



# Utilization of *Chlorella vulgaris* and Sediments after N-NH<sub>4</sub> Removal Containing Clinoptilolite for Sorption of Heavy Metals from Wastewater

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## 1. Introduction

Industrial wastewater contains high concentration of inorganic toxic contaminants e.g. ammonium and heavy metals to be treated before being discharged into the environment. Heavy metals can create undefined mineral and organometallic connections in the aqueous and land environment [22]. Such contaminants may produce long-term environmental impacts without proper treatment. The composition of the wastewater is complicated depending on the types of industry. The coal coking wastewater contains high concentration of ammonium and electroplating or processing plants of metals contains high concentration of heavy metals. The wastewater produced by zinc smelter and mining and processing plants of Zn-Pb and ores electroplating usually content many heavy metals, such as Cr(VI), Ni(II), Zn(II) and Pb(II) that are specified in the regulation of the Minister of the Environment of 15th March 2008 amending the regulation on substances particularly harmful to water environment that discharging of these substances in industrial wastewater to sewage system requires granting the water-legal permit [11].

There are several methods for N-NH<sub>4</sub> removal from wastewater but these technologies are energy consuming and costly. Conventional methods for removal of heavy metals from water solutions include physicochemical methods [3]. Usually wastewater treatment is based on the precipitation methods that produce huge amounts of sludge. The management of such sludge is required.

Algae can grow in N-rich wastewaters. Moreover, recently micro-algae have been introduced for wastewaters treatment because of their high sorption capacity of heavy metals. Algae show fast and continuous growth at minimum environmental requirements and irrespective from seasons [24]. Algae cultivation in wastewater has numerous advantages, such as: low cost and non-toxic treatment without chemicals.

According to the literature, *C. vulgaris* can shift between autotrophy and heterotrophy. It was indicated that this algae are capable of growing on CO<sub>2</sub> in light and on certain forms of organic carbon during darkness [4]. It allows to grow algae in dark coloured (opaque) wastewaters [5]. Molasses is a low cost carbon source and could be used as a nutrient that supports the algal heterotrophic growth.

The adsorption capacity of clinoptilolite for the removal of ammonium ions demonstrates its potential application in the treatment of wastewater [25]. Removal of heavy metals can also be achieved with sorption on activated carbon or zeolite [18]. Clinoptilolite is also known for its favorable sorption capacity especially for heavy metals [26].

Little information is however available on the performance of combined industrial wastewater treatment by utilization of biomass from sediments after N-NH<sub>4</sub> assimilation from wastewater for heavy metal bioaccumulation from other wastewater contaminated with metals. This is the first study to investigate the recycling of sediments containing mixture of *C. vulgaris* and clinoptilolite for N-NH<sub>4</sub> and then heavy metal removal.

Two phases of experiments were conducted. The goal of the first phase of the presented research was to investigate the effects of algae and algae enriching by natural clinoptilolite on removal of ammonium ions from synthetic wastewater. The aim of the second phase was to assess the potential applications of algae and sediments after ammonium ions assimilation for heavy metal bioaccumulation by algae and sorption by clinoptilolite. In order to shift between autotrophy and heterotrophy of *C. vulgaris* the utilization of molasses was performed. The heterotrophic growth (night) was stimulated followed by a period of autotrophic growth (day). It has been hypothesized that the addition of clinoptilolite would enhance the removal efficiency of N-NH<sub>4</sub> and heavy metals by *C. vulgaris*.

This study will deliver the uptake of N-NH<sub>4</sub> and heavy metals and factors that affect it.

## 2. Materials and methods

Synthetic wastewater, algal living biomass of *C. vulgaris* and natural zeolite (clinoptilolite) were used for the experiment. Synthetic wastewater was prepared according to the PN-72/C-04550.09 [19].

Natural zeolite (clinoptilolite) of granulation 0.0–0.2 mm was used. Studies were carried out using clinoptilolite in untreated, powdered form. *C. vulgaris* living biomass was used. The algae was cultured in 250 ml flasks at an irradiance of  $80 \pm 5 \mu\text{mol photons m}^2/\text{s}$  in a climatic chamber and a light/dark period of 12/12 h at temperature  $29 \pm 0.5^\circ\text{C}$  at day and  $25 \pm 0.5^\circ\text{C}$  at night in order to increase the biomass production. Algae were cultivated for 3 months and then used for the present investigation.

Two phases of experiments were conducted. During the first phase of the research  $\text{NH}_4\text{Cl}$  was used as nitrogen source. The solution of  $\text{NH}_4\text{Cl}$  was prepared in volume of 1000 ml to obtain concentration of:

- 100 mg/L N- $\text{NH}_4$  – sample 1A, 1B,
- 500 mg/L N- $\text{NH}_4$  – sample 2A, 2B,
- 1000 mg/L N- $\text{NH}_4$  – sample 3A, 3B,
- 5000 mg/L N- $\text{NH}_4$  – sample 4A, 4B.

The nitrogen solutions dissolved in the synthetic wastewater were added to the samples containing:

**Algae** – 1% of *Chlorella vulgaris* living biomass

There were 2 different series of wastewater with additives prepared:

- A** – no molasses,
- B** – 2% of molasses.

The experiment was conducted until the N- $\text{NH}_4$  content reached value below 10 mg/L, the values below the maximum permissible concentration for N- $\text{NH}_4$  in wastewater, according the Polish standards [12]. 2% of clinoptilolite was added to this container in which the final concentration of ammonium nitrogen hadn't reached values below the maximum permissible concentration.

Molasses was added every day of the experiment at the beginning of the night phase. Molasses on an average contains about 25% glucose, 25% fructose and 30% sucrose [4].

Dissolved oxygen concentration (DO), pH and N-NH<sub>4</sub> content measurements were made every 24h of contact time. Biochemical oxygen demand (BOD) was monitored on the samples. Many physical and biological factors (e.g. temperature, salt concentrations) affect the amount of dissolved oxygen measured in units such milligrams O<sub>2</sub> per liter (mg/L) in solution. Therefore, DO results was presented in %, because the used wastewaters in each phases have different salt concentrations.

The pH determination in wastewater was done using the pH-meter. Cells growth was monitored by measuring the dissolved oxygen concentration (DO). N-NH<sub>4</sub> content was measured by titration. The BOD determination was achieved using manometric method.

During the second phase the heavy metals: Cr(VI), Ni(II), Zn(II) and Pb(II) as: K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, Ni(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, Pb(NO<sub>3</sub>)<sub>2</sub> solutions were prepared in the forms of salts to obtain concentration of 10 mg/L in suitable container of 1000 ml. Monometallic system was used. The experiment lasted 4 days. The experiment was performed in triplicates.

The heavy metals solutions dissolved in the synthetic wastewater were added to the samples containing:

**Algae** – 1% of *Chlorella vulgaris* living biomass, samples No 1–4,  
**sediment** – 1% of sediment after N-NH<sub>4</sub> removal which include: 1% of *Chlorella vulgaris* living biomass and 2% of clinoptilolite, samples No 6–9.

