Plant Archives Vol. 20 Supplement 1, 2020 pp. 3469-3475 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF CYANOBACTERIA EFFICIENCY IN NUTRIENT REMOVAL FROM AL-RUSTAMIAH WASTEWATER TREATMENT PLANT, SOUTH OF BAGHDAD, IRAQ

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Abstract

This work aims to examine nutrients efficiency removal by two cyanobacteria species (*Oscillatoria limnetica* and *Chroococcus minor*) of pH, NH_3, NO_3, NO_2, PO_4 and COD from the wastewater of Al-Rustamiah treatment plant south of Baghdad city. Wastewater samples were collected and two species of *O. brevis* and *C. minor* were examined. Final sedimentation of Al-Rustamiah treatment plant was used to prepare stock culture of cyanobacteria where the wastewater samples were sterilized by autoclave. In general, it has been found that the mean values of examined variables were varied significantly in terms of algae species and times. Apparently, the nutrients removal was detected after two hours of treatment. However, *O. limnetica* gave better removal result of nutrient and COD than *C. minor*. Both *C. minor* and *O. limnetica* have decreased wastewater content of NH_3 from 20.7 mg/l to 3.4 mg/l at 7 day, 20.3 mg/l to 1.15 mg/l at 10 day, respectively. Also, this study has shown that NO_3 and NO_2 removal rates were lower than that of NH_3 for both algae. In case of wastewater PO_4 content, *O. limnetica* had mean concentration ranging from 5.0 mg/l to 0.0 ± 0.0 mg/l after 2 hours and 14 days respectively while *C. minor* has given mean concentration ranging from 3.4 ± 0.62 mg/l to 1.7 ± 0.84 mg/l at 2 hours and 14 days respectively.

Key words: Cyanobacteria, Nitrogen removal, Phosphorous removal, Wastewater treatment.

Introduction

Treatment of wastewater involves removing high concentrations of organic and inorganic pollutants, such as nitrogen and phosphorus (Sutherland *et al.*, 2015). Microalgae's ability to grow in these waters, which can grow rapidly and live under harsh conditions due to their simple cellular structure and short life cycle (Alrubaie and Al-Shammari, 2018). Simultaneously grow in wastewater and remove organic carbon and inorganic nutrients from wastewater while producing valuable biomass, so microalgae can play as an important role in the phytoremediation of wastewater treatment, particularly during the final tertiary treatment process (Oswald *et al.*, 1957; De la Nou¨e and De Pauw, 1988; Wang *et al.*, 2013; Whitton *et al.*, 2016). The advantages of using algae for this purpose include: low cost of operation, cheaper and more efficient means of nutrient removal than congenital methods (Oswald, 2003). Possibility of recycling assimilated nitrogen and phosphorus as fertilizer in the algae biomass, avoiding sludge handling problems and direct discharge of oxygenated effluent water into the water bodies (Choi and Lee, 2012). The use of several microalgae including *Chlorella vulgaris, Scenedesmus dimorphus, Nostocmuscorum, Anabaena variabilis, Plectonema, Oscillatoria, Phormidium and Spirulina* are examples of the microalgae strains having unique ability in bioremoval many contaminants from agro-industrial wastewaters (Abedia, 2017). The mechanism included in the removal of algal nutrients from wastewater was cell absorption and the removal of ammonia by high pH (Aslan and Kapdan, 2006). Several previous works have focused on the removal of nutrients such as nitrogen and phosphorus by different algae species. Previous work (Al-Rubaiee *et al.,* 2006) has examined the capability of *Oscillatoria pseudogeminata* to treated domestic wastewater and found increased removal of COD, NH_3-N and PO_4-P. The ammonia and active phosphorus were completely removed (100%) after the seventh day of the treatment while 79% of total phosphorus was removed after 10 days but had less ability in bioremoving both

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nitrite and nitrate which were 70% and 80%, respectively. Another work (Beníteza, *et al.,* 2019) has studied the ability of *Chlorella* to treat wastewater that microalgae cultures could successfully remove nitrogen and phosphorus. NH_4 and PO_3 removal efficiencies of 55.6% and 20.4%, as well as NO_3 production efficiencies of 93.1% were reported in aeration photo bioreactors.

