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The evaluation effect of TiO_2 nano particles on different bacterial strains isolated from water purification stations in Baghdad

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Abstract

The antibacterial activities of some nanoparticles, makes them attractive as a new agents against pathogenic bacteria. In this research, the antimicrobial effects of Titanium dioxide-nano-particles against seven bacterial isolates (*E.coli*, *Enterobacter aerogenes*, *Pseudomonas alcaligenes*, *Aeromonas veronii*, *Aeromonas hydrophila*, *Serratia marcescens and Staphylococcus aureus)* being isolated from different Baghdad water purification stations investigated. The physiochemical characters, which influence the quality of the drinking water for the air and water, demonstrated.The characterization of nanoparticles investigated by using Scanning Electrone Microscope, FTIR, and UV-Visible Spectrophotometer. The activity of different concentration of TiO_2 Nps. (50, 100, 150, 200 mg/ ml) measured in liquid medium using the colorimetric analysis and measuring the optical density (OD) for all bacterial species observed. The plate count assay were involved to investigate the effect of TiO_2 Nps. on one model of gram negative bacteria (*E. coli)* and one gram positive bacteria (*S. aureus*.) This study showed that nano-TiO_2 has efficient antibacterial effect in water, and can used as an antibacterial agent for different purposes.

Key words: water purification**,** Tio_{2}- nanoparticles, antibacterial.

تقييم تأثير دقائق ثاني اوكسيد التيتانيوم النانوية على السلالات البكتيرية المختلفة المعزولة من محطات تنقية المياه في بغداد

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خلاصة

الفعالية المضادة للبكتيريا لبعض الدقائق النانوية، يجعلها مميزه لاستخدامها كعوامل جديدة ضد البكتيريا المسببة للأمراض. في هذا البحث، تم تقييم دور دقائق ثاني اوكسيد التيتانيوم النانوية كمضاد للميكروبات ضد سبعة انواع بكتيرية هي (***E.coli, Enterobacter aerogenes***, *Pseudomonas alcaligenes*, *Aeromonas veronii*, *Aeromonas hydrophila*, *Serratia marcescens and Staphylococcus aureus)* التي عزلت من مختلف محطات تنقية المياه في بغداد. تم اثبات الخصائص الفسيولوجية التي تؤثر على نوعية مياه الشرب للهواء والماء. صفات الماده النانويه تم تعيينها باستخدام جهاز المجهرالماسح الالكتروني وجهاز FTIR, جهاز UV–Visible Spectrophotometer., بعدها تم ملاحظة فعالية تراكيز مختلفة من دقائق ثاني اوكسيد التيتانيوم النانوية (50، 100، 150، 200) ملغم/مل التي تم

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قياسها في الوسط الزرعي السائل باستخدام تحليل المطياف وقياس الكثافة البصرية (OD) لجميع أنواع البكتريا المستخدمه. كما استخدمت تقنية العد بالاطباق لمعرفة تأثير TiO_2 Nps على نوع تابع لبكتيريا سالبة لغرام (*E. coli*) ونوع تابع لبكتيريا إيجابية لغرام (*S. aureus*). بينت هذه الدراسة أن فعالية دقائق ثاني اوكسيد التيتانيوم النانويه لها تأثير مضاد للبكتريا الموجوده في المياه، ويمكن استخدامها كعامل مضاد للبكتريا لأغراض مختلفة.

Introduction

Recently, the nanomaterials have crucial role in various field of science such as biology, medicine, pharmacology, and water industry, which makes positive attitude toward this kind of research field more than ever, water borne disease and water quality consider a great issue for scientists all over the world [1]. Due to highly resistance of microbial agents, scientists believe that the nanotechnology is a valuable agent in multi field, properties of nano-materials differ enormously from those of their original molecules, and these differences in characteristics have made nanotechnology multidisciplinary [2].

