

Article

Effectiveness of Nitrification and Denitrification Processes in Biofilters Treating Wastewater from De-Icing Airport Runways

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Abstract: The basic factors determining the efficiency of the removal of nitrogen and carbon compounds from airport wastewater containing de-icing agents are low temperature and the C/N ratio (carbon to nitrogen ratio). Biofilm reactors (biofilters) create better conditions for nitrification and denitrification than suspended biomass reactors. The scope of the study included determination of the influence of the C/N ratio in the wastewater on nitrification, denitrification and organic compound removal in biofilm reactors depending on the temperature. The experiment was performed in 24 circular laboratory biofilters with LECA (Light Expanded Clay Aggregates) filling. The study was divided into three series differing in organic carbon loading. In each series, carried out at the same hydraulic retention time, biofilters were operated at 25, 8, 4 or 0 °C. The study showed the effective removal of nitrogen compounds across a very wide temperature range. The applied filling and properly selected operating parameters of the reactors resulted in effective simultaneous nitrification and denitrification. The highest efficiency of nitrogen removal at 0 °C ($34.57 \pm 4.54\%$) was obtained at the C/N ratio of 0.5 gC/gN. The efficiency of denitrification (the lowest at the temperature of 0 °C) increased as the temperature and C/N ratio increased in the wastewater.

Keywords: pavement de-icing agents; biofilters; LECA (Light Expanded Clay Aggregates); nitrogen and carbon removal; nitrification and denitrification

1. Introduction

Chemical substances used for winter maintenance of airports are an important source of water and land pollution [1,2]. Every winter, a significant amount of chemicals are used at the airport for de-icing of aircraft and airport pavements to facilitate safe air travel. Even in small facilities, an average of several hundred tons of de-icing agents are utilized during the season, the majority of which penetrates with rainwater to the ground and water near the aerodrome. Chemicals commonly used for winter maintenance of airport pavements include: urea, acetate and sodium formate in solid form, and acetate and potassium formate in liquid form. The generated wastewater is contaminated primarily by nitrogen and carbon compounds, while concentrations of phosphorus compounds do not exceed the limit values [2].

The composition of wastewater produced during de-icing of airport pavements is determined by meteorological phenomena, methods used to prevent freezing and de-icing [3] and the systems used for wastewater collection and disposal. This wastewater is characterized by chemical oxygen demand (COD) from several dozen to 630,000 mg O₂/dm³, concentration of total nitrogen (Kjeldahl) from 0 to 600 mg/dm³, concentration of total phosphorus below 1 mg/dm³ [4]. Therefore, the Environmental

Protection Agency chose COD and total nitrogen as quality indicators of wastewater containing de-icing agents [5]. The issue of wastewater treatment containing de-icing agents remains unresolved [6], which results in penetration of most of these substances into the ground and water near airports [7]. Most airports are not equipped with a wastewater treatment system. Only a few airports in the world have wetlands [8], which effectively remove pollution from wastewater. However, wetlands create favorable environmental conditions for birds [9], which may endanger airport operations. A significant environmental problem associated with runoff wastewater containing de-icing agents is eutrophication and oxygen depletion in receiver water [1], resulting in the death of aquatic invertebrates and fish and causing the loss of migratory fish species [10].

The basic factor determining the efficiency of the removal of nitrogen and carbon compounds from wastewater containing de-icing agents is the temperature, because both de-icing and wastewater treatment takes place at low temperatures at the airport. Heterotrophic denitrification is also dependent on access to easily available carbon compounds. The simultaneous use of de-icing agents such as urea and easily decomposable organic compounds such as acetate or potassium formate at the airport might facilitate the effective denitrification process as well as the effective removal of organic compounds [1]. Temperature is one of the most important factors determining the metabolism of nitrifying bacteria [11,12]. The optimal temperature range of wastewater for nitrification is 25–28 °C, at which temperature, *Nitrobacter* sp. are more sensitive to environmental conditions than *Nitrosomonas* sp. [13], thus a temperature below 8–10 °C mainly inhibits the second phase of nitrification, which results in the accumulation of nitrates in an effluent [14]. Nevertheless, nitrification was also observed at very low temperatures of 1 °C or 0.5 °C [14] and reducing the wastewater temperature resulted in a gradual decrease in the efficiency of oxidation of ammonium nitrogen. The nitrification efficiency decreases from 90–94% at 15 °C to about 55% at 2 °C. Despite the possibility of the growth of nitrifying microorganisms at low temperatures being confirmed by other studies, it is commonly accepted that nitrification is effective above 15 °C [15]. Hoilijoki et al. [16] observed that complete nitrification below 5 °C was possible only in a reactor with the immobilized microorganisms that formed biofilm. Rostron et al. [17] noted that a reduction of the temperature from 25 °C to 16 °C decreased 10% of the nitrification efficiency in the reactor with biofilm, while the nitrification efficiency decreased 58% in the reactor with suspended biomass. Literature data also shows that the expanded-clay aggregate applied in biofilm reactors (biofilters) “buffers” changes in pH and redox potential [18,19]. Few studies have investigated the effect of temperature on denitrification which is temperature-dependent as a biochemical reaction [20]. The rate of denitrification increases exponentially to 32–35 °C [21]. The highest rate of the reduction of oxidized forms of nitrogen occurred at 20 °C and a further increase in the temperature did not increase the denitrification rate. The literature presents little data on the influence of low temperature on denitrifying microorganisms. It is commonly accepted that the efficiency of denitrification below 10 °C or above 30 °C decreased significantly. However, the reduction of oxidized forms of nitrogen was noted from 1 °C to 8 °C [20,22]. There are no reports on the efficiency of nitrification or denitrification at 0 °C.

The issue of wastewater treatment containing de-icing agents still remains unresolved. Most of airports in the world are not equipped with wastewater treatment systems. Urea, acetate and sodium formate in solid form and acetate and potassium formate in a liquid form are common chemicals used for the winter maintenance of airport pavements.

The novelty of our work results from thermal conditions, specific wastewater and new biofilter filling. The study was carried out at very low temperatures –0.4 and 8 °C. The subject of research was wastewater generated during the de-icing of airport pavements. Wastewater contains urea and de-icing agents with readily biodegradable organic compounds. Wastewater characterized by a different C/N ratio was treated in biofilm reactors with light weight aggregates prepared from fly ash from sewage sludge thermal treatment (FASST LWA). We expect that the study has proven that the denitrification and nitrification processes may take place even at 0 °C, due to the specificity of LECA filled bioreactors and maintaining the appropriate ratio between nitrogen and carbon compounds in the treated wastewater.

We hope that the results of our studies will help engineers responsible for environmental protection at airports and provide a technological solution to treat wastewater containing de-icing agents from airport pavements and reduce the pollution of the water and ground environment.

