IMPACT OF NOVEL QUORUM QUENCHING STRAINS IN CONTROLLING MEMBRANE BIOFOULING IN MEMBRANE BIOREACTOR

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“Dedicated to my loving parents for their countless support in making a professional that I am today”

Acknowledgement

Alhamdulilah to the Creator Allah Almighty on whom I trusted everyday with perseverance and steadfastness,

I am humbled to have such caring parents who had given me unconditional love and support in my highs and lows throughout my life and in shaping the man and professional that I am today.

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List of Abbreviations

AHL	Acylhomoserine Lactone
AI	Auto-Inducer
AO	Anoxic-oxic
AOBs	Ammonium Oxidizing Bacteria
B-EPS	Bound EPS
BOD	Biological Oxygen Demand
CAS	Conventional Activated Sludge
CEB	Cell Entrapping Beads
CL	Cake Layer
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DSVI	Diluted Sludge Volume Index
EPS	Extracellular Polymeric Substances
F/M	Food Over Mass Ratio
HF	Hollow Fiber
HRT	Hydraulic Retention Time
IN	Inorganic Nitrogen
LB-EPS	Loosely Bound Extracellular Polymeric Substances
MBR	Membrane Bioreactor
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NH_4-N	Ammonium Nitrogen
NMP	N-Methyl-2-Pyrrolidone
OLR	Organic Loading Rate

PAOs	Phosphorous Accumulating Organisms
PB	Pore Blocking
PS	Polysulfone
PVDF	Polyvinylidene Fluoride
QS	Quorum Sensing
QQ	Quorum Quenching
QQMBR	Quorum Quenching MBR
RPM	Revolutions per minute
SMBR	Suspended growth or Submerged MBR
SMP	Soluble Microbial Products
SND	Simultaneous Nitrification & Denitrification
SP	Species
SRT	Solid Retention Time
SS	Suspended Solids
SVI	Sludge Volume Index
TMP	Trans-Membrane Pressure
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorous
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WWTPs	Wastewater Treatment Plants

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Abstract

Membrane bioreactor is a relatively modern treatment technology that not only treats wastewater but is also able to reclaim water that is of high quality. Furthermore, the technology has lower footprint than other conventional technologies and less sludge production. One of the most challenging aspects of this technology is biofouling where membrane filtration ability is compromised with continuously growing slime layer on membrane surface, known as biofouling. One way to tackle biofouling is by studying quorum sensing, that is basically the way microorganisms communicate and talk to each other using signal molecules such AHLs. These exist in various molecular sizes and contribute to a complex chain of bacteria communication. Counter to quorum sensing is quorum quenching where certain strains show ability to destroy these signal molecules and destroy the process of communication known as quorum sensing. These strains are encapsulated in beads into the activated sludge of MBR. These strains work on the signal molecules also known as auto inducers of quorum sensing bacteria. One of the widely studied quorum quenching strain is *Rhodococcus* BH-4 which has been marked as the potential candidate to destroy the mechanism of quorum sensing so far. However new strains have been identified of which the noteworthy have been *Pseudomonas Aeruginosa* and *Bacillus Cereus* who have shown the ability of quorum quenching as well as quorum sensing. These novel strains were encapsulated in PVDF alginate beads and their effect was studied individually in membrane bioreactor along with comparison with *Rhodococcus* BH-4 and vacant beads in conventional membrane bioreactor. Flux of 15 LMH, SRT of 20 days and HRT of about 4.16 hrs was maintained while keeping a mixed liquor concentration of 5-6 grams per litre in membrane bioreactor. It was found out that MBR containing *Pseudomonas Aeruginosa* beads was able to last 13 days in compare to 8 days of *Bacillus Cereus*. Both were able to do significantly well in comparison to conventional MBR and vacant beads run which lasted 5 to 6 days respectively. However, the best strains were still found to be *Rhodococcus* BH-4 where membrane lasted 15 days. Product water quality in quorum quenching beads run when compared side by side with conventional MBR, BOD removal dropped from 96 to 90%, total phosphorous dropped from 80 to 67%. SMP and bound EPS concentration were also found to be lower in *Pseudomonas* and *Rhodococcus* BH-4 in comparison to conventional and vacant MBR. However SVI was high for both of these strains, reported to be 130 and 140 mL/g respectively in comparison to 60 and 80 mL/g of conventional and vacant MBR respectively.

Introduction Chapter 1

1.1 Background

Water is a basic ingredient for the survival for all forms of living beings. It is needed for the development of ecosystem. In recent times however, water scarcity has become an issue for the modern world. Despite being an agriculture country, Pakistan has been declared a water scarce country. It has average water availability less than 1000 meter cube per capita per year which poses an alarming situation (Asian Development Outlook 2013).

As of now, 22 million people in Pakistan don't have access to safe drinking water while 79 million don't have access to adequate sanitation in the country. Meanwhile, 19,500 children under the age of 5 die of diarrhoea annually which is alarming (WaterAid, 2019). The water scarcity issue has become a whole lot worse due to the rise in global population and also because of the limited resources of water. Due to rapid urbanization, a lot of water is required in a concentrated environment where water is not available in huge quantity. This is the part where the need for water recycling becomes an alternative as wastewater is considered now as a resource rather than a liability.

It is also expected that a lot of people are now shifting towards urban locations and cities for better standards of living. Further development of housing schemes and real estate will exert pressure on the available water supply sources. Water is very limited in itself and roughly about 70% of water is used for agriculture purposes. There is a serious competition for water resources where additional groundwater is pumped to meet the scarcity of water. This has caused a shortage in ground water which will soon be depleted even further.

Due to day to day life, wastewater is generated which generally goes untreated in the developing and underdeveloped countries. The wastewater generated goes on to contaminate

freshwater resources which can be deemed unfit for human consumption. This is largely due to lack of control and check on huge sums of wastewater generated from urban settlements.

There are various mechanized techniques used to treat wastewater such as activated sludge, aerated lagoons, sequencing batch reactors (Bolong et al., 2009). Conventional Activated Sludge (CAS) is one of the most popular methods to treat this liability and convert it into a resource. The process has the ability to remove as much as 95% of Chemical Oxygen Demand (COD) and 90% of Biological Oxygen Demand (Sheng et al., 2008). The process of conventional activated sludge can be divided into three parts, (1) the aeration tank where the activated biomass comes into interaction with the wastewater feed, (2) a clarifier where the solids can be separated from the liquids, (3) sludge recycling where a portion of activated biomass is recycled back to the aeration tank and the excessive biomass and sludge is wasted (Drews, 2010; Poostchi et al., 2012; Trussel et al., 2006).

The activated sludge has a few disadvantages which can be stated as, (1) it requires a larger footprint, (2) it requires a higher hydraulic retention time, (3) it can operate at a lower sludge retention time (SRT). For a conventional activated sludge design, the mixed liquor suspended solids (MLSS) has to have a concentration of 2000 to 4000 mg/l to provide an active biological treatment which can be separated from the water later in the clarifier (Wang et al., 2009).

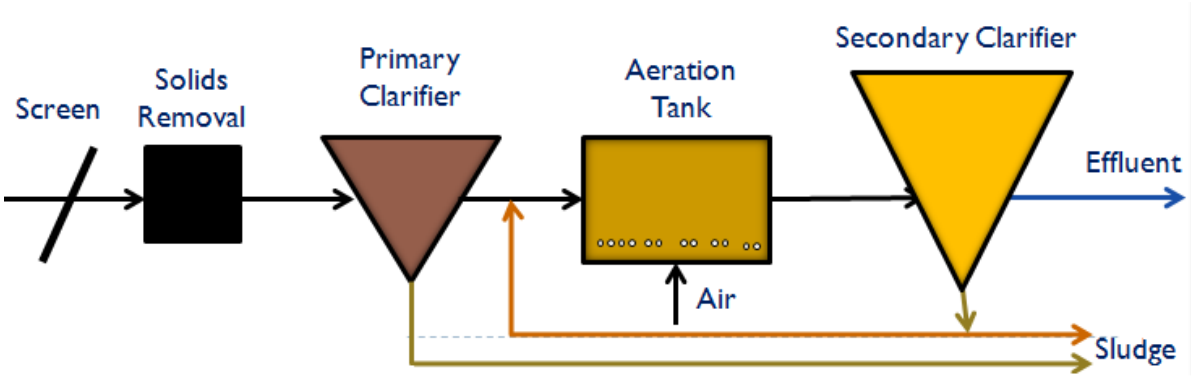

Figure 1.1: Conventional Wastewater Treatment

However, conventional activated sludge and some other biological treatment technologies are not able to treat persistent organic pollutants and they are able to find their way into lakes, rivers and oceans and even back to our drinking water supply (Moreira et al., 2017)

One of the modern activated sludge processes is the membrane bio reactors (MBR). It has a more compact design than the conventional activated sludge process since it involves a biological activated sludge and a separation of solids from liquid in the same compartment. The solid liquid separation is assisted with the help of micro and ultrafiltration membranes which has membrane pores ranging from 30 to 100 nanometres. One of the up sides of this technology is that it produces a very high quality effluent (Jahangir et al., 2012). The other advantages are that (1) it has a high mixed liquor concentration, (2) nutrients removal (3) small footprint, (4) high effluent quality (Cosenza et al., 2013; Masse et al., 2006; Wang et al., 2014).

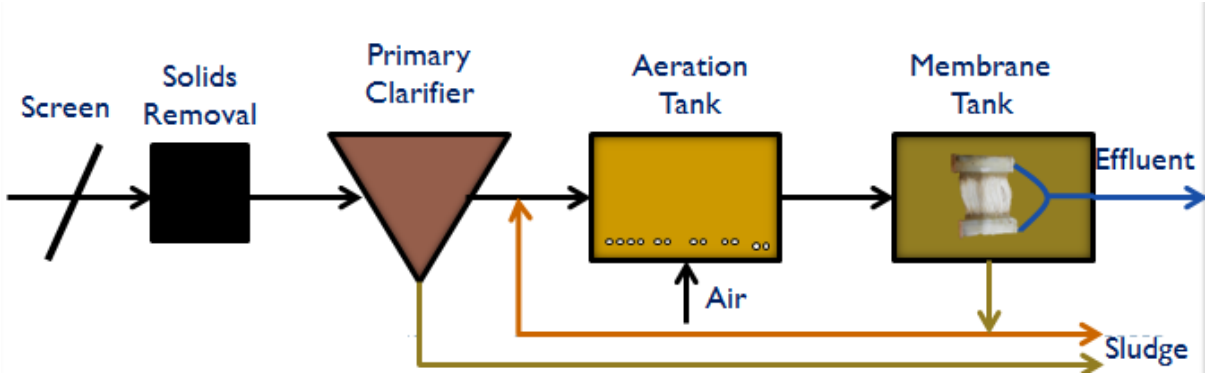

Figure 1.2: Membrane Bioreactors

Due to the compact design of the membrane, it has become an attractive option to treat wastewater. This allows the operation to modify the process with fluctuating wastewater characteristics and flow rate by changing the hydraulic retention time (HRT) and sludge retention time (SRT). Since there is a very high mixed liquor suspended solids (MLSS) concentration which can reach level of 8 to 12 g/l (Lin et al., 2014)

As much as attractive the membrane bioreactor process seems, it also has its downsides which makes it a tough alternative to go with, especially in the developing countries. There is a need of high energy to operate the activated sludge process in an aerobic manner. The other disadvantage is biofouling which occurs due to the small size of membrane and due to the high concentration of mixed liquor suspended solids (MLSS). Due to biofouling, the flux of the membrane bioreactor drops significantly and sooner the membranes have to be cleaned. By tackling these two issues and optimizing the operational control of the membrane bioreactors, its market value is expected to rise to produce fine effluent quality water which can be used for reuse application such as in agriculture, horticulture and even for municipal usage.

The biofouling which clogs the membrane of the MBR can be treated using physical as well as chemical means. The main reasons why the biofouling occurs are due to the formation of the cake layer compromising of soluble microbial products (SMP) and extra polymer

substance (EPS). SMP and EPS are the products which give a sticky nature to the cake layer where microbes bind to membrane surface. These products are usually carbohydrates and proteins which have been observed to be linked with biofouling while other products are also identified such as nucleic acid and lipids whose effect on the biofouling is not deemed significant (Drews, 2010). The cake layer which binds to the membrane and blocks the pores of the membrane which causes a decline in the permeability and the flux of the membrane.

Sludge Retention Time (SRT) is a critical parameter which reflects the time the sludge remains in the system. Prolonging the SRT causes endogenous decay of the sludge which causes it to release dissolve organic substances (DOM) that may also accelerate biofouling process. Optimizing the membrane bioreactor process parameter such as SRT, HRT and MLSS can effectively control the biofouling in membrane bioreactors (Miura et al., 2007).

The main point is the fact that the biocake formation is the main reason of the biofouling which can only be controlled beyond just physical and chemical cleaning means (Yeon et al., 2009). However, these solutions are expensive and a cost effective route needs to be taken.

There are bacteria which are able to communicate with each other using signal molecules. This process is called quorum sensing. These signal molecules produced by the bacteria are organic in nature and are similar in nature to that of Acyl Homoserine Lactones (AHLs). These signal molecules are able to function when they reach a certain concentration and are then able to activate the specific gene for a certain type of group behaviour such as virulence or antibiotic production. It is found out that it is the Extra Polymer Substance (EPS) which is responsible for the agglomeration of bioflocs causing the formation of cake layer on the membrane during the MBR operation. It was found out that the higher concentrations of AHLs are responsible for the formation of Extra polymer substance (EPS) which is needed to be taken care of in order to control the process of biofouling.

1.2 Problem Statement

Despite the numerous installations and increased interest in membrane bioreactors, the major problem of biofouling still persist which needs to be tackled to initiate worldwide acceptance of the MBR technology. Biological processes are a backbone for membrane bioreactor stability and operation and it needs to be addressed. However more research is required on the topic to truly understand the biological processes we are dealing with.

Quorum quenching research has been in interest in the past and only *Rhodococcus* sp. BH-4 strain has been studied extensively. In reality, a lot more strains also show signs of quorum quenching and they also deserve deeper study.

It takes a lot of complex forms of signal molecules from different species to generate a dense, thick cake layer on membrane surface area. A single species alone cannot be studied to completely understand the quorum sensing and quenching mechanisms. Since conditions in membrane bioreactor change from time to time, climate to climate and region to region, more species are needed to be identified to make the technology of membrane bioreactor more feasible for mass adoption.

1.3 Objectives of Study:

The objectives of the research are stated as:

- Establishment of lab-scale membrane bioreactor having a working volume of 5 litres while using a hollow fiber membrane of 0.1 m^2 with a pore size of 0.03 microns.
- Performance evaluation of *Pseudomonas Aeruginosa* and *Bacillus Cereus* in mitigating membrane biofouling.
- Treatment performance of the identified strains with *Rhodococcus* BH-4.

1.4 Scope of Study

The scope of the study will be focused on the performance of membrane bioreactor with a working volume of 5 litres. Membrane run time was studied along with effluent quality parameters. Following set of runs were conducted in the following order:

- Conventional MBR
- Vacant Beads MBR
- *Pseudomonas Aeruginosa* QQ-MBR
- *Bacillus Cereus* QQ-MBR
- *Rhodococcus* BH-4 QQ-MBR

For each run, 0.1 m^2 hollow fiber membrane was used with an operating flux of 15 LMH at an SRT of 20 days. The comparison of each run provided a deeper analysis on how the locally identified strains performed against the widely studied strain of *Rhodococcus* BH-4 in biofouling control to reduce the AHL.

