CONTROL OF MEMBRANE BIOFOULING USING QUORUM QUENCHING BACTERIA IN MEMBRANE BIOREACTOR

By

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List of Abbreviations

Abbreviation	Description
A/O-MBR	Anoxic Oxic 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

Relaxation or backwashing is obligatory for effective operation of membrane module and intermittent aeration is also helpful for nutrients removal. In Phase I- effects of different filtration modes on membrane fouling behavior and treatment in membrane bioreactor (MBR) operated at three modes i.e., 12, 10 and 8 minutes filtration and 3, 2, and 2 minutes relaxation corresponding to 6, 5 and 4 cycles/hour respectively. Various parameters including trans-membrane pressure, specific cake resistance, specific oxygen uptake rate, nutrients removal and sludge dewateriabilty were examined to optimize the filtration mode. TMP profiles showed that, MBR $_{(8+2)}$ with 8 minutes aeration ON and 2 minutes OFF, reduced the fouling rate and depicted long filtration time in MBR, treating synthetic wastewater. MBR $_{(12+3)}$ was more efficient in organic and nutrients removal while denitrification rate was high in MBR $_{(8+2)}$. Dewaterabilty of sludge was high in MBR $_{(12+3)}$ due to longer filtration time. In Phase II- *Rhodococcus sp.* (BH4) entrapped in sodium alginate beads with effective volume of 0.1% of bioreactor, were used for the degradation of AHLs. QQ-MBR (with quorum quenching beads) experienced less biofouling as compared to C-MBR (without beads), leading to significant decrease in AHLs concentration in QQ-MBR. Soluble EPS production was found to be less in QQ-MBR wh. TMP profile of C-MBR was steeper than QQ-MBR, having short filtration duration, hence profound biofouling was observed in C-MBR. No AHL was detected in the extract of QQ-MBR, while presence of C_8HSL in C-MBR was confirmed using high performance liquid chromatography and bioassay. AHLs degradation along with EPS reduction increased the dewaterabilty in terms of capillary suction time and improve the specific cake resistance in QQ-MBR. Efficiency in terms of organics and nutrients removal was found to be same in both MBRs.

Chapter 1

INTRODUCTION

1.1 Background

Water scarcity effects the many countries in world, about 700 million people are under water stress (World Bank, 2007). Pakistan is one of the most water stressed country in hte world, which may be in near future declared as 'water scarce' having water availability less than 1,000m^3 per capita (Asian Development Outlook, 2013). The Situation can be worst if the water resources are not managed properly. Water reuse is need of the time to cope with this problem of scarcity.

Conventional activated sludge process can remove COD up to 95%, in which biomass is cultivated for organic matter degradation. Main three component of CAS Process are 1) aeration tank in which biomass comes into contact with wastewater 2) clarifier where liquid- solid separation take place and 3) Sludge recycling. The major disadvantages of CAS are that, it requires large area, higher HRT, lower SRT so discharge of excess sludge in CAS, MLSS value 2-4 g/L to easily settle the sludge in the secondary clarifier (Wang et al., 2009).

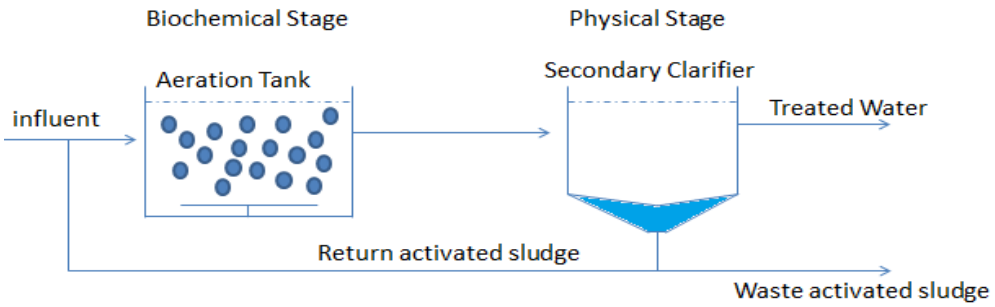


Figure 1.1- Conventional activated sludge process (CAS)

Among the wastewater treatment technologies, membrane bioreactor, a combination of activated sludge and solid-liquid separation by low pressure driven micro-filtration (MF) or ultra-filtration (UF) membrane, is most emerging technology from last two decades because of high effluent quality (Jahangir et al., 2012; Malaeb et al., 2013). Major advantages of MBR over activated sludge process are, (i) small foot prints, (ii) high concentration of mixed liquor, (iii) compact size, (iv) high quality treated water (Judd, 2006; Clech et al., 2010; Fu et al., 2012). High quality effluent from MBR, mostly as aerobic type, is suitable for further polishing by reverse osmosis and nano filtration.

Membrane bioreactor has become a well-established treatment process for both industrial and domestic wastewaters. 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. Nutrient removal is high in MBR because of high SRT necessary for slow growing nitrifiers and some other microorganisms (Metcalf and Eddy, 2003).

Disadvantages of MBR include, high energy cost and membrane fouling due to which short membrane life span and severe flux decline occurs resulting in need physical and chemical cleaning. Membrane biofouling control and energy consumption reduction is one of the major areas issues to increase the MBR market value.

Many researches have been conducted on membrane fouling control by chemical and physical techniques. Although these techniques were found to be efficient in controlling fouling but are able to prolong filtration duration for very short period of time with an extreme permeability loss. Current studies shows that, main constituents

of fouling are cakelayer, EPS and soluble microbial products. This bio-cake stick on the membrane surface and block pores of the membrane resulting in permeability loss.

This suggests that bio cake formation control could be a more relevant solution to befouling control as compared to conventional chemical and physical treatment (Yeon at al., 2009). Bacteria produce signal molecules or auto inducer for cell to cell communication which is called quorum sensing. These molecules are of organic nature with chemical structure of Acyl-Homocerine Lactones (AHLs). When the concentration of signal molecules reach to certain level then these molecules combine with receptor protein to activate specific genes for group behavior e.g., antibiotic production, virulence, EPS production and biofilm formation (Kim et al., 2012; Oh et al., 2011). EPS is considered to be major factor in causing membrane fouling which helps in the agglomeration of microbial flocs and biofilm. AHLs base quorum sensing is responsible for the production of EPS. Novel biological technique for membrane biofouling is to control AHLs concentration in environment so that EPS production can be controlled which is known as quorum quenching. Two types of quorum quenching are being researched nowadays, (i) enzymatic quorum quenching and (ii) bacterial quorum quenching.

1.2. Objectives

1) Establishment of automated Laboratory Scale MBR at IESE-NUST
2) Optimization of the filtration and relaxation mode for the prolong membrane filtration.
3) Preparation and induction of cells entrapped beads (CEBs) having quorum quenching bacteria in the reactor to control membrane bio fouling.

1.3. Scope of Study

1. Established automated MBR with working volume of 35L and PVDF hollow fiber membrane with pore size of 0.1µm and surface area of 0.7m^2.

2. Optimized MBR operation under three filtration and relaxation modes;

   (i) 8min of filtration with aeration and 2 min of relaxation without aeration

   (ii) 10min of filtration with aeration and 2 min of relaxation without aeration

   (iii)12min of filtration with aeration and 3 min of relaxation without aeration

3. Prepared CEBs using Rhodococcus (quorum quenching) bacteria

Chapter 2

Literature Review

2.1. MBR for advance wastewater treatment

Membrane bioreactor (MBR) is an advance technology for wastewater treatment which combines both activated sludge process and separation by membrane filtration unit. Wastewater is supplied to the reactor where microorganisms use this as a substrate for growth, maintenance and metabolism. Biologically treated water is separated by membrane either MF or UF. Activated biomass is recycled back to aeration tank (Drews, 2010; Poostchi et al., 2012;Trussell et al., 2006). The first full scale MBR was established in North America in 1970 and after that in Japan in 1980.

MBR is gaining attention for treating domestic as well as industrial wastewaters with advantages of better effluent quality for reuse as compared to conventional activated sludge process, small foot print, lower waste sludge production, more flexibility and high robustness (Cosenza et al., 2013; Masse et al., 2006; Wang et al., 2007; Yan et al., 2012).

2.2. MBR configuration

In MBR, filtration unit is coupled with bioreactor with one of two basic configurations i.e (i) Side stream MBR and (ii) Submerged MBR (Figure 2.1).

2.2.1. Side stream MBR (SS-MBR)

In SS-MBR, bioreactor is coupled with membrane unit placed outside where MLSS is circulated through the unit. To control deposition of suspended matter on membrane

surface, high cross velocity is required by circulation pump demianding high energy is consumed (Clech et al., 2005), shown in Figure 2.1(a)

2.2.2. Submerged MBR (SMBR)

In SMBR, membrane is submerged in activated sludge. This configuration was found to be more effective then side stream. In SMBR shear stress produce by aeration is high as compared to SS-MBR and can be easily controlled by varying aeration rate which ultimate results in high permeate rate and low membrane fouling (Howell et al., 2004).

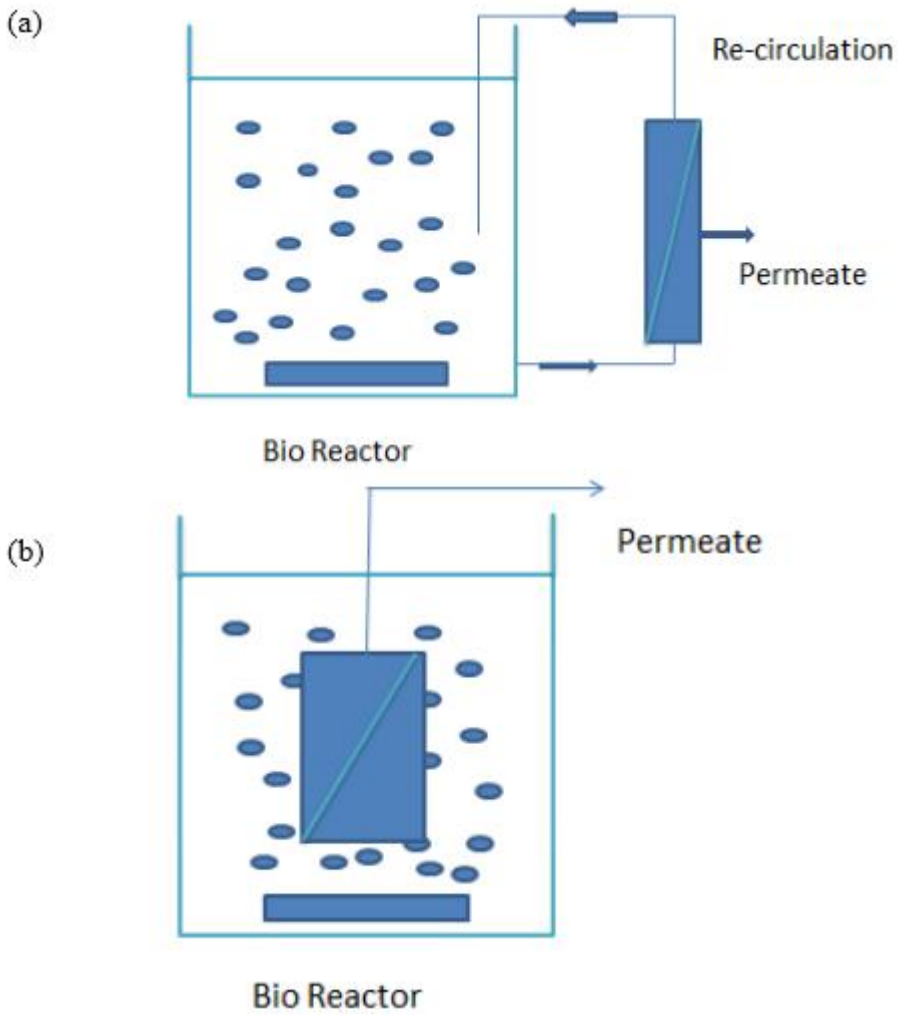


Figure 2.1- (a) side stream membrane bioreactor (b) Submerged membrane bioreactor

Table 2.1-General comparison of submerge and side stream MBRs

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%

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

Table 2.2 -Submerged and side stream MBR commercial suppliers

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

Source: (Yeon, 2009)

2.3. Aerobic and Anaerobic MBRs

Aerobic and anaerobic type of degradation are depend upon the redox conditions depending upon electron acceptor. In aerobic type of MBRs, air is continuously or intermittently supplied, course bubbles help in membrane scouring to avoid rapid membrane fouling and proper environment for microorganism growth. Operational cost of aerobic MBR increase due to the air supply as compare to anaerobic MBR, where air is not needed. Growth rate of aerobic microorganism is high while anaerobic microorganisms are slow growing, therefore retention time increases in anaerobic MBR. Side stream configuration is mostly used for the anaerobic type of MBR.

Table 2.3 Advantages and disadvantages of aerobic and anaerobic MBR

Parameter	Anaerobic MBR	Aerobic MBR
Energy consumption	Low	High
Removal Efficiency (%)	60-90	>95
Sludge production	Low	High
Stability	Low-Moderate	Moderate-High
Alkalinity	High	Low
Bio gas production	Yes	No
Nutrients Removal	Low	Potentially High

Source: (Yeon, 2009)

2.4. Introduction to Membrane Filtration

Membrane can be defined as a barrier which separate two different phases and hinder the transport of many particles through membrane. Membrane filtration is based upon the presence of semi permeable membrane which easily allows the water to pass through and restrict other chemicals. Effectiveness of membrane depends upon two factors (i) selectivity and (ii) productivity. Selectivity is referred to separation and retention and productivity is referred to as water flux.

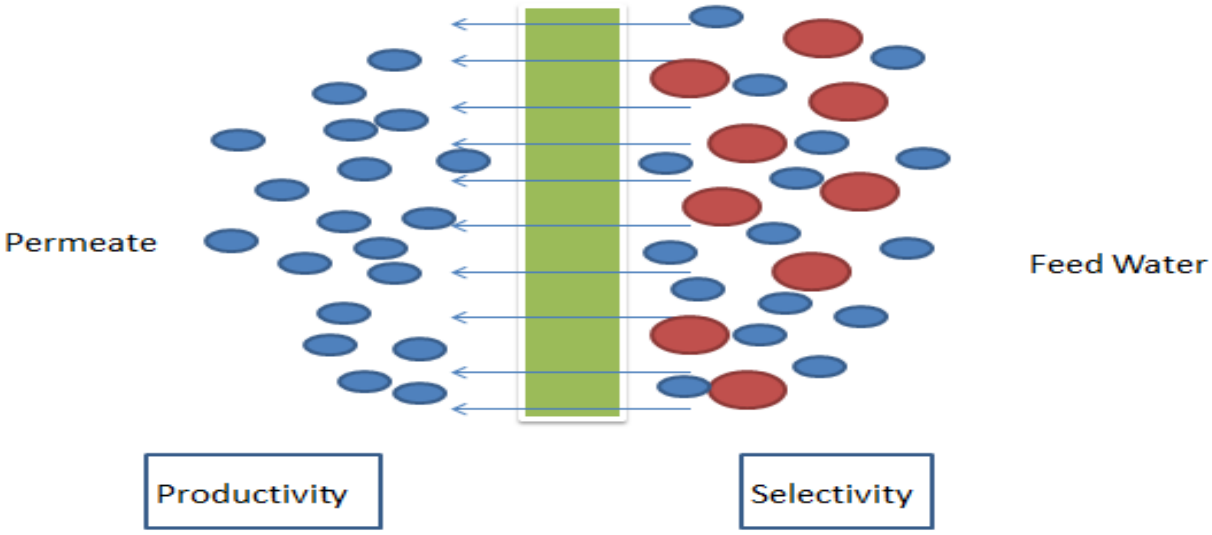


Figure 2.2 Membrane filtration process

Membrane filtration is divided into four categories depending upon pore size.

2.5. Microfiltration

In this type of filtration, membrane has coarse pore size ranging from 0.1 to 10µm. These types of membrane mostly use for separation of suspended particles from dissolved substances. Microfiltration removes all types of bacterial species. Only viruses are not caught in the process. Substances smaller than the pore size of membrane are partially remove. Microfiltration can also be used for the pretreatment of water before reverse osmosis and Nano-filtration.

2.6. Ultrafiltration

Ultrafiltration is used, where complete removal of viruses is required. Pore size rang from 0.001-0.1µm. Ultrafiltration can remove the particle and molecules ranging from 1,000 to 500,000 Daltons. (Lenntech, 2014).

Ultrafiltration membranes can be fabricated in to tabular or flat sheet. The single unit into which membranes are arranged is called membrane module.

2.7. Nano filtration

Nano filtration technique is becoming popular due to its narrow pore size of less than 0.001µm. Nano filtration is mostly used in water treatment for softening, micro pollutant removal and decolorizing. In nano filtration, molecules range from 100-1,000 Daltons can easily be retained.

2.8. Reverse Osmosis

Reverse osmosis (RO) is based upon the fundamental pursuit for balance. Two fluids come in contact with different concentration and are separated by a semi permeable membrane. Water flows from solution having high concentration of water to solution having low concentration till the concentration of both the solution become same, this is osmosis. The difference in head of water column is called osmotic pressure.

By applying pressure on the side where osmotic pressure is gained one can get reverse effect, and water will move from low concentration to high concentration and salt can be retained on membrane. Using this technique almost all salts can be removed.

