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Feasibility test of autotrophic denitrification of industrial wastewater in sequencing batch and static granular bed reactors

by

Yuan Tan

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

Program of Study Committee:
Timothy G. Ellis, Major Professor
Chris Harding
Kaoru Ikuma

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa, USA
2018

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ABSTRACT

The nutrient load at the Cedar Rapids Water and Pollution Control Facility is a significant consideration for future treatment goals. In an effort to include total nitrogen removal, possible electron donors were evaluated to drive denitrification at the plant. Since a portion of the industrial influent to the facility includes pulp and paper wastewater that contains high concentrations of sulfate, reduced sulfur species were considered as an energy source for autotrophic denitrifying bacteria. During anaerobic pretreatment of the industrial wastewater, some of the sulfate is converted to hydrogen sulfide gas which in itself can cause potential safety hazard to the plant operators and an odor nuisance. Autotrophic denitrification is a microbially driven process which can reduce nitrate to nitrogen gas in the presence of reduced sulfur. By having nitrate as the electron acceptor and reduced sulfur as the electron donor during the lab-scale experiment, the nitrogen in the wastewater was effectively removed, and the removal efficiency of nitrate exceeded 95%. Both the sequencing batch and static granular bed reactor exhibited similar performance. *Thiobacillus* and *Shinella* bacteria were determined to contribute to the denitrification process. The process was effective at a near neutral pH. The experimental results illustrated that the autotrophic denitrification process was able to reduce nitrate in industrial wastewater with reduced sulfur as the sole electron donor available. The experimental results also showed that the reduction from nitrate to nitrite was faster than that from nitrate to nitrogen gas during the autotrophic denitrification process.
CHAPTER 1. INTRODUCTION

As society increases industrial development, a significant amount of wastewater is being generated requiring treatment of both the organic and nutrient (nitrogen and phosphorus) loads. Cedar Rapids is a home to pulp and paper, food processing, and other industries which contribute to the influent load to the Cedar Rapids Water and Pollution Control Facility. In a study of the nutrient reduction strategy for the plant, nitrogen removal was identified as a major goal for plant process improvements. Currently, ammonia and organic nitrogen in the influent is converted to nitrate in a separate stage nitrification step. Nitrate is considered as a contaminant in water bodies because of its negative impacts on on the environment from eutrophication as well as human health. For instance, nitrate increases the risks of cancer and methaemoglobinaemia (Lundberg et al., 2004). On the other hand, some of the influent sulfate from the pulp and paper wastewater and other sources is converted to hydrogen sulfide in the anaerobic pretreatment step, which poses potential safety risks to the plant operators. Hydrogen sulfide is known as strong odor and corrosive gas, which is toxic to human beings if the exposure level is high (Vaiopoulou, Melidis and Aivasidis, 2005). The presence of hydrogen sulfide in various process streams at the plant as well as nitrate in the effluent provides a potential opportunity for a novel biochemical transformation process known as autotrophic denitrification whereby sulfide serves as the electron donor and nitrate serves as the electron acceptor.

Autotrophic denitrification is a microbially driven process which can remove both nitrate and sulfide in wastewater (Fajardo et al., 2012). On one hand, there is no need for an external source of organic carbon compared to heterotrophic denitrification. On the other hand, there is much less sludge generated from the process. Thus, autotrophic denitrification
is a more attractive environmental-friendly alternative to treat wastewater with high concentrations of nitrogen and sulfur compounds (Qian et al., 2016).

The Cedar Rapids Water and Pollution Control Facility utilizes the upflow anaerobic sludge blanket (UASB) reactor as a pretreatment step for much of its industrial wastewater. The UASB reactors are able to retain a large quantity of biomass (Shalu and Bishnoi, 2016). However, despite their excellent removal efficiency, the effluent from USAB reactors contains significant organic matter. In addition, nutrients are conserved in the anaerobic environment. Hydrogen sulfide is released in the effluent as a byproduct of the sulfate reducing bacteria as well. Further treatment of the effluent is therefore necessary (Seghezzo, et al., 1998).

There were two types of reactors utilized in testing the feasibility of autotrophic denitrification in this research, a sequencing batch reactor (SBR) and a static granular bed reactor (SGBR). The SBR was operated in an anoxic state, with a sealed gas headspace. Seed material for the SBR included anaerobic granules from the Cedar Rapids Water Pollution Control Facility UASB reactors, biomass from the sulfide scrubber, and biomass from the sulfur oxidation basins. Feed and nutrient solution was prepared regularly. A gas collection cylinder was connected to the SBR for the purpose of collecting and monitoring the gas production. The concentration of nitrate, nitrite, and sulfate in the effluent was measured at regular intervals.

The SGBR is a high rate anaerobic reactor which has high efficiency in converting organic matter into gas predominantly methane and carbon dioxide when operated in an anaerobic environment (i.e., with no oxygen or nitrate present) (Ellis and Evans, 2008). A unique feature of the SGBR is its ability to retain active biomass regardless of the hydraulic
or organic loading rate. Therefore, problems such as solid washout, loss of granular solids and granule deterioration are minimized (Ellis and Evans, 2008). The SGBR was used in this research due to its biomass retention ability and simple operation. Gas collection and effluent testing was performed for the SGBR in a similar fashion to the SBR.

Analysis of the loading rate, hydraulic retention time (HRT) and solids retention time (SRT) was performed. The feed ratio of biomass and the ratio of sulfide to nitrate removal were both studied so that a better understanding of the stoichiometry could be obtained. Gas production was measured to monitor how much nitrogen gas was generated to verify the material balance for nitrogen removal. Environmental conditions such as temperature and pH were monitored as well so that the most favorable environmental conditions could be determined for future application of this unique process.

Autotrophic denitrification highly depends on the characteristics of the microbial community. Most common microbes that contribute to the autotrophic denitrification process are *Thiobacillus*, *Sulfurovum* and *Shinella* bacteria (Qian et al., 2016). Qian et. al. (2016) found that *Thiobacillus* bacteria accounted for 34.2% of all bacteria when thiosulfate was utilized as the electron donor during autotrophic denitrification in an anoxic up-flow sludge bed reactor (AnUSB). They also reported that *Sulfurovum* and *Shinella* bacteria were detected as 4.3% and 2.2%, respectively, in the AnUSB. Beside, Inagaki et al. (2004) commented that *Sulfurovum* bacteria was able to oxide thiosulfate to sulfate during denitrification process for the sake of chemo-lithoautotrophic growth. Bai et al. (2009) reported that *Shinella* was another species that could undertake autotrophic denitrification. Microbial identification tests of the Cedar Rapids seed biomass and SBR biomass samples at different time points were performed in order to determine the type and quantity of microorganisms
present and those communities adapted over the course of the study of the autotrophic denitrification process.
CHAPTER 2. LITERATURE REVIEW

Autotrophic Denitrification

There are multiple treatment strategies to remove contaminants such as nitrogen from water resources. Ion exchange resins, chemical stripping, and distillation are current physical-chemical treatment methods. However, each has specific limitations. Ammonia stripping, for instance, is a commonly used treatment process at some facilities. Although the chemical process is able to treat high level of nitrogen in wastewater efficiently, the discharge of ammonia into the atmosphere becomes a potential air-pollution (U. S. Environmental Protection Agency, 1974). The overall treatment costs will therefore increase. In addition, the physical-chemical treatment process may be difficult to implement for in situ remediation (Lampe and Zhang, 1997.).

