DOI: 10.24996/ijs.2023.64.5.7

ISSN: 0067-2904

Using Microalga *Scenedesmus quadricauda* for the Improvement of Municipal Wastewater Quality

Muzhda Qasim Qader*, Yahya Ahmed Shekha

Environmental Science and Health Department, College of Science, Salahaddin University- Erbil, Iraq

Received: 25/5/2022 Accepted: 20/9/2022 Published: 30/5/2023

Abstract

Microalgae culture is an interesting step in wastewater treatment since it provides a tertiary biotreatment while also producing potentially valuable biomass that may be used for a variety of applications. Microalgae cultures, with their ability to utilize inorganic nitrogen and phosphate for growth, provide an elegant solution to tertiary and quaternary treatments. *Scenedesmus quadricauda* culture was employed with three different doses, 2g/l, 1g/l, and 0.2g/l, to investigate the impacts of microalgae in wastewater. Standard procedures were used to measure samples for physicochemical parameters such as pH, EC, PO_4, NO_3, NO_2, NH_4 and BOD_5 every third day for 21 days. Results showed that a higher dose of $2 g.l^{-1}$ was the most effective for removing the highest rate of nutrients. It was confirmed by significant differences ($p \leq 0.05$) between all doses. Ammonium pass had the highest removal percentage of 97%, followed by NO_3 with 95.7%, and BOD_5 with a range of 90.3 to 93.5 %. Decreases in nutrients were accompanied by a rise in chlorophyll content, with the greatest biomass of $1.52 mg. l^{-1}$ on the 17^{th} day of the experiment. The purpose of this paper is to provide an overview of the methods that microalgae use to bioremediate organic contaminants in aquatic ecosystems.

Keywords: Algal bio-indication, Biomonitoring, Bioremediation, Microalgae, Wastewater treatment.

استخدام الطحالب الدقيقة *Scenedesmus quadricauda* لتحسين جودة مياه الصرف الصحي

موزدة قاسم قادر * ، يحيى أحمد شيخه

قسم علوم البيئة والصحة، كلية العلوم، جامعة صلاح الدين، أربيل،العراق

الخلاصة

تعد زراعة الطحالب الدقيقة مرحلة مهمة في معالجة مياه الصرف الصحي لأنها توفر معالجة حيوية من الخطوة الثالثة بينما تنتج أيضًا كتلة حيوية ذات قيمة كبيرة يمكن استخدامها في مجموعة متنوعة من التطبيقات. توفر مزارع الطحالب الدقيقة ، مع قابليتها لإستهلاك النيتروجين غير العضوي والفوسفات من أجل النمو ، تجهز حلاً عمليا للمعالجات الثالثة والرابعية لمياه الصرف الصحي. تم استخدام مزرعة *Scenedesmus quadricauda* بثلاث جرعات مختلفة (2 غم / لتر ، 1 غم / لتر ، و 0.2 غم / لتر) لتقييم تأثير الطحالب الدقيقة على مياه الصرف الصحي. تم استخدام الطرق المعيارية لقياس العينات لكل من العوامل الفيزيائية والكيميائية مثل الأس الهيدروجيني، التوصيل الكهربائي،الفوسفات، النترات، النتريت،

*Email: Mzhda.qadir@su.edu.krd

الامونيوم ومطلوبية الاوكسجين البايوكيميائية BOD_5 لكل ثلاثة أيام ولمدة 21 يومًا. أظهرت النتائج أن جرعة أعلى من 2 غم / لتر كانت الأكثر فاعلية في إزالة أعلى معدل للمغذيات ، وهو ما تم تأكيده احصائيا بفروقات معنوية ($p \leq 0.05$) بين جميع الجرعات. وسجلت أعلى نسبة إزالة للأمونيوم بنسبة 97٪ ، يليها NO_3 بنسبة 95.7٪ ، ثم BOD_5 بمدى 90.3 إلى 93.5٪. ترافق الانخفاض في المغذيات مع ارتفاع في محتوى الكلوروفيل ، مع أكبر كتلة حيوية قدرها 1.52 ملغم / لتر في اليوم السابع عشر من التجربة. الغرض من هذه الدراسة هو لأعطاء لمحة عامة عن الطرق التي تستخدمها الطحالب الدقيقة للمعالجة الحيوية للملوثات العضوية في النظم البيئية المائية.

1. Introduction

Microalgae have been used in a variety of environmental biotechnology applications, including bioremediation, e.g., phycoremediation. Bioremediation is a branch of environmental biotechnology that treats pollutants through a biological process [1-4].
Phycoremediation is defined as the employment of algae to remove or change contaminants from wastewater, such as nutrients and toxic compounds, while also producing biomass. Due to microalgae's effective photosynthetic uptake of large concentrations of minerals, inorganics and organics, the use of microalgae to treat wastewater and hazardous pollutants is currently of global interest [5, 6]. The relationship between microorganisms and the environment is still not fully understood and more research should be done to determine how various microorganism species can be used to reduce environmental pollution [7]. One of the most important environmental problems that needs to be solved today is the treatment of wastewater produced by pollutants [8].

