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An Innovative Look into Ammonium Nitrogen Removal Using Algae and Zeolites as an Element of a Circular Bioeconomy

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Featured Application: The obtained research results can be used to support the removal of ammonium nitrogen from wastewater in a microalgae culture. Sewage sludge containing natural clinoptilolite and microalgae formed after the assimilation of ammonium nitrogen can be further used, e.g., for the reuse of alginate and wastewater. This new method brings benefits to the environment, the economy, and society.

Abstract: This work focused on the potential of simultaneously removing ammonium nitrogen from industrial wastewater using immobilized microalgae and powdered zeolite. Experiments were performed with different species and doses of microalgae embedded in spherical hydrogels in semicontinuous conditions. Ammonium nitrogen uptake by microalgae promoted the slow release of previously adsorbed ammonium nitrogen from zeolite that was then also absorbed by microalgae. Results showed that immobilized microalgae can reach a removal efficiency of up to 60% (*C. vulgaris*) and 42% (*S. armatus*). A higher removal efficiency was obtained for zeolites and immobilized *C. vulgaris* or *S. armatus* up to 86% and 79%, respectively. Moreover, a higher maximum sorption capacity for *C. vulgaris* (13.8 mg/g) was achieved than for *S. armatus* (5.5 mg/g). The recycling of spent hydrogel, zeolite, and wastewater is possible. Such an approach represents a circular bioeconomy loop.

Keywords: ammonium nitrogen removal; simultaneous sorption; immobilized microalgae; hydrogel beads; alginate; circular bioeconomy

Citation: Zabochnicka, M. An Innovative Look into Ammonium Nitrogen Removal Using Algae and Zeolites as an Element of a Circular Bioeconomy. *Appl. Sci.* **2023**, *13*, 10220. https://doi.org/10.3390/app131810220

Academic Editors: Chang Gyun Kim, Okkyung Choi, Kinjal J. Shah and Hyunook Kim

Received: 11 May 2023
Revised: 17 August 2023
Accepted: 5 September 2023
Published: 12 September 2023

1. Introduction

Wastewaters contains many different contaminants that can even be found in sludges [1]. Therefore, contaminants that are present in large amounts in wastewater should be effectively removed. In wastewater, nitrogen is usually present as organic nitrogen, ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen [2]. Nitrogen compounds in wastewater are hazardous to water bodies and human health. The discharging of nitrogen compounds directly into rivers, canals, and lakes can cause eutrophication of them [3]. High concentrations of ammonium nitrogen are toxic to fish and plants, and to phyto- and zooplankton.

Methods for ammonium nitrogen removal from wastewater are usually unit processes, but there are no data on the removal of ammonium nitrogen from industrial wastewater in integrated processes that are both effective and environmentally friendly. There is a need to develop an innovative method for ammonium nitrogen removal in order to meet the current wastewater treatment regimes, which are more and more restrictive.

The present work aims at the development of an innovative approach to ammonium nitrogen removal from industrial wastewater. Microalgae such as *Chlorella vulgaris* or *Scenedesmus armatus* and zeolite were used. Microalgae can be cultivated in open ponds or closed systems in fresh or marine water [4]. *Chlorella vulgaris* and *Scenedesmus armatus* are very fast-growing microalgae and have a high potential to remove pollutants from the contaminated environment. During the growth of microalgae, CO_2 is absorbed from the air or from industrial sources [5].

Microalgae could be immobilized by many techniques and agents that can form hydrogel beads. According to the literature, hydrogel beads could be utilized to immobilize different microorganisms used for wastewater treatment. Chen et al., 2022 immobilized *Bacillus subtilis* on polyethylene glycol (PEG)-modified polyvinyl alcohol (PVA)/sodium alginate (SA) hydrogel microspheres to treat starch wastewater [6]. An amplification efficiency of the wastewater treatment during the anammox process during utilization of immobilized microorganisms in polyvinyl alcohol/sodium alginate was achieved [3].

Green adsorbents such as hydrogel-bead biomaterials possess functional groups that can act as binding sites for the uptake or removal of many ions from aqueous solutions [7]. For example, alginate gel possesses carboxyl groups, and polyvinyl alcohol has hydroxyl group as binding sites. In addition, the micropores in hydrogel can help the exchange of the substrates between solid and liquid phases [3].

Immobilized algae are easier to remove after wastewater treatment [8]. Moreover, the utilization of immobilized microalgae can help to improve the tolerance of embedded microalgae to the strong environment in industrial wastewater. Thirdly, alginate gel is characterized by its non-toxicity, high porosity, transparency, and mild physical environment for the cells [9].

Zeolites are highly porous natural minerals that can remove various ions from contaminated environments [10,11]. Zeolite is a low-cost sorbent for, e.g., water and wastewater treatment [12–14].

Nowadays, the emphasis is on employing environmental methods that fulfil zero-carbon circular bioeconomy strategies. Microalgae can help to fulfil such aims because during their growth, they absorb carbon dioxide [15]. Microalgae can grow and assimilate ammonium nitrogen during photosynthesis or biosorption. Clinoptilolite can remove ammonium nitrogen by means of adsorption and ion-exchange processes. Natural sorbents such as microalgae and zeolite are environmentally friendly agents in industrial wastewater treatment because they can help to reduce the utilization of chemicals. This also brings benefits to the economy and society. This is directly aligned with the principles of the circular bioeconomy.

Several research articles have been published on algal or zeolite wastewater treatment. However, there are no reports on simultaneous sorption and biosorption of ammonium nitrogen from industrial wastewater in alignment with a circular bioeconomy loop. The present study is highly innovative and aims to develop an integrated method for the removal of ammonium nitrogen from industrial wastewater by using immobilized microalgae and zeolite, and their mixtures, in biological–physical–chemical simultaneous adsorption and biosorption processes. To achieve that aim, an assessment of the effectiveness of ammonium nitrogen removal using the selected sorbents in dynamic conditions and the selection of the appropriate dose, type, and form of the sorbents was carried out. Hydrogel beads were produced to immobilize microalgae. Such an approach represents an entirely new method for wastewater treatment.

2. Materials and Methods

2.1. Sorbent Characterization

Chlorella vulgaris (Cv) and *Scenedesmus armatus* (Sc) strains were used. Algae were cultivated in 250 mL flasks in a climatic chamber. An irradiance of 80 $\pm$ 5 µmol photons m^2/s was performed, with a light/dark period of 12/12 h and at a temperature of 29 $\pm$ 0.5 °C during the light phase and 25 $\pm$ 0.5 °C for dark phase. Algae were harvested in the exponential growth phase.

C. vulgaris and *S. armatus* were obtained from the Culture Collection of Baltic Algae (CCBA), Poland. Microalgae were used suspended in solution and embedded in hydrogel beads. The hydrogel was made of alginate. The sorbents were mixed with alginate to produce small granulate particles of 3 mm diameter. Hydrogels are three-dimensional colloidal systems. They are composed of associated linear or branched polymer chains [16].

Sorption tests were carried out using a mineral sorbent—zeolite (Z). The granulation of zeolite was 0–0.2 mm. Due to the fact that 84% of this zeolite was clinoptilolite, this sorbent was commonly referred to as clinoptilolite. Other minerals in zeolite are as follow: cristobalite—7%, plagiclase—3–4%, loam—4%, others—0.1–0.3%, and quartz < 0.1%. Clinoptilolite was dosed into the wastewater in the form of a powder. A specific surface area of zeolite reached 76 m^2/g.

2.2. Wastewater Characterization

Coke plant wastewater is produced during the process of coal coking and obtaining coal derivatives. Coke wastewater with ammonium nitrogen concentrations of 386 mg $N\text{-}NH_4^+$/L was used. The wastewater was characterized as follows: pH—8.3; dissolved oxygen (DO)—4.2 mg/L; $N\text{-}NO_3$—3.8 mg/L; and Kjeldahl nitrogen—629.0 mg/L; $P\text{-}PO_4^{3-}$—4.0 mg/L; chemical oxygen demand (COD)—552 mg/L; TOC—293 mg/L; biological oxygen demand (BOD_5)—221 mg/L; phenol index—322 mg/L, and volatile fatty acids (VFA)—158 mg/L.

