An-Najah National University

Faculty of Graduate Studies

Understanding Mode of Action of Nanoparticles in Water Disinfection: ZnO in Bacteria Killing vs. Complete Photo-degradation

By

Jumana Fayez Mohammad Ishtaiwa

Supervisors

Dr. Majdi Dwikat

Dr. Samar Al-Shakhshir

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This thesis was Defended Successfully on 23/12/2013 and approved by:

Defense Committee Members:	**Signature**
1. Dr. Majdi Dwikat /Supervisor	.....
2. Dr. Samar Al-Shakhshir / Co.Supervisor	Samar Al Shakhshir .....
3. Dr. Yehia Faidi/ External Examiner	.....
4. Dr. Nidal Za'tar / Internal Examiner	Nidal Zatar .....

Dedication

TO MY FAMILY, BEST FRIEND (HIND MATAR), DR. JABER ABU JOKHA

ALSO MY SONS OSAID, ANAS AND DAUGHTER ALMA, FOR THEIR SUPPORT

WITH MY APPRECIATION

Acknowledgement

I thank Allah who enables me to finish this research.

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الإقرار

انا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Understanding Mode of Action of Nanoparticles in Water Disinfection: ZnO in Bacteria Killing vs. Complete Photo-degradation

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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 الطالبة:**

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List of Abbreviations

Symbol	Abbreviation
P. aeruginosa	***Pseudomonas aeruginosa***
TDS	**Total dissolved solids**
THMs	**Trihalomethane**
WHO	**Word Health Organization**
MCL	**Maximum contaminate level**
TON	**Threshold odor number**
MCLG	**Maximum contamination level goal**
E. coli	***Escherichia coli***
CFU	**Colony forming unit**
TC	**Total coliform**
eV	**Electron volt**
UV	**Ultraviolet light**
TEM	**Transmission electron microscopy**
SEM	**Scanning electron microscopy**
XRD	**X – ray powder diffraction**
TG	**Thermogravimetry**
FTIR	**Fourier transform infrared spectroscopy**
DSC	**Differential scanning calorimetry**
S. aureus	***Staphylococcus aureus***
B. subtilis	***Bacillus subitis***
TOAB	**Tetraoctylammoniumbromide**
ROS	**Reactive oxygen species**
CAT	**Catalyst**

Understanding Mode of Action of Nanoparticles in Water Disinfection: ZnO in Bacteria Killing vs.Complete Photo-degradation

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Abstract

ZnO nanoparticles have been considered to possess potential biological application as efficient antimicrobial agent. In this work, ZnO semiconductor particles were used to disinfect water from bacteria by photo-degrading it with solar sun light. The catalyst 0.1 g was added to 50 mL distilled water pre-contaminated with bacteria. Four different water beakers were prepared each one has 50 mL of ~5 x 10^5 cfu/mL of bacteria. The solutions were magnetically stirred in a 100 mL glass beaker. The first beaker was exposed to the light source for 60 minutes at 30 °C. In the second one, ZnO nano-catalyst was used in the dark to know if it affects bacteria growth. The third sample was also exposed to light without addition of catalyst to examine light effect on bacteria degradation. The fourth beaker was prepared without catalyst under dark condition. ZnO is known to kill bacteria in the dark, without totally degrading it into mineral species. This has been confirmed in this work, as ZnO nanoparticles killed the bacteria in the dark and the organic contents remained therein. It may also photo-catalyze removal of bacteria under light irradiation. This study aims at investigating mode of action of ZnO nanoparticles in disinfecting water. It is assumed that ZnO nanoparticles would totally degrade

Escherichia coli and *Pseudomonas aeruginosa* under light and convert it into CO_2 gas and other minerals. This work showed evidence in this activity, and the bacteria were totally degraded into mineral species. Results of *E. coli* degradation showed 100 % activity. In the dark, using ZnO nanoparticles, there was some decline in bacteria concentration 20.21%.

Under sun light using ZnO nanoparticles, 100% loss of *E. coli* concentration was observed. These results proved that the activity of catalyst in light conditions better than under dark condition. TOC analysis showed that the concentration of TOC is very low 7.27 ppm for

E. coli experiment after using ZnO nanoparticles under sun light condition. This concentration of carbon led to the conclusion that the catalyst and the sun light killed and degraded *E. coli*.

ZnO nanoparticles killed *P. aeruginosa* under solar sun light 100 %.

In the dark condition, the ZnO nanoparticles catalyst killed 50% of bacteria, this result indicates that the activity of the catalyst under sun light condition was better than under dark condition.

In *P. aeruginosa* experiments, the concentration of TOC was very low when the catalyst was used under sun light (8.8 ppm). This result proved that the catalyst has great activity under sun light to kill and degrade *P. aeruginosa* to gases and other minerals. On the other hand, the

concentration of TOC was increased when the catalyst was used without sun light (36.2 ppm).

Some factors affecting photo-degradation reaction of *P. aeruginosa* and catalyst efficiency, such as illumination time, temperature, pH, catalyst amount and bacteria concentration, were studied; All such factors had no effect on *P. aeruginosa* degradation. This shows the wide applicability of the method described there for water disinfection. The study comes out with an important recommendation that "ZnO nanoparticles can be used as a photo-catalyst for complete mineralization of *E. coli* and *P. aeruginosa* under light in different working conditions". More investigations in this direction are therefore needed.

Chapter 1

Introduction

1.1 Clean water properties

Clean water is essential for the survival of humans, animals and life. Clean water is defined as water that doesn't lead to any health risk for the consumers, when used in washing, drinking and other ways [1].

Drinking water should have suitable physical, chemical and biological properties to be considered as acceptable drinking water. There are many physical properties for clean water such as odor, color, taste, pH, temperature, total dissolved solids (TDS) and dissolved oxygen [2]. According to the World Health Organization (WHO) recommendations for drinking water, it shouldn't have any taste, color or odor because these are indicators for the presence of physical, chemical or biological pollutants[1].

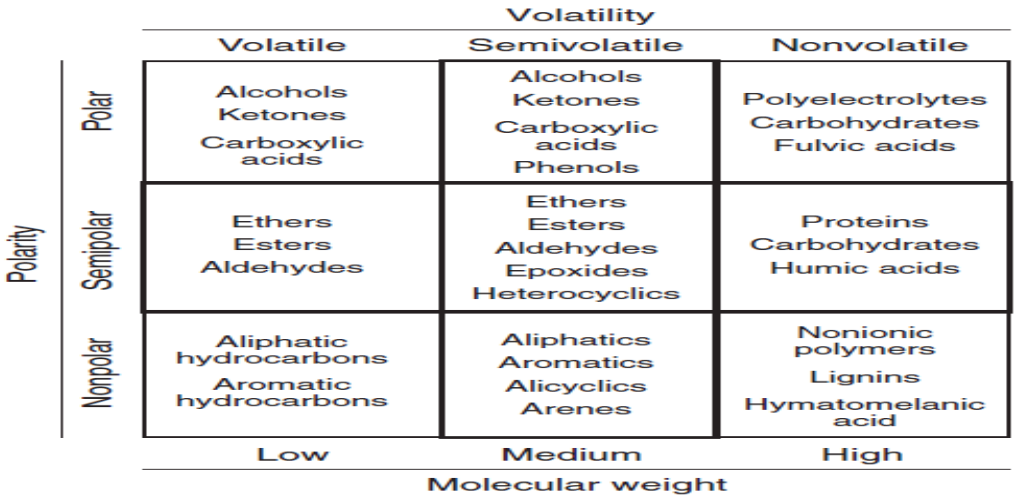
There is no noticeable healthy effect on the temperature of drinking water, but the cold water is more acceptable than the warm water according to the desire of consumer, as warm water is a suitable environment for bacterial growth and the presence of trihalomethane (THMs) decreases with decreasing water temperature [3]. The pH of drinking water should be in the range of 6.5-8 according to WHO drinking water guidelines, although there is no direct health effect resulting from the change in the value of water pH [1]. Table (1.1) shows some physical properties with secondary maximum contaminate level (MCL) [4].

Table (1.1): Physical properties and MCL in drinking water [4].

Contaminant	Secondary MCL	Noticeable effects above the secondary MCL
Color	15 color units	visible tint
Odor	3 TON (threshold odor number)	"rotten-egg", musty or chemical smell
pH	6.5 - 8	low pH: bitter metallic taste; corrosion high pH: slippery feel; soda taste; deposits
Total Dissolved Solids (TDS)	500 mg/L	hardness; deposits; colored water; staining; salty taste
mg/L: milligrams of substance per liter of water		

The chemical constituents of clean water can be divided into organic, inorganic and gaseous components. The organic chemical sources occur naturally and due to human activities, Table (1.2) shows some organic matters found in water that are classified according to molecular weight, polarity and volatility. Inorganic chemicals are found in water as dissolved salts such as chlorides, calcium, magnesium and others. Generally, in the concentration range of 1.0 to 1000 mg/L, gas can be found in water as a result of exposure to the atmosphere such as carbon dioxide (CO_2) and Oxygen (O_2) and decomposed organic matter by bacteria such as methane, CH_4 [5].

Table (1.2): Organic matter found in water was classified according to its molecular weight, polarity and volatility [5].



The last water characteristic is its biological one where it represents the living organisms in water such as protozoa, viruses and bacteria. Microorganisms and their maximum contamination level goal (MCLG) are listed in Table (1.3) [6].

Table (1.3): Microorganisms and their maximum allowed contamination level goal (MCLG).

Microorganism	MCLG (mg/L)
Cryptosporidium	zero
Total coliforms (including fecal coliform and total coliforms)	zero
Viruses	Zero
Giardia lamblia	Zero
Legionella	zero

1.2 Water pollutants

Fresh water percent represents just about 1.1 % of the global water. Therefore, the world faces lack of water sources problems. Another water problem is pollution of water where it can be polluted with different physical, chemical and biological pollutants that cause fatal waterborne diseases [7].

1.2.1 Chemical pollutants

Chemical pollutants can be divided into inorganic, organic and gaseous pollutants. The most important organic compounds that are found in water are phenols, trihalomethane (THMs) and benzene [3].

Inorganic pollutants are found in water in many aspects such as hazardous metal pollutants especially heavy metals, which are considered as the major chemical pollutants. Heavy metals cause lethal effects on the plants, animals and humans [3, 8].

Cadmium (Cd) is one of the most hazardous heavy metal pollutants. That has 2+ oxidation state, and is found in water from many sources such as the industrial effluents of electroplating, batteries and alloying manufactures. Many healthy risk effects of water containing high concentrations of Cd include: kidney damage, enzymes inhibitor and high blood pressure. Therefore, the MCL for Cd in water is very low (less than 1 µg/L) [3, 8].

Lead is another toxic inorganic pollutant that is found in water as Pb^{2+}. The main source of lead is from mining, lead pipes and industrial disposals. Water that contains lead concentrations higher than 0.015 mg/L causes many health effects as hearing problems, headaches and kidney damage [9-11].