Nanotechnology has become one of the most practical technologies, because of unique physical and chemical properties of nanomaterials, such as TiO_2 nanoparticles (TiO_2-NPs), less than 100 nm in diameter, have become a new generation of advanced materials due to their brilliant and interesting optical, dielectric, and photo-catalytic characteristics from size quantization [3]. Titanium dioxide is a photo catalyst and widely utilized as a self-cleaning and self-disinfecting material for surface coating in many applications, titanium dioxide has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect. These properties have applied in removing bacteria and harmful organic materials from water and air, as well as in self-cleaning or self -sterilizing surfaces for places such as medical centers [4].

Nanomaterials used in many research field of science such as antimicrobial agents, medicine and cure of various diseases, and have highly attractive structures as they have high potential for specific functions, providing a safe water resources and reduce the water borne diseases causing by bacterial, viral and parasitic become a vital needs globally [5]. Thus, the annihilation of these microorganisms is an important problem for human survival and has numerously reported as a principal cause. Globally human is facing a great problem for the consumption low sanitation water, diarrhea and water borne disease is major cause of health problems particularly in developing countries [6]. The aim of this study to evaluated of TiO_2 nanoparticles for their applicability in increasing antibacterial activities against seven bacterial isolates from water purification stations in Baghdad.

Materials and Methods

Chemicals

Dry Titanium dioxide nano powder purchased from Sigma Aldrich, USA, (CAS number 637254). The nanoparticles specification was: 99.7% trace metal basis, surface area: 200–220 g/m^2.

Samples collection

Glass container used to collect three samples of water from three different stations in Baghdad (Al-Qadisia, Al karama and Sharq Diglla). Raw and net samples of three stages from water purification stations for Tigris River collected.

Determination of various environmental parameters

Samples of each stage used to examine the physical parameters, Which are including (Temperature, turbidity, pH, Electrical conductivity (EC), Alkalinity, hardness, TDS, Ca^{++}, Mg^{++}, Cl^-, NO_2^-, NO_3^-, NH_3, PO_4^-,SiO_2, Fe^{++}, Fe^{+++}, Al^{+++}, SO_4^{-2}) [7].

Isolation and identification of bacterial strains

Bacterial species isolated from different Baghdad water purification stations (Raw and net samples), microscopic, cultural characterization and biochemical tests done for identification of bacterial isolates. Confirm identification of Bacterial isolates by using Vitek apparatus at Al-Habiebia hospital in Baghdad.

Bacterial species were refreshing by growing on nutrient broth at 37 ^oC for 24 hours, then loop-full of inoculated broth were streaking at nutrient agar, all petri- dishes incubated at 37 ^oC for 24 hours. Growth on the perti-dishes kept in refrigerator until day of experiments [8].

Preparation of TiO_2 NPs dispersion

In 250 ml screw cup bottle, 1g of TiO_2 dissolved in 100 ml of D.W. to prepare 1% of the TiO_2 NPs. suspension as stock solution. The suspension sterilized using the autoclave (121 ºC for 15 min), different concentrations (50, 100,150 and 200) mg/ml of TiO_2 NPs. used in all experiments [9].

Characterization of nanoparticles

Size and shape of nanoparticles illustrated clearly via scanning electron microscopy (SEM), images taken by model (TESCAN-VEGA/USA) with resolution 3nm at 30 kV (Center of Nanotechnology and Advanced Materials, University of Technology/Baghdad), UV-Visible Spectrophotometer (Metertech SP 8001), in the range of (250-450) nm, were used to study optical properties. TiO_2 Nanoparticles are characterized by Fourier Transform infrared spectroscopy (SHIMADZU, IR SPECTAGEL-21), was used to study stretching and bending of bond, which absorbs selected frequencies or energies of infrared radiation [10].

The antimicrobial effect of TiO_2 NPs

The antimicrobial effect of TiO_2 NPs being tested using different analytical methods: growth in liquid medium to determination the optical density for the bacteria before and after the treatment using the colorimeter assay [11], and viable plate count (VPC) methods [12].

