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RESEARCH ARTICLE
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Potential of Tilapia Aquaculture Wastewater for Optimizing Growth and Biomass Composition in New Isolated Strain *Spirulina* sp. AB1

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ABSTRACT

The tilapia aquaculture sector, when cultured using biofloc technology, creates a large amount of inorganic effluents, such as nitrate and phosphate, which are known to be needed for microalgal growth. However, a generally neutral pH and low inorganic carbon supply in aquaculture wastewater can restrict *Spirulina* growth. In this work, we studied the effect of bicarbonate ($NaHCO_3$; 4, 8, 12, and 16g L^{-1}) and Zarrouk medium (50 and 75%) supplementation to aquaculture wastewater on the growth, biochemical composition, and nutrient-removal efficiency of a newly identified strain *Spirulina* sp. AB1. We found that increasing sodium bicarbonate concentration in aquaculture wastewater remarkably increased ($P<0.05$) the growth, biomass, pigment (chl *a*, carotenoid, phycocyanin), and protein content of *Spirulina* sp. AB1. In addition, the highest nitrate (82.59±4.49%) and phosphate (70.11±6.94%) removal among bicarbonate supplementation was obtained in cells treated with 16g L^{-1} and 12g L^{-1} $NaHCO_3$, respectively. Furthermore, the growth, biomass, and biochemical profile of *Spirulina* sp. AB1 were dramatically improved when aquaculture effluent was treated with 75% Zarrouk medium. Interestingly, the addition of 75% Zarrouk medium to aquaculture wastewater was comparable ($P>0.05$) to the control regarding growth, biomass, and biochemical content. Finally, the addition of sodium bicarbonate and Zarrouk medium to aquaculture effluent considerably increased the growth, biomass, and biochemical content of *Spirulina* sp. AB1.

Keywords: Aquaculture wastewater; Pigment; Protein; Sodium bicarbonate; Zarrouk

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INTRODUCTION

Aquaculture activities have exacerbated negative environmental effects by producing huge amounts of waste containing both inorganic and organic effluents. Higher levels of dissolved organic carbon, nitrogenous compounds (ammonia, nitrite, and nitrate), and phosphorus in aquaculture effluents may be detrimental to the ecosystem (Ruiz-Marin et al., 2010). Specifically, aquaculture wastewater includes 3–7mg L^{-1} NH_3, 2-110mg L^{-1} $NaNO_3$, 2–50mg L^{-1} PO_4^{3-} and 100–150mg L^{-1} COD (Lowrey et al., 2015; Nasir et al., 2015; Gao et al., 2016). A variety of techniques have been used to treat aquaculture wastewater, including denitrification and chemical precipitation. However, these procedures generate toxic byproducts and enhance aquaculture production costs (Guldhe et al., 2017).

Surprisingly, this nutrient-rich effluent offers great promise as a nutrition source for microalgae production (Ansari et al., 2017). The use of wastewater as a nutrient substitute in microalgal culture has been presented as a

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practical method for commercializing microalgal biomass (Batista et al., 2015; Mohsenpour et al., 2021). Several microalgae have been successfully cultivated in wastewater, yielding significant amounts of biochemical product (Abdelfattah et al., 2023; Fakhri et al., 2024).

Spirulina is a filamentous, industrially important multicellular cyanobacterium that accounts for 30% of the total algae biomass production globally (Lim et al., 2021). Spirulina biomass is widely used in the pharmaceutical, cosmetic, and food industries (Mohammed et al., 2023; Nakamoto et al., 2023) because it can produce high protein content (up to 70%) (Fernandes et al., 2023), phycocyanin (14.2% DW) (Markou et al., 2019) and also as the source of carotenoid, polysaccharides, and essential fatty acids (Matos et al., 2020). This cyanobacterium is classified as an alkaline species, preferring to grow at pH levels ranging from 8.5 to 11, and can efficiently use $NaHCO_3$ as a carbon source for growth and biochemical synthesis (de Morais et al., 2019; Magwell et al., 2023).

