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Hydrogen Production by Green Alga GAF99 in Sea Water Bioreactor: II Modeling the Effect of Temperature

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Abstract: The aim of this study is to ascertain the influence of temperature on the bio-hydrogen productivity of marine green algae *Chaetomorpha* sp. GAF99 in sea water bioreactor system by utilizing multiple regression analysis. The biohydrogen production by green alga cultures was measured periodically at different incubation temperatures of active biohydrogen production phase to generate a model as well as surface response. The generated model {$P = 26.81015e\ (-0.1[(T_e-22.6)^2+(T_m-24.7)^2])$} relates production of H_2 (mL) as a function of temperature at two daily measurements (i.e., T_m, T_e). The results revealed that the model can give significant correlation ship between biohydrogen production and temperature of incubation according to ANOVA statistical analysis based on F and p tests. The model and surface response curve obtained in this study showed clearly that the optimum biohydrogen production was recorded at the dark period at 23.2°C.

Key words: Marine green algae, *Chaetomorpha* sp. GAF99, biohydrogen production, Gulf of Aqaba, multiple regression analysis, ANOVA statistical analysis

INTRODUCTION

Algae are the fastest growing plants on the planet and have great potential to produce various forms of energy (Demirbas and Demirbas, 2010). They have been shown as an important source of renewable energy; these include biohydrogen (Chader *et al*., 2009; Beer *et al*., 2009), biogas (Vergara-Fernandez *et al*., 2008), bioethanol (Goh and Lee, 2010; Eshaq *et al*., 2010), biodiesel (Miao and Wu, 2004; Wiley *et al*., 2011; Pfromm *et al*., 2011) and bioelectricity (Rosenbaum *et al*., 2010). Moreover these photosynthetic organisms have been known for their other important economical products which are used in cosmetics, bioactive functional compounds, pigments, food and feed (Plaza *et al*., 2010; Spolaore *et al*., 2006; Del-Campo *et al*., 2007; Ming *et al*., 2012).

Biohydrogen production by algae is one of the promising potential biofuel in the future and is a hopeful approach for solving environmental problems associated with fossil fuels. It is viewed as an attractive energy source for transportation by virtue of the fact that it is renewable, considered 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). One important aspect of hydrogen production is the economic cost for production and the required process for utilization of hydrogen which are extremely high per unit of energy when compared to fossil fuel energy sources such as natural gas or gasoline. These issues require consideration in long term plans for high excellence research and development of hydrogen production. The reported results of research work and studies in this field have shown that the production of biohydrogen and other biofuels by biological systems and in particular by algae is an area of promising applications providing optimizing the cultural conditions for higher yield of biohydrogen (Benemann, 1997; Lindblad, 1999; Levin *et al*., 2004; Kotay and Das, 2008). Early studies on the ability of bacteria (Stephenson and Stickland, 1931) and algae (Gaffron and Rubin, 1942) to produce biohydrogen had been mostly a biological curiosity. Subsequently research activities in this subject have shown that biohydrogen can be produced by various genera of blue-green (Ghirardi *et al*., 2000; Dutta *et al*., 2005) and green algae (Guana *et al*., 2004; Markov *et al*., 2006). In this context, green algae or Chlorophyta are the largest phylum of algae can live in diverse habitats, including fresh and sea water (Sandgreen, 1991; Anderson, 2005; Norris, 2010). Recently, the authors were able to isolate and characterize marine green alga *Chaetomorpha* sp. GAF99 from Gulf of Aqaba which produces biohydrogen under anaerobic and dark

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conditions in seawater bioreactor (Ibrahim and Saeed, 2012). The study indicated the importance of light/dark periods, anaerobic condition and temperature of incubations on hydrogen productivity of *Chaetomorpha* sp. GAF99. The role of temperature was found critical in the process of biohydrogen production. Production was shown to drop drastically during production phase at higher incubation temperature 33°C. Thus for an efficient process of hydrogen production in seawater bioreactor requires generating mathematical kinetic model of the process. Such studies will provide useful information on optimization of critical factors for the process effectiveness of biohydrogen production (Palazzi *et al*., 2002; Akande *et al*., 2006). The main argument of present investigation is to provide the required information on the effect of temperature which will assist the simulation and design of future seawater bioreactor for bio-hydrogen production by marine green alga *Chaetomorpha* sp. GAF99.

