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Removal of Bacteria using a Thin Layer of TiO_2 Nanoparticles-based Photo-catalyst for Drinking Water Disinfection

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ABSTRACT

Background: This research examines the removal efficiency of bacteria using titanium dioxide (TiO_2) nanoparticles-based photocatalyst by Photocatalytic for water treatment.

Methods: A nanoparticle of $nTiO_2$-type anatase sol-gel was prepared and coated with a thin glass film to use as the cathode pole of an electrochemical cell connected with the anode pole of platinum. This electrochemical cell was used for treating and disinfecting polluted synthetic water from *E-coli* bacteria with different concentrations from $1*10^3$ to $1*10^7$ cfu/ml. These samples were treated by electrochemical cells and the results showed a significant level of removal efficiency of E-coli bacteria, reaching 95%.

Result: In comparison, the results were reduced to 50% efficiency when the treatment was conducted using direct sunlight as the natural source of ultraviolet light. This occurred because the sunlight was not concentrated enough on the nanoparticle film, which means there was not enough power for the reaction. The results were generally very good for laboratory experiments and need more modification to get high removal efficiency for perfect drinking water disinfection. This result was confirmed using a regression analysis, R^2 was 97.37, 99.80, 98.98, 94.72 for WDE1, WDE2, WDE3 and WDE4, respectively.

Key words: Antibacterial activity, Nanoparticles, Photocatalysis, Regression model, Titanium oxide, Water Disinfection.

INTRODUCTION

Most countries confront serious problems in terms of the viability and availability of potable water, particularly in developing countries (Beauregard *et al.,* 2020). The world faces enormous challenges in increasing the demand for potable water, while surface water began to deplete as a result of increasing drought, increasing population growth and increasing demand for water due to industrial and agricultural activity (Al-Hamdani *et al.,* 2024; Chen and Mao, 2007). While water is the main need for the world population, it is the biggest source of disease when it lacks the basic conditions (Al-Musawy *et al.,* 2021). In India, for example, 80% of diseases are due to bacteria that come from water and arise from species of pathological micro-organisms (bacteria, viruses) (Lazar *et al.,* 2012).

In most countries of the world, the issue of chlorine as a conventional sterilizer leads to the so-called disinfection by-products (DBPs), which are a negative aspect of water treatment plants (Asmath *et al.,* 2022; Kalash *et al.,* 2022). Especially those that use surface water as a source of water, which contains high concentrations of organic materials and often passes through the sand filters to the stage of chlorine disinfection. Their interaction results in the formation of the carcinogenic compounds of Trihalomethane (THMs) (Arularasu *et al.,* 2020; Shrivastava, 2022). Therefore, the search for new, effective disinfection techniques that do not produce harmful by-products is essential for the production of potable water.

One of the most advanced technologies that researchers have begun to prove its activity in water treatment and disinfection is nanotechnology techniques.

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One of these techniques is the use of Photocatalytic with nano-materials for the production of oxidized free radicals that can kill bacteria and remove some other pollutants. These free radicals are affected by some wavelengths of light, such as ultraviolet radiation, as in advanced oxidation techniques (Al-Jadir *et al.,* 2022). Some of these techniques focus on how to reduce the doses or quantities of chlorine used for water disinfection to as little as possible of the chlorine doses added to drink water and thus reduce the chances of producing disinfection by-products (DBPs) (Cevik *et al.,* 2023). The oxidation process is very precise and expensive. However, it is economically feasible in the long term as it provides healthy water that contains no disinfection by-products (DBPs). In the last ten years, photocatalytic materials have been used as sterilizers for drinking water and air and for treating other industries such as dyes and manufacturing canning products (Yan *et al.,* 2016).

Nowadays, several chemical or physical techniques are being used to remove or inhibit the activity of pathogenic microorganisms, such as by adding chlorine and its derivatives. or ultraviolet, ozone and desalination by nano and micro-membranes. In the past and still in recent years, some types of chemicals used for the same purpose, like Bromium and fluorine, cause some problems resulting from the toxicity of these agents when they are used for water treatment and purification (Dalai *et al.,* 2014). It is looking forward to finding more effective and safe water disinfection methods. The use of nanotechnology applications has been started to pay attention to water treatment and disinfection. The higher effectivity of nanoparticles of some fine elements provides a very large surface area with a high ability to react with other components. It is expected that the nanoparticles will have an effective influence on the water treatment because they increase the adsorption ability of sorbent. That is due to the widespread of particles and reactive surface holes, which gave these techniques a unique method in this respect.

