

**PERFORMANCE EVALUATION OF HYBRID BIOLOGICAL
REACTORS FOR TEXTILE WASTEWATER TREATMENT
USING POWDERED ACTIVATED CARBON**



A thesis submitted in partial fulfillment of the requirements for
the degree

of

Master of Science

in

Environmental Engineering

By

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DEDICATION

*I dedicate this thesis to my loving
mother and brothers*

ACKNOWLEDGMENTS

I have been very fortunate working with my co-operative and benign supervisor *Dr. Sher Jamal Khan*. He has been very helping and generous in delivering all his knowledge. Throughout my thesis-writing period, he provided encouragement, sound advice, good teaching, good company, and lots of good and innovative ideas. *Dr. Sher Jamal Khan* provided me a refreshing insight and cynical common sense that was vitalizing and electric

I am especially indebted to my thesis committee members *Dr. Ishtiaq A. Qazi* and *Dr. Zahir-ud-Din Khan*, who have been very supportive. A special thank is addressed to *Mr. Zahoor Ahmad* (Director Drainage WASA-Rawalpindi) for accepting to guide me as an external supervisor.

I am thankful to PhD student *Mr. Saqib Nawaz*, for his support whenever I needed help. I would also like to thank *Ms. Fozia* and *Mr. Ghalib Hasnain* for helping and guiding me throughout my research work. I value my friends and laboratory fellows who always found time for me on their calendar. I would also like to acknowledge my friends and laboratory fellows, *Sumaira Shah, Naushaba Noreen, Anum Imtiaz and Waqas Latif*, for all the moral support and motivation during various stages of my research work.

I feel pride to acknowledge my family members *Dr. Sadiq Noor* and *Dr. Nargis Khattak* (USM- Malaysia) who supported me throughout the entire process, by helping me in knowing various things in my research work.

Engr. Onaira Zahoor

ABSTRACT

Due to the population growth, urbanization, change in human life style and lacking natural water resources, the need and demand of water has become ever greater. To overcome water scarcity problem, wastewater can be treated efficiently by advanced technologies. In Pakistan textile industry has got much importance and is the most attractive sector. The discharge of dye containing effluents from such industry is undesirable for environment. MBR technology is highly promising technique nowadays for biodegrading organic matter in general and dyes in textile wastewater in particular.

Bench scale Hybrid Membrane Bioreactor (H-MBR), Hybrid Sequencing Batch Reactor (H-SBR) and Conventional Sequencing Batch reactor (C-SBR) treating synthetic textile wastewater under similar operating conditions were evaluated for removal of color, Chemical Oxygen Demand (COD) and nutrients at 12 hr hydraulic retention time (HRT) and 30 days solids retention time (SRT). Synthetic textile wastewater was based upon the characterization of real wastewater from Crescent Textile Mill Faisalabad, Pakistan. Treatment of synthetic textile wastewater through H-MBR and H-SBR was supplemented with powdered activated carbon (PAC) as an adsorbent for the removal of dye color and soluble organic and inorganic substances in the reactor. PAC was efficient in removing chemical oxygen demand (COD) and color from textile wastewater. It facilitated in membrane fouling control and reduced soluble extracellular polymeric substances (EPS) in H-MBR. The optimized dose of PAC was observed in the range of 100-150 mg/L. Results showed an improved COD removal of 93% and color removal of 99% in H-MBR as compared to 89% COD and 92% color removal efficiencies in H-SBR.

TABLE OF CONTENTS

| | |
|--|----------|
| Dedication | iii |
| Acknowledgments..... | iv |
| Abstract | v |
| Table of Contents | vi |
| List of Tables | x |
| List of Figures | xi |
| List of Abbreviations | xii |
| 1 INTRODUCTION..... | 1 |
| 1.1 Background..... | 1 |
| 1.2 Ojectives of study. | 3 |
| 1.3 Scope of Study | 3 |
| 2 LITERATURE REVIEW | 5 |
| 2.1 Textile Industries | 5 |
| 2.1.1 Wastewater Discharge in Textile Industries | 6 |
| 2.1.2 Processes in Textile Industries of Pakistan..... | 7 |
| 2.2 Membrane | 11 |
| 2.2.1 Membrane Material..... | 11 |
| 2.2.2 Membrane Morphology and Operation | 12 |

| | |
|---|-----------|
| 2.3 Membrane Bioractor | 12 |
| 2.3.1 Advantages and Disadvantages of MBR | 14 |
| 2.3.2 Moving Biofilm MBR..... | 15 |
| 2.3.3 Membrane Fouling..... | 16 |
| 2.3.4 Membraen Fouling Mitigation | 19 |
| 2.4 Addaition of Powdered Activated Carbon to Bioreactors | 21 |
| 3 METHODOLOGY | 23 |
| 3.1 Materials | 23 |
| 3.1.1 Characteristics of Synthehtic Textile Wastewater | 23 |
| 3.2 Membrane Characteristics | 26 |
| 3.3 Biofilm Carrier..... | 26 |
| 3.4 H-MBR and SBRs Operation..... | 27 |
| 3.4.1 Operating Conditions of the Reactors..... | 28 |
| 3.4.2 Efficiency and Performance Evaluation | 28 |
| 3.4.3 Acclimatization Phase..... | 28 |
| 3.4.4 Analytical Parameters | 30 |
| 3.6 Characteristics of Virgin PAC | 32 |
| 3.7 Performance Evaluation Methods..... | 33 |
| 3.7.1 Sludge Charaterization..... | 33 |
| 3.8 Membrane Fouling Characterization | 35 |

| | |
|--|-----------|
| 3.8.1 Resitance Analysis | 36 |
| 4 RESULTS AND DISCUSSION | 38 |
| 4.1 Performance of MBR and SBRs Systems..... | 38 |
| 4.1.1 Chemical Oxygen Demand | 39 |
| 4.1.2 Nutrients Removal | 40 |
| 4.2.1 Mixed Liqour Suspended Solids, Mixed Lqour Volatilee Suspended Solids..... | 47 |
| 4.2.2 Diluted Sludge Volume Index | 48 |
| 4.2.3 Specific Oxygen Uptake Rate (SOUR)..... | 49 |
| 4.2.4 Extra Polymeric Substances (EPS) | 50 |
| 4.2.5 Particle Size Distribution (PSD) | 52 |
| 4.2.6 Specific Cake Resistancee (SCR) | 53 |
| 4.3 Membrane Fouling During Operation | 54 |
| 4.3.1 Trans Membrane Pressure (TMP)..... | 54 |
| 4.3.2 Resistance Analysis of H-MBR | 55 |
| 4.4 Analysis of PAC Adsorption Behaviour | 56 |
| 4.4.1 Characterization of PAC in Reactors | 56 |
| 5 CONCLUSIONS AND RECOMMENDATIONS..... | 58 |
| 5.1 Conclusions..... | 58 |
| 5.2 Recommendations..... | 59 |
| Refernces..... | 60 |

| | |
|--|-----|
| Appendix A (Design Considerations)..... | 67 |
| Appendix B (Protocols) | 68 |
| Appendix C (Reactors Components and Effluents)..... | 104 |

LIST OF TABLES

| | |
|--|----|
| Table 2.1 Water Consumption in Textile Industry of Pakistan | 7 |
| Table 2.2 Moving Biofilm Carrier Types Used in MBR Systems..... | 16 |
| Table 3.1 Textile Wastewater Chemical Composition | 24 |
| Table 3.2 Dyes Used in the Synthetic Textile Wastewater..... | 25 |
| Table 3.3 HF-PVDF Membrane Characteristics..... | 26 |
| Table 3.4 Sludge Characteristics..... | 31 |
| Table 3.5 Effluent Characteristics..... | 32 |
| Table 3.6 Virgin Powdered Activated Carbon Characteristics | 33 |
| Table 4.1 Chemical Oxygen Demand Removal Statistical Analysis..... | 39 |
| Table 4.2 Ammonium Nitrogen Removal Statistical Analysis..... | 41 |
| Table 4.3 Nitrites Removal Statistical Analysis | 42 |
| Table 4.4 Nitrates Removal Statistical Analysis..... | 43 |
| Table 4.5 Phosphorus Removal Statistical Analysis | 45 |
| Table 4.6 MBR and SBRs System Performance Evaluation | 47 |
| Table 4.7 Resistance Analysis of H-MBR..... | 55 |
| Table 4.8 Properties of Used PAC in Reactors..... | 57 |

LIST OF FIGURES

| | |
|--|----|
| Figure 2.1 Textile Industry Processes | 8 |
| Figure 2.2 Membrane Fouling in MBRs..... | 17 |
| Figure 2.3 Pore Blocking in Membrane..... | 20 |
| Figure 2.4 SEM Images of Sludge (a) without (b) with PAC..... | 21 |
| Figure 3.1 Analytical Parameters..... | 30 |
| Figure 4.1 Chemical Oxygen Demand Time Series Analysis | 39 |
| Figure 4.2 Percent COD Removal Efficiency..... | 40 |
| Figure 4.3 Average Ammonium Nitrogen Concentration in the Effluents..... | 41 |
| Figure 4.4 Average Nitrites Concentration in the Effluents | 42 |
| Figure 4.5 Average Nitrates Concentration in Effluents | 43 |
| Figure 4.6 Total Nitrogen Removal in Reactors..... | 44 |
| Figure 4.7 Percent Phosphorus Removal | 45 |
| Figure 4.8 Percent TOC Removal..... | 46 |
| Figure 4.9 MLSS and MLVSS Concentration in Sludge..... | 48 |
| Figure 4.10 Diluted Sludge Volume Index | 49 |
| Figure 4.11 Specific Oxygen Uptake Rate of Reactor's Sludge | 50 |
| Figure 4.12 Soluble EPS Concentration in Sludge | 51 |
| Figure 4.13 Bound EPS Concentration in Sludge..... | 52 |
| Figure 4.14 Mean Particle size of Sludge | 53 |
| Figure 4.15 Specific Cake Resistances | 53 |
| Figure 4.16 Membrane Fouling Trends | 54 |

LIST OF ABBREVIATIONS

| Abbreviation | Description |
|---------------------|--|
| MLSS | Mixed liquor suspended solids |
| MLVSS | Mixed liquor volatile suspended solids |
| R_m | Intrinsic membrane resistance |
| R_f | Resistance due to pore blocking |
| sEPS | Soluble EPS |
| HMBR | Hybrid membrane bioreactor |
| bEPS | Bound EPS |
| HSBR | Hybrid sequencing batch reactor |
| CSBR | Conventional sequencing batch reactor |
| SOUR | Specific Oxygen uptake rate |
| EPS | Extra polymeric substances |
| RA | Resistance analysis |
| HF | Hollow fiber |
| COD | Chemical oxygen demand |
| sCOD | Soluble COD |
| J | Operational flux |
| μ | Viscosity of permeate |
| CER | Cation exchange resin |
| PAC | Powdered activated carbon |
| DSVI | Diluted sludge volume index |
| SCR | Specific cake resistance |
| DEF | Dead end filtration |

| | |
|-----|-----------------------------|
| TN | Total Nitrogen |
| TOC | Total organic carbon |
| OLR | Organic loading rate |
| DO | Dissolved oxygen |
| F/M | Food to microorganism ratio |
| TMP | Trans-membrane pressure |
| SRT | Sludge retention time |
| HRT | Hydraulic retention time |
| MBR | Membrane bioreactor |
| VSS | Volatile suspended solids |
| BAC | Biological activated carbon |

INTRODUCTION

1.1 Background

Water is not only considered as a resource, but it is a life source. We don't need water only for drinking but it is also required for growing food, crops, generating electricity and for running our industries. Three percent of the water resources is not salty, two third of the water is locked up in glaciers and other ice caps. Only 0.08 percent of remaining water is available for human consumption in the ever increasing demand for sanitation, drinking, manufacturing, industrial use, washing and agriculture. Providing water for irrigation and basic human needs is thus one of the most important problem (*Melijß et al., 1998*). Discharge of wastewater into water bodies and across the watershed causes damage to the environment and hydrology of a place. Global sustainability is affected by the wastewater one way or the other (*Muga et al., 2008*).

Industrial and municipal wastewater contains high level organic compounds and its discharge to the oceans and rivers can have adverse effects on the aquatic life and environment (*Chan et al., 2009*). Nowadays for high treatment performance advanced aerobic biodegradation can be adopted to render the industrial effluents less harmful for environment.

Our concern in the future is sustainability of the current and future water resources allocation. As water is becoming scarce, so the need and importance that

how it should be managed, used and treated is growing vastly. In this situation we cannot think to increase present water resources or to increase the rates of hydrologic cycles occurring for water production. Disposal of waste water from industrial areas, households and other units can cause soil pollution, surface and ground water contamination.

Due to population growth, urbanization, change in human life style and lacking natural water resources, need and demand of water has become ever greater. In wastewater treatment biochemical treatment is mostly preferred and utilized (*Terasaka et al, 2011*). But lower cost and easy maintenance of the technology is mostly considered in developing countries.

In Pakistan textile industry has got much importance and is the most attractive sector. There are more than 670 textile mills in Pakistan and these industries discharge high strength wastewater to water bodies (*Haydar et al., 2009*). The amount of water used for wet processing is approximately 80-150 m³ /1000 kg of the product. The treated water of textile industries helps in recycling of wastewater and keeping the fresh water bodies unaffected. In textile industries different chemicals, acids, grease and bases are used in different processes.

Detergents are added for removal of dirt, waxes and oils, bleaching agents for whitening and brightness, oils are used to improved spinning (*Naveed and Bhatti, 2006*). Dyes and color fixing agents are used to have different shades and arrays in the fabric. Wastewater released from all these processes is highly contaminated and its disposal to environment is unhealthy.

Membrane bioreactor is an advanced technology for wastewater treatment. It is a combined process of biological activities (aerobic process) and membrane filtration. COD removal in MBR is greater than 90% for different type of wastewaters (*Sosa et al., 2011*). It is a promising technology for wastewater treatment as it produces high quality effluent after biotreatment and filtration through membrane (*Chen et al., 2009*).

1.2 Objectives of Study

- Optimization of Powdered Activated Carbon dosage in batch systems
- Comparison of the treatment performance of Hybrid membrane bioreactor (H-MBR), Hybrid sequencing batch reactor (H-SBR) and Conventional sequencing batch reactor (C-SBR) under different biological condition (aerobic, anoxic/oxic and suspended/attached growth).
- Investigation of membrane fouling behavior in Hybrid membrane bioreactor

1.3 Scope of the Study

Hybrid biological reactors were operated at different conditions for organics and nutrients removal along with the investigation of adsorption behavior of PAC. SRT of 30 days and 12 hrs HRT was maintained during the study.

Parameters Analysis

During study the following parameters were analyzed.

- **Effluent Quality Parameters** – This included the Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Nitrates, Nitrites, Ammonium, Total Nitrogen (TN) and Total Phosphorus removal.
- **Membrane Fouling Parameters** – Resistance analysis of fouled membrane and Trans membrane pressure (TMP) during constant flux operation.
- **Sludge Characteristics** – Such as Mixed liquor suspended solids (MLSS), Mixed liquor volatile suspended solids (MLVSS), Diluted sludge volume index (DSVI), Specific cake resistance (SCR), Specific oxygen uptake rate, (SOUR) Extra polymeric substances (EPS) and Particle size distribution (PSD).
- **Adsorption Capacity of PAC** – The adsorption behavior of PAC was studied with the help of Adsorption isotherms.

LITERATURE REVIEW

2.1 Textile Industries

Textile industry being a water intensive sector uses a large amount of processed water throughout the whole operation of cloth making, from washing of fibers to mercerizing, desizing, bleaching, dyeing, printing and finishing. The discharge of dye containing effluents from these processes is undesirable for environment and the discharged water needs to be treated. Textile wastewater adversely affects health of human being and flora of the nearby area (*Valha et al., 2011*). Water consumption in textile mills is very high and it is extremely diverse and heterogeneous. There are many environmental problems associated with the discharge of wastewater of high strength and complex composition from textile industries.

Nowadays due to water scarcity and with regard to globalization wastewater treatment and recycling has gained much importance. A COD of 1000 mg/L is considered high level for petrochemical but it is taken medium level for other industries like food industry. It is due to the reason that chemical industries consists of hard COD or non-biodegradable COD due to the presence of heavy metals in the wastewater. Azo dyes and some other chemicals used in textile industry are carcinogenic in nature (*Selçuk et al., 2006*). Textile wastewater contains different dyes and chemicals. Biodegradation of these dyes and chemicals is necessary for safe discharge to the environment (*Kapdan et al., 2005*).

The remaining color of dye stuffs in textile wastewater causes aesthetic problems, when discharged to water bodies (*Georgiou et al., 2005*). When discharged into the open areas it can also clog the soil, so making it unfit for cultivation and agricultural growth. Treated wastewater may be reused, used for irrigation purpose or it can be discharged to water bodies for keeping aquatic life fresh and safe.

2.1.1 Waste Water Discharge in Industries

By means of ultrafiltration mixed wastewater flow of a textile industry can be recycled. Nanofiltration processes helps in maintaining all requirements of the effluent's reuse. Implementation of these technologies can help in reducing water consumption by 87.5% in the textile industries washing process. These technologies help in COD removal, reducing the industrial emission by 80% and in recycling some washing agents. So consumption for washing process can be lowered by 20%.

Textile industry is an extensive user of dyes. Azo dyes are the most common dyes used. Azo dyes contribute 60–70% of the dyes applied in the printing and dyeing processing. They contain one extra Nitrogen bond called azo-group. This bond is slowly biodegradable under aerobic conditions but under Anoxic/Oxic conditions it can be biodegraded to a major extent (*Spagni et al., 2010*).

From the anaerobic reduction of azo dyes there arise aromatic amines which are reported to be very toxic (*O'Neill et al., 2000*) and their removal from textile wastewater is necessary. Many dyes and their byproducts are harmful to the

aquatic life. Moreover they are also a reason of aesthetic deterioration of water bodies (*Mezohegyi et al., 2010*).

2.1.2 Processes in Textile Industries of Pakistan

There are different processes taking place during the formation of fabric in textile industries. Some processes are called dry, because there is no use of water like ginning, spinning yarn, sizing, weaving, printing and then getting the finished product at the end. Other processes consumes a large amount of water which on getting discharged contains dyes, grease, acids, bases and other pollutants which can severely affect the environment where they flow.

Table 2.1: Water Consumption in Textile Industries of Pakistan.

| Dry processes | Wet processes | Water needed (L/1000Kg) in wet processes |
|---------------|---------------|--|
| Ginning | Singeing | NA |
| Spinning-yarn | Mercerizing | 17000-32000 |
| Sizing | Bleaching | 2500-2500 |
| Weaving | Desizing | 2500-21000 |
| Printing | Dyeing | 10000-300000 |
| Finishing | Washing | 25000-500000 |

Source: *Muhammad Ayaz Shaikh., 2007*

A detailed description of these processes is given below;

Ginning-- In this process the seed is separated and the cotton seed grows into a cotton gin. Removal of dust, leaves and stems also takes place and cotton fibers are separated from seed pods.

Spinning-Yarn-- Ginning is directly followed by spinning process. In this process yarn is developed from cotton fibers which may be of different thickness.

Sizing-- The addition of starch to the manufactured yarn for strengthening of warp takes place which reduces the yarn breakage. Yarn is run through a size solution and dried and it gets some stiffness and strength. The strength gained here helps in resisting friction and withstanding against the abrasion generated in the weaving process.

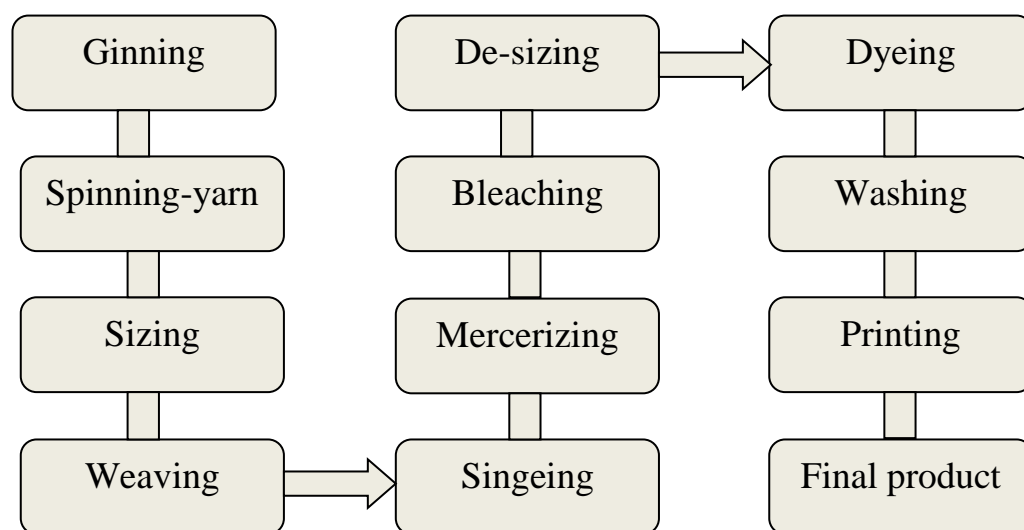


Figure 2.1: Processes in Crescent Textile Mill, Faisalabad – Pakistan

Weaving-- In this process two yarns or threads are interlaced. There are lengthwise and widthwise threads. The threads extending lengthwise are called warp and the

ones of the cross way are called weft. They are connected and interlaced in a way that they can cross each other at right angles hence producing the woven fabric.

