Biodegradation (2009) 20:859–865
DOI 10.1007/s10532-009-9273-z

ORIGINAL PAPER

Comparative study of the nitrification characteristics of two different nitrifier immobilization methods

Zhirong Li · Zhi Zhang · Jun Li · Zhenjia Zhang

Received: 7 December 2008 / Accepted: 8 June 2009 / Published online: 24 June 2009

Abstract The research investigated the nitrification characteristics of two different immobilization methods: nitrifier encapsulation in polyethylene glycol (PEG) gel pellets and nitrifier biofilm attachment on elastic plastic filler. The two carriers were placed in identical reactors. They reached a maximum nitrification rate of 39 and 25 mgN/L·h 30 days after start-up. The results showed that the nitrification efficiency in the PEG reactor was higher than in the biofilm reactor under the same conditions. Variations in temperature decreased the nitrification rate by approximately 55% in the PEG reactor from 28 to 8°C, while 74.2% in the biofilm reactor. When the COD loading rate was increased to 0.8 kg/m^3 day, the nitrification efficiency in the biofilm reactor dropped sharply to 23%, and that of PEG reactor remained over 80%. PEG pellets with a high nitrification rate under all conditions showed promise as an immobilization medium, and are likely to be utilized in the nitrification of high-strength ammonia and COD wastewater during long-term operation.

Z. Li (✉) · Z. Zhang · Z. Zhang
School of Environmental Science & Engineering,
Shanghai Jiaotong University, 200240 Shanghai, China
e-mail: lizr210@hotmail.com

J. Li
School of Municipal and Environmental Engineering,
Shenyang Jian Zhu University, 110168 Shenyang, China

Keywords Immobilized nitrifiers · PEG · Biofilm · Nitrification rate

Introduction

The biological nitrogen removal process is the most common method of removing ammonium from wastewater. The oxidation of ammonia to nitrate is a two-stage reaction. Firstly, there is the oxidation of ammonia attributed to ammonia oxidation bacteria (AOB) such as *Nitrosomonas*. Secondly, there is the oxidation of the ensuing nitrite attributable to nitrite oxidation bacteria (NOB) such as *Nitrobacter* (Bock et al. 1986; Rittmann and McCarty 2001). Nevertheless, nitrifiers grow slowly, and have a low yield. Hence, without long retention times they would be washed out of a continuous reactor unless they are immobilized. Immobilizing the microorganisms either on a support carrier as a fixed biofilm process, or by encapsulating the organisms within a matrix, would ensure that the nitrifiers would be highly concentrated within the treatment system. Thus, volumetric efficiency would be greatly increased. Because of immobilization, relatively small reactors could be used, thus affording protection from extreme conditions. Thus, immobilization would help maintain year-round treatment (Morita et al. 2007).

A fixed biofilm is a viscoelastic layer of microorganisms attached to a solid surface of the support

media. Fixed biofilm systems have generally been the preferred method of immobilization in wastewater treatment because of their simple, reliable and stable characteristics and ease of use in small-scale treatment (Zhu and Chen 2002). The examples of fixed-film reactors including trickle filters (Kamstra et al. 1998), submerged biological aerated filters (Rother et al. 2002), rotating biological contactors (Chen et al. 2006a, b), fluidized bed reactors (Pruden et al. 2003) and suspended carrier biofilm reactors (Wang et al. 2005) have been investigated to remove nutrients and other pollutants from both household and industrial wastewater.

The encapsulation of biomass in polymers results in high cell density and viability. Considerable researches have been conducted using alginate, carrageenan (Kim et al. 2000) and polyvinyl alcohol (PVA) (Cao et al. 2002), and so on. However, such polymers are not sufficiently robust for use in wastewater treatment because the carrier material is required to have low solubility and biodegradability, high stability and diffusivity, and of course, to cost less (Leenen et al. 1996). At present, Encapsulation in polyethylene glycol (PEG) is the only encapsulation immobilization to be used at full-scale (Antonina et al. 1996). Some studies (Qiao et al. 2008; Isaka et al. 2007) on treating ammonia wastewater at high loading rate and low temperature using PEG immobilized nitrifiers has also demonstrated at lab-scale.

