# INFLUENCE OF ANTI-FOULING BACTERIA ON PERFORMANCE EFFECIENCY AND MEMBRANE FOULING OF SUBMERGED MEMBRANE BIOREACTOR

By

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# Abstract

Wastewater reclamation with membrane bioreactor (MBR) technology seems to be a feasible option but membrane biofouling is a critical operational problem that hinders the rapid commercialization of MBRs. Naturally bacteria have the ability to produce signals i.e. N-acyl homoserine lactones (AHLs) which helps them to communicate with each other and form colonies under favorable environmental conditions and produce bacterial byproducts like soluble microbial products (SMP)resulting in biofilm formation on the membrane surface and reduction in membrane permeability. To reduce this natural behavior of microbial interaction, introduction of quorum quenching mechanism in MBR i.e., disruption of signal molecules can significantly decreaseAHLs presenceand extracellular polymeric substance (EPS) production causing reduction in membrane biofouling.In the present study, potential quorum quenching bacteria were screened using a biosensor, Pseudomonas aeruginosa QSIS2 (lasIrhlI double mutant harboring pLasB-SacB1) and applied in MBR. Three lab-scale MBRs in continuous mode were operated in parallel under similar operating conditions. Two QQ-MBRs were inoculated with different QQ bacterial consortium entrapped within polymeric beads. Performance efficiency in terms of membrane permeability, transmembrane pressure (TMP) build up and biofouling retardation rate of QQ-MBRs was investigated and compared with Control-MBR. Both QQ-MBRs experienced three times less biofouling as compared to Control MBR leading to significant decrease in acyl homoserine lactones (AHLs) concentration. Similarly, polysaccharide and protein concentration also significantly decreased in the biocake of QQ-MBRs thereby resulting extension in the time required to reach the TMP of 30 kPa, compared to Control-MBR. More than 90, 45 and 49 % of COD, $NH_4$-N and $PO_4^{-3}$-P removal efficiencies further elucidate that QQ bacterial consortium may efficiently reduce membrane bio fouling by maintaining the performance intact.

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# List of Abbreviations

**Abbreviation Description**

A/O-MBR Anoxic Oxic growth membrane bioreactor

C-MBR control membrane bioreactor

CASP Conventional activated sludge process

CEBs cell entrapping beads

CER Cat-ion exchange resin

C-MBR Conventional Membrane bioreactor

COD Chemical oxygen demand

CST capillary suction time

DI De-ionized water

DO Dissolved oxygen

DSVI Diluted Sludge Volume Index

EPS Extra polymeric substance

F/M Food to microorganism ratio

ft Temperature correction

HF Hollow-fiber

HPLC high performance liquid chromatography

HRT Hydraulic retention time

J Operational flux

MBBR Moving bed biofilm reactor

MB-MBR Moving biofilm Membrane bioreactor

MLSS Mixed liquor suspended solids

MLVSS Mixed liquor volatile suspended solids

NLR Nitrogen loading rates

NTU Naphthalometric turbidity unit

OLR Organic loading rate

PSD Particle size distribution

QQ-MBR Quorum quenching membrane bioreactor

Rc Cake resistance

Rp Resistance due to pore blocking

Rm Intrinsic membrane resistance

Rt Total resistance

RIS Resistance in Series

SCR Specific Cake Resistance

SEM Scanning electron microscopy

SMBR Submerged membrane bioreactor

SMP Soluble microbial product

SND Simultaneous nitrification and denitrification

SOUR Specific oxygen uptake rate

SRT Sludge retention time

TMPTrans-membrane pressure

TN Total nitrogen

TOC Total Organic Carbon

$\mu$ Viscosity of permeate

# Chapter 1

# Introduction

For the people of this earth, water is being a great blessing of God. Canals, rivers and oceans make two-third of the total potion of Water as surface or ground water. Recent growth of residential and commercial communities due to drastic increase of worldwide population, raise the demand of fresh water in every region of the world. From the total sources of what available on earth, 3 % is fresh water, of which 67% comprises of glaciers. Remaining fresh water which is less than 0.5 % is available for human use (World Bank, 2005). According to the World Bank, 700 million citizens are under the waterstressed condition all over the world (World Bank, 2007). In Pakistan per capita availability of water is less than 1000 $m^3$ and this lead the situation towards Water Scarcity condition like other countries including Sudan, Cuba and Venezuela (Asian Development Outlook, 2013). Uneven use of available water resources, water pollution and contamination of water resources made the corridor open for more water shortage conditions. Thus the need of sufficient water got a lions share importance for daily consumption. It has been estimated that to meet the rising demand for horticulture, many cities will face issues to access fresh water within the next 15 to 25 years. The serious question is whether the developing world should follow the advance wastewater treatment technology or there is an alternative-Sustainable-Sanitation

solution (Harleman and Murcott [1]). In Pakistan, 23% population lack access to fresh and safe water for drinking and 30% population lack access to sanitation. The ground water is polluted to a great extend in many areas of Pakistan; the water infrastructure and main barrages are worn out and need proper repair, overall the water distribution system is not sustainable financially (Water Aid). The prevailing situation in country demands the conservation of water resources and requires the treatment of wastewater so that it can be utilized for irrigation, landscaping and ground water recharge purposes. The principal purpose of wastewater treatment is to permit municipal and industrial wastewater effluents to be disposed of in natural water bodies and environment without any hazard to human health or undesirable damage to environment. All the processes which are used to make wastewater meet discharge standards for a suitable end-use are called wastewater treatment systems. The physico-chemical wastewater treatment are costly and raises issues of sludge disposal; which urges us for cost effective treatment processes such as biological treatment systems for removing pollutants and also does not leave chemical sludge. (Kapdan and Oztekin [2]). Biological treatment has capacity to remove the concentration of organic and inorganic compounds and also to transform nutrients. Biological treatment can be divided into two type aerobic and anaerobic treatment. In Activated Sludge Process, aerobic treatment with suspended growth the microorganisms carrying out treatment are kept in liquid suspension in reactor by aeration and mixing. Sufficient amount of air is provided to maintain a minimum of 2 mg/l of dissolved oxygen which is required for microbial growth and degradation of food. This method is the most reliable and efficient one. Removal of COD in activated sludge process (ASP) is 95%, in which degradation of organic matter takes place by the cultivation of biomass. Activated Sludge Process comprises of mainly three components, which are:

- Influent wastewater comes in contact with biomass in **aeration tank**.

- Liquid Solid separation takes place in **clarifier**.
- Sludge **recycle line**

Major drawbacks of conventional activated sludge process includes large area requirement, lower SRT and higher HRT which results in the discharge of excess sludge in conventional activated process, 2-4 g/L valve of MLSS easily settle the sludge in secondary clarifier. (Wang et al. [3]). While considering the treatment technologies for wastewater, membrane bioreactor (MBR), having biological process followed by low pressure driven membrane separation (ultra or micro filtration) for solid-liquid separation, is most emerging technique from last 20 years because of its high quality effluent. High quality of treated wastewater from membrane bioreactor which is generally the result of aerobic condition, is best suited for further refining through Nano filtration or by reverse osmosis.

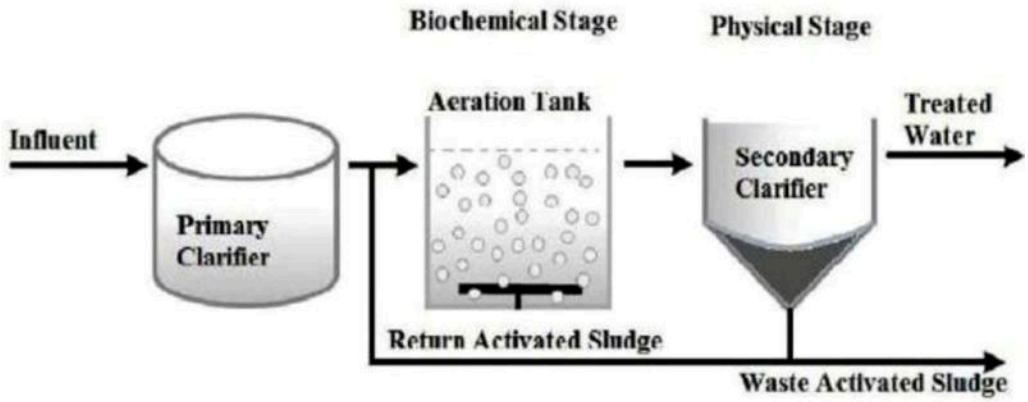



Figure 1.1: Conventional activated sludge process (CAS)

MBR has been a well-reputable treatment technology for the treatment of domestic and industrial wastewaters. Separation process by using membrane in a bioreactor represent a confident hurdleto the flocs of mixed liquor suspended solids, which permit the process to maintain any sludge condition with high reclaimed water. High nutrients removal in MBR is because of

high sludge retention time for slow mounting nitrifying bacteria and some other microorganisms. Limitations regarding MBR may include, high capital and energy cost, membrane fouling which decreases the life span of membrane and severe decline in flux indeed require the chemical and physical cleaning. Control on membrane fouling and reduction of energy consumption is one of the main bottle neck issues for the commercialization of membrane bioreactors. Till now number of researchers have been worked on membrane fouling to found out the effective control using physical and chemical technique. Although these methods are sufficient for the membrane fouling control but these methods enhances the filtration for very short span of time with the loss of extreme permeability. According to the recent studies, soluble microbial products, EPS and formation of cake are the major constituents of membrane clogging. Layer of bio-cake stick on surface of membrane and block holes, which results in permeability loss. From the above discussion it is suggested that the control on formation of cake-layer is the more reliable solution to control biofouling as compared to conventional physical and chemical cleaning (Yeon et al. [4]). Production of signal molecules and auto inducers by bacteria is known as quorum sensing. These molecules are organic in nature having a structure of Acyl-Homoserine Lactones (AHLs). Whenever concentration of these signal molecules arise up to certain level then these molecules combines with receptor protein for the group behavior to activate specific genes, e.g biofilm production, EPS production, antibiotic production and virulence (Kim et al. [5]). Production of EPS is considered as the key factor in a cause of membrane fouling which helps in the accumulation of microbial flocs and biofilm. Quorum Sensing based on AHLs is responsible for the EPS production. Novel biological technology for the control of membrane biofouling by controlling the AHLs production in the environment so that production of EPS can be controlled, that is known as Quorum Quenching. Quorum Quenching are of two types, which are being researched now-a-days.

**a** enzymatic quorum quenching

**b** bacterial quorum quenching

## 1.1 Objectives

1. Establishment of automated laboratory scale MBR setup at IESE-NUST
2. Effect of physic-chemical parameters on fouling rate of submerged membrane
3. Sludge characterization in terms of dewater ability (CST), extra polymeric substances(EPS)
4. Quorum quenching influence on biofouling of membrane

## 1.2 Scope of study

1. Established MBR setup for continuous operation automatically with working volume of 6L and having a PVDF hollow fiber membrane with area of $0.07m^2$ and hole size of 0.05 micrometer.
2. Examined performance efficiency of membrane bioreactor during control as well as QQ operation.
3. Inoculation of indigenous quorum quenching (QQ) bacteria in the form of consortium to evaluate the membrane biofouling reduction.

# Chapter 2

# Literature Review

## 2.1 Membrane Separation

Filtration through membranes is a well-recognized technology, extensively in application for the treatment of wastewater. Membranes generally consist of a support layer with a top dense layer that forms the membrane and this is the membrane that is basically the physical barrier to solids, viruses, bacteria and other undesirable molecules that successfully remove them from water or wastewater. Different kinds of membranes are used for different rationale e.g. for disinfection of water, softening, removal of organic matter, and desalination of water and wastewater. The most extensively used membranes are microfiltration and ultrafiltration , pressure driven processes that are able to separate particles in the size ranges of about 1 to 100 nm and 0.1 to 10 $\mu m$, respectively. Effectiveness of a membrane is mainly dependent on its selectivity and productivity.Cost of membrane based systems is reduced with advancements in membrane filtration technology. Membrane filtration is used to treat water and waste water mainly due to its lower installation cost and mainly because they do not require large land area as compared to conventional systems (W. Richard Bowen [6]).

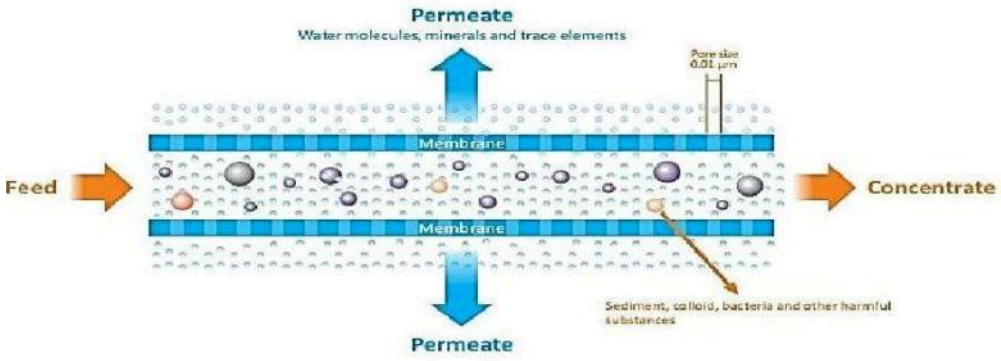



Figure 2.1: Membrane filtration technology

## 2.2 Types of Membranes

Membranes are the physical barrier that only permits the passage of materials only up to a definite size and shape. Membranes processes can be categorized in various categories related to their pore size, molecular weight or the pressure at which they operated. As the pore size of a membrane gets smaller, the pressure applied to the membrane to separate water from other material generally increases. In the Figure 2.1, membrane processes that are pressure driven from micro-filtration to reverse osmosis are specified along with their pore size. Micro-filtration (MF) separation deals with removal of particulate or suspended material with size range of 0.1 to 10 $\mu m$. Ultra-filtration (UF) is usually used to separate macro-molecules having size range of 0.01 to 0.1. Nano-filtration (NF) deals with removal of particles having size range of about 0.001 $\mu m$ to 0.01 $\mu m$. Reverse osmosis (RO) membranes are capable of retaining materials less than 0.001 $\mu m$ size. Sometimes operation of RO requires high pressure of about 150 bar to overcome osmotic pressure (Abdel Kader [7]).