2.3. Sorption Tests

Dynamic (semicontinuous) conditions for wastewater treatment were applied. Air lift columns of 0.6 L capacity were used. An amount of 0.5 L of the reaction volume was applied. The wastewater was diluted by 1:3, and every 10 days, 0.15 L of wastewater was replaced by fresh wastewater. After each replacement of a portion of wastewater, it was necessary to adjust the pH value to 7 in order to maintain optimal conditions for the removal of ammonium nitrogen (at pH~7, the form of ammonium nitrogen is >95%) and to compensate for drops in pH value caused by aeration and photosynthesis processes. The pH was corrected by 1 mol/L HCl or NaOH, according to the literature [17]. The deficiency in the supply of phosphorus was compensated by the use of KH_2PO_4 to achieve a ratio of N:P = 16:1.

All of the sorbents were added to the wastewater suspended (B samples) in the solution or immobilized on hydrogel beads (A samples). The initial sorbent concentrations were as follow: 100 g/L (10CvA, 10CvB samples) and 50 g/L (5CvA, 5CvB samples) of *C. vulgaris* culture; 100 g/L (10ScA, 10ScB samples) and 50 g/L (5ScA, 5ScB samples) of *S. armatus* culture; 100 g/L and 50 g/L (10Z, 5Z samples) of zeolites; 50 g/L of *C. vulgaris* culture with 50 g/L of zeolites (5Cv5ZA, 5Cv5ZB); and 50 g/L of *S. armatus* culture and 50 g/L of zeolites (5Sc5ZA, 5SC5ZB). K—wastewater without sorbents—was a control sample.

Air pumps with a flow of 250 L/h were used to supply air for 6 h/d. The experiment lasted 40 days, based on the previous investigations.

2.4. Analytical Methods

The pH was determined using a pH meter. $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ contents were determined using spectrophotometric methods. The $N\text{-}NH_4^+$ and Kjeldahl nitrogen content were checked by performing titration (Büchi K-355). The total organic carbon (TOC) was measured with a Multi N/C 2100. BOD_5 was determined using the manometric method. Volatile fatty acids, the phenol index, and COD was determined using spectrophotometric methods with a HACH DR4000. Dissolved oxygen was measured using an oxygen meter with a CO-01 Elmetron. The specific surface area (SBET) of zeolite was determined by using a micromeritics accelerated surface area and porosimetry analyzer system with an ASAP 2020, Micromeritics, Atlanta, GA, USA.

The measurements were conducted in triplicate. All data are presented as arithmetical means. The relative standard deviation (RSD) was between 5 and 10%.

The removal efficiency (η) and maximum removal efficiency (η_{max}) of pollutants, expressed as a percentage, were calculated using Equation (1):

$$\eta = \frac{C_i - C_f}{C_i} 100\% \tag{1}$$

where η i η_{max} refer to the removal efficiency (%) and C_i i C_f are the initial and final concentrations of contaminants (mg/L). The sorption capacity of the sorbents (q_{max}) was calculated using Equation (2):

$$q = \frac{(C_i - C_f)}{m} V \tag{2}$$

where q is the sorption capacity (mg/g), C_i i C_f refer to the initial and final concentrations of contaminants (mg/L), V is the sample volume (L), and m is the mass of sorbent (g). The ammonium nitrogen removal rate (η_N) was calculated according to Equation (3):

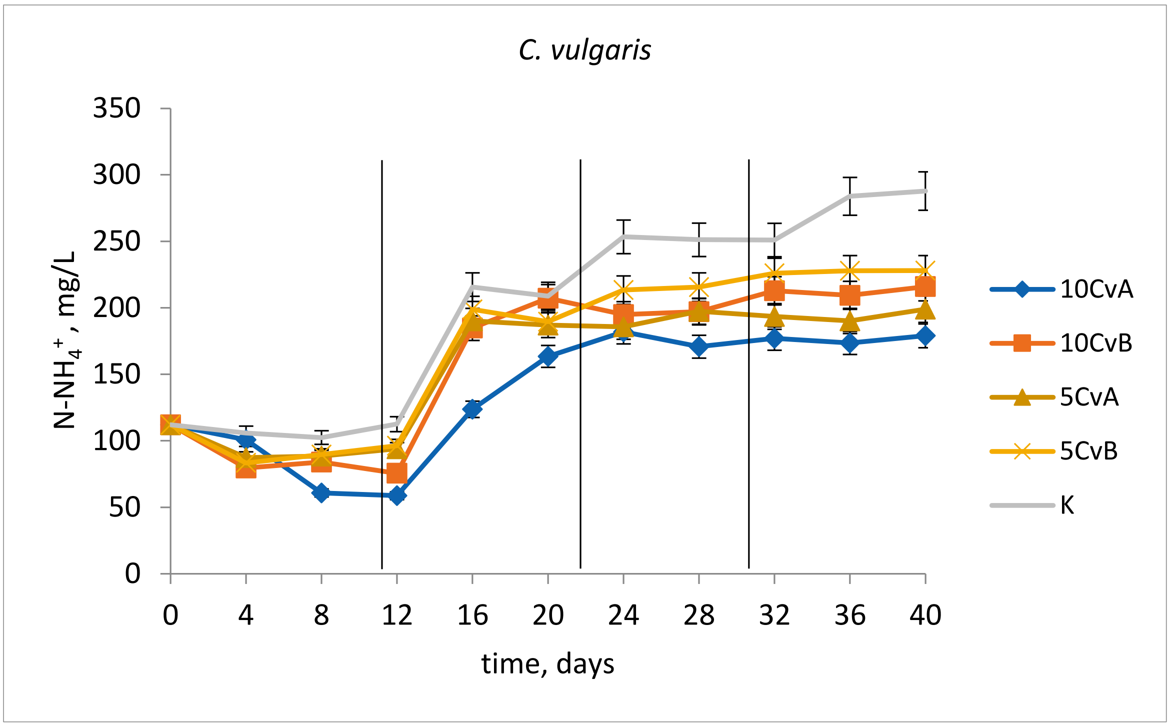
$$\eta_N = \frac{C_i - C_f}{t} \tag{3}$$

where η_N is the removal rate (mg/L d^{-1}), C_i i C_f refer to the initial and final concentrations of contaminants (mg/L), and *t* is the time in days.

