Response of NIPAAm-Ch Gel to Temperature Changes and Its Effectiveness on Nitrification as Medium for Immobilization

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ABSTRACT: Increase in mass transport resistance is one of the major difficulties in immobilization by the use of gels. Functional gels can change their volume and inner structure depending on ambient conditions as stimuli and may reduce the difficulty as a novel immobilization matrix. The response of poly *N*-isopropylacrylamide-co-chlorophyllin (NIPAAm-Ch) gel to temperature changes and its performance on nitrification with nitrifiers immobilized by the gel were investigated in a continuous-flow stirred-tank reactor (CFSTR) with cyclic temperature changes. The gel with an immobilized nitrifier swelled and shrank alternately with 1.2–1.6-fold volume change under a cyclic temperature change of 32–36°C with a period of 2 or 4 h in the reactor. Volume changes of the gel brought periodic changes of its structure that accelerated dissolved oxygen transfer and concentrated ammonium into, and as a result, promoted nitrification compared with that at constant temperature. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 681–686, 2007

Key words: gels; adsorption; stimuli-sensitive polymers; nitrification; immobilization

INTRODUCTION

Immobilized nitrifiers can resist being washed out of a reactor, as well as some adverse impacts on reaction; however, mass transfer resistance between bulk liquid and immobilized nitrifier increases and becomes a limit to nitrification.[1]

During the past two decades, the innovation of functional gels has attracted considerable attention in both research and practice, due to their property of response to ambient conditions. The response is considered effective for mass transfer between the gel and its surrounding environment. Poly (*N*-isopropylacrylamide) (NIPAAm) gel is one of the earliest studied functional gels and has a low critical solution temperature (LCST) at ~ 34°C.[2] When the gel is warmed above its LCST, it shrinks; when cooled below the LCST, it swells, reversibly. As the gel shrinks, water is squeezed out and vice versa (Fig. 1). Cyclic volume change of the gel acting like a "hydraulic pump" was considered to promote mass transfer between the gel/immobilized microorganism and bulk liquid.[3] NIPAAm and chlorophyllin copolymer (NIPAAm-Ch) gel has almost the same LCST as NIPAAm gel, but NIPAAm-Ch gel has a better response to temperature change than NIPAAm gel.[4] In addition, because of its ion radical, the gel is more hydrophilic than NIPAAm gel.

To apply NIPAAm-Ch gel to improve activity of immobilized nitrifier in the future, the response of NIPAAm-Ch gel to temperature stimuli and its usefulness on nitrification as an immobilization medium for the nitrifier were studied in continuous-flow reactors under temperature stimulation and constant temperature in the present study.

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MATERIALS AND METHODS

Nitrifier cultivation and immobilization

Activated sludge from an aeration tank in the East Wastewater Treatment Plant in Fukuoka, Japan was cultivated at 29°C in a cultivation reactor of the laboratory with a culture medium containing 229 mg of NH_4Cl, 706 mg of $NaHCO_3$, 67 mg of K_2HPO_4, and 46 mg of $MgSO_4{\cdot}7H_2O$ in 1 L of tap water. Ammonium nitrogen in influent was ~ 60 mg/L in concentration and was < 10 mg/L in effluent with hydrau-

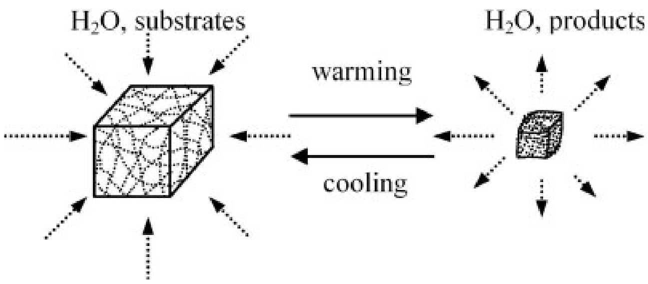

Figure 1 Schematic diagram of NIPAAm-Ch gel responding to temperature stimulus.

lic retention time (HRT) of 8 h as well as nitrate nitrogen was > 40 mg/L. The sludge was centrifuged at 1500*g* for 10 min to obtain nitrifiers for immobilization.

