Kinetic modelling of surface water biodenitrification

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Abstract:
The nitrate removal from surface water of the Cetina river (SW) with the use of natural powdered Croatian clinoptilolite as a carrier of bacterial cells was studied in the batch bioreactor. The removal of 50-250 mg NO_3-N/L from the SW with the bacteria attached to the natural powdered clinoptilolite (Bio-NPC) was monitored. The influence of initial nitrate as well as the temperature impact on the batch biodenitrification process was investigated and modelled. According to parameters obtained in Monod model, the biodenitrification process revealed as zero order reaction. The nitrate removal from the SW (C_0 = 100 mg NO_3-N/L) was monitored in the temperature range of 15-35 ^oC and the denitrification rates increased with the increase of temperature. According to the Arrhenius equation, the activation energy and Arrenius factor were 48.25 kJ/mol and 1.056×10^{10} mg NO_3-N/Lh, respectively. Furthermore, optimal amount of methanol was determined. The model of biological denitrification was developed in accordance with the experimental data and literature. Finally by the use of an alternated differential method (ID algorithm) biological denitrification was determined as the zero order reaction.

Key Words: Activation energy, Clinoptilolite, Denitrification, Nitrate, Surface water, Zero order reaction

1 Introduction

The development and industrial production as well as increased agriculture influenced the environment. The presence of nutrients in water as a consequence of excessive use of fertilizers or other nitrate or phosphate containing compounds nowadays is increasing [1-3]. The negative impact of such substances on the environment is demonstrated among others, through eutrophication of water. The harmful impact of nitrate on water life and humans has been proven [4,5]. Consequently, the purification and protection of waters are gaining increased attention of scientists and legislation [6-8]. The new approaches and methods for nutrient removal are investigated; among them biological methods are frequently used [9,10]. The use of microorganisms that are capable of degrading many chemicals are accordingly often applied for effective removal of pollutants from waters. In order to achieve more stable and efficient biodenitrification, the immobilization or attachment of microbial cells on different carriers was studied. Among many suitable materials, the zeolites as natural or synthetic ion exchanger are well known for their ability to remove ammonium, other cations or many metal ions from water or wastewater and therefore are often investigated in water purification processes [11,12]. In view of the fact that the natural zeolites showed special importance in water purification, adsorption and catalysis primarily due to their high cation-exchange ability as well as molecular sieve properties and nevertheless are easily available in large quantities in many parts of the world as a cheap material, it seems that their application as carrier of microorganisms was the most attractive [13]. Hence, the zeolite was added to a conventional activated sludge unit for improvement of denitrification process and recently clinoptilolite was investigated as a carrier of immobilized microorganisms or biofilm [14,15]. Furthermore, the interaction of surfactant-modified zeolites and phosphate accumulating bacteria was demonstrated in order to improve phosphate removal from wastewater [16].

The biodenitrification process, with respect to bacteria used, was usually conducted in anaerobic or anoxic conditions, so, the denitrifiers enabled a total nitrate removal with observed, usually low nitrite accumulation.

During biological denitrification bacteria use nitrate as terminal electron acceptor in their respiration process and transform nitrate into nitrogen gas and at the same time use carbon for their growth. According to carbon

source used, the process was heterotrophic or autotrophic denitrification. The methanol, ethanol and acetate are the most widely applied organic carbon sources required for heterotrophic denitrification [6]. Among them, the methanol was revealed as cheap and effective carbon source. The partial reaction of biodenitrification with methanol was firstly defined 1969. by McCarty et al. [17]:

electron donor reaction:

$$CH_3OH + H_2O \rightarrow CO_2 + 6H^+ + 6e^- \quad (1)$$

assimilation of nitrate into biomass:

$$NO_3^- + 5CO_2 + 29H^+ + 28e^- \rightarrow C_5H_7O_2N + 11H_2O \quad (2)$$

nitrate reduction (electron-acceptor reaction):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \quad (3)$$

Then, according to partial reactions (1), (2) and (3) a stoichiometric equation was calculated:

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow$$
$$\rightarrow 0.065C_5H_7O_2N + 0.478N_2 + 0.76CO_2 + 2.44H_2O \quad (4)$$

For denitrification, the 1.08 mole of methanol is required for removal of 1 mole of nitrate and conversion to mass ratio revealed that methanol to nitrate nitrogen mass ratio of 2.47 was stoichiometric value. Furthermore, H^+-ions included in denitrification reaction could result in an increase of the pH. Therefore control of pH and determination of necessary amount of methanol was investigated. This process has been well studied, but the use of natural clinoptilolite as a carrier of bacterial cells for improved biodenitrification was not comprehensively reported.

