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Continuous-flow biological denitrification with zeolite as support for bacterial growth

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ABSTRACT

Zeolite particles with bacterial culture (Bio-NPC) were used for nitrate removal from surface water (SW) and effects of Bio-NPC, and the methanol amount and the pH value of SW were investigated in the batch reactor and the process was then monitored in the continuous-flow stirred reactor. The application of Bio-NPC particles (10% w/w) in the batch reactor was efficient for the removal of 100 mg NO_3–N/L from the SW, and the addition of methanol was optimal at CH_3OH/N ratio of 2.5:1. The process was stable in the pH range of 5.85–8.03, but the highest denitrification rate was obtained at the pH value of 7.13. In the continuous-flow stirred reactor, nitrate removal was investigated at different hydraulic retention times (HRTs) (37.04, 23.26, 10.53, 2.33 and 1.32 h) and the efficient nitrate removal was achieved even at HRT of 1.32 h with nitrate and organic removal higher than 99 and 79%, respectively. The volumetric denitrification rates during the first eight days of continuous flow were in the range of 39.2–51.28 mg NO_3–N/L h and then it fluctuated within the range of 63.47–79.90 mg NO_3–N/L h. The use of Bio-NPC was demonstrated as an efficient method for complete nitrate removal from the surface water.

Keywords: Zeolite; Continuous-flow reactor; Surface water; Biological denitrification; Nitrite

1. Introduction

Nitrate concentration in water aquifers has steadily been increasing over the years mainly due to the extensive use of nitrate containing compounds in different industries and their subsequent discharge in industrial, domestic or animal wastes. Nitrate and nitrite toxicity at elevated concentrations is well known and has been linked to several cancers. Thus, for example, nitrosamines are carcinogenic compounds that may be formed from nitrite in the stomach [1,2]. Therefore, lots of countries promulgate specific regulations to set the maximum contaminating levels of nitrate and nitrite in drinking water at values lower than 50 and 0.1 mg/L, respectively [3].

Conventional physical–chemical methods for nitrate removal that are recently being investigated are ion exchange, reverse osmosis and electro-dialysis [4–6]. These processes are effective but expensive and require further treatment or disposal [7]. The biological denitrification as an alternative treatment process converts nitrates to harmless nitrogen gas [8,9] and enables the purification of water contaminated with nitrate due to the high specificity of denitrifying bacteria. Heterotro-

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phic bacteria during denitrification utilize organic substances as a carbon source (methanol, ethanol, sugars and other) and nitrate as the terminal electron acceptor which finally results in the formation of nitrogen gasses and carbon dioxide. In recent years, many different types of denitrification processes have been investigated. The membrane bioreactor is widely used for denitrification in treatment of drinking water, but the presence of air could destroy the anaerobic environment and the membrane can be significantly affected by pressure and hard to clean [10]. A denitrifying submerged filter used for nitrate removal due to the limit of bacterial population could pronounce nitrite accumulation and reverse flush contributed to the main cost of the submerged filter system [11,12]. Generally, the use of different bioreactors is broadly studied and amongst them the use of free dispersed cells could result in the blockage of flow lines and clogging of filters, whilst separation of biomass from the treated effluents, is beset with technical difficulties, rendering the treatment procedure cost-prohibitive [13]. As a consequence, an increase of interest in the entrapment of microbial cells was noted. Karagozoğlu et al. [14] used a fixed-film up-flow column with *Paracoccus denitrificans* immobilized on pumice supporting material for nitrate removal, and the maximum nitrate removal was reported as 97.69% using methanol as carbon sources:

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 7H_2O + 5CO_2 + 6OH^- \quad (1)$$

The use of microorganisms immobilized in hydro gels such as carrageenans and Ca-alginates was investigated in order to improve denitrification and achieve efficient nitrate removal [15–18]. Even these processes revealed several disadvantages that include limitations in the rate of diffusion, insufficient mechanical strength leading to the breakage of gels and dispersal of biomass, lack of open spaces for cell growth and a prohibitive cost of application [19,20]. Such difficulties can be overcome by immobilizing the microbial biomass within the highly porous and strong matrix, such as different polymer granules or natural materials like zeolites [15,21]. The ion exchange capability of zeolite has been extensively studied and used in water purification in many countries all over the world [22–25]. Zeolite has shown a great capacity for metal adsorption (Cu, Cd, Pb and Zn) which enhances the removal of toxics and consequently improves microbial growth in anaerobic digestion [26]. Therefore, zeolite has been reported as a useful material for microbial support [21,27]. The use of small, porous, fluidized media in the reactor enables the retaining of biomass and operation at reduced hydraulic retention times (HRT). The structural physical characteristics of the zeolite as well as the microbial culture used in the colonization step could improve biological denitrification.

