An-Najah National University

Faculty of Graduate Studies

Complete Mineralization of *Enterococcus* and *Proteus mirabilis* Bacteria in Water Using ZnO Nanoparticles Photocatalysts

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Dedication

To my father. As you look down from heaven, I hope you are proud of your little girl.

.. ….

To the woman like no other. Who gave me life, fought for me and supported me to be who I am. My mother.

…..

To the memory of my beloved grandmother.

…..

To my brothers, my sisters. Who have always been there.

…….

I dedicate this work

Acknowledgement

In the name of Allah, the Most Gracious and the Most Merciful, all praises to Allah for the strengths and His blessing in completing this thesis.

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My deepest gratitude to my family and friends; who enlightened my academic path with care and support.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Comparative Study between Commercial Charcoal and *Asphodelus ramosus* Tuber Derived Activated Carbons forAdsorption of Heavy metals from Aqueous Solution

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو 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 or qualification.

Student Name: **إسم الطالبة: أميمة يوسف**

Signature: **التوقيع:**

Date: **التاريخ: 15/6/2018**

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List of Abbreviations

Symbol	Abbreviation
UV	Ultraviolet Light
DBPs	Disinfection By-Products
AOP	Advanced Oxidation Process
eV	Electron Volt
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
(hlk)	Miller's Indices
e-	Electron
h+	Hole
G_{+Ve}	Gram Positive
G_{-Ve}	Gram Negative
°C	Celsius Degree
W	Watt
Cm^2	Square Centimeter
Lx	Lux
M	Molar
mL	Milligrams
g	Grams
nm	Nanometer
cfu	Colony forming unit
D	Crystal size
Å	Angstrom
β	Full width at half maximum of X-ray pattern in radians
C	Correction factor
PL	Photoluminescence
Ls	Luminescence
HPLC	High performance liquid chromatography
h	Planck's constant
q	Charge of electron
Sec	Seconds
J	Joules
INT.	Intensity
Temp	Temperature
Fig.	Figure
#	Number
Cat.	Catalyst

Complete Mineralization of *Enterococcus* and *Proteus mirabilis* Bacteria in Water Using ZnO Nanoparticles Photocatalysts

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Abstract

Semiconductors have been widely used in water disinfection and also used in photodegradation of water-organic contaminants. Zinc oxide (ZnO) semiconductor has a band gap (~3.2 eV). This wide energy gap makes it very effective on microorganisms under sunlight. The ZnO can be activated with the small fraction of UV solar light. It is cheap, stable and safe. In earlier studies, ZnO particles were assessed in killing gram negative bacteria (E. coli) and (P. aeruginosa) followed by complete mineralization. In this research, ZnO was used in photodegradation reaction as a catalyst for water disinfection from *Enterococcus* (gram-positive bacteria) and *Proteus mirabilis* (gram-negative bacteria). Killing and complete mineralization of these two bacteria have not been examined before by photocatalytic systems, to our knowledge. The photodegradation reaction was conducted under solar simulator light. Zinc oxide was lab prepared from the reaction of zinc chloride with sodium hydroxide; the prepared ZnO was characterized using XRD, SEM, photoluminescence (PL) spectra, and electronic absorption spectra. The energy gap was calculated and found to be 3.23 eV. The efficiency of catalyst was assessed based on a bacterial killing percentage; turn over frequency and quantum yield. Effect of different parameters on

reaction was studied at different periods of time for both *Enterococcus* and *Proteus mirabilis.* About 100% degradation percent was achieved in 30 minutes under a light. Control experiments showed that using light and using ZnO separately promote only small percentage of killing.

Other factors that affect photodegradation reaction and catalyst efficiency such as pH, catalyst concentration, contaminant concentration and temperature were studied and discussed.

High-performance liquid chromatography (HPLC) was used to determine organic content in water. For both bacteria under photo-the HPLC results confirm the complete photodegradation of bacteria.

Chapter 1

Introduction

1.1 Overview

The world faces lack of freshwater resources. Freshwater security is one of the global environmental major problems of the 21st century [1]. While water covers about 70 percent of the earth, only ~2.5 % of water volume is fresh, two-thirds of fresh water is trapped in glaciers and snowfields. Only one percent of it is accessible fresh water. A 0.77% of the planet's freshwater is available for human consumption [2]. The population is expected to increase to about 9 billion by 2050 [3] and so more people are expected to lack access to water. Moreover, most of the available water resources are physically, chemically and biologically polluted. With that, the water sources could be a mode of transmitting diseases [4]. Therefore, many efforts have been made to purify water and make it suitable for human consumption.

Disinfection can be physical, chemical or both. Physical disinfection of water is usually done using sunlight, ultraviolet light (UV), microwaves radiation, gamma radiation and both moist heat (autoclave, steam) and dry heat (flame, baking). For chemical disinfection, many chemicals are used to eliminate microorganisms and prevent water born diseases such as chlorine dioxide (ClO_2), hypochlorite (OCl^-), ozone (O_3), halogens including chlorine (Cl_2), bromine (Br_2) and iodine (I_2), metal ions such as copper (Cu^{2+}) and silver

(Ag^+), potassium permanganate ($KMnO_4$), alcohols, soaps, detergents, quarternary ammonium salts, hydrogen peroxide [5].

However, the reaction of chemicals disinfectors with organic matter formed disinfection by-products (DBPs). This issue was discovered in 1970's, therefore, it was necessary to find alternative disinfectants that are effective, cheap and form no by-products [6] .

Remediation of water using semiconducting materials has been studied. Semiconductors were widely used in killing microorganisms in water [7, 8]. However, recent researchers are heading to use semiconductors in photocatalytic degradation of organic pollutants in water [7, 9]. The basic mechanism for this remediation is based on the oxygen defects on the surface of the semiconducting materials which when activated by photon irradiation are used to destroy the organic contaminants. Zinc oxide semiconductor is favorable catalysts as it has a wide energy gap that can be excited by solar radiation [9].

1.2 Photocatalytic degradation

Photocatalysis can be defined as the acceleration of a photoreaction in the presence of a catalyst. *"Photocatalyst is a substance which is activated by absorbing a photon and is capable of accelerating a reaction without being consumed*"[10]. Semiconductor particles commonly used as photocatalysts. Semiconductors have the ability to treat organic contaminants in water in a process known as advanced oxidation process (AOP) [11]. (AOP) mainly depends on the formation of the highly oxidizing radicals ($OH^{\bullet}$). When

oxidants (such as ozone or hydrogen peroxide) combine with ultraviolet (UV) or visible (VIS) radiation and catalysts such as metal ions or semiconductors, radicals are forms. The formed radicals can oxidize a wide range of organic compounds to CO_2, H_2O and others [12].

1.3 Semiconductor

A semiconductor is a material with electrical conductivity properties between a conductor and an insulator, and the conductivity increases with increasing temperature [13].

Semiconductors energy gaps are slightly small, and when they absorb photons with energy higher than the energy gap, the electrons get excited from the valence band to the conduction band leaving a holes in the valence band, figure (1.2). These holes act as positive charges. The electron and hole pairs exist and generate free radicals [14].

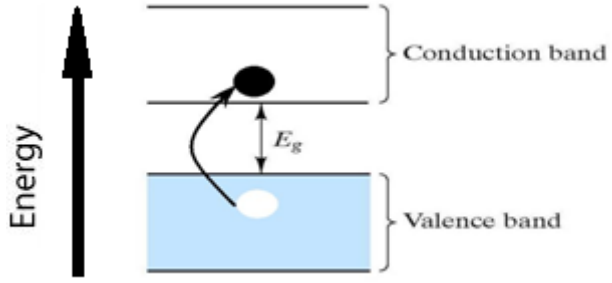

Figure (1.1): Valence electron absorbs photon with energy higher than energy gap and goes to the conduction band leaving a holes in the valence band in a semiconductor [15].

Various semiconductors are used as photocatalysts in photodegradation of organic contaminants. ZnO is widely used because it is chemically stable,

nontoxic, highly efficient and inexpensive. It has a wide band gap and can be excited by UV irradiations [16].

1.4 Zinc oxide

Zinc oxide (ZnO) is a white soft powder inorganic material, insoluble in water and soluble in acids or alkalis. It is used in many industrial applications like in chemicals, rubber, paints, ceramics, glasses, cosmetics, food and so many other industries [17]. Zinc oxide is a good material as a semiconductor; it has been commonly used for its catalytic, electrical, optoelectronic and photochemical properties. It has wide band gap about of 3.37 eV [18]. The nanostructures of ZnO have many preferred features that make it good choice for catalytic reaction processes as photocatalysis in wastewater treatment under sun light. It has relativity large surface area, high UV absorption and reflection with band gap same region of UV spectral, so that excitation emission processes can persist at room temperature, high catalytic activity and high anti-microbial activity [19].The physical and chemical properties of ZnO are affected by the morphology of its nanostructures [20]. The rate of photocatalytic degradation of organic pollutants depends on the particle size and the surface area of the used catalyst [21]. Decreasing the size of ZnO crystals will increase the number of particles and the ZnO surface area. Increasing the number of catalyst particles will increase the number of absorbed photons, at the same time, increasing the surface area of ZnO will lead to absorbing more radiations and heighten reaction rates due to low mass-transfer limitations [22].

Zinc oxide can be characterized using different techniques. The size and the type of ZnO particles are determined by X-ray diffraction (XRD). Scanning electron microscopy (SEM) is used to describe the morphology, size and shape of the particles. Other techniques using UV-Visible absorption spectrophotometry and photoluminescence spectrometry are used for determining the energy bands and the optical properties of the particles [23].

1.4.1 Zinc oxide crystal structure

The crystal structure of ZnO has unique arrangement of atoms. In crystals, atoms are arranged in such a way that their positions are exactly periodic. The structure of crystals can be described in terms of a lattice as shown in figure (1.3). The crystal lattice involves mathematical points have the same geometrical properties as the crystal. So the crystal structure is formed by adding atoms to every lattice, [13].

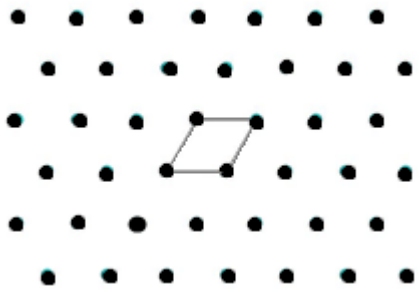

Figure (1.2): Lattice points of space lattice in two dimensions.

The arrangement of atoms can be described in terms of its unit cell. The unit cell is a small box containing locative arrangement of atoms, figure (1.4). The unit cell is given by its lattice parameters, which are the length of the cell edges and the angles between them [13].

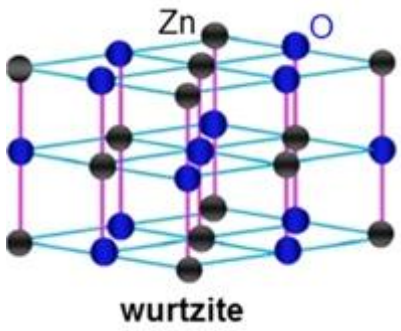

Figure (1.3): Space lattice (zinc oxide wurtzite structure) [24].

A family of lattice planes is determined by three integers h, k, and l, and called the Miller indices (hkl), each index indicate a plane orthogonal in direction to (h, k, l). Hence, Miller indices are used to specify directions and planes in crystals [13].

When a photon with wavelength comparable with the lattice space is incident to crystal, it will diffract in directions differ from the incident direction. So incident beam interfere with one another as they leave the crystal. The study of the diffracted wave is used to study the structure of the crystal using W. L. Bragg's law as following: [25, 26].

$2d \sin \theta = n\lambda$ (eq. 1)

Where, n = is an integer number of wavelengths

λ = wavelength

$2d \sin \theta$ = the path difference

1.5 Disinfection mechanism

The photocatalytic mechanism utilizes the excitation of semiconductors to form electron - hole pairs. When the electrons have enough energy they

move from valence band to conduction band leaving holes in the valence band. Electrons and holes move to the surface of the catalyst and react with other substances [27].

The moved electrons have a reduction power and can react with oxygen molecule to produce O_2^-. The O_2^- transfers through the water and becomes $HO^{\bullet -}$, and then produces H_2O_2. The holes (h^+) have the oxidation power and can react with surface H_2O/OH^- to produce $OH^\bullet$, the $OH^\bullet$ also form H_2O_2 [28].

The h^+, O_2^-, $OH^\bullet$ and H_2O_2 have oxidation effect on bacterial cells and can reduce the chemical oxygen demand (COD) of waste water. They can oxidize the organic pollutants into CO_2 and H_2O as follows [27] :

$ZnO + photon \rightarrow h^+ + e^-$

$H_2O \rightarrow h^+ + OH^-$

$h^+ + OH^- \rightarrow HO^\bullet$

$h^+ + H_2O \rightarrow H^+ + OH^\bullet$

$e^- + O_2 \rightarrow O_2^{-\bullet}$

$O_2^- + h^+ \rightarrow HO_2^\bullet$

$2HO_2^\bullet \rightarrow O_2^\bullet + H_2O_2$

$H_2O_2 + O_2^{-\bullet} \rightarrow OH^\bullet + OH^- + O_2$

$H_2O_2 + photon \rightarrow 2OH^\bullet$

$Organic + OH^\bullet + O_2 \rightarrow CO_2 + H_2O + other\ productions.$

The mechanism is shown in figure (1.4).

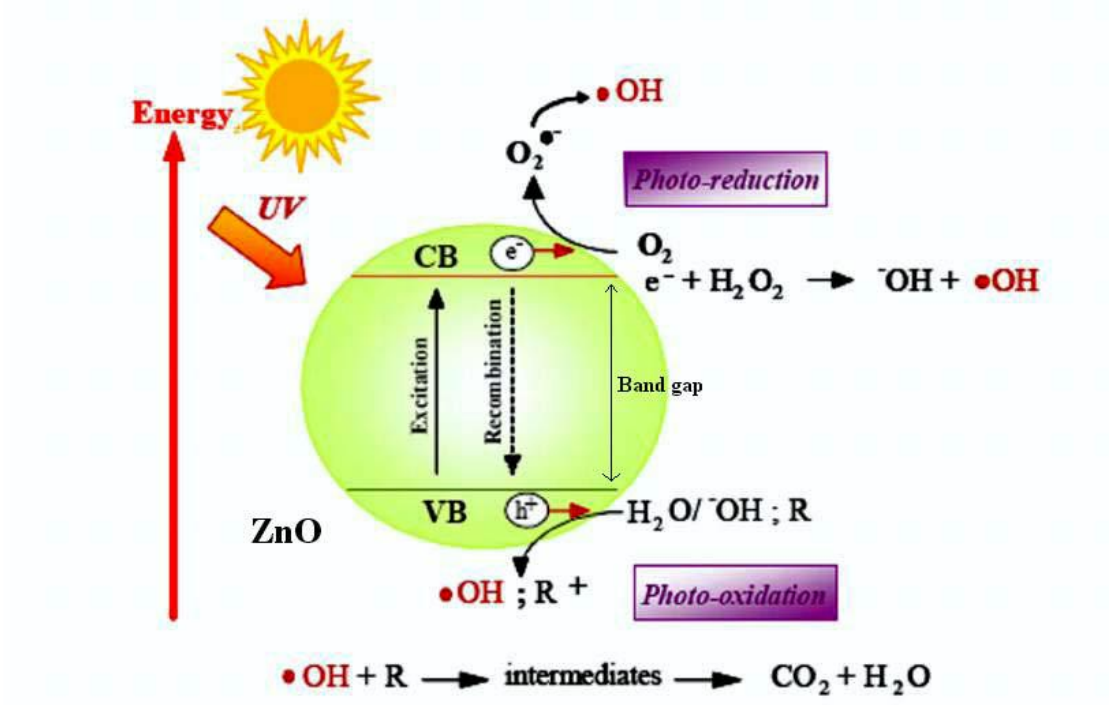

Figure (1.4): Mechanism of photocatalysis [27].

1.6 Pathogenic microorganisms

Microorganisms are microscopic living organisms that surround us and can be found in nature. They are too small to be seen by bare eyes and can exist as contaminants in soil, air, food and water. Most microorganisms are harmless and essential for human body vital processes. However, there are also some microorganisms that cause diseases. These microorganisms are called pathogenic microorganism [29].

In drinking water, pathogenic microorganisms can be divided into; bacteria, fungi; viruses and parasitic protozoa. Bacteria are the most living things that exit on earth. They exists single cell organisms and can be found in different shapes. They exist as individual bacteria or in bacterial chains, bundles or pairs [30]. Most bacteria use organic chemicals for their nutrition, while

some others manufacture their own food by photosynthesis, or derive nutrition from inorganic substances [31].

Bacteria are enclosed in cell walls that are largely composed of a carbohydrate and protein complex called peptidoglycan. It can be divided according to its structure into two types; Gram-positive bacteria (which have thick peptidoglycan layer in their cell wall) and Gram-negative bacteria (which have thin peptidogly layer sandwiched between an inner cell membrane and an outer one). Comparing Gram-negative bacteria with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibodies because of their impenetrable cell wall. Gram-positive bacteria are more receptive to antibiotics due to the absence of the outer membrane [31].

1.6.1 *Enterococci*

Enterococci are Gram-positive bacteria that can be found in water, soil, and plants. They can grow at a temperature range of 10 - 45°C and form resistance to 60 °C. They grow in environments with broad pH values [32]. Enterococci have been considered pathogenic and cause a variety of infections commonly in the urinary tract. They have high resistance to many antibiotics that make treatment difficult [33].

1.6.2 *Proteus mirabilis*

Proteus mirabilis is a Gram-negative, anaerobic, rod shaped bacterium. It is a serious cause of infections in humans. It is widely distributed in soil and

water. *Proteus mirabilis* causes 90% of *Proteus* infections and can be considered as a community acquired infection [34].

Proteus mirabilis bacterium produces urease. Thus, one of the common symptoms of infection with *Proteus mirabilis* is that the patient's urine who suffers from infections becomes alkaline. With no treatment, the increased alkalinity will cause crystals to be formed as kidney stones. If the person is treated with antibiotics for the infection, the bacteria that are found on the kidney stones can lead to the reactivation of the infection [35].

Our approach to solve the problem is by totally killing and degrading the bacteria using zinc oxide as phto-catalyst under sun light.

1.7 Objectives of this work

The main objectives of this study are:

1. Preparing nanoparticles of ZnO and use them as a catalyst in the photodegradation of bacteria in water.
2. Using the catalyst systems in water disinfection by complete killing and degradation of *Enterococcus* (G_{+ve}) and *Proteus mirabilis* (G_{-ve}) bacteria by using a low cost and safe method under solar light. This method is the first time to be used for these bacteria.
3. Achieving complete mineralization of the two bacteria and their organic contents in water. The resulting solution expected to contains no organic compounds.
4. Determination the optimum factors and conditions that affect the photodegradation reaction and catalyst efficiency. Such factors are:

illumination time, temperature, pH, catalyst concentration, contaminant concentration and dissolved organic and inorganic impurities.

5. Give a critical comparison between the two types of bacteria under the photodegradation conditions.

1.8 novelty of this work

Bacterial killing by ZnO nano-particles is well known in literature. However, killing and complete mineralization of the bacteria by photodegradation has been studied recently:

1. Ateeq [36] used sensitized ZnO particles to kill and mineralize gram-bacteria (*E. coli*) under both solar simulator and UV irradiation.
2. Ishtaiwa [37] used naked ZnO nano- particles to kill and mineralize gram-bacteria (*E. coli*) and (*P. aeruginosa*) under UV irradiation from tail of sunlight. Complete Degrading and converting bacteria to gases such as CO_2, N_2, SO_x and H_2O were documented.

The novelty of this work is to experiment ZnO semi-conductor against new types of bacteria namely; *Enterococcus* (G_{+ve}) and *Proteus mirabilis* (G_{-ve}). Complete photodegradation of these bacteria into mineral gas has not been earlier studied to our knowledge.

1.9 Assumptions

This work is based on the following assumptions:

1. Two bacteria are expected to be killed by the ZnO nano-particles, as known in literature.

2. The organic contaminants that result from bacterial killing are expected to be mineralized by light in the presence of ZnO particles (as catalyst).