On the contrary, biological treatment processes, such as heterotrophic and autotrophic denitrification, are able to remove nitrate-nitrogen more efficiently (Lampe and Zhang, 1997.). Heterotrophic denitrification has been highly utilized in nitrate-removal processes. Nevertheless, heterotrophic denitrification requires sufficient organic carbon sources to supply electrons to reduce nitrate as well as carbon source for the metabolism of heterotrophic microorganisms (Zhou et al., 2016). In case of groundwater and surface water, this process will typically lack sufficient organic carbon sources and therefore will require an external organic carbon sources. Moreover, excess organic sources could result in secondary pollution such as overgrowth of algae (Zhou et al., 2016). Clogging of the reactor might occur due to the high level of bacteria. Disposal of the sludge generated by the process becomes another problem (Zhao et al., 2012).
Autotrophic denitrification is a microbially driven process, which reduces nitrate into nitrogen gas and oxidizes sulfide into sulfate or elemental sulfur as shown in Equations (1) and (2) (Fajardo et al., 2012). This process is also able to remove hydrogen sulfide, a toxic and corrosive gas, produced from sulfate reducing bacteria during anaerobic digestion of wastes and wastewaters that contain sulfate (Montalvo et al., 2016).

$$S^{2-} + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O$$ (1)

$$S^{2-} + 0.4NO_3^- + 2.4H^+ \rightarrow S^0 + 0.2N_2 + 1.2H_2O$$ (2)

There are two major types of autotrophic denitrification processes, including hydrogen-based and sulfur-based autotrophic denitrification. Hydrogen-based autotrophic denitrification process involves the use of hydrogen gas as the electron donor. This process is usually not preferred because hydrogen gas is highly flammable and it might cause explosion in contact with open air if it is handled without caution. On the other hand, sulfur-based autotrophic denitrification process is much safer and easier to handle because it mainly utilize sulfur compounds such as sulfide and elemental sulfur as electrons (Zhang and Lampe, 1999; Zhou et al., 2016). Equation (3) shows the stoichiometric equation for the sulfur-based autotrophic denitrification process (Zhang and Lampe, 1997).

$$55S + 20CO_2 + 50NO_3^- + 38H_2O + 4NH_4^+ \rightarrow 4C_5H_7O_2N + 25N_2 + 55SO_4^{2-} + 64H^+$$ (3)

Researchers have found that 110 mg/L sulfide substrate could be completely oxidized and the autotrophic denitrification process could reach 100% removal efficiency of nitrate at the same time. This process have also been determined to be feasible to treat eutrophication by having ceramsite, an artificial developed Chinese sand made from bauxite, as the carrier for microorganism immobilization. The functional bacteria can be enriched and sludge washout
can be prevented as well (Zhou et al., 2016). Sodium thiosulfate was also determined by (Zhou et al., 2016) as the preferred electron donor over elemental sulfur and sodium sulfide. After comparing between the elemental sulfur and thiosulfate, they discovered that thiosulfate had higher solubility and therefore resulted in better electron-providing ability. This resulted in better treatment efficiency. Based on literature (Montalvo et al., 2016), zeolite is able to facilitate the startup process of the autotrophic denitrification process to reach stabilization in both batch and continuous type of reactors. Zeolite is able to fasten the increasing of pH to a neutral value. With zeolite available, pH could reach 7.3 on the fourth day. However, it required 20 days for pH to reach the same value without zeolite. On the other hand, (Oh et al., 2000) determined that nitrite inhibits the autotrophic denitrification process. Fajardo et al. (2012) commented that zeolite can speed up this process by reducing the inhibitory effect of nitrite while posing no effect on nitrogen conversion.

Compared to heterotrophic denitrification, autotrophic denitrification is a better alternative to treat wastewater that contains high level of nitrate and low carbon content (Chung et al., 2014). There are two advantages which autotrophic denitrification has: 1) an external organic carbon source is not required and 2) less sludge is produced which results in lower sludge disposal costs (Mahmood et al., 2007). Regarding the sulfur-based autotrophic denitrification process, the consumption of alkalinity needs to be factored in. There is a significant amount of hydrogen ions generated corresponding to a decrease in alkalinity. There is 3.57 mg CaCO$_3$ alkalinity consumed for each mg NO$_3^-$-N reduced theroretically (Sahinkaya and Kilic, 2014). External alkalinity is therefore necessary if the inherent alkalinity is not sufficient to maintain a neutral pH. The optimal pH for sulfur-based autotrophic denitrification process is approximately 6.5-7.5 (Oh et al., 2000), and the
optimal temperature for this process is approximately 35°C (Montalvo et al., 2016). However, Zhou et al. (2016) suggested that the optimal temperature for this process should be between 24 and 30°C. They also commented on the different optimal temperatures for microbial processes for nitrate versus nitrite reduction. The temperature preferred by nitrate-reducing-bacteria was approximately 30°C or higher. On the other hand, the optimal temperature for nitrite-reducing-bacteria was around 24°C.

Autotrophic denitrification processes utilize a microbial consortium that contains *Thiobacillus*, *Sulfurovum*, and/or *Shinella*, as autotrophic denitrifiers to reduce nitrate into nitrogen gas in wastewater (Qian et al., 2016). An inorganic carbon source, such as carbon dioxide and bicarbonate, can be used by autotrophic denitrifiers as their carbon source (Zhao et al., 2012). *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* have been studied regarding their ability to consume elemental sulfur and reduce nitrate. It was found by the researchers that these two different denitrifying species were able to use elemental sulfur as electron donor while converting nitrate into nitrogen gas (Zhou et al., 2016). However, Fajardo et al. (2012) found that autotrophic denitrifiers have a relatively low growth rate and this limited the autotrophic denitrification process.

**Static Granular Bed Reactor**

Anaerobic processes are similar to anoxic processes in that they operate in an environment devoid of dissolved oxygen and the anaerobic bacteria are slow growing and have a low yield. In their attempt to develop a simple and high rate anaerobic process for treating wastewater, Ellis and Mach (2004) introduced the static granular bed reactor. The
advantages by utilizing an static granular bed reactor (SGBR) in wastewater treatment is that it is able to retain active biomass regardless of the hydraulic or organic loading rate. An additional advantage of anaerobic treatment is the recovery of energy (Lettinga et al., 1980). There are different types of anaerobic granular reactors that have been widely utilized to treat waste sludge. Upflow anaerobic sludge bed (UASB) reactor, for example, is a common reactor that is used to treat high strength municipal and industrial wastewater (Liu et al., 2003). Wastewater treatment plants that utilize UASB reactors consume much less energy than their aerobic counterparts. However, UASB reactors may have difficulties in odor prevention and solids capture. (Van Lier et al., 2010). As with any anaerobic process, nutrients are conserved and direct discharge is not commonplace due to the high level of ammonia and total suspended solids in the effluent. The UASB is also susceptible to solids washout. Due to the high gas generation rate and the upflow velocity of the wastewater, granules can become buoyant and be discharged in the effluent (Evans and Ellis, 2010). This phenomenon can cause the UASB to become unstable during fluctuations in either hydraulic or organic loading. The SGBR system, invented by Ellis at Iowa State University in 2004, aimed to lower the operating cost, enable high solids inventory, and enhance anaerobic treatment efficiency (Evans and Ellis, 2010). They determined that the SGBR was able to treat synthetic industrial wastewater as well or better than the UASB reactor. They also found that the UASB reactor experienced more fluctuations due to erratic solids capture even though it performed well at high organic loading rate. A schematic diagram of a typical SGBR is shown in Figure 1. The main principle of the reactor is that wastewater enters the reactor from the top and then flows by gravity through the active layer that consists of granular anaerobic sludge. An SGBR is able to be operated at long solid retention time (SRT) and
short hydraulic retention time (HRT). Short HRT allows the volume of the static granular bed reactor to be reduced, lowering the capital and operational costs of the reactor. The reactor is also able to maintain a relatively long SRT, allowing for nearly complete transformation of organic matter to carbon dioxide and methane. Studies by Ellis and Mach (2004) have shown that the SGBR achieved over 90% chemical oxygen demand removal at an 8-hour HRT while the concentration of total suspended solids in effluent was less than 100 mg/L.

