Nghiên cứu nuôi tảo xoắn *Spirulina platensis* trong các loại môi trường Zarrouk cải tiến

TÓM TẮT

Nghiên cứu này hướng tới cải tiến môi trường nuôi cấy tảo *Spirulina platensis*, nhằm xác định môi trường dinh dưỡng rẻ tiền phù hợp với việc nuôi cấy loài tảo này. Nghiên cứu được thực hiện trên 4 loại môi trường gồm môi trường Zarrouk cơ bản (M1), môi trường Zarrouk (75%) bổ sung muối Iod (M2), môi trường Zarrouk (50%) bổ sung muối Iod (M3), môi trường Zarrouk (25%) bổ sung muối Iod (M4). Các loại môi trường này được hòa tan bằng nước lọc RO. Định kỳ đo các giá trị pH, độ mặn của các loại môi trường và mật độ sinh khối tảo tại bước sóng 560 nm (OD_{560}). Kết quả cho thấy môi trường M3 có mật độ sinh khối tảo cao nhất sau 22 ngày nuôi (OD_{560} = 1,448). Giá trị pH và độ mặn của các loại môi trường đều nằm trong khoảng thích hợp cho tảo phát triển. Riêng đối với môi trường M4 thì ngày thứ 21 trở đi có giá trị pH >11 nên mật độ tảo giảm mạnh. Tiếp tục khảo sát 2 loại dung môi để hòa tan môi trường M3 là nước lọc RO và nước khoáng thiên nhiên tại Phước Mỹ, Bình Định. Kết quả cho thấy môi trường M3 được hòa tan bằng nước lọc RO có mật độ sinh khối tảo cao hơn so với hòa tan bằng nước khoáng. Vì vậy, môi trường M3 hòa tan bằng nước lọc RO được lựa chọn để nuôi tảo ở quy mô thí điểm nhằm tiết kiệm chi phí cũng như thu được lượng tảo nhiều nhất.

Từ khóa: *Spirulina platensis, Zarrouk, OD_{560}, mật độ sinh khối tảo.*

Research on cultivation of *Spirulina platensis* in modified Zarrouk media

TÓM TẮT

This study aimed to improve the cultivation medium for *Spirulina platensis*, with the goal of identifying a cost-effective nutrient medium suitable for cultivating this algae. The research was conducted on 4 types of media, including basic Zarrouk medium (M1), Zarrouk medium (75%) supplemented with iodized salt (M2), Zarrouk medium (50%) supplemented with iodized salt (M3), Zarrouk medium (25%) supplemented with iodized salt (M4). These media were dissolved with RO water. The pH values, salinity values of the media and the biomass density of the algae are periodically measured at a wavelength of 560 nm (OD_{560}). The results showed that M3 medium had the maximum biomass density after 22 days of cultivation with OD_{560} = 1,448. The pH and salinity values of all media remained within a suitable range for algae growth. However, for the M4 medium, the pH value exceeded 11 after the 21st day, resulting in a significant decline in algae density. Further investigation was conducted on 2 solvents used to dissolve the M3 medium: RO water and natural mineral water from Phuoc My commune, Binh Dinh province. The results indicated that the M3 medium dissolved in RO water had a higher algae biomass density compared to being dissolved in mineral water. Therefore, the M3 medium dissolved in RO water was chosen for pilot-scale algae cultivation to reduce costs while obtaining the highest possible algae yield.

Keywords: *Spirulina platensis, Zarrouk, OD_{560}, algae density.*

1. INTRODUCE

Spirulina platensis (S. platensis) is considered a superfood with high nutritional value, containing a protein content of 60-70%, which is three times higher than that of beef and more than twice that of soybeans. It also contains many essential amino acids for the human body, such as lysine, methionine, phenylalanine, tryptophan, and is rich in minerals like copper, zinc, magnesium, and iron. Additionally, it has a very high vitamin content. Besides its nutritional benefits, *S. platensis* has unique properties such as boosting immunity and helping to prevent diseases like cancer, hepatitis, and diabetes. As a result, the World Health Organization has acknowledged *S. platensis* not only as a safe food source but also as a valuable option for disease prevention and treatment in the 21st century.