Chapter 2

Materials, Equipments and Methods

2.1 Materials

2.1.1 Chemicals

Zinc chloride, sodium hydroxide, barium chloride, sulfuric acid, 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. These chemicals were used in ZnO nano-particles preparation or used for reaction and culturing preparations.

2.1.2 Bacteria

Two types of pathogenic bacteria were chosen to be tested in the research experiments. Clinically isolated *Proteus mirabilis* was chosen as Gram negative bacteria. Meanwhile *Enterococcus faecium* (ATCC 700221) was bought to be representative of Gram positive bacteria. These two bacteria were maintained and cultured on their selective media onto Petridishes to be used later.

2.2 Equipments

2.2.1 Irradiation source

In the photocatalytic experiments solar simulator light was used as visible light irradiation source. Tungeston-halogen lamp (100000 Lux, 0.0146 W/cm2) was used as a light source. Figure (2.1)

Figure (2.1): Solar simulator lamp (tungestun=halogen lamp) (100000 Lux, 0.0146 W/cm2).

2.2.2 Lux Meter

A lux meter (Lx-102) light meter was used to adjust intensity of the light that reaches the water sample from the tungeston-halogen lamp in the photocatalytic disinfection experiments.

2.2.3 Spectrophotometer

A LaboMed, Inc. spectrophotometer was used to determine the concentration of bacteria quantitatively, using turbidometric methods, and to adjust the bacterial suspensions to 0.5 McFarland standard turbidity.

Figure (2.2): Spectrophotometer

2.2.4 pH Meter

A pH meter was used to adjust the reaction mixture pH as desired.

2.2.5 Thermometer

A mercury thermometer was used to measure the temperature.

2.2.6 X- Ray Diffractometer (XRD)

A PANalytical X'Pert PRO X-ray diffractometer (XRD), with CuKa (λ=1.5418Å) at UAE University, was used for XRD measurement and to obtain the crystal size of zinc oxide nano particles.

2.2.7 Scanning Electron Microscope (SEM)

A Jeol-EO Scanning Electron Microscope at UAE University, was used to measure the zinc oxide particles morphology.

2.3 Methods

2.3.1 Catalyst preparation

ZnO nano-particles were prepared 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 250mL distilled water. Aqueous 0.90 M solution of sodium hydroxide (NaOH) was prepared by dissolving 9.0 g NaOH in 250mL distilled water. NaOH solution was poured into a beaker and heated to ~55 °C. Then $ZnCl_2$ 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 powder of ZnO nano-particles precipitated, then the precipitate was cleaned with deionized water, and then air dried and then calcinated at 450 ºC for an hour in inferno air.

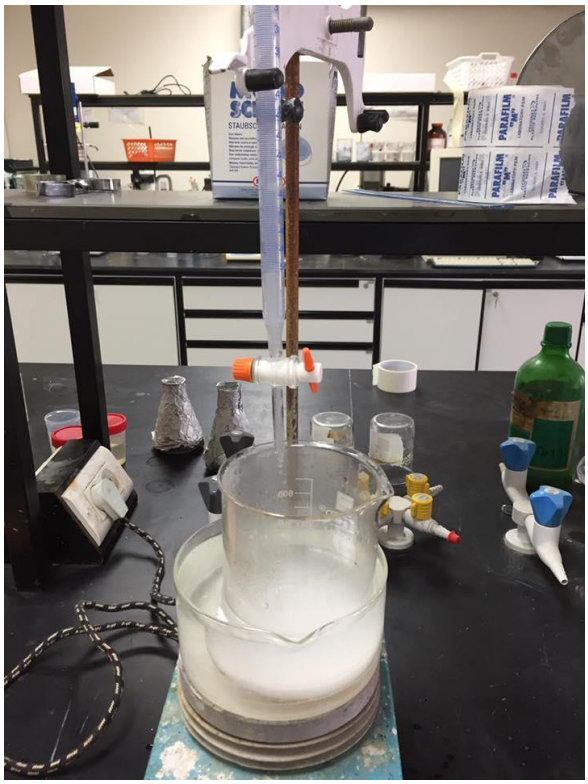

Figure (2.3): Preparation of Zinc oxide by adding zinc chloride to sodium hydroxide solution.

2.3.2 Bacterial culture preparation

2.3.2.1 Nutrient broth

Nutrient broth was used for microorganism inoculum preparations. The broth prepared according to manufacturer instructions by dissolving 20.0 g of broth powder in 1.00 L distilled water then autoclaved.

2.3.2.2 Normal Saline solution

Normal Saline solution (0.9 %) was prepared by dissolving 9.0 g NaCl in 1000mL distilled water, then autoclaved and poured into sterile Perti plates. The saline solution was used to prepare 10mL fold dilutions of contaminated

water samples. The bacterial dilutions were cultured on nutrient agar, and were used to achieve countable number of colonies on the nutrient agar plates.

2.3.2.3 Nutrient agar

Nutrient agar media was prepared according to manufacturer instructions by dissolving 28.0 g of powder in 1.00 L distilled water then autoclaved and used as growth medium for testing the remaining non degraded bacteria after the photodegradation process.

2.3.3 Photocatalyst characterization

2.3.3.1 X-Ray Diffraction

The ZnO particles size and crystal type were determined using (XRD). ZnO nano-powder was exposed to X-ray beam in X-ray diffractometer to get the XRD patterns. Figure (2.4).

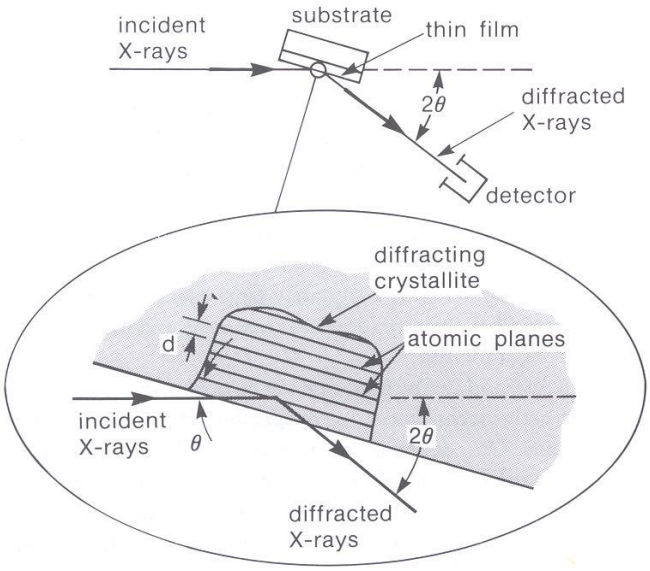

Figure (2.4): Sketch of x-ray diffractometer [38].

The obtained XRD pattern was compared with the published ZnO X-ray diffraction. Figure (2.5)

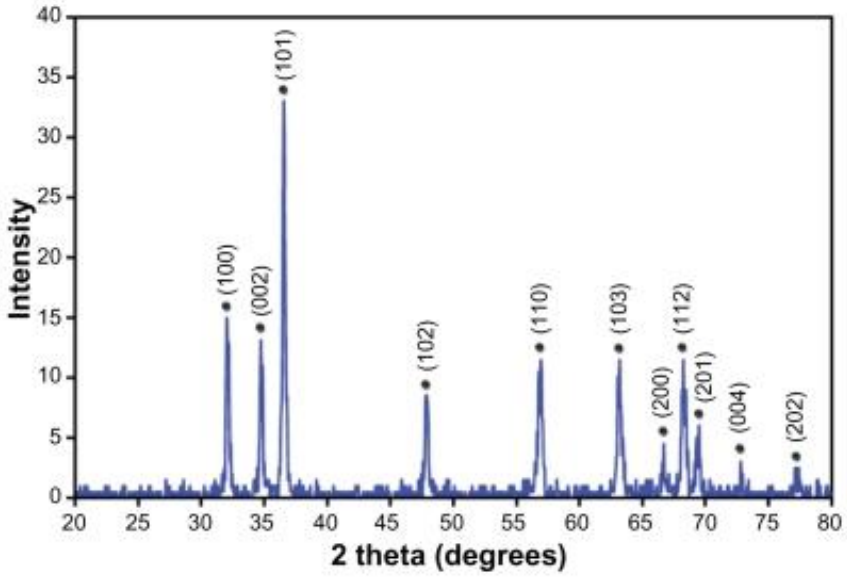

Figure (2.5): Reference X-ray diffraction of ZnO powder [39].

The crystal size was obtained using Scherrer's equation as follows [23]:

$$D = \frac{C\lambda}{\beta \cos\theta} \qquad \text{eq (2.1)}$$

Where, D = crystal size in Å

β = full width at half maximum of X-ray patern in radians

λ = X-ray wavelength

C = correction factor

ZnO X-ray diffraction (XRD) patterns were measured at UAE University; Al-Ain; UAE.

2.3.3.2 Scanning electron microscope (SEM)

SEM micrographs were measured on field emission scanning electron microscope. SEM analysis was mesured at UAE University, Al-Ain; UAE.

2.3.3.3 UV-Visible Spectral Characterization

UV-Visible electron absorption spectra for the ZnO were measured on a Shimadzu UV-1601 spectrophotometer. The spectra were scanned on small amount of fine solid catalyst suspension in a quartz cell.

2.3.3.4 Photoluminescence Spectra (PL)

The prepared nanoparticle Zinc oxide was characterized using fluorescence spectra. A Perkin-Elmer LS50 Luminescence Spectrophotometer was used to measure the emission fluorescence spectra. A suspension of the solid was placed in a quartz cell and was excited by a wave of length (325 nm). The emission spectra was detected and used to calculate semiconductor band gap.

2.3.4 Photocatalytic study

For, gram – *Proteus mirabilis* and gram + *Enterococcus*, the photodegradation reactions were carried out in 100 mL glass beakers. Each

beaker contains 50 mL pre-contaminated water with known volume of 5×10^5cfu/mL bacteria solution. The beakers were placed in a water-bath to keep temperature constant and the aqueous bacteria suspension was magnetically stirred.

For comparison, each experiment was controlled with four different beakers with specific condition:

1- First beaker containing 0.1 g ZnO nano-particles with the contaminated 50 mL water with specific amount of becteria. The beaker was then exposed to light source at specific intensity for 30 min at 30 °C.
2- Second beaker containing 0.1 g ZnO nano-particles was placed in dark to check the effect of catalyst on bacterial growth.
3- The third beaker was similar to the second beaker composition; this beaker was exposed to light without adding catalyst to examine light effect on bacteria.
4- The fourth beaker was left without catalyst under dark condition and to be used as control sample (reference).

Figure (2.6) shows photodegradation system of bacteria under light.

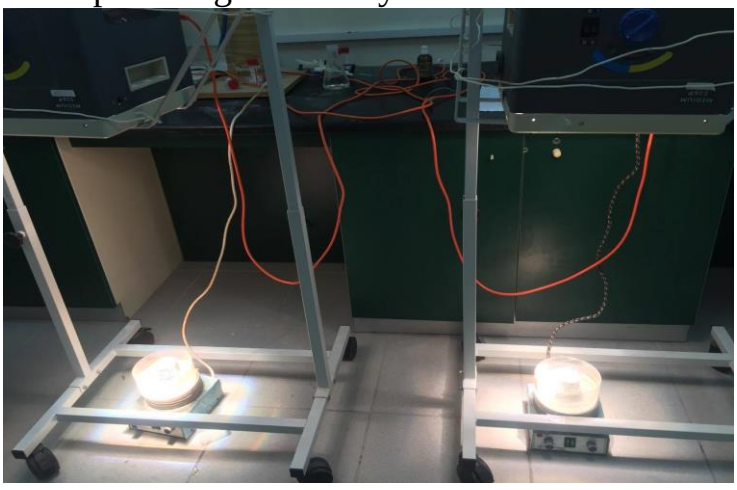

Figure (2.6): Photodegradation process of bacteria under light.

2.3.5 Measuring the remaining concentration of bacteria

After a photodegradaion reaction cession, a 1.0 mL of the treated solution was withdrawn using a micropipette and it was then diluted in a series of saline solutions from 0.1 to 0.01 dilution factor. 100µL of each diluted tube was cultured on two nutrient agar plates. After incubation of the plates at 37 °C for 24 hours, the average numbers of bacterial colonies in the two plates were counted. The average concentration of bacteria in cfu/mL plates was calculated using plate count method as follows:

Bacterial concentration (cfu/ml) =

$$\text{number of counted colonies} \times \text{dilution factor} \times 10 \qquad \text{Eq. (2.2)}$$

The percentage of killed bacterial was calculated as follows:

$$\frac{\text{Bacterial initial concentration} - \text{Bacterial final concentration}}{\text{Bacterial initial concentration.}} 100\ \% \qquad \text{Eq. (2.3)}$$

The initial concentration was known from the control samples.

Figure (2.7) shows the bacterial colonies of *Proteus mirabilis* bacteria on nutrient agar plates after 24 hour of incubation at 37 ° C.

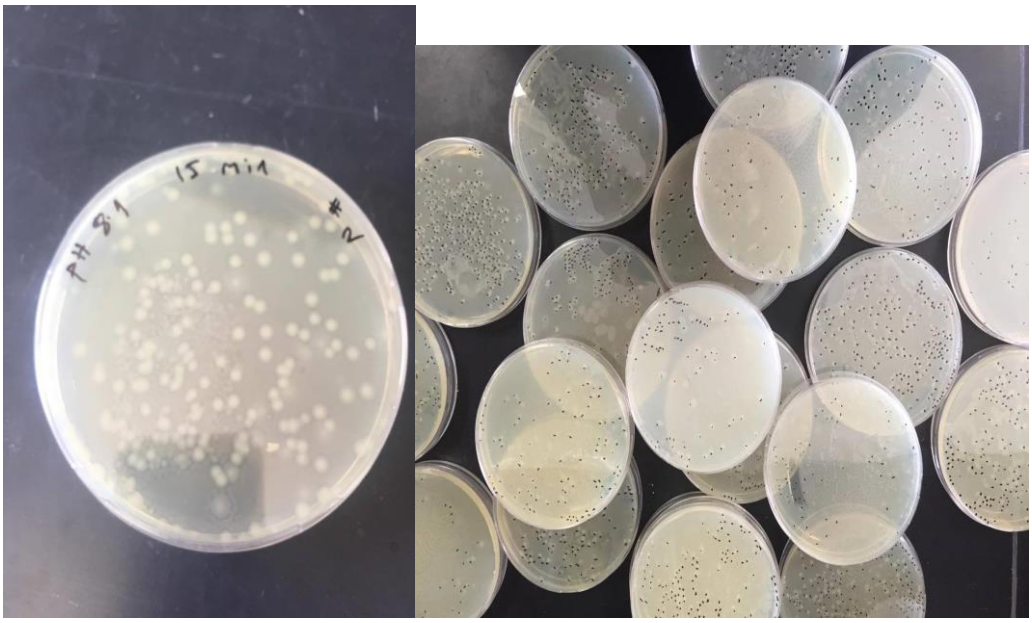

Figure (2.7): *Proteus mirabilis* bacteria after 24 hour, 37 ° C incubation of nutrient agar plates.

2.3.6 Effects of reaction parameters on the photodegradation reaction

To study the effect of different parameters on the degradation reaction, aliquots were taken from the reaction at time periods (5, 10, 15 and 30 minutes) and the concentration of the un-killed bacteria at each time was measured.

Turnover frequency (TOF) and quantum yield (QY) was used to measure the relevant efficiency of the catalyst amount and the light incidence on the photocatalytic reaction.

TOF measured the number of bacteria that was destroyed by ZnO atom per minute as follows:

$$TOF = \frac{\#\ of\ lost\ bacteria}{\#\ of\ ZnO\ atoms} * \frac{1}{time\ (min)} \qquad \text{Eq. (2.4)}$$

$$\#\ \text{of ZnO atoms} = \frac{ZnO\ mass\ (g)}{molar\ mass} * Avogadro's\ numbe \qquad \text{Eq. (2.5)}$$

QY measured the number of bacteria that was destroyed per photons absorbed as follows:

$$Q.Y = \frac{\#\ of\ lost\ bactreia}{\#\ of\ photons} \quad \text{Eq. (2.6)}$$

# of photons (n) was calculated as follows:

$$\text{Energy from solar simulator} = n \times \frac{hc}{\lambda} \quad \text{Eq. (2.7)}$$

where (h) is Planck's constant, (c) is the speed of light

and (λ) the wavelength of UV radiation was 550 nm

Radiation flux from solar simulator was 0.0146 W/cm^2

For 50 mm diameter beaker,

Power = 0.287 Watt

The energy of photons in Joules was measured depending on the irradiation time:

$$\text{Power (Watt)} = \frac{\text{Energy } (Joules)}{Time\ (sec)} \quad \text{Eq. (2.8)}$$

The photons that were used in photo-catalytic reaction were UV photons, UV photons are only about 5% of the total irradiated photons

2.3.6.1 Effect of temperature

Photodegradation of bacteria using 0.1 g of ZnO under solar simulated light was studied at different temperatures with different times.

2.3.6.2 Effect of pH

Photodegradation of bacteria using 0.1 g of ZnO under 29º C was studied at different pH values (Acidic media, neutral media and basic media) at different times.

2.3.6.3 Effect of catalyst amounts

Photodegradation of bacteria under light at specific temperature and media pH was studied with different amounts of ZnO and different times of irradiation.

2.3.6.4 Effect of contaminant concentration

Photodegradation of bacteria using 0.1 g of ZnO, the temperature was controlled at 29º C during the Photodegradation experiment, the media pH was adjust to 7 . The concentration of bacteria and irritation time were varied.

2.3.6.5 Effect of time

The effect of time on photodegradation of bacteria using ZnO under UV light was studied at different parameters.

i. High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) was used for total organic contents analysis.

The HPLC system consisted of a binary water (1525) pump, injection valve (P/N7725i) with 20 µL loop and a (waters 2998) photodiode array detector operating at 250 nm. The HPLC column was 6×250 mm containing 5µm particles. Quantification of peaks was performed using (Breez) integrator. The mobile phase was prepared by adding methanol. The sample were eluted at room temperature at flow rate of 1.0 mL/min.

Chapter 3

Results and Discussion

3.1 Photocatalyst characterization

3.1.1 Absorption Spectra

The Absorption spectra of ZnO nano-powder was recorded in figure (3.1). The spectra had absorption peak at 373 nm**.**

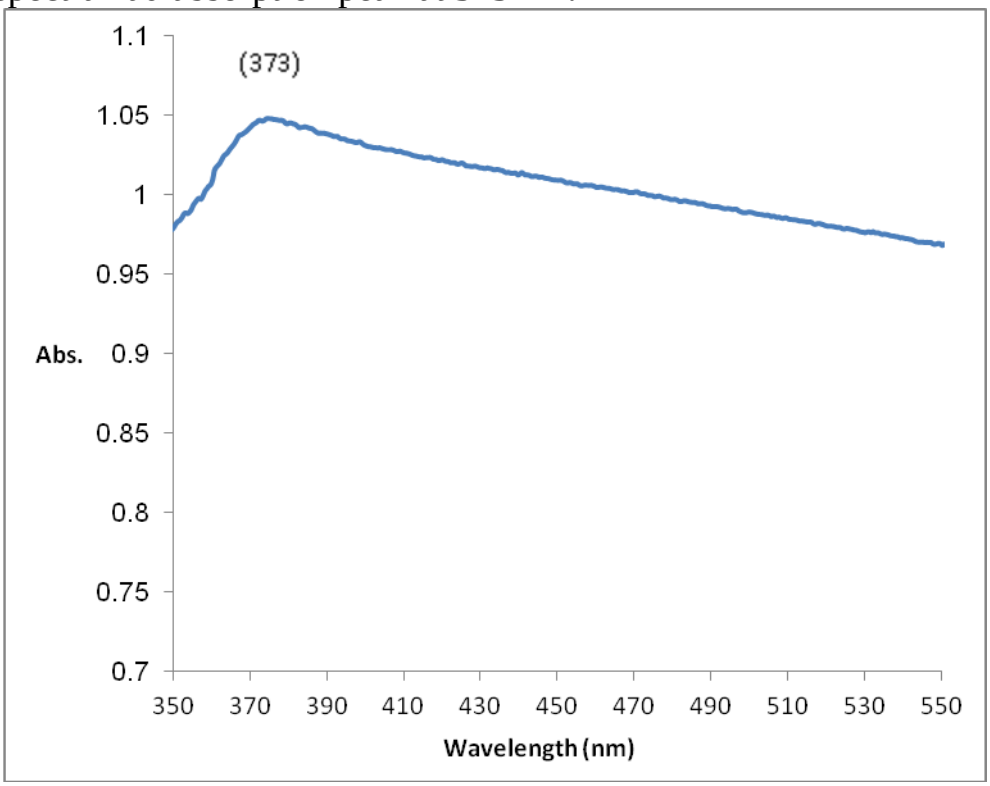

Figure (3.1): Absorption Spectra in the UV-Visible region of ZnO nano-powder.

