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REMOVAL OF NITRATES FROM WASTEWATER USING POND BOTTOM SOIL

The possibility of the final treatment of mine water from chemical leaching has been investigated. Despite water purification, high nitrate levels remain in these waters and must be removed. The main requirements are the lowest economic, operating and staffing levels. These requirements are best achieved by the removal of nitrates using biological methods, in our case using the pond bottom soil. Experiments were carried out in a batch mode. The effect of the environment on the denitrification process, the influence of the type and amount of the organic substrate and basic parameters of the denitrification such as redox potential, pH and dissolved oxygen were studied.

1. INTRODUCTION

Industrial, municipal, and agricultural effluents containing high NO_3-N levels can introduce large amounts of nitrates into surface and groundwaters, and this can end up in water supplies, causing eutrophication and a range of associated effects. Agriculture is a major source of nitrate pollution due to N fertilizers and run-off from animal feedlots [1, 2]. Specific industrial source of nitrate pollution is mining. One possibility of minerals mining is the extraction with leaching acids, which are pushed into the ground through wells to the leaching fields. It is necessary to remove residual acidity after mining in order not to endanger extensive reserves of drinking waters in the mining area above the acceptable limit. There is used a procedure when exhausted acid waters, drained from the underground, are neutralized in the surface treatment plant, usually by calcium hydroxide or barium chloride [3]. Residual amount of nitrates remains in neutralized waters which is necessary to reduce before discharge into receiving waters.

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The main requirements for wastewater treatment processes are the lowest economic, operating and staffing levels. These requirements are best met by biological treatment methods based on natural processes. In addition, companies engaged in mining in Czech Republic have enough free space and capacity resulting from past activities. This is a potential opportunity for natural methods.

Natural methods of wastewater treatment use self-purification processes which occur in soil, water and wetland environments [4, 5]. In general, the process of self-purification of water is a combination of physical, chemical and biological (aerobic and anaerobic) mechanisms. Examples of such treatment plants are generally constructed wetlands and stabilization ponds including aerobic low- and high-load biological ponds/tanks, aerated and anaerobic ponds, sedimentation and accumulation biological flow ponds and special tanks and cascades of aquaculture in open natural and artificial environments [5, 6].

The most common type of pond is a biological stabilization one, characterized by a balance between aerobic and anaerobic processes. Stabilization ponds consist mostly of small pond type tanks, modified to complete the treatment of mechanically and biologically treated wastewater. The ponds are organized in cascades, with the last one being capable of sustaining fish life [7, 8].

In not aerated ponds, the treatment processes take place in the same way as in stagnant or slow-moving water. Oxygen transfer and mixing depend heavily on the climatic and meteorological conditions. In shallow ponds, the large surface area relative to the small depth means that climatic conditions have a strong influence on the body of water. The effects of temperature and wind, which can create waves, can be intense and can have an effect well below the surface of the water [9]. But oxygen supplied by aeration of the surface represents only a small proportion relative to the oxygen caused by the activity of algae [10].

The self-purification ability of water mainly depends on the population density of bacteria, cyanobacteria, algae, aquatic plants and animals (species composition and their number) in water [11]. The most intensive process occurs on the surface of the bodies submerged in water, i.e. the surface of the stones, stalks and leaves of aquatic plants, and the tufts of filamentous algae. A thin layer of slimy sludge formed by fine organic matter where the bacteria, algae and small animals live, first forms on the submerged objects and plants. This layer operates like an activated sludge in biological wastewater treatment plants [12].

In the biological cycle of substances (C, P, N, S, etc.) in ponds, the presence of microorganisms and the composition of water is as important as the composition of the pond bottom soil (sediment). Interaction between the pond bottom soil and water are important regulators in nitrogen biochemistry [13]. Representation of various forms of nitrogen in water is only a momentary external appearance of a dynamic process, during which the nitrogen is transferred from one form to another. The driving forces in this process are various kinds of bacteria and their enzymatic systems [12].

