DEVELOPMENT OF SULFUR-LIMESTONE AUTOTROPHIC DENITRIFICATION PROCESSES FOR TREATMENT OF NITRATE-CONTAMINATED GROUNDWATER IN SMALL COMMUNITIES

FINAL REPORT

Submitted to

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Development of Sulfur-Limestone Autotrophic Denitrification Processes for Treatment of Nitrate-Contaminated Groundwater in Small Communities

Executive Summary. Currently, sulfur-limestone autotrophic denitrification (SLAD) processes have received increasing attention due to their high nitrate removal efficiency and cost-effectiveness. The SLAD processes utilize autotrophic denitrificans to reduce nitrates in contaminated water into nitrogen gas. The objectives of this research are to (1) develop the SLAD processes into a reliable, simple, cost-effective treatment technology for the treatment of nitrate-contaminated groundwater in small communities, and (2) evaluate the economic feasibility of the SLAD process based on the experimental results and related information. To accomplish these objectives, bench-scale column experiments were conducted, together with preliminary economic analyses of the SLAD process based on the results obtained and the mass balance principle. This report presents our results related to (1) the operation and performance, (2) the kinetics, and (3) preliminary economic analysis of the SLAD process.

Performance of the SLAD column reactors was studied under quasi-steady-state conditions of different nitrate-nitrogen loading rates and hydraulic retention times (HRTs). A polynomial linear regression model was developed and can be used to estimate the performance of a SLAD column process with the influent nitrate-nitrogen concentration ranging between 20 and 110 mg/l and the HRT between 2 and 9 hours. The nutrients study showed that 0.00167 mg/l PO_4^{3-} as P per mg/l NO_3-N is enough for obtaining a nitrate removal efficiency > 95% when the nitrate loading rate is < 256.9 g NO_3-N/d-m^3 media. Total organic carbon (TOC) and heterotrophic plate count (HPC) tests of the effluent showed that the SLAD treatment process will not greatly impact the microbiological quality of the water being treated. However, the effluent chemical properties of the SLAD process will be changed due to sulfate and hardness increases. Low nitrate removal efficiencies sometimes occurred during the period of initiating the SLAD columns. Multiple re-inoculations may be used to improve the poor performance.

Kinetic parameters are important for process design. In this research, four kinetic parameters: half-velocity constant, K_s; maximum specific substrate utilization rate, k; bacteria yield coefficient, Y; and bacteria decay coefficient, k_d of autotrophic denitrificans in a SLAD biofilm process were evaluated. The estimation of K_s and k was based on a curve-matching method with kinetic results obtained from several short-term non-steady-state experiments conducted in completely-stirred tank reactors (CSTR). The evaluation of k_d and Y was based on the results obtained from several short-term batch tests with fully-penetrated biofilm cultured in the reactors. The parameters found are as follows: $K_s = 0.398$ mg/L NO_3-N, $k = 0.15\ d^{-1}$, $k_d = 0.12\ d^{-1}$, Y = 0.22 mg VSS / mg NO_3^-.

A preliminary economic analysis was performed for a town of 200 people to add a SLAD unit process in its drinking water treatment system. The construction cost would be about $50,800-58,500 if groundwater wells and the pumping and piping system are not taken into account. This is not unreasonable for a community of the size of 200 people.

The project was funded by the Midwest Technology Assistance Center (MTAC) for small public water systems. Mr. Ken Smothers, the managing director of the MTAC, was the project manager. Mr. Tian C. Zhang at the University of Nebraska-Lincoln (UNL) was the principal investigator, and Ms. Hui Zeng at UNL was the research assistant. The project was conducted at the Civil Engineering Department at UNL from January 1, 2003 to August 31, 2004.

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1. Introduction

1.1 Background Information. Nitrate contamination of ground and surface waters has become an increasingly serious problem in the U.S (Spalding and Exner, 1991). The United States Environmental Protection Agency has set the maximum contaminant level (MCL) at 10 mg/l for nitrate-nitrogen. In Nebraska alone, more than 20% of nitrate-nitrogen concentrations, measured in 5826 wells, exceeded the maximum contaminant level (Exner and Spalding, 1990). Most of the samples exceeding the MCL had concentrations between 10 and 19.9 ppm. Nebraska is not alone in its concern for nitrate contaminated waters. Since groundwater serves as the primary domestic water supply for about 90% of the rural population and 50% of the total population in North America (Power and Schepers, 1989), nitrate removal from groundwater becomes an extremely important practice due to the adverse health affects associated with nitrate (Comly, 1945; Walton, 1951; Crespi and Ramazzotti, 1991; Forman, 1991).

Treatment methods for the removal of nitrates from water resources include (1) physical/ chemical treatment, such as the ion-exchange process, reverse osmosis, electrodialysis, chemical precipitation, distillation, and (2) biological denitrification by heterotrophic and autotrophic denitrifiers. The physical/chemical methods only separate nitrates from one liquid phase to concentrate them in another. The further treatment of the concentrated nitrate solution can be very expensive (Dahab, 1991). Moreover, it is difficult to use the physical/chemical methods for in situ remediation. Heterotrophic biological denitrification is very effective in nitrate removal as long as there is sufficient organic carbon in the system. However, in many situations, such as in groundwater, lakes, and stabilization ponds for tertiary treatment, insufficient organic carbon may limit the application of in situ heterotrophic denitrification unless organic substances [e.g. methanol (CH_3OH), glucose, and glycerol] are added as external carbon sources (Dahab, 1991).

Autotrophic denitrification processes utilize autrotrophic denitrifiers, to reduce nitrate or nitrite to nitrogen gas. The energy source of the autotrophic denitrifying microorganisms is derived from inorganic oxidation-reduction reactions with elements such as hydrogen or various reduced-sulfur compounds as the electron donor, while the carbon sources are inorganic carbon compounds (e.g. CO_2, HCO_3^-). In a sulfur-limestone autotrophic denitrification (SLAD) process, autotrophic denitrificans, such as *Thiobacillus denitrificans* and *Thiomicrospira denitrifican,* can oxidize a wide variety of reduced sulfur compounds (H_2S, S, $S_2O_3^{2-}$, $S_4O_6^{2-}$, SO_3^{2-}), while reducing nitrate or nitrite to elemental nitrogen (Baalsruud and Baalsruud, 1954; Bachelor and Lawrence, 1978a, b, c; Claus and Kutzner, 1985b; Zhang, 2002). As shown in Eq. (1.1), in a SLAD process, the electron donor is elemental sulfur; the carbon source is CO_2; and limestone provides alkalinity and inorganic carbon (Batchelor and Lawrence, 1978a, 1978b; Zhang, 2002).

$$\mathbf{55S + 44CaCO_3 + 50NO_3^- + 18H_2O + 4NH_4^+ \rightarrow 4C_5H_7O_2N + 25N_2 + 55SO_4^{2-} + 44Ca^{2+} + 24HCO_3^-} \qquad (1.1)$$

Therefore, compared with heterotrophic denitrification, the SLAD process has two advantages: (1) no need for an external organic carbon source, i.e. methanol and ethanol; which lowers the cost and risk of the process; and (2) less sludge production, which minimizes the handling of sludge (Claus, Kutzner, 1985a; Flere, 1997; Flere and Zhang, 1999; Koenig and Liu, 2004).

Studies on autrotrophic denitrification processes have been divided into two major directions; that is, hydrogen-based and sulfur-based autotrophic denitrification (Islam and Suidan, 1994; Lee and Rittmann, 2000; Zhang and Shan, 1999; Zhang and Lampe, 1999; Zhang, 2002; Liu and Koenig, 2002). Because it is difficult to handle hydrogen gas and generating

hydrogen (e.g., from methanol) is very expensive, much more attention has been concentrated recently on sulfur-based autotrophic denetrification. Beijerinck (1904), Lieske (1921), and Baalsruud and Baalsruud (1954) are among the first researchers to establish physiological and biochemical studies on sulfur-oxidizing bacteria. Engineering research on the SLAD process for treatment of nitrate-contaminated groundwater started in 1970s (Batchelor and Lawrence, 1978a, b). Most studies on SLAD processes were conducted in Europe and the U.S. in recent two decades.

Although it has been concluded that the sulfur-based autotrophic denitrification process is a reliable and simple process, able to produce bacteriologically and chemically safe water that can meet the guideline, the detailed information about the SLAD processes for above-ground treatment is still unavailable, including the process kinetics, biomass distribution in the system, TOC and biomass in the system effluent, optimal operation conditions, and control of process by-products (e.g, sulfate, hardness increase, and H_2S gas produced in the upper part of the fixed bed of the process, etc.).

1.2. Research Objectives. Currently, there is no simple and reliable method that can be used by small communities or individual farmers in rural areas to treat nitrate-contaminated groundwater. Therefore, the development of a denitrification technology for these people is extremely important. Many of the rural communities belong to fixed- and low-income groups, with a very limited tax base and ability to pay. In such communities, the elected officials are likely to be unpaid, part-time volunteers without strong technical skills in treatment facilities. Therefore, in such communities, issues such as the acceptability of certain technologies are likely to be critical, and the local cost to residents may be paramount.

Based on these considerations, the long term goal of the proposed research is to realize a better and faster technique transfer of the SLAD process such that it can be easily and safely used in small communities or sparsely populated areas. The success of this project would benefit people in rural areas or small communities, and contribute to the long-term improvement of U.S. agriculture. Within this broad goal are the following specific objectives:

(1) To develop a reliable and simple SLAD process for the treatment of nitrate-contaminated groundwater in small communities or sparsely populated areas; and
(2) To evaluate the economic feasibility of the SLAD process based on the experimental results and related information.

To approach these objectives, bench-scale column experiments were conducted, together with preliminary economic analyses of the SLAD process based on the results obtained and the mass balance principle. This report presents our results related to (1) the system performance and optimization, (2) kinetic studies, and (3) preliminary economic analysis of the process.

2. System Performance and Optimization

2.1. Problems Statement, Approaches, and Justification. One important research focus related to the SLAD process is its performance evaluation for optimization. Van der Hoek et al. (1992) conducted studies to optimize the operation of SLAD fixed-bed column reactors. They determined the optimal ratio of sulfur to limestone, and a preferred volumetric nitrate loading rate of 40 g NO_3^-/m^3 media/h at which high nitrate removal efficiency could be obtained. However, designing a field-scale SLAD process requires more information. Claus and Kutzner

(1985a) conducted column studies to evaluate the effects of hydraulic retention time (HRT) on nitrate removal. Thiosulfate was used instead of sulfur stones, which makes their results more difficult to use in design of a SLAD process. In the U.S., Batchelor and Lawrence (1978a), Driscoll and Bisogni (1978) studied the SLAD process where sulfur stones were used but alkalinity was supplied by adding bicarbonate instead of limestone. Lampe (1996) and Flere (1997) determined the optimum sulfur dosage and the loading rate in a SLAD system, the minimum HRT, the nitrate loading rate corresponding to the maximum nitrate removal efficiency, etc, which provided useful information on design criteria of the SLAD processes.

However, even with the contributions of these researchers, the SLAD process, as most of the biological treatment processes, still is not well understood. For example, how are the influent concentration and HRT combined together to influence nitrate removal efficiency? Which factor (HRT or the influent nitrate concentration) is more important in a certain range or the whole range? Can the relationship among these three items be described quantitatively? Currently, the information to answer these questions is not sufficient.

In addition, little work has been conducted on the study of nutrients which are utilized in the SLAD process. In many cases, nutrients are inadequate in the water that is being treated. To add nutrients, most researchers simply followed the recipe of Baalsruud and Baalsruud (1954) or the adapted one by Batchelor and Lawrence (1978a). They both contained a large amount of phosphorus not less than 10 mg P/l. It was also found that P at about 0.1 mg/l was enough to support the cells growing well in our previous study. Therefore, a large amount of phosphorus added as nutrients may just be wasted. It is unknown whether phosphorus is the limiting factor for nitrate removal in a SLAD process.

The objectives of this part of the research were to: 1) establish the quantitative relationship among HRT and the nitrate concentration in influent and effluent in SLAD column systems by developing a multiple linear regression model; 2) study the effects of different phosphorus concentrations on nitrate removal; and 3) evaluate effluent quality and operational problems associated with the SLAD column reactors.

2.2. Materials and Methods. In this section, we will introduce our experimental design and materials and methods used to conduct these experiments.

Design Principle of Response Surface Tests. Response surface methodology (RSM) is an approach to experimental design which allows the investigator to optimize responses obtained from the experiment (Myers, 1971). In this study, the response surface was approximated using a second-order polynomial model shown in Equation 2.1. A second-order polynomial model is more suitable for a system having curvatures and interactions compared with a first-order polynomial model. However, it should be noted that it is impossible for a polynomial model to be an exact representation of the true functional relationship over the whole range of the independent variables. It is likely that the model will work well within a relatively small region. Therefore, limitations of the model are inevitable. The second-order polynomial function is:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_{11} x_{1i}^2 + \beta_2 x_{2i} + \beta_{22} x_{2i}^2 + \beta_{12} x_{1i} x_{2i} + r_i \qquad (2.1)$$

where, y_i is the response for the i^{th} run, β_0 is the y intercept at factor level $x_1 = 0$ and $x_2 = 0$, β_1 is the straight line slope for factor x_1, β_2 is the straight line slope for factor x_2, x_{1i} is the level taken by factor x_1 in the i^{th} run, x_{2i} is the level taken by factor x_2 in the i^{th} run, β_{11} is the second-order parameter for curvature, β_{22} is another second-order parameter for curvature, x_{1i}^2 is the level

taken by factor x_1^2 in the i^{th} run, x_{2i}^2 is the level taken by factor x_2^2 in the i^{th} run, r_i is the residual error for the i^{th} experimental run.

In this study, a rotatable central composite design was used to design a set of column runs. Rotatability means that the variance of the predicted response is constant at all points of interest that are the same distance from the design center (Montgomery, 1991). A central composite design consists of a 2^k factorial with n_f runs {($\pm$ 1, 0, 0, …, 0), (0, $\pm$ 1, 0,…, 0), (0, 0, $\pm$ 1, …, 0) . . ., (0, 0, 0, . . ., $\pm$ 1)}, 2k axial runs {($\pm$ α, 0, 0, …, 0), (0, $\pm$ α, 0,…, 0), (0, 0, $\pm$ α, …, 0) . . ., (0, 0, 0, . . ., $\pm$ α)} and n_c center runs {(0, 0, . . ., 0)}(Montgomery, 1991). A central composite design is made rotatable by letting $\alpha = (n_f)^{1/4}$, where n_f is the number of points used in the factorial portion of the design (Montgomery, 1991). In this study, the response or dependent variable is the effluent nitrate-nitrogen concentration while the influent nitrate-nitrogen concentration and the HRT are the independent variables (i.e., k = 2) which were controlled by the experimenter. Therefore, the rotatable central composite design contains 4 factorial runs (i. e., $n_f = 2^k = 2^2 = 4$), 4 axial runs (i. e., 2k = 4), $n_c = 4$ center runs (Montgomery, 1991). The value of α is 1.414 (i. e., $\alpha = (4)^{1/4} = 1.414$). The specific design is shown in Table 2.1. The software, STATGRAPHICS PLUS 3.0 was used to conduct multiple regression analysis.

Table 2.1 Rotatable central composite design and tests results.