![Diagram of a typical SGBR](image_url)

Figure 1. Schematic diagram of a typical SGBR (Ellis and Mach, 2004).

**Structure and Characteristics of Granule**

Inside the static granular bed reactor, the concentration of granular microbes is relatively high, which reduces the volume of the reactor and enhances the rate of the degradation of microorganism in the reactor. Research has shown that anaerobic granular
sludge is able to remain in the reactor under a high strength and low flow rate of wastewater (Lim and Kim, 2014). The granule surface is full of cavities and holes, which is suitable for transporting substrates, products, and gases. Acidifying bacteria and hydrolytic bacteria remained in the outer layer of granule that grew on lactate or propionate while methanotrophs mainly stayed in the inner layer of the granule (Fukuzaki et. al, 1991). The surface structure of typical granular is displayed in Figure 2.

![Granule Surface Diagram](image)

Figure 2. Diagram of cavities on the surface of a typical granule (Lim and Kim, 2014).

The formation and maintenance of granules highly depend on the extracellular polymer substances. These substances are mainly made up of polysaccharides, proteins, lipids, organic debris, lysed cells etc. Due to the fact that microorganisms usually carry negative charge on their surface, extracellular polymer substances need to maintain positive charges on their surfaces so that they can make the granules stable. Research has shown that extracellular polymer substances are able to protect microorganisms and their interaction with granules accounts for the sludge granulation (Lim and Kim, 2014). Under mesophilic conditions, there are significantly more protein and polysaccharides with less lipid in the
extracellular polymer substrates. However, the amount of protein and polysaccharides is much less under thermophilic conditions. Even though the mechanisms of the formation of granules have not been fully understood yet, most researchers have accepted the idea that the generation of extracellular polymer substances significantly accounts for the formation of granules. Researchers have conducted studies on whether the granular size can affect the denitrification process. Moon et al. (2006) researched the potential effect that granule size has on denitrification process, and they concluded that smaller granules tended to increase the process efficiency. However, they also indicated that granular size must not be too small in order to avoid wash out.

**Anaerobic Reactions**

Industrial wastewater usually contains high concentrations of organic matter contributing to chemical and biochemical oxygen demand. High solids concentrations, pharmaceuticals, and microorganisms may also be present. By increasing the SRT in the SGBR, effluent concentrations of solids and organic matter can be minimized. The high concentration of microorganisms in granular sludge reactors enables the treatment of high strength wastewater without external separation or recirculation (Lim and Kim, 2014). Consistent wastewater flow from the top to the bottom of the anaerobic granular reactor attributes to better treatment performance as well. It is assumed that the buoyancy force accounts for the movement of the granules in the reactor. However, since the reactor is called “static granular bed reactor”, it is more convincible to say that wastewater flows through the reactor with the assistance from biogas that is produced in the reactor.
Sequencing Batch Reactor

The sequencing batch reactor (SBR) operates in a series of process phases, namely fill, react, settle, decant, and idle (Wilderer et al., 2001). Wastewater is fed into the reactor in the fill phase. After reacting for a certain length of time (stirring accompanies the reaction process), the settling is initiated, and the treated wastewater supernatant is discarded in the following decant phase. The idle period is an optional phase when multiple SBRs are used and allows varying flows to be accommodated. Biomass is retained in the reactor to allow for treatment of the next batch of wastewater in a manner that is analogous to the return sludge flow of a conventional flow through process (e.g., activated sludge). A typical sequencing batch reactor is shown in Figure 3. High conversion per unit volume is one of the main advantages of the batch reactor. In a laboratory setting it is easy to clean and maintain as well. The laboratory SBR can be heated or cooled, which can satisfy the temperature requirement of specific microorganisms. However, the batch nature of the reactor requires manual or automated pumping during the fill and decant phases, as well as timed mixing cycles.

Figure 3. Schematic diagram of a typical SBR.
Microbial Community Analysis

There are three main types of microorganisms discovered in the autotrophic denitrification process. *Thiobacillus* has been studied as the most prevalent species during the autotrophic denitrification process (Qian et al., 2016; Oh et al., 2000). These microorganisms reduce nitrate to nitrogen gas when reduced sulfur, such as thiosulfate and sulfide, is available as electron donor. *Shinella* is also able to reduce nitrate to nitrogen gas (Qian et al., 2016; Oh et al., 2000; Bai et al., 2009). *Sulfurovum* has been discovered as another type of microorganisms that can oxide thiosulfate to sulfate during autotrophic denitrification process to facilitate chemo-lithoautrophic growth (Inagaki et al., 2004).

It is important to detect and quantify the species of microorganisms involved in autotrophic denitrification in order to apply this technique to future research on wastewater treatment strategy. Polymerase chain reaction (PCR) is a biological method to make duplicate copies of a segment of DNA. A typical cycle of PCR includes strand separation, the annealing of primers and the extension of primers by DNA synthesis (Tymoczko, Berg and Stryer, 2013).

DNA extraction and purification is necessary proceeding polymerase chain reaction. There are two methods – cell extraction and direct lysis within the soil matrix (Bremen et al., 1999). Compared to the cell extraction technique which collects cellular DNA through cell lysis process, the direct lysis technique is used more often because more DNA can be attained and samples of microorganism community diversity are less biased. But, direct lysis may extract more PCR-inhibitory substances with the desired DNA (Bremen et al., 1999). There are several factors that make the PCR biochemical technique suitable for detecting microorganisms in research. First, researchers need to know the flanking sequences only. A
flanking sequence is the region on either side of the target sequence region. Second, a primer, a short strand of DNA or RNA that acts as the starting point of DNA synthesis, is not necessary to be exactly matched to the flanking sequence. Therefore, detection of variations on the targets is possible. Moreover, primers can be much smaller than the target sequences. Thus, even large size of target sequences can be amplified. Lastly, PCR is extremely specific and sensitive.

Illumina’s MiSeq platform has been widely used in next generation sequencing (NGS) research. Even though community sequencing problems such as PCR primer biases and differential DNA extraction efficiency exist (Caporaso et al., 2012), this technique has high throughput and is able to detect lengths of DNA sequences up to $2 \times 300$ bp with low sequencing costs (Schirmer et al., 2015). In order to fully process the sequencing, four basic steps must be completed (Illumina Inc., 2017): 1) to have the sequencing library ready by fragmenting DNA or cDNA sample and binding with 5’ and 3’ adapter. 2) to capture and amplified the fragments of DNA or cDNA sample. 3) to sequence DNA by detecting single bases as they are incorporated into DNA template strands. 4) to identify sequence reading and align the reading to a reference genome.

