บันทึกข้อความ

ส่วนราชการ ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี โทร.3343

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เรื่อง ขออนุมัติค่าตอบแทนการตีพิมพ์ในวารสารวิชาการระดับนานาชาติ

เรียน รองคณบดีฝ่ายวิจัยและวิชาการ ผ่านหัวหน้าภาควิชาวิศวกรรมเคมี

อ้างถึงประกาศฉบับที่ 40/2550 ประกาศ ณ วันที่ 22 ตุลาคม 2550 คณะวิศวกรรมศาสตร์ เรื่อง "หลักเกณฑ์การจ่ายค่าตอบแทนการตีพิมพ์ผลงานวารสารวิชาการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี" ตามความทราบแล้วนั้น เนื่องด้วยดิฉัน นางสาวสุมนา สิริพัฒนากุล อาจารย์ประจำภาควิชาวิศวกรรมเคมี ได้ตีพิมพ์บทความวิชาการ เรื่อง "Nitrate Removal from Agricultural Infiltrate by Bioaugmented Free and Alginate Entrapped Cells" ในวารสารระดับนานาชาติ Water Environment Research Volume 82 No. 7 pg. 617-621 โดยมีสัดส่วนการทำงานคิดเป็นร้อยละ 70 โดยดิฉันขอรับรองว่าบทความวิชาการที่ขอรับการตอบแทนไม่เป็นส่วนหนึ่งของวิทยานิพนธ์ และเป็นไปตามเกณฑ์ของประกาศฉบับที่ 40/2550 ที่อ้างถึงข้างต้น ดังนั้นดิฉันจึงใคร่ขออนุมัติค่าตอบแทนการตีพิมพ์ผลงานดังกล่าว

จึงเรียนมาเพื่อโปรดพิจารณาอนุมัติ

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อาจารย์ประจำภาควิชาวิศวกรรมเคมี

แบบแสดงหลักฐานการมีส่วนร่วมในการเขียนบทความวิจัย

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ชื่อ-สกุลผู้วิจัย	ปริมาณงานร้อยละ	หน้าที่รับผิดชอบ
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* Corresponding author

ลงชื่อ.....

(ดร.สุมนา สิริพัฒนากุล)

ลงชื่อ.....

(Prof. Dr. Eakalak Khan แทน Carlee J. Pochant)

ลงชื่อ.....

(Prof. Dr. Eakalak Khan)

Nitrate Removal from Agricultural Infiltrate by Bioaugmented Free and Alginate Entrapped Cells

Sumana Siripattanakul[1], Carlee J. Pochant[2], Eakalak Khan[3*]

ABSTRACT: A bench-scale sand column experiment was conducted to investigate nitrate removal from synthetic agricultural infiltrate by denitrifying bacterial cells entrapped in calcium alginate compared to free cells. The effects of methanol as a carbon source and cell loading were examined. Low (0 to 50%) nitrate removal was observed in both entrapped and free-cell columns without methanol supplement. In the presence of methanol, nitrate removals of 90 to 99% and 56 to 75% were obtained for entrapped and free-cell columns, respectively. Nitrate removal followed first-order kinetics. The entrapped-cell columns achieved higher nitrate removal than the free-cell columns because of less bacterial losses. Scanning electron microscopic images supported the nitrate removal results that the denitrifying bacteria proliferated in the entrapment matrix, and several nitrogen gas voids were produced from denitrification. *Water Environ. Res.*, **82**, 617 (2010).

KEYWORDS: Bioaugmentation, calcium alginate, cell loading, denitrification, sand column.

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Introduction

Nitrate contamination in groundwater is mainly from fertilizer and animal waste dissolving into rain and irrigation water, and infiltrating through the unsaturated zone (Mitchell et al., 2003). Treatment of nitrate contamination in agricultural infiltrate is necessary to protect groundwater sources. Entrapped cell bioaugmentation is a promising technique for remediating soil and groundwater contamination (Gentry et al., 2004). The technique can be performed in-situ by adding the entrapped cells to contaminated sites. It provides high contaminant-degrading culture concentration and prevents the transportation of augmented cells away from the site. The entrapment matrix can lessen adverse effects on cells from unsuitable physicochemical conditions such as temperature and pH. The cell entrapment technique has been used to denitrify point-source wastewater. There has been no research on the treatment of nitrate in agricultural infiltrate using the entrapped-cell bioaugmentation technique.

