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Application of Micro-Alga Tetradesmus Nygaardi for Wastewater Quality Improvement

Muzhda Q. Qader*, Yahya A. Shekha

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

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Abstract

Microalgae have been increasingly used for wastewater treatment due to their capacity to assimilate nutrients. Samples of wastewater were taken from the Erbil wastewater channel near Dhahibha village. To investigate the role of algae in wastewater treatment algal samples were collected and used to isolate the most dominant and pollution tolerant algae such as Tetradesmus nygaardi cultures in BG11 broth and used for the treatment. Three doses (2, 1, and 0.2 g. l-1) of micro-algae Tetradesmus nygaardi were applied for this experiment for 21 days, and samples were periodically (every 3 days) analyzed for physicochemical parameters such as pH, EC, phosphate, nitrate, and BOD5 using standard methods, in addition to, chlorophyll a determination. Results showed that the highest dose (2g.l-1) was the most effective for removing the highest rate of nutrients, which was confirmed statistically with significant differences ($p \leq 0.05$) between all doses. Ammonium had the highest removal rate of 96 %, followed by NO_3 at 95%, while BOD5 had a range of 82 to 89.5%. Results showed that the highest dose 2g.l-1 was the most effective dose for removing nutrients, confirmed by significant differences ($p \leq 0.05$) between all doses. Reduced nutrient levels were accompanied by an increase in chlorophyll content, with the greatest biomass production of 1.03 mg. l-1 on the 17th day of the experiment.

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*Corresponding author: mzhda.qadir@su.edu.krd

1. Introduction

Climate change effects become clear during the last two decades, and Iraq is among one of the most affected areas in the middle east [1]. Decreased rains, high temperatures, and the recurrence of the increasing dust storms in Iraq are among the most prominent of these indicators and may develop into a severe water crisis that Iraq may witness in the coming period. Erbil governorate is not different from the rest of the other Iraqi governorates in terms of the climatic impact and its negative secretions in terms of its exposure to drought and lack of rain, which indicates the expectation of scarcity of water resources and water scarcity within the governorate [2, 3]. Therefore, obtaining alternatives to the city's water resources is necessary after increasing its population and water requirements. One of these alternatives is to go to the purification of the city's sewage, which represents a large amount of water that may compensate for part of the daily life requirements of the city [4-6]. The city has no current census and no official population statistics are available; its population is estimated to be around 1329041 people (2022) [7, 8], in addition to immigrants from other Iraqi Governorates. According to [9], water consumption in Erbil city is estimated to be 285 L/ Capita/ day. As a result, domestic water consumption has only reached 378776 m3.day. In addition to other vitality facilities within the city that consumes different quantities of water and there are no statistics for them, as well as for the purpose of irrigation and industries. All these quantities of water are discharged into the Erbil sewage channel, in addition to the rainwater in the wet season. The only logical conclusion is that Erbil City's water demand is insufficient. Wastewater is a water that has been harmed by anthropogenic activities such as home, industrial, commercial or agricultural operations and it is the most concerning in terms of environmental health [10].Surface water pollution caused by high nutrient concentrations is a global problem that affects all countries [11]. Nutrient load monitoring and control is an important aspect of water management [12]. Additionally, dumping wastewater containing a large amount of nutrients into a receiving water body may cause eutrophication, which is defined as a reduction in water oxygen levels [13]. Several researches have proven that micro-algae have a high potential for decreasing wastewater's nutritional burden [14, 15]. This technique also offers a number of advantages, including the fact that it produces No external waste, like activated sludge, necessitates chemical use. agents for phosphorus eliminatio, and allows for good nutrient recovery[16, 17].

Algae are universally acknowledged as playing a very important role in natural water purification process[18, 19]. Thus, the use of microalgae for removal of nutrients from different wastes has been described by a number of authors [13, 20-25].

