

# Denitrification of agricultural drainage line water via immobilized denitrification sludge

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Nonpoint source nitrogen is recognized as a significant water pollutant worldwide. One of the major contributors is agricultural drainage line water. A potential method of reducing this nitrogen discharge to water bodies is the use of immobilized denitrifying sludge (IDS). Our objectives were to (1) produce an effective IDS, (2) determine the IDS reaction kinetics in laboratory column bioreactors, and (3) test a field bioreactor for nitrogen removal from agricultural drainage line water. We developed a mixed liquor suspended solid (MLSS) denitrifying sludge using inoculant from an overland flow treatment system. It had a specific denitrification rate of 11.4 mg NO<sub>3</sub>-N g<sup>-1</sup> MLSS h<sup>-1</sup>. We used polyvinyl alcohol (PVA) to immobilize this sludge and form IDS pellets. When placed in a 3.8-L column bioreactor, the IDS had a maximum removal rate ( $K_{MAX}$ ) of 3.64 mg NO<sub>3</sub>-N g<sup>-1</sup> pellet d<sup>-1</sup>. In a field test with drainage water containing 7.8 mg NO<sub>3</sub>-N L<sup>-1</sup>, 50% nitrogen removal was obtained with a 1 hr hydraulic retention time. Expressed as a 1 m<sup>3</sup> cubically-shaped bioreactor, the nitrogen removal rate would be 94 g NO<sub>3</sub>-N m<sup>-2</sup>d<sup>-1</sup>, which is dramatically higher than treatment wetlands or passive carbonaceous bioreactors. IDS bioreactors for agricultural use and to devise effective strategies for their implementation with other emerging technologies for improved water quality on both watershed and basin scales.

Keywords: Nitrogen, nitrate, denitrification, bioreactor, agricultural drainage, immobilized denitrification sludge.

### Introduction

Nonpoint source nitrogen is recognized as a significant pollutant worldwide.<sup>[1]</sup> It was reported to dominate the riverine pollutant fluxes to the coast of 14 regions of North America, Europe, and Africa.<sup>[2]</sup> These reports are similar to the problematic nitrogen concentrations in 10 major rivers and coastal zones.<sup>[3]</sup> Nitrogen has also been reported to contribute to water quality problems from the Po River discharge where it has impacted phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea.<sup>[4]</sup> Water quality problems associated with hypoxia and nitrogen in the Chesapeake Bay have been well documented.<sup>[5,6]</sup> In the mid-continent of the USA, the problem of nitrogenrelated water pollution in the Gulf of Mexico has been related particularly to the rapid transport of nitrogen by large streams and rivers.<sup>[7,8]</sup>

This nitrogen transport is often exacerbated by subsurface drainage lines that bypass the active riparian zones of agricultural streams.<sup>[9]</sup> The contribution of subsurface drainage to stream water nitrogen is a long recognized problem.<sup>[10–12]</sup> Many factors affect the level of nitrogen in drainage. For instance, drainage intensity has recently been reported by Skaggs et al.<sup>[13,14]</sup> to be a major factor in the loss of nitrogen from fields of both the Southeast and Midwest regions of the USA. Controlled drainage has been long known to reduce the loss of nitrogen from both drainage ditches and tile lines.<sup>[15,16]</sup> Nutrient management strategies can also be used to reduce nitrogen losses, but they can be insufficient in areas of high nitrogen loss potential.<sup>[17–20]</sup>

Thus, some type of additional treatment is needed to reduce the loss of nitrogen from agricultural drainage. Created wetlands have been used to effectively reduce nitrogen loading in portions of the Mississippi river basin.<sup>[21–23]</sup> They projected that created wetlands (about 5% of the watershed area) could reduce approximately half of the nitrogen losses. This is similar to 37% reduction in nitrogen export from an agricultural watershed in the southeastern Coastal Plain by an in-stream wetland which constituted 8% of the watershed.<sup>[24]</sup>

However, these portions of watersheds may often not be available for created wetlands. To decrease the amount of land required for nitrate removal, various types of