The alkaline nature of Spirulina makes it difficult to grow in aquaculture wastewater, which has a relatively neutral pH and fluctuating nutritional profile. This condition also has a major influence on the biomass, growth, and subsequent nutrient removal by microalgae (Lim et al., 2021). Supplementation with certain nutrients, such as sodium bicarbonate ($NaHCO_3$), may be a solution for increasing pH as well as providing inorganic carbon (White et al., 2013). Sodium bicarbonate is the most abundant source of dissolved inorganic carbon (DIC) used by microalgae for photosynthesis (Giordano et al., 2005; Raven & Beardall, 2014). It has been demonstrated that employing sodium bicarbonate in microalgae production reduces the cost of carbon supply while also promoting the growth and synthesis of other essential molecules, such as protein and photosynthetic pigments (Mokashi et al., 2016; Ratomski et al., 2021). Unfortunately, no studies have been conducted to investigate the effects of sodium bicarbonate addition to aquaculture wastewater, especially from the biofloc system, on the growth and biochemical profile of Spirulina.

In the present study, aquaculture wastewater from a tilapia biofloc system was used for *Spirulina* growth medium. We investigated how adding sodium bicarbonate and Zarrouk medium to aquaculture wastewater affected the growth, pigment, and protein content of *Spirulina* sp. AB1. Furthermore, the ability of a recently discovered strain, *Spirulina* sp. AB1, to remove nitrate and phosphate from the medium was studied.

MATERIALS & METHODS

Strain and Cultivation Medium

Spirulina sp. AB1 was isolated from a waste disposal site in Pasuruan District (latitude:-7.6731; longitude:112.7545), East Java, Indonesia. The strain was then cultivated in Zarrouk medium (Costa et al., 2004) without trace element addition (Table 1) at the Laboratory of Aquaculture, Universitas Brawijaya. The morphology of the cells was observed by an inverted microscope (Olympus IX53).

Table 1: Composition of Zarrouk medium

Compound	Amount (g L^{-1})
$NaHCO_3$	16.8
$NaNO_3$	2.5
NaCl	1
K_2HPO_4	0.5
K_2SO_4	1
$MgSO_4.7H_2O$	0.2
$CaCl_2$	0.04
$FeSO_4.7H_2O$	0.01
Na-EDTA	0.08

Aquaculture Wastewater and Water Quality Analysis

Aquaculture wastewater was obtained from an intensive tilapia (*Oreochromis niloticus*) culture. Tilapia was cultivated for 140 days under the biofloc system with a full aeration system and no water exchange. At the end of the tilapia culture, the effluent was collected and filtrated using filters featuring pores with a diameter of 1.2µm. The wastewater suspension was used immediately for the experiment. Finally, the wastewater supernatant was kept at -20ºC to avoid changes in chemical composition.

Inorganic nutrients, including nitrate, nitrite, ammonium, and phosphate, were measured spectrophotometrically according to Boyd (1982). Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), and total organic matter (TOM) were analyzed according to Adams (1991).

Experimental Culture Conditions

In the first investigation, the experiments were performed using 100% aquaculture wastewater supplemented with different concentrations of sodium bicarbonate (4, 8, 12, and 16g L^{-1} $NaHCO_3$). In the second experiment, we grew the cells in aquaculture wastewater supplemented with different concentrations (*w/v*) of the Zarrouk medium: 50 and 75%. Moreover, the cells were cultivated in 100% Zarrouk medium without the addition of aquaculture wastewater, and this condition was determined as a control. The Zarrouk medium composition used in this study is shown in Table 1. *Spirulina* sp. AB1 cells were cultured at 30ºC under continuous illumination (150µmol m^{-2} s^{-1}) for 4 days. The cultures were aerated with ambient air at 0.5mL min^{-1}. The samples were collected daily for growth analysis, while samples for biomass, pigment, and protein determination were taken at the end of the culture period.

Growth and Biomass Determination

The cell growth was determined by measuring optical density at 560nm (OD_{560}) at each 24h interval using a spectrophotometer (GENESYS^{TM} 10S UV-Vis, Thermo Scientific, USA). Biomass (dry weight, g L^{-1}) was determined by filtration after 4 days of cultivation for all treatments, except for aquaculture wastewater treated with 4g L^{-1} $NaHCO_3$ where the sample was measured at day 3. The calculation of the microalgal biomass was conducted according to the method from Janssen et al. (1999) and Fakhri et al. (2021). The specific growth rate (µ) was determined from the following equation (Krzemińska et al., 2014).

$$\mu\ (day^{-1}) = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1} \quad (1)$$

Where µ represents the growth rate per unit amount of cell concentration, x_1 and x_2 are cell concentration at time l (t_l) and time 2 (t_2), respectively.