The aim of the present paper was to investigate the applicability of bacteria attached to the clinoptilolite for improved nitrate removal from the Cetina surface water. The influence of temperature and methanol was studied and optimised values were selected for enhanced nitrate removal. The kinetic equations were applied for nitrate removal modelling.

2 The use of bacteria attached to clinoptilolite for surface water biodenitrification

The aim of the present work is to investigate the features of nitrate degradation from the Cetina surface water by using bacteria attached to Croatian zeolite under batch anoxic conditions. The impact of temperature on the process duration was investigated in the range of 15-35 °C and kinetic equations were applied for the calculation of activation energy (E_A) and Arrhenius factor (A_r). The biodenitrification rates (k_{den}) were determined and a kinetic model for nitrate removal was obtained. Furthermore, the optimization of temperature and MetOH/NO_3-N ratio revealed the decrease of process cost and improved nitrate removal from surface water.

2.1 Materials and methods

The zeolite used was a natural powdered clinoptilolite (NPC), obtained from the Donje Jesenje deposit, Croatia. The chemical composition of clinoptilolite is shown in Table 1. The NPC was washed with redistilled water in order to remove the surface dust, dried at 105 °C for 24 h and used for the attachment of bacteria.

The mixed bacteria culture used and the attachment procedure were described in our previous work in details [18]. After attachment of bacterial cells onto NPC, the obtained wet clinoptilolite with attached bacteria (Bio-NPC) was stored at 4°C until used for denitrification tests.

The surface water sample (SW) was prepared with natural surface water of the Cetina river (Table 2), by addition of 2.5 and 1g/L of K_2HPO_4 and KH_2PO_4, respectively. The SW solution was autoclaved and cooled to room temperature before further use.

Table 1. The chemical composition of clinoptilolite

Component	w/w (%)
SiO_2	63.86
Al_2O_3	14.64
Fe_2O_3	2.28
Na_2O	2.27
K_2O	1.08
CaO	2.84
MgO	0.88
Ignition loss	11.22

For each denitrification test, to sterile SW, the stock nitrate solution ($NaNO_3$ solution containing 10 g nitrate-N/L) and methanol were added separately. The resulting solution contained nitrate-N from 50-250 mg NO_3-N/L and methanol at methanol to nitrate -N mass ratio (MetOH/NO_3-N ratio) of 4.5:1. The excess methanol was used to avoid carbon limited conditions. The influence of

temperature on the denitrification process was investigated in the SW (C_0 = 100 mg NO_3-N/L and MetOH/NO_3-N ratio = 4.5:1) in the range of 15-35°C. All the reagents used during the tests were of an analytical grade level.

Table 2 Physical and chemical parameters of the surface water of the Cetina river

Parameters	
pH	7.25-8.20
CO_2 (free) (mg/L)	4.20-9.80
Dissolved O_2 (mgO_2/L)	9.50-13.9
$KMnO_4$ (mg/L)	4.0-11.6
Total N (mgN/L)	0.02-1.139
NH_3-N (mgN/L)	0.001-0.198
NO_2-N (mgN/L)	0-0.005
NO_3-N (mgN/L)	0.472-0.848
Cl^- (mg/L)	9.5-69.20
SO_4^{2-}(mg/L)	9.4-36.9
PO_4^{3-}P (mg/L)	0.023-0.281
Hardness- $CaCO_3$ (mg/L)	204-256
Ca-$CaCO_3$ (mg/L)	155-204
Mg-$CaCO_3$ (mg/L)	42-63

To study the kinetics of nitrate removal from the SW medium, in the 0.2 L bioreactor, 150 mL of the SW and 15 g of Croatian clinoptilolite with the attached bacterial cells were added. The bioreactor was plugged with a rubber stopper and sealed. The stopper was punctured with two sterile needles with a syringe, one for sampling and the other for draining the produced gas. The bioreactor was placed on a magnetic stirrer with a contact thermometer, at 300 rpm and the samples (2 mL) were taken at the preset time and processed immediately. Liquid samples were taken through the 0.45 μm Chromafil syringe filters and used for determination of the dissolved oxygen concentration, temperature and pH value by the Seven Go dissolved oxygenmeter SG6, Mettler-Toledo (Schwerzenbach, Switzerland) and pH-meter WTW pH 330 (Weilheim, Germany). Nitrate and nitrite concentrations in the water samples during tests were monitored spectrophotometrically on Hach DR/2400 (Hach Company, Loveland, Colorado, USA) by the chromotropic acid method and with α-naphthylamine, respectively [19,20].

