Sàng lọc môi trường Zarrouk cải tiến trong nuôi tảo *Spirulina platensis*

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 NaCl (M2), môi trường Zarrouk (50%) bổ sung muối NaCl (M3), môi trường Zarrouk (25%) bổ sung muối NaCl (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.*

Screening of the modified Zarrouk medium for *Spirulina platensis* cultivation

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ABSTRACT

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 alga. The research was conducted on 4 types of media, including basic Zarrouk medium (M1), Zarrouk medium (75%) supplemented with sodium chloride salt (M2), Zarrouk medium (50%) supplemented with sodium chloride salt (M3), Zarrouk medium (25%) supplemented with sodium chloride salt (M4). These media were prepared with RO water. The pH values, salinity values of the media and the biomass density of the algae were 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 two 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. INTRODUCTION

Spirulina platensis (Arthrospira platensis) is regarded as a superfood due to its exceptional nutritional value and broad potential applications in nutrition and medicine. *Spirulina* contains protein of 60-70%, which is three times higher than beef and more than double that of soybeans, making it one of the most ideal sources of plant-based protein. Additionally, *Spirulina* is rich in essential amino acids such as lysine, methionine, phenylalanine and tryptophan, along with vital minerals like copper, zinc, magnesium and iron, effectively meeting the micronutrient requirements of the human body. Its high vitamin content further enhances the biological value of this alga.

Beyond its nutritional benefits, *Spirulina* has also been proven to boost the immune system and aid in preventing serious conditions such as cancer, hepatitis and diabetes. With its scientifically verified advantages, the World Health Organization (WHO) has recognized *Spirulina* as a safe food source and an effective tool for disease prevention and treatment in the 21st century.

However, the Zarrouk medium used for cultivating algae facilitates optimal *Spirulina* growth but is relatively expensive, limiting its scalability for production. Therefore, researching and developing cost-effective alternative culture media is an urgent requirement. This solution would significantly reduce the production costs of *Spirulina*, enhance its accessibility to the community and create new opportunities for the functional food and modern biomedical industries.

2. MATERIALS AND RESEARCH METHODS

2.1. Materials

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

The chemicals used to prepare Zarrouk medium were purchased from Viet My Chemical Company, Binh Dinh.

Spirulina platensis Strain: The algae strain was sourced from Minh Thien Algae Supply Facility in Bac Ninh province. The morphological characteristics of the *Spirulina*

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 Spirulina

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 sodium chloride salt.

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

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

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 Sodium Chloride Salt solution with 100‰ salinity
2	M3	50% Basic Zarrouk medium (M1) + 15 mL/L of Sodium Chloride Salt solution with 100‰ salinity
3	M4	25% Basic Zarrouk medium (M1) + 15 mL/L of Sodium Chloride Salt solution with 100‰ salinity

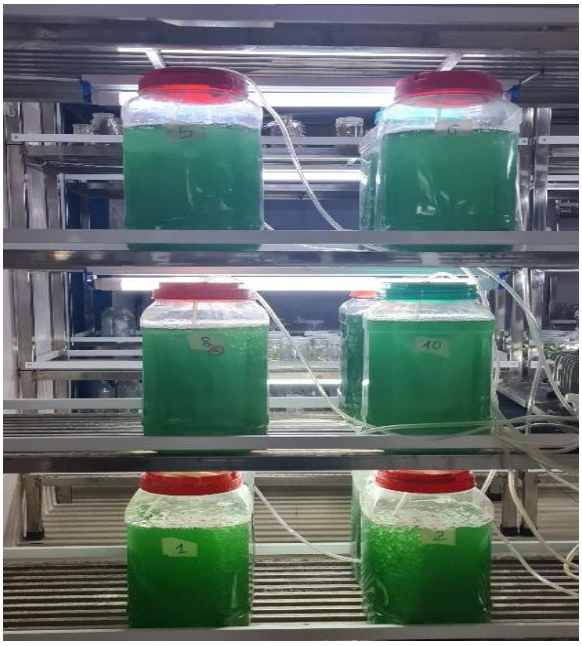

Figure 1. Experimental design.

Each treatment was conducted in a 5-Liter plastic flask. The *Spirulina* strain was inoculated into each flask to achieve an initial cell density of 0.061 OD (0.09 g/L). Continuous aeration at a flow rate of 5 L/min was maintained throughout the cultivation process, with a temperature set at 28 °C and continuous lighting provided by LED lamps at an intensity of 1,100 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 *Spirulina*.

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, including 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 *Spirulina* in each treatment was compared to select the most appropriate solvent.

Table 3. Composition of natural mineral water.

