The use of bacteria attached to Lewatit M600 for denitrification of the Cetina surface water

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Abstract: The selected mixed bacterial culture was attached to the nitrate selective ion exchange resin - Lewatit M600 (NSI) and used for investigation of nitrate removal from the surface Cetina water (SCW). The investigated system enables bonding of nitrate ions and simultaneously, the degradation of bonded nitrate. The nitrate adsorption on NSI was studied and described with the Freundlich isotherm. With the use of bacterial cells attached on NSI, the nitrate (50 - 200 mg NO_3-N/L) was completely removed from the SCW, at a methanol to nitrate-N mass (C/N) ratio of 4.5:1, during 4-7 hours at pH = 7.2 and 25 °C under static anoxic conditions. The influence of initially present methanol on the denitrification process was investigated and the required C/N ratio of 2.5:1 was determined, although the denitrification process was faster in the presence of increased C/N ratios. The applicability of the bacteria attached to the NSI for fast and efficient nitrate removal from the surface Cetina water at different temperatures was further investigated. The denitrification rates were calculated according to the zero -order reaction model. The activation energy, E_A and Arrenius factor, A_r for denitrification were 11.40 kJ/mol and 1790.05 mg NO_3-N/Lh, respectively.

Key Words: Bacteria, Immobilization, Ion exchanger, Nitrate, Nitrite, Denitrification

1 Introduction

Negative environmental influence caused by human activities is encountered through chemical, biological and visual pollution of soil, air and water. Therefore, the environmental protection is ever increasing the attention of public and respected Croatian and international scientists [1-3]. Towards the European Community (EC) there is a requirement for harmonisation of national legislation with the EC directive in the field of water policy [4]. According to it, members of the EC should protect, improve and recover all surface waters in order to achieve quality of water resources during the next 15 years. Furthermore EC directive proposing measures for progressive decrease of toxic substances present in water, resulting in the protection from pollution and the endangering of aquatic ecosystems. Accordingly, the national environmental strategy in the Republic of Croatia contains fifty priorities for resolving the global ecological problems. It determines the highest organizational-investment priorities, and among them water management - water resources have an important role. The impact of waste on surface and ground waters should be an imperative. In industrial and rural areas, the overloading of some pollutants such as phosphate and nitrogen containing compounds is present. They are crucial and essential in appropriate concentrations, but increased quantities cause excessive plant growth resulting in suffocation of life in water.

The presence of nitrate ions in surface waters outcomes from the present pollution of water; it is due to increased production and application of synthetic materials, frequent use of synthetic fertilizers in agriculture and many other industries which include nitrate salts during production or implementation [5]. Accordingly, near the agriculture areas in Croatia, nitrate concentrations in surface and ground waters occasionally were higher than maximum contaminant level for all categories, with a maximal value of 126.0 mg NO_3-N/L [6]. At areas where the nitrate concentration is higher than 50 mg NO_3-N/L, the groundwater is unsuitable for drinking and irrigation purposes [2].

Waters used by the public are generally previously purified by application of selective ion exchangers [7]. Accordingly, nitrate selective ion

exchange resins are used for nitrate removal. The resulting brine contains increased nitrate and NaCl concentrations, so, it should be treated before releasing in the environment. For that purpose, the commonly applied methods are ion exchange, biological treatment and their combination [8,9]. The biological denitrification was successively applied for wastewater treatment and the ion exchange methods for the treatment of drinking and surface waters [10,11]. The main disadvantage of ion exchange is disposal of highly concentrated brine wastewater and used ion exchangers, hence, the combination of biological denitrification and ion exchange was investigated in order to obtain cost effective nitrate removal. The biological method was applied for removal of nitrate from the regeneration solution that was repeatedly used [12]. Usually, for the biological degradation, suspended microbial cells were used but in order to improve performance of the process the use of immobilized cells is being investigated. For that purpose, the immobilisation of bacterial cells on different natural or synthetic support materials was studied [13-15]. The use of ion exchangers as carriers of bacterial cells has many advantages, like their structure and composition properties that enable attachment of bacterial cells. Generally, biological denitrification enables transformation of oxidized nitrogen compounds by a wide spectrum of heterotrophic bacteria into harmless nitrogen gas with the accompanying carbon removal. Based on its price and availability, methanol is most commonly used as an additional carbon source for bacterial denitrification [8,16-17]. The process, according to bacteria used, was usually set in anaerobic or anoxic conditions, so, the true denitrifiers accomplished a complete and rapid nitrate removal with minimum nitrite accumulation. This process has been well studied, but biological denitrification of surface or waste waters is usually slow and lasts several days. The aim of the present paper was to investigate the applicability of bacteria attached to the Lewatit M600 for fast and efficient nitrate removal from the Cetina surface water.

