The possibility of using encapsulated nitrifiers for treatment of reject water coming from anaerobic digestion

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ABSTRACT

Large wastewater treatment plants have to deal not only with the influent wastewater, but also with the highly concentrated reject water coming from anaerobic digestion. The aim of this work was to verify the suitability of using encapsulated nitrifiers in polyvinyl alcohol carrier (so called Lentikats Biocatalyst) at temperatures between 5 and 30 °C. For laboratory nitrification batch tests synthetic wastewater with ammonia nitrogen (Namon) concentration 10–800 mg L^{-1} was used. The system has been proved to operate at the temperature of 10 °C, but not at 5 °C. It was found that the highest specific nitrification rates were observed at 30 °C and with ammonia nitrogen concentrations above 250 mg L^{-1}, which means that separate treatment of reject water by using encapsulated biomass seems to be an effective tool for wastewater treatment plant optimization.

Key words | ammonia nitrogen, encapsulation, immobilization, Lentikats Biocatalyst, nitrification, nutrient removal, reject water, sludge liquor

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INTRODUCTION

Large wastewater treatment plants containing anaerobic sludge treatment have to deal not only with the influent wastewater, but also with the highly concentrated reject water coming from anaerobic digestion of sludge (Appels *et al.* 2008). Various wastewater treatment plants have difficulties to achieve effluent nutrient concentration limits and separate treatment of reject water can be an effective tool for treatment plant optimization (Ladiges *et al.* 2000).

Many intensive methods of high rate ammonia oxidation from highly concentrated reject water already exist, such as nitrification stabilization by using foam blocs (Pickin & Saunders 1994), nitritation of reject water by activated sludge (Gustavsson *et al.* 2008) or advanced nitrogen elimination based on the anammox process (Van Dongen *et al.* 2001).

Application of encapsulated biomass is also one of the intensive techniques of nitrogen removal. The use of the encapsulated biomass brings many advantages, e.g. the intensification of the process connected with reactor-volume savings and decreasing of the reaction time, higher resistance of the bacteria against temperature, substrate concentration or toxic shocks (Sievers *et al.* 2003a; Schlieker & Vorlop 2006). The main advantage of biomass encapsulation is the composition of the pellet biocoenosis, i.e. encapsulation of pure or mixed cultures of microorganisms, e.g., pure cultures or mixture of ammonia oxidizing and nitrite oxidizing nitrifiers (Sievers *et al.* 2003b). As the pore size varies within micrometres and the seeding sludge fills most of the volume of pores during the cultivation period, it is supposed that the bacterial biocoenosis purely comprises the species embedded during the encapsulation process.

The encapsulated nitrifiers could find application for common complete nitrification of reject water coming from anaerobic digestion as well as for partial nitrification. This nitrification step could be followed by a denitrification step, which can take place in a separate tank or can occur in the primary settlement tank or activated sludge tank. Alternatively, nitritation can be followed by the anammox process performed in a separate tank.

The aim of this work was to verify the suitability of using encapsulated nitrifiers at different temperatures and at different initial ammonia concentrations.

MATERIALS AND METHODS

Microorganisms and immobilization method

Pellets used during the experiments contained a mixture of nitrification bacteria *Nitrosomonas europaea* and

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Nitrobacter winogradskyi. The immobilization process was carried out by the company LentiKat's (Prague, Czech Republic). Lens-shaped pellets, so called Lentikat's Biocatalyst, with a diameter of 3–4 mm and thickness of 200–300 μm were produced by mixing of dissolved polyvinyl alcohol (PVA) with small amount of polyethylene glycol and with bacterial suspension. This mixture was dropped on a suitable surface and dried by airflow at temperature gradient 40–30 °C. During the drying, the polymer gelation occurs and the porous structure is created. Then, the pellets were stabilized by solution of sodium sulphate in order to toughen the PVA structure and remove polyethylene glycol from PVA structure (Novák *et al*. 2004). PVA MOWIOL 28-99 (Kuraray America, Inc.) with degree of saponification 99% and relative molecular mass 145,000 g mol^{-1} was used for immobilization.