The Zarrouk medium facilitates favourable algae growth; however, its cost is relatively high. Therefore, researching modified versions of the Zarrouk medium to reduce production expenses is highly important, as it can help lower the overall cost of cultivating *S. platensis*.

2. MATERIALS AND RESEARCH METHODS

2.1. Materials

Algae Culture Medium: Zarrouk medium, iodized salt, RO water, natural mineral water from Phuoc My commune, Binh Dinh province.

Spirulina platensis Algae Strain: The algae strain was sourced from Minh Thien Algae Supply Facility in Bac Ninh province. The morphological characteristics of the *S. platensis* strain (viewed under a MicroBlue optical microscope with a 40X objective) include a helical filamentous shape and a distinctive blue-green color.

2.2. Research methods

2.2.1. Experimental design

The experiment was conducted in the Plant Cell Tissue Culture Laboratory, Faculty of Natural Sciences, Quy Nhon University (Figure 1).

a. Experiment 1: Investigation of optimal nutritional medium for cultivating *S.platensis*

Investigation of the influence of 4 types of nutritional media on the biomass increase of Algae. The media used include:

M1: Basic Zarrouk medium.

M2: Zarrouk medium (75%) supplemented with iodized salt.

M3: Zarrouk medium (50%) supplemented with iodized salt.

M4: Zarrouk medium (25%) supplemented with iodized salt.

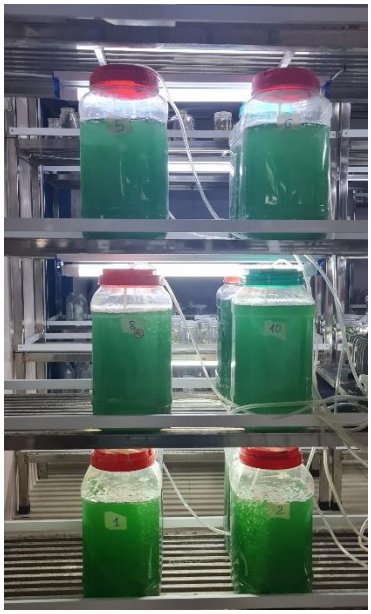

Figure 1. Experimental design

Each treatment was conducted in a 5-liter plastic flask. The *S. platensis* strain was inoculated into each flask to achieve an initial cell density of 0,061 OD. Continuous aeration was maintained throughout the cultivation process, with a temperature set at 28 °C and continuous lighting provided by LED lamps at an intensity of 1100 lux.

Monitored parameters included optical density (OD), pH levels, and salinity of the medium, with measurements taken daily throughout the cultivation process. The biomass density of the algae in each treatment was compared to select the optimal nutritional medium for cultivating *S. platensis*.

b. Experiment 2: Investigation of Solvent Selection for Preparing the Optimal Nutritional Medium for Algae Cultivation from Experiment 1

Investigation of two types of solvents for preparing the nutritional medium chosen in experiment 1: RO water and natural mineral water from Phuoc My commune, Binh Dinh province.

The treatments were performed under the same conditions as those in experiment 1.

Monitored parameters included optical density (OD), pH levels, and salinity of the medium, with measurements taken daily throughout the cultivation process. The biomass density of *S. platensis* in each treatment was compared to select the most appropriate solvent.

2.2.2. Analysis and evaluation methodology

Growth of *S. platensis* was monitored daily by determining the biomass density using the optical density (OD) measurement method. The OD was measured using a spectrophotometer (CECIL - UK) at a wavelength of 560 nm. This method facilitates the quantification of algal biomass, providing a reliable indicator of growth performance over the cultivation period.

Assessment of pH in the *S. platensis* cultivation medium: The pH of the algal cultivation medium was monitored daily and measured using a pH meter (WINLAB - Germany). This measurement helps ensure that the pH remains within the optimal range for the growth of *S. platensis*, as pH fluctuations can significantly affect algal growth and metabolic activity.

Assessment of salinity in the *S. platensis* cultivation medium: The salinity of the algal cultivation medium was monitored daily and measured using a refractometer. This measurement is crucial for ensuring that the salinity levels are appropriate for the growth of *S. platensis*, as variations in salinity can impact algal health and productivity.

