QUORUM QUENCHING BASED ANTI-BIOFOULING MECHANISM IN A SEMI-PILOT SCALE MBR TREATING REAL WASTEWATER

By

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APPROVAL SHEET

Certified that the contents and form of thesis entitled "**Quorum Quenching Based Anti-Biofouling Mechanism in a Semi-Pilot Scale MBR Treating Real Wastewater**" submitted by Hafiz Muhammad Zeshan Arshad have been found satisfactory for the requirement of the Master of Science degree in Environmental Engineering.

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Abbreviation	Description
A/O-MBR	Anoxic growth membrane bioreactor
C-MBR	control membrane bioreactor
CASP	Conventional activated sludge process
CEBs	cell entrapping beads
CER	Cat-ion exchange resin
C-MBR	Conventional Membrane bioreactor
COD	Chemical oxygen demand
CST	capillary suction time
DI	De-ionized water
DO	Dissolved oxygen
DSVI	Diluted Sludge Volume Index
EPS	Extra polymeric substance
F/M	Food to microorganism ratio
ft	Temperature correction
HF	Hollow-fiber
HPLC	high performance liquid chromatography
HRT	Hydraulic retention time
J	Operational flux
MBBR	Moving bed biofilm reactor
MB-MBR	Moving biofilm Membrane bioreactor

MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NLR	Nitrogen loading rates
NTU	Naphthalometric turbidity unit
OLR	Organic loading rate
PSD	Particle size distribution
QQ-MBR	Quorum quenching membrane bioreactor
Rc	Cake resistance
Rp	Resistance due to pore blocking
Rm	Intrinsic membrane resistance
Rt	Total resistance
RIS	Resistance in Series
SCR	Specific Cake Resistance
SEM	Scanning electron microscopy
SMBR	Submerged membrane bioreactor
SMP	Soluble microbial product
SND	Simultaneous nitrification and denitrification
SOUR	Specific oxygen uptake rate
SRT	Sludge retention time
TMP.	Trans-membrane pressure
TN	Total nitrogen
TOC	Total Organic Carbon
μ	Viscosity of permeate

Abstract

Biofouling, due to extracellular polymeric substances and microbial cells, on the membrane surface is a persistent problem in the widespread application of Membrane Bioreactor (MBR) technology. It has already been revealed that many wastewater bacteria rely on N -acyl homoserine lactones (AHLs) mediated quorum sensing via cell to cell communication to synchronize their activities essential for biofilm formation by releasing soluble EPS into the environment. Recently, use of bacterial quorum quenching i.e. disruption of quorum sensing, to control the biofilm formation, by mineralizing the AHLs has successfully been reported using synthetic wastewater. In the present study, biofouling control by using the *Rhodococcus sp*. entrapped in "W" beads in MBR was investigated. Two parallel semi-pilot scale MBRs i.e., QQ-MBR with W-beads and C-MBR with vacant W-beads, were monitored at 0.5% effective volume of the bioreactor using real wastewater. QQ-MBR showed an enhanced anti-biofouling capability i.e. 4 times longer filtration cycle to reach trans-membrane pressure (TMP) of 30kPa, as compared to C-MBR. Less AHLs concentration in the QQ-MBR extract than the C-MBR was observed using high performance liquid chromatography (HPLC) technique. Less soluble EPS concentration in the QQ-MBR (32 mg/L) than in the C-MBR (57 mg/L) along with AHLs degradation reduced the formation of mature and dense biofilm till the 70 d of operation. Moreover, increase in the QQ-MBR sludge dewaterability in terms of capillary suction time (CST) and decrease in the sludge cake compressibility in terms of specific cake resistance (SCR) was found. Removal efficiency of both MBRs in terms of organics and nutrients were found to be comparable with good effluent quality. Study confirms the successful application of quorum quenching as anti-biofouling strategy in MBR treating real domestic wastewater and potential use for pilot and full scale applications.

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Chapter 1

Introduction

1.1 Background

There are many water scarce countries in the world, about 700 million people in conditions of water stress (World Bank, 2007). Pakistan is also facing a serious water problem today and the gap between demand and supply seems to be widening. Rapid population growth, urbanization and unsustainable water consumption practices have placed immense stress on the quality as well as quantity of water resources in the country. The per capita water availability has decreased from 5,000 cubic meters per annum in 1951 to 1,000 m^3 in 2015. Currently, over 35 percent of Pakistan's population does not have access to safe drinking water and this shortage will rise up to 31% by 2025. (Asian Development Outlook, 2013). Reuse of wastewater is one of the solutions to deal with this deficit problem.

Conventional activated sludge(CAS) treatment can remove COD up to 95%, wherein the cultured biomass degrades the organic matter. The main three components of the CAS process: 1) aeration tank, where biomass comes in contact with waste water 2), clarifier where the liquid- solid separation takes place and 3) recycling of sludge for

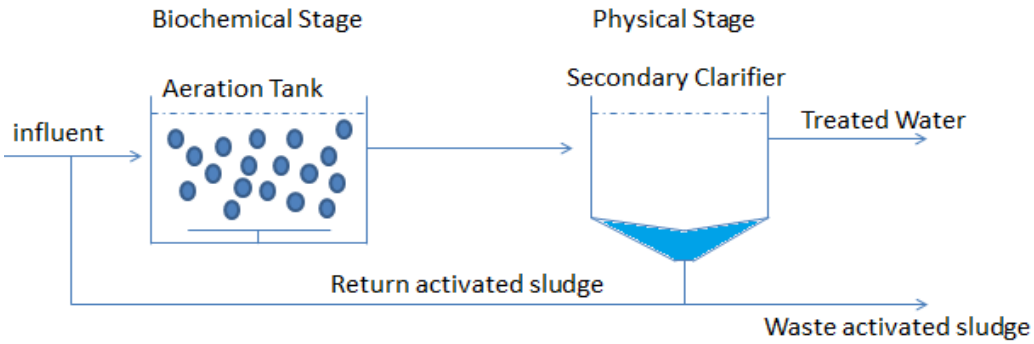

Figure 1.1- Conventional activated sludge process (CAS)

maintaining biomass in the tank. The main disadvantages of the CAS in that it requires a large area, high hydraulic retention time, lower solid retention time, so removal of excess sludge in CAS maintain the value of 2-4 g MLSS / L, which easily settle in the secondary sedimentation tank (Wang et al., 2009)

Among all technologies of wastewater treatment, membrane bioreactor(MBR), the combination of activated sludge and the solid -liquid separation with a low pressure microfiltration (MF) or ultrafiltration (UF) membrane, is a preferred technology from the last two decades because of its high effluent quality (Jahangir et al 2012. Malaeb et al., 2013). The main advantages of membrane bioreactor over activated sludge are (i) small footprints, (ii) a high concentration of mixed liquor, (iii) Compact size and (iv) the high quality of treated water (Judd, 2006; Clech et al 2010; Fu et al, 2012). High effluent quality from MBR, mostly for aerobic type, is suitable for further polishing by Nano filtration and reverse osmosis.

The membrane bioreactor has become well established treatment process for the treatment of domestic and industrial wastewater. Membrane filtration in MBR represents a definite barrier for activated sludge flocs, which allows the operator to maintain any hydraulic and sludge retention time (SRT) with high quality effluent. Organics removal of the MBR is high because of high SRT required for slow growing nitrifying bacteria and other microorganisms (Metcalf and Eddy, 2003).

Primary disadvantages of MBR are, the high operational cost and the fouling of the membrane resulting in short period of the membrane life and a sharp reduction in the flux occurs, as a result of the membrane requires periodic cleaning. Membrane biofouling control and reduction of energy consumption is a major issue area of research to enhance the market value of MBR.

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Many studies have been conducted to control membranes fouling by chemical and physical methods. Although these methods have been effective in dealing with fouling and may extend the filtration cycle and membrane life span to some extent. Modern research shows that the main components of fouling are particulates, colloids and soluble microbial products. These foulants combines together and develop a bio-cake on the membrane surface and progressively which leads to permeability loss.

This suggests that control of the formation of bio cake may be more appropriate solution for biofouling control in MBR as compared to the conventional physical and chemical treatments (Yong et al., 2009). The bacteria produce signaling molecules, or auto inducers for inter cellular communication, called quorum sensing. These molecules are organic in nature and chemical structure is of the Acyl -Homocerine Lactones (AHLs). When the concentration of signaling molecules reach at a certain level, these molecules bind to the receptor protein and activates the specific genes for group behavior, such as the production of antibiotics, virulence, extra polymeric substances production and formation of biofilm (Kim et al, 2012; Oh et al .2011). EPS is considered as an important factor in causing membrane biofouling, which assists in the agglomeration of flocs and microbial biofilms. AHLs based quorum sensing is responsible for the production of extracellular polymeric substances (EPS). New biological method to control membrane biofouling is to monitor and control the concentration of AHLs in the environment, so that the production of EPS can be controlled, which is known as quorum quenching. Two types of quorum quenching have been studied at the moment, (I) enzymatic quorum quenching and (II) bacterial quorum quenching.

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1.2. Objectives of the study

1) Investigate the treatment performance and operational parameters of the Conventional Membrane Bioreactor using real waste water.
2) Evaluate the performance of Quorum Quenching bacteria Rhodococcus.sp BH4 encapsulated in polymer coated beads(W-beads) to mitigate membrane biofouling in quorum quenching (QQ) MBR.

1.3. Scope of Study

(i) Installation of an automated MBR setup with two membrane tanks with 35L working volume having hollow fiber membrane of 0.1µm pore size and 0.7m^2 surface area.

(ii) Two semi pilot scale parallel membrane bioreactors QQ-MBR (inoculated by poly sulfone coated macrocapsules entrapped Rhodococcus.sp bacteria) and C-MBR (containing vacant capsules) was operated using real waste water.

(iii) MBRs were seeded with fully acclimatized activated sludge with 8 g/L concentration.

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Chapter 2

Literature review

2.1. MBR for wastewater treatment

The membrane bioreactor is one of the most advanced technologies in use today for the treatment of sewage or waste water. It is the combination of activated sludge process and the membrane filtration. Wastewater is fed into the reactor and the organics are used as the substrate for microorganisms. Microorganisms use it to grow, maintain and to carry out their general metabolism. Water is treated biologically and then separated using a membrane mainly micro filtration, or sometimes ultrafiltration. Activated biomass is then returned to the aeration tank (Drews, 2010; Poostchi et al 2012; Trussell et al, 2006). The very first full-scale MBR was established in North America in 1970, and then in Japan in 1980. MBR is now gaining popularity as an effective solution for the treatment of municipal and industrial wastewater. Its effluent quality is high as Compared with the CAS. It also leaves the less carbon foot prints and less sludge production along with the highly flexible and robust system.,(Cosenza et al 2013; Masse et al., 2006; Wang et al, 2007; Yang et al 2012).

2.2. Configuration of MBR

The MBR can be configured into two basic configurations, i.e. (I) a side stream MBR and (II) in a submerged MBR (Figure 2.1).

2.2.1. Side stream MBR (SS-MBR)

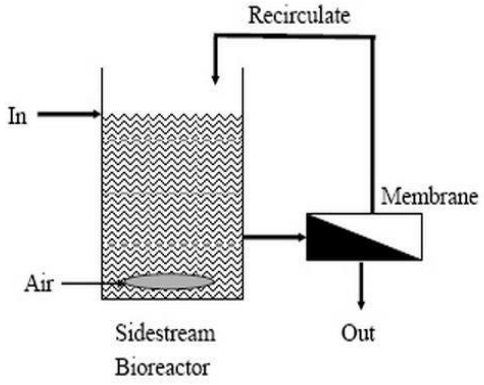
In this configuration, the membrane device is outside and it combines with bio reactor through which MLSS is controlled and circulated. To control the deposition of the suspended matter on the surface of membrane, water is circulated at a high speed. This

intense cross circulation increases the energy demand (Clech et al., 2005), as shown in Figure 2.1(a)

2.2.2. Submerged MBR (SMBR)

In SMBR, the membrane is immersed in the activated sludge. This configuration proved to be more effective than the side stream MBR. In SMBR, shear stress produced by aeration is high as compared with the SS- MBR and can be easily adjusted by changing the aeration rate, which consequently results in high rate of permeate and lower membrane fouling (Howell et al., 2004).