These pellets were cultivated after production at the temperature 30 °C for 1 month in order to increase the number of bacteria inside the pellets and thus to prepare pellets ready for use. The cultivation was performed in double-coated 1 L reactors connected to thermostat and by using synthetic wastewater containing 2,300 mg L^{-1} KH_2PO_4, 2,900 mg L^{-1} $K_2HPO_4 \cdot 2\ H_2O$, 500 mg L^{-1} $NaHCO_3$ with concentrations of ammonia nitrogen (Namon) (as $(NH_4)_2SO_4$) 400 mg L^{-1}. The reactor was operated as sequencing batch reactor (SBR) with cycle length 24 h.

Nitrification activity

The specific nitrification rates were determined experimentally by laboratory kinetic tests.

The $N\text{-}NO_3$, $N\text{-}NO_2$ and Namon concentrations were measured regularly by spectrophotometric methods *Standard Methods for the Examination of Water and Wastewater* (APHA 1998). The amount of encapsulated biomass used in tests was 100 g of wet pellets in 800 mL of synthetic wastewater as described above, with the exception of Namon: NH_4Cl was used as source of Namon and Namon concentrations varied from 10 to 800 mg L^{-1}. During the set of tests the temperature varied from 10 to 30 °C (10, 17, 25, 30 °C); also a few tests at 5 °C were done. No experiment at 10 °C and with initial ammonia nitrogen concentration 800 mg L^{-1} was performed.

During the tests the mixture was stirred continuously by magnetic stirrers and the oxygen concentration was kept above 4 mg L^{-1}. The pH value was kept in range 6.9–7.2 by addition of 0.2 M HCl or 1% Na_2CO_3.

The specific nitrification rates were calculated as the slope of the dependence of the $N\text{-}NO_3$ concentration on the time multiplied by the water volume and divided by the weight of the pellets used in the test.

Each kinetic test was preceded by an adaptation period in order to reach steady-state operation of SBR and to avoid errors caused by growth or inhibition of biomass. The SBR was being operated at desired temperature and Namon initial concentration for three to ten 24 h cycles until it reached the steady-state. This state was controlled by decrease of Namon and increase of $N\text{-}NO_3^-$ concentration in time. Design and operation of SBR as well as synthetic water composition was the same as during kinetic tests. After reaching the steady-state, kinetic test was performed. The adaptation proceeded from low ammonia concentration to high and from high temperature to low temperature. After each kinetic experiment with initial concentration of 800 mg L^{-1} Namon another kinetic test with 10 mg L^{-1} Namon was performed at the same temperature in order to exclude possible growth of biomass in the pellets. These two measured values of specific nitrification rate at 10 mg L^{-1} Namon never differed by more than 5–10%.

The effect of substrate concentration on specific nitrification rate can be expressed by Monod kinetic expression (1) (Sedlak 1991):

$$r = r_{\max} \frac{C_{\text{Namon}}}{K_s + C_{\text{Namon}}} \quad (1)$$

where r is specific nitrification rate, $r_{\max}$ maximal specific nitrification rate, C_{Namon} concentration of ammonia nitrogen and K_s half saturation coefficient.

Half saturation coefficients in Equation (1) were calculated using software STATISTICA 8 (StatSoft, USA).

Temperature coefficient estimation

The temperature dependence of nitrification rate can be described by using an Arrhenius-type Equation (2) (Orhon *et al*. 2000; Sperling 2007):

$$r_{T_1} = r_{T_2} \cdot \theta^{(T_1 - T_2)} \quad (2)$$

where r_{Ti} are specific nitrification rates at the temperature T_i, θ temperature coefficient and T temperature.

The temperature coefficients in Equation (2) were calculated using software STATISTICA 8 (StatSoft, USA).

Protein assay

The pellet pre-treatment was based on previous work (Chen *et al*. 1998; Song *et al*. 2005): 0.25 g of pellets (or 0.25 mL of

cell suspension in case of analysis of organisms before immobilization), 3 mL of 10% sodium dodecyl sulphate and 8 ml of deionized water was put into a 30 mL beaker. The pellets were disintegrated by ultrasonic treatment by UD Ecoson for 5 min; the specific ultrasonic energy was 67,200 kJ kg^{-1} of pellets. The mixture was centrifuged at 9,000 rpm, corresponding to 8,422 *g*, for 10 min to separate proteins and cell debris. Thereafter, the protein content was determined in the supernatant.