2.2 Biodenitrification of the SW with the use of the Bio-NPC

Prior to biodenitrification tests, the adsorption of nitrate to NPC was investigated. The experiments were performed in 0.2 L closed serum bottles. Each clean sterile bottle was filled with 1.0000 g of NPC and 100 mL of SW containing 50 to 250 mg NO_3-N/L. The bottles were plugged and punctured for sample collection and placed on a magnetic stirrer with a contact thermometer for the setting and controlling of the constant temperature. The rotation speed and working temperature were 300 rpm and 25±0.5 °C, respectively. The initial sample and samples collected at 1, 2, 4 and 24 hours were analysed for nitrate concentration.

In the first set of biodenitrification experiments the impact of initially present nitrate was investigated. For that purpose the series of tests were conducted in 200 mL bioreactor containing 150 ml of the SW (C_0=50, 100, 150, 200 and 250 mg NO_3-N/L) and 15 g of Bio-NPC. The methanol previously added to the SW provided MetOH/NO_3-N ratio of 4.5:1. The bioreactor was plugged, punctured with two needles and set up on a magnetic stirrer equipped with a contact thermometer. The rotation speed and working temperature were set to 300 rpm and 25±0.5 °C, respectively. The samples were collected at predetermined time intervals and processed immediately. The kinetic analysis of nitrate removal was the same as previously reported [18,21].

The equation applied for calculation of denitrification rates was:

$$k_{den} \times t = C_{(N)_0} \times X_{(N)} \tag{5}$$

and Monod equation

$$r_D = \frac{\mathrm{d}\, C_N}{\mathrm{d}\, t} = \frac{k_D \cdot C_N}{(K_s + C_N)} \tag{6}$$

where k_{den} is the biodenitrification rate (mg NO_3-N/Lh), $C_{(N)_0}$ and $C_{(N)}$ - initial and nitrate concentration in time (mg NO_3-N/L), $X_{(N)}$ - conversion of nitrate (-), r_D is the rate of nitrate utilization (mg NO_3-N/Lh), k_D - the maximum rate of nitrate utilization (mg NO_3-N/Lh) and K_s - the half-saturation constant (mg NO_3-N/L).

The kinetic parameters of the Monod equation were determined using the Nelder-Mead simplex method of non-linear parameter search incorporated in the Matlab program. The initial guess of the kinetic parameter was entered into the program. Using this set of parameters the response curves were generated by the Runge-Kutta (IV) numerical integration method. Once the optimal kinetic parameters were established, the final optimal theoretical curve was compared with the experimental data plot.

2.3 The impact of temperature and MetOH/NO_3-N ratio on surface water biodenitrification

The initially present nitrate and MetOH/NO_3-N ratio in the SW, during the temperature impact determination were 100 mg NO_3-N/L and 4.5:1 respectively. The tests were set up as previously described and conducted at 15, 20, 25, 30 and 35 ± 0.5 °C under anoxic conditions.

The impact of temperature on the biodenitrification process was primarily considered on the basis of favouring bacterial growth in a predetermined temperature range. Most of the denitrifying bacteria are mesophiles that are known to grow in the range of 20-45 °C. The kinetics of the biodenitrification process include the estimation of activation energy and the temperature coefficient determination that determine the process sensibility and the change of the biodenitrification rates along with the change of the process temperature. The change of the biodenitrification rates along with the temperature was given by the Arrhenius equation [22]:

$$k_{den} = A_r \times e^{-(E_A/R_g T)} \quad (7)$$

where A_r is the Arrhenius factor (mg NO_3^--N/Lh), E_A is the activation energy (J/mol), R_g is the gas constant (8.314J/mol K) and T is temperature (K).

On the basis of this equation by logarithming is obtained the linear form

$$\ln k_{den} = \ln A_r - E_A/R_g \cdot T \quad (8)$$

The graphic plot of ln k_{den} versus $1/T$ is a straight line with a slope of - E_A/R_g and linearity enables the estimation of E_A and A_r. Generally, the E_A measures the change in the potential energy of a pair of molecules that is required to begin the process of converting a pair of reactant molecules into a pair of product molecules. The reactions with a low E_A were less sensitive to the change of temperature. For enzyme catalyzed reactions the E_A was in the range of 16 - 84 kJ/mol and more frequently was 46 kJ/mol [22].