Literature Review Chapter 2

2.1 Membrane Bioreactors (MBR)

Membrane Bioreactor came into light in 1980s and was of much interest. Before its inception, settlings tanks were used to settle the activated sludge which were huge in size and had a big footprint. Moreover, sludge produced in these CASP was in greater volume and costly strategies were taken to reduce its volume, stabilize and dispose.

With rapid urbanization throughout the globe, water resources tend to become scarce and land value become expense with spread of urban cities. There was a growing demand of membrane bioreactor to solve both of these challenging issues and became an interesting prospect for urban environments.

In the beginning, production costs were higher for membranes due to economies of scale and complexity of manufacturing. Membranes were subjected to high fluxes which were unsuitable and a result had shorter life spans.

Higher energy costs was another issue as membrane bioreactors were energy intensive process with need of aerators and pumps. A different approach was required to make the technology economically feasible for mass adoption.

It is a much modern technology to that of conventional activated sludge process and may achieve much higher effluent qualities (Diagne et al., 2012). It involves combination of activated biological process as well as solid liquid separation which may be achieved with the help of membranes. The wastewater is feed into reactor where the substrate is used by activated biomass comprising of microbes. These microorganisms have the capability to use these organics and nutrients for cell growth, metabolism as well as maintenance. The biologically treated effluent is drain out while active biomass stays within reactor. The

activated sludge is continuously aerated to keep the sludge aerobic as well as dislodge particle deposition on the membranes (Le-Clech et al., 2006).

A comparison may be drawn between MBR and other wastewater treatment technologies:

Table 2.1 Wastewater Treatment Technologies

Technology	Process
Soak Pits	Sewage coming from septic tanks, is sent to dry or infiltrate into pits
Aerated Lagoons	Requires aeration to treat wastewater in ponds with some similarities with waste stabilization ponds
Oxidation Ditch	Similar to CASP without primary clarifier or extended aeration
Wetlands	Sewage is channelled through ponds where it is retained for days
Rotating Biological Reactors	A set of rotating circular drums where biofilm grows and treats wastewater
Trickling Filters	A collection of media having biofilm that is contained circular chamber where sewage infiltrates through and is treated
Upflow Anaerobic Sludge Blanket (UASB)	Pollutants absorbed on blanket of bacteria under anaerobic process
Waste Stabilization Ponds	Large ponds where sewage is subjected to decay, dependent highly on temperature

2.2 Types of MBR

The membrane bioreactor may be configured in two ways to perform the operation. They may be stated as:

- Side stream MBR (SS-MBR)
- Submerged MBR (SMBR)

The difference in the two configurations is that in the side stream MBR, the membrane is placed outside of the reactor while the activated biomass is circulated throughout the reactor. The side stream MBR requires higher energy since filtration is cross flow which demands high velocity.

In comparison, the submerged MBR is much easy to control and regulate. It does not require such high energy since the membrane is submerged. It does require high shear stress which is supplied by intense aeration in order to control biofouling (Howell et al., 2004). A general comparison of two technologies is presented in **Table 2.2**.

Table 2.2: Comparison of Submerged vs Side Stream MBR

Items	Units	Submerged MBR	Side stream MBR
Configuration	-	Hollow Fibre (HF) Flat Sheet (FS)	Tubular (TB) Plate & Frame (PF)
Operational Mode	-	Submerged	Crossflow
Operational Pressure	KPa	5-30	30-600
Permeability	LMH/KPa	0.5-5.0	0.07-0.3
Flux	LMH	15-35	50-100
Membrane Cost	$/m2	<50	>1000
Superficial Velocity	m/s	0.2-0.3	2-6

Operating Cost	-	Low	High
Capital Cost	-	Low	High
Cleaning	-	Easy	Difficult
Odour Control	-	High	Low
Share in Market	%	99	1

In terms of operation, membrane bioreactors can be classed as:

- Aerobic MBR
- Anaerobic MBR

The difference between the two processes is the electron acceptor in the end of the redox reaction happening at the molecular level. In aerobic MBR, air is supplied continuously or intermittently to keep a fair concentration of dissolved oxygen. The air is supplied from the base of the reactor to keep an even dissolved oxygen concentration throughout the reactor. The advantage of using coarse bubble aeration over fine is that it prevents the formation of biofilm layer since the air scours up the biofilm formation. The main difference in the cost between both types is due to the cost of aeration provided in the aerobic MBR. However, the plus side of the aerobic MBR is that it has rapidly growing bacteria in comparison to the slow growing bacteria in the anaerobic MBR which will require a higher hydraulic retention time.

The comparison of both of these types of operations is presented in **Table 2.3**:

Table 2.3 Comparison of Anaerobic vs Aerobic MBR (Yeon et al., 2009)

Parameter	**Anaerobic MBR**	**Aerobic MBR**
Energy Required	Low	High
Removal Efficiency (%)	60-90	<95

Stability	Low	High
Production of Sludge	Low	High
Production of Biogas	Yes	No
Alkalinity	High	Low
Removal of Nutrients	Low	High

However, it was studied that aeration rate, position and time has a direct link to mitigate biofouling in membrane bioreactors (Fu et al., 2012).

2.3 Membrane Filtration:

The membrane is material which is used to separate liquid fraction from solid fraction in membrane bioreactor. When effluent passes through fine pores of membrane, almost the entire solid fraction is removed and only water and fraction of dissolved substances is passed through membrane to produce a high quality of the effluent. The performance of membranes depend upon productivity and selectivity of the membrane. The productivity relates to amount of water flux of bioreactor and selectivity relates to separation rate.

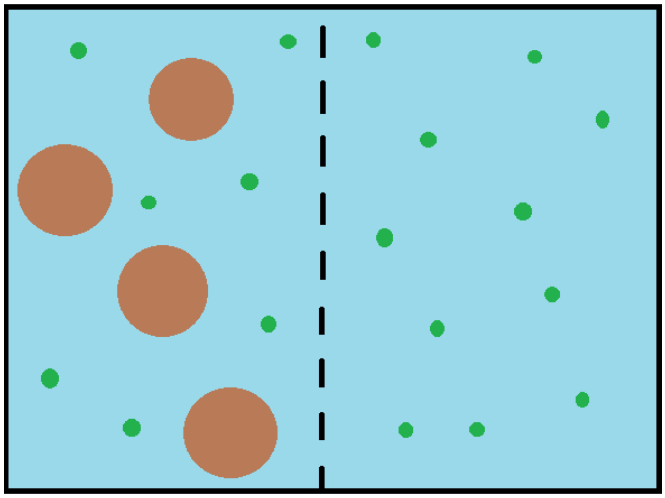

Figure 2.1: Membrane Filtration

The membranes may be further divided on the size of their pores and the substances it may separate as depicted in **Table 2.4**:

Table 2.4 Membrane Pore Sizes

Type of Membrane	Pore Size (Nanometres)
Microfiltration (MF)	100-1000
Ultrafiltration (UF)	5-100
Nanofiltration (NF)	1-5
Reverse Osmosis (RO)	0.1-1
Electrodialysis (ED)	< 0.1

1) **Microfiltration:** The size of membrane in microfiltration ranges from 0.1 to 10 micrometers. This pore size is fine enough to remove suspended particulates from water. It also removes all form of bacterial species, however viruses are not removed in process. This filtration is more used as a pre-treatment technology for further treating water through nanofiltration and reverse osmosis technology.
2) **Ultrafiltration:** This is advanced form of filtration to that of microfiltration. This mode effectively removes all forms of viruses as well with pore size of 1 nanometres to 100 nanometres.
3) **Nanofiltration:** The pore size of membrane in this technology is smaller than 1 nm. At this small pore size, effluent water is effectively softened, decolorized and removes all forms of pollutant from water.
4) **Reverse Osmosis:** It involves use of semi permeable membrane. On simple conditions, water moves from high concentration of water to low concentration. This difference in concentration is known as osmotic potential and it occurs till the concentration on both sides becomes same. The phenomena of reverse osmosis

involves use of energy to move water from a low concentration of water to a high concentration. This typically removes all forms of salts which are retained on membrane and effluent has a clear quality of water passing through it.

5) **Electro dialysis:** It involves use electrodes to separate cations and anions present to produce very high quality treated water, having almost zero dissolved solids.

2.4 Membrane Configuration

The membrane configuration may be divided into two types:

- Cross flow filtration
- Dead end filtration

`1) Cross flow: It uses high cross velocity to maintain shear stress on membrane which may be applied to scour formation of biofilm on the membranes. It removes the formation of cake layer but does not eliminate completely.

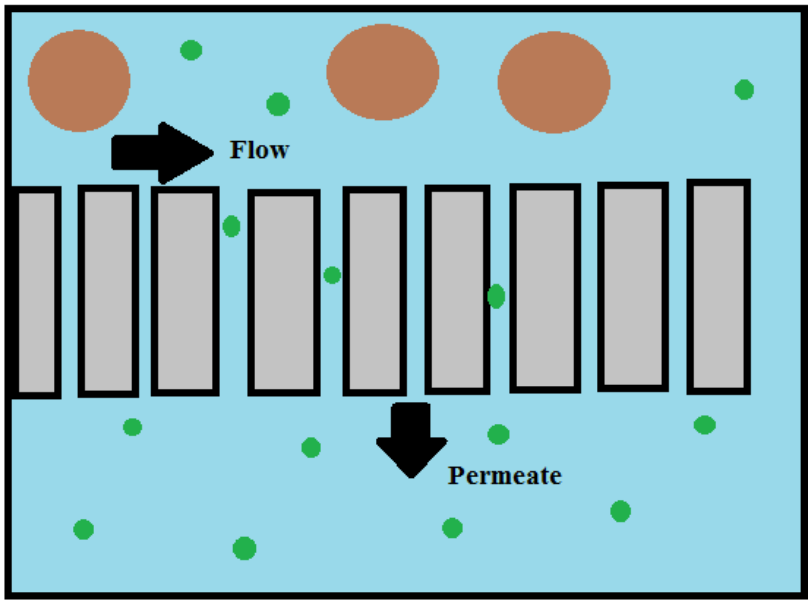

Figure 2.2: Cross Flow Filtration

2) Dead end Filtration: It involves the basic form of filtration where the raw water feed is applied from the top. The coarse particles start to accumulate on the membrane and clog the fine pores. The pressure on the membrane starts to increase with the increased resistance. It goes on until the water flux is reduced so much that it has to be cleaned to retain the same level of performance.

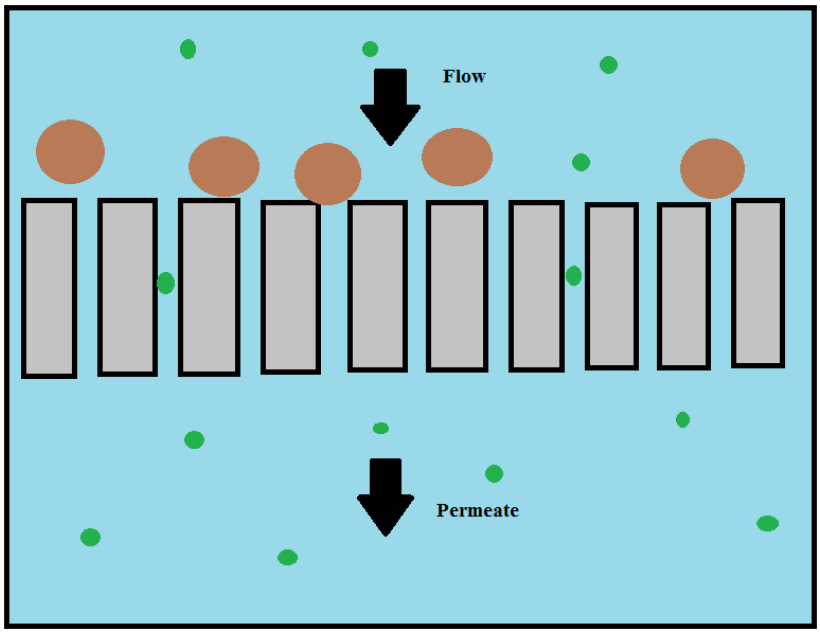

Figure 2.3: Dead End Filtration

2.5 Advantages of Membrane Bioreactor

There are several advantages of MBR over conventional activated sludge treatment process which can make it as an attractive option for biologically treating domestic as well as industrial wastewater biologically. It may be elaborated as:

- **Moderate Energy Requirement:** A membrane bioreactor does not have huge energy demands since it does not involve any kind of phase change as it is observed in the process like distillation process.
- **Large Surface Area:** The submerged membrane bioreactor has a large surface area to the volume of wastewater which as a result provides more sustainable filtration rates while providing high quality effluent water.

- **Treatment Performance:** The membrane bioreactors produce very high quality treated water since it involves a filtration process through a very fine membrane. Generally, about 95% of biological oxygen demand (BOD), 80% of nitrogen and 70% of phosphorous is removed (Maqbool et al., 2015)

  Since the pore size of the membrane is very small; about 0.04 micrometers which completely removes all forms of suspended solids as well as large particulates from the colloidal zone. The activated biomass stays in the reactor along with all forms of bacterial and microbial species which may alter the quality of treated water.
- **Long SRT:** Membrane bioreactor have a longer sludge retention time (SRT) as compared to conventional activated sludge process which results in a lower sludge production. The higher SRT also owes to remove nitrogen and other nutrients since it provides enough time for the growth of slow growing nitrifiers.
- **Simultaneous Nitrification and Denitrification:** Since there is a very long SRT given to the system inside the bioreactor, there is simultaneous nitrification and denitrification of the nitrogen species in the membrane bioreactor (Mustafa et al., 2016).

2.6 Limitations of Membrane Bioreactor

As much as attractive the membrane bioreactor looks, it also has many downsides which need to be taken into consideration while opting it for the treatment of wastewater:

- **Membrane Biofouling:** Due to the very high concentration of biomass which may go as much as 8,000 to 12,000 mg/l and due to a very small size of the membrane, there is a rapid clogging inside the membrane which can decrease the flux of the system and the whole filtration process is hampered as a whole (Amy, 2008),
- **Complexity of the System**: A membrane bioreactor is a much complicated process as compared to conventional activated sludge process. The feed of the wastewater must

not contain any sorts of harmful contaminants which can affect the biological process of the membrane bioreactor.

- **Cost of the membrane:** Though the low space requirement reduces the cost of the membrane bioreactor tremendously, there is still the cost of the membranes which needs to be taken into consideration as well as the availability of it which is not currently produced in many developing countries including Pakistan.
- **Lack of Research:** A membrane bioreactor technology is a modern form of conventional activated sludge process and it deserves more research work. With enough research and projects, issues such as biofouling can be taken care of with a smooth and optimized operation of the membrane bioreactor.
- **High Operational Cost:** A membrane bioreactor has a higher operational cost in comparison to the conventional activated sludge process for the same amount of flow rate. A membrane bioreactor requires a high amount of aeration as well as chemical cleaning of the membrane due to biofouling.
- **Pre-treatment Required:** A membrane bioreactor may be sensitive to the inlet characteristics of the wastewater which needs to be pretreated. Primary sedimentation followed by fine screen will be necessary prior to MBR to avoid clogging of the membranes.