Points Based on Rotatable Central Composite Design							
Variable	Center Points	Semi-range	-1.414	-1	0	1	1.414
NO_3^- - N (mg/l)	50	30	7.6	20	50	80	92.4
HRT (h)	6	3.5	1.1	2.5	6	9.5	10.9

Test Conditions					Results			
Run #	Random #	Points	NO_3^- - N (mg/l)	HRT (h)	Eff. NO_3^- -N (mg/l) (Test)	Eff. NO_3^- -N (mg/l) (Model)	Removal Efficiency, % (Test)	Removal Efficiency, % (Model)
1	8	(-1, -1)	20	2.5	3.86	9.25	80.7	53.7
2	11	(1, -1)	80	2.5	50.56	57.75	36.8	27.8
3	10	(-1, 1)	20	9.5	0.44	1.51	97.8	92.5
4	2	(1, 1)	80	9.5	2.24	5.11	97.2	93.6
5	4	(0, 0)	50	6	3.25	1.81	93.5	96.4
6	5	(0, 0)	50	6	1.02	1.81	97.9	96.4
7	7	(1.414, 0)	92.4	6	39.36	33.95	57.4	63.3
8	1	(-1.414, 0)	7.6	6	0.00	-2.86	100.0	100.0
9	3	(0, 1.414)	50	10.9	0.85	-0.28	98.3	100.0
10	9	(0, -1.414)	50	1.1	49.30	42.00	1.4	16.0
11	12	(0, 0)	50	6	1.09	1.81	97.9	96.4
12	6	(0, 0)	50	6	1.71	9.25	96.6	81.5

Design Principle of Nutrients Studies. Phosphorus is an essential nutrient for cell synthesis and growth. It may at times be a limiting factor. The TOC (total organic carbon): N: P ratio for cell synthesis could be assumed as 20: 5: 1 (LaGrega et al., 2001). In the SLAD system, cells could be assumed as $C_5H_7O_2N$ (Batchelor and Lawrence, 1978a, b). According to Eq. 1.1, 0.343 mg C is used in cell synthesis per 1 mg/l NO_3-N consumed. Thus 0.01715 mg P/l is needed corresponding to reduction of 1 mg/l NO_3-N. Therefore, 0.5145 mg P/l should be enough for the cell synthesis with 30 mg/l NO_3-N in the influent. Based on Flere and Zhang (1999), 0.15 mg/l PO_4^{3-} (i.e., 0.05 mg P/l) was recommended as the reference value in ground water. Therefore, in

this study, two values (i.e., 0.05 and 0.1 mg P/l) that are < 0.5145 mg/l and the other two higher values (i.e., 5 and 10 mg P/l) that are > 0.5145 mg/l were chosen to study the effect of different phosphorus concentrations on nitrate removal when the influent nitrate-nitrogen is 30 mg/l. If the influent nitrate-nitrogen concentration increases, the phosphorus in the feed solution should increase according to the ratio of the influent nitrate-nitrogen to 30 mg/l nitrate-N.

Fixed-bed Column Reactor Systems. Four identical fixed-bed column reactors (H x Φ = 46.6 cm x 6.35 cm) were used to conduct the 12 column runs for response surface. The schematic diagram of the reactor is shown in Fig 2.1. The columns were filled with granular elemental sulfur (Georgia Gulf Sulfur, Bainbrige, GA) and limestone at a ratio of 2:1 volume by volume. This ratio was found to be enough to support bacteria growth (Liu and Koenig, 2002). Sulfur and limestone particles ranged between 2.38 mm - 4.76 mm in size (i.e., U.S. sieve #8 and #4). The height of the packing materials was 36.5 cm. The empty bed volume (including packing media, voids within packing media, and all other empty space) of each column reactor was 1.49 L. In this section, HRT always refers to the empty bed retention time. A Masterflex L/S peristaltic pump (Cole-Parmer, IL, U.S.A.) with four standard Masterflex pump heads (Model 7014-20) was used to provide constant up-flow into the reactors. The effluent was connected to a 1000 ml Erlenmeyer flask full of water to prevent oxygen from getting into the system. The gas outlet was located at the top of the reactor and linked to a gas collector with two plastic cylinders (Fig. 2.1). The slightly smaller cylinder was floating in an acidic salt solution in the bigger one. The acidic salt solution was made by: 200 g Na_2SO_4 + 30 ml 98% H_2SO_4 + 800 ml tap water and used to reduce the effect of oxygen and prevent carbon dioxide absorption.

Seed Source and Inoculation. A continuously-stirred tank reactor (CSTR) has been operated for several years as a seed sludge reactor to culture mixed-population denitrifiers (e.g., *Thiobacillus denitrificans*). It was run at ~ 25 ˚C and an HRT of about eight days under anaerobic conditions. The composition of feed solution for the seed reactor was (Baldensperger and Garcia, 1975): $Na_2S_2O_3$•$5H_2O$, 6.0 g/l; KNO_3, 3.0 g/l; $NaHCO_3$, 1.5 g/l; Na_2HPO_4, 1.5 g/l; KH_2PO_4, 0.3g/l; $MgSO_4$•$7H_2O$, 0.5g/l ; and pH = 7.5. Its effluent was turbid, grey-white liquid containing very low nitrate-nitrogen $\leq$ 10 mg/L. Nitrogen gas was continuously collected in a gas collector similar to the one described above.

At the beginning of the inoculation, 250 ml seed from the above seed reactor was collected and then mixed with about 350 ml feed solution of the seed reactor but without thiosulfate. This mixture then was pumped into the column reactor, which was then left standing still for 2 d, allowing for the denitrificans to acclimate to the sulfur and limestone particles and to form biofilm. After 2 d, the feed solution (without thiosulfate and seed) was replaced daily till the end of the fifth day. All four column reactors were inoculated in the same way. The regular pumping started from the end of the fifth day.

Response Surface 12 Column Tests. The column tests started at the center point of 50 mg/l NO_3-N. The feed solution used was artificial groundwater (Table 2.2) plus varying concentrations of NO_3-N using potassium nitrate (Mallinckrodt Co., Kentucky, U.S.A.). Trace nutrients were made by dissolving a tablet of Centrum (Wyeth Consumer Healthcare, Madison, NJ) into 500 ml tap water. One ml of the nutrient solution was then added into one liter of the artificial groundwater. The reactors were kept operating for about one month until they reached quasi-steady-state conditions. In this study, if the effluent concentration (e.g., NO_3-N, NO_2-N, etc.) from three continuous tests were within 5% of each other, we assumed it reached the quasi-steady-state condition. Meanwhile the sign of biofim growth was obvious, and gas bubbles were accumulated in the reactors.

Once the quasi-steady-state condition was achieved, the column reactors were operated according to the central rotatable composite design listed in Table 2.1. Each run was duplicated in two reactors and the average value is reported in this thesis. For each new test condition (e.g., a new HRT or a new feed nitrate-N concentration, etc.), the column performance was checked to see if it reached its new quasi-steady-state condition. Once reached, 3 to 5 samples were taken within 3 to 5 days for performance analysis. The average of these samples (i.e., 6 – 10 samples in duplicate reactors) is reported in this thesis. Upon completion of each run, the reactor was allowed to return to the center point (i.e., 50 mg NO_3-N / l in feed and HRT = 6 hours) and kept running until the quasi-steady-state conditions were achieved before the next run was conducted in the same way.

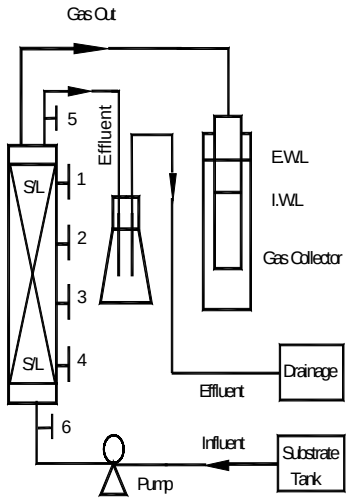

Figure 2.1 Experimental set-up: upflow fixed-bed SLAD column reactor system. S/L is sulfur/limestone; 1, 2, 3, 4, 5 and 6 are sampling ports; E.W.L: external water level; I.W.L: internal water level; there were tap water in effluent flask and acidic salt solution in gas collector.

Table 2.2 Feed solution composition*.

Component	Concentration (mg/L)	Component	Concentration (mg/L)
NO_3^--N	7.6- 92.4	Phosphate (orhto)	0.3
Alkalinity	300	Sulfate	150
Fluouride	0.25	pH	7.8-8.5
Iron	0.3		

* Feed solution made with tap water. Nutrient is added as 1ml/l of feed solution.

Nutrients Studies. Nutrient studies also were conducted in those four column reactors after the 12 column tests for response surface tests were accomplished. The column rectors were backwashed by removing the packing materials out of the reactors and cleaning them with tap

water. After backwash, the reactors were re-inoculated with the same way described above. When regular pumping started, the four reactors were fed with substrate of the same composition shown in Table 2.2 (NO_3-N = 30, 40, and 50 mg/l) except that varying concentrations of phosphorus were used. The phosphorus concentrations (PO_4^{3-}-P) in four reactors 1- 4, respectively were, 0.05, 0.1, 5, and 10 mg/l for 30 mg/l NO_3-N; 0.067, 0.133, 6.67 mg/l for 40 mg/l NO_3-N (no reactor 4 here due to system failure); 0.083, 0.167, 8.33 mg/l for 50 mg/l NO_3-N (no reactor 4 here due to system failure). Phosphorus was made from Na_2HPO_4 (99.93%, Mallinckrodt Co., Kentucky, U.S.A.). The HRT for all the tests was 6 hours. The reactors were kept running until quasi-steady-state conditions were reached. Thus three samples were collected each run for each reactor and analyzed. The average of these three samples is reported.

Analytical Methods. Effluent was analyzed for nitrate, nitrite, sulfate, pH, TOC (total organic carbon), TSS (total suspended solids), VSS (volatile suspended solids), and heterotrophic plate count (HPC). Dissolved oxygen (DO) was monitored in column reactors by a YSI 5000 DO Meter (YSI Incorporated, Ohio, U.S.A.) with a YSI 5010 stirring BOD probe. The DO probe was not small enough to insert into the sampling port. Therefore, samples were taken with a custom-designed bottle, and the DO of the sample then was measured within the bottle. This procedure might increase the DO readings to some extent. Nitrate, nitrite and sulfate were analyzed by a Dionex 500 ion chromatography and high performance liquid chromatography (IC/HPLC) (Dionex, California, U.S.A) equipped with an AS 14A anion column and a CD 20 conductivity detector. pH was measured by an Accumet 925 pH/ion meter (Fisher Scientific Corp.) and a Thermo Orion pH probe. TOC was measured by a Phoenix 8000 UV-Persulfate TOC analyzer (Tekmar-Dohrmann, Ohio, U.S.A.). 25 ml effluent was filtered with 0.45 μm glass microfiber filter paper (Whatman, UK) and then measured for TSS and VSS according to Standard Method (2540) (APHA et al., 1992). The HPC was operated under Standard Method (9215A and B) (APHA et al., 1992).

2.3 Results and Discussion. The major results obtained from this part of the present research are given below.

Response Surface and Its Verification. The operating conditions and results of 12 column tests are listed in Table 2.1. Using STATGRAPH PLUS 3.0, we found the following polynomial second-order equation to represent the response surface:

$$\mathbf{Y = 21.5714 + 0.31171*X_1 - 8.48835*X_2 + 0.0076386*X_1*X_1 + 0.79334*X_2*X_2 - 0.10691*X_1*X_2} \quad (2.2)$$

where Y = effluent nitrate-N concentration, mg/l; X_1 = influent nitrate-N concentration, mg/l; and X_2 = hydraulic retention time, h. As shown in Table 2.1, the model-predicted values of the effluent nitrate-N concentration under different operating conditions are close to the experimental values. Although the model predictions may give some negative values under certain conditions, they are all very small, and therefore, should be rounded up to zero. Table 2.3 lists the coefficients in Eq. 2.2 and the related statistical analysis results. Figs. 2.2 and 2.3 show a response surface based on Eq. 2.2 and an X-Y-Z scatter plot based on experimental results from 12 column runs. The analysis of variance between the model and the experimental results are shown in Table 2.4. Since the p-value in the ANOVA table is 0.0004 less than 0.01, there is a statistically significant relationship between the dependent and independent variables at a 99% confidence level. The R^2 of Eq. 2.2 is 0.9262, which indicates that the model as fitted explains 92.62% of the variability in dependent variable (effluent nitrate-nitrogen concentration).

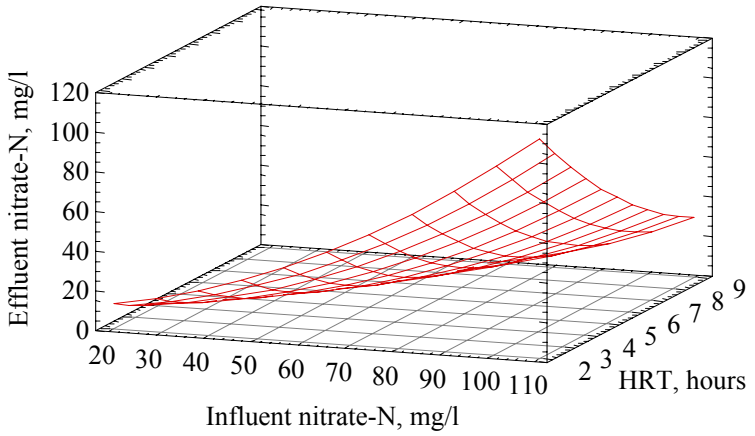

Figure 2.2 Response surface for SLAD column runs.

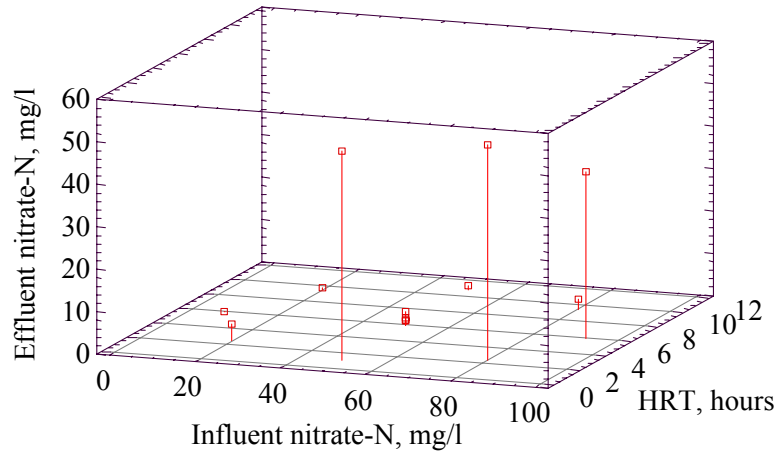

Figure 2.3 Response scatter plot for SLAD column runs.

Table 2.3 Model fitting results based on experimental results in Table 2.1.

Independent Variable	Coefficient	Std. Error	t-value	p-value
constant	21.5714	12.0176	1.79498	0.1228
x_1	0.31171	0.29857	1.04403	0.3367
x_2	-8.48835	2.61652	-3.24413	0.0176
$(x_1)^2$	0.0076386	0.0024416	3.12848	0.0204
$(x_2)^2$	0.79334	0.18211	4.35648	0.0048
$(x_1)(x_2)$	-0.10691	0.026474	-4.03808	0.0068
$R^2 = 0.9262$				

Table 2.4 Analysis of variance (ANOVA).

	Sum of Squares	D_f	Mean Square	p-value
Model	4418.98	5	883.796	0.0004
Residual	185.453	6	30.9089	
Std. error of estimate	5.5596			
Mean absolute error	3.023			

Table 2.5 Experimental and model-predicted results of column tests.

