Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 23(2011) 1808–1815
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Adsorptive property of Cu^{2+}-loaded montmorillonite clays for *Escherichia coli* K_{88} *in vitro*

Tong Guo[1,2,**,*], Shoujun Cao[1,**,*], Rui Su[3], Zhiqiang Li[4], Ping Hu[1], Zirong Xu[2]

1. Beijing Vocational College of Agriculture, Beijing 102442, China. E-mail: sxgtong@163.com
2. College of Animal Science£the Key Laboratory of Molecular Animal Nutrition Ministry of Education Zhejiang University, Hangzhou 310029, China
3. College of Chemical Engineer and Environment, North University of China, Taiyuan 030051, China
4. College Animal Science and Technology, Institute of Grassland Science, China Agricultural University, Beijing 100093, China

Received 21 December 2010; revised 20 May 2011; accepted 25 May 2011

Abstract

The adsorption properties of Cu^{2+}-loaded montmorillonite clays (MMT-Cu) for *Escherichia coli* K_{88} as a function of time, bacteria concentrations, pH, ionic strength and temperature were investigated. The results showed that the bacteria adsorption onto MMT-Cu surface reached equilibrium after 90 min. The percentages of *E. coli* K_{88} adsorbed onto the surfaces of MMT-Cu and montmorillonite clays (MMT) at equilibrium were 88.9% and 56.5%, respectively. Scanning electron microscopy revealed that a lot of *E. coli* K_{88} adhered to the surface of MMT-Cu. The zeta potential of MMT-Cu was relatively high as compared to that of MMT. The adsorptive ability of MMT-Cu for *E. coli* K_{88} was higher than that of MMT ($P < 0.05$). Moreover, pH, ionic strength and temperature produced a strong influence on the extent of *E. coli* K_{88} adsorption to surface of MMT-Cu and MMT. The mechanism of adsorption of *E. coli* onto MMT-Cu may involve electrostatic attraction and physiochemical properties of bacterial cell walls and minerals surfaces.

Key words: Cu^{2+}-loaded montmorillonite clays; adsorption; *Escherichia coli* K_{88}

DOI: 10.1016/S1001-0742(10)60651-1

Citation: Guo T, Cao S J, Su R, Li Z Q, Hu P, Xu Z R, 2011. Adsorptive property of Cu^{2+}-loaded montmorillonite clays for *Escherichia coli* K_{88} *in vitro*. Journal of Environmental Sciences, 23(11): 1808–1815

Introduction

Bacteria are ubiquitous and abundant in natural near-surface aqueous environment (Barns and Nierzwicki-Bauer, 1997). *Escherichia coli* K_{88} is one of the most virulent pathogens. It has been well recognized that diarrheal disease caused by enterotoxigenic *E. coli* that bear the K88+ fimbrial antigen is by far the most common enteric colibacillosis (Yokoyama et al., 1992; Alexander, 1994; Hampson, 1994). A large number of pathogen are present in treated wastewater and can contaminate rivers and seawater. The ingestion of organism via contaminated water may lead to enterotoxigenic *E. coli*. As potable water is scarce, especially in countries of serious lack of water, the cost in obtaining usable water and disposing of wastewater represents a substantial economic expenditure. Therefore, it is very important and necessary to develop novel methods or materials to dispose contaminated water.

Two of the most common chemical disinfectant, ozone and chlorine, have been extensively applied for the treatment of pathogenic bacteria in drinking water and wastewater. Unfortunately, they were not always effective for the removal of waterborne bacteria (Dychdala, 1991; Bitton, 1994).

It is well known that clay minerals possess many unique properties such as a ability to "swell" in aqueous environments, high surface area, and high cationic exchange capacity (Laszlo, 1998; Vaccari, 1998) which lead them ideal adsorbents for multiple applications. Among the many kinds of clay minerals, montmorillonite (MMT) has been recognized as an effective adsorbent due to its large specific surface area (SSA) and high cationic exchange capacity (CEC) (Rong et al., 2008; Chen et al., 2009). Montmorillonite are a subset of aluminosilicate clays having a 2:1 layer structure (Borchardt, 1989). Within the layers of these clays, substitution of other metal ions for silicon or aluminum can occur resulting in a net negative charge on the surface of the clay platelet. This negative charge is offset by hydrated cations, such as Na^+ and Ca^{2+}. The surface chemistry of montmorillonite clays can be altered by exchanging the predominant interlaminar cations with organic materials and inorganic cations that are positively charged.

In recent years, the use of inorganic antimicrobial agents has attracted interest for the control of microbes (Okouchi

* Corresponding author. E-mail: sxgtong@163.com (Tong Guo); CSJ004@sina.com (Shoujun Cao); ** the authors contribute equally to this article.

et al., 1995; Wilczynski, 2000). The key advantages of inorganic antimicrobial agents are improved safety and stability as compared to organic antimicrobial agents. It is well known that cupric ion has bactericidal activity (Domek et al., 1984; Guo and Xu, 2004).

The objective of the present study is to evaluate the adsorptive properties of cupric ion modified MMT (MMT-Cu) for *E. coli* K_{88} *in vitro*. The mechanisms of bacteria adsorption onto MMT-Cu were also evaluated.

1 Materials and methods

1.1 Materials

The MMT sample was collected from Chifeng, China. The sample was purified as previously described (Ma et al., 2004). The structural formula determined from chemical analysis was $[Na_{0.158}K_{0.082}Ca_{0.256}Mg_{0.063}][Mg_{0.376}Fe^{2+}_{0.014}Fe^{3+}_{0.136}Al_{1.474}][Si_{3.87}Al_{0.13}]O_{10}(OH)_2{\cdot}nH_2O$.

