Rapid start-up and efficient long-term nitritation of low strength ammonium wastewater with a sequencing batch reactor containing immobilized cells

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ABSTRACT

Major concerns about nitritation of low-strength ammonium wastewaters include low ammonium loading rates (ALRs) (usually below 0.2 kg/m^3-d) and uncertainty with the long-term stability of the process. The purpose of this study was to test a sequencing batch reactor filled with cell-immobilized polyethylene glycol (PEG) pellets (~2 mm in size) to see if it could achieve efficient and stable nitritation under various environmental conditions. The sequencing batch reactor (SBR) was fed with synthetic ammonium wastewater of 30 ± 2 mg-N/L and pH 8 ± 0.05, maintaining the dissolved oxygen (DO) concentration at 1.7 ± 0.2 mg/L and the temperature at 30 ± 1 °C. The reaction was easily converted to partial nitrification mode within a month by feeding a relatively high ammonium substrate (~100 mg-N/L) in the beginning. We observed stable nitritation over 300 days with high ALRs (as high as ~1.1 kg-N/m^3-d), nitrite accumulation rates (mostly over 97%), and ammonium removal rates (mostly over 95%). DO was the major limiting substrate when the DO concentration was below ~4 mg/L and the NH_4^+-N concentration was above ~5 mg/L, giving an almost linear increase in the ammonium oxidation rate with the bulk DO increase. Low temperatures mainly affected the reaction rate, which could be compensated for by increasing the pellet volume (i.e. biomass). Our results demonstrated that an SBR filled with small cell-immobilized PEG pellets could achieve very efficient and stable nitritation of a low-strength ammonium wastewater.

Key words | ammonium loading rate, cell immobilization, long-term nitritation, sequencing batch reactor, sewage treatment

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INTRODUCTION

Recent advances in biological nutrient removal technology such as nitritation–denitritation or nitritation–anammox, can save significant amounts of energy and electron donor (Joss *et al.* 2009). When the ammonium concentration is fairly high, nitritation can be achieved rather easily by utilizing the selective inhibition force of free ammonia (FA) or free nitrous acid (FNA) on nitrite-oxidizing bacteria (NOB) (Bae *et al.* 2001; Chung *et al.* 2007). When it is low, achieving nitritation is difficult due to the lack of FA/FNA inhibition (Park & Bae 2009). In spite of this difficulty, nitrogen removal via nitritation can be an attractive choice when a low strength wastewater, such as sewage, is treated anaerobically to save energy (McCarty *et al.* 2011).

The goal of this study was to demonstrate very efficient and stable nitritation of an ammonium wastewater of approximately 30 ± 2 mg-N/L – a range that was expected to be in a digested sewage. A sequencing batch reactor (SBR) was used to utilize the relatively high FA (and ammonium) concentration created in the beginning of each batch reaction. For fast settling of cells, we adopted cell immobilization with polyethylene glycol (PEG) polymer. Various dissolved oxygen (DO) concentrations and temperatures were tested to investigate appropriate operation strategies, reflecting practical conditions.

MATERIALS AND METHODS

Immobilization of nitrifying sludge

An activated sludge obtained from a sewage treatment plant was cultivated and maintained in an SBR containing a high concentration of FA to select for ammonia-oxidizing bacteria

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(AOB). PEG was used as an encapsulation material, using a procedure similar to those described elsewhere (Lee *et al.* 2004). The cell-immobilized PEG was cut into $4 \times 4 \times 4$-mm cubes (pellets) initially, and then further cut down to $2 \times 2 \times 2$ mm for this experiment. The pellets had an apparent specific surface area of ~24,000 m^2/m^3, density of 1.02 g/cm^3, micro-pores of mostly less than 1 μm, and contained ~3 mg/cm^3 of activated sludge (dry basis) initially.