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bioreactors have been successfully used to treat drainage waters. These have generally been filled with some type of carbonaceous agricultural product that would provide reaction surfaces as well as carbon for microbial energy and enhanced anaerobic conditions.<sup>[25,26]</sup> A removal rate of 2.5 g NO<sub>3</sub>-N m<sup>-2</sup> d<sup>-1</sup> was reported for a wood-based reactor (surface area of 20  $m^2$  and a reactive media volume of 16 m<sup>3</sup>) by Van Driel et al.<sup>[26]</sup> Insight into broader potential use of these bioreactors can also be gleaned from their use in domestic septic systems for nitrate removal.<sup>[27]</sup> They reported nitrate removal rates greater than 85% for septic systems which used a porous wood-based filter called "Nitrex filter". The surface areas (m<sup>2</sup>)/volume (m<sup>3</sup>) ratios were 21/9, 310/108, and 470/120 with flow rates of 1, 7, and 18 m<sup>3</sup> d<sup>-1</sup>, respectively. The reaction rates were from 7.0 to > 10.0 g NO<sub>3</sub>-N m<sup>-3</sup> d<sup>-1</sup>. The wood-based media provided reaction sites as well as a slow release carbon source.

Ultimately, removal of nitrate from drainage waters in sensitive water locations and under land-limited conditions may require smaller reactors with even higher removal rates. In these conditions, reaction chambers with immobilized denitrifying sludge (IDS) and a source of soluble carbon may be very useful. Yang et al.<sup>[28]</sup> successfully used mixedculture-entrapped microbes to remove nitrate from brine wastewater. A high maximum removal rate,  $K_{MAX}$ , of 8.8 mg NO<sub>3</sub>-N g<sup>-1</sup> pellet  $d^{-1}$  was reported by Ryu et al.<sup>[29]</sup> for denitrification of polluted sea water in a suspended pellet reactor using a methanol and immobilized acclimated marine denitrifying sludge. Expressed as a 1 m<sup>3</sup> cubically shaped reactor volume, the rate was 4.0 kg NO<sub>3</sub>N m<sup>-3</sup>d<sup>-1</sup>. Thus, their reaction column had a removal rate about a thousand times higher than the wood bioreactor. These rates of nitrogen removal by IDS are also in the range of those reported for an advanced sewage treatment process using biological filtration, 0.4 to 0.7 kg NO<sub>3</sub>-N m<sup> $-\bar{3}$ </sup> d<sup>-1</sup>.<sup>[30]</sup>

The objectives of this investigation were (1) to prepare and immobilize an effective denitrifying sludge, (2) to evaluate the nitrate-N removal efficiency of the immobilized denitrifying sludge (IDS) in a column bioreactor; and (3) to determine the in-field performance of an IDS column bioreactor for nitrogen removal from agricultural drainage line water.

## Materials and methods

Investigations were conducted in 1998 and1999 at the Coastal Plain Soil, Water, and Plant Research Center and the Clemson Pee Dee Research and Education Center in Florence, SC.

## Culturing

Denitrifying bacteria were isolated from soil samples collected at an experimental overland flow wastewater treatment site in Duplin County, NC.<sup>[31]</sup> The bacteria were cul-

tured in 15 L polyethylene containers with a modified Wolfe growth medium.<sup>[32]</sup> Cultures were constantly mixed with an Arrow Model 350 stirrer (Arrow Engineering Co., Inc, Hillside, NJ). This provided sufficient oxygen to keep the oxidative reductive potential (ORP) in the denitrification range. Redox potential and pH were measured with an Orion Model 290A and a Model 210A meter (Thermo Electron Corp., Beverly, MA), respectively. The pH was  $6.79 \pm 0.25$ . The ORP was  $103 \pm 54$  mV, and the DO was  $0.25 \pm 0.26$  mg L<sup>-1</sup>. The growth medium was replaced weekly via a fill and draw method to obtain active mixed liquor suspended solids (MLSS).<sup>[29]</sup> After 28 weeks, the denitrifying efficiency of the bacteria culture was determined by treating a 345 mL solution containing 739 mg NO<sub>3</sub>-N L<sup>-1</sup>and 2218 mg methanol-C L<sup>-1</sup> with 40 ml of culture containing (0.0616 g MLSS mL<sup>-1</sup> – dry weight) for 24 h. Hourly samples were collected and analyzed for nitrate-N and nitrite-N with a Bran+Luebbe Auto Analyzer II (Bran+Luebbe, Roselle, IL) using US-EPA method 353.2-1.[33]

