

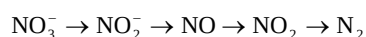
WASTEWATER DENITRIFICATION

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Abstract

Various are the methods for water purification from nitrates, but in significant part of them secondary products are produced. An exception is the biological denitrification in which nitrates are reduced to harmless nitrogen gas in accordance with the following reactionary scheme:



The potential of this method is significant.

Key words: *nitrate denitrification, denitrifying microorganisms*

1. INTRODUCTION

Water is essential for life since the mechanism of metabolism and synthesis are in close relation with the specific characteristics of water. Transport of nutrients inside cells and interactions with the environment without water are impossible. On the other hand, water resources are limited and only 2.66% of the total global water resources comprising groundwater, lakes and rivers, polar ice and glaciers are fresh water. Furthermore, only a small fraction of the fresh water, about 0.6%, is usable as drinking water. For this reason, water resources must be necessarily treated properly and wastewater treatment must be done efficiently (Ghafari et al., 2007, Shimali et al., 2001).

The fast movement of humans towards urbanization, industrialization and agricultural activities introduced various contaminants into the environment.

Nitrogen-containing compounds are examples of those contaminants which can create serious problems when released into the environment, such as eutrophication of rivers, deterioration of water quality and potential hazard to human or animal health.

Nitrate is identified as one of the hazardous contaminants in potable water that may reduce to nitrosamines in the stomach which is suspected to cause gastric cancer (Foglar et. al., 2005). In addition, nitrate exposes infants and pregnant women to danger due to the potential reduction of nitrate to nitrite inside the stomach of foetus after digesting nitrate by the mother. The nitrite reacts with the hemoglobin in blood and converts the hemoglobin into methaemoglobin, which does not carry oxygen to cell tissues. This phenomenon results in a bluish color of infant's skin so called methaemoglobinemia or the blue baby syndrome (Shimali et al., 2001).

Water ground and surface water are contaminated by nitrate in several ways.

Nitrate originated from agriculture is increasingly growing all over the world due to the extreme use of fertilizers. Nitrate salts reach the groundwater as they percolate through the soil. Some other sources of nitrate in ground and surface water are from uncontrolled land discharges of treated or raw wastewater from domestic and industrial wastes, landfills and animal wastes particularly from animal farms.

Various methods are for treatment of water from nitrates, but the majority of them were prepared secondary pollutants. An exception is the biological denitrification, at which nitrates are reduced to harmless nitrogen gas, and side waste products practically do not occur. High the potential of this method. Its flexibility makes it applicable to the treatment of wastewater from households, agriculture and industry nitrate at different concentrations. Restrictions on its wider practical application is the slow growth of microorganisms and their washing of bioreactors for continuous industrial processes.

2. EXHIBITION

2.1. Denitrification methods

Traditional methods for the removal of nitrates

The traditional processes of water treatment including coagulation, filtration, and disinfection (must be applied in water used for drinking). However, these processes do not effectively remove nitrate ion from the water. Therefore, to remove nitrate ion, a supplementary method is necessary. Nitrate removal from water or wastewater can be achieved using two main groups of treatment processes; physicochemical and biological treatment methods. The most common traditional treatment processes used to remove nitrate are reverse osmosis, ion exchange, electro-dialysis and activated carbon adsorption in conjunction with pH adjustment (Shimali et al., 2001, Feleke et al., 2002, Islam et al., 1998). These are traditional methods for the reduction of nitrate (Huang et al., 1998, Choe et al., 2000, Choe et al., 2004, Devlin et al., 2000, Hu et al., 2001, Huang et al., 2003).

Ion exchange is limited by several problems: poor selectivity of nitrates in the regeneration of the resin (Hoek et al., 1987).

There are some disadvantages with these traditional methods that limit their utility due to their expensive operation and subsequent disposal problem of the generated nitrate waste brine or by-products. For instance, ion exchange process removes nitrate and sulfate simultaneously from the water being treated. Subsequent produced wastewater from the resin regeneration process rich in these two anions requires a complementary method before its discharge (Shimali et al., 2001, Hoek et al., 1987). Reverse osmosis, although is able to separate and concentrate the compounds contained in water without any change in their molecular structures, this method is limited due to its high cost and producing concentrated waste brine that may pose a disposal problem for further treatment (Dahab et al., 1987-88, Shimali et al., 2001).

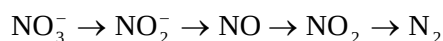
Generally, in the physical and chemical processes to yield a small volume of the waste flows, but with a high concentration of nitrate ions, which require subsequent removal.

