PERFORMANCE EVALUATION OF BENCH SCALE MEMBRANE BIOREACTORS

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APPROVAL SHEET

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DECLARATION

We hereby declare that we (Babar Abbas, Humayun khan Abbasi, Rasikh Habib, Sana Jehan Ansari) are the sole authors of this thesis and the work has not been published anywhere else before. This is the true copy of the thesis, including required any final revisions, as accepted by the examiners.

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This thesis is dedicated to the whole faculty of this pioneer batch of Environmental Engineering at IESE.

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LIST OF ABBREVIATIONS

Abbreviation	Description
COD	Chemical oxygen demand
sCOD	Soluble cod
TN	Total nitrogen
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
C-MBR	Conventional membrane bioreactor
MB-MBR	Moving Bed membrane bioreactor
A/O-MBR	Oxic- Anoxic membrane bioreactor
HF	Hollow-fiber
RBC	Rotating biological contactor
F/M	Food to microorganism ratio
TMP.	Trans-membrane pressure
NLR	Nitrogen loading rates
DO	Dissolved oxygen
HRT	Hydraulic retention time
SRT	Sludge retention time
OLR	Organic loading rate
CASP	Conventional activated sludge process
MBRs	Membrane bioreactors

ABSTRACT

Water is a precious resource for the survival of mankind but we are losing it every day. We can replenish our ground water by treated wastewater recharge. The conventional methods to treat the wastewater are not meeting the recent discharge standards. Membrane bioreactor is the efficient way of treating wastewater by the combination of biological process and membrane technology eliminating the process of sedimentation. Three membrane bioreactors (MBR) were installed at bench scale and the performance parameters were investigated to access the efficiency of MBR technology and to evaluate the quality of treated wastewater for reuse purposes. The activated sludge from I-9 Sewage Treatment Plant, Islamabad was acclimatized with synthetic wastewater for a period of 30 days in MBR along with plastic (Kaldnes) media. Medium strength wastewater was prepared synthetically in the laboratory having a chemical oxygen demand (COD) of 500mg/L and COD:N:P of 100:10:2. The degradation of synthetic wastewater at a hydraulic retention time (HRT) of 8 hours was studied in three separate reactors which included 1) Conventional MBR (C-MBR), 2) Moving Biofilm MBR (MB-MBR) and 3) Oxic-Anoxic MBR (A/O-MBR). The aeration provided to C-MBR and MB-MBR was 5-6 mg/L while 1-2 mg/L was provided in anoxic compartment and 5-6 mg/L in aerobic portion of the A/O-MBR. A pH of 7 to 8 was maintained by using Sodium Bicarbonate ($NaHCO_3$). Sludge retention time (SRT) was maintained at 30 days which resulted in mixed liquor suspended solids (MLSS) concentration between 6000 and 8000 mg/L. The COD removal efficiency above 97% was obtained in all the three MBRs. The Total Nitrogen (TN) removal efficiencies of C-MBR, MB-MBR and A/O-MBR were obtained as 59.81%, 68.82% and 83.18% respectively. Total phosphorous (TP) removal efficiencies of C-MBR, MB-MBR and A/O-MBR were recorded as 46.48%, 59.46% and 69.74%, respectively. Based on these results the performance of A/O-MBR was found efficient in terms of nutrients removal over the other two MBRs due to the production of heterogenic bacteria which are responsible for nitrification, de-nitrification as well as phosphorous removal.

Keywords: Membrane bioreactor (MBR), Kaldnes media, Nutrients removal, Nitrification, De-nitrification, Heterogenic bacteria.

Chapter 1

1. INTRODUCTION

1.1 Background

Water is vitally important and precious commodity for the survival of mankind. Every living thing needs it to live and it is a key component in determining the quality of our lives. The increasing demand of water usage has resulted in water scarcity in Pakistan. Population growth associated water-related pollution and public health problems are major areas of concern. The critical subject is whether the developing world should follow the advance wastewater treatment technology or there is an alternative "Sustainable Sanitation" (Harleman and Murcott, 2001)

Pakistan's urban areas have a need to develop water reuse applications from the existing wastewater sources to overcome the increasing water scarcity and degradation of water sources. Meanwhile water demand is exponentially increasing in urban development and becoming dependent upon availability of high quality water. It is estimated within the next 15 to 25 years a number of cities will face limited fresh water to meet increasing demand for horticulture (Hastuti et al., 2011). We can replenish this precious resource by treating our wastewaters efficiently. The principal objective of wastewater treatment is generally to allow municipal and industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment. Irrigation with treated wastewater is both disposal as well as effective form of utilization.

In present era the conventional wastewater treatment technologies are not meeting the effluent discharge standards. The design of wastewater treatment plants is usually based on the need to reduce organic and inorganic loadings to limit pollution of the environment. Pathogen removal has very rarely been considered an objective but, for reuse of effluents in

agriculture, this must now be of primary concern and processes should be selected and designed accordingly (Hillman, 1988). Treatment to completely remove wastewater constituents is technically possible, but is not economically feasible. However significant progress has been made in developing sound technical and viable economical approaches to producing high quality and reliable water sources from reclaimed wastewater.

The Membrane bioreactors are composed of two primary parts, the biological unit responsible for the biodegradation of the waste compounds and the membrane module for the physical separation of the treated water from mixed liquor. The membrane component of the MBR eliminates the need for a clarifier and is performed using low pressure membranes i.e. Microfiltration (MF) or Ultrafiltration (UF). This technology is suitable for urban area which has limited space for wastewater treatment and ability to remove pathogens, nutrients, and suspended solids (Hastuti et al., 2011). MBR technology is advancing rapidly around the world both in research and commercial applications (Meng et al., 2009). Despite the increasing number of studies and full-scale applications of MBR systems, directions and trends in academic research as well as commercial developments require further investigation (Yang et al., 2006). The MBR with a submerged membrane module can be an attractive choice for the upcoming generations of biological wastewater treatment plants providing two clear advantages, comparatively improved and excellent effluent quality and smaller footprints with minimal aesthetic nuisance. The core application area so far has been to improve the industrial wastewater treatment (Sombatsompop, 2007). Moreover MBRs may actively be employed in domestic wastewater treatment as well because MBRs are operated at high mixed liquor suspended solids (MLSS) concentrations and inhibit the excessive sludge production, resulting in high removal efficiency of chemical oxygen demand (COD), nutrients (NH_3-N, NO_2^{-1}, NO_3^{-1} and PO_4^{-2}) removal and bacterial disinfection(Su et al., 2007). Another useful application of MBRs is to treat the landfill leachate (Yang et al., 2012).

Although MBRs are very efficient in treating wastewater but at the same time we need to develop more suitable set ups for achieving further technological improvements in the system. In this paper the performance evaluation of bench scale MBRs has been evaluated where the treatment performance of MB-MBR using Kaldness media A/O-MBR was compared with C-MBR.

1.2 Microbial Studies

Membrane bioreactor is becoming widely applicable for biological wastewater treatment (Duan et al., 2009). MBRs combine conventional activated sludge treatment with a membrane solid liquid separation process (Bhatti et al., 2009). In such type of biological treatment the biological flocs and biofilms are used for degrading or adsorbing dissolved colloidal, settle able and particulate matter (Henze et al., 2008). Microorganisms are responsible for most of the carbon and nutrient removal from wastewater (Wagner and Loy, 2002) therefore it is important to get an in-depth knowledge of the kind of microorganisms present in biological treatment systems.

Membrane bioreactors can be operated to ensure simultaneous nitrification and denitrification as well as phosphorus removal .Maintenance of higher SRT ensures better treatment of wastewater. Increased MLSS concentration decreases the reactor volume and results in smaller footprint. The effluent can be of greater quality since the higher SRT ensures retaining microorganisms that are important for wastewater treatment such as *Nitrosomonas* and *Nitrobacter*. However the frequent maintenance of MBR, its energy intensiveness and membrane fouling are the drawbacks of membrane bioreactors (Melin et al., 2006).

The advancements in molecular techniques have enabled detection and reliable quantification of bacteria in wastewater (Roberts and Lewis, 2001).Bacterial species of prime importance such as *Nitrobacter, Nitrospira* (Ce´bron & Garnier, 2005), *Nitrosospira, Nitrosomonas*

(Hiorns et al., 1995), *Pseudomonas* (You, 2005), *Bacillus*(Lin et al., 2007) etc. have extensively been evaluated using molecular techniques such as PCR-DGGE and FISH. However the understanding of structure and diversity of bacterial community in MBRs treating municipal waste water is not well understood (Duan et al., 2009). The diversity of all bacterial species and other microorganisms of different membrane bioreactor systems have not been completely evaluated, therefore the aim of this study was to isolate and characterize bacteria present in membrane bioreactor systems and compare it with the performance of the reactor.

1.3 Objectives of study

The main objectives of the study were:

- Performance evaluation of Membrane bioreactor (MBR) in terms of organics and nutrients removal.
- Isolation and identification of bacteria from Membrane bioreactor systems.

The parameters analysed in terms of treatment performance included:

- Temperature
- Dissolved Oxygen (DO)
- pH
- Chemical Oxygen Demand (COD)
- Total Nitrogen (TN) NH_4, NO_2, NO_3
- Total Phosphorus (TP)
- Mixed Liquor Suspended Solids (MLSS)

1.4 Scope of study

The scope of study included:

- Three bench scale MBRs namely: Conventional MBR (C-MBR), Moving biofilm MBR (MB-MBR) and Oxic-Anoxic MBR (A/O-MBR)
- Synthetic wastewater representing medium strength domestic wastewater was used in the study.
- The biological characteristics were analyzed by measuring MLSS and MLVSS.
- The environmental parameters such as pH, DO and temperature were measured daily in all three MBRs.

Chapter 2

2. LITERATURE REVIEW

2.1 Membrane

A membrane is a layer of material which serves as a selective barrier between two phases and remains impermeable to specific particles, molecules, or substances when exposed to the action of a driving force. Some components are allowed passage by the membrane into a permeate stream, whereas others are retained by it and accumulate in the retentate stream (Mulder and Marcel, 1996).

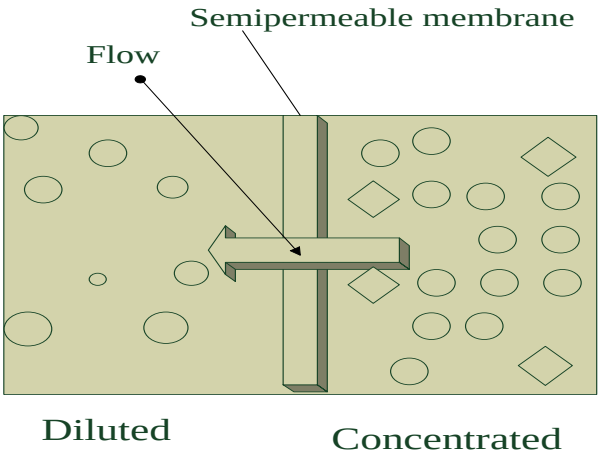

Figure 1 Perm- Selective Membrane

The permeability is dependent on the pore size of the membrane. Although membrane process was introduced in 1960s but the commercialization of membrane increased in 1990s and onward. As it works on the solid liquid separation so, it is appreciable to use membrane technology for water and wastewater treatment which are subsequently used for drinking, non-potable and recharging purposes. With time stringent effluent discharges and legislation for conserving water quality, effective treatment, recycling and reusing the wastewater are the key drivers for the advancement of this technology.

2.2 Membrane Bioreactor

The original process was introduced by Dorr-Olivier Inc. and combined the use of an activated sludge bioreactor with a cross flow membrane filtration loop. The flat sheet membranes used in this process were polymeric and featured pore sizes ranging from 0.003 to 0.01 μm.

The breakthrough for the MBR came in 1989 with the idea of Yamamoto and co-workers to submerge the membranes in the bioreactor. Until then, MBRs were designed with the separation device located external to the reactor (side stream MBR) and relied on high trans membrane pressure (TMP) to maintain filtration. With the membrane directly immersed into the bioreactor, submerged MBR systems are usually preferred to side stream configuration, especially for domestic wastewater treatment. The submerged configuration relies on coarse bubble aeration to produce mixing and limit fouling. The energy demand of the submerged system can be up to 2 orders of magnitude lower than that of the side stream systems and submerged systems operate at a lower flux, demanding more membrane area. In submerged configurations, aeration is considered as one of the major parameter on process performances both hydraulic and biological. Aeration maintains solids in suspension, scours the membrane surface and provides oxygen to the biomass, leading to a better biodegradability and cell synthesis (Judd, 2006). An MBR can replace two physical processes in to one by filtering the biomass by using the membrane while in conventional activated sludge process the wastewater undergoes two stages of treatment: aerobic degradation followed by secondary sedimentation to remove biomass. (Judd, 2006)

A membrane bioreactor (MBR) is advancement to the conventional activated sludge process (CASP). There are two modes of operation for membrane bioreactor i.e., at constant flux and at constant trans membrane pressure (TMP).

A membrane bioreactor (MBR) is one of the applications of membrane technology to wastewater treatments. A submerged MBR is a type of MBR in which membrane modules are directly submerged into a bioreactor. Because the uplifting air flow is assigned the role of cake removal in a submerged MBR, aeration could affect the cake-removing efficiency and hence suction pressure (Tatsuki et al., 1998)

The membrane bioreactor, with an increase of biomass concentrations in the aeration tank, permits work with low F/M ratio and a reduction in sludge production. The membrane bioreactor potentialities have been tested on a laboratory pilot for domestic wastewater treatment. Various operating conditions (HRT, SRT) were applied to investigate organic and nitrogen removal as well as sludge production. (Chaize and Huyard, 1991)

Combining membrane technology with biological reactors for the treatment of municipal and industrial wastewaters has led to the development of three generic membrane processes within bioreactors: for separation and recycle of solids; for bubbles less aeration of the bioreactor; and for extraction of priority organic pollutants from hostile industrial wastewaters. Commercial aerobic and anaerobic membrane separation bioreactors already provide a small footprint alternative to conventional biological treatment methods, producing a high-quality effluent at high organic loading rates (Keith and Tom, 2000)

2.3 Advantages and disadvantages of MBR

Hollow fiber is one of the most popular membranes used in industries. It is because of its several beneficial features that make it attractive for those industries. Among them are:

- **Modest energy requirement:** In hollow fiber filtration process, no phase change is involved. Consequently, need no latent heat. This makes the hollow fiber membrane

have the potential to replace some unit operations which consume heat, such as distillation or evaporation column.

