

# Biological Hydrogen Production from *Amphora* sp. Isolated from Eastern Coast of Thailand

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**Abstract.** The world is finding ways of producing fuel from many sources to replace the fossil fuels. Hydrogen is considered one of the most promising fuels for the future. One biological way of producing hydrogen from solar energy is using photosynthetic microorganisms. The objective of this study is to search for marine algae which produce hydrogen and study the appropriate conditions to produce hydrogen from marine algae. Firstly, the 5 strains of algae were studied the total gas production. *Amphora* sp. was selected and studied the appropriate conditions to produce hydrogen gas. The first condition, we studied the important factors for marine algae which were present and absent sulfur. The second condition was to find the suitable pH for producing hydrogen which were pH 7, pH 8 and pH 9. The last condition, we studied the optimal light intensity which were 481, 1075 and 2085 lux. The result showed that *Amphora* sp. can produce hydrogen gas in present sulfur media, pH 8 and light intensity 2085 lux in volume 495.3 ml per 1 L of algae or the average rate of produce hydrogen is 0.798 ml per g of algae per hour.

## 1 Introduction

When it comes to looking for the energy source that will replace fossil fuels, there is no shortage of options. The hydrogen is an alternative energy that can be used to replace conventional energy. The features of hydrogen are a clean energy because when the fuel cell will not release carbon dioxide out [1-3], so it does not cause global warming [4] to burn high and not cause air pollution [5]. The use of algae in the production of hydrogen is because algae are plants different from extra fuel is generally faster in photosynthesis [6], algae can bring solar energy used in photosynthesis faster than other alternative crops and can be cultured without a lot of trouble [6]. After cultured, algae can be used as fertilizer, animal feed substance, cosmetic or even used as medicine.

Some green microalgae, such as *Chlamydomonas reinhardtii* [7] and *Chlorella salina* [8] produce hydrogen via direct photolysis [9]. Hydrogen gas evolution in algae requires a certain period of anaerobic incubation to induce the reversible hydrogenase. The hydrogenase then functions to combine electrons and protons to form hydrogen gas [10-17]. However, despite extensive research, this process has low yields and is rather feeble. A study by other investigators isolated a unicellular hydrogen-producing green alga, *Tetrasporasp.*, from freshwater pond in Thailand with efficient hydrogen production [18]. However, it is even more preferable to have organisms to generate hydrogen using seawater because they do not require arable land and do not affect fresh water resources, can be produced using the ocean. It is important to find the native species of marine algae

that should suitable for cultivation and hydrogen production. Our study is an initial step designed to fill this gap. In this work, we collected and isolated the marine algae which have the ability to produce the hydrogen gas to find the suitable conditions. The conditions that we tested were sulfur deprivation, pH, and light intensity.

## 2 Material and method

### 2.1. Algal cultures

The hydrogen producing culture was enriched from a sample collected from the eastern coast of Thailand. Many species of marine algae were collected during low tide by hand-picking from the coast of Chanthaburi Province, Thailand and purified by plating, streaking, and single colony picking. The collected marine algae were identified by morphological identification method. All algal cells were grown aerobically under constant shaking at 25 °C under fluorescent light (2085 lux). Marine algal cells were grown under continuous light in Tris-acetate-phosphate (TAP) medium at pH 8.0.

### 2.2. Algal growth conditions

All algal cells were grown aerobically under constant shaking at 25°C under fluorescent light. Marine algal cells were grown under continuous light in TAP medium, pH 8.0.

### 2.3. Hydrogen evaluation

In the linear growth phase ( $15$  to  $25 \mu\text{g Chl}^{-1} \times \text{ml}^{-1}$ ), algal cells were harvested, then transferred into squared glass bottles, sealed with a gas-tight septum and exposed to  $2085 \text{ lux}$ . After total gas production, total gas was used to evaluate the hydrogen volume by replacing a total gas with  $1.2 \text{ mol/l NaOH}$  and  $0.85 \text{ mol/l MnCl}_2$ . Sodium hydroxide and manganese chloride would trap the  $\text{O}_2$  from the total gas [19].

