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Assessment of inorganic nitrogen and phosphorus compounds removal efficiency from different types of wastewater using microalgae cultures

by

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Abstract

The efficiency of ammonium nitrogen (N-NH_4^+) and phosphate (P-PO_4^{3-}) removal from wastewater with different loads of these nutrients was evaluated using *Chlamydomonas reinhardtii* (Chlorophyta) and *Oscillatoria neglecta* (Cyanophyta/Cyanoprokaryota). In addition, functional characteristics of the microalgae under the studied conditions were determined. It was demonstrated that *Ch. reinhardtii* is resistant to a wide range of concentrations of inorganic nitrogen and phosphorus compounds. Microalgae actively participate in the removal of N-NH_4^+ from wastewater (removal efficiency of 49–63%, depending on the initial concentration). At the same time, *Ch. reinhardtii* showed low levels of P-PO_4^{3-} removal (7–18%) from the aquatic environment. *O. neglecta*, unlike *Ch. reinhardtii*, is sensitive to excessively high concentrations of N-NH_4^+ (90–140 mg l^{-1}) and P-PO_4^{3-} (26–90 mg l^{-1}). However, it is characterized by high removal efficiency for both forms of nitrogen (60–61%) and phosphorus (43–55%) at their initial concentrations of 30–50 mg l^{-1} and 7–14 mg l^{-1}, respectively. Therefore, *O. neglecta* is best suited for use in wastewater post-treatment.

Key words: wastewater treatment, ammonium nitrogen, phosphate, microalgae, growth rate, photosynthetic pigments

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1. Introduction

Removal of nitrogen and phosphorus is a priority task when treating wastewater from different sources (Shamanskyi & Boichenko 2018). Reducing the concentration of these nutrients in wastewater is extremely important to prevent anthropogenic eutrophication of water bodies and/or to achieve their satisfactory ecological status (European Commission 2016; Mohsenpour et al. 2020). Considering the negative consequences of eutrophication (harmful algal blooms), strict regulations have been established for the content of nitrogen and phosphorus compounds in wastewater (Silva et al. 2015). Traditional wastewater treatment systems cannot provide sufficient nutrient removal. Therefore, alternative methods of wastewater post-treatment for nitrogen and phosphorus removal are necessary (Mohsenpour et al. 2020).

The most promising alternative technology for wastewater treatment is phytoremediation, which consists in the removal or biotransformation of pollutants, including nutrients, in wastewater using plants and associated microflora (Renuka et al. 2015). Microalgae have become attractive biological systems for wastewater treatment of various origins (Eladel et al. 2019; Mohsenpour et al. 2020). This is due to the fact that they are characterized by higher photosynthetic activity and lower solar energy requirements than higher plants (Bhatnagar et al. 2011; Razzak et al. 2013). In addition, they play a key role in removal of organic and inorganic pollutants from the aquatic environment (Abdel-Raouf et al. 2012). According to Eladel et al. (2019), the rate of nitrogen and phosphorus assimilation by microalgae can reach 24 kg N ha^{-1} day^{-1} and 3 kg P ha^{-1} day^{-1}. In the process of remediation, microalgae effectively remove nitrogen and phosphorus from wastewater to synthesize organic substances necessary to maintain their metabolism (Markou et al. 2014; Madkour et al. 2017). Another advantage of using microalgae to remove nutrients from wastewater is the possibility of recycling assimilated nitrogen and phosphorus into algal biomass, which can be used as a biofertilizer (Solovchenko et al. 2013) or as feedstock for the production of various types of biofuels, including biodiesel, bioethanol, biomethane, biosyngas and biohydrogen (Thomas et al. 2016). It should be noted that the use of microalgae for the production of biodiesel is considered a more promising technology for its production than oilseed crops (sunflower, rapeseed, and soybean), since the yield of oils obtained from algal biomass is much higher (Abou-Shanab et al. 2010). Furthermore, the accumulation of algae biomass in wastewater can significantly reduce the cost of their cultivation due to the presence and high availability of all nutrients there needed for algae growth, which makes this technology cost-effective.

