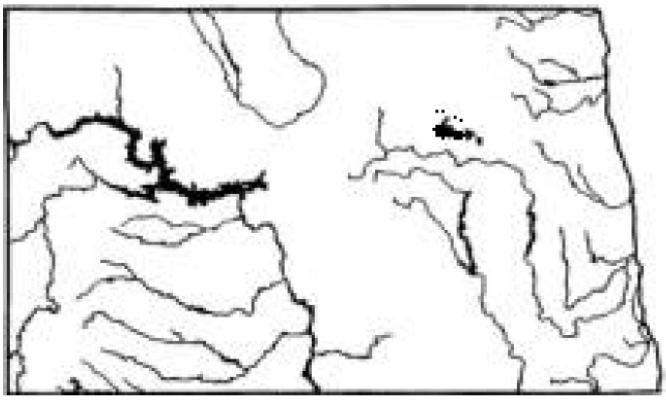


Technical Report No: ND07-03

A COMPARATIVE STUDY OF IMMOBILIZED NITRIFYING AND CO-IMMOBILIZED NITRIFYING AND DENITRIFYING BACTERIA FOR AMMONIA REMOVAL OF SLUDGE DIGESTER SUPERNATANT

by

Christopher B. Hill
Eakalak Khan
Dept. of Civil Engineering, North Dakota State University
Fargo, North Dakota

July 2007

North Dakota Water Resources Research Institute
North Dakota State University, Fargo, North Dakota

Technical Report No: ND07-03

A COMPARATIVE STUDY OF IMMOBILIZED NITRIFYING AND CO-IMMOBILIZED NITRIFYING AND DENITRIFYING BACTERIA FOR AMMONIA REMOVAL OF SLUDGE DIGESTER SUPERNATANT

by

Christopher B. Hill[1]
Eakalak Khan[2]
WRRI Graduate Research Fellow[1] and Associate Professor[2]
Department of Civil Engineering
North Dakota State University
Fargo, ND 58105

July 2007

The work upon which this report is based was supported in part by federal funds provided by the United States of Department of Interior in the form of ND WRRI Graduate Research Fellowship for the graduate student through the North Dakota Water Resources Research Institute.

Contents of this report do not necessarily reflect the views and policies of the US Department of Interior, nor does mention of trade names or commercial products constitute their endorsement or recommendation for use by the US government.

Project Period: March 1, 2006 – February 28, 2007
Project Number: 2006ND99B

North Dakota Water Resources Research Institute
Director, G. Padmanabhan
North Dakota State University
Fargo, North Dakota 58105

TABLE OF CONTENTS

TABLE OF CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	iv
ABSTRACT	1
ACKNOWLEDGMENTS	2
BACKGROUND	3
DESCRIPTION OF THE STATE OR REGIONAL WATER PROBLEM INVESTIGATED	4
SCOPE AND OBJECTIVES	4
MATERIALS AND METHODS	5
Cultivation of Nitrifying and Denitrifying Bacteria	5
Chemicals	5
Cell Immobilization Procedure	6
Experimental Setup and Procedure	6
Scanning Electron Microscopy	6
Analytical Methods	7
RESULTS AND DISCUSSION	7
Kinetics Study	7
Scanning Electron Microscopy	11
CONCLUSIONS	16
REFERENCES	17

LIST OF TABLES

Table 1 Denitrifier cultivation medium 5

Table 2 Average and range of initial NH_3-N concentration 7

LIST OF FIGURES

Figure 1 Average N species concentrations and standard deviations as fractions of initial NH3-N concentration with time for immobilized nitrifying bacteria without methanol addition 8

Figure 2 Average N species concentrations and standard deviations as fractions of initial NH3-N concentration with time for immobilized nitrifying bacteria with methanol addition 8

Figure 3 Average N species concentrations and standard deviations as fractions of initial NH3-N concentration with time for co-immobilized nitrifying and denitrifying bacteria without methanol addition 9

Figure 4 Average N species concentrations and standard deviations as fractions of initial NH3-N concentration with time for co-immobilized nitrifying and denitrifying bacteria with methanol addition 9

Figure 5 pH during replicate no. 4: Nitrifying reactor (Nit); co-immobilized reactor (Co); and control reactor (X) 11

Figure 6 Nitrifying beads immediately after immobilization: a) and b) Surface of bead; and c) Immobilized floc in the interior of bead 12

Figure 7 Nitrifying beads after experiments: a) Surface of bead; b) Surface colony; c) Surface attached bacteria; and d) Full bead interior 13

Figure 8 Co-immobilized beads immediately after immobilization: a) Surface of bead; and b) and c) Bacterial floc in the interior of bead 14

Figure 9 Co-immobilized beads after experiments: a) and b) Gas voids inside the bead; and c) and d) Bacterial colony in center of bead; e) Surface of bead; and f) Bacterial colony rupturing surface of bead 15

ABSTRACT

Bench scale kinetic experiments were conducted to examine the use of cell immobilization in calcium alginate to remove ammonia from the anaerobic sludge digester supernatant of the Moorhead Wastewater Treatment Facility. Two systems, immobilized nitrifiers and co-immobilized nitrifiers/denitrifiers, were studied with and without the addition of methanol. Results indicated that partial nitrification (to nitrite) was achieved in both systems. The co-immobilized reactors did not exhibit the extent of nitrite accumulation observed in the solely nitrifier reactors. The nitrifier reactors were unable to buffer the hydrogen ion production, during the nitrification process, to the level the co-immobilized cells achieved. Both of these differences suggested the occurrence of denitrification in the co-immobilized reactors. The denitrification proceeded via the nitrite pathway due to the lack of nitrate present in the reactors. Scanning electron microscopic images of bacteria immobilized in the alginate spherical beads support the results of the kinetic experiments. Nitrifiers colonized in the 100-200 µm peripheral layer of the beads. Large voids caused by nitrogen gas due to denitrificaiton were found in a number of co-immobilized bead samples. The gas production and heterotrophic nature of the denitrifying bacteria caused a loss in bead integrity.

ACKNOWLEDGMENTS

Stipend support for the Research Fellow, Christopher B. Hill, was provided by the North Dakota Water Resources Research Institute. Appreciations are conveyed to Dr. Robert Zimmerman and staff at the Moorhead Wastewater Treatment Facility for assisting in the collection of wastewater samples used in the following research.

BACKGROUND

The push from regulatory agencies has sent municipal wastewater treatment plants searching for alternative solutions to meet nitrogen (N) limits (Constantine et al., 2005; Wright, 2004**)**. The treatment of sludge digester supernatant is one option to reduce nitrogen emission and has received considerable attention in the past decade. The recycled supernatant stream, which has ammonia nitrogen (NH_3-N) concentration typically in the range of 500-1,500 mg N/L, can account for 15-40% of the nitrogen load to the head of the wastewater treatment plant but only contributes to 5-10% of the hydraulic load (van Dongen et al., 2001; Fux et al., 2003). The appeal of treating the supernatant side-stream is mainly due to the economic aspects such as smaller reactor sizes and lower operational costs (Constantine et al., 2005; Wright, 2004). The high NH_3-N concentration in the recycled supernatant can potentially upset the main treatment process; therefore it is beneficial to reduce NH_3-N concentration to levels found in domestic wastewater, 20 - 70 mg N/L.

