Influence of temperature and C/N ratio on nitrifying and denitrifying bacteria of biofilters treating wastewater from de-icing airport runways

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Abstract. One of the key elements in the operational management of airports is effective de-icing of surfaces. The run-off of precipitation water loaded with pollutants from de-icing is a considerable and costly problem. The aim of our research has been to evaluate the applicability of biofilters filled with light expanded clay aggregate (LECA) generated from incineration of sewage sludge and now serving as a matrix for the development of microorganisms. The tested biofilters treated precipitation water polluted with agents used to de-ice airports. The solution was tested on a laboratory scale, in a range of temperatures from 0 to 25°C, and at concentrations of pollutants from airport de-icing expressed as COD and varying from 503.30 to 3827.50 mg O₂·L⁻¹. The tested filling allowed nitrifying as well as denitrifying bacteria to grow and develop even at low temperatures. Biofilters provide simultaneous nitrification and denitrification, as well as removal of organic compounds, even at 0°C. Biofilters based on the granulate prepared from fly ash from sewage sludge thermal treatment can be a cost-efficient and low-maintenance technology to treat airport surface runoff.

1 Introduction

One of the key elements in the operational management of airports is effective de-icing of surfaces. The run-off of precipitation water loaded with pollutants from de-icing is a considerable and costly problem. De-icing agents are applied on the runway, taxiway and other operational areas to prevent freezing and provide friction for aircrafts and ground handling vehicles. These substances are a significant source of water and soil pollution [1, 2].

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The principal environmental impact of de-icing activities relates to the oxygen demand imposed by the decomposition processes and aquatic toxicity. The following substances are popular agents applied in winter maintenance of airport surfaces: urea, acetate, sodium formate in the solid form, and acetate and potassium formate in the liquid form [3, 4].

A possible way to manage the spent water of this type is by discharging it to municipal sewers to be treated in municipal wastewater treatment plants (WTPs). This is currently the management strategy adopted by 45% of international airports [5]. As a consequence of increased demand for air transport and the projected growth in the global aviation sector, increased application of de-icing fluids will be required in the future. However, long term constraints on sewer network capacity and increasing conveyance, reception and treatment costs are of growing concern, defining the need for sustainable treatment solutions [1]. Large surface areas of airports and the resultant large quantities of discharged pollutants, together with the low temperature of such wastewater, can cause serious problems in the operation of municipal WTPs. Wastewater treatment plants are limited by their capacity and daily hydraulic and organic loading limits could be imposed by the water company. This would require the airport to provide on-site storage during peak events in order to prevent shock loads to the wastewater treatment plants, and to prevent the potential to discharge untreated effluent into the environment [6].

For these reasons, technologies where wastewater treatment facilities are located at a site where wastewater is generated, e.g. aerated lagoons, treatment wetlands (TWs) or ground filters, seem to be a promising solution [2]. However, the efficiency of such facilities suffers when the air and wastewater temperature falls. Reactors with attached-growth biomass, owing to the thermal properties of their filling, have an advantage over other solutions, the fact which has been verified by Hoilijoki et al. [7], who demonstrated that complete nitrification at a temperature below 5°C could only be achieved in a reactor with a filling that enabled immobilisation of microorganisms and consequently the formation of biofilm purifying the wastewater. Moreover, Rostron et al. [8] showed that a drop in temperature from 25°C to 16°C resulted in a decrease in the nitrification efficiency by just 10% in a reactor with immobilised biomass, which was in contrast with a bioreactor with suspended biomass, where the analogous loss of efficiency reached 58%.

Most airports are still not equipped with any systems to treat wastewater containing de-icing agents. Some only have tanks for biodegradation of substances contained in effluents from de-icing of planes. Meanwhile, the airports that have hydrophyte wastewater treatment facilities become colonised by birds, which poses a threat to the safety at these airports [9, 10].

