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Hydrogen Production by Green Alga GAF99 in Sea Water Bioreactor: 1. Isolation of Alga and Evaluation of Environmental Conditions

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Abstract: Green algae are promising source of various types of biofuels and many are capable to catalyze production of hydrogen. In the present work isolation and characterization of green alga collected from Jordan's coastal part of Gulf of Aqaba are reported. Morphological details of the purified green alga GAF99 indicated it belongs to genus *Chaetomorpha*. Potential of the alga for hydrogen production in sea water bioreactor was investigated under various environmental conditions. Hydrogen productivity of *Chaetomorpha* sp. GAF99 was influenced by temperature and light. The main algal capability of hydrogen production occurs during dark period at 23±1°C and it is decreased drastically at higher temperature. Gas chromatography analysis of gaseous mixture obtained under different environmental conditions showed that the percentages of hydrogen gas were in the range 97.767 -99.494. The reported results might indicate that green alga GAF99 has a promising potential for hydrogen production under specific environmental conditions.

Key words: Biofuels, hydrogen , green alga, sea water bioreactor, environmental conditions

INTRODUCTION

Hydrogen is one of the promising future biofuels; it is renewable, does not produce carbon dioxide and is considered friendly to the environment (Nazlina *et al*., 2009; Vijayaraghavan *et al*., 2010). Moreover, hydrogen is viewed as a future attractive energy source for transportation since it liberates large amounts of energy per unit weight (Lindblad, 1999; Yusoff *et al*., 2009). Investigation in this field has shown various ways for hydrogen production, it can be prepared by photoelectrochemical or thermochemical processes and can be produced by biological processes (Skjanes *et al.,* 2008). One of important aspect of hydrogen production is the economic costs for production, which are extremely high per unit of energy when compared to fossil fuel. It might be possible to overcome this problem through development of efficient hydrogen production by algae. Scientists have shown great interest in utilization of algae to develop economically feasible hydrogen production systems (Benemann, 1997; Vijayaraghavan *et al*., 2010). The production of H_2 from algae has been mostly a biological curiosity and it was first reported in 1942 (Gaffron and Rubin, 1942). Further research work showed that hydrogen can be produced by various genera of green and blue green algae, these can live in diverse habitats, including fresh and sea water (Greenbaum *et al*., 1983; Ghirardi *et al*., 2000; Melis, 2002; Dutta *et al*., 2005). In contrast to the widely used freshwater algae, the goal of our research is to ascertain utilization of marine algae based in sea water bioreactor system for hydrogen production. It is presumed that development of such system is cost effective for sustained H_2 production. Thus, the aim of this research was to identify green marine alga collected from Gulf of Aqaba and to ascertain its potential for hydrogen production.

MATERIALS AND METHODS

Isolation and characterization of algae: Algae samples were originally collected in February 2008 from shallow water niches of Gulf of Aqaba shore. The specimens were cleaned by removal of adhering materials like sands particles and other debris. The cultures were purified by micromanipulation and maintained at room temperature in seawater cultures with pH value equivalent to 8.3. Slides were prepared for microscopical examination of algae specimens for characterization of the isolates. Characterization was conducted according to standard methods (http://www.algaebase.org/; Clesceri *et al*., 1998; Bellinger and Sigee, 2010; Norris, 2010).

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Maintenance of algae: For routine maintenance of marine algae, natural seawater was used rather than artificial seawater. The seawater for algae culture maintenance was obtained from open coastal areas away from industrial and housing areas of the Gulf of Aqaba. Sea water was filtered and sterilized before use. The 250 mL, one liter and two-liter pyrex glass flasks were used for cultivation and maintenance algal cultures.

Experimental setup for hydrogen production: The hydrogen production experiments were conducted in sealed sea water bioreactor. The setup is composed of two liter capacity flask containing one liter sea water was used for growing the algal population. Temperature and pH values were measured by suitable submerged temperature sensor and pH electrode. The pH meter and temperature sensor were obtained from Hanna instruments, USA and Digithermo, China, respectively. The algae cultures were exposed to artificial illumination using a light source. The light intensity meter supplied by ELY chemical company limited U.K. was used for measurement of light intensity. Water bath was used to apply artificial control to the temperature.

Quantities of produced hydrogen were collected by displacement method in graduated cylinder. Plastic hose used as a connecting bridge between the flask containing the algal population and the graduated cylinder which was also used to collect gas samples for GC analysis using a syringe.

Cultural conditions of hydrogen production: Hydrogen production was investigated in a sealed sea water bioreactor setup containing *Chaetomorpha* alga under artificial conditions. The sea water of algal culture was changed periodically at start of each experiment. Each experiment usually last for one to two months. The two liters capacity flasks, each containing one liter of sea water, were incubated at 23±1 and 33±1°C. The algal cultures were subjected to periodic exposure to darkness and artificial light (1513 lux). Measurement of the amount of produced gas was carried out daily at 8.30 a.m. and at 17.30 p.m. by water displacement method.

