Preparation, Characterization of Formic Acid Modified Clinoptilolite and Bacteria Adsorption

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Abstract: Acid modified clinoptilolite are usually used as adsorbents for NH_3, heavy metals and organic contaminants. Acid-modified clinoptilolite was prepared using the method of ion exchange reaction at 80°C for 4 h and used for bacteria and toxins adsorption which are significant for providing mechanism for the adsorption of microorganisms *in vitro*. XRD, SEM, AFM, BET and micropore volume were used to characterize parent clinoptilolite and acid modified clinoptilolite. Cation Exchange Capacity (CEC) was used to determine changes of adsorption ability before and after modification. The acid-modified clinoptilolite showed the strong adsorptive in the rang of 20-25 mg g^{-1} when the initial bacterial suspension concentration was 1×10^8 Colony Forming Units (CFU)/mL. The adsorption capacities of acid-modified clinoptilolite for all bacterial suspension increased with an increase of initial bacterial suspension concentration. An increase of adsorption capability of acid-modified clinoptilolite was attributed to the BET and micropore volume. These results indicated the competency of acid-modified clinoptilolite adsorbent for bacteria adsorption.

Key words: Clinoptilolite, formic acid, modified, adsorption, bacteria, China

INTRODUCTION

The incidence of salmonellas outbreaks in poultry farming industry caused by Salmonella enteritidis which is one of the most important pathogens has increased markedly in recent years. Whereas, products of poultry origin such as fowl and eggs is one of the most important food-borne, theirs containing even a small amount of *Salmonella enteritidis* can severely affect the human life due to their toxicity. Therefore, simple and economical methods for the removal of this pathogen in fowl and eggs have been studied and developed including physical, chemical, physic-chemical and biological Methods.

Adsorption is one of the most popular and effective physic-chemical method for *Salmonella enteritidis* removal. Special attention has been paid to various kinds of competent adsorbents (such as clinoptilolite, montmorillonite and other aluminosilicates and their modified forms, etc.) (Tong *et al.*, 2005; Xia *et al.*, 2007). One of the most widely used adsorbent is nonmetallic minerals due to their high adsorption capabilities for various kinds of bacteria and toxin. Natural clinoptilolite is a type of mineral with low cost and abundance; possess a monoclinic crystal structure symmetry which can act as

host materials with high sorption properties. Although, nonmetallic minerals themselves possess the high adsorption capabilities, the modification of their structure can successfully improve their capabilities (Ozdemir *et al.*, 2004).

In the present study, natural clinoptilolite was modified by formic acid to improve the adsorption capability. Adsorption was tested with *Salmonella enteritidis*. The physical properties of natural clinoptilolite and the modified clinoptilolite were investigated.

MATERIALS AND METHODS

The clinoptilolite sample used in this study was collected from the Center of China Geological Survey (Nanjing). After collection, the sample was ground and sieved through a 100 mesh sieve. All chemicals used in the experiments were purchased in analytical purity.

Preparation of acid-modified clinoptilolite: The modified clinoptilolite were prepared using formic acid treatment. Clinoptilolite was first calcinated at around 400°C for 4 h in muffle oven. After that and then certain amounts of clinoptilolite was added into formic acid and the

dispersions were stirred slightly and blended in order to ensure the well dispersion of formic acid at a certain temperature for a certain time. The formic acid solution/mineral mass ratio was 10. Then, the suspension was repeatedly washed with deionized water (dH₂O). The sample was dispersed in water, decanted from the top, water was added, stirred and allowed to settle. The lower sediments were collected and dried in an air oven at around 65°C for 2 h and then ground and sieved through a 100 mesh sieve with an agate mortar.

XRD analysis: The X-Ray Diffraction (XRD) graphs were obtained using ASAP 2400 diffractometer with Cu Kα radiation (λ = 0.154 nm; 40 kV, 30 mA) at room temperature. The diffractograms were scanned from 10-80°C in the 2θ range in 0.02° steps and scanning rate was 5° per min. The samples were studied as powders.