Chlorophyll *a*, Carotenoid, and Phycocyanin Determination

Chlorophyll *a* and carotenoid content were extracted by 90% methanol. The quantification of pigment was determined by a spectrophotometer (GENESYS™ 10S UV-Vis, Thermo Scientific, USA). The chlorophyll *a* was determined at an absorbance 665nm and analyzed as described by Ritchie (2006), while carotenoid quantification was performed at a wavelength of 470nm and calculated as described in Wellburn (1994).

Chlorophyll *a* (µg mL^{-1}) = 12.9447 × (A_{665} − A_{720}) (2)

Carotenoid (µg mL^{-1}) = [1,000 (A_{470} − A_{720}) − 2.86 (Chl *a*)]/221 (3)

A hundred mg dried cell was used for phycocyanin extraction. The phycocyanin was measured at absorbances of 652 and 620nm (Cardoso et al., 2021). Finally, the phycocyanin content was determined as mg g^{-1} DW.

Phycocyanin (mg mL^{-1}) = OD_{620} – 0.474 (OD_{652}) x 5.34 (4)

Determination of Protein

Protein analysis was carried out according to the Lowry method (Lowry et al., 1951) and calibrated using bovine serum albumin (BSA). A BSA concentration range of 0–2,000µg/mL (R^2=0.98) with absorbance readings at 750nm was applied. The protein content was determined as µg mL^{-1}.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS statistic version 27. Data of biomass, protein, and pigment were analyzed using one-way ANOVA followed by Duncan's test with a 95% confidence level (α = 0.05). The values were expressed as the mean ± standard deviation, n=3.

RESULTS & DISCUSSION

Aquaculture Wastewater Characterization and Strain Morphology

To determine the potential of aquaculture effluent as a microalgal growth medium, we investigated macronutrient concentrations and physicochemical parameters (Table 2). In this study, the most common inorganic form of nitrogen was nitrate (NO_3^-) (291.50±30.41mg L^{-1}). Interestingly, the amount of toxic ammonium (NH_4^+) was very low (0.03±0.01mg L^{-1}) and the presence of nitrite (NO_2^-) was negligible (<0.01mg L^{-1}) indicating that the biofloc system worked very well for converting ammonium and nitrite to nitrate. The nitrate concentration is noticeably higher than that of the aquaculture effluent, as reported by multiple earlier investigations (Hawrot-Paw et al., 2020; Lugo et al., 2020; Cardoso et al., 2021). Nitrate is generally used for microalgae growth medium (Grobbelaar, 2004) since it can produce a high growth rate and high biomass productivity compared to other nitrogen sources (Griffiths & Harrison, 2009). Besides nitrate, phosphorus in the form of PO_4^{3-} (95.00±16.97mg L^{-1}) was also obtained in tilapia culture wastewater. It is well known that nitrogen and phosphorus play a vital influence in the growth of microalgae and are regarded as the most critical parameters (Yaakob et al., 2021).

Table 2: Characteristic of tilapia aquaculture wastewater using biofloc system

Parameters	Unit	Value
Nitrate (NO_3^-)	mg L^{-1}	291.50±30.41
Nitrite (NO_2^-)	mg L^{-1}	<0.01
Ammonium (NH_4^+)	mg L^{-1}	0.03±0.01
Phosphate (PO_4^{3-})	mg L^{-1}	95.00±16.97
TOM[1]	mg L^{-1}	36.21±1.92
TOC[2]	mg L^{-1}	59.85±20.02
BOD[3]	mg L^{-1}	1.10±0.28
COD[4]	mg L^{-1}	306.67±133.17
pH	-	7.3

[1]Total Organic Matter; [2]Total Organic Carbon, [3]Biological Oxygen Demand, [4]Chemical Oxygen Demand

Aquaculture wastewater was rich in organic carbon. In this study, we found that the concentration of COD, TOM, and TOC was 306.67±133.17, 36.21±1.92, and 59.85±20.02mg L^{-1}, respectively. The value of COD was similar to the study from Cardoso et al. (2021). In this study, the pH of the tilapia aquaculture wastewater was 7.3, which was normally reported in tilapia culture using biofloc technology (Khanjani et al., 2021).

A new isolated strain, *Spirulina* sp. AB1 was isolated from the waste disposal site. The morphology of the cells is shown in Fig. 1. We observed the cell's length was 60–250µm and the width of the cells was 8–32µm.