3.1.2 Photoluminescence Spectra (PL)

Semiconductor catalyst band gap was measured using fluorescence spectra. PL spectra of ZnO nanopowder are shown in Figure (3.2). The excitation wavelength was 325 nm. The PL spectra showed three emission peaks, one

in the ultraviolent region ~385 nm, and other two peaks located at 423 nm and 487 nm attributed to the presence of singly ionized oxygen vacancies(ref huss). The peak at 385 nm is attributed to ZnO emission photoluminescence peak and used to find band gap as follows: (eq. 5)

$$E_{gap} = \frac{hc}{\lambda * q}$$

Where: h = Plank's constant ($6.626 * 10^{-34}$ Joule sec)

C = Speed of light ($3 * 10^{8}$ m/sec)

λ = Wavelength in nm

q = Charge of electron ($1.60*10^{-19}$ coulombs)

The energy gap was 3.23 eV.

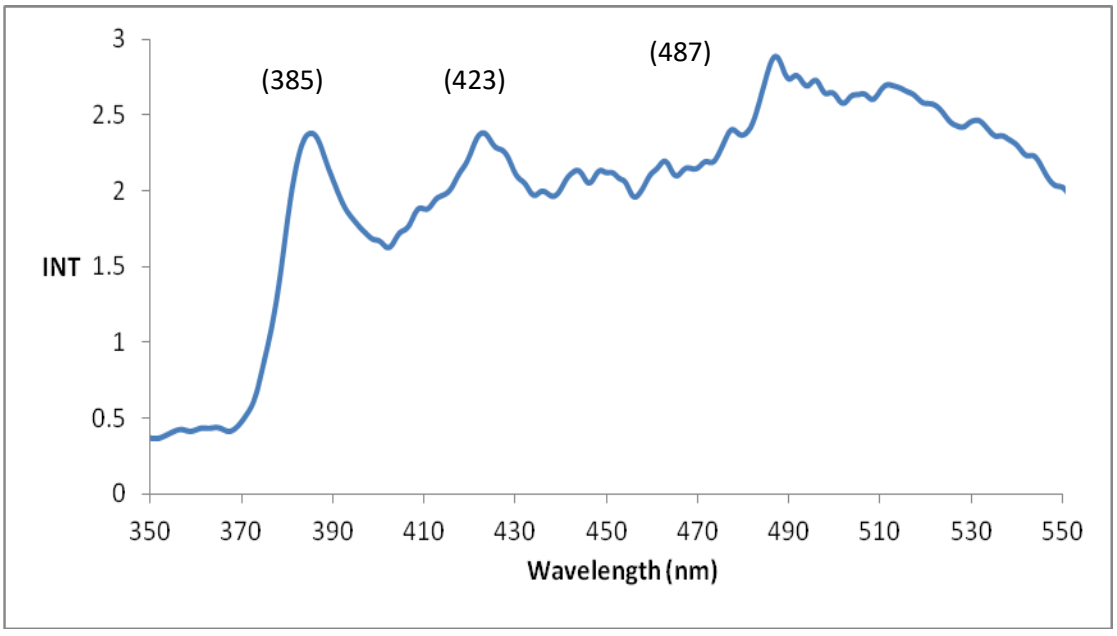

Figure (3.2): Emission Spectra of ZnO nanopowder

3.1.3 X-Ray study

The prepared powder was exposed to x-ray to determine its structural parameters, XRD pattern was obtained by getting glancing angle θ at different intensities, the resulted pattern has matched with the reference X-ray diffraction pattern of ZnO powder and this confirms that the prepared powder is ZnO in crystalline nature. Figure (3.3)

The diffraction peaks were assigned; the highest intensity peak was (101) plane of zinc oxide with other smaller intensity peaks for the plans (100) (002) (102) (110) (103) (112) planes. The average particles size calculated using Scherrer's formula from the plans (100) (002) (101) and (102) was 27.2 nm.

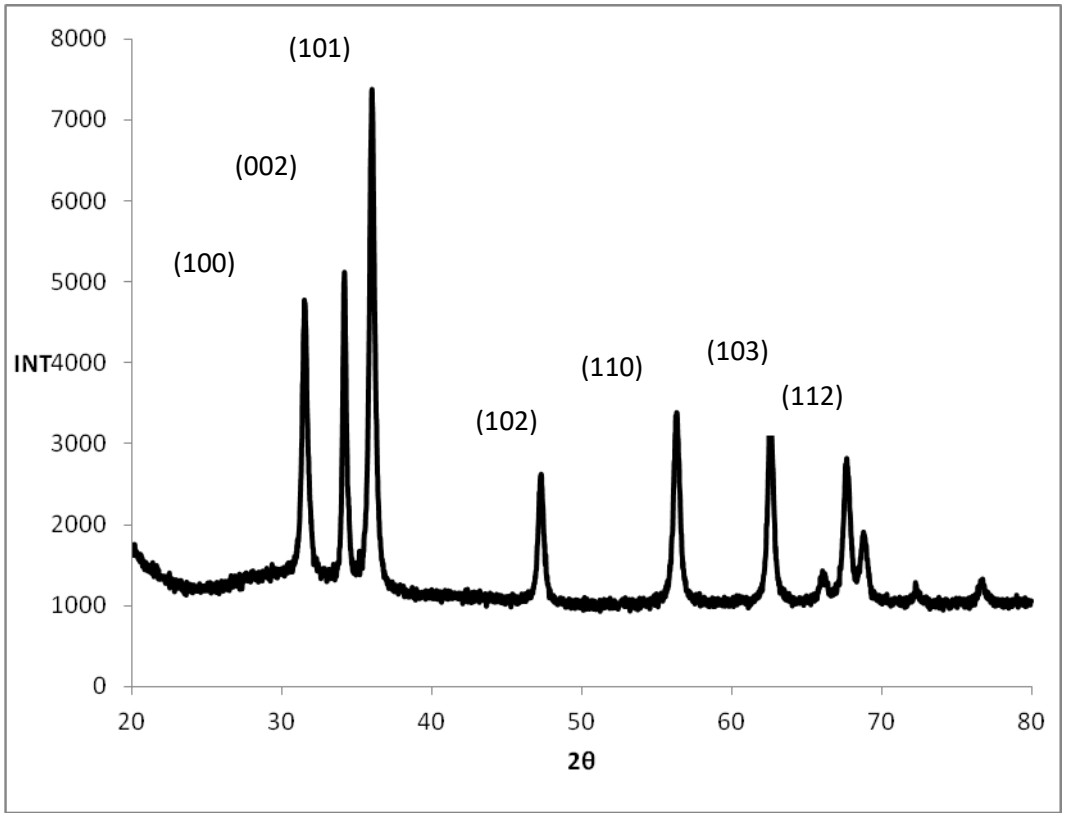

Figure (3.3): XRD patterns of the ZnO annealed at 600º C.

3.1.4 SEM study

"Scanning electron microscopy (SEM) is a convenient technique to study the morphology of the nanostructures"[40]. SEM was used to characterize the surface morphology of the zinc oxide. SEM image for ZnO powder is shown in (Figure 3.3). The SEM image shows agglomeration of nanoparticles of ZnO.

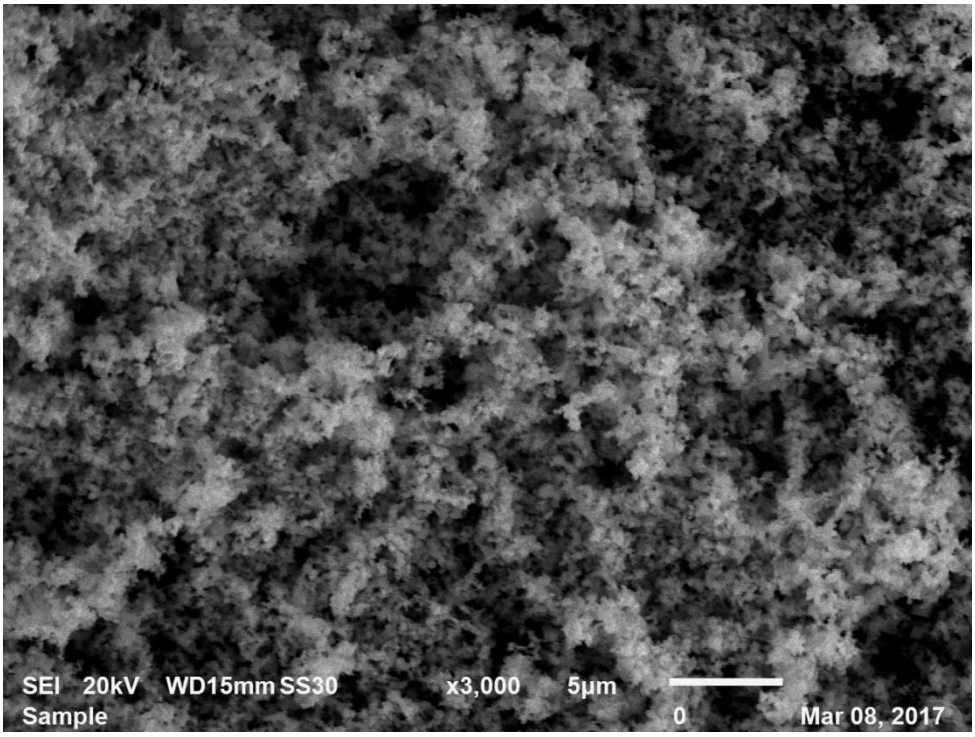

Figure (3.4): SEM image shows the morphology of ZnO.

3.2 The disinfection experiment of *Enterococcus*

In this work, a ZnO catalyst was used in photodegradation of *Enterococcus* bacteria in water under solar simulator irradiation. The catalyst efficiency was measured depending on the percent of killed bacteria. The results show the percentage of killed *Enterococcus* bacteria at 29 °C, pH ~ 7.5 and initial concentration ~ 96000 cfu bacteria after 30 minutes of irradiation in the

presence of catalyst. The percent of killing reached up to 100% under these conditions.

3.2.1 Control experiments of *Enterococcus*

Control experiments were conducted in the absence of catalyst, absence of light and the absence of both light and catalyst. The photodegradation reaction was carried out at ~29 °C, pH ~7.5 and initial concentration of *Enterococcus* bacteria was ~96000 cfu. After 30 minutes of irradiation and in the absence of catalyst of the percent of killing was ~ 23%. When using 0.1 g ZnO catalyst in dark, the percentage of killing of E*ntecococcus* bacteria was ~ 51%. the results are presented in table (3.1) and figure (3.5).

Table (3.1): The loss percentage of *Enterococcus* bacteria after 30 minutes of reaction processing under different conditions, initial concentration of bacteria is 96000 cfu.

	Dilution factor	# of colonies	Remaining bacterial concentration (cfu)	Percentage of loss (±5%)
Cat. + dark	0.01	47	47000	51.04
Light + no cat	0.01	74	74000	22.92
Cat. + light	0.1	3	300	99.7

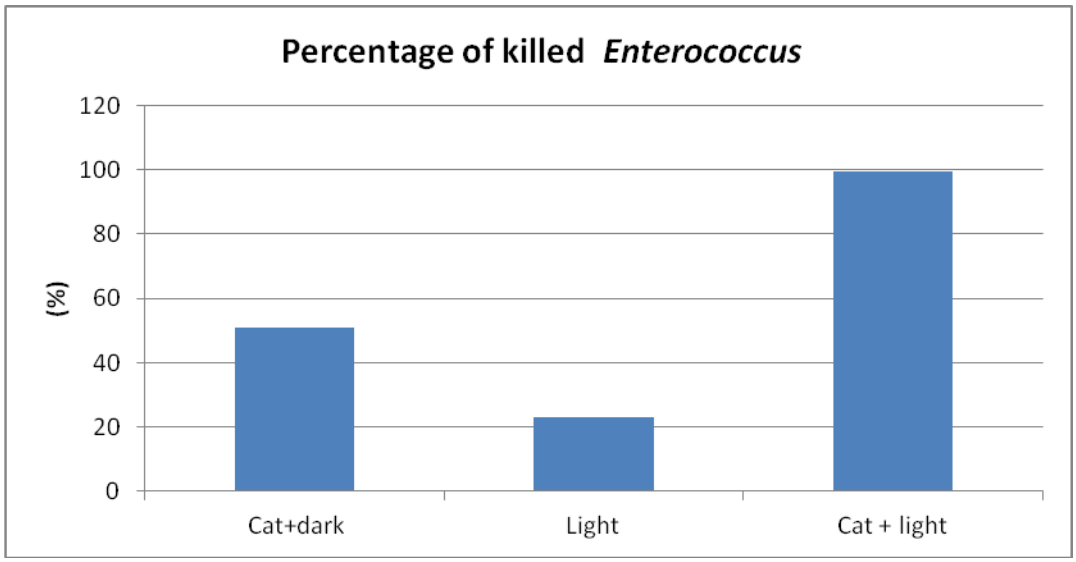

Figure (3.5): The loss percentage of *Enterococcus* bacteria after 30 minutes of reaction processing under different conditions, initial concentration of bacteria is 96000 cfu.

Up to 100% killing percent was achieved in using the ZnO catalyst under solar simulator light. The results also showed that the catalyst is less effective in bacteria killing when used without light, the percentage of bacterial loss declined to only 50%. Exposing the bacteria to light without catalyst also shows reduction in bacteria with a small portion (~20%) figure (3.5).

3.2.2 Parameters affect photodegradation reaction of *Enterococcus*

Effects of different factors on catalyst efficiency were studied at different time periods (5, 10, 15 minutes).

3.2.2.1 The effect of catalyst amount

The effect of ZnO amount on the *Enterococcus* killing percentage was studied at ~29°C and pH ~7.5 with initial concentration (~96000 cfu).

The results of using different amounts of the catalyst showed that the percentage of killing increases with catalyst amount increase. As shown in

table (3.2) and in figures (3.6), (3.7). This is because more amount of the catalyst provides more active sites for photodegradation reaction and achieves higher percent of killing.

However, at the beginning of the reaction, doubling the amount of catalyst from 0.05 g to 0.10 g has increased the *Enterococcus* killing percentage by~10%. At a longer time of irradiation; doubling the catalyst amount increased the killing percent by ~ 6%. Because as the percentage of lost bacteria increases, only a small fraction of catalyst is needed to kill the remaining bacteria, so the more added amount of catalyst will be inactive. This can be proved from table (3.3) which set that TOF decreases as the catalyst amount increases.

The results in tables (3.2) show that the increases in the percent of killing with increasing the catalyst amount is less clear in the process of a long time, Figures (3.6), (3.7). At long time of reaction when using the same amount of catalyst, the efficiency of catalyst on killing the bacteria decreases and turn over frequency (TOF) decreases as shown in the table (3.3).

Table (3.2): The effect of catalyst amount on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation at~29°C, pH 7.5 and initial concentration 96000 cfu.

Amount of catalyst (g)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
0.05	63.54	69.79	86.98	99.8
0.1	70.83	72.92	88.54	99.8
0.15	83.33	86.46	94.27	99.8

Table (3.3): TOF for the process of killing *Enterococcus* bacteria using different amount of ZnO (0.05, 0.1 and 0.15 g) of temperature 29°C, pH 7.5, initial concentration 96000 cfu after (5, 10 and 15 minutes) of irradiation.

the amount of catalyst (g)	TOF 5 min	TOF 10 min	TOF 15 min
0.05	32.9×10^{-18}	18.1×10^{-18}	15.1×10^{-18}
0.1	18.4×10^{-18}	9.47×10^{-18}	7.66×10^{-18}
0.15	14.4×10^{-18}	7.48×10^{-18}	5.44×10^{-18}

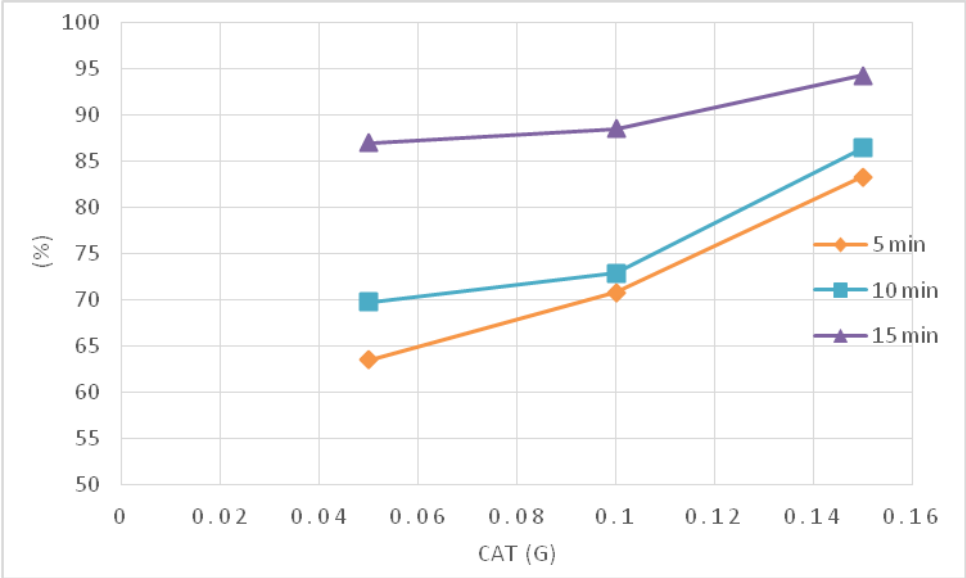

Figure (3.6): The effect of the catalyst amount on killing percentage of *Enterococcus* bacteria after (5,10 and 15 minutes) of irradiation at ~29°C, pH 7.5, initial concentration 96000 cfu.

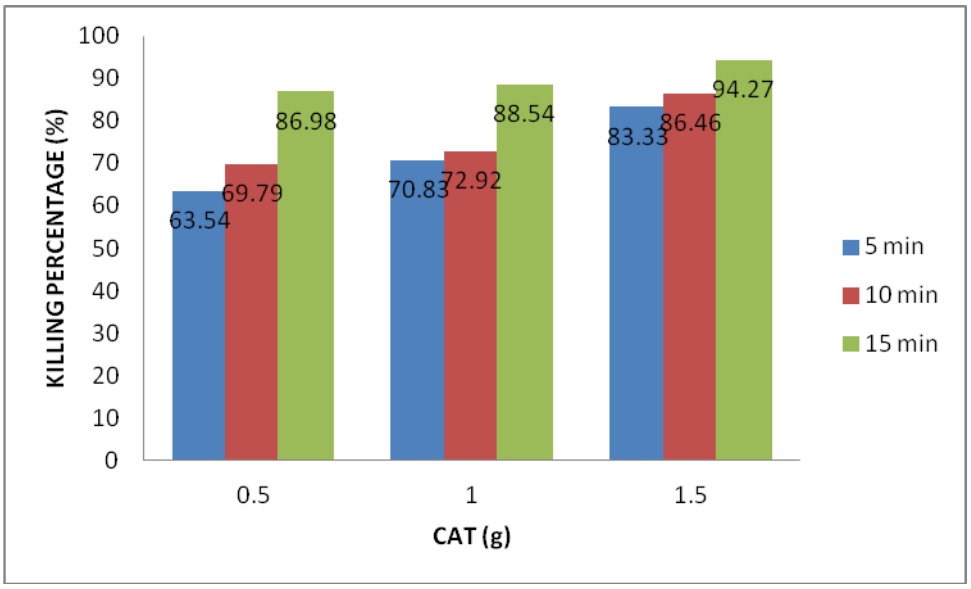

Figure (3.7): The effect of catalyst amount on killing percentage of *Enterococcus* bacteria after (5,10 and 15 minutes) of irradiation at ~29°C, pH 7.5, initial concentration 96000 cfu.

