THE INTERACTION OF ZEOLITE AND BACTERIAL CELLS FOR DENITRIFICATION OF THE CETINA SURFACE WATER

LUCIJA FOGLAR and MARIJA KLJAJIĆ

Division of Industrial Ecology, Faculty of Chemical Engineering and Technology,
University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia
e-mail: lfoglar@fkit.hr

EXTENDED ABSTRACT

Nowadays, nitrate contamination of waters has become a worldwide problem, even in the agricultural areas in northern Croatia, the incremental nitrate concentration values were determined. Therefore, the EU water legislation set the Nitrates directive aimed to protect water quality across Europe by preventing nitrates from agricultural sources polluting ground and surface waters. As a result, the new methods for nitrate removal are studied and among them, the biological methods are often investigated. The use of attached bacterial cells or biofilm was studied in order to establish more stable and effective nitrate removal. Furthermore, for the same purpose, the addition of zeolite in an activated sludge unit was investigated and the improvement of nitrate removal was reported. The low cost and the abundance of natural zeolite in many parts of the world along with their superior characteristics and high exchange capacities amenable the use of clinoptilolite as an inexpensive adsorbent and a carrier of an immobilized biofilm or microorganisms. The use of zeolite was thoroughly investigated for efficient removal of heavy metals, ammonium or other cations; only a few studies were reported for anions removal and a recent one dealt with the use of zeolites and phosphate accumulating bacteria.

The main objective of the present study was the use of zeolite and bacterial cells for establishing the stability and efficiency of the denitrification process and the optimization of the process parameters such as the influence of initially present nitrate, the initial amount of zeolite with bacterial cells, pH and methanol to nitrate-N mass ratio in order to achieve fast and efficient nitrate removal from the Cetina surface water.

The natural powdered clinoptilolite was mixed with bacterial cell suspension and the resulting bio clinoptilolite powder (B-NPC) was filtered and acclimated for nitrate removal from the Cetina surface water (CSW). The influence of the B-NPC amount was investigated and results revealed 15 g of B-NPC (9 %, w/w) as optimal. The obtained denitrification rates increased in the range of 25.85-37.157 mg NO_3-N/Lh. The denitrification of 100 mg nitrate–N/L from the CSW at pH range of 5.82-8.20 and at methanol to nitrate nitrogen (CH_3OH/N) mass ratio of 2.5:1-4.5:1 was achieved during 7 and 4 hours, respectively. The rapid nitrate removal was achieved at pH of 6.85 and 7.22, but a higher pH value was optimal due to lower accumulation of nitrite. The required CH_3OH/N mass ratio was 2.5:1 and the analysis of results showed denitrification as the zero order reaction with denitrification rate of 25.85 mg NO_3-N/Lh. The complete and efficient removal of 100 mg NO_3-N/L from the CSW with B-NPC (9% w/w) was achieved at pH of 7.22, 25 °C and at 300 rpm. Among the bacteria present in B-NPC, the dominant species were isolated and determined as *Pseudomonas stutzeri* and *Paracoccus* sp. Finally, the use of B-NPC was demonstrated as an efficient method for complete nitrate removal from the Cetina surface water.

Keywords: bacteria, zeolite-clinoptilolite, denitrification, methanol, surface water.

1. INTRODUCTION

The presence of nitrate ions in the ground and surface waters is determined and increased values are observed all over the World. Recent studies discuss the presence and removal of nitrates in India, China, the US and Europe (Ayyasamy *et al.*, 2009; Zhou *et al.*, 2009). Even in the agricultural areas in northern Croatia, the higher nitrate concentration values were determined (Vidaček *et al*., 1999). Therefore, the EU water legislation set the Nitrates directive (1991) to reduce water pollution caused or induced by nitrates from agriculture and to prevent further pollution. As a result, the new methods for nitrate removal are studied (Ayyasamy *et al.*, 2009; Zhou *et al.*, 2007). Among them, the biological methods are often investigated for waste, ground and drinking water denitrification (Albuquerque *et al.*, 2009; Park *et al.*, 2007). The majority of biodenitrification studies are conducted with the heterotrophic bacteria that require an external organic carbon source. The use of methanol was favourable as reported Della Rocca *et al.* (2007) in an overview of nitrate removal. Timmermans and Van Haute (1983) reported that biodenitrification with methanol, at optimal pH of 8.3 and at a C/N ratio of 2.52:1 followed a stoichiometric equation:

$$NO_3^- + 1.10\ CH_3OH + H^+ \rightarrow 0.078\ C_5H_7O_2N + 0.46\ N_2 + 0.71\ CO_2 + 2.427\ H_2O \qquad (1)$$