Micro-algae for wastewater treatment isn't a new idea, and various researchers have developed methods to take advantage of the algae's rapid growth and nutrient-removing abilities. Although other nutrient stripping activities such as phosphorus precipitation and ammonia volatilization due to the algae's high pH, also occur, the majority of the nutrient removal attributable to the algae's nutrient assimilation [26]. Algae not only could recycle nutrients into biomass, but they really clean wastewater by releasing oxygen, that could help with heavy metal and xenobiotic chemical bioremediation. The aims of present work is to examine the effectiveness of microalgae Tetradesmus nygaardi in removal inorganic nutirents such as bioremediation of wastewater.

2. Materials and Methods

2.1. Microalgae cultivation

The microalgae, Tetradesmus nygaardi were isolated from Environmental Science and Health Department laboratory according to a previous investigation conducted by Toma and Aziz, 2022 [27]. Molecular identification was conducted through algal DNA extraction, amplification, sequencing, and comparing to the GenBank database, it was identified as Tetradesmus nygaardi (MZ801740). The cells of Tetradesmus nygaardi were cultured in BG11 broth Medium in distilled water with light emitting diode (LED) lamps at ambient temperature. The cultures were incubated at 25°C at constant temperature and continuous light exposure of 3,000 lux for 21 days.

2.2. Experimental design of batch Cultivation

The wastewater samples were collected at Erbil wastewater channel near Dhahibah village. The wastewater samples were autoclaved to eliminate 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 Tetradesmus nygaardi at the start of each series of experiments. Three distinct fractions were prepared with different concentrations of Tetradesmus nygaardi to examine the efficiency of Tetradesmus nygaardi in removing nutrients. Raw wastewater containing 2 g/l (run 1), 1 g/l (run 2), and 0.2 g/l Tetradesmus nygaardi (run 3). The experiments lasted 21 days and used municipal wastewater.

2.3. Analytical Methods

All samplings and measurements were carried out at the same time of day. Biomass was calculated from the microalgal dry weight produced per liter (g/L). The dry cell weight of the microalgal biomass was determined using the following procedure: 100 mL samples were removed once per day, and were centrifuged at 3000 rpm. The percentage of NO3, NO2, NH4-N, PO4-P, BOD5 removal also, K and growth rate was calculated at the interval of 21 days. Daily and at the same time, samples were withdrawn from flasks, and were centrifuged to separate algae. The BOD5 were done according to the standard methods [28], in a certified laboratory. All the other analyses were analyzed photo metrically by using a spectrometer.

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, the cell suspended with 5 ml of diethyl ether. Absorbance value of supernatant was measured using UV-spectrometer at 660 nm and 643 nm [29].

$$\boldsymbol{Chlorophylla = (9.92 \times A660) - (0.77 \times A643)} \quad \dots (1)$$

2.5. Statistical analysis

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

3. Results and Discussion

The mean values of pH, EC, BOD5, PO4, NH4, NO3 and NO2 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 (Figure 1-6). When the wastewater treated with Tetradesemus nygaardi then the pH was increased for each dose of microalgae as compared to control to (8.62, 8.55, 8.43) for run 1, 2 and 3 respectively (Figure 1). It may be due to composition CO2 within batch for photosynthesis process which rise pH value. Similar observation recorded by [30]. Variation in pH values can affect algal metabolism and growth in a variety of ways, including changing the equilibrium of inorganic carbon species, changing the availability of nutrients, and affecting cell physiology directly [31].

High pH values during the treatment period and interpreted the research that the values of the exponent of hydrogen (pH) of the medium increases if the ratio of the absorption of phosphorus into energy that is derived from the process of photosynthesis or respiration as influenced by the absorption of phosphorus by many factors most important of pH, temperature and light intensity as it was observed that the pH increase by precipitating phosphorus [32-35].

