An-Najah National University

Faculty of Graduate Studies

Assessment of TiO_2 as Photocatalyest for Complete Mineralization of Aqueous Bacteria and their Organic Content

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Dedication

My heart pulsates with the thrill for tendering gratitude to those persons who helped in completion for my project. To my mother, father, wife, daughter, son, sister and brother for their continuous support with my appreciation.

The pleasant point of presenting a report is the opportunity to thank those who have contributed to build my knowledge. Unfortunately, the list of expressions of thank no matter how extensive is always incomplete and inadequate. Indeed, this page of acknowledgement shall never be able to touch the horizon of generosity of those who tendered their help to me.

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الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Assessment of TiO_2 as Photocatalyest for Complete Mineralization of Aqueous Bacteria and their Organic Content

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل أو جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية او بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced is the researcher's own work, and has not been submitted elsewhere for any other degree of qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:** 5/7/2022

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List of Abbreviations

Abbreviation	Term
a.u.	Arbitrary unit
ANU	An-Najah National University
AOPs	Advanced oxidation processes
CO_2	Carbon dioxide
CB	Conduction band
eV	Electron volt
E_g	Energy band gap
λ_{EX}	Excitation wavelength
h^+	Holes
OH•	Hydroxyl radical
M	Molarity
SEM	Scanning Electron Microscopy
TiO_2	Titanium dioxide
UV-Vis	Ultraviolet-Visible
VB	Valence band
XRD	X-Ray Diffraction
WHO	World health organization
UV	Ultraviolet light
TOC	Total Organic Carbon
S. aureus	*Staphylococcus aureus*
PL	PhotoLuminescence Spectra
NPs	Nano- particles
G	Gram
e^-	Electron
UAE	United Arab Emirates University
QY	quantum yield
CFU	colony forming units
TN	Turnover Number

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Abstract

Photodegradation is one of the most useful methods of water purification because it reduces pollution levels by eliminating harmful bacteria in the water and includes at a later stage the mineralization of chemical pollutants or pollutants within the water. Photo catalytic activity using titanium dioxide TiO_2 utilizes the longest wavelength located at the end of ultraviolet. In this work water was purified from positive bacteria by complete mineralization using of both types TiO_2 (Rutile) and TiO_2 (Anatase) using UV tail in solar simulated radiation. The results obtained revealed the high efficiency of TiO_2 (Anatase) in the destruction of bacteria and mineralization. This catalyst was able to destroy the bacteria G +ve S. aureus and baptized after exposure to 4 hours of light. The catalyst also proved its ability to inhibit bacteria in the dark but to a lesser extent than under radiation. It is therefore recommended to expand the present study and include other types of biological pollutant.

Chapter One
Introduction

1.1 General

Water is the condition of life. It occupies seventy one percent of earth surface. Only about (2.5%) of earth waters are fresh, round most of this is found in polar areas (~99%),while in rivers, lakes and the atmosphere 0.3% of fresh water is present [1, 2]**.**

Recently, fresh water challenge accrued in many parts of the world, UN statistics estimated that about one billion people on the planet [3] have no effective means of disinfection of water [4]. Non-potable water resulting after human usage is called gray water, which can be treated with ease [5].

Black water is a wastewater that requires comprehensive treatment. water pollution occurs on an individual and industrial level, especially in developing countries [6]**.** Low water quality has harmful effect especially on human health and the environment in general. The World Health Organization (WHO) estimates that about 1.4 million children die annually by diarrhea from contaminated water [7].

Environmental Pollution is one of the dangerous challenges to the human as it negatively affects daily lives. Environmental pollution includes Water pollution due to waste from factories and houses [8]**.** Air pollution caused respiratory diseases by sulfur and nitrogen oxide**s** [9]**.** Purification

may itself cause other problems. Therefore, natural resources must not be harmed from the beginning [10].

However, as contamination is a de facto, we must look for alternatives to address this problem by finding new products that do not cause additional damage to the environment while working to restore the environmental conditions using natural energy sources such as sunlight [11]. Solar light is a renewable low cost nonhazardous energy.

1.1.1 Biological pollution of the aquatic environment

Biological pollution refers to the presence of organisms in the fresh or saline water. Pathogenic microorganism, such as bacteria, viruses, parasites, algae and protozoa , also contaminates water [12, 13]**.**

Pathogens are spared from human and animal waste directly to fresh or salt water bodies, or indirectly through sewage or agricultural water. Such contaminants cause different diseases. Therefor contaminated water should be treated. Examples for treatment are chlorination, ozonation, mechanical filtering and others [4].

Water may contains safe species such as (oocytes, larvae, infectious phases) [14], and some parasites like schistosomiasis, hepatic worms and insets like mosquitoes.

1.1.2 Biological contaminants

Living organisms in water may interact with the aquatic environment, leading to imbalance in the environments, known as biological pollution [15]. Some of these organisms are more widespread than others in certain environments due to the nature and size of those organisms [16] and the

spread or scarcity of natural enemies in addition to competition between organisms in the same environment. For example, one gram of agricultural soil may contain more than 200 million bacterial cells and 300 thousand mushroom Parasites [17]**.**

1.1.3 Bacteria

Bacteria are the most prevalent microorganisms found in animal and human feces. Drinking stool-contaminated water is the major reason of water-borne infections, along with some bacteria. Fortunately only a few cells in water are pathogenic [18].

1.1.4 G+ve and G-ve bacteria

Bacteria are two types based on the ability to color with the Gram staining technique (Gram's negative bacteria and Gram's positive bacteria) [19]. Gram-positive bacteria (G+ve) bacteria maintain the color of the dye and have bacterial cell wall with thick peptidoglycan surface [19]. (Crystal violet to expands in Gram positive bacteria and yields purple gram pigment, making it need iodine to keep the crystal violet trapped inside the layer Peptidoglycan [20]. (Table1) Gram-negative bacteria (G-ve) contain a

thin cell wall of peptidoglycan, which is trapped between the cell membrane and the external bacterial membrane. There is additional protection from proteins and saccharide grease, which makes the bacteria resistant against antibodies due to the impenetrable cell wall [20]. They cause wide range of therapeutic cases (Table1).

Table 1.1: Comparison between G-ve and G+ve bacteria [20-22].

	Gram negative bacteria	Gram positive bacteria
Gram reaction	**Can be decolourized to accept counter stain (Safranin or Fuchsine) stain red or pink , they don't retain the G stain when washed with absolute alcohol and acetone.**	**Retain crystal violet day and stain dark violet or purple, they remain colored blue or purple with G stain when washed with absolute alcohol and water.**
Peptidoglycan layer	**Think (single layered)**	**Thick (multilayered)**
Outer membrane	**Present**	**Absent**
Resistance to physical disruption	**Low**	**High**
Cell wall composition	**The cell wall is 70-120 A Think : two layered . Lipid content is 20-30% (high), Murein content is 10-20% (low).**	**The cell wall is 100-120 A Think : single layered. Lipid content of the cell. Wall is low , whereas Murein Content is 70-80% (higher).**

1.1.5 Staphylococcus aureus

These are Gram positive (**G+ve**) types with non-moving small round shape. They are approximately 0.5-1.5 μm in diameter [23, 24]**.**

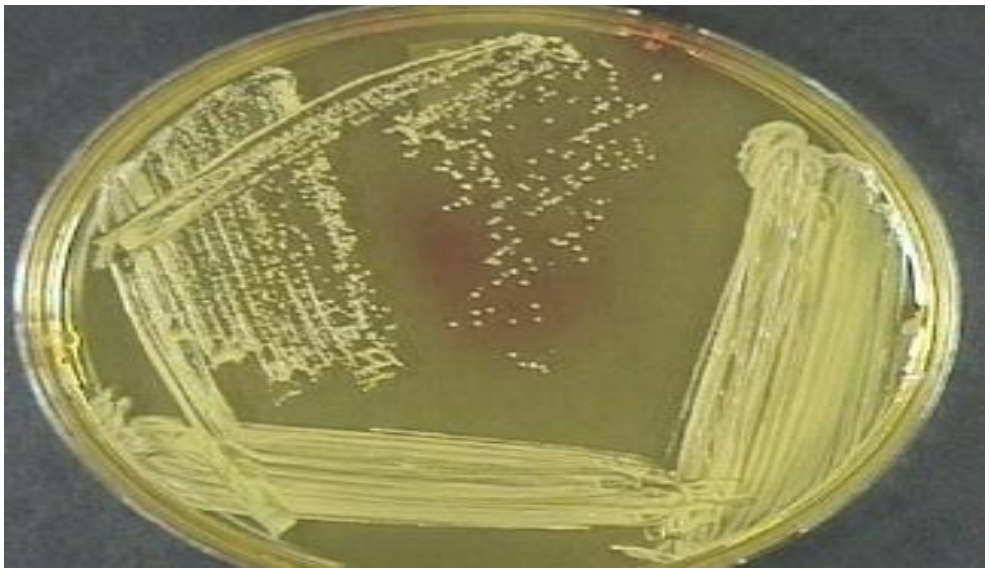

Figure 1.1: G+ve stain S.aureus cell.

Although it does not cause disease all the time , one of the diseases caused by this type of bacterium is the toxic shock syndrome, which leads to a severe disease accompanied by fever, widespread red rash with other organs affected [25, 26]. In environment S. aureus is relatively common but is mainly found on the skin and mucous membranes of animals. In the gastrointestinal track. S. aureus is rarely found and can be detected within sewage [24, 27]. Human contact with water environment like swimming pools, spa pools and other recreational waters may release S. aureus. It was also found in domestic water well [28].

1.1.6 Water purification

Water disinfection means the elimination, deactivation or mineralization of pathogenic microorganisms. Microorganisms are destroyed or deactivated resulting in termination of growth and reproduction. Decontamination is a process related to disinfection [29]. However, during decontamination

process all present microorganisms are killed, both harmful and harmless microorganisms [30, 31]. Disinfection can be chemical or physical. For physical disinfection of water several disinfectants can be utilized such as ultraviolet light (UV), electronic radiation, gamma rays and heat [32]. For chemical disinfection of water several disinfectants can be used such as chlorine dioxide (ClO_2), Ozone (O_3). Hypochlorite ion (OCl^-), halogens including chlorine (Cl_2), bromine (Br_2) and iodine (I_2), metal ions such as copper (Cu) and silver (Ag), potassium permanganate ($KMnO_4$), alcohols, soaps and detergents [31].

Table 1.2: Comparison between main disinfectants [35-[33] .

		Advantages		Linitations	
	Chlorine(Cl_2)	**Very effective for removing almost all microbial pathogens**	**Well-established technology more cost-effective than either UV radiation or ozone disinfection ; chlorine residual can prolong disinfection ; reliable and effective against a wide spectrum of pathogenic organisms effective in oxidizing certain organic and inorganic compounds ; flexible dosing control ; eliminate certain noxious odors**	**Chlorine is dangerous gas that is lethal at concentrations as low as 0.1 percemt air by volume**	**All forms of chlorine are highly corroive and toxic ; create hazardous compounds-disinfection by – products (e.g.,trihalomethanes(THMs))**
	Chlorine Dioxide Clo_2	**Very effective even in low concentration; broad-spectrum; quick and long time disinfection; dose not generate chlorinated phenols and THMs**		**Pungent odor ; should be stored in a dark area**	
	Sodium Hypochlorite Solution	**Easier to handle ; no disinfection by products**		**Very corrosive; decompose and should not be stored for more than one month ; stored in a cool , ark , dry area.**	
	Solid calcium Hypochlorite	**When packaged calcium hypochlorite is very stable allowing long time storage.**			
	Chloramine	**An effective bactericide; produces fewer disinfection by-products; generated onsite; Chloramine forming reactions are 99 percent complete withina few minutes.**		**A corrosive material with a strong odor that requires proper hndling : should be kept away from organic materials; readily absorbs moisture, forming chlorine gas.**	
Ozonation		**Requiring shorter contacttime and dosage than chlorine ; more effective than chlorine destroying viruses**		**Ozone gas is unstable and must be generated onsie ; does not maintain an adequate residual in water ; requiring complicated equipmet and efficient**	

How does disinfection work?

