BIOHYDROGEN PRODUCTION BY ALGAE ISOLATED FROM GULF OF AQABA

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ABSTRACT

Hydrogen is one of the promising futures Biofuel; it is renewable, does not produce carbon dioxide and is considered friendly to environment. 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 Biohydrogen production systems. Scientists have shown great interest in utilization of algae to develop economically feasible hydrogen production systems. The goal of our research is to ascertain the productivity of marine green algae in bioreactor system. Samples of algae were collected from various niches of Gulf of Aqaba shore, and the cultures were maintained in two litres capacity flasks, each contains one litre of sea water. Hydrogen production was measured twice daily to determine the influence of light and temperature. The algae cultures were subjected to periodic exposure to darkness and light. The obtained results showed an interesting response of hydrogen production in presence or absence of light, and temperature of incubation. Variation in hydrogen production was observed, this is expected since hydrogen production is influenced by environmental conditions, and light is known as one of several environmental factors affecting production which included among others: temperature, salinity, nutrient availability, gaseous atmosphere and pH. The research work is in progress to ascertain other factors which might affect hydrogen production. Microscopical examination of algae culture revealed three main predominant genera of algae, Chaetomorpha, Oscillatoria, Anabena. The three types of identified genera are known for their production of hydrogen.

Keywords: biohydrogen, renewable energy, marine algae, Chaetomorpha, Oscillatoria, Anabena

1. INTRODUCTION

Hydrogen gas is viewed as a future attractive energy source for transportation; it does not produce the CO₂ in combustion, and liberates large amounts of energy per unit weight [1]. Thus it is one of the promising potential fuels in the future, and is considered friendly to environment [2]. Investigation in this field has shown various ways for hydrogen production, it can be prepared by photoelectrochemical or thermochemical processes; moreover hydrogen can be produced by biological processes [3]. The production of H₂ from algae has been mostly a biological curiosity [4], and it was first observed by Gaffron and Rubin in 1942 [5]. Research work showed that Biohydrogen can be produced by various genera of algae [6]. In this respect it is worth noting, green algae and blue green algae are known for their ability of hydrogen production, and can live in diverse habitats, including fresh and sea water [7, 8].
In contrast to the widely used freshwater algae, the goal of our research is to ascertain utilization of marine algae based in bioreactor system for Biohydrogen production. It is presumed that development of such system is cost effective for sustained H₂ production. The specific goals of the investigation are to obtain marine algae from Gulf of Aqaba, to characterize the genera which are useful for biohydrogen production, and to ascertain some environmental conditions starting with the influence of light and temperature on hydrogen production.

1.1. Methods

1.1.1. Isolation and characterization of algae

Samples of algae were collected from various niches of Gulf of Aqaba shore. The cultures were maintained at room temperature in two litres capacity flasks, each flask contains one litre sea water with pH value equivalent to 8.3. Microscopical characterization of marine algae was carried out following the procedure of American standards for water analysis [9].

1.1.1. Hydrogen production

Samples of marine algae were inoculated in one litre sea water, incubated at room temperature (24 ± 1.5 °C), and subjected to periodic exposure of sun or artificial light and darkness. Measurement of gas production was carried out daily at 8.30 am and at 17.30 pm. The measurements were performed qualitatively by detection instrument CD100/Kane-May/Kane International Ltd (EN61000-6-3); and quantitatively by GC14/Shimadzu.

1.2. Results and discussion

1.2.1. Characterization of algae

Three genera of green algae have been identified in the samples obtained from Gulf of Aqaba. The predominant observed genera belong to: Chaetomorpha, Oscillatoria and Anabaena. Typical morphology of the three genera (Fig. 1.) was as shown in the American Standards for Water Analysis [9]. The three types of identified genera are known for their production of hydrogen [7, 10].

![Chaetomorpha, Oscillatoria, Anabaena](image)

Fig. 1. Typical microscopical morphology of three identified marine algae isolated from Gulf of Aqaba [9].
1.2.2. *Hydrogen production*

The obtained results in this study showed that new algae culture does not start producing hydrogen for up to eight days from date of inoculation, and then active production will start. These two phases for hydrogen production by marine algae cultures have been observed during the study. This observation indicates that each of new algae culture starts with adaptation period of about eight days, during this period negligible amount of gases are produced. Second phase which last for up to ten days is characterized by relatively active production of hydrogen (Fig. 2).

![Graph showing hydrogen production](image)

**Fig. 2.** Hydrogen production by algae batch culture in sea water.

On the other hand the study revealed that hydrogen production is greatly influenced by duration of light exposure, and raising the temperature from 22 °C to 34 °C caused a sharp decline in hydrogen production (Fig. 3).
It had been reported that hydrogen production is regulated by incubation temperature and light; hydrogen is usually produced during dark period and could be produced during day light under certain conditions [11, 12]. Thus it might be interesting to study the difference during the 24 hours of hydrogen productivity in dark and in comparison with light period of exposure. Gas production was monitored during exposure to day light starting at 8.30 am and at 17.30 pm. The obtained results indicated that batch culture of algae gave low hydrogen productivity when exposed to light; however the productivity was enhanced during the night (Fig. 4).

Several investigators reported the influence of other environmental conditions which affect algae growth and hydrogen production, such as nitrogen limitation, salinity, nutrient availability, pH and others [7, 12, 13, 14]. The research work is in progress to ascertain other factors associated with hydrogen production. It is worth noting that research in the field of Biohydrogen production is now directed on the improvement of algae by genetic engineering methods [15, 16]. Research in this field is considered next essential step following screening hydrogen producing algae in the natural marine habitats.
2. EQUATIONS

Hydrogen production is governed by two types of enzymes, nitrogenases and hydrogenases, the following equations were reported in two excellent reviews [7, 17], these equations demonstrate the biochemical reactions for hydrogen production:

(a) Reactions catalyzed by nitrogenases

\[ 16\text{ATP} + 16\text{H}_2\text{O} + \text{N}_2 + 10\text{H} + 8\text{e} \rightarrow 16\text{ADP} + 16\text{Pi} + 2\text{NH}_4 + \text{H}_2 \]

During this process hydrogen is produced as a byproduct of fixation of nitrogen into ammonia. In this reaction nitrogenases require ATP [7].

(b) Reactions catalyzed by hydrogenases

Das and lu 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:
\[ \text{H}_2\text{O} \rightarrow \text{PSII} \rightarrow \text{PSI} \rightarrow \text{Fd} \rightarrow \text{Hydrogenase} \rightarrow \text{H}_2. \]
\[ \downarrow \]
\[ \text{O}_2 \]

However the authors indicated in their review 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 Lu 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. [17]. It is important to emphasize that hydrogen photo evolution catalyzed by nitrogenases or hydrogenases can only function under anaerobic conditions due to their extreme sensitivity to oxygen [7].

3. CONCLUSIONS

Three genera of marine algae were isolated from Gulf of Aqaba; these showed ability of hydrogen production in sea water culture. Exposure to light has great influence on productivity as well as the incubation temperature. Optimum temperature for hydrogen production is in the range 22-25 °C.

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