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Development of the slowly released molasses barrier system for controlling nitrate plume in groundwater

고체당밀로 구성된 관정형 반응벽체와

종속영양탈질을 이용한 지하수 내

질산염 제거에 관한 연구

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Development of the slowly released molasses barrier system for controlling nitrate plume in groundwater

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Abstract

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This study was objected to identify the applicability of the well-type slowly released molasses barrier system (SRM system) as an *in situ* remedial technique to treat the nitrate-contaminated groundwater by the denitrifying activity of heterotrophic microbes. A SRM material, a solidifying molasses, was made using a molding technique by mixing the liquid phase molasses with paraffin wax, cellulose, and silica sands. This SRM material can continuously release molasses as a carbon source for indigenous heterotrophic denitrifiers over relatively long periods by the diffusion process with decreased release rates when it is placed into groundwater passing through. The developed SRM system could continuously deliver molasses into the groundwater over an extended period of time. Therefore, the SRM system could be an attractive long-term nitrate treatment option, showing nitrate removal efficiencies were estimated to be fairly high as ~85% and ~84% for nitrate of 89 and 142 mg L^{-1} in the column- and pilot-scale experiments. The

removal efficiencies in the field experiment were relatively moderate at ~43% for nitrate of 320 mg L^{-1}. Moderate nitrate removal efficiency in the field might have been caused by the heterogeneity/anisotropy of the aquifer and insufficient molasses dispersion to the denitrifiers. From result of the PCR-DGGE series, nitrite reductase gene fragments were amplified from uncultured isolates in pilot- and field soils, indicating a heterotrophic denitrifying capability in soil is common. Thus, the SRM system can be possibly applied into the nitrate contaminated groundwater with minor consideration for the existence of the denitrifying microbes in soils. Although many constraints related to the heterogeneous nature of aquifers exist, the SRM system can be a useful tool for control of dilute, large, and shallow nitrate contaminated groundwater plume. For achieving on-site remedial goals of the nitrate-contaminated groundwater in the targeted field, changes in the mixing rate of SRM constituents or its volume to prolong effective longevity can be readily modified. Further, the number and shape of the SRM barrier and the array of the SRM rods per a barrier can be changed for attaining remedial goals of the targeted contaminated field.

Keywords: molasses, nitrate, SRM system, heterotrophic denitrification, removal efficiency

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Contents

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION

1.1 Background

Nitrate contamination on shallow groundwater has been found in many parts of rural area worldwide since the last few decades due to the excessive usage of animal manures and nitrogenous fertilizers to enhance crop yields and the land disposal of domestic wastewaters. In Korea, groundwater pollution by nitrate has become increasingly serious. According to statistics of groundwater quality data managed by the Ministry of Environment (MOE, www.me.go.kr), Korea, approximately 22% of contaminated groundwater samples collected from the national groundwater quality monitoring stations was found to be mainly polluted with nitrate, where the effects of human inputs of nitrogen could quickly appear in groundwater through the permeable soils.

Nitrate does not adsorb onto the soil media due to its negative charge property so that it can migrate as the non-reactive solute in groundwater. Therefore, it can diffuse easily in the subsurface environment, resulting in groundwater contamination. Natural nitrate concentrations originated from the soil nitrogen range from 9 to 13 mg L^{-1} (Mueller and Helsel, 1996). If nitrate concentrations are detected more than natural ones in groundwater, it indicates the groundwater quality is affected by human activities. Nitrate can

causes methemoglobinemia in infants and poses other health-related problems (Bouchard et al., 1992) in rural populations who depend on shallow groundwater for water supply. Further, the discharge of nitrate-contaminated groundwater into wetlands, rivers, estuaries, and the coastal environment can contribute to toxic algal blooms in these water bodies that can also cause health problems (Appleyard and Schmoll, 2006).

Conventional technologies such as ion exchange, reverse osmosis, electrodialysis, and distillation are available for treating nitrate from groundwater. However, such technologies are mechanically complex, require periodical maintenance, and generally cost-prohibitive so that those are inhibited to treatment (Moon et al., 2008). On the other hand, biological methods are widely utilized, due to the high solubility and the small trend to coprecipitation and adsorption of the nitrate, which reduces the elimination efficacy with the conventional physicochemical methods (Garcia and Becerril, 1993). Biological denitrification is a process in which the oxidised nitrogen substances, i.e. nitrates and nitrites, are reduced to nitrogen gases, such as N_2O and N_2 (Mora et al., 2003). This method can achieve that process by means of the mechanism of the bacteria. Consequently, nitrate and nitrite replace molecular oxygen as electron acceptors. Thus, these bacteria do not need strict anaerobic conditions, and they can grow more rapidly and adequately when a denitrifying culture is inoculated in aerobic environments. Aerobic growth of the culture results in oxygen consumption and leads to initiation of the denitrifying process, nevertheless, when there is an abundant source of carbon and strict anaerobic conditions, this process mainly results in

the production of ammonia, instead of other gases (Cole, 1991). As a consequence, efficient biological denitrification requires availability of an adequate ratio of carbon to nitrogen and easily degradable carbon sources.

Carbon sources serve two purposes (Schipper et al., 2005): first to reduce the oxygen concentration of the groundwater by stimulating aerobic respiration, and second to provide a carbon to denitrifying bacteria. Many commercially available organic compounds, such as acetic acid (Her and Huang, 1995; Mohseni-Bandpi et al., 1999), glucose (Chou et al., 2003) and methanol (Louzeiro et al., 2002), can serve effectively as carbon sources for denitrification. Of these, methanol is the most commonly used external carbon source due to its cost effectiveness (Louzeiro et al., 2003). However, there is certainly a need to find economical alternative carbon sources to maintain the desired effluent quality. Many researchers have suggested various by-products or waste materials as alternative carbon sources. For example, wine distillery effluents (Bernet et al., 1996), the leachate of food waste (Lee et al., 2002), swine waste (Lee et al., 1997) and hydrolyzed sludge (Æsøy and Ødegaard, 1994; Aravinthan et al., 2001; Barlindhaug and Ødegaard, 1996). Another option that can be considered is the use of molasses. Molasses is a sugar production by-product with high sugar content (48–50%). This by-product is a cheap carbon source used for various industrial fermentations (Miranda et al., 1996; Najafpour and Shan, 2003). Some researchers have used molasses as an external carbon source for denitrification (Boaventura and Rodrigues, 1997; Ten Have et al., 1994).

Permeable reactive barrier system (PRB system) has been used to

treat nitrate contamination from a variety of sources including septic systems, agricultural runoff, landfill leachate, and industrial operations (Robertson et al., 2000). PRB system has the potential to provide complete single-pass nitrate removal using materials that are low cost and, in most cases, locally available. And PRB system can be a successful option to achieve long-term, passive, and in situ attenuation of nitrate. PRB system has proved successful for nitrate removal in a wide range of location including Canada (Robertson and Cherry, 1995; Robertson et al., 2000), New Zealand (Schipper and Vojvodić-Vuković, 1998, 2000, 2001), and Australia (Fahrner, 2002). In PRB system, particulate organic matter is incorporated into subsoil below the water table to enhance denitrification. In those studies, PRB system using cellulose solids (wood mulch, sawdust, leaf compost, cotton burr compost, and sediment) as carbon sources has been tested as a means to remove nitrate from shallow groundwater, indicating high removal efficiency for nitrate in groundwater.

In this study, we developed a well-type reactive barrier system containing slowly-released molasses (SRM) as a reactive material to promote indigenous denitrifying activity as a new semi-passive, and in situ remedial option for treating nitrate in groundwater. To demonstrate this SRM scheme, a laboratory scale column experiment, and pilot scale flow-tank experiments, model applications, and field applications are performed. Results of this study will suggest that the SRM system may provide a practical tool for a long-term treatment option of nitrate plume in groundwater and provide useful information for optimum design and operation of SRM system in practice.

1.2 Objectives

The principal objectives of this dissertation to characterize molasses release pattern from SRM system and identify the effective longevity of SRM system, and describe the nitrate removal efficiency by applying SRM system are as follows:

i. To develop a solidifying molasses called as SRM (slowly released molasses) for a continuous and long-term supply of a carbon to the nitrate contaminated groundwater in aquifer media
ii. To develop a well-type barrier system harboring SRM rods (SRM system) for removing nitrate in groundwater
iii. To verify a long-term molasses release characteristics of a SRM rod with a capability for microbiologic nitrate removal in lab scale experiments
iv. To characterize molasses release pattern of the SRM system in large scale tank experiments and identify the effective longevity by applying a numerical model
v. To determine heterotrophic nitrate removal efficiency by applying the SRM system in large scale tank experiments and identify nitrite reductase genes using a PCR-DGGE technique
vi. To demonstrate a field application result of the SRM system and discuss optimum design and operation of the SRM system in practice

1.3 Dissertation structure

This dissertation consists of seven chapters (Fig. 1-1). Following this introduction, chapter 2 gives a literature review on previous studies. Chapter 3, 5, and 6 present results from lab-, pilot-, and field-scale experiments performed for the objectives of this study, respectively. Chapter 4 covers the simulation providing the effective longevity of SRM system by applying a numeric modeling. The summary of entire thesis, conclusions, and recommendations are described in Chapter 7.

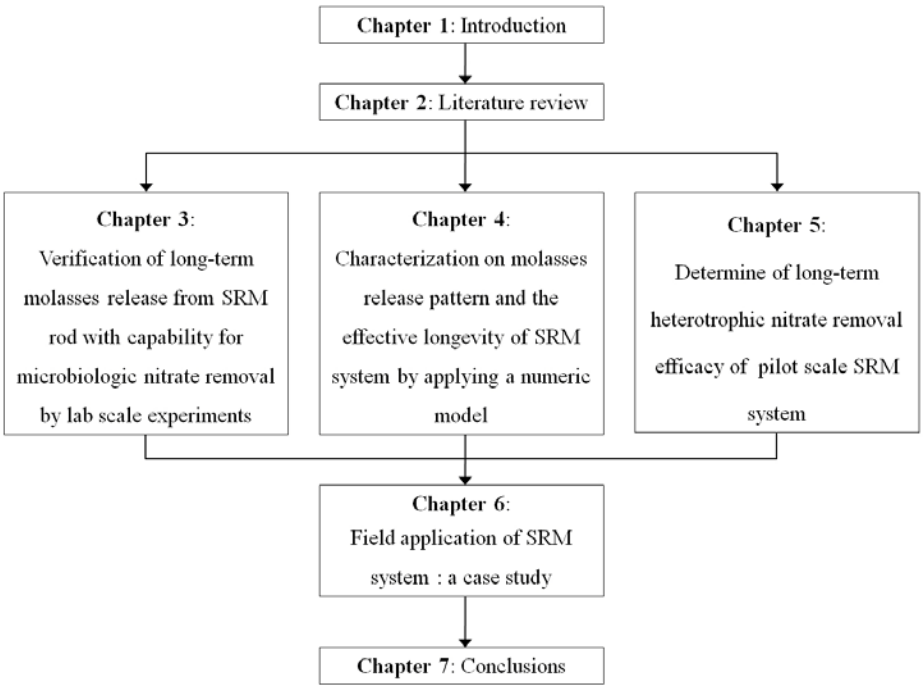

Fig. 1-1. A schematic diagram of the dissertation structure for the SRM system using the heterotrophic denitrification.

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CHAPTER 2

LITERATURE REVIEW

2.1 Organic compounds for denitrification

To enhance biologic nitrate removal in groundwater, some selected studies have suggested commercially available organic compounds such as acetic acid, glucose, ethanol, and methanol (Her and Huang, 1995; Kapoor and Viraraghavan, 1997; Mohseni-Bandpi et al., 1999; Louzeiro et al., 2002; Chou et al., 2003). Even though those materials could successfully induce high nitrate removal efficiency, relatively high price hindered their application to treatment. In order to reduce such costs, other options like biomaterials and liquid organic wastes were considered. In this section, we review some selected manuscripts discussing the usage of the organic compounds for the denitrification.

2.1.1 Sawdust

For treating seepage waste water from septic system, sawdust was used as the reactive material which promotes nitrate attenuation by heterotrophic denitrification (Robertson and Cherry, 1995, 2000). An alternative septic-system design was presented utilizing reactive porous media barriers for passive in situ attenuation of nitrate. Four field trials were

discussed demonstrating two barrier configurations: as a horizontal layer positioned in the vadose zone below a conventional septic-system infiltration bed and as a vertical wall intercepting a horizontally flowing downgradient plume. During one year of operation both barrier configurations have been successful in substantial attenuation from 60 to 100% of input nitrate levels of up to 125 mg N L^{-1}. The horizontal layer configuration could be readily installed during the construction of new infiltration beds, whereas the vertical wall configuration might be more appropriate for retrofitting existing septic systems where nitrate contamination has already occurred. The layer configuration allowed the flexibility of constructing the barrier in the vadose zone by using coarse silt or fine sand matrix material that had the ability to remain tension-saturated, and thus anaerobic, even when positioned above the water table. Advantages of the barrier system were that it was simple to construct, no surface structures or additional plumbing are necessary, and treatment was passive requiring no energy consumption and little or no maintenance. Mass balance calculations and preliminary results suggested that conveniently sized barriers had the potential to last for decades without replenishment of the reactive material.

Schipper and Vojvodić-Vuković (1998, 2000, 2001, 2005) examined the mechanisms by which a denitrification wall removed nitrate from shallow groundwater. The denitrification wall was constructed by digging a trench (35 m length, 1.5 m depth, and 1.5 m width) that intercepted groundwater. The excavated soil was mixed with sawdust (30% v/v) as a C source then returned to the trench. They assessed nitrate removal and

denitrification in the wall for 1 yr. Incoming concentrations of nitrate in groundwater ranged from 5 to 16 mg N L^{-1} but these decreased to <2 mg N L^{-1} in the denitrification wall. Total N in the wall declined during the year demonstrating that N immobilization was not a large sink for nitrate. Denitrifying enzyme activity (DEA) reached a maximum of 906 ng N L^{-1} h^{-1} after 6 mo of operation, indicating that denitrification was an important mechanism for nitrate removal. We calculated a maximum rate of nitrate, removal by denitrification of 3.6 g N m^3 d^{-1}. Substrate-amendment experiments showed that denitrification in the wall was primarily limited by nitrate concentration and not C. During the study there was no significant decrease ($P < 0.05$) in total C but the availability of the remaining C declined. Despite this decrease, the DEA and microbial biomass were stable during the last 6 mo.

2.1.2 Natural calcareous materials

Ashan et al.(2001) and Ashan and Türkman (2003) conducted denitrification tests to assess removal and filtration capacity of waste and natural indigenous materials as treatment mediums e.g., shell, limestone, waste paper mixed with refuse concrete, refuse cement, also processed nitrolite, charcoal-bio and charcoal. Under room temperature condition removal of phosphoric, nitric and ammonium-ions, filtration of suspended substance together with removal of COD in waste water was investigated. Influence of particle size effect for all treatment mediums except for waste

paper was pursued. Significant improvement of waste water quality with respect to SS, phosphate ions and decrease in COD was possible by treating with these filtration mediums. With specific reference to some treatment mediums nitrate and ammonium showed reasonable improvement in quality, although generally removal effect was not very significant. Efficacy of treatment was dependent on the particle size of treatment mediums in general, however, nitrolite for ammonium, charcoal-A for SS and COD, refuse cement mixed with waste paper for phosphate ion removal showed insignificant variability on the particle size effect.

2.1.3 Wood chips

Gilbert et al. (2008) examined the denitrifying permeable reactive barrier system to select a suitable natural organic substrate as a potential carbon source. A number of seven organic substrates including softwood, hardwood, coniferous, mulch, willow, compost, leaves, and native soil were first tested in batch tests. The materials attained varying degrees of success at promoting denitrification. Some of the organic substrates such as softwood and cotton performed very well, achieving complete nitrate removal (>98%), while others were considered unsuitable for a variety of reasons, including insufficient nitrate or nitrogen removal, excessive release of leachable nitrogen from the substrate or excessive reduction of nitrate to ammonium rather than removing it as gaseous N_2. The top performing substrate in terms of denitrification extent (>98%) and rate was then selected

for two bench-scale column experiments in an attempt to simulate the PRB. The inlet concentration was 50 mg dm^{-3} nitrate and the columns operated at two different flow rates. The two columns showed different general patterns, making it clear that the flow rate was a key factor at the nitrate removal. Nitrate was completely removed (>96%) by the passage through Column 1, while only partially removed in Column 2 (66%). The results indicated that softwood was applicable for further use as a filling material for a PRB.

2.1.4 Organic acids (sodium acetate)

The effects of external carbon source and empty bed contact time on denitrification efficiency during simultaneous heterotrophic and sulfur-utilizing autotrophic denitrification were evaluated (Lee et al., 2001). Continuous experiments were conducted with up-flow mode sulfur packed bed reactors (SPBRs) fed with nitrified leachate containing 700–900 mg N L^{-1}. The fraction of nitrate nitrogen removed by heterotrophic denitrification ($HDNR_{fraction}$) for alkalinity production to balance the alkalinity consumption by autotrophic denitrification varied with the type of external carbon source. When sodium acetate was added at $HDNR_{fraction}$ values of 44%, 100% denitrification was achieved without alkalinity addition. The maximum nitrogen removal rate was 5.05 kg N m^{-3} d^{-1} observed with 89% removal efficiency. At short HRT, a clogging problem was observed near the bottom of the SPBR with excess growth of heterotrophic denitrifiers and gas accumulation within the pores of the SPBR.

2.1.5 Molasses

Molasses is a viscous, dark-brown liquid by-product. It is produced while raw cane juice is refined into white sugar, and easily obtained from a lot of sugarcane refineries. In some selected studies, molasses, a cheap sugar-industry waste (~1 L/$), was recommended as a reliable carbon source due to rapid destruction of nitrate, high removal efficiency comparable to the process using methanol, and many kinds of denitrifying microbes using it (Ten Have et al., 1994, Boaventura and Rodrigues, 1988, 1997; Mora et al., 2003; Quan et al., 2005; Ueda et al., 2006; Hamlin et al., 2008; Roy et al., 2010).

Ten Have et al. (1994) demonstrated that full denitrification proved to be impossible with supernatant from manure as the only carbon source under the circumstances tested. Therefore, molasses was used as an extra carbon source. The minimum amount of molasses decreased with increasing predenitrification / nitrification ratio and decreasing recycle factor.

Boaventura and Rodrigues (1988, 1997) examined the fluidized-bed biological reactor for wastewater denitrification. Sand particles were the biofilm support and molasses the carbon source. Denitrification kinetics of a synthetic substrate containing molasses was studied in a rotating disk biofilm reactor. Experimental studies were carried out in order to understand the reactor start-up, changes of biofilm density with biofilm thickness, reactor hydrodynamics and axial concentration profiles of nitrate and nitrite species in the bed. A simple model was developed based on a reaction scheme nitrate >

nitrite > products of two consecutive zero-order reactions. The model also includes mass transport of nitrate and nitrite species by diffusion inside the biofilm. These situations can occur the biolilm is fully penetrated by both species, the biofilm is partially penetrated by nitrate and fully penetrated by nitrite, and the biofilm is partially penetrated by both species. Model calculations of axial concentration profiles show a good agreement with experimental data. A methodology is suggested for the design of fluidized-bed biological reactors including the following steps to get biomass growth rate and denitrification kinetics from batch systems, to check denitrification kinetics and obtain diffusivity measurements and biofilm density as a function of biofilm thickness in a rotating-disc biofilm reactor, and to use previous basic information in the simulation of fluidized-bed biological reactors.

Mora et al. (2003) performed evaluation of sequential batch reactors (SBR) treating sewage, through a process of endogenous biological denitrification. Different operational conditions were carried out, and the behavior under the effects of organic shock loading was examined. Three laboratory scale reactors were operated simultaneously and fed with similar wastewater. The substratum was molasses and nitrate, as carbon and nitrogen sources, respectively. The three reactors were operated during different aeration periods (0, 15 and 30 min). Sudden changes in organic matter concentration were performed during the experiment. Thus, influent load was quickly increased threefold in relation to the original concentration. Results indicated that SBR reactors withstand adequately moderate shock loading. With regard to substratum degradation, nitrate elimination achieved was

approximately 80%, while denitrification rate was approximately 0.87 mg g^{-1} h^{-1}.

Quan et al. (2005) demonstrated that the possibility of hydrolyzed molasses as an alternative carbon source in a biological nitrogen removal process. To increase biodegradability, molasses was acidified before thermo-hydrolyzation. The denitrification rate was 2.9–3.6 mg N g^{-1} VSS h with hydrolyzed molasses, in which the percentage of readily biodegradable substrate was 47.5%. To consider the hydrolysate as a carbon source, a sequencing batch reactor (SBR) was chosen to treat artificial municipal waste water. During the 14 days (28 cycles) of operation, the SBR using hydrolyzed molasses as a carbon source showed 91.6 ± 1.6% nitrogen removal, which was higher than that using methanol (85.3 ± 2.0%). Their results show that hydrolyzed molasses can be an economical and effective external carbon source for the nitrogen removal process.

Ueda et al. (2006) examined a biological denitrification experiment using sugar-industry wastes, namely final molasses as a carbon source and bagasse charcoal pellets as supporting media for denitrifying bacteria. They employed an upflow fixed-bed reactor filled with the pellets and biofilm attached onto them. This was fed with potassium-nitrate and dilutemolasses solutions. Total nitrogen removals of more than 85% were achieved at influent carbon–nitrogen (C/N) ratios between 2 and 4, and hydraulic residence times of more than 0.8 h. This demonstrated that final molasses could be used as an alternative carbon source. On the other hand, final molasses also contained some organic/ammonium nitrogen and refractory

organic matter including colors, both of which were difficult to remove with the reactor. Accordingly, at higher C/N ratios, these substances caused major increases in effluent total-nitrogen and organic-carbon concentrations. Therefore, they suggested that an optimum C/N ratio was found to be around 2.

Hamlin et al. (2008) examined aerobic biological filtration systems employing nitrifying bacteria to remediate excess ammonia and nitrite concentrations. They evaluated the design of a full scale denitrification reactor in a commercial culture RAS application. Four carbon sources were evaluated including methanol, acetic acid, molasses and Cerelose™, a hydrolyzed starch, to determine their applicability under commercial culture conditions and to determine if any of these carbon sources encouraged the production of two common off-flavor compounds, 2-methyisoborneol (MIB) or geosmin. All four carbon sources were able to effectively reduce nitrate to near zero concentrations from influent concentrations ranging from 11 to 57 mg N L^{-1}, and the maximum daily denitrification rate was 670–680 g nitrogen removed m^{-3} media day^{-1}, regardless of the carbon source. Although nitrite production was not a problem once the reactors achieved a constant effluent nitrate, ammonia production was a significant problem for units fed molasses and to a less extent Cerelose™. Turbidity production was significantly increased in reactors fed molasses and to a less extent Cerelose™. Concentrations of geosmin and MIB were not significantly increased in any of the denitrification reactors, regardless of carbon source. Because of its very low cost compared to the other sources tested, molasses may be an attractive carbon source for

denitrification if issues of ammonia production, turbidity and foaming can be resolved.

Roy et al. (2010) suggested one simple method of treating high-nitrogen wastewater using a sequencing batch reactor (SBR). An SBR was a variation of the activated sludge process, which accomplished many treatment events in a single reactor. Removal of ammonia and nitrate involved nitrification and denitrification reactions by operating the SBR aerobically and anaerobically in sequence. Initial SBR operation successfully removed ammonia, but nitrate concentrations were too high because of carbon limitation. An optimization study revealed the optimum carbon to nitrogen (C:N) ratio of 10:1 for successful removal of all nitrogen species from the wastewater. The SBR operated with a C:N ratio of 10:1 with the addition of molasses as carbon source successfully removed 99% of ammonia, nitrate, and nitrite from the shrimp aquaculture wastewater within 9 days of operation.

2.2 Permeable reactive barriers

Over the past decade, permeable reactive barriers have been developed and used to treat groundwater contaminated by inorganic constituents (Blowes et al., 2000). Traditional approaches to treating groundwater contaminated by dissolved inorganic constituents have involved removing the contaminant source, pumping, and treating plumes of contaminated groundwater or isolating the source area with low permeability barriers or covers. The use of permeable reactive barriers provides an alternative in situ approach to replace or supplement these existing techniques.

Permeable reactive barriers are placed in the path of a migrating plume of contaminated groundwater. Reactive materials within the barrier are selected to promote biogeochemical reactions that result in the destruction or stabilization of the groundwater contaminants. Ideally, these materials are sufficiently reactive to treat water for periods of years to decades. Mixtures selected for the attenuation of inorganic species must be designed to maintain their permeability as secondary precipitates accumulate. The barrier design must also ensure that the contaminant will remain immobilized within the aquifer, or can be retrieved with the reactive material following treatment.

The barrier materials should provide treatment at costs that are competitive with other groundwater remediation programs. Costs associated with the implementation of a reactive barrier treatment system include the

initial costs associated with the design, installation, and site rehabilitation, and the continuing costs of monitoring the barrier performance. Costs may also be associated with the recovery and disposal of the reactive material following the completion of the treatment program. Permeable reactive barriers have been developed and demonstrated to be effective for the treatment of dissolved metals (Blowes and Ptacek, 1992; Powell et al., 1995; Cantrell et al., 1995; Blowes et al., 1997), acid-mine drainage (Waybrant et al., 1995; Benner et al., 1997, 1999), and dissolved nutrients (Robertson and Cherry, 1995; Baker et al., 1997; Schipper and Vojvodić-Vuković, 1998, 2000, 2001; Schipper et al., 2005). A wide range of reaction mechanisms can be employed to remove both negatively charged and positively charged inorganic species from flowing groundwater. These include simple adsorption (Morrison and Spangler, 1993), simple precipitation (McMurty and Elton, 1985), adsorptive precipitation (Baker et al., 1997), reductive precipitation (Blowes and Ptacek, 1992), and biologically mediated transformations (Waybrant et al., 1995; Robertson and Cherry, 1995; Benner et al., 1997, 1999). This passage summarizes recent advances in the development of permeable reactive barriers for remediating groundwater contaminated by nitrate.