Bactericidal growth in the presence of nanoparticles in liquid broth

Two ml of the overnight-cultured form each bacterial isolates added to 100 ml of nutrient broth. Two sets of test tube were prepared for this experiment, first for the controls, which were the bacteria without the nano particle, and the second set with (50, 100, 150, and 200) mg/ml of TiO_2 Nps. separately. The bacteria were aerobically cultured at 37ºC for 24 hours. O.D measurements is taken at 600 nm (by UV-visible spectrophotometer) to monitor the percentage of the bactericidal effect of TiO_2 NPs.[11].

Bactericidal growth in the presence of nanoparticles in solid media

To examine the bactericidal effect of TiO_2 Nps on 7 types of tested bacteria, approximately 10^5 colony-forming units (CFU/ml) of each one were cultured on Nutrient agar plates supplemented with TiO_2 NP in concentrations of (50, 100,150,200) mg/ml. TiO_2 NP. free nutrient agar plates cultured under the same conditions used as control. The plates incubated for 3hr. and 24 h at 37 ˚C and the numbers of colonies counted [12, 13]. The effect of bacterial strains calculated, following the equation:

$$\eta = \frac{N_1 - N_2}{N_1} \times 100\%$$

η= is the percentage of bacterial reduction

N_1= is the number of surviving bacterial colonies from the control sample

N_2 = is the number of surviving colonies from test samples

Results and discussion

Determination of various environmental parameters

Most of the results for physical and chemical finding there were no great difference between river and net findings as shown in Table-1. Durairaj, *et al*. 2012 [14] showed results the TDS, total hardness of $CaCO_3$, and chlorides are high, but it has been reduced 50-88% after purification of water.

Isolation and identification of bacterial strains

Seven bacterial isolates (*E.coli, Enterobacter aerogenes, Pseudomonas alcaligenes, Aeromonas veronii, Aeromonas hydrophila, Serratia marcescens and Staphylococcus aureus)* isolated from water purification stations around Baghdad (as mention in material and methods). All bacterial species collected from the raw while the other products were clear of microbes. Water is the common breeding ground for many pathogens. The presence of bacteria is the main indication of water contamination. In countries such as India, 80% of the diseases are due to bacterial contamination in drinking water. The World Health Organization (WHO, 1996) recommended that any water intended for drinking should contain fecal and total coliform counts of 0, in any 100-mL sample. When either of these groups of bacteria encountered in a sample, immediate investigative action should take. The removal or inactivation of pathogenic microorganisms is the last step in the treatment of wastewater [10].

Characterization of (TiO_2) NPs

Figure-1 shows Scanning Electron Microscope (SEM) images of TiO_2 sample derived TiO_2 nanoparticles in anatase phase calcinated at 600 ˚C for 2 hours. Nanoparticle size measurement of samples by SEM indicates that the size nanoparticle is about 76 nm. Scanning electron microscope (SEM) used to investigate the morphological specifications of nano-crystallites of titanium such as, surface morphology, primary size and shape [4]. It should note that the particle diameter always overestimated due to the distortion of SEM images [8]. It is clear that the TiO_2 nanoparticles seen by SEM image consist of number of crystallites, and shows that TiO_2 nanoparticles were agglomerated when the solution stringed in short time [9, 15]. The UV-Vis. absorption spectrum of TiO_2 nanoparticles solution shown in Figure-2 to have an absorption edge in the range of (275-350) nm, indicating that TiO_2 colloidal solution obtained is anatase phase.

Figure-3 shows the FTIR spectra of as prepared TiO_2 sample. The graph is plotted between (%) transmittance and wave number (cm^{-1}). In this, graph different peaks formed at different wave number. It observed in the graph that TiO_2 Nanoparticles have various frequency vibrations, which shown by different peaks formed. The peak in range 1600-1650 cm^{-1} were characteristic of O-Ti-O bond and narrow adsorption bond is observed due to Ti==O bending region. The broad adsorption band is observed at 3000-3600 cm^{-1} is corresponding to O—H stretch region.