Inf. NO_3-N (mg/l)	HRT (h)	Eff. NO_3-N (mg/L) (Test results)	Removal (%)	Eff. NO_3-N (mg/L) (Model prediction)
30	6	0.02	99.9	-3.82
30	6	0.00	100.0	-3.82
30	6	0.00	100.0	-3.82
40	6	0.28	99.3	-1.77
40	6	0.03	99.9	-1.77
40	6	0.17	99.6	-1.77

To verify the response surface model, additional tests that do not belong to factorial or axial or center points were run in three different column reactors with the HRT at 6 h and the feed NO_3^- - N concentrations being 30 mg/l or 40 mg/l. Table 2.5 lists the experimental results and model-predicted values. The effluent nitrate-N concentrations in these 6 tests were very low between 0 and 0.28 mg/l. The model-predicted values are -3.8 and -1.7 mg/l and were assumed to be zero. Therefore, the model is basically consistent with the experimental results under these conditions. As mentioned before, it is inevitable for the polynomial model having a limited region to approximate the experimental results. It is found that this model fits the results better under the operating conditions of $20 < X_1 < 110$ and $2 < X_2 < 9$, where X_1 is the influent nitrate-nitrogen concentration, mg/l, and X_2 is the HRT, hours.

As shown in Table 2.4, the mean absolute error of 3.023 is the average value of the residuals, and the standard deviation of the residuals is 5.5596. Therefore, relatively larger errors may exist in estimation of the effluent concentration when the influent concentration is small, the

HRT is long, and the corresponding effluent concentration is very low or near 0. Thus, the relative errors may be magnified.

As also shown in Table 2.3, the standard errors of the constant (12.0176) and x_1 are relatively large compared with themselves. Those two terms also have a high p-value, 0.1228 and 0.3367, respectively, which means that these terms are not statistically significant in this model at a confidence level > 90%. It should be pointed out that using a multiple linear model to fit a biological process may have a disadvantage because a biological process hardly performs in a regularly continuous way as a chemical process does. Therefore, the model may have some limitations, and further studies are needed.

Phosphorus Effects. As shown in Figure 2.4, after about 38 days running, reactors 1 to 3 reached quasi-steady-state conditions with high nitrate removal efficiencies (> 99%). Reactor 4 (P = 10 mg/l), however, kept a lower nitrate removal efficiency (~ 80-90%). These results seem to indicate that phosphorus at 0.05 mg/l is not a limiting factor for the SLAD process when the influent nitrate-nitrogen concentration is 30 mg/l, the HRT is 6 hours, and the corresponding nitrate-nitrogen loading rate is 154.1g NO_3-N/d-m^3 media (e.g., sulfur and limestone). In this case, 0.05 mg/l P is enough for autotrophic denitrificans to grow well.

The nitrate-nitrogen loading rate was then increased from 154.1 to 204.1 g NO_3-N/d-m^3 media (nitrate-nitrogen concentration in feed = 40 mg/l and HRT = 6 hours) in reactors 1 to 3 at day 47, and the phosphorus concentration in reactors 1, 2, and 3 was accordingly increased to 0.067, 0.133, and 6.67 mg P/l, respectively. Reactor 4 was shut down due to its poor nitrate removal performance caused by unknown reasons. After running for 16 days, these three reactors still kept very high nitrate removal efficiencies (up to 99%). Reactor 2 fed with 0.133 mg/l phosphorus seemed to have the best performance, and reactor 1 with 0.0067 mg/l P and reactor 3 with 6.67 mg/l P performed similarly. As shown in Fig. 2.5, during the period of 17 days, nitrate removal efficiency of each reactor fluctuated within 0.8%. This fluctuation can be neglected since the fluctuation within 5% is considered as the quasi-steady-state condition. No obvious and long-term continuous trends (i.e., either increase or decrease in nitrate removal) were observed. In addition, only a trace amount of nitrite-N (~ 0.3 mg/l) was detected.

The nitrate-nitrogen loading rate was further increased from 204.1 to 256.9 g NO_3-N/d-m^3 media in reactors 1, 2, and 3 (nitrate-nitrogen concentration in feed = 50 mg/l and HRT = 6 hours) at day 63, and the phosphorus concentration in reactors 1, 2, and 3 was accordingly increased to 0.083, 0.167, and 8.33 mg P/l, respectively. As shown in Fig. 2.6, after running for 13 days, reactor 1 fed with 0.083 mg/l P and reactor 2 fed with 0.167 mg/l P performed similarly with their nitrate removal efficiencies > 95%. Reactor 3 fed with 8.33 mg/l phosphorus performed the worst, but the nitrate removal efficiency was still > 95%. During this period, 1-3 mg/l NO_2-N was accumulated in the system. Based on the above results, it is concluded that phosphorus is not the limiting factor of cell growth when the nitrate loading rate is less than 256.9 g NO_3-N/d-m^3 media, and 0.00167 mg/l P per mg/l NO_3-N (i. e., 0.05 mg P/l for 30 mg NO_3-N or 0.083 mg P/l for 50 mg NO_3-N) is enough for obtaining a good nitrate removal efficiency > 95%. This phosphorus concentration is much lower than the value of 10 mg P/l for 30 mg NO_3-N reported by Batchelor (1976).

Effluent Quality of the SLAD Columns. The SLAD process is expected to be a feasible process for nitrate removal in drinking water treatment. In this study, the effluent quality of the SLAD columns was evaluated under quasi-steady-state conditions of different nitrate-nitrogen loading rates and HRTs, and the results follow.

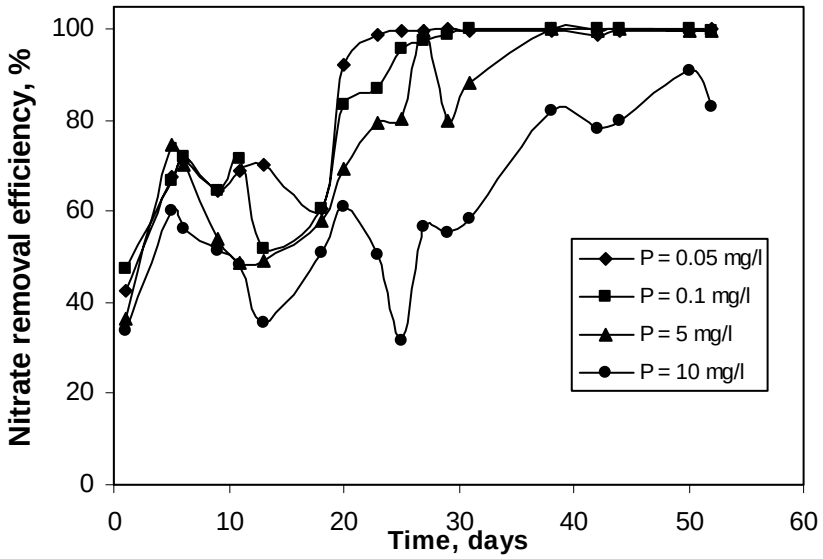

Figure 2.4 Effects of phosphorus concentration in feed solution on nitrate removal efficiency at the influent nitrate-nitrogen concentration of 30 mg/l and the HRT of 6 hours. The feed solution for reactors 1-4 contained 0.05, 0.1, 5, and 10 mg P/l, respectively. Day 0 corresponds to a new inoculation.

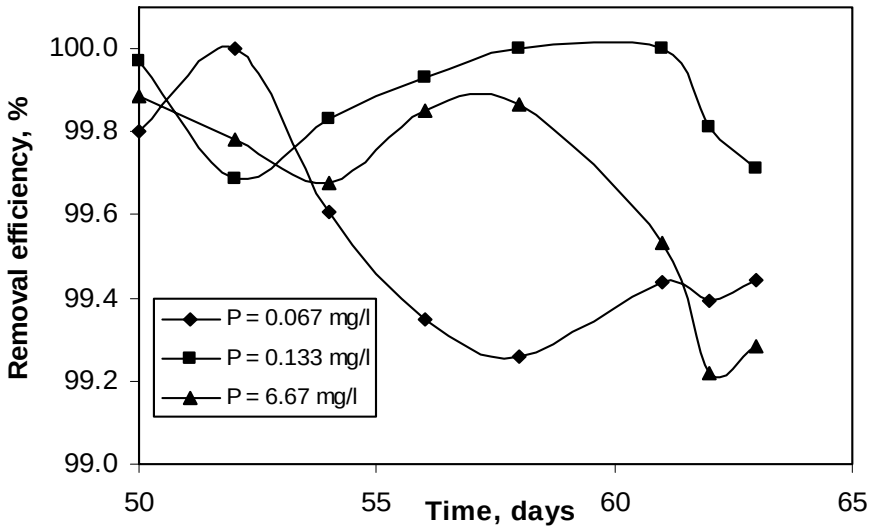

Figure 2.5 Comparison of nitrate removal efficiency corresponding to different phosphorus concentrations after the feed nitrate-nitrogen concentration increased to 40 mg/l NO_3-N and the HRT was kept at 6 hours. The feed solution for reactors 1-3 contained 0.067, 0.133 and 6.67 mg P/l, respectively. Day 0 corresponds to a new inoculation.

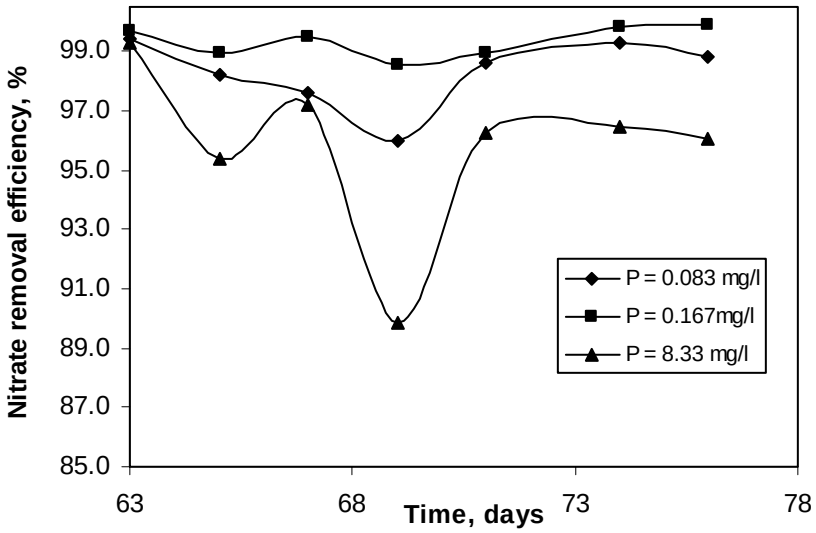

Figure 2.6 Comparison of nitrate removal efficiency corresponding to different phosphorus concentrations after the feed nitrate-nitrogen concentration increased to 50 mg/l NO_3-N and the HRT was kept at 6 hours. The feed solution for reactors 1-3 contained 0.083, 0.167 and 8.33 mg P/l, respectively. Day 0 corresponds to a new inoculation.

Nitrate. As shown in Fig. 2.7, a loading rate up to 260 g NO_3-N /d-m^3 media with HRTs between 6 to 11 hours would result in a high nitrate removal efficiency (~ 95%). Flere and Zhang (1999) reported that the maximum remval efficiency (> 95%) was reached at a loading rate around 175-225 g nitrate-N/ m^3-d. The results of this study are close to but higher than the results of Flere and Zhang (1999). However, a short HRT seems to affect the system performance significantly. When the loading rate was 246 g NO_3-N/d-m^3 media and the HRT was 2.5 hours, the nitrate removal efficiency was about 80%. This is consistent with the report of Claus and Kuntzer (1985a), that is, the nitrate removal efficiency would decrease when the HRT is < 3 hours. Therefore, both the HRT and the nitrate-N loading rate should be considered as design criteria. It is recommended that HRT be > 3 h to achieve high removal efficiency (> 90%). In this study, the maximum nitrate removal rate of 363 g NO_3-N/d-m^3 media was achieved at a loading rate of 987 g NO_3-N /d-m^3 media (the corresponding HRT = 2.5 hrs, and nitrate-N in feed = 80 mg/L).

Nitrite. When the nitrate-nitrogen loading rate was lower than 150 g NO_3-N/d-m^3 media, and the HRT was equal to or longer than 6 h, there was no nitrite detectable in the effluent. Nitrite was accumulated from ~ 0.3 to 1 mg/l NO_2-N when the nitrate-nitrogen loading rate was > 200 g NO_3-N/d-m^3 media at an HRT of 6 h. These results are consistent with previous reports (Flere and Zhang, 1999). Therefore, it is recommended that the HRT be kept equal or longer than 6 h and the nitrate loading rate less than 200 g NO_3-N/d-m^3 media to prevent nitrite accumulation > 1 mg/l NO_2-N (i.e., the MCL of regulated public water systems established by the U.S. EPA).

pH. In a SLAD process, H^+ ions are produced, and the pH in system may decrease. In this study, the influent pH in all tests was between 7.8 and 8.5; the effluent pH was between 6.7 and 8.0. It is believed that limestone would supply sufficient alkalinity at the 2:1 ratio of sulfur to limestone so that the effluent pH was at a relatively steady level.

Temperature. The influent into the SLAD reactors was at a temperature of about 22 ˚C. The temperature of effluent ranged among 20 ~ 25 ˚C. No abnormal fluctuation of temperature was observed during the operation period. No further study on temperature was conducted in this study. Koenig and Liu (2004) reported that the maximum denitrification rate was obtained at 30 ˚C, and sulfur-oxidizing autotrophic denitrifans might be mesothermophilic species that cannot tolerate temperatures > 50 ˚C.

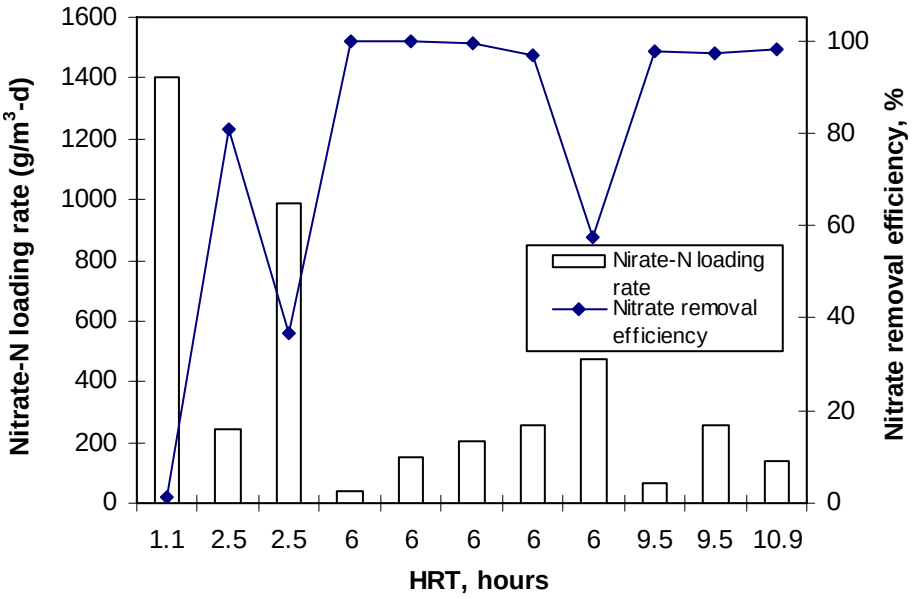

Figure 2.7 Relationship among nitrate removal efficiency, nitrate loading rate, and HRT.

Odor and Color. The effluent always showed normal color of drinking water (i. e., no color). No odor of sulfide was observed during the normal operational time except when the system was already biofouling, and also no precipitate was observed in the effluent.

Microbiological Quality. The column effluent was analyzed for TSS, VSS, TOC, and heterotrophic plate counts under quasi-steady-state conditions when the nitrate-nitrogen loading rate was between 154 and 257 g NO_3-N/d-m^3 media. The effluent biomass was about 9.5 mg TSS/l and 1.0 mg VSS/l; these are very low values. In this study, total organic carbon (TOC) was also used as a criterion for evaluating the microbiological quality of effluent since cells are composed mainly of organic carbon. The effluent TOC was between 4.0 and 9.0 mg/l. Compared with the influent TOC of 3.0 - 5.0 mg/l, this result shows that the SLAD column treatment process will not greatly impact the microbiological quality of the water being treated. In this study, both heterotrophic plate counts and aerobic autotrophic plate counts were conducted to evaluate the effluent microbes. The heterotrophic plate counts indicated that the number of bacteria in the effluent was about 2 x 10^5 CFU/l, which is lower than the regulated level of 5 x 10^5 CFU/l in drinking water (U.S. EPA, 2002).