3. Results and Discussion

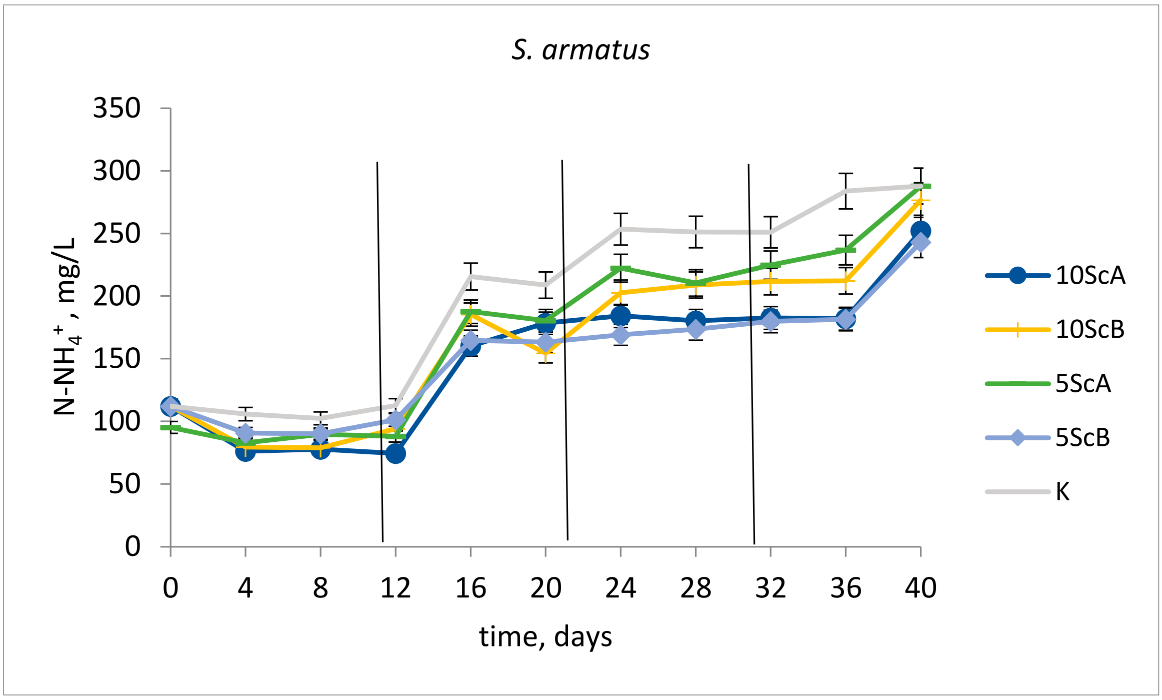
3.1. N-NH_4^+ Removal from Coke Wastewater

The concentrations of ammonium nitrogen in coke wastewater with the use of the sorbents *C. vulgaris*, *S. armatus*, and zeolite, and their mixtures, in dynamic conditions are shown in Figure 1.

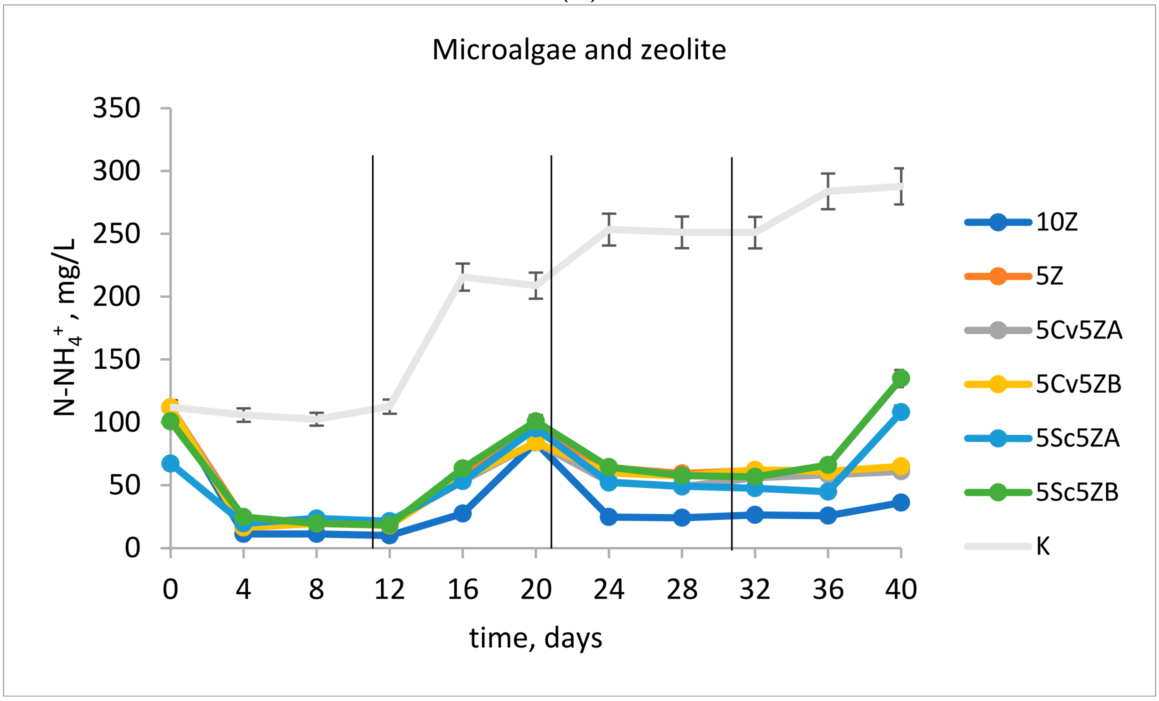


(A)

Figure 1. *Cont.*



(**B**)



(**C**)

Figure 1. Concentration of nitrogen ammonium observed in wastewater: (**A**)—*C. vulgaris*, (**B**)—*S. armatus*, (**C**)—microalgae and zeolite.

The results show that for the first 10 days, the presence of all sorption materials had an impact on the lowering of the N-NH_4^+ concentration. A high efficiency of N-NH_4^+ removal was found from the fourth to the twelfth day in wastewater to which a mixture of microalgae and clinoptilolite had been added (Figure 1C), regardless of whether the microalgae were in suspension (samples marked "B"—samples 5Cv5ZB and 5Sc5ZB) or immobilized on hydrogel beads (samples marked "A"—samples 5Cv5ZA and 5Sc5ZA). At that time, the concentration of ammonium nitrogen in these samples did not exceed 20 mg

$N\text{-}NH_4^+/L$. A high efficiency was also obtained in the sample to which only clinoptilolite had been added at a dose of 100 g/L (sample 10Z); the content of ammonium nitrogen did not exceed 11 mg $N\text{-}NH_4^+/L$. When clinoptilolite was used to remove ammonium nitrogen at a dose of 50 g/L (sample 5Z), the content of ammonium nitrogen did not exceed 22 mg $N\text{-}NH_4^+/L$.

This result is in agreement with some previous studies. It was reported that zeolite is a good material for the adsorption of ammonium from wastewater. Lu et al. (2019) indicated that zeolite used as a pretreatment can mitigate ammonium toxicity for algae.The addition of zeolite can improve the recovery efficiency of ammonium in wastewater. After the adsorption of ammonium by zeolite, the process of slowly releasing ammonium back to the wastewater starts [18,19]. Lu et al. (2019) indicated that such ammonium released by zeolite could be utilized by *Spirulina*. It is worth mentioning that *Spirulina* is a bacterium and not a plant like *Chlorella* sp., and therefore, there are different mechanisms for the growth and assimilation of ammonium by these two strains. Moreover, the innovative approach in the present study is that zeolite and microalgae were both utilized simultaneously at the same time for industrial wastewater treatment.

Lu et al. (2016) [20] also found that the tolerance level of ammonium for *Spirulina* was very low, approximately 127.5 mg $NH_4^+\text{-}N/L$. However, some researchers found that the maximum tolerance level for $NH_4^+\text{-}N$ for *Spirulina* is 100 mg/L, while others stated that *Spirulina* can grow in wastewater with a level of $NH_4^+\text{-}N$ above 100 mg/L. Otherwise, *Chlorella* sp. could grow in wastewater with 600 mg $N\text{-}NH_4^+/L$ [19]. The low level of $NH_4^+\text{-}N$ is insufficient for *Chlorella* sp. growth. Lu et al. (2016) indicated that the limiting factor for algae growth on dairy wastewater was the ammonia nitrogen deficiency. Nitrogen is needed for the synthesis of proteins, which is essential to algae replication. The researchers found that the initial concentrations of $NH_4^-\text{-}N$ (4.96–21.45 mg/L) in diluted dairy wastewaters were extremely low, and there was a need to add nitrogen to increase the microalgal growth [20]. Zheng et al. (2019) also reported that high concentrations of ammonia could be toxic for microalgae in high-strength ammonium wastewaters like manure-free piggery. The researchers found that the removal efficiency of $NH_4^+\text{-}N$, as consumed by *C. vulgaris*, for 27.5, 55, and 110 mg $N\text{-}NH_4^+/L$ was 100%. When the initial concentration of $NH_4^+\text{-}N$ reached 220 mg/L, the removal efficiency was then only 50%. The researchers indicated that such an ammonium concentration inhibited *C. vulgaris* growth, and as a result, limited removal efficiency from piggery wastewater was observed [21].

