An electrochemically enhanced denitrification process using biofilms
by Vijay K Tripathi

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
Many sources of water, especially in areas of intensive agriculture, contain intolerable levels of nitrate concentrations. Applications of biofilms for denitrification purposes has been recommended in the waste - water and drinking water industries. Normally, denitrification refers to the biological process by which microorganisms oxidize a carbon source to CO2 while reducing NO3 to N2 in a 2-3 step biological process. Problems of environmental contamination due to the use of organic carbon sources lead to the suggestions to use H2 in denitrifying biofilm systems. Application of electrochemistry in conjunction with biofilm for denitrification is a relatively recent development. Microorganisms can use the electrolytically produced H2 to reduce NO3 to N2. The research presented in this thesis addresses the application of electrochemical principles to enhance the biological denitrification process. As hydroxide is produced in the denitrification process, there is an inevitable increase in pH. Biofilm activity at various pH was studied under the experimental conditions; biological activity was observed even at pH as high as 13, though loss in biological activity was observed at pH 12 and above. The possibility of a direct electrochemical reduction of nitrate along with the biological denitrification was explored and verified to gain a valuable insight into the mechanism of this process. Microsensors for specific ions (NO3' NH4+, pH) and H2 were developed and used for the measurement of the respective species in the biofilm. Results show that apart from the direct electrochemical reduction of nitrate, a fairly large portion of electric current was used for electrolysis of water to produce H2 which was utilized by the biofilm. The measured net denitrification rates are in qualitative agreement with the mathematical model of biological denitrification available in the literature. A descriptive model based on the hypothesis of a direct electrochemical reduction of nitrate is proposed. Technical feasibility of electrochemically enhanced biological reduction of nitrate is experimentally verified and demonstrated.

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APPROVAL

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Vijay K Tripathi

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date April 14, 1997

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Many sources of water, especially in areas of intensive agriculture, contain intolerable levels of nitrate concentrations. Applications of biofilms for denitrification purposes has been recommended in the waste-water and drinking water industries. Normally, denitrification refers to the biological process by which microorganisms oxidize a carbon source to CO_2 while reducing NO_3^- to N_2 in a 2-3 step biological process. Problems of environmental contamination due to the use of organic carbon sources lead to the suggestions to use H_2 in denitrifying biofilm systems. Application of electrochemistry in conjunction with biofilm for denitrification is a relatively recent development. Microorganisms can use the electrolytically produced H_2 to reduce NO_3^- to N_2. The research presented in this thesis addresses the application of electrochemical principles to enhance the biological denitrification process. As hydroxide is produced in the denitrification process, there is an inevitable increase in pH. Biofilm activity at various pH was studied under the experimental conditions; biological activity was observed even at pH as high as 13, though loss in biological activity was observed at pH 12 and above. The possibility of a direct electrochemical reduction of nitrate along with the biological denitrification was explored and verified to gain a valuable insight into the mechanism of this process. Microsensors for specific ions (NO_3^-, NH_4^+, pH) and H_2 were developed and used for the measurement of the respective species in the biofilm. Results show that apart from the direct electrochemical reduction of nitrate, a fairly large portion of electric current was used for electrolysis of water to produce H_2 which was utilized by the biofilm. The measured net denitrification rates are in qualitative agreement with the mathematical model of biological denitrification available in the literature. A descriptive model based on the hypothesis of a direct electrochemical reduction of nitrate is proposed. Technical feasibility of electrochemically enhanced biological reduction of nitrate is experimentally verified and demonstrated.

CHAPTER 1

INTRODUCTION

Denitrification refers to the biological process by which microorganisms oxidize a carbon source to CO_2 while reducing NO_3^- to N_2 in a 2-3 step biological process. The problems of environmental contamination for a denitrification process in which carbon sources such as ethanol or acetate are added to water are undesirable. It is therefore of interest to seek a denitrification process that does not require addition of a carbon source. A solution to this problem is the use of hydrogen gas as an electron source for the denitrification process.