The present study was focused on the use of zeolite particles as support material for bacterial growth and particles colonized by the bacteria (Bio-NPC) were used for the nitrate removal in the batch and continuous-flow reactor. The investigation was aimed to determine optimal Bio-NPC and methanol amounts and optimal pH value for effective nitrate removal from the surface water (SW) and to optimize denitrification in the continuous-flow stirred reactor.

2. Materials and methods

2.1. Zeolite, microorganisms and the preparation of biozeolite particles

The natural zeolite used as biomass support was natural powdered clinoptilolite (NPC) obtained from a deposit located in Vranjska Banja, Serbia. The chemical composition (% w/w) of the zeolite used was SiO_2 (66.24), Al_2O_3 (14.06), Fe_2O_3 (2.08), CaO (3.12), MgO (1.02), Na_2O (1.16), K_2O (0.94) and loss of ignition 10.28%. The bulk chemical compositions of zeolite were determined by the classical chemical analysis in combination with the atomic absorption spectroscopy technique on the atomic absorption spectrometer PerkinElmer model 3110 as previously described in detail [28]. The NPC was washed with redistilled water in order to remove the surface dust, dried at 105°C for 24 h, and grain size fractions smaller than 0.063 mm were assayed for interaction with the mixed bacterial culture.

The active sludge of the wastewater treatment plant Anamet, Savski Marof, Croatia and the agricultural soil sample (Lastovo, Croatia) were used as a bacterial source. The active sludge (100 mL) and 50 g of the soil were mixed, filtered (blue band filter) and the obtained biomass was washed twice and then diluted to 50 mL with the SW solution. The mixed bacterial suspension was used in the acclimation denitrification tests.

The acclimated suspension of the mixed bacterial culture was pumped and recirculated through a 0.5 L sterile reactor that contained 200 g of NPC with a peristaltic pump for 48 h. The NPC with bacterial cells (Bio-NPC) was filtered and washed with a sterile 0.9% NaCl solution. The wet Bio-NPC particles were stored at 4°C until use for nitrate removal in a batch denitrification study.

2.2. SW solution

The raw SW from the Bjelovar region was used for preparation of polluted water solutions. For that purpose, K_2HPO_4 (2.5 g/L) and KH_2PO_4 (1 g/L) were added into the raw SW and sterilized at 121°C for 15 min. During the second continuous-flow test set, the raw SW was used without addition of phosphate salts and sterilization. The stock nitrate solution ($NaNO_3$ solution containing 10 g NO_3–N/L) and methanol were added separately into prepared SW solutions (SW) to provide an initial nitrate–N concentration of 100 mg NO_3–N/L and designated CH_3OH/N mass ratio, respectively. All the reagents used during the tests were of an analytical grade level.

2.3. Experimental set-up

2.3.1. Batch denitrification tests

The three series of batch denitrification tests were conducted in a 0.2 L sterile reactor containing the Bio-NPC particles and the SW solution up to 150 mL, in order to determine optimal Bio-NPC and methanol amount, and optimal pH value of SW for effective nitrate removal. In the first batch study, the influence of Bio-NPC amount (2.5–20% w/w) was investigated. The initial nitrate concentration and CH_3OH/N mass ratio of 100 mg NO_3–N/L and 3:1 were set by addition of the stock nitrate solution and methanol to the sterile SW solution, respectively. The CH_3OH/N mass ratio of 3:1 was selected in order to avoid carbon-limited conditions [29].

The second test set was aimed to determine the optimal CH_3OH/N mass ratio for removal of 100 mg NO_3–N/L from the SW with use of Bio-NPC (10% w/w). For that purpose, the predetermined methanol amounts were applied during the preparation of the SW samples and the resulting CH_3OH/N mass ratios were 2.0:1, 2.3:1, 2.5:1 and 3.0:1. 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 SW samples prepared by addition of different amounts of K_2HPO_4 and KH_2PO_4 to the raw SW that provided pH values of 5.85, 6.15, 6.66, 7.13, 7.76 and 8.03.