Reactor, feed, and operation

Nitritation was carried out in an SBR with a working volume of 1 L, equipped with a water jacket. Probes for temperature, pH and DO (Orient Star, A216) were used for online measurements in the reactor. Approximately 4.0% (total net volume of wet pellets/bulk liquid volume with pellets) of PEG pellets were contained in the reactor. The composition of the feed wastewater was (g/120 L): $MgSO_4 \cdot 7H_2O$ (1.2), $FeSO_4 \cdot 7H_2O$ (0.13), $CaCl_2$ (0.28), KCl (0.35), $MnSO_4.H_2O$ (0.22), NH_4HCO_3 (30), $NaHCO_3$ (40), and KH_2PO_4 (3.5). The concentration of ammonium in the feed was approximately 30 to 35 mg-N/L. The experiments were performed for 315 days to achieve long term nitritation and to investigate the effects of DO (1.7–3.0 mg-DO/L) and temperature (30–11 °C). In each cycle the aeration was terminated when ammonium was depleting (<5 mg-NH_4^+-N/L) and the DO concentration began to increase sharply. The initial pH in each batch reaction was around 8.0 ± 0.1.

Sampling and analysis

The ammonium, nitrite, nitrate, and alkalinity concentrations were measured according to the standard methods (American Public Health Association 2005). The ammonium removal rate (ARR, %), ammonium loading rate (ALR, kg-N/m^3-d) and nitrite accumulation rate (NAR, (%) = $100 \times \Delta NO_2^-$-N/(ΔNO_2^--N + ΔNO_3^--N), where Δ means the net change) were calculated.

RESULTS AND DISCUSSION

Start-up, achieving nitritation, and transition

The operation of the SBR was started with low ammonium concentration (35 ± 2 mg-N/L) (Phase 1 in Figure 1).

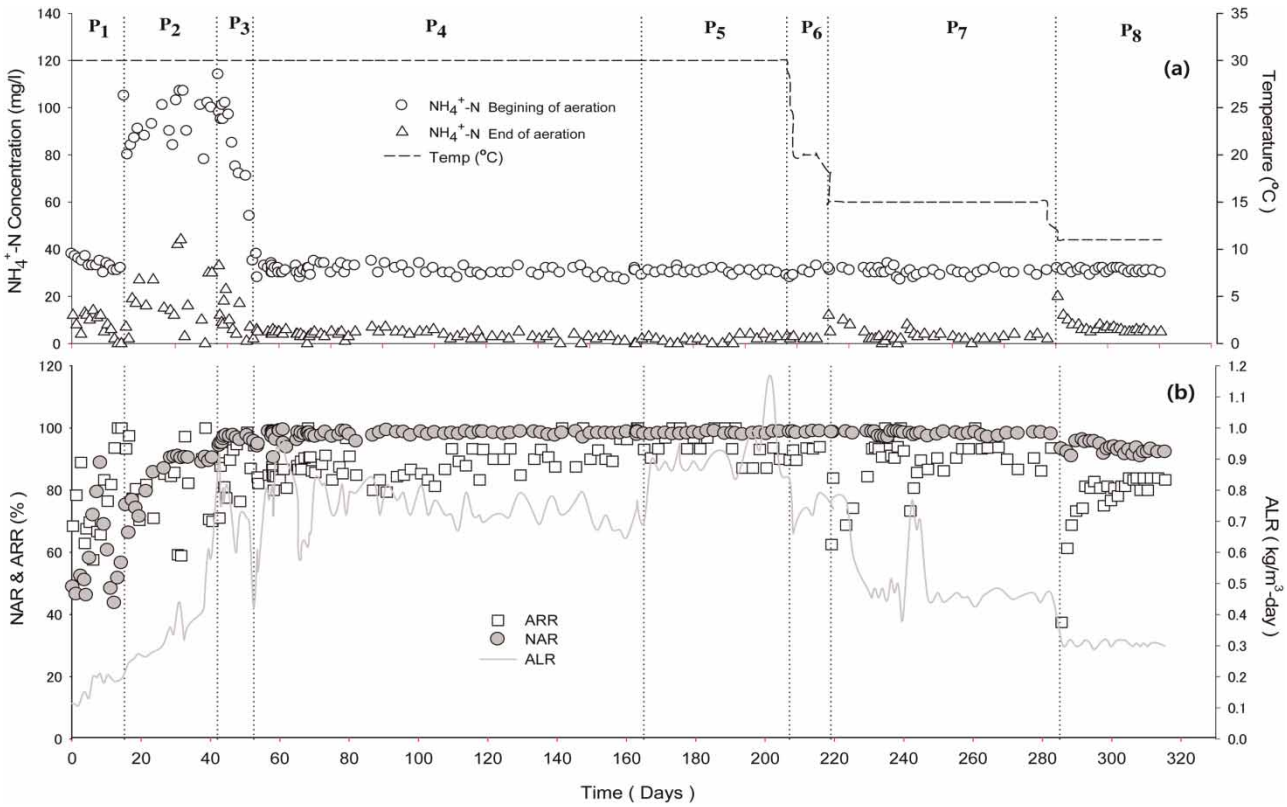

Figure 1 | Overall performance of the SBR throughout the experiment (P1 to P8 are the numbered phases of the experiment).

