공학석사 학위논문

Application of Bacterial Quorum Quenching in a MBR with a Multi-Layer Hollow Fiber module for Wastewater Treatment

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권 혁 필

Abstract

Application of Bacterial Quorum Quenching in a MBR with a Multi-Layer Hollow Fiber Module for Wastewater Treatment

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Membrane fouling is one of the major problems which not only reduce membrane permeability but also increase energy cost in operation of a membrane bioreactor (MBR) for wastewater treatment. Among many approaches for alleviating membrane fouling, the quorum quenching (QQ) has been introduced as simple and promising biological approach; QQ is the process of disrupting cell-to-cell communication of microorganisms, as known as quorum sensing (QS).

Previous studies have revealed that bacterial QQ with lactonase producing bacteria, *Rhodococcus* sp. BH4, mitigates the biofouling in MBR. The mitigation of biofouling was described by delay in trans-membrane pressure (TMP) rise-up.

Also, QQ effect was verified in pilot-scale flat sheet MBR fed with a real wastewater.

In this study, we focused on the potential application of bacterial QQ to a hollow fiber MBR because approximately 80% of MBR plants in operation worldwide are equipped with a hollow fiber (HF) module instead of a flat sheet module. While narrower spacing of fibers are unavoidable in a in a multi-layer HF module, foulants are more likely to deposit in a multi-layer HF module than a flat sheet module. To manipulate such a problem, a lab-scale multi-layer HF module was fabricated with similar spacing of fibers to a commercial one and then QQ-bead entrapping Rhodococcus sp. BH4 was applied. However, QQ effect was not observed in a multi-layer HF module unlike in a mono-layer HF module.

Two new approaches were carried out to improve the QQ efficiency in a multi-layer HF module. Firstly, QQ bacteria (BH4) without any type of entrapment was directly inserted into the MBR, so that QQ bacteria can freely move to decompose signal molecules inside the multi-layer HF module. However, BH4 without entrapment did not show QQ activity. It is speculated that QQ activity of BH4 is suppressed by other microorganisms which are more competitive to survive in MBR

The second approach was to entrap BH4 to protect them against other microorganisms as well as to apply backwashing process to make BH4 contact easier signal molecules inside of the multi-layer hollow fiber module. As entrapped BH4 was introduced to the backwashing system, BH4 could delay TMP rise-up by

1 fold compare to the MBR operated with backwashing but without entrapped BH4. But the QQ effect disappeared when backwashing stopped. It seems like physically removing foulants from inside of multi-layer module could help BH4 to contact signal molecules easier and thus to mitigate biofilm formation on the surface of membrane.

Keywords

Membrane bioreactor, wastewater treatment, quorum sensing, quorum quenching, hollow fiber, membrane fouling,

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Chapter I

Introduction

I.1. Backgrounds and objectives

Slowly but certainly, the water supplies face challenge in worldwide because of increased demand, drought, depletion and contamination of groundwater, and lack of water source. Yet, attention to water reuse have been very small, for example, the proportion of reclaimed and reused water in the US is only 7.4% in 2000, according to the “clean water needs survey 2000 from the report to congress, 2003”. But, total volume of recycled water has been growing 15% per year in US since 2002, which indicates that demand for recycled water will expand as time passes (Miller, 2006).

One of the technologies of water reuse is the activated sludge sewage treatment which is over 100 year old technique and is still at the heart of current wastewater treatment (van Loosdrecht and Brdjanovic, 2014). The activated sludge process utilizes a set of microorganisms which can use contaminants of water as their nutrient source. Also, a membrane has been introduced for the separation of sludge from water instead of separating water by settling of sludge using a gravity alone. A membrane bioreactor (MBR) could improve the quality of effluent compared to that of conventional activated sludge (CAS) process.

Although a MBR yields a better water quality than CAS does, it requires an extra energy for suction and maintenance. Especially the biofouling on membrane increases energy consumption due to increase in filtration resistance caused by a biofilm and the extra bubble supply for biofilm detachment. Actually, extra bubble for membrane cleaning takes of 60% of operating cost (Judd, 2010). A biofouling not only increase operating cost but also requires additional cleaning step that entire membrane module has to be cleaned physically and chemically. In

addition, the membrane loses its lifespan by physical washing and use of chlorine for chemical washing.

Recently, a quorum quenching (QQ) technique is applied to a MBR process which can mitigate biofilm production during the operation. QQ is a method of disrupting quorum sensing (QS) of bacteria which forms biofilm on membrane surface. In 2009, the relationship between biofouling in a MBR and QS has been reported (Yeon et al., 2008). Also, various ways of reducing QS activity using a magnetic carrier containing a QQ enzyme (Yeon et al., 2009), a membrane coated with a QQ enzyme (Kim et al., 2011), and an immobilized bacteria (Oh et al., 2012). Especially, the method of using an entrapped bacteria using a polymer bead showed a promised result in pilot-scale experiments that the bacteria and bead were stable throughout the duration of experiments (Lee et al., 2016).

Previous studies have shown that a QQ could mitigate the formation of biofilm on the membrane surface. Most of QQ method was endogenous degradation of QS signal molecule which is secreted by QS bacteria. However, concentration of QS signal molecule was reported to be very low in MBR supernatant compared to that of biofilm (Emerenini et al., 2015). Thus, monitoring a concentration of AHLs upon addition of QQ bacteria should be carried out. In addition, a real MBR plants are consist of much complex membrane module which inhibits QQ carriers from approaching, thereby possibly reducing effect of QQ. In this study, the examination of QQ efficiency of QQ-MBR with a multi-layer hollow fiber membrane module was carried out in order to manipulate the fouling behavior of actual hollow fiber module used in existing MBR operation systems.

I.2. Objectives

The objective of this study was to examine efficiency of QQ-MBR with a multi-layer hollow fiber module to manipulate change in efficiency of QQ in actual MBR plants using a hollow fiber module. In order to achieve successful QQ effect in MBR with multi-layer hollow fiber module following experiments were performed:

(1) Examination of AHL in MBR

- Continuously monitoring AHLs from the permeate of MBR
- Analysis of correlation between TMP and concentrations of AHLs

(2) Direct application of raw BH4 strain in QQ-MBR

- Comparing TMP rise-up of conventional-MBR with that of QQ-MBR which contains injected BH4 inside of MBR.
- Examination of QQ activity of BH4 which is mixed with activated sludge over time.

(3) Application of backwashing method to QQ-MBR

- Comparing TMP rise-up of conventional-MBR with that of QQ-MBR which contains entrapped BH4 inside of MBR.
- Detection of suspended solids, AHLs and extracellular polymeric substances from the backwashed solution

Chapter II

Literature Review

II.1. Membrane Bioreactor (MBR)

II.1.1. Biological Wastewater Treatment

The increased demand for a water initiated by rapid urbanization and industrialization in the 19th century led to a development of wastewater treatment technology. The activated sludge process is the longest and inevitable technique that is over 100 year old (van Loosdrecht and Brdjanovic, 2014). The conventional activated sludge process (CAS) is developed by E. Ardern and W. T. Lockett that CAS is consist of the reactor where the contaminants are degraded by microorganisms and the settling tank for decanting solid-free water where the mixed liquor suspended solids (MLSS) is settled down due to the Earth gravity. Also, CAS is consist of 4 stream: influent, permeate, sludge recycle, and sludge out which is required for maintaining the concentration of microorganisms inside reactor (McCarty, 2012).

Yet, simple CAS process was unable to get rid of nitrogen and phosphate spices. In 1970s, two-stage CAS system with denitrification reactor (anoxic) was developed. In addition, phosphate removal process (anaerobic) is also coupled with CAS system. The air is not supplied to both anoxic and anaerobic tank to keep the concentration of dissolved oxygen low (Kartal et al., 2010). As CAS system became popular, an effort to decrease cost of CAS was raised. One of successful result was methanogenesis which is process of generating methane gas. However, difficulties in pH control, odor production, and low growth rate of microorganisms are crucial disadvantages of methanogenesis process (McCarty, 2012).

II.1.2. Development of MBR

Although CAS is very simple and reliable process, the problem of MLSS separation is not negligible that development of poor settling is the major problem in CAS operation. Especially, bulking which is caused by the growth of filamentous organisms is one of the cases of poor settling that overflow of sludge blanket can occur. On the other hand unsettled microbes are presented in the product when microorganisms do not form a floc, called a dispersed growth (Jenkins, 1992). When such a problem occur, the effluent water quality become unsuitable for water reuse.

In the late1960s, Dorr-Oliver commercialized the first membrane bioreactor (MBR) which is a process where membraned is added to CAS process, getting rid of settling tank. There are many advantages of MBR of CAS as following (Judd, 2010):

(1) High quality effluent: usually pore size smaller than a size of bacteria is used for a MBR. Thus disperse growth problem does not matter in MBR operation.

(2) Uncoupled HRT and SRT: hydraulic retention time is time for a liquid flowrate which is also related to solid retention time in CAS system. But membrane allows HRT to be uncoupled from SRT by physically separating solids from the liquid. It allows operator to control system more effectively by increasing SRT.

(3) Effective biomass control: since SRT is an independent operating parameter in MBR, concentration of biomass can be increased by increasing SRT. Higher concentration of biomass means cheaper dehydration process for excess sludge. Also, higher SRT usually yields better quality of effluent.

Yet, MBR still have a disadvantages compared to a CAS process as follow:

(1) Higher total energy consumption: using a MBR requires extra energy, usually electricity, for a pump operation. MBR is known to use over 10 times higher energy than CAS does(Yamanoto et al., 1989).

(2) Higher equipment cost: although the capital cost of land could be saved by removing a settling tank, membrane module itself is not a cheap application. However, membrane fabrication cost gets cheaper as a new technologies evolve, while a land cost will not.

(3) Higher maintenance cost: unlike settling tank, membrane has a life time which is usually about 10 years. Also, a fouling on membrane blocks membrane pores, forcing an operator to clean membrane once in a while.

Actually, feasibility of MBR is highly dependent on legislation of water quality that when standard is very strict, usage of MBR is inevitable to satisfy required effluent water quality (Judd, 2010).

II.2. Fouling Control in MBR Process

One of the biggest problem with MBR process is a fouling phenomenon. Especially, bacterial fouling is unavoidable because the membrane is always exposed to growing bacteria inside of the reactor. Various studies on fouling reduction strategies have been made since MBR was introduced. There are two types of fouling that occur on membrane: reversible fouling, the fouling which can be resolved by physical cleaning such as coarse bubble aeration, relaxation and backwashing; irreversible fouling, the fouling which can be removed only by chemical cleaning that tight pore blocking and biofilm formation are typical irreversible fouling (Huyskens et al., 2008). The irreversible fouling are dominant when the TMP jump occurs as shown in Figure II-1 that sudden increase of TMP is observed after 50 hours of operation.

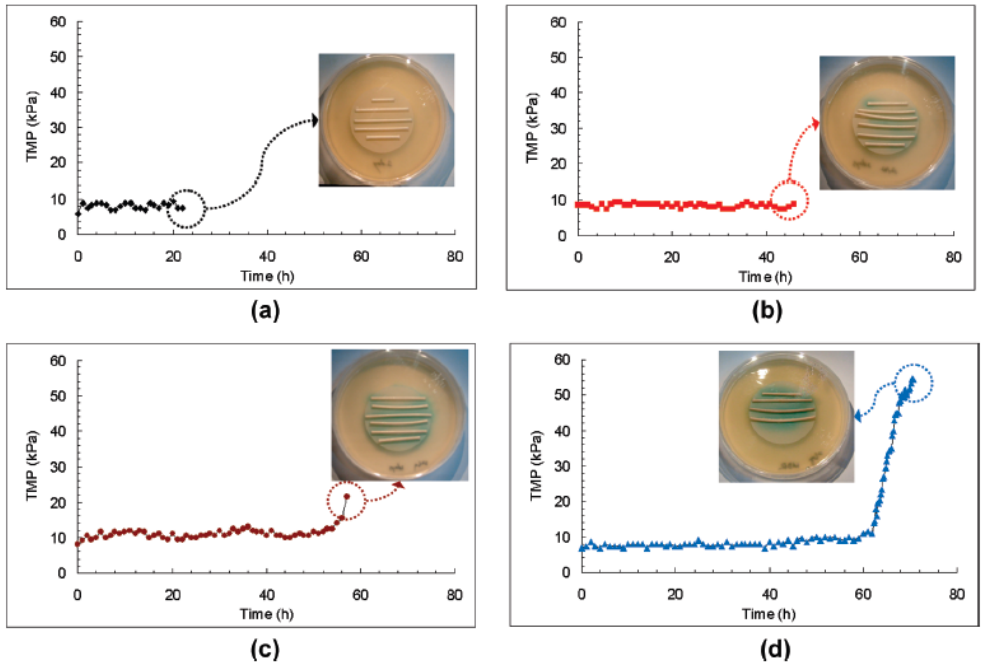


Figure II-1. Occurrence of AHL signals in biocake during continuous MBR operation

(Yeon et al., 2008)

II.2.1. Physical Approach

In addition to aeration for D.O. requisite, a coarse bubble aeration is applied to a membrane surface to detach clogged solids. Also, an addition of a relaxation or backwashing step to an MBR operation increases efficiency of air scrubbing (Ramesh et al., 2006). Besides these technique, modifying a configuration of a membrane module delayed fouling build up rate because of change in hydrodynamics on membrane surface (Hai et al., 2008). Most modules contain a lot of single membranes, thus their packing density influences rate of fouling on membrane but lower packing leads to more space require for a MBR (Günther et al., 2010). Yet, coarse bubble aeration, the most common physical approach in a real MBR plants, takes 35.2% of total MBR energy consumption, which is largest value among components of MBR, as shown in Table II-1. Thus, other approaches such as chemical and biological methods were also considered. It is important to characterize back-washed solution to examine principles of physical washing. It is shown that back-washed solution contains higher fraction of soluble protein that backwashing can successfully remove deposited protein in membrane pores (Wu et al., 2008).

Table II-1. MBR energy distribution

Components of MBR	Energy Distribution (%)
Coarse bubble aeration	35.2
MBR recycle	16.0
Permeate pump	11.6
Bioreactor aeration	10.2
MBR influent pump	5.4
Mixers, acetic pump, Recirculation	7.3
Cleaning in place (CIP)	6.6
Compressors	3.1
Sludge disposal	1.7
Pretreatment	2.6

(Fenu et al., 2010)

II.2.2. Chemical Approach

Even though intensive physical cleaning was applied to a MBR operation, it is still necessary to clean membrane with chemicals such as chlorine to remove residual and irreversible fouling on the membrane. This process is usually carried out twice a year because it is a laborious process that entire membrane is taken out of reactor for the cleaning process. Although increasing temperature increase cleaning efficiency, it is not recommended by membrane manufactures because most membranes are organic material having a low heat. Also many chemicals reported as a good cleaning agents are nitric acid, hydrochloric acid, phosphoric acid, alkaline, carbonates, phosphates, EDTA, sodium hypochlorite, etc (Ramesh et al., 2006). According to Mohammadi et al., combination of sodium hydroxide and sodium hypochlorite, and sodium hydroxide and sodium dodecyl sulphate clean fouled membrane more efficiently than single-agent method (Mohammadi et al., 2003).

