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Microbial Communities in Nutrient-removing Membrane Bioreactors: A Review

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ABSTRACT

During last decade, researches on membrane bioreactors have been focused on nutrient removal. Different aspects of nutrient removal have been studied including different configuration of nutrient removal systems including configuration of nutrient removal systems, hybrid systems, treatment of industrial waste water, influence of hydraulic and solid retention time and dynamics of microbial community structure. A significant number of studies have been focused on microbial communities responsible for enhance nutrient removal in MBR systems. The researchers have applied numerous cultural-independent technologies in last decade. They utilized Fluorescent In Situ Hybridization (FISH), Denaturing Gradient Gel Electrophoresis (DGGE), Phospholipid Fatty Acid (PLFA) analysis and Respiratory quinone profile methods. The impacts of operational conditions such as internal recycling rate, SRT and composition of organic substrate on microbial communities have also studied. Present review summarizes important findings of studies focused on microbial communities in nutrient-removing MBR systems.

Key words: Membrane bioreactors, microbial community structure, nitrogen, phosphorus, molecular microbiological tools

INTRODUCTION

The concept of Membrane Bioreactor (MBR) is a combination of conventional biological wastewater treatment and membrane filtration. The concept of MBR differs from conventional biological wastewater treatment in the separation of activated sludge and treated wastewater. The sedimentation in secondary clarification is replaced by membrane filtration in MBR. Hence, high solid retention time can be achieved in MBR. Therefore, the MBR and conventional systems shows significant technological and biological differences. Due to utilization of membrane in MBRs, it is possible to retain all suspended solids and microbial flocs. Consequently, a longer solid retention time can be achieved in MBR systems which not possible in conventional waste water treatment systems. The prominent advantages of MBR systems are small footprint, enhanced effluent quality, efficient disinfection capability, greater volumetric loading and minimal sludge production (Judd, 2006; Alaboud and Magram, 2008; Alquwaizany *et al.*, 2011).

During last decade, researchers working on membrane bioreactors have been focused on nutrient removal (Song *et al.*, 2010; Ahmed *et al.*, 2007a, b). These systems are composed of alternating anoxic and anoxic conditions in a submerged MBR by intermittently aeration for simultaneously removal of carbon and nitrogen (Kim *et al.*, 2010; Ahn *et al.*, 2003; Ying *et al.*, 2005; Fu *et al.*, 2009; Wang *et al.*, 2009). Different aspects of nutrient removal have been studied including different configuration of nutrient removal systems (Ahn *et al.*, 2003; Kimura *et al.*, 2008; Fu *et al.*, 2009; Zhang *et al.*, 2009), hybrid systems (Shin *et al.*, 2005; Zhang *et al.*, 2005; Ahn *et al.*, 2007), treatment of industrial waste water (Shin *et al.*, 2005;

Ying *et al.*, 2005), influence of hydraulic and solid retention time (Brown *et al.*, 2011; Kim *et al.*, 2010; Song *et al.*, 2010; Ahmed *et al.*, 2007a), removal of micro pollutants (Kim *et al.*, 2009). Some studies have been conducted on microbial community structure (Ahmed *et al.*, 2008; Xia *et al.*, 2010) but have wide difference in approach and results (Choi *et al.*, 2007; Ivnitsky *et al.*, 2007; Bezuidenhout *et al.*, 2008; Arevalo *et al.*, 2009). The studies mostly focused on observing microbial community in systems with nitrification (Li *et al.*, 2006; You and Chen, 2008) denitrification (Lim *et al.*, 2004; Tan *et al.*, 2008) and membrane bio-fouling (Miura *et al.*, 2007a; Molina-Munoz *et al.*, 2009). In this review, the fundamentals of nitrogen and phosphorus have been re-visited and recent studies nutrient removal MBR systems and dynamic of microbial communities are reviewed.

BIOLOGICAL NUTRIENT REMOVAL PROCESSES

Nitrogen removal process: Nitrogen compounds in surface water are well known as one of the causes of eutrophication of aquatic environments. One of the major sources of nitrogen compounds is the discharge from waste water treatment plants. Therefore, removal of nitrogenous compounds should be integrated in conventional wastewater treatment plants. Biological nitrogen removal is carried out with two successive processes: nitrification and denitrification. The possible nitrogen conversions in waste water treatment systems are depicted in Fig. 1.

