COMPARISON OF QUORUM QUENCHING AND BACKWASHING TECHNIQUES FOR BIOFOULING CONTROL IN SUBMERGED MEMBRANE BIOREACTOR

By

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This Thesis is dedicated to my late Grandfather and Grandmother with lots of love and with a praying that Allah Almighty grants them highest ranks in paradise

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List of Abbreviations

MBR	Membrane Bioreactor
PBW	Permeate Backwashing
CEB	Chemical Enhanced Backwashing
QQ	Quorum Quenching
QS	Quorum Sensing
QSI	Quorum Sensing Inhibitors
QQ+PBW	Quorum Quenching + Permeate backwashing
QQ+CEB	Quorum Quenching + Chemical Enhanced backwashing
CST	Capillary Suction time
EPS	Extra Cellular Polymeric Substances
CER	Cation Exchange Resins
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
HF	Hollow Fibre
PVDF	Poly-Vinyl Di-Fluoride
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
J	Operational Flux
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NTU	Nephelometric Turbidity Units
PSD	Particle Size Distribution

Rc	Cake Layer Resistance
Rp	Pore Blockage Resistance
Rm	Intrinsic Membrane Resistance
Rt	Total Resistance
SMP	Soluble Microbial Products
SMBR	Submerged Membrane Bioreactor
SRT	Sludge Retention Time
TMP	Trans Membrane Pressure
NH4-N	Ammonium-N
μ	Viscosity of Permeate
CIP	Cleaning In Place
NaOCl	Sodium Hypochlorite
AHL	Acyl Homoserine Lactose
AI-2	Auto Inducer-II

Abstract

A variety of physical, chemical and biological methods have been adapted for control of biofouling in membrane bioreactors which is considered a major limiting factor in adaptation of MBRs at large scale. This study investigated a variety of biofouling control methods i.e. relaxation, permeate backwashing, chemical enhanced backwashing and Quorum Quenching. MBRs with different biofouling control strategies were operated and their effect on biofouling retardation, change in sludge characteristics and removal efficiency of organics and nutrients in wastewater were compared. Combined quorum quenching (QQ) and chemical enhanced backwashing (CEB) strategy resulted in 3.7 times longer filtration cycle in MBR as compared with that of permeate backwashing (PBW) but QQ+CEB strategy negatively affected the sludge characteristics. In comparison, QQ+PBW approach was found to reveal better sludge characteristics in terms of Extracellular Polymeric Substance (EPS) and capillary suction time (CST) while exhibiting slight difference in biofouling control. COD and NH_4^+-N removal efficiencies were found to be reduced from 93 to 79% and from 64 to 38%, respectively in MBRs having CEB using NaOCl concentration of 3.5 mg/L.

Chapter 1

Introduction

1.1. Background

Membrane bioreactor (MBR) being a combination of microbial degradation and membrane filtration is one of the most promising technologies for treatment of wastewater exhibiting advantages such as excellent effluent quality, small area requirement and low production of sludge as compared to conventional activated sludge process (Cai and Liu., 2016; Piasecka et al., 2015). Biofouling is one of the most challenging issues of MBR technology. This happens when bacteria through a natural process called quorum sensing start communicating through signal molecules known as auto-inducers and start to grow by producing Extracellular Polymeric Substances (EPS) and by attaching themselves to membrane surface, leading to pore blocking, biofilm formation, and sludge cake deposition. This biofouling results in high trans-membrane pressure (TMP) leading to shorter filtration cycles, requiring frequent membrane cleaning and reducing membrane life as shown in Figure 1 (Jiang et al., 2013; Lee et al., 2013; Weerasekara et al., 2016). Wide spread and full scale application of MBR for wastewater treatment is only restricted because of this phenomena of membrane biofouling (Lee et al., 2013, 2012).

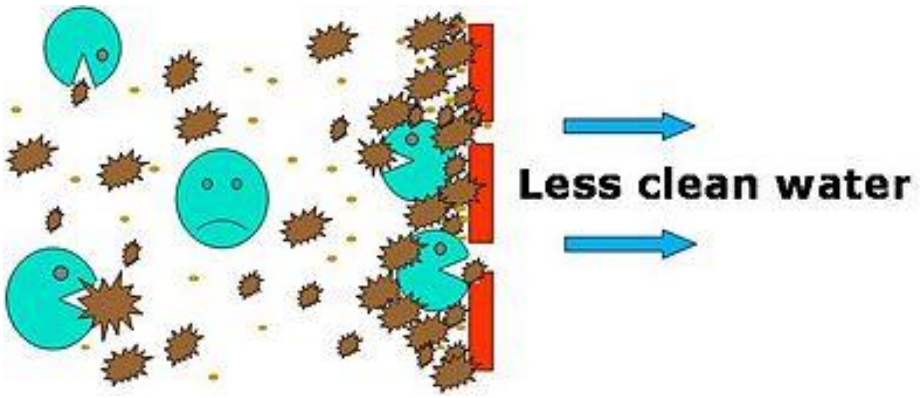


Figure 1.1: Basic illustration of Membrane Fouling

Various physical, chemical and biological methods have been adapted worldwide to retard or control membrane biofouling. Physical cleaning methods such as relaxation and backwashing helps in sustainable filtration operation of membrane bioreactors which is further improved by chemical enhanced backwashing (CEB) or chemical cleaning in place (CIP). A large number of studies have been conducted to determine optimized filtration cycles, relaxation and backwashing durations and frequencies for enhanced permeability, prolonged filtration cycles and delayed TMP (Akhondi et al., 2014; Wu et al., 2008; Zsirai et al., 2012). Relaxation is the intermittent pause in filtration which allows some flux recovery by decreasing trans-membrane pressure (TMP) for submerged MBR (SMBR) (Zsirai et al., 2012). Backwashing is the reverse flow of permeate back into the membrane to open the membrane pores and to detach loosely bound bio-cake on membrane surface as well as reduce reversible fouling. However, backwashing cannot completely reverse membrane fouling as some part of the deposited matter still remains on membrane surface and inside the pores leading to irreversible fouling (Katsoufidou et al., 2007).

This is where chemical enhanced backwashing is employed in which chemicals like sodium hypochlorite are added in permeate for backwashing to reduce irreversible fouling (Lee et al., 2013, 2012). It is to be noted that back pulse frequencies are more effective in fouling control than back pulse durations (Zsirai et al., 2012).

Chemical enhanced backwashing has certain disadvantages as it may affect the microorganisms present in sludge by oxidation and cell-lysis which may cause degradation of sludge characteristics such as reduction in mixed liquor suspended solids (MLSS) and increase in Extracellular Polymeric Substance (EPS) (Lee et al., 2013). Therefore, a more sustainable method such as biological biofouling control have attracted significant attention (Weerasekara et al., 2016). Previous studies have proved that biological

biofouling control using quorum quenching (QQ) approach i.e., inhibition of quorum sensing, is more effective than physico-chemical approaches. Quorum quenching enzyme (acylase enzyme) in free (Yeon et al., 2009a) or immobilized (Jiang et al., 2013; Yeon et al., 2009b) forms have been used to inhibit quorum sensing through N-Acyl Homoserine Lactone (AHL) degradation.

To get rid of the problems of enzymes instability and high cost of enzymes extraction, quorum quenching bacteria were then introduced in the form of an attached QQ bacterial vessel for QQ enzyme production inside the bioreactor for degradation of AHLs (Oh et al., 2012). The microbial vessels were later on replaced by QQ bacteria cell entrapping beads which were freely moving inside the bioreactor with the help of aeration and were found to be more efficient in biofouling control than the microbial vessels because beads had more chances to interact with mixed liquor and biofilm on membrane to catch the signal molecules AHLs more easily (Kim et al., 2013; Maqbool et al., 2015).

1.2. Objectives of Study

Keeping in view the previous studies for control of biofouling in membrane bioreactors, the objectives of this study were:

1. To evaluate the combined effect of quorum quenching (QQ) and chemical enhanced backwashing (CEB) on membrane fouling at optimized filtration cycles.
2. To examine the effects of CEB with sodium hypochlorite (NaOCl) concentration on sludge characteristics and removal efficiencies of organics and nutrients.
3. To investigate QQ influence in replacing CEB with permeate backwash (PBW) for effective biofouling control.

1.3 Scope of Study

- Modified automated MBR with working volume of 35L, submerged PVDF hollow fiber membrane and peristaltic pumps and timers for permeate and chemically enhanced backwashing.
- Filtration cycle was set as
    - 8 minute filtration
    - 1 minute relaxation and 1 minute backwashing
- Two MBRs operated in two phases
    - PBW-MBR (Permeate backwashing MBR)
    - CEB-MBR (Chemical Enhanced Backwashing MBR)
    - QQ+PBW-MBR (Quorum Quenching + Permeate backwashing MBR)
    - QQ+CEB-MBR (Quorum Quenching + Chemical Enhanced backwashing MBR)
- Prepared Quorum Quenching beads using bacterial species Rhodococcus Sp.BH4 to be installed in MBRs.
- Installation of chlorination tanks and peristaltic pumps for a chlorine dose of 3.5 mg/L in chemical enhanced backwashing mode.

Chapter 2

Literature Review

2.1. Membrane Fouling

MBR filtration performance unavoidably declines with filtration duration. This phenomenon occurs because of deposition of soluble and insoluble constituents present in wastewater or activated sludge on membrane surface and in its pores, accredited to the interactions between activated sludge components and the membrane. This is a significant disadvantage and process limitation of MBR and it has been under investigation from a long time but it is still one of the most challenging issues causing inhibition in further development of MBR (Le-Clech et al., 2006).

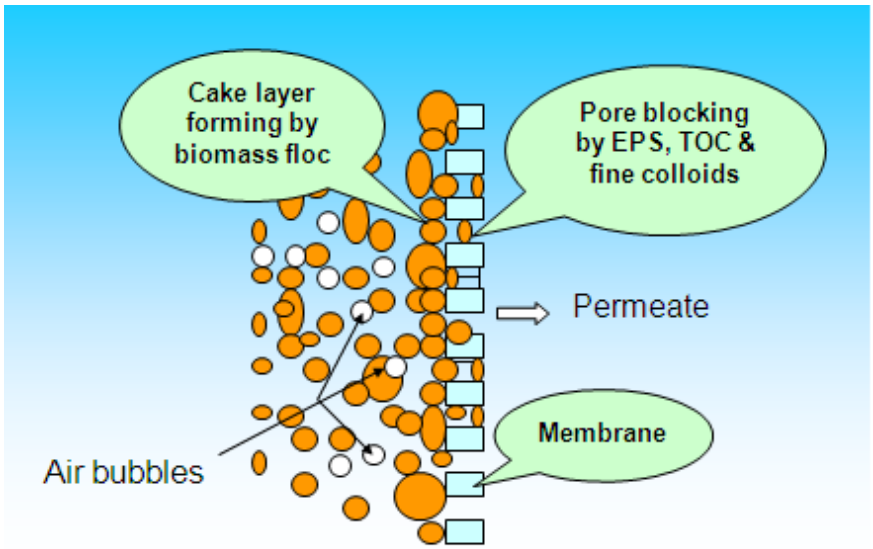


Figure 2.1: Phenomena of membrane biofouling (Wu et al., 2008)

Increase in operational costs, increase in energy demand, more requirement of labour for maintenance purpose, increase in chemical demand for cleaning purpose and shortage in membrane life is due to membrane fouling (Shi et al., 2014).

There are two main factors resulting in lowering of membrane flux over time:

- Concentration polarization

- Membrane fouling

Concentration polarization is a natural process caused due to semi-permeability or selectivity of any membrane. This selectivity results in build-up of some rejected solutes or the particles on the outer layer of membrane surface. It is a common problem during permeation of solutes having lower molecular weights. Once the solutes are being carried towards membrane surface by filtration flow, solvent molecules can pass from the membrane but larger solutes get rejected and are retained on the surface of membrane. The rejected molecules mostly are unable to diffuse again to the bulk solution due to which a concentration gradient is developed just above surface of membrane. The concentration of these molecules near surface of membrane may sometimes reach up to 25–50 times of that in bulk solution. When that much amount of materials accumulate at the surface of membrane, they impede the flow of solvent through membrane (Field, 2010). Concentration polarisation is an inevitable process but it is also a reversible phenomenon which doesn't affect the membrane's intrinsic properties. The flux loss thus can be recovered fully by physical cleaning or permeate backwashing (Zydney, 1997).

The second phenomena responsible for flux decline is membrane fouling which takes place when soluble and particulate matter in inlet feed solution leaves its liquid phase and form a deposit on both membrane surface and inside its pores. Accumulation of materials on surface of membrane is termed as external fouling and deposition in the porous structure is termed as internal fouling of membrane (D'Souza and Mawson, 2005). The major difference between concentration polarisation and membrane fouling is in their fouling nature. Concentration polarization cause reversible loss in permeability and deposition develops only on surface of membrane whereas in membrane fouling both reversible and irreversible loss of the permeability of membrane occurs. Infact, the significant characteristics of fouling is reversibility (Kimura et al., 2004).