1.2.2 Physical pollutants

The physical pollutants are the change in the physical properties of clean water such as the change of taste, odor, color and pH. Any presence of color, taste and odor in drinking water makes it unsuitable for drinking because that indicates the presence of chemical or biological pollutants. In fact it becomes not acceptable for people [11].

1.2.3 Biological pollutants in water

Waterborne diseases are one of the most important results of the drinking water contamination with microbial contaminants. Microorganisms that contaminate the water are viruses, protozoa and bacteria that cause many dangerous diseases such as cholera, diarrhea and typhoid [3, 12]. Pathogenic types with health effects of such micro organism, are listed in Table (1.4) [12].

Table (1.4): Pathogenic groups and types that found to be in eat, with their diseases [12].

Group	Pathogen	Disease or condition
Viruses	Enteroviruses (polio, Echo, coxsackie) Hepatitis A and E Norovirus, Rotavirus	Meningitis, paralysis, rash, fever, mycocariditis, respiratory disease, diarrhea Hepatotis Diarrhea
Bacteria	*Salamonella* *Vibrio cholera* *E. coli*	Typhoid fever, Diarrhea cholera Diarrhea
Protosoa	Naegleria Toxoplasma	Meningoencephalitis Mental retardation, loss of vision
Blue-green algac	Microcystis Anabaena	Diarrhea, possible production of carcinogens
Helminths	Necater americanus Taenia saginata	Hookworm Beef tapeworm

1.3 Bacteria in drinking water.

Bacteria in drinking water are the cause of many diseases such as typhoid and diarrhea that cause millions of deaths. The most important source of bacteria found in drinking water is human and animal feceses. Therefore, the presence of bacteria in water is considered as an indicator for water pollution with human and animal wastes [13]. There are many indicators for the presence of bacteria in drinking water, including total coliform bacteria (TC), *E .coli* and thermotolerant coliform bacteria [1].

1.3.1 Escherichia coli

E. coli is a common type of bacteria that is found to be in human feceses. It belongs to gram-negative bacteria and has rod-shape with length of about 2 µm and width of 0.5 µm. Some categories of *E. coli* are harmless while other categories may cause diarrhea and gastroenteritis. Therefore, the MCL for it in drinking water should be zero. The optimum temperature for the growth of *E. coli* is 37 ºC [14-16].

1.3.2 Pseudomonas aeruginosa

P. aeruginosa is a gram negative bacteria capable of causing serious infections in insects, plants and animals. *P. aeruginosa* is a major cause of nosocomial infections and responsible for the chronic lung infections that affect most cystic fibrosis patients [17].

1.4 Water disinfection

Chemical disinfection is an essential process to inactivate or kill the pathogenic microorganisms in drinking water and to reduce the waterborne diseases. The most widely used chemical disinfectants are: chlorine gas

(Cl_2), free chlorine (NaOCl, $Ca(OCl)_2$), chloramines (monochloramine), ozone and chlorine dioxide. Many advantages of the disinfectants are: high efficiency against the pathogens, low cost and easy to use. While the most disadvantages of some chemical disinfectants are: the formation of

toxic by-products [18, 19]. Some chemical disinfectants with groups of it are listed in Table (1.5).

Table (1.5): Chemical disinfectants options [20].

Disinfectant	Abbreviation /Symbol	Group
Chlorine gas	Cl_2	Halogen
Sodium hypochlorite (liquid bleach)	NaOCl	Halogen compound
Iodine	I_2	Halogen
Bromine	Br_2	Halogen
Ozone	O_3	Peroxygens
Silver	Ag^+	Heavy Metals
Copper	Cu^{2+}	Heavy Metals

1.5 Water disinfection using nanotechnology.

Nanomaterials can be defined as the aggregate of atoms with size range of 1-100 nm. Different morphologies of the nanomaterials were being found such as nanorods, nanoparticles and nanotubes [21]. Nanomaterials have superior chemical and physical properties comparing with large scale materials according to their high surface-to-volume ratio[22].

Nanomaterials nowadays become useful in a lot of applications such as in: biomedicine [23], biosensors [24], solar cells [25] and water disinfection [26]. The materials with nano size and different morphologies can be obtained with different methods such as: electrochemical methods [27], sonochemical [28], alcohothermal synthesis [29] and novel quick-precipitation [30].

Semiconductor nanoparticles such as: CdS, CuO and ZnO have many advantages due to the optical and electrical properties [31-34]. Widely useful applications for the semiconductor nanoparticles such as in: solar cells, gas sensors, catalysis and water treatment [35-38].

1.6 ZnO nanoparticles.

Insoluble white crystalline powder belongs to II-VI semiconductor group, has a wide band gap of 3.2 eV. ZnO has a stable wurtzite structure. It is considered as an n-type semiconductor and has many physical and chemical properties such as: high transmission in the visible range, high stability and it has optical and electrical properties. ZnO is transparent to the light in the region of visible wavelength but absorbs the light of UV below 3655A [39-42].

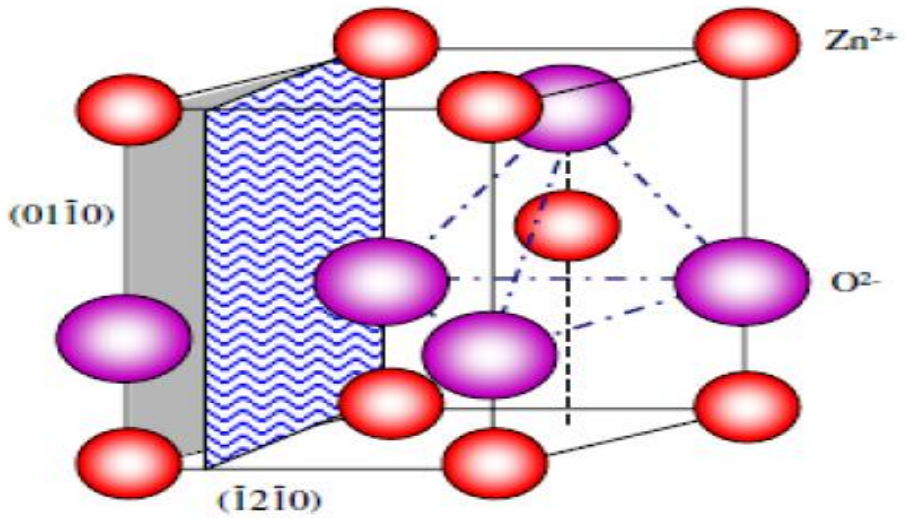


Figure (1.1): Wurtzite structure of ZnO [42]

ZnO nanoparticles are one of the most interested nanoparticles according to its properties and cost. It can be prepared with several methods such as sol-gel method, vapor transport synthesis, electrodeposition method, laser ablation and precipitation method. The shapes that can be obtained of ZnO precipitate are nanoparticles, nanorods and nanospheres [43-50].

Precipitation method is a common method for preparation of ZnO nanoparticles because it produces large quantity of ZnO nanoparticles and the size and shapes of the nanoparticles can be easily controlled using this method [51, 52]. ZnO nanoparticles with different shapes and sizes were obtained by this method using zinc nitrate ($Zn(NO_3)_2$) and ammonium carbonate ($(NH_4)_2CO_3$) as precursor [53].

Other precursors can be used to obtain ZnO nanoparticles such as zinc acetate and ammonium carbonate [54]. The size of about 9.4 nm of ZnO nanoparticles were prepared using $ZnSO_4$ and NH_4HCO_3 [55]. ZnO nanoparticles were prepared using $Zn(NH_3)_4^{2+}$ and sodium oleate and hydrazine to control the size of the nanoparticles, however, the obtained ZnO nanoparticles size were 30–60 nm [56]. Rod-like structure of ZnO nanoparticles can be prepared using zinc acetate and triethanolamine [57].

The size, structure, and morphology of ZnO nanoparticles can be characterized using several techniques such as: transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray powder diffraction (XRD), thermogravimetry (TG), fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) [58-61]. XRD pattern and SEM images for ZnO nanoparticles are shown in Figure (1.2) and (1.3).

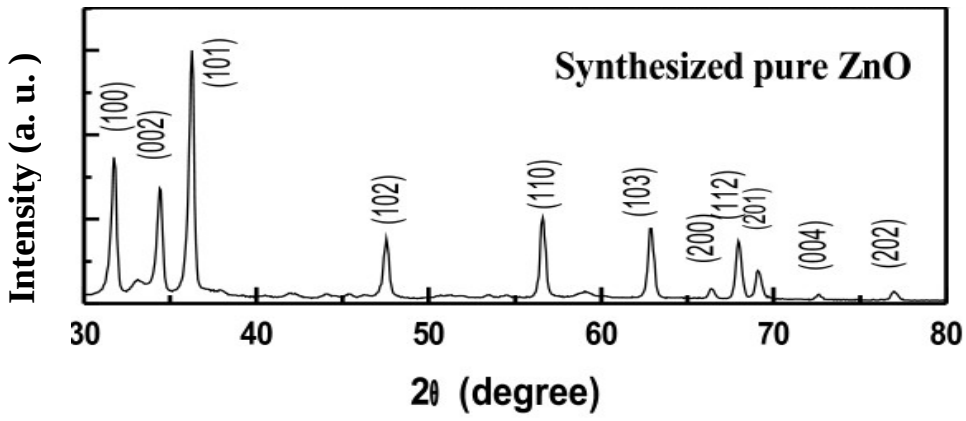


Figure (1.2): XRD pattern for ZnO nanoparticles [62].

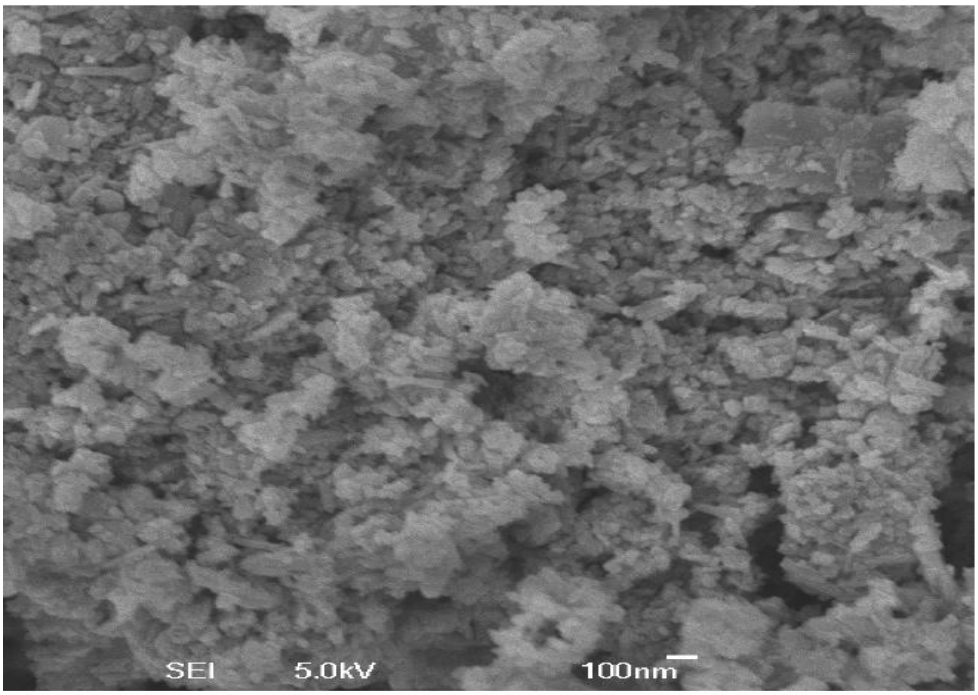


Figure (1.3): SEM image of ZnO nanoparticles [63].