The sensibility of the process to increasing temperature could also be expressed by [23]:

$$k_{denT} = k_{den20} \times 10^{KT(T-20)} \quad (9)$$

where K_T is the temperature constant. Under the studied conditions it was 0.052 (1/°C). The ratio of the specific denitrification rates at different temperatures in the selected range is known as - the temperature coefficient, Q_T:

$$Q_{10} = k_{den(T+10)}/k_{denT} \quad (10)$$

and it determines the change of the denitrification rate along with the change of temperature. During investigation of biodenitrification with methanol Timmermans et al. [23], at optimal pH of 8.3 and MetOH/NO_3-N ratio of 2.52, calculated the temperature coefficient of 3.3 indicating that the increase of temperature for 10 °C caused an increase of denitrification rates for 3.3k_{den}.

The optimal temperature for bacterial growth and therefore for biodenitrification was in the range of 35 - 38 °C, but during the optimization of the process parameters, the cost of temperature maintenance should be considered [24].

To determine the influence of methanol to nitrate-N mass ratio on the denitrification, the selected MetOH/NO_3-N ratios (2.0:1, 2.5:1, 3.5:1, and 4.5:1) were adjusted by addition of predetermined methanol amount during the preparation of the SW samples. The blank test (MetOH/N = 0:1) was set in a parallel. The tests were conducted in sterile bottles containing 15 g Bio-NPC and 150 ml of the SW, on a magnetic stirrer at 300 rpm and 25°C. The samples were taken at preset time intervals with a sterile syringe equipped with a Chromafil filter (0.45 μm) and processed immediately.

All tests were conducted in triplicate and the presented data is reproducible results with an error of less than 5 %. Both the means and standard deviations were determined, using the statistical package within Microsoft® Excel 2003.

3 Problem Solution

3.1 Biodenitrification of the SW with the use of the Bio-NPC

Previous to biodenitrification the adsorption of nitrate from the SW to NPC was investigated. The results obtained during this series of tests were clearly indicated that natural Croatian clinoptilolite did not adsorb nitrate (Fig.1). The nitrate concentrations determined throughout the tests in the SW samples were almost equal to the initial nitrate concentration.

The biodenitrification study dealt with the removal of 50-250 mg NO_3-N/L from the SW by using Bio-NPC. The methanol was used as the only source of organic carbon due to its price and determined efficiency [3]. The amount of added methanol was calculated for each test according

to the defined MetOH/NO_3-N ratio of 4.5:1. During our previous investigations the optimal MetOH/NO_3-N ratio was determined to be 2.5:1, but the excess of methanol was used in order to avoid carbon limited conditions [25].

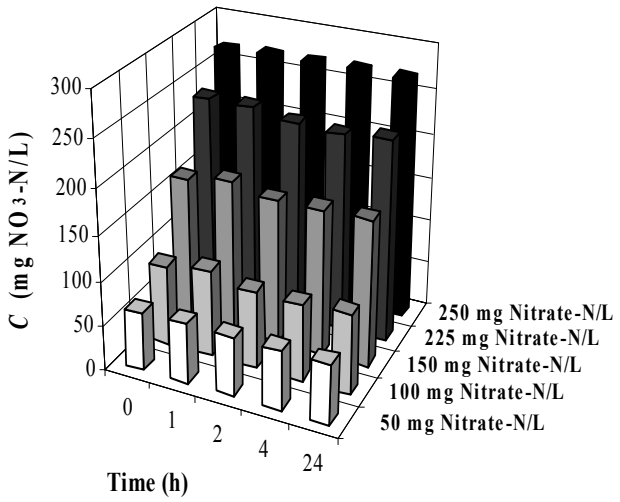

Fig.1 The nitrate concentration in the SW during the adsorption study to NPC.

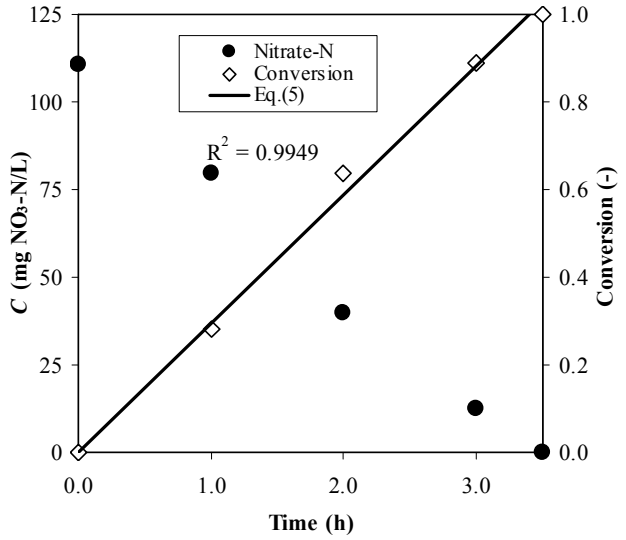

Fig.2 The nitrate concentration in the SW during the biodenitrification of 100 mg NO_3-N/L and the graphic test (integration method) of the zero order reaction model - Eq.(5).

