sciendo

Substrate inhibition and pH effect on denitritation with granular biomass

Stanislava Kecskésová, Zuzana Imreová, Bibiána Kožárová, Ján Derco, Miloslav Drtil

Department of Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinskeho 9, 812 37 Bratislava, Slovak Republic
stanislava.vlckova@stuba.sk

Abstract: Undissociated HNO_2 (up to 2 mg dm^{-3}) was confirmed as substrate inhibitor for granular biomass from a denitritation upflow sludge bed reactor used for biological removal of nitrite. On the contrary, total nitrite nitrogen ($N\text{-}NO_2$ up to 500 mg dm^{-3}) and methanol (COD up to 2000 mg dm^{-3}) were not proven to be inhibitors. pH also affected the denitritation efficiency (optimal pH was 5.9). Reduction of HNO_2 concentration in the reactor by effluent recycling is recommended.

Keywords: denitritation, granulated biomass, methanol, pH, substrate inhibition, undissociated HNO_2

Introduction

Denitritation is a reaction in which nitrite nitrogen ($N\text{-}NO_2$) is biologically removed to gaseous N_2. This process can be used for the treatment of various wastewater types, e.g. industrial wastewater containing $N\text{-}NO_2$ or wastewater after partial nitrification (nitritation), where ammonium nitrogen ($N\text{-}NH_4$) is oxidized only to $N\text{-}NO_2$ with inhibited nitrite oxidizing bacteria (NOB) (Hellinga et al., 1998; Jenicek et al., 2004; Svehla et al., 2014). Denitritation can be realized in various reactor types, including activated sludge reactors with suspended biomass, biofilters with fixed bed biomass, and specific reactors, such as the upflow sludge bed (USB) reactors (Fig. 1) with granular biomass (Galbová et al., 2010; Pagáčová et al., 2009; Pagáčová et al., 2010). In USB reactors, biomass is exposed to a substrate

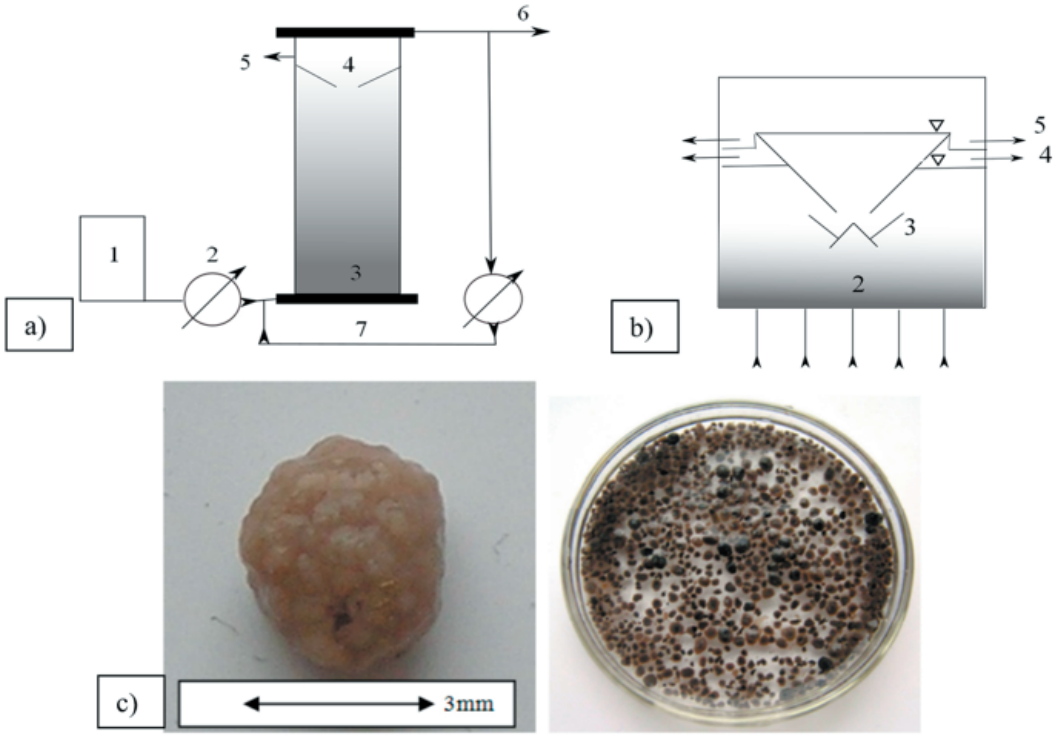

Fig. 1. a) Denitritation USB reactor: 1) substrate (wastewater with $N\text{-}NO_2$ and organic compound e.g. methanol); 2) influent pump; 3) sludge bed with granular biomass; 4) g/l/s separator; 5) denitritation gas (mainly N_2); 6) treated wastewater effluent; 7) recycle of treated wastewater. b) Details of g-l-s separator: 1) influent; 2) sludge bed; 3) separator; 4) denitritation gas; 5) effluent. c) granular denitritation biomass.