(a)



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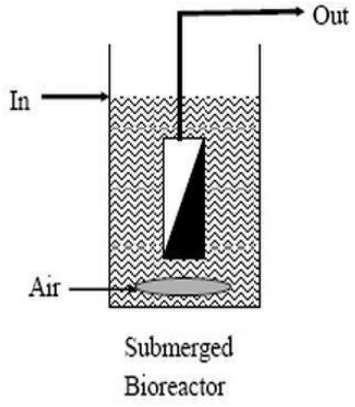

Figure 2.1- (a) Side stream membrane bioreactor (b) Submerged membrane bioreactor

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Item	Unit	Submerged MBR	Side stream MBR
Typical configuration	-	Hollow fibre (HF) Flat sheet (FS)	Tubular (TB) Plate & Frame (PF)
Mode of operation		Submerged	Cross flow
Operating pressure	kPa	5 – 30 (vacuum)	300 – 600
Average Flux	LMH	15-35	50-100
Permeability	LMH/kPa	0.5-5.0	0.07 - 0.3
Superficial velocity	m/s	0.2-0.3	2-6
Membrane cost	$/m^2	<50	>1,000
Capital cost		Low	High
Operating cost		Low	High
Cleaning	-	Difficult	Easy
Odour/VOC emission potential	-	High	Low
Market Share	-	99%	1%

Table 2.1-General comparison of submerge and side stream MBRs

Source: http://onlinembr.info/Membrane%20process/iMBR%20vs%20sMBR.htm

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Submerged	Side stream
Kubota	Degremont
USF	Grontimij
Huber	Weir Envif
Toray	Orelis
Zenon	Norit
Mitsubishi Rayon	Wehrle Werk
Millenniumpore	

Table 2.2 -Submerged and side stream MBR commercial suppliers (Yeon,2009)

2.3. Comparison of Aerobic and Anaerobic MBRs

Aerobic or anaerobic degradation depends primarily on the redox conditions and depends on the electron acceptor in the hand. Under aerobic MBR air is fed either continuously or intermittently. Air supply rate preferably course bubbles than smaller as such bubbles tend to prevent clogging of the membrane by enhancing physical cleaning of the membrane surface and providing an atmosphere that is favorable for the growth of microorganisms. In connection with the provision of air and associated mechanism, aerobic MBR operation cost more than the cost of operation of an anaerobic MBR. The rate at which these microorganisms grow in aerobic settings is greater than the rate at which the organisms grow in anaerobic systems therefore the

retention time in the anaerobic MBR is also high. SS- MBR usually used for anaerobic MBR.

2.4. Membrane Filtration

In this type of filter membrane is used, which basically acts as a barrier, which has the potential to separate materials of different phases from each other, and blocks the movement of some particles which are larger than the pore size of the membrane. All membranes let water pass through them but restrict movement of the solid particles. Efficiency of the membrane largely depends on two major factors; selectivity of the membrane and the membrane performance. The selectivity of the membrane generally relates to the separation and retention of the membrane, and the performance is mainly referred to the water flux.

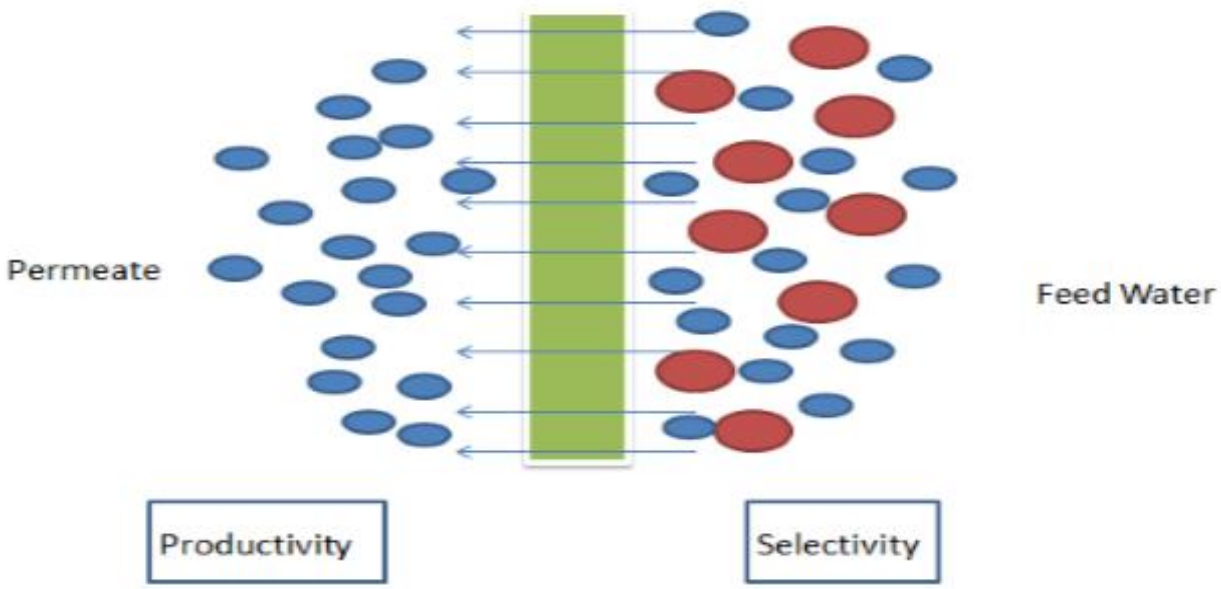

Figure 2.2 Membrane filtration process

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Membrane filtration has four categories depending on their pore size.

2.4.1 Microfiltration

In this type of filtration, the membrane has a coarse pore size ranging from 0.1 to 10 microns. These types of membranes generally used for the separation of suspended particles from the solute. Microfiltration removes all types of bacteria. Only virus is not caught in this process. Substances less than the pore size of the membrane is partially removed. Microfiltration can also be used to pretreat the water prior to the reverse osmosis and Nano filtration.

2.4.2 Ultrafiltration

Ultrafiltration is used when required complete removal of viruses. The range of pore size 0.001-0. 1μm.Ultrafiltration membranes can be made like tabular or a flat sheet. The unit in which membranes are arranged is called membrane module. Ultrafiltration can remove molecules and particles in the range of, from 1,000 to 500,000 Daltons. (Lenntech, 2014).

2.4.3 Nano filtration

Nano filtration technology is becoming popular because of its narrow pore size less than 0.001μm. Nano filtration is mainly used in water treatment for softening, removal of micro pollutants and discoloration. The Nano filtering molecules are in the range of 100-1000 Daltons.

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2.4.4 Reverse Osmosis

Reverse osmosis (RO) based upon the fundamental desire to equilibrium. Two fluids come into contact with different concentrations and are separated by a semipermeable membrane. Water flowing from the solution having a high concentration of water to a solution having a low concentration of the water up to a concentration of both fluids become the same, called osmosis. The difference in water head of the column is called osmotic pressure.

When pressure is applied on the side where the osmotic pressure is obtained, one can get opposite effect, and water will move from a low concentration to the high concentration and salt may be retained on the membrane. Using this method, substantially all the salt can be removed.

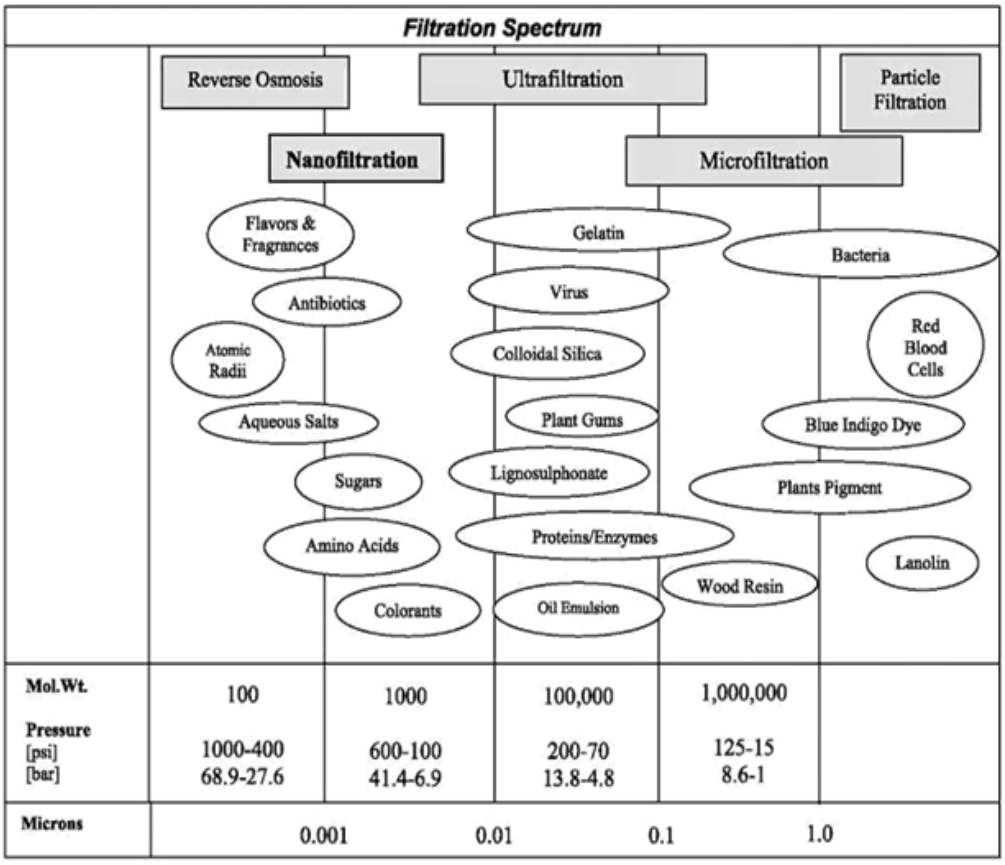

Figure 2.3 various pore sizes of membranes and particles that retained.

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2.5. Membrane configurations

2. 5.1. Dead end Filtration

This is one of the most basic forms of filtering, widely used for the separation of particles from the crude liquid. In this type of filtering particles tend to accumulate on the membrane surface, and this consequently leads to clogging of the pores. Over time, layers of particles or colloids to thicken and form a gel or cake layer. This layer of particles ultimately increases the resistance towards the fluid movement. It also serves as an excellent technique to concentrate the various compounds.

2. 5.2. Cross Flow Filtration

In this filtration, flow is in high speed and in a direction generally parallel or transverse (cross flow) direction to the filter surface. Extreme shear force generated due to cross-flow, which ensures the minimum thickness of the cake layer on the surface of the membrane. But the cross flow does not have the ability to completely wipe out the cake layer. (Figure 2.4)

Dead-end Filtration **Cross Flow Filtration**

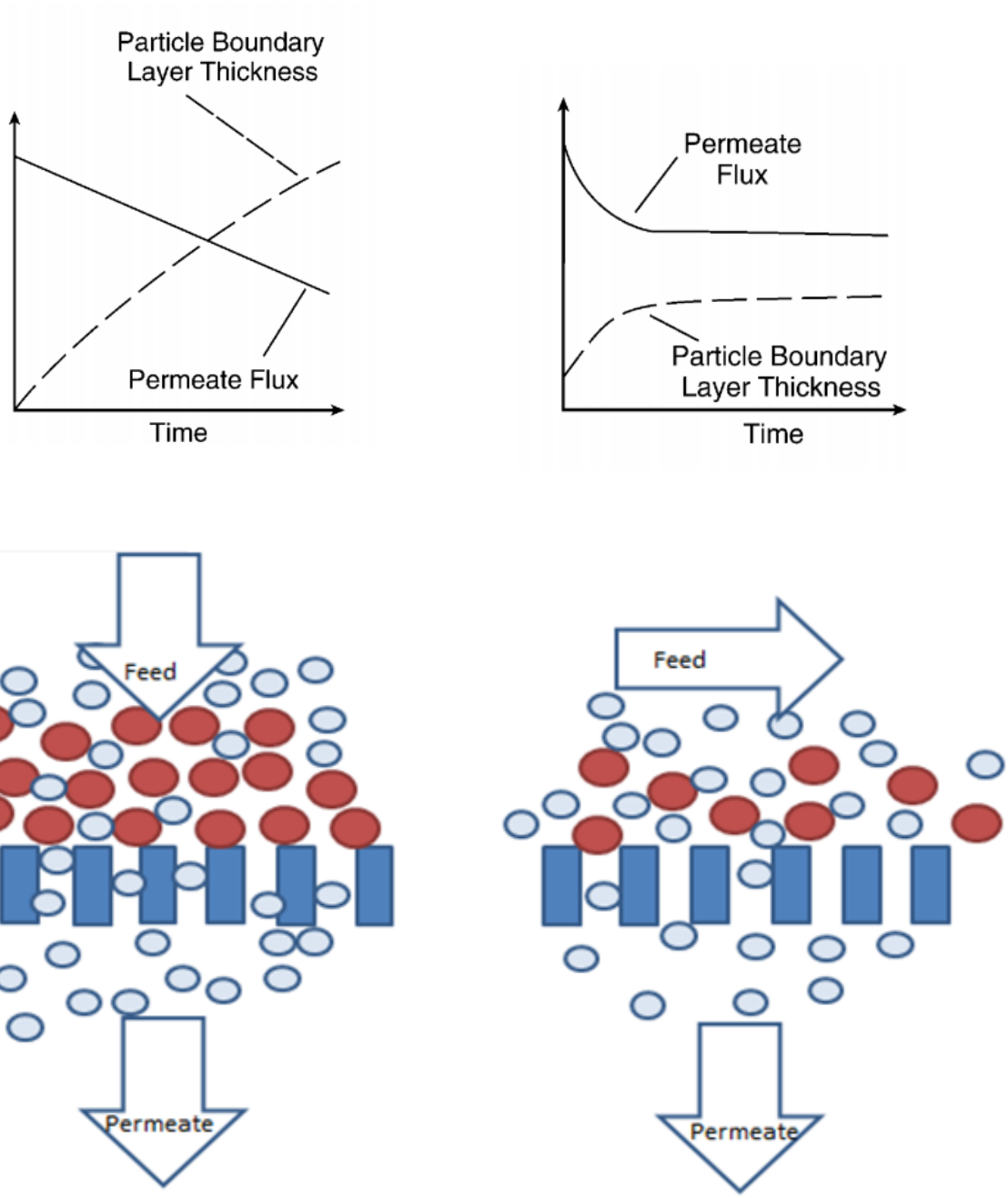

Figure 2.4 Dead End Filtration Vs Cross Flow Filtration (www.onlinembr.info)

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2.6. Membrane materials

Membrane materials always show various fouling tendencies due to their different pore size, hydrophobicity and morphology. Polyvinylidene fluoride (PVDF) membrane is better than the polyethylene (PE) membrane as it prevents the irremovable fouling in MBRs used for municipal wastewater treatment (Yamato et al., 2006). Inorganic membranes such as zirconium, aluminum, and titanium oxide, show excellent hydraulic, chemical and thermal resistance. Stainless steel membrane was also used for the MBR, and the results showed higher permeate flux was obtained (Zhang et al., 2005) and it is a potential alternative for the high temperature waste water treatment (Zhang et al., 2006).