According to literature and the obtained nitrate concentration during the biodenitrification study, it was assumed that nitrate removal was zero order reaction and the graphic test was applied by an integration method and according to Eq.(5) [26]. The experimental values of nitrate concentrations in time and the graphic test presented in Fig.2 clearly confirmed this assumption.

Nitrate concentrations determined during the batch nitrate removal study and lines modelled by the Monod equation - Eq. (6) were depicted in Fig.3. The kinetic parameters in the given model were determined according to the Nedler-Mead simplex method and the response curves are generated by the Runge-Kutta (IV) numerical integration method.

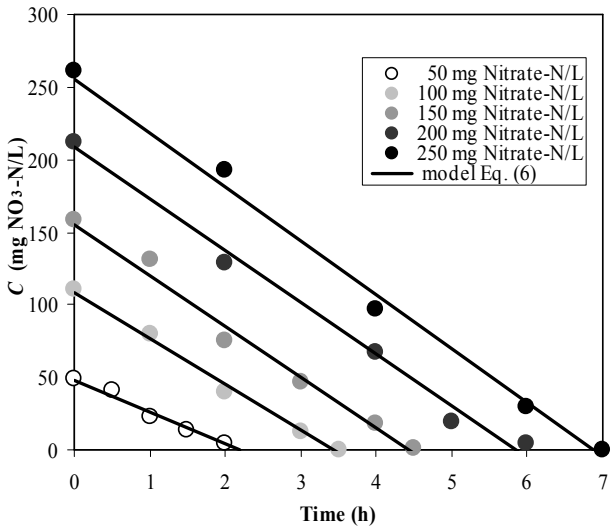

Fig.3 The nitrate-N concentration (experimental - symbols and modelled - line) in the SW during biodenitrification with the use of the Bio-NPC.

The experimental data was in accordance with and the predicted values. The K_S value obtained during modelling was 0.0003 mg NO_3-N/L and in comparison to the initial nitrate of 50-250 mg NO_3-N/L, it could be neglected. Consequently, as cited in literature the nitrate removal reaction was confirmed as a zero order reaction [25,27].

As seen in Fig.3, complete removal of nitrate was achieved during 2-7 hrs. Nitrite concentrations were monitored along with nitrate concentrations and the generation of nitrite was observed (Fig.4).

The highest value of accumulated nitrite of 1.69 mg NO_2-N/L was achieved during two hours of process duration, but a subsequent sharp decrease of nitrite occurred between the 2^{nd} - 4^{th} hrs. The final nitrite concentration in SW was a very important value due to the

nitrite toxicity and the legislation was lower than 0.21 mg NO_2-N/L [3].

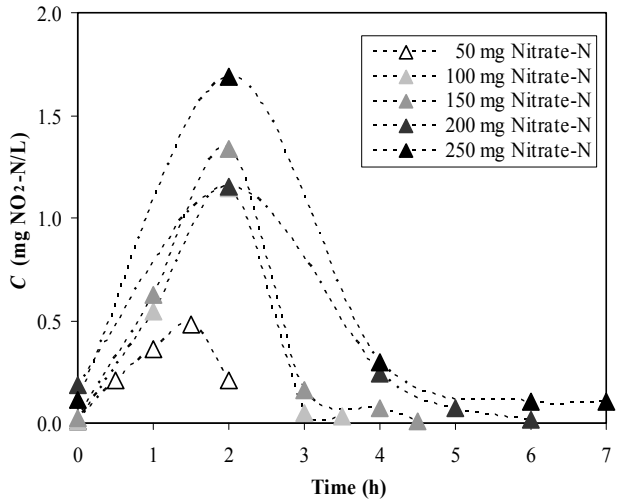

Fig.4 Nitrite-N concentration profile during nitrate removal from the SW by use of Bio-NSI.

