THERMO-MECHANICAL PROPERTIES OF AN ANTI BACTERIA COATING MATERIAL BASED ON A HYBRID COMPOSITE GEOPOLYMER-CHITOSAN

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The main objective of this study was to examine the effect of a chitosan addition on the thermo-mechanical properties and microstructure of a geopolymer-chitosan composite, as well as the growth activity of bacteria on the surface. Chitosan was produced by the demineralisation (DM) and deproteination (DP) of a shrimp shell powder using a dilute sodium hydroxide (NaOH) solution and hydrochloric acid (HCl), followed by deacetylation (DA) using a dilute NaOH solution. Geopolymer pastes were produced through the alkaline activation of metakaolin. The metakaolin was obtained by dehydroxylating kaolin at 750 °C for 4 h. The chemical composition and the functional groups of the chitosan were examined by X-ray diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FTIR), respectively. The results showed that a high-quality chitosan was produced. The hybrid composites were produced by adding chitosan powder into a geopolymer paste at 0.1, 1.5, and 2.0 wt. % of chitosan relative to the mass of the metakaolin. The mixture was then moulded, cured at 70 °C for 2 h and then stored in the open air for 28 days before being subjected to XRD and Scanning Electron Microscopy (SEM) analyses. Differential Scanning Calorimetry (DSC) and Flexural Strength measurements were conducted to investigate the thermo-mechanical properties of the composites. The results showed that the geopolymer paste was a very strong binder to the chitosan and produced composites with high thermal resistance and excellent flexural strength. The Total Count Plate (TCP) tests showed that the addition of 1.5 wt. % of chitosan powder produced the most effective composite to limit the growth of the bacteria.

INTRODUCTION

Bacteria is one of the microbiology cells that has an important role in life and are stated to be the first forms of life. They have existed for four billion years [1] and their existence is huge, for instance, in the small intestine there are 10^9 cell/ml [2], there are about 4×10^7 cells in a gram of soil and 1×10^6 cells in 1 ml of fresh water. Bacteria can also survive in almost all various habitats such as an aquatic environment, in the soil, in the subsurface, in the air, in and on animals, plants or even in extreme conditions and clean room environments [3, 4].

Most bacteria species have no effect on human life, on the contrary several of them have a beneficial role. Unfortunately, other various species have a pathogenic effect that provokes health problems to the other organism or even death [5-7]. In the last case, it is important to develop anti-bacteria agents that can prevent the bacterial infection or even growth. There are many materials have been developed as antibacterial. Natural resources are among these materials. The development of natural resources for the other functional purposes is important especially in a country that has a large variety of natural resources such as Indonesia.

One of the natural resources that have been developed as an anti-bacterial agent is chitosan. Chitosan is a deacetylate form of chitin. Chitin can be extracted from the exoskeleton of a crustacean, for instance a shrimp, a crab or an arthropod such as an insect. Chitin also can be obtained in the cell wall of fungi and yeast [8, 9]. Structurally, chitin is a polymer that mainly consists of a poly(β(14)-2-acetamido-D-glucose monomer unit [10, 11]. Their capability as an anti-bacterial agent is attracting increasing attention, since they can inhibit various species of bacteria including Gram positive or Gram-negative bacteria. Moreover, they can also inhibit yeast and mould growth [12]. The chitosan anti-bacterial activity is conducted through the electrostatic interaction of the chitosan cationic site and the cell-surface negative-site [11]. The chitosan anti-bacterial activity then is applied in various applications including the medical, food or textile industry [9].

One of the chitosan weaknesses that limit its application is its low mechanical strength. The geopolymer is a polymer of SiO_4 and AlO_4 that is built in a tetrahedral structure through a geo-synthesis process [13]. The geopolymer has good mechanical properties and has been applied in the material building industry. The composite of the geopolymer and chitosan is believed that it can

extend the application of the materials. In this study, we investigated the addition of chitosan into a geopolymer which was synthesised by using Indonesian Kaolin. The application of this composite is directed as a building material, which has been reported elsewhere. For instance, Sunendar et. al. [14] has reported the application of chitosan doped Sr composited with a metakaolin geopolymer for dental implants. But they only investigated the mechanical properties of the composite. The study of the influence of the chitosan onto the mechanical, thermal, along with their anti-bacterial properties is still lacking.

In this study, we investigated the influence of chitosan extracted from shrimp shells onto the mechanical, thermal and the anti-bacterial properties composited with a geopolymer made from Indonesian Kaolin.

EXPERIMENTAL

Materials

The materials for the chitosan preparation were a shrimp shell powder and 2 M HCl. Sodium silicate ($Na_2O_3SiO_2$) and metakaolin were used in the geopolymer preparation, while NaOH (pellet, p.a) and distilled water were used for both the chitosan and geopolymer preparation.

