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A Sustainable Environmental Solution for Wastewater Treatment using *Hapallosiphon* sp. Alga

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ABSTRACT

Microalgae-based wastewater treatment is a promising technology for advanced wastewater treatment and nutrient recovery, leveraging the ability of microalgae to remove carbon, nitrogen, and phosphorus from wastewater. In this study, the blue-green alga *Hapalosiphon* sp. was used to treat wastewater from the Hamdan plant, one of the largest treatment facilities in Basrah Governorate, southern Iraq. Three concentrations of wastewater (25%, 50%, and 75%) were prepared, and changes in pH were measured after cultivating the algae. By the end of the experiment, a decrease in the levels of pH was recorded with ranges from 9.41, 9.34, and 8.99 to 8.16, 8.32, and 8.04, respectively. While, the dissolved oxygen (DO) levels were recorded with a significant increase, from 6.2, 3.2, and 1.8mg/ L before treatment to 12.1, 6.4, and 6.5mg/ L at the end of the experiment for the 25, 50, and 75% concentrations, respectively. The main nutrients—carbon, nitrogen and phosphorus—were weekly measured throughout the experiment, with each measurement performed in duplicate. The removal percentages of these nutrients were calculated. The final carbon removal rates were 57, 34, and 26%, while nitrogen removal rates reached 100, 93, and 69.8% for the 25, 50, and 75% wastewater concentrations, respectively. Phosphorus removal showed a steady increase, from initial rates of 14.1, 46.2, and 48.2% at the end of the first week to 92.9, 93.7, and 85.2% by the third week. As a result of the treatment, the algal biomass increased significantly, rising from an initial 4g to 9.56g, 6.5g, and 5.02g for the 25, 50, and 75% wastewater concentrations, respectively.

INTRODUCTION

Due to industrialization and population increase, significant amounts of wastewater are created, and freshwater is utilized. Wastewater that is directly released into the environment poses a major hazard to the ecological system **(Goswami *et al.*, 2021; Salaah *et al.*, 2022)**. According to United Nations reports, ~ 80% of wastewater is discharged into the aquatic environment without appropriate treatment **(Bijekar *et al.*, 2022)**.

The direct dumping of contaminants generated from these plants poses a substantial risk to the environment due to the multiple toxins involved, and the situation is becoming worse. Wastewater contains a variety of chemicals, some of which, in variable levels, are exceedingly toxic to living creatures. Moreover, significant environmental emissions of both organic and inorganic nutrients resulted in elevated levels of chemical and biological oxygen demands (BOD and COD, respectively). The excessive intake of phosphorus (P) and nitrogen (N) in the aquatic environment causes eutrophication, which results in solid waste and annoying emissions, among other environmental problems **(Chen *et al*., 2020, Wang *et al*., 2022)**.

Conventional methods include various mechanical and chemical treatments **(El-Aswar *et al*., 2019; El-Aswar *et al*., 2022)**. Although physical techniques such as filtration, UV light, and adsorption are frequently used for wastewater purification, they have several limitations, such as not removing chemical pollutants and having high energy costs **(Cho *et al*., 2011; Udaiyappan *et al*., 2017)**. Reducing nutrient levels in wastewater can be achieved using traditional treatment methods such as nitrification, sedimentation or coagulation, anaerobic digestion, and denitrification **(Kumar & Pal, 2015)**. However, these methods are neither cost-effective nor sustainable, as they require high energy inputs (ranging from 4 to 391kWh) **(Obotey Ezugbe & Rathilal, 2020)**. Moreover, they have relatively low nutrient removal efficiencies (~10–93%) **(Yilmaz *et al*., 2008; Gurung *et al*., 2018)**. In addition, these methods generate large quantities of sludge and take a longer time **(Li *et al*., 2019)**.

Chemical treatment, which aims to improve water quality by modifying water properties including turbidity (removal of suspended particulates), pH, and dissolved solids, is one of the most efficient wastewater treatment techniques. The most common methods of chemical treatment include ozonation, UV radiation, chlorination, coagulation/flocculation, and chlorination **(Yusuf *et al*., 2020; Al-Tohamy *et al*., 2022)**.