2.2.3. Data processing methodology

The recorded data were processed using Excel and Minitab software. The data were presented as means and underwent statistical analysis to indicate significant differences.

3. RESULTS AND DISCUSSION

3.1. Survey for selecting optimal nutrient media for cultivating *S. platensis*

The basic Zarrouk medium (M1) is formulated with the following components:

Table 1. Composition of basic Zarrouk medium (M1)

No.	Component	Concentration (g/L)
1	$NaHCO_3$	16,8
2	K_2HPO_4	0,5
3	$FeSO_4.7H_2O$	0,01
4	$CaCl_2.2H_2O$	0,04
5	$MgSO_4.7H_2O$	0,2
6	NaCl	1,0
7	K_2SO_4	1,0

8	$NaNO_3$	2,5
9	EDTA	0,08
10	H_3BO_4	0,00286
11	$MnCl_2.4H_2O$	0,00181
12	$ZnSO_4.7H_2O$	0,00022
13	MoO_3	0,00001
14	$CuSO_4.5H_2O$	0,00008

Table 2. Composition of nutrient media M2, M3, and M4

No.	Medium	Composition Description
1	M2	75% Basic Zarrouk medium (M1) + 15 mL/L of Iodized Salt solution with 100‰ salinity
2	M3	50% Basic Zarrouk medium (M1) + 15 mL/L of Iodized Salt solution with 100‰ salinity
3	M4	25% Basic Zarrouk medium (M1) + 15 mL/L of Iodized Salt solution with 100‰ salinity

The *S. platensis* was cultivated in four types of media (M1, M2, M3, M4), dissolved in RO water, at a temperature of 28 °C, with continuous aeration and lighting. The following parameters were monitored: biomass density measured at a wavelength of 560 nm, pH, and salinity of the cultivation medium. The results obtained are as follows.

3.1.1. Changes in algal biomass density during the algal cultivation process

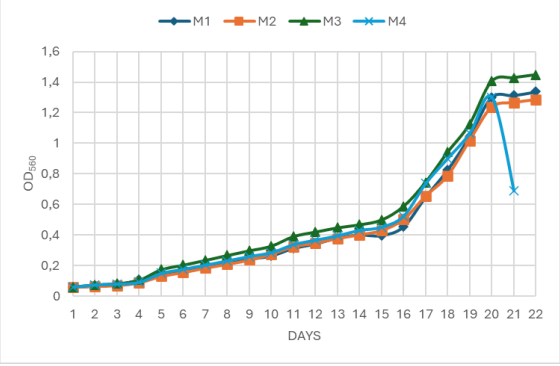

Figure 2. Development of biomass density of *S. platensis* in different nutrient media

The changes in biomass density of *S. platensis* across the four nutrient media are illustrated in Figure 2 and detailed in Table 3.

The results indicate that the biomass density of *S. platensis* increased in all four nutrient media. From Day 1 to Day 15, the increase in biomass density was minimal, corresponding to the lag phase, which is the period required for the algae to acclimate to the culture environment. From Day 16 to Day 20, a significant increase in biomass density was observed, corresponding to the logarithmic phase. After Day 21, biomass density for media M1, M2, and M3 showed negligible increases, indicating the stationary phase. However, in medium M4, there was a sharp decline in biomass density due to the pH of the environment rising above 11. Thus, after 22 days of cultivation, the highest biomass density was recorded in medium M3, which was statistically significant ($p < 0,05$).

According to previous research,[2] it is possible to substitute the sodium bicarbonate ($NaHCO_3$) content with sodium chloride (NaCl) in the cultivation medium for *S. platensis*. Reducing $NaHCO_3$ to a certain level, and fully replacing it, could result in lower productivity for the algae. Therefore, the author suggests investigating an appropriate concentration of NaCl as a replacement to ensure optimal growth of the algae.

Thus, it is feasible to reduce the components in the basic Zarrouk medium by 50% and incorporate iodized salt. This adjustment not only facilitates the healthy growth and development of the algae but also result in cost savings.

3.1.2. Changes in the pH of the media during the algal cultivation process

The change in pH of four types of media during the algal cultivation process is illustrated in Figure 3.