Photocatalytic treatment

The term (Photocatalysis) is used for the chemical reactions that occur on metal oxide in the presence of light. Titanium dioxide nanoparticles demand a light with a wavelength of 320 nm that falls within the wavelengths of ultraviolet radiation and provides enough energy. This light leads to the release of electrons during the reaction from titanium oxide, which is considered a semiconductor. When it is exposed to UV light radiation, it will be negatively charged energy (Litman *et al.,* 2018). The roots are active due to the departure and movement of electrons that lead to the formation of new active sites (gaps) where the electrons depart with oxygen and water molecules (Jasim *et al.,* 2020). This reaction results in releasing negative roots of OH- and O= with additional negative charge. These roots remove and kill the bacteria (Lee and Tan, 2015). Equations (1, 2 and 3) below show the chemical reaction mechanism caused by the released electrons from the water and titanium nanoparticles' thin layer. This step produces new holes (h) that have a positive charge, as shown by the equation below:

$$TiO_2 + hv \rightarrow TiO_2\, e^- + h^+ \quad \text{... (1)}$$

$$H_2O + TiO_2 h^+ \rightarrow TiO_2 + \bullet OH + H^+ \quad \text{... (2)}$$

$$O_2 + TiO_2 e^- \rightarrow TiO_2 + \bullet O_2^- \quad \text{... (3)}$$

MATERIALS AND METHODS

Escherichia coli, isolated from polluted drinking water, was used and diagnosed by using Eosin Methylene Blue culture through the color of the bright green colonies. One colony was taken from the original bacterial culture and cultured on Nutrient Agar, then inoculated 100 ml of the normal saline solution by the bacterial cultures to make the bacterial suspension and then compared with a Macferland solution with a concentration of (0.5), which is mixed with 0.5 mL from 1.175% of barium chloride ($BaCl_2.2H_2O$) with 99.5 ml of 1% sulfuric acid (H_2SO_4) to get a Bacterial suspension contains 1×10^8 CFU\ml. After that, it is placed in the system with the necessary time intervals and then the exposed bacterial suspension is counted the bacteria in the way of Pour Plate account and according to the adopted method in the standard methods of testing water and wastewater before and after the testing (APHA, 2012). All experiments were conducted at the laboratories of the Ministry of Science and Technology in Baghdad-Iraq at the period from 2021 to 2022.

The Pour Plate method was used. The examination steps were carried out in sterile conditions and near the source of the flame. Cumulative dilution about 10^{-1} -10^{-8} was prepared by using normal saline, then transferring 1 ml of the diluted sample to a sterile petri dish and repeating with each dilution. Then, the dissolved and cooled medium was placed at a temperature of 45°C with an amount of 15-20 ml and then incubated at 37°C for 48 hours. After the incubation period, the total number of replicates of the sample was calculated. The ratio was extracted and multiplied in the inverse of the dilution to obtain the total number of bacteria in 1 ml of the sample and the result was recorded in (CFU / ml).

The titanium oxide nanomaterial was prepared using the Sol-Gel method (Pinjari *et al.,* 2015). This method involves the addition and reaction of pure $TiCl_4$ with NH_3Cl in an aqueous medium under highly controlled reaction conditions for a reaction time of about 7-8 hours. Then, the produced solution was left for 24 hours in order to complete the reaction and digestive reactants. Later, the prepared material was sprayed on the surface of a thin soft glass slide after cleaning by an ultrasonic device using acetone and then alcohol. Then, the glass was washed with distilled water for 30 minutes and then dried through hot air at 115°C. After this step, the glass was coated with titanium oxide nanoparticles using an ultrasonic spray device under limited conditions for 5 minutes at 450°C. The film was treated at 500°C, left to cool for a period of 8 to 10 hours and then all visual and electrical tests were conducted to indicate the required specifications of the thin film layer (Ding and Liu, 1997) (Wei-guo *et al.,* 2004).

An electrochemical cell was designed and used in this research. This cell consists of two glass cylinders mounted on each with a small window made to let the UV light penetrate inside the two cylinders. The nanomembrane was made of nano titanium oxide, which analyzes the water into a negatively charged oxygen molecule with the presence of a free electron; it does another job by reacting with water contents to produce hydrogen peroxide, which results from releasing the hydroxide ion. This electron would result in depositing the pure platinum electrode which is placed in the other tube for the purpose of releasing the hydrogen gas. Electrodes were connected from outside by an electric circuit by an electron bridge for electron transmission between electrodes through water. Meanwhile, the ions pass through an intermediate tube connecting the two cylinders installed at the bottom of the system. Measurements of temperature, flow rate and pH were conducted. Samples were taken from a hole in the cells for testing. (Fig 1 and 2) show the main parts of the disinfection system using photocatalytic by UV and titanium dioxide nanoparticles.