Pond bottom soil can be divided into a very thin aerobic layer overlaying a much thicker anoxic layer. Therefore the potential for denitrification in these ponds is very high [13]. The biological pathway for denitrification involves a stepwise conversion of nitrate (NO_3^-) to molecular nitrogen (N_2) through intermediates including nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) [14]. Although the denitrification is an anoxic process, therefore it cannot dominate in an aerobic pond, significant daily variations in the concentration of dissolved oxygen (in relation to the quantity of phytoplankton) provoke a decrease of oxygen to very low levels during the night, allowing denitrification to be possible. Such alternation of aerobic and anaerobic conditions between day time and night time is similar to the controlled conditions in activated sludge systems. The decrease in nitrate and nitrite content may also be caused by plankton uptake, if the otherwise preferred ammonia concentration decreases to a very low value [15]. Nitrogen capturing and retention in the stabilization ponds depend on many factors such as the kind and the extent of contamination, the C:N:P ratio, which is recommended to be 40:10:1, the retention time of water in the pond (14–35 days), the shape and depth of the pond, etc. The retention time of the water in the pond is important, especially for the aforementioned nitrogen uptake by phytoplankton. With a lack of phosphorus, the growth of phytoplankton is not sufficient and nitrogen is bound only partially [16].

2. EXPERIMENTAL

For this project, to evaluate a possible rate of denitrification in model wastewater, pond bottom soil originating from the Labe pond in Hradec Králové, Czech Republic, was used. All the experiments were carried out using twelve glass bottles and, following an earlier study [17], each bottle contained 250 g of pond bottom soil (content of dry matter 19.7%). The bottles containing the soil were then filled with 2 dm^3 of sodium nitrate solution (80 mg NO_3-N/dm^3) prepared from drinking water. To assess the effect of the type and the amount of organic substrate on the denitrification process, two different substrates each with two different concentrations were tested. The addition of the organic substrate is necessary because the organic load of the real mine wastewater is very low, due to its origin. Ethanol was used as the organic substrate for the first batch of six bottles in the ratios of COD:N 4:1 and 8:1. The other batch was filled with commercially available Brenntaplus VP1 (Brenntag CR) organic substrate, hereafter referred to as Brennta (a mixture of alcohols, sugars and proteins) in the same ratios of COD:N as for ethanol, i.e. 4:1 and 8:1. A solution of $Na_3PO_4 \cdot 12H_2O$ was added to all the bottles, giving final ratios of nutrients COD:N:P = 40:10:1, and 80:10:1.

To evaluate a possible influence of the environment on the denitrification, four bottles representing both substrates and nutrient ratios were placed in our laboratory at 19–23 °C. Another four bottles were also left in the laboratory, but in the dark, to inhibit the growth of algae. There was no trace of algae growth in either dark or light conditions.

The latter four bottles were placed in the cold (5–8 °C). During the 25 days of our experiment the samples of model wastewater were regularly tested. The following parameters of denitrification were monitored: oxidation-reduction potential (ORP), pH, the content of dissolved oxygen and temperature. A portable Hach Lange (HQ 30d) device with Intellical ORP/redox probes, an MTC 101103 probe and a PHC 101-03 pH probe were used to measure the ORP and pH. A Hanna HI 9146 oximeter with accessories was employed to assess the dissolved oxygen. Both devices were equipped with a temperature sensor.

Concentration of nitrate nitrogen (NO_3-N) was measured by spectrometric methods using sulfo-salicylic acid. Concentration of nitrite nitrogen (NO_2-N) was determined using molecular absorption spectrometric method. The ammonium molybdate spectrometric method was used to assess the content of phosphorus. And finally the content of COD was determined by the Spectroquant® (Merck) cuvette test based on the standard determination of the chemical oxygen demand index – small-scale sealed-tube method.

Based on the analysis, the concentration of nitrates was added to the same level as at the beginning (it is represented by the dotted line in the figures) as were the concentrations of organic substrate and phosphate to preserve nutrient ratio.

3. RESULTS AND DISCUSSION

The experiments confirmed the effect of temperature on the activity of denitrifying bacteria. At room temperature, the effectiveness of nitrate removal is greater than at 5–8 °C. This corresponds to the well-known dependence of biological processes on temperature. An important role is also played by the temperature dependence of solubility of oxygen in water. At higher temperatures, the solubility is lower, the oxygen is rapidly depleted and the denitrification process is thus facilitated [18].

