

Short communication

Optimizing upflow velocity and calcium precipitation in denitrifying granular systems

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ABSTRACT

The denitrification process was studied in two granular biomass denitrifying reactors (USB1 and USB2). In USB1 large quantities of biomass were accumulated (9.5 g VSS L^{-1}) allowing for the treatment of high nitrogen loads ($3.5 \text{ g NO}_3\text{-N L}^{-1} \text{ d}^{-1}$). As the biomass granulation process is not immediate the effects of different upflow velocities ($0.12\text{--}5.5 \text{ m h}^{-1}$) and calcium contents ($5\text{--}200 \text{ mg Ca}^{2+} \text{ L}^{-1}$) were studied in order to speed up the process. Obtained results indicate that the optimum values for these parameters, which allow for the stable operation of USB1, are of 0.19 m h^{-1} and $60 \text{ mg Ca}^{2+} \text{ L}^{-1}$. Then these optimum conditions were applied to USB2 where the effects of concentrations from 335 to $1000 \text{ mg NO}_3\text{-N L}^{-1}$ were tested. In these conditions nitrate concentrations of $1000 \text{ mg NO}_3\text{-N L}^{-1}$ are required for denitrifying granular biomass formation. Summarizing denitrifying granules can be formed at low upflow velocities and in hard or extremely hard water composition conditions if sufficient high nitrogen loads are treated.

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1. Introduction

Heterotrophic denitrification is an efficient process to remove simultaneously organic matter and nitrogen from industrial wastewater. However when flocculent biomass is used to carry out this process, retention of gas bubbles can cause flotation of the sludge and its wash out from the reactor and, therefore, the efficiency loss of the system [1]. To maintain the stability of the process, biomass retention can be improved by the promotion of biomass granulation which also allows achieving high biomass concentrations, treating high volumetric conversion rates and enhancing biomass dewaterability [2,3]. The formation of granules is widely reported in literature for different kinds of biomass, factors or operational strategies used for promoting granulation depending on the biomass type [3,4]. In the case of denitrifying biomass, two possible strategies are reported to obtain granular biomass: (1) the application of a hydraulic selection pressure such as in the case of anaerobic

systems and; (2) the enhancement of mineral precipitation generally by presence or addition of calcium.

The hydraulic selection pressure strategy consists of imposing a high upflow velocity ($1\text{--}4 \text{ m h}^{-1}$) [5–7] in order to wash out the lighter sludge particles from the reactor and promote only the retention of the heavier ones. This strategy implies the use of reactors with a high height to diameter ratio, like those used in anaerobic digestion, or the application of liquid recirculation, which increase capital and operational costs, respectively [8].

Other possible strategy to promote the formation of denitrifying granular biomass consists of enhancing mineral precipitation. The increase of the pH of the media due to the denitrification process together with the presence of calcium ions promotes the precipitation of this element as CaCO_3 or $\text{Ca}_3(\text{PO}_4)_2$ [9–11]. The occurrence of these precipitates not only favours the formation of granular biomass but also influences its mechanical properties. In the case of calcium phosphate formation, the precipitation process could be also seen as a process to remove phosphorus which can prevent scaling problems or the need of a possible wastewater post-treatment [12,13].

If an alkalophilic environment is favoured inside the denitrifying reactor, most of the CO_2 generated during denitrification will remain dissolved in the liquid phase and high CO_3^{2-} concentrations are expected. Therefore, the formation of calcium carbonate would be possible even at low calcium concentrations if enough organic matter were transformed to CO_2 due to denitrification. However,

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Table 1
Operational parameters of USB1 reactor.

