# Bioflocculant Technology Implementation Using *Navicula* sp for Harvesting of *Arthrospira Platensis* Culture

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## Abstract

Bioflocculation, a microalgae harvesting method utilizing organisms like bacteria and microalgae as flocculant agents, necessitates careful selection and ratio determination of bioflocculant agents. This study seeks to ascertain the optimal balance of bioflocculant, *Navicula* sp., for metabolite production and harvesting efficiency in *Arthrospira platensis* culture. Spanning three one-week cultivation stages, the research commences with *A. platensis* culture under varying salinities (0, 15, 25, and 35 ppt) in the first stage, followed by pH variations (9, 10, 11, and 12) in the second stage. The optimal salinity-pH combination identified in the first two stages is applied in the third stage, involving bioflocculant addition to culture and medium solutions at varying ratios (0.25:1, 0.5:1, 0.75:1, and 1:1 v/v). Precipitation efficiency and pigmentation were measured using spectrophotometry, while lipid content was determined by the Bligh & Dyer extraction method. Carbohydrate content was assessed using the Phenol-Sulphuric Acid assay, and protein content was quantified using the Bradford protein assay. The findings indicate salinity, pH, and bioflocculant influence *A. platensis* growth, metabolite production, and harvesting efficiency. Optimal outcomes are achieved at 15 ppt salinity, pH 9, and a 1:1 ratio of bioflocculant, yielding $1.18x10^{-3}$ g of dry biomass, $3.56x10^{5}$ cells/mL of cell density, $2.9x10^{0}$ g/L of carbohydrates, $3.63x10^{0}$ g/L of lipids, $9.97x10^{-3}$ mg/L of chlorophyll a+b, $8.71x10^{-1}$ mg/L of carotenoids, and $3.04x10^{-1}$ g/L of protein. These results suggest that salinity addition, high pH, and the addition of *Navicula* sp. as bioflocculant increase the harvesting process efficiency of *A. platensis* culture.

Keywords: *Arthrospira platensis,* bioflocculant, harvesting, pH, salinity

## Introduction

*Arthrospira platensis* or *Spirulina,* is a species of microalgae frequently mass cultivated because of its diverse benefits as a highly nutritious food ingredient, cosmetics, and medicine (Saraswathi & Kavitha, 2023). *Spirulina* contains 8-70% w/w of carbohydrates, 3-68% w/w of lipids, 92 mg/L of chlorophyll pigments, and 4-16% w/w of proteins (Rohmawati et al., 2023). Due to its potential, *Spirulina* production is increasing worldwide. Over 30% of the world's microalgal biomass comes from *Spirulina* (Costa et al., 2019). However, harvesting efficiency is still needed to increase *Spirulina* production (Dewayanto et al., 2023).

The harvesting stage dramatically determines the final biomass yield of *Spirulina* obtained during cultivation. Various challenges often restrict this stage, such as environmental conditions, microalgae conditions, and decreased medium quality (Tan et al., 2020). However, the harvesting stage requires up to 20-30% of the total production cost (Dewayanto et al., 2023). Therefore, an economical solution is needed to optimize *Spirulina* harvest yields.

One of the solutions that can be done is the bioflocculation method. According to Tawila et al. (2018), bioflocculation is a process where the mediation of flocculants occurs in the presence of microorganisms or biodegradable macromolecular flocculants released by microorganisms. It is recognized as an active process induced by living cells by generating exopolymeric macromolecules. The initial research of the flocculation process in microorganisms dates back to 1876 when Louis Pasteur reported its occurrence in yeast. In microalgae harvesting, bioflocculation is a technique that utilizes bioflocculant microorganisms (microalgae or bacteria) mixed in non-

flocculant microalgae cultures (Matter et al., 2019). Bioflocculant microorganisms produce exopolymeric macromolecules that help agglomerate particles in cells, suspended solids, and colloidal solids (solid colloid particles) (Tawila et al., 2018). Bioflocculant organisms are relatively safer to use in microalgae cultivation compared to other chemical flocculants such as polyethyleneimine, polyacrylamide, aluminum sulfate, and polyaluminum chloride (Tawila et al., 2018). This technique also has been recognized as an effective and more cost-effective harvesting method because it does not require a particular medium. Compared to using chitosan (recovery efficiency of 91%) in microalgae harvesting, harvesting per 1 kg of biomass will require an additional cost of 51.02 USD (Elcik et al., 2023), While the use of bioflocculants harnesses natural microorganisms like diatoms, requiring low energy, readily available, and without the need for chemical additives (de Morais et al., 2023).

