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Denitrification of Industrial Wastewater

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Abstract

The aim of this thesis was the removal of nitrates from industrial waste water, namely waste water with low organic load. The main requirements for the denitrification process are lowest economic, operating and staffing levels. These requirements are best achieved by the removal of nitrates using natural biological methods. This paper describes the possibilities of denitrification using pond sediment, biological filter, sulfur denitrifier and activated sludge process. All of these methods were experimentally tested in the laboratory scale in both discontinuous and continuous design, especially in bottles and in flow mode in plastic containers. On the basis of the results of the laboratory experiments, pilot tests were carried out in the interconnected lagoons in the Diamo state enterprise, from which a proposal for solving this problem arose. Our interest was mainly targered at the pond sediment, which has proven excellent nitrate removal performance in laboratory scale, and its effectiveness was higher than that of a well-known activated sludge process. Unfortunately, the same results were not achieved during transferring to a pilot scale within a set time. The required output nitrate concentrations were only achieved in two feeders out of a total of seventeen.

Keywords

Denitrification; industrial wastewater; natural method of wastewater treatment; pond sediment; biological filter; sulphur denitrifier; activated sludge

Abstrakt

Cílem této práce bylo odstranění dusičnanů z průmyslových odpadních vod, konkrétně odpadních vod s nízkou organickou zátěží. Hlavním požadavkem bylo, aby byl denitrifikační proces co nejméně ekonomicky, obslužně i personálně náročný. Těmto požadavkům odpovídalo odstraňování dusičnanů prováděné přírodními biologickými způsoby. Předkládaná práce proto popisuje možnosti denitrifikace pomocí rybniční kultury, biologického filtru, sírového denitrifikátoru a aktivovaných kalů. Všechny tyto způsoby čištění odpadních vod zatížených dusičnany byly experimentálně ověřovány v laboratoři jak v diskontinuálním, tak v kontinuálním provedení, tzn. vsádkově především v lahvích a v průtočném režimu v kolonách či nádržích. Na základě výsledků laboratorních experimentů byly provedeny i poloprovozní zkoušky v průtočných lagunách ve s. p. Diamo, od kterého návrh na řešení této problematiky vzešel. Rybniční sediment, na který byla směřována pozornost, prokázal při laboratorních experimentech vynikající účinnost v odstraňování dusičnanů a svoji efektivností předčil i osvědčený proces čištění s aktivovanými kaly. Nicméně stejných výsledků se v rámci stanoveného období nepodařilo dosáhnout při převodu do poloprovozního měřítka. Požadované výstupní koncentrace dusičnanů se podařilo dosáhnout pouze u dvou násad z celkových sedmnácti.

Klíčová slova

Denitrifikace; průmyslové odpadní vody; přírodní čištění odpadních vod; rybniční sediment; biologický filtr; sírový denitrifikátor; aktivovaný kal

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Introduction

Industrial, municipal, and agricultural effluents containing high nitrate 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. Specific industrial source of nitrate pollution is mining. In Czech Republic, mining of uranium underground in-situ leaching with sulfuric acid have been carried out in the periods 1967–1996 in the area of Stráž pod Ralskem. During the extraction have been injected into the ground more then 4 million tons of sulfuric acid and other chemicals. Currently one of the most demanding projects implemented by the Diamo state enterprise is rehabilitation of the rock affected by in situ leaching of uranium in the region of Česká Lípa, consisting in removal of residual technological solutions after uranium mining, especially as a consequence of in situ leaching, from the Cenomanian aquifer and protection of the Turonian aquifer, which is important from the water management point of view. 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. Residual amount of nitrates remains in neutralized waters which is necessary to reduce before discharged to the Ploučnice river or returned underground [1, 2].

In 2011, Diamo state enterprise, contacted our institute with a question about treatment of nitrate containing waters. The requirement was to remove residual concentrations of nitrates from purified mined watter with a low organic substances using a denitrification process. This would be realized in large concrete lagoons, which are available in the company from past activity. The main requirements for wastewater treatment processes are the lowest economic, operating and staffing levels. By the enterprise Diamo was preferred process with pond sediment, where nitrates are used in the production of biomass with lower consumption of other nutrients and organic carbon than in conventional denitrification. Separated nitrogen is thus captured in phytoplankton biomass, the removal of which can be solved by the controlled development of zooplankton and then fishs.

Until then, the wastewater from the neutralization station with an unsatisfactory nitrate concentration was pumped into the lagoons, where they were diluted or chemically modified before being drained to the local rivers. However, any chemical treatment is financially more demanding than a natural process. In the case of the proposed denitrification process, the concrete lagoons could be rebuilt into a natural pond that could be beneficial in the whole of community.

On the basis of these claims, these thesis included laboratory work, where selected suitable denitrification processes with pond sediments, biological filter, sulfur denitrifier and activated sludge were experimentally tested. The laboratory results were subsequently verified by pilot experiments with real industrial wastewater in the system of lagoons in the Diamo Stráž pod Ralskem.

1.1 Denitrification

Denitrification is a process in which there is a gradual conversion of nitrate NO_3^- through various intermediates to the elemental nitrogen N_2. The steps are catalyzed by denitrification enzymes in the bodies of organisms, mainly bacteria [3].
The proces of denitrification can formally be described by the following equation [4]:

$$2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_20$$

Bacteria capable of denitrification are ubiquitous, and thus denitrification occurs widely throughout terrestrial, freshwater, and marine systems [5]. In general, denitrification is favored in anoxic conditions with low dissolved oxygen concentrations, nitrate or nitrite availability, sufficient organic matter, availability of denitrifiers and an optimal range of temperature and pH [6].