Furthermore, discharging wastewater containing an excessive amount of nutrients, e.g. nitrogen and phosphorus, into a receiving water body can cause eutrophication which results in oxygen levels reduction in the water [9]. The limiting nutrient in the freshwater system is usually phosphorus in the form of orthophosphates. However, eutrophication can be caused by runoff wastewater containing high levels of phosphorus [10]. Erbil wastewater can be regarded as weak wastewater [11]. Bitton [12] classified wastewater that ranged from 0-220 mg. l^{-1} as weak to medium wastewater.

Wastewater is 99.9% water, with the materials that must be removed accounting for only 0.1 percent by volume. This solid substance is a mixture of feces, food particles, grease, oils, soap, salts, metals, detergents, plastic, sand and grit. The organic fraction is primarily composed of proteins, carbohydrates and fats, reflecting the diet of the community served by the treatment system [13].

Based on previous studies, it was confirmed that microalgae are classified into two groups based on their cellular organization: microalgae and macroalgae [14]. Microalgae are monocellular due to their organizational structure, and therefore can only be identified with a microscope [15]. Microalgae was observed in oceans many years ago where they expanded rapidly by consuming available carbon dioxide and converting it to oxygen through photosynthesis [16]. Microalgae are being utilized for tertiary wastewater treatment due to assimilation of nitrogen and phosphorus as the algae grow [14, 15]. Additionally, photosynthetic carbon assimilation raises the pH of the water which can lead to phosphate chemical precipitation. This chemical stripping could account for a large portion of the total phosphorus reduction [17-20].

Microalgae cells can remove nutrients including phosphorus, nitrogen and ammonium, as well as heavy metals, from wastewater [21-24]. Therefore, the objective of the study was to evaluate the role of micro-algae *Scenedesmus quadricauda* to improve wastewater quality through removal of high nutrients content.

2. Material and Method

2.1 Microalgae Cultivation

The microalgae, *Scenedesmus quadricauda* was isolated from Environmental Science and Health Department laboratory according to a previous investigation conducted by Toma and Aziz [25] and after identification microscopically [26, 27]. *S. quadricauda* is distinguished by its oblong cylindrical cells which are usually arranged in one series. The outer cells have long curved spines at each pole, while the inner cells lack spines [28].

S. quadricauda measurements: Cell length: 21 width 7.5 µm; average colony length: 84 µm; width: 30 µm [29]. After molecular identification was conducted through algal DNA extraction, amplification, sequencing and comparing to the GenBank database, it was identified as *Scenedesmus quadricauda* (MZ801741). The cells of *S. quadricauda* were cultured in BG11 broth medium in distilled water with light emitting diode (LED) lamps at ambient temperature.

2.2 Experimental Design of Batch Cultivation

Wastewater samples were collected at Erbil wastewater channel near Dhahibah village. The samples were autoclaved for 15 minutes to remove bacteria and protozoa. The experiments were carried out in a batch reactor with 2 L conical flasks. 1200 ml of wastewater was inoculated in the flasks with pre-cultured *S. quadricaudai* at the start of each series of experiments. Three distinct fractions with varied concentrations of *S. quadricaudai* were generated to examine the effectiveness of nutrient removal. Raw wastewater contained 2 g/l (run 1), 1 g/l (run 2) and 0.2 g/l *S. quadricauda* (run 3). The experiments lasted 21 days and used municipal wastewater.

2.3 Analytical Methods

All of the sampling and measurements took place at the same time of day. Dry weight of microalgae produced per liter (g/l) was used to compute biomass. The following approach was used to calculate dry cell weight of the microalgal biomass: once a day, 100 ml samples were taken and centrifuged for 5 minutes at 3000 rpm. At a 21-day interval, the percentage of nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), biological oxygen demand (BOD_5) and potassium (K) removal, as well as the growth rate (chlorophyll-*a* content) were calculated. Samples were taken from flasks every day at the same time and centrifuged to separate algae. The BOD_5 was calculated using standard methods in a certified laboratory [30]. Rest of the analysis used a spectrometer to analyze photometrically.

2.4 Chlorophyll Estimation

For estimation of chlorophyll, 10 ml of culture was taken from each flask of sample and centrifuged at 3000 rpm for 5 min and the supernatant was discarded, and the cells were suspended with 5 ml of diethyl ether. Absorbance value of supernatant was measured using UV-spectrometer (UV–2450, Shimadzu) at 660 nm and 643 nm [31].

Chlorophyll $a = (9.92 * A_{660}) - (0.77 * A_{643})$

2.5 Statistical Analysis

Factorial analysis for parameters was performed by using SPSS version 25 program and Excel spreadsheets. The data was subjected to standard analysis of variance and means were compared at a significant 5% level by the Duncan test.

3. Result and Discussion

The mean values of pH, EC, BOD_5, PO_4, NH_4, NO_3 and NO_2 were 8.09, 912 µS/cm, 775 mg/l, 23.7 mg/l, 42.23 mg/l, 23.7 mg/l and 8.94mg/l respectively before treatment (control) as showed in Figures 1 - 6. When *Scenedesmus quadricauda* was used to treat wastewater, the pH was raised to 8.58, 8.52, and 8.45 for each dose of microalgae (2g/l, 1g/l, and 0.2g/l) when compared to control (Figure 1-7). It may be related to photosynthesis process and CO_2 consumption.