3.2.2.2 The effect of the pH

The effect of the pH on killing percentage was studied using 0.1g of ZnO under solar simulator light at ~29°C with initial concentration ~96000 cfu of *Enterococcus* bacteria. The degradation experiments were studied at three different pH values (4.8, 7.5, and 9.6) at times periods (5, 10 and 15 minutes). The results showed that the killing percentages were decreased in basic medium. This is because the cell membrane of *Enterococcus* has negative charge [41] and at high pH (8.9) ZnO also has negative charge [42]. And this similarity in charge keeps the catalyst particles far away from bacteria cell. The killing percentage was higher in acidic medium. The results are presented in table (3.4) and figures (3.8), (3.9).

Table (3.4): The effect of pH on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1g of ZnO at ~29°C, initial concentration 96000 cfu.

PH	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
4.8	85.42	90.63	93.13	99.8
7.5	81.25	86.97	90.63	99.8
9.6	77.08	83.33	87.51	99.8

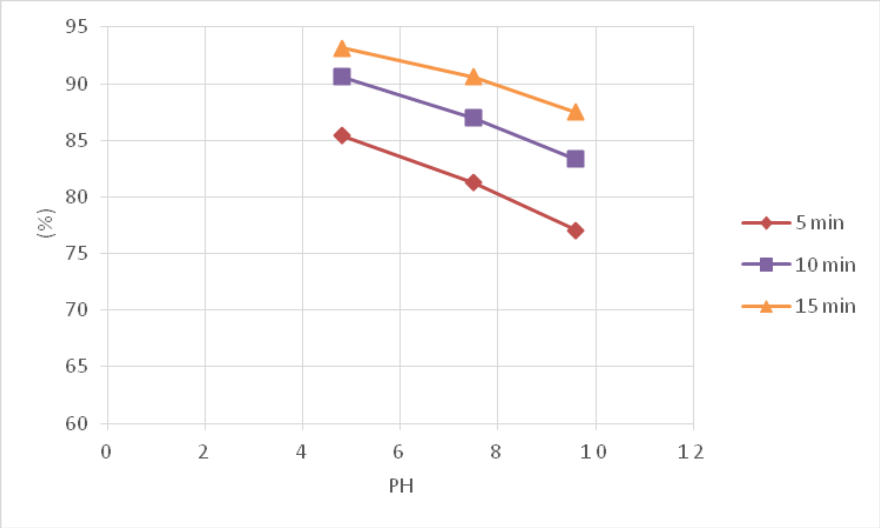

Figure (3.8): The effect of the pH on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation at ~29°C, initial concentration 96000 cfu.

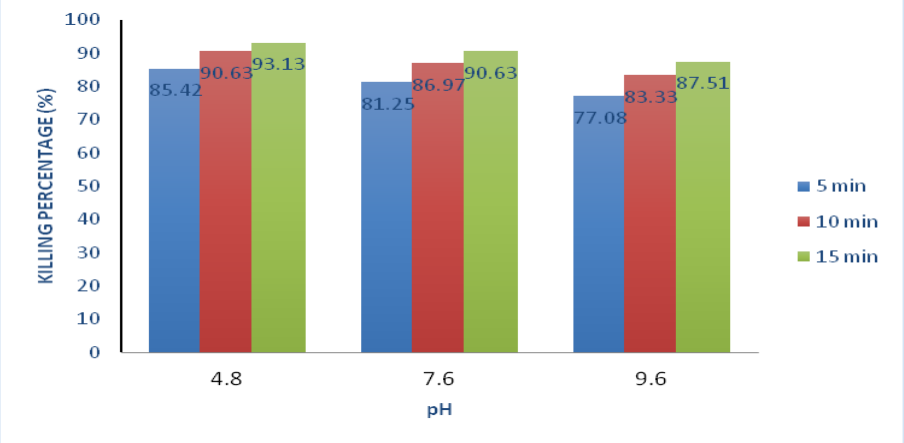

Figure (3.9): The effect of pH on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation at ~29°C, initial concentration 96000 cfu

3.2.2.3 The effect of temperature

The effect of temperature on killing percentage was studied using 0.1g of ZnO catalyst under solar simulator light at neutral pH medium with initial concentration ~36000 cfu *Enterococcus* bacteria. The degradation experiments were studied at three different temperatures (25, 29 and 35 ºC) and time periods of (5, 10 and 15 minutes). The results were nearly similar with slightly lowering in killing percentage at high temperatures. The percent of killing was increased with increasing the time of irradiation. The results are presented in tables (3.12) and figures (3.10), (3.11).

The lower in percent of killing at high temperature is attributed to the decreasing in concentration of oxygen in the reaction media, which lowers the catalyst efficiency as mentioned before.

Table (3.5): The effect of temperature on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1g of ZnO, pH 7.5, initial concentration 36000 cfu.

Temp (°C)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
25	51.39	73.62	74.72	99.8
29	50.0	69.44	73.61	99.8
35	49.12	61.11	70.83	99.8

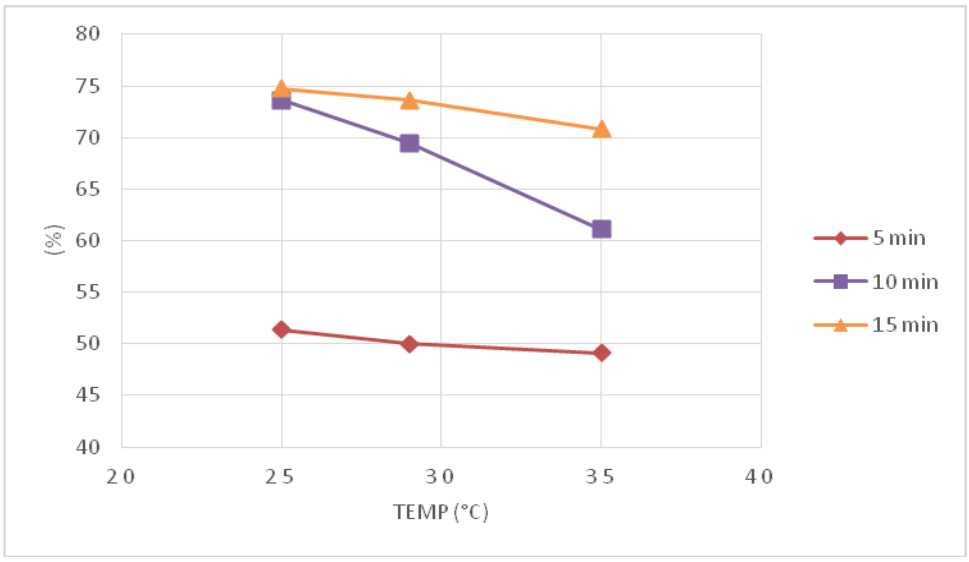

Figure (3.10): The effect of temperature on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation, pH 7.5, initial concentration 36000 cfu

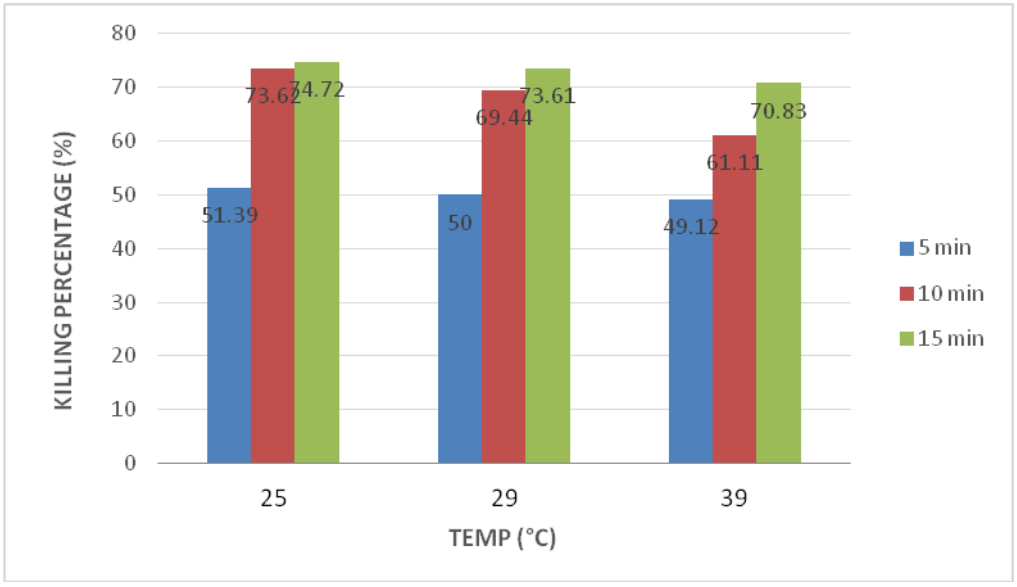

Figure (3.11): The effect of temperature on killing percentage of *Enterococcus* bacteria after (5, 10 and 15 minutes) of irradiation, pH 7.5, initial concentration 36000 cfu

3.2.2.4 The effect of the bacterial initial concentration

The effect of bacteria concentrations on killing percentage was studied using three different initial *Enterococcus* concentration (~7800, ~36000, and ~68000 cfu), with using 0.1g of ZnO catalyst, under solar simulator light and numeral pH at ~29 ºC.

The killing percentage was increased ~40% when the initial concentration was increased from 7800 cfu to 36000 cfu. Increasing the concentration from 36000 to 68000 shows no observable change in killing percentage (~4% of killing percentage was lowered). This behavior was shown at short irradiation time (5 min.), but there is no change in killing percentage at different bacteria concentration when time of irradiation was increased (10 min.) Table (3.6). Figures (3.12), (3.13).

Moreover, when increasing the initial concentration, more amount of bacteria will be killed per photons absorbed, so quantum yield increased as initial concentration increases. At the same time, Q.Y increased ~ 87% as the initial concentration was increases from 7800 cfu to 36000 cfu. While at higher concentration; increasing the concentration from 36000 to 68000 shows smaller change in quantum yield ~44%. Table (3.7).

When bacteria concentration increases, more bacterial cells are killed so the percentage is high, but at higher values of concentrations, more bacteria caused high turbidity and lowered the incident light, this then decreased the catalyst efficiency, so that the killing percentage is lowered and quantum yield increases in a smaller manner.

Also, TOF increases as initial concentration increase table (3.7), because increasing the initial concentration will increase the number of killed bacteria per unit of ZnO atoms.

Table (3.6): The effect of bacteria concentration on killing percentage of *Enterococcus* after (5 and 10 minutes) of irradiation using 0.1g of ZnO at ~29°C, pH 7.5.

Initial concentration (cfu)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 30 min
7800	39.74	67.95	99.8
36000	66.67	71.67	99.8
68000	63.97	71.32	99.8

Table (3.7): TOF and QY for the process of killing *Enterococcus* bacteria using different amounts of initial concentration of bacteria after (5 and 10 minutes) of processing using 0.1 g of ZnO at temperature ~ 29°C, pH 7.5

Initial concentration (cfu)	TOF	QY	TOF	QY
	5 min		10 min	
7800	0.84×10^{-18}	0.26×10^{-15}	0.72×10^{-18}	0.22×10^{-15}
36000	6.49×10^{-18}	2.02×10^{-15}	3.49×10^{-18}	1.08×10^{-15}
68000	11.8×10^{-18}	3.66×10^{-15}	6.56×10^{-18}	2.03×10^{-15}

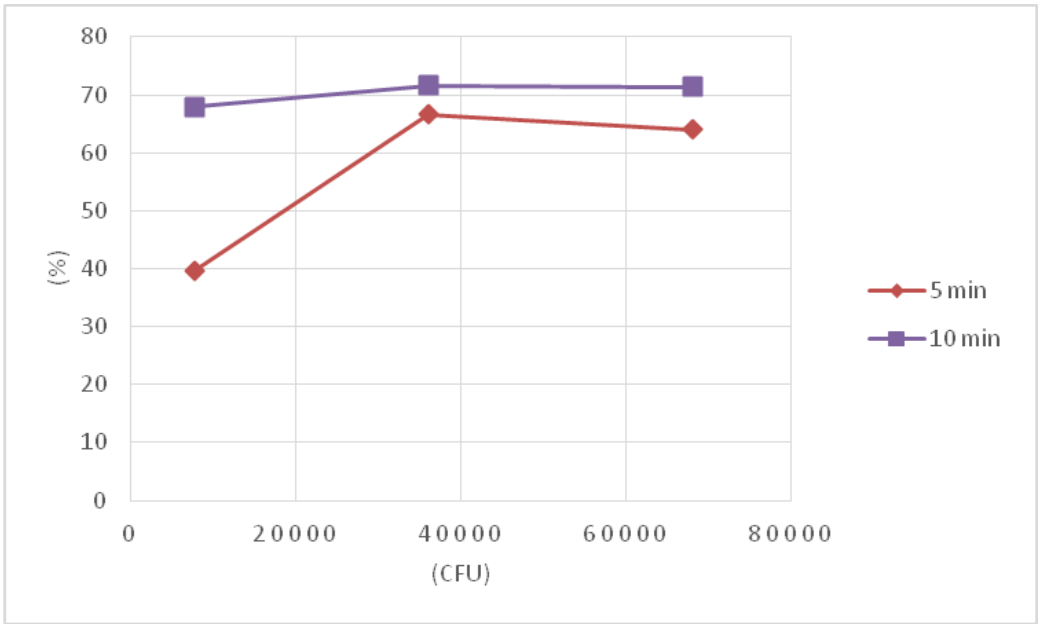

Figure (3.12): The effect of bacterial concentration on killing percentage of *Enterococcus* after (5and 10 minutes) of irradiation, 0.1 g ZnO, ~29°C, pH 7.5

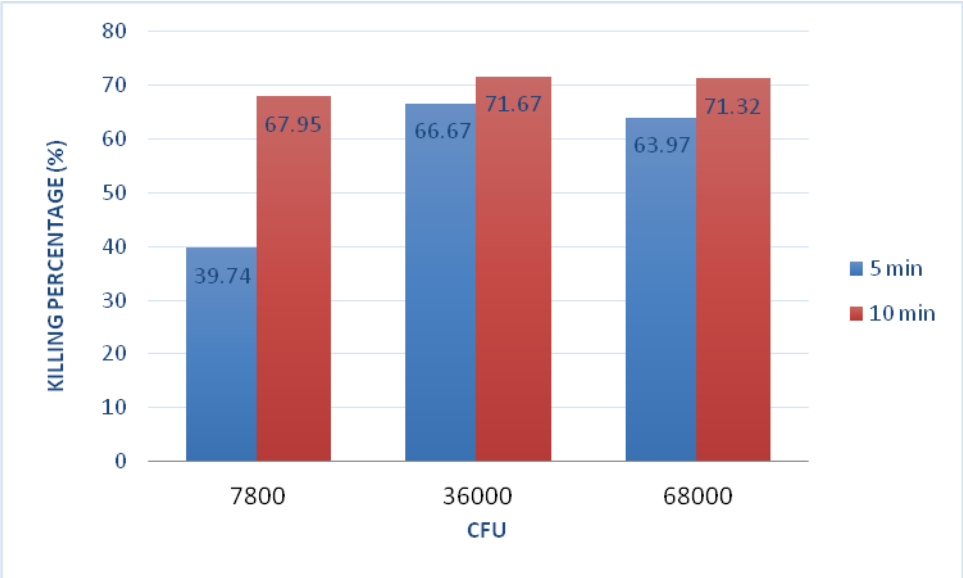

Figure (3.13): The effect of bacteria concentration on killing percentage of *Enterococcus* after (5and 10 minutes) of processing, 0.1 g ZnO, ~29°C, pH 7.5

3.2.2.5 The effect of irradiation time

The effect of time on killing percentage was studied at different conditions. In general killing percentage increased with time, at all conditions the killing percentage reached up to 100% after 30 minutes of reaction processing as shown in table (3.8). At the first period of reaction, the concentration of bacteria was high and so the killing percentage. As time of reaction proceeds, the concentration of remaining bacteria becomes lower and so the killing percentage. TOF and QY decrease with time as well as shown in Table (3.8), because with time, the remaining bacteria is low, so adding more photons and more catalyst becomes unneeded and useless.

Table (3.8): The effect of time on bacterial loss percentage, TOF and QY using 0.1 g ZnO, at temperature 29°C, pH 7.5, initial concentration 96000 cfu *Enterococcus* bacteria.

the amount of catalyst (g)	Bacterial loss percentage (%)	TOF	QY
5 min	70.83	18.4×10^{-18}	5.71×10^{-15}
10 min	72.92	9.47×10^{-18}	2.93×10^{-15}
15 min	88.54	7.66×10^{-18}	2.37×10^{-15}
30 min	99.8	4.33×10^{-18}	0.05×10^{-15}

3.2.3 HPLC

Complete killing and mineralization of *Enterococcus* bacteria by ZnO under solar simulated light has been confirmed by HPLC results.

The bacterial solutions with similar conditions (including 96,000 cfu and broth) were prepared. One solution having no catalyst with no light showed a peak area of ~90,000 µV. sec as shown in table (3.9) and fig (A.1) in appendix A. The peak area is due to the presence of soluble organic compound from the broth. Another solution with ZnO added in the dark, a peak area ~ 74,000 µV. sec was observed for the mixture after 30 minutes. The peak area confirms the existence of organic compound in the bacterial solution. In the dark, bacteria die as observed from fig (3.5), and consumes broth as well. The experiment with no ZnO under illumination for 30 minutes, showed a peak area of ~ 67,000 µV. sec. This means the presence of organic compounds. Moreover, fig (3.5) shows that bacteria was partially killed under the light. Therefore, bacterial killing and consumption are possible.

Under irradiation with ZnO for enough time (4 hours) no organic peaks could be observed. As figure (3.5) shows the complete killing of bacteria, and as

HPLC shows no peaks area for resulting organics, the results indicate that irradiation using ZnO completely kills the bacteria and completely mineralize any resulting organics and completely photodegrades all broth soluble matter.

For short time photocatalytic experiment (30 minutes), an organic peak was observed (150,000 µV. sec). Based on figure (3.5) complete bacterial killing was observed. The results suggest that the 30 minutes are sufficient for bacterial killing which produces high organic compounds in solution. Such organics did not completely degrade in 30 minutes. When left for enough time (4 hours) under photocatalytic conditions fig (A.5) in appendix A and table (3.9), all organics were completely mineralized.

Table (3.9): The loss percentage and HPLC peak area of *Enterococcus* bacteria processing under solar irradiation using ZnO catalyst, initial bacterial concentration 96,000 cfu.

	Percentage of loss (±5%)	Reaction time	Peak area (µV. sec.)
No cat. + dark	0.00	0 min	90,199
Cat. + dark	51.04	30 min	74,021
Light + no cat.	22.92	30 min	67,686
Cat. + light	99.7	30 min	220,528
Cat. + light	100	4 hours	0.00

3.3 The disinfection experiment of *Proteus mirabilis* bacteria

Photodegradation of *Proteus mirabilis* bacteria in water using ZnO catalysts was studied under solar simulator light. The catalysts efficiency was measured depending on the killing percentage of bacteria. Results showed that the percentage of killing for *Proteus mirabilis* bacteria after 30 minutes of processing in the presence catalyst under light radiations reached up to

~100%. The photo degradation reaction was carried out using 0.1 g ZnO catalyst, at ~29°C, pH ~7.5 and initial concentration of *Proteus mirabilis* bacteria ~94000 cfu.

3.3.1 Control experiments of *Proteus mirabilis* bacteria

Control experiments were conducted in the absence of catalyst, the absence of light, and the absence of both light and catalyst. The photodegradation reaction was carried out at ~29 °C, pH ~7.5 and initial concentration of *Proteus mirabilis* bacteria ~ 94000 cfu. After 30 minutes of processing, in the absence of catalyst under light radiation the percentage of killing was about 8%, when using 0.1 g ZnO catalyst in dark, the percentage of killing was about 31%. Results are summarized in Figure (3.14), and are shown in Table (3.10).