concentration gradient, with higher concentrations of substrate (N-NO_2 plus organic compound, e.g. methanol) in the bottom part of the reactor.

N-NO_2 is commonly described as a possible inhibitor of biological processes. However, most attention has been focused on the impact on nitrification (either in the dissociated form of NO_2^- (Buday et al., 1999) or the undissociated form of HNO_2 (Anthonisen et al., 1976; Park et al., 2010)). Here, also methanol was considered as a potential alternative inhibitor of biological processes.

Little information on the N-NO_2 or methanol inhibitory effect (substrate inhibition) on denitritation granular biomass is available. Only the effect of N-NO_2 on denitrification has been reported. According to Bilanovic et al. (1999) and Chen et al. (1991), concentrations of 100—200 mg dm^{-3} and 2000 mg dm^{-3} of total N-NO_2, respectively, did not inhibit denitrification (measured in experiments conducted with adapted biomass). Beccari et al. (1983) and Versefeld et al. (1977) observed denitrification inhibition at 10—150 mg dm^{-3} of total N-NO_2 with non-adapted biomass. According to Abeling et al. (1992), denitrification was inhibited by undissociated HNO_2, with an inhibitory limit of 0.13 mg dm^{-3} of HNO_2. Chen et al. (1991) determined that adapted denitrification bacteria can tolerate 0.02—0.16 mg dm^{-3} of HNO_2.

Potential risk of denitritation substrate inhibition was described in Babjaková et al. (2013). In USB reactors with granular biomass, the efficiency of denitritation with methanol as an external organic substrate was significantly reduced after the interruption of treated water recycle (Fig. 1a, stream 7). One possible explanation is that the inflow was not diluted with the recycle. This dilution reduced the negative influence of the substrate concentration on the denitritation biomass.

For this reason, a series of inhibition tests with granular denitritation biomass were performed. The effects of total N-NO_2 (dissociated plus undissociated form), undissociated HNO_2, methanol concentration, and pH were monitored with the aim to determine substrate inhibition on granular biomass. Methanol is a typical organic substrate used for denitritation or denitrification because it is easily biodegradable, relatively cheap and contains carbon with low oxidation number (–II). Compact granules with diameters from 1 to 3 mm (Fig. 1c) are generally considered to be more resistant to external influence because, compared to ambient water, relatively large volumes with different conditions are likely to occur inside the granules. For example, OH^- and HCO_3^- ions produced by denitritation can increase pH inside the granules (Drtil et al., 1995) or the substrate concentrations might be lower due to lower substrate diffusion into the granules.

In cases where that substrate is not an inhibitor, kinetics of substrate removal can be described by the Monod equation (Eq. 1, Fig. 2a). From this equation it follows that the removal rate increases with the increasing substrate concentration until the maximum removal rate is reached:

$$r_X = r_{X,\max} \cdot S/(K_S + S) \quad (1)$$

where r_x is the specific substrate removal rate (mg g^{-1} h^{-1}); mg of substrate per gram of biomass), $r_{x,\max}$ is the specific maximum substrate removal rate (mg g^{-1} h^{-1}), S is the substrate concentration (mg dm^{-3}) and K_S is the saturation constant (concentration of substrate S with removal rate equal to $r_{x,\max}/2$).