2 The nitrate removal from surface water with the use of bacteria attached to Lewatit M600

The main goal of this study was to investigate possible use of bacteria attached to nitrate selective exchange resin for nitrate removal from surface waters. The use of bacteria attached to NSI was investigated in order to reduce nitrate pollution and to achieve more effective water purification. Furthermore, cells attached on exhausted nitrate selective exchange resin would enable bonding of nitrate ions and simultaneous degradation of bonded nitrate on resin beads. As a consequence, regeneration of saturated resin beads would be avoided and at the same time, the present exhausted resins as a waste material could be further used. Then, the influence of initially present nitrate, temperature and methanol to nitrogen mass ratio on removal of nitrate with bacteria attached on waste resin was investigated in a column at static anoxic conditions.

2.1 Materials and methods

The water sample (SCW medium) contained (g/L): K_2HPO_4 2.5; KH_2PO_4 1 and natural surface water of the Cetina river up to 1 L. The typical composition of the raw Cetina surface water is shown in Table 1. The solutions were autoclaved and allowed to cool at room temperature before adding $NaNO_3$ and CH_3OH. All chemical compounds used were p.a. chemicals.

Table 1 Physical and chemical parameters of the surface Cetina water.

Parameters	Average values
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)	0.001-0.398
NO_2-N (mgN/L)	0-0.05
NO_3-N (mgN/L)	0.472-84.8
Cl^- (mg/L)	9.5-69.20
SO_4^{2-}(mg/L)	9.4-36.9
PO_4^{3-} (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

For each denitrification experiment, nitrate-N from 50-200 mg NO_3-N/L (the stock solution was an aqueous solution of $NaNO_3$ containing nitrate-N 10 g/L) and methanol (at MetOH:N mass ratio of 4.5:1) were added separately. The excess methanol

was used to avoid carbon limited conditions, but than it was optimised to avoid further pollution of the SCW. Phosphate salts in the SCW medium were used as a buffer. This provided unchanged pH (7.2 ± 0.05) of the prepared SCW medium throughout the tests.

2.1.1 The carrier of the bacteria and cell attachment

Microorganisms originated from the active sludge of the wastewater treatment plant Anamet, Savski Marof, Croatia and the agricultural soil sample (Lastovo, Croatia). The active sludge (100 mL) and 50 g of the soil were mixed and filtered (blue band filter). The obtained biomass was washed twice, diluted to 50 mL with the SCW medium, refrigerated at 4 °C and stored until use.

The nitrate selective ion exchanger Lewatit M600 - NSI (Bayer, Leverkusen, Germany) used as a carrier of bacterial cells, was washed with HCl (pH = 2) then with deionised water to achieve neutral pH. The physico-chemical properties and specifications of Lewatit M600, as reported by the supplier are shown in Table 2.

To attach the bacterial cells to the carrier, the 0.5 L sterile serum bottle was filled with 200 g of NSI and the SCW medium with the mixed bacterial culture was pumped and recirculated with a peristaltic pump through a bottle filled with the carrier over 48 hrs. The carrier was then washed with a sterile SCW medium to remove excess bacterial cells. Washing was discontinued when microscopic examination (at 1000×) of the eluate showed that the bacterial cells were brought to zero. The wet NSI beads with attached bacteria were refrigerated at 4 °C and stored for further use.

Table 2. Physical and chemical properties of the Lewatit M600 - NSI

Properties	Values
Type	gel, type II
Matrix	polystyrene
Ionic Form	Cl/OH
Operating temperature (°C)	30-85
Operating pH range	0-11
Total capacity (Eq/L (min))	1.125
Volume change (%)	16
Water retention (%)	45-60
Drying loss at 110 °C (%)	45
Particle size (mm)	0.6

2.2 Adsorption of nitrate on Lewatit M600

Adsorption tests were performed in 0.15 L closed serum bottles. Each clean sterile serum bottle was filled with 1.0000 g of Lewatit M600 and 100 mL of SCW (C_0 = 50–200 mg NO_3-N/L) and closed with a rubber stopper. The stopper was punctured with the disposable syringe by a needle, for sampling.