Each reactor containing the Bio-NPC particles and the SW solution, after preparation, was sealed, punctured with two needles (one for sampling and the other for the removal of produced gas) and placed on the magnetic stirrer at 100 rpm and 25°C under anoxic conditions. At 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 nitrate and nitrite analysis.

2.3.2. The continuous-flow denitrification process

The investigation of the denitrification process in the continuous-flow stirred reactor began as a batch test in a 0.25 L sterile reactor containing 19 g of the Bio-NPC and the SW solution up to 210 mL and after complete removal of nitrate from the SW (C_0 = 100 mg NO_3–N/L; CH_3OH/N mass ratio of 3:1), the overflow pipe was opened and continuous flow of feed began at different flow rates into the bottom of the reactor. The SW used as a feed solution was prepared daily, checked for nitrate–N, nitrite–N and DO concentrations, pH and chemical oxygen demand (COD). The reactor was operated at an ambient temperature (24–26°C) and an agitation speed of 100 rpm under anoxic conditions. The agitation speed of 100 rpm was set for feed SW solution mixing and in order to avoid outflow and loss of Bio-NPC particles.

In a second set of experiments, the nitrate removal was monitored for 22 days with a gradual increase of the HRT. During this experiment after 16 days, an influent SW solution was used without sterilization and addition of phosphate salts. The use of raw SW was investigated in order to determine the impact and the need of phosphate salts addition.

2.4. Analytical methods

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 and by diazotizing with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride, respectively, at the spectrophotometer Hach DR/2400 (Hach Company, Loveland, Colorado, USA) [30,31]. The presence of organic C source (methanol) was monitored indirectly by COD measurement according to the standard methods [30]. The cell numbers present in wet Bio-NPC particles were determined by plate count after crushing of 1 g Bio-NPC in sterile mortar and after repeated decimal dilution with sterile 0.9% NaCl on standard nutrient broth (Biolife, Italy) at 37°C after 48 h and expressed as colony forming units/g Bio-NPC particles (CFU/g Bio-NPC).

3. Results and discussion

Incremental concentrations of nitrate in waste, surface and ground waters, as well as in drinking waters, were caused by the intensive use of nitrate containing compounds such as fertilizers, different amines, amino aromatic substances and many others. The harmful impact of nitrate on water, animals and humans cause great environmental concern [32]. Amongst diverse biological and physical–chemical methods, biological denitrification, chemical reduction or physical adsorption have been studied, but effective nitrate removal is still under consideration. 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 detrimental chemicals from both ground and surface waters and it has been proven that the addition of zeolite improves the performance of the denitrification process [33]. Therefore, in this study, the denitrification of the SW with NPC as a support material for bacterial growth in the batch and continuous-flow reactor was investigated.

3.1. The effect of Bio-NPC and methanol amount, and effect of pH on SW denitrification

A series of batch tests with 3.75–30 g of the Bio-NPC (2.5–20% w/w) were set-up to determine the optimal amount of the Bio-NPC for efficient nitrate removal. The observed results showed that in the presence of 2.5 and 5% of the Bio-NPC, nitrate removal was too low, at the same time, in the presence of the increased amounts of the Bio-NPC (10, 15 and 20%), nitrate removal was 72.57, 76.25 and 100%, respectively (Fig. 1). The comparison of the obtained results and time needed for complete nitrate removal indicated that the use of 10% w/w of Bio-NPC was efficient for the removal of 100 mg NO_3–N/L from the SW.

The organic carbon source is one of the main factors which has a significant influence on the course of nitrate removal. In this study, the influence of methanol to nitrate–N mass ratio on nitrate removal from the SW with the Bio-NPC was demonstrated in Fig. 2. The results obtained at the CH_3OH/N ratio of 2.0:1 and 2.3:1 indicated that the denitrification process was interrupted after 5 h due to the lack of organic carbon; therefore, required CH_3OH/N ratio for the denitrification of 100 mg NO_3–N/L from the SW was higher than 2.3:1. The complete nitrate removal was achieved at CH_3OH/N ratio of 2.5:1, but the use of excess methanol (CH_3OH/N ratio of 3.0:1) did not significantly improve denitrification. In addition, nitrite monitoring revealed that in the presence of the increased amounts of methanol (CH_3OH/N ratio of 3.0:1), the generation of nitrite was increased (up to 0.39 mg NO_2–N/L) in comparison with nitrite generated (up to 0.29 mg NO_2–N/L) at CH_3OH/N ratio of 2.5:1. Nevertheless, the final nitrite concentrations in the SW were lower than 0.07 mg NO_2–N/L, and this could be explained by the presence of an increased number of bacterial cells determined on Bio-NPC particles (6.2×10^9 CFU/g Bio-NPC) that reduce nitrate with a negligible or no nitrite accumulation. Therefore, the addition of methanol at CH_3OH/N mass ratio of 2.5:1 was optimal and a necessary amount of organic carbon needed for complete denitrification. The obtained required amount of methanol was in accordance with practical experience with full-scale denitrification systems using methanol as the organic carbon source [34].