Denitrifying bacteria are biochemically and taxonomically very diverse. Heterotrophs and autotrophs, e.g., paracoccus denitrificans and micrococcus denitrificans, respectively, can reduce nitrate to nitrogen gas through the use of hydrogen gas as an electron donor.

The reaction proceeds according to:

I $$2NO_3^- + 2H_2 \rightarrow 2NO_2^- + 2H_2O \quad (1.1)$$

II $$2NO_2^- + 3H_2 \rightarrow N_2 + 2H_2O + 2OH^- \quad (1.2)$$

Step I requires 0.14 mg H_2/mg NO_3^--N and involves no pH shift. Step II requires one additional mole of H_2 per mole of N or 0.21 mg H_2/mg NO_2^--N. A pH increase results from step II. Various gaseous oxides of nitrogen are intermediates of step II. The overall reaction is:

$$\text{III} \qquad 2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^- \qquad (1.3)$$

The overall requirement is 0.35 mg H_2/mg NO_3^--N for complete reduction. One mole of OH^- is released per mole of NO_3^- reduced [27].

The fact that H_2 is inherently clean and only very slightly soluble in water (1.6 mg/l at 20°C) makes it an excellent reactant for a biological process to denitrify drinking water. The absence of organic carbon requires organisms that can assimilate dissolved carbon dioxide as their carbon source from the water. Normally, autotrophic organisms are relatively slow growing, which suggests a reactor with biofilm retention.

The effectiveness of electrochemical bioreactors to reduce nitrate has been experimentally demonstrated using both an immobilized-enzyme reactor [33] and a denitrifying biofilm reactor [43]. The overall reaction is:

$$2NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O \qquad (1.4)$$

If hydrogen gas is provided by the electrolysis of water,

$$H_2O + e^- \rightarrow \frac{1}{2} H_2 + OH^- \quad (1.5)$$

and then rapidly utilized by a microorganism, nitrate can be reduced to nitrogen gas.

A denitrification process which is driven and controlled by an electric current was demonstrated by Sakakibara and Kuroda [43] and a mathematical model based on this hypothesis was proposed by Sakakibara et al. [42]. In their system, a denitrifying biofilm was attached to a carbon electrode and the hydrogen gas produced by the electrolysis of water was utilized for the reduction of nitrate to nitrogen (see Figure 1.1). Complete denitrification was achieved in the presence of an electric current. The denitrification rate was found to be linear function of current over a range of current density. The reaction at the cathode surface consumes hydrogen ions and produces hydroxyl ions. Since microorganisms are pH-sensitive, electrochemical reactions that produce hydrogen gas may result in an undesirable increase in pH within the biofilm. Overall, the electric current must be controlled to optimize the mass transfer rates, electrochemical reaction rates and biological reaction rates. From the available literature, it appears that a biofilm reactor removes nitrate more efficiently than any other process when an electric current (electrochemical reaction) is used to produce hydrogen necessary for the reduction of nitrate [42, 43].