The purpose of this study was to assess the efficiency of nitrogen and phosphorus removal from different types of wastewater using *Chlamydomonas reinhardtii* P.A. Dangeard and *Oscillatoria neglecta* H.C. Wood, and to determine functional characteristics of microalgae under the studied conditions.

2. Materials and methods

Cultures of green *Chlamydomonas reinhardtii* P.A. Dangeard (strain HPDP-128) and blue-green microalgae *Oscillatoria neglecta* H.C. Wood (strain HPDP-25) were obtained from the Collection of Living Microalgae Cultures of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine.

2.1. Experimental design

Artificial wastewater with different loads of nitrogen and phosphorus was used in this study (Table 1). The composition of the synthetic wastewater was based on a modified formulation of the BG-11 medium. NH_4Cl and KH_2PO_4 were added to the culture medium for microalgae in varying amounts to obtain typical concentrations of ammonium nitrogen and phosphate found in wastewater (Hence et al. 2002; Rawat et al. 2011; Acevedo et al. 2017).

Cultures of microalgae in the late exponential phase of growth (18-day culture) were used to prepare the inoculum. The ratio of inoculum to wastewater was 1:10. Test vessels were flasks with a volume of 500 cm^3. Microalgal cultures were incubated in wastewater for 14 days under artificial lighting (using Philips TL-D 18W 54-765 G13 daylight fluorescent lamps). The light intensity was 50–54 μmol photons m^{-2} s^{-1}; light regime – 16 h of light and 8 h of darkness. The pH and temperature of the test solutions were monitored throughout the exposure period. The pH of the solutions remained in the range of 7.5 to 8.2 and did

Table 1

Concentrations of $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ in wastewater

Variant of the experiment	Initial concentration, mg l^{-1}	
	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$
1	30.00 ± 1.12	7.00 ± 0.18
2	50.00 ± 2.25	14.00 ± 0.56
3	90.00 ± 2.58	26.00 ± 0.47
4	140.00 ± 2.68	41.00 ± 0.67

not change during the exposure period by more than 0.7 units in any test; the temperature of the solutions was 25.0–26.2°C.

2.2. Analytical methods

The concentration of ammonium nitrogen and phosphate in water was analyzed according to Arsan et al. (2006).

The nutrient removal efficiency was calculated as follows:

$$\textit{Removal efficiency (\%)} = \frac{C_0 - C_f}{C_0} \times 100$$

where: C_0 – initial concentration, C_f – final concentration.

The functional activity of the cultures under the studied conditions was assessed based on changes in dry weight and the content of photosynthetic pigments (chlorophyll *a* and carotenoids). For the respective analyses, the biomass of microalgae was concentrated by filtering the cell suspension through cellulose nitrate membrane filters ("Sartorius", pore size 0.45 μm) under low vacuum conditions. Samples for analysis were collected at the beginning and at the end of the experiment.

To determine the dry weight, the filters with concentrated microalgae were dried in a thermostat at a temperature of 105°C to constant weight. Dry matter content was calculated according to the formula (Lim et al. 2010):

$$\text{Dry weight} = \frac{\text{Weight of filter with algal cells - Weight of blank filters (mg)}}{\text{Volume of algal culture (L)}}$$

The specific growth rate (μ) of microalgae was calculated using the equation (Krzemińska et al. 2014):

$$\mu = \frac{ln\left(\frac{N_2}{N_1}\right)}{t_2 - t_1} \ (\text{day}^{-1})$$

where N_1 and N_2 are biomass at time 1 (t_1) and time 2 (t_2), respectively.