There were 2 different series of wastewater with additives prepared:

**A** – no molasses,

**B** – 150 mg/L of molasses.

Based on the results of first phase the quantity of molasses was lowered because of the too deep decrease of pH after adding molasses. That can be due to the not consumption of all quantity of molasses by algae. During the second phase 150 mg/L of molasses at the beginning of night phase was added, according to the Becker (2008) [4]. At first day and then every 24 hours the pH, dissolved oxygen concentration (DO), and heavy metal contents measurements were made.

Heavy metal concentrations was determined by the emission spectrometry with inductively coupled plasma ICP-OES according to the standard procedure PN-EN ISO 11885:2001 [20].

Metal removal from the solution was calculated according to the formula (1):

$$Uptake(\%) = \frac{C_0 - C_k}{C_0} 100 \quad (1)$$

Where  $C_0$  and  $C_k$  are the initial and final metal concentrations (mg/L).

### 3. Results and discussion

#### 3.1. Ammonium nitrogen removal from wastewater

Nitrogen exists in many forms, and the most common nitrogen compounds assimilated by microalgae are ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) [23].

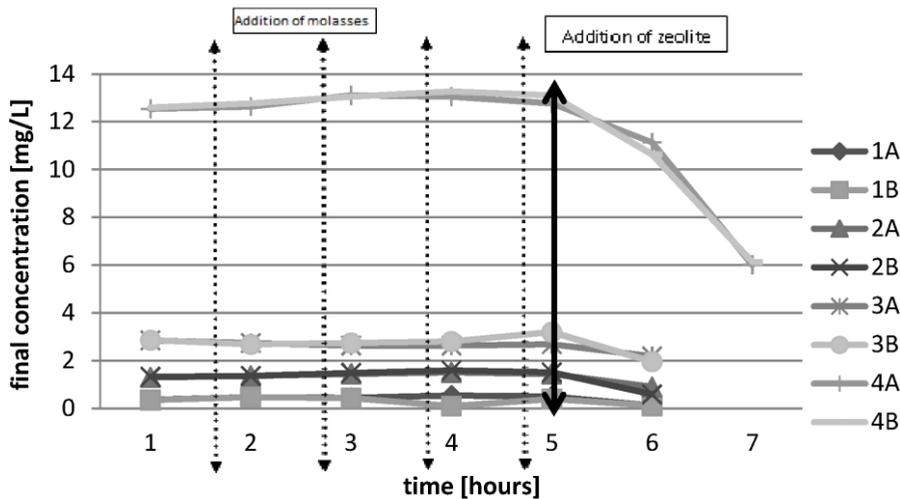
During all experiment pH was  $< 7.6$ . At  $\text{pH} < 9.0$  ammonia ions dominate. The preferred compound is ammonium, and when this is available, no alternative nitrogen sources will be assimilated [23]. However, ammonium concentrations higher than 20 mg/L N- $\text{NH}_4$  are not recommended due to ammonia toxicity [8].

On the 1<sup>st</sup> day of experiment the final concentration of ammonium nitrogen in samples 1–3 reached values below the maximum permissible concentration of N- $\text{NH}_4$  in wastewater (figure 1). N- $\text{NH}_4$  content in sample 4 (initial concentration of 5000 mg/L) significantly decreased but still remained above the maximum permissible level till 5<sup>th</sup> day. Then clinoptilolite was added to enhance removal the ammonium nitrogen from wastewater. After 48 h, on the 7<sup>th</sup> day N- $\text{NH}_4$  concentration decreased to the acceptable value and reached concentration of 6 mg/l.

Recycling of used sediment was done. Sediment consisted of *C. vulgaris* and clinoptilolite, after N- $\text{NH}_4$  removal was used for the second phase of the experiment for heavy metals removal.

Molasses had no effect on removal of ammonium nitrogen.

Many studies have shown that pH is an important factor affecting removal of nitrogen compounds [17]. Nitrogen absorption makes influenced the acidity. If ammonia is utilized as the only nitrogen source, pH of the environment can suddenly drop to such low level as pH 3.0, which has negative effects on algae. The adverse process can be observed during assimilation of nitrate ions, because their absorption leads to pH value increase. Violent pH variations can be a reason for growth inhibition of some algae.



**Fig. 1.**  $N-NH_4$  values observed during the experiment

**Rys. 1.** Stężenia  $N-NH_4$  obserwowane w czasie eksperymentu

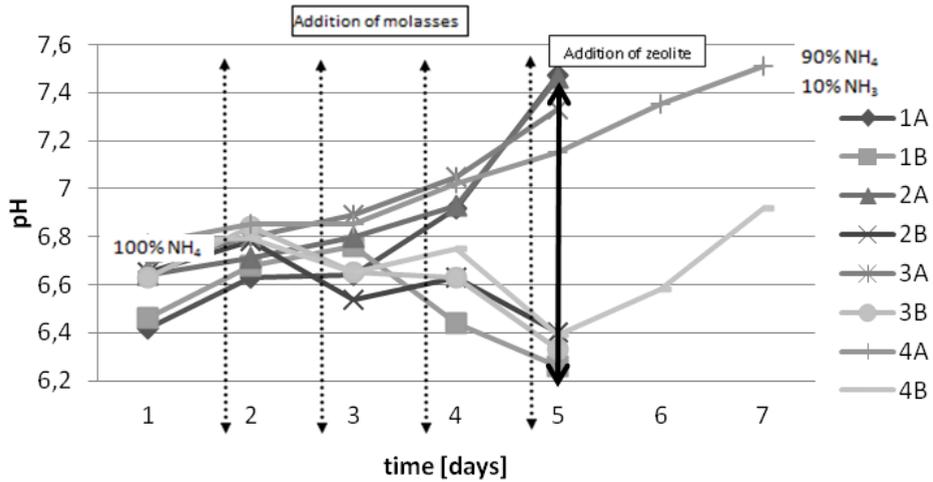
During first 3 days value of pH little varied from 6.4 to 6.9 in all samples (figure 2). The addition of molasses resulted in decrease of pH to the minimum value of  $pH = 6.26$  on the 5<sup>th</sup> day of the experiment.

According to Liang et al. (2009) the range of sewage acidification depends on amount of added compound. After addition of clinoptilolite pH sharply increased which had a positive effect on ammonium ions removal [17].

The oxygen is released during photosynthesis process [6].