Nitrification in immobilization system involves physical, chemical and biological processes that are governed by a variety of parameters such as substrate concentration, hydraulic retention time (HRT), organic matters, temperature, pH and other conditions (Chen et al. 2006a, b). The nitrification characteristics of two different microorganisms immobilization methods of attachment on a support carrier and encapsulation in a polymer matrix applied in two similar reactors have seldom been reported. Little information is available to qualify the effects of operational parameters on the nitrification of two immobilization methods.

In this study, the environmental factors that affect the nitrification characteristics of two types of immobilized cells were investigated. This study presents the results from the nitrification of synthetic ammonia wastewater using both PEG encapsulated nitrifiers and biofilms attached to elastic plastic filler. The aim of the work was to determine whether encapsulation conferred an advantage on reactor operation, and which of the immobilization media tested performed better under ammonia and COD loading conditions and various temperatures.

Materials and methods

Immobilization materials

Artificially immobilized cells and naturally attached cells (biofilms) were used in this research. Two immobilization media were chosen. One was encapsulated nitrifier pellets (Fig. 1a) supplied by Hitachi Plant Engineering and Construction Co., Ltd. The gel carrier contained 10% (w/v) PEG, 0.5% (w/v) promoter, 0.25% (w/v) initiator, and 2% (w/v) microorganisms (Hashimoto and Sumino 1998). The resulting polymerized gel carrier was cut into 3 mm × 3 mm × 3 mm cubes (density 1.02 g/cm^3). The other carrier was elastic plastic filler with specific surface area of about 250 m^2/m^3 (Fig. 1b) attached to active sludge biofilm. Threads made of polyolefins and polyamide were attached to a strong rope and spaced evenly in 3-dimensions. Thus, the biofilm was able to attach to each piece of thread and came into complete contact with both air and liquid.

Description and operation of the reactors

In order to compare the effects of the two immobilization methods, two identical reactors were used, as shown in Fig. 2. 18L reactors made of acrylic glass

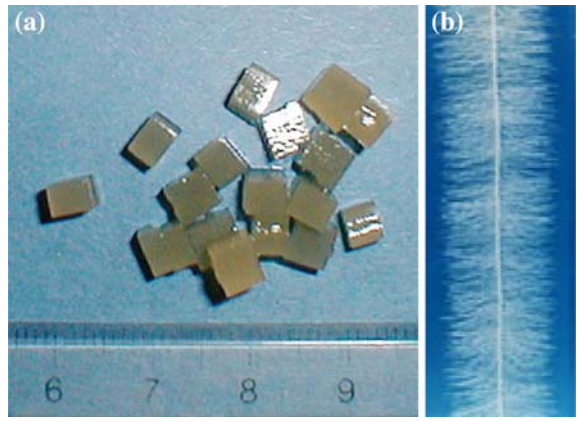

Fig. 1 Carriers of the two reactors. **a** PEG encapsulated nitrifiers; **b** elastic plastic filler

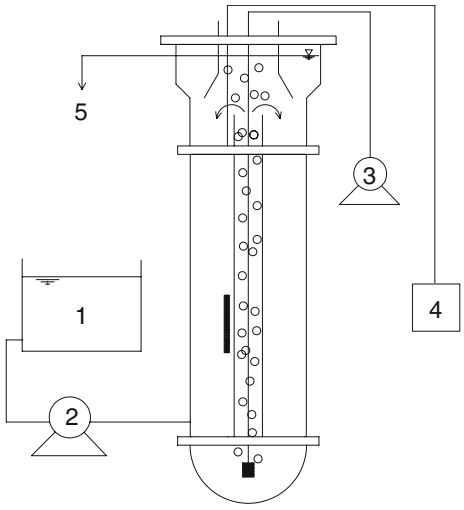

Fig. 2 Schematic diagram of the reactor system. 1, Influent bank; 2, Pump; 3, Aeration pump; 4, Heater and temperature controller; 5, Effluent

were fed with synthetic ammonia wastewater by variable diaphragm pumps (Pulsafeeder, LD54, USA). An inner circulation tube with two open sides was set in the center. Air was supplied through a sintered glass ball at the base of the reactor. Mesh (1 mm × 1 mm) placed at the reactor outlet was used to separate the immobilized particles from the outflow of the treated water. pH was 7.6–7.8 in stable operation. The water temperature was maintained at 27 ± 1°C, except in the experiment investigating the influence of temperature on nitrification.