According to the ZnO nanoparticles properties which are used in many applications such as in: solar cells, gas sensors, optical devices, and antibacterial applications [64-67].

One of the most interested applications for ZnO nanoparticles is antibacterial activity against a lot of bacteria types such as: *E. coli*, *S. aureus* and *Bacillus atrophaeus* [68-69]. Many parameters were investigated to obtain the optimum antibacterial conditions such as nanoparticles size, concentrations, presence of light and pH. However, *Adams et al.* reported that there was no effect of the ZnO nanoparticle size against *E. coli* [70] in contrast with Zhang *et al.* Results showed that the activity of ZnO nanoparticles versus *E. coli* increased with decreasing ZnO nanoparticles size [71]. Antibacterial activity of ZnO nanoparticles against

E. coli under the light and in the dark were investigated and showed higher activity under the light [72]. Another parameter was pH effect, the activity of ZnO nanoparticles showed that the activity against *E. coli* increases with decreasing pH [73].

On the other hand, the activity of ZnO nanoparticles against gram-positive bacteria such as: *S. aureus*, and gram-negative bacteria such as: *E. coli,* were studied. However, the activity against gram-positive bacteria is higher [74].

The activity of ZnO nanoparticles against *E. coli, S. aureus* and *B. subtilis* were studied in the presence and the absence of the stabilizer. The presence of tetraoctylammonium bromide (TOAB) surfactant as stabilizer for ZnO nanoparticles showed higher activity [75]. The activity of ZnO nanoparticles against *E. coli* under solar light in the presence of anthocyanin dye as sensitizer showed good antibacterial activity [76].

1.6.1 The antibacterial mechanism of ZnO nanoparticles

There are many suggestions for the antibacterial mechanism of ZnO nanoparticles. One of the studies was against *Campylobacter* which said that the antibacterial mechanism was through the inducing oxidative stress into the bacteria cell lead to inactivation of the bacteria growth [76]. Also the mechanism against *E. coli* revealed that the bacterial inactivation is due to cell membrane damage [71, 77]. Other proposed mechanism was the

release of high concentrations of reactive oxygen species (ROS) typically hydroxyl radicals and singlet oxygen [78].

1.7 Objectives.

The strategic objective of this work is to disinfect water from bacteria by photo-degradation using solar sun light and a safe and low cost semiconducting material (ZnO) in its nanoparticles form. Evaluation of the process in term of efficiency, cost and environmental point of view will be dicussed. As ZnO nanoparticles are known to kill bacteria in the dark, it is part of this work to see if ZnO nanoparticles can totally photo-degrade bacteria into mineral species not only killing them. Therefore, understanding mode of action of ZnO nanoparticles in water disinfection (killing vs. total photo-degradation) is one major objective of this work. Such study has not been conducted earlier, to our knowledge, except by Sondos Ateeq using *E. coli* [63].

Other technical objectives include:

1- Preparation of semiconducting nano-sized powder to be used in solar sun light degradation of bacteria and water disinfection.

2- Characterization of the ZnO nanoparticles system using XRD and SEM.

3- Using the ZnO nanoparticle semiconductor in photo-degradation of bacteria (*E. coli* and *P. aeruginosa*) in water exposed to solar sun light which contains UV radiations.

4- Studying the effect of bacterial (used in this study) concentration, catalyst concentration, temperature, time and pH on photo-catalyst activity and photo-degradation process efficiency.
5- Studying the total organic carbon after photo-degradation of bacteria by TOC analysis to confirm if total degradation occurs in photo-catalytic experiments.

Chapter 2

Materials and Methods

2.1 Materials.

2.1.1 Zinc oxide.

ZnO nanoparticles were synthesized from $ZnCl_2$ that was purchased from Sigma Co. and NaOH from Frutarom Co, and used as the photo-catalyst for water disinfection. ZnO nanoparticles were prepared and used in the photo-degradation experiment to study the effect of nanoparticles on photo-catalytic activity in sun light – (UV) light.

2.1.2 Other chemicals.

Barium chloride, nitric acid, sulfuric acid, ethanol, sodium, hydroxide and hydrochloric acid were all purchased from either Aldrich – Sigma Co. or Frutarom Co. as analytical grade, and were used as received without further purifications. Culture media (nutrient agar and nutrient broth were purchased from Oxoid Co.

2.1.3 Bacteria.

E. coli and *P. aeruginosa* were used as model organisms for the inactivation studies. Pseudomonas species is one of the most common inhabitants of the human intestinal tract, and is probably the most familiar

organism in microbiology. *P. aeruginosa* is well known for its resistance to anti bacterial agents [79]. This was a reason for using such bacteria in this work. Finding a technique to totally degrade such bacteria is therefore, important.

The microorganisms (used in this study) of undesignated stains or serotype were isolated from clinical specimens and identified in the laboratory of medical laboratory sciences department according to standard diagnostic methods.

2.2 Equipments.

2.2.1 Irradiation sources.

In the photo-catalytic experiments a solar sun was used as the UV light irradiation source.

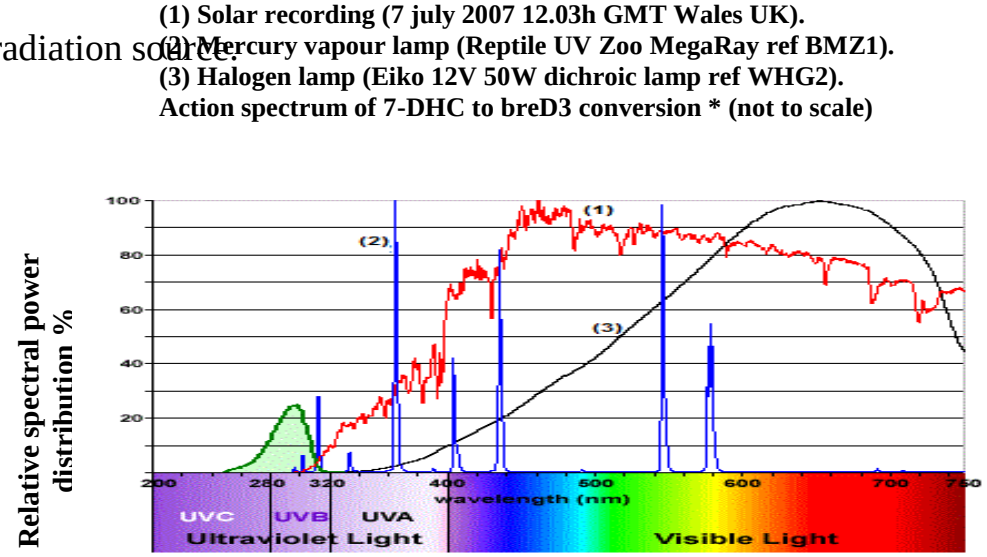


Figure (2.1): Spectrograms for the sun, mercury vapor and halogen lamps [80].

2.2.2 Measuring devices.

2.2.2.1 UV–Visible spectrophotometry.

AlaboMed, Inc, spectrophotometer was used to quantitatively determine bacterial concentration using turbidometric method, and adjust suspensions to 0.5 McFarland standard turbidity.

2.2.2.2 Lux meter.

A lux meter (Lx-102) light meter was used to adjust light intensity that reaches the water sample in the photo-catalytic disinfection experiments. The recorded value using solar sun light was ~ 1000 lux.

2.2.2.3 pH meter.

Controlling the pH of the reaction was necessary for two reasons. First, bacterial growth is affected by pH value. Second, the effect of pH on catalyst efficiency was studied. A pH meter was used to adjust the reaction mixture pH as desired.

2.2.2.4 X-Ray Diffraction.

To study the crystal structure of ZnO nanoparticles size.

2.2.2.5 Scanning electron microscopy (SEM).

To show the surface morphology and estimated size of prepared ZnO nanoparticles.

2.2.2.6 Total organic content (TOC).

To prove photo-degradation of bacteria

2.2.2.7 Other devices .

Vortex, Incubator, micropipette, autoclave and glasses.

Figure (2.2): Chemical photo-catalytic treatment**.**

2.3 Solutions preparation.

2.3.1 Chemical solutions preparation.

A 0.5 M McFarland Standard was prepared from original 1.175 % w/v, (0.096 N) $BaCl_2.2H_2O$ and 1 % v/v (0.36 N) H_2SO_4 solution.

Samples of 9.95 mL of H_2SO_4 solution and 0.05 mL of $BaCl_2.2H_2O$ solutions were mixed together. The solution absorbance was measured spectrophotometrically at 625 nm. It shows typical absorptivity in the

range 0.08 – 0.10. This standard is commonly used as a reference to adjust the turbidity of bacterial suspensions so that number of bacteria will be within a given range [81].

At this standard concentration the bacterial concentration is known to be about 1.5 X 10^8 cfu/mL.

The bacterial solution suspension is then measured and diluted to achieve the same absorbance as the McFarland standard.

NaOH and HCl solutions were prepared and used to adjust the pH as desired. NaOH (0.25 M) solution was prepared by dissolving 1.0 g solid NaOH in 100 mL distilled water and HCl (0.25 M) was prepared by diluting concentrated HCl (33%) in 100 mL distilled water.

2.3.2 Bacterial cultures preparation.

Cultures of *E. coli* and *P. aeruginosa* were prepared similarly. Nutrient agar was used as growth medium for measuring the remaining bacteria after the photo-degradation process. Nutrient broth was used for microorganisms inoculums preparation. Both nutrient agar and nutrient broth were prepared according to manufacturer instructions. The inoculums of microorganisms were prepared using 2-4 h cultured nutrient broth, prepared by inoculating aloopful of each and microorganism in 50 mL nutrient broth incubated at 37^o C. Normal Saline solution 0.9 % was prepared by dissolving 4.5 g NaCl in 500 mL distilled water. The saline solution was used to prepare 10 mL fold dilutions of bacteria in

contaminated water samples for counting bacteria in test and control experiments. The bacterial dilutions were cultured on nutrient agar to achieve countable number of colonies on the plates. Contaminated water samples were prepared by the addition of suitable inoculum of each microorganism to 200 mL sterile distilled water in 500 mL sterile conical flask.

The bacterial count in the flask was adjusted to 5 x 10^5 cfu/mL using Mac. The contaminated water sample was divided equally in four sterile covered 100 mL beakers containing magnetic stirrer.