**Kinetics of Autotrophic Denitrification**

The Michaelis-Menten Model has been utilized by researchers to account for the kinetics of enzymes. This model displaces the relationship between concentration of the substrate and the reaction rate. Figure 4 shows a typical Michaelis-Menten Kinetics Model.
When the curve reaches a stabilized phase, the maximum reaction rate is achieved. The concentration of substrate corresponds to half of the maximum reaction rate is represented by a coefficient, $K_{\text{max}}$. This coefficient is called the Michaelis constant and it is significant for researchers to determine the concentration of substrate for a successful catalysis. In order to determine the kinetics of product formation process, a function of the concentration of product versus time must be determined. Figure 5 shows a typical plot for the kinetics of product generation process.

Figure 4. Michaelis-Menton Kinetics model.
The curve tends to have a steep slope at the beginning and gradually decrease in its slope. A steep slope indicates that enzymes are in their most active stage. A decrease in slope, therefore, means that enzymes are gradually becoming inactive due to possible poisons effect from by-products or substrate is being consumed. Finally, the curve reaches a stabilized phase, which means the reaction is in equilibrium. There will be no more product generated unless more substrate is supplied or poison by-product is removed. The initial reaction velocity can be found by determining the slope of the beginning period of the reaction.

The Monod model had been used by scientists to study the relationship between the growth activity of microorganisms and consumption of substrates. Figure 6 shows a typical curve that can be seen for the Monod model.
The Monod expression had its X-axis as the concentration of substrate while its Y-axis is the specific growth rate of biomass. The model was expressed as

$$
\mu = \mu_{max} \frac{S}{K_S + S}
$$

(4)

where $\mu$ was the specific growth rate of biomass, $\mu_{max}$ was the maximum specific growth rate of biomass, $S$ is the concentration of limiting substrate and $K_S$ is the coefficient that indicates the concentration of substrate at half of maximum specific growth rate of biomass. The model shows a rapid growth at first (approximately linear to the increase in substrate concentration) followed by a gradual decrease in slope where the impact of the half saturation coefficient becomes apparent. Once the maximum activity of microorganisms (substrate saturation) is reached, the slope would remain...
The Monod model was tentatively utilized to model the kinetics of sulfate generation during the autotrophic denitrification process. The equation used to modeling the process was

$$\frac{dS}{dt} = \frac{\mu XS}{Y(K_S+S)}$$

(5)

where $\frac{dS}{dt}$ is the substrate conversion rate in mg/L-hr, $S$ is the concentration of substrate in mg/L, $\mu$ is the specific growth rate, $X$ is the specific biomass concentration in mg/L, $Y$ is the yield and $K_S$ is the substrate concentration at half of the maximum reaction rate in mg/L. Instead of having substrate concentration as X-axis and specific biomass growth rate as Y-axis for the kinetics model, time and concentration of sulfate was used to fit the model. The model should express a deep slope at first to indicate the rapid activity of biomass on converting substrate into sulfate. This was followed by a gradual decrease in slope before the model showed a relatively unchanged concentration of sulfate, which indicated a successful reaction that reached the maximum concentration of sulfate.

Researchers have studied the kinetics of autotrophic denitrification with thiosulfate. Despite the inhibiting concentration of $\text{NO}_2^-$, the concentration of $\text{SO}_4^{2-}$ displayed a gradually increasing tendency (Mora et al., 2015). The concentration of $\text{SO}_4^{2-}$ reached a maximum level after a certain length of time and then maintained the maximum concentration. Bio-kinetics of autotrophic denitrification by denitrifying sulfur bacteria was studied by researchers. Regarding the inhibition of the denitrification
process, gas production versus time with different sulfate concentration as well as nitrate depletion profile was studied. Oh et al. (2000) found that nitrate depletion profile reached zero after approximately 5 hours. At the same time, biomass concentration reached its maximum and became stabilized after 5 hours. Gas production became lower and a longer lag phase was detected when sulfate concentration was greater than 6,000 mg/L. Claus and Kutzner (1985) also determined that the denitrification process was inhibited when sulfate concentration reached 5,000 mg/L. They also reported that the process was inhibited completely when the sulfate concentration reached approximately 20,000 mg/L. Moreover, inhibition of the autotrophic denitrification process was discovered by Campos et al. (2008) when the sulfate concentration reached 1,500 mg/L and the denitrification process was inhibited about 85% when the sulfate concentration was 15,000 mg/L. On the contrary, Chung et al. (2014) reported that there was no inhibition even when the sulfate concentration reached its stoichiometric amount, 11,000 mg/L. They found that nitrate was completely removed during their research.
CHAPTER 3. AUTOTROPHIC DENITRIFICATION OF INDUSTRIAL WASTETATER USING SEQUENCING BATCH AND STATIC GRANULAR BED REACTORS

Modified from a paper to be submitted to WEFTEC

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Abstract

Nutrient removal at the Cedar Rapids Water Pollution Control Facility is a significant consideration for future treatment goals. Alternate electron donors were evaluated for denitrification at the plant to improve removal of total nitrogen. A portion of the industrial wastewater contains copious sulfate and therefore was utilized as an energy source for autotrophic denitrifiers. Oxidation of reduced sulfur species is important since hydrogen sulfide gas generated during anaerobic conditions in the collection system and at the plant can raise occupational safety risk and create an odor nuisance. Autotrophic denitrification is a microbially driven process which can reduce nitrate to nitrogen gas when reduced sulfur is present. The experimental results presented herein illustrate that the autotrophic denitrification process can reduce nitrate in the Cedar Rapids wastewater with reduced sulfur as the sole electron donor available.

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Introduction

Industrial wastewater contains a significant quantity of various environmental contaminants including organic matter, nutrients, and pathogens. In the effort to minimize the nutrient load on receiving streams from point sources, processes such as nitrification/denitrification are required to improve removal of total nitrogen from wastewater. The majority of nitrogen entering sensitive ecosystems is from agriculture. Fertilizer use on farms results in runoff that introduces nitrogen, typically in the form of nitrate, into adjacent surface waters. When surface waters containing nitrate in excess of the regulated level, potential health risks (e.g., methaemoglobinaemia) exist for those using that source for their drinking water supply (Lundberg et al., 2004). Sulfide is another type of environmental pollutant when it exists in the form of hydrogen sulfide (H$_2$S). H$_2$S is known for its strong odor at extremely low concentrations as well as its corrosiveness. If human beings are exposed to high levels of H$_2$S, the toxicity of this gas can cause immediate loss of consciousness and even death (Vaiopoulou et al., 2005). Aside from being a nuisance, reduced sulfur species at wastewater treatment plants may also serve as an energy source (i.e., as electron donors) for autotrophic denitrification.