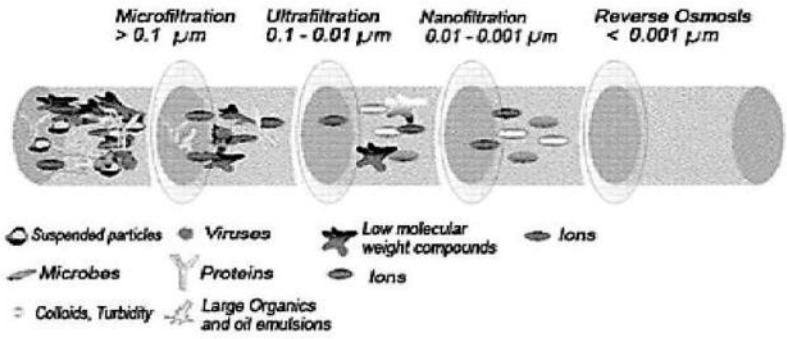



Figure 2.2: Membrane processes with pore sizes

## 2.3 Membrane Configuration

Depending on flow direction, filtration modes are divided into two categories a) cross flow and b) dead-end.

### 2.3.1 Cross flow

To generate the shear stress to scour membrane surface feed in cross flow arrangement moves parallel to the membrane.

### 2.3.2 Dead end

In dead-end arrangement no feed moves toward the filter medium i.e. membrane. All the particles that are filtered by membrane are settled on the membrane surface.

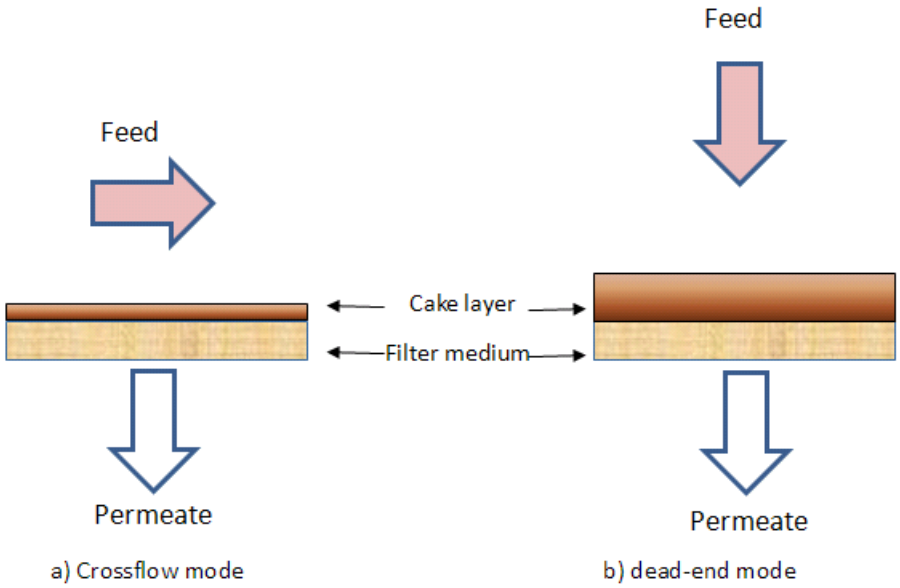



Figure 2.3: Membrane Configuration

## 2.4 Membrane Bioreactor systems

Reactors that produce or convert materials through function of natural living creatures or organisms are known as bioreactors. Typical bioreactor uses enzymes, microorganisms, plants or animal cells. Bioreactors are different from conventional reactors mainly due to the presence of living organisms in the reactor that operates under milder environment of temperature and pressure. The operating conditions ranges within bioreactors are generally determined by the microorganisms working inside. Membrane Bioreactor (MBR) systems basically consists of combination of membrane and bioreactor systems. These MBR systems are the promising technologies for a variety of advanced wastewater treatment processes (Aim [8]). Application of membrane bioreactor to treat municipal and industrial waste water is increasing day by day. The treatment capacity of MBRs for waste water treatment ranges from $< 1m^3$/day to $> 100,000m^3$/day (J. Zhanga [9])

## 2.5 History of MBR

In mid 1960s ultrafilter and microfilter MBRs were introduced at commercial scale. The idea was generated to replace settling tank in conventional activated sludge process with an economically feasible option. Replacement idea of clarifier in the activated sludge process was striking but was hard to substantiate where product had low financially viable value. As a result of this, the center of attention was the attainment of high fluxes mainly due to high cost membranes. Due to the high cost associated with the first generation MBRs they were used only in special needs. Yamamotos in 1980s suggested submerging the membranes in the bioreactor. Now commercially a wide range of MBR systems are available including submerged membranes and external modules are available. MBRs economic feasibility depends on modest energy input with effective membrane fouling control (P. Le-Clech [10]).

## 2.6 MBR process description

A MBR process is an alternative to the conventional treatment of waste water such as activated sludge process in which membrane is responsible to retain biomass inside the bioreactor to carry out the treatment of waste water. A membrane in bioreactor is responsible to retain biomass and impurities is in order to achieve mechanical strength and to sustain a preferred permeate with a high level of selectivity. Membranes with pore size from 0.1-0.4 $\mu$m i.e. microfiltration membranes and membrane with pore size ranging from $2 - 50nm$ i.e. ultrafiltration membranes are the two most important kinds of membrane used to treat waste water.(Abdel Kader [7]) Based on the location of membrane modules in bioreactor configuration of membrane is divided in two categories:

1. Submerged MBR system.

2. External MBR system.

In submerged MBR systems, membrane is submerged in aeration tank and permeate is extracted by suction. (Abdel Kader [7]) Polymeric membranes are usually used for such configuration. The membranes are either hollow fibers oriented vertically or horizontally in tubular or rectangular structure to provide a support, or flat sheets vertically adjusted within a support structure. On the shell side of membrane mixed liquor is located and into the lumen of membrane, effluent is extracted. On the permeate side negative pressure is created as a driving force across the membrane. Module of this arrangement contains considerably additional membrane area / unit volume comparative to external MBR system. Membrane module in this arrangement operates at TMP ranging between 28 to 56 kPa and at a cross-flow velocity $< 0.6$m/s.

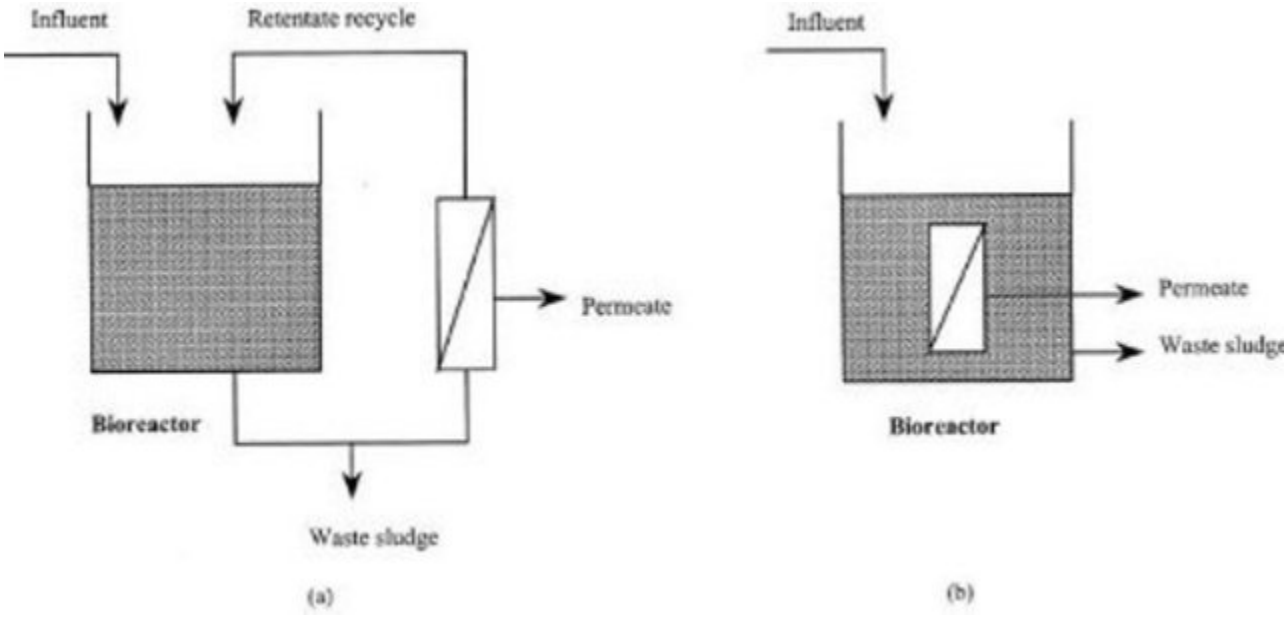



Figure 2.4: MBR process Description

In side-stream (external) configuration membrane bioreactor, membrane is placed outside the reactor and the MLSS/ MLVSS of reactor is recycled back to the reactor. Side-stream or external membrane configuration have high energy requirements as compared to submerged membrane module, mainly due to high operating TMP and high VFR essential to achieve the desired

CFV. On the other hand, external membrane reactors have the advantage that cleaning of membrane may be carried out easily as compared to submerged. Submerged membrane reactors have low energy requirements but the problem is that they function at low permeate fluxes, because they provide low shear of membrane surface. Commercially nowadays submerged membrane reactors are mostly preferred due to low energy requirements (E. Szentgyorgyi [12]).

Table 2.1: General comparison of iMBP and sMBR

| **Item** | **Unit** | **iMBR** | **sMBR** |
|---|---|---|---|
| Typical Configuration | -0.5 | Hollow fiber (HF) | Tabular (TB) |
| | | Flat sheet (FS) | Plate and Frame (PF) |
| Mode of operation | - | Crossflow | Crossflow |
| Operating pressure | KPa | 5-30 | 300-600 |
| Long term average flux | LMH (m/d) | 15-35 (0.36-0.84) | 50-100 (1.2-2.4) |
| Permeability | LMH/KPa | 0.5-5.0 | 0.07-0.3 |
| Recycle ratio | $m^3$ feed/$m^3$ of permeate | - | 25-75 |
| Superficial velocity | m/s | 0.2-0.3 | 2-6 |
| Membrane cost | $\$m^2$ | $< 50$ | $1 > 000$ |
| Capital cost | - | Low | High |
| Operatiing cost | - | -Low | High |
| Cleaning | - | Hard | Easy |
| Odor | - | High | Low |
| Packing Density | - | Low | High |
| Market share | - | 99% | 1% |

## 2.7 MBR for advance wastewater treatment

Existing and future legislations on wastewater discharge has led to the need of improved wastewater treatment processes competent to remove BOD, suspended solids, microorganisms and nutrients like nitrogen and phosphorus.

MBR is the most promising new process involving membrane filtration combined with biological reactor. In an MBR system membrane filtration occurs either inside the bioreactor or externally. The ultrafiltration or microfiltration membrane separates the rejected material, allowing the water to pass through the membrane. (M. Gander [13])

## 2.8 Aerobic and anaerobic MBRS

Due to unique advantages of membrane bioreactor like small footprint, high removal efficiency of organic matter and good effluent quality, MBRs have become very much popular for wastewater treatment (Nuwan A. Weerasekara [14] ). For last few decades membranes are used to treat water and wastewater. MBR technology combines biological treatment of wastewater with membrane filtration to separate solid and liquid. Using micro or ultra-filtration membrane in a reactor allows complete retention of suspended solids and biological flocs in reactor (Pierre Le-Clech [15]).MBRs are mostly used for treatment of wastewater that requires good quality effluent. MBRs allow higher concentrations of sludge and less sludge making and high COD and BOD enable removal efficiency. (Fangang Menga [16]). The use of membrane technology, particularly in combination with biological systems has fascinated a great attention in wastewater treatment. In particular adaptation of membrane together with an aerobic biological process offers the prospect of developing a competent wastewater treatment process being capable of completely retaining biomass inside the bioreactor and producing a good quality effluent. Up till now, some types of anaerobic bioreactor process together with membranes have been studied for the treatment of variety of wastewaters (Cheng Wen [17]).

## 2.9 Drivers and barriers of MBR

There are numerous main points in utilizing a MBR process, the prime ones being the treated water quality, the little foot print the plant, and less sludge generation and adaptability of operation. A primary advantage of MBRs is entire biomass maintenance in the aerobic reactor, which makes SRT free from the pressure driven maintenance time (HRT), permitting MLSS concentration in to rise in reactor. Additionally, the procedure takes out different pre-treatments as in ordinary frameworks and just needs screening (1-3 mm) for removal of bigger solids that could harm the membranes. The aggregate nitrogen removal in MBR is $> 30\%$ than traditional treatment systems. Truly, low membrane flux, low penetrability constrained extensive utilization of the MBR innovation. Likewise, the use of MBRs to wide scale was constrained by its high expenses, both capital and working use primarily because of membrane installation, maintenance and substitution and high energy requirement. This high energy requirement in correlation with a CAS, is nearly connected with techniques for abstaining membrane fouling (Er. Devendra Dohare [18]).