MATERIALS AND METHODS

Marine alga *Chaetomorpha* sp. GAF99: Marine green alga *Chaetomorpha* sp. GAF99 which was isolated originally in February 2008 from various shallow water niches (1-2 m deep) of Gulf of Aqaba shore (Ibrahim and Saeed, 2012) was used in present investigation. The cultures were maintained at room temperature in two liters flasks containing one liter sea water, the pH value of sea water is 8.3.

Biohydrogen production: Biohydrogen production by the marine green alga *Chaetomorpha* sp. GAF99 was followed according to reported method (Ibrahim and Saeed, 2012). Bio-hydrogen production was measured twice daily at 8.30 am (T_m) and at 17.30 pm (T_e) to determine the influence of temperature on productivity at various temperatures. The volume of the generated gas from algal culture incubated in 2 L flask was collected in the graduated glass cylinders and was measured directly by water displacement. The measurements of the composition of produced gases were performed qualitatively by detection instrument CD100/Kane-May/Kane International Ltd. (EN61000-6-3) and quantitatively by GC-2014/Shimadzu.

Model generation: Regression analysis was used to develop a model for the collected data of biohydrogen produced during active production phase at various temperatures. The values of biohydrogen produced in active production phase were subjected to various mathematical models by using MATLAB software package to find the optimum model that defines the behavior of algae as a function to two temperatures. 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

Effect of temperature on biohydrogen production: Biohydrogen production by *Chaetomorpha* sp. GAF99 in sea water bioreactor under anaerobic and dark conditions starts after a period of 10±2 days of adaptation period. The production phase, which continues for another 10±2 days, is influenced greatly by temperature of incubation. The higher incubation temperature (33°C) was found not favorable for hydrogen production. In order to find the optimum correlation that represents the production of biohydrogen (P) as function of temperatures, a statistical analysis was conducted to find an optimum model to show the role of temperatures on biohydrogen production by *Chaetomorpha* sp. GAF99.

Model generation: The model was generated as a function of two temperatures measured at 8:30 AM (T_m) and at 5:30 PM (T_e). The regression model statistics was utilized based on ANOVA test. The numerical estimates of the regression model coefficients were based on 95% confidence limit (significance level (α) = 1-0.95 = 0.05). The significance normally assessed using the t-test statistics (significance F or p-values) which must be $<\alpha = 0.05$ to be significant. The results which are shown in Table 1-3 indicated that the coefficient of determination

Table 1: Regression model statistics

Regression	Statistics
Multiple R	0.885871
R^2	0.784767
Adjusted R^2	0.684767
Standard error	9.848025
Observations	11

Table 2: Adopted model of hydrogen production as a function of two temperatures (T_e and T_m)

SOV	df	Sum of square	Mean of square	F-value	Significance
Regression	1	3536.154	3536.154	36.46136	0.000193
Residual	10	969.836	96.9836		
Total	11	4505.99			

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

					CI 95%	
	Coefficients	SE	t-value	p-value	Lower	Upper
Slope (β)	26.81015	4.439998	6.038324	0.000126	16.91722	36.70308

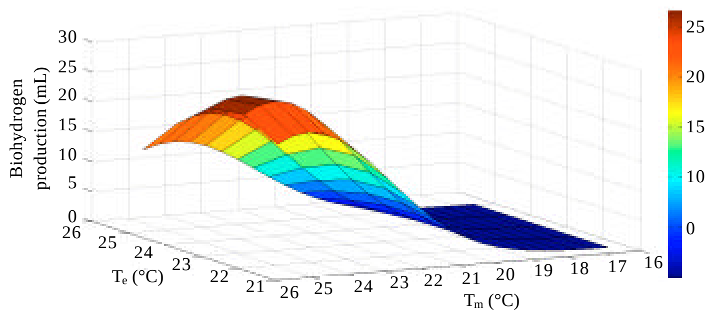

Fig. 1: Three dimensional plot showing the surface response of biohydrogen production as a function of two temperatures T_e and T_m as in Eq. 1

(R^2 = 78.47%) is acceptable. The obtained results also show the significance F value = 0.000193 which is <0.05 and the p value = 0.000126 which is also <0.05. Therefore the overall model given in Eq. 1 is significant.

Equation 1 represents an exponential form for the relation of biohydrogen production (P) as a function of two temperatures (T_e and T_m).

$$P = 26.81015\ e\ (-0.1[(T_e-22.6)^\wedge 2+(T_m-24.7)^\wedge 2]) \quad (1)$$

Furthermore, three dimensional mapping of the surface response (Fig. 1) of Eq. 1 shows clearly that the highest biohydrogen production was recorded at the morning [8:30 a.m.] (T_m) and the least production was recorded at the evening temperature (T_e) which was measured at 5:30 p.m. In other word the main production of biohydrogen production is occurring during dark period at T_m. The statistical analysis showed that the optimum temperature for biohydrogen production is 23.2°C.