No.	Component	Concentration (mg/L)
1	TDS	150 - 300
2	Bicarbonate	150 - 300
3	Sodium	50 - 120
4	Calcium	< 0,1
5	Potassium	< 30
6	Magnesium	< 0,1
7	Iodine	< 1
8	Fluoride	< 1,5

2.2.2. Analysis and evaluation methodology

a. Determination of biomass density

Growth of *Spirulina* was monitored daily by determining the biomass density using the optical density (OD) measurement method. The OD was measured using a spectrophotometer (Aurius CE-2011) 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.

b. Determination of the correlation between the dry weight of algae and the optical density

A correlation graph between the dry weight of algae and optical density was constructed to determine the dry weight of algae in the algal suspension corresponding to optical absorbance.

The algal suspension in the stationary phase was measured spectrophotometrically at a wavelength of 560 nm. Simultaneously, a 50 mL sample of this suspension was filtered using Whatman filter paper. The filter paper containing the algal biomass was then dried at 60°C for 10 hours. The dry weight of the algae was measured using an analytical balance (Radwag AS 220.R1 PLUS).

The algal suspension was serially diluted by factors of 2, 4, 8, and 16. At each dilution level, the optical absorbance at 560 nm was recorded. The dry weight of algae at each dilution level was inferred based on the dry weight determined from the initial suspension.

The correlation between optical absorbance and the dry weight of algae was established using a linear equation ($Y = 1.5918 X – 0.0078$, $R^2 = 0.9962$).

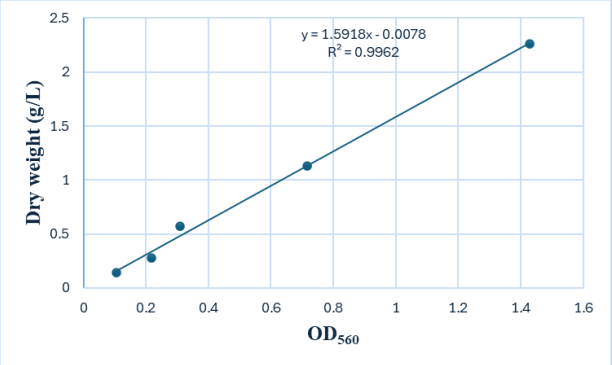

Figure 2. The correlation equation between dry weight and OD_{560}

c. Determination of illumination intensity

The illumination intensity was measured using a lux meter (LX 1330B). Before measurement, the device was calibrated according to the manufacturer's instructions. The sensor was positioned at the desired measurement point, ensuring it was level and facing the light source directly. The device was set to an appropriate range, and the reading was taken once the display stabilized.

d. Determination of pH value

Assessment of pH in the *Spirulina* cultivation medium: The pH of the algal cultivation medium was monitored daily and measured using a pH meter (Winlab W06-614310025). This measurement helps ensure that the pH remains within the optimal range for the growth of *Spirulina*, as pH fluctuations can significantly affect algal growth and metabolic activity.

e. Determination of salinity

Assessment of salinity in the *Spirulina* cultivation medium: The salinity of the algal cultivation medium was monitored daily and measured using a refractometer (Alla ALL35540). This measurement is crucial for ensuring that the salinity levels are appropriate for the growth of *Spirulina*, as variations in salinity can impact algal health and productivity.

f. Determination of protein content

The algae samples are harvested at their maximum biomass in the stationary phase. They are then filtered using a mesh filter and dried at 55 °C for 8 hours. The protein content is analyzed according to TCVN 8125:2009, following the Kjeldahl method. This method determines the total nitrogen content in the sample, which is then used to calculate the protein content by multiplying the nitrogen content by 6.25. The experiment is performed three times, and the average result is recorded.

g. Determination of acid insoluble ash content

The algae samples are filtered using a mesh filter and dried at a temperature of 55 °C for 8 hours. The acid insoluble ash content is analyzed according to TCVN 7765:2007.

2.2.3. Data processing methodology

The recorded data were analyzed using one-way analysis of variance (ANOVA) in Minitab 16 software. When there was a difference between the mean values, the Tukey test was used to determine statistically significant differences at a significance level of $p < 0.05$. Each experiment was repeated three times and 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 *Spirulina*

The *Spirulina* 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

The changes in biomass density of *Spirulina* across the four nutrient media are illustrated in Figure 3 and detailed in Table 4.