Sulfates. In this study, the production rate of sulfate was found to be 7.10 mg SO_4^{2-} per mg NO_3-N reduced (Fig. 2.8). This ratio was calculated based on all the avaiable data (Figure 2.7) collected during the 11-month operational period, including both non-steady-state (i.e., inoculation and acclimatization) and quasi-steady-state conditions. The theoretical sulfate

production ratio based on Eq. 1.1 is 7.54 mg SO_4^{2-} per mg NO_3-N reduced, which is a little higher than those from these experiments. There may be many reasons for this. First, heterotrophic denitrification might occur in this process (Lawrence, 1978; Claus and Kutzner, 1985a; Zhang, 2002). This might result in a low sulfate production ratio since sulfur would not be used in a heterotophic denitrification process. Second, nitrite could be accumulated when the nitrate loading rate was high, which would limit complete reduction of nitrate to nitrogen gas and the complete transformation of sulfur to sulfate. Verifying these mechanisms is beyond the scope of this study.

Several different ratios (all in units of mg SO_4^{2-} per mg NO_3-N reduced) were reported in the literature, such as 11.1 (Schippers et al., 1987), 9.9 (Hashimoto et al., 1987), 7.89 (Koenig and Liu, 1996), 7.64 (Flere and Zhang, 1999), 3.4 (Zhang and Lampe, 1999). According to the sulfate production ratio obtained in this study, when the influent nitrate-nitrogen concentration is > 14 mg/l (assuming 150 mg/l sulfate in ground water being treated), the effluent sulfate will be over 250 mg/l (i.e., the secondary MCL set by the U.S. EPA). Therefore, the high production of sulfate in effluent is a concern of SLAD processes, which may limit its application.

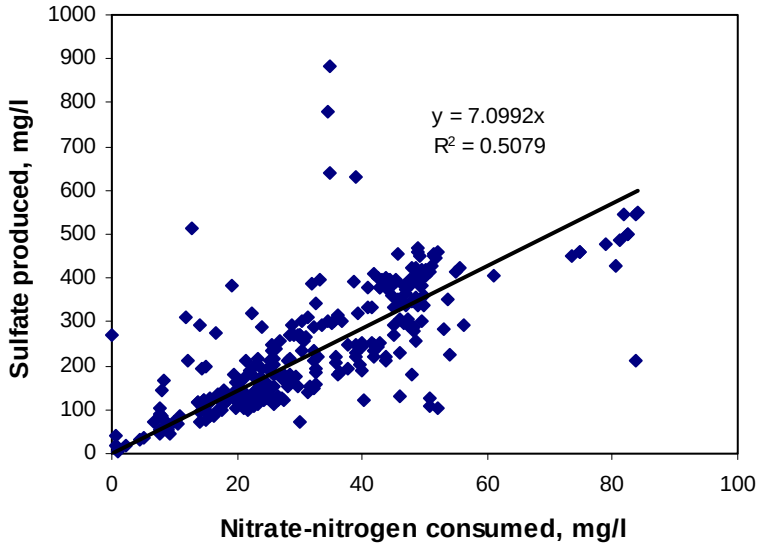

Figure 2.8 Relationship between sulfate production and nitrate-N reduction in all samples.

Operating Problems of SLAD Columns. During the long-term operation of the SLAD column reactors, there were some problems related to the initiation of the reactors. Figs. 2.9 – 2.12 are the 8-month time-course data of those four reactors. In this study, because of biofouling or experimental requirement, the systems were re-inoculated several times (shown in Fig. 2.9-2.12 by arrows) with or without backwashing before inoculating the column(s). Here, backwashing means cleaning the packing media before re-inoculation while no backwashing means no cleaning the packing media and just draining the reactors before re-inoculation. The column tests for the response surface study were started at day 150 (day 1 = the starting pumping day, 05/04/2003). Note that the steep decreases of the nitrate removal efficiency after day 200 in Figs. 2.9 – 2.10 are due to the large increases in nitrate loading rates according to the Central Composite Design (Table 2.1), not because of biofouling or system failure.

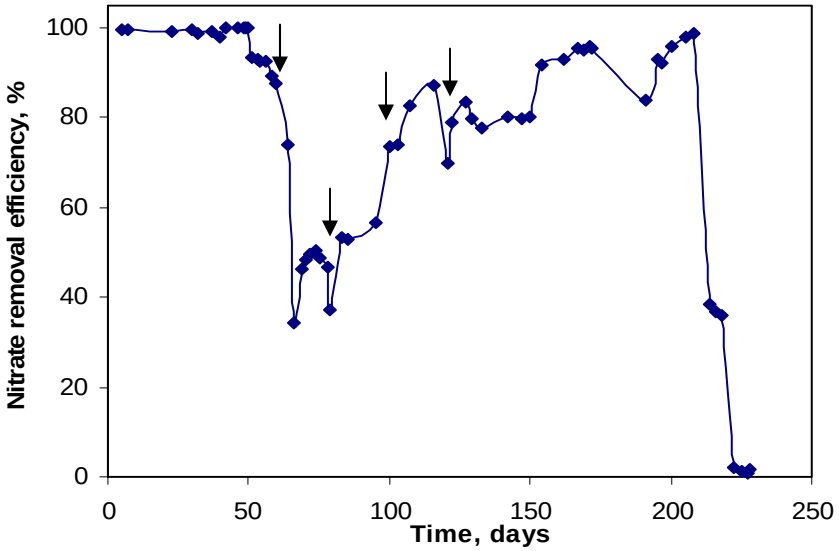

Figure 2.9 Time course of nitrate removal in SLAD column reactor 1 during the operational period (228 d). Arrows represents re-inoculation. Response surface column runs started at day 150. Day 0 corresponds to a new inoculation at the beginning of the test.

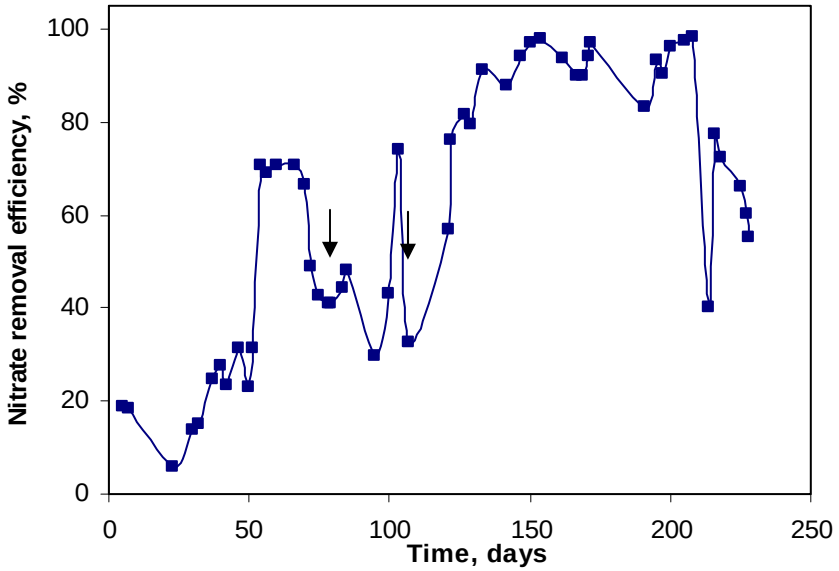

Figure 2.10 Time course of nitrate removal in SLAD column reactor 2 during the operational period (228 d). Arrows represents re-inoculation. Response surface column runs started at the day 150. Day 0 corresponds to a new inoculation at the beginning of the test.

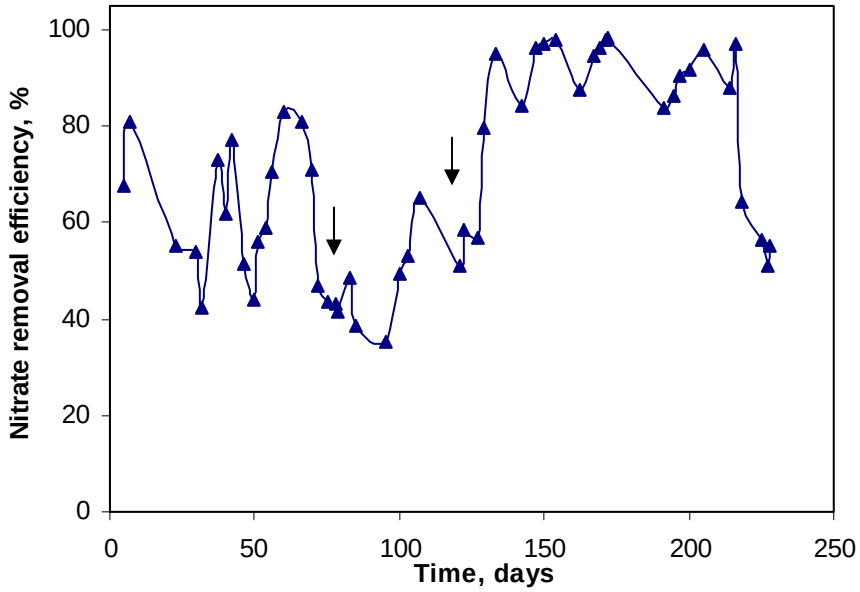

Figure 2.11 Time course of nitrate removal in SLAD column reactor 3 during the operational period (228 d). Arrows represents re-inoculation. Response surface column runs started at the day 150. Day 0 corresponds to a new inoculation at the beginning of the test.

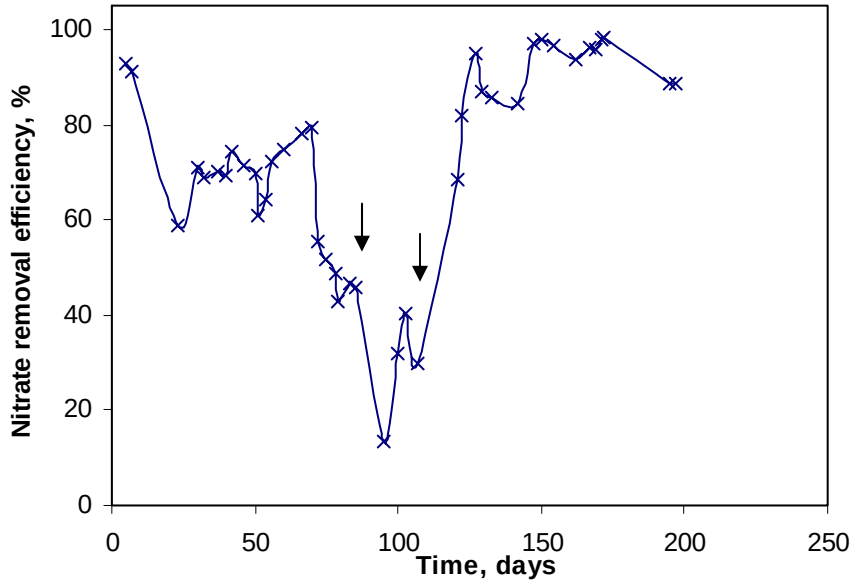

Figure 2.12 Time course of nitrate removal in SLAD column reactor 4 during the operational period (200 d). Reactor 4 shut down due to system failure by accident at day 198. Arrows represents re-inoculation. Response surface column runs started at day 150. Day 0 corresponds to a new inoculation at the beginning of the test.

As shown in the figures, when the four reactors were started with the same method of inoculation, only reactor 1 removed nitrate well with a removal efficiency > 95%; the other three all performed very poorly when the removal efficiency fluctuated in a wide range. Reactor 1 was backwashed at day 64 due to biofouling. After it restarted, it went through four re-inoculations before the nitrate removal efficiency finally reached > 90%. The re-inoculations were at days 65 (with backwashing), 87 (no backwashing), 103 (no backwashing) and 133 (no backwashing). The same situation also happened to reactors 2 to 4. They reached > 90% nitrate removal efficiency after two re-inoculations (i.e., at days 87 (with backwashing) and 121 (without backwashing)). Therefore, multiple re-inoculations may be a way to improve the poor performance of SLAD column reactors.

Up to now, nearly no studies have reported the difficulties of start-up and operation of the SLAD processes as were encountered in this study. In the research of van der Hoek et al. (1994), they reported that nitrate removal was limited by the supersaturation of nitrogen gas in effluent, and this problem was solved by equipping a preceding vacuum deaerator which reduced the nitrogen gas and oxygen in the influent from 18 – 20 and 7 – 8 mg/l to 1 – 2 and 0.5 – 1.0 mg/l, respectively. They explained that supersaturation caused channeling and short-circuiting in reactors, which resulted in the low nitrate removal efficiency. However, there were some differences of experimental conditions between their experiment and ours. The column reactors (H x Φ = 75 cm x 30 cm) used in their study were much longer and had a larger diameter than ours (H x Φ = 36.5 cm x 6.35 cm). Usually smaller reactors suffer more channeling and short-circuiting problems, but the poor nitrate removal performance did not occur in every column reactor though they were operated under the same conditions (i.e., the pump, the flow rate, etc.). Reactor 1 performed well during the period from day 1 to about day 70. Therefore, the channeling and short-circuiting might not be very significant in our case. But short-circuiting and channeling could still be a possible problem because the gas bubbles were observed to be accumulating in reactors which might cause head loss in reactors and thus short-circuiting. In addition, when sulfur and limestone are loaded into reactors, the particles are not uniformly packed, the reactors might more prone to short-circuiting or channeling problem. Since the nitrate removal performance improved after several times of re-inoculation, the inoculation might be very important to the acclimatization of the autotrophs onto sulfur stones. Possibly, if the cells are not well or steadily attached during the standstill period of inoculation, they will have difficulty to grow well under continuously flowing conditions. In this case, replicate inoculations could be helpful on acclimating more cells onto media. So far, the reasons behind this have not been identified.

In the experiment, during the period of days 125 to days 228, the SLAD column reactors were run with artificial ground water at a high nitrate removal efficiency for 3 or longer months without the need of backwashing. Actually the reactors seemed to be able to continue running for a longer time (but the system was shut down after day 228). This is in agreement with previous research (Claus and Kutzner, 1985a; van der Hoek, 1992, 1994; Flere and Zhang, 1999).

2.4. Summary. Based on a rotatable central composite design, 12 column tests were conducted in the response surface study of the SLAD process. A polynomial linear regression model was set up to quantitatively describe the relationship of the effluent and influent nitrate-nitrogen concentration and HRT in the SLAD column reactors. This model may be used for estimating the effluent nitrate-nitrogen concentration when the influent nitrate-nitrogen concentration ranges between 20 and 110 mg/l and the HRT ranges between 2 and 9 hours. This model can be

improved after further calibration. The phosphorus study of nutrients showed that when the nitrate loading rate is less than 256.9 g NO_3-N/d-m^3 media, 0.00167 mg/l P per mg/l NO_3-N is enough for obtaining a good nitrate removal efficiency > 95%. The effluent quality was also evaluated under quasi-steady-state conditions for different nitrate-nitrogen loading rates and HRTs. A loading rate up to 260 g NO_3-N /d-m^3 media with HRTs between 6 to 10.9 hours would result in a high nitrate removal efficiency (~ 95%). It is also recommended that the HRT be kept equal or longer than 6 h and the nitrate loading rate less than 200 g NO_3-N/d-m^3 media to prevent nitrite accumulation to be > 1 mg/l NO_2-N. TOC and HPC tests showed that the SLAD column treatment process will not greatly impact the microbiological quality of the treated water. Multiple re-inoculations of the SLAD column reactor were found to be a way to improve the poor nitrate removal performance of the SLAD reactors.