Biological treatment is generally considered more cost effective for nitrogen removal than physiochemical processes (Metcalf and Eddy, 2003). There have been a number of novel biological processes developed for the removal of N from sludge digester supernatant including, but not limited to the anaerobic ammonium oxidation (Mulder et al., 1995), single reactor high activity ammonia removal over nitrite (SHARON) (Hellinga et al., 1998) and complete autotrophic nitrogen removal over nitrite (CANON) (Third et al., 2001). The SHARON and CANON processes take advantage of partial nitrification (limited to the first step or ammonia oxidation) which reduces the oxygen requirements by 25% and organic carbon required for denitrification by 40%. High NH_3-N concentrations (Anthonisen et al., 1976), low dissolved oxygen (Hanaki et al., 1990) and temperature have been used to inhibit nitrite oxidation or to cause washout nitrite oxidizing bacteria (Hellinga et al., 1998).

The immobilization of bacteria has been studied extensively and found to have numerous benefits over free cells for treatment of domestic wastewater including increased conversion rates, decreased growth rates, higher cell concentrations, no need for cell separation, and elimination of washout possibility (Yang et al., 2002; Wijffels and Tramper, 1995; Cao et al., 2002; Aravinthan et al., 1998; van Ginkel et al., 1983). Some of the studies conducted on the ability of immobilized cells to remove nitrogen have focused on pure cultures treating domestic wastewater (Uemoto et al., 2000; Cao et al., 2004). Immobilized nitrifying and denitrifying mixed cultures have also been studied (Yang and See, 1991, Yang et al., 1997). The co-immobilization of nitrifying and denitrifying bacteria allows for both nitrification and denitrification in the same reactor due to the oxygen gradient through the immobilization matrix (dos Santos et al., 1996a; Cao et al., 2002, Cao et al., 2004).

There has been only one previous study that tested the ability of immobilized cells to nitrify high NH_3-N concentrations as found in sludge digester supernatant (Rostron et al., 2001). The study used a synthetic feed and involved full nitrification. The work presented

in this report investigated the ability of immobilized nitrifiers to partially nitrify high NH_3-N concentrations in real supernatant from a digester of a wastewater treatment plant. A system with only immobilized nitrifiers was compared to a system that had denitrifiers immobilized along with nitrifiers. Although this study focused on nitrification, the co-immobilization could potentially offer benefits such as pH control with complete N removal as a side benefit. This effort correlates well with the need for nitrification only of the wastewater treatment plant where the supernatant sample was collected from. The plant is subjected to only NH_3-N limit for N species. In addition, scanning electron microscopy (SEM) was used to probe the location and propagation of nitrifiers and denitrifiers within and on the surface of the immobilization matrix with time. To the best of our knowledge, these aspects have not been examined in any previous studies on immobilized nitrifiers and/or denitrifiers.

DESCRIPTION OF THE STATE OR REGIONAL WATER PROBLEM INVESTIGATED

The Moorhead WWTF in Moorhead, Minnesota currently treats four million gallons of wastewater per day. It has a capacity to treat six million gallons per day to meet population projected through the year 2020. It relies on high purity oxygen-activated sludge (HPO-AS) process for treating wastewater. Sludge from primary and secondary clarifiers is treated in an anaerobic digester. Digested sludge is applied to farm fields as a soil amendment while the digester supernatant is returned to the HPO-AS process.

The HPO-AS process typically has difficulties in removing NH_3-N due to the toxicity of high oxygen concentration to the nitrifying microorganisms (Uemoto et al., 2000). In addition, the recycling of digester supernatant is a shock load that sometimes upsets the HPO-AS process and/or causes high NH_3-N in the effluent. To accommodate the lack of NH_3-N removal in the HPO-AS process at the Moorhead WWTF, a nitrifying moving bed biofilm reactor (MBBR) was installed in 1994 as a tertiary treatment method after the HPO-AS process. The MBBR process improved the overall NH_3-N removal of the plant but the cyclical introduction of supernatant to the mainstream process still remains a problem in term of meeting a NH_3-N discharge limit. Removing NH_3-N from the digester supernatant before returning will reduce the risk for process upset and will make it easier for the plant to meet the NH_3-N discharge permit for the Red River.

SCOPE AND OBJECTIVES

The main objective of this research is to investigate the use of immobilized cell systems to reduce ammonia discharge to the Red River. The specific objectives are:

- To examine the ammonia nitrification kinetics of the digester supernatant from the Moorhead WWTF by immobilized nitrifiers and co-immobilized nitrifiers and denitrifiers;
- To investigate the benefits of co-immobilized cell system; and
- To investigate the proliferation and spatial location of bacterial colonies immobilized in the matrix through SEM.

MATERIALS AND METHODS

Cultivation of Nitrifying and Denitrifying Bacteria

Nitrifying bacteria were obtained by acclimating mixed liquor suspended solids collected from the Moorhead WWTF in a 20 L sequencing batch reactor (SBR) under aerobic conditions [Dissolved oxygen (DO) = 4-6 mg/L]. The SBR was operated at a hydraulic retention time (HRT) of 2 days and a solid retention time (SRT) of approximately 30 days. The high SRT was used due to the relatively slow growth rate of nitrifying bacteria. The supernatant from the Moorhead WWTF was collected and allowed to settle for 4 hours at room temperature. Its decant was filtered using a paper filter (Schleicher & Schuell, No. 560) and the filtrate was used to feed the SBR. The characteristics of the supernatant, provided by the Moorhead WWTF, based on average values were the 5-day biochemical oxygen demand of 49.3 mg/L, chemical oxygen demand (COD) of 2,426 mg/L and NH_3-N of 2,055 mg/L. The alkalinity of the supernatant was sufficient to buffer pH drop caused by nitrification during the cultivation. The 2 day SBR cycle consisted of filling for 1 minute, aerating for 46 hours, settling for 1 hour 50 minutes, and decanting for 9 minutes. The SRT was controlled by wasting a portion of settled cells during the decantation. Two SBRs were operated for 3 months before the cells were harvested for immobilization. The denitrifying bacteria were cultivated in a 20 L SBR under anaerobic conditions (DO < 0.5 mg/L) fed with a synthetic medium and operated at an HRT of 1 day. The synthetic medium composition is shown in Table 1 and was prepared using distilled water. The denitrifying SBR were operated on a 1 day cycle consisting of filling for 1 minute, periodical mixing for 23 hours, settling for 50 minutes, and decanting for 9 minutes. The denitrifying SBR was operated for 5 weeks without intentional cell wastage prior to cell immobilization.

Table 1 Denitrifier cultivation medium

Component	Concentration (g/L)
Potassium nitrate (KNO_3)	0.36
Methanol (CH_3OH)	0.30
Manganese sulfate ($MnSO_4.H_2O$)	0.0025
Sodium molybdate ($Na_2MoO_4.H_2O$)	0.0025
Sodium phosphate dibasic (Na_2HPO_4)	0.42
Monopotassium phosphate (KH_2PO_4)	0.20
Magnesium sulfate ($MgSO_4.7H_2O$)	0.02
Calcium chloride ($CaCl_2.2H_2O$)	0.01
Iron sulfate ($FeSO_4.7H_2O$)	0.005

Chemicals

All chemicals used for the denitrifying cultivation medium were analytical grade except methanol which was a 98% purity grade. All of them were purchased from VWR. Sodium alginate (Unspecified grade) was manufactured by Pfaltz and Bauer, Inc. and was purchased through VWR.