2.7 Treatment of Domestic Wastewater

Membrane bioreactors are now accepted as one of the best wastewater treatment technologies to date. High effluent quality has led authorities to enforce more strict legislations for domestic and industrial effluents. MBR has the ability to remove almost all of the BOD, alongwith Nitrogen and Phosphorous. It has been more widely accepted in countries where land availability is extremely scarce, such as Singapore where water resources are also limited.

2.8 MBR Operation

Aeration is one of the most vital components of MBR process. Sludge production is also less in MBR due to high Sludge Retention Time (SRT) where microorganisms go under endogenous decay. Besides availability of dissolved oxygen (DO) present in the wastewater, aeration is also responsible for controlling biofouling in membrane bioreactor.

Aeration is used in various stages of MBR processes. Primarily, it is used in the biotank where the heterotrophic microorganisms are originally grown. The heterotrophic microorganisms which go on to degrade the soluble organics require a dissolved oxygen concentration of 2 to 4 mg/l for its effective growth.

Besides the biotank, aeration is also used in controlling biofouling as it causes turbulence in the liquid flow due to the aeration bubbles. This aeration is responsible for driving the membrane foulants away from the membrane surface so to mitigate biofouling on membrane surfaces (Cui et al., 2003). Beside the aeration bubbles, the aeration itself causes movement of the membrane in the lateral direction to the flow of the aeration which lessens the chances of the formation of cake layer on the membrane.

There are two types of aeration modes; coarse bubble and fine bubble aeration. The coarse bubble aeration is inferior in providing effective oxygen transfer efficiency to the mixed liquor suspended solids (MLSS) but manages to keep the dense medium in suspension throughout the MBR process. On the other hand, the fine bubble aeration is not effective in keeping the mixed liquor suspended solids (MLSS) in suspension but is superior in oxygen transfer efficiency and to provide the heterotrophic microorganisms the oxygen it needs to grow and multiply.

Aeration is responsible for 50-80% of the energy expenses in the membrane bioreactor process which needs to be regulated in order to optimize the membrane bioreactor processes

(Gil et al., 2010). Hence it is critically important to develop other methods as well which may allow to optimize aeration rates without compromising the ability to control biofouling in membrane bioreactor processes.

2.9 TMP Increase

It is the pressure which is the driving force for the process of filtration in the MBR. The trans-membrane pressure can be simply related as the difference in the pressure inside and outside of the membrane. As the cake layer starts to form on the membrane, the trans-membrane pressure increases due to the added resistance. The TMP can also be able to predict the flux of the MBR since flux relates to the amount of filtrate passing through per unit time which is dependent of the driving force created by the trans-membrane pressure.

$$Rt = {\Delta P}/{(\mu . J . ft)}$$

Where:

J = Flux, $L/m^2.hr$

ΔP = Trans-membrane Pressure (TMP), KPa

μ = Viscosity of the Permeate

Rt = Total resistance (1/m)

ft = Temperature Correction Factor

2.10 Membrane Fouling in MBR

Fouling may be divided into three stages as:

2.10.1 Conditioning Fouling

Conditioning fouling occurs in the early stages of membrane run. It is caused by organic and colloidal particles that cause irreversible fouling, even reported at no flux at all (Ognier et al.,

2002). Deposition of these substances may affect the surface chemistry and pore distribution size of the membrane. By the end of this stage, cake layer starts to develop on the surface of the membrane.

2.10.2 Steady Fouling

This is considered the longest stage of the membrane run. It proceeds after conditioning fouling has taken place. Microbes tend to temporarily bind to the surface of the membrane and release EPS and SMP. With time, increase concentration of EPS and SMP promote more biomass attachment and TMP continues to increase.

2.10.3 TMP Jump

The last part occurs where transmembrane pressure increase rapidly from about 15 to 20 kPa to 30 kPa in a short time. Fouling rate increases rapidly and majority of the membrane pores are blocked by thick layer of cake.

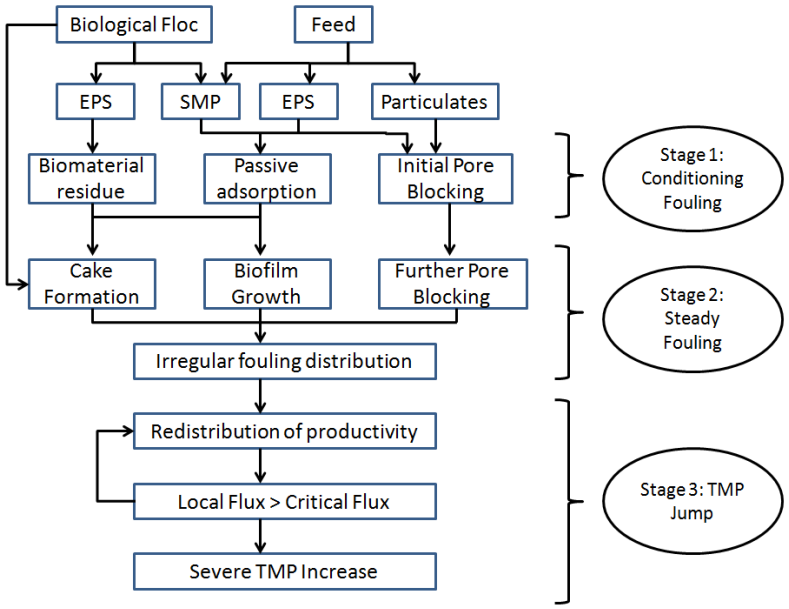

Figure 2.4: Fouling Stages

2.11 Membrane Fouling:

Membrane fouling is the phenomena that is not helping the prospects of membrane bioreactor to achieve large scale adoption. Membrane fouling is linked to the clogging of pores as well

as growth of cake layer on the membrane surface that hampers the permeability of the membrane (Lee et al., 2001). It is also an undesirable attachment of flocs on the membrane surface.

Internal fouling tends to occur because of deposition as well as adsorption of fine particles and solutes in the inner parts of membrane. This causes pore clogging where pores of membrane are narrowed. External fouling is a result of deposition of particles, macromolecules and colloidal particles on the membrane surface. Normally, external fouling can be divided into two parts; cake layer which is caused by solids retained on the membrane surface and a gel layer which is produced by deposition of inorganic solutes, macromolecules (soluble) and colloids (Van Den Brink et al. 2013). External fouling may be as a result of biological, organic or inorganic substances deposition on membrane surfaces and inside the pores (Wang et al. 2014).

2.11.1 Organic Fouling

Most immediate form of fouling is a result of organic fouling where size, shape of molecule and chemical characteristics will affect membrane fouling.

Initially in membrane bioreactors, deposition of amino sugar, proteins, humic acid, polysaccharides and other organic substances which come from the wastewater feed will result in fouling of membrane. Organic fouling is generally considered irreversible and needs chemical treatment.

2.11.1 Inorganic Fouling

Scaling of membranes are a result of inorganic complexes and crystals which deposit on surface of membranes (Costa et al., 2006; Meng et al. 2009). In number of studies, there are metal cation which interact with organic substances which have a functional group to produce chelating polymers (inorganic-organic complexes) (Myat et al. 2014).

Also in cases, there are some inorganic particulates which tend to deposit on membrane surface and results in form of inorganic fouling (Zhang et al., 2012). There are also scenarios where organic substances may interact with inorganic ions to form crosslinking structure which results in a more dense cake layer and speed up fouling (Choo et al. 2008).

2.11.2 Membrane Biofoulng

One of the most challenging aspect of the membrane bioreactor technology is biofouling. It is basically the clogging of the fine pores of the membrane due to the high biomass concentration and the small pore size of the membrane. A cake layer develops on the surface by colonization of microorganisms on membrane surface. Microorganisms release organic matters such as soluble microbial products (SMP) and extracellular polymeric substances (EPS). This results in a decrease in the flux of water and a loss in permeability. It was also reported that characteristics of the wastewater and the parameters of operation such as flux have a distinct impact on the biofouling in the membrane; the sludge retention time may enhance the biofouling characteristics as well. As biofouling increases, permeation rate i.e., flux decreases (Lee et al., 2001)

Biofouling tends to remain the most critical component of fouling. Even if entire cake layer is removed, there are always some cells that remain and they recolonize back to original cake layer as long as organic substances are present in the wastewater feed. Biofouling has various impacts on membrane performance (Murphy et al., 2001), including:

- Decrease in membrane flux due to formation of low permeable biofilm growth on membrane surface.
- Increase in membrane filtration resistance caused by biofilm formation.
- Membrane degradation accelerates due to acidic by-products of biofouling.
- Degradation in quality of produced water due to accumulation of dissolved ions on surface and inside pores of membranes

- Increase in membrane TMP also results in higher energy and chemical demands which hamper the economic feasibility of membrane bioreactors.

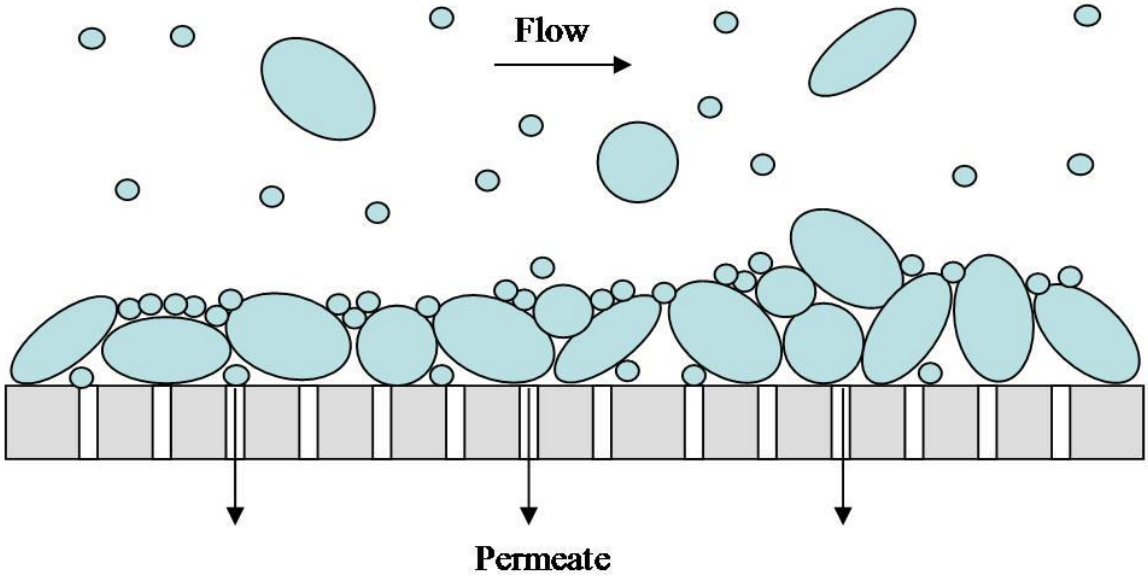

Figure 2.5: Biofouling Mechanism

There are two types of fouling in the membrane bioreactor.

- Physically Reversible Fouling
- Physically Irreversible Fouling

The production of cake layer can decrease the flux by clogging internally as this can easily be removed with physical cleaning and is described as reversible fouling while chemicals are used for treatment of precipitated compounds which completely blocks the pores and is expressed as irreversible fouling (Chang et al., 2002).

The physically reversible fouling may be mitigated with help of surface cleaning or backwashing. The same treatment cannot be applied for the later one which has to be treated with the help of chemical treatment. It is recommended to clean the fouling using physical means as much as possible until chemical cleaning measures are necessary. The chemical

cleaning mechanism takes a toll on the membrane and can significantly lower the life of the membrane.

The components which are responsible for biofouling are soluble microbial products (Kimura et al., 2009). These soluble microbial products depend upon factors such as biomass concentration, organic loading rate and solids retention time.

Furthermore, the fouling mechanism can also be affected by temperature. It was reported that a larger number of polysaccharides were produced which increased the rate of fouling at a lower temperature (Rosenberger & Kraume, 2003).

There is also the presence of extra polymer substances (EPS) which is responsible for the agglomeration of flocs of microorganisms (Jang and Kim, 2006). A relation was developed between the EPS concentration and the sludge cake resistance (SCR) (Choo et al., 2008).

2.12 Factors affecting Membrane Biofouling

2.12.1 Membrane Characteristics and Module Design

The material that makes up the membrane has long been studied to be the sources of biofouling. The hydrodynamic conditions and performance of membrane bioreactors is seriously affected by the module design. The way the fibers are packed, the porosity, density, roughness of membranes, the specific locations where aerators (diffusers) are placed are important considerations.

2.12.2 Feed water Characteristics

Feed water does not impact biofouling directly but it does change the characteristics of sludge on which is fed upon.

- A high strength feedwater enhances the growth of biomass, increase in soluble microbial products (SMP) and turbidity.

- Increase in food to mass (F/M) ratio or also known as organic loading rate (OLR) lowers the sludge filterability by causing the growth SMP and bound EPS as well (Meng et al., 2009)
- High salinity also impacts the properties of activated sludge by increasing the sludge EPS and SMP concentration that as a result impact the membrane permeability (Reid et al., 2006)

2.12.3 Hydrodynamic Conditions

Hydrodynamics conditions, shear stress, flux and air scouring on the membrane bioreactor performance.

The rate at which the air is scoured, the size of the bubble and the location of aerator dictates the hydrodynamic conditions in membrane bioreactor. This also translates to the amount of electricity or energy used. Air scouring has a profound impact in sustaining membrane bioreactor process. It not only supplies oxygen to activated sludge but also significantly reduces biofouling of membranes (Sofia et al., 2014).

The higher the membrane flux, the more MLSS move towards the membrane with the flow. Hence, hydrodynamic conditions are considered the best way to control biofouling by varying the module configuration, intensity of air and bubble size can profoundly impact the performance of membrane bioreactor and its biofouling rate.

2.12.4 Operating Conditions

Operating conditions can heavily impact the performance of membrane bioreactor in terms of biofouling.

High level of aeration or dissolved oxygen concentration can break the sludge flocs and so can reduce the production of EPS and SMP. Meanwhile low dissolved aeration can dictate to

larger molecular compounds and a dense biofilm which significantly impacts the filterability of the membrane.

A decrease in HRT can result in release of extrapolymer substances. Lower HRT can also translate in production of filamentous bacteria with irregular and large floc size. However, having a high HRT is not good as it will increase energy expenditures and foulant products in membrane bioreactor (Wang et al., 2009; Meng et al., 2007)

Sludge retention time (SRT) is another critical operating parameter of membrane bioreactor. It means the time a sludge cell remains in the activated sludge before it is wasted or taken out of the system. A very short SRT can hamper membrane life due to product of EPS and SMP due to high F/M ratio. Deposition of EPS on membrane is much higher in low SRT as compared to high SRT. However increasing the SRT may not only increase the amount of biomass production but it can also increase fouling rate due to increase in sludge viscosity and accumulation of foulants over a long SRT provided. Hence a SRT of 20 to 50 days is proposed which is calculated after taking into consideration of quality of feed water coming into the membrane bioreactor and the HRT of the system (Keskes et al., 2012; Al-Habouni et al., 2008).

2.12.5 Mixed Liquor Characteristic

Mixed liquor is basically the consortium of species in activated sludge. The higher the concentration of mixed liquor, the higher the carbohydrate and protein fractions there would be in SMP and EPS. Hence, the denser the cake layer would lead to higher biofouling. A higher MLSS can also lead to a decrease in size of mean particle size.

Sludge viscosity has a big impact on the membrane performance as well. A higher sludge viscosity leads to lower permeate flow which will result in high transmembrane pressure

(Liao et al., 2004). There also various other parameters like floc size, nutrient availability and dissolved organic matter that may have an impact on the biofouling of the membrane.