Methods of MMT-Cu preparation were described by Ma et al. (2005). Briefly, 10 g MMT was ground and added into 100 mL deionized water and agitated at 300 r/min for 24 hr. The suspension was centrifuged and obtained clay was rehydrated with 100 mL water to which Cu^{2+} ($CuSO_4{\cdot}5H_2O$, analytical grade) was added at a ratio of 1.5 times the cation exchange capacity (CEC) of the MMT. The resulting mixture was then agitated at 300 r/min for 24 hr. The obtained MMT-Cu was separated by centrifugation and washed under agitation (200 r/min) with 100 mL deionized water. The washed material was dried at 80°C, ground and passed through a 400-mesh sieve.

1.2 Characterization of MMT and MMT-Cu

X-ray powder diffraction patterns were obtained with the XD98 automatic X-ray diffraction instrument (XD-98, Philips X light pipe, Netherland) (Cu $K\alpha$, 40 kV, 20 mA, scan speed of 4°/min). The CEC was determined by leaching with 1 mol/L ammonium acetate at pH 7, washing with 90% ethanol, displacing the NH_4^+ with 1 mol/L NaCl and measuring the amount displaced with an autoanalyzer (Theng et al., 1997). The specific surface area was measured on NOVA ver. 3.70n by N_2 adsorption at 77 K and application of BET equation (Stadler and Schindler, 1993). The supernatant was diluted properly and then copper concentration in MMT-Cu was measured by atomic absorption spectrophotometer (Table 1).

1.3 Microorganisms and growth conditions

E. coli ATCC K_{88} was obtained from the American Type Culture Collection (ATCC) and initially cultured in 3 mL of Muller-Hinton Broth (Difco, USA) for 24 hr at 37°C with agitation (200 r/min), then transferred to 1 L of broth and grown for another 8 hr. At the end of the exponential phase, bacteria were harvested by centrifugation (4000 r/min, 10 min), rinsed in distilled deionized water twice, and soaked in 0.01 mol/L $NaNO_3$ for 1 hr. After another rinse in distilled deionized water, the cells were then soaked in 0.001 mol/L EDTA for 1 hr. The cells were rinsed with distilled deionized water three times and soaked in distilled deionized water overnight. Finally, the cells were rinsed twice with 0.01 mol/L $NaNO_3$ (the electrolyte used in the experiments). The wet weight (g) of the bacteria cells was then measured after centrifugation at 7500 r/min for 60 min. All experiments were conducted in nutrient deficient conditions where bacteria are metabolically inactive. Nutrients were not added to maintain constant biomass throughout the experiment.

Table 1 Characterization of the MMT and MMT-Cu

	CEC (mmol/100 g)	BET surface area (m^2/g)	*d* (001) (nm)	Cu (%)
MMT	105.4	760.7	1.544	
MMT-Cu*	129.6	653.2	1.571	3.9

CEC: cation exchange capacity. * Ma et al., 2005.

1.4 Measurement of zeta potential

A known wet weight of bacteria was suspended in electrolyte solution of 0.01 mol/L $NaNO_3$ to reach a final concentration of 1×10^6 CFU/mL by SP-2000UV spectrophotometer (Shanghai, China) at a wavelength of 600 nm. Zeta potentials of *E. coli* K_{88}, MMT and MMT-Cu at different pH and ionic strengths were measured with a Zetasizer 3000HS automated instrument (Malvern Instruments, England) at 25°C. The suspensions of 1×10^6 CFU/mL *E. coli* K_{88}, 0.1 mg/mL MMT and 0.1 mg/mL MMT-Cu in 0.01 mol/L $NaNO_3$ at various ionic strengths and pH value (2–10) which performed with 0.01 mol/L $NaNO_3$ background electrolyte concentration were dispersed overnight. The suspension of 5 mL mentioned above was then introduced in the electrophoretic cell. The value of zeta potential is directly computed from a video analysis of the images obtained under the applied voltage, 80 V. Each assay was performed with ten replicated samples and the values obtained were averages to give the final data with standard deviations.

1.5 Bacteria-mineral adsorption experiments

In this assay, a known wet weight of bacteria was suspended in electrolyte solution of 0.01 mol/L $NaNO_3$ adjusted to 1×10^7 CFU/mL by SP-2000 UV spectrophotometer (Shanghai, China) at a wavelength of 600 nm. Binding was performed with 100 mg of clay (MMT or MMT-Cu) and 1 mL of the bacterial suspension under agitation (160 r/min) at 37°C for 90 min. The samples were fixed with 2.5% glutaraldehyde (Sigma) for 2 hr at room temperature, washed three times with distilled deionized water and postfixed with 1% (*W*/*V*) osmium tetroxide (Sigma) for 60 min at room temperature. Samples were dehydrated twice through a graded series of ethanol (50%, 70%, 80%, 90%, 95% and 100%) for 10 min at each concentration. Final fixed samples were brought to absolute iso-amyl acetate. The samples were dried with a HCP-2 critical point dryer (Hitachi, Japan) using liquid carbon dioxide as a carrier. They were then mounted on specimen stubs and coated with gold in an argon atmosphere using a IB-5 Hummer sputter coating system (Giko, Japan). Samples were viewed and photographed on

a XL30-ESEM (Philips, Netherlands) scanning electron microscope (SEM) at 10 keV. For the assays described below, all bacteria were cultured with gentle rotary mixing to ensure contact with the clay minerals and to prevent sedimentation.