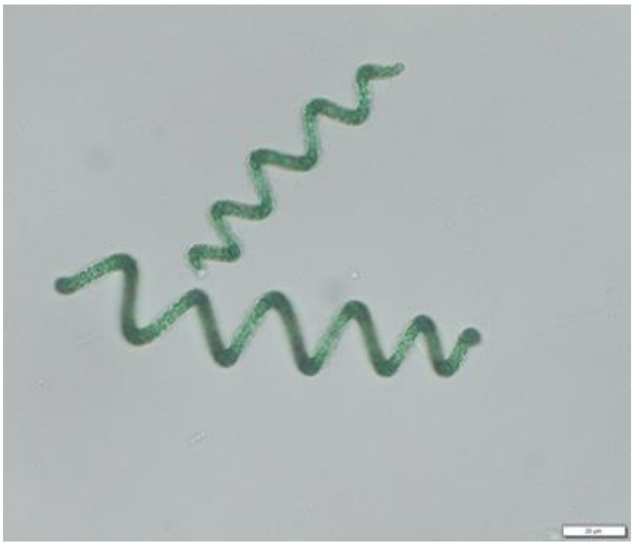

Fig. 1: Morphology of *Spirulina* sp. AB1 isolated from waste disposal site. The cells were observed under inverted microscope with 400x magnification.

Effect of Sodium Bicarbonate and Zarrouk Medium Supplementation on Growth, Biomass and pH Medium of *Spirulina* sp. AB1

In the preliminary study, we grew *Spirulina* sp. AB1 in 100% aquaculture wastewater with an initial pH of 7.0 and we found that the cells still survived but could not grow well with a specific growth rate and maximum OD_{560} of 0.53 day^{-1} and 0.56, respectively. This result suggests that the pH and inorganic carbon might play a significant role in the growth and metabolism of Spirulina.

Therefore, in the first investigation, to achieve the ideal pH and to provide the inorganic carbon for *Spirulina* sp. AB1 growth, we cultured the cells in aquaculture effluent treated with different $NaHCO_3$ concentrations (4, 8, 12, and 16g L^{-1}). The pH of the media after the addition of various sodium bicarbonate concentrations to aquaculture wastewater was initially at around 9 for all treatments and gradually increased with the days (Fig. 2). Moreover, increasing sodium bicarbonate concentration slightly increased the pH of the medium ($P>0.05$) which was still in the range of optimal pH for Spirulina cultivation. The optimal pH for cultivation of *Spirulina* sp. is between 8.5 and 11 (Dineshkumar et al., 2016). pH regulation is critical for microalgae to grow and photosynthesize at their best (Moheimani, 2013).

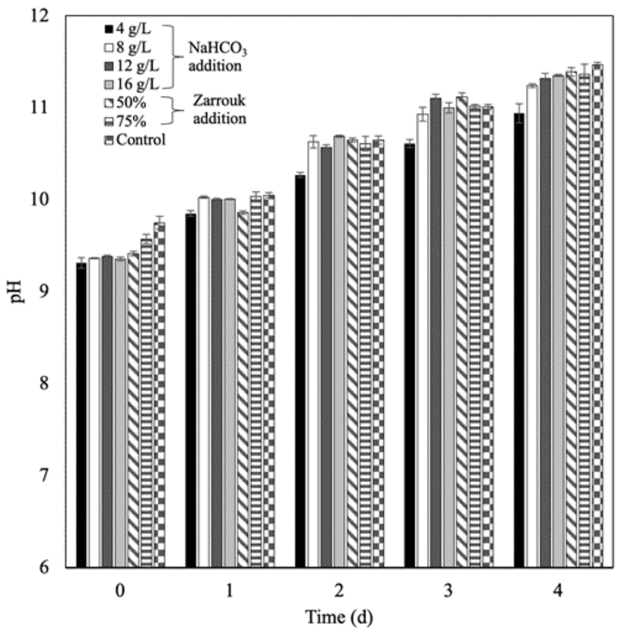

Fig. 2: pH variation during the growth of *Spirulina* sp. AB1 at different supplementation of sodium bicarbonate and Zarrouk medium (50% and 75%). Error bars show standard deviations from the triplicate cultures (n = 3). Same letters shows that there was no significant difference between the values at 95% confidence level.

