CHAPTER ONE

Introduction

1.1 Introduction

Water is a fundamental requirement of life, where there is no water there is no life. Most people in the poor and developing countries suffer from diseases that come from water pollution sustaining activities like drinking, cooking, cleaning and agriculture. Nature has its own mechanism for water recycling to provide us with adequate quantity of fresh water with consumable purity level. Modern human activities have however disrupted the balance between the usage and natural purification processes leading to a shortage of potable water. Most of the natural resources of drinking water are found to be contaminated with diverse toxic materials and pathogenic microorganisms. Reports show that over 700 million people across the globe face water scarcity, and it is estimated that this problem will touch 1.8 billion people by 2025[1].

According to a World Health Organization (WHO) report, water borne diseases kill nearly 12 million people every year [1].

The availability of fresh water is crucial for life over one billion people each year are exposed to unsafe drinking water due to poor source water quality and lack of adequate water treatment. This results in 900 million cases of diarrhea each year. Five out of every 1000 of those exposed to unsafe drinking water will die from diseases carried by the contaminated water; another 2.5 will die from dehydration due to diarrhea [1].

Children are particularly affected; the average child in developing countries has more than two episodes of diarrhea per year. Diarrheal illness results in malnutrition, weakness, and an increase in susceptibility to diseases, and can be life threatening. The estimated number children that die each year due to water related diseases range from 2.5 million to 15 million. The lack of access to good quality drinking water leads to a high risk for waterborne diseases such as diarrhea, cholera, typhoid fever, hepatitis A, amoebic and other diarrheal diseases [1].

1.2 The importance of the use of nano technology:

In recent months concerns have been raised regarding the adverse impact of nano technology on environment. It should be noted that most of the nanotechnological advances are based on known chemicals and materials that are already employed in various application. The novel aspect of nanotechnology has been to organize the molecules into clusters or assemblies so that the properties of the produced nanomaterials are tailored to the desired application.

A recent thrust in this area has been the exploration of size and shape dependent properties of semiconductor and metals. While the contamination caused during manufacture or application of nano materials is a major concern, it should not be difficult to devise environmentally friendly protocols similar to those regulating the manufacture and used of other chemicals and material. On a positive note, semiconductor nanostructure can play an important role in developing smart materials that can simultaneously sense and destroy harmful chemical contaminants from the environment. Such an application seems to be important as the concern over chemical contamination of drinking water and air needs to be dressed. Application of semiconductors nanoparticles as photo catalysts is still limited by the fact that they respond only to UV-excitation. Continuous efforts to extend the response to the visible range have met with limited success. Semiconductor-metal nanocomposites that improve the selectivity and efficiency of the photocatalytic process are expected to draw the attention of future research [2].

1.3 Water

Water contains one oxygen and two hydrogen atoms connected by covalent bonds. Water is liquid at ambient conditions, but it often coexists on earth with its solid state, ice and gaseous state. Water covers 71% of the earth's surface and is vital for all known forms of life. 96.5% of the earth's water is found in oceans, 1.7% in ground water, 1.7%in glaciers and ice caps of Antarctica and Greenland. A small fraction in other large water bodies, and 0.001% in the air as vapor, clouds and precipitation. Only 2.5% of the earth's water is fresh water and 98.8%of that water is in ice and groundwater. Less than 0.3%of all freshwater is in rivers, lakes and the atmosphere, and an even smaller amount of earth, fresh water (0.003%) is contained within biological bodies and manufactured products [2].

1.4 Microbes in water

The major groups microbes in water are: bacteria, fungi viruses and protozoa. All aquatic ecosystems such as: fresh water, ocean water, rainwater etc… contain the micro organisms [2].

1.4.1 Fungi

Fungi are divided into yeasts and moulds. Fungi are used by industry to produce a variety of useful products. They also cause spoilage of fruits, vegetables etc.

Yeasts: are typical unicellular fungi, such as saccharomyces cerevisiae (for baking and brewing beer).

Moulds: are described as filament-like, such as:

Penicillium roqueforti, used in cheese manufacture, and penicillium notatum, first antibiotic [2].

1.4.2 Viruses

Viruses are sub-microscopic small infectious agents that can infect animals, plants and other microorganisms. They can live only in living host cells. They possess DNA or RNA, but never both, and they have three main shapes: helical, icosherdal and complex [2].

1.4.3 Protozoa

Protozoa are single-celled organisms that are ubiquitous in aqueous environments and the soil. Some examples are: malaria parasites, trypanosomes, Giardia lamblia [2].

1.4.4 Bacteria

Bacteria are microscopic, single-celled organisms that are the most numerous organisms on earth. They are so small that over five million could be placed on the head of a pin. Bacteria can live in numerous environments and perform many complex actions, some of which are beneficial and some harmful. Most bacteria, however, are not harmful and do not cause human health problems. Those that are disease producing referred to as pathogenic. Viruses and some protozoans can also be pathogenic. Coliform bacteria are part of the Enterobacteriaceae family and individual cells cannot be seen with the naked eye due to their small size (but colonies can be seen) while some coliform bacteria can be naturally found in soil. The type of coliform bacteria that lives in the intestinal tract of warm-blooded animals and originates from animal and human waste is called fecal coliform bacteria. Escherichia coli (E. coli) is one subgroup of fecal coliform bacteria. Even within this species, there are numerous different strains, some of which can be harmful. However; the release of these naturally-occurring organisms into the environment is generally not a cause for alarm. Other disease causing bacteria, which can include some pathogenic strains of E. coli, or viruses, may also be present in these wastes and pose a health threat [3].

1.5 Water treatment:

(i) Water Storage:

It is a simple method to improve the water quality. Plain sedimentation however cans only fecal pollution. Water storage is only used as pretreatment for surface water.

(ii) Boiling Water:

It is the safest water treatment method. It kills the microorganism present in contaminated water. The main disadvantage of boiling water is large amount of energy required, which makes it relatively expensive and unaffordable for the poorest section of the population in developing countries

(iii) Water Pasteurization: achieve the same effect as boiling at temperatures of only 70c° _ 75c° .Also pasteurization requires large amount of energy.

(iv) Water Filtration: is a simple household filters, such as ceramics candle filters will not remove a high fraction of solid matter, but may not remove all the microorganisms.

(v) Water Disinfection: Chlorine is used to kill microorganisms (bacteria and viruses). Skilled application is necessary as chlorine is hazardous and corrosive substance. Water treated by chlorine has a taste which many users do not appreciate [4].