3. Kinetic Studies

3.1. Problems Statement, Approaches, and Justification. Practical application of the SLAD process requires full understanding of the process kinetics. In the past, most of the fundamental studies (e.g., kinetics) about the SLAD process were conducted using suspended-growth systems. In these studies, batch reactors or CSTRs were fed with thiosulfate ($S_2O_3^{2-}$), instead of sulfur stones, under anaerobic and aerobic conditions with nitrate or nitrite as the limiting substrate (Justin and Kelly, 1978; Claus and Kutzner, 1985b). This is due to the difficulty to completely mix sulfur stones (such as sulfur powders) with others to make a batch or a CSTR reactor work properly as Batchelor (1976) did. While the results obtained from these suspended-growth systems are informative, they can't be used directly for performance evaluation and process design of SLAD biofilm column reactors.

Sikora and Keeney (1976) indicated that although the SLAD process was believed to follow the Monod equation within the biofilm, it showed first-order kinetics in bulk solution because of the relative low nitrate concentration. Batchelor and Lawrence (1978a, b) conducted kinetic studies using a slurry reactor and concluded that, when the nitrate concentration in the SLAD system was high enough to penetrate the whole biofilm, the denitrification rate followed a zero-order reaction in bulk solution. However, when the nitrate could not fully penetrate the biofilm, they found the denitrification reaction to follow a half-order reaction in the bulk solution. In both of these cases, the microbial uptake rate of nitrate in the bioflim was assumed to follow zero-order kinetics. The results of Batchelor and Lawrence (1978a, b) were confirmed by others when the SLAD process was used for nitrate removal in groundwater (Liu, 1992, Koenig and Liu, 2001) and septic tank wastewater (Zhang, 2003). While these studies reported information on rate constants for zero or half-order reaction, critical nitrate-N concentration (Zhang, 2002), effects of the sulfur particle size and nitrate concentration on process performance, etc., Monod-based kinetics of a SLAD biofilm process still are not available. Without these parameters, it is difficult to design and evaluate a SLAD biofilm system (LeCloirec, 1985), and to compare the system with other denitrification technologies.

The objective of this part of the present research was to develop methods to experimentally evaluate the four kinetic parameters, that is, half-velocity constant, K_s; maximum specific substrate utilization rate, k; bacteria yield coefficient, Y and bacteria decay coefficient, k_d in the SLAD biofilm process. Considerable research has been conducted on biofilm kinetics (Rittmann and McCarty, 2001). Determination of kinetic parameters has historically been a tedious and labor-intensive undertaking. The literature search indicates that most studies

estimated biofilm kinetic parameters based on curve fitting techniques, such as fitting a mathematical model with axial substrate concentration profiles along the column, or substrate concentration profiles within the biofilm or immobilized enzyme, or substrate time courses within a batch reactor contained biofilm stripped off from the media or still attached on the media. Rittmann et al. (1986) proposed a method for in-situ determination of K_s and k for biofilms. Their method employs a curve-matching method with kinetic results from several short-term experiments with CSTRs, which would be adapted and used in this study for the evaluation of K_s and k. However, this method is not able to evaluate k_d and Y. Therefore, it was necessary to develop methods of evaluating all four kinetic parameters in a biofilm SLAD reactor which may also be suitable for other biofilm processes.

3.2. Materials and Methods. ***Fixed-bed Column Reactors.*** Two column reactors were used to conduct the kinetics study. One (H x Φ = 19.0 cm x 3.81 cm) was for K_s and k estimation while the other one (H x Φ = 34.0 cm x 3.81cm) was for k_d and Y estimation. These two reactors have the same configurations but the different sizes. The schematic diagram of the reactor system is shown in Fig. 3.1 and the characteristics of the reactors are shown in Table 3.1. The columns were filled with granular elemental sulfur (Georgia Gulf Sulfur, Bainbrige, GA) and limestone at a ratio of 2:1. This ratio was found to support bacteria growth well (Liu and Koenig, 2002). Sulfur and limestone particles ranged between 2.38 mm - 4.76 mm in size (U.S. sieve #8 and #4). The working volume of the reactors is the volume filled with the sulfur/limestone media. The reactors were not filled fully with packing media. The volume above the media was empty to allow nitrogen gas produced during the process to escape from the reactors. A Masterflex L/S peristaltic pump (Cole-Parmer, IL, U.S.A.) with two different standard Masterflex pump heads (Model 7013-20 and Model 7018-20) was used to provide constant flows (including influent flow and recycle flow) into the reactor. The recycle flow was 39.6 times that of the influent flow so that the column reactor became a CSTR reactor. The effluent and gas outlet were both connected with a flask full of water to prevent oxygen from getting into the system. Nitrogen gas was bubbled through the feed solution to eliminate the effect of oxygen on nitrate removal. A vent was provided for extra nitrogen gas to escape from the substrate tank.

Culture Conditions. At the beginning, 50 ml of seed from the seed CSTR (see **Section 2.2**) was mixed with 100 ml of feed solution from the seed reactor but without thiosulfate and then injected into the column reactor. The procedure for inoculation is the same as those in **Section 2.2**. The feed solution used here was artificial groundwater (Table 3.2) plus 30 mg/L NO_3-N from potassium nitrate (Mallinckrodt Co., Kentucky, U.S.A.). Trace nutrients were made by dissolving a tablet of Centrum (Wyeth Consumer Healthcare, Madison, NJ, U.S.A.) into 500 ml tap water. One ml of the nutrient solution was then added into one liter of the artificial groundwater. Before use, each new tank of feed solution was bubbled with nitrogen gas for 30 minutes to remove dissolved oxygen. The nitrogen gas was then kept very gentle bubbling (i.e., 2 bubbles per second) during the experimental period. Note a vent was provided for extra nitrogen gas to escape from the substrate tank. The HRT was maintained at 3.56 hours (Table 3.1) corresponding to the working volume (i.e., sulfur/limestone filled volume); HRTs in this part of the research were all calculated in the same manners. The reactor was run for three to four weeks until it reached the quasi-steady-state conditions (same definition as before).

Non-Steady-State Tests for K_s and k Estimation. After the reactor fed with 30 mg NO_3-N mg/l substrate reached the quasi-steady state, 22 short-term tests were consequently conducted by feeding with artificial groundwater containing 0.4~1200 mg/L NO_3-N.

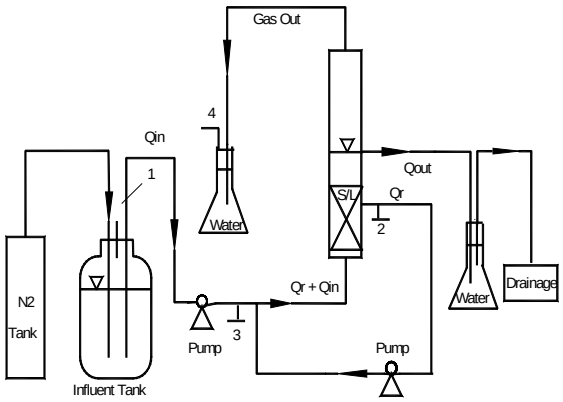

Figure 3.1 Experimental set-up for kinetics study. (Note: 1 and 4 are N_2 gas vents; 2 and 3 are sampling ports.)

Table 3.1 Characteristics of SLAD reactors used for kinetics study.

Characteristics	Reactors		
	Study K_s and k	Study k_d and Y	Measure biomass of 30 mg NO_3-N/l
Length of reactor, cm	19.0	34.0	19.0
Diameter of reactor, cm	3.81	3.81	3.81
Vol. of packing media(S/L), cm^3	65.10	78.63	67.26
Cross-sectional area of reactor	11.40	11.40	11.40
*Diameter of particles, cm	0.246	0.246	0.246
Specific area of particle, a, 1/cm	13.90	13.90	13.90
Influent flow rate,cm^3/min	0.3048	0.307	0.32
Porosity	0.43	0.43	0.43
Recycle ratio	39.7	39.08	39.21
Liquid density, mg/cm^3	998.2	998.2	998.2
Viscosity, mg/hr-cm	36072	36072	36072
D_f, cm^2/hr	0.03744	0.03744	0.03744
D, cm^2/hr	0.0468	0.0468	0.0468
Hydraulic retention time, hrs	3.56	4.27	3.50
L_f, cm	0.0084 ± 0.0060	0.0041 ± 0.0019	0.0084 ± 0.0060
Biomass, mg VSS /mL meida	1.62	1.34	1.62
X_f,mg/cm^3	13.87	23.51	13.87
NO_3-N in feed, mg/l	30	5	30

*d_p is geometric mean of 2.38 mm and 4.76 mm, shape factor = 0.73.

Trace nutrient was the same as in culture conditions. For each run, the feed solution was bubbled with nitrogen gas using the aforementioned procedures. The reactor was first drained of the old feed solution and then washed three times with new feed solution. After that, the reactor was loaded with the new feed solution and started running. The detailed characteristics of the reactor and biofilm are listed in Table 3.1. After running 7 hours, 5 samples were collected. Upon completion of each test, the reactor was allowed to return to the original situation (i.e., 30 mg NO_3-N/L in feed and HRT = 3.56 hours) and kept running until the quasi-steady-state conditions were achieved before the next run was conducted in the same way.

Batch Tests for k_d and Y Estimation. As mentioned before, a similar column reactor (H x Φ = 34.0 cm x 3.81cm) was used to estimate k_d and Y. This reactor was set up, inoculated and cultured in the same way as the one used for K_s and k estimation except that it was fed with 5 mg/l NO_3-N at an HRT of 4.27 hours when started pumping. The other components of feed solution are also shown in Table 3.2. After it reached the quasi-steady state conditions, the column was run as a completely mixed batch reactor by stopping its continuous feed but keeping the same recycle ratio. Information about the reactor and biofilm is shown in Table 3.1. The reactor was drained off and rinsed with 50 mg/l NO_3-N feed solution several times before use. Then 200 ml substrate (same as Table 3.2) with 50 mg/l NO_3-N was quickly injected into the batch reactor. Samples of 2-ml volume each were collected about every 30 minutes for 4 h. The column was then brought back to the CSTR mode with 5 mg/l NO_3-N in the feed after this short-term test. Next, when the column reached quasi-steady-state conditions again, it was starved for 30 hours. During the starving period, feed solution without nitrate but with the same other components as shown in Table 3.2 was fed continuously at the same recycle ratio. After starving, another 4-h batch test was conducted using the same procedure and the same substrate.

The reactor was frequently switched from one feed solution to another of different nitrate concentration. Therefore, to reduce the effect of different substrate, every time the substrate was changed, the reactor was drained off the old substrate and then rinsed with new substrate for several times before it was reloaded.

Table 3.2 Feed solution composition.

Component	Concentration (mg/L)	Component	Concentration (mg/L)
NO_3^--N	0.4 - 1200	Phosphate (ortho)	0.3
Alkalinity	300	Sulfate	150
Fluouride	0.25	pH	7.8-8.5
Iron	0.3		

* Feed solution made with tap water. Nutrient is added as 1ml per liter of feed solution.

Reactor used for Biomass Measurement. In this study, non-steady-state tests for K_s and k estimation were run right after the reactor reached quasi-steady-state conditions. If measuring biomass right after the biofilm reached quasi-steady-state conditions, biofilm would have been destroyed. Therefore, this CSTR was backwashed after all non-steady-state tests had been completed and set up again to measure the biofilm thickness and biomass at quasi-steady-state conditions of 30 mg/l NO_3-N in the feed. This reactor was set up and run in the exact same manner as the one used for K_s and k estimation. Its feed solution was the artificial groundwater (Table 3.2) plus 30 mg/l NO_3-N. It was run for about three weeks and then reached quasi-steady-

state conditions (about 80% nitrate-nitrogen removal). Biofilm thickness and biomass were then measured (Table 3.1).

Analytical Methods. Nitrate, nitrite, DO, were analyzed with the same procedures as in **Section 2.2**. To analyze VSS, 6 samples of about 2 ml (10~20 particles) sulfur and limestone particles were taken out of the packing media throughout the column. The particles were rinsed with deionized water repeatedly until they looked relatively clean by eye sight (Flere and Zhang, 1999). The washed-out biomass was then filtered with a 0.45μm glass microfiber filter paper (Whatman, UK) and analyzed for VSS as per Standard Method 2540E (APHA et al., 1992).

Biofilm thickness was measured with a microscope and a 10-time lens (Leitz wetzlar, Germany). The sample sulfur particle was placed on the glass slide and then added with one drop of deionized water (because the biofilm functioned in water and lack of water might cause inaccuracy in measurement). Under the microscope, the biofilm could be observed at the edge of the particle surface. The interfacial surface of the particle and the biofilm could also be distinguished since the biofilm has a looser structure than sulfur stone does. Therefore, the biofilm thickness was estimated by the minimum scale of 10 μm in the microscope. It was observed that biofilm did not uniformly cover the surface of the sulfur stones. So each particle was turned after one measurement and three values were estimated at different positions. Each time over 20 particles were used to obtain the average biofilm thickness.

Biomass density (X_f) was calculated based on the biomass measured per unit of packing media surface (i.e., VSS) and biofilm thickness (L_f) measured. The equation is: $X_f = M_b /(L_f Va)$, where M_b = total biomass in biofilm as VSS, M; V = volume of sulfur and limestone packed, L^3; a = specific area of sulfur and limestone, 1/L. Its units are mg VSS per ml biofilm where the biofilm includes water and solids.

Procedures for Estimation of K_s and k. The method developed by Rittmann et al. (1986) was used in this study to estimate K_s and k. The detailed procedure is as follows:

The intrinsic kinetic parameters can be estimated from the non-steady-state experimental results using a family of standard curves in the dimensionless domain. The dimensionless domain has only three variables, and is denoted by an asterisk as follows:

$$J^* = J\tau /(K_s D) \qquad (3.1)$$

$$S_s^* = S_s / K_s \qquad (3.2)$$

$$L_f^* = L_f / \tau \qquad (3.3)$$

where J^* = dimensionless substrate flux into biofilm; τ = biofilm depth dimension, $[K_s D_f /(kX_f)]^{1/2}$, L; S_s^* = dimensionless substrate concentration in the interface of biofilm and diffusion layer; L_f^* = dimensionless biofilm thickness. K_s = half-velocity constant, M/L^3; k = maximum specific substrate utilization rate, 1/T; L_f = biofilm thickness, L; X_f = biomass density in biofilm, M/L^3; S_s = substrate concentration in the interface of biofilm and diffusion layer, M/L^3; S_b = substrate concentration in bulk liquid, M/L^3; D = molecular diffusivity of nitrate in bulk solution, L^2/T; D_f = molecular diffusivity of nitrate in biofilm (assuming $D_f = 0.8\ D$), L^2/T; J = substrate flux into biofilm, $M/L^2 \cdot T$.

The family of curves (Fig. 3.2) was generated with Excel software that calculated J^* values for a wide range of S_s^* and L_f^* using solutions presented by Atkinson and Davies (1974) and Rittmann and McCarty (1981 and 1987) for simultaneous reaction with diffusion within a

biofilm. An overlay transparency was made from Fig. 3.2. The following steps were followed to estimate K_s and k (Rittmann et al., 1986):

1) Plot Fig. 3.3 with log J_{exp} (J_{exp} = substrate flux into biofilm) vs. $log\ S_s$ for each run (data shown in Table 3.3) on a separate graph having the same scale as the overlay (Fig. 3.2.).
2) Manipulate the overlay (Fig. 3.2) over the data plot (Fig. 3.3) until the experiment points fit a single overlay curve.
3) Find the point on experimental S_s axis that lines up with the overlay value S_s*=1, this S_s *is* K_s.
4) At the point where the S_s* = 1 line intersects the L_f* curve chosen to fit the data. Read the $log\ J$* and $log\ J_{exp}$.
5) Calculate k from the values obtained in step 4:

$$k= (J_{exp}/J^*)^2\ 2D_f/(K_s\ X_f\ D^2) \qquad (3.4)$$

6) To check if L_f* is consistent with the experimental L_f ,

$$L_f^*= L_f/[2K_s\ D_f/(kX_f)]^{1/2} \qquad (3.5)$$

7) Repeat step 2 to 6 if L_f* is not with experimental L_f until they are consistent.

