An *In Situ* Denitrification and Oxidation Process with Injection of Electrolytic Hydrogen and Oxygen

February 2016

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ABSTRACT

Groundwater is a very important natural water source for humankind. It is widely exploited for different purposes such as drinking water production, irrigation, and industrial usage. Nowadays, nitrate contamination in groundwater - mainly in shallow aquifers - has become a serious global environmental issue. Based on the literature review results, this contamination is mainly due to excessive applications of synthetic nitrogen fertilizers and/or inappropriate management of livestock waste. The impact of nitrate in drinking water has received considerable attention since the 1940s, especially in the past decades. This dissertation evaluates the removal of nitrate by a novel in situ denitrification and oxidation process with injection of electrolytic hydrogen and oxygen. The dissertation consists of five chapters.

Chapter 1 is a general introduction and consists of a literature review and research objectives, focused on the current situation of nitrate contamination and removals by different approaches. This chapter provides an overview of nitrate contamination in groundwater as well as health concerns related to the issue. From the literature review, two different kinds of approaches (i.e., physicochemical approach, biological approach) for removing nitrate from water are discussed. However, for physicochemical approach, besides relatively high process capabilities, the main problems were post treatment and deterioration of catalyst. Thus, a biological denitrification process has been proposed, because it is able to reduce nitrate to a nontoxic product, nitrogen gas. For heterotrophic denitrification, although the removal rate was relatively high, the secondary pollution caused by electron donors such as methanol could not be avoided. Moreover, clogging problems always occurred in aquifer because of high growth rate of heterotrophic microorganisms. In the case of autotrophic denitrification, for instance, hydrogen diffusion via hollow fiber membrane might lead to a relatively high transport efficiency, but clogging always occurred on the surface of the membrane which lead to high cleaning cost as well as difficulty in operation. Moreover, some reactors based on autotrophic denitrification processes with high removal efficiency were also developed.
but were shown not to be suitable for the application in a field situation. Therefore, to overcome the disadvantages of these approaches, a more effective, practical, and inexpensive approach for nitrate removal is required.

Prior to continuous experiment, a start-up phase consisting of the acclimatization of denitrifying bacteria and a batch experiment was introduced in Chapter 2. As bacteria used in denitrification process are usually facultative, they are ubiquitous and could be commonly found in many natural environments; for instance in soil, marine settings, and wastewater treatment plants. Generally, studies related to the ecology of denitrifiers tend to commence with their cultivation. Before introducing the microorganisms into the main experiment, bacteria cultivation was performed in order to enrich appropriate microflora. In this study, the denitrification microflora was obtained by acclimatizing from soil (collected from University’s campus) and obtaining from former studies performed by colleagues of the same laboratory. The denitrification ability of the microorganisms was demonstrated and batch experiments were conducted for a duration of 18 days in order to evaluate performance in artificial aquifer.

In Chapter 3, continuous treatments were conducted in order to evaluate the feasibility of the present in situ process. Synthetic groundwater containing 15.06 ± 0.55 mg-N/L nitrate was fed to the aquifer at the same load. In experiments, electrolytic hydrogen and oxygen were injected upstream and downstream in a laboratory-scale aquifer, respectively, and measurements were performed for nitrate, nitrite, pH, dissolved oxygen, dissolved hydrogen, total organic carbon (TOC), turbidity, and chromaticity. During the initial phase of experiment, nitrite was accumulated in a hydrogen-injected zone; however, it was oxidized to nitrate in subsequent oxygen-injected zone. Moreover, the injection rate of hydrogen gas was changed in a stepwise manner during the experiment. Nitrate concentrations tended to increase, but after the hydrogen injection rate was adjusted to the original state, within one week nitrate concentrations returned to the same level that could be observed prior to the changes. In addition, water quality parameters such as TOC, turbidity, and chromaticity were lower in the effluent than influent, and no clogging problem was observed. The pH was adjusted during the experiment since it was identified to be a limiting factor of the
treatment. From these results, it could be concluded that the present process has several superior performances in terms of stability, effluent water quality, and simplicity in long-term operation.

In Chapter 4, continuous treatments were conducted to evaluate the performance under different hydraulic loadings. At the same time, pH was adjusted to weak acidic based on the results in Chapter 3. Experimental results showed that the aquifer has excellent adaptability in hydraulic loadings variation where the groundwater velocity reached 2.5 m/d, which is relatively high in an actual aquifer. Neutralization of groundwater is possible if the pH of nitrate contaminated groundwater is weak acidic. Other water quality parameters showed a similar trend in Chapter 3.

Finally, overall conclusions of this study and prospects in future works are presented in Chapter 5. This study provides an in situ denitrification process with relatively high efficiency, easy operation, and a wide range of applicability. Some drawbacks are listed for further study.

**Key words:** Denitrification and oxygenation; groundwater; hydrogen; in situ; oxygen
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DISSOLVED HYDROGEN DETECTION

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CHAPTER 1  GENERAL INTRODUCTION

1.1 Background of Nitrate Contamination of Groundwater

1.1.1 Groundwater resources and pollution sources

Groundwater is precious and one of the most important natural water sources for humankind. It is widely exploited for different purposes such as drinking water supply, irrigation, and industrial usage (Raghunath, 2007). Moreover, due to industrial advances over the past two centuries, the population on the Earth has increased from 1 billion to over 7 billion (UN, 2013). Therefore, in addition to surface water, groundwater has become an important source for meeting the increasing drinking water demand all over the world. Nowadays, groundwater has already become one of the most important drinking water sources in many regions, for its high quality, easy extraction (UNESCO, 2015). Groundwater is also an essential water source for the agricultural industry in many regions around the world (Kreins et al., 2015).

At the same time, groundwater pollution, mainly in shallow aquifers, has become a serious environmental issue around the world. The pollution is mainly due to increase in human and industrial activities. Inorganic salts, heavy metals, persistent organic pollutants (POPs), and oil are typical pollutants in groundwater (Alsulaimi et al., 1993; Defo et al., 2015; Lucas and Reeves, 1981; Masih et al., 2014; Saether et al., 1997). Nitrate is one of the main pollutants responsible for groundwater contamination. This pollution is mainly due to the following reasons: excess usage of nitrogen fertilizer, inappropriate management of livestock waste, discharge of domestic and industrial wastewater containing nitrogen, infiltration of solid waste leachate, excessive exploitation of groundwater, and wastewater recharge (Follett, 1989; Keeney, 1986; Tarkalson et al., 2006). These sources of pollution are non-point source in general which makes pollution control difficult.

Nitrogenous compounds are among of the most common and widely used fertilizer nutrients and their use has greatly improved the yield of crops in farmland. However, a large amount of nitrogen fertilizer is not being utilized by crops and the residual
dissolves in precipitation and is then infiltrated into groundwater, causing an increase in nitrate concentration. Thorough investigation on nitrate contamination has been conducted around the world. The results show that groundwater nitrate contamination has become a very serious issue, with nitrate concentration reaching 70 mg-N/L or more in some locations (Canter, 1997; Follett, 1989; Hill, 1991; Zhang et al., 1995). Nitrate accumulation in groundwater below farmland and surrounding areas may become more severe with excessive application of nitrogen fertilizer over time.

Intensive livestock production also has a serious impact on water resources. Livestock waste is usually stored in earthen waste storage ponds. Due to inappropriate management of these ponds, concentration of nutrients including nitrogen tends to increase around farms (Ham and DeSutter, 1999; Kato et al., 2009; Parker et al., 1999). In the past decades, effort has been put into recycling livestock waste as manure and resources for anaerobic digestion and eased the impact of this waste on groundwater and surface water (Maeda et al., 2003; Tarkalson et al., 2006). Once the pond was established, the discharge of nutrients would last for a long period. Thus, a part of these nutrients will be decomposed to nitrate continuously as well as other contaminants (Kato et al., 2007; Kato and Shimura, 2007).

Partial treated sewage water was introduced to irrigation in the 1930s due to the growing shortage of water resources (Chapman, 1935). At present, sewage water irrigation is not only applied in developed countries, but also in a vast number of developing countries, especially in those countries or regions with scarce precipitation (Avnimelech and Raveh, 1974; Fleige et al., 1980; Fujioka and Loh, 1978; Liu et al., 2005; Mathan, 1994; Ryan et al., 2006; Tase, 1992). For instance, in the 1990s, about 100-200 million tons of sewage water was used for the irrigation of over 33 thousand hectares in Beijing, China (Zhu, 1995). The area with sewage water irrigation is still expanding. According to research, the main components of the sewage water are ammonia nitrogen and organic nitrogen as well as small quantities of nitrate and nitrite. Residual of organic nitrogen will be decomposed to ammonia and then converted to nitrate. Finally, the nitrate will be infiltrated to groundwater by means of rainfall and/or groundwater recharge.
Waste landfill is a common approach of municipal solid waste (MSW) in most countries. Usually, the concentration of nitrogen in MSW is relatively high (El-Fadel et al., 2002; El-Mahrouki and Watson-Craik, 2004). The solid waste leachate may infiltrate into groundwater with rain if the landfill is not well treated in advance. Serious groundwater contamination can occur in the vicinity of septic tanks, especially in the countryside, because of this infiltration (Harman et al., 1996; Lu et al., 2008; Zhang et al., 2009). All these factors combined are threatening the safety of residents’ drinking water.

Excessive exploitation of groundwater accompanied by wastewater recharge has exacerbated the situation of groundwater contamination. Once the exploitation of groundwater has exceeded the recovery capabilities, the local groundwater table will drop and a funnel area will be formed. This changes the local dynamics of groundwater and increases the hydraulic gradient. The chemistry of the groundwater environment changes as well. All these will benefit sewage infiltration and lead to an increase of nitrate concentration as well as other contaminants (Bear, 2007; Canter, 1997; Raghunath, 2007).

1.1.2 Health concerns related to nitrate

In the first half of 1940s, a number of comprehensive reports and reviews have been published relating to nitrate contamination in the environment, nitrate toxicity, and potential human health risks (Comly, 1945; Greenberg et al., 1943; Schwartz and Rector, 1940; Stevenson, 1943). Reported health effects included methemoglobinemia. The hazard of nitrate to humans is mainly due to the body’s reduction of nitrate to nitrite (Fraser and Chilvers, 1981; Hegesh and Shiloah, 1982). This reaction takes place in the saliva and stomach of humans of all ages and especially in gastrointestinal tract of newborn infants where the disease is known as “blue baby syndrome” (Schwartz and Rector, 1940; Stevenson, 1943).

Methemoglobinemia refers to an effect in which hemoglobin in blood is oxidized to methemoglobin. With an increase in methemoglobin, the oxygen transport capability
in the blood will be inhibited. If the oxygen content in blood is lower than a certain threshold, cyanosis, in other words oxygen starvation, will occur, and the results of this particular symptom are referred to as methemoglobinemia (Winneberger, 1982). Infants, especially those younger than 3 months, are highly susceptible to gastric bacterial nitrate reduction because they do not have enough gastric acid production and possess low activity of the enzyme which can reduce methemoglobin to hemoglobin (Keeney, 1986; Super et al., 1981). In the case of animals, excessive nitrates in groundwater have led to ruminants (animals with divided stomachs). Sheep and cattle, for instance, can be seriously affected by nitrates from birth to adulthood. It was determined that the formation rate of methemoglobin was high when hemoglobin solutions from ruminants were incubated with freshly prepared sodium nitrite (Canter, 1997; Smith and Beutler, 1966). Moreover, the ruminant hemoglobin solutions gave a much faster rate of methemoglobin formation than did those of man, horse or pig.