The media pH value during biodenitrification has a relevant influence on the behaviour of microorganisms in nitrate reduction. An optimal pH for most denitrifying bacteria was reported in the range of 7-8. The influence of H^+ on the denitrification rate was determined according to:

$$k = k_{max}/(1 + K_I\, I) \qquad (2)$$

where I – is the concentration of H^+ ions and K_I is a constant. The value of the H^+ concentration in Eq. (2) could be replaced by: $I = 10^{(pHmax - pH)} - 1$, and accordingly, the influence of pH-values on biodenitrification could be determined by

$$k_d = k_{dmax}/(1 + K_I\,(10^{(pHmax - pH)} - 1)) \qquad (3)$$

where k_d is the denitrification rate (mg NO_3-N/Lh) and k_{dmax} - the maximal value of k_d.

In the comprehensive study, Glass & Silverstein (1998) reported the influence of the pH ranging from 6.5 to 9 on the denitrification process. They observed that the increase of pH above 7.5 promotes complete removal of increased nitrite concentrations, while at lower pH values (6.5 and 7.0) the denitrification process was inhibited.

The use of attached bacterial cells or biofilm was studied in order to establish more stable and effective nitrate removal. Furthermore, for the same purpose, the addition of zeolite in an activated sludge unit was investigated and the 48% improvement of nitrate removal was reported (Park *et al.*, 2003). The low cost and the abundance of natural zeolite along with their superior characteristics and high exchange capacities amenable its use as an inexpensive adsorbent and a carrier of an immobilized biofilm or microorganisms.

The main objective of the present study was the use of powdered zeolite as a carrier of bacterial cells for establishing the stability and efficiency of the denitrification process. The optimization of the process parameters such as the initially present nitrate, the initial amount of bio-zeolite, the mixing speed, pH and methanol to nitrate-N mass ratio was further investigated in order to achieve fast and efficient nitrate removal from the Cetina surface water.

2. MATERIALS and METHODS

The zeolite used in the study was a natural powdered clinoptilolite (NPC), obtained from the Donje Jesenje deposit, Croatia. The NPC was washed with redistilled water in order to remove the surface dust, dried at 105 °C for 24 h and used for this investigation. The mixed bacteria culture used was described in our previous work in details (Foglar *et al.* 2007). The prepared Cetina surface water medium (CSW) consisted of K_2HPO_4, (2.5 g/L) and KH_2PO_4, (1 g/L) and raw Cetina water up to 1 L (Table 1). Prior to use, the CSW was sterilized at 121 °C for 15 min. The stock nitrate solution ($NaNO_3$ solution containing 10 g

NO_3-N/L) and methanol were added separately to the sterile CSW medium to provide defined nitrate-N concentrations and CH_3OH/N mass ratio, respectively. All the reagents used during the tests were of an analytical grade level.

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

Parameters	
pH	7.25-8.20
CO_2 (free) (mg/L)	4.20-9.80
$KMnO_4$	4.0-11.6
NH_3-N (mgN/L)	0.001-0.198
NO_2-N (mgN/L)	0-0.005
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

The adsorption of nitrate to the NPC was conducted in 0.2 L sterile infusion bottles. Each bottle contained 1 g of NPC and 100 mL of the CSW (C_0 = 50-250 mg NO_3-N/L). The bottles were sealed, punctured for sample collection and placed on a magnetic stirrer at 300 rpm and 25 °C. The initial sample and samples collected at 1, 2, 4 and 24 h were taken with a sterile syringe equipped with a Chromafil filter (0.45 μm) and processed immediately.

2.1. Interaction of the NPC and bacteria for nitrate removal from the CSW

In a 0.5 L sterile reaction bottle 200 g of NPC was weighed and suspension of the mixed bacterial culture was pumped and recirculated with a peristaltic pump through the bottle for 48 h. The NPC with bacterial cells (B-NPC) was filtered (blue band filter) and washed with a sterile 0.9% NaCl solution. The wet B-NPC was stored at 4 °C until use.