1.2 Activated sludge

Activated sludge process is widely used for nutrient removal in wastewater treatment plants (WWTP), which depends on microbial metabolic activity, and it is probably the most versatile and effective of all wastewater treatment processes [7].
Many versions of the basic process exist but all are fundamentally similar. An activated sludge system consists of an equalization basin, a settling tank, an aeration basin, a clarifier, and a sludge recycle line. Wastewater is homogenized in an equalization basin to reduce variations in the feed. Settleable solids are then removed in a settling tank.
Next, wastewater enters an aeration basin, where an aerobic bacterial population is maintained in suspension and oxygen, as well as nutrients, are provided. The contents of the basin are referred to as the mixed liquor. Oxygen is supplied to the aeration basin by mechanical or diffused aeration, which also aids in keeping the microbial population in suspension. The mixed liquor is continuously discharged from the aeration basin into a clarifier, where the biomass is separated from the treated wastewater. A portion of the biomass is recycled to the aeration basin to maintain an optimum concentration of acclimated microorganisms in the aeration basin. The remainder of the separated biomass is discharged or "wasted“. The recycled biomass is referred to as activated sludge. The term "activated" is used because the biomass contains living and acclimated microorganisms that metabolize and assimilate organic material at a higher rate when returned to the aeration basin. This occurs because of the low food-to-microorganism ratio in the sludge from the clarifier [8].
Bacteria in activated sludge in contrast to pure cultures, in which the individual bacteria are mostly freely movable, predominantly occur in the form of zoogleas (a cluster of bacterial cells associated with mucus-derived substances excreted from their surface). Activated sludge differs from most pure microorganism cultures by being able to separate from the liquid phase by simple sedimentation. Efficient flocculation and sedimentation of sludge flocs is one of the most valuable features of this natural culture [9].
Today, the most widespread activated sludge process is such where the microorganism culture provides for the removal of organic matter, while nitrification and denitrification. The aeration tank ensures the nitrification of ammoniacal nitrogen to nitrate and the

nitrates are recycled into the denitrification tank, where the activated sludge is also returned, separated from the water from the settling tank.
In order to achieve the vision of simultaneous nitrification and denitrification in one reactor, strains of bacteria capable of not only aerobic denitrification but also heterotrophic nitrification are increasingly being isolated [10, 11]. New reclassified nitrogen transformation cycles, including aerobic denitrification or denitrification with nitrifying bacteria are known [12].

1.3 Biological filter

Biological filters consist of circular tank (column), which can be operated in either the up or down flow mode. Wastewater is pumped up through the filter bed and, as it passes through the packed media, microorganisms avail themselves of the organics in the waste stream [13].
The principle of aerobic biological treatment of wastewater in biofilm reactors is essentially the same as treatment in activated sludge proces, because it uses all three basic factors of the process, ie. aerobic microorganisms (mainly bacteria), oxygen and organic matter, which is degraded by mineralization processes . The difference against activated sludge is that the microorganisms of the mixed culture are not in flocs suspended in the aerated tank but they are attached to the filter medium as a biological film to the slime layer. In addition to the microbial decomposition of organic matter and other microbial processes (e.g. nitrification), sorptive processes are applied [4, 14].
Fixed film processes offer several advantages relative to suspended growth processes, include the reduced space requirements when constructing or expanding facilities on tight sites and they are stable due to the use of fixed biological films to retain microorganisms in the systems. Disadvantages include poor use of the influent carbon for denitrification leading to increased operating costs associated with the addition of external carbon; in some cases little operator adjustment is possible in response to changing process loading conditions; they possess poor shock load response capabilities since the quantity of biomass is fixed by the biofilm media surface area [15].
Biofilter packing material serves as a biofilm carrier and plays an important role in creating favorable conditions for its development. In order to ensure a more even distribution of the biomass thin layer, materials with a large specific surface are used [14]. Materials used in the past such as gravel, limestone or slag are now replaced by artificial materials, especially PVC, polyester or polyurethane [16], to which microorganisms are inoculated e.g. from activated sludge proces.
In connection with these materials, it has always been the case that biofilter packing is only a material for biofilm attachment, which by its properties does not affect the biochemical processes taking place in the biofilm [9].
In present, insoluble biodegradable polymers were used as biofilm carrier and carbon source simultaneously. Solid substrates were used as alternatives to liquid carbon sources, which are accessible only by microbial enzymatic attack, so it can avoid the risk of overdosing in liquid carbon sources supported denitrification system [17].
According to the arrangement, biofilm reactors can be divided into solid-phase reactors, rotating biological contactors and fluidized bed reactors [4, 9, 14].

1.4 Sulphur denitrifier

The sulfphur denitrifier is also a device of the column-type biofilm reactor, but has not been included in the previous chapter for a different denitrification principle. In this case, denitrification is based on sulfur oxidation and is therefore an autotrophic denitrification. It is usually carried out in fluidized bed reactors or in fixed bed reactors packed with sulfur granules, to which are attached sulfur bacteria, through which the wastewater passes [18].

Main advantages of heterotrophic denitrification are high denitrifying rate and treatment capacity. However, nitrite will be produced and accumulated in water when the added organic is stoichiometrically insufficient. Contrarily, the residual organic compound will pollute the treated water when the added organic is excessive [19]. As an alternative to remove nitrate, the biological autotrophic denitrification process has been receiving more attention recently, due to its two major advantages compared to biological heterotrophic processes: no need for an external organic carbon source which lowers the cost and risk of the process and less sludge production, which minimizes the handling of sludge [20].

Autotrophic denitrification with elemental sulfur is an attractive alternative for treatment of waters contaminated by nitrate and nitrite, especially for those organic-deficient wastewaters [21].

Energy for autotrophic denitrifying microorganisms is derived from the oxidation reactions of inorganic elements such as hydrogen or various sulfur compounds (H_2S, S, S_2O_3). Autotrophic denitrifiers utilize inorganic carbon compounds (e.g., CO_2, HCO_3^-) as their carbon source [22] with the simultaneous reduction of nitrates to elemental nitrogen gas. An example of the oxidation of elemental sulfur using autotrophic microorganisms is given in the following equations.

$$5S + 2\,H_2O + 6\,NO_3^- \rightarrow 5\,SO_4^{2-} + 4\,H^+ + 3\,N_2$$

In order to remove both nitrates and organic matter from the wastewater, the two types of biological denitrification can be combined. These investigation [23] indicated that the mixotrophic column had a higher nitrate removal capacity than the autotrophic column. It was also found that under mixotrophic conditions, some portion of nitrate was removed heterotrophically and the remainder was denitrified by sulfur-utilizing autotrophic bacteria without inhibition by organics. In addition, sulfate production and alkalinity consumption were reduced under mixotrophic conditions. These results suggest that mixotrophic conditions that allow simultaneous reactions of autotrophic and heterotrophic denitrification provide significant advantages in terms of nitrate, sulfate production decrease, and alkalinity consumption over obligate autotrophic or heterotrophic denitrification. In order to take full advantage of heterotrophic denitrification and sulfur autotrophic denitrification, a new combined two-step process of heterotrophic and sulfur autotrophic denitrification processes was developed without residual concentrations of organic substrate and accumulation of nitrites [19].