The growth analysis exhibited that *Spirulina* sp. AB1 experienced no lag phase during the first day of cultivation in all treatments, although cells cultivated in aquaculture wastewater grew slower compared to the control. These results indicate that the cells adapted to the new environment and could utilize the nutrients in aquaculture wastewater medium (Fig. 3A). Fig. 3A also showed the growth of *Spirulina* sp. AB1 increased with increasing $NaHCO_3$ concentration, with a maximum optical density of 1.67 obtained under 16g L^{-1} $NaHCO_3$. The cells were able to grow until the 4th day under all treatments, except for 4g L^{-1} $NaHCO_3$ addition, where cell growth declined after the 3rd day of the culture period. Supplementation of 16g L^{-1} $NaHCO_3$ resulted in 1.53-fold increases in growth compared to 4g L^{-1} $NaHCO_3$ at the end of cultivation. Similarly, White et al. (2013) reported that the addition of sodium bicarbonate into the growth medium enhanced the cell density of *Nannochloropsis salina*. Badger & Price (2003) revealed that bicarbonate is used as a carbon source to avoid the restrictions of using carbon via carbon concentrating mechanisms. However, in this study, the growth of *Spirulina* sp. AB1 under 16g L^{-1} $NaHCO_3$ was 11.1% lower than the control at day 4 of the culture period indicating that only sodium bicarbonate supplementation to aquaculture wastewater is not enough to significantly promote the growth of *Spirulina* sp. AB1.

The biomass concentration of *Spirulina* sp. AB1 cultured on aquaculture wastewater with the addition of $NaHCO_3$ and control is shown in Fig. 3B. There was a significant difference ($P<0.05$) in biomass production of *Spirulina* sp. AB1 among the treatments. The results demonstrated that increasing $NaHCO_3$ concentration increased the dry weight of *Spirulina* sp. AB1. The highest biomass of 1.69g L^{-1} DW was observed under 16g L^{-1} $NaHCO_3$ which was 41.4, 22.7, and 9.2% higher than that of 4, 8, and 12g L^{-1} $NaHCO_3$, respectively. In a similar study, Pancha et al. (2015) reported that increasing sodium carbonate concentration in the medium to some extent increased the dry weight of *Scenedesmus* sp. CCNM 1077 indicates that the addition of $NaHCO_3$ enhances the cell division and metabolic process in microalgae.

To improve the growth and biomass production of *Spirulina* sp. AB1, we cultured the cells in aquaculture wastewater supplemented with 50 and 75% of Zarrouk medium. We found that cell growth increased with increasing the amount of Zarrouk medium (Fig. 3A). Expectedly, when we added 75% Zarrouk medium to the aquaculture wastewater, the growth rate and biomass increased, which were 12.45 and 14.77% higher than 16g L^{-1} $NaHCO_3$, respectively (Table 3, Fig. 3B). On the other hand, the biomass of *Spirulina* sp. AB1 supplemented with 50% Zarrouk medium was similar to the aquaculture wastewater supplemented with 16g L^{-1} $NaHCO_3$. There was a significant difference ($P<0.05$) in biomass between aquaculture wastewater supplemented with 75% Zarrouk medium and 16g L^{-1} $NaHCO_3$. The increased biomass concentration in 75% Zarrouk medium supplementation is due to the higher nutrient contents in this media compared to sodium bicarbonate supplementation alone. Interestingly, there was no significant difference ($P>0.05$) in biomass between control and aquaculture wastewater supplemented with 75% Zarrouk medium (Fig. 3B). These results indicate that the application of aquaculture wastewater as a growth medium can reduce the requirement of Zarrouk by 25%.

Table 3: Maximum specific growth rate (μmax) of *Spirulina* sp. AB1 grown in aquaculture wastewater supplemented with $NaHCO_3$ and Zarrouk medium

Treatment	μmax (day^{-1})
Control	1.02±1.00
$NaHCO_3$ (g L^{-1})	
4	0.59±0.08
8	0.59±0.13
12	0.61±0.03
16	0.65±0.11
Zarrouk addition (*w/v*)	
50%	0.81±0.02
75%	0.96±0.05