During adsorption tests, the bottles entirely immersed in a water bath at 25°C were placed on the magnetic stirrer at 400 rpm. The samples (2 ml) were taken by a disposable sterile syringe supplied with a needle and Chromafil filter (45 μm) at the beginning of the experiment, and after 0.5, 1, 2 and 24 h.

The ion exchange process is usually described by adsorption isotherms. The equilibrium adsorption isotherm is of fundamental importance in the design of adsorption system. Among many isotherm models, the Freundlich adsorption isotherm, as an indicative of surface heterogeneity of sorbent, is commonly used [18]:

$$q_e = K_f C_e^{1/n} \quad (1)$$

where q_e – is the amount of nitrate adsorbed on resin at equilibrium mg NO_3-N/g NSI, C_e - is the equilibrium concentration in the water phase (mg NO_3-N/L), K_f - Freundlich constant that relates to adsorption capacity (mg NO_3-N·L/mg NO_3-N g NSI), and $1/n$ - adsorption intensity (n-dimensionless exponent).

The linear form of the Freundlich equation can be obtained by logarithmic transformation:

$$\log q_e = \log K_f + 1/n \log C_e \quad (2)$$

According to the graphic plot of log q_e versus log C_e, and observed linearity ($R^2 > 0.99$) the Freundlich isotherm constants could be reliably calculated.

However, after saturation exhausted resin had to be regenerated by the use of some regenerant, mostly by NaCl [18]. The resulting solution - brine contained higher nitrate and NaCl concentrations and therefore could not be disposed without previous treatment. This led to the increase of the treatment cost, and consequently, a combination of ion exchange and biological denitrification methods were investigated [19]. According to literature, biological regeneration of saturated resin enables the removal of bonded nitrate ions and the repeated use of regenerated resin [9]. But, there is no data about the investigation of bacteria attached to exhausted resin. Therefore the present work is

aimed to investigate possible adsorption and simultaneous degradation of sorbed nitrate ions from SCW with the use of bacteria attached to NSI.

2.3 Acclimation of bacteria and nitrate removal with bacteria attached on NSI

The acclimation experiments were performed in a 0.25 L closed column (h=20,0 cm; r=2,0 cm). Each column was filled with 37.5 g of NSI with attached bacterial cells and 150 mL of the SCW medium was placed in an air thermostat at a selected temperature. The NSI with attached bacteria was acclimated to nitrate ions (C_0 = 100 mg NO_3-N/L) at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under static anoxic conditions. The samples were taken at the bottom of the column through a pipe equipped with a 0.45 μm filter at a predetermined time and immediately analysed.

The denitrification experiments were performed in columns (h=20,0 cm; r=2,0 cm; V_{scw}=150 mL; m_{NSI}=25 g/100 mL) and the influence of increased initial nitrate concentrations (C_0 = 50-200 mg NO_3-N/L) was determined. Each column was closed with a rubber stopper. The stopper was punctured with a thermometer and a disposable syringe by a needle for drain off the produced gas. The samples were taken at the bottom of the column and immediately analysed.

The denitrification rate at which nitrate was converted to nitrite and finally to nitrogen gas, was calculated according to the general kinetic model [20]:

$$dC_N/dt = -k_{den} \times C_N^{\,n} \quad (3)$$

where C_{N0} and C_N are the initial and nitrate concentrations in time (mg NO_3-N/L), t is time of the process duration, k_{den} is the denitrification rate (mg NO_3-N/Lh) and n is the partial reaction order. Nitrate concentration depending on the time of the partial reaction order (n) is given by

$$\left(\frac{C_N}{C_{N0}}\right)^{(1-n)} = 1 - \frac{(1-n)k_{den}}{C_{N0}^{(1-n)}}t \quad \text{for } n \neq 1 \quad (4)$$

and

$$\frac{C_N}{C_{N0}} = \exp(-k_{den}t) \quad \text{for } n = 1 \quad (5)$$

Also, the nitrate removal could be described according to the widely applied Monod equation [20,21], which is employed for calculating kinetic constants using data from batch tests:

$$r_{den} = \frac{dC_N}{dt} = \frac{K_d \times C_N}{(K_S + C_N)} \quad (6)$$

where r_{den} is the rate of nitrate utilization (mg NO_3-N/Lh), K_S is the half velocity constant (mg NO_3-N/L) and K_d is the maximum rate of nitrate utilization (mg NO_3-N/Lh).