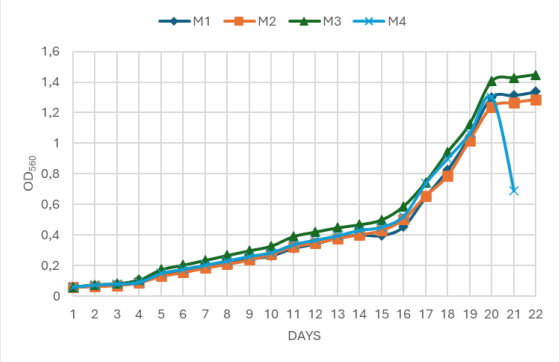

Figure 3. Development of biomass density of *Spirulina* in different nutrient media.

The results indicate that the biomass density of *Spirulina* 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 *Spirulina*. 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 sodium chloride 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 4.

During algal growth and development, the pH of the medium gradually increases as algae assimilate essential compounds such as carbon, nitrogen and minerals for their metabolic processes. Additionally, algae may utilize EDTA as a nitrogen source, which further contributes to the increase in pH. The findings of this study indicate that from Day 1 to Day 16, pH increases at a slow rate, corresponding to the initial slow growth of the algae. However, from Day 17 to Day 22, the pH rises more rapidly, reflecting the accelerated growth and increased consumption of available nutrient sources by the algae.

Furthermore, the marked increase in pH observed in media M4 can likely be attributed to the depletion of the medium's buffering capacity. This effect becomes more pronounced as the algae rapidly deplete the available nutrients, causing a significant shift in pH levels. These observations emphasize the critical importance of maintaining pH within an optimal range to sustain both algal health and the stability of the culture system. Notably, the sedimentation observed in M4 suggests that excessively high

pH may compromise algal cell integrity, leading to cell aggregation and precipitation rather than remaining uniformly dispersed in the medium.

Previous research has identified the optimal pH range for *Spirulina* growth as 8.3 to 11.[5] The pH values in media M1, M2, and M3 (9.29–10.38) fall within this optimal range, supporting robust algal growth and a steady increase in algal density during cultivation. In contrast, in media M4, where the pH exceeds 11 on Days 21 and 22, significant algal mortality is observed, accompanied by sedimentation at the bottom of the culture flask, leading to a sharp decline in algal density.

These results underscore the importance of selecting an appropriate cultivation medium and carefully monitoring pH levels to optimize algal productivity. Maintaining a stable pH environment is essential for maximizing algal biomass yield and ensuring efficient nutrient utilization. Such control is crucial for scaling up algal cultivation for industrial applications. Therefore, understanding the intricate relationship between pH and algal growth is vital for refining cultivation strategies and achieving optimal yields.

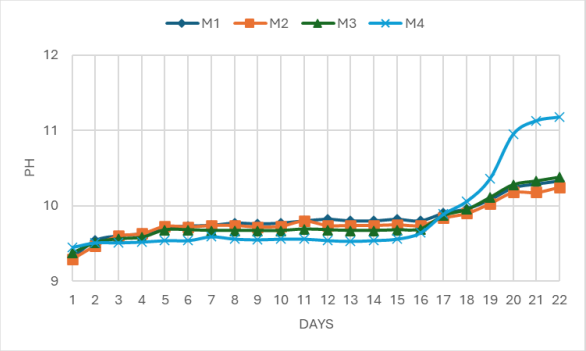

Figure 4. pH values of different algal cultivation media over the days.

Table 4. 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}	$0.081 \pm 0.002^{a.b}$
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}	-

In the same row, the letters written above indicate statistically significant differences ($p < 0.05$).

Table 5. Biomass concentration in different culture media.

Days	Biomass concentration (g/L)			
	M1	M2	M3	M4
1	0.09 ± 0.002^{a}	0.09 ± 0.002^{a}	0.09 ± 0.001^{a}	0.09 ± 0.003^{a}
2	0.1 ± 0.004^{b}	0.1 ± 0.001^{b}	0.11 ± 0.002^{a}	0.11 ± 0.002^{a}
3	0.12 ± 0.002^{b}	0.11 ± 0.002^{c}	0.12 ± 0.004^{a}	$0.12 \pm 0.003^{a.b}$
4	0.16 ± 0.004^{a}	0.13 ± 0.005^{b}	0.16 ± 0.002^{a}	0.14 ± 0.002^{b}
5	0.22 ± 0.003^{c}	0.20 ± 0.003^{d}	0.27 ± 0.002^{a}	0.23 ± 0.004^{b}
6	0.25 ± 0.002^{c}	0.24 ± 0.002^{d}	0.32 ± 0.005^{a}	0.27 ± 0.003^{b}
7	0.31 ± 0.002^{c}	0.29 ± 0.005^{d}	0.37 ± 0.005^{a}	0.32 ± 0.002^{b}