- **No waste products:** Since the basic principle of hollow fiber is filtration, it does not create any waste from its operations except the unwanted components in the feed stream. This can help to decrease the cost of operation to handle the waste.
- **Large surface per unit volume:** Hollow fiber has large membrane surface per module volume. Hence, the size of hollow fiber is smaller than other type of membrane but can give higher performance.
- **Flexible:** Hollow fiber is a flexible membrane, it can carry out the filtration by 2 ways, and either is “inside-out or outside-in".
- **Low operation cost:** Hollow fiber need low operation cost compare to other types of unit operation.
- **Low capital cost:** Reduction in capital cost because less space is required due to elimination of secondary clarifier.
- **Efficiency:** Membrane pore size of ≤ 0.4 μm retains all the biomass within the system resulting in high quality and largely disinfected effluent.
- **High MLSS:** High Mixed liquor suspended solid (MLSS) concentration can be attained unlike in CASP.
- **Removal:** All suspended solids (SS) larger than the membrane pore size are retained in MBR whereas in CASP, removal efficiency of SS depends on the settling characteristics which can be inconsistent.
- **Long SRT:** Long SRT resulting in less sludge production and growth of slow growing denitrifires.
- **Nitrification and denitrification:** Nitrogen removal by Simultaneous nitrification and denitrification (SND).

However, it also has some disadvantages which lead to its application constraints. Among the disadvantages are:

- **Membrane fouling:** Membrane fouling of hollow fiber is more frequent than other membrane due to its configuration. Contaminated feed will increase the rate of membrane fouling, especially for hollow fiber.
- **Greater process complexity** i.e., membrane sensitivity to some chemicals, limitations of dissolved oxygen (DO) and pH.
- Higher capital cost of the membrane.
- Pretreatment of the influent is required.
- Higher operating and maintenance cost which includes frequent membrane cleaning and large aeration demand.
- **Expensive:** Hollow Fiber is more expensive than other membrane which available in market. It is because of its fabrication method and expense is higher than other membranes.
- **Lack of research:** Hollow fiber is a new technology and so far, research done on it is less compare to other types of membrane. Hence, more research will be done on it in future because of its potential.
- **Physically and chemically constraints:** Hollow fiber which made of polymer cannot use on corrosive substances and high temperature condition.

2.4 Microbial Biodiversity

The isolation of microbes depends on adequate information for characterizing the known unit (Hughes et al., 1976). Many methods have been proposed for making such information readily available, some are based on the use of dichotomous keys and others on diagnostic keys and tables. The majority of bacteria in activated sludge belong to gram negative genera (Lightharst et al., 1989). The principal genera are *Achromobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus* and *Pseudomonas.* Others dominating in sludge treating industrial effluents, include *Thiobacillus, Acinetobacter, Achromobacter* and the nitrifying organism *Nitrosomonasr* and *Nitrobacter* (Pick et al., 1995). The population of coliform of raw sewage predominantly consisted of *Escherichia coli,* whereas in activated sludge and effluent, the proportion of this species declined with simultaneous increase in proportion of *Achrobacter* and *Escherichia* other than *Echerichia coli* (Dias et al., 1964). The aim of this study was to isolate and to identify the bacteria which may be present in the Membrane bioreactor.

2.5 Microorganisms responsible for most key processes in MBRs

2.5.1 Microorganisms for removal of soluble organic matter

The removal of soluble organic matter from wastewater has been the major process of biochemical treatment for many years. For typical domestic wastewater streams, which have a biodegradable chemical oxygen demand (COD) range between 50 and 4,000 mg/L, aerobic cultures of microorganisms are especially suitable. The removal is achieved by microorganisms using a portion of the carbon in the wastewater as a substrate, converting it to new biomass and converting the remaining into carbon dioxide (CO_2). The CO_2 is released as a gas, and the biomass is removed by sedimentation. The microorganisms are classified as heterotrophic because they derive their carbon from an organic source, such as the incoming

wastewater, methanol, or ethanol. A number of obligatory anaerobic fermentative bacteria are also known to degrade a variety of organic substrates such as sugars, amino acids, and others, to products such as hydrogen, CO_2, acetate and higher fatty acids and ethanol.

2.5.2 Microorganisms responsible for enhanced biological phosphorus removal

Phosphorus can cause eutrophication (extraordinary growth of algae) when it is excessively discharged into natural water bodies. Therefore phosphorus removal from wastewater is important to prevent eutrophication. Activated Sludge System with alternating anaerobic and aerobic conditions have been successfully used for enhanced biological phosphate removal (EBPR) from wastewater (Liu et al., 2001; Mino et al., 1998). This can also be applied at Oxic/Anoxic MBR, where phosphorous removal has been achieved up to 60% in our results. The EBPR process is as follows: phosphate release occurs in the anaerobic stage followed by an excess of phosphate uptake in the aerobic stage. When wastewater enters the anaerobic phase, specialized organisms, called polyphosphate accumulating bacteria (PAOs) accumulate carbon sources such as internal polymer named (polyhydroxyalkanoate or PHA. The energy to store this polymer is obtained from breakdown of glycogen and hydrolysis of an energy-rich internal phosphorus chain called polyphosphate (poly-P). Since poly-P is broken down to orthophosphate for energy supply, the phosphate concentration in the anaerobic phase increased. Two different models were postulated for the production of the reducing equivalents for this anaerobic metabolism, the details of the two models were stated by (Comeau et al., 1986) and (Mino et al., 1987). The anaerobic phase needs to be followed by an aerobic phase. During this phase, the stored PHA is consumed, generating energy and carbon for replenishment of the glycogen and poly-P pool. Under these conditions, P in wastewater is assimilated by the biomass (sludge), and it is finally removed from the process through the wastage of sludge (Smolders et al., 1994). As phosphorus removal in these processes is achieved by the dominant growth of polyphosphate accumulating bacteria in

MBR too, control of the composition of the microbial population in an MBR is very important for maintaining a sufficient level of phosphorus removal activity.

2.5.3 Microorganisms responsible for nitrogen removal (Nitrification and Denitrification)

In wastewater, four types of nitrogen mainly exist: organic nitrogen, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. These different forms constitute the total nitrogen content. The predominant forms of nitrogen in wastewater are organic nitrogen and ammonia (NH_3). In theory, the nitrogen in wastewater will be converted to harmless nitrogen gas and will be lost to the atmosphere by going through three major biological transformations during removal of nitrogen. The three biological transformations are ammonification, nitrification, and denitrification (Michael et al., 1996; Strous et al., 2002). Organic nitrogen is converted to ammonia in the first step of the nitrogen cycle. In ammonification, microorganisms decompose the organic nitrogen and produce ammonia. In order to remove nitrogen from wastewater, the ammonia must be oxidized to nitrate (NO_3). This process is commonly referred to as nitrification. An oxic environment must be maintained for a sufficient period of time to promote nitrification. Removal of inorganic nitrogen compounds from wastewaters can be accomplished by a combination of the biological processes of nitrification and denitrification (Michael et al., 1996; Strous et al., 2002). Nitrification, the oxidation of ammonia to nitrate via nitrite, is an important step in the full treatment of wastewater. In the first step of nitrification, obligate autotrophic ammonia-oxidizing bacteria convert ammonia to nitrite; subsequently nitrite-oxidizing bacteria catalyse the oxidation of nitrite to nitrate. To avoid the limitation of traditional microbiological methods, an in situ identification technique for ammonia- and nitrite-oxidizing bacteria was developed (Michael et al., 1996).

2.5.4 Microorganism analysis in a full-scale wastewater treatment plant

(Yazdi et al., 2001) examined a wastewater treatment plant in Iran. The comparison of results with the Bergey Manual showed that out of the thirteen Gram-negative bacilli isolated, ten

were identified as genus of *Flavobacterium* and three were identified as genus of *Alcaligenes*. High percentages of similarities were found with *Flavobacteriumaquatile*, more than with other species of the genus, and with *Alcaligenesfaecalis* more than with other species of genus *Alcaligenes*. The other Gram-negative rods were identified as genus *Pseudomonas*. High percentage similarities were found with *Pseudomonas stutzeri*, more than other species of genus *Pseudomonas*. Two strains of Gram positive cocci were identified as genus of *Micrococcus*. High percentage similarities were found with *Micrococcus luteu*, more than with other species of the genus (Sharifi-Yazdi et al., 2001). The results showed that Gram-negative bacilli with a yellow pigment were considered as a major group of the population. In this study, the majority of the isolated Gram-negative bacteria belonged to the genus *Flavobacterium*, whereas 22% of isolated belonged to genus *Psendomonas*. The presence of Gram-positive bacteria has been reported by some workers. In this study the only Gram-positive found from the activated sludge belonged to the genus *Micrococcous*.

2.6 Important parameters affecting the microbial community

2.6.1 Temperature

Temperature is a fundamental factor that affects all living organisms. It influences the rates of enzymatically catalysed reactions and affects the rate of diffusion of substrate into the cells (Grady et al., 1999). Due to the differences in their optimum growth temperatures, the temperature of the wastewater microbial mixture (mixed liquor) strongly influences the population composition of the consortium. The effects of temperature on the efficiency and the kinetics of excess biological phosphorus removal (EBPR) systems have been under investigation, but the studies have yielded contradictory results. Early researchers (Barnard, 1976; Ekama and Wentzel, 1999) reported that EBPR efficiency was greater at lower temperatures than at higher temperatures over the range from 5 to 24°C (Mamais and Jenkins,

1992) showed that there was a wash-out SRT for all temperatures over the range from 10 to 30°C. This introduces the paradox that, even though EPBR system performance becomes more efficient at lower temperatures, if the SRT temperature combination is below a critical value, EBPR ceases before other heterotrophic functions wash-out. A phylogenetically novel aerobic bacterium was isolated from an anaerobic-aerobic sequential batch reactor operated under EBPR conditions for wastewater treatment (Zhangetal et al., 2003). The isolate, designated strain T-27^{T}, was reported to grow at 25–35°C with an optimum growth temperature of 30°C, whereas no growth was observed below 20 or above 37°C within 20 days incubation (Zhangetal et al., 2003).

The effects of temperature variations on aerobic biological wastewater treatment were evaluated by Morgan Sagastume and Allen (2003) with respect to treatment efficiency, solids discharges, sludge physicochemical properties and microbiology. The effects of controlled temperature shifts (from 35 to 45°C; from 45 to 35°C) and periodic temperature oscillations (from 31.5 to 40°C, 6 day period, for 30 days) were assessed in four parallel, lab scale sequencing batch reactors (SBRs)that treated pulp and paper mill effluent. Overall, the temperature shifts caused higher effluent suspended solids (ESS) levels (25-100mg/L) and a decrease (upto20%) in the removal efficiencies of soluble chemical oxygen demand (sCOD). Lower ESS levels were triggered by a slow (2°C/day) versus a fast (10°C/12h) temperature shift from 35 to 45°C, but the sCOD removal efficiencies decreased similarly in both cases (from 66±3% and 65±2% to 49±3% and 51±3%). Temperature oscillations caused an increased deterioration of the sludge settle ability [high sludge volume indices (SVI); low zones settling velocities (ZSV)], high ESS levels, and lower sCOD removals. The temperature transients were associated with poor sludge settle-ability (SVI>100mL/g MLSS, ZSV<1cm/min), more negatively charged sludge (up to −0.35±0.03meq/g MLSS), increased filament abundance (approximately 4 to 4.5, subjective scale equivalent to very common), and

decreased concentrations of protozoa and metazoan (25,000–50,000 microorganisms/mL sludge) (Sagastume et al., 2003).

The seasonal change of microbial population and activities in an existing building wastewater reuse system using membrane separation activated sludge process (MSAS) were investigated, and they were also compared with those in a municipal wastewater treatment plant using conventional activated sludge (CAS) process. The micro fauna in the MSAS process was unstable and changed a lot seasonally, but it would not affect the treatment efficiency. Moreover, the specific activities of nitrification, denitrification, and organic removal fluctuated largely and seasonally, and were lower than those in the CAS process (Zhang and Yamamoto, 1996).

Mehandjiyska (1995) found that the representatives of genus *Pseudomonas* predominated in quantitative aspect among the bacteria from municipal wastewater treatment plant. Actinomycetes were isolated only during the summer months and yeasts were not found. The percentage of *E. coli* was the biggest in *Enterobacteriaceae*. The presence of molds in the activated sludge during all seasons, regardless of their small amount showed that they played certain functions in the bio decomposing treatment processes.

40 g/L neither protozoa nor metazoan survived after 24 h.

2.7 Microorganism analysis in a full-scale wastewater treatment plant

(Sharifi Yazdi et al., 2001) examined a wastewater treatment plant in Iran. The comparison of results with the Bergey Manual showed that out of the thirteen Gram-negative bacilli isolated, ten were identified as genus of *Flavobacterium* and three were identified as genus of *Alcaligenes*. High percentages of similarities were found with *Flavobacterium aquatile*, more than with other species of the genus, and with *Alcaligenes faecalis* more than with other species of genus *Alcaligenes*. The other Gram-negative rods were identified as genus *Pseudomonas*. High percentage similarities were found with *Pseudomonas stutzeri*, more

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Chapter 3

3. MATERIALS AND METHODS

3.1 Experimental Set-up

Three bench scale acrylic made MBRs were set up at IESE wastewater laboratory. MBRs were categorized based on their design and operating conditions as:

1. Conventional MBR (C-MBR)
2. Moving Biofilm MBR (MB-MBR)
3. Oxic-Anoxic MBR (A/O-MBR)

The effective volume of each tank of C-MBR and MB-MBR was 12 L for the study. Perforated plates divided the reactor into three compartments, membrane being installed in the middle one. Perforated plates helped in mixing of the sludge in the reactor as well as maintaining proper aeration in each compartment of the reactor. Hollow fiber (HF) membrane (Mitsubishi Rayon, Japan) was immersed in middle compartments of both reactors having a nominal pore size of 0.1 μm and surface area of 0.2 m^2. The plastic (kaldness) media was used as moving biofilm carrier media and it circulates within all the three compartments of MB-MBR having a dry volume of 20%. The schematic diagram of C-MBR, MB-MBR and A/O-MBR are shown in Figure 3, 4 and 5.