The first reactor was filled with 10% PEG pellets by volume. Aeration caused the pellets to float through the central tube to the top of the reactor, and then gravity caused them to sink to the bottom. In the second reactor, elastic plastic filler with the attached biofilms was wound around the central tube. Thus, inner circulation occured in both reactors, ensuring the complete mixing and contact between the liquid and immobilized nitrifiers.

The synthetic wastewater (40 mgN/L) per liter contained: NH_4Cl, 153 mg; $NaHCO_3$, 468 mg; $Na_2HPO_4 \cdot 12H_2O$, 46.4 mg; NaCl, 20.5 mg; KCl, 9.6 mg; $CaCl_2 \cdot 2H_2O$, 9.6 mg; and $MgSO_4 \cdot 12H_2O$, 33.6 mg. COD in the form of glucose was added.

The reactor was operated until stability was achieved, which occurred after 30 days. Then a batch experiment was carried out to check the maximum nitrification rate of each reactor. Glucose was added to the feed to investigate whether heterotrophs would grow and inhibit the nitrifiers. Also, the temperature was changed in steps from 32 to 8°C, and each step was kept for 10–15 days at each temperature until deviation of nitrification rate was less than 5% in 3 days. Subsequently, the reactors were operated for 10 days at 32°C to reactivate the biomass. Finally, we investigated the nitrification efficiency during a long-term continuous operation.

Reactor pH, DO, temperature and flow rate were monitored daily, while the effluent was analyzed for ammonia, nitrite, nitrate and COD using standard methods (APHA 1995) at least every other day.

Results and discussion

Startup of the reactors

Before the experiment, the encapsulated cell pellets in PEG reactor were black and malodorous because they had been immersed in water and hermetically sealed under room temperature for a long period; thus the initial nitrification activity of the PEG pellets was relatively low.

In the biofilm reactor, seed sludge [mixed liquid suspended solid (MLSS) was 2,000 mg/l] from the anoxic/oxic process of a sewage treatment plant was added. In the early days of cultivation, the ammonia loading was 0.1 kgN/m^3 day, and was enhanced to 0.2 kgN/m^3 day when the nitrification rate of each reactor reached over 80%. However, the nitrifiers could not tolerate a higher concentration, which lead to a sharp decrease in the nitrification rate, as shown in Fig. 3. The nitrification rate gradually returned to

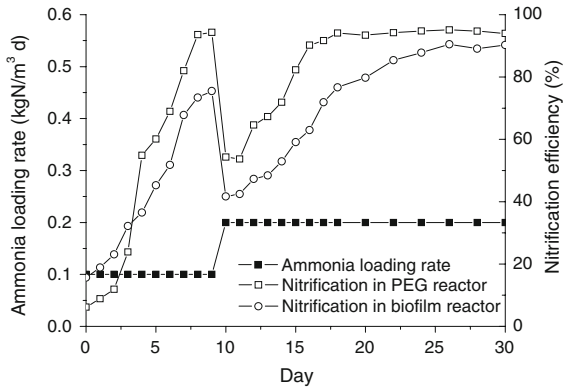

Fig. 3 Start-up of each reactor

the maximum. After 3 weeks' cultivation, the encapsulated pellets turned brown and the nitrification activity remained over 93%. This implied the successful cultivation. After the first 24 h, a brown biofilm appeared on the carrier. Thirty days later, the nitrification rate reached 82%. As the ammonia loading rate increased, nitrification remained stable, which indicated that the startup period of each reactor was over.

Batch experiment

The initial ammonia concentration of both reactors was 250 mg/l. Concentrations of different forms of nitrogen were checked at regular intervals (Fig. 4). In Fig. 4, the nitrification rate of the PEG reactor at 39 mgN/L h was higher than that of the biofilm reactor at 25 mgN/L h. Nitrification is a zero-stage reaction, i.e., ammonia strength is not the limiting factor in nitrification. Therefore, the ammonia concentration will reduce linearly with time if microorganisms can endure the initial concentration. In this experiment, it was difficult to keep the two reactors at the same biomass. However, each reactor eventually achieved its optimal efficiency. Thus, the different nitrification rates reflected the difference between the two immobilization methods, that is, encapsulated nitrifiers were superior to attached biofilm in nitrification.