Despite the fact that these techniques work best in secondary treatment, their high expense and dewatering restrictions, and considerable effort required for maintenance are considered the main disadvantages of these technologies **(Al-Tohamy *et al*., 2022, Daud *et al*., 2022)**.

Conversely, biological methods rely on microorganisms' metabolic activity to break down and transform wastewater contaminants into biomass and related gases (CO_2, CH_4, N_2, and SO_2). Thereby, lowering wastewater's BOD and COD levels and raising their quality **(Dalvi *et al*., 2021)**.

Biological processes can compete satisfactorily with the physical and chemical processes with a good processing performance and relatively low energy consumption as well as low capital/operating costs **(Tan *et al*., 2019)**. Moreover, biological treatment may protect the environment and conserve resources **(Goswami *et al.,* 2021)**. The major purpose of biological treatment, which is typically employed as a secondary treatment method, is to get rid of materials left over from the initial treatment **(Al-Tohamy *et al*., 2022)**. Biological treatment includes biodegradation using various microorganisms, including bacteria, fungi, yeasts, and microalgae **(Al-Tohamy *et al.,* 2020; Ali *et al.,* 2021; Danso *et al*., 2022)**.

In this way, there is a requirement for practical, straightforward, and proficient wastewater treatment frameworks that are fit to satisfy the requirements for treating wastewater and sterilization **(Jayaswal *et al.,* 2018)**. For example, treatments based on microalgae are of great benefit due to the efficient bioaccumulation, nutrient utilization, and high biomass productivity rate **(Abouzied, 2023)**.

Several microalgae species, including *Ankistrodesmus*, *Scenedesmus*, *Euglena*, *Chlamydomonas*, and *Chlorella*, grow well in wastewater **(El-Sheekh *et al.,* 2021)**. In addition, they showed a high tolerance to wastewater toxins such as heavy metals, pesticides, petroleum compounds, and pharmaceuticals. Recently, special attention has been paid to the treatment of urban, industrial, agro-industrial, and livestock wastewater using technologies based on microalgae. Microalgae lessen the possibility of eutrophication since they remove parts of nitrogen and phosphorus **(Priyadharshini *et al.,* 2021)**. Additionally, microalgae collected from certain water bodies for purification may be used for a range of purposes in several sectors, as well as a source of food and raw materials for other products **(Abdelfattah *et al*., 2022)**. When compared to terrestrial plants, microalgae have extraordinary photosynthetic efficiency, which leads to growth rates and biomass output that are sometimes 10 times higher than vascular plants **(Santos *et al*., 2019)**. Compared to conventional wastewater treatment technologies, which often have drawbacks such as high operational costs and secondary contamination caused by chemical reactions **(El-Sheekh *et al.,* 2021)**, microalgae-based wastewater treatment systems offer significant advantages. These systems are more effective at reducing nutrient contamination, and they include built-in disinfection capabilities.

Microalgae wastewater treatment relies on various biochemical processes to directly consume contaminants or nutrient loads in the environment. These processes include bioadsorption, fixation, assimilation, precipitation, and accumulation **(Thakur *et al*., 2020)**.

The objective of this study was to evaluate the ability of a microalgae *Hapalosiphon* sp. belonging to cyanobacteria isolated from Iraqi local water to treat wastewater by withdrawing nutrients, such as carbon, nitrogen, and phosphorous and reducing their concentration in sewage water, therefore, benefiting from them in building biomass.

MATERIALS AND METHODS

Wastewater samples were directly collected from the secondary sedimentation basins of the wastewater treatment plant in Hamdan City, Basrah Governorate, southern Iraq. Three wastewater concentrations (25, 50, and 75%) were prepared by diluting the samples with distilled, deionized water. Each concentration was placed in 500mL conical flasks containing 300mL of the prepared mixture and sterilized using an autoclave. The same amount of *Hapalosiphon* sp. was introduced into each flask, which served as a control sample. Measurements were taken at the end of each week over the three-week experiment, as illustrated in Fig. (1).

The blue-green algae *Hapalosiphon* sp. (Fig. 2) belongs to the order Nostocales, family Hapalosiphonaceae. It was previously cultivated in the laboratory using BG11 medium. The algae were harvested by centrifugation, washed with distilled water, and re-centrifuged 12 times. Subsequently, 4g of wet weight was added to each conical flask.