The main purpose of the study was to determine the effectiveness of removing pollutants from wastewater containing agents for de-icing airport pavements in biofilters. The biofilter filling was made of LECA prepared from fly ash from sewage sludge thermal treatment (FASST LWA), which is characterized by a higher specific density and compressive strength than natural LECA. The specific goals of the study were to determine the effectiveness of nitrification and denitrification processes and carbon and nitrogen removal in biofilters depending on temperatures. The scope of the study also included determination of the influence of the C/N ratio in the wastewater on nitrification, denitrification and removal of organic compounds (COD). Another goal was to determine the effect of temperature and C/N ratio on COD concentration in treated wastewater. In the conducted study, wastewater containing pavement de-icing agents (urea and readily biodegradable organic compounds) was treated, assuming that maintaining a proper relationship between the amount of carbon and nitrogen in the treated wastewater can ensure effective removal of pollutants in low temperatures.

2. Materials and Methods

2.1. Experimental Stand and Organization

The study was performed in 24 circular reactors that are laboratory models of biofilters with the filling in the form of LECA (Figure 1). A bioreactor consisted of a cylindrical polyethylene pipe and a cone-shaped bottom. In the bottom of the reactor, which was the outflow part, a drain valve was used to collect samples of the treated wastewater. The technical parameters of the reactor were: surface area 95 cm^2 , volume 2500 cm^3 and active volume 1552 cm^3 .

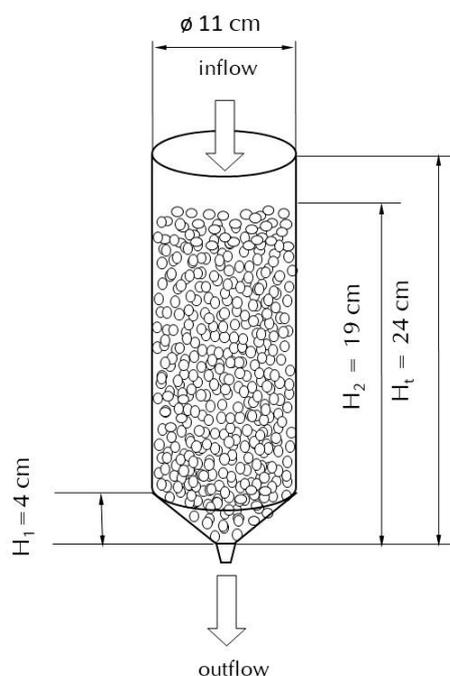


Figure 1. Biofilter with LECA (Light Expanded Clay Aggregates) filling.

The filling of the reactors was granulate. It had a structure of extended-clay aggregate (with a diameter $d_{60} = 8.2 \text{ mm}$) prepared from fly ash from sewage sludge thermal treatment in the “Dębogórze” Wastewater Treatment Plant in Gdynia (Poland). The granulate was prepared using mechanical plasticization and fragmentation of the raw material, followed by firing small balls (average diameter d_{60} of 8.2 mm) into a rotary kiln at 1200 °C [23]. The study was divided into three series

differing in organic carbon dose introduced to the reactor. In each series, four variants with reactors operated at different wastewater temperatures were separated using a thermostatic chamber (POL-EKO APARATURA ST 350/350, EU—Poland) (Figure 2).

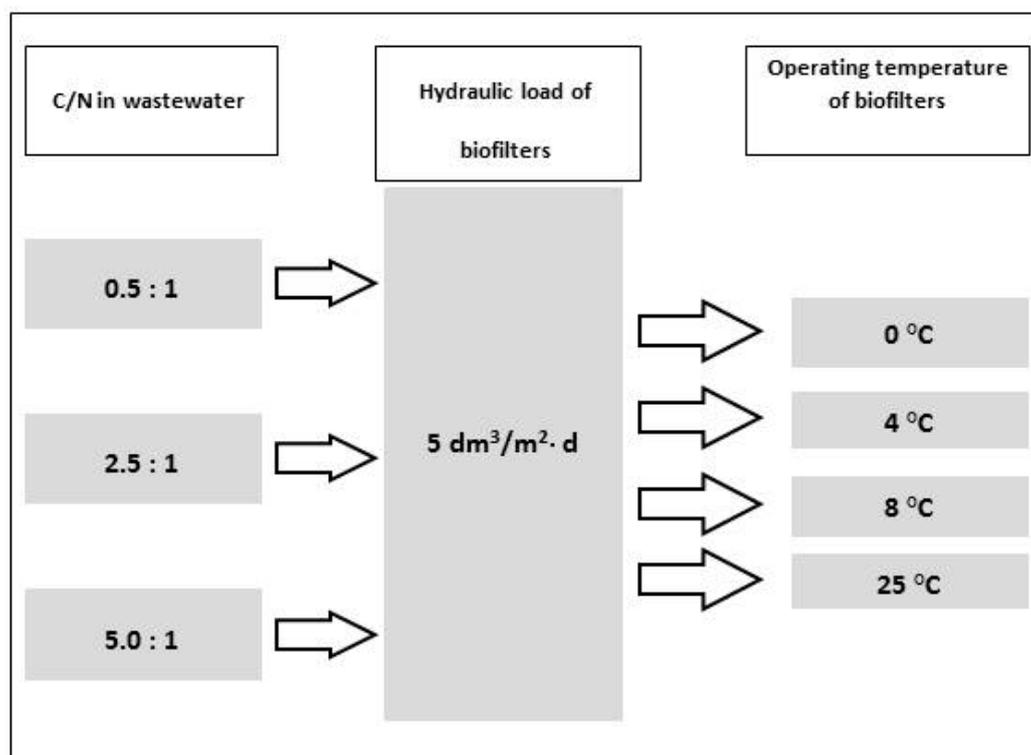


Figure 2. Research scheme.

2.2. Characteristics of Wastewater Fed to Reactors

The synthetic wastewater, prepared from sample weight (using Sartorius CPA225D-0CE, Germany), which included commonly used agents for de-icing airport pavements and tap water, was the substrate for the experiment (Supplementary Table S1).

The experiments were carried out at the same hydraulic retention time. The concentration of organic compounds in the wastewater was increased to obtain higher organic loading in the following biofilters. The indicators of raw wastewater are presented in Table 1.

Table 1. Average (\pm SD) values of raw wastewater concentration in subsequent research series.

Parameter	Series 1	Series 2	Series 3
C/N (gC/gN)	0.5	2.5	5.0
N_{tot} (mg N/dm ³)		71.56 \pm 2.20	
N_{Kjeldahl} (mg/dm ³)		70.80 \pm 3.02	
TOC (mg C/dm ³)	56.96 \pm 1.98	200.80 \pm 4.79	375.71 \pm 6.97
COD (mg O ₂ /dm ³)	100.66 \pm 1.34	386.80 \pm 1.94	765.50 \pm 2.90
Dissolved oxygen (mg O ₂ /dm ³)		6.64 \pm 0.71	
Redox potential (mV)		189.33 \pm 12.23	
pH		7.75 \pm 0.28	

The organic compounds loading in the following series of experiments are presented in Table 2.

Table 2. The average (\pm SD) reactor pollutant loading.

