Cerium-modified cryptomelane: an antibacterial activity against *Pseudomonas aeruginosa*

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Abstract. K-OMS-2 (non-modified sample) and Ce-modified OMS-2 with different Ce-loading amounts were prepared by the refluxed method. The cryptomelane structure and elemental composition of synthesized samples were characterized with X-ray diffraction (XRD) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. ICP-MS analysis revealed ~0.06 – 0.11 of K/Mn molar ratio and the Ce-loading amounts increased from 0 to ~11.27 wt.% under increasing Ce-precursor concentration. Antibacterial activity against *Pseudomonas aeruginosa* was evaluated by the agar well diffusion method. The antibacterial ability against *Pseudomonas aeruginosa* was not recorded over K-OMS-2 (Ce0; 4.5 wt.% of K; 0 wt.% of Ce) and Ce3 (0.89 wt.% of K; 11.27 wt.% of Ce) samples while Ce2 sample (1.89 wt.% of K; 8.7 wt.% of Ce) showed a comparable antibacterial activity against *Pseudomonas aeruginosa* with ~12 mm of inhibition-zone diameter. This suggested the potentiality of using metal-modified cryptomelane in acceleration of antimicrobial ability.

1. Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*) was reported a leading cause of nosocomial infections, ranking second among the gram-negative pathogens reported to the National Nosocomial Infections Surveillance (NNIS) System from January 1990 through March 1996 [1]. *P. aeruginosa* is gram-negative bacteria with straight or slightly curved rods but not helical and is about 0.5 to 1.0 µm in width and 1.5 – 5.0 µm in length. Its motility is by one or several polar flagella; rarely nonmotile and *P. aeruginosa* easily grows on common agar like nutrient agar, blood agar or broth and absolute aerobic. The suitable growth temperature is in the range of 5 - 42°C at pH 4.5 - 9.0 and the best condition is 37°C at pH 7.2 - 7.5 [2]. *P. aeruginosa* from the outside environment could enter the body through open wounds, especially from burns. At the site of penetration, they often cause purulent inflammation and green pus. It was reported that when the bodies’ resistance was reduced, *P. aeruginosa* could invade and caused inflammation of the internal organs (bones, urinary tract, middle ear, bronchi, meninges, ...) or caused systemic diseases (bloodstream infection, endocarditis, ...). Among them, bloodstream infection was reported as an infection with very high mortality. Weinstein *et al.* [3] analyzed NNIS reports from 1986 to 2003 to determine the epidemiology of gram-negative bacilli in intensive care units (ICUs). The results showed that the most frequent types of hospital-acquired infection were pneumonia, surgical site infection, urinary tract infection and bloodstream infection. The types of nosocomial infections mentioned above caused mainly by gram-negative bacilli. Specifically, in 1986, gram-negative bacilli were accounted for 60.1% of the cause of
bloodstream infection, 74.1% of pneumonia episodes, 53.4% of surgical site infections and 78.5% of urinary tract infection. In which, the *P. aeruginosa* was noticed 4.8%, 9.6%, 4.7% and 9.3% of them, respectively. By 2003, the rates tended to decrease for gram-negative bacilli in causing hospital infections, however, the data caused by *P. aeruginosa* increased significantly, they accounted for 3.4%, 18.1%, 9.5%, 16.3% over bloodstream infection, pneumonia episodes, surgical site infection and urinary tract infection, respectively. To prevent infections from open wounds or from human respiratory system, improving airbone quality is one of important ideas, along with applying solid-materials for antimicrobial treatment.