1.5 Natural methods of wastewater treatment

Natural methods of wastewater treatment use self-purification processes which occur in soil, water and wetland environments [24, 25]. 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 stabilization ponds and constructed wetlands [25, 26].

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 [27, 28].

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 [29]. 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 [30].

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 sediment and water are important regulators in nitrogen biochemistry [31]. 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 [30].

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 be-tween 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 [32]. 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 [33].

2 Aims of thesis

For the dissertation thesis "Denitrification of Industrial Wastewater" the following aims were set:

1) Determination of suitable denitrification methods for industrial wastewater with low content of organic matter. The main requirements these processes are the lowest economic, operating and staffing levels.

2) Test suitable denitrification methods on a laboratory scale. Based on the results, choose methods for pilot testing.

3) Pilot testing of selected denitrification methods with real industrial wastewater. The selected denitrification process should meet the requirements of reducing the inflow of wastewater with concentration of 79 $mg.l^{-1}$ $N\text{-}NO_3$ to an outflow concentration of 22 $mg.l^{-1}$ $N\text{-}NO_3$ and 20 $mg.l^{-1}$ COD.

3 Materials and method

3.1 Analytical methods

Concentration of nitrate nitrogen ($N\text{-}NO_3$) was measured by spectrometric methods using sulfo-salicylic acid. Concentration of nitrite nitrogen ($N\text{-}NO_2$) 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. 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 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.

3.2 Pond sediment experiments

Batch experiments with pond sediment were carried out in glass bottles (Fig. 1). The organic substrates ethanol, Brennta (BrenntaPlus VP1 – a mixture of alcohols, sugars and proteins used as a carbon source to increase the activity of microorganisms in wastewater treatment) and glucose and amounts of these substrates were tested with the ratio of nutrients COD : N : P = 40 : 10 : 1 and 80 : 10 : 1 at laboratory conditions (20–25 °C) and cold (5–9 °C) accessing or absence of light and air. Appropriate amounts of sediment were also tested. Experiments were performed with different initial concentrations of $N\text{-}NO_3$.

The changeover from laboratory scale testing in the bottles to more realistic conditions was carried out using the three interconnected plastic containers, each with a volume of 65 l.

Fig. 1 Experimental bottles RS2.1–RS2.9 with pond sediment

3.3 Biological filter experiments

NP-reduction BioPellets (by D. van Houten, Groningen, The Netherlands) were used as the biofilter package. It is originally a commercial product for aquarists. The pellets are in the form of a 100% pure biodegradable polymer (diameter 4 mm, height 2 mm) where immobilized denitrifying bacteria are attached. Pellets have the role of not only bacterial carriers but serve as a source of organic substrate, so there is no need for another external carbon source.

The experimental apparatus (Fig. 2) consisted of a glass column (tube) of an inner diameter of 46,5 mm, closed by a teflon valve. Purified water was introduced into the column by a centrifugal pump from an interconnected 50 liter plastic reservoir with model wastewater. The biopellets in the column were fluidized by the counterflow of water.

In addition to the continuous experiments in the column, discontinuous (batch) experiments in bottles were carried out with biopellets.

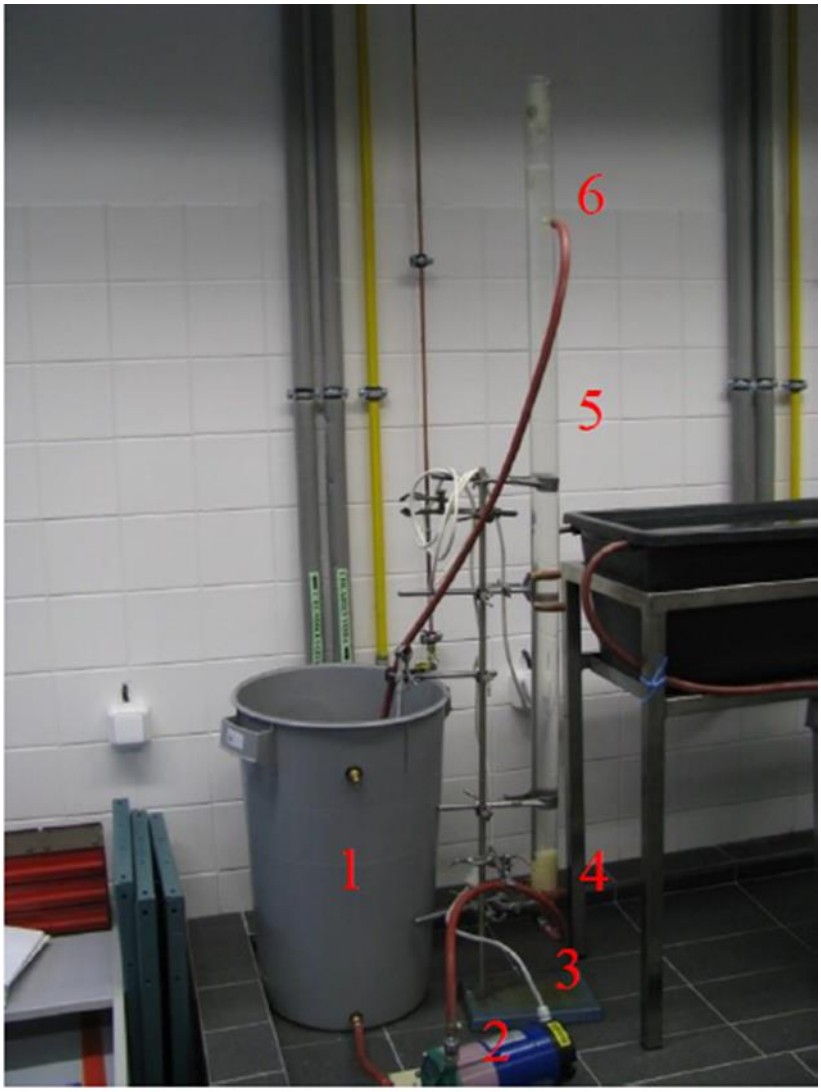

Fig. 2 Fluidized bed reactor: 1 – reservoir with model wastewater, 2 – pump, 3 – inflow of model wastewater, 4 – fluidized biopellets, 5 – column, 6 – overflow of wastewater back into the reservoir