Batch experiments were conducted to measure *E. coli* K_{88} adsorption onto either MMT or MMT-Cu as a function of time, concentration on bacteria, pH, ionic strength and temperature at 25°C. A known wet weight of bacteria was suspended in a 10 mL electrolyte solution of 0.01 mol/L $NaNO_3$, and placed in contact with a 100 mg MMT or MMT-Cu. The pH of electrolyte solution in each batch experiment was adjusted to the desired value (2–11) using 0.1 mol/L NaOH or HNO_3. The ionic strength experiments were performed by suspending the bacteria in ddH_2O instead of electrolytes. Varying amounts of 1.0 mol/L $NaNO_3$ electrolyte were added, and then diluted to a total volume of 10 mL. In each type of experiment, the bacteria-mineral-electrolyte mixture was allowed to equilibrate for 90 min (for varying times for the kinetic experiments) at 25°C (for varying temperature for the temperature experiments).

Separation of the unattached bacteria from the fraction containing mineral power and attached bacteria was accomplished by injecting 3 mL of a sucrose solution (60% by wt.) into the bottom of the mineral-bacteria suspension (Nathan et al., 2000). Because the sucrose is denser than the bacteria suspended in solution, but is less dense than the mineral grains (including those with bacteria attached), the sucrose creates a density gradient. The mineral powder with any adsorbed bacteria sinks to the bottom of the test tube, and the unadsorbed bacteria and aqueous solution float on top of the sucrose layer. After the sucrose separation, the unattached bacterial fraction was extracted with a pipette. The unattached bacterial were re-collected as mentioned above. The wet weight of the unadsorbed bacteria was measured after centrifugation at 7500 r/min for 60 min. The adsorption percentage was determined by subtracting the final unattached bacterial weight from the initial wet weight. Control experiments were performed without the mineral present to determine whether adsorption to the test tubes occurs, and to quantify the efficiency of the separation technique. The control experiments demonstrated that 7% of the bacteria were lost during the separation procedure. Hence, 7% was subtracted from each experiment to account for separation efficiency. Each assay was performed with five replicated samples. The adsorption percentage (R) on the surface of the tested adsorbent was calculated using the following Eq. (1):

$$R = \frac{(W_1 - W_2)}{W_1} \times 100\% - 7\% \quad (1)$$

where, W_1 (g) is the wet weight of initial bacteria including the adsorbed and unadsorbed bacteria, and W_2 (g) is the wet weight of the unadsorbed bacteria.

1.6 Statistical analyses

Data were statistically analyzed using SAS software (version 6, SAS Institute Inc., USA). Values were presented as means ± SD. One-way analysis of variance (ANOVA) and Tuckey test was used in adsorptive assays to compare the MMT with MMT-Cu values. Probability levels of less than 0.05 were considered statistically significant ($P < 0.05$).

2 Results

2.1 Scanning electron microscopy of bacteria-mineral interactions

The interaction between bacteria and clay particles (MMT or MMT-Cu) are visualized by SEM. The electron micrographs of MMT and MMT-Cu after treatment with the bacterial suspension are shown in Fig. 1. In a view of the circled area, multiple aggregates of rod-shaped *E. coli* K_{88} can be seen adhering to the surface of the MMT-Cu (Fig. 1b). A few of *E. coli* K_{88} were seen adhering to the surface of the MMT (Fig. 1a). In the present study, it is showed that both MMT and MMT-Cu had a strong ability to adsorb *E. coli* K_{88} in aqueous solution.

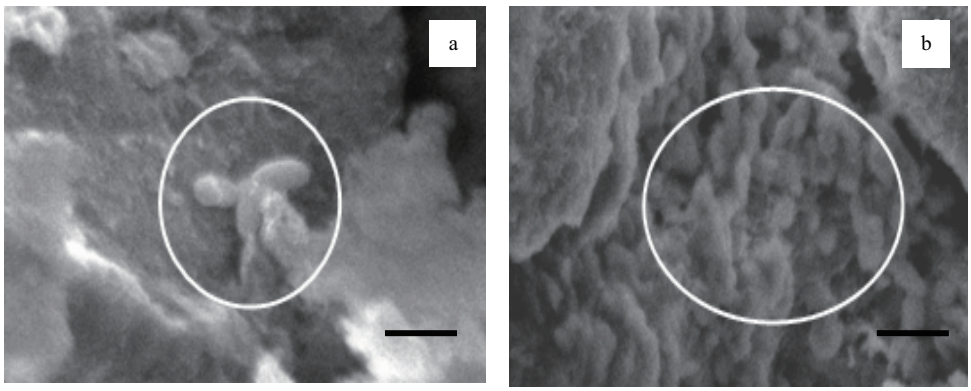

Fig. 1 Scanning electron micrographs of MMT (a) and MMT-Cu (b) after treatment with *E. coli* K_{88} suspension. In a view of the circled area, numerous rod-shaped bacteria can be seen adsorbed on the surfaces of MMT-Cu as compared with MMT. (a) and (b) electron micrographs were taken at × 6,000 magnification.