2.2.1 Biologic PRB system with sawdust

A system for removing nitrate from groundwater affected by discharge from on-site wastewater disposal systems through denitrification

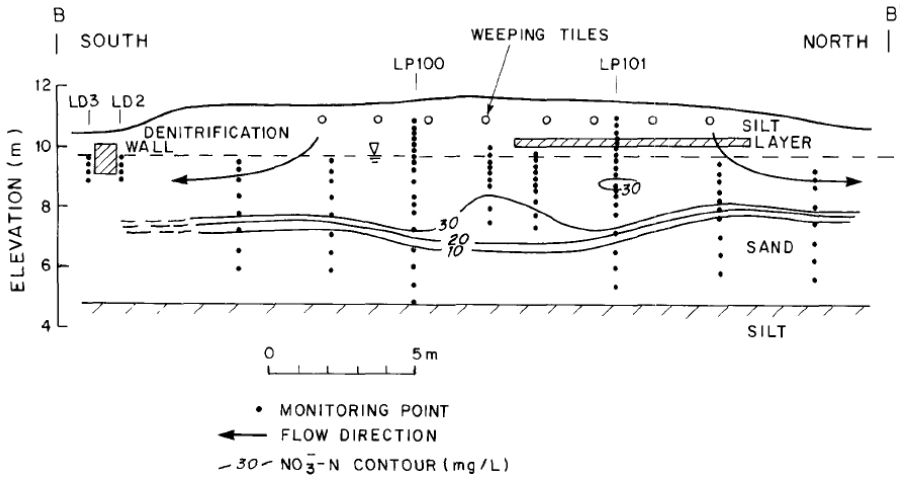

Fig. 2-1. A cross-section diagram of the denitrification wall at Long Point tile bed and nitrate distributions (Robertson and Cherry, 1995).

has been developed by Robertson and Cherry (1995). This system intercepts a plume of nitrate-bearing groundwater with a reactive barrier containing solid-phase organic carbon as sawdust. In the presence of organic carbon, under anaerobic conditions maintained below water cover in the subsurface, reduction of nitrate to N gas is thermodynamically favored. Robertson and Cherry (1995) evaluated permeable reactive barriers for treating nitrate at several domestic and institutional septic systems. The results of these studies indicate that denitrification occurs rapidly, leading to effective treatment. Nitrate is reduced from concentrations typically observed in the effluent of on-site wastewater disposal systems (5–90 mg N L^{-1}) to below the World Health Organization drinking water standard (10 mg N L^{-1}). Long-term monitoring of effluent from several nitrate removal systems indicated continued treatment over a period of several years (Robertson et al., 2000). A similar approach for removing agricultural nitrate was evaluated by Schipper and Vojvodic-Vukovic (1998, 2000, 2001, 2005) who installed a permeable reactive barrier that contained 30% v/v sawdust. Concentrations of nitrate entering this barrier ranged from 5 to 16 mg N L^{-1}. The effluent nitrate concentration was below 2 mg N L^{-1}.

2.2.2 Biologic PRB system with sulfur granules

Moon et al. (2004, 2006a, 2006b, 2008) reported the long-term performance of a sulfur-based reactive barrier system using autotrophic denitrification in a large-scale column. A bacterial consortium, containing

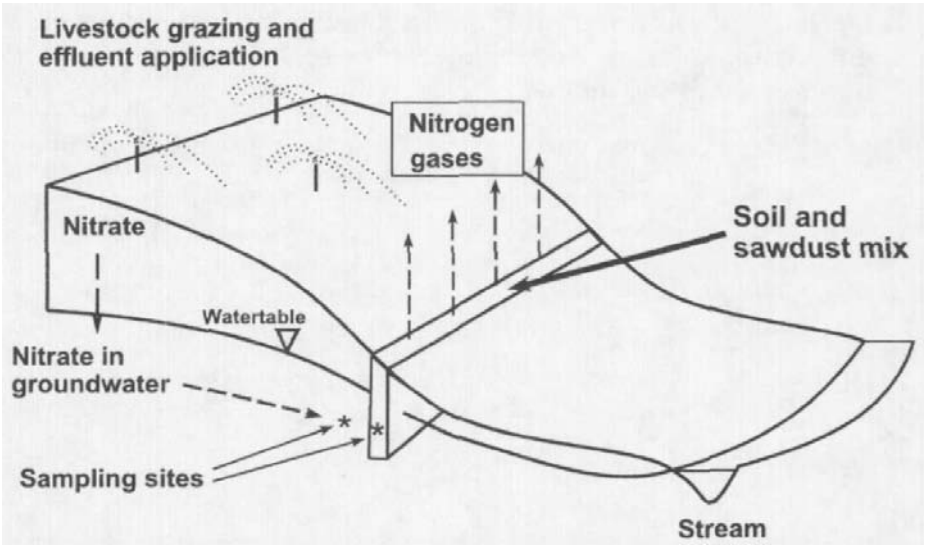

Fig. 2-2. A schematic diagram of a denitrification wall for removing nitrate from groundwater. Sawdust was mixed into shallow groundwater soils as a source of organic matter for denitrification (Schipper and Vojvodic-Vukovic, 1998).

autotrophic denitrifiers attached on sulfur particles, serving as an electron donor, was able to transform 60 mg N L^{-1} of nitrate into dinitrogen. In the absence of phosphate, the consortium was unable to remove nitrate, but after the addition of phosphate, nitrate removal was readily evident. Based on the dinitrogen concentration in the total gas collected, the denitrification efficiency of the tested column was estimated to be more than 95%. After 500 d operation, the hydrodynamic characteristics of the column slightly changed, but these changes did not inhibit the nitrate removal efficiency. Data from a bacterial community analysis obtained from four parts of the column demonstrated the selective a spatial distribution of predominant species depending on available electron acceptors or donors.

2.2.3 Biologic PRB system with molasses

In situ heterotrophic denitrification using molasses for treating nitrate-contaminated groundwater has been proposed as a viable substitute for the conventional remedial techniques (Cunningham et al., 2003; Dutta et al., 2005). In their field-scale studies, subsurface biofilm barriers with injected mixtures of liquid-phase molasses and nutrients were designed for the containment and remediation of nitrate-contaminated groundwater. The injected mixture stimulated and grew denitrifying microbes with almost 99% nitrate removal efficiency. On the other hand, the hydraulic conductivity of the affected area was reduced to 2 orders less than the original one due to the accompanied clogging.

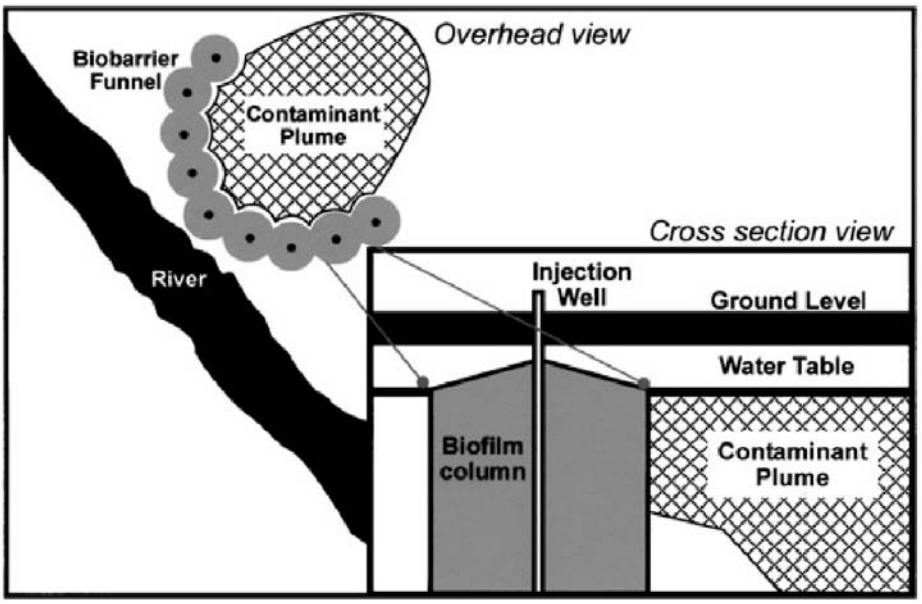

Fig. 2-3. Visualization of a subsurface barrier composed of thick biofilm which plugs aquifer pore space while enhancing contaminant biodegradation. Biofilm barriers could also serve as a means of funneling groundwater through zones of active treatment (Cunningham et al., 2003).

Cunningham et al. (2003) examined an engineered microbial biofilm barrier capable of reducing aquifer hydraulic conductivity while simultaneously biodegrading nitrate at a field-relevant scale. The 22-month demonstration project was conducted in Butte, Montana, which consisted of a 130 ft wide, 180 ft long, 21 ft deep, polyvinylchloride (PVC)-lined test cell, with an initial hydraulic conductivity of 4.2×10^{-2} cm s^{-1}. A flow field was established across the test cell by injecting water up-gradient while simultaneously pumping from an effluent well located approximately 82 ft down gradient. A 30 ft wide biofilm barrier was developed along the centerline of the test cell by injecting a starved bacterial inoculum of *Pseudomonas fluorescens* strain CPC211a, followed by injection of a growth nutrient mixture composed of molasses, nitrate, and other additives. A 99% reduction of average hydraulic conductivity across the barrier was accomplished after three months of weekly or bi-weekly injections of growth nutrient. Reduced hydraulic conductivity was maintained by additional nutrient injections at intervals ranging from three to ten months. After the barrier was in place, a sustained nitrate concentration of 100 mg N L^{-1}, along with a 100 mg L^{-1} concentration of conservative (chloride) tracer, was added to the test cell influent over a six-month period. At the test cell effluent the concentration of chloride increased to about 80 mg L^{-1} while the effluent nitrate concentration varied between 0.0 and 6.4 mg N L^{-1}.

A similar approach for removing agricultural nitrate was evaluated by Dutta et al. (2005). A biofilm barriers was created by stimulating indigenous bacteria with injections of molasses as the carbon donor and a combination of

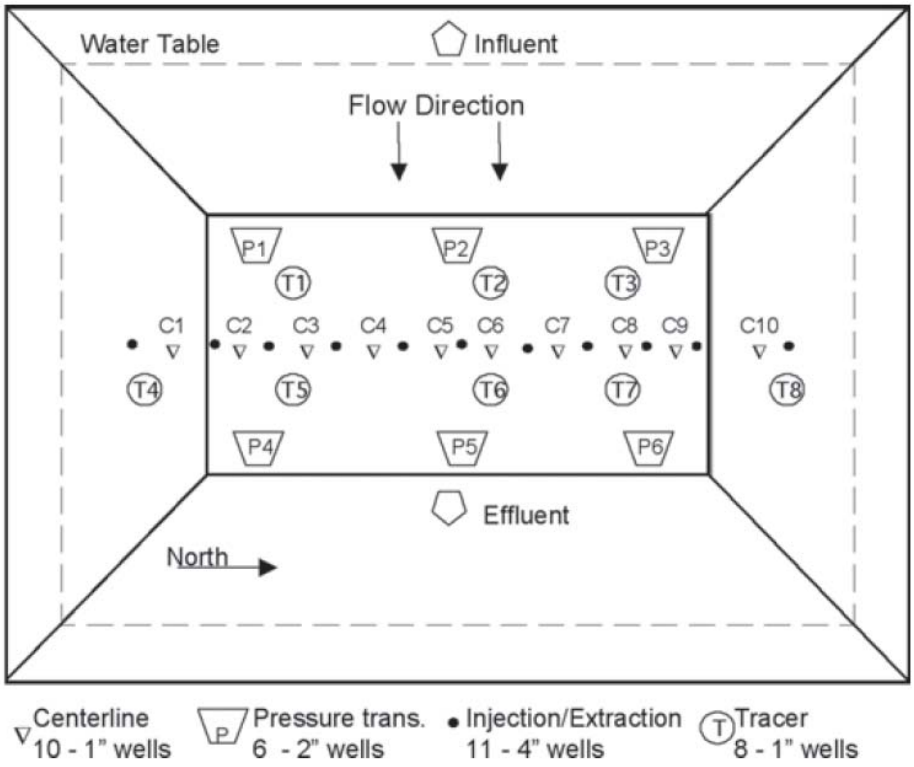

Fig. 2-4. A schematic diagram of relative locations and orientation for the biofilm barrier system including location of injection/extraction and monitoring wells (Dutta et al., 2005).

yeast extract and trimetaphosphate as nutrients. This injection of amendments resulted in bacterial growth in the aquifer, which attached to the sand grains to create a reactive semipermeable biofilm. The biofilm barrier presented reduced the migration of contaminants and provided an active zone for remediation. The cylindrical biobarrier was constructed using eight wells on the perimeter forming a 60-foot-diameter reactive biodenitrification region. Another well at the center was installed to continuously extract the treated water. The intent was to produce a continuous source of nitrate-free water. The system operated for over one year, and during this period, the biobarrier was revived multiple times by reinjecting molasses in the perimeter wells. Nitrate concentrations of treated water decreased from 275 mg N L^{-1} to < 1 mg N L^{-1}.

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CHAPTER 3

DENITRIFICATION BY A HETEROTROPHIC DENITRIFIER WITH THE AID OF THE SLOWLY RELEASED MOLASSES

3.1 Introduction

Conventionally, a reactive barrier system using cellulose solids (wood mulch, sawdust, leaf compost, and cotton burr compost) as carbon sources has been tested as an alternative means to remove nitrates from shallow groundwater (Robertson and Cherry, 1995; Robertson et al., 2000; Schipper and Vojvodić-Vuković, 1998, 2000, 2001; Schipper et al., 2005; Su and Puls, 2007). These studies demonstrated that the barrier system is a viable option to achieve long-term, passive, and in situ attenuation of nitrates. Nevertheless, denitrification rates using these materials appear to be rather slow for practical purposes, due in part to their slow release of the available carbon.

In contrast, a number of studies have employed high-strength liquid organic waste as carbon sources, including brewery waste, whey, yeast, other types of bio-industry waste, along with silage effluents (Monteith et al. 1980; Skrinde and Bhagat 1982). In some cases, they achieved denitrification rates comparable to processes using methanol. Similarly, some textbooks have also proposed final molasses, a sugar industry waste product, as a carbon source

(Eckenfelder, 1989; Horan, 1990). Nevertheless, little experience has been accumulated thus far on denitrification using final molasses (Ueda et al., 2006). Molasses is a viscous, dark-brown liquid by-product. It is produced when raw cane juice is refined into white sugar and is easily obtained from sugarcane refineries.

This study was conducted to determine the potential applicability of solidified molasses, i.e., slowly released molasses (SRM), for use as a permeable reactive barrier to treat nitrate-contaminated groundwater as a long-term treatment option. The results can show the possibility that SRM can be used as a reliable, long-term extra carbon source for indigenous heterotrophic denitrifiers.

3.2 Materials and methods

3.2.1 Isolation of a heterotrophic denitrifier

To identify an indigenous heterotrophic denitrifier, 10 g of soil as a sample was collected from the sandy soil of an anonymous agricultural field in Suwon, Korea. The sample was subjected to extraction of the bulk DNA and was then analyzed by PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis). Bulk DNA genes were obtained from the soil using the FastDNA® SPIN for soil kit (MP Biomedicals, USA). To obtain 16S rDNA genes for the microbial community structure analysis, denitrifiers extracted bulk DNA genes were amplified with 27F and 1522R primers using the GeneAmp PCR System 9700 (Applied Biosystems, USA). One μL each of GC-338F and 518R primer solutions (10 pmol μL^{-1} each), 20 ng of the amplified, purified 16S rDNA genes and Accupower HotStart PCR Premix (Bioneer, Korea) were combined to give a final reaction volume of 20 μL. The next steps were as follows: (i) 10 min at 94℃ for initial denaturation, (ii) 30 cycles for 45 sec at 94℃ for denaturation and 45 sec at 55℃ for annealing, and (iii) 45 sec at 72℃ for an extension. Sequence chromatograms of 16S rDNA were compared with database sequences in GenBank. Sequence editing and alignments were performed using CLUSTAL X (Higgins and Sharp, 1988). Phylogenetic analysis of the 16S rDNA was performed using a neighbor-joining algorithm and a distance calculation method (Saitou and Nei, 1987) with MEGA2 (Kumar et al.,

1993). A PCR analysis was also used to amplify the denitrification genes encoding nitrite reductase, including *nirK* and *nirS* from the bulk DNA using the primers F1aCua and R3Cu for *nirK* and cd3aF and R3cd for *nirS* (Hanllin and Lindgren, 1999, Throbäck et al., 2004). The PCR program ran for 2 min at 94℃; 35 cycles for 2 min at 94℃ and 1 min at 51℃; followed by 1 min at 72℃. Sequence chromatograms of *nirK* and *nirS* genes were compared with database sequences in the RCSB PDB (Protein Data Bank, http://www.rcsb.org/pdb/home/home.do).

3.2.2 Identification of the denitrifying capacity

Batch tests were conducted in order to identify the denitrifying capacity of the isolated denitrifier. A liquid medium for the tests contained KNO_3 of 0.722 g L^{-1}, K_2HPO_4 of 0.4 g L^{-1}, KH_2PO_4 of 0.15 g L^{-1}, NH_4Cl of 0.4 g L^{-1}, $MgSO_4 . 7H_2O$ of 0.4 g L^{-1}, a trace element solution SL-10 (DSMZ 320) of 10 mL L^{-1}, and liquid phase molasses of 2.375 g L^{-1}. The initial nitrate and molasses concentrations in the medium were measured as 100 mg N L^{-1} and 2,400 mg COD L^{-1}, respectively, indicating a C/N ratio of 10/1. 100 mL of the medium was poured into a heat-resistant glass bowl with a 160 mL volume. The bowl was purged with argon gas for 20 min in order to remove the oxygen. After purging, it was sterilized at 121 ℃ and 15 psi for 18 min. Approximate 2 mL of the suspension, including the isolated denitrifier, was then placed into the medium. Aliquots (~2 ml) were collected periodically (0, 30, 36, 48, 96, 144, 192, 240, and 288 hrs). To

measure the nitrate concentrations, 2 mL of samples were filtered (0.2 μm) and stored in a refrigerator at 4℃ until they were analyzed by IC (DX-80, Dionex, USA). The COD value as an indirect indication of the molasses concentration was analyzed using a UV-visible spectrophotometer (DR-2800, Hach Co. Ltd., USA) with a TNT821 reagent vial kit (with a detection range of 3~150 mg COD L^{-1}) immediately after the collection of the sample.

3.2.3 SRM manufacture and experimental setup

Batch-scale solidified molasses referred to here as slowly released molasses (SRM) was made by dispersing ~48 g of molasses (25% water content, Hydex Co. Ltd., Korea) into a hydroxypropyl methylcellulose-microcrystalline cellulose-silica sand matrix (~50 g). An acryl column (L x D = 14 cm x 2 cm) was prepared to test the performance of the SRM. Ottawa sand (mesh size, 20 ~ 30; Fisher Scientific, USA) was placed into the column and the porosity was calculated as 0.36. To attach the isolated denitrifier onto the surfaces of the sand particles, liquid medium including a denitrifier (i.e., > 10^8 CFU mL^{-1}) was flowed and circulated into the column using a peristaltic pump (flow rate, 6 mL h^{-1}; velocity, 1.3 m d-^{1}) for 24 hrs. After attaching this system, the column remained for 48 hrs while the inlet and outlet were closed. In order to create synthetic groundwater, one 3.2 L volumetric bowl filled with a nitrate solution of 20 mg N L^{-1} was prepared and sterilized at 121 ℃ and 15 psi for 18 min. A total of 2.3 L of the nitrate solution was constantly flowed into the column for 381 hrs. The solution contained basal salts with

145 mg L^{-1} of KNO_3, 14.5 mg L^{-1} of K_2HPO_4, 5.4 mg L^{-1} of KH_2PO_4, 14.5 mg L^{-1} of NH_4Cl, 14.5 mg L^{-1} of $MgSO_4.7H_2O$, and trace elements of SL-10 (DSMZ 320) at 0.38 mL L^{-1}. To prevent the growth of predatory protozoa, approximately 3.2 mL of cyclohexamide was added to the nitrate solution.

3.3 Results and discussion

3.3.1 Isolation of a heterotrophic denitrifier

Pseudomonas sp. isolate was identified as a possible denitrifier from soil samples with 99% 16S rDNA similarity to *Pseudomonas fluorescens* and was termed *Pseudomonas* sp. KY1 (Fig. 3-1). *Pseudomonas fluorescens* is well known as a typically heterotrophic denitrifier (Tjedje et al., 1989; Zumft, 1992). *Pseudomonas fluorescens* is a gram-negative, rod-shaped soil bacterium. It is an obligate aerobe but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. The optimal temperatures for growth are 25-30 ℃.

Genetic analysis of denitrification usually proceeds from the nitrite reductase step (*nirK* and *nirS*), because the nitrite reduction process is a rate-limiting step in denitrification (Goregues et al., 2005). Generally, denitrifying bacterium with *nirS* is more frequently identified in soil media than it is with *nirK*; however, their functions during denitrification are similar. From a genetic analysis, *nirK* gene fragments (~450 bp) were amplified from *Pseudomonas* sp. KY1.

Overall, *Pseudomonas* sp. KY1 is likely the most indigenous denitrifier in a sandy media. Lee (2010) demonstrated that *Pseudomonas* sp. KY1 transformed nitrate to nitrogen gas.

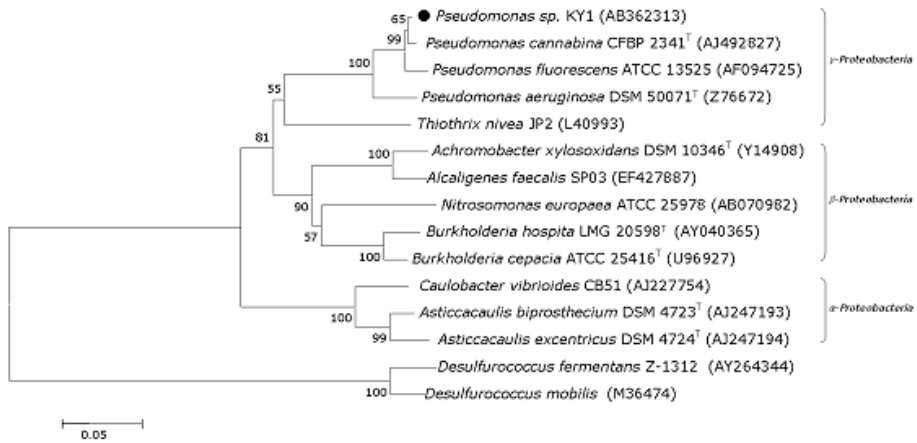

Fig. 3-1. A phylogenetic tree of *Pseudomonas* sp. KY1 and closely related bacteria. *Desulfurococcus fermentans* Z-1312 (AY264344) and *Desulfurococcus mobilis* (M35474) in the *Archaea* domain were selected as the outgroup species. The scale bar represents 0.05 substitutions per nucleotide position. Numbers at the nodes are the bootstrap values.

3.3.2 Denitrifying capacity of the isolated denitrifier

The molasses used to create the SRM material contained ~65% organics (~56% sugar and ~9% non-sugar constituents), ~10% inorganics, and ~25% water. Sucrose was the largest constituent of the sugar in the molasses, accounting for ~38%, while other constituents were glucose at ~9% and fructose at ~9%. Assuming sucrose as the main carbon source in molasses, the denitrification reaction is written as Eq. (3-1) (Boaventura et al., 1997).

$$C_{12}H_{22}O_{11} + 48NO_3^- \rightarrow 24N_2(g) + 60CO_2(g) + 48OH^- \quad (3\text{-}1)$$

From Eq. 3-1, the calculated theoretical C/N ratio is 0.214. However, it is recognized as the optimum value because it does not consider molasses consumption for denitrifier growth but for denitrification. Her and Hwang (1995) reported that denitrification occurs when the C/N ratio is more than 0.214, with the result being 1.9 for acetic acid as a carbon source, and a range of 0.9 to 10.0 for methanol. Lorrain et al. (2004) demonstrated that the C/N ratio increases when a high number of carbon is used for denitrification. In this study, a C/N ratio of 10/1 was applied to the batch test due to identifying denitrifying capacity of *Pseudomonas* sp. KY1. Nitrate of 100 mg N L^{-1} was actively destroyed by the denitrifier *Pseudomonas* sp. KY1, decreasing by 70%, 30%, 10%, and 4% after 30, 36, 48, and 288 hrs, respectively. The initial molasses concentrations of 2,400 mg COD L^{-1} decreased by 87% (up to

1,964 mg COD L^{-1}), 75% (1,530 mg COD L^{-1}), and 48% (788 mg COD L^{-1}) after 36, 48, and 288 hrs, respectively (Fig. 3-2). Based on the change in the nitrate concentration with respect to time, the pseudo-first-order reaction coefficient for the nitrate concentration was calculated to be 0.0033 h^{-1} for 48 hrs. After 48 hrs, a total of 274 mg COD L^{-1} molasses was consumed until the completion of the batch test, while the removed nitrate concentration was no more than 6% (6.1 mg N L^{-1}). Theoretically, 6.1 mg N L^{-1} of nitrate can be removed by denitrification with 3.5 mg COD L^{-1} (Eq. 3-1). As a result, the molasses consumed was approximately 216 times the theoretical amount, indicating that most of the molasses was used for denitrifier growth.

3.3.3 Column test results using the SRM material

SRM could continuously deliver molasses by itself into water over 381 hrs according to the column test. HPMC as an emulsifying agent could hinder the molasses release from SRM material to water, resulting in molasses being slowly released into the water. The average molasses concentrations decreased slowly with time, with the results being ~65, ~12, and ~4 mg COD L^{-1} after 65, 215, and 215 hrs, respectively. The average molasses concentrations according to the measured COD values are presented in Fig. 3-3.

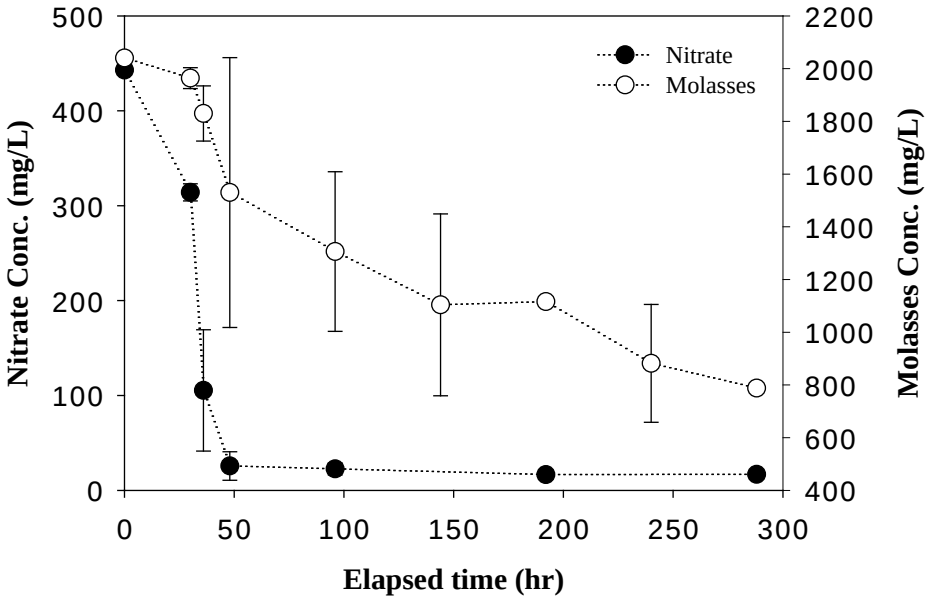

Fig. 3-2. Changes in nitrate and molasses concentrations by *Pseudomonas* sp. KY1 at the C/N ratio of 10/1. Molasses concentrations were expressed as molasses-COD.

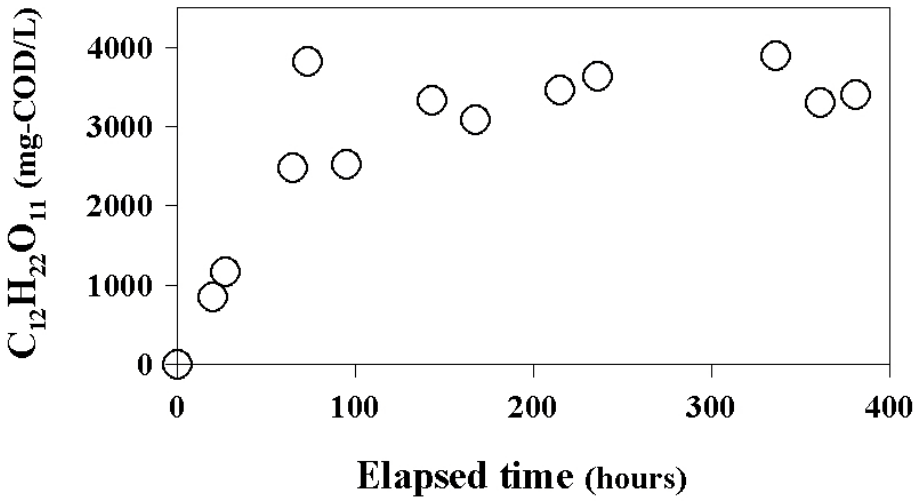

Fig. 3-3. Changes in molasses concentrations from the effluent in the column.

