

SIMULTANEOUS ADSORPTION AND ANTIBACTERIAL ACTIVITIES OF SURFACTANT-MODIFIED KAOLINITE

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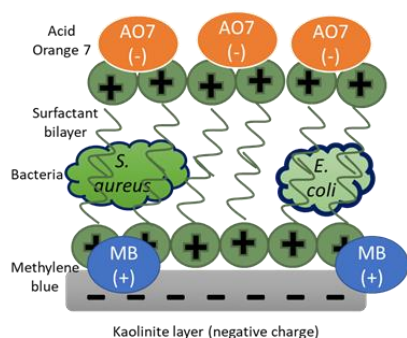
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Graphical abstract



Abstract

Water contamination problems caused by various contaminants (i.e. anionic and cationic compounds, and pathogenic bacteria) necessitate the advanced material development for simultaneous adsorption and antibacterial actions. In this study, kaolinite (Kao) was modified with surfactant hexadecyltrimethyl ammonium bromide (HDTMA-Br) (0.1, 1.0 and 4.0 mM) and they were characterized using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and dispersion behaviour. No structural changes occurred after the adsorption of HDTMA-Br molecules on the Kao. In the simultaneous action study, the samples were tested for their adsorption capacity for cationic (methylene blue, MB) and anionic dyes (acid orange 7, AO7), and for antibacterial activities against Gram-negative (*Escherichia coli* ATCC11229) and Gram-positive bacteria (*Staphylococcus aureus* ATCC6538). HDTMA-modified Kao enhanced the adsorption of AO7 and the trend was correlated with the HDTMA adsorbed on Kao. The modified Kao with the highest HDTMA-Br concentration (KaoH4.0) showed 100% removal capacity for both dyes (AO7 and MB) and reduced the highest amount of both bacterial colonies. Therefore, the kaolinite modified with surfactant simultaneously enhanced the adsorption capacity of both positive and negative compounds, and increased the antibacterial activities against a wide spectrum of bacteria.

Keywords: Kaolinite, surfactant, adsorption, antibacterial activity

Abstrak

Masalah dengan air yang dicemari oleh pelbagai bahan cemar (iaitu sebatian anionik dan kationik, bakteria patogen) memerlukan pengembangan bahan termaju yang boleh melakukan tindakan penjerapan dan antibakteria serentak. Dalam kajian ini, kaolinit (Kao) dimodifikasi dengan surfaktan heksadesitrimetil ammonium bromida (HDTMA-Br) (0.1, 1.0 dan 4.0 mM) dan sampel ini dicirikan menggunakan kaedah pembelauan sinar-X (XRD), spektroskopi inframerah transformasi Fourier (FTIR), dan ciri-ciri penyebaran. Tiada perubahan struktur yang berlaku setelah molekul HDTMA-Br dijerap oleh kaolinit. Dalam kajian tindakan serentak, sampel diuji keupayaan penjerapannya terhadap pewarna kationik (metilena biru, MB) dan pewarna anionik (asid oren 7, AO7), dan aktiviti antibakteria terhadap bakteria Gram negatif (*Escherichia coli* ATCC11229) dan Gram positif (*Staphylococcus aureus* ATCC6538). HDTMA diubah suai Kao meningkatkan penjerapan AO7 dan unjurannya berkorelasi dengan HDTMA yang dijerap pada Kao. Kao yang diubah suai dengan kepekatan HDTMA-Br tertinggi (KaoH4.0) menunjukkan kapasiti penyingkiran 100% untuk kedua-dua pewarna

(AO7 dan MB) dan mengurangkan bilangan koloni bakteria yang paling rendah. Dapat disimpulkan bahawa kaolinit yang diubah suai dengan surfaktan meningkatkan keupayaan penjerapan terhadap bahan positif dan negatif dan meningkatkan aktiviti antibakteria terhadap spektrum bakteria yang luas, secara serentak.