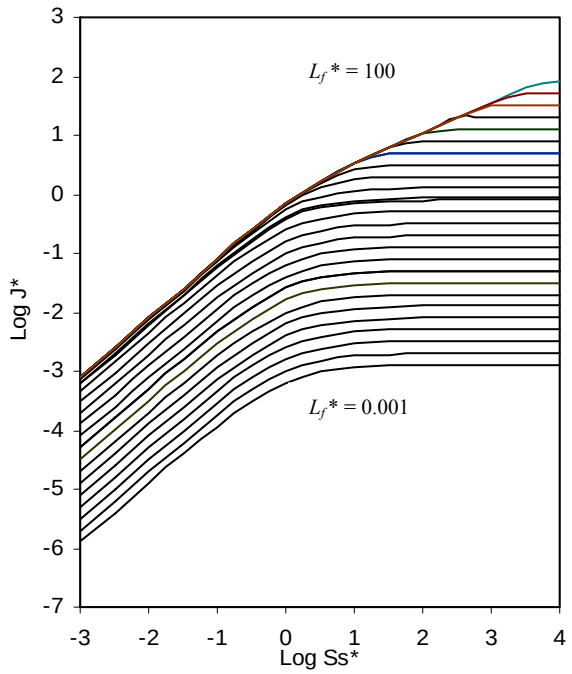

Figure 3.2 Family of dimensionless curves. (From bottom to top L_f* is 0.001, 0.0016, 0.0025, 0.0040, 0.0064; 0.01, 0.016, 0.025, 0.04, 0.064; 0.10, 0.16, 0.25, 0.40, 0.64; 1.0, 1.6, 2.5, 4.0, 6.4; 10, 16, 25, 40, 64, and 100, respectively; the thick black curve L_f* = 1.)

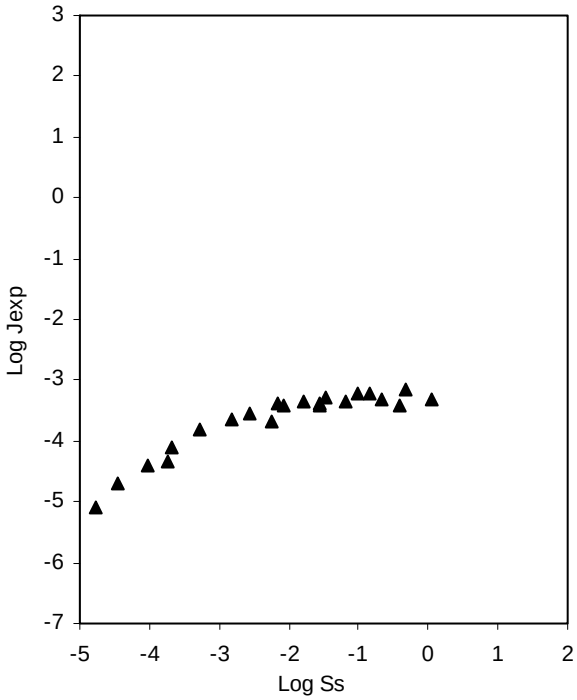

Figure 3.3 Results of non-steady-state tests.

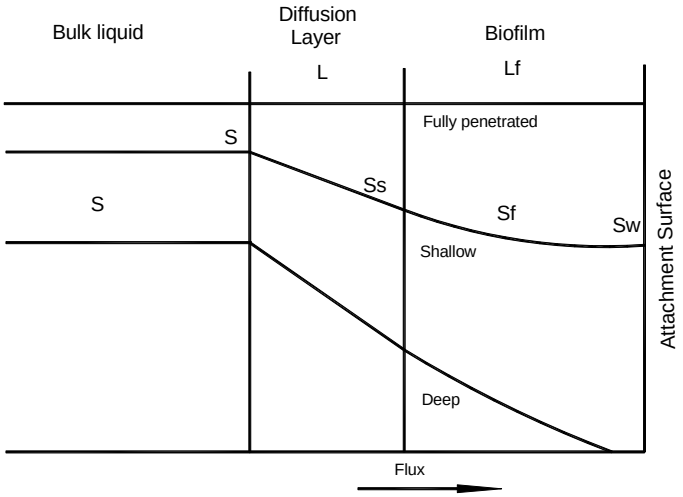

Figure 3.4 Idealized biofilm characteristic concentration profiles.

Procedures for Estimation of k_d and Y. Based on Suidan et al. (1987), a biofilm is fully-penetrated when the substrate concentrations at the outer surface (S_s) and at the attachment surface (S_w) are virtually identical (Fig. 3.4). Therefore, at any position within a fully-penetrated biofilm, substrate is utilized as:

$$r = - kX_f S_f / (K_s + S_f) = - kX_f S_s / (K_s + S_s) \quad (3.6)$$

where r = substrate utilization rate, M/L^3T; K_s = half-velocity constant, M/L^3; k = maximum specific substrate utilization rate, 1/T; X_f = biomass density in biofilm, M/L^3; S_s = substrate concentration in the interface of biofilm and diffusion layer, M/L^3; S_f = substrate concentration at that point in the film, M/L^3; S_w = substrate concentration at the surface of attachment, M/L^3.

Integrating Eq. 3.6 over the biofilm, then

$$r_{avg} = -(1/L_f) \int_0^{L_f} [kX_f S_s / (K_s + S_s)] \, dz = - kX_f S_s / (K_s + S_s) \quad (3.7)$$

where L_f = biofilm thickness, L; r_{avg} = average substrate utilization rate over the biofilm.
If $S_s >> K_s$, the term $S_s / (K_s + S_s) \approx 1$, then

$$r_{avg} = - kX_f \quad (3.8)$$

For a fully-penetrated biofilm and $S_s >> K_s$ (but not being high enough to inhibit the denitrification), the method developed by Lesouf et al. (1992) was adapted to estimate the decay rate coefficient, k_d of the SLAD system. Details of the adapted method are as follows.

When the column was run as a batch reactor that contained the same substrate as shown in Table 3.2 plus 50 mg NO_3-N/l. a reference average nitrate-nitrogen utilization rate, named r_o, was measured as:

$$r_o = - kX_f \quad (3.9)$$

The biofilm was then left without nitrate-N for a sufficient time period, t_d, to allow decay to occur within the biofilm. Assuming the microbial decay follows the first-order kinetics (Lesouf et al., 1992) and can be averaged across the biofilm,

$$\frac{dX}{dt} = -k_d X_f \quad (3.10)$$

Then, after t_d,

$$X_{f\,new} = X_f \, exp(- k_d \, t_d) \quad (3.11)$$

After the starving period, the column was run again as a batch reactor containing the same substrate (Table 3.2). Another average nitrate-nitrogen utilization rate, named r_d, was measured as:

$$r_d = - k \, X_f \, exp(- k_d \, t_d) \quad (3.12)$$

The decay rate coefficient was then calculated by combining Eqs. 3.9 and 3.12

$$k_d = ln\ (r_o/r_d) / t_d \qquad (3.13)$$

Y can be calculated from k_d by employing a biomass balance on biofilm under steady-state conditions (Rittmann and McCarty, 2001):

$$Y = X_f L_f k_d / J \qquad (3.14)$$

It should be pointed out that, in the experiment, a high S_s could be obtained by keeping a high S_b in the reactor because the diffusion layer only made a very small concentration gradient in this case. Moreover, the values of S_s in Eq. 3.7 would be cancelled in the calculation. Then it may be assumed that S_s is equal to S_b. In this study, to evaluate whether the biofilm is fully-penetrated or not, the effective factor η was calculated as follows (Atkinson and Davies, 1974):

$$\eta = 1 - \frac{\tanh(\phi_m)}{\phi_m} \cdot \{\Phi p / tanh(\Phi_p) - 1\} \qquad \text{for } \Phi_p \le 1 \quad \text{or} \qquad (3.15)$$

$$\eta = \frac{1}{\phi_p} - \frac{\tanh(\phi_m)}{\phi_m} \cdot \{1 / tanh(\Phi p) - 1\} \qquad \text{for } \Phi p \ge 1 \qquad (3.16)$$

where $\Phi_p = \Phi_m C_s [2 (1+C_s)^2 (C_s - ln (1+ C_s))]^{-1/2}$; $\Phi_m = [k X_f L_f^2 / K_s D_f]^{1/2}$; $C_s = S_b / K_s$. K_s = half-velocity constant, M/L^3; k = maximum specific substrate utilization rate, 1/T; L_f = biofilm thickness, L; X_f = biomass density in biofilm, M/L^3; S_b = substrate concentration in bulk liquid, M/L^3; D = molecular diffusivity of nitrate in bulk solution, L^2/T; D_f = molecular diffusivity of nitrate in biofilm ($D_f = 0.8\ D$), L^2/T. In this study, the biofilm was fully-penetrated if $\eta \approx 1$.

3.3. Results. ***Estimation of K_s and k.*** The parameters related to the reactor are in Table 3.1. The test results are shown in Table 3.3. The following equations were used to calculate S_s and J_{exp}:

$$S_{avg} = (S_{in}-S_e) / ln(S_{in}/S_e) \qquad (3.17)$$

$$S_{in} = (QS_o+Q_rS_e)/(Q+Q_r) \qquad (3.18)$$

$$J_{exp} = (S_o - S_e)/ (HRT^* a) \qquad (3.19)$$

$$S_s = S_{avg} - LJ_{exp}/D \qquad (3.20)$$

$$L = D (Re_m)^{0.75} (Sc)^{2/3} / (5.7v) \qquad (3.21)$$

where S_{avg}, S_o, S_e = the logarithm average, feed, and effluent concentration of nitrate-N, respectively, M/L^3; S_{in} = actual substrate concentration at inlet of reactor, M/L^3; Q = the feed flow rate, L^3/T; Q_r = the recycle flow rate, L^3/T; L = thickness of the effective diffusion layer, L, which is determined by the empirical formula for porous media (Jennings, 1975; Rittmann and McCarty, 2001); $Re_m = [2\rho d_p / (1- \varepsilon) \mu]$ (Rittmann and McCarty, 2001); ρ = liquid density, M/L^3; d_p = diameter of sulfur particle, L; v = superficial flow velocity, L/T; ε = porosity of media; μ = absolute viscosity of liquid, M/LT, Sc = Schmidt number, $\mu/\rho D$.

Table 3.3 Results and calculated flux for short-term non-steady-state experiments.

Sample Set	S_o (NO_3-N) mg/cm^3	S_e mg/cm^3	S_{in} mg/cm^3	S_{avg} mg/cm^3	J_{exp} mg/cm^2-hr	S_s mg/cm^3	Log J_{exp}	Log S_s
1	0.0025	0.0002	0.000229	0.000199	0.00004654	0.000184	-4.3322	-3.7345
2	0.0156	0.0056	0.005806	0.005682	0.00020229	0.005618	-3.6940	-2.2504
3	0.0459	0.0274	0.027817	0.027588	0.00037527	0.027469	-3.4257	-1.5612
4	0.0597	0.0332	0.033803	0.033476	0.00053674	0.033306	-3.2702	-1.4775
5	0.0866	0.0641	0.064624	0.064347	0.00045530	0.064203	-3.3417	-1.1924
6	0.1256	0.0966	0.097327	0.096970	0.00058616	0.096785	-3.2320	-1.0142
7	0.0010	0.0000	0.000054	0.000041	0.00001960	0.000035	-4.7077	-4.4611
8	0.0476	0.0270	0.027507	0.027253	0.00041629	0.027121	-3.3806	-1.5667
9	0.0280	0.0067	0.007238	0.006973	0.00042993	0.006838	-3.3666	-2.1651
10	0.0280	0.0082	0.008726	0.008481	0.00039912	0.008355	-3.3989	-2.0781
11	0.0388	0.0164	0.016981	0.016704	0.00045247	0.016561	-3.3444	-1.7809
12	0.0172	0.0027	0.003035	0.002853	0.00029347	0.002760	-3.5324	-2.5591
13	0.0081	0.0005	0.000674	0.000576	0.00015281	0.000528	-3.8159	-3.2772
14	0.0124	0.0014	0.001697	0.001558	0.00022195	0.001488	-3.6537	-2.8274
15	0.0040	0.0002	0.000282	0.000231	0.00007776	0.000207	-4.1092	-3.6850
16	0.0020	0.0001	0.000133	0.000108	0.00003868	0.000096	-4.4125	-4.0195
17	0.0004	0.0000	0.000025	0.000019	0.00000804	0.000017	-5.0946	-4.7709
18	0.1707	0.1412	0.141876	0.141512	0.00059615	0.141324	-3.2246	-0.8498
19	0.2337	0.2092	0.209804	0.209502	0.00049591	0.209345	-3.3046	-0.6791
20	0.4159	0.3972	0.397690	0.397460	0.00037790	0.397341	-3.4226	-0.4008
21	0.5207	0.4857	0.486560	0.486130	0.00070669	0.485907	-3.1508	-0.3134
22	1.1773	1.1528	1.153403	1.153101	0.00049511	1.152945	-3.3053	0.0618

Table 3.4 Estimation of K_s and k.

L_f*	logS_s	log$J$*	logJ_{exp}	K_s, mg/ml	k,1/hr	L_f*'(checked)
0.5	-3.4	-0.6	-3.6	0.000398	0.00619	0.45

Table 3.5 Effectiveness factors of biofilms used for k_d and Y estimation.

K_s mg/L	L_f cm	X_f mg/L	D_e cm^2/hr	k 1/hr	S Mg/L	S/K_s	Φ_m	Φ_p	η
*0.398	0.0041	23510	0.03744	*0.0062	50	125.628	0.16139	0.01030	0.99996
*0.398	0.0041	23510	0.03744	*0.0062	40	100.503	0.16139	0.01154	0.99996
**0.045	0.0041	23510	0.03744	**0.176	50	1111.11	0.28914	0.006147	0.99999
**0.045	0.0041	23510	0.03744	**0.176	40	888.889	0.28914	0.006876	0.99998

* K_s and k from the values estimated in this paper; ** K_s and k from Claus and Kutzner (1985b).

Table 3.6 Concentrations and flux for steady-state conditions of 30 mg/L NO_3-N.

Sample Set	S_o (NO_3-N) mg/cm^3	S_e mg/cm^3	S_{in} mg/cm^3	S_{avg} mg/cm^3	J_{exp} mg/cm^2-hr
1	0.03292	0.00523	0.005919	0.005567	0.000569

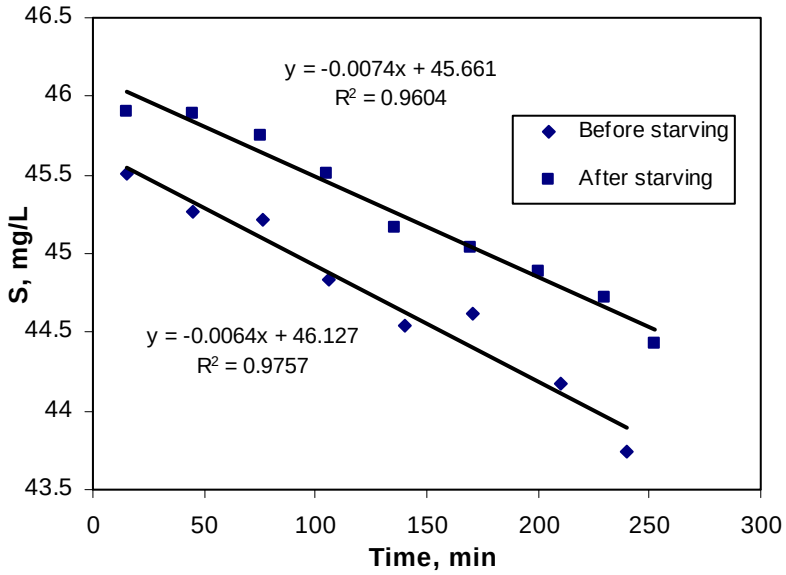

Figure 3.5 Time course of nitrate-N concentration, S, in the batch reactor used for k_d estimation before and after 30-hr starving.