2.12.6 Bacterial Communication

Biofouling is a result of formation of cake layer on membrane surface where solids accumulate and microorganism colonize. It significantly impacts the membrane filterability, increase in the transmembrane pressure and reduce the membrane operational cycle. Not only does the microorganism itself contribute to biofouling and formation of cake layer, also their metabolites contribute significantly to fouling of membranes as well. In order to colonize and form biofilm, bacteria communicate to each other with phenomena known as quorum sensing where signal molecules or auto inducers are released (Xiong & Liu, 2010). Hence, quorum sensing has been directly correlated with membrane biofouling (Yeon et al., 2009).

2.13 BioFouling Control Strategies

The following strategies can be used for fouling mitigations.

- Pretreatment
- Physical/Chemical Treatment
- Patterned Membrane
- Backwashing
- Adding adsorbent and moving media
- Periodic Relaxation
- Air Scouring
- Use of quorum quenching strains

These are physical measures to control biofouling. However, they are not considered an effective strategy as the membrane run time is still limited and does not prolong membrane life (Deng et al., 2016).

2.14 Quorum Sensing

This is basically known as the communication between bacteria at the cellular level by the use of signal molecules known as auto inducers. Whenever these signal molecule concentration increases, the cells show combine behaviour together in unity such as production of antibodies or virulence.

There are many types of quorum sensing of which the most common mechanism is the AHL. It is known as Acyle homoserine lactones (AHL) which are more profound in gram negative bacteria. The AHL has about dozen types, however all of them have common features. They all have homoserine lactones ring which is associated with a fatty acid group and carbon atoms.

The phenomena of quorum sensing occur in the following steps:

- A signal molecule is produced with the help of a protein inside a cell
- The signal molecule start to accumulate in the environment
- A regulatory protein which is responsible for receiving the signal molecule.

These signals molecules help the microbes to interact with different species in the environment. Any species can be communicated who has the specific protein to accept the signal molecules. These molecules play a vital role in the formation of biofilm. These signal molecules are related with the production of EPS which significantly enhances the biofouling occurrence. Hence, these signal molecules are correlated with biofouling.

Quorums quenching are the new measures which can help to solve one of the toughest challenges of MBR by tackling with these signal molecules.

2.15 Quorum Quenching MBR

In a study, it was found that the AHL concentration was reduced by hydrolyzing the AHL molecules using the lactonase and the acylase enzyme. The lactonase was able to degrade the

lactone ring while the later enzyme was successfully able to reduce the acyl amide linkages of the molecule (Yeon et al., 2009). It was found that with concentration of AHL produced lower concentration of EPS which contributed to a delayed TMP rise. Oh et al., (2011) worked on isolating the bacteria responsible for quorum quenching. They were able to found four species of bacteria of which *Paenibacillus* and *Rhodococcus* stains were found out to be most effective. The stain of *Rhodococcus* was encapsulated in form of a microporous membrane which was submerged into a MBR running in parallel with conventional MBR. They reported to find out a significant difference in the rise of TMP with the quorum quenching bacteria which contained the stains of *Rhodococcus* bacteria. A study of microbial dynamics was performed using the quorum quenching bacteria and it was found that the quorum quenching had reduced the production of microbial species responsible for autoinducing; reduction in the production of EPS as a result which caused a delayed TMP (Kim et al., 2012). In another study, the *Rhodococcus* strain was entrapped using cell entrapping beads with the use of sodium alginate and it was found out to be the most effective technique in entrapping the strain of quorum quenching bacteria. In another study carried out by Cheong et al., (2014), *Pseudomonas* sp. 1A1 was inoculated inside a ceramic microbial vessel (CMV) which was later submerged inside a MBR tank and it was found that there was significant decrease in the concentration of AHL as compared to a control MBR which did not have the quorum quenching specie.

Pseudomonas species have reported to show inhibition in membrane biofouling as it has the tendency to produce AHL-acylase and reported to degrade a wide range of AHL (Won et al., 2012).

Bacillus Cereus also have reported to produce AI-2 and recognizes extracellular signal. AI-2 have reported to hamper biofilm formation with increase in its concentration.

In an unpublished work in NUST, strains were identified that show quorum quenching activity. *Pseudomonas Aeruginosa* and *Bacillus Cereus* were among those specie which have identified to produce Lactonase and acylase producing genes. These strains are yet to be tested in membrane bioreactor to show their performance to reduce membrane biofouling in membrane bioreactor (Parveen et al., 2018).

Waheed et al., (2017) also studied effects of quorum quenching and it delayed biofouling by a factor of 3. However when OLR was increased, biofouling retardation dropped to a factor of 1.4 to 1.8 only while EPS and SMP increased 4 times the normal.

2.16 EPS and SMP

Extracellular Polymer Substance (EPS) are the metabolite by-products of microorganisms. These are formed on the surfaces of the cells which allow them to bind with other cells (Liu et al., 2010). These metabolites are sticky in nature and have a positive link to fouling (Lesjean et al., 2004). These metabolites are made up of polysaccharides, proteins, amino acids, humic acid and sugars.

Soluble Microbial Product (SMP) are the solution fraction of EPS. However, major key interest remains in reduction of bound EPS which is more responsible for biofouling.

In initial stages, biofouling occurs when microbes colonize on the surface of the membranes and then followed by release of EPS which allow them to colonize together and grow (Maleab et al., 2013).

The key interest in membrane bioreactors remain in the mineralization of signal molecules which presence has been linked to increase in EPS production that flourishes biofouling (Rasmussen et al., 2005).

Methodology Chapter 3

3.1 Setup

A lab scale membrane bioreactor was used for research placed in IESE Environmental wastewater laboratory. The setup used level sensors to control the level of the tanks throughout the membrane runs and a peristaltic pump to control flux of membrane.

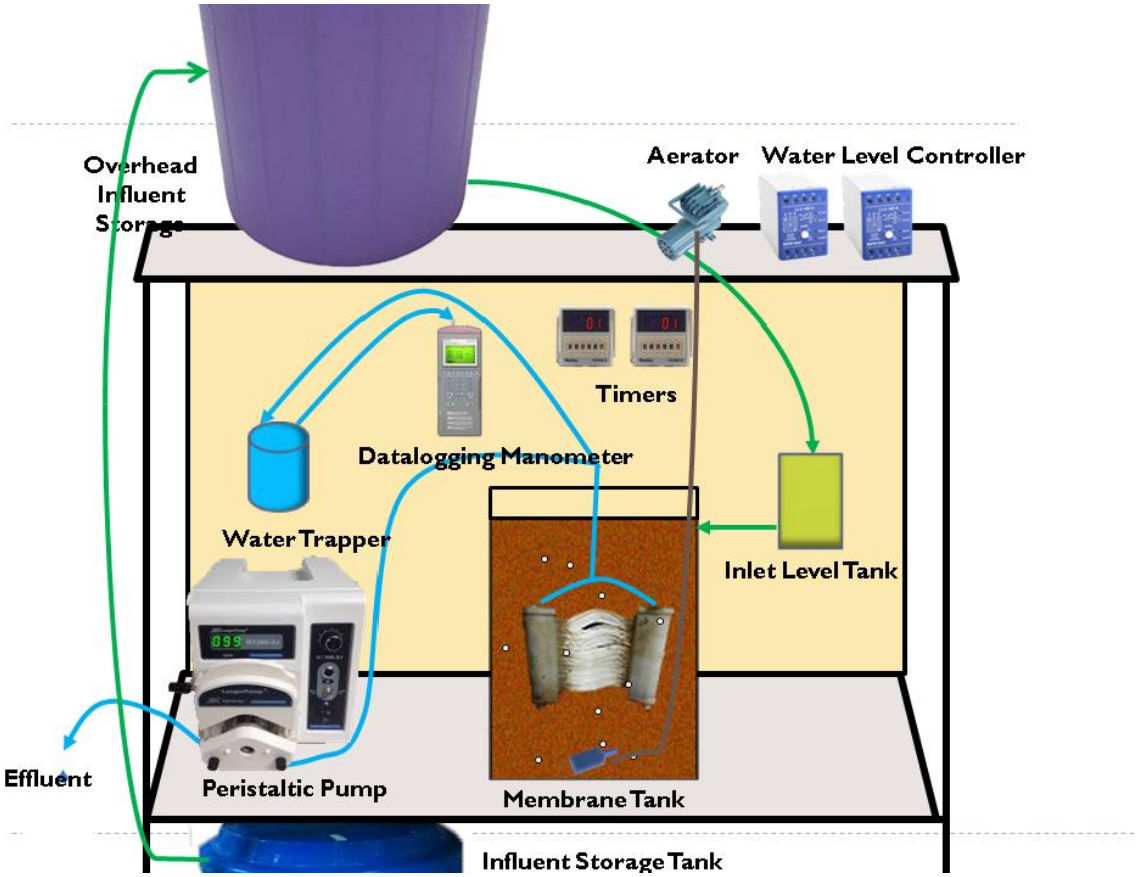

Figure 3.1: MBR Setup

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Figure 3.2: Membrane Bioreactor Setup

The tank volume of the setup was 6L while working volume was kept to 5L using the head of inlet chamber.

The sludge was collected from pilot scale membrane bioreactor at NUST and acclimatized in synthetic wastewater. The concentration of sludge was kept between 5 to 6 g/L while maintaining the sludge retention time (SRT) to 20 days. For 5 litre working volume, 250 ml of sludge was wasted daily.

The following parameters were kept constant during the study as listed:

Table 3.1: Operating Conditions

Parameters	Values	Units
Working Volume	5	L
Total Volume	6	L
HRT	4.16	Hours
SRT	20	Day
MLSS	5-6	g/L
Membrane Surface Area	0.1	m^2
Flux	15	L/m^2-h
Wastewater strength	500	mg/L
Membrane Filtration	8	Min
Membrane Relaxation	2	Min
Air Flow	20	L/min

With the help of timers, membrane filtration was kept at 8 minutes which was followed by 2 minutes of relaxation, this completes filtration cycle. Aeration was kept constant with the help of diffuser to allow coarse bubble aeration not only to maintain dissolved oxygen concentration but also keep the sludge in suspension. Aeration was adequate to avoid any formation of dead zones within tank while majority of aeration was focused on membrane for scouring.

Transmembrane pressure was measured using the Datalogging TMP meter (SPER SCIENTIFIC, 840099, USA). Peristaltic pump ensures constant flux and was kept throughout the run.

3.2 Membrane Specifications:

Hollow fibre membrane was used for the study. The schematics are as depicted on **Figure 3.3**

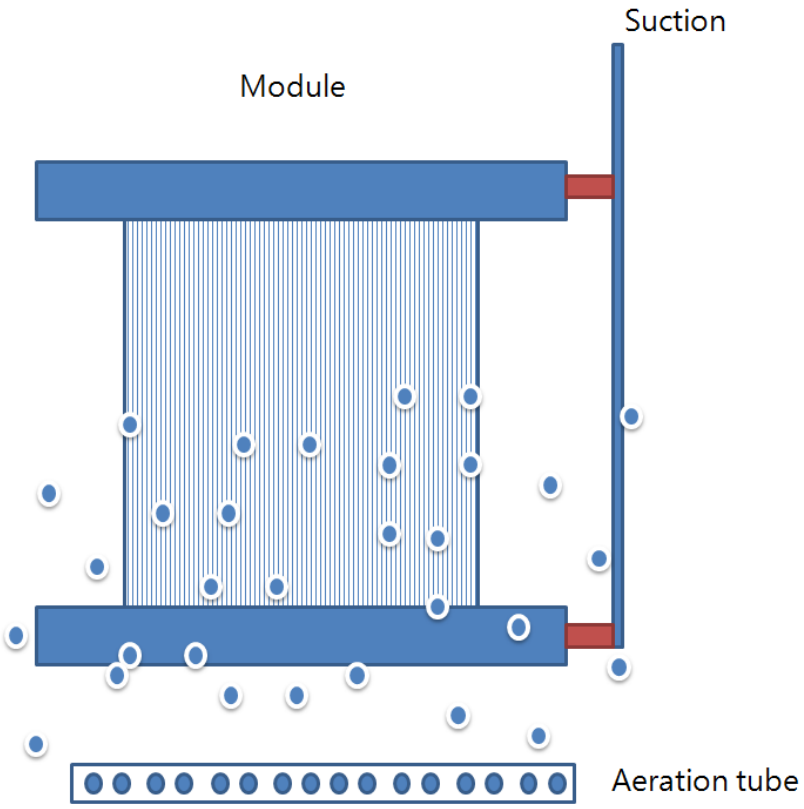

Figure 3.3: Membrane Schematics

The specification of the membrane is stated as presented in **Table 3.2:**

Table 3.2: Membrane Specifications

Product Description	
Membrane Chemistry	High Density Polyethylene
Membrane Type	Hollow Fiber
Membrane Pore Type	Slit pore and asymmetric structure
Membrane Pore Size	0.4 um
Membrane Outer/Inner Diameter	0.65/0.41 mm
Average Operating Flux	12.5-20 LMH (0.3-0.5 m^3/m^2-d)
Chlorine Resistance	1,000,000 ppm hrs

Operating Pressure	0.7-8.7 psi (0.05-0.6 bars)
Allowable pH Range	2-13
Operating Temperature Range	5-40℃

Figure 3.4: Membrane Module

3.3 Wastewater Composition:

Synthetic wastewater was kept at 500 mg/l strength in terms of chemical oxygen demand (COD). Following compounds were used to prepare synthetic composition as listed:

Table 3.3 Wastewater Composition

Compounds	Values	Units
$(C_6H_{12}O_6)n$	500	mg/l
Ammonium Chloride	191	mg/l
Sodium Bicarbonate	100	mg/l

Calcium Chloride	4.87	mg/l
Potassium dihydrogen Phosphate	23.85	mg/l
Magnesium Sulfate	4.87	mg/l
Ferric Chloride	0.5	mg/l
Nickel Chloride	0.05	mg/l
Zinc Chloride	0.05	mg/l
Cobalt Chloride	0.05	mg/l

3.4 Membrane Resistances

Hollow Fiber Membrane was first checked for intrinsic resistances by using different rpm using the peristaltic pump. This was performed in distilled water and then followed by sludge in which membrane runs were performed.

Resistances was measured to investigate the amount of fouling caused by individual runs and impact of quorum quenching species on membrane fouling as a whole.

$$Rt = \Delta P / (\mu . J . ft)$$

Where.

Rt = Total Hydraulic Resistance (1/m)

ΔP = Transmembrane Pressure (Pa)

J = Operational Flux of Permeate (m^3/ m^2.s)

μ = Viscosity of Permeate

ft = Temperature Correction Factor to 20 °C

ft = exp^(0.0239*Temp)

Rt = Rc + Rp + Rm

Rm = Intrinsic Resistance

Rc = Cake Resistance

Rp = Pore Resistance

Basic intrinsic resistance occurs naturally due to fine pores and movement of water through it. Pore resistance occurs when flocs at microbial level starts to block pores till end of the run. Cake resistance occurs due to the layer of sludge formed as a cake on fibres of membrane.

Rm is measured by finding resistances in distilled water after chemical cleaning. Rp is measured by subtracting intrinsic resistance from pore resistance. Later Rc is measured by subtracting Rp and Rm from total resistance measured. Total resistance is then sum of Rc, Rp and Rm combined.