Several studies have shown that simultaneous ingestion of nitrite (or nitrate) with amines results in cancers of many organ systems. It is reported that the N-nitroso compounds are presumed as the ultimate carcinogenic substances (Correa, 1983; Kleinjans et al., 1991). However, the cause and effect relationship between exposure and incidence of certain cancers is still not clear because the relationship model between nitrate in groundwater and cancer is very difficult to be established. According to the investigation results mentioned in last section, nitrate concentration in some locations reached 70 mg-N/L or more, and these values are relatively high. Therefore, in order to reduce the risk of excessive exposure to nitrate in potable water, international organizations and various countries have set the nitrate and nitrite standards. These and other relevant standards are excerpted and shown in Table 1.1 because others are usually maintained normal (European Commission, 2007; Ministry of Health Labour and Welfare, 2003; Standard Administration of the People's Republic of China, 2006; USEPA, 2012; WHO, 2011).
Table 1.1 Drinking water standard (Excerpt)

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>WHO</th>
<th>EU</th>
<th>P. R. China</th>
<th>Japan</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (mg/L, as N)</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>10 *</td>
<td>10</td>
</tr>
<tr>
<td>Nitrite (mg/L, as N)</td>
<td>0.9</td>
<td>0.15</td>
<td>0.3</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>1 **</td>
<td>**</td>
<td>1</td>
<td>2</td>
<td>5 ***</td>
</tr>
<tr>
<td>Chromaticity (PCU)</td>
<td>15 **</td>
<td>**</td>
<td>15</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>pH</td>
<td>6.5-8.5</td>
<td>6.5-9.5</td>
<td>6.5-8.5</td>
<td>7.5</td>
<td>6.5-8.5</td>
</tr>
</tbody>
</table>

Notes: * Nitrite nitrogen is consolidated
** Acceptable to consumers and no abnormal change
*** At no time can turbidity go above 5 NTU

In order to ensure the drinking water safety, achieve the standards, and ease the health risk, protection of groundwater quality should have the highest priority (Follett, 1989; Perk, 2014; Winneberger, 1982). If groundwater is suffering nitrate contamination, cutting off the pollution source is the optimum choice. For groundwater that is already contaminated, remediation is the best choice (Nyer, 2009; Suthersan and Payne, 2005). Besides, for agricultural region, rational fertilization and irrigation, improving fertilizer efficiency, and the use of nitrification inhibitors are very important in reducing nitrate contamination caused by agricultural sources. It is also very important that farmers recognize that excessive fertilization does not increase crop yield (Follett, 1989). For nitrate removal from contaminated groundwater, it should be depended on some nitrate removal technologies which will be discussed in the following section.
1.2 Nitrate Removal Technologies

As mentioned in the last section, the demand for safe and clean drinking water is an urgent issue in many places around the world. Over the past decades, abnormal weather caused by global warming has resulted in uneven distribution of rainfall which affects groundwater recharge (Labraga and Villalba, 2009; LeHouerou, 1996). Thus, in order to ensure a stable and continuous drinking water source, it is necessary to both protect and remediate the underground sites contaminated by nitrate and other contaminants.

There are many kinds of remediation technologies investigated for nitrate removal in groundwater. Based on the nitrate removal mechanism, these technologies can generally be divided into physicochemical removal and biological removal approaches. Methods can also be divided into in situ and ex situ remediation technologies according to the site of treatment. In this study, in situ denitrification means that the denitrification occurs in aquifer contaminated with nitrate (Canter, 1997; Janda et al., 1988). Some of these approaches have been applied in practical processes (Archna et al., 2012; Ghafari et al., 2008).

1.2.1 Physicochemical removal approach

There are four conventional physicochemical nitrate removal approaches in general, which are ion exchange, reverse osmosis, electrodialysis, and catalyst. These approaches have been studied gradually since the 1970s (Canter, 1997). Table 1.2 shows a brief comparison of these approaches.
Table 1.2 Comparison of physicochemical nitrate removal approaches

<table>
<thead>
<tr>
<th>Technology*</th>
<th>Principle</th>
<th>Merit</th>
<th>Defect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>By using strong basic anion exchange resin.</td>
<td>High removal rate, facilitate management.</td>
<td>Relatively poor selectivity, high cost, post treatment is needed. Usually pump and treat.</td>
<td>(Dahab, 1988)</td>
</tr>
<tr>
<td>RO</td>
<td>By using reverse osmosis membrane.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED</td>
<td>Remove electrically through an anion exchange membrane.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalyst</td>
<td>Remove nitrate via metal reduction or catalyst method.</td>
<td>Nitrate is reduced to nitrogen gas. Reaction can be conducted under atmospheric - normal temperature and pressure – conditions.</td>
<td>Ammonia/ammonium salt accumulation and the deterioration of catalysts will occur.</td>
<td>(Murphy, 1991)</td>
</tr>
</tbody>
</table>

* IX = ion exchange; RO = reverse osmosis; ED = electrodialysis
Ion exchange is a technology that involves the exchange of ions in a solution (i.e., contaminated groundwater) with chemically equivalent numbers of ions associated with the exchange material (i.e., the resin). In the case of nitrate removal in groundwater, nitrate ions are typically replaced by chloride ions when the groundwater is passed through the resin (Lauch and Guter, 1986; Nur et al., 2015; Wiegleb and Baeck, 1981). Nitrate removal using ion exchange usually utilizes a strongly basic anion exchanger. The nitrate removal efficiency of this exchanger may be affected by the presence and the concentration of other anions. Lauch and Guter (1986) reported that sulfate has a higher selectivity in exchange than nitrate. At the same time, the sulfate concentration is usually higher than nitrate or at the same level and this may affect the processing performance of ion exchange (Lauch and Guter, 1986).

Reverse osmosis is a technology which is commonly and widely applied in seawater desalination (Archna et al., 2012). Its mechanism is to force the solution through a semipermeable membrane by applying high pressure and leave behind the nitrates as well as other ions in the solution (Richards et al., 2011). This is usually achieved by subjecting the water supply in a reverse osmosis cell in which the pressure exceeds its corresponding osmotic pressure. These cells have to be able to endure high system pressures (i.e., up to 6.9 MPa when used in desalinating seawater) (Dahab et al., 1990). Generally, these cells do not have a significantly high selectivity for any given ions present in the solution. As a consequence, this process results in removal of many ionic species, including nitrates. Other potential important issues associated with this process include, for instance, the need for post treatment of the concentrated solution after the process has ended (Huxstep and Sorg, 1987).

Electrodialysis refers to an electrically driven unit operation in which ions are selectively transported through semipermeable membranes from one solution to another under the influence of a direct current electric field (Dahab et al., 1990). The membranes are generally set in cell pairs; the pairs are alternately placed as cation (i.e., sodium, potassium, and magnesium) and anion (i.e., nitrate, chloride, and sulfate) transfer membranes. Ions will be selectively transferred through the correspondent membranes when a direct current is applied on the membranes array (Indusekhar et al.,
During the process, the polarity of the electrodes will be reversed two to four times to ease deposition and desalination on the cathode. Operating these measures is generally very complex, and therefore the overall maintenance of the system is difficult (Rautenbach et al., 1987).

The chemical approach usually means removal of nitrate via metal reduction or catalyst method and it could be conducted under normal conditions (Murphy, 1991). In this process, nitrate is reduced to nitrogen gas by using platinum or copper or binary metal (i.e., Pd/Cu-Al₂O₃) as a catalyst in the reaction of dissolved hydrogen (DH). Of all the physicochemical approaches, this is the sole method that can reduce nitrate to nitrogen gas. No concentrated solution or sludge will be produced during the process; thus, it can be considered a practical and useful nitrate removal approach. It has been reported that ammonia/ammonium salt accumulation and the deterioration of catalysts occurred in the course of this process (Hörold et al., 1993). This may cause the approach to be inconvenient and not appropriate for practical application and operation.

In summary, these approaches can achieve high removal rate or even can reduce nitrate to nitrogen gas, but they need post treatment or lead to deterioration of catalysts. These defects have limited their wide practical application.

1.2.2 Biological removal approach

Since the physicochemical approaches all have some disadvantages (especially in operation and/or post treatment), many researchers have tried to develop a more convenient and practical approach to remove nitrate from groundwater. In order to overcome the disadvantages of these approaches, biological processes, known as biological denitrification, were introduced to biochemically convert constituents of concern (i.e., nitrate, phosphorus) to end-products that are innocuous or can be used as a resource (Archna et al., 2012; Sun et al., 2015).

Biological denitrification is a respiratory process in which bacteria utilize nitrate or nitrite as a terminal electron acceptors (Ghafari et al., 2008). Some microorganisms, usually facultative, are capable of utilizing compounds other than oxygen as an electron
accepter when the dissolved oxygen (DO) content of an environment is absent or insufficient (Ghafari et al., 2008; Karanasios et al., 2010). This condition can occur under anoxic conditions – a certain low level or absence of DO (Bitton and Gerba, 1984; Sprent, 1987). When nitrate is utilized as an electron acceptor, it is finally reduced to a nontoxic product: nitrogen gas (N₂) (Dahab, 1988). Biological denitrification is generally divided into heterotrophic (1.2.2.1) and autotrophic (1.2.2.2) denitrifications, based on different types of carbon sources (Archna et al., 2012).

1.2.2.1 Heterotrophic denitrification

Heterotrophic denitrification utilizes organic compounds as electron donors. Methanol, ethanol, acetic acid, and sucrose are usually selected as carbon source in drinking water treatment (ÆsØy et al., 1998; Constantin and Fick, 1997; Du Toit and Davies, 1973; Janda et al., 1988; Lewandoswki, 1982; Mercado et al., 1988). The first three compounds are in liquid form and need special attention regarding secondary pollution. In order to ease secondary pollution caused by these organic compounds, it is very important to control and adjust the ratio of C/N in the influent and this is difficult to achieve (Buttiglieri et al., 2005; Nuhoglu et al., 2002; Sobieszuk and Szewczyk, 2006). Therefore, further advanced treatment should be introduced to improve water quality.

The stoichiometric relationships for these processes are shown as the follows (take methanol as an example) (Dahab and Lee, 1988).

Bacterial energy reaction, step 1:

\[
6 \text{NO}_3^- + 2 \text{CH}_3\text{OH} \rightarrow 6 \text{NO}_2^- + 2 \text{CO}_2 + 4 \text{H}_2\text{O} \tag{1-1}
\]

Bacterial energy reaction, step 2:

\[
6 \text{NO}_2^- + 3 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 3 \text{CO}_2 + 3 \text{H}_2\text{O} + 6 \text{OH}^- \tag{1-2}
\]

Overall energy reaction:

\[
6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 5 \text{CO}_2 + 7 \text{H}_2\text{O} + 6 \text{OH}^- \tag{1-3}
\]

Bacterial synthesis reaction:

\[
3 \text{NO}_3^- + 14 \text{CH}_3\text{OH} + \text{CO}_2 + 3 \text{H}^- \rightarrow 3 \text{C}_3\text{H}_5\text{O}_2\text{N} + \text{H}_2\text{O} \tag{1-4}
\]

Besides these substances, other solid state carbon sources such as cotton, straw, and sawdust have been studied in drinking water treatment (Ines et al., 1998; Rocca et
al., 2005; Schipper et al., 2004; Schipper and Vojvodic-Vukovic, 2000). It should be noted that even though these denitrification processes were capable of reducing the nitrate concentration in the influent from as high as 100 mg-N/L to levels within the 1.0 mg-N/L range, they affected some other water quality parameters, such as increasing total organic carbon (TOC) and chromaticity in effluent. The efficiency of these processes is influenced by temperature changes. One common feature of all these processes is that the growth rate of microorganisms is relatively high, which may lead to sludge and/or clogging problems in pumps. For instance, Mercado et al. (1988) conducted an in situ biological groundwater denitrification experiment using sucrose as a carbon source (Mercado et al., 1988). In this study, part of the experimental sites were clogged immediately after the experiment started.

Extraction membrane bioreactor has been introduced to overcome the shortcomings. Mansell and Schroeder (1999) investigated a continuous flat membrane reactor study at bench-scale and methanol was applied as the carbon source. The overall nitrate removal rate of this process is up to 90% (Mansell and Schroeder, 1999). Ergas and Rheinheimer (2004) developed a hollow fiber membrane bioreactor in which the carbon source was also methanol. The overall nitrate removal rate in this case has been stated as more than 99% (Ergas and Rheinheimer, 2004). Residual methanol could be detected in effluent of these processes; it could be due to some methanol diffused through the membrane. Both cases show that secondary pollution caused by organic carbon is still the main problem in these processes. Table 1.3 shows performances of some heterotrophic denitrification approaches.

