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REMOVAL OF BIOGENS FROM SYNTHETIC WASTEWATER BY MICROALGAE

Removal of biogens (P, N) from synthetic wastewater by microalgae *Chlorella vulgaris* was investigated. The study was carried out under static conditions (batch tests). Significant differences were found in the removal of nutrients by algae. The removal of biogens was >50% for ammonium ions and >80% for nitrates and phosphates. N and P removal through assimilation by microalgal *C. vulgaris* species can be considered an ecological alternative for current methods applied for removal of these substances from wastewater. It seems that using algae for N and P removal from wastewater can be affordable in comparison to other methods. The efficiency of biogens removal depends on the type of a biogen to be removed and the concentration of N and P in solutions. Optimization of N:P ratio can have some positive influence on the removal of biogens from wastewater by *C. vulgaris*.

1. INTRODUCTION

Microalgae are aquatic and microscopic organisms that can live in marine, fresh water, and also in various wastewaters. These organisms can be grown in various systems such as open ponds, closed and/or hybrid systems [1–3]. Algae can grow biomass using various nutrients, mostly N and P that are present in liquid phase of municipal, agricultural and industrial wastewater [4, 5]. The results of numerous studies show that algae can utilize selected compounds such as N and P from wastewater and use them for growth of biomass [10, 13]. Some algae species such as *Chlorella vulgaris* can be used for removal of biogens during wastewater treatment [4, 6–8]. Mahapatra et al. [9] investigated the role of algae in treatment of domestic wastewater and

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found that algae removed nitrogen compounds from wastewater. Zhu et al. [10] observed efficient removal of pollutants from piggery wastewater by algae grown for biodiesel production. Tantanasarin et al. [11] reported that phytoplankton quickly remove nutrients from solution when the external supply is suddenly increased in order to assimilate for binary fission. Phytoplankton cells are able to accumulate large quantities of unassimilated nutrients in larger amounts than needed. They have the ability to store such nutrients in large internal vacuoles and later assimilate them. He et al. [12] found that increasing NH_4^+-N from 17 to 207 mg/dm^3 yielded additional short-chain and saturated fatty acids in algal cells. Therefore algae can be considered a promising technology for the removal of biogens (N, P) from wastewater.

Wastewater is generated from a variety of sources. The chemical composition of wastewater is very complex and usually differs in the type and concentrations of pollutants despite the fact that the same technological processes are applied [14, 15]. Generally, industrial wastewater is more difficult to treat rather than domestic wastewater. There are a number of methods applied for treatment of industrial wastewater, including chemical, physical and biological methods. For many years there has been a growing interest in biological methods for wastewater treatment, and they are considered to have a great potential for treatment of different types of wastewater [16].

Nitrogen as a contaminant can be present in the environment in various forms. Four types of nitrogen that can be found in wastewater: organic nitrogen, ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen, making up the total nitrogen content. The predominant forms of nitrogen in wastewater are organic nitrogen and ammonium ion. Ideally, the removal of nitrogen from wastewater should mimic the transformations of nitrogen that occur in nature, however with much higher efficiency. Therefore the process parameters should be adjusted in order to obtain required efficiencies of N removal [17]. During the treatment of wastewater, nitrogen compounds undergo a variety of biochemical conversions. Nitrogen introduced with wastewater into the biological wastewater treatment plant can be transformed into other forms or completely removed. The conversion of nitrogen can occur through [17]:

- ammonification – nitrogen is bound to organic compounds (organic nitrogen) which leads to ammonium ion production,
- nitrification – ammonium nitrogen is converted into oxidative form (nitrites and nitrates),
- denitrification – nitrogen is converted into nitrates and gaseous nitrogen,
- assimilation – ammonium nitrogen is assimilated by cells of an active sediment, and then separated from wastewater.

These biochemical transformations depend on many factors such as pH, alkalinity, nitrogen concentration, age of sediment, concentration of dissolved oxygen and toxic substances.

Removal of phosphorus from wastewater is possible due to the ability of some bacteria or algae to store in the cells significant quantities of phosphorus that exceed physiological needs of these living organisms.

The overall goal of the presented study was to evaluate the removal of biogens (P, N) from synthetic wastewater by microalgae *C. vulgaris* during batch tests.

2. EXPERIMENTAL

Materials. Synthetic wastewater was used through the experiments. It was prepared according to the PN-72/C-04550.09 [18]. NH_4Cl, $NaNO_3$, K_2HPO_4 and KH_2PO_4 were used as nitrogen and phosphorus sources.

The strains of unicellular green microalgae *C. vulgaris* were obtained from the Culture Collection of Baltic Algae (CCBA), Institute of Oceanography, University of Gdansk, Poland. Algae cells were of spherical shape and 2–10 μm in diameter. Their chloroplast contained chlorophyll a and b.

