POLISHING OF MEMBRANE BIOREACTOR EFFLUENT (MBR) USING NANOTECHNOLOY

By

SYEDA SARAH GILANI

NUST201261041MSCEE65212F

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

Master of Science

in

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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It is certified that the contents and forms of the thesis entitled

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has been found satisfactory for the requirements of the degree of
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This thesis is dedicated to my Parents

For their endless affection, support and encouragement

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Sarah Gilani

Table of Contents

LIST OF ABBREVIATIONS

$^{\bullet}O^{-}_{2}$	Oxygen Radical
$^{\bullet}OH$	Hydroxyl Radical
e-	Electron
EDS	Energy Dispersive Spectroscopy
EPA	Environmental Protection Agency
eV	Electron Volt
GPR	General Purpose Reagent
h^{+}	Hole
OH^{-}	Hydroxyl Ion
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
TiO_2	Titanium Dioxide
UV	Ultra Violet
WHO	World Health Organization
XRD	X - Ray Diffraction
Λ	Wavelength

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Membrane bioreactor (MBR) is one of the advanced wastewater treatments; however, the MBR effluent has high microbial count. Bacterial investigation was done in MBR effluent among microbes in effluent, *Enterobacteriaceae* are most prominent. Hence gram negative bacteria were prominent, further investigation was done using analytical profile index (API) kit. The dominant species found were *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Pseudomonas fluorescens/putida*, *Aeromonashydrophila/caviae/sobria*, *Raoultellaplanticola* and *Serratia odorifera.* Two model bacteria *Pseudomonas aeruginosa* and *Pseudomonas putida* were selected and their disinfection was studied by photocatylsis of TiO_2 nanoparticles. Acrylic setup was designed to maintain the flow of effluent. The TiO_2nanoparticles were prepared by liquid impregnation method and characterized by SEM, XRD and EDS. The photocatalysis of MBR effluent was studied under visible and sunlight. The results showed that if 5 liters of membrane bioreactor effluent is allowed to pass through the setup at flow rate of 42 ml/sec then 7 log reductions is shown in bacterial colonies with 45 mins of visible light exposure; however, it takes 60mins in sunlight.

Chapter 1

Introduction

1.1 Background

Water is a resource for human civilization and most essential substance for all life on earth. One of the basic humanitarian goal and major global challenge is reliable access to clean and affordable water.

As indicated by WHO 780 million individuals need access to enhanced drinking water sources. Our momentum water supply confronts enormous difficulties such as the progressively stringent water quality guidelines, exacerbated by developing contaminants. These difficulties brought about another take a gander at the current water treatment and appropriation frameworks broadly settled in developed nations. The quickly developing worldwide populace and the change of expectations for everyday comforts constantly drive up the interest. Besides, worldwide environmental change highlights the effectively uneven conveyance of crisp water, destabilizing the supply. Because of developing weight on water supplies, capricious water sources (e.g., tempest water, polluted new water, bitter water, wastewater and seawater) are turning into another standard.

1.2 Membrane Bioreactor

Wastewater treatment is becoming a necessity with increase in use of water and one of the most advanced wastewater treatment techniques involves the use of Membrane Bioreactors (MBR). It is a combination of activated sludge treatment and filtration. Microbes involved in the digestion of organic waste in MBR are bacteria, algae, fungi and protozoa, but bacteria make up

95% of all microorganisms. A variety of bacteria is involved in wastewater treatment that can be classified as filamentous bacteria, methanogenic bacteria, and polyphosphate-accumulating bacteria, nitrifying and denitrifying bacteria. Most of the bacteria are gram negative hetrotrophic rod shaped and survive in aerobic conditions like *Pseudomonas* and *Chromobacter,* etc. Some of these bacteria are pathogenic in nature and are responsible for many diseases like cholera, typhoid. Due to their presence in MBR; a large number of microbes are discharged in the final effluent. MBR effluent is discharged into streams assuming that self-purification will take care of it, but the receiving bodies are already overcharged and accumulation of more bacteria is of great environmental concern. Disinfection of MBR effluent may therefore be needed to protect the water bodies from further contamination.

1.3 Conventional Disinfection Methods

Disinfection of effluent can be done by different processes. Use of chloramines, free chlorine, ozone, UV-A and UV-C for disinfection of effluent are fairly common, but by-products formed by some of these techniques are not desirable. Many bacteria have developed resistance against these disinfection techniques and are specific in their action, targeting specific species.

Nanotechnology enabled highly efficient, modular, and multifunctional processes whichare envisaged to provide high performance, affordable water and wastewater treatment solutions (Qu *et al*., 2013).

1.4 Photo Catalytic Disinfection

Photocatalytic disinfection of wastewater, which is an emerging technology and TiO_2/UV process has been shown to successfully inactivate many microorganisms including bacteria such as *Escherichia coli*.

Titania (TiO_2) being a metal oxide and semi conductor is very effective for photocatalysis. Several methods used to improve the photocatalytic efficiency of Titania involve increase of the surface area of TiO_2 through tailoring the particle size. TiO_2 nanoparticles are extensively used for bacterial disinfection because of their photocatalytic activity under ambient temperature, high stability, low cost and no formation of secondary pollutants. TiO_2 nanoparticles, when coupled with light, can oxidize organic pollutants into nontoxic materials such as CO_2 and water, it can also disinfect bacteria.

1.5 Proposed Solution

An inexpensive approach to synthesize TiO_2 photo catalyst is developed, improving the efficiency of visible light-induced disinfection of wastewaters, and introducing a new generation of catalysts for this application. For water treatment applications, photocatalytic disinfection has been studied and implemented for drinking water production using novel reactors and solar irradiation.

In this study, the disinfection of bacteria is investigated in membrane bioreactor effluent, using titania in a lab scale setup under visible light and sunlight. *Enterobactericeae* like *Pseudomonas aeruginosa* and P*seudomonas putida* are selected as model bacteria because they are most prominent in effluent. The objective of this study was to develop a technique by couplingnanotechnology with solar light for MBR effluent treatment so that the bacterial count in the effluent should be fairly reduced before discharge.

1.6 The Present Study

The aim of this study was to polish the effluent from membrane bioreactor using nanotechnology. Based on the following:

- Characterization of microbes present in membrane bioreactor effluent
- Identification of *Enterobacteriaceae* species using API kit
- Preparation of titania nanoparticles
- Characterization of prepared titania nanoparticles
- Designing a setup and optimizing flow rate for disinfection studies
- Lab studies for disinfection of prepared culture
- Disinfection of effluent under visible and sunlight
- Monitoring of disinfection at different flow rates under visible and sunlight

Chapter 2

Literature Review

2.1 Membrane Bioreactor

To provide an advanced level removal of organic and suspended solids, conventional biological treatment processes is combined with membrane filtration and is known as membrane bioreactor. An advanced level of nutrient removal is achieved by this combination. In an MBR system, an aerated biological reactor is used to submerge the membranes. The membranes have porosities ranging between micro and ultra filtration, depending on the manufacturer the pore size may range from 0.035 microns to 0.4 microns.

High quality effluent is drawn through the membranes by this level of filtration. It also eliminates the sedimentation and filtration processes typically used for wastewater treatment. Because the need for sedimentation is eliminated, the biological process can operate at a much higher mixed liquor concentration (Kiera *et al*., 2008).

2.1.1 Advantages over Other Treatments

Compared to Extended Aeration (EA) and Sequencing Batch Reactor (SBR) systems, membrane bioreactor has several distinct advantages. One of the key advantages of MBRs are that they are much more resistant to fluctuating influent flows and provide a higher level of treatment (Duan *et al*., 2009).

The basic goal of water treatment is to removal of undesired constituents from water. Membrane provides a physical barrier for constituents depending upon their sizes, allowing use of unconventional water sources. Membrane bioreactors are the key component of water

treatmentand reuse, they require less land and chemical use, provide high level of automation, and the modular configuration allows flexible design (Qu *et al*., 2013).

2.1.2 Membrane Technology

The major challenge of the membrane technology is inherent tradeoff between membrane permeability and selectivity. There are wide applications of pressure driven membrane processes but the barrier is high energy consumption. The complexity of the process design and operation is owing to the effects of membrane fouling and high energy consumption. Furthermore, membrane fouling reduces the life of membranes and membrane modules. The membrane material greatly decides the performance of membrane. By the assimilation of functional nanomaterials into membranes, there are great opportunities to improve the membrane permeability, mechanical, fouling resistance, and thermal stability, as well as to deliver new functions for self cleaning and contaminant degradation.

2.2 Filtration

Filtration may be done at various levels namely:

1. Micro filtration
2. Ultra filtration
3. Nano filtration
4. Reverse osmosis

Micro filtration can achieve greater than 90% reduction in turbidity and its pore size ranges from 0.1 to 1 micron. Turbidity can be reduced to greater than 99% with ultrafiltration as it has a reduced pore size of 0.001 to 0.1 micron. Separation of salts and sugars from water is done by nano filtration, thus it is used for water softening. Ultrapure water for potable usage can be obtained by reverse osmosis. It can remove almost all the contaminants from water.

The operating pressure increases for microfiltration and utrafiltration because of the smaller pore size and the operational cost increases (Naveed *et al*., 2006). Usually the low pressure micro or ultra filtration is used with membrane being immersed in the aeration tank (Bhatti *et al*., 2009). Details of membrane filtration, their sizes and pathogen removal potential are given below(Allgeier, 2003).

- Micro filtration (MF) 0.1µm: Removes suspended or colloidal particles and can retain bacteria
- Ultra filtration (UF) 0.01 µm: Removes organic macro molecules and has the ability to remove viruses
- Nano Filtration (NF) 0.001 µm: Removes organic macro molecules and has the ability to remove viruses
- Reverse Osmosis (RO) 0.0001 µm: Designed to remove dissolved contaminants and remove almost everything from water

2.3 Ancillary Treatments for MBR

The prior discussion focused on the membrane system process. In addition to the membrane process, the ancillary processes are also significant, it includes:

- Primary Treatment
- Sludge Stabilization
- Sludge Dewatering & Disposal
- **Disinfection**

2.3.1 Primary Treatment

Many conventional treatment plants utilize primary clarification, screening and grit removal prior to the biological process. For MBR systems, undue wear and breakage of membrane is protected by screening. To protect the membranes a screen with less than 3 mm is generally required. The screen is designed by using rear cleaning d with multiple rakes that travel up the screen and discharge over the back, for construction of screen all non-corrosive materials are used.

2.3.2 Sludge Stabilization

An aerated sludge holding tank is used for sludge stabilization. The proposed means of disposal and biological treatment system selected decides the size and configuration of this tank. Generally warm climate is chosen for aerobic stabilization because it is the least odiferous means for stabilization.

2.3.3 Sludge Dewatering & Disposal

If disposal is to be done at a long distance from the plant then sludge dewatering system is needed, if dewatered sludge is less costly to dispose of, or if there is no means for off-site sludge disposal. Dewatering of the sludge is done by many means; however, most are expensive and requires high machinery. For smaller plants, where odors are not a major concern and land is available, the best option is dewatering bed.

2.3.4 Disinfection

Effluent sterilization is suggested after any natural treatment process, unless gushing is discarded utilizing subsurface release. For non-layer frameworks, to acquire legitimate

sterilization, gushing filtration ought to be given; generally microscopic organisms are protected by huge particles from sanitization.

Chlorine cleansing is regularly prescribed since chlorine lingering is effortlessly observed, Especially in ranges that can't routinely check effluent for microbes. Tablet chlorinators are regularly set on the gushing channel to give nonstop cleansing before release in small plants. Generally blanch or sodium hypochlorite can be conveyed through little concoction metering pump. Moreover, UV obliges "clean" power and the UV tubes oblige regular checking and cleaning so chlorine has a tendency to give a more dependable method for purification.

2.4 Need of Disinfection

Presence of bacteria and their disinfection are directly related to public health. Conventional treatment plants have a variety of microorganism that are emerging pathogens like viruses (hepatitis E and A, echoviruses, coxsackie viruses, adenoviruses, and Norwalk viruses), protozoan (Giardia and Cryptosporidium) and bacteria (*Helicobacter* and *Legionella*), (Theron *et al*., 2010). Several of these pathogens are etiologic agents linked with drinking water.

2.4.1 Microbes in Wastewater

Bacteria, protozoa, metazoa, algae and fungi are major organisms in wastewater. About 95% of all the wastewater microorganisms are bacteria in activated sludge and have important role in wastewater treatment (Gerardi, 2006).