Qualitative and quantitative analysis of produced gasses: The measurements of produced hydrogen in various experiments were performed qualitatively by detection instrument CD100/Kane-May/Kane International Ltd. (EN61000-6-3). In addition, samples of produced gases were analyzed quantitatively by GC-2014/Shimadzu to measure the amounts of hydrogen, oxygen, nitrogen methane and carbon dioxide.

Statistical analysis: Mean, median, mode and standard deviation are calculated according to reported statistical methods (Montgomery and Runger, 2007). A low standard deviation indicates that the data points tend to be very close to the mean, whereas high standard deviation indicates that the data points are spread out over a large range of values.

RESULTS

Characterization of algae in collected specimens: Three genera of algae, *Chaetomorpha, Oscillatoria* and *Anabaena,* have been identified in the samples obtained from Gulf of Aqaba.

Blue-green algae *Anabaena* sp. showed characteristic easy to recognize trichome, thread-like series of cells, exclusive of the sheath. The observed filaments were curved and the appearance of cells is barrel-shaped string. Heterocysts, distinctive enlarged cells with thickened walls, were also observed. The other identified blue green alga, *Oscillatoria* sp., showed typical cap cell at end of filament and distinctive purple color (Clesceri *et al*., 1998; Bellinger and Sigee, 2010).

The other identified alga, *Chaetomorpha*, was found dominant in the collected specimens. This alga showed the characteristic green unbranched filamentous phenotype of the family Cladophoraceae. The filaments were found uniseriate and intertwining forming extensive mats. Further investigation showed that the alga had the taxonomic features of the species *C. linum*; two types of filaments were observed, filaments attached to the substratum by rhizoid extending from the basal cell and forming clumps and tufts, while the other type were unattached floating filaments (Fig. 1). Our investigation showed the importance of presence of small marbles in the bioreactor for growth of *Chaetomorpha*. These marbles formed the base for growth of the substratum. The identified green alga was designated *Chaetomorpha* sp. GAF99.

The cultures of this green alga were investigated for their ability to produce hydrogen, since our extensive review of literature showed no published reports on hydrogen production by *Chaetomorpha* sp.

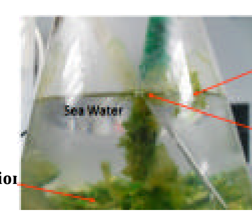

Fig. 1: Two types of algal growth demonstrated by green alga GAF99 in sea water bioreactor, floating and attached intertwining filamentous mats

Hydrogen production: The obtained results in this study showed that the green alga *Chaetomorpha* sp. GAF99 culture requires adaptation period before starting hydrogen production. The algal culture does not start producing hydrogen for up to eight days from date of inoculation and then active production phase will start. These two phases were observed during the study (Fig. 2).

The influence of temperature and light on hydrogen production was investigated during six days of the hydrogen production phase in comparison with hydrogen production of the day which showed optimum productions. The study revealed that hydrogen production is greatly influenced by light exposure and temperature. The data showed that main production of hydrogen by *Chaetomorpha* sp. GAF99 is during the dark period (Fig. 3). The cultural conditions were 12 h of light exposure followed by 12 hours of incubation in dark, the incubations temperature was 23±1°C. Exposing the algal culture to 24 h light period followed by 24 h dark period resulted in drastic decline in hydrogen production especially in day six of production phase (Fig. 4).

The influence of higher temperature on hydrogen production showed clear negative impact on hydrogen production. The obtained results showed that incubating algal culture at 33±1°C ceased production during six days of production phase (Fig. 5).

The obtained statistical analysis of the results shown in Figures 3, 4 and 5 are demonstrated in Tables 1, 2 and 3. In our case it is obvious that the value of Standard Deviation (SD) is less than the value of the mean as shown in Table 1 for the production of H_2 in the presence and absence of illumination although the value of SD in the absence of illumination is less than the value of SD in the presence of illumination, indicating greater hydrogen production in absence of illumination. But, in both cases production of hydrogen gas was noticed. On the other hand it is obvious that the values of SD in Tables 2 and 3 are greater than the values of mean which reflects that the data points were spread out over a large range of values.