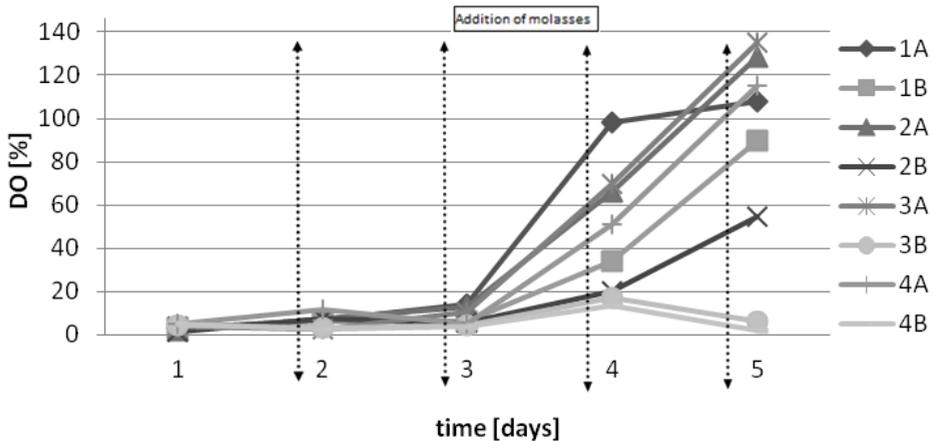
The increase in DO started on the 3<sup>rd</sup> day and the process prolonged till the end of the experiment (figure 3). In samples with addition of molasses (B samples) the DO value was lower than in A samples. On the 5<sup>th</sup> day it reached maximum value of 135% for 3A and minimum of 20% for 4B.

The maximum permissible level of BOD is 25 mg  $O_2/L$  [12]. On the 1<sup>st</sup> day the BOD level in samples 1, 2, 3 and 4 was 350, 100, 300 and 100 mg  $O_2/L$ , respectively. The value of BOD decreased in all samples. On the 5<sup>th</sup> day the BOD in samples 1,2,3 and 4 was 0, 0, 50, 100 mg  $O_2/L$ , respectively. This phenomena indicates that the wastewater treatment in samples 1 and 2 was very effective [8].



**Fig. 2.** pH values observed during the removal of  $\text{N-NH}_4$  from wastewater. Initial concentration of  $\text{N-NH}_4$ : 1A, 1B – 100 mg/L, 2A, 2B – 500 mg/L, 3A, 3B – 1000 mg/L, 4A, 4B – 5000 mg/L

**Rys. 2.** Wartości pH obserwowane w czasie usuwania azotu amonowego. Stężenia początkowe  $\text{N-NH}_4$ : 1A, 1B – 100 mg/l, 2A, 2B – 500 mg/l, 3A, 3B – 1000 mg/l, 4A, 4B – 5000 mg/l



**Fig. 3.** Dissolved oxygen concentration observed during the removal of  $\text{N-NH}_4$

**Rys. 3.** Stężenie tlenu rozpuszczonego w czasie usuwania azotu amonowego

### 3.2. Heavy metal removal from wastewater

In the present study the bioremoval of Cr (VI), Ni (II), Zn (II) and Pb(II) ions from synthetic wastewater by means of algae *Chlorella vulgaris* and by sediment (after N-NH<sub>4</sub> removal) consisted of *Chlorella vulgaris* and clinoptilolite was examined.

There are several factors that affect biosorption process. The most important are metal ion initial concentration, temperature, biomass concentration in solution, presence of other cations and anions and pH [9, 16]. According to literature the process is not disturbed at the temperature in the range of 20–35°C and the absolutely essential factor in the biosorption process is pH. It is known that pH value affects the activity of the functional groups, solution chemistry of metals and the competition of the metallic ions [9]. According to the Polish standards, the maximum permissible value of pH is 6.5–9.0.

The uptake of investigated heavy metals was observed. Some of the metal ions were released back into the water while the DO level drastically declined. This suggests that the algae did not perform photosynthesis process since the production of DO was extremely low.

Afkar et al. (2010) demonstrated the toxicity of heavy metals on algae species [1]. Low concentrations of metals could have stimulating effects on algae although too high concentrations led to total cell's death.

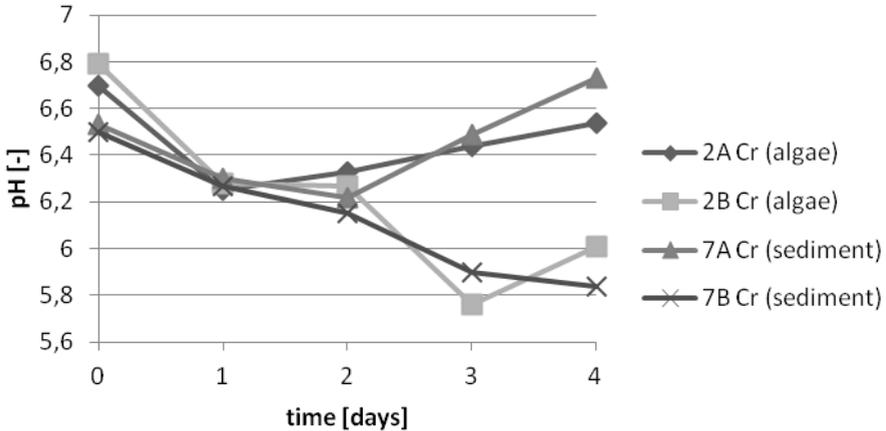
#### Chromium (VI) ions removal

Many authors state that adsorption of chromium by green algae was improved at lower pH values ranging from pH = 3.5 to pH = 5.5, while the best removal efficiency for chromium (VI) occurs at the pH = 2 and it decreases with the further raise in pH value [7, 13, 21]. With the increase of pH to higher than pH = 3 algal wall of the cell acquires more functional groups that have a net negative charge which leads to rejection of the anions [13]. Cr (VI) could be more effectively removed only if reduced from hexavalent chromium to trivalent form Cr (III) [21].

According to the literature, pH is the most important factor that affects chromium(VI) ions uptake from metal solution. With decreasing pH (below 6), the sorption of chromium increases due to the higher degree of protonation [2].

For chromium(VI) ions pH value decreased in 2B samples from pH = 6.79 to pH = 6.01 and in 7B samples from pH = 6.50 to pH = 5.84 (fig-

ure 4). The values of pH in the sample 2A changed from pH = 6.70 to 6.54 and in the sample with sediment (7A) it increased from pH = 6.53 to pH = 6.73. The decrease of pH in samples 'B' could have been caused by not consuming of all molasses by algae.