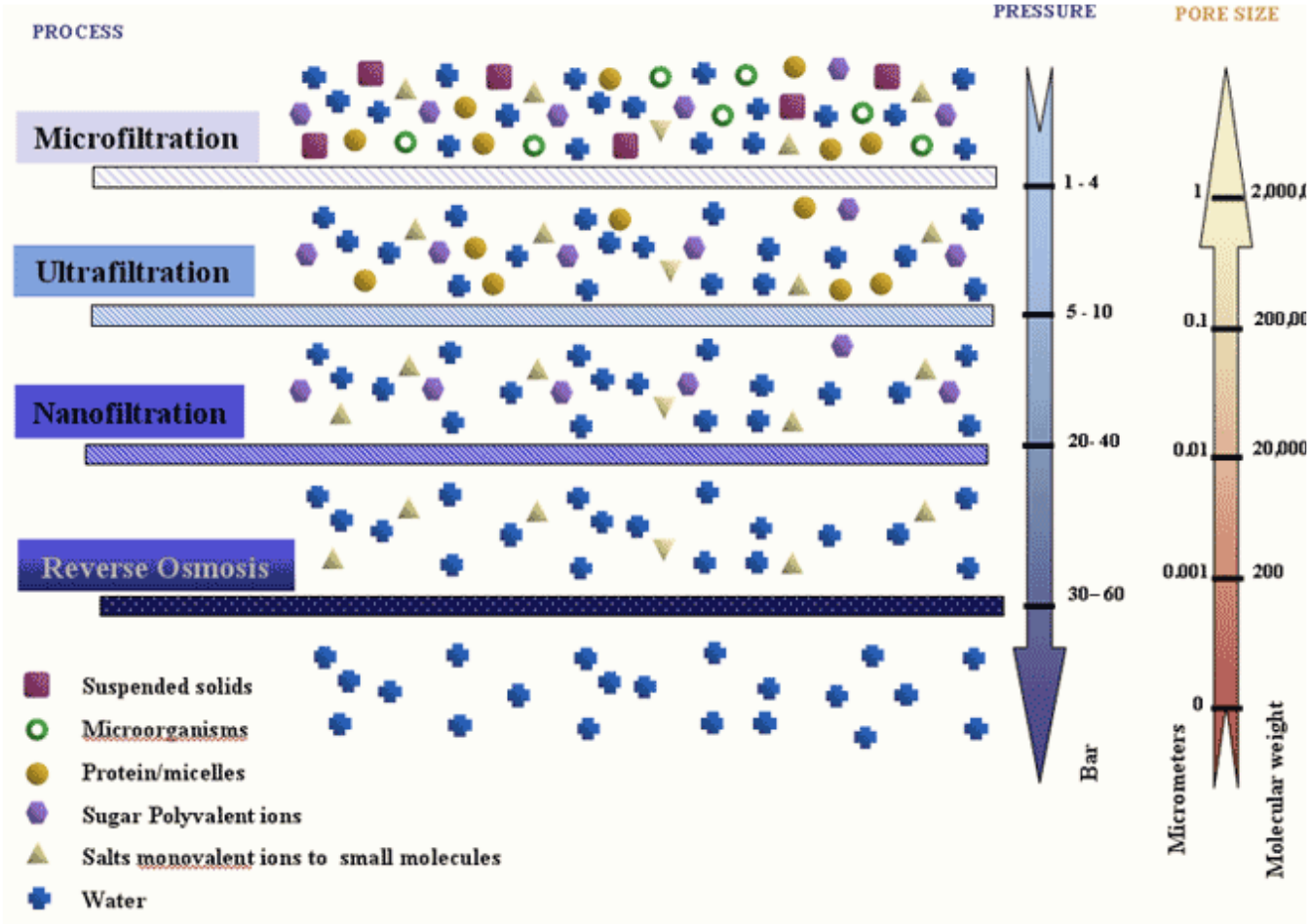


Figure 2.3 Types and categories of membrane filtration (Koch Membrane Systems, www.kochmembrane.com)

2.9. Membrane configurations

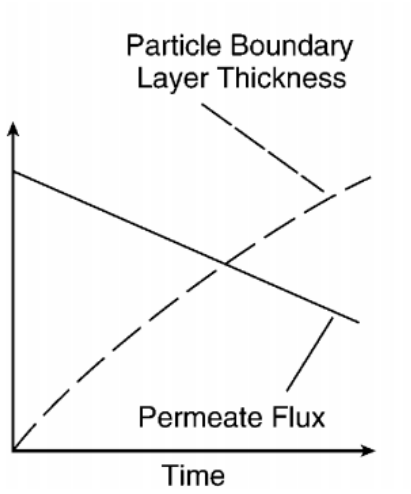
2.9.1. Dead end Filtration

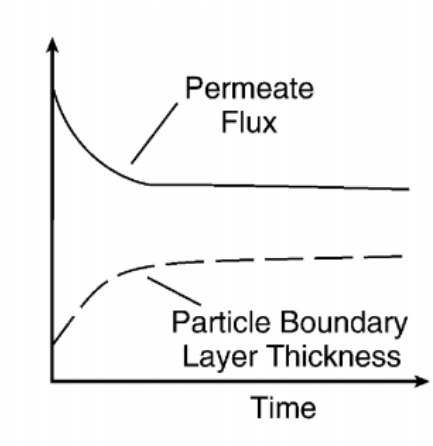
It is a basic form of filtration, which is mostly used for the separation of coarse particle from water or some other fluids. In Dead end filtration, coarse particles are accumulated on the filter and with passage of time clog the pores of filter or membrane. Particles make a thick layer on the filter called cake layer, which increases resistance to fluid flow. Filter or membrane can be reused after removal of cake layer. Dead-end filtration can be decent technique to concentrate the compounds.

2.9.2. Cross Flow Filtration

In cross flow filtration, high velocity of flow is parallel or cross flow to the filter medium surface. Cross flow filtration uses shear force created by the high velocity to maintain the cake layer minimum on surface of filter media. Although cross flow filtration does note eliminate cake layer completely. (Figure 2.4)

Dead-end Filtration **Cross Flow Filtration**





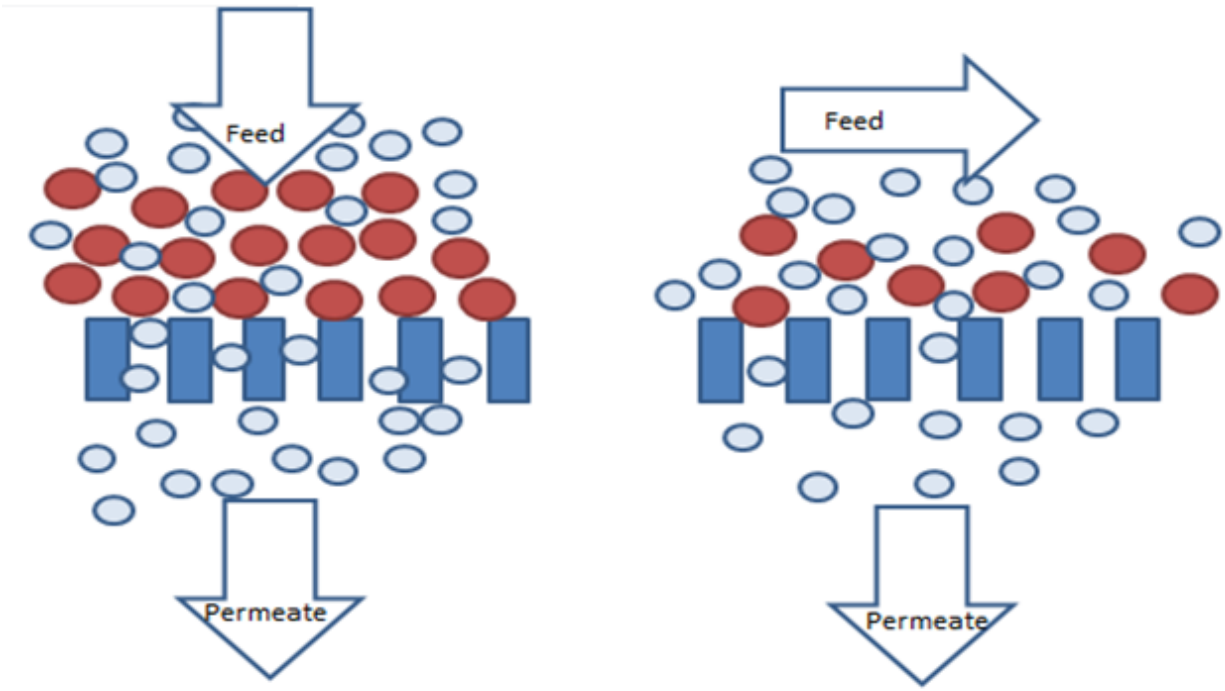


Figure 2.4 Dead and cross flow filtration (www.onlinembr.info)

2.10. Membrane materials

The membrane materials always show different fouling propensity due to their different pore size, morphology and hydrophobicity. Polyvinylidene fluoride (PVDF) membrane is superior to polyethylene (PE) membrane in terms of prevention of irremovable fouling in MBRs used for the treatment of municipal wastewater (Yamato et al., 2006). Inorganic membranes, such as aluminum, zirconium, and titanium oxide, show superior hydraulic, thermal, and chemical resistance. A stainless steel membrane was used for MBR, and the result showed that the stainless steel membrane could obtain a higher permeate flux (Zhang et al., 2005), and it is a potential alternative for the treatment of high temperature wastewater (Zhang et al., 2006).

2.11. Membrane operational parameters

2.11.1. Trans-membrane Pressure, flux and Resistance

Trans-membrane Pressure (TMP) is the basic driving force behind the filtration process. TMP is basically difference of pressure of in and outside of membrane, as the cake start to build on surface of membrane medium, which enhance the resistance of the material and TMP starts to increase. TMP is also used to predict the flux of membrane; flux is the fluid coming out of membrane per unit time.

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

J = flux, L/m^2.hr

ΔP = Trans membrane Pressure, kPa

μ = viscosity of permeate, Pa.s

R_t = total resistance, 1/m

2.12. Membrane Fouling

Membrane fouling is the real obstruction that prevents the fast commercialization of MBR. Membrane fouling is ascribed to both pore clogging and thick cake layer formation on the surface of membrane medium which is typically dominant form of fouling. As the fouling rate increases permeate flux start to decrease (Lee et al., 2001). Fouling is the undesirable attachment of microorganisms to the membrane surface and into pores. (Fangans et al., 2009).

Fouling can occur due to the following reasons enlisted below:

(i) Adsorption of colloids on membrane

(ii) Adhesion of sludge on membrane medium

(iii) Thick cake layer formation

(iv) Temporal changes in foulants

2.12.1. Stages of fouling

Typically fouling prospenityy can be divided into three stages:

Stage 1: Conditioning fouling

Conditioning fouling stage which is initial fouling caused by the interaction between EPS and SMP present in MLSS with membrane surface. Ognier et al. (2002) observed rapid irreversible fouling during initial stage, passive colloids and organics adsorption were also found even at zero flux operation. Ma et al. (2005) coupled vacuum pump instead of suction pump with air backflushing and was able to reduce the conditional fouling due to colloidal adsorption. Intensity of passive adsorption

may affect the pore size distribution and surface chemistry of membrane. Cake layer start to develop on the membrane surface which not effect flux in the initial stage but over the time cake partially or completely block the membrane pores which result in TMP rise.

Stage 2: Steady fouling

Even at below critical flux operation of membrane in biomass, temporally attached flocs on membrane surface can contribute to second stage of slow fouling. Most of the membrane surface already covered with EPS/SMP will promote more biomass flocs and colloidal attachment.

Stage 3: TMP jump

The abrupt rise of TMP or "Jump" is the result of filtration at constant flux and several mechanisms can be proposed for the TMP jump. Inhomogenous fouling (area loss)

model, Inhomogenous fouling (pore loss) model, Critical suction pressure model, Percolation theory, Inhomogenous fibre bundle model (Judd, 2006)

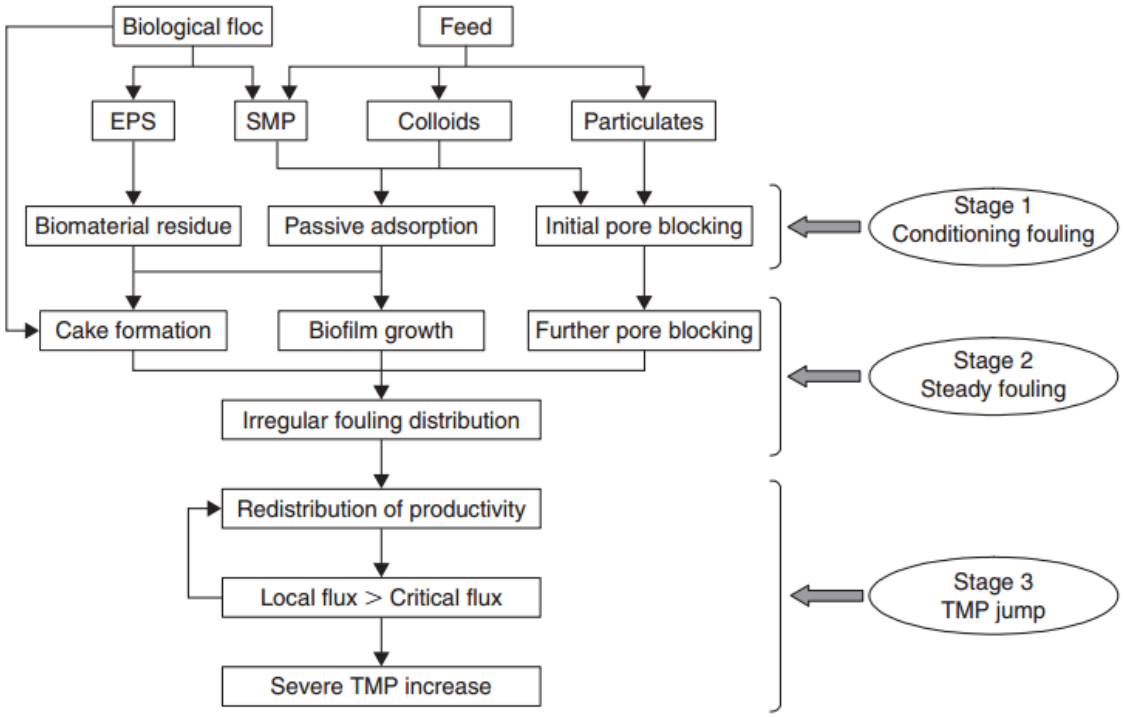


Figure 2.5 Fouling mechanisms during MBR operations (Zhang et al., 2006)

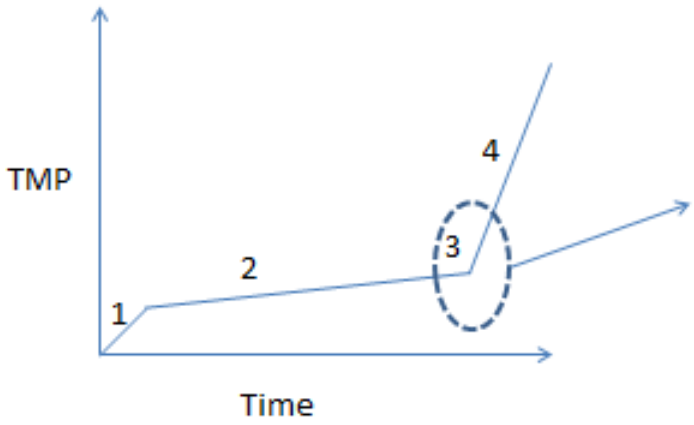


Figure 2.6 Fouling stages of membrane

2.12.2. Classification of membrane fouling

Membrane fouling is a very complex phenomenon as it occurs due to multiple reasons depending upon sludge flocs size, nature of foulants, and colloids, and hydrodynamics condition in membrane tank. Particles smaller than pore size of membrane either get absorbed on the membrane wall or constrict the pores. Particles having size larger than pore size form cake layer on the membrane surface. Fouling can be classified as per following three categories (Meng et al., 2009).

(i) Removable fouling

(ii) Irremovable fouling

(iii) Irreversible fouling

Removable fouling

Removable fouling is caused due to attachment of cake layer on the membrane surface; it can removed by physical cleaning of membrane or by backwashing.

Irremovable fouling

Some colloidal particles, solute and microbes enter the pore and cannot be removed by physical cleaning. At the same time, some inorganic particle also deposit on surface of membrane. For such irremovable fouling different chemical cleaning protocols are followed depending upon the material of membrane (Figure 2.6).

Irreversible fouling

Some particles deposited within pores of membrane cannot be removed by even chemical cleaning which tend to cause permanent irreversible fouling of membrane.

Excessive Irreversible fouling cause severe flux decline and ultimately membrane unit needs to be replaced.