Parameter	Series 1	Series 2	Series 3
C/N (gC/gN)	0.5	2.5	5.0
N _{tot.} (mg N/m ² ·d)		357.8 \pm 11.00	
N _{Kjeldahl} (mg N/m ² ·d)		354.00 \pm 15.10	
N-NH ₄ (mg N/m ² ·d)		0.00	
COD (mg O ₂ /m ² ·d)	503.30 \pm 7.19	1934.00 \pm 9.47	3827.50 \pm 14.50

The study was carried out using biofilters with vertical flow. During the first three months, they were operated with synthetic wastewater (adaptive phase). During this period, the temperature of the biofilters dropped 1 °C/d until the assumed operating temperature was reached. The study was performed at a low hydraulic loading of 5 dm³/m²·d, which facilitated nitrification [22] and a hydraulic retention time of 4 d, which corresponded to the dosing frequency of de-icing agents at airports of 4 d. The adaptive phase lasted until stabilization of composition of the treated wastewater. After adaptive period, each of biofilters was operated at the temperature of 25, 8, 4 or 0 °C. The reactor operated at the temperature of 25 °C was the control reactor. Biofilters were placed in a thermostatic chamber which maintained the temperature with an accuracy of \pm 0.1 °C.

The wastewater was dispensed to biofilters once every four days in a way that ensured an even distribution of the wastewater on the surface of the filling.

2.3. Analytical Procedures

Physicochemical analyses of raw and treated wastewater included: nitrate concentration (spectrophotometric method) [24], nitrite concentration (spectrophotometric method) [25], ammonium nitrogen concentration (spectrophotometric method) [26] using VWR UV-3100PC Spectrophotometer (China) Kjeldahl total nitrogen (titrimetric method) [27] using a Buchi SpeedDigester K-436 (Switzerland) and Buchi KjelFlex K-360 (Switzerland), organic compound concentration (COD) (titrimetric method) [28] using a Gerhardt KI 16 (Germany) laboratory heater, concentration of total nitrogen (TN) and total organic carbon (TOC) with a Shimadzu Corporation TOC-L CPN and TNM-L analyzer (Japan), dissolved oxygen, pH, redox potential and temperature with a HACH HQ440d multi-analyzer (USA).

The results were calculated on the basis of approx. 30 measurements of pollution indicators in the raw and treated wastewater.

2.4. Statistical Analysis

To determine any statistically significant differences between variants, a one-way analysis of variance was carried out. Significant differences in the mean values of the measured parameters were estimated using the ANOVA analysis, with a post-hoc reasonable significant difference (RIR) Tukey test. If the assumptions of the ANOVA analysis were not met, a non-parametric Kruskal–Wallis test was used as well as the post-hoc test of multiple comparison test (Dunn's test). The significance level of $\alpha = 0.05$ was used. The statistical analyses were performed in the Statistica 13.1 PL package. Details of the statistical analysis are presented in Supplementary Tables S2–S10.

3. Results and Discussion

The study tested oxidation reduction potential, pH and concentration of dissolved oxygen in raw and treated wastewater (Tables 1 and 3).

Table 3. Physicochemical properties of treated wastewater.

C/N [gC/gN]	Temperature [°C]	pH	Oxidation-Reduction Potential [mV]
0.5	0 °C	8.37–8.55	127.30 ± 4.84
	4 °C	8.42–8.62	118.50 ± 3.00
	8 °C	8.36–8.66	114.80 ± 3.04
	25 °C	8.61–8.79	104.90 ± 3.22
2.5	0 °C	8.32–8.50	117.20 ± 3.88
	4 °C	8.39–8.61	112.08 ± 4.21
	8 °C	8.41–8.65	105.65 ± 3.07
	25 °C	8.40–8.72	93.74 ± 1.94
5.0	0 °C	8.41–8.67	107.80 ± 3.21
	4 °C	8.46–8.72	96.03 ± 2.87
	8 °C	8.48–8.76	92.28 ± 2.24
	25 °C	8.62–8.78	86.38 ± 3.31

The concentration of dissolved oxygen in the treated wastewater was 0.00 mg O₂/dm³ independent of the series of the experiment. The redox potential in the treated wastewater was lower than in the raw wastewater (Table 1). In all series of experiments, a low temperature of reactor operation increased redox potential in the treated wastewater in the same technological conditions. In all series of experiments, the pH of wastewater increased during the treatment process. At low temperatures, the pH values in the treated wastewater were lower than at higher temperatures.

The pH of wastewater increased during treatment in biofilters. The low temperatures of biofilter operation decreased the values of pH in the treated wastewater.

Nitrogen compound transformations in the biofilm occurred as shown in the Figure 3 and described by Equations (1)–(5). This corresponded to the removal efficiency shown in the Supplementary Table S11. The mass balance of the overall wastewater treatment process is shown in Table 4.

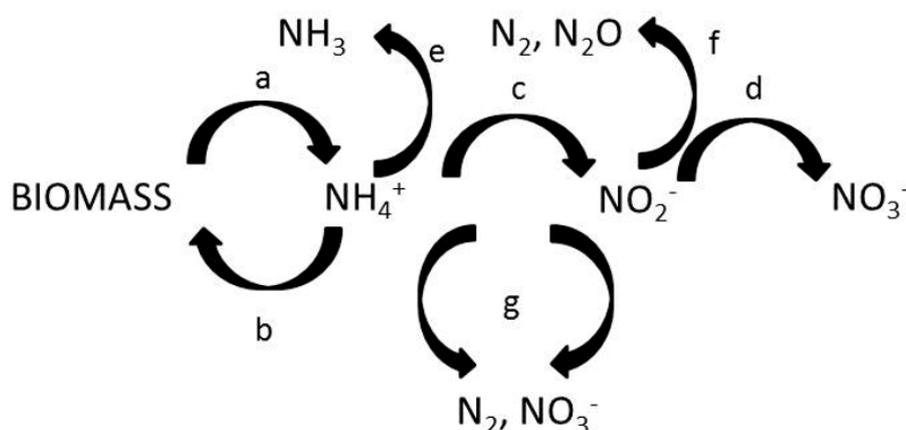


Figure 3. Transformation of nitrogen compounds in a biofilm: a—ammonification, b—immobilization, c—first phase of nitrification, d—second phase of nitrification, e—release to atmosphere, f—denitrification, g—anaerobic oxidation of ammonium nitrogen [29].

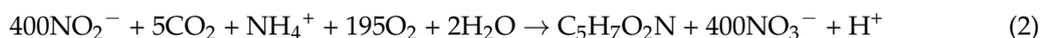
Mass balance in nitrogen compound transformations in the biofilm:

Nitrification (including biomass production— $C_5H_7O_2N$)

-I stage:



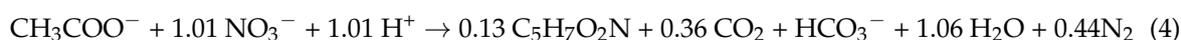
-II stage:



summary:



Denitrification (including biomass production - $C_5H_7O_2N$ and acetate as carbon source)



Anaerobic oxidation of ammonium nitrogen (including biomass production - $CH_2O_{0.5}N_{0.15}$)



Table 4. Mass balance of the overall wastewater treatment process.