N-isopropylacylamide (7.8 g), *N*,*N*′-methylene-bis-acrylamide (0.133 g), and chlorophyllin coppered trisodium salt (0.361 g) were dissolved in 100 mL of distilled water. Nitrifiers with volatile suspended solid of 600 mg were suspended in the solution. Polymerization was initiated by adding 0.4 mL of *N*,*N*,*N*′,*N*′-tetramethylenediamine and 0.4 g of ammonium persulfate. This mixture was then transferred immediately into a mold with a size of 250 mm (L) × 120 mm (W) × 4 mm (H); the mold was sealed and kept at 7°C for 24 h. The polymer was removed from the mold and cut into cubes with a dimension of 3–4 mm for use.

Reactor

Two sets of CFSTR with a volume of 4 L were prepared (Fig. 2). Each was set in a water bath whose temperature was controlled automatically. Dissolved oxygen (DO), temperature, and pH value were monitored by an on-line system. Temperature in Reactor A was cyclically changed from 32°C to 34°C or 36°C with regulated periods. Temperature in Reactor B was kept constant at 32°C as the reference of Reactor A. In the reactors, pH was maintained at 7.4–7.5.

Wastewater

The composition of synthetic wastewater used in this experiment was the same as that in the nitrifier cultivation mentioned above. The concentration of ammonia nitrogen in the solution was changed through the experiment, but the ratio of C : N : P remained unchanged.

Observation of the gel with electronic microscopy

Gels under cyclic temperature change were used for electronic microscopy observation. After quickly removing water at the gel surface by soaking with filter papers, they were frozen with liquid nitrogen and dried by a freeze drier (VD-800F; TAITEC. Koga, Fukuoka, Japan). The dried gels were cut into slices, and their sections were observed by scanning electronic microscopy (SEM)(SS-550; Shimatsu. Kyoto, Japan).

Adsorption of ammonium and nitrate by the gel

Gels without immobilizing nitrifier, taken from deionized water of 20°C or 40°C, were wiped to remove excessive water on the surface. After weighed, the gels were put into NH_4NO_3 solution at 20 ± 0.5°C or 40 ± 0.5°C with a volume ratio of solution to the gel of < 50. Concentrations, the volume of the solution, and the weight and volume of the gel were measured at both the beginning and end of the experiment.

Response of the gel to temperature stimulus

Gels of 30 pieces with nitrifier immobilized in the reactor at different temperature were weighted (W). When the temperature was lowered from 36°C or 34°C, the swelling ratio of the gels was calculated by

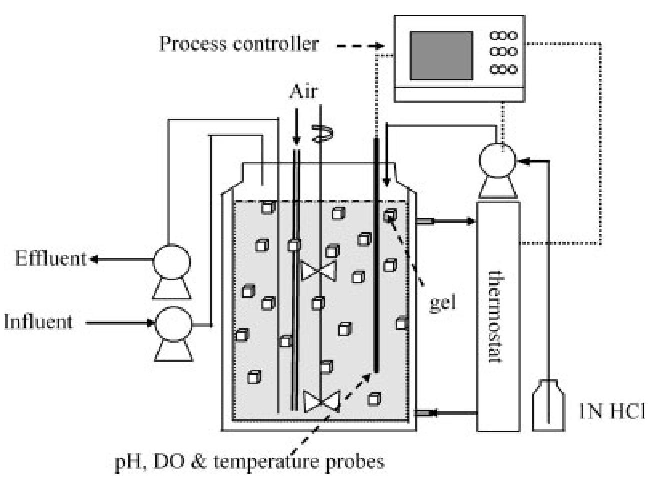

Figure 2 Schematic diagram of the reactor and its thermoregulated system.

