International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 1
ISSN: 2088-5334

The Effect of pH on Contamination Reduction and Metabolite Contents in Mass Cultures of *Spirulina* (*Arthrospira platensis* Gomont)

Anita Mufidatun [a], Mochamad D. Koerniawan [b], Ulfah J. Siregar [c], Lucia T. Suwanti [d], Arief Budiman [e], Eko A. Suyono [a,*]

[a] *Faculty of Biology, Universitas Gadjah Mada, Sleman, D.I. Yogyakarta, 55281, Indonesia*
[b] *School of Architecture, Planning and Policy Development, Institut Teknologi Bandung, Bandung, Jawa Barat, 40123, Indonesia*
[c] *Faculty of Forestry, Institut Pertanian Bogor, Bogor, Jawa Barat, 16680, Indonesia*
[d] *Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Jawa Timur, 60115, Indonesia*
[e] *Department of Chemical Engineering, Universitas Gadjah Mada, Sleman, D.I. Yogyakarta, 55284, Indonesia*
Corresponding author: [*]eko_suyono@ugm.ac.id

***Abstract*— The microalgae *Spirulina* (*Arthrospira platensis* Gomont) is already cultured commercially using open ponds. The obstacle to mass cultivation of *Spirulina* is maintaining the monoculture without any contamination that can affect biomass products and their metabolites. The tolerance of *Spirulina* to environmental changes, such as changes in pH conditions, can be used as a method to overcome contamination in *Spirulina* mass cultivation. The growth contaminant can be avoided or controlled by giving mechanical stress by modifying the pH to alkaline levels. The efficient use of cost-effective materials in mass cultivation prevents contamination and maintains Spirulina's productivity. This study investigated the optimal pH parameters of 7–11 for 10 days. Cell density and dry biomass were measured daily using a hemocytometer and filter paper Whatman. The growth rate of contaminant microorganisms was carried out every five days along ten days of cultivation using the Total Plate Count (TPC) method. Using pH 9 effectively increased the cell density significantly (9.12±1.02%) and dry biomass (17.31±4.19 g.mL−1), reducing the contaminants in *Spirulina* mass cultures. The metabolite content was measured, including total protein using the Kjeldahl method, total lipid using the Soxhlet method, and pigmentations (such as chlorophyll, carotene, and phycocyanin) using spectrophotometry. The pH scale 8–10 can increase protein, lipid, and pigmentations. However, the pH 11 decreased almost entirely as a result of the metabolite contents of *Spirulina*.**

***Keywords*— Contamination; mass cultivation; *Arthrospira platensis* Gomont; pH.**

Manuscript received 4 Nov. 2021; revised 20 Jul. 2022; accepted 18 Sep. 2022. Date of publication 28 Feb. 2023.

I. INTRODUCTION

The cultivation approach of several microalgae species has been carried out on a mass scale to produce industrial products, such as food and feed supplements, pharmaceuticals, cosmetics, and, more recently, biofuels, as well as wastewater treatment and mitigation of atmospheric CO_2 build-up. These microalgae produce many bioproducts, including polysaccharides, proteins, lipids, pigments, antioxidants, vitamins, bioactive compounds, and many others. In addition, industrial microalgae cultivation is becoming popular because of the ability of microalgae, which is very versatile in its utilization [1].

Microalgae production using open ponds (rather than photobioreactors) is often considered the most cost-effective approach, and cultures are typically produced in a pH environment close to neutral [2]. Microalgae cultivation systems with open ponds in a non-aseptic environment will expose microalgae directly to the surrounding environmental conditions. Utilizing the surrounding environmental condition can cause contamination of several biological agents, which is the main problem of this cultivating system [3]. The presence of other organisms (contaminants) growing in the culture can be considered a pest. These contaminant organisms can interfere with culture development, decrease productivity, and sometimes result in the loss of microalgae populations or desired end products [4].

Contamination is a common and significant issue in the mass production of *Spirulina* (*A. platensis*). As they are open to the environment, they are vulnerable to contamination from other microorganisms, including other microalgal strains [5]. The strategies to avoid or control culture contamination can be monitored using psychical or chemical control systems.

One method of chemical control for suppressing the growth of contaminating organisms in *Spirulina* production is by raising the pH to alkaline levels [4] [6]. *Spirulina* can adapt to alkaline solutions by fixing photosynthetic carbon and utilizing HCO_3^- as an inorganic carbon source. It is known that phototropic utilization of HCO_3^- is facilitated through a carbon concentration mechanism (CCM) consisting mainly of membrane-bound and intracellular enzyme carbonic anhydrase, which converts HCO_3^- to CO_2 in cells [7].

Environmental pH conditions in alkaline levels can also influence the production and accumulation of valuable bio-products such as antioxidant compounds. Applied stress can cause oxidative stress, which is characterized by an imbalance between prooxidant and antioxidant activity within a cell due to an increase in prooxidants. Oxidative stress causes *Spirulina* to produce valuable secondary defensive metabolites with antioxidant potential [8].

In a previous study by Mehar *et al.* [9], risk contamination can be reduced by increased pH using CO_2 feeding. Efforts to increase the pH in the alkaline range were carried out to reduce the risk of contamination in the *Spirulina* microalgae mass culture. Microalgae culture conditions with alkaline pH can also cause an increase in CO_2 mass transfer, which produces high HCO_3^- ions to encourage *Spirulina* growth quickly. Based on this description, modifying the pH with specific ranges to an alkaline pH state to inhibit the growth of contaminant organisms but using a cost-effective approach such as commercial-based materials and its effects on the growth rate and yield of *Spirulina* metabolites.

II. Material and Method

A. Cultivation of Spirulina

Spirulina monoculture seedlings were taken from Nogotirto Algae Park and then cultured into open culture tanks filled with water and fertilizer as a mass culture medium (Table I) [10]. The treatment pH parameters were 7, 8, 9, 10, and 11. The justification of pH using the addition of caustic soda and vinegar was adjusted to the pH parameter scale in each treatment [11]. *Spirulina* growth measurements were carried out microscopically using a hemocytometer and the dry weight measurement for ten days.

