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Antibacterial activity of bactericidal ceramisite filler immobilized quaternary phosphonium

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ABSTRACT

A novel insoluble bactericidal ceramisite filler was obtained by immobilizing 3-(trimethoxysily1)-1-propanamine onto the surface of ceramisite and reacting with bromo propyl triphenyl phosphonium bromide. The intermediate and product were characterized by Fourier transform infrared spectrometer, thermo gravimetric analyzer, and environment scanning electron microscopy. The antibacterial activity of bactericidal ceramisite against heterotrophic bacteria in the simulated industrial recirculation water was tested by plate count method. The result shows that when the prepared dosage of bactericide ceramisite filler is 0.2 g/mL, the sterilizing ratio can be up to 99.3% in 45 min of operation time. After regenerating the ceramisite filler for one time, its bactericidal ability could be recovered to above 97.2%. When the initial bacterial concentration of 7.48×10^6 (colony-forming units (CFU))/mL with 3.1 L water was treated by 90 g of bactericide ceramisite filler in 6 h, the heterotrophic bacteria concentration kept below 5×10^5 CFU/mL. After being regenerated, the reused bactericide ceramisite could still treat 2.5 L water as well.

Keywords: Ceramisite; Quaternary phosphonium salt; Sterilization; Regeneration

1. Introduction

Nowadays, studies focus on industrial water reusable rate. Interest had grown in the research about cationic fungicide, since the bactericidal effect of long-chain alkyl quaternary ammonium salt was found [1]. Various quaternary ammonium and quaternary phosphonium [2–6] have been tested and developed. Considering pro-environment and sustainable development, more and more studies focus on insoluble bactericidal material obtained by immobilization of bactericidal group onto the surface of carrier [7,8].

Different materials, including organic materials (such as chloromethylated polystyrene, cellulose fiber, etc) [9–12], and inorganic materials (such as silica gels, glass, etc) [13] were studied and applied. In recent years, application of clay ceramsite mainly composed of SiO_2 and Al_2O_3 has been tested for their advantages of good absorption property and high mechanical strength. Clay ceramsite and ceramic products like ceramic membranes [14–16] are mainly used in

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industry and daily life [17] as filter material, bio-carrier, and filler of cooling tower. The conventional method preparing bactericidal materials is impregnating the ceramsite with antibacterial agents (silver ions, cupric ions, and quaternary ammonium compounds) [18,19]. Seckin immobilized polyvinyl pyridinium onto the clay and discussed the antibacterial activity [20]. In addition, Ya grafted the papain onto modified ceramic particles with silane coupling agent [21].

In this paper, A novel insoluble bactericidal ceramisite filler was prepared by immobilizing 3-(trimethoxysily1)-1-propanamine onto the surface of ceramisite and reacting with bromo propyl triphenyl phosphonium bromide. This bactericidal ceramisite filler was proposed and tested for industrial water treatment.

2. Experimental

2.1. Materials and instruments

Different particle sizes of clay ceramisite filler were purchased from Yiyuan Industrial Corporation, Jiangxi province, China. High qualities of 1,3-dibromopropane, triphenylphosphine and all other chemicals and solvents were from Sinopharm Chemical Reagent Corporation, Shanghai, China and used without further purification. The water sample is taken from SINOPEC Yangzi Petrochemical Corporation, Nanjing, China.

To investigate whether bactericidal ceramisite held any organic on its surface, Fourier transform infrared spectroscopy (FT-IR), thermogravimetry (TG), and environmental scanning electron microscope (ESEM) were used. The chemical structure of compounds was characterized with FTIR (AVTAR-380, Thermo Nicolet company, USA). Surface images of materials were analyzed by ESEM (QUANTA200, Philips company, Holland). Results of weight loss were collected on TG (STA409, Netzsch company, Germany), and some easily oxidized materials were dried in ZK-82BB Vacuum Drying Oven (ZK-82BB, Shanghai instrument plant Corporation, China). Temperature control meter (XMTA-3001, XinLing Electric Co, Ltd, Zhejiang province, China) was used to regulate the temperature in our experiments.

2.2. Preparation of bactericidal ceramisite filler

Clay ceramisite with particle diameter of 1–2 mm (D90) was washed with distilled water, dried, and placed in a solution of 5 mL methanesulfonic acid in 95 mL of water, followed by stirring at 102℃ for 4 h [22].