C/N [gC/gN]	Influent			Effluent	
	Temperature [°C]	COD [mgO ₂ /m ² ·d]	Total Nitrogen [mgN/m ² ·d]	COD [mgO ₂ /m ² ·d]	Total Nitrogen [mgN/m ² ·d]
0.5	0	503.30 ± 6.70	357.80 ± 11.00	187.98 ± 30.50	234.11 ± 16.24
	4			164.83 ± 26.12	231.10 ± 12.20
	8			115.76 ± 13.04	216.86 ± 16.82
	25			102.17 ± 8.56	194.18 ± 10.16
2.5	0	1934.00 ± 9.70	357.80 ± 11.00	1523.80 ± 38.10	269.85 ± 8.27
	4			1467.52 ± 38.87	231.64 ± 10.41
	8			1445.08 ± 21.27	228.96 ± 10.52
	25			866.63 ± 152.59	170.74 ± 12.56
5.0	0	3827.50 ± 14.50	357.80 ± 11.00	3109.84 ± 100.66	274.75 ± 9.48
	4			3018.37 ± 101.43	256.26 ± 11.34
	8			2981.62 ± 65.07	245.31 ± 16.78
	25			1446.08 ± 203.24	165.73 ± 13.99

3.1. Nitrification Efficiency

The experiments determined the influence of temperature on the efficiency of ammonium nitrogen oxidation and the relationship between temperature and the efficiency of nitrification under different concentrations of organic carbon compounds in wastewater fed to biofilters.

In the first series, the organic carbon to nitrogen ratio was 0.5 gC/gN and the efficiency of nitrification was $47.68 \pm 2.26\%$ in the control reactor (operated at the temperature of 25 °C). The low temperatures of biofilter operation significantly ($p < 0.05$) decreased the efficiency of the process compared with the control reactor while maintaining the same technological conditions (Figure 4a). In the biofilter operated at 0 °C, the nitrification efficiency was $36.88 \pm 6.29\%$.

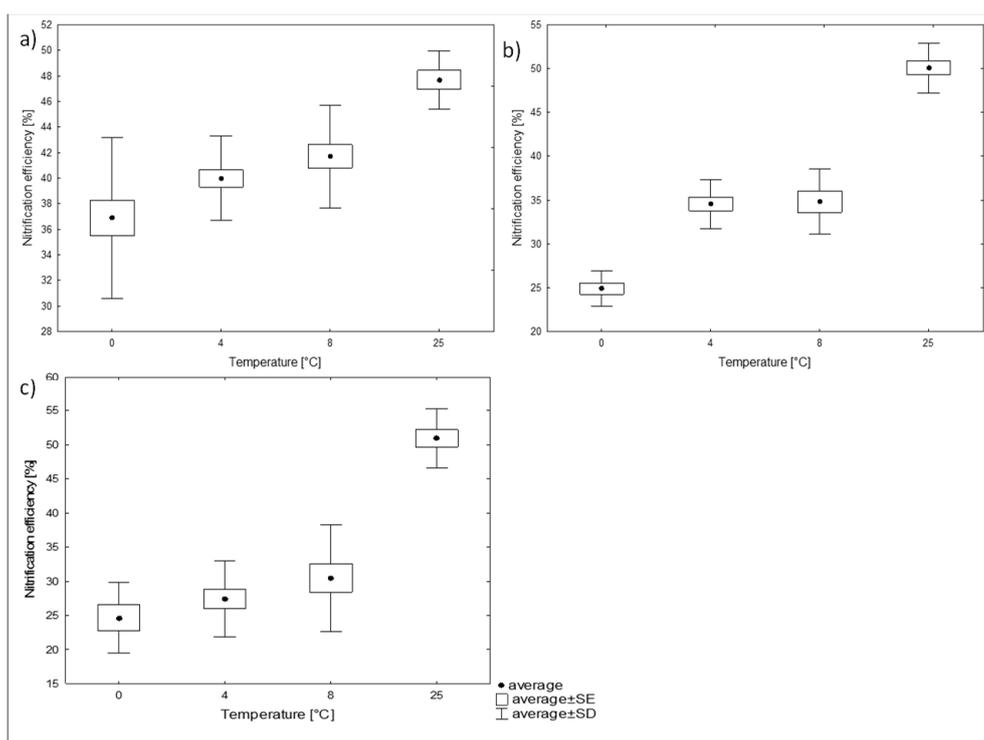


Figure 4. Influence of temperature on the nitrification efficiency in: (a) series 1 (C/N = 0.5 gC/gN), (b) series 2 (C/N = 2.5 gC/gN), (c) series 3 (C/N = 5.0 gC/gN).

A multiple comparison test showed that in the biofilters operated at low temperatures, the efficiencies of oxidation of ammonium nitrogen were similar and did not significantly differ ($p < 0.05$). In the reactors operated at 4 °C and 8 °C the percentage of ammonium nitrogen that was oxidized was $39.98 \pm 3.31\%$ and $41.70 \pm 4.02\%$, respectively.

The changing of the technological parameters of reactor operations in the second series, by increasing the amount of organic carbon in the influent to 2.5 gC/gN, did not significantly change the efficiency of nitrification in the control reactor, in which it was $50.08 \pm 2.86\%$. The efficiency of oxidation of ammonium nitrogen in the other reactors was significantly lower than in the control reactor. In this series of experiments, the efficiency of nitrification was from $24.88 \pm 1.99\%$ in the reactor operated at 0 °C to $34.81 \pm 3.72\%$ in the reactor operated at 8 °C (Figure 4b).

The changing of the technological parameters of reactor operation in the third series, by increasing the amount of organic carbon in the influent to 5.0 gC/gN, caused that in the reactor operated at the temperature of 25 °C, the efficiency of nitrification was $50.93 \pm 4.35\%$. However, this efficiency was not significantly higher than in the other series. In the third series, the low temperatures of the biofilter operation significantly decreased the nitrification efficiency. The lowest efficiency of nitrification ($24.65 \pm 5.15\%$) was noted in the biofilter operated at 0 °C. The efficiency of ammonium nitrogen oxidation was $27.46 \pm 5.58\%$ in the reactor operated at 4 °C and $30.49 \pm 7.83\%$ in the reactor operated at 8 °C (Figure 4c).

The obtained results and the literature data indicate that the efficiency of nitrification depends on the temperature conditions. Many authors have also identified temperature as the main factor determining the metabolism of nitrifying bacteria, which inhibited nitrification [11]. In this study, the operation of a biofilter at 0 °C significantly ($p < 0.05$) decreased the nitrification efficiency compared with the control reactor in each series. These results confirmed the study carried out by Zhang et al. [30] which showed a rapid decrease in the nitrification efficiency to 12.7% at the temperature of 2.5 °C and a study carried out by Hoang et al. [31] which showed the nitrification activity of microorganisms at a temperature below 1 °C. In the present study, the influence of the temperature on the nitrification

efficiency was the highest in the third series, in which the wastewater was characterized by the C/N ratio of 5.0 gC/gN. In this series, dropping the temperature of the reactor operation from 25 °C to 0 °C decreased the efficiency of ammonium nitrogen oxidation by more than 26%. The highest efficiency of nitrification of $36.88 \pm 6.29\%$ in the biofilter operated at the temperature of 0 °C was noted in the series in which the wastewater was characterized by the lowest organic compound concentration.