Influent to the Cedar Rapids Water Pollution Control Facility contains a significant amount of sulfate primarily from pulp and paper and food processing industries in the city. A portion of the sulfate is reduced to hydrogen sulfide in the collection system prior to entering the plant. An additional portion is reduced to hydrogen sulfide by sulfate reducing bacteria in the anaerobic pretreatment system for the industrial portion of the influent. Cedar
Rapids utilizes an upflow anaerobic sludge bed (UASB) reactor for pretreatment. The UASB has been widely used in industrial wastewater treatment for high strength wastewater where the majority of the organic load is soluble. It has the ability to grow and retain a large quantity of granular biomass (Shalu and Bishnoi, 2016). Despite its high removal efficiency due to the large biomass concentration, there will be some residual organic concentration in the effluent as well as suspended solids and reduced sulfur species, i.e., hydrogen sulfide. Anaerobic treatment is traditionally followed up with aerobic treatment to remove the residual organic concentration and to remove nutrients. During aerobic treatment sulfides will be oxidized to sulfate, and ammonia will be oxidized to nitrate if the SRT is sufficiently long to sustain a nitrifying population. Heterotrophic denitrification is a commonly used treatment strategy to remove nitrate from wastewater, and separate stage denitrification requires an external organic carbon source such as methanol, ethanol, glucose, glycerol, acetic acid or starch (Zhao et al., 2012). Single sludge systems utilize the organics in the influent as the electron donor and require recirculation of the nitrates from the aerobic zone to an anoxic zone. Heterotrophic denitrification will generate additional biomass, which increases the cost for further treatment and disposal. Autotrophic denitrification, instead, is able to remove nitrate from wastewater without the need for an external carbon source. It also produces less sludge than heterotrophic denitrification, and therefore minimize the disposal cost (Mahmood et al., 2007). Autotrophic denitrification is a microbially driven process, which reduces nitrate into nitrogen gas and oxidizes sulfide into sulfate or sulfur as shown in Equation (6) and (7) (Fajardo et al., 2012). The following equations illustrate that the autotrophic denitrification process with sulfide as the electron donor is able to generate alkalinity, which is beneficial as alkalinity is essential to the process.
\[ S^{2-} + 1.6\text{NO}_3^- + 1.6H^+ \rightarrow \text{SO}_4^{2-} + 0.8\text{N}_2 + 0.8\text{H}_2\text{O} \quad (6) \]
\[ S^{2-} + 0.4\text{NO}_3^- + 2.4H^+ \rightarrow S^0 + 0.2\text{N}_2 + 1.2\text{H}_2\text{O} \quad (7) \]

When thiosulfate serves as the electron donor, the stoichiometry becomes
\[ 5\text{S}_2\text{O}_3^{2-} + 8\text{NO}_3^- + \text{H}_2\text{O} \rightarrow 4\text{N}_2 + 10\text{SO}_4^{2-} + 2\text{H}^+ \quad (8) \]

This reaction produces hydrogen ions instead. Therefore, it is important to supply an external source of alkalinity to maintain a neutral pH favored by autotrophic denitrification processes.

The make-up of the microbial community is important for autotrophic denitrification to occur. *Thiobacillus*, *Sulfurovum* and *Shinella* bacteria are the common types of autotrophic denitrifiers seen in the autotrophic denitrification process. Qian et al. (2016) found that *Thiobacillus* bacteria accounted for 34.2% of all bacteria when thiosulfate was utilized as the electron donor during autotrophic denitrification in an anoxic up-flow sludge bed reactor (AnUSB). They also reported that *Sulfurovum* and *Shinella* bacteria was detected as 4.3% and 2.2%, respectively, in the AnUSB. Beside, Inagaki et al. (2004) commented that *Sulfurovum* bacteria was able to oxide thiosulfate to sulfate during denitrification process for the sake of chemo-lithoautrophic growth. Bai et al. (2009) reported that *Shinella* was another species that could undertake autotrophic denitrification.

This study looked at the feasibility of autotrophic denitrification at the Cedar Rapids Water Pollution Control Facility which receives a significant amount of industrial wastewater including food processing and cardboard recycling wastewater. A sequencing batch reactor (SBR) was used in this research to enrich a culture of bacteria capable of autotrophic denitrification. This culture was then used to seed a static granular bed reactor (SGBR) for flow through treatment of plant wastewater containing both nitrate from plant effluent and reduced sulfur from the pretreatment (UASB) effluent. Analysis of the loading rate, hydraulic
retention time (HRT) and solids retention time (SRT) was conducted. The feed biomass ratio, concentration of generated products, and kinetics of sulfate generation process were studied in order to determine the optimal operating parameters and treatment strategy. The pH and alkalinity were monitored to determine the optimal environmental conditions for the process. Analysis of the microbial community was conducted to further understand the process and assist future research on and use of autotrophic denitrification. This paper presents and discusses the results obtained from the lab-scale autotrophic denitrification using SBR and SGBR bioreactors.

Materials and Methods

Laboratory Setup

Two types of lab-scale reactors, a sequencing batch reactor (SBR) and static granular bed reactor (SGBR), were utilized in these experiments. The SBR utilized a glass reaction vessel (Bioflo II fermentor, New Brunswick Scientific, Edison, NJ), and the SGBR was fabricated from Plexiglass. The temperature for the entire experiment was fixed at 33℃ ± 0.5℃. Two identical Masterflex peristaltic pumps (Masterflex, 7553-00, Chicago, IL) with standard pump heads were utilized for each reactor. A 6-L plastic gas collector was installed for each reactor system in order to collect and measure the amount of nitrogen gas generated. Automatic time controllers (ChronTrol, San Diego, CA) were used to control the cycling of pumps and stirring mechanism. Both reactors were filled with 1-L biomass included anaerobic granules from the UASB, biomass from the sulfide scrubber, and biomass from the sulfur oxidation basin at the Cedar Rapids Water Pollution Control Facility. The feed
solution composition for both reactors were prepared according to Lampe and Zhang (1997) with slight modifications: KNO$_3$, 3.00 g/L; Na$_2$S$_2$O$_3$·5H$_2$O, 4.20 g/L; NaHCO$_3$, 1.50 g/L; Na$_2$HPO$_4$, 1.50 g/L; KH$_2$PO$_4$, 0.30 g/L; MgSO$_4$·7H$_2$O, 0.40 g/L. Each liter of feed solution was mixed with 1 mL of stock trace nutrient solution, which was made from: NH$_4$Cl, 5.74 g/L; K$_2$HPO$_4$, 5.60 g/L; FeCl$_2$·6H$_2$O, 1.00 g/L; MnSO$_4$·H$_2$O, 1.00 g/L; and CaCl$_2$, 1.00 g/L (Lampe and Zhang, 1997). Room temperature tap water was used to make the entire solution. Feed solution (influent) and effluent was stored in a refrigerator.

**Analytical Methods**

Before determining the concentration of nitrate and sulfate in the supernatant, samples of effluent were filtered using glass microfiber filters with a fine nominal particle retention of 1.2 μm. A Seal AQ2 Automated Discrete Analyzer (Mequon, WI) was utilized to measure the concentration of sulfate in the effluent supernatant, and a Spectronic Genesys 2 (Thermo Electron, Madison, WI) was utilized to determine the concentration of nitrate in the effluent supernatant. The amount of nitrogen gas generated was determined by observing the gas volume in the gas collector. Suspended solids concentrations were measured according to Standard Methods (APHA, 2012). The pH was determined by using a Fisherbrand™ accuT™ AB15 Basic and BioBasic™ pH/mV/°C meter (Fisher Scientific, Pittsburg, PA). The alkalinity was measured following Standard Methods (APHA, 2012). Microbial analysis of autotrophic denitrifiers was performed through DNA sequencing. Invitrogen PureLink™ Microbiome DNA purification kit (Thermo Fisher Scientific, Carlsbad, CA) was utilized to extract DNA from centrifuged biomass samples (UASB granules, oxidation basin biomass, sulfide scrubber biomass, and SBR biomass). The 16S
rRNA gene (v4-v5 region) was amplified and sequenced using Illumina MiSeq (Illumina, Inc., San Diego, CA) for microbial community profiling.