Disinfection commonly takes place by damaging the cell wall of bacteria, demoralize proteins or nucleic acids synthesis, changing cell membrane permittivity and effecting enzymes action. These dis-arrangements in cell activity inhibit microorganisms to grow [34, 35]. Oxidizing disinfectants also remove organic matter from water, and remove nutrients Figure (1.2) shows a sketch for the photo-catalytic inactivation of a bacterial cell process [36, 37].

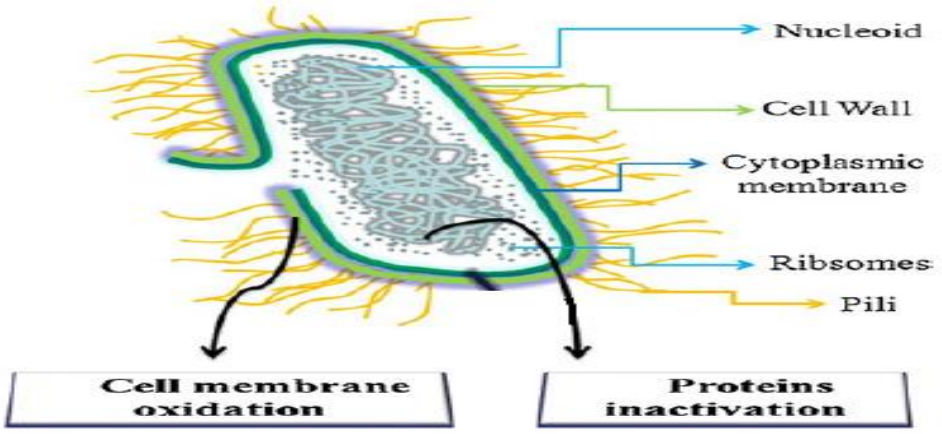

Figure 1.2: Inactivation of a bacterial cell [36, 37].

Disinfection by oxidation processes.

Advanced Oxidation Processes (AOP) are among the modern chemical techniques used for water disinfection [38]. Abiotic degradation (such as wet oxidation, chemical oxidation or acid-base hydrolysis) and photo-degradation by H_2O_2/UV,O_3/UV or O_3/H_2O_2/UV processes, solar photolysis, processes in vacuum ultraviolet or photo-catalysis are studied [34, 39].

What does Photo -catalysis Mean?

The word photo-catalysis is two parts. Part one "photo" means "light" and part two "catalysis" means "stimulation "of the catalysis process by a material. This material is considered a catalyst. It increases the rate of reaction by decreasing the energy needed to activate reaction [40]. Photo catalysis is therefore a process of light activation to increase the rate of chemical reaction without having role in the reaction itself. Photo-catalyst functions and uses can be divided into basic categories.. Water purification, Prevention of contamination, antibacterial, removing unpleasant odors, air purification [41, 42]. All of these applications in the presence of photo-catalyst depend on sunlight or ultraviolet light from any source [43].

Semiconductors have been chosen as photo-catalysts because they have relatively small energy gaps between the valance band and the conduction band [44, 45] as shown in Table (1.3).

Table 1.3: Band gap width for common semiconductor [46, 47].

semiconductor	Eg(ev)
Si	**1.1**
Fe_2O_3	**2.3**
TiO_2 (rutile)	**2.5**
TiO_2 (anatase)	**3.0**
ZnO	**3.2**
SnO_2	**(3.2-3.7)**

In photo catalysis, the semiconductor absorbs energy from the rays of the sun, or from an ultraviolet source that is at least equal to the energy gap [48].

A positive hole result and can oxidize molecules. In photo-catalysis, radiation excites the catalyst and the reactants are oxidized by oxygen as shown in Figure (1.3).

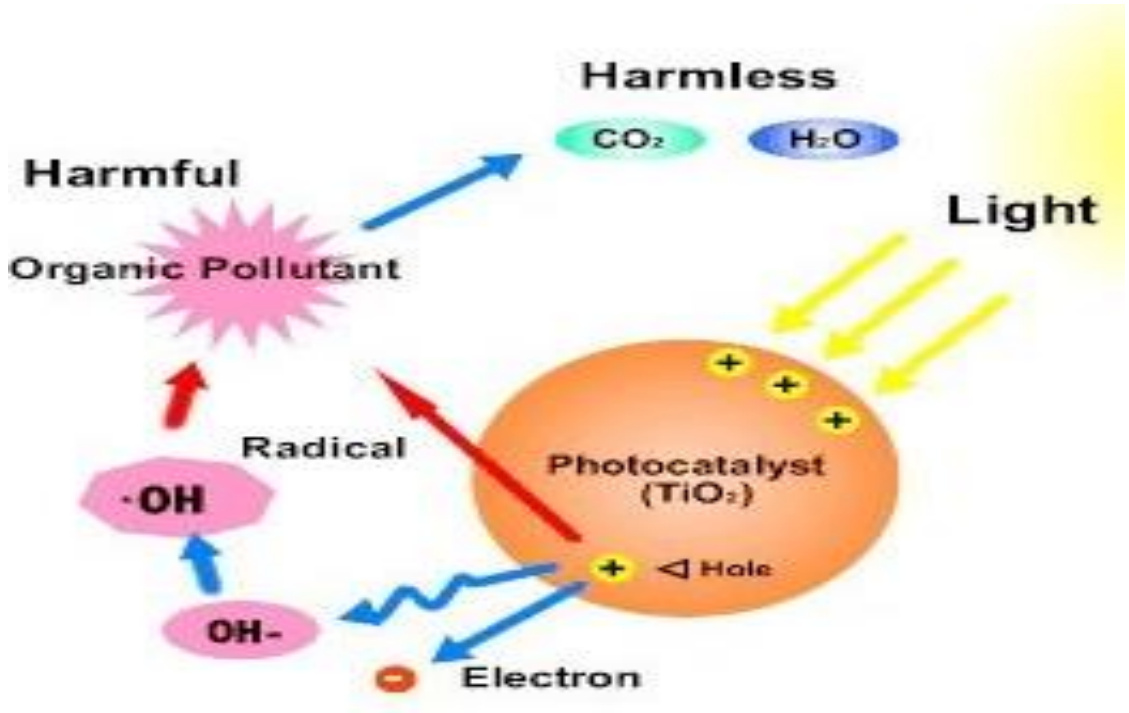

Figure 1.3: The role of titanium dioxide in photo-catalytic degradation [49, 50].

1.1.7 Titanium dioxide

Among semiconductors that can be used as a photo-catalysts is titanium dioxide, with the chemical symbol TiO_2 and energy gap Eg=3.2 Ev [51, 52].

Figure 1.4: Titanium dioxide powder TiO_2.

Titanium dioxide is best suited for several advantages as a photo-catalyst.

For example it is inert, corrosion resistant and needs less refining and preparation than other semiconductors, It is available at a low price under normal conditions.

Titanium dioxide absorbs ultraviolet light with enough energy to create electron and a positive hole. The electron in the titanium dioxide becomes excited and the electron e^- travels to the conduction band leaving a positive hole (h^+**)** valence band**.** In this case, dioxide of titanium becomes excited.

The titanium dioxide hole h^+ turns the water molecule into hydrogen and hydroxyl radical. The electron e^- reacts with dissolved oxygen and creates a very strong oxidizing anion O_2^-.This process goes on with radiation [53].

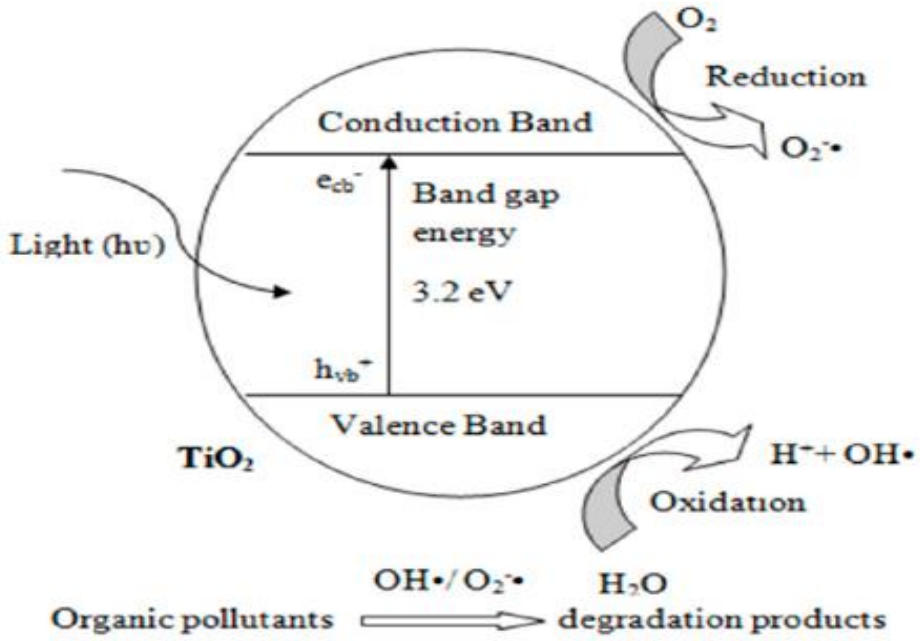

Figure 1.5: The mechanism of semiconductor photo-catalyst [54][55].

The reaction mechanisms are widely known and can be understood by Equations [55-57].

$$TiO_2 + h\upsilon \rightarrow e_{cb}^{-} + hv_b^{+} \quad (1)$$

$$h_{vb+} + H_2O \rightarrow H^+ + HO^{\bullet} \quad (2)$$

$$h_{vb+} + R \rightarrow \text{intermediates} \rightarrow CO2 + H2O \quad (3)$$

$$e_{cb}^{-} + O_2 \rightarrow O_2^{\bullet -} \quad (4)$$

$$HO^{\bullet} + R \rightarrow \text{intermediates} \rightarrow CO_2 + H_2O \quad (5)$$

It is generally known that the photo-catalytic disinfection of water requires exposure to irradiation of equivalent or higher energy than the band gap of the semiconductor.

Zinc Oxide is classified as a semiconductor in group II- VI, whose covalence is on the boundary between ionic and covalent semiconductors. A broad energy band (3.2-3.37 eV) with high thermal and mechanical stability at room temperature makes it attractive for potential uses [25].

Titanium dioxide(TiO_2), has unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photo-stability [58].

Titanium dioxide (TiO_2), has emerged as an excellent photo-catalyst material for environmental purification and it used in the area of photo catalysis, mainly photo catalytic air purification, sterilization and cancer medication [48].

1.2 Literature review

In 2015, various morphologies of ZnO Nano-materials were synthesized and used as photo-catalyst for self-sensitized photo-degradation of Malachite Green (MG) under solar light ZnO flowers exhibited highest photo-catalytic activity, with complete mineralization of dye to produce CO_2 [59].