Cell Immobilization Procedure

The nitrifying culture was immobilized by itself and in combination with the denitrifying culture in calcium alginate according to the procedure described in van Ginkel et al. (1983). The cultured cells were harvested during exponential growth phase by centrifuging the contents of the SBRs at 7,000 rpm for 15 minutes. Sodium alginate was dissolved in de-ionized water at a concentration of 2% (w/w). The centrifuged cells were added to the sodium alginate solution at a concentration of 3.2 g volatile suspended solids (VSS)/L and the mixture was mixed to form a homogeneous solution. Using a peristaltic pump (Masterflex 7553-60, 7013 pump head, 6409-13 tubing), the mixture was dropped into a 3.5% calcium chloride ($CaCl_2$) solution to form calcium alginate beads (through sodium and calcium exchange). The beads were stirred in the $CaCl_2$ solution for 3 hours and immediately placed in a reactor. The nitrifiers and denitrifiers were co-immobilized at a mass ratio of 1:1.5 within the optimum range reported in a previous study (Cao et al., 2002). Control beads were prepared in the same manner as immobilized cells, omitting the cell addition step.

Experimental Setup and Procedure

The nitrifier, combined nitrifier-denitrifier, and control beads, were placed in separate reactors. Each reactor was operated at room temperature as a SBR with a reactive volume of 1.3 L. The bulk volume of the beads in the reactor was 700 mL for all three reactors. The reactors were subjected to an 8 hour batch kinetic test. One liter of supernatant was treated during each test. The 8 hr time was based on a preliminary test on the ability of supernatant to buffer pH reduction caused by nitrification to a limit of 6.75. The two reactors with immobilized cells were operated under aerobic conditions supplying air at a rate of 20 mL/min (DO = 4.0-6.7 mg/L). The air supplied to the reactor also provided mixing. Samples were taken from the reactors every hour during the kinetic tests and were analyzed for NH_3-N, nitrite N (NO_2-N), nitrate N (NO_3-N), pH, DO, VSS and COD. Each of the reactors, immobilized nitrifiers and co-immobilized nitrifiers-denitrifiers, were tested with and without the addition of methanol. Four replicates of each reactor and each methanol condition were conducted. The control reactor was tested once without and once with the addition of methanol.

Scanning Electron Microscopy

The beads with cells were collected and stored in a 0.1 M $CaCl_2$ solution at 4^oC. For SEM sample preparation, the beads were rinsed in 0.1 M $CaCl_2$ two times (15 min each), and then placed in a solution consisting of 2.5% glutaraldehyde and 0.1 M $CaCl_2$ for 1 hour. The beads were rinsed again, twice in 0.1 M $CaCl_2$ (15 min each) and dehydrated using 30% ethanol in 0.07 M $CaCl_2$, 50% ethanol in 0.05 M $CaCl_2$, 70% ethanol in 0.03 M $CaCl_2$, 90% ethanol in de-ionized water, and twice in 100% ethanol (30 min each step). After the dehydration, the bead samples were then critical point dried using an autosamdri-810 critical point drier with liquid carbon dioxide as the transitional fluid. The samples were attached to aluminum mounts by silver paint and coated with gold/palladium using a Balzers SCD 030 sputter coater. Images were obtained using a JEOL JSM-6300 Scanning Electron Microscope.

Analytical Methods

Ammonia and nitrate were analyzed using ion selective electrodes (Thermo Orion 250A+, VWR SympHony Ammonia Combination Electrode, VWR SympHony Nitrate Ion Selective Electrode, VWR SympHony Double Junction Reference Half-Cell) in accordance with *Standard Methods* (APHA et al., 1998). Nitrite was analyzed colorimetrically using standard HACH NitriVer®2 reagents according to the method specified by the reagent manufacturer (HACH). Total suspended solids, VSS, COD, pH (Thermo Orion 250A+pH Electrode) and DO (Thermo Orion 850A meter, Thermo Orion DO 083005D probe,) were all determined according to *Standard Methods* (APHA et al., 1998).

RESULTS AND DISCUSSION

Kinetics Study

Figure 1 through 4 presents average nitrogen species (NH_3-N, NO_2-N, and NO_3-N) concentrations normalized by initial NH_3-N concentrations with time for each of the immobilized cell and control reactors, with and without methanol addition. The error bars represent the standard deviation of the normalized concentrations based on the replicate experiments. The average and range of initial NH_3-N concentration are shown in Table 2.

For all configurations, the accumulation of nitrite appears to follow zero order kinetics and nitrate remained relatively constant. The ammonia removal kinetics also follows zero order kinetics for all but the first hour. The nature of the hydro-gel polymer calcium alginate can be used to explain the higher drop in ammonia concentration in the first hour. The initial drop in ammonia was contributed by nitrification and sorption due to the difference in the initial NH_3-N concentrations of the bead and supernatant. Since NH_3-N concentration of the bead was lower than that in the surrounding supernatant at the beginning of the test, sorption of NH_3-N occurred.

Table 2 Average and range of initial NH_3-N concentration

Experiment	Initial NH_3-N concentration (mg N/L)	
	Average	Range
Nitrifiers	920	727 - 1245
Nitrifiers with methanol	938	740 - 1120
Co-Immobilized	1008	866 - 1326
Co-Immobilized with methanol	959	840 - 1090
Control	850	N/A
Control with methanol	719	N/A

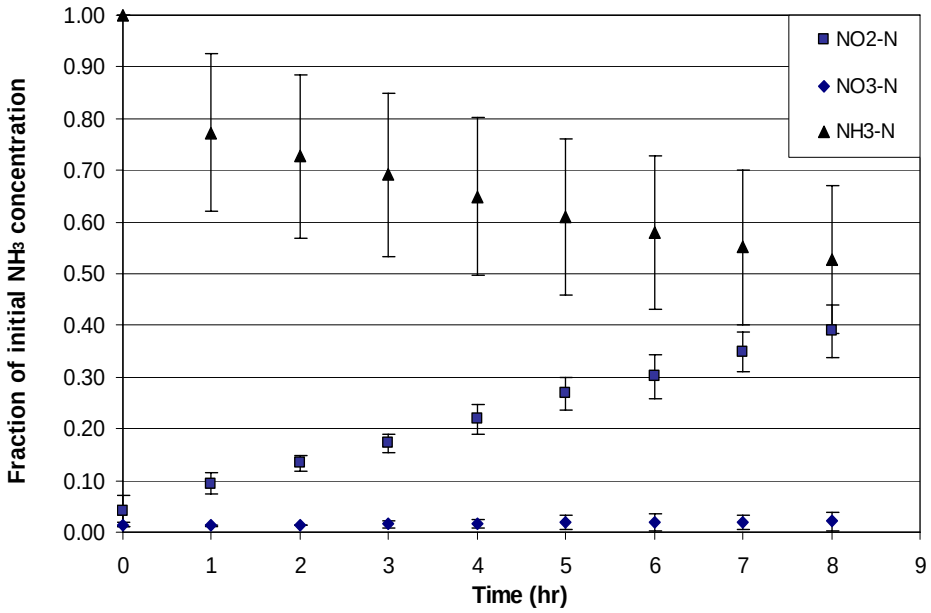


Figure 1 Average N species concentrations and standard deviations as fractions of initial NH_3-N concentration with time for immobilized nitrifying bacteria without methanol addition

