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Nitrate Removal in a Continuous-Flow Stirred Reactor

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The presence of excess nitrate in natural water resources is determined worldwide and therefore the methods for reduction and prevention of water pollution are still being investigated. The main objective of the present study was the use of natural zeolite that was interacted with bacterial cells to obtain biozeolite particles (Bio-ZPs). The resulting Bio-ZPs were applied for nitrate removal from the surface water (SW) in a continuous-flow stirred reactor. In the first set of experiments, the nitrate removal was monitored at dilution rates (*D*) of 0.027, 0.043, 0.095, 0.429, and 0.755 h^{-1} in order to achieve the optimal dilution rate. During the second test set, the removal of 100 mg NO_3^--NL^{-1} from the SW with Bio-ZPs was monitored at 0.429 and 0.755 h^{-1} during 21 days. The use of Bio-ZPs in the continuous-flow stirred reactor was demonstrated as an efficient method for nitrate removal from the SW.

Key words:

Clinoptilolite, continuous-flow stirred reactor, denitrification, mixed bacterial culture, nitrite accumulation

Introduction

The presence of nitrate in water is an increasingly important global issue as nitrogen compounds flowing through ecosystems have multiple impacts on terrestrial, aquatic and atmospheric environments.[1,2] In agricultural areas, the excess nitrate can leach into groundwater and ultimately enter surface waters through different discharges like under-field tile drainage and ditches or the discharge of shallow groundwater to surface waters. Accordingly, in order to reduce pollution, many land management approaches have been proposed,[3–5] but the removal of nitrate and nitrite ions from waters are still being investigated.[6–9] Low-cost and simple nitrogen removal technologies are essential considering its well known harmful impacts. High inorganic nitrogen concentrations in the aquatic environment could lead to many environmental problems including acidification of freshwater bodies, eutrophication and associated hypoxic zones, adverse health effects for aquatic organisms, as well as production of greenhouse gas such as N_2O.[10] In addition, high nitrate and nitrite concentrations in drinking water can also be hazardous to human health. They can cause cancer in the digestive system through the formation of nitrosamines or nitrosamides or the loss of the oxygen-carrying ability of haemoglobin (methaemoglobinemia or blue baby disease) in newborn infants.[11,12] The epidemiologic evidence for the linkages between drinking water nitrate and the risk of specific cancers, adverse reproductive outcomes, and other health outcomes in the context of the current regulatory limit for nitrate in drinking water was reviewed.[13]

The most commonly used treatment methods to remove/reduce nitrate ions include adsorption, ion exchange, reverse osmosis, electrodialysis, chemical and catalytic and biological denitrification, and among them the World Health Organization has suggested biological denitrification and ion exchange as the preferred nitrate removal methods.[6,7,9,12,14] Biological denitrification is considered economically effective and feasible, and thus is a

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widely applied process for nitrate removal. Heterotrophic denitrification relies on heterotrophic microorganisms which convert nitrate (NO_3^-) to N gases using a carbon (C) source as the electron donor and for growth.[7,12] The process has mostly been studied in different reactors (denitrifying bioreactors) that contain microbes and carbon substrates added into the contaminated water to provide a C and energy source for microbial growth, enabling the conversion of nitrate to nitrogen gases through a sequence of enzymatic reactions. At the microbial scale, the denitrification rate is generally controlled by concentrations of oxygen (O_2), nitrate, nitrite and carbon.[15]

Organic C present in the water during denitrification provides an anoxic environment through the oxidation of organic compounds and acts as an electron donor, which promotes denitrification. The availability of a degradable organic C source is crucial for this process and in order to improve efficiency, it could be supplemented as liquid C sources such as acetate, ethanol or methanol.[16,17] Furthermore, to improve reactor performance in water treatment systems the presence or addition of phosphate salts was investigated since phosphorus is an essential element in cell growth and function.[18]