The aim of this study was to evaluate the efficiency of nutrients removal by two cyanobacteria *(O. limnetica* and *C. minor)* were tested to remove the nutrients (NH_3, NO_3, NO_2, PO_4 and COD) from the wastewater of Al-Rustamiah treatment plant south of Baghdad city.

Material and Method

Algae isolation and identification

Two strains of cyanobacteria were used in this study, *O. brevis* and *C. minor* strains were isolated from Mustansiriya University Gardens according to previous study (Stein, 1973). These cyanobacteria was identified microscopically according to previous work (Desikachary, 1959). It was cultivated in BG11 medium under laboratory conditions such as constant 268 μE/m²/s, 25±2 Co and 16:8 light : dark condition following the method of previous study (Rippka *et al.,* 1979).

Wastewater treatment with cyanobacteria

Following the way kassim and Al-Lami, (1999) final sedimentation tank was taken before being discharged into the river. 900 ml of wastewater were sterilizing by autoclaving, the wastewater was placed in 1.5 ml transparent glass bottles and then add 400 ml inoculated for each species. Put all the culture in the light incubator. Each treatment was conducted with three replicates.

Physio-chemical characterization of wastewater

Physiochemical wastewater variables were determined using the standard method suggested by earlier work (Greenberg *et al.*, 1992). The parameters studied included pH, NH_3, NO_3, NO_2, PO_4 and COD. However, the present amount of nutrient content was measured in wastewater samples before and after inoculation of the culture of cyanobacteria.

Biomass calculation

Biomass was calculated as dry weight produced by both examined algae species. The dry cell weight of the microalgae biomass was determined by filtering 125 ml wastewater sample using (0.45μm) Wattman filter paper with air vacuum device and left to dry in electric oven at 105-110°C. The dry weight was measured daily using the following formula:

Dry weight mg/l = (A-B) × 1000) / (volume of sample)

A=Paper weight after filtration

B=Paper weight before filtration

Statistical Analysis

The obtained results were subjected statistical analysis tests such as Chi-square test, analysis of variance and least significant difference using SAS programme (SAS, 2012).

Results and Discussion

The effect of cyanobacteria on wastewater

Mean values ± SD of pH, COD, NH_3, NO_3, NO_2 and PO_4 of wastewater before and after using both *O. limnetica* and *C. minor* are illustrated in table 1.

In case of pH, *C. minor* had mean value higher than that of control (6.045 ± 0.015) and varied from minimum value of 7.86 ± 0.21 after 2 hours to maximum value of 9.2 ± 0.48 after 14 days while *O. limnetica* gave mean value lower than that of control (7.6 ± 0.0) particularly for the first three samples (2 hour, 3 and 7 days) which ranged from 7.14 ± 0.2 after 2 hours to 7.19 ± 0.1 after 7

Table 1: Mean ± SD of pH, COD, NH_3, NO_3, NO_2, and PO_4 of wastewater before and after using cyanobacteria.