Table 1- Physicochemical characters of samples collected from different water purification station.

Parameters	**S1- East of Tigris river**		**S2- Al-Karama**		**S3- Al-qadisia**	
	River	net	River	Net	River	Net
Air Temp. (˚C)	15.5	15.5	16.5	16.5	16	16
Water temp (˚C)	15.5	15.5	15	15	15	`15
Ph	7.88	7.48	8	7.6	7.8	7.4
Electrical conductivity (EC) MS/cm	1104	1092	1162	1162	1135	1148
Turbidity (NTU)	30	30	25	1.3	24	1.4
Alkalinity (mg/L)	148	145	144	138	156	142
Hardness (mg/L)	383	380	452	456	447	447
TDS (mg/L)	740	753	805	805	802	800
Ca^{++} (mg/L)	107	107	122	123	121	124
Mg^{++} (mg/L)	31	31	36	36	35	34
Cl^{-} (mg/L)	97	97	100	101	95	95
NO_2^{-} (mg/L)	0.004	NIL	0.006	0.002	0.005	0.002
NO_3^{-} (mg/L)	0.9	0.89	1.36	1.45	1.30	1.20
NH_3 (mg/L)	0.03	0.01	0.02	0.01	0.08	0.05
PO_4^{-} (mg/L)	0.015	0.004	0.04	0.01	0.05	0.02
SiO_2 (mg/L)	1.5	1.4	4.0	4.0	3.32	3.21
Fe^{+++} (mg/L)	3.1	0.19	0.9	0.21	1.5	0.001
Fe^{++} (mg/L)	0.12	0.08	0.14	0.11	0.08	0.05
AL^{+++} (mg/L)	0.02	0.04	0.01	0.06	0.0	0.05
SO_4^{-2} (mg/L)	255	255	270	271	260	260

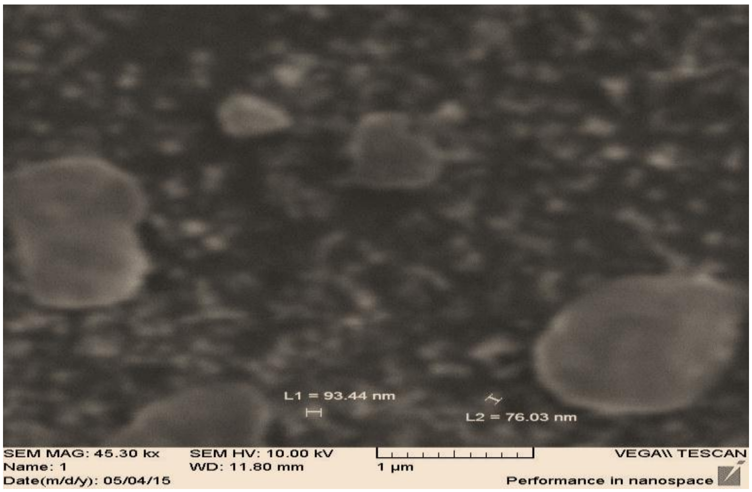

Figure 1- SEM image of TiO_2 nanoparticle for corresponding sample.

