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RESEARCH ARTICLE

Antibacterial activity of copper exchanged zeolite Y synthesized from rice husk ash

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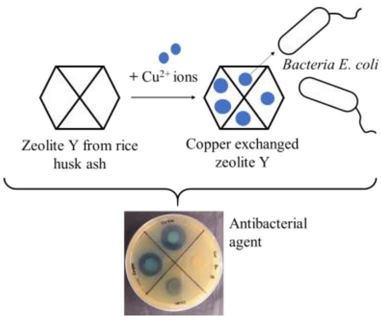
Graphical abstract



Abstract

Increasing problems with antibacterial agent primarily bacterial resistance and environmental pollution due to the high release of antibacterial agents in water necessitates the development of new and effective antibacterial agents. One of the techniques that can be used to overcome these problems is by immobilizing antibacterial compounds or any related compounds on the carrier system such as by using zeolite. In this study, zeolite Y was synthesized from rice husk ash as silica source by using hydrothermal technique and it was used as a carrier system for antibacterial copper (Cu) ions. A series of Cu-exchanged zeolite Y was then prepared by loading with different concentrations of Cu ions (100 ppm, 600 ppm and 900 ppm of the $Cu(NO_3)_2$) on the synthesized zeolite Y. The Cu-exchanged zeolite Y was characterized by X-ray diffraction (XRD) and Fourier Transform Infrared spectroscopy (FTIR). These characterization techniques showed that the zeolite Y was synthesized in pure phase and had a good degree of crystallinity. Whereas, from the characterization results, zeolite Y was successfully loaded with different concentrations of Cu ions and no structural changes happen after modification. The antibacterial activity of the samples was determined through disc diffusion technique (DDT) against Gram positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212) and Gram negative bacteria (*Escherichia coli* ATCC 11229 and *Pseudomonas aeruginosa* ATCC 15442). Based on the antibacterial results, the synthesized zeolite Y loaded with 900 ppm of Cu^{2+} showed the highest antibacterial activity compared to that of loaded with 100 ppm and 600 ppm of Cu^{2+}. The higher the Cu concentration on the zeolite Y resulted in the higher antibacterial activity against wide spectrum of bacteria. As a conclusion, synthesized zeolite Y from rice husk ash could be a carrier system for antibacterial Cu ions and it has the potential for the application as antibacterial agents.

***Keywords*: Zeolite Y, copper ion, rice husk ash, hydrothermal, antibacterial agent

INTRODUCTION

The increasing emergence of microbial resistant to antibiotics and diseases associated to it has been a major problem to global health nowadays. In the future, there must be new unknown symptoms or advanced diseases related to this problem. Besides monitoring of antibiotic use and surveillance on local outbreak of antibiotic resistance symptoms, developing a new and effective antimicrobial agent is important to counter this problem (Mölstad *et al*., 2017). Recent advancement is by using zeolite as a microcarrier system containing antibacterial agents such as silver, zinc, copper *etc*. (Alswat *et al*., 2016).

Zeolites are crystalline aluminosilicates with a three-dimensional framework structure. It composed of TO_4 tetrahedral (T = Al, Si) joined into 3-dimensional frameworks having pores of molecular dimensions to accommodate other molecules (Erdem *et al*., 2004). They are widely used for many applications such as catalysts, adsorbents, and ion exchangers. Both natural and synthetic zeolites show strong affinities for water and one of the most excellent drying agents even at extremely low partial pressure of water and temperature (Ghasemi *et al*., 2011). Recently, there has been interest in the synthesis of nano-sized zeolites due to their advantages over the micro-sized of zeolites (Charkhi *et al*., 2012). Zeolite structure has emerged as an ideal supports to accommodate one or even two metals, due to their high cation exchange capacity especially zeolites X, Y and A (Ferreira *et al*., 2016).

Zeolites can be synthesized using various silica resources. Silica can be found in agricultural waste materials such as rice husk ash (RHA) and coal fly ash (Yao *et al*., 2015). Rice husk resources are abundant in Malaysia due to extensive rice plantation nationwide. The amorphous silica forms the main component (83–90%) of RHA (Pode, 2016). Open burning of rice husk producing rice husk ash which may cause serious environmental and human health problems. Several ways are being performed for disposing RHA by making it to a value added product. The combustion of rice husk at a proper temperature will form amorphous silica which can be used as a starting material to synthesis zeolite (Yusof *et al*., 2010). For high purity of specific types of zeolite either zeolites Y, X or A, the chemical composition ratio of the Na_2O/SiO_2, SiO_2/Al_2O_3 and H_2O/Na_2O must be used.