Kata kunci: Kaolin, surfaktan, penjerapan, aktiviti antibakteria

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1.0 INTRODUCTION

Human beings face various pathogenic microorganisms, including bacteria that can cause various diseases. In order to overcome this critical situation, there are many types of products containing antibacterial agents available in the market to promote a hygienic lifestyle. Besides that, the increasing development of textile industries creates problems to the environment because of the dyes molecules released into the waters. The presence of dyes in water streams threatens the natural ecosystem and causes detrimental effects to humans as dyes inhibit the photosynthetic activities of aquatic biota and they are highly mutagenic to living things [1]. Thus, a material that can simultaneously remove pathogenic bacteria and dyes in the water is important to be studied and developed.

The issues of bacterial resistance against antibiotics or antibacterial agents necessitate the development of enhanced and effective antibacterial agents. Some researchers have studied the kaolinite as a clay material that acts as a suitable carrier system for antibacterial agents [2-4]. Natural kaolinite is chosen as a carrier material because it is cheap, abundant and easy to be handled. Clay mineral has some benefits or advantages such as the ion-exchange capacity, high surface area, sorption capacity, negatively charged, low in toxicity and chemical inertness [5]. However, clay has low antimicrobial activity. Despite of that, kaolinite can be modified with cationic surfactants, creating organo-clay to enhance its antibacterial activities. The immobilization of surfactant molecules such as cetylpyridinium bromide (CPB) or hexadecyltrimethyl ammonium bromide (HDTMA-Br) on the clay improves the antibacterial activities of the kaolinite [6]. The optimum amount of surfactant attached on the clay needs to be carefully analysed to optimize the efficacy of the antibacterial activity of the organo-clay [6].

Some examples of techniques used to remove dyes from wastewaters are flocculation, sedimentation, and filtration. However, these techniques are unable to treat various classes of dyes and inefficient to completely remove dyes from aqueous phase [7]. The adsorption technique is an alternative method to improve the treatment process in order to produce safe, treated water. Modification

of clay surface with cationic surfactants yields significant surface properties to the clay and thus, it can be used in many applications. For example, due to its hydrophobicity, it has been applied as a water filter material for water purification [8]. Other than adsorbent for various adsorbates, kaolinite can also act as a carrier system for surfactants to enhance the antibacterial activities [6]. Therefore, in this study, surfactant-modified kaolinite was prepared, characterized and analysed for its simultaneous actions which were adsorption of positive and negative dyes, and antibacterial activities against Gram-positive and negative bacteria.

2.0 METHODOLOGY

Kaolinite was supplied from Kaolin (M) Sdn. Bhd located in Perak, Malaysia while the cationic surfactant, HDTMA-Br was purchased from Sigma-Aldrich. For the preparation of HDTMA-Br modified kaolinite with three different concentrations of HDTMA-Br (0.1, 1.0 and 4.0 mM), 2.0 g of kaolinite was added into 200 ml of each HDTMA-Br solution in a conical flask. The three HDTMA-Br concentrations were selected based on the Critical Micelle Concentration (CMC) of the HDTMA-Br (0.93 mM) [9] which was below (0.1 mM), near (1.0 mM) and above (4.0 mM) the CMC value. The mixture was stirred by using a magnetic stirrer for 16 hours. After that, the suspension was filtered, and the solid portion was dried at 80°C while the liquid portion was kept for the analysis of leftover HDTMA-Br. The dried solid was crushed into a powder by using a mortar and pestle. The prepared materials were characterized by Fourier transform infrared (FTIR) spectroscopy (Thermo Fisher Scientific Nicolet iS5 with Smart iTR Diamond crystal FT-IR spectrometer) and X-ray diffraction (XRD) technique (Bruker D8 Advance X-ray diffractometer). The analysis of HDTMA-Br concentration in the solution could be referred to the previous work [6]. The amount of HDTMA-Br adsorbed on the kaolinite was calculated based on the concentrations before and after the adsorption process according to equation below:

$$\text{Amount of HDTMA-Br adsorbed} = \frac{([HDTMA]_i - [HDTMA]_f)V}{W_k} \quad (\text{Eq. 1})$$

Where the amount of HDTMA-Br adsorbed is in mmol/kg, $[HDTMA]_i$ and $[HDTMA]_f$ are concentration of HDTMA before and after adsorption (mmol/L), respectively, V is volume of the HDTMA-Br solution (L) and W_k is the weight of kaolinite (kg). The dispersion behaviour of the samples in the water and hexane mixture was performed according to the previous paper [10].