However, these data were monitored from a limited volume (3.2 L) of water in the water tank test and cannot therefore explain the molasses release rate in a groundwater aquifer. Further study, like a pilot study or field tests in a groundwater aquifer, is required. The molasses concentrations at the inlet ranged from 1,278 to 6,744 mg COD L^{-1}, while those at the outlet ranged from 1,169 to 3,406 mg COD L^{-1}. Therefore, the molasses consumption amounts with respect to time could be calculated by subtracting the outlet concentrations from the inlet amounts (Table 3-1).

The molasses concentrations at the inlet after 27 hrs were found to be 1,278 mg COD L^{-1}, while those at the outlet were 1,169 mg COD L^{-1}, indicating that 100 mg COD L^{-1} of molasses was consumed by *Pseudomonas* sp. KY1 in the column. Further, these values at the inlet after 73, 336, and 381 hrs were determined to be 5,706, 6,131, and 6,744 mg COD L^{-1}, while at the outlet the values were 3,818, 3,306, and 3,406 mg COD L^{-1}, respectively. These results demonstrate that amounts of 1,888, 2,825, and 3,338 mg COD L^{-1} of molasses were consumed by *Pseudomonas* sp. KY1 after 73, 336, and 381 hrs, respectively.

The molasses consumption amounts at the late stage were larger than they were at the early stage, most likely due to the fact that the denitrifier population growth rate at the late stage was higher than it was at the early stage. After the completion of the column test, *Pseudomonas* sp. KY1 attached onto an Ottawa sand particle was observed using FE-SEM (JSM 5410LV, JEOL, Japan) (Fig. 3-4).

Table 3-1. Changes in molasses concentrations by *Pseudomonas* sp. KY 1 in the column.

Elapsed Time (hr)	**Molasses conc. expressed as COD (mg L^{-1})**		**Uptake by *Pseudomonas* sp. KY 1 (mg L^{-1}) ****
	Influent*	**Effluent**	
27	1,278	1,169	109
73	5,706	3,819	1,888
361	6,131	3,306	2,825
381	6,744	3,406	3,338

* Molasses concentration released from the molasses-releasing test reservoir with time.

** The difference of COD between influent and effluent concentrations.

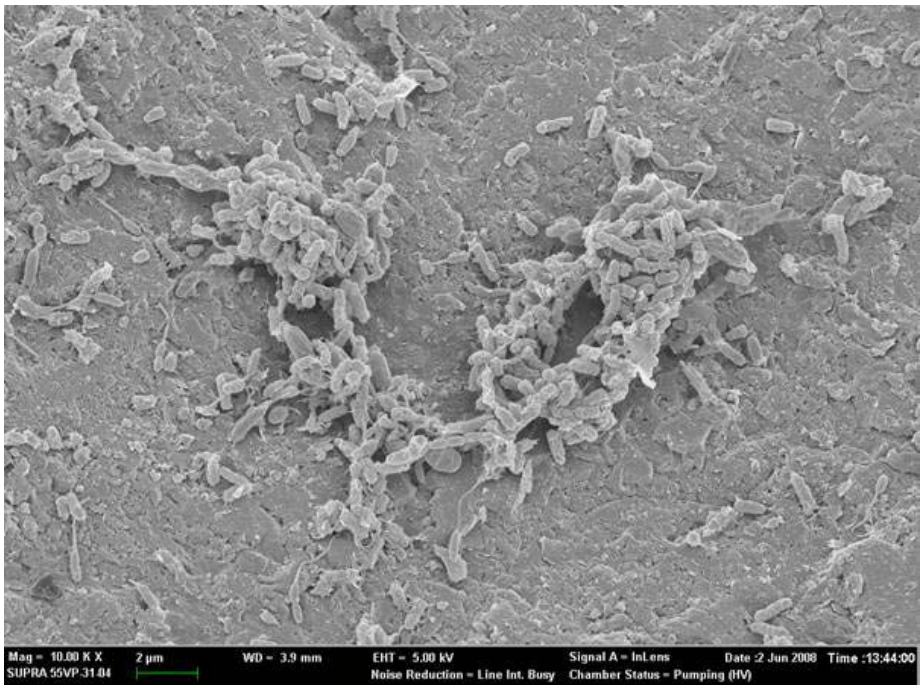

Fig. 3-4. A scanning electron microscopy image of *Pseudomonas sp.* KY1 on Ottawa sand granules (× 10,000).

Nitrates were continuously destroyed by *Pseudomonas* sp. KY1 in the column during the test of time. The initial nitrate concentration of 20 mg N L^{-1} decreased by 53% (up to 11 mg N L^{-1}), by 44% (9 mg N L^{-1}), and by 14% (3 mg N L^{-1}) after 65, 167, and 361 hrs, respectively. On the other hand, nitrite, the intermediate product of nitrate reduction, was also observed. The nitrite concentrations increased during the test of time, showing values of 3, 16, and 21 mg N L^{-1} at the same monitoring times, respectively (Fig. 3-5). As nitrate is converted to nitrite or ammonium by the denitrifying bacteria or by nitrogen fixing bacteria (ammonifying bacteria), the decrease in the nitrate concentration cannot be evidence of denitrification (Dandie et al., 2007). Nonetheless, the *nirK* gene was found in *Pseudomonas* sp. KY1. Therefore, the results of a nitrate decrease and a nitrite increase were based on the denitrifying activity. The reason why nitrite was not further reduced to nitrogen gas may depend on a number of factors, such as the type of carbon source, the biofilm composition, the oxygen content, the pH, the phosphate concentration (Gibert et al., 2008). In this study, nitrite accumulation was probably caused by its relatively low reduction rate compared with nitrate reduction one. As one good reference, Dhamole et al. (2008) demonstrated nitrite could be accumulated when nitrite reduction rate was less than nitrate reduction one. They performed a denitrifying experiment with a 42 L airlift reactor and estimated specific rates of nitrate and nitrite reduction to be 49 ± 3 and 15 ± 1 N/g MLSS/L/h, respectively. In their study, nitrite concentrations were built up during the time when nitrate was removed and nitrite reduction began when the nitrate concentration was completely

removed. In addition, Moon et al. (2004) reported nitrite accumulation in their large-scale column (D x L = 7 cm x 70 cm) study of autotrophic denitrification. In their study, nitrite accumulated in the column up to 14 days but decreased after 16 days. As a result, if the column operation time in this study would be long enough, the nitrite reduction could be possibly observed.

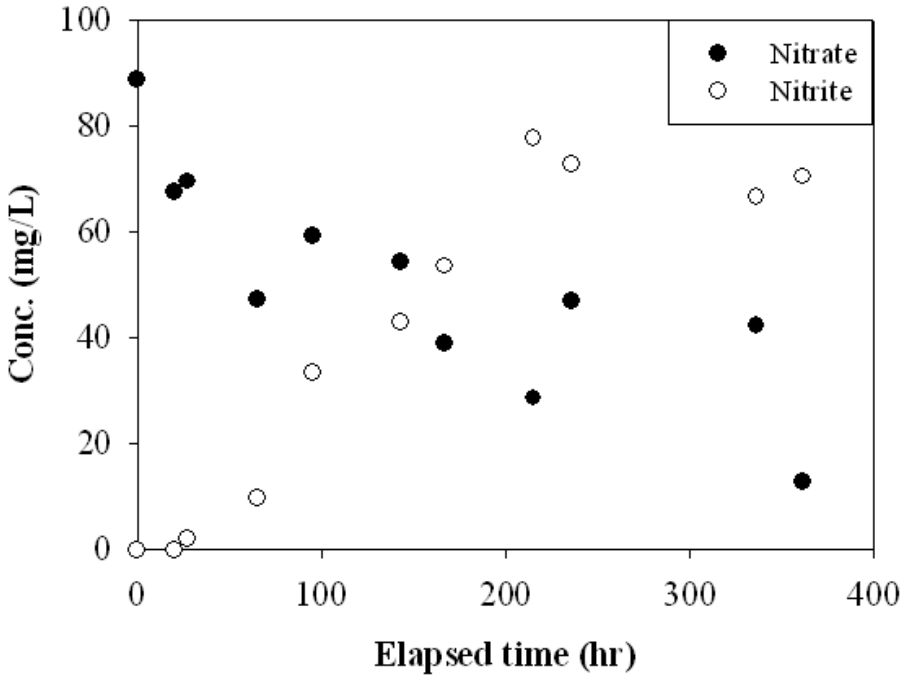

Fig. 3-5. Changes in nitrate and nitrite concentrations during denitrification in the column.

3.4 Conclusions

This study was conducted to determine the potential applicability of slowly released molasses (SRM) to treat nitrate-contaminated groundwater. SRM was made by dispersing molasses in a hydroxy propyl methyl cellulose-silica-microcrystalline cellulose matrix. A column test indicated that SRM can continuously release molasses with slowly decreasing release rates of 64.6 mg COD L^{-1} h^{-1} up to 65 hrs, 12.1 mg COD L^{-1} h^{-1} up to 215 hrs, and 4.4 mg COD L^{-1} h^{-1} up to 361 hrs. A batch test using the isolated indigenous heterotrophic denitrifier *Pseudomonas* sp. KY1 with nitrite reductase (*nirK*) and liquid molasses demonstrated that the bacterium decreased 100 mg N L^{-1} of nitrate to less than 10 mg N L^{-1} for a C/N ratio of 10/1 in 48 hours. In a *Pseudomonas* sp. KY1-attached Ottawa sand column which continuously received molasses from a SRM-containing reservoir, the bacterium successfully removed nitrates, from the initial 20 mg N L^{-1} to 3 mg N L^{-1} during 381 hours of column operation. These results show the possibility that SRM can be used as a reliable, long-term extra carbon source for indigenous heterotrophic denitrifiers.

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CHAPTER 4

RELEASE CHARACTERISTICS OF MOLASSES FROM A WELL-TYPE BARRIER SYSTEM IN GROUNDWATER: A LARGE TEST TANK STUDY FOR NITRATE REMOVAL

4.1 Introduction

In heterotrophic denitrification, the nitrate removal efficiency generally depends on the carbon source used as an electron donor. A readily degradable organic compound as a carbon source may be a limiting factor for successful nitrogen removal (Æsøy et al. 1998; Mora et al. 2003). Although commercially available organic compounds such as acetic acid, glucose, ethanol, and methanol can be used to enhance biologic nitrate removal (Her and Huang 1995; Kapoor and Viraraghavan 1997; Mohseni-Bandpi et al. 1999; Louzeiro et al. 2003; Chou et al. 2003), efforts to reduce the operation cost continues. As an alternative, molasses, a cheap sugar-industry by-product, has been recommended as a reliable carbon source comparable to commercial carbon sources (Ten Have et al. 1994; Boaventura and Rodrigues 1997; Lee et

al. 2001; Quan et al. 2005; Hamlin et al. 2008; Roy et al. 2010).

One good example for groundwater is the use of molasses to treat nitrate as an *in situ* heterotrophic denitrification medium (Cunningham et al. 2003). In their field-scale studies, subsurface biofilm barriers with injected mixtures of liquid-phase molasses and nutrients were designed for the containment and remediation of nitrate-contaminated groundwater. The injected mixture stimulated the growth of denitrifying microbes with almost 94% of nitrate removal efficiency. A similar approach for removing nitrate was conducted by Dutta et al. (2005). A cylindrical biofilm barrier system was created by stimulating indigenous bacteria with an injection of liquid-phase molasses as a carbon source and a combination of yeast extract and trimetaphosphate as nutrients. The system was operated for over 1 yr, during which the nitrate concentration of the treated water decreased from 1,217 to less than 5 mg L^{-1}.

A well-type reactive barrier system has been recently developed as a semi-passive, long-term treatment option for diluted plumes of chlorinated solvents using solidifying $KMnO_4$ materials referred to as controlled-release $KMnO_4$ (CRP) in aquifers (Lee and Schwartz 2007a, 2007b; Lee et al. 2008a, 2008b, 2009). The dynamics and efficacies of the system have been demonstrated by a series of proof-of-concept tests, lab-scale tests, and pilot-scale flow tank tests, and by modeling approaches. From pilot-scale tank tests, the removal efficiencies were estimated to be fairly high as 65-74% for a trichloroethylene (TCE) plume of 87-172 μg L^{-1} that persisted over a long period of time while creating low pore plugging and little hydraulic

disturbance (Lee et al. 2009).

In this study, a well-type reactive barrier system containing solidifying molasses rods, named slowly released molasses (SRM) as a reactive medium to promote indigenous denitrifying activity, is proposed as an *in situ* remedial option for treating nitrate contaminated groundwater. The proposed SRM system is operated with periodic additions of SRM rods into the well-type reactive barriers without hydraulic disturbance. As groundwater flows through the wells, molasses is continuously released, resulting in the enrichment of heterotrophic denitrifiers. In a column-scale experiment with the SRM rods, the heterotrophic denitrifier *Pseudomonas* sp. KY1 having nitrite reductase (*nirK*) successfully removed nitrate from 89 to less than 13 mg L^{-1} over a period of 361 hrs (Lee et al. 2010a). For field applications, the performance of the SRM rods needs to be verified with an *in situ* barrier system. This study addresses the molasses dissolution pattern of the SRM system using a large-scale flow tank implemented with well-type barriers. Simulation results with an aid of the upscaled mass transfer function (MTF) model are presented to describe how the SRM system can create a long-term denitrifying zone in the subsurface. The MTF model was developed to describe a field-scale DNAPL source dissolution with advective-dispersive plume transport (Parker and Park, 2004; Park and Parker, 2005). In this study, this model offers a pragmatic means to estimate the molasses mass flux and to predict the longevity of the SRM system.

4.2 Materials and methods

4.2.1 Preparation of the SRM rod and the SRM release test

A prototype SRM rod (OD x L = 4 cm x 30 cm) was made by dispersing 177 g molasses (Hydex, Korea) in 360 g of a paraffin wax-cellulose-silica sands matrix (215 g paraffin wax, 109 g cellulose, and 36 g silica sands) in a cylindrical mold at an ambient temperature. The paraffin wax-cellulose-silica matrix was used to prevent the instant dissolution of molasses from the rod. Lee and Schwartz (2007a, 2007b) reported that an inert organic crystalline matrix can ensure the slow dissolution and diffusion-controlled transport of molasses. The apparent molasses solubility of a SRM rod was identified as approximately 6,000 mg L^{-1} as chemical oxygen demand values (COD L^{-1}) from a batch-type release test conducted for 112 d (Lee et al. 2010b) (Fig. 4-1).

A pilot-scale flow tank (L x W x D = 8 m x 4 m x 2 m, 95 m^3 of sands, bulk density of 1.47 g cm^{-3}, and porosity of 0.45) was prepared to test the performance of the SRM system. Three discrete barriers were installed at 1-m interval in the tank (Fig. 4-2). In each barrier, there were forty-screened polyvinylchloride (PVC) wells (OD x D = 10 cm x 150 cm) arrayed in a zigzag shape, as shown in Fig. 4-2. The input and output chambers located in the upstream and downstream ends, respectively, were kept at constant levels and let the groundwater flow in a longitudinal direction at a constant rate. The flow velocity was regulated by the hydraulic head of each end and the flow

rate was controlled at 1,402 L d^{-1}. At this flow rate, the transport of nonreactive solute was predominantly controlled by advection and by the longitudinal dispersion in the test tank, resulting in little lateral bypass flow among wells (Lee et al. 2008a). Transverse dispersion might possibly occur at a boundary between System A and B in the center of the tank, but its effect on molasses concentrations of both Systems was probably small. The hydrologic and geochemical parameters of the test tank are as follows: flow velocity = 120 cm d^{-1}; hydraulic conductivity = 8.01×10^{-2} cm s^{-1}; average aquifer thickness = 110 cm; and total organic carbon content = 0.11% based on measurements. Thirty SRM rods were placed to form a straight line in System A and sixty SRM rods were placed in a zigzag manner to form double straight lines in System B (Fig. 4-2). The two SRM systems were operated simultaneously for 96 d.

4.2.2 Water sampling and chemical analysis

A total of twelve monitoring wells were installed 0.25 m downstream of each barrier to monitor the released molasses concentrations (Fig. 4-2). The monitoring wells placed 2.25 m downstream collected the molasses released from the first barrier while wells at 3.25 m collected the molasses which accumulated from the first and second barriers. The wells at 4.25 m collected the accumulated molasses concentrations from the first, second, and third barriers. The monitoring wells were constructed with ~0.7 mm stainless steel diffusion stones (OD x L = 1.5 cm x 10.5 cm) attached to the end of

polyurethane tubing (ID = 8 mm). Sampling points were set at a depth of 100 cm below the top of the sand medium. Approximately 20 mL of groundwater sample was pumped out from each sampling point using a peristaltic pump and was subjected to chemical analyses. The COD value as an indirect indicator of the molasses concentration was determined using a UV-visible spectrophotometer (DR-2800, HACH, USA) with TNT821 reagent vial kit (with a detection range of 3~150 mg COD L^{-1}) immediately after each sampling. Water samples were collected twelve times during the test period.

4.2.3 Model application

While the SRM rods are placed into the wells installed in the sand tank to form the SRM system, molasses is slowly dissolved and released by diffusion into the groundwater passing through. The molasses dissolution mechanism from the SRM rods can be explained in terms of conventional mass transfer models such as the stagnant film model (Sherwood et al. 1975), in which the rate of mass transfer is proportional to the concentration difference between the boundary layer and the adjacent aqueous phase (Miller et al. 1990). However, the uncertainty in the SRM surface change upon dissolution interferes with the direct application of conventional models of the mass flux delineation; thus, a lumped scale mass transfer model was used in this study. The lump scale mass transfer model has long been used to predict DNAPL (Dense Non-Aqueous Phase Liquid) dissolution (Miller et al. 1990; Parker and Park 2004).

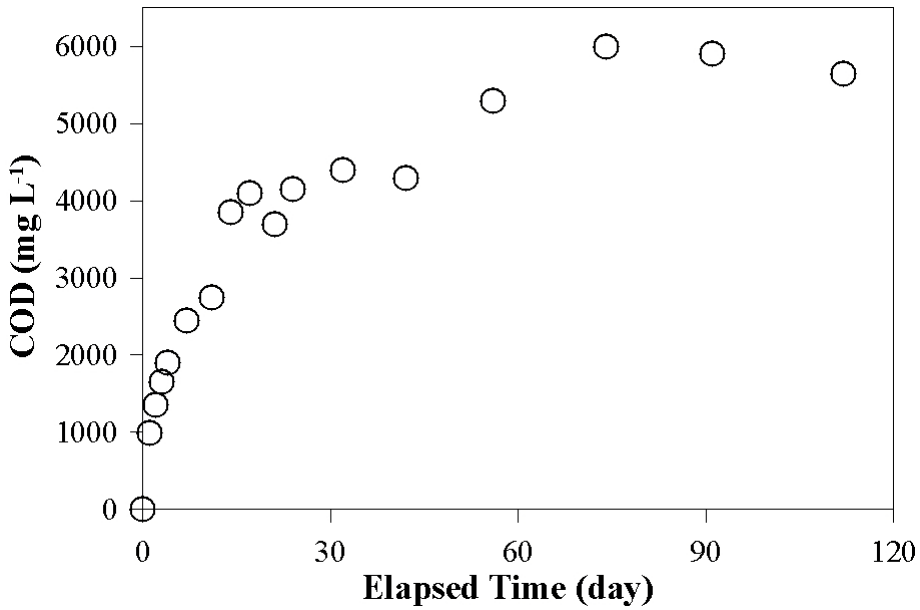
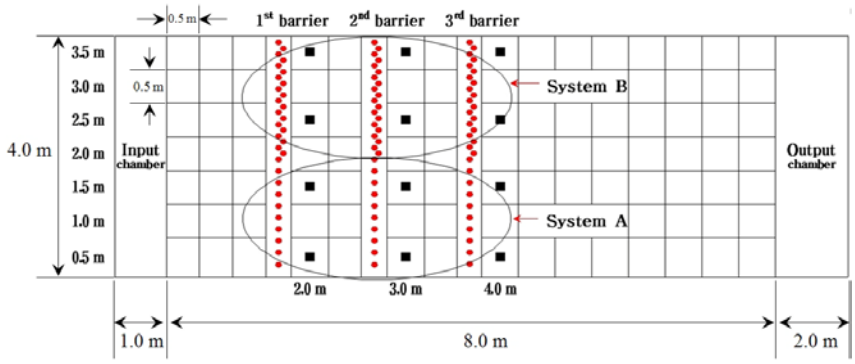
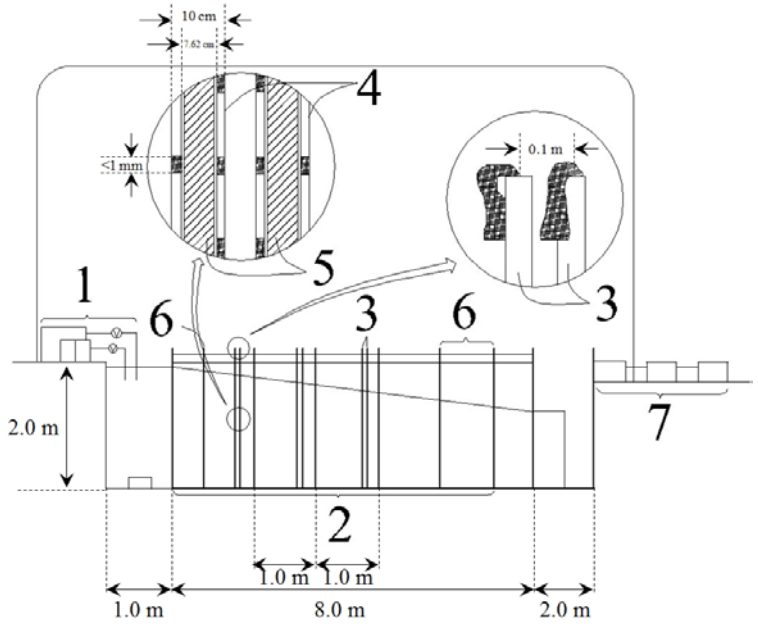


Fig. 4-1. Apparent solubility of the slowly released molasses rod using a batch scale test.



(a)



(b)

Fig. 4-2. A schematic diagram of SRM rods and barriers in the large test tank: (a) Top view, (b) Side view; ① the facility for composing artifically contaminated groundwater, ② a flow-tank, ③ a well-type barrier, ④ SRM well, ⑤ SRM material, ⑥ multi-level monitoring wells for water sampling, ⑦ a sewage system.

Parker and Park (2004) and Park and Parker (2005) developed a mass transfer function to solve field-scale DNAPL dissolution kinetics problems. They also provided closed-form equations for the mass depletion of the source zone and the mass flux leaving from the source zone over time (Park and Parker 2008). In this study, the molasses mass flux and the longevity of the SRM system is addressed by employing the solutions developed by Parker and Park (2004) and Park and Parker (2008). The upscaled mass transfer function (MTF) model used in this study is given by Parker and Park (2004) and Park and Parker (2005, 2008). The molasses mass flux and the field-scale mass transfer coefficient are expressed as follows:

$$J = -\kappa_{eff}(C_{eq} - \overline{C}) \tag{4-1a}$$

$$\text{where, } \kappa_{eff} = \kappa_0 \left(\frac{\overline{q}}{\overline{K}_S} \right) \left(\frac{M}{M_0} \right)^{\beta} \tag{4-1b}$$

where J is the molasses mass flux per unit volume of source region [$MT^{-1}L^{-3}$], κ_{eff} is the effective mass transfer coefficient [T^{-1}], $\overline{C}$ is the aqueous phase concentration [ML^{-3}], C_{eq} is the equilibrium concentration [ML^{-3}], $\overline{K}_s$ is the averaged saturate hydraulic conductivity of the source region [LT^{-1}], M_o is the initial molasses mass [M], M is the molasses mass as a function of time [M], κ_o is a rate coefficient related to the initial mass transfer rate [T^{-1}], β is a mass depletion exponent that accounts for the non-linearity of the mass transfer kinetics [-], and $\overline{q}$ is the average groundwater Darcian

velocity in the region including the SRM system [LT^{-1}].

Based on a mass balance, two closed-form mass flux solutions can be derived and conditioned on the model parameter of β (Park and Parker 2005).

$$J_T = \frac{\kappa_0 C_{eq} V_S \overline{q}}{\overline{K}_s} \exp(-Bt) \qquad \text{for } \beta=1 \qquad (4\text{-}2a)$$

$$J_T = \frac{\kappa_0 C_{eq} V_S \overline{q}}{\overline{K}_s} \left(\frac{((\beta-1)Bt + {M_0}^{1-\beta})^{1/(1-\beta)}}{M_0} \right)^{\beta} \qquad \text{for } \beta\neq 1 \qquad (4\text{-}2b)$$

$$\text{where,} \quad B = \kappa_0 C_{eq} V_s {M_o}^{-\beta} \overline{q} / \overline{K}s \qquad (4\text{-}2c)$$

where V_s is the gross volume of the SRM system [L^3]. Also t denotes the time [T].

The longevity at which the mass remaining in the SRM system reaches the minimal effective mass of M_{min} can be computed from

$$t(m_{ref}) = \frac{M_0(1-{m_{ref}}^{1-\beta})}{B{M_o}^{\beta}(1-\beta)} \qquad \text{for } \beta=1 \qquad (4\text{-}3a)$$

$$t(m_{ref}) = \frac{\ln(m_{ref})}{B} \qquad \text{for } \beta\neq 1 \qquad (4\text{-}3b)$$

where the relative mass index of m_{ref} [-] is identical to M_{min}/M_0. With Eq. 4.1~4.3, the molasses mass flux from the SRM system and the longevity

of the SRM system can be delineated once the model parameters are calibrated through field observations. However, the down-gradient effectiveness and the longevity of the SRM system are another problem which is mainly controlled by advection and the mechanical dispersion of the emanating molasses plume. To address this problem in more conservative manner, a two-dimensional analytical solution instead of one-dimensional stream tube ones, by considering a fully penetrating SRM system, on advective-dispersive solute transport with a volumetric source (Park and Zhan 2001) and associated FORTRAN code (Park and Parker 2005) was applied:

$$C(x,y,t)=\frac{1}{4L_xL_y\phi_a\sqrt{\pi RA_Lv}}\int_0^t J(t-\tau)\exp\left(-\frac{(Rx-v\tau)^2}{4RA_Lv\tau}\right)$$
$$\times\left[erfc\left(\frac{y+L_y/2}{2\sqrt{A_Tv\tau/R}}\right)-erfc\left(\frac{y-L_y/2}{2\sqrt{A_Tv\tau/R}}\right)\right]\frac{d\tau}{\tau^{1/2}} \qquad (4\text{-}4)$$

Where L_x is a length of the SRM system along the flow direction [L], L_y is a length transverse to the flow direction [L] which should be less than the sum of the capture width (W_c) of each well in one barrier (Sec. 5.3.3), A_L is the longitudinal dispersivity [L], A_T is the transverse dispersivity [L], φ_a is the aquifer porosity, v is the mean aquifer groundwater pore velocity downgradient of the SRM system [LT^{-1}], R is the retardation factor [-], τ is the integration variable, x [L] is the distance from the SRM barrier along the down-gradient direction, and $J(t\text{-}\tau)$ is the molasses mass flux leaving the SRM system through the down-gradient plane at time t-τ as computed from Eq. 4-2.