Many researches differentiate both types of fouling (reversible & irreversible), based on their relative resistances to different methods of cleaning.

2.1.1. Reversible and Irreversible Fouling

The fouling which can be removed by cleaning is called reversible fouling whereas fouling which cannot be removed by cleaning is called irreversible fouling. There are also further sub-divisions in that for example the fouling which can be removed only by physical cleaning is called hydraulic reversible fouling, the fouling which can be removed by chemical cleaning is called chemically reversible fouling and the fouling which cannot be removed even after chemical cleaning is called chemically irreversible fouling (Shi et al., 2014).

2.1.2. Forms of Fouling

Several mechanism are responsible for rise of fouling in membrane processes namely adsorption, pore blockage and cake layer formation.

Adsorption occurs due to specific interaction of solutes and particles with membrane surface. The interactions generally are of three types

- Weak van der wall forces
- Chemical bonding
- Electrostatic attraction

The type of interaction depends on type of functional group involved. Sometimes a layer of solute can also be developed on membrane even in the absence of filtration due to these interactive forces. This phenomena happens commonly during separation of macromolecules of proteins and humic acids and the fouling caused due to this phenomena

is mostly irreversible which means the membranes experiencing this type of biofouling cannot be cleaned without chemical cleaning (Jones and O'Melia, 2000). These adsorbed materials can have certain negative impacts on membranes such as change in its surface characteristics and charge on membranes (Baker, 2004).

2.1.3. Concept of Membrane Fouling

Membrane filtration processes involves many steps of mass transport in terms of water permeation and separation of particles. Due to mass transfer, accumulation, attachment and adsorption of different materials on and within membrane pores occur which results in increase of hydraulic resistance with time resulting in fouling of membrane (Gander et al., 2000; Zhou and Smith, 2002).The most crucial mechanisms which result in membrane fouling are as following (Kabsch-Korbutowicz, 1992):

1) When minerals start depositing on membrane surface in the form of excess product or crystals, it results in scaling which is also called crystalline fouling

2) Fouling occurred due to deposition of grease, oil, humic acid and lipids is known as organic fouling

3) Fouling due to position of clay, silica, debris which is also known as colloidal fouling

4) Fouling induced due to accumulation of microorganisms on membrane surface termed as biofouling

2.1.4. Factors Affecting MBR Fouling

The factors which are responsible for the rate and extent of fouling in membrane consists of (Hillis, 2000):

a) The properties of Membrane: material, type of module and pore distribution and size

b) Mixed liquor suspended solids physiognomies: Biomass concentration, Extracellular polymeric substances, particle size distribution

c) MBR design and operating parameters: flux, velocity of crossflow, sludge retention time, HRT, and aeration intensity

Table 2.1: Types of fouling phenomena and their causes

Phenomena	**Physical Cause**	**Description**
Cake layer fouling	Boundary layer resistance	Deposit of particles larger than the membrane pore size onto the membrane surface
Complete blocking	Pore blocking	Occlusion of pores by particles with no particle superimposition
Intermediate blocking	Long-term adsorption	Occlusion of pores by particles with particle superimposition
Standard blocking	Direct adsorption	Deposit of particles smaller than the membrane pore size onto the pore walls, reducing the pore size

Source: (Field, 2010)

Table 2.2: Different types of foulants and their fouling modes

Foulants	**Fouling modes**
Large suspended particles	Particles present in the original feed or developed due to aggregation can form a cake layer and/or block module channels
Small colloidal particles	Colloids present in recovery of cells from fermentation broth can form a dense cake layer. They can also block the entrance of a membrane pore or clog inside of it
Inert macromolecules	Gel or cake formation on membrane
Adsorptive macromolecules	Proteins and HAs are known to adsorb on to surfaces on membranes or in the pores
Small molecules	Some small organic molecules tend to have strong interactions with some polymeric membranes (e.g., anti-foaming agents, such as polypropylene glycols used during fermentation, adhere strongly to certain polymeric membranes)
Biological substances	The growth of biologically active organisms such as bacteria and their excreted material aka EPS form biofilms on membrane surfaces
Cations	Precipitation of salts and hydroxides to form scaling. Certain cations such as calcium can facilitate macro-molecular foulings

Source: (Field, 2010)

2.2. Fouling Prevention

Instead of dealing with the hectic process of membrane cleaning and restarting the process afterwards or to apply complex methods of biofouling control some methods can also be applied to prevent biofouling even before its occurrence. Some of which are as follows

- Pre-treatment of influents
- Development of antifouling properties in membrane by chemical modification
- Optimisation of operational parameters (D'Souza and Mawson, 2005)

To prevent primary adhesion of macromolecules, organic acids and lipopolysaccharides on the membrane surface, much research has been carried out on membrane surface modification. These modifications include surfactant pretreatment (Yamagiwa and Tasaka, 1994), ozone induced membranes (Wang et al., 2000), UV assisted graft polymerization, photochemical modification (Kilduff et al., 2000), surface fluorination (Sedath et al., 1993), and coating with bactericidal substance (Hardorfer and Hartel, 1999).

2.3. Fouling Control:

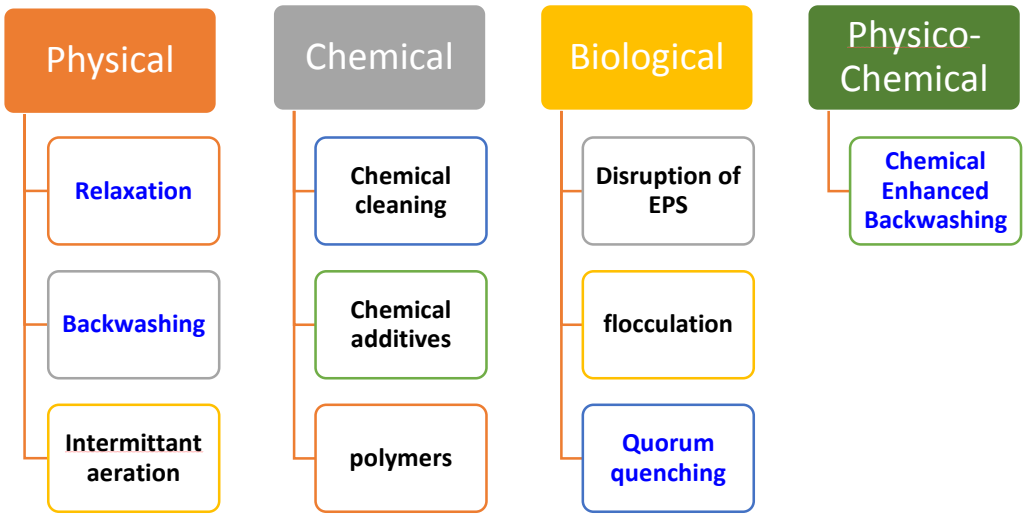


Figure 2.2: Types of fouling control methods

2.3.1 Physical Control Measures:

Duration and frequency of backwashing are generally considered as significant cleaning measures for membrane fouling. Less frequent but long backflush interval (600 sec filtration / 45 sec backflush) was found to be more effective than short backflush duration and more frequent filtration (200 sec filtration / 15 sec backflush) (Jiang et al,. 2005). High Backflushing rate is usually witnessed to be more effective for removing the membrane fouling than both high aeration intensity and back flushing duration in hollow fiber immersed membrane bioreactor (HF-iMBR) (Schoeberl et al., 2005). Stronger, more frequent and long duration of backflushing is required as effectual and efficient control measure for membrane fouling. These objective can be obtained by designing the commonly controlled system having automatically adjusted backflush episodes according to the continuously monitored TMP profile. However, such kind of findings does not taken an account of the loss in productivity, utilizing permeate water during backflushing.

Backflushing can also be affected by aeration (Sun et al., 2004) and aeration intensity can enhance the backflushing with permeate water. About 400% increase in flux was attained over the continuous MBR operation, using an air backflush (Visvanathan et al., 1997), however, 15 min air backflush duration is demanded with 15 min of filtration in this case. On the other hand, parallel to these advantages air backflushing also offered some disadvantages. Air backflushing is unquestionably effective, circumstantial signals advised that it may cause the fractional drying out of the membrane pores, which creates embrittlement, resulting in membrane disintegrity.

Membrane relaxation also acts as control measure strategy for the fouling of membrane. It encourages the diffusive back transportation of foulants (organic and inorganic) away from the surface of membrane, under the concentration gradient. This process is additionally

boosted-up by shearing force produced by air scouring (Chua et al., 2002). Extensive details about TMP performance during this kind of operation has shown that, though the fouling rate of membrane is higher than for non-stop filtration, relaxation however, allows the membrane filtration to be maintained for long duration prior to the demand of chemical cleaning (Ng et al., 2005). Now a days, relaxation technique is nearly ubiquitous in modern full-scale membrane bioreactors and studies evaluating maintenance practises, tended to integrate the backflushing with that of relaxation to achieve finest results (Vallero et al., 2005: Zhang et al., 2005). Additionally, an organised comparison of integrated condition of relaxation and backflushing was suggested during short-term filtration duration of 24 hours (Wu et al., 2008). However, the total fouling rate of membrane (in terms of TMP rise) was comparable under different operating conditions. Studies showed that the nature of the emerging membrane fouling varied considerably with the filtration modes.

In practice, protocols of membrane physical cleaning generally follow the recommendations of suppliers. Normally relaxation is applied for the duration of 1-2 min for every 8-15 min duration of filtration, depending upon the adjustment of cycle in both flat sheet and hollow fibre systems. In MBRs containing hollow fibre membranes, backflushing is normally applied with the flux of 2-3 times of the water flux. However, the MBR operation without backflushing increase the rate of foulants accumulation on or with in the pores of membrane, resulting in the development of biofilm on the surface of membrane, which gave a degree of protection. This kind of a fouling layer developed on membrane surface is considerably more selective and less permeable than the membrane, which can be advantageous to the whole MBR process provided that it does not offers any excessive amount of resistance (Judd et al., 2010).

2.3.2 Chemical Control Measures:

For the removal of irreversible and residual fouling, physical methods of membrane cleaning are accompanied with chemical cleansing. This kind of cleaning method tends to contain some integration that are shown in Figure 2.3.

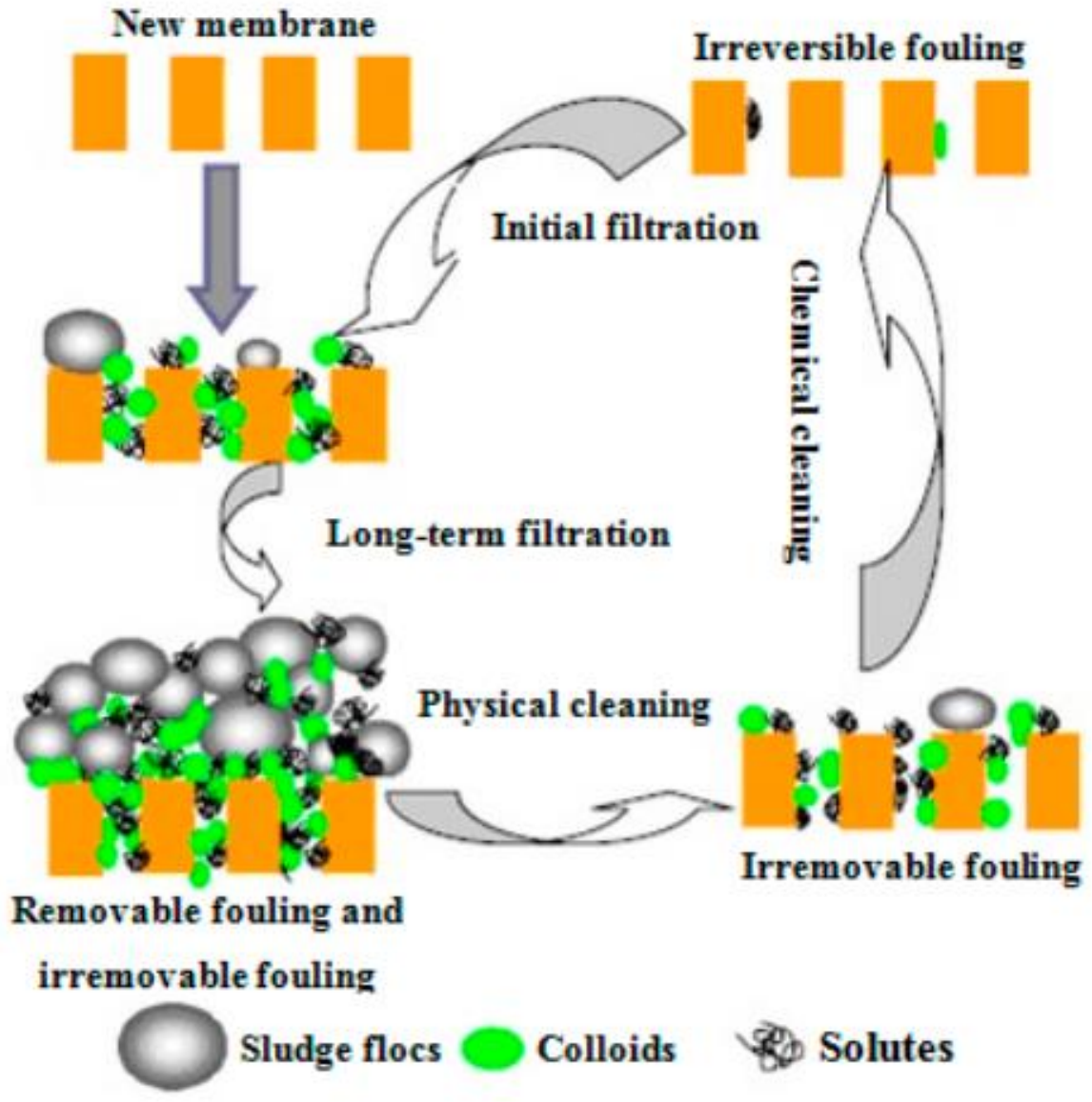


Figure 2.3: Fouling and Cleaning (Meng et al., 2009)

- Maintenance cleaning with adequate concentrations of chemicals, twice for weekly to monthly basis is actually planned for the removal of residual fouling.
- Recovery or intensive chemical cleaning is generally design to get rid of the so-called irreversible fouling.