In 2016, Venieri and his team worked on doping titania with non-metals to decrease its band gap energy to less than value of 3.2 eV. Disinfection effect of cation-doped titania in S. aureus elimination in aqueous samples under artificial and natural sunlight was investigated. For Fe, Al and Cr doped TiO_2, the relative activity for S. aureus inactivation decreased in the

order Fe-TiO_2> Al-TiO_2> Cr-TiO_2 with 99.9% deactivation of S. aureus population after 60 min [60].

In 2017, a novel combined solar system with a TiO_2 thin-film photo reactor and a pasteurizer operating under continuous flow was tested. both bacteria inactivation and organics degradation where studied [61]. In 2017, electrochemical oxidative degradation of diazo dye amido black 10B (AB10B) as model pollutant in water has been studied by using nanostructured ZnO-TiO_2 thin films deposited on graphite felt (GrF) substrate as anode [62].

In 2009 Gurudev Sujatha and his team studied the applicability of UV photo catalysis for the treatment of coffee processing wastewater with TiO_2 as a catalyst. The experimental runs were carried out with, wastewater produced by an Indian company. In addition to the applicability of the photo-catalysis, parameters were tested, namely, first wastewater pH, load of catalyst, UV light potential, kind of catalyst and oxidant loading. Finally, the use of solar light instead of UV was tested , and the performances obtained in both cases were compare the use of photo-catalysis [56].

1.3 Objectives

The main purpose of this work is "Disinfection of water by complete deactivation and mineralization of G+ve bacteria by using a low cost and safe process of photo mineralization".

1.4 Novelty of this work

Literature showed that TiO_2 nano-particles can inhibit bacteria under solar light However literature does not describe what happens to the inhibited bacteria. As bacteria involve organic matter, which can be hazardous to humans, such matter may remain in water. Literature did not describe how to remove such organic materials from drinking water.

This proposed work is a novel. It will the assess ability of TiO_2 photo-catalysts to completely inhibit bacteria and mineralize their organic content under direct solar light. Such study has not been described in literature to our knowledge.

Chapter Two
Materials and Methods

2.1 Materials

2.1.1 Titanium dioxide (TiO_2)

Two types of titanium dioxide granules (Anatase and Rutile) were used. Commercial powders (catalog no 22,422-7 and 1317-80-2) were taken from (Sigma Co.) and used in photo-degradation of contaminated water samples.

2.1.2 Bacteria

Positive Gram+ bacteria named S.aureus was used. This type of bacteria was isolated from clinical sample obtained from Department of Medical Science laboratory at an-Najah National University.

2.1.3 Other chemicals

Ethanol, sodium hydroxide and hydrochloric acid were purchased from Aldrich –sigma Co. or from Frutarom Co.

Nutrient broth was purchased from Hi Media Lab Pvt. Ltd, India and nutrient agar was bought from USA company Sparks, Becton, Dickinson.

2.2 Instruments

2.2.1 UV–visible spectrophotometry

A Labo Med, Inc. 1601spectrophotometer was used to measure bacterial concentration. Quantitative determination of the bacterial concentration was made through the use of tubative method. By measuring the amount of light that passed through bacterial contaminated water. The more suspended bacterial cells show more turbidity, and more turbidity means less light passing through the suspension.

The wavelength 630 nm was used in the absorption range (0.08-0.1) where the initial concentration of bacteria equals 1.5×10^8 CFU\mL. This is consistent with the standard McFarland turbidity.

2.2.2 Photoluminescence spectra device (PL)

Perkin-Elmer LS50 Luminescence Spectrophotometer was used to measure the emission fluorescence spectra for TiO_2 (0.1g) in 50 ml water anatase and rutile. Excitation wavelength was (360) nm.

The emitted spectrum was used to calculate the semiconductor catalyst band gap. The gap was compared with the literature [46, 47, 63-65] where the spectrum was studied for each of commercial anatase and commercial rutile.

2.2.3 Light sun simulator

An intermediate type sunlight simulator lamp is used. Maximum 400 watts. The intensity of the radiation passing through the water was controlled in order to obtain accurate result.

2.2.4 Lux-meter

A Lux-meter (Lx-102 light meter) was used to measure the intensity of light or radiation pass through tested polluted water to determinate the efficiency of naked catalyst. and was set to ~100000 lux (similar to solar light intensity about 1,360 watts per square meter).

2.2.5 XRD

X-ray diffraction was used to identify and examine crystallization of the catalyst. The patterns for dry powder were recorded using Philips XRD XPERT PRO diffractometer with CuK with X-ray wavelength (λ=1.45 Å). The analysis was kindly performed at UAE University, Al Ain, UAE.

2.2.6 SEM

SEM photos of the samples were taken using a Jeol Model JSM-6700F. The analysis was performed at UAE University, Al Ain, UAE.

2.2.7 TOC

The Total Organic Carbon (TOC) was used as an equivalent to the organic content of water in order to assess the degree of mineralization of treated aqueous solution. Inorganic carbon was determined by the acidification of the sample in the rate 1% hydrochloric acid was measured before injection and organic carbon was burned ~at 800 Cº. The total Organic Carbon (TOC) was calculated using a SHIMADZU Model TOC-L CSH\CSN. Each measurement was repeated three times, and the average was taken for nearest values. The analysis was kindly performed by Faculty of Agricultural Science and Technology at Palestine Technical University.

2.3 Preparations

2.3.1 Preparations related to bacterial treatment.

G+ve S.aureus bacteria were used. Nutrient agar were prepared according to manufacturer's instructions and poured after sterilized in dishes in a regular manner in order to facilitate the counting process The nutrient broth was prepared according to the manufacturers. Instruction and then poured into the test tube 5ml of each tube sterilize by autoclave.

Saline solution was prepared in concentration of (0.9 %) by dissolving 9.00 g of NaCl salt in 1000_{mL} distilled pure water and then poured into test tube at rate of 9 $_{mL}$ each tube then sterilized by autoclave. The previously prepared saline solution is used to prepare fold dilutions of bacteria in polluted water samples to count bacteria.

All prepared materials and tools were autoclaved at 121 ^{o}C under 1.5 atm, and kept in retreater (~4ºC) for the sake of different analysis.

In order to prepare the bacteria we take , a few bacterial colonies were taken and put them in 5 mL nutrient broth tube and incubated for period of 2-4 hours at 37 ºC [66]. Then the sample was analyzed by using UV–Visible spectrophotometry at 630 nm wavelength and then bacteria were diluted until device reading was in the range (0.08 - 0.10) [67]. It expresses the concentration of McFarland were concentration of bacteria is 1.5×10^{8} CFU/mL [68, 69].

In all experiments 1×10^{6} CFU/mL bacterial concentration was treated. This concentration was obtained by the following equation :

$$(\text{Molarity}_1 \times \text{Vol}_1)_{con} = (\text{Molarity}_2 \times \text{Vol}_2)_{dil}.$$

$$(1.5 \times 10^{8}\ CFU/mL_1 \times \text{Vol}_1)_{con} = (1\times 10^{6}\ CFU/mL_2 \times 50mL_2)_{dil}.$$

Vol_1 taken from concentrated bacteria solution = 0.330 mL.

Where, Moarity_1 is McFarland concentration which obtained by UV–Visible spectrophotometry at 630 nm wavelength (1.5×10^{8} CFU/mL).

Vol_1 is taken from McFarland concentration. Molarity_2 is needed bacterial concentration to be treated in 50 mL sample. Vol_2 is sample volume (50 mL).

2.4 Catalytic experiment

2.4.1 Bacterial disinfections

As shown in Fig. (3.1), each container includes 50 mL of distilled and sterile water. The first was called a control sample that contained only bacteria. The second container contained bacteria treated with TiO_2 anatase. where both pots were exposed to sun simulated light in specific and multiple period of time and two other pots were made each containing 50 ml of sterile water the first cup contains bacteria only, the other contains bacteria treated with (TiO_2) anatase where placed in the dark under same condition and in specific and multiple time period.

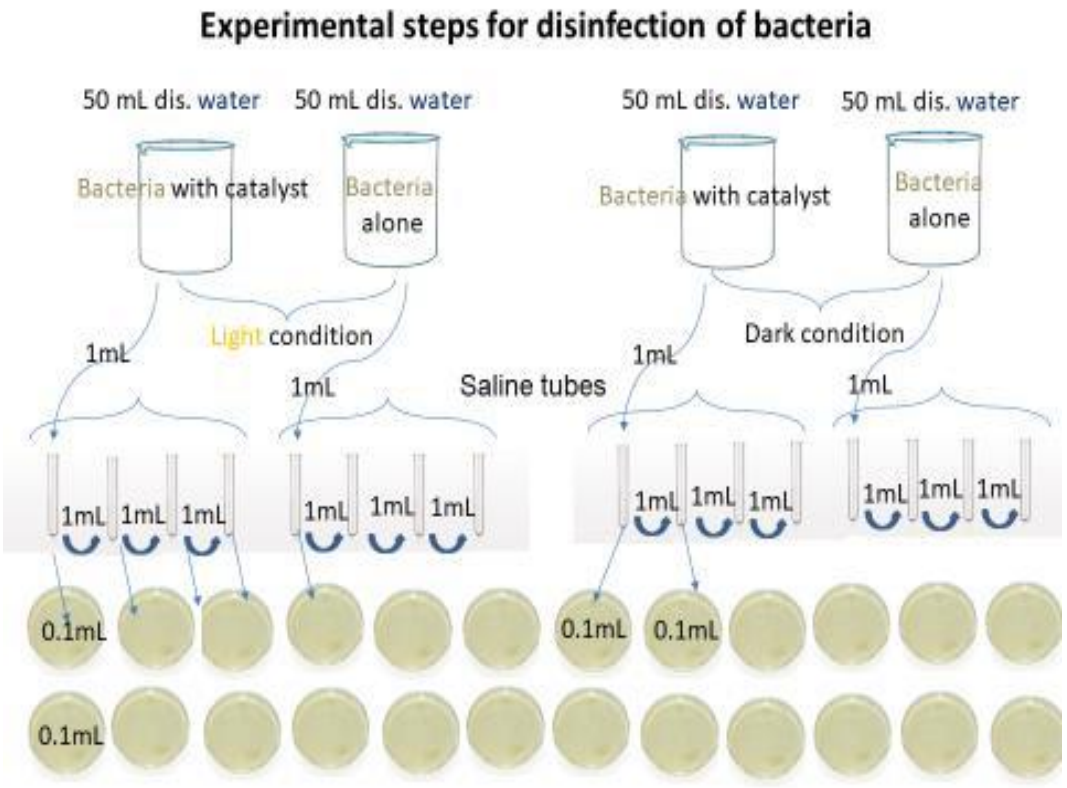

Figure 2.1: Experimental steps for bacterial deactivation by using several catalyst systems [70].

Finally, for all experiments and at the end of selected time, 1000 μL of each treated sample was withdrawn using a micropipette and diluted in a series of saline solution tubes with different dilutions (0.1, 0.01, 0.001 dilutions) [71].

From each diluted, aliquots of 0.1 mL were taken by yellow tip and were spread on two nutrient agar Petri dishes using bent glass rod, then incubated for 24 hours at 37 °C. After incubation, the bacteria were grown and colonies in each plate were counted using plate count method, only plates which have colonies between 30 and 300 colonies were considered.

The average number of each two plates for each dilution was calculated and the bacteria concentration as CFU/mL unit was reported according to the following equation:

Bacterial conc = (Average number of colonies × Dilution factor ×10) [72].

$$\text{The \% deactivation} = (\text{Conc. initial} - \text{Conc. final}) \div \text{Conc.intial} \times 100\%)$$

- Conc.final is bacterial final concentration.
- Conc.initial is bacterial initial concentration.

where the initial concentration was taken from samples contained bacteria only under sacrifice condition (control sample), and the final concentration was taken from samples treated with TiO_2. Experiments were tested on a G+ve .S.aureus bacteria.

