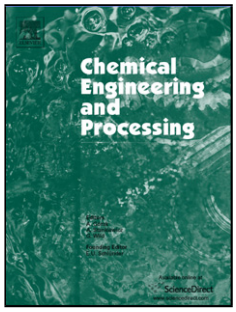

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Nitrate removal in an innovative up-flow stirred packed-bed bioreactor

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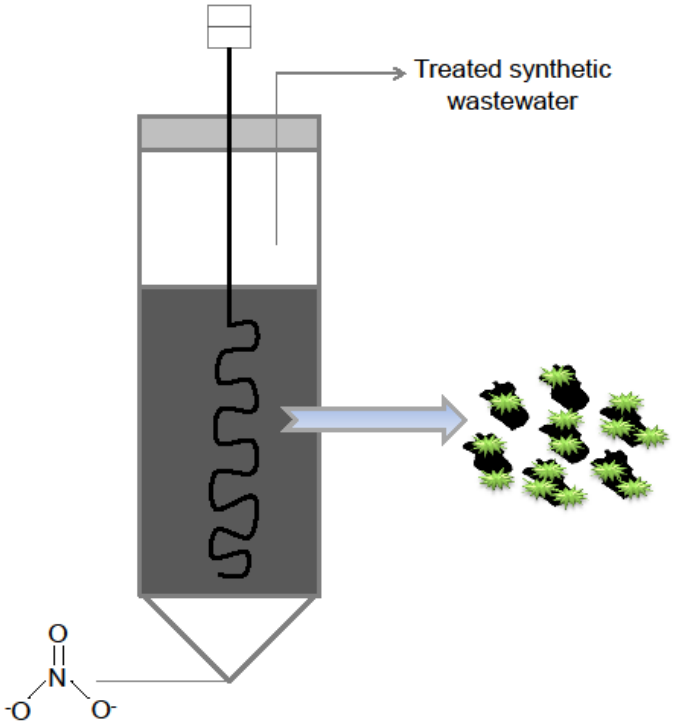
Graphical abstract



Highlights

- **An anaerobic up-flow stirred packed-bed reactor for nitrate removal is proposed.**
- **Sludge carbonaceous material was used as support material.**
- **High nitrate removal efficiency was achieved at very short space time.**

- **Michaelis–Menten model was found to describe the denitrification process rather well at low initial nitrate concentration.**
- **An effective and promising method for nitrate removal was demonstrated using a cheap and environmental friendly reactor.**

Abstract

The anaerobic removal of nitrates was studied in a continuous up-flow stirred packed-bed reactor (USPBR) containing biological sludge carbonaceous material (BSCM). The carbonaceous material (CM), obtained from exhausted sludge, was used as support to grow the biofilm and solid electron mediator for nitrate reduction in the bioreactor. In a continuous USPBR-BSCM system feed with treated synthetic wastewaters, nitrate removal efficiency was 99% at very short space times (τ) 2 min/hydraulic residence times (HRT) 6 min. By comparing these results with the hydraulic residence times applied in other classical denitrification technologies, it seems that USPBR-BSCM requires the shortest times to achieve almost complete nitrate removal. In addition, a high denitrification rate of $3.67 \cdot 10^{-3}$ mmol$NO^{3-} \cdot min^{-1} \cdot g^{-1}$ was observed in the USPBR-BSCM, whilst in the USPBR-biological activated carbon (BAC) it was $2.04 \cdot 10^{-3}$ mmol$NO^{3-} \cdot min^{-1} \cdot g^{-1}$. The kinetic analysis of the systems showed a good fit with the Michaelis–Menten model and the kinetic parameters estimated were k_1= 3.37 mmol$\cdot L^{-1} \cdot min^{-1}$, k_2= 0.58 mmol$\cdot L^{-1}$ for USPBR-BSCM and k_1= 2.50 mmol$\cdot L^{-1} \cdot min^{-1}$, k_2= 0.27 mmol$\cdot L^{-1}$ for USPBR-BAC. Results indicate that SCM is a potentially low cost catalyst and effectively competes with commercial ones; USPBR-BSCM is an inexpensive and advantageous process for nitrate removal and also an effective system for denitrification.

Keywords: Anaerobic denitrification; Biological sludge carbonaceous material; Biological activated carbon; Packed-bed reactor; Continuous-flow reactor.

1. Introduction

Groundwater is generally polluted by nitrate as a result of industrial processes, agricultural runoff and unexploded ammunition, and has become an important environmental problem. Usually, an excess of nitrates in water is related to public health problems such as methemoglobinemia and carcinogenesis. Therefore, the European Environment Agency (EEA) regulations and the United States Environmental Protection Agency (USEPA) have established specific global rules to address this problem. For example, drinking water should not contain more than 11.3 mgNO_3^--N/L (EEA) and 10 mgNO_3^--N/L (USEPA), respectively [1: USEPA,2: EEA]. For this reason, nitrate removal processes need to be incorporated into waste treatment plants.

There are many different technologies for removing nitrates from drinking water and wastewaters, but mainly used for drinking waters. For example, reverse osmosis, ion exchange, membrane process and electro-dialysis. They all have their individual advantages and disadvantages. Ion exchange has been developed principally to treat high concentrations of nitrate, but it is limited because there are few resins with high selectivity for nitrate and all of them present high problem of regeneration [3,4]. Reverse osmosis, is capable of separating and concentrating the compounds in wastewater without making any changes to their molecular structures, but its main disadvantage is the low selectivity of the membranes used for nitrate [5,6]. Membrane biotechnology is widely used for denitrification, but the membrane can be considerably damaged by pressure and easily contaminated [2,7]. Electro-dialysis removes nitrate from wastewater without the use of additional chemicals, but is sensitive to iron, manganese, hydrogen sulfide (H_2S), chlorine and hardness [8,9]. Of all of the existing techniques, biological denitrification is considered to be economically effective and feasible, and is widely used for nitrate removal for wastewaters [10].

