Chapter I

GENERAL INTRODUCTION

Nitrogen Removal from Wastewater by Biofilm Method and Research of Condition in a Biofilm

1.1 INTRODUCTION

Diverse microorganisms form complex microbial communities and commonly attach to solid surfaces as biofilms in natural environments. In engineered systems such as wastewater treatment systems, various types of microorganisms usually exist as biofilms on supporting materials, flocs in an activated sludge and granules. However, the conventional approach for the investigation of microbial ecology in biofilms, which does not allow exact determination of the localization of specific bacterial cells, has not contributed to the understanding of actual microbial ecology. Therefore, a bioreactor for a wastewater treatment system has to date been regarded as a 'black-box', although wastewater treatment systems are the most common bioengineering processes.

The recent use of new approaches, such as the application of molecular techniques, has enabled in situ monitoring of microbial biofilm communities. The monitoring involves the identification phylogenetic affiliations, determination of spatial distribution, elucidating functions and activities and establishing coordination in microbial biofilm communities. The next challenge in this field is to elucidate the mechanism of treatment activities based on microbial ecology and applying this to the development of a new wastewater treatment system or the improvement of process performance in terms of treatment activity and process stability.

This paper introduces the development of and advances in new methods for the investigation of the spatial organization of biofilm communities and describes application to the analysis of biofilm communities in engineered systems using nitrifying biofilms in wastewater treatment processes as an example. The major purpose of this paper is to present and discuss the effectiveness and limitations of the use of these techniques for advancing environmental biotechnology processes such as wastewater treatment systems based on the understanding of the spatial organization and the activity of biofilm communities.

1.2 BACKGROUNDS

1.2.1 Nitrogen Removal

Nitrogenous pollutants from domestic and industrial wastewaters are responsible for promoting the eutrophication effect in ponds and lakes [1]. Thus, the removal of nitrogen compounds from wastewater is of increasing importance. Biological nitrogen removal involves two successive processes, i.e., nitrification and denitrification. Nitrification transforms ammonia to a more oxidized nitrogen compound such as nitrite or nitrate which is then converted to nitrogen gas in the subsequent denitrification process [2]. These two processes are usually carried out in different reactors because nitrification occurs under an aerobic condition while denitrification prevails in the absence of oxygen [3]. However, the two processes are complementary in many ways, i.e., 1) nitrification produces nitrite or nitrate which is a reactant in denitrification, 2) nitrification reduces the pH that is raised in denitrification, and 3) denitrification generates the alkalinity that is required in nitrification [4]. Therefore, there exist obvious advantages to performing simultaneous nitrification and denitrification in a single reactor.

Nitrification:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

Denitrification:

$$2NO_3^- + 2H^+ \rightarrow 2NO_2^- + 2OH^- \quad (3)$$

$$2NO_2^- + 4H^+ \rightarrow N_2 \uparrow + 2H_2O + 2OH^- \quad (4)$$

1.2.2 Biofilm method

There are two advantages of biological wastewater treatment using a fluidized bed reactor with a biofilm fixed on particles: 1) the reactor has active transfer phenomena accelerated by fluidizing materials, and 2) the reactor retains 5-10 times higher biomass concentration than conventional activated sludge reactors [5, 6]. This technique had been successfully applied to the removal of nutrients and harmful compounds from domestic and industrial wastewater [7-11]. In wastewater treatment using a biofilm, bacteria inside the biofilm degrade substrates that have diffused into the biofilm. In this process, a shortage of oxygen for biological oxidation is frequently observed because nominal oxygen is soluble in water. Therefore, the spatial distribution of oxygen in biofilms is very important for the operation of reactors.

1.2.3 Analysis inside biofilm

Currently, new methods of evaluating the physical and microbial properties of a biofilm are being developed, using a needle-type microelectrode with a tip diameter of 3-20 micrometers to measure the substrate concentration distribution in a biofilm [12]. Liquid ion-selective (LIX) microelectrodes can be applied to measure pH and the concentrations of ammonium, nitrite and nitrate ions [13]. Moreover, a fluorescent in situ hybridization (FISH) method is highly effective for analyzing complex microbial communities in a biofilm, because specific bacterial cells are detected using 16S-rRNA-targeted oligonucleotide probes labeled with a fluorescent compound [14-16]. A hybrid analysis of using with microelectrodes and the FISH method clarifies the microenvironment inside a biofilm in a rotated-disk type reactor and a fluidized bed reactor, which is important in the design of a biofilm reactor system [17, 18].