The pH value of water is another parameter that played a major role on the denitrification and as reported in literature, an optimal pH for most denitrifying bacteria

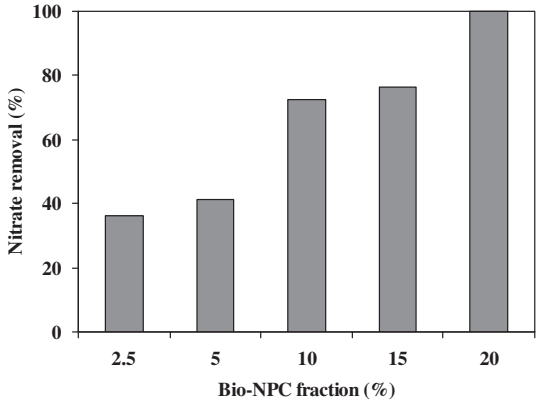

Fig. 1. The nitrate removal during the SW denitrification in the presence of different amounts of the B-NPC particles.

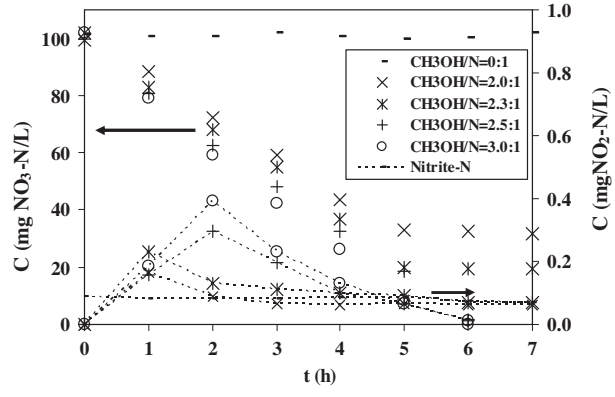

Fig. 2. The nitrate concentrations in the SW during the influence study of CH_3OH/N mass ratios on the denitrification course.

was reported in the range of 7–8 [35]. Already in 1983, Timmermans and Van Haute [36] reported that denitrification with methanol (CH_3OH/N ratio of 2.52:1) was optimal at pH of 8.3:

$$NO_3^- + 1.10\ CH_3OH + H^+ \rightarrow 0.078\ C_5H_7O_2N + 0.46\ N_2 + 0.71\ CO_2 + 2.427\ H_2O \quad (2)$$

The pH value has a relevant influence on the behaviour of microorganisms in nitrate reduction. Thus, the denitrification was studied in the predetermined pH range of 5.85–8.03, and the complete removal of 100 mg NO_3–N/L from the SW at pH of 7.13 and 7.76 was observed during 6 h. The nitrite generation during the denitrification was low and final nitrite concentration in the SW was lower than 0.04 mg NO_2–N/L. At lower investigated pH values (5.85, 6.15 and 6.66) nitrate removal lasted 7–9 h with similar nitrite generation and even at pH value of 8.03 nitrate removal was achieved during 8 h. During this study, according to literature and previous investigations, the denitrification process was confirmed as zero order reaction and the denitrification rates were calculated as described previously in detail [37]. The observed values (Fig. 3) were in the range of 11.03–16.82 mg NO_3–N/L h and were similar to previously published data [37,38] as shown in Table 1. In addition, volumetric denitrification rates obtained during nitrate removal in different reactors with different bacteria and different support materials presented in Table 1 indicated that the use of Bio-NPC enable effective denitrification. According to the obtained results, the fast and efficient denitrification of the SW was achieved at a pH value of 7.13. Similar optimal reduction of nitrate at pH of 7.4 was reported in a study conducted with methanol at 30°C, but denitrification rates were significantly decreased in the pH range 5–9 [42]. On the contrary, as seen in Fig. 3, in the pH range of 5.85–8.03, denitrification rates were slightly decreased, indicating that biological denitrification with Bio-NPC particles in the selected pH range was a stable process. Additionally, the presence of phosphate salts in the SW regulated pH and therefore just a slight increase of pH was recorded, although according to the general denitrification equations (Eqs. (1) and (2)) a higher increase of pH was expected.