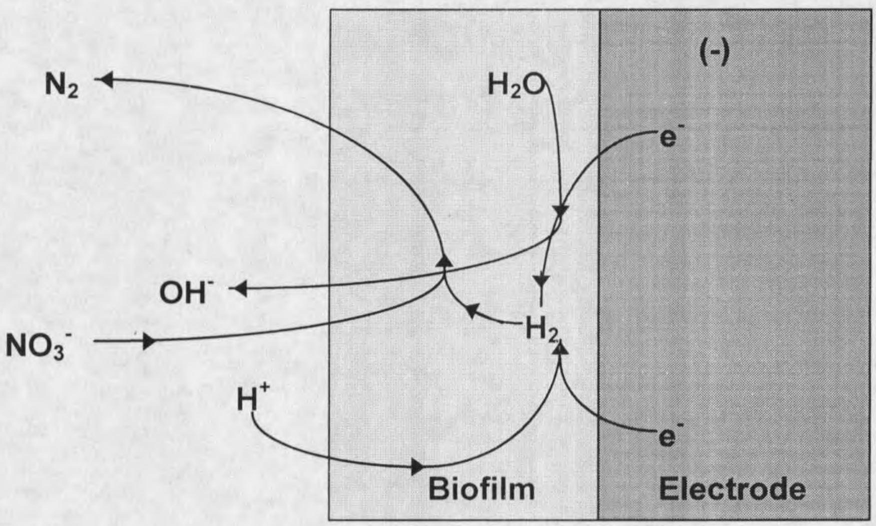


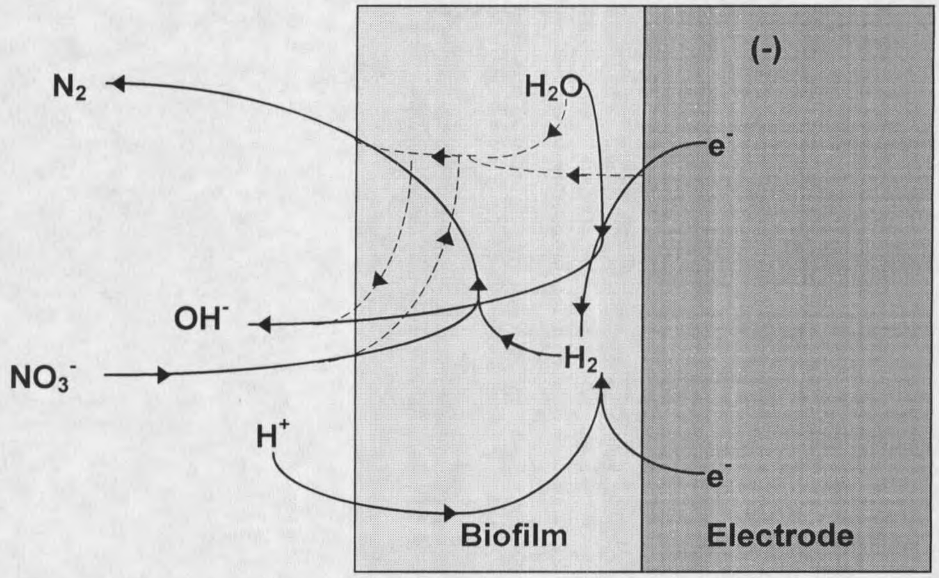
Figure 1.1 A schematic of the mathematical model proposed by Sakakibara et al. [42].

Though hydrogen is an obvious electron donor in a denitrification process, the possibility of a parallel direct electron transfer process can not be over-looked. Nitrate can be reduced electrochemically by the direct electron transfer due to the passage of electric current. This direct electrochemical reduction of nitrate during the process may influence the mechanism and hence, the kinetics of the denitrifying process. Part of the electrons supplied through the electric current may contribute to the direct electrochemical reduction and the remaining part for producing hydrogen gas through electrolysis. Only a limited part of the hydrogen gas, which is soluble in the solution, can be consumed by the bacteria as an electron source for the reduction of nitrate. Nitrate can be reduced electrochemically through a direct electron transfer process given by:

$$NO_3^- + 3H_2O + 5e^- \rightarrow \frac{1}{2} N_2 + 6OH^- \quad (1.6)$$

This possibility of a direct electron transfer for the reduction of nitrate was the hypothesis of this research work. This hypothesis along with the biological reduction of nitrate, is depicted in Figure 1.2 as a complete pathway model for the electrochemically enhanced denitrification process.