(vi) Fluoridation: Fluoride component which are put in water are expensive as well as risky to human health. As the study published in Brain research shows that rats drinking only part per million fluorides (NaF) in water had histological lesions in their brain similar to Alzheimer's disease and dementia. In addition, evidence was seen pointing to possible damage to the blood brain barrier from extended fluoride exposure [5].

1.6 TiO2 for water treatment

Water is a fundamental requirement for life. The availability of fresh water is very important for life supporting activities like drinking, cooking, cleaning, agriculture, etc. Most of the natural resources of drinking water are found to be contaminated with different toxic materials and pathogenic bacteria.

One of the main applications of Titanium dioxide (TiO_2) nanoparticles are using in environmental purification as photocatalysts to water treatment.

Photocatalyst coupled with UV lights can oxidize organic pollutants into nontoxic materials such as water and can disinfect certain bacteria. This technology is very effective in removing further hazardous organic compounds and killing a variety of bacteria and some viruses in the secondary waste water treatment. Pilot projects demonstrated that photocatalytic detoxification systems could effectively kill fecal coli form bacteria in secondary waste water treatment [6].

1.7 Solar water disinfection (SODIS)

It is applied at household level with a great potential to reduce diarrhea incidence of users. The method is very simple and the only resources required for its application are transparent PET plastic bottles (or glass bottles) and sufficient Sunlight: microbiologically contaminated water is filled into the bottles and exposed to the full sunlight for 6hours. During Solar exposure the diarrhea causing pathogen are killed by UV –A radiation of Sunlight.

Solar disinfection (SODIS) is a simple and low cost technique used to disinfect drinking water, where water is placed in transparent containers and exposed to sunlight for 6 hours. There are a number of parameters which affect the efficacy of SODIS, including the solar irradiance, the quality of the water, and the nature of the contamination. One approach to SODIS enhancement is the use of semiconductor photocatalysis to produce highly reactive species that can destroy organic pollutants and inactivate water pathogens.

In recent year interest of photocatalysis has focused on the use of semiconductor materials as photocatalysis for removal of organic and in organic species from aqueous or gas phase systems in environmental clean-up, drinking water treatment. The ability of TiO_2 to oxidize organic and inorganic substrates in air and water through redox processes TiO_2 has not only emerged as one of the most fascinate.

Many industrial waste streams are not suitable for biological processing due to their inherent toxicity. On the other hand, TiO_2 photocatalysts has been shown to decompose contaminants in water efficiently because a strong oxidizing ability is generated when irradiated by appropriate band gap illumination. Increased attention is being paid to heterogeneous .

Nano and Micro size particles of P25 TiO_2 and ZnO semiconductors have nearly the same bandwidth and often used for their comparative studies for water purification application for hazardous heavy metals for health, such as, Cr, dyes, and E-Coli bacteria , TiO_2 is more favored than other semiconductor oxides and it had a heavy literature in water treatment than ZnO for example that were studied kind of lately . TiO_2 is also cheap and it's a mixed phase semiconductor oxide is relatively more active in photocatalysts that's why it is been preferred for water purification [7].

1.8 Some Applications of TiO2

Titanium dioxide TiO_2 exhibits good photo catalytic properties, hence is used in antiseptic and antibacterial compositions degrading organic contaminants and germs As a UV-resistant material manufacture of printing ink, self-cleaning ceramics and glass, coating. Making of cosmetic products such as sunscreen creams, whitening creams, morning and night creams, skin milks, etc. Used in the paper industry for improving the opacity of paper [8].

1.9 The scope of the present work

This thesis includes a brief introduction about water pollution and treatment. Chapter 2 presents theoretical background relevant to crystal structure and physical properties of TiO_2 in addition to the photocatalyst effect. The characterization techniques employed to this study is presented in chapter 3. Chapter 4 provides details of the experimental results, and this is followed by conclusions and suggestion for future work in chapter 5. References are given at the end of the thesis.

CHAPTER TWO

Theoretical Background

Titanium dioxide (TiO_2), also known as Titania, is a naturally occurring oxide of titanium. The property of titanium dioxide includes high refractive index, light absorption, non-toxicity, chemical stability and relatively low-cost production. Titanium dioxide (TiO_2), nanoparticles have attracted attention in the fields of environmental purification, solar energy cells, photo catalysts, gas sensors, photo electrodes and electronic devices. It has been widely used as a pigment in paints, ointments and toothpaste [9].

The performances of TiO_2 are strongly influenced by the crystalline structure, the morphology and the size of the particles [10]. In the process of synthesis of TiO_2, the shape and crystal structure of the product significantly depends on the raw material used.

2.1 Structure of TiO_2

TiO_2 belongs to the family of transition metal oxides. There are three commonly known phases of TiO_2 found in nature, namely anatase (tetragonal), brookite (orthorhombic), rutile (tetragonal) [11].

Rutile has the highest thermodynamic stability and thus is the form most commonly found in nature [12]. Anatase and brookite transform into rutile upon heating, and the abundance of rutile rapidly increases as the temperature reaches 600°C[13].

The heat treatment of amorphous titanium dioxide(TiO_2) converts it to a crystalline anatase structure at temperature below 400^0C which is further converted to rutile form when heated between 600^0C- 1100^0C [14]. The anatase to rutile phase transformation is envisaged as a transformation from a metastable state to stable state. However no unique phase transformation temperature is reported [15].

The transformation of anatase to rutile is suppressed due to lack of oxygen and only3% of anatase is transformed to rutile at 900^0C. while rutile is the more stable phase at high temperatures, the following transformations are also seen:

- Anatase to brookite to rutile.
- Brookite to anatase to rutile.
- Anatase to rutile and brookite to rutile [16].

The oxygen atoms in rutile are arranged in a slightly distorted hexagonal close-packing, while a cubic-close packing applies for anatase and brookite.

Half of the octahedral interstices are occupied by titanium atoms in all three structures. Rutile and anatase are both tetragonal, respectively containing 6 and 12 atoms per unit cell, with anatase being the more elongated structure [17]. The TiO_6 octahedra are slightly distorted; the lengths of two {Ti-O} bonds are slightly greater than the other four. Brookite has an orthorhombic structural geometry, formed by joining together the, sharing three edges. The Fermi level in anatase is higher than that of rutile by about 0.1 eV, resulting in different electrical and optical properties [18].