Substituting Eq. 4-2 into 4-4 and integrating the resulting expression numerically yields the vertically-averaged dissolved concentration at a given down-gradient location and time due to the decrease in molasses concentration of the SRM system zone with time. In the solution, it is assumed that the first-order decay of molasses is minimal. This is therefore ignored.

4.2.4 Boundary conditions

Simulation assumed the pseudo-steady state: constant dispersivities; a perfect sink condition (i.e., release in flowing groundwater); homogeneous initial agent distribution; no matrix degradation or swelling; and diffusion as the rate-controlling step (i.e., negligible dissolution kinetics). Source zone permeability for System A and B was assumed to be equal. The aquifer domain was discretized into 128 cells following an arrangement of the monitoring wells. One grid sized 50 cm x 50 cm (L x W), assuming one monitoring well was affected by molasses concentrations released from the SRM wells located in the same width of one barrier. To represent the molasses concentrations of the lumped SRM barrier, the monitoring wells should be located farther from the barrier. Suitability of the monitoring well locations was confirmed by a model approach by Lee et al. (2008a), indicating solute concentrations in the one monitoring wells reflect the mixed concentrations released from the front barrier. The left and right boundaries were impermeable to groundwater. No flow is permitted through lateral

boundaries parallel to the mean groundwater flow direction. To simplify the transport part, only molasses concentrations are calculated. The transport of molasses involves processes of advection and dispersion. Biodecay within the dissolved plume downgradient of the SRM system is not considered. In the application of Eq. 4-4, the maximum molasses solubility was assumed to be 6,000 mg COD L^{-1} based on the result of an apparent solubility test (Lee et al. 2010b). The retardation factor was determined to be 1.04 by a linear sorption isotherm using experimental values (bulk density, 1.68 g cm^{-3}; porosity, 0.45; organic carbon content, 0.11%) and a reference value (organic carbon normalized distribution coefficient of sucrose, 10 L kg^{-1}). Aquifer hydraulic parameters of the longitudinal and transverse dispersivities (0.1 and 0.01 m for a_L and a_T), average Darcian velocity (1.2 m d^{-1}), and permeability (70 m d^{-1}) were assigned from an earlier study based on the same sand tank (Lee et al., 2009). Dispersivity values were determined through previous non-reactive tracer tests for the sandy tank (Lee et al., 2009). For the calibration of the model parameters (κ_o and β), the observed and the predicted concentrations are fitted using a nonlinear regression method (Doherty and Hunt, 2010).

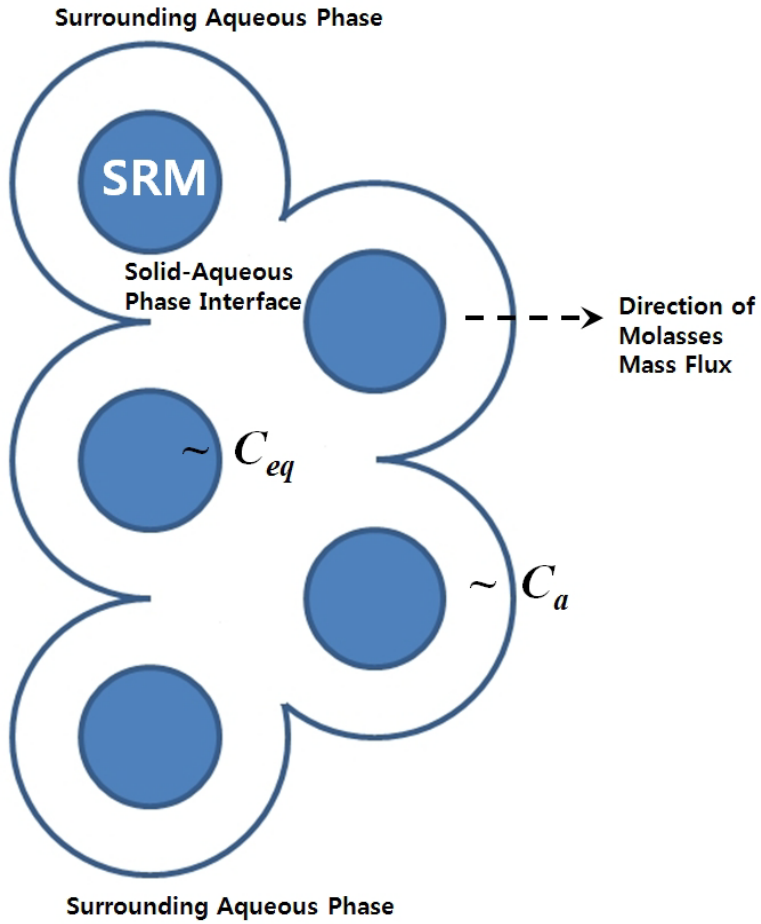


Fig. 4-3. A schematic view of molasses release from the SRM system with the lumped scale mass transfer model.

Table 4-1. Aquifer and SRM source parameters applied to the MTF model.

Model Parameters	Values
Dispersivities, a_L and a_T (m)	0.1, 0.01
Thickness of the aquifer (m)	1.1
Porosity of the aquifer (-)	0.45
Mean darcian velocity of the aquifer (m d^{-1})	1.2
First-order decay coefficient of the aquifer (d^{-1})	0.0
R, retardation factor of the aquifer (-)	1.04
z_0, z_1, source dimension along vertical axis (m)	0, 0.3
Permeability of the aquifer (m d^{-1})	70
Apparent solubility of molasses (mg L^{-1})	6,000
Mean darcian velocity of source zone (m d^{-1})	1.2

4.3 Results and discussion

4.3.1 SRM release test

In SRM system, diffusion controls the release process (Langer 1990; Lee and Schwartz 2007a, 2007b). Diffusion occurs in the wax-cellulose-sand matrix, where molasses is dispersed through mixing. The dissolution of molasses in the matrix develops secondary porosity and permeability, through which dissolved molasses is released by diffusion into groundwater passing through. By the diffusion process, the SRM material can continuously release molasses over relatively long periods with decreased release rates (Lee et al. 2010a).

In this study, the average molasses concentrations as COD values measured from the monitoring wells are presented in Fig. 4-4. The average molasses concentrations from the downstream monitoring wells were greater than those from upstream wells. For System A, the concentrations were determined to be 288, 565, and 763 mg COD L^{-1} after the first, second, and third barriers, respectively, after 5 d. As the release of molasses continued, the values at the same locations decreased with time, finally decreasing to 49, 74, and 95 mg COD L^{-1} after 96 d, respectively. For System B, they were 440, 495, and 1,150 mg COD L^{-1} after 5 d, and decreased to 69, 101, and 183 mg COD L^{-1} after 96 d, respectively. The average molasses concentrations in System B were approximately 1.7 times higher than those in System A because the number of SRM arrays of System B was double that of System A.

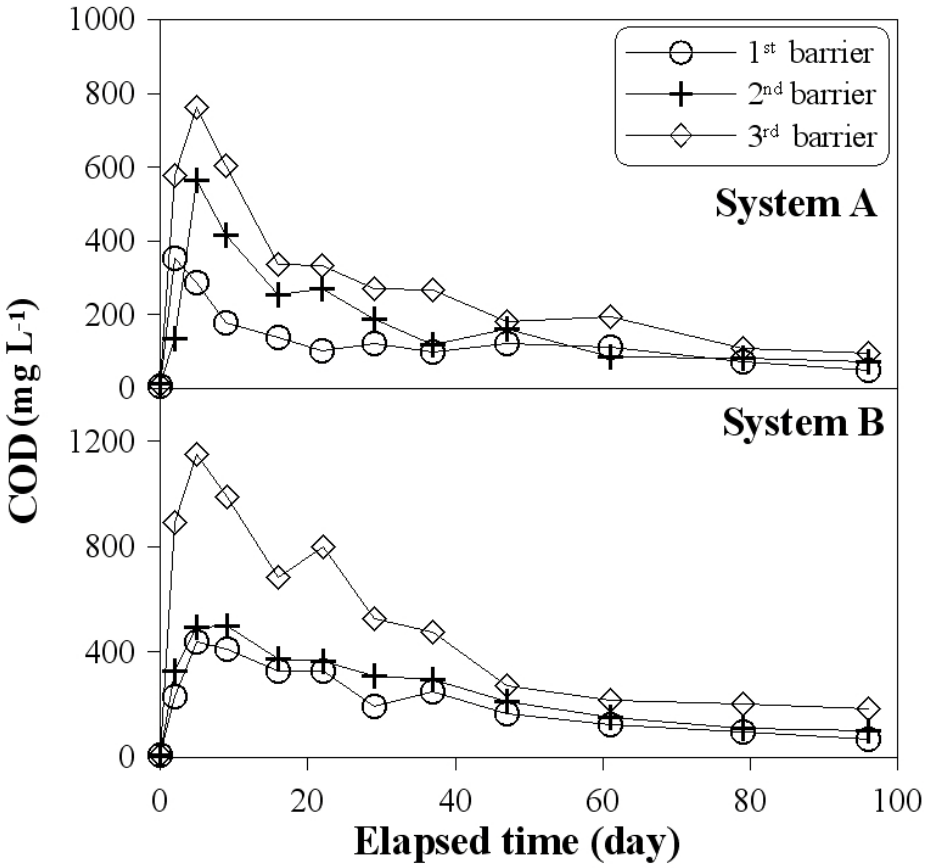


Fig. 4-4. Temporal changes of the mean COD concentrations in SRM systems.

For System B, the decrease in the molasses concentrations from the second barrier was similar to that from the first barrier. The SRM rods of the second barrier probably contained less molasses than the others due to the mixing uncertainties of the constituents during the SRM manufacturing process. Otherwise, the sampling points after the second barrier of System B may have been positioned between individual molasses plumes emitted from individual SRM rods, in which samples with relatively low concentration could be obtained. Overall, the monitoring results demonstrated that the SRM system could continuously supply molasses on the subsurface over an extended period of time.

4.3.2 Model simulation

The long-term molasses release from the SRM system was simulated with MTF model in Eq. 4-4. To yield the best-fit values of unknown model parameters, in this case κ_o and β, the modeled data were calibrated against the measured data using PEST (the parameter estimation code) (Doherty and Hunt 2010) (Fig. 4-5). The initial mass transfer rate coefficient κ_o was identified as 110, 190, and 260 d^{-1} for the first, second, and third barrier for System A, respectively, indicating nonlinear correlation between the κ_o values and the SRM barrier numbers due to the accumulated effect of three independent SRM barriers. Specifically, a κ_o value of 110 d^{-1} represented the initially lumped mass transfer rate coefficient for the first barrier. Also, the values of 190 and 260 d^{-1} represented those for the first and

second barriers, and for a total of three barriers, respectively. For System B, these values were determined to be 105, 110, and 220 d^{-1}, respectively, which shows close similarity indicating the resemblances of the source structures of the two systems. As mentioned in Sec. 4.3.1, if the molasses concentrations from the second barrier of System B were considered as abnormal values due to the manufacturing uncertainty of the SRM rods and/or the little representative sampling points, a nonlinear correlation may exist in System B. The mass depletion exponent β was identified to be 4.6 and 4.8 for System A and B, respectively. The high κ_o value indicates a large release of molasses at the early stages and the high β value indicates slow mass depletion progress (Park and Parker 2008). The calibrated model parameter κ_o values of System A were larger than those of System B, while the β value of System A was smaller than that of System B. These results denote that the molasses mass depletion progress of System B was relatively slow compared to that of System A (Fig. 4-6). Thus, it could be expected that longevity of System B was longer than that of System A. The computation results from Eq. 4-3 indicate that 90, 70, and 50% of the molasses mass remains 12, 63, and 267 d after the release for System A, respectively, while these values are 12, 65, and 291 d for System B (Fig. 4-6). With the calibrated model parameters and the semi-analytical solution in Eq. 4-4, the molasses mass flux of System A was determined to be 36, 62, and 85 mg COD d^{-1} in 1 d after the first, second, and third barrier, respectively.

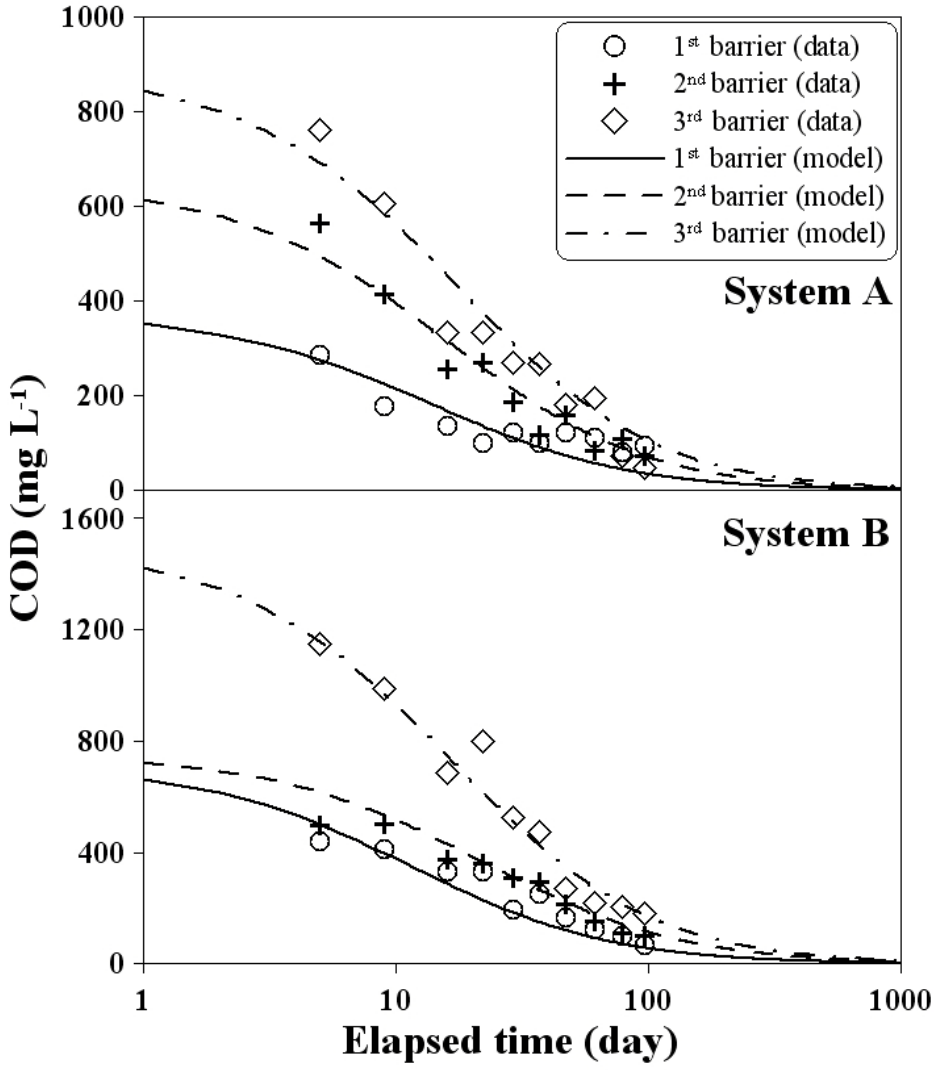


Fig. 4-5. Molasses release from each SRM barrier expressed as COD concentrations.

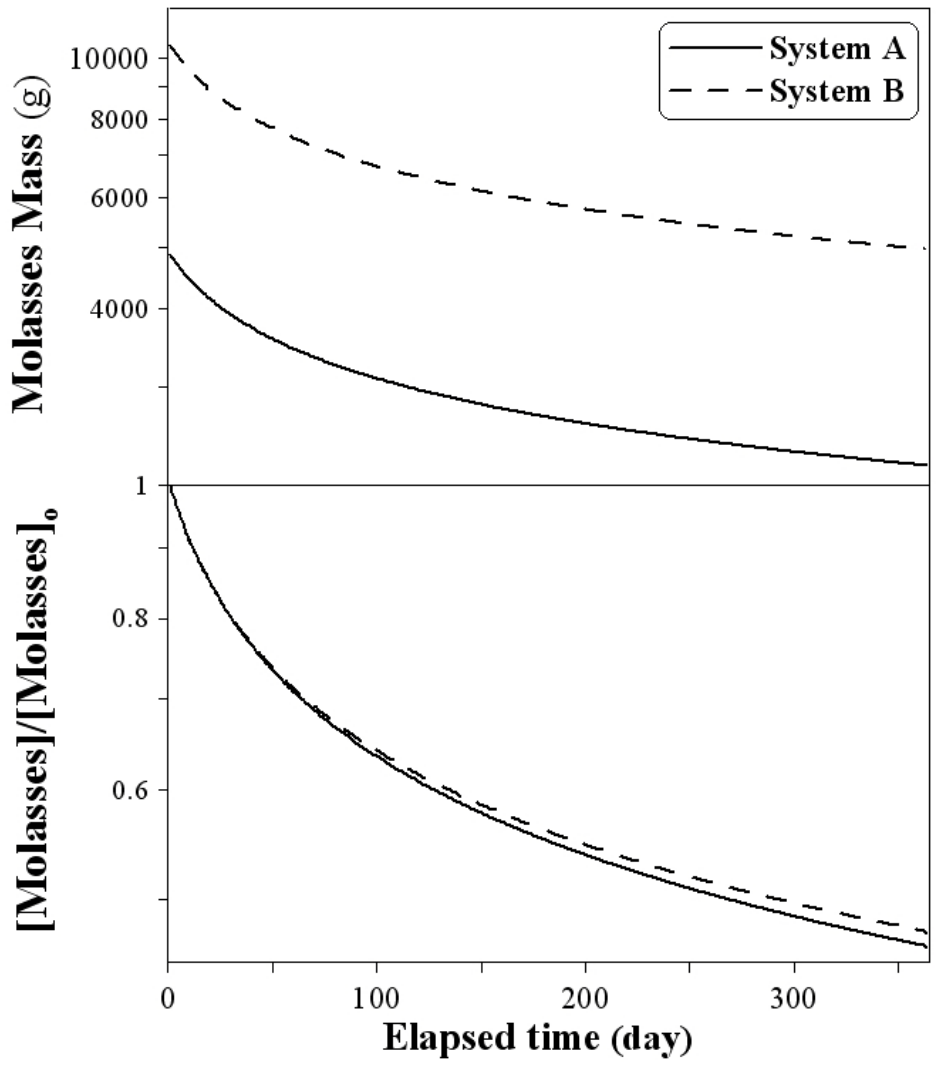


Fig. 4-6. Predicted molasses mass remaining in SRM systems.

These values decreased up to 22, 40, and 57 mg COD d^{-1} in 10 d, 3, 7, and 11 mg COD d^{-1} in 100 d, and 0.8, 1.6, and 2.5 mg COD d^{-1} in 1 yr, respectively (Fig. 4-7). For System B, these values were determined to be 99, 107, and 212 mg COD d^{-1} in 1 d; 56, 77, and 138 mg COD d^{-1} in 10 d; 8, 17, and 25 mg COD d^{-1} in 100 d; and 2, 4, and 6 mg COD d^{-1} in 1 yr, respectively (Fig. 4-7). These results demonstrated that the daily molasses release amounts of System B were larger than those of System A.

4.3.3 Longevity of the SRM system

To determine how long the SRM system can provide a carbon source (i.e., dissolved molasses) sufficient to transform nitrate microbially, the longevity values of both SRM systems were calculated from simulation results and the heterotrophic denitrifying stoichiometry. Assuming that sucrose is the main component of molasses (Table 4-2), the denitrification reaction is written as Eq. 4-5 and 4-6 (Hamlin et al. 2008).

$$O_2 + 0.0832C_{12}H_{22}O_{11} + 0.144NO_3^- \rightarrow 0.144C_5H_7O_2N + 0.048CO_2 + 0.229H_2O \quad (4\text{-}5)$$

$$0.088C_{12}H_{22}O_{11} + NO_3^- + 1.52H^+ \rightarrow 0.159C_5H_7O_2N + 0.42N_2 + 0.33CO_2 + 3.72H_2O \quad (4\text{-}6)$$

Aerobic growth of the denitrifier results in oxygen consumption and leads to initiation of the denitrifying process (Mora et al. 2003). Dennitrification is a microbial process in which the oxidized nitrogen substrates (i.e. nitrate and nitrite) are reduced to nitrogen gases. As a result, nitrate and nitrite replace molecular oxygen as electron acceptors.

Aerobic growth of the denitrifier results in oxygen consumption and leads to initiation of the denitrifying process is written as Eq. 4-5 (Mora et al. 2003). Denitrification is a microbial process in which the oxidized nitrogen substrates (i.e. nitrate and nitrite) are reduced to nitrogen gases, as written in Eq. 4-6. As a result, nitrate and nitrite replace molecular oxygen as electron acceptors. One mole of nitrate is transformed into 0.42 mole of nitrogen gas when 0.088 mole of sucrose as a sole carbon source is only consumed for denitrification by denitrifying microbes (Eq. 4-6). They can possibly decrease 2.1 mole of nitrate (30 mg-N L^{-1}) to 0.7 mole (10 mg-N L^{-1}, i.e., WHO drinking water quality guideline), consuming 0.126 mole (43 mg L^{-1}) of sucrose. Sucrose is the largest constituent of the sugar in molasses used for this study, accounting for 65%. Therefore, 43 mg L^{-1} of sucrose are included in 66 mg L^{-1} (74 mg COD L^{-1}) of molasses. Consequently, 74 mg COD L^{-1} of molasses is microbially used for removing 20 mg-N L^{-1} of nitrate. From Fig. 4-8, the calculated longevity of the System A used for removing 20 mg-N L^{-1} of nitrate was identified to be 47, 94, and 139 d for the first, second, and third barrier, respectively. The replacing period of the SRM rods was calculated to be 47 d when only one barrier (i.e., the first barrier) of System A was placed in this aquifer. The replacement period after

Table 4-2. Molasses constituents.

Classification			Relative Composition to Total Mass (%)	Relative Composition to Total Organic Mass (%)
Organics	Sugar	Glucose	5-10	8-17
		Sucrose	30-40	46-67
		Fructose	5-10	8-17
		Total Sugar	48-56	75-82
	Non-sugar	Crude protein	3	5
		Starches & Polysaccharides	4	6-7
		Methoxy group	3-4	5-7
		Organic acid	3	5
		Total Non-sugar	9-12	18-25
	Total Organics		60-65	100
Ash	Inorganics	Macronutrients (Na, K, Ca, Cl, P, S, Mg)	>5	
		Micronutrients (Cu, Fe, Zn, Mn, Co, Pb, Cd, As, Al)	<1	
		Total inorganics	8-12	
	Others		3-7	
	Total Ash		10-15	
Total Solids			75	
Moisture			25	

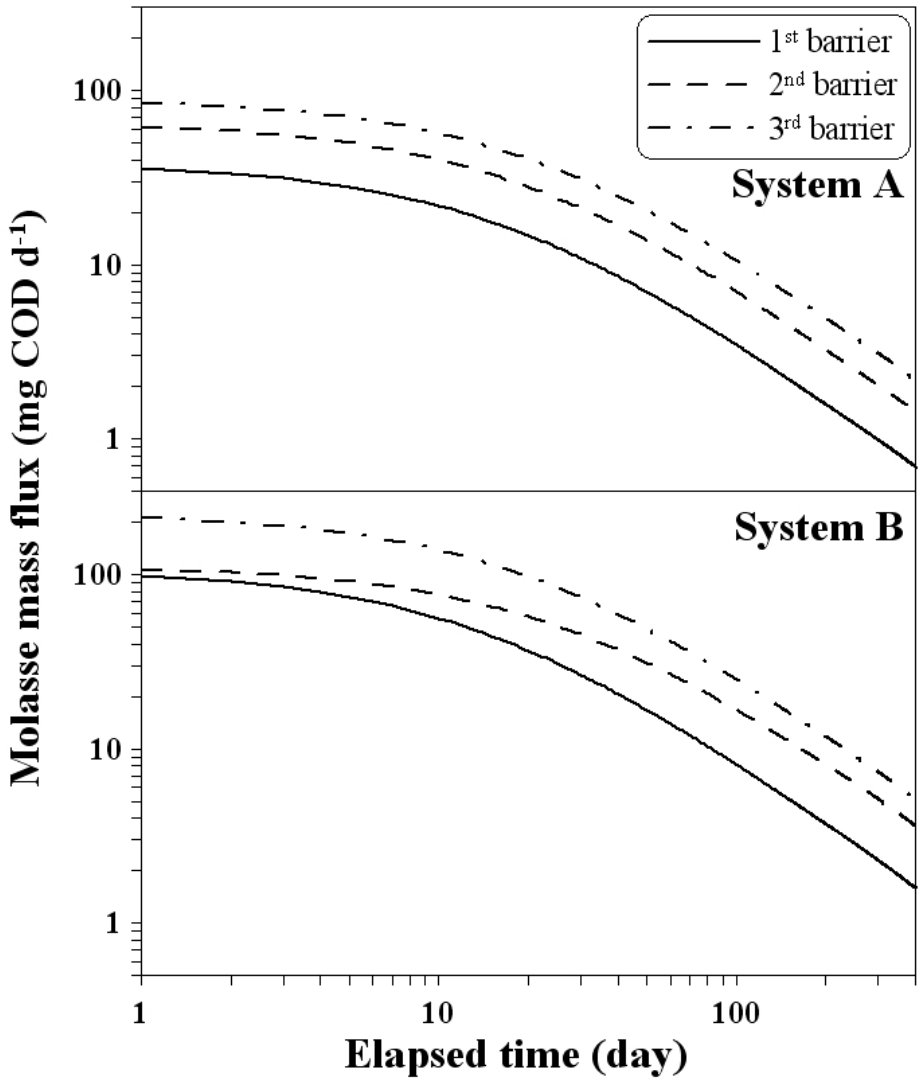


Fig. 4-7. Predicted molasses mass flux in each barrier of SRM systems.

the second and third barriers would be prolonged due to the accumulated molasses concentrations released from the first and second barriers, and all three barriers, respectively. For System B, the replacing period of the first, second, and third barrier was identified to be 76, 152, and 213 d, respectively. Therefore, the longevity values of System A and B could be considered to be 139 and 213 d, respectively, reflecting the total molasses concentration released from the three barriers. The longevity results suggest that two single barriers (i.e., the first and second barriers of System A) provide dissolved molasses for a long time than one double barrier (i.e., the first barrier of System B) despite the same number of SRM rods being placed. System B is better than System A in terms of the longevity. For a field application, the number of SRM barriers should be designated while considering the longevity.

The simulation results indicate that the longevity of the SRM system can be predicted by obtaining the site-specific calibrations of the κ_o and β values. For instance, if the κ_o and β of one SRM system are identified respectively as 190 d^{-1} and 4.6, the predicted longevity for decreasing 133 mg L^{-1} of nitrate to meet the drinking water quality guidelines is 94 d (i.e., 3 months). These two parameters can be determined by calibrating modeled data against measured data at the early stages, as shown in Fig. 4-5. Further, site-specific κ_o and β values can provide a useful guidance for determining the number of barrier required to comply with a target cleanup level (i.e., 10 mg-N L^{-1}) of nitrate-contaminated groundwater at a site of interest.