The bioflocculant microorganism that was used in this study was *Navicula* sp. *Navicula* sp. is a unicellular diatom most often found in various places, including freshwater, seawater, and groundwater (Pane et al., 2023). Morphologically, *Navicula* sp. has a cell wall composed of silica without partition in the inner layer, oval-shaped, and brown (Pane et al., 2023). *Navicula* sp. can act as a bioflocculant organism because it can produce Extracellular Polymeric Substances (EPSs) as adhesive mucilage capable of agglomerating various substances and cells (Kurnia & Panjaitan, 2020). EPSs are categorized as polysaccharides, microcapsular, slime, inorganic polysaccharides, polyesters, and polyimides (Jahandideh et al., 2021). Within the bioflocculation process, EPSs contribute to regulating cell-cell recognition, such as cell adhesion, and adherence to surfaces. They also aid in water retention to prevent cell desiccation, exogenous organic compounds sorption, and inorganic ions' absorption. Additionally, EPSs are involved in enzymatic activities and the interaction of polysaccharides with enzymes (Melo et al., 2022).

EPSs from *Navicula* sp. will form an aggregate with microalgae cell, which has a higher density than water. EPSs also comprise essential elements like carbon, nitrogen, and various organic compounds, including primary amines, aromatic compounds, aliphatic alkyl groups, and carbohydrates (Jimoh & Cowan, 2017). Therefore, it is assumed that EPSs from *Navicula* sp. can agglomerate *Spirulina* cells (Chen et al., 2019).

The presence of contaminants is also one of the factors causing the decline in crop yields. Contaminant organisms will damage microalgae cells and inhibit the growth rate, resulting in decreased metabolite production (Zhu et al., 2020). This problem can be overcome through the manipulation of pH and salinity. The pH conditions during cultivation must be stable to maintain $CO_2$ absorption and always be conditioned at the optimum pH range for *Spirulina* growth (pH range of 8-11) (Kawaroe et al., 2019; Nurafifah et al., 2023). In microalgae growth, pH variations often impact biomass, primary, and secondary metabolites (Nurafifah et al., 2023). pH 9 is known to maximize cell density and dry biomass in *Spirulina* and effectively reduce contaminants in *Spirulina* mass cultures. At the same time, the production of lipids, proteins, and pigments reached its optimal point in the pH range of 8-10 (Mufidatun et al., 2023). In previous research by Suyono et al. (2015), Kakarla et al. (2018), Yun et al. (2019), Mirizadeh et al. (2020), Fal et al. (2022), and Timotius et al. (2022), salinity can increase the growth and production of primary metabolites such as carbohydrates, lipids, pigments, and proteins in microalgae. Studies regarding using *Navicula* sp. as a bioflocculant for the *Spirulina* mass harvesting, with salinity and pH treatment for growth, metabolite production, and settling efficiency, have never been done before. Therefore, this is the first time that research with *Navicula* sp. as a bioflocculant in *Spirulina*.

Thus, this study aims to determine the effect of salinity and pH variations on *Spirulina's* cell density, dry biomass, and metabolites. In addition, this study was also conducted to determine the effect of variations in the ratio of bioflocculants *Navicula* sp. on the cell density, dry biomass, and metabolites of *Spirulina*.

## Material and Methods

### Materials

The materials used in this study are *Spirulina* microalgae culture stock obtained from Nogotirto Algae Park, Sleman, Special Region of Yogyakarta, Indonesia, and *Navicula* sp. culture stock obtained from Pertamina Research & Technology Innovation (RTI), Cakung, Special Region of Jakarta, Indonesia. A 2.5 g/L Sodium Chloride (NaCl), 2 g/L Sodium Hydroxide (NaOH), and 2 g/L Hydrochloride Acid (HCl) were purchased from Merck for salinity and pH manipulation. Bold Basal Medium that was used in this study consisted of macronutrients 25 g/L Sodium Nitrate ($NaNO_3$) was purchased from Nanyang Chemical, 7.5 g/L Magnesium Sulphate Heptahydrate ($MgSO_4 \cdot 7H_2O$), 2.5 g/L NaCl, 7.5 g/L Potassium Hydrogen Phosphate ($K_2HPO_4$), 17.5 g/L Potassium Dihydrogen Phosphate ($KH_2PO_4$), and 2.5 g/L Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$), 11.42 g/L Boric Acid ($H_3BO_3$), trace elements such as 8.82 g/L Zinc Sulphate Heptahydrate