8	0.35 ± 0.002^{c}	0.33 ± 0.002^{d}	0.42 ± 0.001^{a}	0.36 ± 0.003^{b}
9	0.38 ± 0.012^{c}	0.37 ± 0.006^{c}	0.47 ± 0.003^{a}	0.41 ± 0.004^{b}
10	0.41 ± 0.001^{d}	0.43 ± 0.002^{c}	0.51 ± 0.001^{a}	0.45 ± 0.002^{b}
11	0.49 ± 0.008^{d}	0.51 ± 0.004^{c}	0.61 ± 0.003^{a}	0.53 ± 0.003^{b}
12	0.54 ± 0.004^{d}	0.55 ± 0.003^{c}	0.66 ± 0.007^{a}	0.58 ± 0.006^{b}
13	0.60 ± 0.003^{c}	0.59 ± 0.005^{c}	0.71 ± 0.005^{a}	0.62 ± 0.008^{b}
14	0.63 ± 0.012^{c}	0.63 ± 0.006^{c}	0.74 ± 0.001^{a}	0.68 ± 0.01^{b}
15	0.63 ± 0.005^{d}	0.68 ± 0.003^{c}	0.79 ± 0.005^{a}	0.71 ± 0.002^{b}
16	0.72 ± 0.005^{d}	0.79 ± 0.003^{c}	0.93 ± 0.007^{a}	0.83 ± 0.002^{b}
17	1.02 ± 0.003^{d}	1.03 ± 0.002^{c}	1.18 ± 0.002^{a}	1.17 ± 0.002^{b}
18	1.31 ± 0.003^{c}	1.25 ± 0.011^{d}	1.49 ± 0.014^{a}	1.43 ± 0.01^{b}
19	1.66 ± 0.002^{c}	1.61 ± 0.004^{d}	1.78 ± 0.003^{a}	1.69 ± 0.002^{b}
20	2.06 ± 0.013^{b}	1.96 ± 0.003^{c}	2.23 ± 0.005^{a}	2.06 ± 0.015^{b}
21	2.08 ± 0.008^{b}	2.01 ± 0.075^{c}	2.27 ± 0.002^{a}	1.09 ± 0.004^{d}
22	2.12 ± 0.01^{b}	2.04 ± 0.02^{d}	2.30 ± 0.016^{a}	-

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

The change in salinity of four types of media during the algal cultivation process is illustrated in Figure 5.

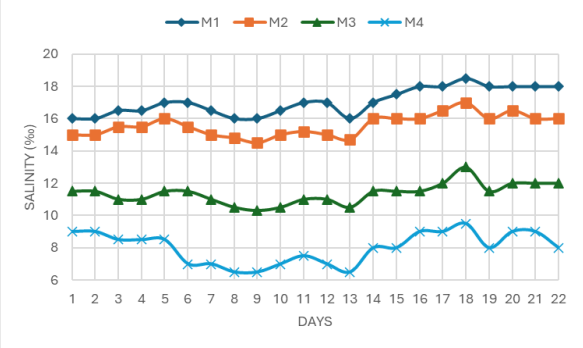

Figure 5. Salinity of different algal cultivation media over the days.

Based on Figure 5, the salinity in the media shows slight fluctuations over the days. During growth and development, *Spirulina* have the ability to absorb salts while releasing certain byproducts of metabolic processes, which leads to changes in the salinity of the medium.

Previous research has shown that when the salinity of the medium reaches 20‰, *Spirulina* growth slows significantly and cells die within 5–6 days.[8,9] This is attributed to the detrimental effects of high salinity on cellular osmotic pressure, which disrupts essential physiological processes such as photosynthesis and respiration, ultimately reducing the cell growth rate.

Experimental findings indicate that the salinity levels of all tested media remain within the permissible range for *Spirulina* growth and development, specifically between 9‰ and 18.5‰. Notably, medium M3 maintains a salinity range of 10.3‰ to 13‰, supporting a higher algal density compared to the other media. These results highlight the importance of maintaining optimal salinity conditions to enhance algal productivity and ensure the stability of cultivation systems.

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

The cultivation of *Spirulina* was performed using the M3 medium selected from Experiment 1, with two solvent types include RO water and natural mineral water sourced from Phuoc My commune, Binh Dinh province. The cultivation conditions were maintained consistent with those of Experiment 1.

Table 6. 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).

The results detailing changes in pH, salinity and algal biomass density across the media are presented in the following Tables 6 and Figures 6, 7, 8.

