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DIOXIDE TITANIUM NANOPARTICLES EFFICIENCY ON REMOVING WATER HETEROTROPHIC BACTERIA POPULATION

Elham Asrari *, Nader Cheraghpour

Department of Civil Engineering, Payame Noor University (PNU), Tehran, Iran·
*Corresponding author Email: e_ asrari@pnu.ac.ir

Abstract

Water source pollution is increasing in recent years and use of new methods of contaminants removal is required. Nanotechnology is a suitable technique for environmental applications. Titanium dioxide is one of most commonly used nanoparticles containing interesting properties for water and wastewater treatment. Purpose of this study was determination of nanoparticles efficiency for removing of heterotrophic bacteria.Spread plate method was used for enumeration of heterotrophic bacteria. Variables of pH, nanoparticle concentration, and contact time were investigated to remove heterotrophic bacteria. pH 7, contact time 90 minutes and heterotrophic population 305 were optimum condition for maximum removal efficiency. Investigation showed also suitability of titanium dioxide nanoparticles for real water samples. It showed that TiO_2 nanoparticles have good antibacterial features, so that they were able to remove heterotrophic bacteria.

Keywords: *heterotrophic bacteria population, TiO_2 nanoparticles, water pollution, spread plate method*

Introduction

During last decades, more and more pollutants are disseminated into aquatic environment which is deleterious for safety of drinking water. Although common water treatment processes are able to remove some of pollutants from water, the produced disinfection by products such as *trihalomethanes* (THMs) are risky for human health. Nowadays, various processes have been used for removal of bio-organic pollutants in water such as physical processes (adsorption, distillation and filtration), biological processes (activated sludge), chemical processes (flocculation and chlorination), electromagnetic radiation, photocatalytic processes and etc. Nanotechnology is a rapidly growing industry and constantly extending application of nano-enabled products reach from technical, medical and research sectors to a wide range of consumer products (Ahmadi, 2008). Metal oxide nanoparticles have drawn more attention due to their antimicrobial properties and their potential utilization in wastewater treatment, surface disinfection and active food packaging. The metal oxides are included TiO_2, Ag, ZnO, Al_2O_3 and Fe_2O_3. Among the metal oxides, Titanium dioxide is mostly investigated. Characteristics such as high stability, high temperature resistance, high surface area and low solubility make it

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as a good candidate for environmental applications (Boker and Boison, 2009). Nanoparticles damage bacterial cells through damage to protein and DNA and bacterial cell destruction (Heinlaan et al., 2008; Zhang et al., 2010a). Heterotrophic Plate Count (HPC) is a typical test for monitoring of microbiological water quality in distribution system. Heterotrophic bacteria use organic matter for growth and reproduction. There are reports regarding health importance and presence of damaged bacteria in drinking water and pathogenic characteristic of heterotrophic bacteria from treated and untreated drinking water. High HPC may be indication of some insufficient treatment processes especially disinfection and pollution events in distribution system. Also, HPC can be used as early sign of water quality deterioration. High HPC in water may, interfere with total coliform test by lactose-based media. Largely for this interference; a standard of 500 CFU/ml has been accepted as an operational norm (WHO, 2002a, b). Malakootiyan investigated effects of TiO_2, ZnO, and CuO nanoparticles on removal of gram positive and Gram negative bacteria from urban wastewater. Results showed direct association between nanoparticle concentration and bactericidal power. Also, no nanoparticles are more efficient than TiO_2 (Malakoutian and Toolabi, 2013). The performance of silver nanoparticles has been studied for disinfection drinking water in Hamedan city studied by Samadi (2013). Results indicated silver nanoparticles were able to remove water bacteriaconsiderably (Samadi, 2013). Saadat studied antibacterial activity of titanium dioxide on *Pseudomona saueroginosa* that results were indication of titanium nanoparticles high efficiency (Saadat, 2011). Toxicity and antibacterial properties of TiO_2 and CuO nanoparticles has been compared by Bina (2013). Findings showed that *Staphylococcus aureus* and *Escherichia coli* have respectively highest and lowest sensitivity against mentioned nanoparticles (Bina, 2013). Long investigated photocatalystic disinfection mechanism of TiO_2 nanoparticles on *Salmonella typhi* and *Listeria monocytogen* and found that TiO_2 nanoparticle has very high influence on pathogens which grow on meat products (Ling, 2013). Zhang studied photocatalystic disinfection of TiO_2 nanoparticles on *Pseudomonas* and *Macrococcus caseolyticus* and found that TiO_2 nanoparticle influence is dependent on primary bacterial pollution, TiO_2 concentration and UV light intensity (Zhang, 2010). Purpose of present research included determination of Titanium dioxide nanoparticles performance for removal of drinking water heterotrophic bacteria population. Results of this study can be used for water and wastewater treatment applications.