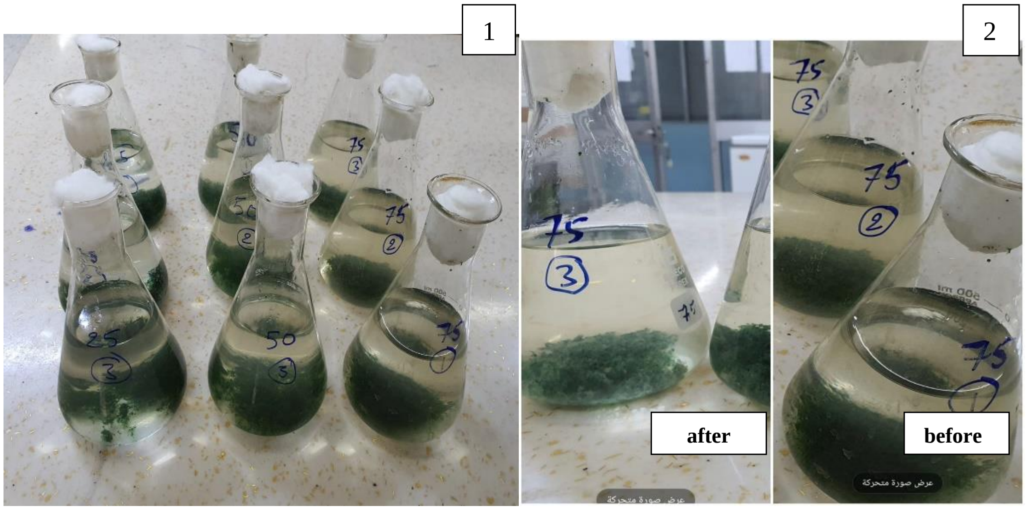

Fig. 1. (1) Three concentrations of wastewater with algae; (2) Before and after treatment

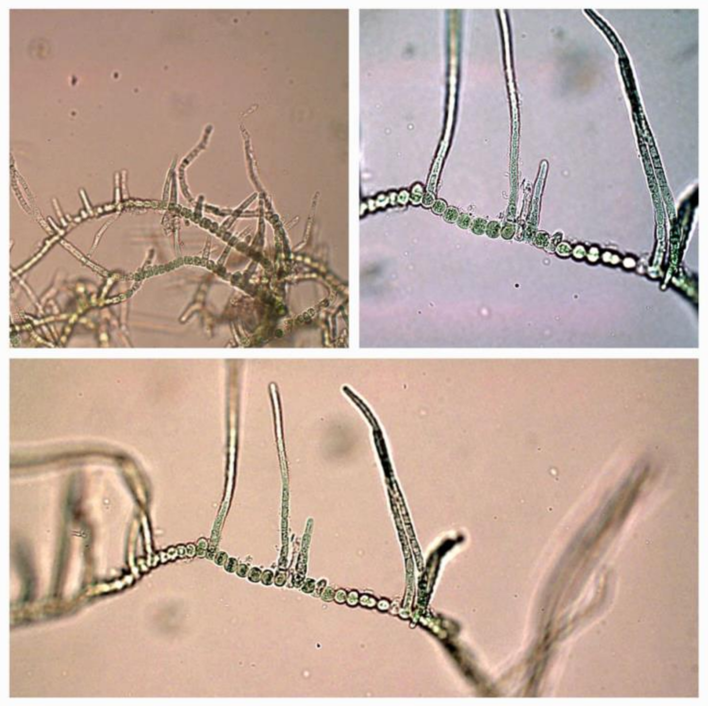

Fig. 2. Light microscopy micrographs of *Hapalosiphon* sp.

The pH of all treatments was weekly measured for 3 weeks using a German-made Lovibond pH meter after calibration with standard Buffer solutions.

All chemical analyses were performed according to **APHA (2005)**. The DO was measured using the iodometric method, which was considered the most precise and reliable titrimetric procedure for DO analysis. The COD was measured using the closed reflux titrimetric method. To calculate organic nitrogen, the macro-Kjeldahl was utilized (N-org). The total nitrogen (TN) was computed by adding the ammonium and organic nitrogen from the aforementioned phases, whereas the total phosphorus (TP) was determined using the ascorbic acid method.