If $K_S << C_N$ in Eq. (6), K_S is insignificant compared to C_N, and the Monod equation turns to a zero-order reaction model [22]. The integration form of the zero order kinetic model equation is given by

$$C_N - C_{N0} = C_{N0} \times X_N = -k_{den}t \quad (7)$$

where X_N is the conversion of nitrate (-) and k_{den} is the denitrification rate (mg NO_3-N/Lh).

2.4 The influence of temperature and methanol to the nitrate-nitrogen ratio on the denitrification process

The denitrification tests were conducted in the column as previously described at an initial nitrate concentration of 100 mg NO_3-N/L, at pH = 7.2 and 25 °C under static anoxic conditions. The influence of temperature on the process was investigated in the range of 15-35 °C.

Denitrification rates increased with temperature, depending on the activation energy of the reaction, as given by the Arrhenius equation:

$$k_{den} = A_r e^{-\frac{E_A}{R_g T}} \quad (8)$$

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

The overall relationship between the nitrate concentration and temperature can be expressed as [23]

$$\ln k_{den} = \ln A_r - E_A/R_g \cdot T \quad (9)$$

Generally, the obtained activation energy, E_A for biological denitrification was in the activation energy range of enzyme-catalysed reactions, which were usually 16 - 84 kJ/mol [24].

The biological denitrification of SCW (C_0 = 100 mg NO_3-N/L) with bacteria attached on NSI in the presence of different predetermined amounts of methanol (MetOH:N mass ratios were in the range of 2.0:1 to 4.5:1) was further investigated. The tests

were conducted in the column as previously described.

2.5 Analytical methods

To study the kinetics of nitrate removal from the SCW medium, the bottle and the column contents were sampled at the preset time and processed immediately.

The concentration of the dissolved oxygen and pH of water samples were monitored by the Seven Go dissolved oxygen meter SG6, Mettler-Toledo (Schwerzenbach, Switzerland) and pH-meter WTW pH 330 (Weilheim, Germany). Liquid samples were filtered through the 0.45 μm sterile syringe filters immediately after sampling and used for nitrate and nitrite analysis.

Nitrate and nitrite concentrations in the water samples during experiments were monitored spectrophotometrically on Hach DR/2400 (Hach Company, Loveland, Colorado, USA) by the chromotropic acid method and with sulfanylic acid and α-naphthylamine, respectively [25,26].

The number of bacterial colonies (CFU) attached to the NSI was determined by a plate count on the standard nutrient agar, after repeated dilution with NaCl (m/V ratio = 9 g/L). The NSI beads were weighed (1 g-wet weight) and crushed in sterile mortar. The crushed beads were transferred into the sterile Erlenmeyer flasks and 100 mL of a sterile 0.9% NaCl solution was added. The flask was agitated vigorously with vortex over 30 min on the magnetic stirrer in order to remove the bacteria from the NSI beads. The resulting suspension was serially diluted in the sterile 0.9% NaCl solution and triplicate aliquots were plated on the standard nutrient agar (Biolife, Italy). After incubation over two days at 37 °C, all plates containing 30–150 discrete colonies were selected for determination of the cell number by plate count. The number of bacteria attached to the NSI was expressed as CFU/g NSI.

3 Problem Solution

3.1 Adsorption of nitrate on Lewatit M600

At first the adsorption of nitrate from the SCW (C_0 = 50–200 mg NO_3-N/L) was investigated in batch reactor at 400 rpm and 25 °C. According to obtained results, the nitrate adsorption equilibrium was achieved during 1 hour. For all investigated nitrate concentrations during 24 h, 65-70% of the nitrate was adsorbed (Fig. 1).

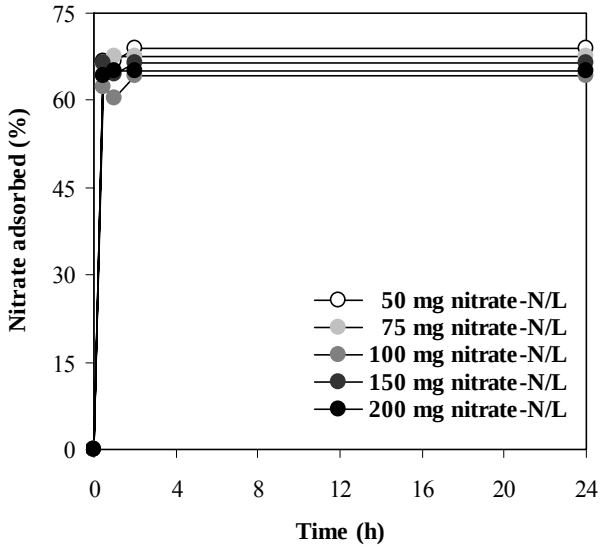

Fig.1 The determination of nitrate-N adsorption equilibrium in the SCW.