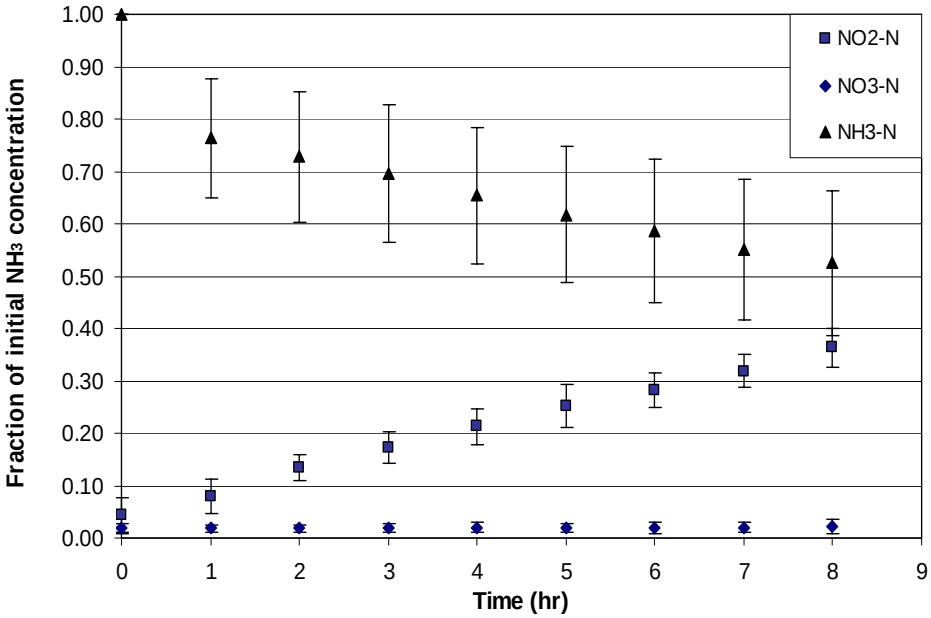


Figure 2 Average N species concentrations and standard deviations as fractions of initial NH_3-N concentration with time for immobilized nitrifying bacteria with methanol addition

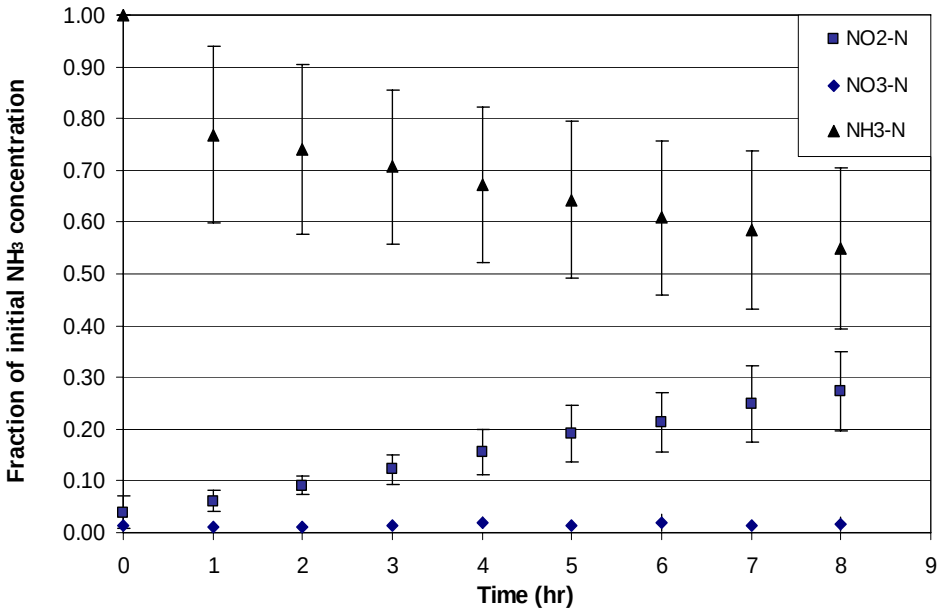


Figure 3 Average N species concentrations and standard deviations as fractions of initial NH_3-N concentration with time for co-immobilized nitrifying and denitrifying bacteria without methanol addition

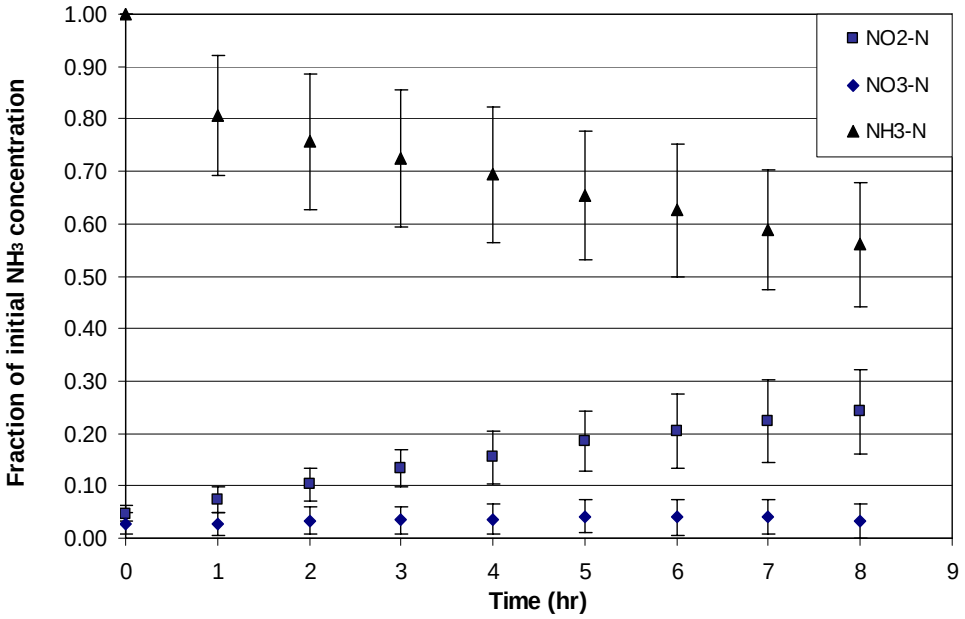


Figure 4 Average N species concentrations and standard deviations as fractions of initial NH_3-N concentration with time for co-immobilized nitrifying and denitrifying bacteria with methanol addition

Methanol is typically used as a readily biodegradable carbon source for denitrification in wastewater treatment processes. Based on the results above, it may appear that the augmentation of a carbon source to both the nitrifying and co-immobilized reactors had minor influence over the removal of ammonia or the accumulation of nitrite. With further investigation of the COD data, it was found that the alginate itself was leaching carbon (Data not shown). The carbon supplied from the alginate could not be controlled. Therefore, regardless if methanol was supplied, the microorganisms had a carbon source and the effect of methanol addition could not be justified.