The maximum percentage removal of K, EC, and TDS occurred on day 21st of experiment were 68.18%, 61.56%, and 73.96% respectively (Figure 7). The maximum and minimum removal of TDS , EC by Tetradesemus nygaardi mainly due to the nutrients and other growth limiting factors such as the concentrations or form of carbon source available in the culture medium and other organic growth promoters [36]. Also algae use the biosorption and adsorption mechanisms to lower TDS to a base level. [37]. Present results are higher than that observed by [38], which showed treatment of industrial dairy wastewater by Spirulina sp. removed 54 % of TDS in 10th days.

The potential of microorganisms to convert organic material to CO2 and water using molecular oxygen as an oxidizing agent is exploited by biological oxygen demand. The effects of BOD5 reduction in the presence of Tetradesemus nygaardi in wastewater were determined and graphically displayed in (Figure 4). Under the present experimental specification, the average percentage of BOD5 removal was 82.25 % for run 1, 87.09 percent for run 2, and 89.51 % for run 3 (Figure 7). The CO2 produced from BOD5 reaction, with light and carbonic inhydrite enzyme Tetradesemus nygaardi through photosynthesis process increases algal biomass and pH values in batch [39]. Similar BOD5 removal percent was recorded by several authors through using different microalgae [40], reported 84% in BOD5 reduction. [41], reported BOD5 reduction from 75% to 95%.

Biological treatment of domestic wastewater using algae indicated 68.4% BOD5 and 67.2%. [42], reported 89% in BOD5 reduction using C. vulgaris at 30°C during 48 hrs. It is clear from the results that increased doses of microalgae have inverse or no effect on BOD5 removal percentage. Even a small dose of Tetradesemus nygaardi is sufficient to efficiently reduce BOD5 in wastewater. The optimal dose for removing BOD5 was 0.2 mg. l-1.

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and 48.2 mg/L, respectively; (Figure 5 and 6). Data indicated that the efficiency of NO3 removal was observed to be 95.46, 95.43, 94.88% for run 1, run 2, and run 3, respectively, and for NH4 were 96.74 for run 1 and 96.61, 96.41% using the varied doses of Tetradesemus nygaardi (Figure 7). The highest dose (2g.l-1) showed a slightly higher removal percentage of NO3 and NH4 in comparison to the other doses. The total NO2 removal was obtained as 85.45 for run1, 82.99 and 81.26% for run 2,and run 3, respectively. [44], obtained 93% removal efficiency for NH4 by C. vulgaris in the treatment of synthetic wastewater in 8 days.

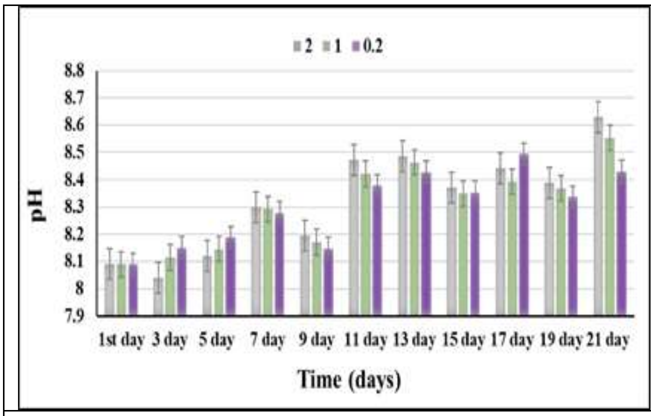


Figure 1. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 g. l-1) on pH values of wastewater.

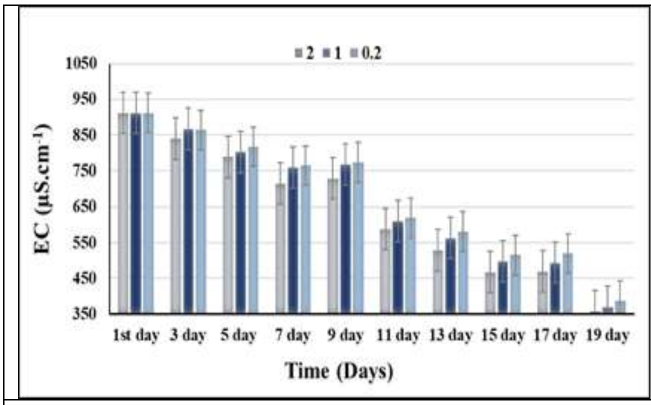


Figure 2. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 g. l-1) on electrical conductivity values of wastewater.