It can be seen from Fig. 4 that nitrite accumulation took place in each reactor when ammonia concentrations decreased. When ammonia concentration was close to zero, nitrite concentration began falling. Some researches (Anthonisen et al. 1976; Abeling and Seyfried 1992) has revealed that the inhibition concentration of free ammonia (FA) to NOB is 0.1–1.0 mg/l, and that the inhibition concentration of FA to AOB is 10–40 mg/l. In this experiment, the concentration of FA was 20–30 mg/l, according to the following formula:

$$\mathrm{FA} = \frac{17}{14} \times \frac{[\mathrm{NH_4}^+ - \mathrm{N}] \times 10^{\mathrm{pH}}}{K_\mathrm{b}/K_\mathrm{w} + 10^{\mathrm{pH}}}$$

in which K_b dissociation constant of ammonia and K_w is the ionization constant of water. FA inhibits NOB, while AOB could metabolize and reproduce normally. This is why nitrite accumulation occured even when DO was sufficient.

However, accumulation of nitrite in the PEG reactor was more obvious than in biofilm reactor. In the encapsulated pellets, diffusing resistance was from double block of penetrating through the biomass and the carrier. *Nitrobacter* with a lower cell yield (0.042 mg cells/mgN) than that of *Nitrosomonas* (0.142 mg cells/mgN), may grow in the inner layer (Wiesman 1994). Also the NOB used the products of the AOB as its substrate. Thus, the double resistance in mass transfer blocked contact between most NOB and the substrates, while there was little effect on AOB. In the biofilm reactor, microorganisms grew on the surface of the carrier and liquid flew passing the biofilm like a film (Montràs et al. 2008), so that substrates were blocked only by biofilm from surface to inside. Over time, more NOB were bred and hence involved in the metabolization process converting nitrite into nitrate (Montràs et al. 2008).

During the nitrification process, there were some discrepancies between the influent ammonia and the total effluent nitrogen levels. These nitrogen losses could have been due to nitrogen being incorporated into the biomass during growth, absorbed on the surface of the carrier or biofilm and ammonia stripping at high pH. Otherwise, the reaction in each reactor was heterogeneous and there must have been a mass transfer limitation. Low DO concentration and poor turbulent flow existed inside the biofilm where aerobic denitrifiers may have breed to reduce nitrogen or to release nitrogen in gaseous forms (NO and N_2O) before conversion into nitrate.

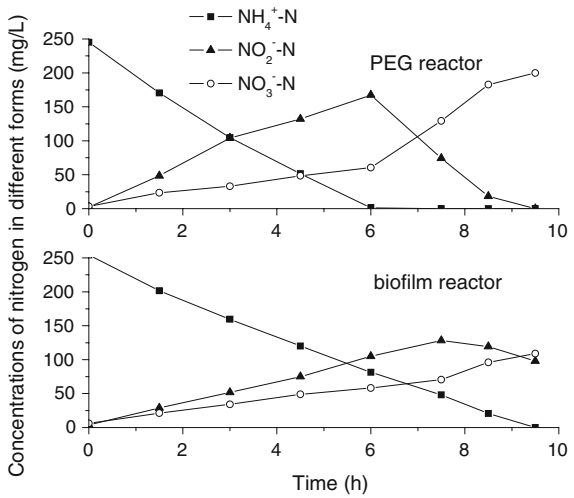

Fig. 4 Ammonia, nitrite and nitrate profiles in each reactor

Influence of temperature on nitrification

Nitrifying bacteria are known to be sensitive to temperature, and nitrification is sometimes considered impossible at low water temperature (Zhu and Chen 2002).

As seen in Fig. 5, the PEG reactor had a higher nitrification rate than the biofilm reactor. Maximum activity appeared at 28°C. A decrease in nitrification of approximately 55% was seen in the PEG reactor from 28 to 8°C, while nitrification decreased 74.2% in the biofilm reactor. Likewise, a decrease of nitrification of 2.5% was seen in the PEG reactor from 28 to 32°C, while nitrification decreased 19.4% in the biofilm reactor. Nitrification rate in biofilm reactor decreased sharply when temperature was below 13°C while in PEG reactor diffusion continue to mask the microbiological rates down to about 10°C. PEG reactor seemed to be superior to biofilm reactor between 10 and 32°C, although below 10°C both reactors are losing performance at a similar rate.

