

# Nitrate removal from the Cetina surface water by using bacteria attached to Lewatit M600

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*Abstract:* The applicability of the bacteria attached to the exhausted exchange resin - Lewatit M600 (NSI) for fast and efficient nitrate removal from the surface Cetina water (SCW) was investigated. The investigated system enables simultaneous bonding of nitrate and degradation of bonded nitrate ions. The nitrate adsorption on NSI was described with the Freundlich isotherm. The nitrate (50-200 mg NO<sub>3</sub><sup>-</sup>-N/L) was completely removed from the SCW during 4-7 hrs at pH = 7.2 and 25 °C under anoxic conditions. Activation energy,  $E_A$  and Arrhenius factor,  $A_r$  for nitrate reduction were 11.40 kJ/mol and 1790.05 mg NO<sub>3</sub><sup>-</sup>-N/Lh, respectively. The required MetOH:N ratio was 2.5:1 although the denitrification process was faster in the presence of increased MetOH:N ratios.

*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. Towards the European Community (EC) there is a requirement for harmonisation of national legislation with the EC directive in the field of water policy [1]. 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. 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<sub>3</sub><sup>-</sup>/L [2].

Waters used by the public are generally previously purified by application of selective ion exchangers [3]. 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 [4,5]. Usually, for the biological degradation, suspended microbial cells were used, but the use of immobilized cells is being investigated, too. 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 [4,6-8]. The process, according to bacteria used, was usually set in

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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 wastewater 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. It was investigated in order to achieve more effective water purification. Ion exchangers as carriers of bacterial cells have many advantages, like their structure and composition properties. Furthermore, cells attached on exhausted nitrate selective exchange resin would enable simultaneous bonding of nitrate ions and degradation of nitrate bonded 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.

### 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 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.

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. Phosphate salts in the SCW medium were used as a buffer. This provided unchanged pH ( $7.2 \pm 0.05$ ) of the prepared SCW medium throughout the tests. The influence of temperature on the denitrification was determined in the range of 15-35 °C. The influence of methanol to nitrate-nitrogen ratio on the denitrification process was investigated in the presence of 100 mg  $NO_3^-$ -N/L in the SCW and at predetermined MetOH:N ratios (2.0:1 - 4.5:1).

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  $\mu$ m 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  $\alpha$ -naphthylamine, respectively [9,10].

#### 2.1.1 The carriers 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.

Lewatit M600 (NSI) used as a carrier of bacterial cells, was washed with HCl (pH = 2) then with deionised water to achieve neutral pH. 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. The wet NSI beads with attached bacteria were placed in a refrigerator at 4 °C. Heterotrophic bacteria were counted by standard plating techniques on nutrient agar ("Biolife", Milano, Italy) with incubation at 37 °C for 3 days, as previously described [8]. The results are expressed in colony forming units (CFU/g NSI).

#### 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 (Bayer, Leverkusen, Germany) 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 ion exchange process is usually described by adsorption isotherms. Among many isotherm

models, the Freundlich adsorption isotherm, as an indicative of surface heterogeneity of sorbent, is commonly used [11]:

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

where  $q_e$  - is the amount of nitrate adsorbed on resin at equilibrium mg NO<sub>3</sub><sup>-</sup>/g NSI,  $\gamma_e$  - is the equilibrium concentration in the water phase (mg NO<sub>3</sub><sup>-</sup>-N/L),  $K_f$  - Freundlich constant that relates to adsorption capacity (mg NO<sub>3</sub><sup>-</sup>-N/g), and  $1/n$  - adsorption intensity ( $n$ - dimensionless exponent). The Freundlich equation can be linearised by logarithmic transformation:

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

According to the graphic plot of  $\log q_e$  versus  $\log \gamma_e$  the Freundlich isotherm constants were calculated.