The acclimation tests and the denitrification tests with the use of the B-NPC were conducted in 0.2 L sterile reaction bottles containing 15 g of the B-NPC and 150 mL of the CSW medium. The initial nitrate concentration and CH_3OH/N mass ratio of 100 mg NO_3-N/L and 4.5:1 were set by addition of the stock nitrate solution and methanol to the sterile CSW medium, respectively. The tests were conducted at 300 rpm and 25 °C in anoxic conditions. The process was investigated in the presence of 100-500 mg NO_3-N/L for the determination of initial nitrate influence and the effect of the B-NPC amount on nitrate removal was determined in the presence of 3.75-30.00 g B-NPC. The influence of CH_3OH/N mass ratio on the denitrification of the CSW was determined by the addition of the predetermined methanol amount during the preparation of the CSW samples. The selected CH_3OH/N mass ratios were 2.0:1, 2.5:1, 3.5:1, and 4.5:1. The blank test (CH_3OH/N=0:1) was set in a parallel. The effect of pH on the denitrification process was studied with the use of buffered CSW samples, which were prepared with the use of K_2HPO_4 and KH_2PO_4 at different amounts that provided pH values of 5.82, 6.30, 6.85, 7.22 and 8.25. After preparation, the reaction bottles containing 150 mL CSW and B-NPC were sealed, punctured with 2 needles (one for sampling and the other for removal of produced gas) and placed on the magnetic stirrer at 300 rpm and 25 °C. During the predetermined time intervals, the samples were taken with a sterile syringe, filtered through a Chromafil filter (0.45 μm), immediately processed for pH and dissolved O_2 (DO) measurements, and then used for the nitrate and nitrite analysis.

Immediately after sampling, the DO concentration, pH and temperature were monitored by the Seven Go dissolved oxygen meter SG6, Mettler-Toledo (Schwerzenbach, Switzerland) and pH-meter WTW pH 330 (Weilheim, Germany), respectively. Nitrate and nitrite concentrations were determined by the chromotropic acid method (Standard methods, 1989) and by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-

ethylenediamine dihydrochloride (Höll, 1979) respectively, at spectrophotometer Hach DR/2400 (Hach Company, Loveland, Colorado, USA). The number of bacterial colonies (CFU) in the B-NPC was determined by a plate count on the standard nutrient agar, after repeated dilution with NaCl (m/V ratio = 9 g/L) and expressed as CFU/g B-NPC. The procedure was previously described in detail (Foglar *et al.* 2007).

The denitrification kinetic analysis applied for the calculation of the denitrification rates during this study was given in our previous paper (Foglar *et al.* 2005). The integration form of the zero order kinetic model given by

$$k_d\, t = C_{nitrate\text{-}N} - C_{nitrate\text{-}N0} = C_{nitrate\text{-}N0} \times X_N \qquad (4)$$

was used (Gomzi, 1997). In the Eq. (4) the X_N is the conversion of nitrate (-), $C_{nitrate\text{-}N}$ and $C_{nitrate\text{-}N0}$ represented nitrate concentrations in time and the initial concentration (mg NO_3-N/L), respectively.

3. RESULTS and DISCUSSION

The intensive use of fertilizers and other numerous nitrate containing compounds cause a incremental concentrations of nitrate in waste, surface, ground and in drinking waters. Their harmful effects on water life and even on humans are well known and therefore, nitrate removal is of great environmental concern. Although diverse biological and physico-chemical methods (chemical reduction or physical adsorption) have been employed, no simple solution is available for nitrate removal.

The zeolite according to its high ion exchange abilities, molecular sieve properties, ease of availability, and special importance in many water purification processes is widely used for removal of harmful chemicals from both ground and surface waters and it has been proven that the addition of zeolite improves the performance of the denitrification process (Park *et al.*, 2003). Therefore, in this study the interaction of natural powdered clinoptilolite and bacterial cells for the denitrification of the CSW in the batch reactor was investigated. The bacteria, originating from an industrial plant, was selected for the interaction with zeolite (B-NPC) and used in the denitrification process study, in order to achieve a more stable and efficient removal of nitrate from the CSW.

Prior to the denitrification tests, the NPC was investigated for adsorption of nitrate and the results presented in Fig. 1a, revealed that during 24 h, no nitrate was adsorbed. The NPC was then interacted with a selected bacterial culture and as a result the B-NPC was formed. Subsequently, the B-NPC was applied for the acclimation process for the nitrate removal from the CSW.