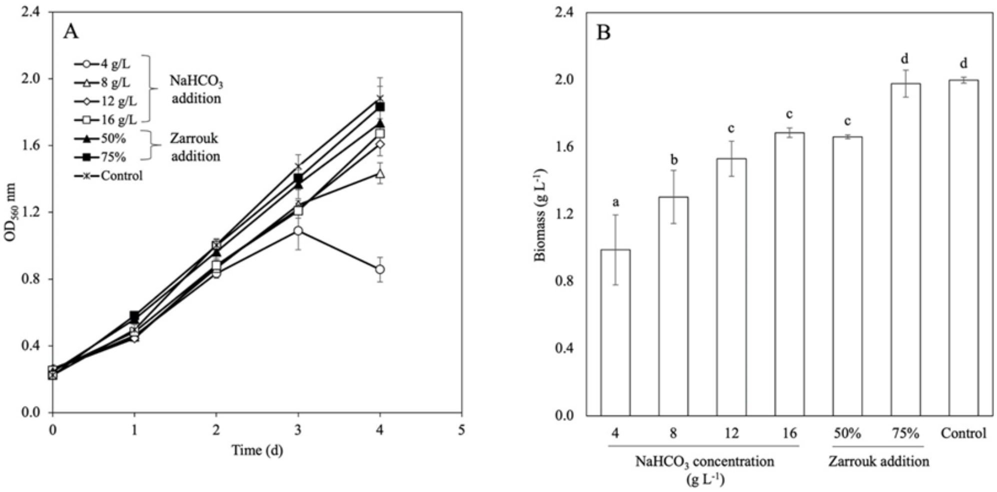

Fig. 3: Growth of *Spirulina* sp. AB1. A. Changes in optical density at 560 nm (OD_{560}); B. Biomass of *Spirulina* sp. AB1 under various $NaHCO_3$ concentrations and Zarrouk supplementation. Error bars show standard deviations from the triplicate cultures (n=3). Same letters show that there was no significant difference between treatment at 95% confidence level.

Protein Synthesis under Supplementation of Various $NaHCO_3$ Concentrations and Zarrouk Medium

We investigated the influence of aquaculture wastewater supplemented with $NaHCO_3$ and Zarrouk medium on the protein content of *Spirulina* sp. AB1 (Fig. 4). The synthesis of protein increased remarkably as a response to $NaHCO_3$ concentrations. Among the $NaHCO_3$ treatment, the maximum protein content of 772.67μg mL^{-1} was obtained at 16g L^{-1} (Fig. 4), which was 2-fold higher than 4g L^{-1} $NaHCO_3$ ($P<0.05$). Similar to our results, Pancha et al. (2015) also found a significant increase in the protein content of *Scenedesmus* sp. CCNM 1077 with increasing sodium bicarbonate concentration. Microalgal cells require a consistent supply of inorganic carbon for photosynthesis, carbon fixation, and the synthesis of protein (Singh et al., 2022).

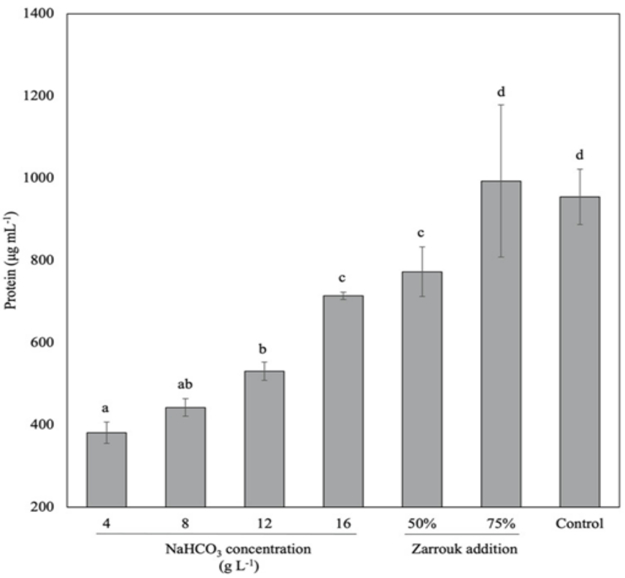

Fig. 4: Protein of *Spirulina* sp. AB1 grown in 100% aquaculture wastewater supplemented with different sodium bicarbonate concentration and 50% and 75% Zarrouk medium. Error bars show standard deviations from the triplicate cultures (n=3). Same letters shows that there was no significant difference between the values at 95% confidence level.

Our further investigation revealed that the protein content under 16g L^{-1} $NaHCO_3$ was significantly lower ($P<0.05$) than that of the control. There was a 28.10% enhancement in protein content in *Spirulina* sp. AB1 grown in 75% Zarrouk medium vs. 16g L^{-1} $NaHCO_3$. Interestingly, there was no significant difference ($P>0.05$) in protein content between control and supplementation of 75% Zarrouk medium to aquaculture wastewater. Some studies have revealed that the concentration of nitrogen in the medium has a significant effect on the accumulation of proteins in microalgae (Markou, 2015; Fakhri et al., 2021).