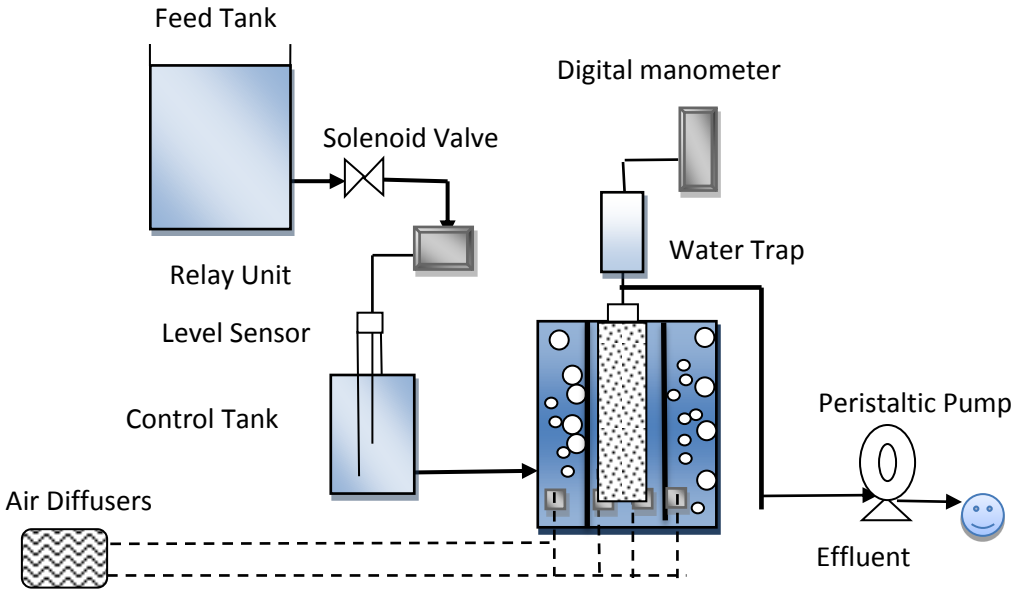


Figure 2 Schematic diagram of C-MBR

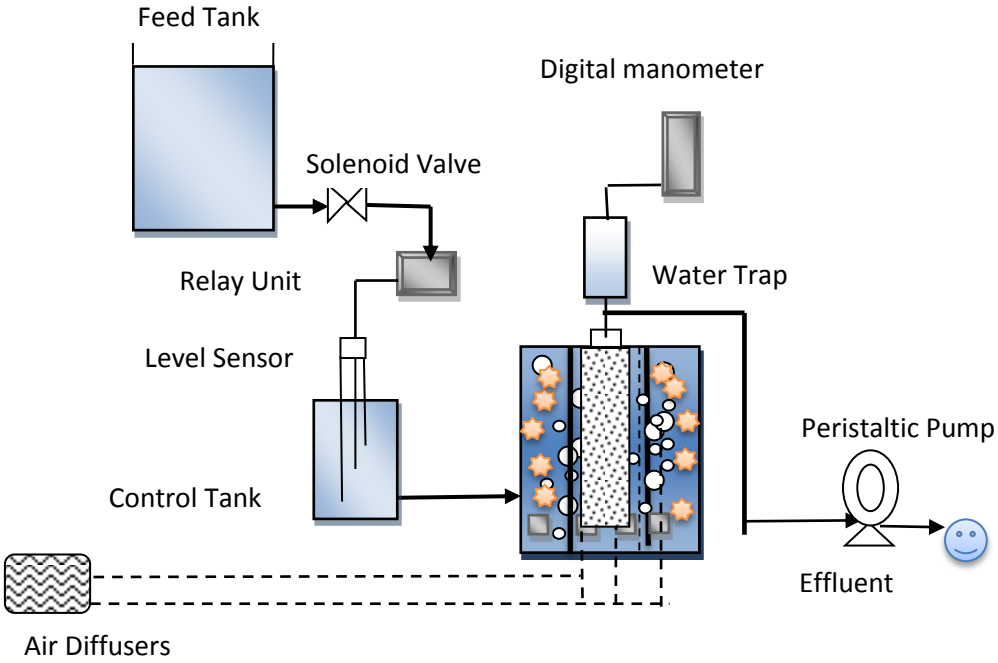


Figure 3 Schematic diagram of AG- MBR

A third laboratory scale acrylic made MBR was set up, having a 15 L volume tank for the study. Perforated plate divided the reactor into two Compartments, with a ratio of 1:2 of the total volume. Membrane was installed in the smaller compartment as shown Figure 2. The plastic (Kaldness) media with 20% effective volume was introduced in both compartments. A

mechanical mixer (Cole-Parmer, Model 50007-25, USA) was installed in the larger compartment to make anoxic condition as well as to keep media in suspension. The mechanical mixer was operated in cyclic mode as 2 minutes OFF and 10 minutes ON.

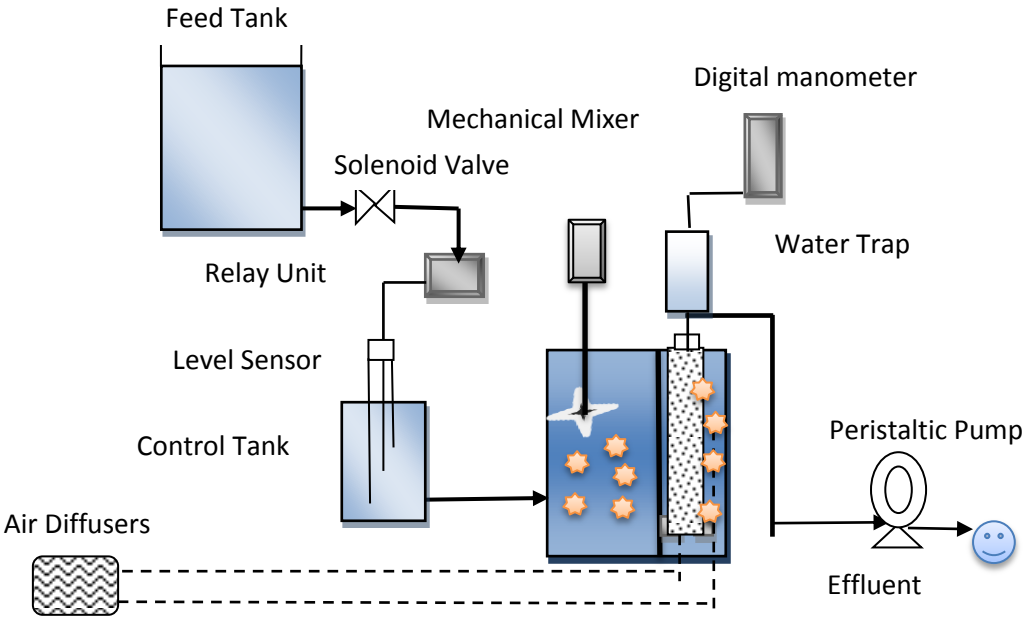


Figure 4 Schematic diagram of A/O-MBR

3.2 Acclimatization of sludge and media with synthetic wastewater

Wastewater was prepared synthetically in the laboratory having a COD of 500 mg/L and COD:N:P of 100:10:2. To maintain a pH of 7-8, $NaHCO_3$ was used as pH buffer. The activated sludge from I-9 Sewage Treatment Plant, Islamabad was acclimatized with synthetic wastewater for a period of 30 days in MBR along with Kaldness media. The chemical composition of synthetic wastewater is given in table 1.

Table 1 Chemical composition of synthetic wastewater

Chemicals	Formula	Quantity (mg/L)
Hydrated Glucose	$C_6H_{12}O_6.H_2O$	500
Ammonium Chloride	NH_4Cl	191
Potassium Di-Hydrogen Phosphate	KH_2PO_4	54.85
Calcium chloride	$CaCl_2$	5
Magnesium Sulphate	$MgSO_4.7H_2O$	5
Ferric Chloride	$FeCl_3$	1.5
Manganese chloride	$MnCl_2.4H_2O$	1
pH buffer	$NaHCO_3$	142.5

The specific properties of the Kaldness media used during the research study are as listed in Table 2.

Table 2 Specific Properties of Plastic (Kaldness) Media

Properties	Description
Dimensions	1 cm dia.
Dry Volume	20 %
Wet Volume	8 %
Material	K3 Type Plastic

3.3 Membrane characteristics

The main features of the membrane modules used in the MBRs are presented in Table 3.

Table 3 Hollow-fiber (HF) membrane characteristics

Item	**Characteristic**
Manufacturer	Mitsubishi Rayon Engineering Co. Ltd., Japan
Membrane material	Polyethylene
Pore size	0.1 μm
Filtration area	0.2 m^2
MLSS	5,000-12,000 mg/L recommended (3,000 - 15,000 mg/L)
Filtration flow rate	Constant
Suction pressure	5-30 kPa
Intermittent suction	Operating time ≤ 13 min; relaxing time ≥ 2 min
Temperature	15-35^oC

3.4 MBR operational conditions

- Peristaltic Pump (Master Flex, Cole-Parmer, USA) was used to periodically draw permeate at a cycle of 10 min filtration, 2 min relaxation, maintaining HRT of 8 hrs. Sludge retention time (SRT) was set to 30 days and nitrogen loading rate (NLR) of 0.15 Kg/m^3/d organic loading rate (OLR) was kept at 1.5 Kg/m^3/d.
- Air pumps provided sufficient air flow rate to keep the media in suspension, scour the membrane fibers along with maintaining dissolved oxygen (DO) concentration of 5-6 mg/L except the anoxic zone of A/O-MBR where DO concentration was maintained at 2 mg/L.
- Diffused aeration was provided in the reactor by the help of air diffusers.

- Flow meter was used to monitor the aeration rate at 7 L/min. (3 L/min in the membrane compartment and 4 L/min in the side compartments in C-MBR and MB-MBR). Similarly 3 L/min aeration rate was maintained in the membrane compartment of A/O-MBR.
- Trans-membrane pressure (TMP) was recorded using Data logging manometer (Sper-Scientific 840099, Taiwan) as indicator of membrane fouling tendency. The membranes were operated till the TMP reached to a limit of 30 KPa.

The MBR set up was operated for 140 days under the following conditions:

Table 4 Operating conditions

Parameter	**Condition**
SRT	30 days
HRT	8 hours
OLR	1.5 $Kg/m^3/d$
NLR	0.15 $Kg/m^3/d$
F/M	0.2
pH	6-7
MLSS	6-8 g/L

3.5 Analytical Methods

The parameters that were investigated, the technique adopted to determine each parameter and the equipment/material used are reported in Table 5.

Table 5 Analytical parameters, methods and equipment

Parameter	Method	Equipment/Material	Reference
MLSS/ MLVSS	Filtration-Evaporation	1.2 µm (GF/C, Whatman); 105^{o}C oven (MLSS); 550^{o}C Muffle Furnace (MLVSS)	APHA , 2005
COD	Close reflex	COD tube/vial; 150^{o}C oven	APHA , 2005
NH_4^+-N, NO_2^--N, NO_3^-N	Hach Reagents	Spectrophotometer (DR 2010, Hach)	APHA , 2005
PO^{-4}-P	Molybdovanadate	Spectrophotometer (DR 2010, Hach)	APHA , 2005

3.6 Methodology for microbial analysis

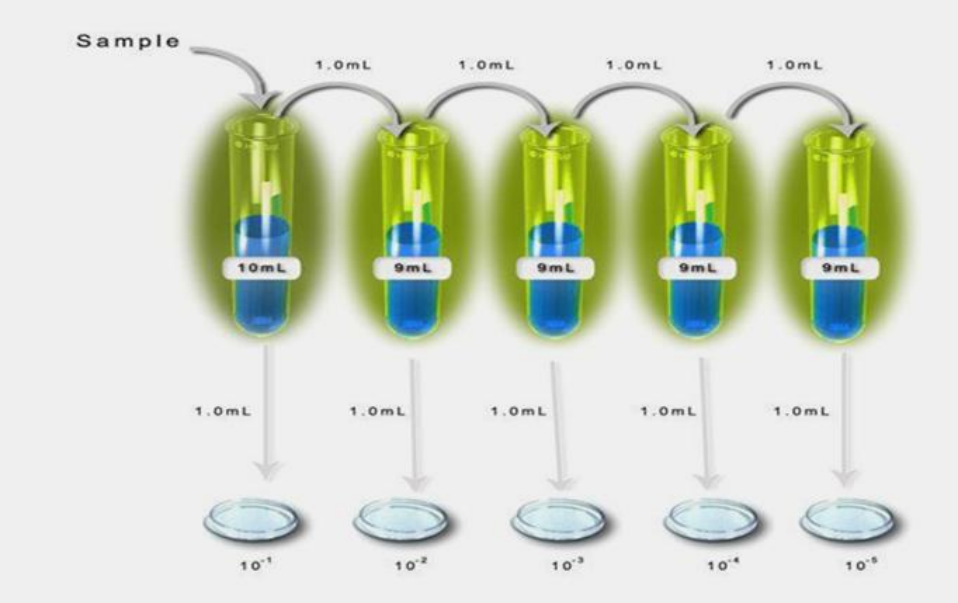


Figure 5 Isolation of bacteria

Samples were collected from membrane bioreactors at the same time and relatively same place of the bioreactor. Serial dilutions were performed up to 10^{-7} and were plated on nutrient agar plates. Morphologically different colonies were marked and picked for further isolation and streaked until obtaining a pure colony.

3.6.2 Plating methods

Spread plate method was followed for plating the sample on nutrient agar plates. Dilutions were mixed and 0.1 ml of all dilutions was added on nutrient agar plate and evenly spread with the help of a glass spreader. The petri plates were incubated in inverted position at 37ºC for 24 hours. Colonies with different morphological characteristics were isolated through streak plate method. The marked colonies were picked and purified on separate nutrient agar plates. The colonies were observed for colour, size, shape, elevation, texture, margin and pigmentation. Their cell morphology was found using Gram staining.

The isolates were then grown on 1) MacConkey agar, 2) EMB agar, 3) Simmons Citrate agar, 4) Brilliant Green agar and 5) Citrimite agar.

3.6.3 Identification of bacteria

The Gram negative isolates were identified by API 20 E (biomeurix, Canada) according to the instructions provided by the manufacturer. The seven digit code was added into API web software for isolate identification.

Chapter 4

4. RESULTS AND DISCUSSIONS

The previous studies (Jamal et al., 2011) and (Jamal et al., 2012) have already evaluated the performance of MBRs. These studies proved MBR technology's capability to treat the wastewater for reuse purposes. Previously, the studies by (Jamal et al., 2011) were carried out with sponge media as biofilm carrier having 20% dry volume of the reactor. In the present study, the sponge media was replaced by plastic (Kaldness) media and A/O-MBR was introduced. Results revealed that the nutrients (TN and TP) removal was more efficient in A/O-MBR due to the production of nitrifying and denitrifying bacteria in anoxic zone of A/O-MBR. The anoxic zone may have also facilitated the growth of *phosphorous accumulating microorganisms* (PAOs).

4.1 COD removal

All the three MBRs gave almost more than 95% COD removal for entire period. There is the slight difference in COD removal of all three MBRs however A/O-MBR shows relatively better COD removal among the three MBRs as shown in the following figure.