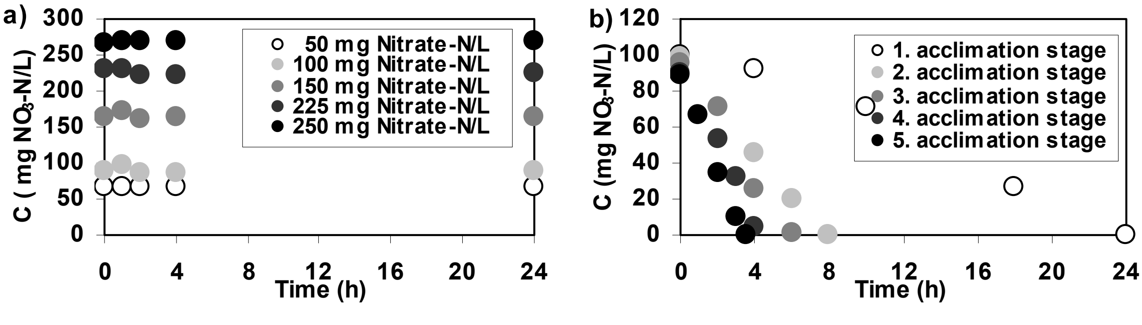

Figure 1: The nitrate-N concentration in the CSW during the adsorption on the NPC (**a**) and the acclimation of B-NPC for nitrate removal (**b**).

The nitrate concentrations determined within tests, presented in Fig. 1b showed that in the first acclimation stage, the B-NPC completely removed 100 mg NO_3-N/L during 24 hours. This B-NPC, used in the second and afterwards in the third stage of the acclimation, removed all initially present nitrates in the CSW during 8 and 6 hours, respectively. At the end of acclimation, the denitrification of 100 mg NO_3-N/L from the

CSW lasted 3.5 hours. Apparently, the bacteria originating from the industrial plant, seem to be favourable for the interaction with zeolite and the use in the denitrification study.

In order to determine the influence of initially present nitrate in the CSW on the denitrification process, a series of tests were conducted. An increase in initial nitrate (100-500 mg NO_3-N/L) prolonged nitrate removal from the CSW from 3 to 8 h (Fig. 2a).

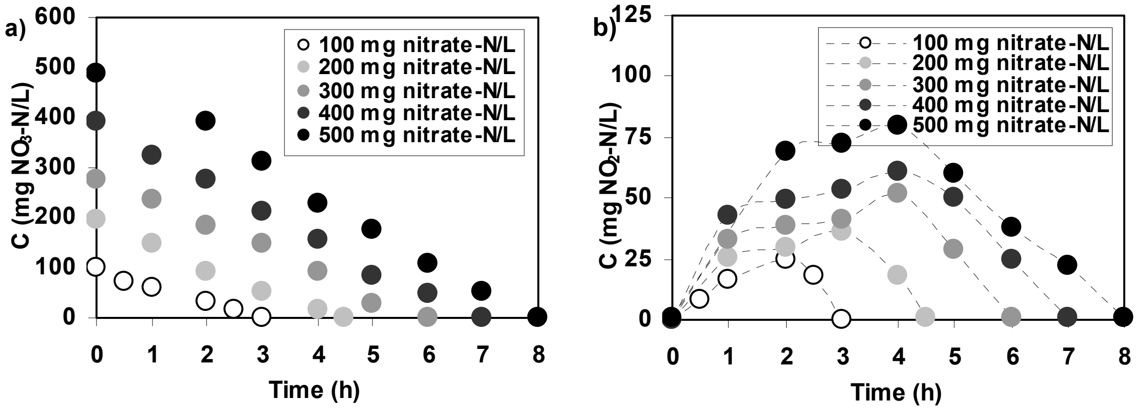

Figure 2: The time course of the initially present nitrate (100-500 mg NO_3-N/L) in the CSW (**a**) and nitrite concentration profile (**b**) during denitrification with the B-NPC.