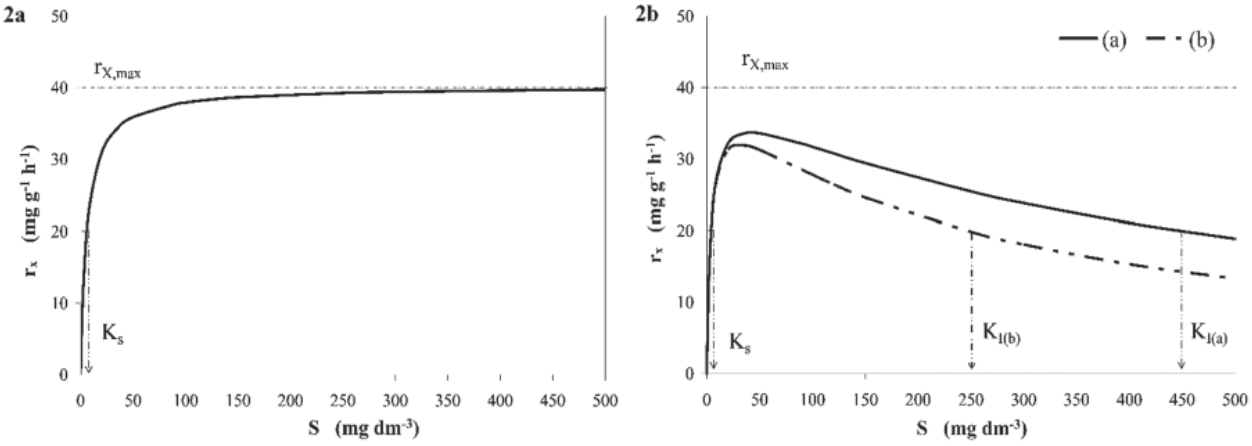

Fig. 2. Typical dependence of substrate kinetics according to the Monod equation (Fig. 2a, Eq. 1. No substrate inhibition: $r_{X,\max}$ = 40 mg g^{-1} h^{-1}, K_S = 10 mg dm^{-3}) and the Haldane equation (Figure 2b, Eq. 2. Substrate exhibits inhibition. Full line: $r_{X,\max}$ = 40 mg g^{-1} h^{-1}, K_S = 10 mg dm^{-3}, $K_{I(a)}$ = 450 mg dm^{-3}, intermittent line: $r_{X,\max}$ = 40 mg g^{-1} h^{-1}, K_S = 10 mg dm^{-3}, $K_{I(b)}$ = 250 mg dm^{-3}).

If the substrate is an inhibitor, r_x values are lower due to inhibition and r_x begins to decrease from a certain *S*. The Haldane equation is most commonly used to describe the substrate inhibition kinetics (Eq. 2, Fig. 2b) (Carrera et al., 2004):

$$r_X = r_{X,\,max} \cdot S/(K_S + S + S^2/K_I) \quad (2)$$

where r_x is the specific rate of substrate removal (mg g^{-1} h^{-1}), $r_{x,\,max}$ is the specific maximum substrate removal rate (mg g^{-1} h^{-1}), *S* is the substrate concentration (mg dm^{-3}), K_S is the saturation constant (concentration of substrate *S* with removal rate equal to $r_{x,\,max}/2$) and K_I is the inhibitory constant. The substrate inhibition is inversely correlated with the inhibitory constant K_I. Figure 2b shows that inhibition is more intensive if $K_{I(b)}$ (intermittent line) is lower than $K_{I(a)}$ (full line).

Materials and methods

Denitritation inhibition tests were realized with granular biomass samples taken from a laboratory USB reactor (Fig. 1) already adapted to denitritation with methanol (wastewater containing 500 mg dm^{-3} of $N\text{-}NO_2$ and 1500 mg dm^{-3} of $COD_{methanol}$ was treated with denitritation efficiency higher than 95 % at the loading of 2 kg $N\text{-}NO_2$ m^{-3} d^{-1} and the recycle ratio of 1). The biomass sample was mixed half a day before the test to reach endogenous conditions without exogenous substrate and was then repeatedly washed with drinking water without O_2 (water after nitrogen sparging). Initial biomass concentration in the test was 8 g dm^{-3} and its change during the experiment was negligible.