Algae Species	Treatment	Mean ± Standard deviation					
		pH	COD mg/l	NH_3 mg/l	NO_3 mg/l	NO_2 mg/l	PO_4 mg/l
Oscillatoria limnetica	Control	7.6 ± 0.0	67.0 ± 6.0	20.3 ± 0.1	13.5 ± 0.1	3.5 ± 0.5	5.8 ± 0.5
	2 hours	7.14 ± 0.2	51.0 ± 2.0	17.5 ± 0.1	13.05± 0.05	3.2 ± 0.4	5.0 ± 1.0
	3 days	7.17 ± 0.2	20.5 ± 1.0	13.6 ± 0.3	12.5 ± 0.1	2.55 ± 0.35	4.2 ± 0.9
	7 days	7.19 ± 0.1	17.0 ± 1.0	8.6 ± 1.4	11.35 ± 0.05	1.9 ± 0.2	3.4 ± 1.0
	10 days	8.01 ± 0.05	10.5 ± 1.0	1.15 ± 0.35	10.45 ± 0.15	1.3 ± 0.1	2.7 ± 0.9
	14 days	8.14 ± 0.01	7.5 ± 0.5	0.0 ± 0.0	8.15 ± 0.05	0.6 ± 0.01	0.0 ± 0.0
Chroococcus minor	Control	6.045 ± 0.015	26.5 ± 1.5	20.7 ± 2.6	16.85 ± 0.45	3.5 ± 0.5	3.95 ± 0.15
	2 hours	7.86 ± 0.21	19.0 ± 0.9	13.0 ± 0.0	16.3 ± 0.4	3.2 ± 0.4	3.4 ± 0.62
	3 days	7.91 ± 0.18	11.5 ± 1.5	10.55 ± 3.45	15.4 ± 2.26	2.55 ± 0.7	2.85 ± 0.73
	7 days	8.38 ± 0.16	7.5 ± 1.62	3.4 ± 1.86	14.9 ± 1.94	1.7 ± 0.9	2.2 ± 0.92
	10 days	8.68 ± 0.62	4.5 ± 0.92	0.0 ± 0.0	13.75 ± 2.34	1.5 ± 0.5	1.85 ± 0.67
	14 days	9.2 ± 0.48	2.5 ± 0.64	0.0 ± 0.0	11.35 ± 2.11	1.5 ± 0.6	1.7 ± 0.84

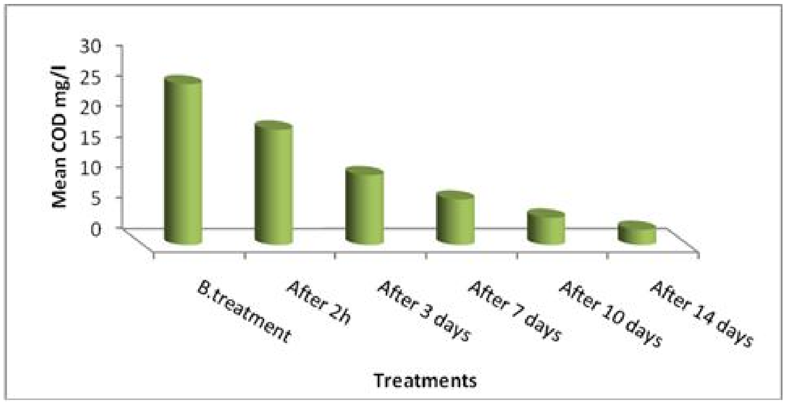

Fig. 1: Mean wastewater COD value (mg/l) before and after treatment by *C. minor*.

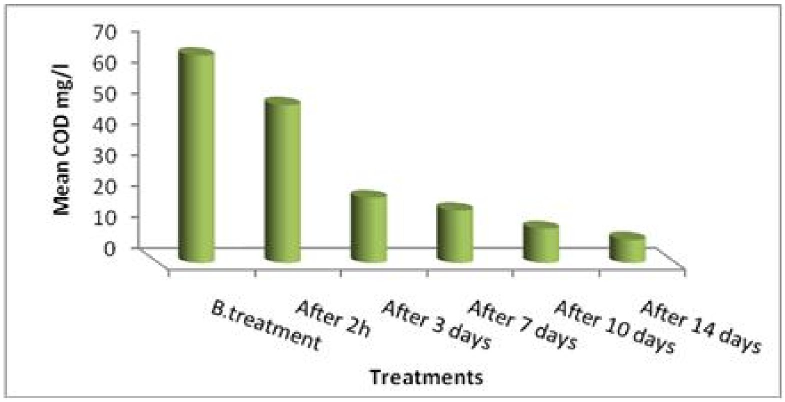

Fig. 2: Mean wastewater COD value (mg/l) before and after treatment by *O. limnetica*.