Autotrophic nitrification: Biological nitrification is generally accomplished with the oxidation of ammonia to nitrite through hydroxylamine (NH₂OH) by *Nitrosomonas* and subsequent oxidation of nitrite to nitrate by *Nitrobacter*. Both of these genera classified as autotrophic organisms derive energy from the oxidation of the inorganic nitrogen compound. 58-84 Kcal mole⁻¹ and 15.4-20.9 Kcal mole⁻¹ of free energies are release by oxidation of ammonia and nitrite, respectively (US-EPA, 1993). The overall process can be expressed as in following equations:

Nitrosomonas:



Nitrobacter:

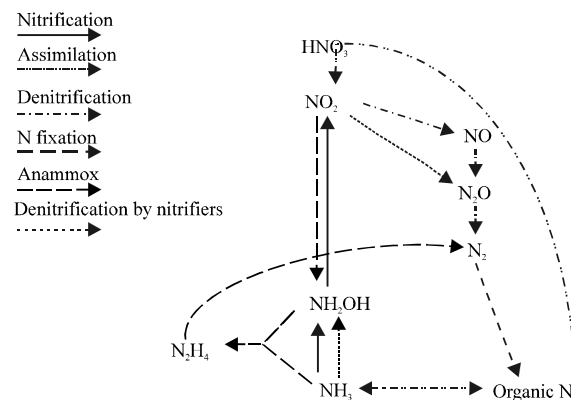


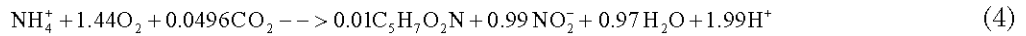
Fig. 1: Possible microbial nitrogen conversions (Van Loosdrecht and Jetten, 1998)

Overall:

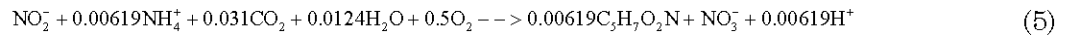


Inorganic carbon (carbon dioxide) is used for synthesis these bacterial groups. The yield coefficient for *Nitrosomonas* is 0.04-0.13 g VSS g⁻¹ NH₄⁺-N and for *Nitrobacter* is 0.02-0.07 gSS g⁻¹ NO₂⁻-N (US-EPA, 1993). Incorporating the synthesis average yield values the oxidation equation can be re-written as:

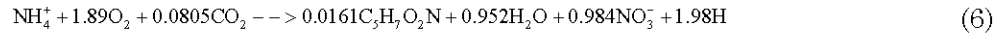
Nitrosomonas:



Nitrobacter:



Overall:

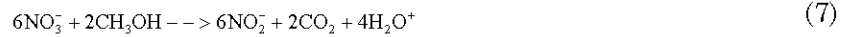


The favorable environmental conditions for autotrophic nitrifiers are; a) aerobic conditions at a minimum dissolved oxygen level of 2.0 mg L⁻¹, b) sufficient alkalinity and pH, optimum pH for the growth of nitrifying bacteria is in the 8 to 9 range, with pH levels below 7 causing a substantial reduction in nitrification activity, c) Finally, though nitrification occurs over a wide range of temperatures, a reduction in temperature produces a slower rate of reaction.

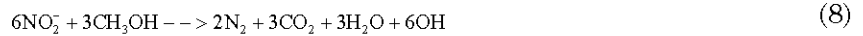
Heterotrophic denitrification: Nitrate conversion takes place through both assimilatory and dissimilatory cellular functions. In assimilatory denitrification, nitrate is reduced to ammonia, which then serves as nitrogen source for cell synthesis. Thus, nitrogen is removed from the liquid stream by incorporating it into cytoplasmic material. In dissimilatory denitrification, nitrate serves as the electron acceptor in energy metabolism and is converted to various gaseous end products but principally molecular nitrogen, N₂, which is then stripped from the liquid stream. Because the molecular yield under anoxic conditions is considerably lower than the under aerobic conditions, a relatively small fraction of the nitrogen is removed through assimilation. Dissimilatory denitrification is, therefore, the primary means by which nitrogen removal is achieved, accounting 70 to 75% of total nitrogen removal (Benfield and Randall, 1980).