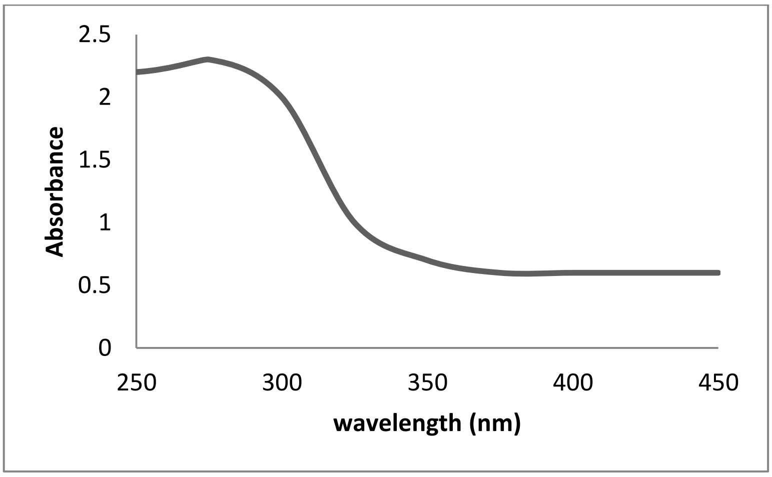

Figure 2- UV-Vis absorption spectrum of TiO_2 nanoparticle.

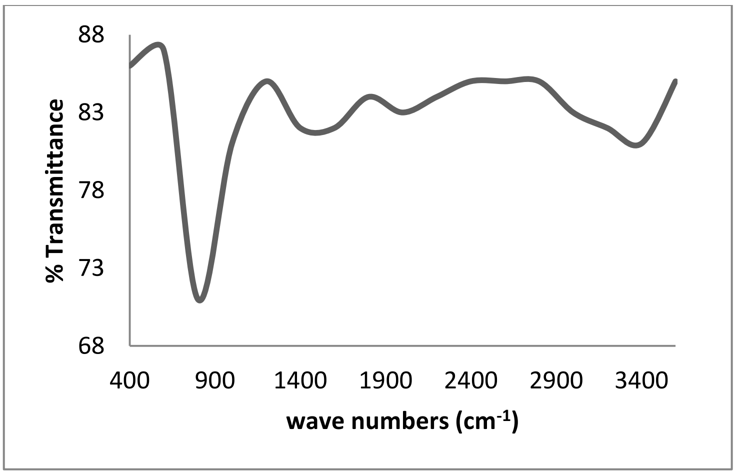

Figure 3- FTIR spectra of TiO_2 nanoparticle for corresponding sample.

Bactericidal growth in the presence of nanoparticles in liquid broth

The efficiency of the antimicrobial effect by using different TiO_2 NPs concentration (50, 100, 150 and 200) mg/ml shown in Figure-4. The optical density of the medium investigated as the number of bactericidal cells after contact with nanoparticles. Figure-4-A demonstrated the effect of 50 mg/ml of nano TiO_2 on the growth and killing kinetics of seven bacterial isolates, while Figure-4-B demonstrated the effect of 100 mg/ml of nano TiO_2, also Figure-4-C demonstrated the effect of 150 mg/ml of nano TiO_2, and Figure-4-D demonstrated the effect of 200 mg/ml of nano TiO_2. It has showed that 150 mg/ml of nano TiO_2 and 200 mg/ml of nano TiO_2 have the best effect on the seven bacterial isolates of this study, while 50 mg/ml of nano TiO_2 and 100 mg/ml of nano TiO_2 have little antibacterial effect.