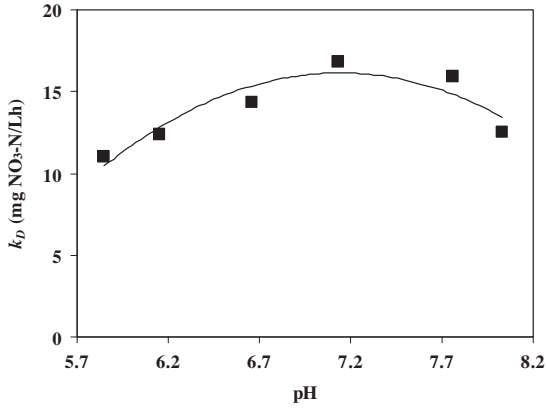

Fig. 3. The denitrification rates as a function of pH.

3.2. The denitrification in continuous-flow stirred reactor

Nitrate removal in the continuous-flow stirred reactor was monitored in the first set of experiments at different HRTs (37.04, 23.26, 10.53, 2.33 and 1.32 h) in order to achieve the optimal dilution rate and efficient nitrate removal (Fig. 4). Each test started as a batch and when nitrate was completely reduced (in approximately 6–7 h), continuous flow of the feed solution began. Nitrate–N and nitrite–N in the feed solution were usually 99–122 mg NO_3–N/L and 0–0.02 mg NO_2–N/L, respectively. The DO was between 6.50–7.50 mg O_2/L and average pH and COD values were 7.28 and 540 mg O_2/L, respectively. In the first test set, nitrate ions were completely reduced in the reactor at almost all investigated HRTs. Nitrite ions were generated up to 4 mg NO_2–N/L during the start up of the first test conducted at an HRT of 37.04 h (Fig. 4(A)), whilst during eight days of continuous-flow nitrite generation was lower than 0.06 mg NO_2–N/L. In the course of subsequent tests, the maximum of nitrite generation was up to 1.08 mg NO_2–N/L, observed at HRT of 1.32 h but the nitrite was subsequently completely reduced or at the end of the process in the SW, the presence of 0.01 mg NO_2–N/L was determined (Fig. 4(B)). Generally, accumulated nitrite was low in comparison with the initially present nitrate and has no significant impact on the denitrification, as revealed from an earlier study [43].

The methanol was a source of organic C for the bacterial growth and its removal was monitored throughout the COD measurement. During this set of tests, as shown in Fig. 5, the COD removal was in the range of 65.06–86.76%. The comparison of values observed at HRT of 37.04 and 10.53 h indicated that 3 × increase of nitrate and COD loading rates have no significant influence on nitrate removal since it was higher than 99%. On the contrary, the COD removal achieved in a steady state was increased from an average value of 65.06% (HRT = 37.04 h) to an average value of 86.74% achieved at HRT of 10.53 h, but

Table 1
Volumetric denitrification rates by some denitrifying reactors

Denitrifying reactor	Medium/*Bacteria*	Carbon source	Volumetric denitrification rates (mg NO_3–N/L h)	Reference
Membrane feeding substrate reactor	Silicon tube/*Alcaligenes eutrophus*	CO_2	0.67–2.25	[16]
Packed gel envelopes	Na alginate beads/*Pseudomonas butanovora*	Ethanol and acetic acid	63.75 and 67.92	[16]
Glass column	Na alginate beads/*Pseudomonas stutzeri* and *Comamonas testosteroni*	Fusel oil	58.75 and 69.17	[16]
Stirred-tank reactor	NPC/*mixed culture*	CH_3OH	15.45–63.09	[29]
Denitrification filters	Plant material/*Activated sludge*	CH_3OH	45.83	[34]
Tank reactor	Zeolite sand/*mixed culture*	CH_3OH	3.96–28.5	[37]
Hollow fibre membrane bioreactor	Polypropylene	CH_3OH	1.25–32.08	[38]
Column bioreactor	Na alginate beads/*Pseudomonas butanovora*	Ethanol and acetic acid	22.5–36.67	[39]
Packed bed	Biodegradable polymers	$(C_6H_{10}O_2)_n$	21–166	[40]
Municipal solid waste reactor	municipal solid waste	organic (leachate)	35	[41]
Stirred-tank reactor	NPC/*mixed culture*	CH_3OH	11.03–79.90	This study