*Pseudomonas, Chromobacter, Achromobacter, Alcaligenes*and *Flavobacterium*are Gram negative heterotrophic rod shaped in aerobic conditions commonly found in wastewater treatment plants. Coliforms are not indigenous and they are said to enter wastewater through

influent. Wastewater also contains nitrifying bacteria as well as filamentous bacteria (*Beggiatoa, Thiothrix and Sphaerotilus)*. Other types of bacteria are as follows:

- Filamentous bacteria
- Methanogenic bacteria
- Polyphosphate accumulating bacteria
- Sulfate reducing bacteria
- Nitrifying bacteria
- Denitrifying bacteria

2.5 Bacteria

Prokaryotic microorganisms lacking the nucleus characteristic of cells of higher plants and animals (eukaryotic cells) are known as bacteria. The nuclear membrane around DNA is absent, there is no separation of genetic material from the cytoplasm. Prokaryotic cells do not have differentiated cytoplasm, lacking distinguishable units for specialized functions; for example, cell membrane is used for respiration. Bacteria have thousands of different species, but most of them fall into three general morphological categories: spherical, rod or spiral. The usual size of bacteria ranges from 0.5 to 5 microns in maximum dimensions.

2.5.1 Types of Bacteria

Depending upon the ability of bacterial cell wall to be stained by the Gram stain, bacteria are divided into two major classes (Gupta, 2000): Gram-positive and Gram-negative organisms.

2.5.2 Gram-positive Bacteria

The cell wall of gram positive bacterial is mainly composed of peptidoglycan with thickness ranging from 20 - 80 nm in thickness. The thick peptidoglycan layer accounts for nearly 80% of cell wall components in certain bacteria. Other components may be 10 to 20 % of lipopolysaccharides , teichoic acids, and traces of proteins and lipids. Gram-positive bacteria are differentiated from gram negative bacteria as they take crystal violet during bacterial differentiation. The cell membrane is encased by the thick peptidoglycan which retains the stain, showing definitive identification by appearing crystal violet (Medigan, 2005).

2.5.3 Gram-negative Bacteria

Gram-negative bacteria have chemically more complex cell wall. Here only 10% of the cell wall is composed of peptidoglycan layer and it is very thin ranging from 2-6 nm in thickness. The outermost layer/ outer membrane of Gram-negative bacteria, is about 6 to 18 nm thick and accounts for the rest of the cell wall. It mainly consists of lipopolysaccharides, phospholipids, and lipoproteins. The outer membrane long with peptidoglcan provides mechanical protection to cell morphology and sensitivity to phage infection, similar to the functions of Gram-positive cells.

Permeability of many moderate or large size molecules is also influenced by cell wall of bacteria. The crystal violet stain used in bacterial differentiation during gram staining, is not retained by gram negative bacteria . The thin peptidoglycan layer of their cell wall is sandwiched between an inner cell membrane and a bacterial outer membrane (Medigan, 2005).

2.5.4 Resistance of Bacteria

The hardware for the oxidative phosphorylation and electron-transport responses is available in procaryotic cell layer. With a specific end goal to couple the free vitality change to ATP union, the proteins and electron transporters responsible for the redox responses must be effectively united on the layer. In this manner, any intrusion to the cell layer structure will diminish film capability of vitality generation and will force impeding impacts on cell.

The external film has included hindrance of material availability which clarifies the explanation behind resistance of Gram-negative microscopic organisms than Gram positive microorganisms towards certain chemicals under distinctive circumstances. Cytoplasmic or plasma layer is available underneath the cell mass of all microscopic organisms, plasma memebrane speaks the truth 7.5 nm thick, it is made bilayer out of the phospholipid. For keeping up reasonability of cells, the cytoplasmic film is critical. This layer has the remarkable property of specific porousness permitting the stream of specific metabolites all through cells while barring different mixes. The cytoplasmic film contains the important chemicals for the blend, get together, and transport of cell divider segments, it likewise keeps up osmotic harmony of the bacterial cell.

The Gram-negative cells have periplasmic space , where Gram-positive cells doesn't have this space, it lies between the cell divider and cytoplasmic layer. Numerous proteins, electron arbiters and catalysts are available in this periplasmic space, it is exceptionally alterable and has gel like environment. Any vast estimated materials picking up section by means of the external layer and neglects to penetrate through the cell film are handled by hydrolytic catalysts. The cell divider and external film parts are likewise turned here, and recently shaped materials are carried through this space before coming to inside the cell. Numerous electron go betweens and compounds included in vitality yielding responses are situated in this periplasmic space. Since

this segment is in a split second influenced by an organism's outside surroundings, it assumes a basic part in protecting the cell against remote operators. Numerous different structures and materials, for example, sheaths, s-layers, sludges, and cilia are available on the outside surface of some microscopic organisms. The case or ooze layer of certain microscopic organisms is exceptionally goes about as a protecting covering to the phone divider and is gooey in nature. The put away nourishment for the life forms and expand the infective capacity for pathogenic life forms is served by case. The capacity of microscopic organisms to colonize as biofilms on strong surfaces or to connect to tidy or air channels, and go in air is additionally because of thick ooze layer. Some or these structures may be shaped because of ecological jolts and can be truant in microscopic organisms developed in a generally kind research center society. These marvels absolutely will have a noteworthy impact on their survival. Any treatment procedure would need to join suitable reactions in the outline to work adequately.

2.6 Conventional Disinfection Methods

Free chlorine, chloramines and ozone are chemical disinfectants commonly used by the water industry, they have certain drawback and they can react with an assortment of compounds in natural water to result into disinfection by-products (DBPs), many of DBPs are carcinogenic in nature.

According to Krasner *et al*., 2006, more than 600 DBPs have been reported in the literature. In 1993 Trussell stated that considering the mechanisms involved in formation of DBP , it has been predicted that DBPs will be formed any time chemical oxidants are used in water treatment. Furthermore, the resistance of some pathogens, such as Giardia and Cryptosporidium, to conventional chemical disinfectants requires extremely high disinfectant dosage, leading to aggravated DBP formation. Therefore, there is an urgent need to reconsider

the conventional disinfection methods and to reflect on innovative approaches that enhance the reliability and robustness of disinfection while avoiding DBP formation.

2.7 Potential of Nanotechnology in Disinfection

The water industry is facing a difficult challenge by the dilemma created between effective disinfection and formation of toxic disinfection by-products (DBPs).Now it's well known that toxic DBPs (e.g., Halogenated disinfection byproducts, carcinogenic nitrosamines, bromate etc.) can be formed by the conventional disinfectants like chlorine disinfectants and ozone.To reduce the production of DBPs, UV disinfection has emerged as an alternative for oxidative disinfection, but it requires proper set up and energy. These limitations urge the development of alternative methods that can enhance the robustness of disinfection while avoiding DBP formation.

Nanotechnology in disinfection and microbial control is reviewed by Li *et al*., 2008. It was found that nanomaterials such asnano-Ag, nano-ZnO, nano-TiO_2, nano-Ce_2O_4, carbon nano tubes (CNTs), and fullerenes, show antimicrobial properties without strong oxidation, which results in less DBPs formation. The antimicrobial mechanisms of these nanomaterials, their merits, limitations, and usage for water treatment, and the critical research needs have thoroughlybeen reviewed.

2.7.1 Antimicrobial Nanomaterials

Antimicrobial nanomaterials can discover their applications in three basic difficulties in water/wastewater frameworks: purification, film biofouling control, and biofilm control on other significant surfaces.

Water quality observing is the real test for water/waste water treatment. It is a direct result of the to a great degree low convergence of specific contaminants, the absence of pathogen identification and the high many-sided quality of the water/waste water lattices.

The quick improvement in nanotechnology has impelled impressive enthusiasm for the natural uses of nanomaterials. Specifically, its capability to modernize exceptionally old routine water treatment procedures has been pronounced of late (USEPA, 2007; Shannon *et al*., 2008). Nanomaterials are uncommon adsorbents, sensors and impetuses because of their extensive particular surface region and high reactivity. All the more as of late, solid antimircrobial properties have been demonstrated by a few common and designed nanomaterials including photocatalytic TiO2 (Cho *et al*., 2005), silver nanoparticles (nAg), chitosan (Qi *et al*., 2004) and carbon nanotubes (CNT). These antimicrobial nanomaterials are not solid oxidants and are moderately latent in water unlike other synthetic disinfectants. Hence they won't have the capacity to deliver destructive DBPs. On the off chance that these antimicrobial nanomaterials are legitimately utilized as a part of the treatment forms, they have the ability to just supplant or atleast improve the customary cleansing systems.

2.8 Photochemistry

Photochemistry is the study of ‘chemical change developed by the action of light’. ‘Light’ means electromagnetic radiation in the visible and ultraviolet range and ‘chemical change’ includes mostly those events that occur at the molecular level after absorption of a photon even if it’s not an overall chemical change. According to Kopecky, photochemistry involves reactions of molecules when their electrons are in ground state i.e. the lowest electronic

energy state. A molecule absorbs a photon or a quantum of light energy and goes to a higher energy state known as excited state.

A molecule in excited state is more reactive than a ground state molecule and therefore can react entirely differently following several different reaction pathways.

2.9 Photocatalysis

Photocatalytic oxidation is used to remove traces of contaminants and microbial pathogens by advanced oxidation process. It is a useful pretreatment to increase the biodegradability of hazardous and non-biodegradable contaminants. Photocatalysis is also useful for further treatment of recalcitrant organic compounds. Due to limited light influence and photocatalytic activity, the process possess slow kinetics which is a major barrier for its vast application.

2.9.1 Semiconductor Photocatalyst

Semiconductors go about as sensitizers in light instigated redox-forms because of the electronic structure of the metal molecules in synthetic mix. It is described by a filled valence band and a vacant conduction band.

The collaboration of electrons and gaps created in an initiated strong with the encompassing medium causes the photolysis by semiconductors. Actuation is the aftereffect of light ingestion. The deterioration of contaminants is then done by the electron gap matches that are framed in the strong molecule which recombine or take part in reductive and oxidation responses.

2.9.2 TiO_2 as a Semiconductor

Among many semiconductors investigated, in anatase form has become a standard in environmental applications because it proved to be titanium dioxide (TiO_2) is an efficient photocatalyst, biologically and chemically inert, resistant to photo-corrosion and chemical corrosion, inexpensive, highly photoactive, non toxic,, recyclable. It has suitable band gap (Eg = 3.2 eV) shows redox potential of the H_2O/•OH couple (-2.8 eV). Due to all the above properties, TiO_2in anatase form has become a standard in environmental applications and is a main candidate for wide applications.

2.9.3 Photocatalytic Disinfection Efficiency of TiO_2

Minimal cost of TiO2, its steady nature in water and being non-poisonous by ingestion makes it suitable for applications in water treatment. Among distinctive nanomaterialsTiO2 is the most generally utilized and the most concentrated on semiconductor photocatalyst. Countless on the photocatalytic sterilization effectiveness of TiO2 have demonstrated the capability of TiO2 for water purification. Few of these are examined here. At the point when enacted by UV-An illumination, the photocatalytic properties of TiO2have been connected in various ecological applications for the removalof contaminants from both water and air (; Murray *et al*., 2007; Gelover*et al*., 2006). Throughout the most recent 20 years, there has been an abundance of data procured on TiO2 photocatalytic inactivation of microorganisms (]. Gram positive microorganisms are less delicate due to their capacity to frame spores. TiO2 can murder both Gram-positive microscopic organisms and also Gram-negative microorganisms (Wei *et al*., 1994). Nano-sized TiO2can additionally execute infections including hepatitis B infection (Zan*et al*., 2007), poliovirus, Herpes simplex infection, and MS2 bacteriophage (Cho *et al*., 2005). The convergence of TiO2necessary to eliminate microscopic organisms ranges from 100 and 1000

ppm, contingent on the particles' measure and the power and wavelength of the light utilized (Wei *et al*., 1994). The antibacterial movement of TiO2 is connected with the ROS creation, particularly hydroxyl free radicals and peroxide which are framed under UV-A light by means of oxidative and reductive pathways, individually (Kikuchi *et al*., 1997).

Study by Gelover*et al*. (2006) uncovered that finish inactivation of fecal coliforms was refined 45 minutes before utilizing an exposing so as to star convergence of 3000 cfu/100 mL water in TiO2-covered plastic compartments to daylight. Though the same inactivation for the most part obliges 60 min with uncoated compartments. All the more vitally, it was likewise found in the same study that the microorganisms presented to TiO2photocatalytic sanitization was not able toself-repair. In any case, oblivious bacterial deathoccurred which shows that other obscure systems may be included (Adams *et al*., 2006).

Moreover, because of vicinity of little portion of UV-An in sun oriented radiation, TiO2 based sunlight based sterilization is for the most part a moderate procedure. Consequently, accomplishment in examination on metal or nitrogen doping to build noticeable light absorbance of TiO2 or UV-A movement is intense to the use of TiO2 sun oriented cleansing. It was uncovered that silver doping of TiO2significantlyenhancedphotocatalytic bacterial inactivation by UV-An actuated TiO2 (Page *et al*., 2007; Reddy *et al*., 2007). TiO2 can either be connected as a suspension in a slurry UV reactor, as a major aspect of a layer channel or a slim film coatedon a reactor surface (Benabbou*et al*., 2007; Belhacova*et al*., 1999; Kwak*et al*., 2001).

2.9.4 Combining Current Technologies with Nanotechnology

One potential use of antimicrobial nanomaterials is in cross breed procedures in blend with existing cleansing advancements. For instance, photosensitive nanomaterials can be effectively connected to UV reactors to enhance UV purification.