The DO concentrations and temperature were set at ~3 mg/L and 30 °C, respectively. The immobilized cells adapted quickly and reached ~100% of ARR on day 15, but the NAR was low (~50%). The accumulation of nitrite was achieved by suppressing the growth rate of NOB through FA inhibition (Phase 2). The influent ammonium concentration was increased to ~100 mg-N/L (as FA, ~10 mg-N/L) on day 16. Then the NAR gradually increased to 90% on day 40. Phase 3 was a transition period in which initial ammonium concentrations were gradually lowered from 100 to ~30 mg/L. The DO concentration was also reduced from ~3.0 to ~1.7 mg/L in order to suppress NOB growth through DO limitation. The nitrifiers adapted to the new ammonium and DO concentrations very rapidly, resulting in an ARR above 88% and a NAR over 95%. Because the cells were tightly immobilized in the tiny internal pores of PEG pellets, it was inferred that they grew or decayed *in situ*, but did not migrate (Sumino *et al.* 1992). The probable mechanism for AOB selection, therefore, was inactivation or net decay of NOB due to low growth potential by FA inhibition and insufficient DO (Lee *et al.* 2004; Park *et al.* 2009). Assuming that the NO_3^- production potential (estimated from 100 minus NAR (%)) was proportional to the active biomass of NOB in the reactor, the time required to reduce active NOB mass to a half of the original (half life of NOB) was approximately a week.

Long-term successful nitritation under varying DO concentrations

Nitritation remained stable from day 53 to 165 with DO 1.7 ± 0.2 mg/L (Phase 4). The ARR and NAR were mostly above 90% and 97%, respectively. Oxidation of nitrite to nitrate was effectively prevented with aeration control by terminating aeration when ammonium was depleted (Peng *et al.* 2012). Figure 2 presents typical performance data during an aeration period in Phase 4 (taken on day 163). A rapid oxidation of ammonium to nitrite could be observed. DO appeared to be the main limiting substrate for nitritation since the removal rate of ammonium was almost independent of its concentration until it became very low (e.g. less than 2–3 mgN/L) (according to Monod). Once DO became no more limiting, its concentration began to increase sharply near the end of aeration, generating a 'DO elbow'. The results indicated that the 'DO elbows' were not sharp enough to indicate a completion of ammonium oxidation, especially at higher DO levels (e.g. above 4.1 mg-DO/L). Such type of increased DO (although

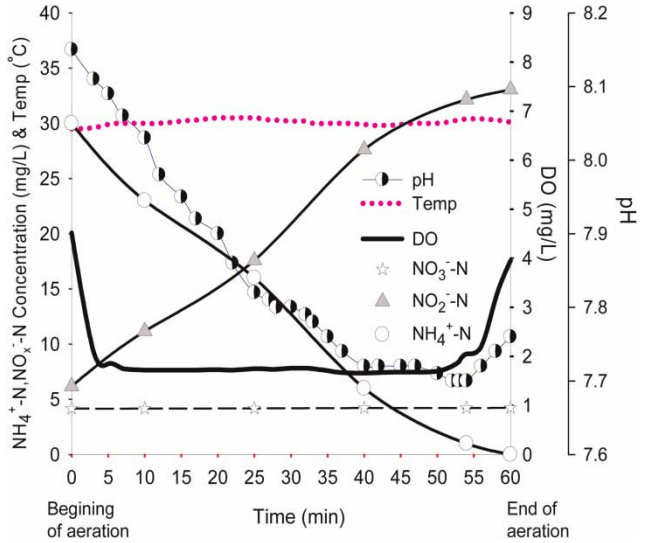

Figure 2 | Typical changes in nitrogen species and control parameters during the 'react' period in the SBR on day 163.

for a short time, e.g. 5–10 min) can be favourable for NOB (when there are 24–36 cycle/d). So cycle duration was optimized for that effluent concentration.

