Title

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ABSTRACT

For biological treatment of drinking water, several crucial issues need to be addressed: firstly, microorganisms used in the treatment must be confined and free from leaking into the bulk water; secondly, certain nutrients, particularly, organic carbon source must be provided for optimal microbial growth; finally, the end products of biological conversions must be non-toxic to humans and animals. These objectives are difficult to attain in a typical water treatment plant, as a result, most water treatment technologies employed in America have not been out of scope of physical-chemical treatment. In this study, however, application of artificially-immobilized microorganisms in alginate gel beads to drinking water treatment has proved to be a viable technology in solving these problems associated with the removal of high concentrations of soluble pollutants, such as nitrate in raw water sources. Naturally-derived alginate beads were used as support materials for immobilized microorganisms from activated sludge. Calcium tartrate was co-immobilized into the gel structure and it functions both as an organic carbon source and as a stabilizing agent for the gel structure. Several batches of denitrification experiments were carried out to test the feasibility of this immobilization technology. The results of these experiments show that the nitrate removal rate is very high. There was very low concentrations of residual nitrite in the treated water. The alginate beads containing microorganisms survived the harsh hydrodynamic environment with high biomass retention both in treatment experiments and the stability testing experiment. The alginate beads are also recyclable and are very easy to use in immobilization procedures.

KEY WORDS

Biological control and treatment, Microorganisms, Nitrate,Water treatment, Water quality

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INTRODUCTION

The use of immobilized microorganisms in nitrate removal has been gaining ground both in research and in wastewater treatment plants in the last decades, and is evidently successful. However, application of this technology to drinking water treatment has been slow and less enthusiastic. This reluctance stemmed from the fact that the quality of raw water in most municipalities is traditionally good and from public objection to the idea of introducing microorganisms into drinking water treatment processes due to health concerns. As a result, most water treatment plants in North America employ only physical and chemical processes. These processes are adequate in terms of achieving water quality standard as long as the quality of raw water remains consistently good, free from significant amount of soluble pollutants, such as nitrate. However, increasing demand for water consumption resulting from population growth and industrialization and ubiquitous water pollution problems around world eventually may force many water treatment operators to look for ways to deal with the problems of ammonia, nitrate, and soluble organics present in raw water sources. Physical and chemical processes, which characterize water treatment technologies for centuries in this country, are no longer viable for current and future drinking water treatment needs. Biological removal of pollutants, such as nitrate that has been well-established practice in wastewater treatment industry, seems to provide an inexpensive alternative to eliminating nitrate or/and other soluble pollutants from drinking water. As a matter of fact, many European countries have applied this technology to drinking water treatment for years.

In order to facilitate biological processes to be included as part of a drinking water treatment scheme, two major problems need to be tackled; first, the amount of "free" microorganisms entering the treated water should be minimized and hopefully, absent; second, nutrients, particularly, organic carbon source, should be sufficient enough to sustain microbial growth during the biological phase

of water treatment. Luckily, these obstacles, no matter how formidable, can be overcome by employing cell immobilization technology. Why immobilize? The simplest answer may be: Immobilization of microorganisms along with organic carbon such as insoluble calcium salts of fatty acids allows for creation of optimum conditions for microbial activity and for retention of microorganisms in a bioreactor. While the use of immobilized whole cells has already been known for a long time, application of this technology to drinking water treatment is new. Much of research on this subject has been focused upon evaluating an array of cell carriers and organic carbon sources. In this study, calcium tartrate (although there are many calcium salts of fatty acids that are considered as excellent choices for organic carbon sources/stabilizing agents, their low densities prevent them from being used in co-immobilization of calcium salts of fatty acids and microorganisms) was chosen as the organic carbon source after a long, painstaking evaluation and testing of many calcium salts of fatty acids considered in the study. The reason for choosing calcium salts of fatty acids is that not only do they play roles in supplying carbon sources (The benefit of this approach is two-fold: first, it provides organic carbon source which is scarce in the raw water; and second, it makes utilization of the carbon easier, eliminating the constraint of diffusion of the compound containing organic carbon into the beads) for microbial growth, but also in the case of alginate gel beads, serve as stabilizing agents to prevent the beads from swelling and/or disintegration.

Lewandowski *et al.* (1987) successfully co-immobilized calcium carbonate and autotrophic denitrifiers into their alginate beads. The calcium carbonate serves merely as a stabilizing agent, while using the organic carbon source in the feedstock in a continuous operation mode. It is by no means the only way to improve alginate gel stability against chelating compounds like phosphates and citrates or high Na^+ concentrations in the water. Leenen *et al.* (1996), for instance, developed a method in which alginate beads were formed by dripping discrete Na-alginate droplets into a $BaCl_2$

and $CaCl_2$ mixed solution.

Isolated culture such as *Pseudomonas denitrificans* are frequently cited as denitrifying microorganisms used in many denitrification study (e.g., Nilsson *et al.*, 1980). Others like *Thiobacillus denitrificans* (Lewandowski *et al.*, 1996) and *Alcaligenes* (Krul, 1976) were also mentioned in the literature. In this study, a mixed culture from activated sludge of a wastewater treatment plant was used as an inoculum because it was felt that it is more relevant to actual drinking water treatment precesses.