2.4 Catalyst preparation.

ZnO nanoparticles, which were used as catalyst throughout this work, were prepared as described earlier [82]. By precipitation at room temperature as follows: 0.45 M aqueous solution of zinc chloride ($ZnCl_2$) was prepared by dissolving 15.231 g in 200 mL distilled water. The solution was then diluted to 250 mL with distilled water using a volumetric flask. An aqueous solution of 0.9 M sodium hydroxide (NaOH) was prepared by dissolving 9.0 g NaOH in 200 mL distilled water, and the solution was then diluted to 250 mL with distilled water.

The NaOH solution was then poured into a 500 mL beaker and heated to $\sim$ 55 °C. The $ZnCl_2$ solution was added over a period of

40 minute, to the heated NaOH solution under high speed stirring (magnetically). The beaker was sealed at this condition for 2 hours. The

white fine ZnO nanoparticles precipitate was cleaned with deionized water and ethanol successively, and then dried in air atmosphere at about 60 °C.

2.5 Catalyst characterization.

2.5.1 XRD Characterization.

ZnO X-ray diffraction (XRD) patterns were measured at ICMCB laboratories at the University of Bordeaux using a Philips XRD XPERT PRO diffractometer with Cu Kα radiation (λ = 1.5418 Å) as a source. Particle size was calculated from XRD diffraction pattern measured for ZnO particles using Scherrer equation [83]:

$$d = K \lambda / (B \cos \theta)$$

where K is the shape factor that has a typical value of about 0.09, λ is the X-ray wavelength, B is the line broadening at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle; **d** is the mean size (averaged dimension of crystallites in nm) of the ordered (crystalline) domains [84], which may vary at different particles.

2.5.2 SEM Characterization.

Field emission scanning electron microscopy was measured on a Jeol microscope Model JSM-6700F, using the energy dispersive spectroscopic FE-SEM/EDS technique. SEM shows the surface morphology and an estimated size of the prepared ZnO nanoparticles.

2.6 The disinfection experiments of *E. coli.*

A ZnO nanoparticles was used as a photo-catalyst for bacteria inactivation experiment. Four different beakers were prepared each one has 50 mL of ~5 x 10^5 cfu/mL of bacteria solution. The solutions were magnetically stirred in a 100 mL glass beaker. The first beaker which contains 0,1 g ZnO nanoparticles was exposed to the light source for 60 minutes at 30 °C. In the second one, 0,1 g ZnO nanoparticles was used in dark to know if it affects *E. coli* growth. The third sample was also exposed to light without addition of catalyst to examine light effect on bacteria degradation. The fourth beaker was prepared without catalyst under dark condition. After 60 minutes (end of the selected time) 1 mL of each treated sample was withdrawn using a micropipette and diluted in a series of saline tubes with different dilutions to get 10 fold dilutions, 0.1, 0.01, 0.001 times. Aliquots of 100 µL from each diluted, were spread on two nutrient agar media plates using bent glass rod and incubated at 37 °C for 24 hours. After incubation of the plates at 37 °C for 24 hours, the bacterial colonies in each plates were counted, only plates which have colonies between 30 and 300 colonies were considered in the experiment.

The average number for each tow plates for each dilution was reported as cfu/mL according to the following formula: average number of colonies multiply dilution factor multiply 10 [85].

2.6.1 Control experiments of *E. coli.*

ZnO nanoparticles were used in absence of sun light to study its activity as photo-catalyst and anti bacterial properties without light effect.

Another sample was also exposed to light without addition of catalyst to examine light effect on bacteria degradation. Other control experiment was made without catalyst and without light to know the exact initial concentration of the added bacteria.

2.6.2 The disinfection experiment of *P. aeruginosa* .

A ZnO nanoparticles was used as a photo-catalyst for bacteria inactivation experiment. Four different beakers were prepared each one has 50 mL of ~5 x 10^5 cfu/mL of bacteria solution. The solutions were magnetically stirred in a 100 mL glass beaker. The first beaker which contains 0.1 g ZnO nanoparticles was exposed to the light source for 60 minutes at 30 °C. In the second one, 0.1 g ZnO nanoparticles was used in dark to know if it affects *P. aeruginosa* growth. The third sample was also exposed to light without addition of catalyst to examine light effect on bacteria degradation. The fourth beaker was prepared without catalyst under dark condition. After 60 minutes (end of the selected time) 1 mL of each treated sample was withdrawn using a micropipette and diluted in a series of saline tubes with different dilutions to get 10 fold dilutions, 0.1, 0.01, 0.001 times. Aliquots of 100 µL from each diluted, were spread on two nutrient agar media plates using bent glass rod and incubated at 37 °C

for 24 hours. After incubation of the plates at 37 °C for 24 hours, the bacterial colonies in each plates were counted, only plates which have colonies between 30 and 300 colonies were considered in the experiment.

The average number for each tow plates for each dilution was reported as cfu/mL according to the following formula: average number of colonies multiply dilution factor multiply 10 [85].

2.6.3 Control experiments of *P. aeruginosa*.

ZnO nanoparticles were used in the absence of sun light to study its activity and anti bacterial properties without light effect.

Another sample was also exposed to light without addition of catalyst to examine light effect on bacteria degradation. Other control experiment was made without catalyst and without light to know the exact initial concentration of the added bacteria.

Cut-off filter eliminating 400 nm and shorter wave lengths without any catalyst was used to study effect of the visible light on *P. aeruginosa* growth.

2.6.4 Study of different parameters of *P. aeruginosa* degradation.

Effect of different parameters on the photo-catalyst efficiency and photo-degradation process was studied, and the results are shown herein.

2.6.4.1 Effect of time on catalyst efficiency.

A liquots were pipeted out of the reactor at different reaction times (15, 30, 45, 60 minutes), and the concentration of the contaminants was measured.

2.6.4.2 Effect of temperature on catalyst efficiency.

Photo-degradation of bacteria, using ZnO nanoparticles catalyst, under solar sun light was studied at different temperatures, ranges between 26-38°C.

2.6.4.3 Effect of pH on catalyst efficiency.

Photo-degradation experiments were conducted on pre-contaminated water samples under different pH values. Acidic (pH 4.5), neutral (pH 7.04) and basic media (pH 9) all used. Control experiments were also conducted at these pH values to see if pH itself affects bacterial growth in the contaminated sample, few drops of 0.1 M NaOH solution were added to the first sample to make it basic, the pH of the second sample was measured without any addition, and the third sample was made acidic by adding a drop of 0.25 M HCl solution.

2.6.4.4 Effect of catalyst amount its efficiency.

The added amount of the ZnO nanoparticles catalyst to the contaminated water sample under solar simulator was varied. Catalyst

weights of (0.0, 0.05, 0.10, 0.15 g) were added to 4 water samples with the same volumes of same bacterial concentrations under similar conditions.

2.6.4.5 Effect of contaminant concentration on catalyst efficiency.

Different concentrations of bacteria were used to study the effect of contaminant concentration. The used concentrations were 2.5×10^5, 5×10^5, and 10×10^5 cfu/mL. Control Samples were made for each used concentration to calculate the degradation percent.

2.7 Photo- catalytic system and irradiation sources.

2.7.1 Photo-catalytic system.

The photo-degradation reaction was carried out in 100 mL beaker containing the water sample, contaminated with bacteria and the ZnO nanoparticles catalyst. The beaker was placed in a thermostated water-bath to prevent sample temperature changes. Temperature was measured through the reaction time and adjusted by manipulating the water-bath when needed. The reactor was stirred magnetically and throughout the reaction time to make good distribution of the catalyst through the sample. Sun light was adjusted above the reactor. The default temperature was 30 °C and the default pH was 8. In order to reconfirm an earlier study by Ateeq [63], *E. coli* was examined as one part of this work.

2.8 Total organic content analysis

The determination of total organic carbon (TOC) content in water is useful as a measure of pollution. It is an analytical process which is validated and it demonstrates that it is suitable for its intended purpose.

Total organic content was measured in Al-Quds Pharmaceutical Company Laboratory in Ramallah.

Chapter 3

Results and Discussion

3.1 Introduction.

In this work, ZnO nanoparticles semiconductor particles were used to disinfect water from bacteria by complete photo-degradation with solar sun radiation. UV irradiation of sunlight was used in all experiments. The effect of different parameters on reaction progress was studied. Evaluation of the process in terms of efficiency, cost, environment, economic point and analysis of bacteria to organic content were studied. ZnO nanoparticles under sun light were used in water disinfection from *E. coli* and *P. aeruginosa*. Complete 100 % degradation was achieved in 60 minutes under sun light radiation. Control experiments showed only small percent of degradation. Some factors affecting photo-degradation reaction on *P. aeruginosa* and catalyst efficiency, such as illumination time, temperature, pH, catalyst amount, bacteria concentration were studied.

It is known that photo-degradation of organic contaminates and microorganisms occur due to generation of reaction hydroxyl radicals on the surfaces of the semiconductor particles [86]. When semiconductor crystals are exposed to light with wavelengths suitable for semiconductor band gap, electrons transfer from the valance band to the conduction band leaving holes in the valance band. Presence of electron acceptors such as

O_2 molecules prevents electrons-holes recombination, by accepting the electron and giving $O_2^{\cdot -}$. Holes oxidize hydroxyl groups (OH^-) and adsorb H_2O molecules to form $OH^{\cdot}$ radicals. The $O_2^{\cdot -}$ species also reacts with H^+ ions and form hydroxyl radicals. The hydroxyl radicals have the power to oxidize and degrade organic molecules [87].

$$MO + h\nu \rightarrow MO\ (e^- + h^+) \quad (3.1)$$

$$MO\ (e^-) + O_2 \rightarrow MO + {}^{\bullet}O_2^-, \quad (3.2)$$

$$MO\ (e^-) + {}^{\bullet}O_2^- + {}_2H^+ \rightarrow MO + H_2O_2, \quad (3.3)$$

$$MO\ (e^-) + H_2O_2 \rightarrow MO + {}^{\bullet}OH + OH^-, \quad (3.4)$$

$${}^{\bullet}O_2^- + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2, \quad (3.5)$$

$${}^{\bullet}O_2^- + H^+ \rightarrow {}^{\bullet}OH_2, \quad (3.6)$$

$$MO\ (e^-) + {}^{\bullet}OH_2 \rightarrow MO + HO_2^-, \quad (3.7)$$

$$HO_2^- + H^+ \rightarrow H_2O_2, \quad (3.8)$$

$$2{}^{\bullet}OH_2 \rightarrow O_2 + H_2O_2. \quad (3.9)$$

While the oxidation reactions initiated by the photo- generated holes are:

$$MO\ (h^+) + H_2O \rightarrow MO + {}^{\bullet}OH + H^+, \quad (3.10)$$

$$MO\ (h^+) + H_2O \rightarrow MO + {}^{\bullet}OH + H^+, \quad (3.11)$$

$$MO\ (h^+) + OH^- \rightarrow MO + {}^{\bullet}OH. \quad (3.12)$$

The reactions are terminated as:

$$^{\bullet}OH + H^{+} + 2e^{-} \rightarrow H_2O, \quad (3.13)$$

$$1/2\ O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O. \quad (3.14)$$

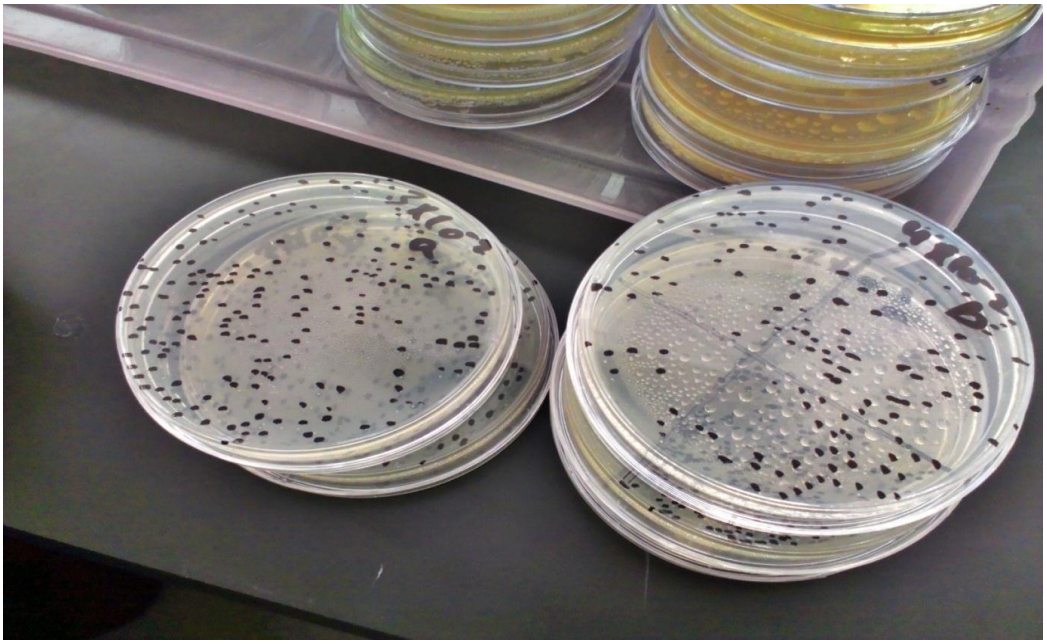


Figure (3.1): *P. aeruginosa* experiment result after chemical treatment.

3.2 Catalyst characterization results.

3.2.1 XRD Study.

ZnO nanoparticles were characterized using XRD technique. Particle size was calculated from XRD diffraction pattern for ZnO nanoparticles, using Scherrer equation [83].

$$d = K\lambda / (B\cos\theta)$$

Where K is the shape factor that has a typical value of about 0.9; λ is the X-ray wavelength, B is the line broadening at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle; **d** is the mean size (averaged dimension of crystallites in nm) of the ordered (crystalline) domains, which may vary of different particles [84].

The X-ray pattern for the prepared ZnO particles showed a hexagonal wurtzite crystal type, Figure (3.2) coincides with our results for XRD pattern of synthesized pure ZnO.

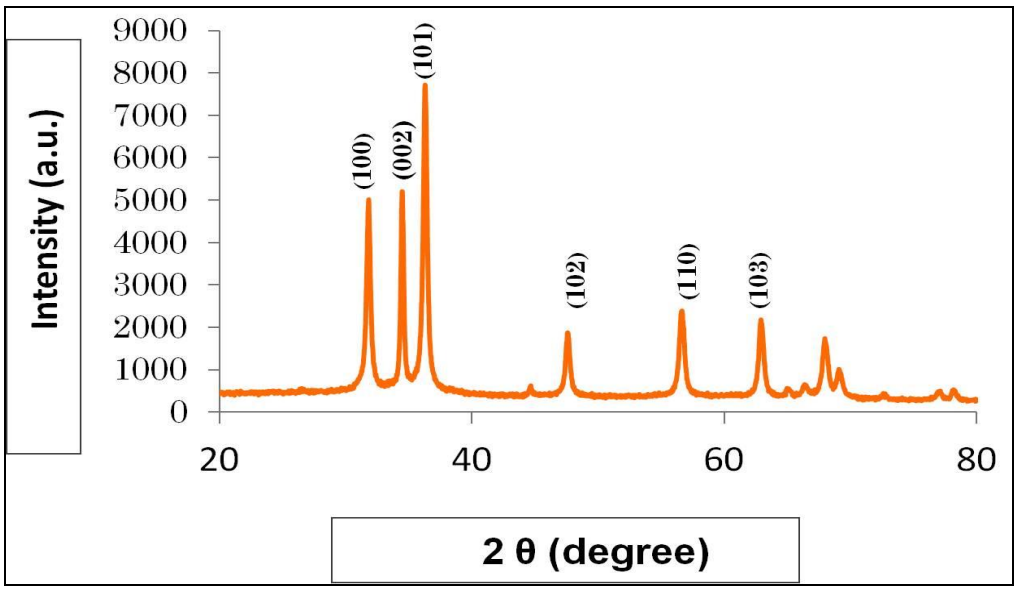


Figure (3.2): X-ray diffraction patterns of nano size zinc oxide (ZnO) particles [63].

Based on 3 different XRD peaks at (102), (110), and (103) planes, the average ZnO diameters was 20 nm. Other peaks are due to other species.

3.2.2 SEM Results

SEM characterization was used to show the surface morphology and estimated size of the prepared ZnO particles. SEM images showed elongated nanorods (rice-shaped) for the obtained ZnO particles with about 25 nm in diameter and 140 nm in length. Surface morphology of the nanoparticles is shown in Figure (3.3).

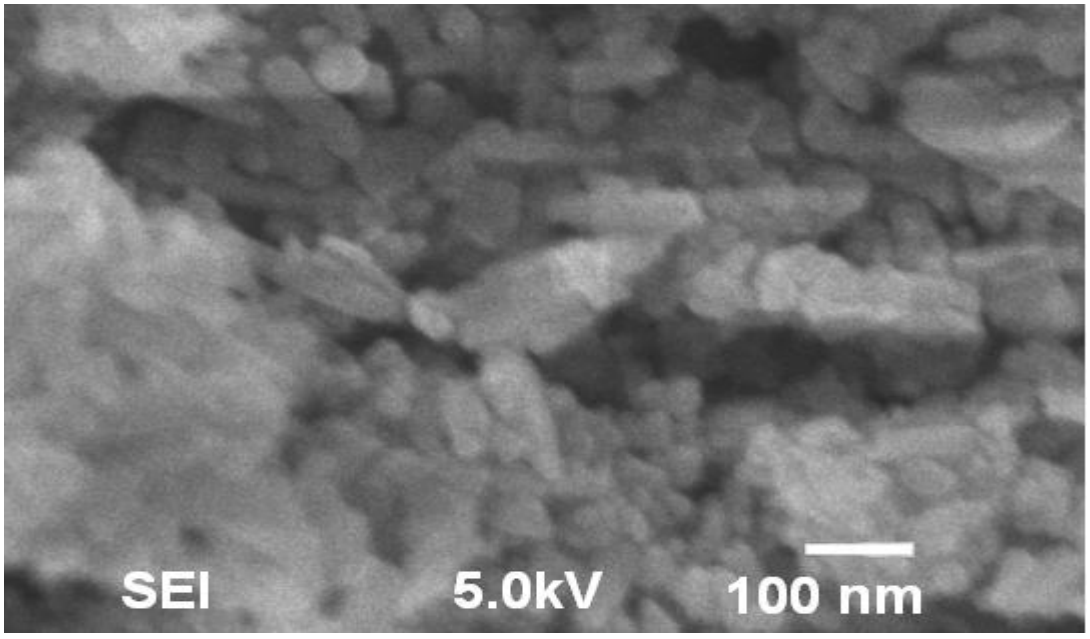


Figure (3.3): SEM images for the prepared ZnO nanoparticles [63].

3.3 Solar sun irradiation experiments.

Photolysis experiments showed that UV light can kill and degrade *E. coli* and *P. aeruginosa*, in sample having the catalyst and sample without the catalyst. DNA molecules inside the bacteria cell creating new linkages between adjacent nucleotides on the same DNA strand. If this damage is

unrepaired, DNA replication is blocked. This blocking in DNA leads to cell death [88].

ZnO nanoparticles were used in bacteria photo-degradation under solar sun radiations. Efficiencies of catalyst were measured by degradation percent.

3.4 *E. coli* experiment results.

ZnO nonoparticles showed powerful oxidizing activity with a wide band gap 3.2 eV that needs UV irradiation 390 nm to be excited. Results of *E. coli* degradation showed 100 % activity. In the dark, using ZnO nanoparticles, there was some decline in bacteria concentration 20.21 %.

Under sun light using ZnO nanoparticles, 100% loss of *E. coli* concentration was observed. These results proved that the activity of catalyst in light conditions better than under dark condition.

3.4.1 Control experiments of *E. coli*

Control experiments were conducted in the absence of light, absence of catalyst or absence of both. Concentration of *E. coli* in the absence of light was decline 20.21 % which is due to anti bacterial activity of ZnO nanoparticles that could accumulate in the cell membrane and make disruption of the membrane [89].

Concentration of bacteria in the absence of catalyst under sun light condition shows small loss, due to the exposure to the sun light only, the

solar sun light contain UV tail that affected the bacteria cell and changes in DNA sequence [88].

The decrease of concentration of *E. coli* in the absence of catalyst and sun light due to unsuitable condition for growth of bacteria such as food, light and temperature. All of *E. coli* experiment results are shown in Table (3.1).

Table (3.1): *E. coli* loss under catalylic condition in dark and light within 60 minutes, original concentration of bacteria is 5 x 10^5 cfu/mL.

Entry Number	Conditions	*E. coli* concentration after treatment (cfu/mL)	*E. coli* loss (%)
1	ZnO/ light	0	100
2	No catalyst/ light	4.95 x 10^4	0
3	ZnO / Dark	3.75 x 10^4	20.21
4	No catalyst / dark	4.7 x 10^4	0

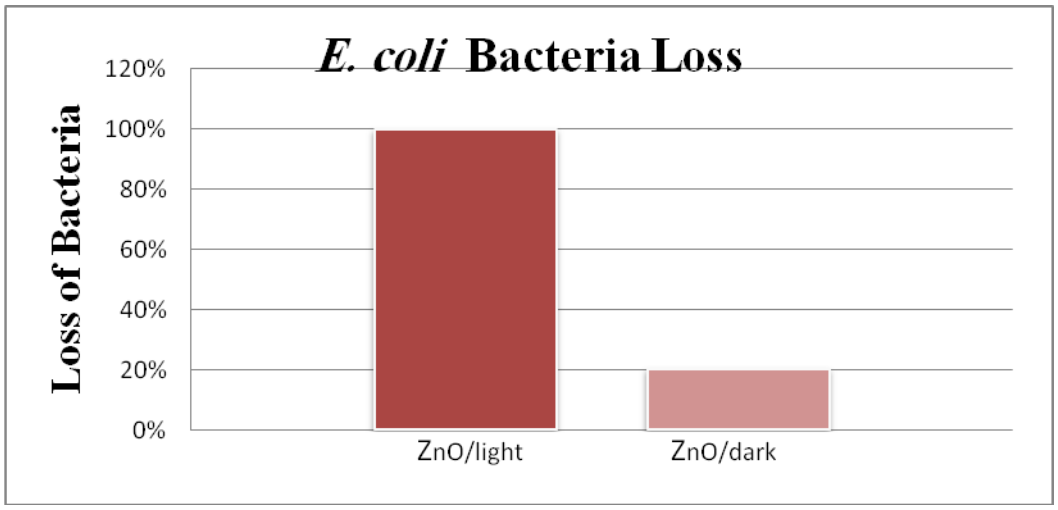


Figure (3.4): *E. coli* loss under catalytic condition in dark and light within 60 minutes, original concentration of bacteria is 5 x 10^5 cfu/mL.