## 2.10 Membrane fouling

Membrane bioreactors (MBR) are advanced innovation for wastewater treatment. However membrane fouling is a major constraint to the cost effective operation of MBR. Roughly 30% of MBR publications deal with membrane fouling (V. Iversena [19]). Formation of biofilms or biocake on membrane surfaces that causes clogging of membrane pores is a bottleneck for the widespread use of membrane bioreactors. (Kim Sang ryoung [20]).Membrane fouling has been and will keep on being a noteworthy issue for the operators as well as designers of membrane bioreactors. Mostly MBR plants are worked at very steady flux keeping in mind the end goal to back off the fouling rate

of membrane and to lessen recurrence of cleaning. To comprehend the way of membrane fouling a broad effort has been made lately to control it. The level of fouling in a MBR is dictated by three fundamental fouling components:

Properties of membrane

Nature of feed

Hydrodynamic environment

Fouling of membrane can be biological, inorganic or organic. Limit between these fouling groupings is not fixed so their definitions may overlap. For instance, inorganic fouling can be a straight result of biologically actuated mineralization between biopolymers and salts and internal fouling brought about by the adsorption in layer pores of broke down natural and inorganic matter in MBRs is known to occur parallel with biofouling (Lilian Malaeb P. L.-C. [21])

## 2.11 Membrane fouling mechanism

Membrane fouling is the adsorption or trapping of materials present in the fluid passing through it in the pores of a membrane. Fouling may be chemical or physical phenomena. Typical foulants of membrane are proteins, bacterias and lipids. The basics of membrane fouling are studied by number of researchers. According to the study of different researchers it is found that membrane fouling happens due to following means:

- Formation of dynamic membrane/ filter cake

  In the event of ultrafiltration membrane, fouling happens at the layer surface

- Fouling within the membrane structures

  Occurrence of ultrafiltration membrane fouling at the membrane surface

is more when contrasted with the membrane fouling inside the membrane structure. In case of microfiltration there is a greater deposition of foulants inside the pores as compared to the deposition at membrane surface (Zhao Yan-jun [22])

## 2.12 Types of Membrane Fouling

Fouling of layer is characterized by the kinds of foulants. The primary contrast between the sorts of fouling (colloidal fouling, natural fouling, scaling and biofouling) is the way of the particles that causes the fouling. Furthermore, membrane fouling can be separated into reversible and irreversible fouling taking into account the connection force of particles to the surface of membrane. Reversible clogging can be uprooted by method of backwashing. Development of a solid fouling layer with the solute in filtration procedure results in reversible fouling that is then changed into irreversible fouling layer. Sturdy connection of particles causes irreversible fouling, which is illogical to be uprooted by physical cleaning method.

### Colloidal fouling

Colloids are fine particles with size range of 1-1000 nm. These fine particles have a strong propensity to foul membranes in pressure-driven membrane systems, bringing a considerable loss in water mobility and producing water with a bad quality (Chuyang Y. Tanga [23])

### Organic fouling

Fouling linked with in both drinking water and wastewater is known as organic fouling. It is a most important limitation to application of membranes in water and wastewater treatment.(Amy [24])

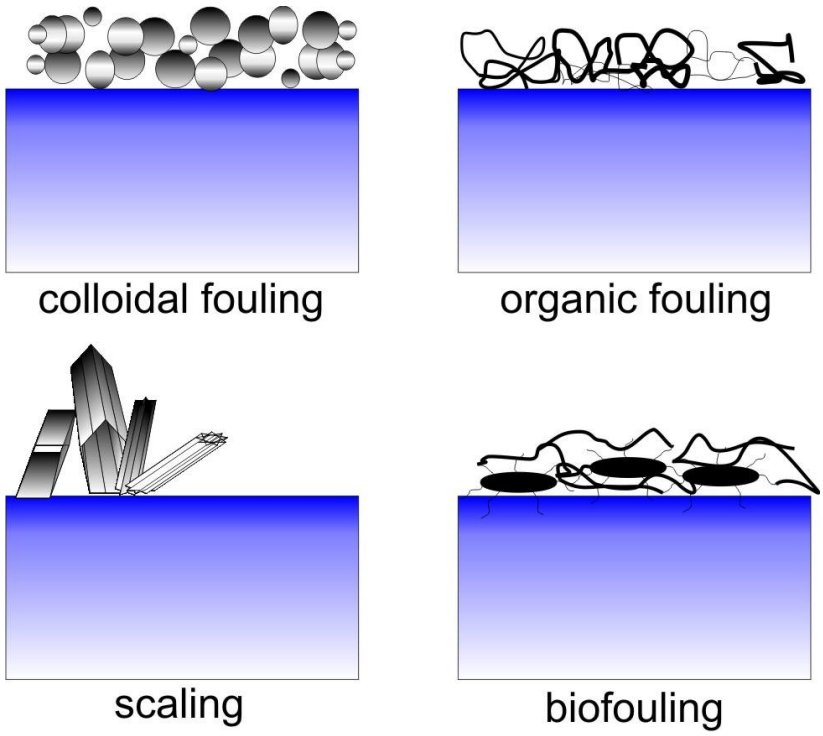



Figure 2.5: Types of membrane fouling

**Scaling**

Deposition of particles on a membrane causing it to plug is referred as scaling. Scaling results in high energy use and reduces a life span of the membranes. Scaling causes the reduction in nominal flux. This makes the membrane water treatment process much more expensive. Addition of acids can prevent the precipitation of salts thus will result into decrease in scaling (Rotterdamseweg [25])

**Biofouling**

For over 20 years membrane bioreactors (MBRs) have been in commercial use, membrane biofouling on the membrane surface still remains a major constraint that confines their extensive application. Biofouling is defined

as unwanted collection over time of microorganisms at a transition phase, which happens by growth and deposition of bacterial cells on the membranes. Following steps are usually concerned in the biofilm creation.

1. Formation of a conditioning film (macromolecules, proteins, etc.);
2. Planktonic cells attachment onto membrane surfaces
3. Micro colonies formation by primary bio adhesion
4. Mature biofilm development

Biofilms could possibly consistently cover the membrane surface and comprises of various layers of living and dead microorganisms and their extracellular items. Microorganisms' aggregate on layer by growth and development (Wenshan Guoa [26]).

## 2.13 Factors contributing or affecting membrane fouling

In MBR forms every one of the parameters included in the operation and configuration of MBR process affect membrane fouling. Typically, membrane fouling is brought on by three kind of factors characterized as, membrane, attributes of membrane module, feed qualities and parameters of biomass and working conditions (J. Zhanga [9]).

## 2.14 The impact of membrane material and its physicochemical properties

Two characteristics of membrane membranes have great influence on membrane fouling

**a)** Porosity, pore size and morphology of membrane

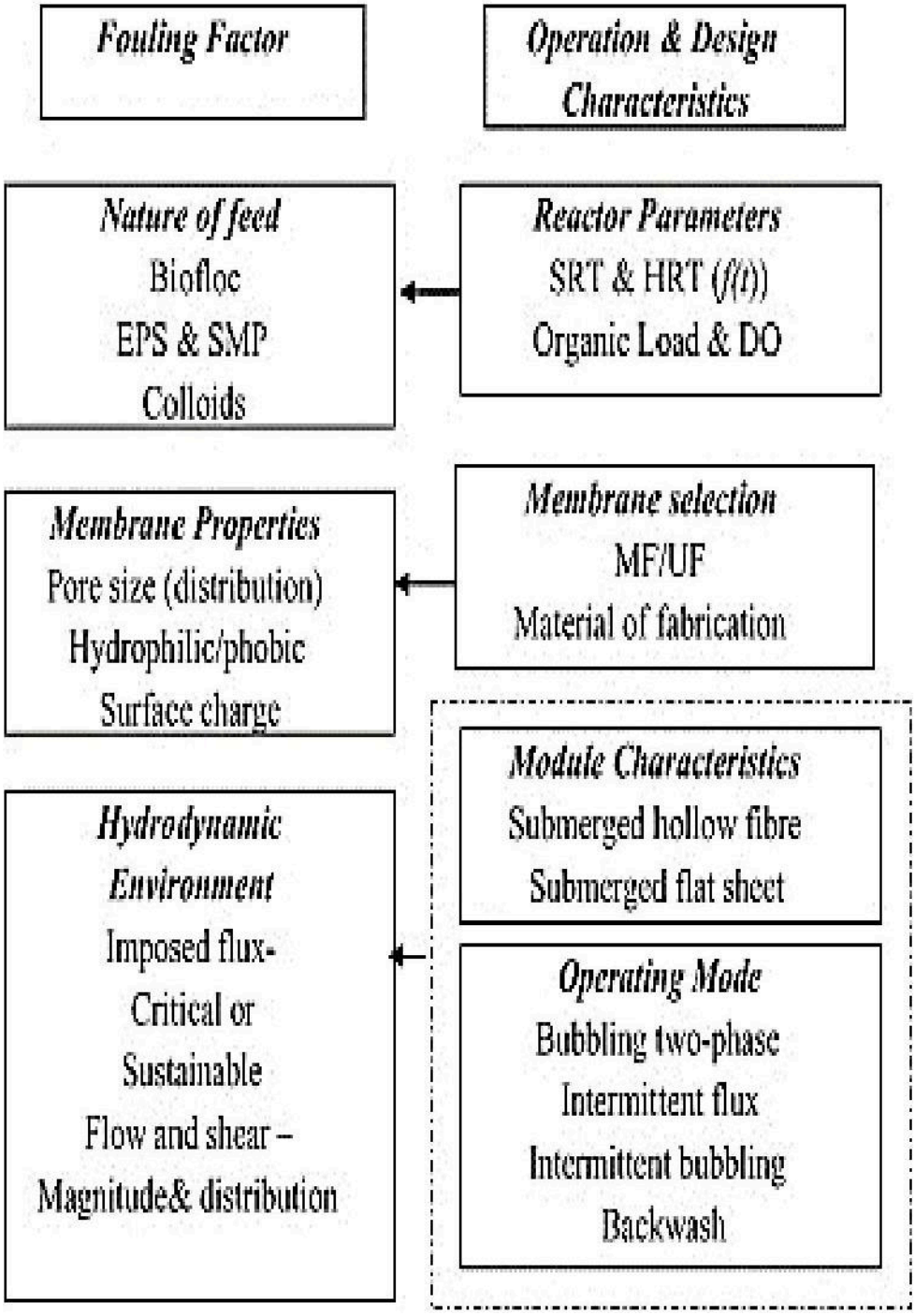



Figure 2.6: Factors affecting membrane fouling

**b)** Physico chemical properties of membrane

Extracellular polymeric substances (EPS) in bound or colloidal structure are at present considered as the actual reason of membrane fouling in a MBR. Therefore, two aspects need to be thought about: the occurrence and concen-

tration of SMP, from one viewpoint, and their properties like atomic weight and fouling potential, on the other. As to previous, a few components like the kind of wastewater , microbial growth sludge age, MLSS concentration, sludge loading rate, and mechanical stress are considered to have impact on the concentration of EPS and SMP(A. Drewsa [27]).

***I.*** **Pore size and distribution**

There is an ideal pore size of a membrane beneath which it limits the flow of permeate or beyond which membrane fouling lessens the permeate flow. The impact of pore size on membrane fouling is identified with the qualities of a feed and especially the PSD. Results of literature has reported inverse pattern that if molecule size is smaller than pore size of a membrane then pore blocking is expected. Accordingly it is normal that MF membrane with bigger pore would display higher fouling inclination when contrasted with UF membranes (Pierre Le-Clech [15])

***II.*** **Porosity/ Roughness**

Mostly UF and MF membrane have broad pore size distribution (PSD). The permeate flow is dominated by the flow through the largest pores and as an end result the permeate flux is very much responsive to foul the large pores(Herbert H.P. Fang [28])

***III.*** **Membrane arrangement**

The current pattern in MBR design support submerged arrangement over side stream in common of the readingsallocating with treatment of greywater. In view of critical flux tests, evaluation between sunken and sidestream MBRs showed when the two designs were worked at apparent gas velocity (UG) of 0.070.11 m/s for submerged and apparent fluid velocity (UL) of 0.250.55 m/s for sidestream. An increment of superficial gas velocity in the sunken MBR was establish to have extra impact on evacuation of fouling than a raise of critical flow velocity in the sidestream arrangement (P. Le-Clech [10]) Performance of flat sheet and

hollow fiber submerged MBR for sewage treatments were compared. The differences were observed in fouling rates of both types of membranes mainly due to the different working and repairing conditions rather than the design of a module. Price of hollow fiber systems is estimated to be 25% lower than that of flat sheet systems. But membrane fouling rate repairs and operation of hollow fiber based systems are generally less than flat sheet based systems (Judd [29])

**b) Physico Chemical properties**

***IV.*** **Hydrophobicity**

Membrane fouling occurrence in hydrophobic membrane is more serious than hydrophilic membranes on account of the hydrophobic associations between microbial cells, solute and membrane. Mostly in studies it is reported that with membrane modifications such as change in morphology and pore size, membrane hydrophobicity often occurs. (Pierre Le-Clech [15]). According to a study conducted the estimation of contact angle demonstrated that the hydrophobicity of polyether sulfone (PES) membranes diminished with the increment in sub-atomic weight cut-off.

***V.*** **Charge effect**

Charges on membrane surface are greatly dependent on pH and ionic strength of feed solution and membrane material. The effect of feed properties

***VI.*** **Feed concentration and nature**

Wastewater effect on membrane fouling is unquestionable. Most of the researchers have found that with rise in concentration of wastewater, permeate flux abatements and it has impact on membrane retention qualities, aside from in the event that when part sizes changes with shape. Increase in feed concentration has little impact on irreversible membrane fouling where surface fouling happens yet purposes an increment in reversible gel formation. In the event that when internal

membrane fouling rules, membrane fouling increases with rise in feed concentration (M. Gander [13]).