## 3 Result

### 3.1. Total gas production

Total gas production volume for each alga was measured by water replacement method. The information generated was used in selecting the best algae for hydrogen production. Production of total gas by *Anacystis sp.*, and *Amphora sp.* increased only slightly during 0-20 h (Figure 1). Total gas production increased rapidly during 20-30 hours, and finally became stable in 30-40 hours. *Anacystis sp.* was the highest gas producing strain of the group at the last stage. The total gas production of *Cheatoseros sp.* increased during 0-3 hours and became stable thereafter, followed by a small final increase. *Tetraselmis sp.* and *Nitzschia sp.* cultures did not show any gas production. *Amphora sp.*, was selected to be tested for further experiment because of the high volume of biological hydrogen production at the first stage (0-40 hours).

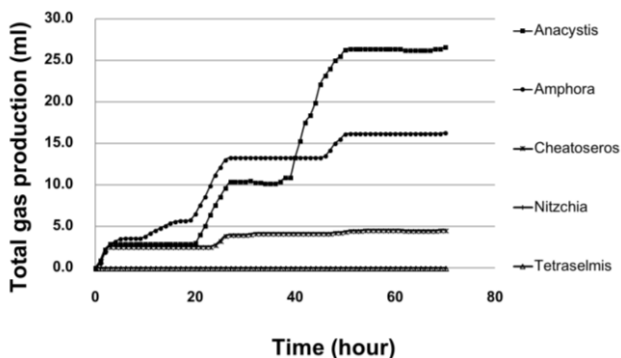


Fig. 1. The relationship between the gas volume and time for all the algae.

### 3.2. Effect of composition of sulfur in the medium on hydrogen yield

A suitable method, developed in order to produce sustainable hydrogen, has been reported where the process is handled in 2 stages. Algae were grown in TAP medium, washed and transferred to TAP or TAP-S (S-deprived) medium and pH were 8.3. From Figure 2, a graph showed hydrogen production of *Amphora sp.* every 1 hour. Results showed that there were 3 stages of hydrogen production in unchanged medium. The first stage was 0-24 hours, the second stage was 25-47 hours, and third stages was 48-67 hours. The highest hydrogen

production time was at 67 hours which produced 148.6 ml. However, the hydrogen increased continuously at all stages in unchanged medium. On the other hand, previous studies have shown an increased hydrogen production in cells cultivated in S-deprived conditions during the production phase [20].

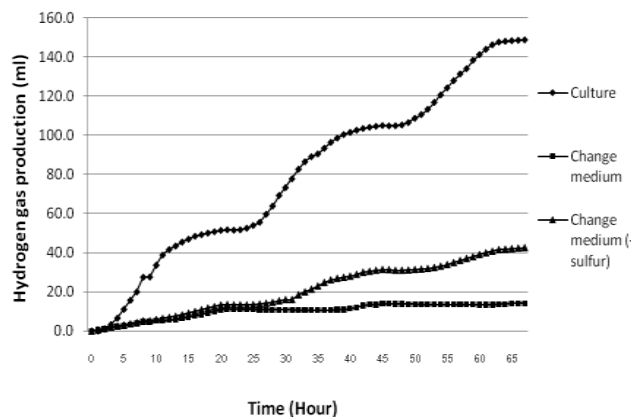


Fig. 2. The graph shows the relationship between time with the amount of hydrogen of *Amphora sp.* in the medium that has no sulfur and sulfur.

### 3.3. Effect of pH on hydrogen yield

To examine the dependence of hydrogen evolution on pH, *Amphora sp.* cells were grown in TAP medium for 5 days, washed and transferred to medium at desired pH (7, 8, 9) and then illuminated under the light. This experiment was designed to know the condition of acid-base which effects on hydrogen production. In this test, we adjusted the pH of the medium to 7, 8 and 9 before inoculation. From Figure 3, a graph showed hydrogen production of *Amphora sp.* every 1 hour in the different pH. The hydrogen production was highest at pH 8. In this condition, the hydrogen gas was 68.5 ml in 60 hours or the average rate of produce hydrogen is 1.151 ml per g of algae per hour.