The problem of how to manage and treat wastewater containing agents used for de-icing of airport surfaces is still unresolved, which means that most of these substances penetrate soil and waters. A possible solution could be the implementation of biofilters with a special filling, situated in the immediate vicinity of taxiways, runways and parking spaces at airports [11]. Owing to this solution, the precipitation water flowing from these hardened surfaces would undergo treatment regardless of the weather conditions. As well as being a safe solution generating low maintenance costs, this would reduce the amounts of pollutants permeating the environment. For biofilters to perform their function, it is essential to create the right conditions for the development of microorganisms that ensure adequate efficiency of degradation of pollutants at low temperatures.

The aim of our research has been to evaluate the applicability of biofilters filled with light expanded clay aggregate (LECA) generated from incineration of sewage sludge and now serving as a matrix for the development of microorganisms. The tested biofilters treated precipitation water polluted with agents used to de-ice airports. The solution was tested on a laboratory scale, in a range of temperatures from 0 to 25°C, and at

concentrations of pollutants from airport de-icing expressed as COD and varying from 503.30 ± 7.19 to 3827.50 ± 14.50 mg $O_2 \cdot L^{-1}$.

2 Methods

2.1 Biofilters and wastewater

Samples of the biofilm used for the tests were taken from laboratory bioreactors treating wastewater from airport de-icing. The experiments were performed in laboratory models of biofilters with the filling composed of LECA and with a vertical flow. The technical parameters of each biofilter were the following: surface area 95 cm^2 , volume 0.25 m^3 and active volume 0.155 m^3 . The filling of the biofilters was granulate. It had a structure of expanded-clay aggregate (with a diameter $d_{60} = 8.2 \text{ mm}$) prepared from fly ash originating from incineration of sewage sludge.

The study was performed at a low hydraulic loading of $5 \text{ L} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, which facilitated nitrification, and at a hydraulic retention time of 4 d, which corresponded to the dosing frequency of de-icing agents at airports of 4 d. The study was divided into three series differing in organic loading (series 1 – 503.30 ± 7.19 ; series 2 – 1934.00 ± 9.47 ; series 3 – 3827.50 ± 14.50 mg $\text{COD} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$), which corresponded to the C:N ratios of 0.5, 2.5 and 5.0, respectively. Total nitrogen loading in each series was 357.8 ± 11.00 mg $\text{N} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (Kjeldahl nitrogen – 354.00 ± 15.10 mg $\text{N} / \text{m}^2 \cdot \text{d}$). In each series, four variants with biofilters operating at different wastewater temperatures (0, 4, 8 and 25°C) were separated using a thermostatic chamber. The reactor set at a temperature of 25°C was the control reactor. The first three months of the experiment were adaptive period. It lasted until stabilization of composition of the treated wastewater. After this period each series also has lasted for three months.

The model wastewater used in the experiment contained popular agents for de-icing airport pavements and tap water. The composition of the model wastewater was as follows: in series 1 – 150.00 ± 0.10 mg $\text{CH}_4\text{N}_2\text{O} \cdot \text{L}^{-1}$, 136.00 ± 0.10 mg $\text{HCOONa} \cdot \text{L}^{-1}$ and 49.00 ± 0.10 mg $\text{CH}_3\text{COOK} \cdot \text{L}^{-1}$; in series 2 – 150.00 ± 0.10 mg $\text{CH}_4\text{N}_2\text{O} \cdot \text{L}^{-1}$, 657.00 ± 0.10 mg $\text{HCOONa} \cdot \text{L}^{-1}$ and 237.00 ± 0.10 mg $\text{CH}_3\text{COOK} \cdot \text{L}^{-1}$; and in series 3 – 150.00 ± 0.10 mg $\text{CH}_4\text{N}_2\text{O} \cdot \text{L}^{-1}$, 1326.00 ± 0.10 mg $\text{HCOONa} \cdot \text{L}^{-1}$ and 478.00 ± 0.10 mg $\text{CH}_3\text{COOK} \cdot \text{L}^{-1}$.