The percentage removal of COD, TN, and TP was computed using the subsequent formula.:

% removal = ((concentration at t=0 - concentration at time t) / concentration at t= 0) * 100 **(Wuang *et a*l., 2016)**

The statistical analysis of the data was conducted using SPSS v-19.0 software. Means were compared using ANOVA. A *post-hoc* test with a less significant difference, (LSD) set at $P \leq 0.05$, was used to identify the significant changes.

RESULTS

1. pH

Table (1) shows the pH values before and after treatment with algae for 3 weeks for concentrations 25, 50, and 75%, where the values ranged between 8.04 and 8.32 after the third week of treatment with algae and for 75% concentration. However, it was increased to 8.52 after the second week of treatment with 50% concentration. There were significant differences between the concentrations (LSD = 0.322) and significant differences between the periods (LSD = 0.913). Furthermore, significant differences were detected between the pre-treatment period and the third week of treatment under the probability level of $P \leq 0.05$.

Table 1. pH values before and after treatment with *Hapalosiphon* sp.

Conc.	Before treatment	After the first week of treatment	After the second week of treatment	After the third week of treatment	Std. deviation
%25	9.41	9.34	9.32	8.16	0.599
%50	9.34	8.97	8.52	8.32	0.530
%75	8.99	8.95	8.88	8.04	0.452
Std. Deviation	0.225	0.220	0.327	0.140	

2. Dissolved oxygen

Table (2) shows the DO values of wastewater before treating it with algae, which were 6.2, 3.2, and 1.8mg/ L for 25, 50, and 75% concentrations, respectively. After treatment for 3 weeks, it showed an increase in the DO values with the progress of the experiment. The DO values gradually increased to reach 12.1 after the third week with 25% concentration, and the DO values increased from 3.2 to 6.4mg /L in the 50% dilution at the end of the third week. At 75% concentration, the dissolved oxygen value increased from 1.8 mg/L to 6.5 mg/L by the end of the experiment. Significant differences were observed between the concentrations (LSD = 5.725) and the treatment periods (LSD = 2.633). However, no significant differences were detected between the pre-treatment period and the first week of treatment. In contrast, significant differences were noted between the pre-treatment period and the second and third weeks of treatment at a probability level of $P \leq 0.05$.

Table 2. DO values (mg/L) before and after treatment with *Hapalosiphon* sp.

Conc.	Before treatment	After the first week of treatment	After the second week of treatment	After the third week of treatment	Std. deviation
%25	6.2	11	12	12.1	2.794
%50	3.2	4.1	4.7	6.4	1.349
%75	1.8	2	3.5	6.5	2.170
Std. Deviation	2.248	4.708	4.600	3.262	

3. COD

The percentages of COD removal from wastewater by *Hapalosiphon* algae, as shown in Table (3), increased across all concentrations during the experiment. In the 25% concentration, the removal percentage rose from 28 to 57%. Similarly, in the 50% concentration, it increased from 18 to 43%, while in the 75% concentration, it grew from 11 to 26%. Significant differences were observed between the concentrations (LSD = 41.101) at a probability level of $P \leq 0.05$, as well as between the treatment periods (LSD = 66.976). Fig. (3) illustrates the COD concentrations in the three treatments (25, 50, and 75%).

Table 3. The percentage removal (%) of COD before and after treatment with *Hapalosiphon* sp.

Conc.	Percentage of removal after the first week	Percentage of removal after the second week	Percentage of removal after the third week
%25	28	34	57
%50	18	37	43
%75	11	21	26