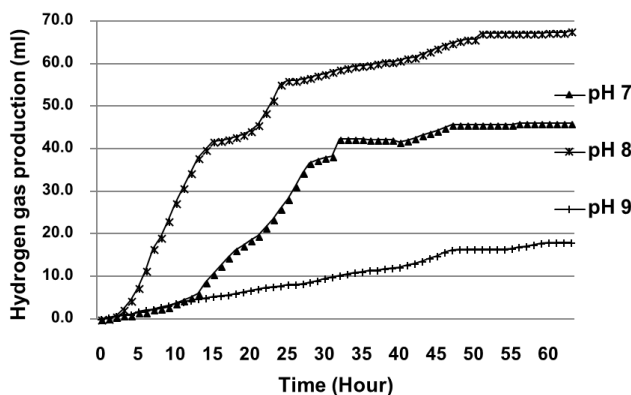
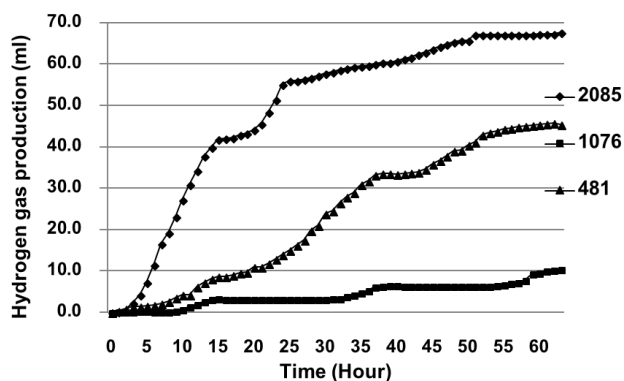


Fig. 3. The graph shows the relationship between time with the amount of hydrogen of *Amphora sp.* in the different pH.

### 3.4. Effect of light intensity on hydrogen yield

This experiment was to find the suitable light intensity because of the amount of light effect on the growth and photosynthesis of the algae. To find the suitable light intensity for optimal production of hydrogen, the algae were subjected to light exposure at various intensities (481, 1076, 2085 lux). From Figure 4, the light intensity of 2085 lux *Amphora sp.* algae could produce hydrogen at the highest volume.



**Fig. 4.** The graph shows the relationship between time with the amount of hydrogen of *Amphora sp.* in the different light intensity.

#### 4 Discussion and Conclusion

The optimum condition of *Amphora sp.* to have the high volume of hydrogen gas was a medium which was continued from the medium growth. The result showed that *Amphora sp.* grown in unchanged medium in Figure 2 was the best condition to produce hydrogen gas in pH 8 and light intensity 2085 lux in volume 495.3 ml per 1 L of algae or the average rate of produce hydrogen was 0.798 ml per g of algae per hour.

In many cases, the certain dark period was also necessary for conversion of sugars into alcohol and other metabolite formation including hydrogen. Generally certain species and strains of *Chlorella*, *Chlamydomonas*, *Nannochloropsis* etc. tolerate light fluctuations and exhibits the high growth rate including yield [21]. Generally, acidic condition alters the morphology of algal cells and limits the motility of *Euglena mutabilis* and *Chlamydomonasapplanata* [22]. Low pH condition limits the nutrient uptake which leads to the reduction of photosynthetic ability thereby decreasing the accumulation of total carbon and evolution of oxygen [23]. However, a significant method of hydrogen production is not developed for the massive level. The enhancement of biological hydrogen production would be focused on using various environmental and biochemical approaches because it would help us to understand the response of *Amphora sp.* in different environmental conditions with respect to hydrogen production.

#### 5 Acknowledgement

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