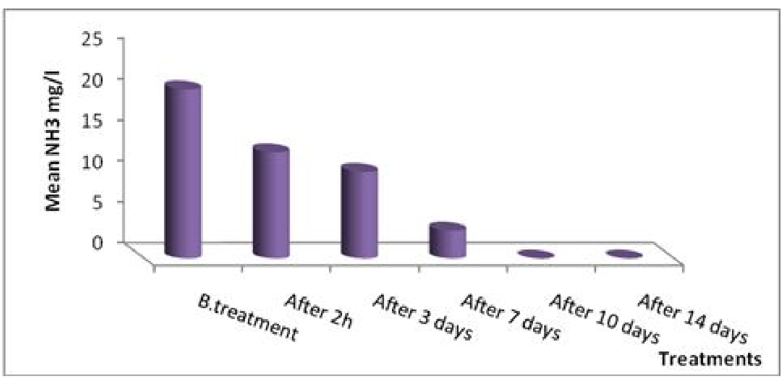

Fig. 3: Mean wastewater NH_3 value (mg/l) before and after treatment by *C. minor*.

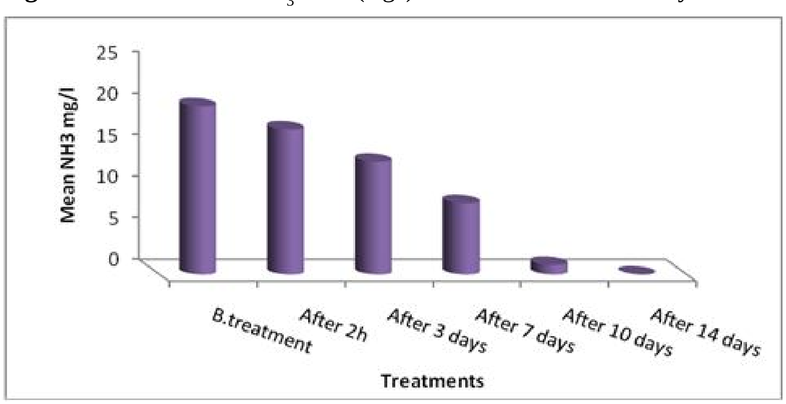

Fig. 4: Mean wastewater NH_3 value (mg/l) before and after treatment *by O. limnetica*.

days, but showed rather higher mean value in examined samples of 10 and 14 days which were 8.0 ± 0.05 and 8.4 ± 0.01respectively (Table 1).

The pH values are important factors that determine the validity of the water, number and species of organisms occurred. It is well known that been pH has multiple effects on algal growth and certain nutrient removal from wastewater where increased algae growth may increase pH due to the use of CO_2 by algae during photosynthesis (Larsdotter, 2006). At these pH values, most dissolved inorganic carbon is in the form of bicarbonate and with increasing pH an increasing fraction of is converted to carbonate. Under these conditions, CO_2 availability may act as an important limiting factor for photosynthesis (Ji *et al.*, 2017).

Regarding wastewater COD, the obtained results showed that both examined cyanobacteria gave high efficiency to a decrease in COD content from the first day and up to fourteenth days. However, *C. minor* has decreased COD concentration significantly from 26.5 mg/l of control sample to 2.5 mg/l in tested sample after 14 days while *O. limnetica* has similarly decreased COD content from 67.0 ±6.0 mg/l of control sample to 7.5 ± 0.5 mg/l after 14days (Fig. 1 and 2).