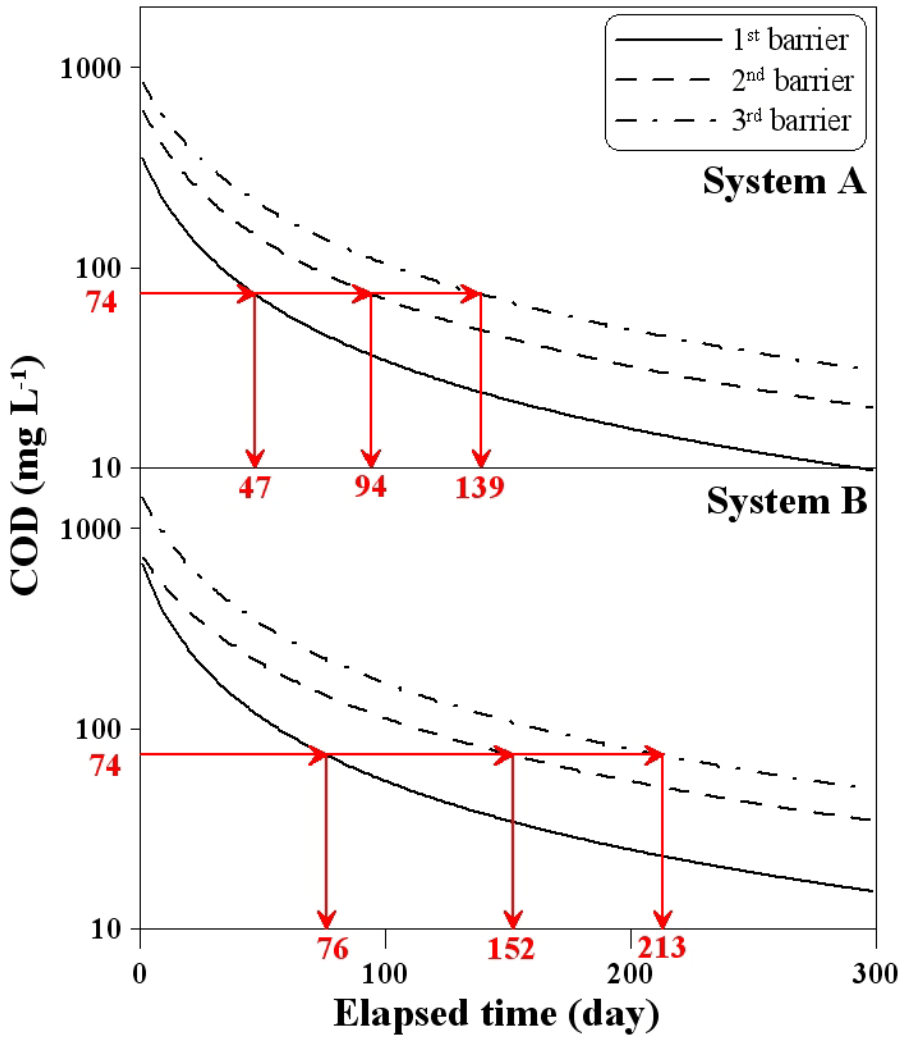


Fig. 4-8. Longevity of each SRM system to decrease 133 mg L^{-1} of nitrate up to drinking water quality guideline (44 mg L^{-1}).

4.3.4 Suitability of model application

Model simulation is a useful tool to characterize the SRM system in a quantified manner. It is efficient to estimate the longevity of the SRM system and to optimize design to various concentration/time requirements. Roseman and Higuchi (1970) developed a model to describe slowly-released behavior of a tablet via dissolution-diffusion in the field of pharmaceutical study. And it applied to describe the permanganate releasing behavior from the solidifying $KMnO_4$ of cylindrical form (Lee and Schwartz, 2007a). Molasses releasing behavior from a finite-height SRM rod can be possibly described with the pharmaceutical/permanganate models. Nonetheless, a limited problem regarding the heterogeneous distribution of molasses within SRM rod is still remained to be unsolved when those models are applied. Thus, a lumped scale mass transfer model (MTF) was selected to describe the slowly-released behavior of molasses from the SRM system itself in this study. Simulation results of MTF model is not affected by the homogeneous/heterogeneous distribution of molasses within the individual SRM rod but the arrangement of SRM rods within the SRM system. In addition, MTF model can provide the optimum arrangement of SRM rods to various nitrate concentrations/time requirements. Therefore, if the molasses concentrations at the individual monitoring points after each discrete barrier of the SRM system are similarly observed, the homogeneous distribution of molasses within the individual SRM rod can be possibly accepted despite it is not evenly distributed. Simulation results using molasses concentrations at

individual monitoring points after each discrete barrier demonstrated that the difference between two monitoring points after each barrier was around ±15% and ±20% for System A and B, respectively. Therefore, if individual values at the individual monitoring points were applied into the MTF model, it might result a little difference on the simulation result when average ones were applied (Sec. 4.3.1~4.3.3). Nonetheless, it is considered that the difference may not affect on predicting the longevity of the SRM system.

4.4 Conclusions

A pilot scale study was conducted to characterize the performance of molasses release from a well-type barrier system harboring solidifying molasses named the slowly released molasses (SRM) as a reactive medium to promote indigenous denitrifying activity. Two SRM systems harboring 30 and 60 SRM rods, named as System A and B, respectively, were constructed in a large flow tank (L x W x D = 8 m x 4 m x 2 m) filled with natural sands. These two systems continuously delivered molasses with groundwater flow over 96 d. Results from pilot-scale flow-tank experiments and model simulations demonstrate that the SRM system can supply molasses for heterotrophic denitrification in groundwater over an extended period of time. With the aid of a MTF model simulation, the molasses mass flux and the longevity of the SRM system can be controlled by adjusting the number of SRM barriers and the array of the SRM rods per barrier. This is important not only to optimize the heterotrophic denitrification process but also to minimize possible secondary groundwater contamination by the extra carbon source provided. Further studies regarding quantification of molasses release concentration within SRM source zone are required in order to achieve more precise result to the MTF model validation. Therefore, water samples from the SRM wells should be pumped, analyzed, and applied to the MTF model in the further studies. In the field, removal efficiency would depend on the dispersive mixing degree between the released molasses and the nitrate plume. Lee et al. (2008a) demonstrated that limited transverse dispersion led

to incomplete mixing and a decrease in the removal efficiency. They suggested the additional placement of doublet injection/withdrawal wells to facilitate lateral spreading and mixing, which is an important issue for the SRM method from an engineering perspective. The high β value indicates slow mass depletion progress. Even though β value ranges from 4.6 to 4.8 for two SRM systems in this study, the effort to increase β value for achieving more long-term remedial requirements of the contaminant plume. Further studies regarding changes of the mixing rate of SRM constituents, size, and their volumes to maximize treatment periods are required.

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CHAPTER 5

THE SLOWLY RELEASED MOLASSES BARRIER SYSTEM FOR CONTROLLING NITRATE PLUME: A LARGE TEST TANK STUDY

5.1 Introduction

Nitrate contamination of shallow groundwater has been found in many rural areas worldwide over the last few decades due to the excessive usage of animal manure and nitrogenous fertilizers to enhance crop yields and due to the land disposal of domestic wastewaters. Nitrates can cause methemoglobinemia in infants, and they pose other health-related problems in rural populations who depend on shallow groundwater as a water supply (Bouchard et al., 1992). The discharge of nitrate-contaminated groundwater into wetlands, rivers, estuaries, and the coastal environment can contribute to toxic algal blooms in these water bodies which can in turn cause various health problems (Appleyard and Schmoll, 2006).

Conventional technologies such as ion exchange, reverse osmosis, electrodialysis, and distillation are available for treating nitrate in groundwater. However, such technologies are mechanically complex,

require periodical maintenance, and are generally cost-prohibitive (Moon et al., 2008). As an alternative, molasses, a cheap sugar-industry by-product, has been recommended as a reliable carbon source comparable to commercial carbon sources (Ten Have et al., 1994; Boaventura and Rodrigues, 1997; Lee et al., 2001; Quan et al., 2005; Hamlin et al., 2008; Roy et al., 2010). For the remediation of nitrate-contaminated groundwater, subsurface biofilm barriers using liquid-phase molasses and a heterotrophic denitrifier have been demonstrated (Cunningham et al., 2003; Dutta et al., 2005).

Recently, a heterotrophic bacterial denitrification combined with a well-type permeable reactive barrier system using solidifying molasses known as slowly released molasses (SRM system) has been developed as a long-term semi-passive, *in situ* remedial option for the treatment of nitrates in groundwater (Lee et al., 2010a, 2012). The SRM system is operated with the periodic addition of slowly released molasses (SRM) rods into well-type reactive barriers without hydraulic disturbance. As groundwater flows through the wells, molasses is continuously released, resulting in the enrichment of heterotrophic denitrifiers. The dynamics of the SRM system were investigated by a series of a batch tests, a lab-scale column test, pilot-scale sandy tank tests, and by means of transport modeling. In the column-scale experiment with the SRM rods, the heterotrophic denitrifier *Pseudomonas* sp. KY1 with nitrite reductase (*nirK*) successfully removed nitrate from 89 to less than 13 mg L^{-1} over a course of 361 hrs (Lee et al. 2010a). From the results of pilot-scale longevity tests of the SRM system

and model simulations, the SRM system was found to be able to supply molasses continuously for heterotrophic denitrification in groundwater over a course of 96 d (Lee et al., 2012). One of next steps in developing this remedial approach is to demonstrate remedial efficiencies in a pilot-scale flow tank. In this study, pilot-scale SRM system operations were performed to determine the heterotrophic nitrate removal efficiency levels and to identify the optimum injection amounts of SRM rods in order to minimize remaining molasses

5.2 Materials and methods

5.2.1 Preparation of the SRM rods

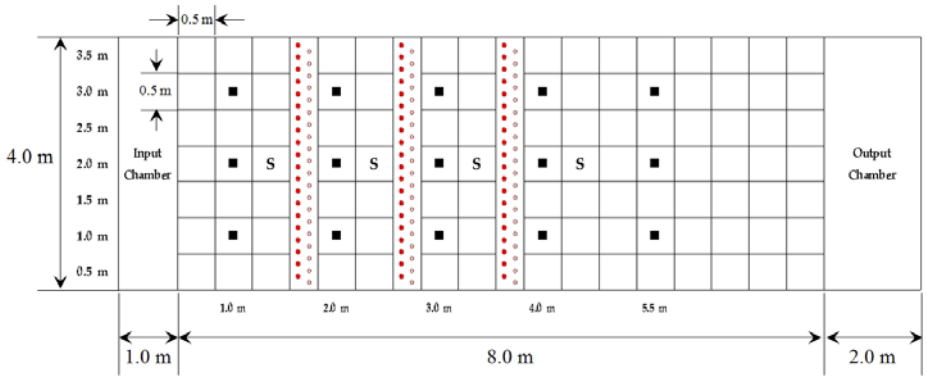
A prototype SRM rod (OD x L = 4 cm x 30 cm) was made by dispersing 177 g of molasses (Hydex, Korea) in a 360 g paraffin wax-cellulose-silica sand matrix (215 g paraffin wax, 109 g cellulose, and 36 g silica sand) in a cylindrical mold at an ambient temperature. The paraffin wax-cellulose-silica matrix was used to prevent the instant dissolution of molasses from the rod. Lee and Schwartz (2007a, 2007b) reported that an inert organic crystalline matrix can accomplish slow dissolution and the diffusion-controlled transport of molasses. The apparent molasses solubility of a SRM rod determined that the chemical oxygen demand values (COD L^{-1}) were approximately 6,000 mg L^{-1} from a batch-type release test conducted for 112 d (Lee et al. 2010b).

5.2.2 Flow-tank setup and SRM system operation

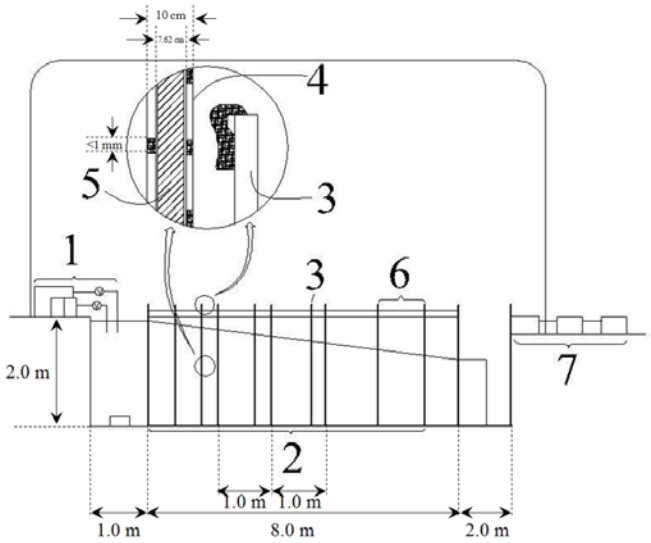
Details of the pilot-scale flow tank facility used for this study are described in Chapter 4 and are summarized below. A total of 120 polyvinylchloride (PVC) screened wells (OD x L = 10 cm x 150 cm) consisting of three layers of discrete barriers were installed at 1-m intervals in the natural sandy media of the flow tank (L × W × D = 8 m × 4 m × 2 m). In each barrier, forty wells were arrayed in a zigzag shape vertical to the

flow direction to could deliver molasses to the groundwater (Fig. 5-1). The input and output chambers at the upstream and downstream ends, respectively, of the tank were kept at constant levels to let the groundwater flow in a longitudinal direction at a constant rate. The flow velocity was regulated by the hydraulic head of each end. Hydrologic and geochemical parameters were estimated as follows: flow velocity = 120 cm d^{-1}; hydraulic conductivity = 8.01×10^{-2} cm s^{-1}; porosity = 0.45; total organic carbon content = 0.11%; with a bulk density of 1.47 g cm^{-3} based on the measurements. The SRM system could continuously deliver molasses by itself into groundwater over 96 d, while decreasing the average molasses concentrations from 763 to 95 mg COD L^{-1} for the single-straight-line type of SRM system, as shown in Fig. 5-1a, and 1,150 to 183 mg COD L^{-1} for the double-straight-line type of SRM system, as shown in Fig. 5-1b, respectively. After the completion of the molasses releasing experiments, the SRM rods were removed from the wells. The flow tank was flushed with tap water for 5 months before being drained.

After the tap water flushing procedure, nitrate removal tests using the SRM system were conducted. To formulate synthetic groundwater, four 1 L volumetric flasks filled with 78 g L^{-1} of nitrate solution were prepared. These solutions were diluted 250 times with tap water to yield 1,000 L of 312 mg L^{-1} of nitrate solution in a large nitrate storage tank connected to an input chamber for their introduction into the flow tank. The nitrate flux from the storage tank was regulated using an automated pumping system.

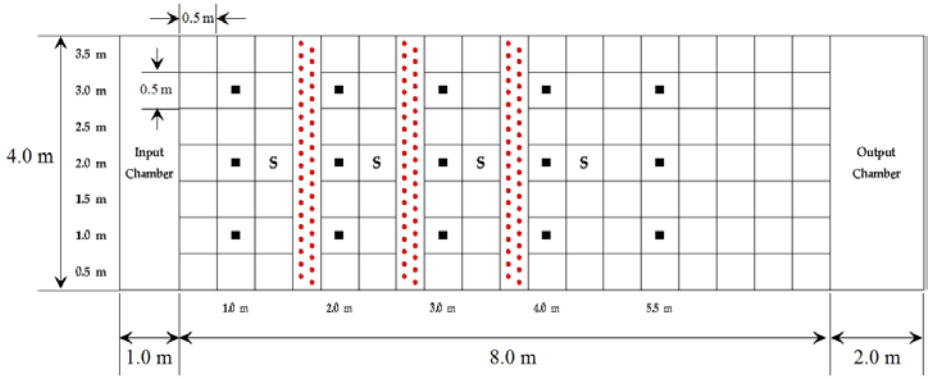


(a)

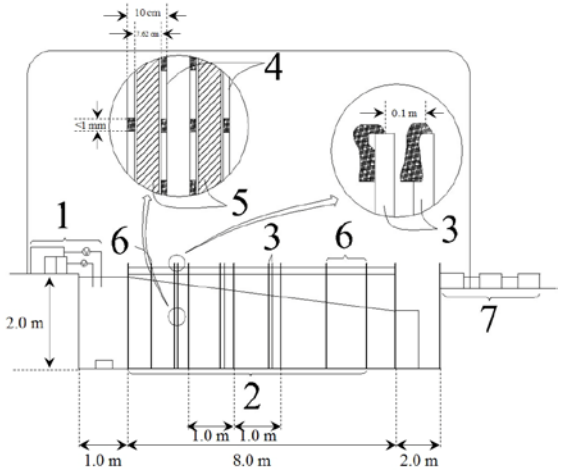


(b)

Fig. 5-1. A schematic diagram of the a large test tank setup: (a) Top view, (b) cross-section view of single straight line type of SRM system for test I, (c) Top view, (d) cross-section view of double straight lines type of SRM system for test II and III.



(c)



(d)

Closed circle (●), closed square (■), and letter (S) represent for SRM rod, groundwater-, and soil sampling point, respectively. ① the facility for composing artifically contaminated groundwater, ② a flow-tank, ③ a well-type barrier, ④ SRM well, ⑤ SRM material, ⑥ multi-level monitoring wells for water sampling, ⑦ a sewage system.

The input chamber was then filled with 142 mg L^{-1} of nitrate solution by introducing both tap water (600 L d^{-1}) and diluted nitrate solution (500 L d^{-1}) from a water supply line and from a nitrate storage tank, respectively. Mixing was facilitated by two underwater circulators in the input chamber. The synthetic groundwater contained basal salts of 19 mg L^{-1} of NH_4Cl, 7 mg L^{-1} of KH_2PO_4, 19 mg L^{-1} of K_2HPO_4, and 19 mg L^{-1} of $MgSO_4$. The natural sandy media in the flow tank was saturated by 142 mg of L^{-1} nitrate solution for 50 d before the placement of the SRM rods. When the nitrate concentrations in the flow tank were stabilized at a level of 142 mg L^{-1}, the SRM rods were placed in the wells to construct the SRM system (L × W × D = 3 m × 4 m × 1.5 m). The flow velocity in the flow tank was regulated so that it was 120 cm d^{-1}. Three SRM systems were operated at room temperature, as follows: (i) System A: a single-straight-line type of SRM system containing 60 SRM rods with a test period of 13 d, (ii) System B: a double-straight-line type of SRM system containing 120 SRM rods with a test period of 21 d, (iii) System C: a double-straight-line type of SRM system containing 120 SRM rods with the addition of the external heterotrophic denitrifier *Pseudomonas* sp. KY1 (Lee, 2010) in order to increase the removal efficiency with a test period of 55 d.

5.2.3 Sample collection and chemical analysis

Water samples were collected from 15 multi-level monitoring wells screened at depths of 1.0 and 2.0 m using a peristaltic pump (Eijkelkamp Co.

Ltd., Netherland). The SRM rods were placed up to 1.5 m below the surface of the sandy tank; therefore, the SRM system could only supply molasses within a depth of 1.5 m in the sandy tank. Water samples collected from 1.0 and 2.0 m depths were used as experimental and control groups, respectively. To measure the nitrate amounts and the nitrite concentrations, 40 mL water samples were filtered (0.2 μm) and stored in a refrigerator at 4℃ until an IC analysis (DX-80, Dionex, USA). Additional water samples (20 mL) for measuring the COD values as indirect molasses concentrations were collected in amber vials and were analyzed using a UV-visible spectrophotometer (DR-2800, Hach Co. Ltd., USA) immediately after sample collection. The on-site properties of the temperature, pH, and dissolved oxygen value (DO) were immediately measured by a portable multi-sensor (Thermo Orion 3-star series, USA). In addition, the electric conductivity (EC) value was measured by a portable conductivity meter (TOA, CM-14P, Japan).

5.2.4 Identification of nitrate reductase and denitrifiers

A genetic analysis of denitrification usually proceeds from the nitrite reductase (*nirK*) step, because the nitrite reduction process is a rate-limiting step in denitrification (Goregues et al., 2005). In order to identify the *nirK* gene, soil samples were collected from four points (1, 2, 3, and 4 m downstream from the input chamber) in the sandy tank (Fig. 5-1). The samples were subjected to extraction of the bulk DNA and were then

analyzed by PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis). Bulk DNA genes were obtained from the soil using the FastDNA® SPIN kit for soil (MP Biomedicals, USA).

PCR was used to amplify denitrification gene encoding nitrite reductase (*nirK*) from the bulk DNA using the primers F1aCua and R3Cu (Hanllin and Lindgren, 1999, Throbäck et al., 2004). The PCR program ran for 2 min at 94℃, 35 cycles for 2 min at 94℃, 1 min at 51℃, and 1 min at 72℃. Using these PCR products, a DGGE analysis was performed following the standard protocol of the D-Code™ system (Bio-Rad, USA). Gels consisted of 1.0-mm thick 9% polyacrylamide, with a denaturant gradient of 45~55% urea-formamide solution (100% of the concentration of the denaturants was 7 M urea and 40% deionized formamide). Electrophoresis was performed in 1 × TAE (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA; pH 8.3) at 60℃ and at 100 volts for 7 hours. The DGGE gels were stained with 1 × GreenStar staining dye solution (Bioneer, Korea) and the bands were visualized using a UV illuminator (302 nm, Vilber Lourmat, France). The DGGE band intensities were quantified using Bio-Rad Quantity One software (v.5.2). Sequence chromatograms of the *nirK* gene from DGGE were compared with the database sequences in GenBank. For System C, the heterotrophic denitrifier *Pseudomonas* sp. KY1 (Lee, 2010) was enriched in a 1,000 L volumetric tap-water tank filled with a liquid medium containing 582 mg L^{-1} of KNO_3, 19 mg L^{-1} of NH_4Cl, 7 mg of L^{-1} of KH_2PO_4, 19 mg L^{-1} of K_2HPO_4, and 19 mg L^{-1} of $MgSO_4$ at 25℃ during 15 days. Then, it was inoculated into the flow-tank sand to promote a

synergistic effect on the nitrate removal efficiency with the indigenous denitrifier.

PCR was also used to identify indigenous heterotrophic denitrifiers. To obtain 16S rDNA genes for the microbial community structure analysis, the extracted bulk DNA genes were amplified with 27F and 1522R primers using the GeneAmp PCR System 9700 (Applied Biosystems, USA). Another round of PCR was performed with the amplified 16S rDNA genes for a DGGE analysis. One μL each of GC-338F and 518R primer solutions (10 pmol μL^{-1} each), 20 ng of the amplified, purified 16S rDNA genes, and Accupower HotStart PCR Premix (Bioneer, Korea) were combined to give a final reaction volume of 20 μL. The next steps were as follows: 10 min at 94℃ for initial denaturing, 30 cycles of denaturation for 45 sec at 94℃, 45 sec at 55℃ for annealing, and 45 sec at 72℃ for an extension. Using these PCR products, a DGGE analysis was performed following the standard protocol of the D-Code™ system (Bio-Rad, USA). Sequence chromatograms of 16S rDNA were compared with database sequences in GenBank. Sequence editing and alignments were performed using CLUSTAL X (Higgins and Sharp, 1988).

5.3 Results and discussion

5.3.1 Nitrate removal efficiency

In the SRM system, diffusion controls the release process (Langer 1990; Lee and Schwartz 2007a, 2007b). Diffusion occurs in the wax-cellulose-sand matrix, where molasses is dispersed by mixing. The dissolution of molasses in the matrix develops secondary porosity and permeability, through which the dissolved molasses is released by diffusion into groundwater passing through. Due to the diffusion process, the SRM material can continuously release molasses over relatively long periods of time with decreased release rates (Lee et al. 2012). The molasses used for creating the SRM rod contained ~65% organics (~56% sugar and ~9% non-sugar constituents), ~10% inorganics, and ~25% water. Sucrose was the largest constituent of the sugar in molasses, accounting for ~38%, while the other constituents were glucose at ~9% and fructose at ~9%. Assuming sucrose as the main carbon source in the molasses, the denitrification reaction is written as Eq. 5-1 and 5-2 (Hamlin et al., 2008).

$$O_2 + 0.0832C_{12}H_{22}O_{11} + 0.144NO_3^- \rightarrow 0.144C_5H_7O_2N + 0.048CO_2 + 0.229H_2O \quad (5\text{-}1)$$

$$0.088C_{12}H_{22}O_{11} + NO_3^- + 1.52H^+ \rightarrow 0.159C_5H_7O_2N + 0.42N_2 + 0.33CO_2 + 3.72H_2O \quad (5\text{-}2)$$

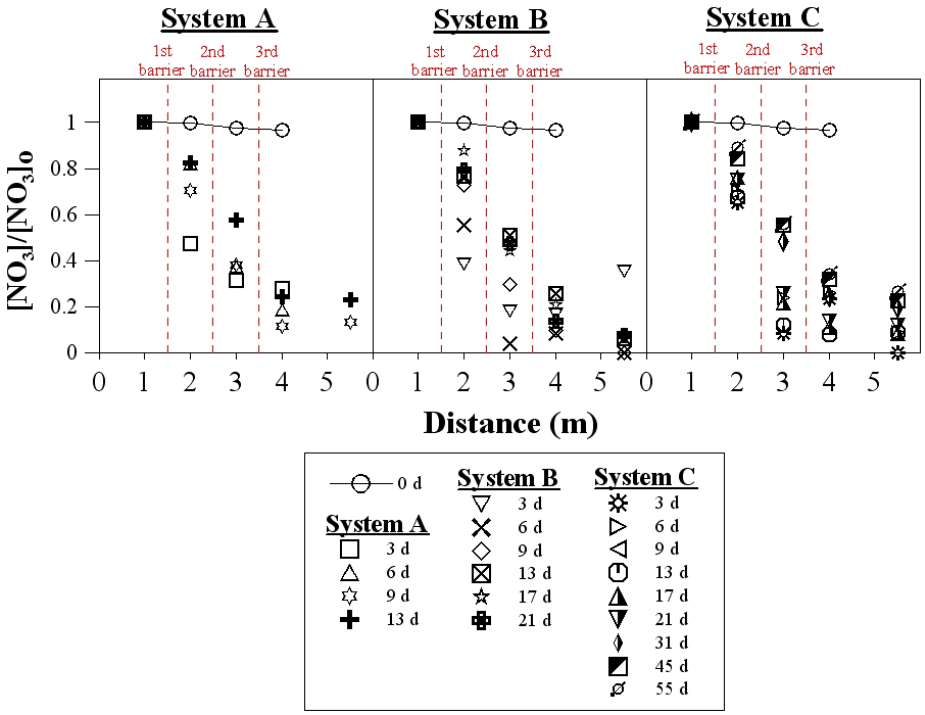


Fig. 5-2. Temporal and spatial changes of mean nitrate concentrations monitored at 25 cm downstream of each barrier.