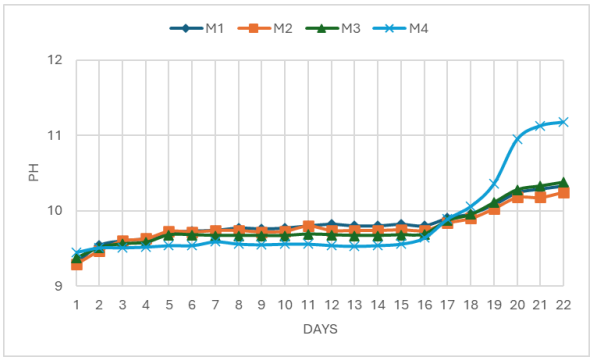

Figure 3. pH values of different algal cultivation media over the days

Table 3. Biomass Density Changes Over Time

Days	Biomass Density (OD_{560})			
	M1	M2	M3	M4
1	0,061 ± 0,002[a]	0,061 ± 0,001[a]	0,061 ± 0,001[a]	0,061 ± 0,002[a]
2	0,067 ± 0,003[b]	0,065 ± 0,001[b]	0,076 ± 0,001[a]	0,075 ± 0,002[a]
3	0,078 ± 0,001[b]	0,071 ± 0,002[c]	0,083 ± 0,003[a]	0,081 ± 0,002[a,b]
4	0,107 ± 0,004[a]	0,089 ± 0,003[b]	0,106 ± 0,001[a]	0,092 ± 0,002[b]
5	0,145 ± 0,002[c]	0,131 ± 0,002[d]	0,174 ± 0,001[a]	0,15 ± 0,003[b]
6	0,164 ± 0,001[c]	0,156 ± 0,001[d]	0,204 ± 0,003[a]	0,177 ± 0,002[b]
7	0,198 ± 0,001[c]	0,185 ± 0,003[d]	0,235 ± 0,003[a]	0,204 ± 0,001[b]
8	0,222 ± 0,001[c]	0,21 ± 0,001[d]	0,267 ± 0,001[a]	0,232 ± 0,002[b]
9	0,244 ± 0,008[c]	0,238 ± 0,004[c]	0,298 ± 0,002[a]	0,261 ± 0,003[b]
10	0,265 ± 0,001[d]	0,276 ± 0,002[c]	0,328 ± 0,001[a]	0,286 ± 0,002[b]
11	0,314 ± 0,005[d]	0,324 ± 0,002[c]	0,391 ± 0,002[a]	0,338 ± 0,002[b]
12	0,343 ± 0,003[d]	0,348 ± 0,002[c]	0,42 ± 0,004[a]	0,367 ± 0,004[b]
13	0,382 ± 0,002[c]	0,378 ± 0,003[c]	0,448 ± 0,003[a]	0,396 ± 0,005[b]
14	0,402 ± 0,008[c]	0,403 ± 0,004[c]	0,468 ± 0,001[a]	0,431 ± 0,007[b]
15	0,398 ± 0,003[d]	0,43 ± 0,002[c]	0,499 ± 0,003[a]	0,452 ± 0,001[b]
16	0,455 ± 0,003[d]	0,504 ± 0,002[c]	0,588 ± 0,004[a]	0,525 ± 0,002[b]
17	0,648 ± 0,004[d]	0,655 ± 0,001[c]	0,745 ± 0,001[a]	0,738 ± 0,001[b]
18	0,826 ± 0,002[c]	0,789 ± 0,007[d]	0,942 ± 0,009[a]	0,901 ± 0,006[b]
19	1,045 ± 0,001[c]	1,019 ± 0,002[d]	1,126 ± 0,002[a]	1,065 ± 0,001[b]
20	1,301 ± 0,008[b]	1,236 ± 0,002[c]	1,405 ± 0,003[a]	1,3 ± 0,009[b]
21	1,313 ± 0,005[b]	1,267 ± 0,047[c]	1,429 ± 0,001[a]	0,69 ± 0,003[d]
22	1,337 ± 0,006[b]	1,285 ± 0,013[d]	1,448 ± 0,010[a]	-

During the growth and development of algae, the pH of the media gradually increases due to the algae utilizing inorganic carbon from $NaHCO_3$. According to the research findings, from Day 1 to day 16, the pH increases slowly due to the slow growth of the algae. From Day 17 to Day 22, the pH rises rapidly as the algae grow quickly and utilize more carbon sources.