2.7. Operational parameters of membrane

2.7.1. Trans-membrane Pressure (TMP), flux and resistance

Trans - membrane pressure (TMP) is the main driving force behind the filtration process. TMP is primarily the pressure difference inside and outside of the membrane, as the cake layer start to build on the membrane surface, which increases the resistance of the material and the TMP starts increasing. TMP is also used to predict the flux of the membrane; flux is the liquid stream coming from the membrane per unit time per unit membrane surface area.

$$J = \frac{\Delta P}{\mu R t}$$

J = flux, L/m^2.hr

ΔP = Trans- membrane Pressure, kPa

μ = Viscosity of permeate, Pa.s

$\mathbf{R_t}$ = Total hydraulic resistance, 1/m

2.8. Membrane Fouling

Membrane fouling is the real obstruction that prevents the fast commercialization of MBR. Fouling is the undesirable attachment of microorganisms to the membrane surface and into its pores. Membrane fouling is caused by clogging of membrane pores due to bio-cake formation on the surface of membrane. As fouling rate increases the rate of flow through the membrane begins to decrease.

Fouling can mainly occur due to a number of reasons, those reasons are enlisted below:

(i) Adsorption of solutes and colloids on membrane

(ii) Adhesion or attachment of sludge on the membrane.

(iii) Cake layer formation.

(iv) Variation of foulants with time.

2.8.1. Stages of fouling

There are three stages of membrane fouling:

Stage 1: Conditioning fouling

The initial fouling stage occurs due to the interaction of extracellular substances (EPS) and soluble microbial products (SMP), with the surface of the membrane. It has been reported that fouling is mostly irreversible and adsorption of colloids and organics is most common even when the operating flux is minimum or near zero. A combination of a suction pump with vacuum can be used to avoid the conditional fouling due to adsorption of materials.

The measure of the intensity of such adsorption affects the distribution of the pore size and the chemistry of the membrane surface. The cake layer begins to form in this stage, but in this stage it does not have a huge effect on the flux. But once the cake forms it can either partially or completely block the pores which can lead to a subsequent rise in TMP (Chang et al., 2002).

Stage 2: Steady fouling

Even when the operational flux is below the critical value, the flocs that are temporarily attached onto the surface of the membrane can cause the second stage of fouling known as steady fouling. During this stage majority of the surface of the membrane is covered with particulates, colloids. EPS and SMP further enhances the floc and colloidal adhesion on the surface of the membrane (Judd et al., 2006).

Stage 3: TMP jump

The immediate increase of TMP or in other words "Jump" is caused when filtration exceeds critical flux. There are several mechanisms that can cause the TMP jump (Judd et al., 2006).

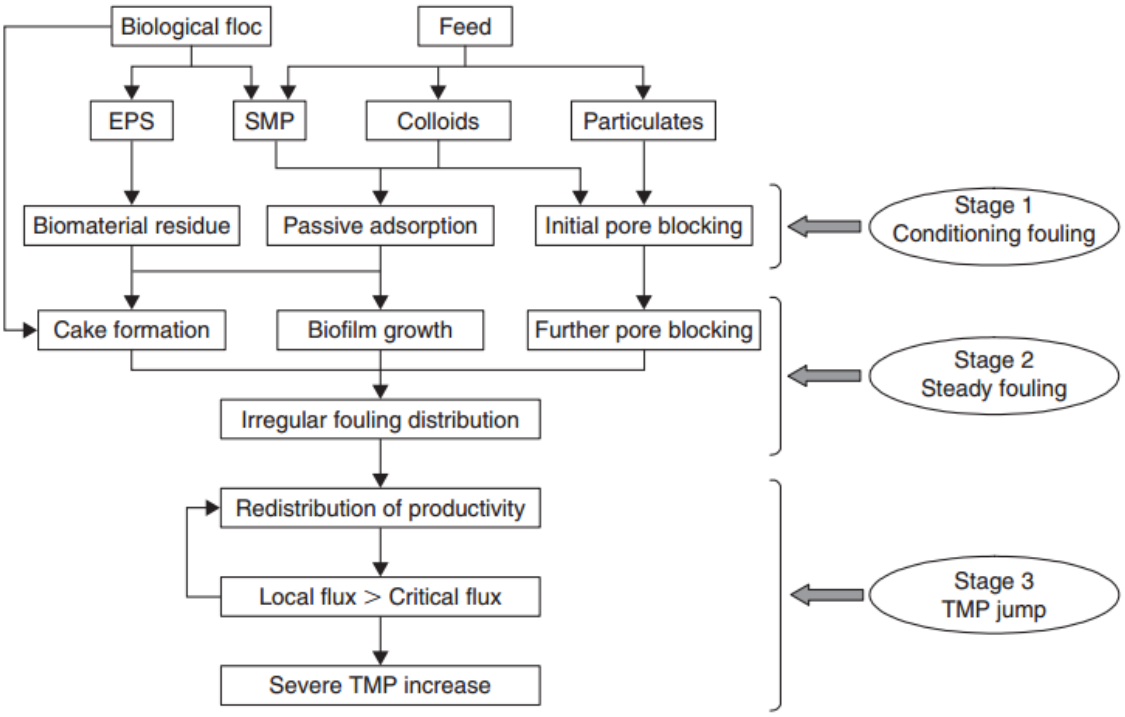

Figure 2.5 Fouling mechanisms during MBR operations (Zhang et al., 2006)

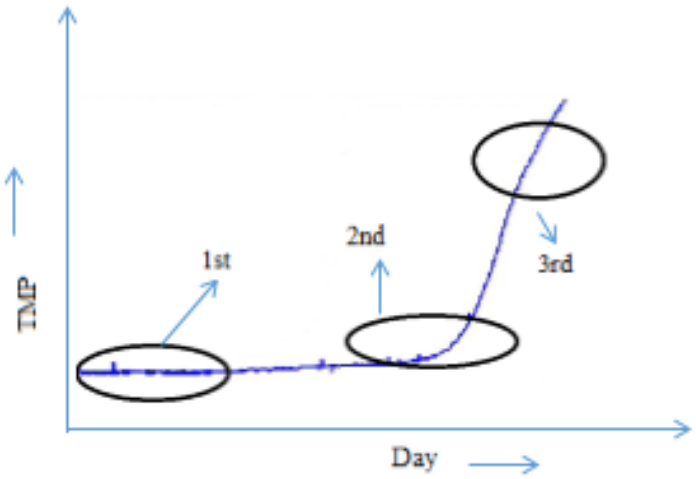

Figure 2.6 Fouling stages of membrane

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2.8.2. Classification of membrane fouling

Membrane fouling is one of the most complex phenomenon occurring in the MBR. It primarily occurs due to number of reasons like the size of the sludge flocs, the typical nature of the foulants and the colloidal particles and the existing hydrodynamic conditions. Particles that are smaller than the size of the membrane pores are either absorbed on to the wall of the membrane or they ultimately reduce the size of the pores. Particles that are bigger than the size of the pore form a layer of cake on the surface of the membrane.

Fouling can be classified into three types (Meng et al., 2009).

(i) Removable fouling

(ii) Irremovable fouling

(iii) Irreversible fouling

Removable fouling

It occurs when the cake layer is attached onto the surface of the membrane. It can easily be taken off using physical methods like a rake or by using the backwashing mechanism.

Irremovable Fouling

There are some colloidal particles that are very small and upon entering into the pores cannot be taken out using physical means of cleaning. Along with these particles there are certain inorganic particles that can easily be deposited on to the membrane surface. Chemical cleaning is required to remove such fouling. The chemical protocols followed should be according to the membrane manufacturer recommendation as per material.

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Irreversible Fouling

There are some particles that get stuck either on the membrane surface or within the pores and cannot be removed even by using chemical means and methods, they tend to cause irreversible fouling. Intense irreversible fouling can decrease the flux by an immense rate and thus prompts that the membrane be changed immediately (Kim et at., 2006).

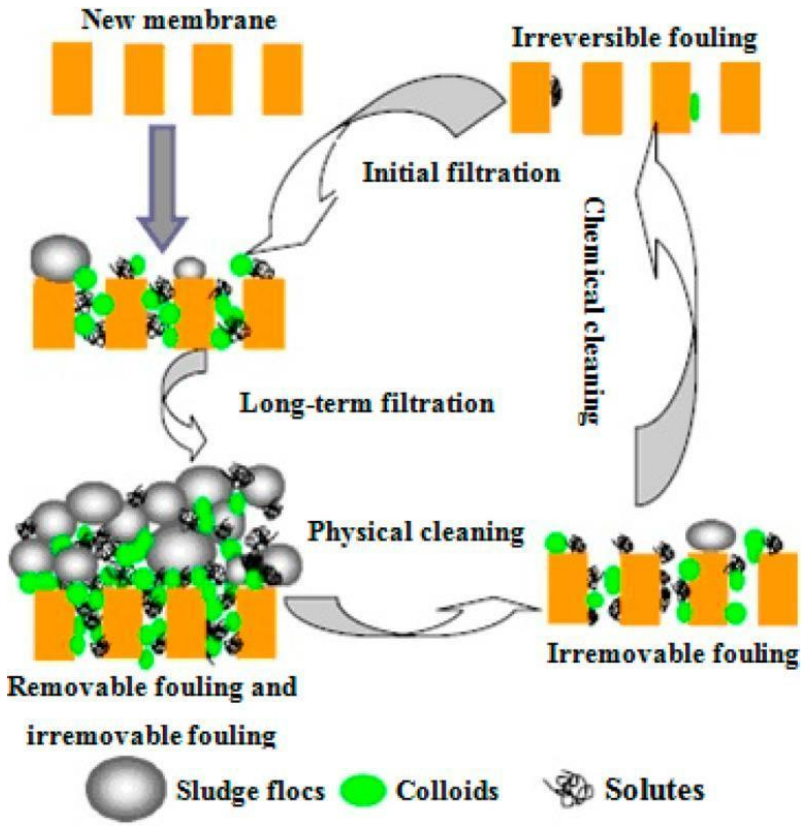


Figure 2.7 Deposition of foulants on membrane surface (Kim and Jang, 2006)

2.9. Factors affecting fouling

There are a number of factors that play a part in membrane fouling like

1) Hydraulic retention time (HRT)

2) Sludge retention time (SRT)
3) Extracellular Polymeric substances (EPS)
4) Distribution of Pore Sizes
5) Organic Loading Rate (OLR)
6) Food to Microbe Ratio (F/M)
7) Dissolved Oxygen (DO)
8) pH

All these characteristics of sludge and the operational conditions are the main factors that affect the fouling of membrane.

2.9.1. Extracellular Polymeric Substances (EPS)

Extracellular Polymeric Substances (EPS) are basically biopolymer items produced from the excretion of microbes and cell-lysis. They are generally composed of proteins, carbohydrates and humic substances. EPS play an important part in the formation of flocs and bio-film, they also tend to work like glue and form a layer of protection on the biofilm that shields the microbes against toxic or harmful substances. EPS can either be present in bound or soluble form. They generally fill the voids between cells and form the matrix that cells need to live in. There is a linear relation between EPS and fouling and are generally referred to as the basic reason for fouling of membrane.