Chitosan extraction

Chitosan was prepared from a shrimp shell powder in three stages, namely deproteination (DP), demineralisation (DM) and deacetylation (DA). Deproteination was carried out by immersing the shrimp shell powder in a 0.1 M sodium hydroxide solution. The ratio of the shrimp shell powder mass to the volume of the sodium hydroxide solution was 10 ml:1 g. The mixture was stirred at 57-65 °C for 2 h and then filtered and washed using distilled water until the pH of the filtrate was 7. The resulting powder (chitin) was dried at 70 °C for 10 h before being demineralised.

Demineralisation was performed to remove any minerals from the DP stage. The process was carried out using a 2 M hydrochloric acid (HCl) solution. The ratio between the resulting powder to the HCl solution was 1 g:10 ml. The mixture was stirred at a temperature between 23 and 30 °C for 30 min, followed by a filtration process. The resulting precipitate was then washed using demineralised water until a neutral pH was reached and then dried at 70 °C for 10 h. The process produced a demineralised chitin powder.

The last stage was deacetylation. For this process, ten portions of a 20 % sodium hydroxide solution were added into the obtained demineralised chitin powder. The mixture was stirred at a temperature between 90 and 100 °C for 30 min. The resulting chitosan powder

was filtered, washed until a neutral pH was reached and then dried at 70 °C for 10 h.

Geopolymer synthesis

The geopolymer paste was prepared by using the alkali activation method. The alkali solution was prepared by dissolving sodium silicate ($Na_2O_3 \cdot 3SiO_2$) and a sodium hydroxide pellet (NaOH) into distilled water. The compositions were adjusted to reach a $Na_2O:SiO_2$ and $H_2O:Na_2O$ ratio of 0.8 and 10, respectively. The activator solution was then mixed manually with metakaolin to obtain a homogenous geopolymer paste. Half of the paste was moulded into bars for the flexural strength test while the rest were moulded into a specially made glass mould. The inner part of the glass mould was coated with chitosan powder. After the geopolymer paste was poured into the mould, the surface of the geopolymer paste was covered with the chitosan powder. The addition of the dried chitosan powder is meant to coat the geopolymer paste with chitosan at 0.0 %, 1.0 %, 1.5 % and 2 % chitosan mass relative to the mass of the metakaolin. The mixture was left to set for several minutes and then cured at a temperature of 70 °C for two hours.

Characterisation

The resulting geopolymer-chitosan composites were characterised using X-ray diffraction (XRD), a Fourier Transform Infra-Red (FTIR) spectrometer and Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS). The XRD analyses were carried out to investigate the phase and chemical compositions of the resulting chitosan and geopolymer-chitosan composites. FTIR was used to study the functional groups of chitosan and the composite while SEM-EDS was used to study the morphology of the chitosan and geopolymer- chitosan composites.

The thermo-mechanical properties of the geopolymer-chitosan composites were studied by means of Differential Scanning Calorimetric (DSC) and flexural strength measurements. The antibacterial properties of the material were tested using the Total Count Plate (TCP) method according Ahsan et al. [15].

RESULTS AND DISCUSSION

Chitosan extraction

Figure 1a shows the photograph of the resulting chitosan particles taken using an optical microscope. The particles appear transparent, reasonably homogenous, irregular in shape and size. Further examination on the morphology of the chitosan using SEM is shown in Figure 1b. The figure shows inhomogeneous flake-like chitosan particles with a size smaller than 50 μm. The

surface morphology and the size of chitosan particles from the shrimp shells produced in this study are very similar to those reported by [18, 19] using a similar raw material.

a)
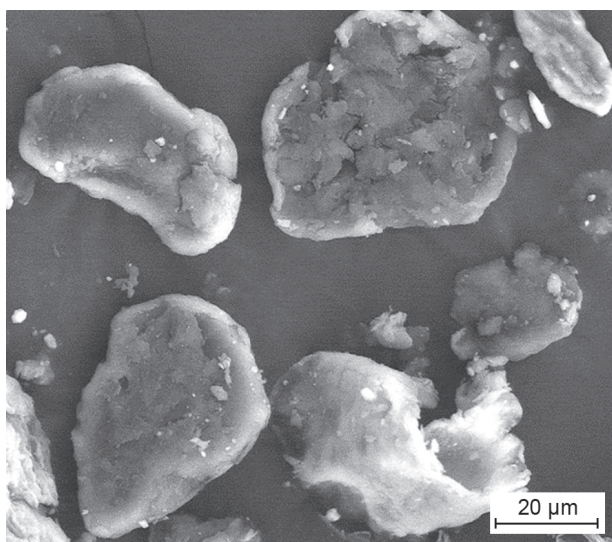
b)

Figure 1. Optical microscope (a) and SEM images (b) of the chitosan particles.
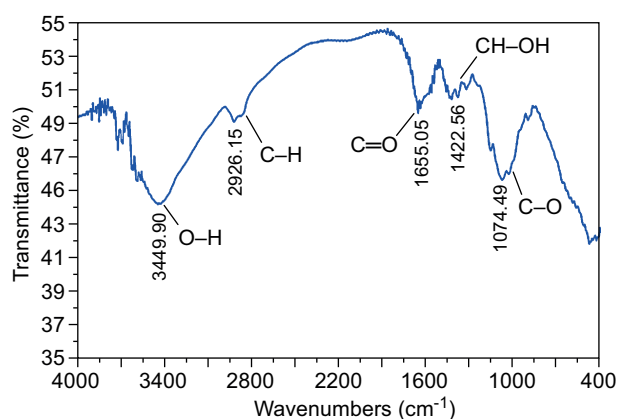

Figure 2. FTIR spectrum of the as-prepared chitosan.