2.4.2 Impact of various parameters on bacteria mineralization

The effects of various parameters on photo-mineralization efficiency were studied as follows:

2.4.2.1 Time effect

Different reaction times (10, 30 and 60 min) were tested. Experiments are made with constant (0.1g) catalyst amount and bacterial concentration 1×10^6 CFU\mL at temperature 28 ºC and (pH~ 7) both TiO_2 anatase and rutile were used against S.aureus and under irradiation using sun light simulator. After 10 minutes the differences were observed in ability to destroy bacteria using TiO_2 anatase and TiO_2 rutile system. After 60 minutes total bacterial deactivation was achieved

2.4.2.2 Influence of bacteria concentration

Effect of concentration of bacteria (1×10^6 , 3×10^6 , 5×10^6 CFU\mL) on the mineralization efficiency of S.aureus studied [73].

2.4.2.3 Effect of pH

Based on earlier literature PH values (5.7, 7.3, and 8.08) these pH values are suitable for bacteria growth were used to study the effect on the mineralization of bacteria loss [34, 74-76].

2.4.2.4 Temperature effect

Bacteria can live at (20 ºC,30 ºC, and 37 ºC) these temperatures used to study the effect of temperature on the mineralization of bacteria [25, 67, 77].

2.4.2.5 Effect of catalyst amount

Catalyst quantities (0.1,0.05 and 0.025g) TiO2 anatase were used for economical purposes. A constant concentration of bacteria (1×106) CFU/mL at 28 ºC and pH =7.3 under the sun–simulation irradiation was used. measurements were made after (10, 30, and 60 min).

2.4.2.6 Catalyst reuse

After the completion of the mineralization process, the treated solution was completely sterilized. Then it was contaminated with a quantity of bacteria (1×10^6) CFU/mL. The process was repeated again twice in order to study the catalyst, recovery and reuse.

Chapter Three
Results and Discussion

3.1 Preliminary remarks

The main aim of this research was to disinfect water by complete deactivation and mineralization of a G+ve (S. aureus) bacteria by using a low price, secure method passed on with solar light. Naked TiO2 anatase and rutile were characterized using PL, Electronic Absorption Spectra, SEM, and XRD instruments.

TiO_2 systems were then used to catalyze the photo-mineralization of G+ve

(S. aureus) using simulated light. Complete mineralization of bacteria and their organic contents was tested by measuring TOC in reaction mixture. Finally, factors impacting photo-mineralization reaction and catalyst efficiency were studied.

3.2 Catalyst characterization

3.2.1 Photoluminescence spectra (PL)

The photoluminescence (PL) emission spectra were measured for commercial (TiO_2 anatase) and (TiO_2 rutile). The purpose is to find E_g for each TiO_2 system. As shown in Fig.(3.1), PL spectra collected using 300 nm excitation wavelength show maximum wave lengths.

PhotoLuminescence (PL) spectra for TiO2 both system

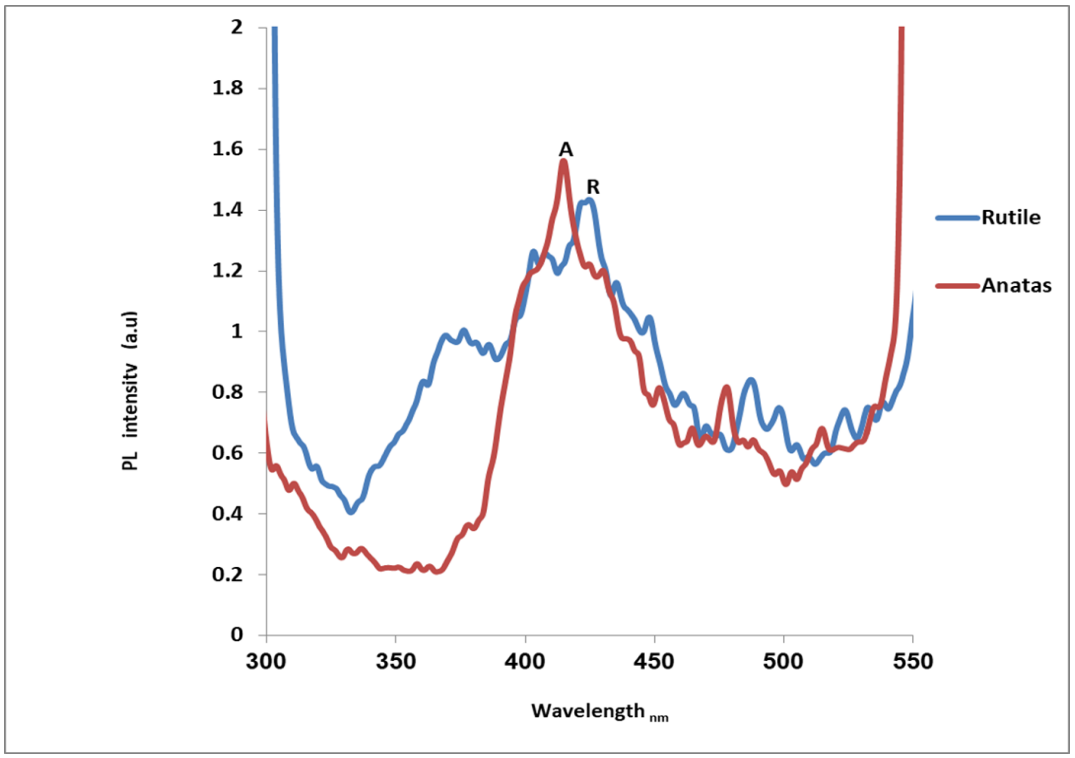

Fig 3.1: (PL) spectra of 0.1 g TiO_2 dispersed in 50 mL distilled water under 300 nm UV excitation wavelength, for commercial anatase (A) and rutile (R) system**.**

By using equation (E_g (eV)= h×c ÷ λ max $_{(nm)}$) or (eV=1.2398÷ λμ_m max) [78, 79] were h is blank constant ,c is light speed in space and λ is maximum wavelength recorded and intense emission peaks shown in Fig.(4.1).

The E_g values for TiO_2 systems were calculated using (Eg (eV)= h×c ÷ λ max (nm)) or (eV=1.2398÷ λμm max)**,** as brief in Table(4.1).

Table 3.1: Wavelengths (nm) found on highest point in PL spectra and energy Band Gap determined by Eg equation, for TiO_2 systems.

	Commercial TiO_2 anatase	Commercial TiO_2rutile
λ(nm)	415	425
Eg(eV)	2.987	2.91

As in Table (4.1) TiO_2 systems give (2.987, 2.91 eV) band gap values. Our results are within the literature range of TiO_2 (2.98-3.05 eV). Eg gives indication about particle size.

When Eg value increases, particle size decreases Since the small particle has low band gap energy and thus shifted to the absorption to the lower wavelength, somehow, a higher particle size behaves contrarily [80-82].

3.2.2 SEM results

Both commercial TiO_2 (anatase and rutile) were studied using Scanning Electron Microscopy.

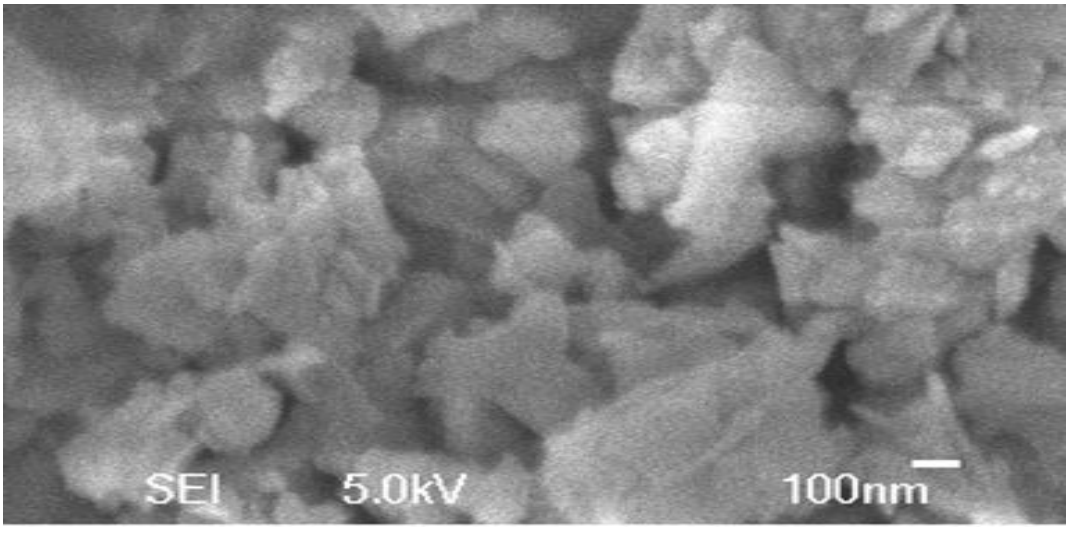

Figure 3.2.a: SEM images for solid TiO_2 powders commercial anatase.

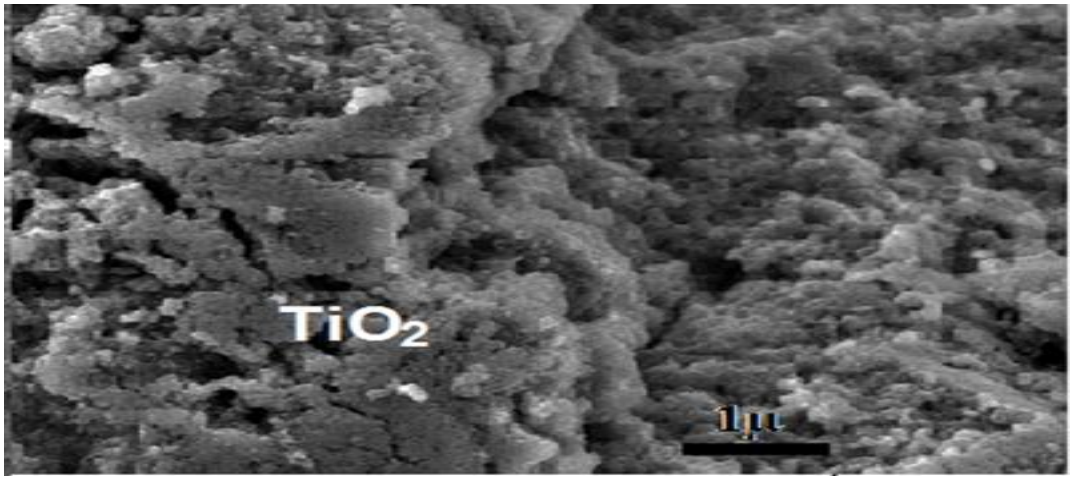

Figure 3.2.b: SEM images for solid TiO_2 powders commercial rutile.

The micrographs show that the commercial rutile TiO_2 has large agglomerates (~230 nm), involving nanoparticles (average ~50 nm) as confirmed by XRD below. Based on SEM, the anatase TiO_2 has agglomerates (~250 nm), with average nanoparticles in the range 45-50 nm as confirmed by XRD(smaller particle), as shown below.

3.2.3 XRD results

The average crystallite sizes of the samples were studied by using Debye Scherer's equation $DP=0.94\lambda\backslash\beta\cos\theta$ [83], using the full width at half maximum height of the X-ray diffraction peaks, Where, Dp = Average Crystallite size, β = Line broadening in radians, θ = Bragg angle, λ = X-Ray wavelength.