For wastewater NH_3 content, the current study has found significant reduction in NH_3 content when treated by both examined algae species. Highest mean values were found in control (untreated) sample which was 20.7 ± 2.6 mg/l while the lowest mean value (0.0 ± 0.0 mg/l) was detected in treated samples at the end of the test. So, *C. minor* algae had mean values varied from 13.0 ± 0.0 mg/l to 0.0 ± 0.40 mg/l at 2 hours and 10 days respectively (Fig. 3). On the other hand, *O. limnetica* algae gave similar pattern of mean values which were very high in control sample (20.3 ± 0.1mg/l) whereas treated samples had mean value varying from maximum value of 17.5 ± 0.1 to minimum value of 0.0 ± 0.0 mg/l after 2 hour and 14 days respectively (Fig. 4).

Nitrogen is the most important element for the growth of microalgae, as it contains 10% of the biomass of microalgae (Abdulsada, 2014). Nitrogen participates in essential biomass biochemical compounds, such as nucleic acids

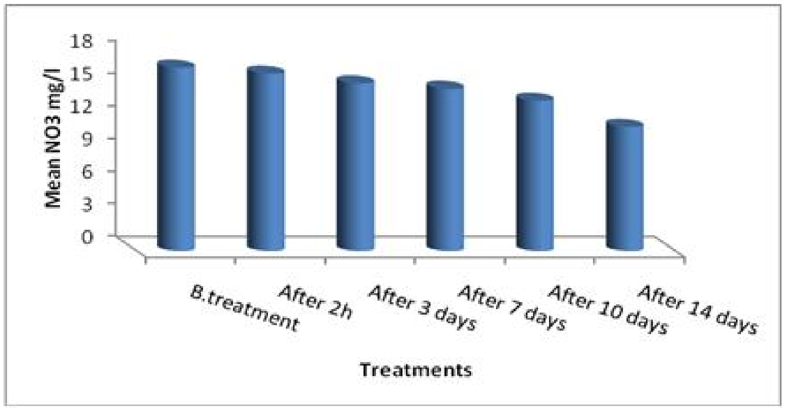

Fig. 5: Mean wastewater NO_3 value (mg/l) before and after treatment by *C. minor*.

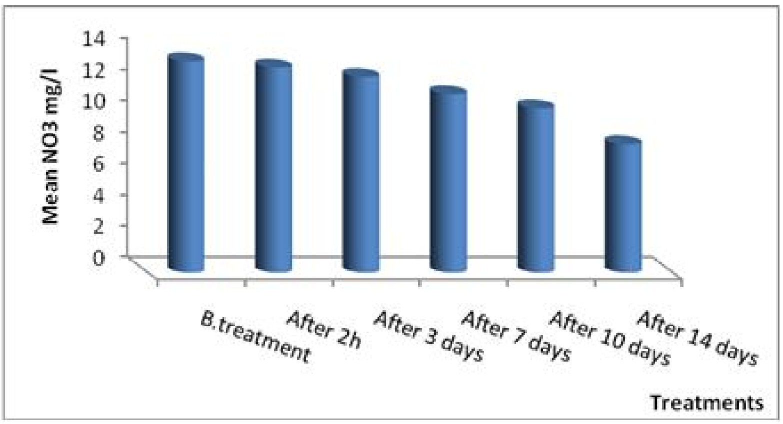

Fig. 6: Mean wastewater NO_3 value (mg/l) before and after treatment by *O. limnetica*.

