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# Development of nitrate elimination by autohydrogenotrophic bacteria in bio-electrochemical reactors – A review

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## Introduction

The inappropriate discharge of raw and treated wastewater containing excess inorganic nitrogen, such as ammonium, nitrate and nitrite into natural waters causes the excessive growth of algae that eventually promotes eutrophication of lakes and rivers and decreases the quality of drinking water resources [1]. Among the nitrogen components, nitrite and nitrate are widely known to be challenging contaminants in ground and surface water [2–11]. Nitrate and nitrite in drinking water threaten human health and other life forms. The uninterrupted consumption of water containing high levels of nitrate can be the source of certain diseases, such as methemoglobinemia in the foetus [8,12–14].

Groundwater is one of the main resources for the supply of drinking water, and nitrate/nitrite contamination in groundwater has been reported in numerous countries, such as India, China, Japan, the United Kingdom, United States of America, Saudi Arabia and some parts of Europe [8]. The high stability and solubility of nitrate ions enables them to percolate to groundwater, making their removal complicated [13], which explains why dramatically high concentrations of nitrate are seen in some cases. In order to protect consumers from the unpleasant effects of high nitrate ingestion, standards have been established by different organizations to limit nitrate/nitrite concentrations in drinking water. Among them, the World Health Organization (WHO) in 1985 declared that the acceptable concentration of nitrate was 45 mg $NO_3^-$ $L^{-1}$ [11,13–16].

The inefficiency of conventional water and wastewater treatment methods has led to a wide range of nitrate removal techniques in recent years. The efficiency of these new techniques has been compared to the objectives of controlling ecological problems by avoiding the inappropriate discharge of wastewater into the receiving water bodies and attaining the acceptable concentration of nitrate [17,18].

Nitrate removal techniques for water and wastewater are classified as biological processes, chemical processes, and physicochemical technologies, which involve many different methods [18]. Among them, some well-known methods applied for nitrate removal include ion exchange [13,19,20], reverse osmosis [13], electro-dialysis [21], catalytic [17,18], activated carbon adsorption [22], as well as microbial treatment processes [13]. The advantages and disadvantages for bio-electrochemical and alternative treatment methods are shown in Table 1. Physico-chemical methods are broadly used; however, these methods are associated with some drawbacks, such as low efficiency, waste brine disposal which implies a post treatment, high capital and operating costs, and generation of more toxic by-products through catalytic processes (i.e., $NO_2^-$ and $NH_4^+$) [17,18,21,23]. Researchers reported biological denitrification as the most favorable approach to treat nitrate contaminated water [17,24,25].

Biological processes are cost effective, have high potential for the elimination of nitrate, are relatively easy to control, and can show high stability and reliability [12,26,27]. The main weaknesses are that biological denitrification is generally a slow method, especially for industrial wastewaters, and may not be suitable for highly contaminated water and wastewater [12,28]. Researchers have attempted to speed up the biological treatment by finding ways to provide better contact between the nitrate and the microorganisms [12,29]. There has been focus on biofilm systems, which are favorable for denitrification because they are more compact. Fixed-bed reactors are more favored compared to suspended and fluidized bed reactors, due to simple operation, smaller reactor volumes, less sludge, greater stability, and capacity to handle shock loading [30]. Other methods namely packed beds, rotating biological contactors, soil-aquifer denitrification systems, granular activated carbon (GAC) beds, membrane biofilm techniques [22], and bioelectrochemical reactor (BER) [12,24], have also been studied. The bio-electrochemical reactor as an integrated system is considered as a new method. The advantages of electrochemical nitrate removal (high efficiency, no sludge production, use of a small reactor, and low investment costs) make this a promising method to integrate with biological processes to overcome some of the disadvantages of the biological process (slow process, organic residues), providing an alternative method to eliminate nitrate [31].

The term “bio-electrochemical reactor” commonly refers to the process where an electric current passes through a proper electrode system, which enhances the biological degradation of the pollutants, such as nitrate, due to the generation of appropriate energy source “$H_2$” and direct immobilization of denitrifiers on the surface of the electrode [24,32,33]. Apart from nitrate/nitrite treatment, BERs have been used for simultaneous removal of other pollutants, such as heavy metals and biological dechlorination [33–35], simultaneous removal of nitrate and pesticides [36], copper ion [32] or ammonium nitrogen [37,38].