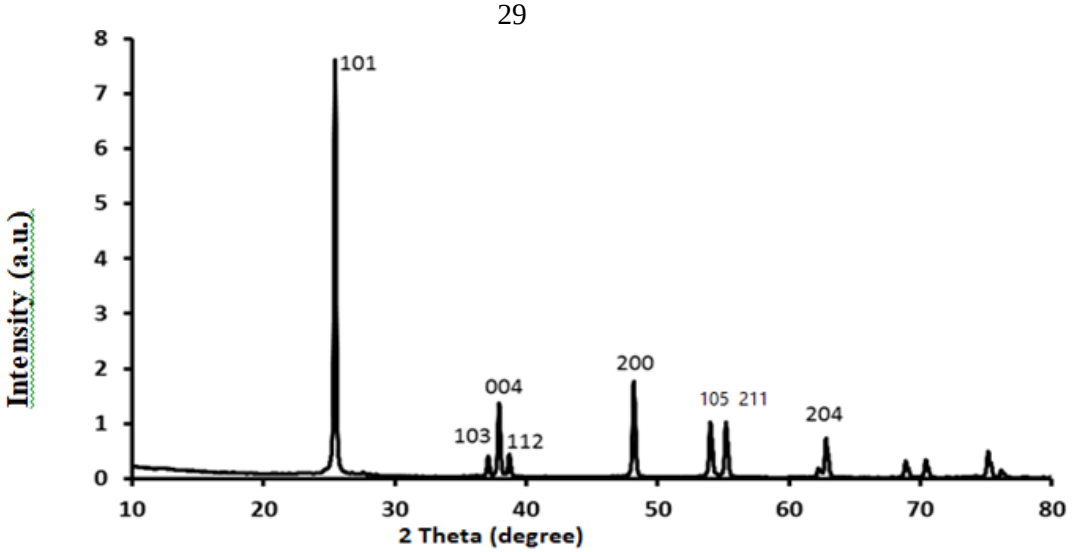

Figure 3.3: XRD patterns for commercial (TiO_2 anatase systems).

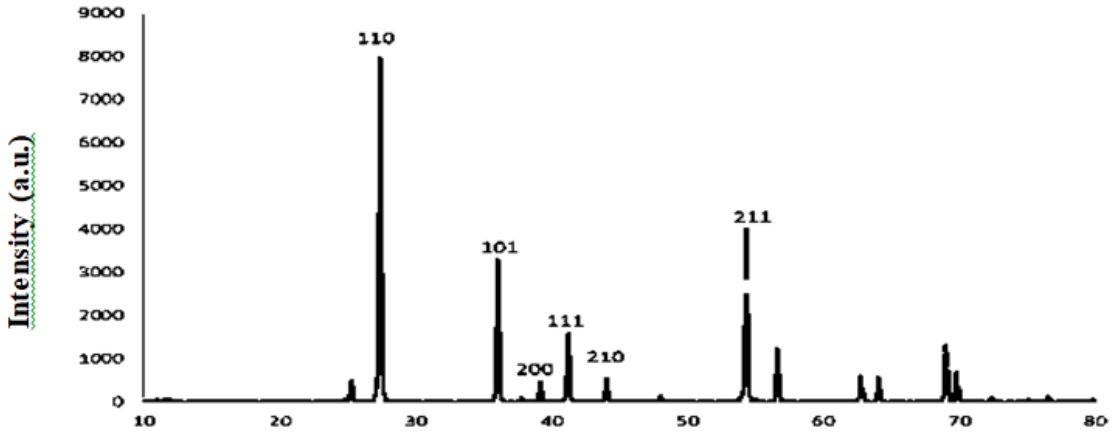

Figure 3.4: XRD patterns for commercial (TiO_2 rutile systems).

Table (3.2) summarizes Crystallite size in D (nm) for both commercial TiO_2 catalysts, based on Debye Scherrer's equation [84-88].

Table 3.2: Average crystallite size of commercial TiO_2 anatase and rutile calculated by XRD.

X-ray diffraction peaks	Crystallite size, D(nm) for commercial TiO_2 anatase	X-ray diffraction peaks	Crystallite size, D(nm) for commercial TiO_2 rutile
101	41.53	110	48
200	46.12	111	53.01
211	47.76	101	51.15
	Average = 45.13		Average = 50.72

Table (3.2) shows that average crystallite size for commercial TiO_2 anatase catalyst was (45.13 nm) and for commercial TiO_2 rutile catalyst was (50.72 nm). Smaller size for anatase means higher surface area of catalyst which increases its efficacy in disinfection of polluted water.

By these results we expect that anatase catalyst will be more efficient than rutile commercial catalyst in deactivating bacteria.

Both SEM and XRD results summarize the particle size for both TiO_2 systems. Both SEM and XRD results show that anatase powder has smaller crystallite size than commercial rutile.

3.3 Simulated solar irradiation experiments

Both TiO_2 anatase and TiO_2 rutile catalysts were used against G+ve S. aureus bacteria under solar simulated radiations. Performances of TiO_2 anatase catalysts were found with quantum yield (QY), turn over frequency (TF) and deactivation percent, which are simply calculated by following methods:

QY = bacterial colonies concentration lost experimentally/number of incident photons (n)

Energy (J) = Incident power per unit area × Exposure time (s) × exposed area (cm^2).

Assuming average wavelength of incident light is 630 nm, then υ=c/ λ, and E(J) = nhυ

Where:

M. wt of TiO_2 = 79.866 g/mol.

Incident power per unit area for solar light = 0.0146 w/cm^2

exposed area = 23.75 cm^2 , c = 3×10^8 , h = 6.62×10^{-34}

Average wavelength of incident light for UV light = 630 nm, n = number of photon.

Turnover Number (TN) = Number of deactivated bacteria / unite of TiO_2 [89] , TF =TN / Time.

deactivation percent = [Number of deactivated bacteria / Number of initial bacteria]×100%.

Incident power per unit area for solar light = 0.0146 w/cm^2 [90].

3.3.1 Impact of time on bacterial deactivation

Fig. (3.5) illustrates the influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) of G +ve S. aureus bacteria treated by 0.1 g of (anatase and rutile TiO_2) in separate experiments under light.

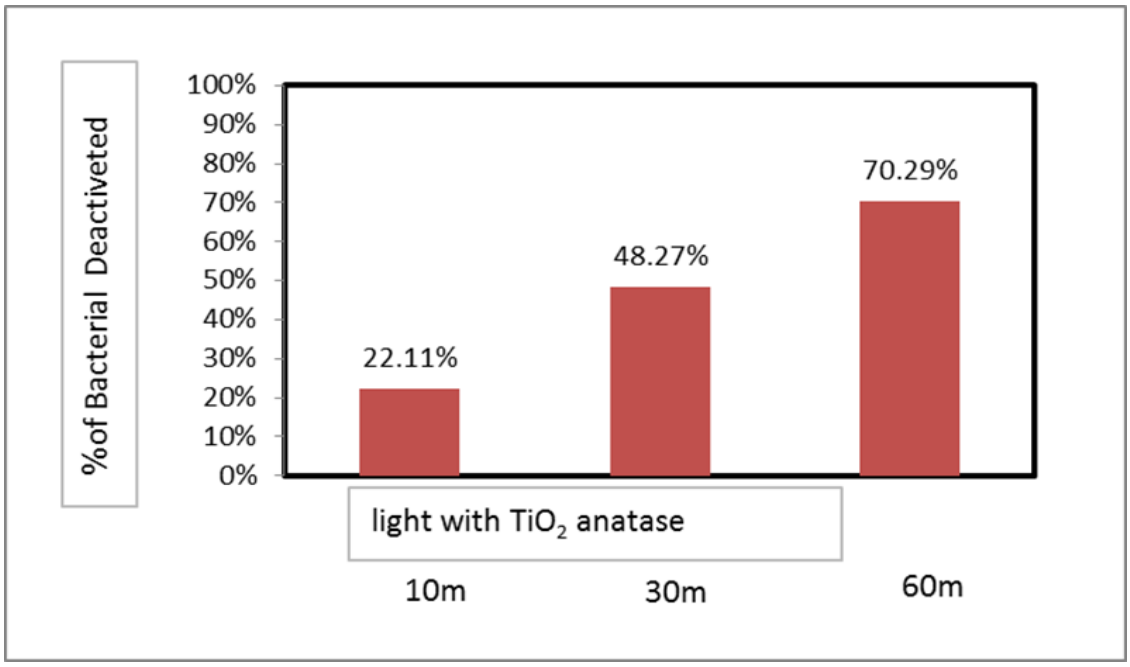

Figure 3.5.a: Influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) S. aureus bacteria, catalyst loading 0.1g (anatase TiO_2 catalyst) at 28 ºC under light condition and pH=7.4

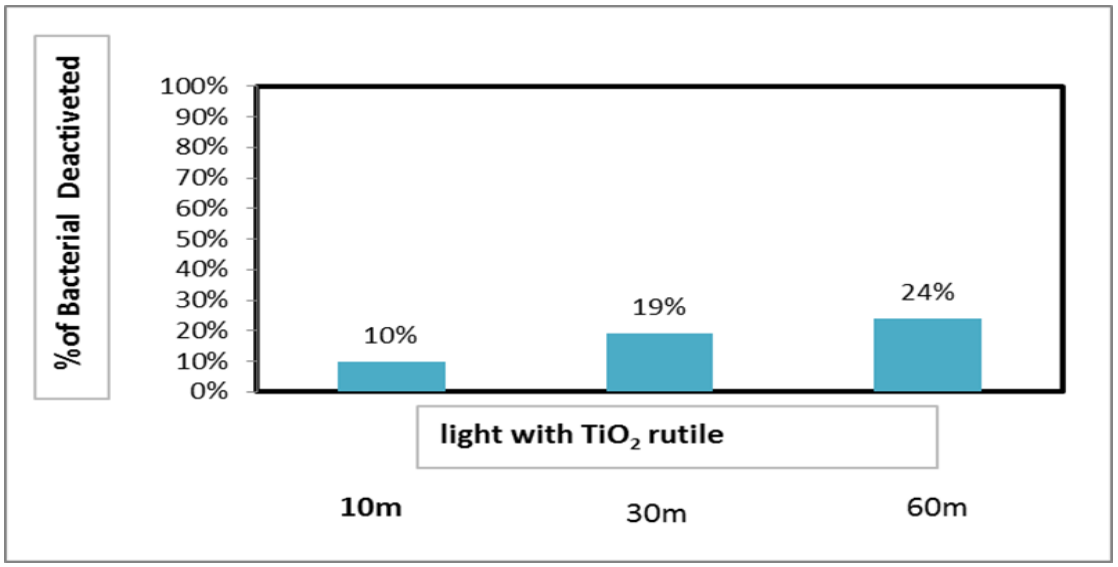

Figure 3.5.b: Influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) S. aureus bacteria, catalyst loading 0.1g (rutile TiO_2 catalyst) at 28 ºC under light condition and pH=7.4

Fig. (3.5.a) and Fig.(3.5.b) shows that after 60 min 70.29% (S.aureus bacteria) were deactivated when we use anatase TiO_2. only 24% was deactivated when we use rutile TiO_2 .Therefore, shorter exposure time (10 min) and (30 min) was necessary to compare the effectiveness of (anatase TiO2) and (rutile TiO2). The deactivation percent by anatase TiO_2 which was 48.27% after 30 mint compared to Rutile TiO_2 which was (only 19%) for S. aureus treatment.