Biological denitrification

Biological nitrogen removal in wastewater treatment plants is achieved through bacterially mediated oxidation of ammonia to nitrate and reduction of nitrate to nitrite, nitrous oxide, nitric oxide, or dinitrogen. Complete biological reduction of nitrate to dinitrogen is termed denitrification and is carried out by heterotrophic, facultative aerobic bacteria.

Biological denitrification seems the most promising process for nitrate removal. The potential of this method is significant. Compared with traditional methods fail to remove nitrates, but separate them into new more concentrated flows accompanied by-products, biological methods provide complete elimination of the pollutant by complete reduction of nitrate. For removing nitrate, although aerobic nitrification has been reported (Joo et al., 2005, Robertson et al., 1984, Zart et al., 1998, Chiu et al., 2007) most of the reported denitrification investigations are done by facultative anaerobes in the absence of oxygen (Rijn et al., 1996). Biological denitrification is a mechanism by which denitrifying bacteria use nitrate as terminal electron acceptor in their respiratory process in the absence of oxygen.

Complete reduction from nitrate to nitrogen gas (N₂) involves four consecutive steps that are catalyzed by different enzymes (Choe et al., 2004).



The enzymes are susceptible towards changes in pH, dissolved oxygen, carbon and energy source dose and the presence of inhibitors (Devlin et al., 2000), which may cause the accumulation of intermediates.

Denitrifying bacteria, in this method, reduce inorganic nitrogen compounds such as nitrite and nitrate into harmless elemental nitrogen gas (Prosnansky et al., 2002). Also, unlike some contaminants which are in need for a certain microbe to be treated, denitrifying bacteria are ubiquitous in nature and numerous researchers cultivated them using mix cultures taken from wastewater treatment plants as seeds (Gamble et al., 1977, Szekeres et al., 2001).

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facultative aerobic bacteria. Although denitrification under aerobic conditions has been reported, the rate of denitrification is maximized under anoxic (dissolved oxygen absent, nitrate present) conditions (Tiedje, 1988). Therefore, to achieve nitrogen removal in a single-sludge system, a mixed population of nitrifying and denitrifying bacteria must be exposed to alternating aerobic and anoxic conditions. To maximize the rate of nitrogen removal, the bacteria must be able to adapt quickly to these changes (Casasusa et al., 2005).

Moreover, microbial removal of nitrate may be the most economical strategy for the reclamation of nitrate polluted waters and wastewaters. As a result, there has been a huge interest towards microbial removal of nitrate as the most environmentally friendly and cost-effective method, although biological denitrification is usually slow and lasts long time particularly for industrial wastewaters containing high concentrations of nitrate (Islam et al., 1998). Later studies tried to speed up biological denitrification by applying new mechanisms through which better connection provided between the nitrate and the microorganisms in the water stream. The inorganic Contaminants Research Committee of USA (Islam et al., 1998) reported that different denitrification strategies, such as the use of granular activated carbon (GAC), packed beds, rotating biological contactors and soil-aquifer denitrification systems have been undertaken by various researchers. Also, efforts are still ongoing and some novelties in combination of biological and other abiotic methods, such as membrane biofilm reactors (MBR), were recently manifested (Terada et al., 2003, Ghafari et al., 2007).

The disadvantage of biological denitrification is the risk of bacterial contamination of the treated water. This risk is expected and disinfection is essential. A further drawback of biological processes is the low rate of growth of the microorganisms.

Denitrifying bacteria

Many bacteria are capable of growing anaerobically by reducing ionic nitrogenous oxides to gaseous products (Koike et al., 1975, Michalski et al., 1984, Painter et al., 1970, Herbert et al., 1982). This respiratory process, in which nitrates or nitrites serve as terminal electron acceptors instead of oxygen, results in generation of ATP (Bengston et al., 1989) and is termed denitrification or dissimilatory nitrate reduction (Ritter et al., 1988, Krul et al., 1976, Koerner et al., 1989, Roberson et al., 1988, Hochstein et al., 1988).

When electrons are transferred from the donor to the acceptor, the organism gains energy which can be applied for the synthesis of a new cell mass and the maintenance of the existing cell mass (Matějík et al., 1992).

The most detailed investigation into denitrification have involved a limited group of specialized bacteria. This has resulted in a view that denitrification can only occur under anaerobic conditions. However, it has been shown that denitrification can occur in the presence of oxygen in certain species too (Aida et al., 1986, Frunzke et al., 1986, Mateju et al., 1992, Hulse et al., 1988, Zumft et al., 1987).