2.2 Respiratory activity of the biofilm

The amount of oxygen absorbed by microorganisms in the processes of organic compound decomposition and nitrification was determined. The results were used to determine the percentage of nitrifying bacteria in the biomass of the biofilm. The tests were supported by a respirometric set Oxi-Top, made by WTW, which for 96 hours registered changes in the partial pressure in the measuring chamber, caused by the consumption of oxygen due to microbial respiration. Changes in the pressure were converted to amounts of oxygen consumed, expressed in mg $O_2 \cdot L^{-1}$. On the basis of the respirometric assays, an estimate of the mass of nitrifying bacteria in the reactor was made, and the percentage of nitrifying bacteria in the biomass of the biofilm was calculated.

2.3 Determination of the most probable number (MPN) of bacteria

Samples of the biofilm from biofilters were collected three times during the study and submitted to analysis in order to quantify the presence of nitrifying and denitrifying bacteria. The following were determined:

- number (MPN·100 cm⁻³) of bacteria of the 1st step of nitrification, oxidising N-NH₄ to N-NO₂, and of the 2nd step of nitrification, oxidising N-NO₂ to N-NO₃, on Meiklejohn medium with the addition of (NH₄)₂SO₄ and NaNO₂, respectively, after 14 and 19 days of incubation at a temperature of 25°C,
- number (MPN·1cm⁻³) of denitrifying bacteria reducing NO₃-N to N₂O and N₂, on Giltay medium in Durham test tubes after 7 days of incubation at 25°C.
- number (MPN·1cm⁻³) of bacteria reducing N-NO₃ to N-NO₂, on Giltay medium after 7 days of incubation at 25°C.

In the case of nitrifying bacteria, the presence of N-NO₂ (1st step) or N-NO₃ (2nd step) was ascertained after the incubation period in cultures. N-NO₂ was detected with the help of the dry Griess reagent, while the presence of N-NO₃ was attested using diphenylamine in concentrated H₂SO₄, having first decomposed all non-oxidised N-NO₃ remaining in the medium with the help of urea and sulphuric acid.

2.4 Physicochemical analyses

Determinations of nitrate concentration, nitrite concentration, ammonium nitrogen concentration, Kjeldahl total nitrogen, organic compounds concentration (COD) were carried out according to the APHA [12]. Concentration of total nitrogen (TN) was analysed on a Shimadzu Corporation TNM-L analyzer (Japan) with the “oxidative combustion-chemiluminescence” method.

2.5 Statistical analysis

Statistical analysis was performed using the Statistica 13.1 PL package. The results are presented in the form of mean values ± standard deviation from using one-way analysis variance (ANOVA). The significance level of $\alpha = 0.05$ was used.

3 Results and discussion

The influence of temperature and the concentration of pollutants on the development of nitrifying and denitrifying bacteria was assessed through the determination of changes in the concentrations of particular forms of nitrogen, efficiency of the removal of nitrogen and organic compounds, measurements of respiratory activity of microorganisms, and number (MPN) of nitrifying and denitrifying bacteria growing on the filling in a reactor.

This study has demonstrated that organic pollutants and nitrogen compounds can be removed from wastewater originating from de-icing of airport surfaces at low temperatures in biofilters with the filling composed of LECA granulate. The results indicate that this type of a filling can be beneficial for wastewater treatment processes, and that it creates suitable conditions for the development of microorganisms and for simultaneous nitrification and denitrification even at low temperatures.

A drop in the temperature maintained in working biofilters, in addition to an increase in the content of organic carbon in wastewater, led to a decrease in the percentage of nitrifying bacteria in the biomass of the biofilm, and in the MPN of 1st and 2nd step nitrification bacteria (Fig. 1). Regardless of the temperature, the highest share of nitrifying bacteria was

detected on the biofilm in bioreactors of the first series ($C/N = 0.5$). The impact of the quantity of organic carbon in dosed wastewater as a factor that depressed the share of nitrifying bacteria in the biofilm biomass was the least pronounced at a temperature of 0°C . In this variant, the number of bacteria oxidising ammonia nitrogen decreased from 12.10% in the first series to 7.17% of the biofilm biomass in the third series ($C/N=5$). Similar relationships were found when analysing the MPN of nitrifying bacteria, both involved in the 1st and in the 2nd nitrification step. In each series of experiments, a decrease in the temperature in biofilters contributed to a decrease in the share of nitrifying microorganisms in the biofilm biomass in comparison with the same parameter in the control bioreactor (temp. = 25°C). The activity of nitrifying microbes, which to a large extent depends on thermal conditions, did not change in response to lower temperatures so much as to inhibit completely the process of ammonia nitrogen oxidation.