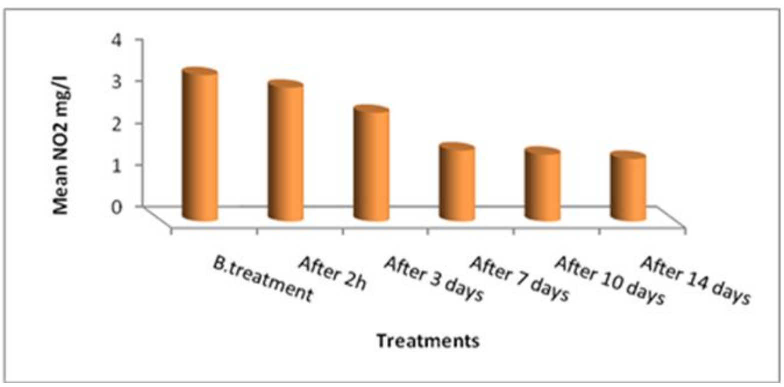

Fig. 7: Mean wastewater NO_3 value (mg/l) before and after *C. minor*

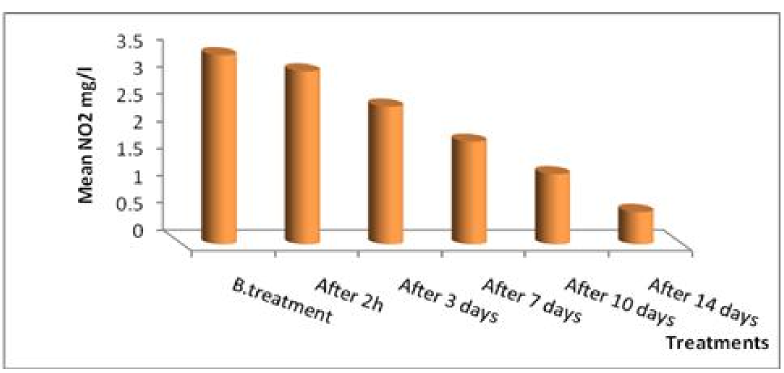

Fig. 8: Mean wastewater NO_3 value (mg/l) before and after by *O. limnetica*.

(DNA, RNA), amino acids (proteins) and pigments such as chlorophylls and phycocyanin, enzymes, vitamins and hormone (Jin *et al*., 2014). It is well documented that nitrogen is involved in the biogeological cycle that produces compounds with different oxidation states available to plants, algae and microbes. Nitrate, nitrite and ammonium form organic nitrogen including amino acids, urea and proteins while ammonium is energetically more favorable and is the preferred nitrogen source when it is available (Cai *et al*., 2013). Also, algae tend to prefer ammonium over nitrate and nitrate consumption does not occur until the ammonium is almost completely consumed (Harrison *et al*., 1990). Previous work (Collo and Harrison, 2014) has showed a ranking of sensitivity to high levels of ammonium/ammonia (39-1.2 mM) where the order of tolerance was: Chlorophyceae > Cyanophyceae, Dinophyceae, Diatomophyceae and Raphidophyceae.

Regarding wastewater NO_3 and NO_2 contents, the current work has found that there was slight decrease in NO_3 content treated by both algae species (Table 1). However, highest mean values were recorded in untreated samples while the lowest mean values were detected in treated samples. Furthermore, these values were varied from 16.3 ±0.4 mg/l to 11.35 ± 2.11 mg/l, 13.05 ± 0.05mg/l to 8.15 ± 0.05 mg/l for *C. minor* ,*O. limnetica* at 2 hours and 14 days respectively while control samples of these examined algae had mean value of 16.8 ± 2.6 and 13.5 ± 0.1 respectively (Fig. 5 and 6).

Apparently, *C. minor* was the best in decreasing wastewater NO_3 content followed *O. limnetica*. Nitrate is the most commonly used mineral for microalgae and cyanobacteria cultivation on synthetic media. Nitrate is taken up by active mechanisms and therefore consumes energy (Graham and Wilcox, 2000). Nitrate does not display toxic effects on cells and microalgae can tolerate concentrations of up to 100 mM of nitrate (Jeanfils *et al*., 1993). To assimilate the inorganic nitrogen, nitrate and nitrite must be reduced to ammonium by nitrate reductase (NR) and nitrite reductase (NiR), respectively (Jia and Yuan, 2016).The most important path for nitrogen assimilation is through the glutamine synthesize enzyme system, by which glutamate reacts with ammonium (driven energetically by ATP) to form the amino acid glutamine (Markou *et al.*, 2014).