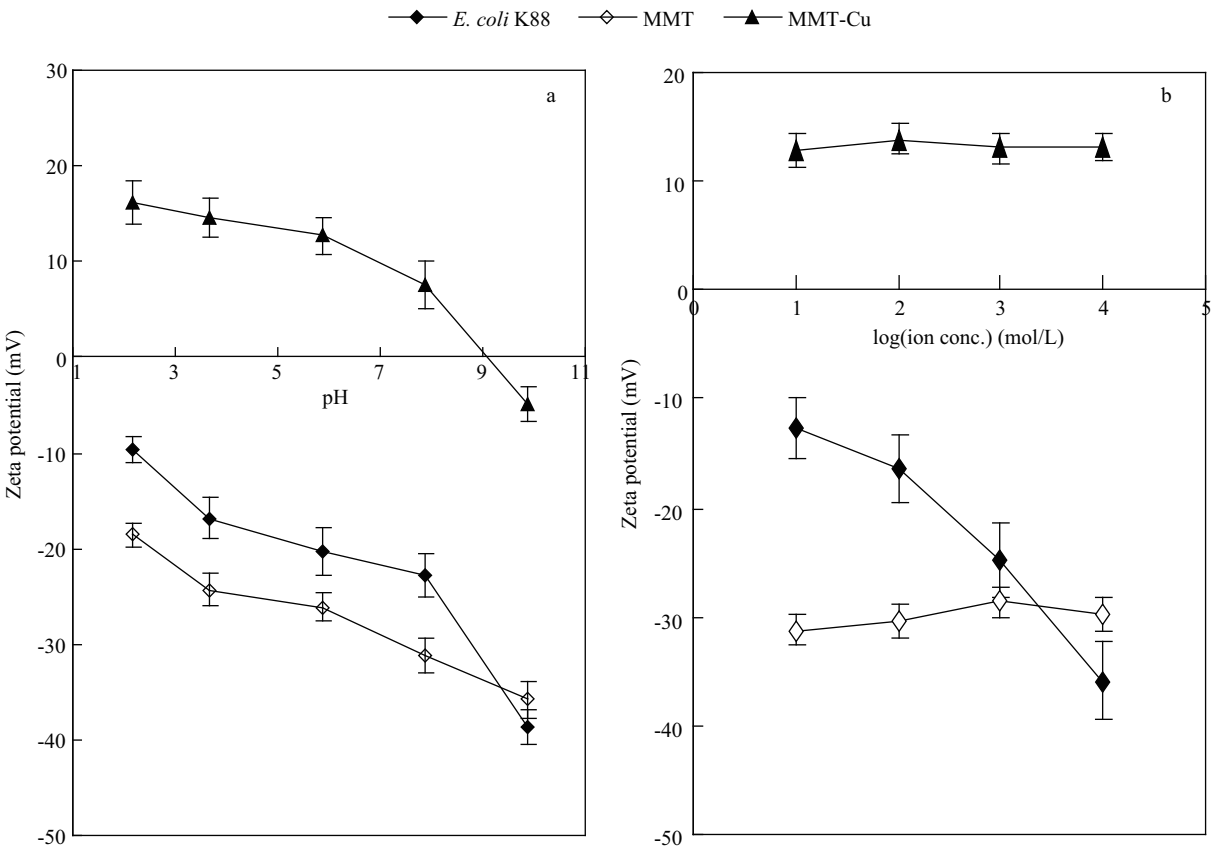

Fig. 2 Zeta potential of *E. coli* K_{88}, MMT and MMT-Cu as a function of pH (0.01 mol/L $NaNO_3$) (a) and as a function of of ionic strength (pH 6.5) (b). Error bars represent standard deviations.

2.2 Zeta potential of *E. coli* K_{88}, MMT and MMT-Cu

The zeta potentials of *E. coli* K_{88}, MMT and MMT-Cu as a function of pH in 0.01 mol/L $NaNO_3$ medium and as a function of the ionic strengths are given in Fig. 2a, b, respectively. Figure 2a shows that the zeta potentials of *E. coli* K_{88}, MMT and MMT-Cu decreased with increasing pH value. Both *E. coli* K_{88} and MMT had a negative zeta potential at the tested pH range, while MMT-Cu had a positive zeta potential at pH values less than 9. Figure 2b exhibits that ionic strength did not influence significantly the zeta potentials of MMT and MMT-Cu, whereas *E. coli* K_{88} zeta potential decreased with increasing ionic strength.

2.3 Factor effects

The effects of time, bacteria concentration, pH, ionic strength, and temperature on the adsorption of *E. coli* K_{88} onto MMT OR MMT-Cu are shown in Fig. 3.

The time effect on the interaction between *E. coli* K_{88} and the tested minerals are shown in Fig. 3a. It indicated that *E. coli* K_{88} adsorption onto the surface of either MMT-Cu or MMT reached equilibrium after 90 min. The percentage of *E. coli* K_{88} adsorbed on MMT-Cu was 88.9%, while only about 56.5% of bacterial cells were adsorbed by MMT after 90 mins. Data showed that the ability of MMT-Cu to adsorb *E. coli* K_{88} was significantly ($P < 0.05$) high as compared to MMT.

Figure 3b illustrates that the extent of bacterial adsorption onto MMT or MMT-Cu did not change significantly with increasing bacteria concentrations. Figure 3b also shows that *E. coli* K_{88} displayed a much higher ($P < 0.05$) adsorption to MMT-Cu than to MMT in the entire tested bacteria concentrations range.