The simultaneous action of surfactant-modified kaolinite involved the adsorption studies of acid orange 7 (AO7) (Sigma-Aldrich) and methylene blue (MB) (Sigma-Aldrich), and the antibacterial activities against Gram-negative ATCC (American Type Culture Collection) bacteria (*Escherichia coli* ATCC 11229) and Gram-positive ATCC bacteria (*Staphylococcus aureus* ATCC 6538). Each bacterium was mixed with two dyes solution, AO7 and MB for the simultaneous action study of all samples (Kao, KaoH0.1, KaoH1.0 and KaoH4.0).

A single loop of the pure bacterial culture was inoculated in a 100 ml of Luria-Bertani (LB) broth and incubated at 37°C for 12 h with agitation rate of 200 rpm. The overnight culture (10%) was then transferred into 90 ml of fresh LB medium and incubated in an incubator shaker for 3 h at 37°C (200 rpm). The optical density (OD) of the bacteria was examined at λ_{550} nm using a visible spectrophotometer (BUCK 100 Vis Spectrophotometer, Jenway) until the value reached between 0.6 and 0.8. Then, the bacterial culture was centrifuged at 4000 rpm at low temperature (4°C) for 15 min. The supernatant was discarded, and the cells were washed 3 times using distilled water (DW). The bacterial culture was mixed with 50 ml of 10 ppm dye solution in a falcon tube and then, it was shaken to disperse the bacteria. About 20 ml of bacterial culture and dye solutions were mixed with the solid sample in a ratio of 0.1 g/L. The mixture was shaken to disperse the sample before incubating at 27°C for 30 min with an agitation rate of 100 rpm. After that, the solid samples were separated from the solutions by a single filtration technique through Macherey-Nagey filter paper (125 mm). The solution was measured for its absorbance using a visible spectrophotometer at λ_{max} 487 and 661 nm for AO7 and MB, respectively. The removal percentage of dyes (%Dye Removal) by the adsorbent was calculated based on the different concentrations of dyes before and after the experiment. Meanwhile, the viability of the bacteria treated with the Kao, KaoH0.1, KaoH1.0 and KaoH4.0 samples was determined by serial dilution technique using a dropped plate technique. For the serial dilution technique, the filtrate solution was diluted until 8 dilution factors. Accurately, 10 μ L of each sample was dropped on a nutrient agar plate that was divided into four quadrants, where each quadrant was prepared for one dilution factor. The plates were kept in an incubator at 37°C for 18 h. The number of colonies was measured by each spot of each quadrant. The experiment was performed in triplicates.

3.0 RESULTS AND DISCUSSION

Figures 1 and 2 show the FTIR spectra and XRD diffractograms, respectively for the HDTMA-Br modified kaolinite at different HDTMA-Br concentrations. In the FTIR spectra, the general O-H stretching absorption bands for kaolinite were shown at 3686, 3653 and 3618 cm^{-1} [11]. The Si-O-Si vibration of tetrahedral sheet and Si-O-Al stretching vibration of the octahedral sheet were shown at bands below 1000 cm^{-1} [12]. The FTIR spectra of kaolinite modified with 1.0 and 4.0 mM of HDTMA-Br showed additional absorption bands at 2920 and 2851 cm^{-1} . The former was attributed to symmetric and the latter to asymmetric stretching vibration of C-H in the alkyl chain of HDTMA-Br [6, 13]. These peaks were evidence of modified kaolinite by surfactants. Based on the FTIR results, the kaolinite was successfully modified with HDTMA-Br.

The structure of the kaolinite before and after modification with HDTMA-Br was characterized by XRD (Figure 2). Two intense reflections in the XRD pattern at 7.1 and 3.6 Å showed that the kaolinite structure [14] remained the same even after loading with HDTMA-Br at different amounts. This result proved that the modification of kaolinite with surfactants only involved the cation-exchange process and hydrophobic interactions among the HDTMA-Br molecules without affecting the original framework structure of the kaolinite [15].

