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Hydrogen Production by Green Alga GAF99 in Sea Water Bioreactor: III Use of Modeling and Three Dimensional Plot to Investigate Critical Influence of pH

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Abstract: Biohydrogen production by green algae is effected by environmental conditions. The aim of this study was to show the influence of pH on biohydrogen production by *Chaetomorpha* sp. GAF99, green alga isolated from Gulf of Aqaba, in seawater bioreactor under anaerobic condition. It was possible to generate a model {HP = 40.52896 e $(-15\times[(T(C)-24.8)^2+(pH-8.18)^2])$} and building three dimensional plot in order to show the critical influence of various pH values on the biohydrogen production by *Chaetomorpha* sp. GAF99.The results revealed that the model can give significant relationship between biohydrogen production and pH according to ANOVA statistical analysis based on F and P-tests. The model and three dimensional plot obtained in this study showed clearly that the optimum biohydrogen production was recorded at pH values in the range of 8.2 to 8.7.

Key words: Three dimensional plot, biohydrogen production, green alga, *Chaetomorpha*

INTRODUCTION

For over 70 years marine and fresh water green algae have fascinated scientists and the public for their ability to produce bio-hydrogen (Gaffron and Rubin, 1942; Melis, 2002; Ji *et al*., 2010; Maneeruttanarungroj *et al*., 2010). Green algae are unique in their ability to metabolize hydrogen beside photosynthesis; the other eukaryotic organisms cannot express both activities (Melis, 2002). Bio-hydrogen is a metabolic product of hydrogenases, these enzymes are widespread in prokaryotic and lower eukaryotic organisms (Vignais *et al*., 2001; Yusoff *et al*., 2009) and were first reported in 1931 (Stephenson and Stickland, 1931). The available results on hydrogenases show that they are diverse in their protein structure and in the type of electron carrier they use (e.g., ferredoxins, rubredoxins and quinines), thus according to their metal content, three kinds of hydrogenases have been described: namely, the [NiFe]-hydrogenases (including the subfamily of [NiFeSe]-hydrogenases), the [Fe]-hydrogenases and the metal-free hydrogenases, among these, the [NiFe]-hydrogenases are the most abundant (Vignais *et al*., 2001). Enzymes which belong to [Fe]-hydrogenases are widely distributed in green algae and are responsible for ability of biohydrogen production (Winkler *et al*., 2002; Happe *et al*., 2002). The Fe-hydrogenases of green algae are extremely sensitive to oxygen, moreover it is reported that their gene expression is inhibited by oxygen (Melis, 2002). Thus, anaerobic green algal cultures are required for biohydrogen production; this is performed in sealed bioreactor. Recently marine microfilamentous green alga *Chaetomorpha* sp. GAF99 was shown to produce bio-hydrogen in sea water bioreactor under anaerobic condition (Ibrahim and Saeed, 2012; Saeed and Ibrahim, 2012). Furthermore, these studies indicated that the productivity of green alga was also affected by other environmental conditions, e.g., light, dark and temperature. On the other hand, various studies have shown the influence of pH values on the biohydrogen production by various organisms e.g., bacteria (Fang and Liu, 2002; Khanal *et al*., 2004) and algae (Maneeruttanarungroj *et al*., 2010). It had been suggested that pH values have critical effects on the metabolic activities of bio-hydrogen producing organisms; these are associated with hydrogenases (Dutta *et al*., 2005; Ni *et al*., 2006). Thus, in the following work surface response analysis was used to investigate the critical role of pH on biohydrogen productivity of green alga *Chaetomorpha* sp. GAF99 in sealed sea water bioreactor.

MATERIALS AND METHODS

Cultivation and maintenance of *Chaetomorpha* sp. GAF99: The green alga *Chaetomorpha* sp. GAF99 used in the present study was originally obtained in January

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2008 from shallow water of Gulf of Aqaba (Ibrahim and Saeed, 2012). Algal culture was cultivated and maintained at range of temperatures and pH values in 2 L open glass bioreactor containing one liter sea water obtained from Gulf of Aqaba (Ibrahim and Saeed, 2012). The sea water of algal culture was changed periodically at start of each experiment. Each experiment usually last for one to two months with new sea water.

Biohydrogen production: Biohydrogen production by marine green alga *Chaetomorpha* sp. GAF99 was followed in glass sealed 2 L bioreactor containing sea water of Gulf of Aqaba according to the reported methods (Ibrahim and Saeed, 2012). The algal cultures were incubated at in water bath and pH values were determined daily at 8:30 AM and at 17:30 PM. The volume of the generated gas from algal culture was collected in graduated glass cylinders and measured directly by water displacement. The composition of produced gases was analyzed quantitatively by GC-2014/Shimadzu.