Desizing-- In sizing processes different chemicals are used for strengthening the cloth. The sizes are removed from warp threads of the fabric. The size chemicals and materials are resistant to the dyes and other chemicals which are to be applied later. Desizing is necessary for cloth before it is ready for other processes. For desizing the fabric is immersed in an aqueous solution of an enzyme called amylase and is kept for a duration of 8 hours. The sizes get removed after this and cloth is washed for cleaning.

Bleaching-- It is a step in the pretreatment of textile material. It helps in removal of trace colors from fabric and whitens the cloth material. Different chemicals which are used for bleaching purpose in textile industries are mainly Hydrogen peroxide, Sodium hypochlorite, and Sodium perborate.

Mercerizing-- This process involves development of luster in fabrics. For causing swelling in the fabric to improve its affinity for dyes absorption and for getting enough strength the fabric is treated with a solution of caustic soda.

Singeing-- Singeing is process of burning off the superficial and protruded threads to get smoothness in fabric. After Singeing fibers gets well prepared for printing of fine intricate patterns with high clarity.

Dyeing--This process involves application of dyes to the fabrics for changing their color. Cotton being an adsorbent fiber readily responds to the coloration and dyeing process. Vat and reactive dyes are mostly used for improved rubbing. For

printing and dyeing purpose every year approximately 7×10^5 metric tons of dyes and dyes reagents are produced worldwide (*Kong et al., 2008*). Textile wastewater is characterized by strong color, low biodegradability of dyes, high pH and high COD (*Liakou et al., 1997*).

Washing-- To remove non-fibrous impurities and natural wax from fibers washing is done. It also removes added soiling or dirt along with washing of some dyes which are needed to be removed from cloth surface to have a uniform surface texture.

Printing-- The cloth after all the processes of smoothness, preparation, bleaching and washing is ready for printing. In this process color is applied to the surface of fabric in the form of paste of variety of colors or ink. Printing is related to dyeing but in dyeing process whole fabric or cloth is colored while in printing the fiber is colored with different colors for having different patterns in different shades.

Finished Product-- It is the final step in wet processing. Before marketing some finishing features are needed to be added to the textile product. In this process the properties of fabric are altered. The cloth is supposed to be durable, resistant to sharp wear and tear and is comfortable as a human wear. In this process a variety of chemicals are used for adding different properties to fabric.

Along with chemical treatment of the cloth some mechanical techniques are also used which helps in little consumption of chemicals and improved strength of the cloth (*Snowden-Swan, 1995*). Finishing is usually a continuous process that produces little or no wastewater (*Noyes, 1993*). At this stage the cloth is prepared to be dispatched to the market.

2.2 Membrane

2.2.1 Material

Polyvinylidene fluoride (PVDF) is microporous in nature and is very attractive in membrane industry. Its molecular structure comprises of alternating CH_2 and CF_2 groups with PVDF homopolymer. These two combined with a polymer chain makes a unique polymer and membrane module (*Yuliwati et al., 2011*). PVDF membranes are resistant to mechanical abrasions because of having extraordinary mechanical properties. Moreover they have good thermal stability and are resistant to chemicals. Polyvinylidenedifluoride (PVDF) has gained attraction of users because of its excellent thermo stability and piezoelectric properties and is used in many fields for wastewater treatment like textile, food, distillery and dairy industries (*Meng et al., 2011*).

The structure and preparation of PVDF hollow fiber membranes depends on the permeability, wetting pressure, morphology and contact angle of liquid. The modification of membrane surface or morphology can help in better suction of liquids (*Naim et al., 2012*). PVDF is more hydrophobic than Polyethylene (PE), Poly-ether-Sulfone (PES) and Polyimide (PI). PVDF is preferred for microfiltration and ultrafiltration in wastewater treatment. PVDF has greater chemical, thermal and oxidation resistance and can resist acids, bases and halogens etc (*Zhang et al., 2012*).

2.2.2 Morphology and Operation Process

PVDF membrane is superior quality membrane because it resists and prevents irreversible fouling to a major extent in MBRs during wastewater treatment (*Gabelman, et al., 2005*). The membrane material show different fouling behaviors because of its different pore sizes, hydrophobicity and morphology. PVDF membranes are highly hydrophobic and they have greater capability against chemicals and acids. With the help of phase inversion, membrane modification into hollow fibers is making them very attractive for use (*Sukitpaneenit et al., 2011*). As compared to flat sheet, hollow fiber membranes are advantageous in many ways. PVDF hollow fiber membranes have larger membrane area, high flexibility and a good self supporting structure.

Permeability of membrane decreases with clogging and fouling. The removal of solids accumulated on the membrane's surface by chemical and physical cleaning helps in regaining and maintaining the filtration rate (*Buzatu et al., 2012*). Sometimes dissolved solids or colloidal solids may block and clog the pores of membrane and causes irreversible fouling (*Tay et al., 2007*). These suspended solids affect the critical flux through MLSS concentration and particle size. Permeate is sucked through one side module of membrane while the other side of the module is sealed and closed.

2.3 Membrane Bioreactor

Membrane bioreactor (MBR) is a type of wastewater treatment technology which comprises of biotreatment by microbes and membrane filtration process. The important use of MBR is treatment of wastewater of high strength either

industrial or municipal wastewater. Membrane bioreactor is feasible for wastewater treatment at high biomass concentration up to 20g/L (*Holler et al., 2001*). According to different compositions and characteristics of waste water membrane technology combined with biotreatment can be used for treating high strength wastewater in various conditions. Textile wastewater is a complicated mixture because of the dyes used and rinsed water from different processes having low biodegradability (*Sabrina et al., 2012*). Textile wastewater contains oil, fats, grease and many other compounds (organic or inorganic) in greater amount depending on the types of processes occurring during cloth formation.

Important factors like hydraulic retention time (HRT), solid retention time (SRT), mixed liquor suspended solid (MLSS), food to microorganism ratio (F/M), Trans membrane pressure (TMP) and flux are taken into account for studying characteristics of MBR and its performance.

Conventional treatment process may not cope with the water re-use regulations therefore membrane bioreactor technology is used nowadays for wastewater treatment. Membrane bioreactor is advantageous over conventional treatment technologies due to good and stable effluent quality, high volumetric loading rate and decreased solids production.

Membrane fouling causes a decrease in filtration rate and for this purpose the system is halted and flux is controlled by membrane backwashing. This results in the high maintenance and good flux (*Liu et al., 2010*).

2.3.1 Advantages and Disadvantages of Membrane Bioreactor

Advantages

Membrane bioreactor (MBR) is a small footprint technology with characteristics of high quality effluent. It has combined processes of biotreatment with membrane separation. Mainly MBRs are used for treating domestic wastewater but now it has become an attractive option for treating wastewater of different industries along with drinking water pretreatment in many areas.

Membrane bioreactor is becoming widely applicable for biological wastewater treatment because of various advantages. It reduces soluble and suspended organic solvents to a very high extent with great efficiency (*Duan et al., 2009*). MBR is advantageous over other treatment technologies because it lacks secondary treatment which leads to the overall small size of treatment plant (*Xing et al., 2008*).

Moreover increase in the contact time between activated sludge and solute (organic substances) can result in effective removal of low biodegradable contaminants. Water treated by MBR is bacteria free and has a potential in municipal and industrial reuse because of better quality.

Disadvantages

Pre-treatment, high capital cost (membrane modules) and maintenance cost (membrane cleaning) are the major factors which are effecting attraction of MBR. Membrane fouling is the most important factor that is challenging nowadays (*Guo et al., 2010*). Fouling of membrane is hindrance to large scale application of MBR

(Li *et al.*, 2006). Due to fouling the TMP rises and suction pressure increases which may result in lower filtration efficiency of MBR and low quality effluent.

Membrane fouling is limiting the use of MBR technology, although this technology has gained much importance and attention in the last 10 years (Farizoglu *et al.*, 2006). Soluble extra polymeric substances and soluble microbial products (SMP) cause severe membrane fouling. Fouling results in low filterability and efficiency at higher suction pressure (Johir *et al.*, 2012). Mitigation of membrane fouling can increase demand for this small footprint technology.

2.3.2 Moving Biofilm MBR

For improved removal efficiency and effective wastewater treatment hybrid membrane bioreactors are preferred nowadays which are a combination of activated sludge and biofilm carrier in the form of adsorbents or plastic (kaldness®) media.

Hybrid MBR operates at lower MLSS concentration hence helps in reducing membrane fouling tendency. Leiknes *et al.*, (2007) has reported higher removal efficiency of hybrid membrane bioreactor with a good filtration rate. Because of nitrification and denitrification process which results from biofilm growth the quality of effluent is improved and more total nitrogen removal occurs (Díaz *et al.*, 2013).

Activity of ammonium and nitrite oxidizing bacteria increases with application of moving biofilm in MBR which increases nutrients removal efficiency (*Yang et al., 2009*). Polyurethane sponge use in membrane bioreactor as a biofilm carrier results in effective removal of nutrients as well as it reduces membrane fouling (*Jamal Khan et al., 2012*). Nowadays hybrid systems are using different media, assessing the plant in nutrients removal capability and good oxygen transfer.

Table 2.2: Moving Biofilm Carrier Types Used in MBR Systems

| Reference | Media Type | Specification | Effect |
|-----------------------------|---------------------|-----------------------|----------------------|
| Artiga <i>et al.</i> , 2008 | Kaldnes® (K-3) | 950 Kg/m ³ | Reduced Fouling |
| Ying <i>et al.</i> , 2006 | PAC | 0.75Kg/m ³ | Decreased EPS |
| Xing <i>et al.</i> , 2011 | Polyurethane porous | 30Kg/m ³ | Less cake resistance |

2.3.3 Membrane Fouling

Sludge filamentous bulking and sludge deflocculation can cause problems in membrane filtration. This can lead to deterioration of effluent quality. Deflocculation is the absence of floc formation or small activated sludge floc formation. Problem associated with deflocculation includes, disperse growth of filamentous bacteria and pin point floc. Sometimes activated sludge flocculates slowly and poorly hence results in increase of suspended solids in the supernatant water which may affect the effluent's quality (*Meng et al., 2007*). This may be caused by change in operating conditions or other environmental problems such as

temperature, toxic compounds, pH, substrate loading, nutrient characteristics and dissolved oxygen concentration.

Performance and efficiency of a bioreactor, its nature and effectiveness of a reaction is influenced by operating conditions and physical properties of the membrane. Membrane fouling and scaling are the two problems which are causing difficulties in using membrane bioreactor but they are now mitigated by use of adsorbents and some other advanced mechanisms.

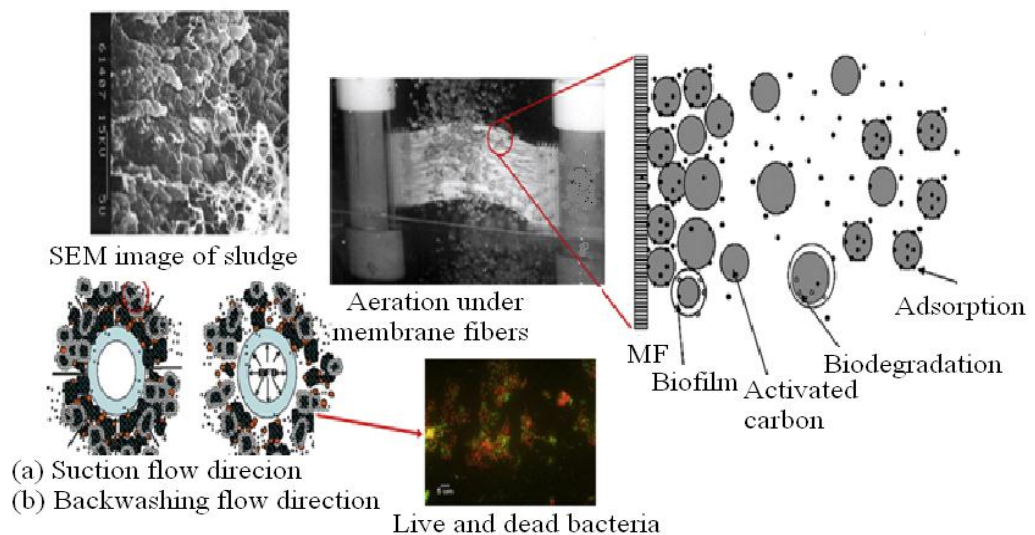


Figure 2.2: Membrane Fouling in MBR

The absence of filamentous bacteria in sludge flocs leads to severe biofouling and pore blocking, because they are contributing to a high soluble EPS release (*Meng et al., 2006*). Release of EPS in larger amount is the result of excess and rapid growth of filamentous bacteria. This also leads to higher hydrophobicity of sludge flocs which ultimately have a negative effect on membrane filtration. The formation of filamentous bacterial colonies and their concentration has become very important in the MBRs operation.

Membrane fouling is main problem in a membrane bioreactor which is reduced by different methods (*Wen et al., 2010*). Growth of filamentous bacteria and cake layer formation on membrane surface can result in a rapid TMP rise. Filamentous bacteria produce more EPS than floc forming bacteria resulting in rapid fouling of the membrane (*Jamal et al., 2012.*) Due to overgrowth of filamentous bacteria sludge settling characteristics decreases and sludge thickens very poorly, so this overgrowth is considered as a fouling indicator.

SMP and EPS which are secreted by bacteria can also cause biofouling, which gets started by the deposition and sticking of individual bacteria on membrane layer. Higher the SMP greater are the chances of bio-fouling. The filterability of membrane decreases with increase in concentration of soluble and extractable EPS (*Kim et al., 2009*). Due to increase in nitrogen loading rate soluble EPS production increases which results in rapid membrane fouling (*Jamal et al., 2013*).

Attached biofilm is having the ability to absorb biological flocs and some colloidal matter, mostly extracellular polymeric substances (EPS) which are identified as main foulants in MBR operation. The fouling rate is also affected by backwashing and relaxation rate (*Wu et al., 2008*). Pore blocking in membranes is more likely due to proteins than the carbohydrates. The cake layer formation due to growth of filamentous bacteria imparts a major contribution in membrane fouling. Many studies have been conducted for membrane fouling mitigation in which most important is hybrid membrane bioreactor supported by suspended media.

Hybrid MBR has additional biomass hence total biomass increases which helps in increase in the biodegradation and also enhances the nitrification process.

Colloidal and organic solute particles along with some biological flocs in sludge are mostly absorbed by the biofilm. The biofilm also helps in absorbing and reducing the concentration of EPS which are considered as main foulants in MBR operation (*Liu et al., 2010*). Membrane replacement in an immersed membrane bioreactor is also dependent on the characteristics of feed water, pre-treatment efficiency and cleaning methods. Organic fouling also has a great influence on membrane replacement (*Ayala et al., 2011*).

2.3.4 Membrane Fouling Mitigation

Because of the presence of mixed culture communities in MBR's activated sludge, biofouling solution is yet difficult to find. The disruption of signal molecule by bacteria using a quorum quenching enzyme called Acylase can help in membrane biofouling mitigation in MBRs. But mechanism of membrane biofouling mitigation by enzymatic quorum quenching is still not known (*Woo Kim et al., 2012*). Use of Cell entrapping beads (CEBs) with entrapping quorum quenching bacteria (*Rhodococcus sp. BH4*) can help in mitigating biofouling. TMP rise is 10 times lesser in an MBR having CEBs than other without CEBs.

Due to use of CEBs in the reactor the biofilm in activated sludge produces less extra polymeric substances hence results in loosely bound membrane cake layer. Due to enhanced collision between CEBs and membrane a frictional force raises which also helps in detachment of cake layer from the membrane.

Hence along with reduced extra polymeric substances, development of this frictional force is also another important factor in membrane fouling mitigation (*Kim et al., 2012*).

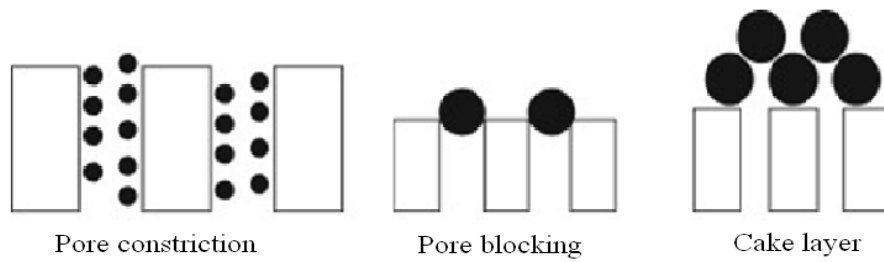


Figure 2.3: Pore Blocking in Membranes

There are many factors which affect fouling mechanism in membrane filtration system. The most important of which is particle sizes of pollutants present in wastewater. Sometimes flocculants are smaller than membrane pores so they can enter in membrane pores and can clog it. In case the flocculants are larger than membrane pores, they form a cake layer on membrane surface (*Chae et al., 2006*). Biofouling is a major problem in waste water treatment, because some flocculants in MBRs are larger than the membrane pore size causing membrane fouling. EPS are considered to be the major factor in membrane fouling because they consist of flocs of organic compounds such as polysaccharides, amino acids and proteins.

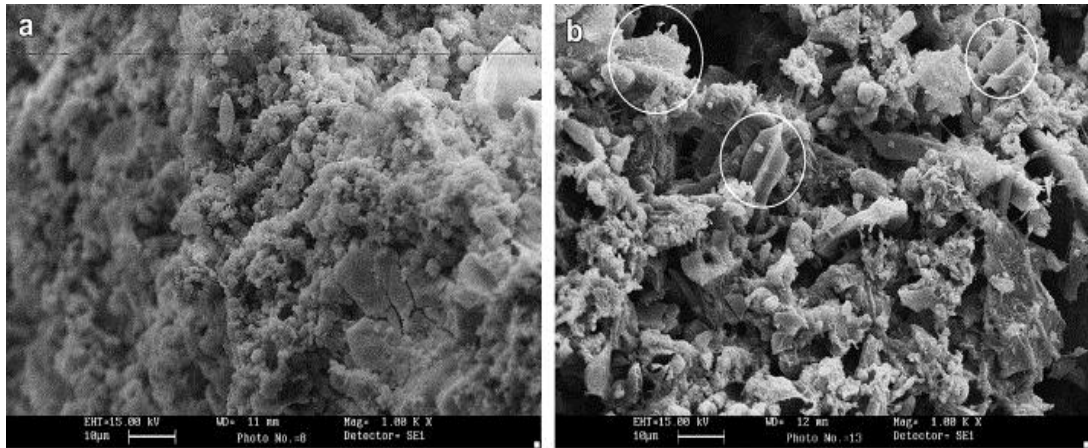


Figure 2.4: SEM Images of Sludge (a) without (b) with PAC

2.4 Addition of Powdered Activated Carbon to Bioreactors

Addition of Powdered Activated Carbon in bioreactors has got much attention, because more dissolved organic carbon (DOC) and disinfection byproducts (DBPs) removal and mitigation in membrane fouling can be achieved by reducing organic loading, to membrane adsorbing organic matters. PAC addition results in greater than 95% COD removal under anaerobic conditions (Akram *et al.*, 2008).

PAC has simultaneous functions of biodegradation as well as adsorption to improve DOC and COD removal efficiencies. On the surface of PAC adsorbed organics are biodegraded by biomass creating further sites for organics adsorption. MBR with PAC has lower TMP as compared to other reactors because of the direct adsorption of organics on the PAC surface rather than on membrane module, thereby mitigating membrane fouling (Guo *et al.*, 2008). Ying *et al.*, (2006) reported that lower dosage of PAC facilitates reduction of EPS deposition on membrane surface, hence resulting in lower membrane fouling. A study conducted by Jamal Khan *et al.*, (2012) depicts that presence of biological activated carbon

(BAC) on membrane cake layer results in decreased cake resistance, assessing greater fluid permeability.

PAC has improved catalytic degradation of organic pollutants by their oxidation in textile wastewater and its absorbance capacity helps in color removal (*Türgay et al., 2011*). The effect of PAC on flux and membrane fouling is studied by many researchers. Submerged membrane adsorption bioreactor (SMABR) exhibits better results in terms of MLSS, growth, COD removal (over 97%), trans membrane pressure (TMP) and oxygen uptake rate. In this system nearly 100% of bacteria and 100% of total coliforms are removed. PAC addition can maintain the critical flux at lower TMP value (*Guo et al., 2008*).

PAC is used to adsorb color and remove heavy metals from wastewater. At initial stage the adsorption rate is fast (*Ahmad et al., 2009*) because of more surface area and exterior pores, but with the passage of time the surface area decreases and adsorption as well.

The porosity of cake layer on membrane surface increases (*Satyawali et al., 2009*) by PAC addition to the reactors, resulting in improved sludge dewaterability and settleability. Excellent decoloration of textile wastewater takes place and along with biodegradation of the dyes to an extent, their adsorption also takes place on the open surface of PAC (*Taimur et al., 2103*). At higher HRT filtration rate increases because of the decrease in membrane fouling (biofouling) due to reduced soluble EPS.