When the DO concentration was increased from ~1.7 to ~3.0 mg/L (Phase 5), the ALR could be increased from ~0.7 kg/m^3-d to ~0.9 kg/m^3-d. Although the FA concentration (below 3 mg-N/L) was less than the threshold concentrations (3.5–10 mg-N/L) for NOB suppression (Bae *et al.* 2001; Li *et al.* 2012), the NAR remained above 95% (Phase 5 in Figure 1). This observation was different from that reported by Bernet *et al.* (2001) and Garrido *et al.* (1997) where nitrite oxidation resumed quickly when DO was recovered. The major reason for the stability in our system might be attributed to the structure of the pellets: if the NOB had been surviving deep inside the pellets, escaping from the FA toxicity (Park *et al.* 2010), they should have experienced more severe DO limitation.

To investigate short term effects of DO concentration, the SBR was operated at different DO levels (i.e. 1.7, 2.2, 3.1, 3.6, 4.1, 4.7, 5.4 and 6.1 mg-DO/L) at 30 °C in the later stage of Phase 5 (days 190 to 200). The reaction rate increased notably as DO increased, and the aeration time required to remove ~30 mg-N/L of ammonium decreased from ~55 min to less than 20 min. The increased reaction rate allowed an elevation of ALR. Figures 3(a) and 3(b) represent the two-dimensional contour plots for ALR and NAR, based on the collected data at 30 °C. The short-term influence of elevated DO did not lead to nitrate production as NAR always remained above 96%.

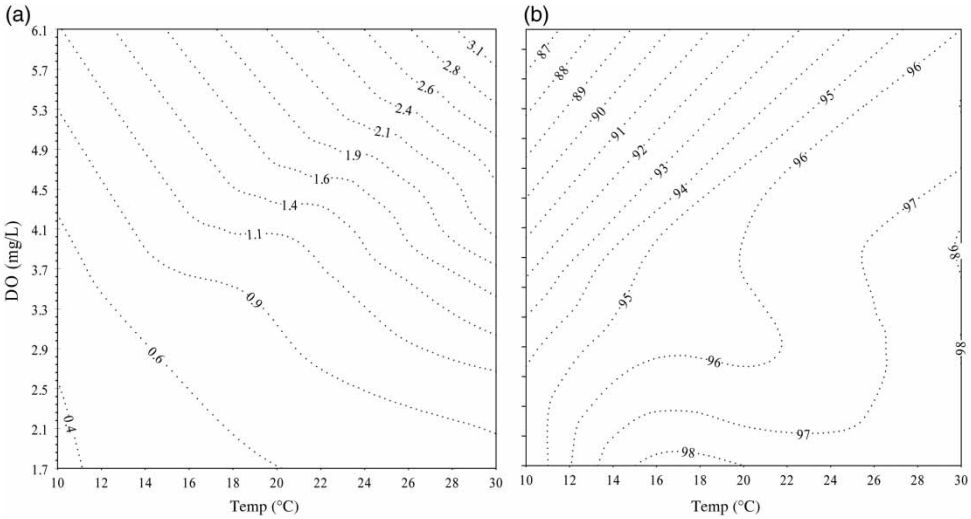

Figure 3 | Effect of temperature and DO concentration on (a) ALR (kg-N/m^3.d) and (b) NAR (%).

Effect of low temperature

During Phases 6 to 8, the reactor temperature was gradually decreased from 30 to 11 °C while keeping the DO concentration at 1.7 ± 0.1 mg/L (days 207 to 315 in Figure 1). Nitrite oxidation was apparent as temperature decreased below 15 °C, although the average NAR was still above 94%. This indicated that such low temperatures should not be allowed for too long, unless other provisions for NOB suppression are applied (e.g. heat shock treatment) (Isaka *et al.* 2008). A more critical disadvantage of lower temperature was observed in the reaction rate, as it decreased to a third at 11 °C compared to 30 °C. This problem could be mitigated by increasing the volume of pellets in the reactor. When the apparent volume ratio of the pellets against the reactor volume was increased from 3 to 5% the removal rate of ammonium increased almost proportionally (Figure 4). Further investigations were needed to optimize the pellet volume based on reaction rate and cost.

To investigate the combined effects of DO and temperature, the SBR was operated at varying DO levels (same as before) at 20, 15 and 11 °C during phase 6 to 8. The results are shown in Figure 3(a). To achieve a target ALR (e.g. 1.0 kg-N/m^3-d) at 10 °C, a higher DO (~5.5 mg/L) was necessary while ~2.5 mg/L was enough at 30 °C (Figure 3(a)). A higher DO at low temperature would reactivate NOB, as hinted by the decreased NAR in Figure 3(b), which indicated that maintaining nitritation at low temperature would be very difficult.