In summary, although heterotrophic denitrification approaches can achieve high nitrate removal rate, they lead to some problems including secondary pollution, clogging in sites. Thus, these approaches are not practical for an in situ groundwater denitrification treatment.
Table 1.3 Performances of heterotrophic denitrification approaches

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Style</th>
<th>Nitrate concentration (mg-N/L)</th>
<th>Nitrite out (mg-N/L)</th>
<th>Carbon source residual</th>
<th>DO (mg/L)</th>
<th>pH</th>
<th>Clogging</th>
<th>Others</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Reactor</td>
<td>25</td>
<td>NG</td>
<td>Y</td>
<td>0</td>
<td>8.75±0.2</td>
<td>NG</td>
<td>Alkalinity increase</td>
<td>ÆsØy 1998</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Reactor</td>
<td>1,610</td>
<td>0-</td>
<td>0</td>
<td>Y</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>Constantin 1997</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Reactor</td>
<td>1,386</td>
<td>37.5</td>
<td>Trace</td>
<td>Y</td>
<td>&lt;1</td>
<td>7.6-9.3</td>
<td>NG</td>
<td>Du Toit 1973</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Reactor</td>
<td>9.2-11.7</td>
<td>0.2-7.3</td>
<td>&lt;0.32</td>
<td>Y</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>Janda 1988</td>
</tr>
<tr>
<td>Methanol</td>
<td>Reactor</td>
<td>14.5</td>
<td>NG</td>
<td>Trace</td>
<td>Y</td>
<td>&lt;1</td>
<td>8.0±0.2</td>
<td>NG</td>
<td>Mercado 1988</td>
</tr>
<tr>
<td>Sucrose</td>
<td>in situ</td>
<td>30</td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>Y</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>Buttiglieri 2005</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Reactor</td>
<td>16-123</td>
<td>NG</td>
<td>Y</td>
<td>NG</td>
<td>8.0±0.2</td>
<td>NG</td>
<td>NG</td>
<td>Nuhoglu 2002</td>
</tr>
<tr>
<td>Straw</td>
<td>Reactor</td>
<td>23</td>
<td>&lt;5</td>
<td>&lt;3</td>
<td>N</td>
<td>NG</td>
<td>NG</td>
<td>DOC increase</td>
<td>Ines 1998</td>
</tr>
<tr>
<td>Cotton</td>
<td>Reactor</td>
<td>13.9</td>
<td>2.1</td>
<td>NG</td>
<td>N</td>
<td>NG</td>
<td>NG</td>
<td>TOC increase</td>
<td>Rocca 2005</td>
</tr>
</tbody>
</table>

Notes: Y = Yes, N = No, NG = Not given
1.2.2.2 Autotrophic denitrification

Autotrophic denitrification is an approach in which microorganisms utilize inorganic substances (i.e., sulfur, hydrogen gas) as electron donors in their metabolism (Batchelor and Lawrence, 1978; Kurt et al., 1987). The electron donors employed in this process exist in solid or gaseous state and it is therefore assumed that they will not remain in effluent after treatment. In addition, compared to heterotrophic denitrification microorganisms, autotrophic ones utilize bicarbonate or carbon dioxide as a carbon source which subsequently leads to a longer generation period, less biomass, and less metabolites (Ren et al., 2007).

Sulfur is used as an electron donor for it is cheap, non-toxic, insoluble in water, and stable under atmospheric conditions (normal temperature and pressure) (Soares, 2002). The reactions for sulfur-dependent denitrification are shown as the follows.

\[
5 \text{S} + 6 \text{NO}_3^- + 2 \text{H}_2\text{O} \rightarrow 5 \text{SO}_4^{2-} + 3 \text{N}_2 + 4 \text{H}^+ \quad (1-5)
\]

\[
55 \text{S} + 50 \text{NO}_3^- + 38 \text{H}_2\text{O} + 20 \text{CO}_2 + 4 \text{NH}_4^+
\rightarrow 4 \text{C}_2\text{H}_7\text{O}_2\text{N} + 55 \text{SO}_4^{2-} + 25 \text{N}_2 + 64 \text{H}^+ \quad (1-6)
\]

Alkalinity will be consumed because of the release of hydrogen ions during the process. An amount of 4 mg alkalinity (in the form of CaCO3) is required for the complete removal of 1 mg nitrate nitrogen. The activity of microorganisms can be significantly influenced by pH, so limestone is often introduced into such systems in order to maintain the pH as well as to provide a carbon source (Koenig and Liu, 2002; Liu and Koenig, 2002). During the process, 7.54 mg of sulfate will be generated for the complete removal of 1 mg nitrate nitrogen. Therefore, this approach is not suitable for groundwater which already contains high sulfate concentration.

Compared to sulfur, hydrogen gas (H2) is an ideal electron donor due to its relatively low cost, clean, non-toxic, and low solubility in water (1.6 mg/L at 20 °C, 1 atm). The hydrogenotrophic denitrification reactions are represented by the following equations (Kurt et al., 1987; Lee and Rittmann, 2002).

Nitrate reduction:

\[
\text{NO}_3^- + \text{H}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \quad (1-7)
\]
Nitrite reduction:

\[
\text{NO}_2^- + 0.5 \text{H}_2 \rightarrow \text{NO}_2(\text{g}) + \text{OH}^- \quad (1-8)
\]

Nitric oxide reduction:

\[
2 \text{NO}_2(\text{g}) + \text{H}_2 \rightarrow \text{N}_2\text{O}(\text{g}) + \text{H}_2\text{O} \quad (1-9)
\]

Nitrous oxide reduction:

\[
\text{N}_2\text{O}(\text{g}) + \text{H}_2 \rightarrow \text{N}_2(\text{g}) + \text{H}_2\text{O} \quad (1-10)
\]

Equations (1-8) to (1-10) can be simplified as the follows (NO\text{\textsuperscript{2-}} to N\text{\textsubscript{2}}):

\[
2 \text{NO}_2^- + 3 \text{H}_2 \rightarrow \text{N}_2 + 2 \text{H}_2\text{O} + 2 \text{OH}^- \quad (1-11)
\]

Overall denitrification reaction from NO\text{\textsuperscript{3-}} to N\text{\textsubscript{2}}:

\[
2 \text{NO}_3^- + 5 \text{H}_2 \rightarrow \text{N}_2 + 4 \text{H}_2\text{O} + 2 \text{OH}^- \quad (1-12)
\]

Stoichiometric reaction among e- donor, e- acceptor, and biomass:

\[
\text{NO}_3^- + 3.03 \text{H}_2 + 0.229 \text{CO}_2 \rightarrow 0.0458 \text{C}_3\text{H}_7\text{O}_2\text{N} + 0.477 \text{N}_2 + 2.37 \text{H}_2\text{O} + \text{OH}^- \quad (1-13)
\]

Based on Equation (1-7), nitrate is reduced to nitrite, 0.14 mg H\text{\textsubscript{2}} is required per 1 mg nitrate nitrogen involves no pH shift; in Equation (1-11), nitrite is further reduced to nitrogen gas, 0.21 mg H\text{\textsubscript{2}} is required per 1 mg nitrite nitrogen and a pH shift could be observed from this step. For overall denitrification reaction, it demands 0.35 mg H\text{\textsubscript{2}} per 1 mg nitrate nitrogen for complete reduction. One mole of OH\textsuperscript{-} is released while one mole of NO\text{\textsuperscript{3-}} is reduced. Similar with heterotrophic denitrification 3.57 unit of alkalinity will be released for complete removal of 1 mg nitrate.

Many factors including DO, pH, and temperature may affect the processing efficiency of biological denitrification.

Different microorganisms have different optimal pH values, for denitrifying bacteria, the optimum range of pH is 6.5-7.5. If the pH is below 6 or above 8, the reaction rate will be reduced significantly (Karanasios et al., 2010; Tang et al., 2011). As pH may affect the production of intermediate product, for instance, nitrous oxide (Pan et al., 2012). The activity of microorganisms and sedimentation of inorganic ions are influenced by pH, thus there is still no conclusion on optimum pH range. pH values around 7.0 are most common (Haugen et al., 2002; Lee and Rittmann, 2002; Mansell and Schroeder, 2002). It is reported that nitrite will be accumulated significantly if the
pH is above 8.6, and the total removal efficiency will decrease (Lee and Rittmann, 2003).

Denitrifying bacteria are usually facultative and are capable to utilize nitrate/nitrite only if the environment is anoxic. Once the DO exceeds a certain threshold concentration, the denitrification process will be inhibited. Although former studies agreed that the DO should be maintained at a certain low level, the range of DO varies from below 0.5 to several mg/L. Moreover, denitrification is available even if the DO is relatively high, but more hydrogen amount is required (Visvanathan et al., 2008). For example, Schnobrich et al. (2007) reported that 0.9 mg/L of extra DH was required for an influent with a DO concentration of 5.5 mg/L (Schnobrich et al., 2007). Therefore, the threshold of DO on denitrification is still not clear yet.

An appropriate temperature range for denitrification is 20 - 40 °C. The growth of microorganisms as well as their metabolism and denitrification rates will be inhibited if the temperature is lower than 15 °C. The impact of temperature is mainly manifested in the following three aspects: 1: The activity of nitrate reductase is only at 30% in case of 20 °C compared to 42 °C - the temperature at which the nitrate reductase shows the highest efficiency (Kurt et al., 1987). 2: Hydrogen mass transfer coefficient decreases by 20% if the temperature is lowered from 20 °C to 10 °C. Given about 7% increase of hydrogen saturation concentration, the overall transfer coefficient decreases about 10% (Haugen et al., 2002). 3: Optimum pH varies in connection to temperature. For example, the optimum pH is 9.5 at 25 °C while it is 8.5 at 12 °C (Rezania et al., 2005).

In the past decades, many ex situ and in situ hydrogenotrophic denitrification studies have been conducted. Table 1.4 summarizes the performances of some hydrogenotrophic denitrification approaches, including in situ and ex situ ones.
Table 1.4 Performances of hydrogenotrophic denitrification approaches

<table>
<thead>
<tr>
<th>Style</th>
<th>Nitrate concentration (mg-N/L)</th>
<th>Nitrate out (mg-N/L)</th>
<th>DO in (mg/L)</th>
<th>pH (range)</th>
<th>Duration (d)</th>
<th>HRT or Velocity</th>
<th>Others</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluidized-bed</td>
<td>16-27.6</td>
<td>&lt;1</td>
<td>NG</td>
<td>7.1-9.8</td>
<td>60</td>
<td>53, 400min</td>
<td>DH&gt;0.2mg/L</td>
<td>Chang 1999</td>
</tr>
<tr>
<td>MBR</td>
<td>10, 12.5</td>
<td>&lt;2.4</td>
<td>&lt;0.1</td>
<td>7-7.2</td>
<td>NG</td>
<td>42min</td>
<td>DOC increased</td>
<td>Lee 2000</td>
</tr>
<tr>
<td>MBR</td>
<td>20-40</td>
<td>0.8-3.2</td>
<td>NG</td>
<td>7</td>
<td>~55**</td>
<td>NG</td>
<td>-</td>
<td>Mansell 2002</td>
</tr>
<tr>
<td>In Situ</td>
<td>8.2, 16.4</td>
<td>0*</td>
<td>Removed</td>
<td>7</td>
<td>140</td>
<td>0.3 m/s</td>
<td>TOC&lt;0.5mg/L</td>
<td>Haugen 2002</td>
</tr>
<tr>
<td>MBR</td>
<td>14-72</td>
<td>0, 20</td>
<td>0</td>
<td>7.4-8</td>
<td>180</td>
<td>9, 12h</td>
<td>DOC&gt;8mg/L</td>
<td>Mo 2005</td>
</tr>
<tr>
<td>Reactor</td>
<td>28</td>
<td>1.1</td>
<td>&lt;0.1</td>
<td>7.1-7.7</td>
<td>191</td>
<td>2h</td>
<td>-</td>
<td>Smith 2005</td>
</tr>
<tr>
<td>In Situ</td>
<td>24</td>
<td>0-10</td>
<td>0-24</td>
<td>5.5</td>
<td>270</td>
<td>0.5m/d</td>
<td>-</td>
<td>Schnobrich 2007</td>
</tr>
<tr>
<td>Reactor</td>
<td>50</td>
<td>&lt;5.7</td>
<td>&lt;1.1</td>
<td>Removed</td>
<td>~43</td>
<td>2-9h</td>
<td>-</td>
<td>Visvanathan 2008</td>
</tr>
</tbody>
</table>

Notes: *: Not detected, **: Several Runs were conducted, the longest one, NG: Not given
From Table 1.4, it could be concluded that many studies on hydrogenotrophic denitrification have been conducted, but on *in situ* hydrogenotrophic denitrification, there is still a lack of knowledge. The impact of pH on nitrite accumulation is not clear for some nitrite accumulation observed even the pH was supposed to be the most optimum values. In addition, the impact of DO on denitrification was also not clear especially in denitrification zone. Moreover, some water quality parameters in effluent such as TOC/DOC as well as turbidity could not yet meet the drinking water guidelines. Finally, the durations of all these experiments were relatively short (no more than 200 d in general), thus the stability of a long-term treatment is not clear enough, which is one of the most important indicators for applying the technology to a field treatment.