Experimental methods. The experiments were conducted under static conditions in 1 dm^3 flasks in a batch mode. The flasks were filled with 1 dm^3 of medium. The experiment was conducted in two phases. During the former phase, 3 g of algal biomass as well as NH_4^+-N of the initial concentrations of 50 mg/dm^3 (sample 50N + 3A) and 100 mg/dm^3 (sample 100N + 3A), 20 mg/dm^3 of NO_3^--N, 80 mg/dm^3 of KH_2PO_4 and 32 mg/dm^3 K_2HPO_4 were applied. In the latter phase, in order to enhance the NH_4^+-N removal, the concentration of algae was increased two-fold and 6 g (sample 100N + 6A) of algal biomass was used. The NH_4^+-N concentration was 100 mg/dm^3, the initial concentrations of NO_3^--N, KH_2PO_4, K_2HPO_4 were increased two-fold in order to maintain their constant ratios to algal biomass. The exposure time for the biomass was 7 days. Because the highest removal efficiency of nutrients was observed until fourth day, the second phase lasted only 4 days. The experiments were conducted in the 12/12 hour day/night cycles at room temperature (20±2 °C). The initial pH was 7.55 and the turbidity 111 NTU.

Analytical methods. The samples were monitored for ammonium nitrogen, the total nitrogen, orthophosphates (PO_4^{3-}-P), the total phosphorus, pH, turbidity, chemical oxygen demand (COD) and biological oxygen demand (BOD). The measurements were determined in clear supernatant. The concentration of NO_3^--N was determined by spectrophotometric methods (Hach, Lange). The total P and PO_4^{3-}-P concentrations were determined according to the PN-EN ISO 6878/2006 [19]. The total N

(PN-73/C-04576.12) [20] and NH_4^+-N (PN-73/C-04576.02) [21] contents were determined by titration. The standard deviations ranged from ±0.02 to ±0.07.

pH was measured with a pH-meter (Cyberscan pH11, Eutech Instruments, PN-91 /C-04540.05 [22] whereas turbidity with a densitometer (WTW Turb 355 IR/T, Germany).

The determination of COD in wastewater was performed by the spectrophotometric method (PN-74/C-04578.03) [23] whereas the BOD was determined by the manometric method (PN-84/C-04578.04) [24]. The measurements of physicochemical parameters performed during the second phase in three different time intervals within light phase allowed the evaluation of the influence of photosynthetic phases on the investigated parameters.

Initial and final concentration of total carbon was measured with a Multi N/C 2100, Analytik Jena. The initial total carbon was 145 mg C/dm^3 in the sample 50N + 3A, 149 mg C/dm^3 in the sample 100N + 3A and the final concentrations were 45 mg C/dm^3 and 36 mg C/dm^3 in both samples, respectively. Initial and final content of total carbon in the sample 100N + 6A was 152 mg C/dm^3 and 71 mg C/dm^3, respectively.

The percentage removal (%) of NH_4^+-N, NO_3^--N, $PO^{3-}{}_4$-P, total N, total P was calculated according to the formula:

$$\text{Removal [\%]} = \frac{C_0 - C_f}{C_0} 100 \quad (1)$$

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

3. RESULTS AND DISCUSSION

3.1. pH VARIATIONS DURING THE PROCESSES

Figure 1 presents the time dependences of pH during the removal of biogens by *C. vulgaris* from the investigated synthetic wastewater during the first and second phase. Addition of algal biomass resulted in the pH increase from 7.45 (50N + 3A) and 7.59 (100N + 3A) to the maximum value of 8.52 (50N + 3A) and 8.36 (100N + 3A) on the second day. pH increase could be due to photosynthetic inorganic carbon uptake. Moreover, nitrate consumption by denitrification could also increase with pH [13]. According to the literature, the concentration of nutrients in solution can be affected by pH. Ammonia stripping or phosphate precipitation in the form of calcium phosphate could be observed at high pH values of 9–11 [5].

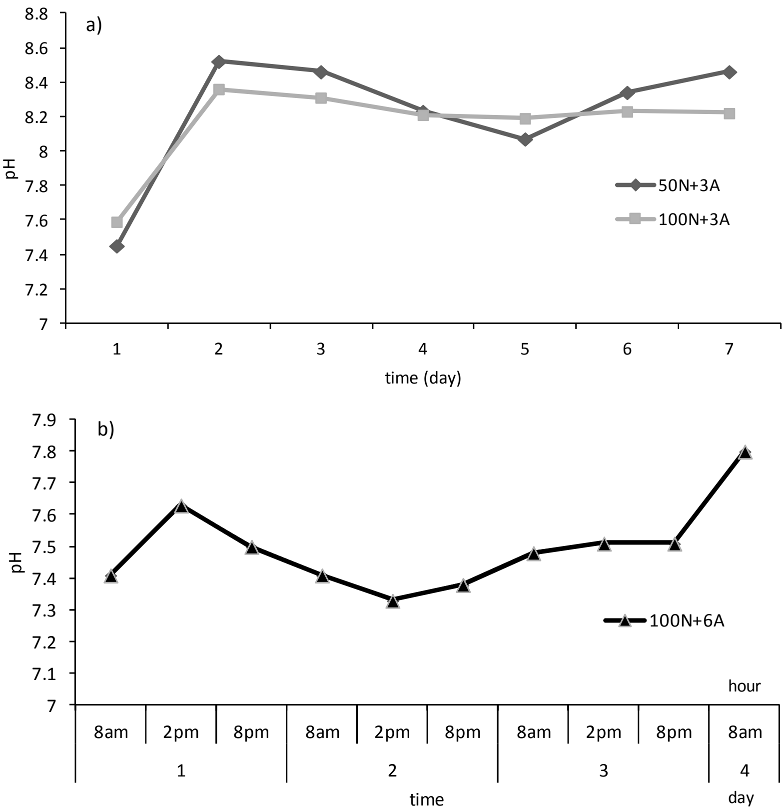


Fig. 1. pH values determined during the first (a) and second (b) phase of the experiments