***VII.*** **PH and ionic strength**

Function of pH and ionic quality on membrane fouling is essential. The pH and ionic quality of the feed or wastewater has an extraordinary influence on the charge of the membrane and the charge of the particles. Along these lines variation in pH and ionic quality of wastewater changes the charge on membrane and, and in this way impacts the adhesiveness of particles on membrane surface and thus effects the size of the cake. (A. Abdelrasoul [30])

***VIII.*** **Interaction of Components**

Occurrence of bigger particles in feed causes a hindrance to the flow of small particles through membrane. Mainly because these bigger particles form a active surface over the membrane surface causing a change in porosity of membrane (Herbert H.P. Fang [28])

***IX.*** **SMP and EPS**

EPS and MPSS have been given more value among distinctive foulants parameters of membrane fouling. The EPS forms a hydrated gel-like, and frequently three-dimensional, charged biofilm grid, in which microorganisms are settled in (J [31]). For microbial cells EPSs have a considerable measure of utilities. The EPS may capture, tie and concentrate organic materials nearly to the cells. Extracellular chemicals that are additionally confined near the cells can hydrolyze the sorbed organic matter (B.E [32]). MPSs are beneficial for the microbial growth but MPSs are the most important membrane foulants in MBRs used for wastewater treatment. In many studies their connection with membrane fouling were reported. EPS was reported to be key factor of membrane fouling and in this way utilized as an indicator of membrane fouling (C.M [33]) Especially main indicator of membrane fouling was reported

to be carbohydrate concentration of SMP and to have a strong connection with critical membrane flux filtration resistances specific cake resistance and fouling rate. Protein concentration of SMP was also reported to play a significant role in membrane fouling (Frolung [34])

**Impact of processing variables**

***X.*** **Transmembrane pressure (TMP)**

Increasing TMP increases in permeate flux but in addition results in increased fouling rate. In microfiltration raise in membrane fouling with boost in TMP is greater than in case of ultrafiltration. At a low pressure and low concentration with increase in membrane fouling, membrane resistance increases. There is an optimum pressure to maximize the permeate flow, below this pressure driving force is too low and may cause a large reduction in membrane flux (Zhao Yan-jun [22])

## 2.15 Membrane biofouling

Biofouling is an active, composite and moderate process among a wide range of fouling. As an aftereffect of two components biofouling occurs:

1. Microorganisms colonization on membrane surface.
2. Creation of foulants by microorganisms.

It has been proved by studies that both microorganisms and their products add to membrane fouling furthermore these studies upgraded membrane permeability. Quorum quenching focuses on the microorganisms and foulants, for example, EPS and SMP. It is difficult to verify what of the two systems by using analytical approaches. However, with progression in analytical categorization instruments it will be conceivable to conduct methodical studies that clear up the key players in the biofouling process and connect the qualities of the microbial groups and their products to biofouling rates (Lilian Malaeb

P. L.-C. [21]). Biofouling represents limitation of the membrane process in light of the fact that microorganisms duplicate after some time; regardless of the possibility that 99.9% of them are uprooted still there are sufficient cells remaining which can keep on developing. Biofouling is a causative element to more than 45% for fouling of membrane and in this way stated as a remarkable issue. Biofouling can have a few unfavorable effects on membrane, for example,

Membrane flux reduction.

Increase in feed and differential burden is required.

Membrane biodegradation owing acidic by-products e.g. cellulose acetate.

Increase in passage of salt through membrane and reduction in water quality

Increase in energy consumption due to high resistance to flow (Thang Nguyen [35])

### 2.15.1 Factors affecting biofouling

The important factors affecting biofilm formation on membrane depends on carbon: nitrogen: phosphorus ratio, redox potential, pH and temperature. Extracellular polysaccharide (EPS) is the substance produced by microorganism is responsible for the slimy nature of biofilms (Baker and Dudley [36]).

Table 2.2: Factors disturbing microorganism connection to membrane surfaces

| Microorganism | Surface | Feed water |
|---|---|---|
| Species | Chemical composition | Temperature |
| Composition of mixed population | Surface charge | pH |
| Population density | Hydrophobicity | Dissolved inorganics |
| Growth phase | Conditioning film | Suspended matter |
| Nutrients status | Roughness | Viscosity |
| Hydrophobicity | Porosity | Shear forces |
| Charges | | Boundary layer |
| Physiological responses | | Flux |

## 2.16 Fouling control Strategies

A number of methods to control of membrane biofouling have been created throughout the most recent two decades. Membrane fouling control and obstructing particles is for the most restricted to following principle procedures:

Application of pretreatment to the feed water

Employing proper physical or chemical cleaning

Flux reduction

Aeration

Modification of mixed liquor chemically or biochemically modifying

Membrane surface transformation (Petros K. Gkotsis [37])

Permeate backwashing

Using gas to scour membrane surface (B. Siembida [38])

Novel biological approach using quorum quenching bacteria.

Various innovations such as microporous membranes replacement by low cost mesh filters, addition of coagulants and flocculants such as aluminum

and ferric chloride for feed pre-treatment and powdered activated carbon have been attempted to improve the economics of membrane systems(Yamini Satyawali [39]).

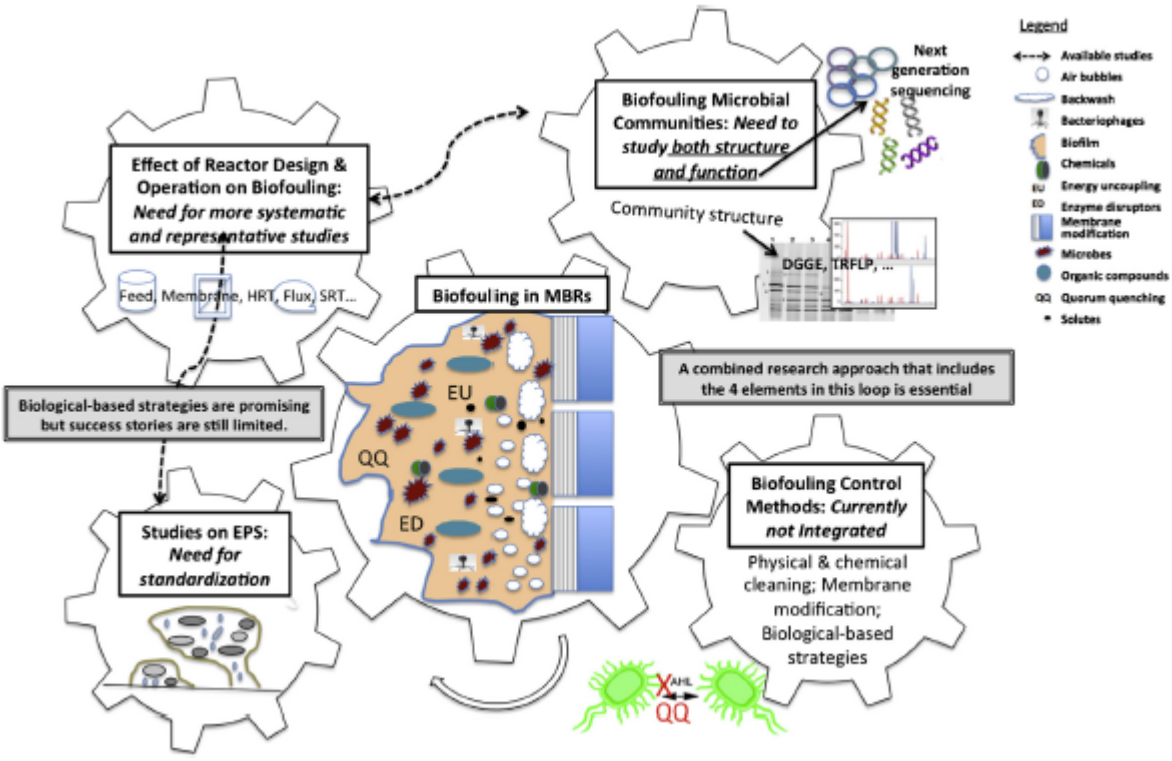



Figure 2.7: Membrane biofouling: a comprehensive overview for an integrated control

## 2.17 Quorum Sensing

Cellcell correspondence between microorganisms is known as majority detecting, which decides phenotypes, for example, discharge of extracellular polymeric substances (EPS), biofilm development and destructiveness. To convey and asses the population, microscopic organisms utilizes the dialect of signaling molecules called auto inducers a procedure known as quorum sensing. The method of sensing depends on the creation, discharge and uptake of auto inducers in the encompassing medium, whose concentration corresponded to

Table 2.3: Membrane biofouling control approaches

| **Method** | **Description of biological based antifouling strategy used in MBR** | **Type of MBR (flux)** | **Enhancement of permeability in terms of TMP(compared to ) control MBR)** |
|---|---|---|---|
| ED | Bioassy with Agrobacterium tumefaciensA136 supplemented with spectinomycin and tetracycline to maintain two plasmids that provide the AHL response system | Batch MBR with total recycle mode [15 Lmh] | 32 h to reach 40 KPa; [20h in control] maximum TMP 48 KPa at at 40 h [70 KPa at 23 h for control] |
| ED | Magentic enzymes carriers prepared by immobalizing the QQ Porcine kidney acylase I on a magnetic carrier and recycled back from the redrawn sludge | Batch MBR with total recycle mode [15 Lmh] Continous MBR [15 Lmh] | Maximum TMP 36-39 KPa [76-79 KPa in control] in 3 operation cycles (15-20h) TMP 10 KPa throughout the experiment [30KPain 48h for control] |
| QQ | QQ bacteria encapsulated inside a porous vessel (microbial-vessel) recirculation at 7.5-30 ml/min | Continous MBR [30Lmh], filtration (60 min),relaxation (1 min),MLSS | Maximum TMP 30 KPa at 68h [48h in control] |

the density of secreting microorganisms in the region. The essential system of QS is the connection of Autoinducer specifically or through actuation of sensor kinaswe with a transcriptional controller. Gram-positive and Gram-negative microscopic organisms use particular auto inducers for quorum sensing actuation. To communicate population densities in microorganisms local sensors function as a QS signaling molecules. Receptors have been exten-

sively partitioned into three noteworthy classes: N-acyl homoserine lactones (AHLs) created by Gram-negative microscopic organisms; oligopeptides or auto inducing peptides (AIP), comprising utilized by Gram-positive microorganisms; autoinducer-2 (AI-2), a ribose subsidiary utilized by Gram-positive and Gram-negative microbes for correspondence.

## 2.18 Quorum Quenching

In recent times, novel organic methodologies have been endeavored to control biofouling by utilizing quorum sensing.The quorum quenching is a method through which quorum sensing is broken up. Quorum sensing assists bacterias with communicating and organize, yet is not key for development of bacteria's. Consequently, obstruction with quorum sensing may prompt the hindrance of arrangement of biofilms. Since quorum sensing is included in arrangement of biofilms along these lines focusing on quorum sensing has offered a novel approach to decrease film biofouling without bacterial development (Harshad Lade [40]).

## 2.19 Role of QS in biofilm

QS systems plays a prominent role in biofilm development. Biofilm development is a procedure that includes bond of cells to layer surface, development of micro colonies and development, and separation of matured microorganisms. QS systems are involved in all stages of biofilm formation (Matthew R. Parsek [41]). There are several microbial factors that have been shown to have a great influence on biofilm formation, including EPS production, motility and surface appendage expression. The impact of QS on biofilm formation was described by Davies et al in 1998 in Pseudomonas aeruginosa. However, successive studies showed that the effect of QS in biofilms structure

was dependent on experimental conditions (Lin Feng [42])

## 2.20 Quorum quenching control strategies for quorum sensing

A few quorum quenching methodologies are accessible through which the procedure of quorum sensing can be interfered. It incorporates;

- AHL amalgamation hindrance by blocking t LuxI-sort synthase proteins.
- AHLs particles enzymatic pulverization by AHL-acylase and AHL-lactonase that will keep their gathering.
- AHL/LuxR complex development blockage or obstruction with sign receptors. Notwithstanding this, quorum quenching method has been beforehand ended up being an objective for both quorum sensing signal synthase and sensors or response controller proteins. These systems can be connected to hinder AHLs-interceded quorum sensing in Gram-negative and AIPs-intervened quorum sensing in Gram-positive microscopic organisms (Harshad Lade [43]).

## 2.21 Relevant studies carried out on quorum quenching (QQ)

(Xiang-Ning Song [44]) In his study he found an acyl homoserine lactones-debasing enzymatic movement, a QQ impact, in activated sludge and discovered it to influence the QS recognition results. Bacterial screening and denaturing inclination gel electrophoresis examination affirmed the concurrence of QS and QQ microscopic organisms in activated sludge. (Muhammad Faisal Siddiquia [45]) Researched the counter quorum sensing movement of PBE from the Piper beetle was identified to relieve bio-

fouling of layer in MBR. Agrobacterium tumefaciens strain NTL4 was utilized to decide the generation of AHLs in. The biocake displayed AIs movement, which demonstrated that QS was in pleasant association with film biofouling. PBE was affirmed to moderate layer biofouling through AIs generation restraint. It was likewise found that the expansion of PBE diminished the measure of EPS in biocake; while the expansion of HHL expanded the measure of EPS arrangement. In this manner discoveries of this study uncovered that PBE could be a novel operators to target AIs for film biofouling control. (Sang-Ryoung Kim [20]) Arranged and described "macrocapsule" encapsulation of QQ bacteria and afterward explored their soundness in an unforgiving synthetic condition furthermore its possible for the control of membrane clogging in the persistent membrane bioreactor bolstered with genuine greywater. The QQ microscopic organisms capturing macrocapsules demonstrated a fantastic against biofouling limit in the nonstop layer bioreactor (MBR) bolstered even with genuine wastewater. Results demonstrated that full scale containers were skilled of keeping up QQ action more securely than alginate dots under unforgiving natural conditions. (SangRyoung Kim [20]) In this study, quorum quenching microscopic organisms captured in free-moving globules were connected to the hindrance of biofouling in a MBR. Permeable microstructure cell ensnaring globules (CEBs) were readied by entangling quorum quenching microscopic organisms (Rhodococcus sp. BH4) into alginate dabs. The moderation of biofouling was credited to both physical (contact) and organic (quorum quenching) impacts of CEBs, the last being a great deal more vital. Results demonstrated that due to the CEBs with ensnared quorum quenching microorganisms, EPS generation from microbial cells in the biofilm was lower, and in this manner empowered biofilm to quagmire off from the film surface all the more effectively.