Cell protein was measured using the modified Lowry method (Lowry *et al*. 1951). The standard reagent consisted of 1 volume of reagent A, 1 volume of reagent B and 100 volumes of reagent C. Reagent A contained 2 g of potassium tartrate and deionized water to achieve a final volume of 100 mL. Reagent B comprised 1 g $CuSO_4 \cdot 5H_2O$ and deionized water to achieve a final volume of 100 mL. Reagent C composition was as follows: 2 g NaOH and 10 g Na_2CO_3 dissolved in 500 mL of deionized water. 0.5 mL of sample was mixed with 2 mL of standard reagent and incubated for 10 min at laboratory temperature. Then 0.2 mL of 1 N Folin-Ciocalteu reagent was added and the test tube contents were vigorously mixed for 2 s. Absorbance at 750 nm was measured after 30 min of colour development. Human serum albumin was used as the calibration standard.

The cell protein concentration was measured in triplets at the beginning of experiments from pellets and in the suspension of nitrifying organisms before immobilization. The biomass content in pellets was calculated on the basis of knowledge of protein content in bacterial suspension and inside the pellets. This method has already been used by Song *et al*. (2005) and Chen *et al.* (1998) as the protein content related to total suspended solids (TSS) remains essentially constant (Simon & Azam 1989).

RESULTS AND DISCUSSION

Nitrification course at different temperatures

Nitrification is a two-step process involving oxidation of ammonia to nitrite, so-called nitritation, and oxidation of nitrite to nitrate, nitratation. During all of the experiments, ammonia, nitrite and nitrate profile of the reaction was being observed.

Ammonia concentration decreased and nitrate nitrogen increased almost linearly in all of the tests with the exception of experiments performed at 5 °C. At 5 °C, even after the 2-month adaptation of the pellets to this temperature, the observed decrease of ammonia concentration was negligible and also nitrification rates were close to zero (the fastest 0.03 mg kg^{-1} h^{-1} ($N\text{-}NO_3$, wet pellets)). This finding indicates that this kind of pellet is not able to nitrify at 5 °C. Typical nitrification course at 5 and 25 °C and at initial Namon concentration of 250 mg L^{-1} is given in Figures 1 and 2, respectively.

Only low accumulation of nitrite was detected during all the tests: the highest peak of 200 mg L^{-1} $N\text{-}NO_2$ was detected during the test with initial ammonia nitrogen concentration of 800 mg/L at temperatures 17 and 30 °C, while at 25 °C and the same initial ammonia concentration nitrite nitrogen concentration never exceeded 60 mg L^{-1}. On the contrary, the lowest nitrite nitrogen peak was observed at the tests at low initial ammonia concentration (10 mg L^{-1}), where the highest peaks never exceeded 0.5 mg L^{-1} at any temperature.

The nitrite accumulation occurs as the coincidence of difference in the rates of ammonia and nitrite oxidation. Degree of nitrite accumulation can be expressed as the ratio between the areas under the curves of ammonia

