

## EXPERIMENTAL CULTIVATION OF *Spirulina platensis* USING MY AN MINERAL WATER, THUA THIEN HUE PROVINCE

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### ABSTRACT

*Spirulina platensis* was experimentally cultivated by semi-continuous method at 20 m<sup>2</sup> scale pilot for the effective use of the available bicarbonate and minerals in My An mineral water, Phu Vang district, Thua Thien Hue province in order to reduce production costs. Analysis results of My An water quality showed that this mineral water source was suitable to *Spirulina platensis* cultivation after removing H<sub>2</sub>S. The average yield of the alga reached 10 g dry biomass/m<sup>2</sup>.day. The harvested algal biomass contained high nutritive compositions and was a suitable source for functional food (68.32 % protein, 7.32 % lipids and low heavy metal concentration). The obtained results indicated that the My An mineral water source is indeed a suitable water source for producing *Spirulina platensis* biomass as a functional food for human and animal feed.

**Keywords:** algal biomass, functional food, My An mineral water, semi-continuous cultivation, *Spirulina platensis*.

### 1. INTRODUCTION

*Spirulina platensis* Geitl. (cyanobacterium) is a multicellular filamentous form, belonging to the Oscillatoriaceae, Cyanophyceae, Cyanophyta and is known as a rich-nutrient food source. Since 1970s, the studies on biochemical composition of *Spirulina* showed that this alga contained protein, carbohydrate, essential fatty acids, vitamins, microminerals, carotene, chlorophyll a and phycocyanin [1, 2]. The alga grows very fast and its biomass is doubled every 5 days. The productivity of harvesting protein is much higher in this algae than the one in soybean, corn and cow [3]. For this reason, *Spirulina platensis* is commonly used as a feedstock for aquaculture, functional food for human, and as a source of bioactive substances for medicine

and cosmetics. *Spirulina* is widely used in many countries in the world such as Germany, Brazil, Chile, Spain, France, Canada, Belgium, Egypt, USA, India, Japan, China, Vietnam, etc. Some famous companies producing *Spirulina* include Earthrise farms (USA), Cyanotech (USA), Hainan DIC microalgae Co., Ltd (China), Marugapa chettir research center (India), Genix (Cuba) and Solarium Biotechnology (Chile) [4]. Currently, there are many different methods of algal cultivation including the open or close systems through continuous or semi-continuous cultivation [5].

There are some factors which can influence the successful cultivation of *Spirulina platensis*. Among these factors, nutrient compositions, temperature, and light are key factors. They could influence the algal growth and metabolism processes, and nutrient compositions of algal biomass, which can lead to reducing the accumulation of algal biomass components [6]. Among nutrient factors, carbon is an extremely essential nutrient, accounting up to 45 % of *Spirulina platensis* dry biomass. Therefore, it is necessary to add carbon sources in cultivation process in order to meet the growth needs of algae, and reduce the cost of cultivation.

In Vietnam, *Spirulina* has been studied since 1970s. Institute of Biology (renamed as the Institute of Biotechnology) under the guidance of Prof., Nguyen Huu Thuoc studied effects of external conditions on the growth and development of *Spirulina*. The cultivation medium and simple technological process were successfully developed and applied at scale of 5000 m<sup>2</sup> at Binh Thuan province using Vinh Hao mineral water containing a high content of sodium bicarbonate. Therefore, the cost of cultivation medium could be reduced significantly [7, 8, 9]. Currently, the algal productivity at Vinh Hao Company is about 8-10 g of dry biomass/m<sup>2</sup>.day, and the production area is expanding. The biomass of *Spirulina* is mainly used as a functional food for humans and food supplements for domestic animals [10].

Nguyen Thi Bich Ngoc [11] researched successfully the process of *Spirulina* cultivation with high productivity using mineral water to produce foods for human and aquatic animals. Duong Duc Tien [12] also conducted a trial production of rich-nutrient food from *Spirulina* in Ha Noi through a research project funded by Ha Noi Department of Science and Technology. At present, a number of small and medium-sized installations of *Spirullina* cultivation functions for above-mentioned purposes in Ha Noi, Nghe An, Thanh Hoa [10].

In our study, we cultivated *Spirulina platensis* using My An mineral water to utilize the available bicarbonate and minerals of My An mineral water. The composition of mineral water, algal growth, and nutrient compositions of algal biomass were measured and analyzed in laboratory.

## 2. MATERIALS AND METHODS

### 2.1. Algae

*Spirulina platensis* GEITL was selected from the Algal collection of Institute of Environmental Technology, Vietnam Academy of Science and Technology (VAST).

### 2.2. Mineral water source

My An mineral water, Phu Duong commune, Phu Vang district, Thua Thien Hue province.