Period	1	2	3	4	5
Time (days)	0–20	21–50	51–90	91–160	161–200
HRT (h)	8.0 ± 2.0	4.3 ± 2.0	2.7 ± 0.4	8.9 ± 2.3	11.7 ± 0.5
NO ₃ ⁻ -N (mg L ⁻¹)	545 ± 70	436 ± 90	330 ± 3	330 ± 3	330 ± 3
CH ₃ COO ⁻ -C (mg L ⁻¹)	827 ± 85	613 ± 20	467 ± 17	468 ± 25	482 ± 11
ν_{up} (m h ⁻¹)	5.50 ± 0.02*	0.47 ± 0.17	0.46 ± 0.05	0.15 ± 0.05	0.12 ± 0.01
Ca ²⁺ (mg L ⁻¹)	189 ± 18	194 ± 22	100 ± 20	53 ± 11	5 ± 3

* The first 4 operational days the ν_{up} was 0.5 m h⁻¹.

most of the existing research works focused on the formation of denitrifying granular biomass by calcium carbonate precipitation use wastewater with high hardness or add calcium compounds in order to maintain a high Ca²⁺ concentration [14]. This strategy can be not optimal since an excess of precipitates decreases the physical stability of aggregates and also inhibits the biomass activity [15,16] as it was observed in the case of anaerobic and Anammox processes [17,18]. Recently, Eldyasti et al. [15] and Liu & Sun [16] studied the effect of calcium concentration on the formation of denitrifying aggregates and found that concentrations of 50–120 mg Ca²⁺ L⁻¹ were enough to obtain big size and fast-settling denitrifying granules or biofilms.

In spite of the fact that the formation of calcium carbonate precipitate would be enough to generate the granular biomass, all authors using this strategy applied simultaneously a hydraulic selection pressure strategy and high values of imposed settling velocities larger than 1 m h⁻¹ in their systems [14].

Having this in mind the novelty of the present research work is to study the possibility of generating stable denitrifying granules at low upflow velocities and low calcium concentrations in order to decrease pumping requirements for liquid recirculation and to minimize the costs related to external calcium addition. The effects of the fed nitrogen as nitrate concentration will be also evaluated to determine the limits of the operation.

2. Materials and methods

2.1. Experimental setup

Two anoxic upflow sludge blanket reactors (USB1 and USB2) with an effective volume of 2.8 and 2.3 L, respectively, were used. The reactors were provided in the upper zone with a 3-phase (gas–liquid–solid) separator. The inner diameters of the lower and upper parts of the reactors were: 5.4 cm and 11.2 cm for USB1 and 4.2 and 11.2 cm for USB2, respectively. Biogas production was measured using a liquid displacement device [19]. Feeding solution was pumped at the bottom of the reactors by means of a peristaltic pump and the effluent was removed from the top. Part of the respective effluents was recycled to each reactor together with the feeding flow in order to achieve the desirable liquid upflow velocity. Both reactors were kept inside a thermostated chamber to maintain a constant temperature of 37 ± 1 °C. The pH value was never controlled and ranged from 8.5 to 9.9.

The feeding of both reactors consisted in a synthetic solution composed by NaNO₃ and CH₃COONa·H₂O with a carbon to nitrogen (C/N) ratio of 1.4 ± 0.1 g C g N⁻¹, which is a value larger than 0.94 g C g N⁻¹, the theoretical stoichiometric amount needed for complete denitrification with acetate. Nitrate and acetate feeding solutions were freshly prepared every 1–3 days in tap water and kept in separate collapsible containers to avoid degradation. Tap water used had the following composition: 3 mg Ca²⁺ L⁻¹ and 1 mg Mg²⁺ L⁻¹ which accounts for 0.23 meq L⁻¹ (as Ca²⁺ + Mg²⁺) and can be classified as soft water according to van der Aa [20].

The synthetic feeding was supplemented with phosphate (0.16 mg KH₂PO₄ mg N⁻¹), ammonium (0.50 mg (NH₄)₂

SO₄ mg N⁻¹, 0.42 mg NH₄Cl mg N⁻¹) and micronutrients (14.11 × 10⁻² mg MgSO₄ mg N⁻¹, 11.41 × 10⁻⁶ mg CuCl₂ mg N⁻¹, 15.78 × 10⁻⁵ mg CoCl₂ mg N⁻¹, 15.78 × 10⁻⁶ mg NiCl₂ mg N⁻¹, 3.00 × 10⁻⁵ mg MnCl₂ mg N⁻¹, 3.68 × 10⁻⁵ mg Na₂MoO₄ mg N⁻¹, 15.81 × 10⁻⁴ mg FeSO₄ mg N⁻¹) in constant proportion to the nitrate concentration. The concentration of calcium (added as CaCl₂) in the feeding of USB1 was variable (Table 1) while it was maintained constant for USB2 (Table 2). The composition of this synthetic media fits to hard (60 mg Ca²⁺ L⁻¹) and extremely hard (200 mg Ca²⁺ L⁻¹) water.