### 1.3 Objectives and Contents of This Dissertation

The objective of this study is to verify the long-term practicality of the *in situ* denitrification process, the impact of pH on denitrification and nitrite accumulation, and the effect of DO related to denitrification and some water quality parameters such as TOC. Therefore, a novel *in situ* denitrification and oxidation process with injection of electrolytic hydrogen and oxygen was investigated; focusing on denitrification performance of nitrate contaminated shallow aquifers as well as water quality parameters related. Figure 1.1 illustrates the conceptual diagram of this study.

**Figure 1.1** Illustration of conceptual diagram.
The contents of this dissertation are shown as the follows:

1. In Chapter 1, the background and the objectives of this study are discussed.

2. In Chapter 2, hydrogenotrophic denitrifying microorganisms were cultivated and then the batch experiment was conducted over a duration of 18 days in order to verify the denitrification capability of these microorganisms in the apparatus. At the same time, problems caused by pH and DO were re-confirmed and compared with the reviewed results.

3. Since denitrification was available based on the results of Chapter 2, in Chapter 3, experiments of continuous treatment were studied to verify the feasibility of the present system. Water quality parameters were collected and then compared with drinking water guidelines to make sure whether they could meet the standard or not.

4. In order to examine applicable operating conditions, a long-term continuous treatment experiment with different hydraulic and nitrate loading conditions were conducted. The performances of nitrate removal as well as water quality parameters were evaluated.

5. In Chapter 5, the results of this study were summarized. At the same time, prospect of future work was mentioned.
CHAPTER 2  BATCH EXPERIMENT

2.1 Introduction

As mentioned in Chapter 1, microorganisms exist all around the biosphere, including atmosphere, hydrosphere, and pedosphere, and the bacteria used in denitrification process are usually facultative (Ren et al., 2007). They could be commonly found in many natural environments, for instance, soil, marine, and wastewater treatment plants. Studies related to the microorganisms usually commence with their cultivation. Gamble et al (1977) performed one of the first and most comprehensive studies in exploring soil denitrifier communities (Gamble et al., 1977).

Since many problems including secondary pollution and aquifer clogging occurred in heterotrophic denitrification processes, researchers tried to develop processes which could counter these shortcomings (Du Toit and Davies, 1973; Janda et al., 1988; Mercado et al., 1988). Studies on autotrophic denitrification with hydrogen gas as electron donor have been conducted and developed since the 1980s (Kurt et al., 1987; Lee and Rittmann, 2000; Rezania et al., 2007; Till et al., 1998). All of them indicated the availability of autotrophic denitrification to utilize hydrogen and some of them were able to demonstrate a high speed denitrification rate as well (Ergas and Rheinheimer, 2004; Hasar and Ipek, 2010; Komori, 2010; Komori and Sakakibara, 2008; Nuhoglu et al., 2002). Before applying these microorganisms to a main experiment, bacteria cultivation was concluded to get appropriate microflora. Widely accepted and common methods of inoculation of bacteria were developed during these studies.

In this study, part of the microflora was obtained and acclimatized from soil (collected from University’s campus) and a part was taken from former studies. In this chapter, the acclimatization of denitrifying bacteria, inoculation of bacteria to apparatus, batch experiment, and performance of batch experiment are introduced.
2.2 Materials and Methods

2.2.1 Thermostatic chamber

As mentioned in Chapter 1, the activity of microorganisms might be affected by temperature which then leads to performance variation. In order to realize a thermally stable subsurface environment and avoid temporal influence as much as possible, a thermostatic chamber was constructed in the laboratory with air conditioner inside near ventilators. With this chamber, a thermally stable subsurface environment where temperature variation is relatively small (20 ± 2 °C, monitored by using temperature recorder, TR-71D, T&D Corp.) could be imitated.

2.2.2 Denitrifying bacteria cultivation apparatus

Figure 2.1 illustrates the apparatus used for batch bacteria cultivation. The volumes of the hydrogen gas container and culture bottle were 760 and 1,100 mL, respectively. Saturated sodium chloride solution was filled in order to restrain hydrogen gas escaping from the container. To imitate a subsurface environment and avoid the influence of illumination, the culture bottle was covered by bilayer aluminum film and light shielding nylon tubes were used to connect the containers. Leather hose clips were used to connect the tubes in order to assure the tightness. A magnetic stir was put inside the culture bottle and then the bottle was placed on an electromagnetic stirrer to assure a homogenous solution.

Water electrolysis is a common way to obtain hydrogen gas. Conventional electrolytic methods usually use separate anode and cathode for electrolysis which is known to lead to sediment of inorganic salts on cathode. Furthermore, the oxygen gas produced at the anode may hinder denitrification process. Additionally, high current density is usually applied to the electrodes in order to get higher electrolysis rate and this may lead to large power consumption which may become one of several limiting factors in practical application. If graphite electrodes are used instead of metal ones, carbon dioxide will be produced which is then able to act as an inorganic carbon source and buffer (Sakakibara et al., 1994). But at the same time, the exchange of electrode is
required in this setting due to electrode consumption which may subsequently result in complicated maintenance as well as additional cost.

Figure 2.1 Illustration of bacteria cultivation apparatus.

To solve these shortcomings, solid polymer electrolyte membrane electrode (SPEME) were then developed and introduced for water electrolysis. Figure 2.2 indicates the conceptual diagram of SPEME (Komori, 2010) (Doctoral dissertation). SPEME is a relatively new technology and some studies related have been conducted in the past decades (Millet et al., 1993; Sawada et al., 2008; Tanaka et al., 2003). It acts in the role of a charge movement media during electrolysis and consumes smaller electric energy than other electrodes as well. Thus such electrode can help overcoming the shortcomings mentioned above. From the figure, it could be seen that the water of anode and cathode is separated by the electrode.
The oxygen produced in the electrolysis could not move to the cathode since the separation of water is ensured by SPEME itself. The half reaction of anode could be shown as the following equation:

$$\text{H}_2\text{O} \rightarrow 0.5 \text{O}_2 + 2 \text{H}^+ + 2 \text{e}^- \quad (2-1)$$

For the cathode, it could be considered that the hydrogen gas will not be produced unless the oxygen on the surface of SPEME is completely consumed (Sakakibara et al., 1997). The half reactions are shown as the follows:

$$0.5 \text{O}_2 + 2 \text{e}^- + \text{H}_2\text{O} \rightarrow 2 \text{OH}^- \quad (2-2)$$

$$2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{H}_2 \quad (2-3)$$

From equation (2-2), it could be indicated that the increase of alkalinity on the surface of cathode is negligible, and salt sediments could be avoided since no water molecule is electrolyzed. Moreover, the voltage applied on the electrodes is very low.
(i.e., several volts) and the resistance of the membrane very small. Finally, conductivity almost does not have any impact on the voltage and a stable voltage could be achieved. These superior properties make low voltage/high current density available which leads to smaller energy consumption in electrolysis.

2.2.3 Batch experiment apparatus

Figure 2.3 shows the experimental setup of the laboratory-scale aquifer used for the batch experiment. This aquifer imitated a part of underground (suggested model shown in Figure 1.1). The dimensions of the aquifer were 1,950 mm in length, 25 mm in width, and 780 mm in height. The aquifer was filled with glass beads (diameter \( \varphi = 2 \) mm, hydraulic conductivity \( k = 2.0 \pm 0.1 \) m/d) as carrier of the microorganisms. The hydraulic conductivity \( k \) was determined according to Darcy’s law (Bear, 2007; Sato and Iwasa, 2000). The glass beads applied in this study were equivalent to clean sand or sand/gravel, which approximately ranged from 0.05 to 4 mm in diameter (Bear, 2007). The total and effective liquid volumes of the aquifer were about 40 L and 16 L, respectively. An amount of 18 sampling ports were allocated in horizontal and vertical directions with a 300 mm interval as shown in Figure 2.3. To avoid algal growth, the aquifer and every tube were covered with a light-shielding curtain or aluminum foil. For batch experiment, the peristaltic pumps were stopped during the experimental period (see Figure 2.3).
2.3 Denitrifying Bacteria Cultivation

2.3.1 Inoculum

Table 2.1 and Table 2.2 show the composition of nutrient solution and inoculum composition for main experiment, respectively. An amount of 20 g surface soil was collected from the university’s campus and put into 1,000 mL of pre-cooled boiled tap water. A supernatant of this soil/water mixture was taken and mixed with other solutions (see Table 2.2).
Table 2.1 Composition of nutrient solution for bacteria cultivation

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$_2$HPO$_4$</td>
<td>220</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>260</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>500</td>
</tr>
<tr>
<td>NaCl</td>
<td>120</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>140</td>
</tr>
<tr>
<td>FeCl$_3$·6H$_2$O</td>
<td>240</td>
</tr>
</tbody>
</table>

Table 2.2 Composition of bacteria

<table>
<thead>
<tr>
<th>Source</th>
<th>Contents</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant of soil/water mixture</td>
<td>Soil from campus 350 mL</td>
<td>20 g</td>
</tr>
<tr>
<td></td>
<td>Boiled tap water 1,000 mL</td>
<td>1,000 mL</td>
</tr>
<tr>
<td>Nutrient solution</td>
<td>NaNO$_3$ 350 mL</td>
<td>15 mg-N/L</td>
</tr>
<tr>
<td></td>
<td>Nutrient solution (Table 2.1)</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>Boiled tap water 333 mL</td>
<td>333 mL</td>
</tr>
<tr>
<td>Bacteria solution</td>
<td>Obtained from former study 350 mL</td>
<td>(Komori and Sakakibara, 2008)</td>
</tr>
<tr>
<td>Total</td>
<td>1,050 mL</td>
<td></td>
</tr>
</tbody>
</table>

2.3.2 Cultivation

The cultivation system was set up as shown in Figure 2.1 and the culture bottle was filled with the bacteria containing solution as shown in Table 2.2. Once the nitrate and nitrite levels reached 0, another 91 mg of NaNO$_3$ (about 15 mg-N/L) was added to the culture bottle. An amount of 750 mL of hydrogen gas was produced and stored in the hydrogen gas container; it was consumed over time by denitrifying bacteria via the connecting tube. Once the amount of hydrogen gas was below 50 mL, the electrolysis of water would be conducted to supply hydrogen gas. Moreover, in order to provide enough buffer and keep a stable pH, an amount of 3 g NaHCO$_3$ were added to the culture bottle whenever the pH was higher than 9.
2.3.3 Denitrification performance

A period of 118 days of cultivation was conducted to enrich the bacteria. Weekly measurement for nitrate, nitrite, pH, and DO was performed. Liquid samples were taken from the culture bottle by using a syringe. Samples for nitrate and nitrite analysis were filtered using a 0.45 μm syringe-driven membrane filter (25CS045AN, Advantec), while no filtration was made for other samples.

Nitrate and nitrite were measured with ion chromatography (HIC-10A, Shimadzu). pH and DO were measured with pH meter (pH 510, Eutech instruments) and DO meter (Ultra DO meter, Central Kagaku Corp.), respectively.

Figure 2.4 illustrates the denitrification performance of denitrifying bacteria during cultivation. At the start up, the nitrate concentration was 17.6 mg-N/L, while no nitrite was detected. The next day, a slight decrease of nitrate was observed without nitrite accumulation. On day 15, both nitrate and nitrite could not be detected anymore. From day 20 to 70, almost no nitrate or nitrite could be detected which concluded that complete denitrification was achieved by these denitrifying bacteria. From day 71 until the end of bacteria cultivation, there was no nitrate but some nitrite accumulated.

Figure 2.5 indicates the temporal changes of pH values. The pH was kept around 8.5 until day 30. Thereafter, pH increased and was kept around 9. Although NaHCO₃ was added when the pH was higher than 9, it was still difficult to maintain the pH at a lower level. As mentioned above, nitrite accumulated from day 71, which could be due to a relatively high pH of the solution, and therefore it could be considered as a limiting factor of complete denitrification (Rust et al., 2000; Tang et al., 2011).
Figure 2.4 Temporal changes of nitrate and nitrite.

Figure 2.5 Temporal changes of pH.
Figure 2.6 shows temporal variation of DO. It could be concluded from the figure that DO was maintained at a relatively low level, around 2 mg/L. Thus an anoxic circumstance was achieved. It was reported that denitrification could occur in an anoxic environment if DO concentration is low enough (i.e., <2 mg/L) (Doi and Sakakibara, 2004; Du Toit and Davies, 1973; Sprent, 1987). The results gotten from this bacteria cultivation coincides with these former studies.