Some of the bacteria are known for: *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Aquaspirillum*, *Azospirillum*, *Bacillus*, *Beggiatoa*, *Chromobacterium*, *Clostridium*, *Desulfovibrio*, *Erythrobacter*, *Gallionella*, *Halobacterium*, *Halomonas*, *Hyphomicrobium*, *Janthinobacterium*, *Neisseria*, *Paracoccus*, *Protonibacterium*, *Pseudomonas*, *Rhizobium*, *Rhodobacter*, *Thiobacillus*, *Thiosphaera*, *Xanthomonas*.

The enzymes associated with denitrification are synthesized when conditions become advantageous for denitrification (Coyne et al., 1989). Synthesis of denitrifying enzymes is typically a highly regulated process. It is generally held that the denitrifying enzymes are inducible. Their synthesis occurs under anaerobic conditions (Shapleigh et al., 1985), although denitrification can occur in the presence of oxygen (Zumft et al., 1987, Frunzke et al., 1986). In some cases enzyme induction may even require low concentration of oxygen (Hulse et al., 1988, Shapleigh et al., 1987). Significant levels of enzyme may be present as a consequence of anaerobiosis even in the absence of nitrate or other nitrogenous oxides (Bazylnski et al., 1985).

Reduction of nitrate to nitrogen gas proceeds in four steps. Each step is catalysed by enzyme system. Dissimilatory reduction of nitrate to nitrite is important to a number of bacteria, since the process involves energy conservation through generation of a proton motive force (Schauer et al., 1998). This step is catalyzed by membrane-bound nitrate reductases. The dissimilatory reduction of nitrite is carried out by two distinct nitrite reductases (Downes et al., 1988, Manoharan et al., 1989, Brauer et al., 1988).

One enzyme contains a Cu center and the other contains two hemes. Both seem to carry out the same physiological reaction (Murray et al., 1990). Nitric oxide is typically produced from nitrite, but under some conditions nitrous oxide is also produced (Shapleigh et al., 1985).

Nitrite reductases are membrane-bound as well as cytoplasmatic enzymes (Nishio et al., 1988). The reduction of nitric oxide is the least well-characterized of the enzymatic steps associated with denitrification. But there are

observations that support the notion that nitric oxide reductase is present in bacterial membranes (Rice et al., 1988). The fractions that exhibited nitric oxide reduction to nitrous oxide were isolated from membranes of *Pseudomonas halodenitrificans*. Shapleigh et al. examined the production of nitrous oxide from nitrite by membranes prepared from various bacterial strains. They also concluded that nitrite reduction to nitrous oxide proceeds via nitric oxide and is catalysed by two discrete enzymes, a nitrite reductase and a nitric oxide reductase. The last denitrification step, the reduction of nitrous oxide to dinitrogen, is catalysed by nitrous oxide reductase. This step is coupled to ATP formation (Christensen et al., 1990, Kim et al., 2004).

From detailed studies of the enzyme systems of some bacterial species it can be concluded that factors affecting induction and repression of these enzymes are not universal because denitrifying bacteria as a group are genetically diverse and metabolically versatile. The influence of oxygen concentration (Killingstad et al., 2002, Kim et al., 2002, Szekeres et al., 2002), pH (Gomez et al., 2003), temperature (Lemoine et al., 1988), electron donor, and nitrate and intermediate concentration (Hoek et al., 1988, Galvez et al., 2003, Chiu et al., 2003, Lewandowski et al., 1985, Hamsch et al., 1989, Boussid et al., 1989) on denitrification performance of some bacterial species has been investigated in detail. On the contrary, very little is known about regulatory interdependence of the reductase involved.

Bio-electrochemical denitrification

Especially progressive approach is a bio-electrochemical decomposition of nitrate ions. In biological denitrification occurring under action electric current can theoretically be accomplished *in situ* hydrogen production directly on the cathode surface. Using cathode produced hydrogen hydrogenotrophic microorganisms reduce nitrate in nitrogen gas (Zhang et al., 2005, Sakakibara et al., 1993).

A proper contact between microorganisms and hydrogen improves biological denitrification. BERs involving direct immobilization of denitrifiers on the surface of the cathode, where hydrogen is being produced, facilitates appropriate access of hydrogen to the microorganisms (Park et al., 2005). BERs provide immobilized microorganisms with a compulsory supply, rather than accidental contact, of electron donors which results in faster denitrification. This is in addition to the advantages of the hydrogenotrophic method and *in situ* hydrogen production by electrolysis of water. Bioelectro reactors may be fed by organic or inorganic carbon sources which serve heterotrophic and autotrophic hydrogenotrophs, respectively (Ghafari et al., 2007).

CONCLUSION

From the known methods for practical denitrification most appropriate and progressive are the biological, combined with the stimulating effect of constant electric field. They are characterized by high velocity and lack of secondary and waste products.

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