MATERIAL AND METHODS

Materials

Sodium alginate (Protanal SF40) was obtained from Protan, Incorporated. It is commercially derived from brown seaweed and characterized by viscosity and percentage of guluronic-acid group that determines the gel strength. Other reagents were of analytical grade and were purchased from commercial sources. 0.45 μm syringes filters from Gelman were used to prefilter the samples prior to injecting to the Dionex Ion Chromatography in nitrate and nitrite concentration determination.

Mixture of Trace Elements

MgO, 10 g; $CaCO_3$, 2g; $FeCl_3 \cdot 6H_2O$, 5.4 g; $ZnSO_4 \cdot 7H_2O$, 1.44 g; $MnSO_4 \cdot 4H_2O$, 1.11 g; $CuSO_4 \cdot 5H_2O$, 0.25 g; $CoSO_4 \cdot 7H_2O$, 0.28 g; H_3BO_3, 0.062 g; and $Na_2MoO_4 \cdot 2H_2O$, 0.49 g; dissolved in 1000 ml deionized water plus few drops of concentrated HCl to give a stock solution of 200 times working strength (Gherhardt *et al.*, 1981).

Denitrification Medium

Sodium Acetate, 4.0 g (Calcium Tartrate, 0.5 g); KNO_3, 1.0 g; K_2HPO_4, 0.5 g; $MgSO_4$

$7H_2O$, 0.2 g; $CaCl_2$ $2H_2O$, 0.1 g; $FeCl_3$, 0.2 g; Yeast Extract, 0.5 g; Trace Element Solution, 60 ml; Distilled Water, 1000 ml.

Organic Carbon Source

Calcium salts of organic acids are chosen because they can provide calcium ions that act as a cross-linking agent stabilizing alginate gel and organic carbon for microbial growth. A list of possible candidates includes calcium salts of higher fatty acids such as palmitate, stearate, or of organic acids such as tartrate. All these compounds are slightly soluble or insoluble, which make them ideal candidates for being co-immobilized into the beads. However, an extensive laboratory trials ruled out most of calcium salts (calcium stearate and calcium palmitate) of higher fatty acids category due to their low densities, which makes bead-forming very difficult. Calcium tartrate with a density more than 1 g/cm^3, on the other hand, is more suitable for the research purpose. The solubility of calcium tartrate (0.036 g/100 g saturated solution at temperature of 25 °C) did cause some problem in the initial trials of bead-making. The problem started when calcium tartrate powder were mixed into the alginate solution in order to produce homogenous calcium tartrate-alginate suspension for co-immobilization of calcium (stabilizing the beads) and organic carbon into final beads. Instead, small fraction of calcium tartrate dissolved and its calcium ions reacted with the alginate, starting the gelation process prematurely. The problem was solved after it was realized that sodium tartrate could be added to reduce the solubility of calcium tartrate, however, caution must be taken as how much of sodium tartrate need to be added. Excessive amount of sodium tartrate would weaken the strength of beads and result in beads with large pores.

Microorganisms

The microorganisms used in this study were directly come from the sample of the activated sludge in a municipal wastewater treatment plant in Richmond, California. The activated sludge

sample was maintained in the refrigerator and no cultivation or/and isolation of the bacteria were performed.

Immobilization of Cells

4 g of calcium tartrate and 0.6 g of sodium tartrate were blended into a 1.5 % (w/v) sodium alginate solution of 100 ml by a magnetic stirrer. The blending process usually takes 4 hours to achieve its uniformity of the suspension. An inoculum of 0.1 ml of mixed culture of bacteria was uniformly dispersed into the calcium tartrate/sodium alginate suspension by vigorous agitation for about 5 minutes, and their mixture was subsequently extruded into the stirred calcium chloride solution through a syringe. The beads were immediately formed upon contacting the $CaCl_2$ solution. The beads were subsequently cured for 24 hours for complete cross-linking of the beads.

Experiment

Each of three flasks was filled with 300 ml denitrification medium, inoculum, and the organic carbon source. The first one contained sodium acetate; the second one included calcium tartrate as organic carbon source; the last one held approximately 500 alginate beads with co-immobilized microorganisms and calcium-sodium tartrate source inside. The experiment was carried out under strict anoxic condition. Nitrogen gas was introduced to purge the air trapped in the headspace of the flask. The sample was taken periodically to determine the concentrations of nitrate and nitrite. Observations were also made in terms of turbidity and coloring of the medium during the course of the experiment. The duration of the experiment was pre-determined to be 7 - 14 days to reflect the fact that most of calcium-sodium tartrate was leaked from the beads (see also the "Stability" part of RESULTS AND DISCUSSION section). The samples from three batch flasks were analyzed using ion chromatography.

Determination of Nitrate and Nitrite

Nitrate and nitrite concentrations were determined using an ion chromatography (model 2000i, Dionex) following Standard Methods for the Examination of Water and Wastewater (1995).

Assay of Nitrate Reduction Rate

Immobilized cells: 0.1 ml activated sludge sample containing mixed culture of denitrifying and non-denitrifying bacteria was stirred into each of five flasks filled with 100 ml of growth medium, containing sodium acetate, calcium stearate, sodium stearate, sodium palimtate and calcium tartrate, respectively. Nitrogen gas is bubbled through the suspension to remove oxygen and nitrate reduction is initiated by adding the substrate KNO_3. The concentration of nitrate-nitrogen used is in the range of 10 - 100 mg/l NO_3-N. The progress of the reaction was recorded continuously at 23 ^{0}C using the ion chromatography, and nitrite reduction was also monitored with the ion chromatograpy. Nitrate reduction rate was determined as the consumption of mg of NO_3^--N/min/g gel (wet wt) for immobilized cells.