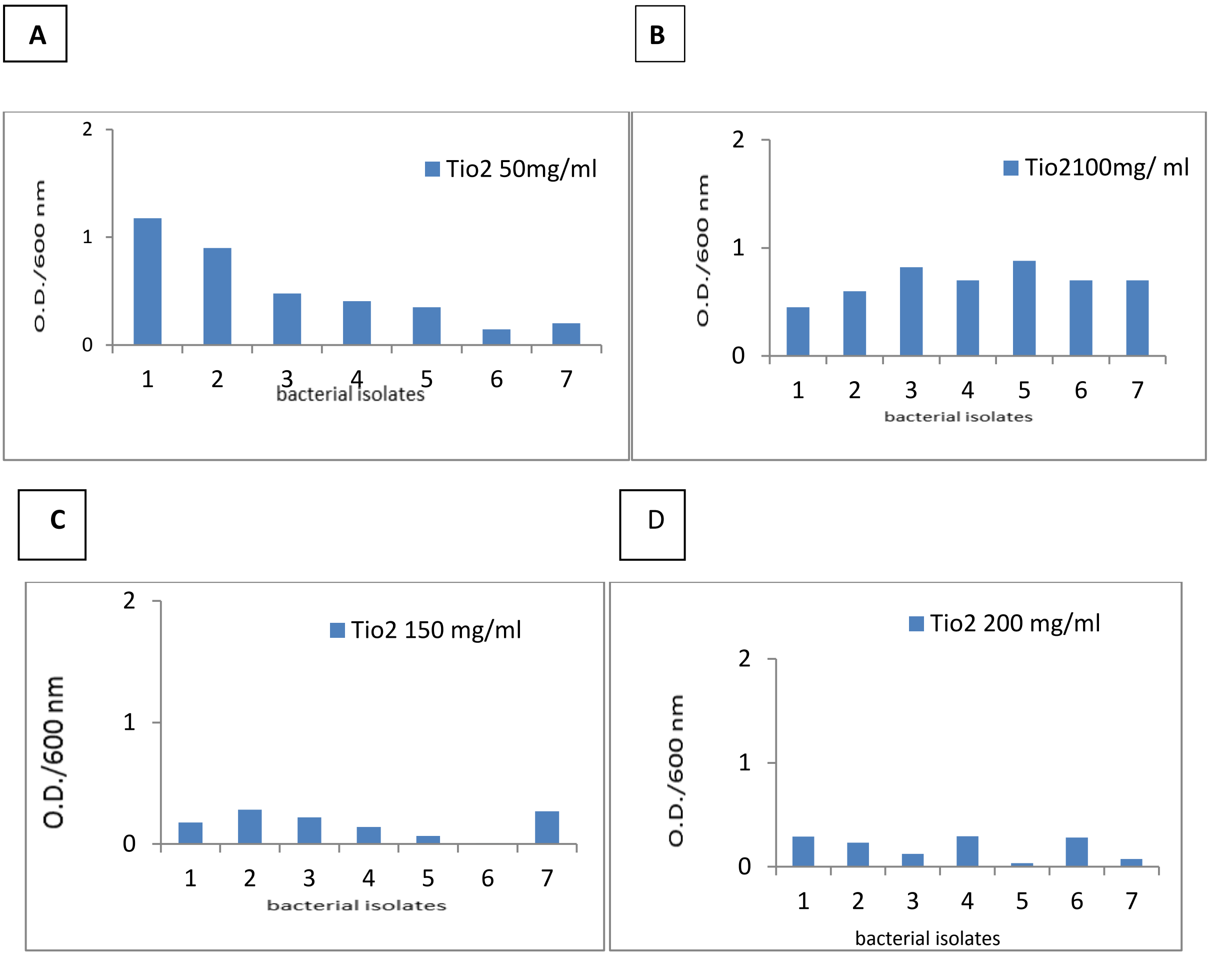

Figure 4- Antibacterial efficiency for the different TiO_2 Nps concentration on bacterial isolates. A- TiO_2 50 mg/ml, B-TiO_2 100 mg/ml, C-$TiO_2$150 mg/ml, D- TiO_2 200mg/ml. [(1) *E.coli (2) Enterobacter aerogenes* , (3) *Pseudomonas alcaligenes*, (4) *Aeromonas veronii*, (5) *Aeromonas hydrophila, (6)Serratia marcescens, (7) staphylococcus aureus*].

Researchers in [16] showed that the decrease in Optical density (OD) for *E. coli*, observed with nano-TiO_2 concentration increase (0.225, 0.218, 0.158, 0.075, 0.031). [17] Explain that effect of different concentrations of nano-ZnO also evaluated on the growth of *S. dysenteriae*, 0.01% nano-ZnO did not have anti bactericidal effect while 0.5 and 1% nano-ZnO were highly efficient in inhibiting the bactericidal growth.