# Chapter 3

# Materials and Methodology

## 3.1 Experimental setup

Two bench scale membrane bioreactors were installed at IESE-Wastewater laboratory (Fig 3.1).The volume of each reactor was 6 liters. The acclimatized sludge was taken from the pre-installed membrane bioreactor setup in NUST. The initial concentration of sludge was 8 g/L and kept constant through the study by keeping Solids retention time (SRT) of 20 days by wasting daily sludge 250 ml calculated from the following formula

$$Sludgedischarge(Q)(ml) = \frac{Volume(ml)}{SRT(days)} \tag{3.1}$$

Table 3.1: Working parameters for the lab scale MBR

| **Parameter** | **Value** |
|---|---|
| Working volume | 5 L |
| MLSS | 8 g/L |
| SRT | 20 days |
| HRT | 4 hours |
| Flux | 20 $L/m^2/h$ |

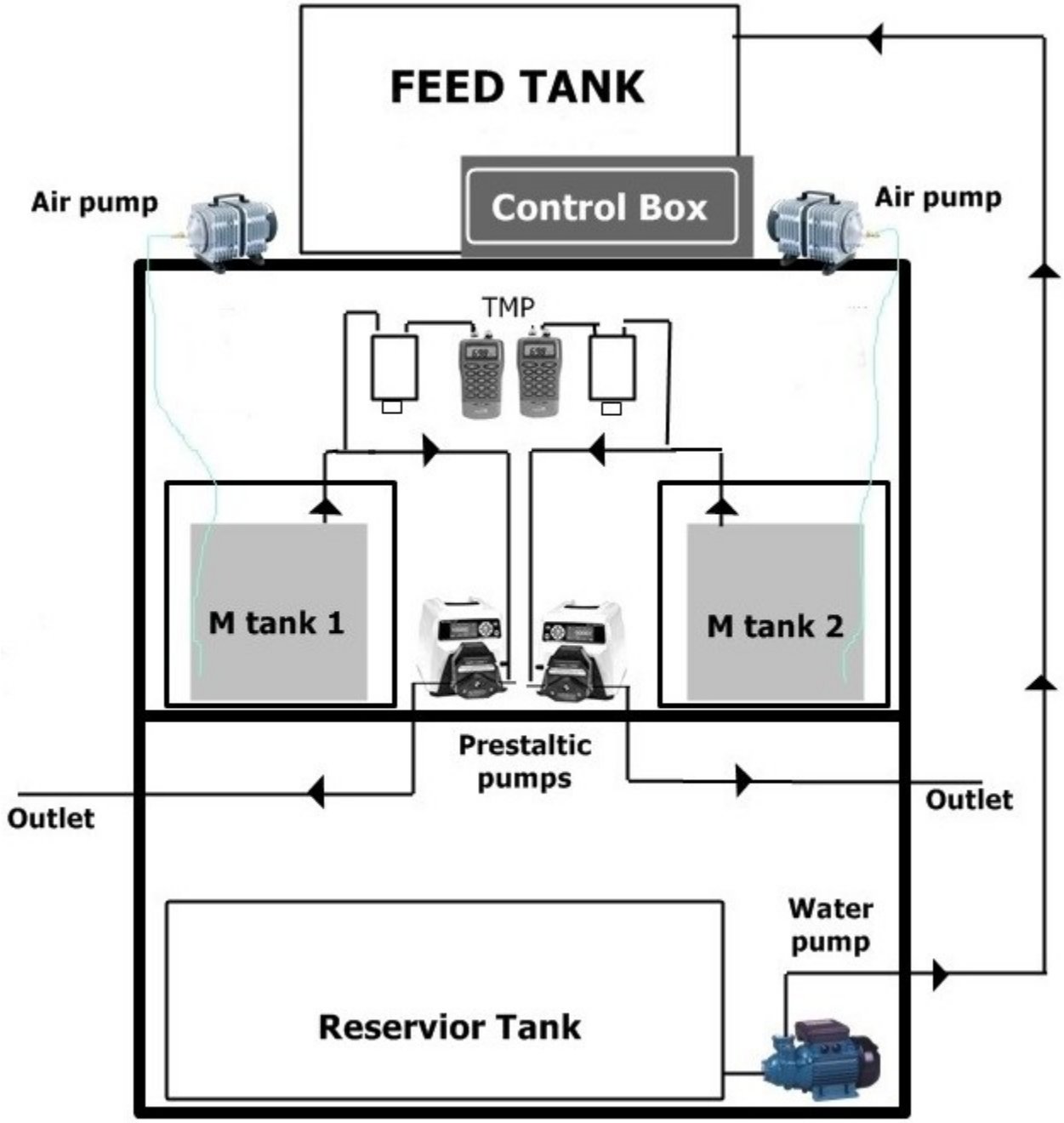



Figure 3.1: Lab Scale MBR

Both MBRs were operated with optimized filtration and relaxation mode, 8 min filtration with aeration and 2 min relaxation without aeration using peristaltic pumps (Master flex, USA). Air was supplied with the help

Figure 3.2: Lab Scale MBR without sludge

of air compressor (HAILEA ACO-208) at a rate of 8L/min for coarse bubbling throughout the membrane bioreactor to maintain dissolved oxygen (DO) concentration for microbes to grow, to degrade the wastewater, to create turbulence for membrane scouring and to avoid dead zones at the bottom of bioreactor. To maintain the sludge concentration of 6-8 g/l the extra sludge

was wasted to keep SRT of 20 days, with HRT of 4h at a flux of 20 LMH (Table 3.1).Water trapper with TMP meter was used to find the TMP profile throughout the study. Relay units with water controller level used to maintain the level of water in the bioreactor and feed water stored in storage tank from where it pumped to the overhead tank.

## 3.2 Wastewater composition

The recipe for the synthetic feed wastewater was: glucose (514 mg/L), $NH_4Cl$ (190mg/L), $KH_2PO_4$ (55.6 mg/L), $CaCl_2$ (5.5 mg/L), 5.7 mg/Litre of Magnesium sulfate, $FeCl_3$ (1.5 mg/L), $MnCl_2$ (1 mg/L) and $NaHCO_3$ to keep pH 7.0-7.5.

Table 3.2: Synthetic Wastewater Composition

| **Chemicals** | **Formula** | **Quantity (mg/L)** |
|---|---|---|
| Glucose | $C_6H_{12}O_6$ | 514 |
| Ammonium chloride | $NH_4Cl$ | 190 |
| Calcium chloride | $CaCl_2$ | 5.7 |
| Potassium di-Hydrogen phosphate | $KH_2PO_4$ | 55.6 |
| Magnesium sulphate | $MgSO_4.7H_2O$ | 5.7 |
| Ferric chloride | $FeCl_3$ | 1.5 |
| Manganese chloride | $MnCl_2.4H_2O$ | 1 |
| Sodium bicarbonate | $NaHCO_3$ | 120 |

## 3.3 Membrane characteristics

Poly vinyl di fluoride (PVDF) membranes were used because of their resistance to acid and base chemicals and higher infiltration rate. The membrane module was developed by Mitsubishi rayon, Japan. A single membrane con-

Table 3.3: Membrane characteristics

| | |
|---|---|
| Manufacturer<br>Membrane material | Mitsubishi rayon, Japan<br>PVDF |
| Pore size | 0.05 $\mu m$ |
| Filtration area | $0.07m^2$ |
| Suction pressure | 10-30 KPa |
| Temperature | 15-35 $^{\circ}C$ |

sists of a bundle of hollow fibers (HF). The fibers are horizontally connected to module on both the ends. The membrane type was outside-in because water flows from the outside of fibers in to the hollow fibers.

## 3.4 Continuous operation of Setup

Both the reactors were filled with distilled water and filtration cycle was operated to check membrane resistance rm. This method was also used to check the flux of membrane as per RPM of peristaltic pump and then RPM was set for the required flux rate in both pumps. Sample of sludge was taken to determine initial characteristics of sludge (MLSS, MLVSS, EPS, AHL, SCR, CST etc.) so that difference may be spotted between initial sludge characteristics and change in them after membrane rejection.

### 3.4.1 Resistance evaluation

Resistance was used to check the membrane fouling potential of both MBRs.

$$Rt = \frac{\Delta P}{\mu . J . ft} \tag{3.2}$$

Where,

$Rt$ =total hydraulic resistance (1/m)

$\Delta P$ = TMP (Pa)

$\mu$ =permeate dynamic viscosity (Pa.s)

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

$ft$ = temperature correction factor correspond to 20°C, $ft = \exp^{0.0239(T20)}$

$R_t = R_c + R_p + R_m$

$R_m$ = basic membrane objection/resistance

$R_p$ = holeobstacleobjection/resistance

$R_c$= resistance by cake layer

$R_c$ was developed because of cake developed on the membrane surface, $R_p$ was because of small microbial flocs which blocked the membrane pores, $R_t$ was calculated from $R_m + R_p$, cake from membrane surface was removed and membrane was placed in distilled water followed by TMP and flux measurement and $R_c$ was measured by subtracting $R_p + R_m$ from $R_t$, $R_m$ was measured after cleaning membrane chemically and passing DI water (Wang et al. [46]). Each type of resistance and their effect was compared in both MBRs.

### 3.4.2 Preparation and inoculation of macrocapsules

1. Entrapment of bacterial species in alginate matrix separately.
2. Cultured QQ bacterial species are centrifuged at 12000 rpm for 15 min.
3. Re Suspend in 10 ml distilled water.
4. Mix suspension (1) with 90 ml of alginate solution 2% w/v.
5. Drop mixture (2) into 500 ml of $CaCl_2$ solution 4% w/v.
6. Stirring for 30 min.
7. Dissolve Polysulfone pellets in NMP at $60°C$ for 12 hours.
8. Stirring of polymeric solution for 24 hours.
9. Immerse alginate beads in polymeric solution for 30 seconds.
10. Immerse beads in a water bath for 1 hour.
11. Macro capsules were splashed and kept at $4°C$.

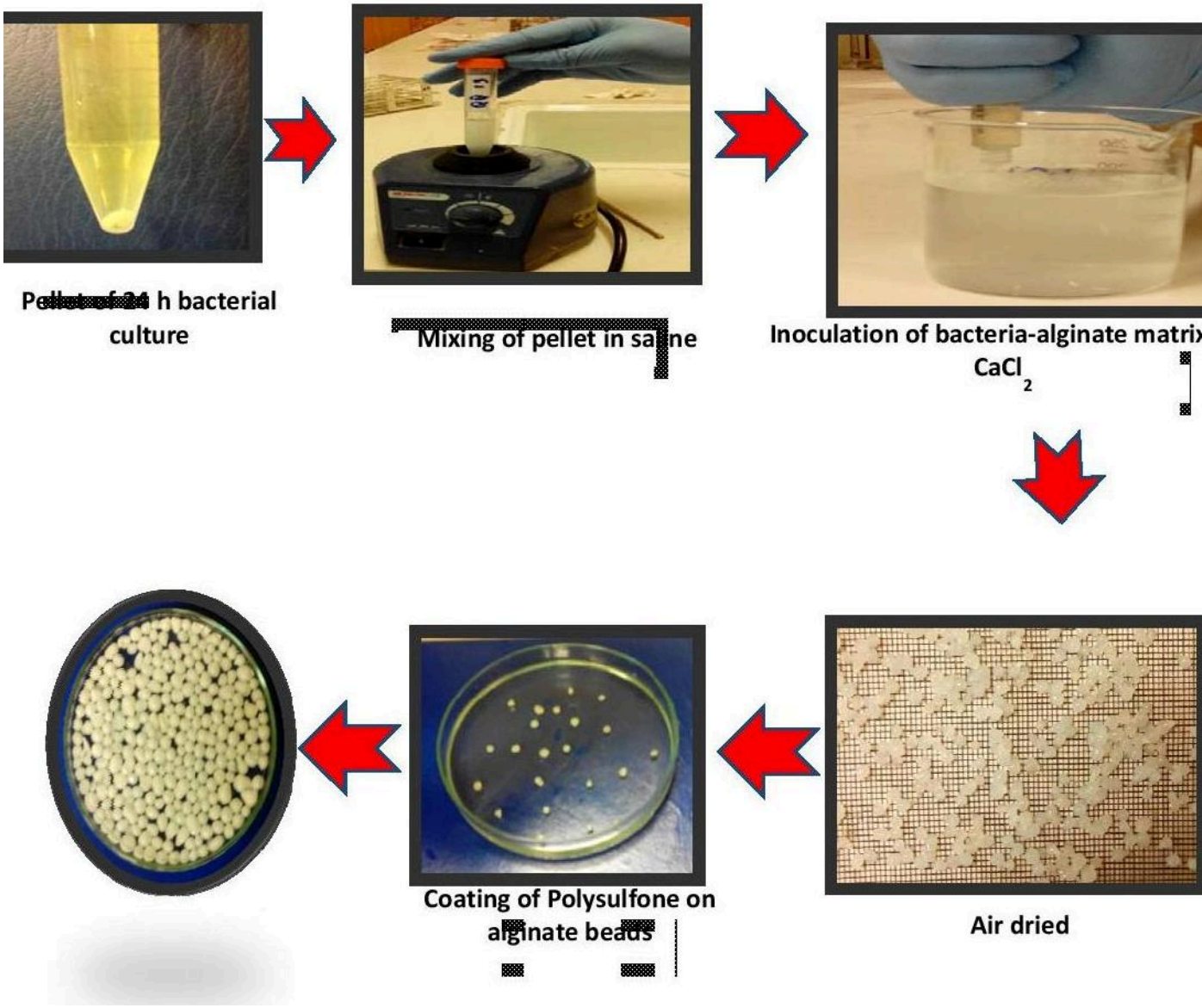
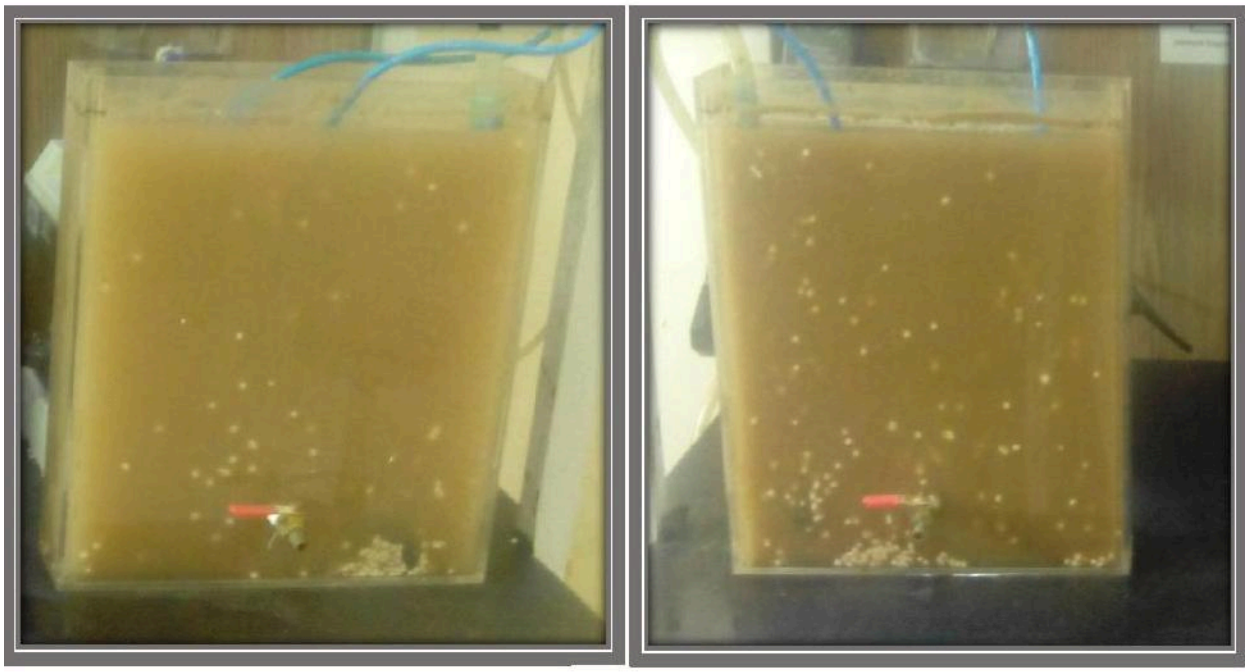