Table 1 compares nitritation performances in this study to the literature for low strength ammonium wastewaters.

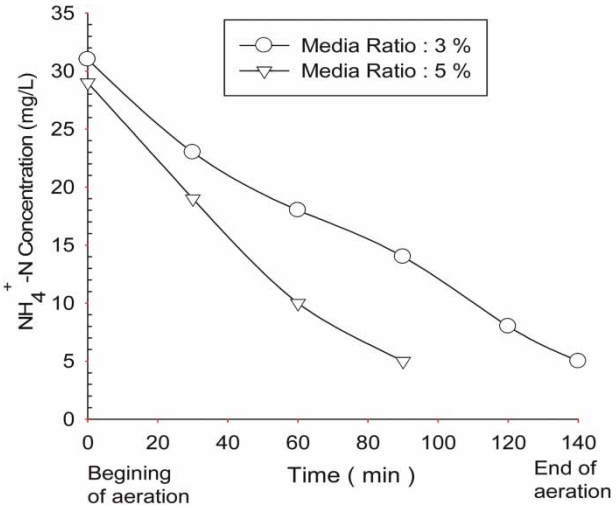

Figure 4 | Effect of pellet ratio on nitrogen removal performance at 11 °C and ~1.7 mg-DO/L.

Although long-term nitritation was achieved in all cases in the literature, the ALR values were low, mostly less than 0.2 kg-N/m^3-d. The ALRs observed in this study were three to four times higher than those in the literature under comparable reaction conditions.

Efficacy of the process

Although an SBR type of reactor is advantageous as it provides substantial FA inhibition at the beginning of each cycle, the settling time becomes a critical parameter that governs the hydraulic selection of sludge particles (i.e. a shorter settling time permits retention of only the good

Table 1 | Comparison of nitritation performances with relatively low ammonium concentrations[a]

Feed ammonium (mg-N/L)	ARR (%)	NAR (%)	ALR (kg-N/m^3-d)	Operational days	VER[b]	Temp (°C)	DO (mg/L)	Reactor type	References
50	100.0	80%	0.23	30	–	28 ± 0.5	1.5–3.0	SBR	Wu *et al.* (2007)
79	100.0	93.6%	0.16	80	0.65	23 ± 1	< 2.5	SBR	Yongzhen *et al.* (2007)
32	100.0	91.4%	0.15	60	–	18–25	~ 3.5	SBR	Guo *et al.* (2010)
40	100.0	96.2%	0.11	74	–	12–17	~ 3.5	SBR	
60	95.0	95.7%	0.33	17	–	25 ± 1	2.8 ± 0.7	CSTR	Peng *et al.* (2012)
40	100.0	83.3%	0.13	44	–	15.8	> 2.5	SBR	Yang *et al.* (2007)
40	95.0	99%	0.10	101		11.9	> 2.5	SBR	
30	96.0	87.7 ± 3.7	0.15	60	0.50	18–25	2.5–3.0	SBR	Guo *et al.* (2009b)
30	92.3	96.3%	0.14	–	–	–	< 3.0	SBR	Guo *et al.* (2009a)
~ 30	100.0	~ 98%	0.72	113	0.95	30	1.7 ± 0.1	SBR	This study
~ 30	100.0	~ 98%	~ 1.1	40	0.95	30	3.0 ± 0.1	SBR	This study
~ 30	> 95	~ 98%	0.66	13	0.95	20	1.7 ± 0.1	SBR	This study
~ 30	> 95	~ 98%	0.45	60	0.95	15	1.7 ± 0.1	SBR	This study
~ 30	> 87	~ 95%	0.30	30	0.95	11	1.7 ± 0.1	SBR	This study
~ 30	~ 85	~ 95%	0.45[c]	–	0.95	11	1.7 ± 0.1	SBR	This study

[a]Calculated on the basis of information given in articles.
[b]VER stands for volume exchange ratio, the ratio of the liquid volume decanted per cycle to the effective reactor volume.
[c]Pellet volume increased to 5%.