About 20.2 g of 1,3-dibromopropane was put into a 250-mL three-neck flask and added the solution of 26.2 g of triphenylphosphine, and 100 mL of dimethylbenzene was added dropwise with effective stirring and protecting with nitrogen. After reacting for 24 h, the white solid was filtered, rinsed with diethyl ether, and dried in a vacuum oven at 60℃. Then, the resultant 3-(bromopropyl) triphenylphosphonium was purified by recrystallized with ethanol and dried in a vacuum oven at 60℃ for 2 h [23].

The activated clay ceramisite was rinsed with distilled water and dried in a vacuum oven at 60℃ for 5 h. In a 250-mL three-neck flask, 10 g of clay ceramisite and 50 mL of toluene were added and heated to 110℃. Fifty millilitres of toluene solution of KH 551 (of which 40 mL of toluene and 10 mL KH 551) was then added dropwise within 40 min combined with stirring and protecting with nitrogen and reacted for 24 h in the same conditions. When the reaction was over, the resultant N-alkylated ceramisite was rinsed with solvents of methanol, and dichloromethane several times and dried in a vacuum oven at 60℃ for 2 h.

The N-alkylated ceramisite and 3-(bromopropyl) triphenylphosphonium were placed in a solution of 30 mL of tetrahydrofuran and 30 mL of ethanol. One millilitre of triethylamine was added, followed by stirring and reacting under refluxing for 24 h. After washed with methanol and hot water and dried in a vacuum oven at 60℃ for 2 h, the bactericidal

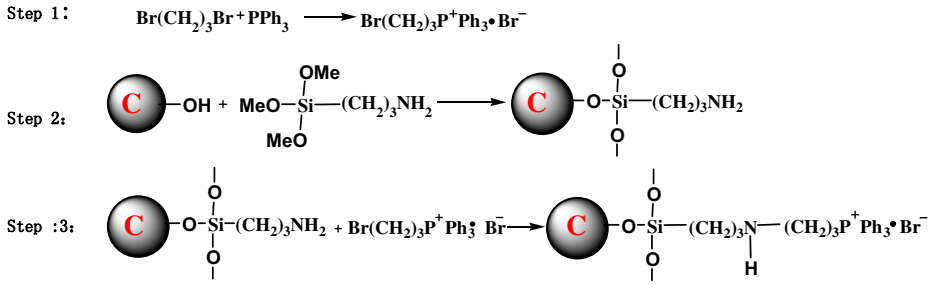

Fig. 1. Preparation of bactericidal ceramisite immobilized quaternary phosphonium salt (C : ceramisite).

ceramisite filler immobilized quaternary phosphonium was obtained. Experimental steps were shown in Fig. 1.

2.3. Determination of antibacterial activity

Since the vast majority of bacteria in cooling water of industrial recirculaion are heterotrophic bacteria [24], the antibacterial activity can be evaluated by testing the number of the heterotrophic bacteria in the water before and after sterilization. In this article, the test of microbicidal activity was conducted with the method of plate count for bacteria colonies.

Cooled nutrient broth after being boiled and the water sample containing heterotrophic bacteria were added into a bucket and cultured for a few days. Then, the bacteria were prepared. Saline solution, melted nutrient agar, and the articles used in this test were sterilized at 120°C, with high pressure steam for 15 min. In a conical flask, 10 g of bactericidal ceramisite filler and 50 mL of bacteria suspension were stirred for 1 h, and then, 1 mL of the treated bacteria suspension was gradient diluted. The diluted suspension was put in a culture dish, and the melted nutrient agar that was cooled to about 45°C was added. The mixture was shaken gently and then cultured at 29°C for 72 h. Then, the number of survival bacteria and sterilizing rate were calculated. Sterilizing rate was calculated as follows:

$$R(\%) = (N_1 - N_2)/N_1 \times 100 \quad (1)$$

where R represents sterilizing rate, while N_1 is the number of bacteria in initial water sample, N_2 is the number of bacteria in water sample after being sterilized.

Sixty gram of bactericidal ceramisite filler was placed in the column. The bacteria suspension in elevated tank passed through the bed from the bottom up, and the flow was adjusted by valve. After sterilizing for 6 h, samples were taken every 2 h, and the bactericidal rate was tested.

After being used for 6 h, the filler was dipped in the solution of 2% sodium hypochlorite for 30 min, while being irradiated by ultrasound and then used for the evaluation of bactericidal activities after being washed twice with ethanol and dried at a temperature below 80°C for 2 h. Thus, the bactericidal ceramics was regenerated and could be used in water sterilization repeatedly.

