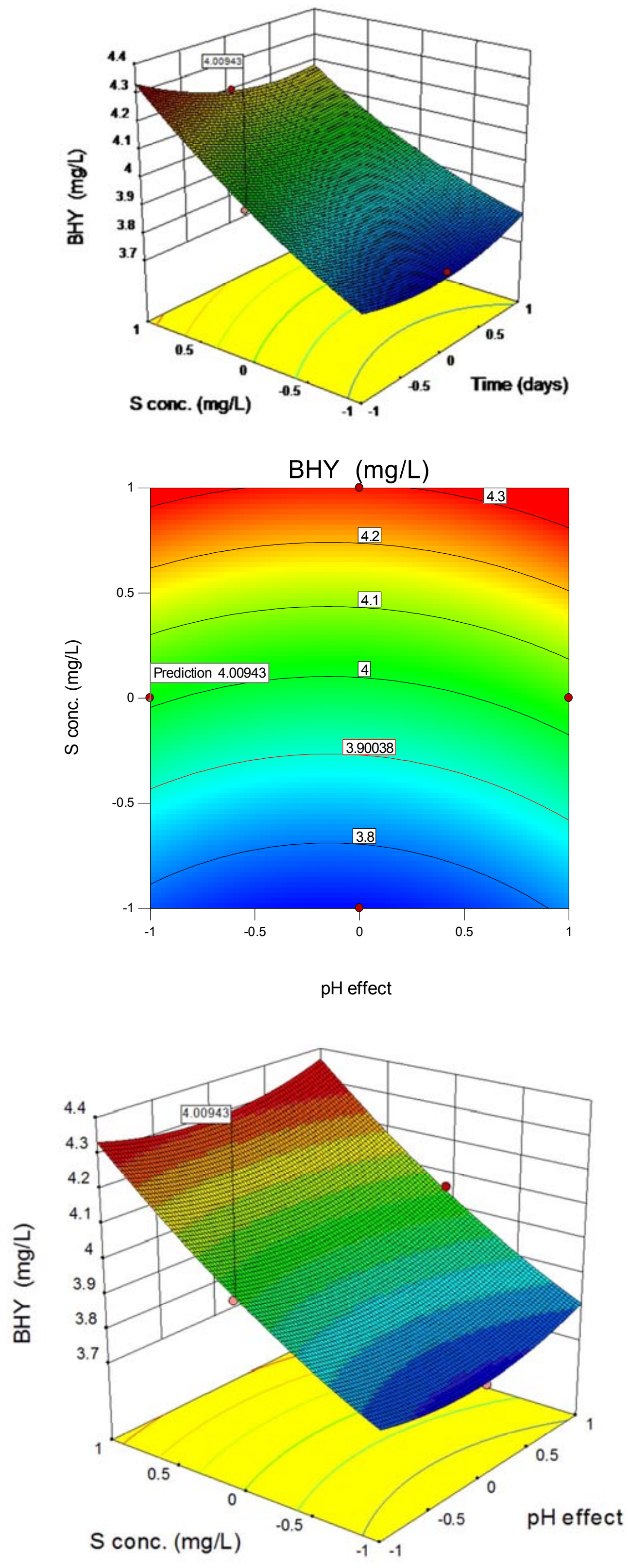


Fig. 2. *Contour and the 3-D plots.*

3.2. Physicochemical Properties of Agro Waste Water

Physicochemical properties of an agro waste water was carried out, the results shows that the initial low pH acidity of

5.46 at 24.3°C, total diffuse solid (TDS) of 3.93 mg/L, total hardness of 185 mg/L, conductivity of 0.786 ms/cm, chemical oxygen demand (COD) of 0.25 mg/L and biochemical oxygen demand (BOD) of 0.16 mg/L was measured. The analysis also showed the dissolve oxygen of 1.44 mg/L with alkalinity of 310 mg/L, ammonium and nitrate values were 0.47 and 2.08, respectively. The Carbon/Nitrogen ratio of the agro waste water was 0.36, which far less than the optimum between 20:1 and 30:1 for biohydrogen production from biomass. Insufficient carbon may have contributed to retarded effective gas generation at some point during the production. The phosphate value was 101.1 mg/L, calcium was 38 mg/L, potassium was 41 mg/L, and magnesium, sulfate, iron, zinc and nickel were 50, 73, 4.4, 19.5, 13.4 mg/L respectively. Other factors such as chlorine, aluminum and fluoride were also found.

