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Nitrification using immobilized nitrifiers on NIPAAm, NIPAAm-AA and NIPAAm-MA gels

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1. Introduction

Nitrification is a fragile process because nitrifiers are sensitive and extremely susceptible to a wide variety of inhibitors that inhibit their growth and action. In this study, nitrifiers were immobilized on gels. We chose N-Isopropylacrylamide(NIPAAm) gel consisting of a polymer network and pure water. NIPAAm gel undergoes a volume-phase transition in response to temperature change while N-Isopropylacrylamide acrylic acid(NIPAAm-AA) gel and N-Isopropylacrylamide methacrylic acid(NIPAAm-MA) gel in response to pH and temperature changes. By using the volume-phase transition of the gels which shrink and swell reversibly, nitrification may be simulated and a higher rate of nitrification might be obtained. Together with physical properties of the gels, that can be used repeatedly and continuously operate for a long period and can not be simultaneously decomposed by harmful substances in waste water, so that immobilized nitrifiers are stable compared with intact cells. In the present works, nitrifiers were immobilized on NIPAAm, NIPAAm-AA and NIPAAm-MA gels. Gelpolymerizaion of all gels was investigated systematically. Experimental results indicate that the immobilized nitrifiers remain viable and nitrification is effectively accomplished on NIPAAm and NIPAAm-MA gels. Furthermore, for NIPAAm-AA gel, it can be attributed to acrylic acid being toxic to the immobilized nitrifiers.

2. Material and methods

2.1 Gelpolymerization

Nitrifiers collected from a cultivation reactor were suspended in physiological saline. To the suspension, NIPAAm, a monomer of polymerization and N,N'-Methylene-bis-(acrylamide)(BIS), a cross-linking reagent (and AA for NIPAAm-AA gel or MA for NIPAAm-MA gel, a copolymer of polymerization) were added and mixed. To the mixture, 2.2% Ammonium persulfate, an initiator of polymerization and 6% NNN'N'-tetramethylethylene diamine, an accelerator of polymerization were added and mixed. The mixture was allowed to put into microtubes(5 mm in diameter). After reacting at 37°C for 30 min, the resulting stiff gel was granulated to a suitable cylindrical size(5 mm in diameter and 5 mm in length) and used for experimental observation. Components of gelpolymerization are given in Table 1.

2.2 Experiments

Cultivated gels made from the previous steps were reincubated for 2-3 days. Contents of the culture medium are given in Table 2. Experimental sampling was conducted to measure the concentration of ammonium($\text{NH}_4\text{-N}$ mg/L) which was consumed by nitrifiers and the concentrations of nitrite ($\text{NO}_2\text{-N}$ mg/L) and nitrate($\text{NO}_3\text{-N}$ mg/L) which were produced by nitrifiers. In this study, blank tests(in which gels contained dead bacterial cells) were executed.

Table1. Components of Gelpolymerization

Component	NIPAAm	NIPAAm-AA	NIPAAm-MA
NIPAAm (g.)	2.388	2.3164	2.3164
Acrylic acid (g.)	-	0.0716 (3%)	-
Methacrylic acid (g.)	-	-	0.0716 (3%)
BIS (mg.)	80	400	400
6% NNN'N'-tetramethylethylene diamine(ml.)	1.0	1.5	1.0
2.2% Ammonium persulfate(ml.)	1.0	1.5	1.0
Saline Solution (ml.)	8.0	7.0	8.0
Nitrifiers wet weight (g.)	0.5	0.5	0.5

Table 2. Contents of the culture medium

Content	mg/L
$\text{NH}_4\text{-N}$	20
KH_2PO_4	200
K_2HPO_4	1000
NaHCO_3	180
NaCl	300
MgSO_4	200

3.Results and discussion

By immobilized nitrifiers, nitrification occurred on all studied gels(refer to Fig 1,3 and 5). Removal rates of ammonium nitrogen ($\text{NH}_4\text{-N}$) were different in each experiment. On NIPAAm gel, $\text{NH}_4\text{-N}$ was removed from 30 to 0 mg/L in 3 days. By batch experiment, $\text{NH}_4\text{-N}$ was removed completely within 1 day by the first and second new substrate addition. On NIPAAm-AA gel, $\text{NH}_4\text{-N}$ was removed from 30 to 0 mg/L for more than 7 days and on the new substrate addition, it took more than 3 days for immobilized nitrifiers to consume. But on NIPAAm-MA gel, the gel which response to pH and temperature changes similar to NIPAAm-AA gel, $\text{NH}_4\text{-N}$ was removed from 30 to 0 mg/L in 5 days and on the new substrate addition, it took only 2 days for complete consumption by immobilized nitrifiers.

Considering immobilization, nitrifiers were immobilized on the gels so that the nitrifiers were not lost from gel lattice, while low molecular substrates could pass freely through the 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 toxicity of the acrylic acid(AA) effecting directly to immobilized nitrifiers.

Blank tests(refer to Fig 2,4 and 6), showed a loss of nitrogen compounds when time passed due to the adsorption effect of ammonium ion (NH_4^+) which occurred through ion-exchange reaction with charged particles on all studied gels.

4.Conclusion

Experiments conducted indicate that nitrification can be accomplished by the immobilized nitrifiers on all studied gels, especially immobilized nitrifiers on NIPAAm gel for application of the gel which response to temperature and on NIPAAm-MA gel for the application of the gel which response to pH and temperature. It showed good results and is advantageous for application use. But in case of NIPAAm-AA gel, it can be attributed to acrylic acid being toxic to immobilized nitrifiers. Blank tests also suggest that the adsorption effect of ammonium ion(NH_4^+) occurs on all studied gels.

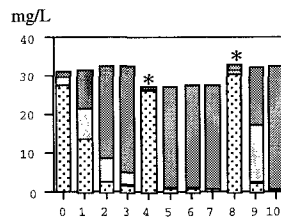


Fig1. Nitrification by immobilized nitrifiers on NIPAAm gel

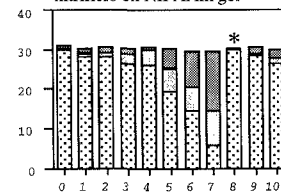


Fig3. Nitrification by immobilized nitrifiers on NIPAAm-AA gel

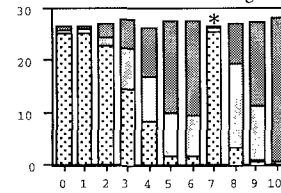


Fig5. Nitrification by immobilized nitrifiers on NIPAAm-MA gel

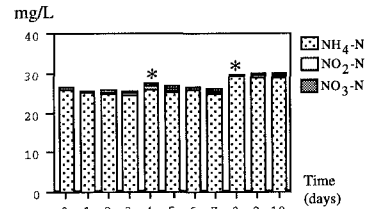


Fig2. Effect of NIPAAm gel on nitrification by blank test

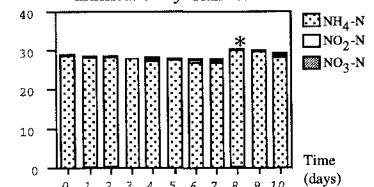


Fig4. Effect of NIPAAm-AA gel on nitrification by blank test

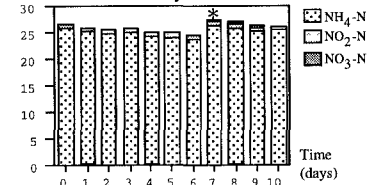


Fig6. Effect of NIPAAm-MA gel on nitrification by blank test

NOTE ; " *" indicates substrate addition point