($ZnSO_4 \cdot 7H_2O$), 1.44 g/L Manganese Dichloride Tetrahydrate ($MnCl_2 \cdot 4H_2O$), 0.71 g/L Molybdenum Trioxide ($MoO_3$), 1.57 g/L Copper Sulphate Pentahydrate ($CuSO_4 \cdot 5H_2O$), and 0.49 g/L Cobalt Nitrate Hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$), EDTA solution (consisted of 50 g/L Ethylenediaminetetraacetic Acid Disodium (EDTA $Na_2$), and 31 g/L Potassium Hydroxide (KOH)), and iron solution (consisted of 4.96 g/L Ferrous Sulphate Heptahydrate ($FeSO_4.7H_2O$) and 1 mL/L Sulphuric Acid ($H_2SO_4$)) were purchased from Merck. The materials used to measure microalgae's density and biomass were Whatman filter paper number 40 (purchased from GE Healthcare Life Sciences), 70% Alcohol (purchased from Teknosains Indonesia), and aquadest (purchased from Visindo Kimia). In contrast, the materials used in the carbohydrate analysis were Ethanol ($C_2H_5OH$; purchased from SINKA Laboratories), Sulphuric Acid ($H_2SO_4$), and 5% Phenol solution (purchased from Merck). The lipid analysis test required Chloroform ($CHCl_3$), Methanol (MeOH), and NaCl, purchased from Merck. The materials needed in the protein analysis test are Bovine Serum Albumin (BSA), 10% Sodium Dodecyl Sulphate (SDS), and Bradford reagent purchased from MitraLab Indonesia, whereas Acetone (purchased from Teknosains Indonesia) was used in the pigmentation analysis test.

## Methods

### Experimental Design and Set-Up

The research utilized a factorial pattern within a completely randomized design (CRD) framework. It comprised three stages, each lasting for one week of cultivation. The initial stage explored salinity variations (0, 15, 25, 35 ppt), followed by pH treatments (9, 10, 11, 12) in the subsequent stage using the optimal value of salinity acquired in the first stage. The previous stages' combination of salinity and pH optimum value was used in the third stage, where *Navicula* sp. bioflocculant (1:0.25, 1:0.5, 1:0.75, 1:1 in v/v ratios of the total volume of the medium solutions) was added to the culture. Each stage involved four treatment variations with three replications, resulting in 36 samples.

The *Spirulina* culture in this study employs Bold Basal Medium as the nutrient source. The Bold Basal Medium ingredients are put into the volumetric flask according to the standard recipe order in Table 1.

A-1000 mL distilled water was then added to the volumetric flask. The volumetric flask containing the medium solution was shaken for three minutes. Next, the homogeneous medium was put into a 400 mL

Table 1. Bold Basal Medium compositions (Andersen, 2005).

| Component | Stock Solution (g/L) | Quantity Used (mL) |
|---|---|---|
| Macronutrients | | |
| $NaNO_3$ | 25 | 10 |
| $CaCl_2.2H_2O$ | 2.5 | 10 |
| $MgSO_4.7H_2O$ | 7.5 | 10 |
| $K_2HPO_4$ | 7.5 | 10 |
| $KH_2PO_4$ | 17.5 | 10 |
| NaCl | 2.5 | 10 |
| Alkaline EDTA Solution | | 1 |
| EDTA | 50 | |
| KOH | 31 | |
| Acidified Iron Solution | | 1 |
| $FeSO_4.7H_2O$ | 4.98 | |
| $H_2SO_4$ | | 1 |
| Boron Solution | | 1 |
| $H_3BO_3$ | 11.42 | |
| Trace Metals Solution | | 1 |
| $ZnSO_4.7H_2O$ | 8.82 | |
| $MnCl_2.4H_2O$ | 1.44 | |
| $MoO_3$ | 0.71 | |
| $CuSO_4.5H_2O$ | 1.57 | |
| $Co(NO_3)_2.6H_2O$ | 0.49 | |

culture bottle. The pH of the media in the culture bottles was then measured with a pH meter (Digital Water Tester for pH EZ-9909). Preparation of Bold Basal Medium was repeated until 36 culture bottles containing 400 mL of medium were obtained.

The bottles filled with 400 mL of medium were sterilized in an autoclave at 121 °C for 15 minutes at a pressure of 1 atm. Each culture bottle cultivated at a particular stage was then added with 100 mL of *Spirulina* culture. The culture bottles were connected to an aeration hose that circulates 850-860 ppm $CO_2$ and is stored in a room with a temperature of 26-27 °C and a TL lamp illumination (HINODE model MNL5-35W with 220-240 V of voltage, 35 W of power, and 50/60 Hz of frequency) of 1590-1600 lux under 24 hours light exposure.

## Cell Density Analysis

*Spirulina* growth rate was measured by the growth rate calculation formula specific (µ). *Spirulina* density data was calculated using the Haemocytometer brand Neubauer. Microalgae samples in 900ìL volume were taken with a micropipette in the Laminar Air Flow under aseptic conditions. The samples were then put into a microtube containing 100 µl of 70% alcohol and homogenized. The homogeneous samples then dripped through the sidelines of the hemocytometer with a cover slip on it. The cell density was determined using the following formula (Andersen, 2005).:

$$d = \frac{10^4 xQ}{v} \quad \text{.....(1)}$$

Note: *d* (cell density in mg/mL), *v* (sample volume in mL), *Q* (counted cells)