From the fourth to the twelfth day of the study, in wastewater inoculated with microalgae (without clinoptilolite) (Figure 1A,B) at a dose of 100 and 50 g/L (samples: 10CvA/10ScA, 10CvB/10ScB, 5CvA/5ScA, and 5CvB/5ScB), regardless of the form of their occurrence, the concentrations of ammonium nitrogen varied within a range of 59–100, 76–101, 87–101, and 83–101 mg $N\text{-}NH_4^+/L$, respectively. At that time, the concentration of ammonium nitrogen in the control samples was 102–112 mg $N\text{-}NH_4^+/L$.

The first portion of wastewater was replaced after ten days of testing. The microalgae without clinoptilolite (Figure 1A,B) needed few days to adapt to the new environment with a higher concentration of contaminants each time after the addition of the new portion of fresh wastewater. During that time, the concentration of ammonium nitrogen remained at a high level for both microalgal strains because no assimilation processes occurred. On the contrary, it was found that in the case of a mixture of microalgae with clinoptilolite and in wastewater with clinoptilolite (without microalgae, Figure 1C), the concentration of $N\text{-}NH_4^+$ in wastewater was below 63 mg $N\text{-}NH_4^+/L$. Clinoptilolite had an impact on the lowering of the concentration of nitrogen ammonium in those samples. Concentrations of clinoptilolite stayed the same during the experiment, and clinoptilolite sorbed $N\text{-}NH_4^+$ by means of fast physicochemical reactions. The algae needed more time to assimilate $N\text{-}NH_4^+$ during their growth.

At that time, the concentration of ammonium nitrogen in the control samples was 112–215 mg $N\text{-}NH_4^+/L$.

The next (second) portion of wastewater was replaced after 20 days, and thereafter, the concentration of ammonium nitrogen only in samples with a mixture of microalgae and clinoptilolite dropped below 65 mg $N\text{-}NH_4^+/L$ (Figure 1C), and in wastewater with clinoptilolite, the concentration of ammonium nitrogen did not exceed 64 mg $N\text{-}NH_4^+/L$.

In wastewater samples with immobilized microalgae (Figure 1A), the concentration of ammonium nitrogen did not exceed 197 mg $N\text{-}NH_4^+/L$ for *C. vulgaris* and 222 mg $N\text{-}NH_4^+/L$ for *S. armatus* when the dose of both microalgae strains was 50 g/L, and it did not exceed 182 mg $N\text{-}NH_4^+/L$ for *C. vulgaris* and 184 mg $N\text{-}NH_4^+/L$ for *S. armatus* in the sample where the content of microalgae was 100 g/L. At that time, the least effective removal of ammonium nitrogen using microalgae in the form of suspension was at the level of 195–215 mg $N\text{-}NH_4^+/L$. At that time, the concentration of ammonium nitrogen in the control samples was 251–253 mg $N\text{-}NH_4^+/L$.

The third portion of wastewater was replaced after 30 days. A concentration of ammonium nitrogen below the value of 65 was observed only in wastewater samples with the addition of a mixture of *C. vulgaris* and clinoptilolite, and with the addition of clinoptilolite (Figure 1C). The mixture of *S. armatus* and zeolite facilitates a concentration of ammonium nitrogen below 135 mg $N\text{-}NH_4^+/L$. In wastewater samples with the addition of microalgae immobilized in both doses (50 and 100 g/L) on the carrier, the concentration of ammonium nitrogen did not exceed 199 mg $N\text{-}NH_4^+/L$. At that time, the concentration of ammonium nitrogen in wastewater samples containing microalgae in suspension was below 228 mg $N\text{-}NH_4^+/L$ for *C. vulgaris* and 276 for *S. armatus* mg $N\text{-}NH_4^+/L$ (Figure 1A,B). At that time, the concentration of ammonium nitrogen in the control samples was 284–288 mg $N\text{-}NH_4^+/L$.

Microalgae can act as a pollutant sorbent only after the end of their period of adaptation to new environmental conditions. This time is longer the more polluted the environment is. In all the samples containing microalgae, the ammonium nitrogen removal efficiency was lower than in the samples containing a mixture of microalgae with clinoptilolite, and with the addition of clinoptilolite. The observed lower efficiency of $N\text{-}NH_4^+$ removal from wastewater using microalgae was caused by the increasing concentration of pollutants after adding successive portions of wastewater and, as a result, causing microalgae stress. This could be caused by an intracellular oxidative stress [22,23].

The concentration of nitrogen in the form of $N\text{-}NO_3^-$ in raw wastewater was low and amounted to 3.7 mg $N\text{-}NO_3^-/L$. In all the tested samples of treated wastewater, the concentrations of nitrate nitrogen (V) and nitrates (III) were below the level of determination (<0.5 mg $N\text{-}NO_3^-/L$).

3.2. Other Indicators of Wastewater Treatment

Microalgae had an impact on pH value fluctuations due to metabolic processes. Over time, in all samples, the pH values of the wastewater decreased from the initial values to the final values of pH = 6.6–7.7. These results are in agreement with some previous studies. It was reported that low pH (5.0) as well as high pH (9.0) strengthened the ammonia toxicity of *C. vulgaris* [21]. The researchers found that the maximum biomass concentration and the highest cell viability for cell growth was pH = 7.0. Lu et al. (2019) also indicated that during their studies, the ammonium removal efficiency reached peak value at pH = 8.0. When the pH of wastewater was lower (<6.5) or higher (9.5), the ammonium removal efficiency decreased [19].

On three occasions, the replacement of a portion of wastewater with raw wastewater had an impact on the fluctuations of the pH value of the wastewater, and temporary increases and decreases in pH value were observed.

In the samples of treated wastewater, the concentration of oxygen dissolved during the tests was monitored. The observed dissolved oxygen concentrations were a reflection of the ongoing photosynthesis process. A decrease in the content of dissolved oxygen in the wastewater was noted, from 9.3 mg/L to 11.8 mg/L to 4.8–5.8 mg/L. This was because the adaptation processes of the microalgae to the new environment (wastewater) occurred. The increase in the concentration of DO observed in the following days indicated the beginning of the active phase of assimilation of ammonium nitrogen.

From the 30th day, after replacing the last portion of wastewater, the concentration of dissolved oxygen began to decrease in all samples and reached its lowest values in the range of 3.4–6.2 mg/L whichwas unfavorable for microalgae culture and N-NH_4^+ binding.

In the control sample (K), the trends in changes in the concentration of dissolved oxygen in the wastewater to the 28th day were also observed. The concentration of dissolved oxygen varied from 8.8 mg/L on the first day to 6.3 mg/L on the 28th day. Then, a decrease in dissolved oxygen concentration to 1.5 mg/L was observed, which was determined after 40 days. In the remaining samples (with the addition of sorbents), the concentration of dissolved oxygen in the treated wastewater was higher than in the control sample, and at the end, it was 3.4–6.4 mg/L.

In wastewater samples treated with sorbents, the concentration of volatile fatty acids was determined (Figure 2).