Silver zeolites are being used in biomedical and biological area (Saengmee-anupharb *et al*., 2013). As an ion exchanger, zeolite could be loaded with other metal ions such as copper and zinc that were applicable for stopping or slowing bacterial and fungal growth in medical treatment, water treatment, wood preservation and other areas (Malachova *et al*., 2011). According to Salim and Malek (2016), the application of synthetic zeolite incorporated with silver was used as an antibacterial agent because it is well known that some kind of metallic nanoparticles such as silver, copper and zinc have antibacterial capabilities to reduce health risks.

Silver ions loaded into zeolite Y had been investigated (Salim and Malek, 2016) but there is lack of studies for Cu-exchanged zeolite Y as antibacterial agent. According to Yoon *et al*. (2007), antibacterial activity of copper was more effective compared to the silver using single representative strains of *Escherichia coli* and *Bacillus subtilis*. Therefore, the objectives of this research is to synthesis and characterize zeolite Y from rice husk ash as silica source, to prepare Cu-exchanged zeolite Y with different Cu ions loading and characterize them and study the antibacterial activity of Cu-exchanged zeolite Y against Gram positive and negative bacteria.

EXPERIMENTAL

Materials

Rice husk that was obtained from Bernas (Beras National) milling, Selangor, Malaysia had undergone pre-treatment of washing, drying under sunlight and dried before physical combustion in a Plug Flow Combustor (PFC) located at the Solid State Laboratory, Universiti Teknologi Malaysia (UTM) at 600°C and constant pressure for one hour. The rice husk ash (RHA) was ground using mortar into powdered form.

Synthesis of zeolite Y from rice husk

Zeolite NaY was synthesized using RHA through hydrothermal method aiding with seeding and ageing technique following method by previous work (Yusof *et al*., 2010). The RHA was initially calcined at 600°C for 4 hours. After calcination, seed gel was prepared by 24 hours ageing and feed stock gel was also prepared. Fig. 1 and 2 show the process of the preparation of seeding and feedstock gels, respectively.

After that, both gels were mixed together and stirred vigorously with magnetic stirrer for 2 hours and inserted into Teflon bottle before ageing at room temperature for 24 hours. After the ageing process, the samples were placed in an oven at 90°C for another 24 hours. The mixture was taken out from the oven and the cap was opened rapidly at room temperature. Then, the solid was separated using suction filtration. The separated solid was then washed with distilled water until pH of the solution reach below 8 and dried in an oven at 100°C overnight. The dried solid sample was then crushed by mortar and pestle before sieved.

Preparation of Cu-exchanged zeolite Y

Copper ion (Cu^{2+}) was prepared to react with zeolite Y to obtain Cu-exchanged zeolite Y. About 3.7980 g of $Cu(NO_3)_2$ was dissolved in 900 ml distilled water and stirred until completely dissolved. The solution was then filled with distilled water up to the mark in 1000 ml volumetric flask. A series of Cu-exchanged zeolite Y was prepared by ion exchange with different concentration of Cu ions (100, 600 and 900 ppm). The mixture of zeolite Y and $Cu(NO_3)_2$ was stirred for 16 hours. After the stirring process, the solid and liquid fractions were separated. The liquid was discarded by filtration using Whatman No. 1 filter paper and the solid was dried in an oven at 80°C overnight.

X-ray diffraction (XRD)

The structure of the synthesized and Cu-exchanged zeolite Y was identified using X-ray Diffraction (XRD) technique on a Bruker D8 Advance X-ray diffractometer. This machine was operated using a step scan programme with scanning speed of 0.05° per second, in the range of 2θ= 5 to 45°. The XRD patterns was recorded with monochromated Cu-Kα radiation at λ = 1.5418 Å, at 40 kV, 20 mA.