2.10. Membrane biofouling

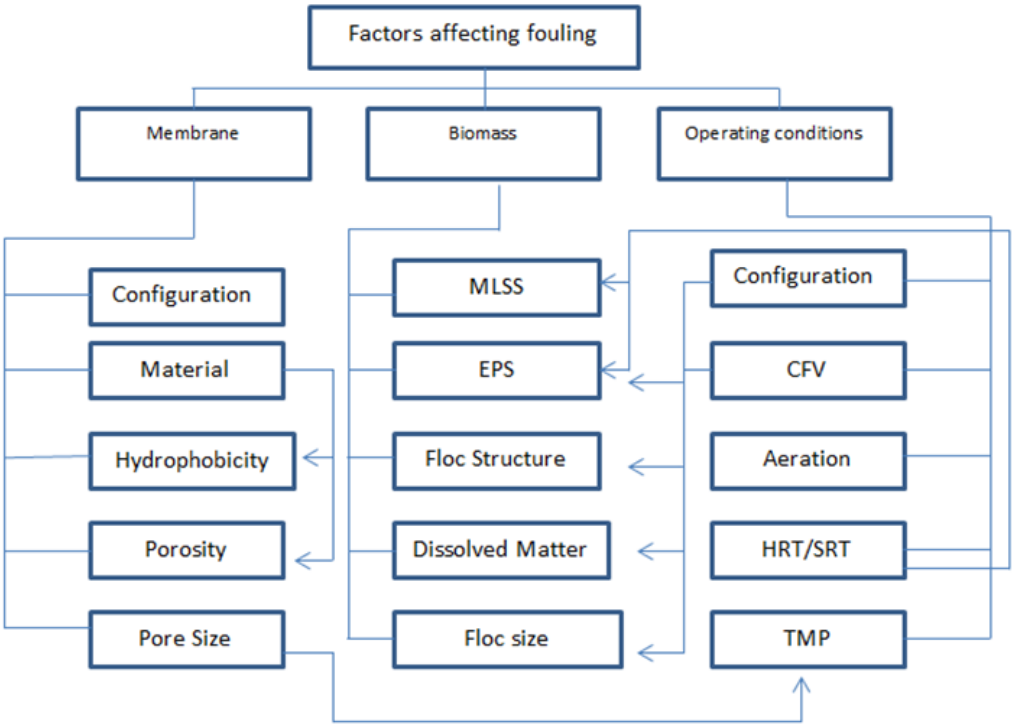
Membrane biofouling in its strict form is the coverage of the membrane surface (external and internal) by microbial deposits which adsorb or simply accumulate during operation. It causes significant permeability loss in all kinds of MBRs (Lee et

al., 2007) (Kayung et al., 2008). It was reported that membrane biofouling the cake layer consists of bio-flocs that have been rejected but are still active and thus excretes slimy substances (e.g., EPS) that are glue like in nature as opposed to inactive inorganic substances. Bioflocs residues that are irreversibly attached along with planktonic bacteria serve as the growing nutrition for biofilm on the surface of membrane. Maleab et al. (2013) observed that biofouling can occur due to a variety of mechanisms that includes: (i) Growth of colonies of microorganisms on the membrane's surface (ii) The release of foulants by microbes. Operational as well as wastewater characteristics affect the membrane biofouling and a greater SRT increases the filterability. Significant increase in SRT will intensify the biofouling (Meng et al. 2009).

The concentration of extracellular polymeric substances (EPS) and soluble microbial products (SMP) are also two main causes of biofouling (Gao et al., 2010). EPS, due to its ability to act as glue, plays a huge part in agglomeration to the microbial flocs (Kim et al., 2006). Soluble EPS or SMP includes soluble protein, humic acids and polysaccharides (Drews et al., 1999). Cho et al. (2004) showed that the specific cake resistance increases as the concentration of the EPS rises. When compared to mixed liquor, it was found that EPS had a greater potential to cause biofouling. Studies have also shown that soluble EPS and loosely bounded EPS played a greater role in causing fouling as compared to EPS that is more severely bound.

The effects of aerator position, aeration rate and aeration time was investigated by Fu et al. (2012), and reported that aeration time and aeration rate has positive effect than aeration position in term of permeate quality and fouling control.

Biocake layer contributes mainly in flux decline than other clogging, as cake layer can be removed easily by physical cleaning while chemical cleaning is needed for the removal of internally deposited compounds which causes pore blockage, is named as irreversible fouling (Chang et al., 2002).



Factors affecting membrane fouling are shown in Figure 2.8 (Chang et al., 2002)

2.11. Fouling control methods

There are four basic strategies to control biofouling in MBRs that can yield effective results:

1) Membrane fabrication.
2) Physical
3) Chemical
4) Biological

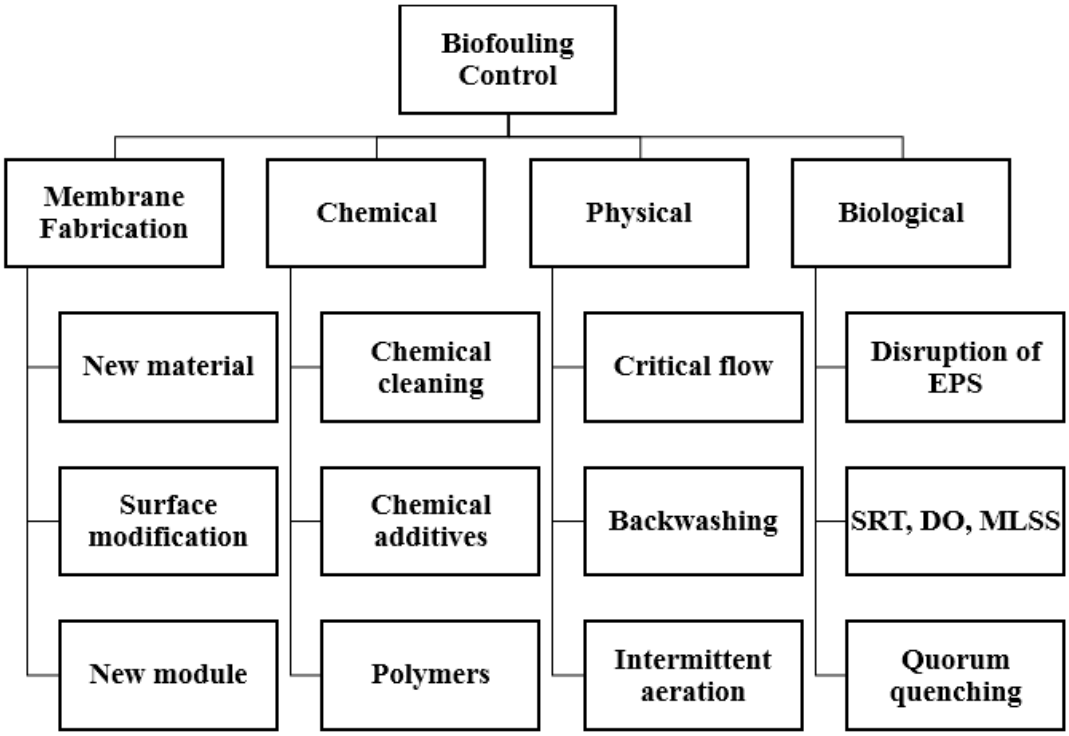


Figure 2.9. Biofouling control methods

The most prominent experiment to show the reduction of membrane biofouling using patterned membrane was done by Won et al. (2012) where patterned membrane was used by employing the lithographic method and found that there was a significant decrease in the amount of microbial flocs that settled on the surface when compared to the ordinary membrane. Significant researches have also been conducted on other methods and strategies but all of them were found to have been effective for a very limited duration and delayed the TMP for few days.

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2.12. Quorum sensing

It has been studied that bacterial cells communicate with each other using a mechanism known as quorum sensing. This mechanism employs signal molecules that are known as auto inducers. These molecules accumulate in their surrounding environment over time, When the concentration reaches a critical value then they tend to exhibit associated manner i.e. Secretion of polysaccharide, virulence and proteins. Different types of bacterial quorum sensing have been identified. Quorum Sensing that exhibits acyle homoserine lactones (AHLs) is one of the most significant and widely used type of quorum sensing caused by the gram negative bacteria found in wastewater. AHLs, the signaling molecules can further be divided into 12 more types. A single AHLs molecule contains a ring of homoserine lactone which is attached to a molecule of fatty acid and has some carbon molecules attached to the ring also.

2.12.1. Role of QS in biofilm formation

Nerenberg and Shrout (2012) described the concept of quorum sensing and its different steps. These steps are enlisted below:

1) The cell produces protein to form a signal molecule.
2) The concentration of signal molecule is maintained in the surrounding environment.
3) A regulatory protein is unleashed whose main purpose is to accept signal molecules and ensure successful communication.

Bacteria that produce signals are called donor and the bacteria that accept signals are called receivers. Receivers play a significant role in development of biofilm. They excrete EPS after receiving signal molecules. EPS plays a huge part in agglomeration of flocs and biofilm formation. A direct relation of AHLs concentration and EPS

production was reported by many researchers. A high AHLs concentration can lead to a high amount of bio film production.

2.13. Quorum sensing control strategies:

Three different points of attack on the AHLs molecules that can be used to control membrane biofouling.

1) Attack on the cells that generate the signal molecules.

2) Attack on the cells that receive the signal molecules.

3) The signal molecules that have been generated.

A very novel approach to prevent biofilm formation is quorum quenching and it tends to control the biofilm production by either reducing the concentration of AHLs or by deactivating the AHLs molecules.

Quorum Quenching is a relatively new technique to control the production of biofilm and thus in preventing biofouling in the MBR. According to one study the AHLs were hydrolyzed by the hydrolysis of either the lactone rings using lactonase or the acyl-amide links using acylase. Research proved that acylase in immobile form was always better at inhibiting the production of biofilm as compared to acylase in mobile form. Oh et al. (2012) did work on isolating the quorum quenching bacteria (the bacteria that produce the quorum quenching enzymes) and found out that there were a total of four species. Out of these *Rhodococcus* and *Panibaccilus* were found to be the most effective. *Rhodococcus* bacteria when encapsulated in alginate beads and submerged in a MBR that was running parallel to a conventional MBR contained empty alginate beads performed effectively.

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2.14. Quorum Quenching based biofouling control studies

Quorum quenching is a revolutionary technique resolving the most critical issue of biofilm formation in membranes.The deactivation of AHLs by hydrolyzing the lactone ring by lactonase and acyl-amide linkage by acylase was studied by Yeon at al. (2009). Procine kidney enzymes were used and the reduction of AHLs, less EPS production and delayed TMP rise in MBR (with acylase) was observed. Yeon at el., (2009) compared the performance of immobilized and free enzymes and their results proved that immobilize acylase performed better than free moving acylase in same quantity. Acylase was immobilized on magnetic carrier enzymes which were prepared by Yeon at el., (2009) to resolve the issue of stability of free enzymes.

Oh et al., (2011) investigated the isolation of quorum quenching bacteria to find out the most prominent species which produce quorum quenching enzymes. The studies showed that Rhodococcus and Panibaccilus stains were the most effective. Two parallel MBRs were operated, one having an encapsulated Rhodococcus sp.BH4 in microporous membrane submerged in MBR and a control MBR having a similar filtration mode. The comparison of their TMP profiles showed substantial difference between both QQ-MBR (having quorum quenching bacteria) and C-MBR (control MBR).

Jahangir et al. (2012) worked on the positioning of microporous membrane having quorum quenching bacteria encapsulated in it and concluded that biofouling was less in MBR in which the microporous membrane was placed in the membrane tank as compared to the MBR in which the membrane is placed in a separate bio-tank with

sludge recirculation, Furthermore, it was also reported that the quorum quenching activity was also dependent on the rate of recirculation. Kim et al., (2013) studied the effect of quorum quenching on the microbial dynamics in MBR, which proved that QQ decreases the auto-inducers which produce microbial species hence reducing the EPS production resulting in less biofouling.

Kim et al. (2013) prepared cell entrapping beads (CEBs) of sodium alignate injected with *Rhodococcus sp.BH4* and an MBR of batch type was installed for analysis. This technique proved to be the most effective than all the others. Cheong et al. (2014) inoculated *Pseudomonance sp.1A1* in ceramic microbial vessel (CMV) and these vessels were submerged in MBR. The result was compared with a conventional MBR without CMV and an MBR with CMV having inactivated quorum quenching bacteria. The EPS production in MBR having CMV with activated bacteria was the least.

Maqbool et al. (2015) prepared *Rhodococcus sp.BH4* entrapped alginate beads to mitigate biofouling in MBR using synthetic wastewater and reported that QQ-MBR (having beads) exhibited a deferred TMP rise while in C-MBR (with no beads) showed rapid TMP rise to 30 Kpa within 10 to 14 days of operation. C-MBR showed 7 times higher fouling rate than QQ-MBR. However, they observed that alginate beads disintegrated during continuous operation and need to be added after 45 days.

Kim et al. (2015) studied the macro-encapsulation of QQ bacteria (Rhodococcus sp.BH4) into alginate beads with polymeric membrane layer and its application for biofouling control in MBR. Different polymers such as poly sulfone (PS), poly (vinyl Dene) fluoride (PVDF) and polyether sulfone PES was coated on alginate beads by phase inversion method. It was found that membrane coated with poly sulfone layer

prevented QQ bacteria from leaking out of the macro-capsules in a harsh chemical and hydrodynamic environment.