3.5 Beads Preparation

Polysulfone beads were prepared using the following protocol:

- Quorum quenching species were freshly grow on agar plates for 24 hours.
- 2% alginate solution was autoclaved.
- 1 µL wire loop was used to pour bacteria film into the alginate solution.
- Alginate solution was then poured drop wise into 4% calcium chloride solution.
- 1 to 2 hours was given for beads to form shape.
- 10% polysulfone solution was made in N-Methyl-2-pyrrolidone.
- Beads were solidified by passing them through polysulfone solution and then distilled water
- Beads were kept at 4℃ refrigeration for 24 hours to completely solidify.

Figure 3.5: PVDF Alginate

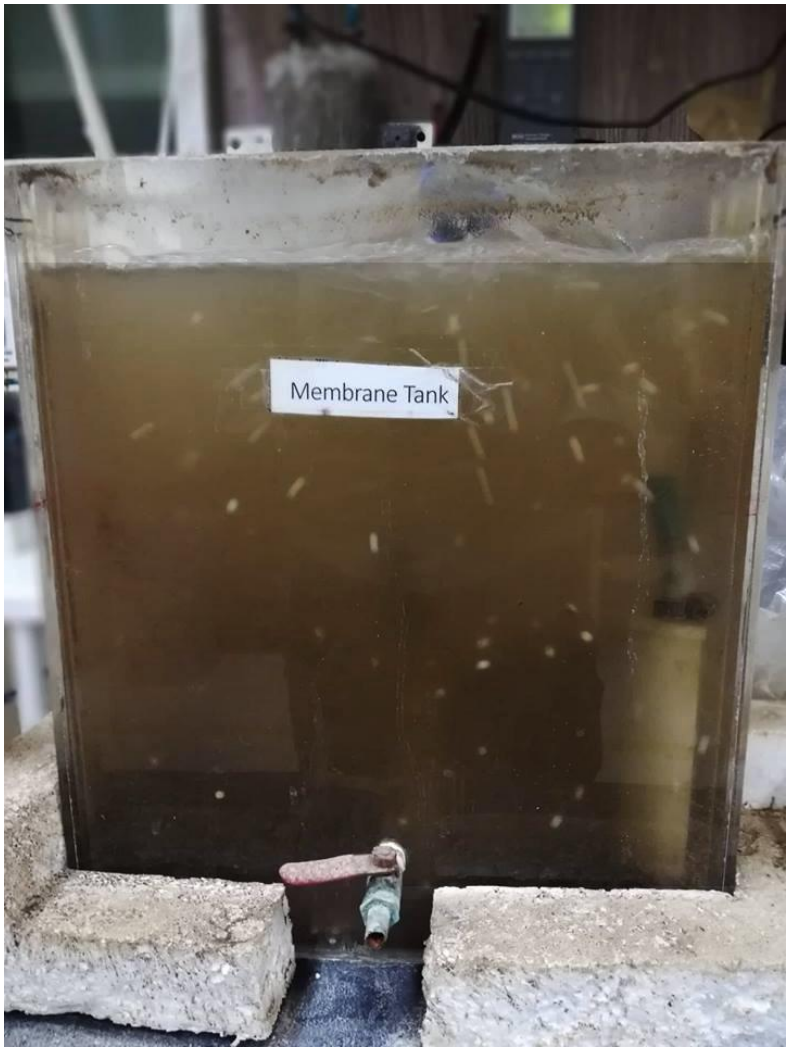

Figure 3.6: Beads in Membrane Bioreactor

3.6 Quorum quenching bacteria inoculation

Quorum quenching species were identified during previous studies and were used to study their quorum quenching abilities on membrane bioreactors. They were following as stated:

- *Pseudomonas Aeruginosa*
- *Bacillus Cereus*
- *Rhodococcus BH-4*

In a note of comparison, *Rhodococcus* BH-4 was also studied as it was widely regarded as very effective quorum quenching specie.

3.7 Analytical Methods

Following analytical tests were carried out to monitor the performance of membrane bioreactors and the impact of quorum quenching species has on them. The following tests were:

- Chemical Oxygen Demand (COD)
- Biological Oxygen Demand (BOD)
- Mixed Liquor Suspended Solids (MLSS)
- Mixed Liquor Volatile Suspended Solids (MLVSS)
- Ammonium Nitrogen (NH_4-N)
- Kjeldahl Nitrogen (TKN)
- Inorganic Nitrogen (IN)
- Orthophosphate (PO4-P)
- Total Phosphates (TP)
- Trans membrane Pressure (TMP)
- Extra Polymer Substances (EPS)
- Specific Oxygen Uptake Rate (SOUR)

3.7.1 Chemical Oxygen Demand

For analysis of chemical oxygen demand, closed reflux titrimetric method where vials were prepared by using 2.5 ml sample, 1.5 ml potassium dichromate and 3.5 ml sulphuric reagent. Vials were subjected to digestion at 150℃ for 2 hours to allow complete oxidation of any organic or inorganic matter (Baird et al., 2012).

Followed by digestion, the vials were titrated with Ferrous Ammonium Sulphate (0.1 N) solution until color changed from yellowish to first shade of brown.

The analytical formula of COD:

$$COD = (A - B)x\ N\ x \frac{8000}{Sample.vol}$$

Where;

A = Amount of FAS used by Blank (ml)

B = Amount of FAS used by Sample (ml)

N= Normality of FAS (N)

3.7.2 Biological Oxygen Demand:

Biological Oxygen Demand is the measurement of activity of microorganism that relies aerobically to degrade organic matter. Biological Oxygen Demand is the fraction of chemical oxygen demand. It is measured by measuring activity of microbes over a 5 day period when incubated. The procedure is as stated:

- First, measure COD first to get an estimate of BOD to expect.
- Decide the dilution factor on the basis COD. The higher the COD will be, the higher the dilution factor.
- Prepare dilution water for BOD by adding 1 ml of each reagent required.
- Add dilution water into the BOD bottles.
- Add the required amount of sample set according to the dilution factor, for example 3 ml sample in 300 ml BOD bottle if dilution factor is found to be 100.
- Measure the initial concentration of dissolved oxygen in the dilution water.
- Incubate the BOD bottles for 5 days at 20 Celsius centigrade.
- Measure the dissolved oxygen in each sampling BOD bottle after incubation ends.

- Calculate the difference in concentration of dissolved oxygen and multiply it by the dilution factor to measure the biological oxygen demand.

$$BOD = \ (A - B)x\frac{300}{V}$$

A = Dissolved Oxygen Before incubation (mg/l)

B = Dissolved Oxygen after incubation (mg/l)

V= Sample Volume (ml)

3.7.3 Orthophosphates:

Phosphorous is measured in the form of orthophosphates and total phosphorous. The measurement of orthophosphate uses UV visible spectrophotometer. Following steps are performed:

- Take 10 ml sample.
- Add 2 ml molybdate Vanadate solution.
- Thoroughly mix and allow standing for 5 minutes for color to form.
- Check absorbance in UV spectroscopy at 470 nm.

3.7.4 Total Phosphorous

Total phosphorous uses the same procedure as that of orthophosphate but uses digestion prior to adding molybdate vanadate. The procedure for digestion is as stated:

- Take 50 ml sample.
- Add 1 drop of phenolphthalein indicator.
- Allow red colour to appear.
- Add sulphuric reagent drop wise to disappear the red color.
- Add further 1 ml sulphuric reagent.

- Add 0.4g Ammonium Per Sulfate.
- Boil till final volume becomes 10 ml.
- Dilute to 30 ml mark using distil water.
- Add 1 drop of phenolphthalein.
- Neutralize to a faint colour of red with NaOH.
- Complete procedure by following orthophosphate procedure.

3.7.5 Inorganic Nitrogen

Inorganic Nitrogen exists in the form of nitrates and nitrites. They are important parameters for assessing nitrification and denitrification process. Inorganic nitrogen as a whole is measured by cadmium reduction method. The method is stated as:

- Adjust the sample pH between 7 and 9.
- Remove all forms for suspended particles.
- Take 25 ml sample and mix with 75 ml ammonium chloride EDTA.
- Add the total volume into column and discharge at a rate of 7 to 10 ml/min through the column.
- Discard the first 25 ml.
- Store the next 75 ml of sample.
- Prepare color reagent by adding 100 ml (85%) phosphoric acid into 800 ml distil water. Add 10 gram sulphanilamide, followed by 1 gram N-(1-napthyl)-ethylene diamine dihydrochloride. Thoroughly mix and dilute to 1000 ml mark.
- Add 1 ml of color reagent into 25 ml sample.
- Allow 10 minutes for colour to form.
- Measure the absorbance at 543 nm at UV spectrophotometer.

Figure 3.7: Cadmium Reduction Column

3.7.6 Ammonium-N:

Ammonium Nitrogen is one of the important parameters of membrane bioreactors. The method to measure is as stated:

- Measure 50 ml sample and add 20 ml borate buffer.
- Dilute the total volume to 150-200 ml mark.
- Adjust the pH to 9.5 using alkali/acid.
- Take 50 ml mixed indicator in the condensate point of Kjeldahl apparatus and start recirculation of coolant water.
- Add the sample in the heating flask of Kjeldahl flask and start the equipment.
- Wait until the condensate volume increases from 50 ml to 110 ml.
- Titrate the condensate with 0.02 N sulphuric acid until faint pink color appears.
- Use the titrated volume to calculate ammonium nitrogen concentration:

$$Ammonium\ Nitrogen = \frac{(A-B)x280}{V}$$

Where;

A = Titrant volume for sample (ml),

B = Titrant volume for blank (ml)

V= Sample Volume (ml)

3.7.7 Mixed Liquor Suspended Solids (MLSS)

Mixed Liquor Suspended Solids is basically known as total suspended solids of sludge concentration. Bacteria exist in micron size and cannot pass the 0.4 micron filter pore size and are hence measured in terms of suspended solids. The procedure is as stated:

- Preheat the GFC filter.
- Measure the mass of GFC filter (B).
- Insert the filter in the filtration assembly and tightly close it with hinges.
- Take 10 ml sludge sample and add onto the GFC filter.
- Start filtration assembly and let the sludge pass through for 2 minutes approximately.
- Stop filtration and gently take out the filter.
- Heat the filter in oven at 105℃ for 1 hour.
- Measure the mass of filter (A) after drying it in oven.
- Calculate the concentration of MLSS by stated formula:

$$MLSS = (A-B)x\frac{1000}{V}$$

A= After Drying the sludge sample (g)

B= Mass of Sample after preheat (g)

V= Volume of sample (ml)

3.7.8 Mixed Liquor Volatile Suspended Solids

Mixed Liquor Volatile Suspended Solids is a fraction of Mixed Liquor Suspended Solids where a fraction of suspended solids consists of living microbes and no form of inert suspended solids. It is carried out after measuring MLSS. It is measured using the following procedure:

- Measure the mass of GFC filter (A) with dried sludge.
- Place it in muffle furnace at 550℃ for 20 minutes.
- Measure the mass of filter (B) after muffle furnace.
- Calculate the concentration of MLVSS by stated formula

$$MLVSS = (A - B)x\frac{1000}{V}$$

A= Mass of filter paper before putting in muffle furnace (g)

B= Mass of filter paper after taking out from muffle furnace (g)

V= Sample volume (ml)

3.7.9 Bound Extracellular polymer Substances/Soluble Microbial Products

Extrapolymer substances are measured in soluble, loosely and tightly bound form. The higher the EPS is in the sludge sample, the more the tendency is for biofouling to occur. Following is the method used:

- 50 ml sludge sample is taken.
- Sludge is centrifuged at 5000 rpm for 15 minutes.
- Supernatant is taken for as a soluble fraction for EPS.
- Buffer solution is used for the remaining portion to restore 50 ml volume.

- Stir the restored volume at 300 rpm for 2 hours in a hotplate mixer.
- Centrifuge the sample then at 5000 rpm for 15 minutes.
- Separate the supernatant for loosely bound fraction for EPS.
- Prepare cation exchange resins (CER) on basis of 0.05 l x 70 grams x MLVSS (g/l) for tightly bound EPS.
- Soak the CER in buffer solutions for 2 hours before usage.
- Remove the buffer solution and add the sludge sample.
- Stir at 300 rpm for 2 hours and then followed by centrifuge at 5000 rpm for 15 minutes.
- Separate the resins and flocs and centrifuge at 5000 rpm for 15 minutes.
- Take out the supernatant when all flocs have been settled.

Following codes are used for the strain in results:

i. *Pseudomonas Aeruginosa* (QQ-1)

ii. *Bacillus Cereus* (QQ-3)

iii. *Rhodococcus* (BH-4)

4.1 Trans membrane Pressure Profile

Transmembrane pressure was the most critical parameter of the research study to analyze the performance of each strain in terms of membrane filtration cycle. It was found out that *Rhodococcus* BH-4 run remained supreme over the newly identified strains and showed a life of 15 days to reach the terminal pressure of 30 kPa with an average membrane fouling rate of 2.0 kPa/day.

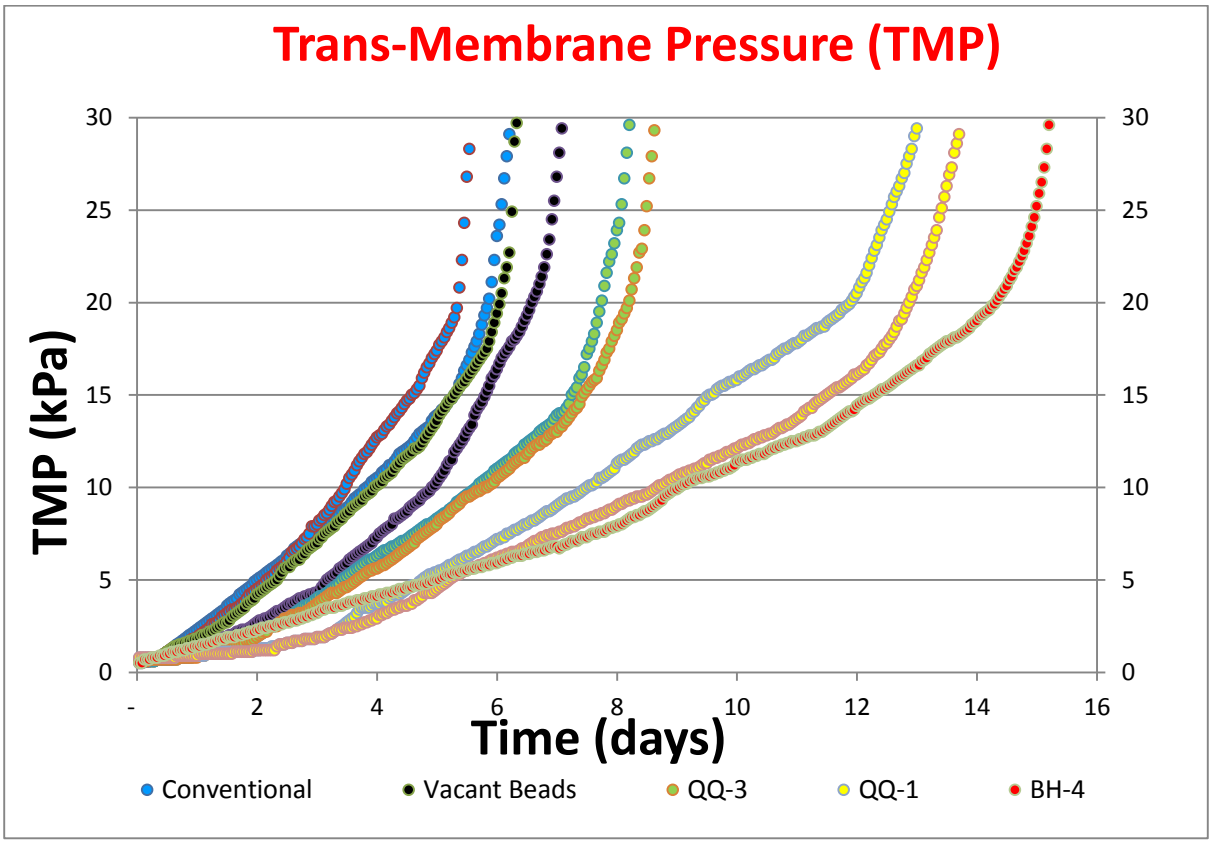

Figure 4.1: Trans Membrane Profile

Pseudomonas Aeruginosa (QQ-1) remained closely and showed a membrane life of 13 days with an average membrane fouling rate 2.31 kPa/day. The second identified strain; *Bacillus Cereus* (QQ-3) was not as good as (QQ-1) and showed a membrane lifespan of 8 days with an average membrane fouling of 3.75 kPa/d. However, in case of conventional and vacant beads, membrane lifespan was reported to be 5 and 6 days respectively with an average membrane fouling rate of 6 and 5 kPa/d respectively.