A similar findings were reported by Rajasulochana *et al.*[32]. Sharma and Khan [33] observed that the pH of the wastewater sample treated with *Chlorella minutissima* drifted from 8.01 to 8.82, while the pH of the wastewater sample treated with *Scenedesmus sp*. shifted from 8.01 to 9.09. The increased photosynthetic activity of algae or the chemical composition of water may cause an alkaline inclination in general [33-35]. Increase of pH caused by photosynthesis can further accelerate the removal of nutrients by ammonia stripping or phosphorous precipitation [36]. Highest applied dose (2g/l) played more effective role for raising pH values than other doses (Figure 1).

Biological phosphorus and nitrogen reduction are two processes that generate conductivity variations in many treatment plants [37]. On day 21 of the treatment, EC values decreased to 342.5 µS/cm, 356.5 µS/cm and 364 µS/cm (Figure 2), which comprised 62.4, 60.9 and 60.04% (Figure 3). On the other hand, the maximum removal of K in wastewater by *Scenedesmus quadricauda* was 69.195% (run 1), 68.18% (run 2), and 66.66% (run3) accordingly, and TDS was reduced to 75.39, 73.99 and 73.02%. The highest applied dose (2g/l) was more effective than the other doses in reducing K, TDS and EC values (Figure 3). Higher concentration of microalgae has more ability to remove salts and other products that benefit the algae for growth and its metabolic activities. Levlin concluded that conductivity is a broad indicator of water quality that varies when the amount of dissolved salt changes throughout wastewater treatment processes. Conductivity changes as a result of total salt content. Measurements of conductivity can be used to assess the processes that cause conductivity changes in wastewater treatment, such as biological phosphorus and nitrogen removal. The concentration of total dissolved solids (TDS) is directly proportional to the EC [37].

BOD_5 levels of treated effluent were reduced significantly (Figure 4). BOD_5 is an indicator measurement of substances that can be degraded biologically, consuming dissolved oxygen in the treatment during 21 days. BOD_5 level was reduced to 90.3, 91.9 and 93.5% by *Scenedesmus quadricauda*. Aziz *et al.* investigated the feasibility of using an activated-algal process to treat wastewater and discovered that by using C. vulgaris they were able to remove 80-88 percent of BOD_5 after a 15-day retention period [38]. The high algal growth rate and intense photosynthetic activity of C. vulgaris resulted in a progressive drop in the effluent's BOD_5 value [39]. A study by Zhang *et al*. reported that *Scenedesmus* sp. has a high inorganic nutrient removal efficiency from domestic effluents [40]. Using *C. vulgaris*, the removal efficiency of BOD_5 was 89.60 % [41]. The lowest applied dose (0.2g/l) had a more effective role in reducing BOD_5 than other doses.

In the present study, removal of nitrate, nitrite and ammonium by using *Scenedesmus quadricauda* from wastewater was also determined. The maximum removal from wastewater were 95.75%, 86.01 and 96.74% respectively. The maximum PO_4 removed after 21 days was 93.83, 93.12 and 92.89% for applied doses (Figure 3).

In incubation of *Scenedesmus quadricauda* in wastewater, the minimum and maximum removal of NO_3 occurred on the 3rd and 21st days respectively. Larsdotter et al. reported that the lowest reduction of NO_3 could be attributed to the nitrification process; algae prefer to assimilate N in the form of ammonia since it is a passive and energetically less expensive type of assimilation than nitrate uptake, which showed that *Monoraphidium* sp. removed NO_3 in wastewater by 51% and 95% in 5 days [42]. Hammouda *et al.* [35] reported that NO_3 removal was 87.6 % respectively by *Chlorella* sp. in wastewater. The maximum removal of NO_2 occurred at a $2g.l^{-1}$ dose was 86% (Figure 3) on the 21st day which was probably due to an increase in pH values caused by photosynthesis. This can accelerate the removal of nutrients via ammonia stripping or phosphorous precipitation which increases phosphate adsorption on microalgal cells [43-47]

.

Similar results were reported by Gantar *et al.* who concluded that *Chlorella and Scenedesmus* seem to be the most effective algae strains for removing phosphate from a mixture of municipal and refinery wastes [48]. Only algae can effectively remove high levels of nitrogenous compounds in wastewater [49]. A study conducted by Narkthon on the efficiency of nitrogen and phosphorus removal from swine wastewater by *C. vulgaris* showed that 77-86 % of nitrogen and 53-75 % of phosphorus were removed with a retention period of 8 days [50]. González *et al*. [51] reported that *C. vulgaris and Scenedesmus sp.* removed 95% of ammonium nitrogen and 50% of phosphorus from wastewater.

The results of our study showed that use of *Scenedesmus quadricauda* in wastewater treatment resulted in better phosphate removal rates. Similar observation was recorded by Chevalier and De la Noüe [52]. On the 20th day of the experiment, *C. vulgaris* removed 58.7% of phosphate in wastewater, with a maximum removal capability of 91.9%, while *S. abundance* and *S. quadricauda* removed 80% of phosphate from wastewater on the 15th day [48, 53]. Chemical precipitation has been reported to remove over 90% of total phosphorus within the 10 days of algal cultivation [17, 54]. *Scenedesmus quadricauda* had a total phosphorus and nitrogen removal efficiency of more than 94% [55]. According to Zheng *et al.* [56], under the optimal condition, within 11 days the microalgae could eliminate 78% active phosphorus, 62.3% nitrite nitrogen, 84.7% nitrate nitrogen and 100% ammonia nitrogen from the wastewater. Our results revealed that the highest applied dose (2g/l) had more effective role in removing PO_4, NH_4, NO_3 and NO_2 than other doses (Table 1).