METHODOLOGY

3.1 Materials

Textile wastewater was prepared in laboratory synthetically having a COD of 1000 mg/L and COD: N: P was maintained at 100:20:6. Composite sample obtained from Crescent Textile mill, Faisalabad-Pakistan was analyzed for characterization. The pH was maintained in range of 7-8. For this purpose sodium bicarbonate was added as buffer to the feed. Activated sludge was taken from Sewage Treatment Plant I-9 Islamabad with initial MLSS concentration of 2000 mg/L and it was acclimatized for two months with synthetic wastewater. At the end of acclimatization period MLSS remained in the range of 8000-10000 mg/L.

3.1.1 Characteristics of Synthetic Textile Wastewater

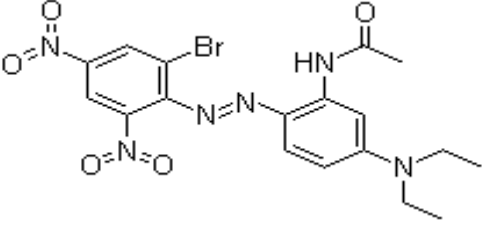
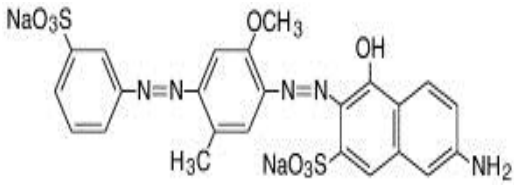
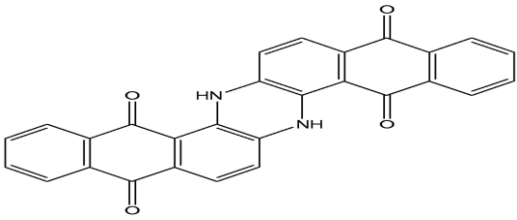
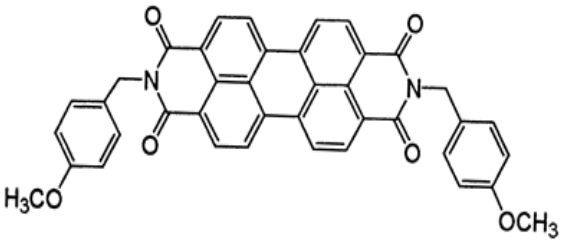
The reactors were fed with synthetic textile wastewater daily at a uniform organic loading rate (OLR). Stock solution was prepared having a total volume of 7L. Textile wastewater was high strength, having a COD of 1000 mg/L. Synthetic textile wastewater was comprising of Hydrated D-Glucose (1000 mg/L), Ammonium Chloride (760 mg/L), Potassium di-Hydrogen Phosphate (220 mg/L), Calcium Chloride (10 mg/L), Magnesium Sulphate (10 mg/L), Ferric Chloride (3.3 mg/L), Magnesium Chloride (2 mg/L) and Sodium bicarbonate (800 mg/L). Sodium bicarbonate was added as a buffer to adjust pH. The feed tank was daily

fed with feed and water in a ratio of 1:50 (i.e. 1 liter of feed solution and 50 liters of tap water) to make high strength textile waste water.

Table 3.1 Chemical Composition of Textile Wastewater

| Chemicals | Formula | Quantity (mg/L) |
|---------------------------------|--------------------------------------|------------------------|
| Ammonium Chloride | NH ₄ Cl | 760 |
| Potassium di-Hydrogen Phosphate | KH ₂ PO ₄ | 220 |
| Calcium Chloride | CaCl ₂ | 10 |
| Magnesium Sulphate | MgSO ₄ .7H ₂ O | 10 |
| Magnesium Chloride | MgCl ₂ | 2 |
| Ferric Chloride | FeCl ₃ | 3.3 |
| Sodium Bicarbonate | NaHCO ₃ | 800 |

Table 3.2: Dyes in Synthetic Textile Wastewater

| Name | Quantity Used (mg/L) | Structural Formula |
|----------------------|----------------------|--|
| Disperse Violet (93) | 100 |  |
| Direct Violet | 100 |  |
| Vat Blue | 10 |  |
| Pigment Black 32 | 10 |  |

3.2 Membrane Characteristics

Due to high filtration rate and resistance to acids and bases, hollow fiber PVDF membranes were selected for treatment of textile wastewater. There is a bunch of hollow fiber membranes which forms membrane module. The characteristics of hollow fiber membrane module are given in Table 3.3.

Table 3.3: Hollow Fiber-PVDF Membrane Characteristics

| Item | Characteristics |
|------------------|---|
| Material | Polyethylene |
| Manufacturers | Mitsubishi Rayon Engineering Co.Ltd Japan |
| Temperature | 15-35C° |
| Suction pressure | 30kPa |
| MLSS | 4000-15000mg/L |
| Pore Size | 0.1µm |

3.3 Biofilm Carrier

Kaldnes® media was introduced in H-MBR, 10% by volume during the study for improved nutrients removal. These are composed of polyethylene plastic material, of very high density mostly cylindrical in shape. On the inner side they have cross and on the outer side media have a rough tooth. The size of each cylindrical shaped media is 10 mm in diameter and 7 mm in height.

3.4 H-MBR and SBRs Operation

Three biological reactors, Hybrid membrane bioreactor (H-MBR), Hybrid sequencing batch reactor (H-SBR) and Conventional sequencing batch reactor (C-SBR) were operated at 12 hrs HRT and 30 days SRT during this study. The three acrylic reactors were separated by perforated plates/baffles into two or three compartments.

A peristaltic pump was used to suck permeate from hybrid membrane bioreactor through hollow fiber PVDF membrane, connected to the discharge line. The pump was operated in intermittent mode with 10 minutes operation and 2 minutes relaxation time. H-MBR consisted of two zones separated by perforated plates with the ratio of 1:2, having mechanical mixer (Cole Parmer) installed in the larger portion, air diffusers and membrane installed in the smaller portion. Continuous aeration was provided to all reactors for keeping the sludge in suspension and providing dissolved oxygen for microbial growth.

Anoxic/Oxic conditions in H-MBR helped in more COD and nutrients removal as compared to conventional aerobic MBRs or SBRs. For measuring trans membrane pressure (TMP) in H-MBR a data logging manometer was connected to membrane which helped in generating a complete profile of the pressure variation through the membrane. Membrane module was operated till the TMP reached 30kPa and there was a continuous desired flux rate. As a result of the deposition of cake layer on membrane surface fouling occurred due to which TMP raised above 30 kPa. At this point the membrane was physically and chemically cleaned followed by a new filtration cycle.

3.4.1 Operating Conditions of Reactors

Reactors were designed to be operated on 12hrs HRT and an SRT of 30 days. Except anoxic portion of H-MBR, aeration was provided to all the portions at a rate of 6L/min. Organic loading rate for all the reactors was maintained 3000 mg/L/day and the reactors were fed daily. The Food to Microorganism ratio (F/M) was slightly different in all reactors. F/M to ratio of 0.25 ± 0.03 , 0.17 ± 0.03 , 0.20 ± 0.03 was noted for H-MBR, H-SBR and C-SBR respectively.

3.4.2 Efficiency and Performance Evaluation

The efficiency of reactors was determined and analyzed regularly in terms of Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) and Total Nitrogen (TN) measured by TOC analyzer, nutrients (NO_3^- -N, NO_2^- -N, and NH_4^+ -N) and PO_4^{3-} -P measured using spectrophotometer (DR/2010, HACH, USA). Dissolved Oxygen and pH were measured using pH/DO meter (Oakton PD 300, USA). Trans membrane pressure was monitored by sper scientific data logging manometer (840099, Taiwan) having capacity of storing 12000 readings to the maximum.

3.4.3 Acclimatization Phase

Sludge was acclimatized for two months duration. Sludge color was “Khaki” in start but it turned yellowish brown after 3 weeks. There was a continuous and rapid foaming in beginning of the acclimatization of sludge because of rapid microbial activity. MLSS in the beginning was 2000 mg/L which increased to 8000mg/L after complete acclimatization of sludge.

Addition of PAC (100 mg/L) to sludge resulted in reduced foaming activity of bacteria. Despite smaller dosage, PAC was effective in organics and color removal. Increase in MLSS was also noted due to PAC addition. H-MBR and H-SBR were supplemented with PAC and C-SBR was operated without addition of PAC. The supernatant obtained from SBRs was followed by microfiltration, through Dead end filtration system which improved the quality of effluents.

The reactors were operated for a time span of 180 days. During this phase performance of reactors was evaluated for organics and nutrients removal from textile wastewater.

3.4.4 Analytical Parameters

Following parameters were analyzed in terms of sludge characteristics, removal efficiency and membrane fouling characteristics.

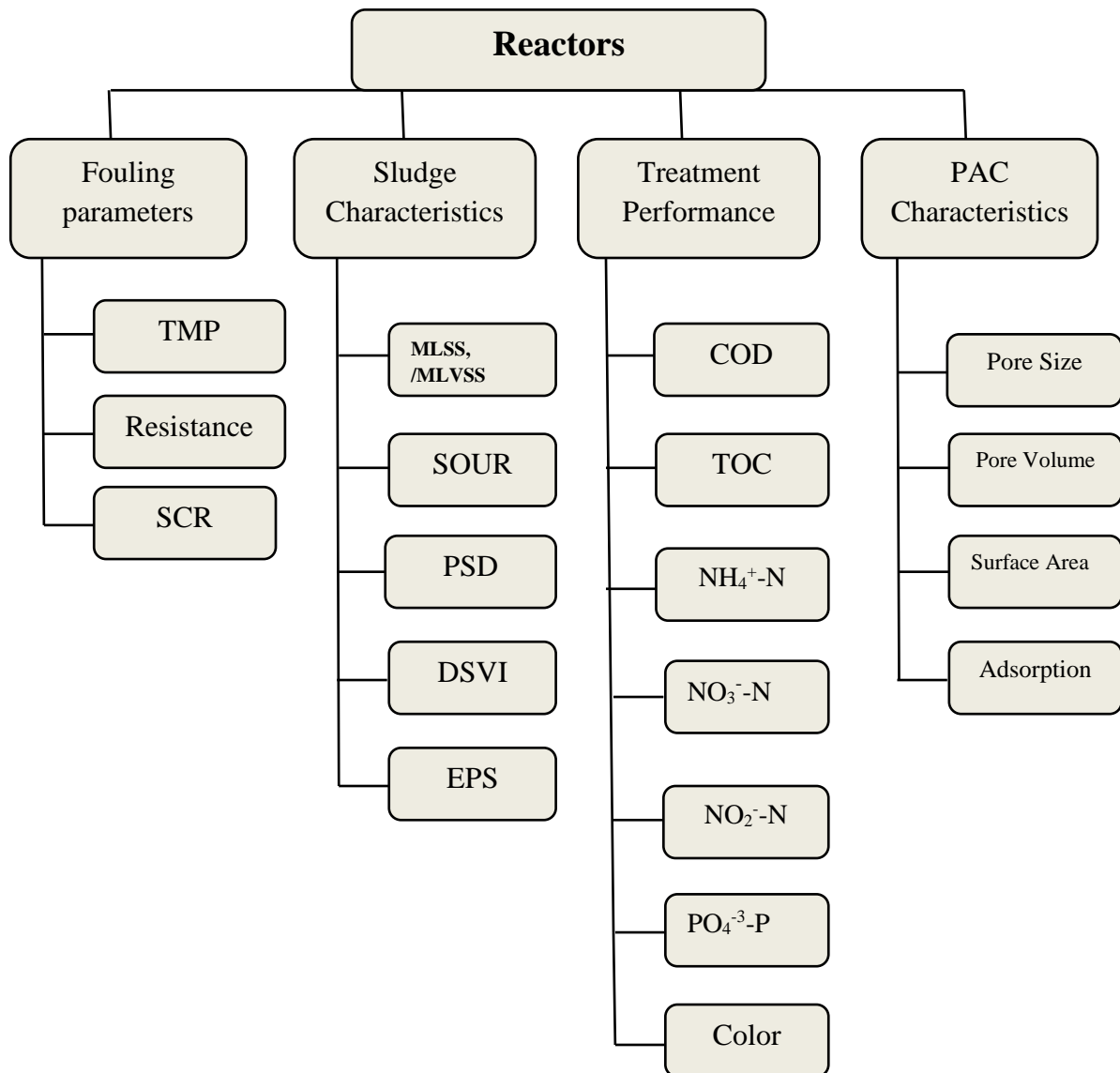


Figure 3.1: Analytical Parameters

Most of the analytical techniques used in this study were adopted from Standards Methods (APHA et al., 2005).

Table 3.4: Sludge Characteristics

| Parameter | Method | Model |
|-------------------------------|----------------------------------|---|
| MLSS/MLVSS | Filtration- Evaporation | 1.2µm (GF/C, Whatman) Filter, 105°C (MLSS) 550°C (MLVSS), APHA (2005) |
| SOUR | DO Depletion | DO meter (YSI, Model 5100) , APHA (2005) |
| Soluble EPS | Centrifugation | Centrifuge (Sigma Laborzentrifugen 204, Germany) <i>Zhang et al., 2006</i> |
| Bound EPS | CER Method | Centrifuge (sigma Laborzentrifugen 204 Germany), <i>Frolund et al., 1997</i> |
| Carbohydrate concentration | Colorimetric method | Spectrophotometer (DR/2010, HACH ,USA) <i>Dubois et al., 1956</i> |
| Protein concentration | Colorimetric method | Spectrophotometer (DR/2010, HACH ,USA) <i>Lowery et al., 1951</i> |
| PSD | Laser light | Horiba LA-300 Japan, LA-300 manual |
| TMP (kPa) | Monitoring TMP Change | Sper scientific data logging manometer (840099), Manometer manual |
| SCR | Membrane flux at constant TMP | Dead end filtration setup (Amicon 8400, USA), <i>Wang et al., 2007</i> |

Table 3.5: Effluent Characteristics

| Parameter | Method | Equipment and Reference |
|--|-------------------------|--|
| COD and sCOD | Close reflux | COD Digester (tubes digestion),APHA,2005 |
| TOC,TN | TOC differential method | TOC/TN Analyzer multi C/N Analytikjena, Germany. TOC manual |
| NO ₂ -N,NO ₃ -N,NH ₄ -N | HACH method | Spectrophotometer (DR/2400, HACH, USA), APHA 2005 |
| PO ₄ -P | Molybdovanadate method | Spectrophotometer (DR/2400, HACH, USA), APHA 2005 |
| Color | Colorimetric method | Colorimetric method , DR 2010 , HACH |

3.6 Characteristic of Virgin Powdered Activated Carbon

Industrial effluents must be treated before they are discharged. *Harrelkas et al.*, (2009) reported that about 50% of dyes do not get fixed on the fiber surface and they are released in discharged water. PAC was analyzed for its properties including pore size, pore volume and its surface area following the method of nitrogen adsorption with Micromeritics (Surface porosity and sample Degas system) Gemini VII 2390t, USA.

Table 3.6: Properties of Virgin PAC

| Property | Value |
|---------------------------|--------------------------|
| Single point surface area | 544.65 m ² /g |
| BET Surface area | 539.84m ² /g |
| Langmuir Surface area | 759.27 m ² /g |
| Pore volume | 0.25cm ³ /g |
| Micro pore area | 295.34 m ² /g |
| Pore Size (BET) | 19.21 Å |
| Micro pore volume | 0.15cm ³ /g |

3.7 Performance Evaluation Methods

Chemical oxygen demand (COD) was analyzed regularly according to close reflux method protocol in standard methods. Total organic carbon (TOC) and total nitrogen (TN) was measured by TOC analyzer. Performance evaluation parameters like NO₃⁻-N, NO₂⁻-N, NH₄⁻-N and PO₄-P were measured by absorption method using Spectrophotometer (DR/2010, Hach, USA) as per HACH protocols. DO and pH of all the reactors were measured by DO/pH meter (Oakton PD 300, USA). Trans-membrane pressure was monitored using Sper scientific digital data logging manometers (840099, Taiwan) for every 2 minute reading.

3.8 Sludge Characterization Methods

The reactors MLSS and MLVSS were quantified and evaluated thrice a week. Measurements of Specific Oxygen Uptake Rate (SOUR) were helpful in determining microbial respirometric activity with the rate of dissolved oxygen depletion in a specific aeration time of sludge (30 minutes). The analysis was

performed by using DO meter (YSI, Model 5100, USA), the method described by *Xing et al.*, 2001.

Soluble EPS was analyzed by centrifugation of sludge sample using Centrifuge machine (Laborzentrifugen, Sigma 204, Germany) while for bound EPS extraction, Cation exchange resin (CER) method was adopted, method by *Frolund et al.*, (1996). Carbohydrate and protein concentration were measured using spectrophotometric methods performed by *Dubois et al.*, (1956) and *Lowry et al.*, (1951), respectively. The settleability of sludge was revealed by determining its diluted sludge volume index (DSVI) and it was measured by 50 % dilution of 1 liter sludge in Imhoff Cone and allowing the sludge to settle for 30 minutes. The settled volume in mL/L determined the DSVI.

Addition of PAC resulted in lower DSVI values in the two reactors. It was because of the fact that larger flocs were formed when solids of sludge surrounded the PAC particles. A higher DSVI is indication of low sludge settleability, hence resulting in reduced filtration and vice versa. DSVI was measured according to protocols given in standard methods (*APHA et al.*, 2005).

Particle size distribution (PSD) was performed to determine the mean size of sludge particles. Enhanced aeration resulted in smaller particle size because of flocs breakage, while a greater particle size was obtained at normal aeration. Moreover, PAC addition was also responsible for slight increase in particle size, due to adsorption of particulates and soluble substances. PSD was determined using Particle size analyzer (LA-300, Horiba, Japan) through ultra-sonication followed by laser light scattering.

Specific cake resistance analysis was performed on Dead end filtration unit (Model 8400, Amicon, USA). A flat sheet cellulose membrane (GVWP 09050, Millipore, USA) was used having pore size of 0.22- μm (*Jamal et al., 2009*). Nitrogen gas cylinder was fitted with apparatus and the set up was operated at TMP of 30Kpa. Weight of permeate per second was recorded using a weighing balance, filtered through the membrane.

SCR was calculated using the following formula as reported by *Jamal Khan et al., (2009)*.

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

Where,

- α = Specific cake resistance (m/kg)
- A = Membrane area (m^2)
- ΔP = Trans membrane pressure (kPa)
- t = Time of filtration (s)
- V = Volume of filtration (m^3)
- μ = Viscosity of permeate ($\text{N}\cdot\text{s}/\text{m}^2$)
- C = MLSS concentration (kg/m^3)

3.9 Membrane Fouling Characterization

Membrane fouling propensity was measured by data log manometer connected to H-MBR. The meter had the capability to store readings of trans membrane pressure, during operation of membrane and permeate suction through peristaltic pump. These values were plotted against time using MatLAB 7.0 software by importing values from Sper scientific software data log.

3.8.1 Resistance Analysis

Membrane filtration resistance is an important parameter for understanding filtration capacity and membrane resistance after fouling. The total resistance offered by membrane to activated sludge was measured, denoted by R_t . After physical cleaning another filter run was performed at incremental flow rates, to calculate the resistance by pore clogging and membrane itself i.e. R_m+R_p . A third run was carried for calculating resistance offered by membrane itself. For calculating this resistance membrane was chemically cleaned and after 8 hours, tap water was passed through membrane. The resistances were evaluated using following equations *Lee et al.*, (2001).

$$J = \frac{\Delta P}{(\mu \cdot f_t \cdot R_t)} \quad \dots\dots a$$

$$R_t = R_m + R_f + R_c \quad \dots\dots b$$

Where,

$J =$ Operational

Flux ($L/m^2.s$)

$\Delta P =$ Applied TMP (kPa)

$f_t =$ Temperature Correction to 2020°C, $f_t = e^{-0.0239(T-20)}$

$R_t =$ Total Hydraulic Resistance (m^{-1})

$R_c =$ Reversible Cake Resistance (m^{-1})

$R_p =$ Irreversible Pore Blocking (m^{-1})

$\mu =$ Viscosity of Permeate ($N\cdot s/m^2$)

R_m, R_c and R_p are calculated as described by *Poostchi et al., (2012)*

$$R_m = \Delta P / \mu J_w$$

$$R_p = \Delta P / \mu J_{wf} - R_m$$

$$R_c = \Delta P / \mu J_{AS} - R_m - R_p$$

Where,

$$J_w = \text{Initial tap water flux}$$

J_{wf} = Initial tap water flux after physically washing the membrane and removing the cake layer

$$J_{AS} = \text{Flux of activated sludge at steady state}$$

RESULTS AND DISCUSSION

This study was conducted for the comparison of Hybrid MBR and SBR systems performance for textile wastewater treatment, using Powdered Activated Carbon (PAC). The presence of PAC in reactors enhanced operational flux and removal efficiency, which was due to the adsorptive nature of PAC. Reactors were evaluated on the basis of effluent quality and sludge characteristics, along with study of membrane fouling behavior.