Most of chemicals mentioned above are hazardous chemicals, therefore mild and environmentally friendly cleaning chemicals have been reported. Especially, enzyme is most studied agent because of its selectivity and various functions. Protease A and lipase A are reported that they are not only able to degrade proteins adsorbed on membrane but also restore distorted contact angle of membrane surface (Maartens et al., 1996). Also, an environmental friendly surfactants such as CTAB (cetyl-trimethyl-ammonium bromide) and TAZ (Terg-A-Zyme) were able to clean fouled membrane (Munoz-Aguado et al., 1996).

The cationic biopolymers were got attention from the researchers because extracellular polymeric substance (EPS) and soluble microbial product (SMP) were found to be important factors for membrane fouling. An examples of EPS and SMP are protein, polysaccharide, lipid, nucleic acid and humic acid. The cationic biopolymers were known to effective for alleviating membrane fouling caused by EPS and SMP which are negatively charged polymers. One of cationic polymer is MPE (Membrane Performance Enhancer) which is able to reduece the level of polysaccharide (Guo et al., 2008).

II.2.3. Material Approach

One of fundamental approach is developing a new membrane material because membrane surface is where the fouling is occurring. A membrane can be categorized to two type; hydrophilic and hydrophobic membranes. The relationship between hydrophilicity and fouling rate was found that hydrophilic membranes have slow fouling rate (Futamura et al., 1994). The simple method of changing membrane hydrophilicity is surface treatment such as ozone or plasma coating (Kawakami et al., 1984; Sainbayar et al., 2001). However, the stability of coating method is questionable because a membrane must be washed with concentrated chlorine for washing. Recently, addition of graphene oxide (GO) on hydrophobic membrane enhanced anti-biofouling effect and increased its hydrophilicity as shown in Figure II-2. According to Lee et al., anti-biofouling effect is caused be charge-charge repulsion between negatively charged membrane surface and EPS, SMP, and microorganisms.

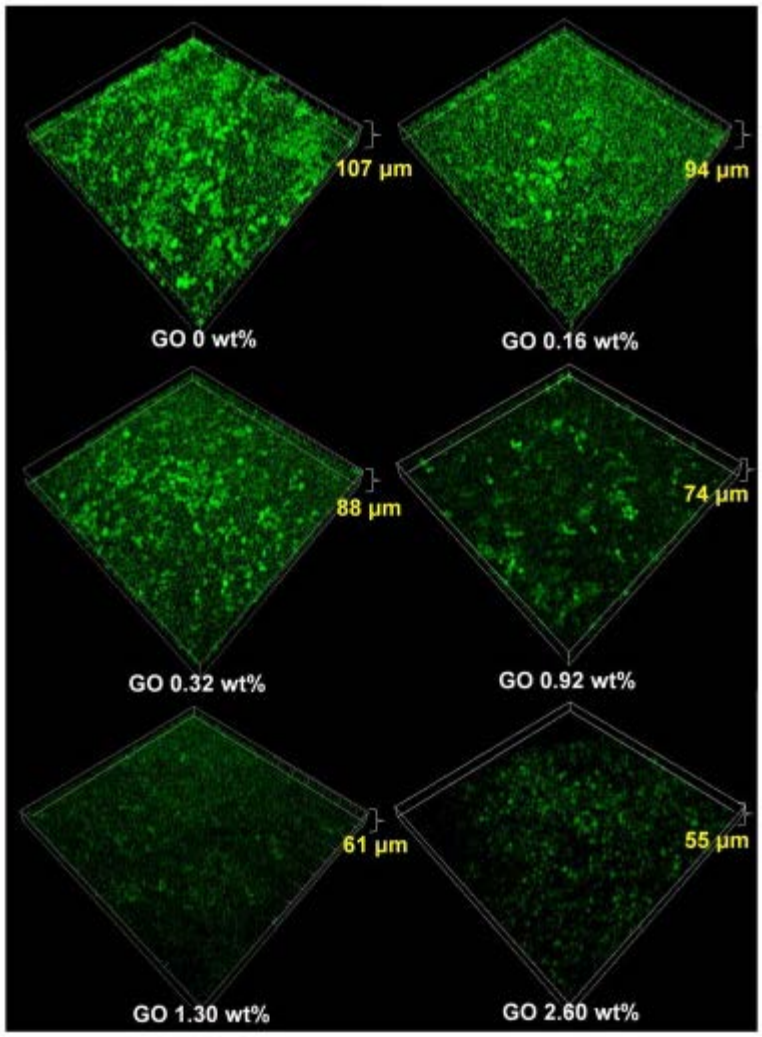


Figure II-2. Anti-biofouling effect of GO composite membrane

II.2.4. Biological Approach

Another fundamental approach is targeting a microorganisms in a MBRs because they not only produced EPS and SMP but also make a biofilm on the membrane surface. Recently, extensive research has been pursued to examine the applicable methods to prevent or mitigate membrane biofouling (Xiong and Liu, 2010). Nitric oxide (NO) is biologically important gas molecule for the growth regulation of both human cell and microorganisms (Sarti et al., 2002). Addition of NO at a low concentration caused dispersion of *P. aeruginosa* biofilm without harming the bacteria (Barraud et al., 2006). Also, its ability of inducing a dispersal of biofilm was also confirmed with multi-species experiments that it might be a suitable for MBR process (Barraud et al., 2009). But its low solubility and stability in water hindered application of direct addition of NO in MBR (Wang et al., 2005).

The most powerful method of biofilm prevention is killing microorganisms on the membrane surface; usage of hydrolytic enzymes of cell wall have shown to be able to not only reduce biofilm but also hinder attachment of microorganisms (Xiong and Liu, 2010). But hydrolytic enzymes are very sensitive to pH and temperature which cannot be well controlled in MBR process. An energy uncoupling method was suggested to be effective way of inhibiting biofilm formation by disrupting ATP synthesis process. Most energy uncouplers are chemical such as 3,3',4',5-tetrachlorosalicylanilide (TCS), dinitrophenol (DNP), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Fletcher, 1977; Klebensberger et al., 2006; Xu and Liu, 2010). But chemical uncouplers are aromatic compounds that can be potentially toxic to human. The novel technique called quorum quenching will be discussed in detail.

II.3. Quorum Sensing (QS) System

The QS is known as cell-to-cell communication between bacteria: when the concentration of QS molecule exceeds a certain thresh hold, they act as a group. Microorganisms produce “an extensive repertoire of secondary metabolites” such as QS molecules to survive in their environment (Keller and Surette, 2006). Most of biological approaches are accompanied by death or slowdown of growth of microorganisms. In 2008, the first paper on application of quorum quenching (QQ) was introduced that QQ is the process of disrupting QS system in MBR (Yeon et al., 2008). The QQ technique is very fascinating that it does not interfere with the process of biological degradation of contaminants and the growth of microorganisms. The importance of QS in biofilm is explored by the researchers in various fields of science.

II.3.1. Metabolic Cost of QS Molecules

The QS molecule and proteins related to QS various by different category of microorganisms as shown in Figure II-3. Among variety of QS signal molecules, three types are the most widely studied ones:

[1] N-acyl homoserine lactone (AHL or HSL) for gram-negative bacteria

[2] Oligopeptides (PQS) for gram-positive bacteria

[3] Autoinducer-2 (AI-2) for both gram-negative and –positive bacteria

These QS molecules are produced by bacteria, in other words, the bacteria have to use an energy to produce QS signals. Thus, the opportunity cost for survival and evolution has been studied for a deeper understanding: among 3 most studied QS molecules, AI-2

requires the lowest and PQS requires the highest metabolic cost. Although the metabolic cost is important, the specificity of signal molecule is very important to maximize the efficiency of the QS molecule. Interestingly, a specificity is highest for PQS and lowest for AI-2: specificity compensate metabolic cost (Keller and Surette, 2006). Thus, one sort of QS is not dominant of the other in terms of opportunity cost; as a result, there are many categories and derivatives of QS molecules exist in nature.

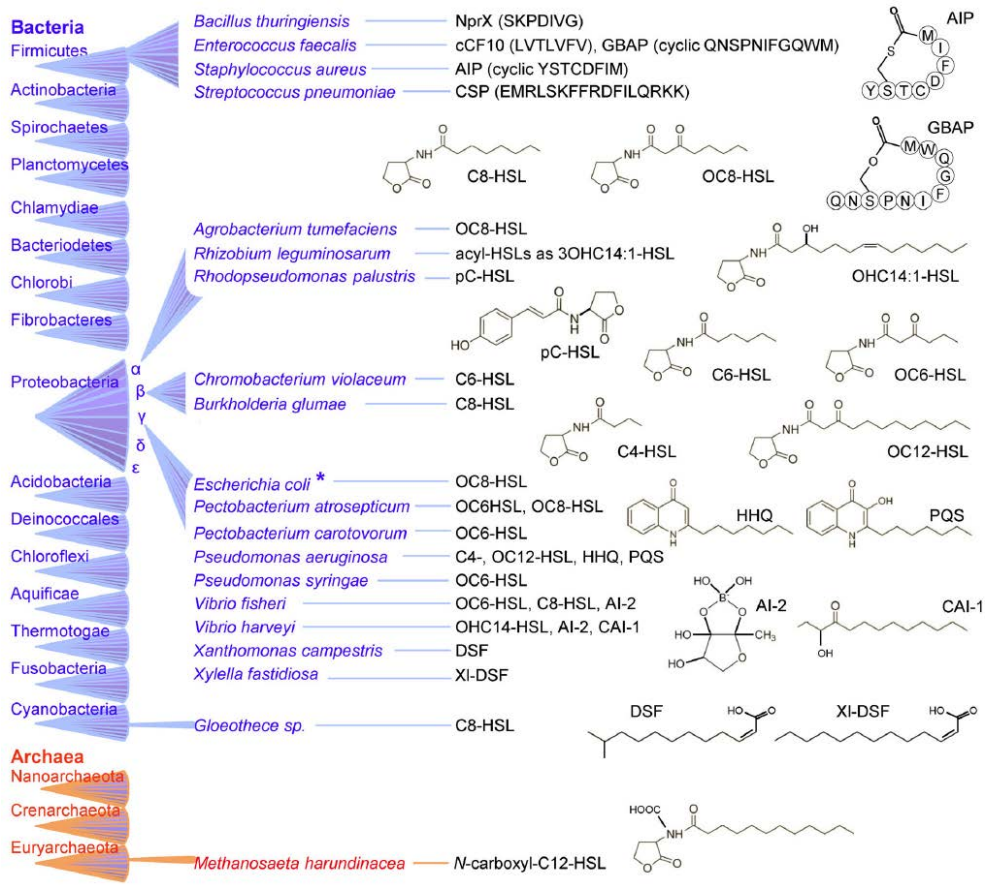


Figure II-3. Various known QS signals of microorganisms

(Grandclément et al., 2015)

II.3.2. Role of QS in Biofilm Formation

Although QS is a molecule used for communication, its “cue” is simple that it either activates a certain protein or not. But such a simple sign could activate different sort of behavior depending on a situation, such as stage of biofilm. The QS circuit is promoted according to the concentration of QS molecule that QS does not work until it reaches a threshold value; therefore QS is kwon for population sensing system of same species (Kreft, 2004). The cell density is higher in biofilm than any other place, and QS activity is active. One of famous bacteria that are making a biofilm in relation to QS is. The factors related to each stage of biofilm formation and the strategy of biofilm control is listed in Table II-2. QQ is one of the option for biofilm control for mutation stage. Another study have shown role of QQ on *Pseudomonas aeruginosa* biofilm as shown in Figure II-4; biofilm with and without lasI which is a gene responsible QS signal uptake showed structural difference that QS and biofilm is related. However, QS does not always worsen the biofilm formation that it promotes dispersion of biofilm as shown in Figure II-5. Thus it is necessary to conduct actual experiment to find out whether QQ will mitigate biofiouling in MBR or not because MBR is consist of numerous species.

Table II-2. Biofilm control strategy of *Pseudomonas aeruginosa*

Stage	Implicated Factors	Regulation Target
I. Reversible attachment	c-di-GMP	Promotion of planktonic life style
II. Irreversible attachment	flagella, adhesins, Psl, Pel	Reduction of initial adhesion
III. Maturation (microcolony)	QS, Psl, Pel, eDNA, rhlA, fimbriae, Lectin A & B	QQ
IV. Maturation (maintenance)	QS, psl, pel, eDNA, rhlA	QQ, antibiotic
V. Dispersion	Rhamnolipids	Promotion of dispersion

Biofilm control strategy of each stage of biofilm formation (Rasamiravaka et al., 2015).

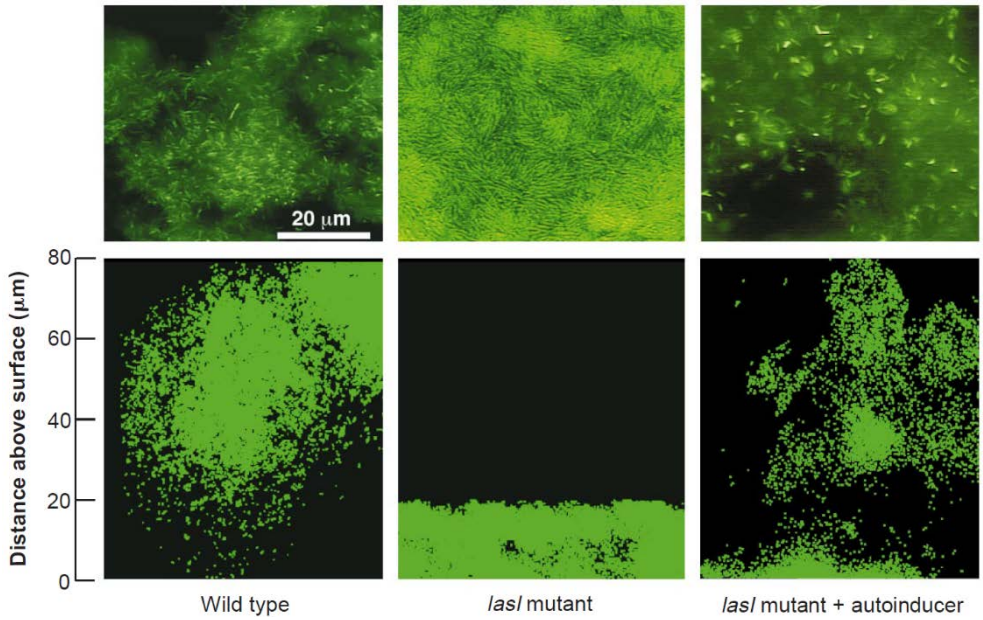


Figure II-4. Structure of Pseudomonas aeruginosa biofilm and role of QS (Davies et al., 1998)