$logJ_{exp}$ *vs.* $log\ S_s$ in Table 3.3 was plotted in Figure 3.3. Table 3.1 shows biomass data obtained at steady-state conditions when the reactor was fed with 30 mg/L NO_3-N. Table 3.4 shows the estimation and the check of K_s and k. The estimated $K_s = 0.398\ mg/l\ NO_3\text{-}N$, and the estimated $k = 0.15\ g\ NO_3^-\text{-}N / g\ VSS\text{-}d$.

Estimation of k_d and Y. Table 3.1 shows the characteristics of the column reactor used for short-term batch tests for estimation of k_d and Y. The biofilm thickness and biomass density were measured at steady-state conditions with the nitrate concentration in the feed solution being 5 mg/L NO_3-N. Table 3.5 lists the corresponding effectiveness factors for the short-term batch tests. Both the K_s and k values estimated in this study and reported by Claus and Kutzner (1985b) were used to estimate the effectiveness factors. As shown in Table 3.5, the effectiveness factors are all very close to 1 no matter which set of the kinetic parameters is chosen. This, therefore, confirms that the biofilm was fully-penetrated during the tests in this study.

Figure 3.5 shows the difference between the reference average substrate utilization rate, r_o and the rate after decay, r_d. The r_o and r_d were calculated by the change of nitrate-N concentration per unit time (assuming the average substrate utilization rate is not changed within a short period of time). Based on Fig. 3.4 and Eq. 3.13, we estimated the decay rate coefficient $k_d = ln\ (0.0074 / 0.0064\ ) / 30\ hours = 0.00484\ hr^{-1} = 0.12\ d^{-1}$. The concentrations and flux at the steady-state conditions were calculated based on Table 3.2 and are shown in Table 3.6. Based on Eq. 3.14, we estimated $Y = X_f L_f k_d / J_{exp} = 0.22\ mg\ VSS / mg\ NO_3^-$.

3.4. Discussion. Previous studies on autotrophic denitrification by *Thiobacillus denitrificans* did not provide much kinetic information. Moreover, few studies have been focused on the kinetics of biofilm processes. From the literature, the recommended ranges of kinetic parameters of autotrophs including nitrifying bacteria are listed in Table 3.7: $\mu_m = 0.005 - 0.104\ h^{-1}$; $k_d = 0.05 -$

0.15 d^{-1} (Zeng, 2004). Our μ_m and k_d are within the recommended range in literature. The most complete kinetic information comes from Claus and Kutzner's work as shown in Table 3.7. The kinetic parameters evaluated in this study are not in agreement with those. This might be attributed to several reasons.

Table 3.7 Comparison of kinetic parameters.*

Parameters	Sources			
	Claus & Kutzner (1985b)	Hashimoto et al. (1987)	This study	Recommended range for autotrophs
Form of bacteria	Suspended	Suspended	Biofilm	
Reactor	CSTR	CSTR	CSTR and batch	
S source	$S_2O_3^{2-}$	S^0-acclimated activated sludge	Elemental sulfur particles	
K_s	0.045 mg/l NO_3-N	-	0.398 mg/l NO_3-N	-
k	0.176 g NO_3-N/ g cells-h	-	0.0062 g NO_3-N/ g VSS-h	-
μ_m	0.11 h^{-1}	-	0.006 h^{-1}	# 0.005 – 0.104 h^{-1}
Y	0.129 g cells/ g NO_3	**0.15 g cells/ g NO_3	0.22 g VSS/ g NO_3	-
k_d	-	0.058 d^{-1}	0.12 d^{-1}	0.05 – 0.15 d^{-1}

* From Zeng, 2004. ** Converted from 0.33 mg-TOC/mg NO_3-N assuming cell formula $C_5H_7O_2N$. # From Botrous's thesis (1999). "-" means no reference available.

First, Claus and Kutzner (1985b) used a strain of pure culture of *Thiobacillus denitrificans*, while we used a mixed culture in this study. The mixed culture might contain several strains of *Thiobacillus* such as *Thiobacillus denitrificans*, *Thiobacillus thioparus, Thiobacillus heaplitanus, and Thiobacillus intermedius,* etc. Moreover, some heterotrophic denitrificans might be able to grow in our reactors as was reported previously (Claus and Kutzner, 1985a; Zhang, 2002). Second, they used bacteria in suspended-growth mode in experiment while kinetic parameters of a biofilm process might have been altered from those of suspended-growth process. The physiology of cells may be changed with the aggregate of cells, the exposure to different concentrations of substrate, or the kinetic parameters behave as the average of those exposed to fresh substrate and those near to the attachment surface. Third, $S_2O_3^{2-}$ was used in Claus and Kutzner's experiment instead of S^0. Sulfur is not easily dissolved in room-temperature water. Therefore $S_2O_3^{2-}$ may be more easily utilized by denitrificans. Lawrence (1978) reported that the specific denitrification rate ranged from 0.6 to 2.0 mg NO_3^--N/ mg-organic N-d under continuous denitrification conditions using S^0-acclimated activated sludge. These rates can be converted to values ranging from 0.07 to 0.25 mg-N/ mg-cells-d if the

cell formula ($C_5H_7O_2N$) is used. Koenig and Liu (2004) reported that the average specific denitrification rate obtained from batch tests of wastewater using elemental sulfur particles was 0.15 g NO_3^--N/g VSS-d, which is similar with our result of k, maximum specific denitrification (nitrate utilization) rate, 0.15 g NO_3^-- N/g VSS-d. This similarity makes sense because Koenig and Liu (2004) used an influent of 100 mg NO_3^--N/l in their tests. This influent concentration was much bigger than K_s (~ 0.398 mg NO_3^--N/l or even smaller); therefore, the specific denitrification rate in Koenig and Liu's tests (2004) was very possibly close to or the same as the maximum specific denitrification rate, k. In other words, all these values are much lower than 27.1 mg-N/ mg-cells-d reported by Claus and Kutzner (1985b) using $S_2O_3^{2-}$ as the substrate. Apparently, denitrificans are more easily acclimated to and then to utilize thiosulfate as compared with elemental sulfur, which may explain why a higher K_s and a lower k were obtained in this study as compared with Claus and Kutzner's (1985b).

It should be pointed out that to use the method developed in this study for estimation of the four kinetic parameters, biofilm thickness (L_f) and biomass density (X_f) are very important. Table 3.8 lists how the kinetic parameters respond to the variances of L_f. Because X_f is calculated based on L_f, L_f is assumed to be an independent variable while the others are dependent variables. As shown in Table 3.8, k is the most sensitive with L_f while K_s is the second most sensitive, but k_d and Y are not very sensitive. Since the estimated biofilm thickness fluctuated around the average value as much as up to ± 70%, k value could be in the range of 0.10 – 0.22 d^{-1}.

Table 3.8 Sensitivity tests of L_f on kinetic parameters estimation.

	Variable		Dependent Variables					Change			
Difference %	L_f cm	X_f mg/ml	L_f*	K_s mg/ml	k 1/d	k_d 1/d	Y	K_s %	k %	k_d %	Y %
-70	0.0025	46.61	0.16	0.000447	0.099	0.12	0.22	12.2	-33.4	0.0	0.0
-30	0.0059	19.75	0.25	0.000447	0.117	0.12	0.22	12.2	-21.2	0.0	0.0
-10	0.0076	15.33	0.45	0.000447	0.144	0.12	0.22	12.2	-3.1	0.0	0.0
0	0.0084	13.87	0.5	0.000398	0.149	0.12	0.22	0.0	0.0	0.0	0.0
10	0.0092	12.67	0.5	0.000447	0.183	0.12	0.22	12.2	22.9	0.0	0.0
30	0.0109	10.69	0.55	0.000398	0.193	0.12	0.22	0.0	29.8	0.0	0.0
70	0.0143	8.15	0.6	0.000398	0.220	0.12	0.22	0.0	48.3	0.0	0.0

* Note: L_f was first assumed by different percentages, and then other dependent variables were calculated in the exact same way as the experimental results were obtained in this study.

In this study, biomass measurement is not easy. First, sulfur and limestone particles are fragile, sulfur evaporates when heated and particle surfaces are totally not smooth. All of these factors created difficulties in taking biomass off the surface of sulfur stones. Second, it was observed that biomass was not fully covering the particle surface which could cause the inaccuracy of the estimation of L_f. Finally, during the relatively long period of biofilm growth (~ 40 days), a portion of the biofilm in our reactor should have consisted of non-active cells which were not able to be differentiated from the active portion of the cells. Therefore, the measured X_f could overestimate the actual active biomass. In summary, L_f and X_f could affect the results possibly to a great extent. The accuracy of the estimated kinetic parameters could be improved should these problems be solved. In the batch tests for k_d and Y estimation, specific

denitrification rates were calculated ranging between 0.04 to 0.07 mg NO_3-N / mg VSS-d when fed with artificial groundwater of 50 mg/l NO_3-N. These values are a little lower than those reported by Batchelor and Lawrence (1978a) and Hashimoto et al. (1987). The difference can be explained by the very thin biofilm cultured by 5 mg/l NO_3-N in this study. Combining these four kinetic parameters evaluated in this study, the minimum substrate concentration (Rittmann et al., 1986), S_{min} (M/L^3) = $K_s\ k_d\ /\ (\mu_m - k_d)$, for sustaining steady-state biomass is 2.4 mg/l NO_3-N, which was satisfied in this study.

3.5. Summary. Kinetic parameters are important for process design. In this research, four kinetic parameters: half-velocity constant, K_s; maximum specific substrate utilization rate, k; bacteria yield coefficient, Y; and bacteria decay coefficient, k_d of autotrophic denitrificans in a SLAD biofilm process were evaluated. The estimation of K_s and k was based on a curve-matching method with kinetic results obtained from several short-term non-steady-state experiments conducted in completely-stirred tank reactors (CSTR). The evaluation of k_d and Y was based on the results obtained from several short-term batch tests with fully-penetrated biofilm cultured in the reactors. The parameters found are as follows: $K_s = 0.398$ mg/L NO_3-N, $k = 0.15\ d^{-1}$, $k_d = 0.12\ d^{-1}$, Y = 0.22 mg VSS / mg NO_3^-.

3.6. Nomenclature.

a	=	specific area of sulfur and limestone, 1/L.
D	=	molecular diffusivity of nitrate in bulk solution, L^2/T;
D_f	=	molecular diffusivity of nitrate in biofilm (assuming D_f= 0.8 D), L^2/T;
d_p	=	diameter of sulfur particle, L;
HRT	=	hydraulic retention time, T.
J	=	substrate flux into biofilm, M/L^2T.
k	=	maximum specific substrate utilization rate, 1/T;
k_d	=	bacteria decay coefficient,1/T;
K_s	=	half-velocity constant, M/L^3;
L	=	thickness of the effective diffusion layer, which is determined by the empirical formula for porous media, L;
L_f	=	biofilm thickness, L;
M_b	=	total biomass in biofilm as VSS, M;
Q	=	the feed flow rate, L^3/T;
Q_r	=	the recycle flow rate, L^3/T;
r	=	substrate utilization rate, M/L^3T;
Re_m	=	$[2\rho d_p / (1- \varepsilon)\ \mu]$;
S_{avg},, S_o, S_e	=	the logarithm average, feed, and effluent concentration of nitrate-N, respectively, M/L^3;
S_b	=	substrate concentration in bulk liquid, M/L^3;
Sc	=	Schmidt number, $\mu/\rho D$;
S_f	=	substrate concentration at that point in the film, M/L^3;
S_{in}	=	actual substrate concentration at inlet of reactor, M/L^3;
S_{min}	=	minimum substrate concentration for sustaining biomass growth, M/L^3;
S_s	=	substrate concentration at the interface of biofilm and bulk liquid, M/L^3;

S_w = substrate concentration at the surface of attachment, M/L^3;
v = superficial flow velocity, L/T;
V = volume of sulfur and limestone packed, L^3;
X_f = biomass concentration in biofilm, M/L^3;
Y = bacteria yield coefficient;
ε = porosity of media;
η = biofilm effectiveness factor;
μ = absolute viscosity of liquid, M/LT;
μ_m = maxium specific growth rate,1/T;
ρ = liquid density, M/L^3;
τ = biofilm depth dimension, $[K_s D_f/(kX_f)]^{1/2}$, L;
Φ_p , Φ_m, Cs = parameters related with η;

4. Preliminary Economic Analysis

4.1. Introduction. In this research, we did a simple economical analysis for a town of 200 people to add a SLAD unit process in its drinking water treatment system. The analysis is for the construction of the SLAD reactor only, not including any analysis for groundwater wells, water transport pipelines, secondary treatment systems or water distribution system. The analyses for two scenarios are as follows.

4.2. Economic Analysis 1. In this scenario, the system is designed for continuous flow with no by-passing, and a reduction of nitrate contamination by ≥ 90% in the groundwater. The design is for a drinking water system that would supply water to a town of 200 people.

Table 4.1 Assumptions in economic analysis 1.

National average water use per capita	600 L/capita day
Nitrate-N concentration in groundwater	20 mg nitrate-N/L
Sulfate concentration in groundwater	100 mg sulfate/L

4.2.1. Water use per day. Daily water use in town of 200 people = 120,000 L/day

4.2.2. SLAD system design.

1) Design of hydraulic retention time (HRT). Nitrate-N loading rate = 200 g NO_3^--N/m^3/day (based on our results)

$$HRT = \frac{\text{Co}}{\text{Loading rate}} = \frac{20 g/m^3}{200 g/m^3/day} = 0.1 day = 2.4 h$$

2) Determination of effluent quality. Assuming 90% nitrate removal efficiency and a sulfate production rate of 7.1 mg SO_4^{2-} per 1.0 mg NO_3^--N removed.

The effluent quality would be equal to: Nitrate-N = 2 mg NO_3^--N/L

Sulfate conc. (mg/L) = Influent conc. (mg/L) + Nitrate removed*7.1 = 227.8 mg SO_4^{2-}/L

This is less then the 250 mg SO_4^{2-}/L MCL. Sulfate does not control the design.

3) Water volume of the reactor. Volume = HRT*Q = 120,000 L/day * 0.1 day = 12,000 L

4) Total tank volume. Assume porosity of the media is 30%.

$$\text{Total tank volume} = \frac{\text{water volume (L)}}{\textit{porosity}} = \frac{12{,}000\ \text{L}}{0.3} = 40{,}000\ \text{L}$$

Multiplying by a safety factor of 1.5, the total tank volume = 60,000 L = 60 m^3.

5) Sizing on reactor (cylinder), assuming height = 10 ft = 3.048 m

$$\text{Diameter} = \sqrt{\frac{4 * \text{Volume } (m^3)}{PI * \text{Height (m)}}} = 5.0\ \text{m} = 16.4\ \text{ft}$$

So the reactor will have a diameter of 17 feet and a height of 10 feet.

4.2.3. Amount of sulfur and limestone.

1) Volume of total sulfur and limestone needed.

$$\text{Volume of S/L} = \text{Tank volume (L)} - \text{Water volume (L)} = 60{,}000\ \text{L} - 12{,}000\ \text{L} = 48{,}000\ \text{L}$$

The sulfur and limestone is mixed in a 3:1 ratio so:

Sulfur needed = 36,000 L = 1271.3 ft^3; and limestone needed = 12,000 L = 428.3 ft^3.