In H-MBR the study was mainly focused on the adsorptive behavior of PAC, extra polymeric substances (EPS) in the sludge and trans membrane pressure (TMP) during operation.

4.1 Performance of MBR and SBR Systems

Influent textile wastewater prepared in laboratory synthetically having a COD of 1000 mg/L and COD: N: P ratio 100:20:6 was treated through MBR and SBRs. PAC concentration of 100 mg/L was added for effective removal of organics and dyes. Performance of the systems was investigated in terms of COD, color /dyes degradation and nutrients removal. Soluble EPS concentration in sludge was determined which is an important factor in membrane fouling behavior. A detailed discussion of the performance evaluation is given below.

4.1.1 Chemical Oxygen Demand (COD)

COD analysis was performed throughout the study and the influent COD was maintained 1000 mg/L. PAC addition to the two reactors (H-MBR and H-SBR) helped in COD removal from high strength textile wastewater. The two hybrid reactors showed high COD removal.

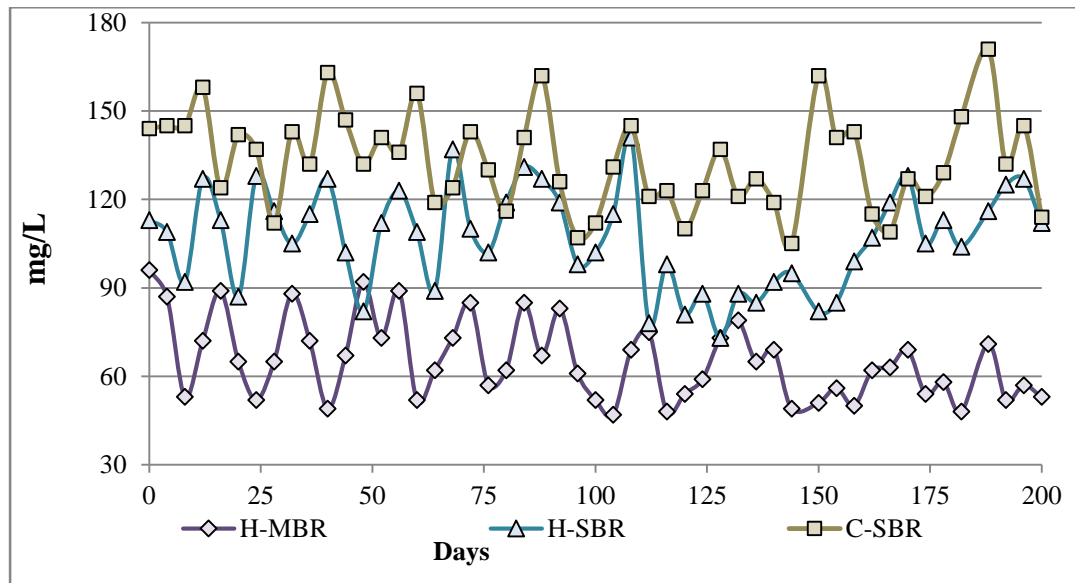


Figure 4.1: Time Series COD Concentrations

Table 4.1: Chemical Oxygen Demand Removal Statistical Analysis

| Analysis | H-MBR | H-SBR | C-SBR |
|----------------------|-------|-------|-------|
| Standard Deviation | 13 | 17 | 15 |
| Mean Value (mg/L) | 65 | 105 | 133 |
| Maximum Value (mg/L) | 96 | 141 | 171 |
| Minimum Value (mg/L) | 48 | 73 | 109 |

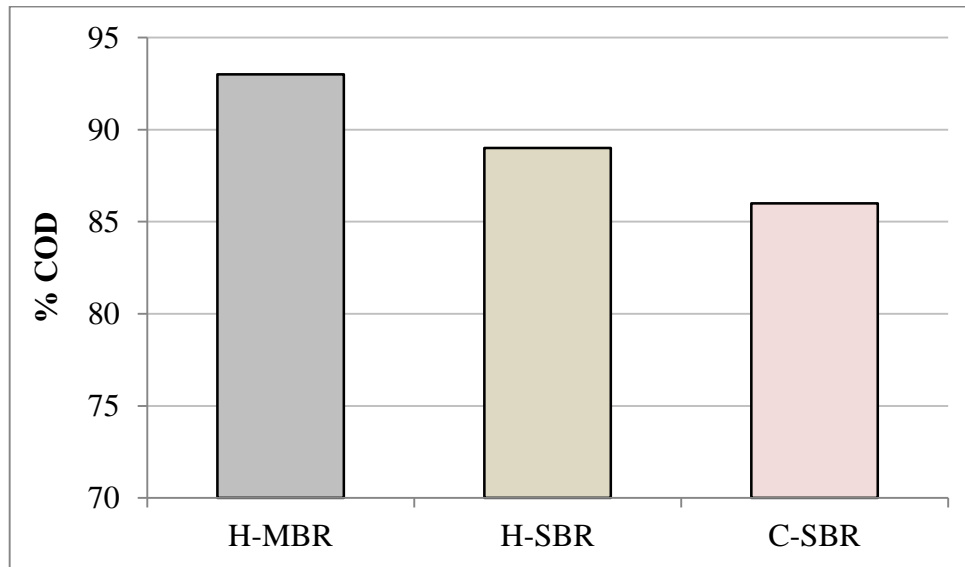


Figure 4.2: COD Removal (%)

4.1.2 Nutrients Removal

Ammonium Nitrogen Removal

$\text{NH}_4^+\text{-N}$ concentration was measured during the study. Results showed that ammonium nitrogen concentration was less in H-MBR and H-SBR as compared to C-SBR. Nitrifiers are very strict aerobes and their growth is inhibited at low DO level. Therefore anoxic zone in H-MBR was a reason to cause increase in average effluent $\text{NH}_4\text{-N}$ concentration.

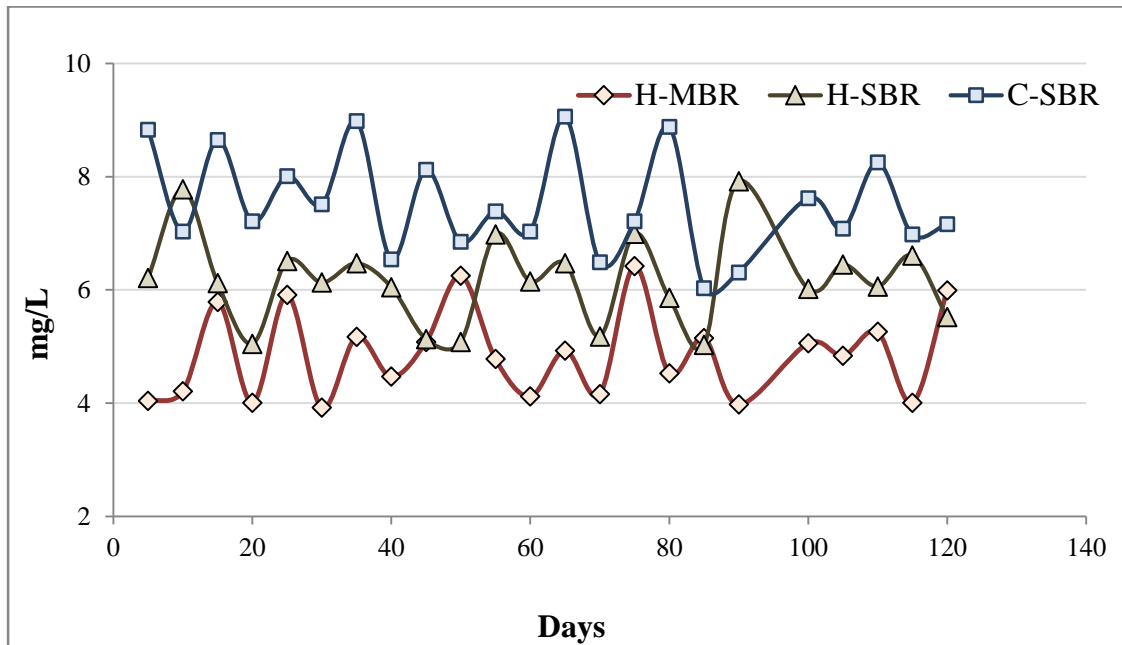


Figure 4.3: Effluents Average Ammonium Nitrogen Concentration

Table 4.2: Ammonium-N Removal Statistical Analysis

| Analysis | H-MBR | H-SBR | C-SBR |
|----------------------|-------|-------|-------|
| Standard Deviation | 0.82 | 0.56 | 0.93 |
| Mean Value (mg/L) | 4.87 | 6.15 | 7.53 |
| Maximum Value (mg/L) | 6.42 | 7.92 | 9.06 |
| Minimum Value (mg/L) | 3.92 | 5.03 | 6.31 |

Nitrites Removal

Effluent average Nitrites concentration in H-MBR was lower than SBRs because of anoxic conditions which favored denitrification. Denitrification is favored by low dissolved oxygen (Yang *et al.*, 2010) hence SBRs were less effective in nitrites and nitrates removal than H-MBR.

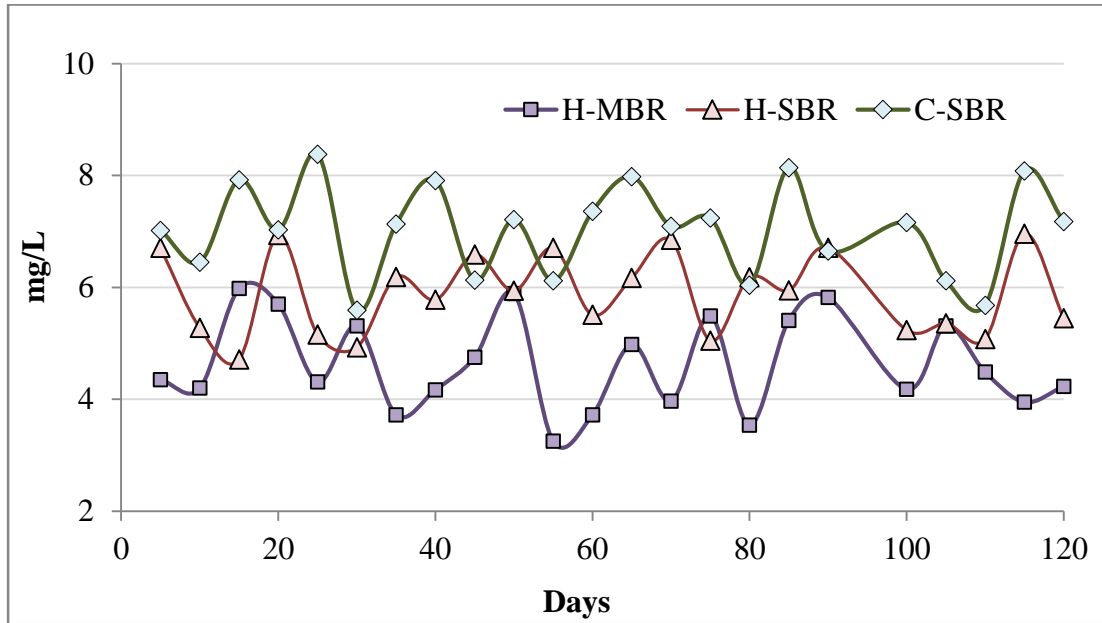


Figure 4.4: Effluents Average Nitrites Concentration

Table 4.3: Nitrites Removal Statistical Analysis

| Analysis | H-MBR | H-SBR | C-SBR |
|----------------------|-------|-------|-------|
| Standard Deviation | 0.82 | 0.80 | 0.81 |
| Mean Value (mg/L) | 4.67 | 5.83 | 7.02 |
| Maximum Value (mg/L) | 5.98 | 6.96 | 8.38 |
| Minimum Value (mg/L) | 3.25 | 4.71 | 5.59 |

Nitrates Removal

Anoxic conditions maintained by mechanical mixing resulted in lesser effluent's nitrates concentration in H-MBR. The effluent followed by microfiltration through Dead end filtration unit showed a remarkable change in decreasing the concentration of nutrients and organic particulates in the final

effluent. SBRs showed an improved effluent quality when the supernatant was passed through micro filter /membrane.

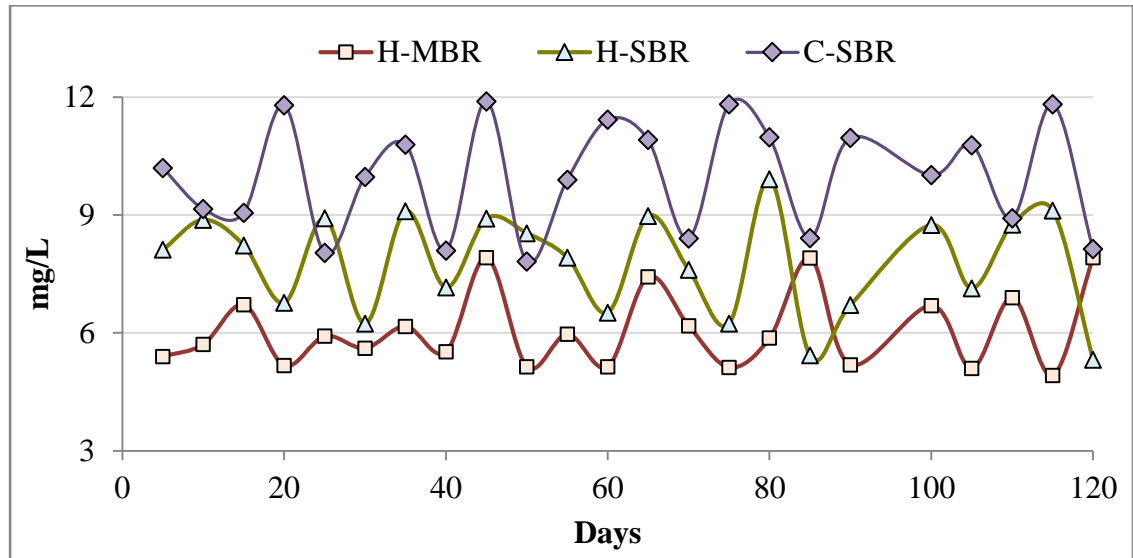


Figure 4.5: Average Nitrates Concentration in Effluents

Table: 4.4 Nitrates Removal Statistical Analysis

| Analysis | H-MBR | H-SBR | C-SBR |
|----------------------|--------------|--------------|--------------|
| Standard Deviation | 0.92 | 1.20 | 1.46 |
| Mean Value (mg/L) | 5.98 | 7.83 | 10.06 |
| Maximum Value (mg/L) | 7.92 | 9.92 | 11.82 |
| Minimum Value (mg/L) | 5.19 | 5.32 | 7.82 |

Total Nitrogen

Favorable conditions for growth of denitrifying bacteria resulted in smaller effluent's TN concentration in H-MBR than in SBRs. On average 70% TN removal was noted in H-MBR as compared to 68 and 62% in H-SBR and C-SBR respectively.

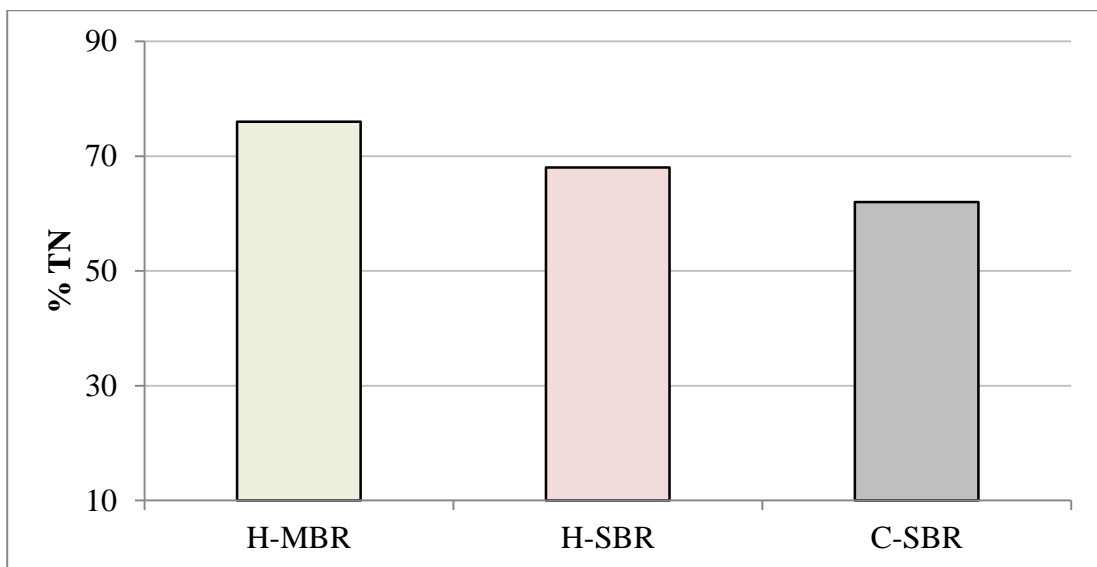


Figure 4.6: Total Nitrogen Removal in Reactors

Due to anoxic conditions and PAC addition H-MBR resulted in lowest TN among the biological reactors. *Chu et al.*, (2005) investigated that with the decrease of ammonium level in sludge total nitrogen level also decreases which declares nitrification as the rate-limiting factor.

Phosphorus Removal

High concentration of phosphorus causes excessive growth of algae which results in eutrophication. Hence it is very important parameter to be removed from

wastewater. H-MBR and H-SBR showed a greater removal of phosphorus from influent textile wastewater supplemented with Powdered Activated Carbon. H-MBR gave 78% phosphorus removal and both the SBRs gave a removal greater than 64%.

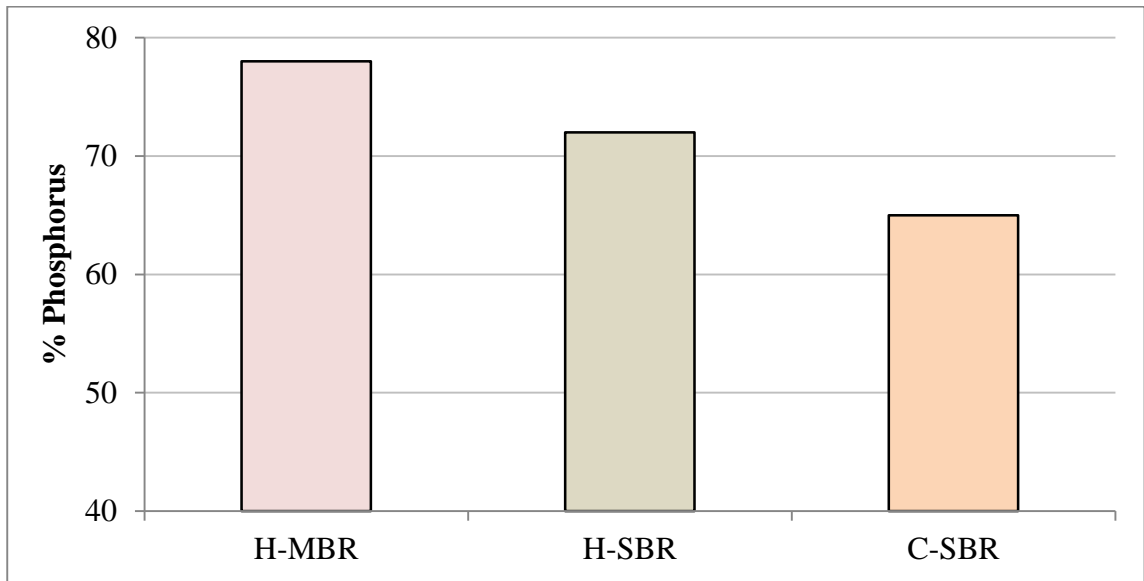


Figure 4.7: Phosphorus Removal

Table 4.5: Phosphorus Removal Statistical Analysis

| Analysis | H-MBR | H-SBR | C-SBR |
|----------------------|--------------|--------------|--------------|
| Standard Deviation | 0.94 | 0.83 | 1.24 |
| Mean Value (mg/L) | 2.74 | 5.57 | 8.2i |
| Maximum Value (mg/L) | 3.42 | 6.84 | 8.74 |
| Minimum Value (mg/L) | 2.32 | 4.40 | 6.08 |

Total Organic Carbon

Total Organic Carbon (TOC) concentration in the influent wastewater was about 400 mg/L. H-MBR and C-SBR were efficient in removing TOC from influent wastewater while H-SBR showed a lower efficiency in removing TOC. TOC removal was more than 90% for all the reactors with effluent minimum TOC concentration of 30 mg/L for H-MBR.