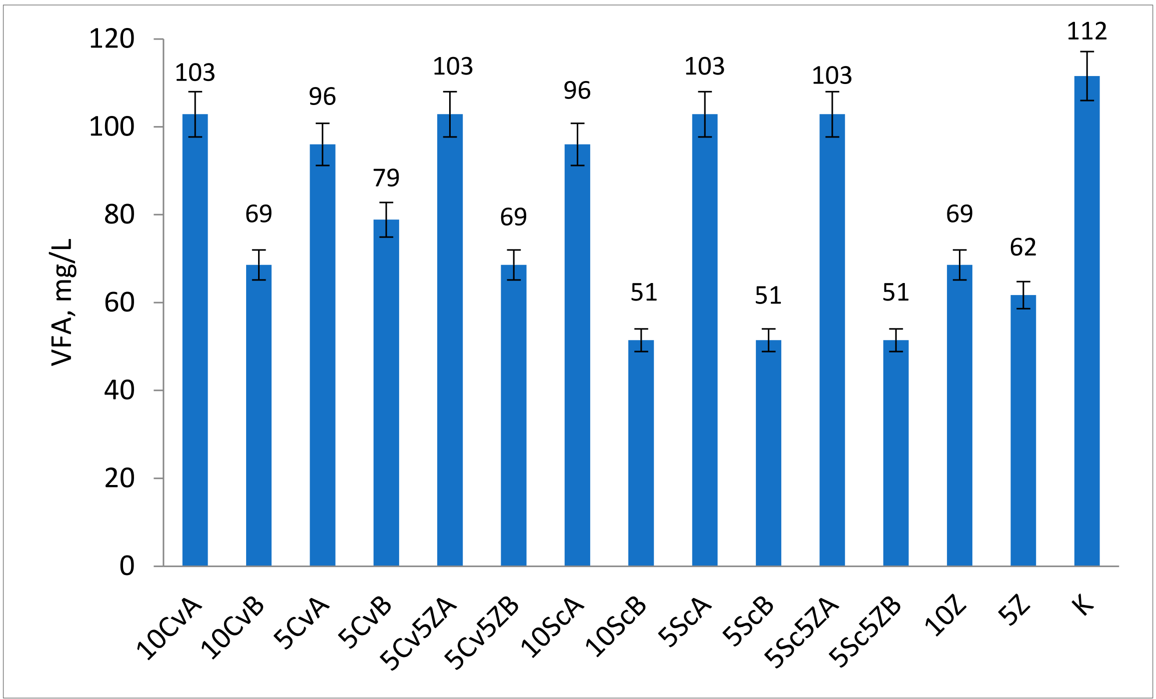


Figure 2. Volatile fatty acids after the experiment.

The initial concentration of volatile fatty acids in the wastewater was 158 mg CH_3COOH/L. After the tests, the concentration of volatile fatty acids in the treated wastewater was lower. In the samples in which microalgae were used in the form of a suspension, the concentrations of volatile fatty acids were in the range of 69 to 79 mg CH_3COOH/L for samples CvB and 51 mg CH_3COOH/L for samples ScB. In the wastewater treated with both strains (CvA and ScA) of immobilized microalgae, the VFAs were higher at 96 to 103 mg CH_3COOH/L. These results indicated that microalgae can use an external organic carbon source (e.g., the volatile fatty acids present in wastewater), which enables their growth in a wastewater with high turbidity. The carbon contained in volatile fatty acids is more easily absorbed when the microalgae are present in suspended form. This can be explained by the better access to the components dissolved in the wastewater. These results are in agreement with the achievements of other researchers that indicate that volatile fatty acids (VFAs), thanks their simple chemical structure, are a source of easily assimilable carbon for microorganisms. Therefore, they play a key role in biological wastewater treatment processes [24].

The research showed that the use of clinoptilolite also reduced the concentration of volatile fatty acids in wastewater. These observations are consistent with the results obtained by Banel and Zygmunt (2009) [24], who proved that volatile fatty acids can be sorbed on alkaline materials. Numerous authors emphasize that during volatile sorption of fatty acids by the alkaline sorbent, the pH value of the solution increases (Banel and Zygmunt, 2009). An example of research on the removal of ammonium ions from wastewater by clinoptilolite in the presence of organic compounds is the research conducted by Jorgensen and Weatherley (2003) [25]. These authors found that in the presence of organic compounds, the efficiency of ammonium ion removal was higher (Jorgensen and Weatherley, 2003) [25].

The results presented in Figure 2 show that when the concentration of clinoptilolite was lower (50 g/L—sample 5Z), the concentration of volatile fatty acids decreased to a level of 62 mg CH_3COOH/L, while at a concentration of 100 g/L (sample 10Z), the final concentration of volatile fatty acids was 69 mg CH_3COOH/L. In the control wastewater (K) on the last day of the process, the concentration of volatile fatty acids remained at a level of 112 mg CH_3COOH/L.

A macroscopic view of the structure of sewage sludge formed during the sewage treatment process in dynamic conditions is shown in Figure 3.

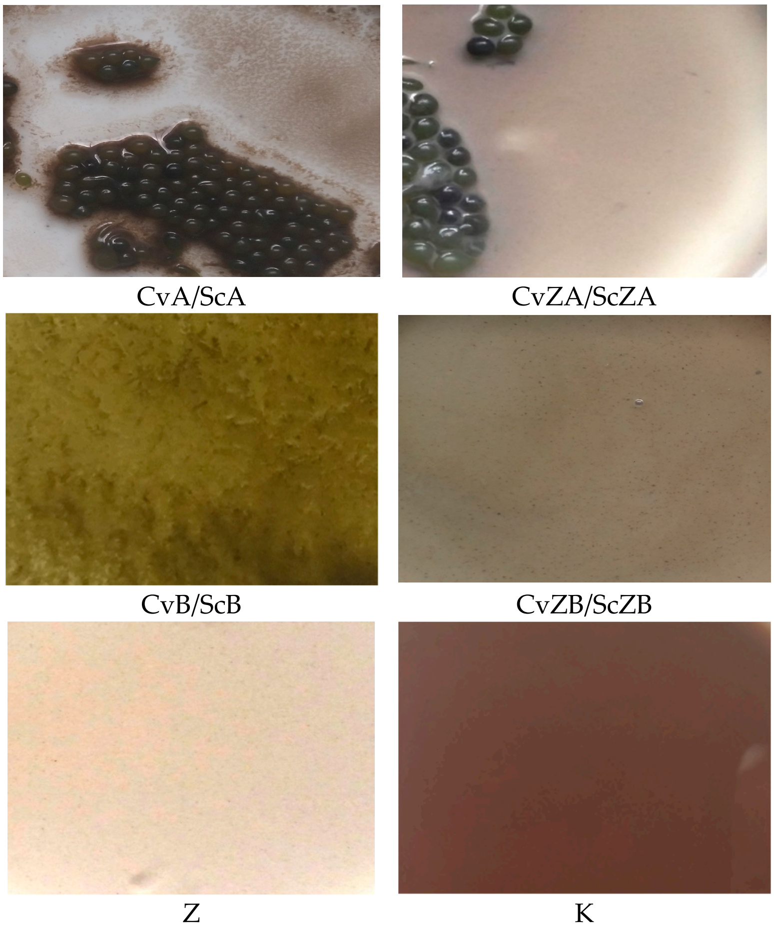


Figure 3. Photographs of sludges after the sorption processes.

The photographs show that the microalgae were effectively immobilized on polysaccharide beads with good biomass-binding properties, which allowed them to be easily separated from the treated wastewater. In turn, clinoptilolite and microalgae constituted a homogeneous suspension.

Micrographs of sewage sludge after the assimilation of $N\text{-}NH_4^+$ from wastewater carried out in dynamic conditions are shown in Figure 4.