RESULTS AND DISCUSSION

Treatment by Titanium Oxide Nano Particles Photo-catalyst for Water disinfection has many applications, including the removal of bacteria and organics as well as the possibility of producing panels or surfaces that have the possibility of self-disinfection and cleaning (Cheng *et al.,* 2016).

Several laboratory experiments have been conducted using different operational conditions to treat bacteria-contaminated water, a synthesized solution with concentrations ranging from $3*10^3$ to $3*10^7$ cfu/ml. This synthesized water was prepared at the laboratory to study the effect of Titanium Oxide nanoparticles on the activity of the microorganisms. different operating conditions were selected and experiments were conducted under the source of ultraviolet radiation to stimulate the titanium particles.

Fig 3 shows the effect of ultraviolet on the reaction for laboratory experiments which were conducted without using a UV light source, only the TiO_2 NP for disinfection of the contaminated water in order to compare the results with that applied with UV source light, condition of the experiments were conducted at different intervals of retention time (40, 60, 90, 220) minutes and pH values (4, 6, 8, 10), results obtained for the removal efficiency E- colie bacteria which calculated by equation (1) below. Results indicated that the limits were within less than 40% because of the weak activity and low rate of free active hydroxy radicals. This is

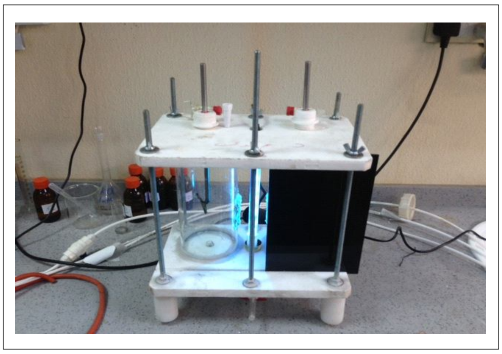

Fig 1: The electrochemical cell.

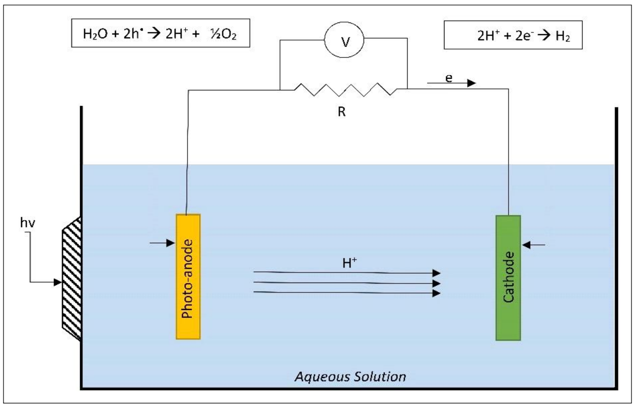

Fig 2: Electrochemical mechanisms of electrodes with water contaminants.

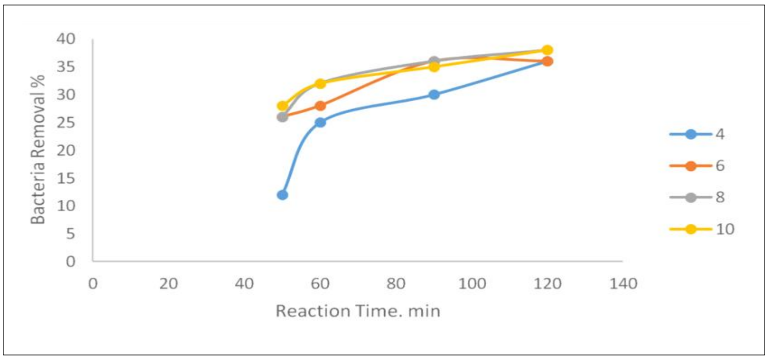

Fig 3: Disinfection efficiency using titanium nanoparticles without ultraviolet radiation at different pH.

due to the lack of sufficient energy to stimulate the movement of the electrons from the titanium nanoparticle layer to activate the oxidized radicals (•OH- and •O -), which confirms the effect of the catalytic radiation by increasing the formation and mobility of electrons between the auxiliary material and the ultraviolet radiation.

$$\text{Bacteria(conc.in) \%} = 1 - \frac{\text{Bacteria (conc.out)}}{\text{Bacteria (conc.in)}} \times 100 \quad ...(1)$$

Other experiments were conducted to study the influence of titanium nanoparticles on disinfection efficiency; these experiments were carried out without the use of titanium nanoparticles and under the same conditions using the same concentrations of bacteria in water ($3 * 10^7$) cfu/ ml with applying ultraviolet radiation as the main disinfectant and for the same time intervals used in previous experiments (30, 60, 90 and 120) minutes and pH values (4, 6, 8, 10).