Experimental findings reveal that the pH levels of media M3 and M3', range between 9.37 and 10.38, while salinity fluctuates from 10.3‰ to 14‰, which is suitable for the growth and development of *Spirulina*. The algal biomass density in both media exhibits an upward trend over the cultivation period. During the initial phase (Day 1 to Day 3), the algal density is comparable in both media. However, from Day 4 onward, medium M3 demonstrates a consistently higher biomass density compared to medium M3'.

The observed difference in algal density can be attributed to the elevated salinity levels in medium M3', which likely impose osmotic stress, thereby slowing algal growth. These results suggest that using RO water as the solvent for preparing the cultivation medium provides more favorable conditions for algal growth compared to natural mineral water. This highlights the importance of controlling salinity levels in the medium to optimize algal productivity.

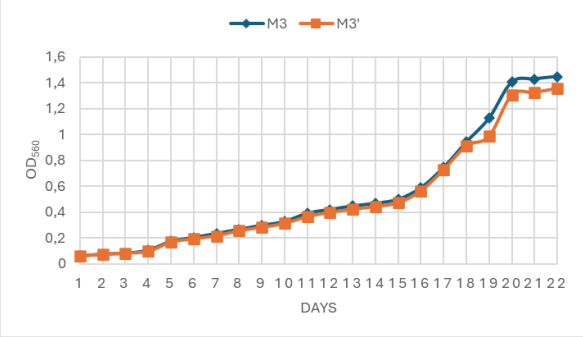

Figure 6. Growth of algal biomass density in two types of media over the days.

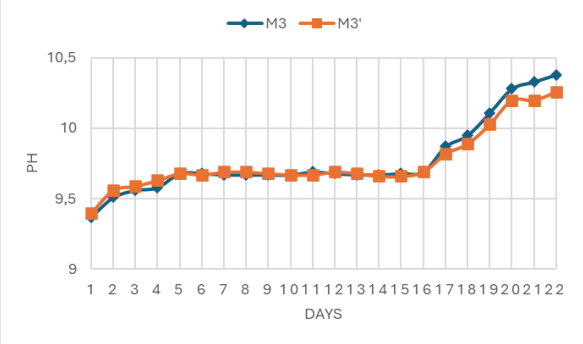

Figure 7. pH values of the two types of algal cultivation media over the days.

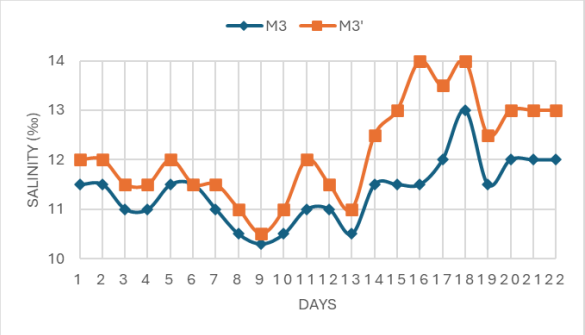

Figure 8. Salinity of two types of algal cultivation media over the days.

3.3. Protein content and acid insoluble ash content of *Spirulina*

The analysis results show that the protein content of *Spirulina* reached 64.7%, which is consistent with previous studies, where the protein content of the algae ranged from 60% to 70%.[10-12] Compared to another study, which reported a protein content of 51.98% to 54.53%,[15] the algae obtained in this study have a significantly higher protein content. This demonstrates that cultivating algae in M3 medium not only yields algae with high protein content but also provides economic benefits, while offering a valuable source of nutrition for humans.

The measured acid insoluble ash content is very low, at 0.11%, indicating a high degree of purity and quality of the *Spirulina* biomass. This low level of insoluble ash suggests that the algae are free from contaminants such as heavy metals, sand, or dust, which are potential concerns for the safety and applicability of the product.

Previous studies have reported an insoluble ash content of 0.28% and 2.09%,[17,18] which was still considered acceptable for use in medical, functional, and therapeutic foods. Therefore, the *Spirulina* obtained in this study demonstrates superior purity, offering enhanced product quality compared to the *Spirulina* from earlier research.

3.4. Observing the morphology of *Spirulina* 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. *Spirulina* has a filamentous structure, a blue-green color, and exhibits movement, with many evenly spaced helical turns and numerous vacuoles containing air.

Figure 9. Morphology of *Spirulina* observed under a microscope.

4. CONCLUSION

The optimized algal cultivation medium is the Zarrouk medium at a 50% ratio, supplemented with sodium chloride salt at a salinity of 100‰ and a concentration of 15 mL/L, using RO water to prepare the medium. The maximum algal biomass density was reached on Day 22 (2.30 g/L). *Spirulina* exhibits a blue-green color and a twisted filamentous structure, with a high protein content (64.7%) and low acid insoluble ash content (0.11%). 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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