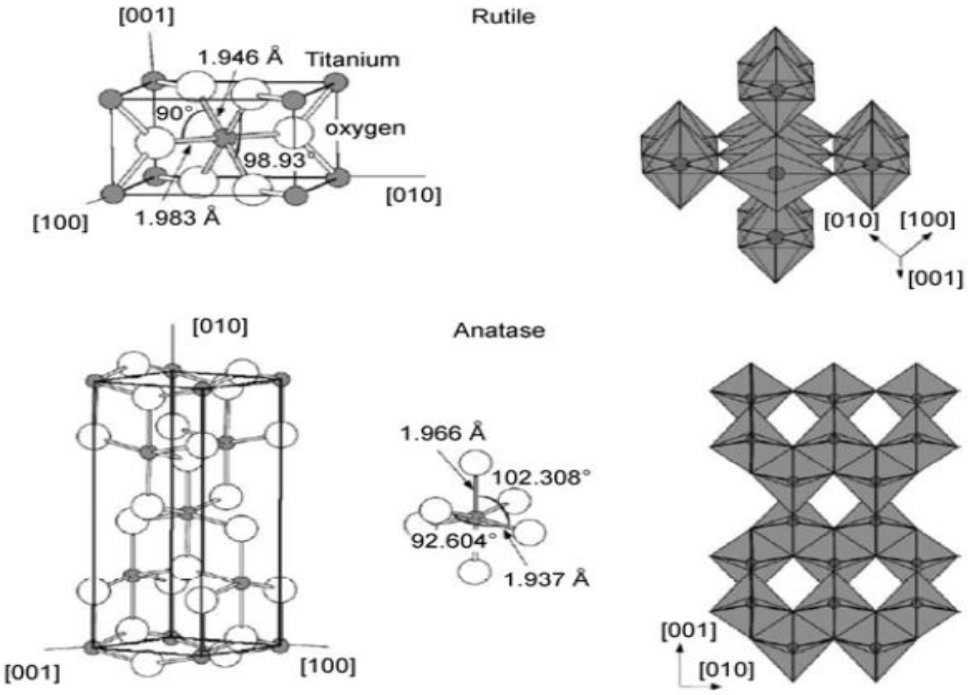

Figure 2.1 Crystal structures of the rutile and anatase phases of TiO_2 [19]

2.2 Photo Catalytic Reactions

Heterogeneous photocatalysis using UV/TiO_2 is one of the most common photocatalytic process and is based on absorption of photons with energy higher than 3.2 eV (wavelengths lower than ~390 nm) resulting in initiating excitation related to charge separation event [20]. Generation of excited high-energy states of electron and hole pairs occurs when wide band gap semiconductors are irradiated higher than their band gap energy. It results in the promotion of an electron in the conduction band (eCB −) and formation of a positive hole in the valence band (hVB+) as presented in equation (1) [21]. The hVB+ and eCB− are powerful oxidizing and reducing agents, respectively.

The hVB+ reacts with organic compounds resulting in their oxidation, producing CO_2 and H_2O as end products in equation (2). The hVB+ can also oxidize organic compounds by reacting with water to generate •OH in Eq. (3). Hydroxyl radical (•OH) is produced as the second highest oxidation potential (2.80 eV), which is only slightly lower than the strongest oxidant, fluorine. Due to its electrophilic nature (electron preferring), the •OH can non-selectively oxidize almost all electron rich organic molecules, eventually converting them to CO_2 and water as described in equation (4).

$$TiO_2 + hv\ (<387\ nm) \longrightarrow e_{CB}^{-} + h_{VB}^{+} \qquad (1)$$

$$h_{VB}^{+} + R \rightarrow \text{intermediates} \rightarrow CO_2 + H_2O \qquad (2)$$

$$H_2O + h_{VB}^{+} \longrightarrow \bullet OH + H \qquad (3)$$

$$\bullet OH + R \rightarrow \text{intermediates} \longrightarrow CO_2 + H_2O \qquad (4)$$

Where R represents the organic compound. The conductive band can react with O_2 forming an anion radical superoxide as shown in Eq. 5. Further reaction can lead to the formation of hydrogen peroxide which lead to the formation of •OH [22].

$$e_{CB}^{-} + O_2 \longrightarrow O_2^{-} \qquad (5)$$

The presence of dissolved oxygen is extremely important during photocatalytic degradation as it can make the recombination process on TiO_2 (eCB−/hVB +) difficult which results in maintaining the electro neutrality of the TiO_2 particles [19]. In other words, it is important for effective photocatalytic degradation of organic pollutants that the reduction process of oxygen and the oxidation of pollutants proceed simultaneously to avoid the accumulation of electron in the conduction band and thus reduce the rate of recombination of eCB− and hVB+ [24].

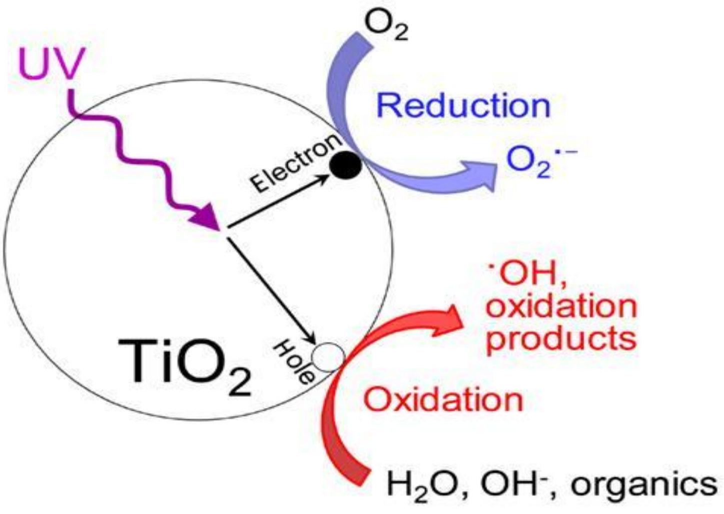

Fig 2.2: The mechanism of charge separation and photocatalytic activity of the TiO_2 (Fujishima, Akira 2005)

2.3 Physical properties of TiO_2:

Pure titanium metal can exist as a dark gray, shiny metal or as a dark gray powder. It has a melting point of 1.677°C and a boiling point of 3.277°C. Its density is 4.6 grams per cubic centimeter. Titanium metal is brittle when cold and can break apart easily at room temperature.

At higher temperatures, it becomes malleable and ductile. Malleable means capable of being hammered into thin sheets. Ductile means capable of being drawn into thin wires. Titanium has an interesting physical property. Small amounts of oxygen or nitrogen make it much stronger [8].

2.4Chemical properties of TiO_2

In general, titanium tends to be quite unreactive. It does not combine with oxygen at room temperature. It also resists attack by acids, chlorine, and other corrosive agents. A corrosive agent is a material that tends to vigorously react or eat away at something.

Titanium becomes more reactive at high temperatures. It can actually catch fire when heated in the presence of oxygen.