TABLE I
Spirulina Growth Medium Composition on a Mass Culture

Composition	g.L^{-1}
NaCl	5
Urea Fertilizer (CH_4N_2O)	0.05
Nitrogen, Phosphate, Kalium Fertilizer	0.03
Ammonium Sulphate ($(NH_4)_2SO_4$)	0.15
Soda Ash Dense (Na_2CO_3)	0.075

B. Spirulina Growth Rate and Biomass Measurement

The growth rate of *Spirulina* culture in this study was carried out every day using a Neubauer Improved hemocytometer because the cell size was microscopic (2–10 µm in diameter). Calculations were carried out on four blocks, and the results were divided by the total number of counting space blocks with a microscope magnification of 100x [12]. Furthermore, *Spirulina* biomass was calculated by weighing 15 mL of *Spirulina* culture, filtering it through Whatman filter paper, and rinsing it three times with distilled water to dissolve any remaining excess salt. The filter paper was then dried, and the dry weight was calculated using a g.L^{-1} unit weight [13]. Furthermore, the daily density data was used in the formula for calculating the specific growth rate (µ) using Equation (1) [14], [15]:

$$\mu = \frac{\ln N_t - \ln N_0}{T_t - T_0} \quad (1)$$

μ: Specific growth rate; N_t: cell density/biomass at observation time; N_0: initial cell density/biomass; T_t: observation time; T_0: initial time.

C. Measurement of the Growth Rate of Contaminant Microorganisms

The Total Plate Count (TPC) method was used to measure the growth rate of contaminant microorganisms every five days. The sample was filtered with sterile filter paper and gradually diluted until 10^{-6} in a test tube. Each 100 µL diluted sample was collected aseptically, inoculated into Plate Count Agar (PCA) media, and incubated at 28°C for 24 hours. Colony counts in a Petri dish ranged between 30-300 Colony Forming Units (CFU) [16], [3].

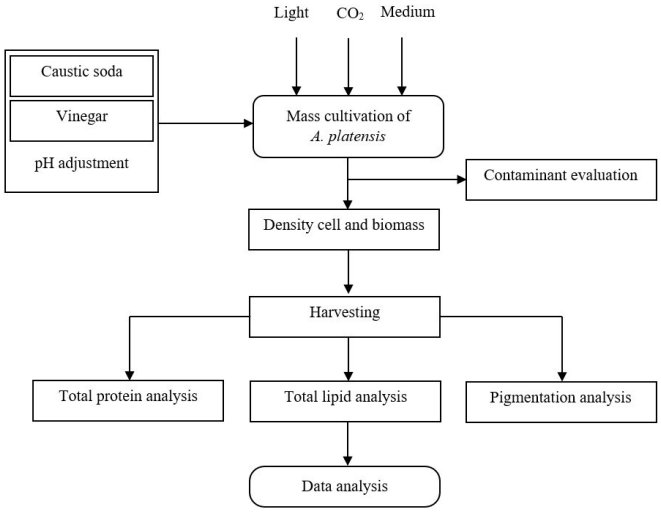


Fig. 1 Schematic flow chart of methods used in this study.

D. Total Protein Analysis

The Kjeldahl method was used to determine total protein content. A 0.1-gram sample was placed in a Kjeldahl flask, and 5 grams of a catalyst mixture of sodium sulfate (Na_2SO_4) and $CuSO_4$ was added (2:1). Destruction with 2 mL of concentrated sulfuric acid (H_2SO_4) was heated at high temperatures (200–600°C) until the mixture boiled and became clear. After 30 minutes, the sample was transferred to a distillate flask, diluted with 100 mL of distilled water, and conditioned under alkaline conditions using NaOH. The distillate flask was connected to the condenser and brought to a boil so that the water vapor could be collected in an Erlenmeyer flask containing 5 mL of 0.1 N boric acids that had been dripped (2 drops) with a methyl red indicator. Furthermore, 50 mL of distillate liquid was titrated with 0.02 N HCl until the solution turned pink. The volume of HCl in the titration was used to calculate the total N content using the following Equation (2) [17], [18]:

$$[\%N = V\ HCl \times N\ HCl \times 14{,}008 \times 100\%] \quad (2)$$

%N: percentage of nitrogen; V HCl: volume of HCl used for titration; N HCl: HCl normality.

E. Total Lipid Analysis

Total lipid content was analyzed using the Soxhlet method [19], [20], [21] with a 0.5–1 gram sample (A) placed in a porous thimble and inserted into a Soxhlet tube. After that, the flask (the empty flask has been weighed (B)) was heated, then 100 mL of solvent (ether) was evaporated and transferred to the condenser. This extraction process lasts for 3 hours. After the extraction, the solvent was evaporated by drying in an oven at 100°C for 24 hours, and the remaining lipid (C) mass was measured and used for analysis. The total lipid was calculated using the following Equation (3):

$$[\%\ \text{total lipid} = \frac{(C-B)}{A}\ x\ 100\%] \quad (3)$$

A: the weight of the sample; B: the weight of the empty flask; C: the weight of the final extracted flask.

F. Pigmentation Analysis

The pigmentation was analyzed using 30 mg of dried biomass sample dissolved and homogenized with 10 mL of pure methanol and incubated at 50°C for 3 hours in a water bath. Then, it was centrifuged for 10 minutes at 3000 rpm. The supernatant was transferred to a cuvette for spectrophotometric pigment analysis. The wavelength and formulation for the analysis of chlorophyll, carotenoids, and phycocyanin [22], [15] refer to Equation (4) below:

$$\begin{aligned}
&\text{Chlorophyll } a\ (\text{mg.L}^{-1}) = [(16.72 \times A_{665,2}) - (9.16 \times A_{652,4})] \\
&\text{Chlorophyll } b\ (\text{mg.L}^{-1}) = [(34.09 \times A_{652,4}) - (15.28 \times A_{665,2})] \\
&\text{Carotenoids } (\text{mg.L}^{-1}) = [(1000\ A_{470} - 1.63\ \text{Chlorophyll a} - 104.96\ \text{Chlorophyll b}) / 221] \\
&\text{Phycocyanin (mg.L-1)} = [(A620 - 0.474A652) / 5.34]
\end{aligned} \quad (4)$$

where A is Absorbance.