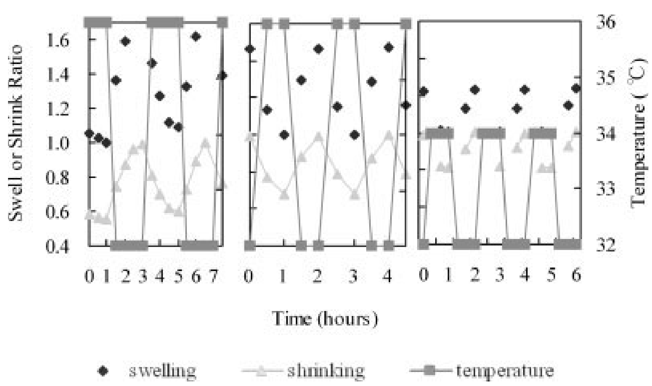

Figure 3 Changes of shrinking and swelling ratio of the NIPAAm-Ch gels responding to three temperature stimuli in Reactor A. Left: 32°C : 36°C, 2 h : 2 h. Middle: 32°C : 36°C, 1 h : 1 h. Right: 32°C : 34°C, 1 h : 1 h.

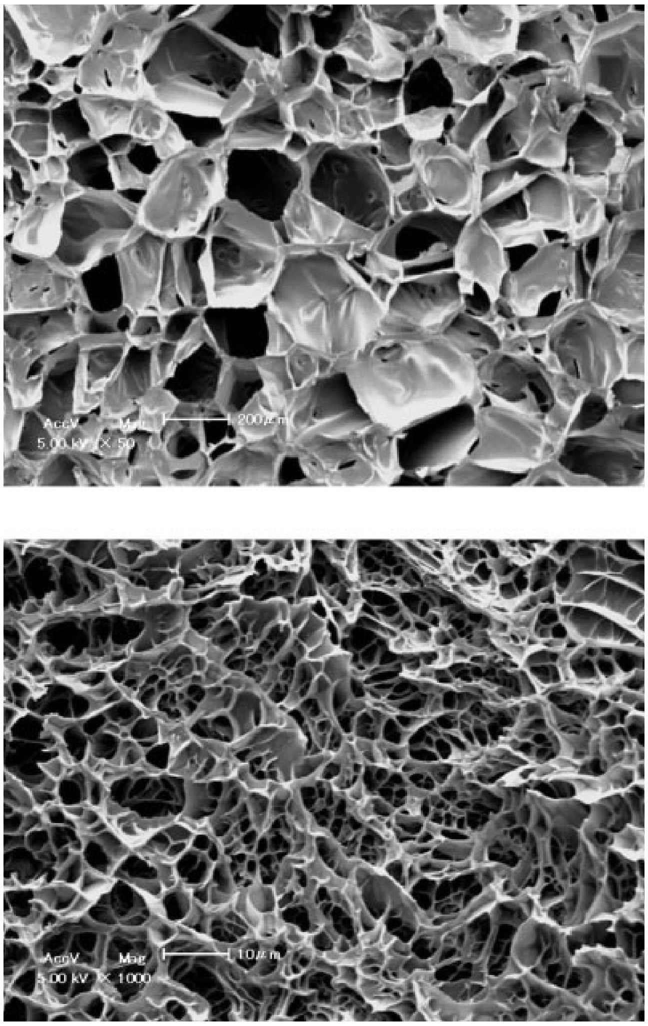

Figure 4 Electronic microscope of the NIPAAm-Ch gels at 25°C (top) and 36°C (bottom).

W/W_{36}°C or W/W_{34}°C, in which W_{34}°C and W_{36}°C represent the minimal weight of the gels sampling at 34°C and 36°C, respectively. The shrinking ratio of the gel was calculated by W /W_{32}°C in the case of the temperature rise from 32°C, in which W_{32}°C represents the maximal weight of the gel sampling at 32°C.