The sludge was encapsulated in polymer gels according to the PVA-freezing method.<sup>[34]</sup> Polyvinyl alcohol (PVA) was mixed with deionized water and heated to 95°C with a Castle Thermatic 60 steam sterilizer (Getings USA, Inc., Rochester, NY) to produce a 20% (weight basis) PVA solution. The solution was cooled to 40°C with continuous stirring by an Arrow Model 350 stirrer. For production of 10 batches of denitrifying pellets, the bacteria from the culture medium were concentrated by sedimentation for 1hr. After concentration, the sludge had a dry weight of 43  $\pm$ 14 g L<sup>-1</sup> and a wet weight of  $1003 \pm 25$  g L<sup>-1</sup>. One unit of the concentrated denitrifying bacteria was mixed with one unit of the PVA suspension on a wet weight basis. The PVAbacteria suspension was placed in Immulon MicroElisa (Dynatech Laboratories, Inc., Alexandria, VA) plates and frozen overnight at  $-4^{\circ}$ C. After rapid thawing, the capsules were removed from the MicroElisa plates, weighed, and placed in the modified Wolfe growth medium to maintain viability.

Pellets were produced at the rate of  $817 \pm 8$  g (wet weight) pellets/1000 g of sludge-PVA suspension (Fig. 1). They contained 26  $\pm$  8 mg sludge (dry weight) g<sup>-1</sup> pellets (wet weight). The average size of the pellets was 6-mm diameter by 10-mm length. The denitrification rates of the pellets were checked by both chemical and enzymatic methodologies. This provided information for (1) confirmation of biological denitrification; (2) comparison of chemical analysis with denitrification enzyme activity (DEA); and (3) comparison of sludge DEA to that of sludge from treatment wetlands. Denitrification enzyme activity was measured by the acetylene inhibition technique.<sup>[35,36]</sup> The nitrate-N solution was measured colorimetrically before and after incubation with the denitrifying bacteria by EPA method 353.2.<sup>[33]</sup> This comparison was done 11 times to establish the correlation between the two procedures.

 Table 1. Denitrifying efficiency of immobilized bacteria as influenced by nitrogen load, flow rates and methanol: N ratios in a laboratory study.

N concentration and load				NO3-N removal		
$mg NO_3 - N L^{-1}$	$mg NO_3$ - $N d^{-1}$	Flow rate mL min <sup>-1</sup>	Methanol/ N ratio	$mg NO_3$ - $N d^{-1}$	%	Efficiency mg N $g^{-1}$ pellet $d^{-1}$
7.5 <sup><i>a</i></sup>	38	3.5	219:1	24	64	0.09
$7^a$	43	4.3	143:1	28	66	0.10
$7.8^{a}$	180	16	58:1	90	50	0.35
$8^a$	184	16	5:1	105	57	0.42
$8^a$	184	16	6:1	94	51	0.39
$10^{a}$	230	16	20:1	147	64	0.57
$8^a$	317	28	14:1	159	50	0.61
$10^a$	432	30	16:1	138	32	0.53
$8^a$	691	60	4:1	435	63	1.66
$12^{a}$	1037	60	2:1	425	41	1.64
$30^{b}$	1296	30	4:1	868	67	2.03
$12^{a}$	1866	108	3:1	709	38	2.68
$95^{b}$	1915	14	5:1	689	36	1.60
100 <sup>c</sup>	2304	16	3:1	1359	59	1.55
$82^{b}$	3995	34	5:1	919	23	2.14
98 <sup>b</sup>	4939	35	5:1	1435	29	3.33

<sup>*a*</sup>262 g IDS pellets, 1 reactor.

<sup>b</sup>430 g IDS pellets, 4 parallel reactors.

<sup>c</sup>877 g of pellets, 4 reactors.