Table-2 showed that the specific characterization for the nanoparticles is effective for killing microorganisms. That was clear the percentage of inhibition were closely to 100%, when the bacterial growth of cells treated with 150 and 200 mg/ml TiO_2-NPs inhibited, after 3 h, almost all treated bacterial cells were dead. The bacterial growth of the cells treated with 100 mg/ml TiO_2-NPs was also slightly lower than that of cells in the control group. At concentration of 100mg/ml, antibacterial efficiency of gram-positive (***staphylococcus aureus***) is 95.1% were for gram-negative (***E.coli***) is 99%.

Table 2- The efficiency of antibacterial for the different TiO_2 NPs analysis using plate count method.

Species / Conc. mg/ ml	*S.aureus* η (%)		*E.coli* η (%)	
	3h	24 h	3 h	24 h
(Control)	0	0	0	0
50	53.8	99.9	58	99
100	95.1	100	99	100
150	100	100	100	100
200	100	100	100	100

In study [10] exhibited that TiO_2 nanoparticles has stronger effect and more efficient on *Staphylococcus aureus* with 10^{-3} concentration than *E.coli*, while the efficient concentration of *E.coli* bacteria is 10^{-5}.

These differences could explain based on the structure of their cell walls. The gram-negative bacteria have a layer of lipopolysaccharide at the exterior, followed underneath by a thin (about 7–8 nm) layer of peptidoglycan. Although the lipopoly-saccharides are composed of covalently linked lipids and polysaccharides, they lack strength and rigidity [18]. On the other hand, the cell wall in gram-positive bacteria is principally composed of a thick layer (about 20–80 nm) of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides to form a three dimensional rigid structure. The rigidity and extended cross-linking not only endow the cell walls with fewer anchoring sites for the silver nanoparticles but also make them difficult to penetrate [19].

The mechanism of action of this nanoparticles on bacterial cell, inhibit protein or polysaccharides (Mucopeptides) synthesis. These particles may affect cell division by modifying the cellular environment but induce damages through a direct action on the cell wall and plasma membrane which become weaker region which suspected that dividing cells [20,21].

Conclusions

The results in this study indicated that using the nanoTiO_2 material as an antibacterial agent for 7 bacterial species isolated from water purification stations were almost effective. The major size of silver nanoparticles was less than 100 nm via SEM images, and The UV-Vis measurements showed that these as-synthesized samples have absorbance peak at 275-350 nm, and the antibacterial efficiency increased when increasing the concentration of TiO_2 solutions.

References

1. Greenberg, A. E, Clesceri, L. S. and Andrew, D. E. **1992**. *Standard Methods for examination of water and wastewater*. American Public Health Association ,18th Edition.
2. Zhang, W.X. **2005**. *Nanotechnology for Water Purification and Waste Treatment.* Frontiers in Nanotechnology US EPA Millennium Lecture Series. Washington D.C.
3. Phong, N.T, Thanh, N.V. and Phuong, P.H. **2009**. Fabrication of antibacterial water filter by coating silver nanoparticles on flexible polyurethane foams. *Journal of Physics*: Conference Series 187.
4. Sadiq, M., Dalai, S., Chandrasekaran, N. and Mukherjee, A. **2011**. Ecotoxicity study of (TiO_2) NPs on two microalgae species: *Scenedesmus* sp. and *Chlorella* sp. *Ecotoxicology and Environmental Safety*, 74, 1180–1187.
5. Mansoori, G.A. Bastami,T. Ahmadpour, Z. and Eshaghi, A. **2008**. *Environmental application of nanotechnology. Annual Review of Nano Research, Vol.2, Chap.2*