Figure 3.3: Schematic for thepreparation of macrocapules

### 3.4.3 Inoculation of QQ bacteria

In both the membrane bioreactors the quorum quenching bacteria was added in the form of consortium. Rhodococcus with the addition of Delftia and Pseudomonas species were introduced in bioreactor 1 while in bioreactor 2 Enterobacter was the leading specie with the addition of Delftia and Pseudomonas.

The effective volume of beads was almost 1% as compared to reactor working volume.



| $M_1$ | $M_2$ |
|---|---|
| **QQ-MBR$_R$** | **QQ-MBR$_I$** |
| *Rhodococcus sp.* | *Enterobacter cloacae* |
| *Delftia sp.* | *Delftia sp.* |
| *Pseudomonas sp.* | *Pseudomonas sp.* |

Figure 3.4: QQ bacterial consortium in MBRs

## 3.5 Analytical methods

Following tests were done to check performance efficiencies of different parameters involved in study of MBRs.

**Effluent quality**

COD

Ammonia

Nitrate

Nitrite

Phosphate

pH

**Sludge characteristics**

Mixed liquor suspended solids (MLSS)

Mixed liquor volatile suspended solids (MLVSS)

Sludge volume index (SVI)

Capillary suction time (CST)

Specific cake resistance (SCR)

Extra cellular polymeric substances (EPS)

N-Acyl homoserine lactones (AHLs)

**Membrane performance** Trans membrane pressure profile (TMP)

**Microbial activity**

Specific oxygen uptake rate (SOUR)

DO

**Resistance analysis**

$R_t$ = total hydraulic objection/resistance

$R_m$ = intrinsic membrane objection/resistance

$R_p$ = holeobstacle objection/resistance

$R_c$ = resistance by cake layer

## 3.6 Effluent quality analysis

### 3.6.1 COD

COD of samples was done by close reflux method in which COD a vile was prepared as follow

2.5 ml sample

1.5 ml $K_2Cr_2O_7$ solution(0.0166 M)

3.5 ml $H_2SO_4$ reagent

These vials were then digested for 2 hours at 1500C.after digestion the sample was titrated with ferrous ammonium sulphate FAS (0.25M) with ferroin indicator till the color changes from yellow to brown. COD (mg/L) of sample was calculated by:

$$COD = \frac{A - B \times 8000 \times M}{Sample\ volume} \tag{3.3}$$

where

A= for the titration of blank, FAS used (by volume)

B= for the titration of sample, FAS used, M= molarity of FAS

### 3.6.2 Nitrates and phosphates

Nitrates and phosphates in samples of wastewater and treated effluent were analyzed using UV-visible spectrophotometer which works on principle of beer lambert law i.e.Absorbance is directly proportional to concentration. Standards of known concentrations were prepared and their absorbance was measured to make a standard curve between absorbance and concentration. Absorbance of influent and influent samples were then plotted on graph to find their concentration using $y = mx + c$.

The main principle for both nitrates and phosphates is explained as above. The only exception is change in reagents used for making standard solutions as recommended by APHA (2012).

## 3.7 Sludge Characteristics Analysis

### 3.7.1 MLSS/MLVSS

**Apparatus:**

Filtration assembly, 10 ml pipette, whattman filter paper, china dish, oven, muffle furnace, stop watch.

**Procedure:**

Whattman filter paper is first dried in oven at 105°C for 10 minutes to remove moisture from filter paper if any.

It is then placed in desiccator till it cool down to room temperature and initial weight of paper is noted.

Then it is fitted on filtration assembly.

10 ml of sludge sample is taken from reactor during aeration to obtain uniform sludge sample.

Pour that 10 ml sludge sample on filter paper that is fitted on filter paper and start the filtration assembly.

When all the water drains out remove the filter paper. A sludge cake will be developed on filter paper.

Put that filter paper in china dish and put it in oven at 105°C for 1 hour.

After 1 hour take it out note the weight of filer paper again after it cools down to room temperature in a desiccator.

Then put it in muffle furnace at 550°C for 15 minutes.

Weigh the filter paper again after cooling it down in desiccator.

MLSS and MLVSS will be calculated by following formulas

$$MLSS = \frac{(A - B) \times 1000}{Volume\ of\ sample} \tag{3.4}$$

Where

A= weight of filter paper + residues after drying at 105°C

B= weight of empty filter paper after drying

$$MLVSS = \frac{(A - B) \times 1000}{Volume\ of\ sample} \tag{3.5}$$

Where

A= weight of filter paper + residues after drying at 105°C

B= weight of filter paper + residues after ignition at 550°C

MLVSS to MLSS ratio was calculated to find out percentage of biomass present in sludge sample.

### 3.7.2 SVI (Sludge Volume Index)

Sludge volume index is parameter used to check settling characteristics of sludge.it is actually the volume occupied per gram of activated sludge.

**Apparatus:**

Settling column, stop watch

**Procedure:**

- Determine the MLSS concentration of sludge sample.
- Then pour well mixed 1 liter sludge sample in settling column.
- Let it settle for 30 minutes.
- Note the settled sludge volume.

SVI is then calculated as:

$$SVI = \frac{Settled\ sludge\ volume(ml)}{MLSS(mg/L)} \tag{3.6}$$

### 3.7.3 CST (Capillary Suction Time)

This test measure the water releasing rate from sample of sludge. The method gives a quantitative measurement of how quickly a sludge will release water. The results can be used to help in determining sludge dewaterability.

**Apparatus**

- CST apparatus
- CST filter paper
- Pipette, 10 ml
- Thermometer

**Procedure**

1. Power on and then reset CST meter. Dry the CST test block and the reservoir.
2. Put a new CST filter paper on the lower test block and put rough side up and then grain parallel to the side which is 9-cm.
3. Now add upper test block, enter sludge reservoir in the test block and set it using low pressure and turn quarter to prevent leaks from surface.
4. Check and note temperature of sludge. Pipette 6.4 ml of sludge into the test cell reservoir, if pipetting is not easy due to sludge thickness, add a representative sample of sludge in the cell till it is full.
5. CST apparatus will start to note time as liquid which is drawn into paper reach the inside pair of electrical contractors.
6. Timing will end to be measured when the water at outer contacts is reached.
7. Record CST which appears on display screen.
8. Now remove the remaining sludge from the reservoir and put out and discard CST paper which is used. Rinse with DI water and dry both test block and the reservoir.
9. Sample volume and temperature may affect results of CST. Make sure that all analyses must be done under similar conditions.

### 3.7.4 AHLs (Acyl homoserine lactones)

AHLs are the basic enzymes responsible for quorum sensing in other words, more the amount of AHLs more will be EPS production and more will be biofouling. So their analysis is very important in biofouling studies.

**AHL extraction**

Take 20 ml sludge and centrifuge it for 20 minutes.

Separate supernatant and mix it with equal volume of ethyl acetate.

Vortex the solution at 120 rpm for 2 hours.

Put this solution in separating funnel and separate organic layer.

Now again centrifuge this separated solution for 10 minutes at 40C at 4000 rpm to remove any suspended matter

Evaporate that solution in rotary shaker at $300°C$ and put 300L methanol in the residue left behind after evaporation.

Store the sample.

**AHL detection in HPLC**

Procure N-octanoyl homoserine lactose from sigma Aldrich.

Dissolve it in methanol to obtain 1000 ppm stock solution.

Mix 20 $\mu L$ stock solution with 980 $\mu L$ of methanol having dissolved 0.1% formic acid.

Mobile phase used should have water methanol ration of 35:65.

Column 18 of HPLC is used for detection and AHL sample was injected at 0.8 ml per min.

210 nm wavelength was set for UV detector.

### 3.7.5 SCR (Specific Cake Resistance)

Specific cake resistance test is used for the measuring of cake resistance on the surface of membrane. For this purpose dead end filtration assembly (Amicon, 8400, USA) was used. Permeate weight was continuously measured with help

of balance which was coupled to a computer. PVDF membrane filter having opening size 0.22m and effective surface area of 90mm2 was employed. A continuousforce of 30kPa was used by inactive gas which was nitrogen in this case. SCR as measured by (Jamal et al. [47]) has the following formula

$$\alpha = \frac{2000.A2.P.t/V}{\mu.C.V} \tag{3.7}$$

Here,

$\alpha$ = symbol of specific cake resistance, m/kg

$\Delta P$ = amount pressure applied, 30kPa

A = area of PVDF membrane, 0.0042m$^2$

(t/V) / V = line slope, sec/m

$\mu$= viscosity of effluent, Ns/m$^2$

C = MLSS concentration, kg/m$^3$

### 3.7.6 EPS (Extra-Polymeric substances)

The EPS is usually measured in the form of soluble, loosely and tightly bound EPS. Both these forms of EPS were extracted by the following procedure

1. Sludge sample from bioreactor was taken (50ml).
2. The sludge sample was rotated at 4,000 rpm at 40°C (20 minutes).
3. Now upper portion was stored at 40°C for analysis of soluble form of EPS.
4. Use buffer solution to re-suspend settled sludge flocs to original volume.
5. Stir the sample for 1hr at 300 rpm.
6. Now Centrifuge the sample for 15min at 5,000 rpm.
7. Separate supernatant to be used for analysis of loosely bound EPS.
8. Add resin 70 g/g VSS 0.05 L x 70g x MLVSS g/L = required weight in grams.

9. Now stir sample at for 2h at 300 rpm, at ambient temperature.
10. Now Centrifuge the sample for 10min at 5,000 rpm at 40°C.
11. Now separate cation exchange resins and components in flocs.
12. Centrifuge the sample for 20min at 5,000 rpm at 40°C.
13. Then Remove remaining components of flocs.
14. Supernatant was stored at 40°C for analysis of tightly bound EPS.

# Chapter 4

# Results and discussion

Both the bioreactors were fed with the same sludge having initial MLSS concentration of 7 g/L. Before the addition of QQ bacteria in both the reactors the physio-chemical and sludge parameters were analyzed and also after addition of QQ bacteria in the form of consortium the effect on physio-chemical as well as on sludge parameters were analyzed and are presented below in tables and in graphs. In reactor 1, consortium of three bacterial species were inoculated in the form of beads, species were Rhodococcus, Delftia and Pseudomonas while in reactor 2 the predominant specie was Enterobacter with the addition of Delftia and Pseudomonas.

## 4.1 Performance analysis

The COD removal, ammonium, nitrate and phosphorus during control and with QQ bacteria are shown intable 4.1.Removal efficiency for COD during the control operation was 92.5 $\pm$0.5 while during the QQ operation of both the MBRs the efficiency were 93.6 $\pm$1.4 and 94.7$\pm$1 .1, respectively showing that quorum quenching bacteria had no adverse effect at all on the performance efficiency.

Table 4.1: Physico-chemical parameters analysis

| **Parameters** | **Control** | **M1** | **M2** |
|---|---|---|---|
| **COD** | 92.5 ± 0.5 | 93.6 ± 1.4 | 94.7 ± 1.1 |
| $\mathbf{PO_4^{-3}}$ | 50 ± 1.5 | 48 ± 2.5 | 49 ± 1.1 |
| $\mathbf{NH_{+4}N}$ | 47 ± 2.0 | 52 ± 1.5 | 50 ±2.5 |
| $\mathbf{NO_3N}$ | 91.6 ± 2.5 | 92.5 ± 2.1 | 93 ± 1.5 |

### 4.1.1 Effect on COD and Ammonia removal

COD and ammonia removal efficiency was examined during the control as well as during the steady state while quorum quenching bacteria was introduced. Quorum quenching doesnt affect the removal efficiency in both the MBRs depicting that it has no adverse effect at all on the performance efficiency of membrane bioreactor.