Stability

The stability of alginate beads was investigated in the solution which has the following composition of the typical surface water found in Sacramento River, California: Ca^{2+}, 12 mg/l; Mg^{2+}, 8 mg/l; Na^+, 5 mg/l; CO_3^{2-},20 mg/l; SO_4^{2-}, 2 mg/l; Cl^-, 2 mg/l, NO_3^-. The purpose of this line of tests was to examine whether the beads made of alginate and various co-immobilized regents can retain their functionality for certain period of time in the water whose chemical (and biological) composition is similar to the one in a packed beds biofilm reactor. Five batches of beads were made with the following ingredients: (1) alginate only; (2) alginate + $CaCO_3$; (3) alginate + Ca-Tartrate + Na-Tartrate, Ca/Na = 6.08; (4) alginate + Ca-Tartrate + Na-Tartrate, Ca/Na = 5.32; (5) alginate + Ca-Tartrate + Na-Tartrate + $CaCO_3$. More than three hundred alginate beads for each batch were shaken

in an Erlenmeyer flask at 100 rpm, and the solution was drained and the flask was refilled with the same amount of the above-mentioned solution every other day. Periodically the mean diameters of beads and the weight of 50 beads were measured.

The stability of these beads was also measured by a combination of visual observation and compression which was done by using a flat glass slide placed against the testing bead with known weights. The relative deformability serves as an indicator of the severity of the damage due to compression, which in turn provides the qualitative information about the stability of the beads. The tensile test of beads under elongational forces was not carried out in the research laboratory, as opposite to common practice in testing rheological properties of materials. It was felt that the prospective application of our research does not warrant this type of test.

RESULTS AND DISCUSSION

Stability of Alginate Beads

Because alginate beads are intended to use in an open system such as a water treatment facility, loss of Ca^{2+} to the surroundings with a concomitant destabilization and swelling of the gel beads could jeopardize their usefulness in drinking water treatment. Among many methods of combating this problem, three major approaches have been emerged to be effective in stabilizing the gel: they are (1) adding barium ions (Vogelsang and Østgaard, 1996), (2) continuous addition of Ca^{2+} (Nilsson and Ohlson, 1982; Wijffels and Tramper, 1995), and (3) immobilization of $CaCO_3$ in the gel beads (Lewandowski *et al.*, 1987). What sets this approach apart from the above-mentioned methods is that the organic carbon was supplied within the beads by co-immobilizing calcium salts of tartaric acid, which eliminates the need of adding carbon source into the substrate or relying solely upon the existing organic carbon in the water/wastewater. However, the effectiveness of these calcium salts

in terms of contributing to the stability of the beads had to be investigated. Several batches of tests were carried out for five groups of beads made of different ingredients and methods. The first group was of pure alginate (G1); the second group was made of $CaCO_3$ and alginate (G2); the third one was formed using alginate-calcium tartrate with small amount of sodium nitrate for easy mixing (G3); the fourth one was similar to the third one except the amount of sodium tartrate has been increased (G4); the last group was a mixture of calcium tartrate, $CaCO_3$, alginate, and again, small amount of sodium tartrate (G5).

Alginate Beads Stability Experiment

All five groups of beads were separately placed in five labeled flasks filled with the solution whose chemical composition (see Table 1) is similar to the one in Sacramento River. The flasks were mounted on a shaker at 100 rpm. The water in the flasks was changed every other day and the size and weight of these beads were also being measured periodically. In addition, the shapes and texture as well as compression strength were also monitored, employing some simple techniques such as observing the coloring of the beads (only the beads made of pure alginate are semi-transparent), texture and densities (terminal velocities in the water during free settling). The beads are considered as being destabilized if there are observations and/or measurements indicating swelling and breakage in the bead structures for significant number of beads in the flask.

The beads containing calcium tartrate usually lost their calcium salts (and small amount of sodium tartrate) after 8 - 13 days (13 days for G3 and 8 days for G4) and became very much like the beads of pure alginate. However, the presence of $CaCO_3$ (G5) strengthens the bead structures a little, and as expected, G2 retains its strength and calcium salt inside structures after four month long test. The amount of sodium ion present in G3 and G4 structures has some negative effect upon the stability of beads, which comes no surprise to the investigators. All in all, the beads in every flask have

survived the long test and no swelling and breakage have been observed in the stability test. However, it should been noted that the environment for these beads in a real experiment is far more hostile and harsh than the one in the stability test. There are several additional factors that need to be reckoned with: first, the microbial degradation to the beads, second the nitrogen gas as result of denitrification which might swell or disrupt the gel structure; finally, the weakening of the gel structure due to the dissolution of calcium ions from biotranformed calcium tartrate in the gel. These factors need to examined in the future denitrification experiments in conjunction with the information obtained from the stability test.