Analysis method

Nitrogens of ammonium, nitrite, and nitrate were analyzed using an Autoanalyzer 3 (Bran + Luebbe, Saxonburg, PA).[5] The measurement method of DO in the gel with a microelectrode was based on that described by Satoh et al.[6]

RESULTS

Response of the gel to temperature stimuli

Figure 3 shows shrinking and swelling ratios of the gel in three kinds of temperature stimuli in Reactor A. When temperature rose from 32°C to 36°C, the gel shrank and became lighter. When temperature dropped from 36°C to 32°C, the gel swelled and became heavier. Under a cyclic thermal operation of 36–32°C, the weight of the gel changed ~ 1.4–1.6 times. When the temperature was changed from 36°C to 34°C, the change in gel weight reduced to ~ 1.2 times. The bigger the change of temperature, the greater the weight difference was. When the period of temperature stimulus was shortened from 4 to 2 h, the response of the gel lessened, possibly because the gel had less time to shrink and swell thoroughly. This result shows that the gel had good response to cyclic temperature stimulus for longer periods.

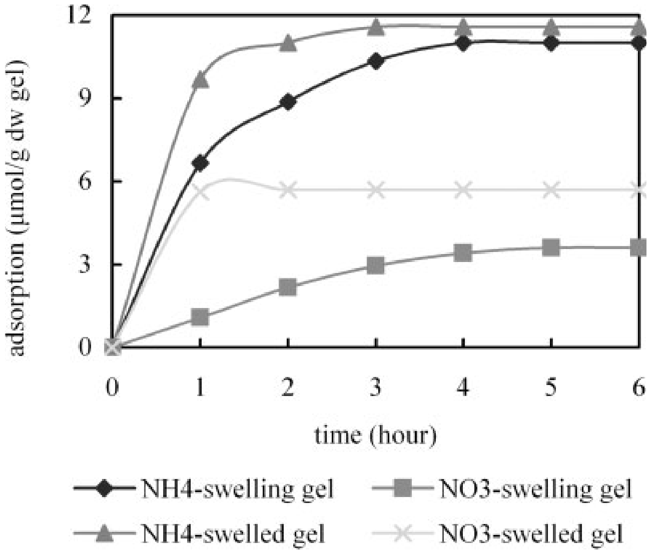

Figure 5 Adsorption of the NIPAAm-Ch gel on ammonium and nitrate ions.

Structural change of the gel

SEMs of the gel at 25°C and 36°C are shown in Figure 4. The gel is shown to be porous. Pores in the shrunken gel are smaller than those in the swollen gel. Whenever the gel shrank and swelled alternately, the pores also

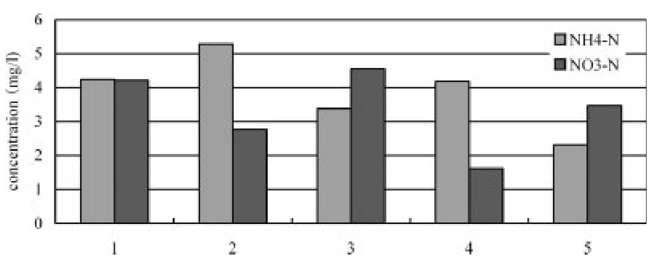

Figure 6 Concentrations of ammonium and nitrate ions in solution and the gels: 1, original solution without gel; 2, the gel that was swelling in the solution; 3, the remaining solution after the swelling gel drew water in and adsorbed ions; 4, the swollen gel that did not draw water in and change its volume in the solution; and 5, the remaining solution after the swollen gel adsorbed ions only.

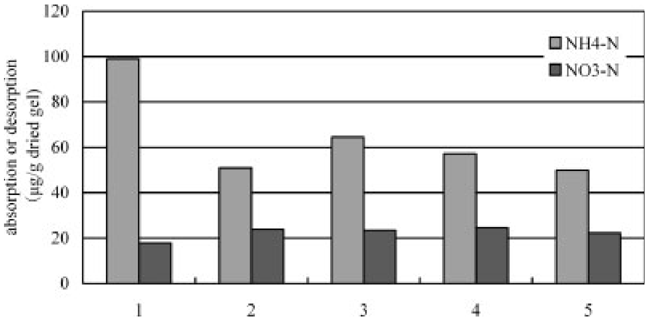

Figure 7 Quantity of ammonium and nitrate ions adsorbed in the gel and discharged into bulk solution when it swelled and shrank alternately in the solution responding to temperature changes. 1 (adsorbed-swelling) → 2 (discharged-shrinking) → 3 (adsorbed-swelling) → 4 (discharged-shrinking) → 5 (adsorbed-swelling).

changed reversibly. Their reversible change responding to temperature is a piece of evidence on the benefit to mass transfer.