The internal physical features of biofilms and microbial distribution in biofilms have been clarified [19, 20]. By physical analysis, the structures of biofilms and substrate distributions in biofilms were measured using a confocal laser scanning microscope (CLSM) and microelectrodes [21, 22]. By microbial analysis, bacterial species and their distributions inside biofilms were determined using molecular biology techniques such as fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) analysis followed by denaturing gradient gel electrophoresis (DGGE) [23, 24]. Moreover, biofilm characteristics were mathematically modeled taking into account of complex phenomena occurring in biofilms [25]. These extensive research studies clarified the microbial ecology inside biofilms. In particular, biofilms have distinct microbial distributions of heterotrophic bacteria and autotrophic nitrifying bacteria [26, 27]. These distributions are caused by the competition between both bacterial populations for oxygen uptake [28]. Therefore, the oxygen distribution in biofilms should be elucidated.

The internal features of biofilms differ greatly with age, thickness, density, porosity and tortuousity. In particular, the substrate and microbial distributions in biofilms change with biofilm thickness. These distributions are closely related to mass transfer phenomena [29]. In fact, the diffusion coefficient of biofilms, which is lower than that of water, is influenced by their density, porosity, pore size, convection, type of extracellular polymeric substances (EPSs) and minerals [30].

1.2.4 Biofilm modeling

Nutrient removal from wastewater is necessary to prevent eutrophication of the receiving waters. Fluidized bed bioreactors have been found to remove nutrients and other pollutants both from domestic and industrial wastewaters. Most of the previous works on biofilm process assumed that the reaction follows Monod-type, first order or zero order equation. Even though the simplest rate equation was employed, biofilm models are still relatively

complex because the microbial conversion of substrate is coupled with the diffusive transport of soluble substrates inside the biofilm. Thus, the substrate concentration varies not only with time but also with location within the biofilm. Biomass balances in bulk solution and inside the biofilm result in coupled simultaneous partial differential equations that are mathematically complex even for steady state single-substrate single-microorganism biofilm system. The difficulty is further augmented in dynamic systems. Early biofilm modeling approaches simplified the process by assuming that the process is at steady state, the biofilm thickness is constant and the microbial distribution within the biofilm is predetermined [31-35]. Microbial species distribution both in time and space was considered in the models of Kissel *et al.* [36] and Wanner and Gujer [37]. These models made the prediction of microbial species distribution as a function of substrate flux possible. The model of Rittmann and Manem [38] predicts species distribution within the steady state multispecies biofilm. The heterogeneous structure of the biofilm was considered in the researches of Lewandowski *et al.* [39], Zhang and Bishop [40], and Bishop [41]. Quantitative analysis of biofilm heterogeneity was performed in the mixed culture biofilm model of Wanner and Reichert [42]. Lewandowski *et al.* [43] defined textural entropy, areal porosity, fractal dimension and maximum diffusion distance as the quantitative parameters for describing the structure of a biofilm. With the current trend in biofilm modeling, the model continuously becomes complex as attempts are made to make it more realistic. However, as pointed by Holmberg and Ranta [44] modeling must be a compromise between making the model extensive enough to be realistic and reducing the number of parameters to a level at which they can be estimated from available data.

Methods to obtain the solution to these models were developed. The effectiveness factor method of Fink *et al.* [45] was applied in biofilm model. The effectiveness factor corrects for the effect of mass transport when the reaction rate at any point within the bioreactor is defined by the intrinsic reaction rate expressed in terms of the bulk concentration. Numerical values of this factor can be obtained from the plot of overall effectiveness factor against modified Thiele modulus at various Biot numbers [46]. Saez and Rittmann [47] developed the simplified pseudoanalytical solution for steady state biofilm. This solution is based on the analysis of the numerical results of differential equations. It eliminates the need for repetitiously solving a set of nonlinear differential equations. Using pseudoanalytical approach to solve for the substrate flux associated with various bulk substrate concentrations, Heath *et al.* [48] introduced the normalized loading curve approach to biofilm reactor analysis and design. In process identification, Rittmann *et al.* [49] developed an *in situ* kinetic parameter determination using curve matching technique.

Another approach to solve the problem was using direct numerical integration of the simultaneous partial differential equations. Software packages that can perform numerical integration are currently available (*e.g.*, MATLAB) [50]. Simulation programs specifically for water systems had been developed such as AQUASIM [51]. Horn and Hempel used direct numerical integration to simulate the long-term experiment on autotrophic biofilm growth and auto-/heterotrophic biofilm system. With fast computers, direct simulation is

easy if the kinetic parameters and other system constants were all known [52, 53]. However, for process identification purposes, finding the kinetic parameters and system constants for a system of nonlinear partial differential equations from sets of experimental data is still a difficult and tedious task. Simplifying assumptions have to be employed so that process identification is possible.