## Dry Biomass Analysis

Measurement of biomass was carried out by Gravimetric method (Ramaraj et al., 2013). Under aseptic conditions, 10 mL samples were taken from the culture. The collection was carried out in the Laminar Air Flow with the help of a measuring pipette and a conical tube. The samples were then brought to the vacuum pump kit. Under the Glass Micro Fiber GF/C, Filter Paper Whatman 1822-047 was placed (filter paper had been dried in the oven and weighed first). The samples were poured into the glass funnel, and the conical tube was rinsed three times with distilled water. Next, filter paper (with filtrate) was collected and dried in an incubator at 33 °C for one day (Esco Isotherm). Finally, each filter paper is weighed, and its dry biomass is calculated using the following formula.

$$B(g/L = \frac{Wi - Wb}{V} \quad \text{.....(2)}$$

Note: *B* (dry biomass), $W_1$ (filter paper weight+filtrate), $W_0$ (initial filter paper weight)

## Carbohydrate Content Analysis

Carbohydrate content analysis was performed using the Phenol-Sulphuric Acid method (Kochert 1978). The standard solution used was Glucose dissolved in a 2 N $H_2SO_4$ solution. At 27 °C, 15 mL of microalgae samples were centrifuged at 3300 rpm for 10 minutes (LW Scientific Ultra 8S with fixed-angle rotor). The supernatant was discarded, while the pellet in the conical tube was added with 0.5 mL of 5% phenol. The sample solutions were vortexed and left for ten minutes. Next, 1 mL $H_2SO_4$ was added through the wall of the tube, and it vortexed again. Homogenized sample solutions were then left for 20 minutes. The sample's absorbance was measured using a spectrophotometer at a wavelength (ë) of 490 nm (UV/VIS Genesys 150).

## Protein Content Analysis

Protein content was determined using the Bradford method. In aseptic conditions, 2 mL of microalgae samples were taken. Then, the samples were centrifuged at 3000 rpm for 10 minutes (LW Scientific Ultra 8S with fixed-angle rotor). The supernatant was taken and discarded with a measuring pipette, while the pellet was added with 1 mL of 10% SDS and vortexed for one minute. Next, samples were incubated in a water bath at 95 °C for five minutes (Memmert Waterbath WNB 7). After five minutes, the samples were incubated at 1 °C in the freezer for five minutes. Finally, 8 ìL of incubated samples were inserted into the 500 ìL microplate and added with 200 ìL Bradford reagent. The sample's absorbance was analyzed with ELISA Plate Reader (BioTek 800 TS) at 595 nm wavelength.

## Lipid Content Analysis

Measurement of lipid content was carried out using the Bligh & Dyer method. First, 10 mL of microalgae culture samples were centrifuged at 4000 rpm for 15 minutes. The supernatant was discarded, while the pellet was added 1 mL of $CHCl_3$ and 2 mL of MeOH. The conical tube containing the sample solutions then vortexed for one minute. The solutions were added with 1 mL aquadest and 1 mL $CHCl_3$. The conical tube

was vortexed again for one minute and centrifuged for 15 minutes at 1500 rpm (LW Scientific Ultra 8S with fixed-angle rotor). Lipids at the bottom of the conical tube were placed on a petri dish inside the oven. The $CHCl_3$ evaporation process was carried out in an open petri dish placed in an incubator for one hour at a temperature of 33 °C (Esco Isotherm). Lipid content can be identified from the difference between the total weight and the initial weight of the petri dish and multiplied by the total volume of the sample.

## Pigmentation Analysis

Pigmentation analysis was carried out by adapting the method from Ilavarasi et al. (2012). Dry algal biomass in 30 mg of volume was added with 10 mL of MeOH. The mixture was then incubated in a water bath at 50 °C for 30 minutes (Memmert Waterbath WNB 7). Next, the aliquots were centrifuged at 3000 rpm three times for 10 minutes (LW Scientific Ultra 8S with fixed-angle rotor). Finally, the supernatant was transferred by pipette into a cuvette for pigment analysis. The pigmentation content of the sample (chlorophyll and carotenoid pigments) was determined by the spectrophotometric method at a wavelength of 470, 645, and 662 nm with UV/VIS spectrophotometer (Genesys 150), and analyzed by Ilavarasi et al. (2012) analysis formula. Samples pigmentations were calculated using the following formula:

$$C_a\ (\text{g/L}) = \frac{[11.75 \times (A_{662}) - 2.350 \times (A_{645})]}{1000} \quad \text{.....(3)}$$

$$C_b\ (\text{g/L}) = \frac{[18.61 \times (A_{645}) - 3.960 \times (A_{662})]}{1000} \quad \text{.....(4)}$$

$$C_{a+b}\ (\text{g/L}) = C_a + C_b \quad \text{.....(5)}$$

$$C_{x+c}\ (\text{g/L}) = \frac{[1000 \times (A_{470}) - 2.270 \times C_a - 81.4 \times C_b / 227]}{1000} \quad \text{.....(6)}$$

Note: $C_a$ (chlorophyll a), $C_b$ (chlorophyll b), $C_{a+b}$ (chlorophyll a+b), $C_{x+c}$(carotenoid content), $A$ (absorbance).