Young et al. [14] showed that as nitrification efficiency decreased due to the decreased temperature, the accumulation of nitrate nitrogen (III) occurred in the treated wastewater. In the present study, the accumulation of nitrate nitrogen (III) associated with a decrease in the nitrification efficiency was not observed despite the decrease in ammonia nitrogen oxidation efficiency. These results are consistent with recent literature reports, which showed that ammonium oxidizing bacteria are just as sensitive [32] to a temperature drop below 20 °C as nitrite oxidizing bacteria, or even more so.

Many authors have noted the dependency of the nitrification efficiency on the concentration of immobilized biomass in the biofilter. It is commonly accepted that a drop in the temperature of 10 °C will result in a 50% decrease in microorganism activity [33]. However, numerous authors have also noted that an immobilized biomass is less sensitive to the temperature drop than suspended biomass due to limited effect of diffusion on mass transfer processes [16]. Rostron et al. [17] showed that dropping the temperature from 25 °C to 16 °C decreased the nitrification efficiency by 10% in the biofilter with immobilized biomass, while a 58% decrease in nitrification efficiency was observed in a reactor with suspended biomass. The insufficient alkalinity of wastewater results in a decrease in pH, which inhibits the nitrifying microorganisms, mainly bacteria, of the second phase of nitrification since they are more sensitive to changes in pH [34]. A literature review showed that the expanded-clay aggregate used in the BAF (Biological Aerated Filter) system “buffered” changes in pH and redox potential [18], which is consistent with the results obtained in the present study, and the pH in all experiments was maintained at a constant level of 8.41–8.70. This level facilitated nitrification [19].

Young et al. [14] observed that the unfavorable effect of high C/N ratio in the wastewater on the ammonium nitrogen removal increased at the low temperatures. The current study showed that the nitrification efficiency in the series of the study was not directly related to the C/N ratio in the wastewater. Therefore, the high nitrification efficiency observed with high organic compound concentrations in the wastewater resulted from heterotrophic nitrification. Guo et al. [35] observed that many heterotrophic microorganisms (not only bacteria, but also fungi and plants) were capable of nitrifying organic and inorganic nitrogen compounds. These microorganisms grew faster, required less oxygen and tolerated a higher C/N ratio in the wastewater than autotrophic nitrifying bacteria.

3.2. Denitrification Efficiency

Although the concentration of oxidized ammonium nitrogen indicates the efficiency of nitrification, the difference between the concentration of oxidized ammonium nitrogen and the concentration of nitrites and nitrates in the treated wastewater is the amount of nitrogen removed during denitrification. The study results suggest simultaneous nitrification and denitrification in the biofilm.

In the first series, in which the wastewater was characterized by an organic carbon-to-nitrogen ratio of 0.5 gC/gN, the efficiency of denitrification was $89.83 \pm 0.96\%$ in the biofilter operated at 25 °C. The low temperatures of the biofilter operation resulted in decreased and slightly increased denitrification efficiency. However, a statistical analysis showed that only in the biofilter operated at 4 °C was the process efficiency significantly different ($p < 0.05$) than in the control biofilter. In this reactor, the efficiency of denitrification was $84.69 \pm 2.72\%$ (Figure 5a).

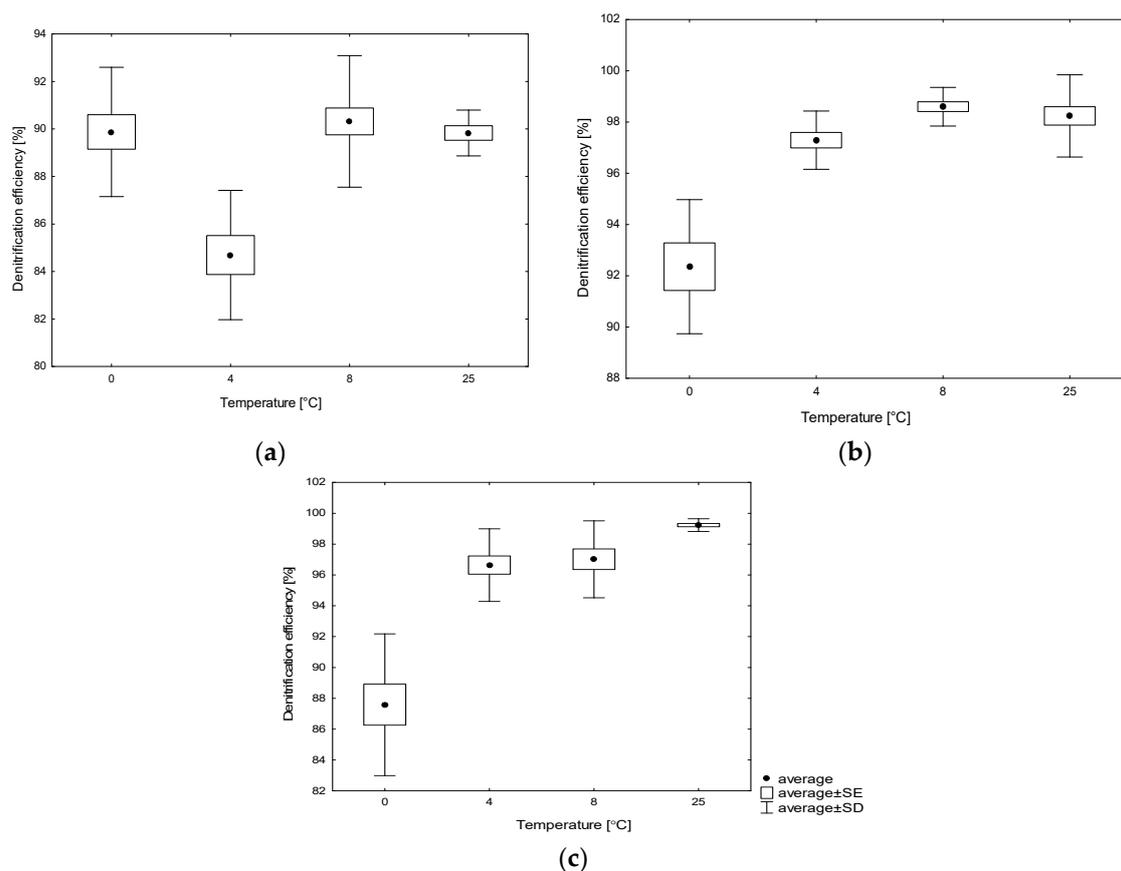


Figure 5. Influence of temperature on the denitrification efficiency in: (a) series 1 (C/N = 0.5 gC/gN), (b) series 2 (C/N = 2.5 gC/gN), (c) series 3 (C/N = 5.0 gC/gN).