### 2.3. Methods

#### 2.3.1. Measurement of the algal growth

The growth of *Spirulina platensis* was indirectly determined by measuring OD at 445 nm wavelength on spectrum UV-Vis 2450 Shimatzu (Japan).

### 2.3.2. Analysis of $\text{HCO}_3^-$ và $\text{CO}_3^{2-}$ in the algal culture tank

The algal suspension was filtered through filter paper GF/C to remove algal cells, and then 50 ml of suspension was put into the 100 ml flask. Two drops of phenolphthalein 0.1 % in ethanol were added into the flask, and the suspension was titrated by HCl 0.1N. The amount of HCl 0.1 N used to remove the pink of suspension was V1. Two drops of orange methyl phenolphthalein 0.1% in ethanol then were added into the flask, and the suspension was titrated with HCl 0.1N. Volume of HCl 0.1N used to convert the solution from yellow to orange was V2. The amount of  $\text{HCO}_3^-$  và  $\text{CO}_3^{2-}$  was calculated by following formulas:

$$\text{CO}_3^{2-} \text{ (mg/L)} = (V1 * 60 * 0.1 * 1000) / 50$$

$$\text{HCO}_3^- \text{ (mg/L)} = [(V2 - V1) * 61 * 0.1 * 1000] / 50$$

### 2.3.3. Analysis of the nutrient composition of algal biomass

The amount of crude protein was determined by the Kjeldahl method, and then multiplied with 6.25. Total N (%), glucide, polysaccharide, ash, humidity were determined by Association of Official Analytical Chemists (AOAC) method. The composition of lipids, fatty acids, phycocyanin, and carotenoid were determined by UV-Vis spectrophotometry. Pb (mg/kg), Cd (mg/kg), Hg (mg/kg), As (mg/kg) were determined by Atomic Absorption Spectrometric (AAS) method. Chlorophyll-a was determined using the method described by Lorenzen [13].

### 2.3.4. Algal tank operation

The raceway tanks were used to cultivate *Spirulina platensis* biomass in many countries, including Vietnam. In our study, the raceway tank (Figure 1) was built in My An Joint Stock Company of Tourism, Phu Duong commune, Phu Vang district, Thua Thien Hue province. The tank had a total surface area of 20 m<sup>2</sup>, 10 m length, 2 m width, 0.5 m height. The two ends of tank were rounded to reduce the hindrance of water movements. In addition, two inoxidable tanks (3 m<sup>3</sup>) were prepared to store the mineral water and regularly clean the algal tank. The plastic containers (200 L) were used for algal breeding and preparing nutrient medium. The cultivation system was covered by white nylon.

Semi-continuous method is a mode of cultivation in which the nutrient medium is added after each time of algal biomass harvesting. Algal suspension column is 30 cm height. When suspension OD reaches 1.2 - 1.4, we can harvest the algal biomass until the OD reduces to 0.5 - 0.6, follow the nutrient supplying. Under this condition, the algal cells grow rapidly and can be harvested every 2-3 days. The advantages of this cultivation mode is that we can adjust timely and reasonably the nutrients supplying during the process, optimize the yield, quality and quantity of algal biomass.

+ The algal cultivation medium used in our study was Zarrouk's nutrient medium.

+ Mineral water taken from the Company was stored in the inoxidable tanks and left overnight or aerated to remove H<sub>2</sub>S.

+ Protective measures should be taken to prevent the algae from photoinhibition under intense lighting conditions while the suitable temperature for the growth of *Spirulina platensis* was from 28 °C to 35 °C.

+ Stirring the suspension medium was implemented by paddle wheel with suspension flow velocity of 18 cm/s.

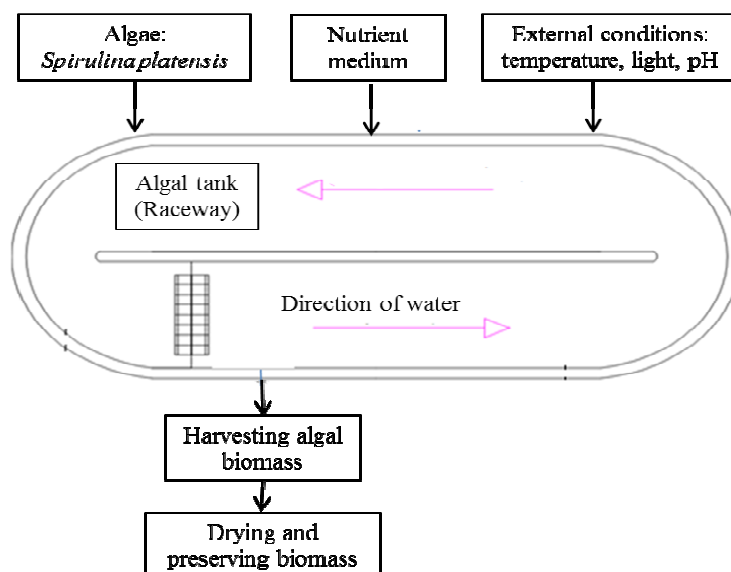


Figure 1. The raceway tank for *Spirulina* cultivation using My An mineral water.