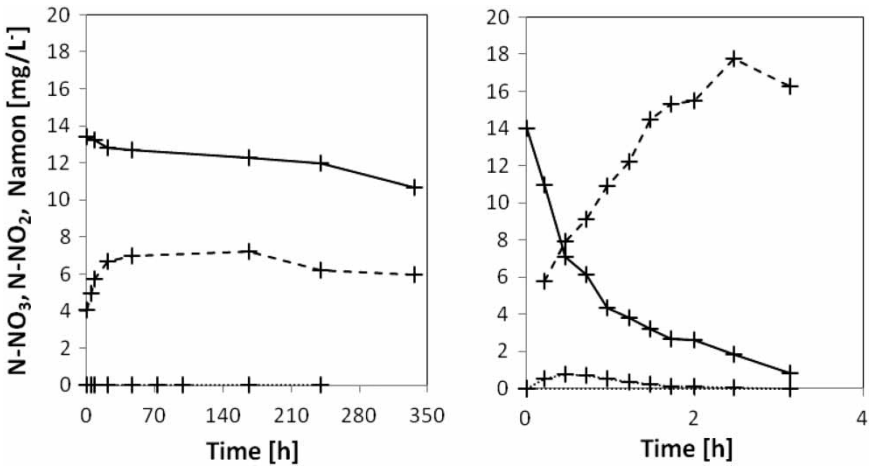

Figure 1 | Course of nitrification at 5 °C (left) and at 25 °C (right) with initial Namon concentration 15 mg L^{-1} and 250 mg L^{-1} (B) . Dotted line: $N\text{-}NO_2$; dashed line: $N\text{-}NO_3$; solid line: Namon.

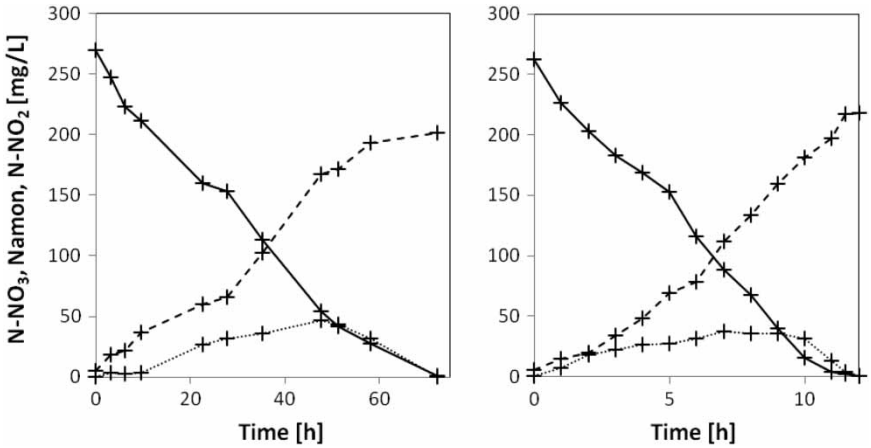

Figure 2 | Course of nitrification at 10 °C (left) and at 25 °C (right) with initial Namon concentration 250 mg L^{-1}. Dotted line: $N\text{-}NO_2$; dashed line: $N\text{-}NO_3$; solid line: Namon.

nitrogen concentration versus time and nitrite nitrogen concentration versus time (Figure 3).

Relative nitrite accumulation was strongly affected by initial ammonia concentration as well as by the temperature. The highest accumulation was observed at 17 °C and initial ammonia nitrogen concentration 400 mg L^{-1}, the lowest at 400 mg L^{-1} Namon and 10 °C. Inconsistency of nitrite accumulation at different conditions indicates that each immobilized organism, *N. europaea* and *N. winogradskyi*, is affected differently. Conditions more favourable for the former microorganism than for the latter result in higher nitrite nitrogen production than consumption and thus nitrite accumulation.

Lower nitrite peaks and low nitrite accumulation are associated with lower probability of nitrite-caused inhibition of nitrification (Anthonisen *et al.* 1976; Král 2009). This inhibition is more probable at low pH values, as the toxicity is assumed to be caused mainly by the unionized nitrous acid (Anthonisen *et al.* 1976).

On the other hand, high production of nitrite at the expense of nitrate with combination with denitritation (Zeng *et al.* 2010) or anammox process (Fux *et al.* 2002) can be a desired useful tool to reduce running costs of wastewater treatment. Presented results indicate that used immobilized microorganisms are not suitable to perform partial nitrification of synthetic wastewater.

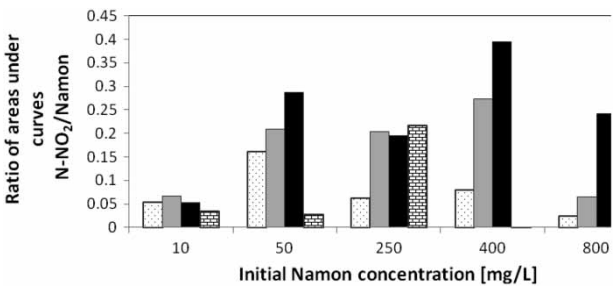

Figure 3 | Ratio between the areas under the curves of ammonia nitrogen concentration versus time and nitrite nitrogen concentration versus time in dependence on initial Namon concentration and temperature. Dotted: 30 °C; grey: 25 °C; black: 17 °C; bricks: 10 °C.