The deactivation percent of anatase TiO_2 was to 22.11% after 10 mint compared to Rutile TiO_2 (only 10% after 10 min) for S. aureus treatment.

We conclude from this result that (rutile TiO_2) is less effective than (TiO_2 anatase) in the process of deactivate bacteria. Therefore anatase will be used in the subsequent processes due to its high efficiency to deactivate this type of bacteria. Tables (4.4) give summery values of quantum yield (QY), turnover frequency (TF) and deactivation percent of (1×10^6 CFU\mL) of S. aureus bacteria using 0.1 g of TiO_2 anatase in light for (10, 30 and 60) min.

Table 3.3: Values of QY, TF and deactivation percent for different exposure times. All experiments were done using 0.1 g of TiO_2 anatase against S.aureus (1×10^6 CFU\mL) at 28 ºC and light condition.

Time	QY	TF$\times10^{-17}$ min^{-1}	TN$\times10^{-16}$	% bacterial deactivation
10 m	1.377×10^{-38}	1.327	1.372	22.2%
30 m	1.28×10^{-44}	1.239	3.712	48.27%
60 m	1.06×10^{-44}	1.169	9.416	70.29%

Table (3.3) shows that OY and TF values. Were higher at the penning of treatment, due to higher bacterial concentrations, which promoted more deactivation for both bacteria types. As the reaction begins, bacterial

concentration was lowered which caused decrease in the deactivation percent per time unit [91, 92]. Another reason is also possible. As reaction progressed, organic molecules resulting from deactivated bacteria become more and catalyst functioned to decompose both these organic molecules and the remaining bacterial cells [25, 93]. Fig. (3.6.a) and (3.6.b) shows the influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) of G+ve S.aureus pneumonia bacteria using 0.1g of (both rutile TiO_2) and (anatase TiO_2) in separate experiment dark conditions.

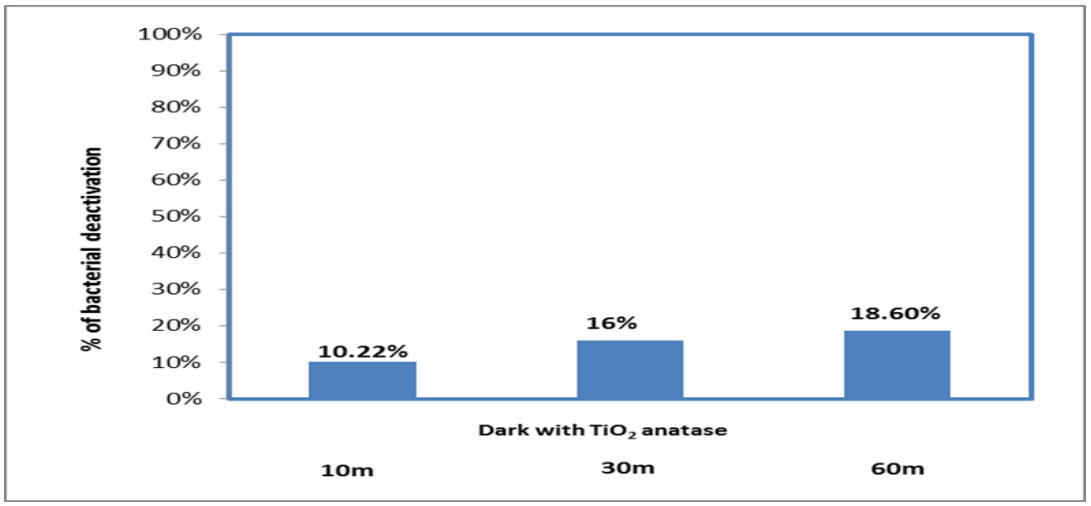

Figure 3.6.a: Influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) bacteria S. aureus bacteria , catalyst loading 0.1 g from anatase at28ºC and pH=7.4 in dark.

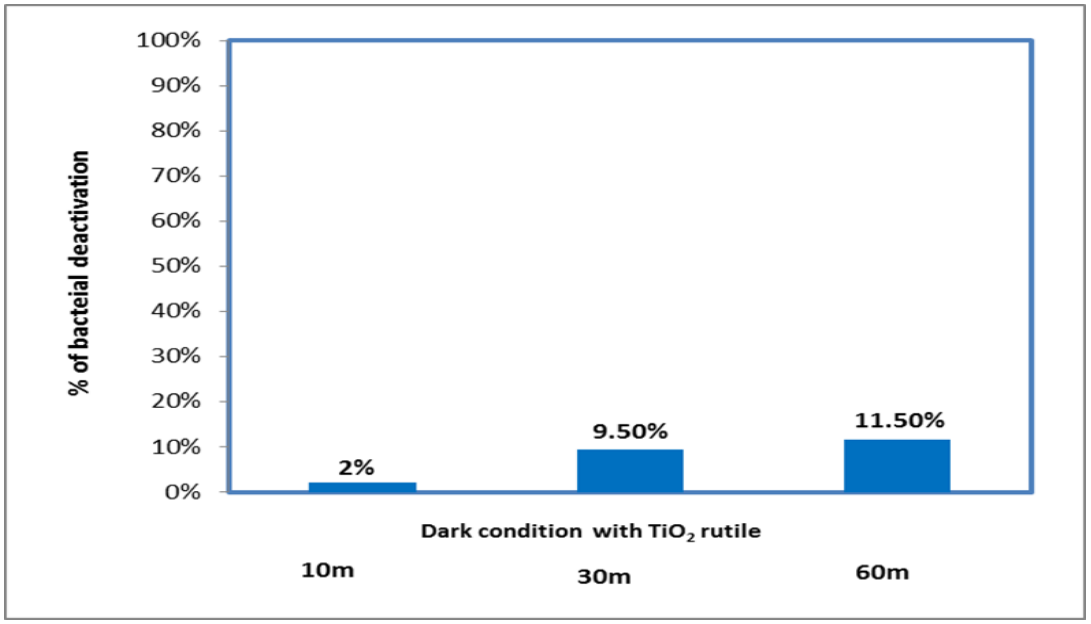

Figure 3.6.b: Influence of exposure time on the deactivation percentage of (1×10^6 CFU\mL) bacteria S. aureus bacteria , catalyst loading 0.1 g from rutile at28ºC and pH=7.4 in dark.

As shown in Fig.(3.5) and (3.6) the deactivation percent was Sharp decline When comparing the two experiences in the dark and under light between (79.29 - 18.6) % for bacteria after 60 min in TiO_2 anatase catalyst .

The percent decreased to ~ 32% for anatase catalysts in dark experiments for G +ve S.aureus after 30 min, whereas the percent decreased to 10% for rutile catalysts in dark experiments for G+ve S.aureus after 30 min and both catalyst not effective at 10 min agents G+ve S.aureus.

Detailed mechanism in dark for TiO_2 anatase work is still not known yet. Most widely accepted mechanism suppose changing in bacterial membrane permeability and dissipation as a result of accumulation and attachment of TiO_2 anatase particles on the bacterial membrane. Bacteria showed big resistance when treated with rutile and the percentage of deactivation was

very low in both light and dark condition. There for anatase will be used in the flowing studies

3.4 TOC results

4.3.1-TOC results By oxidizing organic carbon supply from deactivated bacteria, the Total Organic Carbon could be perceive and quantified by calculating the amount of oxidized carbon (CO_2). As shown in Table (4.6) samples from mineralization test (using 0.1g of TiO_2 anatase) against (1×10^6 CFU\mL) of S. aureus bacteria were taken after 4 hrs. treatment.

Table 3.4: TOC results for bacterial aqueous mixtures remaining from experiment after 3 hrs. treatment with 0.1 g TiO_2 anatase against (1×10^6 CFU\mL) of S. aureus in light and dark at 28 °C

Sample number	Catalyest appearance	Conditon	Time	T OC mg/L
1 , 4	TiO_2	light	4h	28 , 26
2 , 5	No –ctalyest	light	4h	46 , 47
3 , 6	TiO_2	dark	4h	39 , 40
7 , 8	No –ctalyest	dark	4h	43 , 42

Table 3.4 show that for S. aureus experiments a clear decrease in organic carbon occurred in light. The waste water treated with TiO_2 showed TOC value 28 mg\L compared to the sample without catalyst which showed 47 mg\L for TOC

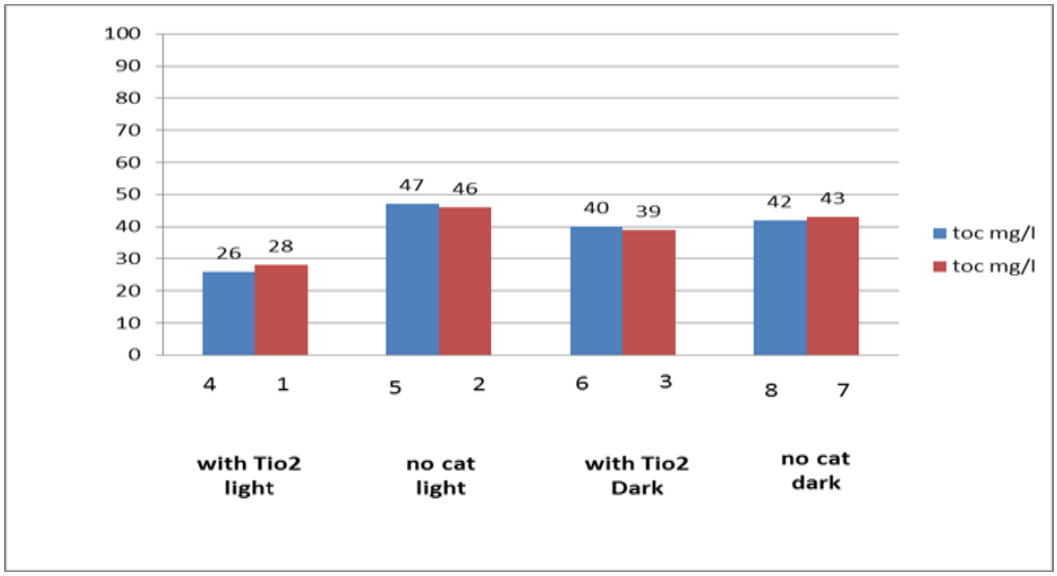

Figure 3.7: samples from mineralization experiments (using 0.1g of naked TiO_2 anatase) against (1×10^6 CFU\mL) of S. aureus were taken after 3 h treatment at 28 ºC

This difference between the two values confirms that TiO_2 deactivates bacteria, and mineralizes their organic matters under radiation. In dark, the TOC analysis gave almost the same TOC value~ 42 mg\L with and without using TiO_2 .The ability of TiO_2 catalyst in dark to form free radicals is not possible. In dark TiO_2 only deactivates the bacteria with no mineralization.

3.5 Impact of concentration of bacteria

Impact of bacterial concentration of S.aureus on bacterial deactivation was studied using 0.1 g naked TiO_2 anatase in treatment process. In Fig. (3.8) 0.1g TiO_2 anatase of was used to treat different concentrations of S.aureus bacteria in separate experiments in light and dark for 60 min at 28Cº.