4. Conclusion

This work demonstrates the use of response surface methodology to determine the optimum yield of biohydrogen from cassava waste water. The highest BHY was 4.25 ml at a coded factors of X_1= 0, X_2= 1 and X_3= 1, but the RSM statistical software predicted BHY of 4.009 ml at X_1= -1, X_2= -1.0 and X_3= -0.011 at desirability of 0.706. The study concluded that the cassava waste water is suitable for biohydrogen production.

References

[1] Hahn-Hagerdal, B. M., Gable, M. F., Gorwa-Graslund, G. Liden and G. Zacchi. (2006). Bioethanol– the fuel of tomorrow from the residues of today. *Trends in Biotechnology,* 24(12): 549-556.

[2] Wahab, A. K. A., Azwar M. Y., Hussain, M. A. (2014). Development of biohydrogen production by photobiological, fermentation and electrochemical processes: A review. *Renewable and Sustainable Energy Reviews*, 31:158-173.

[3] Van Ginkel, S., Sung, S. W., Lay, J. J. (2001). Biohydrogenproduction as a function of pH and substrate concentration. *Environmental Science & Technology,* 35(24), 4726-4730.

[4] Das, D., Verziroglu, T. N. (2001). Hydrogen production by biological process: A survey of literature. *International Journal of Hydrogen Energy*, 26(1), 13-28.

[5] Pandu, K., Joseph S. (2012) Comparisons and limitations of biohydrogen production processes: A Review. *International Journal of Advances in Engineering & Technology*, 2:342-356.

[6] Amorim, E. L. C., Barros, A. R., Damianovic, M. H. R. Z., Silva E. L. (2009). Anaerobic fluidized bed reactor with expanded clay as support for hydrogen production through dark fermentation of glucose. *International Journal of Hydrogen Energy*, 34(2), 783-790.

[7] Kapdan, I. K., Kargi, F. (2006). Bio-hydrogen production from waste materials. Enzymes and Micro Technology, 38:569-582.

[8] González, L. J. R., Dávila, I. M. M. M., García, Y. G., De la Garza, J. A. R., Martínez J. R. (2011). Biohydrogenproduction from diary processing wastewater by anaerobic biofilm reactors. *African Journal of Biotechnology*, 10(27):5320-5326.

[9] Ravi, K. P., Parihuar, K. U. (2015). Production of bio-hydrogen gas from waste water by anaerobic fermentation process: A Review. *International Journal Chemical Studies*, 3(3): 07-14.

[10] Luo, G., Xie, L., Zou, Z., Wang, W., Zhou, Q. (2010a). Exploring optimal conditions for thermophilic fermentative hydrogen production from cassava stillage. *International Journal of Hydrogen Energy*, 35(12), 6161-6169.

[11] 2016 Cassava production in Nigeria. http://en.wikipedia.org/wiki/cassava_production_in_Nigeria(accessed October 15, 2016)

[12] Cappelletti, B. M., Reginatto, V., Amante, E. R., Antônio, R. V. (2011). Fermentative production of hydrogen from cassava processing wastewater by *Clostridium acetobutylicum*. *Renewable Energy,* 36(12), 3367-3372.

[13] Mohan S. V., Babu V. L., Sarma P. N. (2007). Anaerobic biohydrogen production from dairy wastewater treatment in sequencing batch reactor (AnSBR): Effect of organic loading rate. *Enzyme and Microbial Technology*, 41:506-515.

[14] Montgomery, Douglas C. (2005). Design and Analysis of Experiments: Response surface method and designs.

[15] 2016 Response Surface Methodology. http://en.wikipedia.org/wiki/response_surface_methodology (accessed October 16, 2016)