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STUDY ON THE ANTIMICROBIAL PROPERTIES OF TiO_2 NANOPARTICLESBY POUR PLATE CULTURE METHOD FOR APPLICATION IN WATER TREATMENT

Su Su Hlaing[1], Khin Lay Thwe[2], May Thu Aung[3]

Abstract

TiO_2 nanoparticles were synthesized by the sol-gel combustion hybrid method using acetylene black as a fuel. The starting materials were: titanium isopropoxide, isopropanol and acetylene black which was obtained by the decomposition of acetylene and ammonium hydroxide. The obtained TiO_2nanopowderswere examined by X-ray Diffraction (XRD). The sizes of the particles were estimated by using Scherrer's equation. The as-prepared TiO_2nanoparticles synthesized at 500°C were applied to study the antimicrobial behaviour. The activity of TiO_2 nanoparticle against Escherichia coli (E.coli) using a serial dilution and pour plate culture method were investigated for application in water treatment.

Keywords: Antimicrobial behaviour, E.coli, Pour plate culture method, Sol-gel combustion hybrid method, TiO_2nanoparticles.

Introduction

The unique, unusual and interesting physical, chemical and biological properties of nanometer-sized materials have recently attracted a great deal of interest in the scientific community(Choi. S.Y.et al, 2004). There is a need to develop a simple and efficient method to obtain TiO_2 nanoparticles with a narrow size distribution and high crystallinity (Rana. N.et al, 2016).Sol-gel combustion is a novel method that uses a unique combination of the chemical sol-gel process and combustion. The sol-gel combustion method is based on the gelling and subsequent combustion of an aqueous solution containing salts of the desired metals and an inorganic fuel such as acetylene black and it yields a voluminous and fluffy product with alarge surface area (Yun. H. S. et al, 2013). This process has the advantages of inexpensive precursors, a simple preparation method and the ability to yield nanosized powders.

E.coli is a microscopic organism that indicates fecal contamination of drinking water. Most of the drinking water originates from rivers and lakes near where people live. Depending on where it is, the water may pick up bacteria and other microorganisms as well as pollutants from industry, agriculture, roadways, and other sources.

Some of water contaminants may cause acute illness, such as what might occur from bacteria or other microbes, like *E.coli*, which may cause illness and even death(Kormann. C. et al, 1988). It is important that waterpollution should be concerned and act upon it. Good thing that there are processes that can get rid of these microbes(Prado. A. G. S. et al, 2008).

In the present work, the TiO_2 nanoparticles were synthesized by sol-gel combustion hybrid method. The as-prepared TiO_2 nanoparticles were used to study the antimicrobial behaviour against Escherichia coli (*E.coli*) using a serial dilution and pour plate culture method(Han. C. H. et al, 2007)for application in water treatment.

[1] Dr, Assistant Lecturer, Department of Physics, University of Yangon, Myanmar
[2] Dr, Lecturer, Environment and Water Studies Department, University of Yangon, Myanmar
[3] Dr, Department of Physics, University of Yangon, Myanmar

Experimental procedures

(i) Synthesis of Nanocrystalline TiO_2 Powder

TiO_2 nanoparticles have been synthesized using various methods. Among the various methods, the sol-gel combustion hybrid method is found to have many advantages over others. This method uses a unique combination of chemical sol-gel process and combustion. It is based on the gelling and subsequent combustion of an aqueous solution containing salts of desired metals and an inorganic fuel such as acetylene black. With the sol-gel method, it is advantageous to yield nanopowders of high purity and good homogeneity at low processing temperature while with the combustion method, requirements of simple equipments, low energy and short operation time are advantages as this method uses a sustainable exothermic solid-solid reaction among the raw materials. Consequently, the sol-gel combustion hybrid method has the advantages such as needs of inexpensive precursors and simple method of preparation to yield nanosized powder with considerably short operation time. It may also yield a voluminous fluffy product with a large surface area. Figure1shows the flow scheme of the combustion processes used for the synthesis of nanocrystalline TiO_2 powder.