Lower acidities were recorded in the second experimental phase which could result from the presence of larger amounts of nitrogen compounds in wastewaters. The maximum value of pH (7.8) was observed on the fourth day. In the case of using ammonia as a sole nitrogen source, pH will decrease due to the release of H^+ ions [25]. It is worth mentioning that under alkaline conditions ammonium ions release ammonia that is toxic at high concentrations [26, 27].

3.2. TURBIDITY VARIATIONS DURING THE PROCESSES

The obtained values of turbidity confirmed the increase of algal population during the experiment (Fig. 2).

Figure 2b presents the changes in turbidity with time during the second phase of the experiment. The increase in turbidity with time was also observed during that phase and varied along with the change of photosynthetic phases of algal cells.

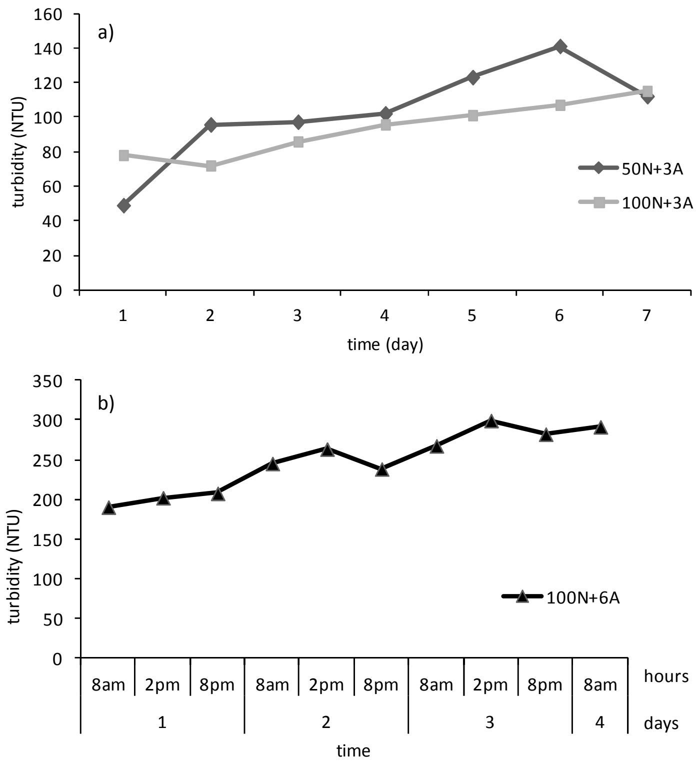


Fig. 2. Turbidity values determined during the first (a) and the second (b) phase of the experiments

3.3. CHEMICAL OXYGEN DEMAND AND BIOLOGICAL OXYGEN DEMAND

Chemical oxygen demand (COD) and biological oxygen demand (BOD) parameters were measured at the beginning and at the end of the experiment. Their values decreased which indicated sufficient degree of wastewater treatment. Figure 3 presents the values of COD and BOD measured at the beginning and at the end of the first and second phase of the experiment.

There were found almost 10-fold decreases in BOD and COD in all samples. Particularly, the COD values for the sample 50N + 3A decreased from the initial level of 415 mg COD/dm^3 to 43.5 mg COD/dm^3. In the sample 100N + 3A, the COD decreased from the initial 386 mg COD/dm^3 to the final concentration of 35 mg COD/dm^3. The COD in the second phase of the experiment in the sample 100N + 6A decreased from the initial value of 752 mg COD/dm^3 to the final value of 178 mg COD/dm^3. The observed decrease in COD values during the entire experiment could have resulted from exhausting the oxygen needed for nitrates oxidation.

In the case of BOD in the sample 50N + 3A, the values decreased from the initial level of 225 mg BOD/dm^3 to 23.5 mg BOD/dm^3. In the sample 100N + 3A, the BOD value decreased from 210 mg BOD/dm^3 to 18.9 mg BOD/dm^3 (Fig. 3b). On the first day of the second phase, the initial concentration was of 350 mg BOD/dm^3 whereas the final concentration decreased to 60 mg BOD/dm^3.