Biological denitrification is accomplished by two-step process; a) first nitrate convert to nitrite and process, b) then nitrite is reduced to nitrogen gas in second step. Traditionally, the denitrification process is considered to carry out by facultative heterotrophic bacteria including *Bacillus*, *Acromobacter*, *Brevibacterium*, *Entrobacter*, *Pseudomonas*, *Spirillum*, *Lactobacillus*, *Micrococcus* and *Paracalobacterium* in the absence of oxygen. The two-step denitrification process using methanol as carbon source is illustrated by following equations (Benfield and Randall, 1980).

- First step:



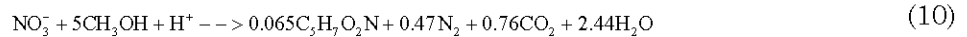
- Second step:



- Overall:

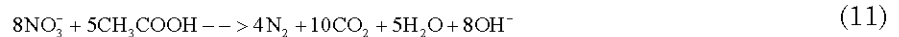


Using data obtained from laboratory studies, McCarty has proposed that the overall nitrate removal reaction (including both assimilation and dissimilation reactions) can be described by the following empirical equation (McCarty *et al.*, 1969):



The overall reaction using acetic acid as the electron donor can be expressed as shown in following stoichiometric equation (Barnard and Stensel, 1992):

- Overall:



The activity of the denitrifying enzyme and hence denitrification is suppressed in presence of Dissolved Oxygen (DO). An oxygen concentration of 0.2 mg L⁻¹ and above has been reported to inhibit denitrification in pure cultures (Dawson and Murphy, 1972). However, the denitrification has been reported to proceed in presence of DO in concentration 0.3 to 1.5 mg L⁻¹ (US-EPA, 1993). The pH should also be in limited range of 7 to 8 for optimum denitrification (US-EPA, 1993). Nitrogen removal has also been studied using simultaneous sulfur utilizing autotrophic and heterotrophic denitrification (Oh *et al.*, 2001).

Biological phosphorus removal process: Biological phosphate removal from wastewater is considered to be achieved in two ways: a) stoichiometric coupling in the biomass (Riding *et al.*, 1979), enhanced storage in the biomass as polyphosphate (poly-P). The latter was formerly called “luxury uptake” (Levin and Shapiro, 1965) and key mechanism in the Enhanced Biological Phosphorus Removal (EBPR). The enhanced biological phosphorus removing process in activated sludge has been reviewed in detail by Toerien *et al.* (1990), Wentzel *et al.* (1991) and Mino *et al.* (1998).

The mechanisms of EBPR: The biological phosphorus removal in wastewater treatment plants, biomass first passes through an anaerobic phase, before entering a phase where an electron

acceptor is present, i.e., an anoxic phase where nitrate is present or an aerobic phase where oxygen is present (Mino *et al.*, 1998). The concentration profiles of the main measurable components for EBPR operated under anaerobic-aerobic conditions (Yeoman *et al.*, 1988).

When the waste water enters the anaerobic phase, specialized organisms, called Poly-phosphate Accumulating bacteria (PAOs) accumulate carbon sources as an internal polymer called PolyHydroxyAlkanoates (PHAs). The main forms of these PHAs are Poly-beta-HydroxyButyrate (PHB) and Poly-beta-HydroxyValerate (PHV). The energy to store this polymer is obtained from breakdown of glycogen and hydrolysis of an energy rich internal phosphorus chain called poly-Phosphate (poly-P). Since poly-P is broken down to ortho-phosphate for energy supply, the phosphate concentration in the anaerobic phase increases. The anaerobic phase needs to be followed by an oxygen or nitrate rich phase, i.e., an anoxic phase (anoxic P-removal) or an aerobic phase (aerobic P-removal). During this phase the stored PHB is consumed, generating energy for growth, for uptake of ortho-phosphate from the liquid phase and generating energy and carbon for replenishment of the glycogen and poly-P pools. Under these conditions the ortho-phosphate concentration thus decreases. Most importantly, since the amount of biomass containing large amounts of poly-P-PAOs are able to store up to 10% of their dry weight (Yeoman *et al.*, 1988), is increasing under these conditions, a net phosphorus removal occurs with the wasted sludge.