The content of pigments was analyzed by an extractive spectrophotometric method (SCOR-UNESCO, 1966). For extraction of pigments, the filter with algae was thoroughly homogenized with the addition of quartz sand and 90% acetone. The resulting extract was separated by centrifugation. In samples of green microalgae, which contained chlorophyll *a*, chlorophyll *b*, and carotenoids (Rowan, 1989), the optical density of extracts was determined at 647, 664, and 480 nm, respectively. In the culture of blue-green microalgae, containing only chlorophyll *a* and carotenoids (Rowan, 1989), the optical density of extracts was determined at 664 and 480 nm, respectively. Non-specific light absorption by extracts at a wavelength of 750 nm was also measured. Chlorophyll *a* concentration was calculated according to the equations of Jeffrey & Humphrey (1975), and the total carotenoid content was calculated according to the formula of Parsons & Strickland (1963).

2.3. Statistical analysis

All measurements were conducted in triplicate. Statistical processing of the research results was performed using SPSS Statistics software (version 17). The Dunnett test was used to assess the differences between the values of the indicators. A difference was considered significant at $p \leq 0.05$. All data were presented as mean ± standard deviation (M ± SD).

3. Results

3.1. Removal of nitrogen and phosphorus from synthetic wastewater by microalgae cultures.

During the cultivation period, the efficiency of ammonium nitrogen and phosphate removal by *Ch. reinhardtii* in the experiment variant with their lowest concentrations (30 mg N-NH_4^+ l^{-1} + 7 mg P-PO_4^{3-} l^{-1}) was 63% and 18%, respectively (Table 2). *Ch. reinhardtii* is distinguished by high efficiency of nitrogen removal at its significantly higher concentrations in wastewater, 50, 90 and 140 mg l^{-1} (removal efficiency of 60%, 56%

Table 2

Final concentration of nutrients in wastewater (mg l^{-1}) during the microalgae cultivation period, M ± SD

Experiment variant	*Chlamydomonas reinhardtii*		*Oscillatoria neglecta*	
	N	P	N	P
1	11.00 ± 0.25	5.70 ± 0.13	12.00 ± 0.50	3.15 ± 0.11
2	20.03 ± 1.00	13.00 ± 0.36	19.50 ± 1.21	8.00 ± 0.44
3	40.00 ± 2.50	24.00 ± 1.48	62.00 ± 3.45	19.30 ± 0.82
4	71.00 ± 2.25	38.00 ± 1.66	168.10 ± 5.40	50.00 ± 2.55

and 49%, respectively). As for $P\text{-}PO_4^{3-}$, the efficiency of its removal by microalgae was low and at higher concentration of $P\text{-}PO_4^{3-}$ did not exceed 7%.

For *O. neglecta* in the experiment variant with 30 mg $N\text{-}NH_4^+$ l^{-1} + 7 mg $P\text{-}PO_4^{3-}$ l^{-1}, the efficiency of their removal on day 14 was 60% and 55%, respectively, while in the variant with 50 mg $N\text{-}NH_4^+$ l^{-1} + 14 mg $P\text{-}PO_4^{3-}$ l^{-1} – 61% and 43%, respectively (Table 2). The results obtained indicate the ability of the blue-green algae culture to significantly reduce both nitrogen and phosphorus concentrations in the analyzed wastewater. However, at higher concentrations of nutrients, the percentage of their removal by *O. neglecta* decreases rapidly. In the experiment variant with 140 mg $N\text{-}NH_4^+$ l^{-1} + 41 mg $P\text{-}PO_4^{3-}$ l^{-1}, an increase in the concentrations of nitrogen and phosphorus was observed on day 14 compared to their initial values. This is obviously due to the fact that the culture under the studied conditions died and was lysed.

3.2. Changes in the growth rate and content of photosynthetic pigments in microalgae during 14-day cultivation in wastewater.

A reliable indicator of the metabolic activity of microalgal cells is the growth rate. Figure 1 shows the changes in dry weight of *Ch. reinhardtii* and *O. neglecta* during the period of their cultivation in wastewater with different loads of ammonium nitrogen and phosphate. The maximum specific growth rate of *Ch. reinhardtii* (0.091 day^{-1}) was observed at a concentration of 140 mg $N\text{-}NH_4^+$ l^{-1} and 41 mg $P\text{-}PO_4^{3-}$ l^{-1}, with slightly lower rates (0.087–0.082 day^{-1}) at lower nitrogen and phosphorus concentrations. Thus, as the concentration of nutrients increases, the growth rate of *Ch. reinhardtii* also increases.