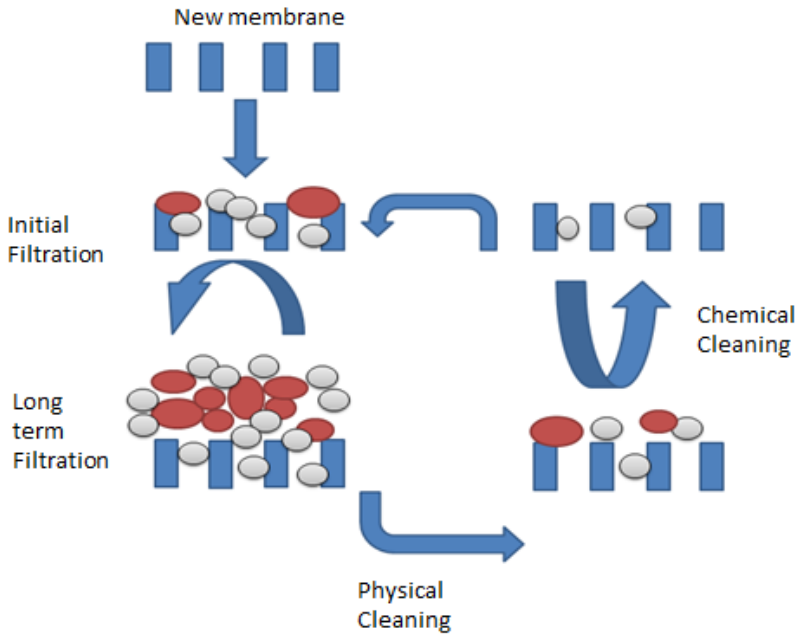


Figure 2.7 Deposition of foulants on membrane surface

2.13. Factors affecting fouling

Hydraulic retention time (HRT), sludge retention time (SRT), extracellular polymeric substance (EPS), pore size distributions, organic loading rate (OLR), and food to microbe ratio (F/M), dissolved oxygen (D.O) and pH play important role in membrane fouling. Sludge characteristics and operational conditions are the main factors affecting membrane fouling propagation.

2.13.1. Extracellular Polymeric Substances (EPS)

EPS are the biopolymer products of microbial excretion and cell-lysis. EPS are basically proteins, carbohydrate and humic substances. EPS are important in terms of

flocs and bio film formation, act like glue and protective layer on biofilm which protect the microorganism against toxic compounds. EPS are present in bound and soluble forms. EPS fill the space between cells, and form the matrix in which the cells live (Liu et al., 2001). EPS are main cause of fouling in membrane bioreactor and a linear relation is found between EPS and fouling (Lesjean et al., 2004).

2.14. Membrane biofouling

Membrane biofouling is associated with cake layer formed on the surface of membrane. Membrane biofouling causes permeability loss in MBRs (Kayung et al., 2008). Lee et al. (2007) reported, although the biofouling layer on the membrane in MBR consists of rejected microbial flocs, they are active to excrete slimy, glue-like substances (e.g., EPS) rather than inactive inorganic particles. It is also probable that irreversibly attached bioflocs residues and planktonic bacteria act as the seed for biofilm growth on the membrane surface. Maleab et al. (2013) reported that biofouling occur due to mechanisms: (i) colonization of membrane surface with microorganisms, (ii) production of membrane foulants by microorganisms. Meng et al. (2009) reported that operational parameter and wastewater characteristics also affect the membrane biofouling and higher SRT increases filterability. However further increase in SRT will intensify the biofouling.

EPS, soluble microbial products (SMP) concentrations and membrane material are related factors in causing biofouling (Gao et al., 2010). EPS play a major role in agglomeration of microbial flocs (Kim and Jang, 2006). Soluble EPS or SMP refers to soluble microbial products as soluble protein, polysaccharides and humic acids (Drews, 1999). Cho et al. (2004) investigated that specific cake resistance (SCR)

increases as the EPS concentration rise and developed relation between the SCR and EPS. Wang et al. (2009) found that EPS has a strong potential for biofouling as compared to mixed liquor in MBR, and found that soluble EPS and loosely bound (LB)-EPS plays a major role in fouling than tightly bound TB-EPS.

Fu et al. (2012) examined the effects of aeration rate, aerator position and aeration time and found that, aeration time and rate effect positively than aeration position in term of effluent quality and low fouling. Major factors which play a role in fouling are shown in Figure 2.7.

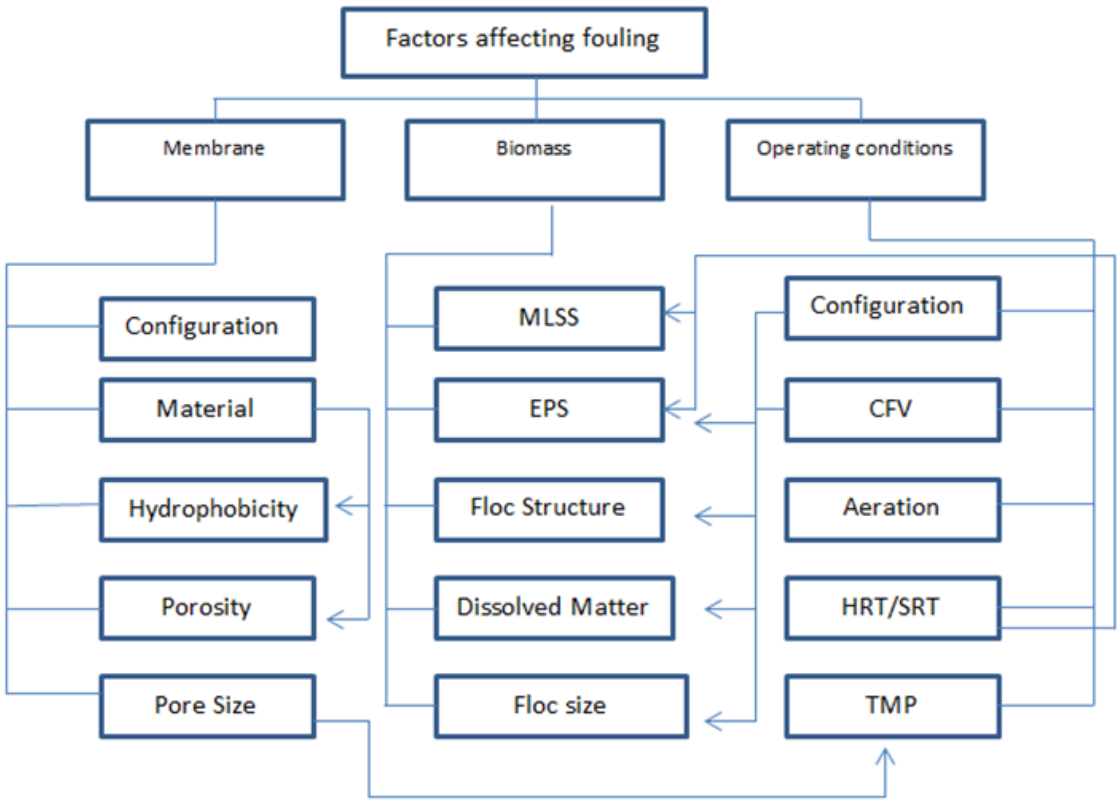


Figure 2.8 Factors affecting membrane fouling (Chang et al., 2002)

Cake layer contributes more in flux decline than internal clogging as cake layer can easily be removed by physical cleaning and classified as reversible fouling while chemical cleaning used for the removal of internally precipitated compounds which causes pore blockage, is classified as irreversible fouling (Chang et al., 2002).

2.15. Fouling control strategies

- Patterned membrane
- Addition of moving media and adsorbent
- Backwashing
- Air scouring
- Periodic relaxation
- Novel biological approach using quorum quenching bacteria.

Won et al. (2012) prepared a patterned membrane using lithographic method to mitigate the membrane biofouling and found significant reduction in deposition of microbial flocs as compared to conventional membrane. Many researches have been conducted on different moving media and adsorbents, backwashing with different flow rates, air flow rates and periodic relaxation were examined but all these strategies controlled the fouling up to short time and delayed TMP rise for few days than conventional MBR (Deng, et al., 2014; Wu, et al., 2008).

2.16. Quorum sensing

Bacteria show some behaviors in associated manner by cell to cell communication called quorum sensing and communicate with each other by signal molecules called

autoinducers. These signal molecules accumulate in the environment and when a critical concentration is achieved then associated manner is showed i.e virulence, secretion of polysaccharide and protein.

More than half dozen of quorum sensing types have been described in bacteria. Acyle homoserine lactones (AHLs) based quorum sensing is most common in gram negative bacteria in wastewater where more than 25 species are identified. AHLs are the signaling molecules which are of twelve types. AHLs consist of homoserine lacton ring attached with fatty acid side, having varying number of carbons.

2.16.1. Role of QS in biofilm

Shrout and Nerenberg (2012) described the phenomenon of quorum sensing and its main steps (i) protein to produce signal molecule by cell (ii) signal molecule concentration in environment and (iii) regulatory protein to accept the signal molecule and to complete the communication.

Mostly signal producing and receiving species are different, but some microbes show both the phenomenon simultaneously. Signal molecules receiving microbes play an important role in biofilm formation, those secreted EPS on receiving signal molecules. EPS helps in agglomeration of microbial flocs and biofilm formation. Researcher found a direct relation of AHLs concteration and EPS production. High AHLs concentration led to high membrane biofouling.

2.17. Quorum sensing control strategies:

Membrane biofouling can be controlled by three different point of attack aiming on AHLs:

(i) Signal molecules generator cells

(ii) Generated signal molecules

(iii) Signal receptor cells

Recently, a new term quorum quenching (QQ) is introduced for the inhibition of biofilm formation by deactivating or reducing AHLs concentration.

The most investigated strategy for biofouling control is the deactivation or hydrolyzing of generated signal molecules and found to be the most appropriate (Rasmussen et al., 2005).

2.18. Relevant studies carried out on quorum quenching (QQ)

Quorum quenching is an innovative technique for biofouling control in MBR. It brought revolution in mitigating the most concerned problems of this technology.

Yeon at al. (2009) deactivated the AHLs by hydrolyzing at lactone ring by lactonase and at acyl-amide linkage by acylase. They used procine kidney enzymes I (EC 3.5.1.14) and found reduction in AHLs concentration, EPS production and delayed TMP rise in MBR having acylase. Yeon at el. (2009) prepared the magnetic enzymes carrier (MEC) on which enzyme (acylase) was immobilized to resolve the problem of stability of free enzymes and results showed that immobilized acylase perform better than the free moving acylase with same quantity. Oh et al. (2011) worked on the isolation of quorum quenching bacteria (those produce qq enzymes) and found four species out of which *Rhodococcus* and *Panibaccilus* stains found to be more effective, they encapsulated the *Rhodococcus sp*.BH4 in micro-porous membrane and submerged in MBR run parallel to the control MBR operated at similar filtration mode. TMP profile showed substantial difference between MBR with QQ bacteria

encapsulated in membrane and control MBR. Jahangir et al. (2012) found a suitable position of micro porous membrane having encapsulated QQ bacteria and they reported that biofouling was less in MBR having micro porous membrane in membrane tank as compared to the condition in which micro porous membrane was place in separated bio-tank with recirculated sludge and QQ activity was also dependent on the rate of recirculation. Kim et al. (2012) worked on effect of QQ on microbial dynamics in MBR, QQ reduce the autoinducer producing microbial species which ultimately reduce the EPS production and results in less biofouling. Kim et al. (2013) prepared cell entrapping beads (CEBs) of sodium alginate having entrapped *Rhodococcus sp.*BH4, MBR setup installed for the analysis was of 1.6L batch type. This type of engineered quorum quenching mechanism was found to be most effective than others. Cheong et al. (2014) inoculated the bacteria, *Pseoudomance sp.1A1* in ceramic microbial vessel (CMV) and submerged these in MBR and compared the result with control MBR (C-MBR) and MBR with CMV having inactivated QQ bacteria. They found substantial reduction of AHLs concentration and EPS production in MBR having CMV with activated bacteria.

In this study,

Phase I - bench scale MBR was operated at three different relaxation modes with different filtration and relaxation cycle, i.e 12min, 10min and 8min filtration time with aeration and relaxation time of 3min,2min and 2min respectively without aeration corresponding to 4,5, and 6 cycles per hour.

Phase II- The batch scale work of Kim et al. (2013) was expanded to continuous bench scale MBR setup with a working volume of 35L over a 90 days of operation on synthetic wastewater.

Chapter 3

Materials and Methodology

3.1. Wastewater composition

Synthetic waste water with a medium strength having COD: N: P as 100:10:2 was used as substrate. Synthetic wastewater composition included glucose 514 mg/L, ammonium chloride (NH_4Cl) 190mg/L, Potassium di-Hydrogen phosphate (KH_2PO_4) 55.6 mg/L, Calcium Chloride($CaCl_2$) 5.7mg/L, Magnesium Sulfate ($MgSO_4.7H_2O$) 5.7mg/L, Ferric Chloride ($FeCl_3$) 1.5mg/L and Manganese Chloride($MnCl_2.4H_2O$) 1mg/L. pH of wastewater was maintained by sodium bicarbonate ($NaHCO_3$) at 7-7.5 for optimum function of activated sludge. Seed activated sludge was collected from the return line of full scale activated sludge wastewater treatment plant (capacity 17MGD, I-9 Islamabad, Pakistan) and acclimatized for one month period with synthetic wastewater of COD 500mg/L. Organic loading rate (OLR) of 2 Kg/m3/d and nitrogen loading rate (NLR) of 0.2kg/m3/d was maintained in the MBR.

Table 3.1 Synthetic wastewater composition

Chemicals	**Formula**	**Quantity (mg/L)**
Glucose	$C_6H_{12}O_6.H_2O$	514mg/L
Ammonium Chloride	NH_4Cl	190 mg/L
Potassium di-Hydrogen Phosphate	$KH2PO_4$	55.6 mg/L
Calcium Chloride	$CaCl_2$	5.7 mg/L
Magnesium	$MgSO_4.7H_2O$	5.7 mg/L
Ferric Chloride	$FeCl_3$	1.5 mg/L
Manganese Chloride	$MnCl_2.4H_2O$	1
Sodium bicarbonate	$NaHCO_3$	120 mg/L

3.2. Membrane material and types

Poly vinyldine fluoride (PVDF) hollow fibe (HF) I shaped membrane was used procured from Memstar, Chana. Fiber are vertically arrange having water collector on upside with built-in aerator. PVDF membrane has high robustness than other materials and forbearance to acid and basic chemical for cleaning. Detailed membrane characteristics are presented in Table 3.2 and membrane is shown in Figure 3.1.

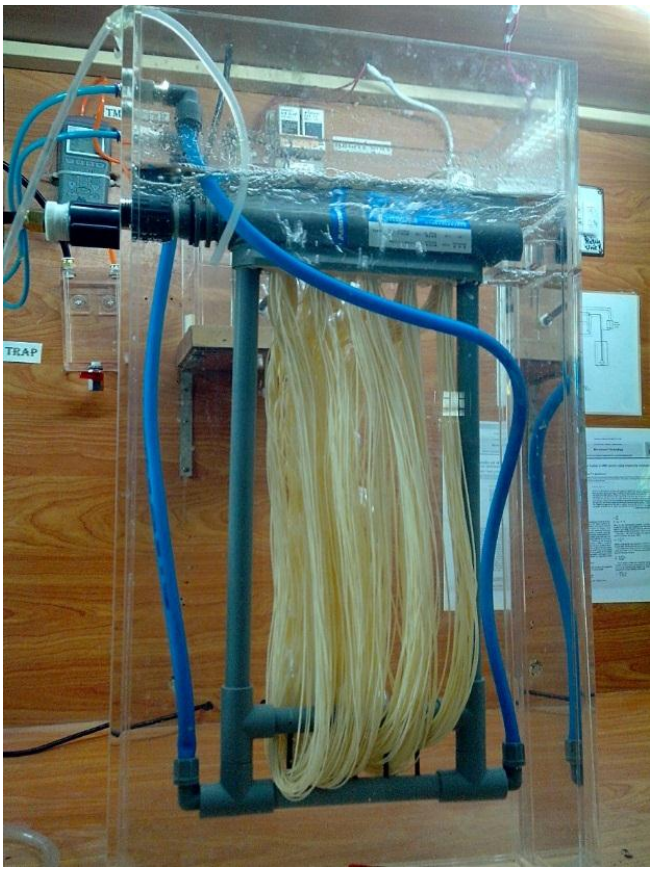


Figure 3.1 PVDF MBR membrane

3.3. Bench-scale MBR

3.3.1. Phase I

The bench-scale MBR setup with a 35 L of working volume was established in Water and Wastewater Laboratory, IESE-NUST as shown in Figures 3.2 and 3.3. Operating conditions are presented in Table3.1. MBR was operated at three different filtration and relaxation modes, i.e $MBR_{(8+2)}$ with 8min filtration and aeration ON and 2min for relaxation and aeration OFF, $MBR_{(10+2)}$ with 10min filtration and 2 min relaxation, and

$MBR_{(12+3)}$ with 12min filtration 3 min OFF for relaxation. For aeration air compressor (Masterflex ,Cole Parmer, USA) was used and air flow controller was used to maintain desired dissolved oxygen (DO) concentration in bio-tank. Peristaltic pump (Masterflex ,Cole Parmer, USA) with flow controller was used to maintain the 15 LMH. Synthetic wastewater contents were kept well mixed using mixer (Stir Park, Cole Parmer, USA).