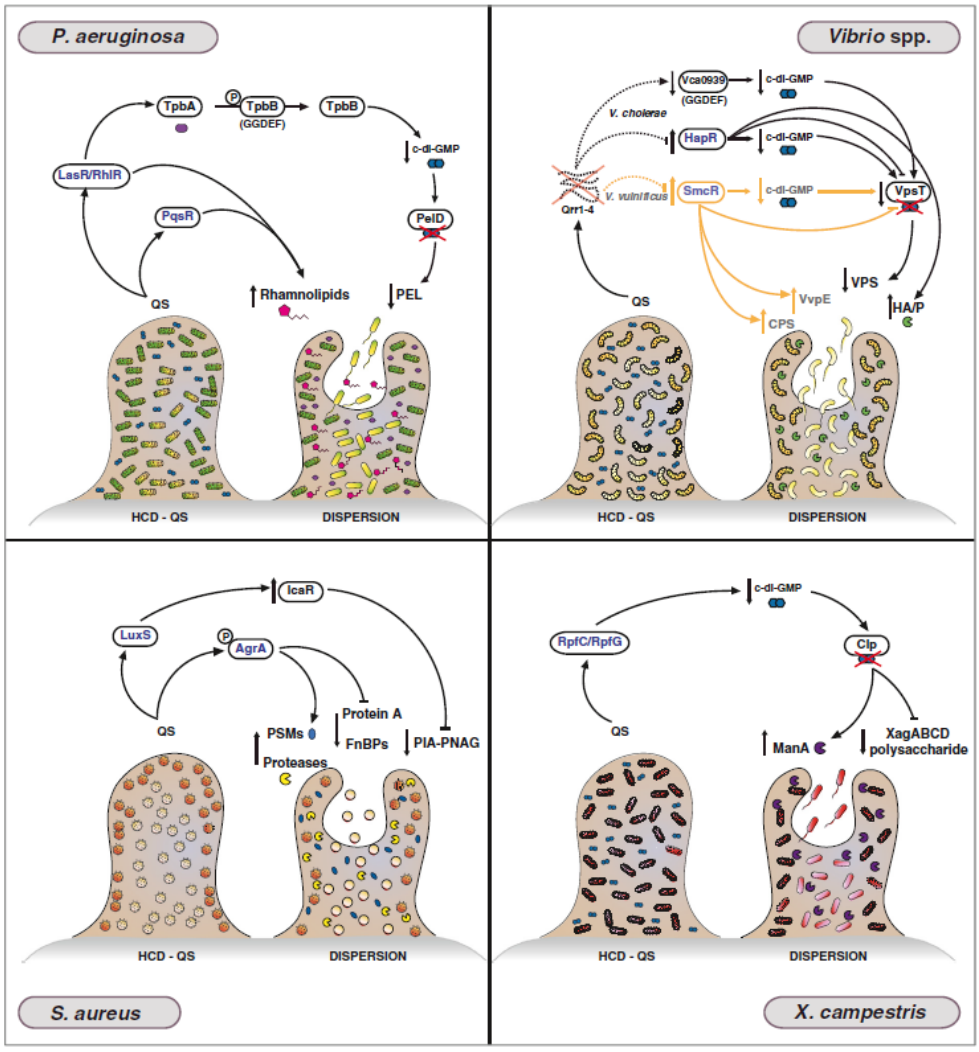


Figure II-5. Schematics of QS induced biofilm dispersion (Solano et al., 2014)

II.4. Quorum Quenching

II.4.1. QQ Molecules and QQ Target Sites

A lot of QQ molecules have been reported based on 4 target sites of QS circuit as shown in Figure II-6. The first target site is QS molecule itself that distortion of QS molecule hinders QS activity. Most of known QQ molecules for this purpose are enzymes and antibodies. In other words, QS molecules generally have low chemical activity. The product of enzymatic QQ of QS molecules are shown in Figure II-7 that QS molecules are reformed or degraded by enzymatic QQ. The second target is QS signal reception which is consist of QS-sensor turn-over and QS-signal/sensor complex formation. The chemicals listed in Figure II-7 have shown that they can reduce QS signal recognition function, thereby reducing QS activity. The third target is QS molecule synthesis, that disconcerting QS signal synthesis gene resulted in low production of QS molecule. The last target is QS-signal transport that mimics or blocking QS transport channel decrease awareness of QS concentration surrounding the bacteria. Interestingly, some mimic compounds are produced by host organisms such as plants (Bauer and Mathesius, 2004). Among 4 targets, it is the best to disturb QS-signal synthesis process because QS signal is produced by bacteria and it will be zero if no bacteria produced a QS signal molecule. But, it is hard to target genes of different organisms living in MBRs. Thus QQ of QS molecule is practical approach in MBR system.

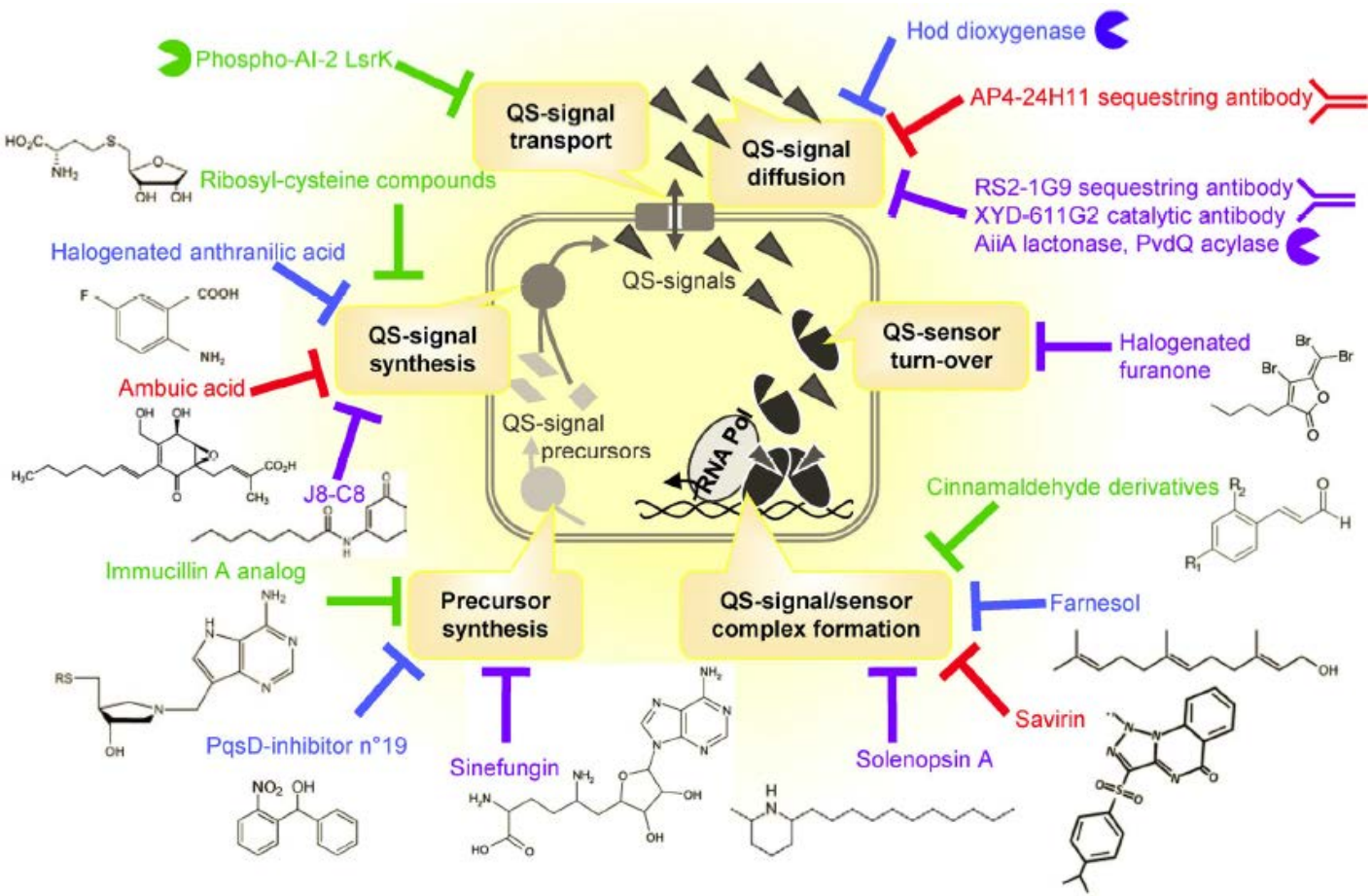


Figure II-6. Reported QQ molecules and its target in QS circuit (Grandclément et al., 2015)

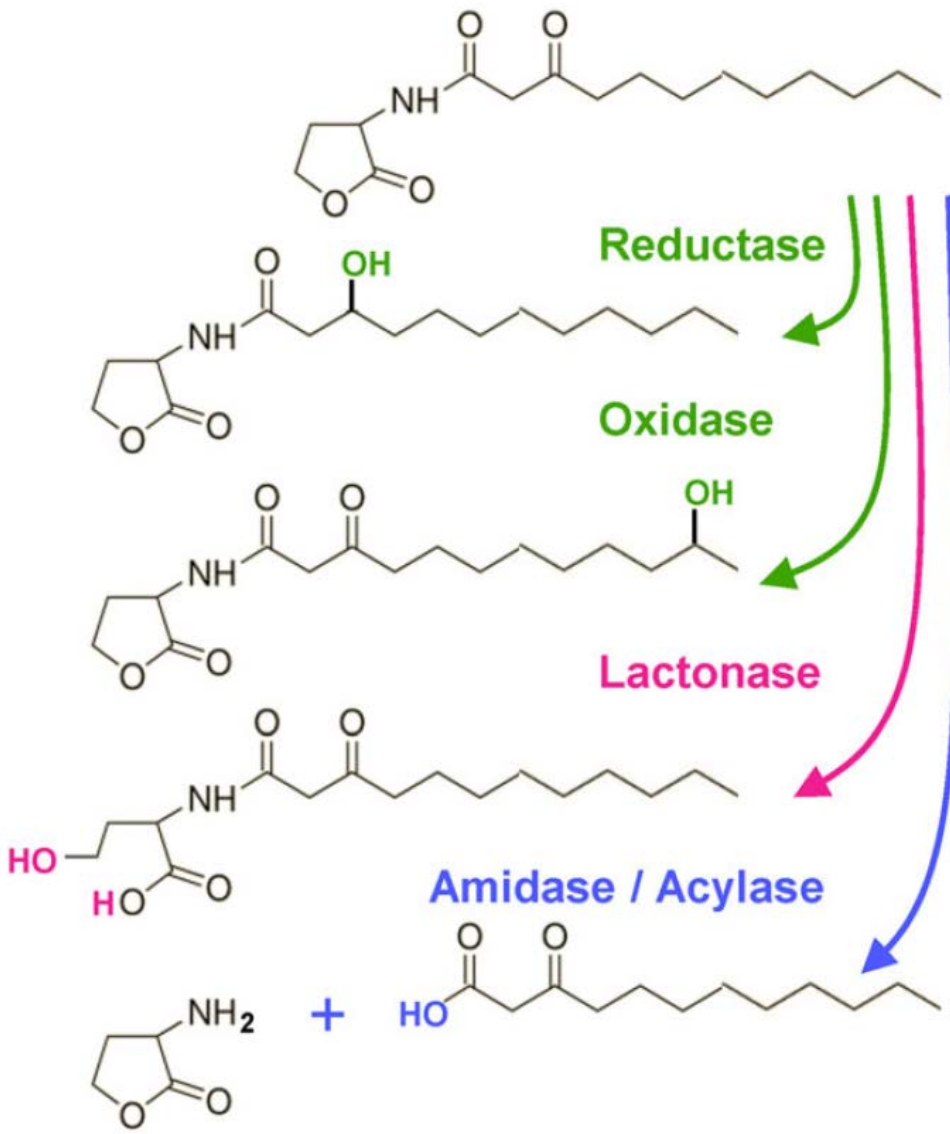


Figure II-7. Mechanism of enzymatic QQ: reductase, oxidase, lactonase and acylase (Grandclément et al., 2015)

II.4.2. Application of QQ in MBRs.

The MBRs are consist of complex microbial community that many different genera of bacteria are found in real MBR plants as shown in Figure II-8. Thus, outcome of applying QQ in real MBR process is questionable. However, a lots of laboratory and pilot scale experiments have shown that QQ can delay a rise-up period of transmembrane pressure (TMP) which is proportional to the degree of membrane fouling. In 2008, the first report of effective QQ application in MBR was reported that addition of acylase which disturbs a structure of QS signal molecule delayed TMP rise-up (Yeon et al., 2008). Different approaches of enzymatic QQ was applied in form of enzyme coated bead carrier (Figure II-10) and enzyme coated membrane (Figure II-11).

However, application of enzyme was not favorable because of the fact that enzyme is not only costly but also very sensitive to environment such as pH and temperature (Battistel et al., 1998). Thus, bacterial QQ was introduced; bacteria QQ is a process of addition of bacteria capable of QQ, such as *Rhodococcus* sp. BH4 by entrapping a bacteria in a carrier (Oh et al., 2012). Later, a new type of carrier, a moving carrier was reported that it not only shown QQ effect but also physical effect of delaying TMP rise-up (Figure II-12.) To verify stability and effectiveness of bacteria QQ in real wastewater plant, a pilot scale test using a real wastewater was conducted. The pilot scale MBR was A/O system, and same wastewater that is fed to real wastewater treatment plant was fed. Yet, QQ effect and the contamination removal efficiency were stable over period of experiment (Lee et al., 2016).

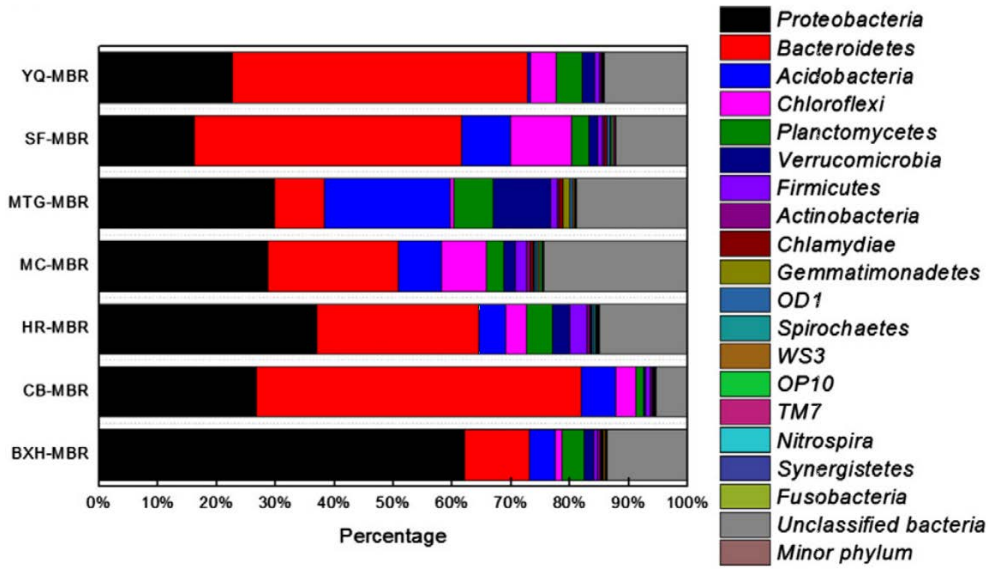


Figure II-8. Composition of microbial community in real waste water plants (Hu et al., 2012)