Effects of volume change on mass transfer

Adsorptions of ammonium and nitrate ions by the swelling and swollen gels are shown in Figures 5. Both gels were found to adsorb more ammonium ion than the nitrate ion, and the swelling gel was more apt to adsorb ammonium ion than the swollen gel. Figure 6 shows the concentrations of the two ions in the gels and solutions after the gels were put in. The concentration of ammonium ion in the gels was higher than in the bulk solution, i.e., ammonium ion was concentrated in the gels. On the contrary, that of nitrate was not. The concentration of ammonium ion in the swelling gel was higher than that in the swollen one. While the gel was made to swell and shrink alternately, almost all nitrate ion and part of ammonium ion adsorbed by the gel during its swelling were discharged into the bulk solution with water during the shrinking process (Fig. 7). This finding suggests that while the gel was swelling or shrinking, the gel drew in mainly the ammonium ion and discharged drew in mainly the nitrate ion. This result was also supported by the observation that temperature cyclic change enhanced to transport substrates and products from a temperature stimulus responsive gel.[7] Because of the similarity between the nitrite ion and the nitrate ion, it is considered that the adsorption of nitrite ion by the gel under temperature stimuli would be the same as nitrate ion.

DO diffusion profiles in the gel under temperature stimulus

DO diffusion profiles in the gel with nitrifier immobilized under several conditions during swelling and shrinking are shown in Figure 8. Oxygen reached a

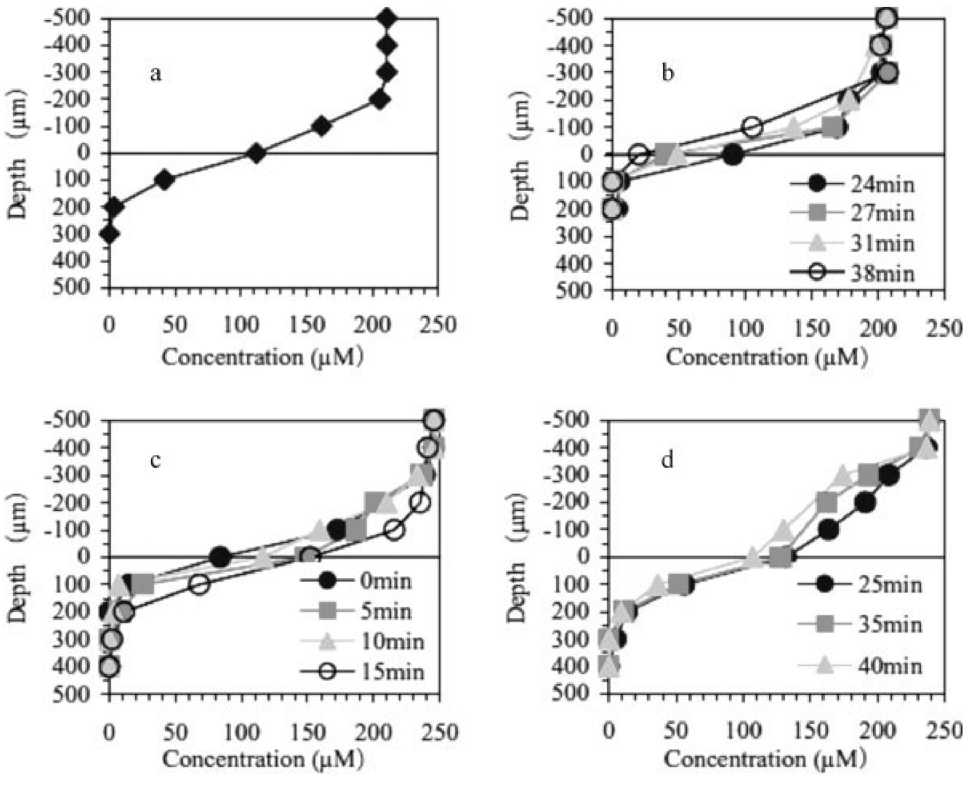

Figure 8 Concentration profiles for DO in the gels under different situations. (a) 27°C. (b) temperature increased from 27°C to 35°C. (c) temperature lowered from 36°C to 28°C. (d) temperature lowered from 36°C to 28°C. The gel surface is at zero. Minus sign indicates the direction toward solution.