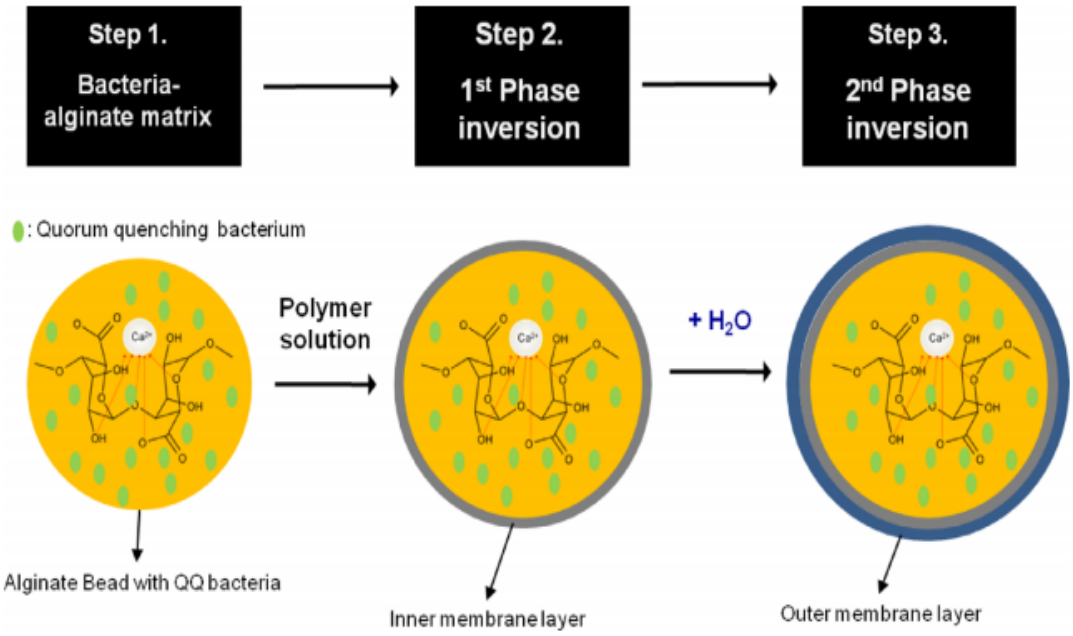

Figure 2.10 polymer coating of alginate beads kim et al. (2015)

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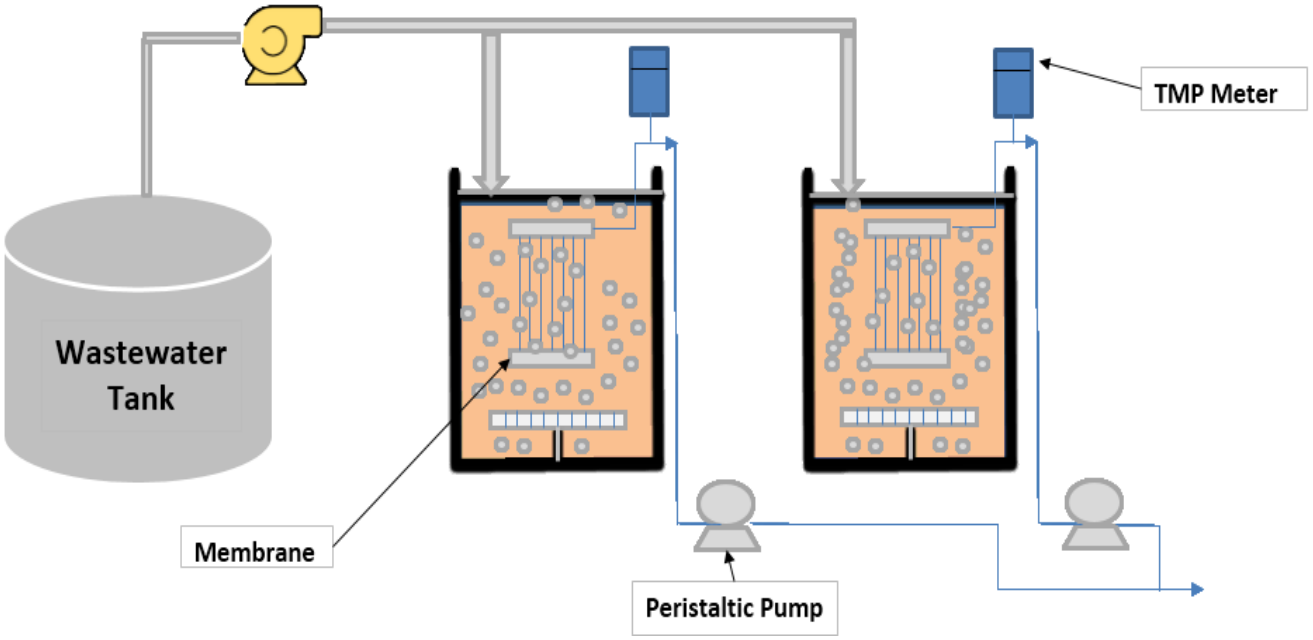
Chapter 3

Materials and Methodology

3.1. Wastewater composition

Low strength real wastewater of NUST, H-12 campus Islamabad, was fed to the both reactors after screening and settling to remove suspended solids (SS) for better treatment or degradation of dissolved organics. Seed activated sludge taken from the return line of full-scale wastewater treatment plant installed at I-9 Islamabad, Pakistan of 17MGD capacity, Real wastewater of 270 mg/L average COD and 186 mg/L average BOD was supplied to the microbes and concentration of activated sludge (MLSS) was maintained as 8 g/L in the MBRs. Average organic loading rate (OLR) of 1.5 Kg/m^3/d fed to the MBRs.

Figure 3.1 Flow diagram of Membrane bioreactor



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3.2. Membrane material and types

Poly vinyl dine fluoride (PVDF) hollow fiber (HF) membrane was imported from Memstar, China. PVDF membrane has a high reliability than other materials and patience in acidic and basic chemical conditioning for cleaning. Detailed membrane characteristics are presented in Table 3.1.

Table 3.1 Membrane material and characteristics

Item	**Characteristics**
Membrane type	Hollow fiber
Manufacturer	Memstar, China
Membrane material	PVDF
Pore size	0.1µm
Filtration area	$0.7m^2$
Suction pressure	<30kPa
Temperature	15-45 ^{0}C

3.3. Lab-scale MBR

Two parallel semi pilot-scale MBRs with working volume of 35 L each was installed at MBR NUST lab as shown in Figures 3.2 and 3.3. One of MBRs was quorum quenching MBR (having QQ bacteria entrapped beads) and the second one was conventional MBR (containing vacant beads). Both MBRs were seeded with same activated sludge and operated at optimized filtration and relaxation mode of 10 min cycle with 8 min filtration and 2 min relaxation, Operational parameters and conditions are listed in Table 3.2. Air compressor was used for aeration and peristaltic pump (Masterflex, Cole Parmer, USA) with flow controller was used to maintain permeate flux at 16.5 LMH and desired dissolved oxygen (DO) was maintained by air flow controller in bio-tank.

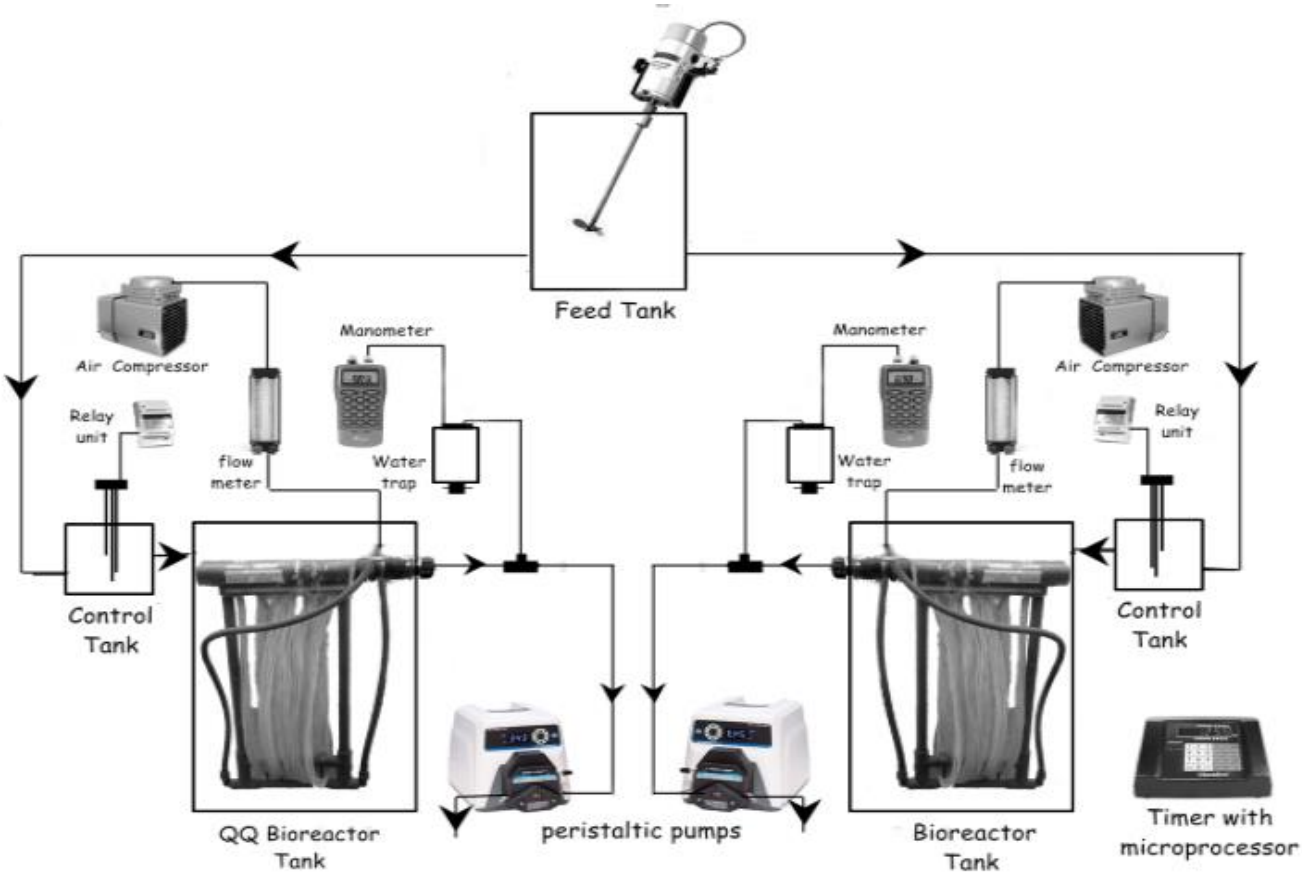

Figure 3.2-Process flow diagram of lab scale MBR

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Working MBR

Figure 3.3 Semi pilot-Scale MBR at NUST water and wastewater lab

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Table 3.2 Working conditions of MBR

Operating conditions	**Value**
Working volume	35 L
flux	16.5 LMH
HRT	3 hrs
SRT	40 days
MLSS	8-10 g/L
Membrane type	PVDF hollow fiber
Beads concentration	0.5% tank volume

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3.4. Analytical methods

Influent and effluent water quality of both MBRs were analyzed regularly, Parameters included COD, Ammonium-N and Phosphate-P. Sludge characteristics involved MLSS, MLVSS, CST (capillary suction time) for dewaterabilty and diluted sludge volume index (DSVI) for settle ability were monitored as per Standard Methods (APHA et al., 2012). Multi-meter (pH/DO 300 series, Oakton, USA) was used to measure DO and pH. Activity of microbes was determined in term of specific oxygen uptake rate (SOUR) using DO meter (YSI 5010, Cole Parmer, USA)**.** TMP data logger (Super scientific, 84009, Taiwan) was used to measure Continuous trans-membrane pressure (TMP).

3.5. Specific cake resistance

To calculate the cake resistance on the membrane surface dead end filtration unit (Amicon, 8400, USA) was used and this test is known as Specific cake resistances (SCR) test in which weight of permeate was continuously monitored by weight balance (Shimadzu, UW6200H, Japan) connected to a computer. A PVDF filter (Millipore, GVWP09050, and USA) of 0.22µm pore size and effective surface area of 90 mm was used. Nitrogen gas (as an inert gas) was supplied with a Constant pressure of 30kPa. Specific cake resistance was calculated by the formula given below (khan et al., 2012).

$$\boldsymbol{\alpha = \frac{2000.A^2.\Delta P}{\mu.C} . \frac{t/V}{V}}$$

Where,

α = specific cake resistance, m/kg

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A = PVDF membrane area, 0.0042m^2

ΔP = constant pressure applied, 30 kPa

μ = dynamic viscosity of effluent, N-S/m^2

C = concentration of mixed liquor, kg/m^3

$\frac{\mathbf{t/V}}{\mathbf{V}}$ = slope of line, sec/m^6

3.6. Membrane chemical cleaning protocol

Before the filtration run, the membrane was chemically cleaned using NaOH and NaOCl called basic cleaning. 2% aqueous solution of sodium hydroxide (Sigma Aldrich, USA), and sodium hypochlorite having an effective chlorine concentration of 2g / L were used to remove bacterial deposits on the surface of foul membrane, in Acid cleaning 1% solution of concentrated HCl was used to remove inorganic foulants. After physical cleaning to remove the biocake, the membrane was submerged in an alkaline solution for 8 hours and filtered for 30 minutes. Finally, the membrane was washed with tap water and then was immersed, and filtered tap water for 30 minutes.

3.7. Filtration resistance analysis

Fouling potentials of both MBRs was evaluated using resistance-in-series (RIS) hydraulic filtration model based upon Darcy's Law:

$$R_t = \frac{\Delta P}{\mu . J}$$

Where R is hydraulic resistance (1/m), ΔP is TMP rise (Pa), J is operational flux ($m^3/m^2/s$) and μ is permeate dynamic viscosity (Pa s).