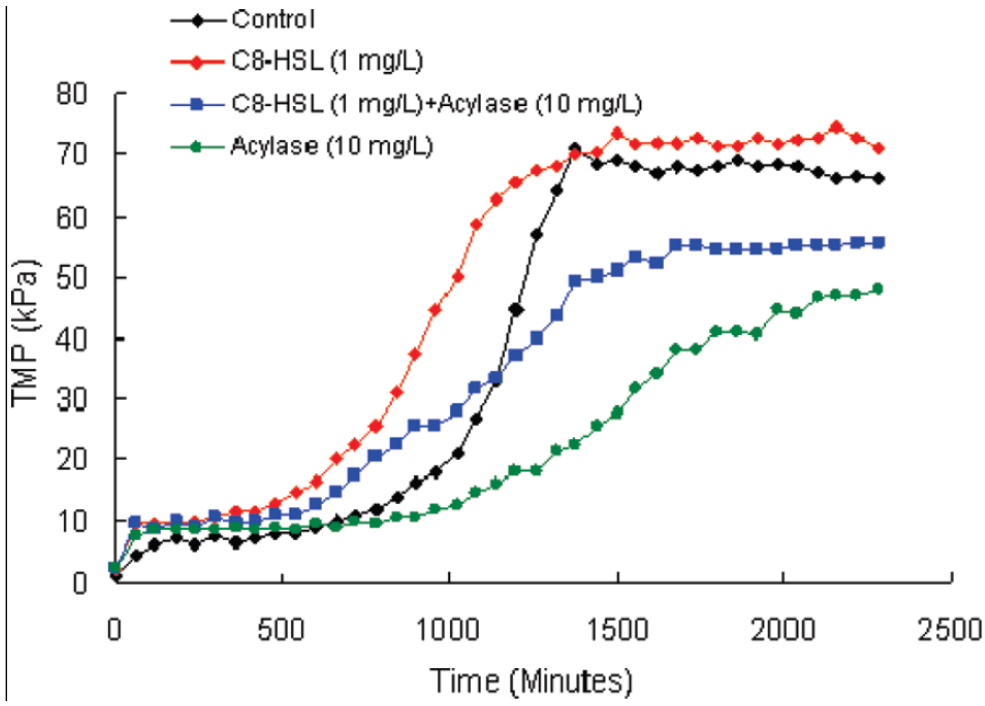


Figure II-9. TMP profile of MBR in response to addition of QS and QQ molecule

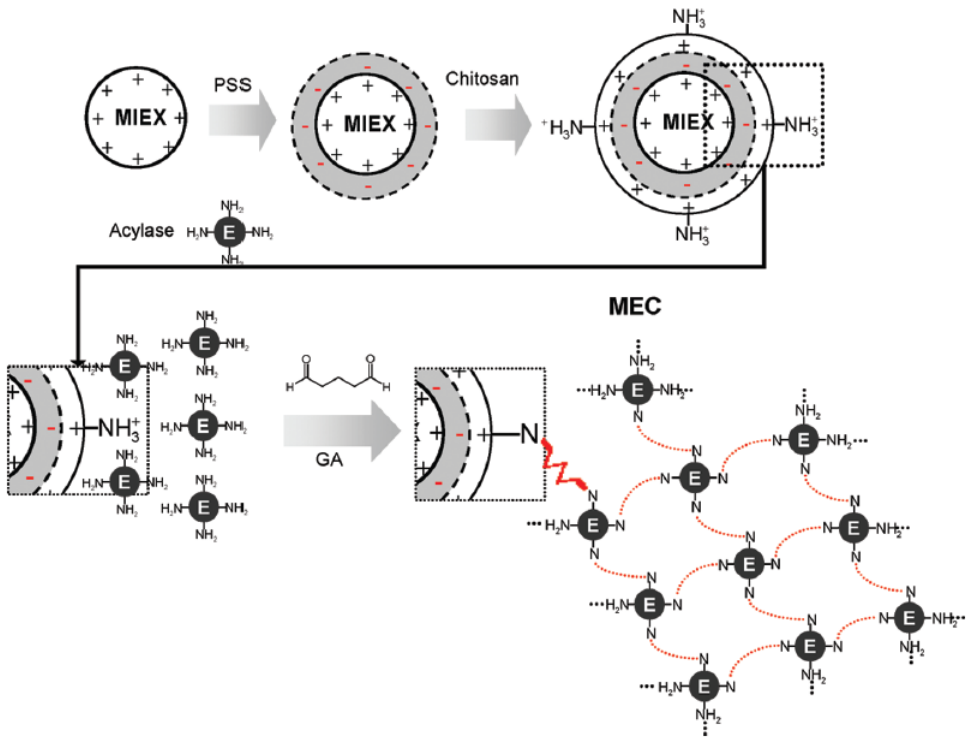


Figure II-10. Schematics of QQ Enzyme coated magnetic carrier (Yeon et al. 2009)

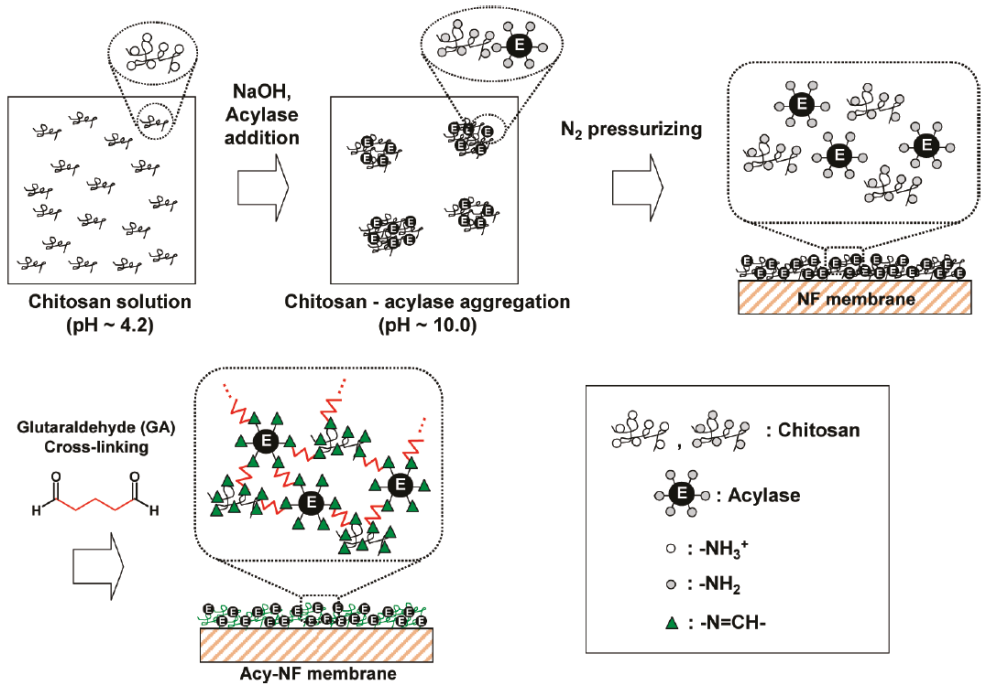


Figure II-11. Schematics of QQ Enzyme immobilized NF membrane (Kim et al., 2011)

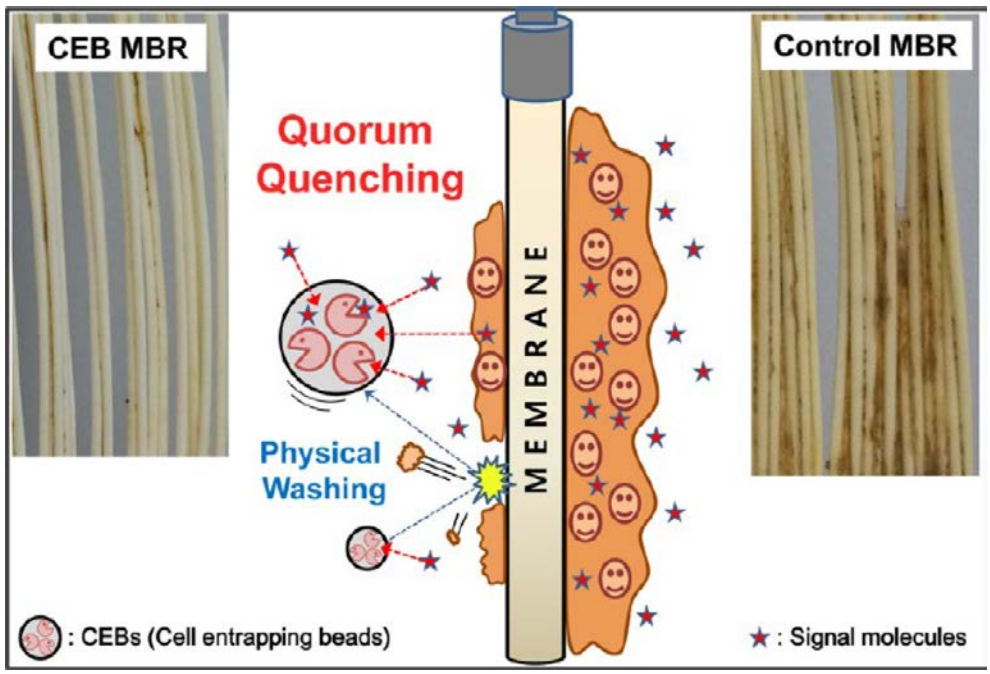


Figure II-12. Schematics of QQ-bead, QQ bacteria entrapping bead (Kim et al., 2013)

II.4.3. QS Signal Distribution

Although the QQ-bead is simple and stable application in MBR, concentration profile of QS signal molecule in MBR is unknown. One research on bacterial QQ and its effect related to its location has been reported (Jahangir et al., 2012). He constructed two bioreactor so that QQ-vessel could be located either in membrane tank or in bioreactor tank. As shown in Figure II-14, QQ effect was increased as recirculation rate was increased and was higher when QQ-vessel was located in membrane tank. The result implies that concentration gradient of QS signal does exist in biofilm because the QQ effect is proportional to the distance between QQ-vessel and membrane and to the rate of recirculation which is the convection of QS molecule from biofilm to the surrounding.

In other words, QQ effect can be improved upon utilization of QS gradient in MBR. Actually, QQ-bead is one of the best approach because it not only physically detach biofilm but also carries endogenous QQ bacteria, the bacteria containing QQ enzyme inside cell, near membrane surface where the concentration of QS signal is the highest. But real MBR plants using a hollow fiber membrane uses such a packed and complicated module as shown in Figure II-15: QQ-carrier containing an endogenous bacteria cannot easily move though hollow fiber membrane module because of packed strand of membrane. Especially, a strand located in center of each bundle is more likely to have fast biofouling rate because the coarse cannot come in contact with membrane surface (Böhm et al., 2012). Although flat sheet membrane module does not have such a problem, it is still necessary to consider hollow fiber membrane because of the fact that 75% of MBRs use hollow fiber membrane (Cote et al., 2012).

There were comparison between intracellular and extracellular enzyme production of biodiesel and the performance varied depending on the condition of environment (Ranganathan et al., 2008). In addition, the concentration of QS signal molecule in MBR should be carefully considered to improve performance of QQ-carriers. As shown in Figure II-16, the concentration of AHL from supernatant of SBR is known to be very small (0 ~ 1 nM) and unsteady overtime of the operation (Tan et al., 2014). In a polymeric gel, such as biofilm, diffusion of solute can be slowed or even confined due to small spacing of matrix and elevated *Donnan potential* of EPS (Decho et al., 2010). In fact, the diffusion coefficient of solutes in biofilm can be slowed according to the molecular weight of solute as shown in Figure II-13 that diffusion coefficient of AHL (MW: 200~300) can be slowed in biofilm. However, the diffusion of the extracellular QQ molecules also could be inhibited by biofilm matrix.

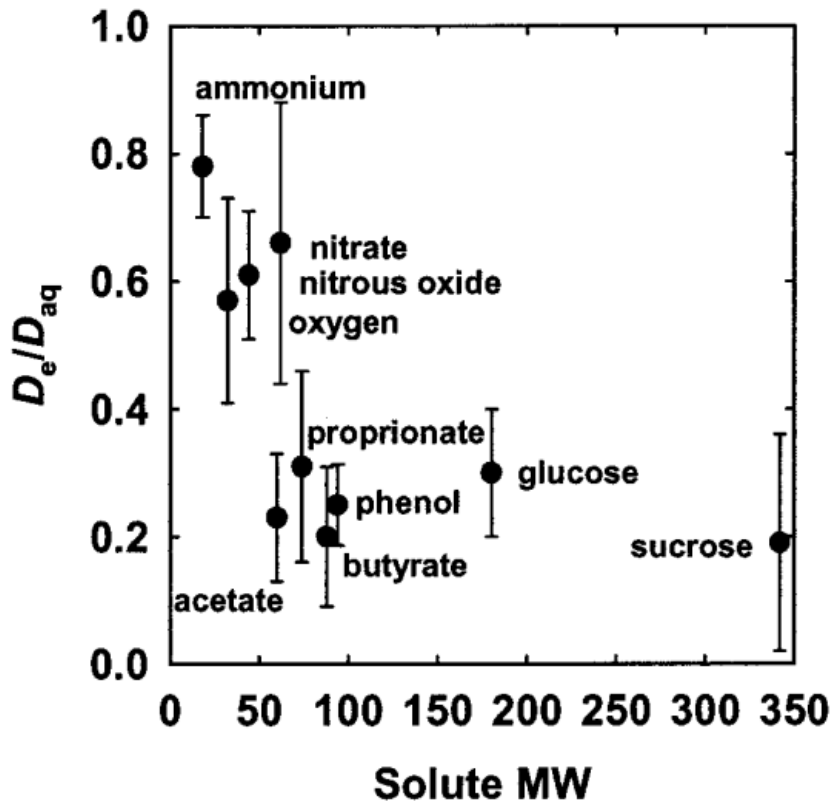


Figure II-13. Relative effective diffusion coefficient of solutes in biofilm (Stewart, 2003)

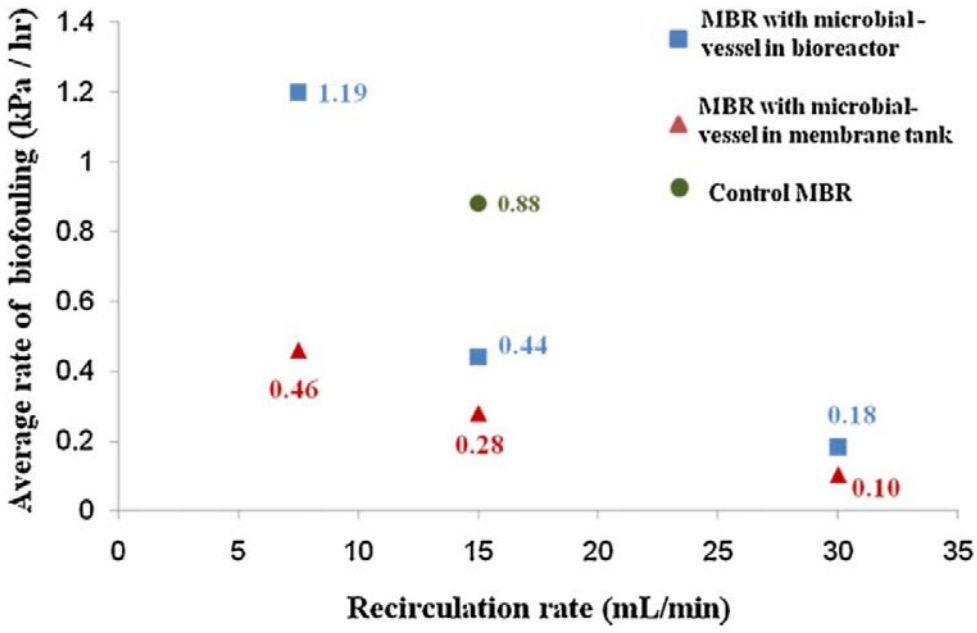


Figure II-14. Biofouling rate as a function of recirculation rate (Jahangir et al., 2012)