An increase of nitrate could inhibit the removal process (Zhou *et al.* 2007), however, by the acclimation of denitrifying bacteria on the increased nitrate concentrations, complete removal of 100-500 mg NO_3-N/L was achieved during 2 to 6 h (Wang *et al.,* 1995; Foglar *et al.*, 2005). Furthermore, a recent study reported that the nitrate removal was not inhibited by the increase of initial nitrate from 80 to 500 mg NO_3-N/L, and that nitrate-N reduction rates were increased from 0.57 to 35 mg NO_3-N/Lh (Wu *et al.*, 2009).

During the course of tests, nitrite was proven as the main intermediate of denitrification; it was gradually formed and reached the maximum of 79.52 mg NO_2-N/L by the 4th h, and then degraded (Fig. 2b). The observed nitrite profile was typical for biological denitrification (Wu *et al.*, 2009), but the final nitrite concentration in the CSW was lower than 0.9 mg NO_2-N/L and of the maximum contaminant level of 1 mg NO_2-N/L (Della Rocca *et al.*, 2007). Generally, accumulated nitrite was low in comparison to the initially present nitrate and has no significant impact on the denitrification, as revealed from some earlier study (Lemmer *et al.*, 1997). In the course of denitrification, due to the impact of hydrogen ions that are involved in the denitrification stoichiometric equation (Eq. 1.), the increase of pH was expected, but, the observed pH values were negligible higher, since the presence of phosphate salts (K_2HPO_4 and KH_2PO_4) that acted as a buffer compensated the pH increase. The measurements of the dissolved oxygen in the CSW samples indicate that the initially present O_2 (up to 6 mg O_2/L) was quickly consumed by the present bacteria and after 1 h, the DO in the CSW was not detected, confirming the denitrification, as an anaerobic or at least anoxic process.

To optimize the amount of the B-NPC for efficient nitrate removal, a series of tests with 3.75-30 g of the B-NPC (2.4-16.67 % w/w) were set up. The observed results showed that in the presence of 3.75 and 7.5 g of the B-NPC, nitrate removal lasted 7 and 6 h, respectively (Fig. 3a). Obviously, these B-NPC amounts were too low for the efficient removal of 100 mg NO_3-N/L from the CSW. Simultaneously, in the presence of the increased amounts of the B-NPC (15, 22.5 and 30 g), total nitrate removal was achieved during 3.5, 3 and 2.5 h, respectively. In addition, the monitoring of nitrite (data not shown) revealed that in the presence of the increased amounts of B-NPC, the generation of nitrite was decreased (up to 9.61 mg NO_2-N/L) in comparison to the nitrite generated (up to 27.35 and 19.13 mg NO_2-N/L) at the presence of 3.75 and 7.5 g B-NPC, respectively. The final nitrite concentrations in the CSW were lower than 0.30 mg NO_2-N/L. The final low nitrite could be explained by the presence of increased B-NPC amounts that are accompanied by an increased number of bacterial cells (8.4×10^9 CFU/g B-NPC) which are capable of reducing nitrate with a little or no nitrite accumulation.

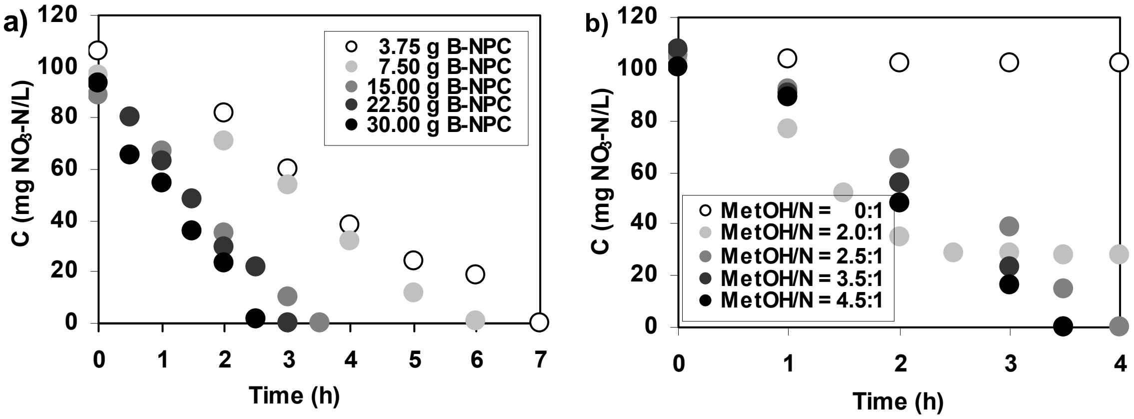

Figure 3: The time course of nitrate concentration during the CSW denitrification in the presence of different amount of the B-NPC (**a**) and at different CH_3OH/N mass ratios (**b**).