Materials and Methods

R_2A agar medium was made according to manufacturer instructions and was sterilized in temperature 121^{o}, 15 psi pressure for 15 minutes (Reasoner and Geldreich, 1985). Spread plate method was used for detection of heterotrophic bacteria. water samples was prepared synthetically in this way: Bacterial cells were collected from blood agar medium surface and were mixed into sterile phosphate buffered-salin for production of a sample containing turbidity of 0.5 McFarland

and bacterial concentration equal to 1.5×10^8. Sample absorbance was measured in 620 nm wavelength and absorbance value was adjusted in $0.8-1$ range. Five microbial concentrations were made by use of water sample dilution (APHA, 2005). Water sample containing 600 mg/L nanoparticle concentration and a given HPC, was examined in different pH in order to determine. Percentage of bacterial reduction calculated by this formula:

$$\text{Reduction Percentage} = [(N_0-N_1)/ N_0] \times 100 \qquad [1]$$

where:
N_0: Heterotrophic Bacteria Population in sample water;
N_1: Heterotrophic Bacteria Population after reduction by nanoparticle.

Optimum pH

The pH values 3, 5, 7, 9, 11 were chosen. Contact time was 30 minutes in a shaker with 200 rpm.Purity degree of TiO_2 nanoparticle was 99 percent and particle sizes ranged from 10 to 20 nanometers. Nanoparticle concentrations of 3, 6.9,15,20,25 and 50g/lit were selected.One milliliter of water and nanoparticle suspension was cultured on R_2 agar medium. Incubation was carried out and then based on reduction of colonies on medium, colony forming unit (CFU) per milliliter and removal percentage was estimated. A blank sample was also used during examinations (ISIRI, 2007).

Optimum concentration

Different concentrations of nanoparticle at optimum pH and constant heterotrophic population and contact time of 30 minutes were examined for determination of most efficient nanoparticle concentration.

Contact time

At optimum pH and nanoparticle concentration, different contact times (15, 30, 60, 90 and 120 min) were surveyed in order to suggest optimum contact time.

Different population

Optimum pH, nanoparticle concentration and contact time were obtained; removal percentage of different heterotrophic populations was estimated.

Results

Optimum pH determinations

Removal percentage of heterotrophic bacteria in different pH has been illustrated in table 1. Optimum pH has been found 7.

Optimum nanoparticle concentration

Efficiency of different concentrations of Tio_2 for removal of heterotrophic bacteria has been shown in table 2. Because of low difference between removals efficiency

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of 20, and 50 mg/L nanoparticle concentrations, optimum concentration has been suggested to be 20 g/L.

pH	Removal (%)
3	25.0
5	29.2
7	31.2
9	29.2
11	27.2

Table1
Relation between pH and Heterotrophic bacteria removal.
(Heterotrophic population of 240 CFU/ml, contact time 30 minutes and nanoparticles concentration 6g/L).