The denitritation tests were performed similarly to the batch kinetic tests. PO_4-P (KH_2PO_4) was added at the start of each test so that the weight ratio $COD_{methanol}$: P was 100 : 1, while pH was adjusted to the desired value with 1 M and 0.1 M HCl or NaOH. Subsequently, the mixture was sparged with nitrogen for 30 minutes to completely remove dissolved oxygen, and specific amounts of $N\text{-}NO_2$ and methanol were added to this mixture. Concentrations of $N\text{-}NO_2$ in the tests were in the range from 5 to 500 mg dm^{-3}, and those of $COD_{methanol}$ were in the range from 20 to 2000 mg dm^{-3}. The pH range in the tests was from 4.6 to 8.5. The relevant concentrations of undissociated HNO_2 were calculated according to Anthonisen et al. (1976) from the concentration of $N\text{-}NO_2$ and pH (Eqs. 3 and 4). Concentration range of HNO_2 was from 0.0002 to 2 mg dm^{-3}.

$$HNO_2 = (47/14) \cdot N\text{-}NO_2/(K_a \cdot 10^{pH}) \quad (3)$$

where HNO_2 and $N\text{-}NO_2$ are the concentrations in mg dm^{-3}, and K_a is the ionization constant of HNO_2.

$$K_a = e^{(-2300/T)} \quad (4)$$

where T is the temperature in Kelvin.

A total of 21 denitritation tests were performed. The biomass was mechanically stirred in 300 mL closed flasks during the tests. The stirrer speed was up to 100 min^{-1} (sufficient for mixing of granular biomass without its mechanical destruction). With regard to the production of OH^- in the denitritation, pH was continuously adjusted to the initial value by additions of 0.1 and 0.01 M HCl.

Activity of denitritation biomass was evaluated from the specific denitritation rates, r_X (mg of $N\text{-}NO_2$ per gram of biomass per hour) measured at various concentrations of methanol, $N\text{-}NO_2$, and at different pH values. The rates were calculated from the linear part of the $N\text{-}NO_2$ concentration decrease curve. Samples with the volume of 5 mL were taken in 10- to 30-min intervals, they were filtered immediately, and the $N\text{-}NO_2$ concentration was determined by a spectrophotometric method (APHA, 2005). Temperature in the tests was between 18—21 °C.

Results and discussion

Specific denitritation rates, r_X, are summarized in Figures 3—5. Dependencies in these figures were compared with the Haldane kinetics model (their correspondence with Eq. 2 and Fig. 2b). The correlation coefficients show the compliance with this equation (the closer the correlation coefficient is to 1, the higher the conformity with Eq. 2). Similarly, the substrate inhibition of nitrification was evaluated in Buday et al. (1999) and Carrera et al. (2004).

Comparing Figures 3 and 4 with Figure 2b, it is evident that $N\text{-}NO_2$ and methanol do not show any dependence, which confirms substrate inhibition. This conclusion was also confirmed by the very low calculated correlation coefficient values. Correlation coefficient for $N\text{-}NO_2$ was only 0.13 (other parameters from Eq. 2 were: $r_{x,\,max}$ = 2.1 mg g^{-1} h^{-1}, K_S = 5 mg dm^{-3}, K_I = 1950 mg dm^{-3}) and that for COD was 0.11 ($r_{x,\,max}$ = 2.5 mg g^{-1} h^{-1}; K_S = 76 mg dm^{-3}; K_I = 2670 mg dm^{-3}).

Substrate inhibition, which correlates with the Haldane kinetics and Eq. 2, was observed only with undissociated HNO_2 (Fig. 5). In this case, denitritation activity increased with the increasing HNO_2 concentration; reaching a maximum at the concentration of 0.01—0.1 mg dm^{-3}; then it begun to decrease with the increasing HNO_2 concentration. The calculated correlation coefficient was 0.79, which confirms the dependence according to Eq 2. Therefore, in Figure 5, experimentally measured values were compared with the dependence calculated according to Eq. 2 (intermittent