Model generation: MATLAB software package was used to generate the model. Regression analysis was used to evaluate the developed model for the collected data of biohydrogen produced during active production phase at various pH values. The statistical analysis based on ANOVA test (Montgomery and Runger, 2007) was used to show the significance of the model based on F and T tests and P values with 95% confidence limit.

RESULTS

Influence of pH on biohydrogen production: During biohydrogen production cycle in sea water bioreactor, active production phase was observed after 10±2 days of lag period. This behavior expressed by *Chaetomorpha* sp. GAF99 was observed at specific pH values of culture. The obtained results showed that biohydrogen production starts in the production phase accompanied with changes in pH values of sea water in the sealed bioreactor. At start of new culture the pH value of seawater is 8.3, then during adaptation phase pH value decreased to 7.7. Then at this pH value biohydrogen production phase starts and continues to increase to maximum pH value of 8.822.

Building the model and three dimensional plot: The model which is shown in Eq. 1 correlates the influence of pH and temperature on the biohydrogen production (HP):

$$HP = 40.52896\ e\ (-15*[(T(C)-24.8)^2+(pH-8.18)^2]) \quad (1)$$

Multiple regression analysis based on ANOVA was utilized to show the significance of Eq. 1 based on F and t-tests as well as p-values as shown in Table 1, 2 and 3. This argument is based on the obtained results which indicated that the coefficient of determination (R^2 = 650661) is acceptable. Moreover, the obtained results also showed the significance F-value = 2.59007 E^{-60} which is <0.05 and the p-value = 2.11589E^{-60} which is also <0.05.

Three dimensional plot was built (Fig. 1) for the results of biohydrogen measurements during biohydrogen production phase by green alga *Chaetomorpha* sp. GAF99. The results showed three levels of biohydrogen produced during production phase, these were low, medium and high levels. At lower pH values (7.7-7.9) minimum production of biohydrogen (less than 5 mL) were recorded. Moderate production of biohydrogen (5-15 mL) was observed when pH values were between 8.0 and 8.2. On the other hand, the measured amounts of produced biohydrogen at pH values between 8.342 and 8.822 were within range 15 to 35.5 mL. However, the results of statistical analysis, as illustrated in the three dimensional plot in Fig. 1, showed the optimum amounts of produced hydrogen by *Chaetomorpha* sp. GAF99 were at pH values in the range of 8.2 to 8.7.

GC analysis of produced gasses: The results of measurements of gaseous mixture composition produced by *Chaetomorpha* sp. GAF99 indicated that the highest

Table 1: Regression model statistics

Regression	Statistics
Multiple R	0.806635
R^2	0.650661
R^2_{adj}	0.646754
SE (δ)	2.010705
Observations	275

Table 2: Adopted model of hydrogen production as a function of pH values and temperatures

	df	Sum of square	Mean of square	F or f_o	p-value
Regression	1	1927.719	1927.719	476.8119123	2.59007^{E-60}
Residual	256	1034.991	4.042934		
Total	257	2962.71			

Table 3: Numerical estimates of the regression model coefficients based on p-value with 95% confidence limit

					CI 95%	
	Coefficients	SE (β)	t-value	p-value	Lower	Upper
Slope (β)	40.52896	1.856059	21.83602	2.11589E^{-60}	36.87386874	44.18405

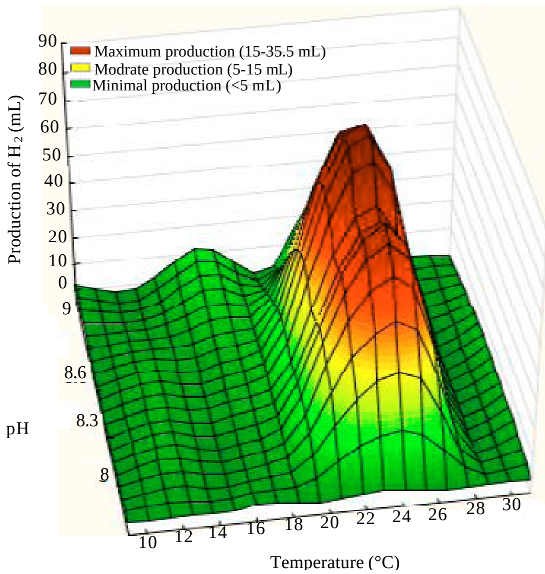

Fig. 1: Three dimensional plot showing the effect of pH and incubation temperature on the production of *Chaetomorpha* GAF 99

percentage of produced biohydrogen under anaerobic dark conditions in the gas mixture was 99.691 at pH value of 8.822 and temperature of 23.2; whereas the percentage of biohydrogen was lower (97.767) in early stage of production phase when the pH value was 7.42.