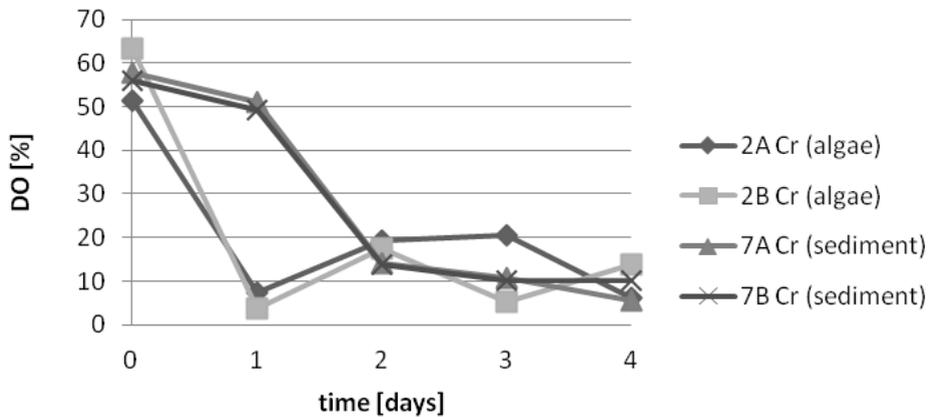


**Fig. 4.** pH values observed during the sorption of Cr(VI) ions

**Rys. 4.** Wartości pH obserwowane w czasie sorpcji jonów Cr(VI)

According to the literature green algae are more resistant to Cr than brown algae [7]. In the present studies, in samples with sediment, on the first day of the experiment, the DO remained constant at 50% (figure 5). Photosynthesis was not affected because the algae did not absorb metal ions. Adsorption process proceeded very slowly. On the second day of the experiment the concentration of metal decreased from 10 mg/L to 9.13 to 7.99 mg/L in samples 2A and 2B for wastewater with algae, and to 6.46 and 6.64 mg/L in the samples 7A and 7B respectively for wastewater with sediment.

Oxygen remained at the level of 10%, meaning that the algae were still carrying out the process of photosynthesis, and the amount of chromium(VI) ions sorbed was not completely toxic to them. Cervantes et al. (2006) concluded that low concentrations of metal are tolerated by algae. Although chromium(VI) ions results in lengthening in the lag growth phase (algae growth stoppage) [7].



**Fig. 5.** Dissolved oxygen concentration observed during the sorption of Cr(VI) ions  
**Rys. 5.** Stężenie tlenu rozpuszczonego w czasie sorpcji jonów Cr(VI)

The sorption of chromium(VI) ions by sediment was more efficient than by algae after six hours, at 1<sup>st</sup> and 3<sup>rd</sup> day of contact time. After 6 hours the removal efficiency of chromium(VI) ions by algae was of 0%. Utilization of molasses enhanced removal efficiency of chromium(VI) ions every 2 days in samples with algae as well as with sediment. It could be due to the growth-enhancing effect (stimulation of heterotrophic growth) of molasses on *C. vulgaris*.

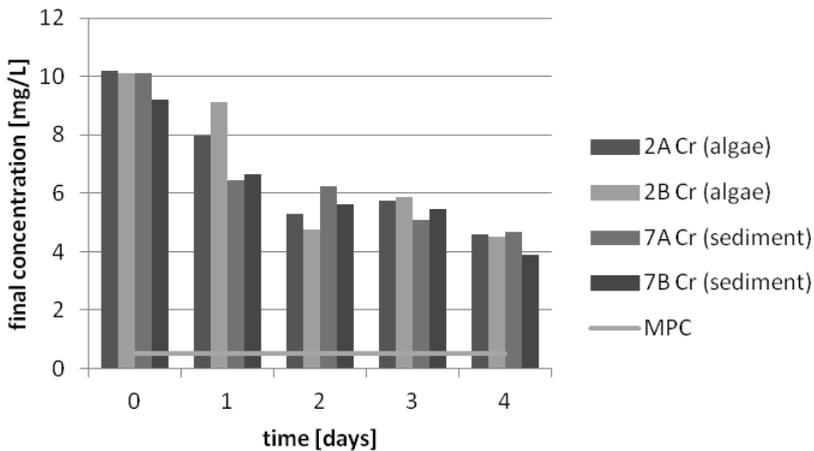
According to Polish standards, the maximum permissible concentration of chromium is 0.1 mg/L [12]. The highest uptake of chromium(VI) ions was observed in 4<sup>th</sup> day, but still above the maximum permissible concentration of chromium in wastewater.

The sorption proceeded gradually decreasing from 10 mg/L to 4.5 mg/L for samples 2A and 2B in wastewater with algae to 4.6 mg/L and 3.8 mg/L for samples 7A and 7B in wastewater with sediment (figure 6). Lowering concentration values of chromium(VI) ions in the sample 7A was due to the presence of clinoptilolite which could enhance the sorption of Cr(VI) ions.

### Nickel (II) ions removal

In wastewater containing nickel(II) ions decrease in pH was observed during the first 2 days, and in the next days of experiment it increased to pH = 7. Literature states that the optimum value of pH at

which a high uptake of nickel occurs is equal to  $\text{pH} = 5$ . It is an important factor that affects the final concentration of metal, therefore the uptake was not as effective [15]. In sample with molasses (8B) the decrease on the 4<sup>th</sup> day was observed because of not utilization of molasses, which acidified the wastewater (figure 7). However, molasses was better utilized by algae from wastewater containing nickel(II) ions than from wastewater containing chromium(VI) ions, zinc(II) and nickel(II) ions. It could be due to the stimulation by molasses the heterotrophic growth of *C. vulgaris*.