Different bioreactors have been investigated and among them the use of free dispersed cells could cause operational problems due to the blockage of flow lines and clogging of filters, while separation of biomass from the treated effluents is beset with technical difficulties, rendering the treatment procedure cost-prohibitive.[19] This has led to greater interest in the entrapment and immobilization of microbial cells. Therefore, some studies have investigated microorganisms immobilized in hydrogels such as carrageenans and Ca-alginates, in order to achieve efficient nitrate removal.[20–23] But even gel entrapment involves several disadvantages associated with limitations in the rate of diffusion, insufficient mechanical strength that causes breakage of gels, lack of open spaces for cell growth, and the prohibitive cost of application.[24,25] These 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.[26] Zeolites, as naturally occurring porous aluminosilicate minerals, have a large specific surface area and cation exchange capacity.[8,26] Additionally, due to their mechanical strength, abundance in many regions, and low cost, zeolites are favourable for application in many different water treatment processes. Nitrate removal by adsorption or ion exchange on modified zeolite was recently studied.[27,28] The zeolite was used even as a fertilizer carrier to control nitrate release and

the interaction of zeolite and microorganisms was applied for phosphate removal.[29,30] Therefore, the use of clinoptilolite-rich zeolite as a support material for bacterial growth seems to be promising for heterotrophic denitrification and the removal of nitrate from water.

In the present paper, the previously acclimated microbial culture interacted with natural zeolite and the resulting biozeolite particles were used for investigation of nitrate removal in a continuous-flow stirred reactor. To investigate denitrification in a continuous-flow stirred reactor, the predetermined CH_3OH/NO_3-N mass ratio was applied and a simplified kinetic analysis was performed for quantitative comparison of nitrate degradation and methanol consumption. At first, different dilution rates (*D*) were investigated, and then in the second set of experiments, the efficiency and stability of the process were monitored for 22 days. In order to determine the impact of phosphate addition on microbial community functions and the denitrification performance, raw surface water (without addition of phosphate salts) was used in the last part of the second set of experiments.

Materials and methods

Microorganisms, zeolite, and surface water

Bacterial culture originated from the active sludge of the wastewater treatment plant Anamet, Savski Marof, Croatia and the agricultural soil sample from 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 surface water (SW), used in the acclimation tests and in a batch denitrification study.[31] The acclimated mixed bacterial culture was refrigerated at 4 °C and stored until use.

The zeolite used in the study was a natural powdered zeolite containing 71.1 % clinoptilolite, obtained from the Zlatokop deposit in Vranjska Banja, Serbia. The natural powdered clinoptilolite was washed with redistilled water to remove the surface dust, dried at 105 °C for 24 h, and grain size fractions smaller than 0.063 mm were used for interaction with the mixed bacterial culture.

The SW sample was prepared using raw surface water from the Bjelovar region with the addition of K_2HPO_4, (2.5 g L^{-1}) and KH_2PO_4, (1 g L^{-1}), and sterilized at 121 °C for 15 min. During the last part of the second test set, the raw surface water from the Bjelovar region was used without addition of phosphate salts and sterilization. The stock nitrate solution (NaNO_3 solution containing 10 g

$NO_3^- $-$NL^{-1}$) and methanol were added separately to the sterile SW or to the raw SW to provide initial nitrate-N concentration of 100 mg $NO_3^- $-$NL^{-1}$ and CH_3OH/NO_3-N mass ratio of 3:1, respectively. All the reagents used during the tests were of an analytical grade level.

Preparation of biozeolite particles (Bio-ZPs)

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

Experimental set-up

The denitrification tests with the use of the Bio-ZPs were conducted in a 0.25 L sterile reactor containing 19 g of the Bio-ZPs and the SW medium up to 210 mL. The stock nitrate solution and methanol were added to the sterile SW to obtain initial nitrate concentration and CH_3OH/NO_3-N mass ratio of 100 mg $NO_3^- $-$NL^{-1}$ and 3 : 1, respectively. The CH_3OH/NO_3-N mass ratio of 3 : 1 was selected according to previous investigations and in order to avoid carbon-limited conditions.[31] The reactor was sealed, punctured with 2 needles (one for sampling and the other for removal of produced gas) and placed on the magnetic stirrer at 1.667 s^{-1} (Fig. 1). The agitation speed for the feed SW mixing was set at 1.667 s^{-1} to avoid outflow and loss of Bio-ZPs. The investigation of the denitrification process in a continuous-flow stirred reactor began as a batch test and after complete removal of nitrate from the SW, 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 feed solution was prepared daily and checked for nitrate-N, nitrite-N, chemical oxygen demand (COD) and dissolved oxygen (DO) concentrations. Incubation was conducted at 16–25 °C, pH 7.2 and an agitation speed of 1.667 s^{-1} under anoxic conditions.