## Laboratory studies

The laboratory studies were conducted in 1998 and 1999 to evaluate the denitrifying efficiency of immobilized bacteria in relation to varying nitrate-N loading rates and methanol rates (Table 1). Immobilized denitrifying bacteria (262, 430, and 877 g) were placed into polyvinyl chloride cylinders (10-cm diameter  $\times$  40-cm length; 3.14 L). Some loading conditions used a single IDS column bioreactor and some had four parallel IDS column bioreactors; the number of reactors is listed in Table 1. For this experiment, a nitrate-N solution was prepared by passing deionized water through



Fig. 1. A picture of the immobilized denitrifying sludge.

a sand column to provide water that was similar to drainage water. It was enriched with NO<sub>3</sub>-N in the form of KNO<sub>3</sub>. The nitrate-N was loaded at rates from 38 to 4939 mg NO<sub>3</sub>-N d<sup>-1</sup>. The loading rate was varied by changing the concentrations and flow rates. A carbon source, methanol, was added at methanol-C to NO<sub>3</sub>-N ratios of 2:1 to 219:1.

The solution was pumped into the column bottoms via Masterflex (Barnant Co., Barrington, IL) peristaltic pumps. The solution was allowed to flow upward through the denitrifying capsules and exit at the top of the column. The whole column was not fully occupied by pellets, and they were fluidized within the reactor. This meant that there was space for additional pellets and treatment. However, for treatment efficiency, we made the conservative assumption that the entire volume of the reactor was involved in the nitrogen removal. The nitrate-N concentration of the inflow and outflow was measured with a Bran+Luebbe Auto-Analyzer II. Redox potential and pH were measured with an Orion Model 290A and a Model 210A meter, respectively. This study was conducted for 44 days. Flow rates, nitrate-N concentrations, and methanol to nitrate-N ratios were varied as presented in Table 1. The effluent water had a dissolved oxygen concentration of  $0.25 \pm 0.50$  mg L<sup>-1</sup>, and the oxidative reductive potential (ORP) was  $-10 \pm 19$  mV. The pH was  $6.47 \pm 0.07$ .

## Field bioreactor prototype

One of the columns used in the laboratory investigation was relocated to the Clemson University Pee Dee Research and

NO NO<sub>2</sub>

- NO<sub>v</sub>

N concentration and load Flow rate Hydraulic retention time NO<sub>3</sub>-N removal  $mg NO_3-NL^{-1}$  $mg NO_3-N d^{-1}$  $mL min^{-1}$ days % $7.52 \pm 0.10$  $38 \pm 1$ 3.5 0.623  $98 \pm 2$  $6.87\pm0.20$  $42 \pm 1$ 4.3 0.513  $98 \pm 1$  $87 \pm 1$ 7.7  $89 \pm 5$  $7.85\pm0.14$ 0.283  $179 \pm 2$  $76 \pm 14$  $7.78 \pm 0.11$ 16 0.136  $190 \pm 4$  $83 \pm 12$  $8.38 \pm 0.68$ 16 0.136  $6.45 \pm 0.28$  $334 \pm 15$ 36 0.061  $49 \pm 4$ 

Table 2. Denitrifying efficiency of immobilized bacteria as influenced by nitrogen load, flow rates and methanol:N ratios in field study.

Education Center for the field investigation. A schematic of the system is presented in Figure 2. The column bioreactor contained 400 g of immobilized bacteria. Water from a field drainage tile was captured in a 15-L polyethylene container and subsequently pumped through the column bioreactor via a peristaltic pump. The drainage line water contained  $7.8 \pm 0.59$  mg NO<sub>3</sub>-N L<sup>-1</sup>. Inflow rates to the bioreactor varied from 3.5 to 36.0 mL min<sup>-1</sup> (Table 2). These flow rates constituted bioreactor hydraulic retention times of 0.05 to 0.60 d. A methanol solution (180 mg methanol  $L^{-1}$ ) was added at methanol:nitrate ratios of 2:1, 10:1, and 20:1 to both promote anaerobic conditions and provide an energy source for the denitrifying sludge.

Redox potential and pH were measured with an Orion Model 290A meter and an Orion Model 210A meter, respectively. The effluent dissolved oxygen content was  $1.07 \pm 0.66$ mg L<sup>-1</sup>, and the ORP was  $-53\pm80$  mV. The pH was  $6.21\pm$ 0.23. Sampling rate for the inflow and outflow of the column bioreactor was 200 mL h<sup>-1</sup>. Samples were composited ev-

**PVC** Pipe (10 x 40 cm) **Peristalic Pump** IDS Drainage Tile Line **Methanol Injection** 

Fig. 2. A schematic of the field IDS bioreactor for removal of nitrogen from agricultural drainage line water. (Hydraulic retention time 0.05 to 0.6 d and NO<sub>3</sub>-N concentrations of 7.8 mg  $L^{-1}$ .)

ery four hours and acidified to pH 2 with H<sub>2</sub>SO<sub>4</sub>. They were collected from the ISCO automated samplers, transported to the laboratory, and stored at 4°C. The nitrate-N concentration of the samples was measured with a Bran+Luebbe Auto-Analyzer II. This study was conducted for 36 days.