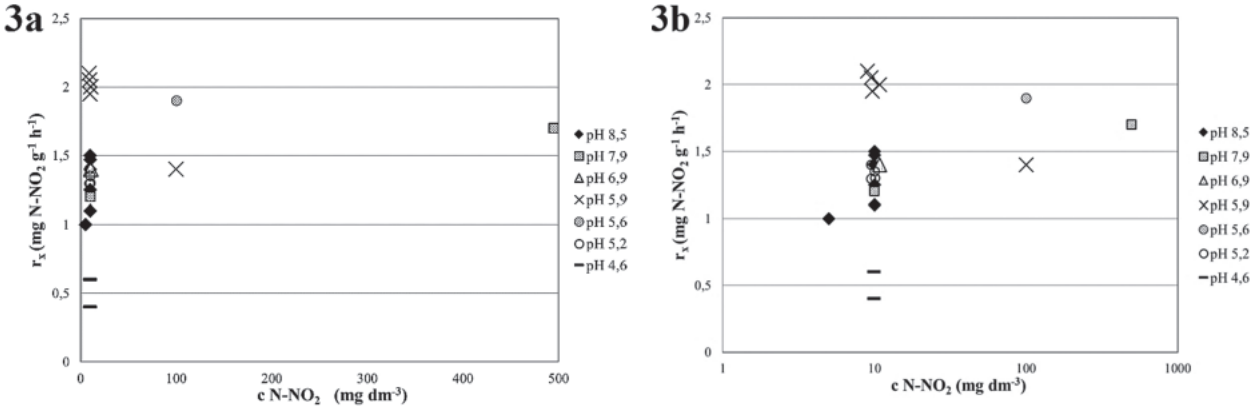

Fig. 3. Effect of N-NO_2 concentration on denitritation rate, r_X (3a). Graph with logarithmic scale (3b) is introduced to better distinguish r_x values at low N-NO_2 concentrations.

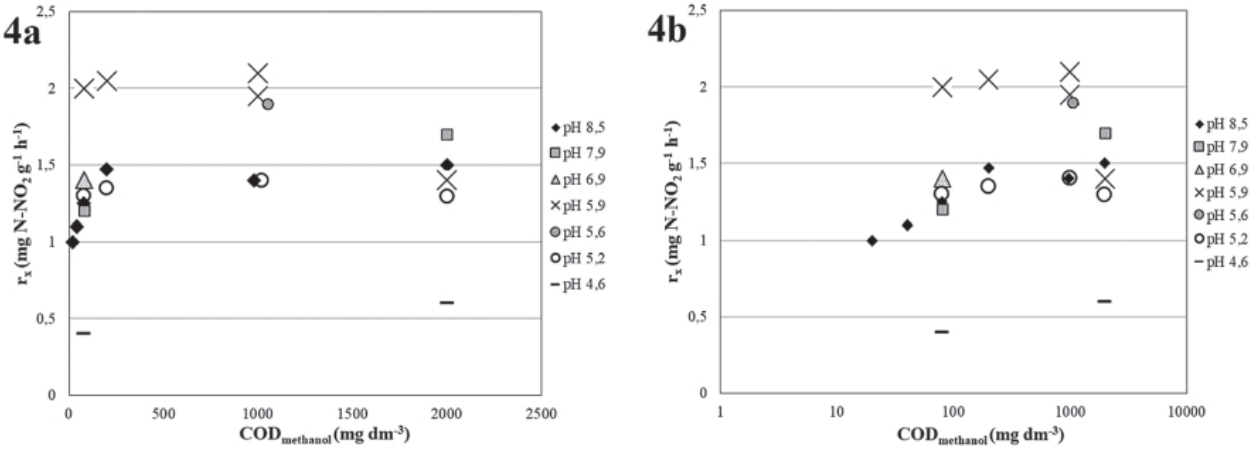

Fig. 4. Effect of methanol concentration on denitritation rate, r_X (4a). Graph with logarithmic scale (4b) is introduced to better distinguish r_x values.

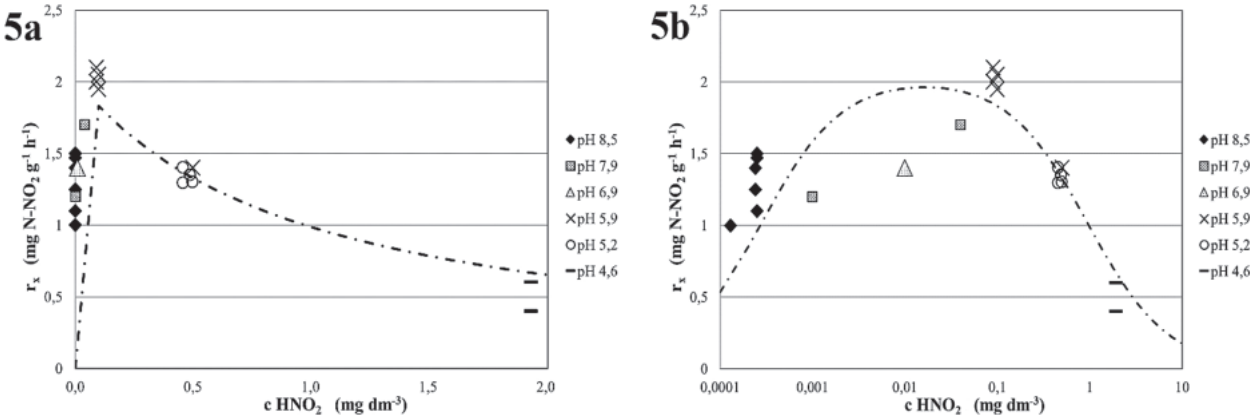

Fig. 5. Effect of HNO_2 concentration on denitritation rate, r_X (5a). Graph with logarithmic scale (5b) is introduced to better distinguish r_x values at low HNO_2 concentrations.