In a second set of experiments the nitrate removal was monitored for 22 days with a gradual increase in the dilution rate. During this experiment, after 16 days, the influent SW was used without sterilization or addition of phosphate salts in order to determine the impact and the need to add phosphate salts.

Analytical methods

At predetermined time intervals 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, nitrite and COD 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 and by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride respectively, at spectrophotometer Hach DR/2400 (Hach Company, Loveland, Colorado, USA).[32,33] COD was determined according to the Standard methods.[32]

Calculation

During denitrification in a continuous-flow stirred reactor, the nitrate loading rate (*NLR*), organic loading rate (*OLR*), nitrate removal rate, COD removal rate, and volumetric denitrification rate (*VDR*) were calculated as follows:

$$NLR = (\gamma_{\text{Nitrate} - \text{N}})_{\text{infl.}} \cdot D(\text{mg NO}_3^- - \text{NL}^{-1}\text{h}^{-1})$$

$$OLR = (\text{COD})_{\text{infl.}} \cdot D(\text{mg O}_2\text{L}^{-1}\text{h}^{-1})$$

Nitrate removal rate $=$

$$= \frac{(\gamma_{\text{Nitrate} - \text{N}})_{\text{infl.}} - (\gamma_{\text{Nitrate} - \text{N}})_{\text{effl.}}}{(\gamma_{\text{Nitrate} - \text{N}})_{\text{infl.}}} \cdot 100\,(\%)$$

$$\text{COD removal rate} = \frac{(\text{COD})_{\text{infl.}} - (\text{COD})_{\text{effl.}}}{(\text{COD})_{\text{infl.}}} \cdot 100\,(\%)$$

$$VDR = [(\gamma_{\text{Nitrate} - \text{N}})_{\text{infl.}} - [(\gamma_{\text{Nitrate} - \text{N}})_{\text{effl.}}] \cdot D(\text{mg NO}_3^- - \text{NL}^{-1}\text{h}^{-1})$$
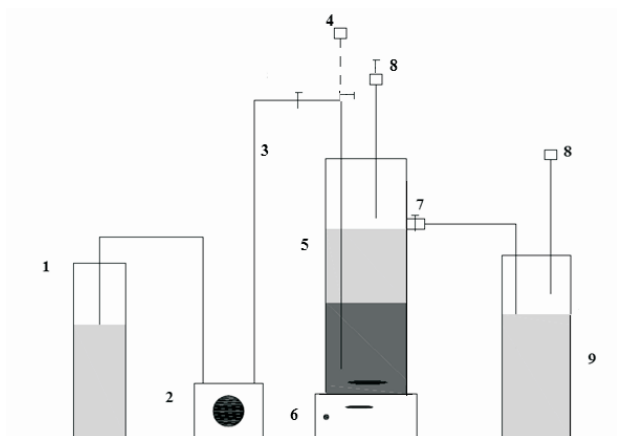

F i g . 1 – *Scheme of denitrification in the continuous-flow stirred reactor: 1 – Feed SW; 2 – Peristaltic pump; 3 – Influent flow tube; 4 – Sampling tube; 5 – Reactor; 6 – Magnetic stirrer; 7 – Effluent pipe; 8 – Gas drain pipe; 9 – Effluent collector*

where $(\gamma_{Nitrate-N})_{infl.}$, $(\gamma_{Nitrate-N})_{effl.}$, $(COD)_{infl.}$ and $(COD)_{effl.}$ represented the influent and effluent nitrate nitrogen concentrations (mg NO_3^--NL^{-1}) and COD (mg O_2 L^{-1}), respectively. The dilution rate, D (h^{-1}) was calculated from the influent SW flow rate value, FR (L h^{-1}) and the void (working) reactor volume, V (L): $D = FR/V$ (h^{-1}).

Results and discussion

In order to achieve the optimal dilution rate and efficient nitrate removal, in the first set of experiments, the denitrification of the SW in the continuous-flow stirred reactor was monitored at the dilution rates (D) of 0.027, 0.043, 0.095, 0.429 and 0.755 h^{-1}. Each test started as a batch and after complete reduction of nitrate ions (in approximately 6–7 h), continuous flow of feed SW was turned on. Nitrate-N and nitrite-N in feed SW were usually 99 – 122 mg NO_3^--NL^{-1} and 0 – 0.02 mg NO_2^--NL^{-1}, respectively (Fig. 2a). The DO was between 6.00 – 8.20 mg O_2 L^{-1} and average pH and COD values were 7.28 and 540 mg O_2 L^{-1}, respectively. In the first test set, nitrate ions in the reactor were completely reduced at almost all investigated dilution rates. The nitrate concentration of 0.12 mg