3.4 Sulphur denitrifier experiments

The main part of the experimental apparatus was the Sulfur Nitratereductor SR 400 (by Aqua Medic, Bissendorf, Germany) (Fig. 3). It is originally a commercial product for aquarists. The dimensions of the sulfur denitrifier are: column diameter 70 mm, bed length 230 mm, volume 0,88 l, dry sulfur 1050 g (sulfur granules are impregnated with lyophilized bacteria). Purified water was introduced into the column by a pump from an interconnected 5 liter plastic reservoir with model wastewater. Sulphur denitrifier was operated as a fixed bed reactor.

In addition to the continuous experiments in the column, discontinuous (batch) experiments in bottles were carried out with sulphur granules.

Fig. 3 Sulphur denitrifier apparatus (flow mode on the left, circulation mode on the right): 1 – reservoir with model wastewater, 2 – pump, 3 – column packed with sulphur granules, 4 – inflow of model wastewater, 5 – outflow from column, 6 – overflow of wastewater

3.5 Activated sludge experiments

The first series of experiments was studied in batch experiments using 1 l polyethylene bottles filled with solution of $NaNO_3$, which were inoculated with diluted sludge from the WWTP denitrification unit focused on the removal of nitrate (denitrification unit SBU Nitrocellulose Synthesia Pardubice) (Sludge 1), the second half of the number of bottles were inoculated with diluted sludge from classical biological WWTP (Sludge 2). Bottles of both types of sludge were placed in the refrigerator (7 °C), simulating the winter conditions, remaining bottles from both types of sludge were left in the laboratory at room temperature (20 – 23 °C).

Another experiment to verify the effect of various organic substrates on the activity of the activated sludge process, the sludge, which was gradually created in the column of our experimental biofilter and contained clusters of adapted denitrification bacteria, was used for inoculation of bottles with $NaNO_3$ solution.
Subsequently, batch experiments of denitrification process on a larger scale were studied in rectangular plastic containers (simulating aeration tanks) with a capacity of 65 litres and barrels with capacity of 50 litres.

3.6 Pilot scale of denitrification in lagoons

For pilot scale testing a cascade of three interconnected concrete lagoons was used in the site of Diamo enterprise - Pustý in Hamr na Jezeře (Fig. 4). The lagoons had a total volume of 480 m^3 with dimension of 16 x 10,5 x 0,9 m for each one. At the bottom of the lagoons there was 2–3 cm of pond sediment dosed in 2009. The lagoon system was not externally mixed, but each lagoon was equipped with 14–20 carps, which had to contribute to the natural mixing. Real wastewater treatment from the neutralization unit should then be carried out in large lagoons. These are concrete rectangular tanks with bevelled walls with internal dimensions of 20 x 103 x 2,5 m with a volume of 5883 m^3.

Fig. 4 The Pusty area with a cascade of three lagoons used for pilot experiments (1, 2, 3 – lagoons, 4 – circular tank for preparation of model wastewater, 5, 6, 7 – large lagoons used for real wastewater treatment)

The model wastewater for pilot testing was prepared by Diamo's personnel in a stirred circular tank and gravitationally driven by plastic hoses to the first of the lagoons. A total of 17 feeders were prepared.
The results of the pond sediment pilot tests revealed that the pond sediment under these conditions and arrangements did not have much precondition for intensifying the denitrification process and therefore it was decided in 2012 to replace the pond sediment with activated sludge. In 2013, a continuous regime was tested.

4 Results and discusion

4.1 Pond sediment experiments

The last experiment with pond sediment in bottles was focused on different initial concentrations of nitrate. Table 1 shows the total amount of nitrate nitrogen removed in 24 days in individual bottles RS4.1–RS3.8.
Table 1 also shows how the actual amount of nitrate dosed into individual bottles, as well as the theoretical amount that would be dosed in the case of 100% removal after each replenishment. In the case of bottles RS4.3–RS4.7, both the actual and the theoretical dosed amounts of nitrate were identical. In addition to the $N\text{-}NO_3$ removal efficiency related to the actual and theoretical total amount of $N\text{-}NO_3$, the average efficacy since the 6th day of the experiment is also shown. This is the efficiency of nitrate removal, which was achieved in individual bottles after system stabilization, respectively after adaptation of the bacteria.

Table 1 Efficiency of $N\text{-}NO_3$ removal in experimental bottles RS4.1–RS4.8 in 24 days

Bottle	Initial concetration ($mg.l^{-1}$)	Dosed actual/theoret. ($mg.l^{-1}$)	Actually removed ($mg.l^{-1}$)	Efficiency (%)	Average efficiency since 6th exp. day (%)
RS4.1	80	400 / 960	241,1	60,3 / 25,1	29,7
RS4.2	80	400 / 960	269,8	67,5 / 28,1	41,2
RS4.3	160	1920 / 1920	1716,2	89,4	94,7
RS4.4	160	1920 / 1920	1815,9	94,6	99,0
RS4.5	240	2880 / 2880	2441,4	84,8	95,8
RS4.6	240	2880 / 2880	2614,5	90,8	96,4
RS4.7	320	3840 / 3840	3559,1	92,7	95,8
RS4.8	320	3520 / 3840	3352,9	95,3 / 87,3	98,9

4.2 Biological filter experiments

Testing of the biofilter took a total of 89 days (Fig. 5 – blue line). After increasing the initial concentration to 79,1 $mg.l^{-1}$ $N\text{-}NO_3$ (350 $mg.l^{-1}$ NO_3^-), the nitrate content declined slowly for a period of 69 days by a total of 23,0 % of the initial concentration. Then a significant break occurred, and a final concentration of 2,64 $mg.l^{-1}$ $N\text{-}NO_3$ was measured on the 89th day of the experiment, a decrease of 99,3 % relative to the initial concentration. This significant improvement in denitrification process was achieved by sealing the top of the glass tube with a rubber stopper and the overflow hose being introduced deep below the surface nitrate solution in reservoir (the outlet was located about 20 cm above the bottom of model wastewater reservoir).
These modifications led to a significant decrease in the dissolved oxygen concentration, which had so far been around 7 $mg.l^{-1}$, and the present microorganisms preferentially used this oxygen as an electron acceptor instead of the oxygen contained in the nitrate

molecules. The only transport of oxygen to the model wastewater was only possible by diffusion at the surface – air in the reservoir and the dissolved oxygen concentration reached 0,41 mg.l^{-1} at the end of the experiment. In this context redox potential decreased from aerobic conditions to anoxic conditions. The pH of the system was around 7.