UV sterilization is progressively utilized for drinking water treatment because of its viability against blister framing protozoa, for example, Giardia and Cryptosporidium. In any case, some pathogenic infections, for example, adenoviruses are exceptionally impervious to UV purification, obliging high measurements (Yates *et al*., 2006). A mix of UV with photocatalytic nanomaterials (e.g.; fullerol and TiO2) that give extra inactivation instruments may have the capacity to defeat this basic boundary. It has been demonstrated that UV reactors inside covered with TiO2 can improve the cleansing rate (Sunada *et al*., 1998; Belhacova *et al*., 1999). This, in blend with its capacity to corrupt natural contaminants (Hoffmann *et al*., 1995) and additionally normal natural matter (Huang *et al*., 2008b; Murray *et al*., 2007; Eggins *et al*., 1997), makes TiO2 a standout amongst the most encouraging nanomaterials for application in expansive scale water treatment frameworks.

2.10 Mode of action of TiO_2

Anatase and rutile are the two crystalline forms of TiO_2 which shows photocatalytic activity. The band gap of anatase is 3.2 eV and 3.0 eV for rutile. The most active form is anatase. A sharp decrease in activity is shown above about 385 nm in action spectrum of anatase. The highly reactive species produced during chemical steps of photocatalytic process are responsible for causing damage to microorganisms. The formation of the following species is involved:

- Hydroxyl radical
- Hydrogen peroxide
- Superoxide
- Conduction band electron
- Valence band hole

Development of singlet oxygen on lighted TiO2 has additionally been accounted for however is not generally thought to be available under the standard states of sanitization responses. The responsive oxygen species (ROS) may disturb or harm different cell or viral capacities or structures. The prevalence of confirmation on photocatalytic science in watery arrangement proposes that the hydroxyl radical shaped by opening exchange does not diffuse from the surface of the TiO2 into mass fluid stage. Figure 2.1 demonstrates the procedure of photocatalyst activity

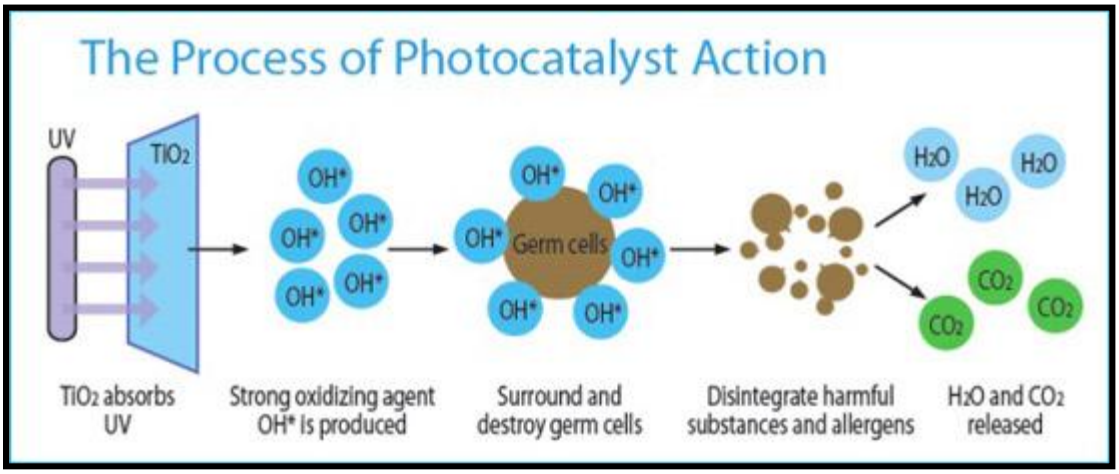

Figure 2.1: The Process of Photocatalyst Action (Je-Wen Liou *et al.*, 2012)

2.11 Nano-Photocatalyst Optimization

In water/wastewater treatments the semiconductor TiO_2 is the extensively used photocatalyst due to following properties

- Low toxicity
- Chemical stability
- Low cost
- Natural abundance

An electron/hole pair is generated in TiO_2 upon absorbing a photon, which is later either migrated to the surface and s reactive oxygen species (ROS) is formed or it undergoes undesired recombination.

By optimizing/changing particle size and shape the photoactivity of nano-TiO_2 can be readily improved, electron/hole recombination can be reduced by doping with noble metal, maximizing reactive facets, and to enhance contaminant adsorption surface treatment is also done. In solid-phase transformation, electron hole dynamics and sorption the size of particle plays vital role.

TiO_2 has three crystalline structures, among them rutile with size 35nm is the most stable for particles of larger size, where anatase is the suitable for particles smaller than 11nm and it is more capable in generating ROS (Fujishima *et al*., 2008). A main reason for the sluggish reaction kinetics of TiO_2 photocatalysis is the rapid recombination of electron and hole.

Lessening TiO_2 particle size decreases recombination rate of electron and hole, and enhances interfacial charge carrier transfer (Zhang *et al*., 1998). However, if particle size is reduced to several nanometers, surface recombination dominates, diminishing photocatalytic activity. Therefore, the photocatalytic activity of TiO_2 is maximum in the specific nanometer range due to the interplay of the aforementioned mechanisms.

2.12 Potential Applications in Water Treatment

The operational parameters of the photo reactor and design emphatically impacts general effectiveness of a photocatalytic water treatment. Slurry reactors setup and reactors utilizing immobilized TiO2 are two regularly utilized. Assorted sorts of impetus immobilization methods are being executed to expand its adequacy. Broad examination on working parameters has been completed with research facility or pilot scale frameworks. The impacts of water quality

alongside an extensive variety of working parameters including broke down oxygen, TiO2 stacking, temperature, contaminant sort and focus, pH, light wavelength and power are as of late looked into by Chong in 2010.

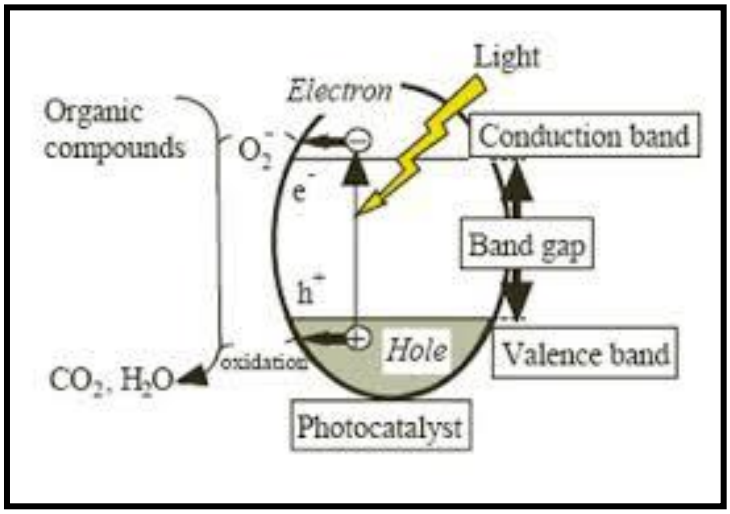

Figure 2.2: Mechanism of Photocatalysis (Amy L. Linsebiglerw *et al*., 1995)

A business item, Purifics Photo-Cat_ framework, has treatment limit as high as 2 million gallon for each day with a little foot shaped impression of 678 ft2. Pilot tests demonstrated that the Photo-Cat_ framework is profoundly effective for uprooting organics without delivering waste streams and it works with generally low particular force utilization of around 4 kWh/m3 (Al-Bastaki, 2004; Benotti *et al*., 2009;Westerhoff *et al*., 2009).

2.13 Nano-TiO_2 Facilitated Solar Disinfection

As an ease, maintainable and natural agreeable water treatment innovation, photocatalysis is known not incredible potential. Nano-TiO_2 encouraged sun based sterilization (SODIS) are generally tried and gives off an impression of being a practicable alternative to create safe savoring water remote zones of creating nations. The SODIS framework can be scaled up to medium size sun powered compound illustrative authorities or little scale for one individual.

In any case, vast scale use of nanomaterials likewise confront a few difficulties, for example,

1) Improving quantum yield or to using noticeable light for catalyst optimization;

2) Immobilization procedures;

3) Selectivity for response

TiO2 and CeO2 are metal oxide nanomaterials, their carbon nanotubes are likewise mulls over for reactant properties in heterogeneous procedures, for example, ozonation that similarly finish and quick corruption of natural toxins.

High synergist action of nanomaterials are because of substantial unmistakable surface territory and a promptly available surface, ozone disintegration into hydroxyl radicals is likewise advanced by some nanomaterials, radical-intervened courses encourage corruption process through nanomaterials (Orge *et al*., 2011)

Chapter 3

Materials and Methods

3.1 Materials and Reagents

Titanium (IV) dioxide (Sigma-Aldrich Labor chemikalien) was used to prepare TiO_2 nanoparticles by liquid impregnation method. The solvent used in this process was distilled water. Two bacteria strains *Pseudomonas aeruginosa* and *Pseudomonas putida* were purchased from Microbiologics, Inc., USA. Agar, sodium chloride, and dilution tubes, pyrex petri dishes and other glassware were of Merck grade. Ceramic tiles were placed in a setup made up of acrylic sheets of 8mm thickness.

3.2 Bacterial Studies in MBR Effluent

The bacterial study was done on effluent of membrane bioreactor (MBR) installed at Water and Wastewater lab IESE, SCEE NUST.

Figure 3.1: Membrane Bioreactor (MBR) at Water and Wastewater lab IESE

3.2.1 Collection of Effluent Samples

To check the presences of bacteria, samples were collected from MBR effluent in sterilized flasks from the membrane bioreactor installed at Water and Wastewater lab IESE, SCEE NUST (Fig 3.1).

3.2.2 Isolation of Bacteria from Effluent

Samples of MBR effluent were taken in sterilized flasks. Serial dilution technique was performed as per standard procedure. Nutrient agar plates were prepared using the standard procedure, specific for different species of bacteria. After serial dilution of the effluent samples up to 10^{-6}mL, spread plate technique was performed by pouring 0.1mL of diluted samples on nutrient agar plates and incubated for 24 hours at 37^{o}C. Colonies were marked and presence of specific bacteria was studied on different agar plates.

3.2.3 Purification of Bacteria

Maximum possible bacteria were marked based on their morphological characteristics such as shape, size and color and isolated on fresh agar plates. Selected colonies were single colony streaked (Fig 3.2). Streak plate technique was performed to isolate the colonies. Plates were incubated for 24-48 hours at 37°C. Colonies were streaked for 3-5 rounds or more till assured of having obtained a pure colony. Each pure colony was stored in the refrigerator for further use.

3.2.4 Gram Staining

Gram Staining was performed for all the isolated samples using standard procedure (*Gephardt et al.,* 1981). Gram-negative bacteria retained safranin whereas Gram-positive bacteria retained crystal violet. Spirit lamp, burner, alcohol-cleaned microscope slide and water

was used during staining. Reagents used were Crystal violet, Gram's iodine solution, decolorizing solution and safranin solution shown in Fig 3.3. Steps involved in gram staining are detailed in Annexure A.

Figure 3.2: Streak Plate Technique to Isolate Colonies

Figure 3.3: Reagents used in Gram Staining

3.2.5 Analytical Profile Index Kit

Analytical Profile Index (API) kit was used for the identification of bacterial samples. For the identification of enteric and other non-fastidious bacteria API 20E (Biomeurix, Canada) test kit was used. It consists of a plastic strip that has 20 mini cupules in it (Fig 3.4). Specific medium

for biochemical characterization is present in each cupule. A saline suspension (0.85% NaCl) was prepared and autoclaved, 24 hours fresh culture was used to perform the test.

Figure 3.4: API Kit and Reagents

Fresh colonies were used with saline solution to prepare suspension. The suspensions were added in the cupules of API strips to the end except for citrate utilization (CIT), voges–proskauer (VP) and gelatin liquefaction (GEL), where the cupule was filled completely. To avoid drying out a drop of mineral oil was added in the cupules filled to neck. The strip was covered with the lid provided and placed in incubator overnight. Color changes were noted and results were recorded (Table 3.1). Yellow color showed for the carbohydrates test indicated fermentation and acid production. However, few cupules needed with reagents, provided by the manufacturer. TDA reagent is added into TDA cupule. James/Kovacs reagent was added to IND while VP1 and VP2 were added to VP. All the reagents were provided by the manufacturer. The test was allowed to develop for a few mins and results were recorded.

To develop seven digit code required for API web software, an additional oxidase test was performed. Tryptic soy agar plates were prepared to perform this test. Colonies were grown and 1% *N*,*N*-dimethyl-*p*-phenylene diamine dihydrochloride was added. A color change to purple was noted as positive while no color change as negative result.