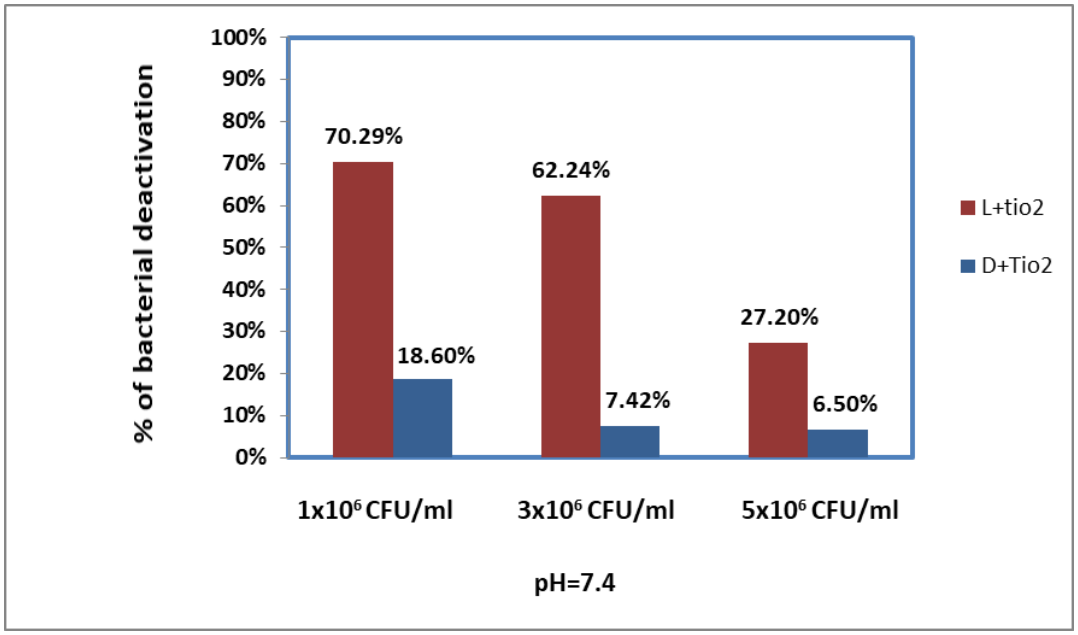

Fig 3.8: Impact of increasing the concentrations of S.aureus pneumonia bacteria on the deactivation percentage using 0.1 g of naked TiO_2 in light and dark for 60 min at 28 ºC with pH=7.4

Fig. (3.8) shows that when the concentration of S.(aureus) bacteria increases the deactivation percentages decrease. The results show that under light 70.29%, 62.24% and 27.2% of S.aureus respectively, are deactivated in 60 min, from 1×10^6 CFU\mL, 3×10^6 CFU\mL to 5×10^6 CFU\mL solution .

On the other hand, in dark when bacterial concentrations became extremely high the TiO_2 deactivation percent reached only (6.5%) in case of 5×10^6 CFU\mL of S. aureus.

Table (3.5) illustrate values of QY, TF and deactivation percent values for Different bacterial concentrations of (S. aureus pneumonia bacteria) using 0.1 g of TiO_2 anatase in light for 60 min.

Table 3.5: Values of QY, TF and deactivation percent of increasing the concentrations of S.aureus using 0.1 g of TiO2 anatase in light after 60 min, 28ºC.

Concentrations CFU\mL	QY	TN min^{-1}	TF	% deactivated bacteria
1×10^6	1.664×10^{-44}	9.416×10^{-16}	1.569×10^{-17}	70.29%
3×10^6	2.571×10^{-44}	1.48×10^{-15}	2.467×10^{-17}	62.24%
5×10^6	4.832×10^{-45}	2.78×10^{-16}	4.63×10^{-18}	27.20%

Table (3.5) show that when increasing the concentration of bacteria from (1×10^6 CFU\mL to 3×10^6 CFU\mL**)** the value of QY and TF will increase due to the surface of the catalyst is exposed to a greater amount of bacteria, which causes a greater amount of deactivated bacteria.

On other hand when increasing the bacterial concentration more than 3×10^6 CFU\mL. The QY, TF will decrease, contrary to what is expected, and this is due to the fact that a large concentration of bacteria performs a process of blocking the sun's rays, which causes a decrease in these values.

3.6 Impact of TiO_2 anatase catalyst loading

Lowering and increasing the loading of TiO_2 anatase is necessary for economic reasons. Impacts of this reduction on the deactivation percentage of 1×10^6 CFU\mL (S. aureus) in light and dark were studied. Fig. (4.9) shows the results.

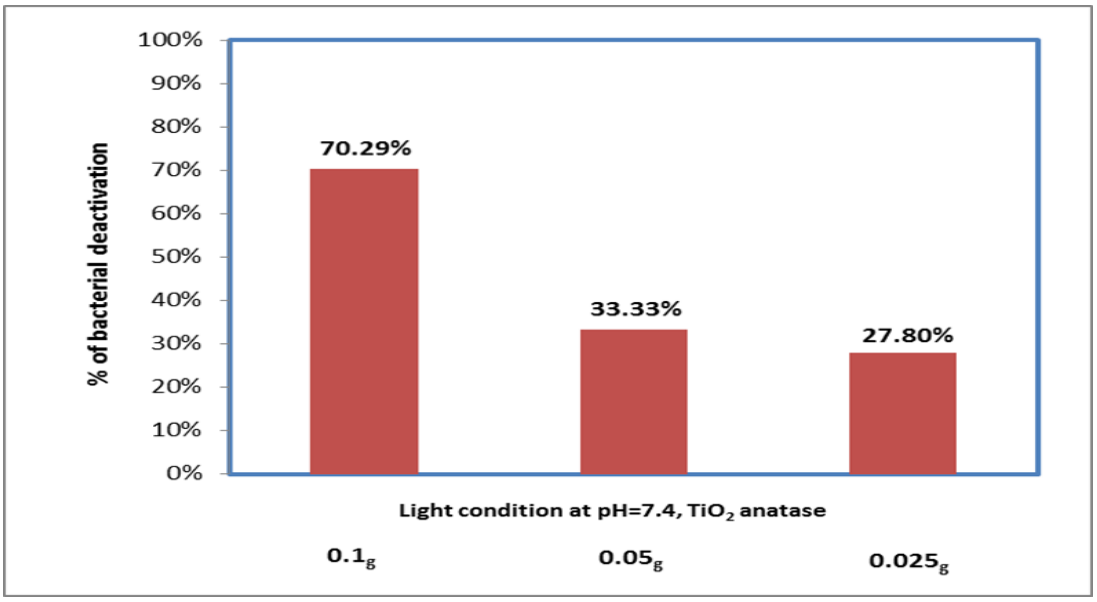

Figure 3.9.a: Impact of catalyst loading on the deactivation percentage of S. aureus (1×10^6 CFU\mL), after (60) min at 28 ºC in light at pH=7.4

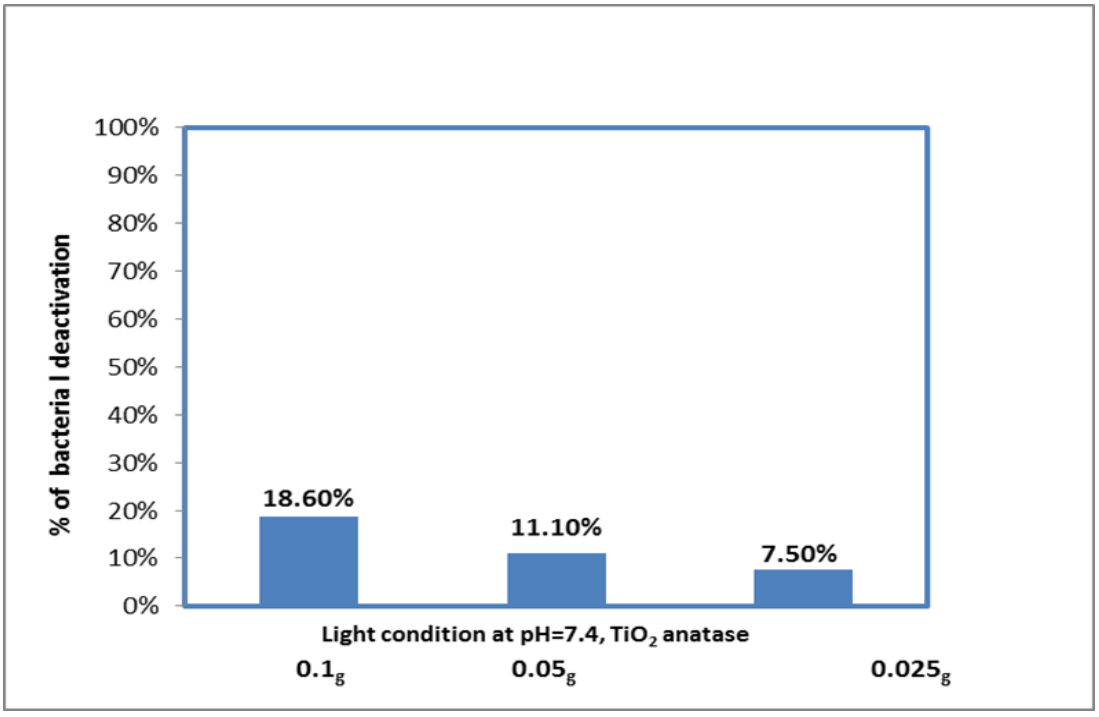

Figure 3.9.b: Impact of catalyst loading on the deactivation percentage of S. aureus (1×10^6 CFU\mL), after (60) min at 28 ºC in dark at pH=7.4

Fig.(3.9.a) and (3.9.b) shows that in light and dark deactivated percentages were lowered after 60 min to (33.3%, 27.8%) and (11.1%, 7.5%) when 0.05g and 0.025g of TiO_2 were used against S.aureus respectively. By reducing the catalyst weight from 0.1 g to 0.025g catalyst can still remove bacterial.

Table (3.6) shows values of QY, TF and deactivation percent calculated for experiments tested using different TiO_2 anatase loads against 1×10^6 CFU\mL of (S. aureus) in light after 60 min.

Table 3.6: Values of QY, TF and % deactivation, for experiments with different TiO_2 anatase loading against 1×10^6 CFU\mL of S. aureus in light after 60 min.

loading	QY	TN	TF	% deactivated bacteria
0.1 g	**1.664×10^{-44}**	**9.416×10^{-16}**	**1.569×10^{-17}**	**70.29%**
0.05 g	**9.184×10^{-45}**	**1.061×10^{-15}**	**1.768×10^{-17}**	**33.3%**
0.025 g	**5.74×10^{-45}**	**1.326×10^{-15}**	**2.211×10^{-17}**	**27.80%**

In Tables (3.6) show QY values increase as catalyst weight increases with (1.664×10^{-44} ,9.184×10^{-45}, 5.74×10^{-45}) when (0.1, 0.05, 0.025g) were used against S.aureus bacteria respectively as catalyst loading increased extra photons are used for deactivation.

In other hand table show TF values decreased as catalyst weights increased. This means that relative efficiency of the TiO_2 catalysts decreased with higher loading. This is due to tendency of TiO_2 particles to screen each other from UV light.

3.7 Impact of temperature on photo-mineralization reaction

For S.aureus bacteria, the suitable temperature range for growth is between (7 to 48 °C), with an optimum at 37 ºLiterature thus confirm that within the range (20-37 ºC), the temperature does not have impact on bacterial deactivation. In this work, the Temperature 28 ºC was used for technical reasons [51,52] . Due to exposure to light, water temperature stabilizes at 28 Cº (slightly higher than room Temperature 25 ºC). For this reason , we used the 28 ºC [94].

3.8 Impact of pH

Impact of pH of was studied on S.aureus bacterial deactivation using 0.1 g TiO_2 anatase in treatment process. In Fig. (4.10) , 0.1 g TiO_2 anatase was used with different pH in separate experiments in light and dark for 60 min at 28ºC.