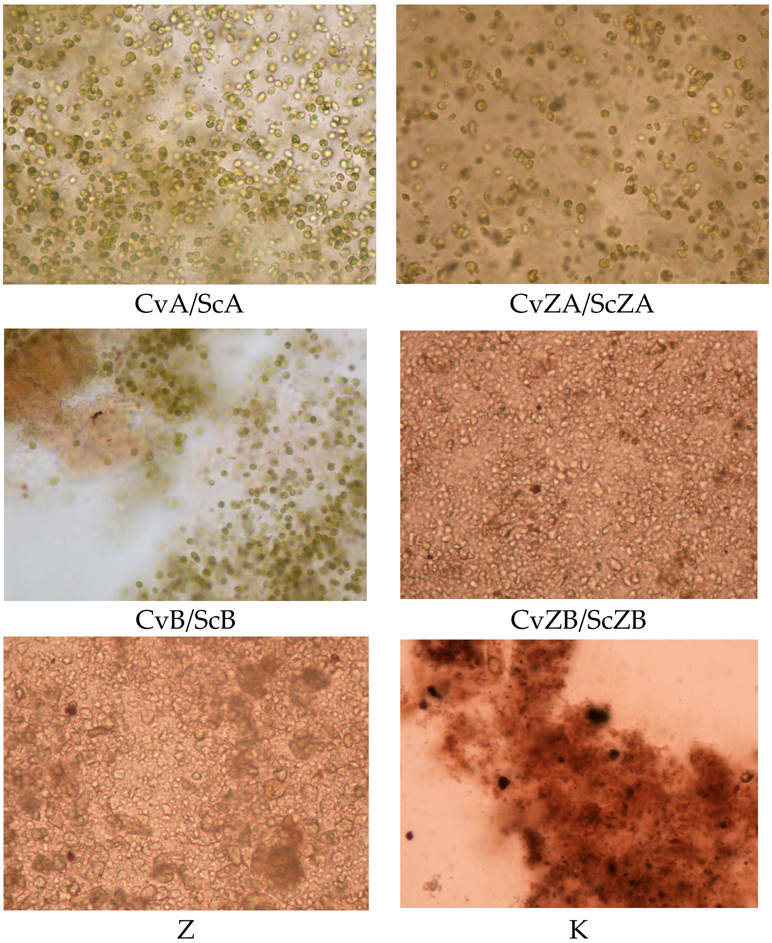


Figure 4. Microscopic images of sludges after the sorption processes (400×).

The sewage sludges are uniform and consist of sorbent residues. The locations of microalgae in clinoptilolite are shown as green, round-shaped particles. In the structure of sludge containing immobilized microalgae, a much higher cell density is visible compared to sludge with microalgae in the form of a suspension. These results are in agreement with the literature that states that the immobilizing of algae results in the obtaining of a several times greater biomass than the suspended system [26,27]. Therefore microalgae immobilized in alginate are proposed for removal of contaminants and easier biomass harvesting [28].

In the control sample, dark clusters of impurities in the form of flocs are noticeable.

3.3. The Effectiveness of Removing Ammonium Nitrogen from Wastewater

The results of the research confirmed that ammonium nitrogen can be removed from wastewater using the microalgae *C. vulgaris* and *S. armatus*, clinoptilolite, and their mixture. An analysis of the data shows that clinoptilolite plays a decisive role in the removal of ammonium nitrogen. In wastewater to which clinoptilolite was added in a mixture with microalgae, gradual decreases in N-NH_4^+ concentration were observed throughout the time (Figure 5C). Jiang et al. (2022) [29] improved the biological treatment of nitrogenous wastewater using a hybrid embedding of materials synthesized and applied in microbial immobilization to form novel biocomposites. Polyvinyl alcohol/sodium alginate (PVA/SA) beads were prepared. By means of the addition of layered double hydroxides (LDHs) to the hydrogel beads, the authors provided effective protection for the proliferation of immobilized microorganisms. The efficient removal of nitrogen was achieved by the immobilized microorganisms thanks to the processes of the simultaneous nitrification/denitrification and assimilation in the bioreaction system [9]. The adsorption of ammonium nitrogen onto alginate is caused mainly by electrostatic attraction between functional groups and the nutrient ions in the aqueous solution [9].

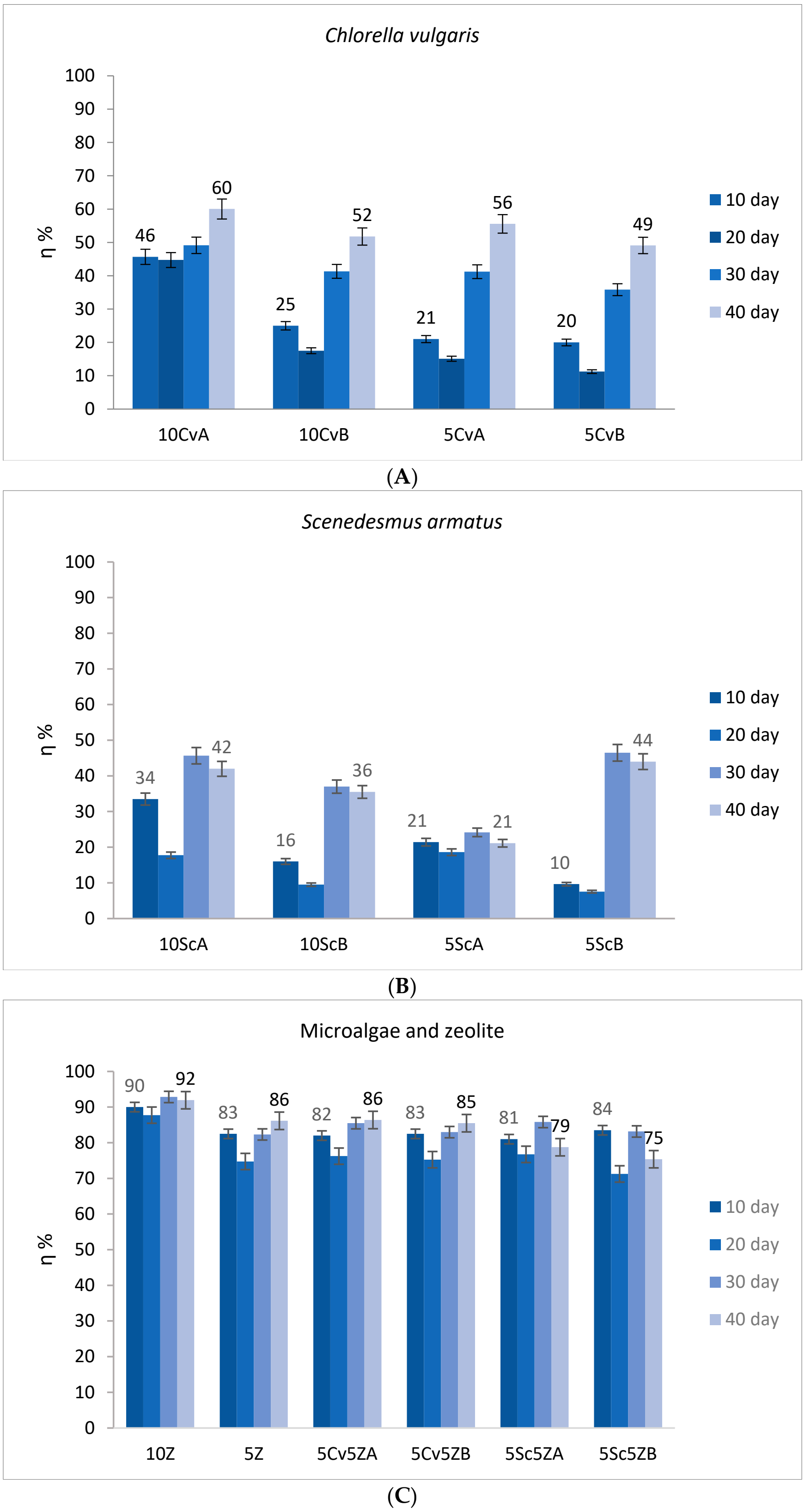


Figure 5. Removal efficiency of ammonium nitrogen: (**A**)—*C. vulgaris*, (**B**)—*S. armatus*, (**C**)—microalgae and zeolite.