3.2.6 Isolation of *Entereobacteriaceae*

The *Enterobacteriaceae* are a substantial group of Gram-negative microscopic organisms that incorporates bacterial species, for example, *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella* and *Shigella*. To recognize diverse genera of *Enterobacteriaceae*, a progression of tests can be completed in the lab like; Tryptone soup, Phenylalanine agar for discovery of generation of deaminase, which changes over phenylalanine to phenylpyruvic corrosive. Catalase test on supplement agar tests for the generation of catalase protein, parts hydrogen peroxide and discharges oxygen gas. Oxidase test on supplement agar tests for the chemical's creation oxidase, which responds with a fragrant amine to deliver a purple shading. To affirm the vicinity of *Enterobacteriaceae* in MBR gushing , the microscopic organisms in emanating were permitted to develop on distinctive agars and vicinity was affirmed by investigating the shade of settlements. Formula and sorts of specific media utilized are demonstrated as a part of Table 3.2.

3.2.7 Preparation of Plates

Specific amount of each selective media (Table 3.2) is mixed in 1 liter warm distilled water with slow mixing by glass rod. After complete dissolution of agar, flasks were autoclaved for complete sterilization at 121°C for 15 mins. To avoid solidification the flasks were placed in hot water bath at 47°C. Finally, nutrient agar solution poured into petri plates (autoclaved at

121°C for 15 mins) under completely sterile laminar flow hood cabinet and allowed to cool down. To observe their sterility the prepared petri plates, after solidification were transferred into incubator (for 24 hours at 37°C).

Table 3.1: Cupule Medium of API

Cupule Medium	**Results**	
	Positive	**Negative**
O-Nitrophenyle-B-D-galactoside (ONPG)	Light yellow to yellow	Colourless
Arginine Dehydrolase (ADH)	Light to dark red	Yellow
Lysine Decarboxylase (LDC)	Light to dark red	Yellow
OnthinineDecarbolyxase (ODC)	Light to dark red	Yellow
Citrate Utilization (CIT)	Blue green to Blue	Pale green to yellow
HydregenSulfide (H_2S)	Black	Grey to colorless
Urease (URE)	Pink	Colorless/pale green/yellow
Tryptophan deaminase (TDA)	Deep red	Brown
Indole (IND)	Pink	Colorless/pale green/yellow
Voges–Proskauer (VP)	Red/Pink	Colorless/ slight pink
Gelatin liquefaction (GEL)	Goes Black (digested)	No change
Glucose (GLU)	Yellow	Yellow green, green, blue
Mannitol	Yellow	Yellow green, green, blue
Inositol	Yellow	Yellow green, green, blue
Sorbitol	Yellow	Yellow green, green, blue
Rhamnos	Yellow	Yellow green, green, blue
Sucrose	Yellow	Yellow green, green, blue
Melibiose	Yellow	Yellow green, green, blue

Amygdaline	Yellow	Yellow green, green, blue
Arabinose	Yellow	Yellow green, green, blue

Table 3.2: Selective Media used to Identify *Enterobacteriaceae*

Selective Media	Bacterial Species	Recipe
Cetrimide	*Pseudomonas aeruginosa*	4.53/100mL
Salmonella Shigella Agar	*Salmonella Shigella*	5.20/100mL
Eosin Methylene Blue	*Escherichia coli*	3.6/100mL
MacConkey agar	*Enterobacteriaceae*	5/100mL
Tryptone Soya Agar	For Oxidase Test	4/100mL

3.3 Synthesis of TiO_2 Nanoparticles

TiO_2 nanoparticles were prepared by using liquid impregnation method byfollowing steps given below:

- 20 g of TiO_2GPR was added to 100mL of deionized water
- The slurry was stirred well for 24hrs using a magnetic stirrer
- The slurry was then allowed to dry in oven at 105^0C for 12hrs
- After drying the prepared material was crushed to powder using pestlee and mortar
- The powder was allowed to calcinate in a muffle furnace for 6hrs at 450^0C in order to get anatase phase of titania

3.4 Characterization of TiO_2 Nanoparticles

3.4.1 X-ray Diffraction

X-ray Diffractometer (Theta-Theta, Store, Germany) was utilized to dissect gem structure of the TiO2 nanoparticles. Standard conditions for XRD estimations were room temperature with CuKα radiation (λ = 0.15478 nm) at 60 keV and 15mA.

Diffraction impacts are watched when electromagnetic radiation encroaches on occasional structures with geometrical varieties on the length size of the radiation's wavelength. The interatomic separations in precious stones and particles add up to 0.15–0.4nm, which relate to the electromagnetic range, with the wavelength of x-beams having photon energies somewhere around 3 and 8keV. In like manner, marvels like valuable and dangerous obstruction ought to end up recognizable when crystalline and atomic structures are presented to x-rays.

3.4.2 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was utilized for geology, concoction piece, crystalline structure portrayal of the TiO_2.

A scanning electron magnifying instrument (SEM) is a sort of electron magnifying lens that creates pictures of a specimen by filtering it with an engaged light emission. The electrons interface with particles in the specimen, delivering different signs that can be distinguished and that contain data about the example's surface geography and arrangement. The electron pillar is for the most part filtered in a raster sweep example, and the shaft's position is consolidated with the recognized sign to create a picture. SEM can accomplish determination superior to anything 1 nm. Examples may be seen in high vacuum, in low vacuum, in wet conditions, and at an extensive variety of cryogenic or raised temperatures.

3.4.3 Energy Dispersive Spectroscopy

Vitality Dispersive Spectroscopy (EDS) implanted inside JEOL JSM 6490A was utilized for distinguishing proof of the components present in the nanoparticles, and for determination of its compound structure. Portrayal of covered and uncoated earthenware tiles was additionally done utilizing the same strategies.

EDS is a systematic procedure utilized for the basic investigation or concoction portrayal of an example. It depends on a communication of some wellspring of X-beam excitation and an example. Its portrayal capacities are expected in substantial part to the major rule that every component has an one of a kind nuclear structure permitting extraordinary arrangement of crests on its X-beam discharge range.

3.5 Immobilization of TiO_2Nanoparticles on Tiles

Immobilization of Ag-TiO_2 nanoparticles was done over ceramic tiles after etching them, water based coating method was used.

3.5.1 Etching of Tiles

Tiles were carved with weaken hydrofluoric corrosive (20%v/v) for 2 hours, and washed completely with deionized water, improving an unpleasant surface for contact of TiO_2 on the surface.

3.5.2 Coating of Tiles

Immobilization Immobilization of the nanoparticles over earthenware tiles was finished by water based technique. To expel natural and inorganic materials from the substrate's surface, they were treated with $(CH_3)_2CO$ and refined water and dried under air conditions.

For immobilization of TiO2distilled water was utilized as dissolvable. TiO_2 slurry was readied with 1.5 g of TiO_2 in 200mL of deionized water, and the suspension was put in a ultrasonic shower for 15 mins for uniform scattering. Tiles were drenched in the resultant slurry of TiO_2 for 60 minutes, after which the tiles were expelled from the suspension, and put in a broiler for 2 hours at 150◦C. The tiles were then set in a heater for 5 hrs at 5000C. The covered substrates were altogether washed with twofold refined water to uproot any free TiO_2 particle

3.6 Dimensions of Setup/Batch and Flow Rate of Effluent

The setup was designed using AutoCad and shown in Figs 3.5 and 3.6. It was designed to easily retain 5 liters of water.

The set up was made up of acrylic sheet of 8 mm thickness for easy passage of light and the base was made up of ceramic tiles coated with titania nanoparticles. Two valves were attached, each at both ends for continuous flow of water and to regulate proper flow rate.

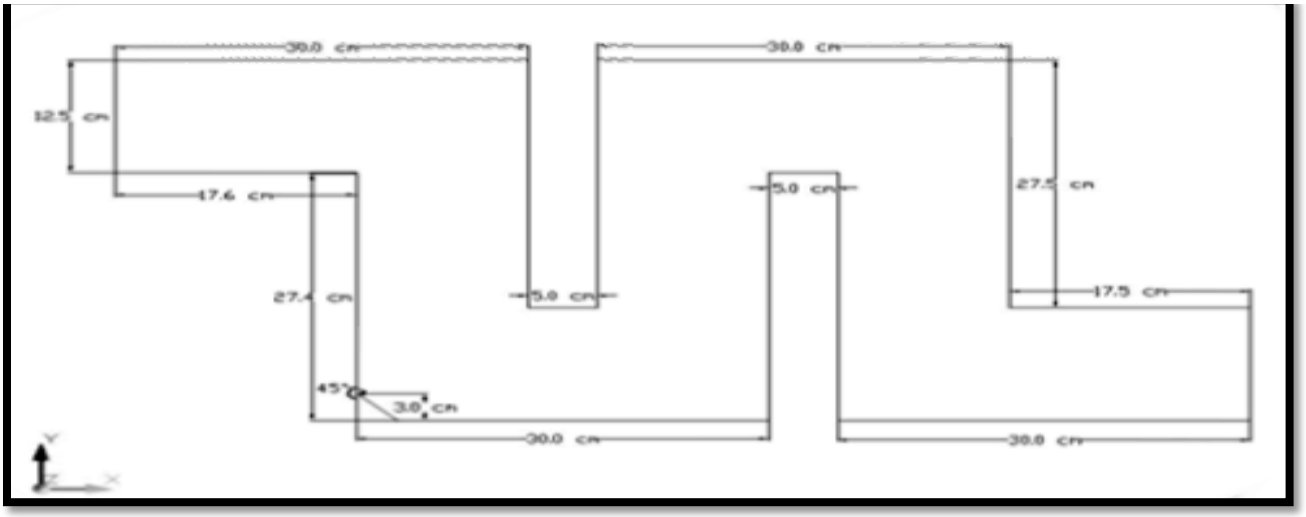

Figure 3.5 : Dimensions of Setup

Flow rate is the measure of liquid streaming in a given time. It is communicated in gallons per meter (gpm) or liters per meter (lpm). It is given by Q = Av; where An is stream zone and v is

stream speed. Stream rate can likewise be communicated as the limit of liquid put away (C) in a given time (t).

$$Q = \frac{C}{t}$$

where C is the capacity of fluid stored, t is the time taken to flow. Three flow rates were set Q1, Q2 and Q3 based on above formula, Q1=125ml/sec, Q2=83ml/sec and Q2=42ml/sec

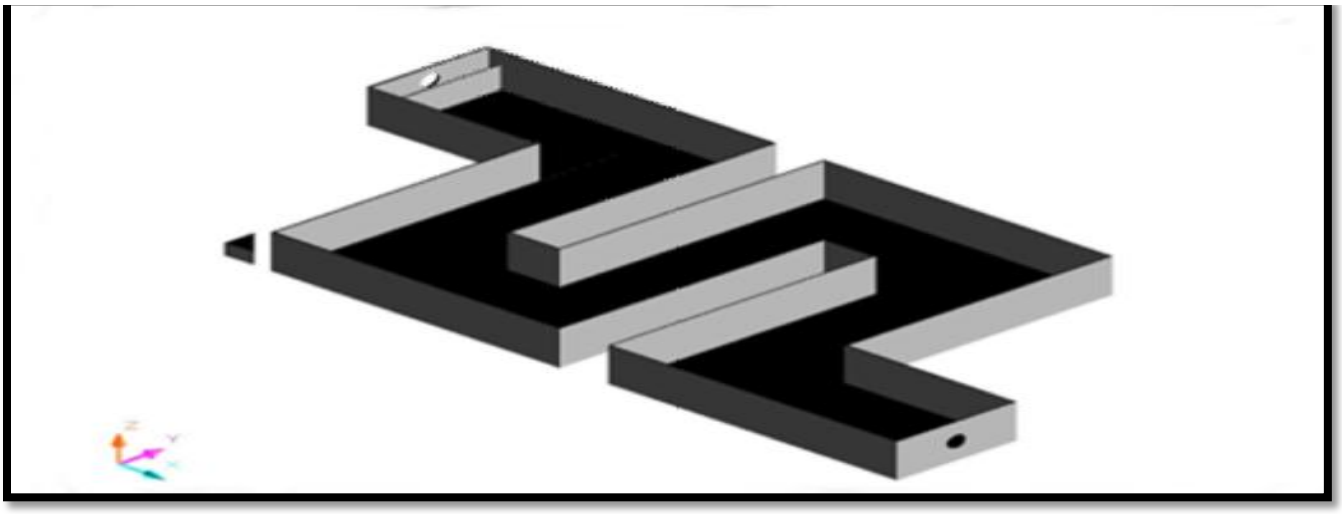

Figure 3.6: Autocad Images of setup

3.7 Disinfection Using Titania Nanoparticles

Model bacterial strains of *P. aeruginosa* and *P. putida* were used in this study. Liquid culture of *P. aeruginosa* and and *P. putida* were grown aerobically in nutrient broth (NB) at 37°C for 16 hours in an incubator. The cell suspensions used for antibacterial activity were approximately 8×10^9 colony forming units (cfu/mL). The bacteria concentration was also determined by a viable count procedure on nutrient agar plates after serial dilutions of the culture in 0.85% saline solution.

3.7.1 Bacterial Culture Preparation

One mL of each pure bacterial cultures of *P. aeruginosa* and and *P. putida* were inoculated in 100mL of Nutrient Broth. The suspension was incubated at 37^{o}C for 16 hours.