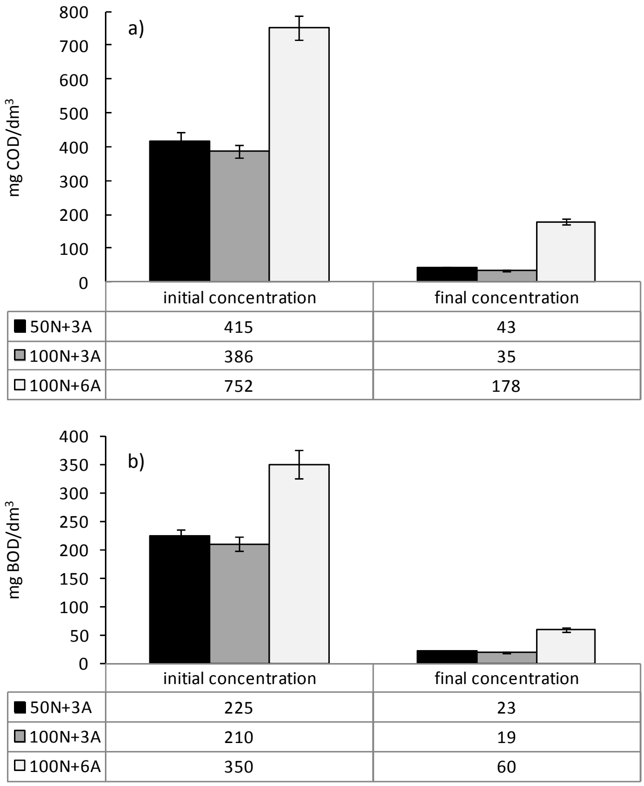


Fig. 3. Initial and the final concentrations of COD (a) and BOD (b): C_i – initial concentration, C_f – final concentration on the seventh day of the first and on the fourth day of the second experimental phase

The relationship between COD and BOD in the samples 50N + 3A and 100N + 3A was within the recommended range at the beginning and the end of the experiment. The BOD/total P and BOD/total N ratios in the samples 50N + 3A and 100N + 3A were lower than the recommended values at the beginning and the end of the processes (Table 1).

The COD/BOD ratio in the sample 100N + 6A was above the recommended values. The BOD/total N ratio in the sample 100N + 6A was within the recommended value at the beginning of experiment and it decreased below the recommended level at its end. The BOD/total P ratio in the samples 100N + 6A did not exceed the recommended values (Table 1).

Table 1

Ratios of COD/BOD, BOD/total N, and BOD/total P

Sample	COD/BOD (recommended <2)	BOD/Total N (recommended >3–4)	BOD/Total P (recommended >20)
50N + 3A – initial	1.8	2.25	13.53
50N + 3A – final	1.8	0.76	1.80
100N + 3A – initial	1.8	1.92	12.16
100N + 3A – final	1.8	0.47	1.07
100N + 6A – initial	2.1	3.80	17.50
100N + 6A – final	2.9	0.93	3.60

3.4. REMOVAL OF NH_4^+-N, NO_3^--N AND TOTAL N

Algae are capable of utilizing nitrates, ammonia, and other nitrogen sources such as urea. Nitrites can also be absorbed but they are toxic at higher concentrations. Some blue-green algae are able to assimilate atmospheric nitrogen. Moreover, nitrites and nitrates have to be added in larger amounts because prior to building into cellular structures, they have to be reduced. The optimum nitrogen quantity for the majority of algae is ca. 5–50 mM [25].

According to the literature, the order of using a nitrogen source by most microalgal species is as follows: ammonium ion > nitrate > nitrite > urea. Assimilation of nitrates and ammonia is closely associated with pH. The ammonium ion removal could be due to algal uptake and ammonia stripping at high pH value [25, 29]. If ammonia is utilized as the only nitrogen source, pH of the environment can suddenly decrease to such low level as 3.0 which has negative effects on algae. The adverse process can be observed during assimilation of nitrate ions because their absorption leads to increase of pH. pH variations can be a reason for growth inhibition of some algae [25].

The percentage ratio of NH_4^+-N and NH_3 in solution varied with pH [30]. Depending on pH, within the whole experiment duration, the ratio of NH_4^+-N to NH_3 in the investigated wastewater varied most significantly. At the beginning of the first day, the content of NH_4^+-N was ca. 100% in the sample 50N + 3A at pH = 6.62. The content of NH_4^+-N ions at pH 6.22 was also ca. 100% at the beginning of the first day in the sample 100N + 3A. The lowest ratio of NH_4^+-N to NH_3 content was recorded for the sample 50N + 3A on the third and seventh day at pH 8.46 (ca. 85% of NH_4^+-N) while the percentage of NH_3 amounted to ca. 15% that time. In the sample 100N + 3A, the lowest ratio of NH_4^+-N to NH_3 was observed on the second day at pH = 8.36 (ca. 90%). Differences in NH_4Cl concentrations in the investigated wastewater had insignificant impact on the change in nitrogen forms during the entire experiment (the NH_4^+-N forms predominated).

The increase in the NH_4^+-N concentration was observed on the first day and the process continued until the third day for both initial concentrations (50 and 100 mg/dm^3). It could have resulted from some decrease in algal population and/or nitrate reduction leading to the enhanced NH_4^+-N percentage in wastewater at its worse assimilation. Then, the increase in algal culture affected the efficiency of elevated NH_4^+-N concentrations removal. At the fourth day (sample 50N + 3A) and the fifth day (sample 100N + 3A) the highest removal of NH_4^+-N was observed (Fig. 4a).