NO_3^--NL^{-1} was recorded at steady state in the effluent at $D = 0.755$ h^{-1} (Fig. 2b). In the course of tests, the maximum of nitrite generation was up to 0.11 mg NO_2^--NL^{-1}. However, the nitrite was subsequently reduced and at the end of the process (at steady state) the presence of less than 0.01 mg NO_2^--NL^{-1} in the SW was determined. Generally, nitrite accumulation was low in comparison to the initially present nitrate and has no significant impact on the denitrification, as revealed from an earlier study.[34,35] During the first set of experiments conducted at different dilution rates, the volumetric denitrification rates (*VDR*) obtained at steady state were increased from 2 to 74 mg NO_3^--NL^{-1} h^{-1} (Fig. 3).

The role of methanol addition was to provide an external C source for the bacterial denitrification and throughout the test, it should be reduced. Since feed SW contained methanol as the sole organic carbon, the measuring of COD in filtered samples could be considered as a measure of methanol consumption. As shown in Fig. 3, the COD removal was in the range of 65.06 – 86.76 % and the highest COD removal was achieved at D of 0.095 h^{-1} and 0.755 h^{-1} along with nitrate removal higher than 99.52 %. During this set of experiments, the nitrate and organic loading rate were gradually increased for almost 25 times and the nitrate was still almost completely removed. The presence of Bio-ZPs and the increased activity of mixed bacterial culture that interacted with clinoptilolite could be the reason for the observed high efficiency of the reactor. Similarly, it has been demonstrated that the addition of zeolite improved the performance of the denitrification process.[36] Obviously, this interaction of bacteria and zeolite enabled effective nitrate removal.

In the second test set, denitrification started at the dilution rate of 0.429 h^{-1}, according to the previously observed presence of nitrate in the effluent at the dilution rate of 0.755 h^{-1}. Nitrate monitoring revealed that during 8 days of continuous flow, the effluent nitrate-N was lower than 0.9 mg NO_3^--NL^{-1}, and therefore on the 9th day, D was increased to 0.755 h^{-1} (Fig. 4). Within the following 24 hrs, the nitrate concentration in the effluent increased to 11 mg NO_3^--NL^{-1} but during the next few days it was lowered to 0.5 mg NO_3^--NL^{-1}. After 12 days of continuous operation, the system reached steady state, and the process was monitored over another 10 days. The nitrate-N and nitrite-N concentrations in the effluent fluctuated throughout the first 12 days and the values observed after reaching steady state were in the range of 0.2 – 0.47 mg NO_3^--NL^{-1} and 0 – 0.06 mg NO_2^--NL^{-1} respectively (Fig. 4a). The steady state values of nitrate and COD removal were in the range of 87.77 –
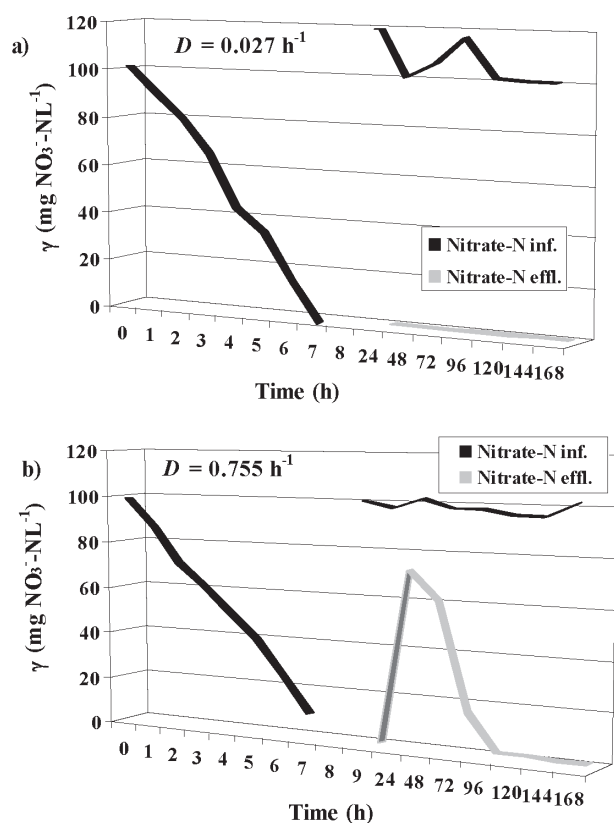