![Figure 2.6 Temporal changes of DO.](image)

From the results of cultivation, it could be concluded that the microorganisms cultivated might be capable and suitable for denitrification process in further batch/continuous experiments.
2.4 Batch Experiment

2.4.1 Inoculum

Besides anoxic bacteria, aerobic bacteria was obtained from a supernatant which were produced by mixing 20 g soil from University’s campus (the same location for collecting denitrifying bacteria) in 1,000 mL cooled boiled tap water. 500 mL of both mixtures were injected into the aquifer through the ports according to the flow chart shown in Figure 2.7. Table 2.3 shows the composition of synthetic groundwater used for batch experiment.

The hydrogen gas produced via electrolysis was stored in a gas container (as shown in Figure 2.3) and hydrogen gas was injected to the aquifer via peristaltic pump at the bottom of port 6 once the gas bubbles in the aquifer almost invisible. The interval of hydrogen gas injection was about 72 hours. The oxygen gas produced simultaneously in batch experiment was discharged to the atmosphere directly. At the top of the aquifer, a port was set for gas effluent as shown in Figure 2.3.

![Flow chart of initial set up of batch experiment.](image)

**Figure 2.7 Flow chart of initial set up of batch experiment.**
Table 2.3 Composition of synthetic groundwater

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃ (as N)</td>
<td>15.06</td>
</tr>
<tr>
<td>Inorganic carbon (as C)</td>
<td>10.20</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.76</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2.08</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>4.00</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.96</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.12</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>1.92</td>
</tr>
</tbody>
</table>

2.4.2 Results

Nitrate, nitrite, and DO were monitored every day and the ports monitored were shown in Figure 2.8, while pH of every port was observed only for start and end.

Daily nitrate and nitrite variation are shown in Figures 2.9 and 2.10, respectively. Moreover, Figure 2.11 depicts the daily variation of total nitrogen. The days hydrogen injected was marked as arrows in Figure 2.10.
Figure 2.9 Temporal changes of nitrate in batch experiment.

Figure 2.10 Temporal changes of nitrite in batch experiment.
Figure 2.11 Temporal changes of total nitrogen in batch experiment.

Figure 2.12 shows daily DO variation in batch experiment. Ports 4-6 were the hydrogen injection zones as shown in Figure 2.3. An amount of 630 mL hydrogen gas was injected at the beginning of this experiment, and DO in hydrogen injection zone increased to about 5 mg/L on day 5. A significant decrease in visible gas bubbles was observed, thus it could be indicated that the hydrogen gas injected was consumed. This could be due to the insufficient supply of hydrogen gas. To maintain a relatively low level of DO around these ports, an amount of 500 mL of hydrogen gas was injected every three days. The appearance of hydrogen gas bubbles just after injection (day 5) and before injection (day 8) were shown in the left and right sides in Figure 2.13, respectively.

Table 2.4 illustrates the pH values at the beginning and end of batch experiment.
Figure 2.12 Temporal changes of DO in batch experiment.

Figure 2.13 Hydrogen gas bubbles: Immediately after injection and before injection.
Table 2.4 Comparison of pH of start and end of batch experiment

<table>
<thead>
<tr>
<th>Port</th>
<th>Start</th>
<th>End</th>
<th>Port</th>
<th>Start</th>
<th>End</th>
<th>Port</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.10</td>
<td>8.30</td>
<td>7</td>
<td>8.64</td>
<td>9.87</td>
<td>13</td>
<td>9.02</td>
<td>9.63</td>
</tr>
<tr>
<td>2</td>
<td>7.10</td>
<td>8.76</td>
<td>8</td>
<td>8.01</td>
<td>9.86</td>
<td>14</td>
<td>8.93</td>
<td>9.65</td>
</tr>
<tr>
<td>3</td>
<td>7.11</td>
<td>9.04</td>
<td>9</td>
<td>8.01</td>
<td>9.79</td>
<td>15</td>
<td>8.50</td>
<td>9.46</td>
</tr>
<tr>
<td>4</td>
<td>7.54</td>
<td>9.29</td>
<td>10</td>
<td>8.90</td>
<td>9.90</td>
<td>16</td>
<td>9.17</td>
<td>9.64</td>
</tr>
<tr>
<td>6</td>
<td>7.86</td>
<td>9.60</td>
<td>12</td>
<td>8.21</td>
<td>9.49</td>
<td>18</td>
<td>8.88</td>
<td>9.66</td>
</tr>
</tbody>
</table>

2.4.3 Discussion

Figures 2.9 and 2.11 depict the denitrification ability of the microorganisms in the aquifer. Nitrate decreased significantly from day 5, especially in ports 4-6 - where hydrogen gas was injected - DO below 2 mg/L, in some incidents the DO was even less than 1 mg/L. Denitrification occurred in these locations as well. Moreover, DO at both sides of hydrogen injection zone only decreased to around 5 mg/L, but the nitrate concentration still could be observed. It was reported that denitrification could occur even when the DO concentration was about 2 mg/L (Doi and Sakakibara, 2004; Sprent, 1987). In this experiment, the results demonstrated that a slight nitrate removal was possible even the DO was as high as 5 mg/L, which is much higher than former studies. At the end of batch experiment, the total nitrogen at ports 4-6 decreased to lower than 3 mg-N/L on average, which indicated promising denitrification ability.

A maximum nitrite accumulation of 10.8 mg-N/L was observed during the batch experiment, which indicated that the denitrification was not carried out completely. Insufficient hydrogen supply and relatively high DO concentration (i.e., 5 mg/L) could be considered as possible reasons for the incomplete denitrification. Moreover, pH could be considered as another important factor since it is reported that a neutral or weak base environment was required for the denitrification process (Rust et al., 2000; Tang et al., 2011). In the batch experiment, the pH increased close to 10 due to the denitrification process (Equation (1-12)), and the pH at the beginning was around 7.5-8 which means a weak base environment. As mentioned in Chapter 1, appropriate pH range for denitrification is 6-8, and nitrite will be accumulated when pH is higher than
8.6. The results of batch experiment generally agreed with former studies, but the boundary of nitrite accumulation was not clear yet.

In Figure 2.12, a slight DO decrease could be observed. At the same time, a slight decrease of nitrate without nitrite accumulation was visible at ports 11, 14, and 17 from Figures 2.9 and 2.11. As mentioned in section 2.4.1, aerobic bacteria contained mixture were injected at ports 10-18. Thus it could be indicated that the nitrate decrease in this area was due to assimilation uptake by the bacteria.

2.5 Conclusions

Based on experimental results of bacteria cultivation and batch experiment, the following conclusions were made:

1. A thermostatic chamber was constructed and a light shielding environment was set up; this equipment ensured a thermally stable subsurface environment and the elimination of algal growth.

2. The cultivated bacteria were capable of denitrification process.

3. The denitrification capability of the microorganisms in the aquifer was demonstrated via batch experiment. Since the conditions for continuous experiments are different from the batch one, further studies should be conducted.

4. Nitrite accumulation and pH shift to weak alkalinity condition were observed in the batch experiment. Thus, further studies should be conducted to clarify the relationship between nitrite accumulation and pH.
CHAPTER 3  FEASIBILITY OF CONTINUOUS TREATMENT

3.1 Introduction

As introduced in Chapter 1, hydrogen is a nontoxic substance and a clean and non-hazardous material to the environment. In the past decades, attempts have been made to utilize hydrogen as an electron donor for microbial metabolism in groundwater remediation and drinking water treatment (Doi and Sakakibara, 2004; Haugen et al., 2002; Kurt et al., 1987; Till et al., 1998). All these studies demonstrated the capability of hydrogen utilization in denitrification process and showed many superior advantages. At the same time, its limitation of wide application is significant and simple: an appropriate supply method for its low solubility in water.

As mentioned in Chapter 1, high hydrogen diffusion efficiency as well as high rate treatment has been achieved, for instance, via hollow fiber biofilm membrane which was expected to be applied in an in situ site (Lee and Rittmann, 2000). Besides the advantages, for the hollow fiber biofilm membrane, the clogging problem on the surface of the hollow fiber and periodic high cleaning cost might be accounted as limitation for its application in practice. In addition, it was reported that there was usually a slight increase of TOC in effluent after the treatment which may lead to inconvenience in water quality (see Table 1.4) (Lu, 2009; Schnobrich et al., 2007). Moreover, all these studies only lasted for no more than 270 d (see Table 1.4), which is not long enough to ensure its practical application in a real situation.

Therefore, in this chapter, in addition to batch experiment, in order to improve the shortcomings, focusing on nitrate contaminated groundwater, especially in shallow aquifers, an in situ denitrification process using electrolytic hydrogen and oxygen gases were established and conducted for evaluating the denitrification and oxygenation performances. The gases were injected to the apparatus directly but not via hollow fiber membrane or a comparable method. In addition, other relevant water quality parameters were also monitored and the utilization performances of hydrogen and oxygen were
assessed as well.

3.2 Materials and Methods

3.2.1 Experimental apparatus

Figure 3.1 illustrates the experimental setup of the laboratory-scale aquifer used for continuous treatment of nitrate contaminated groundwater. The dimensions, sampling ports, and filler of the aquifer were the same as introduced in section 2.2.3. The hydraulic retention time (HRT) was calculated based on the effective liquid volume.

To achieve a uniform flow, synthetic groundwater was fed and withdrawn continuously using peristaltic pumps, as shown in Figure 3.1. The flow rate of each peristaltic pump was controlled almost every day. The pumps were not set for two withdrawal lines to adjust the groundwater flow rate of the influent and effluent at a constant value. The effluent from aquifer was discharged through an overflow weir to maintain the water level.

In these experiments, hydrogen and oxygen gases were produced using a solid polymer electrolyte membrane electrode (SPEME) (Komori and Sakakibara, 2008), then injected at the bottom of ports 6 and 15 using peristaltic pumps. To avoid algal growth, the aquifer and every tube were covered with a light-shielding curtain or Aluminum foil. The apparatus was set in a thermostatic chamber in which the temperature was maintained around 20 °C (section 2.2.1).
Figure 3.1 Schematic diagram of experimental apparatus.
3.2.2 Operating conditions

Synthetic groundwater for continuous treatment was prepared by dissolving sodium nitrate at 15.06 ± 0.55 mg-N/L and trace nutrients with inorganic carbon (IC) as NaHCO₃ or CO₂ in deionized water (Chang et al., 1999; Sahu et al., 2009; Ye et al., 2013). All experiments were conducted until steady-stable conditions were achieved. Conditions of the continuous experiments are shown in Table 3.1. The composition of the synthetic groundwater is shown in Table 3.2.

As shown in Table 3.1, 5 different runs (Runs 1 to 5) were conducted with a constant temperature and influent nitrate concentration, while other conditions such as the flow rates of liquids, gases and IC were changed. Concentrations of NaHCO₃/CO₂ (i.e., 101 mg-C/L) were set referring to a concentration range of 2.81-484.8 mg-C/L in groundwater (Cai et al., 2003; Herczeg et al., 1991a; Herczeg et al., 1991b).

<table>
<thead>
<tr>
<th>Table 3.1</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run No.</td>
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</tr>
<tr>
<td>Flow rate (Q) (L/d)</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Inorganic Carbon (as C, mg/L)</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>H₂ gas (mL/d)</td>
<td>360 ± 20</td>
</tr>
<tr>
<td>O₂ gas (mL/d)</td>
<td>220 ± 60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3.2</th>
<th>Composition of synthetic groundwater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>Concentration (mg/L)</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>15.06</td>
</tr>
<tr>
<td>Inorganic Carbon</td>
<td>10-250</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.76</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2.08</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>4.00</td>
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<tr>
<td>NaCl</td>
<td>0.96</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.12</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>1.92</td>
</tr>
</tbody>
</table>
During the treatments, the mass balance of hydrogen and oxygen was evaluated in zone ‘ANAERO’ and zone ‘AERO’ near the injection ports of hydrogen and oxygen, as shown on the left and right sides of Figure 3.1, respectively.

### 3.2.3 Sampling and analytical methods

Liquid samples were taken from the influent (inlet, 0.00 m), ports 1-18, and the effluent (outlet, 2.00 m), while gas samples were collected from gas influent injection points and the gas outlet point. Moreover, liquid samples from the aquifer were obtained by mixing the same amount of liquids produced along three vertical sampling ports (as shown in Figure 3.1). In conclusion observed results are mean values of the three ports. Liquid samples for the ion chromatography analysis were filtered using a 0.45-μm syringe-driven membrane filter (25CS045AN, Advantec), while no filtration was done for other samples.