DISCUSSION

Biohydrogen production by algae is one of the promising potential biofuel in the future and is considered a hopeful approach for solving environmental problems associated with fossil fuels (Lindblad, 1999; Das and Veziroglu, 2001; Chader *et al*., 2009; Beer *et al*., 2009). Moreover, it is viewed as an attractive energy source for transportation by virtue of the fact that it is renewable and friendly to environment since it does not produce the CO_2 in combustion and as a consequence reduces air pollution (Balat, 2008; Nazlina *et al*., 2009; Vijayaraghavan *et al*., 2010).

Our previous study (Ibrahim and Saeed, 2012) showed that new cultured *Chaetomorpha* sp. GAF99 in anaerobic sea water bioreactor required adaptation period of approximately 10±2 days before starting the biohydrogen production stage which last for 10±2 days. In this study we report an interesting observation of very active production at certain phases during biohydrogen production stage. This observation was found related to the optimum pH value(s) for biohydrogen production. Accordingly, we postulate there is a certain critical value of alkaline pH which may be not optimum for algal growth but is suitable for biohydrogen production under anaerobic in dark environment. These results might be explained taking into account the ancient history of evolution of algae on earth. In other words, suitable conditions for biohydrogen production in sea water by green alga *Chaetomorpha* sp. GAF99 might be an ancient property associated with the requirement of alkaline optimum pH.

The results of present investigation showed that utilizing ANOVA statistical analysis and three dimensional plot revealed an optimum range of pH values for biohydrogen production. The given relationship in Eq. 1 showed a significant behavior of biohydrogen production in relation with given data of pH and temperature values. This was demonstrated by the p-value which is equal to 2.11589 E^{-60}, which was less than alpha value (α) of 0.05. In this respect, it is well known that pH values and temperature are important factors for optimum activities of various types of enzymes including hydrogenases which are responsible for biohydrogen production. It is possible to link the influence of pH on hydrogenases and biohydrogen production from various studies which investigated this biological phenomenon in various organisms. It had shown in bacteria, e.g., biohydrogen production by *Clostridium* sp. was highest in the pH range of 5.5-5.7 (Khanal *et al*., 2004), whereas others showed that the optimal pH for H_2 production by mixed culture of bacteria was 5.5 (Fang and Liu, 2002). On the other hand, higher pH values were found optimal for green algae; newly isolated green alga *Tetraspora* sp. CU2551 gave better biohydrogen productivity when the pH value was increased from 5.75 to 9.30 during the production phase (Maneeruttanarungroj *et al*., 2010). In this context it might be useful in this discussion to show the biochemical mechanism of biohydrogen production catalyzed by hydrogenase. Das and Veziroglu (2001) had reviewed in detail hydrogen production by hydrogenases. They reported that in a direct biophotolysis reaction, electrons flow from water through the two photosystems (PSII and PSI) of plant photosynthesis, to the hydrogen evolving enzyme hydrogenase via electron carrier Ferredoxin (Fd), as follows:

$$\begin{array}{l} H_2O \rightarrow PSII \rightarrow PSI \rightarrow Fd \rightarrow Hydrogenase \rightarrow H_2 \\ \downarrow \\ O_2 \end{array}$$

However the authors indicated that the rate of hydrogen production in this biochemical reaction is lower than typical rate for CO_2 reduction. This is because small amount of O_2 inhibits the hydrogenase activity during biophotolysis reaction which reduces hydrogen evolution. Furthermore Das and Veziroglu (2001) indicated that many microalgae, in particular species classified as "green algae", produce hydrogen after a period of anaerobic conditions in the dark, during which the hydrogenase enzyme is activated and synthesized and small amounts of hydrogen production are observed. It is important to emphasize that hydrogen photo evolution catalyzed by hydrogenases can only function under anaerobic conditions due to their extreme sensitivity to oxygen. Our data presented in this work and our previous studies (Ibrahim and Saeed, 2012; Saeed and Ibrahim, 2012) indicate the importance of temperature, anaerobic condition and dark period for providing the right condition for hydrogenase(s) of green alga *Chaetomorpha* sp. GAF99. These conditions facilitate hydrogen production by green alga *Chaetomorpha* sp. GAF99. Moreover, it might be possible to relate the optimum conditions for biohydrogen production to the activities of hydrogenases and their requirements to optimum pH. As mentioned earlier in this study, it is well known that pH values are important factors for optimum activities of various types of hydrogenases. In addition and taking in the account the possibility of designing more active hydrogenases by new molecular technologies (Ibrahim, 2012), it is expected that hydrogenases activity could be improved by new invented synthetic biology methodology.