Figure 3c shows the pH-dependent adsorption behavior in the *E. coli* K_{88}-MMT and *E. coli* K_{88}-MMT-Cu system. It clearly shows that the pH values in medium produced an influence on bacterial adsorption to surface of MMT or MMT-Cu. The adsorptive percentages of *E coli* K_{88} onto each adsorbent in the acid medium were higher than in alkaline medium. However, the percentage of the bacteria adsorbed onto the clay surface was relatively low at pH 2. Figure 3c also shows that *E. coli* K_{88} displayed a much higher ($P < 0.05$) adsorption to MMT-Cu than to MMT in the entire tested pH range.

Figure 3d describes the ionic strength-dependent adsorption behavior in the *E. coli* K_{88}-MMT and *E. coli* K_{88}-MMT-Cu system. It was clearly observed that the percentage of *E. coli* K_{88} adsorption onto MMT-Cu or MMT decrease significantly with increasing $NaNO_3$ concentration in the medium, range from 88.2% to 62.5% and from 56.4% to 42.2%, respectively. Moreover, Fig. 3d also shows that *E. coli* K_{88} displayed a much lower ($P < 0.05$) adsorption to MMT than to MMT-Cu in the entire tested ionic strength range.

The percentages of adsorbed on each adsorbent were measured at the standard temperature for refrigeration (4°C), room temperature (25°C), body temperature (37°C), and 45°C. The effect of temperature on the adsorption of bacterial cells on MMT or MMT-Cu is illustrated in Fig. 3e. *E. coli* K_{88} adsorption increased from 4 to

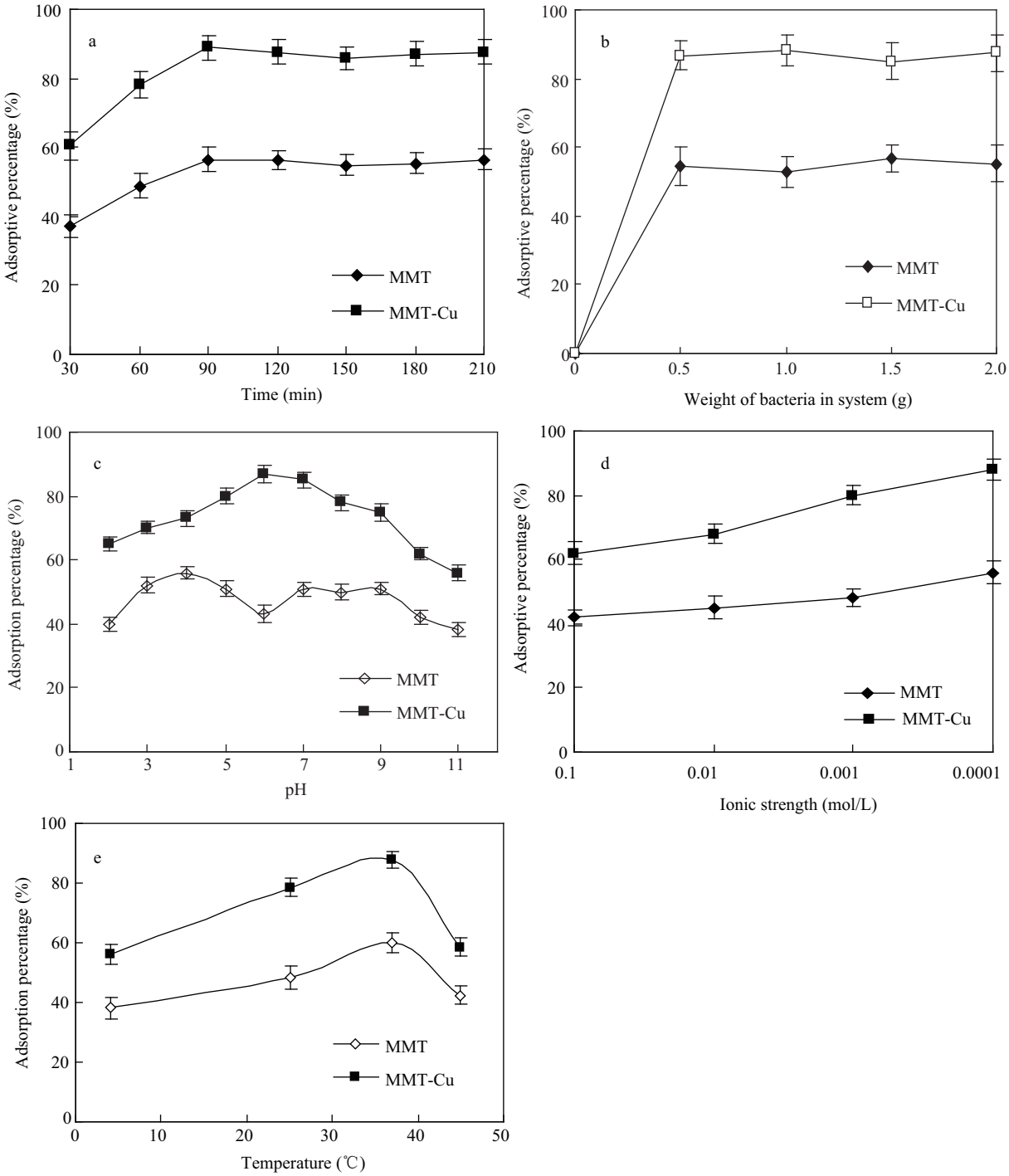

Fig. 3 *E. coli* K_{88} adsorption onto the surfaces of MMT and MMT-Cu as a function of time (a), bacteria concentration (b), pH (c), ionic strength (d) and temperature (e). Conditions: mineral powder10 g/L; bacteria 2 g/L (wet weight); pH (6.5); $NaNO_3$ electrolyte solution 0.01 mol/L. Among these factors, one is varied as a function factor. The error bars represent standard deviations.