## Flocculation or Precipitation Efficiency Measurement

The flocculation efficiency was calculated from the difference value in cell density before and after the flocculation process. Changes in cell density were measured by spectrophotometric method at a wavelength of 680 nm (UV/VIS Genesys 150). The flocculation efficiency(the percentage of microalgae biomass removed from the suspension) was calculated using the method by Praharyawan & Putri (2017) in the following formula.

$$\text{Ef} = \frac{O_{dsb} - O_{dst}}{O_{dsb}} \times 100\% \quad \text{.....(7)}$$

Note: *Ef* (flocculation efficiency), $O_{dsb}$ (the optical density before flocculation), $O_{dst}$ (the cell density after flocculation).

## Results and Discussion

Based on Figure 1a., the culture with 15 ppt salinity treatment had the most optimal dry biomass among the other treatments. On the other hand, the control treatment (0 ppt) had the lowest dry biomass among the other treatments. Figure 1b. also showed the same thing; the 15-ppt salinity treatment had the most optimal cell density among the other treatments. The treatment with the lowest cell density was 35 ppt. The 15-ppt salinity treatment had sufficient sodium ions to support the regulation of osmotic balance, and electron transport activity in photosynthetic reactions. If the salinity is too low, then electron transport and photosystem II activity will not be optimal. However, high salinity stress will be followed by deceleration of cell division, immobilization of the cells, escalation in the amount of energy for osmotic balance regulation, and inhibition of electron transport activity in photosynthetic reactions and reduction of cell size, which ultimately causes a decrease in the growth rate of *Spirulina* (Shetty et al., 2019). The optimal *Spirulina* growth at 15 ppt is in accordance with the results of research conducted by Rohmawati et al. (2023). These results indicate that *Spirulina* growth increases at moderate salt levels.

Figure 1c. shows that the highest carbohydrate content can be found in the 35 ppt treatment, while the lowest carbohydrate content was found in the 0-ppt treatment. High salinity stress also led to the accumulation of lipids in *Spirulina*, as shown in Figure 1d. The 35-ppt treatment found the highest lipid content, followed by 25-ppt, 15-ppt, and 0-ppt treatment. Rohmawati et al. (2023) stated that high salinity stress increased carbohydrate and lipid metabolisms. High salinity stress on *Spirulina's* cells triggers the synthesis of sugar in the form of osmoticum and the accumulation of various fatty acids (mainly in the form of oftriacylgliseride) to maintain osmoregulation and the osmotic pressure (Rangkuti et al., 2023). According to Shetty et al. (2019), Spirulina's synthesized lipids are used as an energy source.

Figure 1e. illustrates that the highest levels of chlorophyll a+b were found in the 0-ppt treatment, followed by 15-ppt, 25-ppt, and 35-ppt treatment, the lowest chlorophyll a+b content. The carotenoid levels in *Spirulina* are inversely proportional to the levels of chlorophyll a+b, with the highest carotenoid content found at a salinity of 35-ppt, decreasing gradually at salinities of 25-ppt, 15-ppt, and reaching the lowest at