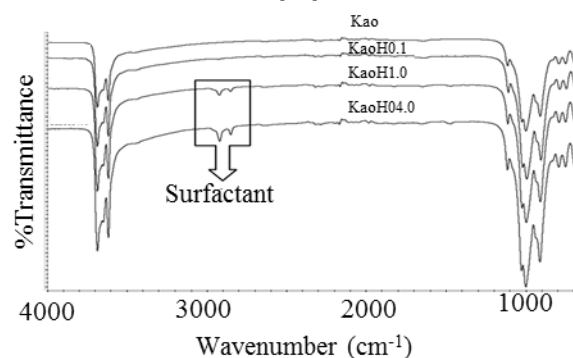


Figure 1 FTIR spectra of surfactant-modified kaolinite

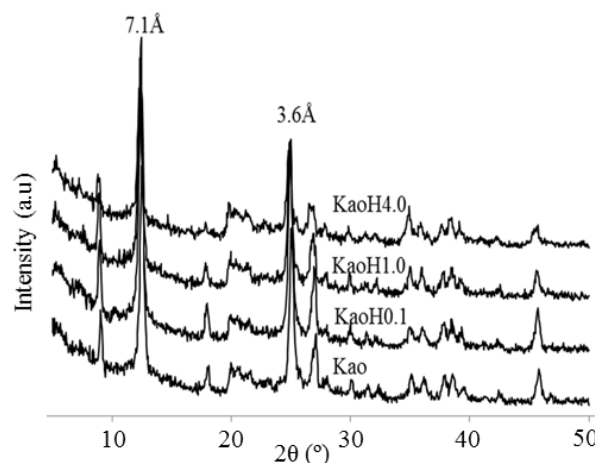


Figure 2 XRD diffractogram of surfactant-modified kaolinite

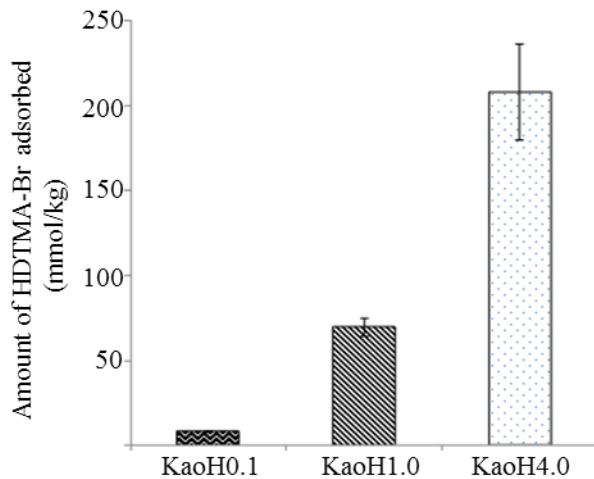


Figure 3 Amount of HDTMA-Br adsorbed on kaolinite

Figure 3 shows that the amount of HDTMA-Br adsorbed by kaolinite increased when the initial concentrations of HDTMA-Br increased. Based on previous studies, the affinity of surfactant towards kaolinite is caused by the electrostatic interactions between the charged groups on the surfaces of kaolinite and HDTMA-Br molecules [16]. The result of this study showed that the positively charged groups of surfactants interacted with the negatively charged surface of the kaolinite. The adsorption of HDTMA-Br on the kaolinite was possible due to the cation-exchange property of the kaolinite and hydrophobic interactions among the HDTMA-Br molecules [17].

The relative position and dispersion behaviour of unmodified and modified kaolinite particles in a water-hexane mixture are shown in Figure 4.