DISCUSSION

Ever since the discovery that the green alga *Scenedesmus* was able to produce hydrogen during anaerobic conditions (Gaffron and Rubin, 1942), efforts have been made to investigate the influence of various environmental factors on hydrogen production. Temperature is one of important environmental factors which have critical effect on biohydrogen production by various tested organisms. It had shown that biohydrogen production by *Anabaena* was highest at 27°C (Prabina and Kumar, 2010). Whereas other study investigated production by *Chlamydomonas* sp. MGA161, a marine green alga, when cultivated at a high CO_2 concentration (15% CO_2) and low temperature (15°C), under these conditions the total productivity was significantly improved (Miura *et al*., 1993). On the other hand, hydrogen production was studied using immobilized green alga *Chlorella* sp., the rate of hydrogen evolution was increased as temperature increased from 37-40°C (Song *et al*., 2011). In this context, our previous study (Ibrahim and Saeed, 2012) showed that production of hydrogen is almost ceased at 33°C. As part of our efforts to understand the role of environmental factors in biohydrogen production by *Chaetomorpha* sp. GAF99 culture, the collected data of biohydrogen produced at various temperatures during production phase were subjected to regression analysis to generate the model shown in Eq. 1 (see the result section) which represents the biohydrogen productivity of *Chaetomorpha* sp. GAF 99 population for various periods during 2010 and 2011. It was possible to demonstrate in the present investigation the correlation between hydrogen productivity of *Chaetomorpha* sp. GAF 99 in sea water bioreactor and incubation temperature. The given relationship in Eq. 1 showed a significant behavior of biohydrogen production in relation with the given data of T_e and T_m. This was demonstrated by the R square value which is equivalent to 78.4767% (Table 1) and the p value which is equal to 0.000126 and was less than alpha value (α) of 0.05.

It is worth to mention that our study gave further support to utilize sea water for biohydrogen production. In addition and in contrast to the widely used freshwater green algae, the goal of our research was to ascertain utilization of marine green algae-based bioreactor system for biohydrogen production by developing a mathematical model to correlate biohydrogen production with incubation temperature. It is presumed that generating such system will demonstrate the cost feasibility effectiveness for sustained H_2 photo-production. Thus by utilizing seawater as the substrate and solar light as the source of energy and right temperature beside other environmental conditions it might be possible to develop economically feasible system for biohydrogen production. The specific objectives of this study could be met by developing such mathematical models. These include higher production at lower cost in sustainable manner.

The results of this study and our other two studies (Ibrahim and Saeed, 2012; Saeed and Ibrahim, 2012) and those reported by other investigators (Palazzi *et al*., 2002; Prabina and Kumar, 2010; Song *et al*., 2011) show the importance of more standardized careful study which requires modeling and statistical analysis to define the effect of temperature as a crucial environmental factor in addition to other environmental factor (i.e., pH) in the production of biohydrogen by marine green alga *Chaetomorpha* sp. GAF99. Furthermore and taking in the account the influence of temperature on the activity of hydrogenases, key enzymes for biohydrogen production by green algae (Saeed and Ibrahim, 2012), it is possible to suggest designing improved hydrogenases for efficient production of biohydrogen at higher temperature by utilizing new molecular technologies (Ibrahim, 2012).

CONCLUSION

Marine green algae have been shown as promising candidates for future biohydrogen production. In this study *Chaetomorpha* sp. GAF99 was found dominant species in specimens obtained from habitats of Gulf of Aqaba. The green alga produced biohydrogen in bioreactor contained natural sea water and showed an interesting response to temperature. The study revealed the critical role of temperature on the amounts of biohydrogen produced during active production phase. The model and surface response curve obtained in this study showed clearly that the optimum biohydrogen production was recorded at the dark period at 23.2°C.

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REFERENCES

Akande, A., A. Aboudheir, R. Idem and A. Dalai, 2006. Kinetic modeling of hydrogen production by the catalytic reforming of crude ethanol over a co-precipitated Ni-Al_2O_3 catalyst in a packed bed tubular reactor. Int. J. Hydrogen Energy, 31: 1707-1715.