Decreasing the temperature to 0 °C in the biofilter did not significantly change the efficiency of denitrification ($p < 0.05$). In this biofilter, $-89.88 \pm 2.72\%$ of oxidized nitrogen was reduced, while in the biofilter operated at 8 °C $-90.32 \pm 2.77\%$ of oxidized nitrogen was reduced.

The change in the technological parameters of the reactor operation in the second series, by increasing the amount of organic carbon in the influent to 2.5 gC/gN significantly increased the denitrification efficiency to $98.24 \pm 1.56\%$ ($p < 0.05$) compared with the first series. A significantly lower concentration of reduced nitrogen compared with the control reactor was only observed in the biofilter operated at 0 °C, in which the efficiency of denitrification was $92.36 \pm 2.62\%$ (Figure 5b).

The efficiency of the reduction of oxidized nitrogen forms in the other reactors ranged from $97.29 \pm 1.14\%$ at 4 °C to $98.60 \pm 0.76\%$ at 8 °C.

The changing of the technological parameters of reactor operation in the third series, by increasing the amount of organic carbon in the influent to 5.0 gC/gN, resulted in the denitrification efficiency of $99.23 \pm 0.42\%$ in the control reactor, although this efficiency was not significantly higher than in the second series ($p < 0.05$).

In the third series, the low temperatures of the reactors operation significantly decreased the efficiency of denitrification compared with the control reactor ($p < 0.05$). In the biofilter operated at 0 °C, the denitrification efficiency was $87.59 \pm 4.60\%$. A multiple comparison test showed that the denitrification efficiency was significantly higher than in the biofilter operated at 0 °C ($96.64 \pm 2.36\%$ and $97.02 \pm 2.50\%$) than in the biofilters operated at 4 °C and 8 °C, respectively (Figure 5c).

It is assumed that the activity of the heterotrophic denitrifying bacteria is less dependent on the temperature than nitrifying bacteria. Denitrification was observed at temperatures from 1 °C to 5 °C [36]. In the present study, the highest denitrification efficiency of $99.23 \pm 0.42\%$ was observed in the control reactor in the third series (C/N = 5.0 gC/gN). In this series, the denitrification efficiency

was significantly reduced ($p < 0.05$) in the biofilters operated at low temperatures. In the other series, a significant relationship between the efficiency of denitrification and the temperature was not observed. Therefore, if the temperature was not the main factor determining the denitrification efficiency, the formation of anoxic zones as a result of oxygen depletion in the biofilm was decisive. At higher temperatures, oxygen consumption was faster in the outer, more active layers of the biofilm, which resulted in the formation of anoxic zones and a greater share of denitrification in the removal of nitrogen from wastewater. The dependency of the formation of anoxic zones on the temperature was confirmed by Liu et al. [37]. The authors observed that at low temperatures, the penetration of oxygen into the biofilm was higher. In the present study, the porous structure of the filling could contribute to the formation of specific oxygen-anaerobic conditions that were favorable to simultaneous nitrification and denitrification [38]. Microscopic analysis of extended-clay aggregates showed the immobilization of microorganisms, both on their surface and in their micropores, intensified biological wastewater treatment processes. On the other hand, in the biofilm operated with a high content of organic compounds in the wastewater, the high number of heterotrophs produced a greater amount of exopolymers, which could increase the thickness and density of the biofilm. A higher density biomass (and lower porosity) may limit effective oxygen diffusion, even in the case of a thin biofilm [39].

3.3. Efficiency of Nitrogen Compounds Removal

In the biofilters, the nitrogen compounds were removed as a result of biomass growth and reduction of oxidized nitrogen to nitrogen oxides and nitrogen gas.

A statistical study showed the significant influence of the temperature of the biofilter operation and the C/N ratio in the wastewater on the efficiency of nitrogen removal from wastewater ($p < 0.05$) (Figure 6). At 25 °C, the lowest efficiency of nitrogen removal of $45.73 \pm 2.85\%$ was noted at the C/N ratio of 0.5 gC/gN. Significantly higher efficiency of nitrogen removal of $52.28 \pm 3.51\%$ was noted at the C/N = 2.5 gC/gN and $53.68 \pm 3.91\%$ at the C/N = 5.0 gC/gN than in the first series.

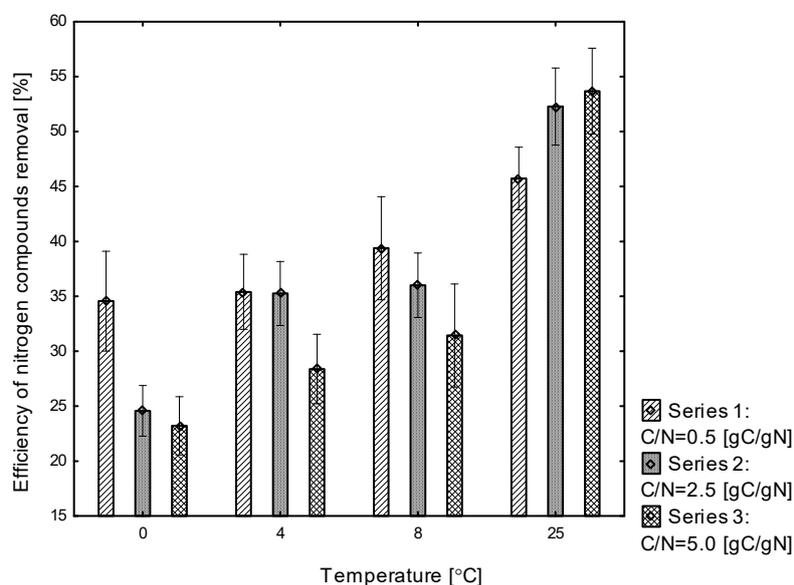


Figure 6. Temperature influence on the efficiency of nitrogen compound removal, depending on the C/N ratio.

Since the efficiency of nitrogen removal from the wastewater at the C/N = 0.5 gC/gN was the most resistant to the low temperatures, in this series the significantly highest efficiency of nitrogen removal from wastewater of $34.57 \pm 4.54\%$ was observed at 0 °C ($p < 0.05$). The statistical study showed a significant decrease in the efficiency of nitrogen removal with an increase in the C/N ratio

at the low temperature of biofilter operation. However, in the control reactors, the reverse tendency was observed.

In the first series, a multiple comparison test showed that in the reactors which were operated at low temperatures, the reduction of the nitrogen compounds was similar and did not significantly differ. The statistical study also did not show significant differences in the share of biomass growth and denitrification observed at low temperatures of the biofilter operation ($p < 0.05$). Only at 25 °C was the share of biomass growth in the removal of nitrogen compounds significantly increased to $6.36 \pm 0.99\%$, which corresponded to a concentration of $2.08 \pm 0.34 \text{ mg N/dm}^3$. Figure 7a presents the concentration of removed nitrogen (determined as the concentration of removed nitrogen compounds) resulting from biomass growth and denitrification in the first series.