37°C and then decreased. The maximum amount of adsorption for MMT or MMT-Cu was observed at 37°C. In addition, significantly lower ($P < 0.05$) bacterial adsorption was found at 4 and 45°C. At 4°C, the percentage of bacterial adsorption onto MMT or MMT-Cu was 38.3% and 56.2%, respectively. At 37°C, the percentage of bacterial adsorption was 60.8% and 87.8%, respectively, and at 45°C, was 42.5% and 68.9%, respectively. From these data can be seen that the degree of adsorption of bacteria adsorbed onto surface of MMT-Cu was higher ($P < 0.05$) than that onto MMT surface.

3 Discussion

3.1 Bacteria-mineral interactions

SEM images revealed that *E. coli* K_{88} were adherent to the surface of the MMT or MMT-Cu and adsorption equilibrium reached after 90 min. It is well known that both cation exchange capacity (CEC) and specific surface area (SSA) are important parameters to illuminate the adsorption capacity of the clay. Generally, the exchanged interlayer cations increase with increasing CEC value of the clay. A large SSA reflects that the clay has a high

surface tension and van der Waals force. Data showed the SSA of MMT-Cu was smaller than that of MMT, this signifies that both the surface tension and van der Waals force of MMT-Cu are low compared to those of MMT. But the MMT, loaded with Cu^{2+}, had a strong ability to adsorb *E. coli* K_{88}. This suggests that interaction between *E. coli* K_{88} and the adsorbents may be controlled not only by surface tension and van der Waals force, but also by other factors such as electrostatic attraction.

It has been accepted that cell surface characteristics play significant roles in bacterial adherent to clays (Rutter and Vincent, 1984). Under physiologic conditions, bacterial cell walls are negatively charged due to functional groups such as carboxyls, phosphate and hydroxyl present in lipoproteins at the surface (Stotzky, 1989). In our present studies, the zeta potential of *E. coli* K_{88} displayed a negative charge in the pH range (2–10). Moreover, absolute value of zeta potential of *E. coli* K_{88} increased with increasing pH value (Fig. 2a). MMT had negative zata potential at the tested pH range (Fig. 2a), which is consistent with the result of Nzengung et al. (1996). Under these conditions, *E. coli* K_{88} would not be significantly adsorbed to MMT due to mutually repel each other. The data obtained from our laboratory showed that when produce MMT-Cu from MMT the surface charge changed from negative to positive, that is to say, becoming positively charged. Thus, MMT-Cu significantly increases adsorption ability to *E. coli* K_{88}. The following mechanisms may account for the results. On the one hand, between MMT-Cu and *E. coli* K_{88} and between the bacterial surface carboxyl groups and Cu^{2+} exist electrostatic attraction. On the other hand, there are lipophilic components of the bacterial cell walls, such as lipoproteins, liposaccharides which made them easier adsorbed by MMT-Cu. Results suggest that electrostatic attractions and properties of bacterial cell walls and mineral surfaces may play important roles in controlling the adsorption of *E. coli* K_{88} onto the surface of MMT-Cu.

3.2 Effect of bacteria concentrations

The extent of *E. coli* K_{88} adsorption onto MMT or MMT-Cu did not change significantly at different *E. coli* K_{88} concentrations. It was suggested that the mineral surface was not saturated with respect to adsorbed bacteria in the entire tested concentration range. But it is impossible to determine if adsorption would become site-limited at higher bacteria concentration. A similar result was obtained by Wang and Han (2010), who investigated the effect of concentrations on *R. Palustris* adsorption onto the montmorillonite.

3.3 Effect of pH

Previously experimental studies demonstrated that the pH of the medium strongly influenced the electrostatic attraction between bacteria and clays by changing the surface charge properties of the bacteria and clays (Rong et al., 2008). In the present study, the data showed that medium pH exhibited some effect on interaction between *E. coli* K_{88} and the clays (Fig. 3c). The adsorptive percentages of *E coli* K_{88} onto MMT or MMT-Cu in the acid medium were higher than in alkaline medium and the ability of MMT-Cu to adsorb bacteria was higher ($P < 0.05$) than MMT at the experimental pH range. However, the percentage of the bacteria adsorbed onto the clay surface was relatively low at pH 2. Previous investigations demonstrated that the bacterial cell walls were neutrally charged at pH less than 2 due to the deprotonation of organic functional groups on the cell walls (van Loosdrecht et al., 1987; Yee et al., 2000). Under this condition, the electrostatic attraction between *E. coli* K_{88} and the tested adsorbent was relatively low. In the acidic medium, positive charge on the MMT and MMT-Cu surface decreased with pH increasing, and thereby decreases electrostatic attraction between MMT-Cu and *E. coli* K_{88}. In the medium of pH higher than 9, the interaction between copper ion and hydroxide ion can produce precipitation of cupric hydroxide, as described by the following Reaction (2):

$$Cu^{2+} + 2OH^- = Cu(OH)_2 \quad (2)$$

Therefore, the reaction mentioned above decreases the positive charge on the surface of MMT-Cu, and then results in the decrease in adsorption capacity of MMT-Cu for *E. coli* K_{88}.

In our present study, *E. coli* K_{88} adsorbed onto surfaces of MMT or MMT-Cu showed a minimum at a certain pH of the medium. This phenomenon might be related to the point of zero charge (PZC) on the surface of the clay, because chemical adsorption is lowest when the medium pH is equal to the PZC of clay.