An opposite trend was observed for the blue-green alga *O. neglecta*: as the concentration of $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ in the aquatic environment increases, the dry matter content decreases. The maximum specific growth rate of *O. neglecta* was observed at a concentration of 30–50 mg $N\text{-}NH_4^+$ l^{-1} + 7–14 mg $P\text{-}PO_4^{3-}$ l^{-1}, reaching 0.083–0.084 day^{-1}. The minimum values of this indicator (0.054 day^{-1}) were observed when microalgae were grown in wastewater with 140 mg $N\text{-}NH_4^+$ l^{-1} + 41 mg $P\text{-}PO_4^{3-}$ l^{-1}.

When microalgae are used for wastewater treatment, their high photosynthetic activity is of paramount importance, because the rate of photosynthetic processes in algal cells determines the rate of biomass accumulation, nutrient assimilation, and photosynthetic aeration (Solovchenko et al. 2013). An important characteristic of the photosynthetic apparatus, determining its functional activity, is the

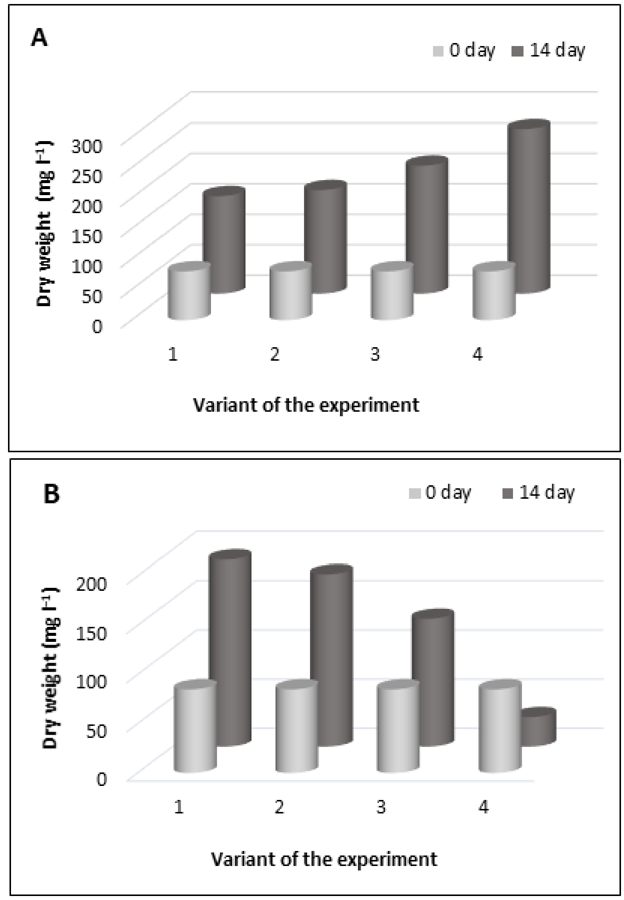

Figure 1

Changes in dry weight of *Chlamydomonas reinhardtii* (A) and *Oscillatoria neglecta* (B) during their cultivation in different types of wastewater: 1 – 30 mg $N\text{-}NH_4^+$ l^{-1} + 7 mg $P\text{-}PO_4^{3-}$ l^{-1}; 2 – 50 mg $N\text{-}NH_4^+$ l^{-1} + 14 mg $P\text{-}PO_4^{3-}$ l^{-1}; 3 – 90 mg $N\text{-}NH_4^+$ l^{-1} + 26 mg $P\text{-}PO_4^{3-}$ l^{-1}; 4 – 140 mg $N\text{-}NH_4^+$ l^{-1} + 41 mg $P\text{-}PO_4^{3-}$ l^{-1}.