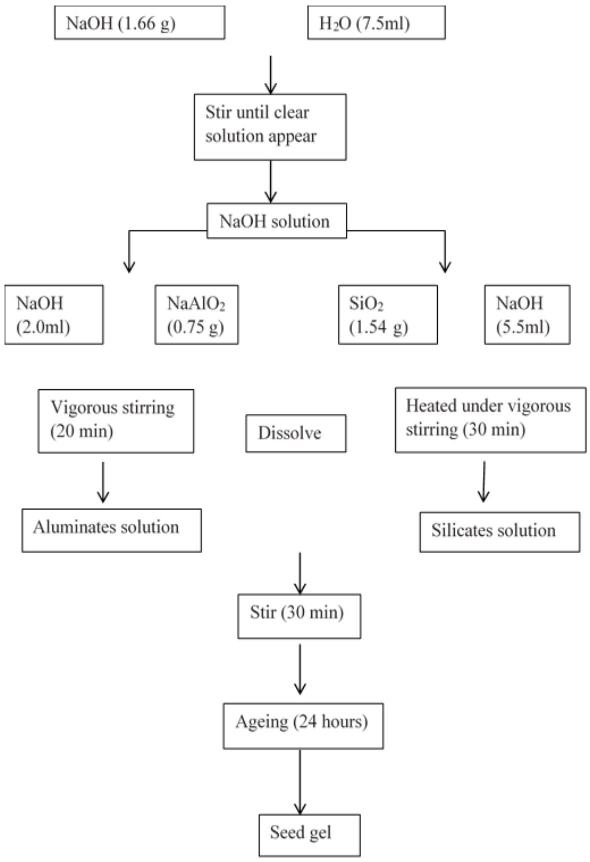


Fig. 1 Flowchart for the preparation of seed gel.

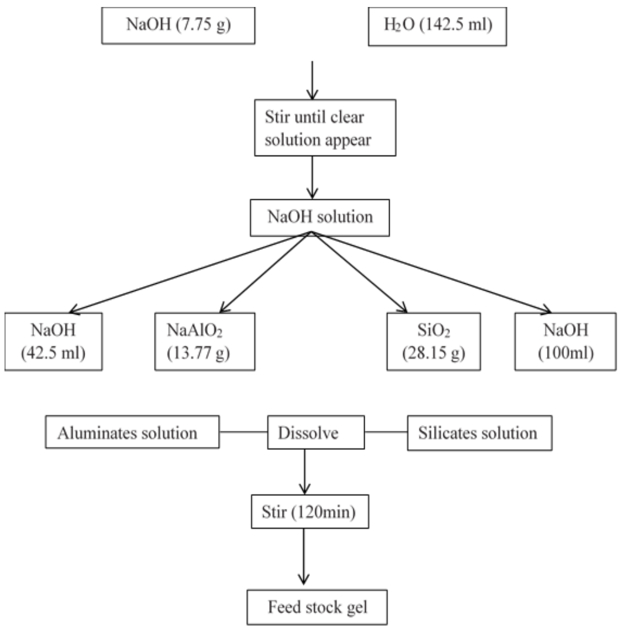


Fig. 2 Flowchart for the preparation of feed stock gel.

Fourier Transform infrared spectroscopy (FTIR)

The structural analysis of zeolite Y and Cu-exchanged zeolite Y with different concentration of Cu ions (100, 600 and 900 ppm) was determined by FTIR spectrophotometer model Thermo Scientific Nicolet IS5-IR spectrometer equipped with OMNIC™ software using KBr pressed disc method. All samples were scanned for their FTIR spectra in the range of 4000–450 cm^{-1} with a wavenumber resolution of 4 cm^{-1}.

Disc diffusion technique (DDT)

The antibacterial activity of the prepared samples was studied against different species of Gram positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212) and Gram negative bacteria (*E. coli* ATCC 11229 and *Pseudomonas aeruginosa* ATCC 15442) based on DDT. Each bacterium was cultured on nutrient agar at 37°C overnight for obtaining a single colony. Then, three to five colonies were picked and diluted with sterile 0.9% saline solution to match with 0.5 McFarland standard solutions. A sterile cotton bud was then used to swab bacteria culture onto Muller Hinton Agar (MHA) plate by rotating and swabbing plate at every 60° turn to ensure homogenous bacteria growth. Then, the pellets were placed on top of the bacteria surface and the inhibition zone was measured after 24 hours of incubation at 37°C.

RESULTS AND DISCUSSION

Characterization

In this study, the characterization of the materials is important to understand the physical and chemical properties of the studied samples. In the synthesis of the zeolite from rice husk ash at every batch, the seeding technique and ageing the sample for 24 hours at room temperature was consistently performed in order to ensure high purity and reproducibility of the zeolite Y. In this study, XRD and FTIR were used to determine the structure of the synthesized material and also changes happen after loaded with Cu ions.