Several parameters changed with temperature, including diffusivity, $K_L a$, the kinetics of nitrification, and equilibrium DO concentration. Each parameter will influence the reaction rates, especially in immobilized systems. All parameters should be considered when attempting to explain temperature effects. There are several reports in the literature of immobilized biomasses being immune to adverse temperatures (Leenen et al. 1997; Asano et al. 1992). Substrate diffusion limitation was the probable reason for this (Zhu and Chen 2002; Isaka et al. 2007). Zhu and Chen (2002) concluded that although the diffusion coefficient itself increases as temperature increases, the diffusion process limited the mass flux into the immobilized biomass and thus DO can become a limiting factor within the biomass. It seems that diffusion through the matrix or biofilm can help make biomasses immune to adverse conditions by creating micro-environments within the gel matrix.

Influence of organic substances on nitrification

Wastewater is always composed of nitrogen compounds and organic substances (measured with COD). Even with pretreatment of COD removal, the residual COD will influence the nitrification activity of autotrophic nitrifiers in traditional treatments (Ling and Chen 2005).

At the operational HRT of 10.5 h glucose was added to the feed in order to investigate whether heterotrophs would grow and inhibit the nitrifiers (Fig. 6). COD loading hardly affected the nitrification activity of the PEG reactor obviously, but it did affect the biofilm reactor. When the COD loading rate increased to 0.8 kg/m^3 day, the nitrification efficiency of the biofilm reactor dropped sharply to 23%, whereas that of the PEG reactor remained over 80%. This may be explained by the following. After the long-range acclimation with high strength ammonia wastewater, the majority of the pores of encapsulated pellets were filled with nitrifiers. When COD was added, there was no space for heterotrophs to grow. Most would be washed out of the reactor.

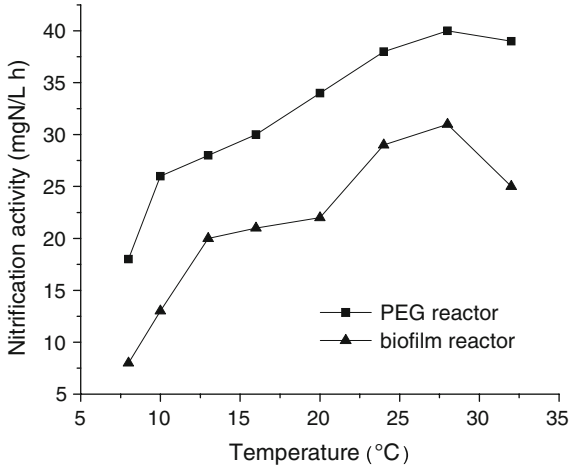

Fig. 5 The temperature effect on nitrification rates

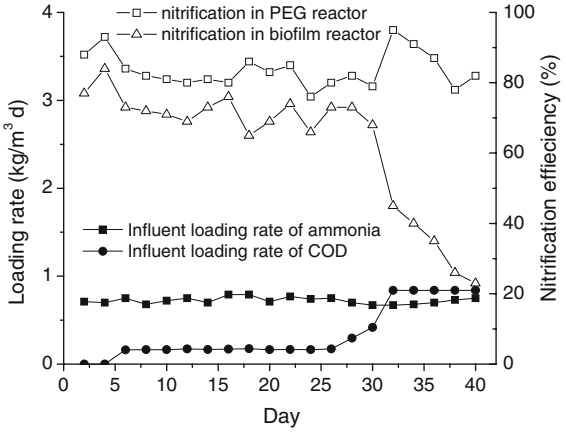

Fig. 6 Ammonia removal under continuous operation in each reactor

Meanwhile, more heterotrophs would be attached to the surface of the biofilm formerly composed of nitrifiers. As heterotrophs reproduce much faster than nitrifiers and have a strong affinity with oxygen, after a period of operation with COD feed, the nitrifiers in the biofilm reactor were greatly inhibited.