content of photosynthetic pigments, chlorophyll *a* and carotenoids in particular (Nezbrytskaya et al. 2015). The highest content of these pigments in the biomass of *Ch. reinhardtii* was recorded at the maximum concentration of $N\text{-}NH_4^+$ (140 mg l^{-1}) and $P\text{-}PO_4^{3-}$ (41 mg l^{-1}; Table 3). The results obtained indicate that the content of photosynthetic pigments and dry weight increase in *Ch. reinhardtii* with increasing concentration of nutrients, which indicates the intensification of the functional activity of the microalga and its resistance to a high load of inorganic nitrogen and phosphorus compounds in the aquatic environment. In the experiment variants with 30–90 mg $N\text{-}NH_4^+$ l^{-1} + 7–41 mg $P\text{-}PO_4^{3-}$ l^{-1}, the content of chlorophyll *a* and carotenoids was almost at the same level ($p > 0.05$, Dunnett's test) in terms of dry weight of algae.

Table 3

Changes in the content of photosynthetic pigments in *Chlamydomonas reinhardtii*, M ± SD

Experiment variant	Chlorophyll *a*, mg g^{-1} DW		Carotenoids, mg g^{-1} DW	
	0 days	14 days	0 days	14 days
1	4.89 ± 0.25	8.66 ± 0.53	1.08 ± 0.04	2.17 ± 0.10
2	4.89 ± 0.25	9.01 ± 0.45	1.08 ± 0.04	2.22 ± 0.11
3	4.89 ± 0.25	10.47 ± 0.58	1.08 ± 0.04	2.48 ± 0.11
4	4.89 ± 0.25	14.89 ± 0.66	1.08 ± 0.04	3.50 ± 0.20

In the biomass of the *O. neglecta* culture, a high content of chlorophyll *a* and carotenoids was recorded in the experiment variant with 30–50 mg N-NH_4^+ l^{-1} + 7–14 mg P-PO_4^{3-} l^{-1} (Table 4). With increasing concentration of nutrients in blue-green algae, a noticeable decrease in the content of the main photosynthetic pigments ($P \leq 0.05$, Dunnett's test) was observed, which is consistent with a change in the growth rate and removal of nutrients. At the maximum concentration of nitrogen and phosphorus, the values of indicators of the content of chlorophyll *a* and carotenoids were even lower than their initial values, which indicates the death of culture cells.

Table 4

Changes in the content of photosynthetic pigments in *Oscillatoria neglecta*, M ± SD

Experiment variant	Chlorophyll *a*, mg g^{-1} DW		Carotenoids, mg g^{-1} DW	
	0 days	14 days	0 days	14 days
1	4.00 ± 0.12	7.00 ± 0.33	0.62 ± 0.02	2.40 ± 0.09
2	4.00 ± 0.12	6.76 ± 0.24	0.62 ± 0.02	2.10 ± 0.10
3	4.00 ± 0.12	4.60 ± 0.16	0.62 ± 0.02	1.12 ± 0.04
4	4.00 ± 0.12	1.03 ± 0.04	0.62 ± 0.02	0.47 ± 0.01

4. Discussion

The results of the study show that *Ch. reinhardtii* is resistant to a wide range of concentrations of inorganic compounds of nitrogen and phosphorus. The microalga actively participates in the removal of ammonium nitrogen from wastewater. However, *Ch. reinhardtii* is characterized by low efficiency of phosphate removal from the aquatic environment. Similar results were obtained by other authors, Kong et al. (2010) in particular. They found that during a 10-day incubation period, *Ch. reinhardtii* removes from 42.2% to 55.0% of N-NH_4^+ from wastewater and only 12.5–15.4% of P-PO_4^{3-} (Kong et al. 2010).