TiO_2 nanoparticles were synthesized by the sol-gel combustion hybrid method using acetylene black as a fuel. The starting materials are: titanium isopropoxide, isopropanol, acetylene black which was obtained by the decomposition of acetylene and ammonium hydroxide. First, 3.5mL of titanium isopropoxide was dissolved in 10mL of isopropanol and 0.2 g of acetylene black was added to the titanium solution followed by an aqueous NH_4OH solution dropwise under constant stirring until it was transformed into a sol at ambient conditions. The sol was heated at 100ºC to obtain a dry gel. The gel was ignited in air at 500ºC resulting in an auto-combustion process that yielded TiO_2 powders.

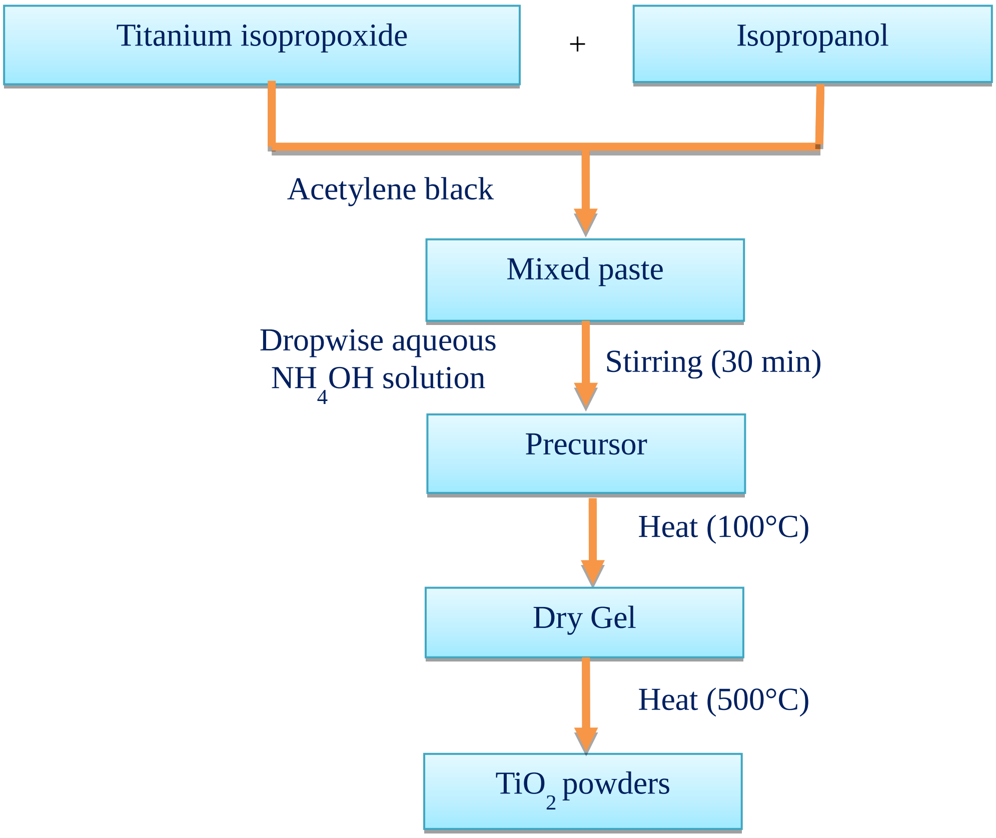


Figure 1 Flow scheme of the combustion process for the synthesis of NanocrystallineTiO$_2$ powders

The obtained TiO_2nanopowders were examined by powder X-ray diffraction (XRD: RIGAKU-RINT 2000 X-ray diffractometer).The crystallized sizes of these nanoparticles were estimated by using Scherrer's equation. The synthesis processes of TiO_2nanopowder were shown in Figure 2 (a), (b), (c), (d), (e) and (f).

(a) Titanium isopropoxide and isopropanol

(b) Acetylene black

(c) Dropwise aqueous NH_4OH solution

(d) Heating the sol at 100ºC

(e) Home-made furnace and temperature sensor

(f) Annealing the gel at 500ºC

Figure 2 The synthesis processes of TiO_2nanopowder

(ii) Antimicrobial Activity of TiO_2 Nanoparticlesby Pour Plate Culture Method

The as-prepared TiO_2 nanopowders synthesized at 500°C were applied to study the antimicrobial behavior. The activity of TiO_2 nanoparticle against Escherichia coli (E.coli) were investigated by using a serial dilution and pour plate culture method.