Aerobic growth of the denitrifier results in oxygen consumption and leads to the initiation of the denitrifying process (Mora et al., 2003). Denitrification is a microbial process in which the oxidized nitrogen substrates (i.e., nitrate and nitrite) are reduced to nitrogen gases. As a result, nitrate and nitrite replace molecular oxygen as electron acceptors. Organisms with a denitrification capacity are widely distributed and exist at high densities in nature (Tiedje et al., 1982). In agricultural soils, for example, the denitrifier population is often $1\sim5 \times 10^6$ organisms per g^{-1} of soil (Gamble et al., 1977). Temporal and spatial variations in nitrate concentrations measured from the monitoring wells are presented in Fig. 5-2. Clearly, nitrates were actively destroyed by the indigenous heterotrophic denitrifier in the SRM system. The removal efficiencies generally increased downstream as the nitrate plume flowed through the three discrete SRM barriers. For System A with an experimental group with a depth of 1.0 m, the initial 142 mg L^{-1} nitrate concentrations decreased by an average of 29% (up to 101 mg L^{-1}), 59% (58 mg L^{-1}), and 80% (28 mg L^{-1}) after the first, second, and third barriers during 13 days, respectively. For System B, the nitrate concentrations decreased by an average of 32% (97 mg L^{-1}), 68% (45 mg L^{-1}), and 84% (23 mg L^{-1}) after the first, second, and third barriers during 21 days, and for System C, the average decreases were 25% (107 mg L^{-1}), 71% (41 mg L^{-1}), and 79% (30 mg L^{-1}) during 55 days, respectively. At 5.5 m downstream (i.e., 1.75 m behind the third barrier), the nitrate concentrations decreased by an average of 81% (27 mg L^{-1}), 90% (14 mg L^{-1}), and 88% (17 mg L^{-1}) for Systems A, B, and C, respectively. Removal efficiencies at 5.5 m

downstream were 1~9% higher than those after the third barrier. The indigenous denitrifier at 5.5 m removed the remaining nitrate using the remaining molasses behind the SRM system. Unfortunately, the effect of the extra heterotrophic denitrifier *Pseudomonas* sp. KY1 on the nitrate removal efficiencies was not identified, showing the removal efficiencies after the first, second, and third barrier of System B were similar to those of System C. It was assumed that the populations of indigenous denitrifiers in the sands were plentiful enough to remove nitrate in groundwater during the test periods. Therefore, it resulted in the little effects of the *Pseudomonas* sp. KY1 on the denitrification for System C. For the control group at a depth of 2.0 m, the nitrate concentrations did not decrease for Systems A, B, and C, as no denitrifying activity occurred corresponding to the lack of a molasses supply from the SRM systems.

The nitrate removal efficiencies slowly decreased with time due to the decreasing released molasses concentrations. These values for Systems B and C slowly decreased compared with System A because the mass fluxes of Systems B and C were higher than those of System A at the same monitoring time (Lee et al., 2012). The removal efficiencies decreased from 89% (9 d) to 76% (13 d) for System A, from 90% (9 d) via 74% (13 d) to 86% (21 d) for System B, and from 81% (9 d) via 92% (13 d) and 86% (21 d) to 66% (55 d) for System C. At 5.5 m downstream, these values were identified to exist in a range of 87% (9 d) and 77% (13 d) for System A, from 99% (9 d) via 94% (13 d) to 93% (21 d) for System B, and 97% (9 d) via 91 (13 d) and 88% (21 d) to 74% (55 d) for System C. Nitrite-nitrogen concentrations were

observed within a small range of 0.1 to 8.2 mg L^{-1} in the SRM systems (Fig. 5-3). In denitrifying process, nitrite accumulation could possibly occur at the initial stage due to relatively low nitrite reduction rate compared with nitrate reduction one (Sec. 3.3.3). In Fig. 5-3, the nitrite-nitrogen concentrations were high at the initial stage of the each System, but gradually decreased to the end of the test periods. This indicated that a complete denitrification of nitrate to nitrogen gas was the primary reaction taking place on the subsurface, and a complete denitrification pathway arose in the SRM systems. During the tests, groundwater levels were daily monitored in order to identify a pore clogging problem due to microbial growth. The levels have rarely changed during the test, indicating little occurrence of a pore clogging. It demonstrates the well type barriers using slowly release material can be applied for the long-term nitrate treatment without the clogging problem.

5.3.2 Remaining molasses concentrations

The average COD concentrations as an indirect indicator of the remaining molasses concentrations simultaneously measured with the nitrate concentrations (Fig. 5-4). For the experimental group at a depth of 1.0 m, these generally increased downstream due to the cumulative effect of the three discrete barriers, indicating averages of 81, 105, and 179 mg COD L^{-1} after the first, second, and third barriers for System A during 13 d, averages of 329, 287, and 377 mg COD L^{-1} for System B during 21 d, and averages of 148, 167, and 228 mg COD L^{-1} for System C during 55 d, respectively.

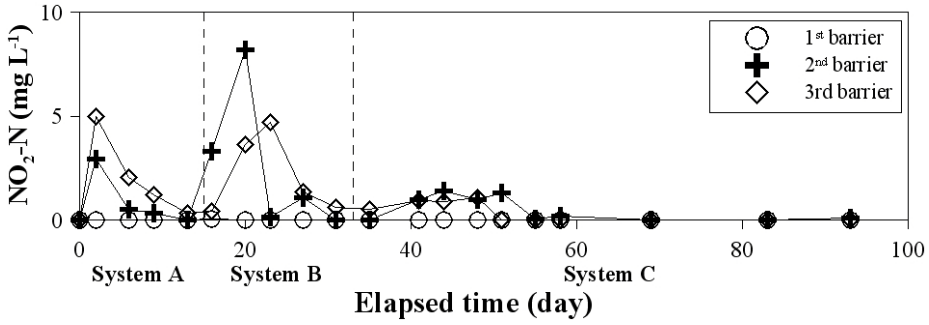


Fig. 5-3. Temporal and spatial changes of nitrite-nitrogen concentrations monitored at 25 cm downstream of each barrier.

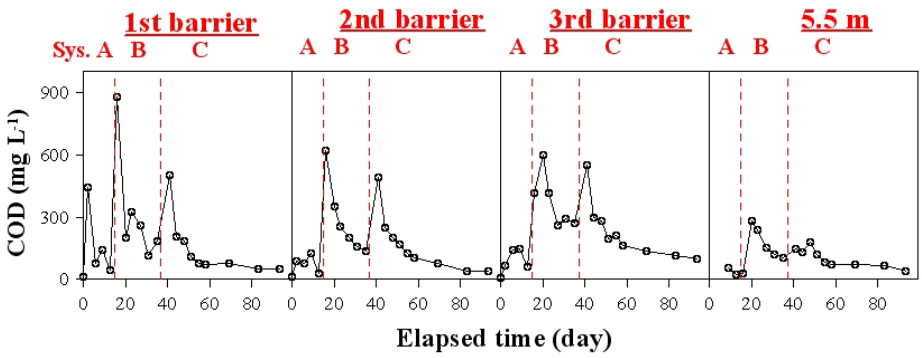


Fig. 5-4. Temporal and spatial changes of mean molasses concentrations monitored at 25 cm downstream of each barrier. Molasses concentrations were expressed as molasses-COD.

For the control group at a depth of 2.0 m, the mean COD concentration was 9 mg L^{-1} for Systems A, B, and C, which was similar to the natural value of 8 mg L^{-1} in the sandy media.

The remaining molasses concentrations slowly decreased with time due to the decreasing molasses concentrations of SRM systems (Lee et al., 2012). These values slowly decreased with time, going from 598 (3 d) to 141 mg COD L^{-1} (13 d) for System A, from 1,917 (3 d) via 721 (13 d) to 595 mg COD L^{-1} (21 d) for System B, and from 1,547 (3 d) via 470 (13 d) and 340 (21 d) to 193 mg COD L^{-1} (55 d) for System C. The remaining molasses resulted in a slight increase in the turbidity of the groundwater to the naked eyes. Turbidity is the cloudiness or haziness of a fluid caused by small suspended matters such as sedimentary particles, organic substances, bacteria, planktons, algae and so on. These matters consisting of small particles will settle very slowly or not at all if the particles are colloidal, which cause the liquid to appear turbid. In this study, turbidity was caused by the remaining molasses, which might be considered as hardly biodegradable materials (Sec. 5.3.3). Turbidity exceeding the permissible groundwater quality guideline can have a detrimental effect on human health and well-being. If the issues of turbidity can be resolved, molasses may be a more attractive carbon source for denitrification due to its very low cost and high bioavailability (Hamlin et al., 2008).

5.3.3 Actual nitrate removal and molasses consumption

Although the SRM system resulted in relatively high nitrate removal efficiency, it did not achieve 100% nitrate removal efficiency. To identify the reason approximate 20% nitrate did not be removed, the suitability of the design of the SRM system was evaluated by numeric analysis (Lee, 2011), which can explain the optimum well arrangement. For inducing the inflow of the nitrate plume into the all wells in the one barrier, the transverse distance (d_t) between two wells in the first array should be less than twice of the capture width (W_c) of the well in second array (Eq. 5-3; Fig. 5-5).

$$d_t \leq 2W_c \qquad (5\text{-}3)$$

The relationship between capture width (W_c) and well diameter (d) was determined by the ratio between well hydraulic conductivity (k_w) and aquifer hydraulic conductivity (k_{aq}), shown in Fig. 5-6. In addition, the longitudinal interval between the first straight line of the zigzag array and the second one should be shortened to attain more retention time of the nitrate plume within the well (Lee, 2011). In this study, k_{aq} value (8.01×10^{-2} cm s^{-1}) of the sandy aquifer was applied as an experimental data. k_w value (3.945×10^{-1} cm s^{-1}) was referred from Lee (2011). The ratio between k_w and k_{aq} was determined to be 5.0, corresponding to the ratio 1.63 between W_c and d. The well diameter is measured to be 7.62 cm, as a result, the capture width could be determined 12 cm. Therefore, d_t value should be less than 24 cm.

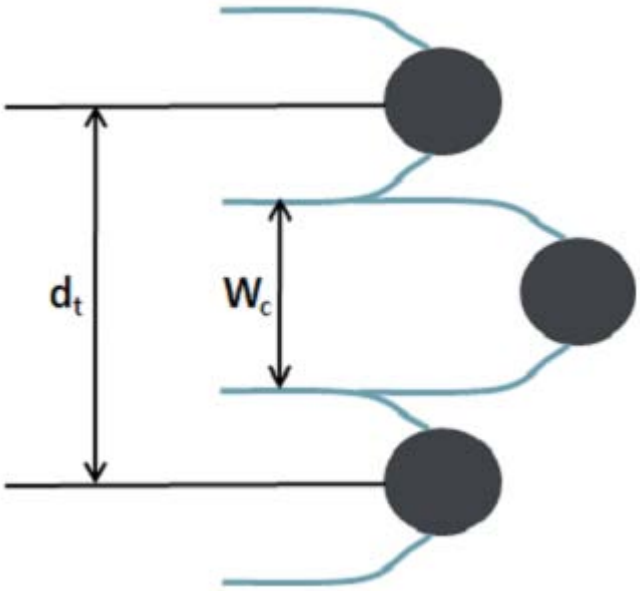


Fig. 5-5. Top view of zigzag well array demonstrating W_c and d_t (Lee, 2011).

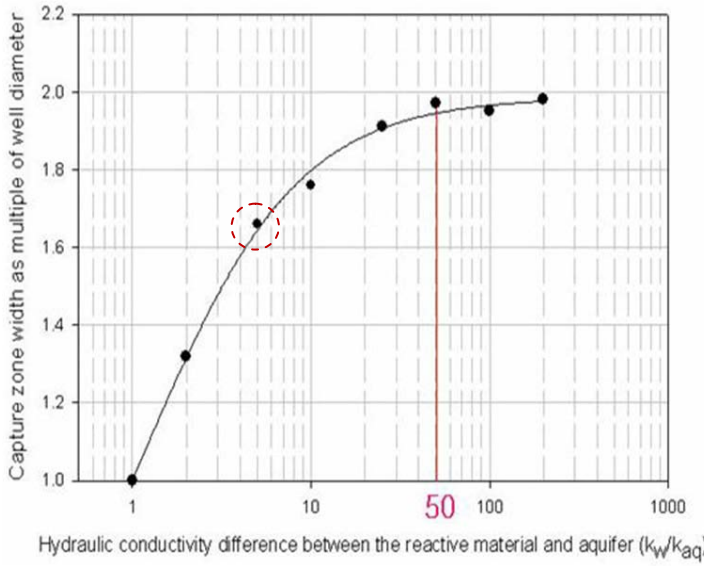
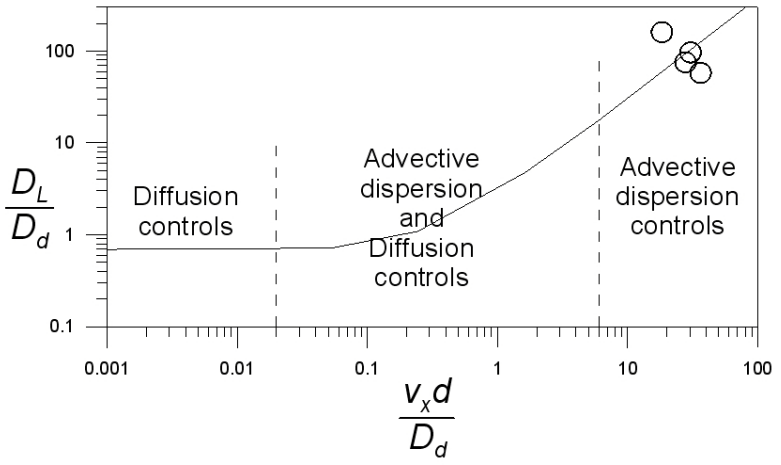


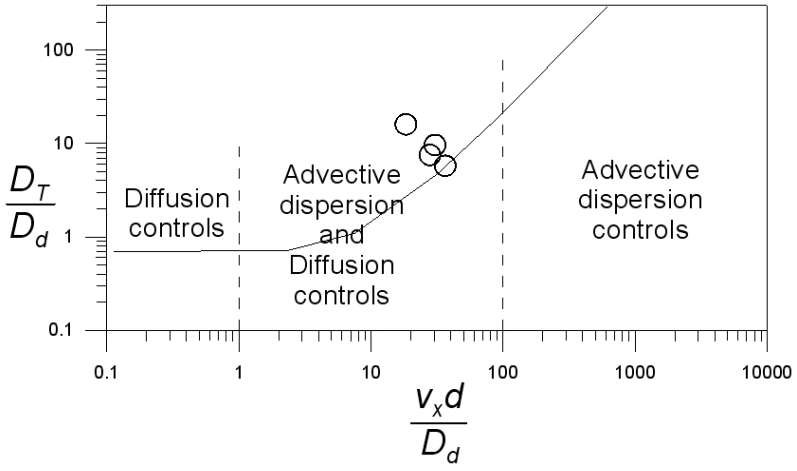
Fig. 5-6. The effect of hydraulic conductivity of the capture width (Lee, 2011).

The d_t value of the SRM system is 20 cm, which can be considered that the nitrate plume flowed into the all wells. And the longitudinal interval is 10 cm, having relatively longer retention time. Overall, the design for the well arrangement of the SRM system was suitable for all nitrate plume to inflow into the wells..

Lee et al. (2009) conducted a tracer test to describe solute transport in the flow tank. Bromide tracer solution (40 L; 400 mg L^{-1}) as a conservative tracer was injected from three upstream delivery wells (1.0 m). Samples were collected from the monitoring wells downstream of the injection points for one week. Calculated longitudinal dispersivity values (a_L) ranged from 0.09 to 0.19 m. These values were within the similar range of the values (0.01–0.30 m) estimated for similar travel distances in the sandy Borden aquifer, Canada (Sudicky and Cherry, 1979). Transverse dispersivity values (a_T) were assumed to be 0.1 a_L. The Peclet number estimated using these values ranged from 18.3 to 27.9, where longitudinal dispersion coefficient over diffusion coefficient (D_L/D_d) and transverse dispersion coefficient over diffusion coefficient (D_T/D_d) values ranged from 76 to 161 and 8 to 16, respectively. These data suggested that solute transport in the sandy media was predominantly constrained by advection and longitudinal dispersion (Fig. 5-7). Lee et al. (2008) simulated the permanganate plume growth within the same test tank with respect to time, indicating barrier inefficiencies because of the inability of transverse dispersion to fully fill in the gaps between release sources.



(a)



(b)

Fig. 5-7. The calculated Peclet number by the tracer test: (a) longitudinal number, (b) transverse number.

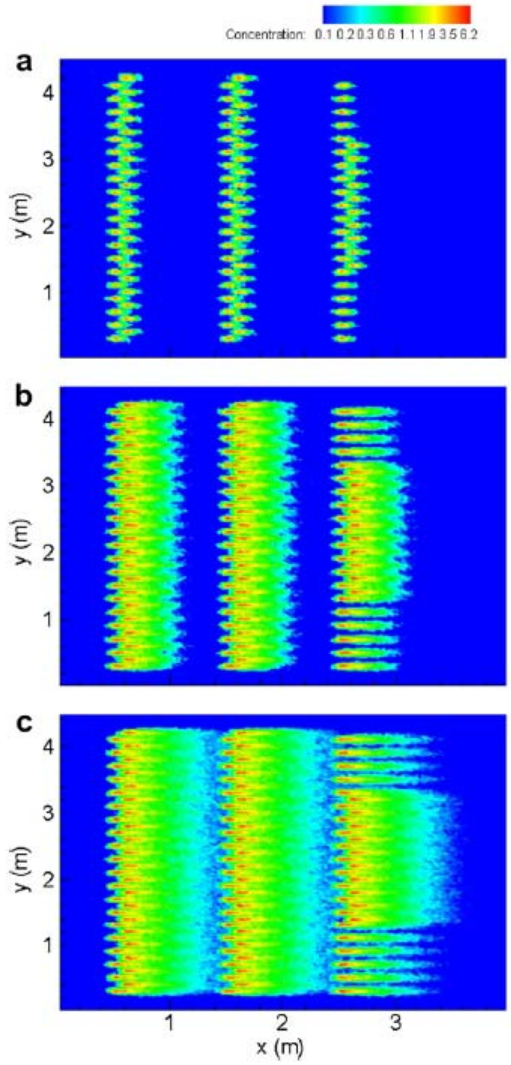


Fig. 5-8. Simulation results of a pilot-scale remediation experiment: (a) After 1 h, (b) after 6 h, (c) after 1 d (Lee et al., 2008).

Fig. 5-8 showed that materials released from the single straight line type barrier migrate to the end of the tank without mixing each other due to the lack of transverse dispersion. Meanwhile the ones released from the double straight line type barrier can possibly migrate and spread themselves over the whole aquifer. Consequently, the suitability of the barrier design regarding well arrangement for nitrate removal was confirmed again. Well arrangement design, the tracer test result, and plume transport simulation demonstrated that all released molasses from the SRM system have been predominantly transported from the upstream to the downstream by the longitudinal advection. And it has spread itself over the whole aquifer due to a suitable well arrangement. It means that molasses can be practically supplied itself to the most part denitrifiers in the sandy tank. Nonetheless, it did not achieve 100% nitrate removal efficiency. Therefore, the considerations on chemistry and microbiology were demanded in order to explain the reason approximate 20% nitrate did not be removed.

Daily nitrate-nitrogen removal rates per volume of the SRM system can be estimated using Darcy's law, shown in Eq. 5-4 (modified after Schipper et al., 2005).

$$NO_3\text{-N removal} = (v \times A \times [\Delta\ NO_3\text{-N}]) / (\text{soil volume} \times \text{porosity}) \qquad (5\text{-}4)$$

where v is the groundwater velocity (LT^{-1}), A is the cross sectional area conducting ground water (L^2), [Δ NO_3-N] is the decrease in nitrate-nitrogen concentration (ML^{-3}), and soil volume is volume of wall the nitrate

travels through (L^3). Porosity (0.45) and average Darcian velocity (1.2 m d^{-1}) were assigned from an earlier study based on the same sand tank (Lee et al., 2009). From Eq. 5-4, the nitrate-nitrogen removal rates represented as the denitrifying capacities were determined to be 35, 33, and 30 g m^{-3} d^{-1} on day 1 for System A, B, and C, respectively. As the release of molasses continued, the values at the same locations decreased with time, finally decreasing to 29, 30, and 28 g m^{-3} d^{-1} on day 10, 24, 27, and 27 g m^{-3} d^{-1} on day 100, 20, 25, and 26 g m^{-3} d^{-1} on day 365, respectively (Fig. 5-9). Nitrate species formed the primarily limiting factor for denitrification in practical applications (Boaventura et al., 1997; Schipper et al., 2005). In this study, an initial nitrate plume of 142 mg L^{-1} was constantly supplied to the sandy aquifer at a flow velocity of 120 cm d^{-1} during the test periods. These constant nitrate concentrations with this flow velocity may have resulted in the specific nitrate removal rate by the indigenous denitrifiers. The removal rate of System A was identified range from 35 to 20 g m^{-3} d^{-1} during 365 d. Meanwhile, those values of System B and C ranged from 33 to 25 g m^{-3} d^{-1} and 30 to 26 g m^{-3} d^{-1} during the same period, respectively. The removal rates of Systems B and C were higher than those of System A because the mass fluxes of Systems B and C were higher than those of System A at the same monitoring time.

Scarcely biodegradable molecular substrates of molasses could possibly induce the occurrence of approximate 20% remaining nitrate. A scarcely biodegradable and high molecular substrate can induce low efficiency due to the slow release of available carbon from it (Quan et al, 2005; Ueda et al., 2006). Although the majority (～69%) of molasses used in

these experiments consisted of readily biodegradable substrates (i.e. sugar), substrates with a high molecular weight (~31%), such as starches (2%) and polysaccharides (2%), can be involved (Lee, 2010). Several selective studies demonstrated that 100% nitrate removal efficiency could not be achieved due to effect of these high molecular substrates (Lee et al., 2001; Mora et al., 2003; Quan et al., 2005). The actual molasses consumption for denitrification in the SRM systems can be calculated by subtracting the remaining molasses amounts in the nitrate removal experiments from the observed molasses amounts in the molasses release experiments (Chapter 4) at the same sandy tank. This was roughly calculated as an average of 1.3, 1.6, and 1.7 mM for Systems A, B, and C (Fig. 5-10), respectively. These values were calculated as somewhat 60% of injected molasses for each system, which was similar to the portion of the readily biodegradable substrates in molasses. This result demonstrated that dentirifiers can remove 79~84% when 1.3~1.7 mM of molasses were injected. If the more molasses were injected into the well, it may possibly attain the complete nitrate removal due to effect of the more amounts of readily biodegradable substances, even though significant amounts of molasses were used for anonymous microbe which does not take part in denitrification, It is certain that indigenous denitrifiers not only consumed molasses but also numerous microbes did as well in the sandy media. Therefore, the molasses amounts only for denitrification were most likely less than the reported values (i.e., an average of 1.3~1.7 mM). Additional detailed investigations regarding the optimum injected number of SRM rods are required.

As the nitrate plume flowed through the three discrete barriers of the SRM system, the ratio between the organic carbon in the molasses and the nitrate-nitrogen (C/N ratio) increased along the flow direction due to the gradual decrease of the nitrate concentrations and the increase of the molasses concentrations. In this study, nitrate less than 1.5 mM was removed after each barrier regardless of the C/N ratio (Fig. 5-11).

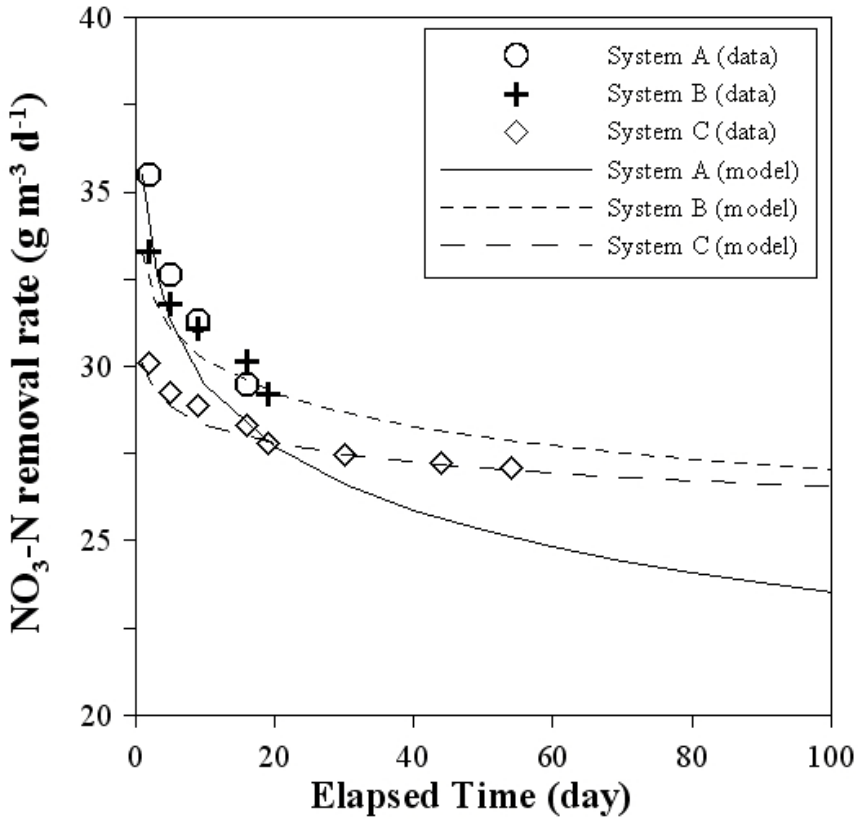


Fig. 5-9. Nitrate removal rates from each SRM system.

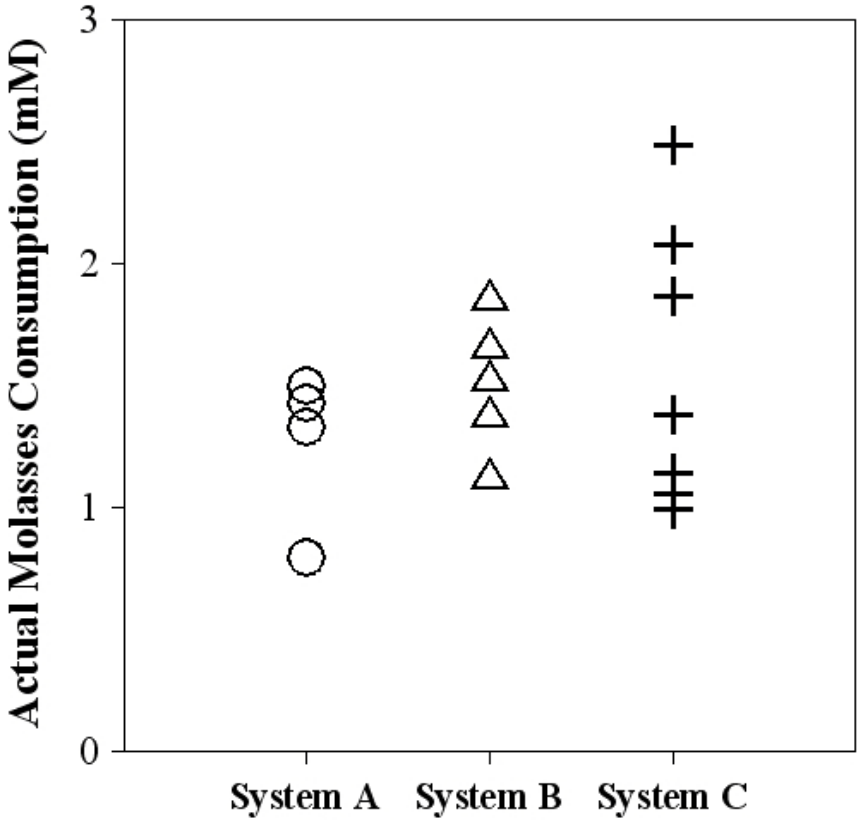


Fig. 5-10. Actual molasses consumption amounts by the indigenous microorganism in sandy soil media.