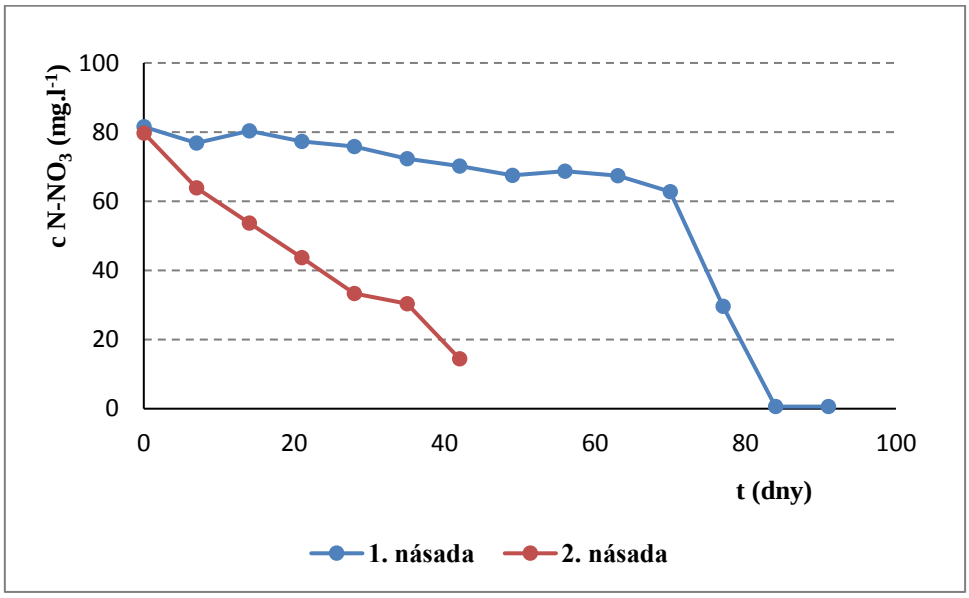

Fig. 5 Course of nitrate removal in the biofilter during 1st and 2nd test

Subsequently, testing was repeated (Fig. 5 – red line). In contrast to the first test, the nitrate removal reaction time was reduced to half. In addition to setting the appropriate conditions at the end of the first test, the result can also be affected by the fact that the denitrification rate increases with the gradual adaptation of the bacteria to the environment, especially to the organic substrate. This applies to both continuous and batch reactions [34, 35]. The C : N ratio is closely linked to the organic substrate, which can stimulate the growth of bacterial populations with suitable enzymes, thereby increasing the denitrification rate [36].

Discontinuous bottle experiments took place for 81 days. The enzymatic system of bacteria did not properly start due to the presence of dissolved oxygen, as in the case in the previous experiment in the column. From the results it can be concluded that biopillets are useless to use in a discontinuous way because, in contrast to the pond sediment, intensive contact with purified wastewater is needed to effectively removal of nitrates.

4.3 Sulphur denitrifier experiments

The first series of testing lasted 119 days. The course of nitrate removal is shown in Fig. 6 (the blue line). From the beginning the concentration of nitrates slowly decreased and after 62 days, the removal efficiency was 25,0 %. Over the next 60 days, total nitrate removal occurred with an efficiency of 92,58 % relative to the initial concentration 80,2 mg.l^{-1} N-NO_3 (355 mg.l^{-1} NO_3^-).

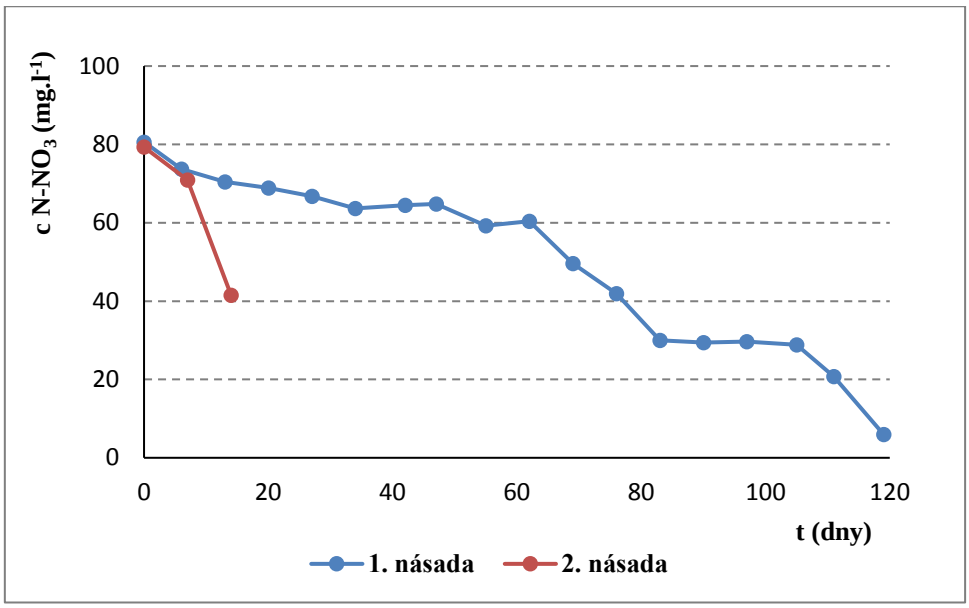

Fig. 6 Course of nitrate removal in the sulphur denitrifier during 1st and 2nd test

The second experiment series is shown in Figure 6 – red line. The nitrate concentration was supplemented with 80,2 mg.l^{-1} N-NO_3 (355 mg.l^{-1} NO_3^-). For technical reasons, the experiment only lasted for 14 days. However, during this time the concentration of nitrates fell by almost half, the efficiency of denitrification was 47,6 %. The same efficiency was achieved by the 76th day of the experiment on the first test. It can be assumed that, as in the case of the biofilter column, bacterial adaptation has occurred over time and hence increased denitrification rate, although parameters such as redox potential and dissolved oxygen concentrations have still been shown to aerobic environment in system. The pH of the system remained, as in the case of the first test, in the acidic area.