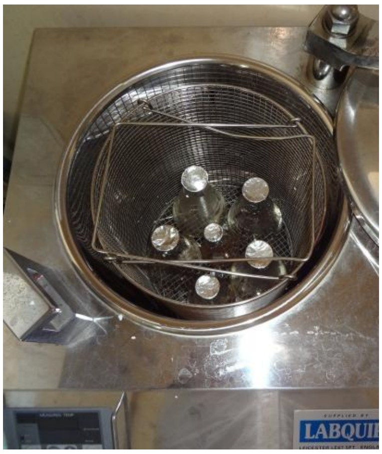
The nutrient broth (1g) and plate count agar (5g) were added to 50 ml of distilled water. This solution was added to the conical flask (each conical flask per 25 mL). The agar solution was heated on the hotplate until boil to well dissolve. These conical flasks were added to the autoclave at 121°C for 45 min. After 45 min, these conical flasks were cooled about 30 min. Then TiO_2 nanopowders (0.005, 0.01, 0.05, 0.1, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5g) were mixed to the liquefied agar, respectively. The initial dilution was made by transferring 1mL of E.coli sample to a 9 mL sterile saline with water (1:10 or 10^{-1} dilution). The 10^{-1} dilution was then shaken by grasping the tube between the palms of both hands and rotating quickly to create a vortex. This serves to distribute the bacteria and break up any clumps. Immediately after the 10^{-1} dilution has been shaken, uncap it and aseptically transfer 1 mL to a second 9 mL saline with water (1:100 or 10^{-2} dilution) through to 1:10000 or 10^{-5}. Since the third was a 10^{-3} dilution, the fifth represents a 10^{-5} dilution of the original sample. Then 1 mL of 10^{-3} dilute saline solution containing E.coli was placed into the ten sterilized petri dishes each 1 mL. After that, 50 mL of ager solution was poured into 10^{-3} petri dishes each 25 mL. The bacterial agar solution and sample were immediately mixed gently moving the plate. This process for the remaining ten plates of the 10^{-5} dilution was repeated. After the pour plates have cooled and the agar has hardened, they were moved into the incubator at 37°C for 24 hours. The process of antimicrobial test as shown in Figure 3(a), (b), (c), (d), (e), (f), (g) and (h).

At the end of the incubation period, all of the petri plates containing between 30 and 300 colonies were selected. Plates with more than 300 colonies cannot be counted and were designated too many to count (TMTC) and plates with fewer than 30 colonies were designated too few to count (TFTC). Then the colonies on each plate were counted. From the counted number of colonies numbers, numbers of bacteria per mL were calculated by the following formula.

$$\frac{number\ of\ colonies\ (CFUs)}{dilution \times amount\ of\ specimen\ added\ to\ liquified\ agar} = number\ of\ bacteria/mL$$

(a)Plate count agar

(b)Heating agar solution on the hotplate



(c)Placing in the autoclave

(d) Adding TiO_2nanoparticles

(e)*E. coli* dilution

(f)Adding *E. coli* dilution

(g) Pouring the agar solution

(h)Incubating at 37°C for 24 h

Figure 3 The processes of antimicrobial test

Results and Discussion

3.1 Analysis of TiO_2 Nanopowders by XRD

The TiO_2 nanopowders were examined by powder X-ray diffraction (XRD: RIGAKU-RINT 2000 X-ray diffractometer). The X-ray diffraction data were recorded by using CuKα radiation (λ= 0.154056 nm). The average grain size of the samples was estimated with the help of Scherrer's equation using the diffraction intensity of the peak.

$$\mathrm{D} = \frac{0.9\lambda}{B\cos\theta}$$

Where λ is the wavelength, B is the full width at half- maximum (FWHM) and $\theta$is the diffraction angle. The powders calcined at 500°C showed with particle size between 20-30 nm. The XRD patterns of TiO_2 nanopowder scalcined at 500° was shown in Figure 4.