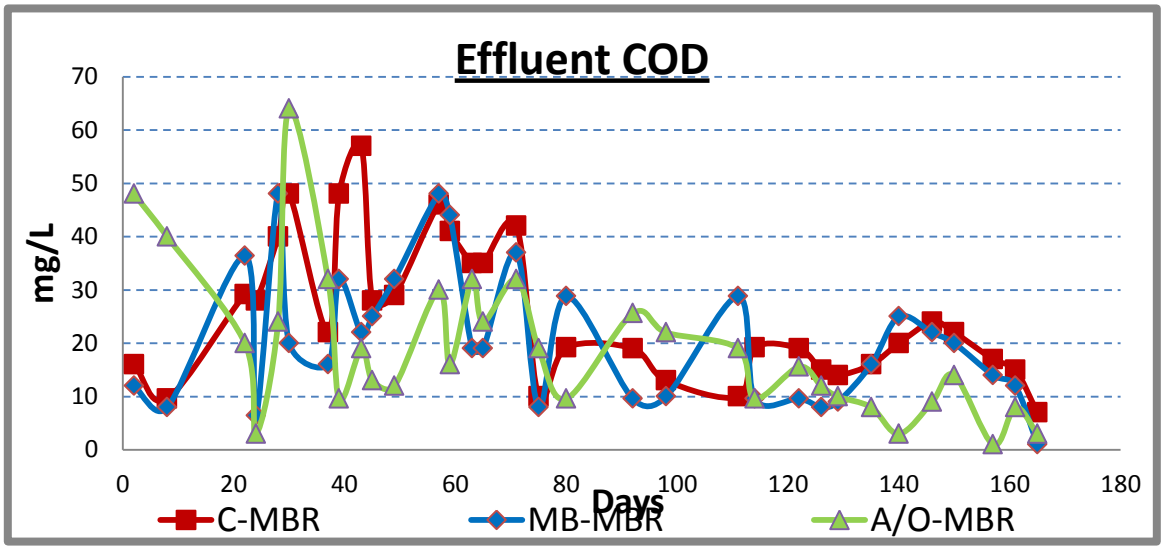


Figure 6 Effluent COD of all MBRs

4.2 Nutrients removal

4.2.1 NH_4-N removal

The C-MBR was able to remove 90% NH_4-N from influent synthetic wastewater. The MB-MBR gave 95% NH_4-N removal due to nitrification by the biofilm developed on the plastic media. A/O-MBR exhibits relatively high quality effluent, almost 96% removal due to nitrification followed by de-nitrification in the anoxic zone. Nitrification converts NH_4-N into $NO_2^{-1}$and NO_3^{-1} i.e. low concentration of NH_4-N in A/O-MBR and MB-MBR's effluents. Figure shows the influent and effluent trend in all MBRs.

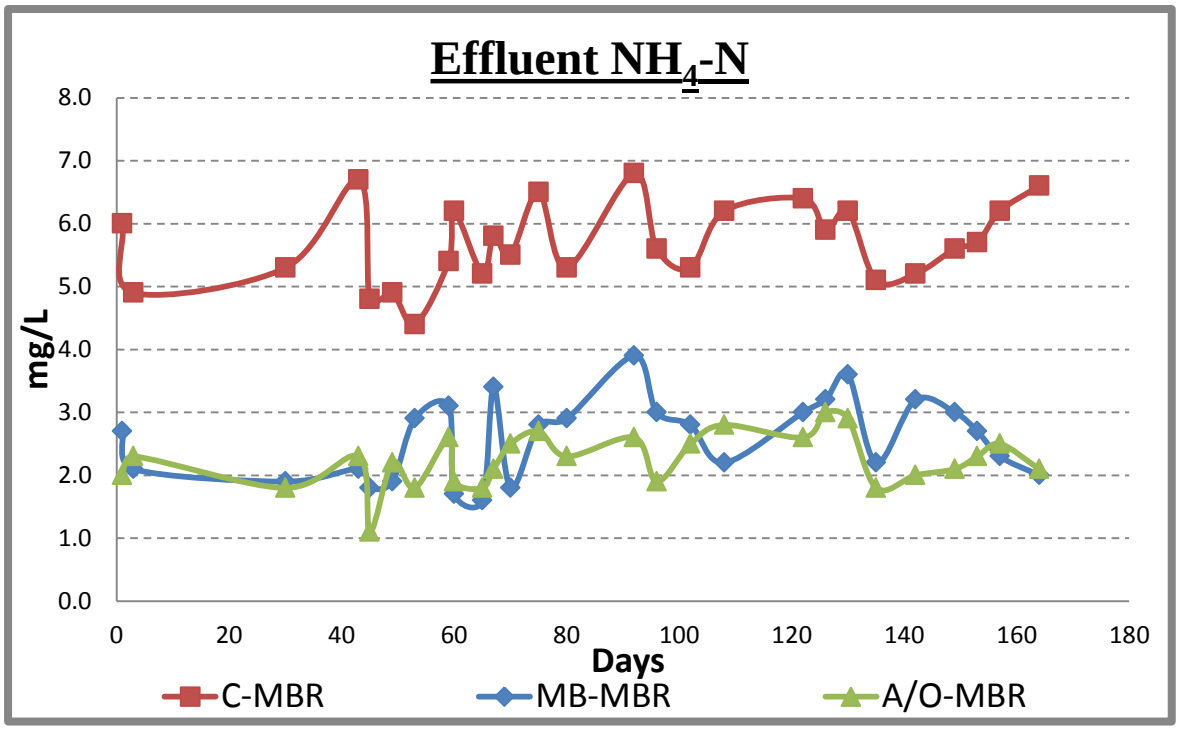


Figure 7 Effluent NH_4-N of all MBRs.

4.2.2 NO_2^{-1} removal

A/O-MBR gave maximum NO_2^{-1} removal than the other two MBRs due to enhanced nitrification process in anoxic conditions.

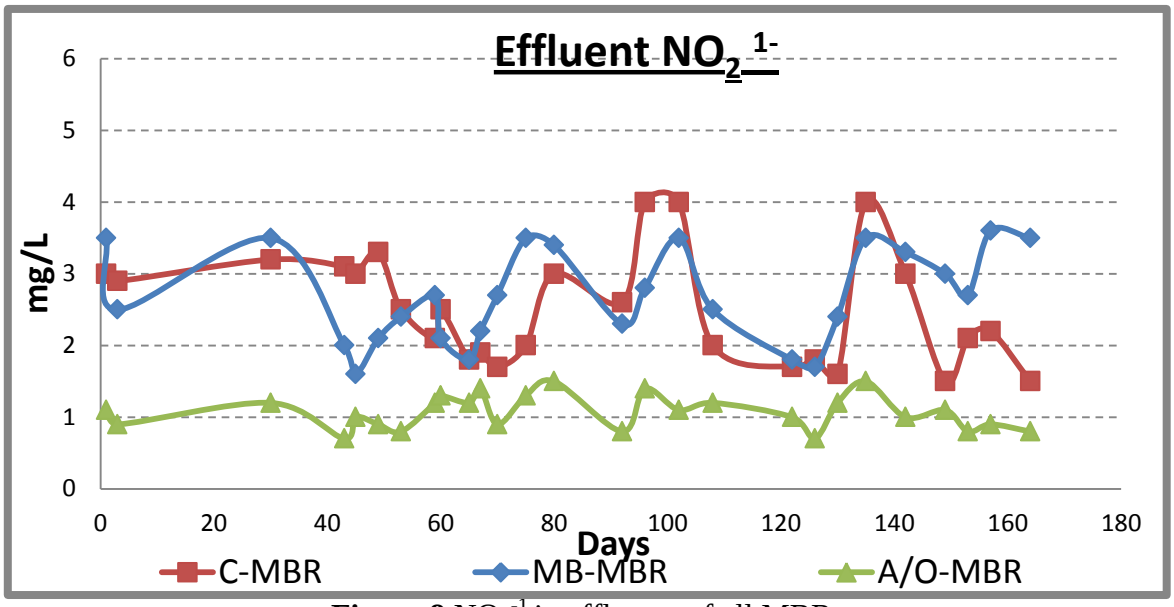


Figure 8 NO_2^{-1} in effluents of all MBRs

4.2.3 NO_3^{-1} Removal

AO-MBR was most efficient in terms of NO_3^{-1} removal due to nitrification and de-nitrification in anoxic zone. AO-MBR gave 5 mg/L of NO_3^{-1} in effluent on average. While C-MBR and MB-MBR effluents were 12 mg/L and 10 mg/L, respectively.

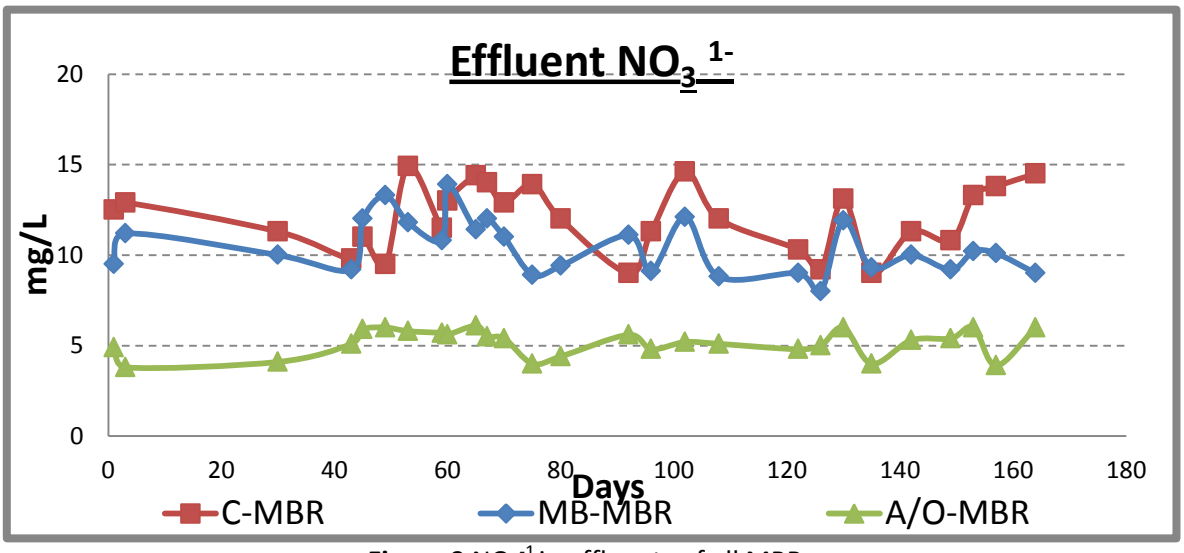


Figure 9 NO_3^{-1} in effluents of all MBRs

4.2.4 TN Removal

It was found that the maximum TN removal (83.2 %) was in A/O-MBR followed by 69 % TN removal in MB-MBR while 60 % in C-MBR. Better TN removal in A/O-MBR was due to the appropriate production of nitrifying and denitrifying bacteria.

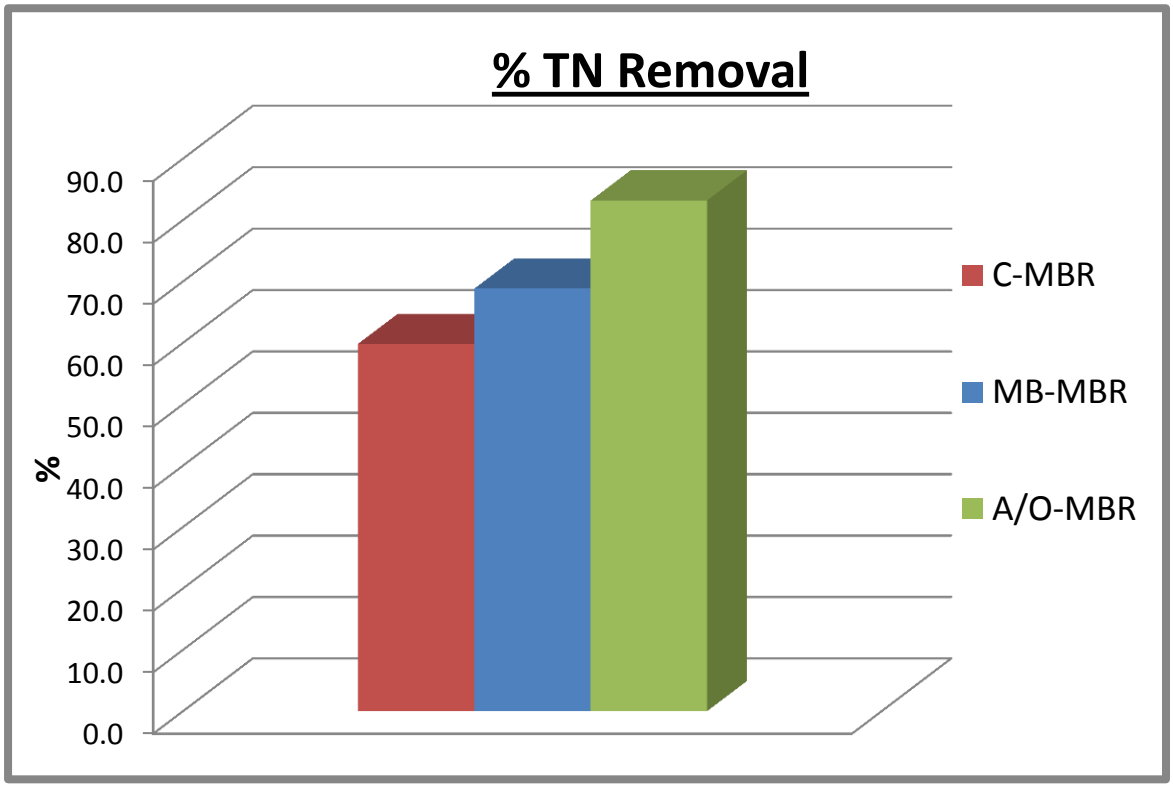


Figure 10 TN removal in all three MBRs

4.2.5 PO_4^{-3}– P Removal

The PO_4^{-3}-P removal is very important parameter in wastewater treatment as it can cause eutrophication along with NO_2^{-1} and NO_3^{-1}while eutrophication causes the extra ordinary growth of *Algae* and *Algae* results in anoxic conditions. This happens because the *Algae* consumes all the oxygen present and this leads to the destruction of microbes as well as biological treatment. The effluent quality with least concentration of PO_4^{-3} was found in the A/O-MBR. Phosphorous removal efficiencies of C-MBR, MB-MBR and A/O-MBR were as 46.48%, 59.46% and 69.74% respectively on average basis. C-MBR showed least removal efficiency because it had no anoxic zone and moving biofilm carriers in it.