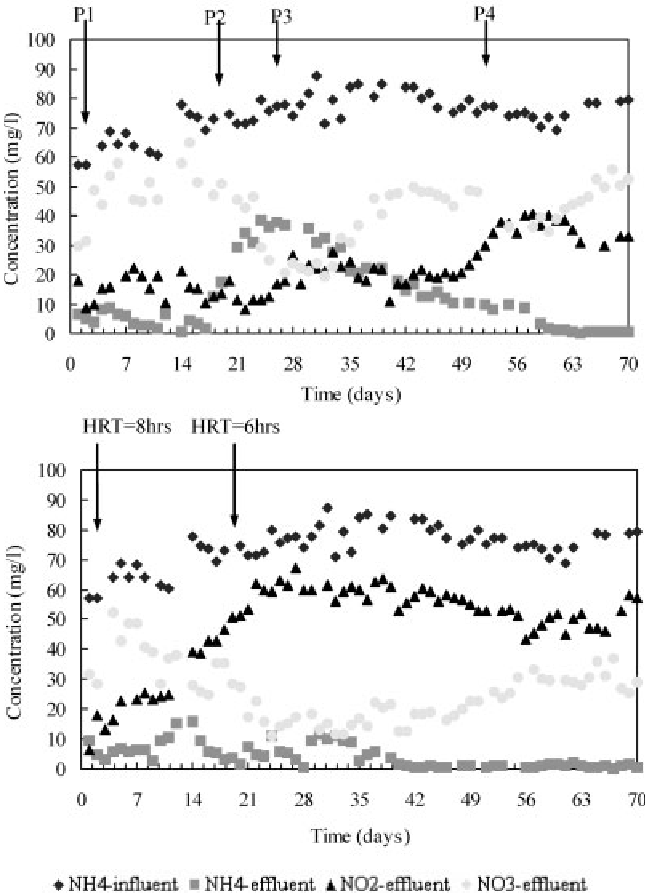

Figure 9 Nitrification in the two reactors under different operations. Top: Reactor A. Bottom: Reactor B. (P1) 32°C : 36°C, 2 h : 2 h, HRT, 8 h. (P2) 32°C : 36°C, 2 h : 2 h, HRT, 6 h. (P3) 32°C : 36°C, 1 h : 1 h, HRT, 6 h. (P4) 32°C : 34°C, 1 h : 1 h, HRT, 6 h.

maximum depth of ~ 300 μm in the swelled gel at 27°C. When the gel began to shrink at 35°C, the depth of oxygen reaching into the gel decreased to ~ 200 μm after 38 min. Oxygen diffused deeper into the gel and reached a maximum depth of ~ 400 μm after 40 min in the swelling phase.

Declining DO in the solution with temperature rise shortened the distance of oxygen transfer into the gel, but while the gel was shrinking, not only the distance of oxygen transfer, but also the concentration of DO at the surface of the gel decreased. As the gel was swelling, the distance of oxygen transfer and concentration of DO at the surface of the gel increased as time went on. DO changes in the gel differ from any other results of a biofilm and media immobilized microorganism.[8,9] Alternately, swelling and shrinking promoted oxygen diffuse deeper into the gel. Although the measurements were done in batch tests where temperature stimuli differed slightly from those in the reactors, the results obtained still clearly suggested that there was a difference between swelling and shrinking gels in oxygen concentration.