Reactor performance

The PEG reactor had greater nitrification efficiency than the biofilm reactor. This was probably because the PEG reactor supported more biomass and/or the encapsulated bacteria were better protected from substrate inhibition effects. After a long period of operation, part of the biofilms in the biofilm reactor regularly fell off the carrier, thus increasing the turbidity of the effluent. The nitrification in this research compares favorably with those reported in Table 1. The encapsulated pellets were mechanically stable. At present, PEG is the only encapsulation immobilization media to be used at full-scale (Antonina et al. 1996). The rates achieved in the PEG reactor could be further improved with particle and reactor optimization to improve mass transfer. The use of biomasses immobilized in PEG appears very promising in treating wastewater of higher ammonia and COD loading rate in long-term operation.

Conclusions

Nitrifier encapsulation and biofilm attachment to elastic plastic filler are efficient methods in the nitrification process. Each exhibited good nitrification activity and operational stability. The reactors using these two immobilization methods took 30 days to reach their respective maximum nitrification rates of 39 and 25 mgN/L h.

Nitrite accumulation occurred in each reactor. The PEG reactor yielded more nitrite due to the cell yields of *Nitrosomonas* and *Nitrobacter* and the substrate transfer mechanism. Nitrogen loss indicated that there was probably a denitrification reaction inside the carrier and biomass.

Temperature variations affected nitrification activity: going from 28 to 8°C resulted in approximately a 55% decrease in the PEG reactor from and a 74.2% in the biofilm reactor. PEG reactor seemed to be superior to biofilm reactor between 10 and 32°C, although below 10°C both reactors are losing performance at a similar rate. It seems that diffusion through the matrix or biofilm offers protection from adverse conditions by creating micro-environments within the gel.

The impact of COD and ammonia loading hardly affected nitrification in PEG reactor, while the nitrification rate of the biofilm reactor dropped to 23% after a period of operation with a COD feed. Heterotrophs reproduced easily on the biofilm surface and consumed most of the oxygen, which restrained the ability of the nitrifiers.

The PEG encapsulated pellets showed high nitrification ability and more stability over the long term, thus promising to be a preferred immobilization method for treating wastewater of higher ammonia and COD loading rate in long-term operation and achieving quick start-up.

Table 1 Nitrification rates with immobilized biomasses reported in the literature

Nitrification rate (mgN/L h)	Wastewater	Immobilization method	Reference
27.1	Seawater 40 mgN/L	Porous cellulose carrier	Antonina et al. (1996)
10	Synthetic wastewater (lab scale) 20 mgN/L	Suspended carrier (biofilm)	Wang et al. (2005)
8.3	Synthetic wastewater (lab scale) 35 mgN/L	Membrane aerator (biofilm)	Brindle and Stephenson (1996)
10.4	Synthetic wastewater (lab scale) 50 mgN/L	Calcium alginate (encapsulation)	Van Ginkel et al. (1983)
29.2	Synthetic wastewater (lab scale) 500 mgN/L	PVA (encapsulation)	Rostron et al. (2001)
22.5	Wastewater from a sludge drying plant (full-scale)	PEG, 3 mm cubes (encapsulation)	Tanaka et al. (1994)
29.6	Landfill leachate (lab scale at 10°C) 200 mgN/L	PEG, 3 mm cubes (encapsulation)	Isaka et al. (2007)

Acknowledgments Thanks to Hitachi Plant Engineering and Construction Co., Ltd for supplying the PEG pellets and financial support.

References

Abeling U, Seyfried CF (1992) Anaerobic–aerobic treatment of high-strength ammonium wastewater nitrogen removal. Water Sci Technol 26(3):1007–1015

Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. J Water Pollut Control Fed 48(5):835–852

Antonina M, Sakairi C, Yasuda K, Matsumura M (1996) Nitrogen removal in seawater using nitrifying and denitrifying bacteria immobilized in porous cellulose carrier. Water Sci Technol 34(7–8):267–274

APHA (1995) Standard methods for the examination of water and wastewater, 19th edn. American Publishers Health Association, Washington

Asano H, Myoga H, Asano M, Toyao M (1992) A study of nitrification utilizing whole micro-organisms immobilized by the PVA-freezing method. Water Sci Technol 26(3):1037–1046

Bock E, Koops HP, Harns H (1986) Cell biology of nitrifying bacteria. In: Prosser JI (ed) Nitrification. IRL Press, Oxford, pp 17–38

Brindle K, Stephenson T (1996) Nitrification in a bubbleless oxygen mass transfer membrane bioreactor. Water Sci Technol 34(9):261–267