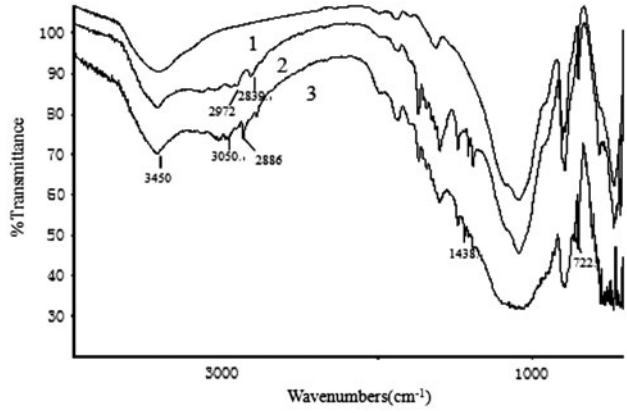

Fig. 2. FT-IR spectra of activated clay ceramisite (1), N-alkylated ceramisite (2) and clay ceramisite coated with quaternary phosphonium (3).

3. Results and discussion

3.1. Preparation of bactericidal ceramisite

The FT-IR spectra of activated clay ceramisite (1), N-alkylated ceramisite (2), and clay ceramisite coated with quaternary phosphonium (3) are shown in Fig. 2. At 3,450 cm^{-1} is the Si–OH bands, and the one at 1,100 cm^{-1} was –Si–O–Si–O– stretching vibrational bands. The C–H vibrational bands are at 2,972 cm^{-1} and 2,839 cm^{-1} (Fig. 2 (2)) and the N–H bending vibrational bands at 1,400–1,600 cm^{-1} (Fig. 2(2)) showed that KH 551 after being hydrolyzed was grafted onto the surface of activated ceramisite through the condensation reaction of Si–OH in KH 551 with Si–OH in SiO_2 on the surface of activated ceramisite. The FTIR spectra of the final product (Fig. 2(3)), which indicated the presence of peaks at 722 cm^{-1} for bending vibration of C–H, at 3,050 and 3,072 cm^{-1} for stretching vibration of unsaturated C–H, and at 1,438 cm^{-1} for vibrating peaks of aromatic rings, confirmed the formation of the quaternary phosphonium salts.

The TG curves for activated ceramisite and bactericidal ceramisite were shown in Fig. 3. Since the clay ceramisite was obtained by calcination at 1,100°C, curve for clay ceramisite was almost flat below 600°C. The curve for the activated ceramisite showed a obvious weight loss at 230°C. It was indicated that the bactericidal groups on the surface of the ceramisite were decomposed.

The surface images of the activated ceramisite (A_1 and A_2) and the bactericidal ceramisite (B_1 and B_2) were examined by ESEM in Fig. 4. The comparison between the scanning electron photomicrographs of activated ceramisite and bactericidal ceramisite showed great difference. Many dots fixtures appeared

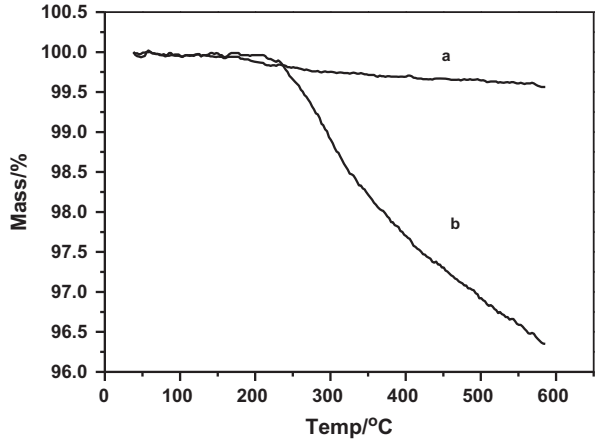

Fig. 3. The TG curves of activated ceramisite (a) and bactericidal ceramisite (b).

in B_1 and B_2 of Fig. 4. It could be deduced that the bactericide was immobilized to the surface of the ceramisite.

3.2. Antibacterial activity of the bactericidal ceramisite in water treatment

Twenty gram of bactericidal ceramisite (1 and 2 mm of particle diameter(D90)) was put into 100 mL of bacterial suspensions with initial bacteria concentration of 1.41×10^{10} (colony-forming units (CFU))/mL and used for the test of microbicidal activity. The effect of sterilizing time on antibacterial activity was explorated, and the results were shown in Fig. 5(a). From the plot (Fig. 5(a)), it was found that extending the sterilizing time within 45 min enhanced the sterilizing rate, 45 min later and the sterilizing rate reduced slightly above 99% as the sterilizing time increased. It indicated that the antibacterial activity did not monotonously increased as the sterilizing time increased, due to the limited absorption capacity of bactericidal ceramisite (1–2 mm particle diameter) and bacteria contamination from the external environment.