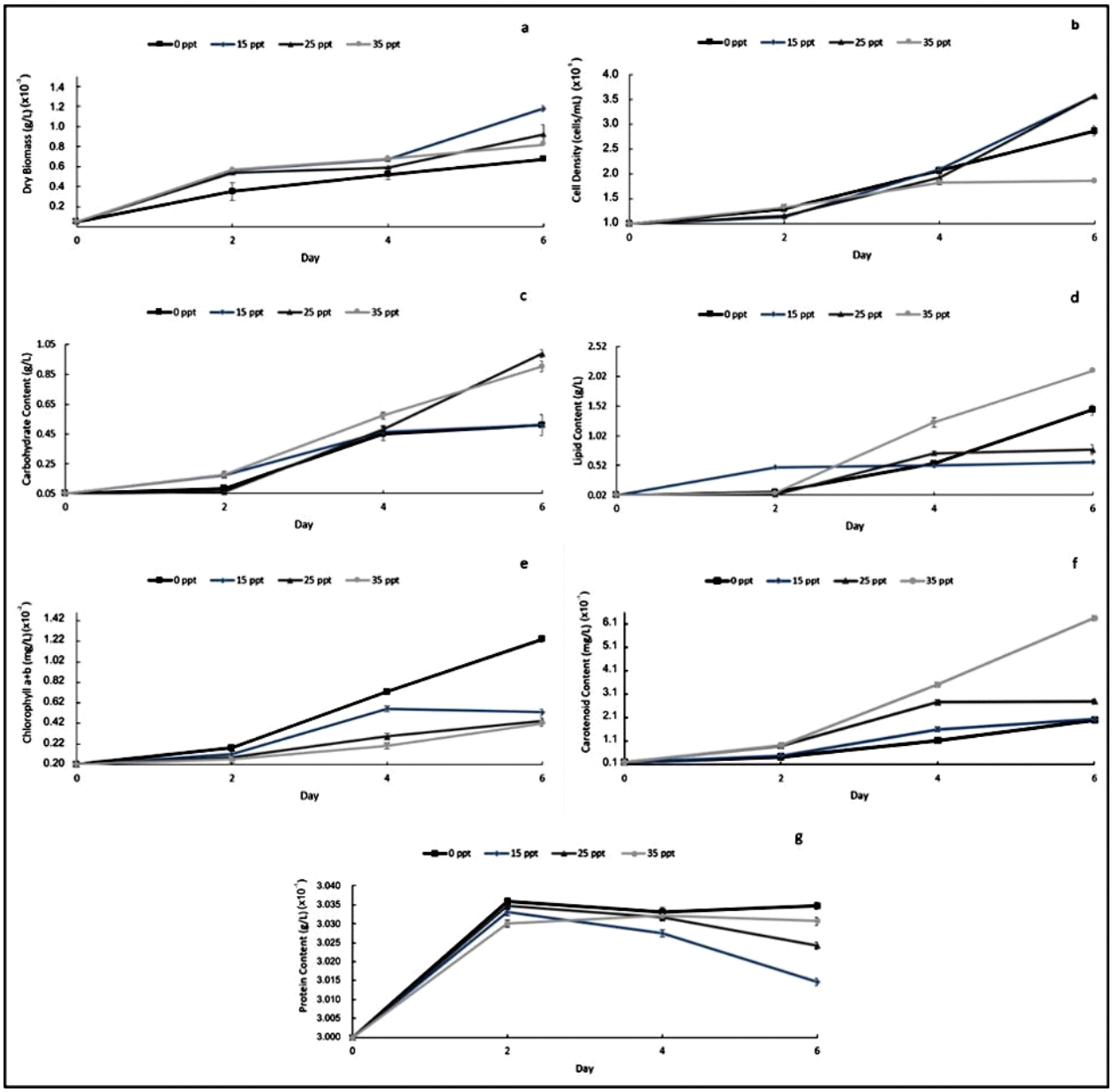


Figure 1. Average of a. dry biomass, b. cell density, c. carbohydrate content, d. lipid content, e. chlorophyll a+b, f. carotenoid, and g. protein content in *Spirulina* cultured on salinity variations (Error bars serve as standard deviation indicators).

0-ppt. These results are correlated to Mutawie (2015) and Sujatha & Nagarajan (2014), that reported the production of chlorophyll a+b in *Spirulina* cells will increase at low salinity levels, while the production of carotenoid pigments will increase at high salinity levels.

Figure 1e. shows high salinity stress conditions, starting from 15 to 35 ppt, reducing chlorophyll content. This is because high salinity decreases chloroplast size, damages the chloroplast envelope, and alters the lamella and grana structures (Hameed et al., 2021), so high salinity decreases chlorophyll production (Rasouli et al., 2021). From Figure 1f., it is observed that a salinity of 35-ppt exhibits the highest carotenoid levels. This finding aligns with Mutawie (2015) and Sujatha & Nagarajan (2014), where carotenoid pigments act as defense agents in *Spirulina* cells against salinity stress conditions; thus, its production correlates with the salinity increase.

Figure 2 shows that the pH 9 treatment increased dry biomass, cell density, carbohydrates, lipids, and pigmentation (chlorophyll a+b and carotenoid). These results correspond with research from Mufidatun et al. (2023) and Kawaroe et al. (2019), which state that the optimal pH for *Spirulina* ranges from 9-9.5. These pH ranges have the most optimal carbonate levels and support the photosynthesis of *Spirulina*, while pH ≥11