3.7.2 Disinfection of Test Solution

This bacterial suspension was added to the effluent; samples were collected after intervals of 30mins, and were observed until the cfu/mL was below detectable limit. The readings were taken for different flow rates.

3.8 Monitoring of Bacterial Disinfection

Bacterial disinfection was done under visible light (Fig 3.7) and sunlight for 15 days each. Samples were collected after intervals of 30 mins and bacterial colonies were counted. The bacterial colonies were counted separately for different flow rates.

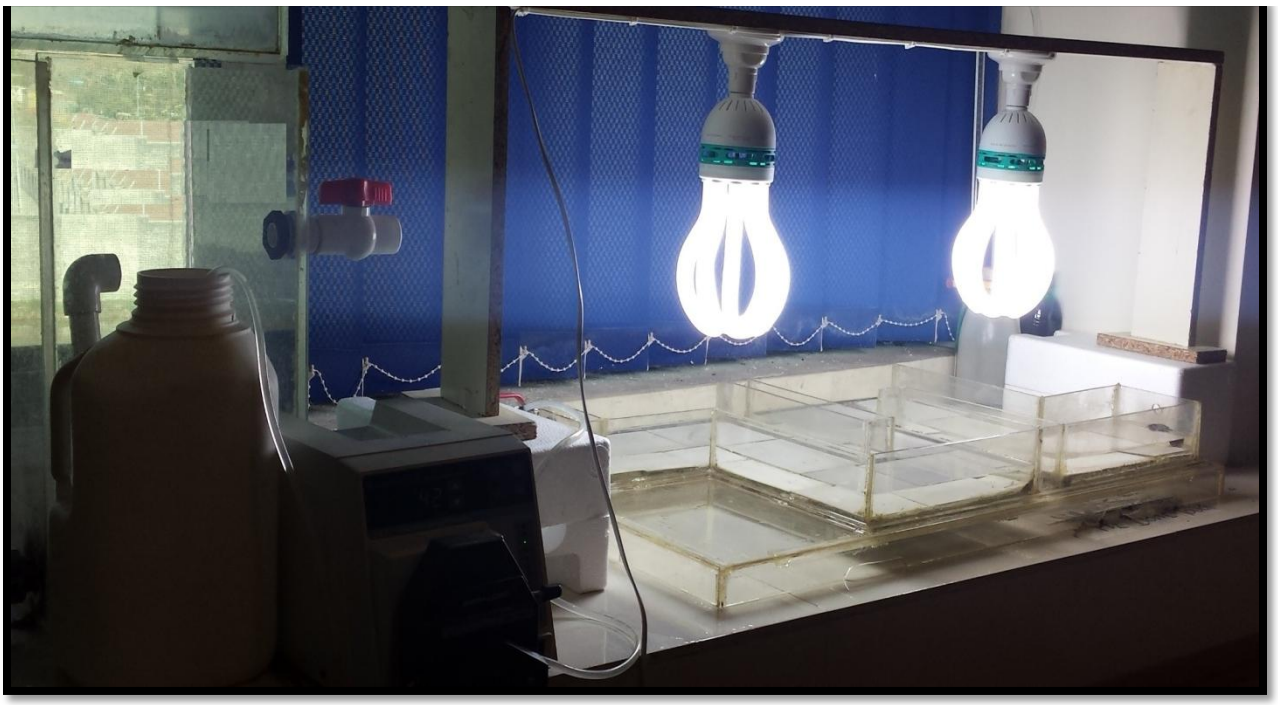

Figure 3.7: Bacterial Disinfection under Visible Light

Chapter 4

Results and Discussion

4.1 Bacteria in Effluent

Both kinds of bacteria, i.e.; gram positive and gram negative, were present in MBR effluent, which was confirmed by gram staining. The gram-negative microbes have complex cell divider comprised of a slim layer of peptidoglycan layer and two cell layers, an external film, and a plasma layer though grampositive microscopic organisms have single plasma layer and cell divider made out of numerous layers of peptidoglycan polymer. The external film of the gramnegative microbes cells changes the penetrability of numerous particles, which makes gramnegative bacteria more resistant than the gram positive ones. *Enterobacteriaceae* family (gram negative bacteria) was selected for further studies as these are of more importance and are proven to be more resistant to disinfection.

4.2 API Results

Gramnegative bacteria colonies were further identified using API kit. By observing the color change in API (Table 4.1), seven digits codes were generated for each isolated colony.

These codes were used to identify the bacterial isolates using API web software (Table 4.2).For identification of dominating microorganisms API 20E system may be applied as proposed by Juang and Morgan (2001).*Enterobacteriaceae* were identified among the isolatesusing API. Various pathogenic bacteria such as *E. coli, Salmonella, Shigella, Yersina*along with many harmless symbiotic bacteria are included in this family (Williams *et al*., 2010).

Table 4.1: API Results to Generate Seven Digit Code

Cupule Medium	Results					
	E1	**E2**	**E3**	**E4**	**E5**	**E6**
O-Nitrophenyle-B-D-galactoside (ONPG)	Positive	Negative	Positive	Positive	Positive	Negative
Arginine Dehydrolase (ADH)	Positive	Positive	Negative	Positive	Negative	Negative
Lysine Decarboxylase (LDC)	Negative	Negative	Positive	Negative	Positive	Negative
OnthinineDecarbolyxase (ODC)	Positive	Negative	Negative	Negative	Negative	Negative
Citrate Utilization (CIT)	Negative	Positive	Positive	Negative	Positive	Positive
HydregenSulfide (H2S)	Negative	Negative	Negative	Negative	Negative	Negative
Urease (URE)	Negative	Negative	Positive	Negative	Negative	Positive
Tryptophan deaminase (TDA)	Negative	Negative	Positive	Positive	Positive	Positive
Indole (IND)	Positive	Positive	Negative	Negative	Negative	Negative
Voges–Proskauer (VP)	Positive	Positive	Negative	Positive	Negative	Positive
Gelatin liquefaction (GEL)	Positive	Positive	Positive	Negative	Positive	Positive
Glucose (GLU)	Negative	Negative	Positive	Negative	Positive	Negative
Mannitol	Negative	Negative	Positive	Negative	Positive	Negative
Inositol	Positive	Negative	Negative	Negative	Negative	Negative
Sorbitol	Positive	Negative	Positive	Negative	Positive	Negative
Rhamnos	Positive	Negative	Positive	Negative	Positive	Negative
Sucrose	Positive	Negative	Positive	Negative	Positive	Negative
Melibiose	Positive	Negative	Positive	Negative	Positive	Negative
Amygdaline	Positive	Negative	Positive	Negative	Positive	Negative
Arabinose	Positive	Negative	Positive	Negative	Positive	Negative

Isolates	Seven Digit Codes	Significant Species
E1	2243000	*Pseudomonas aeruginosa*
E2	5216673	*Klebsiellapneumoniae*
E3	2243004	*Pseudomonas fluorescens/putida*
E4	7243677	*Aeromonashydrophila/caviae/sobria*
E5	5217677	*Raoultellaplanticola*
E6	7243673	*Serratiaodorifera*

Table 4.2 Seven Digit Code and API web software results

The following species were found in the effluent, the isolates of thesebacteria may have entered the MBR effluent after it passed through the membrane.

a) Pseudomonas putida

b) Klebsiellapneumoniae

c) Aeromonashydrophilia

d) Pseudomonas aeruginosa

e) Serratamarcesscans

f) *Raoultellaplanticola*

Pseudomonas individuals show qualities as they are pole molded, gram-negative, one or more polar flagella, giving motility, high-impact and non spore shaping. They give positive catalase test and positive oxidase test.

Pseudomonas aeruginosa is a noteworthy denitrifying microscopic organisms, are known for their differing qualities and their development in a wide range of environment, Pseudomonas putida, an individual from the fluorescent gathering of Pseudomonas, is a whipped, gram-negative pole that is found all through the indigenous habitat. Case reports in the writing portray an extensive variety of conditions that have prompted maladies, including pneumonia, catheter-

related circulatory system diseases, intense cholecystitis and cholangitis, tonsillitis, thrombophlebitis, and skin and delicate tissue contaminations (SSTIs).

4.3 Characterization of TiO_2Nanoparticles

4.3.1 X-ray Diffraction

To investigate the crystal phase composition and the crystallite size of TiO_2 nanoparticles -ray diffraction was used. Pure anatase phase was confirmed from the patterns of XRD. There was no presence of rutile in XRD patterns .The average crystallite sizes of the anatase phase of TiO_2 were in the range of 26 to 48 nm. The XRD patterns are shown in Fig 4.1

4.3.2 Scanning Electron Microscope (SEM)

For size and morphology of prepared particles, SEM JEOL JSM-6460 was used. Fig 4.2 and Fig 4.3 show SEM images of TiO_2nanoparticles at 20,000and 30,000 magnifications, respectively.

Fig 4.3 shows presence of porous sponge like structure of high complexity with average size ranging from 40 to 71nm.

Fig 4.4 and 4.5 show the images of titania nanoparticles by JEOL JSM-6460 at 250 and 50,000 magnifications. The structure of TiO_2 in SEM images shows high roughness and surface area, confirming they are suitable for photocatalytic degradation.