Figure II-15. Pictures of Zeeweed hollow fiber membrane module for MBR (Cote et al., 2012)

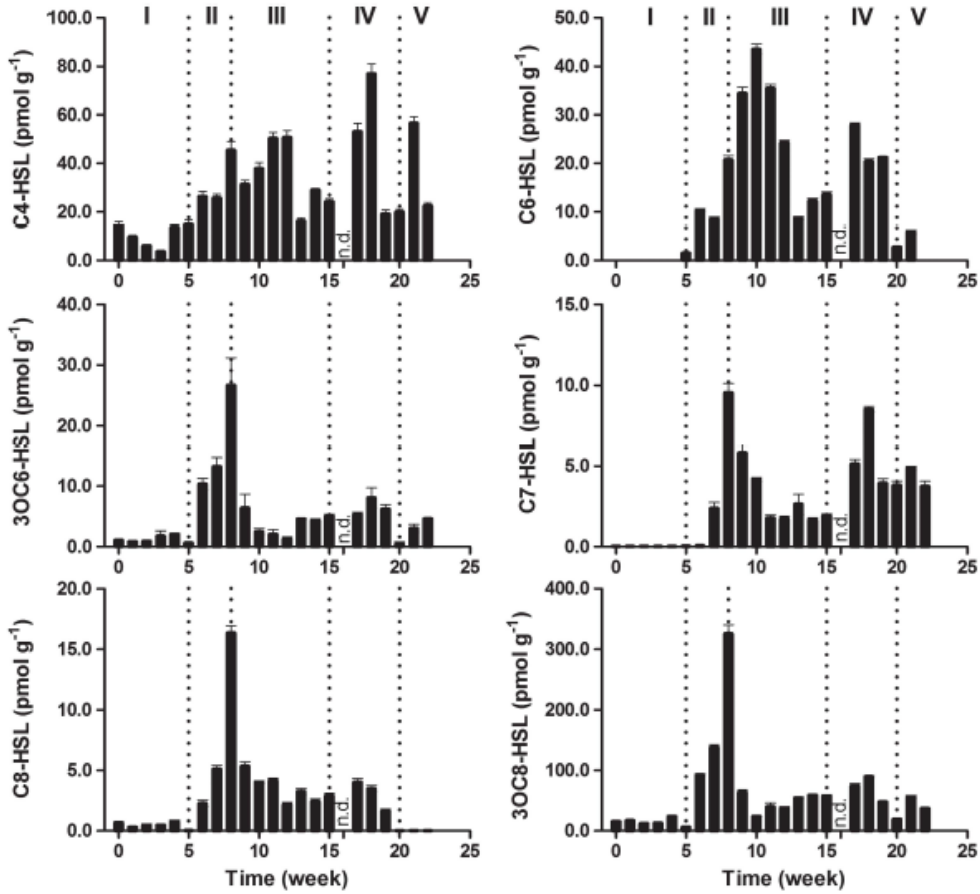


Figure II-16. Concentration of AHL signal molecules per g-cell in MBR permeate (Tan et al., 2014)

Chapter III

Materials and Methods

III.1. Analysis of QS Signal Molecule (AHL)

To concentrate AHL molecules, it was extracted using equivalent volume of ethyl acetate: triple extraction of one third volume used (Wang et al., 2011). For the detection of AHL molecules from the permeate, 1 L of permeate is collected; then, 1 L of ethyl acetate was used to extract AHL from the permeate; then, ethyl acetate was evaporated at 40 ^{o}C using a rotary evaporator; then, solute was re-dissolved in a solution, either 30 mM Tris-buffer or 50 % methanol (methanol/water).

III.1.1. Detection of AHL using LC-MS/MS

The concentrated analyte was examined using LC-MS/MS (Accela 1250 UPLC^{TM} and TSQ Quantum Access Max QQQ, Thermo Fisher Scientific, USA) and commercial AHL molecules were used to find the transition ion with the highest intensity as listed in Table III-1: C6, *N*-Hexanoyl-*L*-HSL; 3-oxo-C6, *N*-(3-oxo-hexanoyl)-*L*-HSL; C7, *N*-Heptanoyl-*L*-HSL; C8, *N*-Octanoyl-*L*-HSL; 3-oxo-C8, *N*-(3-oxo-octanoyl)-*L*-HSL. The flow rate was 0.3 ml min^{-1} (unison UK-C18 column, Imtakt) with linear gradient (10-100%) of water and acetonitrile with 0.1% formic acid. Positive mode and SRM method was used to examine intensity of AHL signal molecule.

III.1.2. Detection of AHL using a bioassay

The analyte was also examined using bioassay, a luminescence method (Lee et al., 2016). Briefly, analyte is dissolved in 50 mM Tris-HCL buffer (pH 7); then,

reporter strain Agrobacterium tumefaciens A136 (Ti-)(pCF218)(pCF372) is diluted to OD 0.1 using LB-broth (with 0.1 v/v% tetracycline and 0.5 v/v% of spectinomycin); then, 95 μL of A136 and 5 μL of analyte is loaded in microwell plate and reacted for 90 minutes at 30 °C; then, 30 μL of Beta-Glo® is added and reacted for 30 minutes; then, luminescence was measured by a luminometer (synergy2, Biotek, U.S.A.). The luminescence and a concentration of standard AHL molecule is used to construct a calibration curve for a quantification of AHL molecule.

The samples were prepared for measuring QQ activity of entrapped BH4 as following. Fabricated carrier was put into 20 mL of 50 mM Tris-buffer containing 200 nM of C8 AHL signal molecule; then, samples were taken at 0, 30, 60, and 120 minutes; then, each samples were raised up to 90 °C in water bath for enzyme deactivation; then, its supernatant was analyzed with bioassay as described above.

Table III-1. The transition ions of AHL signal molecules for LC-MS/MS

AHL	Q1 (m/z)	Q3 (m/z)	Voltage (V)	Retention Time (min)
C6	200.12	56.08	5	6.4
3-oxo-C6	214.10	154.09	17	6.2
C7	214.10	196.02	6	7.4
C8	228.12	57.26	15	8.2
3-oxo-C8	242.12	141.18	6	7.1

III.2. Examination of Backwashed Solution and BH4

III.2.1. QQ activity Test for BH4, mixed in Sludge

The QQ activity was measured using the centrifuged pallets of activated sludge from the MBR. The 10 mL of broth sample was taken before BH4 addition, and right after BH4 addition, 10 hours later BH4 addition and 20 hours later BH4 addition. 250 mg BH4 was added to the MBR that 1 mg BH4 is expected to be presented in 10 mL of sample. Thus 1 mg of pure BH4 pallet was chosen for the comparison. The QQ activity is represented as the degradation rate of 1000 nM C8-HSL in 50 mM Tris-HCl buffer for 30 minutes. The concentration of C8-HSL molecule was measured using a bioassay method described in III.1.2.

III.2.2. Analysis of Backwashed Solution

The membrane operated without backwashing was taken out of the reactor at TMP of 20 kPa. The 50 mL backwashed solution was collected by backwashing a membrane at 40 LMH (13 mL/min) with DI water. Concentration of suspended solid, EPS, and AHL were measured according to the methods described above. For the measurement concentration of AHL, solution was extracted with ethyl alcohol for 100 times concentration using a method described above.

III.3. MBR Operating Conditions

III.3.1. Characterization of MBR

The concentration of MLSS was measured by the standard method that dry weight of 5 ml of broth sample on 47 mm glass microfiber filter was measured (Association and Association, 1981). The chemical oxygen demand (COD) was measured by spectrometry with reagent kits (Hach, USA); briefly, 2 mL of permeate and a kit is reacted at 150 ^{o}C for 2 hours; then, cooled to room temperature; then, DR/4000U spectrophotometer (Hach, USA) was used for the measurement. Average floc size (volume) was measured by Mastersizer 2000 (Malvern, UK).

The preparation of samples for measurement of the concentration of EPS (extracellular polymeric substances) were conducted as the reported procedure (Lee et al., 2016). Briefly, samples containing particles and cells were centrifuged at 6000 rpm for 10 minutes; then, supernatant was filtered through a 0.45 μm syringe filter. And concentration of protein and polysaccharide in filtered sample were measured according to Bradford assay and the phenol-sulfuric acid method, respectively (Dubois et al., 1956).

III.3.2. Module Fabrication

The commercial hollow fiber with pore size of 0.04 μm was used: poly(vinylidene) fluoride (PVDF), Lp_0=1300 L/(m^2 h bar), ZeeWeed 500, GE-Zenon, USA. Each module have 25 strands of 13 cm of hollow fibers, having

0.0194 m^2 of total effective filtration area. The 3-D printer (Stratasys Object 30, USA) was used to fabricate parts for multilayer module assembly. The dimensions and shapes of each printed parts are described in Figure III-1. Also a visual comparison of a single-layer module and multi-layer module is shown in Figure III-2 that total effective filtration area were same.

III.3.3. MBR operating parameters

Two lab-scale submerged aerobic MBRs with 2.5 L of working volume were operated either with or without backwashing. The real wastewater feed was obtained from the cafeteria of Seoul National University, Korea. The HRT was fixed to 8 hours, resulting a flowrate of feed to be 5.2 ml/min. A peristaltic pump was used for both feed and suction pump. The air was supplied at the bottom of reactor from the circular airstone disk, which covers entire cross-sectional area of the reactor, at rate of 1.2 L/min. A digital TMP gauge was used to receive real-time data which is then recorded by LabVIEW: averaged value of 300 points which was taken every 6 seconds was recorded. When backwashing was applied, twice of suction flowrate is applied (20 minutes suction and 30 seconds backwashing) without addition of chlorine in backwashing step. The diagram of MBR reactor is described in Figure III-3. The chemical oxygen demand (COD) of feed was ranged from 120 to 250 mg/L.

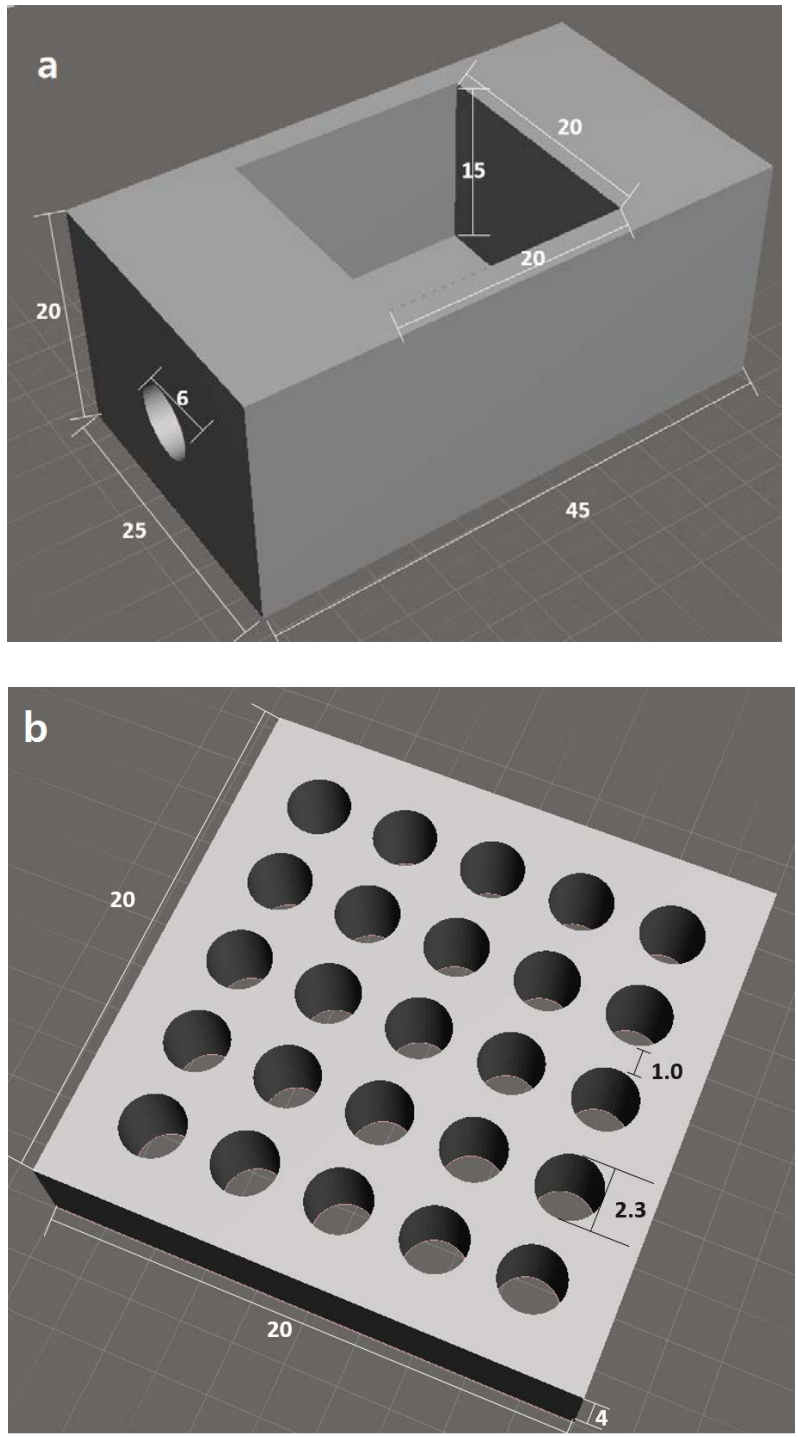


Figure III-1. 3-D printed parts for multi-layer module assembly

(a) the part where one point suction can be applied to 25 fibers

(b) the spacer to keep each fiber in same distance (mm).