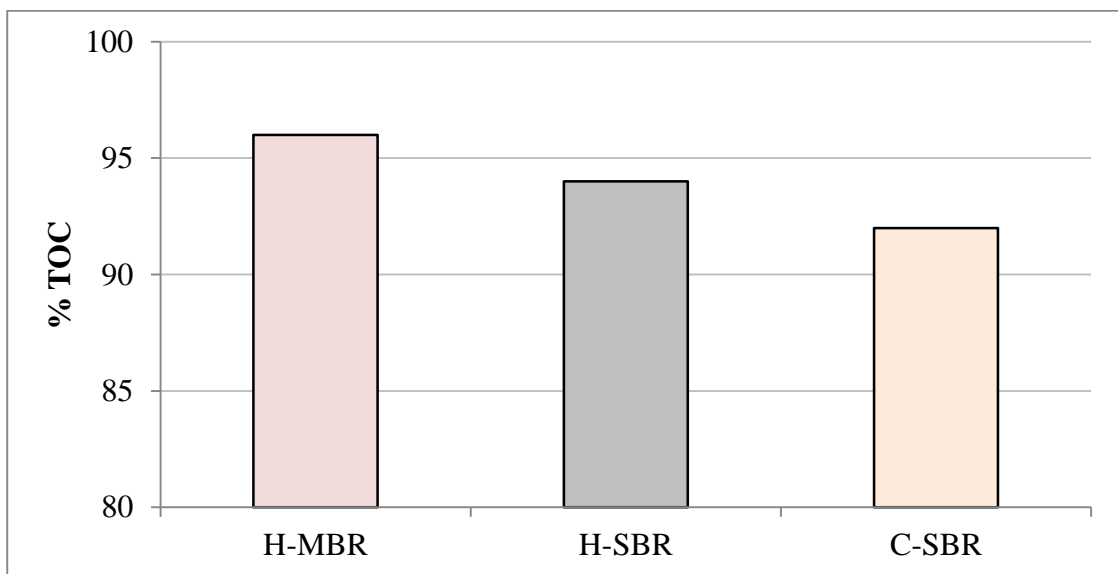


Figure 4.8: TOC Removal

Table 4.6: Overall MBR and SBRs System Performance Evaluation

| Effluent Quality Parameter | H-MBR | H-SBR | C-SBR |
|--|--------------|--------------|--------------|
| COD (mg/L) | 65 | 107 | 133 |
| COD Removal (%) | 93 | 89 | 86 |
| NH ₄ ⁺ -N (mg/L) | 4.87 | 6.15 | 7.53 |
| NO ₂ ⁻ -N (mg/L) | 4.63 | 5.81 | 7.02 |
| NO ₃ ⁻ -N (mg/L) | 5.98 | 7.84 | 10 |
| TN (mg/L) | 18 | 24 | 10 |
| TN Removal (%) | 76 | 68 | 62 |
| PO ₄ ⁻³ -P (mg/L) | 2.71 | 5.57 | 8.23 |
| PO ₄ ⁻³ -P Removal (%) | 78 | 72 | 66 |
| TOC (mg/L) | 30.2 | 45.2 | 36.8 |
| TOC Removal (%) | 96 | 94 | 92 |

4.2 Sludge Characterization

4.2.1 Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile

Suspended Solids (MLVSS)

Addition of PAC in low dosage to the reactors resulted in slight increase of MLSS and MLVSS values, ranging between 8-13 g/L. H-MBR and H-SBR containing PAC had larger values of MLSS and MLVSS than C-SBR which was operated without PAC addition. *Ferreira et al., (2010)* found that activated sludge in MBRs having MLSS > 10 g L⁻¹ is able to entrap solute particles smaller in size than 20 µm, which as a result decreases membrane resistance.

High MLSS concentration helps in the formation of porous and spongy media and loosely bound cake layer on membrane, which results in decreases of membrane resistance and good filterability (*Le-Clech et al*). However *Broeck et al.*, (2011) has reported optimal concentration of MLSS for better and efficient membrane operation.

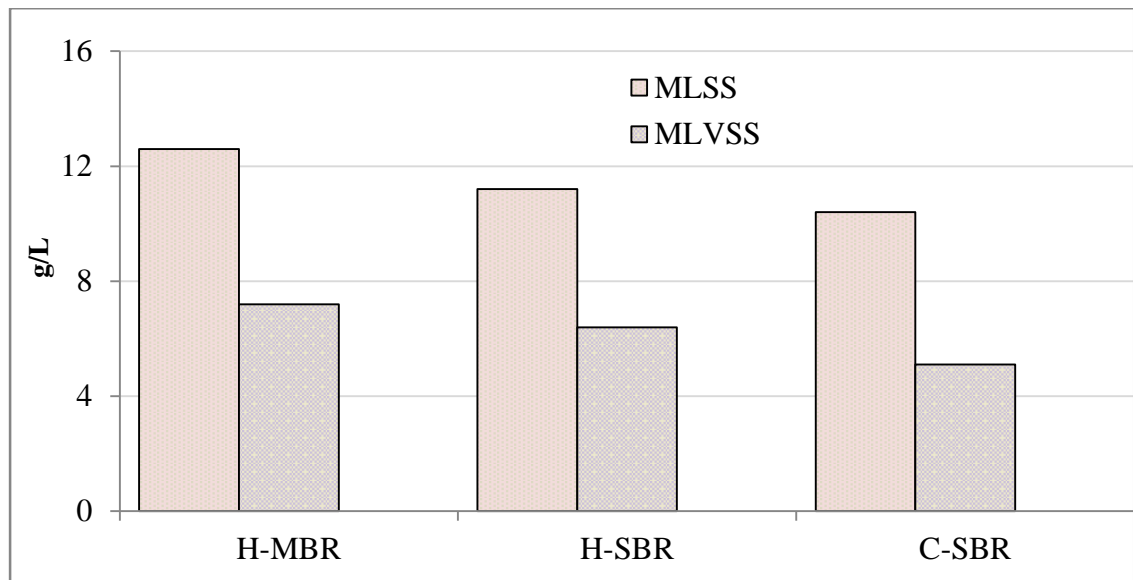


Figure 4.9: MLSS and MLVSS Concentration in Sludge

4.2.2 Diluted Sludge Volume Index (DSVI)

Higher DSVI was noted for H-MBR during analysis phase. The SBRs showed improved DSVI having similar biological conditions. PAC addition altered sludge characteristics and resulted in lowest DSVI in H-MBR because of increased and improved sludge settling rates. Rapid sludge settling was observed due to larger and heavier flocs formation by organics and PAC particles.

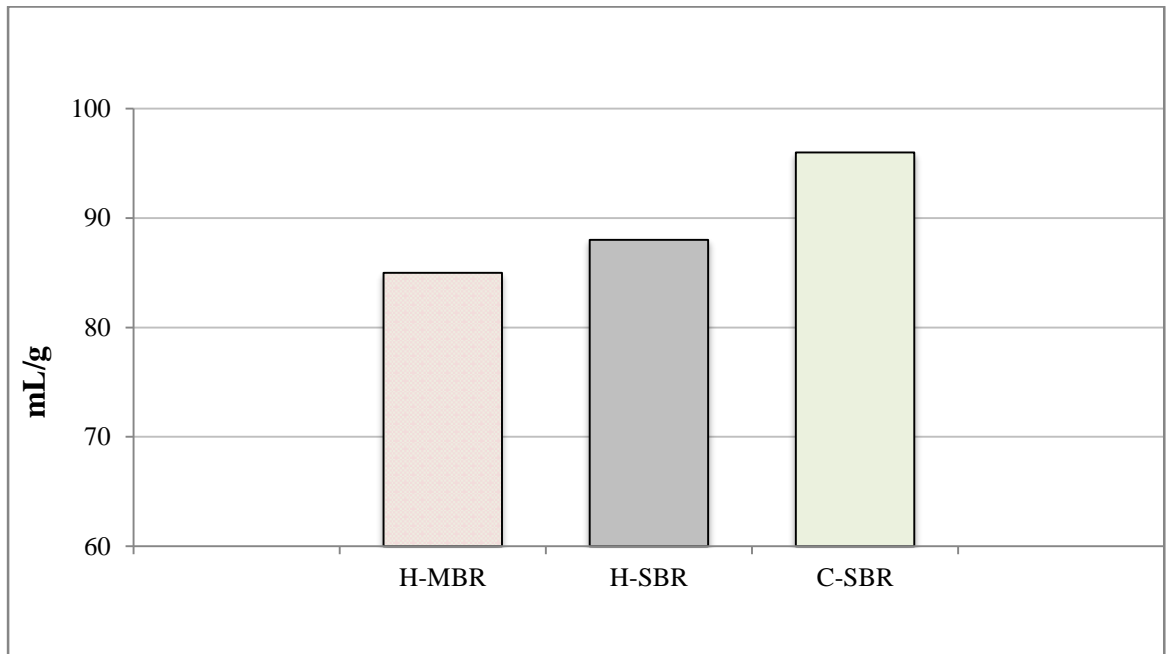


Figure 4.10: Diluted Sludge Volume Index of H-MBR and SBRs

4.2.3 Specific Oxygen Uptake Rate (SOUR)

Specific oxygen uptake rate is metabolic indicator of cells respirometric and physiological activity in bioreactors. During study high MLSS in all the reactors depicted more microbial respirometric activity and high substrate consumption. Average SOUR values in the study were found to be 72, 77 and 92 mg O₂/gVSS/h for H-MBR, H-SBR and C-SBR respectively.

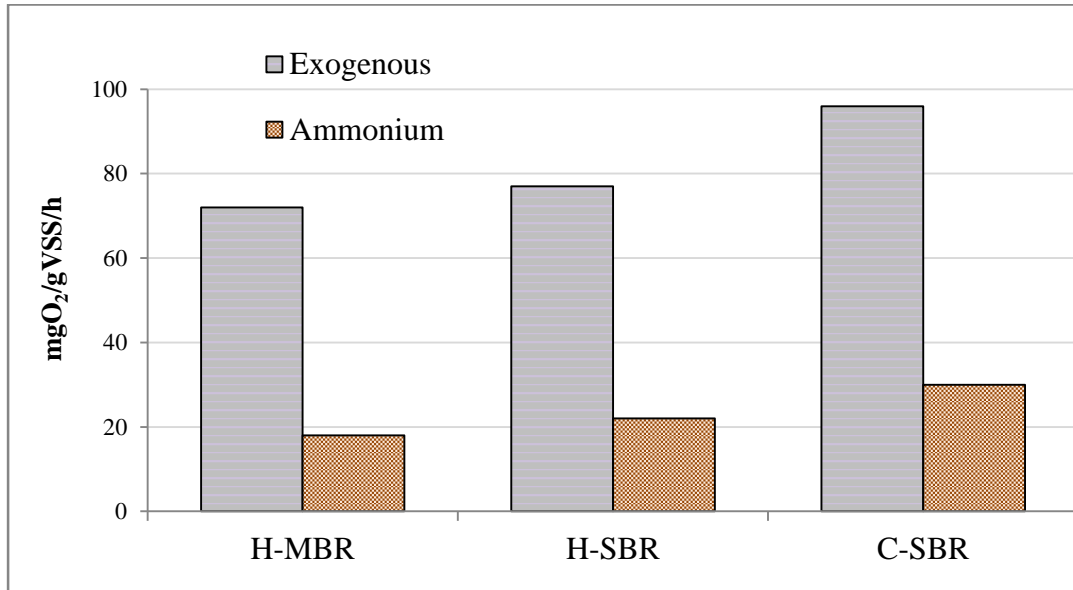


Figure 4.11: Specific Oxygen Uptake Rate

4.2.4 Extra Polymeric Substances (EPS)

Extra polymeric substances in reactors were released as a result of microbial activity. EPS was categorized as soluble and bound, which was found in the sludge because of cell lyses, cells growth and their continuous decay. The reactors contained lesser soluble EPS content than bound EPS due to the presence of PAC. Bound EPS in higher concentration resulted in greater specific cake resistance which caused membrane fouling earlier.

Because of the gel like behavior EPS leads to formation of more tightly bound cake layer by strongly bonding the floc particles, resulting higher membrane resistance (*Metzger et al., 2007*). In soluble EPS protein contents are found in larger concentration than carbohydrates. H-MBR showed a more improved reduction in soluble EPS because of the anoxic conditions along with PAC addition.

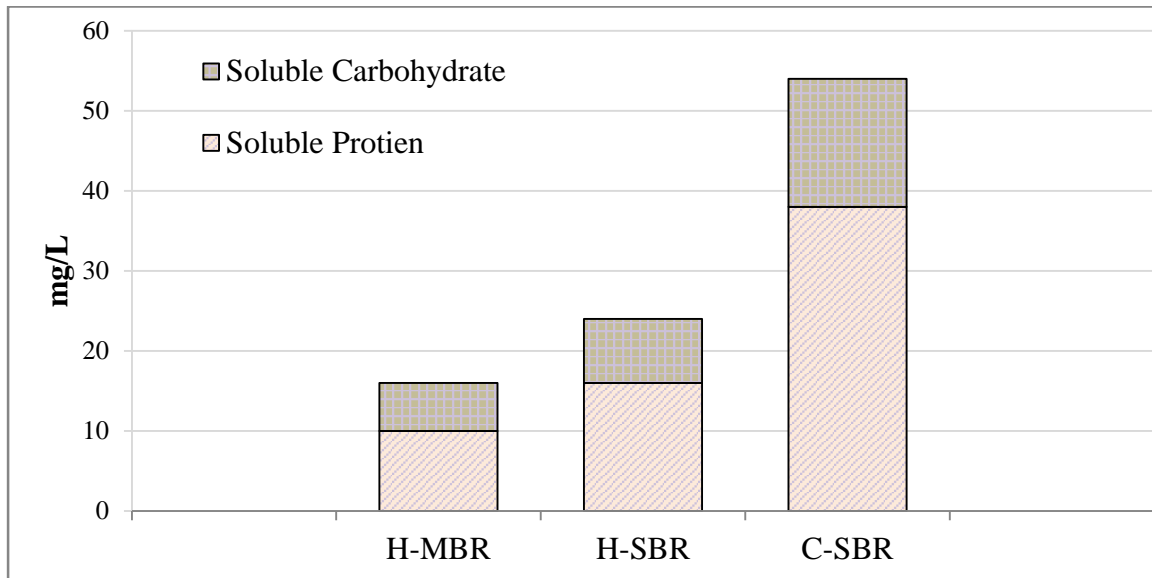


Figure 4.12: Soluble EPS Concentration in Sludge

Protein is found as the major foulant in membrane bioreactors operation (Wingender *et al.*, 2001). Moreover filtration capacity of membrane bioreactor is highly effected by greater release of EPS as found by Xuan *et al.*, (2010).

Significant decrease was noted in soluble EPS concentration because of the adsorption of proteins and carbohydrates by PAC, which resulted in slight increase of bound EPS release. EPS are those containing carbohydrates, proteins and glutamic acid (having glue like behavior), but adsorption of released EPS contents over PAC surface diminished and decreased the glue like behavior which was helpful in mitigating biofouling of the membrane, resisting microbial colonial formation by sticking to each other.

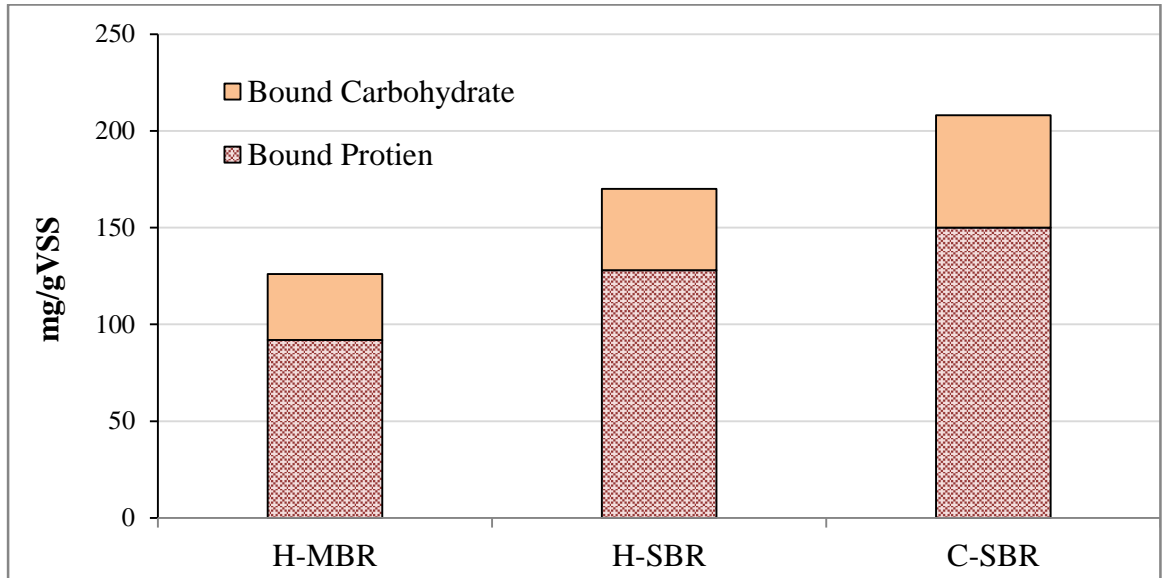


Figure 4.13: Bound EPS Concentration in Sludge

4.2.5 Particle Size Distribution (PSD)

Particle size distribution varied in all reactors due to different hydrodynamic and biological conditions. Enhanced aeration causes flocs breakage which results in smaller average particle size. Under controlled aeration larger average particle size was noted, facilitated by PAC addition as well.

Use of Kaldnes® media in H-MBR resulted in flocs breakage, so the particle size of H-MBR was noted less than C-SBR and H-SBR. Reduction in the particle size is also a function of shear stresses offered by any media used.

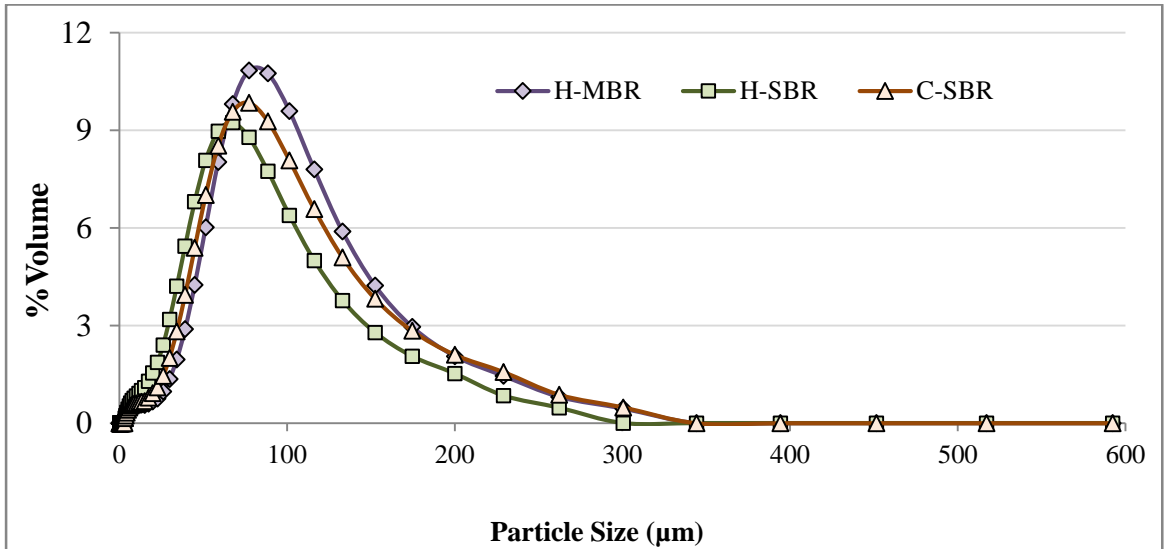


Figure 4.14: Mean Particle Size Distribution of Activated Sludge

4.2.6 Specific Cake Resistance (SCR)

Specific cake resistance of SBRs sludge was higher than that of H-MBR. H-MBR sludge gave a less cake resistance of activated sludge due to the presence of kaldnes® media facilitated by PAC as well. Kaldnes® media also helped in reducing biomass load over membrane surface hence helped in better flow rate.

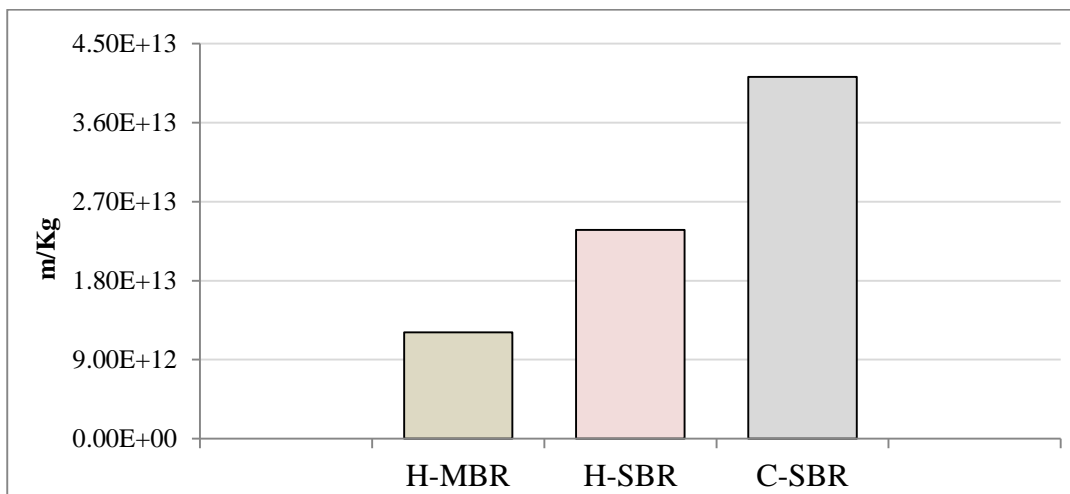


Figure 4.15: Specific Cake Resistance

Juang *et al.*, (2011) investigated that the cake layer causing inner fouling is composed of proteins and carbohydrates. This cake layer increases with decrease in particle size because of the interaction of smaller particles.