However, after saturation exhausted resin had to be regenerated by the use of some regenerant, mostly by NaCl [11]. 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 [12]. There was no data about the investigation of bacteria attached to exhausted resin in literature. 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 adjusted to nitrate ions ( $C_0 = 100$  mg NO<sub>3</sub><sup>-</sup>-N/L) at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under 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 (50-200 mg NO<sub>3</sub><sup>-</sup>-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 collecting and measuring 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 was calculated according to the zero-order reaction model [13]:

$$dC/dt = -k_{den} \quad (3)$$

and its integration form

$$C_N - C_{N0} = -k_{den}t \quad (4)$$

where  $C_{N0}$  and  $C_N$  are the initial and nitrate concentrations in time (mg NO<sub>3</sub><sup>-</sup>-N/L) and  $k_{den}$  is the denitrification rate (mg NO<sub>3</sub><sup>-</sup>-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<sub>3</sub><sup>-</sup>-N/L, at pH = 7.2 and 25 °C under 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:

$$k_{den} = A_r e^{-\frac{E_A}{R_g T}} \quad (5)$$

where  $A_r$  is the Arrhenius factor (mg NO<sub>3</sub><sup>-</sup>-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). The overall relationship between the nitrate concentration and temperature can be expressed as [13]

$$\ln k_{den} = \ln A_r - E_A/R_g \cdot T \quad (6)$$

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 [14].

The biological denitrification with bacteria attached on NSI in the presence of different amounts of methanol (MetOH:N mass ratios were in the range of 2.0:1 to 4.5:1) was further investigated.

## 3 Problem Solution

### 3.1 Adsorption of nitrate on Lewatit M600

According to obtained results, the nitrate adsorption equilibrium was achieved during 1 hour (data not shown). For all investigated nitrate concentrations during 24 h, 75% of the nitrate was adsorbed.

The graphic plot of  $\log q_e$  versus  $\log \gamma_e$  (Eq.2) enables the calculation of Freundlich isotherm constants (Fig.1). As shown in Fig.1 the Freundlich constant,  $K_f$  was 0.298 mg NO<sub>3</sub><sup>-</sup>-N/g NSI and  $n$  was 1.131. The obtained  $n$  value between 1 and 10

represents favourable adsorption, as in accordance to literature [15].

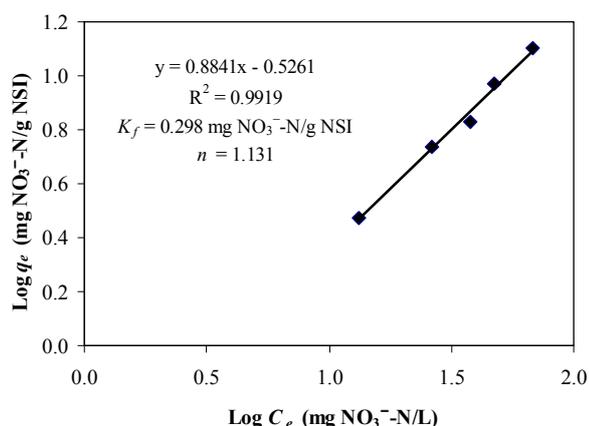


Fig.1 The Freundlich isotherm plot for the nitrate adsorption on NSI.

### 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.2). Finally, 100 mg  $\text{NO}_3^-$ -N/L were completely removed during 4 hrs at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under anoxic conditions. The initial dissolved  $\text{O}_2$  value of 5.9 mg  $\text{O}_2$ /L was quickly consumed by bacteria and after 1 h, it was only 0.02 mg  $\text{O}_2$ /L. The number of bacterial cells attached on NSI at the beginning of the tests was  $1.8 \times 10^7$  CFU/g NSI, while at the end of acclimation, it was  $6.0 \times 10^9$  CFU/g NSI.

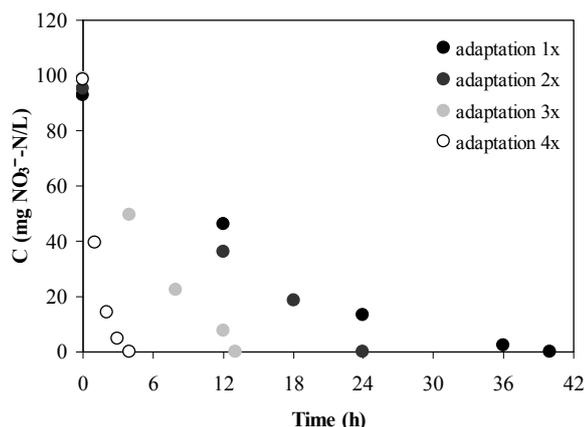


Fig.2 The decrease of nitrate concentration in SCW during the acclimation of bacteria.