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$$R_t = Rc + Rp + Rm$$

Total hydraulic resistances (Rt) is composed of three types of resistances, cake resistance (Rc), pore blockage resistance (Rp) and intrinsic membrane resistance (Rm). Rt was calculated at the end of operation, for Rc measurement cake from the membrane was sponged and submerged in de-ionized (DI) water, flux and TMP were recorded and Rc value was obtained by the subtraction of Rm + Rp from Rt, while Rm was found after chemical cleaning of membrane. Contribution of each component of resistance was compared in both MBRs.

3.8. Extraction and quantification of EPS

Extraction of extra polymeric substances (EPS) from MBR sludge was carried out using Cation exchange resins (CER) (Dowex, USA). 50 mL sludge sample was collected from both reactors and centrifuged at 4000 rpm at 4 ^{0}C using refrigerated centrifuge (K2015R, Pro-Research, Britain) for 15 min to separate supernatant for soluble EPS. For loosely bond EPS (LBEPS) extraction, sludge pellets were mixed in phosphoric buffer solution, stirred for 1 h and centrifuged for 15 min at 4^{0}C and preserve the supernatant. For TB-EPS (tightly bond EPS), sludge pellets were again mixed in buffer solution and CER were added to make up the 50 mL volume and stirred for 1 h.

Lowry method was used to determine Protein (PN) concentration by using Folin–ciocalteu phenolic reagent and absorption was taken on 750 nm using a spectrophotometer (T60UV, PG Instrument, Britain). Polysaccharides (PS) quantity was measured by using standard curve of bovine serum albumin (BSA) (Sigma-

Aldrich). Dubois (phenol– sulfuric acid) method was employed For further quantification of total polysaccharides (PS), solution turned yellow on addition of phenol and sulfuric acid, absorption was taken at 490 nm and standard curve of glucose was used to determine the PS concentrations.

3.9. Preparation of beads

CEBs were prepared as per method developed previously by Kim et al. (2013) with some modifications. Already isolated bacterial stain *Rhodococcus sp.* BH4 was grown in LB agar medium. Bacterial suspension was prepared in D.I water at OD600nm.5 % sodium alginate and 4% $CaCl_2$ solutions were prepared in D.I water. 5ml bacterial suspension then was mixed in sodium alginate after that sodium alginate solution was dripped in $CaCl_2$ solution using peristaltic pump with flow controller at flow rate of 1ml per minute. Number of beads per minute was counted and 2000 CEBs were prepared. These beads were left in $CaCl_2$ solution for 8 hrs for gelation period before inoculating in QQ-MBR. Average CEBs size (diameter) and density was found to be 3.3 mm and 1.6 g/ml respectively which made 0.1% of total working volume as shown in figure 3.4.

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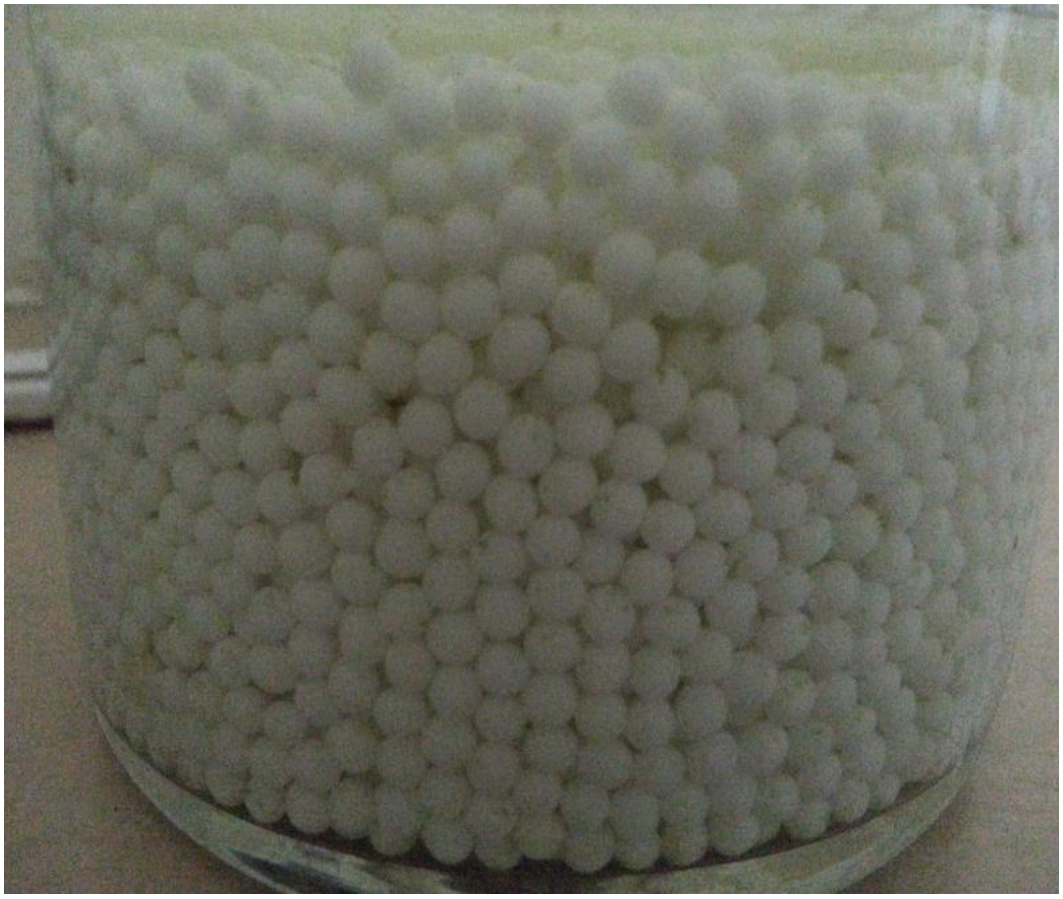

Figure 3.4- Polymer coated QQ-bacteria entrapped beads

3.10. Extraction of AHLs from activated sludge

Activated sludge sample (20 mL) from both reactors was centrifuged to remove large flocs and supernatant was mixed with an equal volume of ethyl acetate for 2 h at 120 rpm. Organic layer was separated out by separating funnel and colloidal removal was achieved by centrifugation at 4000 rpm for 10 min. Supernatant was dried in rotary evaporator at 30°C and residue was dissolved in 300 µL of methanol.

3.11. Detection of AHLs using HPLC

N-octanoyl homoserine lactone (C8HSL) was procured from Sigma-Aldrich. Standard (C8HSL) was dissolved in methanol to obtain 1 mg/mL stock solution. Working

solution was prepared by mixing 20 mL of stock solution with 980 mL of methanol having 0.1% formic acid. Analysis was performed using a water/methanol composition of 35:65 as a mobile phase and the UV detector was set at 210 nm. Column C18 was used for the high performance liquid chromatography (HPLC) system (Waters, Breeze system, USA). AHL standard/extract was injected at a flow rate of 0.8 mL/min.

3.12. Bioassay for in situ AHL detection

Presence of AHLs was confirmed within the system using original bioassay consisted of *Agrobacterium tumefaciens* A136 (Ti -) (pCF218) (pCF372), a filter paper and the test sample. The extracted samples were applied on LB agar plates, containing A136 culture, antibiotics (Spectinomycin 50ug/ml, tetracycline 4.5 ug/ ml) and 40ug /ml X-Gal for visualization of AHLs.

A136 carries fusion trai - LacZ (pCF218) (pCF372) plasmids and capable of generating a blue color from the hydrolysis of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside by β-galactosidase, in response to C8-HSL, C12-HSL, C10-HSL, 3-oxo-C12-HSL3-oxo-C8-HSL and C14-HSL exogenous AHLs molecules.

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Chapter 4

Results and discussion

4.1. Evaluation of TMP Profile

Both MBRs were seeded with activated sludge and 8g/L MLSS was maintained throw-out the study. Operating conditions were also same except QQ-MBR was inoculated with polymer coated beads having QQ-bacteria (Rhodococcus**)** and C-MBR containing vacant beads with 0.5 % of the working volume (35L) of reactor. Average diameter of beads were 3.73mm and have smooth surface. TMP profile was used as an indicator of membrane fouling behavior and it directly relates to filterability of membrane. TMP profiles of QQ-MBR and C-MBR were compared in Figure 4.1 and significant differences in fouling behavior and filtration duration was noticed. C-MBR exhibited a rapid TMP rise to 30kPa in 12 to 15 days of operation while in QQ-MBR 55 days' operation was observed. The average fouling rate of both membranes were calculated and found that QQ-MBR has 4 time less fouling rate than C-MBR, 0.5kPa/day and 2.14kPa/day respectively. This shows that cell entrapped beads reduced the biofouling in QQ-MBR.

QQ-MBR showed less fouling and longer filtration time than C-MBR and was operated continuously for more than 55 days to reach 30 kPa. Intrinsic membrane resistance (***Rm***) of C-MBR membrane was increasing after every chemical cleaning and showed permanent fouling over time. TMP jump was also slow in QQ-MBR as compared to C-MBR.as shown in figure 4.1.

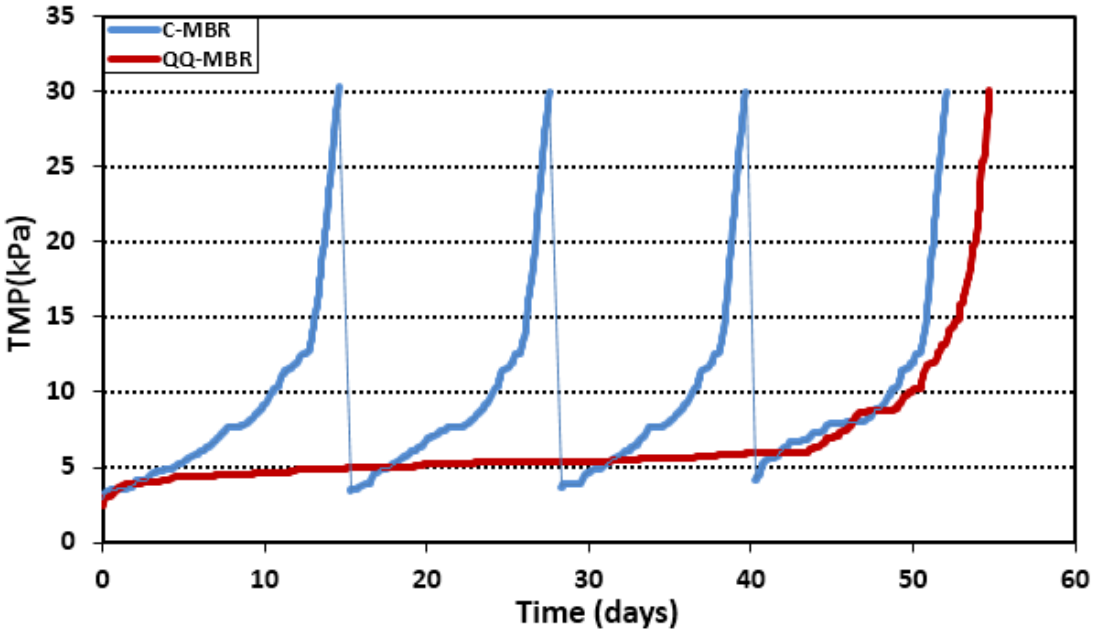


Figure 4.1 TMP profile of QQ-MBR and C-MBR membranes

4.2. Evaluation of filterability and dewaterability of sludge

Specific cake resistance (SCR) indicating the filterability as porosity of sludge layer deposited on the membrane surface and the capillary suction time (CST) is a convenient tool for the determination of sludge dewaterabilty. CST and SCR can be used for the characterization of membrane fouling (Wang et al., 2006; Wu et al, 2007). Chao and his co-workers observed a direct relationship between the EPS production and SCR, and reported that SCR increased as EPS increased. The bio cake layer deposited on the membrane surface plays an important role in membrane fouling with a high degree of hydraulic resistance.

SCR in QQ - MBR was 5.84 x 10^{13}(m/kg), which was 63% of that SCR of C- MBR, which was found to be 9.18x 10^{13}(m/kg) as shown in Figure 4.3. Lee and Yang (2007) found that LB-EPS has a negative effect on the sludge sedimentation, sludge dewatering and biofloculation and excess LB-EPS concentration causes flocs breakage that result in poor settelability and dewaterability. Improved capacity for sludge

dewatering has been found in QQ- MBR, in terms of the CST.QQ- MBR exhibited low CST of 15.7 seconds, while the C MBR showed greater CST of 21.85 seconds as shown in figure 4.2. Both SCR and CST confirmed the efficiency of CEBs in attenuating membrane filtration conditions, improved dewaterability and increased permeability. These advantages of QQ-beads make the QQ- MBR more acceptable.