The experiments were divided three group: (a) adding amounts of 0.5 g/mL and concentration of 1.41×10^{10} CFU/mL, (b) sterilizing time of 45 min and concentration of 1.41×10^{10} CFU/mL, (c) sterilizing time of 45 min, adding amounts of 0.5 g/mL and concentration of 1.41×10^{10} CFU/mL.

Increasing the adding amounts of filler from 15 to 90 min increased contact between bacteria and bactericidal groups and enhanced the antibacterial activity. So the sterilizing rate increased as the adding amounts of bactericidal ceramisite increased, and it would over 99% when the adding amounts more than 0.2 g/mL, as shown in Fig. 5(b).

Twenty grams of filler with different particle diameter (D90) was taken and used for the test of microbicidal activity. As shown in Fig. 5(c), bactericidal

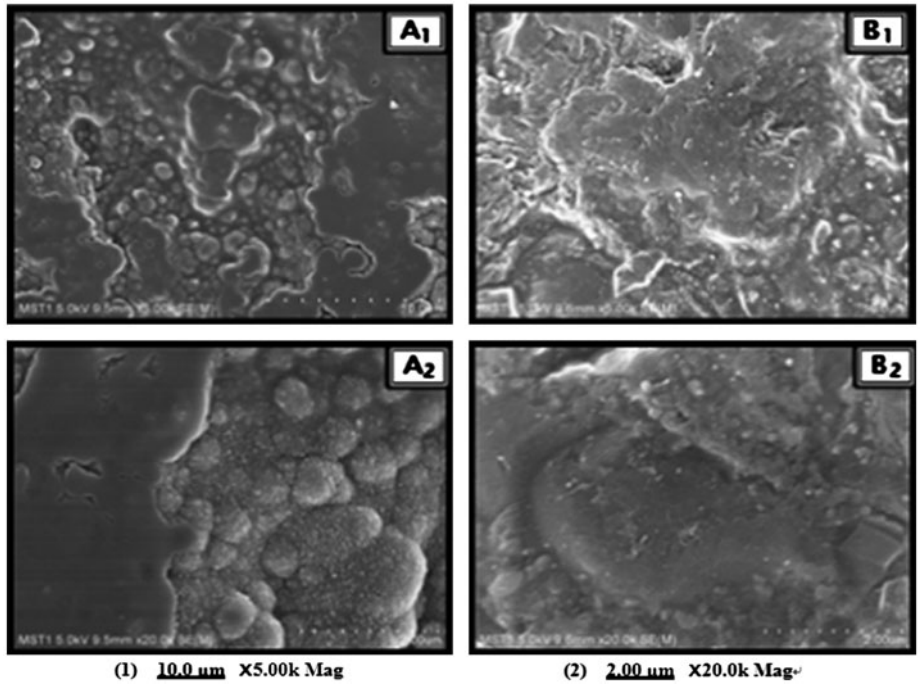

Fig. 4. ESEM of the activated ceramisite (A_1, A_2) and the bactericidal ceramisite (B_1, B_2) (A_1, B_1: 10.0 μm × 5.00 k Mag, A_2, B_2: 2.00 μm × 20.0 k Mag).

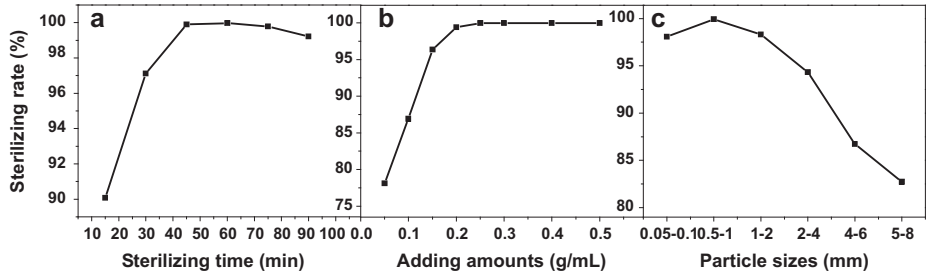

Fig. 5. Effect of sterilizing time (a), adding amounts (b) and particle sizes (c) on bactericidal rate.

ceramisite with 0.5–1 mm of particle diameter was found to be most suitable for sterilization when tested by single factor experiment. The micro–pores of the ceramisite with particle diameter less than 0.05–0.1 mm did not allow the microorganism in. While the low specific surface area of the ceramisite with particle diameter more than 2–4 mm reduced contact between bacteria and bactericidal groups. Therefore, particle diameter of the ceramisite above 2–4 mm or below 0.05–0.1 mm promoted weak adsorbability to micro-organism, and further do harmful to sterilization. It could be deduced the structure of the micropore and high adsorptive capability of the clay ceramisite promoted sterilization.