Figures 1-6 show the removal rates of nitrate and the changes of nitrite concentrations in the liquid media during the experiment. As demonstrated in Figures 1, 3 and 5, the nitrate concentrations in all batches of the experiment reduced to almost zero just after less than three days of operation. The batch containing acetate, as expected, comes out as a leader in terms of rate of nitrate reduction. The two remaining batches, which all utilize tartrate as carbon and calcium sources, show some interesting result; First, the batch with alginate beads (co-immobilized microorganisms with tartrate) has a better nitrate removal rate than the one with tartrate dispersed in the suspension. It is worth noting that the performance of acetate-containing medium in terms of nitrate transformation is close to the one with tartrate-immobilized alginate beads. The explanation for this phenomenon lies in the fact that contrary to tartrate in the suspension, which is more mass transfer barriers, the tartrate in the gel structure is readily available to the entrapped microorganisms and can be fully utilized. Figures 2, 4 and 6 reveal the dynamics of nitrite concentrations. The nitrite concentrations of the two batches, one with acetate and the other with alginate beads, peak out after two days. The figures for these two batches clearly show the similarity in the removal rate, this time it is of nitrite, although the batch with alginate beads has its nitrite concentration climbed to 170 mg/l , which is 10

mg/l more than the highest concentration of acetate-based batch. Once again, the batches with beads and acetate perform better than the batch with the dispersed tartrate. Unlike nitrate reduction part of transformation processes, there is about 10 mg/l of residual nitrite in the medium of every flask after eighteen days of batch operation. The relatively small amount of nitrite residual still causes our concern since one of our objectives of this study is nitrogen elimination, which means biological transformation of nitrate into nitrogenous gas. Furthermore, the existence of secondary nitrite concentration could defeat our original goal for the nitrate removal operation: elimination of harmful effects of nitrate in drinking water on human health, because nitrite is more toxic than nitrate. This issue should be looked at carefully in the next phase of this study.

CONCLUDING REMARKS

Successful application of cell immobilization technology requires the development of a feasible and cost-effective operational protocol which includes the choice of medium and carrier material, bead-making procedures and duration of the operation (both batch and continuous). It is also equally important to formulate a feasible technology to recycle and re-use these alginate beads. Based upon the first phase of our experiment, It was found that calcium tartrate is an excellent carbon source for denitrifying bacteria to grow, and the scheme of co-immobilizing calcium tartrate into alginate beads works extremely well in batch mode of denitrification operation, because the existence of calcium tartrate in the proximity of entrapped microbial culture does enhance the biological transformation and achieve a lower residual nitrite concentration. The jury is still out on the issue of the role of calcium tartrate in the stability of alginate. It is not clear, however, based upon the stability tests and observation of the denitrification batch operation, that the calcium tartrate in the gel structure actually improve the gel stability. The remedy for this shortcoming could be mixing calcium carbonate with

calcium tartrate first and then co-immobilizing with denitrifying bacteria to achieve better stability. we also need to realize that excessive stability of alginate beads are maybe not desirable since there is still a need of recycling the beads in order to reduce the cost associated with this technology.