settling and heavier sludge flocs whereas the lighter and poor settling ones wash out, in a suspended-cell reactor). A short decanting time (resulting in a high flow rate) can also lead to the cell washout from the reactor. In particular, when the process involves slow growing microorganisms such as nitrifiers fed at low substrate concentrations, longer settling and decanting time is crucial for stable operation. An extension of the settling/decanting period reduces the ALR, hence affecting cost-effectiveness of the system. The higher ALRs in this study were partly the result of rapid settling and decanting. The use of 2 mm pellets permitted the reactor to sustain a stable and high biomass concentration with excellent settling compared to a suspended growth system. Our system also achieved robust nitritation caused by high surface area as compared to conventional 4 mm pellets, thus resulting in the reduction in the pellet ratio (usually 15–20%) (Isaka *et al.* 2007), while in our case only 4.0%). The 2 mm pellet size allowed excellent settling and decanting. The time requirements were as short as 1–2 and 2–3 min respectively, which were incomparable to the usual 1–2 h settling (Irvine *et al.* 1997; Rim *et al.* 1997) and 30–40 min decanting for suspended flocs (Yang *et al.* 2007). More importantly, as the pellets settled and remained stationary at the bottom, up to 95% of the cleared supernatant (suspended solids < 3 mg/L) could be decanted at the end of each cycle giving a volume exchange ratio (VER) of 0.95. This level of VER was far higher than the commonly observed VERs of less than 0.5 (Table 1). A larger VER allowed higher FA availability in an SBR as well as an increased ALR.

Moreover the enhancement of the start-up process is a highly appropriate topic in the field of biological wastewater treatment, specifically when the process comprises slow-growing microbes. For a field application, the start-up is very advantageous, as it can otherwise be a highly time- and cost-consuming process. This study successfully exhibited the rapid start-up procedure of partial nitrification of low-strength wastewater. Reduced temperature (e.g. 11 °C) affected the NAR, which it reduced to 94%, due to the favorable growth conditions of NOB over AOB at lower temperatures. So at lower temperatures when substantial FA and FNA inhibition is also absent, other factors should be considered for efficient nitritation, such as heat shock treatment. Also for field application, when there are significant variations in the influent concentrations, controlling the reaction time (to have a residual ammonium concentration of 2–3 mg/L) is very important for nitrite accumulation. This problem can be mitigated by using the real-time control strategy (Guo *et al.* 2009b). Based on these results, we have been successfully operating a

pilot-scale plant for several months, nitritating anaerobically digested sewage, whose ammonium concentration is ~25 mg-N/L. The pilot plant comprises pre-denitrification and post-denitrification, in addition to nitritation, the results of which will be reported in the near future.

CONCLUSIONS

With a laboratory-scale SBR containing cell-immobilized PEG pellets (~2 mm cubes), very efficient nitritation of low-strength ammonium wastewater (30 ± 2 mg-N/L) was successfully demonstrated for over 300 days. The reaction was easily converted to partial nitrification mode within a month by feeding a relatively high ammonium substrate (~100 mg-N/L). Excellent ALRs (as high as ~1.1 kg-N/m^3-d), NARs (mostly over 97%), and ARRs (mostly over 95%) could be attained. DO was the major limiting substrate in this system; when the DO concentration was below 4 ± 0.1 mg/L and the NH_4^+-N concentration was above 5 ± 2 mg/L. The ammonium oxidation rate increased almost linearly with the bulk DO increase under these conditions. It suggested an optimum DO concentration of around 3 mg/L, considering the system stability and the oxygen transfer efficiency. Low temperatures mainly affected the reaction rate, but had little effect on nitritation stability. By increasing the pellet volume in the reactor, the system's performance could be boosted even at low temperatures.

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REFERENCES

American Public Health Association 2005 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Bae, W., Baek, S., Chung, J. & Lee, Y. 2001 Optimal operational factors for nitrite accumulation in batch reactors. *Biodegradation* **12** (5), 359–366.

Bernet, N., Dangcong, P., Delgenes, J. P. & Moletta, R. 2001 Nitrification at low oxygen concentration in biofilm reactor. *Journal of Environmental Engineering* **127** (3), 266–271.

Chung, J., Bae, W., Lee, Y.-W. & Rittmann, B. E. 2007 Shortcut biological nitrogen removal in hybrid biofilm/suspended growth reactors. *Process Biochemistry* **42** (3), 320–328.