Chlorophyll *a* concentration in all batches cultured with *Scenedesmus quadricauda* treatments had increased during experimental period. The initial value of chlorophyll *a* for all doses were 0.58 mg/l, 0.32 mg/l and 0.28 mg/l. Then on day 24 it reached to the maximum values of 1.52 mg/l, 1.20 mg/l and 1.130 mg/l respectively for all doses. On the 27th day of experiment, in all treatments, obvious and significant reduction in the biomass and chlorophyll *a* was observed which indicated that the algae enter the death stage, and the number of algae cells decreased and chlorophyll *a* content dropped to 1.32 mg/l, 1.03 mg/l and 0.94 mg/l respectively (Figure 8). Study has shown that increasing and decreasing biomass is primarily due to the amount of nutrients in the environment, especially nitrogen, and then it is also related to light [57]. This was due to the light intensity in the environment which remained constant throughout the experiment. The decrease in biomass in the environment can be attributed to the low nutritional environment.

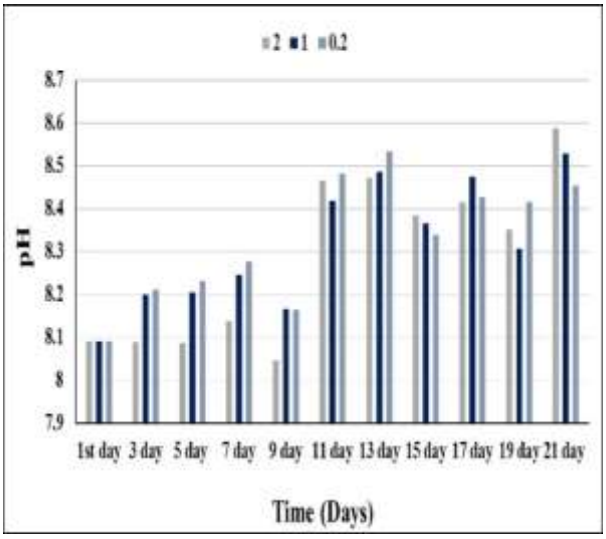

Figure 1: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 g. l^{-1}) on pH of the wastewater.

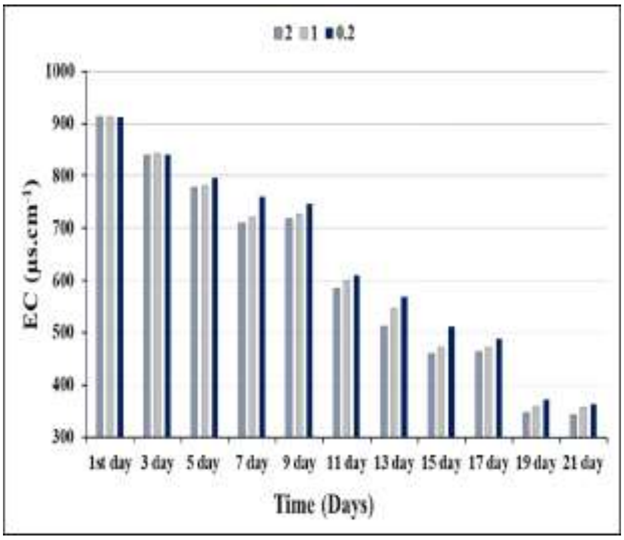

Figure 2: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 g. l^{-1}) on the electrical conductivity of the wastewater.

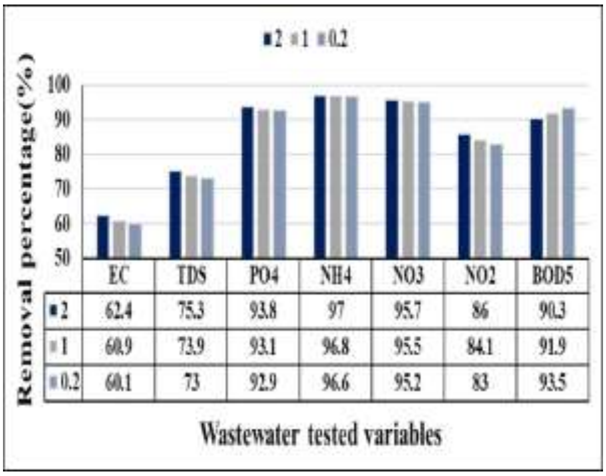

Figure 3: Percent removal from wastewater tested variables after adding three doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2g. l^{-1}).

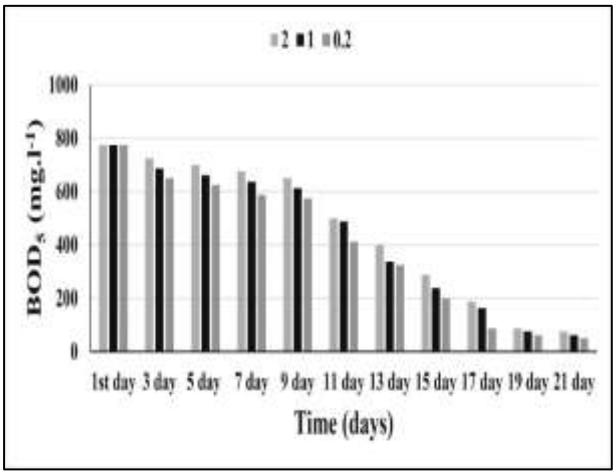

Figure 4: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 g. l^{-1}) on BOD_5 values of the wastewater.