**SBR Batch Tests**

A dilution solution without sulfate and nitrate source was used to remove as much residual sulfate in the SBR following a decant cycle. The dilution solution was prepared by using the following chemicals: \( \text{Na}_2\text{HPO}_4 \), 1.50 g/L; and \( \text{KH}_2\text{PO}_4 \), 0.30 g/L. Each liter of dilution solution was combined with 1 mL of stock trace nutrient solution, which was made from: \( \text{NH}_4\text{Cl} \), 5.74 g/L; \( \text{K}_2\text{HPO}_4 \), 5.60 g/L; \( \text{FeCl}_2 \cdot 6\text{H}_2\text{O} \), 1.00 g/L; and \( \text{CaCl}_2 \), 1.00 g/L. Four dilution runs were performed before performing a kinetic test using the synthesized wastewater. Excess nitrate source was provided by having \( \text{KNO}_3 \) concentration of 5.00 g/L for the synthesized wastewater. Sampling of effluent was performed every half an hour for the first 7 hours and every one hour for the following hours. Before determining the concentration of sulfate and nitrite in the supernatant, samples of effluent were filtered using glass microfiber filters with a fine nominal particle retention of 1.2 μm. Seal AQ2 Automated Discrete Analyzer (Mequon, WI) was utilized to detect the concentration of sulfate in the effluent supernatant. Spectronic Genesys 2 (Thermo Electron, Madison, WI) was utilized to determine the concentration of nitrite in the effluent supernatant.
Figure 7. Schematic diagram of the SBR system.

Figure 8. Schematic diagram of the SGBR system.
Results and Discussion

During the course of the SBR laboratory experiment, a high concentration of sulfate and low concentration of nitrate was observed in the effluent. During the first month, the concentration of sulfate was somewhat unstable. Figure 9 shows that the concentration of sulfate in the supernatant of the SBR effluent was around 6 mg/L as S at the lowest and about 1,664 mg/L as S at the highest. After the first 30 days, the concentration of sulfate produced in the reactor stabilized in between 1,350 – 1,400 mg/L as S, which was close to the theoretical concentration based on stoichiometry for 100% conversion. The concentration of sulfate suddenly decreased from day 50 to day 70. This dramatic decrease in sulfate concentration was due to the intentional change of the nitrate concentration in influent. The concentration of nitrate was 0.68 g/L as N originally; however, it was changed into 0.47 g/L as N on day 50 to observe the effect of a lower concentration on the biological response. After day 70, it was changed back to the original concentration again, and therefore the concentration of sulfate produced in the reactor increased and stabilized between 1,240 – 1,310 mg/L as S. The concentration of nitrate in the supernatant of effluent stabilized after about 10 days operation. From Figure 9, similar to the concentration of sulfate, the nitrate concentration was unstable during the first 10 days of the system start-up. It was around 28 mg/L as N at the lowest and about 282 mg/L as N at the highest. The concentration of nitrate gradually stabilized by day 10 and remained constant with 20 mg/L as N.

The nitrate removal efficiency fluctuated during the first 10 days of system start-up as the system acclimated to the feed characteristics and a stable population of autotrophic denitrifiers could be developed. After day 10, nitrate removal efficiency increased above 95% and was relatively stable during the rest of the operating period. Once the system
stabilized, the highest nitrate removal efficiency was 99.5%, and the lowest nitrate removal efficiency was around 90.8%.

Figure 9. Concentration of sulfate and nitrate in the SBR effluent.

Figure 10. Nitrate removal efficiency and effluent concentration in SBR.
During the entire experimental period, routine results for pH and alkalinity were determined according to Standard Methods. From Figure 11, pH and alkalinity both dropped dramatically during the first 10 days of operation. This was due to the activity of the autotrophic denitrifiers. The stoichiometry of the reaction predicts a reduction of alkalinity. Sodium bicarbonate, which provided alkalinity to the influent wastewater, was consumed by the autotrophic denitrifiers. The pH remained neutral after day 10 while alkalinity stabilized at approximately 500 mg/L as CaCO₃ after day 35.

According to Table 1, the mixed liquor suspended solids (MLSS) in the SBR was around 7,294 mg/L after operating the SBR system on day 30. It dropped approximately 1,077 mg/L 15 days later. When the system was operated for 60 days, MLSS decreased to about 5,467 mg/L, which was approximately 1,827 mg/L lower than the results obtained on day 30. Furthermore, 17 days later, MLSS decreased to 5,423 mg/L which was 43 mg/L
lower than the result measured previously. The decrease in MLSS in the reactor suggests that the biomass concentration after seeding was higher than necessary and the system was reaching an equilibrium with respect to the biomass concentration. After several months of operation the MLSS concentration became stable, and after 137 days, the MLSS in the SBR dropped to 4,443 mg/L. Compared to the result on day 30, there was about 39% less mixed liquor suspended solids in the SBR. The mixed liquor volatile suspended solids (MLVSS) in the SBR was 3,550 mg/L after operating the SBR system for 30 days. Unlike MLSS at 45 days, the MLVSS concentration increased about 461 mg/L after the same length of operation time. After operating about 60 days, the MLVSS decreased from 4,011 mg/L to 3,727 mg/L. The MLVSS decreased to 3,067 mg/L after operating for 137 days.

Table 1. Mixed liquor suspended and volatile suspended solids of the SBR biomass.

<table>
<thead>
<tr>
<th>Day</th>
<th>Mixed Liquor suspended solids (mg/L)</th>
<th>Mixed liquor volatile suspended solids (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>7,294</td>
<td>3,550</td>
</tr>
<tr>
<td>45</td>
<td>6,217</td>
<td>4,011</td>
</tr>
<tr>
<td>60</td>
<td>5,467</td>
<td>3,727</td>
</tr>
<tr>
<td>77</td>
<td>5,423</td>
<td>3,107</td>
</tr>
<tr>
<td>137</td>
<td>4,443</td>
<td>3,067</td>
</tr>
</tbody>
</table>

All experimental results above illustrated that the biological environment in the SBR became relatively stable after about 50 days. Therefore, the same biomass seeding was applied to the SGBR in order to test whether the same biological process was feasible with an alternative reactor design. The SGBR was started with the synthetic feed solution after the SBR was operated for 54 days.

The pH and alkalinity in the SGBR is shown in Figure 12. During the initial 10 days, pH decreased from 7.67 to 7.31 as the autotrophic population acclimated to the feed. Accordingly, alkalinity dropped from 1,500 mg/L as CaCO$_3$ to 600 mg/L as CaCO$_3$. The pH
remained stable around 7.50 after day 10, with small fluctuations, and the alkalinity was relatively stable at about 700 mg/L as CaCO₃. Similar to pH, alkalinity had slight variations where the highest concentration was 780 mg/L as CaCO₃ and the lowest was 600 mg/L as CaCO₃.

Starting on day 103, the feed was changed from synthetic feed solution to plant wastewater. Table 2 shows the time when the feed and HRT in the SGBR were changed. This resulted in the pH and alkalinity both increasing due to the lower nitrate concentration in the influent. Corresponding data were within the orange and green dash lines shown in Figure 12. The pH increased to 8.00 and was highly stable following the feed change. The alkalinity increased from 700 mg/L as CaCO₃ to 1,100 mg/L as CaCO₃ within the same period of time.
Table 2. Feed type and hydraulic retention time of the SGBR.

<table>
<thead>
<tr>
<th>Day</th>
<th>Feed type</th>
<th>Hydraulic retention time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Synthetic feed</td>
<td>48</td>
</tr>
<tr>
<td>103</td>
<td>Plant wastewater</td>
<td>48</td>
</tr>
<tr>
<td>153</td>
<td>Plant wastewater</td>
<td>36</td>
</tr>
</tbody>
</table>

Less nitrate in the feed resulted in less production of hydrogen ions and less consumption of alkalinity. After day 153 (green dash line represented the time point on Figure 12 and 13) and for the rest of the duration of the study, the HRT was reduced from 48 hours to 36 hours to evaluate the effect of HRT on the autotrophic denitrification process. As a result, the pH increased again from 8.00 to about 8.40, while alkalinity decreased from 1,160 mg/L as CaCO$_3$ to 1,120 mg/L as CaCO$_3$.