Pigment Content under Supplementation of Various $NaHCO_3$ Concentrations and Zarrouk Medium

The effect of different concentrations of $NaHCO_3$ and Zarrouk medium supplementation to aquaculture wastewater on chl *a*, carotenoid, and phycocyanin was studied. An increasing concentration of $NaHCO_3$ in the aquaculture wastewater led to a significant increase ($P<0.05$) in the chl *a*, carotenoid, and phycocyanin content of *Spirulina* sp. AB1, with the highest chl *a*, carotenoid, and phycocyanin content of 5.51μg mL^{-1}, 1.79μg mL^{-1}, and 8.77mg g^{-1} DW was obtained at 16g L^{-1} $NaHCO_3$, respectively (Fig. 5A and 5B). Similar to our results, Pancha et al. (2015) also observed an increase in chl *a* and carotenoid content of microalgae *Scenedesmus* sp. CCNM 1077 with increasing sodium bicarbonate supplementation. They suggested that an increase in the culture pH as a result of bicarbonate addition might be the reason for higher pigment content. Fig. 5 also demonstrated that the chl *a*, carotenoid, and phycocyanin in $NaHCO_3$-supplemented cultures were remarkably lower ($P<0.05$) compared to that of the control. There was a 36.7, 55.4, and 73.7% increase in chl *a*, carotenoid, and phycocyanin content, respectively, in *Spirulina* sp. AB1 grown in 16g L^{-1} $NaHCO_3$ vs. control.

In the present study, the chl *a* production is directly correlated with the growth, biomass, and protein content of microalgae which was also reported by several workers (Shanthi et al., 2021; Fakhri et al., 2024). These findings suggest that chl *a* synthesis is part of microalgae's main metabolism (Fakhri et al., 2017). Cardoso et al. (2021) indicated that phycocyanin is closely related to nitrogen and carbon availability during culture. When there is no nitrogen or carbon constraint, phycocyanin is frequently produced in abundance. Perez-Garcia et al. (2011)

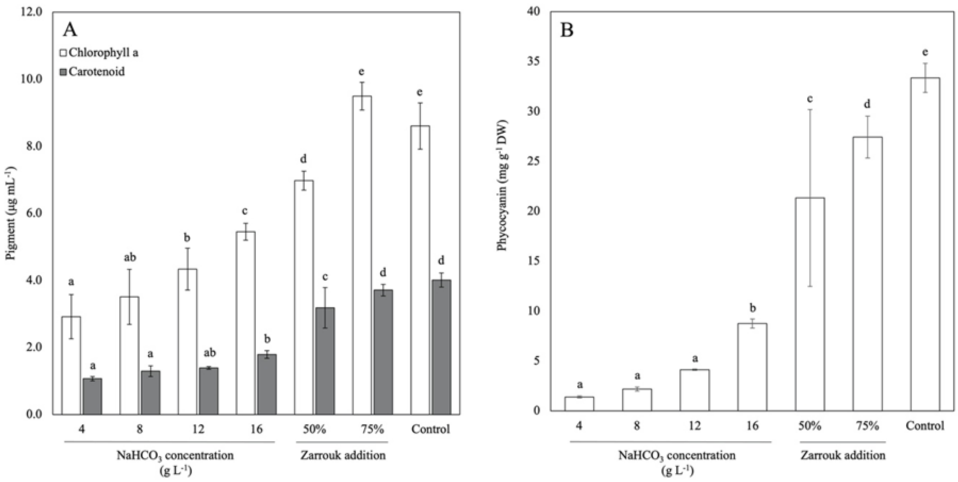

Fig. 5: Pigment content of *Spirulina* sp. AB1 in 100% aquaculture wastewater supplemented with different $NaHCO_3$ concentration and 50% and 75% Zarrouk medium. A. Chlorophyll *a* and carotenoid; B. Phycocyanin. Error bars show standard deviations from the triplicate cultures (n=3). Same letters shows that there was no significant difference between the values at 95% confidence level.

proposed that during stress, phycocyanin is one of the intracellular nitrogen sources that cells might reduce and employ for growth.

We observed higher pigment content in *Spirulina sp*. AB1 when aquaculture wastewater is supplemented with 50% and 75% Zarrouk medium. There was a 1.72-fold and 2.2-fold increase in chl *a* and carotenoid content, respectively, in cells treated with 75% Zarrouk than 16g L^{-1} $NaHCO_3$ addition.