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	15-50˚C

Table 3.2 Membrane characteristics

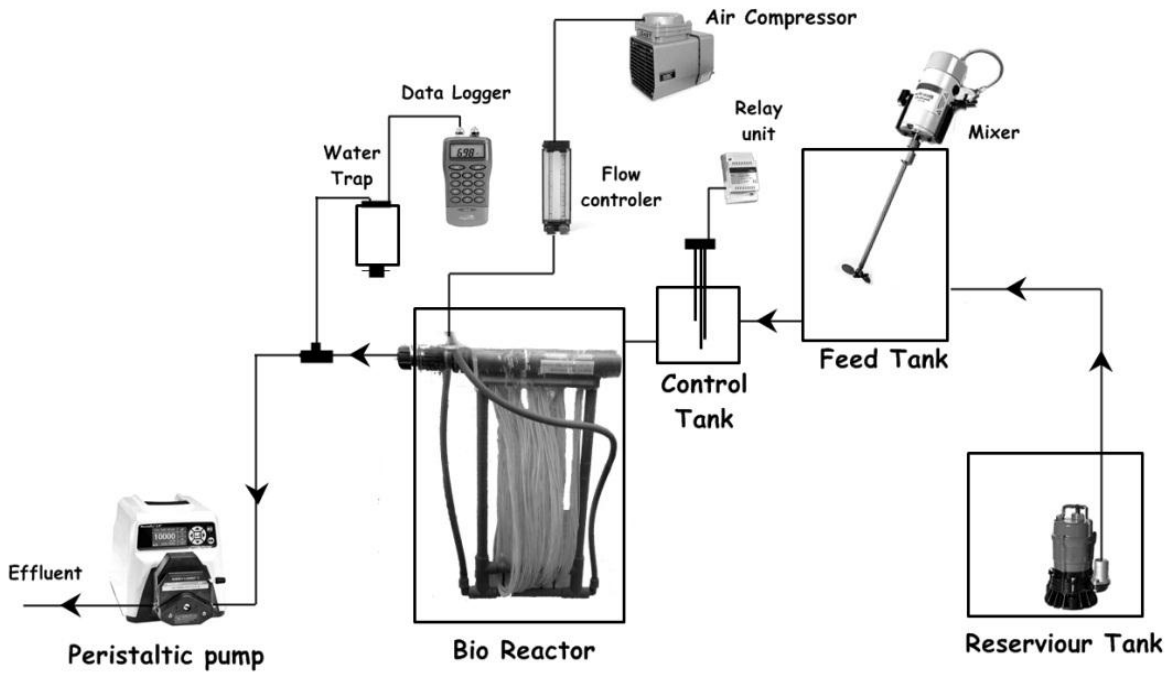


Figure 3.2-Process flow diagram for lab scale MBR during Phase I

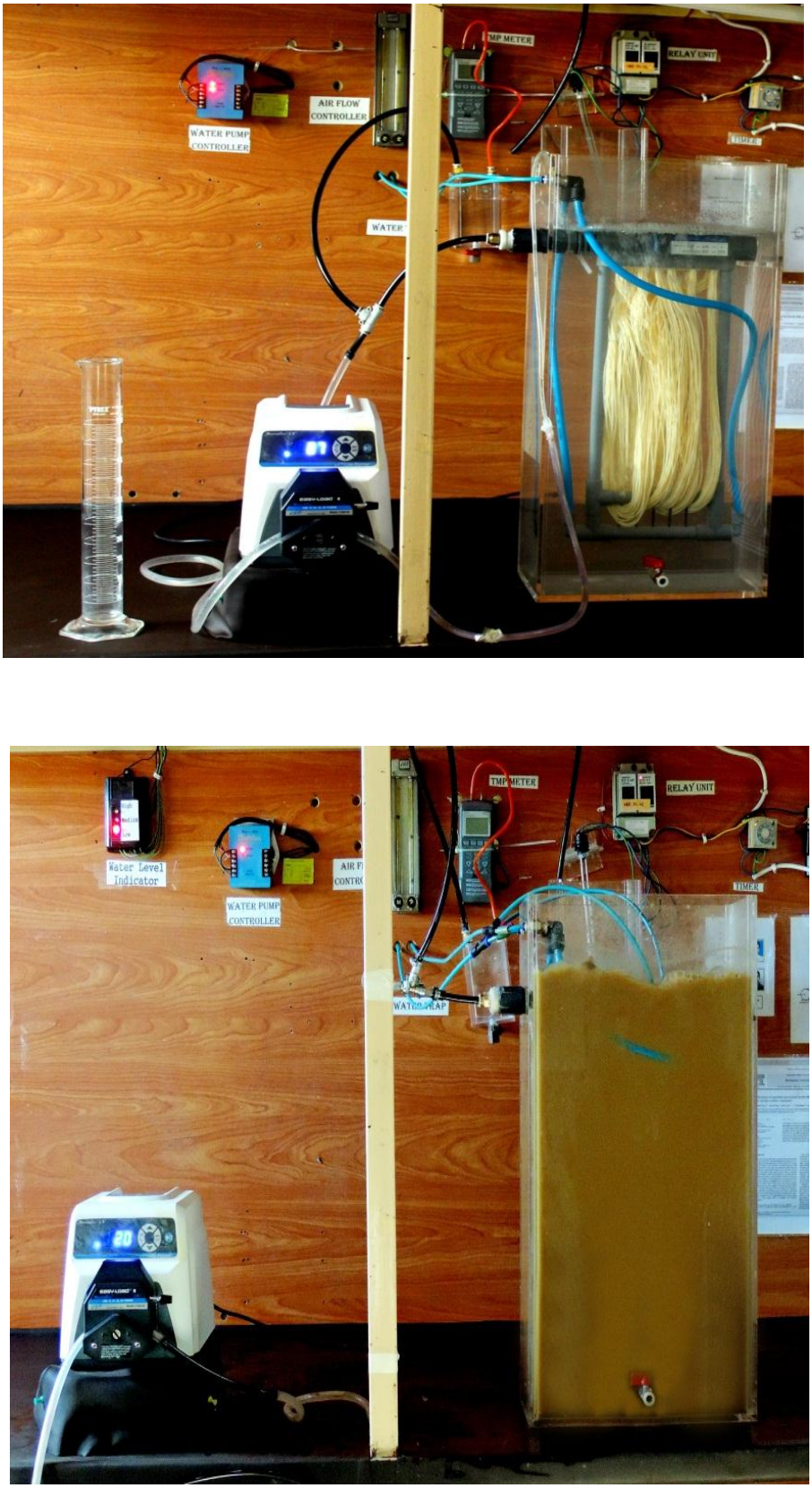


Figure 3.3- Lab scale MBR (a) without sludge (b) with sludge during Phase I

Table 3.3 -Working condition in MBR

Working conditions	
Working volume	35L
Water flux	15 LMH
HRT	4 hrs.
SRT	20days
MLSS	10-11g/L
Membrane Type	PVDF Hollow fiber

3.3.2. Phase II

Two parallel bench-scale MBR with 35 L of working volume of each were installed. One of MBRs was control MBR (C-MBR) and the second one was quorum quenching MBR (QQ-MBR). C-MBR was operated as conventional on optimized filtration and relaxation mode of 8 min filtration and 2 min relaxation. While QQ-MBR was inoculated with CEBs, all other operating parameters of QQ-MBR were similar to that of C-MBR.

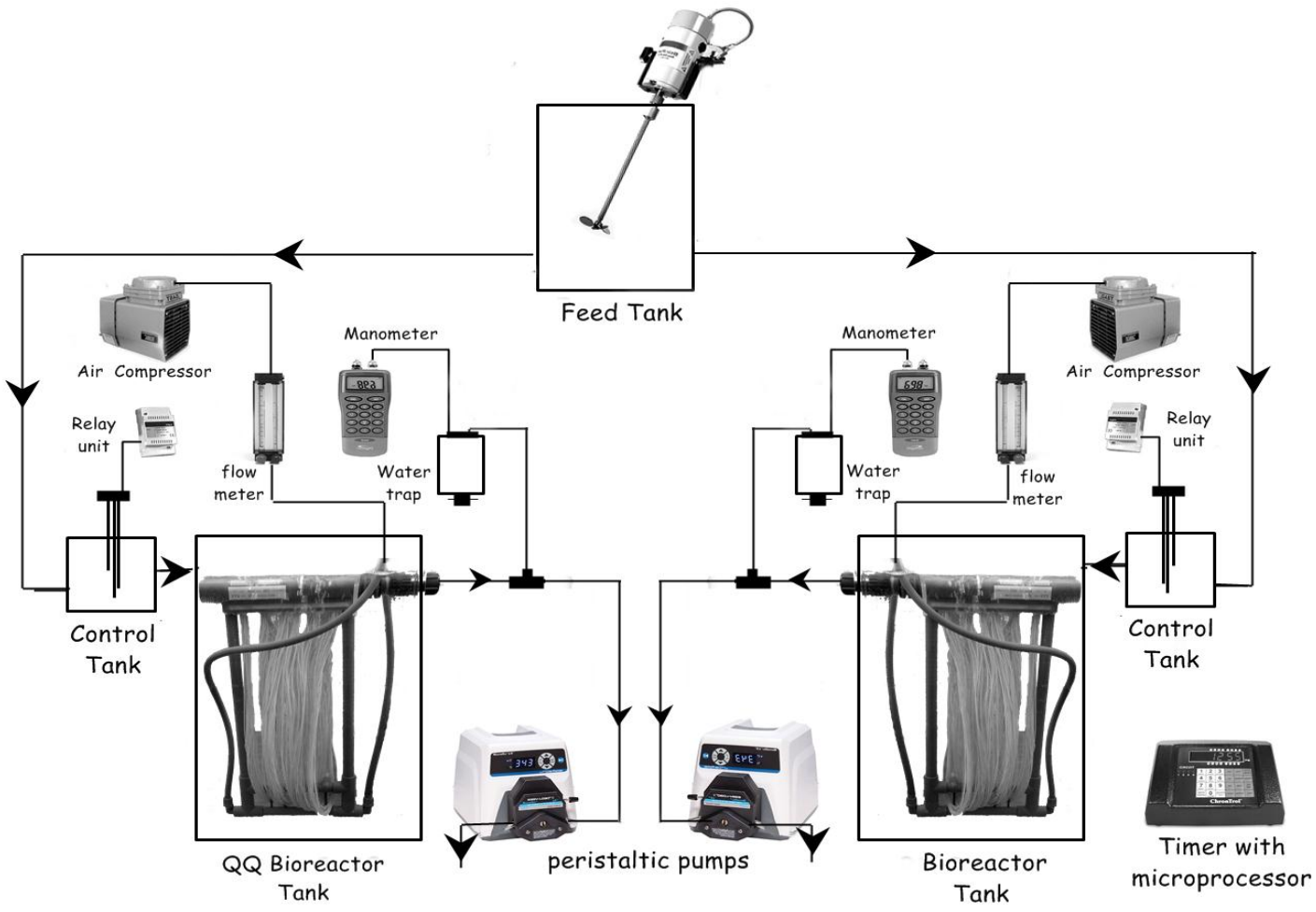


Figure 3.4-Process flow diagram of lab scale MBR during phase II

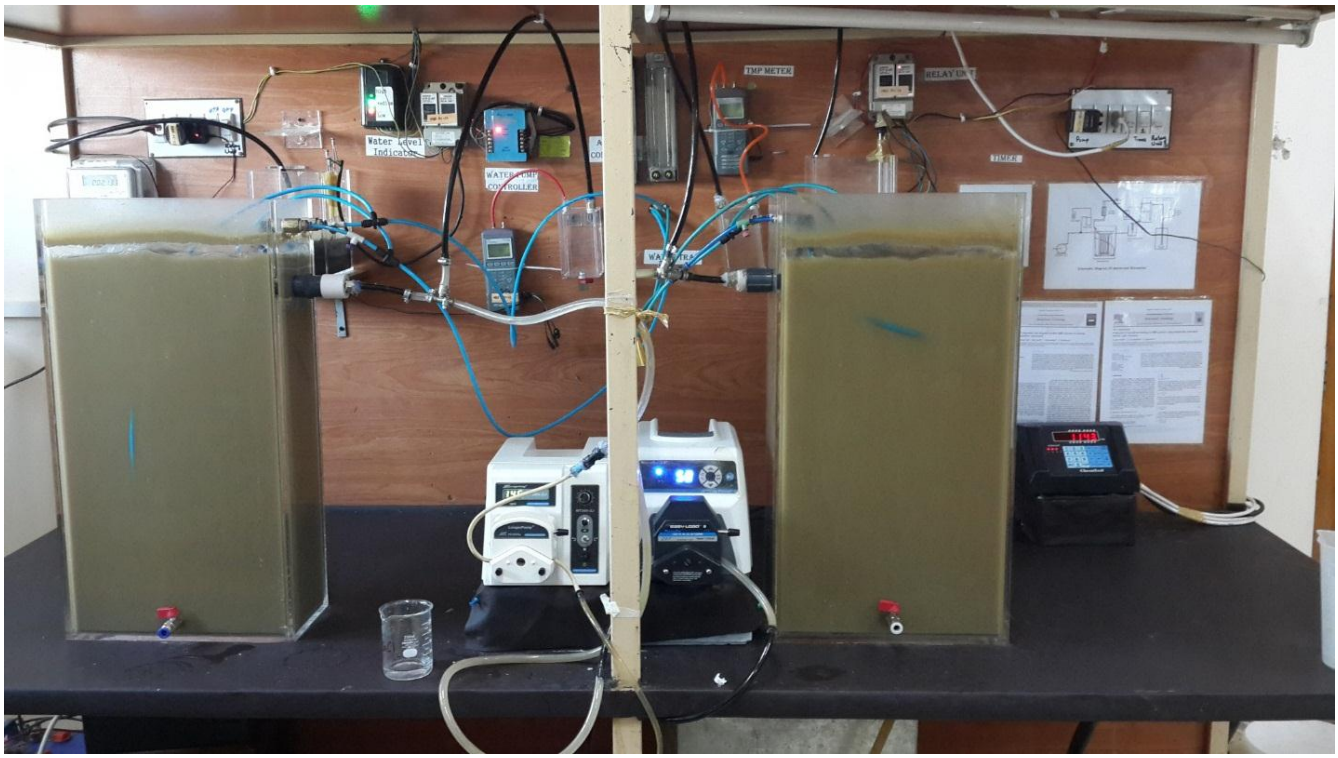


Figure 3.5-Lab-Scale MBR with sludge during Phase II

3.4. Analytical methods

Effluent quality parameters analyzed included COD, ammonia, nitrite, nitrate and phosphate. Sludge characteristics investigated include MLSS, MLVSS, capillary suction time (CST) for dewaterabilty and sludge volume index (SVI) for setteleabilty were measured as per Standard Methods (APHA et al., 2005). pH and DO were measured using multi-meter (pH/DO 300 series, Oakton, USA) . Microbial activity was determined in term of specific oxygen uptake rate (SOUR) using DO meter (YSI 5010, Cole Parmer, USA)**.**. Continuous trans-membrane pressure (TMP) was measured by TMP data logger (Super scientific, 84009, Taiwan).

3.5. Specific cake resistance

Specific cake resistances (SCR) test is used to determine to the cake resistance on the membrane surface for which dead end filtration cell (Amicon, 8400, USA) was used. Weight of permeate was continusoly measured using weight balance (Shimadzu, UW6200H, Japan) connected with computer. PVDF membraen filter (Millipore, GVWP09050, USA) with a pore size of 0.22µm and effective surface area of 90mm was used. Constant pressure of 30kPa was applied by nitrogen inert gas. SCR calculated by (Jamal et al., 2012).

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

where,

α = specific cake resistance, m/kg

A = PVDF membrane, 0.0042m2

ΔP = pressure applied, 30kPa

µ = dynamic viscosity of effluent after, N-S/m2

C = mixed liquor concentration, kg/m3

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

3.6. Membrane chemical cleaning protocol.

Prior to start to filtration run, membrane was cleaned chemically NaOH and NaOCl were used for basic cleaning to remove microbial deposit on membrane surface fouling 2% aqueous solution of sodium hydroxide (Sigma Aldrich, USA) along with sodium

hypochlorite having effective chlorine concentration of 2 g/L, while for acidic cleaning 1% HCl solution was used to remove inorganic foulants . After physical cleaning to remove cake, membrane was soaked in basic solution for 8 hrs and circulated with basic solution 30min. Lastly, membrane was rinsed with tap water and then was submerged and filtered with tap water for 30min.

3.7. Resistance Analysis

Resistance in series (RIS) model was used to evaluate the fouling potential of both MBRs.

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

$$R_t = Rc + Rp + Rm$$

Where,

R_t = total hydraulic resistance (1/m)

ΔP = TMP (Pa)

J = operational flux of permeate ($m^3/m^2/s$)

μ = permeate dynamic viscosity (Pa.s)

ft = temperature correction factor correspond to 20°C, $ft = e^{-0.0239(T-20)}$

Rc = cake layer resistance (1/m)

Rp = pore blockage resistance (1/m)

Rm = intrinsic membrane resistance (1/m)

Rc was produced by the cake developed on the surface of membrane, Rp due to small microbial flocs which constricted the membrane pores, R_t was calculated at the end of operation, for $Rm + Rp$, cake on membrane was removed and place the membrane in deionized (DI) water followed by flux and TMP measurement and Rc was determined by subtracting $Rp + Rm$ from R_t, Rm was measured after chemical cleaning of membrane and filtration of DI water (Wang et al., 2009). Contribution of each component of resistance was compared in both MBRs.