The intracellular poly-P pool in PAOs is divided into two sub-fractions, i.e., the intracellular "volutin" granules and a fraction located in the periplasmic space and/or loosely bounded to the cell membrane (Streichan and Schon, 1991). The terminology "volutin granules" has been introduced due to their characteristics to changes in pigmentation of certain dye as well as they were first described in *Spirillum volutans*. Poly-P granules (e.g., volutin granules) can be easily observed under bright-field or phase-contrast microscopy or by staining with many basic dyes such as toluidine blue (Brock and Madigan, 1991) or by staining with high 4'-6-diamidino-2-phenylindole (DAPI) concentrations (Kawaharasaki *et al.*, 1999).

Another storage compounds in microorganism is glycogen. It is a starch like polymer of glucose sub-units. Glycogen granules are usually smaller than PHB granules and can only be seen by electron microscope. However, the presence of glycogen in a cell can be detected in the light microscope because the cell appears as red-brown when treated with dilute iodine, due to a glycogen-iodine reaction (Brock and Madigan, 1991).

Nitrogen and phosphorus removal in MBR systems: The nitrogen removal in MBR system conceptualized and studied just after introduction of submerged membrane bioreactor (Chiemchaisri *et al.*, 1992). In the early studies submerged membrane was introduced in an aeration tank and intermittent aeration was applied to achieve enhanced total nitrogen removal. After then, the MBR coupled biological denitrification process was studied with intention for system improvements at bench scale (Chiemchaisri and Yamamoto, 1993; Delanghe *et al.*, 1994) as well as pilot scale (Cote *et al.*, 1997; Cicek *et al.*, 1998). The majority of studies conducted in that era i.e., late 1990s were focused on intermittent aeration mode of denitrification processes (Yeom *et al.*, 1999; Nah *et al.*, 2000) and without phosphorus removal. Enhanced biological phosphorus removal in a membrane bioreactor was first attempted by Adam *et al.* (2002). Although, the system configuration adopted at bench-scale and pilot scale reactors showed efficient enhanced phosphorus removal (Lesjean *et al.*, 2002, 2003; Adam *et al.*, 2003) but complex configuration were adopted. Furthermore the treatment efficiencies were not high enough to meet the legal future restrictions on wastewater discharge. An innovative simple configuration was developed by Ahn *et al.*

(2003) for both nitrogen and enhanced phosphorus removal termed as SAM. Owing to its simple configuration, compactness, higher treatment efficiencies, the SAM process gained popularity and a pilot plant study was conducted to up scale the SAM process and to test on real domestic wastewater (Cho *et al.*, 2005).

Microbial community structure in MBR systems: The understanding of processes occurs in MBRs particularly membrane fouling, nitrification, denitrification and phosphate removal is not complete without studying of the microbial community structure responsible for such phenomena (Luxmy *et al.*, 2000; Cicek *et al.*, 2001). The researchers started to investigate the microbial community structure in MBRs and applied numerous cultural-independent technologies in last decade of the past century. They utilized Fluorescent *In situ* Hybridization (FISH), Denaturing Gradient Gel Electrophoresis (DGGE) (Luxmy *et al.*, 2000; Klatt and LaPara, 2003), Phospholipid Fatty Acid (PLFA) analysis (Cicek *et al.*, 2001), Respiratory quinone profile method (Lim *et al.*, 2004, 2005).