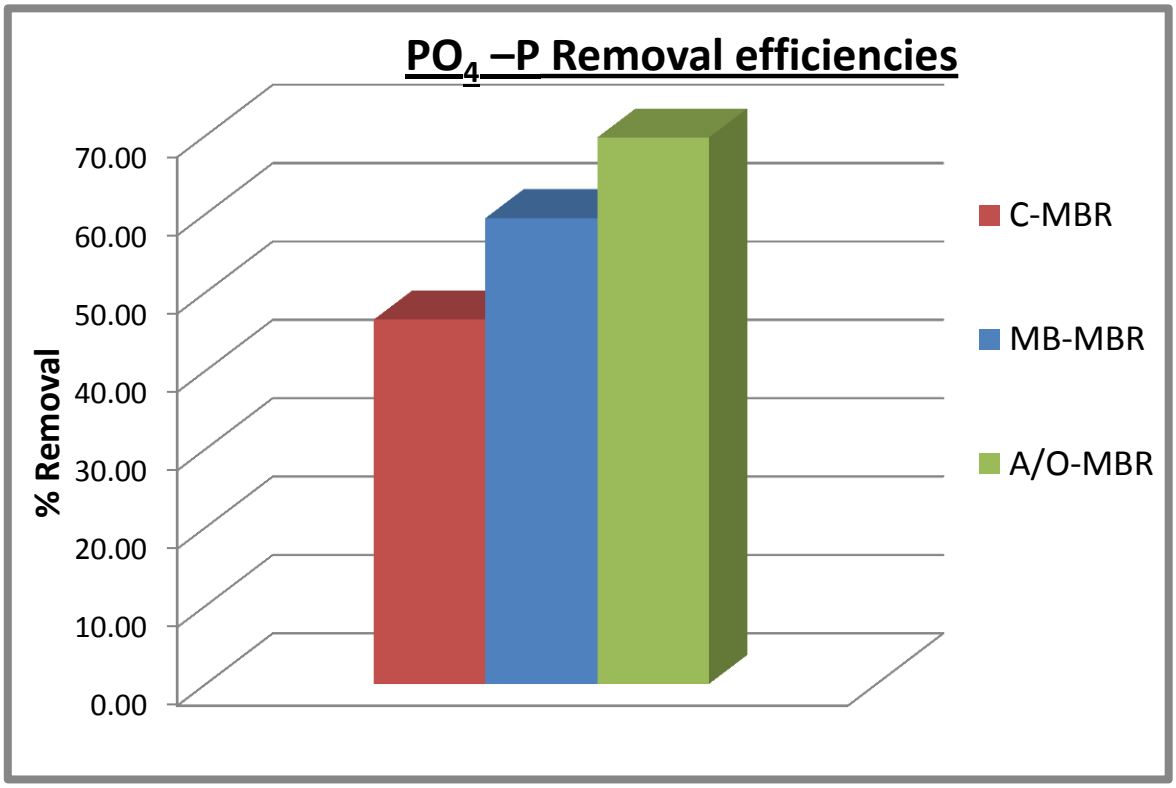


Figure 11 PO_4^{-3} removal in all three MBR

4.3 Results for microbial analysis

4.3.1 Colony Count

The results obtained for colony counting showed that A/O-MBR allows the growth of maximum microorganisms as compared to that of MB-MBR and C-MBR (Table 2).

$$\text{Number of Bacteria/ml} = \frac{\text{Number of Colonies (CFU)}}{\text{(Dilution x amount plated)}}$$

4.3.2 Reactor

Table 6 Comparison of colony counts for all three reactors

Reactor	CFU/ml
C-MBR	5.1×10^7
MB-MBR	1.5×10^8
A/O-MBR	8.3×10^8

4.3.3 Effluent

Effluent of all reactors was plated on EMB agar and the following results were obtained

Table 7 Bacterial Count of effluent

Reactor	Number of bacteria
C-MBR	2×10^4
MB-MBR	6.1×10^5
MMMB-MBR	1.7×10^6

It can be seen that increased bacterial count in reactor also influences the number of microbes in the effluent as the results are same as before.

4.3.4 Gram Staining

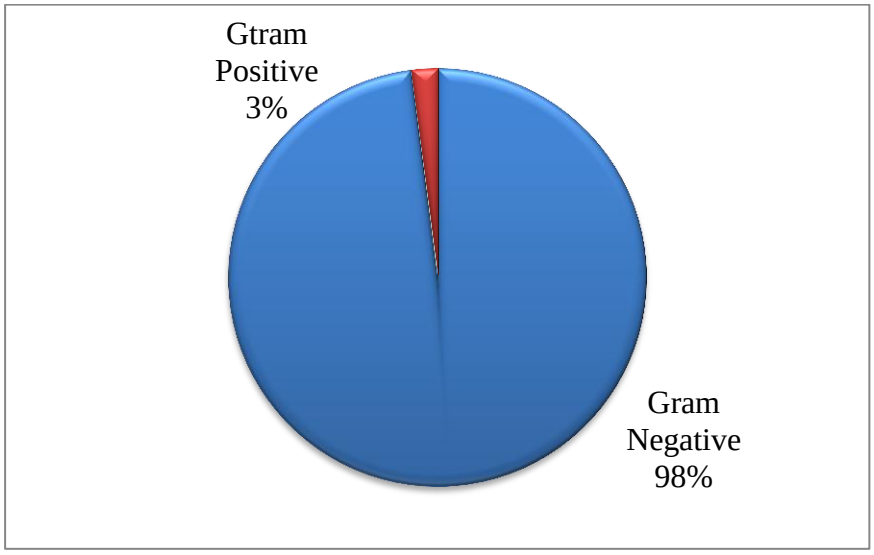


Figure 12 Gram staining of bacterial isolates obtained from C-MBR

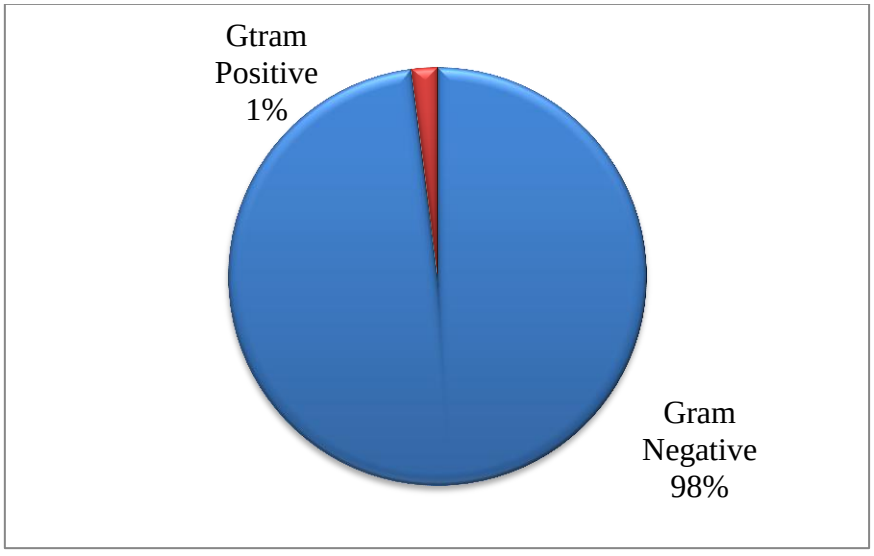


Figure 13 Gram staining of bacterial isolates obtained from MB-MBR

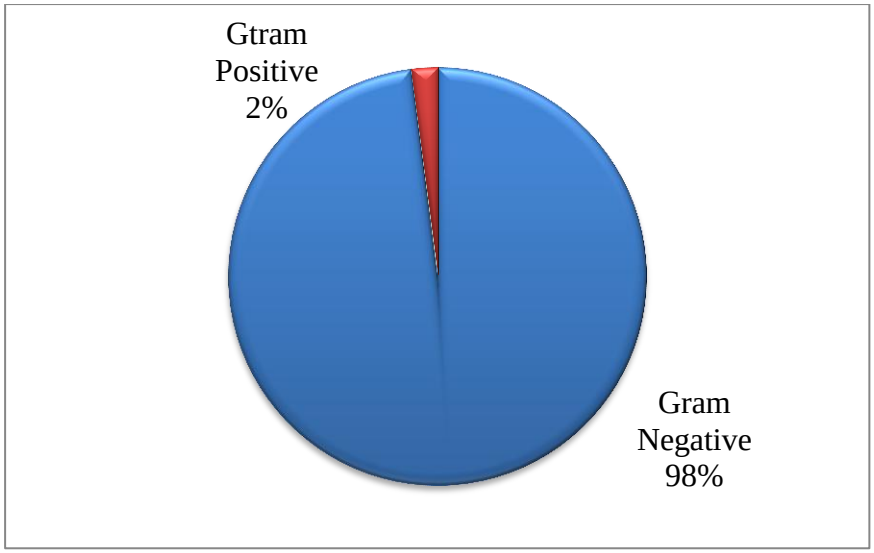


Figure 14 Gram staining of bacterial isolates obtained from A/O-MBR

4.3.5 Colony Morphology

The morphological examination of isolates revealed that the colonies of the C-MBR were mostly circular in form, had raised elevations and the margins were mostly smooth, although some were found to be lobate and undulate. A majority of them looked opaque, varied in colour from off-white to dark yellow, were glistening and their sizes ranged from 0.1 to 0.2 cm.

In contrast to this, all MB-MBR colonies were smooth and circular in shape, were mostly pasty in texture and off-white in colour that appeared golden brown under a light microscope. The colonies of A/O-MBR were similar to those of C-MBR in terms of shape, elevation, texture and colour. The sizes of these colonies were different, however, as they varied from <0.1cm to 0.4cm. The sizes of the colonies of C-MBR, MB-MBR were more of less similar, with the smallest colonies found in the MB-MBR. Whereas the cell morphology showed only one isolate in each reactor to be Gram positive with the remaining being Gram negative. The sizes, elevation and shape of the bacteria in all the three reactors is shown in figures 15, 16 and 17 respectively.

The detailed colony morphology is given for each of the reactors in the graphs below:

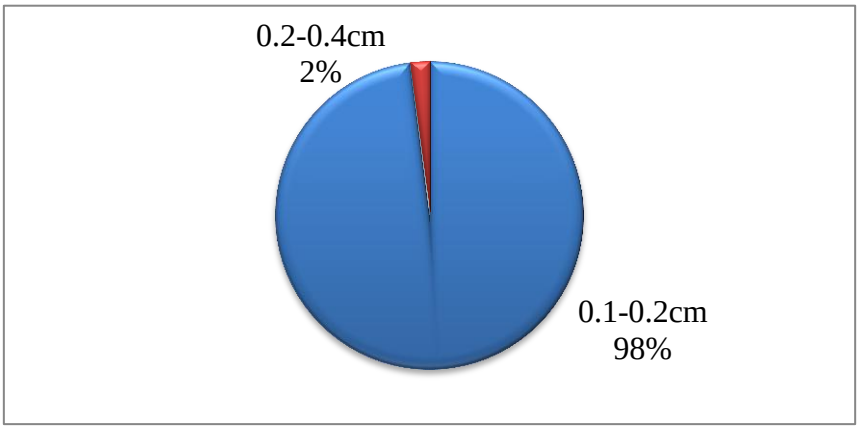


Figure 15 Sizes of bacterial colonies obtained from all three Reactors

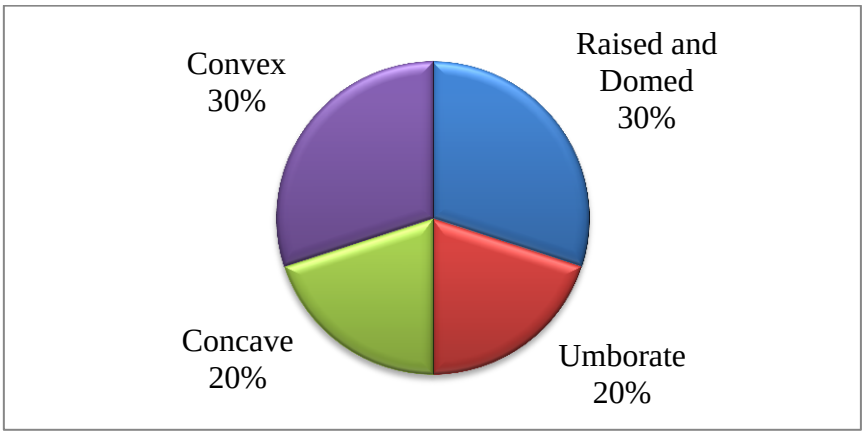


Figure 16 Elevation of bacterial colonies obtained from all three Reactors

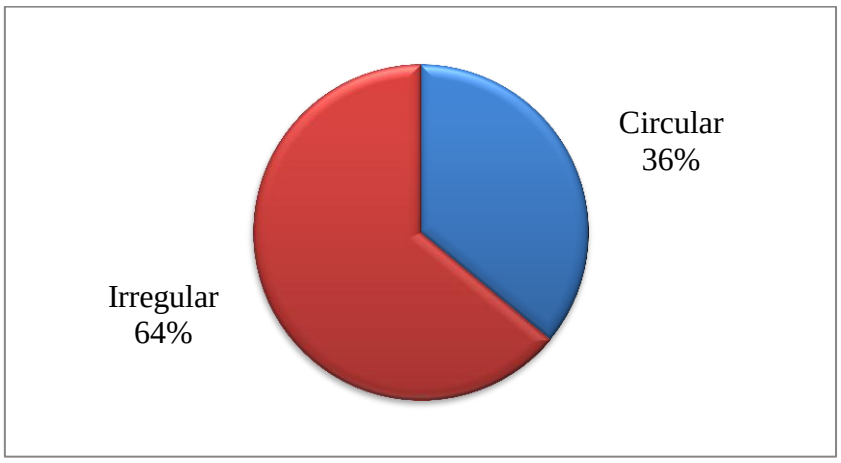
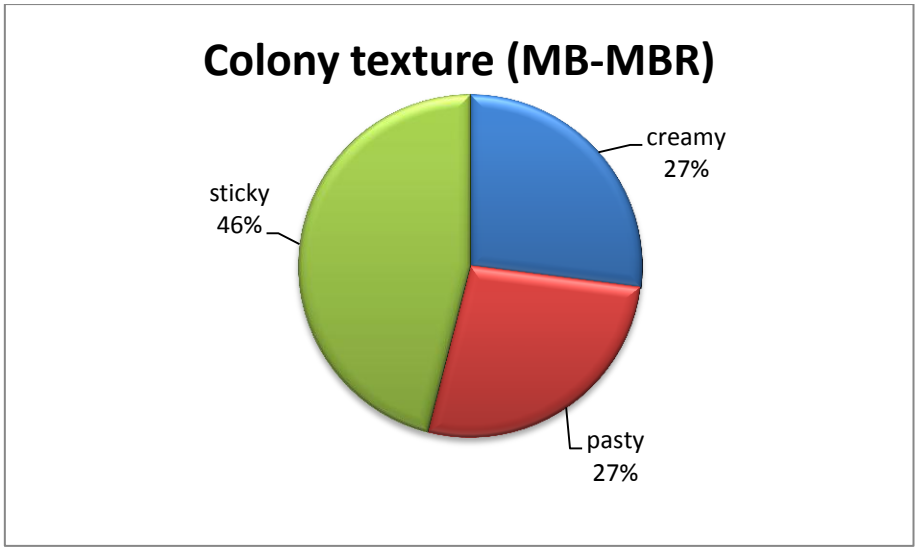
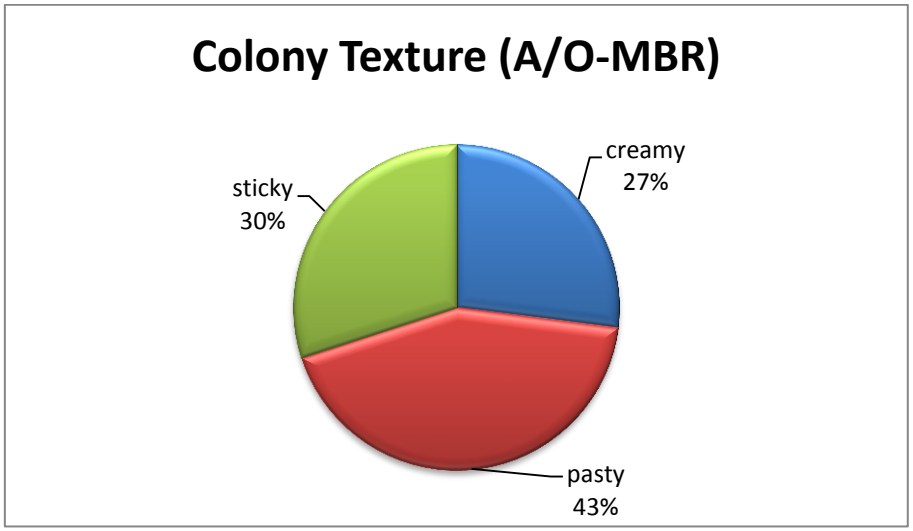