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CAPTIONS OF FIGURES

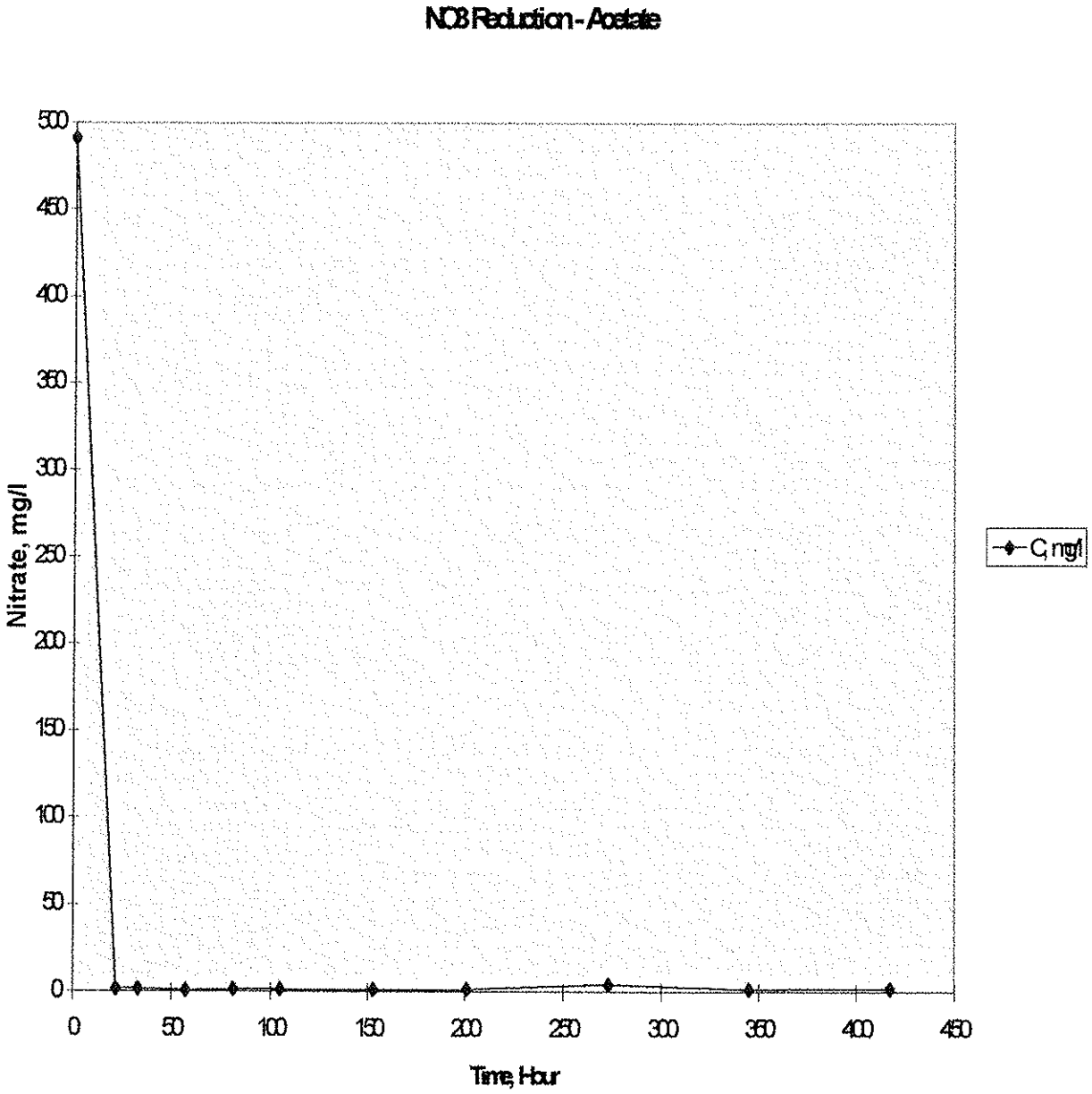

Figure1 Dynamics of nitrate concentration of the acetate-based denitrification experiment

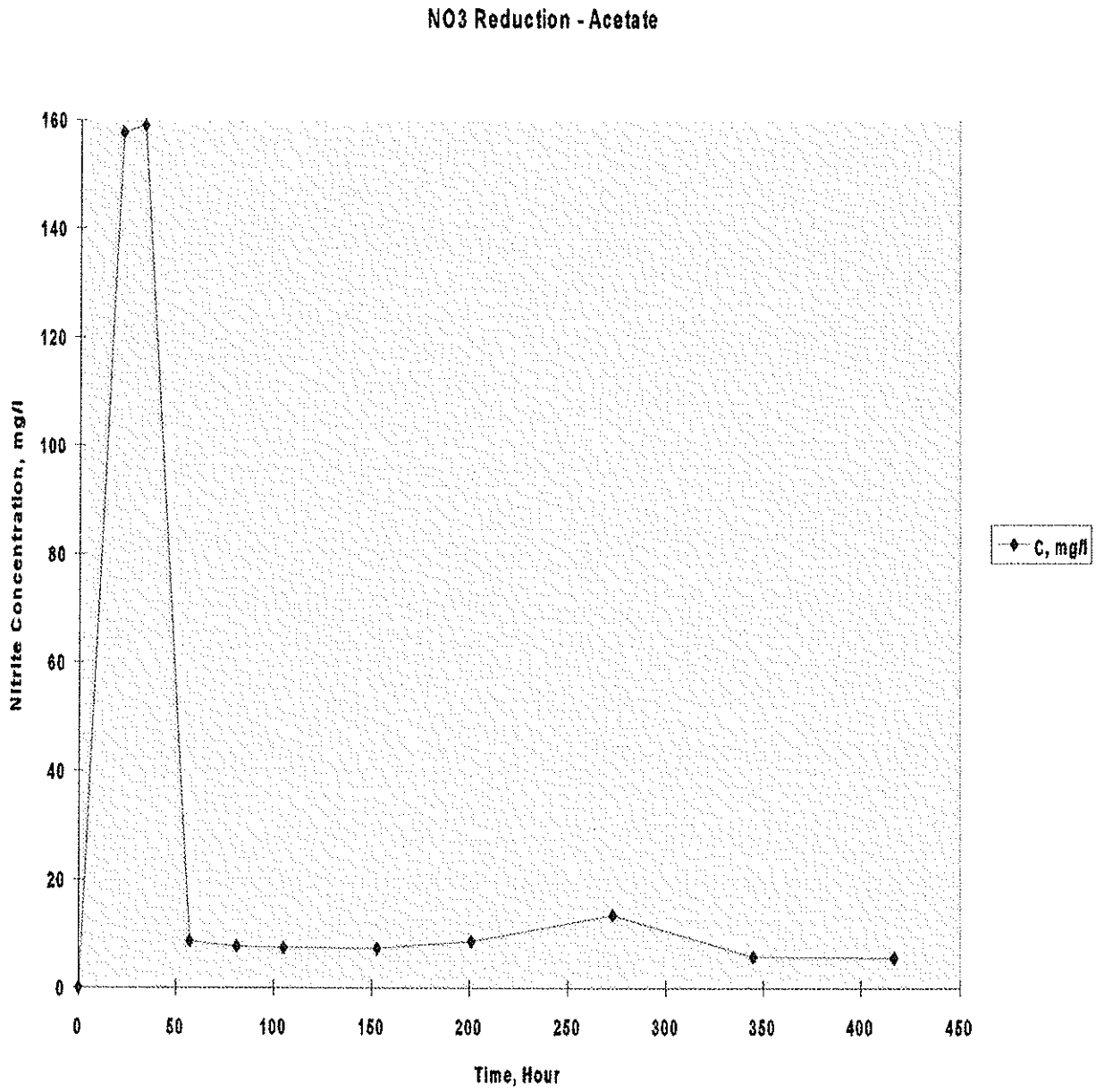

Figure 2 Dynamics of nitrite concentration of the acetate-based denitrification experiment