To study the effect of Ultraviolet on the removal of E-Colie bacteria from contaminated water samples, the results obtained can be described in (Fig 4), which shows that the efficiency per cent of removing bacteria is not at the level sufficient to disinfect contaminated water, so we can see that the optimum reading does not exceed 70% at a time about 90 minutes. We can also see that when the time is more than 100 minutes, readings of bacteria concentration rise again because bacteria grow again and affect the process of disinfection.

While results of removal eff.% rise to a level of more than 95% when the system of equipped with UV as can be seen in (Fig 5), which illustrates the effect of the detention time(exposure time) on the rate of reduction or killing of bacteria in the case of use of an ultraviolet radiation source with a wavelength of 380 nm to stimulate the chemical reaction on the surface layer of titanium dioxide nanoparticles to treat the contaminated water and remove coliform bacteria. The obtained results indicated a high activity of TiO_2 NP for disinfection efficiency reached more than 95% after time about 120 mins. That's due to the oxidation effect of free radicals (•OH- and •O-) that are generated on the surface layer of TiO2NP.these experiments were conducted at different pHs (4, 6, 8, 10) of polluted water, which affected the photocatalytic reaction significantly.

Table 1: Values of the studied variables based on the prediction model.

Sample	Reaction time	WDE1	WDE2	WDE3	WDE4
1	30	0.57	0.25	0.74	0.23
2	60	0.9	0.33	0.9	0.28
3	90	0.95	0.34	0.9	0.13
4	120	0.94	0.23	0.64	0

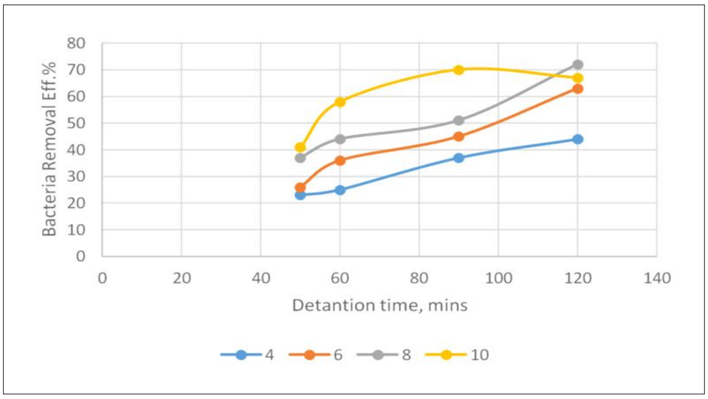

Fig 4: Water disinfection efficiency using ultraviolet radiation without titanium dioxide nanoparticles at different pH.

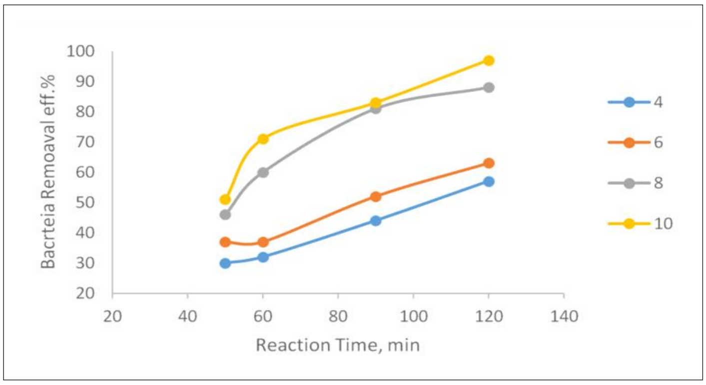

Fig 5: Disinfection efficiency using Ultraviolet radiation and titanium dioxide nanoparticles at different pH.

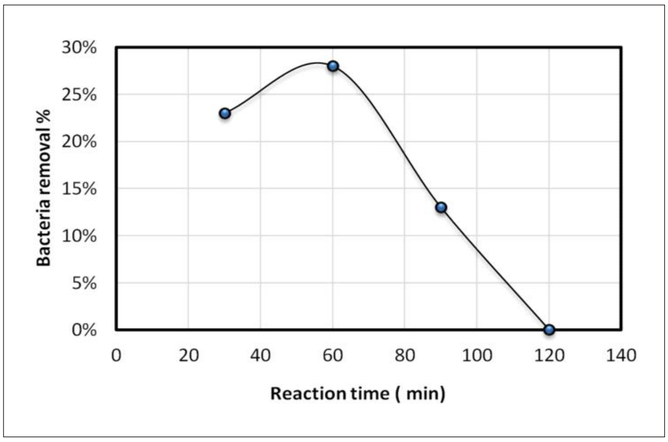

Fig 6: The use of sunlight in Photocatalysis of titanium nanoparticles in water disinfection.