Titanium dioxide, predominantly in the anatase phase with an average grain size of 5–10 nm, has been synthesized by the hydrazine method. These nanocrystalline particles show a blue shift in the absorption edge of the diffuse reflectance ultraviolet spectrum of about 10 nm compared to that of commercially available Titania. Synthesized samples were characterized by X-ray diffraction and transmission electron microscopy for their structural properties and UV-Vis absorption spectroscopy for the band gap studies. The absorption spectra show a linear fit for the direct transition. The optical and electrical properties of the samples have been studied and the Arrhenius plots of electrical conductivity both for the as-prepared anatase TiO_2 and the one subsequently reduced in hydrogen atmosphere at 673 K show a distinct difference in the activation energy. The hydrogen-annealed sample shows a typical semiconducting behavior whereas the as-prepared sample indicates a phonon contribution to the conductivity around 300k [1].

2.5 Contamination of Water by Escherichia coli (E. coli) Bacteria

E.coli is currently the most specific indicator for fecal contamination of a water source; its presence in high numbers also allows for more likely determination of water contamination than if they were only found in small numbers in water samples .E. coli is more resistant to disinfection than other enteric bacteria and organisms. .The World Health Organization has set the worldwide guideline for safe drinking water as no detectable E. coli or thermotolerant bacteria in any 100 ml sample .The number of bacteria coliform enumerating using colony count. It is traditionally performed manually using a pencil and a click-counter [1].

CHAPTER THREE

Experimental Aspects

3.1 Characterization techniques:

This section describes the characterization techniques that used to evaluate the nanoparticle in details and some of the devices that used are mentioned. The samples properties evaluates optically using Infrared Spectroscopy (IR), while the crystal structure evaluates by X-ray Diffraction (XRD), and electron microscope (SEM) uses to examine The surface morphology.

3.1.1 X-Ray Diffraction (XRD)

XRD is a well-known analysis method for the characterization of crystal struwctures. In this research XRD diffractometer: Bruker AXS diffractometer D8 (wavelength 1.504 A°), serial number: 202899, made by P76181 Karlsruhe, Germany was used to study crystal structure of TiO_2 nano particles, Fig 3-1.

Diffraction effects are observed when an electromagnetic radiation impinges on the structure at the level of the radiation wavelength. XRD was developed from Bragg's law which is described as follows:

$$\boldsymbol{2d\,\sin\theta = n\lambda} \qquad \textbf{(3.1)}$$

Where:

n ≡Positive integer

λ ≡ Wavelength of incident wave

θ ≡ Scattering angle

d ≡Distance between atomic layers in a crystal.

Figure 3.1: XRD system.

Although Bragg's law was used to explain the interference patterns of X-rays scattered by the crystals, the diffraction has been developed to study the structure of all the states of the matter with any beam; e.g. ion electrons, neutrons, and protons, with a wavelength similar to the distance between the atomic or the molecular structures of interest. Because a pure substance has a unique diffraction pattern, the powder diffraction method is suitable for the characterization and identification of the polycrystalline phases. The sample is pressed into a sample holder to create a smooth flat surface [25].

When an X-ray beam hits an atom, the electrons around the atom start to oscillate with the same frequency as the incoming beam; this results in constructive or destructive interference, depending on the structure. Planes going through areas with high electron density will reflect strongly, planes with low electron density will give weak intensities.

3.1.2 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectroscopic analysis is used to determine the chemical functional groups in the samples. FTIR used in this research is SHIMADZU, Model**:** FTIR 8400S. It is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. At temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other. When the frequency of a specific vibration is equal to the frequency of the IR radiation directed on the molecule, the molecule absorbs the radiation. Infrared radiation spans a section of the electromagnetic spectrum having wave numbers from roughly 13,000 to 10 cm^{-1}, or wavelengths from 0.78 to 1000 μm. It is bound by the red end of the visible region at high frequencies and the microwave region at low frequencies [26].

Figure 3.2: FT-IR spectroscopy

3.1.3 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons, Figs. 3.3-3.4. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, in low vacuum, in wet conditions (in environmental SEM), and at a wide range of cryogenic or elevated temperatures [25].

The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons depends on the angle at which beam meets surface of specimen, i.e. on specimen topography. By scanning the sample and collecting the secondary electrons with a special detector, an image displaying the topography of the surface is created.

Figure 3.3: scanning electron microscope (SEM)

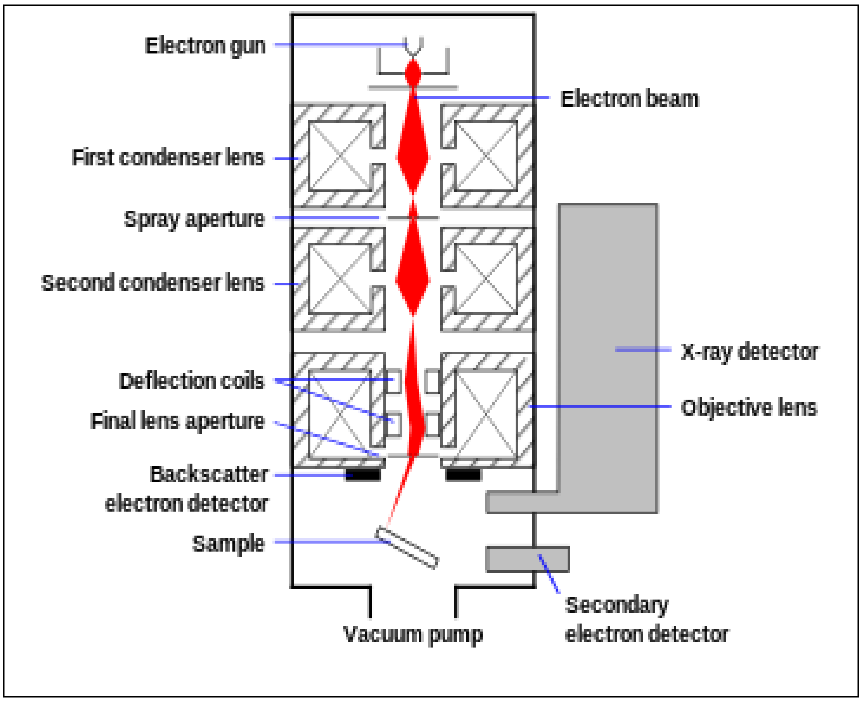

Figure 3.4: Scanning process and image formation (Schematic of SEM.) [21].