curve with calculated $r_{x,\ max}$ = 2.03 mg g^{-1} h^{-1}, K_S = 0.00028 mg dm^{-3}, K_I = 0.95 mg dm^{-3}, correlation coefficient = 0.79). Similar inhibition limit for denitrification (0.13 mg dm^{-3} HNO_2) was measured also by Abeling et al. (1992). The range of HNO_2 concentrations still tolerated by denitritation granular biomass (up to 1.9 mg dm^{-3} according to Fig. 5) is wider than the range reported by Chen et al. (1991) (up to 0.16 mg dm^{-3} HNO_2). However, the decrease of denitritation activity with the increasing HNO_2 concentration is significant (35 % inhibition compared to the maximum r_X at 0.5 mg dm^{-3} of HNO_2 and 75 % at 1.9 mg dm^{-3} of HNO_2; Fig. 5).

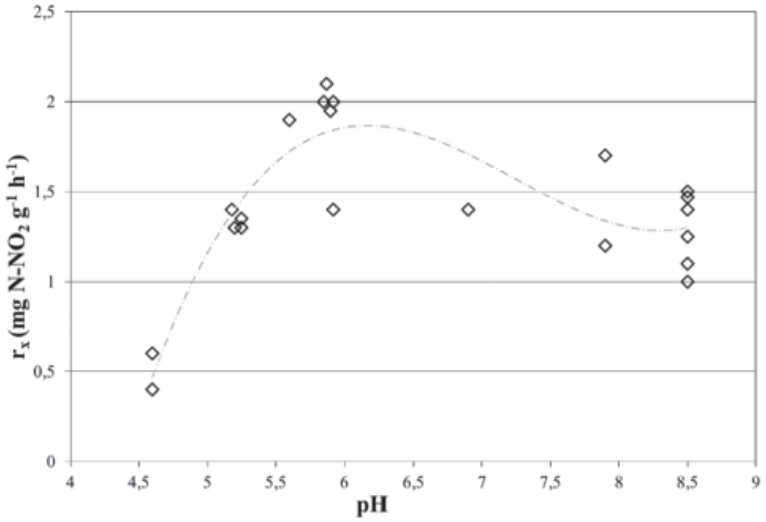

Fig. 6. Effect of pH on denitritation rates, r_X.

Inhibition found for undissociated HNO_2 explains the reduced denitritation efficiency in USB reactors without effluent recycle (Fig. 1a, without stream 7) Babjaková et al. (2013). Recycle dilutes the influent and reduces the HNO_2 concentration in the reactor, especially in the bottom part with the sludge bed. Therefore, effluent recycle is recommended for any upflow reactor with substrate concentration gradient.

The dependence of r_X and pH (Fig. 6) summarized from all tests shows that pH 5.9 is optimal for denitritation. The dependence of r_x and pH in Figure 6 is also highlighted by a trend line (intermittent curve with a calculated correlation coefficient of 0.93).

It is interesting that denitritation was observed also at very low pH = 4.6. This can be explained by higher internal pH in big and compact granules with diameters of up to 3 mm (Fig. 1c) compared to ambient water (due to denitritation producing OH^- and subsequently HCO_3^- ions, Drtil et al., 1995). Such internal conditions with pH different from ambient wastewater was detected also in the experiments with biomass fixed in polyurethane cubes used as biomass carrier (cubes with the size from 0,75 cm up to 1,5 cm, with fixed biomass concentration of up to 13.3 g dm^{-3}, Drtil et al., 1994). Simultaneous denitrification in deeper parts of the biomass carriers improved nitrification at acid pH of 4.2—6.2.