As shown in Figure 13, the effluent concentration of sulfate decreased gradually from about 1,375 mg/L as S to 1,082 mg/L as S within the first 102 days, and then remained relatively stable around 1,100 mg/L as S. The feed solution was changed from the synthetic solution to a mixture of plant and UASB effluent on day 103. The effluent concentration of sulfate dramatically decreased to about 170 mg/L as S. This was because that the concentration of thiosulfate and nitrate was much lower in the feed solution containing treatment plant wastewater than in the synthetic feed solution. During the first 10 days, the concentration of nitrate was unable to be measured because of the interference effect resulted from the high concentration of thiosulfate. After day 10, the concentration of nitrate was able to be measured. The nitrate concentration decreased gradually from about 24 mg/L as N to 8 mg/L as N. It was relatively stable around 10 mg/L as N within this period of time. After day 103, the concentration of nitrate dramatically decreased from about 12 mg/L as N to 2 mg/L as N. The nitrate concentration was stable after the feed was changed. The data points within
the section in between the orange and green lines represent the corresponding results. After day 153, the nitrate concentration started to increase to about 5 mg/L as N. This was due to the change of HRT from 48 hours to 36 hours.

Figure 11. Effluent nitrate and sulfate concentrations in the SGBR. Influent nitrate is indicated for the period that plant wastewater was fed to the SGBR.

Figure 12. Nitrate removal efficiency and effluent concentration in the SGBR. Influent nitrate is indicated for the period that plant wastewater was fed to the SGBR.
The removal efficiency of nitrate in the SGBR was quite stable. Prior to day 103, it was stable around 98% with relatively small variations. It dropped from 98% to 93% after day 102 because of the lower concentration of reduced sulfur species (i.e., hydrogen sulfide and supplemented thiosulfate) in the influent after the feed solution was changed from synthetic solution to a mixture of plant and UASB effluent. Likewise, the removal efficiency of nitrate stabilized around 93% within the period when feed solution was changed and HRT was shortened. After day 152, the removal efficiency of nitrate continued to decrease to the value lower than 90%, with a sudden change since day 153. Variations appeared since the day when HRT was shortened.

Table 3 shows the results from the MiSeq analysis for *Thiobacillus* and *Shinella* bacteria contained in feed biomass samples in the SBR. There was no *Thiobacillus* bacteria measured in the biomass from sulfide scrubber and anaerobic granules from the UASB. However, there were 0.21% of total operational taxonomic units (OTUs) *Thiobacillus* bacteria detected in the biomass from the sulfur oxidation basin. *Thiobacillus* therefore was possibly the dominant species in the plant biomass from the sulfur oxidation basin. During the experiment, there was 0.0096% of total OTUs *Thiobacillus* bacteria presented in the SBR biomass during the first month. After the first month, there was no *Thiobacillus* bacteria observed. *Shinella* bacteria, however, was not observed in any seed biomass. However, this bacteria appeared in the SBR biomass after the first month. It therefore indicated that there must have been trace amounts of *Shinella* bacteria contained in the feed biomass/granules. The reason why there were no *Shinella* bacteria detected in the feed biomass samples might be that the amount of the bacteria in each sample was extremely low and the MiSeq analyzer was not able to detect the low concentration.
Table 3. Thiobacillus and Shinella bacteria in the feed biomass samples.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Thiobacillus (% of total OTUs)</th>
<th>Shinella (% of total OTUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed biomass from sulfide scrubber</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feed granules from the UASB</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feed biomass from sulfur oxidization basin</td>
<td>0.21</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4 shows the prevalence of *Thiobacillus* and *Shinella* bacteria that were observed in SBR biomass samples collected at different times during the entire experiment. *Thiobacillus* bacteria appeared in the SBR biomass after operating for 30 days. However, *Thiobacillus* was not observed after the initial 30 days of the experiment. *Shinella*, on the contrary, appeared in all samples collected at different times during the experiment. The highest amount of *Shinella* detected occupied about 0.22% of total OTUs in the entire biomass while the lowest amount of this bacteria was determined to be 0.020%. Based on the results shown in Table 4, *Shinella* bacteria was the major species that contributed to the sulfur-based autotrophic denitrification process. Even though it did not appear in any plant biomass initially, there had to have been trace amounts of *Shinella* bacteria present in the seed biomass for it to appear in the SBR biomass. The environmental conditions in the SBR enriched the growth of *Shinella* bacteria.

Table 4. *Thiobacillus* and *Shinella* bacteria in the SBR biomass.

<table>
<thead>
<tr>
<th>Day</th>
<th>Thiobacillus (% of total OTUs)</th>
<th>Shinella (% of total OTUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>0.0096</td>
<td>0.13</td>
</tr>
<tr>
<td>49</td>
<td>0</td>
<td>0.049</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>127</td>
<td>0</td>
<td>0.020</td>
</tr>
<tr>
<td>133</td>
<td>0</td>
<td>0.11</td>
</tr>
</tbody>
</table>
The SRT values were around 17-19 days and the HRT values were kept constant for the SBR, as shown in Table 5. This range of SRT was sufficient to provide the necessary growth conditions for the autotrophic nitrifiers to flourish. While the system was not operated with intentional SRT control, it is apparent that the SRT was sufficient for the growth and development of the denitrifying biomass, in this case *Shinella* bacteria.

Table 5. HRT and SRT of the SBR.

<table>
<thead>
<tr>
<th>Day</th>
<th>Hydraulic retention time (day)</th>
<th>Solids retention time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>48</td>
<td>18.9</td>
</tr>
<tr>
<td>77</td>
<td>48</td>
<td>18.1</td>
</tr>
<tr>
<td>137</td>
<td>48</td>
<td>17.1</td>
</tr>
</tbody>
</table>

By having different concentration of sodium thiosulfate as substrate in the feed solution, several different initial reaction rates were determined and displayed in Figure 15.

Figure 13. Experimental results of the initial reaction rate at different concentration of substrate (sodium thiosulfate).
The initial reaction rate increased as the concentration of substrate increased. Specially, a clear positive slope of the curve was observed when concentration of substrate was increased from 4 mg/L to 6 mg/L. When concentration of substrate increased from 8 mg/L to 10 mg/L, the initial reaction rate was still increasing; however, the slope of the curve illustrated that there was a potential for the initial reaction rate to reach its maximum.

From Figure 16, the change of sulfate concentration in the supertanant of the effluent over time did not represent a typical curve for the rate of substrate utilization. After five times of dilution, the initial concentration of sulfate was 131.69 mg/L as N. Therefore, at time 0, sulfate concentration was about 154 mg/L instead of zero. Within 9.5 hours, sulfate concentration kept increasing. However, it stopped increasing after 9.5 hours and tended to become stabilized. One and half hour later, the sulfate concentration started to increase again.
for about 2 hours and then it became stabilized. This indicated that it was not feasible to model the generation of sulfate by only using a single equation such as the Monod equation. It was possible that the reaction was a two step process. If nitrite was not converted to nitrogen gas fast enough and it was theorized that nitrite accumulated during the batch test, then it would accumulate as an intermediate. Therefore, it was not possible to test on the kinetics of sulfate generation by using a one step method. Instead, we determined the concentration of nitrite in the supertanant of the effluent versus time and the same operating conditions was utilized for the continuous testing on the kinetics of the process.

