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Short Communication

Reduction of Microbial Contamination in Kaolin from Belitung Island, Indonesia

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ABSTRACT

Kaolin is often used as an excipient in pharmaceutical and cosmetic industries as well as an anticaking, bulking, absorbent, and opacifying agent. It is also required in some cosmetic industries with a limited number of microbial contaminations, but no study has explored the reduction of these contaminant microbes based on the requirements. Therefore, this study aims to produce kaolin that complies with the microbial contamination specifications. Several methods were used in this study to reduce the microbial content, including heating, sterilization using an autoclave, as well as soaking with ethanol, methanol, and chloroform. The number of microbial contaminations was then measured using the Petri dish method. The result showed that the 2 h heating treatment was not sufficient to eliminate microbial contamination even at a temperature of 250°C, hence, it was combined with the addition of sodium hypochlorite to obtain a better outcome. Furthermore, autoclave treatment at 121°C for 20 min with or without pre-treatment successfully reduced the population of microbes to <10^2 per gram and this met the standard requirement for cosmetic industries.

***Keywords*:** Kaolin, Microbial Contamination, Heating Treatment, Autoclave, Sterilization.

Introduction

Kaolin is one of the minerals produced in Indonesia with Belitung island as the biggest deposition site in the country. It has been widely used as paper coating, paper filling, paint extender, ceramic ingredient, rubber filler, plastic filler, ink extender, cracking catalysis, fiberglass, cement, adhesives, enamels, pharmaceuticals, crayon, and molecular sieves.[1] Furthermore, several techniques have been employed to improve its quality, such as separation with the magnetic field, which helps to reduce magnetable minerals as well as improve the brightness.[2] This separation technique has also been used for purification through the elimination of metallic contaminants, such as Fe_2O_3[3]. Several studies have reported the application of bleaching of clay using organic acid, microbial culture media, and oxalic acid-rich media from the cultivation of *Aspergillus niger* with biodiesel processing waste as culture medium.[4–8] Calcination of kaolin is often carried out to improve its quality, as well as reduce the usage of titanium oxide in the paper and paint industries. The process is performed at different temperatures, such as 650°C, 1050°C, or 1300°C to produce meta-kaolin, mullite, or refractory grog, respectively. Calcination also increases whiteness, hardness, electrical properties, adsorption capacity, alter size, and shape of particles.[9,10] Low-grade kaolin can be improved by removing Fe(III) impurities using the iron-reducing bacteria, where its whiteness increased, while the redness decreased after treatment.[11–14]
Kaolin is widely used in pharmaceutical, cosmetic, and food industries, where it serves as an abrasive, absorbent, anticaking, bulking, and opacifying agent. Although microbial contamination is not regulated in Pharmacopoeia, certain cosmetic industries require kaolin with limited microbe content.

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Several efforts have been made by producers to reduce the contaminants through drying as well as the use of hypochlorite as a disinfecting agent, but they were ineffective. Incomplete drying also leaves residual smell of sodium hypochlorite on the material.
In this study, several attempts have been made to reduce the number of microbial contaminants, including heating of the kaolin powder and the addition of disinfectants, such as sodium hypochlorite. Rinsing with other disinfectant and sterilization using an autoclave were also performed in this study.

Materials and Methods

Sample collection
Kaolin was obtained May 2017 from Belitung by PT Aneka Kaolin Utama.

Heating treatment
A total of 10 g kaolin was spread on a metal container to produce 5 mm thickness, followed by heating in an air oven at 100, 200, and 250^{0}C for 2 h. Subsequently, the sample was taken to analyze the microbial counts in each gram. Heating treatment was also performed at 105°C overnight.

Treatment with ethanol, methanol, and chloroform
Grade 70% ethanol is often used as a disinfectant, and methanol with similar properties is expected to have the same function. Water saturated with chloroform is also frequently used to prevent microbial contamination through extraction using cold water. Therefore, these solvents were also applied in this study to reduce the population of microbe in kaolin. A total of 10 g of the sample was soaked with 50 ml solvent for 24 h, followed by the removal of the solvent and drying of kaolin.
A total of 10 g of kaolin was placed in each vial, where one of them was soaked in ethanol for 24 h and the other was left without any treatment. The sample was then sterilized using an autoclave at 121^{0}C for 20 min. Subsequently, the supernatant was removed and kaolin was dried using an oven for 2 h at 105^{0}C.

Measurement of the microbial contamination
Each of the dried and treated kaolin was dissolved in water to obtain different concentrations. The suspension produced was then dispersed

in 15 ml nutrient agar media in a Petri dish and incubated overnight at 37°C. Furthermore, the total colonies that grew in the sample were then counted.

Results and Discussion

Kaolin used in this experiment was in a fine powder form and its particle cannot be clearly observed under a light microscope due to the formation of aggregates. However, Figure 1 shows that the particle can be observed under SEM, because the size was less than 10 µm and mostly < 1 µm.

Heating treatment

The effect of heating treatment on the number of microorganisms is presented in Table 1. In this experiment, kaolin was spread at 0.5 cm thickness and heated at 100, 200, and 250°C for 2 h. Furthermore, Figure 2 shows the substantial decrease in the microbial population after the treatment. It was surprising that after heating at 200 °C and 250 °C, some of the microbes still survive. This was possibly caused by two factors, namely the surviving microorganisms were thermophilic, or the heat had not spread evenly throughout the sample. This indicates that a longer period of heating is needed to overcome this problem and obtain better results. The previous study by Drew showed that kaolin clay treatment using heating at 104°C was unable to remove the microbial contamination,[15] and this is consistent with the present study. Therefore, it can be concluded that the use of only this treatment method cannot reduce microbial growth in the kaolin sample.