The graphic plot of log q_e versus log C_e (Eq.2) enables the calculation of Freundlich isotherm constants (Fig.2). As shown in Fig.2 the Freundlich constant, K_f was 0.298 mg NO_3-N/g NSI and n was 1.131. The obtained n value between 1 and 10 represents favourable adsorption and is in accordance to literature [27].

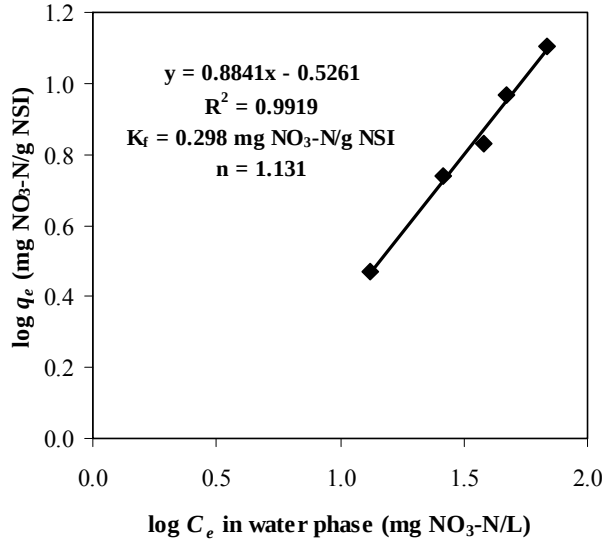

Fig.2 The Freundlich isotherm plot for the nitrate adsorption on NSI.

3. 2 Acclimation of bacteria and nitrate removal with bacteria attached on NSI

During the investigation of bacteria acclimation for efficient nitrate removal, a significant decrease of the process duration was observed (Fig.3). Finally, 100 mg NO_3-N/L were completely removed during 4 h at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under static anoxic conditions.

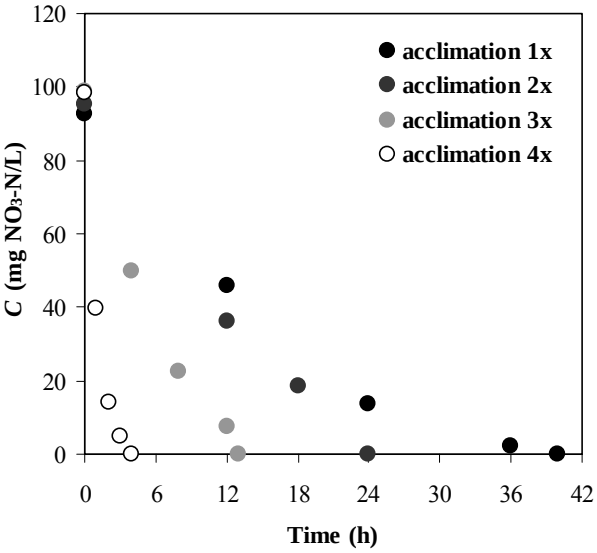

Fig.3 The decrease of nitrate concentration in SCW during the acclimation of bacteria.

The initial dissolved O_2 value of 5.9 mg O_2/L was quickly consumed by bacteria and after 1 h, it was only 0.02 mg O_2/L. The number of bacterial cells attached on NSI at the beginning of the tests was 1.8×10^7 CFU/g NSI, while at the end of acclimation it was 6.0×10^9 CFU/g NSI.

The influence of initially present nitrate (C_0 = 50 - 200 mg NO_3-N/L) on the process with bacteria attached on NSI was studied in the column at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under static anoxic conditions (Figs. 4 and 5). Increased initial nitrate concentrations prolonged complete nitrate removal from SCW from 4.5 to 9 h. The results indicated that the presence of higher nitrate concentrations led to slower denitrification.