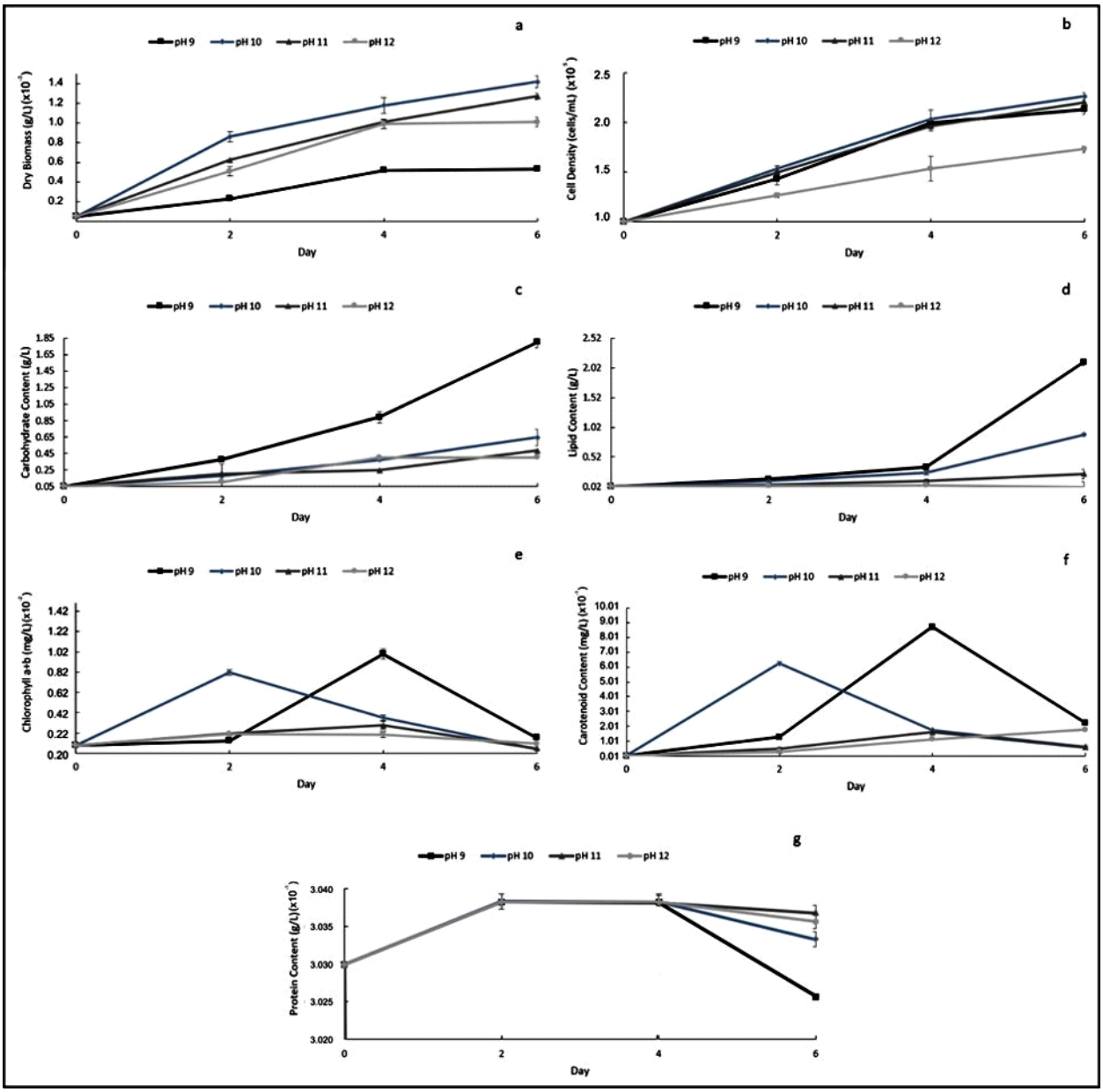


Figure 2. Average of a. dry biomass, b. cell density, c. carbohydrate content, d. lipid content, e. chlorophyll a+b, f. carotenoid, and g. protein content in *Spirulina* cultured on pH variations (Error bars serve as standard deviation indicators).

will harm cells by triggering oxidative stress. Oxidative stress will cause an increase in the amount of ROS in cells, for example, hydrogen peroxide ($H_2O_2$). If this happens continuously, the cell organelles' function will fail, and eventually, the cell will rupture (Babu et al., 2020). Figure 2 also shows that changes in cell density, dry biomass, and metabolites occurred slowly due to *Spirulina*'s adaptation to medium conditions.

Table 2 shows the differences in dry biomass, cell density, metabolites, and precipitation efficiency of *Spirulina* when added with *Navicula* sp. bioflocculant. The addition of *Navicula* sp. with the same v/v ratio as *Spirulina* (1:1) had the highest dry biomass, cell density, carbohydrate content, lipid content, and precipitation efficiency, among the other treatments. This result is due to *Navicula* sp.'s capability to produce extra polymeric substances (EPSs), which act as a source of carbon and nitrogen for *Spirulina*. Specifically, EPSs contain primary amines, aromatics compounds, aliphatic alkyl groups, and carbohydrates (Melo et al., 2022). EPSs also act as an absorbent for humic acids contained in aquatic systems, such as culture medium (Jimoh & Cowan, 2017).

Humic acid in the *Spirulina* culture is caused by the decomposition process of *Spirulina* dead cells (Sharma & Anthal, 2016). The humic acid contained