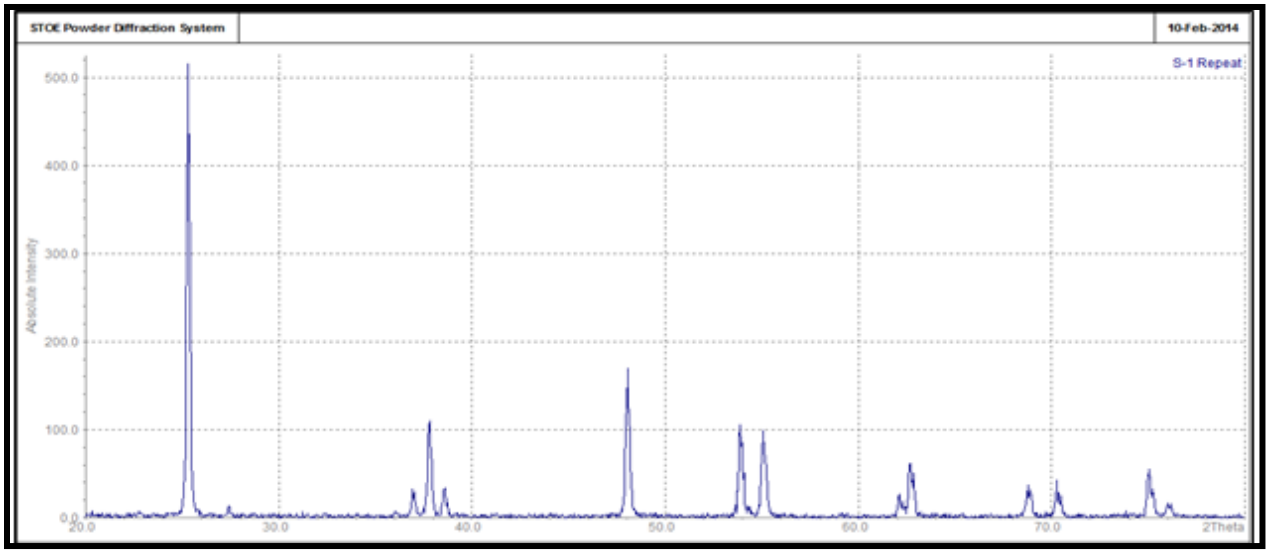

Figure 4.1: XRD pattern of TiO_2 nanoparticles

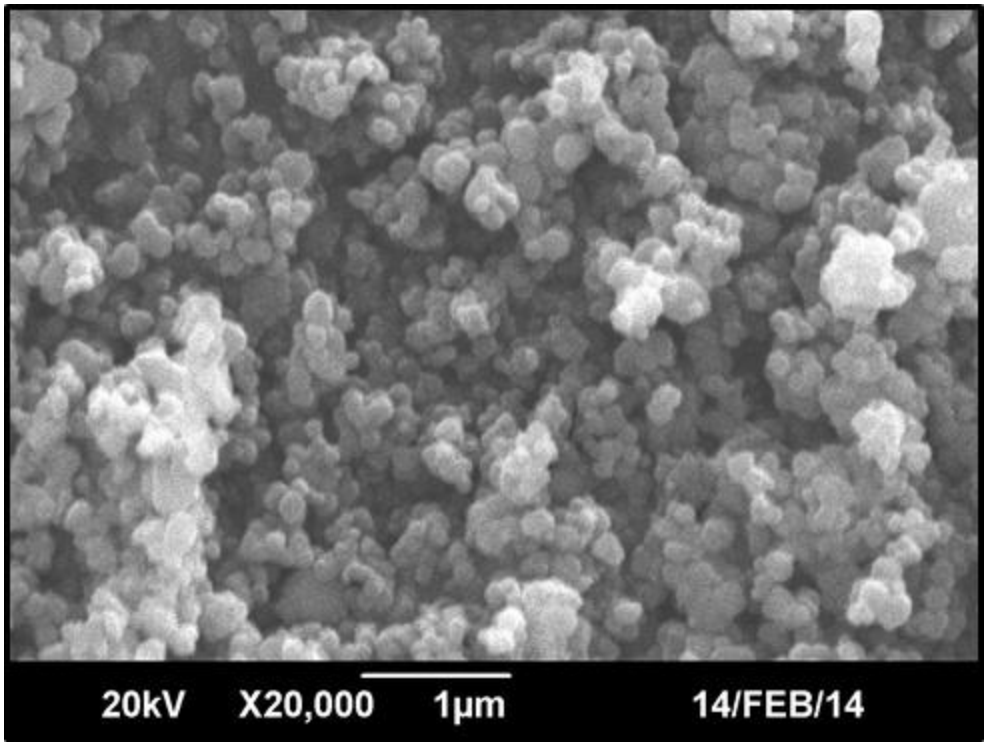

Figure 4.2: SEM image of TiO_2at 20,000 Magnification

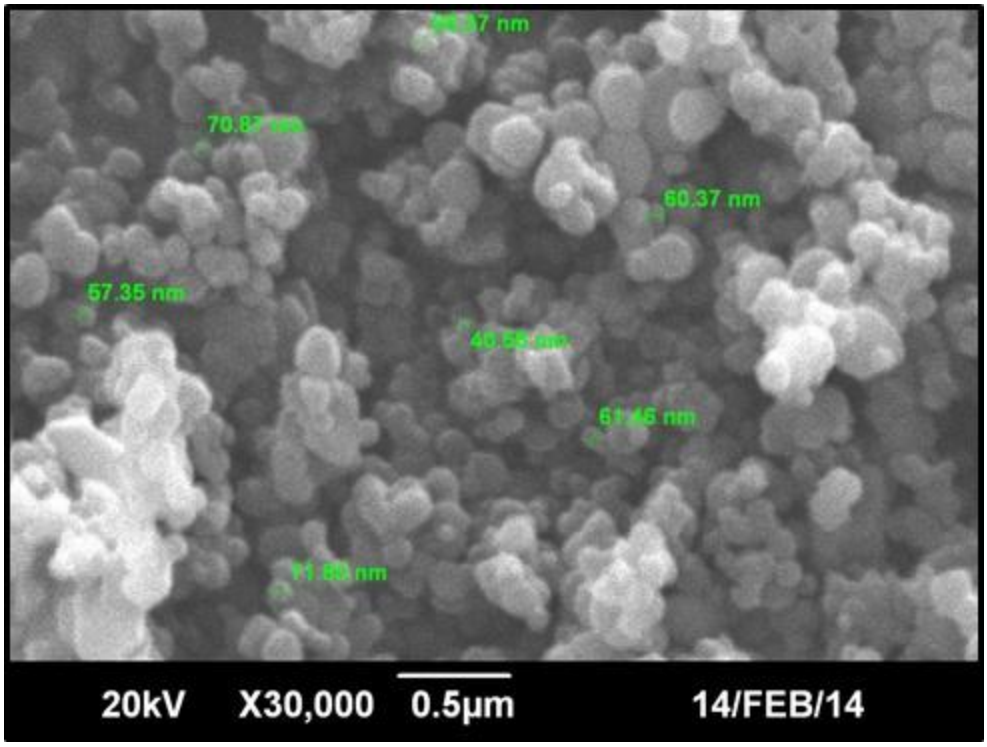

Figure 4.3: SEM image of TiO_2at 30,000 Magnification

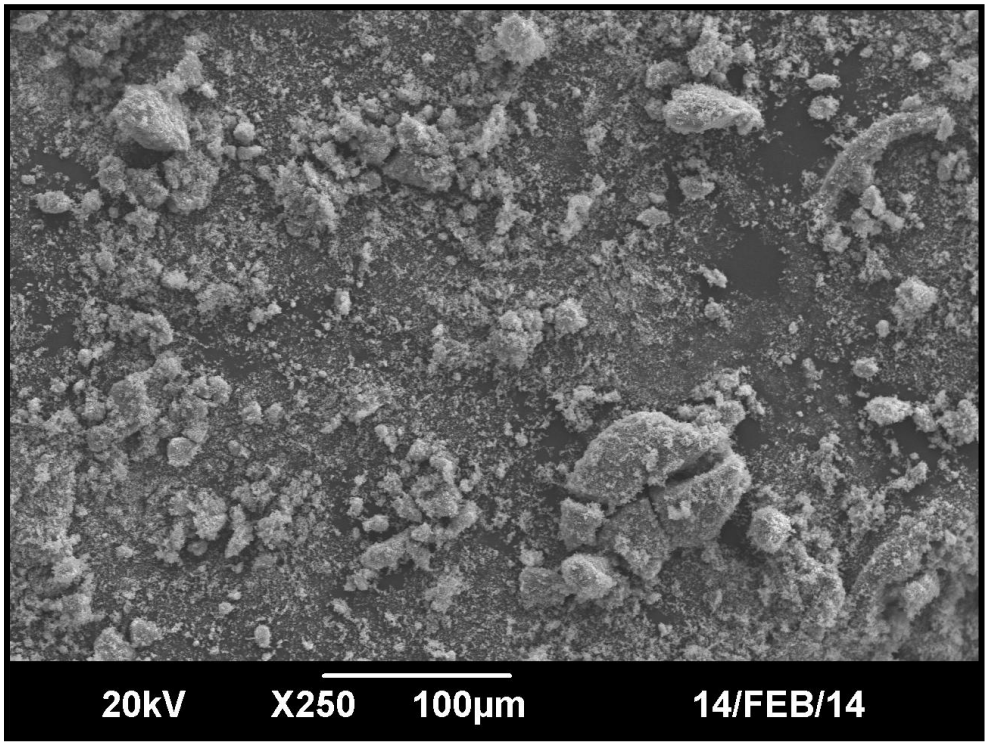

Figure 4.4: SEM image of TiO_2at 250 Magnification

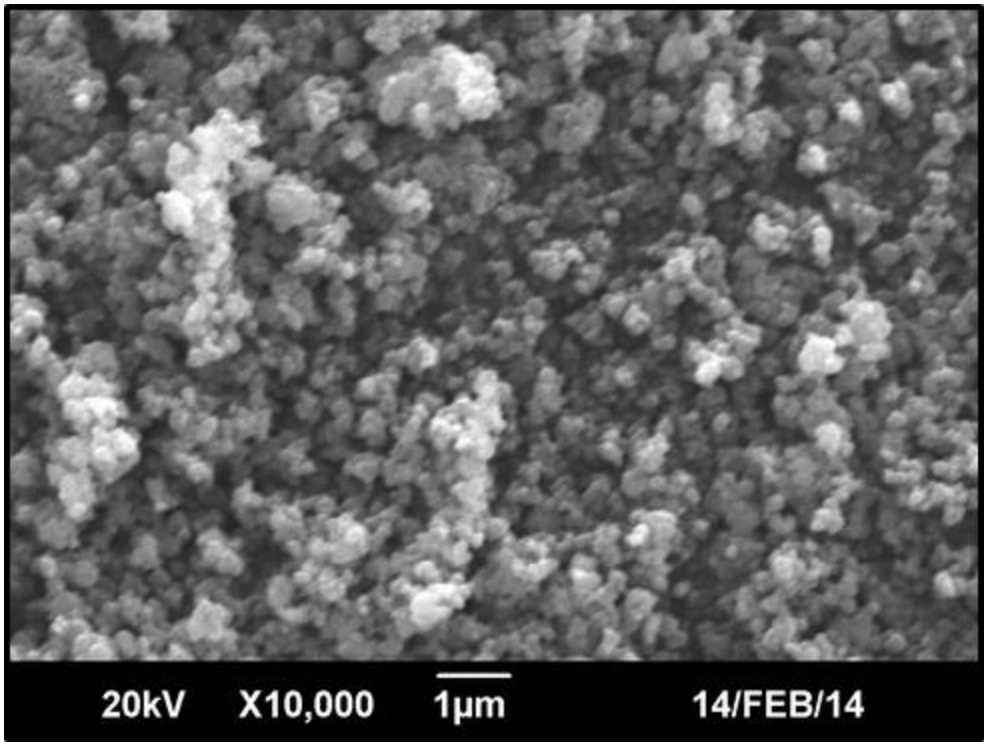

Figure 4.5: SEM image of TiO_2at 10,000 Magnification

4.3.3 Electron Dispersive Spectroscopy (EDS)

The composition of particles was studied using Energy Dispersive Spectroscopy (EDS). The EDS analysis (Fig. 4.6) indicated presence of titanium and oxygen only in particles samples prepared by liquid impregnation method. There was no other elements present.

4.4 Characterization of Coated and Uncoated Tiles

4.4.1 SEM

Fig 4.7 and Fig 4.8 show the difference between coated and uncoated tiles at 50 and 250 magnifications, respectively. The ceramic tiles have rough surface and after etching it becomes rougher.

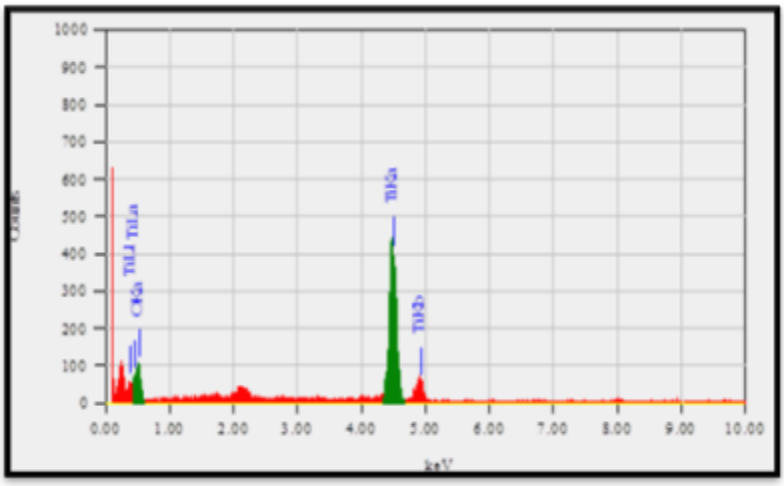

Figure 4.6 EDS of TiO_2 Nanoparticles

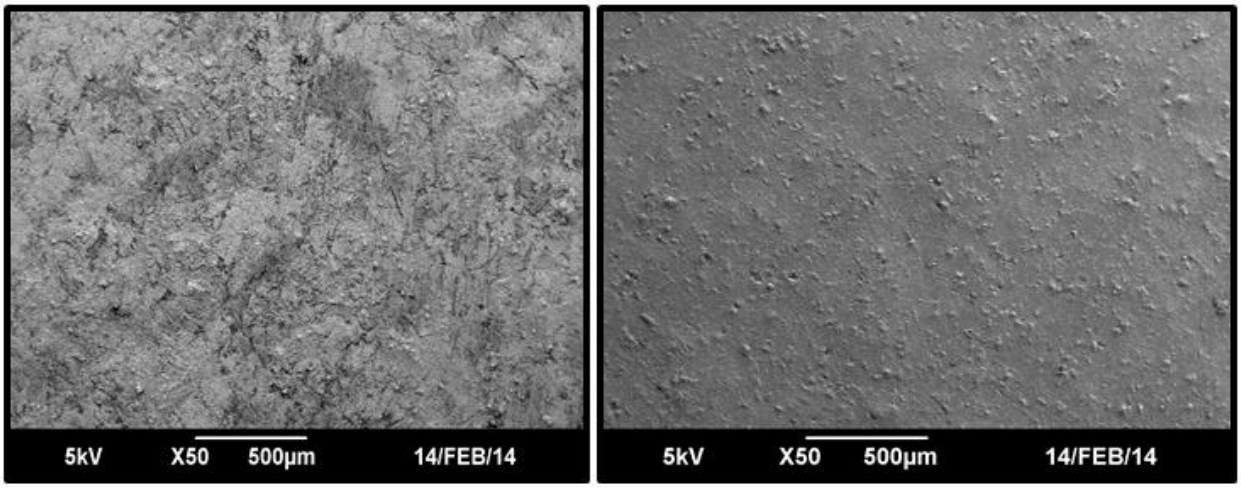

Figure 4.7: SEM Images of Coated and Uncoated Tiles at 50 Magnifications

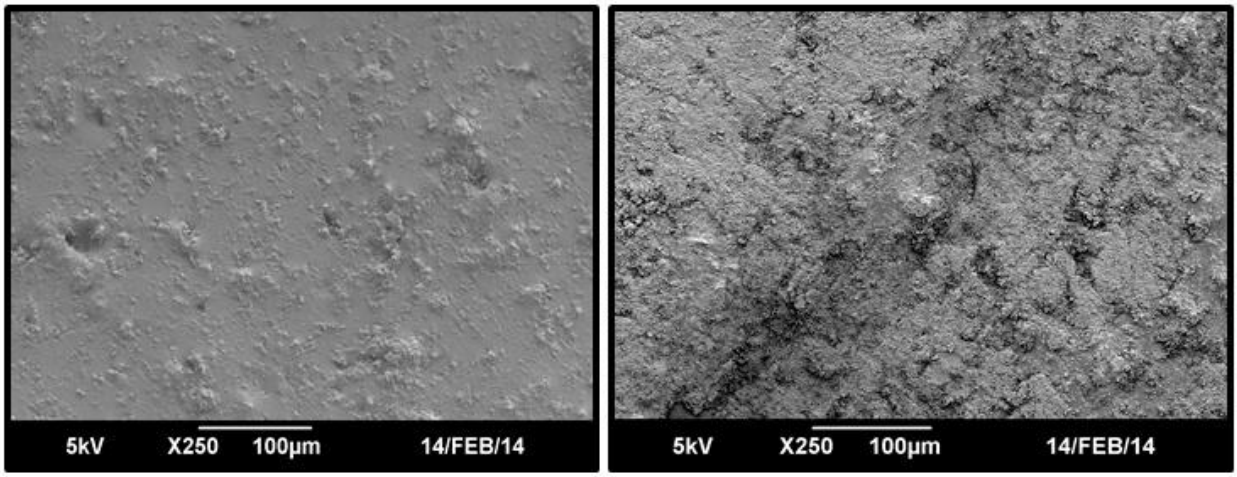

Figure 4.8: SEM Images of Coated and Uncoated Tiles at 250 Magnifications

Nanoparticles cover the surface of tiles completely as shown in Fig 4.9 and 4.10. SEM images showed thick layer of particles over surface of tiles, providing surface for photocataytic activity. The danger of coating to run off with water is very low because of thick layer of titania and this coating is advantageous because titania itself doesn't decompose.