The observed low values of nitrite concentrations in SW were a consequence of the presence and growth of denitrifying bacteria on the Bio-NPC and more important due to the presence of denitrifying enzymes in the bacteria that were necessary for complete reduction of nitrate [28].

The monitoring of dissolved O_2 revealed that almost all initially present dissolved O_2 (4.60±0.5 mg O_2/L) was consumed by the bacteria attached to the NPC during the first hour of biodenitrification and accordingly subsequent analysis of dissolved O_2 in the SW showed no dissolved O_2. This observation confirmed a general description of biodenitrification as an anaerobic or anoxic process [29].

The initial pH values of the SW were 7.17±0.03 and during the process were slightly raised (up to 7.4±0.12). The negligible increase of pH, is in conformity to the reaction of the denitrification with methanol that includes H^+ ions -equation (4) [23]. At the same time, the presence of phosphate salts that act as a buffer (K_2HPO_4 and KH_2PO_4) in the SW probably compensate higher increase of pH.

3.2 The impact of temperature and MetOH/NO_3-N ratio on surface water biodenitrification

The initially present nitrate (C_0 = 100 mg NO_3-N/L) in the SW, subject to temperature conditions was degraded in 6 hrs.

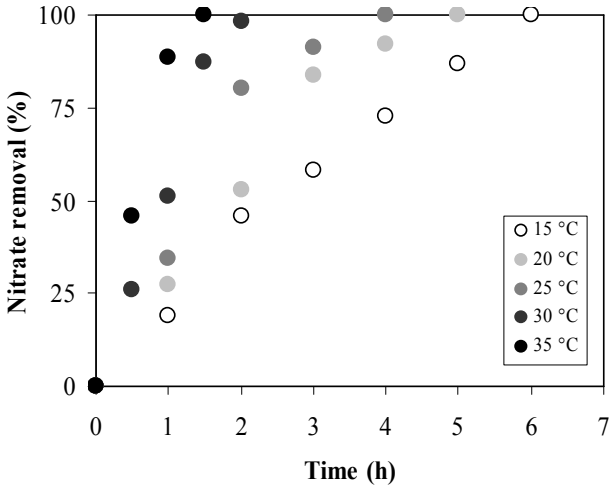

Fig.5 Nitrate reduction during biodenitrification of the SW by use of Bio-NSI.

At the highest investigated process temperature (35 °C) total nitrate removal was obtained after 1.5 hrs (Fig.5). Simultaneously, within 1.5 hour only 26%, 34% and 43% of nitrate were reduced at 15, 20 and 25 °C, respectively. The slower removal of nitrate noticed at 15 °C was the result of slower growth of bacteria at that temperature. At 25 °C, the nitrate was completely removed within 4 hrs and the observed accumulation of nitrite ions, in 1 h was 0.54 mg NO_2-N/L (Fig.6). During this investigation removal of 100 mg NO_3-N/L was accomplished with low generation of nitrite (up to 0.72 mg NO_2-N/L at 15 °C) and formed nitrite was than reduced to less than 0.015 mg NO_2-N/L. Apparently this low generation of nitrite, as a results of the existing denitrification enzymes, was favourable for fast and efficient removal of nitrate since the presence of increased nitrite concentrations proved to inhibit denitrification [28].

The values of dissolved O_2 and pH were similar to values that were obtained during the previous study. Dissolved O_2 was quickly brought to zero (during 1 h) and subsequent monitoring of O_2 in SW showed no dissolved O_2. The pH values of SW were slightly increased from 7.19±0.04 to 7.4±0.06. The dependence of the biodenitrification rates on temperature change was used to determine the impact of temperature on the biodenitrification process.

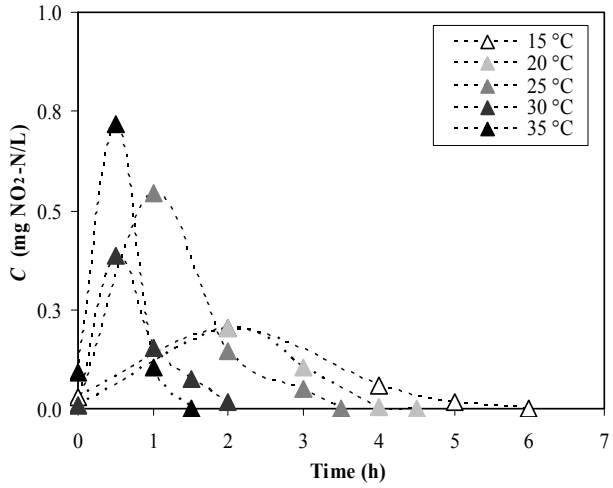

Fig.6 Nitrite concentration profile during the temperature impact determination on the SW biodenitrification.