Table 1: Effect of adding different doses (2, 1, and 0.2 g. l^{-1}) of micro-algae *Scenedesmus quadricauda* on some tested wastewater variables data represented (Mean±S.E).

Doses (g.l^{-1})	pH	EC (µS.cm^{-1})	TDS (mg. l^{-1})	K (mg. l^{-1})	NH_4 (mg. l^{-1})	NO_3 (mg. l^{-1})	NO_2 (mg. l^{-1})	PO_4 (mg. l^{-1})	BOD_5 (mg. l^{-1})	Chlorophyll *a* (mg. l^{-1})
D1 (2)	8.28±0.001^{c}	605.36±0.64^{c}	383.95±0.49^{c}	5.01±0.012^{c}	25.103±0.01^{c}	18.54±0.019^{c}	4.38±0.008^{c}	10.45±1.07^{c}	460.22±1.07^{c}	1.076±0.004^{c}
D2 (1g)	8.31±0.001^{b}	617.04±0.64^{b}	395.03±0.49^{b}	5.18±0.012^{b}	25.17±0.01^{b}	18.66±0.016^{b}	4.28±0.008^{b}	10.60±1.7^{b}	430.68±1.7^{b}	0.797±0.004^{b}
D3 (0.2)	8.33±0.001^{a}	633.36±0.64^{a}	403.53±0.49^{a}	5.36±0.012^{a}	25.43±0.01^{a}	18.75±0.016^{a}	4.74±0.008^{a}	10.76±1.6^{a}	395.45±1.6^{a}	0.671±0.004^{a}

Note: Values in each column with different letters are significantly different at P<0.01. Values in rows with same letters are not significantly different.

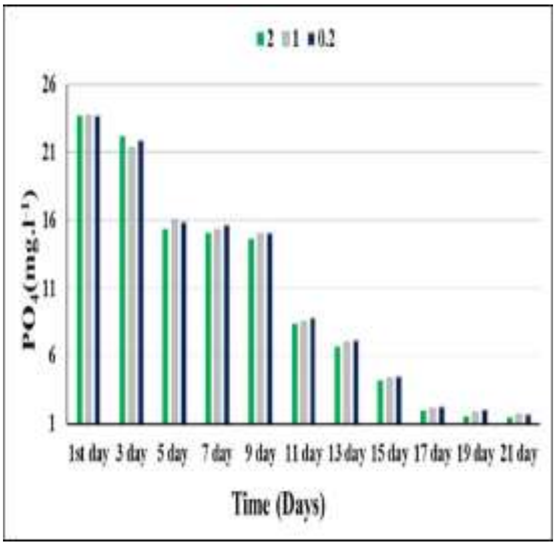

Figure 5: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 g. l^{-1}) on phosphate content of wastewater.

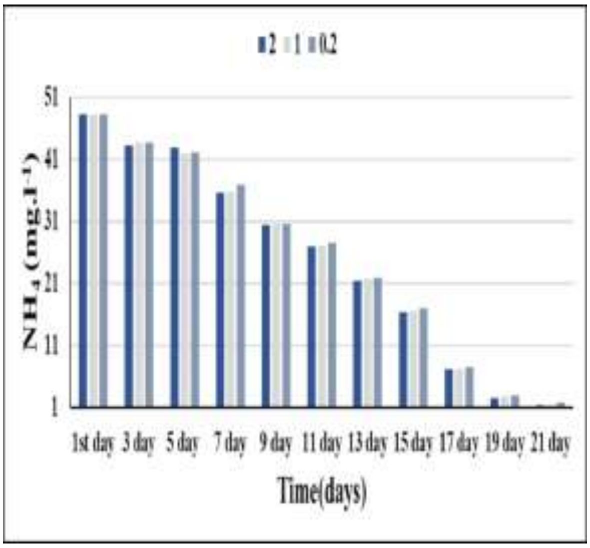

Figure 6: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 g. l^{-1}) on ammonium content of the wastewater.

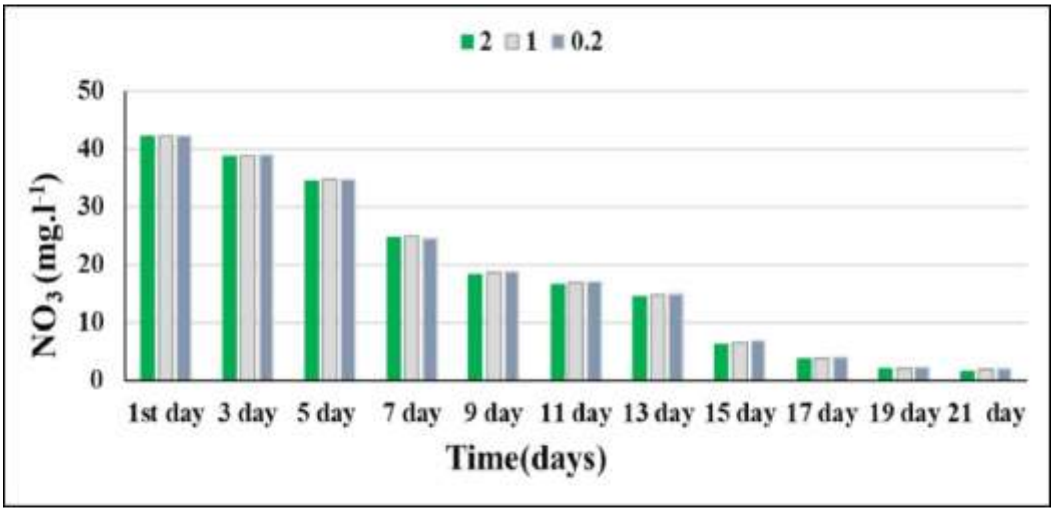

Figure 7: Effect of adding different doses of micro-algae *Scenedesmus quadrica udai* (2, 1, and 0.2 g. l^{-1}) on nitrate content of the wastewater.