## **Results and discussion**

1250

1000

750

500

250

0

0

1

Concentration (mg  ${
m L}^{-1}$ )

(15 L)

#### Bacteria cultures and immobilized pellets

The denitrifying sludge was very effective in removing nitrate from the test solution which contained both nitrate and methanol (Fig. 3). The nitrate was initially converted to nitrite, and the conversion of nitrite to di-nitrogen was slower than nitrite production. Thus, there was an initial accumulation of nitrite followed by its complete removal. The availability of nitrite in the system may allow for even more effective denitrification via immobilized autotrophic denitrifiers which would not require methanol.<sup>[37]</sup> The potential use of this nitrite in future systems will be noted later in the paper.

The specific denitrification rate of the bacteria culture was 11.4 mg NO<sub>3</sub>-N  $g^{-1}$  MLSS  $h^{-1}$ (Fig. 4). These reaction rates were  $10^3$  greater than the denitrification rate of floating sludge of constructed wetlands that treated swine



Time (hr)

4

5

6

3

2



1080



Fig. 4. Reaction rate of the denitrifying sludge MLSS ( $R^2 = 0.82$ ; the specific rate of activity for nitrate removal was 11.4 mg NO3-N  $gm^{-1}$  MLSS  $h^{-1}$ ).

wastewater.<sup>[36,38,39]</sup> They were nearly 10<sup>4</sup> greater than the denitrification of the wetland detritus layer and 10<sup>5</sup> greater than denitrification of the wetland soil.<sup>[38]</sup> The reaction rate was in the range observed by Tchobanoglous and Burton.<sup>[40]</sup> Additionally, it was similar to that reported for the denitrifying sludge used by Ryu et al.<sup>[29]</sup> (16.1 mg NO<sub>3</sub>-N g<sup>-1</sup> MLSS  $\tilde{h}^{-1}$ ).

The immobilized denitrification sludge pellets produced in our lab had similar denitrification reactivity when measured by either chemical or enzymatic methodologies ( $R^2$ = 0.94; Chemical N removal = 0.94 DEA + 0.08). This verified that the nitrogen removal was via denitrification. Thus, we were successful in the first objective to culture and immobilize an effective denitrifying sludge.

#### Laboratory bioreactor reaction kinetics

The principal goal of these experiments was to obtain denitrification kinetics information for the IDS pellets in bioreactor columns using different NO<sub>3</sub>-N loading rates and concentrations (Table 1). The varying conditions were as follows: (i) flow rates ranged from 3.5 to 108 mL min<sup>-1</sup>; (ii) influent concentrations of NO<sub>3</sub>-N ranged from 7 to 100 mg  $L^{-1}$ ; and (iii) nitrogen load ranged from 38 to 4,939 mg NO<sub>3</sub>-N d<sup>-1</sup>. The nitrogen removal efficiency ranged from 23 to 67%, and the specific reaction rates of the IDS pellets ranged from 0.09 to 3.33 mg NO<sub>3</sub>-N  $g^{-1}$  pellet  $d^{-1}$ . Methanol ratios of 3:1 seem to be sufficient for denitrification at all of the loading rates.

A good fit was obtained ( $R^2 = 0.89$ ) for the regression of specific denitrification rate of the IDS pellets vs. the bioreactor nitrogen load rate using the Monod kinetic mathematical form (Fig. 5). Two kinetic parameters of the Monod kinetic equation (i.e.,  $K_{MAX}$  and  $K_S$ ) were estimated. This was obtained by regression which solved for both  $K_{MAX}$  and  $K_S$  simultaneously via non-linear regression analysis using GeoPad Prism (GeoPad Software Inc., CA). The KMAX was



Fig. 5. Specific reactivity vs nitrogen loading rate for IDS column bioreactors in a laboratory investigation ( $R^2 = 0.89$ ).