The major advantage of treating sludge digester supernatant is that it is easy to accomplish partial nitrification. The accumulation of nitrite and lack of accumulation of nitrate leads to extreme cost savings during operation of wastewater treatment facilities (Wright, 2004). Figures 1 through 4 illustrate that both the nitrifying and co-immobilized reactors accomplished partial nitrification. Although insignificant in comparison to nitrite, it should be noted that nitrate accumulation of above 30 mg/L as N was seen in all reactors only during replicate no. 3 experiments (Data not shown). The presence of nitrite oxidizing bacteria acclimated to high ammonia concentration in the supernatant could be the cause of this occurrence. During the following replicate no. 4 experiments, nitrate accumulation did not occur in any of the reactors. It is likely that the nitrate oxidizing bacteria were washed out of the reactor along with the supernatant tested in replicate no. 3 experiments. The lack of nitrate production during the replicate no. 4 experiments showed how unwanted microorganisms can be controlled in immobilized cell systems.

Previous studies conducted on domestic wastewater reported the ability of immobilized denitrifying bacteria to accomplish nitrogen removal in aerobic environments. The immobilization matrix creates an oxygen gradient throughout the bead. Therefore, the denitrification process will proceed within the center of bead where oxygen is limited (dos Santos et al., 1996a; dos Santos et al., 1996b). Originally, COD was to be utilized to study denitrification activity but the event of carbon leaching from the alginate matrix produced an unattainable challenge to this method. An alternative method was established using pH and nitrite accumulation for evaluating if denitrification activity was occurring.

During denitrification, alkalinity is produced and is used to partially control the hydrogen production during nitrification of sludge digester supernatant (Fux et al., 2003). Figure 5 is a plot of the pH during replicate no. 4. Sludge digester supernatant naturally has a large amount of alkalinity. During the aeration of the supernatant, carbon dioxide gas was stripped from the system causing an increase in pH until equilibrium was reached with the supplied air. All of the reactors exhibited this trend initially. Due to the lack of nitrification, pH of the control reactor reached a plateau. Both nitrifying and co-immobilized reactors rebounded from the rising pH once the hydrogen production during nitrification neutralized the alkalinity. It was observed during all experiments that the nitrifying reactor had lower final pH than the co-immobilized reactor. This is believed to be evidence of denitrification activity.

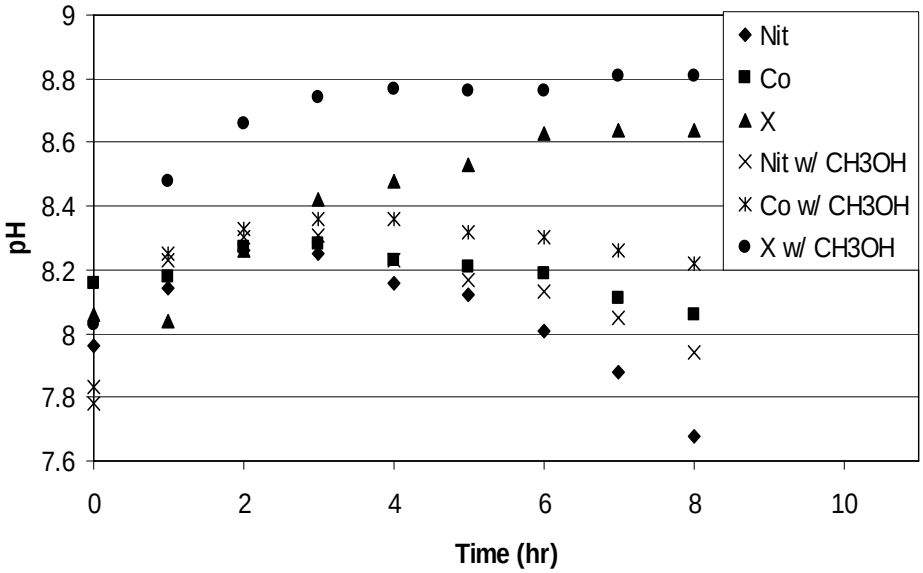


Figure 5 pH during replicate no. 4: Nitrifying reactor (Nit); co-immobilized reactor (Co); and control reactor (X).

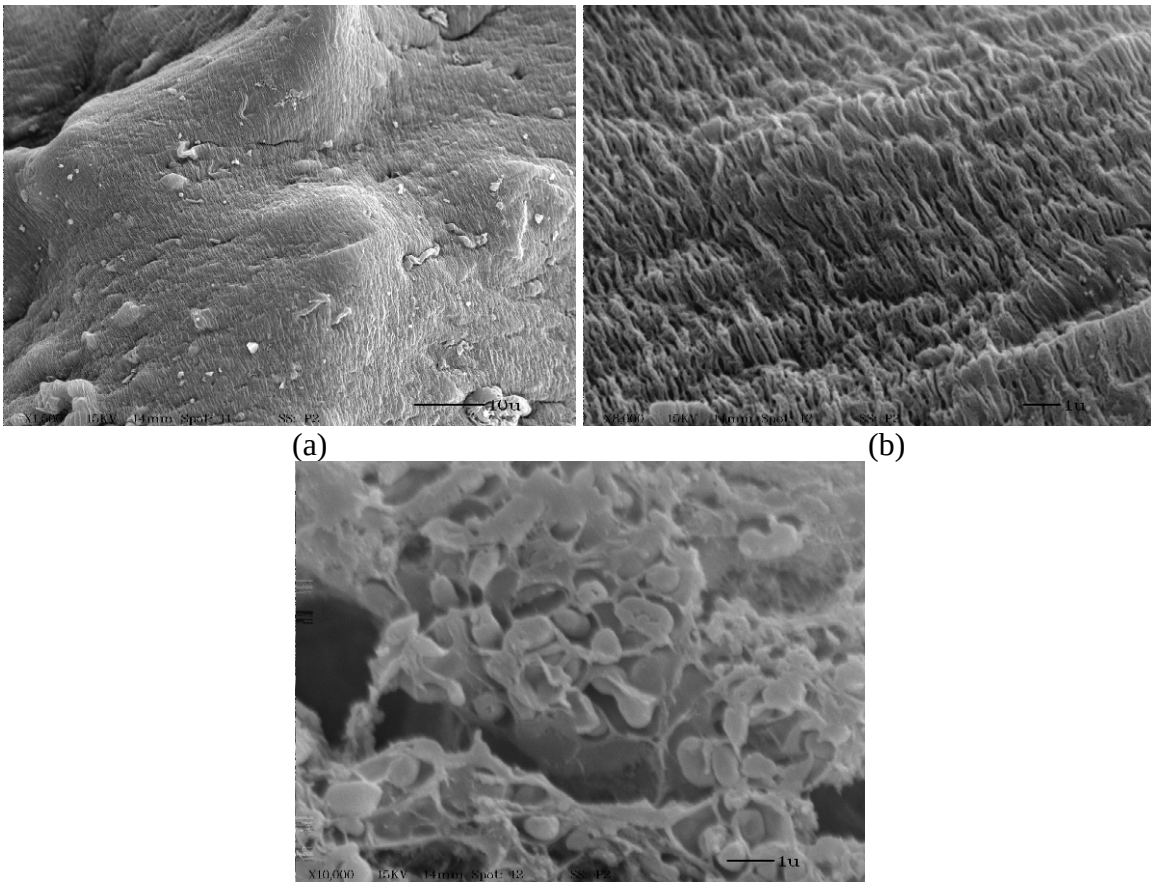
Nitrite accumulation was also an indicator of denitrification activity. The average ammonia removal of the nitrifying reactors was 47% with and without the addition of methanol during the 8 hour test period. The average nitrite accumulation was 68% and 74% of the ammonia removed for the reactors with and without methanol, respectively. In comparison, the co-immobilized reactors provided the ammonia removal of 45% and 44% while the average nitrite accumulation was 51% and 43% of the average ammonia removed with and without methanol, respectively. The co-immobilized cells performed similar to the nitrifying cells in terms of ammonia removal, but denitrification activity reduced the accumulation of nitrite. Assuming most of N in the supernatant was contributed by NH_3-N, NO_2-N, and NO_3-N, the total nitrogen concentrations in the reactor, which could be estimated by the sum of these N species, at 0 and 8 hours were quite balanced. Based on these conditions, the co-immobilized cell reactors removed on average 8.5% more total nitrogen than the immobilized nitrifier cell reactors. Scanning electron microscopy provided further evidence of denitrifying activity.