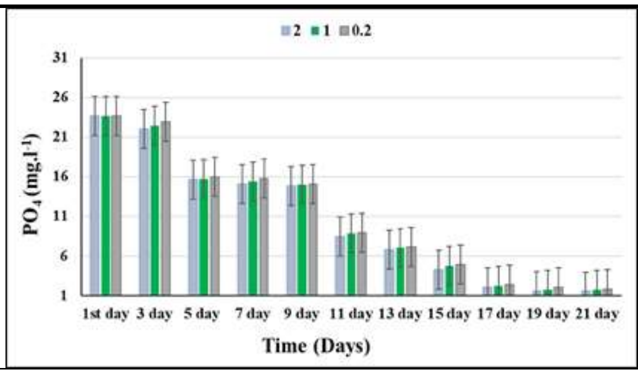


Figure 3. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2,1, and 0.2 g. l-1) on phosphate content of wastewater.

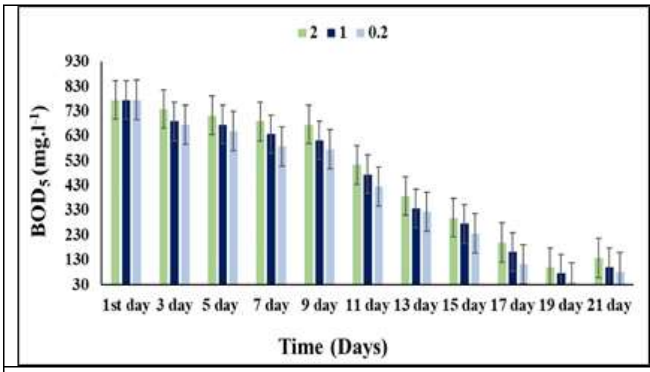


Figure 4. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 g. l-1) on BOD5 values of wastewater.

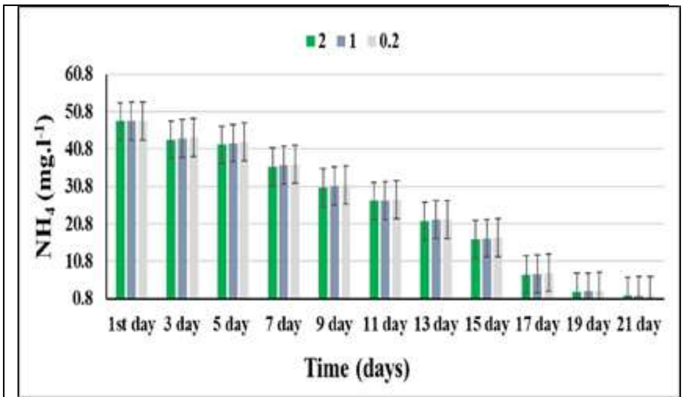


Figure 5. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 g. l-1) on ammonium content of wastewater.

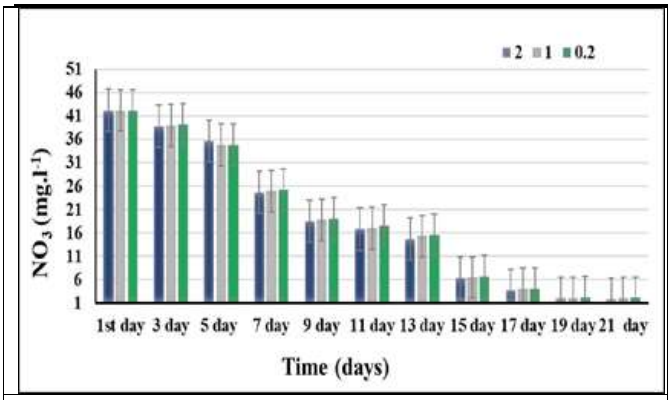


Figure 6. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 g. l-1) on nitrate content of wastewater.