The influence of initially present nitrate (50-200 mg  $\text{NO}_3^-$ -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 anoxic conditions (Figs. 3 and 4).

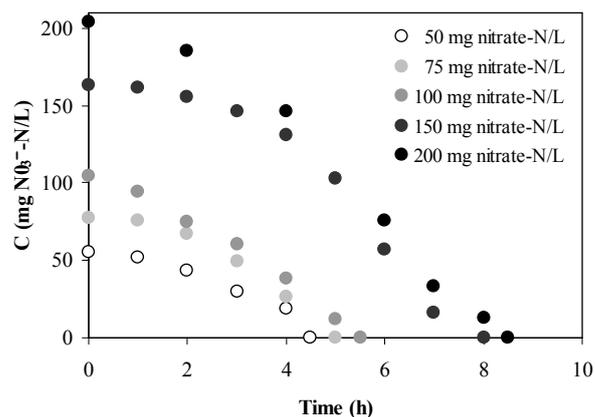


Fig.3 Nitrate-N removal from the SCW with use of bacteria attached on NSI.

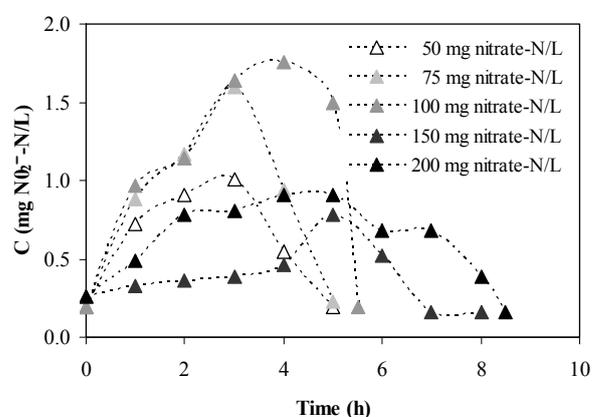


Fig.4 Nitrite-N concentration profile during nitrate removal from the SCW with use of bacteria attached on NSI.

The complete removal of 100 mg  $\text{NO}_3^-$ -N/L was achieved during 5.5 hrs at MetOH:N = 4.5:1, pH = 7.2 and 25 °C under anoxic conditions. During that process, nitrite ions were generated up to 1.76 mg  $\text{NO}_2^-$ -N/L, but they were quickly reduced and at the end of process the final nitrite concentration was 0.19 mg  $\text{NO}_2^-$ -N/L (Fig 4). 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.

The results suggest that nitrite accumulation was insignificant and that denitrification was not inhibited by nitrite ions. The selected mixed culture originating from an industrial wastewater treatment plant seemed to be more advantageous for fast denitrification.

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

Nitrate removal from the SCW (100 mg NO<sub>3</sub><sup>-</sup>-N/L) at 15°C, was very slow and lasted for 7 hrs (Fig. 5). 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. As shown in Fig. 5, 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. The maximum nitrite accumulation of 1.98 mg NO<sub>2</sub><sup>-</sup>-N/L was observed during 3 hrs, but after that nitrite was reduced reaching final concentration of 0.13 mg NO<sub>2</sub><sup>-</sup>-N/L. The initial number of bacterial cells attached to NSI was 3×10<sup>7</sup> CFU/g NSI, while at the end of denitrification, it was 7×10<sup>9</sup> CFU/g NSI. Observed values were similar to previously published data [8]. During this study dissolved O<sub>2</sub> in the SCW was quickly consumed by bacteria and after 1 h there was not any dissolved O<sub>2</sub> in the SCW. The pH was continuously checked and the observed values were 7.20 ± 0.05. The presence of phosphate salts, K<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub> that act as a buffer obviously enable control of pH in the SCW.