To assess the effect of the initial concentration of nitrates on the course of autotrophic denitrification in batch mode, eight bottles S2.1–S2.8 with a initial concentration range of 10, 50, 100, 150, 200, 250 and 300 mg.l^{-1} NO_3^- were prepared. For all bottles, the bacteria system started between the 28th and 35th days of the experiment, when the first decrease in NO_3^- concentration was measured. According to the total nitrate removal efficiency, shown for all bottles in Table 2, it can be deduced that an initial concentration of 100 mg.l^{-1} NO_3^- (22,6 mg.l^{-1} N-NO_3) appears to be appropriate under the conditions set in our experiment.

Tab. 2 Total efficiency of nitrate removal in bottles S2.1–S2.8 in 62 days

Bottle	Initial concentration of NO_3^- ($mg.l^{-1}$)	Efficieny of removal NO_3^- (%)
S2.1	10	55,8
S2.2	50	85,9
S2.3	100	97,3
S2.4	150	84,2
S2.5	200	82,9
S2.6	250	57,8
S2.7	300	46,5
S2.8	350	46,4

4.4 Activated sludge experiments

The denitrification process of the bottles can be assessed on the basis of the total amount of nitrate that has been dosed into the bottles during the experiment and subsequently removed. In the case of Sludge 1 placed at room temperature, 277,5 $mg.l^{-1}$ $N\text{-}NO_3$ was dosed within 25 days and 244,5 $mg.l^{-1}$ $N\text{-}NO_3$ was removed. One half of the nitrates amount (exactly 119 $mg.l^{-1}$ $N\text{-}NO_3$) was dosed into the bottled with Sludge 1 placed in cold due to the lower nitrate removal efficiency and only 52 $mg.l^{-1}$ $N\text{-}NO_3$ were removed. In the case of the Sludge 2 bottle placed at room temperature, a total of 52 $mg.l^{-1}$ $N\text{-}NO_3$ was dosed and the same amount was removed. 143 $mg.l^{-1}$ $N\text{-}NO_3$ was dosed into the bottles placed in cold and the Sludge 2 was able to remove 134 $mg.l^{-1}$ $N\text{-}NO_3$.

The comparison of the results shows that in our set experimental conditions was more efficient the sludge from the classical biological waste water treatment plant (Sludge 2). It can be concluded that the mixed bacterial cultures contained in this type of sludge are better able to adapt and are less sensitive to changes in the environment than cultures derived from a special denitrification unit (Sludge 1).

4.5 Pilot scale of denitrification in lagoons

The results from the pilot denitrification experiments were evaluated for the feeders 4–17 (Fig. 7 graph with the feeder no. 5), because the initial three feeders encountered a technical problem in the form of leakage of model wastewater from the circular preparatory tank. There was also an insufficient height gradient between the circular tank and the lagoons.

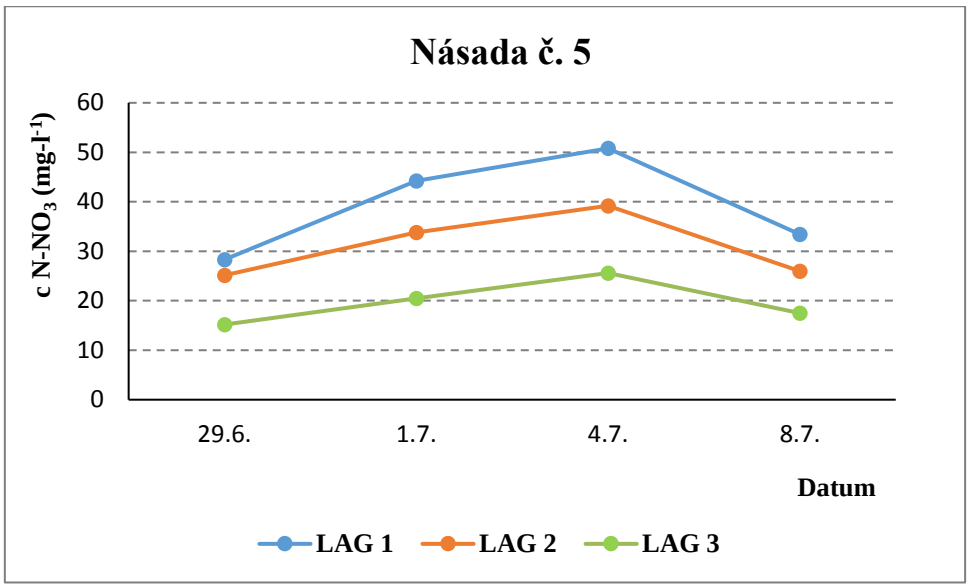

Fig. 7 Course of the N-NO_3 concentration in feed no. 5 during time, 29.6.2011 was given an initial concentration of 62,6 mg.l^{-1} N-NO_3

In the following year 2013, a continuous regime was tested in the lagoon systém (Fig. 8). Effective results have been achieved since the start of the testing. During August 2013, the nitrate nitrogen content decreased to a concentration of 23,7 mg.l^{-1} in the 1st lagoon, 23,3 mg.l^{-1} in the second and 28,0 mg.l^{-1} in the third lagoon.
Denitrification process took place at an average temperature of 20 °C, a phosphorus concentration in the range of 0,4–2 mg.l^{-1} and a COD of about 50 mg.l^{-1}. At the end of August, unfortunately, a neutralization unit pump crash and there was a failure to deliver real wastewater to the first lagoon. After that, successful denitrification efficiency has not been restored.