The objective of this study was to examine nitrate removal from agricultural infiltrate using bioaugmented entrapped denitrifying bacteria compared to the corresponding free cells in a sand column setup simulating a field condition. Calcium alginate was chosen as a cell entrapment matrix. The effects of methanol as a carbon source and cell loading were studied. Leaching of free and entrapped cells from the columns was monitored. The entrapment matrix and entrapped cells were examined before and after the experiment via scanning electron microscopy (SEM).

Methodology

Chemicals. Sodium alginate (unspecified grade) produced by Pfaltz and Bauer (Waterbury, Connecticut) was obtained through VWR International Co. (West Chester, Pennsylvania). All other chemicals were analytical grade and were purchased from VWR International Co.

Denitrifying Bacteria Acclimation. Denitrifying bacteria were enriched from mixed liquor suspended solids (MLSS) from the Moorhead wastewater treatment plant, Moorhead, Minnesota. The sludge was acclimated in a 20-L reactor for three months under anoxic condition. The reactor was operated at hydraulic and sludge retention times of 1 and 17 days, respectively. The growth medium contained 50 mg/L as N of nitrate and 300 mg/L of methanol (Hill and Khan, 2008).

Calcium Alginate Cell-Entrapment Procedure. The denitrifying bacteria were entrapped using a calcium alginate technique adapted from Hill and Khan (2008). The acclimated sludge was centrifuged at 7000 rpm for 10 minutes to obtain concentrated denitrifying bacterial cells. The concentrated cells of 5 and 10 g were homogenously mixed with 50 and 100 mL of 2% (w/v) aqueous sodium alginate solutions, respectively, representing two different cell loadings. The mixtures were dropped into a calcium chloride solution of 3.5% (w/v) using a peristaltic pump at a flow rate of 3 mL/min (bead size of 2 mm). The droplets remained in the solution for 2.5 hours to form and harden spherical beads.

Synthetic Agricultural Infiltrate, Sand, and Column Preparations. Two synthetic agricultural infiltrates, with and without supplemented methanol, were prepared for the denitrification kinetic experiment. Infiltrate with methanol was the growth medium, and infiltrate without methanol was the growth medium excluding methanol. Composition of the growth medium is described elsewhere (Hill and Khan, 2008). The infiltrate was sterilized by autoclaving before applying to sand columns.

Silica sand was used as a model soil medium. The sand was washed with tap water and dried at 105°C for 24 hours. The cleaned sand was sieved to obtain the grain sizes between 0.42

[1] Department of Chemical Engineering, Faculty of Engineering and National Center of Excellence for Environmental and Hazardous Waste Management, Ubon Ratchathani University, Thailand.

[2] Belcourt High School, Belcourt, North Dakota.

[3] *Department of Civil Engineering, North Dakota State University, Fargo, ND, 58108; e-mail: eakalak.khan@ndsu.edu.