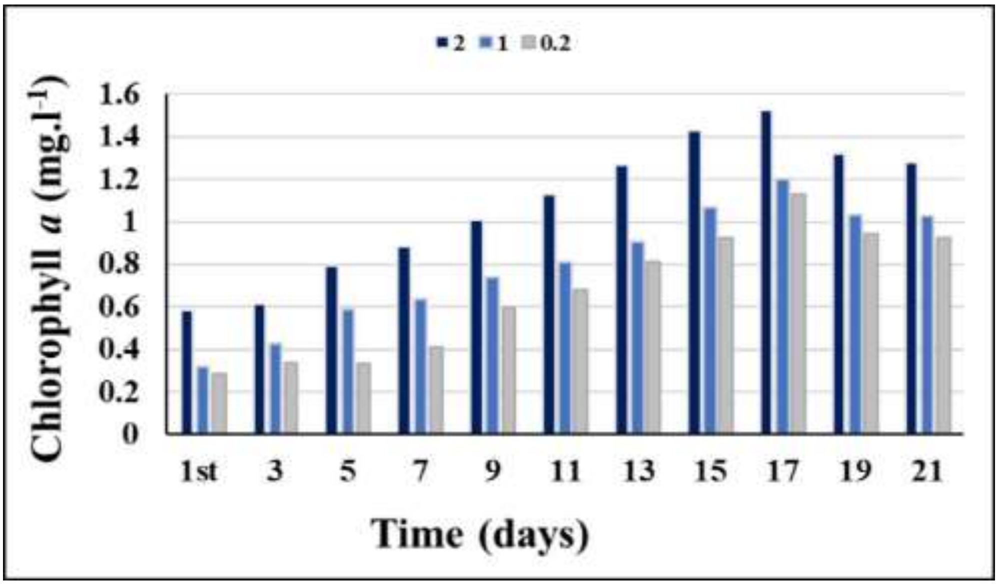

Figure 8: Effect of adding different doses of micro-algae *Scenedesmus quadricaudai* (2, 1, and 0.2 mg. l^{-1}) on chlorophyll and wastewater**.**

1. Conclusion

It was clear from this study that when the reduction rate of different pollutants or nutrients increases the growth rate (chlorophyll-*a*) content of *Scenedesmus quadricauda* in the wastewater also increases. Removal percent of nutrients showed by order ia as following: NH_4>NO_3>PO_4>BOD_5>NO_2. Whereas the highest applied dose (2g/l) had the highest reduction percent during 21 days of treatment. These findings support the idea that *Scenedesmus quadricauda* is an effective nutrient remover.

References

[1] P. Rao, R. R. Kumar, B. Raghavan, V. Subramanian, and V. Sivasubramanian, "Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility," *Water Sa*, vol. 37, no. 1, 2011.

[2] E. J. Olguín, "Phycoremediation: key issues for cost-effective nutrient removal processes," *Biotechnology advances*, vol. 22, no. 1-2, pp. 81-91, 2003.

[3] P. Gani *et al.*, "Experimental study for phycoremediation of Botryococcus sp. on greywater," in *Applied Mechanics and Materials*, 2015, vol. 773, pp. 1312-1317: Trans Tech Publ.

[4] R. Boopathy, "Factors limiting bioremediation technologies," *Bioresource technology*, vol. 74, no. 1, pp. 63-67, 2000.

[5] N. Mohan, N. Balasubramanian, and V. Subramanian, "Electrochemical treatment of simulated textile effluent," *Chemical Engineering & Technology: Industrial Chemistry‐Plant Equipment‐Process Engineering‐Biotechnology*, vol. 24, no. 7, pp. 749-753, 2001.

[6] X. Zeng, M. K. Danquah, C. Zheng, R. Potumarthi, X. D. Chen, and Y. Lu, "NaCS–PDMDAAC immobilized autotrophic cultivation of Chlorella sp. for wastewater nitrogen and phosphate removal," *Chemical Engineering Journal*, vol. 187, pp. 185-192, 2012.

[7] A. A. A. Al-Hisnawi, Y. K. Yasser, N. H. Kadhum, and J. M. Mustafa, "Hydrocarbon degradation test among the microbial community in oil-contaminated soil of power generators in Kerbala city, Iraq," *Iraqi Journal of Science*, pp. 2900-2913, 2022.

[8] A.-S. Hussein and D. E. AL-Mammar, "Adsorption of Biebrich Scarlet Dye into Remains Chromium and Vegetable Tanned Leather as Adsorbents," *Iraqi Journal of Science*, pp. 2814-2826, 2022.