Previous studies[11] have shown that the optimal pH for the growth of *S. platensis* is between 8.3 and 11. The pH values in media M1, M2 and M3 (9.29–10.38) were suitable, supporting favorable algal growth and and increase in algal density during cultivation. In contrast, for media M4, the pH exceeds 11 on Days 21 and 22, resulting in significant algal mortality and sedimentation at the bottom of the culture flask, leading to a sharp decrease in algal density.

3.1.3. Changes in salinity of the media during the algal cultivation process

Based on Figure 4, the salinity in the media shows slight fluctuations over the days. *S. platensis* has the ability to absorb salt and tolerate high salinity due to its enhanced carbohydrate metabolism within the cells. According to studies,[9,10] when the salinity of the media reaches 20‰, the algae grow very slowly and die after 5-6 days. This occurs because high salinity alters the osmotic pressure of the cells, inhibiting photosynthesis and respiration, which leads to a reduction in the cell growth rate.

The experimental results indicate that the salinity levels of all media fall within the permissible range (9‰ to 18.5‰) for the growth and development of algae. The M3 medium has a salinity range of 10.3‰ to 13‰ and exhibits a higher algal density compared to the other media.

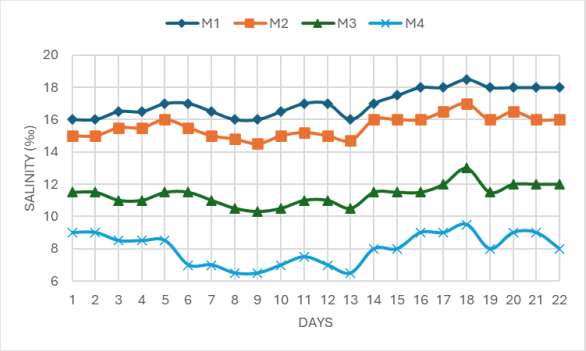

Figure 4. Salinity of different algal cultivation media over the days

3.2. Survey on the selection of solvents for preparing the optimal nutrient medium

The cultivation of *S. platensis* was conducted using the selected M3 medium from Experiment 1, utilizing two types of solvents: RO water and natural mineral water from Phuoc My commune, Binh Đinh province. The cultivation process was carried out under conditions similar to those in Experiment 1. The results regarding changes in pH, salinity, and algal biomass density

in the media are presented in the following tables and figures.

Table 4. OD values of the two types of algal cultivation media over the days

Days	OD_{560}	
	M3	M3’
1	0,061 ± 0,001[a]	0,061 ± 0,002[a]
2	0,076 ± 0,001[a]	0,074 ± 0,003[a]
3	0,083 ± 0,003[a]	0,083 ± 0,005[a]
4	0,106 ± 0,001[a]	0,097 ± 0,005[b]
5	0,174 ± 0,001[a]	0,168 ± 0,002[b]
6	0,204 ± 0,003[a]	0,194 ± 0,003[b]
7	0,235 ± 0,003[a]	0,217 ± 0,002[b]
8	0,267 ± 0,001[a]	0,257 ± 0,001[b]
9	0,298 ± 0,002[a]	0,283 ± 0,002[b]
10	0,328 ± 0,001[a]	0,313 ± 0,006[b]
11	0,391 ± 0,002[a]	0,365 ± 0,011[b]
12	0,42 ± 0,004[a]	0,398 ± 0,010[b]
13	0,448 ± 0,003[a]	0,422 ± 0,002[b]
14	0,468 ± 0,001[a]	0,443 ± 0,003[b]
15	0,499 ± 0,003[a]	0,472 ± 0,003[b]
16	0,588 ± 0,004[a]	0,565 ± 0,004[b]
17	0,745 ± 0,001[a]	0,727 ± 0,004[b]
18	0,942 ± 0,009[a]	0,912 ± 0,019[a]
19	1,126 ± 0,002[a]	0,985 ± 0,009[b]
20	1,405 ± 0,003[a]	1,304 ± 0,002[b]
21	1,429 ± 0,001[a]	1,324 ± 0,004[b]
22	1,448 ± 0,010[a]	1,356 ± 0,003[b]