Figure (3.4) shows the difference between *E. coli* loss under two catalyst condition (light and dark). The loss of *E. coli* with ZnO nanoparticles under sun light was 100 % loss. But the loss of *E. coli* with ZnO nano particles without sun light (dark) was 20.21 % loss. These results proved the activity of the catalyst with UV light. Further analysis was done using total organic content (TOC) which proved that ZnO nanoparticles under dark condition killed *E. coli* only, but using ZnO nanoparticles under sun light killed and degraded the *E. coli* completely.

3.4.2 TOC Results of *E. coli* experiments

Environmental impact of total organic carbon (TOC) is the sum of organically bound carbon present in water, bonded to dissolved or

suspended matter, including organisms, organic compounds, cyanate, elemental carbon and thiocyanate .

By using TOC measurements, a number of carbon-containing compounds in a source can be determined. This is important because knowing the amount of carbon in a freshwater stream is an indicator of the organic matter of the stream [90, 91].

In our work, the main aim of using TOC measurements is to determine the ability of the ZnO nanoparticles to kill *E. coli* and convert it into gases. The concentration of the organic content indicates the efficiency of the nanoparticles under sun light to convert the organic content to gases, and purify the water.

TOC analysis shows that the concentration of TOC is very low 7.27 ppm for *E. coli* experiment after using ZnO nanoparticles under sun light condition. This concentration of carbon led to the conclusion that the catalyst and the sun light killed and degraded the *E. coli* bacteria. But when water was treated with catalyst only the concentration of carbon was large 27.19 ppm; the result showed that the catalyst without sun light killed *E. coli* bacteria only without degrading it.

The TOC results proved activity of ZnO nonoparticles under sun light condition against *E. coli* in killing and degrading to gases and minerals, then purify the water from *E. coli* 100 %.

Table (3.2) shows the original concentration of *E. coli* 35.43 ppm and other concentrations and conditions. This result proved the activity of catalyst

under sun light against *E. coli*. The TOC experiment of *E. coli* result is shown in Table (3.2)

Table (3.2): TOC analysis for *E. coli* after zinc oxide nanopaticles treatments at pH (8), temperature (30 °C) and original TOC (35.43 ppm) within 60 minutes.

Entry number	Condition	Organic concentration (ppm)
1	ZnO/ light	7.27
2	ZnO/ dark	27.19
3	No catalyst/ light	31.36
4	No catalyst/ dark	35.43

3.5 *P. aeruginosa* experiments

Photolysis experiments showed that UV light can degrade *P. aeruginosa* with and without catalyst in the light, ZnO nanoparticles killed *P. aeruginosa* under solar sun light 100 %. This experiment was repeated four times to confirm the effect of sun light on the degradation of *P. aeruginosa*. In the dark condition, the ZnO nanoparticles catalyst killed the bacteria 50%. This result indicates that the activity of the catalyst under sun light condition was better than under dark condition.

3.5.1 Control experiments of *P. aeruginosa*

Control experiments were conducted in the absence of catalyst, absence of light, absence of both or used cut-off filter. In the absence of catalyst and light, the concentration of bacteria slightly decreased. This loss may be due to several reasons: no nutrients, no suitable temperature and light. But in the absence of light, the ZnO nanoparticles killed some bacteria and the concentration was decreased to 50%.

The concentration of these bacteria in the absence of the catalyst under sun light condition was decreased due to the effect of UV light on bacteria growth. Control experiment was made and showed that without catalyst no effect on growth of *P. aeruginosa* under cutting-off UV light. (Using cut-off filter eliminating 400 nm and shorter wavelengths). All of the above results are shown in Table (3.3).

Table (3.3): *P. aeruginosa* loss under catalytic condition in light and dark within 60 minutes, original concentration of bacteria is 5 x 10^5 cfu/mL.

Entry number	Condition	*P. aeruginosa* after treatment concentration (cfu/mL)	*P. aeruginosa* loss (%)
1	ZnO/ light	0	100
2	No catalyst/ light	10.5 x 10^4	0
3	ZnO / Dark	16.75 x 10^4	50
4	No catalyst/ dark	22.2 x 10^4	0
5	No catalyst/filter light	25.25 x 10^4	0

This table shows the concentrations of *P. aeruginosa* after treatment with catalyst under light and in the absence of light. The concentration of these bacteria was zero when we used catalyst under sun light and the percent of degradation was 100% and the concentration of these bacteria when the catalyst was used under dark condition was 16.75 x 10^4 and the degradation was 50%. These results showed that the activity of the catalyst under sun light better than its activity in the dark.

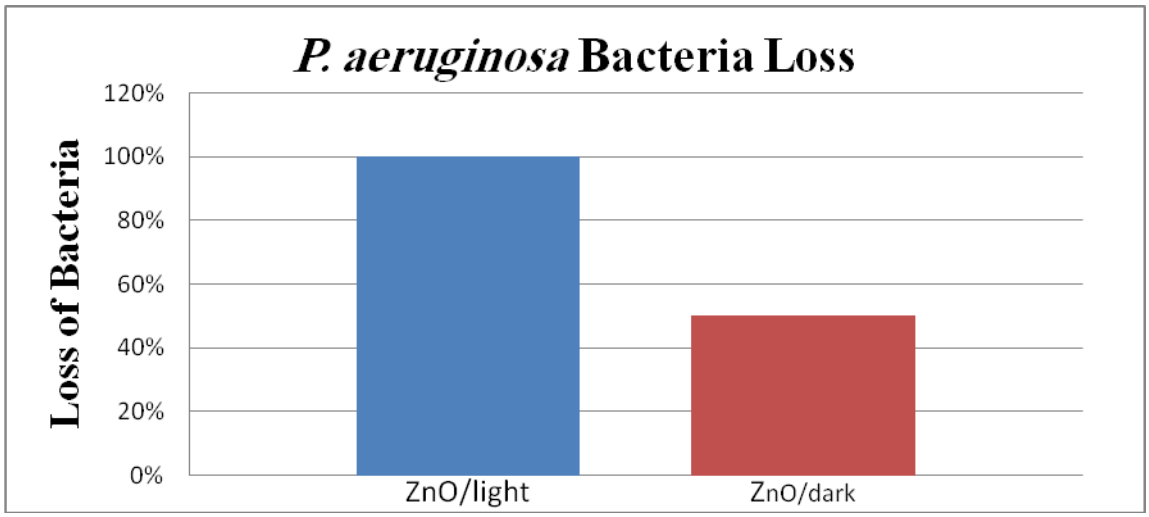


Figure (3.5*):* *P. aeruginosa* loss under catalytic condition in light and dark within 60 minutes, original concentration of bacteria is 5 x 10^5 cfu/mL.

The previous figure showed that the activity of catalyst under sun light caused 100 % loss of concentration of *P. aeruginosa*. This activity of the catalyst decreased without sun light to 50 %.

3.5.2 TOC Results of *P. aeruginosa* experiments.

The higher the carbon or organic content concentration, the more oxygen is consumed. A high organic content means an increase in the growth of microorganisms which contributes to the depletion of oxygen supplies. It affects biochemical processes, nutrient cycling, biological availability, chemical transport and interactions. It also has direct implications in the planning of wastewater treatment and drinking water treatment.

Organic matter content is typically measured as TOC and it is essential component of the carbon cycle. Organic matter in water consists of

thousands of components, including macroscopic particles, colloids, dissolved macromolecules and specific compounds [92].

In *P. aeruginosa* experiments, the concentration of TOC was very low when the catalyst was used under sun light (8.8 ppm). This result proved that the catalyst has great activity under sun light to kill and degrade *P. aeruginosa* to gases and other minerals. On the other hand, the concentration of TOC was increased when the catalyst was used without sun light (36.2 ppm).

In our work, the main aim of using TOC measurements was to determine the ability of the ZnO nanoparticles to kill and degrade *P. aeruginosa* and convert it into gases.

The concentration of the organic content in *E. coli* and *P. aeruginosa* experiments indicate the efficiency of the nano-catalyst under sun light to convert the bacteria to gases and other minerals. The results were shown in Table (3.4). These results proved the activity of the catalyst without light in killing the bacteria only. But this activity is increased when we used the catalyst under sun light in killing and degrading bacteria to gases and minerals then water was purified.

Table (3.4): TOC analysis for *P. aeruginosa* after zinc oxide nanopaticles treatments.

Entry number	Condition	Organic concentration (ppm)
1	ZnO/ light	8.8
2	ZnO/ dark	36.2

3.6.1 Control experiments

ZnO nanoparticles showed a powerful oxidizing activity with a wide band gap 3.2 eV that needs UV irradiation 390 nm to be excited. Results of *P. aeruginosa* degradation showed 100 % activity.

Control experiments were conducted in the absence of catalyst, absence of light or absence of both. *E. coli* and *P. aeruginosa* were affected with photolysis, in the absence of catalyst, as shown in Table (3.3).

3.6.2 Photo catalytic experiments

Some bacteria were affected by photolysis, in the absence of catalyst showing loss of bacteria due to sun light only. The solar light contains UV tail that affects the bacterial cell and causes changes in DNA sequence [88].

The *P. aeruginosa* is one of the known bacteria that are sensitive to UV irradiation [93]. In the presence of catalyst under sunlight, loss of bacteria in the *E. coli* and *P. aeruginosa* experiments was 100 %. The TOC analysis proved that ZnO nanoparticles killed *E. coli* and *P.*

aeruginosa and degraded them into gases. The lower concentration of organic content indicates the efficiency of the ZnO nanoparticles to convert the organic content to gases, and purify the water as shown in Tables (3.2) and (3.4). Treatment of water with ZnO nanoparticles in dark condition didn't degrade bacteria as observed in Tables (3.2) and (3.4). TOC analysis showed that the concentration of the remaining organic content is high.

The 100% degradation was achieved by ZnO nanoparticles. The powerful oxidizing ability of the ZnO, is due to its wide band gap of

3.2 eV and a wavelength equals 390 nm. Therefore, it can completely mineralize the remaining organic compounds to simple molecules, when excited by suitable radiations.

Excitation of electrons from the valance band to the conduction band creates holes, in the valance band, which lead to hydroxyl radical formation. The radicals oxidize the bacterial cell wall together with internal contents, and consequently cause cell death and degradation.

The creation of more holes and hydroxyl radicals would increase the percent of degradation when used ZnO nanoparticles system under sunlight.