The USB1 was inoculated with flocculent sludge, collected from a nitrifying/denitrifying unit of a WWTP, at an initial solids concentration of 5.5 g TSS L⁻¹ and with an ash-fraction of 38%. The USB2 was started up with 1.71 g VSSL⁻¹ of alkaliphilic denitrifying sludge (55% of ash fraction) collected from USB1 after 116 days of operation and after mechanical disintegration.

2.2. Operational strategy

The first experiment was performed in the USB1 reactor in order to optimize the upflow velocity value (ν_{up}) and calcium concentration applied to the process, necessary to obtain a stable denitrifying granular sludge. The operation was divided into different stages (Table 1) according to the different values fixed of both parameters that were progressively decreased. During Stage 1 the upflow velocity (5.5 m h⁻¹) and calcium concentration (189 mg L⁻¹) were kept high and later in Stage 2 the upflow velocity was decreased to 0.47 m h⁻¹ maintaining the calcium concentration in the previous value. In Stage 3 the Ca²⁺ concentration in the feeding was decreased from 200 to 100 mg L⁻¹ while the upflow velocity was maintained at values around 0.46 m h⁻¹. In Stages 4 and 5 both parameters were diminished, the upflow velocity to values of 0.15 and 0.12 m h⁻¹ and the calcium concentration to values of 53 and 5 mg L⁻¹ for each stage, respectively.

Following the results obtained in this experience the second reactor (USB2) was operated with a constant calcium concentration (60 mg L⁻¹) in the feeding and an upflow velocity between 0.1 and 0.2 m h⁻¹, that corresponded to the best values observed on the performance of USB1 which guarantee to maintain denitrifying granular biomass in stable conditions. The operation of this second reactor lasted 147 days. It was also divided into different stages, depending on the concentration of nitrate in the influent (Table 2), which ranged between 335 and 1000 mg NO₃⁻-N L⁻¹. These variable concentrations were applied in order to study the effects of the stepwise increase of substrate concentration in the granular denitrifying system.

2.3. Calculations

The yield coefficient of biomass from acetate was calculated by means of a carbon mass balance:

$$C_{biomass} = C_{inlet} - C_{outlet} - C_{precipitated}$$

Table 2
Operational parameters of USB2 reactor.

Period	1	2	3	4
Time (days)	0–39	40–58	59–89	90–147
HRT (h)	31.0 ± 3.8	11.5 ± 0.9	12.0 ± 1.9	13.1 ± 2.3
NO ₃ ⁻ -N (mg L ⁻¹)	333 ± 1	563 ± 75	749 ± 3	986 ± 38
CH ₃ COO ⁻ -C (mg L ⁻¹)	455 ± 16	732 ± 98	973 ± 38	1287 ± 46
<i>v</i> _{up} (m h ⁻¹)	0.08 ± 0.02	0.21 ± 0.04	0.19 ± 0.01	0.18 ± 0.01
Ca ²⁺ (mg L ⁻¹)	60	60	60	60

where C_{biomass} is the amount of carbon daily built-in biomass (g C d⁻¹); C_{inlet} and C_{outlet} is the carbon (as acetate) flow in the influent and effluent (g C d⁻¹), respectively, and $C_{\text{precipitated}}$ is the amount of carbon daily precipitated as CaCO₃ (g C L⁻¹).

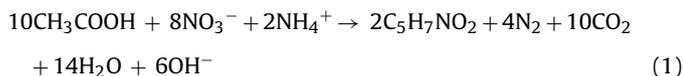
C_{inlet} and C_{outlet} values were calculated taking into account the concentration of acetate and total inorganic carbon (TIC) and the feeding media flow rate. $C_{\text{precipitated}}$ was calculated considering that calcium removed inside the reactor corresponded to that present in the formed CaCO₃. Due to the operational pH values, CO₂ flow was considered negligible.