Figure 17 Colony Shape of bacterial colonies obtained from all three Reactors





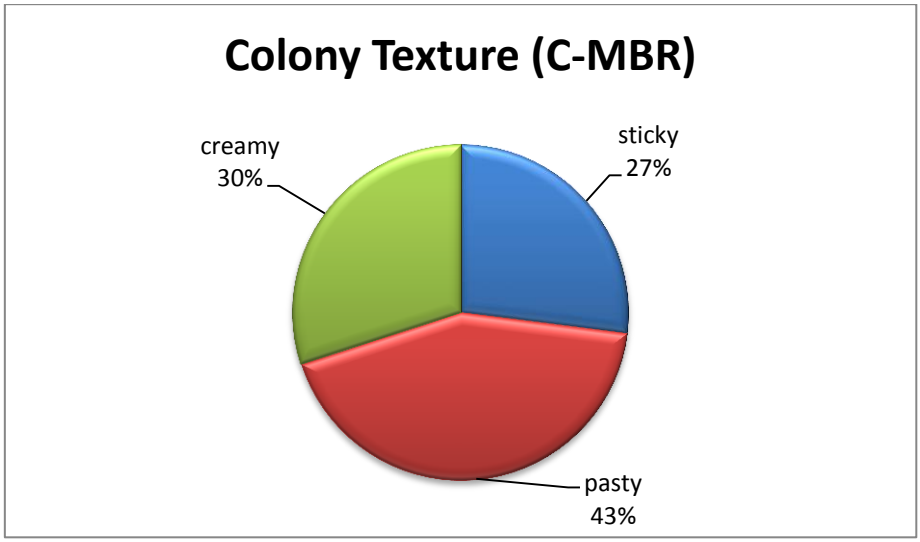


Figure 18 Colony Texture of bacterial colonies obtained from all three Reactors

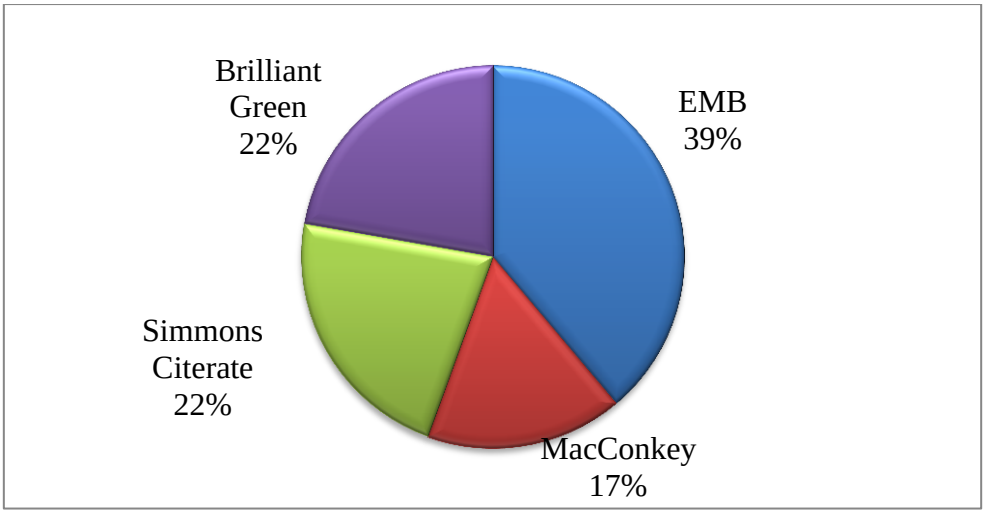


Figure 19 Biochemical Test Results for MB-MBR

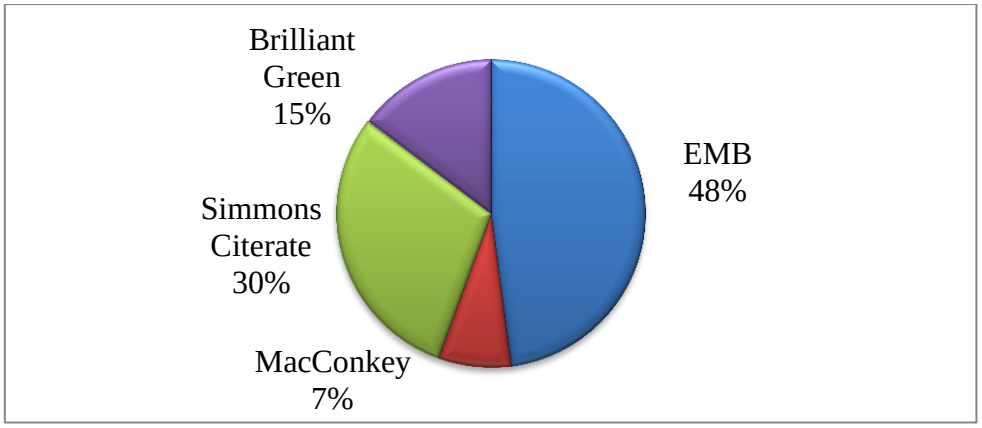


Figure 20 Biochemical Test Results for A/O-MBR

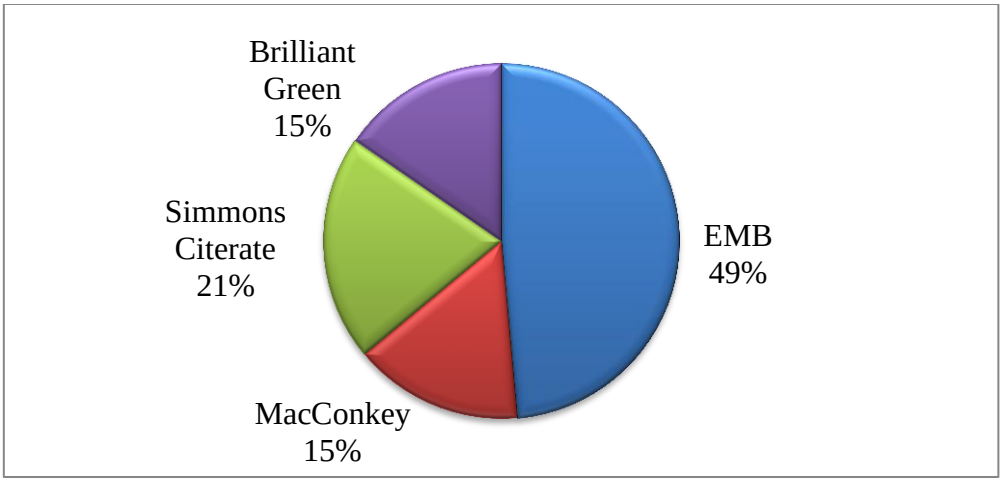


Figure 21 Biochemical Test Results for C-MBR

4.4 Discussion

The bacteria are responsible for the degradation of organic and inorganic compounds. They derive their nutritional requirement from the compounds presented to them in the influent waste. They derive their energy from oxidizing either organic compounds (chemoorganotrophic metabolism), or inorganic compounds (chemolithotrophic metabolism), such as reduced sulphur or nitrogen compounds.

The maximum COD removal was found to be occurring in the A/O-MBR, which may be attributed to the fact that most rod-shaped or the bacteria of the genera Bacillus that are attributed to COD removal, were in this reactor.

When attempting to identify unknown bacteria, it is important to note the cultural characteristics that the organisms exhibit on and in various types of media. Proper isolation of individual species enabled us to examine the colonial shape and appearance, as well as other factors such as pigmentation and appearance.

To do this, a solid agar-based media was used to identify colonial characteristics (shape, size, elevation, margin type), and differentiate between two or more different species. In this study, we used the Nutrient Agar as an enriched medium for general bacterial isolation since most common species and even some fastidious forms will grow on this medium. The colonies in all three reactors were either creamy or pasty, although 2 and 3 colonies in C-MBR and A/O-MBR were stick too. The creamy colonies formed threads when picked with inoculating loop, showing that they may contribute to EPS formation in the reactor. Pasty colonies are, however, difficult to emulsify and subculture and such strains grow well on media that contains glycerol as a main carbon source and asparagine as a nitrogen source. The distribution of colony texture in MB-MBR, A/O MBR and C-MBR is shown in Figure 18.

Table 8 Growth of isolates on various media

Reactor	No of isolates	Eosin Methylene Blue Agar	Simmons Citrate Agar	MacConkey Agar	Brilliant Green Agar	Citrimite Agar
C-MBR	11	8	8	8	3	nil
MB-MBR	11	7	7	4	4	nil
A/O-MBR	11	8	8	5	5	yes

Eosin methylene blue (**EMB**) is a slightly selective stain for Gram-negative bacteria (Lavine et al., 1981), which is why all the bacteria that turned out to be gram positive via gram staining did not grow in this media, hence confirming our results. As seen in table 8, seven to eight colonies in the reactors turned out to be those of the lactose fermenting bacteria, of which two to three gave green metallic sheen (GMC), a distinctive feature of the *Enteriobacteria (E.Coli),* and some species of *Citrobacter* and *Enterobacter*. Lactose fermenting bacteria degrade large, complex organic molecules such as polysaccharides, lipids and proteins to smaller and simpler compounds through step-by-step biochemical reactions by a diversity of lactose fermenting bacterial groups. However, no colonies in any of the three reactors showed clear colonies, which means non-lactose fermenting and gram negative bacteria, giving rise to the possibility that all isolated colonies were lactose fermenting.

Using the **Simmons' Citrate Agar**, it was found that number of colonies that utilized citrate as a sole carbon source and ammonium ions as the sole nitrogen source producing basic by-products were equal in all the three reactors. However, it was found that the fermentation that produced acidic by-products, such as lactose fermentation, was taking place only in the C-MBR, and the pH in this rector was slightly lower than the other two reactors too.

Plating the isolated colonies on **MacConkey Agar** showed that the minimum number of lactose fermenting bacteria was found in the MB-MBR, while an equal number of them were found in the other two.

Using the **Brilliant Green Agar,** it showed that at least one genus of Salmonella, such as *Salmonella typhimurium* were present in all three reactors.

Colonies only appeared on the *Citrimite agar* when plated from the A/O-MBR reactor, showing that the denitrifying bacteria *Pseudomonas aeruginosa* existed in significant amounts in this reactor. This result was consistent with the percentage of nitrogen and phosphorous removal in this reactor as it was maximum here.

Most of these mediums support growth of gram negative bacteria only. Growth of the isolates can be related to their activity where A/O-MBR and C-MBR isolates showed similar activities followed by MB-MBR isolates. Several platings of the sample on citrimide agar allowed isolation of *Pseudomonas aeruginosa* from A/O-MBR only showing that maintenance of lesser DO allows the growth of important denitrifying microorganisms.

Parallel to the microbial analysis, performance of the reactors was evaluated in terms of nutrients (P, TN) removal and Nitrate and Nitrite removal. The results as percentage removal are shown in Table 9.

Table 9 API 20 E identification of the isolates

C-MBR	**MB-MBR**	**A/O - MBR**
Pseudomonas fluorescens/putida **(denitrifying bacteria)**	*Klebsiellapneumoniaessp pneumoniae* **(lactose fermenting)**	*Burkholderiacepacia*
		Pseudomonas aeruginosa **(denitrifying bacteria)**
Pseudomonas oryzihabitans **(denitrifying bacteria)**	*Grimontiahollisae*	*Pseudomonas fluorescens/putida* **(denitrifying bacteria)**
Klebsiellaoxytoca **(lactose fermenting)**	*Pseudomonas oryzihabitans* **(denitrifying bacteria)**	*Yersinia ruckeri* (possibility)
Pantoeaspp	*Pasteurellapneumotropica*	*Raoultellaterrigena*
Klebsiellapneaess pozaenae **(lactose fermenting)**	*Klebsiellaoxytoca* **(lactose fermenting)**	*Klebsiellaoxytoca* **(lactose fermenting)**
	Non fermentor	*Myroidesspp/ Chryseobacteriumindologenes*
Erwiniaspp (possibility)	*Vibrio fluvialis* (possibility)	*Bordetella/Alcaligenes /Moraxellaspp*

Pseudomonas are known for their diversity and their growth in all kinds of environment (Peixet al., 2009) similarly from all three membrane bioreactors almost all kinds of

Pseudomonas species were isolated that include *Pseudomonas fluorescens/putida, Pseudomonas oryzihabitans* and *Pseudomonas aeruginosa* with the first two isolated from all reactors while last from A/O-MBR only, as shown in Table 9. This implies that *Pseudomonas fluorescens/putida, Pseudomonas oryzihabitans*can grow at higher DO as compared to *Pseudomonas aeruginosa.*

Klebsiellaoxytoca has been studied (Abd-al-haleem et al., 2007) for its ability to reduce nitrite in wastewater and is therefore nitrifying bacteria. This specie was isolated from wastewater of all three reactors. Chen et al., (2008) studied *Klebsiellaoxytoca* for its ability to degrade cyanide, so along with nitrification it can perform other important functions as well. Similarly *Pantoeaspp* has been evaluated for biosorption of Cr, Cd, Cu and other industrially important metals (Ozdemir et. al., 2004). Majority of the colonies belonged to the *Enterobacteriaceae* family such as *Klebsiella pneumonia sspozaenae, Erwiniaspp, Raoultellaterrigena, Klebsiellaoxytoca* and *Pantoea spp.* Members of this family are Gram negative rods and are facultative anaerobic in nature and non-spore formers.

Chapter 5

5. CONCLUSIONS

5.1 MBR performance

This study revealed the following specific conclusions:

a. COD removal efficiency above 95% was achieved in all the three MBR systems namely C-MBR, MB-MBR and A/O-MBR
b. NH_4-N removal was found to be maximum in A/O-MBR due to effective nitrification process in anoxic zone and biofilm carriers.
c. A/O-MBR was efficient in the removal of NO_3^{1-} due to abundance of de-nitrifying bacteria's as compared to the other MBRs.
d. PO_4 –P removal was highest in A/O-MBR due to anoxic zone attained by optimum mechanical mixing and biofilm carriers. It was also better in MB-MBR due to biofilm formation on moving biofilm carriers.

5.2 MBR Microbial diversity

The abundance of creamy in all three reactors may conclude the EPS formation in the reactors, while the pasty colonies conclude that chemolithotrophs and nitrifying bacteria are present, along with a possibility of some sulphur reducing bacteria all the three reactors.

Activated sludge in all three reactors is dominated by *Pseudomonadaceae* family followed by *Enterobacteriaceae***,** one *Vibrionaceae* species and so on. Overall it can be suggested that Membrane Bioreactor of any kind is helpful in retaining microorganisms important for wastewater treatment and are indeed a better option as compared to activated sludge treatment systems. There in not much difference in the microbes isolated

from the reactors while their overall performance in terms of organic removal is also more or less the same except that addition of media and mechanical mixer placement and therefore lesser DO maintenance has enabled better treatment performance by supporting other important wastewater microorganisms such as *Pseudomonas aerugunisa*. Species identified by API recommend that the reactors are suited to treatment of industrial wastewater as well, as the isolates are potential species found in industrial wastewater treatment.