further decrease of HRTs to 2.33 and 1.32 h did not improve COD removal and observed organic removal efficiencies were 84.10 and 85.39%, respectively. The highest COD removal was achieved at HRT of 10.53 and 1.32 h along with the nitrate removal higher than 99.52% (Fig. 5(A)). Furthermore, the increase of the nitrate loading rate along with the efficient nitrate removal resulted in significant enhancement of the volumetric denitrification rates (k_{Dvol}) that were increased from 2 to 74 mg NO_3–N/L h. The similar results were obtained with methanol as electron donor in hollow fibre membrane bioreactor and denitrification rates increased to 32 mg NO_3–N/L h at an influent NO_3–N concentration of 145 mg NO_3–N/L and a hydraulic residence time of 4.1 h (Table 1) [38].

The monitoring of pH and the DO throughout the experiments indicated that the influent pH values were slightly raised, and DO was almost completely depleted (Fig. 5(B)) confirming that the course of the process was in accordance with the general denitrification equation. The results of this study were similar to previously published data [44]. This recently conducted study of different HRTs on biological denitrification in the biofilm reactor indicated that the influent flow of 100 mg NO_3–N/L in the presence of methanol at a CH_3OH/N ratio of 3.0:1 could be effectively removed at an HRT of 8 h. At the same time, the optimal pH range of 7–7.5 was determined, but increase or decrease of pH in the range of 5–9 and the decrease of HRT to 2 h resulted in reduced nitrate removal efficiency.

The high efficiency of the bioreactor could be explained by the presence of Bio-NPC particles or by the increased growth of mixed bacterial culture. The bacterial numbers (CFU/g Bio-NPC) determined prior to the beginning and immediately after each test were increased from the range of 3.3×10^8–5.3×10^9 CFU/g Bio-NPC to the range of 6.59×10^8–7.5×10^9 CFU/g Bio-NPC. The bacterial cells that interacted with clinoptilolite particles obviously enabled efficient nitrate removal. In addition, a similar observation has been demonstrated and the addition of zeolite improved the performance of the denitrification process [33].

In the second test set, denitrification started at the HRT of 2.33 h, since the previous test had shown that the effluent at HRT of 1.32 h contained nitrate. During eight days of continuous-flow, effluent nitrate–N was lower than 0.9 mg NO_3–N/L and nitrate removal was higher than 99.5%, therefore on the ninth day the HRT was decreased to 1.32 h (Fig. 6). Nitrate concentration in the effluent increased within the following 24 h to 11 mg NO_3–N/L with a decrease of nitrate removal to 87.77%, but in the next few days it raised to 99.5%. After 12 days of continuous operation, the system reached the steady state and the process was monitored over another 10 days. After some fluctuations during the first 12 days, the nitrate–N and nitrite-N concentrations in the effluent were