G. Data Analysis

ANOVA was used to analyze all of the data from each treatment, followed by the Duncan Multiple Range Test. The significance of data was determined by $p < 0.05$. All the summarized methods are illustrated in Figure 1.

III. Result and Discussion

A. The Growth Rate of Spirulina

The characteristics of microalgae growth rates are highly dependent on cultivation conditions. Microalgae cultivation conditions need to provide a source of nutrients and energy that can produce variations in cell density and biomass productivity. Environmental factors in cultivation can also influence microalgae biomass's growth and biochemical properties [23]. The treatment of various pH parameters was given to the *Spirulina* (*A. platensis*) cultivation media, which was one of the variations in the conditions of the cultivation media carried out to increase productivity. Based on the treatment carried out, the results of measuring the growth rate of *Spirulina* have been obtained, which can be seen in Figure 2.

According to Figure 2, the pH 9 treatment can increase the growth rate of *Spirulina* cell density and dry biomass with the same pattern of result, with both of them having the highest growth rate of *Spirulina*. Table 2 shows Spirulina's specific growth rate (SGR) in all pH treatments. The highest result shows density cell's specific growth rate at pH 9 treatment is (9.12±1.02%), as well as its dry biomass is (17.31±4.19 $g.mL^{-1}$), and the pH 11 treatment yields have the lowest result [10]. The increased growth rates were obtained at pH 8, 9, and 10 due to the high content of HCO_3^- and the decreased concentration of free CO_2 in the *Spirulina* culture medium, which affected the growth rate and accumulation of a higher rate of photosynthesis [24]. However, the decreased cell density and dry biomass of *Spirulina* in pH 11 can be explained based on Wahyuni *et al*. [25] using *Arthrospira maxima* cultivated with Thoriq Eko Arief (TEA) medium, which showed the possible reduced density of the cells that may occur because of the yellowish discoloration in the culture that leads to the death of some cell cultures.

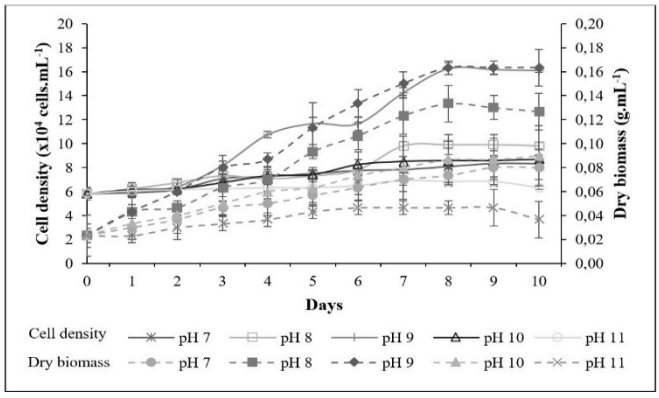


Fig. 2 The growth pattern of *Spirulina* from cell density and dry biomass in pH treatments of 7, 8, 9, 10, and 11 over 10 days of cultivation.

An experiment by Liu et al. [26] used CO2 concentration to control pH parameters on Spirulina (A. platensis) culture media at pH 11 had a slower growth rate than pH 9 and 10. Based on Jangir *et al*. [27], the recommended pH in the growth medium for cyanobacteria strains ranged from 9 to 9.5. A high pH level in a culture medium can result in high concentrations of CO_3^{2-}, which can denature cell proteins and be toxic via cellular mechanisms. The photosystem II (PS-II) cell mechanism remains active even at very high pH levels (pH>11), and the photosynthetic process that produces electrons is then used for NO_3^- reduction when the availability of HCO_3^- is limited [28].

TABLE II
SPECIFIC GROWTH RATE (SGR) BASED ON *SPIRULINA* CELL DENSITY AND DRY BIOMASS

pH	Cell density (x10⁴ cells.mL⁻¹)	Dry Biomass (g.mL⁻¹)
7	3.55±1.21[a]	12.46±1.44[ab]
8	6.65±1.41[b]	15.35±6.48[b]
9	9.12±1.02[c]	17.31±4.19[b]
10	4.07±0.94[a]	13.65±3.37[ab]
11	2.48±1.47[a]	6.32±3.19[a]

The specific growth rate of *Spirulina* can vary according to the conditions of the growth factors present in the growth medium. *Spirulina* can grow in freshwater (pH 7) and in alkaline water environments at pH 9 to 11 in tropical and subtropical regions [29]. According to these results, alkaline pH (pH>10) favored CO_2 (i.e., HCO_3^-) consumption and

dissolution into nutrient solutions in biomass production. On the other hand, these environmental conditions can inhibit microalgae growth and biomass production. It is caused by a reduction in C and P bioavailability at these pH levels due to limited microalgae assimilation of CO_3^{-2} forms, which predominate at pH >10.33 [30]. These research results show that the optimal pH for the maximum specific growth of *Spirulina* (*A. platensis*) is pH 9, and the lowest result at pH 11. According to previous studies, *Spirulina* is an alkalophilic organism, but the initial pH of 11.5 can strongly inhibit biomass production. Higher pH limited the availability of CO_2 for *Spirulina*. This further inhibited the growth of cells. In these studies, at an initial pH of 11, *Spirulina* flocks gathered at the bottom of the cultivation. The aggregation of cells commonly causes limited light transmission and oxygen permeation, which further threatens the growth of *Spirulina*. In these conditions, it was observed that the photosynthetic activity became very low and the cultures gradually lost their natural color [31].