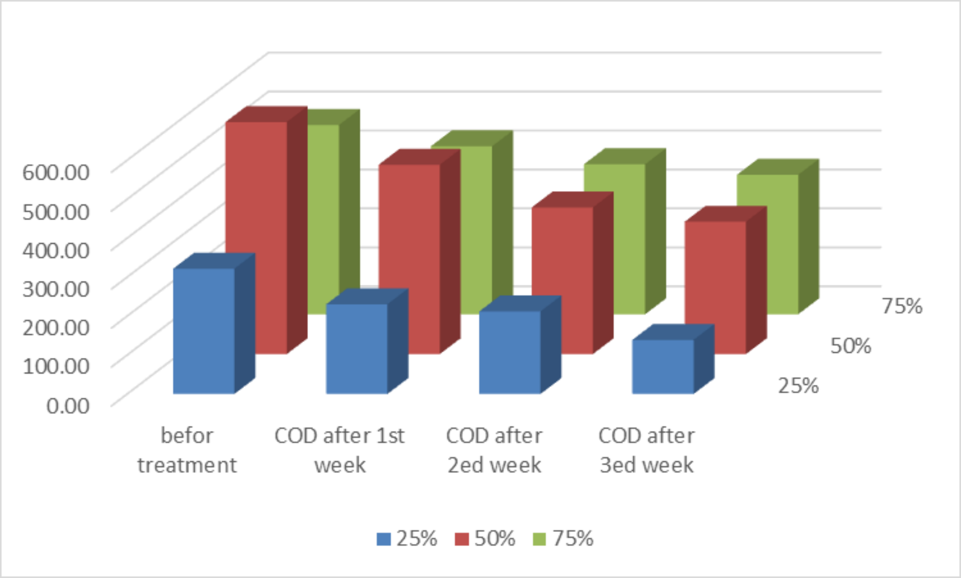

Fig. 3. COD concentrations before and after treatment of the three W.W. concentrations

4. TN

The removal percentage of TN by *Hapallosiphon* alga (Table 4) indicates that the removal percentage increases with the exposure period. At a 25% wastewater concentration, the removal percentage started at 28.2%, and then increased to 64.1% by the end of the second week, reaching 100% by the end of the experiment. Similarly, at a 50% wastewater concentration, the removal percentage was 40.6% at the end of the first week, rising to 70.3% and then 93.2% by the end of the third week. At a 75% wastewater concentration, the percentage increased from 17.7 to 51.1%, reaching 69.8% by the end of the experiment.

Fig. (4) shows the TN concentrations before and after treatment for the three wastewater concentrations. The TN concentrations decreased to zero after 21 days of the experiment. Before treatment, the TN concentration was 7.8 at the 25% concentration of wastewater, while it reached 1.9 and 9 for the 50 and 75% wastewater concentrations, respectively.

Table 4. The percentage removal (%) of TN before and after treatment with *Hapalosiphon* sp.

Conc. of wastewater	Percentage of removal after the first week	Percentage of removal after the second week	Percentage of removal after the third week
%25	28.2	64.1	100
%50	40.6	70.3	93.2
%75	17.7	51.1	69.8

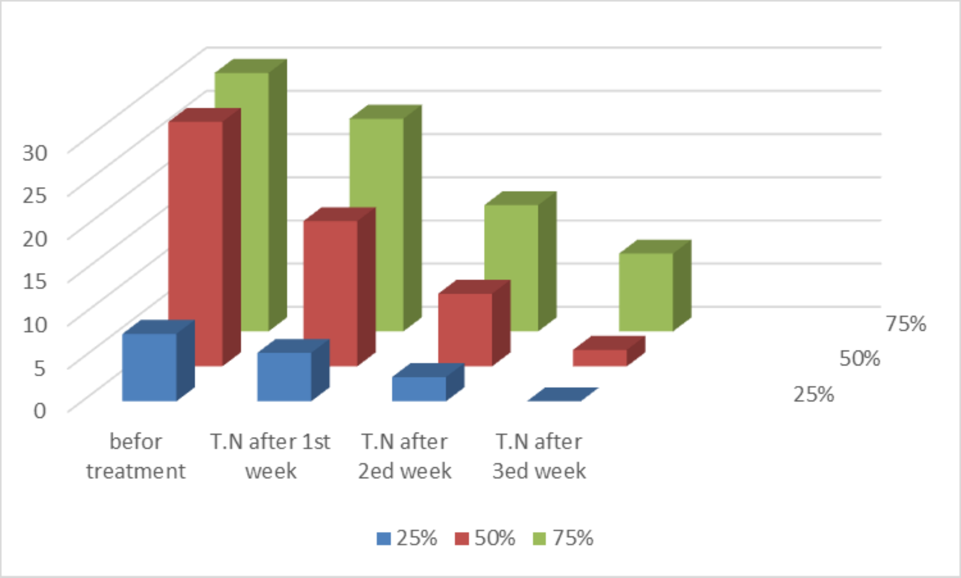

Fig. 4. T.N concentrations before and after treatment of the three W.W. concentrations