4.3 Membrane Fouling

4.3.1 Trans Membrane Pressure (TMP)

TMP profiling helps in understanding membrane fouling behavior during the operation of MBR. When suction pressure of permeate by membrane reaches to 30kPa at constant flux then membrane is considered to be fouled. During the study, fouled membrane in H-MBR was physically and chemically cleaned after each fouling cycle and then was re-connected to the system for normal filtration.

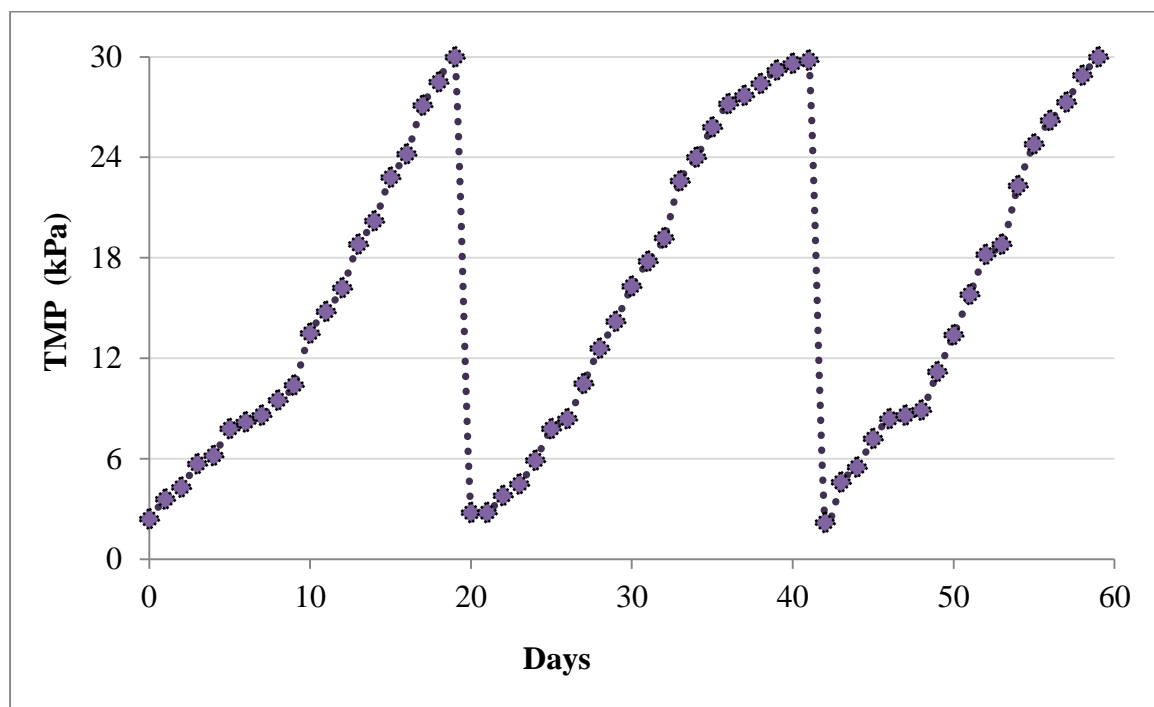


Figure 4.16: Membrane Fouling Trends

PAC addition enhanced filtration capability of the membrane but after filtration period, increase in TMP was noticed. Membrane fouled after 19 days on the average. Moreover due to anoxic conditions, PAC and kaldnes® media addition, membrane showed mixed fouling responses with variation in the number of days it fouled. The membrane fouling period varied between 15-21 days.

4.3.2 Resistance Analysis of H-MBR

H-MBR showed less total resistance because of porous and loosely bound cake layer formation on membrane surface due to addition of PAC which resulted in lower TMP rise. *Sarioglu et al.*, (2012) found that total resistance is mostly caused by intrinsic membrane resistance (R_m) and fouling resistance (R_f).

Table 4.7: Resistance Analysis of H-MBR

| Resistance x ($10^{12}m^{-1}$) | H-MBR |
|---|-------|
| Total hydraulic resistance (R_t) | 6.07 |
| Reversible cake resistance (R_c) | 2.14 |
| Irreversible pore blocking (R_f) | 2.79 |
| Intrinsic membrane resistance (R_m) | 1.17 |

4.4 Analysis of PAC Adsorption Behavior

Effluent of textile industry needs to be treated before its discharge to water bodies or nearby places, because some dyes have a toxic effect and results in damage of the aquatic life. PAC was analyzed for its properties including pore size, pore volume and its surface area using Nitrogen adsorption with Micromeritics (Surface porosity and sample Degas system) Gemini VII 2390t, USA.

All these parameters were noted for virgin PAC before its introduction to reactors. After the reactors were supplemented with PAC (100 mg/L), adsorption of PAC was found in terms of decrease of its surface area, pore size and pore volume which were reduced drastically. For this purpose sludge containing PAC was taken, oven dried at 105C° and then analyzed for surface porosity and volume. The results obtained for used PAC were different from virgin PAC. Decrease in surface area, pore size and pore volume revealed that there has been a greater adsorption of the solute particles and dyes contained in influent wastewater which resulted in greater removal efficiency.

4.4.1 Characterization of PAC in Reactors

Sludge combined with PAC was taken from the reactors and was filtered. The sludge taken from the reactors was oven dried (105C°) for 2 hours after filtering it.

The dried residue was weighed and the samples were tested for measuring the surface area and pore size of the PAC in the three reactors which was found to be a function of adsorptive capacity of PAC under different conditions.

The surface area, pore size and pore volume of PAC in the three reactors is given in the table below;

Table 4.8: Properties of Used PAC in Reactors

| Property | H-MBR | H-SBR |
|---|--------------|--------------|
| Single point surface area (m ² /g) | 3.31 | 1.57 |
| BET Surface area (m ² /g) | 3.61 | 1.71 |
| Langmuir Surface area (m ² /g) | 5.57 | 2.61 |
| Pore Volume (cm ³ /g) | 0.001 | 0.007 |
| Pore Size (Å) | 17.32 | 17.42 |

CONCLUSIONS AND RECOMMENDATIONS

This study consisted of performance evaluation of hybrid biological reactors for textile wastewater treatment supplemented with Powdered Activated Carbon. Comparison of hybrid biological reactors with conventional sequencing batch reactor without PAC showed that PAC is effective in efficient treatment and results in slower membrane fouling. The diverse environment in H-MBR due to Anoxic/Oxic zone and kaldnes® media application was important factor in enhanced treatment efficiency. Addition of media should be avoided as it causes breakage of membrane fibers. However application of kaldnes® media in a separate compartment can overcome this problem.

5.1 Conclusions

- 1) Addition of PAC resulted in reduced soluble EPS and specific cake resistance.
- 2) PAC was effective in organics and nutrients removal under Anoxic/Oxic conditions.
- 3) Optimum dosage of 100 mg/L of PAC exhibited about 90% COD, more than 80% total phosphorus and 70% total nitrogen removal in hybrid reactors.
- 4) Replacement of PAC with Biological activated Carbon (BAC) with the passage of time resulted in lower adsorption capacity.

- 5) Due to higher adsorption rate, the surface area, pore size and pore volume of PAC in hybrid reactors got decreased drastically, compared to values of these parameters for virgin PAC.

5.2 Recommendations

- 1) PAC dosages lower than 100 mg/L should be investigated and optimized for cost effective treatment of domestic and industrial wastewater.
- 2) Biological reactors supplemented with PAC should be operated at lower SRT to investigate PAC replacement by BAC in better way.

References

- Ahmad, A.A. and Hameed, B.H. (2009). Reduction of COD and color of dyeing effluent from a cotton textile mill by adsorption onto bamboo-based activated carbon. *Journal of hazardous materials*, 172:1538-1543
- Akram, A. and Stuckey, D.C (2008). Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Journal of process biochemistry*, 43: 93–102
- Al-Malack, M.H. (2007). Performance of an immersed membrane bioreactor (IMBR) King Fahd University of Petroleum & Minerals (KFUPM), Box 1150, Dhahran 31261, Saudi Arabia, 214: 112–12
- Artiga, P., Toriello, G.G., Méndez, R. and Garrido, J.M. (2008). Use of a hybrid membrane bioreactor for the treatment of saline wastewater from a fish canning factory. *Journal of Desalination*, 221: 518–525
- Aryal, A. (2010). Effect of different flocculants on short-term performance of submerged membrane bioreactor. *Journal of Separation and Purification Technology*, 70: 274–279
- Ayala, D.F., Ferre, V. and Judd, S.J. Membrane life estimation in full-scale immersed membrane bioreactors. *Journal of membrane Science*, 378: 95–100
- Buzatu, P., Zsirai, T., Aerts, P. and Judd, S.J. (2012). Permeability and clogging in an immersed hollow fiber membrane bioreactor. *Journal of Membrane Science*, 421-422: 342–348
- Carneiro, P.A., Umbuzeiro, G.A., Oliveira, D.P., Valnice M. and Zanoni, B. (2010). Assessment of water contamination caused by a mutagenic textile effluent/dyehouse effluent bearing disperse dyes, 174: 694–699
- Chari, I., Feki, M., Medhoub, M., Jalelbouid, Fakhfakh, E and Jamoussi, F. (2009). Adsorption of a textile dye intermediate blue RS (C.I Vat blue 4) from aqueous solutions onto smectite-rich clayey rock. *Journal of hazardous materials*, 172:1623-1628
- Chae, S-R., Ahn, Y-T., Kang, S-T. and Shin H-S. (2006). Mitigated membrane fouling in a vertical submerged membrane bioreactor (VSMBR). *Journal of Membrane Science*, 280: 572–581
- Chan, Y.J., ChongChung, M.F., Law, L. and Hassell, G. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater, 155: 1–18
- Chen, Z-B., Hu, D-X., Ren, N-Q., Tian, Y. and Zhang, Z-P. (2009). Biological COD reduction and inorganic suspended solids accumulation in a pilot-scale membrane bioreactor for traditional Chinese medicine wastewater treatment. *Chemical Engineering Journal*, 155: 115–122

- Chu, L-B., Zhang, X-W., Li, X. and Yang, F-L. (2005). Simultaneous removal of organic substances and nitrogen using membrane bioreactor seeded with anaerobic granular sludge under oxygen-limited conditions. *Journal of Desalination*, 172: 271–280
- Díaz, J.C-L., Calderón, K., Rodríguez, F.A., González-López, J., Hontoria, E. and Poyatos, J.M. (2013). Comparative kinetic study between moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrients removal. *Journal of Biochemical Engineering*, 77: 28–44
- Duan, L., Andrade, I.M., Huang, C-L., Xia, S. and Hermanowicz, S.W. (2009). Effects of short solids retention time on microbial community in a membrane bioreactor. *Journal of Bioresource Technology*, 100: 3489–349
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem*, 28: 350–356
- El-Rahim, W.M.A., El-Arady, O.A.M. and Mohammad, F.H.A. (2009). The effect of pH on bioremediation potential for the removal of direct violet textile dye by *Aspergillus niger*. *Journal of Desalination*, 249: 1206–1211
- Farizoglu, B. and Keskinler, B. (2006). Sludge characteristics and effect of crossflow membrane filtration on membrane fouling in a jet loop membrane bioreactor (JLMBR). *Journal of Membrane Science*, 279: 578–587
- Ferreira, M.L., Geilvoet, S., Moreau, A., Atasoy, Krzeminski, P., Nieuwenhuijzen, A.V. and Graaf, J.V. (2010). MLSS concentration: Still a poorly understood parameter in MBR filterability. *Journal of Desalination*, 250: 618–622
- Frølund, B., Palmgren, R., Keiding, K. and Nielsen, P.H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Journal of Water Research*, 30: 1749–175
- Gabelman, and Hwang, S.T. (2005). Experimental results versus model predictions for dense gas extraction using a hollow fiber membrane contactor, 35: 26–39
- Georgiou, D., Hatiras, J. and Aivasidis, A. (2005). Microbial immobilization in a two-stage fixed-bed-reactor pilot plant for on-site anaerobic decolorization of textile wastewater. *Journal of enzyme and microbial technology*, 37: 597–605
- Guo, W., Vigneswaran, S., Ngo, H-H., Xing, W. and Goteti, P. (2008). Comparison of the performance of submerged membrane bioreactor (SMBR) and submerged membrane adsorption bioreactor (SMABR). *Journal of Bioresource Technology*, 99: 1012–1017
- Harrelkas, F., Azizi, A., Yaacoubi, A., Benhammou, A. and Pons, M.N. (2009). *Journal of Desalination*, 235: 330-339

- Haydar, S. and Bari, A. (2009). Characterization and Study of Correlations among Major Pollution Parameters in Textile Wastewater. *Mehran University Research Journal of Engineering & Technology* Volume: 30
- Holler, S. and Trösch, W. (2001). Treatment of urban wastewater in a membrane bioreactor at high organic loading rates. *Journal of Biotechnology*, 92: 95–101
- Jiang, T., Kennedy, M.D., Meer, W.G.J., Vanrolleghem, P.A. and Schippers, C. (2003). The role of blocking and cake filtration in MBR fouling. *Journal of Desalination*, 157:335–343
- Jiang, T., Kennedy, M.D., Yoo, C.K., Nopens, I., Meer, W.V., Futselaar, H., Schippers, J.C. and Vanrolleghem, P.A. (2007). Controlling submicron particle deposition in a side-stream membrane bioreactor. A theoretical hydrodynamic modeling approach incorporating energy consumption. *Journal of Membrane Science*, 297: 141–151
- Johir, M.A.H., George, J., Vigneswaran, S., Kandasamy, J., Sathasivan, and Grasmick, A. (2012). Effect of imposed flux on fouling behavior in high rate membrane bioreactor. *Journal of Bioresource Technology*, 122: 42–49
- Juang, Y.C., Su, A., Fang, L.H., Lee, D.J. and Lai, J.Y. (2011). Fouling with aerobic granule membrane bioreactor, 64:1870–1875
- Juliastuti, S.R., Baeyens, J., Creemers, C., Bixio, D. and Lodewyckx, E. (2003). The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge. *Journal of hazardous materials*, 100: 271–283
- Kapdan, I.K. and Alparslan, S. (2005). Application of anaerobic–aerobic sequential treatment system to real textile wastewater for color and COD removal. *Journal of Enzyme and Microbial Technology*, 36: 273–279
- Khan, M.T., Takizawa, S., Lewandowski, Z., Rehman, M.H., Komatsu, K., Nelson, S.E., Kurisu, F., Camper, A.K., Katawama, H. and Ohgaki, S. (2013). Combined effects of EPS and HRT enhanced biofouling on a submerged and hybrid PAC-MF membrane bioreactor. *Journal of Water Research*, 47: 747–757
- Khan, S.J., Ilyas, S. and Rehman, Z-U. (2013). Impact of nitrogen loading rates on treatment performance of domestic wastewater and fouling propensity in submerged membrane bioreactor (MBR). *Journal of Bioresource Technology*, 141: 46–49
- Khan, S.J., Visvanathan, C. and Jegatheesan, V. (2009). Prediction of membrane fouling in MBR systems using empirically estimated specific cake resistance. *Journal of Bioresource Technology*, 100: 6133-613
- Khan, S.J., Rehman, Z-U., Visvanathan, C. and Jegatheesan, V. (2012). Influence of biofilm carriers on membrane fouling propensity in moving biofilm membrane bioreactor. *Journal of Bioresource Technology*, 113: 161–164

- Khan, S.J., Visvanathan, C. and Jegatheesan, V. (2010). Effect of powdered activated carbon (PAC) and cationic polymer on biofouling mitigation in hybrid MBRs. *Journal of Bioresource Technology*, 113: 165–168
- Kim, H-W., Oh, H-S., Kim, S-R., Lee, K-B., Yeon, K-M., Lee, C-H., Kim, S. and Lee, J-K. (2012). Microbial population dynamics and proteomics in membrane bioreactors with enzymatic quorum quenching
- Kim, S-R., Oh, H-S., Jo, S-J., Yeon, K-M., Lee, C-H., Lim, D-J., Lee C-H. and Lee J-K. (2012). Biofouling Control with Bead-Entrapped Quorum Quenching Bacteria in Membrane Bioreactors
- Kim, M. and Nakhla, G. (2009). Comparative studies on membrane fouling between two membrane-based biological nutrient removal systems. *Journal of Membrane Science*, 331: 91–99
- Kong, H. and Wu, H. (2008). Pretreatment of textile dyeing wastewater using an anoxic baffled reactor. *Journal of Bioresource Technology*, 99: 7886–7891
- Krzeminski, R.V.P., Dierdonck, J.V., Gins, G., Ferreira, M.L., Impe, J.F.M., Graaf, J.H.J.M., Smets, I.Y. and Lier, J.B. (2011). Activated sludge characteristics affecting sludge filterability in municipal and industrial MBRs: Unraveling correlations using multi-component regression analysis. *Journal of membrane science*, 378: 330–338
- Le-Clech, P., Jefferson, B. and Judd, S.J. (2003). Impact of aeration, solids concentration and membrane characteristics on the hydraulic performance of a membrane bioreactor. *Journal of membrane science*, 218:117-129
- Leiknes, T. and Ødegaard, H. (2007). The development of a biofilm membrane bioreactor. *Journal of Desalination*, 202: 135–14
- Li, X. and Wang, X. (2006). Modeling of membrane fouling in a submerged membrane bioreactor. *Journal of Membrane Science*, 278: 151–161
- Liakou, S., Kornaros, M. and Lyberatos, G. (1997). Pretreatment of azo dyes using ozone *Journal of Water Science and Technology*, 36: 155–163
- Liu, Q., Wang, X.C., Liu, Y., Yuan, H. and Du, Y. (2010). Performance of a hybrid membrane bioreactor in municipal wastewater treatment. *Journal of Desalination*, 258: 143–147
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent, 193: 265–275
- Meliß, M., Neskakis, A., Marliani, J-P., Lange, C., Hövelmann, A. and Schumacher, J. (1998). Waste water recycling supplied by renewable energies - basic conditions and possible treatment technologies. *Journal of Renewable Energy*, 14: 325-331

- Meng, F., Zhang, H., Yang, F., Li, Y., Xiao, J. and Zhang, X. (2006). Effect of filamentous bacteria on membrane fouling in submerged membrane bioreactor, 272: 161–168
- Meng, F. and Yang, F. (2007). Fouling mechanisms of deflocculated sludge, normal sludge, and bulking sludge in membrane bioreactor. *Journal of Membrane Science*, 305: 48–56
- Meng, J-Q., Chen, C-L., Huang, L-P., Du, Q-U. and Zhang, Y.F. (2011). Surface modification of PVDF membrane via AGET ATRP directly from the membrane surface. *Journal of Applied Surface Science*, 257: 6282–6290
- Metzger, U., Le-Clech, P., Stuetz, R.M., Frimmel, F.H. and Chen, V. (2007). Characterization of polymeric fouling in membrane bioreactors and the effect of different filtration modes. *Journal of membrane science*, 301: 180–189
- Mezohegyi, G., Goncalves, F., Orfao, J.M., Fabregat, Fortuni, A., Font, J., Brengoa, C. and Stuber, F. (2010). Tailored activated carbons as catalysts in biodecolourisation of textile azo dyes, 94:179-185
- Muga, H.E. and Mihelcic, J.R. (2008). Sustainability of wastewater treatment technologies. *Journal of Environmental Management*, 88: 437–447
- Naim, R., Ismail, A.F. and Mansourizadeh, A. (2012). Preparation of microporous PVDF hollow fiber membrane contactors for CO₂ stripping from diethanolamine solution. *Journal of Membrane Science*, 392-393: 29–37
- Naveed, S. and Bhatti, I. (2006). *Journal of Research (Science)*, Bahauddin Zakariya University, Multan, Pakistan , 17: 155-164
- Neill, C. O., Hawkes, F.R., Hawkes, D.L., Esteves, S. and Wilcox, S.J. (2000). Anaerobic–aerobic biotreatment of simulated textile effluent containing varied ratios of starch and azo dye. *Journal of Water Research*, 34: 2355–2361
- Noyes, E. R. (1993). *Pollution Prevention and Technology Handbook*. New Jersey: Noyes Publications.
- Poostchi, A.A., Rezvani, F.A. and Sarrafzadeh, M.H. (2012). Low-cost monofilament mesh filter used in membrane bioreactor process: Filtration characteristics and resistance analysis. *Journal of Desalination*, 286: 429–435
- Ren, N., Chen, Z., Wang, A. and Hu, D. (2005). Removal of organic pollutants and analysis of MLSS–COD removal relationship at different HRTs in a submerged membrane bioreactor. *Journal of International Biodeterioration & Biodegradation*, 55: 279–284
- Sabrina, N., Mutamim, A., Zainon, Z., Ariffin, N.M., Hassan, A. and Olsson, G. (2012). Application of membrane bioreactor technology in treating high strength industrial wastewater: a performance review . *Journal of Desalination*, 305: 1-11