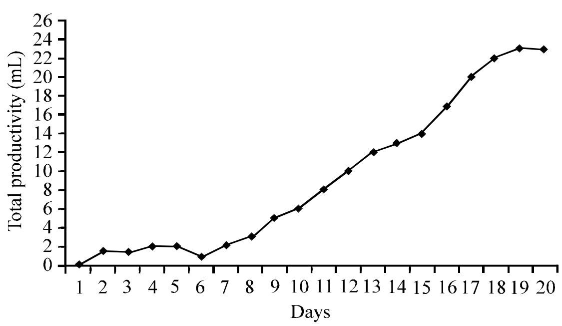

Fig. 2: Hydrogen production by green alga GAF99 in sea water bioreactor at 23 C±1°C

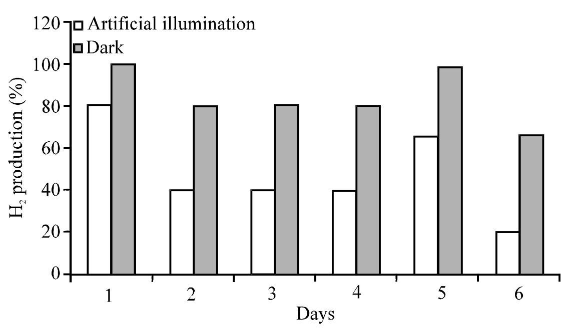

Fig. 3: Percentages of hydrogen produced by green alga GAF99 during six days of active production phase as compared with the highest amount produced during dark period of day five. Green alga was exposed to 12 hours periods of light/dark incubations at 23±1°C. Days of incubation represent the days of active production phase shown in Fig. 2

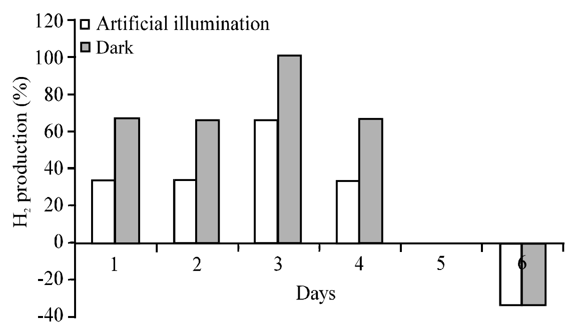

Fig. 4: Hydrogen production by green alga GAF99 when exposed for 24 h to light/dark incubation periods at 23± 1°C

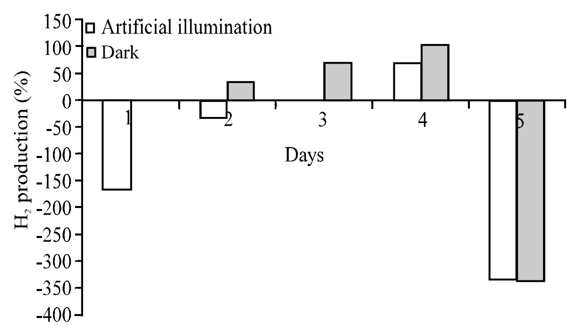

Fig. 5: Hydrogen production by green alga GAF99 when exposed for 24 h to light/dark incubation periods at 33±1°C

Table 1: Data of statistical analysis for results shown in of Fig. 3

	H_2 (%)	
Statistics	Artificial illumination	Dark
Minimum	20.00	66.67
Maximum	80.00	100.00
Mean	47.78	84.44
Median	40.00	80.00
Mode	40.00	80.00
Standard Deviation	21.67	13.11

Table 2: Data of statistical analysis for results shown in of Fig. 4

	H_2 (%)	
Statistics	Artificial illumination	Dark
Minimum	-33.33	-33.33
Maximum	66.67	100.00
Mean	22.22	44.44
Median	33.33	66.67
Mode	33.33	66.67
Standard Deviation	34.43	50.18

Table 3: Data of statistical analysis for results shown in of Fig. 5

	H_2 (%)	
Statistics	Artificial illumination	Dark
Minimum	-333.30	-333.30
Maximum	66.67	100.00
Mean	-93.33	-53.33
Median	-33.33	33.33
Mode	-333.30	-333.30
Standard deviation	158.80	180.40

Table 4: GC analysis of the gaseous mixtures produced by *Chaetomorpha* species GAF99 in anaerobic sea water bioreactor

Production phase (Day)	O_2 (%)	N_2 (%)	CH_4 (%)	CO_2 (%)	H_2 (%)
5	0.470	1.598	0	0.165	97.767
7	0.358	1.224	0	0.124	98.294
9	0.330	0.173	0	0	99.494

In other word it is clear that GAF99 started producing H_2 and then stopped due to the unfavorable operating condition.