Table (3.10): The loss percentage of *Proteus mirabilis* bacteria after 30 minutes of reaction processing under different conditions, initial concentration of bacteria is 94000 cfu.

	Dilution factor	Number of colonies	Remaining bacterial concentration (cfu)	Percentage of loss (±5%)
Cat. + dark	10^{-2}	65	65000	30.85
Light + no cat.	10^{-2}	86	86000	8.51
Cat. + light	10^{-1}	2	200	99.79

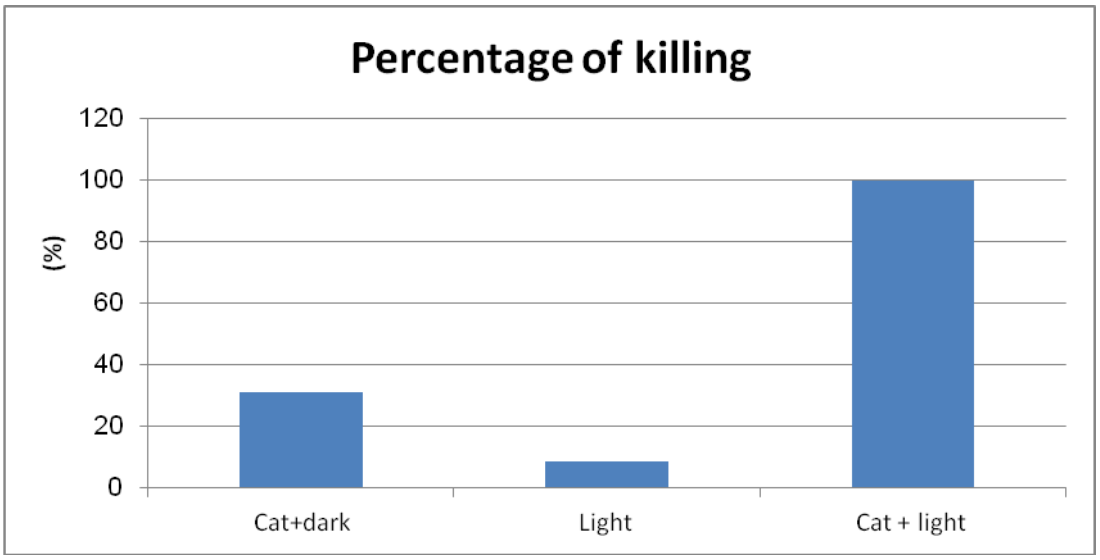

Figure (3.14): The loss percentage of *Proteus mirabilis* bacteria after 30 minutes of processing under different conditions. Initial concentration of bacteria is 94000 cfu.

Up to 100% killing percent was achieved by the ZnO catalyst under solar simulator light. The results also showed that the catalyst is less effective in absence of light as the percentage of bacteria declined only in a small portion. Exposing the bacteria to light without using any types of catalysts also has decreased the bacteria concentration about only 8%.

3.3.2 Parameters affecting photodegradation reaction of *Proteus mirabilis* bacteria

Effects of different factors on the catalyst efficiency were studied at different periods of time (5, 10, and 15 min.).

3.3.2.1 The effect of catalyst amount

The effect of the amount of catalyst on killing percentage at ~29°C and pH ~7.5 with initial concentration of *Proteus mirabilis bacteria* of ~94000 cfu was studied.

Results show that increasing the catalyst quantity can increase the bacterial killing because more amount of the catalyst provides more active sites for photodegradation reaction and will achieve higher percent of killing. However, at the beginning of the reaction, changing the amount of catalyst from 0.05g to 0.1g has increased the killing percentage by about 10%, but increasing the amount of catalyst from 0.1 g to 0.15 g has less efficient on killing percentage.

After 15 minutes of irradiation, using 0.1g of catalyst showed similar percentage of killing to 0.15 g catalyst under the same conditions. This might happen because adding more catalyst may limit the amount of light that reaches the reaction and so decreases the efficiency of photocatalysis reaction. The results are showed in table (3.11) and figures (3.15), (3.16).

Moreover, table (3.12) shows that (TOF) decreases as time of reaction increases, it is also decreases when using higher amounts of catalyst, this indicates that using high amount of catalyst for long time will decrease its efficiency on killing the bacteria.

Table (3.11): The effect of the ZnO amount on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation at~29°C, pH 7.5, initial concentration 94000 cfu.

Amount of catalyst (g)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
0.05	68.02	84.19	89.53	99.8
0.1	75.53	84.53	93.14	99.8
0.15	74.89	87.21	93.02	99.8

Table (3.12): TOF for the process of killig *Proteus mirabilis* bacteria using different amount of ZnO (0.05, 0.1 and 0.15g) at temperature 29°C, pH 7.5, and initial concentration 94000 cfu after (5, 10 and 15 minutes).

the amount of catalyst (g)	TOF 5 min	TOF 10 min	TOF 15 min
0.05	37.0×10^{-18}	21.7×10^{-18}	15.2×10^{-18}
0.1	19.2×10^{-18}	10.9×10^{-18}	7.89×10^{-18}
0.15	13.1×10^{-18}	7.48×10^{-18}	5.29×10^{-18}

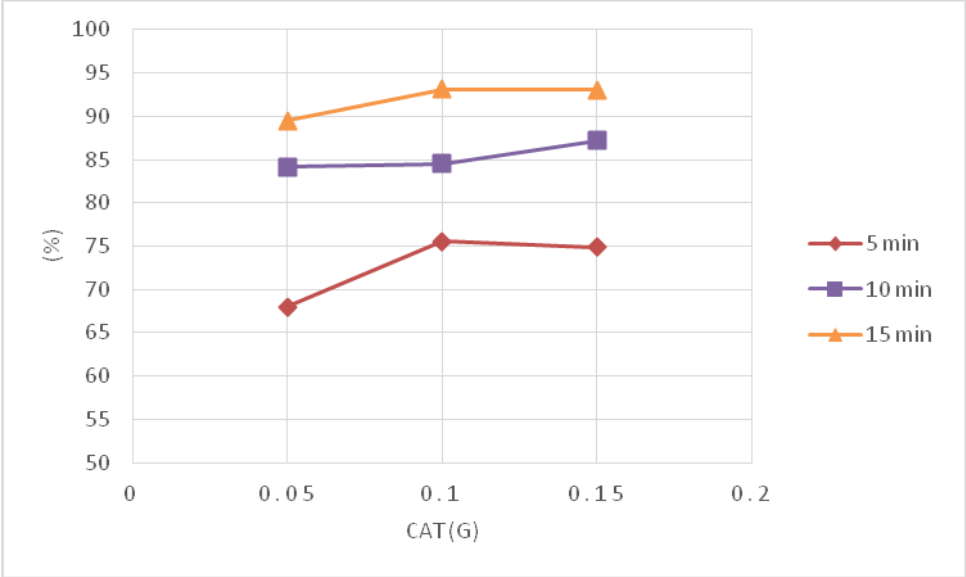

Figure (3.15): The effect of ZnO amount on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation at ~29°C, pH 7.5, initial concentration 94000 cfu.

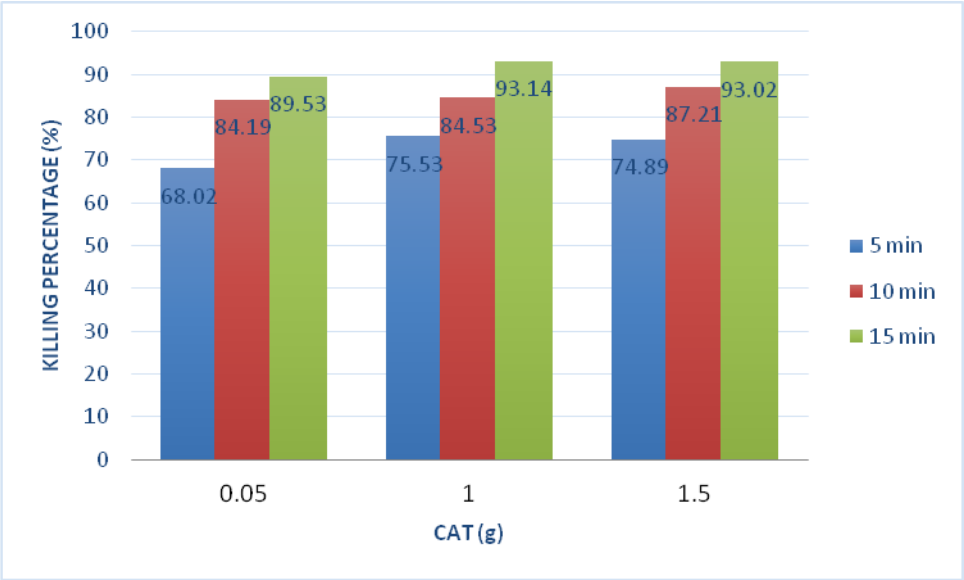

Figure (3.16): The effect of ZnO amount on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation at ~29°C, pH 7.5, initial concentration 94000 cfu.

3.3.2.2 The effect of the pH

The effect of the pH on killing percentage was studied using 0.1g of ZnO catalyst under solar simulator at ~29°C with initial concentration of *Proteus mirabilis* bacteria ~69000 cfu. The killing experiments were studied pH (4.3, 7.4, and 8.9) at times periods (5, 10 and 15 minutes). The results showed that killing percentages were almost the same for both acidic and neutral medias, while in basic media (pH 8.9) , the killing percentage was lower because the cell membrane of *Proteus mirabilis* bacteria has negative charge [43] and at high pH (8.9) ZnO also has negative charge so that in basic media repulsive force acts as inhibiting factor for reaction between bacteria and ZnO. While acidic and neutral media shows better photodegradation results. Results are presented in table (3.13) and figures (3.17), (3.18).

Table (3.13): The effect of pH on killing percentage of *Proteus mirabilis* bacteria after (5,10 and 15 minutes) of irradiation using 0.1 g ZnO at ~29°C, initial concentration 94000 cfu.

pH	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
4.3	91.88	94.49	97.39	99.8
7.4	86.23	95.07	96.52	99.8
8.9	76.52	81.89	82.75	99.8

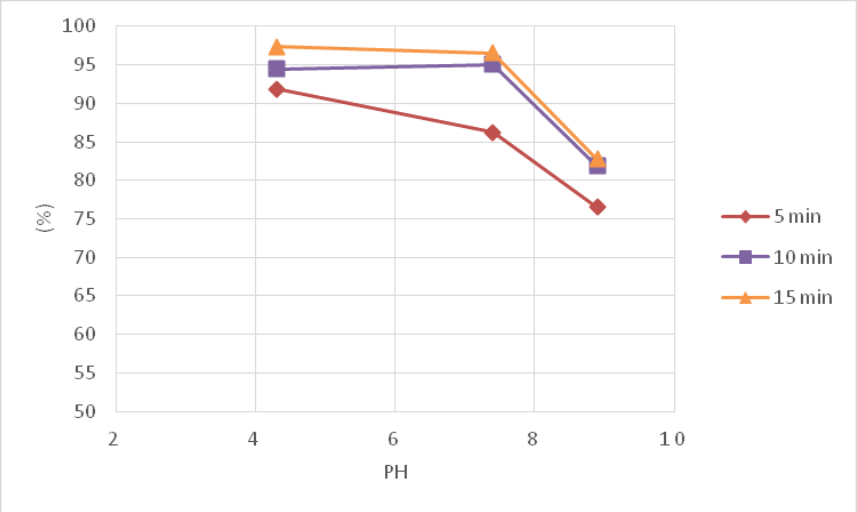

Figure (3.17): The effect of pH on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1 g ZnO at ~29°C, initial concentration 94000 cfu.

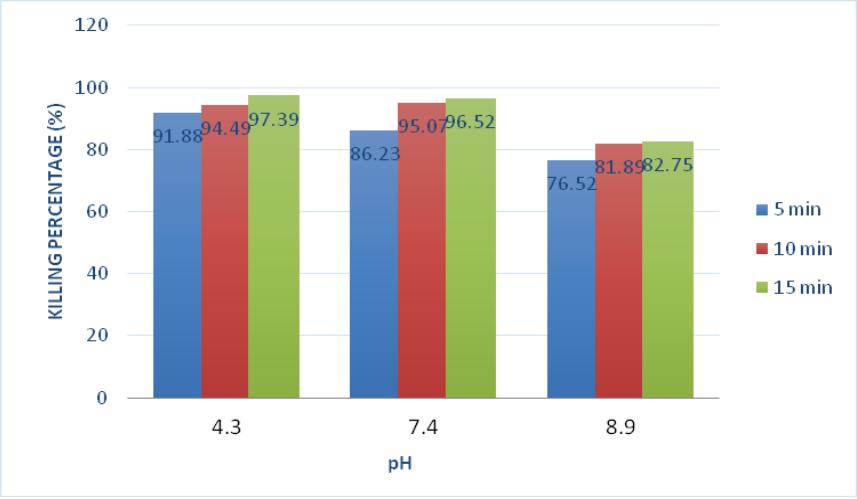

Figure (3.18): The effect of pH on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1 g ZnO at ~29°C, initial concentration 94000 cfu.

3.3.2.3 The effect of the temperature

The effect of the temperature on killing percentage was studied using 0.1g of ZnO catalyst under solar simulator light and neutral pH with initial concentration of *Proteus mirabilis* bacteria ~ 69000 cfu. The degradation experiments were studied at three different temperatures (25, 29 and 38 ºC) and three periods of times (5, 10, and 15 min.). The results were approximately similar at the three different temperatures, the results are presented in table (3.14) and figures (3.19) and (3.20).

When temperature increases, the concentration of oxygen molecules in the reaction media decreases, and thus the catalyst efficiency was decreases. On the other hand, at high temperature, the bacterial mobility increases and so its ability to reach OH radicals increases, this means higher killing percentage, and this could be the reason why increasing the temperature has no clear effect on degradation percentage.

Table (3.14): The effect of temperature on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1 g ZnO, pH 7.5, initial concentration 69000 cfu.

Temp (°C)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 15 min	Percentage of loss (±5 %) 30 min
25	93.10	95.95	97.86	99.8
29	92.62	95.00	98.33	99.8
38	93.34	96.67	97.62	99.8

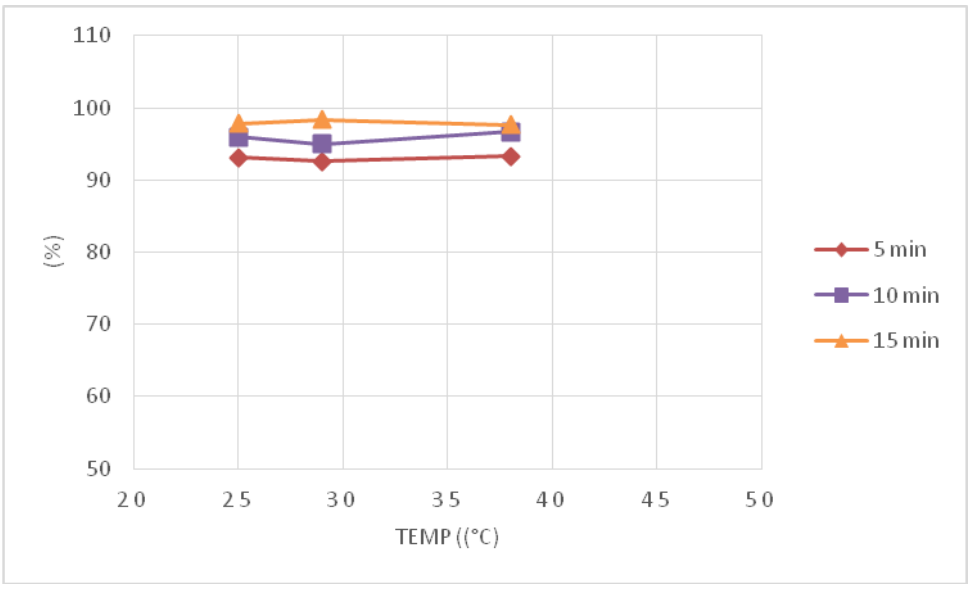

Figure (3.19): The effect of temperature on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1 g ZnO, pH 7.5, initial concentration 69000 cfu.

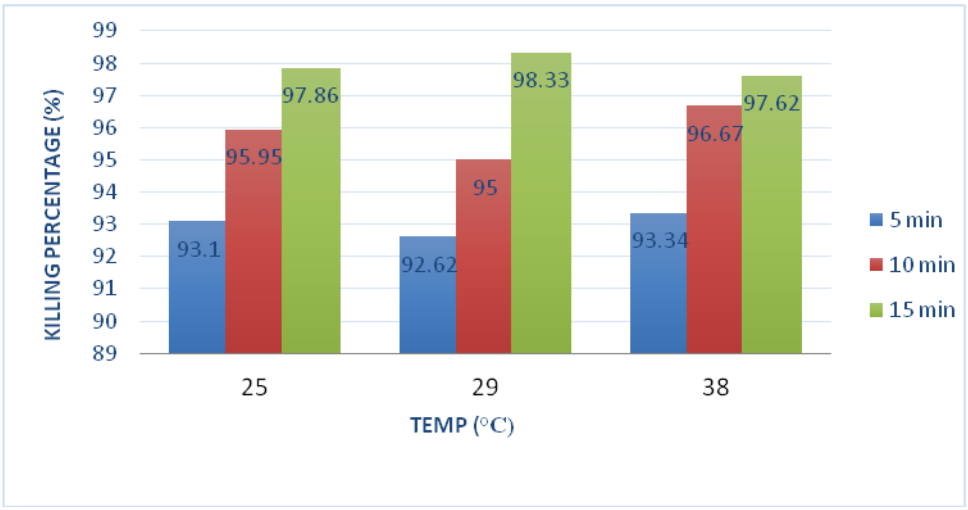

Figure (3.20): The effect of temperature on killing percentage of *Proteus mirabilis* bacteria after (5, 10 and 15 minutes) of irradiation using 0.1 g ZnO at ~ 29°C, pH 7.5, initial concentration 69000 cfu.

3.2.2.4 The effect of the initial concentration of bacteria

The effect of bacteria concentrations on killing percentage was studied using three different initial concentrations (15000, 69000, and 130000 cfu), using 0.1g of ZnO catalyst under solar simulator light at neutral pH and ~29 ºC**.** The killing percentage slightly increased when the initial concentration was increased from 7800cfu to 36000 cfu. On the other hand, at higher concentration, increasing the concentration from 69000 to 130000 gave almost equal killing percentages, tables (3.15) and figures (3.21) and (3.22). When bacteria concentration increases more bacterial cells killed so the percentage is high, but at higher values concentrations, more bacteria caused high turbidity that lowered the incident light and decreased the catalyst efficiency.

Quantum yield increased as initial concentration increases as shown in table (3.16) this means that at higher bacterial concentrations more bacteria will be killed per photons absorbed. Also, TOF increases as initial concentration increase table (3.16), because increasing the initial concentration increase the number of killed bacteria per unit of ZnO atoms.

Table (3.15): The effect of initial concentration of bacteria on killing percentage of *Proteus mirabilis bacteria* after (5 and 10 minutes) of irradiation using 0.1 g ZnO at ~29°C, pH 7.5

Initial concentration (cfu)	Percentage of loss (±5 %) 5 min	Percentage of loss (±5 %) 10 min	Percentage of loss (±5 %) 30 min
15000	74.67	82.00	99.8
69000	86.23	95.07	99.8
130000	88.77	95.85	99.8

Table (3.16): TOF and QY of the process of killing *Proteus mirabilis bacteria* after 10 minutes of irradiation, using 0.1 g ZnO at temperature 29°C, pH 7.5.