Under dynamic conditions, the mixing process took place evenly throughout the column volume, which was conducive to the removal of ammonium nitrogen from the wastewater. Numerous authors confirm the beneficial effect of mixing on the increase in the efficiency of $N\text{-}NH_4^+$ removal. Silva-Benavides and Torzillo (2012) noted on the last day of research the total removal of ammonium nitrogen from wastewater by *C. vulgaris*, with an initial concentration of 33.6 mg $N\text{-}NH_4^+/L$ in the case of mixing samples. The authors showed that in unmixed samples, the ammonium nitrogen was not removed [17]. Similar results were obtained by Karadag et al. (2008), who showed that the mixing time and the initial concentration are the most important factors affecting the removal of ammonium nitrogen by clinoptilolite. The above-mentioned authors showed not only the beneficial effect of mixing on the ammonia removal efficiency, but also that the best ammonia removal efficiency is achieved after only 3 h [30]. This is confirmed by the result recorded by the author of this paper that clinoptilolite was the fastest in sorbing $N\text{-}NH_4^+$ compared to the other sorbents used in this research.

The effectiveness of removing ammonium nitrogen from wastewater with high concentrations of ammonium nitrogen by employing the sorption materials used is shown in Figure 5.

These results are consistent with the results obtained by Tam and Wong (1996) [31]. The researchers studied the effect of nitrogen on the growth of microalgae of the *C. vulgaris* species and found that the removal of ammonium nitrogen depends, among other factors, on its initial concentration in wastewater, algal biomass concentration, strain, and chlorophyll a content [31]. However, the efficiency of ammonium nitrogen removal obtained by the above-mentioned authors did not exceed 54%.

In dynamic conditions, the addition of successive portions of wastewater every 10 days increased temporarily the concentration of this pollutant in wastewater, which influenced the efficiency of $N\text{-}NH_4^+$ removal from wastewater using microalgae of the species *Chlorella vulgaris*, *Scenedesmus armatus*, and clinoptilolite.

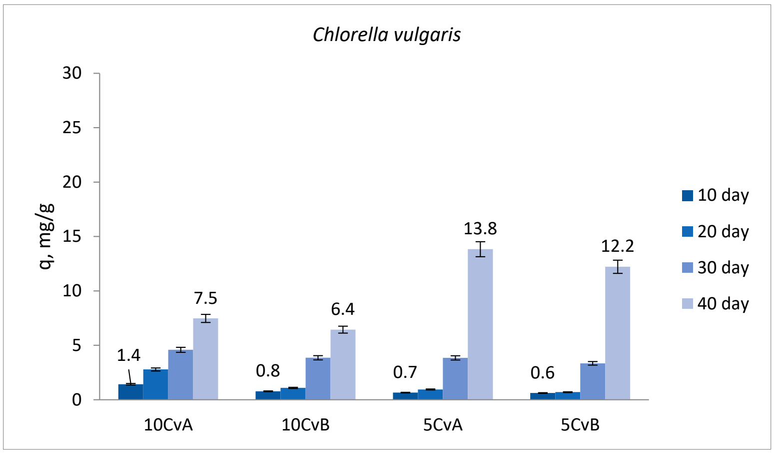
After replacing the second portion of wastewater (from day 20), the efficiency of $N\text{-}NH_4^+$ removal from wastewater by all applied sorbents increased. The best results were achieved after using a mixture of both microalgal strains with clinoptilolite (75–86%) or clinoptilolite alone (86–92%) (Figure 5C).

The use of immobilized microalgae resulted in an efficiency of ammonium nitrogen removal at a level of 21–60%, and the use of microalgae in the form of a suspension was lower. It was at a level of 10–52% (Figure 5A,B). Moreover, the application of *Chlorella vulgaris* (Figure 5A) helped to achieve better a maximum removal efficiency of $N\text{-}NH_4^+$—60% for sample 10CvA than after the addition of *Scenedesmus armatus*—44% for sample 5ScB (Figure 5B).

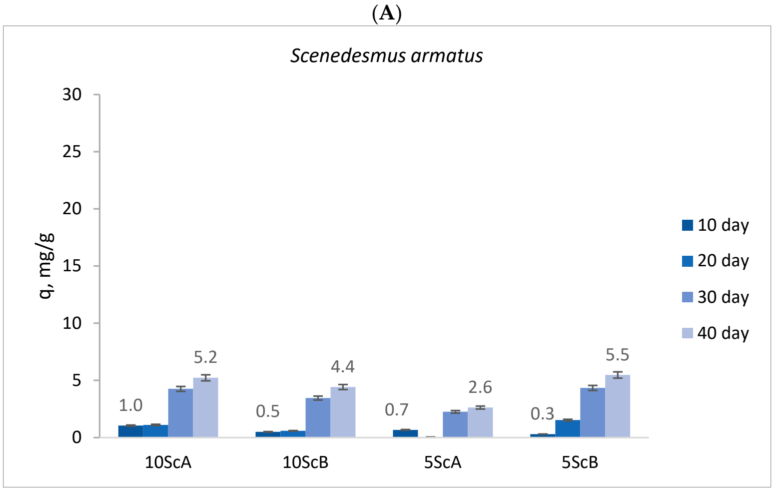
3.4. Sorption Capacities of Used Sorbents

The obtained sorption capacities of microalgae and clinoptilolite, and their mixtures, towards $N\text{-}NH_4^+$ during the process carried out in dynamic conditions are shown in Figure 6.

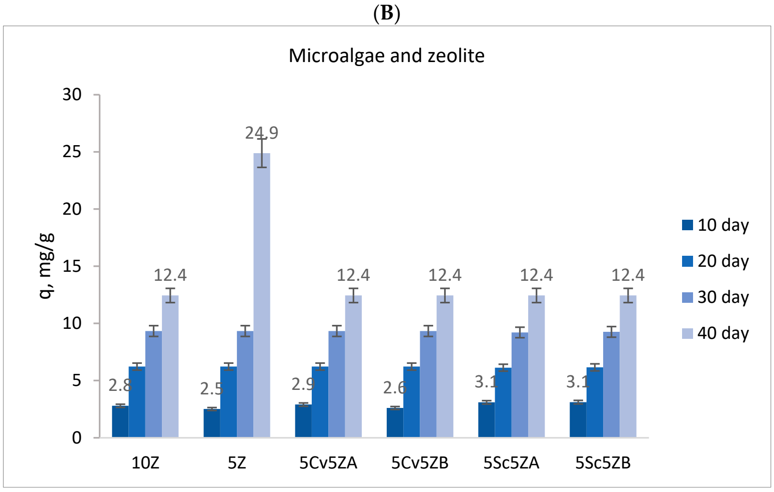
Karadag et al. (2008) showed that the initial concentration is one of the most important factors influencing the obtained values of clinoptilolite adsorption capacity towards ammonium nitrogen [30]. These authors obtained for the initial ammonium nitrogen concentration of 3750 mg $N\text{-}NH_4^+/L$ a clinoptilolite adsorption capacity of 20.4 mg/g and an ammonium nitrogen removal efficiency of 58% at pH = 7. At lower initial concentrations of ammonium nitrogen, e.g., 402 and 200 mg $N\text{-}NH_4^+/L$, the adsorption capacity was correspondingly lower and amounted to 5 and 3 mg/g, respectively. In the author's research, similar relationships were obtained during tests conducted in dynamic conditions (Figure 6). The authors showed that the higher the initial concentration of ammonium nitrogen in relation to the concentration of clinoptilolite and *C. vulgaris*, the higher the sorption capacity obtained for these sorbents. The results presented in Figure 6 show that when the concentration of clinoptilolite was lower (50 g/L—sample 5Z), the maximum sorption capacity was the highest at 24.9 mg/g, and at a concentration of 100 g/L (sample 10Z), the sorption capacity was only 12.4 mg/g. For all mixtures of microalgae and zeolites, the maximum sorption capacity was 12.4 mg/g. The maximum sorption capacity for N-NH_4^+ by the utilization of immobilized *C. vulgaris* as a sorbent was higher at 7.5 mg/g and 13.8 mg/g than after using immobilized *S. armatus*—2.6 mg/g and 5.2 mg/g. The same was noted after the utilization of microalgae in the form of a suspension. For suspended *C. vulgaris*, the maximum sorption capacity for N-NH_4^+ was at a level of 6.4 mg/g and 12.2 mg/g, and for suspended *S. armatus* it was 4.4 mg/g and 5.5 mg/g.