Nutrient Removal

To avoid eutrophication, nutrients should be removed from wastewater before it is released (Lananan et al., 2014). Through the phytoremediation process, microalgae have demonstrated the ability to remove nutrients from wastewater effectively (Caporgno et al., 2015). In this study, we assessed *Spirulina* sp. AB1's ability as a bioremediation agent by measuring nitrate and phosphate removal from the medium. An increase in sodium bicarbonate concentration in wastewater medium significantly ($P<0.05$) enhanced the nitrate and phosphate consumption of *Spirulina* sp. AB1 (Fig. 6). The highest nitrate (82.59±4.49%) and phosphate (70.11±6.94%) consumption among bicarbonate supplementation was found in cells cultivated with 16g L^{-1} and 12g L^{-1} $NaHCO_3$, respectively. According to Lee & Lee (2002), increased nitrate consumption in *Chlorella kessleri* may be caused by improved cell absorption efficiency of nitrate. Interestingly, there was no significant difference between the supplementation of 16g L^{-1} $NaHCO_3$ and the control. In addition, the nitrate consumption was higher in aquaculture wastewater treated with 16g L^{-1} than in 75% Zarrouk medium which has 12g L^{-1} $NaHCO_3$. These results indicate that the addition of sodium bicarbonate improved the consumption of nitrate in *Spirulina* sp. AB1.

Fig. 6 also revealed that a further increase in sodium bicarbonate concentration to 16g L^{-1} showed reduced phosphate removal by 6.8% compared to 12g L^{-1} $NaHCO_3$. This finding was consistent with that of Pancha et al. (2015), who found that increasing the sodium bicarbonate content to 1.5g L^{-1} reduced phosphate intake in *Scenedesmus* sp. CCNM 1077. Guihéneuf & Stengel (2013) explained lower nutrient consumption with high bicarbonate supplementation could be attributed to an increase in the pH of the growth media, since many salts in the culture medium precipitate and become unavailable for absorption by microalgae.

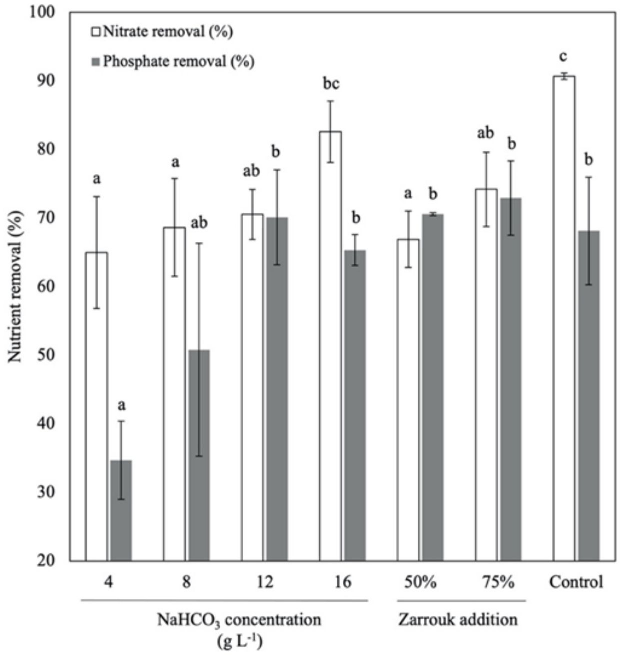

Fig. 6: Nitrate and phosphate removal of *Spirulina* sp. AB1 under supplementation of sodium bicarbonate and Zarrouk medium to aquaculture wastewater. Error bars show standard deviations from the triplicate cultures (n=3). Same letters shows that there was no significant difference between the values at 95% confidence level.

Conclusion

This study indicated that sodium bicarbonate supplementation to aquaculture wastewater enhanced the initial pH of growth medium to the optimal value, which in turn led to increased growth, biomass, nutrient removal, and biochemical composition of *Spirulina* sp. AB1. In addition, supplementation of Zarrouk medium to aquaculture wastewater significantly enhanced the growth, biomass, and biochemical content of the cells. Interestingly, using aquaculture wastewater as a Spirulina growing medium can lower Zarrouk requirements by 25%. To our knowledge, this is the first report of the utilization of aquaculture wastewater from a bio floc system as an alternate medium for Spirulina culture.

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