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APPENDIX A

Date	Day	MB-MBR			C-MBR			A/O-MBR		
		MLSS (g/L)	MLVSS (g/L)	MLVSS/MLSS	MLSS (g/L)	MLVSS (g/L)	MLVSS/MLSS	MLSS (g/L)	MLVSS (g/L)	MLVSS/MLSS
17-Aug-11	2	**7.7**	**6.2**	0.81	6.36	5.06	0.80	6.3	5.15	0.82
19-Aug-11	4	7.84	6	0.77	6.02	5.14	0.85	6.18	4.86	0.79
22-Aug-11	7	7.46	6.24	0.84	6.2	5.4	0.87	6.86	5.42	0.79
24-Aug-11	9	8.02	6.4	0.80	7.98	6.38	0.80	7.98	6.34	0.79
29-Aug-11	14	8.36	6.32	0.76	8.16	6.12	0.75	6.76	5.42	0.80
04-Sep-11	20	9.12	5.88	0.64	6.02	4.68	0.78	8.18	7.24	0.89
06-Sep-11	22	8.02	6.48	0.81	5.7	4.82	0.85	6.52	5.32	0.82
08-Sep-11	24	7.87	6.67	0.85	5.47	4.82	0.88	6.72	5.56	0.83
12-Sep-11	28	7.72	6.08	0.79	4.56	3.9	0.86	6.68	5.66	0.85
14-Sep-11	30	7.54	5.76	0.76	4.94	3.86	0.78	7.14	5.32	0.75
15-Sep-11	31	7.78	6.22	0.80	4.04	3.33	0.82	6.9	5.52	0.80
20-Sep-11	36	8.66	7.1	0.82	4.08	3.36	0.82	5.42	4.4	0.81
21-Sep-11	37	7.72	6.6	0.85	4.7	4	0.85	5.92	5.06	0.85
26-Sep-11	42	7.62	7	0.92	4.88	4	0.82	6.96	6.3	0.91
29-Sep-11	45	8.92	7.1	0.80	4.9	3.92	0.80	6.9	5.52	0.80
03-Oct-11	49	8.97	7.2	0.80	6.44	4.38	0.68	7.26	5.38	0.74
05-Oct-11	51	9.12	7.7	0.84	5.5	4.18	0.76	7.15	5.47	0.77
06-Oct-11	52	9.04	6.8	0.75	6.06	4.96	0.82	6.6	4.88	0.74
10-Oct-11	56	8.42	5.98	0.71	5.42	4.54	0.84	7.22	5.8	0.80
12-Oct-11	58	9.6	6.9	0.72	5.9	5.1	0.86	7.94	7.2	0.91
14-Oct-11	60	8.6	6.1	0.71	7	5.9	0.84	6.8	6	0.88
17-Oct-11	63	6.84	5.8	0.85	5.8	5.4	0.93	7.2	6.06	0.84
19-Oct-11	65	7.78	5.8	0.75	6.72	5.6	0.83	6.98	5.2	0.74
21-Oct-11	67	7.46	5.22	0.70	6.46	5.3	0.82	6.92	5.54	0.80
24-Oct-11	70	7.76	5.3	0.68	6.34	5.54	0.87	7.34	5.7	0.78
26-Oct-11	72	8.5	6.58	0.77	6.74	5.88	0.87	7.08	6.02	0.85
28-Oct-11	74	7.94	6.12	0.77	5.84	4.38	0.75	6.1	3.88	0.64
30-Oct-11	76	7.12	5.54	0.78	5.76	4.12	0.72	5.96	4.3	0.72
01-Nov-11	78	7.84	5.7	0.73	6.2	5.04	0.81	6.34	4.42	0.70
03-Nov-11	80	7.98	5.92	0.74	6.26	4.91	0.78	6.44	4.46	0.69
15-Nov-11	92	7.72	6.4	0.83	5.06	4.66	0.92	5.82	4.7	0.81
17-Nov-11	94	8.14	7.26	0.89	6.68	5.62	0.84	6.54	5.28	0.81
19-Nov-11	96	6.72	5.9	0.88	6.26	5.47	0.87	6.3	5.1	0.81
21-Nov-11	98	6.78	5.86	0.86	5.94	5.07	0.85	6.46	5.3	0.82
23-Nov-11	100	8.7	7.1	0.82	6.28	5.23	0.83	6.62	5.6	0.85
25-Nov-11	102	7.6	5.94	0.78	5.16	4.24	0.82	6.26	5.58	0.89
27-Nov-11	104	7.9	5.58	0.71	5.54	4.56	0.82	7.66	5.72	0.75
29-Nov-11	106	9.6	7.36	0.77	5.56	4.58	0.82	6.92	4.76	0.69
01-Dec-11	108	8.3	5.2	0.63	6.72	4.72	0.70	5.92	4.02	0.68

03-Dec-11	110	9.4	6.22	0.66	5.92	4.88	0.82	6.66	4.58	0.69
05-Dec-11	112	7.6	5.86	0.77	6.26	5.3	0.85	6.4	4.42	0.69
07-Dec-11	114	7.94	6.42	0.81	6.84	4.38	0.64	6.96	4.88	0.70
16-Dec-11	122	8.9	6.9	0.78	6.99	5.4	0.77	6.41	4.8	0.75
19-Dec-11	126	7.84	5.7	0.73	6.2	5.04	0.81	6.34	4.42	0.70
23-Dec-11	130	8.14	7.26	0.89	7.68	6.12	0.80	6.54	5.28	0.81
27-Dec-11	134	8.5	7.3	0.86	8.1	5.62	0.69	6.98	5.78	0.83
31-Dec-11	138	8.4	6.4	0.76	8.16	6.12	0.75	6.76	5.42	0.80
04-Jan-12	142	7.68	6.6	0.86	7.2	5.8	0.81	7.1	6.2	0.87
07-Jan-12	145	9.5	8	0.84	6.1	5.4	0.89	5	4.32	0.86
11-Jan-12	149	8.87	6.8	0.77	5.9	5	0.85	5.9	5.2	0.88
16-Jan-12	154	9.2	6.08	0.66	6.4	5.1	0.80	5.8	5.1	0.88
19-Jan-12	157	8.5	6.3	0.74	7.2	6.1	0.85	5.7	5	0.88
23-Jan-12	161	9.3	8.1	0.87	8.4	7.1	0.85	5.4	4.2	0.78
26-Jan-12	164	8.2	6.6	0.80	8.5	7.2	0.85	5.8	4.9	0.84
AVERAGE		8.2	6.4	0.8	6.2	5.1	0.8	6.6	5.3	0.8
S.D		0.72	0.67	0.07	1.04	0.83	0.06	0.65	0.70	0.07

Date	Day	Influent NH_4-N (mg/L)	MB-MBR Effluent (mg/L)					C-MBR Effluent (mg/L)					A/O-MBR Effluent (mg/L)				
			NH_4-N	NO_2^{1-}	NO_3^{1-}	TN	% TN Removal	NH_4-N	NO_2^{1-}	NO_3^{1-}	TN	% TN Removal	NH_4-N	NO_2^{1-}	NO_3^{1-}	TN	% TN Removal
16/8/11	1	52.9	2.7	3.5	9.5	15.7	70.3	6.0	3	12.5	21.5	59.4	2.0	1.1	4.9	8.0	84.9
18/8/11	3	53.1	2.1	2.5	11.2	15.8	70.2	4.9	2.9	12.9	20.7	61.0	2.3	0.9	3.8	7.0	86.8
14/9/11	30	48.7	1.9	3.5	10	15.4	68.3	5.3	3.2	11.3	19.8	59.3	1.8	1.2	4.1	7.1	85.4
27/9/11	43	50.8	2.1	2	9.2	13.3	73.8	6.7	3.1	9.8	19.6	61.4	2.3	0.7	5.1	8.1	84.0
29/9/11	45	47.6	1.8	1.6	12	15.4	67.6	4.8	3	11	18.8	60.5	1.1	1	5.9	8.0	83.2
03/10/11	49	53.3	1.9	2.1	13.3	17.3	67.5	4.9	3.3	9.5	17.7	66.8	2.2	0.9	6	9.1	82.9
07/10/11	53	51.6	2.9	2.4	11.8	17.1	66.9	4.4	2.5	14.9	21.8	57.8	1.8	0.8	5.8	8.4	83.7
13/10/11	59	48.4	3.1	2.7	10.8	16.6	65.7	5.4	2.1	11.5	19.0	60.7	2.6	1.2	5.7	9.5	80.4
14/10/11	60	45.3	1.7	2.1	13.9	17.7	60.9	6.2	2.5	13	21.7	52.0	1.9	1.3	5.6	8.8	80.6
19/10/11	65	47.2	1.6	1.8	11.4	14.8	68.6	5.2	1.8	14.4	21.4	54.7	1.8	1.2	6.1	9.1	80.7
21/10/11	67	53.1	3.4	2.2	12	17.6	66.9	5.8	1.9	14	21.7	59.1	2.1	1.4	5.5	9.0	83.1
24/10/11	70	51.0	1.8	2.7	11	15.5	69.6	5.5	1.7	12.9	20.1	60.6	2.5	0.9	5.4	8.8	82.7
29/10/11	75	51.0	2.8	3.5	8.9	15.2	70.2	6.5	2	13.9	22.4	56.1	2.7	1.3	4	8.0	84.3
03/11/11	80	52.0	2.9	3.4	9.4	15.7	69.8	5.3	3	12	20.3	61.0	2.3	1.5	4.4	8.2	84.2
15/11/11	92	51.3	3.9	2.3	11.1	17.3	66.3	6.8	2.6	9	18.4	64.1	2.6	0.8	5.6	9.0	82.5
19/11/11	96	56.8	3.0	2.8	9.1	14.9	73.8	5.6	4	11.3	20.9	63.2	1.9	1.4	4.8	8.1	85.7
25/11/11	102	52.6	2.8	3.5	12.1	18.4	65.0	5.3	4	14.6	23.9	54.6	2.5	1.1	5.2	8.8	83.3
01/12/11	108	47.4	2.2	2.5	8.8	13.5	71.5	6.2	2	12	20.2	57.4	2.8	1.2	5.1	9.1	80.8
15/12/11	122	52.0	3.0	1.8	9	13.8	73.5	6.4	1.7	10.3	18.4	64.6	2.6	1	4.8	8.4	83.8
19/12/11	126	49.0	3.2	1.7	8	12.9	73.7	5.9	1.8	9.2	16.9	65.5	3.0	0.7	5	8.7	82.2
22/12/11	130	54.0	3.6	2.4	11.9	17.9	66.9	6.2	1.6	13.1	20.9	61.3	2.9	1.2	6	10	81.3
28/12/11	135	44.0	2.2	3.5	9.3	15.0	65.9	5.1	4	9	18.1	58.9	1.8	1.5	4	7.3	83.4
04/1/12	142	50.2	3.2	3.3	10	16.5	67.1	5.2	3	11.3	19.5	61.2	2.0	1	5.3	8.3	83.5
11/1/12	149	49.3	3.0	3	9.2	15.2	69.2	5.6	1.5	10.8	17.9	63.7	2.1	1.1	5.4	8.6	82.6
15/1/12	153	48.0	2.7	2.7	10.2	15.6	67.5	5.7	2.1	13.3	21.1	56.0	2.3	0.8	6	9.1	81.0
19/1/12	157	53.3	2.3	3.6	10.1	16.0	70.0	6.2	2.2	13.8	22.2	58.3	2.5	0.9	3.9	7.3	86.3
26/1/12	164	51.0	2.0	3.5	9	14.5	71.6	6.6	1.5	14.5	22.6	55.7	2.1	0.8	6	8.9	82.5
AVERAGE		50	2.6	2.7	10	15	68.8	5.7	2.5	12	20	59.8	2.2	1.1	5.2	8.5	83

Phosphate Readings

Date	Day	**PO_4-P influent (mg/L)**	**MB-MBR Effluent**		**C-MBR Effluent**		**A/O-MBR Effluent**	
			PO_4 – P (mg/L)	% Removal	PO_4 – P (mg/L)	% Removal	PO_4 – P (mg/L)	% Removal
16-Aug-11	1	14.6	6.7	54.1	8.2	43.8	4.8	67.1
18-Aug-11	3	15	6.3	58.0	6.5	56.7	6	60.0
14-Sep-11	30	15.3	5.4	64.7	10.3	32.7	1.2	92.2
27-Sep-11	43	13.5	5.6	58.5	9	33.3	5.4	60.0
29-Sep-11	45	16	6.1	61.9	9.3	41.9	5.2	67.5
03-Oct-11	49	17.6	7.4	58.0	8.6	51.1	3.8	78.4
07-Oct-11	53	18.1	7	61.3	8	55.8	5	72.4
13-Oct-11	59	20	8.6	57.0	9.1	54.5	7.1	64.5
14-Oct-11	60	11.2	5	55.4	5.7	49.1	3.7	67.0
19-Oct-11	65	14.5	6	58.6	7	51.7	2.65	81.7
21-Oct-11	67	16.5	7.8	52.7	8.7	47.3	5.3	67.9
24-Oct-11	70	18	7	61.1	10	44.4	6	66.7
29-Oct-11	75	12.6	3.8	69.8	6.9	45.2	2.5	80.2
03-Nov-11	80	16	7.1	55.6	8.5	46.9	5.7	64.4
15-Nov-11	92	9	3.24	64.0	5.4	40.0	3.7	58.9
19-Nov-11	96	12	4.9	59.2	5.6	53.3	3.6	70.0
25-Nov-11	102	15	5.8	61.3	8.7	42.0	5	66.7
01-Dec-11	108	14.5	6.2	57.2	7.7	46.9	4.7	67.6
15-Dec-11	122	16.3	5.8	64.4	7.6	53.4	2.5	84.7
19-Dec-11	126	17	5.1	70.0	9.3	45.3	6.2	63.5
22-Dec-11	130	13	5.84	55.1	6.7	48.5	4	69.2
28-Dec-11	135	13.1	5.7	56.5	6.5	50.4	4.1	68.7
04-Jan-12	142	9.6	4.4	54.2	5.9	38.5	3.1	67.7
11-Jan-12	149	11	4.7	57.3	6.9	37.3	3.4	69.1
15-Jan-12	153	12.2	5.1	58.2	5.6	54.1	3.8	68.9
19-Jan-12	157	10.6	4.3	59.4	5.9	44.3	2.9	72.6
26-Jan-12	164	13.1	5	61.8	7	46.6	4.5	65.6

AVERAGE		14.27	5.77	59.46	7.58	46.48	4.29	69.74
SD		2.76	1.23	4.38	1.45	6.49	1.37	7.70

COD Readings:

Date	Day	Influent COD (mg/L)	MB-MBR Effluent		C-MBR Effluent		A/O-MBR Effluent	
			COD (mg/L)	% Removal	COD (mg/L)	% Removal	COD (mg/L)	% Removal
17-Aug-11	2	420	**12**	**97**	16	96	48	89
23-Aug-11	8	536	8	99	9.6	98	40	93
06-Sep-11	22	492.8	36.4	93	29.2	94	20	96
08-Sep-11	24	560	6.4	99	28	95	3	99
12-Sep-11	28	528	48	91	40	92	24	96
14-Sep-11	30	640	20	97	48	93	64	90
21-Sep-11	37	620	16	98	22	96	32	95
23-Sep-11	39	456	32	93	48	90	9.6	98
27-Sep-11	43	595	22	96	57	90	19	97
29-Sep-11	45	496	25	95	28	94	13	97
03-Oct-11	49	492	32	94	29	94	12	98
11-Oct-11	57	523	48	91	46	92	30	94
13-Oct-11	59	488	44	91	41	92	16	97
17-Oct-11	63	528	19	96	35	94	32	94
19-Oct-11	65	492	19	96	35	93	24	95
25-Oct-11	71	520	37	93	42	92	32	94
29-Oct-11	75	520	8	98	10	98	19	96
03-Nov-11	80	450	28.8	93.6	19.2	95.7	9.6	97.8
15-Nov-11	92	496	9.6	98	19	96	25.6	94
21-Nov-11	98	530	10	98	13	97	22	97
04-Dec-11	111	520	28.8	93.6	10	98	19	96
07-Dec-11	114	450	9.6	93.6	19.2	95.7	9.6	97.8
15-Dec-11	122	496	9.6	98	19	96	15.6	97
19-Dec-11	126	530	8	98.4	15	97	12	98
22-Dec-11	129	510	9	98	14	97	10	98
28-Dec-11	135	480	16	98	16	97	8	98
02-Jan-12	140	520	25	95.2	20	96.2	3	99.4
08-Jan-12	146	512	22	95.7	24	95.3	9	98.2
12-Jan-12	150	492	20	95.9	22	95.5	14	97.2
19-Jan-12	157	540	14	97.4	17	96.9	1	99.8
23-Jan-12	161	481	12	97.5	15	96.9	8	98.3
27-Jan-12	165	526	1	99.8	7	98.7	3	99.4

AVERAGE		513.7	20.5		25.4	95.1	19.0	96.4

Summary:

Tests performed	**MB-MBR**	**C-MBR**	**A/O-MBR**
MLSS (g/L)	8.20	6.21	6.61
MLVSS (g/L)	6.40	5.05	5.26
COD (mg/L)	20.51	25.41	18.97
COD % Removal	95.87	95.06	96.38
NH_4-N (mg\L)	2.59	5.69	2.24
NO_2^{1-} (mg\L)	2.69	2.52	1.07
NO_3^{1-} (mg\L)	10.45	12.07	5.16
TN (mg\L)	15.73	20.28	8.47
TN % Removal	68.82	59.81	83.18
PO_4 –P (mg/L)	5.77	7.58	4.29
PO4 –P % Removal	59.46	46.48	69.74

Results for Shape and gram staining

Table No. 3.13: Gram staining of bacterial isolates obtained from Conventional Membrane Bioreactor (C-MBR)

Bacterial Isolates	**Gram Staining**	**Shape**	**Arrangement**
SG-1	Gram negative	Bacilli	Diplo &strepto- bacilli
SG-3	Gram negative	Cocci	Single &strepto-cocci
SG-4	Gram negative	Bacilli	Diplo &strepto- bacilli
SG-5	Gram negative	Cocci	Strepto-cocci
SG-6	Gram negative	Cocci	Strepto-cocci
SG-7	Gram negative	Cocci	Strepto-cocci
SG-8	Gram negative	Cocci	Single &strepto-cocci
SG-9	Gram negative	Cocci	Staphylococci
SG-10	Gram negative	Cocci	Tetrad &staphylococci
SG-11	Gram negative	Cocci	Staphylococci
SG-12	Gram positive	Cocci	Diplo &strepto-cocci
SG-13	Gram negative	Cocci	Strepto-cocci

Table 3.14Gram staining of bacterial isolates obtained from Moving Bed Membrane Bioreactor (MB-MBR)

Bacterial Isolates	**Gram Staining**	**Shape**	**Arrangement**
AG-2	Gram negative	Cocci	Diplo &strepto-cocci
AG-3	Gram negative	Cocci	Strepto-cocci
AG-4	Gram negative	Bacilli	Strepto- bacilli

AG-5	Gram negative	Bacilli	Strepto- bacilli
AG-6	Gram negative	Cocci	Diplo &strepto-cocci
AG-7	Gram negative	Cocci	Diplo &strepto-cocci
AG-8	Gram negative	Cocci	Staphylococci
AG-9			
AG-11	Gram positive	Cocci	Diplo,tetrad &staphylococci
AG-12	Gram negative	Bacilli	Strepto- bacilli

Table 3.15 Bacterial isolates obtained from Moving Bed & Mechanically Mixed Membrane Bioreactor (MBMM-MBR)

Bacterial Isolate	**Gram Staining**	**Shape**	**Arrangement**
MM-1	Gram negative	Bacilli	Strepto- bacilli
MM-3	Gram negative	Bacilli	Diplo &strepto- bacilli
MM-4	Gram negative	Bacilli	Strepto- bacilli
MM-5	Gram negative	Cocci	Strepto-cocci
MM-6	Gram negative	Cocci	Diplo &strepto-cocci
MM-8	Gram negative	Cocci	Strepto-cocci
MM-9	Gram negative	Bacilli	Diplo-bacilli
MM-10	Gram negative	Cocci	Diplo &strepto-cocci
MM-11	Gram negative	Cocci	Tetrad &staphylococci
MM-12	Gram negative	Cocci	Single
MM-13	Gram positive	Cocci	Tetrad &staphylococci

Results of Colony Morphology

Table 3.9 Colony Morphology of bacterial isolates obtained from (C-MBR)

Isolate	**Shape**	**Size (cm)**	**Margin**	**Elevation**	**Texture**	**Color**	
						Naked eye	**Microscope**
SG-3	Irregular	0.2	Undulate	Umborate	Pasty	Luminescent yellow	Dark golden brown
SG-4	Irregular	0.1-0.3	Lobate	Concave	Sticky	Dirty off-white	Dark golden brown
SG-5	Circular	0.2-0.4	Smooth	Raised	Pasty	Off-white	Soil brown
SG-6	Irregular	0.1-0.2	Lobate	Concave	Sticky	Dirty off-white	Dark golden brown
SG-7	Irregular	0.1-0.2	Undulate	Umborate	Sticky	Yellowish off-white	Dark olive green
SG-8	Circular	0.1	Smooth	Raised	Creamy	Off-white	Dark grey
SG-9	Circular	0.2	Smooth	Flat	Creamy	Off-white	Dark grey
SG-10	Circular	0.1	Smooth	Dome	Pasty	Lemon yellow	Golden brown
SG-11	Circular	0.1	Smooth	Flat	Pasty	Off-white	Soil brown
SG-12	Circular	0.1	Smooth	Flat	Pasty	Dark yellow	Soil brown
SG-13	Circular	0.1-0.2	Smooth	Convex	Creamy	Yellow	Soil brown

Table 3.10 Colony Morphology of bacterial isolates obtained from (MB-MBR)

Isolate	**Shape**	**Size (cm)**	**Margin**	**Elevation**	**Texture**	**Color**	
						Naked eye	**Microscope**
AG-3	Circular	0.2-0.3	Smooth	Convex	Creamy	Off-white	Golden brown
AG-4	Circular	<0.1	Smooth	Flat	Creamy	Lemon yellow	Dark golden brown
AG-5	Circular	0.1	Smooth	Flat	Creamy	Dirty offwhite	Golden brown
AG-6	Circular	≤0.1	Smooth	Flat	Pasty	Off-white	Soil brown
AG-7	Circular	≤0.1	Smooth	Flat	Pasty	Off-white	Soil brown
AG-8	Circular	0.1	Smooth	Raised	Pasty	Milky offwhite	Soil brown
AG-9	Circular	0.3-0.4	Smooth	Convex	Pasty	Off-white	Dark brown
AG-11	Circular	<0.1	Smooth	Convex	Pasty	Milky offwhite	Dark grey
AG-12	Circular	0.1-0.2	Smooth/ undulate	Umborate	Creamy	Luminescent Off-white	
AG-13	Circular	0.1-0.3	Smooth	Raised	Pasty	Off-white	Golden brown

Table 3.11 Colony Morphology of bacterial isolates obtained from (MBMM-MBR)

Isolate	**Shape**	**Size (cm)**	**Margin**	**Elevation**	**Texture**	**Color**	
						Naked eye	**Microscope**
MM-1	Irregular	0.1-0.2	Undulate	Umborate	Sticky	Yellowish Off-white	Soil brown
MM-3	Circular	<0.1	Smooth	Convex	Sticky	Dirty off-white	Dark soil brown
MM-4	Irregular	0.2-0.4	Undulate	Concave	Sticky	Dirty Yellow	Light golden brown
MM-5	Irregular	0.1-0.2	Undulate	Raised	Sticky	Yellowish Off-white	Dark golden brown
MM-6	Circular	0.1	Smooth	Convex	Pasty	Light Off-white	Golden brown
MM-8	Irregular	0.2-0.4	Undulate	Raised	Pasty	Off-white	Dark brown
MM-9	Circular	0.1-0.2	Smooth	Flat	Creamy	Light dirty Off-white	Light golden brown
MM-10	Circular	0.2-0.4	Smooth	Convex	Pasty	Off-white	Dark grey
MM-11	Circular	<0.1	Smooth	Flat	Pasty	Milky offwhite	Soil brown
MM-12	Circular	<0.1		doom	Pasty	dirty Off-white	
MM-13	Circular	0.2	Smooth	Flat	Creamy	Milky off-white	Dark grey

Table 3.12 Colony Morphology of Isolates obtained from Effluent and Cake layer

Isolate	**Shape**	**Size (cm)**	**Margin**	**Elevation**	**Texture**	**Color**	
						Naked eye	**Microscope**
MF-SG-1	Circular	< 1	rhizoidal	Flat	Pasty	offwhite	soil brown
MF-SG-2	circular	≤ 1	smooth	Flat	creamy	Translucent off-white	Light golden brown
MF-SG-3	irregular	2-3	Smooth	Flat	Pasty	Off-white	Dark grey brown
MF-AG-1	circular	1-2	Dentate	Flat	Creamy	Luminescent yellow	Light golden brown
MF-AG-2	Irregular	2	rhizoidal	Flat	creamy	off-white	Dark grey brown
MF-MM-1	irregular	3-4	rhizoidal	Flat	Creamy	Dirty Off-white	Golden brown
MF-MM-2	irregular	3-4	Smooth	Flat	Creamy	Dirty Off-white	Dark grey
MF-MM-3	irregular	2	rhizoidal	Flat	Pasty	Off white	Soil brown
ESG-1	Circular	1-2	Smooth	raised	creamy	Translucent yellow	Very light Soil brown
EAG-1	Circular	< 1	Smooth	raised	creamy	Translucent yellow	Colorless
EMM-1	Circular	< 0.1	Smooth	raised	pasty	Yellow	Soil brown
EMM-2	Circular	< 0.1	smooth	concave	pasty	Yellow	Golden brown

Results of Biochemical Tests

Table 3.16 Growth of isolates on Eosin Methylene Blue and MacConkey agar

Isolate	**EMB**	**MacConkey Agar**	
		Growth	**Medium**
AG2	PK	PK	Y
AG3	GMS	SP	PK
AG4	-	-	-
AG5	PL		
AG6	-	-	-
AG7	-	-	-
AG8	PL	-	-
AG9	GMS	SP	PK
AG11	-	-	-
AG12	PL	-	-
AG13	GMS	SP	PK

Isolate	**EMB**	**MacConkey Agar**	
		Growth	**Medium**
SG1	PL	PK	Y
SG3	PK	PK	Y
SG4	PL	PK	Y
SG5	GMS	SP	P
SG6	PK	PK	Y

SG7	PK	PK	Y
SG8	PK	PK	Y
SG9	GMS (M)	PK	-
SG10	-	-	-
SG11	-	-	-
SG12	-	-	-

Isolate	**EMB**	**MacConkey Agar**	
		Growth	**Medium**
MM1	PK	PK	Y
MM3	PK	PK	Y
MM4	PK	PK	Y
MM5	PK	PK	Y
MM6	PK	OW	Y
MM8	GMS (M)	SP	-
MM9	PL	PL	Y
MM10	GMS	SP	PK
MM11	-	-	-
MM12	-	-	-
MM13	-	-	-

GMS Green Metallic sheen

PL purple PK Pink SP Shocking Pink OW Off- White

Y Yellow

Table 3.17 Growth of isolates on Simmon Citrate Agar and Brilliant Green agar

Strain	SCA		BGA	
	Growth	**Medium**	**Growth**	**Medium**
AG2	CL	B	PK	SP
AG3	OW	B	OW	-
AG4	Y	-	-	-
AG5	Y	-	-	-
AG6	-	-	-	-
AG7	-	-	-	-
AG8	-	-	-	-
AG9	OW	B	OW	-
AG11	-	-	-	-
AG12	Y	-	-	-
AG13	OW	B	OW	-

Strain	SCA		BGA	
	Growth	**Medium**	**Growth**	**Medium**
SG1	CL	B	PK	SP
SG3	YG	B	-	-
SG4	YG	B		
SG5	SP	P	OW	-
SG6	YG	B	-	-
SG7	PK	Y	-	-
SG8	PK	Y	-	-

SG9	PK	-	OW	-
SG10	-	-	-	-
SG11	-	-	-	-
SG12	-	-	-	-
SG13	-	-	-	-
SG14	-	-	-	-

Strain	**SCA**		**BGA**	
	Growth	**Medium**	**Growth**	**Medium**
MM1	OW	B	PK	SP
MM3	OW	B	-	-
MM4	OW	B	-	-
MM5	OW	B	-	-
MM6	-	-	PK	SP
MM8	Y (OW)	-	OW	-
MM9			-	-
MM10			OW	-
MM11	-	-	-	-
MM12	-	-	OW	-
MM13	-	-	-	-

OW Off-white PK Pink B Blue Y Yellow

SP Shocking Pink CL Colorless

API Strip Results

AG2	000404
AG3	121577
AG5	004100
AG6	000502
AG7	000502
AG8	731112
AG9	704577
AG13	524577

SG1	000004
SG3	020000
SG4	020004
SG5	725577
SG6	524577
SG7	000404
SG8	000100
SG9	100416
SG10	101317
SG11	301417

MM1	000104
MM3	000200

MM4	000004
MM6	731300
MM8	500777
MM9	001200
MM10	525577
MM11	000202

ESG1	621107
MFA2	611245
EMM2	000577
EMM1	000073
MFSG2	725577
MFSG1	725577
MFA1	733657
MFSG3	621300
EAG1	221100