*Tank operation:*

*Step 1:* Multiplying algal inoculate in flasks, and then transferring the alga step by step to the larger containers of 1 L; 2 L; 20 L; 100 L; 200 L (Figure 2).

*Step 2:* Preparing cultivation medium, and then transferring it into the raceway tank (Figure 1). New nutrient medium should be gradually added to the tank for alga adaptation to new condition during this process until the algal suspension reached  $6 \text{ m}^3$ . The temperature, pH, and water level were monitored everyday (Figure 3).



Figure 2. Growth of algae in plastic container of 200 L.

*Step 3:* The algal biomass was harvested by a filter net (0.02mm) when the OD reached 1.2

- 1.4, follow the nutrient supplying. The biomass then was dried by spraying dryer.

### 3.1. RESULTS AND DISCUSSION

#### 3.1. Chemical composition of My An mineral water

Table 1. Chemical composition of My An mineral water, Phu Vang district, Thua Thien Hue province.

No.	Parameters	Unit	Values
1	pH	-	7.78
2	Hardness (CaCO <sub>3</sub> )	mg/l	328.0
3	SiO <sub>2</sub>	mg/l	25.058
4	H <sub>2</sub> S	mg/l	0.48-1.39
5	Ammonia (NH <sub>4</sub> <sup>+</sup> )	mg/l	0.05
6	Sodium (Na)	mg/l	134.79
7	Potassium (K)	mg/l	58.67
8	Calcium (Ca)	mg/l	94.40
9	Magnesium (Mg)	mg/l	22.08
10	Iron II (Fe <sup>2+</sup> )	mg/l	< 0.05
11	Iron III (Fe <sup>3+</sup> )	mg/l	< 0.02
12	Aluminium (Al)	mg/l	< 0.02
13	Chlorine (Cl)	mg/l	250
14	Sulfate (SO <sub>4</sub> <sup>2-</sup> )	mg/l	2.577
15	Nitrate (NO <sub>3</sub> <sup>-</sup> )	mg/l	< 0.02
16	Nitrite (NO <sub>2</sub> <sup>-</sup> )	mg/l	< 0.02
17	HCO <sub>3</sub> <sup>-</sup>	mg/l	1055
18	Fluorine (F)	mg/l	0.887
19	Arsenic (As)	mg/l	0.00370
20	Cadmium (Cd)	mg/l	0.00019
21	Lead (Pb)	mg/l	0.00121
22	Mercury (Hg)	mg/l	0.00010
23	Copper (Cu)	mg/l	0.00374
24	Zinc (Zn)	mg/l	0.061
25	Phosphate (PO <sub>4</sub> <sup>3-</sup> )	mg/l	0.086

Analysis of mineral water (Table 1) showed that My An mineral water contained many minerals such as Mg, Na, K,... and especially high amount of bicarbonate (1055 mg/l). These minerals are the important nutrient sources for *Spirulina platensis* [14].  $\text{HCO}_3^-$  plays a significant role in maintaining a stable buffer system ( $\text{CO}_2\text{-H}_2\text{CO}_3\text{-HCO}_3^-$ ), and maintaining the pH at a suitable level for growth of the alga as well as minimizing the contamination. Furthermore,  $\text{HCO}_3^-$  provides carbon for photosynthesis of algae. Because amount of  $\text{CO}_2$  in the air cannot meet the carbon demand, then the supplement of  $\text{CO}_2$  or  $\text{HCO}_3^-$  during algal cultivation was very essential for high-yielding algal biomass system. The availability of  $\text{HCO}_3^-$  in My An mineral water was one of good conditions for growth of the alga, and for cutting down the cultivation cost. However, the sulfur hydrogen ( $\text{H}_2\text{S}$ ) in My An mineral water should be removed before cultivating the algae.

### 3.2. Productivity and quality of *Spirulina platensis*

#### 3.2.1. Productivity

The OD was measured every three days in order to evaluate the growth and productivity of *Spirulina platensis*. Figure 4 illustrates the results of OD taken at 445 nm wavelength.



(a) (b)  
Figure 3. The raceway tank before (a) and during algal cultivation (b).