3.8. Extraction and quantification of EPS

EPS from MBRs sludge was extracted according to method developed by (Froelund et al., 1996) with some modification using Dowex cations exchange resins (Sigma-aldrich) for which 50ml activated sample was collected from each MBR and centrifuged using refrigerated centrifuge (K2015R, Pro-Reseearch, Britain) at 4,000g and 4◦C for 15min, supernatant having soluble EPS was separated form biomass pellet having bound EPS. For lightly bound (LB)-EPS extraction, biomass pellets was suspended in phosphoric buffer solution and stirred on magnetic stirrer for 1 hour after which centrifuged at 4,000g and 4◦C for 15min and lastly supernatant was removed for LB-EPS analysis. TB-EPS was extracted by re-suspending sludge pellet in buffer solution and adding Dowex cations exchange resins and stirred for 1 hour and after centrifugation supernatant containing TB-EPS was obtained.

Protein production was measured by Lowery method using the Folin-ciocalteu phenolic reagent which measures the copper ions reacting with peptide bond as the aromatic protein oxidize in alkaline solution (Lowry et al., 1951; Kunacheve and Stuckey, 2014) and absorption was taken on 750nm, bovine serum albumin (Sigma-alrich) was used for the preparation standard curve. For the quantification of total polysaccharide, Dubois method was employed in which sulfuric acid and phenol were used. Phenol and sulfuric acid addition turned the solution to yellow and absorption was taken at 490nm and standard glucose was used for the determination of total polysaccharide in the sample (Dubois et al., 1956).

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% CaCl2 solutions were prepared in D.I water. 5ml bacterial suspension then was mixed in sodium alginate after that sodium alginate solution was dripped in CaCl2 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 CaCl2 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. Figure 3.6.

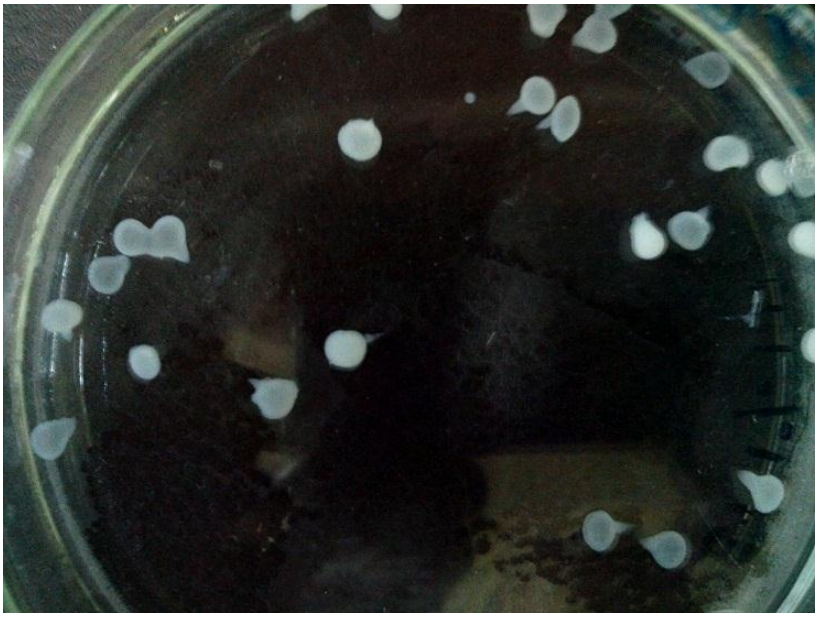


Figure 3.6- Cell entrapping beads

3.10. Extraction of AHLs from activated sludge

20 ml of sludge sample was centrifuged to separate supernatant from large microbial flocs then shacked with an equal volume of ethyl acetate. This mixture was vortexed at 120 rpm for 2 hrs. Organic layer was separated out using separating funnel. Further small flocs were removed by centrifugation at 4000g at 4˚C for 10 min. Supernatant was removed and dried in rotary evaporator at 30˚C and dissolved residue in 300 µl of methanol.

3.11. Detection of AHLs using HPLC

N-octanoyl homoserine lactones (C8-HSL) standard was procured from Sigma-Aldrich. C8-HSL standard was dissolved in methanol to obtain 1000 ppm stock solution. 1 mg/L solution was prepared as working solution by mixing 20 µl of standard stock solution with 980 µl of methanol having dissolved 0.1% formic acid. Analysis was performed using a water/methanol (35:65) as a mobile phase, and the

UV detector was set at 210 nm. Column, C18, HPLC (Waters, Breeze system, USA) was used for analysis. AHL standard/extract was injected at a flow rate of 0.8 ml/min.

3.12. Bioassay for in situ AHL detection

AHLs presence within the system was confirmed using an original bioassay consisted of Agrobacterium tumefaciens A136 (Ti-)(pCF218)(pCF372), a sterilized filter paper and sample to be tested. Extracted samples were overlaid on LB agar plates containing A136 culture, antibiotics (spectinomycin 50 µg/ml and tetracycline 4.5 µg/ml) and 40 µg/ml of X-gal for the imaging of the AHLs.

A136 bears the traI-lacZ fusion (pCF218) (pCF372) plasmids and capable of producing a blue colour from the hydrolysis of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside by the β-galactosidase activity, in response to C8-HSL, 3-oxo-C8-HSL, C10-HSL, C12-HSL, 3-oxo-C12-HSL and C14-HSL exogenous AHLs molecules.

Results and discussion

4.1. Phase I

4.1.1. Membrane fouling tendencies

TMP profiles show the membrane fouling behaviors of $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ as shows in Figure 4.1. A substantial difference was found in profiles of $MBR_{(8+2)}$ and $MBR_{(12+3)}$.

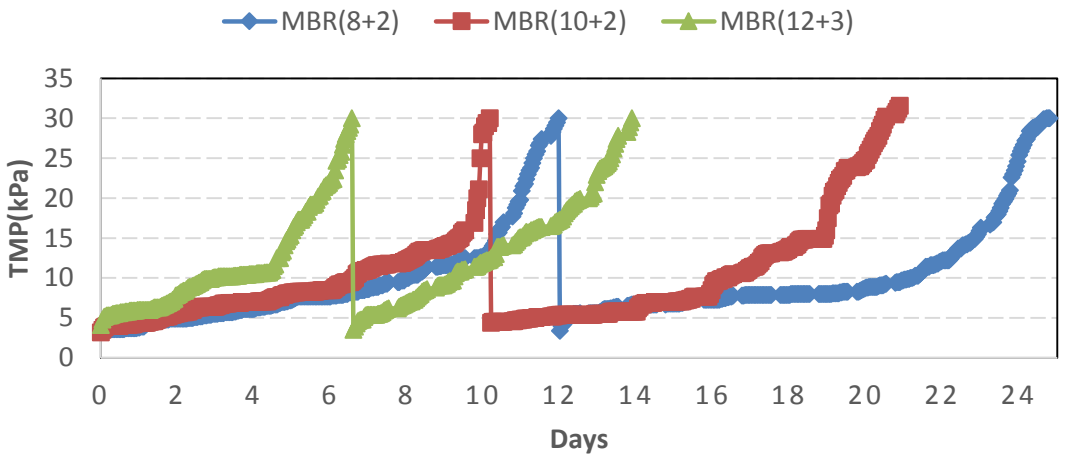


Figure 4.1- TMP profiles with different filtration modes

$MBR_{(8+2)}$ exhibited longer steady state and TMP jump phase periods with moderate gradual increase in TMP in steady phase followed by rapid rise as TMP jump. $MBR_{(8+2)}$ had relatively more cycle per hour i.e., 6 cycles per hour including 8 min of filtration with aeration and 2 min relaxation without aeration under each cycle, while $MBR_{(10+2)}$ and $MBR_{(12+3)}$ had 5 and 4 cycles with 10 min and 12 min of filtration and 2 and 3 min of relaxation respectively. Aeration is turned off during relaxation periods under all three filtration modes.

Membrane fouling rate (dTMP/dt) in terms of rise of TMP per unit time for all three different operational modes in three phase, maturation, steady and TMP jump phase is computed as

$$\frac{\mathrm{dTMP}}{\mathrm{dt}} = \frac{\mathrm{TMPf} - \mathrm{TMPi}}{\mathrm{dt}}$$

Where TMPf is final and TMPi is the initial TMP during filtration of membrane and dt is time duration. Membrane fouling rates are presented in Table 4.1, clearly illustrating that $MBR_{(8+2)}$ exhibited low value in all three phases of operation while $MBR_{(12+3)}$ found to be with high membrane fouling rates. During TMP jump fouling rate increased quickly, and during steady phase TMP rise was gentle for all type of MBRs with different filtration and relaxation modes. In $MBR_{(8+2)}$ during short filtration runs under each cycle, the TMP was not allowed to increase as compared to that in $MBR_{(12+3)}$.

Table 4.1 - Fouling rate in maturation, steady TMP jump phase and average for a complete fouled membrane

Filtration modes	Fouling rate in Maturation Phase (kPa/day) (3.5 kPa -6 kPa)	Fouling rate in Steady phase (kPa/day)(6 Kpa-8kPa)	Fouling rate in TMP jump phase (kPa/day)(8KPa -30KPa)	Average Fouling rate (kPa/day)
$MBR_{(8+2)}$	0.70	0.20	4.30	2.03
$MBR_{(10+2)}$	1.05	0.30	6.40	2.40
$MBR_{(12+3)}$	2.50	0.50	7.10	3.31

4.1.2 Evaluation of fouling resistance

Results of membrane resistance analysis for all three different filtration modes are summarized in Table 4.2

Table 4.2- Membrane fouling resistances in MBR under different filtration modes

Resistance(10^{12})	**$MBR_{(8+2)}$(1/m)**	**$MBR_{(10+2)}$(1/m)**	**$MBR_{(12+3)}$(1/m)**
Total hydraulic resistance, R_t	2.7 ± 0.5	2.88±0.25	3.51 ± 0.3
Cake layer resistance, R_c	1.09 ± 0.1	1.38 ± 0.25	1.90 ± 0.2
Pore blocking resistance, R_p	0.88 ± 0.05	0.81 ± 0.06	0.9 ± 0.1
Intrinsic membrane resistance , R_m	0.67 ± 0.2	0.72 ± 0.2	0.7 ± 0.1
R_c/R_t (%)	40	48	54
R_p/R_t (%)	33	28	26

According to Table 4.2, cake layer resistance decreased with decrease in filtration time which i.e reduction from 12min to 8min filtration and consequently increase in cycles per hour. $MBR_{(12+3)}$ experienced high Rc and Rp value as compared to other two MBRs. Due to longer filtration time in $MBR_{(12+3)}$, compressibility of cake increased and cake porosity decrease as compared to that in both $MBR_{(10+2)}$ and $MBR_{(8+2)}$ with short filtration time.

4.1.3. Performance analysis

The COD removal, ammonium, nitrate, and phosphorus with different filtration and relaxation modes are presented in Table 4.3. The COD removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ was found to be 93.3 ± 0.7%, 94.4 ± 0.4% and 95.1 ± 0.6% respectively. $MBR_{(12+3)}$ having longer filtration and aeration time per cycle has high COD removal as compared to $MBR_{(8+2)}$ having shorter filtration and aeration time. There are two process which nitrogen in wastewater could be removed either assimilatory or nitification-dinitrification process.

Table 4.3- Performance evaluation parameters

Parameters	**$MBR_{(8+2)}$**	**$MBR_{(10+2)}$**	**$MBR_{(12+3)}$**
COD (% removal)	93.3 ± 0.7	94.4 ± 0.4	95.1 ± 0.6
NH_4^{+1}-N (% removal)	53.6 ± 2.7	64.1 ± 1.9	69.9 ± 1.7
NO_3-N (% removal)	95.2 ± 2.0	67.8 ± 1.1	57.5 ± 4.3
PO_4^{+3} (% removal)	41.3 ± 11.8	47.8 ± 1.2	48.9 ± 3.9

(Han et al., 2005) found that lengthier aeration time impede the denitrification process, and also slow down the assimilation process for nitrogen removal.

The ammonium (NH_4^{+1}) removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ was 53.6 ± 2.7%, 64.1 ± 1.9% and 69.9 ± 1.72% respectively, and nitrate removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ was found to be 95.2 ± 2.0%, 67.8 ± 1.06and 57.5 ± 4.3%

respectively. High denitirtiifcation rate was observed in $MBR_{(8+2)}$ and low in $MBR_{(12+3)}$ which may be due to shorter aeration time per cycle in $MBR_{(8+2)}$ and more intermittent filtration which enhanced the growth of denitrifier and due to longer aeration time inhibited their growth in $MBR_{(12+3)}$.

In activated sludge process phosphorus can be removed by two methods either (i) assimilation or by (ii) luxury uptake (uptake beyond need). In former mechanism, growth phase of microbes is more important for phosphorus removal while in luxury uptake, phosphorus is removed when substrate alternately passes through anaerobic and aerobic bioreactors (Rosenberger et al., 2002).

Phosphorus removal by three different operational modes, $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ were found to be 41.3 ±11.8, 47.8 ± 1.18 and 48.9 ± 3.94% respectively. The main reason for low nutrients removal in MBR was because of relatively short hydraulic retention time (4 hrs).

4.1.4. Evaluation of compressibility, activity and dewaterability

SCR is a unique parameter for the characterization of cake compressibility deposited on the membrane surface. Cake deposited depends upon suspended solids concentration, particle size distribution, cake porosity, aeration and filtration (Chang and Kim, 2004). SCR for $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$ was found to be 0.98 x 10^{14},1.27 x 10^{14} and 1.75 x 10^{14}(kg/m) respectively, which depicts low permeability with high thickness of cake deposited on the membrane surface in $MBR_{(12+3)}$ and consequently causing high cake layer resistance (Rc).specific oxygen uptake rate (SOUR) is mostly used for the assessment of microbial activity in activated sludge in

bioreactor. Microbial activity was high in $MBR_{(12+3)}$ as compared to other two filtration modes.

Dewaterbility is associated with rate of water release from activated sludge. Dewaterability of activated sludge depends upon sludge viscosity, flocs size and presence

of mono and divalent cations and also correlated with the filterability (Guglielmi et al., 2010). CST apparatus is used for determination of dewaterability in which filter having

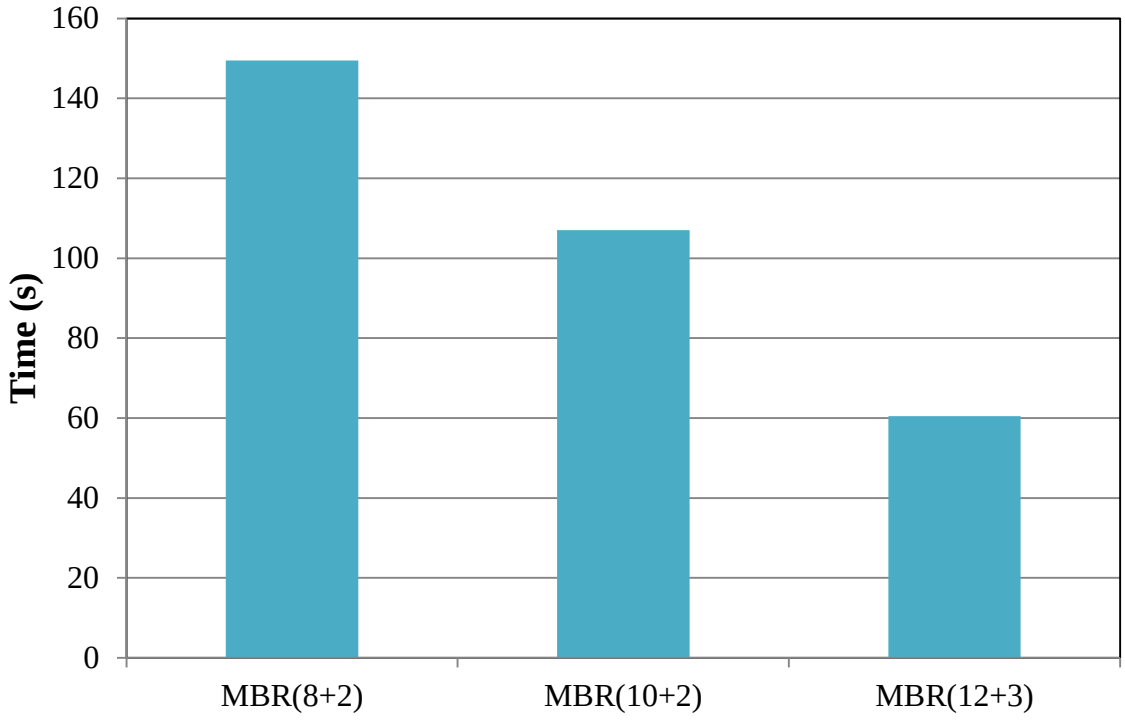


Figure 4.2- Capillary suction time for different filtration mode

capillary is used and timer results in seconds.