- Sarioglu, M., Insel, G. and Orhon, D. (2012). Dynamic in-series resistance modeling and analysis of a submerged membrane bioreactor using a novel filtration mode. *Journal of Desalination*, 285: 285–294
- Satyawali, Y. and Balakrishnan, M. (2009). Effect of PAC addition on sludge properties in an MBR treating high strength wastewater. *Journal of Water Research*, 43: 1577–1588
- Selçuk, H., Eremektar, G. and Meriç, S. (2006). The effect of pre-ozone oxidation on acute toxicity and inert soluble COD fractions of a textile finishing industry wastewater. *Journal of Hazardous Materials*, 137: 254–260
- Shaikh, M.A. (2007). *Water conservation in textile industry*, Pakistan.
- Snowden-Swan, L. (1995). *Industrial Pollution Prevention Handbook*. Harry M. Freeman Ed. McGraw Hill. New York.
- Sosa, D.M., Helmreich, B., Netter, T., Paris, S., Bischof, F. and Harald, H. (2011). Anaerobic submerged membrane bioreactor (AnSMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. *Journal of Bioresource Technology*, 102: 10377–10385
- Spagni, A., Grilli, S., Casu, S. and Mattioli, D. (2010). Treatment of a simulated textile wastewater containing the azo-dye reactive orange 16 in an anaerobic-biofilm anoxic–aerobic membrane bioreactor *Journal of International Biodeterioration and Biodegradation*, 64:676-681
- Standard Methods for the Examination of Water and Wastewater APHA, 2005
- Sukitpaneent, P. and Chung, T.S. (2011). Molecular design of the morphology and pore size of PVDF hollow fiber membranes for ethanol–water separation employing the modified pore-flow concept. *Journal of Membrane Science*, 374: 67–81
- Tay, J.H., Yang, P., Zhuang, Q., Tay, S.T.L. and Pan, Z.H. (2007). Reactor performance and membrane filtration in aerobic granular sludge membrane bioreactor. *Journal of Membrane Science*, 304: 24–32
- Terasaka, K., Hirabayashi, A., Nishino, A., Fujioka, S. and Kobayashi, D. (2011). Development of microbubble aerator for waste water treatment using aerobic activated sludge. *Journal of Chemical Engineering Science*, 14: 3172–3179
- Türgay, O., Ersöz, G., Atalay, S., Forss, J. and Welander, U. (2011). The treatment of azo dyes found in textile industry wastewater by anaerobic biological method and chemical oxidation, 79: 26–33
- Valh, J.V., Marechal, A.M.L., Vajnhandl, S., Jerič, T. and Šimon, E. (2011). Water in the Textile Industry. *Journal of Earth system and Environmental Sciences*, 4: 685–706

- Wen, G., Ma, J., Zhang, L. and Yu, G. (2010). Membrane Bioreactor in Water Treatment Journal of Comprehensive Membrane Science and Engineering , 4: 195–209
- Wingender, J., Strathmann, M., Rode, A., Leis, A. and Flemming, H-C. (2001). Isolation and biochemical characterization of extracellular polymeric substances from *Pseudomonas aeruginosa*. Journal of Methods in Enzymology, 336: 302–314
- Wu, J., Le-Clech, P., Stuetz, R.M., Fane, A.G. and Chen, V. (2008). Effects of relaxation and backwashing conditions on fouling in membrane bioreactor, 324:26-32
- Xing, C.H., Tardieu, E., Qian, Y. and Wen, X.H. (2008). Ultrafiltration membrane bioreactor for urban wastewater reclamation. Journal of Membrane Science, 177: 73–85
- Xing, W., Ngo, H.H., Guo, W.S., Listowski, A. and Cullum, P. (2011). Evaluation of an integrated sponge – Granular activated carbon fluidized bed bioreactor for treating primary treated sewage effluent. Journal of Bioresource Technology, 102: 5448–5453
- Xuan, W., Bin, Z., Zhiqiang, S., Zhigang, Q., Zhaoli, C., Min, J., Junwen, L. and Jingfeng, W. (2010). The EPS characteristics of sludge in an aerobic granule membrane bioreactor. Journal of Bioresource Technology., 101: 8046–8050
- Yang, S., Yang, F., Fu, Z. and Lei, R. (2009). Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal. Journal of Bioresource Technology, 100: 2369–2374
- Yang, Q-Y., Yang, T., Wang, H-J. and Liu, K-Q. (2009). Filtration characteristics of activated sludge in hybrid membrane bioreactor with porous suspended carriers (HMBR). Journal of Desalination, 249: 507–514
- Yang, S., Yang, F., Fu, Z., Wang, T. and Lei, R. (2010). Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment. Journal of Hazardous Materials, 1 75: 551–557
- Ying, Z. and Ping, G. (2006). Effect of powdered activated carbon dosage on retarding membrane fouling in MBR. Journal of separation and purification technology, 52:154–160
- Yuliwati, E., Ismail, A.F., Matsuura, T., Kassim, M.A. and Abdullah, M.S. (2011). Effect of modified PVDF hollow fiber submerged ultrafiltration membrane for refinery wastewater treatment. Journal of Desalination, 283: 214–22
- Zhang, X., Wang, Y., You, Y., Jianghua, H. and XinxinXu (2012). Preparation, performance and adsorption activity of TiO₂nanoparticles entrapped PVDF hybrid membranes. Journal of Applied Surface Science, 263: 660–665

APPENDIX A

H-MBR and SBRs Setup

Hydraulic Retention Time (HRT) Design

Volume of reactors = 16 L

Flow rate of H-MBR = 25mL/min

Peristaltic pump relaxation time = 2min

Peristaltic pump operating time = 10min

So one operational hour = 50min

Flow rate = 25×50

1.25L/hr

HRT = V/Q

$16/1.25$

= 12.8hrs (HRT was maintained 12hrs for all reactors)

Sludge Retention Time (SRT) Design

Volume of reactors = 16 L

Sludge wasted per day = 530ml

= 0.53L, SRT = Volume of reactor/sludge wasted

$16/0.53 = 30$ days

APPENDIX B

Protocols

Chemical Oxygen Demand (COD)

For COD analysis Distilled water is taken as blank and samples are collected from S.G reactor and effluent, A. G reactor and effluent and Mechanical Mixer reactor and effluent While, the influent is diluted five times for the study

Sample Preparation:

Table 1.1

| Digestion Vessel | Sample <i>mL</i> | Digestion Solution (K ₂ Cr ₂ O ₇) <i>mL</i> | Sulfuric Acid Reagent <i>mL</i> | Total Final Volume <i>mL</i> |
|---------------------------------|---------------------|---|------------------------------------|---------------------------------|
| Standard 10-mL Ampules/vials | 2.50 | 1.50 | 3.5 | 7.5 |

Procedure

Determination of COD

- Wash culture tubes and caps with 20% H₂SO₄ before first use to prevent contamination.
- Oven dry the tubes in pre-heated oven at 150°C for 1 hr, don't put the caps in oven.
- Take tubes out of oven and let them stay to cool.
- Refer to Table 1.1 for proper sample and reagent volumes.

- Tightly cap tubes or seal ampules, and mix completely.
- Place ampules (10 ml vials) in COD reactor preheated to 150°C and reflux for 2 h behind a protective shield.
- Cool to room temperature and place vessels in test tube rack.
- Remove culture tube caps and transfer contents to a larger container (flask) for titration and add small TFE-covered magnetic stirring bar.
- Add 0.05 to 0.10 mL (1 to 2 drops) ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10N FAS.
- The end point is a sharp color change from blue-green to reddish brown.
- In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

Calculation

$$\text{COD (mg/L)} = (A-B) (N) \times 8,000 / \text{sample volume (mL)}$$

Where:

A = mL FAS used for blank,

B = mL FAS used for sample,

M = molarity of FAS, and

8000 = milliequivalent weight of oxygen × 1000 mL/L.

CAUTION:

- Wear face shield and protect hands from heat produced when contents of vessels are mixed. Mix thoroughly before applying heat to prevent local heating of vessel bottom and possible explosive reaction.
- These sealed vessels may be under pressure from gases generated during digestion so wear face and hand protection when handling. If sulfuric acid is

omitted or reduced in concentration, very high and dangerous pressures will be generated at 150°C.

MLSS (Mixed Liquor Suspended Solids)

Procedure

Preparation of filter paper

- Warm-up drying oven and adjust the temperature to 103⁰ - 105⁰C.
- Heat GFC filter paper in a clean evaporating dish in the oven for 1 hour at 103⁰ - 105⁰C.
- Place the dish in a desiccator to cool.
- Weigh the dish on balance and note the weight.

Determination of MLSS

- Assemble filtering apparatus and place filter paper on filter disc with wrinkled side up and begin suction.
- Wet filter with a small volume of reagent-grade water to seat it
- Pipet a measured volume onto the seated glass-fiber filter.
- Carefully remove filter from filtration apparatus and transfer to china dish and put it in oven and dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh.

Calculation

$$MLSS \frac{mg}{L} = \frac{(A - B) \times 1000}{Sample Volume, mL}$$

where:

A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

MLVSS (Mixed Liquor Volatile Suspended Samples)

- After weighing for MLSS Place the filter paper with residue in Muffle furnace at 550°C for 15 minutes
- Allow it to cool and weigh

Calculation

$$MLVSS \text{ mg/l} = \frac{(A - X) \times 1000}{\text{Sample volume, ml}}$$

Sample volume, ml

Where:

A = weight of filter + dried residue, mg, and

X = weight of the filter paper + residue after muffle furnace treatment

Membrane cleaning

Base cleaning

1. Disconnect the suction line and remove the membrane unit from the aeration tank.
2. Wash the inside and outside of the membrane unit with tap water to remove activated sludge.

3. Immerse the membrane unit completely in a chemical cleaning tank containing 4%wt/vol aqueous sodium hydroxide and sodium hypochlorite (effective chlorine concentration= 3,000mg/L).
4. Keep the membrane in solution for about 6 hours.
5. After 6 hours, filter chemical solution from the membrane for 30 minutes.
6. Remove the unit from the cleaning tank and rinse thoroughly with water to remove chemicals.
7. Immerse again the membrane in distilled water and filter in for next 30 minutes.
8. Return the membrane to aeration tank.
9. For first 30 minutes, operate at a half flux of standard.
10. After that return to the standard flux and resume operation.
11. Discard the used chemical solution after chemically reducing the solution.

Cleaning with acid

1. If the trans-membrane pressure is not sufficiently recovered by the ordinary chemical cleaning methods, cleaning with acid may be effective for restoring TMP.
2. Rinse thoroughly with tap water after 6 hours immersion in the basic solution.
3. Immerse the membrane unit completely in another chemical cleaning tank containing hydrochloric acid (0.5mol/L or 1.8%).
4. Immerse membrane in this solution for 10 hours.
5. Follow steps 6 to 11 of the above procedure.

Ammonium, Nitrite and Nitrate Nitrogen and Phosphorus determination

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N₂), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons. Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. It does not include all organic nitrogen compounds. Typical organic nitrogen concentrations vary from a few hundred micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

NO₃⁻ -N determination by Electrode method

Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as nitrogen/ L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient

Determination of nitrate (NO₃⁻) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques. The NO₃⁻

ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger.

Calibration

1. Install the Ammonium Sulfate Electrolyte Gel Cartridge in the Platinum series Combination Nitrate Electrode.
2. Connect the Combination Nitrate Electrode to the meter.
3. Prime the electrode by pushing the dispenser button until gel comes out of the reference junction. Rinse excess gel from the tip and the outlet.
4. In 100mL beakers, prepare three 50mL standard solutions of 1, 10, 100 mg/L NO_3^- -N. Use a TenSette Pipet to pipet 25mL of standard into a beaker. Pipet 25mL of liquid Ionic Strength Adjuster (ISA) into the beaker.
5. Turn on the meter by pressing I/O. Press **SETUP**. Press **ENTER** until **BNC** appears. Press **EXIT**.
6. Press the **ISE mV** key until the display shows concentration units.
7. Press **CAL**. The display will show **CAL?**, concentration units, and the active keys. The units will be flashing. Use the arrow keys to select mg/L and press **ENTER**.
8. Add stirrer bar to each beaker. Place the 1.0 mg/L standard on an electromagnetic stirrer and stir at a moderate rate.
9. Place the electrode in the 1.0 mg/L standard.
10. The display will show: **Standard 1?** And the value from the previous calibration. Press **ENTER** to accept the numerical value or use the number pad to edit the display to read 1 mg/L and press **ENTER**.
11. The display shows **stabilizing...** until the reading is stable

12. The display will show: **standard 2?**. Remove the electrode from the beaker, rinse and blot dry.
13. Place the beaker containing the 10mg/L standard on stirrer. Place the electrode in the standard.
14. Use the number pad to edit the display to read 10.0 mg/L. Press **ENTER** after the correct concentration is displayed.
15. The display will show **standard 3?**. Remove the electrode from the beaker, rinse, and blot dry. Place the beaker containing the 100 mg/L standard on stirrer. Place the electrode in the standard.
16. Use the number pad to edit the display to read 100.0 mg/L. Press **ENTER** after correct concentration is displayed.
17. After the third calibration point is entered and the display reads **Standard 4?** Press **EXIT**.
18. **Store?** Will be displayed. Press **ENTER** to accept the calibration or **EXIT** to leave the calibration mode without storing the calibration values.
19. Press **review**. Use the up arrow key to scroll to the second slope value. the slope should be -58 ± 3 mV/decade. Press **EXIT** to return to measurement mode.
20. Remove the electrode from the last standard, rinse with deionized water, and blot dry with a paper towel.

Measurement

1. Accurately measure 25 mL of sample into a 100mL beaker.
2. Pipet 25mL of liquid ISA into the beaker with the sample.
3. Add stir bar to the sample. Place the sample on a stirrer and stir at a moderate rate. Place the electrode into the sample.
4. The display will show **Stabilizing...**until reading is stable. Record or store this value.
5. Remove the electrode from the sample after reading and store the electrode in a nitrate standard of concentration similar to samples to be analyzed during next use.

NH_4^+ -N determination

Ammonia is present naturally in surface and wastewaters. Its concentration generally is low in ground waters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to form a combined chlorine residual. Ammonia concentrations encountered in water vary from less than 10 μg ammonia nitrogen/L in some natural surface and ground waters to more than 30 mg/L in some wastewaters.

Preliminary Distillation Step

General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be used.

Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

Reagents

a. Ammonia-free water: Prepare by ion-exchange or distillation methods: We will use the distillation method

Distillation—Eliminate traces of ammonia in distilled water by adding 0.1mL conc. H₂SO₄ to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/ L and redistill after standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water. Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

b. Borate buffer solution: Add 88mL 0.1N NaOH solution to 500 mL approximately 0.025M sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$) solution (9.5 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ /L) and dilute to 1 L.

c. Sodium hydroxide, 6N.

d. Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500mL sample.

e. Neutralization agent.

1) Sodium hydroxide, NaOH, 1N.

2) Sulfuric acid, H_2SO_4 , 1N.

f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in water and dilute to 1 L.

g. Indicating boric acid solution: First prepare mixed indicator solution by dissolving 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Now for indicating boric acid solution dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

h. Sulfuric acid, 0.04N: Dilute 1.0 mL conc H_2SO_4 to 1 L.

Procedure

a. Preparation of equipment: Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6N NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. Sample preparation: Use 500 mL dechlorinated sample or a known portion diluted to 500 mL with water. When $\text{NH}_3\text{-N}$ concentration is less than $100\mu\text{g/L}$, use a sample volume of 1000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter. Add 25 mL borate buffer solution and adjust to pH 9.5 with 6N NaOH using a pH meter.

c. Distillation: To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04N H_2SO_4 for the ammonia-selective electrode method and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that the end of the delivery tube is free of contact with the liquid and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water. When the phenate method is used for determining $\text{NH}_3\text{-N}$, neutralize distillate with 1N NaOH solution.

d. Ammonia determination: Determine ammonia by the titrimetric method.

Titrimetric Method

The titrimetric method is used only on samples that have been carried through preliminary distillation. The following table is useful in selecting sample volume for the distillation and titration method.

| Ammonia Nitrogen in Sample <i>mg/L</i> | Sample Volume <i>mL</i> |
|--|-----------------------------------|
| 5–10 | 250 |
| 10–20 | 100 |
| 20–50 | 50.0 |
| 50–100 | 25.0 |

Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

Reagents

Use ammonia-free water in making all reagents and dilutions.

a. Mixed indicator solution: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

b. Indicating boric acid solution: Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

c. Standard sulfuric acid titrant, 0.02N: Prepare and standardize as directed in Alkalinity, Dilute 200.00 mL 0.1000N standard acid to 1000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05N Na_2CO_3 according to the procedure of ¶ 3b; 1 mL = 1.00 mg CaCO_3 . For greatest accuracy, standardize titrant against an amount of Na_2CO_3 that has been incorporated in the indicating boric acid solution to reproduce the actual conditions of sample titration; 1.00 mL = $14 \times \text{normality} \times 1000 \mu\text{g N}$. (For 0.02N, 1.00 mL = 280 $\mu\text{g N}$.)

Procedure

1. Proceed as described in distillation step using indicating boric acid solution as absorbent for the distillate.
2. Titrate ammonia in distillate with standard 0.02N H_2SO_4 titrant until indicator turns a pale lavender.
3. *Blank:* Carry a blank through all steps of the procedure and apply the necessary correction to the results.

Calculation

a. Liquid samples: $\text{mg NH}_4\text{-N/L} = (A-B) \times 280/\text{ml of sample}$

where:

A = volume of H_2SO_4 titrated for sample, mL, and

B = volume of H_2SO_4 titrated for blank, mL.

$\text{NO}_2\text{-N}$ Determination

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines ($\text{RR}'\text{NH}$) to form nitrosamines ($\text{RR}'\text{N-NO}$), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Colorimetric Method

1. General Discussion

Nitrite (NO_2^-) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 $\mu\text{g NO}_2^-$ /L. Photometric measurements can be made in the range 5 to 50 $\mu\text{g N/L}$ if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 $\mu\text{g N/L}$ with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

Remove suspended solids by filtration.

2. Apparatus

Colorimetric equipment: One of the following is required:

- a. *Spectrophotometer*, for use at 543 nm, providing a light path of 1 cm or longer.
- b. *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

3. Reagents

a. *Nitrite-free water:* If it is not known that the distilled or demineralized water is free from NO_2^- , use either of the following procedures to prepare nitrite-free water:

1. Add to 1 L distilled water one small crystal each of KMnO_4 and either $\text{Ba}(\text{OH})_2$ or $\text{Ca}(\text{OH})_2$. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 mL of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent indicates the presence of permanganate.
2. Add 1 mL conc H_2SO_4 and 0.2 mL MnSO_4 solution (36.4 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}/100$ mL distilled water) to each 1 L distilled water, and make pink with 1 to 3 mL KMnO_4 solution (400 mg KMnO_4/L distilled water). Redistill as described in the preceding paragraph. Use nitrite-free water in making all reagents and dilutions.

b. *Color reagent:* To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

c. Sodium oxalate, 0.025M (0.05N): Dissolve 3.350 g $\text{Na}_2\text{C}_2\text{O}_4$, primary standard grade, in water and dilute to 1000 mL.

d. Ferrous ammonium sulfate, 0.05M (0.05N): Dissolve 19.607 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize it against standard $\text{K}_2\text{Cr}_2\text{O}_7$. Dilute 25.00 mL standard $\text{K}_2\text{Cr}_2\text{O}_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Molarity of FAS solution.

= (Volume 0.04167M $\text{K}_2\text{Cr}_2\text{O}_7$ solution titrated (ml)/Volume of FAS used in titration) x 0.2500

e. Stock nitrite solution: Commercial reagent-grade NaNO_2 assays at less than 99%. Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine NaNO_2 content, add a known excess of standard 0.01M (0.05N) KMnO_4 solution, discharge permanganate color with a known quantity of standard reductant such as 0.025M $\text{Na}_2\text{C}_2\text{O}_4$ or 0.05M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, and back-titrate with standard permanganate solution.

1. Preparation of stock solution—Dissolve 1.232 g NaNO_2 in water and dilute to 1000 mL; 1.00 mL = 250 μg N. Preserve with 1 mL CHCl_3 .
2. Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.01M (0.05N) KMnO_4 , 5 mL conc H_2SO_4 , and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while adding stock NO_2^- solution. Shake gently and warm to 70 to 80°C on a hot plate.

Discharge permanganate color by adding sufficient 10-mL portions of standard 0.025M Na₂C₂O₄. Titrate excess Na₂C₂O₄ with 0.01M (0.05N) KMnO₄ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

If standard 0.05M ferrous ammonium sulfate solution is substituted for Na₂C₂O₄, omit heating and extend reaction period between KMnO₄ and Fe²⁺ to 5 min before making final KMnO₄ titration. Calculate NO₂⁻-N content of stock solution by the following equation: where:

$$A = (B \times C) - (D - E) \times 7/F$$

A = mg NO₂⁻-N/mL in stock NaNO₂ solution,

B = total mL standard KMnO₄ used,

C = normality of standard KMnO₄,

D = total mL standard reductant added,

E = normality of standard reductant, and

F = mL stock NaNO₂ solution taken for titration.