Notice that here we assume the ratio is 3 to 1 not 2 to 1 because we want to provide some safety margin for our design.

2) Amount of sulfur. 1) Crude lump sulfur is 1 in or less in diameter
2) Bulk density is 75 - 115 lb/ft^3 --will use 100 lb/ft^3
3) Price is $16 per ton

$$\text{Sulfur (ton)} = 1271.3\ \text{ft}^3 * 100\ \text{lb/ft}^3 * \frac{1\ \text{ton}}{2{,}000\ \text{lb}} = 63.6\ \text{ton}$$

The tonnage needed is 64 tons of granular sulfur. Price ($) = 64 tons * $16/ton = $1024. Note that the price for sulfur stone may not high, but transportation fee (not considered in this analysis) can be high because the town may not be able to obtain sulfur stone nearby.

3) Amount of limestone. 1) Dirty crush is 0.25 in to 1 inch in diameter
2) Bulk density is 165 lb/ft^3
3) Price is $7.75 per ton

$$\text{Limestone (ton)} = 423.8\ \text{ft}^3 * 165\ \text{lb/ft}^3 * \frac{1\ \text{ton}}{2{,}000\ \text{lb}} = 35\ \text{ton}$$

The tonnage of limestone needed is 35 tons. Price ($) = 35 tons * $7.75/ton = $271.

4) Concrete for the reactor system. The walls of the reactor are all 1 foot thick and the reactor base is 2 feet thick. The reactor is built on a foundation slab that is 5 foot wider than the reactor wall and the slab is 2 feet thick. Table 4-3 shows concrete cost for forms, reinforcing, man-hours and concrete in 1985 dollars. The end of the table shows the amount in 2004 dollars by computing the dollar change from the Engineering News Record (ENR, 2004).

2) Cost for concrete. Total concrete cost can be determined by using Tables 4-2 and 4-3.

Price ($) = $326/cy*28.3 cy + $501/cy*20.9 cy + $262/cy*62.3 cy = $37,038

Table 4.2 Amount of concrete needed.

Construction	Amount of concrete	
	cubic feet	cubic yards
Reactor floor and ceiling	763.4	28.3
Reactor side walls	565.5	20.9
Reactor foundation	1682	62.3

Table 4.3 Concrete Cost.

Construction	1985 $s	2004 $s*
Reactor walls and ceiling	205/cy	326/cy
Reactor side walls	315/cy	501/cy
Reactor foundation	165/cy	262/cy

* determined by multipling 1985 $s by 1.59

4.2.4. Development of Capital Costs. The total material cost ($) = sulfur + limestone + concrete = $1024 + $271 + $37038 = $38,333. By using the material costs, an estimate of total cost can be calculated (Qasim, 1994). A cost of $38,333 will be used for the development of capital costs. The total estimated cost of the project is approximately $59,417 (use $59,500).

Table 4.4. Development of capital costs.

Cost	Percentage of materials cost	Total materials cost in dollars	Final Cost in dollars
Backwashing and piping	20%	38333	7667
Site preparation	5%	38333	1917
Enginering and construction supervision	15%	38333	5750
Contingencies	15%	38333	5750
		subtotal	21084
		Material	38333
		Total	59417

4.3. Economic Analysis 2. In this scenario, the system is designed for flow through the reactor with by-pass included in the system to lower the construction cost. The reactor will remove 90% of the nitrate contamination, but the by-pass will blend water together to produce an effluent nitrate contamination of 5 mg NO_3^--N/L. The design is for a drinking water system that would supply water to a town of 200 people.

Table 4.5 Assumptions in economic analysis 2.

National average water use per capita	600 L/capita day
Nitrate-N concentration in groundwater	20 mg nitrate-N/L
Sulfate concentration in groundwater	100 mg sulfate/L

4.3.1. Water use per day. Daily water use in town of 200 people = 120,000 L/day

4.3.2. SLAD system design.

1) Design of hydraulic retention time (HRT).

Nitrate-N loading rate = 200 g NO_3^--N/m^3/day (Based on our results)

$$HRT = \frac{Co}{\text{Loading rate}} = \frac{20\ g/m^3}{200\ g/m^3/day} = 0.1 day = 2.4\ h$$

2) Determination of system flow. Mass balance equations are below:

$$C_vQ_v + C_rQ_r = C_{eff}Q_t \quad => \quad 2\ mg/L * Qv + 20\ mg/L * Qr = 5\ mg/L * 120{,}000\ L/d$$
$$Q_v + Q_r = Q_t \quad => \quad Q_v + Q_r = 120{,}000\ L/d$$

where, C_v = reactor effluent nitrate conc. (2 mg/L); Q_v = flow in reactor (unknown); C_r = nitrate conc. in by-pass (20 mg/L); Q_r = flow in bypass (unknown); C_{eff} = nitrate conc. in effluent (5 mg/L); and Q_t = total flow (120,000 L/day). Using simultaneous equations the flow of the reactor and the by-pass was found. The by-pass (Q_r) is 20,000 L/day. Therefore, the flow in the reactor (Q_v) is 100,000 L/day.

3) Determination of effluent quality. Assuming 100% nitrate removal efficiency in the reactor and a sulfate production of 7.1 mg SO_4^{2-} per 1.0 mg NO_3^--N removed. Concentrations would equal: Nitrate-N = 5 mg NO_3^--N/L. By using a mass balances on the system at the blending point, the sulfate concentration in the effluent can be found.

$$S_cQ_v + S_{ci}Q_r = S_{sc}Q_t$$

where, S_c = sulfate conc. from reactor (228 mg/L); Q_v = flow from reactor (100,000 L/day); S_{ci} = groundwater sulfate conc. (100 mg/L); Q_r = flow in by-pass (20,000 L/day); S_{cf} = sulfate in effluent (unknown); and Q_t = total flow (120,000 l/day). Sulfate conc. = 206.7 mg SO_4^{2-}/L. Therefore, the sulfate production will not control the process design.

3) Water volume of the reactor.

Volume = HRT*Q = 100,000 L/day * 0.1 day = 10,000 L

4) Total tank volume. Assume porosity of media is 30%.

$$\text{Total tank volume} = \frac{\text{water volume (L)}}{\textit{porosity}} = \frac{10{,}000\ \text{L}}{0.3} = 33{,}333\ \text{L}$$

Multiplying by factor of safety of 1.5, the total tank volume = 50,000 L = 50 m^3.

5) Sizing on reactor (cylinder), assuming height = 10 ft = 3.048 m

$$\text{Diameter} = \sqrt{\frac{4 * \text{Volume } (m^3)}{PI * \text{Height (m)}}} = 4.57\ \text{m} = 15\ \text{ft}$$

So the reactor will have a diameter of 15 feet and a height of 10 feet.

4.3.3. Amount of sulfur and limestone.

1) Volume of total sulfur and limestone needed.

Volume of S/L = Tank volume -Water volume = 50,000L - 10,000L = 40,000L

The sulfur and limestone is mixed in a 3:1 ratio so:

Sulfur needed = 30,000 L = 1059.4 ft^3; and limestone needed = 10,000 L = 353.1 ft^3.

2) Amount of sulfur. 1) Crude lump sulfur is 1 in or less in diameter
2) Bulk density is 75 - 115 lb/ft^3 --will use 100 lb/ft^3
3) Price is $16 per ton

$$\text{Sulfur (ton)} = 1059.4\ \text{ft}^3 * 100\ \text{lb/ft}^3 * \frac{1\ \text{ton}}{2{,}000\ \text{lb}} = 53\ \text{ton}$$

The tonnage needed is 53 tons of granular sulfur. Price ($) = 53 tons * $16/ton = $848.

3) Amount of limestone. 1) Dirty crush is 0.25 in to 1 inch in diameter
2) Bulk density is 165 lb/ft^3
3) Price is $7.75 per ton

$$\text{Limestone (ton)} = 353.1\ \text{ft}^3 * 165\ \text{lb/ft}^3 * \frac{1\ \text{ton}}{2{,}000\ \text{lb}} = 29.1\ \text{ton}$$

The tonnage of limestone needed is 30 tons. Price ($) = 30 tons * $7.75/ton = $232.5.

4) Concrete for the reactor system. The walls of the reactor are all 1 foot thick and the reactor base is 2 feet thick. The reactor is built on a foundation slab that is 5 foot wider than the reactor wall and the slab is 2 feet thick. Table 4-7 shows concrete cost for forms, reinforcing, man-hours and concrete in 1985 dollars. The end of the table shows the amount in 2004 dollars by computing the dollar change from the Engineering News Record (ENR, 2004).

2) Cost for concrete. Total concrete cost can be determined by using Tables 4-6 and 4-7.

Price ($) = $326/cy * 25.2 cy + $501/cy * 18.6 cy + $262/cy * 54 cy = $31,682

Table 4.6 Amount of concrete needed.

Construction	Amount of concrete	
	cubic feet	cubic yards
Reactor floor and ceiling	680.9	25.2
Reactor side walls	502.7	18.6
Reactor foundation	1458	54

Table 4.7 Concrete Cost.

Construction	1985 $s	2004 $s*
Reactor walls and ceiling	205/cy	326/cy
Reactor side walls	315/cy	501/cy
Reactor foundation	165/cy	262/cy

* determined by multipling 1985 $s by 1.59

4.3.4. Development of Capital Costs. The total material cost ($) = sulfur + limestone + concrete = $850 + $235 + $31682 = $32,767. A cost of $32,767 will be used for the development of capital costs. The total estimated cost of the project is approximately $50,788 (use $50,800).

Table 4.8 Development of capital costs

Cost	Percentage of materials cost	Total materials cost in dollars	Final Cost in dollars
Backwashing and piping	20%	32767	6553
Site preparation	5%	32767	1638
Enginering and construction supervision	15%	32767	4915
Contingencies	15%	32767	4915
		subtotal	18021
		Material	32767
		Total	50788

4.4. Annual Costs of Sulfur. Elemental sulfur stones will be used up over time. The rate of sulfur used can be found by using the stoichiometric equation (Eq. 1.1) for SLAD processes. From this we can find that 2.51 mg S will be used for every 1 mg NO_3^--N reduced.

Economic Analysis 1. The cost was estimated for a SLAD system that would treat all of the water in the town (120,000 L/day). A yearly cost can be found for the replacement of used granular sulfur.

Sulfur used/year = flow rate in reactor (L/yr) * nitrate removed (mg/L) * 2.51
$= 4.38*10^7$ L/yr * 18 mg/L * 2.51 = 1978.9 kg/yr = 4362.7 lb/yr

Therefore, 2.2 tons of sulfur would be used per year in the reactor. Every year that amount would theoretically need to be replaced in the system that treated all of the water in the town. At $16 per ton for sulfur, the cost to replenish the amount will be $36. Please notice that the change in the sulfur price and the transportation cost are not considered here.

Economic analysis 2. The cost was estimated for a SLAD system that would treat part of the water and blend in some water at attain a nitrate effluent concentration of 5 mg NO_3^--N/L. The daily flow through the reactor was found to be 100,000 L/day. A yearly cost needs to be found for the replacement of used granular sulfur.

Sulfur used/year = flow rate in reactor (L/yr) * nitrate removed (mg/L) * 2.51
$= 3.65*10^7$ L/yr * 18 mg/L * 2.51 = 1649.1 kg/yr = 3635.6 lb/yr

Therefore, 1.8 tons of sulfur would be used per year in the reactor. Every year that amount would theoretically need to be replaced in the. At $16 per ton for sulfur, the cost to replenish the amount will be $28.8.

4.5. Summary. For a community, the size of 200 people, this cost would not be unreasonable. A cost comparison needs to be conducted in the future between the SLAD process and the other denitrification methods to determine the actual cost advantages.

The two economic analyses determined the cost of the two different systems. The first system removes 90% of the nitrate-N and does not include a by-pass. The system was determined to cost $59,500. Meanwhile, economic analysis 2 determined the cost of a system including by-pass with an effluent nitrate-N concentration after blanking of 5 mg NO_3^--N/L. This system was estimated at a cost of $50,800. However, the second system would need some extra pipes or valves, etc. and therefore, may not save cost at all.

5. Conclusions and Suggestions

5.1. Conclusions. Based on the results of this study, the following conclusions can be drawn:

1. Ex-situ fixed-bed SLAD columns with a ratio of sulfur to limestone equal to 2:1 (v/v) are a very efficient method for removing nitrate from groundwater.
2. A loading rate up to 260 g NO_3-N /d-m^3 media with HRTs between 6 and 11 hours would achieve high nitrate removal efficiency (~ 95%).
3. A maximum nitrate removal rate of 363 g NO_3-N/d-m^3 media was achieved at a loading rate of 987 g NO_3-N /d-m^3 media (at HRT = 2.5 h and nitrate-N in feed = 80 mg/L).

4. Both the HRT and the nitrate-N loading rate should be considered as design criteria. An HRT > 3 h should be considered to achieve high removal efficiency (> 90%).
5. To prevent nitrite accumulation to be > 1 mg/l NO_2-N, the HRT should be kept equal or longer than 6 h and the nitrate loading rate less than 200 g NO_3-N/d-m^3 media.
6. TOC and HPC tests of the effluent showed that the SLAD column treatment process will not greatly impact the microbiological quality of the water being treated.
7. The sulfate production rate was found to be 7.10 mg SO_4^{2-} per mg NO_3-N reduced.
8. Low nitrate removal efficiencies sometimes occurred during the period of initiating the SLAD column reactors. Multiple re-inoculations may improve the poor performance.
9. The following equation was found to quantitatively describe performance of the SLAD column system:

$$\mathbf{Y = 21.5714 + 0.31171*X_1 - 8.48835*X_2 + 0.0076386*X_1*X_1 + 0.79334*X_2*X_2 - 0.10691*X_1*X_2}$$

   where Y, X_1 = effluent and influent nitrate-N concentration, respectively, mg/l; and X_2 = hydraulic retention time, h. This model fits better for the situation when X_1 = 20 - 110 mg/l and HRT = 2 - 9 h.
10. Phosphorus is not the limiting factor of cell growth when the nitrate loading rate is less than 256.9 g NO_3-N/d-m^3 media. For obtaining a nitrate removal efficiency > 95% at a nitrate loading rate of less than or equal to this, 0.00167 mg/l P per mg/l NO_3-N (i. e., 0.05 mg P/l for 30 mg NO_3-N or 0.083 mg P/l for 50 mg NO_3-N) is enough.
11. Four kinetic parameters of autotrophic denitrificans in the SLAD biofilm process were evaluated as: half-velocity constant, K_s = 0.398 mg/L NO_3-N; maximum specific substrate utilization rate, k = 0.15 g NO_3^-- N / g VSS-d; bacteria decay coefficient, k_d = 0.12 d^{-1}; and bacteria yield coefficient, Y = 0.22 mg VSS / mg NO_3^-.
12. The cost analysis for a SLAD reactor system shows that the system is inexpensive.

5.2 Suggestions. Further research is needed, and the suggested areas are as follows:

1. Sulfate in the effluent needs to be controlled to polish the treated water.
2. Further studies are needed to identify the reasons why poor nitrate removal performance sometimes occurs during the initiation time of SLAD column reactors.
3. More tests should be conducted on the microbiological quality of effluent from SLAD column reactors if this technology is to be used for drinking water treatment.
4. Nitrite accumulation should be monitored in a wider range of different nitrate loading rates to determine the maximum applicable nitrate treatment rate of SLAD column processes in order to prevent the nitrite concentration in effluent being too high.
5. Better methods to accurately measure the biofilm thickness and completely rinse off biomass from sulfur stones would improve the accuracy of evaluating those kinetic parameters. Further research is needed to experimentally obtain and verify fully-penetrated biofilm.

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