Cao GM, Zhao QX, Sun XB, Zhang T (2002) Characterization of nitrifying and denitrifying bacteria coimmobilized in PVA and kinetics model of biological nitrogen removal by coimmobilized cells. Enzyme Microb Technol 30(1):49–55

Chen SL, Ling J, Blancheton JP (2006a) Nitrification kinetics of biofilm as affected by water quality factors. Aquac Eng 34:179–197

Chen ZQ, Wen QX, Wang JL, Li F (2006b) Simultaneous removal of carbon and nitrogen from municipal-type synthetic wastewater using net-like rotating biological contactor (NRBC). Process Biochem 41(12):2468–2472

Hashimoto N, Sumino T (1998) Wastewater treatment using activated sludge entrapped in polyethylene glycol prepolymer. J Ferment Bioeng 86(4):424–426

Isaka K, Yoshie S, Sumino T, Inamori Y, Tsuneda S (2007) Nitrification of landfill leachate using immobilized nitrifying bacteria at low temperatures. Biochem Eng J 37(1):49–55

Kamstra A, Van der Heul JW, Nijhof M (1998) Performance and optimization of trickling filters on eel farms. Aquac Eng 17:175–192

Kim SK, Kong I, Lee BH, Kang L, Lee MG, Suh KH (2000) Removal of ammonium-N from a recirculation aquacultural system using an immobilized nitrifier. Aquac Eng 21:139–150

Leenen EJTM, dos Santos VAP, Grolle KCF, Tramper J, Wijffels RH (1996) Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment. Water Res 30(12):2985–2996

Leenen EJTM, van Boxtel AMGA, Englund G, Tramper J, Wijffels RH (1997) Reduced temperature sensitivity of immobilized *Nitrobacter agilis* cells caused by diffusion limitation. Enzyme Microb Technol 20(8):573–580

Ling J, Chen SL (2005) Impact of organic carbon on nitrification performance of different biofilters. Aquac Eng 33:150–162

Montràs A, Pyke B, Boon N, Gòdia M, Mergeay M, Hendrickx L, Pérez J (2008) Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in an autotrophic biofilm reactor as depicted by molecular analyses and mathematical modelling. Water Res 42:1700–1714

Morita M, Kudo N, Uemoto H, Watanabe A, Shinozaki H (2007) Protective effect of immobilized ammonia oxidizers and phenol-degrading bacteria on nitrification in ammonia- and phenol containing wastewater. Eng Life Sci 7:587–592

Pruden A, Sedran M, Suidan M, Venosa A (2003) Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor. Water Sci Technol 47(9):123–128

Qiao XL, Zhang ZJ, Chen QX, Chen YJ (2008) Nitrification characteristics of PEG immobilized activated sludge at high ammonia and COD loading rates. Desalination 222:340–347

Rittmann BE, McCarty PL (2001) Environmental biotechnology: principles and applications. McGraw-Hill, New York, p 470

Rostron WM, Stuckey DC, Young AA (2001) Nitrification of high strength ammonia wastewaters: comparative study of immobilisation media. Water Res 35(5):1169–1178

Rother E, Cornel P, Ante A, Kleinert P, Brambach R (2002) Comparison of combined and separated biological aerated filter (BAF) performance for pre-denitrifcation/nitrification of municipal wastewater. Water Sci Technol 46(4–5):149–156

Tanaka K, Nakao M, Mori N, Emori H, Sumino T, Nakamura Y (1994) Application of immobilized nitrifiers gel to removal of high ammonium nitrogen. Water Sci Technol 29(9):241–250

van Ginkel CG, KChAM Luyben, Klapwijk A (1983) Characterisation of *Nitrosomonas europaea* immobilised in calcium alginate. Enzyme Microb Technol 5(4):297–303

Wang RC, Wen XH, Qian Y (2005) Influence of carrier concentration on the performance and microbial characteristics of a suspended carrier biofilm reactor. Process Biochem 40(9):2992–3001

Wiesman U (1994) Biological nitrogen removal from wastewater. In: Fiechter A (ed) Advances in biochemical engineering/biotechnology, vol 51. Springer, Berlin, pp 113–155

Zhu SM, Chen SL (2002) The impact of temperature on nitrification rate in fixed film biofilters. Aquac Eng 26:221–237