Nitrate (NO₃⁻), nitrite (NO₂⁻), chloride ion (Cl⁻), and sulfate (SO₄²⁻) were measured by ion chromatography (HIC-10A, Shimadzu). Dissolved hydrogen and a potential intermediate product - nitrous oxide gas - were measured by gas chromatography (GC-8A, Shimadzu). TOC, DO, and pH were measured by TOC analyzer (TOC-5000A, Shimadzu), a DO meter (Ultra DO meter, Central Kagaku Corp.), and pH meter (pH510, Eutech instruments), respectively. Turbidity and chromaticity were measured by a digital turbidity/chromaticity meter (WA-PT-4DG, Kyoritsu Chemical Check Lab. Corp.). The temperature in the thermostatic chamber was monitored using a temperature recorder (TR-71D, T&D Corp.).

### 3.2.4 Mass balance of hydrogen and oxygen

In this study, the mass balance of hydrogen and oxygen were evaluated according to flow rate and concentrations at zone ‘ANAERO’ and zone ‘AERO’ shown in Figure 3.1. The overall denitrification reaction was represented by Equation (1-12), and oxygen uptakes were represented by the following reaction (Eggers and Terlouw, 1979; Kurt et al., 1987; Szekeres et al., 2001):
\[ 2 \text{H}_2 + \text{O}_2 \rightarrow 2 \text{H}_2\text{O} \] (3-1)

Hydrogen mass balance equation is shown as follows:

\[ Q_{\text{H}_2} = Q_{\text{De}} + Q_{\text{O}_2} + Q_{\text{Ex}} + Q_{\text{Ex.gas}} \] (3-2)

Where \( Q_{\text{H}_2} \) is the amount of hydrogen gas injected per day (mmol/d), \( Q_{\text{De}} \) and \( Q_{\text{O}_2} \) are equivalent amounts of hydrogen used for denitrification and oxygen utilization (mmol/d), and \( Q_{\text{Ex}} \) and \( Q_{\text{Ex.gas}} \) are those discharged with bulk liquid and gas effluent (mmol/d), respectively. \( Q_{\text{Ex}} \) and \( Q_{\text{Ex.gas}} \) were measured directly, and the amount of \( Q_{\text{De}} \) and \( Q_{\text{O}_2} \) was calculated by the following equations:

\[ Q_{\text{De}} = 2.5 \times \frac{Q \times (C_{\text{Nin}} - C_{\text{Nout}})}{14} \] (3-3)

\[ Q_{\text{O}_2} = 2 \times \frac{Q \times (DO_{\text{in}} - DO_{\text{out}})}{32} \] (3-4)

Where \( Q \) is the liquid flow rate (L/d), and \( C_{\text{Nin}}/C_{\text{Nout}} \) and \( DO_{\text{in}}/DO_{\text{out}} \) are the mean nitrate concentration (mg-N/L) and DO (mg/L) of the influent and effluent for the zone ‘ANAERO’ shown in Figure 3.1.

Oxygen mass balance equation is shown as follows:

\[ Q_{\text{O}_2} = Q_{\text{O}_2\text{ox}} + Q_{\text{Ex.gasO}_2} + Q_{\text{H}_2\text{dis}} + Q_{\text{TOCde}} + Q_{\text{Ni}} \] (3-5)

Where \( Q_{\text{O}_2} \) is the amount of oxygen gas injected per day (mmol/d), \( Q_{\text{O}_2\text{ox}} \) and \( Q_{\text{Ex.gasO}_2} \) are the amount of DO increased in zone “ANAERO” and discharged to gas effluent (mmol/d), respectively. \( Q_{\text{H}_2\text{dis}}, Q_{\text{TOCde}} \) and \( Q_{\text{Ni}} \) are the equivalent amounts of utilized hydrogen discharged from the zone ‘ANAERO’, TOC degradation, and nitrification from nitrite to nitrate (mmol/d), respectively. \( Q_{\text{O}_2\text{ox}} \) and \( Q_{\text{Ex.gasO}_2} \) are measured directly (via DO value), and the amount of \( Q_{\text{H}_2\text{dis}}, Q_{\text{TOCde}} \) and \( Q_{\text{Ni}} \) (mmol/d) were calculated by the following equations:

\[ Q_{\text{H}_2\text{dis}} = 0.5 \times Q_{\text{Ex}} \] (3-6)

\[ Q_{\text{TOCde}} = Q \times \frac{(\text{TOC}_{\text{in}} - \text{TOC}_{\text{out}})}{12} \] (3-7)

\[ Q_{\text{Ni}} = Q \times \frac{(C_{\text{Nin}} - C_{\text{Nout}})}{28} \] (3-8)

where \( \text{TOC}_{\text{in}} \) and \( \text{TOC}_{\text{out}} \) are influent and effluent concentrations of TOC in the zone ‘AERO’, \( C_{\text{Nin}} \) and \( C_{\text{Nout}} \) represent for mean nitrite of influent and effluent in the zone ‘AERO’ in Figure 3.1.
3.3 Results and Discussion

3.3.1 Performance of flow rates

Figure 3.2 illustrates temporal changes of liquid flow rates variation, as well as the injection rates of hydrogen and oxygen gases. It could be indicated from the figure that both the liquid and gas flow rates were as stable as they were set. An exception was shown for the hydrogen gas injection rate which varied in Run 4. In this Run, the injection rate of hydrogen gas was modified in a stepwise manner (as marked by a cycle in Figure 3.4) to verify the stability of the \textit{in situ} denitrification process.

![Figure 3.2 Temporal changes of liquid and gas flow rate of Runs 1 to 5.](image)

3.3.2 Removal of nitrate and nitrite

Figures 3.3 and 3.4 show temporal changes of nitrate and nitrite in Runs 1 to 5, while Figures 3.5 and 3.6 depict temporal changes of total nitrogen and steady-stable profiles of total nitrogen, respectively. In Run 1, nitrate concentration decreased at a place 0.75 m down from the inlet, and increased with increasing distance downstream. Concentrations of nitrite increased at 0.75 m down from the inlet and decreased...
thereafter. The total nitrogen at these positions was maintained almost constant but around the standard (Table 1.1). This means that incomplete denitrification with nitrite accumulation occurred in a hydrogen-injected zone (i.e., at the bottom of port 6), but nitrite was almost oxidized to nitrate in the downstream oxygen-injected zone (i.e., at the bottom of port 15). The accumulation of nitrite was due to a relatively high pH value (see section 3.3.4) as well as insufficient supply of hydrogen, as shown in Tables 1.3 and 1.4. Since nitrite is much more toxic than nitrate (Amenu and Kumar, 2008; Comly, 1945), oxidation of nitrite to nitrate is advantageous from the viewpoint of reducing the toxicity of the effluent.

![Figure 3.3 Temporal changes of nitrate in Runs 1 to 5.](image-url)
Figure 3.4 Temporal changes of nitrite in Runs 1 to 5.

Figure 3.5 Temporal changes of total nitrogen in Runs 1 to 5.
In Runs 2 to 5, the concentration of inorganic carbon was increased from 10.2 to 250 mg-C/L. In Run 2, the accumulation of nitrite shrunk and rarely observed, and very stable denitrification was achieved. From Run 3 to Run 5, the injection rate of hydrogen gas was increased while the liquid flow rate was kept constant. In Run 3, at around day 280, effluent nitrate concentrations decreased further and then stabilized, and the concentrations were below the standards (Table 1.1). It may be due to the current in situ denitrification process adapted to the new condition.

In Run 4, the injection rate of hydrogen gas was changed in a stepwise manner (as indicated by a circle in Figures 3.3 and 3.5) in the middle of the run. In such a case, nitrate concentrations tended to increase while nitrite was rarely detected, but after the hydrogen injection rate was changed to the original state, within one week the nitrate concentrations returned to the same level that has been observed before. Therefore, the stability of the process could be proven.

Based on Figure 3.4, it could be indicated that nitrogen concentration slightly decreased at the position 0.15 m (position A) followed by a rapid reduction until the
position 0.75 m (position C). It also could be indicated from the figure that the reduction rate from position A to position 0.45 m (position B) was slightly higher than from position B to position C. It could be due to that at position B (ports 4-6) and its surroundings was the part where the denitrification rate was the highest. It could be due to that hydrogen gas was injected under the port 6 and the boundaries of hydrogen bubbles were about 10 cm of both sides of position B. This area has the highest hydrogen concentration. Thereafter, the nitrogen concentration slightly increased and achieved a local maximum value at positions 1.35m (position E) or 1.65 m (position F) and afterwards slightly decreased in the effluent. The concentration in the effluent was approximately the same as position 1.05 m (position D). These variations could be due to microorganisms’ decomposition in increase and nitrate utilization in decrease, respectively. An exception was observed in Run 5, the reason was unknown, but it might be because of the impact of high inorganic carbon (i.e., bicarbonate at 100 mg-C/L and carbon dioxide at 150 mg-C/L).

### 3.3.3 Oxygenation

Figures 3.7 and 3.8 illustrate temporal changes and steady-stable profiles of DO in Runs 1-5, respectively. In every experiment, the influent synthetic groundwater contained about 8.0 mg/L DO except in Run 5, where DO in influent was relatively low, around 5 mg/L. This may be due to the dissolution problem of carbon dioxide in the influent which made a part of DO stripped. DO decreased to values around 2.0 - 3.0 mg/L at 0.75 m and 1.05 m down from the inlet of the aquifer. The effluent contained more than 15 mg/L DO due to the oxygen gas injected at port 15 (1.65 m down from inlet), and complete oxygenation was achieved. In summary, the DO was consumed and oxygenated by injected hydrogen in the ‘ANAERO’ zone and injected oxygen in the ‘AERO’ zone (as shown in Figure 3.1), respectively.

Moreover, it could be seen from Figure 3.8 that DO is slightly higher at 1.05 m from inlet than at 0.75 m. It should be considered that due to diffusion in the aquifer, part of the injected oxygen was transported upstream. But this has not been confirmed
yet.

Figure 3.7 Temporal changes of DO in Runs 1 to 5.

Figure 3.8 Profiles of DO in Runs 1 to 5.
DO increased significantly from around 2.0 - 3.0 mg/L to more than 15.0 mg/L, and a local maximum value was achieved between the oxygen injection port and effluent (1.65 m down from inlet) and then decreased in effluent. This decrease in effluent could be considered that oxygen injected was consumed by aspiration of aerobic microorganisms in the oxygen injection zone.

### 3.3.4 Other water quality parameters

During the experiments, conventional water quality parameters including turbidity, chromaticity, TOC, and pH were monitored. Some of the data in Runs 1 and 2 was not shown because machinery malfunction or detection method error occurred.

Figure 3.9 shows temporal changes of turbidity and chromaticity in Runs 2 to 5. Although the turbidity and chromaticity fluctuated in the influent, which might have been caused during the influent preparation, both parameters decreased with the distance downstream and finally reached 0.2 NTU and 1.5 PCU, respectively. These values meet the current drinking water standard (Table 1.1). Since glass beads have a poor adsorption capacity for every constituent in groundwater (Table 3.2), it was assumed that suspended solids as well as soluble and colloidal constituents exerting chromaticity were adsorbed by, attached to, or decomposed by biofilms that formed on the glass beads (Wik, 1999).
The influent solution used in this study contained about 2.4 mg/L TOC on average according to experimental results. Although data variation was observed, especially in influent, the TOC difference between the influent and the effluent was about −0.65 mg/L on average, indicating that TOC contained in the influent or produced in the aquifer was reduced during the treatment. As Schnobrich et al. (2007) and Lu (2009) reported that there were slight accumulation of TOC (i.e., $0.387 \pm 0.25$ mg/L as C) in hydrogenotrophic denitrification processes, thus it could be considered that TOC produced in zone ‘ANAERO’ was removed aerobically in the oxygen injected zone.
Figures 3.11 and 3.12 illustrate the temporal changes and steady-stable profiles of pH in Runs 1 to 5. Values of pH increased from 7.7 up to around 10 at 1.05 m down from inlet in Run 1. As mentioned before, nitrogen decreased at this position. Thus it could be indicated that denitrification started immediately after the continuous experiment began. Value of pH then decreased to around 8.5.

After the IC concentration was adjusted from 10 to 101 mg-C/L by dissolving sodium bicarbonate, the initial pH increased from 7.7 to 8.6 while the maximum pH decreased from around 10 to 9. Thus the pH variation was significantly reduced because the buffer capacity was increased. In all these Runs, pH values exceeded the standards (Table 1.1).

In Run 5, the pH values were significantly lower than the former Runs (i.e., lower than 6.0 in influent), which could be due to excessive dissolution of carbon dioxide into the influent. In this Run, the pH values still showed the same trend that pH increased slightly in the zone ‘ANAERO’ and then decreased in the ‘AERO’. As mentioned in Chapter 1, a low pH may lead to lower denitrification rates as well as some hazardous
by-products. Moreover, these pH values were lower than the standard (Table 1.1). Therefore, it is necessary to adjust the pH in the continuous experiment.