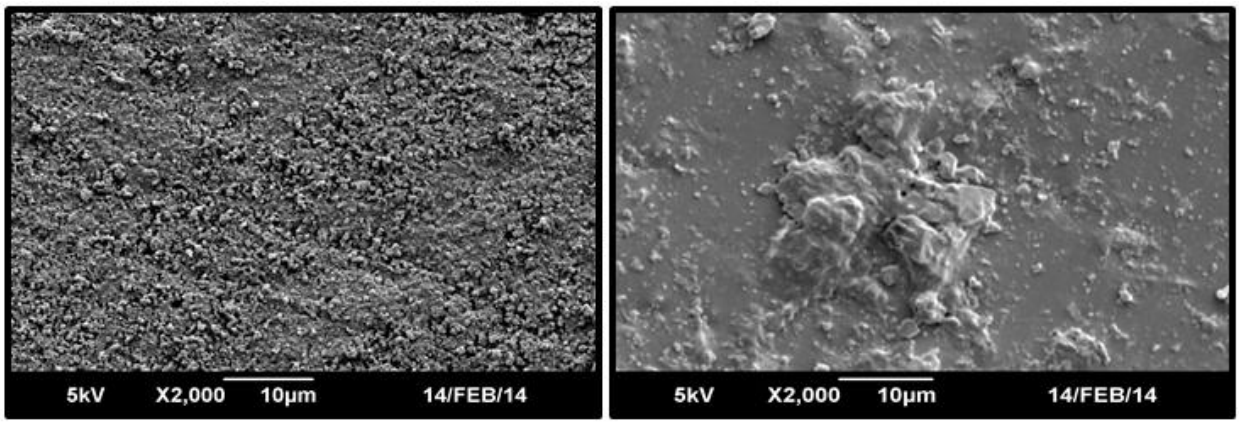

Figure 4.9: SEM Images of Coated and Uncoated Tiles at 2,000 mMagnifications

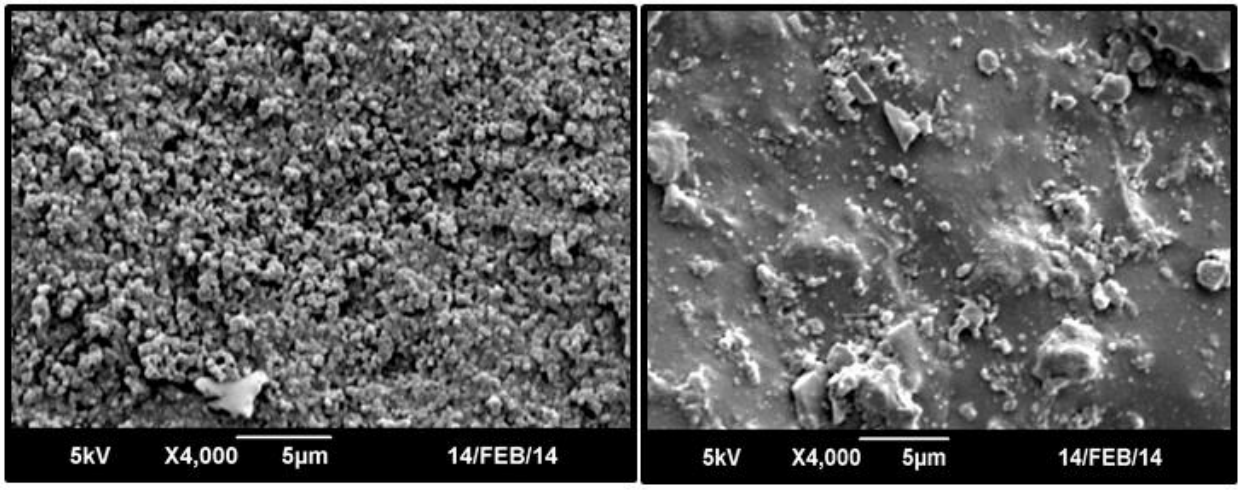

Figure 4.10: SEM Images of Coated and Uncoated Tiles at 4,000 Magnification

4.4.2 EDS

Energy Dispersive Spectroscopy was used to authenticate presence of titania layer on tiles after coating. Elements like silica, carbon, calcium and oxygen were present on the surface of tiles initially (Fig 4.11) but after coating titanium and oxygen was observed Figure (4.12).

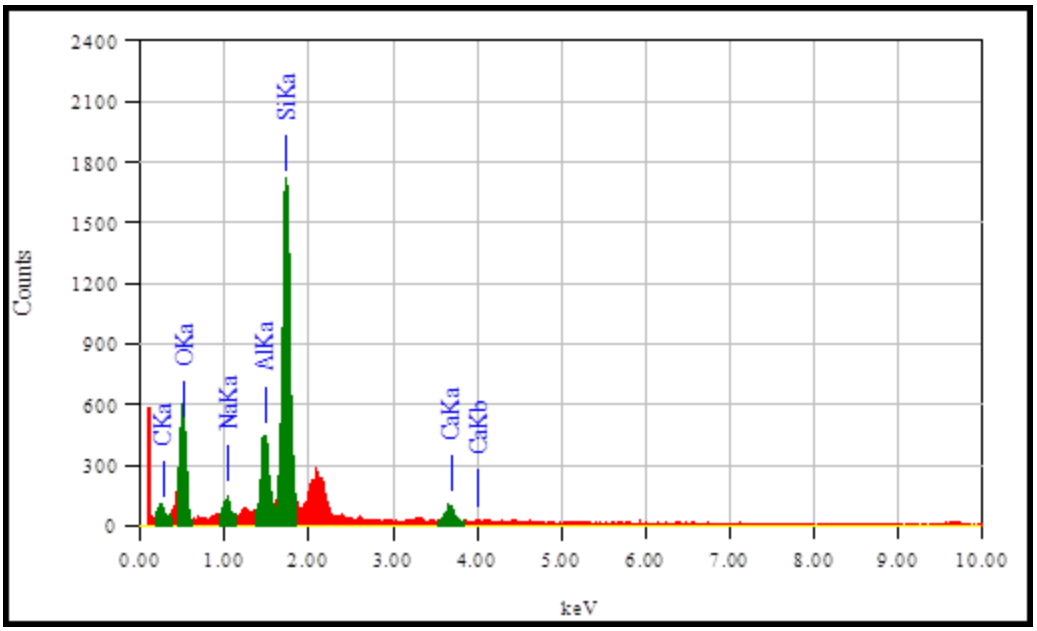

Figure 4.11: EDS of Uncoated Ceramic Tile

Table 4.3 shows that uncoated tile has no Ti , the mass of Si is 38.39% and other elements have larger percentage; however, after coating Si becomes 11.56% and Ti is 43%, the percentage of other elements also significantly decreases. This shows the presence of TiO_2after coating.

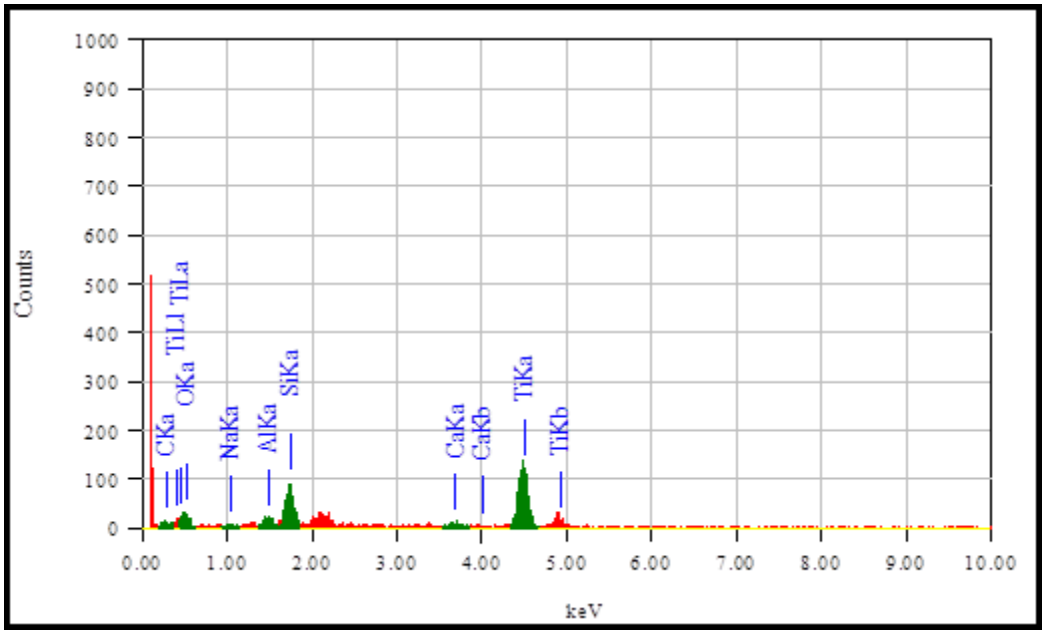

Figure 4.12: EDS of Coated Ceramic Tile

Table 4.3: Percentage mass of elements in coated and uncoated tiles

Element	Mass % Coated Tile	Mass % Uncoated Tile
C	2.23	5.27
O	37.16	41.48
Na	1.74	3.1
Al	1.92	8.8
Si	11.56	38.39
Ca	1.48	2.97
Ti	43.91	0

4.5 Photocatlytic Disinfection of *Pseudomonas* Cultures

The bacterial disinfection property of prepared setup with titania nanoparticles coated tiles was first tested for bacterial cultures of *P. aeruginosa* and *P. putida* prepared in the laboratory. Colony forming units determined the disinfection of bacterial cultures after 24 hours of incubation at 37 ^{o}C. Initially the bacterial concentration was 8×10^{9}cfu/mL, the survival of bacteria started decreasing with increase in retention time. Retention time was increased by interval of 15 mins and samples were collected. Colony counting was done after 18 to 24 hours of incubation. There was significant drop in numbers of bacteria after photocatalytic reaction with TiO_2 nanoparticles. At higher flow rates the disinfection was lower and at lower flow rate disinfection was higher. The number of bacteria became below detectable limit within 45mins of exposure.

4.6 Monitoring of Bacterial Disinfection

The result shows that there was no inhibition of bacteria in control solution. For maximum disinfection of *P. aeruginosa* and *P. putida* the retention time was 45mins, at this time

the antibacterial effect showed 100% killing efficiency. The disinfection results of overall consortium in the effluent; however, showed an increase in disinfection time. This illustrates that (Anatase) TiO_2 nanoparticles have inhibitory behavior to bacterial growth in the presence of light.

4.7 Bactericidal Effect of TiO_2Nanoparticle in Visible Light

The bactericidal effect on effluent treated via the designed setup was studied under visible light at different flow rates as shown in Fig 4.13.

The bacterial colonies in the setup were significantly decreased as a function of time, at different flow rates. When the initial bacterial count was 8×10^9 cfu/mL, the bacterial load was reduced by almost 6 logs under visible light within 45 mins of exposure to light. Bacterial decontamination was observed at all the flow rates. Almost 6 log reduction was achieved within 45mins at 42 ml/sec, where the colonies were still present at flow rate of 125 ml/sec. Control samples showed no reduction in bacterial load as a function of time.

4.8 Bactericidal Effect of TiO_2Nanoparticle in Sunlight

The bactericidal effect on effluent treated via the designed setup was studied under sunlight at different flow rates and it is in shown in Fig 4.14

The bacterial colonies in setup were significantly decreased as a function of time, while colonies in sunlight proliferated well at all flow rates. The graph shows that disinfection rate increases quickly at slower flow rate as it gets more retention time and hence more contact time with surface of titania nanoparticles. Relatively at higher flow rate, the bacterial colonies show less disinfection. Control samples showed no reduction in bacterial load as a function of time.