5. T.P

With regards to the ability of algae to remove phosphorous, Table (5) shows that these percentages increase upon increasing the exposure periods. In 25% concentration, the percentage increased from 14.1 to 78.7% on the last day of the second week to 92.9% on the last day of the third week. For the 50% concentration, the percentage of removal increased from 46.2% to 92.4% and reaching 93.7% on the last day of the third week. With respect to the 75% concentration, the percentage of removal was 48.2% at the end of the first week, which increased to 82.5%, reaching 85.2% at the end of the third week.

Table (4) shows that the percentage of removal increased with an increase in the concentration of phosphate in the medium, where we found that at the end of the first week, the percentage of removal was 14.1% in 25% concentration; whereas in 50% concentration, the removal percentage became 46.2%, and the ratio was 48.2% in 75% concentration.

Table 5. The percentage of TP removal (%) before and after treatment with *Hapalosiphon* sp.

Conc.	Percentage of removal after the first week	Percentage of removal after the second week	Percentage of removal after the third week
%25	14.1	78.7	92.9
%50	46.2	92.4	93.7
%75	48.2	82.5	85.2

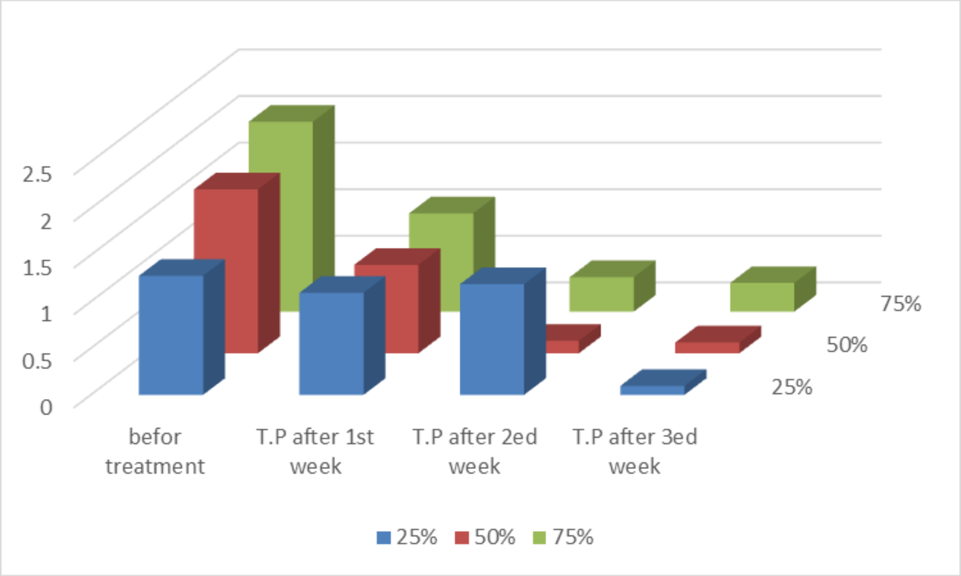

Fig. 5. T.P concentrations before and after treatment of the three W.W. concentrations

6. Biomass

Biomass increased from 5.1g at the end of the first week to 7.2g at the end of the second week, and then reached 9.6g by the end of the third week. In the 50% wastewater concentration, biomass increased from 5.5g at the end of the first week to

6.5g at the end of the third week. In the 75% wastewater concentration, biomass gradually increased from 5 to 5.6g, reaching 6.4g by the end of the experiment. Significant differences were observed between the periods (LSD = 1.033); however, no significant differences were found between the concentrations nor between the last day of the second and third weeks of treatment.