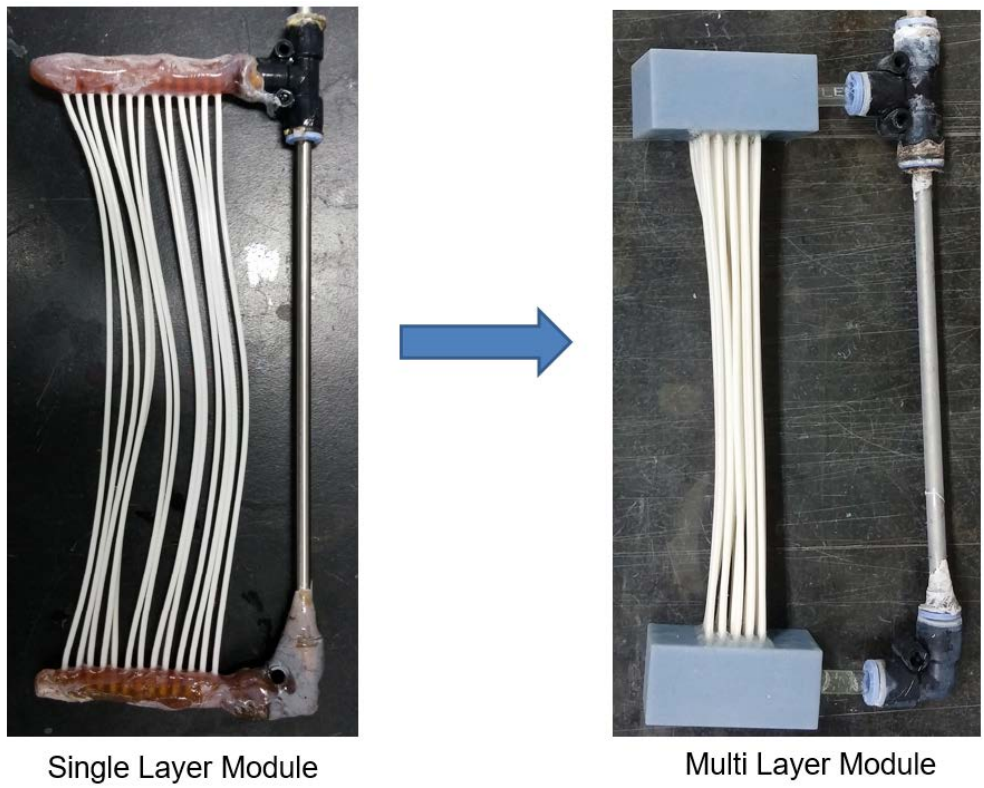


Figure III-2. Visual comparison of single and multi-layer module

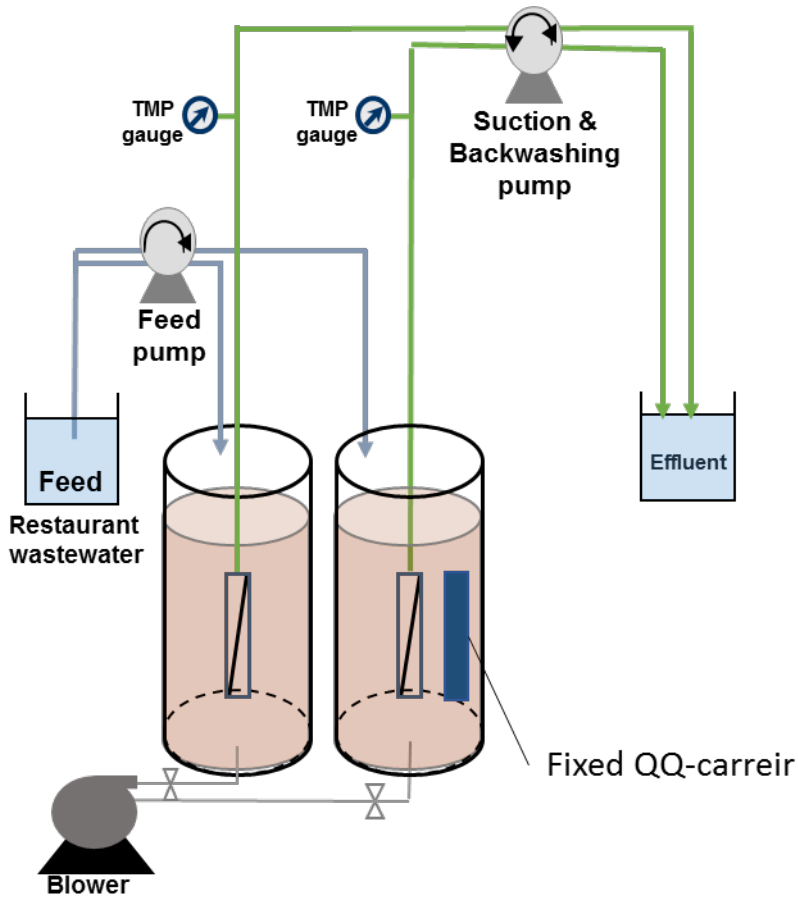


Figure III-3. Schematics of MBR system

Chapter IV

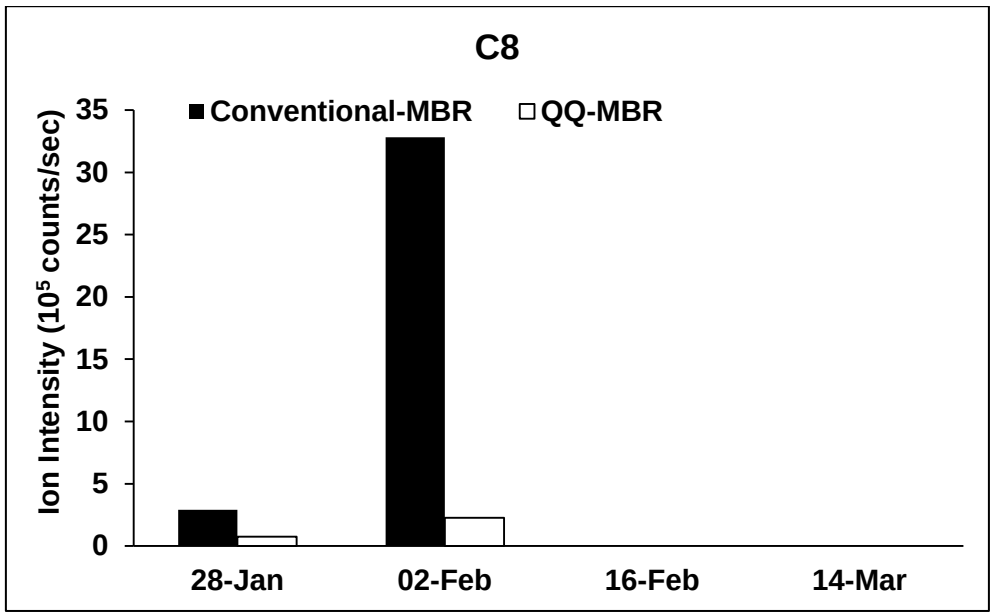
Results and Discussion

IV.1. Analysis of AHLs in MBR Permeate

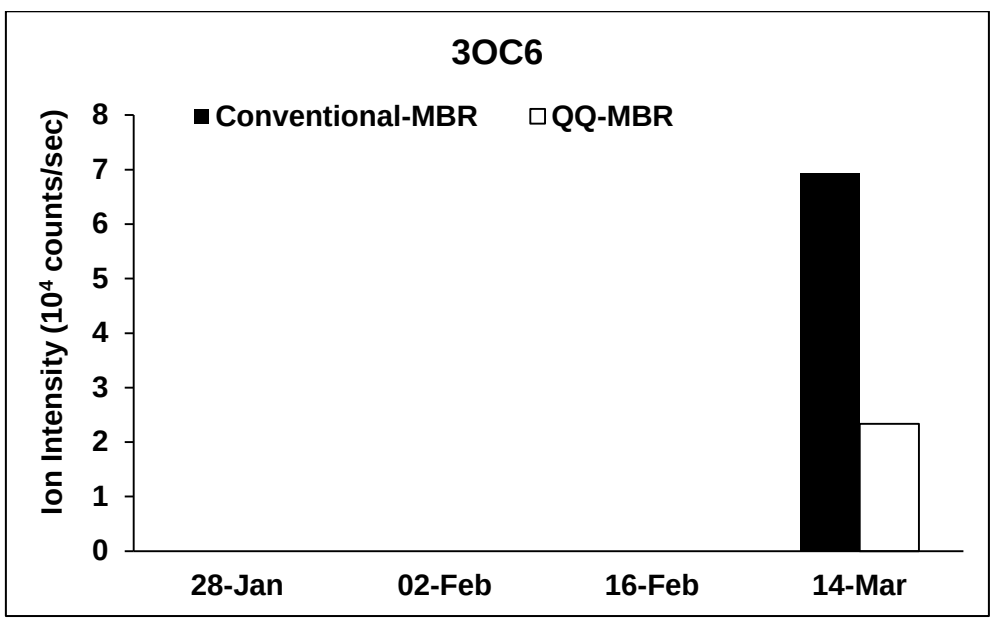
Although the previous studies have used QQ enzyme or QQ bacteria for the mitigation of biofouling on the membrane surface, a real time observation of the concentration of QS signal molecule was not presented (Kim et al., 2013; Lee et al., 2014; Lee et al., 2016; Yeon et al., 2009). Recently, a method for monitoring a concentration of AHLs were introduced that permeate can be used for extraction of AHLs without disrupting MBR operation (Yu et al., 2016). Before applying a multi-layer membrane module in MBR system, the correlation between the concentrations of AHLs in permeate and TMP of MBR was examined.

As described in section III.1.1., 5 AHL molecules were monitored using LC-MS/MS from the permeate of the MBR operated with single layer hollow fiber module and fed with synthetic wastewater. The permeate was chosen over the supernatant of the MBR broth sample because the concentration of AHL is expected to be same as that of permeate and over 1000 times concentration of AHL molecule was necessary for the detection and the large volume of permeate could be easily obtained (Tan et al., 2014; Yu et al., 2016). Although the permeate solution was concentrated 1000 times, only two AHL molecules, *N*-(3-oxo-hexanoyl)-*L*-HSL (3-oxo-C6) and *N*-Octanoyl-*L*-HSL (C8), were detected as shown in Figure IV-1. Although the quantification of each AHL molecule signals were not conducted, the degree of difference in the intensity of AHL molecule between conventional-MBR and QQ-MBR is very clear for both C8 and 3-oxo-C6. The reduced concentration of AHLs in MBR permeate upon introduction of BH4 strain was firstly observed in this study.

In addition, the intensity of AHL molecule and the TMP of the MBR were not correlated as shown in Table IV-1 that highest amount of AHL was detected on February 2nd while the lowest TMP was recorded on the same day. And none of five AHL molecules were detected on the February 16th. Thus there is little correlation between TMP and the concentration of AHL in MBR permeate. This result implies that the increase in concentration of AHLs in biofilm formed on membrane surface could be contained within biofilm matrix (Decho et al., 2010; Emerenini et al., 2015; Yeon et al., 2008). The examination of QQ efficiency in multi-layer module where the fouling is severe at inner part of module should be performed to be able to apply QQ technique to MBR with a hollow fiber module.



(a)



(b)

Figure IV-1. Ion intensity of AHL molecules from the MBR permeate.

(a) *N*-Octanoyl-*L*-HSL (C8) and (b) *N*-(3-oxo-hexanoyl)-*L*-HSL (3-oxo-C6)

Table IV-1. The value of TMP at the sampling date.

Day	Conventional-MBR (kPa)	QQ-MBR (kPa)
01/28	33	5
02/02	5	6
02/16	5	9
03/04	7	4

IV.2. Application of QQ-Beads in MBR with Multi-Layer Hollow Fiber Module

The multi-layer hollow fiber module was made of 25 fibers (5 by 5) with 1 mm distance (same as module made of hollow fiber provider) between each fibers. The flux was 25 LMH and the resulting permeate flow rate was 8 mL/min. Since the study of QQ effect on multi-layer hollow fiber was not reported, the most recent application method of QQ bacteria, the QQ-bead entrapping *Rhodococcus* sp. BH4 was applied to MBR with multi-layer hollow fiber module for the first trial (Lee et al., 2016). As shown in Figure IV-2, a little delaying of TMP-rise up was observed at the first TMP jump, but it too small that QQ-beads were replaced to MBR1 from MBR2 to reproduce QQ effect (0 – 4 days). During the second TMP rise-up (4 – 6 days), delay of TMP rise-up was not observed and QQ-beads were replaced to MBR2 again. Last two TMP rise-up (6 – 8 days) also did not show significant delay in TMP rise-up. Although total of 4 TMP rise-up was observed, no QQ effect was observed. As discussed in a previous section, the concentration of AHLs could be higher at membrane surface but QQ-beads could not get into the inner part of multi-layer module because the spacing of module (1 mm) was much smaller than the diameter of QQ-beads (4 mm). Thus difference in efficiency of QQ-beads in this study and the previous study might have been caused by the portion of membrane area that QQ-bead could come in contact.

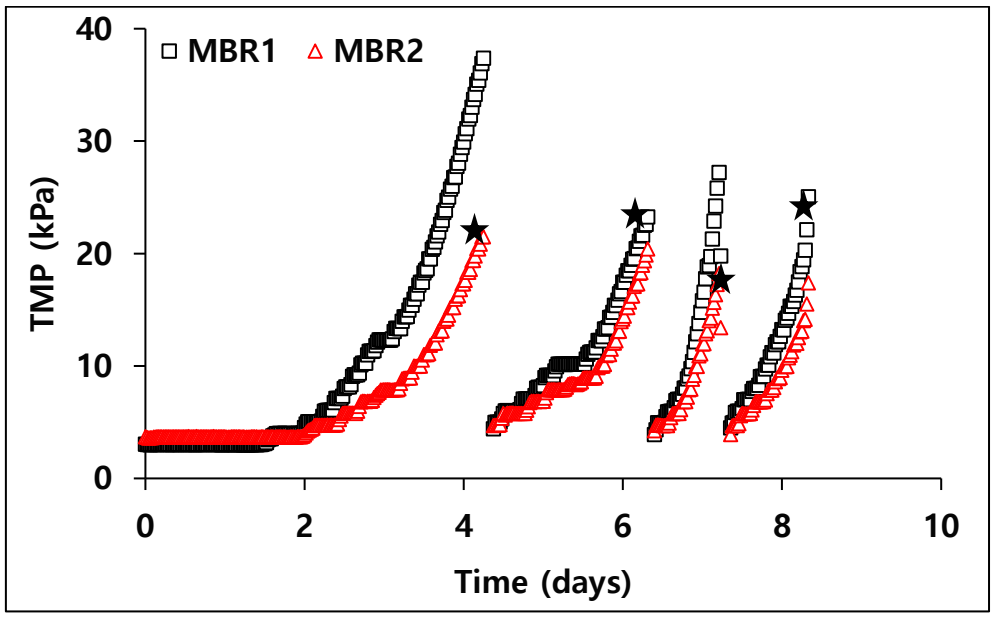


Figure IV-2. TMP profile for multi-layer module with BH4-QQ-beads

QQ-beads were initially placed in MBR2 then removed from MBR2 and placed in MBR1 and repeated switching locations once both MBR reached TMP of 20 kPa.

IV.3. Biofilm Mitigation Approaches for MBR with Multi-Layer Hollow Fiber Module

In order to make a QQ-bead to reach inner part of multi-layer module, it was necessary to come up with another methods of applying QQ bacteria because it is hard to make a QQ-beads with diameter smaller than 1 mm. Also, the difference of QQ performance according to the specific location of QQ-vessel was reported (Jahangir et al., 2012). Two methods of quenching a QS molecule were proposed: let BH4 freely swim to inner part of multi-layer hollow fiber module or apply an external force to distribute foulants and QS molecules from inner part of multi-layer hollow fiber module.

IV.3.1. Direct Addition of Raw BH4 Strain into MBR

The direct addition of unentrapped BH4 strain was conducted that raw BH4 strain might be able to interact with the microorganisms on membrane surface directly. But, the growth rate of BH4 in MBR is unknown and is hard to determine because massive gene sequencing should be accompanied in order to separately analyze amount of BH4 (125 mg) from other organisms presented in MBR. It was assumed that amount of active BH4 in MBR will not increase or decrease during the operation period. Also, excess sludge was removed every day and 4.2 mg of BH4 is expected to be removed with this process. To ensure same amount of BH4 to be presented in MBR broth, additional 4.2 mg of BH4 was added to MBR. As

shown in Figure IV-3, the delay of TMP rise-up in QQ-MBR compared to conventional-MBR where BH4 was not added was not observed. It was unclear whether low QQ activity of BH4 is a property of multi-layer module or there were problems associated with the way of applying bacteria to MBR. Thus, same experiment was carried out with single layer module which have already shown a good QQ activity with entrapped BH4 (Kim et al., 2013).