In the biological denitrification, the organic carbon source is one of the main factors and it has a significant influence on the course of nitrate removal. In this study, the influence of CH_3OH/N mass ratio on nitrate removal from the CSW with the B-NPC was demonstrated in Fig. 3b. The required (CH_3OH/N) ratio for the denitrification of 100 mg NO_3^--N/L from the CSW was higher than 2.0:1 since at that ratio, the denitrification process was interrupted after 2.5 h due to the lack of organic carbon. In view of the observed nitrate removal, that was only 1 h faster than the nitrate removal achieved at CH_3OH/N ratio of 2.5:1, the use of excess methanol (CH_3OH/N ratios of 3.5:1 and 4.5:1) did not significantly improve denitrification. Therefore, as the optimal and necessary amount of organic carbon needed for the complete denitrification CH_3OH/N mass ratio of 2.5:1 was determined. The obtained required amount of methanol was previously reported (Park *et al.* 2007).

The pH value of water played a major role on the denitrification rates and as reported in literature, the process might be favoured in the pH range of 7-8 (Wang *et al.*, 1995). Thus, the denitrification was studied in the predetermined pH range of 5.8 - 8.25 (Fig. 4a) and the complete removal of 100 mg NO_3-N/L from the CSW (at pH 5.82 ± 0.08) was observed during 6.5 h. The high nitrite, up to 60 mg NO_2-N/L was generated, but the final nitrite concentration in the CSW was 1.47 NO_2-N/L (Fig. 4b). The increase of pH to 6.30 ± 0.06 resulted in nitrate removal during 5 h with similar nitrite generation. During the denitrification tests that were conducted at pH value of 6.85 ± 0.04 and 7.22 ± 0.04, the nitrate removal was achieved during 3.5 h. In the course of tests, the maximum of nitrite generation at pH of 6.85 observed at 2 h was up to 50 mg NO_2-N/L, while significantly lower accumulation of nitrite ions (up to 15.6 mg NO_2-N/L) were recorded during the denitrification of the CSW with a pH of 7.22. The nitrite was subsequently reduced and at the end of process in the CSW, the presence of 0.2 and 0.1 mg NO_2-N/L was determined, respectively. A high nitrite generation was observed at low pH, as previously reported (Lemmer *et al.* 1997). The highest investigated pH value of the CSW was 8.25 ± 0.03 and complete removal of 100 mg NO_3-N/L was observed during 5.5 h. According to the results obtained during this study, fast and complete removal of 100 mg NO_3-N/L from the CSW was achieved at the pH value of 6.85 ± 0.04 and 7.22 ± 0.04. In respect to the nitrate removal that was achieved with a lower accumulation of nitrite, the pH value of 7.22 was selected as optimal for fast and efficient denitrification of the CSW. The denitrification rates calculated according to the Eq. (4) were in the range of 15.6 - 29.84 mg NO_3-N/Lh and were similar to previously published data (Wu *et al.*, 2009). The influence of pH-values on the denitrification rates was depicted in Fig. 4c. The experimental data was in good agreement with the theoretic values (Eq. 3).