Initial concentration (cfu)	TOF	QY	TOF	QY
	5 min		10 min	
15000	0.30×10^{-17}	0.94×10^{-15}	1.66×10^{-18}	0.53×10^{-15}
69000	1.61×10^{-17}	5.00×10^{-15}	8.87×10^{-18}	2.76×10^{-15}
130000	3.12×10^{-17}	9.70×10^{-15}	16.8×10^{-18}	5.23×10^{-15}

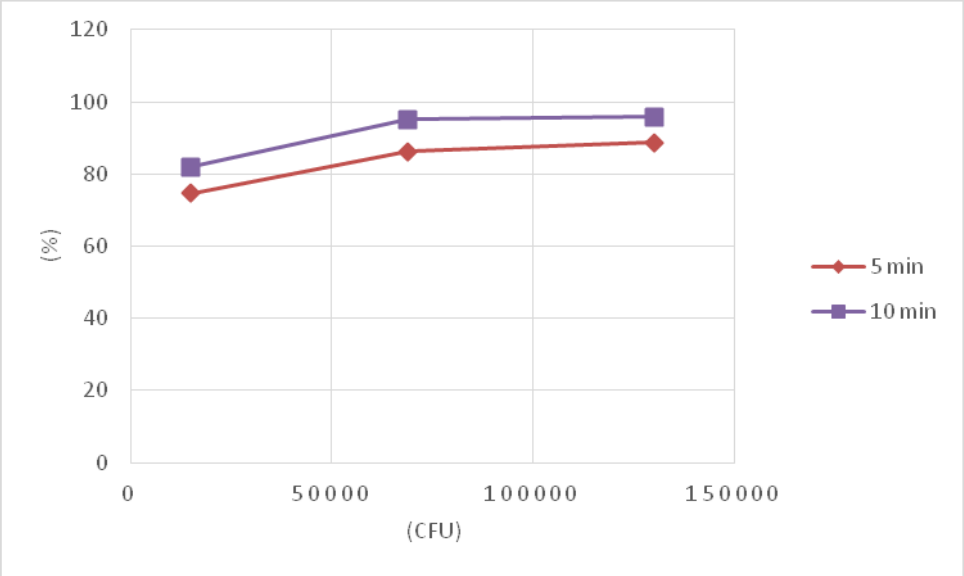

Figure (3.21): The effect of initial concentration of bacteria on killing percentage of *Proteus mirabilis* bacteria after (5 and 10 minutes) of irradiation using 0.1 g ZnO at ~29°C, pH 7.5.

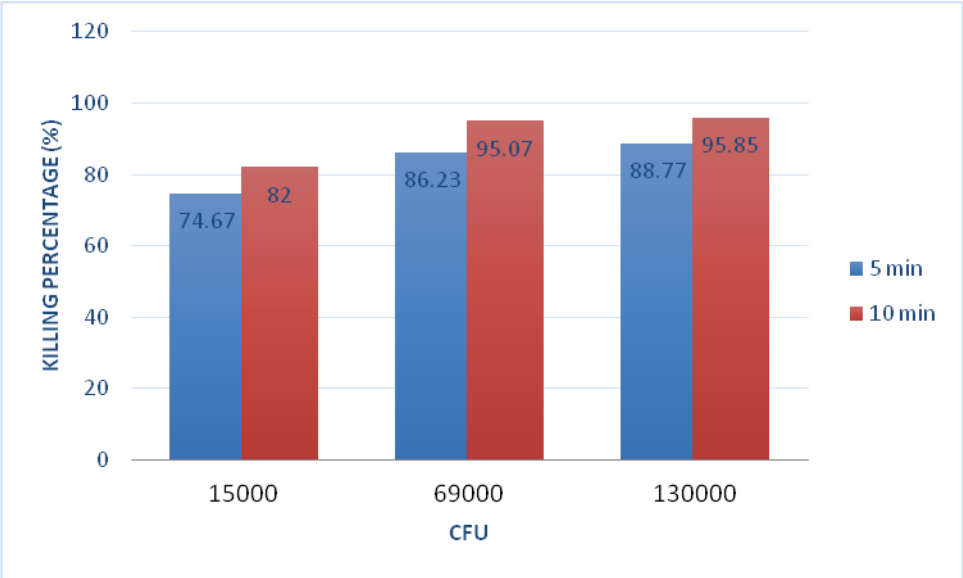

Figure (3.22): The effect of initial concentration of bacteria on killing percentage of *Proteus mirabilis* bacteria after (5 and 10 minutes) of irradiation using 0.1 g ZnO at~29°C, pH 7.5.

3.3.2.5 The effect of irradiation time

The effect irradiation time on killing percentage was studied at different conditions. Results in table (3.17) show that the percentage of killing increases as time of reaction proceeds. The killing percentage reached 100% after 30 minutes of reaction processing in every reaction condition. However, at the beginning of reactions, the bacterial concentration decreased fast; about the half at the first five minutes. As time proceeded the change in the concentration of bacteria starts to decrease because at the first period of reaction, the concentration of bacteria was high and so the killing percentage. With time the concentration of bacteria becomes lower and so the killing percentage.

Table (3.17): The effect of time on bacterial loss percentage, TOF and QY using 0.1g ZnO, at temperature 29°C, pH 7.5, initial concentration 94000 cfu of *Proteus mirabilis* bacteria.

the amount of catalyst (g)	Bacterial loss percentage (%)	TOF	QY
5 min	75.53	1.92×0^{-17}	5.97×10^{-15}
10 min	84.53	1.09×10^{-17}	3.39×10^{-15}
15 min	87.21	0.79×10^{-17}	2.46×10^{-15}
30 min	99.8	0.42×10^{-17}	1.31×10^{-15}

3.3.3 HPLC for *Proteus mirabilis* bacteria.

Complete killing and mineralization of *Proteus mirabilis* bacteria by ZnO under solar simulated light has been confirmed by HPLC results.

The bacterial solutions with the same conditions (including 94,000 cfu and broth) were prepared. The solution with no catalyst added and kept in dark sowed a peak area of ~33,760 µV. sec as shown in table (3.18) and fig(B.1) in appendix B. The peak area refers to the presence of soluble organic compound from the broth. Another solution was treated with Zno but exposed to no light; it showed a peak area ~ 42,690 µV. sec after 30 minutes. The peak area confirms the existence of organic compound in the bacterial solution. Because in the dark, some of the bacteria die (as shown in Fig (3.6) but the resulted organic compounds from the killed bacteria still in the solution. The experiment with no ZnO under illumination for 30 minutes, showed a peak area of ~ 60,800 µV. sec. This means that the solution contains organic compounds refers to the organic compound from the broth and the killed bacteria. At the same time, fig (3.14) shows that bacteria were partially killed under light. Therefore, bacterial killing and consumption are possible.

For photocatalytic experiment and after 30 minutes of irradiation, organic peak about (140,400 µV. sec) was observed. Meanwhile, Figure (3.14) confirms that after 30 minutes of photocatalytic experiment complete bacterial killing was achieved. This means that the 30 minutes are sufficient for bacterial killing which produces high organic compounds in solution, but this time is not enough to completely degrade the organics.

When left for enough time (4 hours) under irradiation with ZnO, no organic peaks were observed. As Figure (3.14) shows complete killing of bacteria, and HPLC Table (3.18) shows no peaks area for resulting organics, we conclude that irradiation using ZnO completely kills *Proteus mirabilis* bacteria and completely mineralize any resulting organics and completely photodegrades all broth soluble matter.

Table (3.18): The loss percentage and HPLC peak area of *Proteus mirabilis* bacteria processing under solar irradiation using ZnO catalyst, initial bacterial concentration 94,000 cfu.

	Percentage of loss (±5%)	Reaction time	Peak area (µV. sec.)
No Cat. + dark	0.00	0 min	33,765
Cat. + dark	30.85	30 min	42,690
Light + no cat.	8.51	30 min	60,794
Cat. + light	99.79	30 min	140,402
Cat. + light	100	4 hours	0.00

Conclusions

1. The prepared nanoparticles of ZnO catalyst showed high activity in killing and complete photodegradation (mineralization) of *Enterococcus* (gram positive bacteria) and bacteria *Proteus mirabilis* (gram negative bacteria) in water under solar simulator light.
2. ZnO nano particles are partially killed *Enterococcus* (gram positive bacteria) and bacteria *Proteus mirabilis* (gram negative bacteria) in the dark.
3. UV alone can kill *Enterococcus* (gram positive bacteria) and bacteria *Proteus mirabilis* (gram negative bacteria) but to lesser extent.
4. All the results showed that ZnO has achieved complete bacterial killing after 30 minutes of irradiations.
5. *Proteus mirabilis* bacteria has shown higher resistance to ZnO activity compared with *Enterococcus* due to differences in the structure of their cell wall. Gram-positive bacteria have a cell wall which surrounds the cell membrane, the cell wall made of peptidoglycan layer, teichoic and lipoteichoic acids. The cell wall of Gram-negative bacteria is more complex because of the presence of an outer membrane that composed of lipopolysaccharide (LPS), in addition to a thin peptidoglycan layer[44]. The outer membrane of Gram-negative bacteria acts as permeation barrier and reduced the influx of ZnO into the bacterial cell.
6. Increasing amounts of catalyst increased percent of bacterial killing.
7. Changing the pH value showed that the catalyst is less effective in basic medium.

8. Changing temperature in acceptable range had no adverse effect on the catalyst efficiency.
9. Increasing the initial concentration of bacteria enhanced the catalyst activity in terms of turnover frequency and quantum yield.
10. In case of light, ZnO completely mineralized the resulted organic matter from the killed *Enterococcus* (gram positive bacteria) and bacteria *Proteus mirabilis* (gram negative bacteria) within four hours of irradiation.

Suggestion for Further Work

Based on our observations, bacterial complete degradation needs UV light due to ZnO wide band gap. In order to enable ZnO particles to photodegradation bacteria in the visible light (major component of sunlight), the band gap needs to be lowered. We suggest doping ZnO particles with different types of safe metal ions. Doped ZnO particles can be used as catalyst.

References

[1] C.J. Vörösmarty, P. Green, J. Salisbury, R.B. Lammers. ***Global water resources: vulnerability from climate change and population growth***. Science. 289 (2000) 284-288.

[2] P.H. Gleick. **W*ater in crisis: a guide to the worlds fresh water resources***. Oxford University Press. (1993).

[3] P.H. Gleick, M. Palaniappan. ***Peak water limits to freshwater withdrawal and use***. Proceedings of the National Academy of Sciences. 107 (2010) 11155-11162.

[4] J.C. Dooge. ***Fresh Surface Water***. Eolss Publishers Company Limite. 2009.

[5] G. Dvorak. ***Disinfection 101***. Center for Food Security and Public Health. Iowa State University. Ames. IA. (2005).

[6] National Research Council (US) Safe Drinking Water Committee.

The Chemistry of Disinfectants in Water: ***Reactions and Products.*** National Academies Press (US). (1980).

[7] A. Zyoud, M. Dwikat, S. Al-Shakhshir, S. Ateeq, J. Shteiwi, A. Zu'bi, M.H. Helal, G. Campet, D. Park, H. Kwon. ***Natural dye-sensitized ZnO nano-particles as photo-catalysts in complete degradation of E. coli bacteria and their organic content***. Journal of Photochemistry and Photobiology A: Chemistry. 328 (2016) 207-216.

[8] A.H. Zyoud, M. Dwikat, S. Al-Shakhshir, S. Ateeq, J. Ishtaiwa, M.H. Helal, M. Kharoof, S. Alami, H. Kelani, G. Campet. ***ZnO nanoparticles in complete photo-mineralization of aqueous gram negative bacteria***

and their organic content with direct solar light. Solar Energy Materials and Solar Cells. 168 (2017) 30-37.

[9] A. Gupta, J.R. Saurav, S. Bhattacharya. S***olar light based degradation of organic pollutants using ZnO nanobrushes for water filtration***. RSC Advances. 5 (2015) 71472-71481.

[10] Fox. M. ***Photocatalytic Oxidation of Organic Substances, In: Kluwer (ed.) Photocatalysis and Environment***: ***Trends and Applications***. New York Academic Publishers (1988) 445–467.

[11] M. Umar, H.A. Aziz. **Photocatalytic degradation of organic pollutants in water.** Organic Pollutants-Monitoring, Risk and Treatment. InTech. (2013).

[12] P. de Abreu, E. Lopes Pereira, C.M. Montenegro Campos, F. Luiz Naves. ***Photocatalytic Oxidation Process (UV/H2O2/ZnO) in the treatment and sterilization of dairy wastewater***. Acta Scientiarum. Technology. 35 (2013).

[13] C. Kittel. I***ntroduction to solid state physics***. Wiley, (2005).

[14] A.C. Melissinos, J. Napolitano. ***Experiments in modern physics***. Gulf Professional Publishing. (2003).

[15] B. El-Kareh. ***Fundamentals of semiconductor processing technology.*** Springer Science & Business Media. (2012).

[16] A. Zyoud, H.S. Hilal, I. Aboasba, L. Badwan. ***Comparative Assessment of ZnO and TiO2 Nano-Particles in Solar Simulated Light*** Mineralization of Methylene Blue in Aqueous Media. (2016).

[17] D. Montalvo, F. Degryse, R. da Silva, R. Baird, M.J. McLaughlin. ***Chapter Five-Agronomic Effectiveness of Zinc Sources as Micronutrient Fertilizer***. Advances in Agronomy. 139 (2016) 215-267.

[18] A. Janotti, C.G. Van de Walle. ***Fundamentals of zinc oxide as a semiconductor***. Reports on progress in physics. 72 (2009) 126501.

[19] Tan Siew Yen, Magdalene. ***Long-term effects of zinc oxide nanoparticles on wastewater treatment in a membrane bioreactor (MBR) process***. National University of Singapore. (2015).

[20] S.S. Kumar, P. Venkateswarlu, V.R. Rao, G.N. Rao. ***Synthesis, characterization and optical properties of zinc oxide nanoparticles***. International Nano Letters. 3 (2013) 30.

[21] C.P. Poole Jr, F.J. Owens. I***ntroduction to nanotechnology.*** John Wiley & Sons (2003).

[22] N. Daneshvar, S. Aber, M. Dorraji, A. Khataee, M. Rasoulifard. ***Preparation and investigation of photocatalytic properties of ZnO nanocrystals: effect of operational parameters and kinetic study.*** evaluation. 900 (2008).

[23] B. Sonage, P. Mohanan. C***haracterization of Zinc Oxide Nanoparticles used for Preparation of Nanofluids***. Procedia Materials Science. 5 (2014) 1160-1164.

[24] G. Modi. ***Zinc oxide tetrapod: a morphology with multifunctional applications***. Advances in Natural Sciences: Nanoscience and Nanotechnology. 6 (2015) 033002.

[25] C. Suryanarayana, M.G. Norton. ***X-ray diffraction: a practical approach.*** Springer Science & Business Media. (2013).

[26] L.V. Azaroff, M.J. Buerger. ***The powder method in X-ray crystallography***. Mc-Graw-Hill. 342 (1953).

[27] M. Zhao, J. Zhang. ***Wastewater treatment by photocatalytic oxidation of nano-ZnO***. Global Environmental Policy in Japan 12 (2009) 88.

[28] D. Wang, J.R. Bolton, S.A. Andrews, R. Hofmann. ***Formation of disinfection by-products in the ultraviolet/chlorine advanced oxidation process***. Science of the Total Environment. 518 (2015) 49-57.

[29] R.V. Tauxe, P.A. Blake. ***Epidemic cholera in Latin America***. JAMA. 267 (1992) 1388-1390.

[30] J.P. Cabral. ***Water microbiology. Bacterial pathogens and water.*** International Journal of Environmental Research and Public Health. 7 (2010) 3657-3703.

[31] T.J. Silhavy, D. Kahne, S. Walker. ***The bacterial cell envelope***. Cold Spring Harbor Perspectives in Biology. 2 (2010) a000414.

[32] L. Devriese, B. Pot, M. Collins. ***Phenotypic identification of the genus Enterococcus and differentiation of phylogenetically distinct enterococcal species and species groups***. Journal of Applied Microbiology. 75 (1993) 399-408.

[33] C.W. Hoge, J. Adams, B. Buchanan, S.D. Sears. ***Enterococcal bacteremia: to treat or not to treat, a reappraisal.*** Reviews of Infectious Diseases. 13 (1991) 600-605.

[34] S.M. Jacobsen, M.E. Shirtliff. ***Proteus mirabilis biofilms and catheter-associated urinary tract infections***. Virulence. 2 (2011) 460-465.

[35] G. Gonzalez, M. Bronze. P***roteus infections***. Medscape. (2015).

[36] S. Ateeq. ***Water Disinfection by Photo-Degradation of Microorganisms Using Natural Dye-Sensitized ZnO Catalyst***. An-Najah National University (2012).

[37] J.F.M. Ishtaiwa. ***Understanding Mode of Action of Nanoparticles in Water Disinfection: ZnO in Bacteria Killing vs. Complete Photo-degradation***. An-Najah National University. Nablus. Palestine (2013).

[38] R.A. Silva-Molina. ***Boron Nitride and Carbon Nanostructures: Synthesis, Characterization and Ab Initio Calculations***. The Autonomous University of San Luis Potosí. Mexico. (2015).

[38] A.K. Zak, R. Razali, W.A. Majid, M. Darroudi. ***Synthesis and characterization of a narrow size distribution of zinc oxide nanoparticles***. International Journal of Nanomedicine. 6 (2011) 1399.

[40] P. Taunk, R. Das, D. Bisen, R. kumar Tamrakar. S***tructural characterization and photoluminescence properties of zinc oxide nano particles synthesized by chemical route method***. Journal of Radiation Research and Applied Sciences. 8 (2015) 433-438.

[41] H. Roy. ***Tuning the properties of the bacterial membrane with aminoacylated phosphatidylglycerol***. IUBMB Life. 61 (2009) 940-953.

[42] A. Akyol, H. Yatmaz, M. Bayramoglu. ***Photocatalytic decolorization of Remazol Red RR in aqueous ZnO suspensions.*** Applied Catalysis B: Environmental. 54 (2004) 19-24.

[43] G. Czerwonka, A. Guzy, K. Kałuża, M. Grosicka, M. Dańczuk, Ł. Lechowicz, D. Gmiter, P. Kowalczyk, W. Kaca. ***The role of Proteus mirabilis cell wall features in biofilm formation***. Archives of Microbiology. 198 (2016) 877-884.

[44] R.M. Epand, R.F. Epand. ***Lipid domains in bacterial membranes and the action of antimicrobial agents***. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1788 (2009) 289-294.