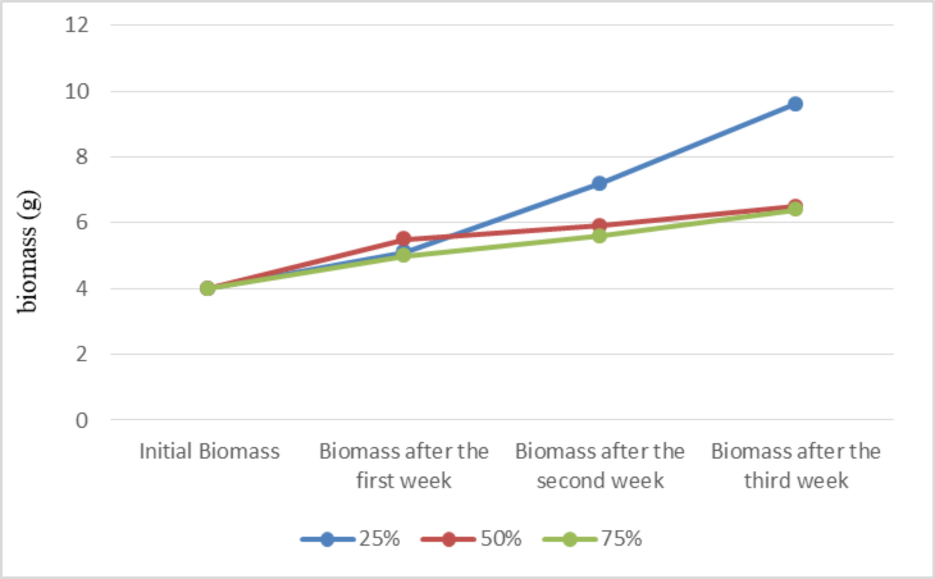

Fig. 6. Biomass production (g) of *Hapalosiphon* sp. during the experiment

DISCUSSION

pH

It was noticeable that the values began to decrease as the treatment period increased. The reduction in pH may be linked to the H+ ions produced during the removal of nutrients and organic materials. Studies by **Wang and Lan (2011)** and **AlMomani and Örmeci (2020)** demonstrated that the removal of ammonium from wastewater leads to a decrease in the pH of the culture.

The optimal pH range for most freshwater microalgae species is between 7 and 9 **(Richmond, 2004 ; Kumar *et al*., 2010; Pérez *et al*., 2013)**.

DO

An increase in DO concentration indicated that the number of microalgae had increased. This was observed by **Amini *et al.* (2020)**, who found that microalgae alone, without mechanical aeration, generated a significant amount of dissolved oxygen, as demonstrated by the results of batch and semi-continuous cultures.

COD

AlMomani and Örmeci (2020) suggested that microalgae in the samples were primarily responsible for the removal of COD, while heterotrophic bacteria did not significantly contribute to this process. Phosphorus has been mineralized by heterotrophic microalgae using COD as a carbon source in dark environments. Furthermore, heterotrophic microalgae utilizing COD created CO_2 in the dark, which autotrophic microalgae could utilize during the day **(AlMomani & Örmeci, 2020)**. The maximum removal rate was 57% in 25% concentration after the third week of treatment, and this difference may be due to the type of algae and its ability to remove. Significant variations were evident between the pre-treatment period and between the end of the first, second, and third weeks of treatment, while there were no significant differences between the second and third weeks, as noted by **Su *et al*. (2012)** elucidating that the cultures with only algae and only sludge achieved removal rates of less than 74%. The cultures of *Spirulina* sp. (71.7%) and *Chlorella vulgaris* (51.6%) outperformed those of *C. minutissima* (42.9%) and *Chlorella pyreneidosa* (37.5%) in terms of COD removal efficiency **(Chawla *et al*., 2020)** where the higher the COD concentration in the medium, the higher the algae consumption of it. The possible reason is the large ability of algae to mineralize organic carbon, but the percentage of removal decreases as the concentration increases due to competition for active sites in the cell wall for the assimilation of pollutants **(Ardila *et al*., 2017; Amini *et al*., 2020)**.

T.N.

Several studies have indicated that the success of using algae to purify urban wastewater was due to algae being rich in nitrogen and phosphorus compounds collected at different stages of the treatment process **(Li *et al*., 2011; Zhou *et al*., 2011)**. There were significant differences between the concentrations (LSD = 9.800) at the level of probability ($P \leq 0.05$) and between periods (LSD = 12.033). No significant variations existed between the pre-treatment period and the end of the first week of treatment. As shown by **de Souza Celente *et al.* (2019)**, up to 99.2% and 70% of N-NH3 and TN, respectively, were removed from wastewater with an algae turf scrubber. According to research, *Chlorella* has also been demonstrated to have an overall nitrogen removal efficiency ranging from 23 to 100% and an overall phosphorus removal efficiency ranging from 20 to 100% **(Cai *et al*., 2013)**. The results of **Tran *et al.* (2021)** showed that after 14 days of incubation, *C. vulgaris* TH03 generated biomass of 1520–1720mg/ L and decreased the quantity of TP (97–100%), TN (94–96.1%), and COD (75–90%). However, these percentages differed according to the different concentrations of nutrients in the used wastewater.