Figure 17 illustrated that nitrate was converted to nitrite continuously and quickly within 7 hours operation. However, the generation of nitrite started to decrease after 8 hours. The nitrite concentration decreased to about 7.5 mg/L as N after 42 hours operation.

For further research, nitrite could be used as the substrate instead of nitrate to look at
the rate of nitrite conversion to nitrogen gas using the same operational condition. The concentration of nitrite and sulfate should be measured in order to calculate the rate of nitrite reduction and sulfate generation.

**Conclusion**

By utilizing the autotrophic denitrification process with optimal operating parameters, it was feasible to reduce nitrate to nitrogen gas and oxidize sulfide into sulfate in the wastewater from Cedar Rapids Water Pollution Control Facility. The pH remained between 7.5 and 8.0, and the alkalinity was stable at approximately 500 mg/L as CaCO₃. The lowest effluent nitrate concentration measured from the SBR was around 2.0 mg/L as N. The denitrification process in the SBR was also able to convert thiosulfate into sulfate; the effluent concentration of which was around 1,240 – 1,310 mg/L as S. The removal efficiency reached as high as 99% and stabilized between 95% and 99%. Using the seed biomass used to inoculate the SBR, the SGBR was able to decrease the concentration of nitrate to as low as 8 mg/L as N within 100 days. The corresponding removal efficiency of nitrate was around 98%. The concentration of sulfate in the SGBR effluent was gradually decreased from the beginning and became relatively stable around 1,100 mg/L as S within the same period of time. The pH for the SGBR was around 7.5 and the alkalinity was approximately 700 mg/L as CaCO₃. During the 5-month operation of SBR, MLSS decreased from 7,294 mg/L to 4,443 mg/L, and the MLVSS decreased from 3,550 mg/L to 3,067 mg/L. The dominant bacteria that facilitated the autotrophic denitrification process in the SBR was originally *Thiobacillus* and *Shinella*. Eventually *Thiobacillus* washed out of the system and *Shinella*
predominated. The seed biomass contained 0.21% of total OTUs *Thiobacillus* in the plant biomass from the sulfur oxidation basin. No *Thiobacillus* bacteria was found in the plant biomass from the sulfide scrubber and granules from the UASB. *Thiobacillus* was 0.0096% of the total OTUs of biomass in the SBR within the first month of operation; however, it did not appear in the SBR after this point. *Shinella* bacteria was not observed in any feed biomass. Nevertheless, it was detected in the SBR biomass samples during the experiment, with a percentage ranging from to 0.020 to 0.22 % of the total OTUs. Thus, it was concluded that *Shinella* was the primary species responsible for autotrophic denitrification in this study. It was not feasible to model the generation of sulfate by only using one single equation of a typical Monod model. The highest concentration of nitrite during the entire process reached to 21 mg/L as N after 8 hours operation and continuously decreased afterwards. Therefore, the reduction from nitrate to nitrite was faster than the conversion from nitrate to nitrogen gas during the batch study of autotrophic denitrification process.

**References**


CHAPTER 4. ENGINEERING SIGNIFICANCE

The autotrophic denitrification process is a feasible alternative to remove nutrients in wastewater, namely nitrate. By applying autotrophic denitrification process to a wastewater treatment facility, less sludge disposal is required, which results in more cost efficient operation. Moreover, compared to heterotrophic denitrification process that is commonly used, autotrophic denitrification does not require an organic carbon supply, which results in less material cost.

Regarding to the Cedar Rapids Water Pollution Control Facility, autotrophic denitrification process is able to solve the safety risks brought by the high level of hydrogen sulfide at the treatment plant. Accordingly, plant operators can work in a much safer occupational environment after lowering the level of hydrogen sulfide at the treatment plant. Besides, nitrate can be treated and mostly converted to nitrogen gas by using the autotrophic denitrification process. Nitrogen gas is harmless to the environment and therefore the potential risks to environmental contamination can be prevented. By determining the kinetics of the entire reaction, the basis for future facility planning can be provided and therefore improve the treatment plant efficiency for nutrient removal. A diverse group of autotrophic denitrifiers in the plant biomass contribute to the anaerobic treatment process and can be helpful in determining the future application to autotrophic denitrification.

While the SBR provided useful information on operating conditions, treatment results, and reaction kinetics, the SGRB was used in order to test the feasibility of treating the plant wastewater on a continous basis. The SGRB had the ability to retain solids for a longer time and retain active biomass active for a longer time. It differs from other reactors in its downflow configuration as well as the use of anaerobic granular sludge (Evans and Ellis,
According to these design features, solids and organic removals are highly efficient. Therefore, lower effluent solids and nitrate concentrations were able to be achieved. By switching from other type of reactor system to SGBR, it is possible for the plant wastewater effluent to contain less solids and to improve the overall quality of effluent before discharging.
CHAPTER 5. CONCLUSION

This experimental research was feasible to reduce nitrate into nitrogen gas and convert sulfide into sulfate in the industrial wastewater from Cedar Rapids Water Pollution Control Facility by using autotrophic denitrification process. By having a neutral pH and an alkalinity around 500 mg/L as CaCO₃, the denitrification process could be optimized. After running the experiment for several months, we were able to remove most of the nitrate and have the nitrate concentration as low as 2.0 mg/L as N in the SBR effluent. The corresponding removal efficiency of nitrate reached about 99% and became quite stable between 95 and 99%. In the meantime, thiosulfate was completely converted to sulfate. Approximately 1,240 – 1,310 mg/L as S of sulfate was generated during the entire denitrification process in the SBR.

By utilizing the same strategy in the SGBR, we were able to reduce nitrate to as low as 8 mg/L as N within 100 days. The corresponding removal efficiency of nitrate was around 98%. The sulfate concentration in the SGBR effluent gradually dropped from day 1 and finally became relatively stable at 1,100 mg/L as S within the same period of time. The optimal pH and alkalinity for the SGBR was about 7.5 and 700 mg/L as CaCO₃. While the SGBR showed a slightly higher optimal alkalinity during the autotrophic denitrification process, a neutral pH was significant to optimize the autotrophi denitrification process in both reactors.

Within the operational period of SBR, both MLSS and MLVSS decreased. MLSS dropped from 7,294 mg/L to 4,443 mg/L while MLVSS decreased from 3,550 mg/L to 3,067 mg/L.
Thiobacillus and Shinella bacteria was the dominant microbe that facilitated the autotrophic denitrification process in the SBR. There was 0.21% of total OTUs Thiobacillus bacteria presented in the plant biomass from the sulfur oxidation basin. No Thiobacillus bacteria was found in the plant granules from the UASB and the biomass from the sulfide scrubber. Shinella bacteria, however, was not detected in any of the plant biomass samples. Regarding the SBR biomass samples, there was 0.0096% of total OTUs Thiobacillus bacteria presented in the samples within the first month of operation. However, no more Thiobacillus bacteria was detected after this. Shinella bacteria was found in all SBR biomass sampled during the experiment. The highest amount of Shinella bacteria detected was 0.22% of total OTUs and the lowest amount of this bacteria was measured as 0.020% of total OTUs. Therefore, Shinella bacteria became the dominant species to facilitate the autotrophic denitrification process.

By conducting the kinetics test, the generation of sulfate could not be modeled by using one single equation of the Monod model. The highest concentration of nitrite during the entire process reached to 21 mg/L as N after 8 hours operation and continuously decreased afterwards. Therefore, the reduction from nitrate to nitrite was faster than the conversion from nitrate to nitrogen gas during the autotrophic denitrification process.
REFERENCES