BET surface area and micropore volume measurement: The specific surface areas of samples were measured by multipoint BET Method on an ASAP 2400 surface analyser. Samples were outgassed at 133.322 K for 5 h at about 10⁻⁴ tore. The micropore volume can be determined from the BET and t-Plot Method (Rouquerol *et al.*, 1999). The specific surface areas (SBET) were estimated by the Brunauere-Emmette-Teller (BET) equation (Sing *et al.*, 1985) and the micropore volume and limited micropore volume were calculated by the adsorption data (Table 1).

Scanning electron microscopic measurement: A scanning electron microscope (HitachiS-3000N) was used for the electronic scanning of the samples. For Scanning Electron Microscopic (SEM) analyses, the samples were sprayed on mica foil and sputter coated with gold layers.

Atomic force microscopy measurement: The surface structure of clinoptilolite and its morphological changes was obtained by using Atomic Force Microscope (AFM). AFM (SPM-9500J3) was used to take the AFM images.

Microorganism adsorption: Adsorption of *Salmonella enteritidi*s K533 (obtained from National Institute for the Control of Pharmaceutical and Biological Products, China) was carried out in a batch process under ambient conditions by varying adsorptive concentration (10, 15, 20, 25, 30, 35, 40, 50 mg mL⁻¹). Trypticate Soya Broth (TSB) was used as a growing medium for the microorganism. The bacteria suspension was adjusted to 1×10⁸ Colony Forming Units (CFU)/mL. A sample was mixed in 10 mL centrifuge tube and was shaken for a period of time at room temperature. The mixture was allowed to settle, centrifuged at 4000 rpm for 15 min alkali treatment. The concentrations of bacteria protein were measured in a UV-visible spectrophotometer at wavelength of 550 nm. The OD values were converted into concentration of *Salmonella enteritidis* cells (CFU per mL). The amounts of *Salmonella enteritidis* K533 adsorbed on nonmetallic minerals was calculated by subtracting the final concentration from the initial concentration of bacteria solutions. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

X-Ray Diffraction (XRD): XRD pattern of natural and acid-modified clinoptilolite sample was shown in Fig. 1a and b. In the present study, d value of two ray peak at 3.20817 and 3.34574 were observed. The formic acid treated natural clinoptilolite in general the diffraction peak positions move to the high angle region which was attributed to the basal spacing of modified clinoptilolite. With the cation exchange by formic acid, constriction of the clinoptilolite framework occurred. The reason for the constriction can be attributed to deglutination by the cationic surfactant (Xi *et al.*, 2004, 2005; Churchman, 1990). On the other hand, the acid-modified clinoptilolite did not show much change on XRD patterns compared to natural clinoptilolite. It shows that internal composition of clinoptilolite crystal has no much change after acid treatment.

Table 1: BET surface area and micropore volume of natural clinoptilolite and acid-modified clinoptilolite

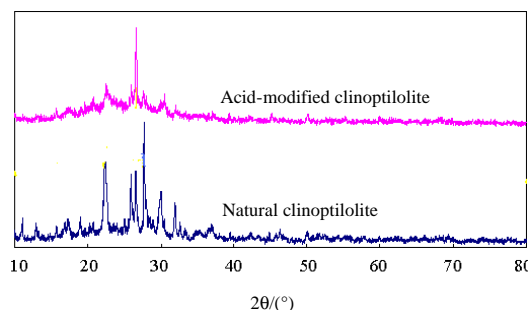| Parameters	Samples	
	Natural clinoptilolite	Acid-modified clinoptilolite
Single point surface area at p/p° (m² g⁻¹)	19.893300	24.995400
BET surface area (m² g⁻¹)	19.485200	24.993100
Longmuir surface area (m² g⁻¹)	29.396400	33.926800
Microspore surface area: (m² g⁻¹)	19.062996	26.820009
micropore volume (cm³ g⁻¹)	0.002344	0.005049
Limiting micropore volume (cm³ g⁻¹)	0.008468	0.012301