The functional groups of the resulting chitosan were examined by means of FTIR as shown in Figure 2. Based on the FTIR spectrum shown in Figure 2, the functional groups of chitosan are identified and listed in Table 1.

Table 1. Functional groups of the as-prepared chitosan.

Groups	Wave number (cm^{-1})
–OH	3448.8
–CH	2922.3
–C=O (carbonyl in amide (NH))	1653.0
NH (R–NH$_2$)	1547.0
–C–O	1066.7

All the characteristic functional groups of chitosan are detected. The results confirmed that the resulting chitosan from the shrimp shell powder is in accordance with the chemical structure of chitosan as shown in Figure 3.
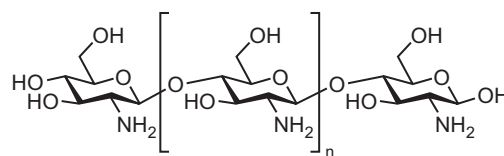

Figure 3. The chemical structure of chitosan.

It is also known that chitosan is a semi-crystalline organic compound [16] and, therefore, it has a certain X-ray diffraction pattern. The X-ray diffraction pattern of the resulting chitosan is shown in Figure 4. The figure shows the main peaks of the chitosan at 9-10° and 19-20° 2θ, identified as the (020) and (110) planes, respectively. The intensity of these two peaks shows that the produced material was chitosan and the broad diffraction peaks 10° and 20° 2θ confirmed that the chitosan is a semi-crystalline organic material, and normally used to calculate the crystallinity index of the chitosan. A similar XRD pattern of the chitosan produced from shrimp shells was also reported by [20].
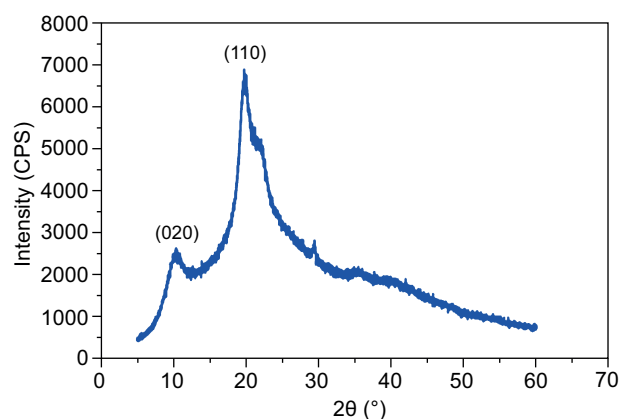

Figure 4. Diffractogram of the as-prepared chitosan.

Geopolymer paste and
Geopolymer-chitosan composite

The geopolymer paste was produced from meta-kaolin that was produced by the dehydroxylation of kaolin (CV Citra Persada, Indonesia) at 750 °C for 4 h. The elemental composition of the metakaolin was examined by Energy Dispersive Spectroscopy (EDS) and the result is shown in Figure 5. It was found that the main composition of the metakaolin was 57.15 wt. % SiO_2 and 42.85 wt. % Al_2O_3. The sodium silicate ($Na_2O\cdot3SiO_2$) which was used as activator in this study is comprised of 30.1 % SiO_2, 9.4 % Na_2O and 60.5 % H_2O. The production of the geopolymers was performed by adjusting the molar oxide ratios of $SiO_2/Al_2O_3 = 3.0$, $Na_2O/SiO_2 = 0.2$, and $H_2O/Na_2O = 10$. Figure 6a and 6b show the samples of the geopolymer-chitosan composites produced in this study. The samples were used for the flexural strength measurement and anti-bacterial test.

a)

b)

Figure 6. Geopolymer-chitosan composite samples for: a) the flexural strength measurements with a dimension of 14 × 2 × × 1 cm and b) the anti-bacterial test with an OD of 4.0 cm, an ID of 3.0 cm and 4.0 cm in height.