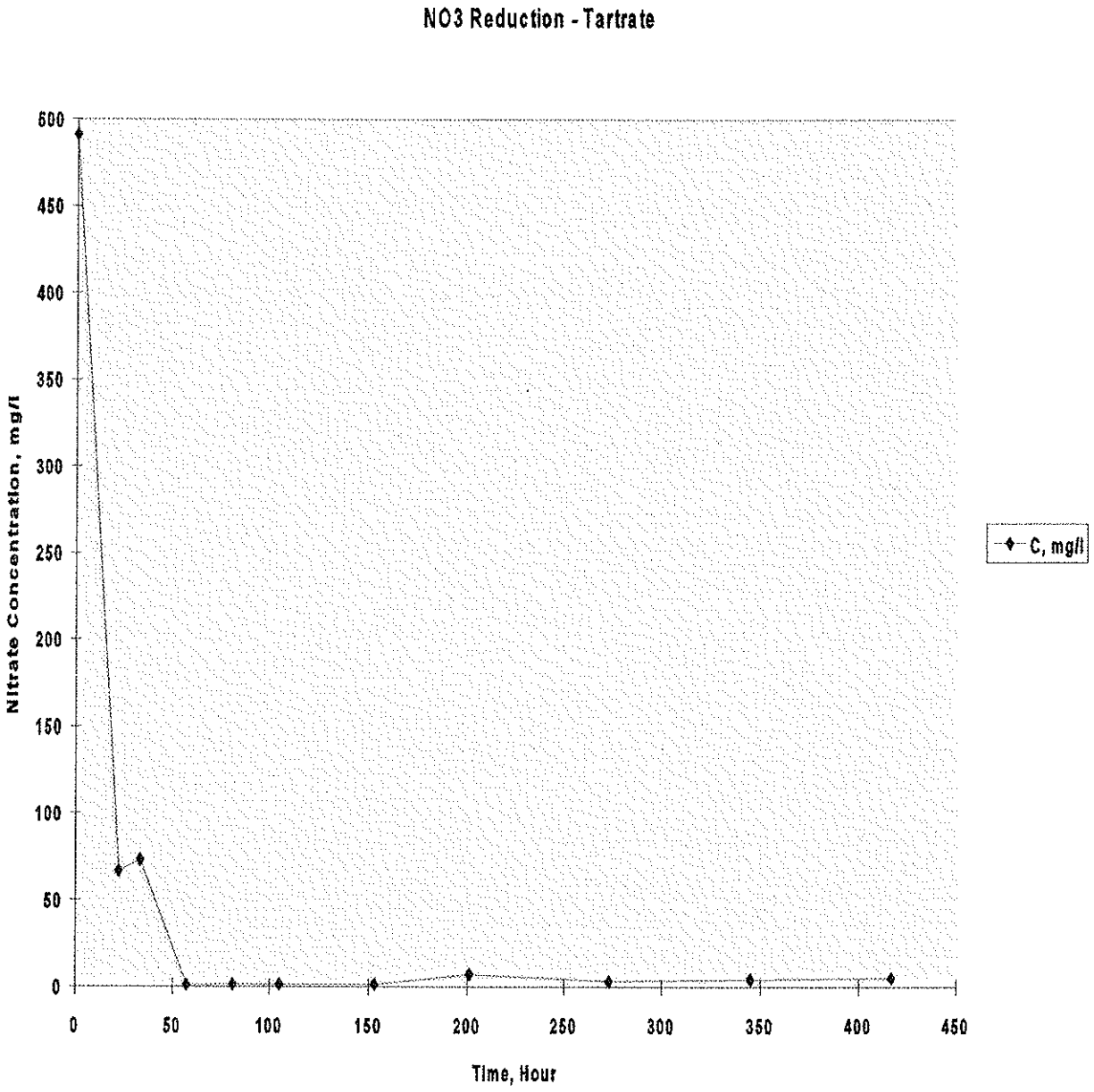

Figure 3 Dynamics of nitrate concentration of tartrate-based denitrification experiment