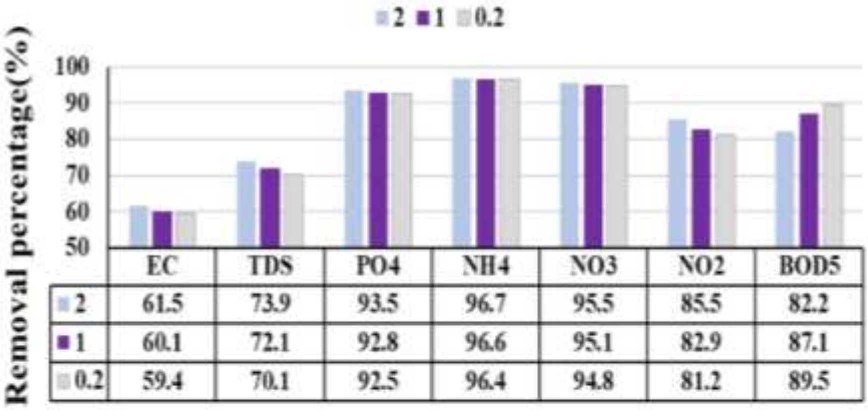
Tetradesemus nygaardi at various concentrations removes PO4 (Figures 3 and 7). The reported phosphorus uptake efficiency varies based on the media composition and environmental variables, such as initial nutrient concentration, light intensity, nitrogen/phosphorus ratio, light/dark cycle, and algal species [45]. When compared to some other studies, the efficiency of phosphorus uptake achieved in this study was higher. Lower PO4 removal percent was recorded in different studies [42, 46], by using C. vulgaris and Scenedesmus dimorphous with 28% and 55% respectively. On the 21st day of the experiment, a maximum of 93.56, 92.805, and 92.53 percent of PO4 was eliminated for the original concentration of PO4 (23.7mg/l).

Microalgae growth is believed to be essential for nitrogen removal through uptake, degradation, and sedimentation because nitrogen was required by microalga cells to synthesize proteins, nucleic acids, and phospholipids [47], moreover, the microalgal process of denitrification and nitrification [48]. The lowest NO3 removal might be due to nitrification process, algae prefer to assimilate N in the form of ammonia because it is a passive way of assimilation and energetically less expensive than uptake of nitrate [49], or may be due to gradually consuming certain nutrient components, like nitrate, Nitrate concentrations decrease as C. vulgaris and Monoraphidium sp. growth. The amount of chlorophyll a in the Tetradesemus nygaardi treatments is increased during experimental period. The initial value of chlorophyll a for all doses was (0.58 mg/l, 0.31 mg/l and 0.28 mg/l), then on day 17th reached the maximum value for all doses (1.51, 1.16 and 1.10 mg/l) respectively. Meanwhile, on the 21st day of the experiment all treatments confront a significant reduction in the biomass and chlorophyll a which indicated that the algae enter the stage of death and the number of algae cells decreased to 1.017 mg/l, 0.86 mg/l and 0.91 mg/l respectively (Figure 8). According to [50], the amount of nutrients in the environment (particularly nitrogen) and then light play a role in increasing and decreasing biomass, Because the light intensity in the environment has not change during the experiments, the low nutrient content can be behind for the reduction of biomass in the batch.

Table 1. Effect of adding different doses (2, 1, and 0.2 g. l^{-1}) of micro-algae *Tetradesmus nygaardi* on some tested wastewater variables (all unit in mg. l^{-1}, except pH and other were mentioned).