The biomass yield coefficient (Y) was calculated according to the following equation:

$$Y = \frac{C_{\text{biomass}}}{C_{\text{inlet acetate}} - C_{\text{outlet acetate}}}$$

where $C_{\text{inlet acetate}}$ and $C_{\text{outlet acetate}}$ are the inlet and outlet acetate carbon mass loads (g C d⁻¹).

The theoretical biomass yield coefficient was considered of 0.5 g C g C⁻¹ taking into account Eq. (1).



2.4. Analytical methods

The solids concentration as total and volatile suspended solids (TSS and VSS) were determined according to the Standard Methods [21]. The calcium ion (Ca²⁺) was determined by titrimetric method using EDTA (method 3500-Ca D of the Standard Methods). The total carbon (TC) and inorganic carbon (TIC) were analyzed in a Total Carbon Analyser Shimadzu TOC-5000, while the total organic carbon (TOC) was determined as the difference between TC and TIC. The TC concentration was determined from the amount of CO₂ produced during the combustion of the sample at 680 °C, and the IC concentration was obtained from the CO₂ produced in the chemical decomposition of the sample with H₃PO₄ (25%) at room temperature. Nitrite and nitrate were measured by capillary electrophoresis using a Waters Capillary Ion Analyzer (CIA). Biogas composition (N₂, CH₄, CO₂ and N₂O) was analyzed with a gas chromatograph Hewlett-Packard 5890 series II. The stainless steel column was 2 m long with an external diameter of 1/8" and it was filled with Porapak Q (mesh 80/100). The temperatures of the injector, column and detector were 110, 35 and 110 °C, respectively. Sludge composition in C, N, O, S and H was measured based on the complete and instantaneous combustion of the sample and the determination of the gases from the combustion through a thermal conductivity detector model CHNS FISOONS EA 1108 (for C, H, N and S) and model CARLO ERBA EA 1108 (for oxygen). Settling velocity of individual granules was measured in a glass 250 mL cylinder (internal diameter of 40 mm) filled with tap water at 20 °C. Single granules were introduced in the upper zone of a cylinder and then the time required for them to settle and reach the bottom of the cylinder (distance of 30 cm) was measured with an accuracy of ±0.5 s. Settling tests were performed for each individual granule in

duplicate and the average value was determined for a total amount of 50 granules. The denitrification activity was measured by tracking the N₂ production in batch tests using an initial concentration of 100 mg NO₃⁻-N L⁻¹ and according to the method developed by Buys et al. [22].

3. Results and discussion

3.1. USB1: optimization of the upflow velocity and the calcium concentration

The USB1 reactor was operated throughout 200 days in five different stages. The denitrification efficiency remained around 100% during most of the operational period (Fig. 1a). The pH value inside the reactor ranged between 8.5 and 9.9. Under these alkalophilic conditions the denitrification process took place successfully. Nitrite was sporadically measured in the effluent only at concentrations lower than 10 mg NO₂⁻-N L⁻¹. The removed nitrogen loading rate (NLR) reached values up to around 3.5 g NO₃⁻-N L⁻¹ d⁻¹. The produced biogas was mainly composed of N₂ (>98%) with traces of CO₂ and CH₄. The fed organic carbon as acetate was completely consumed during the whole operational period.

In the first 4 days of operation the upflow velocity was fixed at 0.5 m h⁻¹ to avoid the biomass washout and then it was increased to values as high as 5.5 m h⁻¹. The application of high upflow velocities is reported as a good strategy to promote the granulation process [4,5]. In the case of anaerobic UASB reactors the upflow velocities range typically from 0.10 to 10 m h⁻¹ [23]. At lower upflow velocities the granulation process does not occur due to the lack of selection of good settling biomass. The upflow velocity (*v*_{up}) is therefore an important parameter during sludge granulation. In Stage 2, once the granules were formed the upflow velocity was decreased to values of around 0.5 m h⁻¹, in order to keep the granular sludge inside the reactor, as well as to reduce the energy consumption of the system due to liquid pumping.