All of the membranes had exhibited a typical membrane profile where there is a steady rise in transmembrane pressure and by 15-20 kPa, cake layer starts to develop and a TMP jump is observed.

Membrane life was shorter in all cases largely due to hydrodynamic conditions and the operating conditions. A flux of 15 LMH proved to be good for QQ-1 and BH-4 which showed good membrane cycle. With time irreversible fouling also starts to occur on membrane after each run since there was no backwashing in the operation. Backwashing or Chemical backwashing has a profound impact in increasing the membrane life which was absent in this study. Furthermore, the membrane used in this study was more compact and had a much higher fiber density as compared to the membrane used for previous researches (Maqbool et al., 2015). Hence, membrane run were brief but still comparable due to the similar conditions used.

The thickness of cake layer on QQ-1 and QQ-3 were also in comparison thinner as compared to vacant and conventional run that resulted in higher pore resistance instead of cake resistance for the strains identified.

However, no single specie alone can mitigate biofouling to a very large extent as quorum sensing mechanism is linked by countless biofilm forming bacteria for example proteobacteria (Shrout and Nerenberg, 2012).

4.2 Membrane Resistances

In a comparison of the best strains with that of control and vacant MBR, significantly less cake layer was observed which as result lead to a longer membrane run. Less cake layer formation would mean less cake resistance and more pore resistance as the membrane run extends in quorum quenching runs.

Rhodococcus BH-4 had 49% of cake resistance out of total while QQ-1 showed 63.8% of cake resistance which was far less in comparison to conventional MBR and vacant MBR which showed cake resistance portion of 80.3 and 76.2% respectively. This is believed to be due to the release of enzymes by quorum quenching bacteria that causes mineralization of signal molecules which is responsible for the thick cake layer on the membrane surface.

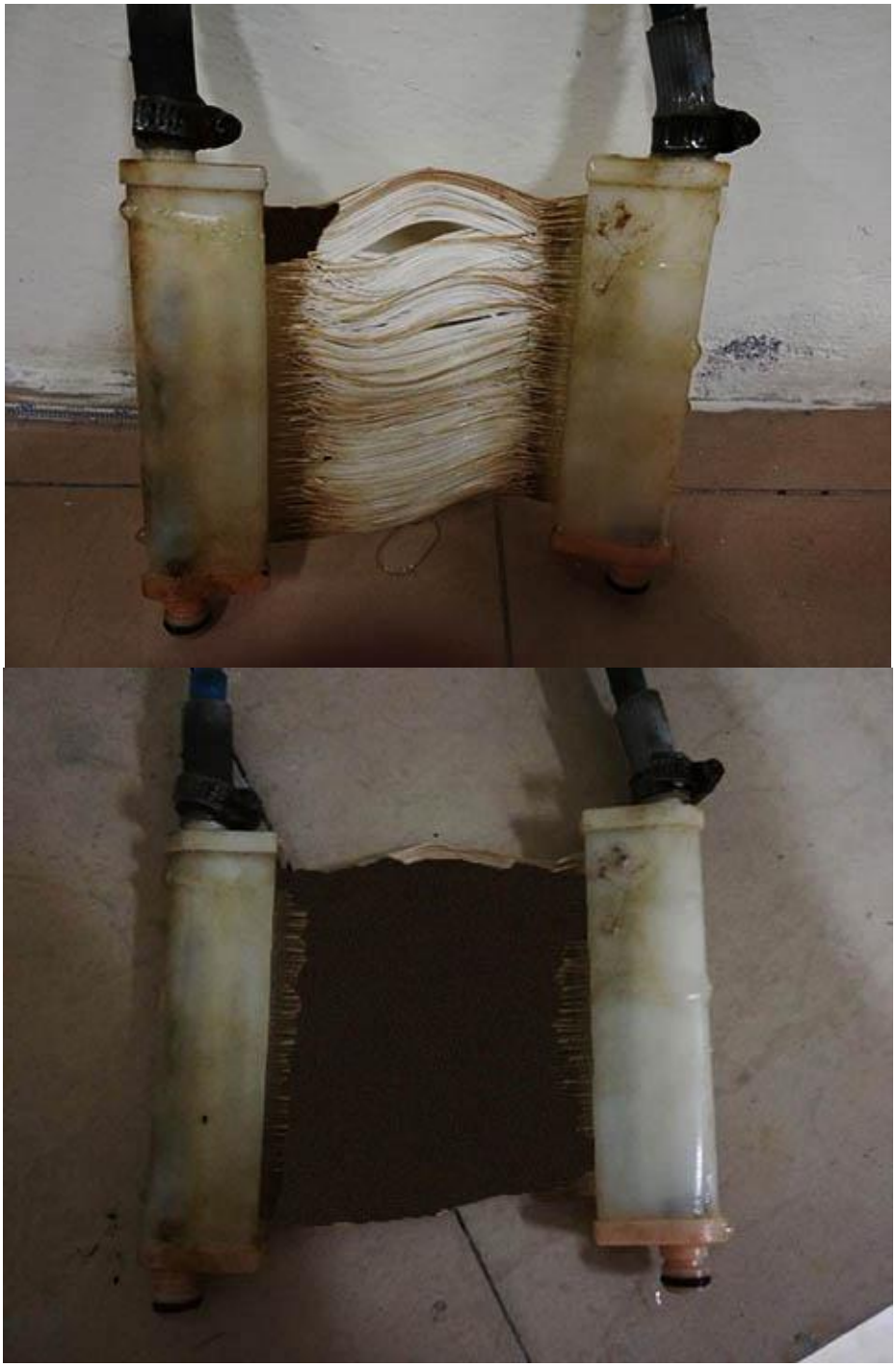

Figure 4.2: Conventional vs *Pseudomonas Aeruginosa*

Table 4.1: Membrane Resistances

Parameters	Units	Conventional	Vacant	QQ-1	QQ-3	BH-4
Total Resistance (Rt)	(10^12)/m	10.0	7.21	5.25	6.06	4.41

Cake Resistance (Rc)	(10^12)/m	8.06	5.50	3.35	4.27	2.16
Pore Resistance (Rp)	(10^12)/m	1.56	1.16	1.07	1.24	1.04
Intrinsic Resistance (Ri)	(10^12)/m	0.421	0.55	0.833	0.541	1.21
Rc/Rt	-	0.803	0.762	0.638	0.705	0.490
Rp/Rt	-	0.155	0.161	0.203	0.205	0.236

Resistances were calculated for each run and compared side by side. Cake Resistance show a high 80 and 76% for conventional and vacant beads respectively while it was only 63 and 49% for *Pseudomonas Aeruginosa* (QQ-1) and *Rhodococcus* (BH-4) respectively. This shows that in QQ-1 and BH-4 runs cake layer dominated less which lead to higher membrane run time.

4.3 Biological/Chemical Oxygen Demand (BOD/COD)

Biological Oxygen Demand in effluent concentrations were less than 20 mg/L and showing atleast 90% removal in all the strains and samples tested. The highest removal efficiency observed was in conventional MBR where it was 96% while the least reported was 90% in BH-4 strain.

For chemical oxygen demand (COD), the same trend was witnessed similar to BOD. Lowest effluent concentration was found in conventional MBR at 10.26 mg/L while the highest effluent concentration for COD was 32.91 mg/L for BH-4.

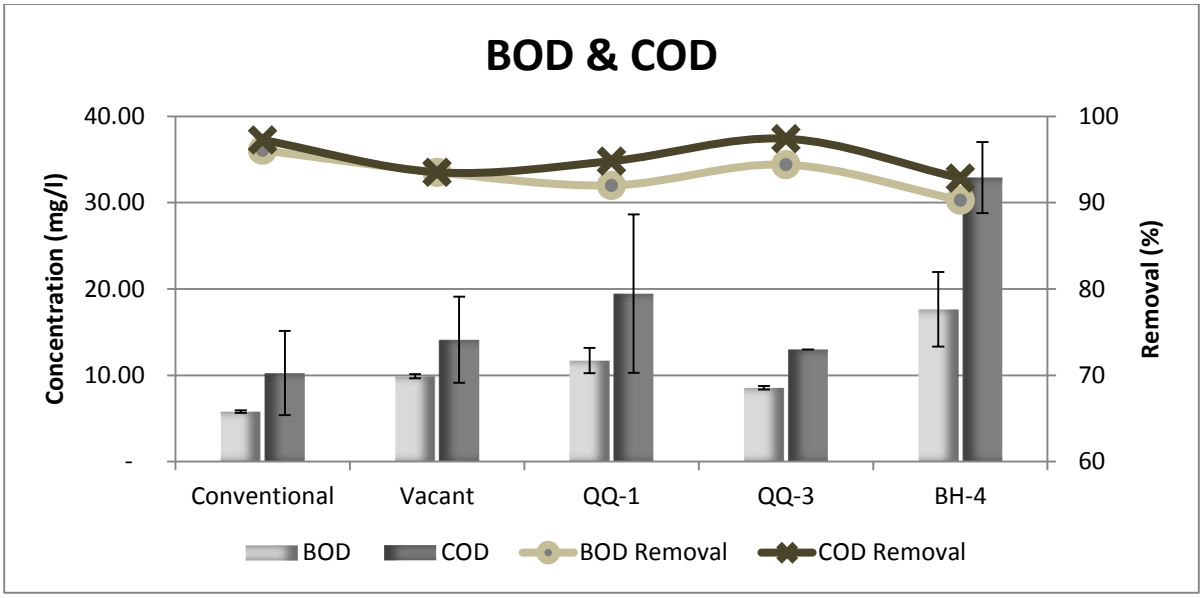

Figure 4.3: Biological/Chemical Oxygen Demand

It can be concluded that heterotrophic bacteria in MLSS readily degraded most of the BOD and COD in the beginning but no significant decrease was observed in the removal efficiency for Biological and Chemical Oxygen Demand.

4.4 Ortho Phosphorous and Total Phosphorous

Phosphorous was measured both in terms of orthophosphate and total phosphorous. Effluent concentration in all the runs for orthophosphate was less than 12.53 mg/L while in case of total phosphorous was less than 25.46 mg/L.

The removal efficiency for orthophosphate ranged from 64.2% in QQ-1 run to 76.2% for conventional MBR. This shows the activity of heterotrophic bacteria which assimilate phosphorous for cell growth (Jiang et al., 2013).

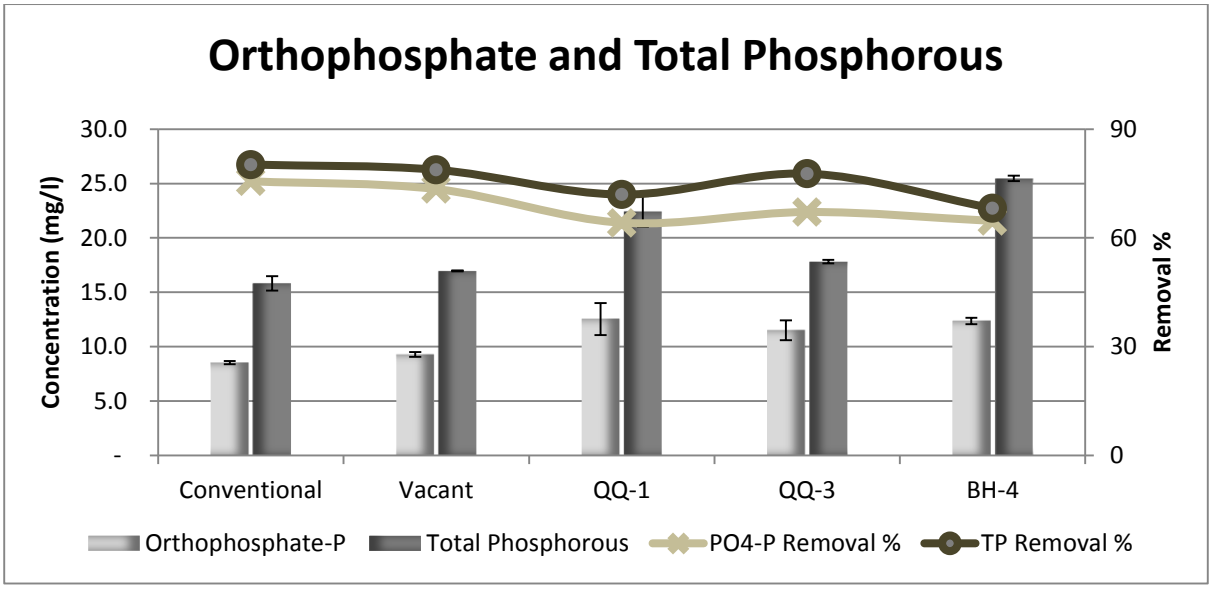

Figure 4.4: Orthophosphate and Total Phosphorous

4.5 Ammonium and Kjeldahl Nitrogen

Nitrogen was measured in terms of ammonium nitrogen and kjeldahl nitrogen. Ammonium nitrogen concentration in effluent water ranged from 12 mg/l in conventional MBR to 16 mg/l in case of BH-4. Ammonium removal was not observed to be high as aeration is not the same as in pilot or full scale MBR that will allow full nitrification of ammonium nitrogen.

Kjeldahl Nitrogen (TKN) exhibited similar trend as compare to ammonium nitrogen as a much bigger portion of influent nitrogen was ammonium nitrogen and very little organic nitrogen was present in the synthetic wastewater feed. TKN removal was highest in vacant beads run where it was as high as 79% while it was as low as 60% for QQ-3. In comparison with other aerobic MBR, removal efficiencies in terms of Nitrogen and Phosphorous were the same.

Inorganic nitrogen was also measured to calculate total nitrogen. TN is the sum of Kjeldahl nitrogen, nitrite and nitrate nitrogen. Inorganic nitrogen was found to be mostly on par to each other. Highest inorganic nitrogen was reported at 1.37 mg/l and the lowest has been 1.04 mg/l for QQ-3. This depicts the performance of nitrification and denitrificaiton processes

which were retarded due to lack of anoxic zone present which is present in large scale MBR before aeration tanks (Jiang et al., 2013).