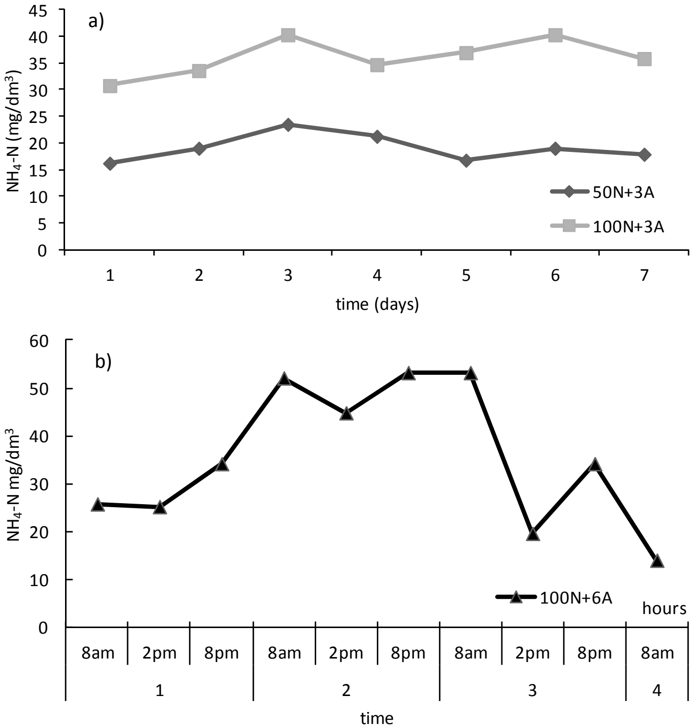


Fig. 4. NH_4^+-N values determined during the first (a) and the second (b) phase of the experiment

Like in the first phase of the experiment, similar pattern was observed during the second phase. Depending on the pH, the percentage of NH_4^+-N and NH_3 varied through the entire experiment. The percentage of NH_4^+-N at pH 7.41 reached up to ca. 99% at the beginning of the first day. The lowest proportion of NH_4^+-N was recorded on the fourth day at pH 7.87 (ca. 95%) whereas the share of NH_3 was ca. 5% (Fig. 4b).

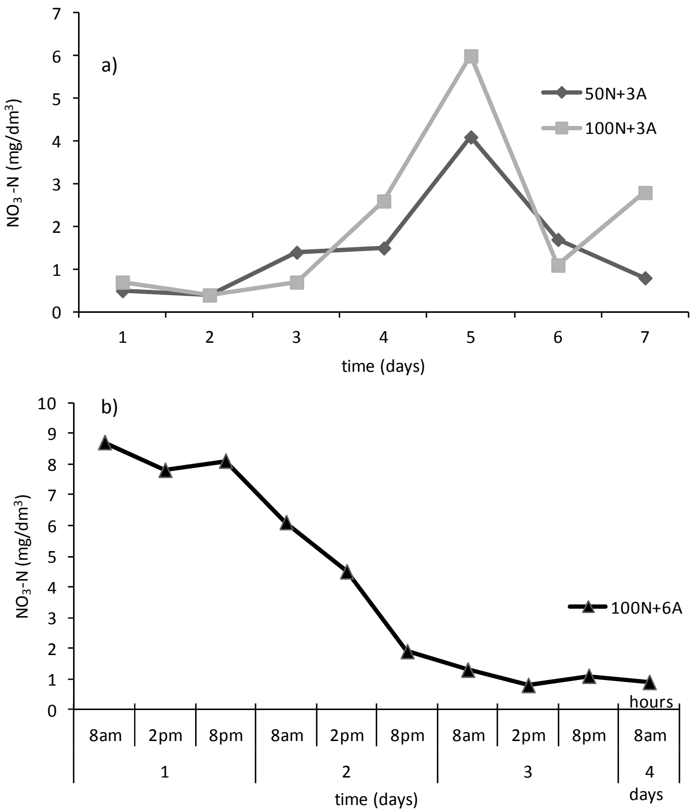


Fig. 5. The NO_3^--N values determined during the first (a) and the second phase (b) of the experiments

The initial concentration of NO_3^--N in the first phase 20 mg/dm^3. Figure 5a shows changes in nitrate concentration during the first phase. Nitrates were removed at the peak efficiency from the fifth day until the end of the experiment. The NO_3^--N consumption in wastewater is dependent on the turbidity of the wastewater. The higher turbidity, the lower NO_3^--N consumption observed is. During the investigation, the turbidity increased and the consumption of NO_3^--N decreased since the third day. Photosynthetic organisms consume energy to reduce nitrate to nitrite and to ammonia by enzymes nitrate and nitrite reductases [25]. The utilization of nitrate as a nitrogen source starts when ammonia is consumed [26]. Ammonium in high concentration will inhibit assimilation of nitrate because ammonium represses the synthesis of nitrate reductase. Reduction of nitrates to NH_3 and NH_4^+-N will affect the increase of these ions in wastewater [28].