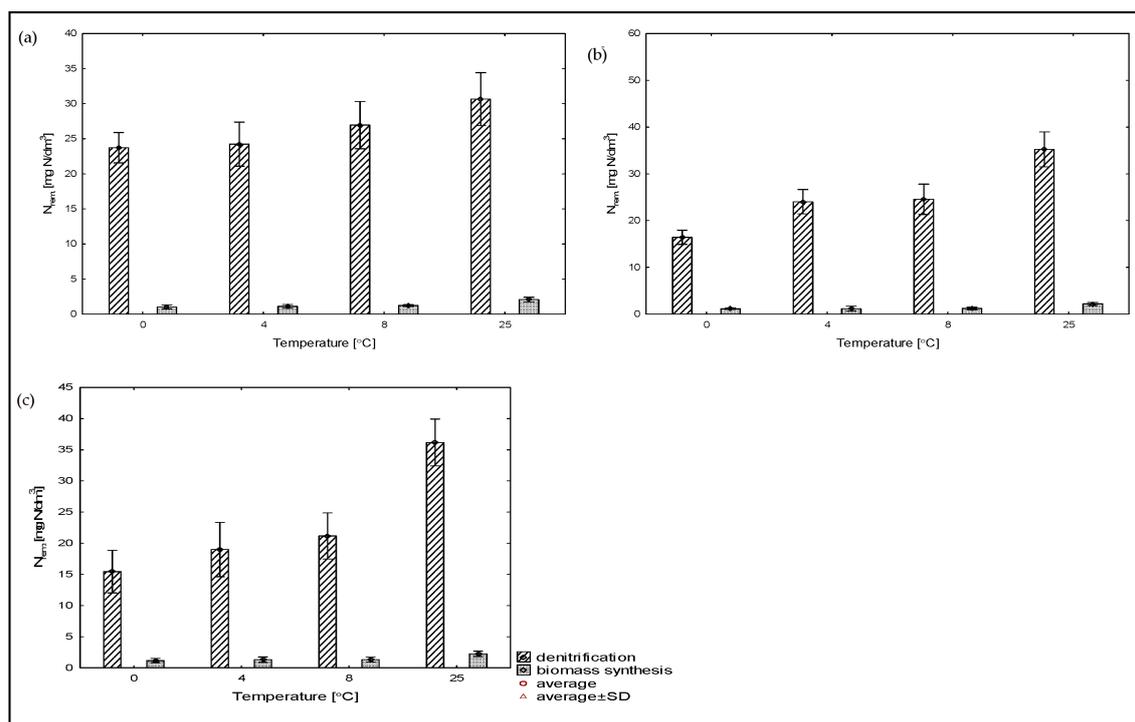


Figure 7. Concentration of removed nitrogen as a result of biomass synthesis and denitrification processes in: (a) series 1 (C/N = 0.5 gC/gN), (b) series 2 (C/N = 2.5 gC/gN), (c) series 3 (C/N = 5.0 gC/gN).

A multiple comparison test showed that in the reactors operated at low temperatures, the share of biomass synthesis and denitrification in nitrogen compound removal was similar and did not significantly differ in the second series ($p < 0.05$). Under these conditions, denitrification predominated in nitrogen compound removal, whereas only $4.68 \pm 0.19 - 6.54 \pm 1.12\%$ of nitrogen was removed (due to the biomass growth). The significantly highest share of biomass growth in the nitrogen compound removal in this series was observed only in the control reactor, in which $5.80 \pm 0.30\%$ of the removed nitrogen (i.e., $2.17 \pm 0.32 \text{ mg N/dm}^3$) was used for biomass synthesis. The concentration of nitrogen compound removal resulting from biomass growth and denitrification in the second series is presented in Figure 7b.

A multiple comparison test showed that in the third series, independent of the variant of the experiment, denitrification predominated in the removal of nitrogen compounds (Figure 7c). The share of biomass growth and denitrification was similar and did not significantly differ at the temperatures of 0 $^{\circ}\text{C}$, 4 $^{\circ}\text{C}$ and 8 $^{\circ}\text{C}$ ($p < 0.05$). The biomass synthesis resulted in the removal of $5.91 \pm 0.15 - 6.98 \pm 1.56\%$ of nitrogen from the wastewater. In the control reactor, the biomass synthesis removed $5.57 \pm 0.21\%$ of nitrogen, which corresponded to concentration of nitrogen removed

of 2.24 ± 0.44 mg N/dm³. The share of denitrification in the removal of nitrogen compounds from wastewater was significantly higher at 25 °C than at the lower temperatures.

A low temperature of biofilter operation significantly decreased the nitrogen removal efficiency from the wastewater ($p < 0.05$). In all biofilters, nitrogen was removed as a result of biomass growth and a reduction of oxidized nitrogen forms. The obtained results suggest that the technological parameters of biofilter operations caused the formation of anoxic zones in the biofilm, which resulted in simultaneous nitrification and denitrification. The possibility of simultaneous nitrification and denitrification in the biofilm has been widely documented in the literature [40]. If both nitrifying and denitrifying bacteria are present in the biofilm and the amount of oxygen is sufficient for the oxidation of organic compounds and nitrification, yet low enough to ensure denitrification, these processes may proceed simultaneously. Puznava et al. [41] determined that the concentration of dissolved oxygen at the level of 0.5–3.0 mg O₂/dm³ reduced the penetration of oxygen and denitrification was possible in its internal layers. The authors obtained an efficiency of denitrification of 71% with simultaneous nitrification reaching 96–98%. However, Zinatizadeh and Ghaytoolin [42] used a dissolved oxygen concentration of 2.5–3.0 mg O₂/dm³, depending on the filling of the MBBR (moving bed biofilm reactor) reactor, and observed the efficiency of nitrogen removal of 46–50% due to simultaneous nitrification and denitrification. Choo and Stensel [43] used a dissolved oxygen concentration of 2.8 mg O₂/dm³ and observed a 27% process efficiency. However, after a reduction of the oxygen concentration below 0.6 mg O₂/dm³, the efficiency of simultaneous nitrification and denitrification increased to 47%.

3.4. Efficiency of Organic Compounds (COD) Removal

The study tested the concentration of organic compounds (COD) in the raw and treated wastewater. The results showed that the efficiency of organic compound removal depended on the C/N ratio in the wastewater and the temperature of the biofilter operation.

The efficiency of organic compound removal was significantly influenced by the C/N ratio in the wastewater ($p < 0.05$) (Figure 8).