Fig. 1. Most probable number (MPN) of nitrifying bacteria: A – MPN of 1st step nitrification bacteria in the biofilm; B – MPN of 2nd step nitrification bacteria in the biofilm; C – results of tests on the respiratory activity of the biofilm (percentage of nitrifying bacteria in the biofilm).

Regardless of the temperature, the highest number of denitrifying bacteria was determined in bioreactors in the third series ($C/N = 5.0$) (Fig. 2). The effect of an increase in the organic carbon content of wastewater as a factor that raised the number of denitrifying bacteria was most evident in the control reactor, where the MPN of denitrifying bacteria rose from 140 MPN·cm⁻³ (series 1) to 45,000 MPN·cm⁻³ (series 3). In all series, the highest number of denitrifying bacteria was determined in bioreactors set at a temperature of 25°C .

The working temperature of bioreactors and the C/N ratio had a significant effect on the activity of microorganisms, simultaneously affecting the final efficiency of the removal of nitrogen and carbon compounds from wastewater (Tab. 1). The highest efficiency of organic pollutant removal in reactors, irrespective of the temperature, was found at the lowest C/N. Although the lowering of the bioreactor working temperature led to a considerable decrease in the achieved efficiency of the oxidation of organic compounds, a high (over 60%) removal rate of organic substances was achieved in series 1 even at

a temperature of 0°C. The current results are in accord with the literature data, which implicate that at a higher load of organic pollutants, the COD in treated wastewater increases [13]. A possible way to improve the efficiency of wastewater treatment at low temperatures is by diminishing the load of organic pollutants [14]. In turn, Yadu et al. [15] demonstrated a decrease in the efficiency of the removal of ammonia nitrogen as the C/N-NH₄ rose from 2 to 10. These researchers showed that the C/N ratio equal 4 (at HRT=41 h) is the optimal one for the removal of organic pollutants and nitrogen compounds. Values of COD of the wastewater discharged from the bioreactors suggest that especially when the bioreactor's working temperatures are low, the C/N ratio in raw wastewater above 0.5 gC/gN is associated with high concentrations of organic compounds in treated wastewater. Based on the results obtained from this study, it can be assumed that a value of the C/N ratio in wastewater undergoing treatment equal 0.5 does not have a negative effect on the activity of nitrifying bacteria, and still ensures suitable conditions for the simultaneous nitrification and denitrification in the tested bioreactors.

Fig. 2. Most probable number (MPN) of denitrifying bacteria A-C/N = 0.5; B-C/N = 2.5; C-C/N = 5.0.

It is indicated that the activity of denitrifying bacteria is less dependent on temperature than that of nitrifying bacteria [16]. Although the temperature around 10°C partly inhibits the reduction of nitrates, it is possible to maintain the efficiency of this process on a high level by supplying easily available organic carbon [17]. In this study, the dominant forms of nitrogen in the wastewater discharged from bioreactors were ammonia nitrogen and organic nitrogen. The share of oxidated nitrogen compounds did not exceed 6% (Tab. 1). The tests showed an improved efficiency of denitrification achieved as the percentage of organic compounds increased, but even in the series with the lowest C/N ratio (0.5 gC/gN) a high (89.88 ± 2.72%) efficiency of denitrification at a temperature of 0°C was obtained. Data presented in the literature concerning denitrifying bacteria suggest a considerable dependence between the ratio of organic compounds to nitrogen in wastewater and the efficiency of nitrate respiration [18]. Dhamole et al. [19] obtained complete denitrification at a C/N ratio of 2.0 gC/gN-NO₃. When the value of this ratio fell below 2, treated wastewater was observed to contain ammonia nitrogen.

The results obtained in this study suggest that the applied technological parameters of the bioreactors ensured the process of simultaneous nitrification and denitrification (SND). Possible occurrence of SND on a biological membrane is broadly documented in the literature [20, 21]. If both nitrificants and denitrificants are present in the biofilm, and the amount of oxygen is sufficient to oxidise organic compounds and to carry out nitrification, but low enough to ensure denitrification, then these processes can run concurrently.