B. Microorganism Contamination

Contaminant management in open ponds creates pond environmental conditions that can encourage the growth of *Spirulina* microalgae by reducing contaminant organisms in the cultivation media. Several strategies have been implemented to mitigate the impact of this contamination; the most effective approach actively deployed in the commercial production of microalgae using open pond technology involves maintaining an extreme culture environment, such as high salinity, high alkalinity, or high nutritional status. Many strategies involve deploying a chemical into the pond that alters the pond environment to give the target algae strain a competitive advantage or to disadvantage the undesirable contaminant. By manipulating the pond environment, such as the pH of the culture environment, the growth rate of *Spirulina* can be maximized while the growth rate of existing contaminants is reduced [32].

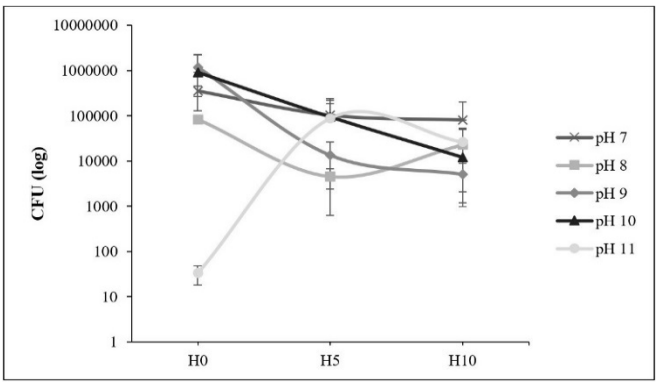


Fig. 3 The number of microorganism contaminations in *Spirulina's* mass culture in the 0th, 5th, and 10th day of cultivation.

Figure 3 shows the results of contamination measurements were analyzed using the Total Plate Count (TPC) method in *Spirulina* mass culture with pH treatments of 7, 8, 9, 10, and 11 were carried out every 5 days over 10 days of cultivation. The pH treatment for 10 days was able to reduce the number of contaminations compared to the 0th day. The pH 9 treatment had the most significant reduction of contamination in *Spirulina* culture [10]. According to Fanka *et al.* [33]., the optimal pH range for large-scale *Spirulina* cultivation is 9.8 to 10.5. The benefits of an alkaline pH environment in *Spirulina* cultivation can prevent contamination by bacteria and fungi, which grow optimally in neutral or acidic pH environments.

Based on the results obtained, the alkaline pH parameter treatment can reduce the contamination rate of cultivation. However, on the 5th day, there was an increase in contamination. The cultures are gradually becoming yellow or clear because the *Spirulina* cell is affected by contaminants [34]. According to Pleissner *et al.* [35], compromising two or more microbes' environmental conditions results in conditions that are not optimal for all microbes but are more unfavorable to one than the other. The least affected strain will survive. For this study, it seems that when *Spirulina* cells grow slowly, they become prey for predators, which can lead to the collapse of the entire culture. Cells grow slowly under nutrient deficiency and light limitation and when environmental conditions are unfavorable.

C. Total Protein Content

The total protein content of *Spirulina* in the pH treatments of 7, 8, 9, 10, and 11 are presented in Figure 4. The results show that the total protein content of *Spirulina* increased at pH 8, then gradually decreased at the higher level of pH (9, 10, and 11 parameters). The highest content of total protein was (73.34±9.07%) at pH 8 treatment compared to other pH treatments [10]. The results from this study show a nearly similar pattern to the previous study by Mehar *et al.* [9], that the maximum protein content in *Spirulina* was obtained at pH 8.5, which was cultivated using Zarrouk standard media. The concentration of protein increased due to CO_2 metabolism in the *Spirulina* cell. The CO_2 metabolism can use CO_2 and bicarbonate as substrates for the active transport of inorganic carbon, which accumulates bicarbonate and stimulates internal carbonic anhydrase [36].

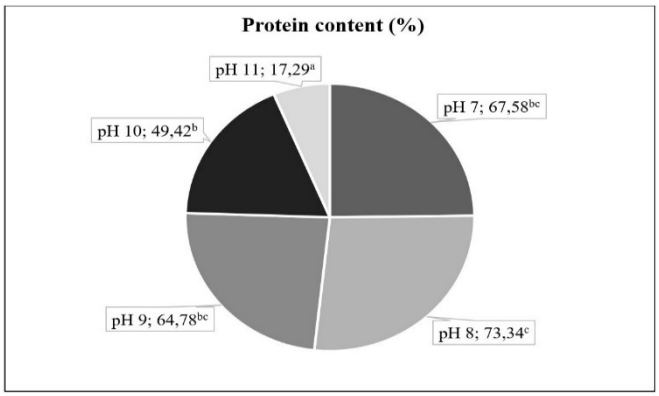


Fig. 4 The total protein content of *Spirulina* in pH treatments of 7, 8, 9, 10, and 11.

Spirulina's decreasing total protein content occurred at the higher level of pH treatment or alkaline conditions in culture. Environmental conditions with high alkaline levels will increase the concentration of OH^-. The OH^- ions will attack the cell wall structure, causing it to become looser and the cellular components to be released more easily. Sequentially, proteins are released in the aqueous phase. However, the higher concentration of OH^- will attack the cell wall molecules, causing them to lose their integrity, so the protein is more easily degraded. In the aqueous phase, high protein content occurs at a pH level of 7.0 to 14.0 [37]. The decrease

in the protein content of *Spirulina* when the pH is too high (pH < 11) can denature the protein cells, or the high concentration of CO_3^{2-} in *Spirulina* growth media can be toxic in a cell mechanism [28].

D. Total Lipid Content

Figure 5 shows the total lipid content of *Spirulina* cultivated for ten days with pH 7, 8, 9, 10, and 11. It found a significant increase in lipid content, and the highest result (0.0507±0.0047%) was at pH 9 [10]. The result of increasing total lipid content has also been previously reported by Eldiehy *et al*. [38], stating that *Spirulina* under alkaline conditions (pH 9) significantly increases lipid productivity. Based on Mehar *et al*. [9], at pH 8.5 using standard cultivation conditions (Zarrouk's media), *Spirulina* lipid accumulation occurred optimally. *Spirulina's* lipids are known to have important nutraceutical compounds due to gamma-linoleic acid. Based on the studies that have been carried out, the total lipid content of *Spirulina* is smaller than the total protein content. *Spirulina's* low lipid content occurs because cells modulate carbon flow for carbohydrate synthesis, thus preventing lipid accumulation [36].