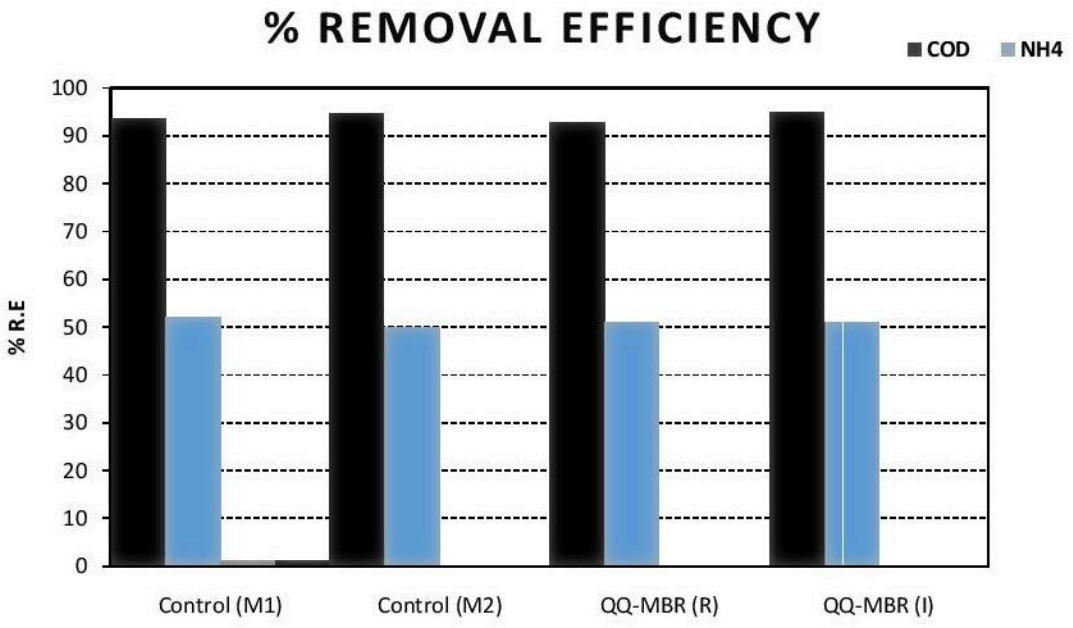



Figure 4.1: % Removal efficiency of COD and Ammonia

### 4.1.2 Effect on nutrients removal

To check that the quorum quenching bacteria have either negative effect on the performance efficiency or not, we analysis the nutrients removal as well as COD removal regularly during the whole cycle of operation. Results showed that there is no difference in the performance efficiency in both the MBRs during the preliminary and in steady phase depicting that quorum quenching had no adverse effect at all. Nutrients removal is very essential because eutrophication is the major problem caused by the nutrients if they are present in wastewater and receiving water bodies. Nitrification process involves conversion of nitrate to nitrite and denitrification converts nitrite into nitrogen gas. Phosphorus removal from wastewater occurs with the help of phosphorus accumulating substances (PAO). Increase in the aeration time decreases the process of nitrification as well slow down the process of denitrification.

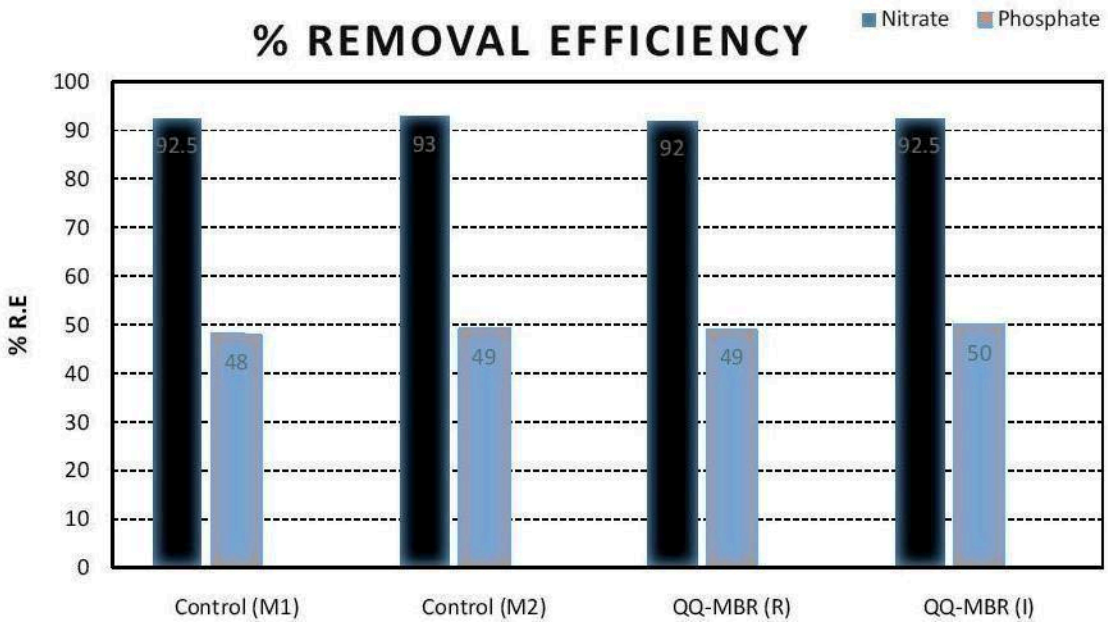



Figure 4.2: % Removal efficiency of Nitrate and Phosphate

## 4.2 Effect on membrane resistance

Effect of filtration resistance of both the MBRs, resistances in each phase during preliminary and steady state was examined and found that during preliminary stage Rt was high because of cake deposition on the membrane surface while during steady phase quorum quenching reduces the production of soluble microbial products, hence decrease in the cake layer resistance resulting in total hydraulic resistance to be less. According to Table 4.2, during

Table 4.2: Resistance analysis during control and QQ

| | **Control** | **Control** | **QQ** | **QQ** |
|---|---|---|---|---|
| Resistance ($10^{12}$) | M1 (1/m) | M2(1/m) | M1(R) | M2(I) |
| Total hydraulic | 0.6±0.2 | 0.66±0.25 | 0.5±0.2 | 0.54±0.1 |
| Cake Layer | 0.4±0.1 | 0.3±0.15 | 0.25±0.1 | 0.2±0.1 |
| Pore blockage | 0.2±0.01 | 0.3±0.1 | 0.3±0.1 | 0.4±0.1 |
| Virgin membrane | 0.1±0.01 | 0.2 ± 0.1 | 0.25 ± 0.01 | 0.32 ± 0.1 |
| $R_c/R_t$ (%) | 66 | 50 | 50 | 37 |
| $R_p/R_t$ (%) | 33 | 45 | 60 | 74 |

control operation of membrane bioreactor the Rc increases showing that soluble microbial products (SMP) helps the bacteria to attach with themselves and so on the surface of membrane causing decrease in filtration and increase in membrane resistance while during the operation of membrane bioreactor with the addition of quorum quenching bacteria the Rc reduces as the concentration of AHLs decreases and also the production of soluble microbial products decreased which increases the filterability of membrane thrice as compared with control operation of MBR. The ratio of Rp/Rt increased as cake porosity increased.

## 4.3 Effect of QQ on Trans-membrane pressure (TMP) profile

The increase in TMP profile is an indication of membrane fouling. During control operation when no quorum quenching bacteria was added in both the MBRs, the value of 30 KPa reaches within 12-13 days of operation while with the addition of QQ bacteria, delay in TMP profile was observed (Fig.4.3) which increases the days of operation (36-39 days) of both the MBRs hence increase in membrane filterability duration was achieved which supports the QQ effect on inhibition of membrane fouling.

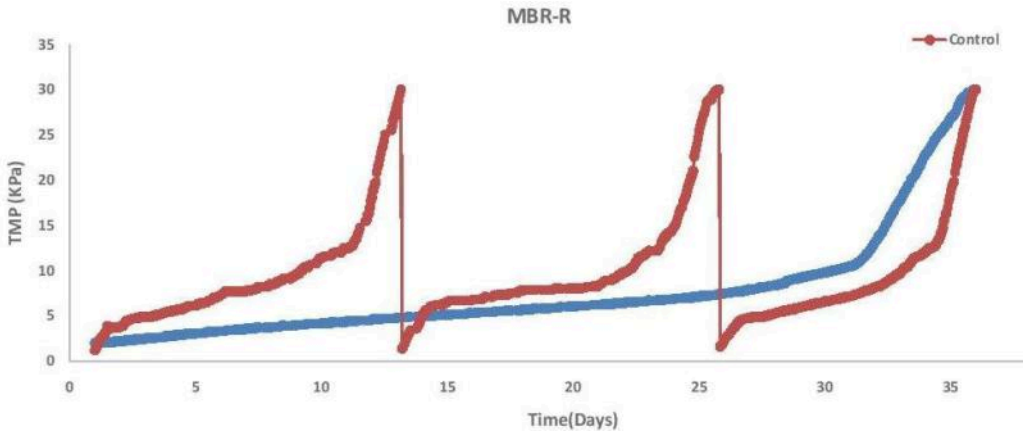



Figure 4.3: TMP profiles during control and QQ MBR-R

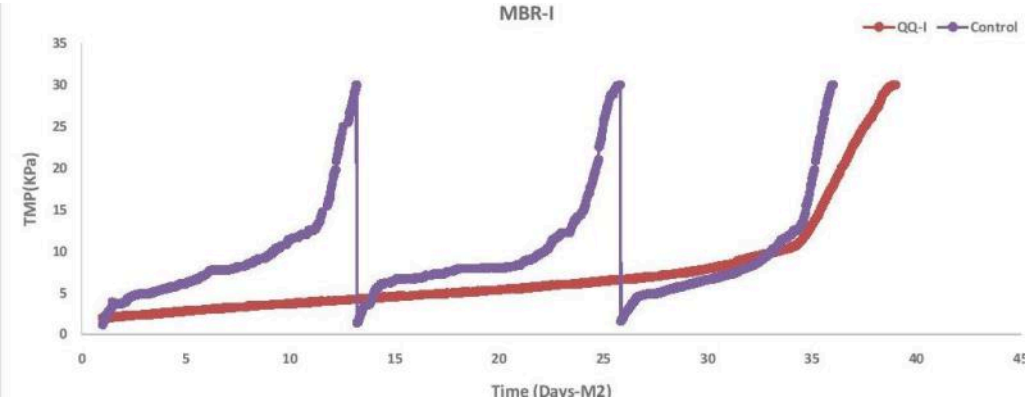



Figure 4.4: TMP profiles during control and QQ MBR-I

## 4.4 Effect on Extra-polymer substances (EPS) production

EPS and SMP plays a main part in cake layer formation on surface of membrane. EPS constitute of two major components of polysaccharides and proteins. Considering the consequence of EPS on fouling of membrane it was divided into three parts: (1) Soluble-EPS or SMP (2) Loosely bound EPS (3) Tightly bound EPS and then role of each type of EPS was examined and found to be that during control operation soluble PN concentration was high which increases the hydrophobicity of mixed liquor causing fouling of membrane abruptly while addition of QQ bacteria consortium reduces the production of soluble PN causing retardation of membrane fouling as hydrophobicity increases the attachment of microbial flocs on the surface of membrane causing abrupt increase in TMP. As both the bioreactors were seeded with the sludge having initial concentration of MLSS similar. During the control operation of bio-reactors when no bacterial consortium were added the EPS shows an increasing trend which depicted the membrane fouling and also decreases in

filterability. Soluble microbial products is main reason for the membrane fouling, increase in the production of SMP would increases the chances of membrane fouling and also decreases the days of operation. During the control study the membrane was choke within 12-13 days of operation because of the rise in the production of soluble microbial products while with addition of quorum quenching bacteria the days of operation increases 3 times. Quorum quenching bacteria disrupts the production of signaling molecules thus reducing the binding of flocs together hence decreases the chances of microbial flocs to combine with each other and reduces the biofilm formation