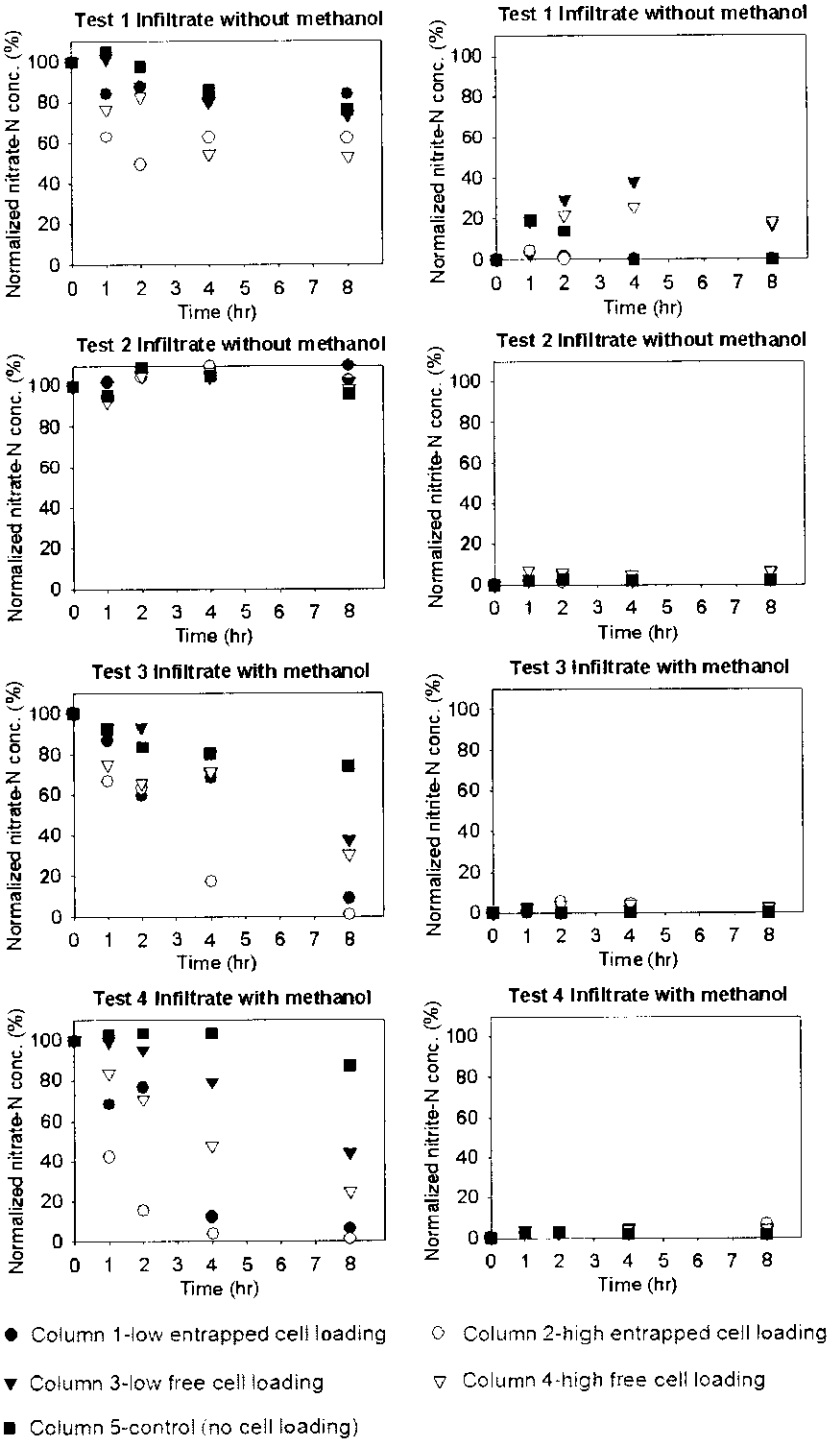

Figure 1—Normalized nitrate-N and nitrite-N concentrations in infiltrate samples versus time.

and 0.84 mm. The sieved sand was autoclaved at 121°C for 30 minutes twice within two consecutive days. The void ratio (v/v) of sieved sand loosely packed in a 400-mL graduate cylinder was 0.30.

The entire sand column was modeled as the top soil layer, where the cells would be augmented in practice. Five down-flow sand columns were used to represent: (1) low entrapped-cell loading, (2) high entrapped-cell loading, (3) low free-cell loading, (4) high free-cell loading, and (5) control (no cells). Each column was 6.35 cm in diameter and 23 cm in length. Each column had an effluent sampling port at the bottom. All columns were rinsed with 70% isopropanol and autoclaved deionized water before use. For

Table 1—Descriptions of entrapped cell, free cell, and control columns and denitrification kinetics.

	Column description						Kinetic results	
No.	Cell type	Cell loading (mg wet cells/mL empty bed volume)	Cell mass (g wet cells)	Bulk dry sand (mL)	Bulk bioaugmented volume (mL)	Total empty bed volume (mL)	Denitrification kinetic equation[3]	R^2
1	Low entrapped cell loading	12.5	5	350	50[1]	400	$c = c_0\exp(-0.281t)$	0.884
2	High entrapped cell loading	25.0	10	300	100[1]	400	$c = c_0\exp(-0.534t)$	0.937
3	Low free cell loading	12.5	5	400	-[2]	400	$c = c_0\exp(-0.096t)$	0.889
4	High free cell loading	25.0	10	400	-[2]	400	$c = c_0\exp(-0.139t)$	0.784
5	Control (no cells)	0	0	400	0	400	$c = c_0\exp(-0.022t)$	0.777

[1] Volume of immobilized denitrifying bacteria and matrices.
[2] Volume of the bacteria is negligible.
[3] Equations are based on the third and fourth tests and c, c_0, and t are nitrate concentration at t hr (mg/L), initial nitrate concentration (mg/L) and time (hr), respectively.

columns 1 to 4, the bioaugmentation procedure was as follows. The concentrated free cell suspension or entrapped cells in a phosphate buffer solution were poured into the sterile sand in a sterile beaker. The mixture was stirred using a sterile glass rod for five minutes and then poured into the column. The control (column 5) contained only the sterile sand. Table 1 shows the contents of the five sand columns.