It is normal to implicitly assume that the pH in the interior of biofilms or flocs in biological waste water treatment plants is about the same as in the bulk liquid. This is however not always the case. Riemer and Harremoes [34] showed theoretically that the alkalinity production can create a pH inside the biofilm up to 12. Accordingly a difference of two to four pH units between the interior of the biofilm and the bulk liquid may result, particularly in case of low alkalinity waters. The pH-phenomenon is important in several respects because the pH has a significant role in, for example, precipitation processes and strongly influences biological reactions, including inhibition.



—— Biological pathway as suggested by Sakakibara et al. [42]

--------- Electrochemical route for reduction of nitrate.

Figure 1.2 Schematic of a complete pathway model of an electrochemically enhanced denitrification process.

A pH-effect may occur in the denitrifying biofilms caused by the production of OH^- as a result of electrolysis. This pH rise is not desirable. An appropriate buffer in enough quantities should be added to nullify the pH rise. The pH-profile inside a growing denitrifying biofilm should be measured and would help in better understanding of the nature of the denitrifying biofilm and the processes involved.

In pure culture, as well as in natural systems, the denitrification rate is related to pH, with an optimum in the range of 7.0 to 8.0. Denitrification has been shown to occur in wastes up to pH 11 [41]. Due to the pH gradient between the biofilm and the bulk, a delayed pH increase can be expected in the bulk too.

The research work presented in this thesis is an attempt towards the better understanding of the denitrification process under the influence of electric current. The question regarding the nature of an enhancement in the denitrification rate due to the passage of electric current is addressed. What portion of this enhancement is due to the direct electrochemical reduction (if at all), and how this influences the mechanism and the path of the denitrification reaction were objectives. Based on the results a conceptual complete pathway model for the electrochemically enhanced denitrification process is proposed. This model focuses on the mechanism of electrochemical electron transfer reaction for the reduction of nitrate along with the biological denitrification resulting from the donation of electrons by hydrogen. Finally, suggestions are made for future studies in this field to refine the knowledge of this process and understand the intricacies of the mechanism involved, which may have applications in the modification of industrial practices for denitrification.

CHAPTER 2

THEORY

Study of denitrification process in presence of electric current requires the knowledge of certain areas of microbiology, biofilms, electrochemistry, and related technologies. This chapter mainly describes the physiology and microbiology of the denitrifying biofilms, principles of electrochemistry, and construction of ion-selective microsensors.

DENITRIFICATION

Introduction to Denitrification process

Nitrate is one of the major ions in natural waters. The nitrate in underground and surface water comes from a variety of sources, many of which are dependent to some extent on biological activity and so vary between regions and time. The main nitrate sources are rainfall, sewage or animal excreta, biological fixation in the soil and various agricultural practices. Nitrate is the end product of the microbial break-down of the nitrogenous compounds in the excreta. It is also released from plant material by microbial action, and so an apparently innocuous process, such as ploughing a field of pasture, can have major consequences for the nitrate content. The public health standard for nitrate in drinking water is set at 10 mg/l NO_3^--N (0.7 mM) due to its toxicity and health effects. When necessity arises denitrification offers a relatively inexpensive, natural method for removing unwanted nitrate. Normally, denitrification refers to the biological process by which microorganisms oxidize a carbon source to carbon dioxide, reducing nitrate to nitrogen.

Denitrification refers to the dissimilatory reduction, by essentially aerobic bacteria, of one or both of the ionic nitrogen oxides (nitrate and nitrite) to the gaseous oxides (nitric oxide and nitrous oxide), which may themselves be further reduced to nitrogen. Nitrogen oxides act as terminal electron acceptors in the absence of oxygen. The gaseous nitrogen species are the major products of these reductive processes. A dissimilatory reduction of NO_3^- and NO_2^- may occur, however, in which the major product is ammonia (NH_4^+). Such reactions occur in many of the enterobacteriaceae, bacilli, and clostridia and are reported in soils and marine sediments under very anaerobic conditions. At least some of these dissimilatory reductions to NH_4^+ can yield small amounts of N_2O as a minor product. However these reactions are not denitrification in the strict sense [25].