In the past few years, several novel and cost-effective bioelectrochemical reactors have been developed, including different configurations such as biofilm electrode reactor, up flow bioelectrochemical reactor, three-dimensional (3D) electrode, and combined bio-electrochemical with other biological systems. Significant attempts have been made to improve designs to overcome certain shortcomings by using previous configurations, such as those that create a better and longer contact between the substrate and biomass, increase delivery of $H_2$ to biomass, adjust pH around optimum value, to make high rate nitrate removal possible. The effects of different environmental and operational parameters, such as temperature, dissolved oxygen, nitrate concentration, and salinity, on the denitrification process are clear. In addition, the pH, carbon source, hydraulic retention time (HRT), and current value (I), as important and efficient factors, were considered in different studies to determine the best and optimum range value of those parameters to attain economic and high rate elimination of nitrate.

This paper reviews recent developments of BERs for nitrate elimination including applied microbial processes, the applied structure and configurations, as well as the main and important factors concerning the actual performance of the reactor.

**Microbiology of denitrification process**

The conventional biological degradation of nitrogen components in water and wastewater comprise two main steps, namely, nitrification and denitrification. Within the nitrification process, microorganisms oxidize ammonia to nitrite and then to nitrate. Eqs. (1) and (2) explain the two steps of aerobic nitrification for ammonia accomplished by bacteria (e.g., Nitrosomonas and Nitrobacter) [9,47–49].

Ammoniaoxidizing bacteria

*Please refer to the full text*

Assimilation and dissimilation are two vital mechanisms of biological nitrate reduction. During assimilatory nitrate reduction (built-in proteins and nucleic acids), nitrate is transformed to nitrite and then to ammonium by plants and microorganisms. Dissimilatory nitrate reduction, known as denitrification, is referred to as the biological stepwise reduction of nitrate to nitrogen gas through nitrite, nitric oxide, and nitrous oxide as the intermediate components [50]. The subsequent reduction steps are carried out by denitrifying bacteria, which utilize nitrate and/or nitrite as electron acceptors under anoxic conditions, as shown below Eq. (3) [34,41,51].

*Please refer to the full text*

Denitrifiers are a ubiquitous group of bacteria because of their variety, and great metabolic diversity [52], and are generally found in soil, groundwater, surface water, and the sludge of wastewater treatment plants [53]. Denitrifiers are originally aerobic bacteria; however, they change to facultative anaerobes in the absence of free oxygen, when they use the oxygen in nitrate and/or nitrite ion as a primary oxygen source [50,54]. The two main sub-reactions that take place in the denitrification process are shown in Eqs. (4) and (5) [55].

*Please refer to the full text*

Denitrifying bacteria belong to several taxonomic and physiological groups of bacteria, such as organotrophs, lithotrophs, and phototrophs. Denitrifiers belong to the gram-negative alpha and beta classes of the Proteobacteria as the common group, such as Paracoccus, Alcaligenes, Pseudomonas, and Thiobacillus. Also the following genera: Bacillus, Spirillum, Hyphomicrobium, Agrobacterium, Acinetobacter, Propionobacterium, Rhizobium, Corynebacterium, Cytophaga, are responsible for denitrification. Furthermore, the most common genera are probably Pseudomonas (P. fluorescens, P. aeruginosa, P. denitrificans) and Alcaligenes, which are frequently found in soils, water, and wastewater [50,56–58]. Also, a few halophilic archaea, like Halobacterium, and Haloferax as well as gram positive bacteria like Bacillus and Jonesia are capable of denitrification [58]. Some species of denitrifying bacteria are able to carry out denitrification in the presence of oxygen (at dissolved oxygen concentrations up to 7 mg $L^{-1}$) [59,60]. In this situation, oxygen and nitrate are co-respired. Anammox bacteria are another type of microorganisms that oxidize ammonia as the electron donor to nitrite, and, subsequently, utilize generated nitrite as the electron acceptor, when oxygen and organic matter are not available in the environment [61]. Eq. (6) shows the general reaction that occurs in the Anammox process:

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