The process of denitrification involves reducing nitrate to dinitrogen gas by anaerobic facultative bacteria that use nitrate as an electron acceptor [11]. Denitrifies are classified into two main groups: heterotrophs and autotrophs [12]. Heterotrophic denitrification has been used in conventional wastewater treatment plants (WWTPs), while autotrophic denitrification has been studied on a laboratory scale. Heterotrophs

are microorganisms that use organic matter as electron donors [13,14], whilst autotrophic denitrifiers are microbes that can use inorganic reduced compounds (e.g. sulfur-reduced compounds, ferrous iron, hydrogen gas, pyrite and arsenite) as electron donors by fixing inorganic carbon [15,16]. These substrates are not always present in the wastewater to be treated and need to be added. Consequently, the biological removal of nitrogen from wastewaters containing about 1000 mgN/L can be a problem, because of the low concentration of electron donors necessary for the denitrifying bacteria [17]. Therefore, various researchers have found that the addition of certain electron donors (acetate, methanol, ethanol, starch or glucose) can favourably improve denitrification in organic carbon-limited wastewaters [18,19]. For example, if acetate (CH_3COO^-) is used as an electron donor, the stoichiometric reaction for denitrification can be described as follows [20]:

$$8\,NO_3^- + 5CH_3COO^- + 15H_2O = 4N_2 + 10CO_2 + 13OH^- + 16H_2O \qquad (1)$$

On the basis of the above stoichiometric reaction, the CH_3COO^-/NO_3^- mass ratio for complete denitrification is 0.59. The substrate selected depends on criteria such as availability, the carbon compound which produces the fastest denitrification rate and cost [21–23].

The only serious disadvantage of the traditional anaerobic biological systems is the requirement of long hydraulic residence times (HRT). As an alternative, biofilm systems such as fixed-bed or fluidized-bed reactors have been used for nitrate reduction. These biofilm systems use support media with a high surface area to sustain biomass concentrations up to 10 times more than the typical concentration commonly used in conventional active sludge systems [24]. The use of high biomass concentration gives the biofilm systems a high volumetric removal capacity and the benefit of having a more compact system [25]. Although effective for the denitrification process, the use of a fluidized-bed for nitrate removal could be extremely costly because more energy is needed to suspend the attached biomass in the reactor. Therefore, it seems that fixed-bed reactors have the potential to comply with the above requirements. One of their main characteristics is simplicity, and low construction, operation and maintenance costs. They have no problems in separating the catalyst from the reactor effluent, which is another of the disadvantages of several fluidized-bed systems, because recovering the

catalyst can be difficult and requires considerable equipment costs [26]. Besides, as has been mentioned above, fixed-bed reactors have a large surface area for biological growth, so appropriate packing material should be selected. Of the solid supports used, activated carbon offers the advantages of a large adsorptive capacity and an irregular shape, and shelters bacteria from high fluid shear forces [27]. Nevertheless, commercially available activated carbons are expensive so it is better to find low-cost solid carriers for use in wastewater treatment production of activated carbons from available sources. Research, then, has mainly focused on producing activated carbons from such sources as waste from the production of cereals, nut shells, olive stones, etc. [28]. Sewage sludge is also potentially appropriate for the production of carbons because of its wide availability, carbonaceous structure, low cost, and rich content of organic materials [29]. For this reason, many studies using sewage sludge as catalytic support material have focused on the production of CMs for adsorbing organic pollutants [30–32].

Our research group has studied the biodegradation of azo dyes in an up-flow stirred packed-bed reactor (USPBR) using AC and CMs prepared from exhausted-sludge materials, as catalytic support materials for the biomass. The results demonstrated that filling USPBR with BSCM and BAC was an effective and promising system for azo dye degradation [32–34].

Biological nitrate removal has been widely studied in the literature, but this paper is the first study of an USPBR-BSCM being used for the biological reduction of nitrate. The aim of the research was to study anaerobic nitrate removal using a continuous USPBR-BSCM system. This system was compared with a continuous USPBR filled with AC, as catalytic support material, for anaerobic nitrate removal. The significance of applying special agitation to the BSCM and BAC beds was studied. The influence of nitrate concentration on the denitrification rate and nitrate removal was investigated, in order to determine the maximum nitrate concentration degraded in our systems. Furthermore, the Michaelis-Menten kinetic model was applied to describe the denitrification process in USPBR-BSCM and USPBR-BAC systems.

Obviously, this new process only applies to sewage or general wastewater, because the presence of microorganisms makes it not suitable for drinking water.

2. Materials and methods

2.1. Chemicals

Potassium nitrate (KNO_3, 99.4%) was selected as the NO_3^- source. Sodium acetate (CH_3COONa, 99%) was used as the co-substrate and was at once the carbon source for sludge and the electron donor for nitrate reduction. Both compounds were purchased from Sigma-Aldrich. Sewage sludge from a municipal WWTP was used to prepare the sludge carbonaceous material (SCM) [32] and to obtain the mixed culture. SCM was crushed and granules with a mesh size of 25–50 were separated. Activated carbon (Merck, granules of 1.5 mm, ref. 1025141000) was crushed and sieved into sizes of 0.3-0.7 mm. Carborundum granules obtained from Carlo Erba Reagents were used as inert diluent for SCM and AC. The standard solutions of the basal media contained the following composites ($mg \cdot L^{-1}$): $MnSO_4 \cdot H_2O$ (0.155); $CuSO_4 \cdot 5H_2O$ (0.285); $ZnSO_4 \cdot 7H_2O$ (0.46); $CoCl_2 \cdot 6H_2O$ (0.26); $(NH_4)_6Mo_7O_{24}$ (0.285); $MgSO_4 \cdot 7H_2O$ (15.2); $CaCl_2$ (13.48); $FeCl_3 \cdot 6H_2O$ (29.06); NH_4Cl (190.9); KH_2PO_4 (8.5); $Na_2HPO_4 \cdot 2H_2O$ (33.4); K_2HPO_4 (21.75). These chemicals were obtained from Sigma-Aldrich.