Doses	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})
D3 (2g/l)	8.29 ±0.002^{b}	613.2 ±0.7^{a}	390.6 ±0.37^{a}	5.46 ±0.04^{a}	25.14 ±0.012^{a}	18.97 ±0.024^{a}	4.41 ±0.009^{a}	10.95 ±0.009^{a}	475 ±1.4^{a}	0.97 ±0.003^{a}
D2 (1g/l)	8.3 ±0.002^{a}	637 ±0.7^{b}	398.688 ±0.3^{b}	5.44 ±0.04^{b}	25.42 ±0.012^{b}	18.78 ±0.024^{b}	4.32 ±0.009^{b}	10.75 ±0.009^{b}	437.5 ±1.14^{b}	0.753 ±0.003^{b}
D1 (0.2g/l)	8.29 ±0.002^{b}	647.68 ±0.7^{a}	408.403 ±0.37^{a}	5.58 ±0.04^{a}	25.59 ±0.012^{a}	18.97 ±0.024^{a}	4.75 ±0.09^{a}	10.94 ±0.009^{a}	407.38 ±1.4^{c}	0.688 ±0.003^{c}

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.



	EC	TDS	PO4	NH4	NO3	NO2	BOD5
2	61.5	73.9	93.5	96.7	95.5	85.5	82.2
1	60.1	72.1	92.8	96.6	95.1	82.9	87.1
0.2	59.4	70.1	92.5	96.4	94.8	81.2	89.5

Figure 7. Percent removal from wastewater tested variables after adding three doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2g. l-1).

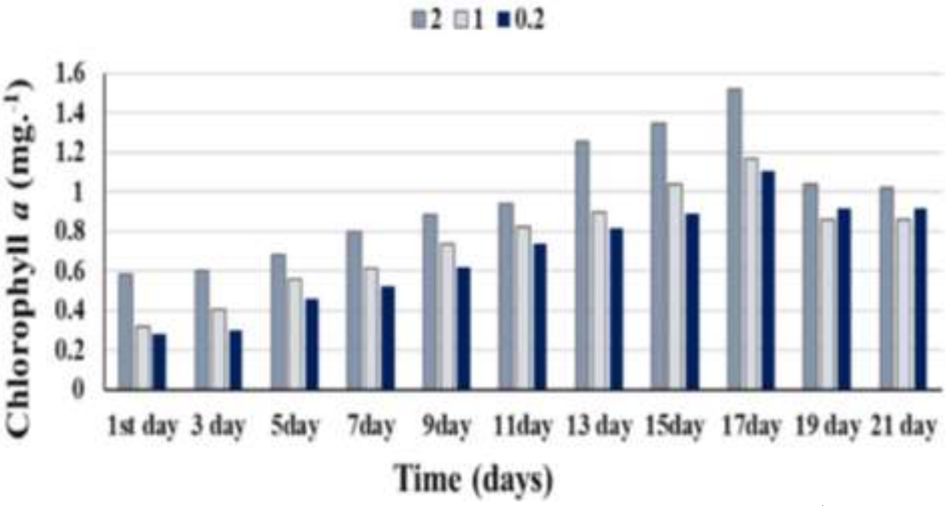


Figure 8. Effect of adding different doses of micro-algae Tetradesmus nygaardi (2, 1, and 0.2 mg. l-1) on chlorophyll and wastewater.

4. Conclusions

As a result, algae contributed to a considerable reduction in all nutrient concentrations, as well as an increase in algae biomass. Furthermore, ammonium nitrogen was found to be a more desirable form of nitrogen for algae, with a higher percentage of nitrogen that could be removed than other forms of nitrogen. BOD5 and dissolved salts (EC value) were both removed in significant amounts. Removal percent of nutrients, showed by order: NH4>NO3>PO4>BOD5>NO2. Whereas the highest applies dose (2g/l) had the highest reduction percent during 21 days of treatment. It may be preferred for future work to use fungi with algae together to know the efficiency of mixed culture in improving wastewater quality.

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