To maintain the membrane permeability, maintenance cleaning is planned, resulting in reduced frequency of intensive cleaning. This type of cleaning is either performed with the membrane in situ, a usual CIP or in the situation of submerged membrane bioreactor (with drained membrane tank), generally refers to the cleaning in air (CIA). Intensive or recovery cleaning method applied ex situ or either in the drained Membrane tank, in this kind of cleaning membranes are soaked in highly concentrated chemical reagent. Because of reduced permeability, when further filtration is no more bearable, recovery or intensive membrane cleaning is applied.

Protocol that suppliers generally recommend for the intensive chemical cleaning are based on the integrated composition of hypochlorite, usually at 0.1-0.5 wt% for the removal of organic matter attached on the surface of the membrane and carbon-based acid (generally oxalic or citric with the targeted pH of almost 3) for the removal of inorganic scaling from membrane surface. Some studies have been conducted to find out the impact of chemical cleansing on microbial communities in the membrane bioreactor (Lim et al., 2003). On the other hand, no systematic research have been conducted to find the comparison of range of chemicals or cleaning conditions on the recovery of membrane permeability in MBR systems. Some studies have been performed with amplified cleaning techniques, such as sonically improved practise for the removal of fouling from membrane surface (Lim et al., 2003: Fang et al., 2005). Ultrasonic cleaning of membrane undoubtedly increase the flux

retrieval, as this study was conducted in portable water, suggest the impact of cleaning on the integrity of membrane (Masselin et al., 2001).

A complete cycle of 30-120 min is used for the maintenance cleaning, generally applied on every 3-7 days with cleaning reagent (NaOCl) having concentration of 200-500 mg/L for conventional aerobic membrane bioreactors. On the contrary, higher concentrations of 0.2-0.3 wt% NaOCl as a chemical reagent were applied for the recovery or intensive cleaning, integrated with acidic concentrations i.e. 0.5-1 wt% of oxalic acid or 0.2-0.3 wt% of citric acid (Judd. 2010).

Ramos et al., (2014) conducted the experiments by applying different chemical cleaning protocols on anaerobic MBR having submerged hollow fiber membranes treating the wastewater with high content of oil and grease: submerged chemical cleaning, chemical cleaning in air and chemically enhanced backwashing. Cleaning reagent NaOCl with the concentrations of 500-2000 mg/L and the volume per unit area of the membrane 3-17.5 L/m^2 were applied. Submerged chemical cleaning gave best results that allows the better spreading of chemical reagent in suspension, resulting into the increased cleaning effect on the different fouled portions of the hollow fiber membrane. However, chemically enhanced backflux driven technique results into considerably reduced cleaning efficacy, where there is non-uniform fouling appeared on the module.

Vanysacker et al., (2014) investigated the performance of cleaning protocols on three different kinds of MF membranes using NaOCl and Citric acid as cleaning reagents. Different biofoulant organisms with the increasing complications (monospecies-duospecies-complex communities) were used for the fouling of membranes for this purpose.

Efficiency of the cleansing protocol was determined in terms of exopolyomeric substance and the density of bacterial cells. Citric acid was found to be less effective, as it mostly kills the bacterial cells, especially in the case of complex activated sludge used for the fouling of membranes. Effect of both cleaning reagents were found on the properties of membrane surface, such as increase in porosity and pore size of the membrane. On the contrary, no change in surface chemistry and hydrophobicity of membrane surface was witnessed. Irrespective of the membrane type and biofoulant used, highest efficiency of membrane cleaning was found with NaOCl.

2.3.3 Biological Control Measures

Control of biofouling is very difficult because it is a natural biological process and it is very important to have basic knowledge of biofouling and its causes to suggest any biological control measures.

2.3.3.1 Quorum Sensing

Quorum sensing is a mechanism used by bacteria to coordinate definite behaviours such as virulence and biofilm formation, this communication is based on the local population density of bacteria as shown in Figure 2.4. It is not species dependent and can occur within same as well as within bacterial consortium. Different types of molecules can be used for communication purpose or as signals depending on type of bacteria e.g. signalling molecules used by Gram positive bacteria are oligopeptides, those by Gram negative bacteria are N-acyl homoserine lactones pronounced as AHLs. Another type of signalling molecules known as autoinducer-2 (AI-2) are also used by both Gram-positive and Gram-negative bacteria (Miller and Bassler, 2001)

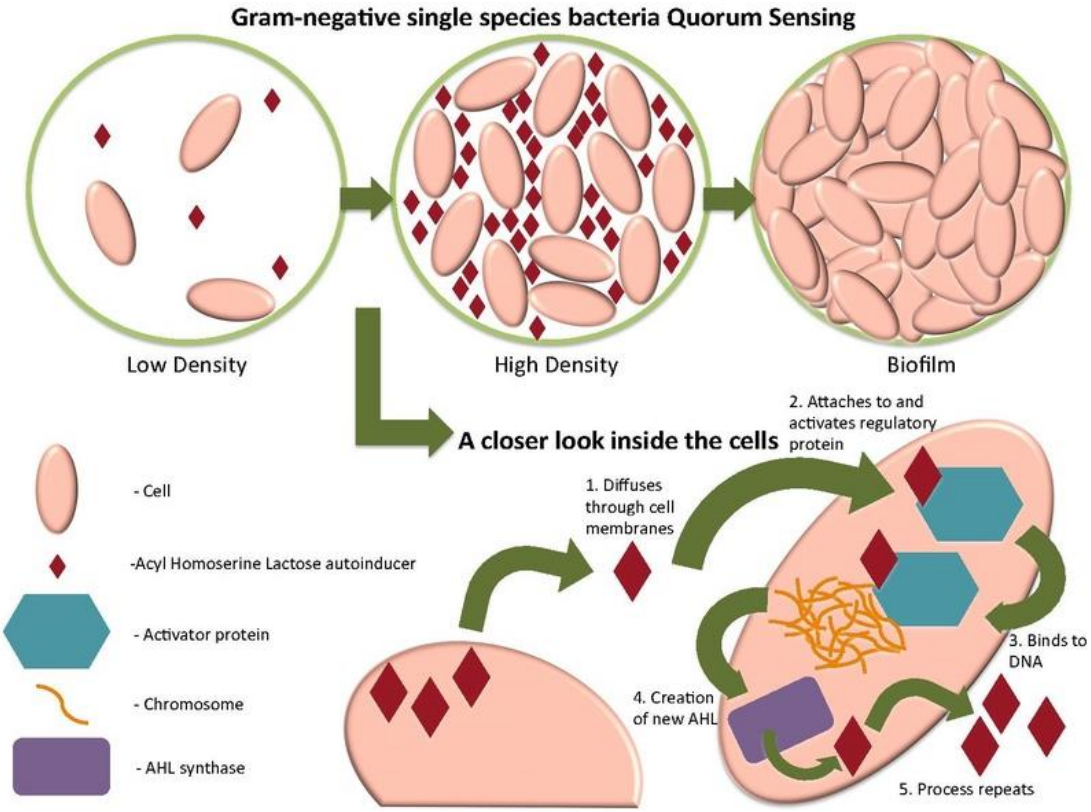


Figure 2.4: Quorum sensing in a gram negative Cell (Galloway et al., 2011)

Some of the biological processes for biofouling control are being established in the recent past and are proven to be very much effective as compared to chemical and physical control measures (Weerasekara et al., 2016). They are also found out to be less toxic and more sustainable than other control approaches (Xiong and Liu, 2010).

2.3.3.2 Changing Floc Size of Sludge

It is a well-known fact that sludge floc size has a significant impact on filtration characteristics of sludge as well as biofouling on the membranes. Large floc size is generally more favourable for MBR operations. Van den Broeck et al., (2010) studied on sludge deflocculation and reflocculation and found out that by using a high ratio of monovalent cations to polyvalent cations in the influent will result in deflocculation hence

resulting in low floc size and ultimately increased fouling on membrane surface. Whereas if a low monovalent to polyvalent cation ratio is used in t influent sludge will makes flocs again and sludge characteristics will start to improve after a certain amount of time.

2.3.3.3 Enzymes for EPS Degradation

Some enzymes have been successfully applied in recent past for detachment of biofilm including proteolytic enzymes which are being used for protein hydrolysis (e.g. trypsin and proteinase K), and polysaccharases are used for the polysaccharides hydrolysis (e.g. dextranase and Mutanase) (Chaignon et al., 2007; Guezennec et al., 2012). A protein named lysozyme which is a Hydrolytic enzyme has been used for prevention of microbial attachment and is found to act more explicitly than conventional biocides (Xiong and Liu, 2010). Studies on efficiency of di-nitro-phenol (DNP) have also been conducted and Xu and Liu (2011) found out signs of suppressed synthesis of ATP and AI-2 by using di-nitro-phenol DNP which helps in reduction of biofilm on surfaces of nylon membrane. It was a promising technique for removal of biofilm but had certain limitation of use only incase of nylon membrane which are flat sheet and follow dead end microfiltration in a batch reactor and can only be fed synthetic wastewater.

2.3.3.4 Bacteriophage

Bacteriophages (a virus that infects bacteria and replicates inside it) are also being used for control of biofilm growth and is found to be very promising technique because of their specificity, rapid growth and very limited infectivity to prokaryotic organisms (Lu and Collins, 2007). Biofilm prevention has also been achieved effectively for multi consortia by use of Polyvalent phages (Jensen et al., 1998).

Some modified phages were produced through continuous application of bacteriophages e.g. polyvalent phage K on bacterial strain and were found to have improved lytic properties. (O'Flaherty et al., 2005). In some studies genetic engineered phages were produced using biofilm-degrading enzymes and were proved to degrade EPS more effectively (Lu and Collins, 2007).

Goldman et al. (2009) determined that the microbial attachment on the UF membranes were reduced to 40-60% by addition of bacteriophage. This study was performed on MBR treating wastewater containing only three species of bacteria. They demonstrated that for wastewaters containing a wide range of bacterial species, a coupling or combination of different bacteriophages should be applied for more efficiency. The drawback of this method is that due to their small sizes, phages cannot be completely retained and appeared in permeate which can cause serious environmental and health concerns.

2.3.3.5 Quorum Quenching Enzymes

Enzymatic cleaning with the help of protease was used by Poele and Graaf (2005) for removal of protein biofouling in Ultra filtration membranes. The results showed a higher efficiency of enzymatic cleaning as compared to conventional alkaline cleaning. Biological antifouling strategies in MBRs were pioneered by Yeon et al. (2009a, b).They evaluated the capability of acylase I enzyme found in porcine kidney as well as AHL-acylase enzymes to inhibit MBR biofouling by quenching of AHL autoinducers. In another study AHL-acylase was directly immobilized on a membrane (NF) to prevent formation of mature biofilm because of reduced secretion of EPS (Kim et al., 2011).

2.3.3.6 QQ Bacteria

Enzymes always had cost and stability related issues in operation of MBRs. Oh et al. (2012) proposed a solution to this problem by evaluation of QQ bacteria for antifouling purpose and suggested that they could be more practical, do not require purification of enzymes and also have a longer life span than enzymes. Rhodococcus sp. producing N-acyl homoserene lactonase was obtained from a MBR and was encapsulated in a microbial vessel inside hollow fiber membrane setup which was found to efficiently control biofouling. The QQ activity of microbial-vessel was maintained over almost 100 days of operation because of continuous regeneration of QQ bacteria. Recirculation rate of sludge between membrane tank and bioreactor majorly determined the QQ effect (Jiang et al., 2013). Higher recirculation rates were favoured because they gave more chances of auntoinducers inside biofilm and mixed liquor to come in contact with microbial vessel.