Scanning Electron Microscopy

Scanning electron microscopy images of the beads were used to qualitatively investigate the proliferation and spatial location of bacteria in the alginate beads. The images verified the presence of microorganisms after immobilization and the production of gas due to denitrification.

The SEM images of immobilized nitrifying alginate beads immediately after immobilization are shown in Figure 6. The bacteria were found in floc homogenously dispersed within the matrix (Figure 6c). No bacteria or colonies were found on the surface of the bead (Figure 6a and b). The SEM of the bead post kinetic experiments

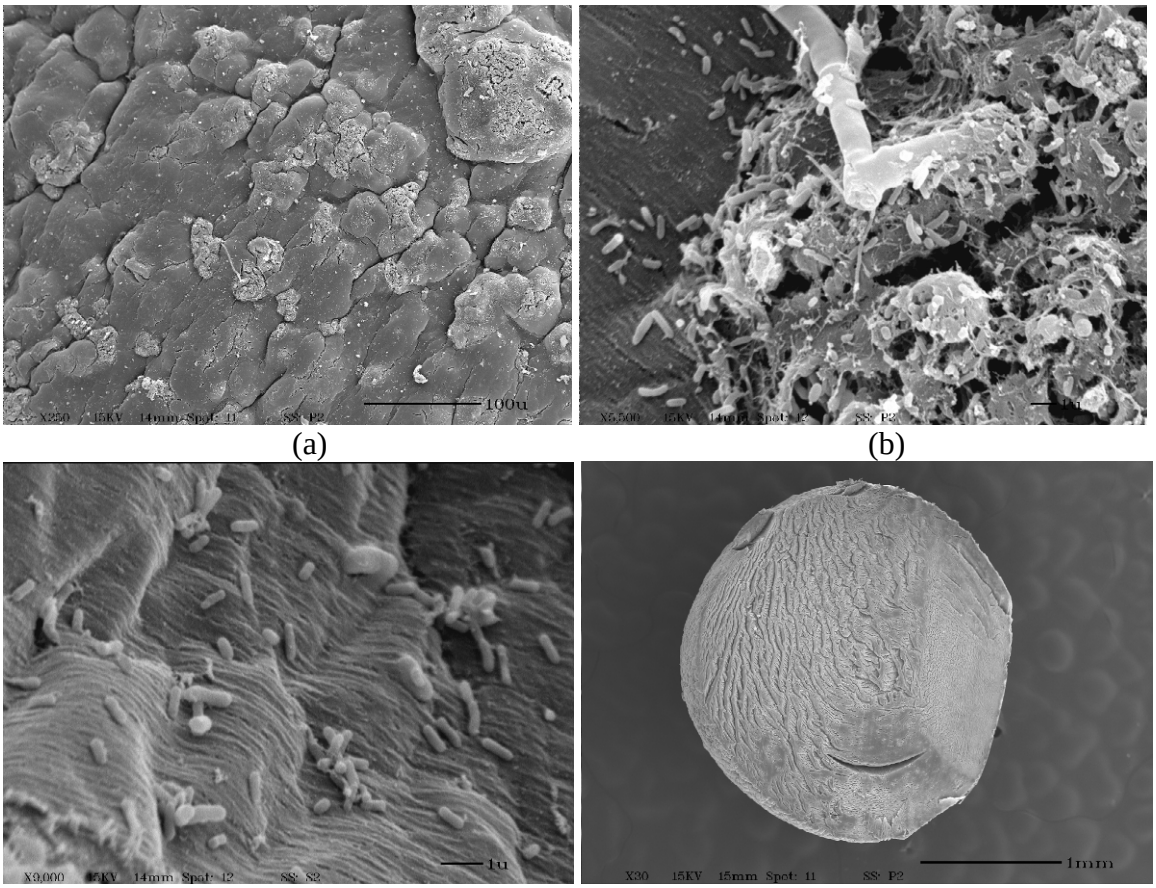
confirmed a definite change in cell concentration of the beads. The growth of colonies near the surface of the bead was so extensive that some colonies ruptured to the exterior (Figure 7a, b, and c). In addition, bacteria slightly dispersed over the entire surface of the nitrifying bead. Besides the substantial amount of bacteria within approximately 100-200 µm distance of the surface of the bead, the interior of the nitrifying beads remained relatively unchanged compared to the initial images (Figure not shown). The spatial location of bacteria and colony eruption found coincide with previous studies on immobilized nitrifying microorganisms (Wijffels and Tramper, 1995, dos Santos et al., 1996a). Although the rupturing of colonies has been reported in previous studies, this study is the first to capture the rupturing bacterial colonies with SEM images.



(a) (b)

(c)

Figure 6 Nitrifying beads immediately after immobilization: a) and b) Surface of bead; and c) Immobilized floc in the interior of bead