Nitrification: Initially the purpose of MBR was treatment of domestic wastewater and nitrogen conversion was limited to nitrification process. Therefore, studies investigated microbial community structures in MBRs were also focused on nitrification. For example (Luxmy *et al.*, 2000) analyzed microbial community structure in pilot plant scale MBR fed with raw sewage using FISH and PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) techniques. They found that α - and β -subclasses of *proteobacteria* were the most dominant group. The ammonia oxidizing group was also identified and found in group present in the form of clusters or aggregates. The clusters formed by *Nitrobacter* sp. were found smaller than those of ammonia-oxidizing groups. They also showed by numerical analysis on the band pattern of DGGE that MBR bacterial communities were different from the communities found in conventional activated sludge system. Liebig *et al.* (2001) investigated nitrification performance of a membrane-assisted MBR at pilot scale for treatment of sludge reject water with $\text{NH}_4\text{-N}$ concentration up to 600 mg L^{-1} and low organic content. The microbial community structure found in MBR was found different than in chemostate. The nitrifier species were found 23.2-27% of identifiable cells in the MBR. Klatt and LaPara (2003) operated laboratory scale MBR fed with a mixture of starch, gelatin and polyoxyethylene-sorbitan monooleate to simulate the polysaccharide, protein and lipid component of municipal wastewater. They found substantial community shift occurred within the first 7 days operation and *Flavobacterium*-like bacterial population dominated the community and continued to do so for rest of the study. The observation of community shift was probably due to type of organic compounds in influent but it support previous studies that microbial community structure found in MBR differs from sludge of conventional activated sludge (Li *et al.*, 2006). They operated a MBR supplied with inorganic ammonium-bearing wastewater and complete nitrification was demonstrated. FISH analysis showed that *Nitrosomonas* sp. and *Nitrospira* sp. were dominant nitrifying genera responsible for ammonia and nitrite oxidation, respectively. More recently, it was showed that influent wastewater composition had a larger impact on bacterial community structures (Miura *et al.*, 2007a).

Denitrification: An alternating anoxic-oxic membrane reactor was operated by Sofia *et al.* (2004) for removal of nitrogen from sewage. The system was capable to remove nitrogen from raw wastewater through typical nitrogen removal transformation (i.e., aerobic ammonia oxidation and

anoxic nitrate reduction). The characterization of bacterial community showed that *Nitrosopira* spp. and *Nitrospira* spp. were the dominant groups of ammonia and nitrite oxidizing groups, respectively. Along with these species members of *Paracoccus* spp. were found metabolically functional in nitrogen removal. In another study, two Modified Ludzack-Ettinger (MLE)-type MBRs were investigated for COD and nitrogen removal (Ghosh and LaPara, 2004). Bacterial community analysis of ammonia oxidizing bacteria by a nested PCR-DGGE demonstrated that two *Nitrosomonas*-like populations and one *Nitrosopira*-like species were present.

Quinone profile: Lim *et al.* (2004) used respiratory quinone profile method to study the microbial community structure in an intermittently aerated submerged membrane bioreactor treating domestic wastewater. They found ubiquinone (UQ)-8 type followed by UQ-10 and menaquinone (MK)-6 and concluded that *Nitrosomonas* species, *Alcaligenes* species and *Thiobacillus* were actively contributed to the biological nitrification/denitrification in operated MBRs. The microbial diversities of suspended microorganisms on the molar fraction basis of all quinone composition were in the range 8.79-11.82. In another study (Lim *et al.*, 2005) investigated effect of in-line cleaning chemicals on microbial community in biofilm on membrane surface of submerged MBR using quinone profile method. They found that dominant quinone types of biofilm were UQ-8, -10 followed by MK-8(H4), -7 and UQ-9 but those of suspended microorganism were UQ-8, -10 followed by MK-8(H4), -7 and -11. Quinone profile in a submerged MBR supplied with inorganic ammonium-bearing wastewater, which showed completed nitrification, indicated that UQ-8 was dominant (66-84%) followed by UQ-10, -7 and -9. the dominant menaquinone in the MBR was MK-7 followed by MK-6, MK-8 and MK-8(H2) and with the prolonged operation percentage of menaquinone increased from 8 to 14%.

PCR-DGGE: The PCR-DGGE has been also used to understand relationship between bacterial enzyme activities, feed composition and bacterial community structure (LaPara *et al.*, 2002, 2006; Klatt and LaPara, 2003). It was found that the microbial community structure was dynamic even after the biomass had reached a quasi-steady state with respect to physiological measurements. In the MBR fed with yeast cells, respiratory potentials increased 2- to 5-fold during the initial portion of the BRR run and α -glucosidase and β -glucosidase activities increased 2- to 4-fold. Substantial bacterial community shifts were also detected in both the rDNA and rRNA profiles, indicating that this community was also structurally dynamic. These experiments suggested that phylogenetically different bacteria sustained the functional activities in these ecosystems in response to increasingly stringent nutrient limitation (LaPara *et al.*, 2002). In another study, the bacterial community substantially increased its α -glucosidase affinity (>1000-fold), while the leucine aminopeptidase and heptanoate esterase affinities increased slightly (<40-fold) or remained relatively constant (LaPara *et al.*, 2006). Concomitant to these physiological adaptations, shifts in the bacterial community structure in MBRs were detected by PCR-DGGE. Four of the bacterial populations detected by PCR-DGGE were isolated and exhibited specific growth rates in batch culture ranging from 0.009 to 0.22 h⁻¹. It was suggested that bacterial communities growing under increasingly stringent nutrient limitation adapt their enzyme activities primarily for the nutrients provided but that there is also a more subtle response not linked to the substrates included in the feed medium. It was demonstrated that MBRs can support relatively complex bacterial communities even on simple feed media.