F i g . 2 – *Nitrate profile during denitrification in the continuous-flow stirred reactor. Nitrate concentrations in influent and effluent: a) at dilution rate of 0.027 h^{-1} and b) at dilution rate of 0.755 h^{-1}*
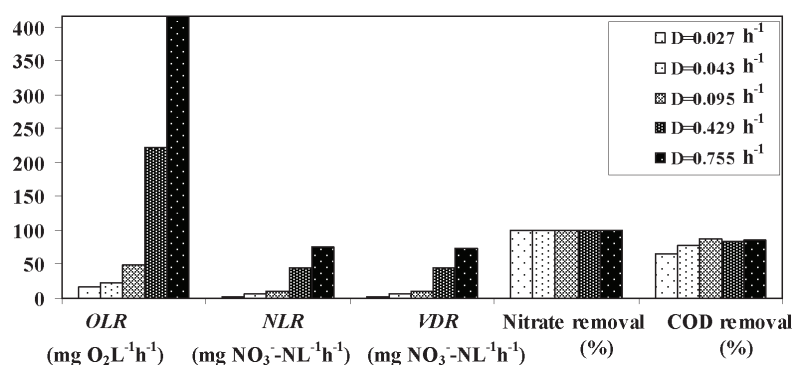
F i g . 3 – *Parameters determined during denitrification at different dilution rates in the continuous-flow stirred reactor: Organic and nitrate loading rates, volumetric denitrification rates, nitrate and COD removal*
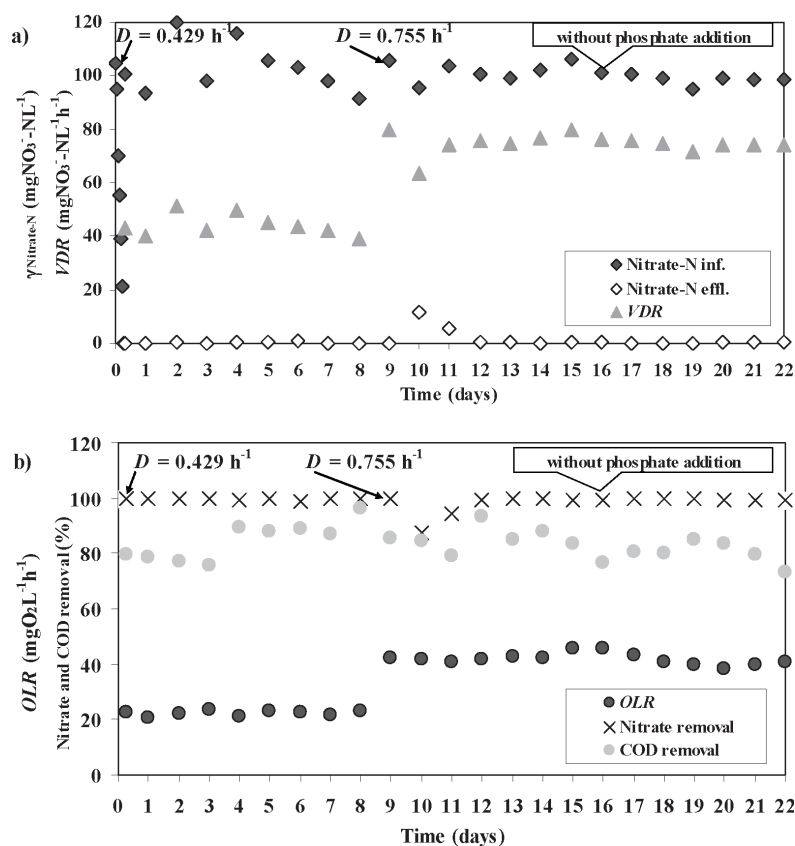

F i g . 4 – *Time course of a continuous-flow denitrification: a) Influent, effluent nitrate-N and volumetric denitrification rates and b) organic loading rates, nitrate and COD removal*

99.91 % and 75.72 – 96.33 %, with average values of 98.42 % and 84.96 %, respectively (Fig. 4b). The COD removal observed during this prolonged denitrification was generally higher than the values observed during the previous test set.