The pH treatment under alkaline conditions in Figure 5 has higher yields than the pH 7 treatment. At alkaline pH or high pH, there is a bicarbonate content that benefits lipid accumulation. *Spirulina* lipid accumulation depends on the environmental conditions of the culture. Under stress conditions (e.g., nitrogen starvation, extreme salinity, extreme pH, abnormal temperature, or high light penetration), cells can divide actively to store energy in the form of lipids. The lipid content also depends on its cell size, and species with larger cell sizes and broad tolerant properties could increase biomass and lipid content. According to Cahyani *et al*. [39], lipid content differed in each growth period and the diversity of species in the culture. In a previous study by Peng et al. [40], freshwater microalgae Neochloris oleoabundans cultivated with a combination of 160 mM $NaHCO_3$ and a cultured control pH of 9.5 can allow the accumulation of lipids with high cell productivity and prevent contamination of protozoa that may be present in cultivation.

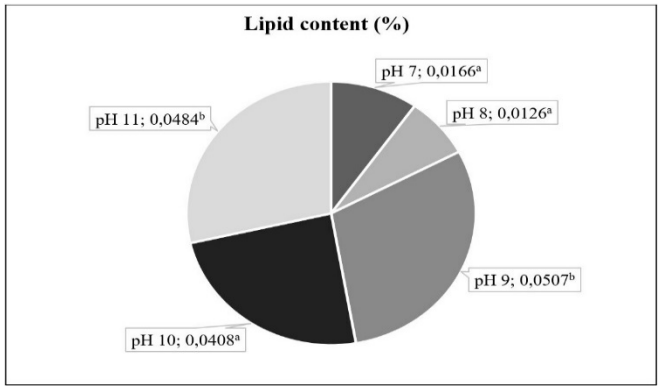
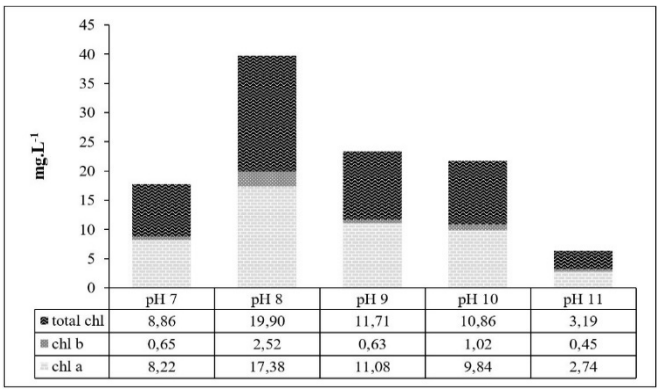


Fig. 5 The total lipid content of *Spirulina* in pH treatments of 7, 8, 9, 10, and 11.

E. Pigmentation

The chlorophyll, carotenoids, and phycocyanin contents of *Spirulina* in the pH treatments 7, 8, 9, 10, and 11 are presented in Figures 6a, 6b, and 6c.

Based on Figure 6a results, the pH 8 parameter treatment showed the highest content of chlorophyll *a* (17.38±1.43 $mg.L^{-1}$), chlorophyll *b* (2.52±0.99 $mg.L^{-1}$), and total chlorophyll (19.90±1.04 $mg.L^{-1}$). However, the pH 11 treatment showed the lowest content of chlorophyll *a* (2.74±0.71 $mg.L^{-1}$), chlorophyll *b* (0.45±0.43 $mg.L^{-1}$), and total chlorophyll (3.19±0.68 $mg.L^{-1}$) [10]. These results follow previous research by Ismaiel *et al*. [41] that pH 8-8.5 has the highest chlorophyll content but low results in the other pH treatments. The decreased content of chlorophyll *a* is primarily due to a decrease in the free carbon dioxide concentration in the medium at high pH (>10) because at this pH, the carbonate form predominates, and the bicarbonate form is the one utilized by *Spirulina* [42].



	pH 7	pH 8	pH 9	pH 10	pH 11
total chl	8,86	19,90	11,71	10,86	3,19
chl b	0,65	2,52	0,63	1,02	0,45
chl a	8,22	17,38	11,08	9,84	2,74

Fig. 6a The chlorophyll contents of *Spirulina* in pH treatments of 7, 8, 9, 10, and 11.

Chlorophyll *a* is also found to be much more abundant than chlorophyll *b*. This finding supports the important role of chlorophyll *a* as an antenna for capturing light required for photosynthesis [43]. The process of photosynthesis at high pH significantly affects the absorption and utilization of dissolved organic carbon (CO_2+HCO_3^-) by *Spirulina* cells [44]. High pH in the media culture will affect the decreased pigment content, which can be explained if bicarbonate is the only source of carbon. Bicarbonate raises the pH of the culture and eventually causes free CO_2 concentrations to become limiting. Because of the stress from carbon dioxide deficiency at the high pH, the free radicals or ROS levels in algal cells may increase, which causes the algal cells to undergo oxidative stress [41].

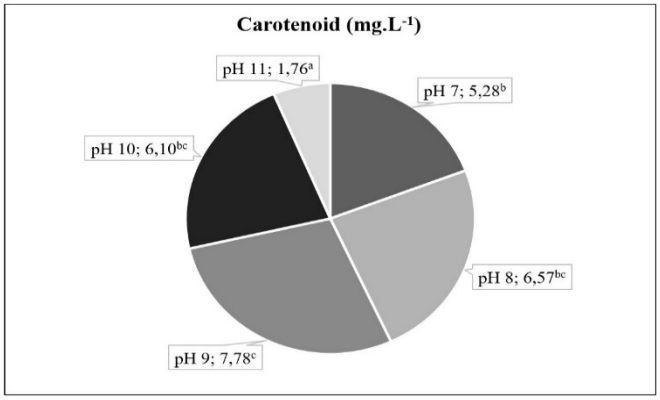


Fig. 6b The carotenoid contents of *Spirulina* in pH treatments of 7, 8, 9, 10, and 11.