In case of wastewater NO_2 content, there was clear decrease in NO_2 concentrations in all wastewater samples treated with both algae species, but it seems that samples treated with *O. limnetica* were much better than these of another algae. With *C. minor*, these mean values were found to range from 3.2 ± 0.4 mg/l to 1.5 ± 0.6 mg/l at 2 hour and 14 days respectively while the control sample had 3.5 ± 0.5 mg/l (Fig. 7).

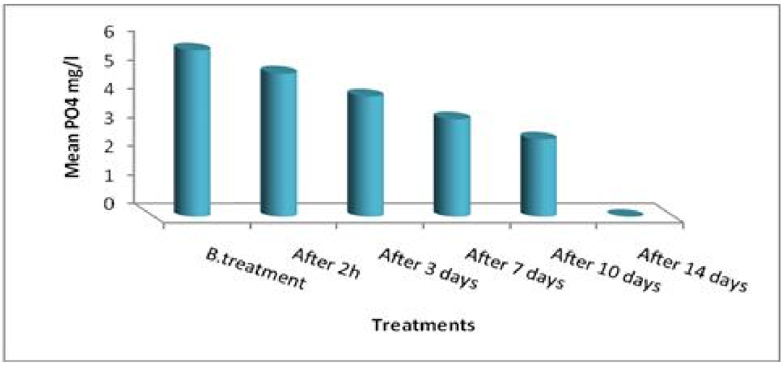

Fig. 9: Mean wastewater PO_4value (mg/l) before and after by *O. limnetica*.

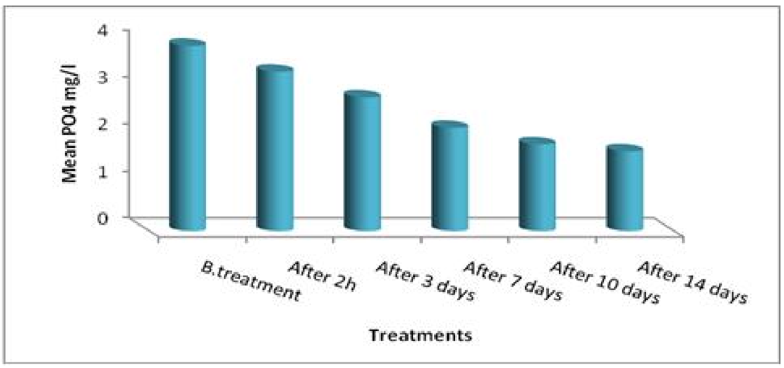

Fig. 10: Mean wastewater PO_4value (mg/l) before and after by *C. minor*.

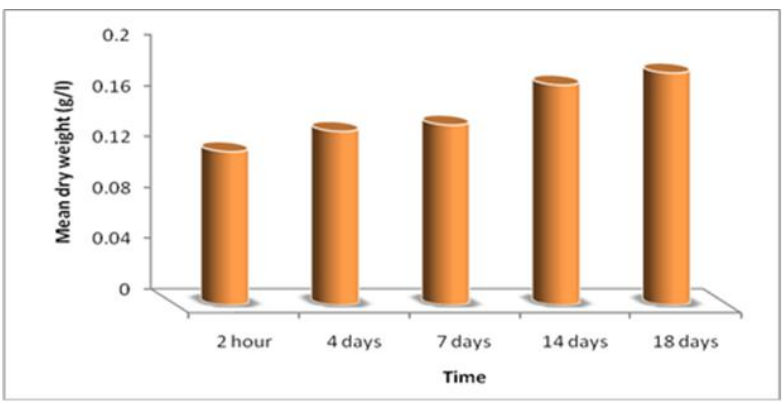

Fig. 11: Mean dry weight of *O. brevis* during various times.