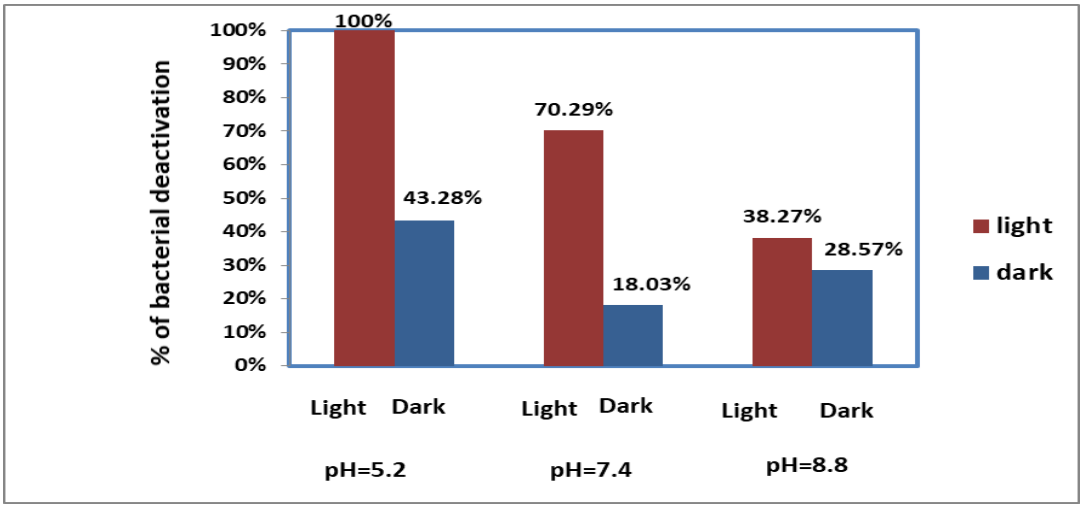

Fig 3.10: Impact of pH on S.aureu bacteria deactivation percentage using 0.1 g of TiO_2 in light or dark for 60 min at 28 ºC at several (pH=5.2 ,7.4 and 8.8).

The results show the values of deactivated percentage of bacteria are higher in acidic medium than in neutral medium. The neutral medium shows higher degradation than basic medium .This indicates that photo-degradation efficiency increased as lower pH value. This is become the cell membrane of bacteria has negative charge and at pH more than (8.8), TiO_2 also has negative charge therefor in basic media repulsive force act as inhibiting factor for reaction between bacteria and TiO_2. Acidic and neutral media show better photo degradation results.

Values of T.N, Q.Y and degradation percentage were calculated after 60 minutes as shown in Table (4.7).

Table 3.7: Values of QY, TN and % deactivation, for experiments with different pH against 1×10^6 CFU\mL of S. aureus in light after 60 min anatase.

pH	% of deactivated Bacteria	TN	QY
5.2	100%	1.06×10^{-15}	1.83×10^{-44}
7.4	71.92%	9.46×10^{-16}	1.63×10^{-44}
8.8	88.27%	8.228×10^{-16}	1.42×10^{-44}

The results show that the values of T.N, Q.Y and degradation percentage in acidic medium are higher than in neutral and basic media.

3.9 Impact of recovery

At the end of each round and after sterilizing the samples by autoclave in order to remove of bacteria, new 0.33 ml of bacteria was added to the catalyst was present, light was irradiated for 1 hour. Dark experiments were also conducted for them.

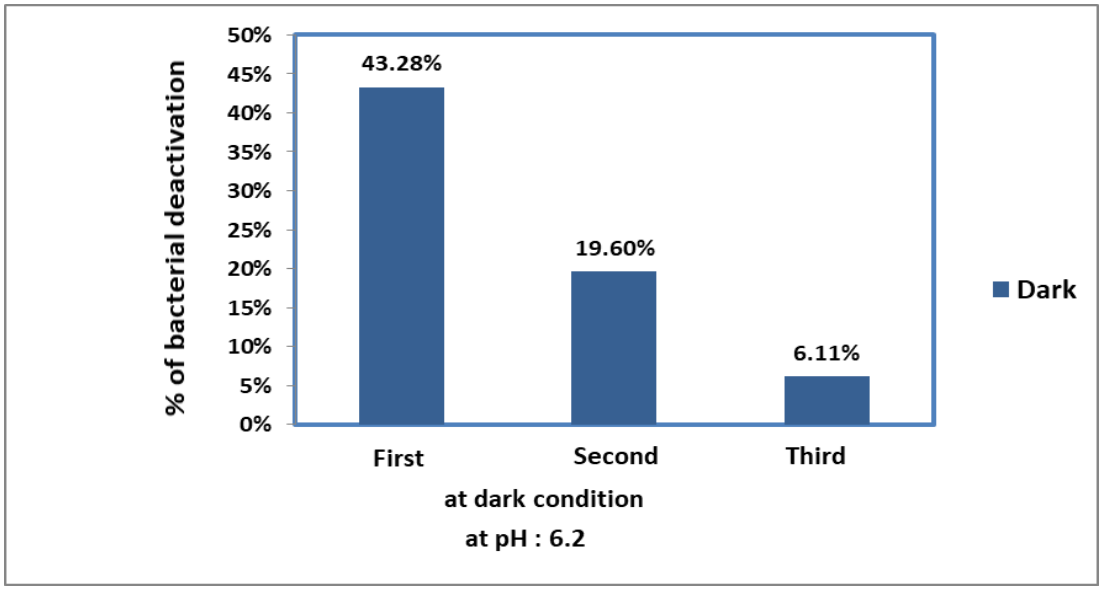

Fig 3.11.a: 0.1g of TiO_2 anatase were reused for 3rd time against of S. aureus (1×10^6 CFU\mL), for 60 min. at 28 ºC dark condition.

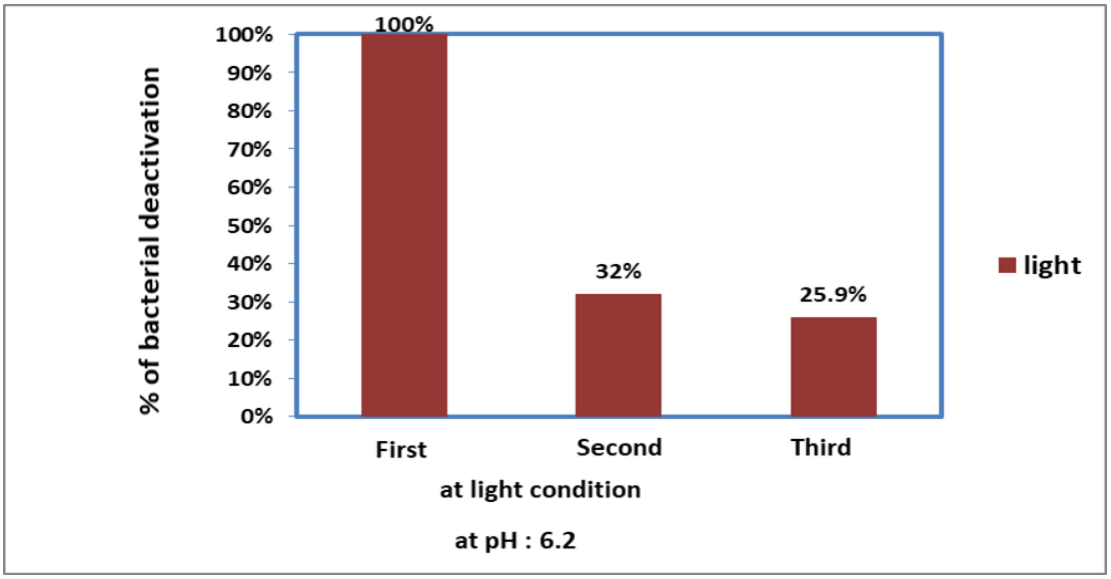

Fig 3.11.b: 0.1g of TiO_2 anatase were reused for 3rd time against of S. aureus (1×10^6 CFU\mL), for 60 min. at 28 ºC at light condition.

Fig.(4.11.a) and (4.11.b) shows possibility to reuse the catalyst for third time in light and in dark.

in light condition deactivation percent lowering from 100% to 32% in second round and lowering to 25.9% in third round, in dark condition deactivation percent lowering from 43.28 % to19.6% in second round and lowering to 6.11% in third round

noting the decrease in the efficiency and ability of the catalyst to gradually inactivate the bacteria whenever it is reused.

3.10 Conclusion and Recommendations

The results show that TiO_2 anatase can be effective catalyst to deactivate G +ve S. aureus bacteria in both dark and light experiments. Under light, mineralization of bacteria occurs for S. aureus (43.47%) for the first time TiO_2 catalysts can also be recovered and reused for second time.

The results show the potential of using TiO_2 catalyst system in future waste water disinfection. For future work, we suggest applying the TiO_2 catalyst against other types of bacteria and other species such as viruses and algae Applying the TiO_2 catalyst on sewage water, in cooperation with the Water Authority is recommended. The study should involve both organic contaminants and microorganisms.

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جامعه النجاح الوطنية

كلية الدراسات العليا

تقييم الدور الحفزي الضوئي لدقائق اكسيد التيتانيوم في التحطيم الكامل للبكتيريا ومحتوياتها العضوية في الماء

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إشراف

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في برنامج الكيمياء، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2021

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الملخص

تنقية المياه وتطهيرها من الملوثات المتعددة أصبح ضرورة لحياة كريمة للفرد، حيث يتم إتباع عدة استراتيجيات وطرق مختلفة في دول عدة من أجل تنقية المياه من هذه الملوثات، ان أفضل الطرق هي الطرق الآمنة على صحة الإنسان وذات الكفاءة العالية والتكلفة المنخفضة.

إن التحطيم الضوئي للبكتيريا يعتبر من أكثر الطرق كفاءة و جذبا لاهتمام الباحثين في هذا المجال حيث يتم تحفيز أشباه الموصلات باستخدام الضوء والذي يقوم بدوره بالتحطيم الضوئي للملوثات البيولوجية والكيميائية المتواجدة في الماء حيث أصبحت حبيبات أكسيد التيتانيوم النانوية (TiO_2) تستخدم على نطاق واسع لكثير من الملوثات العضوية في الماء.

إن حبيبان ثاني اكسيد التيتانيوم النانوية (TiO_2) التي تمتلك فجوة طاقة (Band gap) واسعة من 2.5 الى 3 الكترون فولت تحتاج إلى طول موجة يتواجد في منطقة الأشعة فوق البنفسجية حيث يقتصر النشاط التحفيزي على أطوال موجات أقصر تقع فهذه المنطقة.

في هذا العمل تم تعقيم المياه عن طريق التحطيم الكامل لنوع البكتيريا إيجابية الغرام (Staphylococcus aureus) مع مكوناتها العضوية وذلك باستخدام جزيئات ثاني أكسيد التيتانيوم النانوية (TiO_2 anatase) حيث لوحظ انه بعد 60 دقيقة تم التخلص من البكتيريا بنسبة 70,29%، وبعد 4 ساعات لوحظ انخفاض كبير في المكونات العضوية الكربونية بنسبة 55% ولقد تمت دراسة أثر بعض العوامل على فعالية الحفاز وسير تفاعل التحطيم الضوئي مثل درجة الحرارة والفترة الزمنية للتعريض الضوئي ودرجة الحموضة وتركيز كل من الحفاز والملوثات هذا وقد تبين إن الحفاز يمكن إعادة استخدامه اكثر من مرة.