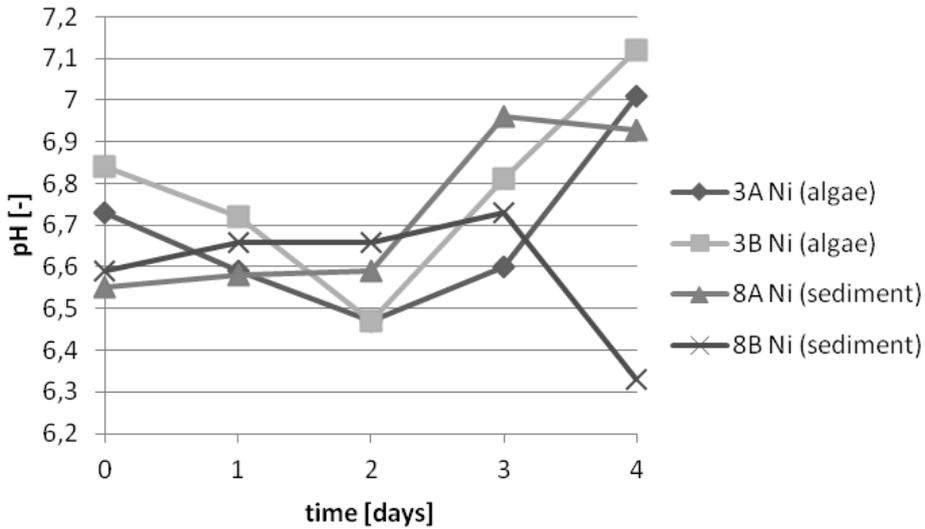


**Fig. 6.** The final concentration of Cr(VI) ions. MPC = maximum permissible concentration

**Rys. 6.** Stężenia końcowe jonów Cr(VI). MPC = maksymalne dopuszczalne stężenie

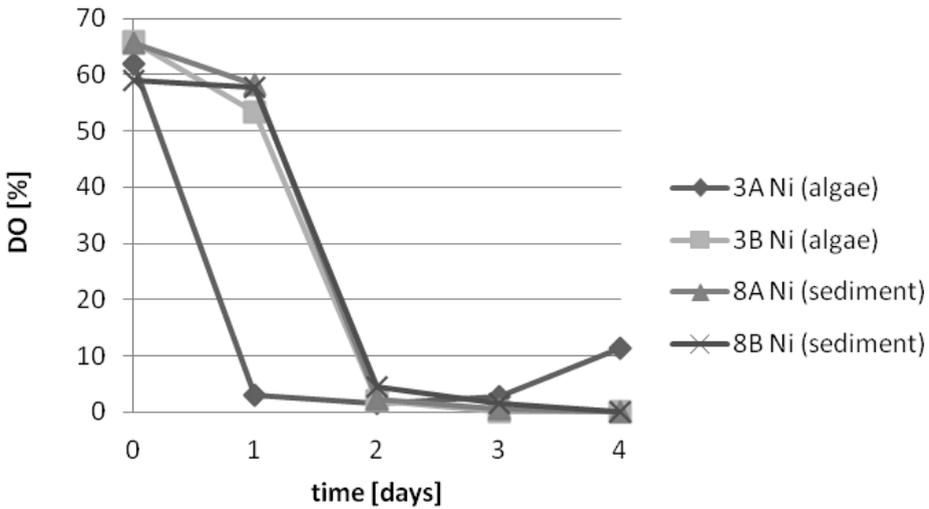
The DO remained at the level of 50–60% in the first day of experiment, however it decreased rapidly afterwards (figure 8).

In wastewater with sediment constant decrease of final concentration was noticed. This could be due to the nickel adsorption by clinoptilolite, which supported the absorption of this metal by algae. The final concentration changed from 10 mg/L to 2.32 mg/L for the 8A sample (figure 9).



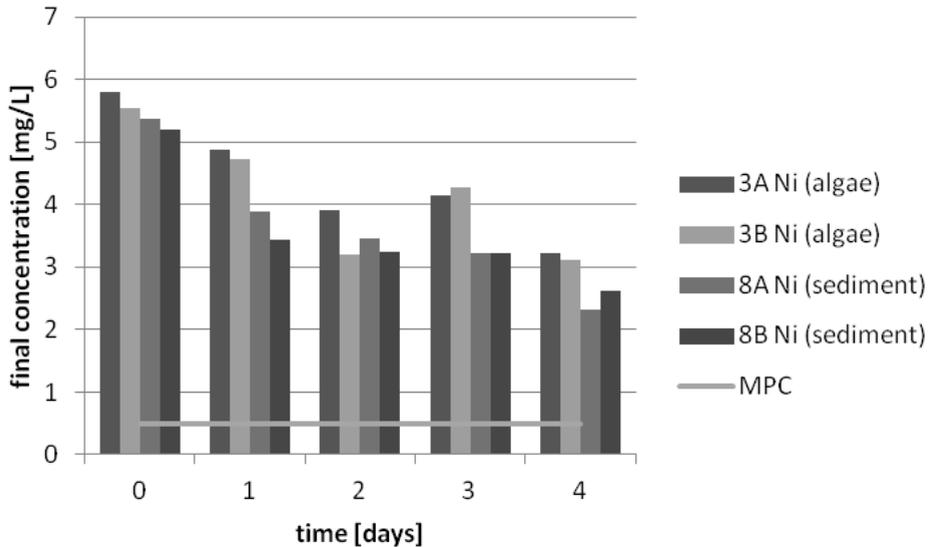
**Fig. 7.** pH values observed during the sorption of Ni(II) ions

**Rys. 7.** Wartości pH obserwowane w czasie sorpcji jonów Ni(II)



**Fig. 8.** Dissolved oxygen concentration observed during the sorption of Ni(II) ions

**Rys. 8.** Stężenie tlenu rozpuszczonego w czasie sorpcji jonów Ni(II) ions



**Fig. 9.** The final concentration of Ni(II) ions. MPC = maximum permissible concentration

**Rys. 9.** Stężenia końcowe jonów Ni(II). MPC = maksymalne dopuszczalne stężenie

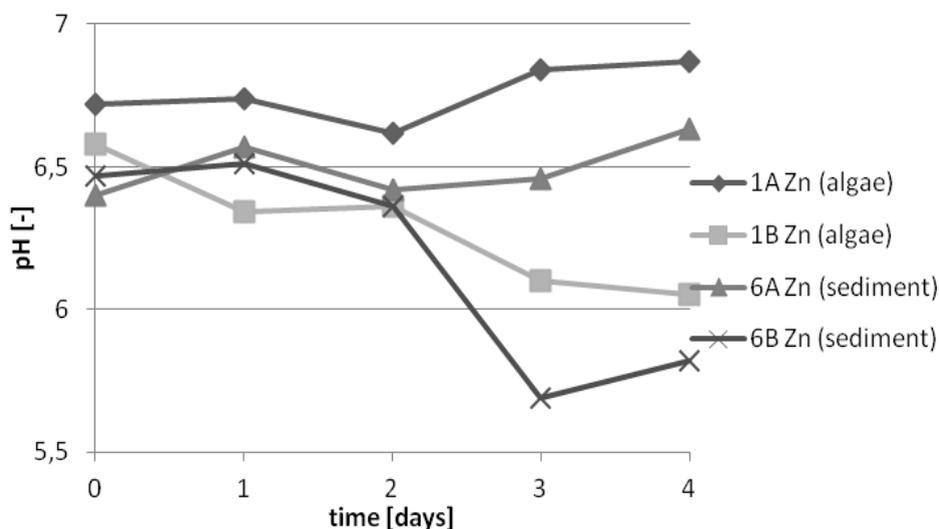
The maximum permissible concentration of nickel is 0.5 mg/L [12]. Nickel(II) ions were removed with the highest efficiency at 4<sup>th</sup> day but the final concentration of nickel(II) ions still remained above the maximum permissible concentration.

None of the samples achieved this level. It could be caused by environmental factors which are relevant for nickel(II) ions uptake effectiveness such as pH, exposure time, light, metal concentration. According to the literature, the percentage removal of nickel(II) ions increases with the increase of algae concentration [29].

The sorption of nickel(II) ions by sediment was more efficient than by algae. The stimulation of heterotrophic growth of *C. vulgaris* by molasses was observed. Utilization of molasses by algae enhanced removal efficiency of nickel(II) ions from wastewater with algae from the beginning till the end of the experiment and in wastewater with sediment after 3 days of contact time molasses didn't enhance the removal of nickel(II) ions.

## Zinc (II) ions removal

Figure 10 presents changes in pH values with time during removal of zinc(II) ions by *C. vulgaris* and sediment after biogens removal. For zinc(II) ions, (1A sample), during the first two days of the experiments, the pH remained at a level around  $pH \geq 6.7$ . The initial pH in 1B sample decreased from  $pH = 6.52$  to  $pH = 6.05$  and in sample with microalgae and clinoptilolite (6B samples) the pH was from  $pH = 6.47$  to  $pH = 5.82$ . Since 2<sup>nd</sup> till 4<sup>th</sup> day of the experiment pH decreased in samples with addition of molasses because of the decreasing in consumption of molasses because of the decreasing in photosynthesis as shown by the DO decrease (figure 11).