2.2. Experimental set-up

Figure 1 shows the USPBR system. The operating parameters used in this study are similar to those described by [34]. The total reactor volume was 10 mL. A set of two reactors was prepared and they were started up in parallel. Each reactor was filled with the mixture of 9 g of carborundum granules and 1 g of SCM or AC as catalyst. During the first period of operation, the entering feed was 50 $mgNO_3^-/L$ solution containing CH_3COO^-/NO_3^- with a mass ratio of 2:1 (in order to avoid the carbon-limiting condition) and the basal media with microelements (1 mL of each basal media per litre of feeding solution). A stirring system was incorporated into the USPBR so that the agitation in the biological SCM or AC bed was slow (1 rph) to avoid outflow and loss of biomass.

In another series of assays, the effect of different nitrate concentrations (25–700 $mgNO_3^-/L$) on the denitrification rate and nitrate removal was examined. The

flow rate of the feed was varied between 25 and 350 $mL \cdot h^{-1}$ and was ensured by a peristaltic pump.

2.3. Biological system

Anaerobic sludge with mixed culture was obtained from the wastewater treatment plant from Reus, Tarragona, Spain. The sludge was filtered by a micro filter with a pore size of 20–25 μm so that only single cells and spores remained. The mixed culture was pumped through the two anaerobic USPBR for a week, one filled with SCM and the other with AC. The anaerobic conditions were maintained by bubbling helium and temperature was kept constant at 35ºC. During this period the biofilm was immobilized on the surface of the SCM and AC resulting in the so-called biological sludge carbonaceous material (BSCM) and biological activated carbon (BAC), respectively. The biofilm was adapted to NO_3^- in both USPBRs, by a continuous flow of the synthetic solution containing the basal media and carbon source through the reactor.

2.4. Denitrification kinetic analysis

2.4.1. Determination of reaction rate

In continuous USPBRs the decisive factor is the quantity of catalyst rather than the volume of the reactor. Therefore, it is more suitable to consider the conversion value as a function of space time instead of hydraulic residence time (HRT). Thus, the space time (τ, min) is defined by the following equation:

$$\tau = \frac{m_c}{F_v \cdot \rho} \quad (2)$$

where m_c (g) is the amount of catalyst in the reactor, F_v ($mL \cdot min^{-1}$) is the volumetric flow rate of nitrate solution, and ρ ($g \cdot mL^{-1}$) is the density of the solution [33].

In packed-bed reactors the mole balance is given by equation 3:

$$\frac{dF_{NO_3^-}}{dm_c} = -r_{NO_3^-} = \frac{d(C_{NO_3^-} \cdot F_v)}{d(\tau \cdot F_v \cdot \rho)} \quad (3)$$

where $F_{NO_3^-}$ (mmol·min^{-1}) is the molar flow of nitrate solution, m_C (g) is the amount of catalyst in the bioreactor, $C_{NO_3^-}$ (mmol·L^{-1}) is the nitrate concentration, F_v is the volumetric flow, τ (min) is the space time, ρ (g·L^{-1}) is the density of solution and $r_{NO_3^-}$ (mmol·min^{-1}·g^{-1}) is the nitrate removal rate. If the density of the solution is equivalent to the density of water and the flow rate of nitrate solution is kept constant, the denitrification rate will finally be:

$$\frac{dC_{NO_3^-}}{d\tau} = r_{NO_3^-} \quad (4)$$

The rate of denitrification can be described by a Michaelis-Menten model [35]:

$$r_{NO_3^-} = \frac{dC_{NO_3^-}}{d\tau} = -\frac{k_1 \cdot C_{NO_3^-}}{k_2 + C_{NO_3^-}} \quad (5)$$

where k_1 (mmol·L^{-1}·min^{-1}) is the maximum removal rate and k_2 (mmol·L^{-1}) is half the maximum rate.

Equation 5 was solved using MATLAB® R2012a. The algorithm uses the fifth-order Runge-Kutta subroutine to solve the differential equation coupled to a nonlinear least-squares fitting algorithm (lsqnonlin; algorithm: Trust-Region-Reflective), to estimate the values of the parameters in the equation.

2.5. Analytical methods

Samples of influent and effluent were collected every day and filtered with syringe filters with a pore size of 0.45µm. Nitrate and nitrite concentrations were determined by ultraviolet spectrophotometric screening and the colorimetric method, respectively, according to Standard Methods [36], and using the 8500 Spectrophotometer UV-VIS (DINKO Instruments). Ammonium was analysed using a HI83099 COD and Multiparameter Photometer (Hanna Instruments).

3. Results and discussion

3.1. Denitrification in continuous USPBR

In USPBR-BSCM and USPBR-BAC the denitrification was started at a space time (τ) of 2 min (HRT of 6min) in both bioreactors. The results are shown in figure 2. In an experiment of ten days of continuous-flow, effluent nitrate was lower than 25 $mgNO_3^-/L$ and nitrate removal was about 60% for both systems. After some fluctuations during the first 20 days of continuous operation, the reactors reached steady state and the processes were controlled for another 55 days. The nitrate and nitrite concentrations in the effluent of USPBR-BSCM fluctuated between 0.50–32.50 $mgNO_3^-/L$ and 0.30–0.86 $mgNO_2^-/L$, respectively; the removal rates for nitrate were 35.64 $gNO_3^- \cdot L^{-1} \cdot d^{-1}$ (0.57 mol $NO_3^- \cdot L^{-1} \cdot d^{-1}$). In USPBR-BAC the nitrate and nitrite concentrations in the effluent, for the same period of operation, fluctuated between 0.50–29.50 $mgNO_3^-/L$ and 0.36–0.86 $mgNO_2^-/L$, respectively; the removal rates for nitrate were 34.92 $gNO_3^- \cdot L^{-1} \cdot d^{-1}$ (0.56 $molNO_3^- \cdot L^{-1} \cdot d^{-1}$). The ammonium concentrations in influent and effluent samples were also monitored for both systems, because of the addition of a huge amount of this compound to the model water (64.40 $mgNH_4^+/L$) as micronutrient for microorganisms. As we can see in figure 2a and 2b, the consumption of this compound in the bioreactors is very low.