As shown in Figure 4.3 $MBR_{(8+2)}$ exhibited less dewaterability with high CST of 149.5s and dewaterability in $MBR_{(12+3)}$ was high having less CST value of 69.5s, these results did not show any direct relation of dewaterability with SCR.

4.2 Phase II

4.2.1. Evaluation of TMP Profile

Activated sludge with MLSS of 10g/L was used for both C-MBR and QQ-MBR with an effective volume of 35L. QQ-MBR was inoculated with CEBs having entrapped *Rhodococcus* with effective volume of 0.1%, CEBs were spherical in shape with smooth surface. TMP profiles are determined as indicators of membrane fouling behavior and directly relate to membrane filterability. TMP profiles of both C-MBR and QQ-MBR were compared Figure4.4 and substantial differences in fouling behavior and duration were found. QQ-MBR exhibited a deferred TMP rise while in C-MBR rapid TMP rise to 30kPa within 10-14 days was observed. Average membrane fouling rate ($\Delta P/\Delta t$) of membrane in QQ-MBR was found to be 0.3kPa/day, while higher in C-MBR , 2.3kPa/day which was 7 times higher, this shows that CEBs reduced the biofouling in QQ-MBR. Membrane of C-MBR was washed physically and chemically TMP reached 30kPa. At this stage, it was taken out of bioreactor for the physical cleaning by removing the cake layer followed by soaking in NaOH and NaOCl solution for basic cleaning. For acidic cleaning 1% HCL solution was used afterward if considered necessary. Membrane of QQ-MBR operated for more than 90 days to reach 30kPa with delayed steady phase, and showed less fouling and longer filtration time than C-MBR. Rm of C-MBR membrane was increasing after every chemical cleaning and induced some permanent fouling. After which TMP jump was also slow as compared to C-MBR. Figure 4.4

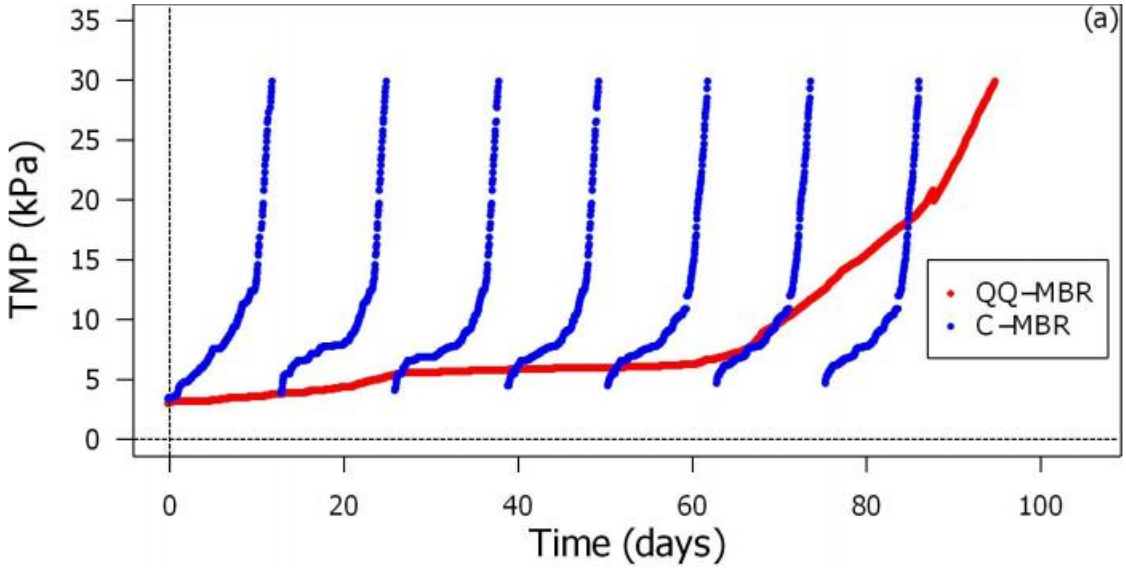


Figure 4.3 TMP profile of QQ-MBR and C-MBR membranes

4.2.2. Evaluation of compressibility, activity and dewaterability

SCR indicates filterability in the form of the porosity of cake deposited on membrane surface and CST is a convenient tool for the determination of dewaterabilty of excess sludge and both CST and SCR can be used for characterizing membrane fouling (Jin et al., 2004; Rosenberger and Kraume, 2002; Wang et al., 2006; Wu et al., 2007). Chao et al., (2004) observed a direct relation between SCR and EPS production, and reported that SCR was found to increase as EPS increased. The deposited cake layer on membrane surface play a major role in membrane fouling with a large share of hydraulic resistance. SCR in QQ-MBR was 6.2 x 10^{13}(m/kg), which was 56% that of SCR of C-MBR which was found to be 1.1x 10^{13}(m/kg) as shown in Figure 4.5. Li and Yang, (2007) found that LB-EPS has negative effects on sludge settling, sludge deaterability and bioflocuulation and excess LB-EPS cause flocs structure deterioration which results in poor settelability and sludge-water separation. Improved dewaterability of sludge was found in QQ-MBR, in terms of CST. QQ-

MBR exhibited a low CST of 47 seconds, while C-MBR showed a substantially high value CST with 89 seconds. Both SCR and CST confirmed the performance of CEBs in terms alleviating membrane filtration, improved dewaterability and enhanced permeability. These merits of CEBs make QQ-MBR more acceptable. It is also inferred from the results that CST and SCR are important parameters that has direct relation with membrane fouling.

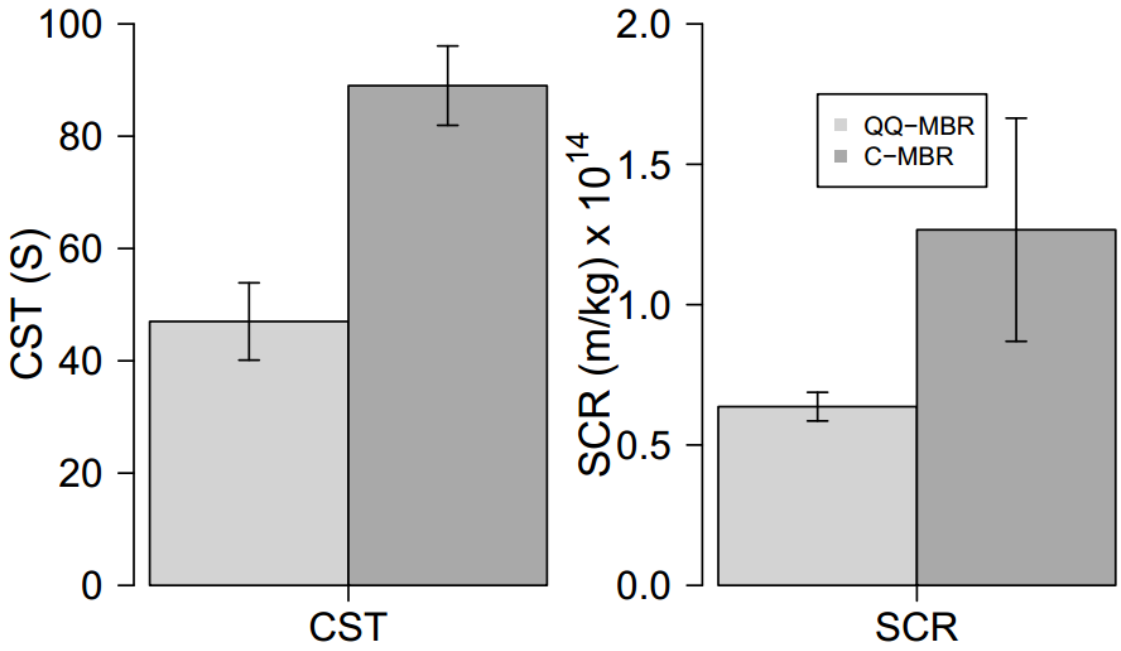


Figure 4.4 CST and SCR of QQ-MBR and C-MBR

4.2.3. Performance parameter

Both MBRs showed similar pollutant removal performance with slight difference. Overall COD and nitrogen removal was less due to low HRT i.e 4 hrs because over main focus was to investigate the performance of quorum quenching activity of

Rhodococcus to mitigate biofouling. Negligible difference of COD and nutrient removal was found in both MBRs as reported in Table 4.4. From results it can be concluded that, quorum quenching mechanism does not adversely affect the MBR operation. Microbial activity in terms of SOUR were also found to be same in both MBRs, by which it can be inferred that microbial activity remained intact by the CEBs application.

Table 4.4 Performance evaluation parameters

Parameters	**C-MBR**	**QQ-MBR**
COD(% removal)	94.3 ± 1.3	94.6 ± 1.7
PO_4^{+3}(% removal)	50.8 ± 2.9	50.0 ± 3.9
NH_4^{+1}-N(% removal)	53.4 ± 2.5	55.4 ± 2.3
NO_3-N(% removal)	94.7 ± 2.1	93.6 ± 1.1

4.2.4. Resistance analysis

Results of membrane resistance analysis showed that, QQ-MBR had a less total hydraulic resistance (Rt) even after the 95 days of continuous operation, while C-MBR showed high Rt after merely 10-12 days of operation. Rt was measured using resistance-in-series model (RIS) and resistance analysis was performed when the membrane approached to TMP of 30kPa. Intrinsic membrane resistance Rm of C-MBR was found to be continuously increasing after every chemical cleaning Figure 4.5, which indicates permanent fouling of membrane.

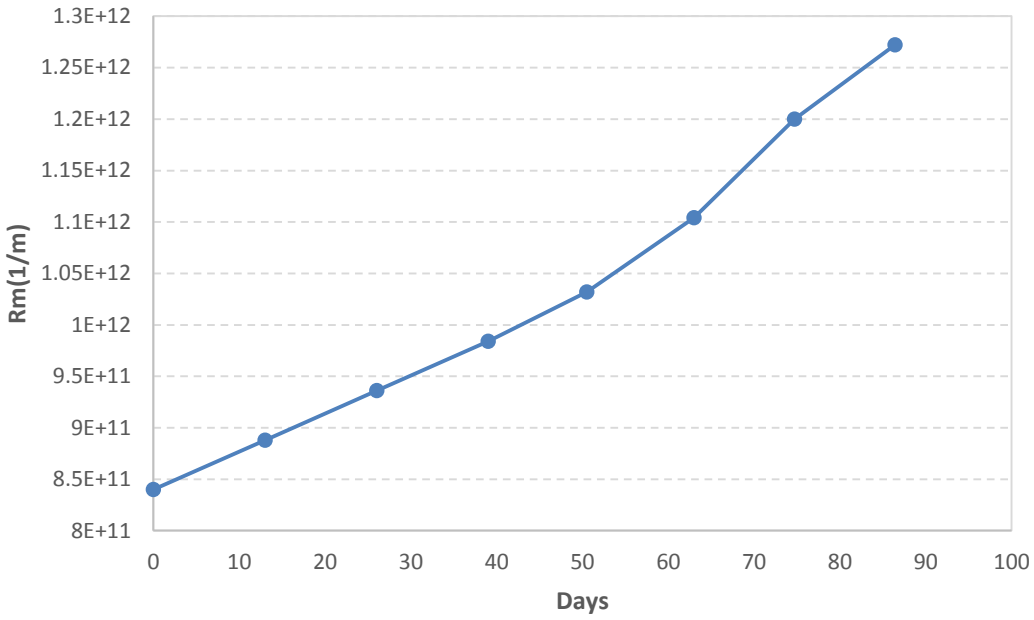


Figure 4.5- Intrinsic resistance (Rm) rise in C-MBR membrane

Cake layer resistance (Rc) was found to contribute major share of resistance in both type of MBRs (Table 4.5). Jiang et al., (2013) examined that, more biocake induced high concentration polarization. Cake layer is comprised of many components, including microorganism, organic and inorganic substances including EPS (Lee et al., 2001). Rc is considered to be removable by physical cleaning while pore blockage resistance (Rp) is irreversible by physical means and requires chemical cleaning. Rp value was found to be high in QQ-MBR which accounted for 32.5% of Rt in QQ-MBR while less in C-MBR with 19.7% of Rt. Results reported in Table 4.5 reveal that, due to long term filtration exposure of QQ-MBR membrane, soluble organic compounds directly adsorbed on the membrane surface and inside the membrane pores in the absence of dense cake layer. Wu et al. (2011) claimed Rc due to the suspended solids and Rp, constriction of pore, due to solute and colloids. From these results direct

relation can be established between Rc and SCR along with CST and improved sludge behavior by the addition of CEBs in QQ-MBR.

Table 4.5- Membrane fouling resistances in of QQ-MBR and C-MBR.

Resistance	QQ-MBR(1/m)	C-MBR(1/m)
Total hydraulic resistance, Rt	4.27E+12	4.87E+12
Cake layer resistance, Rc	2.11E+12	2.64E+12
Pore blocking resistance, Rp	1.39E+12	9.6E+11
Intrinsic membrane resistance , Rm	7.68E+11	1.27E+12
Rc/Rt(%)	49.4	54.1
Rp/Rt(%)	32.5	19.7
Rm/Rt(%)	17.9	26.1

4.2.5. Effect of quorum quenching on EPS production

Polysaccharide (PS) and protein (PN) are considered to be two major components of EPS that play a role in membrane fouling. EPS act as a scaffolding and provide a habitat for microorganism to agglomerate by polymer entanglement on membrane surface. Higher PN concentration causes higher hydrophobicity of activated sludge due to the amino acid with more hydrophobic groups and cause high fouling (Deng et al., 2014).

Xiong and Liu (2013) reported that less EPS production and change of PN- PS composition would cause breakage of PN-PS interbiopolymeric chain. Considering the important role of EPS in membrane biofouling it can be divided into three categories, (i) soluble EPS also called SMP (ii) loosely bound EPS (LB-EPS) and (iii) tightly

bound EPS (TB-EPS). The influence of each type of EPS in membrane biofouling was investigated and effect of CEBs on all three types of EPS production was determined.

As both the reactors were fed with same activated sludge, initial sludge EPS concentrations were similar as shown in Figure 4.6 and 4.7. QQ-MBR demonstrated a substantial decrease in both PS and PN soluble EPS, and become stable after 10 days of operation. Quorum quenching reduced the production of both PN and PS manifolds than C-MBR. While on other hand, no significant reduction in LB-EPS and TB-EPS were found QQ-MBR than C-MBR.Q-MBR as shown in Figure 4.8. Results revealed a direct relation of soluble EPS with membrane fouling and addition of *Rhodococcus* led to decrease in EPS production.

Recent studies found quorum sensing a major factor in aerobic granular formation which produce more polysaccharide than other conventional aerobic flocs (Liu et al., 2010). Li et al. (2014) found that AHLs make the aerobic granular more stable and AHLs-acylase inactivate the AHLs and decrease their concentration and EPS matrix collapsed due to the reduced production of PS and PN, which caused dispersion of aerobic sludge granules and weakens the attachment potential.

Based upon the results obtained, it is inferred that reduction in EPS production control the membrane biofouling and quorum quenching was found to be proficient in reducing EPS production. PN concentration was found to be very low in QQ-MBR which indicate less hydrophobicity of activated sludge flocs and inhibit the biofilm formation on surface of membrane.

Le-Clech et al., (2006) found that, more hydrophobicity causes enhance attachment of microbial flocs on membrane surface which results in high TMP and also reported that, PN make the sludge flocs more hydrophobic than PS.