The calcium concentration supplemented to the reactor feeding in Stages 1 and 2 was high (around 190 mg Ca²⁺ L⁻¹) in order to promote the granulation process. The calcium concentration, measured in the effluent in these stages, was lower than 20 mg Ca²⁺ L⁻¹ which indicates that calcium precipitation occurred. This observation correlates in this period (Stages 1–2) with the large amounts of white precipitates, presumably of CaCO₃, observed as deposits in the biomass. These formed precipitates provoked clogging problems in the influent tubes at the bottom of the reactor, which originated sporadic problems with the entrance of the feeding and as a consequence fluctuations of the NLR fed. In order to minimize this phenomenon and to reduce the cation addition the calcium concentration supplemented in the feeding was progressively decreased in the following stages from 100 mg Ca²⁺ L⁻¹ (Stage 3) to no addition (Stage 5). The calcium concentration fed in Stage 5 (5 mg Ca²⁺ L⁻¹) corresponded to the calcium concentration of the tap water used to prepare the synthetic feeding. However, in this stage, the calcium concentration measured in the effluent was higher than in the influent, due to the redissolving of the precipitated calcium in the liquid media.

Table 3
Specific denitrifying activity (37 °C) of different kinds of sludge in USB1 reactor at the end of the operation.

Type of biomass	Solids concentration (g VSS L ⁻¹)	Specific activity (g N g ⁻¹ VSS d ⁻¹)
Flocculent (top)	2.53–6.18	0.85–0.87
Granules (1 mm × 1 mm × 1 mm)	0.87 ± 0.11	0.18 ± 0.09
Granules (1.5 mm × 1.5 mm × 1.5 mm)	1.14 ± 0.08	0.19 ± 0.03
Granules (1 cm × 1 cm × 1 cm)	1.33 ± 0.14	0.22 ± 0.05
Large granules (3 mm × 1 mm × 1 mm)	1.07 ± 0.19	0.05 ± 0.02
Crushed large granules	1.05 ± 0.23	0.18 ± 0.07

The carbon balance was calculated in order to determine if the amount of calcium precipitated correlated with the amount of inorganic carbon disappeared. Calculations performed in the range of concentrations in the feeding from 200 to 60 mg Ca²⁺ L⁻¹ (Stages 1–4) indicated that the amount of carbon that was not justified from the balance corresponded to the theoretical amount necessary to precipitate the calcium removed from the liquid phase. When low calcium concentrations were used in Stage 5 no precipitation was detected.

The formation of the first granules from the inoculated sludge was observed after 24 days of operation, which was considered a fast start up of the granulation process. The upflow velocity was of 0.47 m h⁻¹ and the calcium concentration fed of 194 mg Ca²⁺ L⁻¹. Taking into account the low imposed upflow velocity the large applied calcium concentration was assumed to be the main factor for the granulation process enhancement. Liu & Sun [16] also observed that the granular sludge needed around 30 days to reach

a mean size of 150 μm in a denitrifying reactor supplemented with a high calcium concentration (100 mg Ca²⁺ L⁻¹), while without calcium addition 100 days were needed to reach the same size.

From the experimental results the biomass yield was calculated (Fig. 1b). It experienced a decrease from 0.25 g C_{biomass} g C⁻¹_{influent} corresponding to the first 90 days of operation (Stages 1–3) to 0.15 g C_{biomass} g C⁻¹_{influent} corresponding to the rest of the operational period (Stages 4–5). These values are inferior to those theoretically obtained from Eq. (1) of 0.5 g C_{biomass} g C⁻¹_{influent}. The low values of biomass yield obtained can be related to the fact that the biomass was aggregated in granules, since the effect of the granulation on the decrease of apparent biomass yield values has been already reported [24–26].

The settling velocity of the granular sludge on day 33 was of 87 m h⁻¹ for the smallest granules (mean size diameter of 0.8 mm) and 323 m h⁻¹ for the largest granules (mean size diameter of 4.5 mm). These values are much larger than the imposed upflow velocities and in the range of values obtained in previous works [15]. These high values of the biomass settling velocity implicate that the granules cannot easily be washed out from the reactor. Small particles were also present in the reactor (Fig. 1c), but they were washed out with the effluent, where solid concentrations between 40 and 100 mg TSS L⁻¹ were measured. The average size of the granules increased throughout time and the largest granules (diameter of 10 mm) were observed at the end of Stage 3.