3.1.4 UV-visible Spectroscopy

UV-visible absorption spectroscopy involves the spectroscopy of photons in the UV-visible region. This means it uses light in the visible, near ultraviolet (UV) and near infrared (NIR) ranges. The absorption in the visible ranges directly affects the color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions [23]. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. The wavelength of light that a compound will absorb is characteristic of its chemical structure. Primarily used for quantitative analysis of known compounds, UV-Vis is one of the most popular techniques in the pharmaceutical, foods and paints Industries, as well as water laboratories [20].

From literature many ways were used to test TiO_2 by UV-Visible spectroscopy, the difficulties that follow this process is that TiO_2 don't solve in water or alcohol as methanol or ethanol and it doesn't solve in water, some of the ways that used is by using $BaSO_4$ as a reference material by putting $BaSO_4$ in a circular sample holder and press it to make it uniform. Some others used TiO_2 as a paste and others like in this study used warm water for UV test, unfortunately no good result were found.

The system used in this study is shown in Figure 3.5. This system consists of a dark holder room, a UV and visible source, detector and screen to show the results. Also it was connected to a computer for data analysis.

Fig 3.5: Ultra-violate spectroscopy.

Data analysis:

Transmittance: $T = P/P_0$

Absorbance: $A = -\log^{10} T = \log^{10} P_0/P$

The Beer-Lambert Law: $A = ebc$

Where the absorbance A has no units, since $A = \log^{10} P_0 / P$

P_0: is the initial radiant power

P: is the radiant power after light passes through sample

e : is the molar absorptivity with units of L mol^{-1} cm^{-1}

b : is the path length of the sample in cm

C: is the concentration of the compound in solution, expressed in mol L^{-1}.

The fundamental absorption, which corresponds to electron excitation from the valence band to conduction band, can be used to determine the value of the optical band gap. The relation between the absorption coefficient (α) and the incident photon energy (hν) can be written as

$$\alpha h\nu = A\,(h\nu - E.g\,)^n$$

Where, A is a constant, E.g is the band gap of the material and exponent n depends on the type of transition. The value of optical band gap is calculated by extrapolating the straight line portion of $(\alpha h\nu)^2$ vs. (hν) [21, 22].

3.2 Material and Methods

3.2.1 Equipment

- Oven
- Autoclave or pressure cooker
- Bottles
- Culture tubes (test tubes)
- Incubator
- Laminar
- Spreader
- Petri plate (Petri dishes)
- Sterilized tips
- Thermometer

3.2.2 Materials

- Bacteria
- Ethanol
- The media (Nutrient Agar)
- Bunsen burn
- Distilled water
- Deaionized water
- TiO_2 nanopowder

3.2.3sterilization:

Sterilization is a process that eliminates (removes) or kills (deactivate) all forms of life and other biological agents. All equipment and materials were sterilized before being used, because any contamination can change the results. The experiment was carried out in a well-equipped laboratory.

3.2.4 Media preparation:

Soyabean casein digest agar were been dissolved in1000 ml of deionized water, sterilized in autoclave at 121 °C and 15 Ibs for15 minutes, it was taken into a laminar before it used for culturing bacteria. 20 ml of the media was put in each plate to be ready for the culturing of bacteria.

Agar is used throughout the world to provide a solid surface containg media for bacteria and fungi growth.

3.2.5 Culture of bacteria

20 ml from the liquid media was poured in each plate, and then 0.1 ml from the sample was added to liquid, to culture the bacteria. The loop was put in ethanol then entered slowly into the flame and moved all apart through the flame until it turned red hot, it was waited until it cool down, and then was put in the plate where the sample were put, and the sample was spread all over the plate.

3.2.6 Samples preparation:

Plates were sterilized in oven at 160 C° and for two hours before it had been used

Two glass bottles were filled by 500 ml of distilled water and tips were put in the autoclave to be sterilized at temperature 121 C° and 15 Ibs for 15minutes.

The laminar surface was sterilize by ethanol and dry air to remove any possible contamination. All the sterilized equipment and materials were taken into the laminar. Bunsen burner used as a heat source to sterilize the tools and the air around the work area. Two plastic bottles were filled with 500 ml by the sterilized distilled water. 0.1 ml of standard E.colie was added into each one of the bottles, and 0.05 gram of TiO_2 powder was added into one of the bottles. The two bottles were then shacked well. 0.1ml was taken from each bottle and cultured at the Petri dishes.

The samples were taken outdoor on sunlight for one hour, then back into the laminar and then 0.1 ml was taken from each sample and cultured in another plates. The procedure was repeated for six hours. The temperature was measured every hour.

The cultured plates were put in the incubator for 24 hours, and then the bacteria colonies were counted.

CHAPTER FOUR

Results and Discussion

4.1 XRD results of TiO_2 Powder

XRD model Burker's X-Ray diffraction instrument with Cu-Kα radiation of wave length of 1.54 A°, was used. XRD patterns exhibited strong diffraction peaks at 25°. Fig. 4.1 shows the intensity at angles of incidence $^{\theta}$ indicating TiO_2 is in the anatase phase. All peaks are in good agreement with the standard spectrum.