Figure 6b shows that the highest carotene content was in the pH 9 treatment (7.78±0.70 $mg.L^{-1}$). However, the pH 11

treatments show the lowest carotene content (1.75±0.44 mg.L^{-1}) [10]. The pH 9 treatment in this study can significantly increase *Spirulina's* carotene content because of its optimal pH in photosynthetic and respiratory capacities. Photosynthetic and respiratory capacities are related to the enzymatic activity for biosynthesis and the production of several important chemical substances such as carotene and tocopherols [45]. Based on this study, pH 9 was the maximum value for increasing carotenoid. In the previous study by Khalil *et al*. [46], using *Dunaliella bardawil* species has the maximum value in the pH 7.5 treatment. Meanwhile, the carotene content significantly decreased in the lower or higher pH values. The effect of medium pH on pigmentation indirectly exerts growth and physiological changes rather than a direct effect on carotenoid biosynthesis.

Spirulina naturally contains antioxidants, one of which is carotene, a source of dietary supplements with therapeutic characteristics [41]. Several studies have shown that carotenoids are important antioxidants in microorganisms, and oxygen radicals are important inducers of carotenoid biosynthesis. It can be concluded that the pH of the medium can affect the formation of reactive oxygen species (ROS), which in turn will affect the quantity and composition of carotenoids in *Spirulina* [46].

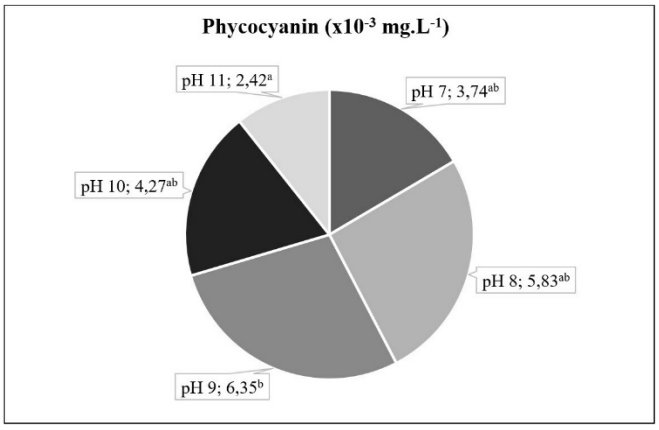


Fig. 6c The phycocyanin contents of *Spirulina* in pH treatments of 7, 8, 9, 10, and 11.

Figure 6c shows that the phycocyanin yield increased to a maximum at pH 9 (0.0064±0.0012 mg.L^{-1}) and then gradually decreased to the lowest yield at pH 11 (0.0024±0.0021 mg.L^{-1}) [10]. *Spirulina* contains phycocyanin, a pigment that is extremely sensitive to alkaline or high pH [27]. The presence of salt and alkaline stress in *Spirulina's* natural habitat causes genetic and physiological tolerance. According to Mehar *et al*. [9], a comparison of several pH treatments given in *Spirulina* culture revealed the best metabolite production at pH 9.5. In fact, a high pH is associated with a high CO_2 gradient under alkaline conditions and the possibility of free CO_2 from the atmosphere.

IV. Conclusion

This study shows that pH modification to alkaline slightly decreased the microorganism contamination in the mass culture of *Spirulina*. *Spirulina's* growth rate increased significantly when the pH level was raised to 8-10. Nonetheless, pH levels 8 and 9 are significantly more suitable candidates for maximum *Spirulina* metabolite yields. The pH level of 11 is significantly lowermost than the yield of *Spirulina* and only suitable for maximizing lipid content.

Acknowledgment

This study was a part of a master's student thesis and supported by The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia.

References

[1] J. Masojídek, K. Ranglová, G. E. Lakatos, A. M. S. Benavides, and G. Torzillo, "Variables governing photosynthesis and growth in microalgae mass cultures," *Processes*, vol. 9, no. 5, 2021, doi: 10.3390/pr9050820.

[2] A. Vadlamani, B. Pendyala, S. Viamajala, and S. Varanasi, "High Productivity Cultivation of Microalgae without Concentrated CO_2 Input," *ACS Sustain. Chem. Eng.*, vol. 7, no. 2, pp. 1933–1943, 2019, doi: 10.1021/acssuschemeng.8b04094.

[3] F. Di Caprio, "Methods to quantify biological contaminants in microalgae cultures," *Algal Res.*, vol. 49, no. December 2019, p. 101943, 2020, doi: 10.1016/j.algal.2020.101943.

[4] D. Molina, J. C. de Carvalho, A. I. M. Júnior, C. Faulds, E. Bertrand, and C. R. Soccol, "Biological contamination and its chemical control in microalgal mass cultures," *Appl. Microbiol. Biotechnol.*, vol. 103, no. 23–24, pp. 9345–9358, 2019, doi: 10.1007/s00253-019-10193-7.

[5] T. Lafarga, A. Sánchez-Zurano, S. Villaró, A. Morillas-España, and G. Acién, "Industrial production of spirulina as a protein source for bioactive peptide generation," *Trends Food Sci. Technol.*, vol. 116, no. June, pp. 176–185, 2021, doi: 10.1016/j.tifs.2021.07.018.

[6] K. P. Papadopoulos *et al.*, "Cultivation of *Arthrospira platensis* in Brewery Wastewater," *Water (Switzerland)*, vol. 14, no. 10, 2022, doi: 10.3390/w14101547.

[7] J. A. Raven, C. S. Cockell, and C. L. De La Rocha, "The evolution of inorganic carbon concentrating mechanisms in photosynthesis," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 363, no. 1504, pp. 2641–2650, 2008, doi: 10.1098/rstb.2008.0020.

[8] M. R. Gauthier, G. N. A. Senhorinho, and J. A. Scott, "Microalgae under environmental stress as a source of antioxidants," *Algal Res.*, vol. 52, no. October, p. 102104, 2020, doi: 10.1016/j.algal.2020.102104.