The study by Xu and Liu (2011), which used DNP to remove biofilms from nylon membrane surfaces is promising but was conducted for hydrophilic flat-sheet nylon membranes used in dead-end microfiltration (MF) filtration of batch-fed synthetic wastewater.

2.3.3.7 Predation of Microorganisms

Biofouling control may also be achieved by using prokaryotic microorganisms for predation, purposes. An effective reduction in biocake layer and thus an enhaced biofouling control was observed in MBR due to a prokaryotic population commonly known as metazoans composed primarily of oligochaete worms and rotifiers (Luxmy et al., 2001) but it was observed that activity of these organisms might be suppressed if there is a high concentration of MLSS (mixed liquor suspended solids) in MBR. Predation is particularly suitable in systems working without the cross-flow mechanism because it can

provide appropriate conditions for development of larger eukaryotic organisms (Derlon et al., 2012).

2.3.3.8 Combined Control Measures

To increase the efficiency of biofouling control, several studies have been done in which a combination of biofouling control strategies have been used to retard biofouling, enhance sludge characteristics and membrane permeability. Some of the used combination are as follows

1. Physico chemical cleaning or chemical enhanced backwashing i.e. including physical cleaning of backwash and chemical cleaning through NaOCl (Lee et al., 2012).
2. Quorum quenching beads for QQ activity along with physical scouring with help of beads (Lee et al., 2013)
3. Quorum Quenching and permeate backwashing for retarding cake layer fouling as well as reduction in pore blockage resistance (Hasnain et al., 2017).
4. Quorum Quenching and chemical enhanced backwashing for biofouling control through EPS reduction due to QQ activity as well as improved reduction in pore blockage resistance due to oxidizing effect of chlorine (Weerasekara et al., 2016)

Chapter 3

Materials and Methods

3.1 Lab Scale Setup

Two submerged membrane bioreactors having working volume of 35 L each were used for experimental study. The membrane used was hollow fiber PVDF membrane which is shown in Figure 3.1 and whose specification are presented in Table 3.1.

Table 3.1: Characteristics of membrane used in study

Item	Characteristics
Membrane Type	Hollow Fiber
Membrane Manufacturer	Hinada treatment tech, China
Membrane material	Polyvinyl di fluoride
Membrane surface area	$0.7m^2$
Pore size	0.1 µm
Temperature Range	5-45 ^oC

Figure 3.1: Membrane with module

Four peristaltic pumps (Baoding Longer BT300-2J, USA) were used for the setup. Two pumps were used for extraction of permeate from HF membranes and the other two for back-pulsing of NaOCl in backwash lines of MBR. Air Compressor (Model 07054-05, Gast, USA) was attached for aeration and membrane scouring purposes. Four (Omron DH48S-S, China) timers were used for cyclic operation of filtration and backwash cycles for MBRs and one (Chrontrol XT) timer was used for operation of chlorination pumps and for diffused aerators. Two (2) data logging manometers (Sper Scientific 840098) were used to measure trans-membrane pressure (TMP) of membrane modules as shown in Figure 3.2

Both of the reactors were fed with real domestic wastewater from residential area, departmental buildings and student hostels of National University of Sciences and Technology (NUST), Islamabad, Pakistan. Sludge used in the reactors was taken from full scale MBR plant installed at NUST, Islamabad, Pakistan. SRT of sludge was kept at 20 days to maintain MLSS concentration of 8-10 g/L. The range of raw wastewater parameters are listed in Table 3.2. QQ beads were prepared using the method prescribed in (Kim et al., 2013) with slight modifications and were used as 0.5% of bioreactor working volume.

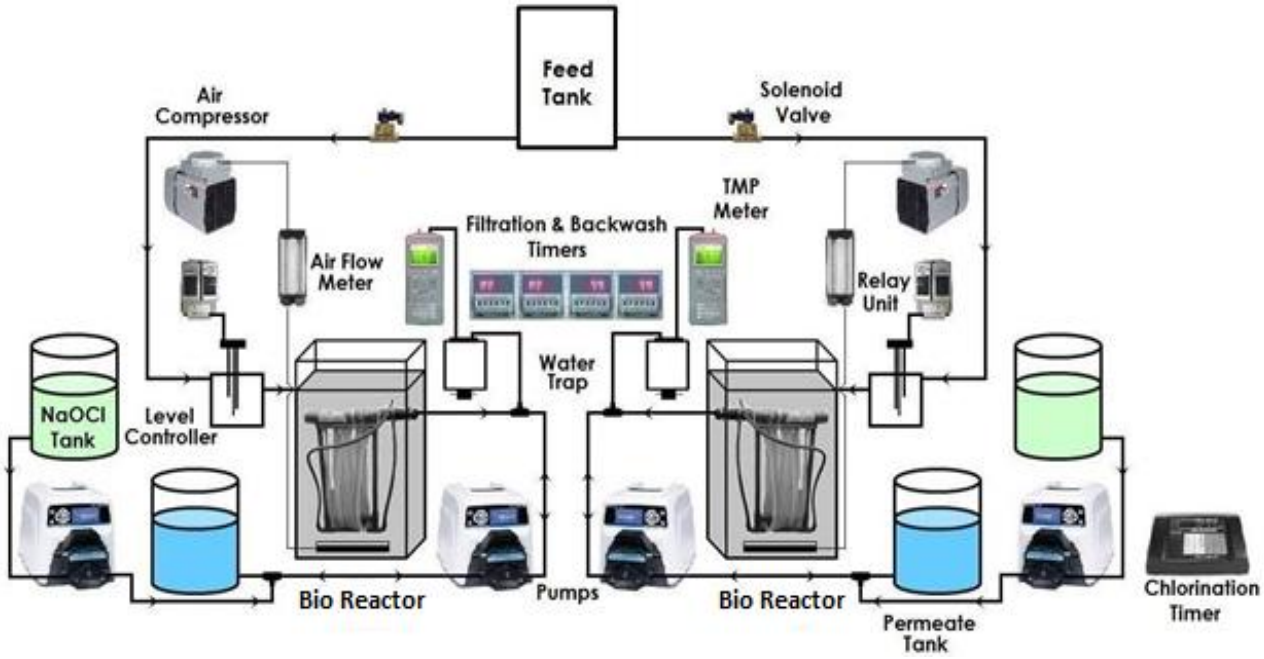


Figure 3.2: Schematic of Lab Scale MBR

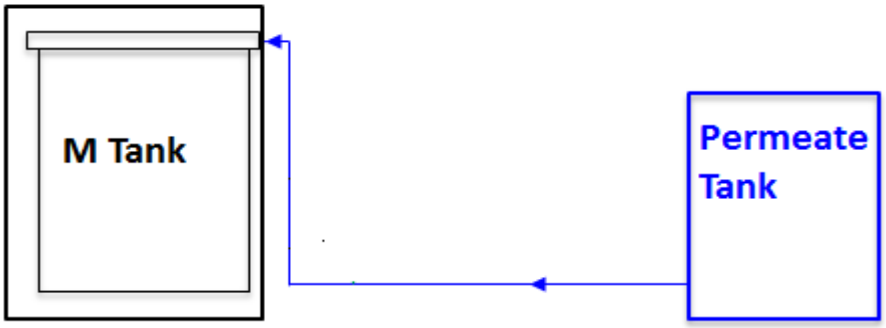
Table 3.2: Real Wastewater Characteristics

Parameter	**Range (mg/L)**
Chemical Oxygen Demand (COD)	90-230
Biochemical Oxygen Demand (BOD)	65-170
Ammonium-N (NH_4-N)	11.4-21.8
Phosphate-P (PO_4-P)	10.4-19.6
Total Suspended Solids (TSS)	434- 1308
Total Dissolved Solids (TDS)	205-1245
pH	6.9-8.4

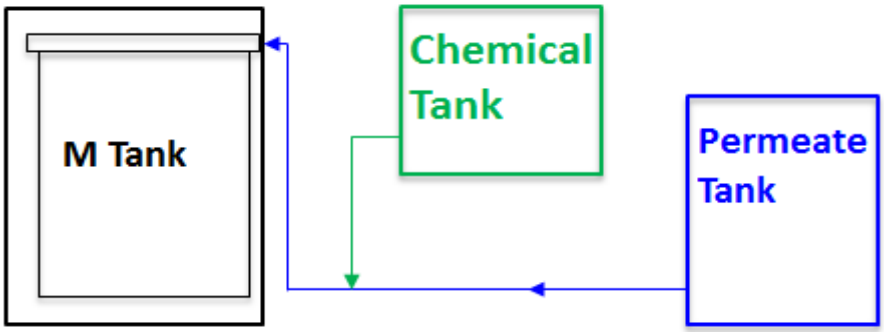
3.2 Operating Conditions

Both MBRs were operated at similar flux of 16.5 LMH to maintain HRT of 3 hours. The 10 minute operational cycle of both MBRs was maintained at 8 min filtration, 1 minute relaxation and 1 minute permeate backwashing. In the first phase, MBR with permeate backwash was called PBW-MBR and the one with chemical enhanced backwashing (CEB) was called CEB-MBR. In the next phase, QQ beads were added to both the MBRs and called QQ+PBW-MBR and QQ+CEB-MBR. CEB was introduced by using sodium hypochlorite and mixing it with permeate backwash water in a ratio achieving NaOCl concentration of 3.5 mg/L.

3.2.1 Types of MBRs Used



(a)



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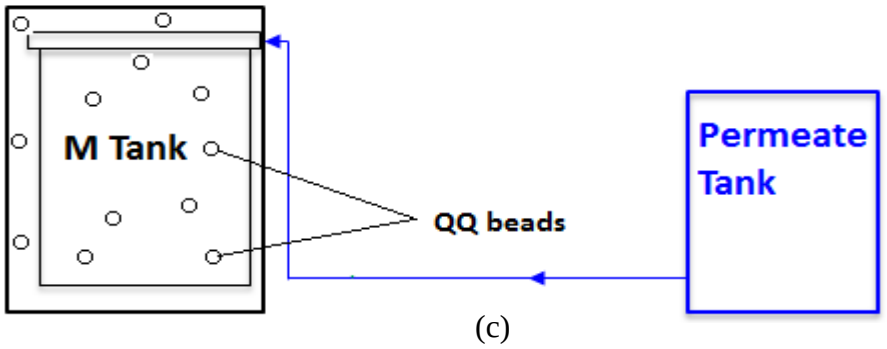
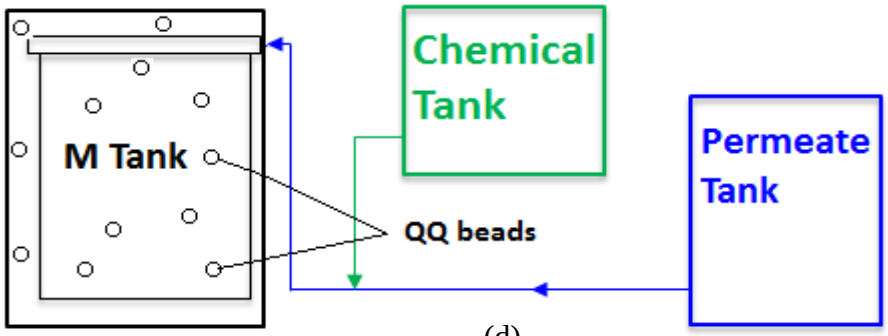


(c)



(d)

Figure 3.3: (a): Permeate backwashing MBR (PBW-MBR), (b): Chemical enhanced backwashing MBR (CEB-MBR), (c): Quorum Quenching + Permeate backwashing MBR (QQ+PBW-MBR), (d): Quorum Quenching + Chemical Enhanced Backwashing MBR (QQ+CEB-MBR)

3.3 Analytical Methods

Chemical oxygen demand (COD), biological oxygen demand (BOD), ammonium-N and phosphate-P were used to measure the treatment performance efficiency of MBRs using Standard Methods (APHA et al., 2012). Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), capillary suction time (CST), particle size distribution (PSD) and extra-cellular polymeric substances (EPS) were used to measure sludge characteristics. Extraction and quantification of EPS were performed as per method described in previous study (Frolund et al., 1996). CST was measured using CST apparatus (304B Triton, Canada). Mean particle size was measured using particle size analyser (LA 300, Horiba, Japan).

