

### Characteristics of nitrification

#### by adhesived and immobilized nitrifiers on NIPAAm-gel

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#### 1. Introduction

Nitrification is a biological process by which ammonium is converted first to nitrite and then to nitrate. Two bacteria genera responsible for nitrification are *Nitrosomonas* (oxidizes ammonium to nitrite) and *Nitrobacter* (oxidizes nitrite to nitrate). Nitrification is often a problem because nitrifiers are sensitive and extremely susceptible to a wide variety of inhibitors and cause a slow rate of nitrification. Therefore it is necessary to investigate other effective nitrification processes. In this study, nitrifiers were adhesived or immobilized by NIPAAm-gel and NIPAAm-AA gel. For adhesion, gel works as attached medium to nitrifiers which grow in the same way as in the attached-growth biological treatment process. For immobilization, nitrifiers are immobilized in gel with retention of their catalytic activity. Functional gels, shrink and swell when temperature changes (for NIPAAm gel) or pH-value changes (for NIPAAm-AA gel), and a higher rate of nitrification can be obtained by use of it. Together with physical properties of the gels, that can be used repeatedly, can continuously operate for a long period and can not be simultaneously decomposed by harmful substances in waste water, adhesived and immobilized nitrifiers are stable compared with intact cells. Nitrifiers can exist and nitrification can be accomplished on both studied gel.

#### 2. Material and methods

##### 2.1 Gelpolymerization

-Gelpolymerization for adhesived nitrifiers; all components were mixed and then added into tubes to make a suitable sized gel (5 mm in diameter and 5 mm in length). Stand the mixture at 60° c for 90 min to obtain a stiff gel. (Components of gelpolymerization for adhesion are given in Table 1.) Nitrifiers were collected from a cultured reactor and suspended solution were made. With the suspension, gels were mixed and shaken at 100 time/min 25° c. After being shaken for 24 hrs, nitrifiers adhesived on the gels.

-Gelpolymerization for immobilized nitrifiers; nitrifiers collected from a cultured reactor were suspended in physiological saline. To the suspension, N-Isopropylacrylamide (NIPAAm), a monomer of polymerization, and N,N'-Methylene-bis-(acrylamide) (BIS), cross-linking reagent, (and Acrylic Acid (AA), a copolymer of polymerization, in case of NIPAAm-AA-gel) were added and mixed. With the mixture, 6% NNNN'-tetramethylethylenediamine, an accelerator of polymerization, and 2.2% Ammonium persulfate, an initiator of polymerization, were added and mixed. The reaction mixture was allowed to put into microtubes (5 mm in diameter) to make a suitable sized gel (5 mm in diameter and 5 mm in length). Stand at 37° c for 30 min to obtain a stiff gel. (Components of gelpolymerization for immobilization are given in Table 2.)

##### 2.2 Experiment

Cultivated gels made from the previous steps were incubated to obtain more nitrifiers for 2~3 days. (Contents of the culture medium are given in Table 3.) In experiments sampling was conducted to measure the concentration of ammonium (NH<sub>4</sub>-N mg/L) which was consumed by nitrifiers and the concentrations of nitrite (NO<sub>2</sub>-N mg/L) and nitrate (NO<sub>3</sub>-N mg/L) produced by nitrifiers. In this study, blank tests (in which gels contain dead bacterial cells or no bacterial cell) were run.

Table 1: Components of gelpolymerization for adhesion method

Component	NIPAAm-gel	NIPAAm-AA-gel
NIPAAm (g.)	4.0	3.6
Acrylic acid (g.)	-	0.4
BIS (mg.)	80	400
AIBN (mg.)	60	60
DMSO (ml.)	4	4

Table 2: Components of gelpolymerization for immobilization method

Component	NIPAAm-gel	NIPAAm-AA-gel
NIPAAm (g.)	2.388	2.1492
Acrylic acid (g.)	-	0.2388
BIS (mg.)	80	400
6% NNNN'-tetramethylethylenediamine (ml.)	1.0	1.5
2.2% Ammonium persulfate (ml.)	1.0	1.5
Saline Solution (ml.)	8.0	7.0
Nitrifiers wet weight (g.)	0.5	0.5

Table 3: Contents of culture medium

Content	mg/l
NH <sub>4</sub> <sup>+</sup>	20
KH <sub>2</sub> PO <sub>4</sub>	200
K <sub>2</sub> HPO <sub>4</sub>	1000
NaHCO <sub>3</sub>	180
NaCl	300
MgSO <sub>4</sub>	200