The ZnO nanoparticles are sensitive to the 5 % UV tail that exists in the solar sun light. Thus the ZnO nanoparticles absorb the UV tail and behave as a photo-catalyst with good catalytic activity causing 100 % degradation. In our experiment, the light filter (Cutting-off UV light) was

used to prove the effect of UV light in bacteria killing [86]. A cut-off filter (eliminating 400 nm and shorter wavelengths) was placed between the solar sun light and the reactor that containing *P. aeruginosa.* This was to study the role of UV light in *P. aeruginosa* loss. The loss of *P. aeruginosa* was decreased in the reactor with cutting-off UV light as shown in Table (3.3).

A possible mechanism for photo-degradation of bacteria is shown below:

$$\text{Cat.} + \text{UV light} \rightarrow \text{cat.}^{*} \quad (3.15)$$

$$\text{Cat.}^{*} + \text{bacteria} \rightarrow \text{organic matter} + \text{cat.} \quad (3.16)$$

$$\text{Cat.} + \text{UV light} \rightarrow \text{cat.}^{*} \quad (3.17)$$

$$\text{Cat.}^{*} + \text{organic matter} \rightarrow CO_2 + \text{minerals} + \text{cat.} + H_2O \quad (3.18)$$

3.6.3 Total organic content results

Environmental impact of total organic carbon (TOC) is the sum of organically bound carbon present in water, bonded to dissolved or

suspended matter, including organisms, organic compounds, cyanate, elemental carbon and thiocyanate .

By using TOC measurements, a number of carbon-containing compounds in a source can be determined. This is important because knowing the amount of carbon in a freshwater stream is an indicator of the organic matter of the stream [90, 91].

In our work, the main aim of using TOC measurements is to determine the ability of the ZnO nanoparticles to kill *E. coli* and *P. aeruginosa* and convert them into gases (NH_3, CO_2, CO_3). The concentration of the organic content indicates the efficiency of the nanoparticles under sun light to convert the organic content to gases, and purify the water.

The results indicate that ZnO nanoparticles under sun light condition have high efficiency to kill bacteria and convert them into gases. The treated samples in the presence of sun light have proven that our methods was simple, easy, cheap, and efficient method for purifying water from bacteria and their organic wastes by converting them into gases.

3.7 Factors affecting catalyst efficiency.

Effect of different parameters on photo-catalyst efficiency and photo-degradation processes was studied. In general, all these experiment were conducted under solar sun light using 50 mL neutral suspension,

$\sim 5 \times 10^5$ cfu/mL bacteria with 0.1 g nano ZnO catalyst, over a temperature range 26-38 °C for 60 minutes. However, a number of parameters were changed to study their effects, such as: time, temperature, pH, catalyst concentration, bacteria concentration.

3.7.1 Effect of time on catalyst efficiency

Bacteria photo-degradation with time was studied in some experiments, as shown in Figure (3.6). Logically; the results showed that

as time proceeded the bacteria concentration decreased. Values of concentration were higher at the beginning of the reaction.

Table (3.5) and Figure (3.6), show that 100 % bacteria loss was achieved over a period of 60 minutes.

Table (3.5): Time effect on *P. aeruginosa* degradation in light condition.

Entry number	Time by minute	Condition	*P. aeruginosa* concentration cfu/mL	Bacteria degradation (%)
1	Zero	ZnO / light	5×10^5	0
2	15	ZnO / Light	6.8×10^2	99.51
3	30	ZnO / Light	0	100
4	45	ZnO / Light	0	100
5	60	ZnO / Light	0	100

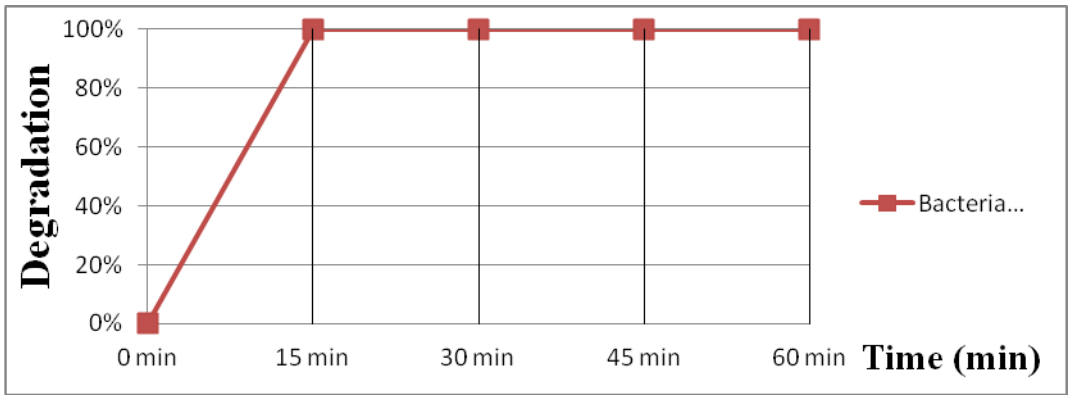


Figure (3.6): Time effect on *P. aeruginosa* degradation in light condition**.**

At the beginning of the reaction the bacteria concentration was higher, which promoted more degradation. As the reaction proceeded bacteria concentration decreased and photo-degradation was 100 % after

15 minutes. The degradation was 100 % when used 0.1 g of ZnO nanoparticles under the sun light.

In a previous study, ZnO nanoparticles caused 90 % reduction of *E. coli* within 12 hours in dark [92].

In our study, the anti bacterial activity of ZnO nanoparticles showed an advantage for the use of sunlight to enhance the activity of the ZnO nanoparticles in very short time (about 15 minutes).

3.7.2 Effect of temperature on catalyst efficiency.

Photo-degradation of bacteria, using ZnO nanoparticles catalyst, under solar sun light was studied at different temperatures, ranges between 26-38 °C. This is known to be suitable for bacteria living and growth. Values of concentration showed no effect of temperature on *P. aeruginosa* degradation and the degradation of *P. aeruginosa* in all of the experiments were 100 %. However, in a previous study, the treatment using ZnO nanoparticles at a higher temperature showed slight effect on the antibacterial activity which leads to lower activity [94]. While in another study, there was no effect of the temperature on the antibacterial activity of the ZnO nanoparticles [63].

The results of the previous studies confirm with our result, that because the mobility of the bacteria increases with increasing temperature, while the oxygen molecules decrease then the activity of the particles decreases. Therefore, the recommended condition for water treatments is at

room temperature where there is no need for energy supply for cooling or heating. Figure (3.7) shows 100 % degradation within 60 minutes for all experiments.

Table (3.6): Temperature effect on *P. aeruginosa* degradation within 60 minutes

Temperature (°C)	*P. aeruginosa* degradation (%)
26	100
28	100
30	100
32	100
34	100
36	100
38	100

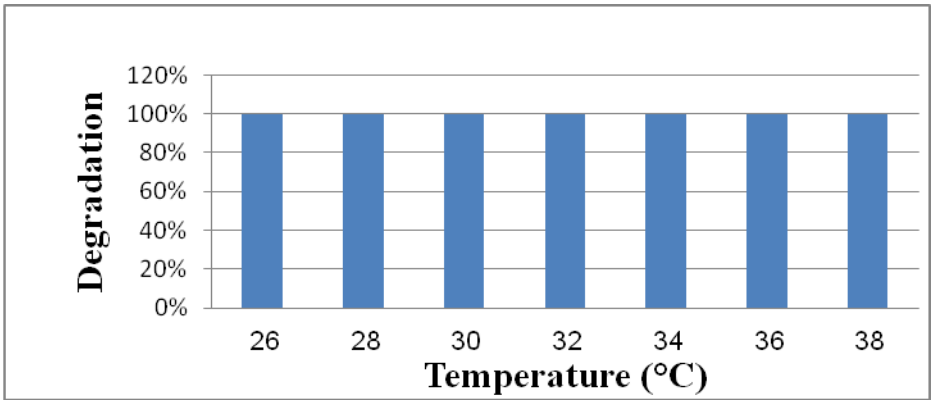


Figure (3.7): Temperature effect on *P. aeruginosa* degradation within 60 minutes**.**

3.7.3 Effect of pH on catalyst efficiency

No effect of pH on catalyst efficiency for *P. aeruginosa* photo-degradation using solar sun light at 28 °C. The photo-degradation reaction was studied using three different pH values, neutral (7.04), acidic (4.5), and basic (9) media.

All these pH values were suitable for *P. aeruginosa* life and do not affect its growth. This was also confirmed from control experiments using no catalyst at all different pH values. These pH values for samples were measured at the start. The reaction process was not much affected with changing the pH value of the reaction. The degradation result was almost the same in three media which is 100 % degradation except for pH 9.0 which showed a slight decrease in degradation. Complete degradation was achieved at pH 4.5 and 7.04. Results for pH effect are presented in Figure (3.8) and Table (3.7).

Table (3.7): pH effect on *P. aeruginosa* degradation within 60 minutes.

Entry number	pH	Condition	*P. aeruginosa* concentration (cfu/mL)	*P. aeruginosa* degradation (%)
1	4.5	ZnO/ light	0	100
2	7.04	ZnO/ light	0	100
3	9.0	ZnO/ light	40	96

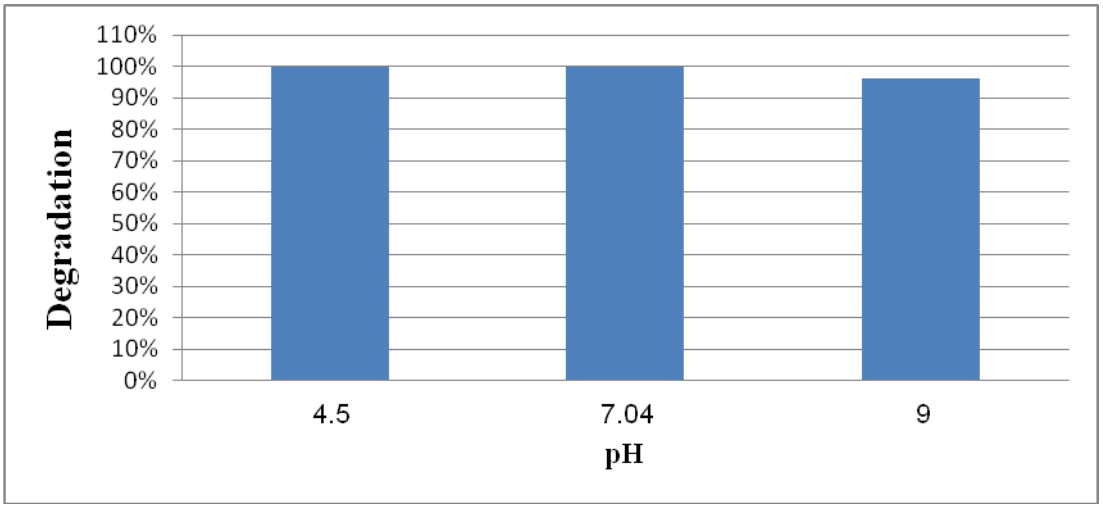


Figure (3.8): pH effect on *P. aeruginosa* degradation within 60 minutes.