3.3.1 Extraction and Quantification of EPS

Cation exchange resin (CER) method was used for extraction of bound extra-cellular polymeric substances (EPS) (Frolund et al. 1996). 50 mL sludge collected from membrane tank was centrifuged for 15 min at 4^{o}C at 4000 rpm using refrigerated centrifuge (K2015R, Pro-Research, UK) after which supernatant was separated from sludge for determination of soluble EPS. In case of bound EPS, the sludge pellets which were extracted in the previous step were mixed with buffer solution and CER for 1 hr using magnetic stirrer, and lastly centrifuged for 15 min to separate supernatant. Protein concentration (PN) was measured by Lowry method by using Folin–ciocalteu phenolic reagent (Lowry et al. 1951). Bovine Serum Albumin (BSA) was used to develop the standard curve for PN. Polysaccharides (PS) concentration was measured by Dubois method (Dubois et al. 1956). Glucose standard curve was used to determine the PS concentrations.

3.3.2 Sludge dewaterability

To determine the rate at which water releases from sludge is called dewateribility and is measured using capillary suction time (CST) apparatus. It provides a quantitative measure, reported in seconds, of how readily a sludge release water. The results may be used to assist in study of sludge dewaterabilty processes; and to indirectly evaluate solid content in sludge.

Procedure

1. Turn on CST meter. Dry CST test block and reservoir.

2. Place a new CST paper on test block having rough side up and grain side parallel to the 9-cm side.

3. Add upper test block, insert sludge reservoir into test block and adjust it using light pressure, then quarter turn it to prevent surface leaks.

4. Temperature of sludge must be recorded before CST analysis. Put 6.4 mL sludge into test cell reservoir; if pipetting is not possible because of sludge consistency, pour a representative sludge sample into the cell until it is full.

5. The CST device will automatically begin time measurement as water being drawn into paper reaches the inner pair of electrical connections.

6. Timing stops when outer contacts are reached.

7. Record the reading of CST showed on digital display.

8. Take out the remaining sludge and used CST paper and discard them. Rinse and dry test block and reservoir.

9. Ensure that all analyses are run at same temperature and same volume.

3.4 Preparation of Beads

QQ beads were prepared using the method prescribed in (Kim et al., 2013) with slight modifications and were used as 0.5% of bioreactor working volume. The bacterial stain of Rhodococcus sp. BH4 were used as QQ specie. 5% solution of sodium alginate and 4% solution of Calcium Chloride ($CaCl_2$) were prepared and bacterial suspension having approx. 5 mL volume was then mixed in sodium alginate solution. This solution of sodium alginate and bacterial strains was then added in $CaCl_2$ solution dropwise using peristaltic pump at a rate of 1 mL/min. The beads prepared as a result of dripping were then given 8 hrs gelation period in $CaCl_2$ solution. These beads were then coated with polymer of 2%

polysulphone solution to prevent rupturing of beads during aeration in QQ-MBR. The beads prepared had an average diameter of 3.7 mm and an average density of 1.4 g/mL.

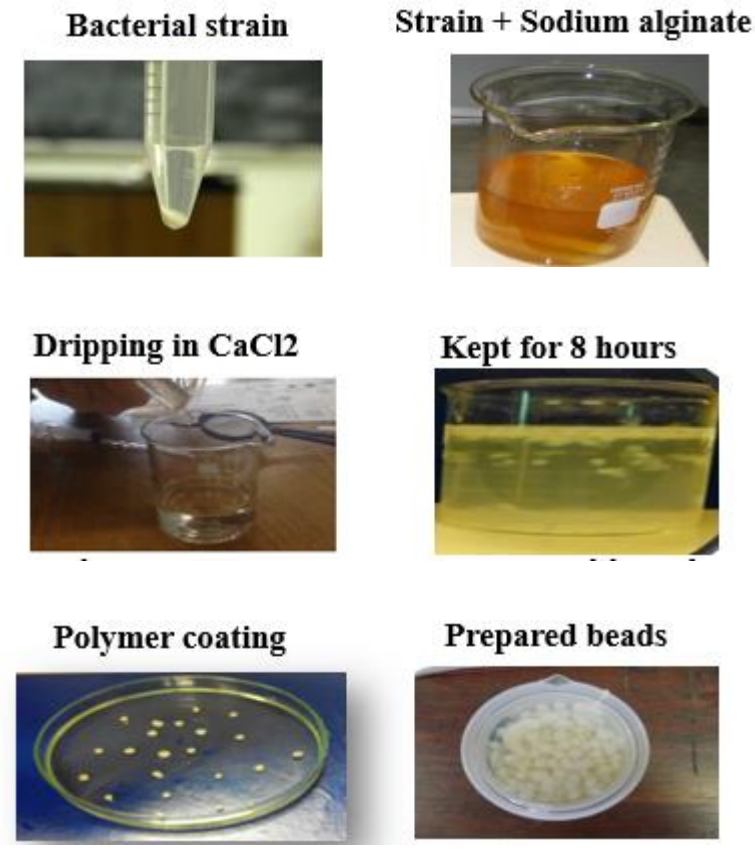


Figure 3.4: Preparation of Quorum Quenching Beads

3.5 Membrane Resistance Analysis

Darcy's Law was used for calculation of resistances offered by membranes at every stage of operation.

$$R_t = \Delta P/J.\mu.ft$$

$$R_t = R_c + R_p + R_m$$

Here,

R_t = total hydraulic resistance (1/m)

R_c = cake layer resistance (1/m)

R_p = pore blockage resistance (1/m)

R_m = intrinsic membrane resistance (1/m)

ΔP = TMP (Pa)

μ = permeate dynamic viscosity (Pa.s)

J = operational flux of permeate (m3/m2/s)

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

R_t is the resistance which the membrane offered when it is completely fouled. It was calculated with distilled water using above equation when membrane pressure reached the maximum allowable limit i.e. 30 kPa i.e. completely fouled.

R_p is the resistance which is offered by membrane to the flow of distilled water due to blockage of its pores. R_p is calculated after removing the biocake layer which is developed on membrane surface. R_m is the resistance which is offered by membrane after cleaning. R_m was calculated before start of every run after chemical cleaning of the membrane and filtration of D.I water for 30 minutes.

R_c cannot be directly calculated so it is calculated using the above equation by subtracting (R_m +R_p) from R_t. Each type of resistance is compared in this study for all the four runs of MBR.

3.6 Sodium Hypochlorite Concentration

A 10 % solution of sodium hypochlorite was used for the purpose of chemical enhanced backwashing (CEB). The concentration of NaOCl was set at 3.5 mg/L for CEB. This concentration was set as per amount of NaOCl used for maintenance cleaning in full scale MBR plant to compare the effects in both MBRs due to change in scale and chlorination

frequencies of both.

3.7 Membrane Cleaning Protocol

When maximum allowable TMP limit of 30 kPa was reached at the end of each run, the membrane was physically as well as chemically cleaned prior to start of the next run. Three steps of cleanings were performed for this purpose. The sludge cake deposited on the fouled membrane fibers was removed manually and measured to determine the total biomass deposited on the membrane fibers. Next, basic cleaning was performed in which the membrane module was dipped in a solution of NaOH and NaOCl for 8 hrs to remove microbial deposition on the membrane surface. The solution prepared comprised of 2% weight to volume ratio of NaOH and 2 g/L of NaOCl concentration. Lastly, after rinsing the membrane module with tap water, it was dipped in acidic solution of 1% HCl for 1 hr to remove inorganic foulants followed by submerging the membrane module in tap water and filtration for 30 min.

Chapter 4

Results and Discussion

4.1 Membrane Fouling:

TMP profiles of the four runs from the different type of MBRs is shown in Figure 4.1. It shows the effect of permeate backwash (PBW), chemically enhanced backwash (CEB) and quorum quenching (QQ) on membrane fouling. It was observed that when no QQ beads were used, MBR having only PBW experienced higher rate of TMP rise (1.5 kPa/day) than the bioreactor having CEB (1.03 kPa/day). This result infers that the fouling rate of PBW-MBR was almost 1.5 times than the CEB-MBR. Both the runs of PBW and CEB were performed twice for confirmation of results. CEB was affective in diminishing fouling propensity because of the fact that NaOCl is a strong disinfectant and it tends to disrupt the deposition of microorganisms on membrane surface as well as inside pores (Saby et al., 2002) thereby lowering the TMP. The CEB-MBR run was almost 29 days as compared to PBW-MBR run of nearly 19 days when the TMP reached threshold of 30 kPa. It was also observed through membrane resistance analysis that Pore blockage resistance (R_p) was much less (**4.67×10^{11} m^{-1}**) as compared to Cake layer resistance (R_c) (**50.44×10^{11} m^{-1}**) in case of CEB-MBR as compared to PBW-MBR (R_p = **25.9×10^{11} m^{-1}**, R_c = **29.7×10^{11} m^{-1}**). Cake layer resistance was thus found to be the major contributor to biofouling in CEB-MBR (Lee et al., 2012). The most probable reason for the prolonged filtration duration of CEB-MBR can be the reduction in pore blockage (irreversible fouling) while cake layer formation (reversible fouling) continued over time despite chlorination (Weerasekara et al., 2016). Physically reversible fouling resistance or cake layer resistance (R_c) in CEB-MBR may be caused due to accumulation of chlorine-resistant bacteria on surface of membrane fibres (Calderon et al., 2011).

In the next phase (Phase 2), QQ beads were added to both the MBRs referred to as QQ+PBW-MBR and QQ+CEB-MBR. A remarkable difference in TMP was observed as compared to previous runs without QQ. Both the runs appeared to be very smooth and stable at the start of operation (Average TMP rate of 0.44 kPa/day for QQ+PBW-MBR and 0.42 kPa/day for QQ+CEB-MBR) and their fouling limits reached very late as compared to Phase 1. The QQ+PBW-MBR fouled after 64 days where as QQ+CEB-MBR fouled after 71 days. After almost 48 days operation, TMPs of both MBRs started to increase rapidly but the TMP profile for QQ+PBW-MBR was steeper than that of QQ+CEB-MBR. As it is well known fact that QQ activity tends to retard biofilm or bio-cake formation hence it is considered as the most effective technique for reducing cake layer resistance (R_c) while the pore blockage resistance (R_p) remains unaltered. In case of QQ+CEB-MBR, cake layer deposition was controlled by QQ activity and pore blockage was minimized by chemical enhanced backwashing causing less steepness of TMP profile of QQ+CEB-MBR than that of QQ+PBW-MBR. The hydraulic filtration resistance analysis of the two MBRs (Phase 2) showed that Pore blockage resistance (R_p) (**5.64×10^{11} m^{-1}**) was very low in fouled membrane of QQ+CEB-MBR and relatively high in membrane of QQ+PBW-MBR (**19.42×10^{11} m^{-1}**) complimenting a previous study (Weerasekara et al., 2016). The slight difference in both TMP profiles depicts that with the addition of QQ beads to MBR, CEB mechanism is not much effective as compared to PBW coupled with QQ mechanism. The CEB not only utilizes additional chemical usage but also may affect the diversity of microbial community as well as sludge characteristics. CEB in MBR as fouling control strategy can only be considered viable in absence of QQ activity where the bioreactor adapts to the chlorination by the growth of chlorine resistant bacteria.

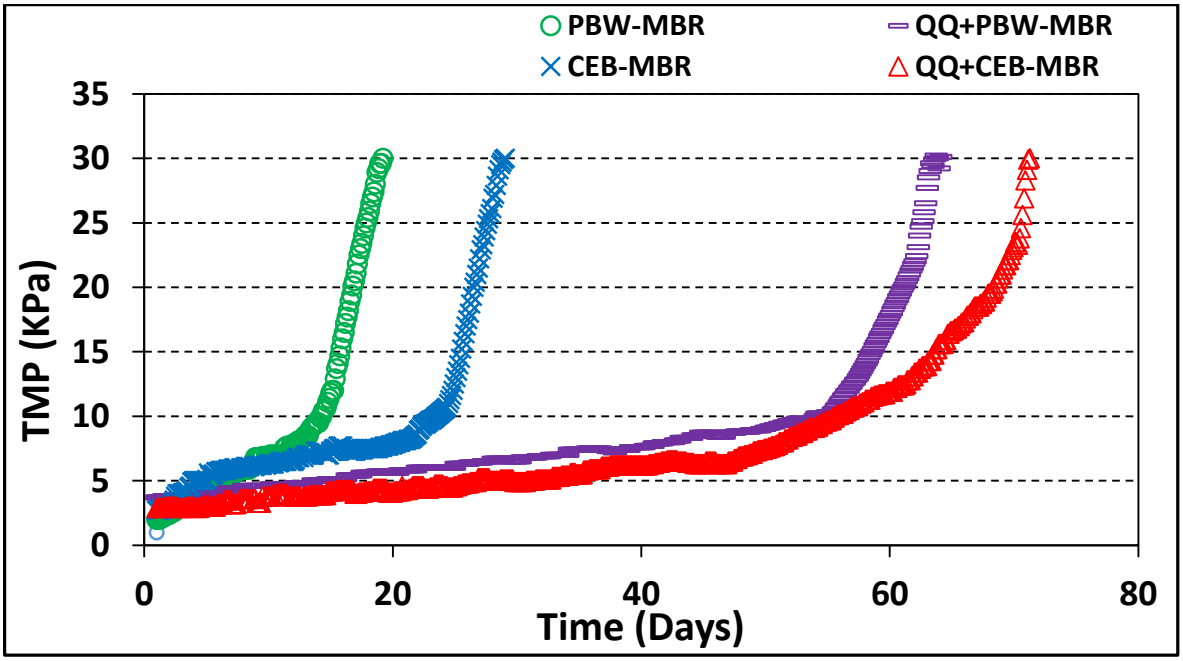


Figure 4.1: TMP profiles of the four MBRs types with different fouling control strategies

However even with QQ bacteria, membranes fouled after sometime showing that biofouling is unavoidable. This is because there are more than 100 types of bacteria capable of biofilm formation and having different types of signaling molecules (Shrout and Nerenberg, 2012). The bacteria used in this study (Rhodococcus sp. BH4), can only inhibit one type of signaling molecule i.e. AHL (Weerasekara et al., 2014).