Figure 3.11 Temporal changes of pH in Runs 1 to 5.

Figure 3.12 Profiles of pH in Runs 1 to 5.
3.3.5 Mass balances of hydrogen and oxygen gases

Nitrous oxide (N\textsubscript{2}O) is an intermediate product during the denitrification process (Knowles, 1982). To confirm the complete reduction of nitrate to nitrogen gas, measurements of dissolved nitrous oxide in addition to nitrite have been performed. However, concentrations of this compound were below the detection limit (0.03%), indicating negligibly small amounts of intermediates.

Figures 3.13 and 3.14 depict the result of mass balance on hydrogen and oxygen in Runs 2 - 5, based on the Equations (3-2) to (3-8). For the machinery reasons, the data for Run 1 and the first half of Run 2 was not shown. In the figures, the percentages of the terms on the right side of Equations (3-2) and (3-5) are shown.

**Figure 3.13** Comparison of mass balance on hydrogen gases in Runs 2 to 5, where 100 % indicates the amount of hydrogen injected.

Part of the injected hydrogen gas was consumed by denitrification and oxygen utilization, and the rest was discharged to bulk liquid and gas effluent. Based on Figure 3.13, it formed that a satisfactory mass balance was obtained, and most data indicated that more than 90% of the injected hydrogen gas was consumed by denitrification \((Q_{De})\) and DO consumption \((Q_{O2})\) in Runs 2 and 3. In contrast, in Runs 4-5, total fractions for
in Equation (3-2) were calculated as about 75%. According to the observation of hydrogen injection to water, it was considered that hydrogen gas reached the head space of the apparatus and escaped from the aquifer.

On oxygen, approximately half of the injected oxygen gas was used for oxygenation. In addition, the rests were discharged to gas phase or utilized for oxidations of incoming hydrogen and TOC. Moreover, total fractions considered in Equation (3-5) were roughly about 85% on average (i.e. 15% smaller than the amount of oxygen injected) and the amount of oxygen utilized for nitrification of nitrite in Figure 3.14 was negligibly small in comparison with that of oxygen injected. The reason was not clear, but it was supposed that a part of DO in effluent escaped to the atmosphere.

Figure 3.14 Comparison of mass balance on oxygen gases in Runs 2 to 5, where 100 % indicates the amount of oxygen injected.
3.4 Conclusions

Based on experimental results in the continuous experiment, the following conclusions can be drawn:

1. Besides batch experiment, the described system is capable for continuous treatment.
2. Except initial startup period, continuous and stable treatments of synthetic groundwater could be achieved by the current experimental system.
3. Nitrate concentration could meet the drinking water standard without significant nitrite accumulation, and very stable denitrification was achieved.
4. Conventional water quality parameters including turbidity, chromaticity, and TOC tended to be smaller in effluent than in influent.
5. More than 90% of hydrogen gas injected was utilized for denitrification and DO consumption and a very small amount of hydrogen gas leaked out from the aquifer.
6. Only one hydraulic load and one nitrate loading were conducted, therefore, it is needed to verify the adaptability of the system under different hydraulic and nitrate loading conditions.
CHAPTER 4  CONTINUOUS TREATMENT UNDER DIFFERENT HYDRAULIC LOADINGS

4.1 Introduction
In Chapter 3, the capability and effectiveness of an in situ process with the injection of hydrogen and oxygen gases was demonstrated. Nitrate concentration was satisfied as well as other related water quality parameters. However, these experiments were conducted under the same hydraulic condition and nitrate loading. Therefore, long-term performances and stability under different hydraulic loadings were still not investigated sufficiently.

In this chapter, the same laboratory-scale aquifer was used for continuous experiments under different hydraulic and nitrate loading conditions, and measurements were also made for water quality parameters such as nitrate, nitrite, pH, TOC, turbidity, and chromaticity to evaluate the performance and stability.

4.2 Materials and Methods

4.2.1 Experimental apparatus and operation conditions
The same experimental apparatus was used for continuous treatment under different hydraulic and nitrate loading conditions as shown in Figure 3.1.

The same synthetic groundwater was used according to Table 3.2. Conditions in detail are shown in Table 4.1. The concentration of NaHCO$_3$/CO$_2$ (i.e., 125 mg-C/L) was set up refer to the previous experiments in Chapter 3.
Table 4.1 Experimental conditions

<table>
<thead>
<tr>
<th>Run No.</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (Q) (L/d)</td>
<td>5.8±0.3</td>
<td>11.3±0.4</td>
<td>22.5±1.1</td>
<td>34.3±2.1</td>
<td>44.8±0.7</td>
<td>35.9±1.4</td>
<td>23.2±1.2</td>
<td>11.4±0.3</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>2.7±0.2</td>
<td>1.4±0.1</td>
<td>0.71±0.04</td>
<td>0.47±0.03</td>
<td>0.36±0.01</td>
<td>0.44±0.02</td>
<td>0.69±0.04</td>
<td>1.4±0.1</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20±2</td>
</tr>
<tr>
<td>Inorganic Carbon (as C, mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>125±10</td>
</tr>
<tr>
<td>H2 gas (mL/d)</td>
<td>580±100</td>
<td>930±85</td>
<td>1,550±80</td>
<td>1,990±80</td>
<td>2,150±30</td>
<td>2,220±60</td>
<td>1,560±40</td>
<td>930±50</td>
<td>575±30</td>
</tr>
<tr>
<td>O2 gas (mL/d)</td>
<td>220±60</td>
<td>430±30</td>
<td>860±20</td>
<td>940±110</td>
<td>1,050±65</td>
<td>1,130±40</td>
<td>790±35</td>
<td>465±60</td>
<td>250±30</td>
</tr>
</tbody>
</table>
4.2.2 Sampling, analytical methods, and mass balance

Liquid and gas samples were taken based on the method as mentioned in Chapter 3, the analytical methods were also the same as in Chapter 3.

In this study, the mass balance of hydrogen and oxygen gases were also evaluated according to the concentration at zone ‘ANAERO’ and zone ‘AERO’, which are shown in Figure 3.1, respectively. The calculation method was the same as Chapter 3 as well.

4.3 Results and Discussion

4.3.1 Performance of flow rates

Figure 4.1 shows temporal changes of liquid and gas variations of the Runs 6 to 14. From the figure the conclusion could be drawn that very stable liquid flow rate was achieved while some fluctuation could be observed in gases’ flow rate. From Runs 6 to 14 (to verify the performance of the system at various hydraulic load), the liquid flow rate was increased to about two, four, six and eight times that of the previous Runs (flow rate of Runs 1-6 was set as one time), and then dropped back to one time, the original flow rate again, while other conditions were almost kept the same.

![Figure 4.1 Temporal changes of liquid and gas flow rate of Runs 6 to 14.](image-url)
Moreover, given the limited gas-holding capacity of the used glass beads in the aquifer, the injection rates of hydrogen and oxygen gases were increased, but by less than about eight times those of the previous Runs.

4.3.2 Removal of nitrate and nitrite

Figures 4.2 and 4.3 illustrate temporal changes of nitrate and nitrite, while Figure 4.4 depicts the steady-stable profiles of total nitrogen in these Runs. In Run 6, where the injection rates of hydrogen gas were sometimes lower than the designed injection rate, some nitrate concentrations in the aquifer increased, but the effluent nitrate was kept constantly at the same level.

Even though a flow rate variation was conducted between Runs 6 and 14, the concentration of nitrate and nitrite in these Runs stabilized soon after conditions changed. An exception could be observed for Run 7, which might be due to a slight fluctuation of hydrogen gas injection. Although nitrite could be detected in the aquifer at some occasions, no nitrite was detected in effluent. Thus the stability of the process could be proven. The trend of the profiles is in conclusion with the previous Runs.

![Figure 4.2 Temporal changes of nitrate in Runs 6 to 14.](image)
Figure 4.3 Temporal changes of nitrite in Runs 6 to 14.

Figure 4.4 Profiles of total nitrogen in Runs 6 to 14.
Figure 4.5 depicts a comparison of the average nitrogen removal and liquid flow rate of each Run, while Figure 4.6 shows nitrate removal ability of present *in situ* denitrification process for Runs 6-14. In these Runs, the relationship between liquid flow and nitrate in the effluent is shown. Generally, the nitrogen in the effluent depicted a direct correlation with the increase and decrease of the liquid flow rate. Moreover, the nitrogen in the effluent of the right side bounded by Run 10 was slightly larger than that of the left part. It should be considered that the liquid flow rate of the right part was slightly higher than that of the former part in Figure 4.5. Furthermore, the nitrogen removal showed an inverse relation to the liquid flow rate in Figure 4.6. In summary, the nitrate removed decreased while the flow rate increased simultaneously, but the stability of the laboratory-scale aquifer with varying hydraulic load has been proven. This means that the present process can be applied to a range of different groundwater velocities (i.e., about 2.5 m/d at most) and it is able to meet the standard (Table 1.1, WHO standard) under the current nitrate concentration.

![Figure 4.5](image_url)  
**Figure 4.5** Comparison of nitrogen removal and liquid flow rate of each Run.
4.3.3 Oxygenation

Figures 4.7 and 4.8 show the temporal changes and steady-stable profiles of DO in Runs 6 to 14. The DO in influent was kept around 7.5 mg/L, slightly lower than in Run 1 to 4, which could be due to the dissolving carbon dioxide in the synthetic groundwater. It could be induced from the figures that DO of Runs 7 to 13 at positions 0.75 m and 1.05 m down from inlet was a slightly higher than that of Runs 6 and 14. This may be appointed to the increase of liquid flow rate.

Moreover, the injection positions of hydrogen and oxygen gases were kept constant during all the experiments, thus it could be induced from Figure 4.8 that the larger the flow rate was, the higher the DO would be at the same position in the aquifer. These results should be due to the faster velocity by higher flow rate.

In the second half of Run 14, the oxygen gas injection rate was adjusted to approximately half of the first half (as marked by dash line). DO in effluent was then decreased to around 13 mg/L and still no nitrite accumulation was observed. Therefore, it could be considered that lower DO does not have significantly impact on nitrification.
Figure 4.7 Temporal changes of DO in Runs 6 to 14.

Figure 4.8 Profiles of DO in Runs 6 to 14.
4.3.4 Other water quality parameters

Figure 4.9 illustrates temporal changes of turbidity and chromaticity in Runs 6 to 14. Although fluctuation in influent, especially in chromaticity, was observed, they decreased with the distance downstream, which agreed with the previous experiments in Chapter 3.

Figure 4.10 shows temporal changes of TOC and TOC difference in Runs 6 to 14. Similar data variations were observed as the previous Runs, but finally the TOC difference between influent and effluent was negative, indicating that TOC contained in the influent or produced in the aquifer was also reduced during the Runs 6 to 14. And these results also agreed with the previous experiments in Chapter 3.

![Figure 4.9 Temporal changes of turbidity and chromaticity in Runs 6 to 14.](image)
Figures 4.11 and 4.12 depict the temporal changes and profiles of pH in Runs 6 to 14. The initial pH was kept around 6.6, which was between Runs 1 to 4 and Run 5. This could be due to the decrease of dissolved carbon dioxide in influent. Acidic groundwater was fed in Runs 6-14, and pH values increased from about 6.6 to around 7. These results indicate that neutralization of groundwater could be achieved. Moreover, all these pH values were in the range of water quality standard (Table 1.1).

In Run 10, total nitrogen concentrations in effluent were higher than 10 mg-N/L but still below the WHO guidelines for drinking water quality (WHO, 2011). This is attributable to an insufficient amount of hydrogen that was injected to achieve Reactions (1-12) and (3-1), and the high liquid flow rate (which led to low HRT in the aquifer), respectively. In other Runs, effluent concentrations of nitrate, nitrite, TOC, turbidity, and chromaticity met WHO guidelines for drinking water quality (WHO, 2011).
Figure 4.11 Temporal changes of pH in Runs 6 to 14.

Figure 4.12 Profiles of pH in Runs 6 to 14.
Moreover, during the continuous experiments over the duration of two years, no clogging problem was observed. In addition, a visible thin film was spotted on the surface of the glass beads. This was considered to be attributed to small net growth rates of autotrophic microorganisms in the aquifer. Based on the experimental results shown in Figures, it can be concluded that the present in situ process has superior performances in terms of stability, effluent water quality, and simplicity of operation. A further kinetic study will be needed to analyze and evaluate the performance in detail under different operating and design conditions.