Apart from the positive effect on the process of wastewater treatment, which arises from the ensurance of appropriate conditions for the growth of microorganisms, the application of granulate made from fly ash originating from sewage sludge incineration brings about an additional pro-ecological effect, such as the management of the waste that previously was mostly stored on dumping sites.

Table 1. Average ($\pm$ SD) values of parameters of treated wastewater.

C/N [gC/gN]	Temp. [°C]	Concentration				
		N-NH ₄ [mgN·L ⁻¹]	N _{organic} [mgN·L ⁻¹]	N-NO ₂ [mgN·L ⁻¹]	N-NO ₃ [mgN·L ⁻¹]	COD [mgO ₂ ·L ⁻¹]
0.5	0	25.46±1.68	20.68±6.46	0.16±0.12	0.51±0.47	37.60±6.10
	4	28.01±3.45	14.01±5.15	1.65±0.72	2.73±1.64	32.97±5.22
	8	23.63±2.35	18.73±6.47	0.83±0.21	2.06±0.41	23.15±2.61
	25	18.02±3.09	21.77±7.68	0.18±0.12	3.29±1.05	20.43±1.72
2.5	0	4.75±1.31	51.71±5.12	0.93±1.13	0.43±0.42	304.75±7.63
	4	13.80±3.01	35.79±4.31	0.39±0.27	0.28±0.26	293.50±7.78
	8	7.91±2.17	44.37±4.90	0.10±0.11	0.25±0.12	289.00±4.24
	25	15.39±3.81	12.83±7.04	0.17±0.12	0.41±1.01	173.33±30.50
5.0	0	6.65±1.75	49.26±8.54	1.90±2.29	0.29±0.26	622.00±20.12
	4	5.25±1.78	50.54±4.28	0.43±0.95	0.23±0.14	603.67±20.31
	8	4.04±1.80	49.23±6.03	0.39±0.74	0.26±0.15	596.33±13.05
	25	9.37±5.15	26.09±5.69	0.07±0.04	0.17±0.14	289.33±40.67

* average ($\pm$ SD) values of raw wastewater concentration: TN = 71.56 $\pm$ 2.20 mg N·L⁻¹; N_{Kjeldahl} = 70.80 $\pm$ 3.02 mg N·L⁻¹; N_{organic} = 70.80 $\pm$ 3.02 mg N·L⁻¹; N-NH₄ < 0.01 mg N·L⁻¹; N-NO₂ < 0.01 mg N·L⁻¹; N-NO₃ < 0.01 mg N·L⁻¹; COD = 100.66 $\pm$ 1.34 mgO₂·L⁻¹ (C/N = 0.5), 386.80 $\pm$ 1.94 mg O₂·L⁻¹ (C/N = 2.5), 765.50 $\pm$ 2.90 mg O₂·L⁻¹ (C/N = 5.0)

4 Conclusions

The research presented in this paper has demonstrated the usefulness of an implementation of biofilters filled with granulate made of ash from incineration of sewage sludge, as this solution decreases the parameters of pollution in wastewater containing de-icing agents used at airports. The tested filling allows nitrifying as well as denitrifying bacteria to grow and develop even at low temperatures. A drop in the temperature in biofilters, in addition to an increase in the content of organic carbon in treated wastewater, led to a decrease in the percentage of nitrifying bacteria. Regardless of the temperature, the highest number of denitrifying bacteria was determined in bioreactors with C/N = 5.0. The highest efficiency of COD removal, irrespective of the temperature, was achieved at the lowest C/N. With the lowering of the temperature, the efficiency of organic compounds removal was decreased. However, a high (over 60%) removal efficiency of organic substances was achieved even at a temperature of 0°C. The study showed an increase of denitrification efficiency with the increase of organic compounds concentration. But even at C/N = 0.5 a high (89.88 $\pm$ 2.72%) efficiency of denitrification at a temperature of 0°C was obtained.

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