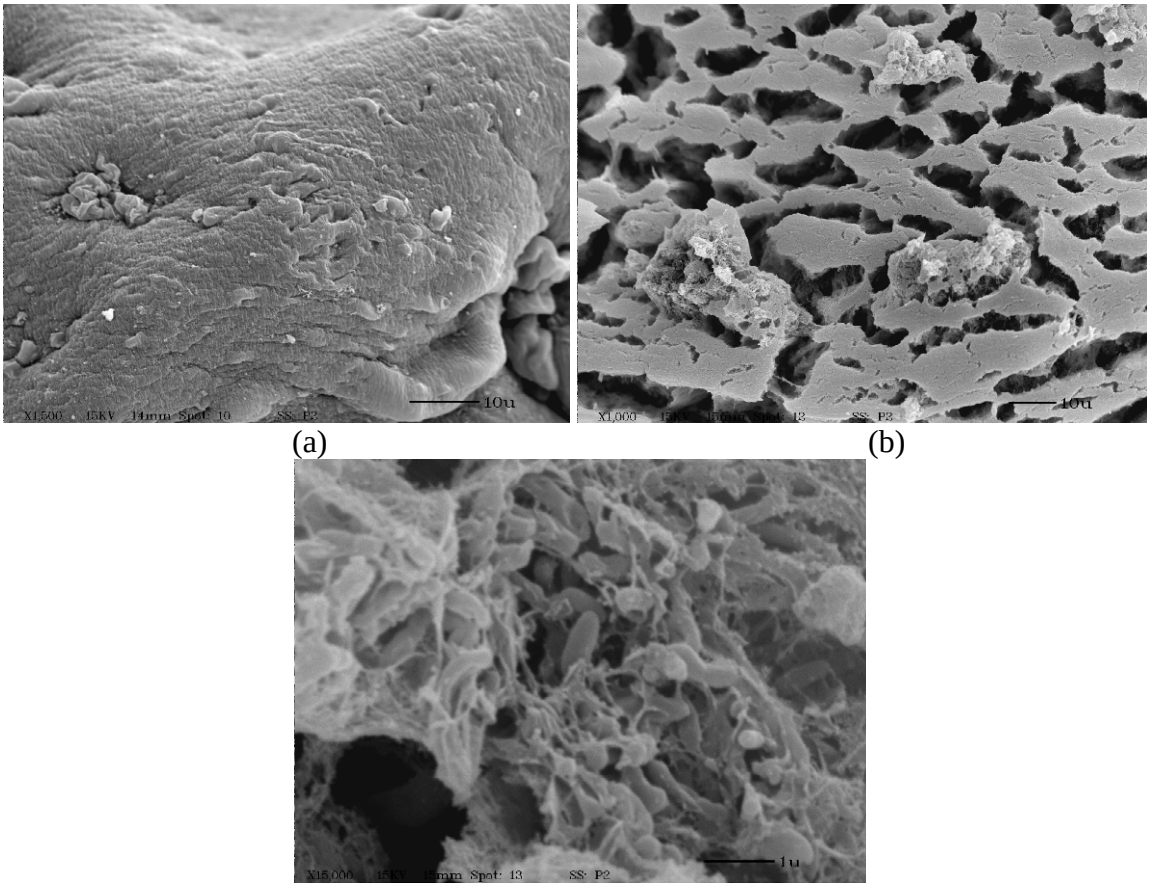


(a) (b)

(c) (d)

Figure 7 Nitrifying beads after experiments: a) Surface of bead; b) Surface colony; c) Surface attached bacteria; and d) Full bead interior

The SEM images of co-immobilized nitrifying and denitrifying alginate beads immediately after immobilization are shown in Figure 8. Similar to the initial nitrifying beads, the bacterial flocs were uniformly distributed within the matrix and no bacteria or colonies were found on the surface of the beads. After the kinetic experiments, the beads were examined. Based on qualitative visual observations, unlike the nitrifying beads, there was evidence of bacterial growth in the core of the bead. In a number of beads, the nitrogen gas produced from denitrification activity formed voids in the beads (Figure 9a and b). Bacteria lined the surface of the voids (Figure 9c and d). It is likely that the bacterial growth congested the pores in the matrix and was the cause of the gas accumulation in the bead. The gas production concurs with the occurrence of denitrification activity. Similar to the nitrifier beads, the presence of rupturing colonies was found on the surface the co-immobilized beads (Figure 9e and f)



(a) (b)

(c)

Figure 8 Co-immobilized beads immediately after immobilization: a) Surface of bead; and b) and c) Bacterial floc in the interior of bead

Throughout the kinetic experiments, the nitrifying bacteria bead maintained their integrity (Figure 7d). On the contrary, the co-immobilized bead became relatively pliable and began to disintegrate during the final replicate experiments. There are two possible reasons for the difference in the final structural characteristics of the co-immobilized bead. First, the gas production from the denitrification caused destructive forces on the beads. In the case of gas accumulation in large voids, this is intuitive but the effect of transporting gas from the interior of the bead to the bulk media may also have an effect on the structural stability of the bead. Second, the denitrifying bacteria utilize organic carbon during their biological processes and alginate is composed of organic carbon. Therefore, it is possible that the denitrifying bacteria exploited the alginate carbon source and undermined the structural stability of the bead.

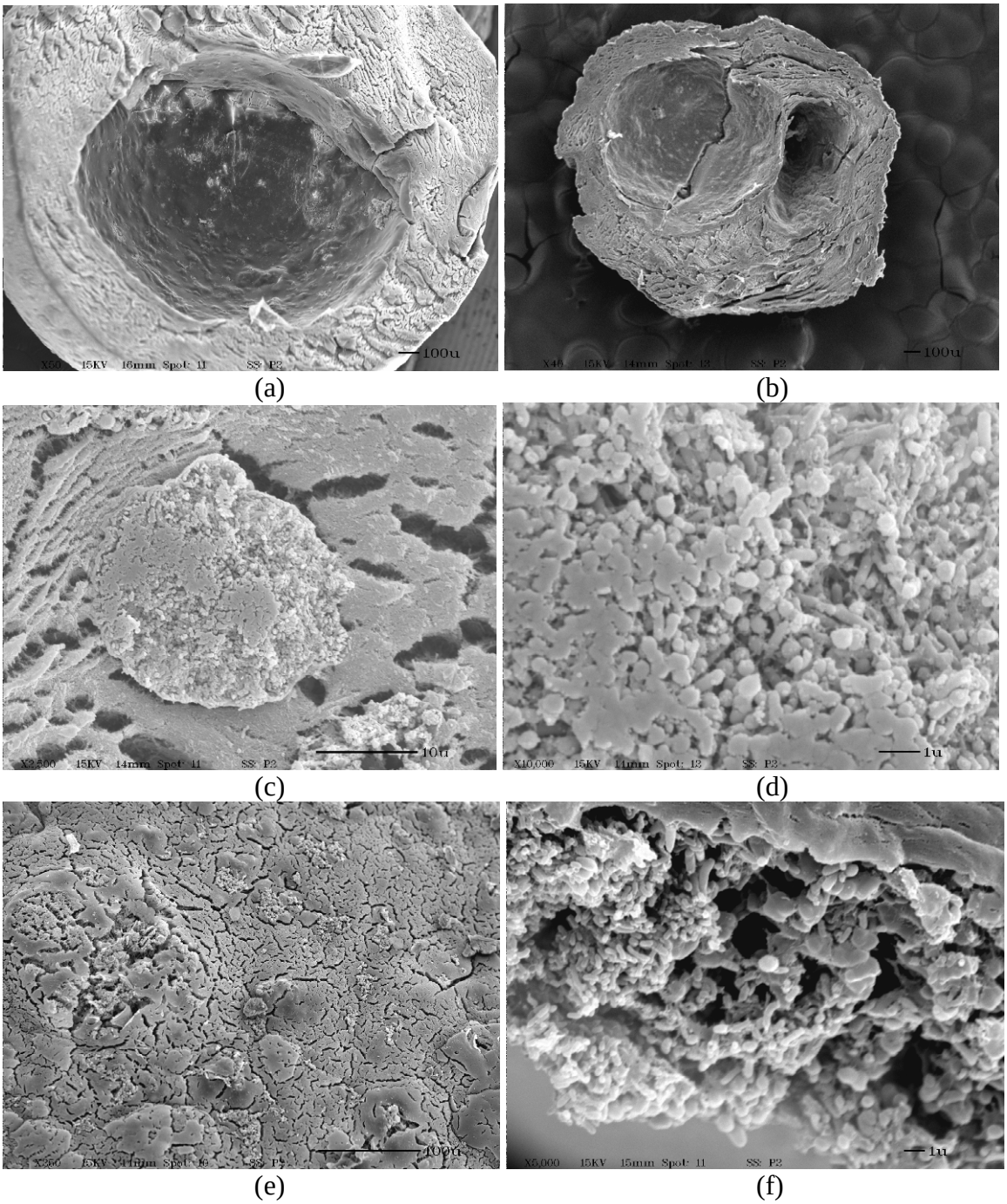


Figure 9 Co-immobilized beads after experiments: a) and b) Gas voids inside the bead; and c) and d) Bacterial colony in center of bead; e) Surface of bead; and f) Bacterial colony rupturing surface of bead