Each 1.00 mL 0.01M (0.05N) KMnO₄ consumed by the NaNO₂ solution corresponds to 1750 µg NO₂⁻-N.

f. Intermediate nitrite solution: Calculate the volume, *G*, of stock NO₂⁻ solution required for the intermediate NO₂⁻ solution from $G = 12.5/A$. Dilute the volume *G* (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 µg N. Prepare daily.

g. Standard nitrite solution: Dilute 10.00 mL intermediate NO₂⁻ solution to 1000 mL with water; 1.00 mL = 0.500 µg N. Prepare daily.

h. Standard potassium permanganate titrant, 0.01M (0.05N): Dissolve 1.6 g KMnO_4 in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure: Weigh to the nearest 0.1 mg several 100- to 200-mg samples of anhydrous $\text{Na}_2\text{C}_2\text{O}_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10 mL 1 + 1 H_2SO_4 and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on distilled water and H_2SO_4 . where:

$$\text{Normality of } \text{KMnO}_4 = \frac{\text{g } \text{Na}_2\text{C}_2\text{O}_4}{(\text{A} - \text{B})} \times 0.35505$$

A = mL titrant for sample and

B = mL titrant for blank.

Average the results of several titrations.

Procedure

- 1. Removal of suspended solids:** If sample contains suspended solids, filter through a 0.45- μm -pore-diam membrane filter.
- 2. Color development:** If sample pH is not between 5 and 9, adjust to that range with 1N HCl or NH_4OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

3. *Photometric measurement:* Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO₂⁻-N concentrations:

| Light Path Length | NO₂⁻-N |
|--------------------------|-------------------------------------|
| <i>Cm</i> | <i>μg/L</i> |
| 1 | 2–25 |
| 5 | 2–6 |
| 10 | <2 |

Calculation

Prepare a standard curve by plotting absorbance of standards against NO₂⁻-N concentration. Compute sample concentration directly from curve.

Phosphorus, Reactive (0 to 45.0 mg/L PO₄³⁻)

Molybdovanadate Method

1. Enter the stored program number **480** for reactive phosphorus, molybdovanadate method.
2. Rotate the wavelength dial until the small display shows: **430 nm**. When the correct wavelength is dialed in, the display will quickly show: **Zero Sample** then: **mg/L PO₄³⁻ MV**.
3. Use a 25-mL graduated cylinder to fill a sample cell with 25 mL of deionized water (the blank).

4. Use another 25-mL graduated cylinder to fill a second sample cell with 25 mL of sample (the prepared sample).
5. Add 1.0 mL of Molybdovanadate Reagent to each sample cell. Swirl to mix.
Note: A yellow color will form if phosphate is present. A small amount of yellow will be present in the blank, because of the reagent.
6. Press: **SHIFT TIMER** A three-minute reaction period will begin.
7. When the timer beeps, the display will show: **mg/L PO₄³⁻ MV**. Place the blank into the cell holder. Close the light shield.
8. Press: **ZERO** The display will show: **Zeroing. . .** then: **0.0 mg/L PO₄³⁻ MV**
9. Place the prepared sample into the cell holder. Close the light shield.
10. Press: **READ** The display will show: **Reading. . .** then the result in mg/L PO₄³⁻P will be displayed.

Oxygen-Consumption Rate

1. General Discussion

This test is used to determine the oxygen consumption rate of a sample of a biological suspension such as activated sludge.

2. Apparatus

a. Oxygen-consumption rate device:

Probe with an oxygen-sensitive electrode (polarographic or galvanic), or

b. Stopwatch or other suitable timing device.

c. Thermometer to read to $\pm 0.5^{\circ}\text{C}$.

3. Procedure (Standard Method)

a. Calibration of oxygen-consumption rate device:

Calibrate the oxygen probe and meter according to the manufacturer's instructions
or

b. Volatile suspended solids determination

c. Preparation of sample: Record temperature. Increase DO concentration of sample by shaking it in a partially filled bottle or by bubbling air or oxygen through it.

d. Measurement of oxygen consumption rate:

- 1) Fill sample container to overflowing with an appropriate volume of a representative sample of the biological suspension to be tested.
- 2) If an oxygen-sensing probe is used, immediately insert it into a BOD bottle containing a magnetic stirring bar and the biological suspension. Displace enough suspension with probe to fill flared top of bottle and isolate its contents from the atmosphere. Activate probe stirring mechanism and magnetic stirrer. (NOTE: Adequate mixing is essential. For suspensions with high concentrations of suspended solids, i.e., >5000 mg/L, more vigorous mixing than that provided by the probe stirring mechanism and magnetic stirrer may be required.)
- 3) After meter reading has stabilized, record initial DO and start timing device. Record appropriate DO at time intervals of less than 1 min, depending on rate of consumption. Record data over a 15-min period or until DO becomes limiting, whichever occurs first.

Limitations

- The oxygen probe may not be accurate below 1 mg DO/L.

- Low DO (≤ 2 mg/L at the start of the test) may limit oxygen uptake by the biological suspension and will be indicated by a decreasing rate of oxygen consumption as the test progresses. Reject such data as being unrepresentative of suspension oxygen consumption rate and repeat test beginning with higher initial DO levels.
- The results of this determination are quite sensitive to temperature variations and poor precision is obtained unless replicate determinations are made at the same temperature. This determination also is sensitive to the time lag between sample collection and test initiation.

4. Calculations

Plots observed readings (DO, milligrams per liter) versus time (minutes) on arithmetic graph paper and determine the slope of the line of best fit. The slope is the oxygen consumption rate in milligrams per liter per minute.

Calculate specific oxygen consumption rate in milligrams per gram per hour as follows:

Specific oxygen consumption rate, (mg/g)/h

$$= \frac{\text{oxygen consumption rate, (mg/L)/min}}{\text{volatile suspended solids, g/L}} \times \frac{60 \text{ min}}{\text{h}}$$

SOUR by 5100 DO meter.

Calibration:

1. Place the probe in to the BOD bottle containing about 1” water.
2. On the instrument
3. Allow the probe to polarize and the temperature to stabilize for at least 15min.
4. Press the [CALIBRATE] soft key.
5. Make sure that the display readings are stable, and then press the [AUTO CAL] soft key.
6. The message “DO calibration saved” will be displayed for seconds.
7. Press [MODE] to return to main mode

Protocol:

1. On the Y5100 DO meter.
2. Press [Mode] to enter into application mode
3. Press [SOUR] soft key.
4. Press [SETUP] to change the SOUR parameters
5. Use the [UP], [DOWN], [DIGIT] and [NEXT] soft keys to set the parameters as necessary

Parameters:

Sample/Total = 1/[Enter the ratio of the sample volume to total volume]

Min time (min) = [minimum time to run the setup]

Max. Time (min) = [when the max time is reached test will stop]

Min. beginning DO (mg/L) = [enter the minimum level of DO allowed at the start of the test. If the DO falls below this level, test will not start]

Min ending DO (mg/L) = [enter the minimum level of DO allowed during the test. If the DO level falls to this level, the test will end]

Solids weight (g/L) = [Enter TSS or TVSS maximum acceptable limit= 31.999g/L]

6. After the parameters have been set, press [ENTER] to save them and return to the SOUR menu
7. Place the probe in the prepared sample and make certain no air bubbles are trapped, turn on the stirring and wait for a few seconds for the temperature readings to stabilize.
8. Press [START] to begin the SOUR measurement.
9. 5 SOUR reading can be saved by pressing [STORE] and can be reviewed again by pressing [REVIEW].
10. Pressing [SEND ON] soft key connects to computer and sends data to it.
11. Data will be sent every 15sec.
12. The instrument will display SOUR by using following formula:

$$SOUR \frac{mg}{g} /h = \frac{DO_{start} - DO_{end}}{T_{elapsed}} \times \frac{3600sec}{1hr} \times \frac{Total\ volume}{Sample\ volume} \times \frac{1}{MLSS\ or\ MLVSS}$$

EPS (Extra Polymeric Substances)

Chemicals and Reagents for EPS Extraction

Cation Exchange Resin (CER)

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

Buffer Solution

| Chemical name | Concentration | Amount in 1 L DI water |
|---|---------------|-----------------------------------|
| Na ₃ PO ₄ .12H ₂ O | 2 mM | $380 \times 2 / 1000 = 0.76$ g |
| NaH ₂ PO ₄ .2H ₂ O | 4 mM | $156 \times 4 / 1000 = 0.624$ g |
| NaCl | 9 mM | $58.5 \times 9 / 1000 = 0.5265$ g |
| KCl | 1 mM | $74.6 \times 1 / 1000 = 0.0746$ g |

Protocol for EPS Extraction

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

1. Take 50 mL sludge sample
2. Centrifuge sample at 5,000 rpm for 20min, 4°C
3. Centrifuge sample at 20,000 rpm for 20 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. Add resin 70 g/g VSS
7. Stir sample at 600 rpm for 1h, room temperature
8. Centrifuge sample at 5,000 rpm for 10min, 4°C
9. Remove CER and floc components
10. Centrifuge sample at 5,000 rpm for 20min, 4°C
11. Remove remaining floc components
12. Supernatant stored at 4°C for Bound EPS analysis

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

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

Principle

Simple sugars, oligosaccharides, polysaccharides and their derivatives give a stable orange-yellow color when treated with phenol and concentrated sulfuric acid.

Under proper conditions, the accuracy of the method is within 2%.

Chemical Reagents

5 w% Phenol solution

Sulfuric acid (H₂SO₄)

D-Glucose for standard solution

Procedure

Standardization:

1. Make all measurements in duplicate

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

Analysis: (Sample for soluble and bound EPS)

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

Measurement of Protein: Lowry method

Principle

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

Chemical Reagents

CuSO₄·5H₂O

Sodium Citrate

Na₂CO₃

NaOH

Folin-Ciocalteu phenol reagent

Bovine Serum Albumin (BSA) for standard solution

Solution A, 100 mL;

0.5 g CuSO₄·5H₂O

1 g Na₃C₆H₅O₇·2H₂O (Sodium citrate)

Solution B, 1L;

20g Na₂CO₃

4 g NaOH

Solution C, 51 mL;

1 mL solution A

50 mL solution B

Solution D, 20mL;

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

Procedure

Standardization:

1. Make all measurements in duplicate
2. Pipette 0.5 mL of BSA solution containing 0, 20, 30, 40, 50, 60, 80 and 100 mg/L of BSA into test tubes
3. Add 2.5 mL solution C
4. Thoroughly mix the solutions using vortex machine and let them stand at room temperature for 10 min
5. Add 0.25 mL Solution D and thoroughly mix again.
6. After 20 min, measure absorbance at 750 nm.
7. Prepare a calibration curve of protein (BSA) concentration (mg/L) versus absorbance.

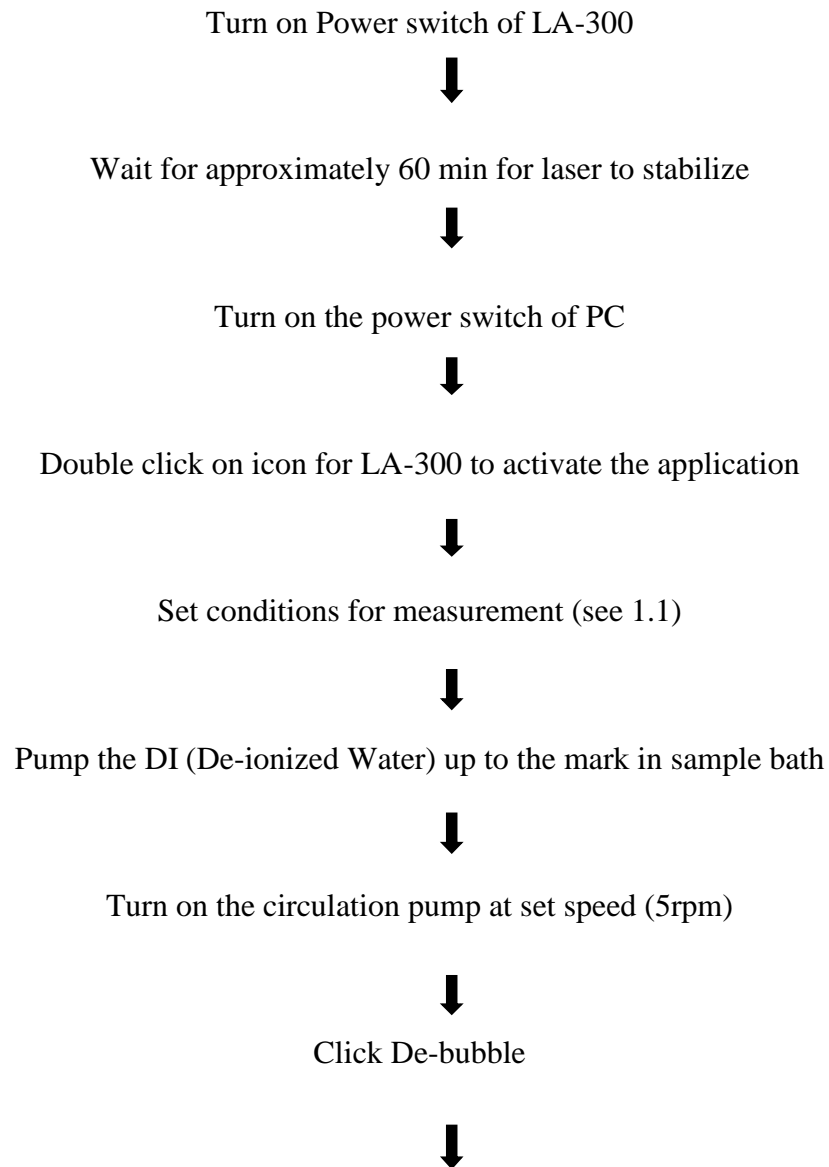
Analysis: (Sample for soluble and bound EPS)

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.

Particle Size Distribution Analysis

1. Particle size distribution analysis and measurement is an important parameter across many industries and in research. In MBR it is very important to know the floc size in order to determine the fouling behavior. To measure the particle size distribution by LA-300 Proceed as following.



Click Alignment



Click Blank



Partial Drain the sample bath



Sonicate for 10 minutes (micro lab sonicator) and Sample injection in sample bath



Sonication (for 3min)



De-bubble



Alignment



Measure

(Measurement Result graph, data table, measurement result data and calculated results are displayed as graphs or values)

Click on “**Measurement Files**” enter “File Name” and select “**Memory Location**” and “**Save**” the file

Note

*Do Initial Alignment before any measurement. Initial alignment is done 20mins after the power switch is turned on; it is done at least once a day.

1.1 System Conditions

Click system condition in conditions menu and select following

❖ Auto functions

| | |
|------------------------------|-----|
| Remarks before measurement | No |
| Automatic printing | No |
| Automatic save | No |
| Auto scale after measurement | Yes |

❖ System condition Blanking

| | |
|---------------------|----|
| Click on Blanking | |
| No. of accumulation | 10 |

❖ Measure condition

| | |
|----------------------------------|-----|
| Sampling Time | 10 |
| U-sonic works during measurement | yes |

❖ Display condition

| | |
|-----------------------------|----------|
| Select for next Measurement | |
| Forms of distribution | Standard |
| R.R Index | PSL |
| Distribution base | Number |

| | | | |
|-------------------------|-----|------|------|
| Ultrasonic | Yes | Time | 3min |
| Waiting after | No | | |
| Auto-concentration | Yes | | |
| Optical axis adjustment | Yes | | |

Screen#4

| | |
|-------------------------|----|
| “Measurement” | |
| Auto-printing after | No |
| Open Print Layout files | No |
| Automatic save | No |
| Repeat Measurement | No |

Screen#5

| | |
|-------------------|----|
| “Drain and Rinse” | |
| Drain all | No |
| Rinse all | No |

Screen#6

| | |
|----------------------------|-------|
| “Wizard File for sequence” | |
| Name of sequence | |
| Comment | |
| Finish | |

3.2.1 Specific cake resistance (α)

Batch filtration tests were performed to determine the specific cake resistance (α) of the sludge samples. The test was conducted in a 400 mL unstirred filtration cell (Model 8400, Amicon, USA) using a 0.22- μm flat-sheet cellulose membrane filter (GVWP 09050, Millipore, USA) as shown in Figure 3.6.

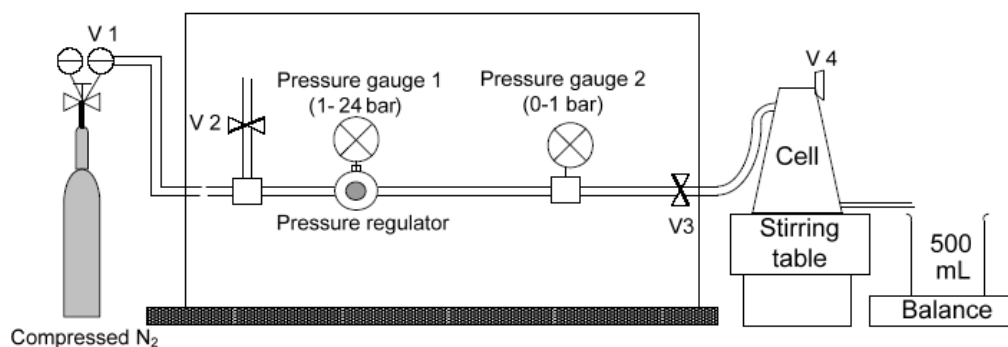


Figure 3.6: Specific cake resistance experimental setup

The cell was filled with 200 mL of mixed liquor sample and a constant pressure of 30 kPa was applied by pressurized nitrogen from a gas cylinder. The filtrate was continuously recorded by an electronic balance connected to a notebook using WINWEDGE software. The specific cake resistance (α) (m/kg) was calculated (Wang et al., 2007) by

$$\alpha = \frac{2000 A^2 \Delta P}{\mu C} \frac{t/V}{V} \quad \text{Equation 3.1}$$

where ΔP is the applied pressure (kPa), A is the filtration area (0.00418 m²), C is the MLSS concentration (kg/m³), μ is the viscosity of permeate (N-s/m²) and $[(t/V)/V]$ (s/m⁶) is the slope of the straight portion of the curve that is obtained by plotting the time of filtration to volume of filtrate (t/V) versus the filtrate volume (V).

APPENDIX C

HMBR and SBRs Effluents / Components



Dead end filtration setup



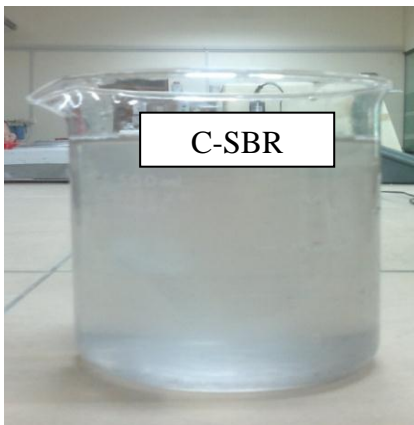
Peristaltic pump



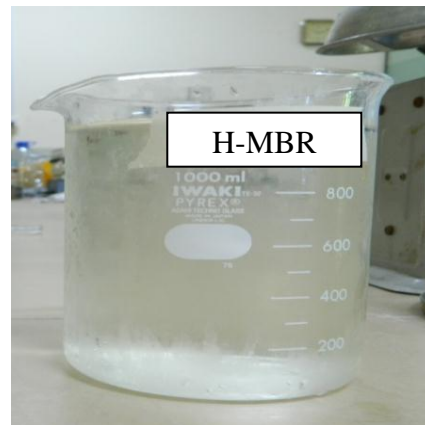
Relay control



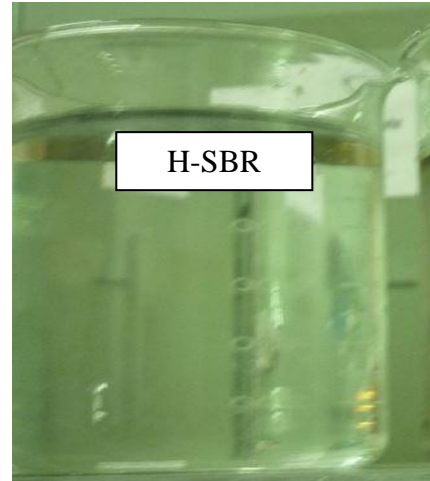
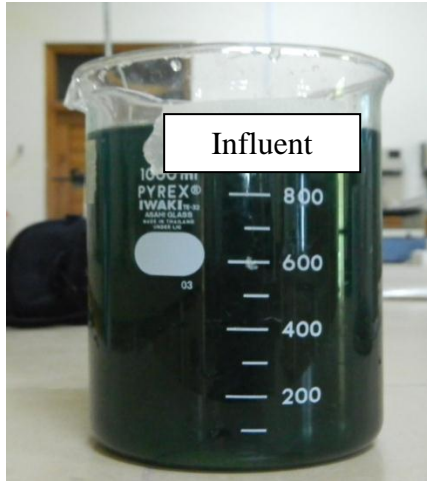
Manometer and air flow meter



C-SBR



H-MBR



Kaldness media after use



Kaldness media before use



Electricity measuring meter



Cake layer on membrane