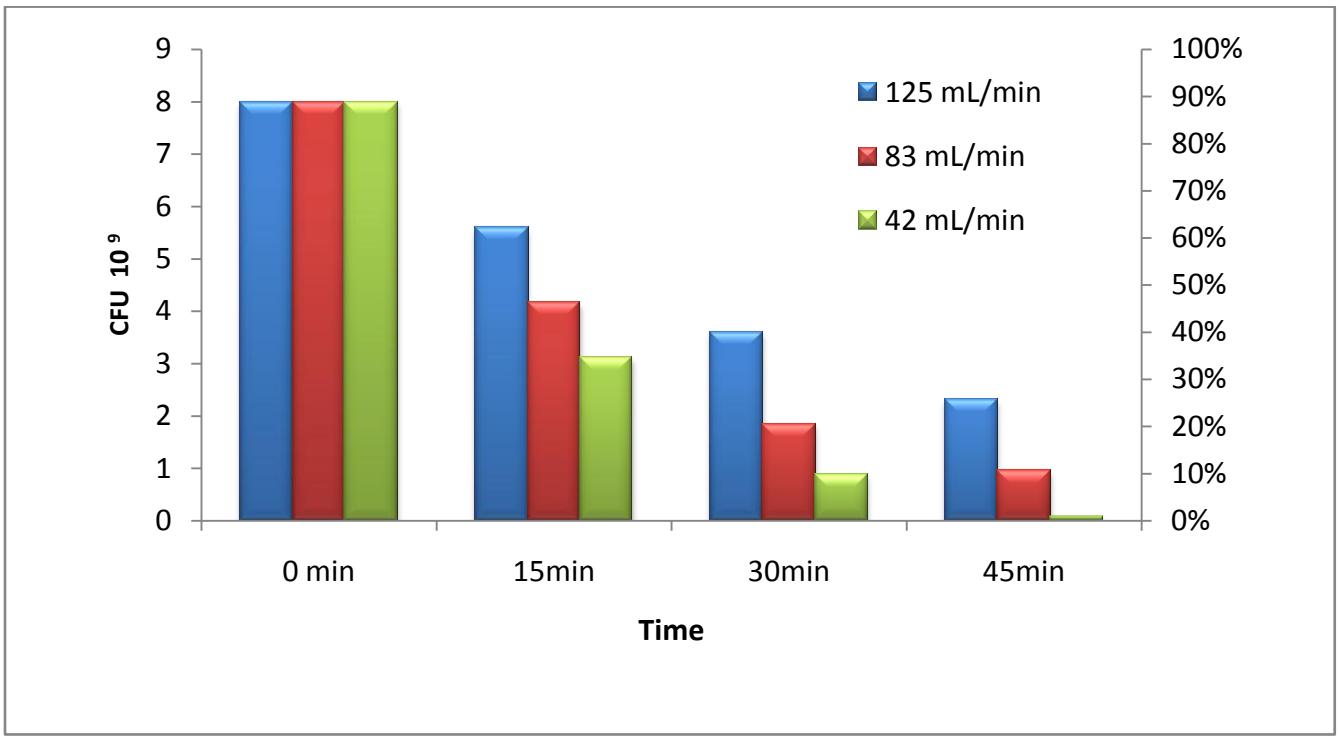

Figure 4.13: Bactericidal Effects under Visible Light

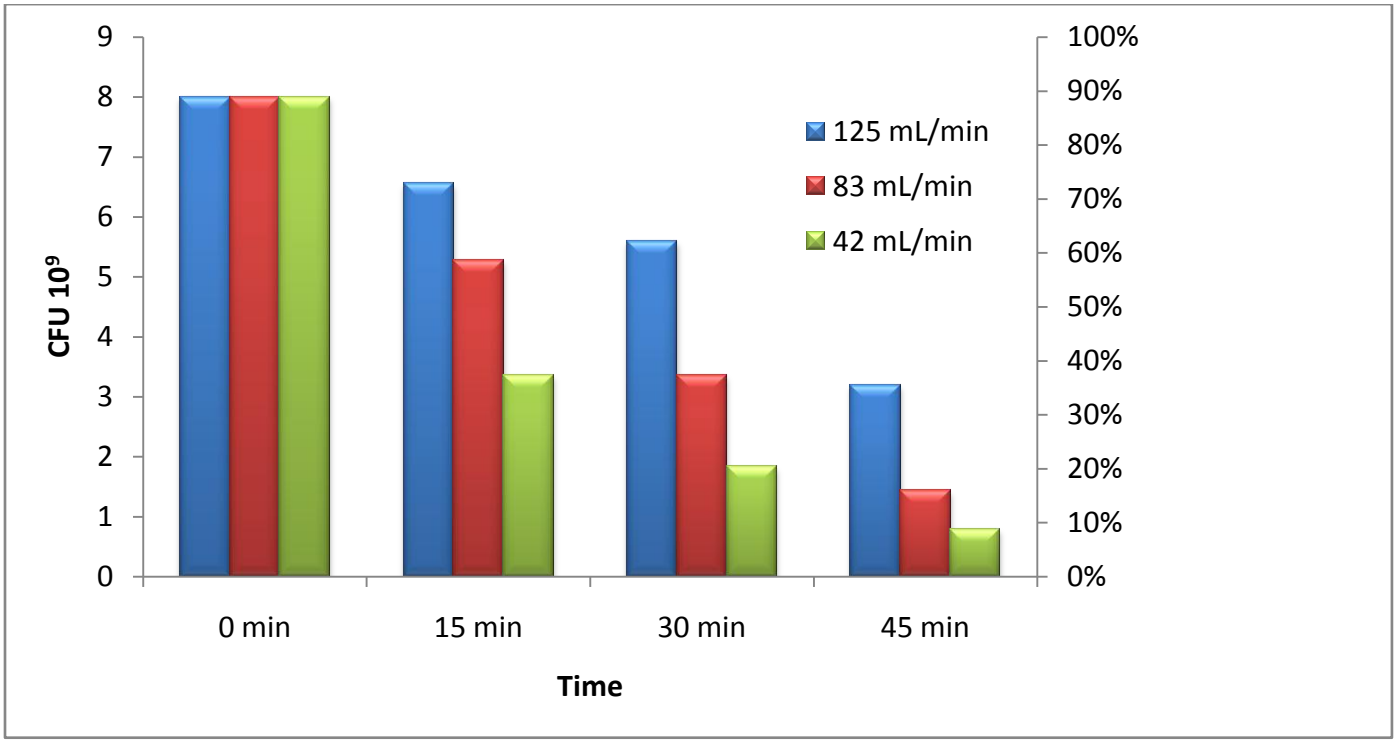

Figure 4.14: Bactericidal Effects under Sunlight

It was observed; however, that the bacterial count did decrease as a function of time. When the initial the bacterial load was 8×10^9 cfu/mL, a 7 log reduction under visible light in 45mins was observed, however it took more than 45mins to show similar results in sunlight. Decontamination was observed within 30 minsexposure of light.In Fig 4.15 shows the graph for bacterial disinfection under visible light and sunlight and it clearly showsthat photokilling under visible light is the most efficient treatment, however, under sunlight bacterial disinfection is also effective.

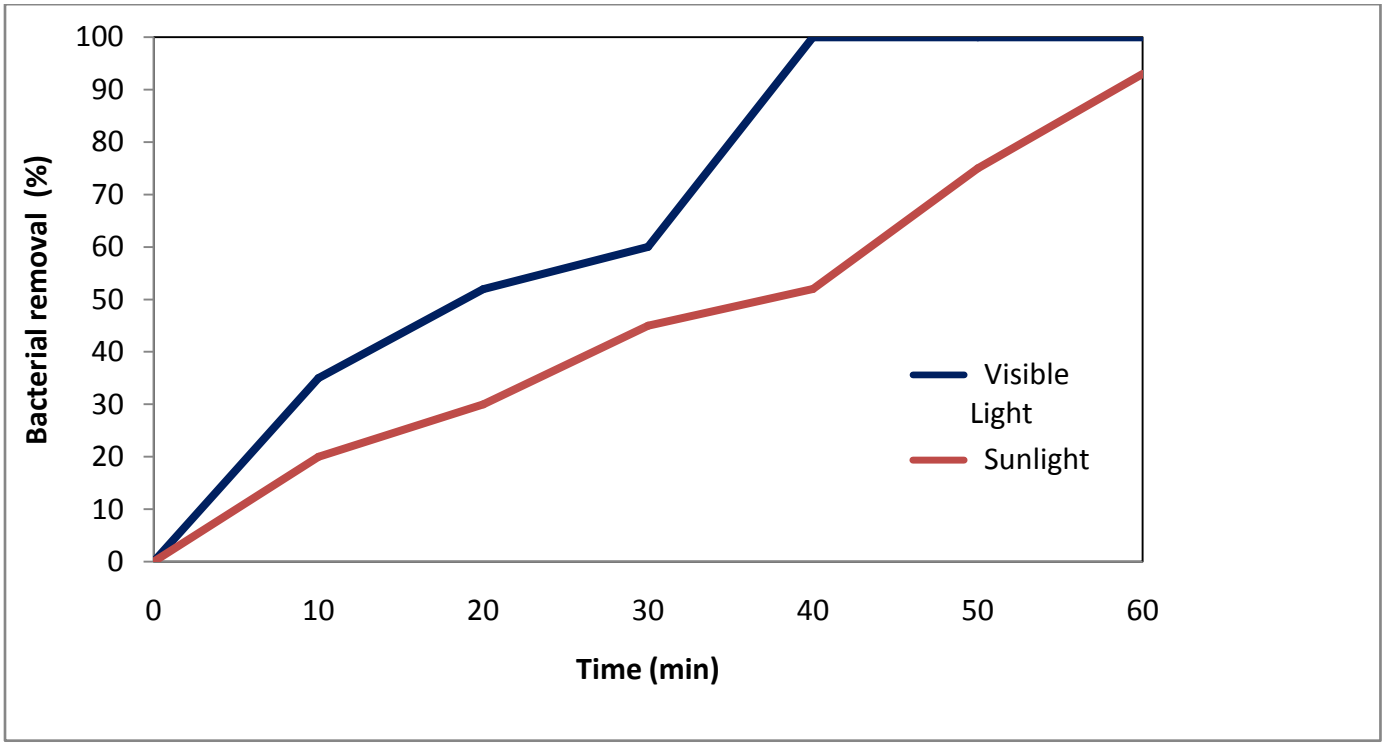

Figure 4.15: Degradation Rate of Bacteria under Visible and Sunlight

Chapter 5

Conclusions and Recommendations

This study investigated bacterial isolates obtained from membrane bioreactor effluent from the water and wastewater laboratory, IESE, NUST. Most of the isolates obtained from streak plate technique had similar characteristics, on gram staining they appear to be Gram-negative bacteria; however, there were certain Gram-positive species. Analytical Profile Index Kit was used for identification of *Enterobacteriaceae* family, as they were dominant in effluent. For disinfection of these bacteria TiO_2 nanoparticles were prepared using the liquid impregnation method. These particles were coated on ceramic tiles incorporated in the disinfection setup designed using AutoCAD. The coating was done using the water based method. Disinfection studies were done under visible light and sunlight and results were observed.

5.1 Conclusions

Following conclusions were drawn from this study:

1. Microbial studies showed that the majority of the bacteria involved in MBR are Gram-negative in nature.
2. The API results showed membrane bioreactor effluent was dominated by *Enterobacteriaceae,* followed by *Pseudomonadaceae* family.
3. For synthesis of anatase TiO_2 nanoparticles, liquid impregnation method is feasible, to prepare particles of desired size, which were characterized using SEM, XRD and EDS.
4. TiO_2 nanoparticles exhibited promising disinfection results in the setup at optimum flow rate, showing significant inhibition of *Enterobacteriaceae* species, *Pseudomonas aeruginosa* and *Pseudomonas putida*.

5. Testing and monitoring of the effluent showed that TiO_2 nanoparticles have 96–100% killing efficiency towards overall bacterial consortium present in MBR effluent.
6. Disinfection using TiO_2 nanoparticles under visible light at different flow rates, showed the most positive results. So, ultimately this setup containing titanium dioxide (TiO_2) nanoparticles readily disinfects the MBR effluent.
7. Disinfection using TiO_2 nanoparticles under sunlight is also effective, it showed positive results within almost 1 hour.

5.2 Recommendations

Following recommendations are noteworthy for further study:

1. Polymerase Chain Reaction (PCR) may be used for identification of bacteria in MBR effluent.
2. Factors effecting the disinfection under visible and sunlight, such as intensity, distance and exposure should be studied.
3. TiO_2 nanotubes and doped particles may be employed for disinfection.
4. The TiO_2 coated channels may be employed with MBR to disinfect the effluent before final discharge to the environment.

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