Denitrification Kinetic Experiments and Scanning Electron Microscopic Observation. As mentioned earlier, this study focused on the effects of cell loading and an additional carbon source in agricultural infiltrate on denitrification. Four consecutive 8-hour tests were performed: (1) tests 1 and 2 used the synthetic infiltrate without methanol; and (2) tests 3 and 4 used the synthetic infiltrate supplemented with methanol. The synthetic infiltrate was filled and maintained at 5 cm above the surface of the sand layer to control anaerobic condition and elevation head. For each test, effluent samples of 20 mL were taken at 0, 1, 2, 4, and 8 hours for measuring nitrate and nitrite. At the end of the test, 1-mL effluent samples were aseptically taken to enumerate bacteria leaching from the columns. Before starting each test, the columns were drained and flushed with the synthetic infiltrate of 200 mL. The entrapped cell beads were taken before test 1 and after test 4 for microscopic examination. They were prepared for SEM observation according to Hill and Khan (2008). A scanning electron microscope (JEOL model JSM-6300, Tokyo, Japan) was used.

Analytical Methods. Nitrate and nitrite were measured according to Standard Methods (American Public Health Association et al., 1998). Nitrate was measured using a VWR® SympHony® Nitrate Ion Selective Electrode and a VWR® SympHony® Double Junction Reference Electrode (VWR). The electrodes were calibrated at least twice a day. Nitrite was analyzed colorimetrically using a standard HACH NitriVer®2 reagent (HACH, Loveland, Colorado). Bacteria enumeration was performed by a spread plate method onto a nutrient agar.

Results and Discussion

Denitrification Kinetics. Normalized nitrate-N and nitrite-N concentrations versus time for each test are presented in Figure 1. In the control column, nitrate concentration decreased by about 20% or less within eight hours. Because the experiment was under a sterile condition, this likely was caused by an abiotic process. Contaminant sorption by alginate beads was reported in previous studies, but it lasted for a very short period after contaminant exposure to the beads (Hill and Khan, 2008; Siripattanakul et al., 2008). Consequently, a control experiment with only alginate beads was not conducted. In the first test (without methanol), nitrate removal was between 20 and 50% for both entrapped and free-cell columns. This indicates that the alginate matrix was not used as a major carbon source by the denitrifying bacteria. The major carbon source could have come from organic carbon on

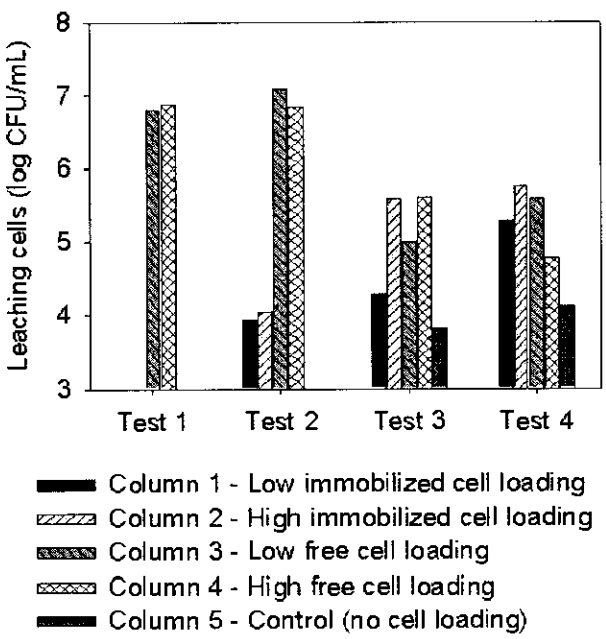

Figure 2—Viable bacteria leaching through sand columns.