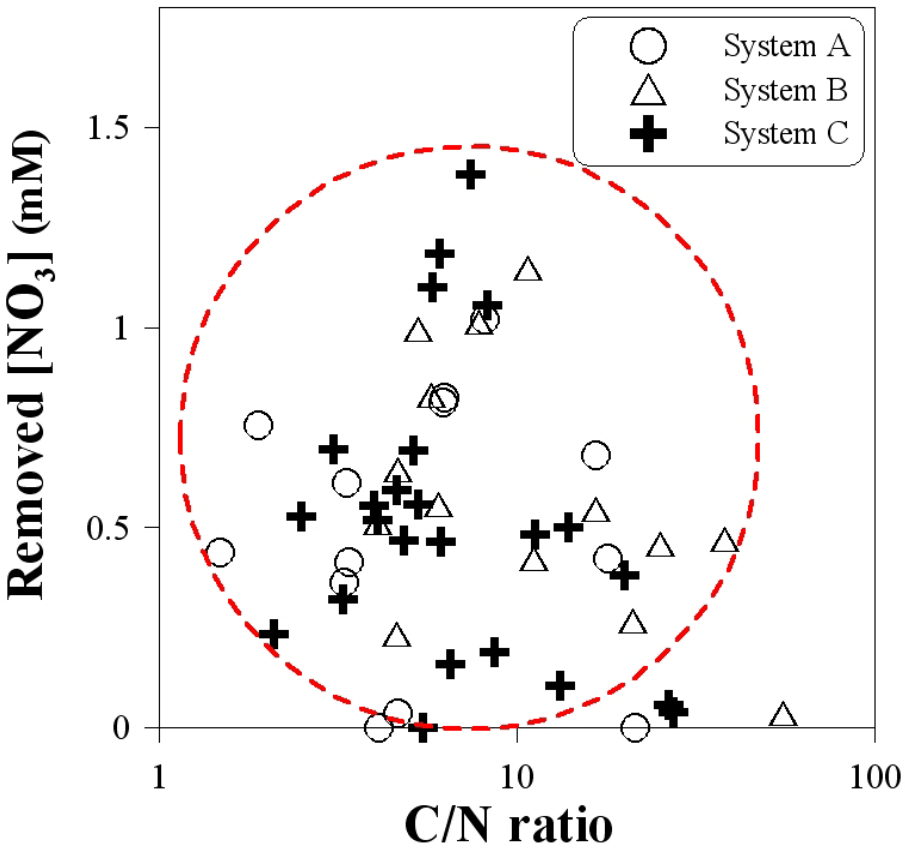


Fig. 5-11. Relationship between removed nitrate concentrations and C/N ratio.

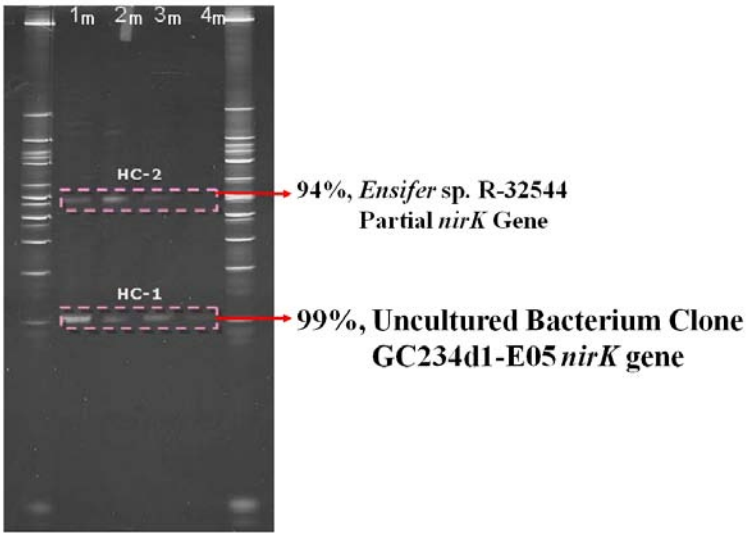
5.3.4 Groundwater chemistry

The nitrate removal efficiency is very sensitive to the pH, and the optimum pH of most denitrifying bacteria is known to be around 7 and 8 (Oh et al., 1999). For Systems A, B, and C, the pH values consistently remained at around 7.1. The electric conductivity (EC) values increased slightly with the distance due to the accumulation effect of the molasses concentration through the SRM systems. Overall, the initially mean EC value of 516 μS cm^{-1} increased to 568, 596, and 646 μS cm^{-1} after the first, second, and third barriers for Systems A, B, and C, respectively. The DO concentrations were initially around 4.0 mg L^{-1} but significantly decreased to a small range from 0.5 and 0.8 mg L^{-1}. Organic matter serves initially to reduce the oxygen concentrations of the groundwater by stimulating aerobic respiration and secondly to provide a carbon source for the denitrifying bacteria (Schipper et al., 2005). Therefore, the released molasses could be the controller to maintain a low DO concentration in the SRM systems at the early stages (Eq. 5-1). The groundwater temperatures were maintained within a range of 19 to 23℃ for Systems A, B, and C, respectively. For the control group at a depth of 2.0 m, the mean pH, EC, DO, and temperature consistently remained at 7.4, 512 μS cm^{-1}, 3.8 mg L^{-1}, and 19℃, respectively.

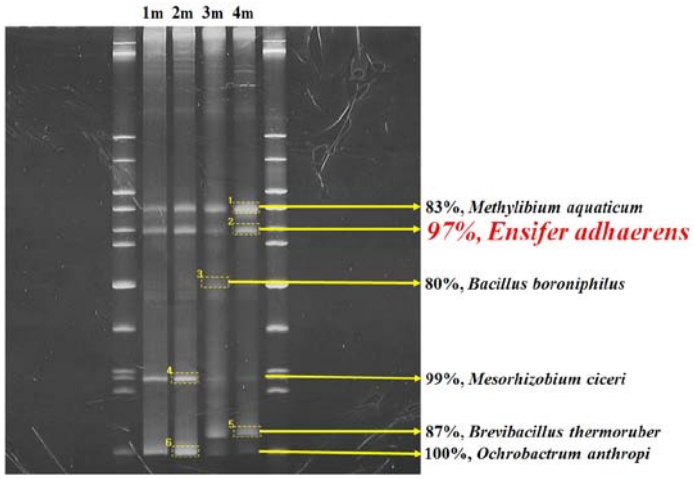
5.3.5 Identification of the *nirK* gene and a denitrifier

From the PCR-DGGE results, the *nirK* gene fragments (~450 bp) were amplified from uncultured and *Ensifer* isolates. Sequence chromatograms of *nirK* gene fragments showed high similarity to an uncultured clone (99%) and *Ensifer* sp. R-32544 (94%) (Fig. 5-12). The results of 16S rDNA sequencing demonstrated that the *Ensifer* sp. isolate was a possible denitrifier from soil samples with 97% 16S rDNA similarity to *Ensifer adhaerens*. *Ensifer adhaerens* is well known as a typically heterotrophic denitrifier having functional denitrification genes including nitrite (*nirK*), nitric oxide (*cnorB*), and nitrous oxide genes (*nosZ*) (Dandie et al., 2007). While typically heterotrophic denitrifiers such as *Pseudomonas, Achromobacter, and Alcaligenes spp.* were not identified in this sandy tank and considering that the *nirK* gene was not amplified in those isolates, the results indicate that the sandy soil had a heterotrophic denitrifying capacity by itself when carbon was supplied into the soil media. Tiedje et al. (1982) demonstrated that denitrifiers are prevalent in various environments, constituting about 20% of the bacterial population capable of anaerobic growth, and that 1 to 5% of the total heterotrophic population can be isolated. On the whole, the results indicate that the SRM system could easily be applied to nitrate-contaminated groundwater with minor consideration of the denitrifiers of the target field. A phylogenetic analysis was done on the basis of the partial 16S rDNA sequence of all isolates and a heterotrophic denitrifier *Pseudomonas* sp. KY1 (Fig. 5-13). Closely matched species of the

isolates were searched for in the GenBank database, and the percent similarities with type strains of each species were calculated using an old distance program in the GCG package. Fig. 5-13 shows that the denitrifying strains used for the nitrate removal experiments were widely distributed in the *Proteobacteria*, indicating that *Ensifer* sp. KY2 and *Pseudomonas* sp. KY1 belonged respectively to the α- and δ-*Proteobacteria*. As a result, the phylogenetic analysis indicated that the sandy soil had a heterotrophic denitrifying capacity by itself when carbon was supplied into the soil.



(a)



(b)

Fig. 5-12. Comparative DGGE analysis of (a) *nirK* gene in sandy soil media and (b) the bacterial consortium.

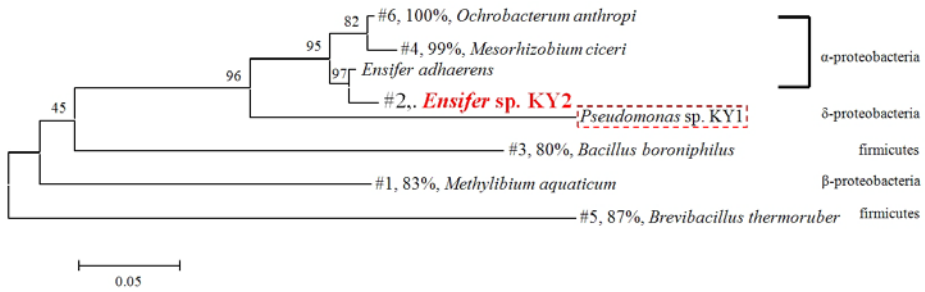


Fig. 5-13. Phylogenetic tree of *Ensifer* sp. KY2 and closely related bacteria. Scale bar represents 0.05 substitutions per nucleotide position. Numbers at the nodes are the bootstrap values.

5.4 Conclusions

The removal efficiencies of a well-type SRM system for nitrates in groundwater were demonstrated through pilot-scale flow tank experiments. The removal efficiencies were estimated to be fairly high as 79-84% for nitrates amounting to 142 mg L^{-1} (32 mg N L^{-1}). This indicated the SRM system could continuously decrease 32 mg N L^{-1} of nitrate nitrogen plume up to 6 mg N L^{-1}, which was below the W.H.O. drinking water quality guideline (10 mg N L^{-1}) during relatively long periods. Nitrate-nitrogen removal rates of System A, B, and C ranged from 35 to 20 g N m^{-3} d^{-1} during 365 d, 33 to 25 g N m^{-3} d^{-1}, and 30 to 26 g N m^{-3} d^{-1}, respectively. Approximate 20% remaining nitrate could be attributed to the specific denitrifying capacity represented as nitrate-nitrogen removal rate in the sandy tank and the scarcely biodegradable molecular substrates of molasses. Actual molasses consumption amounts in the SRM systems were roughly calculated as an average of 1.3~1.7 mM, while the indigenous denitrifiers removed nitrates of less than 1.5 mM after each barrier regardless of the C/N ratio. The nitrite reductase (*nirK*) gene was amplified from uncultured isolate with 99% similarity, demonstrating a heterotrophic denitrifying capacity of the sandy tank and the suitability of the SRM system in field applications. Remaining molasses resulted in a slight increase in the turbidity of the groundwater. An optimizing study to minimize the remaining molasses problem by controlling the C/N ratio is required.

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CHAPTER 6

IN SITU MICROBIOLOGICAL DENITRIFICATION USING THE SLOWLY RELEASED MOLASSES BARRIER SYSTEM: A FIELD APPLICATION

6.1 Introduction

To identify the field applicability of the SRM system, field-scale studies of denitrification were conducted in series at a field test site in Suwon, Korea (Fig. 6-1). Before the nitrate removal test using the SRM system, preliminary studies to identify the hydrogeochemistry, groundwater flow paths, and controlling factors of the nitrate plume were conducted (Lee et al., 2008; Lee et al., 2010b). The groundwater hydrogeochemistry of the field test site was studied in order to identify the influence of cow manure, which is distributed to farmland as organic fertilizer, on nitrate concentrations in shallow groundwater and its spatial and temporal variations (Lee et al., 2008). Groundwater levels were measured using automatic data loggers, and groundwater samples were collected and analyzed in February, April, June and October of 2007.

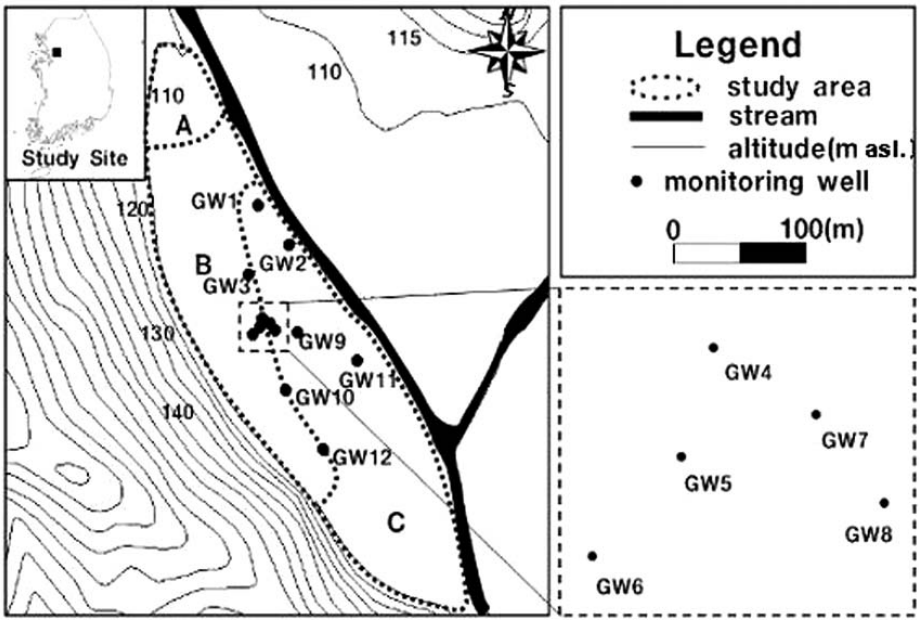


Fig. 6-1. Location of the study site (A: livestock facility area, B: manured crop field area, C: crop field area) with groundwater monitoring wells (Lee et al., 2008).

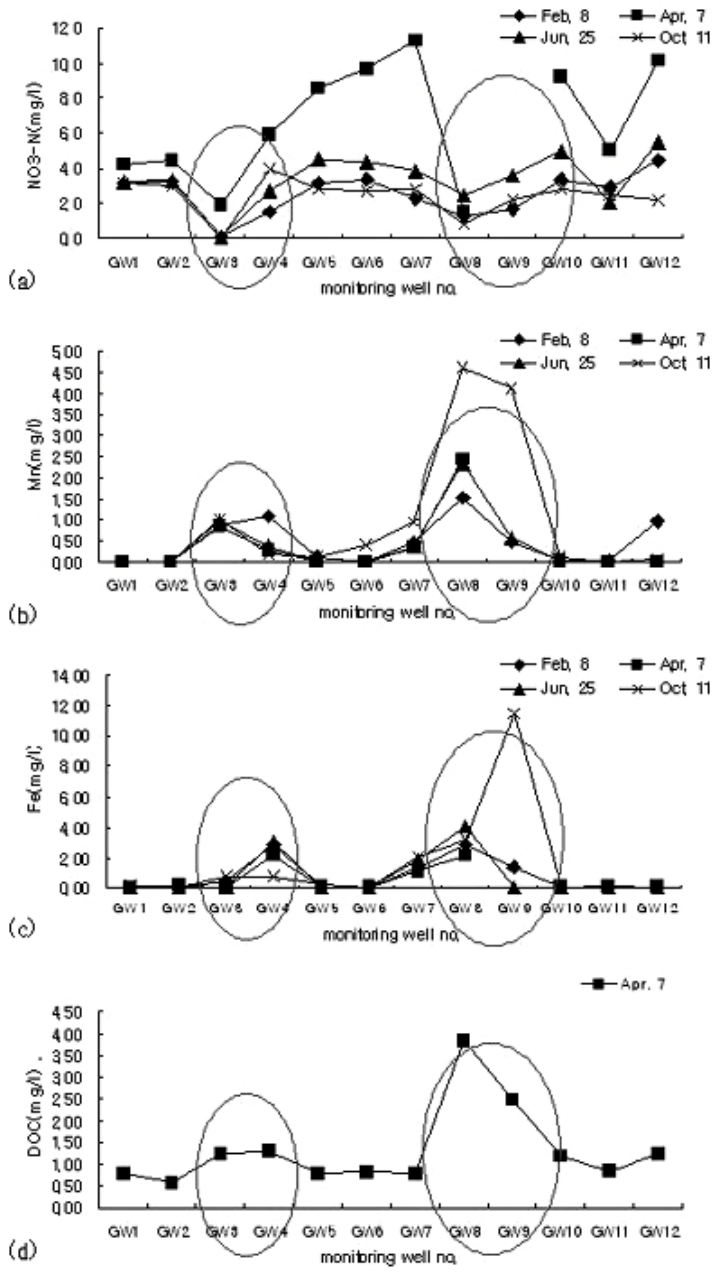
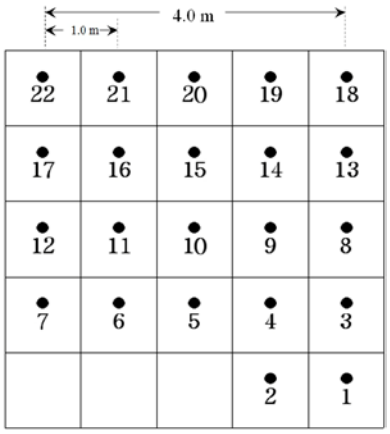


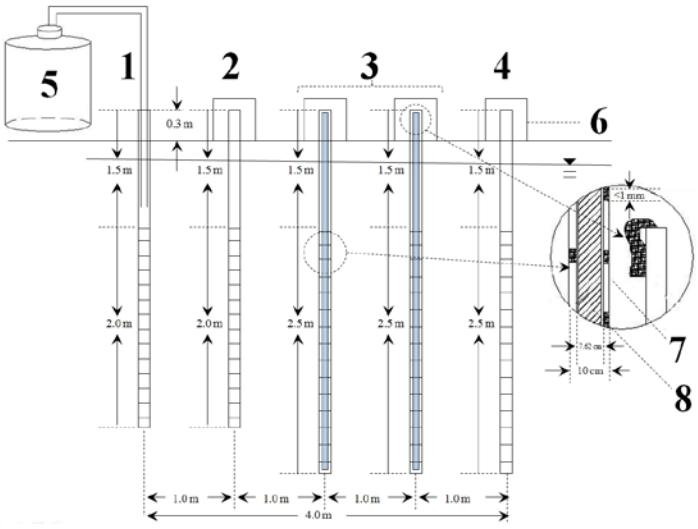
Fig. 6-2. Correlations of (a) nitrate-nitrogen, (b) Mn, (c) Fe, and (d) DOC in groundwater (Lee et al., 2008).

The average electric conductivity and concentration of the nitrates in the groundwater showed the highest levels in April and declined at subsequent sampling times. Decreases in the dissolved oxygen and nitrate concentrations from April to October and corresponding increases in the HCO_3 concentrations indicated denitrification processes by microorganisms. Spatial variation of the nitrate concentration appeared to be affected by the redox conditions of groundwater controlled by the geochemical reactions of the Mn, Fe and DOC contents (Fig. 6-2). After the completion of the site characterization process, 22 wells in total were installed as a grid system in the 5 m × 5 m square area at 1-m intervals at the field test site (Fig. 6-3). Groundwater hydraulic tests including water-level monitoring, water sampling and analysis, pumping and slug tests, and tracer tests were performed in order to identify the characteristics of the aquifer (Lee et al., 2010b). The aquifer appeared to be unconfined with hydraulic conductivities ranging from 2.6×10^{-4} to 9.5×10^{-3} cm s^{-1}. The average linear velocity of the groundwater was estimated to be 2.94×10^{-6} cm s^{-1}, and the longitudinal dispersivity of a conservative tracer was found to be 5.94×10^{-7} m^2 s^{-1}. The groundwater plume moved preferentially through the high hydraulic conductivity zones and the relatively high ion concentrations along the low hydraulic conductivity zones, implying a deterred groundwater flow (Fig. 6-4). The spatial variation of the hydraulic conductivity caused by the heterogeneity of the aquifer and by anisotropy appears to be the most important factor to maximize the effect of the plume treatment system for the application of in-situ groundwater remediation techniques (Fig. 6-5). As

a subsequent study, a nitrate removal test was conducted to identify the field applicability of the SRM system. In this chapter, a field-scale nitrate removal test demonstrates that the SRM system is a viable a long-term remedial technique for nitrates in fields.



(a)



(b)

Fig. 6-3. A field scale SRM system: (a) monitoring well IDs, (b) a cross-section view of the system; ① an nitrate plume injection well, ② a monitoring well to identify initial nitrate concentrations, ③ SRM placing well, ④ a monitoring well to identify nitrate removal, ⑤ a storage tank of nitrate solution, ⑥ a well cap, ⑦ a screened PVC well, ⑧ SRM rods.

Fig. 6-4. A stiff diagram of the groundwater quality in the SRM system. Ion concentrations in squares were higher than others (Lee et al., 2010b).

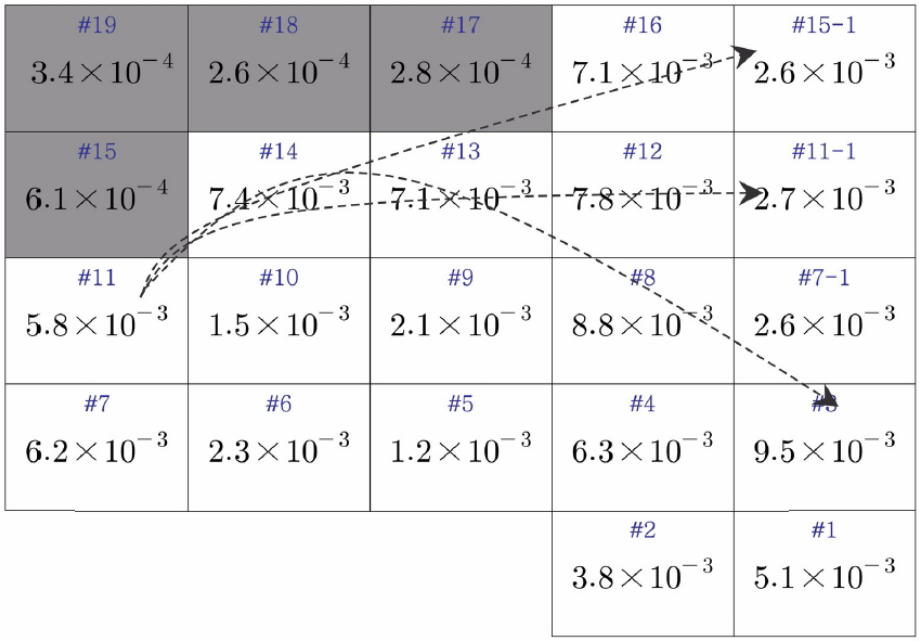


Fig. 6-5. Groundwater flow directions with describing hydraulic conductivity per each well (unit: cm s^{-1}) (Lee et al., 2010b).

6.2 Materials and methods

6.2.1 Identification of nitrate reductase

Soil samples were collected to identify the nitrite reductase genes from four points in the field (Fig. 6-6). The samples were subjected to extraction of the bulk DNA and were then analyzed by PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis). Bulk DNA genes were obtained from the soil using the FastDNA® SPIN kit for soil (MP Biomedicals, USA).

PCR was used to amplify *nirK* and *nirS* genes from the bulk DNA using the primers F1aCua/R3Cu and cd3aF/R3cd, respectively (Hanllin and Lindgren, 1999, Throbäck et al., 2004). The PCR program ran for 2 min at 94℃, 35 cycles for 2 min at 94℃, 1 min at 51℃, and 1 min at 72℃. Using these PCR products, a DGGE analysis was performed following the standard protocol of the D-Code™ system (Bio-Rad, USA). Gels consisted of 1.0-mm thick 9% polyacrylamide, with a denaturant gradient of 45~55% urea-formamide solution (100% of the concentration of the denaturants was 7 M urea and 40% deionized formamide). Electrophoresis was performed in 1 × TAE (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA; pH 8.3) at 60℃ and at 100 volts for 7 hours. The DGGE gels were stained with 1 × GreenStar staining dye solution (Bioneer, Korea) and the bands were visualized using a UV illuminator (302 nm, Vilber Lourmat, France). The DGGE band intensities were quantified using Bio-Rad Quantity One

software (v.5.2). Sequence chromatograms of the *nirK* and *nirS* genes from DGGE were compared with the database sequences in GenBank.

6.2.2 Preparation of the SRM rods

An SRM rod (OD x L = 4 cm x 30 cm) was created by dispersing 177 g of molasses (Hydex Co. Ltd., Korea) in 360 g of a paraffin wax-cellulose-silica sand matrix (215 g paraffin wax, 109 g cellulose, and 36 g silica sands) in a cylindrical mold. The apparent solubility of the SRM rod was determined to be approximately 6,000 mg COD L^{-1} from a batch-type release test over the 112 days of the testing period (Lee et al., 2010a). From the results of a pilot-scale molasses release test, the molasses mass flux slowly decreased with time, exhibiting values of 57, 11, and 3 mg COD d^{-1} after 10, 100, and 365 days in a single straight-line type of SRM system and 138, 25, and 6 mg COD d^{-1} in a double-straight-line type of SRM system, respectively. Additionally, 90, 70, and 50% of the total mass remained after 12, 63, and 267 days in the single-straight-line type of SRM system while 90, 70, and 50% of the total mass remained after 12, 65, and 291 days in the double-straight-line type of SRM system, respectively (Lee et al., 2012).

6.2.3 Experimental setup

Twenty two monitoring wells in total were installed as a grid system in a 5 m × 5 m square area at 1-m intervals at the field test site. The

monitoring well depths ranged from 3.0 to 4.5 m below ground level and the diameter of each well was designated as 7.62 cm (Fig. 6-3). After the completion of groundwater hydraulic tests (Lee et al., 2010b), a nitrate removal test was conducted to identify the field applicability of the SRM system. A total of 70 SRM rods were placed in eight monitoring wells to construct the SRM system (Fig. 6-6). Well numbers of #5, #10, #15, and #20 were designated as the first barrier, and #4, #9, #14, and # 19 constituted the second barrier. A nitrate plume was injected into the #12 well to make a synthetic nitrate-contaminated groundwater field. Well numbers #11, #16, #8, and #13 were selected as the monitoring wells for the nitrate concentrations. Two wells, #11 and #16, were designated for monitoring the inlet nitrate concentrations, while two other wells, #8 and #13, were designated for the outlet nitrate concentrations.

6.2.4 Sample collection and chemical analysis

Before the nitrate removal test by the SRM system, approximately 0.67 m^3 of 760 mg L^{-1} nitrate solution was injected into the #12 well over a course of 24 hours. This solution contained 420 mg L^{-1} of a bromide solution as a tracer. The nitrate and bromide concentrations were continuously detected as 320 mg L^{-1} and 210 mg L^{-1} at the monitoring wells (#8 and #13), respectively, indicating 0.5-fold dilution was naturally occurring in the aquifer media.