Garrido, J., van Benthum, W., van Loosdrecht, M. & Heijnen, J. 1997 Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. *Biotechnology and Bioengineering* **53** (2), 168–178.

Guo, J., Peng, Y., Wang, S., Zheng, Y., Huang, H. & Wang, Z. 2009a Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. *Bioresource Technology* **100** (11), 2796–2802.

Guo, J. H., Peng, Y. Z., Wang, S. Y., Zheng, Y. N., Huang, H. J. & Ge, S. J. 2009b Effective and robust partial nitrification to nitrite by real-time aeration duration control in an SBR treating domestic wastewater. *Process Biochemistry* **44** (9), 979–985.

Guo, J., Peng, Y., Huang, H., Wang, S., Ge, S., Zhang, J. & Wang, Z. 2010 Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. *Journal of Hazardous Materials* **179** (1–3), 471–479.

Irvine, R. L., Wilderer, P. A. & Flemming, H.-C. 1997 Controlled unsteady state processes and technologies – an overview. *Water Science and Technology* **35** (1), 1–10.

Isaka, K., Yoshie, S., Sumino, T., Inamori, Y. & Tsuneda, S. 2007 Nitrification of landfill leachate using immobilized nitrifying bacteria at low temperatures. *Biochemical Engineering Journal* **37** (1), 49–55.

Isaka, K., Sumino, T. & Tsuneda, S. 2008 Novel nitritation process using heat-shocked nitrifying bacteria entrapped in gel carriers. *Process Biochemistry* **43** (3), 265–270.

Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. & Siegrist, H. 2009 Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. *Environmental Science & Technology* **43** (14), 5301–5306.

Lee, W. K., Chung, J., Bae, W., Park, S. J., Kim, Y., Lee, Y. W. & Park, D. W. 2004 Operational factor for nitrite accumulation from a mixed culture by cell-immobilization. *Journal of Industrial and Engineering Chemistry* **10** (6), 959–966.

Li, S., Chen, Y. P., Li, C., Guo, J. S., Fang, F. & Gao, X. 2012 Influence of free ammonia on completely autotrophic nitrogen removal over nitrite (CANON) process. *Applied Biochemistry Biotechnology* **167** (4), 694–704.

McCarty, P. L., Bae, J. & Kim, J. 2011 Domestic wastewater treatment as a net energy producer–can this be achieved? *Environmental Science & Technology* **45** (17), 7100–7106.

Park, S. & Bae, W. 2009 Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid. *Process Biochemistry* **44** (6), 631–640.

Park, S., Bae, W. & Rittmann, B. E. 2009 Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition. *Environmental Science & Technology* **44** (1), 335–342.

Park, S., Bae, W. & Rittmann, B. 2010 Multi-species nitrifying biofilm model (MSNBM) including free ammonia and free nitrous acid inhibition and oxygen limitation. *Biotechnology and Bioengineering* **105** (6), 1115–1130.

Peng, Y., Guo, J., Horn, H., Yang, X. & Wang, S. 2012 Achieving nitrite accumulation in a continuous system treating low-strength domestic wastewater: switchover from batch start-up to continuous operation with process control. *Applied Microbiology and Biotechnology* **94** (2), 517–526.

Rim, Y. T., Yang, H. J., Yoon, C. H., Kim, Y. S., Seo, J. B., Ryu, J. K. & Shin, E. B. 1997 A full-scale test of a biological nutrients removal system using the sequencing batch reactor activated sludge process. *Water Science and Technology* **35** (1), 241–247.

Sumino, T., Nakamura, H., Mori, N. & Kawaguchi, Y. 1992 Immobilization of nitrifying bacteria by polyethylene glycol prepolymer. *Journal of Fermentation and Bioengineering* **73** (1), 37–42.

Wu, C., Chen, Z., Liu, X. & Peng, Y. 2007 Nitrification–denitrification via nitrite in SBR using real-time control strategy when treating domestic wastewater. *Biochemical Engineering Journal* **36** (2), 87–92.

Yang, Q., Peng, Y., Liu, X., Zeng, W., Mino, T. & Satoh, H. 2007 Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities. *Environmental Science & Technology* **41** (23), 8159–8164.

Yongzhen, P., Shouyou, G. A. O., Shuying, W. & Lu, B. A. I. 2007 Partial nitrification from domestic wastewater by aeration control at ambient temperature. *Chinese Journal of Chemical Engineering* **15** (1), 115–121.

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