It is worth mentioned at the end of this discussion the importance of economic side of hydrogen production. The reported results in this study provide an approach to make biohydrogen production more feasible. Since the economic cost for biohydrogen production and the required process for utilization of hydrogen are extremely high per unit of energy when compared to fossil fuel energy sources such as natural gas or gasoline. Thus optimization of biohydrogen production is considered a vital prerequisite from economical view point. This could be achieved by optimizing cultural conditions for the algal species used in biohydrogen production. Furthermore, economic feasibility of biohydrogen production could be further supported considering an integrated approach for biohydrogen production accompanied by utilizing the potential of algae for production other biofuels. It has been reported that various types of algae could be considered as an important source of several forms of renewable energy in addition to biohydrogen. These include biomass (Ilavarasi *et al*., 2011), bioelectricity (Rosenbaum *et al*., 2010), biogas (Vergara-Fernandez *et al*., 2008), bioethanol (Goh and Lee, 2010; Eshaq *et al*., 2010) and biodiesel (Wiley *et al*., 2011; Pfromm *et al*., 2011). The potential promise of green algae as source of renewable energy is further strengthened by the results of present investigation which showed *Chaetomorpha* sp. GAF99 as candidate for future biohydrogen production and other reported results which indicated possible utilization of *Chaetomorpha litorea* biomass in biogas production (Sangeetha *et al*., 2011).

CONCLUSIONS

The critical effects of pH values on the metabolic activities of *Chaetomorpha* sp. GAF99 are found decisive to obtain optimum conditions for biohdrogen production. The results of present investigation showed that by applying three dimensional plot it was possible to determine optimum range of pH values for biohydrogen production by *Chaetomorpha* sp. GAF99 in sea water culture under anaerobic dark condition. Three dimensional plot showed three ranges of pH values associated with the recorded amounts of biohydrogen produced during active production phase of green algal culture. The green alga *Chaetomorpha* sp. GAF99 gives better performance of biohydrogen production in alkaline pH as compared with lower pH values. The study gives further support to possible utilization of the green alga *Chaetomorpha* sp. GAF99 isolated from Gulf of Aqaba as a potential producer of renewable biohydrogen in seawater anaerobic bioreactor.

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REFERENCES

Balat, M., 2008. Potential importance of hydrogen as a future solution to environmental and transportation problems. Int. J. Hydrogen Energy, 33: 4013-4029.

Beer, L.L., E.S. Boyd, J.W. Peters and M.C. Posewitz, 2009. Engineering algae for biohydrogen and biofuel production. Curr. Opin. Biotech., 20: 264-271.

Chader, S., H. Hacene and S.N. Agathos, 2009. Study of hydrogen production by three strains of *Chlorella* isolated from the soil in the Algerian Sahara. Int. J. Hydrogen Energy, 34: 4941-4946.

Das, D. and T.N. Veziroglu, 2001. Hydrogen production by biological processes: A survey of literature. Int. J. Hydrogen Energy, 26: 13-28.

Dutta, D., D. De, S. Chaudhuri and S.K. Bhattacharya, 2005. Hydrogen production by Cyanobacteria. Microb. Cell Fact., Vol. 4.

Eshaq, F.S., M.N. Ali and M.K. Mohd, 2010. Spirogyra biomass a renewable source for biofuel (bioethanol) production. Int. J. Eng. Sci. Tech., 2: 7045-7054.

Fang, H. and H. Liu, 2002. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour. Technol., 82: 87-93.

Gaffron, H. and J. Rubin, 1942. Fermentative and photochemical production of hydrogen in algae. J. Gen. Physiol., 26: 219-240.

Goh, C.S. and K.T. Lee, 2010. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. Renew. Sust. Energ. Rev., 14: 842-848.

Happe, T., A. Hemschemeier, M. Winkler and A. Kaminski, 2002. Hydrogenases in green algae: Do they save the algae's life and solve our energy problems? Trends Plant Sci., 7: 246-250.