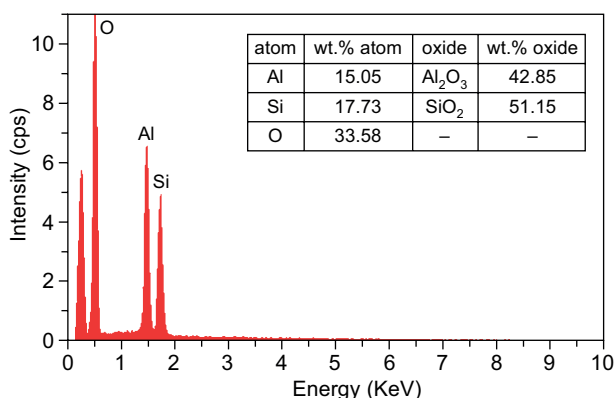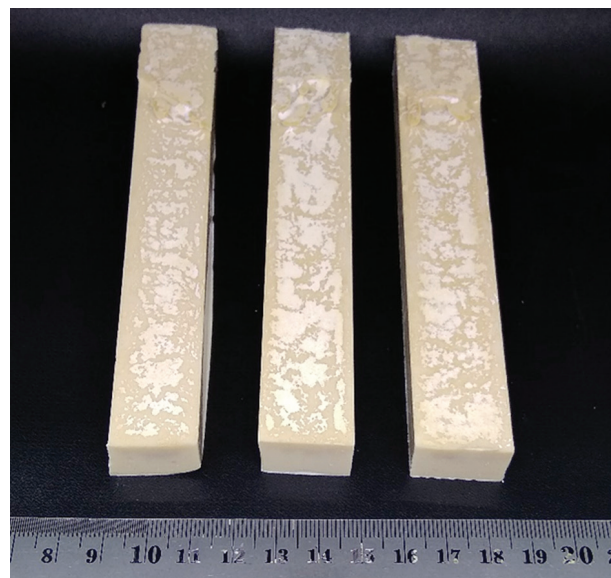| atom	wt.% atom	oxide	wt.% oxide
Al	15.05	Al_2O_3	42.85
Si	17.73	SiO_2	51.15
O	33.58	–	–

Figure 5. EDS spectrum of the EDS metakaolin particles.

The cross-link network of the geopolymer-chitosan composite was examined using FTIR. The geopolymer paste is known as a good binder for organic or inorganic particles forming a composite-like material [17]. Figure 7 shows the FTIR spectra of the geopolymer-chitosan composites. The concentration of chitosan varied from 0.0 to 2.0 % with an interval of 0.5, relative to the mass of the metakaolin.
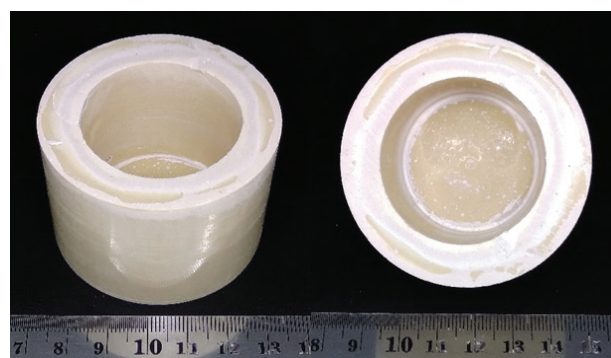
The FTIR result of the geopolymer paste shows the functional groups were found on the broad bands with vibration modes as follows (Figure 7a):

Si–O–Si stretching : 463 cm^{-1}, 1025 cm^{-1}
Si–O–Al stretching : 867 cm^{-1}
H_2O stretching : 3452 cm^{-1}, 2360 cm^{-1}
H_2O bending : 1649 cm^{-1}

The addition of chitosan into the geopolymer network shifted the wave numbers of the vibration modes of Si–O–Si and Si–O–Al stretching and becomes more discernible at the addition of 2 % of chitosan. This result suggests the formation of the geopolymer-chitosan cross-linking to form a new composite as shown schematically in Figure 8.
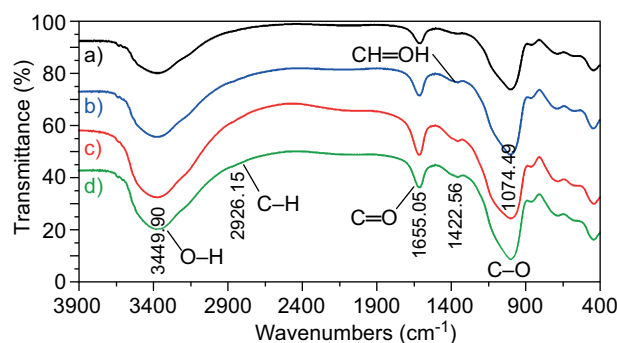

Figure 7. FTIR spectra of the geopolymer-chitosan composites: a) 0 %, b) 1.0 %, c) 1.5 %, d) 2.0 %.