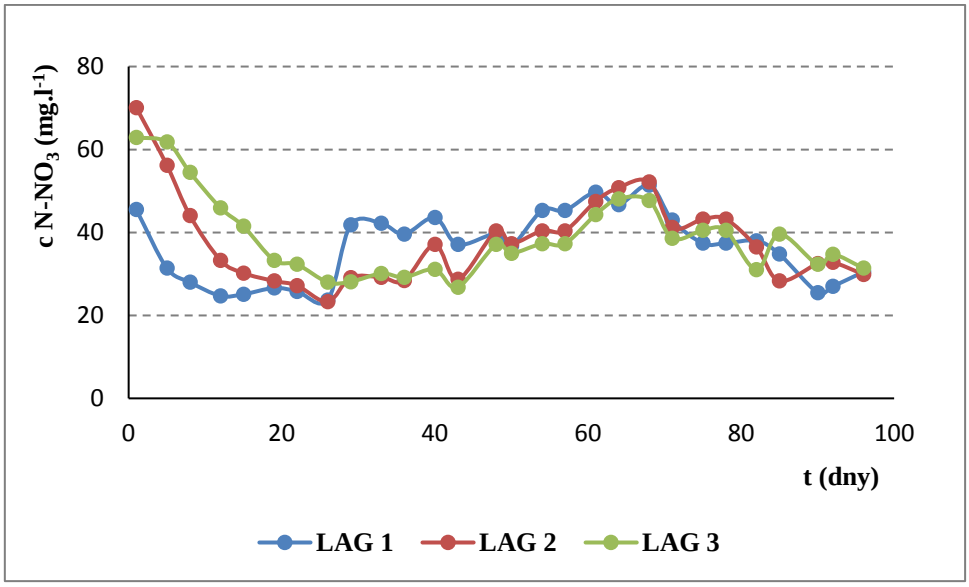

Fig. 8 The course of the concentration of N-NO3 in the system of three lagoons in the period 1.8. – 4.11.2013

5 Conclusion

These thesis aimed to find the possibility of removing nitrates from industrial wastewater with low organic load using natural methods. On the basis of cooperation with the State enterprise Diamo Stráž pod Ralskem, experiments were focused to remove nitrates from model wastewater using pond sediment. The effect of sediment content, organic substrate and phosphorus content, temperature, air and light access to the denitrification process were investigated. At the same time, other denitrification processes were tested using a biofilter and a sulfur denitrifier. For comparison, experiments with activated sludge were also performed as the most widespread wastewater treatment method. The experiments were carried out in both batch and continuous design, i.e. in bottles and in flow mode in columns or containers.

Experiments show the following results. Already during the initial experiments, the nitrate sorption in the pond sediment was not proven. It was further verified that the pond sediment itself, or the bacterium contained therein, is not capable of removing nitrates without the presence of other biogenic elements, i.e. organic carbon and phosphorus. For organic carbon, the results clearly show that its presence is key to denitrification by pond sediment. Experiments did not show a significant effect of pond sediment amount on denitrification, as well as the influence of air and light access under the experimental conditions.

Further experiments focused on the recommended ratios of nutrients taken from the literature and the testing of organic substrates. At the ratio of nutrients COD : N : P = 40 : 10 : 1, nitrates with concentration 79,1 $mg.l^{-1}$ $N\text{-}NO_3$ could be removed from the model wastewater with an average efficiency of 50 %. At ratio 80: 10: 1 under the same conditions, an average of 90 % of nitrates was removed. However, higher dosing of the substrate is associated with higher costs and the need to remove the residual substrate in the next technological step so as not to exceed the limits for discharging into the recipient. Based on our results, the ethanol and Brennta consumption values are similar regardless of the initial COD concentration. At room temperature, average COD removal was 58,4 % for Brennta and 57,4 % for ethanol. Lower removal was measured for bottles placed in cold. In the case of Brennta, the average removal rate was 43,6 % and in the case of ethanol the removal rate was 40,9 % and 30,0 %.

The results also confirmed the effect of temperature on the denitrification process. At temperatures of 5–9 ° C nitrate removal for both tested nutrient ratios was about half that of removal at room temperature. Brennta was shown in comparison to ethanol under these conditions as a more efficient carbon source. Under cold conditions, the Brennta efficiency was 27,2 % for the COD : N : P = 40: 10: 1 and 62,2 % for the COD : N : P = 80 : 10 : 1 ratio. Ethanol, compared to that, reached 18,2 % at a lower nutrient ratio and 32,1 % at a higher ratio.

From the point of view of the formation of intermediates during denitrification, i.e. nitrites, the ratio of nutrients COD : N = 4 : 1 with the initial nitrate concentration of 79,1 $mg.l^{-1}$ $N\text{-}NO_3$ did not again appear optimal. For both ethanol and Brennta, high concentrations of nitrite were measured in tens of $mg.l^{-1}$ $N\text{-}NO_2$.

For a more efficient denitrification, it would be appropriate to increase the ratio for this particular concentration of 79,1 $mg.l^{-1}$ $N\text{-}NO_3$ in favor of COD. However, in a further experiment, the presence of nitrite was observed under the same conditions only when

ethanol was using as substrate. In the case of Brennta, the accumulation of nitrites did not occur at all. For a higher initial nitrate concentration of 160, 240 and 320 $mg.l^{-1}$ $N\text{-}NO_3$, the CHOD : N = 4 : 1 ratio was already sufficient and the nitrite formation was recorded mainly in the first days of the experiment. Subsequently, nitrites fell to the order of the decimal to the hundredth values.

Biofilter experiments have confirmed that the rate of denitrification increases with the gradual adaptation of bacteria to the environment. Bacteria were sensitive to the presence of dissolved oxygen, and it was necessary to provide them with suitable conditions in order to activate their enzymatic system and use the oxygen built in the nitrate molecule. Upon repetition of the experiment, the reaction time was reduced by half compared to the first test, i.e. an 81,8 % efficiency of nitrate removal was achieved within 42 days. The results of bottled experiments show that biopelletes are useless to using them in a batch mode because they need intensive contact with purified wastewater to effectively removal of nitrates (as opposed to pond sediment).