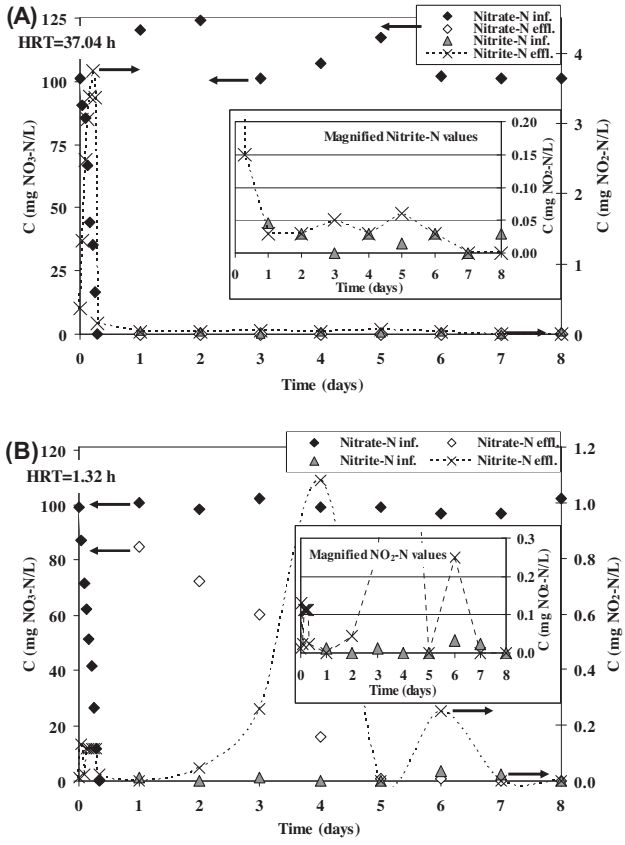

Fig. 4. Nitrate and nitrite profile during denitrification in the continuous-flow stirred reactor at HRT of 37.04 h (A) and at HRT of 1.32 h (B).

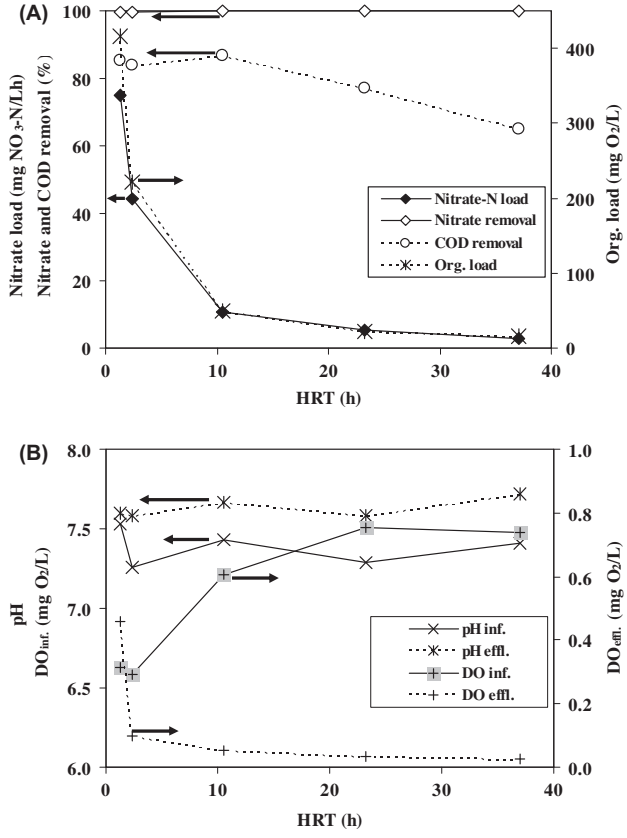

Fig. 5. Parameters determined at a steady state during denitrification at different HRTs in the continuous-flow stirred reactor: Organic and nitrate loading rates and nitrate and COD removal (A) and influent and effluent values of pH and DO (B).

between 0.2–0.47 mg NO_3–N/L and 0–0.057 mg NO_2–N/L, respectively. At that period, nitrate removal and COD removal were in the range of 87.77–99.91% and 75.72–96.33%, with average values of 98.42% and 84.96%, respectively (Fig. 6(A)). The COD removal observed during this prolonged denitrification was generally higher than the values observed during the previous test set.

During the experiment, the DO was regularly checked and the influent values of 6.4–7.5 mg O_2/L were lowered to the effluent values of 0.13 ± 0.08 mg O_2/L (Fig. 6(B)). The temperature and pH were also monitored. Temperature values of the influent solution were always in the range of 20–25°C, but the reactor was operated at 25 ± 1°C. The influent pH values of 7.20–7.42 were increased to an average effluent value of 7.56. The nitrate concentrations were used for calculation of volumetric denitrification rates, k_{Dvol} and during the first eight days, they were in the range of 39.2–51.28 mg NO_3–N/L h and then fluctuated within the range of 63.47–79.90 mg NO_3–N/L h with an average value of 74.37 mg NO_3–N/L h. The decrease of HRTs consequently increased nitrate loading rates, and thus, the observed increase of the k_{Dvol} was supported by the fact that the denitrification rates were dependent on the influent nitrate concentration. That was demonstrated by the presented results, which ranged between 2 and 74 mg NO_3–N/L h during the first set of tests and between 39.29–79.90 mg NO_3–N/L h during the second denitrification test. The obtained denitrification rates as shown in Table 1 compare well with values obtained earlier [16,39], but since our values were somewhat higher, it could be assumed that the interaction of zeolite with the selected mixed culture was favourable for SW denitrification.