The structural change of the geopolymer network due to the presence of chitosan was also examined using XRD. Figure 9 shows the XRD pattern of the geopolymer-chitosan composites with different concentrations of chitosan.
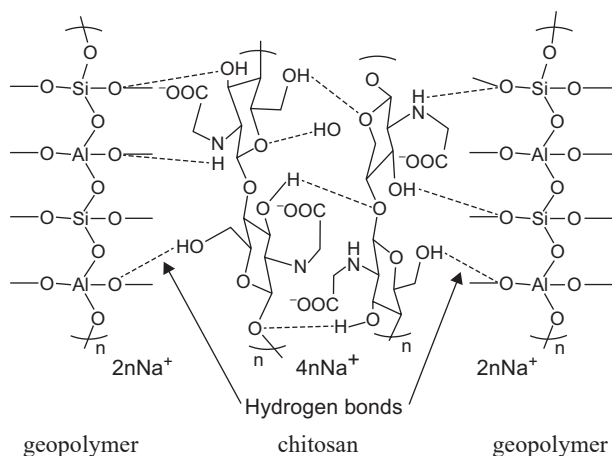
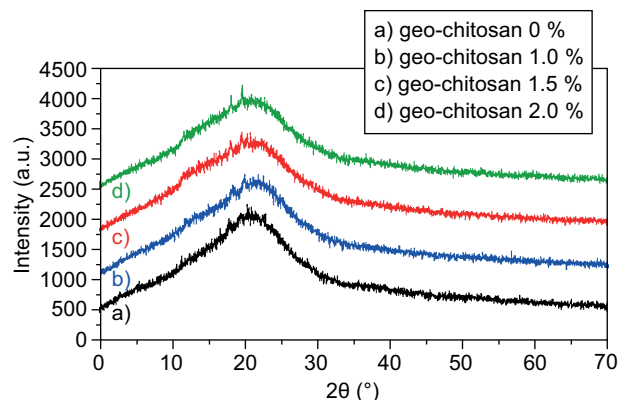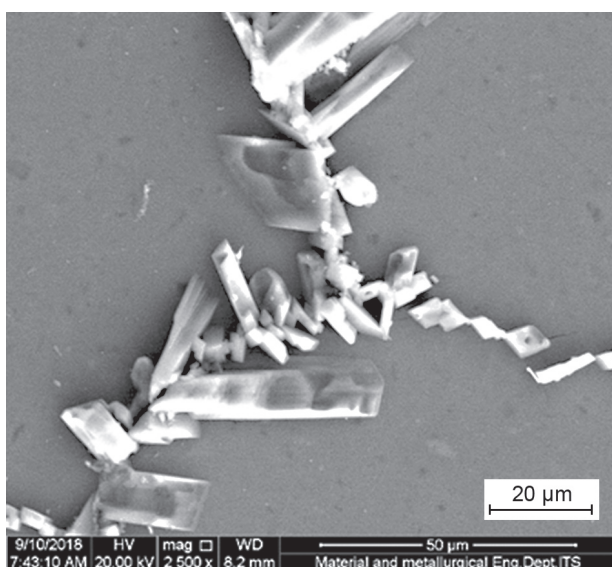Figure 8. Cross-linking model of the geopolymer-chitosan composite (Li et al., 2013)
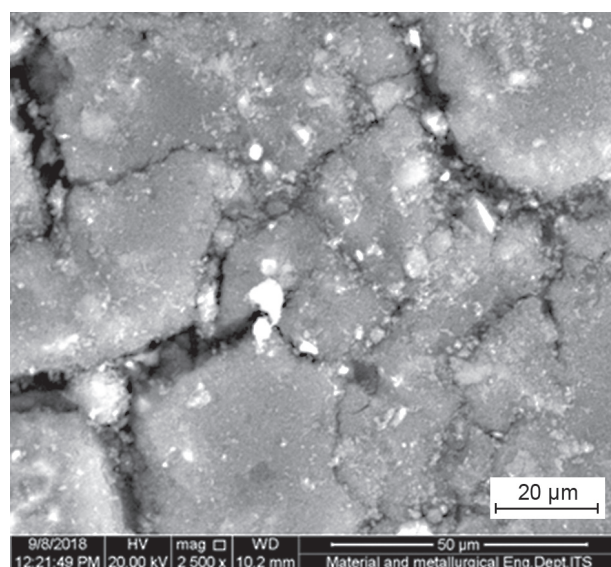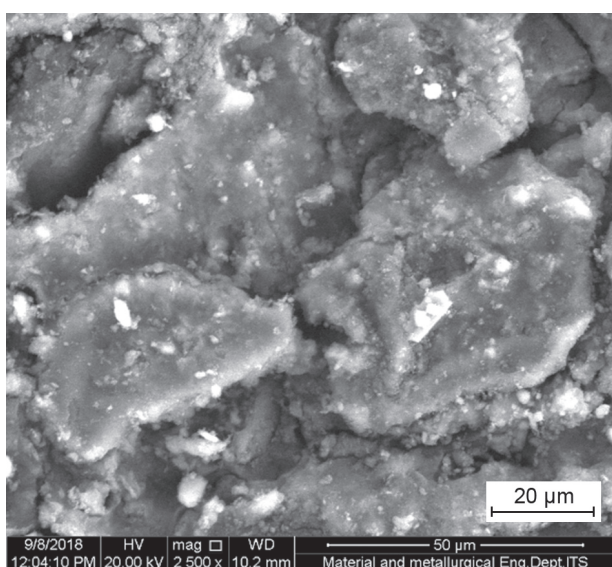

