論文の内容の要旨

Abstract of Dissertation

DEVELOPMENT OF A MEMBRANE BIOFILM REACTOR FOR DENITRIFICATION WITH METHANE

(メタンガスを利用したメンブレンバイオフィルムリアクターによる脱窒)

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ABSTRACT

Nitrate removal from domestic, industrial, and agricultural wastewater is necessary to prevent discharges that contribute to eutrophication of water bodies in the environment. Moreover, overuse of agricultural fertilizers has in many areas around the world resulted in nitrate-contaminated groundwater, which requires treatment before it can be consumed. Thus, there is a need for treatment methods of nitrate-contaminated waters and wastewaters. Nitrate removal from wastewater is typically accomplished by biological denitrification. The denitrification process requires an electron donor and often an external compound must be added. Methane would potentially have several advantages as electron donor for denitrification. It is inexpensive, widely available, non-toxic, and being a gas it is unlikely to remain in solution and contaminate the effluent. Moreover, methane is generated onsite at wastewater treatment plants and on landfills, two places where the methane denitrification process would be particularly useful.

As of yet, no methanotrophic microorganism is known to denitrify. Instead, methane denitrification is accomplished by aerobic methanotrophs oxidizing methane and releasing soluble organics used as electron donors by coexisting denitrifiers. Thus, both oxygen and methane are required in the process. A successful bioreactor setup should not only provide conditions for efficient denitrification, but also avoid hazardous mixtures of methane and oxygen and avoid losses of methane. A bioreactor configuration that potentially meets these challenges is the membrane biofilm reactor (MBfR). In an MBfR, a gaseous substrate (in this case methane) is supplied from the interior of a membrane to a biofilm growing on the membrane surface.

The **objectives of this work** were to (1) develop a membrane biofilm reactor (MBfR) for denitrification with methane, (2) clarify the microbial processes responsible for methane denitrification with particular focus on an MBfR biofilm, and (3) evaluate potential applications for the methane denitrification process.

Experiments were carried out with a microbial culture enriched from activated sludge (A^2O system). The culture was enriched on nitrate mineral salts medium with methane as the sole source of carbon. Denitrification ability was evaluated by measurements of nitrate and nitrite in the liquid phase, mass balances on nitrogen, and the detection of nitrous oxide in the reactor headspace.

The **first objective** of this work was addressed by the development and evaluation of three MBfR configurations with different feeding regimes for methane and oxygen. The first configuration (A) provided both methane and oxygen from the interior of the membrane, which theoretically would lead to the most favorable biofilm development for methane denitrification, with an aerobic methanotrophic biofilm layer forming near the membrane surface and an anoxic denitrifying biofilm layer near the bulk liquid. This configuration, however, require the use of potentially flammable mixtures of methane and oxygen. To avoid such gas mixtures, a second MBfR configuration (B) with oxygen and methane supplied through separate intertwined hollow-fiber membranes was developed. A third MBfR configuration (C) with only methane provided from the membrane and oxygen supplied with the bulk liquid was also evaluated.

MBfRs were constructed using various types of membranes, including a composite hollow-fiber membrane, a microporous membrane, and silicone rubber. Most experiments were conducted with silicone rubber

membranes because they provided a slow, controllable supply of gases through the dense silicone polymer and were the easiest to modify for various laboratory setups.

The most notable feature of the MBfRs was that they achieved nitrate removal with a very high efficiency compared to suspended growth reactors, i.e. the ratio between nitrate removal and methane consumption rates was high. Suspended culture experiment showed that under aerobic conditions, most of the nitrate removal could be attributed assimilation, with a nitrate removal efficiency of approximately 0.079 mol NO_3^- mol^{-1} CH_4. The MBfR experiments, however, frequently achieved nitrate removal efficiencies between 0.33 and 0.63 mol NO_3^- mol^{-1} CH_4. The MBfRs had higher nitrate removal efficiency than suspended growth reactors irrespective of the gas feeding regime (A, B, or C). Similar nitrate removal rates were obtained with varying MBfR configurations, typically ranging from 0.3 to 2.9 g N m^{-2} d^{-1}.
Microsensor measurements of dissolved oxygen concentrations within the biofilms showed that large anoxic portions existed, which may be an explanation for the high nitrate removal efficiencies in the MBfR biofilms compared to suspended growth reactor. Anoxic biofilm segments developed both when oxygen was supplied from the membrane interior (configuration A) and when it was supplied with the bulk liquid (configuration C); although in the former case the DO concentration decreased with distance from the membrane whereas in the latter case it increased.

Though the nitrate removal performance of the various MBfR configurations was similar, some differences could be observed. Configuration A, with methane and oxygen mixed in the membrane lumen, usually gave stable nitrate removal rates. However, it was somewhat sensitive to the ratio between intramembrane oxygen and methane partial pressures. A high oxygen pressure inhibited nitrate removal whereas a low oxygen pressure allowed methane to escape the reactor unutilized. Configuration A also poses a safety concern since methane and oxygen together may form flammable gas mixtures. Intertwined MBfRs (configuration B) always failed to achieve biofilm distributed over both membrane fibers. Instead, biofilm started growing mainly on either the oxygen-permeating or methane-permeating fibers, resulting in an MBfR essentially similar to C, where one substrate is supplied from the membrane and the other is taken up from the bulk liquid. Configuration C, with oxygen supplied from bulk liquid, achieved nitrate removal rates and efficiencies similar to A, though declining rates were sometimes observed, possibly caused by increased biofilm thickness leading to a limitation of oxygen-transport from the bulk liquid to the active methanotrophic zone of the biofilm. Moreover, configuration C MBfRs typically had more non-membrane-attached biomass (loose or attached to tubing) in the reactor, and slightly higher dissolved organics concentrations in the effluent. Despite these disadvantages, C is the recommended MBfR configuration for methane denitrification. It avoids methane and oxygen gas mixtures, is the simplest to set up and operate, and based on the experimental results it typically achieves nitrate removal rates and efficiencies similar to, the philosophically more refined, configuration A.