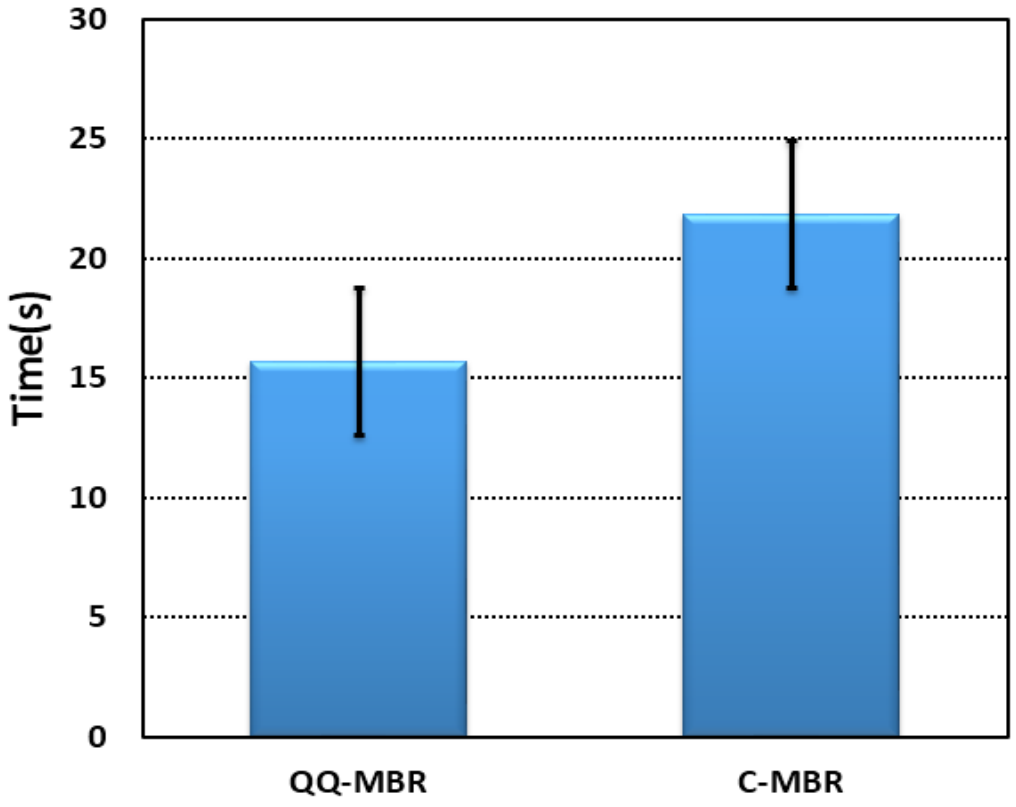


Figure 4.2 CST of QQ-MBR and C-MBR sludge

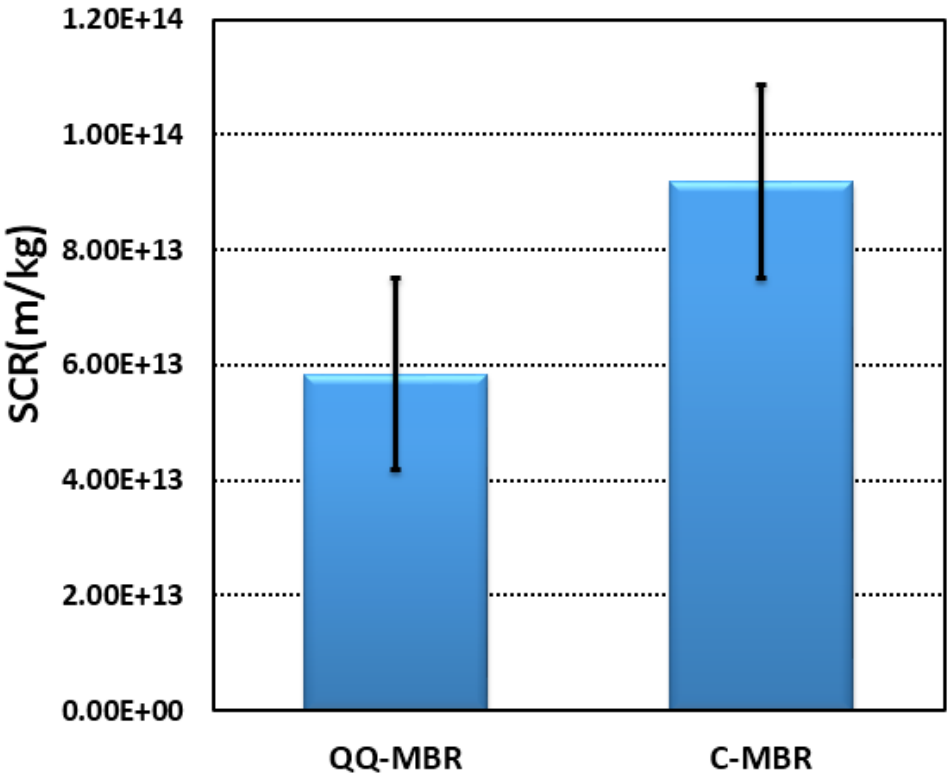


Figure 4.3 SCR of QQ-MBR and C-MBR sludge

4.3. Effect of quorum quenching on EPS production

Protein (PN) and Polysaccharide (PS) are considered two major components of EPS, which play an important role in membrane biofouling. EPS serve as scaffolding and provide a habitat for microorganisms to agglomerate on the membrane surface. Deng et al., (2014) observed that higher proteins concentration caused high hydrophobicity of sludge because of amino groups and cause higher biofouling.

Given the important role of EPS in membrane fouling can be divided into three categories, (i) soluble EPS also called SMP (ii) loosely bound EPS (LB- EPS) and (iii) are tightly bond EPS (TB - EPS). The effect of each type of EPS on membrane fouling was investigated and CEBs effect on all three types of EPS production was determind.

`

Initial EPS concentration of activated sludge of both MBRs was the same as both reactors were seeded with the same activated sludge and almost equal MLSS of 8 g/L, as shown in Figure 4.4. QQ-MBR showed a significant decrease in PS and PN concentration of soluble EPS, and become stable after 23 days of operation. Quorum quenching activity decreased the production of PN and PS by manifolds than C-MBR. On the other side, there was no substantial change in LB-EPS and TB-EPS in both MBRs as shown in Figure 4.6 and 4.7 the results showed a direct relationship of soluble EPS with the membrane biofouling and adding Rhodococcus led to lower production of EPS.

Based on these results it is concluded that the decrease in EPS production control the membrane fouling and the quorum quenching was found to be the major cause in less production of EPS. PN concentration was found to be very low in QQ- MBR,as compared to that of C-MBR which indicate less hydrophobicity of activated sludge flocs and inhibit biofilm formation on the membrane surface. Le-Clech et al. (2006) found that, PN make the flocs more hydrophobic than PS.