Nitrification by nitrifier immobilized in the gel under different temperature stimuli

Nitrification with a nitrifier immobilized in CFSTR under several kinds of temperature stimuli and operating conditions is shown in Figure 9. Under a condition of 8-h HRT and a period of cyclic temperature stimulation (32°C → 36°C → 32°C) of 4 h, the removal ratio of ammonia nitrogen was up to 95%, and the NO_3–N/NO_x–N ratio was near 77% in the effluent of Reactor A. In Reactor B, the removal ratio of ammonia nitrogen was above 90%. Nitrate nitrogen decreased and nitrite nitrogen increased in effluent, the NO_2–N/NO_x–N ratio increased from 24% of the starting to 63% with the increase in ammonium concentration in influent, and nitritation was predominant in Reactor B. It was considered that nitrite oxidation was inhibited due to oxygen deficient in Reactor B. In contrast, the volume change of the gel under cyclical temperature stimulus made nitrite oxidizer obtain enough oxygen to oxidize nitrite to nitrate in Reactor A. It is concluded that under this temperature stimulus and HRT of 8 h, both ammonium removal and nitrite oxidization rates were promoted compared with those without temperature stimulus.

Reducing HRT to 6 h, nitrification deteriorated in Reactor A, though the same temperature stimulus was kept as before. About 48% of ammonia was not oxidized and nitrate nitrogen decreased obviously, but the NO_3–N/NO_x–N ratio was still as high as 67.2% in effluent from Reactor A. In Reactor B, nitrite nitrogen in effluent accumulated continuously (the NO_2–N/NO_x–N ratio was about 80%) after shortening HRT to 6 h. Keeping an HRT of 6 h but shortening the cyclic period of temperature stimulus to 2 h in Reactor A, the concentration of ammonia nitrogen reduced and that of nitrate nitrogen increased simultaneously in effluent. About 4 weeks later, ammonia nitrogen in effluent decreased to 10–20 mg/L in Reactor A. In Reactor B, ammonium was still oxidized mainly to nitrite. When the temperature stimulus was changed to 32°C → 34°C → 32°C, nitrate nitrogen increased and the ammonia nitrogen in effluent decreased to < 10 mg/L, the NO_3–N/NO_x–N ratio in the effluent was about 67%, with an ammonia nitrogen removal ratio of 96.2% in Reactor A, but in Reactor B, the NO_3–N/NO_x–N ratio was about 36.5–40% with an ammonia nitrogen removal ratio of 98.6%. Ammonia removal in both reactors was almost the same, but more nitrite was oxidized to nitrate under temperature stimulation with HRT of 6 h. It is suggested that under the cyclic temperature stimulus of 32°C → 34°C → 32°C with a period of 4 h and HRT of 6 h, nitrification was also better than that at constant temperature.

DISCUSSION

Nitrification in activated sludge generally occurs at a temperature of 15–30°C and the nitrification rate increases with temperature rise, but when temperature rises above 30°C, the nitrification rate of activated sludge declines greatly with the rise in temperature. Immobilization can lower the sensitivity of microorganisms to temperature,[10] so that the nitrification of immobilized nitrifier may be accomplished well at 32–36°C.

In the case of nitrifiers entrapped by NIPAAm-Ch gels, temperature showed complex effects on nitrification. It not only affects the activity of entrapped nitrifier directly, but also leads to significant changes in the properties of the gel ensuing after the changes in activity of the entrapped nitrifier when the temperature was changed through LCST. Park and Hoffman[3] reported that the activity on steroid conversion of *Arthrobacter simplex* immobilized by NIPAAm gel or NIPAAm-AAm (acrylamide), was higher at the thermal cycling operation of 27°C−32°C or 30°C−35°C than at 25°C, 30°C and 35°C, even though the diffusibility of the gel dropped sharply when temperature rose above 30°C. Warinpaisan[11] also indicated that the nitrifying rate of nitrifier immobilized by NIPAAm gel with cyclic thermal operation at 25–35°C was higher than that of a constant temperature of 25°C, 28°C or 35°C in batch tests. Both suggested that without cyclic temperature change, the activity of immobilized microorganisms at temperature either above the gel's LCST or below was lower than that with the temperature stimulus. The results of applying NIPAAm-Ch gel to immobilize the nitrifier in CFSTR were also in agreement, implying that the high activity of immobilized nitrifier at cyclic temperature change did not result from rising temperature but rather from alternate shrinking and swelling of the gel.