Octahedral Molecular Sieves (OMS) including synthetic todorokite (OMS-1) and cryptomelane (OMS-2) were generally built from the basic structural units of manganese oxide octahedra (MnO₆). OMS-1 utilized three MnO₆ octahedra on each side to form a 3×3 square tunnel with a pore size of about 6.9 Å. Whereas, OMS-2 had a 2×2 square tunnel with a pore size of about 4.6 Å [4]. Cryptomelane structure (K-OMS-2, K⁺ cations and H₂O dominated in the tunnels constituted by octahedral unit of MnO₆) was reported as a good and convenient catalyst for oxidation [5]. Additionally, cryptomelane modified with metal like Co-doped OMS-2 was reported as a wastewater tolerant catalyst for CO oxidation [6], or Fe-modified cryptomelane enhanced significant O₂ oxidation in the dry condition [7]. However, a few reports discussed the antibacterial ability of oxide manganese materials. Amjad Khan [8] recently synthesized nanowire cryptomelane type Fe₃Mn₁₋ₓO₄ (for x = 0.00, 0.04, 0.08, 0.12) and reported the effect of Fe amount on inhibitory zones. The anti-bacteria activities of FeₓMn₁₋ₓO₂ against S. aureus and against extensively Drug-Resistant Salmonella Typhi were only recorded while x was in the range of 0.08 – 0.12. This indicated the vital role of metal-doping in the enhancement of anti-bacteria performance over cryptomelane structure. This work presented the antimicrobial ability of modified cryptomelane with various cerium loading against *Pseudomonas aeruginosa*.

2. Materials and methods
All materials from Shanghai Chemical Reagent, Inc. all had been tested and analyzed, can be used without further purification after receiving the product. KMnO₄ solution was added into MnSO₄ solution while concentrated HNO₃ was used to adjust pH < 2. The mixture was then refluxed at 100°C for 24 hours. After filtering, washing and drying at 120°C overnight, K-OMS-2 (Ce0) material was collected. Ce-OMS-2 was prepared by co-precipitation of Ce⁴⁺ (Ce(SO₄)₂·4H₂O) cation in the solution of Mn⁷⁺ and Mn³⁺ cations such that the molar concentration of Ce⁴⁺ is 0.05 (Ce1); 0.10 (Ce2) and 0.15 M (Ce3) in the final mixture. X-ray powder diffraction (XRD) was performed using a Brucker AXS D8 diffractometer over the 20 range of 10-90° and the scan rate was 1°/min. Besides, the elemental compositions of the prepared powders were also determined with inductively coupled plasma mass spectrometry (ICP-MS, Optima™ 8000 ICP-OES).

The antibacterial activity of cerium-modified cryptomelane was tested by the agar well-diffusing method. The Mueller Hinton Broth agar plates were ready prepared, the bacteria were spread on the agar surface and holes (5 mm in diameter) were made. Sequentially, 100 µL of cerium-modified cryptomelane dispersed in Dimethyl sulfoxide ((CH₃)₂SO – DMSO) was added to each hole. After 20-24 hours for the bacteria growth at room temperature, we recorded the inhibitor zone diameter with a concentration of cerium from 0 mg/mL to 10 mg/mL.

3. Results and discussion
X-ray diffraction patterns of the synthesized materials were shown in Figure 1. All samples had characteristic peaks of standard cryptomelane crystal at 2θ of 12.70°; 17.92°; 28.65°; 37.40°; 41.41°; 49.61° and 59.95° (JCPDS 29-1020) [7]. Low intensive diffraction pattern of cryptomelane crystal was observed over Ce2 sample. Beside that two unknown diffraction peaks at 2-theta at 21.43° and 29.88° presented a disfigurement of cryptomelane crystalline over the obtained Ce2 sample.
Figure 1. XRD patterns of Ce0: K-OMS-2 and Ce2: Ce(0.10M)-OMS-2 and Ce3: Ce(0.15M)-OMS-2.

Table 1 showed the elemental compositions of the synthesized samples conducted from ICP-MS analysis. The analysis results showed the presence of K and Mn in all surveyed samples and that of Ce over Ce-OMS-2 samples. The Ce content increased from 0 to 11.27 wt.% by increasing the concentration of Ce(IV) cations from 0 to 0.15 M the precursors. The wt.% of Mn, K elements and the atomic ratio of K/Mn decreased with increase of cerium loading.