4.2 Effect of Sodium Hypochlorite on MLSS and Removal efficiency

It was observed that injection of sodium hypochlorite through chemical backwashing had a negative impact on MLSS concentration. It was found that MLSS started decreasing after 5 days of operation with chemical enhanced backwashing in both CEB-MBR and QQ+ CEB-MBR. It continued to decrease in both the cases till day 21 after which MLSS concentration start to become stable after dropping from 10 to 6 g/L as shown in Figure 4.2. This observation infers that NaOCl inhibits the growth of microorganisms because it has the ability to oxidize organic matter (Cai and Liu, 2016; Lee et al., 2013).

The change in MLSS is also noted to have a negative impact on COD and NH_4^+-N removal efficiencies in both CEB-MBR and QQ+CEB-MBR as depicted in earlier study (Cai and Liu, 2016). After a certain duration both the removal efficiencies started decreasing from their normal ranges but the adverse effect was not immediate and appeared sometime later as observed previously by Lee et al. (Lee et al., 2013). COD removal was decreased from 93 to 78% for CEB-MBR and from 93 to 81% for QQ+CEB-MBR. NH_4^+-N removal was decreased from 64 to 37 % in QQ+CEB-MBR and from 63 to 34 % in CEB-MBR as shown in Figure 4.3 & 4.4. Chlorination of sludge causes oxidation of microbes which increases the overall organic matter and can result in increase of COD within the reactor (Saby et al., 20020. The decrease in removal efficiencies was found to be more drastic for NH_4^+-N removal than that of the COD removal (Figure 4.5 & 4.6). This may be because of the fact that ammonium oxidizing bacteria (AOB) are sensitive to change in pH, temperature, HRT, and many other factors than other heterotrophic bacteria (Lay et al., 2010). No significant effect on removal efficiency of phosphate-P was observed and it remained almost stable suggesting that phosphorus accumulating organisms (PAOs) are more resistant to NaOCl (Saby et al., 2002).

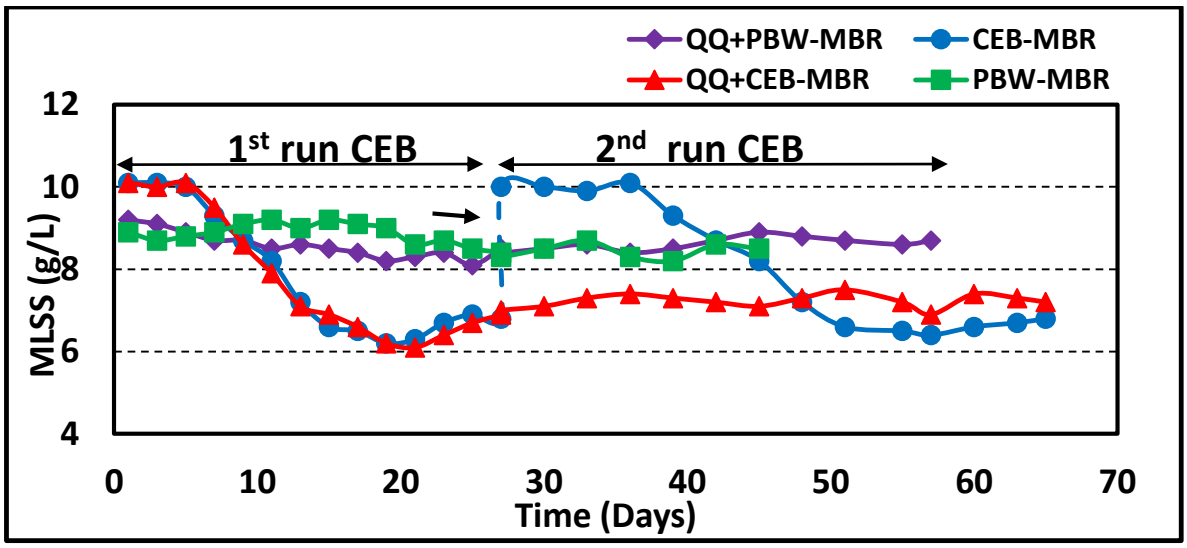


Figure 4.2: Effect of NaOCl injection on MLSS concentration.

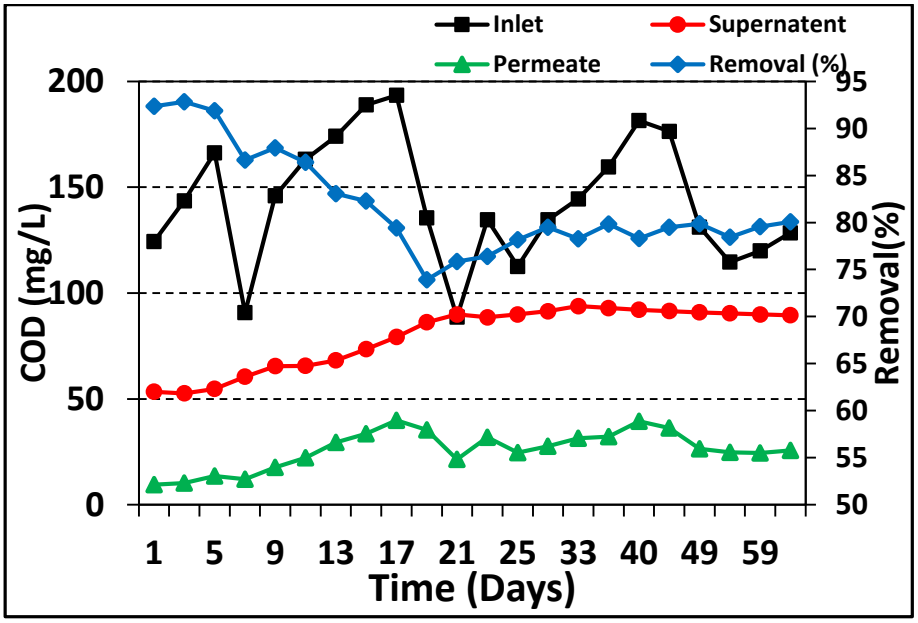


Figure 4.3: Effect of chemical CEB on COD removal in QQ+CEB-MBR

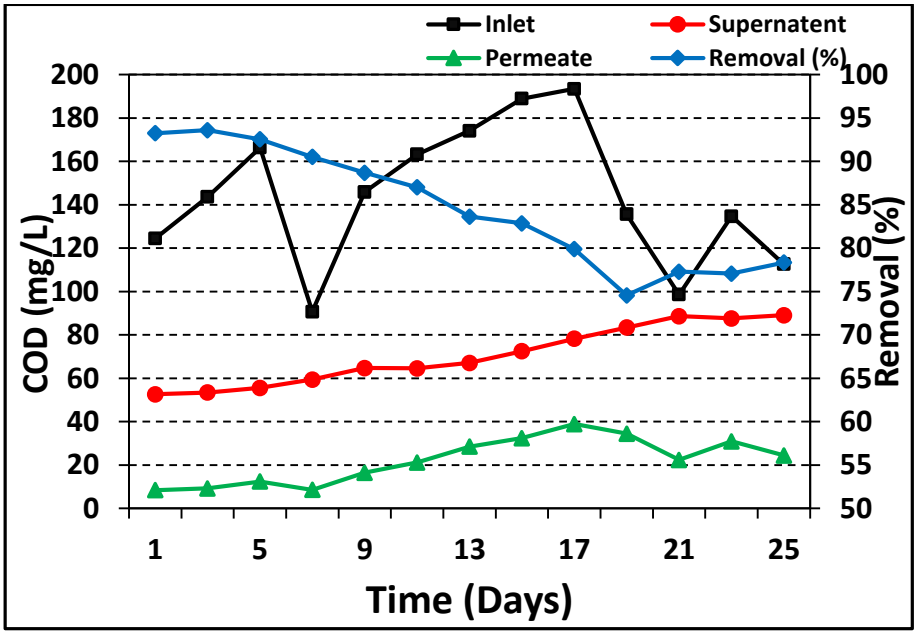


Figure 4.4: Effect of chemical ennhanced backwashing on COD removal in CEB-MBR

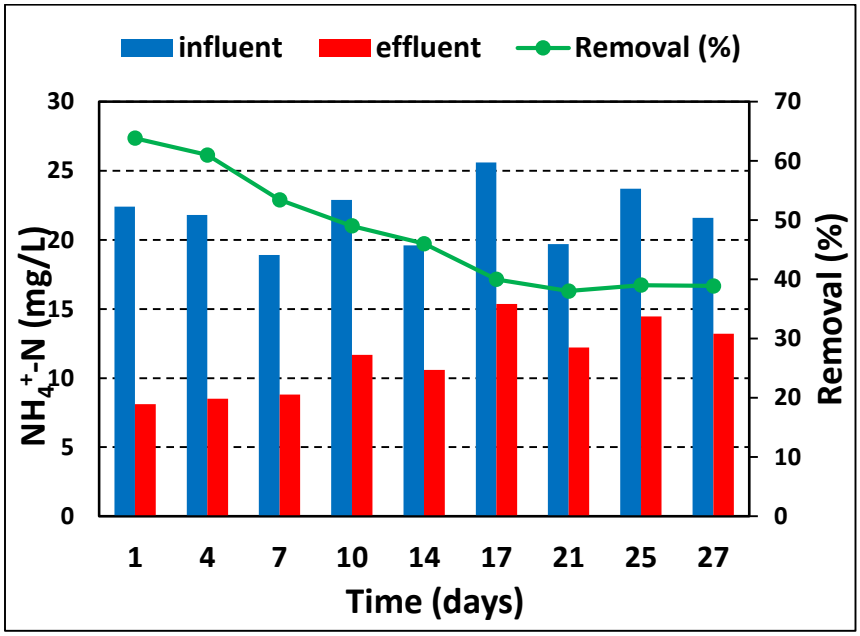


Figure 4.5: Effect on NH_4^+-N removal efficiencies in CEB-MBR

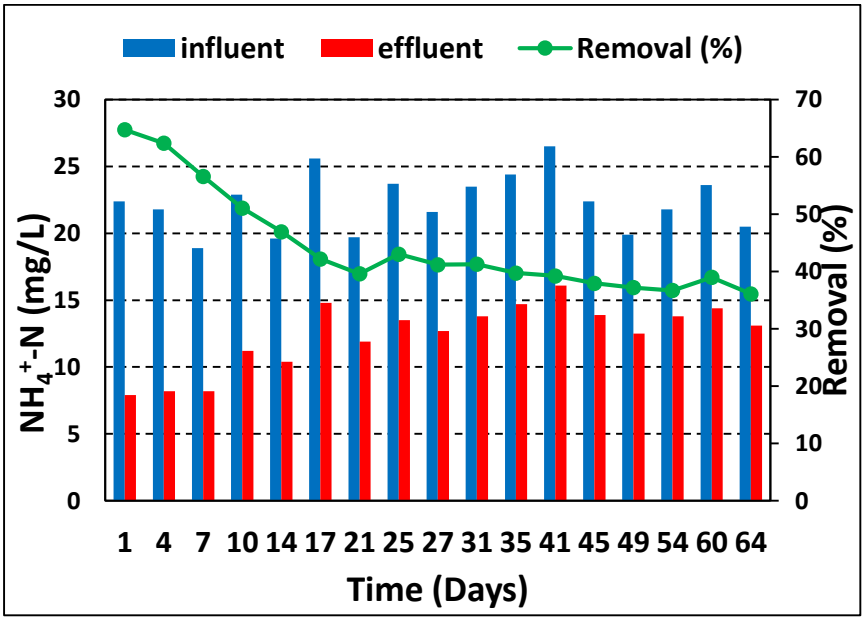


Figure 4.6: Effect on NH_4^+-N removal efficiencies in QQ+CEB-MBR

After almost 21 days of operation the MLSS concentration cease to decrease further and both sludge concentration as well as organic and nutrient removal effeciences started becoming stable which reveals that the prolonged exposure to chlorination causes microorganisms to adapt to the chlorinated environment (Saby et al., 2002).