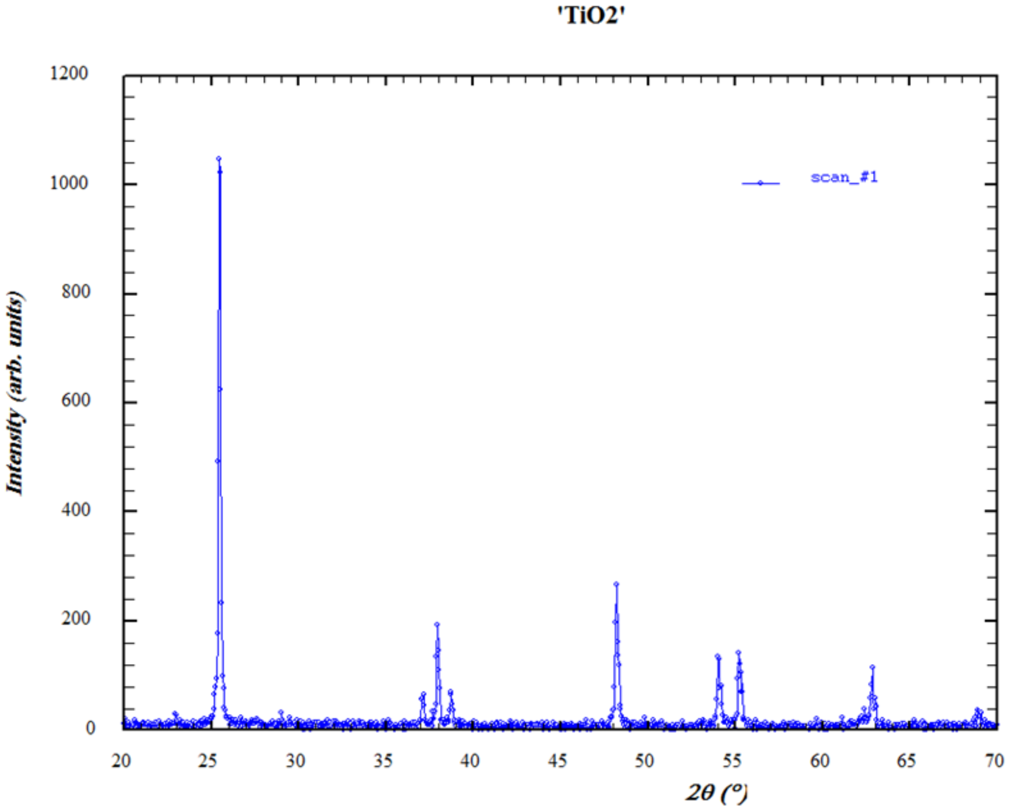

Fig 4.1 X-ray diffraction of anatase TiO_2 nanopowders

4.2 Bacteria counting

The samples were taken outdoor on sunlight for one hour, then back into the laminar and then 0.1 ml was taken from each sample and cultured in another plates. The procedure was repeated for six hours. The temperature was measured every hour.

The cultured plates were put in the incubator for 24 hours, and then the bacteria colonies were counted. The results are shown in Table 4.1.

Table 4.1 Results of bacteria counting

Temperature	Time/hour	Sample 1 With TiO_2	Sample 2 Exposed to the sun only	Sample 3 Control -
18	0 (10:00)Am	∞	∞	0
28	1 (11:00)Am	∞	∞	0
31	2 (12:00)Am	521	∞	0
41	3 (1:00)Am	18	80	0
35	4 (2:00)Am	2	25	0
36	5 (3:00)Am	0	2	0
35	6 (4:00)Am	0	3	0

In the above table the colonies were uncountable at the beginning of the experiment (at zero time) in both samples. The number decreases faster in the sample treated with TiO_2. After two hours of sun exposure the number of colonies decreased into 521 colonies for the sample (on the left) which was treated with TiO_2. For the sample that was exposed to the sun only (on the right) there is no change in the number of colonies, which are uncountable.Fig.4.2.

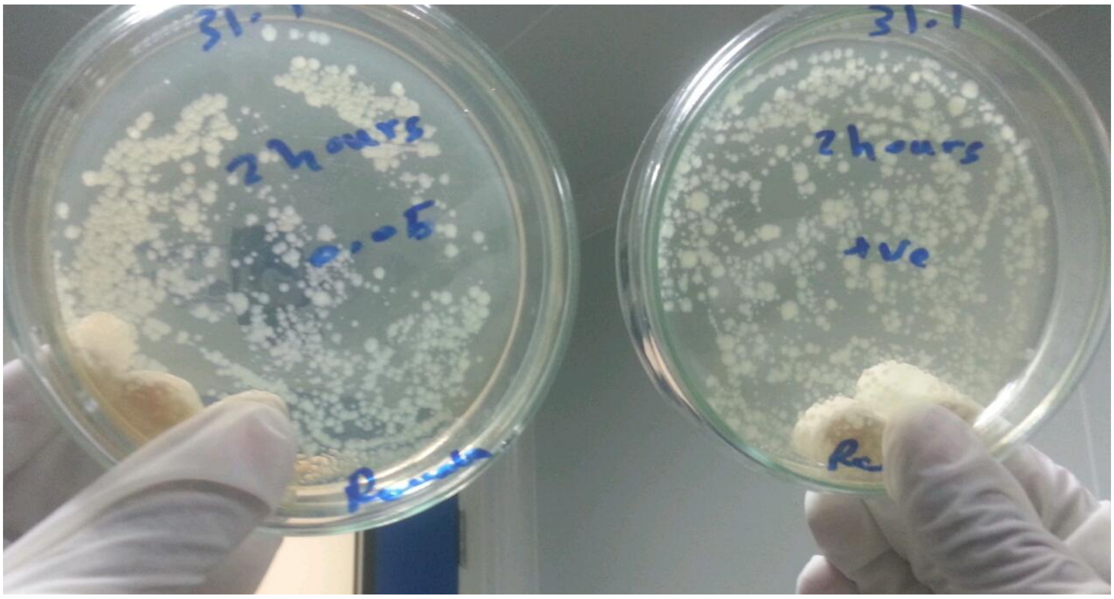

Fig 4.2 number of bacteria colonies after two hours of sun light exposure.

After three hours of sun exposure the number of colonies decreases in the sample treated withTiO_2 to 18 colonies, while in the one exposed to the sun only, the colonies decreased to 80 colonies.Fig 4.3.

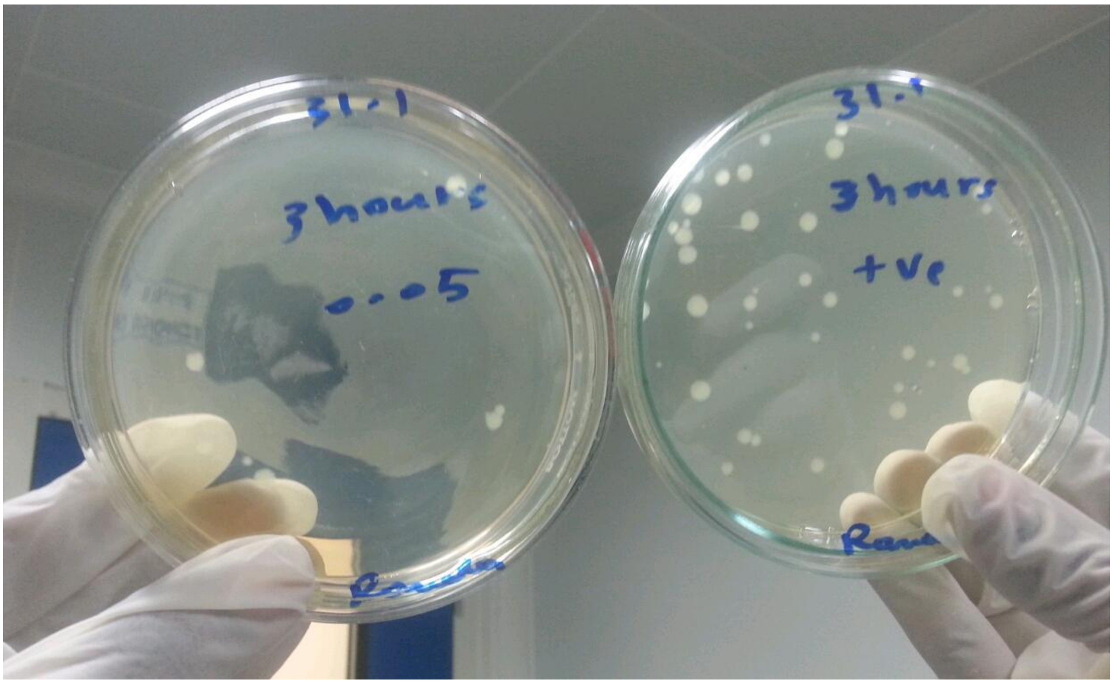

Fig 4.3 number of bacteria colonies after three hours of sun light exposure.