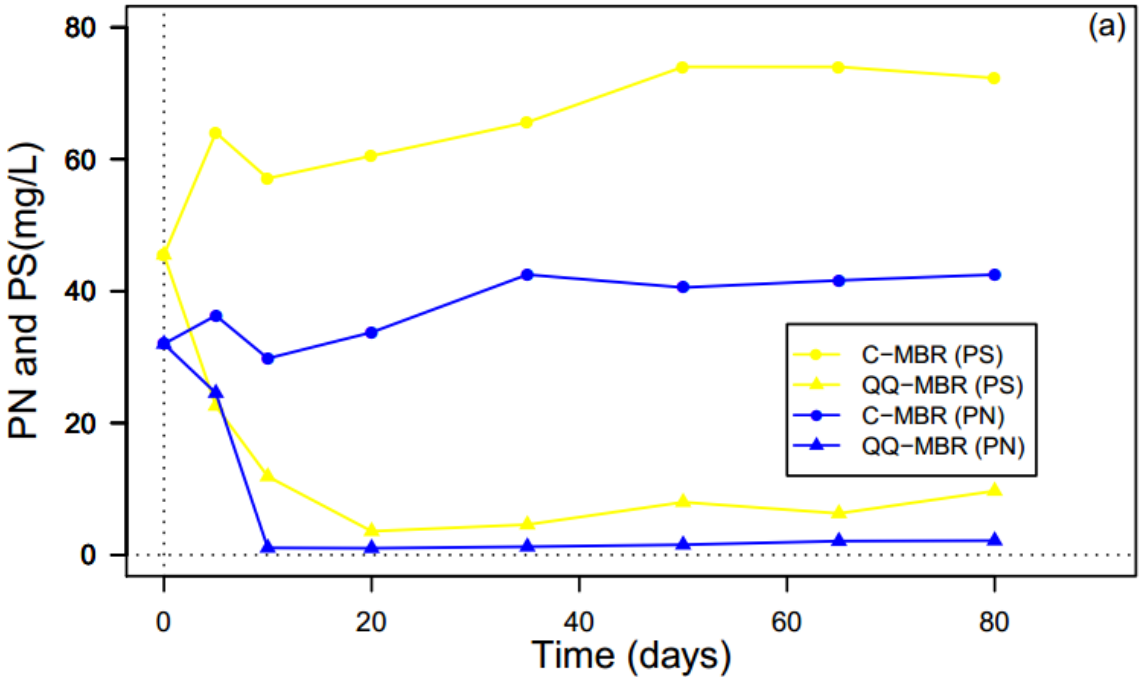


Figure 4.6 PN and PS of soluble EPS C-MBR and QQ-MBR

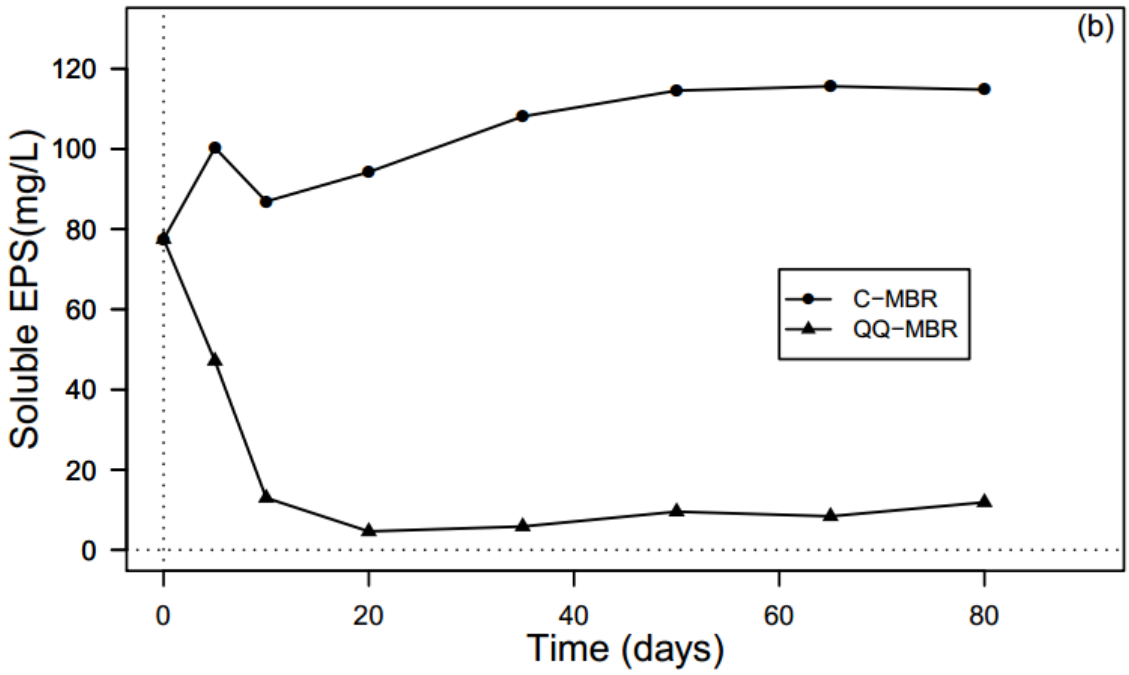


Figure 4.7 Total EPS content in QQ-MBR and C-MBR

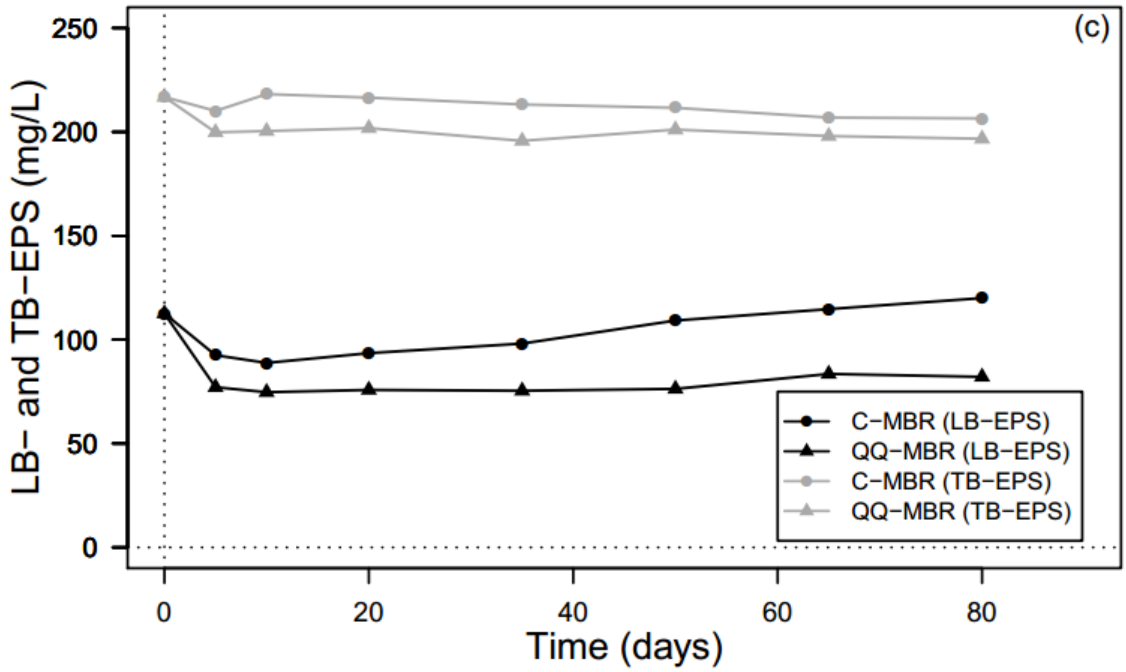


Figure 4.8 LB-EPS and TB-EPS concentration in QQ-MBR and C-MBR

4.2.6. Evidence of AHLs in the MBR

AHLs presence in activated sludge was monitored using bioassay. As shown in Figure 4.11, compounds were detected by overlaying membrane over the surface of indicating agar plate containing *A. tumefaciens* A136. This strain does not produce its own signal molecules, exhibiting a blue zone when supplied to exogenous source of acyl-HSL. Intensified blue color was developed due to high concentration of AHLs in C-MBR, suggesting significant impact of AHL's presence in biofouling. On the contrary, extracts from QQ-MBR did not show any significant AHLs during analysis till 50 days of operation, which could be due to the fact that their concentration is reduced to a level below the detection limit in the bioassay. However, development of small blue zones was observed after 60 d of QQ-MBR operation, indicating less AHLs concentration as compared to C-MBR (Oh et al., 2012). Present results are in good agreement with the previous studies (Kim et al., 2013, Lade et al., 2014) which indicate the performance of CEBs in QQ-MBR.

Presence of important signal molecules was confirmed using HPLC. For this purpose, samples from both MBRs were collected. In the extract of C-MBR, a peak appeared with a retention time of 10.5 min and was identified as C8-HSL, as compared with the peak of standard signal molecules (Figure 4.9). However, a small peak was detected in QQ-MBR confirming the potential of *Rhodococus sp.* in mitigation of biofouling (Kim et al., 2014) via controlling the activity of AHLs through production of lactonase (an AHLs degrading enzyme).

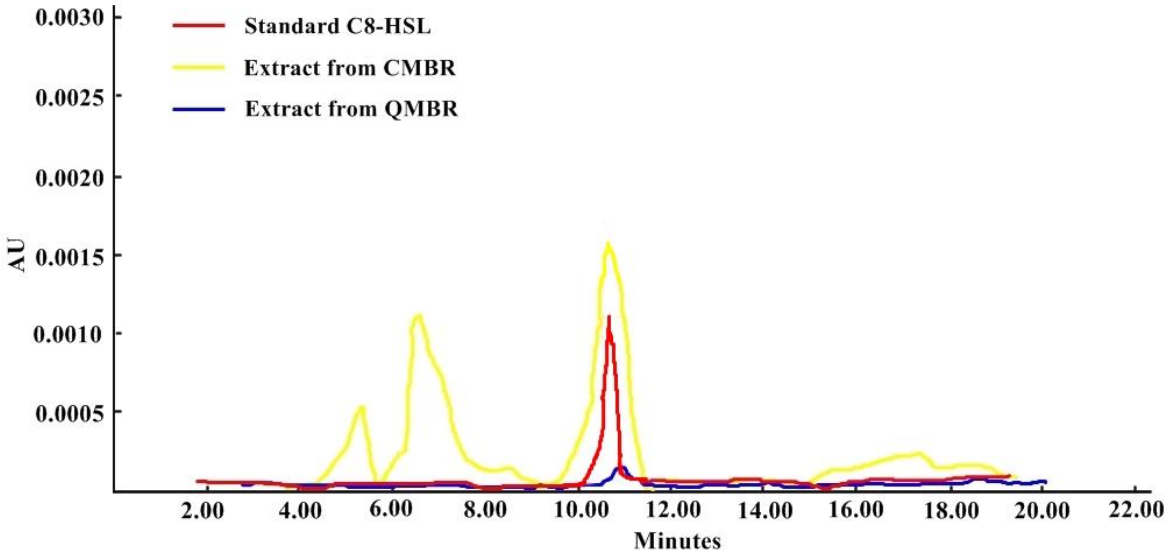


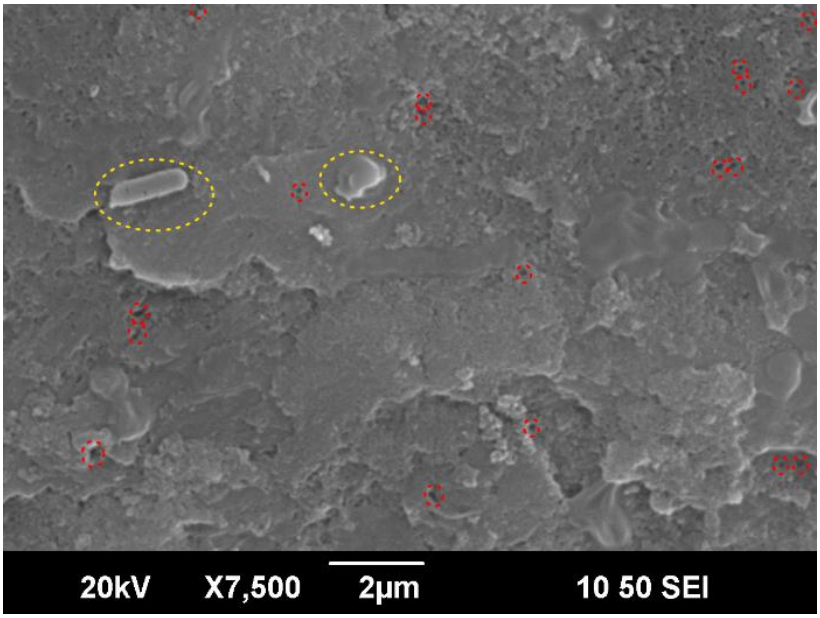
Figure 4.9 Chromatogram of Standard C8-HSL, C-MBR and QQ-MBR extracts

4.2.7. Scanning Electron Microscopy analysis

Although TMP profile and EPS concentration are important indicatiors of biofouling, however, SEM of biofilm formation on membrane surface could be a more indicative parameter in investigating the evidence of QQ based biofouling control. For this purpose SEM profiles of membrane surfaces from MBRs were taken at the end of filtration study as shown in Figure 4.10. Surface of membrane taken from C-MBR was comprised of bacterial cluster along with biopolymers. While the surface of QQ-MBR is covered with few bacterial cells and low deposition of EPS compared to that of C-MBR, exhibiting anti-biofouling effect of *Rhodococus* sp. BH4. One can clearly visualize the reduction in pore size in C-MBR as compared to QQ-MBR. This blockage of pore size strongly depicts the occurrence of fouling layer over the membrane surface

of C-MBR which may ultimately results in permeability reduction (Siddiqui et al., 2012).

(a)



(b)

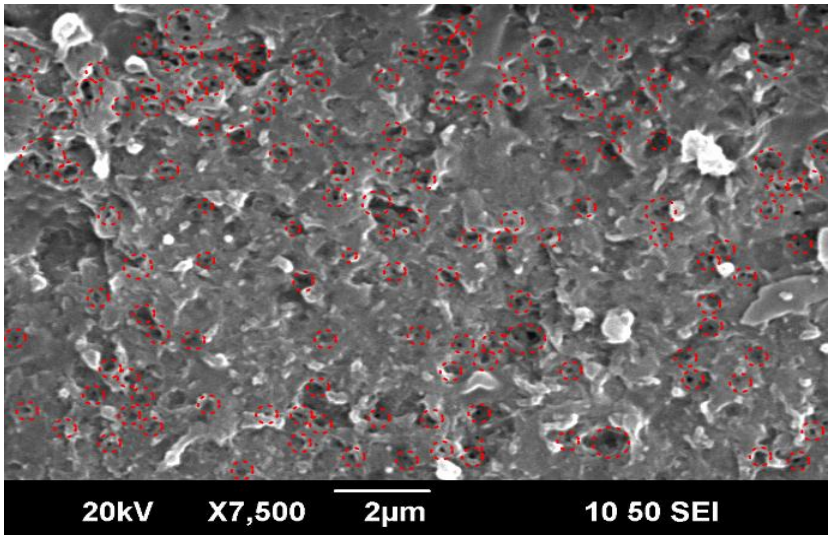


Figure 4.10 SEM of fouled membranes (a) C-MBR (b) QQ-MBR

Conclusions and Recommendations

Conclusions:

Phase I

In this study, submerged bench-scale MBR with three different filtration modes was operated to optimize the system for low membrane fouling and prolong filtration duration. The fouling rate was more profound in $MBR_{(12+3)}$ having short filtration duration and $MBR_{(8+2)}$ exhibited low fouling rate and long filtration duration. SCR showed that cake formed on membrane in $MBR_{(12+3)}$ exhibited high density and less porosity as compared to $MBR_{(8+2)}$ and $MBR_{(10+2)}$ while microbial activity in terms of SOUR was found to be highest in $MBR_{(12+3)}$. Total hydraulic membrane resistance (R_t) was found to be lowest in $MBR_{(8+2)}$ due to its low fouling trend.

Phase II

In this study, two parallel MBRs were operated, C-MBR without cell entrapping beads (CEB) and QQ-MBR with CEBs having quorum quenching *Rhodococcus sp*. CEBs prolonged the filtration duration in QQ-MBR by reducing biofouling, delayed the TMP rise, and reduced the AHLs concentration and minimized the soluble EPS concentrations. CEBs improved the dewaterability of sludge in terms of CST and reduced the specific cake resistance. No adverse effects of CEBs on activated sludge were observed in term of performance. Introduction of CEBs having quorum quenching bacteria led to inhibition of biofilm formation by quorum quenching which improved the filtration and permeability of membrane in QQ-MBR. Filtration duration in QQ-MBR was found to be 7 times more than that of C-MBR.

Recommendations:

(i) Real wastewater replacing synthetic one and then compare TMP rise-up between conventional and QQ MBRs.

(ii) When monitoring TMP rise up, continue for several cycles in both MBRs. Based on TMP pattern, calculate and compare energy consumption per permeate volume at constant flux between the two MBRs.

(iii) Change the aeration rate to minimize energy consumption, while maintain prolong filtration duration and treatment performance in the MBRs.

(iv) Investigate the live/dead ratio of microorganisms at intervals of operation of C-MBR and QQ-MBR.

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Appendix A

Protocols

Extra polymeric substances (EPS) extraction and analyses

Cation exchange resin (CER)

The CER was required to be soaked for 1 h in the extraction buffer solution and dried in room temperature for 1 h before usage.

Buffer solution

Chemical name	Concentration	Amount in 1 L DI water
Na3PO4.12H2O	2 mM	380*2/1000 = 0.76 g
NaH2PO4.2H2O	4 mM	156*4/1000 = 0.624 g
NaCl	9 mM	58.5*9/1000 = 0.5265 g
KCl	1 mM	74.6*1/1000 = 0.0746 g

EPS extraction

The EPS was measured in the form of soluble EPS and bound EPS. The two forms of EPS were extracted by the procedure outlined as follows:

1. Take 50 mL sludge sample
2. Centrifuge sample at 5,000 rpm for 15 min, 4^{o}C
3. Centrifuge sample at 5,000 rpm for 15 min, 4^{o}C
4. Supernatant stored at 4^{o}C for Soluble EPS analysis
5. Re-suspend settled sludge flocs in buffer solution to previous volume
6. Stir sample at 300 rpm for 1hr
7. Centrifuge sample at 5,000 for 15min

8. Remove supernatant for LB-EPS
9. Add resin 70 g/g VSS 70g x MLVSS g/L x 0.05 L= g
10. Stir sample at 300 rpm for 2h, room temperature
11. Centrifuge sample at 5,000 rpm for 10min, 4^{o}C
12. Remove CER and floc components
13. Centrifuge sample at 5,000 rpm for 20min, 4^{o}C
14. Remove remaining floc components
15. Supernatant stored at 4^{o}C for TB-EPS analysis

Carbohydrate and protein fractions of the soluble and bound EPS were measured by the colorimetric methods of Dubois et al. (1956) and Lowry et al. (1951), respectively using spectrophotometer.