The low concentrations of solids present in the effluent during most of the operational period allowed for the accumulation of biomass inside the reactor at concentrations up to 9.5 g VSS L⁻¹ with an ash content as high as 80% at the end of Stage 2. The high ash content of the granular biomass during this period was related to the high added calcium concentration [16], which provoked the formation of precipitates in the core of the granules. This content significantly decrease being at the end of the operational time of around 45%.

Stratification of the granular biomass was observed in height inside the reactor depending on the granules size. At the end of the experimental period, some very large granules (diameter up to several mm) were found at the bottom of the reactor, which were resistant to mild squeezing. The activity of these granules was compared to that corresponding to granules of different sizes and to flocculent sludge collected from the reactor (Table 3). The flocculent sludge from the top of the reactor had a very high specific activity, 0.85–0.87 g N g⁻¹ VSS d⁻¹, possibly caused by the fact that active biomass share in the flocculent biomass is higher than inside the granules. This activity value was similar to those obtained by Jenicek et al. [27], who measured specific activities in granular denitrifying sludge of 0.72 g N g⁻¹ VSS d⁻¹ at 35 °C and using glucose as substrate. The low specific activity of the large granules, 0.046 g N g⁻¹ VSS d⁻¹, was presumably caused by mass transfer limitation. After they were crushed the measured activity was significantly higher and had a value similar to that of the smaller granules (around 0.2 g N g⁻¹ VSS d⁻¹). Among the small granules there was little difference in terms of specific activity.

Results obtained from the operation of USB1 showed that although the formation of denitrifying granules occurred at high calcium concentrations and moderate values of the imposed upflow

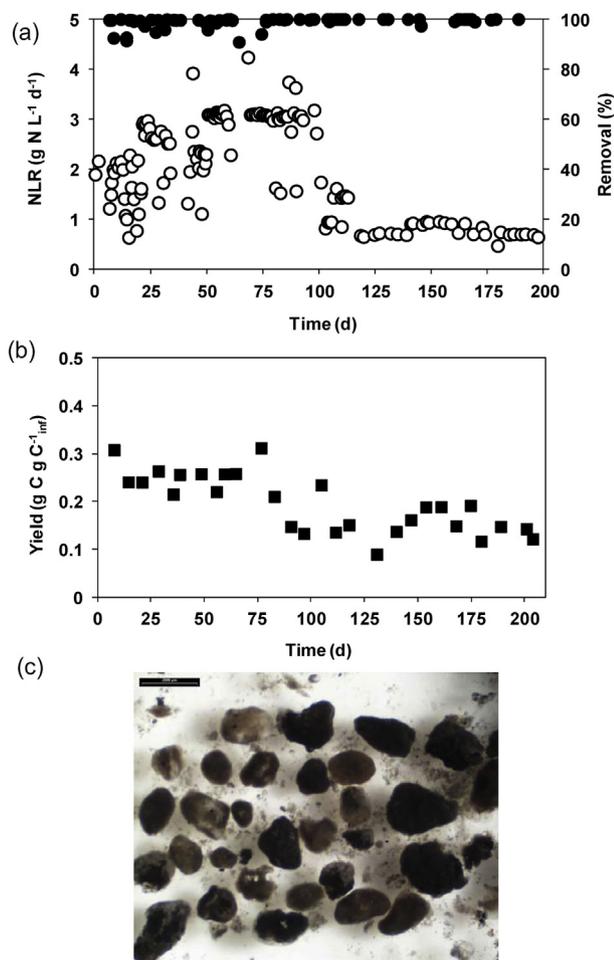


Fig. 1. (a) Operational performance of USB1: nitrogen loading rate fed (NLR) (○) and nitrogen removal efficiency (●); (b) biomass yield coefficient (■) calculated for USB1; (c) image of the anoxic denitrifying granules from USB1 on day 33.