Nitrate removal in USPBR-BSCM and USPBR-BAC was about 95-99% for this short space time in the systems working in continuous. Table 1 compares these results with those of other denitrification bioreactors reported in the literature. It seems that USPBR-BSCM and USPBR-BAC require the shortest time to accomplish almost complete denitrification. This table includes a comparison of dissimilar nitrate environmental problems with different technological solutions. The comparison is only made in terms of literature review. Foglar [10] studied the use of natural zeolite interacted with bacterial cells to obtain bio-zeolite particles for nitrate removal, in a continuous-flow stirred reactor. The removal rate of nitrate was 7.97 $gNO_3^- \cdot L^{-1} \cdot d^{-1}$ (0.13 $molNO_3^- \cdot L^{-1} \cdot d^{-1}$) at a hydraulic residence time (HRT) of 1.32 h. Kesserű [37] evaluated biological denitrification in a continuous-flow pilot bioreactor containing immobilized *Pseudomonas butanovora* cells. The average removal rates were 3.90 $gNO_3^- \cdot L^{-1} \cdot d^{-1}$ (0.004 $molNO_3^- \cdot L^{-1} \cdot d^{-1}$) and 2.39 $gNO_3^- \cdot L^{-1} \cdot d^{-1}$ (0.002 $molNO_3^- \cdot L^{-1} \cdot d^{-1}$) at ethanol–C:nitrate–N ratios of 3:1 and 1.5:1, respectively. Montalvo [38] modified an

up-flow anaerobic sludge blanket (UASB) reactor using zeolite to improve the nitrate removal process. The reactor achieved a nitrate removal efficiency of 92.4% and a removal rate of 6.2 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.10 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) at an HRT of 2.5 h. Isaka [39] evaluated the nitrate removal performance of polyethylene glycol (PEG) gel carriers containing entrapped heterotrophic denitrifying bacteria in a cylindrical reactor. A maximum nitrate removal rate of 22.58 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.36 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) was observed. Jing [40] studied the performance of an anaerobic reactor for simultaneous sulfide and nitrate removal. The removal rate for nitrate was 4.60 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.07 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) at an HRT of 4 hours. Tavares [41] tested an alternative system, in which anaerobic digestion and denitrification take place in the same UASB. The removal rate was 0.19 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.003 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) at an HRT of 6 hours. Barber [42] carried out the nitrate removal in an anaerobic baffled reactor (ABR). Nitrate removal efficiency was 82% and removal rate was 0.98 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.02 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) with a very high HRT of 20 hours. Cai [43] proposed using methane in biogas as an electron donor to facilitate complete nitrate removal with denitrifying anaerobic methane oxidizing (DAMO) microorganisms, in an anaerobic ammonium oxidation (Anammox) reactor. The nitrate removal rate was 3.03 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.05 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) at an HRT of 36 hours. Waki [44] developed a semi-partitioned reactor to study methane-dependent denitrification. The removal rate was 0.27 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.004 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) at an HRT of 72 hours. Islas-Lima [45] obtained a high nitrate removal rate of 31.34 $gNO_3^-\cdot L^{-1}\cdot d^{-1}$ (0.51 $molNO_3^-\cdot L^{-1}\cdot d^{-1}$) in a continuous stirred tank reactor (CSTR). However, the HRT was 48h.

These results are important because AC is costly and using CMs from exhausted-sludge materials as catalysts is an advantage for biological processes. The high nitrate removal rate and efficiency obtained in this study reveal that the use of sludge carbonaceous material (SCM) for denitrification involves a complex process in which biology, chemistry and physics have a synergetic effect. This mechanism has been reported previously [32,33,46], and confirms the role of SCM as a redox mediator for reducing recalcitrant azo dyes.

3.2. Effect of slow agitation on USPBR-BSCM and USPBR-BAC

After the packed-bed reactors had been in operation for some time, nitrate conversion started to decline slowly. This behaviour can be observed in figure 2a and 2b on days 45 and 60 in USPBR-BSCM and USPBR-BAC, respectively. It is caused by the isolation of metabolically active organisms on the sludge carbonaceous material surface and activated carbon with continuous expansion of biofilm over the catalysts [48]. Therefore, BSCM and BAC were slowly agitated in the bioreactors.

The influence of slow agitation was assayed in three up-flow stirred packed-bed reactors, two of which were filled with activated carbon as catalytic support material and the other with sludge carbonaceous material. Figure 3 shows nitrate conversion over the time. In the first 20 days, the three bioreactors were continuously fed so that steady state could be reached. Slow agitation was applied in USPBR-BAC1 on day 60, when a decrease in nitrate conversion had been observed for the first time. It can clearly be seen that nitrate conversion increased when slow agitation was applied in the bioreactor. In USPBR-BAC2 slow agitation was first applied on day 55 in an attempt to prevent nitrate conversion from decreasing. In USPBR-BSCM a decrease in nitrate conversion was observed on day 45. Once agitation was stopped, conversion started to decrease in the bioreactors, which showed the positive influence that stirring BSCM and BAC has on nitrate removal. Nevertheless, conversions of nitrate were different before stirring was applied in the three bioreactors, in the same space time (2min). This is because there were different amounts of biomass in the bioreactors, so it is difficult to control the growth of biomass in biological beds.