Nitrates were slowly consumed during the entire time of algal cultivation due to reduction processes. This tendency was also observed by Ferreira et al. [26]. The removal of nitrates during the second phase began 12 h after the experiment was started (Fig. 5b).

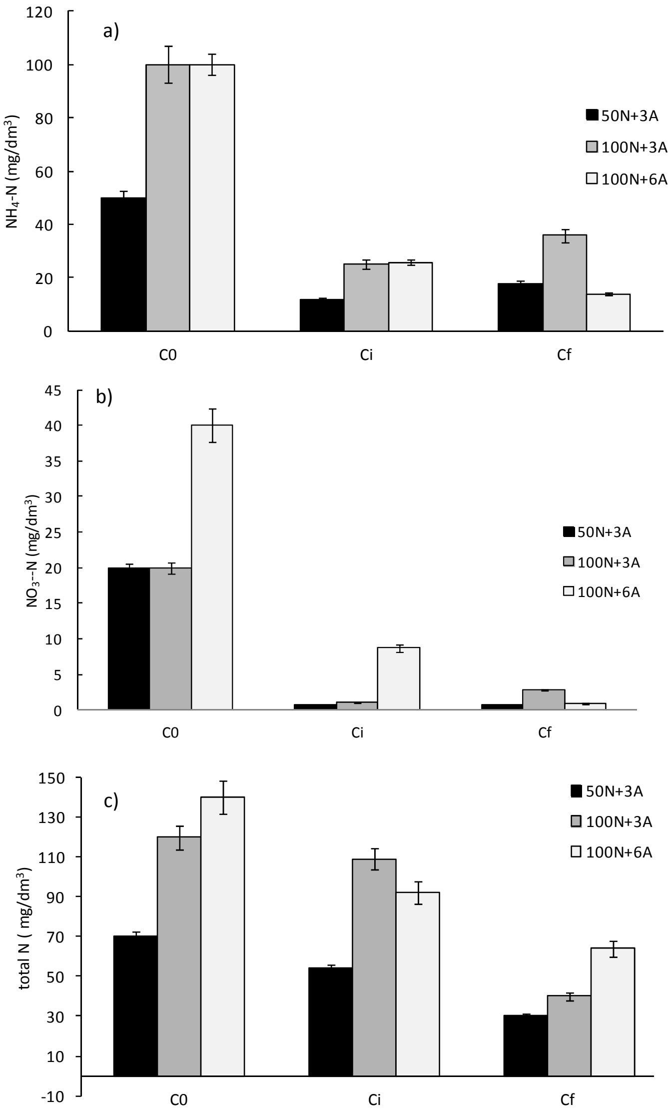


Fig. 6. Raw (wastewater with no algae), initial and final concentrations of NH_4^+-N (a), NO_3^--N (b) and total N (c): C_0 – raw concentration of NH_4^+-N in the wastewater, C_i – the initial concentration, C_f – the final concentration recorded on the seventh day of the first and on the fourth day of the second experimental phase

The largest decrease in the NO_3^--N concentration was observed since the second day which could have resulted from the reduction of nitrates to ammonia according to the reaction sequence stated by Becker [25]:

$$HNO_3 \rightarrow HNO_2 \rightarrow H_2N_2O_2 \rightarrow NH_2OH \rightarrow NH_3$$

or due to the reaction:

$$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$$

An efficient removal of nitrogen compounds from the investigated wastewater after 3 days when nitrogen forms became the most available for algae and the increase in algae population occurred. The increase in the algae population affected the increased demands for nutrients.

Figure 6 presents raw, initial, and final concentrations of NH_4^+-N, NO_3^--N, and total N recorded in the samples. The most efficient removal of NH_4^+-N and NO_3^--N was observed in C_i due to the most efficient assimilation of that compounds by algae at the beginning of experiment during the first phase of the study (Fig. 6a, b). The decrease in the NH_4^+-N and NO_3^--N concentration in the C_f sample during the second phase indicated that the addition of 6 g/dm^3 of algal biomass resulted in higher efficiency of NH_4^+-N and NO_3^--N removal.

Changes in nitrogen forms, their various assimilation degrees, as well as pH changes in the wastewater influenced the concentration of total nitrogen. The decrease in the total N content in C_f sample during the first as well as the second phase was observed (Fig. 6c). The total nitrogen (total N) is determined by dissolved inorganic (nitrites, nitrates and ammonia) and organic nitrogen. High organic nitrogen levels in the environment are due to sewage runoff and decomposition of aquatic life. Inorganic levels are enhanced by erosion and residential runoff (fertilizers) [26].

3.5. REMOVAL OF PO_4^{3-}-P

Algal addition into the investigated wastewater had the influence on changes in PO_4^{3-}-P concentration. Since the moment of adding the algae into wastewater, the decrease in PO_4^{3-}-P concentration lasted until the third day (Fig. 7).

The main phosphorus form available to algae is inorganic phosphate in $H_2PO_4^-$ or HPO_4^{2-} ions. The optimum phosphorus concentration in a medium as well as phosphorus tolerance differ for various species, even if all other nutrients are supplied in appropriate concentrations. Mean phosphate tolerance for the majority of algae is from 50 μg/dm^3 to 20 mg/dm^3.