[9] P. Lau, N. Tam, and Y. Wong, "Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized Chlorella vulgaris," *Environmental Technology*, vol. 18, no. 9, pp. 945-951, 1997.

[10] T. Cai, S. Y. Park, and Y. Li, "Nutrient recovery from wastewater streams by microalgae: status and prospects," *Renewable and Sustainable Energy Reviews,* vol. 19, pp. 360-369, 2013.
[11] Y. A. Shekha and J. K. J. S. J. o. U. o. Z. Al-Abaychi, "Assessment of Monthly Variation of Two Water Bodies in Erbil Governorate," *J Univ Zakho* vol. 1, no. 2, pp. 821-831, 2013.
[12] G. Bitton, *Wastewater microbiology*, 3rd Edition ed. Journal of Environmental Protection,: John Wiley & Sons, 2005.
[13] N. J. W. T. p. Gray, "An introduction for environmental scientists and engineers," 2000.
[14] S. Shivhare, A. Mishra, V. Sethi, and A. Bhadoria, "Growth rate, biochemical and biomass analysis of scenedesmus obliquus algae in Shahpura Lake Bhopal (MP)," *Int J Pharm Chem Sci,* vol. 3, pp. 477-482, 2014.
[15] T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, and F. Bux, "Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production," *Bioresource technology,* vol. 102, no. 1, pp. 57-70, 2011.
[16] Y. Sumi, "Microalgae pioneering the future-application and utilization," NISTEP Science & Technology Foresight Center, Science & Technology Trends 1349-3663, 2009.
[17] M. D. Doran and W. C. Boyle, "Phosphorus removal by activated algae," *Water Research,* vol. 13, no. 8, pp. 805-812, 1979.
[18] F. Mesplé, C. Casellas, M. Troussellier, and J. Bontoux, "Modelling orthophosphate evolution in a high rate algal pond," *Ecological modelling,* vol. 89, no. 1-3, pp. 13-21, 1996.
[19] D. Proulx, P. Lessard, and J. De la Noue, "Tertiary treatment of secondarily treated urban wastewater by intensive culture of Phormidium bohneri," *Environ. Technol.,* vol. 15, no. 5, pp. 449-458, 1994.
[20] T. Moutin, J. Gal, H. El Halouani, B. Picot, and J. Bontoux, "Decrease of phosphate concentration in a high rate pond by precipitation of calcium phosphate: theoretical and experimental results," *Water Research,* vol. 26, no. 11, pp. 1445-1450, 1992.
[21] S.-M. Phang and O. Kim-Chong, "Algal biomass production in digested palm oil mill effluent," *Biological wastes,* vol. 25, no. 3, pp. 177-191, 1988.
[22] N. Abdel-Raouf, A. Al-Homaidan, and I. Ibraheem, "Microalgae and wastewater treatment," *Saudi journal of biological sciences,* vol. 19, no. 3, pp. 257-275, 2012.
[23] M. Aziz and W. Ng, "Feasibility of wastewater treatment using the activated-algae process," *Bioresource Technology,* vol. 40, no. 3, pp. 205-208, 1992.
[24] E. d. Sydney *et al.*, "Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage," *Applied Energy,* vol. 88, no. 10, pp. 3291-3294, 2011.
[25] J. J. Toma and F. H. Aziz, "Antibacterial activity of three algal genera against some pathogenic bacteria," *Baghdad Sc J.* , no. 2022: 7. , pp. 1-9 2022.
[26] J. D. Wehr, R. G. Sheath, and J. P. Kociolek, *Freshwater algae of North America: ecology and classification*. Elsevier, 2015.
[27] D. M. John, B. A. Whitton, A. J. Brook, P. V. York, and L. R. Johnson, *The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae*. Cambridge University Press. , 2002 p. 702
[28] G. J. D. Prescott, Lowa, 997p, "Algae of the western great lake area mc brown comp," 1973.
[29] S. Mayeli, S. Nandini, and S. J. A. E. Sarma, "The efficacy of Scenedesmus morphology as a defense mechanism against grazing by selected species of rotifers and cladocerans," vol. 38, no. 4, pp. 515-524, 2005.
[30] L. S. Clesceri, A. E. Greenberg, and A. D. Eaton, "Standard methods for the examination of water and wastewater," ed: 20th Edition, American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC., 1998, pp. https://www.worldcat.org/title/standard-methods-for-the-examination-of-water-and-wastewater/oclc/40733179.
[31] E. W. Becker, *Microalgae: biotechnology and microbiology*. Cambridge University Press, 1994.
[32] P. Rajasulochana, R. Dhamotharan, S. Murugesan, and A. J. J. o. A. S. Rama Chandra Murthy, "Bioremediation of oil refinery effluent by using Scenedesmus obliquus," vol. 5, no. 4, pp. 17-22, 2009.
[33] G. K. Sharma and S. A. Khan, "Bioremediation of sewage wastewater using selective algae for manure production," *Int J Environ Eng Manag,* vol. 4, no. 6, pp. 573-580 2013.