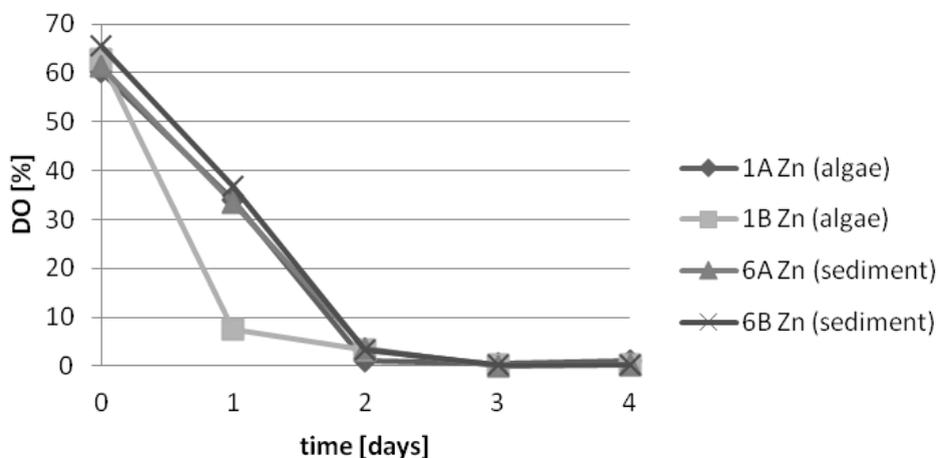


**Fig. 10.** pH values observed during the sorption of Zn(II) ions

**Rys. 10.** Wartości pH obserwowane w czasie sorpcji jonów Zn (II)

Figure 12 presents changes in concentration of zinc(II) ions with time. According to Polish standards, the maximum permissible concentration of zinc in wastewater is 2 mg/L [12]. After 24 h concentration of zinc(II) ions was significantly below the permissible concentration of zinc(II) ions in wastewater till the end of the experiment. After 24 of contact time, the concentration of zinc(II) ions in sample with algae raised which might imply that the dead algae released metal ions back into the

solution. The same trend was noticed for sample consisting of sediment. Despite the release of heavy metals by algae back into the solution, the content of metals in the last day of the experiment still remained at a level not exceeding the maximum permissible concentration of zinc(II) ions, except of 1B sample containing microalgae which was equal to 2.06 mg/L. The bioaccumulation of Zn(II) ions by microalgae vs. sediment was more efficient since 24 of contact time till the end of experiment. In samples with sediment molasses improved the removal of zinc(II) ions till 24h of contact time and within the next day the addition of molasses in these samples and in all samples with algae wasn't improve the removal efficiency of zinc(II) ions.

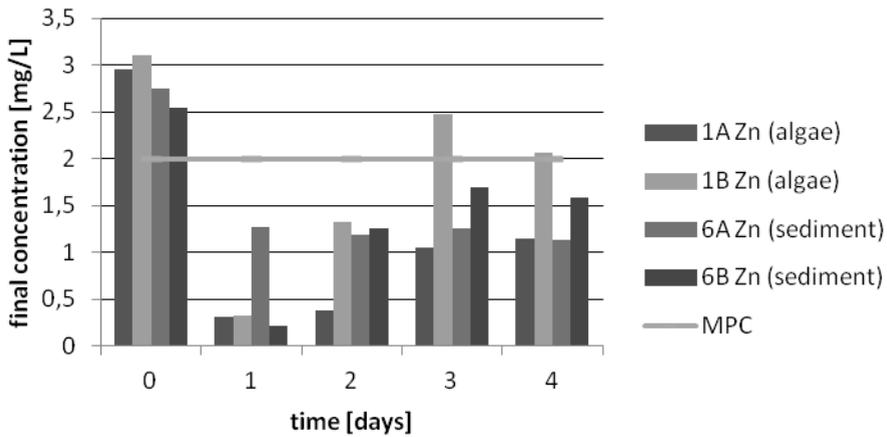


**Fig. 11.** Dissolved oxygen concentration observed during the sorption of Zn(II) ions

**Rys. 11.** Stężenie tlenu rozpuszczonego w czasie sorpcji jonów Zn(II)

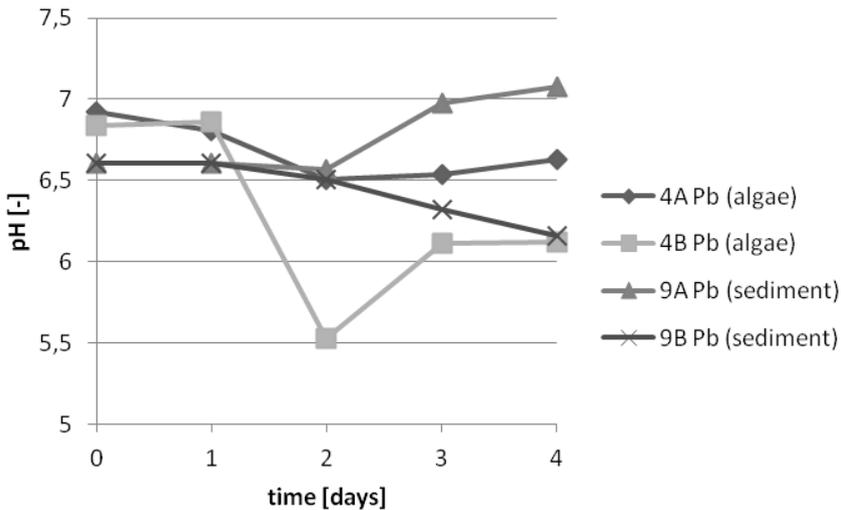
### Lead (II) ions removal

For lead(II) ions removal from wastewater, the pH value in wastewater with algae without molasses remained at the constant level or slightly increased (figure 13). In samples where molasses solution was added pH lowered, because algae was not able to feed on molasses and it start to acidify the wastewater.