As shown in Figure IV-4, delay of TMP rise-up was not observed again. Instead, QQ-MBR showed faster TMP rise-up trend which implied the death of BH4 strain because the debris of the dead microorganism is in charge of faster TMP rise-up (Luintel and Xu, 2010). In other words, low QQ activity of BH4 is caused by the way of applying BH4 that BH4 strain might having a hard time adapting to MBR system along with other microorganisms. Table IV-2 describes relative amounts of *Rhodococcus* genus in 10 real wastewater plants that data was retrieved from reported database (Jo et al., 2016). The average relative proportion was only 0.012% which implies that *Rhodococcus* genus is not a competitive bacteria genus in MBRs. Thus, the viability of BH4 in MBR could be very low.

It is difficult to monitor how BH4 is adapting in MBR in real time. Instead direct observation of BH4, the QQ activity was monitored. As shown in Figure IV-5, activated sludge already shows QQ activity that it could be QQ activity from unknown bacteria in sludge mixture or the adsorption of C8-HSl to sludge surface. As BH4 was introduced to sludge mixture, the QQ activity of activated sludge and BH4 mixture exhibited higher QQ activity than that of activated sludge alone. But the QQ activity of BH4 and activated sludge mixture decreased over short period

of time which might indicate the failure of BH4 strain to adapt in MBR mixture. Interestingly, the equal amount of pure BH4 pallet (1 mg) showed much higher QQ activity than that of BH4 and activated sludge mixture at 0 hours.

Thus, the encapsulation of BH4 is inevitable for achieving a stable QQ effect that entrapment could increase the survival rate of bacteria by protecting bacteria from external stresses such as temperature, antibiotic chemicals and bacteriophage (Rao et al., 1989; Shah and Ravula, 2000; Sheu and Marshal, 1993). It is not clear which component in activated sludge and MBR prevents BH4 from actively degrading AHLs, but surely stable performance of entrapped BH4 have been reported by lots of researchers.

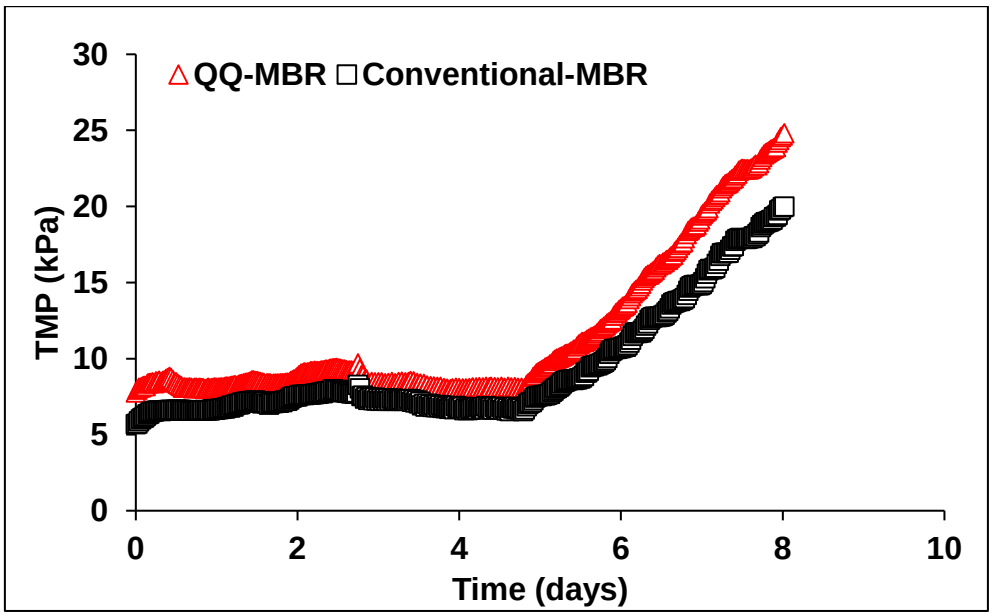


Figure IV-3. TMP profile for multi-layer module with directly applied BH4

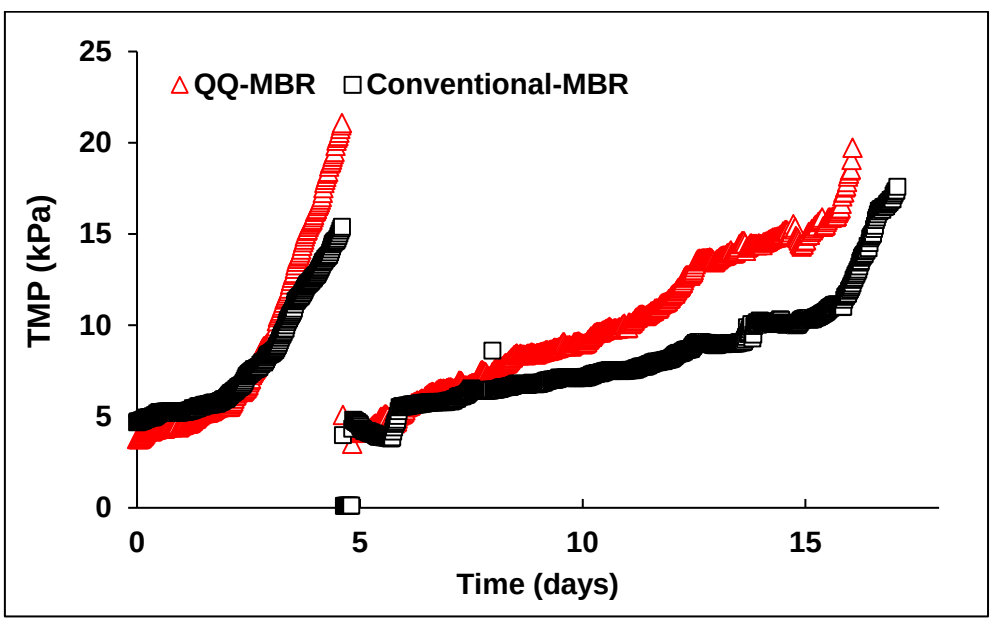


Figure IV-4. TMP profile for single-layer module with directly applied BH4

Table IV-2. Relative proportion (%) of *Rhodococcus* genus in the activated sludge from 10 full-scale MBRs

Sample	1	2	3	4	5	6	7	8	9	10
%	0.001	0.000	0.002	0.006	0.014	0.002	0.002	0.002	0.009	0.078

The data was adapted from the database of reported study average relative proportion of *Rhodococcus* genus was 0.012%

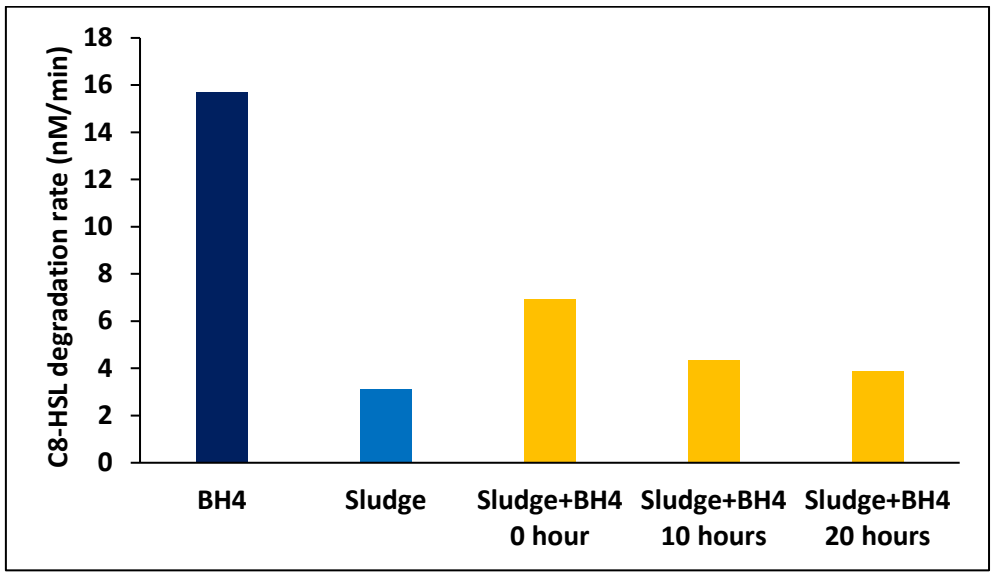


Figure IV-5. QQ activity of activated sludge and mixture of BH4 and sludge.

IV.3.2. QQ Efficiency of fixed QQ-carrier in MBR with a Multi-Layer Hollow Fiber Module

Before applying an external force in multi-layer hollow fiber module, a QQ-carrier was designed to be fixed at certain location in MBR. It was necessary to fix QQ-carrier in order to reduce confusion of beads for the clarification of QQ-effect. Figure IV-6 shows the TMP profiles of the conventional-MBR and the QQ-MBR where 170 mg BH4 was entrapped in QQ-carreir equipped with the multi-layer module. Both QQ-MBR and Conventional-MBR reached TMP of 20 kPa after 4 days of operation that QQ-MBR did not delay TMP rise-up. The possible reasons for disappeared QQ effect is that flocs and particles are more likely to deposit in the inner part of multi-layer module due to reduced convection at inner part of module compare to outer part of the module, as well as the fact that air bubble cannot efficiently remove deposited foulants causing reversible fouling (Böhm et al., 2012). Figure IV-7 is the picture of fouled membrane from the conventional-MBR and the foulants are crowded inside of multi-layer module as suspected.

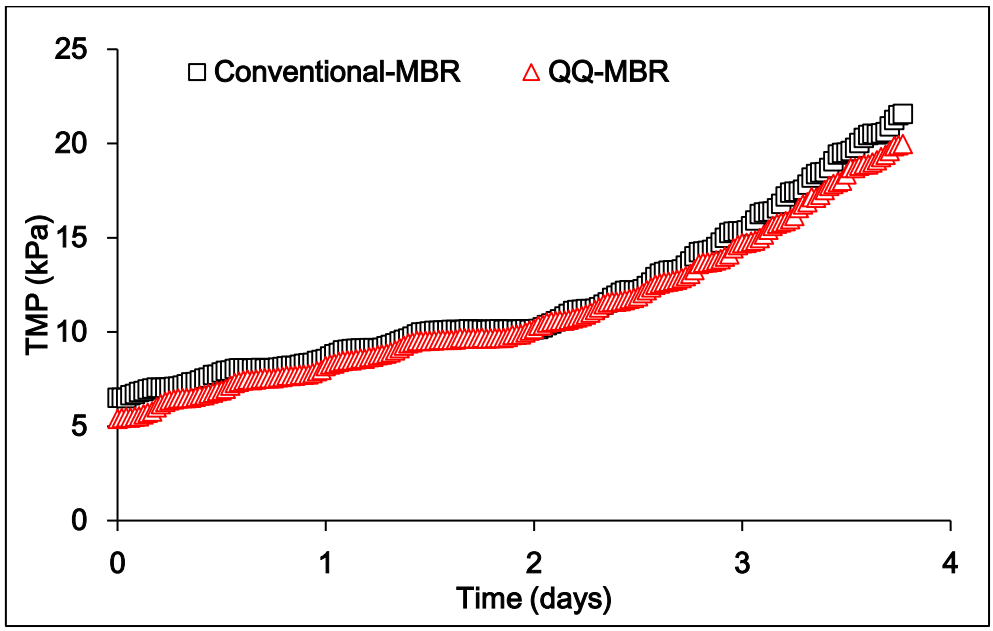


Figure IV-6. TMP profile of multi-layer module

Figure IV-7. Picture of fouled membrane in the conventional-MBR with a multi-layer hollow fiber module

IV.3.3. Quorum Quenching Efficiency under Backwashing

In order to apply external force, backwashing method is applied that some portion of blocked pores are restored by bacwashing, thereby reducing total membrane resistance (Jiang et al., 2003). The characteristics of backwashed solution was reported that soluble EPS of polysaccharide to protein ratio was much less than that of MBR broth (Wu et al., 2008). Thus concentration of EPS, suspended solid (SS) and AHL signal molecule of backwashed solution was examined from two fouled membranes from previous experiment shows the EPS from backwashed solution and the broth of the conventional MBR as shown in Figure IV-9a. The backwashed solution showed different trend of EPS that concentrations of soluble protein and polysaccharide in backwashed solution was higher than that in broth sample as shown in Figure IV-9b. Also, the polysaccharide to protein ratio was much higher in backwashed solution compared to the broth sample. These trend was also observed in previous study; the fouled membrane contains concentrated microbial products compared to the broth and more protein is found at block pores compared to the polysaccharide (Wu et al., 2008). In addition, backwashing could reduce degree of fouling by physically removing mass of fouling substance as shown in Figure IV-10a that considerable amount of suspended solids was detected from backwashed solution. Interestingly, AHL signal molecules were also detected from the backwashed solutions from both membranes as shown in Figure IV-10b: the relative luminescence is proportional to the concentration of AHL signal molecules and detected amounts of relative luminescence from backwashed solution is at the same level of luminescence of

standard C8 AHL molecule between 0 nM and 2.5 nM. Yet, exact amount and type of AHL molecule is unknown because the bioassay detects wide range of AHL molecules and the sensitivity is different from each AHL molecules. Thus, backwashing could not only physically reduce fouling but also distribute AHL signal molecules from the biofilm by convection. These results implies the fact that backwashing could recover performance of QQ-MBR for multi-layer hollow fiber module.

As backwashing was applied to the MBR operation system using a permeate solution, the TMP rise-up of the conventional-MBR was delayed 200% compared to the TMP rise-up time of the conventional-MBR without backwashing as shown in Figure IV-8. This results indicates that the backwashing is successfully applied to MBR system. When backwashing was applied, QQ-MBR was delayed 100% compared to the conventional-MBR as shown in Figure IV-11. Some companies put chlorine and uses a clean water for the backwashing process, but it requires extra instrument and chemical cost. Thus, economic advance of QQ-MBR should be compared with the process having chlorine in backwashing solution. A lot of effort was made to reduce hydraulic hindrance presented inside of hollow fiber module by changing its shape (Ho et al., 2015; Li et al., 2016). Since the performance of the QQ-MBR could be improved by enhanced physical fouling control, combining new module and QQ-MBR might result in improvement of the QQ-MBR.