At the end of the experiment, the percentage for 25% concentration was 100%, 93.2% for 50% concentration, and 69.8% for 75% concentration of the wastewater, as the number of nutrients absorbed can vary depending on the metabolic type and the type of microalgae **(Lee *et al.*, 2021)**. Besides, it varied according to the algae's need for nitrogen compounds. A similar observation by **Lin *et al*. (2007)** reported that the relative rate of NH4+ removal was higher at lower leachate concentrations (10 and 30%) than at higher concentrations (50, 80 and 100%).

Microalgae utilize nitrates, ammonia, and nitrites as major sources of nutrition. **(Cai *et al.*, 2013)**. In order to thrive and contribute to the cleaning of wastewater, microalgae incorporate these nutrients into their cells **(Jalilian *et al.*, 2020)**.

T.P.

The needs of cells and the surrounding environment affect phosphorus absorption. Volutin granules store extra phosphorous, which is then utilized to control the metabolic pathways **(Li *et al.*, 2019)**. Inorganic phosphates play an important role in the growth of algae. Due to the positive effect of N on phosphorus accumulation, sufficiently high concentrations of N are required to ensure the effective removal of phosphorus from wastewater **(Beuckels *et al.*, 2015)**. Phosphorus may have been removed from wastewater by two processes: precipitation brought on by the rising pH and taken up by microalgae **(AlMomani & Örmeci, 2016; Al-Qodah *et al*., 2017; Abdelhay *et al*., 2018)**. The pH levels of the samples did not reach sufficiently high levels, such as 11, for phosphorus to precipitate **(Larsdotter, 2006)**. The primary process responsible for phosphorus removal was most likely the uptake by microalgae.

Therefore, the algae can rapidly withdraw phosphorus when they are at high levels and in quantities exceeding their need for growth in addition to storing it in their cells; the phosphorous polymerizes after being absorbed into the cells in the form of polyphosphate **(Tang *et al.*, 1997)** are used later when phosphorus is lacking from the medium, helping it to continue growing. This phenomenon is known as luxury consumption **(Boney, 1975)**. There were significant differences between concentrations (LSD = 0.203) and between periods (LSD = 0.657), as there were no significant differences between the end of the second and third week of treatment. Similar results were shown in 25 and 50% concentrations, with no significant difference.

The total phosphate concentrations before and after treatment with algae are shown in Fig. (5), where there were high concentrations of phosphate before treatment, which gradually decreased with the increasing period of algae exposure. Two metabolic processes were discussed to describe the mechanism of phosphate removal: (1) phosphate assimilation by algae for growth, and (2) phosphate absorption and storage in algal biomass as polyphosphates **(Hwang *et al*., 2016)**.

Biomass

The productivity of algae is the most important parameter as it reflects the amount of nutrients removed from the wastewater **(Liu *et al*., 2020)**. In each concentration of wastewater, the amount of biomass produced varied.

The algal biomass is a key factor in the removal of pollutants, and the greater the biomass, the more pollutants are eliminated **(Nhat *et al*., 2018)**.

We noted that the growth efficiency decreases with the increasing concentration and exposure period. In each wastewater, there was a different degree of biomass production and nutrient removal **(Xu *et al.* 2015; Kim *et al*. 2016)**.

Wang *et al.* (2016) pointed out the necessity of diluting the highly concentrated sewage water due to the toxicity of ammonia.

CONCLUSION

Wastewater treatment data show that algae have a great potential to remove nitrogen and phosphate pollutants from wastewater, as well as some reduction in the COD. The study also showed that using wastewater as a substitute for algal nutrients would significantly reduce the costs of using the artificial medium, e.g. Chu 10 and BG11. It is preferable to use diluted sewage water for the best growth of algae as high concentrations of it may be toxic to the algae.

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