Appendix

Appendix A:

Enterococcus Bacteria

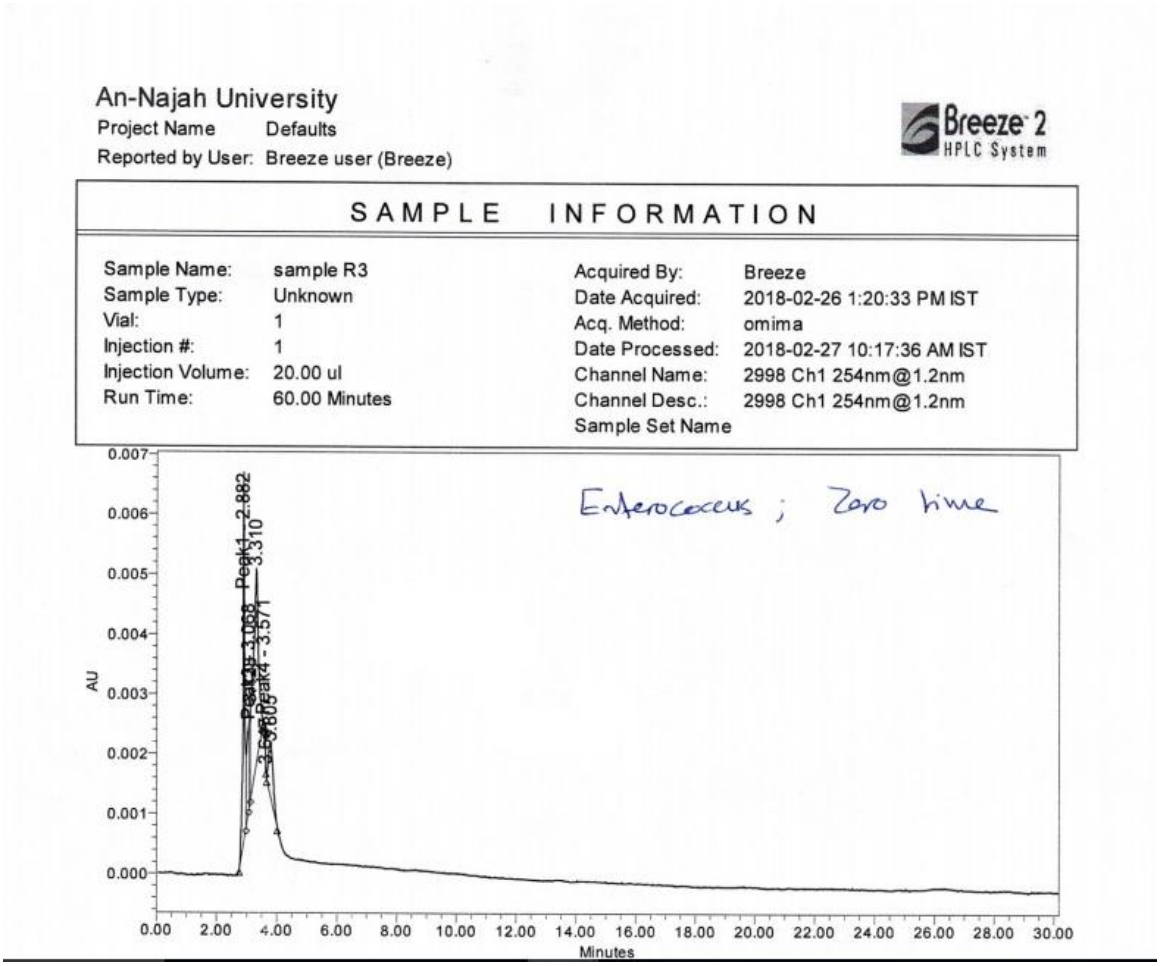

Fig (A.1): The HPLC peaks resulted from water sample pre-contaminated with E*nterococcus* bacteria at zero time of treatment. showed a peak area of ~90,000 µV. sec.

Appendix A:

Enterococcus Bacteria

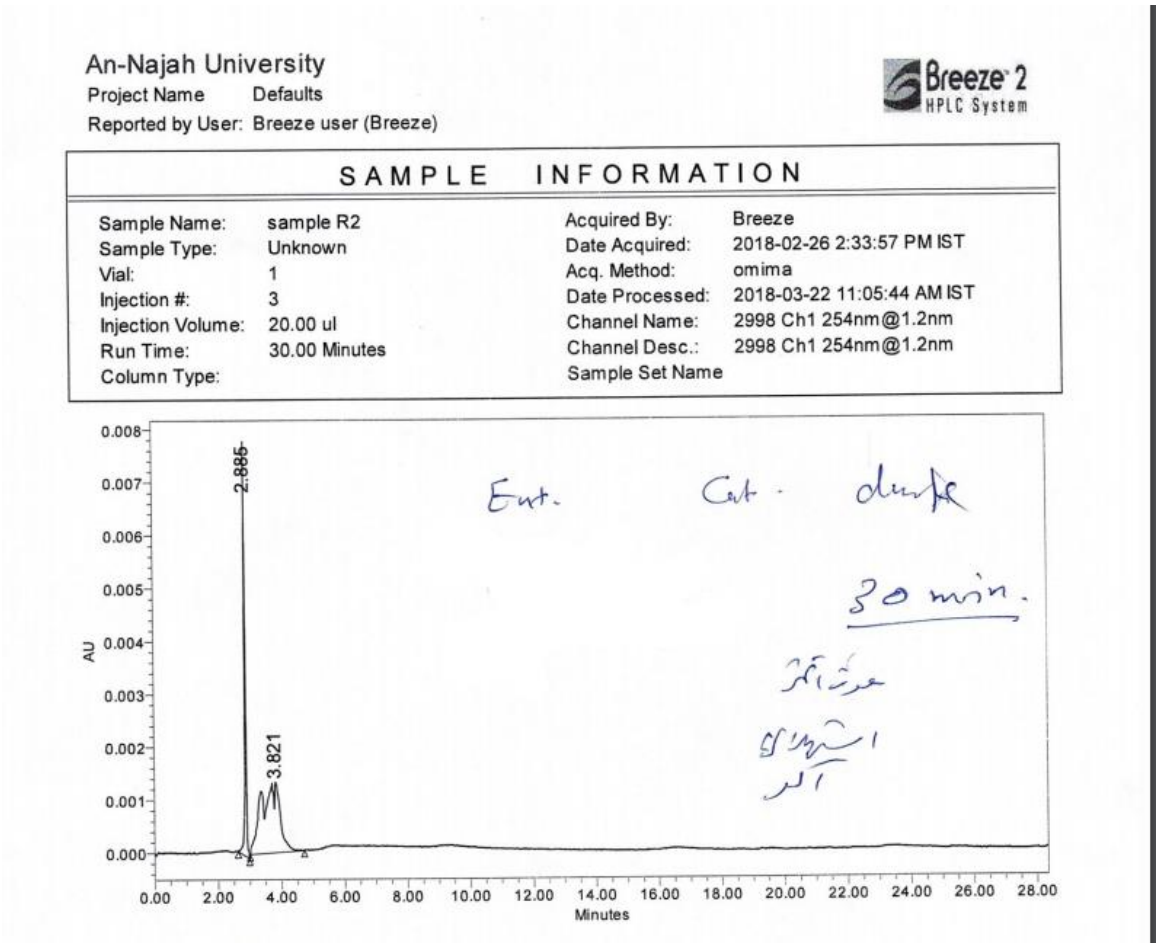

Fig (A.2): The HPLC peaks resulted from water sample pre-contaminated with E*nterococcus* bacteria and treated with ZnO in dark for 30 minutes. showed a peak area of ~74,000 µV. sec.

Appendix A:

Enterococcus Bacteria

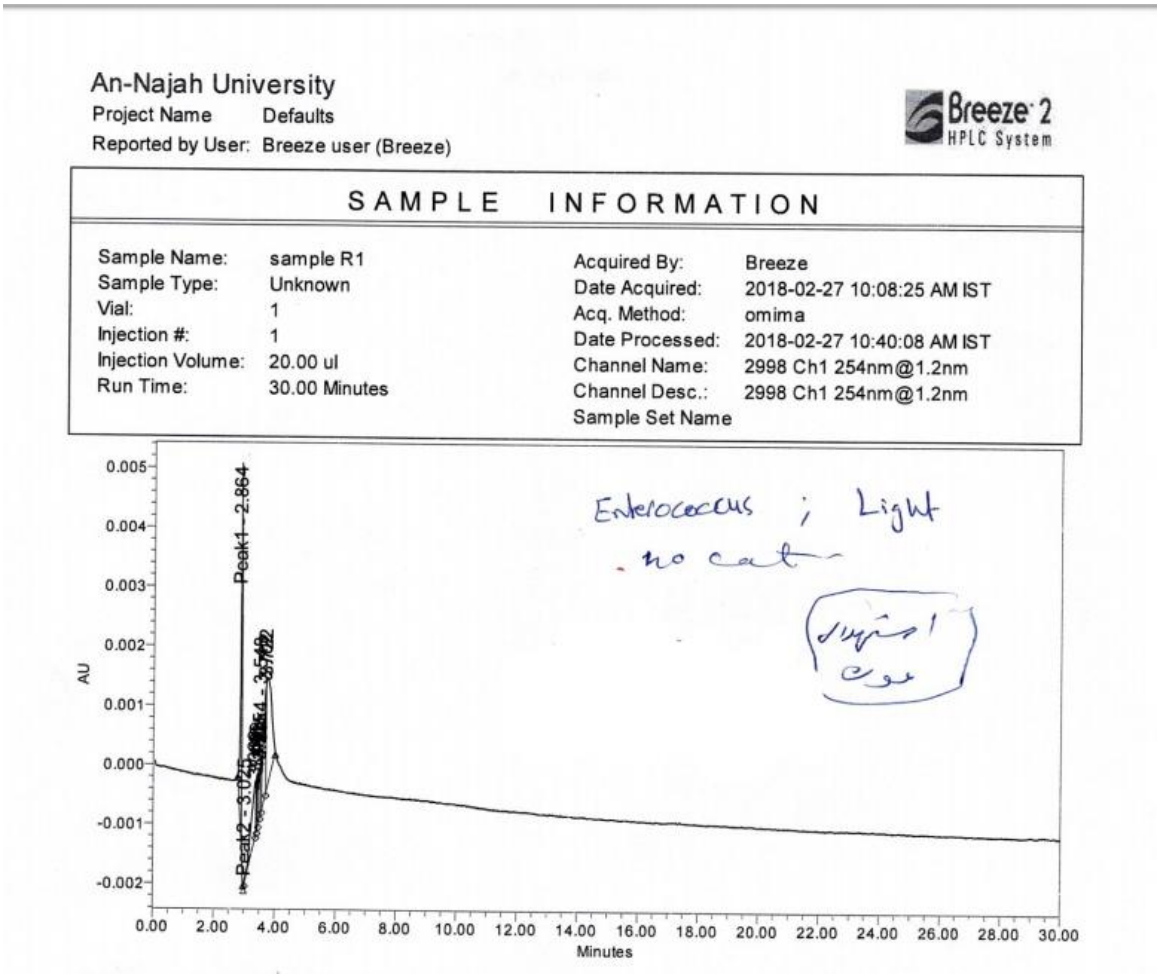

Fig (A.3): The HPLC peaks resulted from water sample pre-contaminated with E*nterococcus* bacteria and exposed to sun light radiations for 30 minutes. showed a peak area of ~ 67,700 µV. sec.

Appendix A:

Enterococcus Bacteria

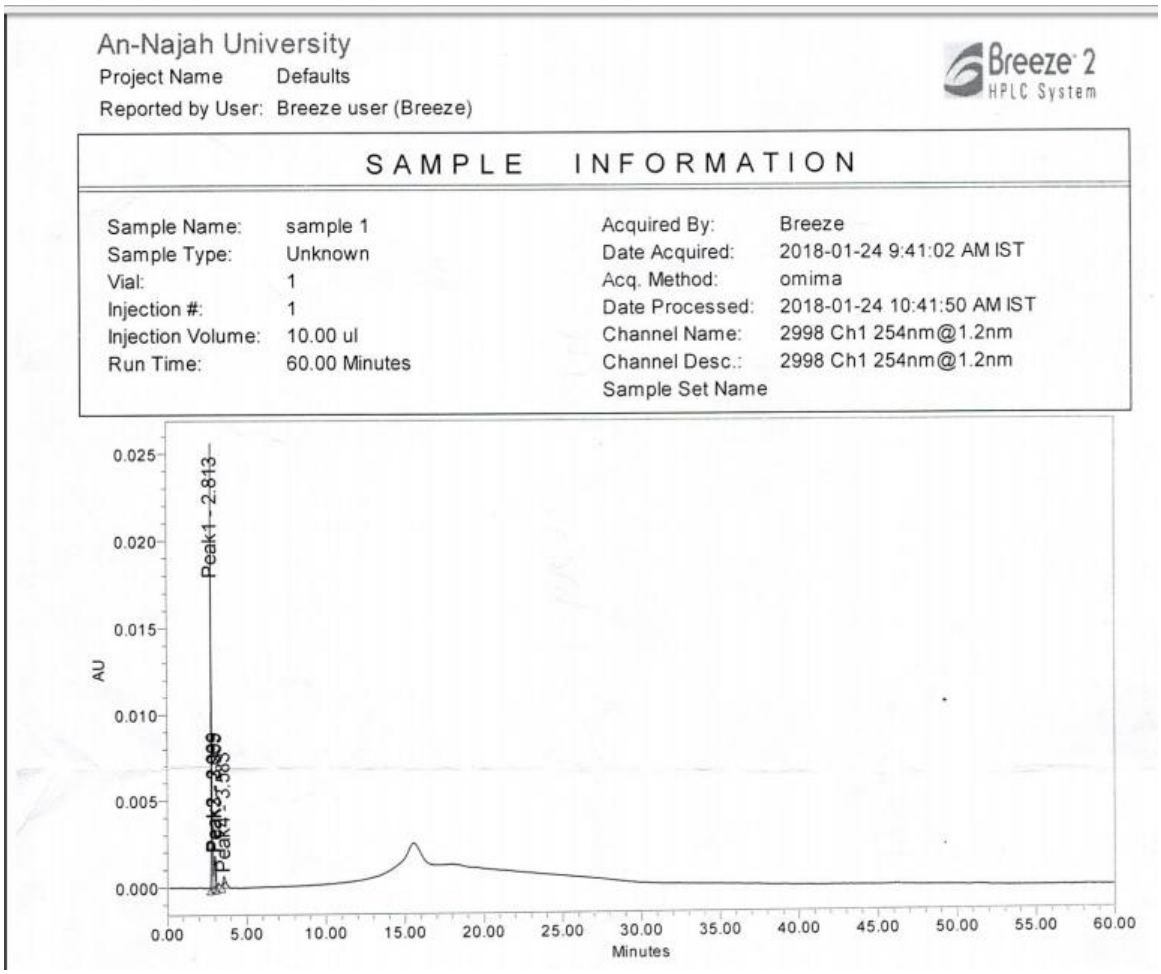

Fig (A.4): The HPLC peaks resulted from water sample pre-contaminated with E*nterococcus* bacteria and treated with ZnO under irradiation for 30 minutes. showed a peak area of ~220,500 µV. sec.

Appendix A:

Enterococcus Bacteria

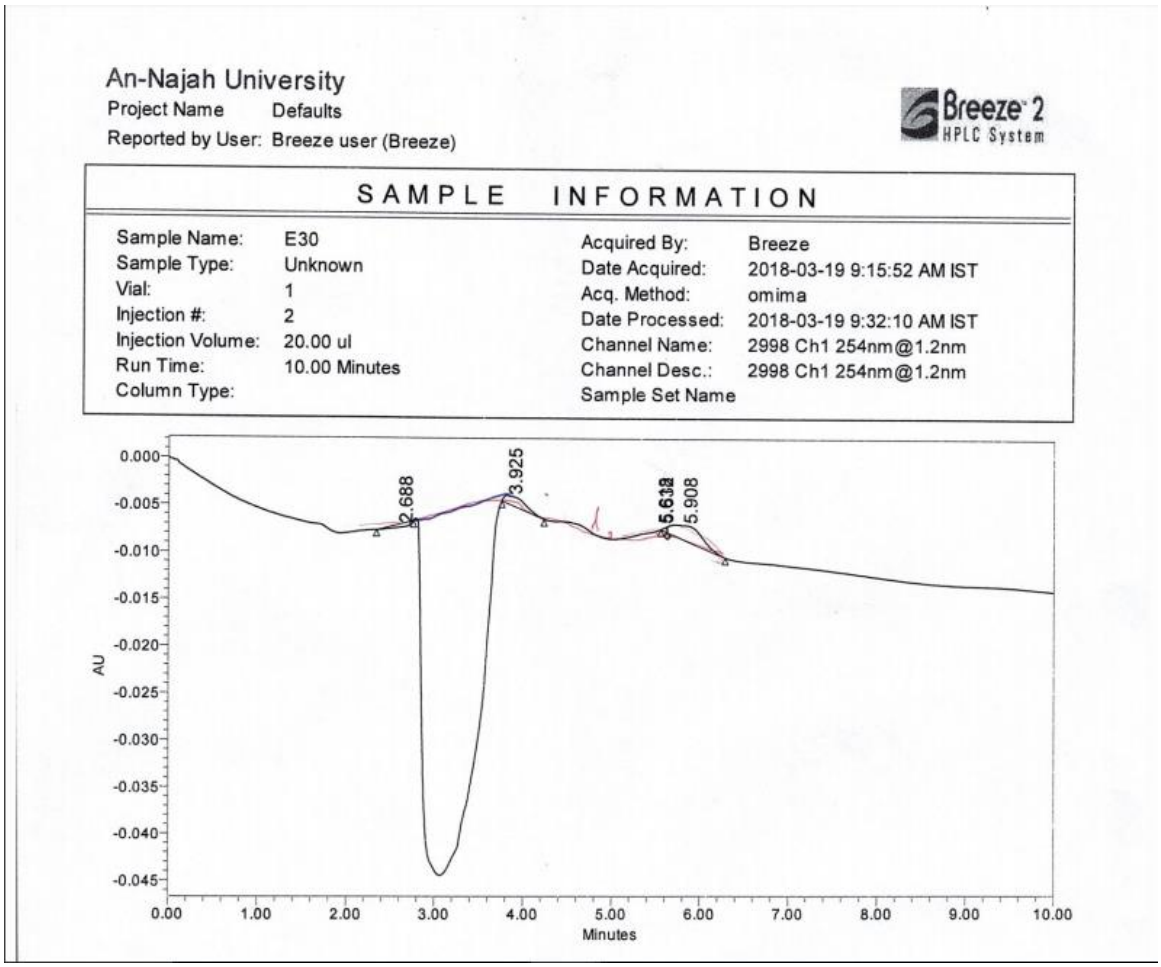

Fig (A.5): The HPLC peaks resulted from water sample pre-contaminated with E*nterococcus* bacteria and treated with ZnO under irradiation for 4 hours. showed a small peak area that was negligible comparing with the previous results.

Appendix B:

Proteus mirabilis Bacteria

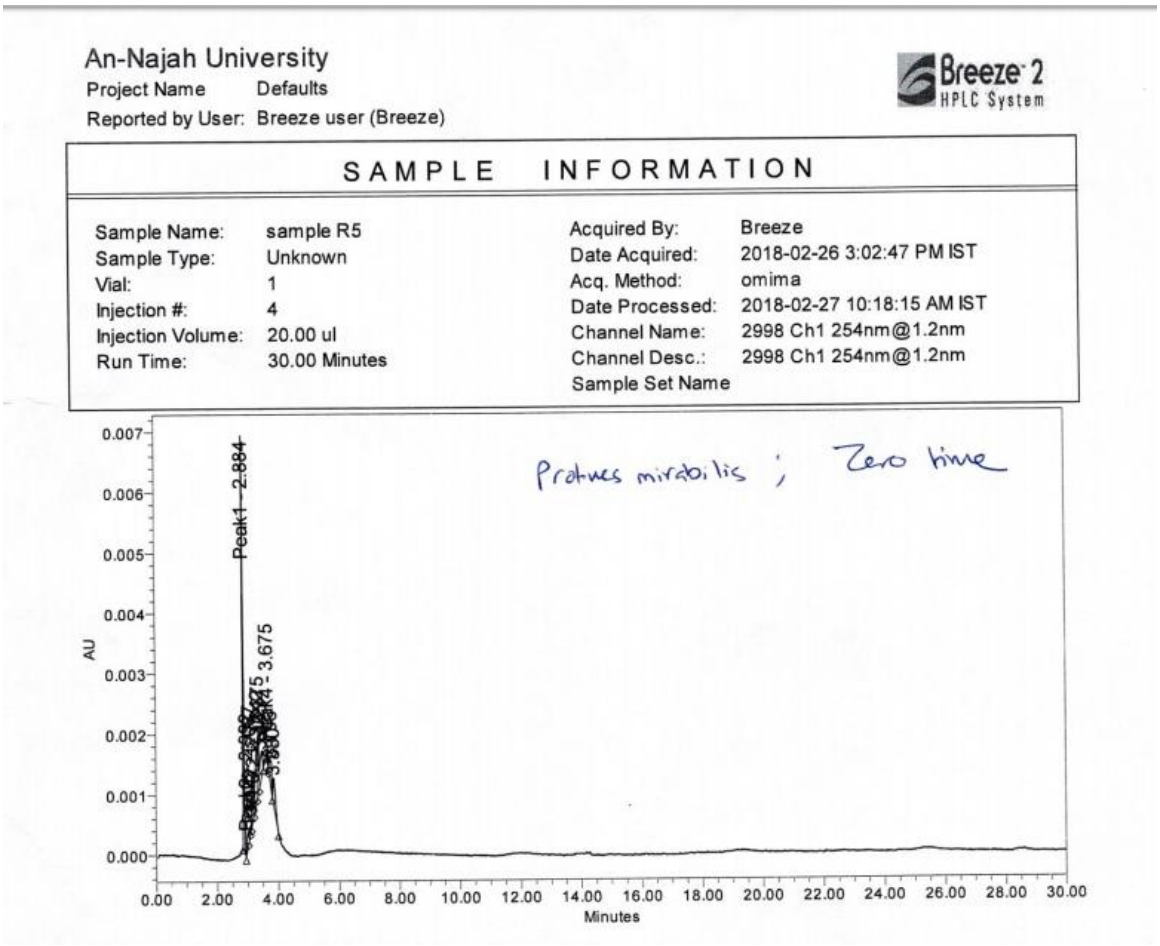

Fig (B.1): The HPLC peaks resulted from water sample pre-contaminated with *Proteus mirabilis* bacteria at zero time of treatment. showed a peak area of ~ 33,760 µV. sec.