### 3. Results and discussion

Ammonium nitrogen( $\text{NH}_4\text{-N}$ ) was removed in every experiment(refer to Fig 1,3,5 and 7) but the removal rate was different in each experiment. On NIPAAm gel (refer to Fig 1 and 3),  $\text{NH}_4\text{-N}$  was removed from 20 to 0 mg/l in 5 days by immobilized nitrifiers and in 7 days by adhesived nitrifiers. On a continuous addition of new substrate,  $\text{NH}_4\text{-N}$  was removed completely by immobilized nitrifiers within 2 days while more than 3 days by adhesived nitrifiers. On NIPAAm-AA gel (refer to Fig 5 and 7), the results was quite different from NIPAAm gel.  $\text{NH}_4\text{-N}$  was removed from 20 to 0 mg/l in 5 days by adhesived nitrifiers and in 7 days by immobilized nitrifiers. On a new dose of substrate, it took more than 3 days for both adhesived and immobilized nitrifiers to consumed.

Considering adhesion by both studied gel, adhesived nitrifiers could escaped from the gel by aeration in experiment, and work efficiently as free nitrifiers, so that nitrification was not occurred exactly by the adhesived nitrifiers but by both of adhesived and free nitrifiers. When the old substrate was discarded before add new substrate, free nitrifiers were also discarded from the system. That caused slower rate of nitrification than the previous one.

Concerning immobilization, nitrifiers were immobilized in gels so that nitrifiers were not lost from gel lattice, while the low molecular substrate could pass freely through gels and nitrification occurred completely by immobilized nitrifiers. But these results were not accompanied with immobilized nitrifiers on NIPAAm-AA gel. The cause may be due to the proportion(10%) and toxicity of Acrylic acid(AA) effecting to immobilized nitrifiers. Blank tests (refer to Fig 2,4,6 and 8) showed a loss of nitrogen compounds when time passed due to the adsorption effect occurred through ion-exchange reaction with charged particle on gel.

### 4. Conclusion

By adhesived and immobilized nitrifiers on NIPAAm gel and NIPAAm-AA gel, nitrification can be accomplished, especially immobilized nitrifiers on NIPAAm gel. It showed good results and is advantageous for practical use. But in case of NIPAAm-AA gel, it can be attributed to Acrylic acid being toxic to adhesived and immobilized nitrifiers, so it needs more investigation on the proportion and characteristics of Acrylic acid. Blank tests also suggest that the adsorption effect occurs on both of NIPAAm gel and NIPAAm-AA gel.

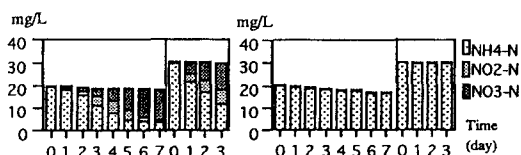


Fig1. Nitrification by adhesived nitrifiers on NIPAAm gel.

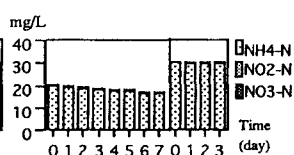


Fig2. Adsorption Effect by NIPAAm gel for Adhesion Method.

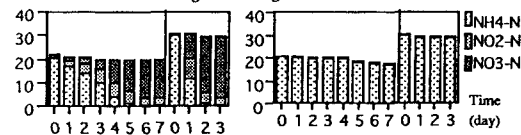


Fig3. Nitrification by immobilized nitrifiers on NIPAAm gel.

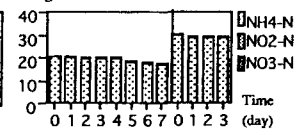


Fig4. Adsorption Effect by NIPAAm gel for Immobilization Method.

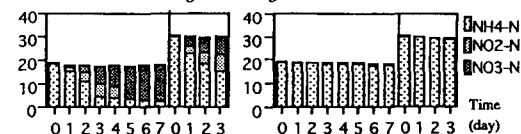


Fig5. Nitrification by adhesived nitrifiers on NIPAAm-AA gel.

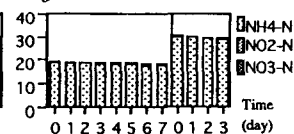


Fig6. Adsorption Effect by NIPAAm-AA gel for Adhesion Method.

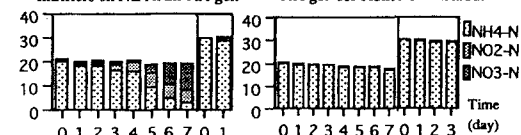


Fig7. Nitrification by immobilized nitrifiers on NIPAAm-AA gel.

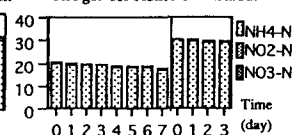


Fig8. Adsorption Effect by NIPAAm-AA gel for Immobilization Method.