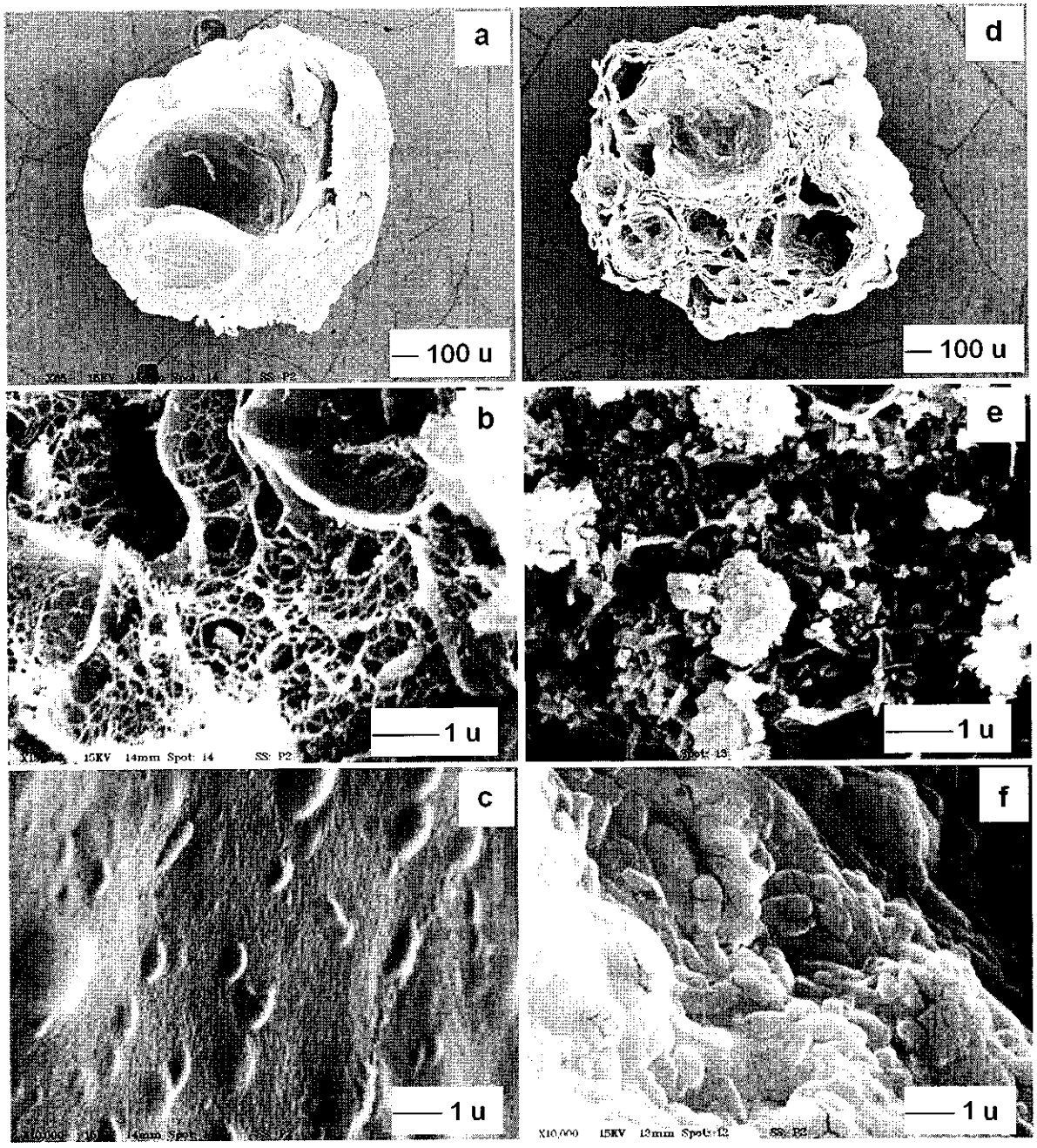

Figure 3—Scanning electron microscopy (SEM) images of entrapped cells: (a) bead cross-section; (b) bacteria entrapped in the interior of a bead; (c) bacteria entrapped in a void before experiment; (d) bead cross-section; (e) bacteria entrapped in the interior of a bead; and (f) bacteria entrapped in a void after experiment.

sand, which was cleaned by washing rather than combustion. In the second test (without methanol), there was no nitrate removal. This suggests that organic substances on sand were in small amounts and were used up during the first test.