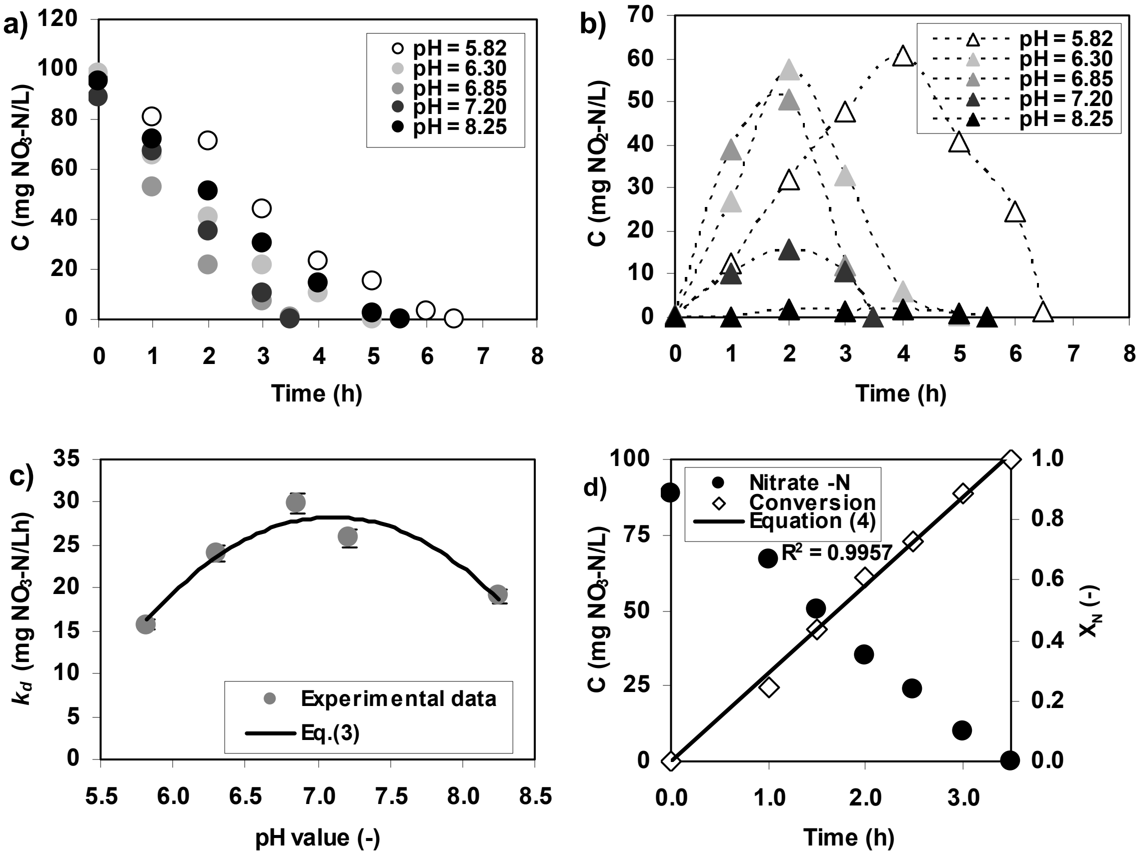

Figure 4: The nitrate (**a**) and nitrite concentration profile (**b**) during the denitrification of the CSW at different pH levels. The denitrification rates as a function of pH (**c**) and the integration test of the zero order reaction model (**d**).

For the denitrification tests conditions, the K_I was 0.093. The observed value was similar to the K_I value of 0.0502 determined by Timmermans & Van Haute (1983). However, with the change of pH in the studied range, the sharp decrease of denitrification rates was not observed, as shown in literature (Timmermans & Van Haute, 1983). Obviously, the presence of selected bacteria in an interaction with zeolite enables rapid nitrate removal in the pH range of 5.8 - 8.25. The integration test of the zero order kinetic model given by the Eq. (4) was applied (Fig. 4d) and the observed high degree of linearity confirmed the denitrification as the zero order reaction (Klas *et al.*, 2006).

During this study, the numbers of bacterial cells present in the B-NPC at the beginning of the denitrification tests were $7.4 \pm 0.3 \times 10^7$ CFU/g B-NPC and at the end of nitrate removal the numbers of the bacterial cells increased to $8.6 \pm 0.5 \times 10^9$ CFU/g B-NPC. Among bacteria present in the mixed culture, the dominant species were isolated and determined as *Pseudomonas stutzeri* and *Paracoccus* sp*.* The use of selected bacteria, their interaction with the NPC and acclimation to nitrate enabled fast and effective nitrate removal from the Cetina surface water.

4. CONCLUSIONS

The bacteria interacted with the NPC (B-NPC) was investigated for nitrate removal from the CSW. The optimal parameters for efficient nitrate removal were determined and the results obtained, revealed that the optimal amount of B-NPC, the optimal pH and the required amount of organic carbon defined by CH_3OH/N mass ratio were 9 % (w/w), 7.22 and 2.5:1, respectively. Throughout all tests, the nitrite accumulation was observed, but terminal nitrite was very low. Additionally, for effective nitrate removal, the process should be set up on a magnetic stirrer at 300 rpm and 25 °C under anoxic conditions. The kinetic analysis of results showed denitrification as the zero order reaction. Finally, the use of B-NPC was demonstrated as efficient method for complete nitrate removal from the Cetina surface water.