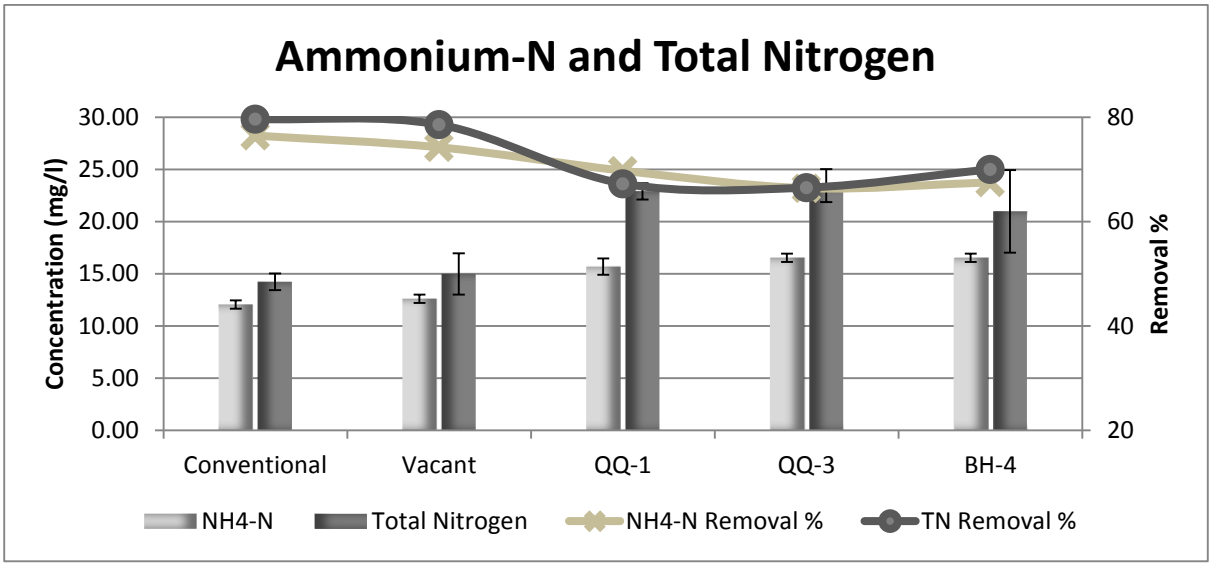

Figure 4.5: Ammonium-N and Total Nitrogen

4.6 Sludge Characteristics

Mixed Liquor Suspended Solids (MLSS) was studied to determine concentration and health of activated sludge. MLSS concentrations varied from 4.55 to 5.3 g/L. MLSS stabilized in quorum quenching runs with SRT kept at 20. However, such a limited SRT could not result in high MLSS concentration.

Sludge Volume Index was measured as well. SVI was lowest in conventional MBR at 60 mL/g while in case of BH-4 it was as high as 140 mL/g. High SVI is attributed to the disturbance in quorum sensing mechanism and inability to form dense aggregate flocs that hamper the settle ability of the sludge.

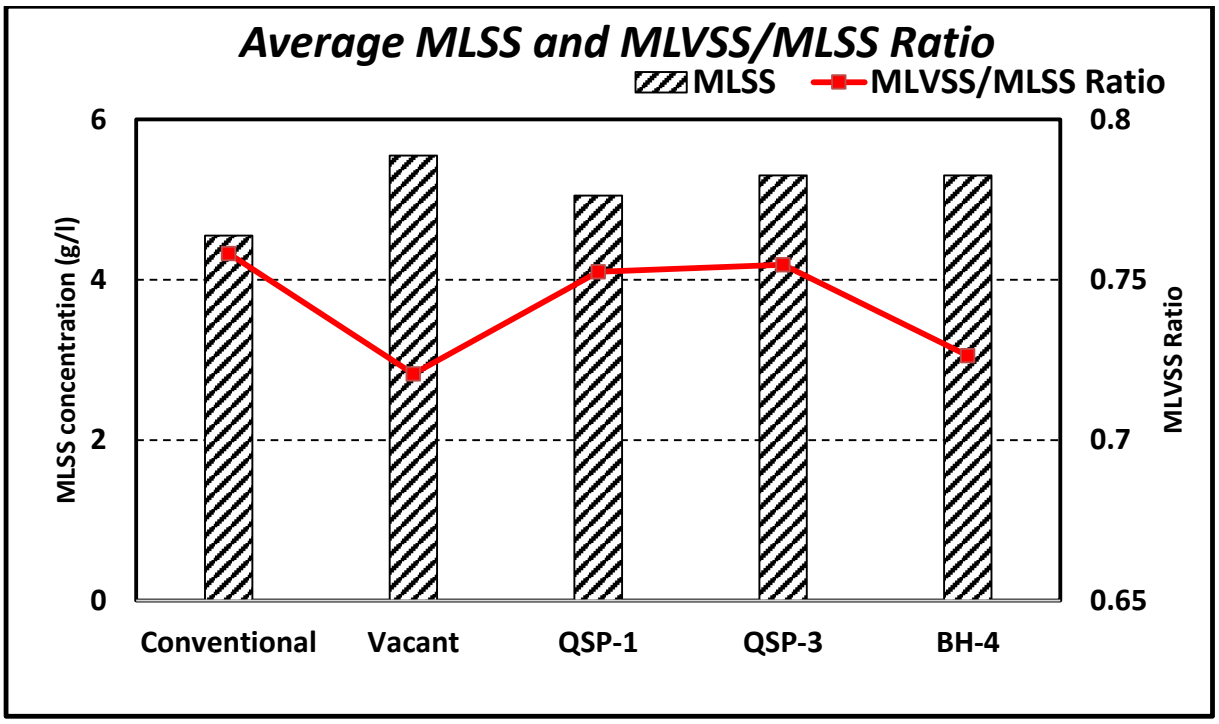

Figure 4.6: Mixed Liquor Suspended Solids

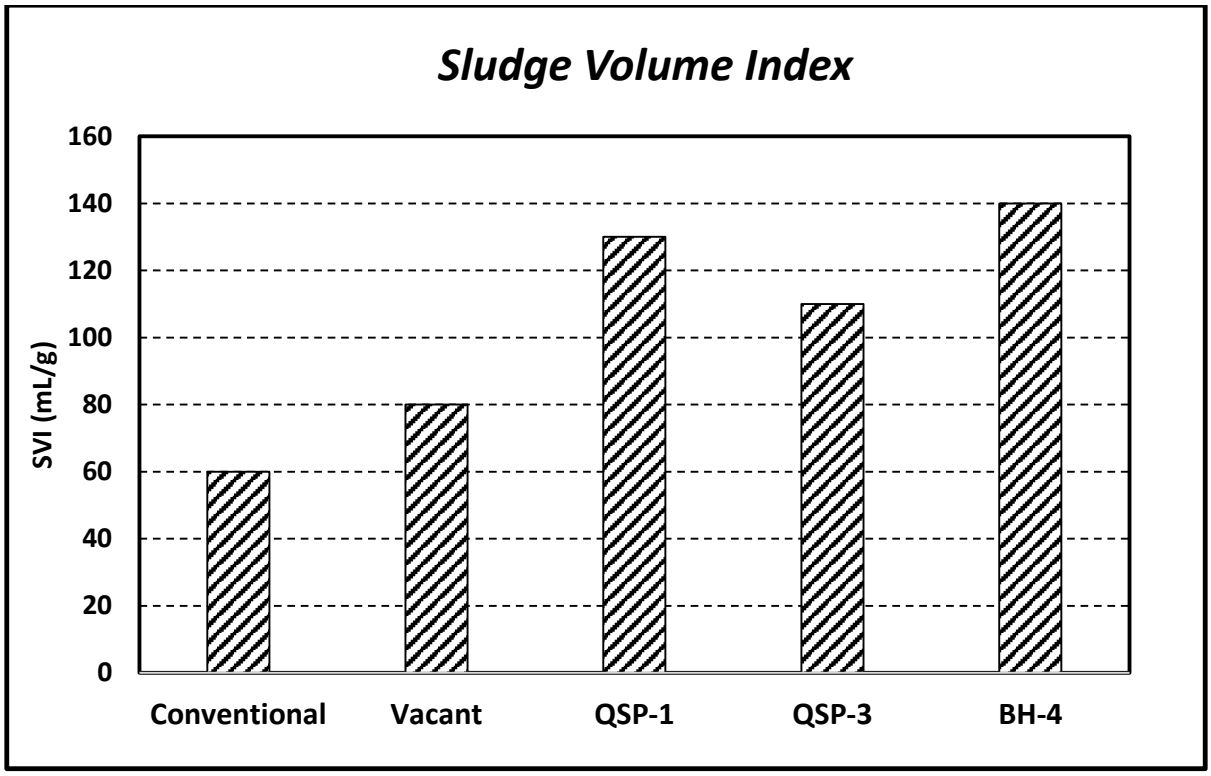

Figure 4.7: Sludge Volume Index

4.7 Soluble Microbial Products (SMP)

SMP are the soluble content of extrapolymeric substances. It is the sum of carbohydrates and proteins. SMP was reported highest in the conventional MBR and was found to be the least in BH-4 run. This is because with more quorum quenching activity and mineralization of signal molecules that SMP tend to decrease. Total SMP was reported highest at 197.04 mg/l while lowest was 104.27 mg/l which was a 47% decrease (Iqbal et al., 2018). The protein to carbohydrates ratio remained in optimal range of 1.43 to 1.60.

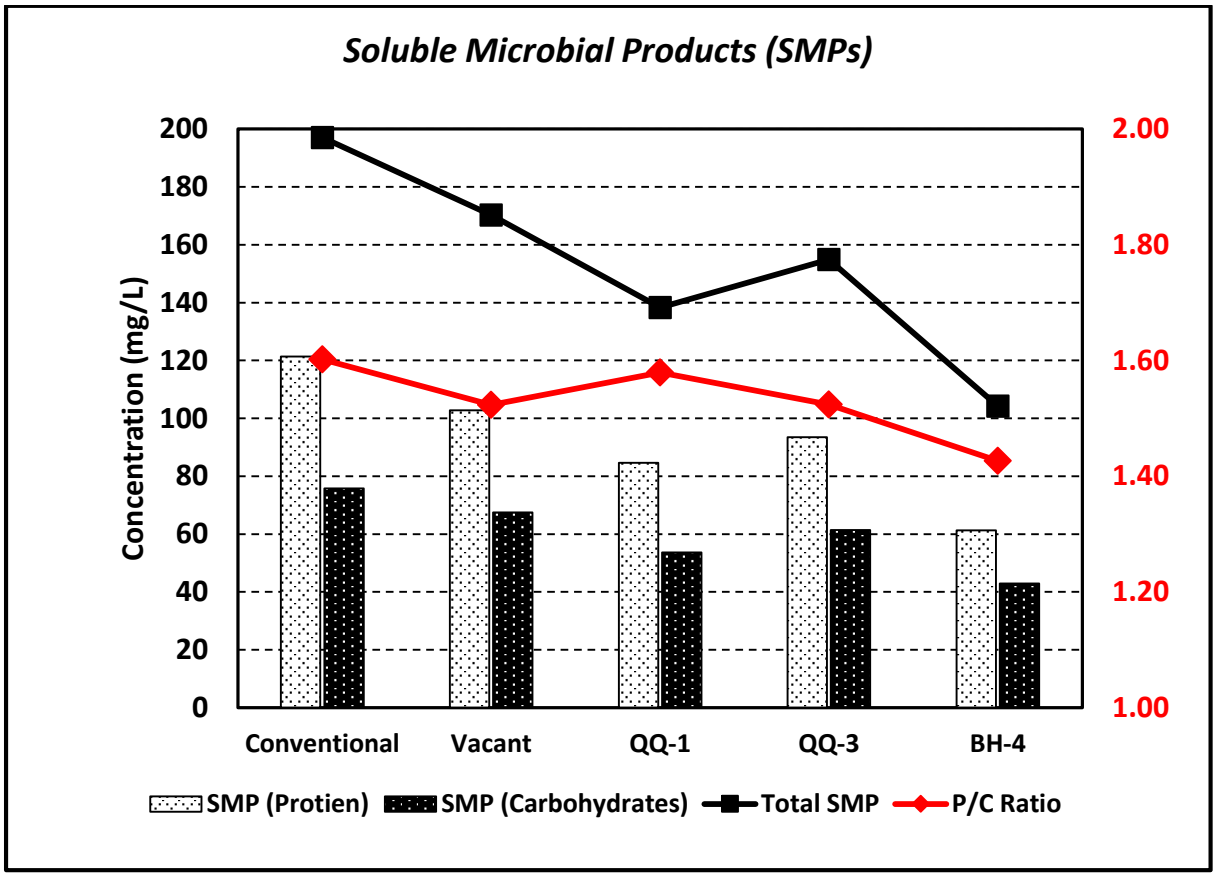

Figure 4.8: Soluble Microbial Products (SMP)

4.8 Extracellular Polymeric Substances (EPS)

Other than SMP, we are left with EPS which is the sum of loosely and tightly bound EPS. Bound EPS correlation has been strongly linked to biofouling. Highest concentration reported was found in conventional MBR at 146.97 mg/g while the lowest was at 67.98 mg/g for BH-4 strain. A 53 and 47% reduction in EPS and SMP respectively show lesser chances of flocs to

bind with membrane surface, owing to lesser cake resistance in case of QQ-1 and BH-4. This marked three times increase in membrane run time while comparing BH-4 with conventional MBR. With more suppression of extrapolymeric substances, membrane run time increases (Iqbal et al., 2018).

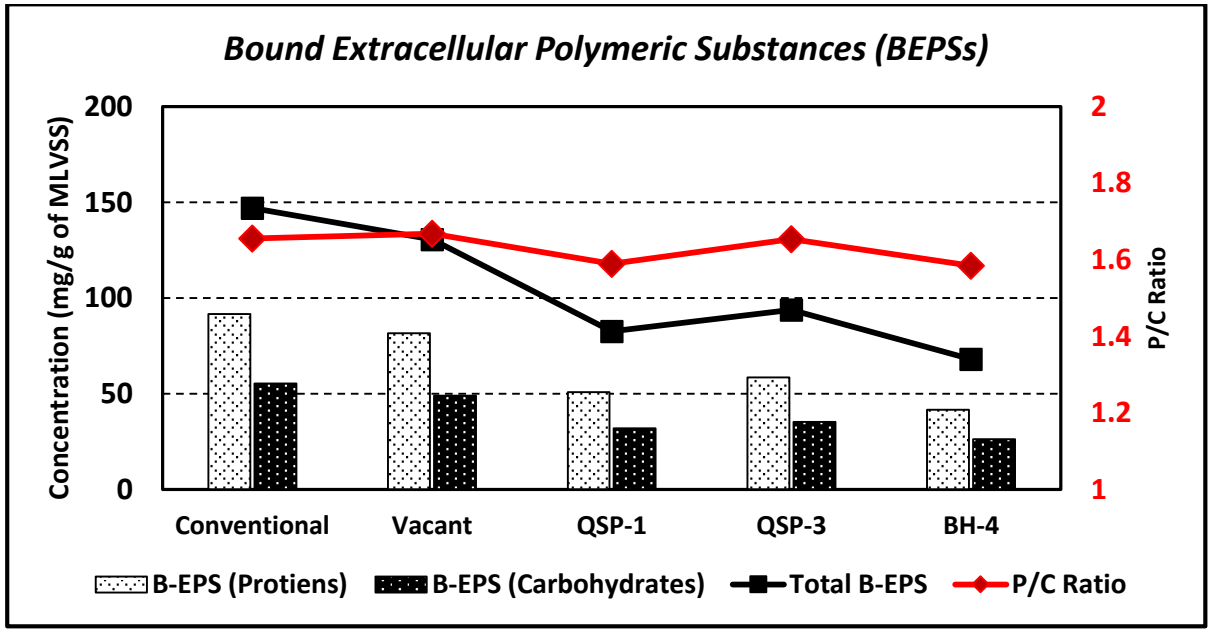

Figure 4.9: Bound EPS

Conclusion and Recommendation Chapter 5

5.1 Conclusions

Two new identified strains were studied in the research as mentioned; *Pseudomonas Aeruginosa* (QQ-1) and *Bacillus Cereus* (QQ-3) which exhibited quorum quenching activities. Initially conventional MBR and MBR with vacant beads were studied to determine the performance of MBR with the operating conditions and hydrodynamic conditions in effect. It was observed that a membrane run time of 5 and 6 days were observed for conventional MBR and MBR with vacant beads. There was a slight improvement in membrane run time but not significant. Later, *Pseudomonas Aeruginosa* (QQ-1) was studied and it showed an excellent membrane run time of 13 days (2.6 times in comparison to conventional MBR) followed by 8 days (1.6 times of conventional MBR) with *Bacillus Cereus* (QQ-3). Both of the strains were able to prolong membrane run but still shorter than *Rhodococcus* BH-4 which still remains the most suitable strain to eliminate a range of signal molecules and diminish the quorum sensing activities of other microbes in activated sludge without showing any quorum quenching ability of their own. *Rhodococcus* BH-4 showed a run time of 15 days, prolonging run time of MBR by thrice as compared to conventional run. However, not much difference in treatment efficiency was observed with addition of QQ beads. Biological Oxygen Demand removal dropped from 96 to 90%. Total Phosphorous removal efficiency dropped from 80 to 67%. Sludge Volume Index (SVI) dropped from 140 down to 60 mL/g. However, all these effluent physiochemical parameters were complying with National Environmental Quality Standards (NEQS).