M3 (M3 medium dissolved in RO water) ; M3’(M3 medium dissolved in natural mineral water)

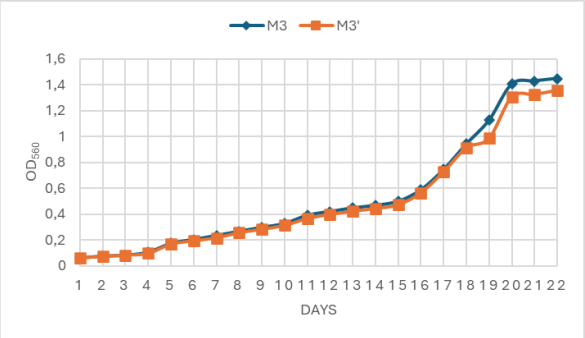

Figure 5. Growth of algal biomass density in two types of media over the days

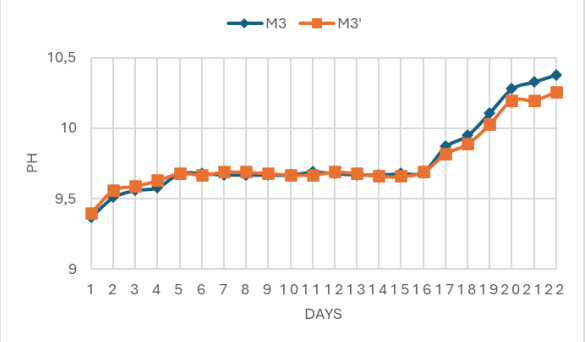

Figure 6. pH values of the two types of algal cultivation media over the days

The results show that the pH of media M3 and M3’ ranges from 9.37 to 10.38, and the salinity of the media is between 10.3‰ and 14‰, which is suitable for the growth and development of *S. platensis*. The biomass density of algae in these two media tends to increase over time. From Day 1 to Day 3, the algal density in both media is similar; however, from Day 4 onwards, the density in medium M3 is higher than that in M3’. This may be attributed to the higher salinity in medium M3’, which slows down algal growth. Therefore, using RO water to prepare the algal cultivation medium is more effective than using natural mineral water from Phuoc My commune, Binh Đinh province.

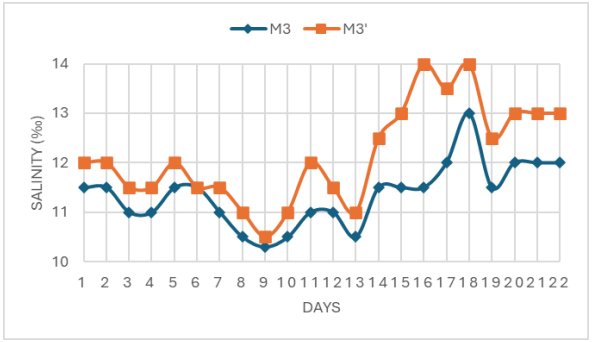

Figure 7. Salinity of two types of algal cultivation media over the days

3.3. Observing the morphology of *S. platensis* using an optical microscope

The algae were cultivated using the selected nutrient medium and solvent to harvest biomass. The algal samples were then observed under an optical microscope using a 40X objective lens. *S. platensis* has a filamentous structure, a blue-green color, and exhibits movement, with many evenly spaced helical turns and numerous vacuoles containing air.

Figure 8. Morphology of *S. platensis* observed under a microscope

4. CONCLUSION

The optimized algal cultivation medium is the Zarrouk medium at a 50% ratio, supplemented with iodine salt at 100‰ salinity and a concentration of 15 mL/L, using RO water to prepare the medium. The maximum algal biomass density was reached on Day 22 (OD_{560} = 1,448). *S. platensis* exhibits a blue-green color and a twisted filamentous structure, with high protein content (64,7%). Therefore, the use of this medium is recommended for further research to enhance the efficiency of algal cultivation while reducing costs.

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