4.3 Capillary Suction Time and Bio-Particle Size

Capillary suction time (CST) which is usually used to represent dewaterability or filterability of sludge and is known to be directly associated with membrane fouling (Lee et al., 2013). In this study capillary suction time was found to be continuously increasing from 25s to almost 36s in the CEB run as shown in Figure 4.7. This means that injection of NaOCl can have a negative effect on sludge stability and can decrease its permeability (Lee et al., 2013). For MBRs having QQ beads, CST was found to be reduced indicating improved sludge conditions due to QQ activity even in QQ+CEB phase where the decrease in permeability due to CEB was countered by the presence of QQ beads.

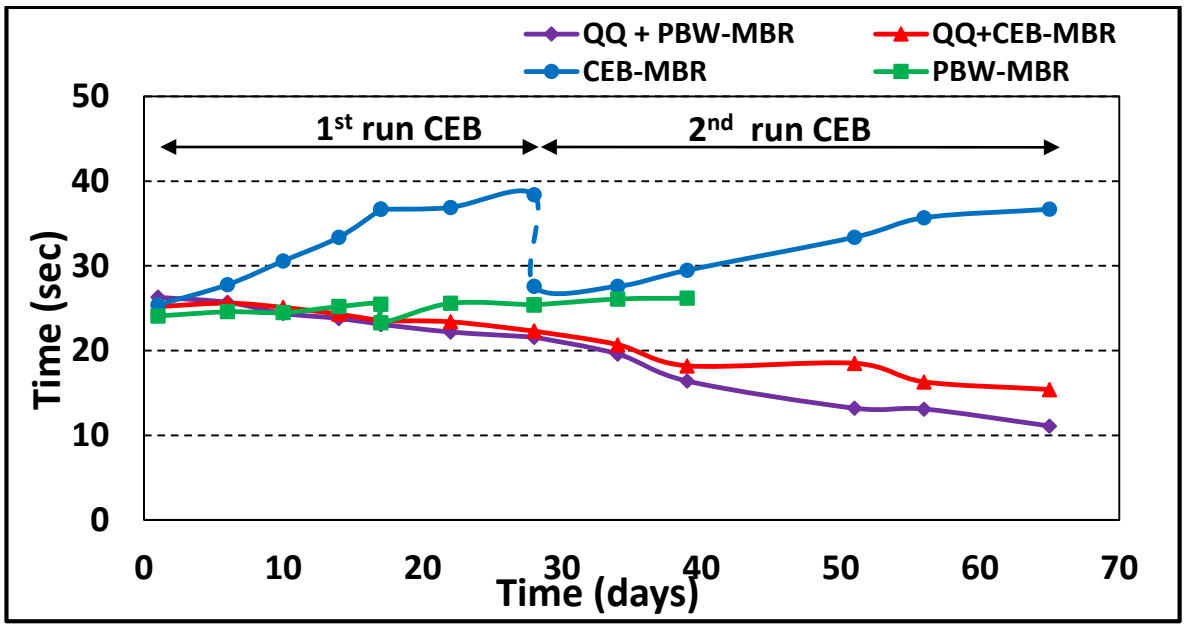


Figure 4.7: Changes in Capillary suction time of all the MBRs with respect to time

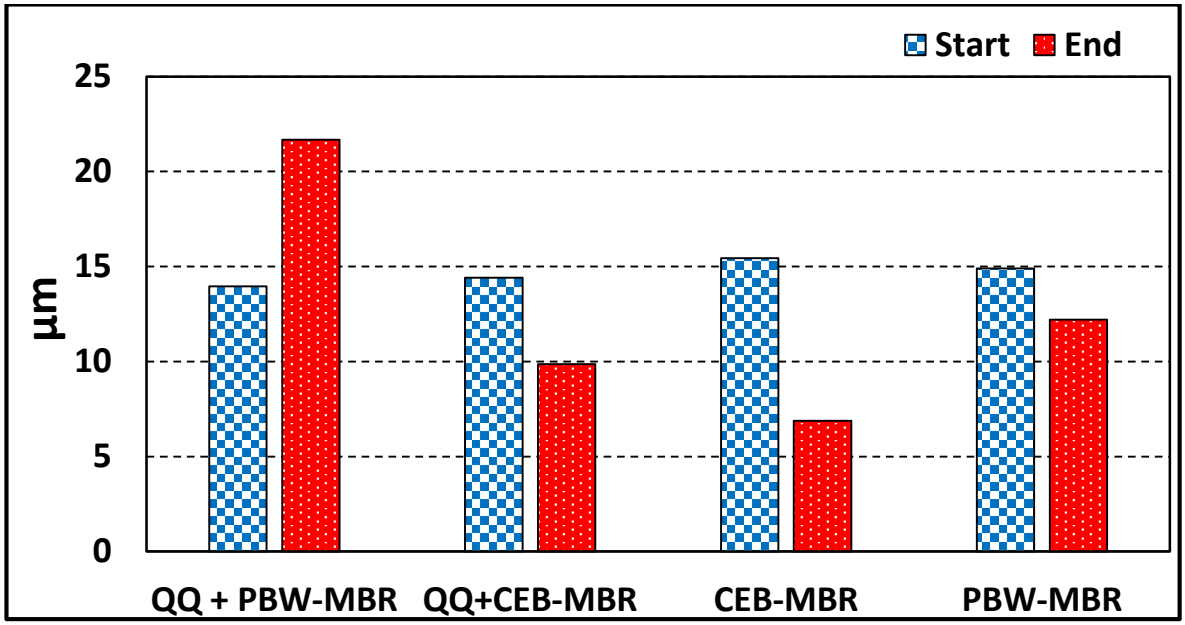


Figure 4.8: Mean Particle Size of 4 MBRs used in study

A large particle size is generally favored for control of membrane fouling and in improving back- transport of particle from surface of membranes (Lee et al., 2013). The mean particle size of sludge decreased from 15.65 to 7.54 µm in case of CEB-MBR indicating that microbial flocs were either disrupted or endured cell lysis [Saby et al., 2002]. It remained almost constant (15.1 to 13.4 µm) for the PBW-MBR and on the contrary increased slightly for the QQ+ PBW-MBR as shown in Figure 4.8. From these results, it may be deduced that there is an inverse relation between the mean particle size and capillary suction time where small particle size can lead to increase in capillary suction time and hence increase in biofouling potential. Thus, increase in floc size in QQ+PBW-MBR resulted in improved sludge permeability.

4.4 Soluble and Bound EPS:

EPS is considered as a major cause in bio-fouling of a membrane in MBR. EPS consists of complex matrix containing proteins, carbohydrates, nucleic acids and humic substance but the most predominant and important forms of EPS that severely affect membrane filtration

are protiens and carbohydrates (Lee et al., 2013). In this study, soluble and bound EPS were investigated. Very small concentration of soluble EPS was observed (Figure 4.9 a) under QQ condition as compared to PBW or CEB mechansim because Rhodococcus sp. in QQ beads play a crucial role by generating lactonase which has the ability to degrade AHL molecules. QQ bacteria helps in biofouling control by opposing the formation of bio-cake layer by decreasing EPS concentration (Weerasekara et al., 2016). As shown in case of PBW-MBR, discernible increase in soluble EPS was observed while during CEB-MBR, the increase was relatively more rapid as compared to that of PBW-MBR. According to a recent study, the increase in NaOCl concentration increases oxidative stress of NaOCl on microorganisms. This stress stimulates the microorganisms' response for production of more signal molecules which may be the major reason for the increase in soluble EPS concentration in CEB phase (Cai and Liu, 2016). The main reason for decrease in EPS for QQ+CEB-MBR was due to dominance of QQ bacteria mitigating the effect of increasing signal molecules due to chlorination. As EPS stimulation factor by CEB was not present in QQ+PBW-MBR, lowest soluble EPS concentration was observed. Another reason of high soluble EPS under CEB condition may be attributed to the fact that CEB tends to cause cell lysis resulting in bio-floc disruption and ultimately higher soluble EPS in bulk solution (Barker and Stuckey, 1999).

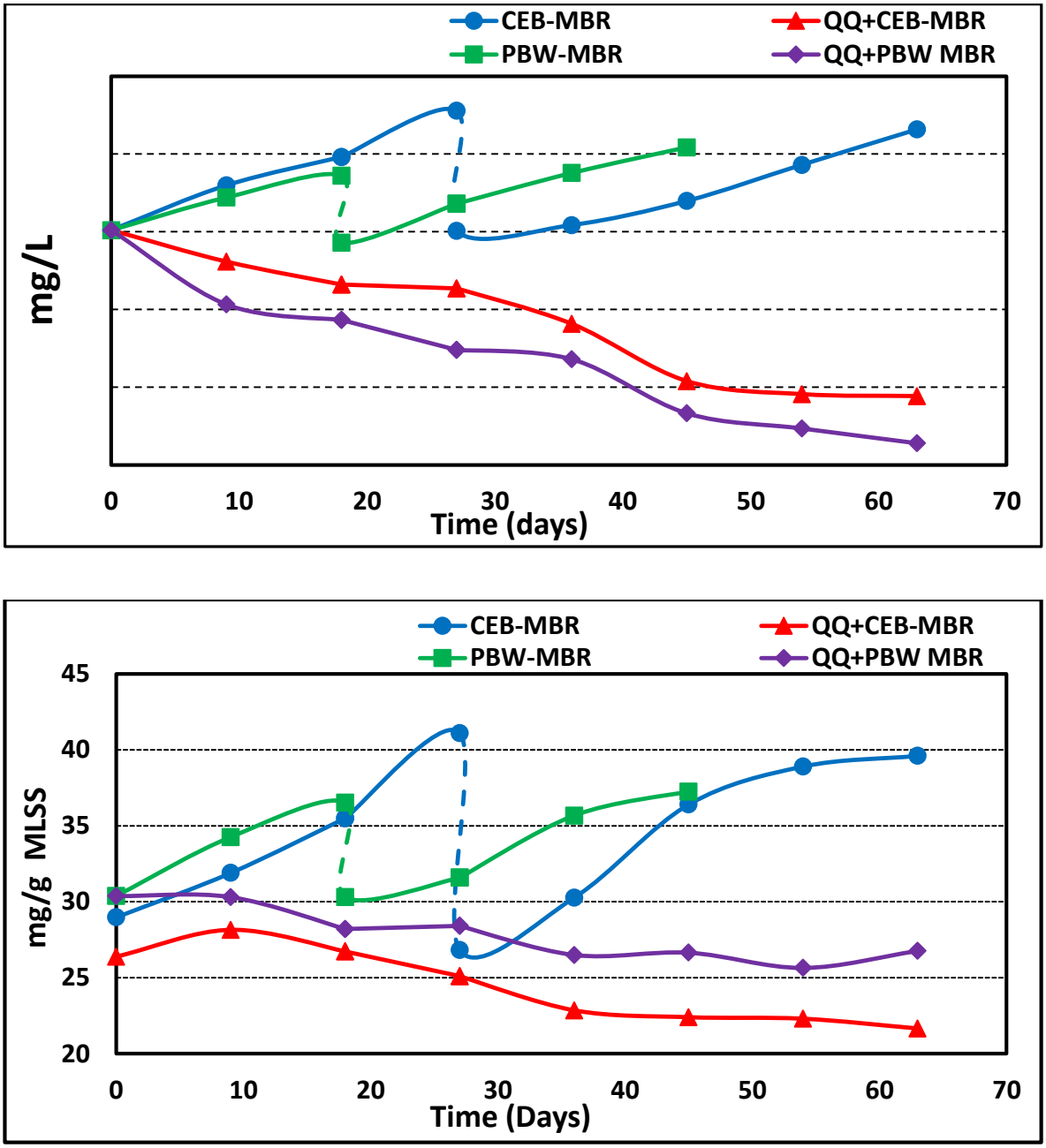


Figure 4.9 (a): Soluble and (b): Bound EPS of sludge of MBRs used in study

Moreover, bound EPS increased with time for PBW-MBR as well as for CEB-MBR. But important aspect to note is that the bound EPS concentration in CEB-MBR was less than that of PBW-MBR (Figure 4.9 b) may be due to de-flocculation i.e., when soluble EPS tends to increase, the bound EPS starts to decrease (Lin et al., 2014). It may also be

observed that as bound EPS was less in CEB-MBR, its value further reduced in QQ+CEB-MBR as QQ was able to degrade most of the remaining bound EPS.

Hence it may be concluded that CEB helps in preventing the membrane pore blockage and to some extent the biofilm formation while QQ mechanism significantly retards formation of bio-cake on the surface of membrane via reduction of soluble and bound EPS. However, in the presence of QQ mechanism, CEB may be replaced with PBW while maintaining effective fouling reduction and prolonged filtration duration. This combination without chlorination may be ever effective in sustaining the microbial community, sludge concentration as well as sludge morphology.