Nitrification rate

The highest nitrification rates were observed at the temperature 30 °C, which is in good agreement with literature: the optimal temperature is 35 °C for *Nitrosomonas* and 38 °C for *Nitrobacter* (Grunditz & Dalhammar 2001). As is shown in Figure 4, the specific nitrification rate was not dependent only on the temperature, but also on the initial concentration of ammonia.

The diffusion limitation was observed at the ammonia nitrogen concentration below 250 mg L^{-1} at temperatures of 10 and 25 °C, below 400 mg L^{-1} at 30 °C and below 800 mg L^{-1}

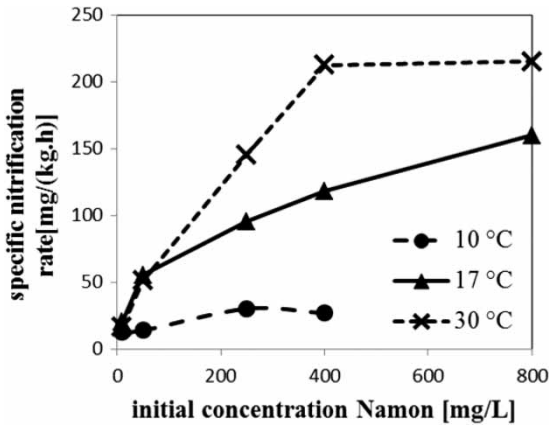

Figure 4 | Dependence of measured specific nitrification rate on initial Namon concentration for selected temperatures.

at 17 °C. These findings suggest that the maximum efficiency is reached at high ammonia concentration, which is very suitable for high strength nitrogen wastewaters such as anaerobic digestion sludge water. The inhibition of nitrification by high substrate concentration was not observed in any test.

The high nitrification rates between 13 and 30 mg kg^{-1} h^{-1} ($N\text{-}NO_3$, pellets) were observed also at 10 °C. It is known that at low temperatures nitrification problems often occur at the wastewater treatment plants. So this technology has been proved to operate without problems at 10 °C.

Dependence of specific nitrification rate on initial substrate concentration can be also described by using Monod kinetic expression. Values of half saturation coefficient obtained in this study are much higher than values given in literature (Table 1). This phenomenon might be caused by great development of nitrifying bacteria during cultivation period, which was performed under relatively high ammonia concentration. At the low initial concentration, the diffusion limitation appears as there is not sufficient gradient of concentration between the solution and the inner parts of pellets. Furthermore, growth of biomass in inner part of pellets worsens the mass transfer over the microcolonies, which also consume ammonia (Wijffels *et al.* 1994) and thus inner bacteria are not able to participate in nitrification at low initial substrate concentration.

Table 1 | Half saturation coefficients (Ks) for nitrification

Reference	Ks [mg L^{-1} Namon]	Temperature [°C]	Type of biomass
This study	101	30	Pure culture immobilized in PVA
This study	81.9	25	Pure culture immobilized in PVA
This study	115	17	Pure culture immobilized in PVA
This study	40.9	10	Pure culture immobilized in PVA
Kapagiannidis *et al.* (2006)	3.1–6.1	NA	Activated sludge
Kihn *et al.* (2000)	2.2	32	Fixed nitrifiers (biofilm)
Sperandio & Espinosa (2008)	0.2–0.6	20	Membrane reactor
Lin *et al.* (2009)	≈ 1	20	Activated sludge
Fang *et al.* (2009)	9.1	NA	Granular sludge
Carvallo *et al.* (2002)	11	23	Biofilm
Wijffels *et al.* (1994)	0.154	NA	Pure culture immobilized in carrageenan

NA – data not available.