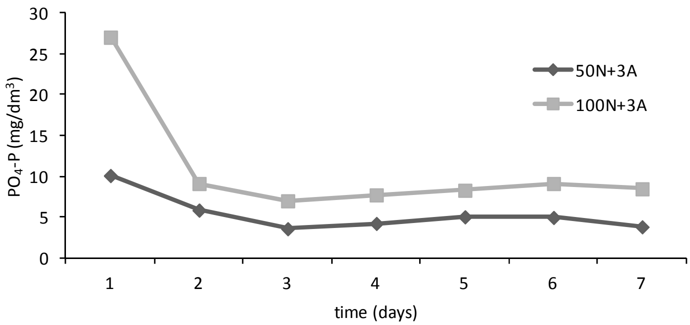


Fig. 7. PO_4^{3-}-P values determined during the first phase of the experiments

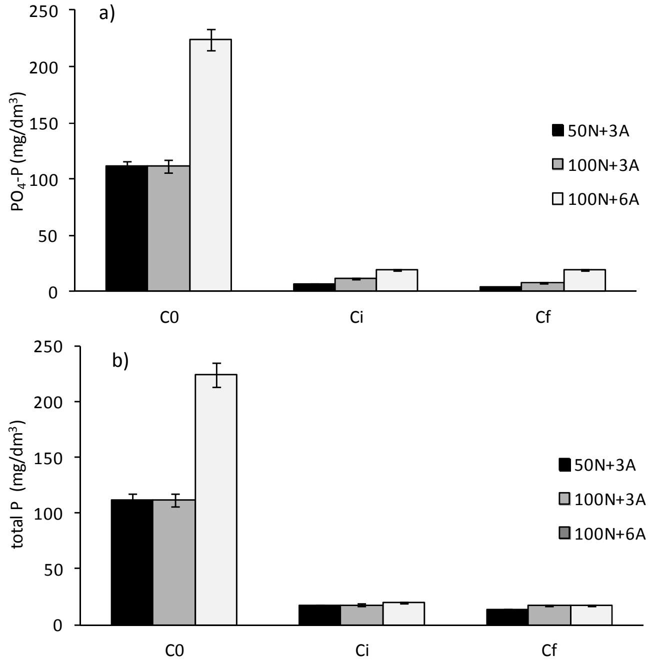


Fig. 8. Raw, initial, and final concentrations of PO_4^{3-}-P (a) and total P (b):
C_0 – raw concentration in synthetic wastewater, C_i – initial concentration recorded after mixing the components (wastewater + algae), C_f – final concentration recorded on the seventh day of the first and on the fourth day of the second experimental phase

The percentage ratio of $H_2PO_4^-$ and HPO_4^{2-} in solution varied with pH [31].The percentage of various orthophosphate ions in the investigated wastewater changed upon pH changes. In the first phase of the study, on the first day, $H_2PO_4^-$ (ca. 33.90%) and HPO_4^{2-} ions (ca. 66.10%) were present at pH = 7.45 in the sample 50N + 3A, while in the sample 100N + 3A it was found at pH = 7.59. The largest share of HPO_4^{2-} was 98.39% at pH = 8.52. Starting from the second day until the end of the experiment, the proportion of HPO_4^{2-} ions amounted to 95.12% while that of $H_2PO_4^-$ ions - ca. 4.88% at pH = 8–9.

For the whole second phase of the experiment, the pH value varied from 7.2 to 7.9. Within that pH range, phosphorus was present as $H_2PO_4^-$ and HPO_4^{2-} ions. At pH 7.2, the $H_2PO_4^-$ ion occurred in ca. 33.9% whereas HPO_4^{2-} in ca. 66.10%. At pH = 8, $H_2PO_4^-$ ion was present in ca. 4.88% while HPO_4^{2-} ion in ca. 95.12%.

The raw concentration of PO_4^{3-}-P in the first phase was 112 mg/dm^3. The removal of orthophosphates started since the beginning of wastewater contact with algae. The results showed that removal of PO_4^{3-}-P and total P by *C. vulgaris* in the sample 50N + 3A was the most efficient (Fig. 8).

The total P included all phosphorus compounds present in the solution. Its concentrations varied with time. On the third day, the decrease in acidity occurred up to pH = 7.28. At that pH, the highest removal of the total phosphorus was achieved. For the other presented experiments, the pH value was above 7.28.

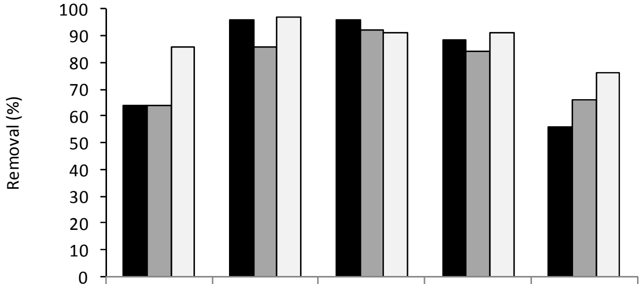
3.6. PERCENTAGE REMOVAL OF INVESTIGATED BIOGENS AND C/N/P RATIOS

Figure 9 presents percentage removal of investigated biogens. The percentage removal of total N was the lowest (56–76%). During the first phase of the study, the percentage removal of NH_4^+-N on the seventh day for both samples 50N + 3A and 100N + 3A and regardless of the initial ammonium ions concentration amounted to 64%. In the second phase of the study, NH_4^+-N ions were most readily removed on the fourth day (up to 86%). The percentage removal from the investigated synthetic wastewater was >55% for total N, more than 85% for NO_3^--N and more that 91% for PO_4^{3-}-P .