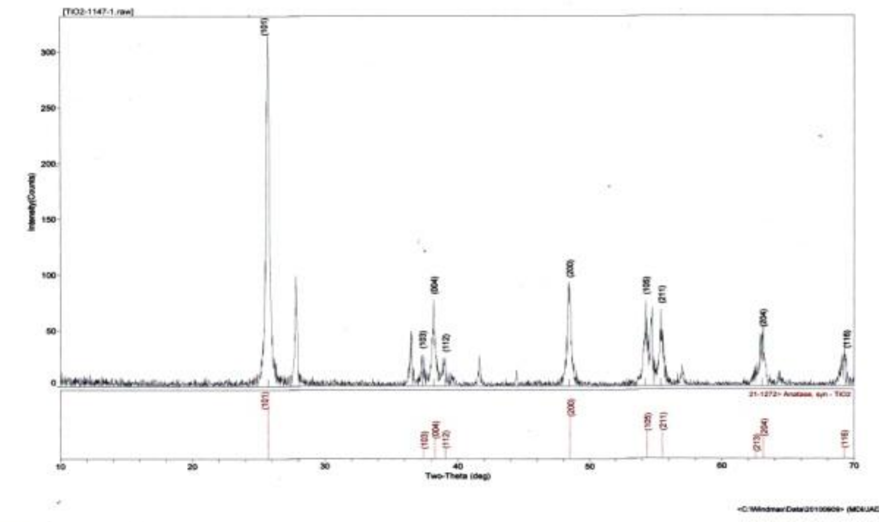


Figure 4 XRD pattern of TiO_2 nanopowders calcined at 500 °C

3.2 Investigation of Antimicrobial Properties

TiO_2 nanopowder synthesized at 500°C was applied to test the antimicrobial activity against Eschericia coli by using a serial dilution and pour plate culture method. E.coli inhibition was observed at 0.005g, 0.01g, 0.05g, 0.1g, 0.25g, 0.3g, 0.35g, 0.4g, 0.45g and 0.5g per 25 mL of bacteria agar solution after 24hours of incubation. After the incubation period, all of the petri plates were selected and counted containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). For the ten samples with 10^{-3} dilution showed too many to count (TMTC) but the ten samples with 10^{-5} dilution, the numbers of colonies were found to be 207, 183, 169, 149, 106, 89, 76, 70, 50 and TFTC respectively. Figure 5 (a) and (b) showed Bacterial count for control 10^{-3} dilution and 10^{-5} dilution and Figure 6(a), (b), (c), (d), (e) and (f) showed bacterial count for 10^{-5} dilution.

The calculation revealed (4140 x 10^6, 1830 x 10^6, 338 x 10^6, 149 x 10^6, 42.4 x 10^6, 29.6 x 10^6, 21.7 x 10^6, 17.5 x 106, 11.1 x 10^6 and TFTC) bacteria per milliliter respectively shown in Table 1. It was found that count decreased as increased the amount of sample.

Figure 7 showed amount of TiO_2(g) Vs number of bacteria/mL in 10^{-5} dilution. This showed that TiO_2 nanoparticles have good antibacterial activity against E-coli.