The complete removal of 100 mg NO_3-N/L lasted 5.5 hrs. During that process, nitrite ions were generated up to 1.76 mg NO_2-N/L, but they were quickly reduced and at the end of process the final nitrite concentration was 0.19 mg NO_2-N/L (Fig. 5).

Monitoring of nitrite concentrations showed that the nitrite followed the typical pattern of biological denitrification: transient increase in nitrite concentrations (nitrite was produced by nitrate reduction) was subsequently followed by nitrite reduction.

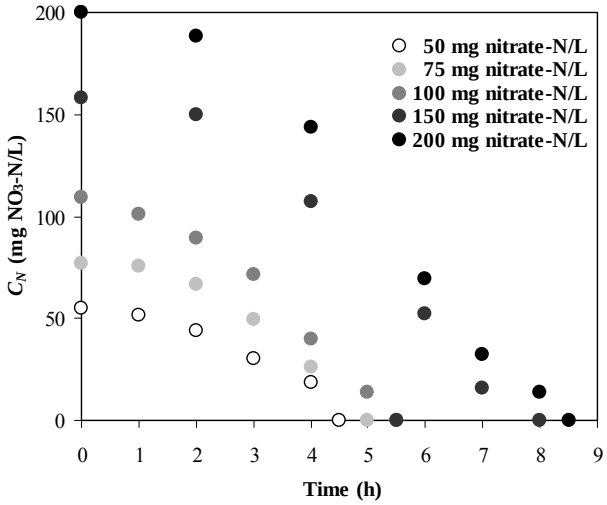

Fig.4 Nitrate-N removal from the SCW with use of bacteria attached on NSI.

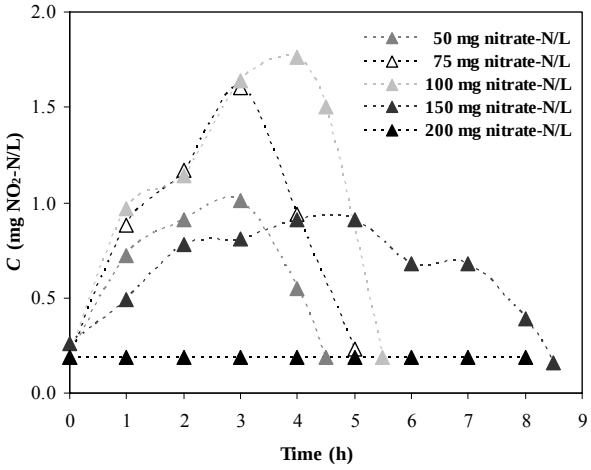

Fig.5 Nitrite-N concentration profile during nitrate removal from the SCW with use of bacteria attached on NSI.

Furthermore, the observed accumulation of nitrite at increased concentrations of initially present nitrate (150 and 200 mg NO_3-N/L) was lower than those observed at 100 mg NO_3-N/L. This could be explained as a consequence of bacterial acclimation to present nitrite ions. Besides, low concentrations of nitrite formed during denitrification of the SCW provided the direct reflow of the SCW in the water recipient

since the observed concentrations were lower than MCL (maximum contaminant level) [28].

The studied denitrification of the SCW was not inhibited by generated nitrite ions. The selected mixed culture originating from an industrial wastewater treatment plant seemed to be more advantageous for fast denitrification. The results clearly indicated that nitrate ions up to 100 mg NO_3-N/L could be effectively removed from the SCW.

3.3 The influence of temperature and methanol to the nitrate-nitrogen ratio on the denitrification process

Nitrate removal from the SCW (C_0 = 100 mg NO_3-N/L) at 15°C, was very slow and lasted for 7 hrs (Fig. 6). At the same time, during the first 4 hrs almost 65% and 80% of nitrate were removed from the SCW at 20 and 25°C, respectively. Complete denitrification was achieved in 6.5 and 5.5 hrs, respectively. At 30 and 35°C there was no significant difference and as shown in Fig. 6, at 35°C complete denitrification was achieved in 4 hrs, which was very fast. Although denitrification at 25°C was slower than at 35°C, for economical reasons the former temperature can be proposed as the operating temperature.