Drew also carried out a study on the application of microbiocide to reduce the population of microbes,[15] but it was ineffective in the clay sample. This finding is in line with this study, where it was predicted that the microbiocide did not penetrate the clay's surface. A previous study revealed that this limitation can be solved through the addition of a surfactant to increase the penetration of the treatment.[16]

As an alternative, Drew proposed a method, which involves heating a kaolin clay slurry for 15 min at 93°C, then cooling to ≤ 35°C, followed by the addition of microbiocide. Therefore, the combination of the heating treatment and the addition of 20 ppm microbiocide was suggested to significantly reduce the microbial contamination in kaolin slurry.[15]

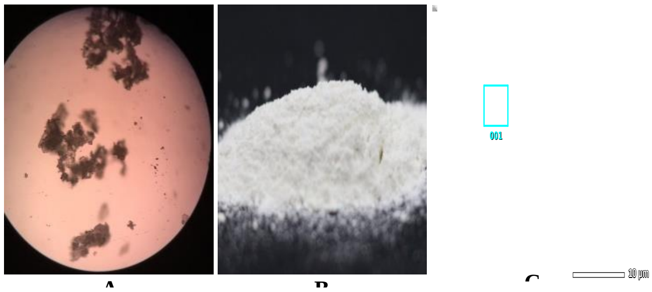

Figure 1: Kaolin powder (A), kaolin particle under light microscope with 100x magnification (B) and under SEM (C)

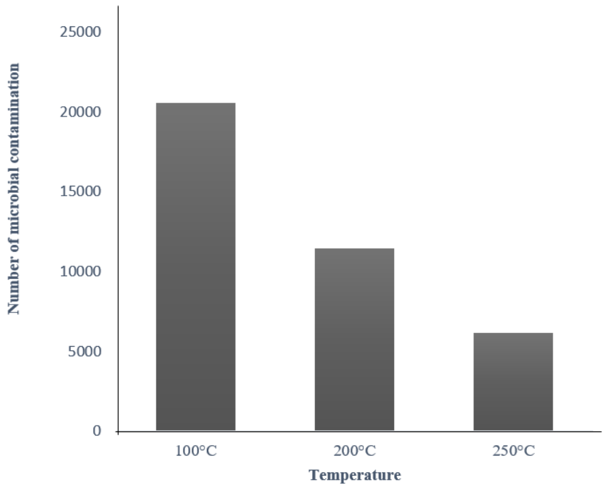

Figure 2: Microbial count of kaolin at elevated heating temperature

Table 1: Autoclave sterilization of kaolin followed by drying

Main treatment	Complementary treatment	Microbial counts /g
Control	Autoclave	4.10^1
	Oven	81.10^1
Soaking in ethanol	Autoclave	3.10^1
	Oven	0
	Air drying	7.10^3
Soaking in chloroform	Autoclave	3.10^1
	Oven	1.10^1
	Air drying	1.10^2
Soaking in bleaching solution	Autoclave	1.10^1
	Oven	2.10^1
	Air drying	0

Apart from bleaching agents, ethanol and chloroform are also widely used to prevent the growth of microrganisms. Grade 100% or 70 ethanol is mostly used for surface sterilization. Saturation of water with chloroform have been reported to be effective in inhibiting the growth of microorganism during the extraction of plant material at ambient temperature for a longer period than overnight.

In this study, kaolin was soaked in a bleaching agent, namely 96% ethanol and chloroform for 24 h followed by autoclave treatment, as well as oven and air drying. Chloroform is often used to lyse microbial cells.[17] The soaking solvent was removed by decantation or filtration using a Buchner funnel. Autoclave treatment was carried out at 121°C (1.1 bar) for 20 min as that used for wet sterilization. Oven drying was also performed on all samples at 105^{0}C for 2 h except the control that was left overnight. Air drying was carried out using hot air stream from a hair dryer, and the results are presented in Table 1.

All the treatments above substantially reduced the microbial counts of kaolin and met the required level for cosmetic industries. Based on Figure 2, the heating of kaolin at 150 °C or 250 °C for overnight is likely to eliminate all the population of microbes that originated from the

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kaolin itself. Furthermore, sterile kaolin cannot be obtained when the overall activities are not performed aseptically.
An observation of the effect of autoclave treatment and gamma sterilization in reducing microbial growth in soil samples was carried out by Berns (2008).[18] The result showed that both of those methods could effectively decrease the number of microbial contamination in soil samples.[18]

Conclusion

Heating of kaolin at > 100°C reduced substantially its microbial counts. The results also showed that heating for 2 h as often used in dry sterilization is not sufficient to eliminate microbial contamination even at a temperature of 250°C. Furthermore, the combination of heat treatment and the addition of sodium hypochlorite was needed to reduce the population of microbes to produce kaolin that met the specification of cosmetic industries. Autoclave treatment at 121°C for 20 min with or without pretreatment also successfully reduced the microbial contamination to lower than 10^2 per gram.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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