As reported in the literature (Markou et al. 2014), the ability of microalgae to remove nutrients from the aquatic environment depends on various factors. The rate and efficiency of these processes are first determined by physiological characteristics of species, in particular their requirements for various nutrients to maintain their vital activity (Fernandes et al. 2017). Thus, for example, the nitrogen content can range from 1% to 14% of dry weight, depending on the species of microalgae (Markou et al. 2014), whereas the phosphorus content can range from 0.05% to 3.3% of their dry weight (Grobbelaar 2004). Nitrogen and phosphorus are involved in many metabolic pathways in algal cells (Madkour et al. 2017). Nitrogen is a component of proteins (amino acids), nucleic acids (DNA, RNA), and pigments (Markou et al. 2014). Phosphorus is a structural component of phospholipids, nucleic acids and an integral component of the unique "energy currency", ATP (Borowitzka et al. 2016). It was shown that the total protein content in *Ch. reinhardtii* is 48% and that of lipids – 21% (Razzak et al. 2013).

Low efficiency of phosphate removal by *Ch. reinhardtii* compared with nitrogen removal could be due to the non-optimal N/P ratio for this species. According to Zabochnicka-Świątek et al. (2014), equally efficient assimilation of nitrogen and phosphorus by microalgal cells can only occur at an optimal N/P ratio. Su et al. (2012) found that the removal of phosphates from wastewater by *Ch. reinhardtii* is more efficient at a N/P ratio of 15:1, and less efficient at a lower ratio of these nutrients (about 5:1). Cai et al. (2013) showed that the optimal N/P ratio is not the same for different cultures of microalgae, which is associated with different peculiarities of their metabolism. It is reported that the N/P ratio in wastewater is often around 3–5:1 (Hence et al. 2002; Zabochnicka-Świątek et al. 2014).

According to the results obtained, *O. neglecta* is characterized by almost the same efficiency of nitrogen and phosphorus removal. It is known that prokaryotic and eukaryotic algae can accumulate nitrogen and phosphorus not only to synthesize important organic substances, but also to store their excess inside the cells (in the cytosol or vacuoles; Mau et al. 2021). Excess supply of phosphorus can be stored in the form of polyphosphate granules, while excess nitrogen – in the form of crystalline guanine rich inclusions (Moudříková et al. 2017; Mau et al. 2021). Excessive absorption of phosphorus allows microalgae to accumulate up to four times more of this element than necessary to maintain their normal metabolism (Powell et al. 2011).

It should be noted, however, that *O. neglecta* showed sensitivity to excessively high nutrient concentrations compared with *Ch. reinhardtii*. According to Aslan & Kapdan (2006) and Solovchenko et al. (2013), unicellular green algae are characterized by the highest tolerance to eutrophic conditions among other algae. One of the important mechanisms of their resistance to excessive concentrations of nutrients is the high activity of enzyme systems involved in their assimilation (Nezbrytskaya et al. 2019; Su 2020). Therefore, green microalgae are promising biosystems for removing high concentrations of nutrients from wastewater.

5. Conclusion

Wastewater from different sources is characterized by varying nitrogen and phosphorus content, as well as the ratio of these nutrients, so it is necessary to carry out preliminary screening of microalgae cultures before using them in wastewater treatment systems with the appropriate chemical composition.

The results of this study indicate that *Ch. reinhardtii* is resistant to a wide range of concentrations of ammonium nitrogen and phosphate in wastewater. It can grow intensively even with an excessive load of these nutrients in the aquatic environment. The efficiency of N-NH_4^+ removal from synthetic wastewater with microalgae is 49–63%, while that of P-PO_4^{3-} removal is 7–18% (depending on the initial concentration of nutrients). Thus, *Ch. reinhardtii* is a promising microalgae species for the removal of ammonium nitrogen from wastewater.

The culture of *O. neglecta* was sensitive to excessively high concentrations of N-NH_4^+ (90–140 mg l^{-1}) and P-PO_4^{3-} (26–90 mg l^{-1}). However, this microalga is characterized by high indicators of functional activity, as well as high efficiency of removal of both ammonium nitrogen (60–61%) and phosphate (43–55%) at their initial concentrations of 30–50 mg l^{-1} and 7–14 mg l^{-1}, respectively. The data obtained indicate that *O. neglecta* can be used for post-treatment of wastewater.

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