Fig. 1 shows XRD patterns of zeolite Y, Cu-100 zeolite Y, Cu-600 zeolite Y and Cu-900 zeolite Y. The diffractogram shows that the peaks are highly matched with the peaks of zeolite Y structure from Powder Diffraction Files (PDF) (Treacy *et al.*, 2007). This confirms that the zeolite Y was successfully synthesized from rice husk ash without major impurities. After loaded with Cu ions, the diffractogram of each samples seem similar to that of synthesized zeolite. This proved that the structure of zeolite Y still remain the same after loading with Cu ions. Furthermore, there are no peaks at 36.56° and 38.83° for Cu nanoparticles (Alswat *et al*., 2017) exist in the XRD patterns of Cu-exchanged zeolite Y. This shows that the Cu ions was 100% loaded successfully with the cations inside the zeolite Y framework without formation of Cu particles and also without distorting the framework structure of zeolite Y (Shen *et al*., 2017).

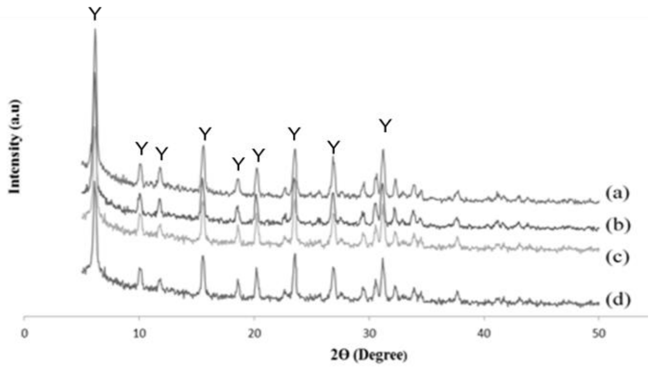


Fig. 3 X-ray diffractograms of (a) zeolite Y, (b) Cu-100 zeolite Y (c) Cu-600 zeolite Y and (d) Cu-900 zeolite Y.

The FTIR spectra of the samples can be seen in Fig. 4. The FTIR spectra show consistent peaks for zeolite Y and confirmed the successful conversion of RHA to zeolite Y. By referring from previous study (Yusof *et al*., 2010), there are 6 significant peaks for zeolite Y which are 1101, 1005, 773, 694, 570 and 464 cm^{-1}. These all peaks can be seen in the FTIR spectra of the synthesized zeolite Y and Cu-exchanged zeolite Y in Fig. 4. The FTIR result also in line with the result from XRD where the structure of the zeolite Y is still remain the same after loaded with Cu ions. These characterization results proved that the Cu ions are located inside the zeolite structure through ion exchanged process.

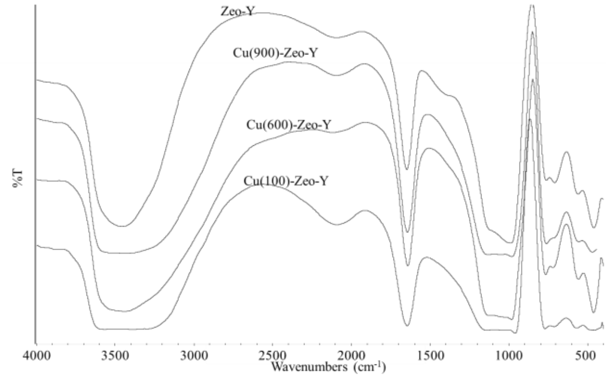


Fig. 4 FTIR spectra of zeolite Y, Cu-100 zeolite Y, Cu-600 zeolite Y and Cu-900 zeolite Y.

Antibacterial activity

The antibacterial activity of raw and modified zeolite Y against Gram-positive bacteria *S. aureus* ATCC 6538 and *E. faecalis* ATCC 29212 and Gram-negative bacteria *E. coli* ATCC 11229 and *P. aeruginosa* ATCC 15442 was determined through disc diffusion technique (DDT). Fig. 5 shows the inhibition zone of zeolite Y, Cu-100 zeolite Y, Cu-600 zeolite Y and Cu-900 zeolite Y pellets on the agar surface after 24 hours of incubation time while Table 1 shows the inhibition zone values from the DDT result. Zeolite Y pellets were used as a control sample to observe the antibacterial effectiveness of Cu ions loaded on the zeolite Y. The colour of Cu was diffused into the agar denoting its activity.