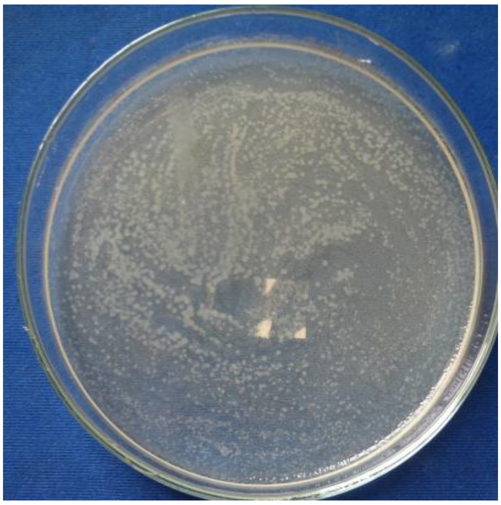


Figure 5(a) 10^{-3} dilution as control

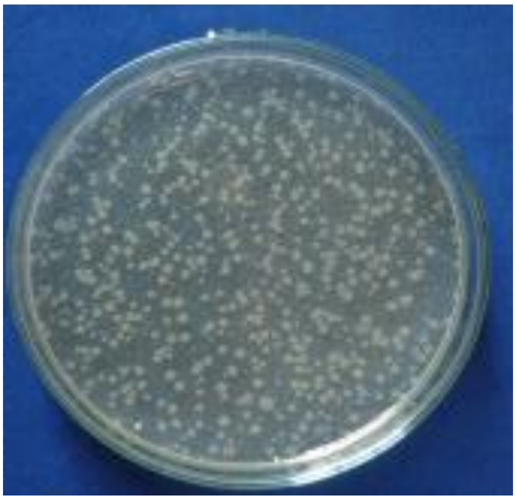


Figure 5(b) 10^{-5} dilution as control

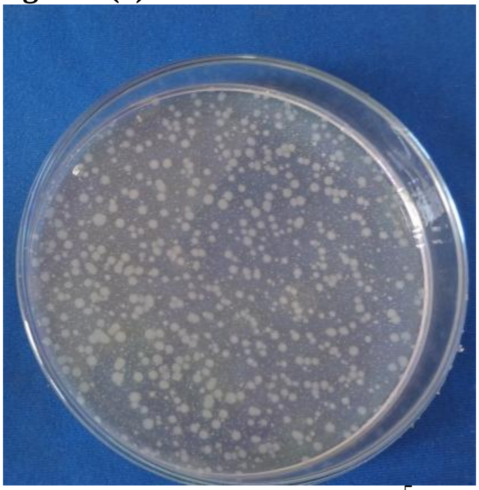


Figure 6(a) Bacterial count for 10^{-5} dilution with TiO_2-0.005g

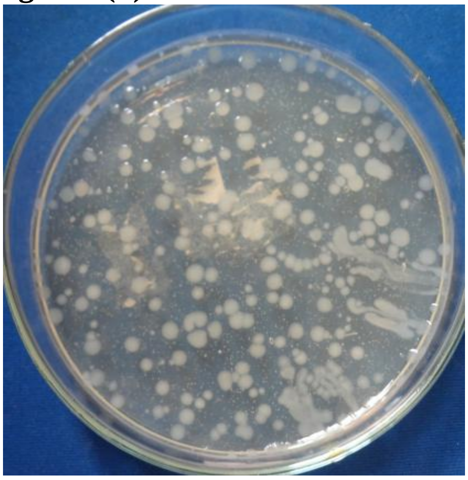


Figure 6(b) Bacterial count for 10^{-5} dilution with TiO_2- 0.01g

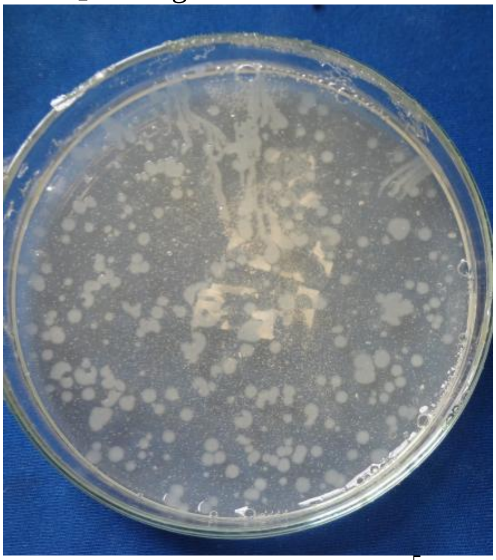


Figure 6(c) Bacterial count for 10^{-5} dilution with TiO_2-0.05g

Figure 6(d) Bacterial count for 10^{-5} dilution with TiO_2- 0.25g

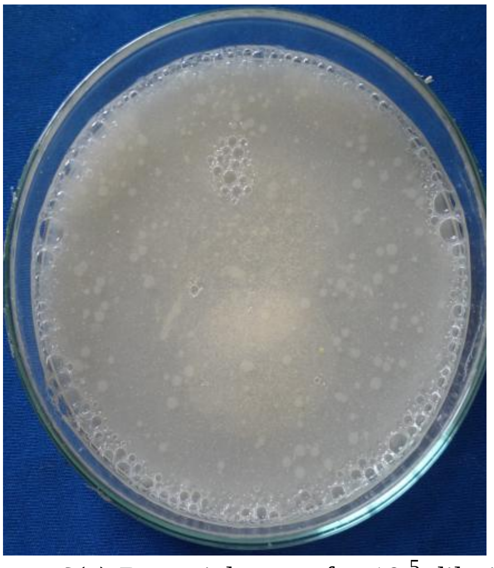


Figure 6(e) Bacterial count for 10^{-5} dilution with TiO_2- 0.35g

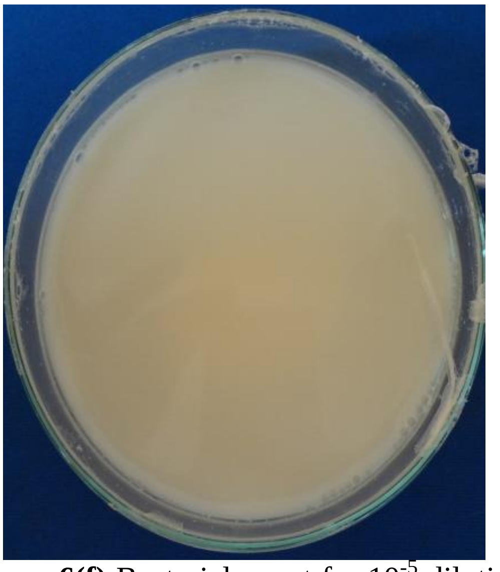


Figure 6(f) Bacterial count for 10^{-5} dilution with TiO_2- 0.5g

Table 1.1 Bacterial counts for 10^{-3} and 10^{-5} dilution

Amount of TiO_2 (g)	**Number of colonies in 10^{-3} dilution**	**Number of colonies in 10^{-5} dilution**	**Number of bacteria/ml in 10^{-5} dilution**
0.005	TMTC	207	4140×10^6
0.010	TMTC	183	1830×10^6
0.050	TMTC	169	338×10^6
0.100	TMTC	149	149×10^6
0.250	TMTC	106	42.4×10^6
0.300	TMTC	89	29.6×10^6
0.350	TMTC	76	21.7×10^6
0.400	TMTC	70	17.5×10^6