Although the diffusion rate of substances generally increases with temperature, and diffusing distance between immobilized nitrifier and bulk liquid becomes shorter when temperature rise causes gel to shrink, the diffusibility of substrates decreased.[3] Diffusions of DO (Fig. 8), ammonium and nitrate (Fig. 6) from bulk liquid to the shrunken gels are less than those to the swelling gels, but during swelling of the gel, which was followed its shrinking, these substances were the ''draw'' phase and were concentrated in the gel. It is the alternate change of gel structure (Fig. 4) that accelerates substance transfer between the gel and main liquid during swelling of the gel. Consequently, the activity of immobilized nitrifier was improved.

At temperatures higher than 34°C, the gels shrink, thus lowering the activity of nitrifier temporally; it was the repeated swelling and shrinking of the NIPAAm-Ch gel stimulated by proper cyclic temperature change, rather than high temperature, that promoted nitrification due to accelerated substance exchange between liquid and nitrifier in the gel.[12] In terms of the volume change of the gel, a high temperature of 36°C was just to make the gels shrink. In actual wastewater treatment that applied nitrifiers immobilized by the gels, it is infeasible to make the temperature of wastewater and the gels in a reactor cyclically change as was done in the reactor described in the present work, but it is possible to warm only part of the gels in the outside of the reactor and to put shrunken gels into the reactor back again. This procedure would make the gels work well to increase the efficiency of treatment.

CONCLUSION

In response to a cyclic temperature change, the gel with immobilized nitrifier swelled and shrank alternately, resulting in changes in the structure and substrate concentrations of the gel. The periodical changes facilitate DO transfer into gels, concentrated ammonium ion in the gel. Cyclic temperature stimulus (36–32°C, a period of 4 h with HRT of 8 h and 34–32°C, a period of 2 h with HRT of 6 h) improved nitrification by immobilized nitrifier compared with that at constant temperature. The temperature–stimulus-responsive gel as a novel kind of immobilization matrix with temperature stimulus is better than non-temperature-stimulus-responsive gels on improving mass transfer and keeping activities of immobilized nitrifier.

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References

1. Wijffels, R. H.; Englund, G.; Hunik, J. H.; Leenen, E. J. T. M.; Bakketun, A.; Gunther, A.; Decastro, J. M. O.; Tramper, J. Biotech Bioeng 1995, 45, 1.
2. Tanaka, T. Phys Rev Lett 1978, 40, 820.
3. Park, T. G.; Hoffman, A. S. Biotech Bioeng 1989, 35, 152.
4. Kumabe, K.; Etoh, M.; Kuba, T.; Kusuda, T. J Jpn Soc Water Environ 2001, 24, 601.
5. Lenore, S. C.; Arnold, E. G.; Andrew, D. E. Standard Methods for the Examination of Water and Wastewater; 20th ed.; American Public Health Association: Washington, DC, 1998.
6. Satoh, H.; Okabe, S.; Yamaguchi, Y.; Watanabe, Y. Water Res 2003, 37, 2206.
7. Park, T. G.; Hoffman, A. S. Biotech Bioeng 1989, 35, 152.
8. Jun, B. H.; Tanji, Y.; Unno, H. J. Biosci Bioeng 1999, 89, 334.
9. Okabe, S.; Satoh, H.; Watanabe, Y. Appl Environ Microsc 1999, 65, 3182.
10. Asano, H.; Myoga, H.; Asano, M.; Toyao, M. Water Sci Technol 1992, 26, 1037.
11. Warinpaisan, B.; Kuba, T.; Irie, M.; Kusuda, T. In Proceedings of the Sixth IWAQ Asia-Pacific Regional Conference, Souel, 1997; 1216.
12. Yuan, L.; Kusuda, T.; Kuba, T. J Environ Sci Health 2004, A39, 1781.