**Fig. 12.** The final concentration of Zn(II). MPC = maximum permissible concentration

**Rys. 12.** Stężenia końcowe jonów Zn(II). MPC = maksymalne dopuszczalne stężenie



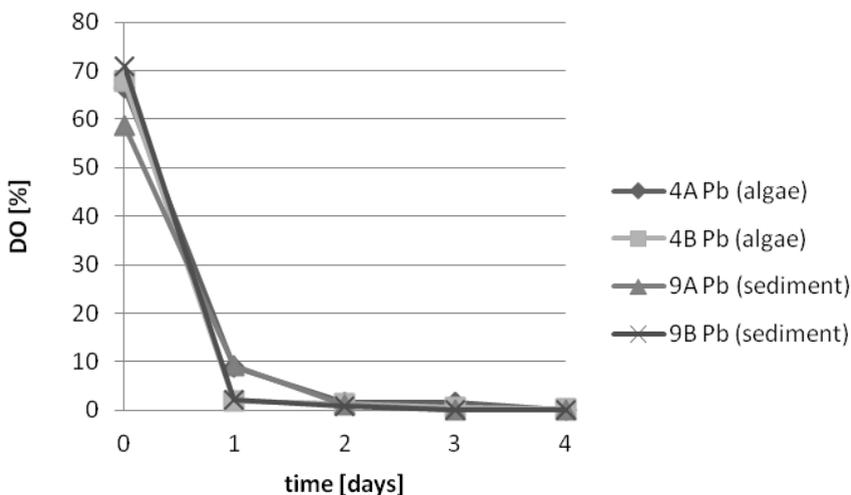
**Fig. 13.** pH values observed during the sorption of Pb(II) ions

**Rys. 13.** Wartości pH obserwowane w czasie sorpcji jonów Pb(II)

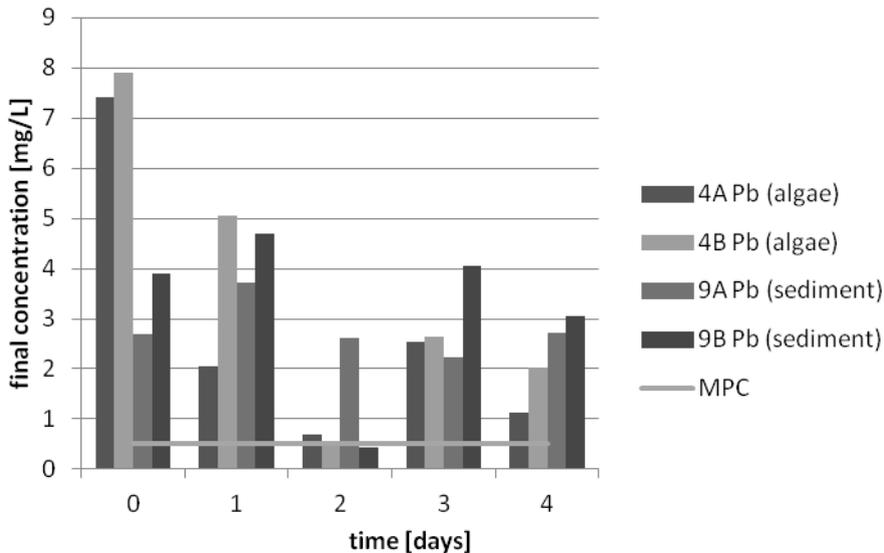
The highest uptake of lead(II) ions was noticed within the first 48 hours of the experiment. In next days the uptake considerably decreased. Like in the case of other metals, some of the metal ions were released

back into the solution since microalgal activity was lowering as shown by the decrease in the DO level in wastewater (figure 14). The algae stopped carrying out the process of photosynthesis and also oxygen production.

For the 4A and 4B samples in the first 48 hours of the experiment the concentration of metal decreased from 10 mg/L to 0.7 mg/L and 0.52 mg/L respectively. It accounted for 93% of uptake. Within the next day DO remained at very low levels close to zero, which indicates the algal cells death. A lot of metal ions were released back to the solution. The literature states that lead(II) ions might hydrolyze which issues in lead(II) ions deposition at high pH. According to the literature, the amount of lead(II) ions removed from sewage reached 81% [10]. Lead(II) ions removal depend also on biomass used for the experiment. Other studies provide highest concentration of lead(II) ions removal for by blue green algae which peaked at 98%. For green algae such as *Chlorella vulgaris* it reached over 80% [14]. According to the Polish standards the maximum permissible concentration for lead is 0.5 mg/L (figure 15) [12]. It was noticed that on the second day of the experiment the concentration fell in two cases slightly below the maximum permissible concentration limit. In the sample with algae (4B) it reached 0.5 mg/L and in wastewater with sediment it reached value of 0.44 mg/L.



**Fig. 14.** Dissolved oxygen concentration observed during the sorption of Pb(II) ions  
**Rys. 14.** Stężenie tlenu rozpuszczonego w czasie sorpcji jonów Pb(II)



**Fig.15.** The final concentration of Pb(II) ions. MPC = maximum permissible concentration

**Rys.15.** Stężenia końcowe jonów Pb(II). MPC = maksymalne dopuszczalne stężenie

The sorption of lead(II) ions by sediment was affected by molasses and the contact time. The utilization of molasses didn't enhance the removal efficiency of lead(II) ions in wastewater with algae as well as with sediment. Only in 2<sup>nd</sup> day the addition of molasses increased the removal efficiency of lead(II) ions.

### 3.3. Uptake of investigated heavy metals

The removal efficiency of investigated heavy metals is presented in Table 1. Based on the conducted results, chromium(VI) ions was sorbed with higher efficiency from the wastewater by algae and sediment with addition of molasses. The highest removal level of Cr(VI) and Ni(II) ions was of 61% (sediment+molasses) and 77% (sediment) on 4<sup>th</sup> day, respectively. The highest uptake of Zn(II) and Pb(II) ions was of 99% (sediment+molasses) on 1<sup>st</sup> day and 96% on 2<sup>nd</sup> day, respectively.

**Table 1.** Removal of Cr(VI), Zn(II), Ni(II) and Pb(II) ions by investigated sorbents  
**Tabela 1.** Usunięcie jonów Cr(VI), Zn(II), Ni(II) i Pb(II) przez sorbenty

Contact time, days	0	1	2	3	4
Uptake, %, Cr (VI)					
Algae	0	20	47	43	54
Algae + molasses	0	9	52	41	55
Sediment	0	35	37	49	53
Sediment + molasses	8	34	44	45	61
Uptake, %, Ni(II)					
Algae	42	51	61	59	68
Algae + molasses	45	53	69	57	69
Sediment	46	61	66	68	77
Sediment + molasses	48	66	68	68	74
Uptake, %, Zn(II)					
Algae	70	97	96	89	88
Algae + molasses	69	96	87	75	79
Sediment	73	87	88	87	89
Sediment + molasses	75	99	87	83	84
Uptake, %, Pb(II)					
Algae	26	79	93	75	89
Algae + molasses	21	49	95	74	80
Sediment	73	63	74	78	73
Sediment + molasses	61	53	96	59	70

#### 4. Conclusions

The results of investigation indicate that *C. vulgaris* is a good additive for ammonium nitrogen absorption in concentration of N-NH<sub>4</sub> from 100 to 1000 mg/L. The process was very quick and on the 1<sup>st</sup> day of experiment the final concentration of ammonium nitrogen reached values below the maximum permissible concentration of N-NH<sub>4</sub> in wastewater.

The obtained results indicate that clinoptilolite is also very good sorbent for N-NH<sub>4</sub> removal. Removal of 5000 mg/L N-NH<sub>4</sub> by *C. vulgaris* was slower than removal of lower concentrations of N-NH<sub>4</sub> but when clinoptilolite was added then the final concentration of ammonium nitrogen reached values below the maximum permissible concentration within 24h. High value of N-NH<sub>4</sub> (1000–5000 mg/L) slow down the process of photosynthesis.