0.450	TMTC	50	11.1×10^6
0.500	TMTC	TFTC	TFTC
Control	**Number of colony in 10^{-3} dilution**	**Number of colony in 10^{-5} dilution**	
-	TMTC	224	

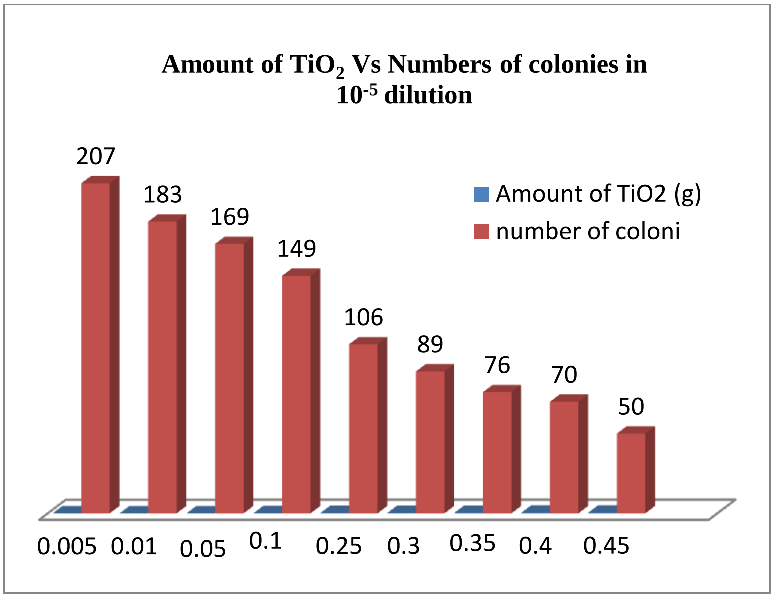


Figure 7 Amount of TiO_2 (g) Vs Numbers of colonies in 10^{-5} dilution

Conclusion

TiO_2 nanoparticles have been synthesized by using the sol-gel combustion hybrid method. Acetylene black was used as a fuel and to prevent increase in the size of the TiO_2 particles. It was found that the particle size of TiO_2 nanopowder scalcined at 500°C was between 20-30 nm. The sol-gel combustion hybrid method would also be useful for the large-scale production of nanosized ceramic materials. TiO_2 nanopowders synthesized at 500°C were applied to study the antimicrobial behavior by using serial dilution and pour plate culture method. For 10^{-3} dilution showed too many to count (TMTC) but the samples with 10^{-5} dilution showed that the amount of TiO_2 increases, the bacterial count decreases. It was found that TiO_2 nanoparticles have good antibacterial activity against E-coli. So TiO_2nanoparticles can be applied in water treatment.

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