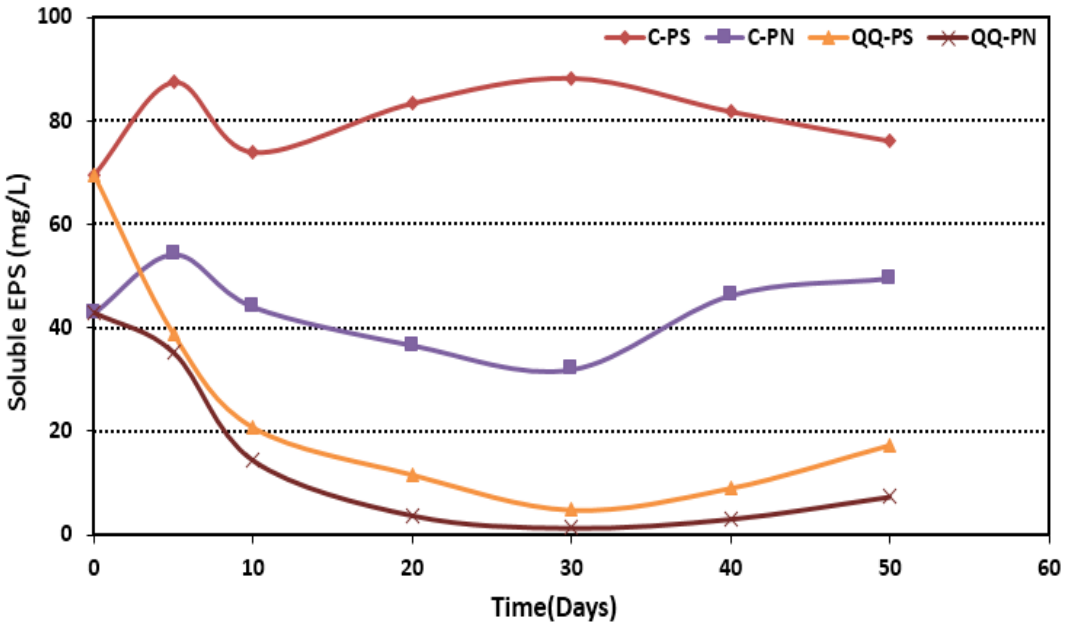


Figure 4.4 Soluble EPS in terms of PS and PN concentration in QQ-MBR and C-MBR

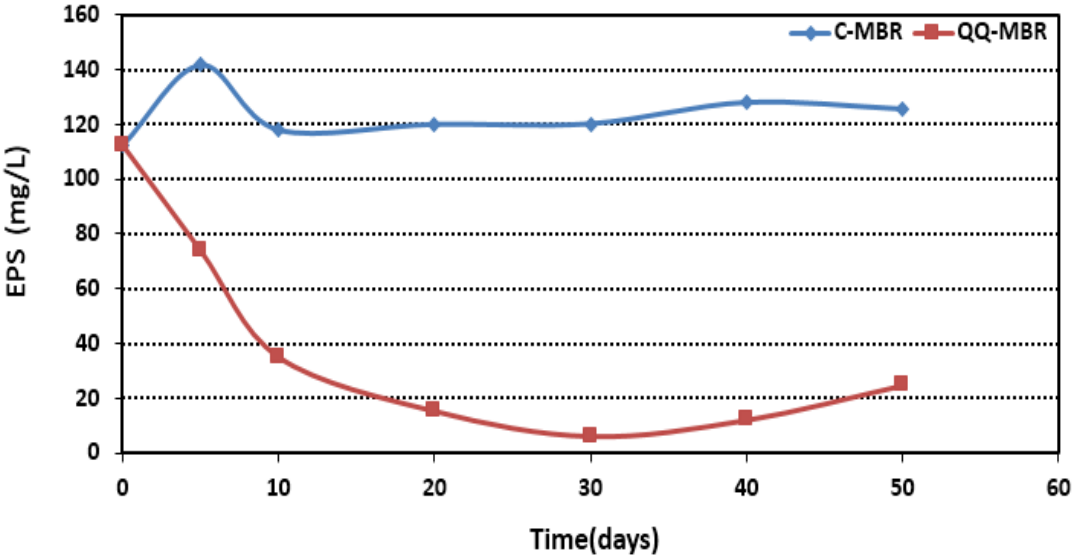


Figure 4.5 Total soluble EPS in QQ-MBR and C-MBR

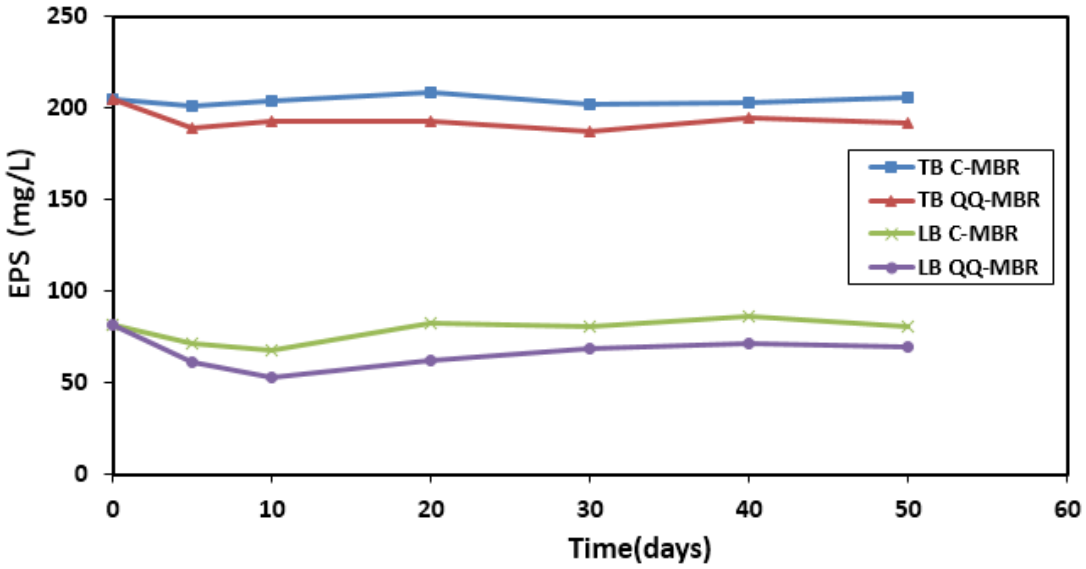


Figure 4.6 loosely bond and tightly bond EPS in QQ-MBR and C-MBR

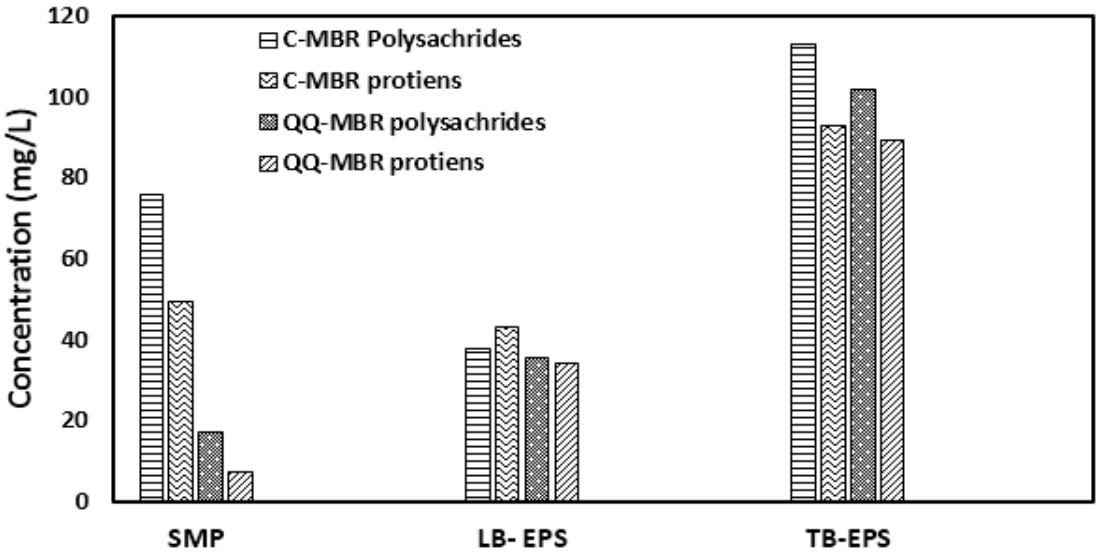


Figure 4.7 Soluble, Loosely and tightly bond EPS in QQ-MBR and C-MBR

4.4. Membrane Resistance analysis

The analysis results showed that the total hydraulic resistance (R_t), of the membrane in QQ-MBR had slightly high overall hydraulic resistance (R_t), after 55 days of continuous operation, while the C-MBR showed less R_t after 13-15 days of operation. Resistance in series model (RIS) was used to calculate total hydraulic resistance (Rt) and resistance analysis was performed when the TMP of membrane approached to 30kpa. The internal resistance Rm of C-MBR membrane was found that continuously increases after each cleaning shown in Figure 4.8, indicating permanent clogging of the membrane.

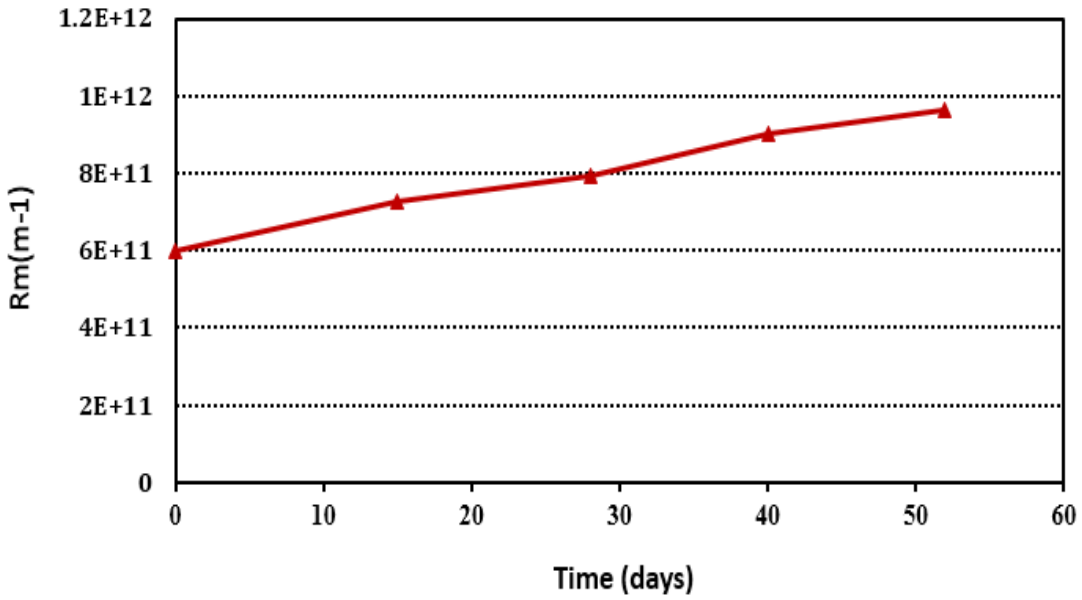


Figure 4.8 Rise in intrinsic resistance of C-MBR

Cake layer resistance (RC) has been found to contribute to the major share of resistance in C-MBR shown in Table 4.1. Jiang and his team (2013) investigated that more biocake induced high concentration polarization. Cake layer consists of many components that includes the microorganisms, organic and inorganic substances including EPS (Lee et al., 2001). Rc can be removed with physical cleaning and pore clogging resistance (Rp) is irreversible by physical means and it required acid base cleaning The Rp value of QQ-MBR was very high which accounted for 51.4% of the

total resistance of QQ-membrane while less pore block resistance was found in C-MBR with 35,3% of Rt. The results are shown in Table 4.1 which show that due to prolonged exposure of QQ-MBR membrane, soluble organic compounds are adsorbed directly onto the membrane surface and inside the pores of the membrane in the absence of a dense layer of sludge. Wu and his partners (2011) studied that Rc because of suspended solids and Rp blockage of pores due to colloids and solutes. From these results, a direct link can be established between the Rc and SCR with the CST and improved the behavior of the sludge by the addition of quorum quenching beads in QQ-MBR.

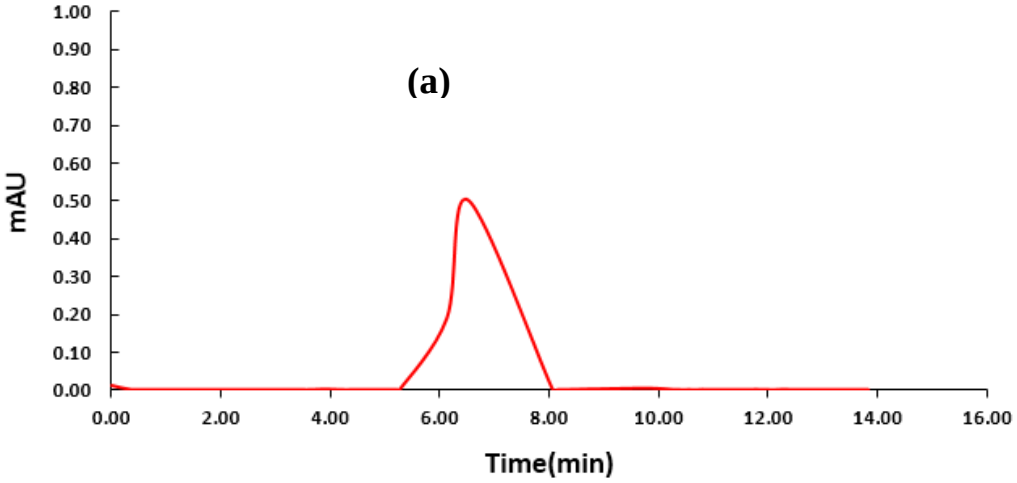
Table 4.1 Fouling resistance comparison of QQ-MBR and C-MBR

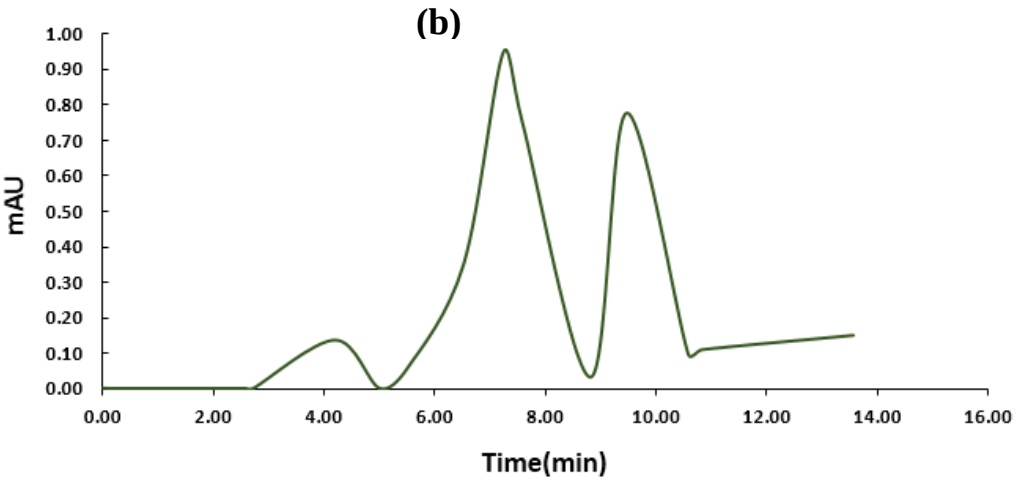
Resistance	**C-MBR** (x^{12} 1/m)	**QQ-MBR** (x^{12} 1/m)
Total hydraulic resistance, Rt	3.06	3.54
Cake layer resistance, Rc	1.14	1.06
Pore blocking resistance, *R*p	1.08	1.82
Intrinsic membrane resistance,Rm	0.904	0.66
Rc/Rt (%)	37 %	30 %
Rp/Rt (%)	35.3%	51.4%
Rm/Rt (%)	29.54%	18.7%

`

4.5. Evidence of AHLs in the MBR

In Figure 4.9 chromatograms from HPLC (a) C8-HSL, standard AHL showed highest peak around 6.9-7.0 minutes, if we look at the chromatogram of C-MBR and QQ-MBR, high absorbance of C-MBR found at 7.2 min which is correspond to the C8-HSL in the activated sludge while similarly less absorbance of QQ-MBR chromatogram showed at 6.8 min which is corresponds to C8-HSl, From these chromatographs we can conclude that quorum quenching bacteria successfully degraded the signal molecules and ultimately cause less biofouling.





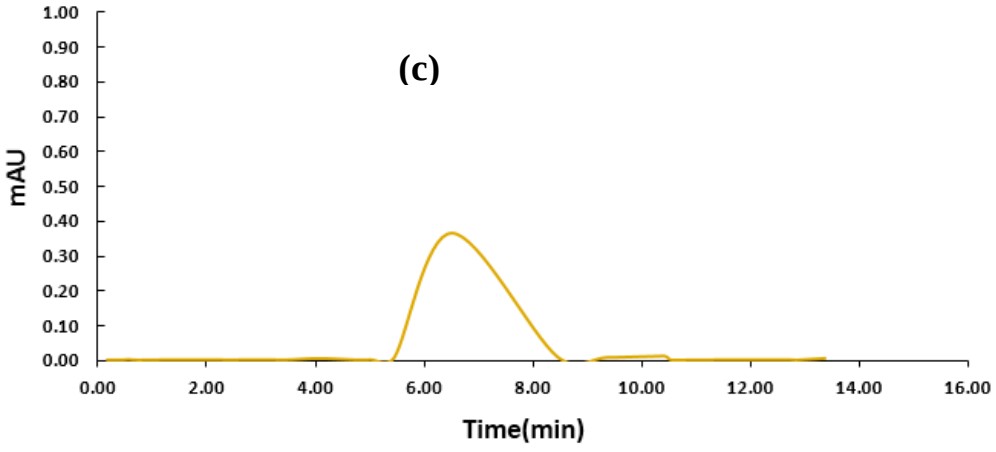


Figure 4.9 (a) standard C8-HSL sample (c) extract of C-MBR (d) extract of QQ-MBR sample

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4.6. Performance parameter

Both MBRs exhibited almost the similar organic removal performance with a minute difference. Our main focus was to investigate the performance of quorum quenching activity of Rhodococcus carrying beads to reduce or control the biofouling. COD and other nutrients removal was monitored three times in week during the study period and slight difference of COD and removal of nutrients was found in both reactors as shown in Table 4.2. From these results it can be concluded that the CEBs (quorum quenching mechanism) does not adversely affect the performance of MBR.

Table 4.2 performance comparison of C-MBR and QQ-MBR

Pollutants(%Removal)	**QQ-MBR**	**C-MBR**
COD	91%	93%
PO_4^{-3}-P	62%	55%
NH_4^{+1}-N	90%	94%

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Conclusions and Recommendations

Conclusions

The study elaborates the presence of vacant beads versus *Rhodococcus sp*. embedded beads in C-MBR and QQ-MBR, respectively treating. It was revealed the presence of CEBs prolonged the filtration cycle of membrane in QQ-MBR by reducing the biofouling, due to decrease in concentration of AHLs consequently and reduced the SMP concentration. The CEBs improved the sludge characteristics (SCR, CST) resulting in enhanced sludge filterability and dewaterability. The polymer coated alginate beads were efficient, stable and effective in handling real wastewater.

Recommendations

i. Investigation of the biofilm on membrane surface for further distribution of AHLs and EPS.
ii. Back washing may also be a good option to increase filtration time and reduce biofouling
iii. Investigate and verify the endogenous QQ bacteria for biofouling control in membrane bioreactor

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