In the third and fourth tests (with methanol), nitrate removal was 90 to 99% for the entrapped cells and 56 to 75% for the free-cell columns. There was a significant difference in the effluent nitrate concentration of the first and second tests compared to those of the third and fourth tests. Moreover, the entrapped-cell columns provided higher nitrate removal than the free-cell columns. The presence of the nitrifying bacteria in the entrapped-cell columns and the adequate substrate (carbon) enhanced nitrate removal. It should be noted that when methanol was sufficiently present, it was unlikely that the alginate matrix was used as a carbon source. Nitrate removal trends followed first-order kinetics (Table 1). Doubling the cell loading increased the denitrification rate by 1.5 and 1.9 times for the free- and entrapped-cell columns. At the same initial cell loading, the free-

cell columns provided lower denitrification rates because of a large bacteria loss (described in the next section).

Nitrite accumulation was observed in the free-cell columns during the first four hours of the first test. After that, nitrite concentration decreased because of the lack of nitrite production (from nitrate reduction) and conversion of nitrite to nitrogen gas. These occurred because initially there were significant cell losses from the free-cell columns, and the nitrite-reducing bacteria required longer acclimation time (Tenokuchi et al., 2006). During the second test, because nitrate was not removed, there was no nitrite build-up. However, in the third and fourth tests, although nitrate decreased substantially, there was no nitrite accumulation.

Bioaugmented Cell Leaching. The amount of leaching bacteria is shown in Figure 2. In the first test, the bacteria leaching from the entrapped cell columns was not observed, while a large amount of bacteria (7 log CFU/mL) leached from the free cell columns. In the second test, bacterial counts were 4 log CFU/mL for the entrapped-cell columns, which gradually increased to 5.5 log CFU/mL in the fourth test. For the free-cell columns, the bacterial counts decreased to a range of 4.5 to 5.5 log CFU/mL in the third and fourth tests. Although the bacteria leaching from both entrapped- and free-cell columns were at the same magnitude in the third and fourth tests, nitrate removal efficiencies of the entrapped cells were higher. There are two possible reasons for this observation. First, in the free-cell columns, a large number of bacteria were flushed off in the first and second tests. Therefore, the bacteria left in the free cell columns were much less than the entrapped-cell columns. Second, in the entrapped-cell columns, most of the denitrifying bacteria were entrapped in the matrices, whereas the leaching bacteria could have come from small amounts of bacteria attached at the surface of the matrices.

Microscopic Observation in Entrapped Cells. The SEM images of entrapped cells before and after the experiment are shown in Figure 3. Figures 3a and b present a dense mesh calcium and alginate cross-linking structure of an alginate bead collected after the cell entrapment but before the experiment. Some beads contained a large void in their center, which was a bubble from the entrapment process (Figure 3a). There were bacteria uniformly entrapped in the entire bead (Figures 3b and c). After the experiment, the bead contained many voids, which were from nitrogen gas produced by denitrification (Figure 3d). Beads containing gas voids are prone to abrasion even in short-term applications and, consequently, their stability needs to be monitored continuously (Gentry et al., 1994). Figures 3e and f show several bacteria growing in the interior of the beads. These SEM results supported the denitrification results presented above. In the entrapped-cell columns, most denitrifying bacteria were entrapped and proliferated in the matrices resulting in high nitrate removal efficiencies.

Conclusions

Calcium alginate entrapped-cell bioaugmentation is a promising technique for nitrate removal from agricultural infiltrate. With methanol supplied as a carbon source, nitrate removal efficiencies by the entrapped and free cells were 90 to 99% and 56 to 75%, respectively, and nitrite accumulation was not observed. The denitrification rate increased 1.5 to 1.9 times when the augmented cell loading was doubled. Bacterial losses resulted in low nitrate removal in the free-cell bioaugmentation but not the entrapped-cell system. The SEM images show denitrifying bacteria proliferation in the alginate matrices and several nitrogen gas voids. For future work, a long-term study is recommended to investigate the effect of nitrogen gas production on the durability of alginate beads. A full nitrogen mass balance and operational condition optimization such as methanol requirement should be examined.

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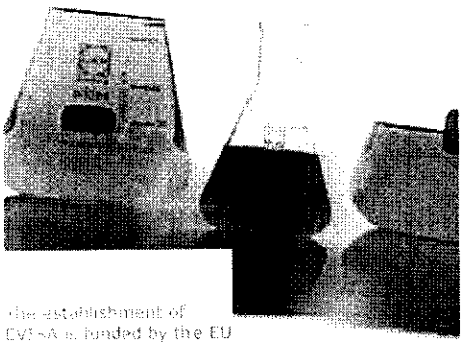

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