Fig. 1: XRD patterns of natural clinoptilolite and acid-modified clinoptilolite

BET surface area and micropore volume: The BET specific surface areas for natural clinoptilolite and acid-modified clinoptilolite were 19.4852 and 24.9931 $m^2\ g^{-1}$, respectively as measured by the BET Method (Brunauer *et al.*, 1938). The micropore volume was 0.002344 and 0.005049 $cm^3\ g^{-1}$. These results showed that the BET specific surface area and micropores volume of clinoptillite increased when modified with formic acid. The increase of BET specific surface area and micropore volume may be attributed to the internal frame research and the micropores of clinoptilolite was dealuminzated and cleared in the formic acid-modified clinoptilolite so more micropores were opened leading to the increase in the micropore surface areas. It was in accordance with the conclusions drawn in literatures (Seki and Yurdakoc, 2005; Burns *et al.*, 2006). This conclusion was confirmed and supported by the SEM and AFM analysis results in this study.

SEM and AFM: Figure 2a and b showed different morphologies of the natural clinoptilolite and acid-modified clinoptilolite. SEM images of natural clinoptilolite show that mineral particle has mass of matrix with number of small holes and rhyolite on its surface. The surface of acid-modified clinoptilolite has more cracks and crevices and the surface becomes very loose and porous flock was

obviously stretched, light transmission increased significantly compared to the natural clinoptilolite so that the specific surface area of acid-modified clinoptilolite greatly increased. Moreover, after natural clinoptilolite was activated by the formic acid has more strong ion exchange capacity to remove the part cations of the clinoptilolite and then adsorption capacity is greatly enhanced (Loizidou *et al.*, 1992; Gu *et al.*, 2010).

Although, Scanning Electron Microscopy (SEM) to examine the surface structure of minerals have been established but it is limited that it use only in a vacuum system (Ono *et al.*, 2001). Atomic Force Microscopy (AFM) has attracted considerable attention as a potential tool that does not has such limitations (Hansma *et al.*, 1988). The surface structures of catalysts have been studied. Figure 3a and b shows the AFM images of the clinoptilolite before and after formic acid loading. It is obvious that the surface morphology has changed greatly after the natural clinoptilolite loaded with formic acid. The surface of natural clinoptilolite is relatively complete, smooth (Fig. 3a). And after loading, the roughness of the surface is remarkably increased and several grooves and structural reorganization can be observed (Fig. 3b).
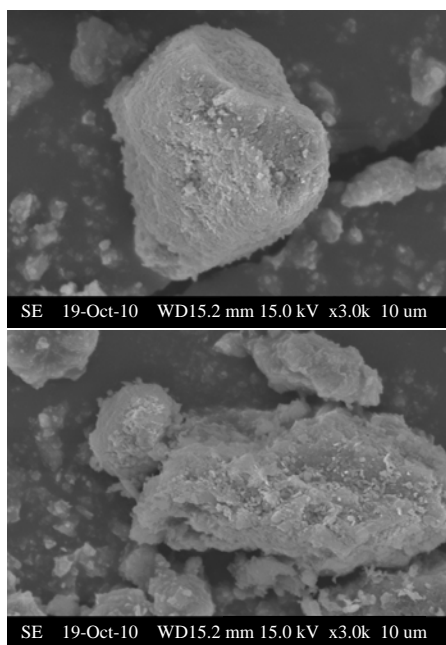

Fig. 2: a) The images of SEM on the surfaces changes for natural clinoptilolite x3.0 k; b) The images of SEM on the surfaces changes for acid-modified clinoptilolite x3.0 k
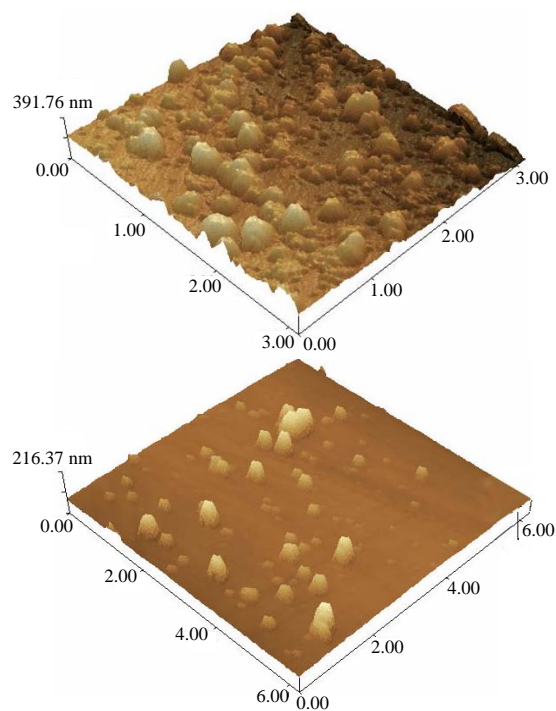