For all these reasons, slow agitation of BSCM and BAC together with a continuous flow of nitrate solution within the packed bed can help decrease the unnecessary amount of biomass from the bioreactor. Agitation since system start-up can also ensure that the amount of biomass in the packed bed is approximately continuous, assuming that there is no significant activated carbon or sludge carbonaceous material wash-out (the reactor systems were built with two filters, at the top and bottom of the bioreactors, to prevent the carbon materials from washing out). This improves the performance of the SCM and AC.

3.3. Influence of nitrate concentration on biological denitrification

The denitrification rate and nitrate removal as a function of nitrate concentration are shown in figure 4. Between 25 and 100 mgNO_3^-/L, the denitrification rate increased and nitrate removal decreased with increasing nitrate concentration. As shown in this figure, similar trends were observed in the two sets of experiments. The highest denitrification rate and nitrate removal obtained in our study in USPBR-BSCM were $3.67 \cdot 10^{-3}$ mmol$NO_3^- \cdot min^{-1} \cdot g^{-1}$ and 99%, respectively, while in USPBR-BAC they were $2.04 \cdot 10^{-3}$ mmol$NO_3^- \cdot min^{-1} \cdot g^{-1}$ and 97%, respectively. The values indicate that nitrate removal by BSCM is a little higher than in BAC.

For both systems, the shape of the denitrification rate curve is very much like the curvature obtained by Vrtovšek and Roš [49] in a biofilm reactor with a mixture of PVC plastic and powdered activated carbon as the support material. Similar results were also reported by Lee [25] in a columnar packed-bed (PB) reactor with glass raschig rings as the support material.

In the same way, Rabah [50] found that nitrogen removal decreased when the nitrogen loading rate increased. We observed similar behaviour in our systems when the nitrate concentration increased.

3.4. Kinetic analysis

Nitrate reduction in the USPBR-BSCM and USPBR-BAC systems can be described by the Michaelis–Menten model (equation 5), since it involves heterogeneous catalysis and biological processes. The model fits the experimental results well for concentrations below 250 mg/L (see figure 5). Similar results were obtained by Foglar [51] for initial nitrate concentrations above 400 mgNO_3^--N/L, in which the nitrate removal predicted by the Michaelis-Menten model is a curve rather than a straight line. Foglar states that denitrification is a complex process and, therefore, that assuming certain parameters and simplifying the model can have some drawbacks for the original model. Table 2 shows the kinetic parameters encountered for this model. As can be seen, the maximum removal rate for USPBR-BSCM (k_1= 3.37 mmol$\cdot L^{-1} \cdot min^{-1}$) is

similar to the maximum rate for USPBR-BAC (k_1= 2.50 mmol·L^{-1}·min^{-1}), which confirms that SCM is a powerful adsorbent that can efficiently remove nitrates from wastewaters. Using the Michaelis-Menten equation for calculating kinetic constants with data from batch experiments, Foglar [52] estimated the denitrification kinetics in a bioreactor filled with zeolite as carrier material, treating salty wastewater. The kinetic parameters obtained were k_1= 0.0844 mgNO_3^--N·L^{-1}·h^{-1} (1 x 10^{-4} mmolNO_3^-·L^{-1}·min^{-1}) and k_2= 5.18 x 10^{-5} mgNO_3^--N·L^{-1} (3.7 x 10^{-6} mmolNO_3^-·L^{-1}). Cao [53] studied denitrification kinetics in a bubble-column bioreactor filled with a gel matrix of polyvinyl alcohol (PVA) to co-immobilize denitrifying bacteria. They certified that the single-stage bio-denitrification process obeyed Michaelis-Menten kinetics. The kinetic parameters for nitrate removal were found to be k_1= 34.5 mgNO_3^--N·L^{-1}·h^{-1} (0.04 mmolNO_3^-·L^{-1}·min^{-1}) and k_2= 303 mgNO_3^--N·L^{-1} (21.6 mmolNO_3^-·L^{-1}). Dinçer [54] studied the kinetics of nitrification and denitrification of synthetic wastewater using two reactors in series: an activated sludge unit for nitrification followed by a down-flow bio-filter for denitrification. The experimental data indicated that denitrification kinetics followed the Michaelis-Menten model and the kinetic parameters for denitrification were found to be k_1= 280 mgNO_3^--N·L^{-1}·h^{-1} (0.01 mmolNO_3^-·L^{-1}·min^{-1}) and k_2= 0.27 mgNO_3^--N·L^{-1} (0.02 mmolNO_3^-·L^{-1}). Therefore, to the best of our knowledge and considering the different synthetic wastewaters used by the authors, denitrification in USPBRs, mainly USPBR-BSCM, is the fastest anaerobic nitrate removal process ever reported, which confirms that SCM can be an efficient alternative for producing low-cost AC and an effective waste management practice.

4. CONCLUSIONS

To our knowledge, this is the first time that a continuous USPBR-BSCM has been used for nitrate removal under anaerobic conditions. In continuous experiments with the USPBR-BSCM, nitrate removal efficiency reached high values of around 95-99% in very short space times. The application of slow agitation in the biological packed-bed ensured high nitrate conversion in the same space time, which shows that the slow agitation of BSCM and BAC has a positive effect on nitrate removal. The denitrification process in USPBRs can be described by the Michaelis-Menten model. The estimated kinetic parameters turned out to be k_1= 3.37 mmol·L^{-1}·min^{-1} and

k_2= 0.58 mmol·L^{-1} for USPBR-BSCM, and k_1= 2.50 mmol·L^{-1}·min^{-1} and k_2= 0.27 mmol·L^{-1} for USPBR-BAC. These are the highest nitrate removal rates ever reported and it is evident that SCM might effectively compete with commercial AC. The high efficiency and removal rates obtained in this study show that SCM plays various roles: it is at once an excellent carrier material for the attachment of microorganisms and a redox mediator for enhanced nitrate bio-reduction in the USPBR. In comparison with other continuous and biological denitrification reactors, USPBR-BSCM has proved to be an effective system and a promising application for nitrate removal.