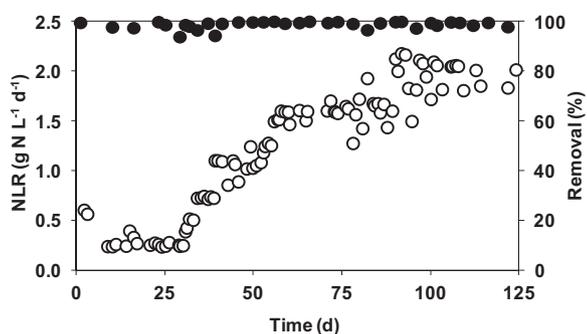


Fig. 2. Operational performance of USB2: nitrogen loading rate fed (NLR) (○) and nitrogen removal efficiency (●).

velocity (Stage 2), the structure of the formed granules was possibly maintained at low values of calcium concentrations and upflow velocity (Stage 4). These conditions are more advantageous when full scale systems are to be operated because less reagents costs and pumping energy consumption are needed. For this reason in the USB2 operation the possibility to form denitrifying granules in these latter conditions was researched.

3.2. USB2 reactor operation: effect of substrate concentration

A second reactor (USB2) was operated during 147 days in four different stages. USB2 was operated at a fixed calcium concentration of $60 \text{ mg Ca}^{+2} \text{ L}^{-1}$. The upflow velocity was maintained at 0.08 m h^{-1} during the start-up (Stage 1) to avoid the wash out of the inoculated biomass. Then the upflow velocity was kept constant at around 0.19 m h^{-1} (Table 2), according to the best values found for this parameter in the operation of USB1 (Stage 4). The nitrate concentration in the influent ranged from 335 (Stage 1) to $1000 \text{ mg NO}_3^- \text{ N L}^{-1}$ (Stage 4). The effects of the stepwise increase of substrate concentration in the feeding to the system were studied.

During the operation of USB2 the nitrate removal efficiency was usually higher than 99%, even at the high values of $1000 \text{ mg NO}_3^- \text{ N L}^{-1}$ in the influent. The nitrogen loading rate treated reached values of $2 \text{ g N L}^{-1} \text{ d}^{-1}$ (Fig. 2). The calculated average biomass yield was of $0.15 \text{ g C g C}^{-1} \text{ influent}$. The C/N ratio was lowered from 1.4 to 1.3 g C g N^{-1} after 36 days of operation. The stepwise increase of nitrate concentration in the influent caused visible changes in the sludge bed, namely from black to light-brown. Clear granulation was not visible in USB2 until day 91 of operation, just before the influent nitrate concentration was raised up to $1000 \text{ mg NO}_3^- \text{ N L}^{-1}$. The biomass concentration in this period was of $3.72 \text{ g VSS L}^{-1}$ with an ash fraction of 68%, higher than the value of the inoculated sludge. These granules were resistant to mild squeezing.

This denitrifying granular sludge system operated stable at low values of upflow velocity (0.19 m h^{-1}) and calcium concentration ($60 \text{ mg Ca}^{+2} \text{ L}^{-1}$), which means savings in energy requirements and substrate addition. Furthermore the application of high values of nitrogen loading rates ($2 \text{ g N L}^{-1} \text{ d}^{-1}$) favoured the granulation process. Therefore the system developed in this study could be applied even in the case of wastewater hard or extremely hard, without the necessity to add extra calcium. This is the case of several zones in the world like more than the 85% of the USA, the prairie provinces in Canada; in Europe: in England, most part of Ireland, southern half of Portugal, Mediterranean region of Spain, etc. Therefore in all of these regions the application of the studied denitrification system would be feasible without reagents addition.

4. Conclusions

The present work shows the feasibility of growing denitrifying biomass in the form of granules using moderate calcium concentrations ($60 \text{ mg Ca}^{+2} \text{ L}^{-1}$) and low values of upflow velocity (0.19 m h^{-1}) without affecting the nitrate removal efficiency. When these conditions are applied minimum nitrate concentrations of $1000 \text{ mg NO}_3^- \text{ N L}^{-1}$ are required to favour the formation and stability of denitrifying granular biomass.

The addition of calcium at moderate concentrations promoted the granulation process in the denitrifying system, because the ash fraction of the biomass was high and this fact favoured the formation of well settling biomass granules.

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