3.4 Effect of ionic strength

In ionic strength experiment (pH 6.5), *E. coli* K_{88} adsorption onto MMT-Cu or MMT decreased significantly with increasing concentration of $NaNO_3$ in medium. This result was in agreement with previous studies in which the adsorption of *B. subtilis* onto corundum decreased significantly with increasing ionic strength (Yee et al., 2000). But it was not in agreement with previous studies (Jiang et al., 2007) showing that the increase in bacterial adsorption on minerals with increasing cation concentration occurs below 100 mmol/L. This difference in adsorption could be attributed to the species and surface characteristics of the experimental bacteria used in the present work in contrast to their study. At pH 6.5, the surfaces of *E. coli* K_{88} and MMT were negatively charged and the surface of MMT-Cu was positively charged. Because pH is constant, the extent to which the two surfaces interact is strongly dependent on ionic strengths. Our present results showed that zeta potentials of MMT and MMT-Cu were constant basically at different ionic strengths. Adsorption phenomenon can be explained by "double layers" theory (Thomas et al., 1999). At low ionic strength, the double layers associated with both surfaces are relatively thick, and the attractive electric fields extend further into solution. This increases the potential for adsorption. As ionic strength increases, the higher concentration of electrolyte ions limits the interaction between the two surfaces. We guess a part of the reason for this peculiar observation may

be the monospecific aggregateion of *E. coli* K_{88} in high ionic strength media, decreasing the bacterial surface area available for the interaction with MMT or MMT-Cu. And another reason may be with increasing $NaNO_3$ electrolyte concentration, Na^+ exchange for copper on the permanent charge sites, causing decreased copper adsorption on these sites. Therefore, adsorption was reduced.

3.5 Effect of temperature

The extent of bacterial adsorption on solid surfaces is affected by the bacterial physiological state, and vigorous bacterial metabolism facilitates their adsorption abilities (Pethica, 1980). In the present study, at 37°C the adsorption of *E. coli* K_{88} onto MMT-Cu or MMT was highest. Below 25°C and above 37°C, the adsorption of *E. coli* K_{88} onto MMT-Cu or MMT decreased significantly. Thus, the greater adsorption might be associated with the physiological state of *E. coli* K_{88} since its activity was optimum in the temperature range (25–37°C). A similar finding was obtained by Jiang *et al*. (2007), who investigated the effect of temperature on *P. putida* adsorption onto clay minerals and showed that the maximum amount of adsorption was reached at 37°C for montmorillonite. When temperature over-high (45°C), *E. coli* K_{88} physiological state and vigorous bacterial metabolism had changed. Therefore, the extent of bacterial adsorption on MMT or MMT-Cu was decreased. When temperature over-low (4°C), between clay particles and bacteria contact insufficiency and at the same time, viscidity of electrolyte solution increased, therefore it leads to a decrease in bacteria adsorption. Moreover, a similarly increasing degree in *E. coli* K_{88} adsorbed on MMT or MMT-Cu was observed with increased temperature, suggesting that temperature has a similar influence on the adsorption capacities of MMT and MMT-Cu.

4 Conclusions

Treatment with Cu^{2+} significantly ($P < 0.05$) increases the adsorption capacity of MMT for *E. coli* K_{88}. Mechanisms of adsorption process may involve both electrostatic attraction and physiochemical properties of bacterial cell walls and mineral surfaces. The adsorption process of *E. coli* K_{88} occurring on MMT and MMT-Cu reached equilibrium after 90 min. The percentages of *E. coli* K_{88} adsorbed onto the tested adsorbents in the acidic medium were relatively high as compared to those in alkaline medium except below pH 2. The extent of bacteria adsorption onto the modified MMT increased with decreasing ionic strength. MMT-Cu showed its high adsorptive ability to *E. coli* K_{88}. In conclusion, MMT-Cu may be a good material for the adsorbed and eliminated pathogenic bacteria from aqueous solution.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 30471255) and the Program for New Century Excellent Talents in University (No. NCET-06-0913). The authors like to acknowledge Dr. Baohua Xu, Dr. Li Xiong, Dr. Xiaogang Yang and Dr. Rongfang Yang for skillful technical assistance. The authors also thank Meisheng Xia and Caihong Hu for their supplying montmorillonite raw mineral. We would like to express our gratitude to Rutie Li for the BET analyses of the mineral powers used in the experiments.

References

Alexander T J L, 1994. Neonatal diarrhea in pigs. In: *Escherichia coli* in Domestic Animals and Humans (Gyles C L, ed.). CAB International, Wallingford. 151–170.

Barns S M, Nierzwicki-Bauer S A, 1997. Microbial diversity in modern subsurface, ocean, surface environments. In: Geomicrobiology: Interactions Between Microbes and Minerals (Banfield J F, Nealson K H, eds.). *Reviews in Mineralogy and Geochemistry*, 35(1): 35–79.

Bitton G, 1994. Water and wastewater disinfection. In: Wastewater Microbiology (Bitton G, ed.). Wiley-Liss, New York. 113–135.

Borchardt G, 1989. Smectites. In: Minerals in Soil Environments, Soil Science of America (Dixon J B, Weed S B, eds.) (2nd ed.). Soil Sci Soc Am, Madison, WI. 675–727.

Chen H, He X M, Rong X M, Chen W L, Cai P, Liang W et al. , 2009. Adsorption and biodegradation of carbaryl on montmorillonite, kaolinite and goethite. *Applied Clay Science*, 46(1): 102–108.