Ibrahim, M.A., 2012. An insight into the use of genome, methylome and gethylome in synthetic biology. Asian J. Applied Sci., 5: 67-73.

Ibrahim, M.A. and F.M. Saeed, 2012. Hydrogen production by green alga GAF99 in sea water bioreactor: 1. Isolation of alga and evaluation of environmental conditions. Biotechnology, (In Press).

Ilavarasi, A., D. Mubarakali, R. Praveenkumar, E. Baldev and N. Thajuddin, 2011. Optimization of various growth media to freshwater microalgae for biomass production. Biotechnology, 10: 540-545.

Ji, C., J. Legrand, J. Pruvost, Z. Chen and W. Zhang, 2010. Characterization of hydrogen production by *Platymonas subcordiformis* in torus photobioreactor. Int. J. Hydrogen Energy, 35: 7200-7205.

Khanal, S.K., W.H. Chen and S. Sung, 2004. Biological hydrogen production: Effects of pH and intermediate products. Int. J. Hydrogen Energy, 29: 1123-1131.

Lindblad, P., 1999. Cyanobacterial H_2 metabolism: knowledge and potential/strategies for a photobiotechnological production of H_2. Biotecnol. Aplicada, 16: 141-144.

Maneeruttanarungroj, C., P. Lindblad and A. Incharoensakdi, 2010. A newly isolated green alga *Tetraspora* sp. CU2551 from Thailand with efficient hydrogen production. Int. J. Hydrogen Energy, 35: 13193-13199.

Melis, A., 2002. Green alga hydrogen production: Progress, challenges and prospects. Int. J. Hydrogen Energy, 27: 1217-1228.

Montgomery, D.C. and G.C. Runger, 2007. Applied Statistics and Probability for Engineers. 4th Edn., John Wiley and Sons, New York, ISBN-13: 978-0470067215.

Nazlina, H.M.Y., A.R.N. Aini, F. Ismail, M.Z.M. Yusof and M.A. Hassan, 2009. Effect of different temperature, initial ph and substrate composition on biohydrogen production from food waste in batch fermentation. Asian J. Biotechnol., 1: 42-50.

Ni, M., D.Y.C. Leung, M.K.H. Leung and K. Sumathy, 2006. An overview of hydrogen production from biomass. Fuel Process. Technol., 87: 461-472.

Pfromm, P.H., V. Amanor-Boadu and R. Nelson, 2011. Sustainability of algae derived biodiesel: A mass balance approach. Bioresoure Technol., 102: 1185-1193.

Rosenbaum, M., Z. He and L.T. Angenent, 2010. Light energy to bioelectricity: Photosynthetic microbial fuel cells. Curr. Opin. Biotech., 21: 259-264.

Saeed, F.M. and M.A. Ibrahim, 2012. Hydrogen production by green alga GAF99 in sea water bioreactor: 2. Modeling of the effect of temperature. Biotechnology, (In Press).

Sangeetha, P., S. Babu and R. Rengasamy, 2011. Potential of green alga *Chaetomorpha litorea* (Harvey) for biogas production. Int. J. Curr. Sci., 1: 24-29.

Stephenson, M. and L.H. Stickland, 1931. Hydrogenase: A bacterial enzyme activating molecular hydrogen. Biochem. J., 25: 205-214.

Vergara-Fernandez, A., G. Vargas, N. Alarcon and A. Velasco, 2008. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass Bioenergy, 32: 338-344.

Vignais, P.M., B. Billoud and J. Meyer, 2001. Classification and phylogeny of hydrogenases. FEMS Microbiol. Rev., 25: 455-501.

Vijayaraghavan, K., R. Karthik and S.P.K. Nalini, 2010. Hydrogen generation from algae: A review. J. Plant Sci., 5: 1-19.

Wiley, P.E., J.E. Campbell and B. McKuin, 2011. Production of biodiesel and biogas from algae: A review of process train options. Water Environ. Res., 83: 326-338.

Winkler, M., A. Hemschemeier, C. Gotor, A. Melis and T. Happe, 2002. [Fe]-hydrogenases in green algae: Photo-fermentation and hydrogen evolution under sulfur deprivation. Int. J. Hydrogen Energy, 27: 1431-1439.

Yusoff, M.Z.M., M.A. Hassan, S. Abd-Aziz and N.A.A. Rahman, 2009. Start-up of biohydrogen production from palm oil mill effluent under non-sterile condition in 50 L continuous stirred tank reactor. Int. J. Agric. Res., 4: 163-168.