Table 1 shows the total quantity of the removed nitrate nitrogen over 25 days in particular bottles for the selected experimental conditions. Nitrates have been most effectively removed in bottle LE80 containing ethanol as organic substrate and the COD:N:P ratio of 80:10:1 under laboratory conditions. This system was able to remove a total of 903.1 mg NO_3-N over 25 days. This was followed by the results for bottle DE80 with the same parameters, but placed in the dark, where the removal of NO_3-N amounted to 857.6 mg. In the bottle LB80 containing the substrate Brennta with the higher COD:N:P ratio 836.9 mg NO_3-N_{was} removed at room temperature, but only 735.5 mg NO_3-N in the dark (bottle DB80). In other bottles (LB40, LE40, DB40, and DE40), with substrates at the lower ratio, nitrate removal during the experiment was from 470.0 to 525.6 mg NO_3-N. The lowest nitrate removal was achieved in the bottle CE40 placed in the cold, where over 25 days a total of 174.5 mg NO_3-N was removed. On the contrary, the surprise was bottle CB80 where 601.1 mg NO_3-N were eliminated despite being left in the cold over

the same time. The results showed that the recommended ratio of nutrients leads to effective removal of nitrates but when using a higher ratio, the denitrification is more effective and faster. On the other hand, it is accompanied by a higher consumption of substrate and therefore involves higher costs.

Table 1

Total quantity of NO_3-N removed over 25 days

Bottle	Conditions	Organic substrate	COD:N:P	NO_3-N [mg/dm^3]
LB40	Laboratory, light, 19–23 °C	Brennta	40:10:1	470.0
LE40		ethanol	40:10:1	525.6
LB80		Brennta	80:10:1	836.9
LE80		ethanol	80:10:1	903.1
DB40	Dark, 19–23 °C	Brennta	40:10:1	507.0
DE40		ethanol	40:10:1	493.4
DB80		Brennta	80:10:1	735.5
DE80		ethanol	80:10:1	857.6
CB40	Cold, dark, 5–8 °C	Brennta	40:10:1	261.5
CE40		Ethanol	40:10:1	174.5
CB80		Brennta	80:10:1	601.1
CE80		ethanol	80:10:1	307.7

The course of the removal of nitrates under the experimental conditions is shown in Figs. 1–3 together with the possible occurrence of nitrites. The accumulation of nitrites during denitrification can occur due to several factors. Nitrites occur in those denitrification processes which are not sufficiently supplied with a source of carbon [19], their occurrence being related to the type of the organic substrate [20, 21]. The selected initial nitrate concentration depending on the C:N ratio can also influence the process [22]. Inhibition of denitrification usually occurs with a nitrite concentration of 200 mg NO_2/dm^3 [23]. During our experiments at the ethanol to substrate ratio COD:N:P of 40:10:1 under laboratory conditions and in the dark, NO_2-N concentrations gradually increased and reached 49 mg/dm^3 (Figs. 1b, 2b). Concentration of NO_2-N in cold conditions reached a maximum of 20 mg/dm^3 (Fig. 3b). The situation was quite opposite with the ratio COD:N:P = 80:10:1. In cold conditions, NO_2-N concentrations reached 45 mg/dm^3 (Fig. 3d), while at normal temperatures, with exposure to daylight or in the dark, NO_2-N values reached a maximum of 5 mg/dm^3 (Figs. 1d, 2d). When using Brennta, the higher ratio COD:N:P reached levels of NO_2-N under all conditions up to 0.4 mg/dm^3 (Figs. 1c, 2c, 3c). At the lower COD:N:P ratio the concentration of NO_2-N under laboratory conditions and in the absence of light was 3.4–7.9 mg/dm^3 and then, towards the end of the experiment, the value fell below 0.2 mg/dm^3 (Figs. 1a and 2a). In the cold, the trend was similar (Fig. 3a).

3.1. IMPACT OF THE OXIDATION REDUCTION POTENTIAL

Generally, nitrate (and nitrite) ions are used for bacterial degradation of organic substrate in an operational condition having a redox potential from 50 mV to –50 mV [18].