1.2.5 Step change analysis

The response of the system to step change in one of the input variables has been widely used to study the transient behavior of the system as well as to determine the constant parameters of the model that describes the system. The transient solution of the set of differential equation governing the system provides dynamic information such as the order of the step response, time constant, damping factor and process gains, which are essential to the design of control devices of the bioreactor. The steady-state solution is also included in the unsteady-state solution as its limit as time approaches infinity [54]. Step change experiment is an effective way for process identification, that is the determination of the form of the model equations as well as the constant parameters in those equations. Tang et al. [55] in their study of the dynamics of draft tube three-phase fluidized bed bioreactor stressed out the importance of the study of dynamic behavior of the fluidized bed bioreactor because the bioreactor operation is always in the transient condition during start-up and shutdown. Aside from that, it is also constantly exposed to disturbances such as diurnal variations and shock loading in inlet concentration and flow rates. Using phenol as the substrate, Tang's model considered external mass transfer resistance, simultaneous diffusion and reaction. As already discussed in [56], these models are theoretically thorough but also mathematically so complex that accurate process identifiably seems to be impossible. Modeling attempts of the transient state operation of the solid–liquid fluidized bed biofilm reactors were done by several researchers [57–59]. Worden and Donaldson [60] studied the dynamics of a fixed film fluidized bed bioreactor. Steady-state characteristics of the three-phase fluidized bed reactors have been investigated by Hirata et al. [61] and the basic design method for plug-flow type fluidized bed bioreactor has been proposed by Hirata and Noguchi [62]. Nevertheless, the standardized kinetic model that could be applied to biofilm processes in a practical use has not yet been established. In addition, it was reported that biofilm also has an active reservoir site for dissolved organic compounds via ion exchange and hydrophobic adsorption by extracellular polymer substances (EPSs) that are composed of polysaccharides, proteins, nucleic acids, lipids and other biological macromolecules [63, 64]. Therefore, it is necessary to illuminate the oxygen concentration profile inside biofilm.

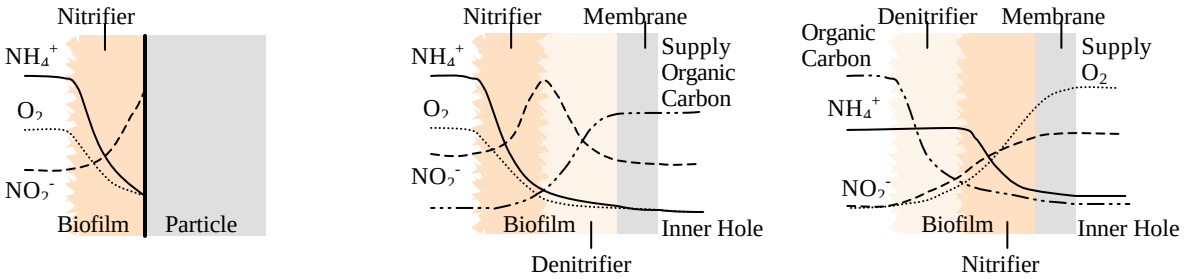
1.2.6 Membrane science

The modification of the surface by the grafting method improved the adhesivity of some bacteria to the polyethylene membrane [65]. To facilitate bacterial adhesion to the

membrane surface, radiation-induced graft polymerization (RIGP) was used in our previous work [66]. This technique enables the modification of various polymeric backbones to open interfaces, which support the "grafting" of functional groups. Our groups successfully promoted bacterial adhesion via electrostatic interaction [69]. This method is expected to make the biofilm form swiftly and firmly, resulting in effective oxygen supply to specific bacteria.

1.2.7 Membrane aeration biofilm reactor

In biofilm processes, an oxygen concentration gradient is created acrossing the aggregated microorganism, so that both aerobic and anaerobic conditions can be established inside a single reactor [67]. A simultaneous nitrification and denitrification system in a single reactor, using a membrane-aerated biofilm reactor (MABR), was proposed. The MABR system can fix a biofilm onto the outside surface of a membrane, and can directly supply oxygen from the inner side to the biofilm. Casey *et al.* described the mechanism of this simultaneous nitrification and denitrification biofilm system [68]. This method contained the advantages: 1) accumulation of bacteria was enhanced by chemical modification of a membrane surface, 2) the amount of oxygen supplied was controlled by the intra-membrane pressure. We have succeeded in promoting the adhesivity and biofilm formation of nitrifying bacteria onto membranes modified with a grafted polymer chain [69]. In the newest research using the MABR, removal of xylene [70], phenol [71], chlorophenol [72] and acetate [73], treatment of hypersaline wastewater [74], nitrification [75], and simultaneous organic carbon removal and nitrification [76] were carried out. The simultaneous nitrification and denitrification using the MABR was suggested [77]. However, it is not directly verified whether the simultaneous nitrification and denitrification occurred inside or outside the biofilm in the MABR.



(a)Conventional Biofilm Structure **(b)New Biofilm Structure Type A** **(c)New Biofilm Structure Type B**

Fig.1.1 Biofilm Structure in Membrane Bioreactor

1.3 OBJECTIVE OF THIS STUDY

The main purpose of this study is to clarify the distribution of substrate concentration inside biofilm, especially the distribution of oxygen concentration in the wastewater treatment processes and applied above information to the improvement of treatment activity and development of new processes.