After four hours of sun exposure the number of colonies decreased in the sample treated withTiO_2 to 2 colonies, while in the one exposed to the sun only, the colonies decreased to 25 colonies. Fig.4.4

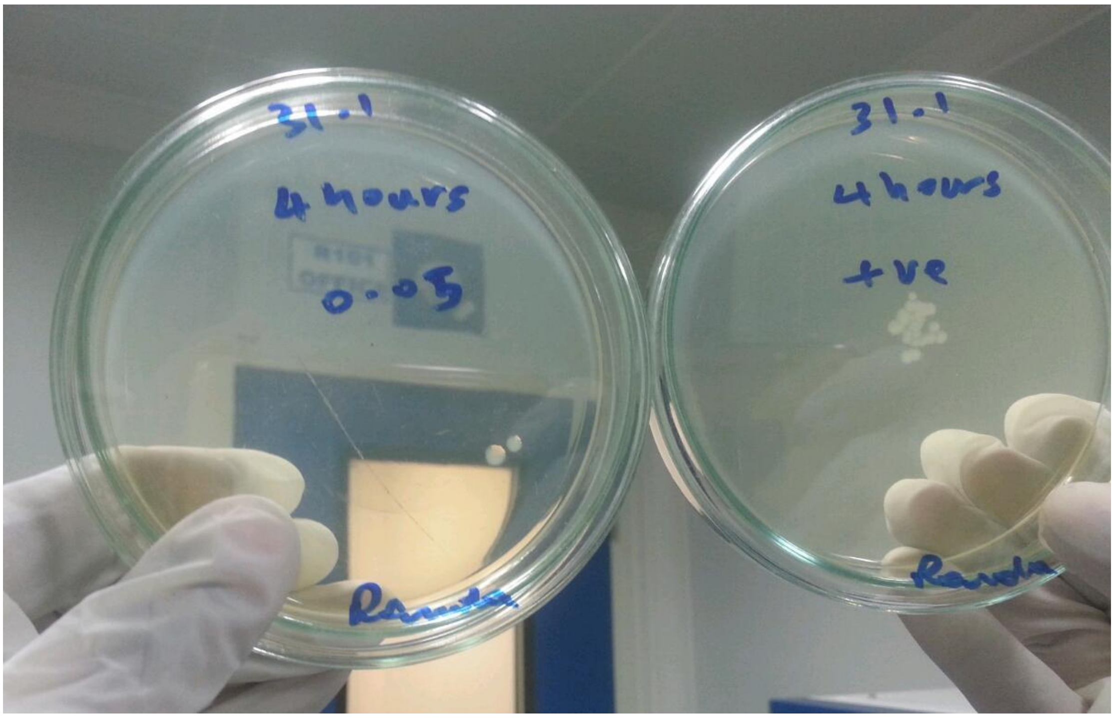

Fig 4.4 number of bacteria colonies after four hours of sun light exposure.

After five hours of sun exposure the number of colonies has completely disappeared in the sample that treated with TiO2, while in the other sample the colonies decreased to 2 colonies, Fig 4.5

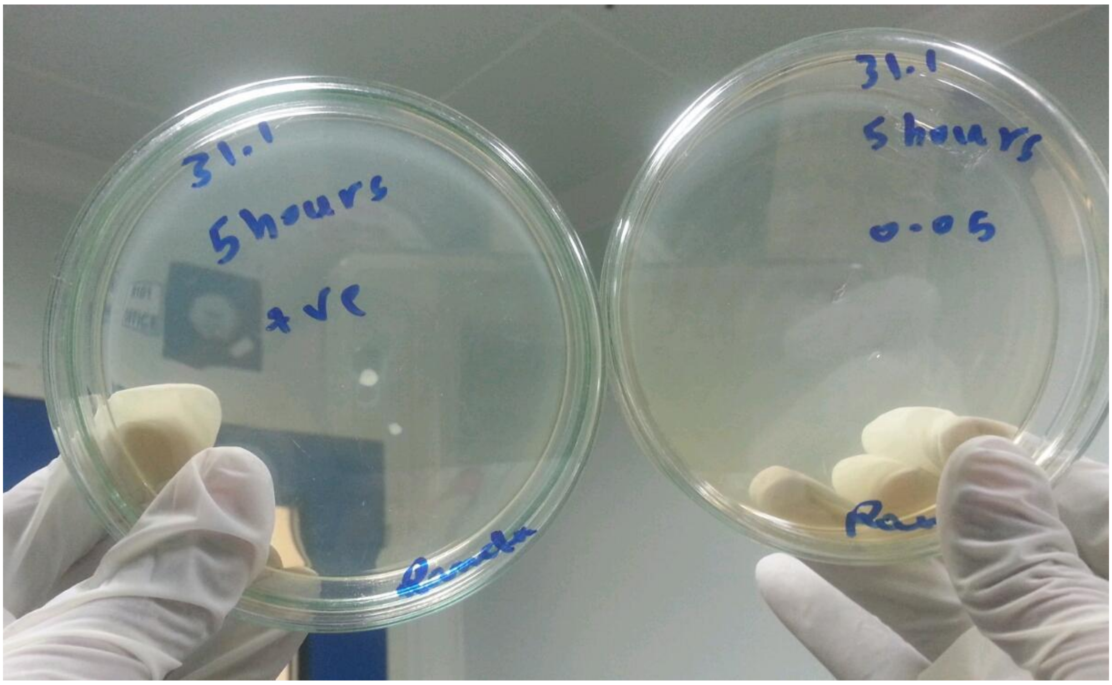

Fig 4.5 number of bacteria colonies after five hours of sun light exposure.

After 6 hours of sun exposure the number of colonies where also zero in sample treated with TiO_2, in the other sample the number of colonies were three. Fig 4.6.