The capability of antibacterial ceramisite was shown in Fig. 6. The concentration of bacteria suspension before and after sterilization and the sterilizing rate were calculated based on the number of bacterial colonies in culture dishes counted. The picture showed that bacterial colonies in dishes B were significantly less than that in dishes A (Fig. 6).

The simulated industrial circulating water containing heterotrophic bacteria (7.48×10^6 CFU/mL) was cultured and used for the dynamic simulating test. When 3.1 L of the simulated industrial circulating water was passed through 90 g of the bactericidal ceramisite filler (the packed bed height was 15 cm) with the velocity at 8.62 mL/min, the bacterial concentration of water fell below 5×10^5 CFU/mL and met the national industrial water criteria in which bacterial concentration of industrial water is below 5×10^5 CFU/mL). (Table 1)

Twenty grams of regenerated bactericidal ceramisite (1–2 mm of particle diameter) was put into 100 mL of bacterial suspensions with sterilizing time of 45 min, and antibacterial activity was tested. The regenerated bactericidal ceramisite after being used was recycled and regenerated again. These steps were repeated several times. The results shown in Table 2 were that after the bactericidal ceramisite being regenerated five times, bactericidal rate could up to above 85.20% and the bacteria concentration fell below 5×10^5 CFU/mL.

As shown in Table 3 and 90 g of bactericidal ceramisite filler after being regenerated one time could treat 2.5 L of simulated industrial circulating water

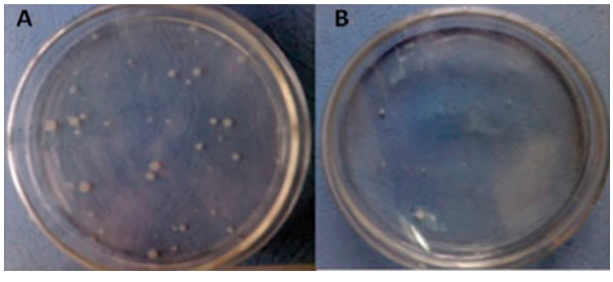

Fig. 6. Bacterial colonies of before sterilization (A, diluted 10^6 times) and after sterilization (B, diluted 10^5 times).

Table 1
Studied on sterilization of fluidized bed in water treatment

Sterilizing time h	Bacteria concentration CFU/mL	Water treatment amounts L
0	7.48×10^6	0
2	6.73×10^3	0.9
4	3.92×10^4	2.0
6	1.41×10^5	3.1

Table 2
Bactericidal activity of the regenerated bactericidal ceramisite

Regeneration times	Initial bacteria concentration: CFU/mL	Bacteria concentration: CFU/mL	Sterilizing rate: %
1	1.33×10^7	3.72×10^5	97.20
2	1.44×10^6	4.54×10^4	96.85
3	1.15×10^6	6.05×10^4	94.74
4	2.12×10^6	2.08×10^5	90.25
5	1.40×10^6	2.07×10^5	85.20

Table 3
Sterilization of fluidized bed with regenerated bactericidal ceramisite in water treatment

Sterilizing time, h	Bacteria concentration, CFU/mL	Water treatment amounts, L
0	8.77×10^6	0
2	5.29×10^3	0.9
4	1.31×10^4	1.7
6	1.16×10^5	2.5

and kept the bacteria concentration fell below 5×10^5 CFU/mL. The results indicated that the bactericidal ceramisite proposed in this paper was regenerable.

4. Conclusion

The antibacterial ceramisite filler was loaded with bromo propyl triphenyl phosphonium bromide and showed significant activity against microbes. The bactericidal ceramisite filler was renewable reuse to avoid secondary pollution, and it showed excellent antibacterial property in simulated industrial circulating water treatment as well. This study provides the foundation for the application of environmental bactericidal materials technology. And the next step of work is improving on carrier to increase efficiency through modification with nano-silica.

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