[9] J. Mehar, A. Shekh, M. U. Nethravathy, R. Sarada, V. S. Chauhan, and S. Mudliar, "Automation of pilot-scale open raceway pond: A case study of CO_2-fed pH control on *Spirulina* biomass, protein and phycocyanin production," *J. CO2 Util.*, vol. 33, no. May, pp. 384–393, 2019, doi: 10.1016/j.jcou.2019.07.006.

[10] A. Mufidatun, "Optimalisasi pH Media Terhadap Kontaminasi, Biomassa dan Kandungan Metabolit pada Kultur Massal *Spirulina* (*Arthrospira platensis* Gomont)," Universitas Gadjah Mada, 2021.

[11] S. C. Silva *et al.*, "*Spirulina* (*Arthrospira platensis*) protein-rich extract as a natural emulsifier for oil-in-water emulsions: Optimization through a sequential experimental design strategy," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 648, no. March, 2022, doi: 10.1016/j.colsurfa.2022.129264.

[12] M. M. El-Sheekh, L. H. S. Hassan, and H. H. Morsi, "Growth enhancement of *Spirulina platensis* through optimization of media and nitrogen sources," *Egypt. J. Bot.*, vol. 61, no. 1, pp. 61–69, 2021, doi: 10.21608/ejbo.2020.27927.1487.

[13] R. Chaiklahan, N. Chirasuwan, T. Srinorasing, S. Attasat, A. Nopharatana, and B. Bunnag, "Enhanced biomass and phycocyanin production of *Arthrospira* (*Spirulina*) *platensis* by a cultivation management strategy: Light intensity and cell concentration," *Bioresour. Technol.*, vol. 343, no. September 2021, 2022, doi: 10.1016/j.biortech.2021.126077.

[14] T. Takahashi, "Routine management of microalgae using autofluorescence from chlorophyll," *Molecules*, vol. 24, no. 24, 2019, doi: 10.3390/molecules24244441.

[15] N. U. F. Niangoran, D. Buso, G. Zissis, and T. Prudhomme, "Influence of light intensity and photoperiod on energy efficiency of biomass and pigment production of *Spirulina* (*Arthrospira platensis*)," *OCL - Oilseeds fats, Crop. Lipids*, vol. 28, 2021, doi: 10.1051/ocl/2021025.

[16] D. T. Boukouvalas, R. A. Prates, C. R. Lima Leal, and S. A. de Araújo, "Automatic segmentation method for CFU counting in single plate-serial dilution," *Chemom. Intell. Lab. Syst.*, vol. 195, no. November, p. 103889, 2019, doi: 10.1016/j.chemolab.2019.103889.

[17] P. Didpinrum, W. Siriangkhawut, K. Ponhong, P. Chantiratikul, and K. Grudpan, "A newly designed sticker-plastic sheet platform and smartphone-based digital imaging for protein assay in food samples with downscaling Kjeldahl digestion," *RSC Adv.*, vol. 11, no. 58, pp. 36494–36501, 2021, doi: 10.1039/d1ra04321h.

[18] S. Arunima and S. Verulkar, "Comparative analysis of different protein estimation methods," *Pharma Innov. J.*, vol. 11, no. 4, pp. 2091–2095, 2022.

[19] S. S. de Jesus, G. F. Ferreira, L. S. Moreira, M. R. Wolf Maciel, and R. Maciel Filho, "Comparison of several methods for effective lipid extraction from wet microalgae using green solvents," *Renew. Energy*, vol. 143, pp. 130–141, 2019, doi: 10.1016/j.renene.2019.04.168.

[20] G. G. Hewavitharana, D. N. Perera, S. B. Navaratne, and I. Wickramasinghe, "Extraction methods of fat from food samples and preparation of fatty acid methyl esters for gas chromatography: A review," *Arab. J. Chem.*, vol. 13, no. 8, pp. 6865–6875, 2020, doi: 10.1016/j.arabjc.2020.06.039.

[21] Z. N. Hussain and A. A. A.-H. Jazie, "Extraction of Bio Oil From Fucus Vesiculosus and Cladophora Glomerata Algal Species," *Kufa J. Eng.*, vol. 12, no. 4, pp. 28–41, 2021, doi: 10.30572/2018/kje/120403.

[22] H. K. Lichtenthaler and C. Buschmann, "Chlorophylls and Carotenoids Measurement and UV-VIS characterization Lichtenthaler 2001," *Curr. Protoc. Food Anal. Chem.*, vol. F4.3.1-F4., no. Supplement 1, pp. 1–8, 2001.

[23] E. Daneshvar *et al.*, "Insights into upstream processing of microalgae: A review," *Bioresour. Technol.*, vol. 329, no. February, 2021, doi: 10.1016/j.biortech.2021.124870.

[24] M. Babu, K. Ashok, J. Senthil, T. Kalaiyarasu, J. S.-A. C. Journal, and U. 2020, "Effect of pH on *Arthrospira platensis* production," *Alochana Chakra J.*, vol. 4, no. 5, pp. 2297–2305, 2020, [Online]. Available: https://www.researchgate.net/publication/341312500.

[25] N. E. Wahyuni, B. Rachmawati, T. T. Samudra, Y. S. Pradana, A. Budiman, and E. A. Suyono, "Variation of Biomass and Lipid Content of a Mixed Culture of Glagah Isolate and *Arthrospira maxima* in Thin-Layer Photobioreactor Using Three Different Media (BBM, Farmpion and TEAM)," *AIP Conf. Proc.*, vol. 2260, 2020, doi: 10.1063/5.0016169.

[26] W. Liu, J. Wang, and T. Liu, "Low pH rather than high CO_2 concentration itself inhibits growth of *Arthrospira*," *Sci. Total Environ.*, vol. 666, pp. 572–580, 2019, doi: 10.1016/j.scitotenv.2019.02.312.

[27] M. M. Jangir, S. Chowdhury, and V. Bhagavatula, "Differential response of photosynthetic apparatus towards alkaline pH treatment in NIES-39 and PCC 7345 strains of *Arthrospira platensis*," *Int. Microbiol.*, vol. 24, no. 2, pp. 219–231, 2021, doi: 10.1007/s10123-021-00160-6.