Fig. 3: a) The images of AFM on the surfaces changes for natural clinoptilolite. The scan size is 391.76 nm; b) The images of AFM on the surfaces changes for acid-modified clinoptilolite. The scan size is 216.37 nm
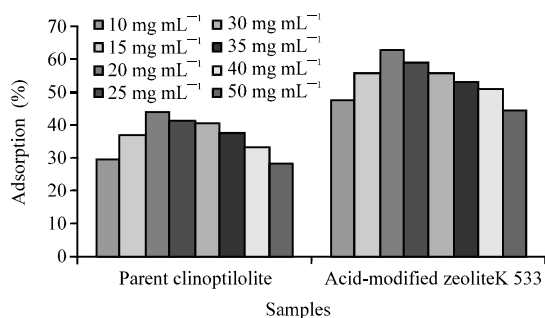
Fig. 4: Percent of *Salmonella enteritains* K533 adsorbed the parent clinoptilolite and acid-modified clinoptilolite in different concentration of nonmetallic minerals particle

As known, acid have strong corrosion capacity. Therefore, researchers suggest that the crevices are affected by formic acid and high temperatures. It means that the corrosion of formic acid mostly takes place at the clinoptilolite surface but not in the pores (Wu *et al.*, 1998).

Microorganism adsorption: The amounts of *Salmonella enteritidis* K533 adsorbed on natural clinoptilolite and acid-modified clinoptilolite with nonmetallic minerals concentration are shown in Fig. 4. The acid-modified clinoptilolite showed the larger adsorption capacity for *Salmonella enteritidis* K533 than those of natural clinoptilolite. The result may be attributed to both its relatively big BET surface area and micropore volume which are very important parameters to illuminate the adsorption capacity of the adsorption agent (Fomina and Gadd, 2002; Marshall, 1969).

Adsorption of *Salmonella enteritidis* K533 increased strongly for natural clinoptilolite and acid-modified clinoptilolite reached an adsorption of about 20 mg mL^{-1}. This increase resulted from the functional groups increase and thereby more surface sites are available at the clinoptilolite surfaces (Scholl and Harvey, 1992; Omoike and Chorover, 2004). The larger pores of acid-modified clinoptilolite facilitated the penetration of the *Salmonella enteritidis* K533 into the interlayer space (micropore volume were 0.005049 cm^3 g^{-1}, Khurana *et al.*, 2005). Thus, the highest adsorption was observed for acid-modified clinoptilolite, indicating the potential of acid-modified clinoptilolite adsorbent for removal of bacteria.

Figure 4 also shows the discrepancy when the different amounts of parent clinoptilolite and acid-modified clinoptilolite were between 30-50 mg mL^{-1} nonmetallic minerals in this study. Because with increasing solid content, the competition among adsorption sites at acid-modified clinoptilolite and natural clinoptilolite surfaces increases and results the concentration of *Salmonella enteritidis* K533 on solid phase decreases. At quite low solid content, the competition is weak whereas the competition becomes strong with increasing solid content (Xu *et al.*, 2008).

CONCLUSION

Modified clinoptilolite were prepared by melt blending with formic acid and clinoptilolite, produced activated clinoptilolite having well developed porosities. XRD analysis was used to evaluate the degree of dispersion of formic acid in the clinoptilolite. The XRD results show that the formic acid modified clinoptilolite may be dealuminzated and constricted the clinoptilolite framework.

Comparing with natural clinoptilolite, acid-modified clinoptilolite increased the BET surface areas and micropore volume as well as adsorption capacities. Moreover, the images of SEM and AFM showed that natural clinoptilolite was modified with formic acid, its surface structure and morphological have much changes.

ACKNOWLEDGEMENTS

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