Figure 9. Diffractogram of the geopolymer-chitosan composites with the concentration of chitosan from 0.0 to 2.0 %, relative to the mass of the chitosan.
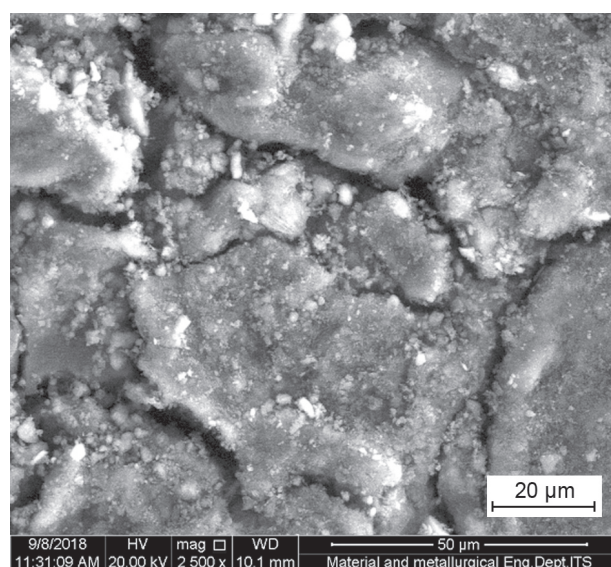

a) without chitosan

b) 1.0 %

c) 1.5 %

d) 2.0 %

Figure 10. SEM images showing the surface morphology of the geopolymer-chitosan composites: a) without chitosan, b) 1.0 %, c) 1.5 %, and d) 2.0 % of chitosan mass relative to the mass of metakaolin.

The geopolymer is an amorphous XRD material. The XRD patterns in Figure 9 show that the presence of the chitosan particles becomes visible at the addition of 1 to 2 % at the diffraction angle between 20-30° 2θ. The structural change of the geopolymer-chitosan composites appears to shift the highest peak position of the chitosan from 19-20° to 26-28° 2θ. This is due to the formation of new chemical bonds between the geopolymer and the chitosan as shown in Figure 8.

The microstructure of the geopolymer-chitosan composite was also examined using SEM. Figure 10a shows the SEM surface morphology of the pure geopolymer without the chitosan addition. The presence of crystalline-like particles on its surface is probably due to the formation of a sodium carbonate crystal when the geopolymer is exposed to air for a long time. The presence of chitosan particles for all concentrations become visible on the surface of the geopolymer with a particle size less than 50 μm, creating a boundary-like condition among the chitosan particles. The SEM results also confirmed that the geopolymer acted as a good binder for the chitosan particles.

The thermal and physical properties of the geopolymer-chitosan composite

The thermal properties of the geopolymer-chitosan composites were examined using differential scanning calorimetry (DSC). The objective of the measurement was to study the ability of the composite to absorb or release thermal energy as well as the phase change of the composite as a function of temperature. The DSC measurements were conducted at a temperature range between 30-400 °C with a heating rate of 20 °C min^{-1}. Figure 11 shows the DSC results of the geopolymer-chitosan composites with different concentrations of chitosan. It is well known that a geopolymer is an exothermic material and the presence of chitosan particles did not change this thermal property of the geopolymer.

The addition of chitosan in the structure of the geopolymer reduces the enthalpy magnitude of the geopolymer as shown in Table 2, indicating that chitosan is an endothermic material. The transition glass temperature at around 280 °C indicates the effect of the chitosan addition. This result indicates that the geopolymer-chitosan composite can still be able to resist temperature up to 400 °C.

The flexural strength of the resulting composites was examined by means of a three-point bending flexural strength measurement. The results are shown in Figure 12.

Table 2. The enthalphy of the geopolymer-chitosan composite (Onset 75 °C, End 237 °C).

No	Sample	ΔH (J·g^{-1})
1	Geo-chitosan 0.0 %	-386.12
2	Geo-chitosan 1.0 %	-355.42
3	Geo-chitosan 1.5 %	-330.41
4	Geo-chitosan 2.0 %	-317.79

Figure 12 showed that the addition of chitosan particles reduced the flexural strength of the geopolymer up to 8 % at the addition of 2 % of chitosan relative to the mass of metakaolin. This result suggests that the ability of the geopolymer paste as a binder decreases as the mass or volume of the chitosan increases. However, the flexural strength of the composite is still high enough for structural applications such as a pre-cast panel for walls.
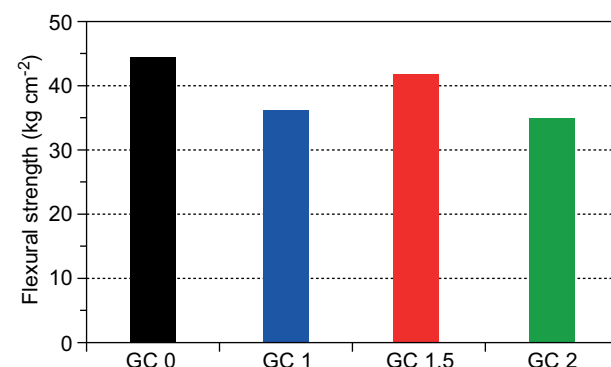