The negative hydrogen measurements (negative signs) which are shown below X-axis might be demonstrated by the displacement method for hydrogen production. Where: ρ1: Water density, ρ2 : Hydrogen gas density, h1: Water head, h2: hydrogen gas level, P_{atm}: atmospheric pressure, P_1: Hydrogen gas pressure, P_2: Water head+atmospheric pressure. it is possible to drive following equations:

$$P_2 = \rho_2 gh_2 \quad (1)$$

$$P_1 = \rho_1 gh_1 + P_{atm} \quad (2)$$

At equilibrium, $P_1 = P_2$ or:

$$\rho_1 gh_1 + P_{atm} = \rho_2 gh_2 \quad (3)$$

Multiplying Eq. 3 by (1/g):

$$\rho_1 h_1 + P_{atm}/g = \rho_2 gh_2 \quad (4)$$

From Eq. 4, it is very clear that h_2 is directly affected when $P_2<P_1$ (when alga culture stops hydrogen production) [Non-equilibrium condition]. In other word we can have readings with negative signs which explain the results obtained in this study.

GC analysis: Three measurements were recorded using gas chromatography analysis of gaseous mixture produced by *Chaetomorpha* sp. GAF99 during various time intervals of production phase in sea water bioreactor. The results which are shown in Table 4 indicated that the percentages of hydrogen were in the range 97.767-99.494%.

DISCUSSION

Algae are promising source of biofuels especially hydrogen which has attracted the most attention among other possible products (Das and Veziroglu, 2001; Vijayaraghavan *et al.*, 2010; Ming *et al.*, 2012). In present study, two of identified algae, *Oscillatoria* and *Anabaena*, are well known producers of hydrogen (Dutta *et al.*, 2005). The other identified alga, *Chaetomorpha* GAF99, has not been reported as producer of hydrogen, although, species of this alga are widely distributed worldwide and occurring mainly in marine and brackish waters and rarely in freshwater (www.algaebase.org). The green alga *Chaetomorpha* GAF99 was collected from shallow water of the Gulf of Aqaba and showed resemblance to *C. linum* with respect to the floating biomass which may be explained by the reported characteristics of *Chaetomorpha* Kutzing (1845). This alga is characterized by unbranched filaments that attached to solid surfaces when young (Blackman and Tansley, 1902; Norris, 2010).

Temperature and light have great effect on hydrogen production by *Chaetomorpha* GAF99. This is expected since hydrogen production is influenced by environmental conditions, and light is known as one of several environmental factors affecting hydrogen production which included among others temperature (Dutta *et al.*, 2005). Hydrogen is usually produced during dark period and could be produced during day light under certain conditions (Phlips and Mitsui, 1983; Miura *et al.*, 1995). It is worth mentioning, other environmental conditions have been reported which affect algae growth and hydrogen production, such as nitrogen limitation, salinity, nutrient availability, pH and others (Atkins, 1922; Phlips and Mitsui, 1983; Dutta *et al.*, 2005; Joint *et al.*, 2011). The results of research work to develop models of affect of temperature on hydrogen production by *Chaetomorpha* sp. GAF99 showed the importance of such modeling analysis in hydrogen production by algae (Saeed and Ibrahim, 2012a). Furthermore surface response analysis was conducted by the same group to ascertain the influence of other environmental factor namely pH which has critical role in hydrogen production by *Chaetomorpha* sp. GAF99 (Saeed and Ibrahim, 2012b). The results presented in present investigation showed clearly the possibility of utilization of marine green alga *Chaetomorpha* sp. GAF99 in sealed sea water bioreactor system for hydrogen production. It is presumed that development of such system is cost effective for sustained H_2 production as compared with other systems, besides its economic importance in production of other biofuels (Sangeetha *et al*., 2011).

The importance of studying algae in hydrogen production comes from extensive and enormous efforts to develop new novel algae strains for biofuels production. Research in the field of hydrogen production is now directed on the improvement of algae by genetic engineering methods (Kruse *et al*., 2005; Melis *et al*., 2007). Moreover, current research in the field of synthetic biology is directed to design bacteria and algae more efficient in biofuels and hydrogen production, this might be achieved by designing genomic and gethylomic circuits (Savage *et al*., 2008; Agapakis *et al*., 2010; Ibrahim, 2012). Research in this field is considered next essential step following screening and identifying efficient hydrogen producing species in the genus *Chaetomorpha*.

CONCLUSIONS

Three genera of marine algae were identified in specimens collected from Gulf of Aqaba; two belong to blue green algae *Oscillatoria and Anabaena* which are well known producers of hydrogen. Whereas the other identified green alga *Chaetomorpha* sp. GAF99 has not been reported as hydrogen producer. Hydrogen is produced by *Chaetomorpha* sp. GAF99 under dark anaerobic conditions at 23±1°C in sea water bioreactor, but incubation at 33±1°C caused drastic decrease in biohydrogen production. The percentages of hydrogen produced by *Chaetomorpha* sp GAF99 during production phase were in the range 97.767 -99.494%.

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