Nitrification rate is closely connected to the hydraulic retention time (HRT) necessary to reach the desired final effluent concentration, which was 2 mg L^{-1} Namon in this study. HRT grew with growing initial Namon concentration and with decreasing temperature (Figure 5). HRT changes at 30, 25 and 17 °C ranged maximally in tens of per cent. The most significant increase of HRT was observed after lowering the temperature from 17 to 10 °C. For example, at initial Namon concentration 400 mg L^{-1}, the HRT increased more than five times at temperature drop from 17 to 10 °C. This fast nitrifying activity drop can be explained by lower probability of effective collisions caused by reduction of kinetic energy of molecules (Ruff & Friedrich 1971), by lowered affinity of microorganisms for substrates (Nedwell 1999) or by inability of proteins and enzymes to perform biochemical reactions (Russell *et al.* 1990).

Temperature coefficient estimation

The value of temperature coefficient expresses the reliance of the nitrification rate on the temperature. The knowledge of temperature coefficients of Arrhenius-type equation is crucial for design of nitrification reactors. The higher the coefficient the more the specific nitrification rate is dependent on the temperature. According to our findings, the temperature coefficients varied depending on the initial concentration of ammonia nitrogen. The coefficients for initial concentrations 10, 50, 250 and 400 mg L^{-1} of ammonia nitrogen were calculated as 1.08, 1.12, 1.10 and 1.10 respectively. These values are much higher than results of

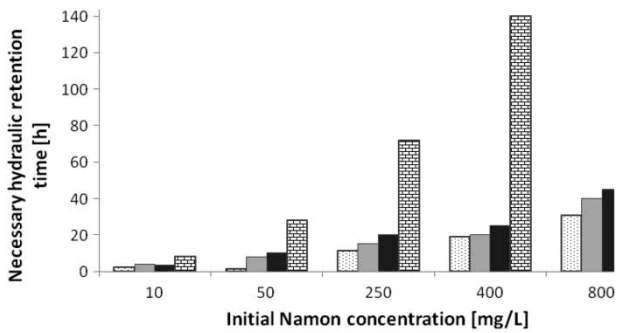

Figure 5 | Dependence of necessary hydraulic retention time on initial Namon concentration and temperature. Dotted: 30 °C; grey: 25 °C; black: 17 °C; bricks: 10 °C.

Rostron *et al*. (2001), who observed the decrease of nitrifying activity of encapsulated nitrifiers of only 10% when temperature declined from 25 to 16 °C, corresponding to the temperature coefficient 1.01. Also in the work of Zhu & Chen (2002) on nitrification by using fixed film biofilters the temperature coefficient can be calculated as 1.01. Higher temperature coefficients were observed by Li *et al*. (2009) for nitrification by using polyethylene glycol entrapped biomass and nitrifier biofilm attachment on elastic plastic filler at 40 mg L^{-1} of ammonia nitrogen: 1.04 and 1.07 respectively. Temperature dependence presented in this study corresponds more to the values related to activated sludge (e.g. temperature coefficient 1.123 (Henze & Loosdrecht 2008)) than to immobilized biomass.

Protein content

The protein content in the mixture of *N. europaea* and *N. winogradskyi* was measured as 360 mg/g TSS. This number is in good agreement with work of Wahman *et al*. (2005), who detected protein content of *N. europaea* 400 mg/g TSS. The average protein concentration in pellets was 0.9% (w/w), i.e. 25 mg TSS biomass/g pellets.

CONCLUSION

This paper shows that the laboratory use of encapsulated nitrifiers *N. europaea* and *N. winogradskyi* is suitable for treatment of wastewater with high ammonia nitrogen concentration within temperature range 10–30 °C. Although the specific nitrification rates strongly depend on temperature as well as on ammonia concentration, use of encapsulated nitrifiers brings many advantages. The most important is the absolute content of nitrifiers in reactors: as the pellets contain pure nitrifying culture, about 32 times higher content of nitrifiers may be reached in the reactor compared to the activated sludge (Bouškova *et al*. 2011). The method can be suitable for reject water nitrification; the additional research is needed to verify those findings on the real wastewater and in pilot plant.

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