ACKNOWLEDGMENTS

This work was jointly supported by funds from Hrvatske Vode and the Ministry of Science, Education and Sports (Scientific Project (125-0000000-1970)), Zagreb, Croatia. Prof. Nađa Dešpalj, Lecturer of English at the Split and Zagreb Universities, translated this paper to English.

REFERENCES

1. Albuquerque, A., Oliveira, J., Semitela, S. & Amaral, L. (2009). Influence of bed media characteristics on ammonia and nitrate removal in shallow horizontal subsurface flow constructed wetlands. *Bioresour. Technol.* **100**, 6269-6277.
2. APHA, (1989). Standard Methods for the examination of water and wastewater. 17th ed. APHA/AWWA/Water Environment Federation, Washington DC, USA.
3. Ayyasamy, P.M., Rajakumar, S., Sathishkumar, M., Swaminathan, K., Shanthi, K. Lakshmanaperumalsamy, P. & Lee, S. (2009). Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. *Desalination* **242**, 286-296.
4. Della Rocca, C., Belgiorno, V. & Meriç, S. (2007). Overview of in-situ applicable nitrate removal processes. *Desalination,* **204**, 46-62.
5. Foglar, L., Briški, F., Sipos, L. & Vuković, M. (2005). High nitrate removal from synthetic wastewater with the mixed bacterial culture. *Bioresour. Technol*. **96**, 879-888.
6. Foglar, L., Sipos, L. & Bolf, N. (2007). Nitrate removal with bacterial cells attached to quartz sand and zeolite from salty wastewaters. *World J. Microbiol. Biotechnol.* **23**, 1595-1603.
7. Glass, C. & Silverstein, J. (1998). Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Water Res.* **32**, 831-839.
8. Gomzi, Z. (1997). Chemical reactors (in Croatian). Hinus, Zagreb, 80-131.
9. Höll, K. (1979). Wasser, 6. auflage Valter deGruyter, Berlin, 41-43.
10. Klas, S., Mozes, N. & Lahav, O. (2006). Development of a single-sludge denitrification method for nitrate removal from RAS effluents: Lab-scale results vs. model prediction. *Aquaculture* **259**, 342-353.
11. Lemmer, H., Zaglauer, A., Neef, A. & Metzner, G. (1997). Denitrification in a methanol fed fixed bed reactor. Part 1: Physico-chemical and biological characterization, *Water Res.* **31**, 1897-1902.
12. Park, S-J., Kim, C.G., Yoon, T-I. & Kim, D.W. (2003). Evaluation of increased denitrification in an anoxic activated sludge using Zeolite. *Korean J. Chem. Eng.* **20**, 492-495.
13. Park, J.B.K., Craggs, R.J. & Sukias, J.P.S. (2007). Treatment of hydroponic wastewater by denitrification filters using plant prunings as the organic carbon source. *Bioresour. Technol.* **99**, 2711-2716.
14. Timmermans, P. & van Haute, A. (1983). Denitrification with methanol. *Water Res.* **17**, 1249-1255.
15. Vidaček, Ž., Sraka, M., Čoga, L. & Mihalić, A. (1999). Nitrates, heavy metals and herbicides in soil and waters of Karašica-Vučica catchment area, *Poljoprivredna znan. smotra* **64**, 143-150.
16. Wang, J.H., Baltzis, B.C. & Lewandowski, G.A. (1995). Fundamental denitrification kinetic studies with *Pseudomonas denitrificans*, *Biotechnol. Bioeng.* **47**, 26-41.
17. Wu, W-X., Hao, Y.-J., Ding, Y. & Chen, Y.-X. (2009). Denitrification capacity in response to increasing nitrate loads and decreasing organic carbon contents in injected leachate of a simulated landfill reactor. *Process Biochem.* **44**, 486-489.
18. Zhou, M., Wang, W. & Chi, M. (2009). Enhancement on the simultaneous removal of nitrate and organic pollutants from groundwater by a three-dimensional bio-electrochemical reactor. *Bioresour. Technol.* **100**, 4662-4668.
19. Zhou, M., Fu, W., Gu, H. & Lei, L. (2007). Nitrate removal from groundwater by a novel three-dimensional electrode biofilm reactor. *Electrochimica Acta* **52**, 6052-6059.