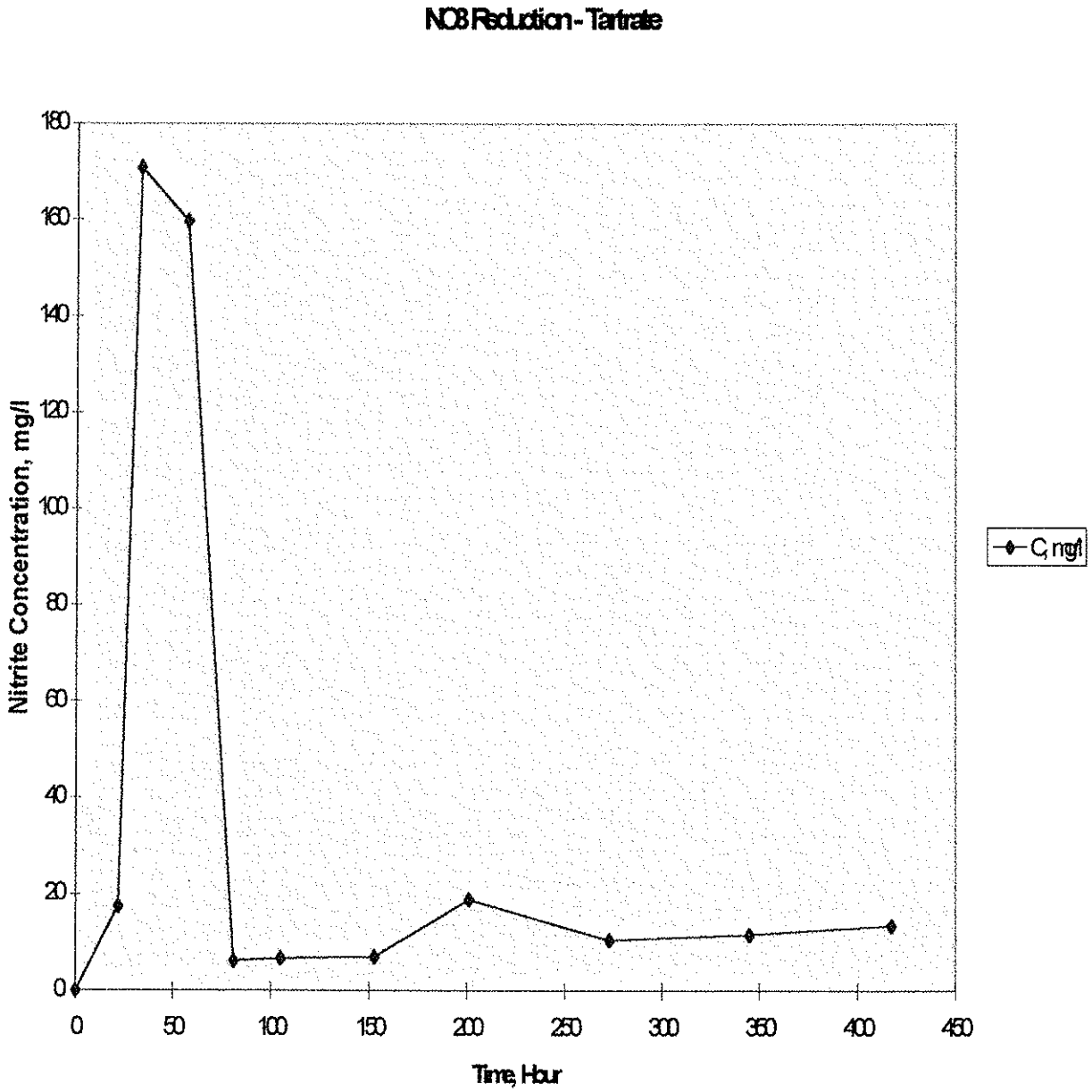

Figure 4 Dynamics of nitrite concentration of the tartrate-based denitrification experiment