The **second objective** of this work was addressed using two strategies, microbial community analysis and mathematical modeling.

Microbial community analysis of the enrichment culture used in the study revealed presence of both type I and II aerobic methanotrophs, as well as other microbes. Type I methanotrophs dominated the culture, particularly during attached-growth. Denitrifiers able to use methanol, acetate, citrate, and glucose were isolated, providing support for the hypothesis that an association of aerobic methanotrophs and facultative heterotrophs is responsible for the methane denitrification process; however, their abundance and role in the mixed culture was not determined.

Microscopic observation of biofilm slices and cross-sections from MBfRs operated under conditions A and C revealed a difference in biofilm structure. In both biofilms, methanotrophs were concentrated near the membrane surface, which provided their source of methane. However, with both methane and oxygen supplied from the membrane (A), large, irregular colonies of methanotrophs grew near the membrane, whereas with oxygen supplied from the bulk liquid (C), a dense, smooth methanotrophic biofilm layer formed near the membrane surface. This difference may have been caused by the methanotrophic growth

rates in the respective biofilms. The DO concentration profiles suggested that the oxygen consumption rate in the configuration A biofilm was significantly higher than that in the C biofilm.

To further elucidate the impact of gas flow regime on biofilm development and MBfR performance, a mechanistic mathematical model, simulating the microbial reactions in a growing membrane-attached methane-denitrifying biofilm, was developed. The modeled space, (1- or 2-dimensional), is divided into grid elements, each containing values for the concentrations of biomass and substrate compounds. The grid element values are updated in discrete time intervals to simulate the growth of the biofilm. The biomass is redistributed throughout the grid using a cellular automaton algorithm, whereas the concentrations of the substrate compounds are updated using numerical solutions to the diffusion-reaction mass balance equations. Two versions of the model, with different assumptions regarding the microbial community structure, were developed. Version 1, the relatively simplistic alternative, considered two types of biomass, active and inert; and three substrate compounds, methane, oxygen, and nitrate. The active biomass was assumed to require methane and oxygen for growth, but capable on endogenous denitrification, i.e. it could reduce nitrate by feeding on itself under anoxic conditions. This view of the microbial culture was supported by a suspended growth experiment in which the culture grew under aerobic conditions reducing nitrate mainly for assimilatory purposes, but continued to reduce nitrate under anoxic decay conditions. Version 2 of the model considered 4 types of biomass, methanotrophs, facultative methanol-utilizers, facultative heterotrophs, and inert; and 5 types of substrate compounds, also including biologically-produced methanol and organics. This view of the microbial community structure was supported by the in-situ detection of aerobic methanotrophs and the isolation of denitrifiers able to use methanol and other organic compounds from the mixed culture. With configuration A, the simulated biofilm showed a high methanotrophic activity near the membrane surface and, depending on the oxygen supply and detachment rate, a large anoxic zone tended to develop in the outer portion of the biofilm. With configuration C, the simulated biofilm's methanotrophic activity tended to be limited by diffusion of methane from the membrane and oxygen from the bulk liquid. Version 2 of the model suggested that methanol-utilizers grow very close to the methanotrophic zone of the biofilm since they are dependent on methanol released by active methanotrophs, whereas facultative heterotrophs can grow and utilize microbial lysis products in anoxic biofilm zones. Simulations in 2D again revealed the difficulties in achieving complete biofilm coverage for the intertwined MBfR (configuration B). Biofilm grows in the interfaces between methane and oxygen permeating fiber, but the growth rate is low compared to configurations A and C due to low substrate availability. However, the model predicted that a configuration consisting of a thin methane-permeating membrane fiber placed between two oxygen-permeating fibers could provide conditions for complete biofilm coverage and prevent methane losses, though such a configuration was not tested experimentally.

The **third objective** of this work concerned potential applications of the methane denitrification process, which may include treatment of domestic, industrial, and agricultural wastewater, landfill leachate, and contaminated groundwater. The high nitrate removal efficiency achieved with the MBfR setups makes methane an economically favorable option compared to methanol, another electron donor for biological denitrification. Regarding groundwater contamination, pesticides often coexist with nitrate as both can originate from agricultural sources; for that reason, simultaneous nitrate and pesticide removal was also investigated in this study. The enrichment culture was able to some extent remove three out of four tested pesticides (aldicarb, alachlor, and malathion; but not atrazine) both when it was grown in suspension and as a membrane-attached biofilm. It should be noted that post-treatment would be required if methane-fed MBfRs were to be utilized for water treatment. The biofilm produces low levels of dissolved organics and detached biomass can be found in the effluent water. An alternative would be to use methane injection for in-situ treatment of nitrate contaminated groundwater, a technique already examined for methanotrophic degradation of trichloroethylene.

A perhaps more attractive application of methane-fed MBfRs would be to use in-situ produced methane for denitrification of wastewater or landfill leachate. Calculations using typical values for methane production and nitrate loads at wastewater treatment plants and landfills suggested that methane produced at wastewater treatment plants may be insufficient for complete denitrification, though it may significantly alleviate the need for an external electron donor. Landfills, on the other hand, likely provide sufficient

methane quantities for complete denitrification of leachate. Furthermore, aerobic methanotrophs are well-known to cometabolically degrade halogenated aliphatics, pollutants that may be present in landfill leachate and groundwater. Thus, simultaneous removal of nitrate and such pollutants is a concept that potentially makes the methane-fed MBfR an even more attractive treatment option.