[34] A. A. Fathi, M. M. Azooz, and M. A. Al-Fredan, "Phycoremediation and the potential of sustainable algal biofuel production using wastewater," *Am J Appl Sci.*, vol. 10, no. 2, p. 189, 2013.
[35] O. Hammouda, N. Abdel-Raouf, M. Shaaban, M. Kamal, and B. Plant, "Treatment of mixed domestic-industrial wastewater using microalgae Chlorella sp," *Am J Sci*, vol. 11, no. 12, pp. 303-315, 2015.
[36] I. Rawat, R. R. Kumar, T. Mutanda, and F. Bux, "Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production," *Applied energy*, vol. 88, no. 10, pp. 3411-3424, 2011.
[37] E. Levlin, "Conductivity measurements for controlling municipal waste-water treatment," in *Proceedings of a polish-Swedish-Ukrainian seminar*, Research and application of new technologies in wastewater treatment and municipal solid waste disposal in Ukraine, Sweden and Poland., 2010, pp. 51-62: Water, Sewage and Waste technology.
[38] M. Aziz and W. Ng, "Industrial wastewater treatment using an activated algae-reactor," *Water science technology*, vol. 28, no. 7, pp. 71-76, 1993.
[39] O. Colak and Z. Kaya, "A study on the possibilities of biological wastewater treatment using algae," *Doga Biyoloji Serisi*, vol. 12, no. 1, pp. 18-29, 1988.
[40] E. Zhang, B. Wang, Q. Wang, S. Zhang, and B. J. B. t. Zhao, "Ammonia–nitrogen and orthophosphate removal by immobilized Scenedesmus sp. isolated from municipal wastewater for potential use in tertiary treatment," vol. 99, no. 9, pp. 3787-3793, 2008.
[41] R. A. Azeez, "A study on the effect of temperature on the treatment of industrial wastewater using chlorella vulgaris alga," *algae*, vol. 8, p. 9, 2010.
[42] K. Larsdotter, J. L. C. Jansen, and G. Dalhammar, "Biologically mediated phosphorus precipitation in wastewater treatment with microalgae," *Environ. Technol.*, vol. 28, no. 9, pp. 953-960. DOI: 10.1080/09593332808618855, 2007.
[43] I. Rawat, R. Kumar, and F. Bux, "12 Phycoremediation by High-Rate Algal Ponds (H RAPs)," *Biotechnological Applications of Microalgae: Biodiesel Value-Added Products CRC Press, Boca Raton*, p. 179, 2013.
[44] A. Ruiz-Marin, L. G. Mendoza-Espinosa, and T. Stephenson, "Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater," *Bioresour. Technol.*, vol. 101, no. 1, pp. 58-64, 2010.
[45] V. Carrillo, B. Fuentes, G. Gómez, and G. Vidal, "Characterization and recovery of phosphorus from wastewater by combined technologies," *Rev. Environ. Sci*, vol. 19, no. 2, pp. 389-418, 2020.
[46] J. Rajesh Banu *et al.*, "Trends in biological nutrient removal for the treatment of low strength organic wastewaters," *Curr. Pollut. Rep.*, vol. 7, no. 1, pp. 1-30, 2021.
[47] R. Tao, "Nutrient and organic matter removal from wastewaters with microalgae," PhD thesis n Environmental Technology, Paris Est.; Tampereen yliopisto, 2019
[48] M. Gantar, S. Gajin, and B. Dalmacija, "The possibility of phosphate elimination by the use of algae in the process of waste water purification," *Acta Biologica Iugoslavica, Series B. Mikrobiologijca*, vol. 21, no. 1, pp. 63-73, 1984.
[49] N. Tam and Y. S. Wong, "The comparison of growth and nutrient removal efficiency of Chlorella pyrenoidosa in settled and activated sewages," *Environmental Pollution*, vol. 65, no. 2, pp. 93-108, 1990.
[50] S. Narkthon, "Nitrogen and phosphorus removal from piggery wastewater by green algae Chlorella vulgaris," *Faculty of Graduated Studies. Mahidol University, Bangkok, Thailand*, 1996.
[51] L. E. González, R. O. Cañizares, and S. Baena, "Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus," *J Bioresource technology*, vol. 60, no. 3, pp. 259-262, 1997.
[52] P. Chevalier and J. De la Noüe, "Efficiency of immobilized hyperconcentrated algae for ammonium and orthophosphate removal from wastewaters," *Biotechnology letters*, vol. 7, no. 6, pp. 395-400, 1985.
[53] T. I. Kassim, "Possible use of microgreen algae to remove phosphate and nitrate from wastewater," in *Proceedings of international symposium on environmental pollution control and waste management*, 2002, vol. 7, no. 10, pp. 628-632.

[54] N. Tam and Y. S. J. E. P. Wong, "The comparison of growth and nutrient removal efficiency of Chlorella pyrenoidosa in settled and activated sewages," vol. 65, no. 2, pp. 93-108, 1990.

[55] R. Xiao, R. Chen, H.-Y. Zhang, and H. Li, "Microalgae Scenedesmus quadricauda grown in digested wastewater for simultaneous CO2 fixation and nutrient removal," *Journal of Biobased Materials Bioenergy,* vol. 5, no. 2, pp. 234-240, 2011.

[56] iculture wastewater by microalgae Isochrysis zhanjiangensis and production of the biomass material," in *Key Eng Mater*, 2011, vol. 460, pp. 491-495: Trans Tech Publ.

[57] G. Samorì, C. Samorì, F. Guerrini, and R. Pistocchi, "Growth and nitrogen removal capacity of Desmodesmus communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: part I," *Water Res.,* vol. 47, no. 2, pp. 791-801. DOI: 10.1016/j.watres.2012.11.006, 2013.