4.5 Extra-cellular Polymeric Substances in Biocake layer

Amount of EPS present in cake layer was measured in terms of proteins and polysaccharides. It may be seen from Figure 4.10 that protein concentrations for all the samples were found greater than polysaccharides. This phenomena of higher protein concentrations in EPS of biocake are observed in many other studies (Weerasekara et al., 2016) A high concentration of EPS was observed in both the reactors without QQ i.e PBW-MBR and CEB-MBR as compared to reactors having QQ bacteria i.e QQ+PBW-MBR and QQ+CEB-MBR. This shows that QQ is mitigating the production of EPS in biocake due to degradation of AHLs present in the biocake. The Rhodococcus BH4 species which is serving as QQ species in this study generates an enzyme name lactonase that has the capacity to degrade AHL thus reducing the EPS production, hindering the ability of microorganisms to communicate with each other and forming biofouling layer on surface of membrane (Weerasekara et al., 2014). It was also observed that mitigation of EPS by QQ was observed both in the presence and absence of chemical enhanced backwashing. Thus it may be deduced that NaOCl injection had no negative effect on efficiency of QQ

(Weerasekara et al., 2016). However there was a slight difference in EPS of both QQ+CEB-MBR and QQ+PBW-MBR which may be attributed to the fact explained in previous section that NaOCl injection cause more production of auto inducers thus more EPS production that's why there is a difference between EPS of both the biocake layers as explained in a previous research (Cai and Liu, 2016).This may also explain the fact why the EPS concentration was highest in CEB-MBR i.e. due to increased production of auto inducers and due to absence of QQ bacteria.

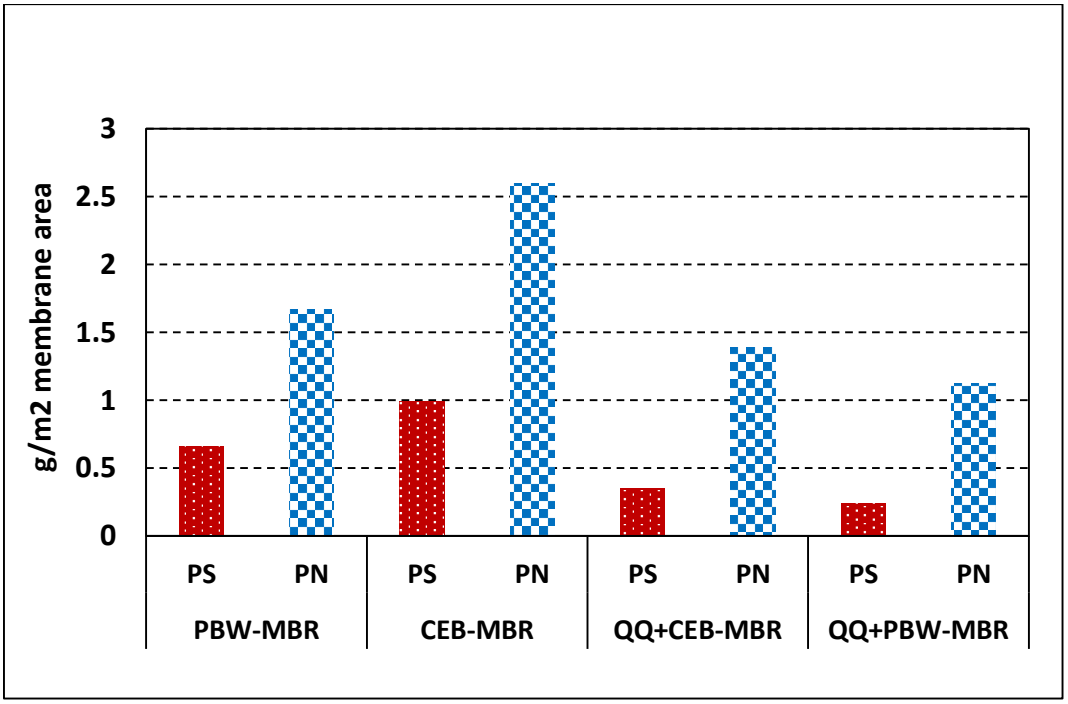


Figure 4.10: EPS present in biocake layer of each MBR

4.6 Total Biomass on Membrane

Biomass accumulated on each membrane was removed and measured after the membrane reached its fouling limit of 30 kPa.

It was observed that a denser biofouling layer was developed on surface of membrane present in CEB-MBR while a comparatively lesser biofouling layer was developed on

surface of membrane which was present in QQ+CEB-MBR as shown in a previous study (Weerasekara et al., 2016). The denser layer explains more production of EPS in CEB-MBR as compared to QQ+CEB-MBR shown in Figure 4.11. The formation of biocake layer despite presence of QQ in QQ+CEB-MBR shows that biofouling is inevitable.

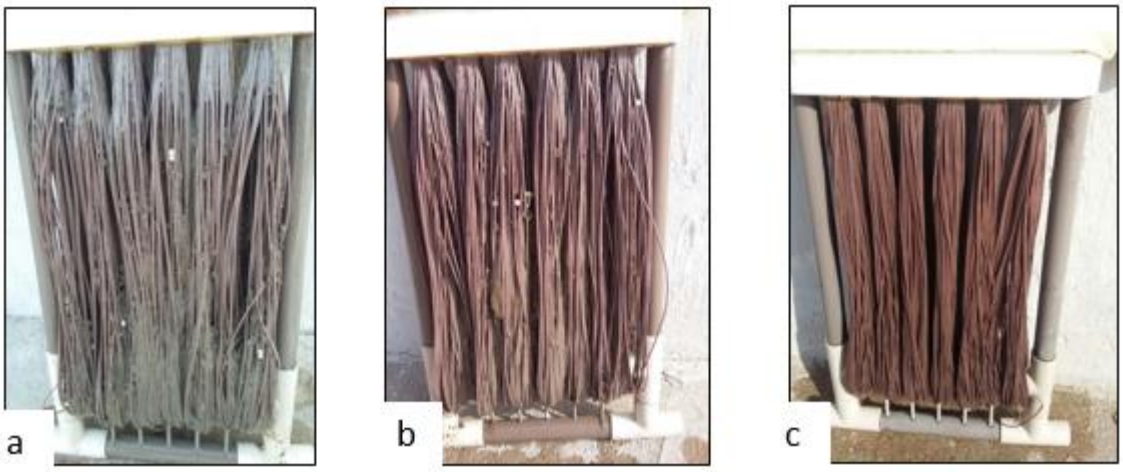


Figure 4.11: (a) Membrane after fouling of CEB-MBR (b) Membrane after fouling of QQ+CEB-MBR (c) Membrane after cleaning

Figure 4.12 shows the total amount of biomass accumulated on each membrane surface after reaching its fouling limit. The amounts of biomasses present on membrane surfaces of MBRs having Quorum Quenching were less than MBRs which were not having quorum quenching mechanism which means QQ is hindering the formation of biofilm in MBRS. Highest amount of biomass was found out to be present in CEB-MBR because of longer duration of runs as compared to PBW-MBR, and because of absence of QQ bacteria as compared to QQ+CEB-MBR. Although the run for QQ+CEB-MBR was of longer duration than QQ+PBW-MBR but the amount of biocake layer was larger in former than latter which explains more production of EPS due to more generation of autoinducers molecules as explained earlier in section 4.4.

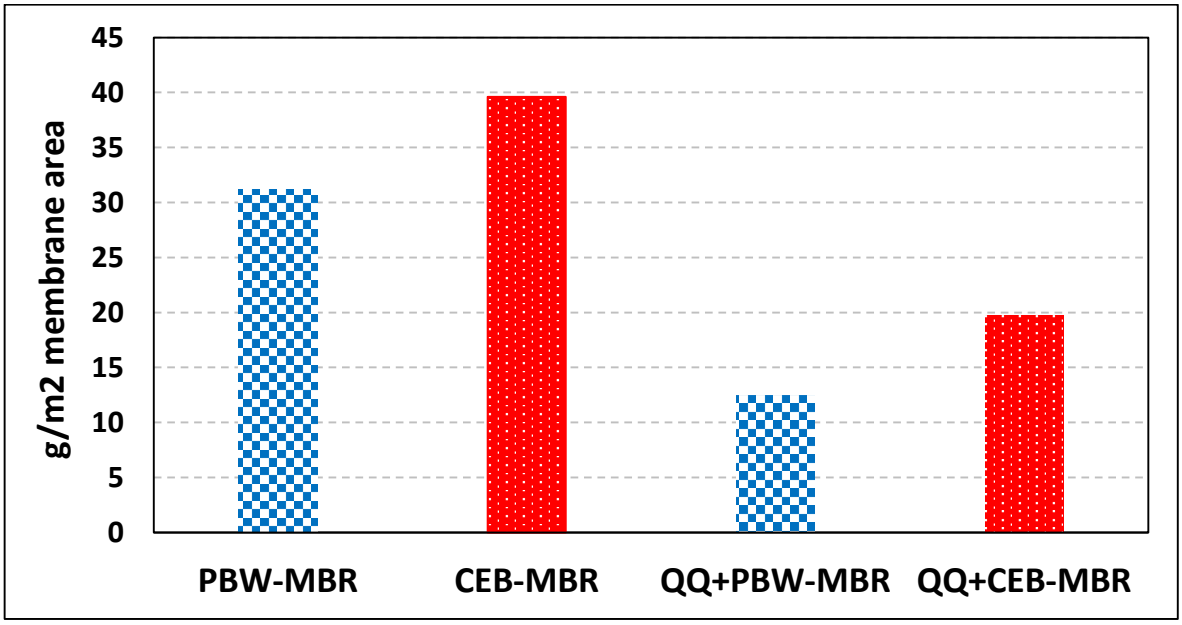


Figure 4.12: Total biomass on membrane accumulated after fouling

4.7 Membrane Resistance Analysis

Resistance Analysis was performed for each membrane as per method described in Chapter 3. Intrinsic membrane resistance was calculated before the start of run with deionized water whereas pore blockage and cake layer resistance were calculated after completion of each run for each MBR. The resistances obtained for each MBR are discussed in Table 4.1. It was observed that least resistance was offered due to pore blockage in case of CEB-MBR because of oxidizing effect of chlorine during backflushing. However it is clearly visible that total resistance in case of CEB-MBR was not reduced as compared to PBW-MBR because cake layer resistance rose to a higher value as compared to PBW-MBR because of higher EPS production and hence thicker biocake layer was observed in case of CEB-MBR. However least resistance was offered to QQ+CEB-MBR because cake layer formation was also hindered due to QQ activity in MBR and thus both types of resistances were low as compared to other MBRs.

Table 4.1: Resistance analysis of MBRs used in study

Resistance * 10^{11} m^{-1}	**PBW-MBR**	**QQ+PBW-MBR**	**CEB-MBR**	**QQ+CEB-MBR**
Intrinsic Membrane Resistance (R_m)	2.38	2.52	2.23	2.34
Total Resistance (R_t)	58.08	32.89	57.34	26.44
Cake Layer Resistance (R_c)	29.7	13.44	50.44	17.46
Pore blockage Resistance (R_p)	25.91	19.45	4.67	5.64
Rc/Rt (%)	51.13	40.86	87.96	66.03
Rp/Rt (%)	44.59	59.13	8.14	21.33
Rm/Rt (%)	4.09	7.66	3.88	8.85

Chapter 5

Conclusions and Recommendations

5.1 Conclusions:

It can be inferred that a combination of quorum quenching (QQ) and chemical enhanced backwashing (CEB) mechanisms may be considered as the most effective technique in terms of biological and physico-chemical methods for biofouling control and prolong operational duration by controlling both cake layer formation and pore blockage which cannot be achieved by QQ or CEB independently. CEB approach caused a harmful impact on the sludge characteristics in terms of extra-cellular polymeric substance (EPS), capillary suction time (CST) and bio-particle size and reduced the removal efficiencies of organics and nutrients in the MBR. As an alternative, CEB may be replaced with PBW in combination with QQ mechanism to achieve considerable bio-fouling control in terms of reversible cake layer fouling as well as irreversible pore-blockage fouling to eliminate the negative affect of chemical usage (chlorination) on sludge characteristics and treatment performance.

5.2. Recommendations:

The chlorine dose used in this study for chemical enhanced backwashing was 3.5 mg/L. This concentration yields certain negative effects as shown in results and discussion section. So the chlorine dose must be monitored on different concentrations to find out the most optimum dose i.e. which may give us maximum fouling control without compromising the organics and nutrients removal efficiency as well as not degrading any sludge characteristics.

Different filtration modes should be tested to examine effect of change in filtration cycles to achieve optimum filtration cycle along with CEB and QQ for maximum biofouling

control. In this study effect of chlorination on removal efficiency as well as sludge characteristics was measured. The direct effect of chlorination on QQ beads, QQ bacterias, and their activity should also be analyzed through advanced equipment not available currently in Pakistan.

Analysis of effect of chlorination on degradation of signal molecules AHLs should also be performed to find out its efficiency as biofouling control strategy.

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