[28] A. Vadlamani, S. Viamajala, B. Pendyala, and S. Varanasi, "Cultivation of Microalgae at Extreme Alkaline pH Conditions: A Novel Approach for Biofuel Production," *ACS Sustain. Chem. Eng.*, vol. 5, no. 8, pp. 7284–7294, 2017, doi: 10.1021/acssuschemeng.7b01534.

[29] F. Jung, A. Krüger-Genge, P. Waldeck, and J. H. Küpper, "*Spirulina platensis*, a super food?," *J. Cell. Biotechnol.*, vol. 5, no. 1, pp. 43–54, 2019, doi: 10.3233/JCB-189012.

[30] J. C. Beltrán-Rocha *et al.*, "Some implications of natural increase of pH in microalgae cultivation and harvest by autoflocculation," *Lat. Am. J. Aquat. Res.*, vol. 49, no. 5, pp. 836–842, 2021, doi: 10.3856/vol49-issue5-fulltext-2691.

[31] Z. Li *et al.*, "Effects of Culture Conditions on the Performance of *Arthrospira platensis* and Its Production of Exopolysaccharides," 2022.

[32] R. C. McBride *et al.*, "Contamination Management in Low Cost Open Algae Ponds for Biofuels Production," *Ind. Biotechnol.*, vol. 10, no. 3, pp. 221–227, 2014, doi: 10.1089/ind.2013.0036.

[33] L. S. Fanka, G. M. da Rosa, M. G. de Morais, and J. A. V. Costa, "Outdoor Production of Biomass and Biomolecules by *Spirulina* (*Arthrospira*) and *Synechococcus* cultivated with Reduced Nutrient Supply," *Bioenergy Res.*, vol. 15, no. 1, pp. 121–130, 2022, doi: 10.1007/s12155-021-10320-1.

[34] Z. Zhu, J. Jian, and Y. Fa, "Overcoming the Biological Contamination in," *Molecules*, 2020.

[35] D. Pleissner, A. V. Lindner, and R. R. Ambati, "Techniques to Control Microbial Contaminants in Nonsterile Microalgae Cultivation," *Appl. Biochem. Biotechnol.*, vol. 192, no. 4, pp. 1376–1385, 2020, doi: 10.1007/s12010-020-03414-7.

[36] L. G. Cardoso *et al.*, "Scaling-up production of *Spirulina* sp. LEB18 grown in aquaculture wastewater," *Aquaculture*, vol. 544, no. June, p. 737045, 2021, doi: 10.1016/j.aquaculture.2021.737045.

[37] W. Chen *et al.*, "Structural insights reveal the effective *Spirulina platensis* cell wall dissociation methods for multi-output recovery," *Bioresour. Technol.*, vol. 300, no. October 2019, p. 122628, 2020, doi: 10.1016/j.biortech.2019.122628.

[38] K. S. H. Eldiehy, M. A. Fawzy, M. Rawway, and U. M. Abdul-, "Optimization of biomass and some metabolites productivity of *Merismopedia tenuissima* and *Spirulina* (*Arthrospira*) *platensis* grown under stress conditions," *Zanco J. Pure Appl. Sci.*, vol. 32, no. 5, 2020, doi: 10.21271/zjpas.32.5.14.

[39] F. Cahyani, A. Ardi, T. T. Samudra, A. Budiman, and E. A. Suyono, "Biomass productivity and lipid content between natural mixed culture consortia and artificial mixed culture," *AIP Conf. Proc.*, vol. 2260, no. September, 2020, doi: 10.1063/5.0016184.

[40] L. Peng, C. Q. Lan, Z. Zhang, C. Sarch, and M. Laporte, "Control of protozoa contamination and lipid accumulation in *Neochloris oleoabundans* culture: Effects of pH and dissolved inorganic carbon," *Bioresour. Technol.*, vol. 197, pp. 143–151, 2015, doi: 10.1016/j.biortech.2015.07.101.

[41] M. M. S. Ismaiel, Y. M. El-Ayouty, and M. Piercey-Normore, "Role of pH on antioxidants production by *Spirulina* (*Arthrospira*) *platensis*," *Brazilian J. Microbiol.*, vol. 47, no. 2, pp. 298–304, 2016, doi: 10.1016/j.bjm.2016.01.003.

[42] G. Sharma, M. Kumar, M. I. Ali, and N. D. Jasuja, "Effect of carbon content, salinity and pH on *Spirulina platensis* for phycocyanin, allophycocyanin and phycoerythrin accumulation," *J. Microb. Biochem. Technol.*, vol. 6, no. 4, pp. 202–206, 2014, doi: 10.4172/1948-5948.1000144.

[43] H. S. H. Munawaroh *et al.*, "Characterization and physicochemical properties of chlorophyll extract from *Spirulina* sp.," *J. Phys. Conf. Ser.*, vol. 1280, no. 2, 2019, doi: 10.1088/1742-6596/1280/2/022013.

[44] D. Yin *et al.*, "Effects of carbon concentration, pH, and bubbling depth on carbon dioxide absorption ratio in microalgae medium," *Environ. Sci. Pollut. Res.*, vol. 26, no. 32, pp. 32902–32910, 2019, doi: 10.1007/s11356-019-06287-4.

[45] A. M. Abd El-Monem, M. M. Gharieb, A.-E. M. Hussian, and K. M. Doman, "Effect of pH on phytochemical and antibacterial activities of *Spirulina platensis*," *Int. J. Appl. Environ. Sci.*, vol. 13, no. 4, pp. 339–351, 2018, [Online]. Available: http://www.ripublication.com.

[46] Z. I. Khalil, M. M. S. Asker, S. El-Sayed, and I. A. Kobbia, "Effect of pH on growth and biochemical responses of *Dunaliella bardawil* and *Chlorella ellipsoidea*," *World J. Microbiol. Biotechnol.*, vol. 26, no. 7, pp. 1225–1231, 2010, doi: 10.1007/s11274-009-0292-z.