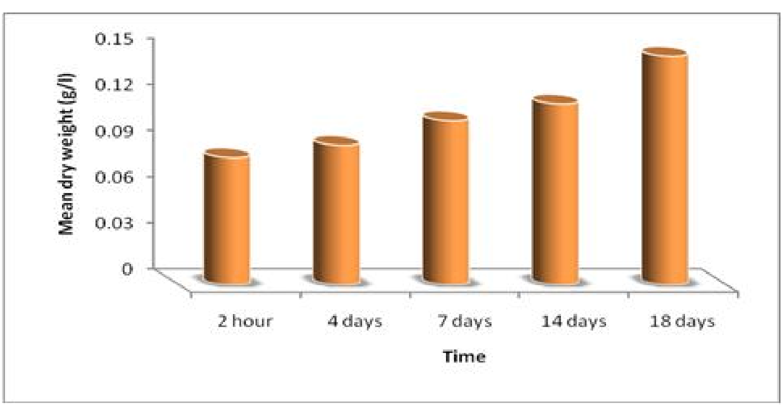

Fig. 12: Mean dry weight of *C. minor* during various times.

The wastewater samples treated with *O. limnetica* contained NO_2 varying from 3.2 ± 0.4 to 0.6 ±1.5 mg/l at 2 hour and 14 days, respectively (Fig. 8).

The main way of nitrite uptake is via active transportation, but diffusion has been also reported for green microalgae and cyanobacteria (Fuggi, 1993). Although nitrite can be taken up and used as a nitrogen source, at high concentrations that has toxic effects (Chen *et al.*, 2012).

Finally, regarding wastewater PO_4 content, there was clear decrease in PO_4 concentrations in all wastewater samples treated with both algae species. However, *O. limnetica* had PO_4 mean concentration ranging from 5.0 ± 1.0 mg/l to 0.0 ± 0.0 mg/l after 2 hour and 14 days respectively (Fig. 9) while *C. minor* has reduced PO_4 mean concentration from 3.4 ± 0.62 mg/l at 2 hour to 1.7 ± 0.84 mg/l (Fig. 10).

Phosphorus is essential for growth and many cellular processes. The preferred form of phosphorus to supply to algae is orthophosphate (PO_4^{-3}), which is incorporated into organic compounds through phosphorylation (Cai *et al.*, 2013). In natural environments as well as in wastewaters, phosphorus is occurred in various forms such as orthophosphate, pyrophosphate, polyphosphate, metaphosphate and their organic forms (Cembella *et al.*, 1982). Phosphorus is often one of the most important growth limiting factors in algal biotechnology because it is easily bound to other ions such as CO_3^{-2} and iron. Therefore, phosphorous can be removed by algae through a combination of adsorption and algae-induced chemical precipitation (Sañudo-Wilhelmy *et al.*, 2004).

Algae dry weight

Table 2 displays the mean dry weight ± SD of all examined algae species during various times. It seems very obvious that dry weight of all examined algae species has increased significantly as the testing time increased.

Mean dry weight of *Oscillatoria limnetica*

Table 2: Mean dry weight ± SD of all examined algae species during various times.

Time	Mean (g/l) ± SD	
	O. limnetica	*C. minor*
2 hour	0.121 ± 0.001	0.083 ± 0.001
4 days	0.137 ± 0.001	0.091 ± 0.003
7 days	0.142 ± 0.002	0.107 ± 0.001
9 days	0.173 ± 0.005	0.118 ± 0.012
14 days	0.183 ± 0.007	0.149 ± 0.003

was found to range from minimum value of 0.121 ± 0.001 g after just 2 hours to 0.183 ± 0.007 g after 14 days (Fig. 11) while C. minor had lowest mean dry weight (0.083 ± 0.001 g) after 2 hours and increased gradually to maximum value of 0.149 ± 0.003 g after 14 days (Fig. 12).

Biomass yield is an expression of organic production, as the dry weight of the organic mass produced over a period of time; it is also used to express microalgae growth. Once the biomass has been harvested and the extracellular water removed, the dry weight concentration is generally around 15-25%. The harvested biomass can be used in the agricultural sector, either as an animal feed or as a fertilizer (Randrianarison and Ashraf, 2017).

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