The heterotrophic growth (night) was stimulated by the growth-enhancing effect of molasses on *C. vulgaris* followed by a period of autotrophic growth (day). The utilization of molasses influenced the removal of the investigated heavy metals during the first 2 days. The observed decrease of pH below the maximum permissible level in “B” samples after 2–3 days was due to the addition of molasses.

The results of investigation indicate that bioremoval of Cr (VI), Ni (II), Zn (II) and Pb(II) ions from synthetic wastewater by means of microalgae *C. vulgaris* and by the utilization of sediments after N-NH<sub>4</sub> removal is very promising technology. Wastewater treatment by clinoptilolite could reduce application of chemicals for precipitation.

The optimum pH conditions as well as the adequate time of the contact and adequate dosage of biosorbents are essential to make the process most effective. The sorption of chromium(VI) ions by sediment was more efficient than by algae after six hours, at 1<sup>st</sup> and 3<sup>rd</sup> day of contact time. The sorption of nickel(II) ions by sediment was more efficient than by algae during all contact time. The biosorption of zinc(II) ions by microalgae vs. sediment was more efficient since 24 of contact time till the end of experiment. The sorption of lead(II) ions by sediment was affected by molasses and contact time.

The obtained results indicate that the removal of heavy metals such as Zn(II), Cr(VI), Ni(II) and Pb(II) ions by microalgae *C. vulgaris* has a great potential, although the Cr(II) ions were removed with lower efficiency. The uptake of investigated heavy metals was influenced by type of metal, type of sorbent and contact time. The highest removal level of Cr(VI) and Ni(II) ions was of 61% (sediment+molasses) and 77% (sediment) on 4<sup>th</sup> day, respectively. The highest uptake of Zn(II) and Pb(II) ions was of 99% (sediment+molasses) on 1<sup>st</sup> day and 96% on 2<sup>nd</sup> day, respectively.

*This scientific work was supported by the BS/PB-401-301/11.*

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## Wykorzystanie *C. vulgaris* oraz osadów zawierających klinoptylolit po usuwaniu N-NH<sub>4</sub> do wychwytu metali ciężkich ze ścieków

### Streszczenie

Ścieki przemysłowe zawierają wysokie stężenia zanieczyszczeń nieorganicznych. Skład ścieków przemysłowych jest uzależniony od rodzaju przemysłu. Dużą uciążliwość stanowią zanieczyszczenia azotem amonowym oraz metalami ciężkimi. Przemysł koksowniczy wytwarza ścieki o znacznych zawartościach azotu amonowego, a ścieki pochodzące z ocynkowni, hut cynku i ołowiu i przemysłu przeróbki metali zawierają duże stężenia metali ciężkich, jak np. chrom, nikiel, cynk czy ołów. Metale te zostały wyszczególnione w Rozporządzeniu Ministra Środowiska dot. substancji szczególnie szkodliwych dla środowiska wodnego (Dz.U. Nr 229, poz. 1538).

Obecnie stosowane technologie do usuwania azotu amonowego ze ścieków wymagają znacznego zużycia energii i dużych nakładów finansowych. Najpowszechniej stosowanymi metodami usuwania jonów metali ciężkich są metody strącaniowe. Stosowanie ich, wiąże się jednak z wieloma trudnościami technologicznymi, takimi jak zagospodarowanie powstających osadów. Ekologicznym sposobem oczyszczania ścieków z tych substancji może być wykorzystanie glonów. Zastosowanie glonów do usuwania azotu amonowego nie wymaga stosowania dodatkowych substancji chemicznych, ponieważ związki te glony asymilują na potrzeby wytwarzania biomasy. Wyhodowana biomasa może następnie zostać wykorzystana do usuwania metali ciężkich w procesie bioakumulacji.

Zawartość substancji powodujący zabarwienie ścieków zmniejsza przenikalność światła koniecznego do prawidłowego wzrostu glonów. Niektóre gatunki glonów charakteryzuje zdolność przestawiania sposobu odżywiania z autotroficznego na heterotroficzny. Glony, takie jak *Chlorella vulgaris* mają zdolność do wykorzystywania węgla organicznego w fazie ciemnej, co jest korzystne ze względu na możliwość prowadzenie hodowli w ściekach o znacznym zabarwieniu.

Zatrzymywanie jonów metali ciężkich na zeolitach jest przedmiotem wielu badań. Klinoptylolit jest najpowszechniej występującym zeolitem, wykazującym zdolność do usuwania azotu amonowego oraz wychwytu kationów wielu metali ciężkich.

W pracy po raz pierwszy zaproponowano wykorzystanie, po wzbogaceniu klinoptylolitem, osadu biomasy glonów z gatunku *C. vulgaris* hodowanej w ściekach zawierających azot amonowy do usuwania metali ciężkich w procesie bioakumulacji.

Badania prowadzono w dwóch etapach. Celem pierwszego etapu badań była ocena możliwości wykorzystania glonów z gatunku *C. vulgaris* do usuwania N-NH<sub>4</sub> ze ścieków syntetycznych w procesie asymilacji azotu amonowego. Klinoptylolit był dodawany do próbek, w których nie osiągnięto założonego poziomu oczyszczenia ścieków. W drugim etapie wykorzystano glony oraz osady z pierwszego etapu badań do usuwania ze ścieków metali ciężkich w procesie bioakumulacji przez glony i sorpcji przez klinoptylolit. Proces ten pozwolił na recykling osadu pozostałego po usuwaniu azotu amonowego. Zarówno w pierwszym jak i w drugim etapie badań do połowy próbek dodawano melasę w celu stymulowania wzrostu heterotroficznego *C. vulgaris*.

Na podstawie uzyskanych wyników badań stwierdzono, iż *C. vulgaris* efektywnie asymilowała N-NH<sub>4</sub> już w pierwszym dniu badań w ściekach zanieczyszczonych N-NH<sub>4</sub> w zakresie stężeń od 100 do 1000 mg/l. Przy stężeniu 5000 mg/l asymilacja przebiegała wolniej, lecz po dodaniu klinoptylolitu w ciągu 24h nastąpiło usunięcie N-NH<sub>4</sub> do poziomu dopuszczalnego dla ścieków wprowadzanych do wód lub do ziemi (Dz.U. 2009 nr 27 poz. 169). W drugim etapie badań stwierdzono, iż usuwanie jonów cynku, ołowiu, niklu oraz chromu przy wykorzystaniu glonów oraz osadów z pierwszego etapu było uzależnione od rodzaju metalu, rodzaju sorbentu i czasu ekspozycji. Uzyskano następujące najwyższe usunięcia jonów metali, wyrażone w procentach: Cr(VI) – 61% dla mieszanki: osad + melasa w 4 dniu badań, Ni(II) – 77% dla osadu w 4 dniu badań, Zn(II) – 99% dla mieszanki: osad + melasa w 1 dniu badań i Pb(II) – 96% dla mieszanki: osad + melasa w 2 dniu badań.