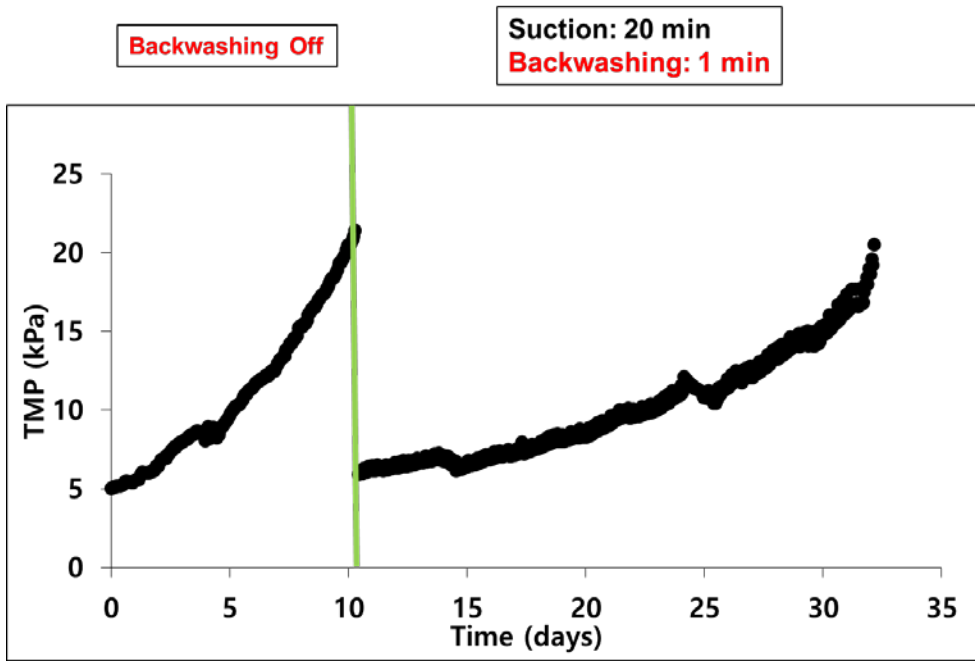
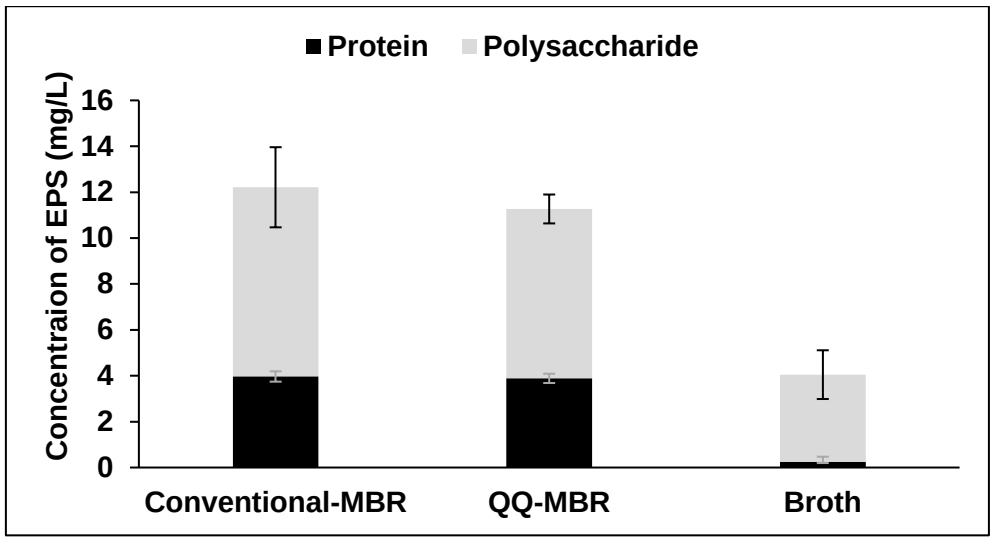
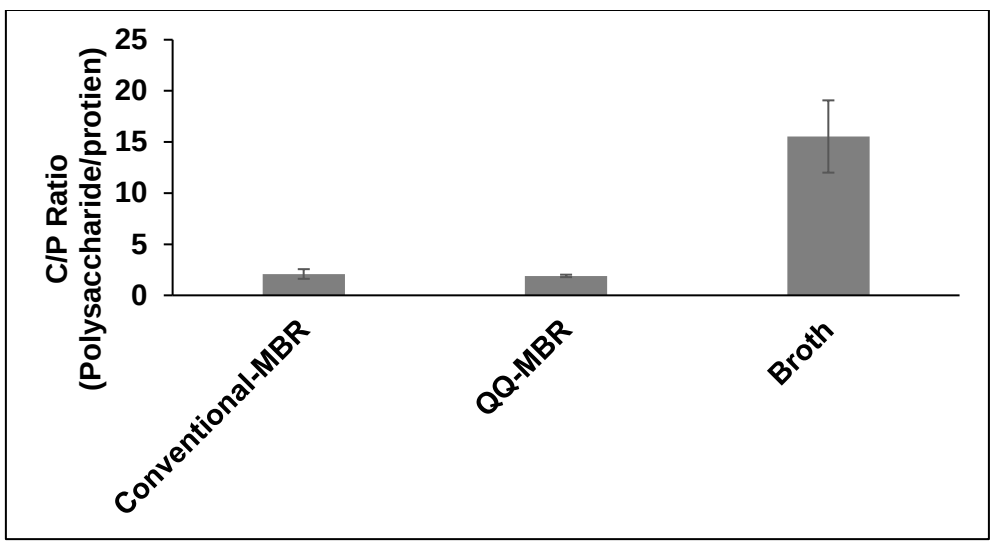


Figure IV-8. TMP profile of conventional-MBR with or without backwashing



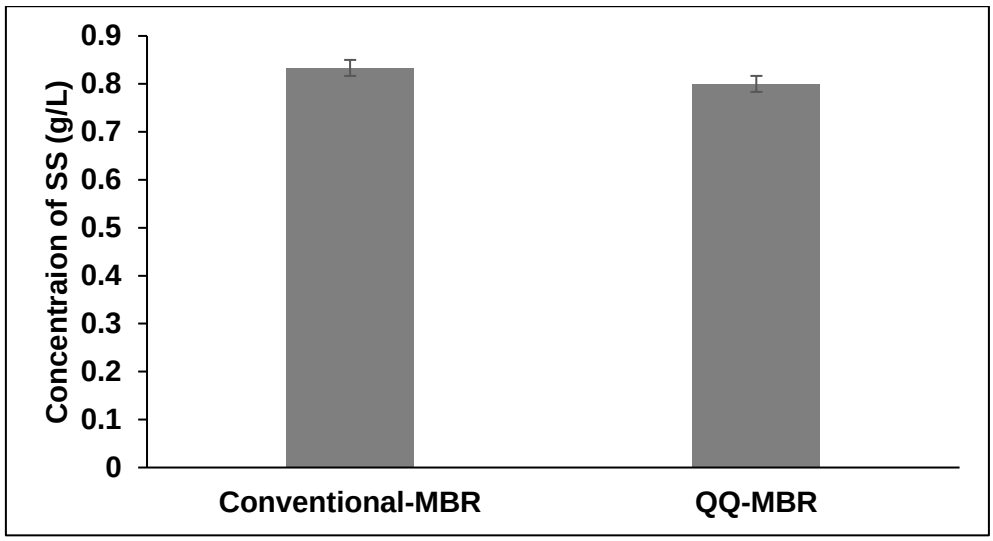
(a)



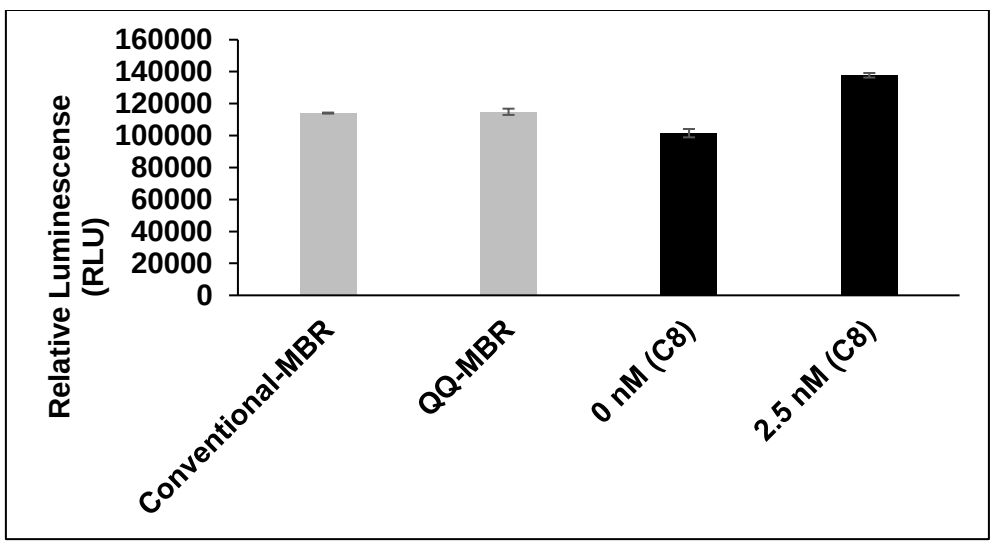
(B)

Figure IV-9. SMP from the backwashed solution and the broth of conventional-MBR.

(a) concentration of soluble protein and polysaccharide (b) polysaccharide to protein ratio



(a)



(B)

Figure IV-10. Detection of AHL and suspended solids from backwashed solution

(a) concentration of suspended solids (b) AHL concentration and the luminescence

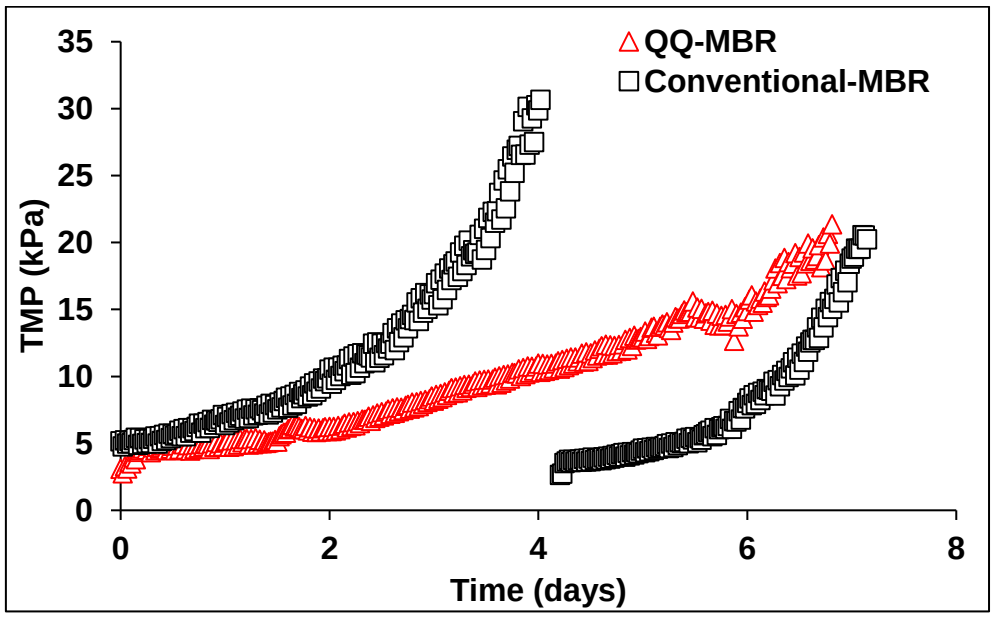


Figure IV-11. TMP profile of MBR with a multi-layer module under backwashing

Chapter V

Conclusion

The objective of this study was to examine efficiency of QQ-MBR with a multi-layer hollow fiber module to simulate change in effect of QQ in real MBR plants with hollow fiber module, and following result were obtained:

(1) Examination of AHL in MBR

- Although concentrations of AHLs in permeate of MBR were irregular, those were always less in permeate of QQ-MBR.
- There were no correlation between TMP and concentrations of AHLs in permeate of MBR.

(2) Direct application of raw BH4 strain in QQ-MBR

- Raw BH4 strain could not delay TMP rise-up.
- QQ activity of raw BH4 strain was decreased upon contact with activated sludge and it was decreased even further as time of contact was increased.

(3) Application of backwashing method to QQ-MBR

- Entrapped BH4 strain could delay TMP rise-up of multi-layer hollow fiber module only when backwashing was accompanied.
- Considerable amount of solids and EPS were detected from the backwashed solution.

국문초록

폐수처리용 다발형 중공사막 생물반응기에 정족수 감지 박테리아 적용

분리막의 오염은 분리막의 수투수도를 감소시킬 뿐만 아니라 수처리용 분리막 생물반응기 (membrane bioreactor, MBR)의 운전 비용을 증가시키는 주요한 원인이다. 분리막 오염을 저감시키기 위하여 생물학적 접근인 정족수 감지 억제 (quorum quenching, QQ)가 간단하며 실현 가능성 높은 방법으로 소개되었는데, QQ는 미생물들 간의 정족수 감지 (quorum sensing, QS)를 방해하는 기술이다.

이전 연구자들은 lactonase를 생산하는 미생물인 *Rhodococcus* sp. BH4 박테리아를 이용한 박테리아 QQ를 도입하였으며, 생물막오염의 정도는 막간차압 (transmembrane pressure, TMP)의 변화를 통하여 나타내었다. 또한 이를 이용한 QQ의 효과는 실폐수를 사용한 pilot-scale의 평막 MBR에서도 확인 되었었다.

본 연구에서는 전제 분리막 생물반응기 전 세계 시장의 약 80% 를 차지하는 중공사막 생물반응기에서의 성능확인을 주안점으로 두었다. 중공사막 모듈은 분리막 사이 간격이 좁은 다발형으로 만들어지게 되는데, 오염원들은 평막 모듈보다 중공사막 이러한 중공사막 모듈에 많이 쌓이게 된다. 이러한 문제를 모사하기 위하여 연구실에서 실험할 수 있는 중공사막 다발형을 실제 모듈의 분리막 사이 간격과 동일하게 하여 제작하였고 이러한 다발형 모듈에 *Rhodococcus* sp. BH4를 적용하였다. 하지만 QQ효과는 기존의 일자형 모듈과

다르게 다발형 모듈에서 나타나지 않게 되었다.

두가지의 새로운 방법들로 다발형 중공사막 모듈에서의 QQ활성을 증대시키기 위하여 시도되었다. 첫번째로 QQ 미생물 (BH4)를 별도의 처리 없이 생물반응기에 집어넣어 QQ 미생물이 비드에 갇히지 않고 자유롭게 반응기 안에서 움직이며 QS 신호분자를 감소 시키도록 하였다. 하지만 이렇게 투입된 BH4는 QQ 활성을 보이지 않았다. 이는 BH4의 활성이 생물반응기 내에서 생존 경쟁에 강한 다른 미생물에 의해 감소되었기 때문으로 추정된다.

두번째 방법은 BH4를 다른 미생물들의 영향으로부터 보호하기 위해 기존 연구와 동일한 재료로 감싸서 적용하되, 역세척 (backwashing) 공정을 추가하여 모듈 안쪽에 쌓인 생물 오염원들이 모듈 밖에 고정되어 있는 BH4와 더욱 잘 반응 하도록 하였다. 역세척이 정용된 공정에 BH4를 적용하였을 때 BH4는 적용이 되지 않고 역세척만 적용된 반응기에 비하여 1배의 지연효과가 있었다. 하지만 두 반응기 모두 역세척을 공정을 제외하자 BH4의 QQ 효과 또한 사라지는 것을 확인하였다. 이는 역세척을 통해 물리적으로 모듈내의 오염원을 제거하여 BH4가 분리막 표면에 생긴 생물막 오염을 저감하는데 도움을 준 것으로 보인다.

주요어 : 분리막 생물반응기, 폐수처리, 정족수감지, 정족수감지 억제, 중공사막, 분리막 오염

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