Domek M J, LeChevallier M W, Cameron S C, McFeters G A, 1984. Evidence for the role of copper in the injury process of coliform bacteria in drinking water. *Applied and Environmental Microbiology*, 48(2): 289–293.

Dychdala G R, 1991. Chlorine and chlorine compounds. In: Disinfection, Sterilization, and Preservation (Block S S, ed.). Lea and Feebiger, Philadelphia, PA. 131–151.

Guo T, Ma Y L, Guo P, Xu Z R, 2005. Antibacterial effects of the Cu(II)-exchanged montmorillonite on *Escherichia coli* K_{88} and *Salmonella choleraesuis*. *Veterinary Microbiology*, 105(2): 113–122.

Guo T, Xu Z R, 2004. Studies on antibacterial mechanism of cupric ions in *Escherichia* coli K_{88}. *Journal of Chinese Preventive Veternary Medicine*, 26(2): 127–130.

Hampson D J, 1994. Postweaning *Escherichia coli* diarrhea in pigs. In: *Escherichia coli* in Domestic Animals and Humans (Gyles C L, ed.). CAB International, Wallingford, UK. 629–647.

Jiang D, Huang Q, Cai P, Rong X, Chen W, 2007. Adsorption of *Pseudomonas putida* on clay minerals and iron oxide. *Colloids and Surfaces B: Biointerfaces*, 54(2): 217–221.

Laszlo P, 1998. Heterogeneous catalysis of organic reactions. *Journal of Physical Organic Chemistry*, 11(5): 356–361.

Ma Y L, Guo T, Xu Z R, Zhao T, 2005. Preparation and characterization of copper-loaded montmorillonite. *Journal of the Chinese Ceramic Society*, 33(2): 1041–1044.

Ma Y L, Xu Z R, Guo T, You P, 2004. Adsorption of methylene blue on Cu(II)-exchanged montmorillonite. *Journal of Colloid and Interface Science*, 280(2): 283–288.

Nathan Y, Jeremy B F, Christopher J D, 2000. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. *Geochimica et Cosmochimica Acta*, 64(4): 609–617.

Nzengung V A, Voudrias E A, Nkedi-kissa P, Wampler J M, Weaver C E, 1996. Organic cosolvent effects on sorp-

tion equilibrium of hydrophobic organic chemicals by organoclays. *Environmental Science and Technology*, 30(1): 89–96.

Okouchi S, Murata R, Sugita H, Moriyoshi Y, Maeda N, 1995. Calorimetric evaluation of the antimicrobial activities of calcined dolomite. *Journal of Antibacterial and Antifungal Agents*, 26(3): 109–114.

Pethica B A, 1980. Microbial and cell adhesion. In: Microbial Adhesion to Surfaces (Berkeley R C W, Lynch J M, Melling J, Rutter P R, Vincent B, eds.). Ellis-Horwood, West Sussex, England. 19–45.

Rong X M, Huang Q Y, He X M, Chen H, Cai P, Liang W, 2008. Interaction of Pseudomonas putida with kaolinite and montmorillonite: A combination study by equilibrium adsorption, ITC, SEM and FTIR. *Colloids and Surfaces B: Biointerfaces*, 64(1): 49–55.

Rutter P R, Vincent B, 1984. Physicochemical interactions of the substratum, microorganisms, and the fluid phase. In: Microbial Adhesion and Aggregation (Marshall K C, ed.). Springer-Verlag, New York. 21.

Stadler M, Schindler P W, 1993. Modeling of H^+ and Cu^{2+} adsorption on calcium-montmorillonite. *Clays and Clay Minerals*, 41(3): 288–296.

Stotzky G, 1989. Surface interactions between clay minerals and microbes, viruses, and soluble organics, and the probable importance of these interactions to the ecology of the soil. In: Microbial Adhesion to Surfaces (Berkeley R C W, Lynch J M, Melling J, Rutter P R, Vincent B, eds.). Ellis Norwood, Chichester, UK. 231–247.

Theng B K G, Hayashi S, Soma M, Seyama H, 1997. Nuclear magnetic resonance and X-ray photoelectron spectroscopic investigation of Lithium migration in montmorillonite. *Clays and Clay Minerals*, 45(5): 718–723.

Thomas F, Michot L J, Vantelon D, Montargès E, Prélot B, Cruchaudet M et al., 1999. Layer charge and electrophoretic mobility of smectites. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 159(2-3): 351–358.

Vaccari A, 1998. Preparation and catalytic properties of cationic and anionic clays. *Catalysis Today*, 41(1-3): 53–71.

van Loosdrecht M C M, Lyklema J, Norde W, Schraa G, Zehnder A J B, 1987. The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology*, 53(8): 1893–1897.

Wang Y B, Han J Z, 2010. Interaction of photosynthetic bacterium, *Rhodopseudomonas Palustris*, with montmorillonite clay. *International Journal of Engineering*, *Science and Technology*, 7(2): 36–43.

Wilczynski M, 2000. Anti-microbial porcelain enamels. *Ceramic Engineering and Science Proceedings*, 21(5): 81–83.

Yee N, Fein J B, Daughney C J, 2000. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. *Geochimica et Cosmochimica Acta*, 64(4): 609–617.

Yokoyama H, Peralta R C, Diaz R, Sendo S, Ikemori Y, Kodama Y, 1992. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. *Infection and Immunity*, 60(3): 998–1007.