(**A**)



(**B**)



(**C**)

Figure 6. Sorption capacity of sorbents: (**A**)—*C. vulgaris*, (**B**)—*S. armatus*, (**C**)—microalgae and zeolite.

To sum up, it can be concluded that in order to determine the optimal levels of sorption capacity of the sorbents used, preliminary tests are necessary with the use of different doses of sorbents and different contact times for the sorbent with wastewater.

3.5. The Removal Rate of N-NH_4^+ Removal from Coke Wastewater

Table 1 presents the values of the removal rate of N-NH_4^+ from coke wastewater. The obtained values of η_N for the mixture of *C. vulgaris* and clinoptilolite were at a similar level (approx. 8.8–10.3 mg/L d), regardless of the form of microalgae used (immobilized or in the form of a suspension).

Table 1. Removal efficiency per day.

Sample	**η_N, mg/L d**			
	Days			
	10	**20**	**30**	**40**
10CvA	3.8 ± 0.04	5.3 ± 0.01	5.7 ± 0.1	6.9 ± 0.1
10CvB	3.1 ± 0.03	2.1 ± 0.03	4.8 ± 0.03	5.9 ± 0.03
5CvA	2.6 ± 0.02	1.8 ± 0.01	4.8 ± 0.1	6.4 ± 0.09
5CvB	2.5 ± 0.01	1.3 ± 0.01	4.2 ± 0.01	5.6 ± 0.03
5Cv5ZA	10.3 ± 0.09	9.0 ± 0.03	9.9 ± 0.02	9.9 ± 0.02
5Cv5ZB	10.2 ± 0.02	8.9 ± 0.02	9.6 ± 0.1	9.8 ± 0.09
10Z	11.2 ± 0.2	10.3 ± 0.03	10.8 ± 0.02	10.6 ± 0.02
5Z	10.3 ± 0.1	8.8 ± 0.1	9.5 ± 0.1	9.9 ± 0.02
10ScA	4.2 ± 0.2	4.4 ± 0.1	17.0 ± 0.2	20.9 ± 0.9
10ScB	2.0 ± 0.03	2.4 ± 0.03	13.8 ± 0.7	17.7 ± 0.5
5ScA	2.4 ± 0.04	7.5 ± 0.4	7.7 ± 0.3	28.4 ± 0.2
5ScB	2.7 ± 0.05	0.2 ± 0.03	9.0 ± 0.2	10.5 ± 0.3
5Sc5ZA	1.2 ± 0.03	6.1 ± 0.7	17.4 ± 0.9	21.9 ± 0.2
5Sc5ZB	10.1 ± 0.2	19.1 ± 0.2	32.0 ± 1.0	39.2 ± 0.1

The addition of successive portions of wastewater affected changes in the rate of N-NH_4^+ removal from coke wastewater. The results showed that after the application of microalgae at a concentration of 100 g/L, higher maximum values of the N-NH_4^+ removal rate from coke wastewater were obtained (6.9 ± 0.1 mg/L d for *C. vulgaris* and 20.9 ± 0.9 mg/L d for *S. armatus*) than when microalgae at a dose of 50 g/L (6.4 ± 0.09 mg/L d for *C. vulgaris* and 28.4 ± 0.2 mg/L d for *S. armatus*) were applied. Similarly, using clinoptilolite at a concentration of 100 g/L resulted in higher maximum η_N values (11.2 ± 0.2 mg/L d) than when clinoptilolite was used at a concentration of 50 g/L, where η_N was 10.3 ± 0.1 mg/L d.

Zeolite with immobilized *C.vulgaris* reached η_N of 10.3 ± 0.09 mg/L d and with *S. armatus* achieved a value for η_N of 21.9 ± 0.2 mg/L d, while zeolite with *C.vulgaris* suspended achieved a value for η_N of 10.2 ± 0.02 mg/L d and with *S. armatus* achieved a value for η_N of 39.2 ± 0.1 mg/L d.

The rate of ammonium nitrogen removal from coke wastewater using microalgae immobilized on a carrier at concentrations of 100 and 50 g/L was higher than the rate of ammonium nitrogen removal from wastewater using microalgae in the form of a suspension. This result is in agreement with other authors. Cao et al. (2022) utilized immobilized algae in an alginate–calcium hydrogel and discovered that the dry weight rose 4.75 times during 2-day semicontinuous cultivation. The authors indicated that immobilized microalgae achieved a high nutrient removal rate from wastewater at continuous operation. The highest removal rate for ammonia was 28.95 mg/L d [32]. The highest concentration of N-NH_4^+ of 72.51 mg/L used by Cao et al. (2022) was lower than in the present study, and that could have resulted in the higher removal rate than they achieved.

The use of natural sorbents, such as microalgae and clinoptilolite embedded in hydrogel, is an alternative way to support the treatment of industrial wastewater with high concentrations of ammonium nitrogen, reducing the consumption of chemical reagents and, at the same time, allowing the recycling of nutrients, such as nitrogen. The solution proposed in this work to support the treatment of wastewater with high concentrations of $N\text{-}NH_4^+$ is beneficial for the environment, as it does not generate waste that needs to be managed. Sewage sludge containing natural clinoptilolite and microalgae formed after the assimilation of ammonium nitrogen can be further tested to determine how it can be used for other applications, e.g., the reuse of alginate and wastewater.

The obtained research results can be used to develop a method to support the removal of ammonium nitrogen from wastewater in a microalgae culture. It should be emphasized that the choice of microalgae cultivation technology is important because, according to data in the literature, it has a decisive impact on the profitability of using biomass for further purposes [33,34].

Due to the lack of available data in the literature on the innovative use of simultaneous methods of ammonium nitrogen fixation from industrial wastewater proposed by the author and involving the integration of biological (microalgae) and physicochemical (clinoptilolite) methods, it is impossible to relate the results of our own research to data in the literature.

4. Conclusions

The simultaneous removal of $N\text{-}NH_4^+$ from industrial wastewater using immobilized microalgae and zeolite is effective. The rate and efficiency of the $N\text{-}NH_4^+$ removal was dependent on the type (zeolite, microalgae), form (suspended or immobilized), and dosage of the sorbent. For each single sorbent, a higher maximum sorption capacity for clinoptilolite (24.9 mg/g) and *C. vulgaris* (13.8 mg/g) was achieved than for *S. armatus* (5.5 mg/g). The higher the initial concentration of ammonium nitrogen in relation to the concentration of clinoptilolite and *C. vulgaris*, the higher the sorption capacity obtained for these sorbents. The removal efficiency of immobilized microalgae reached 60% (*C. vulgaris*) and 42% (*S. armatus*), and that of suspended microalgae reached 52% (*C.vulgaris*) and 44% (*S. armatus*). A higher removal efficiency was obtained for zeolites (86% and 92%) and zeolites with immobilized *C. vulgaris* or *S. armatus* (up to 86% and 79%) than for zeolites with suspended *C. vulgaris* or *S. armatus* (up to 85% and 75%, respectively). The recycling of spent hydrogel, zeolite, and wastewater is possible; this represents a circular bioeconomy loop.

5. Patents

Zabochnicka-Świątek M. (80%), Bień J. (20%). Method for biological treatment of industrial wastewater from heavy metals: No. PL235489.

Zabochnicka-Świątek M. (80%), Bień J. (20%). Method for growing the microalgae biomass: No. PL231040.

Funding: This scientific work was supported by the statute subvention of the Czestochowa University of Technology, Poland.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author declares no conflict of interest.

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