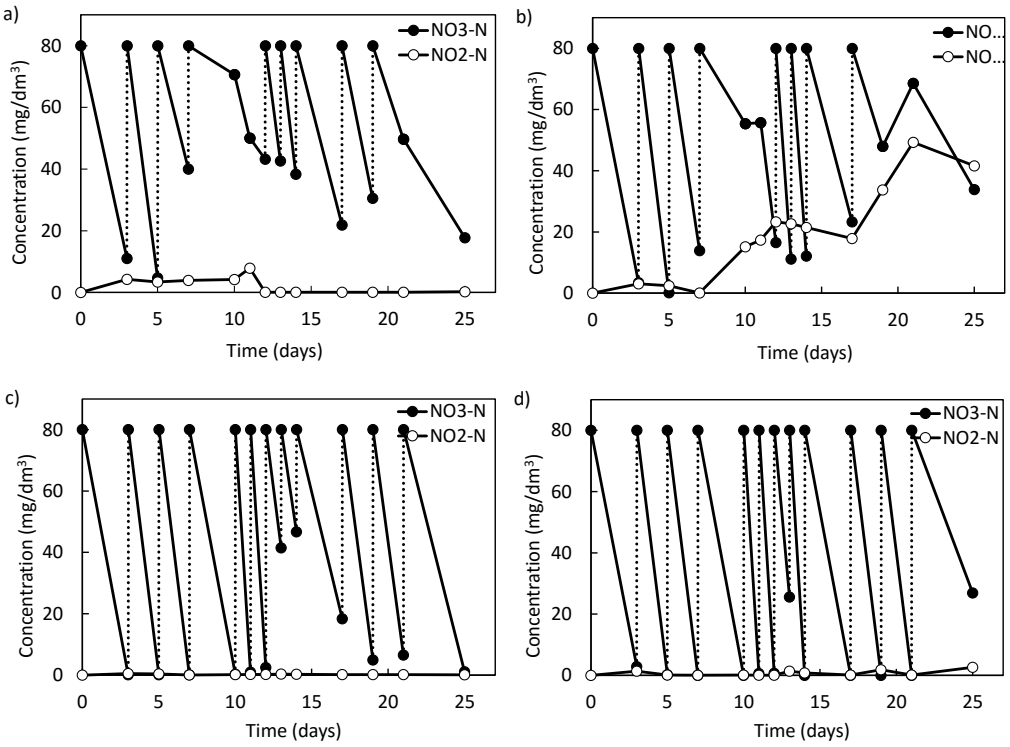

Fig. 1. Course of denitrification under laboratory conditions (presence of light, 19–23 °C); a) substrate Brennta, COD:N:P = 40:10:1, bottle LB40, b) ethanol, COD:N:P = 40:10:1, bottle LE40, c) Brennta, COD:N:P = 80:10:1, bottle LB80, d) ethanol, COD:N:P = 80:10:1, bottle LE80). The dotted line represents an addition of nitrate to the initial solution

At the beginning of the experiments, the ORP reached 200 mV in all bottles due to the preparation of model wastewater. In the case of the bottles placed in the laboratory, the ORP subsequently precipitously fell to –300 mV or –400 mV, except for bottle LB40, for which the value reached only –150 mV. The influence of the substrate was found, however, regardless of its type – this was reflected after the 10th day of the experiment. The ORP in bottles with the lower COD:N:P ratio was in the range from –100 mV to –150 mV, while ORP in the bottles with the higher ratio ranged between –200 mV and –300 mV. In bottles placed in the dark, the trend was similar. In bottles DB40 and

DE40 containing substrate at a lower ratio, the ORP decreased from an initial positive value to zero and gradually fell to –150 mV and –200 mV, respectively, measured at the end of the experiment. Bottles DB80 and DE80 with the higher ratio of substrate showed the ORP from –250 mV to –300 mV. On the other hand, in bottles placed in the cold, the influence of the type of substrate regardless of its amount was observed. The ORP in bottles containing ethanol as organic substrate gradually decreased from initial values to –100 mV (10th day) and then began to increase to positive values. At the end of the experiment, the ORP reached values of 82 mV and 79 mV, respectively. In bottles containing Brennta as an organic substrate, the ORP decreased from the initial value of 200 mV to –100 mV. In the case of the lower ratio of substrate (bottle CB40), this value was maintained until the end of the experiment. For the higher ratio COD:N:P (bottle CB80) although the ORP decreased by the 10th day to –500 mV, on the 13th day of the experiment it reached –100 mV.