The values of 1/T were depicted in relation to ln k_{den} (Fig.7 and Eq. (8)) and the obtained line (y = -5.804x + 23.081) had a high degree of linearity (R^2 = 0.9898). This equation and observed linearity enabled the reliable calculation of the activation energy, E_A and the Arrhenius factor, A_r. In the test conditions, the determined values were 48.25 kJ/mol and 1.056×10^{10} mg NO_3-N/Lh. Obtained values of the activation energy, E_A and the Arrhenius factor, A_r were similar to the previously published data [21,22,24]. Furthermore as mentioned earlier, for the enzyme catalyzed reactions, the more frequently determined E_A was 46 kJ/mol, which was close to the value obtained during this study [22].

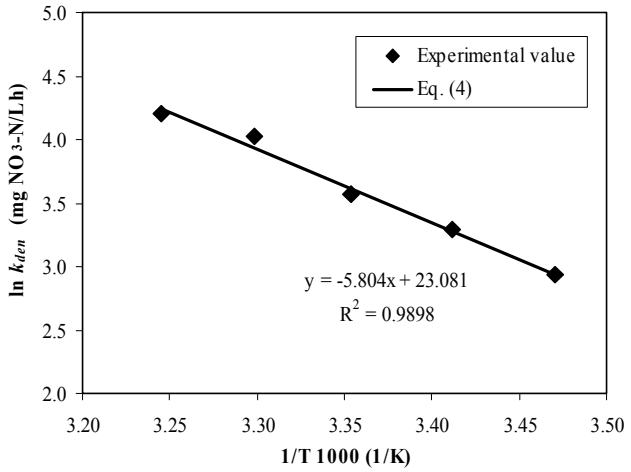

Fig.7 The graphic plot of ln k_{den} versus $1/T$ during biodenitrification of the SW.

The influence of temperature on the biodenitrification rates expressed by Eq. (9) and shown in Fig.8 provides the calculation of the temperature constant K_T. In addition, the temperature coefficient, Q_{10} as the ratio of the denitrification rates at different temperatures in the selected range was determined according to Eq. (10).

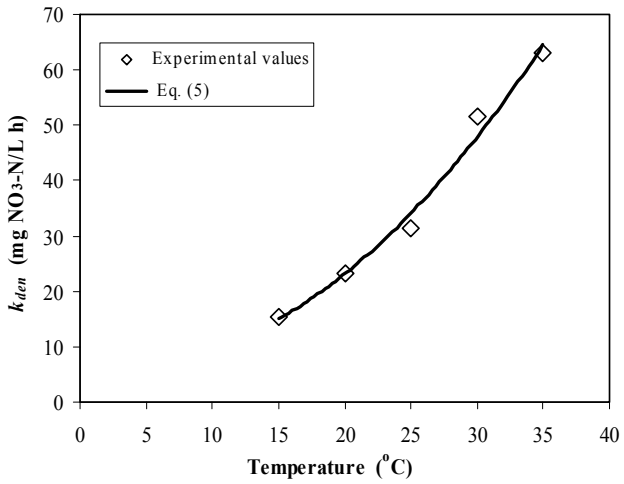

Fig.8 The change of k_{den} vs. temperature during the SW biodenitrification with use of Bio-NPC.

The temperature constant, K_T and temperature coefficient, Q_{10} were 0.031 1/°C and 2.03, respectively. The similar values of K_T and Q_{10} were previously determined during biodenitrification with methanol and ethanol [21,30]. On the contrary, Timmermans et al. during their study reported the higher values [23]. Furthermore, the obtained K_T value was in the range of K_T values of 0.0086–0.0334 1/°C as reported Casey et al. [31]. It is well known that the temperature coefficient determines the sensitivity of the process to the change of temperature, therefore the comparison of the obtained and reported values, revealed that the biodenitrification of SW by Bio-NPC was less sensible to the change of temperature [31].

The influence of methanol on surface water biodenitrification was clearly demonstrated in Fig. 9. As seen from results obtained in the blank test, in the absence of organic carbon (MetOH/NO_3-N= 0:1) nitrate concentration in time remained constant. Obviously methanol was necessary for biological denitrification of SW.