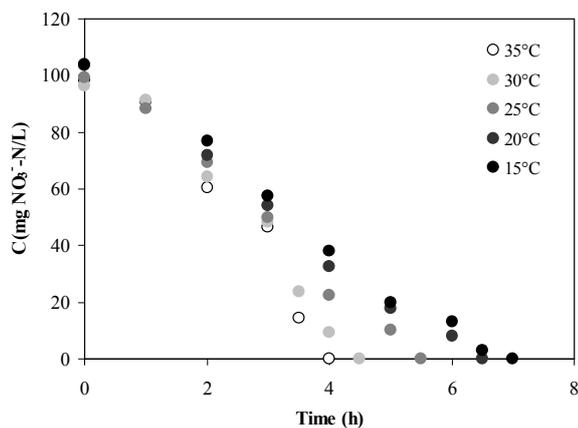


Fig.5 The decrease of nitrate concentrations in the SCW during denitrification tests with bacteria attached on NSI at different incubation temperatures.

According to literature, obtained results and according to the Eqs. 4-6, the denitrification rates ( $k_{den}$ ), the activation energy ( $E_A$ ) and the Arrhenius factor ( $A_r$ ) were determined. The left-hand side of Eq. (6) was determined and plotted against the

reciprocal of temperature as shown in Fig. 6. A high degree of linearity ( $R^2 > 0.99$ ) is known to provide a reliable estimate of the activation energy ( $E_A$ ) and the Arrhenius factor ( $A_r$ ) [16,17]. The activation energy for nitrate reduction was 11.40 kJ/mol and  $A_r$  was 1790.05 mg NO<sub>3</sub><sup>-</sup>-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 [17].

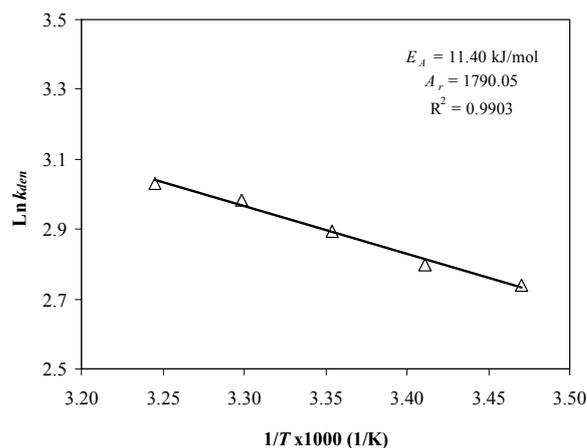


Fig.6 The Arrhenius plot for denitrification of SCW with bacteria attached on NSI.

Methanol was selected as the most suitable external carbon source because it is the least expensive and very efficient in denitrification [4,6] In order to quantify the influence of methanol on denitrification five different MetOH:N mass ratios were tested separately (Fig 7).

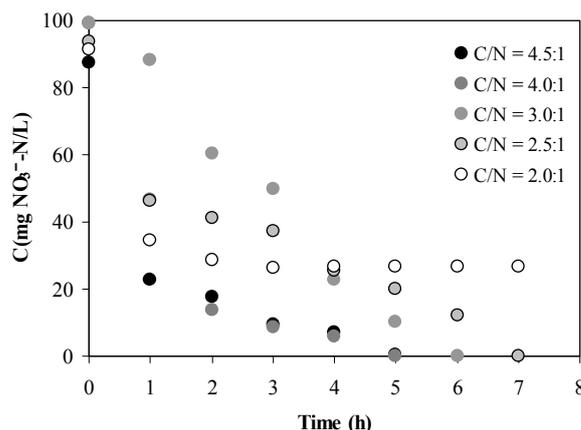


Fig.7 The decrease of nitrate concentration in SCW during denitrification tests with ? at different MetOH:N ratios.

It can be seen from Fig. 7 that nitrate removal continuously increased for all MetOH:N ratios exceeding 2.5 mg CH<sub>3</sub>OH/mg NO<sub>3</sub><sup>-</sup>-N.

At a lower methanol to nitrate-nitrogen ratio (2.0:1) the nitrate removal was incomplete; at 2.5:1 MetOH:N complete denitrification was achieved during 7 hrs. 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 constant. Therefore, it seemed that the MetOH:N ratios of 3.0 mg CH<sub>3</sub>OH:mg NO<sub>3</sub><sup>-</sup>-N were more than sufficient for complete denitrification. Comparison of the time required for complete nitrate removal at MetOH:N ratios of 3.0 and 2.5 suggested that the stoichiometric value was 2.5 under the experimental conditions. This was in accordance with the theoretical value calculated from the equation proposed by McCarthy et al. [18].

#### 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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