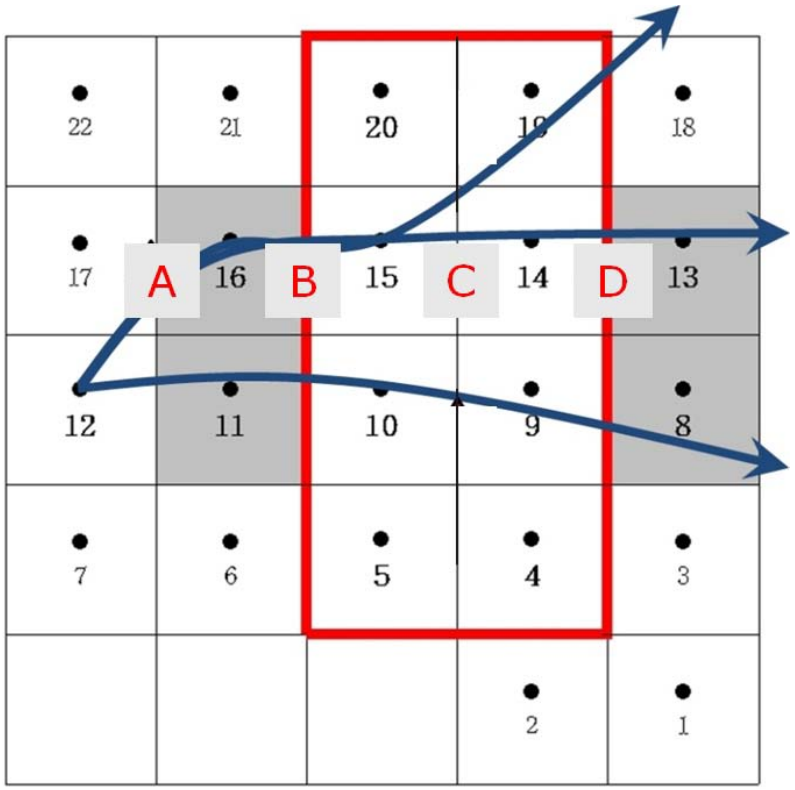


Fig. 6-6. Information on a pilot scale field: Closed circles (●), letters (A, B, C, and D), and arrows (→) represent for monitoring wells, soil sampling points, and groundwater flow path, respectively. Denitrifier (*Pseudomonas* sp. KY1) was identified from the all soil sampling points.

After the nitrate concentrations in the flow tank were stabilized at the level of 320 mg L^{-1}, the SRM rods were placed in the first and second arrays to construct the SRM system. The flow rate was controlled at 5.6 mL s^{-1} using a peristaltic pump, and a total of 2.6 m^3 of synthetic solution was injected into the #12 well over a course of 93 hours. To monitor the change in the groundwater level in accordance with the injections, groundwater level monitoring was conducted until the completion of the test. The synthetic groundwater contained basal salts (19 NH_4Cl, 7 KH_2PO_4, 19 K_2HPO_4, and 19 $MgSO_4$ mg L^{-1}).

To measure the nitrate and bromide concentrations, groundwater samples were collected using the peristaltic pump from the four monitoring wells (the #8, #11, #13, and #16 wells) 3, 19, 21, 25, 42, 48, 65, 71, 74, and 90 hours after the beginning of the test. The samples (80 mL) were filtered (0.2 μm) and stored in a refrigerator at 4℃ until they underwent an IC analysis (DX-80, Dionex, USA). Additional water samples (20 mL) for measurements of the COD values to assess the indirect molasses concentrations were collected in amber vials and were analyzed using a UV-visible spectrophotometer (DR-2800, Hach Co. Ltd., USA) immediately after sample collection. The on-site properties of the temperature, pH, and DO were immediately measured by portable multi-sensors (Thermo Orion 3-star series, USA).

6.3 Results and discussion

6.3.1 Nitrate removal efficiency

The molasses used for making the SRM rod contained ~65% organics (~56% sugar and ~9% non-sugar constituents), ~10% inorganics, and ~25% water. Sucrose was the largest constituent of the sugar in the molasses, accounting for ~38%, while the other constituents were glucose at ~9% and fructose at ~9%. Assuming sucrose as the main carbon source in molasses, the denitrification reaction is written as Eq. 6-1 and 6-2 (Hamlin et al., 2008).

$$O_2 + 0.0832C_{12}H_{22}O_{11} + 0.144NO_3^- \rightarrow 0.144C_5H_7O_2N + 0.048CO_2 + 0.229H_2O \quad (6\text{-}1)$$

$$0.088C_{12}H_{22}O_{11} + NO_3^- + 1.52H^+ \rightarrow 0.159C_5H_7O_2N + 0.42N_2 + 0.33CO_2 + 3.72H_2O \quad (6\text{-}2)$$

Aerobic growth of the denitrifier results in oxygen consumption and leads to the initiation of the denitrifying process (Mora et al., 2003). After oxygen consumption, nitrate and nitrite replace molecular oxygen as electron acceptors. In this study, nitrate of 320 mg L^{-1} was destroyed by the denitrification, decreasing to ~43% at the #8 well after 40 hours and ~30% at the #13 well after the completion of the test (Fig. 6-7).

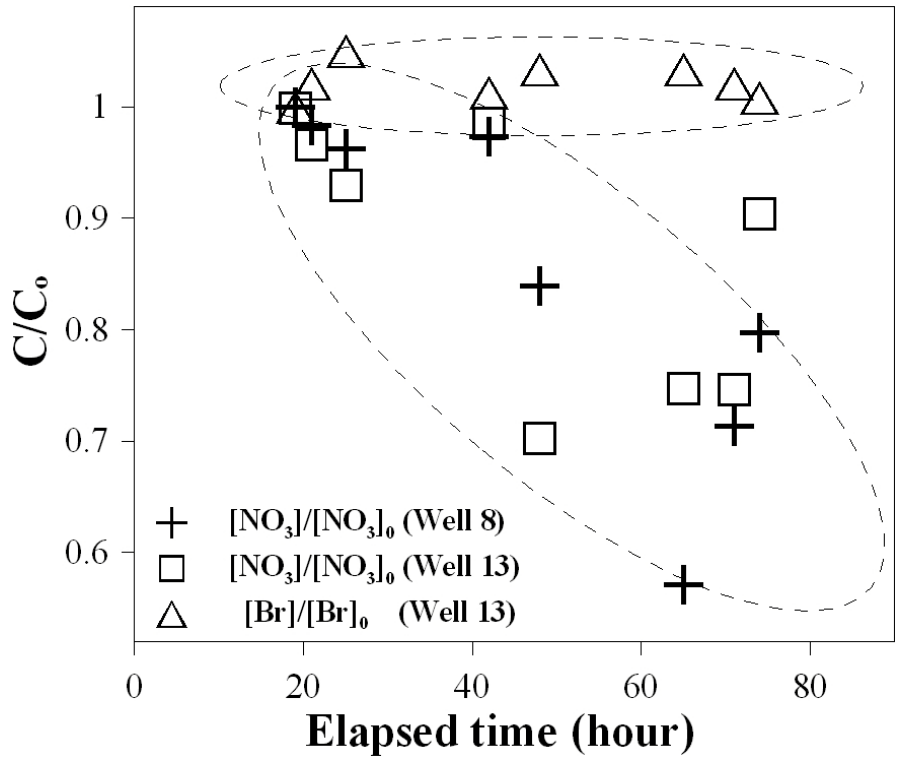


Fig. 6-7. Temporal changes in nitrate and bromide concentrations monitored at 1-m downstream of the SRM system.

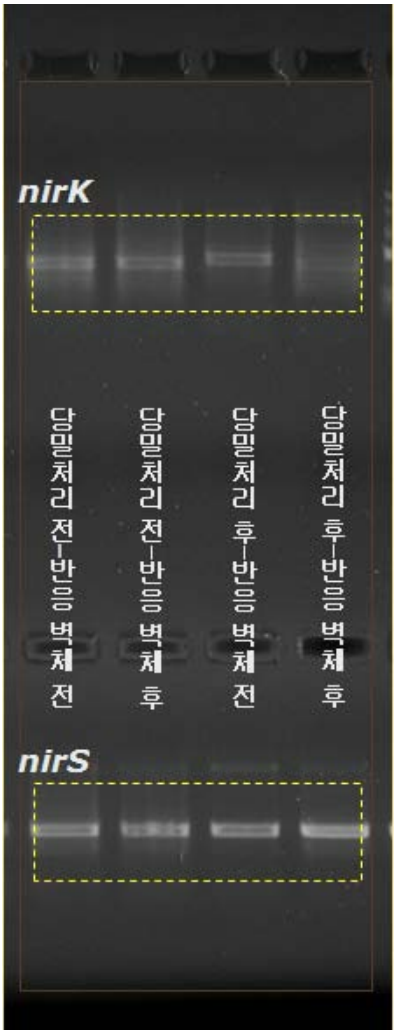


Fig. 6-8. Comparative DGGE analysis of *nirK* and *nirS* gene in the sandy soil media.

The conservative tracer Br level was continuously determined to be around 210 mg L^{-1}, indicating that nitrate was actively removed by the SRM system (Fig. 6-7). From the PCR-DGGE results, the *nirK* and *nirS* gene fragments were amplified from the field soil, which results indicate that the soil had a heterotrophic denitrifying capacity by itself when carbon was supplied into the field soil (Fig. 6-8). The nitrite concentrations were increased at the same monitoring time after 40 hours (Fig. 6-9). Therefore, the results of the nitrate decrease and the nitrite increase were based on its denitrifying activity. Nitrite accumulations were considered as the results from the relatively low nitrite reduction rate during the relatively short test period (93 hrs) (Sec. 3.3.3). If the operation time of this field test would be long enough, the nitrite reduction could be possibly observed.

6.3.2 Remaining molasses problem

Denitrification using molasses can result in a slight increase in the turbidity of groundwater (Hamlin et al., 2008). In addition, it may not achieve complete nitrate removal efficiency due to the scarcely biodegradable molecular substrates of molasses. In denitrification processes, the fraction of readily biodegradable substrates in the carbon source is a very important factor to increase the nitrate removal efficiency (Quan et al., 2005). On the other hand, a scarcely biodegradable and high-molecular substrate can induce low efficiency due to its slow release of available carbon (Ueda et al., 2006). In this study, the COD concentrations were determined to be

~440 mg L^{-1} at the #8 and # 13 monitoring wells, exceeding the drinking water quality guideline (e.g., the WHO guideline of 10 mg L^{-1}). This resulted in the turbidity problem associated with groundwater quality (Fig. 6-10). Moreover, the nitrate removal efficiencies were relatively low, ranging from 30 to 43%, while the remaining molasses concentrations were relatively high (~440 mg L^{-1}). This indicated that too many SRM rods were placed in the barrier. Although the molasses concentrations exceeded the drinking water quality guideline, the SRM system was shown to be able to release molasses over the extended time of the test period. This indicates that the SRM system can undertake long-term molasses release suitably in a field condition. Further studies regarding changes of the mixing rates of the SRM constituents or their volumes to prolong the effective longevity and to meet the cleanup requirements of the target contaminated zone are required. In addition, SRM barrier numbers should be tailored by optimizing the design while with minimizing the risk of secondary contamination and maximizing the destruction efficiency.

6.3.3 Cause of the low nitrate removal efficiency levels

The low nitrate removal efficiency levels may have been caused by (i) the groundwater preferentially moved through the high hydraulic conductivity zones due to the heterogeneity/anisotropy of the aquifer, (ii) insufficient molasses dispersion from the SRM rods to the denitrifier in the aquifer due to relatively far distances among wells placing SRM, and/or (iii)

an unprofitable environment for denitrification due to the relatively low pH and high DO. The most important factor to maximize the removal efficiency for the plume treatment was the well arrangement. Wells should be convergently placed to the flow path of nitrate plume. Even though the wells in this study placed in the pathway of the plume, they were not placed as convergent as possible to the flow path. It resulted in low nitrate removal efficiencies. In addition, the groundwater level of injection well #12 continuously rose by 4 cm compared to the natural level due to the injection of the synthetic nitrate solution, resulting in a steep hydraulic gradient in the aquifer. Therefore, a rapid groundwater velocity three times faster than the original velocity arose. The result was that the molasses movement in the aquifer could not be governed by chemical dispersion but instead by physical transport. Further, molasses released from a relatively long distance (1 m) among the SRM displacement wells could not evenly spread out the aquifer media until arriving at the monitoring wells due to the relatively short distance (1~2 m) between the SRM displaced wells and the monitoring wells. These facts indicate that molasses can be transported within the shortest distance from the injection well to the monitoring wells such that in this case the denitrifiers may not have a chance to use the molasses for denitrification, resulting in low nitrate removal efficiency levels. Lee (2011) demonstrated that that the transverse distance (d_t) between two wells in the first array in one barrier should be less than twice of the capture width (W_c) of the well in second array (Eq. 6-3) in order to induce the inflow of the nitrate plume into the all wells of the barrier.

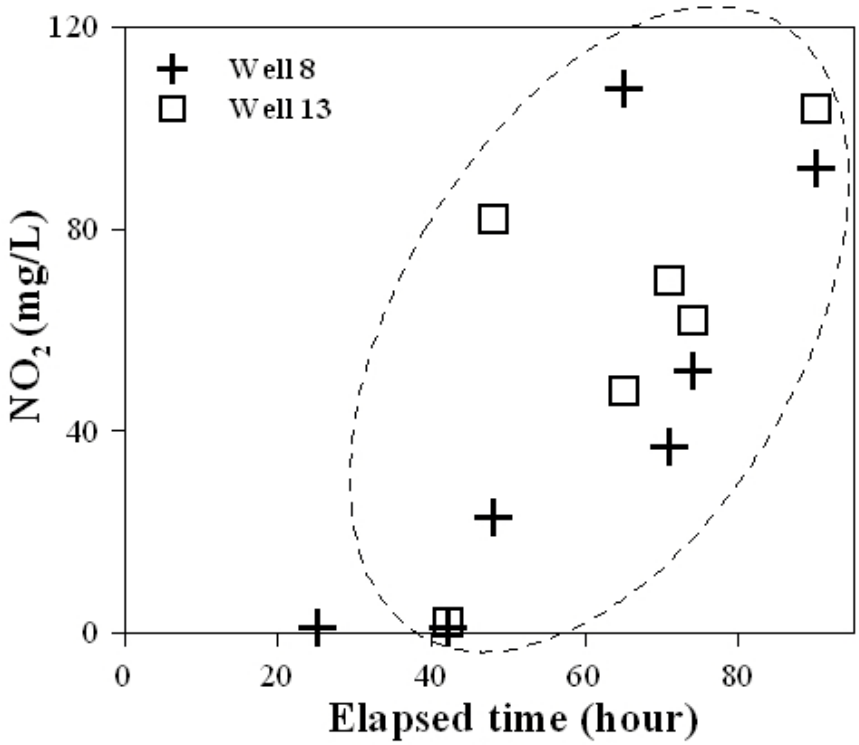


Fig. 6-9. Changes in nitrite concentrations monitored at 1-m downstream of the SRM system during the denitrification.

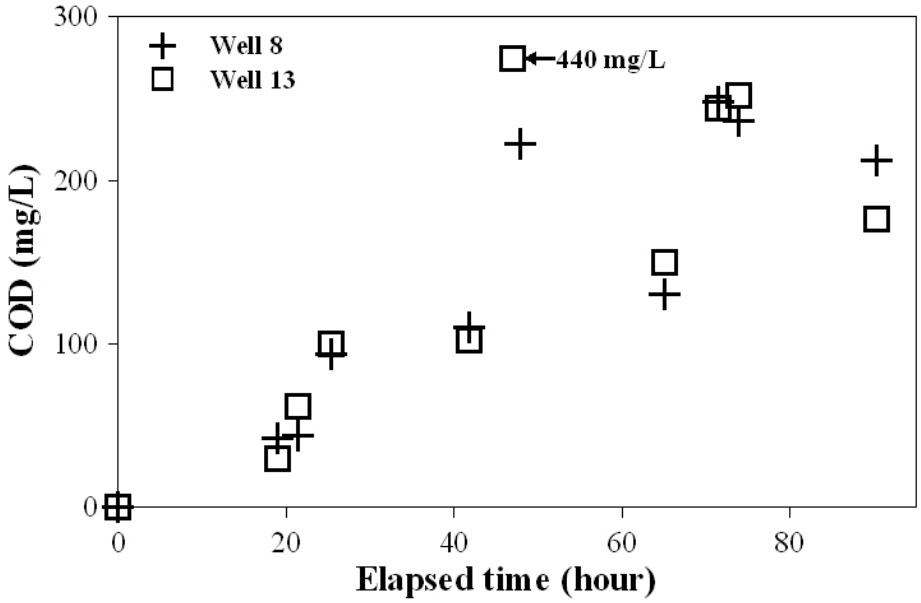


Fig. 6-10. Temporal changes of mean molasses concentrations monitored at 1-m downstream of the SRM system. Molasses concentrations were expressed as molasses-COD.

$$d_t \leq 2W_c \qquad (6\text{-}3)$$

The relationship between capture width (W_c) and well diameter (d) was determined by the ratio between well hydraulic conductivity (k_w) and aquifer hydraulic conductivity (k_{aq}), shown in Fig. 6-11 (Lee, 2011). In this study, k_{aq} value (7.1×10^{-3} cm sec^{-1}) of the sandy aquifer was applied as one result of aquifer pumping tests and k_w value (3.945×10^{-1} cm sec^{-1}) was referred from Lee (2011). The ratio between k_w and k_{aq} was determined to be 56.0, corresponding to the ratio 50~100 between W_c and d. The well diameter value is measured to be 7.62 cm, as a result, the capture width could be determined 14 cm. Therefore, d_t value should be less than 28 cm (Eq. 6-3). The d_t value of the SRM system in the field is 100 cm, which can explain that relatively far distance between the wells resulted nitrate plume did not mixed with released molasses from the well. Overall, the design for the well arrangement of the SRM system in field was not suitable for nitrate removal. While the optimum pH for the denitrifying condition ranged from 7 to 8 (Oh et al., 1999), in this study the pH values were determined to be around 6. The DO values ranged from 0.9 to 2.1 mg L^{-1} before the SRM system and from 0.7 to 1.6 mg L^{-1} after the SRM system due to the injection of the synthetic nitrate solution with oxygen. As oxygen served as an electron acceptor instead of nitrate, the nitrate removal efficiencies could have been low.

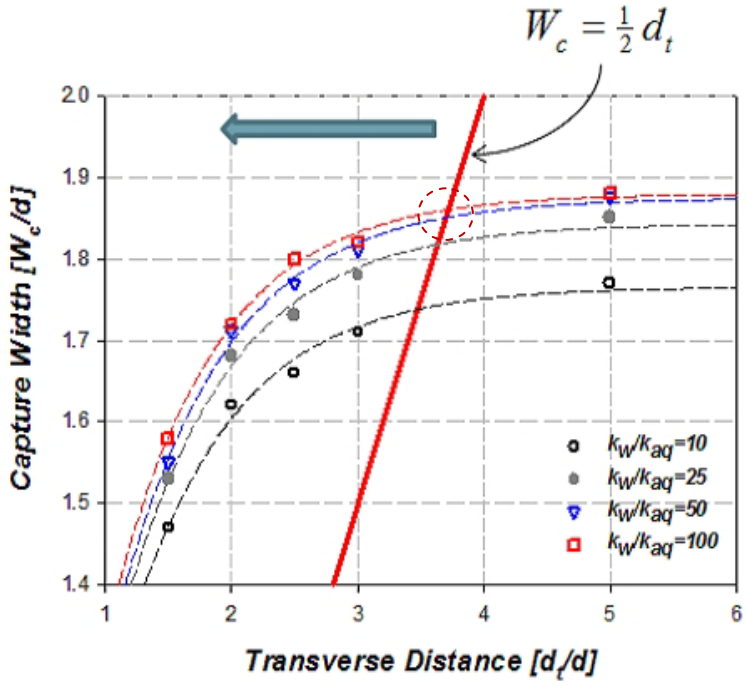


Fig. 6-11. Relationship between capture width and transverse distance (Lee, 2011).

6.4 Conclusions

This study was conducted to identify the field applicability of a well-type barrier system. A total of 22 wells were placed as a grid system in 4 m × 4 m square by 1-m interval in a farming field in Suwon, Korea. Nitrite reductase (*nirK* and *nirS)* genes were identified in the media of the aquifer. The aquifer properties, including the hydraulic conductivities, groundwater flow path, average linear velocity, and longitudinal dispersivity, were identified through various groundwater hydraulic tests. To construct the SRM system, 70 SRM rods in total were placed in 8 wells located in the center of a grid system. A synthetic nitrate plume (320 mg L^{-1} of nitrate) containing 210 mg L^{-1} of bromide as a conservative tracer flowed into the SRM system at a flow rate of 5.9 mL s^{-1}. Changes in the nitrate concentrations were monitored at 4 monitoring wells across the system for 93 hours. The nitrate concentrations decreased by 30～43% after the completion of the test, while the bromide concentrations showed little variation, which indicated that the SRM system promoted the denitrifying activity on the subsurface. The reason approximate 57～70% nitrate did not be reactive was attributed to the rapid groundwater velocity and to the lack of transverse dispersion in accordance with the injection of a synthetic nitrate solution. In addition, the relatively high DO concentrations (～1.9 mg/L) may have inhibited the activity of the denitrification enzyme. In fields, a preferential groundwater flow path and limited transverse dispersion in the aquifer media can lead to incomplete mixing and a decrease in the nitrate

removal efficiency. Thus, high-density deployment of the SRM wells would be required to achieve more effective treatment. Several solutions are feasible. First, the SRM wells in the multiple (sequential) transects can be staggered to provide more complete coverage. Second, the SRM can be simply deployed in a trench (thus, creating a SRM-permeable reactive barrier). Third, a low-flow groundwater recirculation system can be operated to induce lateral mixing, although this takes away some of the advantage of a passive system. In addition, doublet injection/withdrawal wells to facilitate lateral spreading and mixing can be considered as an engineering option to solve the incomplete mixing problem. Further studies regarding the optimizing design of the SRM system to attain on-site remedial goals pertaining to nitrate-contaminated groundwater are required.

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CHAPTER 7

CONCLUSIONS

This dissertation has reported the applicability of the slow-released molasses barrier system (SRM system) to treat nitrate-contaminated groundwater via the denitrifying activity of heterotrophic microbes, performing column-, pilot-, and field-scale nitrate removal experiments. The SRM material, a reactive medium to promote indigenous denitrifying activity in aquifer media, was successfully used as a reliable, long-term extra carbon source for indigenous heterotrophic denitrifiers. Denitrifiers are prevalent in various groundwater aquifers; therefore, SRM can be easily applied to nitrate-contaminated groundwater with minor consideration of the denitrifiers in the targeted field. The developed SRM system is an attractive long-term nitrate treatment option, showing nitrate removal efficiencies that were estimated to be fairly high in the column- and pilot- experiments but moderate in the field experiment, at ~85%, ~84%, and ~43% of nitrates removal efficiencies for 89, 142, and 320 mg L^{-1} in the column-, pilot-, and field-experiments, respectively. In the field, the removal efficiency would depend on the dispersive mixing degree between the released molasses and the nitrate plume. The preferential groundwater flow path and limited transverse dispersion in the aquifer media can lead to low nitrate removal efficiencies. Further studies of an optimized design of the SRM system to attain on-site remedial goals pertaining to nitrate-contaminated groundwater

in the field are required. In addition, the study on a quantifying model using reactive transport models for optimum design of SRM system is also required. The remaining molasses problem in accordance with an excessive supply of SRM rods to the aquifer may also result in an increase in the turbidity of the groundwater. Simulation results demonstrated that the molasses mass flux from the SRM system can be controlled by adjusting the number of SRM barriers and the array of the SRM rods in one barrier. This is important not only to minimize the turbidity but also to optimize the degree of heterotrophic denitrification. Although many constraints related to heterogeneous nature of aquifers exist, this type of slowly released reactive barrier system can be useful for control of dilute, large, or shallow nitrate contaminated groundwater plume. For achieving more long-term remedial requirements of the contaminant plume, size, strength, and duration of the SRM system can be tailored by adjusting the release rates and durations of the SRM products and optimizing the design of SRM barrier system. Further studies regarding changes of the mixing rate of SRM constituents or their volumes to minimize the remaining molasses problem and maximize treatment periods by controlling the C/N ratio are required. Results of this study warrant that the SRM system can provide new approach for a long-term *in situ* treatment of nitrate contaminated groundwater.

국문초록

농촌 지하수의 주오염물질인 질산염에 대한 원위치 정화기술 개발을 위해, 탈질용재로서 지하수 대수층에 주입 시 장기간 당밀방출이 가능한 고체당밀을 개발하였다. 고체당밀은 액상당밀을 미결정셀룰로우스, 무수규사 및 액상 파라핀과 혼합 후 상온에서 건조하여 제조되었다. 고체당밀은 대수층 내에 주입 시 최외각 표면의 당밀부터 순차적으로 용출되어 지하수로 분산되며, 대수층 내 종속영양성 탈질미생물은 당밀을 탄소원으로 이용하여 질산염을 제거한다. 고체당밀의 주기적인 교체를 용이하게 하고, 지하수 대수층의 수리적 교란을 최소화 하기 위하여 관정형 반응벽체를 설계하였다. 고체당밀이 주입된 반응벽체의 적용성을 검토하고자 실험실 컬럼 실험, 파일럿 수리시험장 실험 및 현장 실험을 순차적으로 실시하였다. 경기도의 농경지에서 분리한 종속영양성 탈질미생물을 이용한 실험실 컬럼 실험 결과, 탈질미생물은 381시간 동안 고체당밀에서 꾸준히 방출된 당밀을 탄소원으로 활용하면서 초기농도 20 mg N L^{-1}의 질산염을 3 mg N L^{-1} 이하로 저감시켰다. 3열의 관정형 반응벽이 설치된 파일럿 수리시험장에 30개, 60개의 고체당밀을 주입하여 2개의 반응벽체를 구성하고 내구연한을 평가한 결과, 두 반응벽체는 3개월 이상 당밀을 장기간 방출하였다. 수치모델을 이용한 모사결과, 30개 고체당밀이 주입된 반응벽체의 당밀방출율은 10일, 100일, 1년 후 각 57, 11, 3 mg COD L^{-1}, 60개 반응벽체는 각 138, 25, 6 mg

COD L^{-1}였다. 이 후, 파일럿 수리시험장에 각 60개, 120개, 120개로 구성된 3개의 반응벽체를 구성하여 각기 2주, 3주, 8주간 32 mg N L^{-1} 질산염 오염지하수에 대한 정화실험을 실시한 결과, 3개 반응벽체에서 공통적으로 먹는물 수질기준 이하의 질산염 정화효율(79~84%)이 도출되었다. 경기도 농경지에 22개 관정으로 구성된 반응벽체를 설치하여 70개 고체당밀을 주입하고 현장실험을 실시한 결과, 93시간 동안 72 mg N L^{-1}의 질산염 오염지하수에 대하여 약30~43%의 정화효율이 도출되었다. 상대적으로 낮은 현장정화효율의 원인은 대수층의 불균질성, 상대적으로 넓은 관정간격 등의 영향으로 추정된다. 종합하면, 고체당밀과 관정형 반응벽체를 이용한 종속영양탈질은 질산염 오염지하수의 원위치 장기정화를 위해 효과적으로 사용될 수 있었다. 또한 파일럿 수리시험장 및 현장실험부지 대수층에서 확인된 아질산염 환원효소는 탈질능이 자연계에 흔하게 존재하여 본 고체당밀 반응벽체가 어느 질산염 오염부지에서나 쉽게 적용 가능함을 시사하였다. 향후 고체당밀 반응벽체를 질산염 오염부지 적용 시, 고체당밀의 내구연한은 구성 성분비, 부피 및 형태의 변경으로 조절 가능하다. 또한 해당오염부지의 목표정화수준에 부합하도록 관정 간격, 고체당밀 주입량, 반응벽의 개수 등을 조절하여 설계에 반영한다면 정화효율 제고에 기여할 수 있을 것으로 기대된다.

주요어: 고체당밀, 질산염, 반응벽체, 종속영양탈질, 정화효율

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