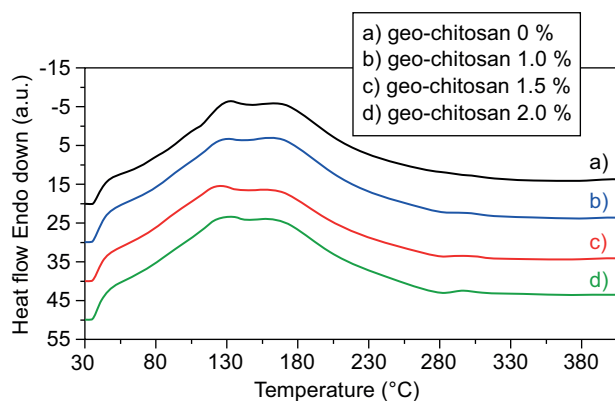Figure 12. Flexural strength of the geopolymer-chitosan composites.

Anti-bacterial examination

The ability of the geopolymer-chitosan composite to limit the growth of bacteria was tested using the Total Plate Count (TPC). This is an enumeration aerobic method, using a mesophilic organism which grows in aerobic conditions at low temperatures in the range of 20-45 °C. The specimens used in these measurements is 10 g of apples as shown in Figure 13. The samples were then homogenized in 90 ml of sterile distilled water. Table 3 showed the results of the TPC with dual repetition and stage dilution from 10-1 to 10-5 for all samples. The formation of bacteria colony as the results of the TPC method is shown in Figure 14.

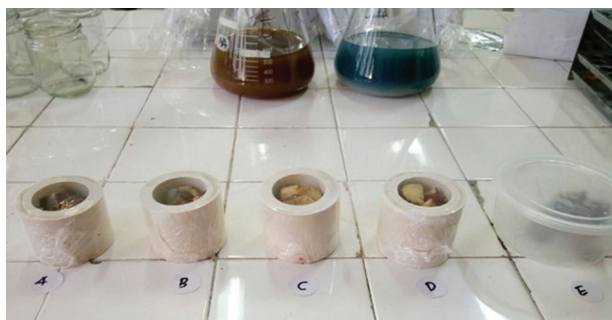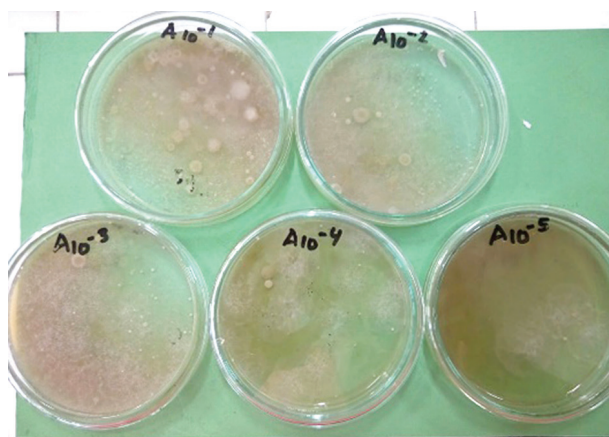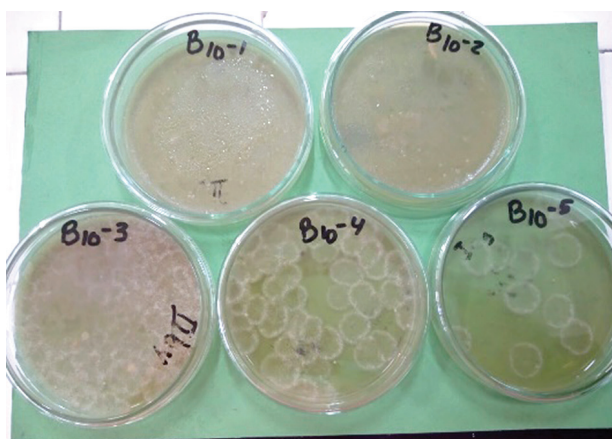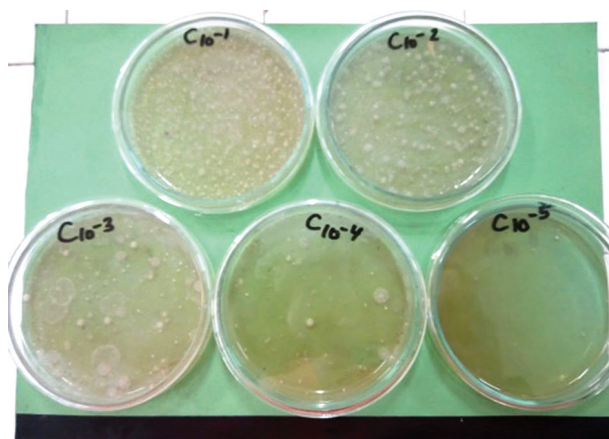Figure 11. DSC results of the geopolymer-chitosan composites.