4.3.5 Mass balance of hydrogen and oxygen gases

Figures 4.13 and 4.14 show the results of the mass balance on hydrogen and oxygen in Runs 6-14, based on the Equations (3-2) to (3-8). In these figures, the percentages of the terms on the right side of Equations (3-2) and (3-5) are shown as well.

From Figure 4.13, it could be concluded that a relatively saturated mass balance was obtained. In Runs 6 to 9 and 13 to 14, approximately 85-90% on average or more of the injected hydrogen gas was consumed by denitrification ($Q_{Dn}$) and DO consumption ($Q_{O_2}$). For Runs 10 to 12, total fractions considered in Equation (3-2) have been calculated as about 60-70%. It could be considered that hydrogen gas could not be kept in the aquifer but reached the head space of the apparatus and then escaped.

For oxygen, about half of the injected oxygen gas was used for oxygenation. Additionally, the rest were discharged to gas phase or utilized for oxidations of incoming hydrogen and TOC. Based on this figure it could be concluded that total fractions considered in Equation (3-5) were 80% on average until the first half of Run 14. In the second half of Run 14 - where the oxygen injection rate was decreased - the fractions were about 120%, which is significantly different from the former Runs.
Figure 4.13 Comparison of mass balance on hydrogen gases in Runs 6 to 14, where 100 % indicates the amount of hydrogen injected.
Figure 4.14 Comparison of mass balance on oxygen gases in Runs 6 to 14, where 100 % indicates the amount of hydrogen injected.
4.4 Conclusions

According to the experimental results in these experiments, the following conclusions were made:

1. In addition to Chapter 3, a stable long-term denitrification and oxygenation process under different hydraulic loadings was achieved and its excellent adaptability to hydraulic and nitrate loadings variation has been proved. The groundwater velocity of this study reaches 2.5 m/d, which is much higher than former studies and relatively high in an actual situation.

2. Observed results demonstrated that, compared with former studies, water quality parameters such as TOC, turbidity, and chromaticity get lower in the effluent than the influent. Furthermore, clogging problem, which was frequently happened in former studies, was not observed during the long-term experiment.

3. Most of the hydrogen gas was consumed by denitrification/oxygen utilization and a small amount of hydrogen gas leaked out from the aquifer, which is in conclusion with Chapter 3.

4. Neutralization of groundwater is possible if the pH of nitrate contaminated groundwater is weakly acidic.
CHAPTER 5 CONCLUSIONS AND PROSPECT

Groundwater is a very important natural water source for humankind. It is widely exploited for different purposes such as drinking water production, irrigation, and industrial usage. Nowadays, nitrate contamination in groundwater - mainly in shallow aquifers - has become a serious global environmental issue. The impact of nitrate in drinking water has received considerable attention since the 1940s, especially in the past decades. This study evaluates the removal of nitrate by a novel in situ denitrification and oxidation process with injection of electrolytic hydrogen and oxygen.

Chapter 1 made a general introduction and consists of a literature review and research objectives and is therefore focused on the current status of nitrate contamination and removals by different approaches. This chapter provided an overview of nitrate contamination in groundwater as well as health concerns related to the issue. From the literature review, two different kinds of approaches (i.e., physicochemical approach, biological approach) for the purpose of removing nitrate from water were discussed. However, for physicochemical approach, beside relatively high process capabilities, the main problems were post treatment and deterioration of catalyst. For heterotrophic denitrification, even though the removal rate was relatively high, the secondary pollution caused by electron donors such as methanol could not be avoided. Moreover, clogging problems always occurred in aquifer due to a high growth rate of heterotrophic microorganisms. For autotrophic denitrification, some reactors based on autotrophic denitrification processes with high removal efficiency were also developed but were shown not to be suitable for the application in a field situation, and some problems on water quality parameters occurred. Moreover, since the duration of former studies are not long enough, it is needed to verify the stability via long-term experiments.

Prior to continuous experiment, a start-up phase consisting of the acclimatization of denitrifying bacteria and a batch experiment was introduced in Chapter 2. A thermally stable subsurface environment and the elimination of algal growth were set
up. Before introducing the microorganisms into the main experiment, bacteria cultivation was performed in order to enrich appropriate microflora. In this study, the denitrification microflora was obtained partly by acclimatizing from soil (collected from University’s campus) and by obtaining from former studies performed by colleagues from the same laboratory. The denitrification ability of the microorganisms was demonstrated and batch experiments were conducted over the duration of 18 days in order to evaluate performance in artificial aquifer.

On the basis of Chapter 2, continuous treatments were conducted in order to evaluate the feasibility of the present in situ process in Chapter 3. Synthetic groundwater containing 15.06 ± 0.55 mg-N/L nitrate was fed to the aquifer at the same load. In the experiments, electrolytic hydrogen and oxygen were injected upstream and downstream in a laboratory-scale aquifer, respectively, and measurements were performed for nitrate, nitrite, pH, dissolved oxygen, dissolved hydrogen, total organic carbon (TOC), turbidity, and chromaticity. During the initial phase of experiment, nitrite was accumulated in a hydrogen-injected zone; however, it was oxidized to nitrate in subsequent oxygen-injected zone. Moreover, the injection rate of hydrogen gas was changed in a stepwise manner during the experiment. Nitrate concentrations tended to increase, but after the hydrogen injection rate was adjusted to the original state, within one week nitrate concentrations returned to the same level that could be observed prior to the changes. In addition, water quality parameters such as TOC, turbidity and chromaticity were lower in the effluent than influent, and no clogging problem was observed. The pH was adjusted during the experiment since it was identified to be a limiting factor of the treatment. From these results, it could be concluded that the present process has several superior performances in terms of stability, effluent water quality, and simplicity in long-term operation.

In Chapter 4, continuous treatments were conducted in order to evaluate the performance of the process under different hydraulic loadings. At the same time, pH was adjusted to weak acidic based on the results in Chapter 3. Experimental results showed that the aquifer has excellent adaptability in hydraulic loadings variations as the groundwater velocity reached up to 2.5 m/d, which is relatively high in comparison
to an actual aquifer. Neutralization of groundwater is possible if the pH of nitrate contaminated groundwater is weakly acidic. Other water quality parameters including TOC, turbidity, chromaticity, and pH showed a similar trend as Chapter 3.

Based on these results, it can be concluded that the present \textit{in situ} denitrification process has an excellent ability to adapt to changes in hydraulic loadings, and it shows outstanding attributes for remediation of weak acidic nitrate contaminated groundwater. Moreover, it was demonstrated that the present \textit{in situ} process showed superior efficiency in terms of long-term performance and stability. At the same time, further study on kinetic analysis will be needed to evaluate the optimum design and operation conditions.
ACKNOWLEDGEMENT

Firstly, I would like to express my sincere respect and gratitude to my supervisor Prof. Sakakibara for the acceptance and the support of my Ph.D study and related research in the past four years. This dissertation will never be completed without his advices.

Besides my supervisor, I would like to express my great appreciation to Waseda University and China Scholarship Council for supporting me a joint scholarship for three years since 21st September, 2011. I may not study here without their financial support.

My sincere thanks also goes to Prof. Song, Prof. Tian, Prof. Shi, and Dr. Komori, for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.

In addition, I would like to thank my lab mates: Miss Morimoto, Miss Nidegawa, Miss Kiga, Mr. Naito, and Mr. Furukawa, for their kind help and hard work in different periods in the past four years.

Furthermore, I would like to give my special thanks to Mr. Leifels and Miss Smith for helping me with English polishing of this dissertation.

Last but not the least, I would like to thank my family: my parents, my grandmother, my grandfather, and other relatives for supporting my study abroad spiritually.
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APPENDIX A DISSOLVED HYDROGEN DETECTION

A.1 Introduction

As mentioned in Chapter 1, dissolved hydrogen (DH) in the liquid phase is one limiting factor during the denitrification process. Thus it is very important to establish a simple and practical detection method to evaluate the hydrogen concentration in the liquid phase.

In this appendix, given the low solubility of hydrogen in water, a DH detecting method was established based on Henry’s Law and a specified Head Space - Gas Chromatograph (HS-GC) method was introduced to achieve the detection.

In the presented study, the constants as well as other parameters involved in calculation, were cited from Chemistry Handbook, edited by The Chemical Society of Japan (The Chemical Society of Japan, 1993).

A.2 Materials and Methods

A.2.1 Analysis method

Hydrogen gas was detected by Gas Chromatograph (GC-8AIT, Shimadzu) with a thermal conductivity detector (TCD). Argon was utilized as the carrier gas. The data was recorded by a Chromatographic pack (C-R8A, Shimadzu). The column was a Molecular Sieve 5A (GL Science Corp.).

Glass bottles with a volume of 27 mL were used for head space samples. The samples were kept in a natural convection oven (DOV-300A, AS ONE Corp.) at 65 °C for two hours.

During the detection, the flow rate of the carrier gas was around 20 mL/min. The column oven was kept at 80 °C while the injection port was kept at 100 °C. An amount of 0.2 mL of sample gas was taken from the sample bottle via a syringe (MS-GAN100, 1 mL; the needle: XX-MS61, Itou Corp.) then injected. The retention time of hydrogen was about 1.0 min.
A.2.2 Experimental apparatus and pre-experiment

Before calibration, it is necessary to obtain a saturated hydrogen gas solution. Huang et al. (2008) used a High-Purity Hydrogen Generator (DGH300) to generate hydrogen gas and aeration in an Erlenmeyer flask. In there, it was kept to get saturated hydrogen gas solution (Huang et al., 2008). In this study, it was rather difficult to utilize such a generator. In order to achieve saturated hydrogen gas solution, a system shown in Figure A.1 was used during the experiment. It is a part of Komori’s experimental apparatus which was simplified by doing some slight modification (Komori, 2010) (Doctoral dissertation). And 2 hours was needed until the saturation of DH.

Figure A.1 Schematic diagram of experimental apparatus for saturated hydrogen gas solution.

The system mainly consisted of three parts: SPEME, circulation pump, and tank. Firstly, the power of SPEME and the circulation pump were turned on. The hydrogen generated was used for liquid phase circulation while oxygen was discharged to atmosphere directly. Moreover, extra hydrogen gas in the circulation system was discharged to atmosphere via the port at the top of the tank.

A preliminary experiment was conducted to evaluate how long it was needed until hydrogen gas get equilibrium between liquid and gas phases. The total volume of a
sample was 15 mL in all. After the sample was taken, it was transferred to the bottle, sealed immediately and then put bottom up in a natural convection oven. Table A.1 shows the compositions of samples in the pre-experiment as well as used in calibration. Two Runs with different experimental periods were conducted for the pre-experiment. In order to determine an appropriate equilibrium time, samples of every composition were taken for 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min in Run 1; and 15, 30, 60, 90, 120, 150, 180, 210, 240, and 270 min in Run 2.

**Table A.1** Compositions of samples in pre-experiment (mL)

<table>
<thead>
<tr>
<th>Liquid volume</th>
<th>Water with DH</th>
<th>Water without DH</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>6</td>
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</tr>
<tr>
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<td>6</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5</td>
<td>13.5</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure A.2 illustrates the results of the preliminary experiment. In Figure a), the 100% and 80% showed an unstable trend while others tended to be stable after 1 hour. Figure b) depicts that from 100% to 40% were very stable from 2 hour. Thus it could be concluded that 2 hours were enough for getting equilibrium status between liquid and gas phases.
Figure A.2-a Results of preliminary experiment, Run 1.

Figure A.2-b Results of preliminary experiment, Run 2.
A.3 Hydrogen HS-GC Calibration Curve

According to the previous sub-section, it was possible to achieve hydrogen equilibrium status in the head space bottle after 2 hours’ standing. After the solution in the apparatus was saturated hydrogen gas, samples were then taken according to Table A.1 and thereafter transferred into the oven until equilibrium was obtained. Afterwards, all of the samples were analyzed via Gas Chromatograph.

Figure A.3 illustrates the calibration curve based on the detection data. For more convenient usage in further experiments, the relationship between X and Y axes were converted to the correlation between percentage (output of gas chromatograph) and hydrogen concentration based on the calculation according to Henry’s Law. The relationships demonstrated that an excellent linear correlation between percentage and hydrogen concentration was achieved.

\[
Y = 0.6165 X \\
R^2 = 1
\]

Figure A.3 Hydrogen HS-GC calibration curve.
A.4 Conclusion

By using this HS-GC method, it is possible to detect the dissolved hydrogen concentration with sufficient precision in the liquid phase. The results showed that it is possible to evaluate the dissolved hydrogen for its excellent linear correlation. Thus, it could be concluded that this is a practical method for dissolved hydrogen evaluation.
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