Anderson, R., 2005. Algal Culturing Techniques. Elsevier Academic Press, New York. .

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.

Benemann, J.R., 1997. Feasibility analysis of photobiological hydrogen production. Int. J. Hydrogen Energy, 22: 979-987.

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.

Del-Campo, J.A., M. Garcia-Gonzalez and M.G. Guerrero, 2007. Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. Applied Microbiol. Biotechnol., 74: 1163-1174.

Demirbas, A. and M.F. Demirbas, 2010. Green Energy and Technology: Algae Energy. Springer-Verlag, London..

Dutta, D., D. De, S. Chaudhuri and S.K. Bhattacharya, 2005. Hydrogen production by Cyanobacteria. Microb. Cell Fact., Vol. 4. 10.1186/1475-2859-4-36

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.

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

Ghirardi, M.L., L. Zhang, J.W. Lee, T. Flynn, M. Seibert, E. Greenbaum and A. Melis, 2000. Microalgae: A green source of renewable H_2. Trends Biotechnol., 18: 506-511.

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.

Guana, Y., M. Denga, X. Yu and W. Zhang, 2004. Two-stage photo-biological production of hydrogen by marine green alga *Platymonas subcordiformis*. Biochem. Eng. J., 19: 69-73.

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. Biohydrogen production by Green Alga *Chaetomorpha* sp. GAF99 from Gulf of Aqaba: 1 isolation and characterization of Alga. Biotechnology, (In Press).

Kotay, S.M. and D. Das, 2008. Biohydrogen as a renewable energy resource-prospects and potentials. Int. J. Hydrogen Energy, 33: 258-263.

Levin, D., L. Pitt and M. Love, 2004. Biohydrogen production: Prospects and limitations to practical application. Int. J. Hydrogen Energy, 29: 173-185.

Lindblad, P., 1999. Cyanobacterial H2 metabolism: knowledge and potential/strategies for a photobiotechnological production of H2. Biotecnol. Aplicada, 16: 141-144.

Markov, S.A., E.R. Eivazova and J. Greenwood, 2006. Photostimulation of hydrogen production in the green alga *Chlamydomonas reinhardtii* upon photoinhibition of its oxygen evolving system. Int. J. Hydrogen Energy, 31: 1314-1317.

Miao, X. and Q. Wu, 2004. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. J. Biotechnol., 110: 85-93.

Ming, L.C., R. Nurliyana, A.B. Syah, M.N. Azizah, H.L. Sim and M.Y. Hirzun, 2012. Identification and biochemical composition of a green microalgae. Asian J. Biotechnol., 4: 38-45.

Miura, Y., W. Yamada, K. Hirata, K. Miyamoto and M. Kiyohara, 1993. Stimulation of hydrogen production in algal cells grown under high CO2 concentration and low temperature. Applied Biochem. Biotecgnol., 39-40: 753-761.

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.

Norris, J.N., 2010. Marine Algae of the Northern Gulf of California: Chlorophyta and Phaeophyceae. Smithsonian Institution Scholarly Press, Washington, pp: 50-51.

Palazzi, E., P. Perego and B. Fabiano, 2002. Mathematical modelling and optimization of hydrogen continuous production in a fixed bed bioreactor. Chem. Eng. Sci., 57: 3819-3830.

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

Plaza, M., S. Santoyo, L. Jaime, G.G.B. Reina, M. Herrero, F.J. Senorans and E. Ibanez, 2010. Screening for bioactive compounds from algae. J. Pharm. Biomed. Anal., 51: 450-455.

Prabina, B.J. and K. Kumar, 2010. Studies on the optimization of cultural conditions for maximum hydrogen production by selected cyanobacteria. ARPN J. Agric. Biol. Sci., 5: 22-31.

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.F.M. and M.A. Ibrahim. 2012. Biohydrogen production by Green Alga Chaetomorpha sp. GAF99 from Gulf of Aqaba: III. Use of modeling and three dimensional plot to investigate critical influence of pH. Biotechnology, (In Press).

Sandgreen, G.D., 1991. Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, Cambridge..

Song, W., N. Rashid, W. Choi and K. Lee, 2011. Biohydrogen production by immobilized *Chlorella* sp. using cycles of oxygenic photosynthesis and anaerobiosis. Bioresource Technol., 102: 8676-8681.

Spolaore, P., C. Joannis-Cassan, E. Duran and A. Isambert, 2006. Commercial applications of microalgae. J. Biosci. Bioeng., 101: 87-96.

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.

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.