The degradation results were almost the same in the three different media (acidic, neutral and basic), with only a slight deviation for the basic medium, as shown in Figure (3.8).

ZnO is considered as an amphoteric oxide. ZnO reacts as a base in acidic solution, and as an acid in basic solutions, as shown in equations 3.12 and 3.13.

In acidic: $ZnO + 2H^{+} \rightarrow Zn^{2+} + H_2O$ (3.19)

In basic: $ZnO + H_2O + 2OH^{-} \rightarrow [Zn(OH)_4]^{2-}$ (3.20)

The increase in the pH value leads to increase in the concentration of the hydroxide layer that formed on the surface of the ZnO nanoparticles. Therefore, the solubility of the ZnO nanoparticles decreases with increasing pH value, then the antibacterial activity of the particles decreases [95, 63].

3.7.4 Effect of catalyst amount on its efficiency

Effects of catalyst amount on its efficiency were studied using three different concentrations of ZnO nanoparticles catalyst (0.05, 0.010, 0.015 g). Our results showed that no effect of catalyst amount on photo-degradation of *P. aeruginosa* as all concentrations used achieved 100% degradation, as shown in Table (3.8) and Figure (3.9).

Table (3.8): Catalyst concentration effect on its efficiency on *P. aeruginosa* degradation within 60 minutes.

Entry number	Weight of catalyst (g)	*P. aeruginosa* degradation (%)
1	0.05	100
2	0.10	100
3	0.15	100

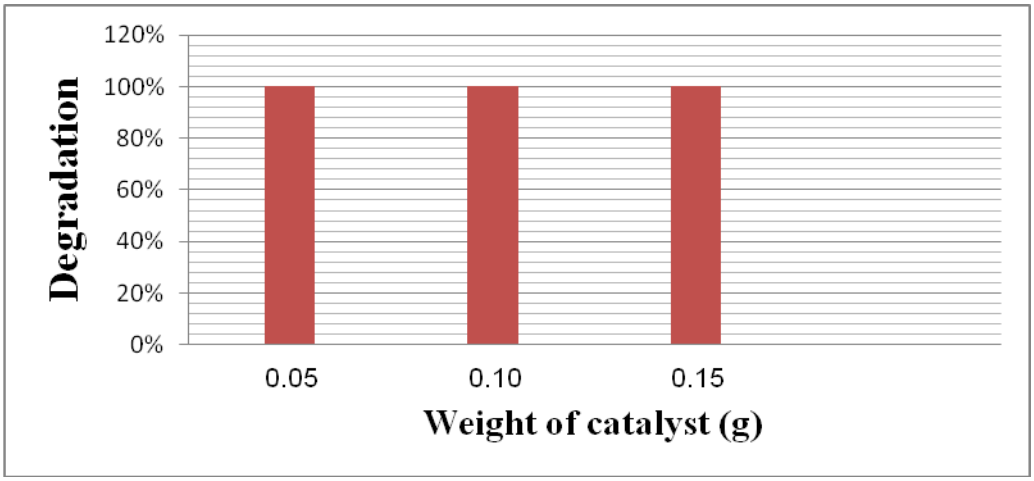


Figure (3.9): Catalyst concentration effect on its efficiency on *P. aeruginosa* degradation within 60 minutes.

3.7.5 Effect of bacteria concentration on catalyst efficiency

At low and high bacteria concentrations the degradation of bacteria percent was 100 % when 0.1 g ZnO nanoparticles were added under solar sun light. And the concentration of bacteria was become zero. The result was shown in Figure (3.10) and Table (3.9).

Table (3.9): Concentration effect on catalyst efficiency on *P. aeruginosa* degradation within 60 minutes.

Entry number	*P. aeruginosa* concentration (cfu/mL)	*P. aeruginosa* degradation (%)
1	2.5×10^5	100
2	5×10^5	100
3	10×10^5	100

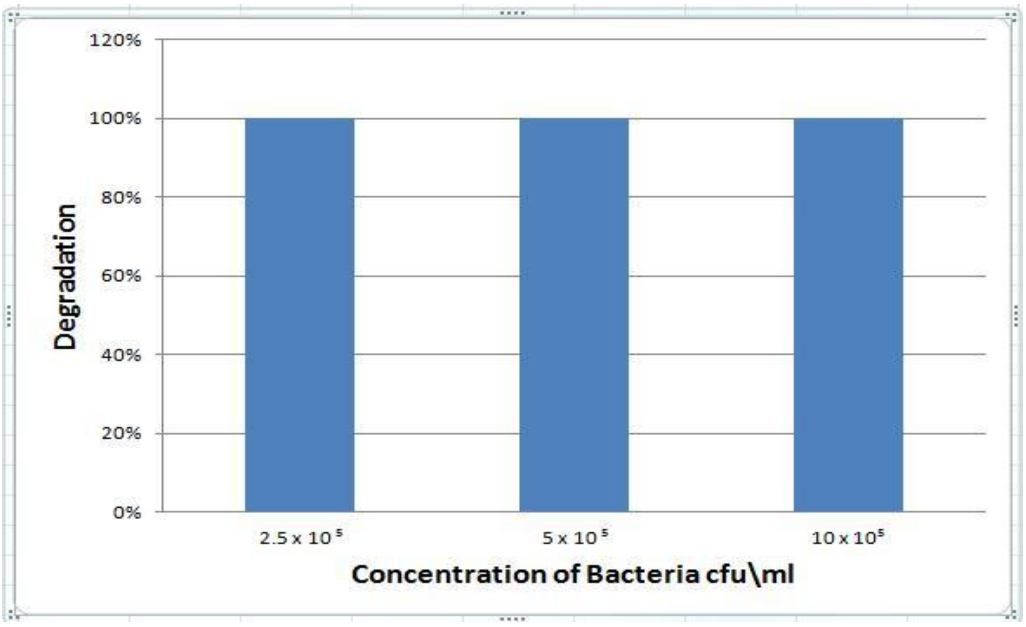


Figure (3.10): Concentration effect on catalyst efficiency on *P. aeruginosa* degradation within 60 minutes.

The results show that ZnO nanoparticles as photo-catalyst for complete degradation of *P. aeruginosa* was effective under different reaction parameters. The catalyst efficiency was not lowered by changing time, temperature, catalyst amount and bacteria concentration. This indicates the importance of using ZnO nanoparticles as photo-catalyst for complete degradation of *P. aeruginosa* in water.

Conclusions

1. ZnO nanoparicles catalyst showed good activity against the two kinds of examined bacteria in the dark condition.
2. ZnO nanoparticles catalyst showed very good activity against the examined bacteria under solar sun light killing and degrading of bacteria and converting the bacteria to gases and minerals.
3. Changing of temperature, pH value, catalyst amount, bacteria concentration and time of experiment didn't affect the catalyst efficiency.
4. TOC analysis proved the efficiency of ZnO nanoparticles under solar sun light, in degradation of *E. coli* and *P. aeruginosa*.
5. ZnO nanoparticles are very good catalyst against *P. aeruginosa* under examined conditions in this research. Also this catalyst has low cost and it is very safe with no side effects on human health.

Suggestions for future work

1. Using the ZnO nanoparticle catalyst against other types of microorganisms.
2. Applying this work on large scale in water (pilot plant testing).
3. Using the ZnO nanoparticle catalyst under solar sun light on soil pollutants.
4. Study effect of ZnO nanoparticles with light against bacteria with time, starting with one minute and more.
5. Study different concentrations of the catalyst lower than that used in this study to determine lower concentration that produces complete photo-degradation.

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جامعة النجاح الوطنية

كلية الدراسات العليا

فهم دور الحبيبات النانوية في تطهير المياه: قتل البكتيريا مقابل تحطيمها الضوئي الكلي بواسطة ZnO

إعداد

جمانة فايز محمد اشتيوة

إشراف

د. مجدي دويكات

د. سمر الشخشير

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية، في نابلس، فلسطين.

2013

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إعداد

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إشراف

د. مجدي دويكات

د. سمر الشخشير

الملخص

تتعرض المياه لملوثات مختلفة منها الفيزيائية والكيميائية والبيولوجية والتي تعرض مستخدميها إلى أمراض وأعراض ربما تكون خطرة، وقد تمت معاجلة المياه بأشكال مختلفة والتركيز على مياه الشرب بشكل خاص، أما من حيث الملوثات البيولوجية التي تشكل خطورة واضحة على مستخدمي المياه والتي تتمثل بالبكتيريا بشكل كبير والتي عولجت بأشكال مختلفة، ولكن الكثير من طرق المعالجة هذه يترتب عليها آثار جانبية خطيرة.

أما الحفاز المستخدم في هذه الدراسة، وهو أول أكسيد الزنك (ZnO) الذي يتمتع بصفات زادت من أهميته من حيث عدم سميته وفعاليته وتواجده بشكل يسير وسهولة التخلص منه، جعلته محط أنظار الدارسين، في هذه الرسالة درس أثر الجزيئات النانوية من (ZnO) على البكتيريا المتواجدة في المياه حيث استخدم ضوء الشمس الذي يحتوي على قدر كاف من الأشعة الفوق بنفسجية لقتل وتحطيم هذه البكتيريا وتحليلها بشكل كامل الى غازات متصاعدة مثل (CO_2). حيث أثبتت الدراسة أنه بالإمكان تطهير المياه بشكل كبير ونهائي من ملوثاتها البيولوجية بهذه الطريقة، حيث وصلت درجة التحطيم الى 100 %.

كما ودرست بعض العوامل على معالجة الحفز مثل مدة التعريض لضوء الشمس ودرجة الحرارة وتركيز الملوث ، وكمية الحفاز ودرجة الحموضة، حيث لم يظهر أي تأثير لهذه

العوامل في تحطيم الحفاز للبكتيريا وكانت نسبة التحطيم غالبا 100 % في معظم التجارب بوجود الحفاز وضوء الشمس ، مما زاد هذا الحفاز الضوئي أهميه وسهولة في الإستخدام.

كما وأجريت تجارب على نوعين من البكتيريا وهما على التوالي *P.aeruginosa* و *E.coli*، حيث أظهرت النتائج القتل والتحطيم لكلتيهما بنسبة 100 % بوجود الحفاز وضوء الشمس (المحتوي على الأشعة الفوق بنفسجية، أما بغياب ضوء الشمس كان هناك تباين بنسبة التحطيم، حيث كانت بنسبة 50 % لل *P. aeruginosa* و20.21% لل *E.coli* ; مما يدل على فعالية الحفاز على بكتيريا *P. aeruginosa* *بشكل أكبر من* *E.coli* في الظروف المعتمة.

وأثبتت هذه الدراسة ايضا، بعد تحليل كمية الكربون في المحلول البكتيري بعد معالجته أن الحفاز لا يقتل البكتيريا فحسب، بل يحللها بوجود الأشعة الفوق بنفسجية، الى غازات متطايرة تخرج من المحلول مما يترك المياه معقمة، ونظيفة بنسبة عالية، حيث لا تحتاج الى عمليات فلترة أخرى.