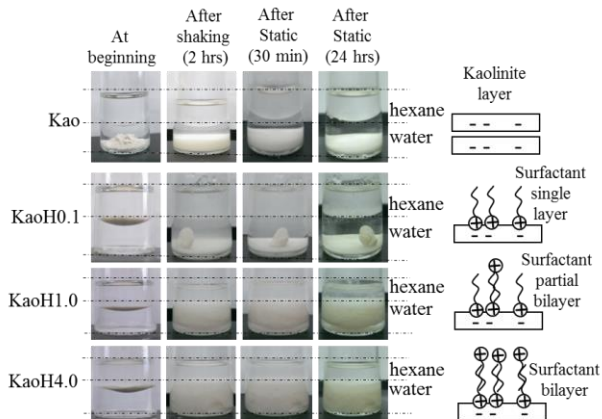


Figure 4 Location of the samples in hexane/water suspension and theoretical formation of surfactant arrangement on the kaolinite at different surfactant loading

At the beginning of the experiment, all samples were located at the middle of the mixture of water-hexane except for raw kaolinite. This scenario

happened because the surfactant molecules were adsorbed at the water-hexane interface, between the hydrophilic group in the water and hydrophobic group in the n-hexane. However, after 24 hours at a static condition, the KaoH0.1 sample formed an aggregation caused by the electrostatic repulsive force that drove the particles apart and by the attractive van der Waals forces [18]. On the contrary, the modified kaolinite (KaoH1.0 and KaoH4.0) dispersed well in the water because the particles of modified kaolinite could not interact with each other due to the blockage by the water molecules. This dispersion behaviour proved that the presence of HDTMA-Br on the kaolinite created particles that had both hydrophobic and hydrophilic properties.

The theoretical formation of surfactant molecules on kaolinite surfaces is shown in Figure 4. As the HDTMA-Br concentration increased, the adsorbed surfactant molecules started to form hemimicelles at the surface of the kaolinite. When approaching or exceeding the CMC value, the adsorbed surfactants formed a bilayer and changed to positively charged surfaces [19]. Hence, the characterization of the surfactant-modified kaolinites using FTIR, XRD, the amount of surfactant adsorbed, and the dispersion behaviour showed that there were changes in the kaolinite surfaces after the modification with different amount of surfactant.

Simultaneous actions, which were the adsorption of dyes and antibacterial activity, were performed by studying the adsorption capacity of unmodified and modified kaolinite towards anionic (AO7) and cationic dye (MB). The antibacterial activity was determined by counting the number of colonies after treated with the samples. The lower number of colonies shows higher antibacterial activities of the sample and thus, exhibits better antibacterial performance. The adsorption of dyes and antibacterial activity for all samples are shown in Figures 5 and 6.

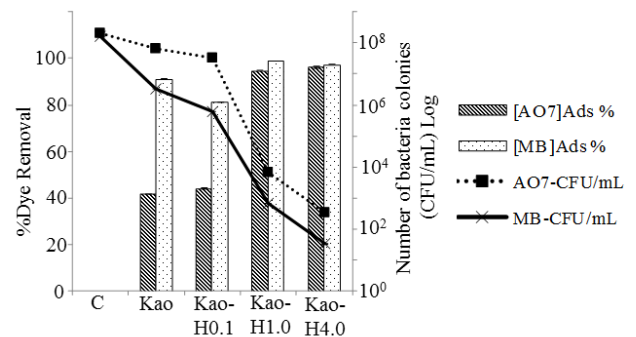


Figure 5 Simultaneous action of adsorption of AO7 and MB, and number of colonies of *E. coli* by Kao and KaoH

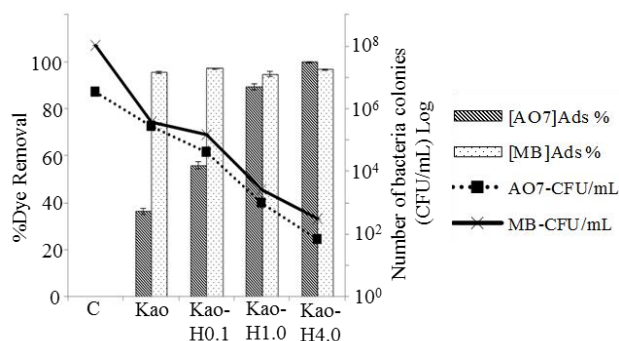


Figure 6 Simultaneous action of adsorption of AO7 and MB, and number of colonies of *S. aureus* by Kao and KaoH

Based on Figures 5 and 6, the modified kaolinite with higher concentration of HDTMA-Br (KaoH4.0) showed the highest adsorption for AO7 and MB and the lowest number of bacterial colonies. Overall, the adsorption of AO7 follows this trend: KaoH4.0 > KaoH1.0 > KaoH0.1 > Kao. This is because, the surface modification process affected the adsorption affinity of the kaolinite towards anionic dye. Theoretically, kaolinite is negatively charged, resulting from the broken edges or structural defects, and it repels the anionic dye due to the similar charge. The modification of kaolinite with HDTMA-Br was performed with the aim to increase the adsorption affinity of kaolinite towards the anionic dye. The critical micelle concentration (CMC) of the surfactant is an important factor in the surface modification process.