Figures 3—6 also illustrate the relatively low denitritation rates of granular biomass from a USB reactor in the range of 0.4 to 2.2 mg $N\text{-}NO_2\ g^{-1}\ h^{-1}$. Similar rates (0.4 mg $N\text{-}NO_2\ g^{-1}\ h^{-1}$) were obtained by Abeling et al. (1992). However, much higher values, up to 7.7 mg $N\text{-}NO_2\ g^{-1}\ h^{-1}$, were reported by Chen et al. (1991). Re-calculating the rates in Figures 3—6 to oxygen equivalents (1 mg of $N\text{-}NO_2$ is equivalent to 1.7 mg of O_2; 1 mole of nitrogen accepts 3 electrons, and 1 mole of oxygen accepts 2 electrons) provides rates equivalent to respiratory rates of 0.7—3.4 mg $O_2\ g^{-1}\ h^{-1}$. Such values are relatively low considering they represent the total rate including endogenous and exogenous denitritation with methanol (e.g., compare with Cech et al. (1984)). However, this fact does not handicap granular biomass in the USB reactor; due to excellent sedimentation properties of the granules, it is possible to maintain extremely high biomass concentrations in the USB reactor (up to 40—50 g dm^{-3}, Pagacova et al., 2010).

Conclusions

A series of inhibition tests have shown the effect of substrate inhibition on adapted granular denitritation biomass only for undissociated HNO_2. Total $N\text{-}NO_2$ and methanol were not confirmed as relevant substrate inhibitors in the tested concentration range. Optimal denitritation pH was 5.9. Denitritation rates higher than 0.4 mg $N\text{-}NO_2\ g^{-1}\ h^{-1}$ (with maximum of 2.2 mg $N\text{-}NO_2\ g^{-1}\ h^{-1}$) were measured with granular biomass at concentrations up to 500 mg dm^{-3} of $N\text{-}NO_2$, 2000 mg dm^{-3} of $COD_{methanol}$ and 2 mg dm^{-3} of undissociated HNO_2.

Acknowledgement

This research is a part of the project VEGA 1/0772/16 and was supported by the Scientific Grant Agency of the Slovak Republic. The Authors are grateful to American Manuscript Editors for English revision.

References

Abeling U, Seyfried CF (1992) Water Science Technology 26/5—6: 1007—1015.

Anthonisen AC, Loehr RC, Prakasam TB, Srinath EG (1976) Journal Water Pollution Control Federation 48/5: 835—850.

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington, DC.

Babjaková L, Drtil M, Imreová Z, Jonatová I (2013) Vodní hospodářství 63/2: 36—41.

Beccari M, Passino RR, Tandoi V (1983) Journal of Water Pollution Control Federation 55/1: 58—64.

Bilanovic D, Battistoni P, Cecchi F, Pavan P, Mata-Alvarez J (1999) Water Research 33/15: 3311—3320.

Buday J, Drtil M, Hutňan M, Derco J (1999) Chemical Papers 53/6: 379—383.

Carrera J, Jubany I, Carvallo L, Chamy R, Lafuente J (2004) Process Biochemistry 39/9: 1159—1165.

Cech JS, Chudoba J, Grau P (1985) Water Science Technology 17/2—3: 259—272.

Chen SK, Juaw CK, Cheng SS (1991) Water Science Technology 23/7—9: 1417—1425.

Drtil M, Németh P, Kucman K, Bodík I, Kašperek V (1995) Water Research 29/5: 1353—1360.

Drtil M, Bodík I, Derco J, Hutňan M (1994) Nutrient Removal from Wastewaters: 103—108.

Galbová K, Pagáčová P, Drtil M, Jonatová I (2010) Chemical Papers 64/2: 132—138.

Hellinga C, Schellen AAJC, Mulder JW, van Loosdrecht MCM, Heijnen JJ (1998) Water Science Technology 37/9: 135—142.

Jenicek P, Svehla P, Zabranska J, Dohanyos M (2004) Water Science Technology 49/5—6: 73—79.

Pagáčová P, Galbová K, Drtil M, Jonatová I (2009a) Bioresource Technology 101/1: 150—156.

Pagáčová P, Drtil M, Galbová K (2009b) Chemical Papers 63/2: 125—130.

Park S, Bae W, Rittmann BE (2010) Environmental Science Technology 44/1: 335—342.

Svehla P, Bartacek J, Pacek L, Hrnciarova H, Radechovsky J, Hanc A, Jenicek P (2014) Chemical Papers 68/7: 871—878.

Versefeld Van HW, Meijer EM, Stouthamer AH (1977) Archives of Microbiology 112: 17—23.