6. Khalid, K., Al-Bayatti, K. H., Al-Arajy, and Al-Nuaemy. S.H. **2012**. Bacteriological and Physico-chemical Studies on Tigris River Near the Water Purification Stations within Baghdad Province. *Journal of Environmental and Public Health*. Vol. 1 : 8 pages.
7. Kim, S., Hyeong, S. L. Ryu, S., Choi, S. J. and Lee, D. S. **2011**. Antibacterial Activity of Silver-nanoparticles Against *Staphylococcus aureus* and *Escherichia coli. Korean J. Microbiol. Biotechnol*.Vol. 39, No. 1, pp.77–85.
8. Mansoori, G.A., Bastami, T. R., Ahmadpour, A. and Eshaghi, Z. **2008**. Environmental application of nanotechnology. *Annual Review of Nano Research*, Vol.2, Chap.2.
9. Hosseinkhani, P., Zand, A.M., Imani, S., Rezayi, M., and Zarchi, R. **2011**. Determining the antibacterial effect of ZnO nanoparticle against the pathogenic bacterium, *Shigella dysenteriae* (type 1). *Int. J. Nano. Dim*. 1(4): pp.279-285.
10. Xiaolei, Q. Alvarez, P. J. and Li, Q. **2013**. Applications of nanotechnology in water and wastewater treatment. *Water research*, 4 7: pp.3 9 3 1-3 9 46.
11. Jameel, Z. N., Haider, A.J. and Taha. S.Y. **2014**. Synthesis of TiO_2 Nano particles by Using Sol-Gel Method and its Applications as Antibacterial Agents. *Eng. & Tech.Journal*. 32 (3) pp. 419-426.
12. Sousa, C. Botelho, C. and Oliveira, R**. 2011**. Nanotechnology applied to medical biofilms control. *Communicating current research and technological advances*. pp. 878-889.
13. Sondi, I. and Sondi, B. A. **2004**. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, 275: pp.177–182.
14. Lotfi, M., Vosoughhosseini, S., Ranjkesh, B., Khani, S., Saghiri, M. and Zand, F. **2011**. Antimicrobial efficacy of nanosilver, sodium hypochlorite and chlorhexidine gluconate against *Enterococcus faecalis. African Journal of Biotechnology*. 10(35): pp. 6799-6803.
15. Negahdary. M., Mohseni, G., Fazilati, M., Parsania, S., Rahimi, G., Rad, S. and Zarchi, S. R. **2012**. The Antibacterial effect of cerium oxide nanoparticles on *Staphylococcus aureus* bacteria. *Annals of Biological Research*, 3(7):3671-3678.
16. Haghi, M., Hekmatafshar, M., Janipour, M. B., Gholizadeh, M. M., Faraz, M. K., Sayyadifar, F. and Ghaedi, M. **2012**. Antibacterial effect of tio2 nanoparticles on pathogenic strain of *E. coli. International Journal of Advanced Biotechnology and Research*, 3(3): pp 621-624.
17. Durairaj, T., Sittaramane, A., Mba, M. E. **2012**. Nano technology based water purification using pro-e. *International Journal of Engineering Research and Applications.* 2(3): pp.2750-2753.
18. lkhagvajava, N., yaşab, I., Celikc, E., koizhaiganovaa, M. And Saria, Ö. **2011**. Antimicrobial activity of colloidal silver nanoparticles prepared by sol-gel method. *Digest Journal of Nanomaterials and Biostructures*. 6(1): p. 149 – 154.
19. Kim, J. S., Kuk, E., Yu, K. N. and Kim, J. **2007**. Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 3: pp. 95– 101.
20. Bhattacharya, S., Saha, I., Mukhopadhyay, A., Chattopadhyay, D., Ghosh, U.C. and Chatterjee, D. **2013**. Role of nanotechnology in water treatment and purification: Potential applications and implications. *International Journal of Chemical Science and Technology*; 3(3): 59-64.
21. Shahab, M., Tabish, A., Zaman, B., Tariq, Z. and Kamran A.**2013**. Characterization and synthesis of nano sized TiO2 particles. *Annals of faculty engineering hunedoara – international journal of engineering*, 3(1).