At concentration below CMC, HDTMA-Br molecules were adsorbed on the surface of kaolinite in a monolayer form. However, as the concentration increased above CMC, the HDTMA-Br molecules rearranged on the surface of the kaolinite to form HDTMA-Br bilayer (Figure 4). Consequently, the surface charge reversed from negative to positive due to the positively charged head of HDTMA-Br [20]. Therefore, KaoH4.0 allowed more uptake of AO7 on its surfaces.

For the antibacterial activity, the trend for the number of bacteria colonies for all samples was as follows: Kao < KaoH0.1 < KaoH1.0 < KaoH4.0. KaoH4.0 had the lowest number of bacteria colonies, proving to be an effective antibacterial agent capable of inhibiting bacterial growth. The HDTMA-Br possesses positively charged quaternary ammonium compound and hence, the presence of cationic surfactant on kaolinite changes the surface properties from hydrophilic to hydrophobic and increases the adsorption capacity towards anionic compounds [21]. Therefore, the positively charged ammonium will attract anions and the negatively charged bacterial cell wall or membrane, eventually killing the bacteria and inhibiting bacterial growth through electrostatic forces [22].

All samples showed higher adsorption capacity for MB because the MB molecules were positively charged, attracting the negatively charged

bacterial membrane. At the same time, the MB molecules, together with the bacteria, were attracted to the positively charged surfaces of modified kaolinite. Biological materials, such as peat, chitosan, yeast, fungi and bacterial biomass, are used as biosorbents to concentrate and remove dyes from the solutions [7]. This is the reason why KaoH4.0 can uptake more MB in the presence of bacteria; the bacterial membrane helps to increase the negative charge of the modified kaolinite and ultimately, enhance the adsorption capacity towards the positively charged compounds.

Figures 5 and 6 also show that the kaolinite do not exhibit any antibacterial activity against both Gram-positive and Gram-negative bacteria. Kaolinite on its own is negatively charged while the bacterial cell walls also have negative charge due to the presence of the phospholipids. Consequently, no changes occur to the bacterial cells since both the kaolinite and bacterial cell walls repel each other. Thus, the kaolinite only acts as a carrier system for the surfactant molecules [22].

Surfactant-modified kaolinite was more effective towards *S. aureus* compared to *E. coli*. This is because, the structural variations of bacterial cell wall between the Gram-positive and negative bacteria; Gram-negative bacteria contain a thin layer of peptidoglycan layer between the outer and inner membrane. The peptidoglycan layer is comprised of covalently linked lipids and polysaccharides and is negatively charged [23]. Gram-negative bacteria have an outer membrane while Gram-positive bacteria do not. This outer membrane acts as an extra barrier that does not allow some of the antimicrobial chemicals to penetrate the bacterial cell walls. Thus, Gram-negative bacteria are more difficult to be inhibited than Gram-positive bacteria.

4.0 CONCLUSION

The results from XRD, FTIR, the amount of HDTMA-Br adsorbed and the dispersion behaviour concluded that the kaolinite was successfully modified with HDTMA-Br and showed different surfactant arrangements on the kaolinite surfaces. The modified kaolinite with 4.0 mM of HDTMA-Br (KaoH4.0) had higher adsorption capacity towards AO7 and MB, and higher antibacterial activities because the attached HDTMA-Br provided positive charges on the surface to uptake more dyes and bacteria. From the results obtained, it could be concluded that the surfactant-modified kaolinite with high concentration of surfactant simultaneously enhanced the adsorption affinity of dyes and increased the antibacterial activities.

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