The following sequences of biogens removal were achieved in the samples with 3g of *C. vulgaris* biomass added (Fig. 9):

- for the sample 50N + 3A: NO_3^--N = PO_4^{3-}-P > total P > NH_4^+-N > total N,
- for the sample 100N + 3A: PO_4^{3-}-P > total P > NO_3^--N > total N > NH_4^+-N,
- for the sample 100N + 6A: N-NO_3> PO_4^{3-}-P = total P > N-NH_4> total N.

In similar experiments, Cai et al. [5] observed that in the case of *Chlorella pyrenoidosa* cultivated in soybean wastewater, the nitrogen was mainly used for algal cell synthesis, whereas 17% of the phosphorus was removed in the process of precipitation rather than by assimilation [5].



	NH4-N	NO3-N	PO4-P	Total P	Total N
■ 50N+3A	64	96	96	88.3	56
■ 100N+3A	64	86	92	84.3	66
□ 100N+6A	86	97	91	91	76

Fig. 9. The percentage removal of NH_4^+-N, NO_3^--N, PO_4^{3-}-P, N_{og}, P_{og}, 50N + 3A measured at the seventh day, 100N + 3A measured at the seventh day, 100N + 6A measured at fourth day

The total N/total P proportion at the beginning of the first phase of the experiment in the samples 50N + 3A and 100N + 3A was 6:1 which suggests that nitrogen was deficient. At the end of the experiment, this ratio reached 2:1 which resulted from nitrogen assimilation. Carbon utilization was also observed. Within the second phase of the study, the total C/N/P proportion was 8:5:1 while at the end of experiment, C and N was used up and the final C/N/P ratio amounted to 4:4:1 (Table 2).

Table 2

Calculated C/N/P ratios

Sample	C/N/P
Wastewater	7:4:1
50N + 3A – initial	9:6:1
50N + 3A – final	3:2:1
100N + 3A – initial	9:6:1
100N + 3A – final	2:2:1
100N + 6A – initial	8:5:1
100N + 6A – final	4:4:1

Simultaneous utilization of both nitrogen and phosphorus could be achieved when the N/P ratio were optimal. Cai et al. [5] found that the optimal ratio differs among cultures due to strain-varying metabolic pathways. The N/P ratio in healthy freshwater environments can be up to 250 and in most wastewater streams the N/P can be around 4–5. The optimal N/P ratio for *C. vulgaris* was reported to be 7. This is in agreement with the N/P ratio of 7.2 that was calculated from the Stumm empirical formula for microalgae ($C_{106}H_{263}O_{110}N_{16}P$). According to that ratio, it could be concluded that the removal rate of nitrogen would be faster than that of phosphate [5]. However, Su et al.

[13] concluded that the recommended ratio between nutrients C/N/P should amount to 106:16:1. The N/P dependence can be a factor that reduces the phytoplankton growth. The N/P ratio is utilized to evaluate, which of these components is deficient. For algae, N/P < 16 means that there is the nitrogen deficiency whereas if N/P > 16, phosphorus is deficient [13].

The researchers also indicated that the removal of phosphate by unicellular microalgae *Chlamydomonas reinhardtii, Scenedesmus rubescens* and *Chlorella vulgaris* compared to that observed for ammonium could be faster from the wastewater when the N/P ratio was around 15:1 and slower when N/P was around 5:1 [13].

4. CONCLUSIONS

The efficiency of biogens removal depends on the type of a biogen to be removed and the concentration of N and P in the solution.

The percentage removal of total N was the lowest (56–76%).

Percentage removal of biogens from the investigated synthetic wastewater was > 55% for total N, higher than 85% for NO_3^--N and higher that 91% for PO_4^{3-}-P.

The percentage removal of NH_4^+-N for both samples 50N + 3A and 100N + 3A regardless of the initial ammonium ions concentration amounted to 64%. In the second phase of the study, the NH_4^+-N removal was up to 86%.

Following sequences of nutrients removal were achieved:

- for the sample 50N + 3A: NO_3^--N = PO_4^{3-}-P > total P > NH_4^+-N > total N,
- for the sample 100N + 3A: PO_4^{3-}-P > total P > NO_3^--N > total N > NH_4^+-N,
- for the sample 100N + 6A: N-NO_3 > PO_4^{3-}-P = total P > N-NH_4 > total N.

Optimization of N:P parameters may have some positive influence on removal of N and P from wastewater by *C. vulgaris*.

Removal of biogens by microalgal *C. vulgaris* species can be a promising alternative for current removal methods of these substances from wastewater.

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