The dissolved O_2 was checked regularly and the influent values of 6.2 – 7.5 mg O_2 L^{-1} were lowered to the effluent values of 0.13 ± 0.08 mg O_2 L^{-1}. The temperature and pH were also monitored in the influent and effluent. Temperature values were always in the range of 21 – 26 °C and the influent pH values of 7.20 – 7.42 were increased to an average effluent value of 7.56 ± 0.05. According to observed results, the volumetric denitrification rates were calculated and during the first 8 days they were in the range of 39.2 – 51.28 mg NO_3^--NL^{-1} h^{-1} and then fluctuated within the range of 63.47 – 79.90 mg NO_3^--NL^{-1} h^{-1} with an average value of 74.37 mg NO_3^--NL^{-1} h^{-1} (Fig. 4a). The increase of *D* to 0.755 h^{-1} consequently increased nitrate loading rates and volumetric denitrification rates. The observed increase of *VDR* was in agreement with the fact that the denitrification rates are dependent on the nitrate concentration. The *VDR* values obtained during the first and second denitrification test set ranged between 2 – 74 mg NO_3^--NL^{-1} h^{-1} and between 39.29 – 79.90 mg NO_3^--NL^{-1} h^{-1} respectively, and comparison of the presented results clearly demonstrated positive correlation of denitrification rates and nitrate concentrations. The obtained denitrification rates compare well with values obtained by Kesserü et al.,[37] but the values observed in this study were somewhat higher, confirming that the interaction of zeolite with the selected mixed culture was favourable for SW denitrification.

On the 16th day, in order to determine the impact of phosphate salts addition, the raw surface water (without addition of phosphate salts and sterilization) was used as the inlet SW. Consequently, the average influent pH value of 7.28 was raised to the average effluent value of 8.55. These pH values were somewhat higher than observed in the effluent during the previous period, but as revealed from the obtained results, the removal of nitrate and COD was still higher than 99.5 % and 79 %, respectively (Fig. 4b). The results obtained during this period indicated that the denitrification with Bio-ZPs in a continuous-flow stirred reactor could be effective even with raw surface water.

Conclusions

Clinoptilolite-rich zeolite was used as support material for bacterial growth, and after interaction, the resulting Bio-ZPs were applied in a continu-

ous-flow stirred reactor for the denitrification study. In order to achieve the optimal dilution rate and efficient nitrate removal from SW, the process was monitored in the presence of external organic C at CH_3OH/NO_3-N mass ratio of 3:1 at dilution rates of 0.027, 0.043, 0.095, 0.429 and 0.755 h^{-1} on a magnetic stirrer at 1.667 s^{-1}, and 16–25 °C under anoxic conditions. The obtained results revealed that Bio-ZPs could effectively reduce 100 mg NO_3^--NL^{-1} present in the SW. Throughout all the tests, the nitrite accumulation was negligible and the effluent nitrite concentration was below 0.02 mg NO_2^--NL^{-1}. The nitrate and COD removal during the continuous denitrification of SW with Bio-ZPs exceeded 99 % and 79 %, respectively. Furthermore, stable and effective nitrate and COD removal was obtained even with the use of raw surface water, demonstrating that the use of Bio-ZPs was an efficient method for complete nitrate removal from the surface water.

ACKNOWLEDGMENTS

Symbols

$(COD)_{infl.}$	– influent COD (mg O_2 L^{-1})
$(COD)_{effl.}$	– effluent COD (mg O_2 L^{-1})
D	– dilution rate (h^{-1})
FR	– flow rate (L h^{-1})
NLR	– nitrate loading rate (mg NO_3^--NL^{-1} h^{-1})
OLR	– organic loading rate (mg O_2 L^{-1} h^{-1})
V	– void (working) reactor volume (L)
VDR	– volumetric denitrification rate (mg NO_3^--NL^{-1} h^{-1})
$(\gamma_{\text{Nitrate-N}})_{infl.}$	– influent nitrate nitrogen concentration (mg NO_3^--NL^{-1})
$(\gamma_{\text{Nitrate-N}})_{effl.}$	– effluent nitrate nitrogen concentration (mg NO_3^--NL^{-1})

Abbreviations

Bio-ZPs	– biozeolite particles
C	– carbon
CH_3OH/NO_3-N	– methanol to nitrate nitrogen mass ratio (–)
COD	– chemical oxygen demand
DO	– dissolved oxygen concentration (mg O_2L^{-1})
SW	– surface water

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