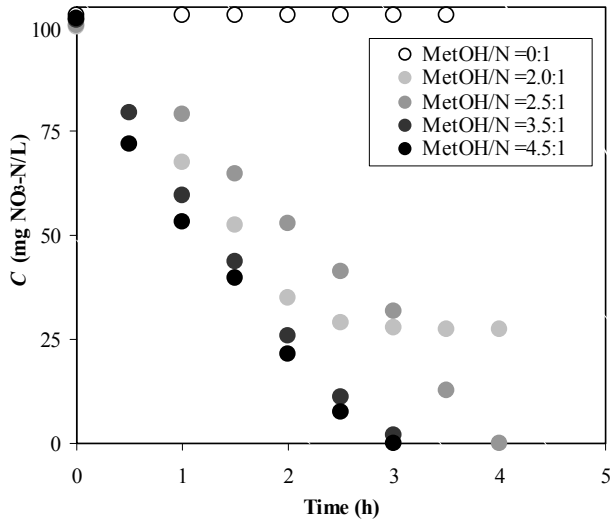

Fig.9 Nitrate concentration during biodenitrification of the SW by use of Bio-NSI in the presence of different amount of methanol.

In addition, the necessary amount of methanol should be higher than those defined through MetOH/NO_3-N ratio of 2.0:1, since at that ratio nitrate removal was incomplete due to the lack of methanol after 3 h. The complete biodenitrification was achieved at MetOH/NO_3-N ratio of 2.5:1 during 4 h. Increased MetOH/NO_3-N ratios (3.5:1 and 4.5:1) enabled complete nitrate removal during 3 h. Considering the cost of methanol and process duration, the use of increased MetOH/NO_3-N ratios was not justified. Therefore, the necessary amount of methanol for effective biodenitrification of SW was defined by MetOH/NO_3-N ratio of 2.5:1. Obtained value was in accordance with stoichiometric denitrification equation (4). Furthermore, the same value was previously determined as optimal for denitrification of surface water with use of bacteria attached to the ion exchanger Lewatit M600 [32]. The accumulated nitrite and final nitrite concentration during this investigation was up to 0.47 mg NO_2-N/L and 0.01 mg NO_2-N/L, respectively. Obviously these very low values enabled fast and efficient nitrate removal.

4 Conclusion

The degradation of nitrate ions present in SW was achieved with the use of mixed bacterial cultures attached to NPC in the batch bioreactor. The preliminary adsorption tests indicated that powdered Croatian clinoptilolite did not adsorb nitrate and therefore during the biodenitrification study with attached bacterial cells only nitrate reduction process was carried out. The complete reduction of 50-250 mg NO_3-N/L from the SW at 25°C was achieved during 2-7 hrs, with neglected nitrite accumulation. The final nitrite in the SW was lower than 0.1 mg NO_2-N/L and was a process priority with respect to legislation.

The application of the Monod equation and the analysis of obtained results revealed that nitrate removal can be well described by the given model. The determined half-saturation constant, K_s was very low and indicated the turn of the model to the zero order reaction model.

The analysis of denitrification rates in relation to the temperature of the studied process conditions enabled the calculation of the temperature constant K_T and the temperature coefficient, Q_{10} that were 0.031 1/°C and 2.03 respectively. The temperature coefficient defines the temperature sensitivity and therefore the studied biodenitrification of the SW by Bio-NPC was less sensitive to temperature change. Furthermore, the obtained activation energy, E_A and the Arrhenius factor, A_r that were 48.25 kJ/mol and 1.056×10^{10} mg NO_3-N/Lh respectively, confirmed the studied process as the less temperature sensitive process. According to obtained results and the process efficiency, 25 °C was selected as the optimal operating temperature.

The results of the tests conducted in the presence of different MetOH/NO_3-N mass ratios clearly indicated that MetOH/NO_3-N mass ratio of 2.5:1 was optimal for investigated biodenitrification process.

Finally, the results of this study demonstrated that complete and efficient removal of 100 mg NO_3-N/L with neglected nitrite generation from the SW with the use of Bio-NPC was achieved at a pH level of 7.20 and 25 °C during 3.5 hours in the batch bioreactor on a magnetic stirrer at 300 rpm in anoxic conditions. The biodenitrification of the SW by using the Bio-NPC was proven as an efficient method for nitrate removal.

Acknowledgments

This paper was jointly supported by funds from Hrvatske Vode and the Ministry of Science, Education and Sports (Scientific Project (125-0000000-1970)), Zagreb, Croatia.

Special thanks to prof. Nadja Dešpalj who translated this paper to English.

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