Figure 4 shows a fluctuation of OD in the algal tank during the cultivation. In the first three days, *Spirulina platensis* grew slowly, OD went up from 0.36 to 0.60. After a period of 10 days of adaptation, the algal density rapidly increased from 0.6 to 1.3 and then declined slightly. The OD reached the peak at 1.44 during the experimental cultivation. Generally, the algal biomass could be harvested when OD exceed 1.2.

During the experimental cultivation, the algal biomass was harvested every 6 days. The obtained results showed that amount of algal biomass in different harvesting times was rather similar. The algal cultivation using My An mineral water reached the productivity of about 10 g dry biomass/m<sup>2</sup>.day. The algal yield in our study is equivalent to average one of Vinh Hao Joint Stock of Algae at large scale cultivation using mineral water [15] and of Dan Phuong Tuynel Brick Factory using  $\text{CO}_2$  separated from the flue gas [10, 16]. Our results are also similar to the results of algal cultivation using Danh Thach mineral water in a close system in Khanh Hoa [11]. The current study indicated good potential of My An mineral water for algal cultivation at large scale.

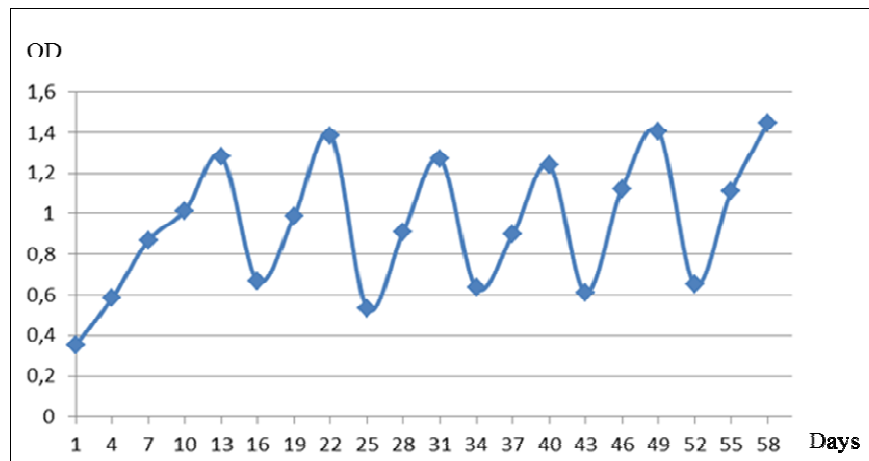


Figure 4. Growth of *Spirulina platensis* using My An mineral water.

### 3.2.2. Quality of algal biomass

Apart from evaluating the growth and productivity of *Spirulina platensis* cultivated in My An mineral water, we also analyzed chemical composition of the harvested algal biomass. The presented data (Table 2) indicated that the amount of crude protein and lipid of the algal biomass was very high, reaching 68.32 % and 7.32 % of dry biomass, respectively. The quality of algal biomass using My An mineral water in the cultivation process, was quite good and equivalent to ones of the dry *Spirulina* produced by Siam Algae Company (SAC) [17], of algal biomass cultivated in Danh Thach mineral water, Khanh Hoa [11], and of the algal biomass produced in Dan Phuong Tuynel Brick Factory [10]. The amount of heavy metals in algal biomass in our study was lower than the limited allowance for functional foods according to the Decision 46/2007/QĐ-BYT and QCVN 8-2:2011/BYT. This was an initial and important scientific basis for utilizing the biomass of *Spirulina* cultivated in My An mineral water as a source of rich-nutrient food.

Table 2. Results of the dried biomass quality of harvested *Spirulina platensis*.

No.	Parameter	Unit	Value	No.	Parameter	Unit	Values
1	Humidity	%	3.4	7	Carotenoids	%	0.42
2	Ash	%	7.46	8	Chlorophyll - a	%	1.34
3	Protein	%	68.32	9	Cadmium (Cd)	ppm	0.005
4	Fiber	%	0.43	10	Arsenic (As)	ppm	0.034
5	Glucide	%	11.31	11	Mercury(Hg)	ppm	0.008
6	Lipid	%	7.32	12	Lead (Pb)	ppm	0.168

#### 4. CONCLUSIONS

My An mineral water was suitable for the growth of *Spirulina platensis* after removing H<sub>2</sub>S. Productivity of *Spirulina platensis* cultivated in My An mineral water was about 10 g/m<sup>2</sup>.day. *Spirulina platensis* biomass had good quality with high amount of crude protein and fatty acids, reaching 68.32 % and 7.32 %, respectively. In addition, the amount of heavy metals detected in algal biomass was lower than the limited allowance for functional foods. Our study indicated that this algal biomass could be used as functional food for human and animal feed.

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