3.64 mg d<sup>-1</sup> and the  $K_{\rm S}$  was 1298 mg d<sup>-1</sup>. The equation for specific denitrification removal (SDR) is presented below in equation 1.

$$SDR = \frac{k_{MAX} \cdot N}{k_S + N} = \frac{3.64 \cdot N}{1298 + N}$$
 (1)

where

- N = N loading rate mg NO<sub>3</sub>-N d<sup>-1</sup>.  $K_{MAX} = 3.64$  mg NO<sub>3</sub>-N g<sup>-1</sup> pellet d<sup>-1</sup>;maximum specific denitrification rate.
- $K_{\rm S} = 1298 \text{ NO}_3$ -N mg d<sup>-1</sup>;half-saturation constant based on the N load.

The  $K_{\text{MAX}}$  value for specific reactivity was lower than the 8.84 mg NO<sub>3</sub>-N g<sup>-1</sup> pellet d<sup>-1</sup> reported by Ryu et al.<sup>[29]</sup> Nonetheless, the reaction rate was sufficient to be very relevant to drainage water nitrogen treatment. For instance, if the  $K_{\rm S}(1298 \text{ NO}_3\text{-N mg d}^{-1})$  for this 3.14 L-reactor was scaled to a cubic meter, the load would be 0.41 kg NO<sub>3</sub>-N  $d^{-1}$ . This rate of nitrogen load would be significant for typical agricultural drainage water.<sup>[41]</sup>

## Field bioreactor performance

The efficiency of nitrate removal in natural systems or constructed bioreactors depends on many factors such as denitrification rate, nitrate and carbon concentrations, temperature, liquid flow rate, and bioreactor size.<sup>[21,24-26,36,39]</sup> Relative to carbon and nitrogen concentration, all 3 of the methanol:nitrate concentrations (2:1, 10:1, and 20:1) seemed to function adequately. However, pushing the ratio lower could result in either lower denitrification or potentially incomplete denitrification.<sup>[42]</sup>

When considering the removal of nitrate from tile drain water, a design factor of paramount importance is hydraulic



**Fig. 6.** Nitrogen removal efficiency vs hydraulic retention time for an agricultural drainage line effluent bio-reactor containing IDS reactor ( $R^2 = 0.56$ ; drainage water contained 7 to 8 mg L<sup>-1</sup> nitrate-N; bioreactor 3.14 L with 430 g IDS pellets).

retention time (HRT). When the HRT was > 0.3 d, the removal efficiency approached 100% (Fig. 6).

$$HRT = \frac{V}{Q} \tag{2}$$

where

HRT = Hydraulic retention time (T), V = volume of bioreactor (L<sup>3</sup>), Q = volumetric flow rate (L<sup>3</sup>T<sup>-1</sup>).

The nitrogen removal efficiency (NRE) curve shown in Figure 6 is nonlinearly fitted as a saturation type with HRT as a system variable.

NRE = 
$$108(HRT)/(0.05 + HRT); R^2 = 0.56$$
 (3)

Although the bioreactor achieved nearly 100% removal efficiency at longer HRTs, this would not be where the bioreactor would be most effectively used to reduce drainage water nitrogen from an agricultural watershed. Long HRTs would necessitate larger bioreactors for typical flow rates from the drainage tile. However, a system could be operated at lower HRT with lower removal efficiencies while still fitting nicely into the overall farm nutrient management plan. For instance, the goal might be to remove approximately 50% of the nitrate as reported for treatment wetlands.<sup>[22-24]</sup> The 50% removal would require, according to equation 3, a HRT of 0.044 d ( $\sim$ 1 h). Thus, a bioreactor of 1 m<sup>3</sup> would be required to treat 1 m<sup>3</sup> of drainage water in 1 h. This HRT would likely be very useful for much of the base and storm flow. For instance, Jaynes and Colvin<sup>[41]</sup> reported tile drain discharges during 2002 to 2005 of 0 to 140 m<sup>3</sup> d<sup>-1</sup>for tile drains in an Iowa field with corn and soybean. On a hourly basis, the discharges would be 0 to  $5.8 \text{ m}^3 \text{ h}^{-1}$ .