Appendix B:

Proteus mirabilis Bacteria

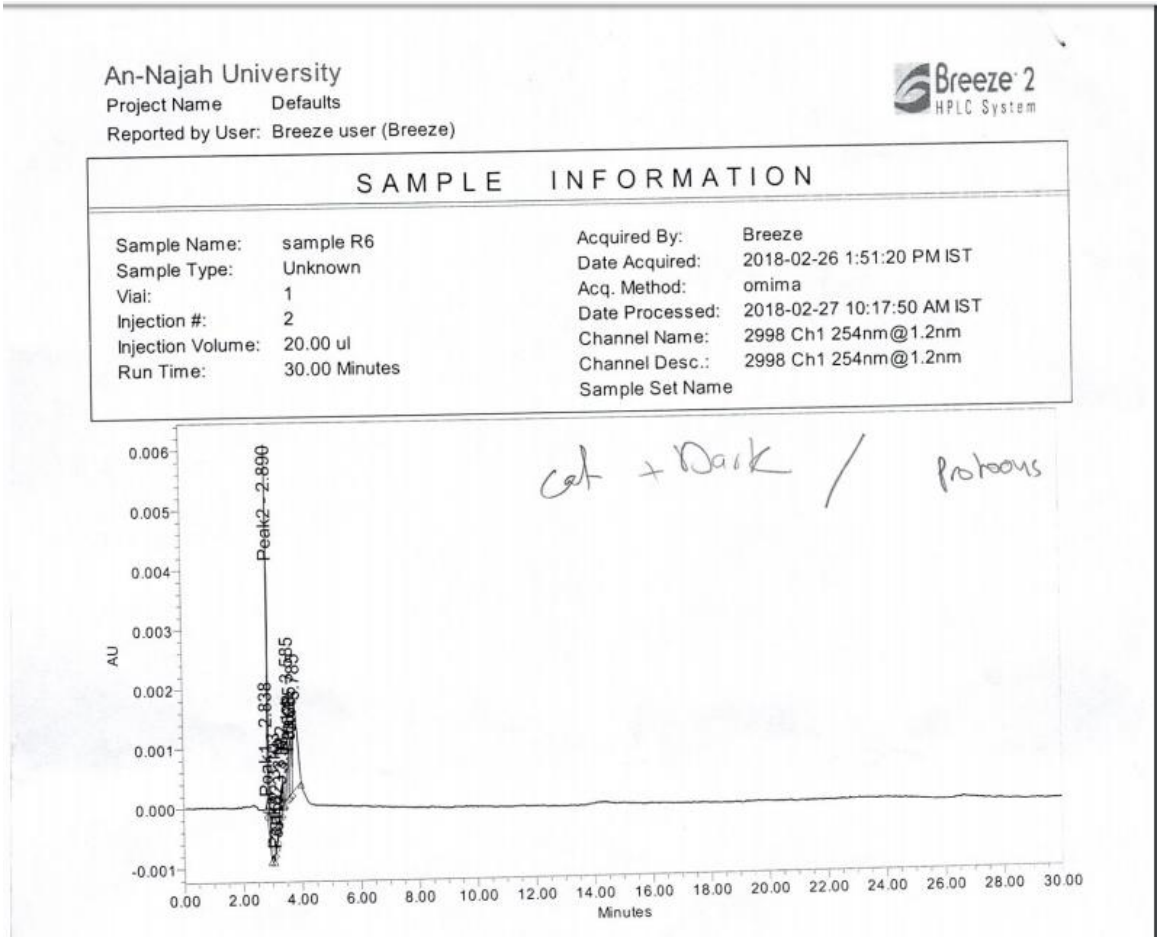

Fig (B.2): The HPLC peaks resulted from water sample pre-contaminated with *Proteus mirabilis* bacteria and treated with ZnO in dark for 30 minutes. Showed a peak area of ~ 42,700 µV. sec.

Appendix B:

Proteus mirabilis Bacteria

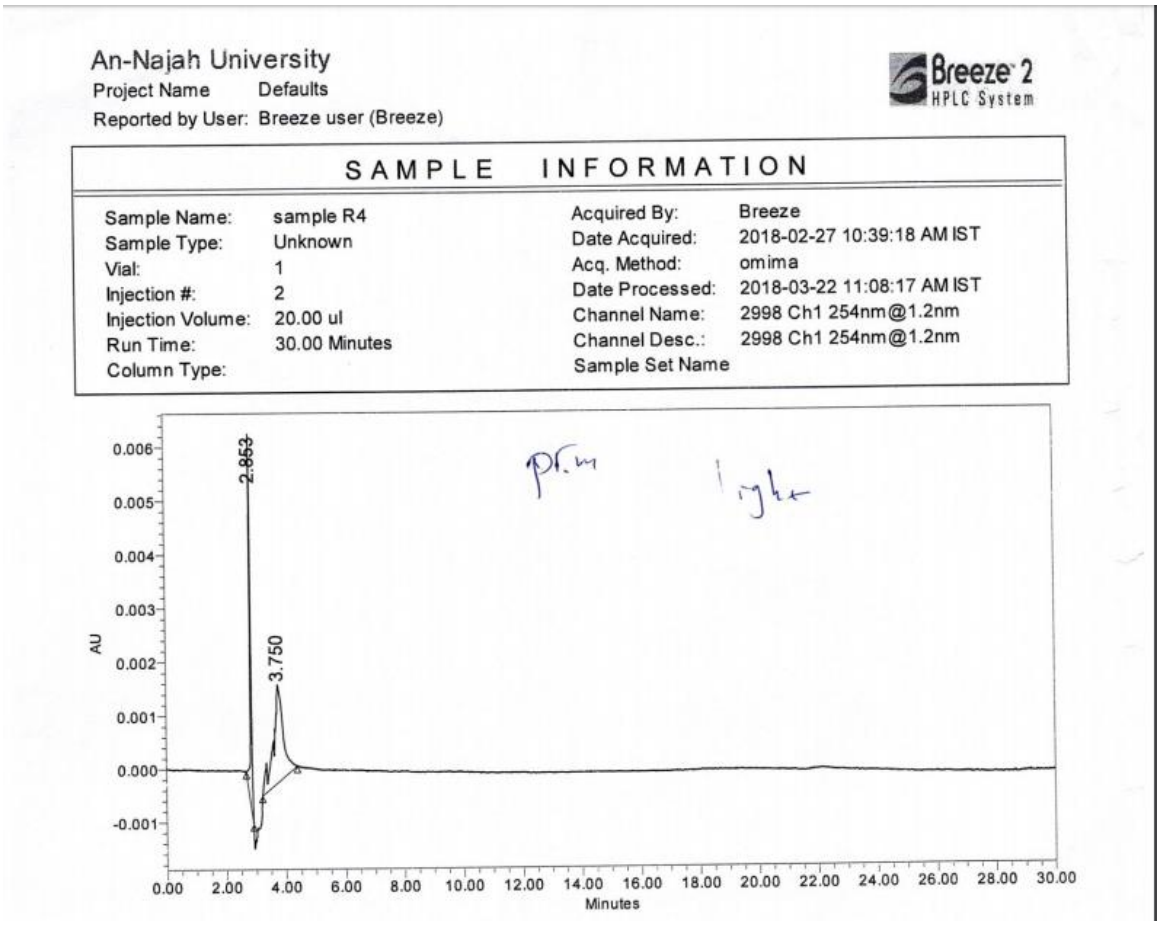

Fig (B.3): The HPLC peaks resulted from water sample pre-contaminated with *Proteus mirabilis* bacteria and exposed to sun light radiations for 30 minutes. Showed a peak area of ~ 60,790 µV. sec.

Appendix B:

Proteus mirabilis Bacteria

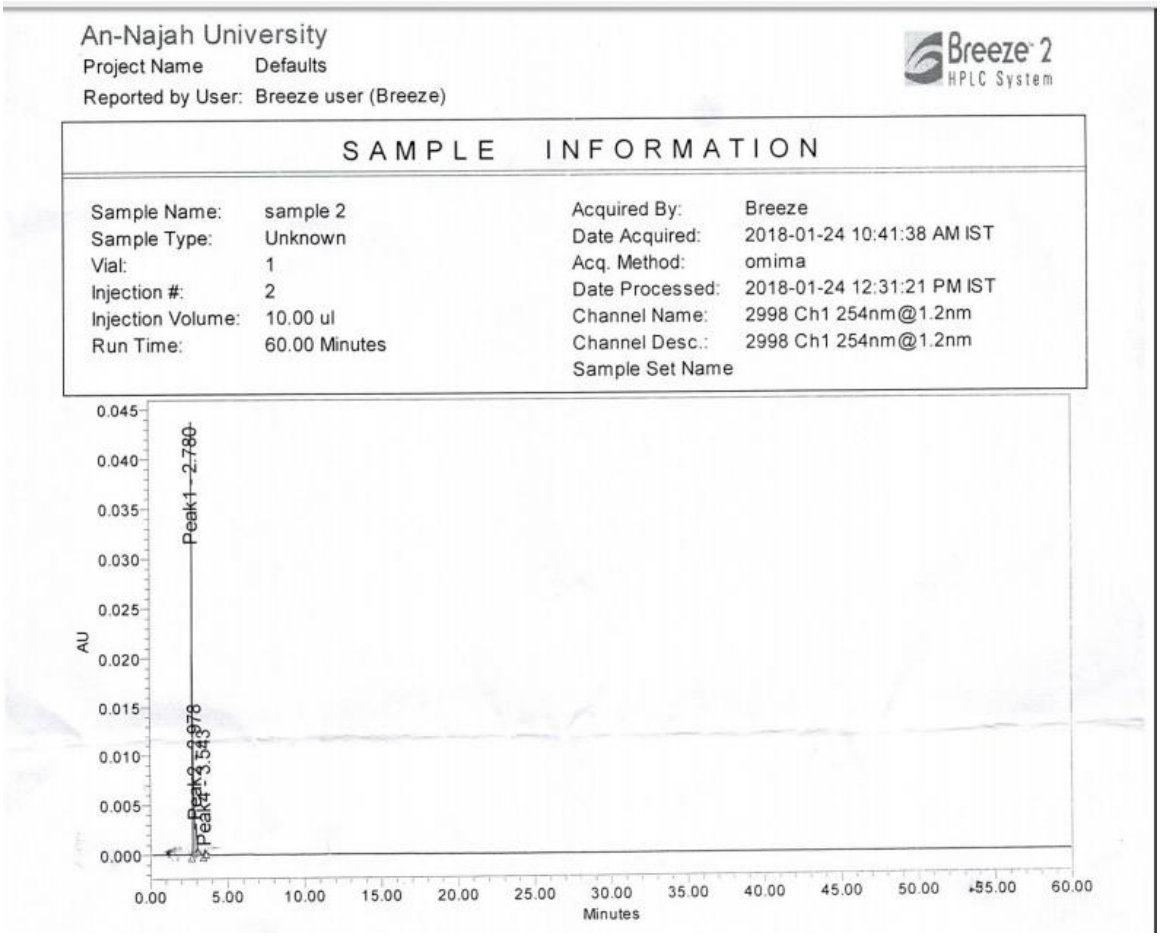

Fig (B.4): The HPLC peaks resulted from water sample pre-contaminated with *Proteus mirabilis* bacteria and treated with ZnO under irradiation for 30 minutes. Showed a peak area of ~ 140,400 µV. sec.

Appendix B:

Proteus mirabilis Bacteria

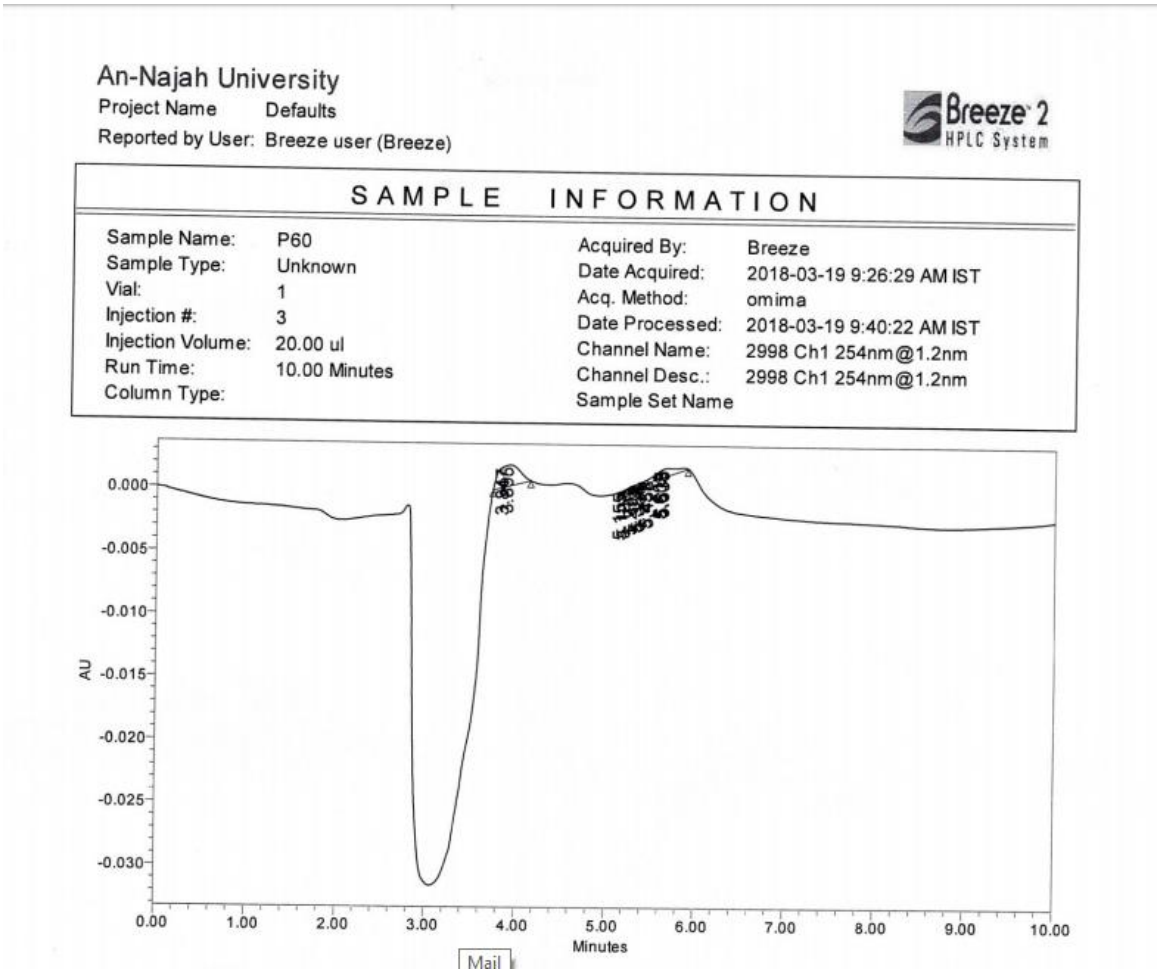

Fig (B.5): The HPLC peaks resulted from water sample pre-contaminated with *Proteus mirabilis* bacteria and treated with ZnO in dark for 4 hours. Showed a small peak area that was negligible comparing with the previous results.

جامعة النجاح الوطنية

كلية الدراسات العليا

تمعدن كامل لبكتيريا انتيروكوكاس و بكتيريا بروتيوس ميرابيليس في الماء باستخدام حبيبات اكسيد الزنك النانوية محفزا ضوئيا

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إشراف

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د. عاهد زيود

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية.

2018

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الملخص

ان تلوث المياه أصبح من أهم المشاكل البيئية حديثا، حيث تشكل مصادر المياه العذبة 2% من نسبة المياه المتوفرة على سطح الارض، ومعظم هذه المصادرة ملوثة او مهددة بالتلوث بسبب النشاط الصناعي والزراعي للانسان. فبالإضافة الى تأثير تلوث المياه على ندرتها فان المياه الملوثة ترتبط بانتشار الامراض والأوبئة، تمت معالجة المياه بعدة طرق لكن أغلب الطرق التي تم استخدامها كان لها آثار جانبية غير مرغوبة. أن ما سبق دفع الى البحث عن طرق جديدة وآمنة من أجل تنقية المياه. في هذه الدراسة تم استخدام حبيبات أكسيد الزنك النانوية بوجود مصدر للأشعة مضاهياً لضوء الشمس من أجل معالجة المياه، أن اكسيد الزنك مادة شبه موصلة غير سامة وغير مكلفة ويسهل التخلص منها بعد انتهاء التفاعل، كما أن خصائصها الفيزيائية أعطتها القدرة على استخدامها كمحفز ضوئي لقتل البكتيريا وتحليلها حيث انه يمكن تحفيز اكسيد الزنك بوجود الاشعة فوق البنفسجية الموجودة في الشمس.

في دراسات سابقة تمت في جامعة النجاح الوطنية تم استخدام حبيبات اكسيد الزنك في قتل بكتيريا سالبة جرام وهي بكتيريا الاشريكية القولونية (E. coli) وبكتيريا الزائفة زنجارية (P. eruginosa) تبعه تمعدن كامل للمواد العضوية الناتجة عن البكتيريا بشكل كامل الى غاز ثاني اكسيد الكربون وماء وغازات اخرى متصاعدة.

في هذا البحث تم استخدام اكسيد الزنك محفزا ضوئيا في تنقية المياه من بكتيريا سالبة الجرام وهي بكتيريا انتيروكوكاس (Enterococcus) وبكتيريا موجبة الجرام وهي بكتيريا بروتيوس ميرابيليس

(Proteus mirabilis) بوجود أشعة محاكية لأشعة الشمس. إن استخدام اكسيد الزنك على هذين النوعين من البكتيريا لم تتم دراسته من قبل.

تم تحضير اكسيد الزنك المستخدم في البحث في المختبر من كلورايد الزنك و هيدوكسيد الصوديم كمواد أولية. تمت دراسة الخصائص الفيزيائية والكيميائية لأكسيد الزنك المحضر باستخدام الاشعة السينية، المجهر الاليكتروني الماسح، كما تم تحديد مستويات الطاقة لأكسيد الزنك من خلال تحليل امتصاص المادة للضوء وتحليل الضوء المنبعث منها بعد ان تتم عملية التحفيز.

أشارت نتائج التجارب الى الحصول على تحطيم بنسبة 100% لكل من بكتيريا انتيروكوكاس و بكتيريا بروتيوس ميرابيليس عند استخدام المحفز تحت الاشعة المحاكية لأشعة الشمس لمدة ثلاثين دقيقة. وعندما تم استخدام كل من اكسيد الزنك والأشعة الضوئية على حدة، تبين أن لكل منهم القدرة على قتل البكتيريا ولكن بنسبة ضئيلة.

تمت دراسة العوامل التي قد تؤثر على كفائة عمل المحفز مثل درجة الحرارة، درجة حموضة الماء، تركيز المحفز المستخدم وتركيز البكتيريا.

تم ايضا فحص نسبة المركبات الكربونية العضوية للمياه التي تمت معالجتها من البكتيريا، لتشير النتائج الى عدم وجود أي مركبات كربونية عضوية مما يثبت ان لأكسيد الزنك القدرة على قتل البكتيريا وعمل تمعدن كامل للمركبات الناتجة عنها.