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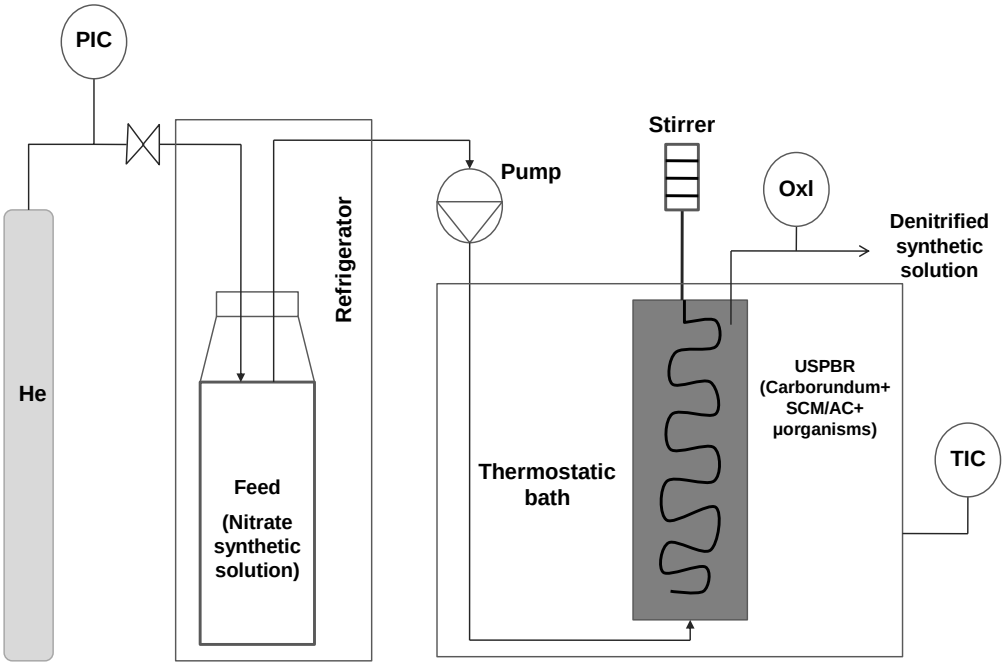
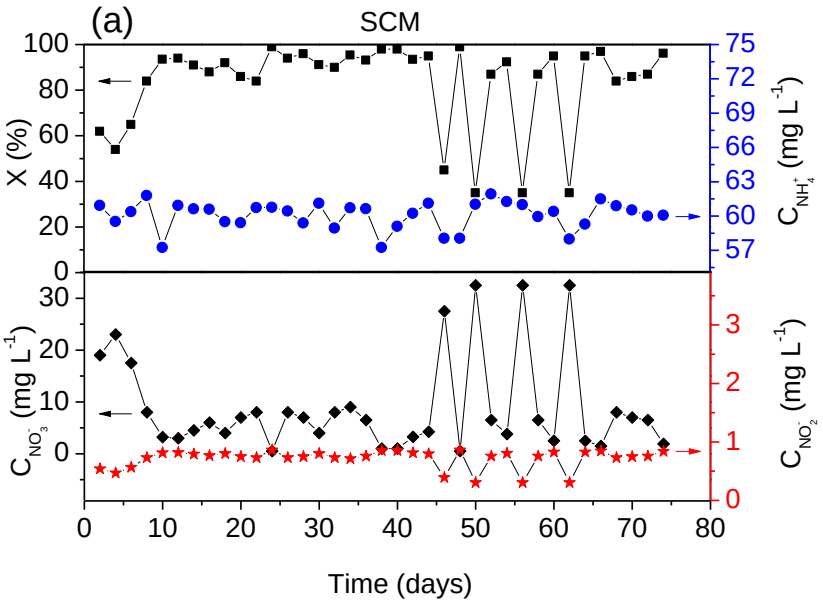


Figure 1. Anaerobic USPBR system



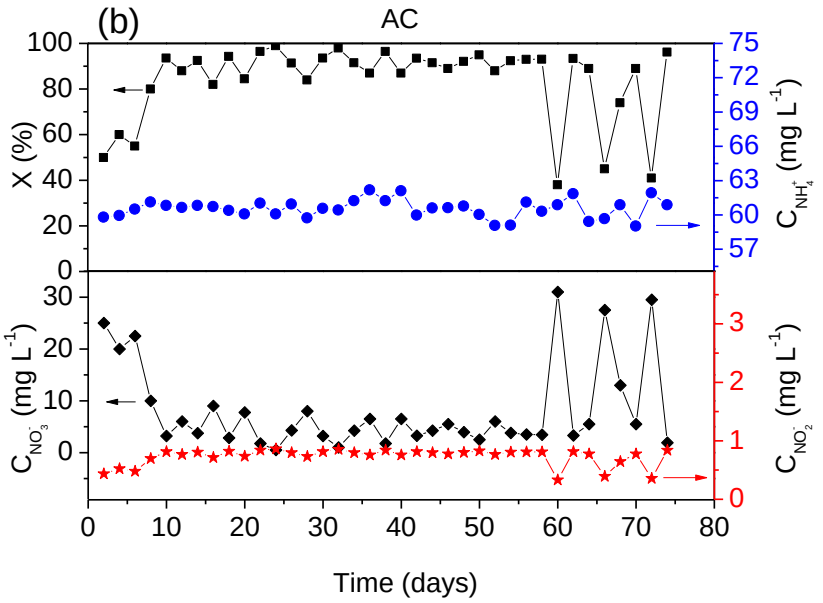


Figure 2. Nitrate (★), nitrite (■), ammonium (●) content of the bioreactor effluent and nitrate removal (X, ◆). (a) Continuous USPBR-BSCM system, (b) Continuous USPBR-BAC system.