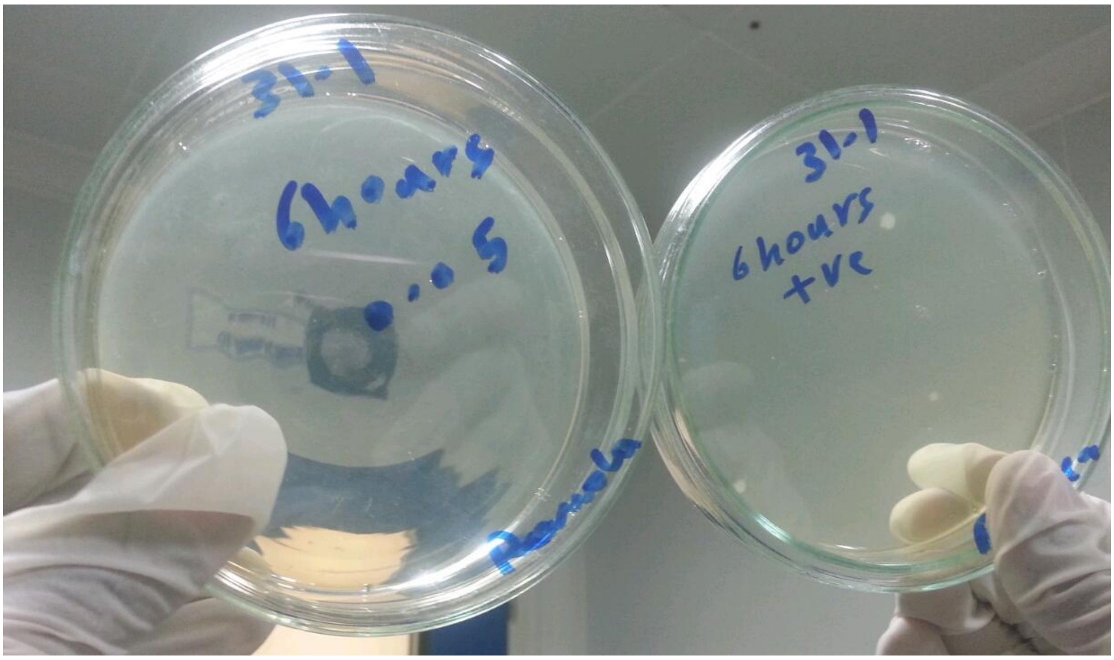

Fig4.6 number of bacteria colonies after six hours of sun light exposure.

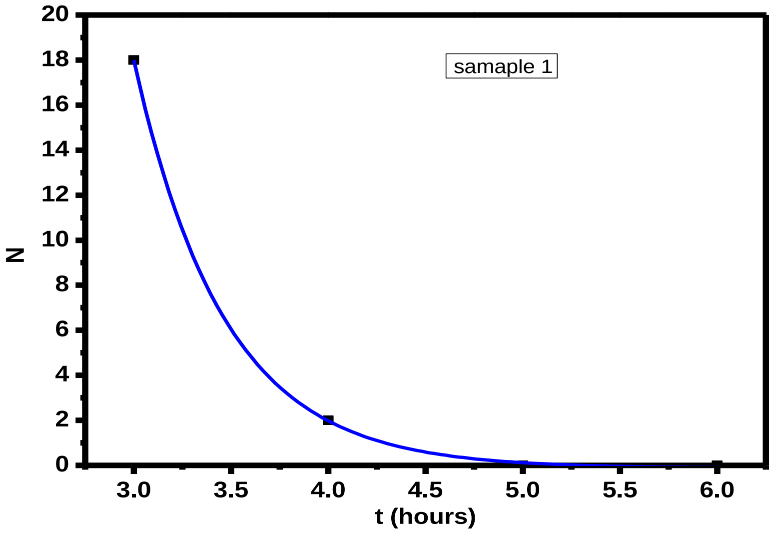

Fig 4.7 Effect of solar radiation with enhancing of TiO_2 on Bacteria E.Coli.

Fig.4.7 shows the change of the number of colonies (N) with time. The results show that the number of colonies decreases exponentially with time, according to the following relationship:

$$N = 12 \times 10^{3} e^{-t/0.46}$$

Where:

N= number of colonies

t= time in hours

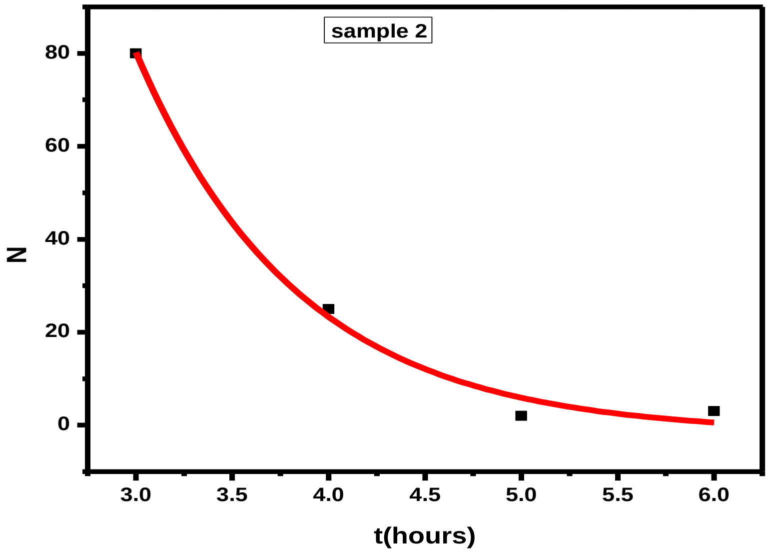

Fig 4.8 Effect of solar radiation only on Bacteria E.Coli.

Fig.4.8 shows the change of the number of colonies (N) with time. The figure shows that the number of colonies decreases exponentially with time, according to the following relationship:

$$N = 3 \times 10^{3} e^{-t/0.84}$$

Where:

N= number of colonies

t= time in hours

CHAPTER Five

Conclusion and Recommendation

5.1Conclusion

In the sample that was treated with ultraviolet radiation and $TiO_{2,}$ the number of colonies decreased from uncountable to 521 colonies in 2 hours of sunlight exposure. The colonies completely disappeared after 5 hours. In the sample without TiO_2 the number of colonies decreased to two colonies in five hours. The results have shown that TiO_2 can disinfect the bacteria effectively.

5.2 Recommendations

- More types of bacteria can be used in the future.
- Different type of sample containers can be used.
- More study about the concentration of TiO_2 can be done to test the effect of concentration on the bacteria colonies.
- Different type of water can be tested for example waste water or river water.
- It’s recommended to test this technique in water stations.

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