During the experiments with the sulfur denitrifier occurred (as in the case of the biofilter column) to adapt bacteria and hence increase the rate of denitrification. Interesting results have been obtained in sulfur experiments in bottles with a concentration range of nitrate (10–300 $mg.l^{-1}$ NO_3^-). Under the conditions set forth in this experiment, 30 g of granulated sulfur and 10 ml of a sulfur denitrifier solution (bacterial inoculation) were added to the bottles with nitrate solution, as a suitable initial nitrate concentration of 100 $mg.l^{-1}$ NO_3^- (22,6 $mg.l^{-1}$ $N\text{-}NO_3$) was evaluated. The efficiency of nitrate removal in this bottle was 97,3 %. In an attempt to purify model wastewater with a higher nitrate content, the removal of NO_3^- was significantly worse. For bottles with a lower initial concentration of 50 and 10 $mg.l^{-1}$ NO_3^-, the nitrate removal efficiency decreased again.

Laboratory experiments with activated sludge proces have confirmed the reliability of this nitrate removal process. In batch bottle tests, the activated sludge at ratio COD : N : P = 80 : 7 : 1 (Brennta substrate) was able to remove 244,5 $mg.l^{-1}$ $N\text{-}NO_3$ at room temperature and 52 $mg.l^{-1}$ $N\text{-}NO_3$ in the cold during 25 days. Another type of activated sludge removed 447 $mg.l^{-1}$ $N\text{-}NO_3$ at room temperature and 134 $mg.l^{-1}$ $N\text{-}NO_3$ in the cold. Compared to the results achieved by the pond sediment, similar nitrate removal efficiency was achieved under the same conditions with a lower ratio of nutrients COD : N : P = 40 : 10 : 1 as for the second type of activated sludge, namely 470 $mg.l^{-1}$ $N\text{-}NO_3$ and 262 $mg.l^{-1}$ $N\text{-}NO_3$ in the cold. At a higher nutrient ratio COD : N : P = 80 : 10 : 1, the pond sediment achieved even double the efficiency of 837 $mg.l^{-1}$ $N\text{-}NO_3$ at room temperature and 601 $mg.l^{-1}$ $N\text{-}NO_3$ in the cold.

With the pond sediment, pilot tests were carried out in the area of Diamo. Cascade of three flow lagoons with a total volume of 480 m3 were used for the experiments, with 2–3 cm of pond sediment. 17 feeders of the specified composition (sodium nitrate, Brennta, phosphate) were prepared. Experiments with individual feeders lasted between 5 and 14 days (average 9 days). However, in the given configuration, denitrification performance has been shown to be low overall. The efficiency of the denitrification process (assessed by comparing the concentration of nitrate nitrogen in the feed to its

concentration in the third lagoon at the end of the experiment) ranged from 72,0 % to 3,7 % (with a 41 % average).
The gradual increase of nitrate concentration in the feeders has probably led (despite ongoing denitrification) to a gradual increase in nitrate ion content in lagoons during the year. This means that the lagoon system has been loaded more than was its actual capacities. The required output concentrations of 22 $mg.l^{-1}$ $N\text{-}NO_3$ were achieved in two feeders (no. 4 and 5). In other cases, the concentration at the third lagoon output ranged from 24 to 74 $mg.l^{-1}$ $N\text{-}NO_3$. The excellent results achieved with the pond sediment in the laboratory, unfortunately, failed to pass to a larger (semi)operating scale.

The pilot system of the lagoons was tested in the following year using activated sludge. Due to the later start of testing and the onset of autumn weather experiments were realized with only three feeders, which were carried out 11, 9 and 10 days. Efficiency of the removal of $N\text{-}NO_3$ (relative to the initial concentration) was 13,0 % for the first feeder, 17,0 % for the second feeder and 23,2 % for the third feeder.
In the following year (2013), a continuous regime was tested in the lagoon system. Each day a feeder was discharged into the first lagoon and real wastewater was continuously supply from the neutralization unit. In the first month of testing, the nitrate nitrogen content decreased to 23,7 $mg.l^{-1}$ in the 1st lagoon, 23,3 $mg.l^{-1}$ in the second and 28,0 $mg.l^{-1}$ in the third lagoon. Subsequently, a neutralization unit pump crash and there was a failure to deliver real wastewater to the first lagoon. The temporary shutdown lasted for 7 days and during this period the level of denitrification deteriorated to about 40 $mg.l^{-1}$ $N\text{-}NO_3$, i.e. a decrease in target efficiency to 68 %, respectively absolute efficiency to 49 %. The cause was considered to be (with a fall in temperature during the onset of autumn to an average of 10 °C) the excessive growth of green algae in the lagoons, which in their growth produce oxygen, thus preventing the formation of a sufficiently low concentration of oxygen suitable for denitrification. For pilot experiments, the required COD 20 $mg.l^{-1}$ output values also failed to be achieved. The location of the activated sludge in the second lagoon is also considered. In the continuous mode of the treatment, it would be desirable for the 1st lagoon to remain the organic substrate content at a certain level so as not to be a limiting factor for denitrification in the event of its decline. With the decrease it would be possible to count in the second lagoon and the third lagoon would only serve as sedimentary.

Due to the fact that the operation of the lagoon system is primarily dependent on the nitrate content flowing in the wastewater from the neutralization unit, which thanks to the present technology achieves very good parameters (from the point of view of nitrogen pollution and COD), solutions to remove $N\text{-}NO_3$ peak concentrations are available in the form of a sulfur denitrifier column or selective nitrate ion exchangers. This would be an investment more demanding solution than the operation of a biological treatment plant, but nowadays the wastewater purification using the denitrification step paradoxically increases the monitored parameters due to the addition of nutrients necessary for the activation process. Another possibility could be that wastewaters with high $N\text{-}NO_3$ concentrations will be discharged into large lagoons and treat here not actively, but will be left to natural purification without the support of any additional feedstock. However, it could not be a flowing system, as the water residence time could be extended to several weeks. The lagoon's capacity would be sufficient, as overdose

concentrations are rarely encountered. Diamo enterprise currently does not use these recommendations and adjusts wastewater with excess nitrate concentrations to the required values by dilution with "clean" water.

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