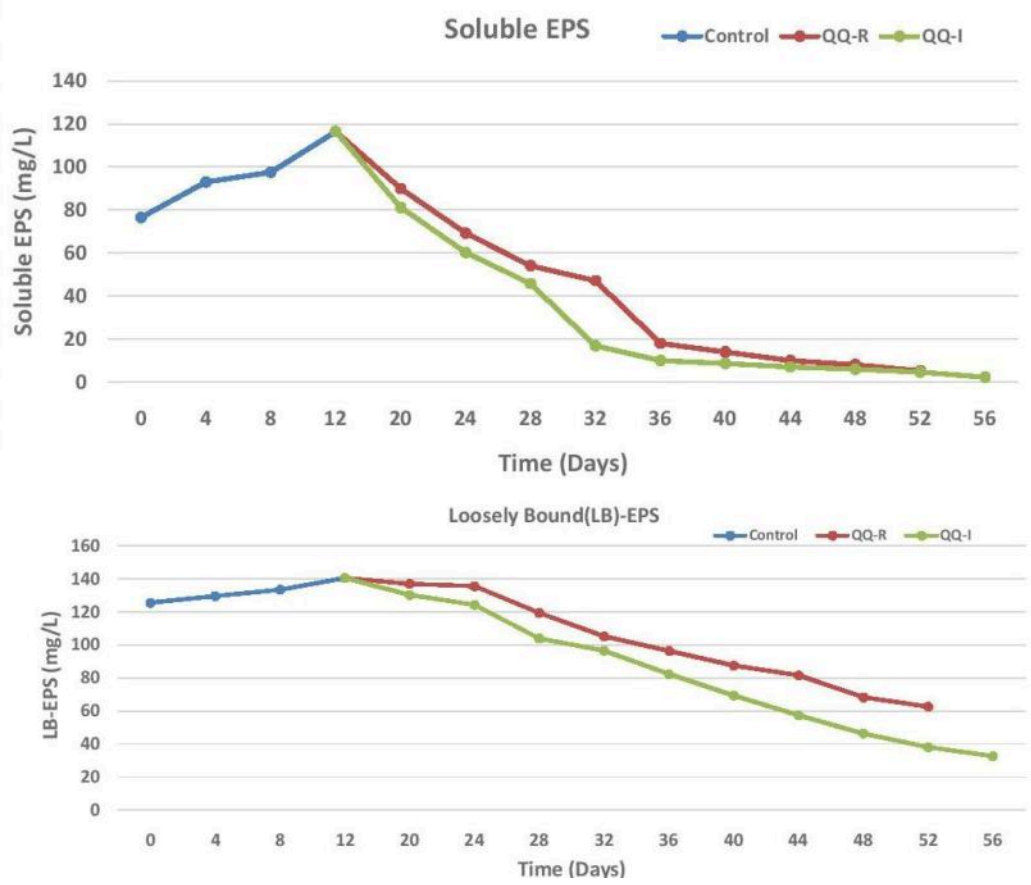



Figure 4.6: Loosely bound EPS production during control and QQ

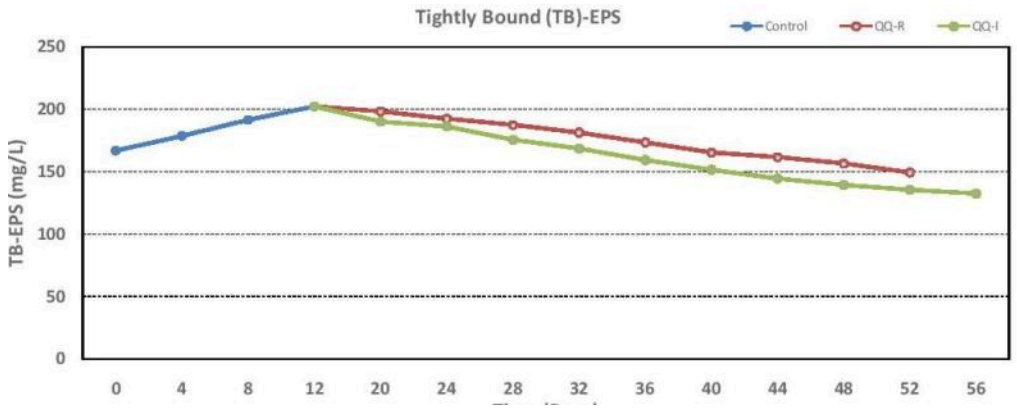



Figure 4.7: Tightly bound EPS production during control and QQ

## 4.5 AHLs concentrations effect by QQ

For the confirmation of AHLs in the sludge high performance liquid chromatography (HPLC) technique was used. Sample for the preparation HPLC analysis goes from the following protocol. 50 ml of sludge was collected from the biotank. 20 ml of sludge then pour into small flask and placed the flasks in the centrifugation assembly for 20 min. after the centrifugation of sludge, remove the supernatant in conical flask. Add same amount of ethyl acetate as of supernatant in conical flask. After this place the conical flask in shaker for 2 h. now place the sample in separating funnel for 10-15 min. the formation of two layers will occur, discard the lowest layer and put the upper layer again in beaker. Now turn on the rotary evaporator and place the sample in it. Set temperature $30°C$ and wait until sample evaporates. Now mix 300ml in the sample left. Now the sample is ready for the HPLC analysis. After the HPLC analysis the results showed that in C-MBR the peak was large showing that the AHLs concentration was nor decreases as the operation started and it tends to increasing showing the possible reason for biofilm formation and hence decrease in the filterability of membrane module.

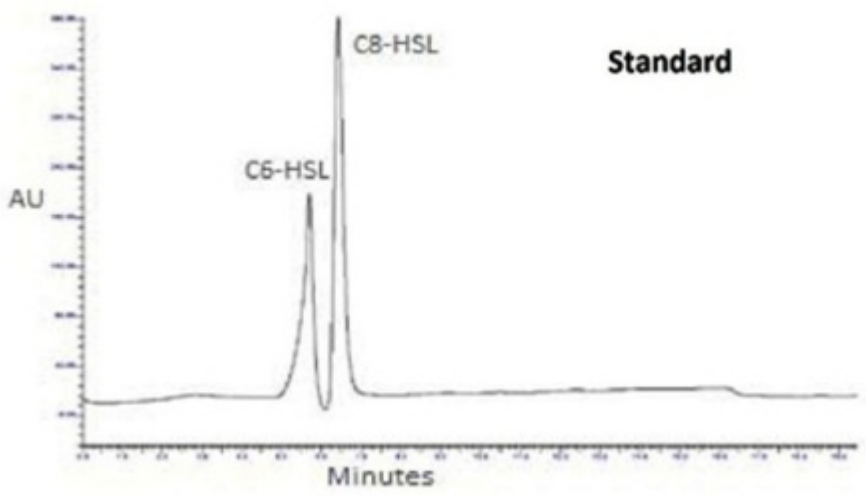



Figure 4.8: Chromatogram of Standard C8-HSL

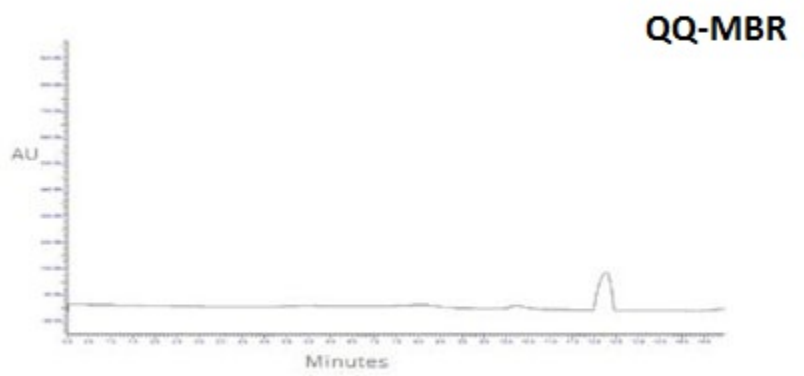



Figure 4.9: Chromatogram of Standard QQ-MBR$_R$

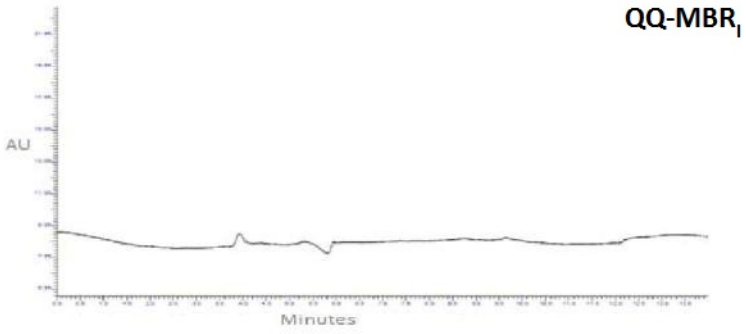



Figure 4.10: Chromatogram of Standard QQ-$MBR_1$

# Chapter 5

# Conclusions and Recommendations

## 5.1 Conclusions

In this study, submerged membrane bioreactor (SMBR) was fed with sludge having initial concentration of 8 g/L. In bioreactor 1 the consortium comprised of Rhodococcus, Delftia and Pseudomonas species whereas in bioreactor 2 the consortium consists of indigenous species including Enterobacter, Delftia and Pseudomonas.Efficiency of quorum quenching mechanism the system was operated initially without the addition of QQ bacterial consortium and found that the membrane was choke within 12-13 days of operation showing that soluble EPS concentration was enlarged and hence lessen the number of operating days while with the addition of bacterial consortium the operating days increased thrice(36-39 days) showing decrease in cake formation on the surface of membrane and confirming less production of AHLs (almost 3 times less production). Quorum quenching improved the dewaterability of sludge by 50% and also reduced the specific cake resistance 55%. No substantial effect of QQ bacteria were found on the performance efficiency. So it may be concluded that indigenous bacteria may be used for retardation of membrane

biofouling.

## 5.2 Recommendations

1. Real wastewater replacing synthetic one and then compare the TMP increase between conventional and indigenous bacteria.
2. Addition of backwash effect in membrane bioreactor.

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# Appendix A

## Protocols

**Extra polymeric substances (EPS) extraction and analyses**

**Cation exchange resin (CER)**

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

| **Buffer solution** | **Concentration** | **Amount in 1L DI water** |
|---|---|---|
| Chemical name | | |
| $Na_3PO_4.12H_2O$ | 2 mM | $380 * 2/1000 = 0.76g$ |
| $NaH_2PO_4.2H_2O$ | 4 mM | $156 * 4/1000 = 0.624$ g |
| NaCl | 9 mM | $58.5 * 9/1000 = 0.5265$ g |
| KCl | 1mM | $74.6 * 1/1000 = 0.0746$ g |

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

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

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

Carbohydrate and protein fractions of the soluble and bound EPS were measured by the colorimetric methods of Dubois et al. [48] and Lowry et al. [49] respectively, using spectrophotometer.

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

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

**Chemical reagents**

5 w% Phenol solution

Sulfuric acid ($H_2SO_4$)

D-Glucose for standard solution

**Procedure**

**Standardization**:

1. Make all measurements in duplicate
2. Pipette 2 mL of sugar solution (D-Glucose) containing 0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 mg/L of glucose into test tubes

3. Add 1 mL of the 5% phenol solution and 5 mL of the concentrated sulfuric acid to the test tubes. The addition should be rapid. In addition, direct the stream of acid against the liquid surface, rather than against the side of the test tube for good mixing.
4. Allow the tubes to stand 5 min.
5. Thoroughly mix the solutions using vertex machine.
6. Cool again by standing for 5 minutes.
7. Measure absorbance at 490 nm in HACH spectrophotometer.
8. Prepare a calibration curve of concentration of sugar (Glucose-D) versus absorbance.

**Analysis: (Sample for soluble and bound EPS)**

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

**Measurement of Protein: Lowry method**

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

**Chemical reagents** $CuSO_4.5H_2O$

Sodium Citrate

$Na_2CO_3$

NaOH

Folin-Ciocalteu phenol reagent

Bovine Serum Albumin (BSA) for standard solution

**Solution A, 100 mL;**

0.5 g $CuSO_4.5H_2O$

1 g $Na_3C_6H_5O_7.2H_2O$ (Sodium citrate)

**Solution B, 1L;**

20g $Na_2CO_3$

4 g NaOH

**Solution C, 51 mL;**

1 mL solution A

50 mL solution B

**Solution D, 20mL;**

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

**Procedure**

**Standardization:**

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

**Analysis: (Sample for soluble and bound EPS)**

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

**Capillary Suction Time (APHA, 2012)**

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

**Apparatus**

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

**Procedure**

1. Turn on and reset CST meter. Dry CST test block and reservoir.
2. Place a new CST paper on lower test block with rough side up and grain parallel to the 9-cm side.
3. Add upper test block, insert sludge reservoir into test block and seat it using light pressure and quarter turn to prevent surface leaks.

4. Measure and record temperature of sludge. Pipet 6.4 mL sludge into test cell reservoir; if pipetting is difficult because of sludge consistency, pour a representative sludge sample into cell until it is full.
5. The CST device will begin time measurement as liquid being drawn into paper reaches the inner pair of electrical contacts.
6. Timing ends when the outer contacts is reached.
7. Record CST on digital display.
8. Empty remaining sludge from reservoir and remove and discard used CST paper. Rinse and dry test block and reservoir.
9. Temperature and sample volume can affect CST results. Ensure that all analyses are run under same conditions.

### Sludge Volume Index (APHA, 2012)

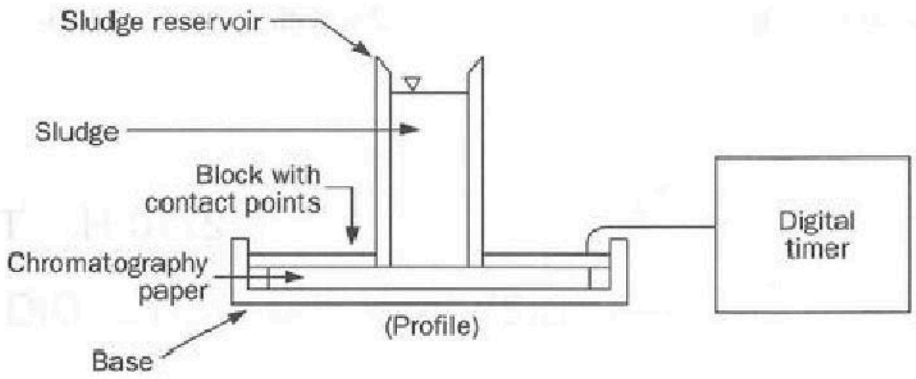
**General Discussion** The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of suspension after 30min settling. SVI typically is used to monitor settling characteristics of activated sludge.

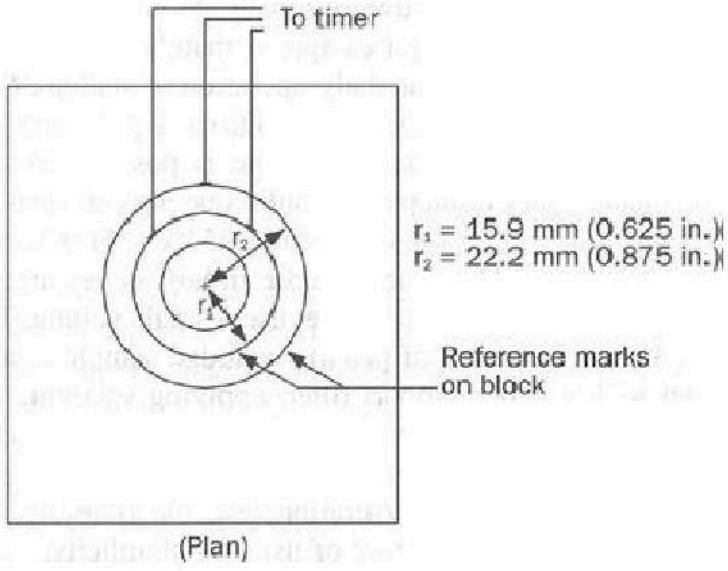
**Apparatus**:

1. Settling column
2. Stopwatch
3. Thermometer

**Procedure**:

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




**Calculations**:

$$SVI = \frac{Settled\ sludge\ volume\ (mL/L) * 1000}{Suspended\ solids\ (mg/L)} \tag{5.1}$$