Measurement of carbohydrate: Phenol-sulfuric acid method (Dubois method)

Principle

Simple sugars, oligosaccharides. Polysaccharides and their derivatives give a stable orange-yellow color when treated with phenol and concentrated sulfuric acid. Under proper conditions, the accuracy of the method is within 2%.

Chemical reagents

5 w% Phenol solution

Sulfuric acid (H2SO4)

D-Glucose for standard solution

Procedure

Standardization:

1. Make all measurements in duplicate
2. Pipette 2 mL of sugar solution (D-Glucose) containing 0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 mg/L of glucose into test tubes
3. Add 1 mL of the 5% phenol solution and 5 mL of the concentrated sulfuric acid to the test tubes. The addition should be rapid. In addition, direct the stream of acid against the liquid surface, rather than against the side of the test tube for good mixing.
4. Allow the tubes to stand 5 min.
5. Thoroughly mix the solutions using vertex machine.
6. Cool again by standing for 5 minutes.
7. Measure absorbance at 490 nm in HACH spectrophotometer.
8. Prepare a calibration curve of concentration of sugar (Glucose-D) versus absorbance.

Analysis: (Sample for soluble and bound EPS)

1. Soluble and bound EPS were determined with dilution factor 2 i.e. 1 mL sample and 1 mL deionized (DI) water were pipetted into the test tubes.
2. Remaining procedure was identical to the one followed for carbohydrate standardization mentioned above.
3. Measured absorbance of sample solution at 490 nm was correlated to the carbohydrate concentration in the sample using the carbohydrate standard curve and straight line equation.
4. Carbohydrate concentration was reported in mg/L for soluble EPS and mg/gVSS for bound EPS.

Measurement of Protein: Lowry method

Principle

This is a standard and quantitative method for determining protein content in a solution. Lowry method is a reliable method for protein quantification and little variation among different proteins has been observed.

Chemical reagents

CuSO4.5H2O

Sodium Citrate

Na2CO3

NaOH

Folin-Ciocalteu phenol reagent

Bovine Serum Albumin (BSA) for standard solution

Solution A, 100 mL;

0.5 g CuSO4.5H2O

1 g Na3C6H5O7.2H2O (Sodium citrate)

Solution B, 1L;

20g Na2CO3

4 g NaOH

Solution C, 51 mL;

1 mL solution A

50 mL solution B

Solution D, 20mL;

10 mL Folin-Ciocalteu phenol reagent + 10 mL DI water

Procedure

Standardization:

1. Make all measurements in duplicate
2. Pipette 0.5 mL of BSA solution containing 0, 20, 30, 40, 50, 60, 80 and 100 mg/L of BSA into test tubes
3. Add 2.5 mL solution C
4. Thoroughly mix the solutions using vertex machine and let them stand at room temperature for 5 min
5. Add 0.25 mL Solution D and thoroughly mix again.
6. After 20 min, measure absorbance at 750 nm in dark condition.
7. Prepare a calibration curve of protein (BSA) concentration (mg/L) versus absorbance.

Analysis: (Sample for soluble and bound EPS)

1. Soluble EPS was determined with no dilution while bound EPS was determined with dilution factor 2 i.e. 1 mL sample and 1 mL deionized (DI) water were pipetted into the test tubes.
2. Remaining procedure was identical to the one followed for protein standardization mentioned above.
3. Measured absorbance of sample solution at 750 nm was correlated to the protein concentration in the sample using the protein standard curve and equation of straight line.
4. Protein concentration was reported in mg/L for soluble EPS and mg/gVSS for bound EPS.

Capillary Suction Time (APHA, 2005)

General discussion

The capillary suction time (CST) test determines rate of water release from sludge. It provides a quantitative measure, reported in seconds, of how readily a sludge release water. The results can be used to assist in sludge dewaterabilty processes; to evaluate sludge conditioning aids and dosages.

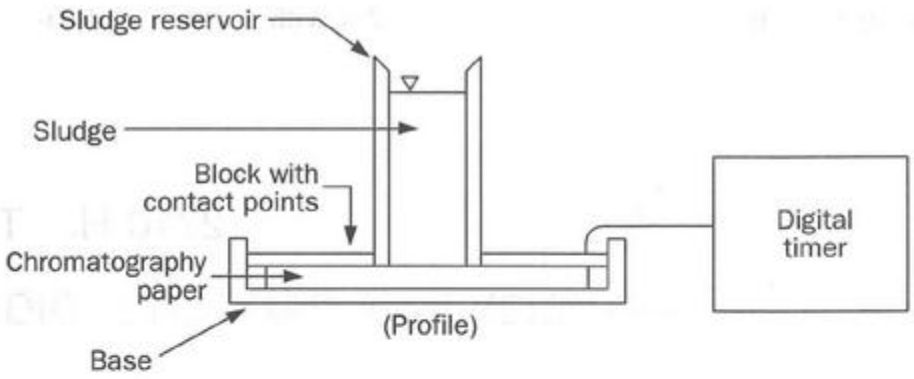
Apparatus

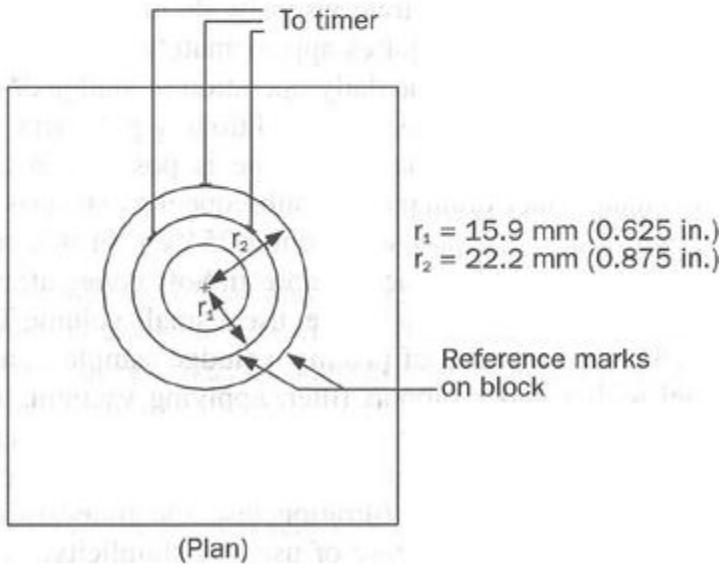
1. CST apparatus including reservoir 18mm ID and 25-mm height.
2. CST paper
3. Thermometer
4. Pipet, 10-mL

Procedure

1. Turn on and reset CST meter. Dry CST test block and reservoir.
2. Place a new CST paper on lower test block with rough side up and grain parallel to the 9-cm side.
3. Add upper test block, insert sludge reservoir into test block and seat it using light pressure and quarter turn to prevent surface leaks.
4. Measure and record temperature of sludge. Pipet 6.4 mL sludge into test cell reservoir; if pipetting is difficult because of sludge consistency, pour a representative sludge sample into cell until it is full.
5. The CST device will begin time measurement as liquid being drawn into paper reaches the inner pair of electrical contacts.
6. Timing ends when the outer contacts is reached.
7. Record CST on digital display.

8. Empty remaining sludge from reservoir and remove and discard used CST paper. Rinse and dry test block and reservoir.
9. Temperature and sample volume can affect CST results. Ensure that all analyses are run under same conditions.





Sludge Volume Index (APHA, 2005)

General Discussion

The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of suspension after 30min settling. SVI typically is used to monitor settling characteristics of activated sludge.

Apparatus

1. Settling column
2. Stopwatch
3. Thermometer

Procedure

1. Determine the suspended solids concentration of a well-mixed sample of the suspension
2. Place 1.0L sample in settling column by covering the top and inverting cylinder three times.
3. Determine the 30min settled sludge volume

Calculations

$$SVI = \frac{settled\ sludge\ volume\left(\frac{mL}{L}\right)\ x\ 1000}{suspended\ solids\ (\frac{mg}{L})}$$

Appendix B

Results Phase I

Table B-1: COD Removal of $MBR_{(8+2)}$, $MBR_{(10+2)}$ and MBR (12+3)

$MBR_{(8+2)}$ (mg/L)				$MBR_{(10+2)}$ (mg/L)				$MBR_{(12+3)}$ (mg/L)			
Test	in	out	% removal	Test	in	out	% removal	Test	in	Out	% removal
1	500	27.5	94.5	1	50	25.9	94.8	1	50	20	96.0
2	500	30.0	94.0	2	50	30.0	94.0	2	50	20.9	95.8
3	500	33.0	93.4	3	50	26.4	94.7	3	50	24.5	95.1
4	500	36.4	92.7	4	50	26.8	94.6	4	50	26.3	94.7
5	500	38.2	92.4	5	50	30.9	93.8	5	50	27.0	94.6
6	500	34.5	93.1	6	50	25.3	94.9	6	50	27.2	94.5
Average			93.3				94.4				95.1
Std. Dev.			0.72				0.40				0.58

Table B-2: Nutrients Removal by MBR$_{(8+2)}$

Nutrients Removal (8+2)									
Phosphate				Ammonia			Nitrate		
Test	in(mg/L)	out(mg/L)	% removal	in (mg/L)	out (mg/L)	% removal	in (mg/L)	out (mg/L)	% removal
1	37.4	28.1	24.9	44	19.5	55.7	12.3	0.7	94.3
2	37.4	17.7	52.7	44	19	56.8	13.5	0.9	93.3
3	35.9	24.4	32.0	44	22	50.0	10	0.2	98.0
4	38.7	26.2	32.3	44	21	52.3			
5	33.9	16.3	51.9						
6	35.3	16.3	53.8						
Averg.			41.3			53.7			95.2
Std. Dev.			11.8			4.0			2.0

Table B-3: Nutrients Removal by MBR$_{(10+2)}$

Nutrients and COD Removal (10+2)									
Phosphate				Ammonia			Nitrate		
Test	in (mg/L)	out(mg/L)	% removal	in (mg/L)	out (mg/L)	% removal	In (mg/L)	out (mg/L)	% removal
1	32.3	16.4	49.2	44	17	61.4	10.2	3.2	68.6
2	34.4	18.2	47.1	44	15.5	64.8	10.2	3.2	68.6
3	31.4	16.8	46.5	44	15	65.9	11	3.7	66.4
4	34.8	18	48.3						
5	31.2	16.2	48.1						
Average			47.8			64.01515			67.87285
Std. Dev.			1.0			1.39			1.067172

Table B-4: Nutrients Removal by MBR$_{(12+3)}$

Nutrients and COD Removal (12+3)									
	Phosphate			Ammonia			Nitrate		
Test	in (mg/L)	out (mg/L)	% removal	in (mg/L)	out (mg/L)	% removal	in(mg/L)	out(mg/L)	% removal
1	34	19	44.1	44	12.2	72.3	11.2	4.2	62.5
2	34	16	52.9	44	14	68.2	12	5	58.3
3	30.8	15.5	49.7	44	13.5	69.3	13.5	6.5	51.9
4	33.5	18.1	46.0						
5									
Average			48.2			69.9			57.6
Std. Dev.			3.4			1.4			4.4

Table B-5: Capillary Suction Time of $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$

MBR (8+2)		MBR (10+2)		MBR (12+3)	
Test	CST(s)	Test	CST(s)	Test	CST(s)
1	159	1	114	1	58
2	140	2	110	2	63
Avg.	149.5		112		60.5

Results Phase II

Table B-6: COD removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$

COD Removal						
	QQ-MBR			C-MBR		
Test	in(mg/L)	out(mg/L)	%removal	in(mg/L)	out(mg/L)	%removal
1	500	34	93.2	500	33	93.4
2	500	28	94.4	500	30	94
3	500	28.9	94.2	500	39	92.2
4	500	14	97.2	500	28	94.4
5	500	27.6	94.4	500	28.5	94.3
6	500	23	95.4	500	24.5	95.1
7	500	18	96.4	500	24	95.2
8	500	23.7	95.2	500	11	97.8
9	500	28.5	94.3	500	30	94
10	500	28.6	94.2	500	32	93.6
11	500	28.9	94.2	500	22	95.6
12	500	12	97.6	500	32	93.6
13	500	39	92.2	500	32	93.6
14	500	45	91.0	500	34	93.2
Avg.			94.6			94.3
Std. Dev.			1.8			1.3

Table B-7: Phosphate removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$

Phosphate Removal						
	C-MBR			QQ-MBR		
Test	in (mg/L)	out(mg/L)	% removal	in(mg/L)	out(mg/L)	%removal
1	39	19.6	49.7	39	21.2	45.6
2	38	19.7	48.2	38	19.7	48.2
3	37.5	18.2	51.5	37.5	18.1	51.7
4	36.6	18.1	50.5	36.6	18.1	50.5
5	36.2	17	53.0	36.2	20	44.8
6	39.3	18.5	52.9	39.3	18.2	53.7
7	34.7	18.8	45.8	34.7	18.4	47.0
8	33.1	17	48.6	33.1	16.9	48.9
9	37.2	16.3	56.2	37.2	16	57.0
10	39	19	51.3	39	18.2	53.3
Avg.			50.8			50.1
Std. Dev.			2.9			3.9

Table B-8: Nitrate removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$

Nitrate Removal						
	C-MBR			QQ-MBR		
Test	in (mg/L)	out(mg/L)	% removal	in (mg/L)	out(mg/L)	% removal
1	12	0.9	92.5	12	0.7	94.1
2	12.4	0.5	95.9	12.4	0.5	95.9
3	10.2	0.9	91.1	10.2	0.7	93.1
4	10.4	0.6	94.2	10.4	0.6	94.2
5	10.3	0.4	96.1	10.3	0.4	96.1
6	11.2	0.9	91.9	11.2	0.6	94.6
Avg			93.6			94.7
Std.Dev			2.1			1.1

Table B-9: Nitrate removal by $MBR_{(8+2)}$, $MBR_{(10+2)}$ and $MBR_{(12+3)}$

Ammonium Removal						
	QQ-MBR			C-MBR		
Test	in(mg/L)	out(mg/L)	%removal	in(mg/L)	out(mg/L)	%removal
1	44	18.7	57.5	44	19.5	55.6
2	44	18.2	58.6	44	19	56.8
3	44	21	52.2	44	22	50.0
4	44	20.1	54.3	44	21	52.2
5	44	19.8	55.0	44	20.5	53.4
6	44	19.8	55.0	44	21	52.2
Avg.			55.45455			53.4
Std. Dev.			2.286323			2.4

Table B-10: Rise in intrinsic resistance (Rm) of C-MBR membrane

Intrinsinc Rm(1/m)	
Days	Resistance
0	8.4E+11
13	8.88E+11
26.04	9.36E+11
39	9.84E+11
50.48	1.03E+12
63	1.1E+12
74.7	1.2E+12

Table B-11: Concentration of PS,PN and Total EPS in C-MBR and QQ-MBR

Soluble EPS Results (mg/L)						
	QQ-MBR			C-MBR		
Days	PS	PN	Total	PS	PN	Total
0	45.5	32	77.5	45.5	32	77.5
5	22.6	24.55	47.15	64.02	36.295	100.315
10	11.9	1.075	12.975	57.1	29.8	86.9
20	3.6	1.025	4.625	60.5	33.75	94.25
35	4.6	1.25	5.85	65.6	42.5	108.1
50	8	1.55	9.55	74	40.55	114.55
65	6.3	2.1	8.4	74	41.625	115.625
80	9.7	2.175	11.875	72.3	42.5	114.8

Table B-12: Concentration of Loosely bound (LB)-EPS

Loosely bound EPS						
	QQ-MBR			C-MBR		
Days	PS(mg/L)	PN(mg/L)	Total	PS(mg/L)	PN(mg/L)	Total
0	57.1	55.5	112.6	57.1	55.5	112.6
5	40.2	36.9	77.1	43.5	49	92.5
10	38.5	36.2	74.7	41.9	46.9	88.8
20	36.8	39	75.8	40.2	53.3	93.5
35	38.5	36.9	75.4	46.9	51.2	98.1
50	35.1	41.2	76.3	53.7	55.5	109.2
65	40.2	43.3	83.5	57.1	57.6	114.7
80	41.9	40.2	82.1	58.8	61.2	120
Avg.	41.0375	41.15	82.1875	49.9	53.775	103.675
Std. dev.	6.38591	5.8811138	11.88554	7.118462	4.367994	11.0623

Table B-13: Concentration of Tightly bound (TB)-EPS

	QQ-MBR			C-MBR		
Days	PS(mg/L)	PN(mg/L)	total	PS(mg/L)	PN(mg/L)	Total
0	124.8	92	216.8	124.8	92	216.8
5	110.8	89	199.8	115.9	94.2	210.1
10	107.4	93	200.4	114.2	104	218.2
20	110.8	91	201.8	117.5	99	216.5
35	105.7	90	195.7	120.9	92.3	213.2
50	109.1	92	201.1	114.2	97.4	211.6
65	104	94	198	107.4	99.5	206.9
80	105.7	91	196.7	107.4	99.1	206.5
Avg.	109.7875	91.5	201.2875	115.2875	97.1875	212.475
Std. Dev.	6.128915	1.5	6.194844	5.635255	3.848194	4.20409