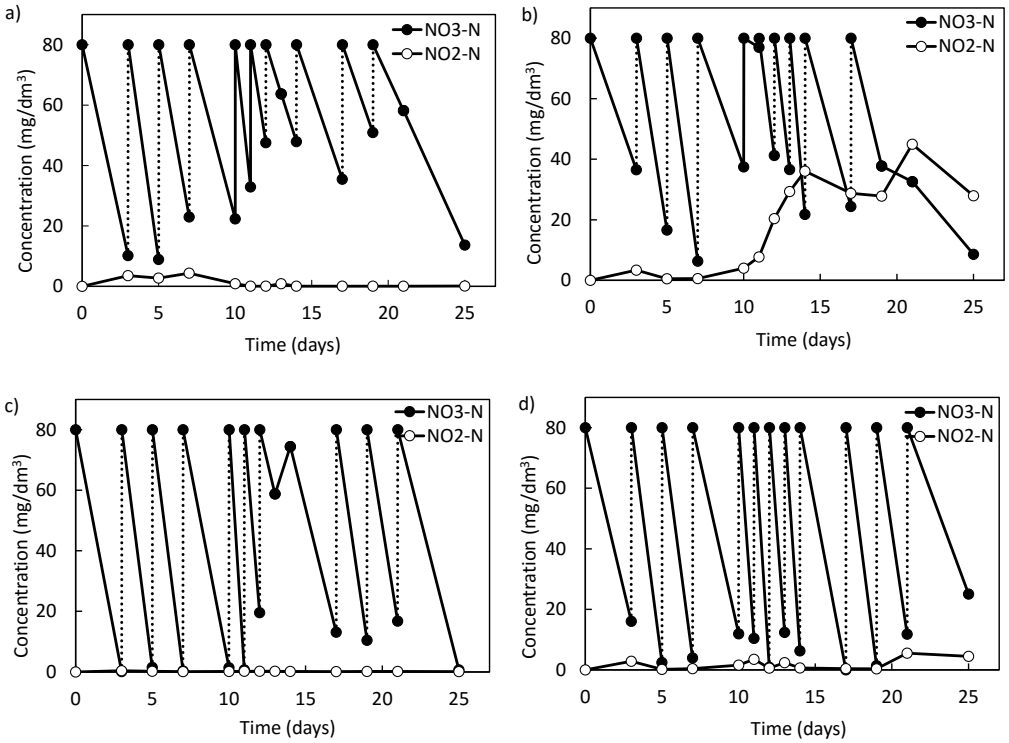

Fig. 2. Course of denitrification in dark, 19–23 °C):
a) substrate Brennta, COD:N:P = 40:10:1, bottle DB40, b) ethanol, COD:N:P = 40:10:1, bottle DE40, c) Brennta, COD:N:P = 80:10:1, bottle DB80, d) ethanol, COD:N:P = 80:10:1, bottle DE80. The dotted line represents an addition of nitrate to the initial solution