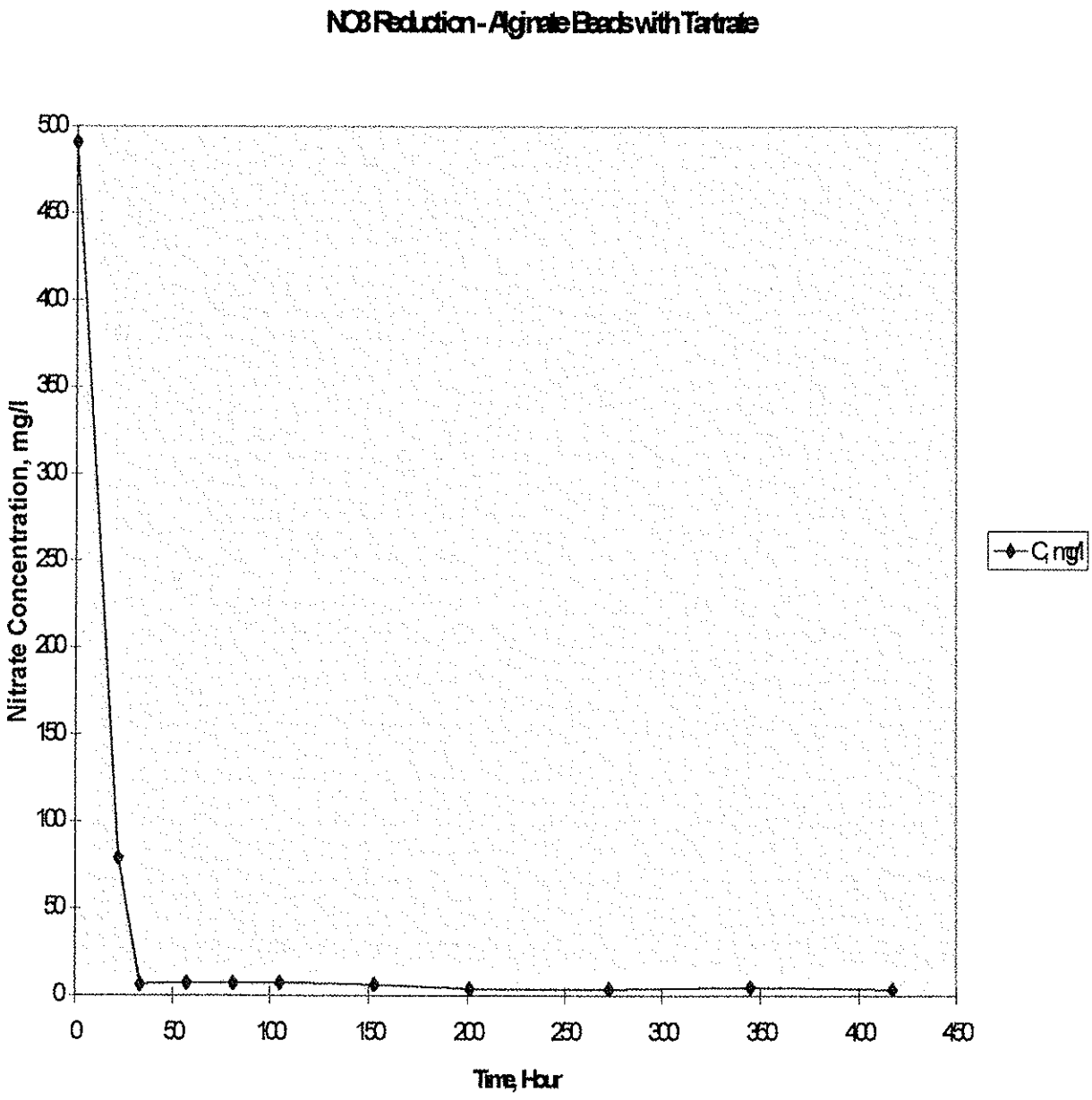

Figure 5 Dynamics of nitrate concentration of the experiment with tartrate-alginate beads

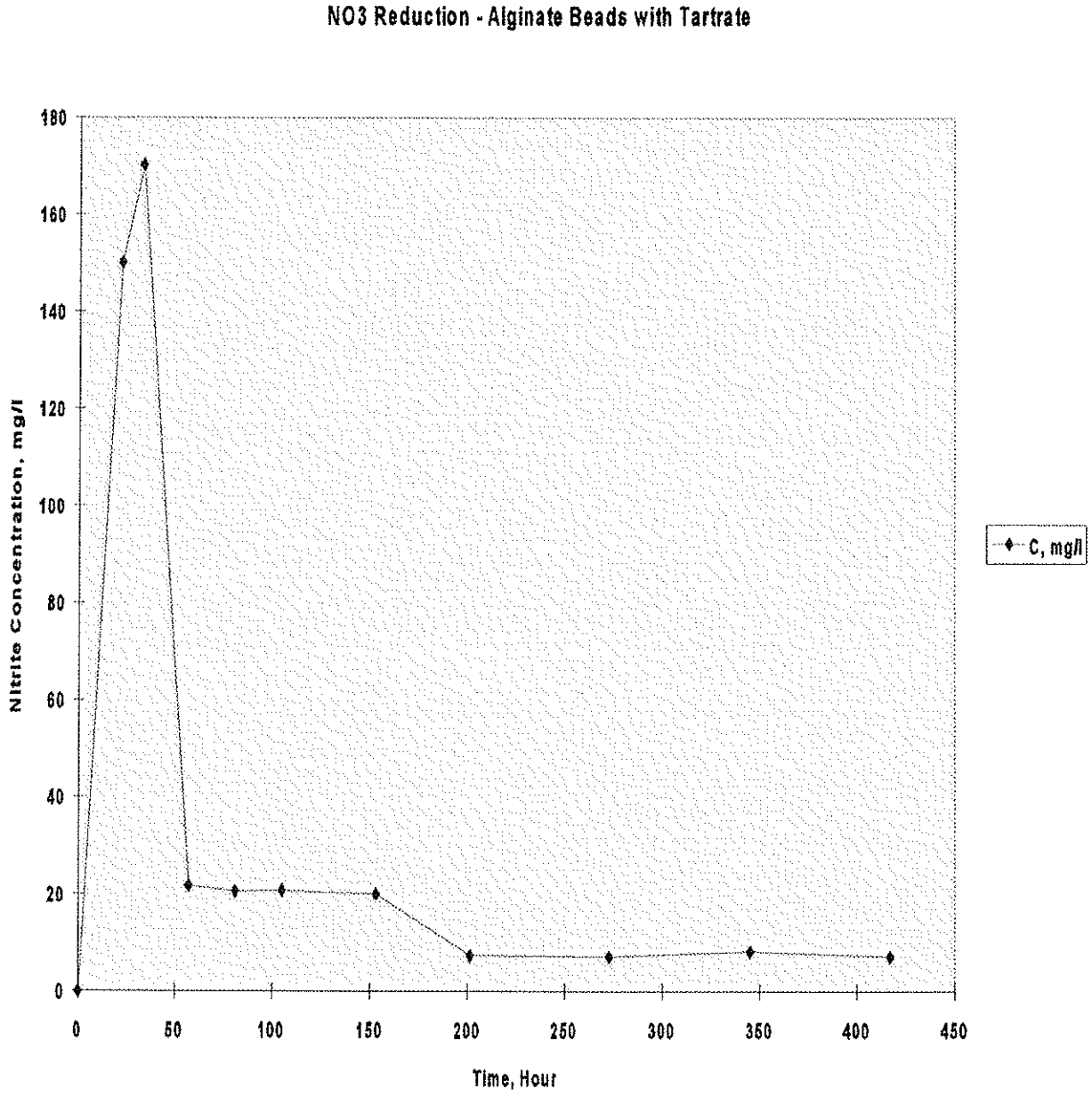

Figure 6 Dynamics of nitrite concentration of the experiment with tartrate-alginate beads