5.2 Recommendations:

Following recommendations were proposed:

- A comparison study of MBR performance of the potential identified strain (*Pseudomonas Aeruginosa* at 20 LMH) against conventional MBR (at 15 LMH). The

study will justify improved economic feasibility of MBR as more product water may be extracted at a given time with the same surface area of the membrane.

- A comparison study of MBR performance of the potential identified strain (*Pseudomonas Aeruginosa*) operated at a lower aeration rate as compared to conventional MBR operated at the normal aeration rate. This study will also help to understand the economic feasibility from a different perspective.

References

Al-Halbouni, D., Traber, J., Lyko, S., Wintgens, T., Melin, T., Tacke, D., ... & Hollender, J. (2008). Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena. *Water Research*, 42(6-7), 1475-1488.

Amy, G. (2008). Fundamental understanding of organic matter fouling of membranes. *Desalination*, 231(1-3), 44-51.

Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *Standard methods for the examination of water and wastewater* (Vol. 10). E. W. Rice (Ed.). Washington, DC: American Public Health Association.

Bolong, N., Ismail, A. F., Salim, M. R., & Matsuura, T. (2009). A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination*, 239(1-3), 229-246.

Chang, I. S., Le Clech, P., Jefferson, B., & Judd, S. (2002). Membrane fouling in membrane bioreactors for wastewater treatment. *Journal of Environmental Engineering*, 128(11), 1018-1029.

Cheong, W. S., Kim, S. R., Oh, H. S., Lee, S. H., Yeon, K. M., Lee, C. H., & Lee, J. K. (2014). Design of quorum quenching microbial vessel to enhance cell viability for biofouling control in membrane bioreactor. *J. Microbiol. Biotechnol*, 24(1), 97-105.

Choo, K. H., Chang, D. I., Park, K. W., & Kim, M. H. (2008). Use of an integrated photocatalysis/hollow fiber microfiltration system for the removal of trichloroethylene in water. *Journal of Hazardous Materials*, 152(1), 183-190.

Cosenza, A., Di Bella, G., Mannina, G., & Torregrossa, M. (2013). The role of EPS in fouling and foaming phenomena for a membrane bioreactor. *Bioresource Technology*, 147, 184-192.

Costa, A. R., de Pinho, M. N., & Elimelech, M. (2006). Mechanisms of colloidal natural organic matter fouling in ultrafiltration. *Journal of Membrane Science*, 281(1-2), 716-725.

Cui, Z. F., Chang, S., & Fane, A. G. (2003). The use of gas bubbling to enhance membrane processes. *Journal of Membrane Science*, 221(1-2), 1-35.

Deng, L., Guo, W., Ngo, H. H., Zhang, H., Wang, J., Li, J., & Wu, Y. (2016). Biofouling and control approaches in membrane bioreactors. *Bioresource Technology*, 221, 656-665.

Diagne, F., Malaisamy, R., Boddie, V., Holbrook, R. D., Eribo, B., & Jones, K. L. (2012). Polyelectrolyte and silver nanoparticle modification of microfiltration membranes to mitigate organic and bacterial fouling. *Environmental Science & Technology*, 46(7), 4025-4033.

Drews, A. (2010). Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures. *Journal of Membrane Science*, 363(1-2), 1-28.

Fu, H. Y., Xu, P. C., Huang, G. H., Chai, T., Hou, M., & Gao, P. F. (2012). Effects of aeration parameters on effluent quality and membrane fouling in a submerged membrane bioreactor using Box–Behnken response surface methodology. *Desalination*, 302, 33-42.

Gil, J. A., Túa, L., Rueda, A., Montaño, B., Rodríguez, M., & Prats, D. (2010). Monitoring and analysis of the energy cost of an MBR. *Desalination*, 250(3), 997-1001.

Howell, J. A., Chua, H. C., & Arnot, T. C. (2004). In situ manipulation of critical flux in a submerged membrane bioreactor using variable aeration rates, and effects of membrane history. *Journal of Membrane Science*, 242(1-2), 13-19.

Iqbal, T., Lee, K., Lee, C. H., & Choo, K. H. (2018). Effective quorum quenching bacteria dose for anti-fouling strategy in membrane bioreactors utilizing fixed-sheet media. *Journal of Membrane Science*, 562, 18-25.

Jiang, Q., Rentschler, J., Perrone, R., & Liu, K. (2013). Application of ceramic membrane and ion-exchange for the treatment of the flowback water from Marcellus shale gas production. *Journal of Membrane Science*, 431, 55-61.

Keskes, S., Hmaied, F., Gannoun, H., Bouallagui, H., Godon, J. J., & Hamdi, M. (2012). Performance of a submerged membrane bioreactor for the aerobic treatment of abattoir wastewater. *Bioresource technology*, 103(1), 28-34.

Kim, E. S., Hwang, G., El-Din, M. G., & Liu, Y. (2012). Development of nanosilver and multi-walled carbon nanotubes thin-film nanocomposite membrane for enhanced water treatment. *Journal of Membrane Science*, 394, 37-48.

Kimura, K., Naruse, T., & Watanabe, Y. (2009). Changes in characteristics of soluble microbial products in membrane bioreactors associated with different solid retention times: Relation to membrane fouling. *Water Research*, 43(4), 1033-1039.

Laspidou, C. S., & Rittmann, B. E. (2002). A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research*, 36(11), 2711-2720.

Le-Clech, P., Chen, V., & Fane, T. A. (2006). Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, 284(1-2), 17-53.

Lee, J., Ahn, W. Y., & Lee, C. H. (2001). Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor. *Water Research*, 35(10), 2435-2445.

Lesjean, B., Rosenberger, S., Schrotter, J. C., & Recherche, A. (2004). Membrane-aided biological wastewater treatment—an overview of applied systems. *Membrane Technology*, 2004(8), 5-10.

Liao, B. Q., Bagley, D. M., Kraemer, H. E., Leppard, G. G., & Liss, S. N. (2004). A review of biofouling and its control in membrane separation bioreactors. *Water Environment Research*, 76(5), 425-436.

Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., & Zhang, Y. (2013). A review on anaerobic membrane bioreactors: applications, membrane fouling and future perspectives. *Desalination*, 314, 169-188.

Lin, H., Zhang, M., Wang, F., Meng, F., Liao, B. Q., Hong, H., ... & Gao, W. (2014). A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: characteristics, roles in membrane fouling and control strategies. *Journal of Membrane Science*, 460, 110-125.

Liu, J., Nguyen, D., & Paice, M. (2010). Aerobic granule formation in a sequencing batch reactor treating newsprint effluent under low phosphate conditions. *Water Science and Technology*, 62(11), 2571-2578.

Malaeb, L., Le-Clech, P., Vrouwenvelder, J. S., Ayoub, G. M., & Saikaly, P. E. (2013). Do biological-based strategies hold promise to biofouling control in MBRs?. *Water Research*, 47(15), 5447-5463.

Maqbool, T., Khan, S. J., Waheed, H., Lee, C. H., Hashmi, I., & Iqbal, H. (2015). Membrane biofouling retardation and improved sludge characteristics using quorum quenching bacteria in submerged membrane bioreactor. *Journal of Membrane Science*, 483, 75-83.

Massé, A., Spérandio, M., & Cabassud, C. (2006). Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Research*, 40(12), 2405-2415.

Meng, F., Chae, S. R., Drews, A., Kraume, M., Shin, H. S., & Yang, F. (2009). Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. *Water Research*, 43(6), 1489-1512.

Miura, Y., Hiraiwa, M. N., Ito, T., Itonaga, T., Watanabe, Y., & Okabe, S. (2007). Bacterial community structures in MBRs treating municipal wastewater: relationship between community stability and reactor performance. *Water Research*, 41(3), 627-637.

Moreira, F. C., Boaventura, R. A., Brillas, E., & Vilar, V. J. (2017). Electrochemical advanced oxidation processes: a review on their application to synthetic and real wastewaters. *Applied Catalysis B: Environmental*, 202, 217-261.

Murphy, A. P., Moody, C. D., Riley, R. L., Lin, S. W., Murugaverl, B., & Rusin, P. (2001). Microbiological damage of cellulose acetate RO membranes. *Journal of Membrane Science*, 193(1), 111-121.

Mustafa, G., Wyns, K., Buekenhoudt, A., & Meynen, V. (2016). New insights into the fouling mechanism of dissolved organic matter applying nanofiltration membranes with a variety of surface chemistries. *Water Research*, 93, 195-204.

Myat, D. T., Stewart, M. B., Mergen, M., Zhao, O., Orbell, J. D., & Gray, S. (2014). Experimental and computational investigations of the interactions between model organic compounds and subsequent membrane fouling. *Water Research*, 48, 108-118.

Nguyen, T., Roddick, F., & Fan, L. (2012). Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures. *Membranes*, 2(4), 804-840.

Ognier, S., Wisniewski, C., & Grasmick, A. (2002). Characterisation and modelling of fouling in membrane bioreactors. *Desalination*, 146(1-3), 141-147.

Oh, H. S., Yeon, K. M., Yang, C. S., Kim, S. R., Lee, C. H., Park, S. Y., ... & Lee, J. K. (2012). Control of membrane biofouling in MBR for wastewater treatment by quorum

quenching bacteria encapsulated in microporous membrane. *Environmental Science & Technology*, 46(9), 4877-4884.

Perveen, S. (2018). *Detection of Quorum Quenching Enzymes Producing Genes in Membrane Bioreactor* (Doctoral dissertation, IESE NUST Islamabad.).

Poostchi, A. A., Mehrnia, M. R., Rezvani, F., & Sarrafzadeh, M. H. (2012). Low-cost monofilament mesh filter used in membrane bioreactor process: Filtration characteristics and resistance analysis. *Desalination*, 286, 429-435.

Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M., Kristoffersen, P., Köte, M., ... & Givskov, M. (2005). Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *Journal of Bacteriology*, 187(5), 1799-1814.

Reid, E., Liu, X., & Judd, S. J. (2006). Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor. *Journal of Membrane Science*, 283(1-2), 164-171.

Rosenberger, S., & Kraume, M., 2003. Filterability of activated sludge in membrane bioreactors. *Desalination* 151, 195–200.

Sheng, G. P., Yu, H. Q., & Cui, H. (2008). Model-evaluation of the erosion behavior of activated sludge under shear conditions using a chemical-equilibrium-based model. *Chemical Engineering Journal*, 140(1-3), 241-246.

Shrout, J. D., & Nerenberg, R. (2012). Monitoring bacterial twitter: does quorum sensing determine the behavior of water and wastewater treatment biofilms?. *Environmental Science & Technology*, 46(4), 1995-2005.

Sofia, A., Ng, W. J., & Ong, S. L. (2004). Engineering design approaches for minimum fouling in submerged MBR. *Desalination*, 160(1), 67-74.

The Express Tribune. (2016). Less than 8% of urban waste water is treated in Pakistan, says report | The Express Tribune. [online] Available at:

https://tribune.com.pk/story/1362083/world-water-day-less-8-urban-waste-water-treated-pakistan-says-report/ [Accessed 23 Apr. 2019].

The Express Tribune. (2018). Pakistan's water crisis | The Express Tribune. [online] Available at: https://tribune.com.pk/story/1911457/6-pakistans-water-crisis-2/ [Accessed 23 Apr. 2019].

Trussell, R. S., Merlo, R. P., Hermanowicz, S. W., & Jenkins, D. (2006). The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research*, 40(14), 2675-2683.

Van Den Brink, P., Vergeldt, F., Van As, H., Zwijnenburg, A., Temmink, H., & van Loosdrecht, M. C. (2013). Potential of mechanical cleaning of membranes from a membrane bioreactor. *Journal of Membrane Science*, 429, 259-267.

Waheed, H., Xiao, Y., Hashmi, I., Stuckey, D., & Zhou, Y. (2017). Insights into quorum quenching mechanisms to control membrane biofouling under changing organic loading rates. *Chemosphere*, 182, 40-47.

Wang, X., Wu, Z., Wang, Z., Yin, X., & Du, X. (2009). Floc destruction and its impact on dewatering properties in the process of using flat-sheet membrane for simultaneous thickening and digestion of waste activated sludge. *Bioresource Technology*, 100(6), 1937-1942.

Wang, Z., Ma, J., Tang, C. Y., Kimura, K., Wang, Q., & Han, X. (2014). Membrane cleaning in membrane bioreactors: a review. *Journal of Membrane Science*, 468, 276-307.

Wateraid.org. (2019). The crisis | WaterAid Pakistan. [online] Available at: https://www.wateraid.org/pk/the-crisis [Accessed 23 Apr. 2019].

Won, Y. J., Lee, J., Choi, D. C., Chae, H. R., Kim, I., Lee, C. H., & Kim, I. C. (2012). Preparation and application of patterned membranes for wastewater treatment. *Environmental Science & Technology*, 46(20), 11021-11027.

Xiong, Y., & Liu, Y. (2010). Biological control of microbial attachment: a promising alternative for mitigating membrane biofouling. *Applied Microbiology and Biotechnology*, 86(3), 825-837.

Yeon, K. M., Lee, C. H., & Kim, J. (2009). Magnetic enzyme carrier for effective biofouling control in the membrane bioreactor based on enzymatic quorum quenching. *Environmental Science & Technology*, 43(19), 7403-7409.

Zhang, G., Zhang, J., Wang, L., Meng, Q., & Wang, J. (2012). Fouling mechanism of low-pressure hollow fiber membranes used in separating nanosized photocatalysts. *Journal of Membrane Science*, 389, 532-543.