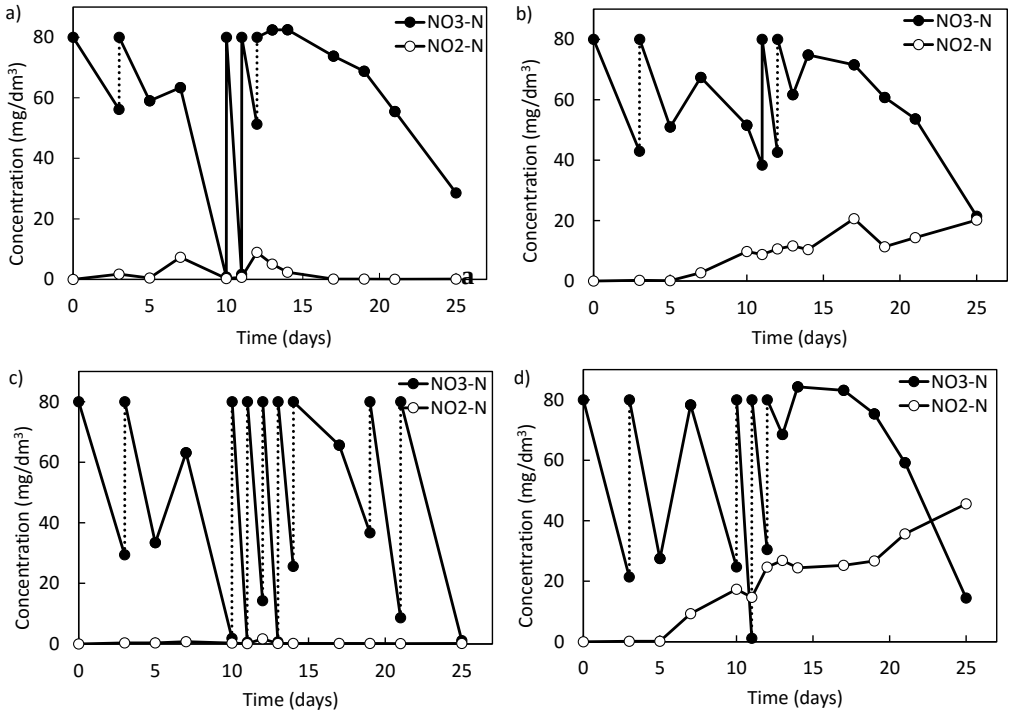

Fig. 3. Course of denitrification in cold, absence of light, 5–8 °C):
a) substrate Brennta, COD:N:P = 40:10:1, bottle CB40, b) ethanol, COD:N:P = 40:10:1, bottle CE40
c) Brennta, COD:N:P = 80:10:1, bottle CB80, d) ethanol, COD:N:P = 80:10:1, bottle CE80.
The dotted line represents an addition of nitrate to the initial solution

3.2. IMPACT OF PH

Denitrification can occur over a wide range of pH values with the optimal pH range of 6.5–7.5 [24]. However, hydroxide ions are released during the denitrification leading to alkalization of the environment, as demonstrated in our experiments. The pH values during denitrification were influenced by the type and quantity of substrates used. pH in the bottles containing ethanol placed in the laboratory in the dark gradually increased from neutral to the alkaline area and on the 5th day of the experiment was at 8.7 for the ratio COD:N:P = 40:10:1. In the case of a higher ratio samples, pH reached 9.2 and stayed around this value until the end of the experiment. In bottles placed in the cold, pH increased more slowly and the alkaline reaction was reached on the 10th day of the experiment. For the ratio COD:N:P = 40:10:1, the pH range was around 8.3 and around 8.6 for the higher ratio. In bottles with Brennta placed in the laboratory in the dark, pH increased very slowly, with maximum values being about 8.4 for the ratio COD:N:P of 40:10:1 and, for the higher ratio pH values were about 8.0. However, these values were achieved only at the end of the experiment. For bottles placed in the cold, pH increased

more slowly compared with those in other bottles and reached its final maximum value of 7.7 for both ratios.

3.3. IMPACT OF DISSOLVED OXYGEN

Concentration of the dissolved oxygen (DO) at the beginning of the experiment was considerable (up to 6.0 mg/dm^3) due to the preparation of model wastewater. However, by the 3rd day of the experiment, the DO concentration decreased to zero, except for bottles placed in the cold, where the level zero was recorded on the 5th day of the experiment for the Brennta substrate and on the 7th day for the ethanol substrate. Thereafter, through to the end of the experiment, DO concentrations ranged from zero to 0.4 mg/dm^3. At these low concentrations (lower than 1.0 mg/dm^3), free molecular oxygen does not compete with the oxygen bound in the nitrate and nitrite, which can be reduced to elementary nitrogen by bacteria [25].

4. CONCLUSION

In this project, the possibility of nitrate removal from wastewater using pond bottom soil has been studied. Experiments were carried out in a batch mode. The results have demonstrated that pond bottom soil possesses significant potential for denitrification. Denitrification was effectively carried out at room temperature in the presence of light using ethanol as an organic substrate with a COD:N:P ratio = 80:10:1. The results confirmed the effect of temperature on the removal of nitrate, while the presence of light as a major factor was not demonstrated. The occurrence of nitrite during denitrification was observed when using ethanol as an organic substrate, especially at lower COD:N:P ratios. In the case of the Brennta organic substrate a significant accumulation of nitrites has not been observed during the experiments.

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