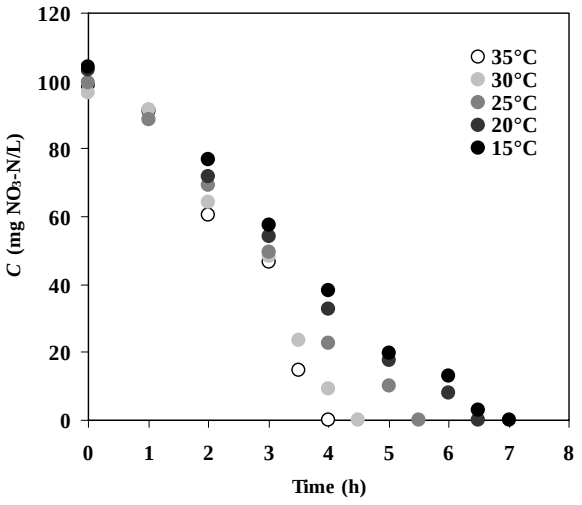

Fig.6 The decrease of nitrate concentrations in the SCW during denitrification tests with bacteria attached on NSI at different incubation temperatures.

The maximum nitrite accumulation of 1.98 mg NO_2-N/L was observed during 3 hrs, but after that nitrite was reduced reaching final concentration of 0.13 mg NO_2-N/L. The initial number of bacterial cells attached to NSI was 3×10^7 CFU/g NSI, while at the end of denitrification, it was 7×10^9 CFU/g NSI. Observed values were similar to previously published data [16].

During this study dissolved O_2 in the SCW was quickly consumed by bacteria and after 1 h there was not any dissolved O_2 in the SCW. The pH was continuously checked and the observed values were 7.20 ± 0.05. The presence of phosphate salts, K_2HPO_4 and KH_2PO_4 that act as a buffer obviously enable control of pH in the SCW.

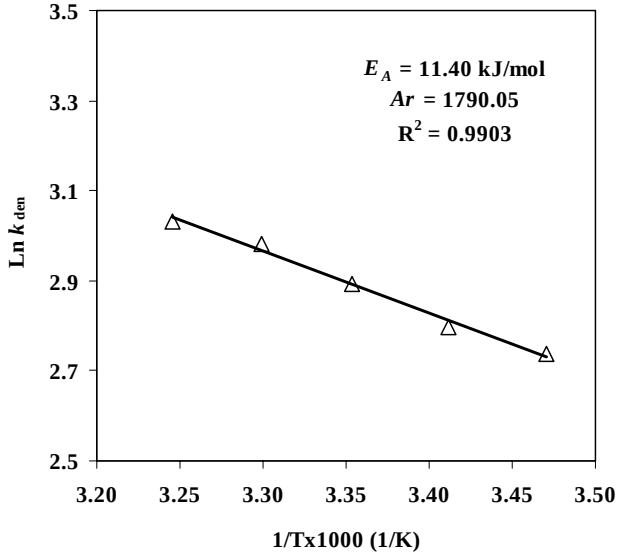

Fig.7 The Arrenius plot for denitrification of SCW with bacteria attached on NSI.

According to literature, obtained results and according to the Eqs. 7-9, the denitrification rates (k_{den}), the activation energy (E_A) and the Arrenius factor (A_r) were determined. The left-hand side of Eq. (9) was determined and plotted against the reciprocal of temperature as shown in Fig. 7. A high degree of linearity ($R^2 > 0.99$) is known to provide a reliable estimate of the activation energy (E_A) and the Arrenius factor (A_r) [29,30]. The activation energy for nitrate reduction was 11.40 kJ/mol and A_r was 1790.05 mg NO_3-N/Lh. Accordingly, this E_A value is in very good agreement with the findings of Kumar et al., who reported an activation energy value of 17.7 kJ/mol [30].

The nitrate -N concentration values observed during the denitrification test that was conducted at 25 °C was used for the determination of kinetic

reaction order. According to the equation (7), these values were used for the testing of the zero order kinetic model by an integration method. The graphic plot (Fig.8) indicated that the observed linearity ($R^2 > 0.99$) confirmed the investigated denitrification process as a zero order reaction. The determined order of the denitrification process was previously established [17,31,32].