On Day 16, raw SW (without addition of phosphate salts and sterilization) as the inlet SW solution was used in order to determine the impact of phosphate salts addition. The average influent pH value of 7.28 was raised to the average effluent value of 8.55 (Fig. 6(B)), confirming that phosphate salts previously added into the SW acted as a buffer. Accordingly, their absence contributed to a slight

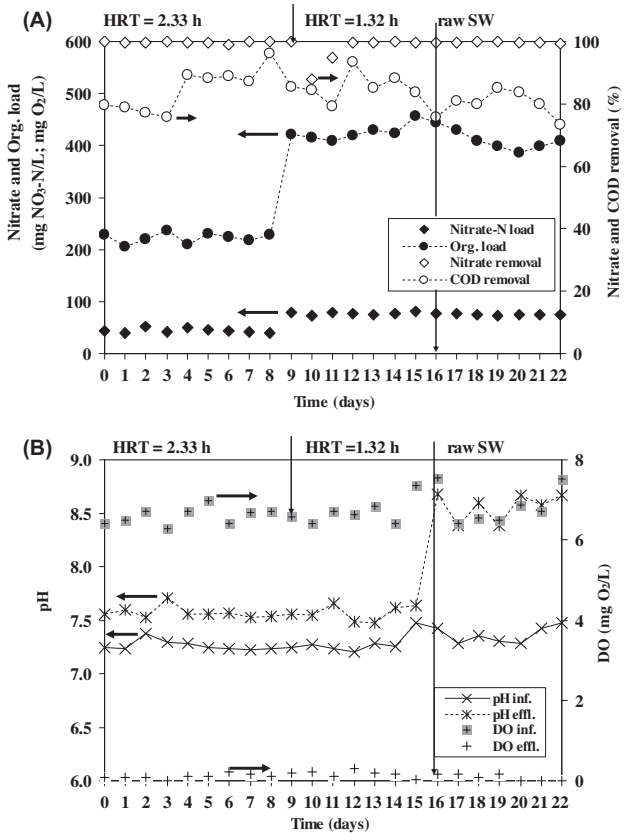

Fig. 6. Time course of continuous-flow denitrification: Nitrate and organic loading rates, nitrate and COD removal (A) and influent and effluent values of pH and DO (B).

increase of effluent pH as expected from the general denitrification equations (Eqs. (1) and (2)). Although the pH values were in some extent higher than observed in the effluent during the previous period, nitrate removal and COD removal were still higher than 99.5 and 79%, respectively (Fig. 6(A)). The observed results demonstrated that the denitrification of SW with Bio-NPC in the continuous-flow stirred reactor could be effective even without addition of phosphate salts.

4. Conclusions

A series of batch tests indicated that the use of 10% w/w of Bio-NPC was efficient for the removal of 100 mg NO_3–N/L from the SW and that the addition of methanol at CH_3OH/N mass ratio of 2.5:1 was optimal and necessary for the complete denitrification. The process was stable in the pH range of 5.85–8.03, but the highest efficiency was obtained at the pH value of 7.13.

The denitrification with Bio-NPC in the continuous-flow stirred reactor was investigated at different HRTs (37.04, 23.26, 10.53, 2.33 and 1.32 h) in order to achieve the optimal HRT and efficient nitrate removal from the SW. The efficient nitrate removal was determined even at a HRT of 1.32 h and the obtained results revealed that Bio-NPC particles in the presence of external organic carbon at CH_3OH/N mass ratio of 3:1 could effectively reduce 100 mg NO_3–N/L from the SW. Additionally, for effective nitrate removal, the process should be set-up on a magnetic stirrer at 100 rpm and 20–25°C under anoxic conditions. Throughout all tests, the nitrite accumulation was negligible and effluent nitrite was below 0.02 mg NO_2–N/L. The results indicate that during continuous denitrification with the Bio-NPC particles, nitrate and COD removal exceeded 99 and 79%, respectively. Even with the use of raw surface water, the process was stable and effective. Finally, the use of Bio-NPC was demonstrated as an efficient method for complete nitrate removal from the surface water.

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