| Sample | Element | wt.% | Molar ratio of K/Mn | Molar ratio of Ce/Mn |
|--------|---------|------|----------------------|---------------------|
| Ce0    | K       | 4.54 | -                    | -                   |
|        | Mn      | 59.59| 0.11                 | -                   |
|        | Ce      | -    | -                    | -                   |
| Ce2    | K       | 1.89 | 0.06                 | 0.08                |
|        | Mn      | 43.11| -                    | -                   |
|        | Ce      | 8.27 | -                    | -                   |
| Ce3    | K       | 0.89 | 0.03                 | 0.11                |
|        | Mn      | 41.32| -                    | -                   |
|        | Ce      | 11.27| -                    | -                   |
Figure 2. Mueller Hinton Broth agar plates of the antibacterial activity against *Pseudomonas* over Ce0 – Ce3 samples at different catalyst concentrations in DMSO solvent ([X]). The signals of 1 – 5 on the plates were presented for 6, 7, 8, 9 and 10 mg/mL of [X], respectively.

Figure 2 showed the antibacterial activity of the as-prepared samples against *P. aeruginosa* by using the agar well-diffusing method. Ce0 showed inactivity against *P. aeruginosa* with a large range of [X] (6-10 mg/mL). A similar less activation in antibacterial performance against *P. aeruginosa* over Ce3 (11.27 wt.% of Ce loading) was also recorded. Whereas, Ce1 and Ce2 samples presented enlargement of inhibitor zones with an increase of [X] from 6-10 mg/mL. Ce1 material gave a clear inhibitor diameter of ~11 mm at 10 mg/mL of [X] while Ce2 sample (8.27 wt.% of Ce loading) exhibited a remarkable inhibitor zone of ~12 mm of diameter in the [X] range of 6-7 mg/mL. Increasing the [X] of Ce2 up to 10 mg/mL did not expand the inhibitor zone (~10-12 mm) confirming that the Ce2 had good antibacterial activity against *P. aeruginosa*, among four synthesized cryptomelane samples.
Besides, the filtrate from the mixture of Ce2-dispersed DMSO ([X] = 7 mg/mL) was collected and tested the anti-bacteria ability that was shown in Figure 3. The filtrate clearly gave ~5mm of the inhibitor zone diameter presenting a deactivation in antibacterial treatment against \textit{P. aeruginosa} over the filtrate. This confirmed that the anti-bacteria performance against \textit{P. aeruginosa} from the suspension of Ce-OMS-2 in DMSO was only activated over solid Ce-doped cryptomelane material. In comparison with non-doped cryptomelane sample (Ce0; containing 4.5 wt.% of K and 0 wt.% of Ce), Ce3 sample (0.89 wt.% of K and 11.27 wt.% of Ce) showed the similar evidences of deactivation in antibacterial treatment against \textit{P. aeruginosa} proving that the exchangeable cerium cations properly resided in the tunnels of cryptomelane structure might not take part in the inhibition of \textit{P. aeruginosa} growth. A noticeable inhibitor zone (~12 mm of diameter) Ce2 sample (1.89 wt.% of K and 8.27 wt.% of Ce) should be, therefore, assigned from numerous reactive oxygen species on the surface of cerium-modified cryptomelane materials; in agreement to reports from Khan \textit{et al.} \cite{8} and Li \textit{et al.} \cite{9}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{The antibacterial activity against \textit{Pseudomonas} over the filtrate from the mixture of Ce2-dispersed DMSO. The signals of 1 – 5 on the plates were presented for 6, 7, 8, 9 and 10 mg/mL of [X], respectively.}
\end{figure}

\textbf{4. Conclusions}
Cerium-modified cryptomelane with various cerium loading amounts (from 0 to 11.27 wt.%) were prepared by refluxed method. 0 and 11.27 wt.% of cerium (Ce0 and Ce3 samples) showed a deactivation in antibacterial activity against \textit{P. aeruginosa} recorded from the agar well-diffusing method. 8.27 wt.% of Ce-modified cryptomelane presented remarkable antibacterial performance against \textit{P. aeruginosa} with the encircled diameter of inhibitory zone of ~12 mm providing a feasibility of applying Ce-OMS-2 solid materials in antimicrobial activity.

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