Table 2. Average of cell density, dry biomass, metabolite, and precipitation efficiency of *Spirulina* with the addition of *Navicula* sp. as a bioflocculant in v/v ratio (in 1 week period of cultivation)

| S:N | DB (g) | CD (cells/mL) | Carb (g/L) | Lipid (g/L) |
|---|---|---|---|---|
| 1:0.25 | $2x10^{-4} \pm 3x10^{-5}$ | $5.67x10^{4} \pm 1.73x10^{4}$ | $2.8x10^{-1} \pm 9x10^{-2}$ | $6.4x10^{-1} \pm 1.02x10^{-2}$ |
| 1:0.5 | $2.9x10^{-4} \pm 8x10^{-5}$ | $3.7x10^{4} \pm 7x10^{3}$ | $3.4x10^{-1} \pm 4x10^{-2}$ | $8.1x10^{-1} \pm 3.07x10^{-2}$ |
| 1:0.75 | $3.8x10^{-4} \pm 1.5x10^{-5}$ | $5.7x10^{4} \pm 2.12x10^{4}$ | $4.5x10^{-1} \pm 1.14x10^{-1}$ | $7.8x10^{-1} \pm 3x10^{-2}$ |
| 1:1 | $6.6x10^{-4} \pm 2.2x10^{-5}$ | $1.14x10^{5} \pm 1x10^{4}$ | $7x10^{-1} \pm 1.55x10^{-1}$ | $2.29x10^{0} \pm 3.29x10^{-2}$ |

| S:N | P (g/L) | $C_{a+b}$ (mg/L) | $C_{x+c}$ (mg/L) | PE (%) |
|---|---|---|---|---|
| 1:0.25 | $3.03324x10^{-1} \pm 5.7x10^{-4}$ | $4.5x10^{-3} \pm 4.1x10^{-5}$ | $2.77x10^{-1} \pm 3.5x10^{-3}$ | $1x10^{-1} \pm 1.41x10^{-1}$ |
| 1:0.5 | $3.03174x\ 10^{-1} \pm 3.5x10^{-4}$ | $4.6x10^{-3} \pm 1.1x10^{-5}$ | $2.85x10^{-1} \pm 2.1x10^{-3}$ | $2.3x10^{-1} \pm 5.77x10^{-2}$ |
| 1:0.75 | $3.03138x\ 10^{-1} \pm 1.5x10^{-4}$ | $4.67x10^{-3} \pm 1.3x10^{-5}$ | $2.89x10^{-1} \pm 1.4x10^{-3}$ | $3.7x10^{-1} \pm 1.15x10^{-1}$ |
| 1:1 | $3.03237x\ 10^{-1} \pm 2.8x10^{-3}$ | $6x10^{-3} \pm 3.5x10^{-5}$ | $3.08x10^{-1} \pm 5.1x10^{-3}$ | $5x10^{-1} \pm 3.6x10^{-1}$ |

Note: S:N (*Spirulina* : *Navicula* sp. ratio), DB (dry biomass), CD (cell density), Carb (carbohydrate content), P (protein content), $C_{a+b}$ (chlorophyll a+b content), $C_{x+c}$ (carotenoid content), PE (precipitation efficiency). Values: Mean ± standard deviation.

in the *Spirulina* culture will be absorbed by the EPS of the *Navicula* sp. bioflocculant (Jimoh & Cowan, 2017). During the agglomeration process, the humic acid absorbed by the EPS will supply dissolved organic carbon to the *Spirulina* cells. This dissolved organic carbon enables *Spirulina* to increase biomass, cell density, carbohydrate, and lipid production. However, if the amount of humic acid absorbed by the EPS is too high, it will cause damage to the protein content due to oxidative stress. The 1:1 v/v ratio has the highest precipitation efficiency among other treatments. This was because the number of EPSs produced was sufficient to agglomerate most *Spirulina* cells. EPSs as bioflocculants from *Navicula* sp. will aggregate *Spirulina* cells, which have a higher density than water (Kurniawan et al., 2022). *Spirulina* cells agglomeration by *Navicula* sp. occurs through a bridging mechanism, where the EPSs as bioflocculant produced by *Navicula* sp. will act as a connector between each cell and then form an aggregate which has a higher density than water until finally, the *Spirulina* cells form flocs (Kurniawan et al., 2022). This process makes it easier to harvest *Spirulina*. Based on this research, bioflocculant organisms like *Navicula* sp. only need to be introduced into the microalgae culture and left for some time. Subsequently, *Navicula* sp. at specific ratios can influence the growth and metabolites of *Spirulina* and induce flocculation processes, thus increasing harvesting efficiency.

## Conclusion

Based on the results, the salinity treatment of 15 ppt, pH 9, and addition of *Navicula* sp. bioflocculant with a ratio of 1:1 showed the optimal results among other treatment combinations with $1.18x10^{-3}$ g of dry biomass, $3.56x10^{5}$ cells/mL of cell density, $2.9x10^{0}$ g/L of carbohydrate content, $3.63x10^{0}$ g/L of lipid content, $9.97x10^{-3}$ mg/L of chlorophyll a+b content, $8.71x10^{-1}$ mg/L of carotenoid content, and $3.04x10^{-1}$ g/L of protein content. Results showed that moderate levels of salinity and pH and using *Navicula* sp. positively impacted *Spirulina* cultivation. Furthermore, the bioflocculant addition and manipulation of environmental factors, such as salinity and pH, can be utilized to obtain the desired growth and microalgae metabolites in further experiments.

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## Supplementary Materials

Supplementary materials is not available for this article

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