Microbial community and membrane bio-fouling: The study of membrane bio-fouling in relation with microbial community structure gained focus of researcher very recently. In initial phase, the composition of the planktonic and sessile microbial communities inhabiting in laboratory-scale MBR systems were compared using Amplified Ribosomal DNA Restriction Analysis (ARDRA) and 16S ribosomal DNA gene sequencing (Zhang *et al.*, 2006). The ARDRA results suggested that the microbial communities on membrane surfaces could be very different from the ones in the suspended biomass. Phylogenetic analysis based on the 16S rRNA gene sequences provided a list of bacteria that might be the pioneers of surface colonization on microfiltration membranes. Present results further suggested that research on the mechanisms of cell attachment in such an engineering environment could be critical for future development of appropriate biofouling control strategies. Choi *et al.* (2006) examined the impact of biological, chemical and physical properties of activated sludge on membrane filtration performance in laboratory-scale MBRs. Various filtration resistances were used to investigate membrane fouling characteristics and molecular tools targeting 16S ribosomal DNA gene sequences were used to identify predominant bacterial populations in the sludges or attached to the fouled membranes. They showed that the tendency of membranes to biofoul depended upon membrane operating conditions as well as the properties of the activated sludge in the MBR systems. Specific bacterial populations, which were not dominant in the activated sludges, were selectively accumulated on the membrane surface leading to the development of irreversible biofouling. (Xie *et al.*, 2006) highlighted importance of protein profiles of Extracellular Polymeric Substances (EPS) and activated sludge in MBR using 2-Dimensional Gel Electrophoresis (2DGE) and studied metabolisms of microbial communities at metaproteomic level.

The influence of filamentous bacteria on membrane fouling in microfiltration of activated sludge wastewater has also been studied (Meng *et al.*, 2006). It was demonstrated that the sludge flocs with negligible filamentous bacteria led to severe membrane pore blocking and the sludge flocs with filamentous bacteria created the formation of a non-porous cake layer on the membrane surface. The excess growth of filamentous bacteria resulted in much more release of EPS, lower zeta potential, higher hydrophobicity of sludge flocs and more irregularly shaped flocs which did great harm to membrane filtration. The sludge flocs with a small quantity of filamentous bacteria had a positive effect on membrane permeation. It is important to control filamentous bacteria concentration in the operation of MBRs.

Fundamental understanding of mechanisms of membrane bio-fouling was attempted to acquired using FISH, 16S rRNA gene sequence analysis and scanning electron microscopy (Miura *et al.*, 2007b). The study was conducted using sludge from full-scale submerged MBRs fed with real municipal wastewater delivered from the primary sedimentation basin of a municipal waste water treatment facility. The compositions of planktonic and biofilm microbial communities in the MBR. The FISH results revealed that the microbial communities on membrane surfaces were quite different from those in the planktonic biomass in the mixed liquor. Moreover, a specific phylogenetic group of bacteria, the β -Proteobacteria, was considered responsible for a major role in development of the mature biofilms, which led to the severe irreversible membrane biofouling.

CONCLUSION

A significant number of studies have been conducted on microbial community structure but have wide difference in approach and results. The studies mostly focused on observing microbial community in systems with nitrification, denitrification or membrane bio-fouling. The impact of

operational conditions such as internal recycling rate, SRT and composition of organic substrate on microbial communities were relatively neglected. Furthermore, few studies have found which demonstrated simultaneously nitrogen and phosphorus removal in relation with understanding of microbial community structure responsible for nutrient removal in MBR systems.

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