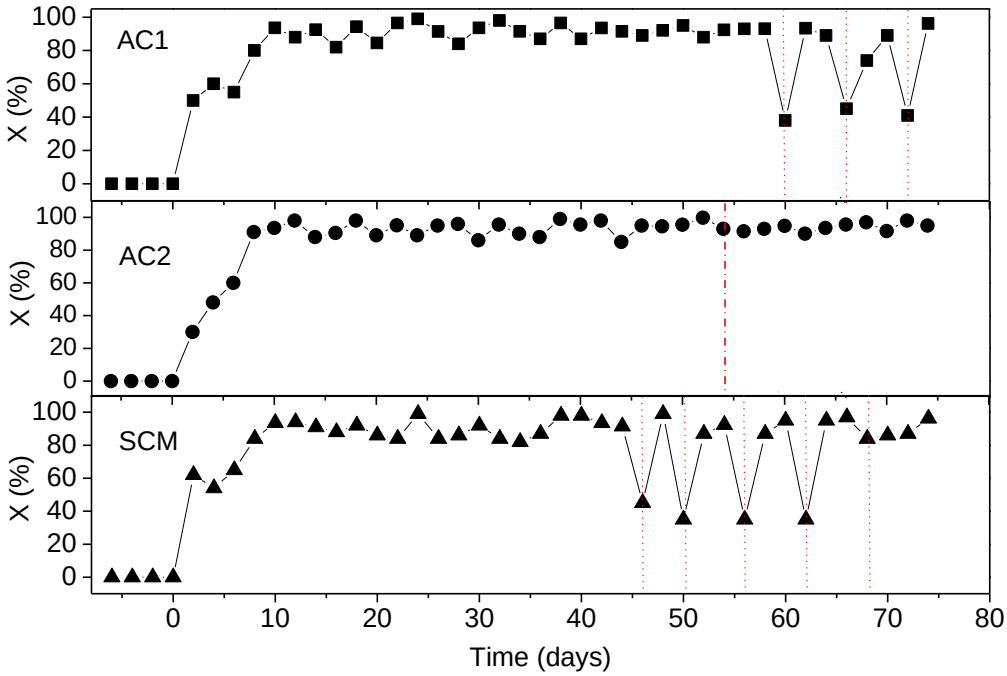


Figure 3. Effect of slow agitation in three up-flow stirred packed-bed reactors, two of which were filled with activated carbon as catalytic support material (USPBR-BAC1 and USPBR-BAC2) and the other with sludge carbonaceous material (USPBR-BSCM): nitrate removal (X); the dashed line shows the agitation applied on some days.

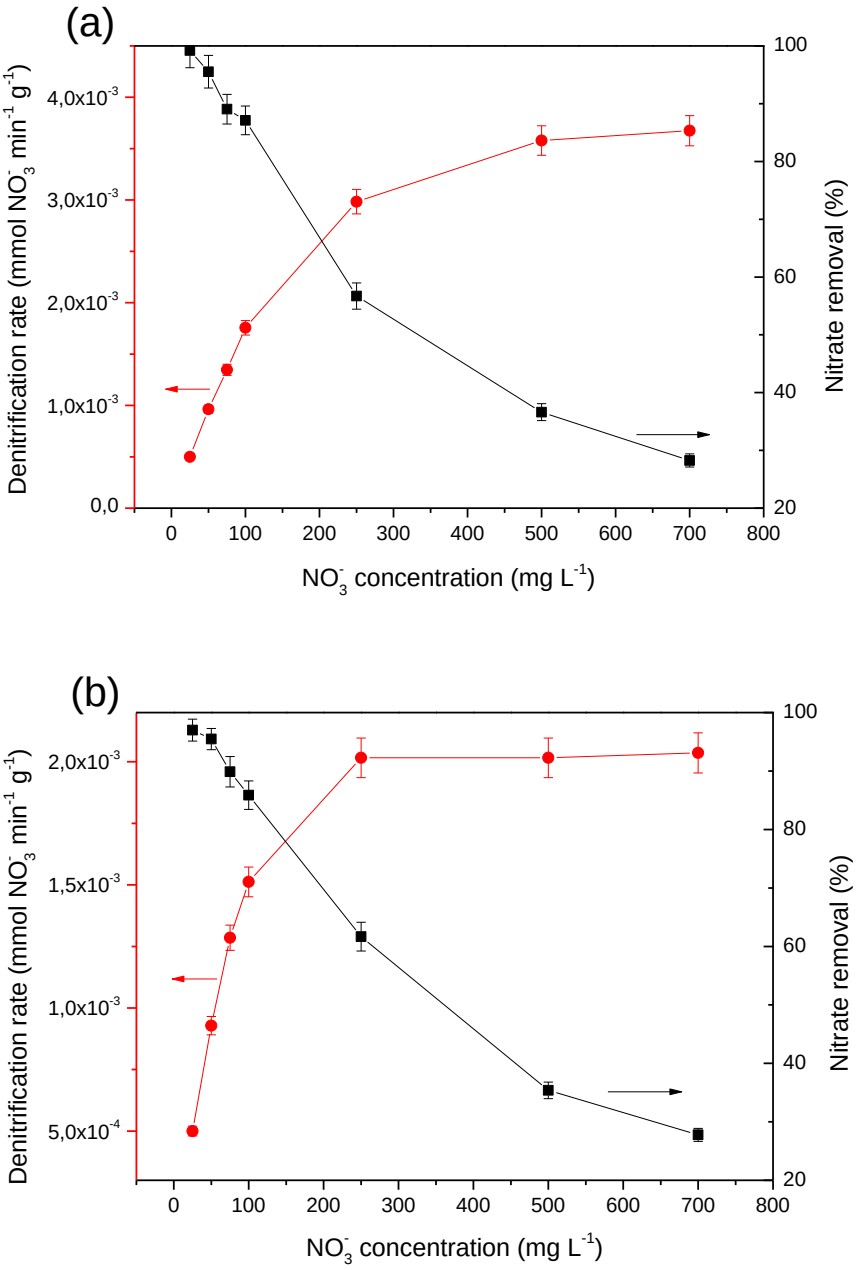


Figure 4. Denitrification rate ( ) and nitrate removal ( ) versus nitrate concentration. (a) USPBR-BSCM, (b) USPBR-BAC.