Additionally, (Fig 5) shows that the disinfection efficiency is reduced and the bacterial activity is increased again over time due to the inability of UV radiation to complete disinfection for a long time, especially in the high concentrations of bacterial activity, which soon begins to grow and multiply over time (Lazar *et al.,* 2012).

To benefit from the sunlight in providing the energy needed to stimulate electrons from titanium oxide particles and to form strong, free and oxidized roots of (OH-), it provides a good opportunity to invest in securing small, low-cost water purification systems, especially in rural areas of Iraq or in developing countries where the sun is available suitably. The use of solar energy in photocatalytic processes requires a special design that can provide or focus sunlight to a degree that can stimulate electrons. This could be done by using solar collectors to focus the light, which suffers from dispersion in various directions when it falls on the surfaces. While this may add some costs, it is considered feasible compared to the good efforts or possibilities in providing potable water (Cai *et al.,* 2014).

This contrasts with the results obtained from experiments outside the laboratory and under direct sunlight for using ultraviolet rays from the sunlight to complete the disinfection process, whereby the laboratory system was not equipped with solar collectors. (Fig 6) displays the results, indicating that the disinfection efficiency does not exceed 50%. Thus, these results cannot be relied upon to disinfect potable water. Hence, some modifications and additions are required to increase the efficiency of the system in disinfection in case of using direct sunlight.

For comparison purposes, experiments were conducted for the same types of bacteria but without the source of ultraviolet radiation and other experiments without the particles of titanium oxide to know the effect of ultraviolet radiation and nanoparticles on the efficiency of the disinfection process.

A prediction model for the water disinfection efficiency using ultraviolet radiation and titanium dioxide nanoparticles (WDE 1) is established by taking the water disinfection efficiency as the dependent variable and the input variables (Reaction Time) as independent variables. Using Minitab software, a model by regression is used based on 4 experiments for one factor, as shown in (Table 1).

Regression Equation

$$\text{WDE 1}= 0.133+0.01772 \times 1-0.000092 \times 1*\times 1 \ (R^2=97.37\%)$$

Where $\times 1$: = Reaction Time.

The prediction model for disinfection efficiency using titanium nanoparticles without ultraviolet radiations (WDE2) was made by taking water disinfection efficiency as the dependent variable. While the reaction Time l is an independent variable (input) as follows Regression Equation:

$$\text{WDE2}= 0.0550+0.008133 \times 1-0.000056\times 1*\times 1 \ (R^2=99.8\%)$$

Where $\times 1$: = Reaction time.

The prediction model for Disinfection efficiency using Ultraviolet radiation (WDE3) was made by taking water disinfection efficiency as the dependent variable. The ratio of the reaction Time as an independent variable (input) is as follows in the Regression Equation :

$$\text{WDE3}= 0.3450+0.01650 \times 1-0.000117\times 1*\times 1(R^2=98.98\%)$$

Where $\times 1$: = Reaction time.

The prediction model for Disinfection efficiency using sunlight in Photo catalysis of titanium nanoparticles (WDE4) was made by taking water disinfection efficiency as the dependent variable. The Reaction Time as an independent variable (input) is as follows in the Regression Equation:

$$\text{WDE4}= 0.145+0.00470 \times 1-0.000050\times 1*\times 1(R^2=94.72\%)$$

Where $\times 1$: = Reaction time.

CONCLUSION

Photocatalytic of the TiO_2 nanoparticles have many effective applications for water disinfection, such as the removal of organic compounds and some biological contaminants, as well as the possibility of producing hydroxy radicals that have the possibility of self-disinfecting to remove biological pollutants, for example, E-Colie Bacteria. The results indicate a high activity in the disinfection efficiency reached more than 95% after about 90 minutes. The results were stable and reached high removal efficiency at this level of disinfection in the case of using ultraviolet radiation to stimulate electrons from titanium oxide nanoparticles. Disinfection of contaminated water with a pH level of (8-10) indicates a high percentage of bacteria removal 95-97. Was it reduced to 50% in the case of using a UV light source for Titanium nanoparticle stimulation? using direct sunlight as a source of ultraviolet (UV) radiation did not give encouraging results. To get useful results, we need to enhance this process with solar collectors to collect and focus the radiation on the nanoparticle plates.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript

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