Based on the field investigation, a 1 m<sup>3</sup> bioreactor would have removed 50% of 7.8 mg NO<sub>3</sub>-N L<sup>-1</sup> in the drainage water in 1 h (3.9 g of NO<sub>3</sub>-N d<sup>-1</sup>). If we assume a cube shape for the bioreactor, the bioreactor footprint would be 1 m<sup>2</sup>. Its daily nitrogen removal rate would be 94 g NO<sub>3</sub>- N m<sup>-2</sup>d<sup>-1</sup>. This rate of treatment is dramatically higher than those reported for in-stream wetlands, treatment wetlands, or wood-based bioreactors.<sup>[21,24,26]</sup> As such, the IDS bioreactors offer the potential for requiring small bioreactor footprints, treating nitrate concentration hot spots, and treating moderate storm flows. Furthermore, the reaction rate is conservative relative to the potential for IDS reactors. The bioreactor pellet content could likely be doubled while still maintaining good fluidity within the reactors (one of the laboratory reactors had this content).

The research results reported in this paper indicate that bioreactors with IDS can be used to reduce the nitrogen loading from drainage lines. However, this field experiment provided performance data of a small reactor during a short time period. Full size testing is needed to address aspects such as extreme drought or flows from prolonged storm patterns. Moreover, information is needed on how IDS reactors can be best used in combination with technology such as good nutrient management plans, controlled drainage, treatment wetlands, and passive carbonaceous reactors.<sup>[16,22,25,26,41,43]</sup>

There is good reason to be optimistic that future research will produce even more effective lower cost reactors. One promising area is the immobilization and simultaneous use of both denitrifying heterotrophic and autotrophic sludges. Sumino et al.<sup>[37]</sup> has reported the enhanced denitrification by the inclusion of both immobilized anaerobic ammonia oxidization (ANAMMOX) sludge and heterotrophic sludge. Their wastewater contained both nitrate and ammonia; the heterotrophic sludge both consumed nitrate and produced nitrite. Thus, they had both the nitrite and ammonia needed for effectively autotrophic denitrification by the ANAMMOX. The IDS of our study also produced nitrites as shown in Figure 3. In the case of drainage water, ammonia would also be needed for the ANAMMOX reaction. The possibility of such ammonia production via dissimilatory nitrate reduction to ammonium by ANAMMOX bacteria has recently been reported by Kartal et al.<sup>[44]</sup>

If this phenomenon could be bioengineered into an ANAMMOX-IDS, it would allow for a very effective reactor design which would be similar to that of Sumino et. al.<sup>[37]</sup> Such a system could be used for effective removal of nitrate-N from drainage line water with a lower need for a carbon source. Thus, our research indicates that 1) bioreactors with IDS can be used to remove nitrate-N from drainage water, and 2) more research is needed to devise effective strategies for their implementation with other emerging technologies for improved water quality on both watershed and basin scales.

## Conclusions

An effective IDS was prepared by use of PVA and a denitrifying sludge that had a specific denitrification rate of 11.4 mg NO<sub>3</sub>-N  $g^{-1}$  MLSS  $h^{-1}$ . When placed in a column bioreactor and loaded with variable nitrate-N, the IDS had a  $K_{MAX}$  of 3.64 mg N  $g^{-1}$  pellet  $d^{-1}.$  The  $K_S$  value was 1298 mg NO<sub>3</sub>-N d<sup>1</sup>. When an IDS column bioreactor (3.14 L) was used to treat agricultural drainage line water with a NO<sub>3</sub>-N concentration of 7.8 mg  $L^{-1}$  and HRT values of 0.05 to 0.6d, it removed from 50 to 99% of the NO<sub>3</sub>N. A 1 m<sup>3</sup> bioreactor would remove 50% of 7.8 mg NO<sub>3</sub>-N  $L^{-1}$  in the drainage water in 1 hr (3.9 g of NO<sub>3</sub>-N). With a cube shape for the bioreactor, daily nitrogen removal rate would be 94 g NO<sub>3</sub>-N m<sup>-2</sup>d<sup>-1</sup>, which is dramatically higher than treatment wetlands or solid passive carbonaceous bioreactors. IDS bioreactors offer potential for reducing nitrogen discharge from agricultural drainage lines especially if employed in combination with technologies such as good nutrient management plans, controlled drainage, treatment wetlands, and passive carbonaceous reactors. More research is needed to develop the bioreactors for agricultural use and to devise effective strategies for their implementation with other emerging technologies for improved water quality on both watershed and basin scales.

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