Nanoparticle concentrations (g/lit)	Removal (%)
3	14.6
6	31.2
9	45.8
15	70.8
20	75.0
25	75.0
50	77.1

Table2
Relation between nanoparticles concentration and bacteria removal (Heterotrophic population of 240 CFU/ml, contact time 30 minutes and pH 7).

Optimum contact time

Contact time was also varied in order to determine optimum contact time. Results have been exhibited in table 3. Lower contact time was selected as optimum contact time, because contact times of 90 and 120 minutes did not differ significantly in removal efficiency. Therefore, optimum particle concentration, pH and contact time are respectively 20 g/L, 7 and 90 minutes by end of third stage.

Contact times (min)	Removal (%)
15	60.4
30	75.0
60	89.6
90	97.9
120	97.2

Table 3
Relation between contact time and HPC removal.
(Heterotrophic population 240 CFU/ml, pH 7, nanoparticles concentration 20g/lit)

Optimum heterotrophic bacteria population

Some experiments were also carried out on different bacterial populations. Results showed in table 4. It was concluded that best removal efficiency was obtainable at pH 7, contact time 90 minutes and heterotrophic bacterial population 305 CFU/ml. Removal efficiency values of 95 percentages has been obtained.

Real samples

Also, five water samples from Fereydoon Shar city were taken and examined. Results have been shown in table5.

Table 4
Relation between Heterotrophic population and bacteria removal (Contact time 30 minutes, pH 7and nanoparticles concentration 20g/lit)

Heterotrophic population (CFU/ml)	Removal (%)
110	95.4
210	97.6
305	95.2
420	91.7
510	90.2

Table 5. *Heterotrophic bacteria removal in real samples*

Source	pH	HPC (CFU/ml)	Result (CFU/ml)	Removal (%)
Mirza Spring of Sardab	7.25	225	5	97.8
SarabSpring of Fereydoon Shahr	7.33	185	5	97.3
Spring of Chogha	7.18	1\80	5	97.2
Spring of Soroushjan	7.23	235	5	97.9
Spring of Daresib	7.51	305	10	96.7

Discussion and conclusions

American drinking water standard for heterotrophic bacteria has set guideline of 500CFU/ml. Because conventional water treatment processes are able to reach admissible range of heterotrophic bacteria, nanoparticles can also be used for removal of these bacteria.

Results indicate a direct correlation between nanoparticle concentration and efficiency of heterotrophic bacteria removal. Most removal was obtained at pH 7. Also finding showed that at pH 7 and nanoparticle concentration 20g/lit with 90 minutes contact time, removal value of heterotrophic bacteria as much as 95 percent is possible. So, Tio_2 nanoparticles were efficient for removal of heterotrophic bacteria. It likes to result of research of Saadatmand. He found that the high efficiency of E.Coli bacteria removal with concentration of 2% TiO_2 nano particles from real samples (Saadatmand and Yazdanshenas, 2012). Also result of Antimicrobial Efficacy of Zinc Oxide nanoparticles suspension against Gram Negative and Gram Positive Bacteria by Hossienzadeh showed that the best antibacterial remove with ZnO nanoparticles occurred on pH7.5 (Hoseinzadeh and Samarghandi 2012).

In recent years, use of nanoparticles containing new structure, advanced physical, chemical and biological properties have been increased considerably.

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Nanoparticles can cause toxicity effects for bacteria and inhibited their growth or kill them. A few studies are available regarding antibacterial power of nanoparticles. An investigation on ceramic powder (Zn nanoparticles) showed that *Staphylococcus aureus* has lower resistance than *Escherichia coli* (Kumar, 2014). Another study on TiO_2 toxicity in aquatic environments indicated that positive gram bacteria are more resistant against nanoparticles and this ability was attributed to spore formation ability and cellular structure (Rincon, 2004). Present study showed that TiO_2 nanoparticles have good antibacterial features, so that they are able to remove heterotrophic bacteria efficiently provided optimum conditions of pH, contact time and nanoparticle concentration are established.

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