CONCLUSIONS

The City of Moorhead, Minnesota currently has the challenge of managing sludge digester supernatant of its WWTF. The high ammonia concentration in the supernatant created difficulties in meeting discharging limits into the Red River. Two immobilized cell systems, immobilized nitrifying and co-immobilized nitrifying/denitrifying bacteria, were examined to treat the supernatant before recycling to the head of the plant. This was the first time immobilized cells were used to remove ammonia from real supernatant. The two systems performed similarly in terms of ammonia removal. Although, two key differences were established between them. First, the co-immobilized reactors did not exhibit the extent of nitrite accumulation seen in the solely nitrifier bead reactors. Second, the nitrifier bead reactors consistently were unable to buffer the hydrogen ion production, during the nitrification process, to the level the co-immobilized cells achieved. Both of these differences support the denitrification activity of the co-immobilized cells. Furthermore, it is believed the denitrification proceeded via the nitrite pathway due to the lack of nitrate present in the reactors.

SEM images of bacteria immobilized in the alginate matrix support the results of the kinetic experiments. Nitrifying microorganisms colonized in the 100-200 µm peripheral layers of the beads. Large voids caused by denitrification gas production were found in a number of co-immobilized bead samples. The gas production and heterotrophic nature of the denitrifying bacteria caused a loss in bead integrity. The application of immobilized cell systems to remove nitrogen in sludge digester supernatant is a viable alternative. There is a need to explore or develop a more durable immobilization matrix to make this process feasible for full scale operation.

REFERENCES

APHA; AWWA; WEF (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; Washington, D.C.

Anthonisen, A. C.; Loehr, R. C.; Prakasam, T. B. S.; Srinath, E. G. (1976) Inhibition of Nitrification by Ammonia and Nitrous acid. *J. Water Pollut. Con. F.,* **48** (5), 835.

Aravinthan, V.; Takizawa, S.; Fujita, K. (1998) Factors Affecting Nitrogen Removal from Domestic Wastewater Using Immobilized Bacteria. *Water Sci. Technol.,* **38** (1), 193.

Cao, G.; Zhao, Q.; Sun, X.; Zhang, T. (2002) Characterization of Nitrifying and Denitrifying Bacteria Coimmobilized in PVA and Kinetics Model of Biological Nitrogen Removal by Coimmobilized Cells. *Enzyme Microb. Tech.,* **30** (1), 49.

Cao, G.; Zhao, Q.; Sun, X.; Zhang, T. (2004) Integrated Nitrogen Removal in a Shell-and-Tube Co-immobilized Cell Bioreactor. *Process Biochem.,* **39** (10), 1269.

Constantine, T.; Murthy, S.; Bailey, W.; Benson, L.; Sadick, T.; Daigger, G. (2005) Alternatives for Treating High Nitrogen Liquor from Advanced Anaerobic Digestion at the Blue Plains AWTP. *Proceeding 78th Annual Water Environment Federation Conference & Exposition*, Oct. 29-Nov. 2, 2005, Washington, D.C.

dos Santos, V. A. P. M.; Bruijnse, M.; Tramper, J.; Wijffels, R. H. (1996a) The Magic-Bead Concept: An Integrated Approach to Nitrogen Removal with Co-immobilized Microorganisms *Appl. Microbiol. Biot. ,* **45** (4), 447.

dos Santos, V. A. P. M.; Tramper, J.; Wijffels, R. H. (1996b) Modeling and Evaluation of an Integrated Nitrogen Removal System with Microorganisms Co-immobilized in Double-Layer Gel Beads. *Biotechnol. Progr.,* **12,** 240.

Fux, C.; Lange, K.; Faessler, A.; Huber, P.; Grueniger, B.; Siegrist, H. (2003) Nitrogen Removal from Digester Supernatant via Nitrite–SBR or SHARON? *Water Sci. Technol.,* **48** (8), 9.

Hanaki, K.; Wantawin, C.; Ohgaki, S. (1990) Nitrificaiton at Low levels of Dissolved Oxygen with and without Organic Loading in a Suspended-Growth Reactor. *Water Res.,* **24** (3), 297.

Hellinga, C.; Schellen, A. A. J. C.; Mulder, J. W.; van Loosdrecht, M. C. M.; Heijnen, J. J. (1998) The SHARON Process: An Innovative Method for Nitrogen Removal from Ammonium-Rich Waste Water. *Water Sci. Technol.,* **37** (9), 135.

Metcalf and Eddy (2003) *Wastewater Engineering: Treatment, Disposal and Reuse*. 4th ed.; McGraw-Hill Inc., New York.

Mulder, A.; van de Graaf, A. A.; Robertson, L. A.; Kuenen, J. G. (1995) Anaerobic Ammonium Oxidation Discovered in a Denitrifying Fluidized Bed Reactor. *FEMS Microbiol. Ecol.,* **16** (3), 177.

Rostron, W. M.; Stuckey, D. C.; Young, A. A. (2001) Nitrification of High Strength Ammonia Wastewater: Comparative Study of Immobilisation Media. *Water Res.,* **35** (5), 1169.

Third, K. A.; Sliekers, A. O.; Kuenen, J. G.; Jetten, M. S. M. (2001) The CANON System (Completely Autotrophic Nitrogen-Removal over Nitrite) under Ammonium Limitation: Interaction and Competition between Three Groups of Bacteria. *Sys. Appl. Microbiol.,* **24** (4), 588.

Uemoto, H.; Ando, A.; Saiki, H. (2000) Effect of Oxygen Concentration on Nitrogen Removal by *Nitrosomonas europaea* and *Paracoccus denitrificans* Immobilized with Tubular Polymeric Gel. *J. Biosci. Bioeng.,* **90** (6), 654.

van Dongen, U.; Jetten, M. S. M.; van Loosdrecht, M. C. M. (2001) The SHARON®-Anammox® Process for Treatment of Ammonium Rich Wastewater. *Water Sci. Technol.,* **44** (1), 153.

van Ginkel, C. G.; Tramper, J.; Luyben, K. C. A. M.; Klapwijk, A. (1983) Characterization of *Nitrosomonas europa* Immobilized in Calcium Alginate. *Enzyme Microb. Tech.,* **5** (4), 297.

Wijffels, R. H.; Tramper, J. (1995) Nitrification by Immobilized Cells. *Enzyme Microb. Tech.,* **17** (6), 482.

Wright (2004) New York City Pushes High-Tech, Low-Cost Sound Investment. *Eng. News-Rec.,* **253** (13), 24.

Yang, P. Y.; See, T. S. (1991) Packed Entrapped Mixed Microbial Cell Process for Removal of Phenol and its Compounds. *J. Environ. Sci. Heal. A,* **26** (8), 1491.

Yang, P. Y.; Zhang, Z. Q.; Jeong, B. G. (1997) Simultaneous Removal of Carbon and Nitrogen Using an Entrapped-Mixed-Microbial-Cell Process. *Water Environ. Res.,* **31** (10), 2617.

Yang, P. Y.; Cao, K.; Kim, S. J. (2002) Entrapped Mixed Microbial Cell Process for Combined Secondary and Tertiary Wastewater Treatment. *Water Environ. Res.,* **74** (3), 226.