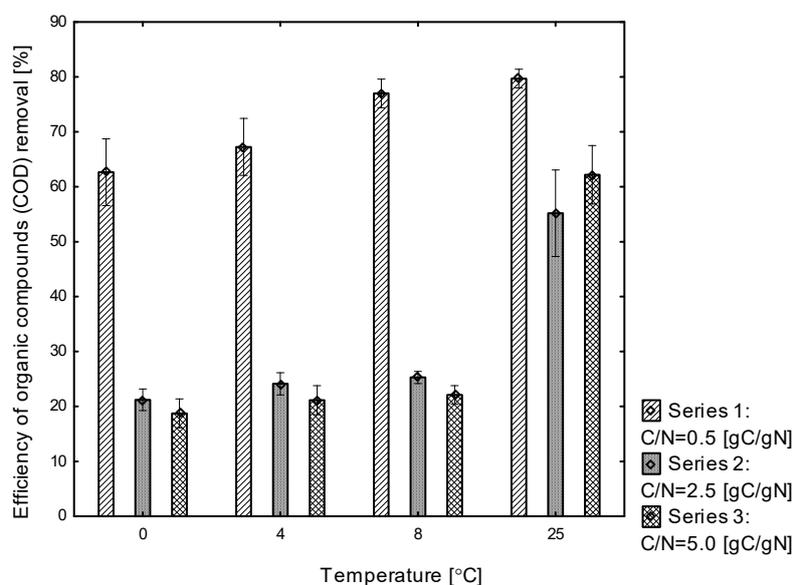


Figure 8. Efficiency of organic compound removal depending on the C/N ratio.

The significantly highest efficiency of organic compound removal, independent of the temperature, was noted in the biofilters operated at the lowest C/N ratio in the wastewater ($p < 0.05$). The most resistant to the low temperatures was the efficiency of organic compound removal in the first series,

i.e., at a C/N ratio of 0.5 gC/gN. For each temperature, increasing the concentration of organic carbon decreased the efficiency of its removal.

A statistical analysis showed a significant effect of the C/N ratio in the wastewater on the concentration of organic compounds in the treated wastewater (Figure 9). The increased concentration of organic compounds in the raw wastewater in the following series of the study resulted in a significant deterioration of the treated wastewater quality, which resulted from a significant increase in the concentration of organic compounds expressed in the COD of the treated wastewater ($p < 0.05$).

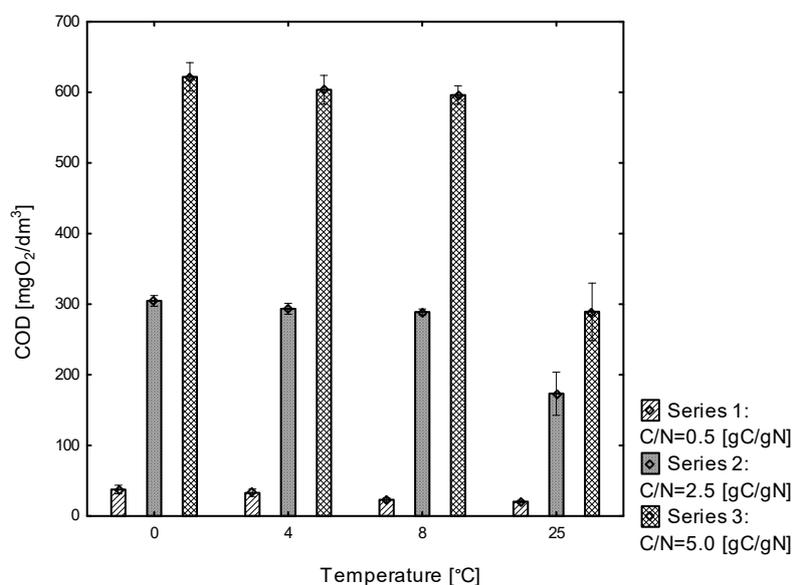


Figure 9. Influence of the C/N ratio on the organic compound concentrations in treated wastewater.

The concentration of organic compounds expressed in the COD in the treated wastewater showed that the application of a C/N ratio above 0.5 gC/gN in the wastewater at the low temperature resulted in a high concentration of organic compounds in the treated wastewater.

The present study showed an increased denitrification efficiency which was associated with an increased concentration of organic compounds in the wastewater. Even in the series with the lowest concentration of organic compounds in the wastewater, the denitrification efficiency was $89.88 \pm 2.72\%$ in the biofilter operated at 0 °C. The high denitrification efficiency resulted from low nitrification efficiency and a low concentration of available NO_x^- . In the first series, the concentration of removed nitrogen compounds was $23.72 \pm 2.17 \text{ mg N/dm}^3$. In the second series, the efficiency of denitrification was $92.36 \pm 2.62\%$, while the concentration of removed nitrogen compounds was $16.44 \pm 1.52 \text{ mg N/dm}^3$. In the third series, the denitrification efficiency was $87.59 \pm 4.60\%$, while the concentration of removed nitrogen compounds was $15.45 \pm 3.41 \text{ mg N/dm}^3$. The decrease in the efficiency of nitrogen removal at a lower concentration of organic compounds most probably resulted from the lower amount of available organic compounds and the better oxygenation of the biofilm. This was confirmed by the nitrogen removal in the control reactor in the first series, in which the process efficiency was $45.73 \pm 2.85\%$ and the nitrification efficiency reached almost 50%, which indicated good oxygen conditions inside the membrane.

4. Conclusions

The study showed that the effective removal of nitrogen compounds from wastewater occurred across a very wide temperature range. The applied filling and properly selected operating parameters of the reactors resulted in effective nitrification and reduction of oxidized nitrogen forms to gaseous nitrogen. Therefore the removal of nitrogen compounds was possible due to simultaneous nitrification and denitrification, in which carbon sources were organic compounds contained in the wastewater.

Maintaining a proper relationship between the amount of carbon and nitrogen in the treated wastewater, through the simultaneous application of de-icing agents containing urea and easily biodegradable carbon compounds, can ensure the effective removal of pollutants at low temperatures. Table S7. Dependent variable: concentration of removed nitrogen as a results of biomass synthesis, qualitative variable: temperature. Table S8. Dependent variable: efficiency of organic compounds removal, qualitative variable: temperature. Table S9. Dependent variable: efficiency of organic compounds removal, qualitative variable: ratio C/N. Table S10. Dependent variable: concentration of organic compounds in treated wastewater, qualitative variable: ratio C/N.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/11/3/630/s1>, Table S1. Composition of model wastewater. Table S2. Dependent variable: nitrification efficiency, qualitative variable: temperature. Table S3. Dependent variable: denitrification efficiency, qualitative variable: temperature. Table S4. Dependent variable: efficiency of nitrogen compounds removal, qualitative variable: temperature. Table S5. Dependent variable: efficiency of nitrogen compounds removal, qualitative variable: ratio C/N. Table S6. Dependent variable: concentration of removed nitrogen as a result of denitrification, qualitative variable: temperature. Table S7. Dependent variable: concentration of removed nitrogen as a results of biomass synthesis, qualitative variable: temperature. Table S8. Dependent variable: efficiency of organic compounds removal, qualitative variable: temperature. Table S9. Dependent variable: efficiency of organic compounds removal, qualitative variable: ratio C/N. Table S10. Dependent variable: concentration of organic compounds in treated wastewater, qualitative variable: ratio C/N. Table S11. Nitrification and denitrification efficiencies and processes in different bioreactor configurations under the conditions tested.

Author Contributions: J.R. developed the research idea and planned the research activities. K.O. carried out the research, including collecting the input data and prepared manuscript. W.J. supervised the research activities and reviewed the final draft of the manuscript. A.M. carried out a statistical analysis of the obtained results. All authors have read and approved the final manuscript.

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