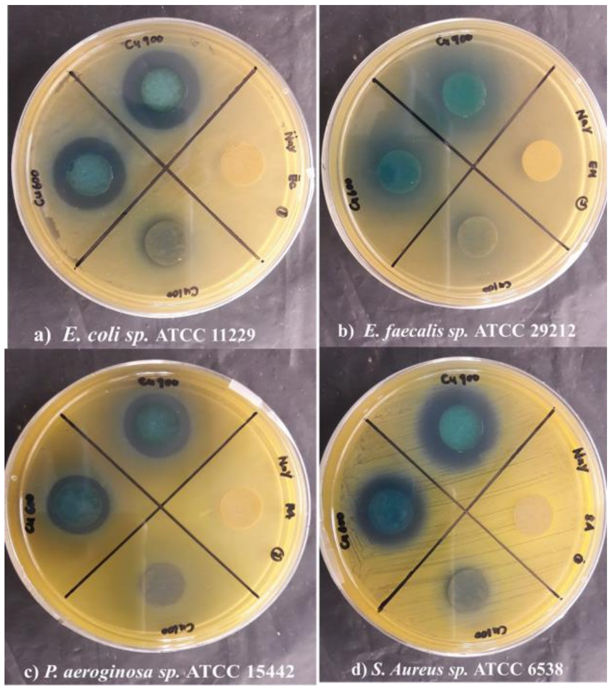


Fig. 5 The images of the plate from DDT against (a) *E. coli* ATCC 11229, (b) *E. faecalis* ATCC 29212, (c) *P. aeruginosa* ATCC 15442 and (d) *S. aureus* ATCC 6538.

Table 1 Inhibition zone of zeolite Y, Cu-100 zeolite Y, Cu- 600 zeolite Y and Cu-900 zeolite Y against four different ATCC bacteria.

Samples	Inhibition zone (mm)			
	EC	PA	SA	EF
Zeolite Y	0.00	0.00	0.00	0.00
Cu-100 Zeolite Y	1.60	1.55	1.45	1.50
Cu-600 Zeolite Y	2.15	2.00	1.90	1.95
Cu-900 Zeolite Y	2.40	2.25	2.20	2.25

*Note: EC: *E. coli* ATCC 11229, PA: *P. aeruginosa* ATCC 15442, SA: *S. aureus* ATCC 6538, EF: *E. faecalis ATCC* 29212

From the DDT result, raw zeolite Y does not have antibacterial activity against all of the studied bacteria since there are no formations of inhibition zone around the pellet of the zeolite Y. The framework structure of zeolite NaY used in this study composed of aluminosilicate structure and Na as its cation that balance the negatively charge of its aluminosilicate framework. All of these elements creating the zeolite framework are not listed as antibacterial metal and do not have antibacterial properties. Most of the previous studies related to antibacterial activity of raw zeolite also showed the same result where raw zeolite does not have antibacterial activity against all bacteria (Hanim *et al*., 2017, Salim and Malek, 2016, Salim and Malek, 2017, Malek *et al*., 2014). However, these previous studies showed that the antibacterial activity of zeolite was enhanced by loading with other antibacterial agents in the zeolite framework.

The DDT results (Table 1 and Fig. 5) revealed that all of Cu exchange zeolite Y have antibacterial activities against all of the studied bacteria and there is no significant different among the inhibition zone values for each of the bacteria. This shows that the samples can kill or inhibit a wide spectrum of bacteria including gram-positive and negative bacteria. Furthermore, there is a good correlation between the inhibition zone values and also the amount of Cu in the zeolite Y where the higher amount of Cu resulted in a higher antibacterial activity of the sample. The possible antibacterial mechanism by Cu-exchanged zeolite involves the release of Cu ions into the agar plate and kills the bacteria or inhibit the bacterial growth. The insertion of Cu ions into the cells eventually change the ion concentration and cause leakage of DNA, RNA and inactivation of enzyme thus kill the bacteria (Cai *et al*., 2015). There are also other previous studies that showed similar result where Cu ions immobilized on inorganic materials such as kaolinite (Saad *et al*., 2016) and zeolite (Milenkovic *et al*., 2017) also showed antibacterial activity.

CONCLUSION

High purity of zeolite Y has been successfully synthesized from rice husk ash as silica source without significant impurities or other phases. The Cu-exchanged synthesized zeolite Y showed antibacterial activity aginst wide spectrum bacteria and the antibacterial activity was related to the Cu ions that kill or inhibit the bacteria. This antibacterial agent could be used as an alternative inorganic antibacterial agent to combat with pathogenic bacteria and also, zeolite Y synthesized from rice husk ash is a suitable carrier for antibacterial metal ions.

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