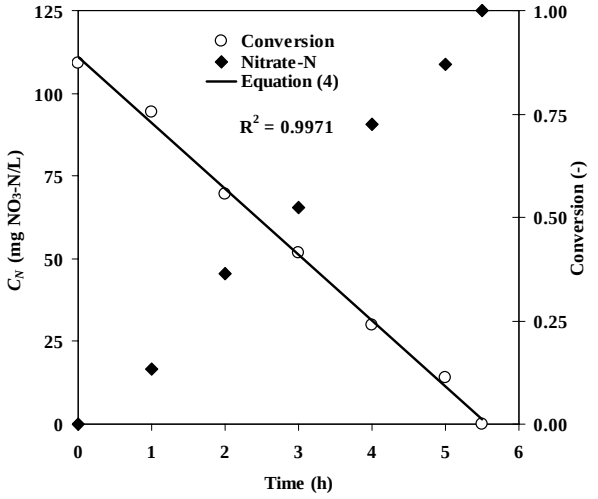

Fig.8. Nitrate concentrations and the conversion versus time.

Methanol was selected as the most suitable external carbon source because it is the least expensive and very efficient in denitrification [8,16]. In order to quantify the influence of methanol on denitrification five different MetOH:N mass ratios were tested separately (Fig 9). It can be seen from Fig. 9 that nitrate removal continuously increased for all MetOH:N ratios exceeding 2.5:1 mg CH_3OH/mg NO_3-N. At a lower methanol to nitrate-nitrogen ratio (2.0:1) the nitrate removal was incomplete. Due to the lack of methanol, after 3 h of process duration, nitrate concentration in the SCW samples remained constant. At MetOH:N mass ratio of 2.5:1 and 3.0:1 complete denitrification was achieved during 7 and 6 hrs, respectively. Total nitrate removal at MetOH:N ratios of 4.0:1 and 4.5:1 lasted for 5 hrs. Results obtained at MetOH:N ratios over 3.0 indicated that the time for complete nitrate removal remained almost constant. Therefore, it seemed that the MetOH:N ratio of 3.0:1 was more than sufficient for complete denitrification. Comparison of the time required for complete nitrate removal at MetOH:N ratios of 3.0:1 and 2.5:1 suggested that the stoichiometric value was 2.5:1 under the experimental conditions. This was in accordance with the theoretical value calculated from the equation proposed by McCarthy et al. [33].

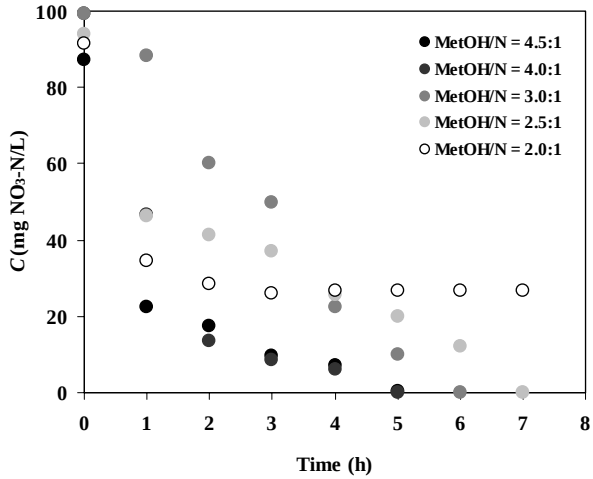

Fig.9 The decrease of nitrate concentration in SCW during denitrification tests at different MetOH:N ratios.

Furthermore, monitoring of nitrite concentrations in the SCW indicated that the accumulated nitrite of 0.46 mg NO_2-N/L was insignificant in comparison to the initially present nitrate and revealed no influence on denitrification of the SCW. Simultaneously, dissolved oxygen in the SCW was consumed by bacteria during the first hour of denitrification and afterwards there was no dissolved oxygen in the SCW. The pH monitoring indicated once again that the addition of K_2HPO_4 and KH_2PO_4 to the SCW enabled the constant pH values of 7.2 ± 0.05 in the SCW samples throughout the tests.

In addition, MetOH:N ratio of 2.5:1 was selected as an optimal value, since the increase of methanol amount increased the process cost, but the time for complete nitrate removal was not significantly decreased.

4 Conclusion

The use of mixed bacterial cultures attached to NSI resulted in adsorption of nitrate and simultaneous degradation of sorbed nitrate ions. The complete reduction of nitrate from the SCW at 25°C was achieved during 4-7 hrs, with neglected nitrite accumulation. The required MetOH:N ratio was 2.5:1 although the denitrification process was faster in the presence of increased MetOH:N ratios. Furthermore, the regeneration of saturated resin beads was avoided and at the same time, the

present exhausted resin was used as a waste material. The optimisation of process parameters enabled fast and efficient nitrate removal from the Cetina surface water.

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