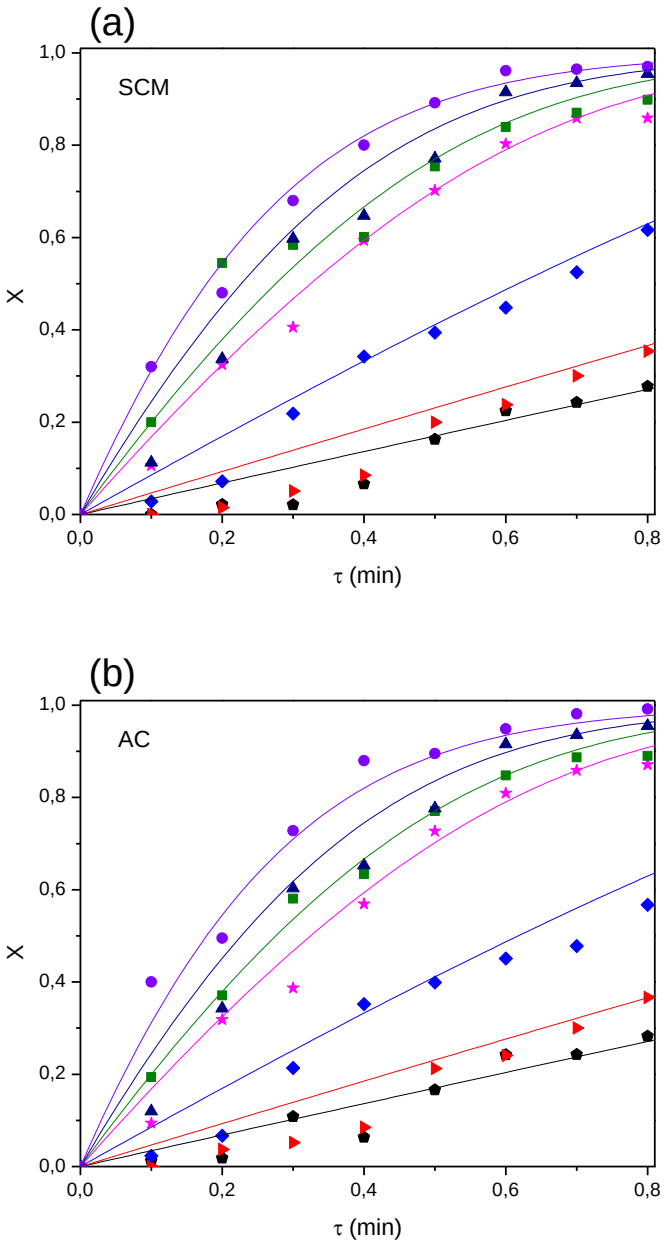


Figure 5. Kinetic modelling of nitrate removal in (a) USPBR-BSCM, (b) USPBR-BAC. Nitrate concentrations: 25 mg·L^{-1} (◆), 50 mg·L^{-1} (▶), 75 mg·L^{-1} (●), 100 mg·L^{-1} (⬟), 250 mg·L^{-1} (▲), 500 mg·L^{-1} (■), 700 mg·L^{-1} (★); lines represent the fit to the Michaelis-Menten model. X: nitrate removal rate (mmol·min^{-1}·g^{-1}), τ: space time (min).

Table 1. Results of studies reported in the literature using several denitrifying reactors

Reactor type	Concentration ($mgNO_3^-·L^{-1}$)	Rate ($mgNO_3^-·L^{-1}·d^{-1}$)	Nitrate removal (%)	Reference
Continuous-flow stirred reactor with zeolite as biomass support material	443	7968	99	[47]
Continuous-flow pilot bioreactor with *Pseudomonas butanovora*	50	3896 2390	100	[37]
Up-flow anaerobic sludge blanket reactor	2300	6200	92.4	[38]
Cylindrical reactor with gel carrier cubes	2430-4860	22577	92	[39]
Anaerobic up-flow reactor with biomass retention	95.5	4604	N.R	[40]
Up-flow anaerobic sludge blanket reactor	53	191	90	[41]
Anaerobic baffled reactor	1000	984	82	[42]
Anaerobic ammonium oxidation reactor	4427	3028	92	[43]
Semi-partitioned reactor mixed cultures	1210	266	N.R	[44]
Continuous stirred tank reactor	600	31342	99.3	[45]
Up-flow stirred packed-bed reactor	50	34920	95-99	This study

Table 2. Kinetic parameters of the Michaelis-Menten model in USPBR-BSCM and USPBR-BAC

Reactor	**k_1 (mmol·L^{-1}·min^{-1})**	**k_2 (mmol·L^{-1})**	**σ^a**
USPBR-BSCM	3.37 (±0.34)	0.58 (±0.11)	0.22
USPBR-BAC	2.50 (±0.26)	0.27 (±0.09)	0.34

[a]Standard deviation associated with the model fitting: $\sigma = \frac{\sqrt{\sum(X - X^{MOD})^2}}{n-1}$, where n is the number of experimental points.