Figure 13. Sample preparation for the anti-bacteria measurement using the TPC method: a) GC 0.0, b) GC 1.0 %, c) GC 1.5 %, d) GC 2.0 % of chitosan, and e) control.
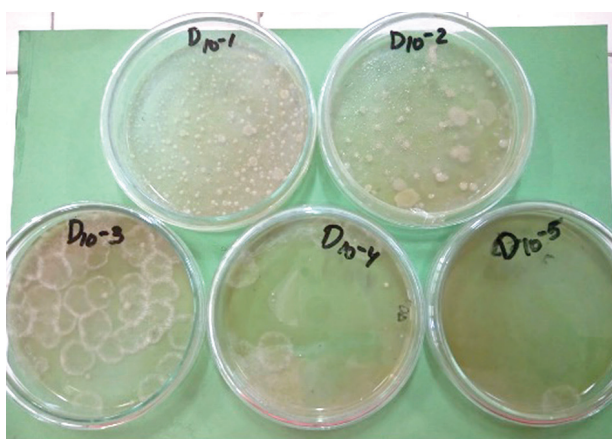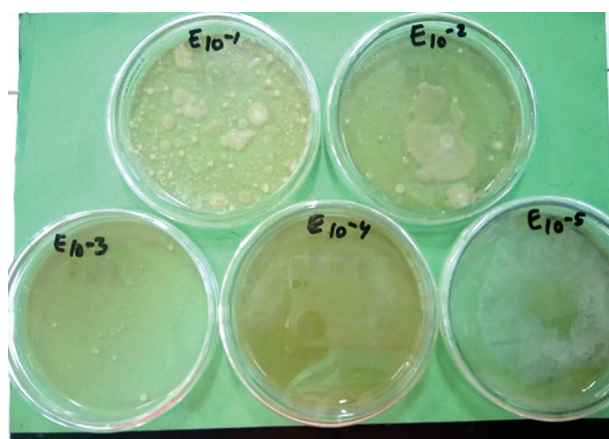

a)

b)

c)

d)

e)

Figure 14. Bacteria colony based on the TCP measurement of the geopolymer-chitosan composite..

Table 3. Data of the total plate count (TPC) method (TBUD = too much to count).

Sample	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}		TPC (cfu·ml^{-1})
	U1	U2	U1	U2	U1	U2	U1	U2	U1	U2	
A (GC 0.0)	TBUD	TBUD	TBUD	TBUD	251	238	80	57	37	30	1408 × 103
B (GC 1.0)	TBUD	TBUD	TBUD	TBUD	104	110	56	75	26	21	378 × 103
C (GC 1.5)	TBUD	TBUD	TBUD	TBUD	TBUD	TBUD	207	196	29	28	20 × 103
D (GC 2.0)	TBUD	TBUD	TBUD	TBUD	85	67	29	29	3	2	76 × 103
E (Control)	TBUD	TBUD	TBUD	TBUD	161	136	32	23	3	6	234 × 103

Figure 15. Photographs of the geopolymer-chitosan composites after heat treatment at 750 °C.

Based on Table 2, it can be seen that the presence of chitosan particles was able to limit the growth of the bacteria colony. The TCP value of the pure geopolymer is 1408×10^3 cfu·ml^{-1}. The addition of 1 % of chitosan resulted in a TCP value of 378×10^3 cfu·ml^{-1}. The lowest TCP value was found at the addition of 1.5 % chitosan, namely 20×10^3 cfu·ml^{-1}. The results confirmed that chitosan is an effective organic material to limit the growth of bacteria.

Geopolymer are well known to be a fire and heat resistance material and are able to withstand temperatures up 1200 °C. As a prototype material for limiting the growth of bacteria, the geopolymer-chitosan composites were subjected to a high temperature test, up to 700 °C for several hours. It was expected that up to this temperature, the composites are still able to retain their integrity even though the chitosan particle on the geopolymer surface will possibly get burnt. Figure 15 shows the condition of the geopolymer-chitosan composite after subjecting it to 750 °C for 4 h.

It can be seen that all samples remained dimensionally stable although significant cracks developed on the surface of the samples. Crack development on the surface of the samples was reduced at the addition of 1.5 % and 2 % of chitosan. The results suggest that chitosan is working as a heat absorber in the structure of the geopolymer composite.

CONCLUSIONS

Based on the microstructure as well as thermo-mechanical examinations of the resulting geopolymer-chitosan composites, it can be concluded that:

- Shrimp cells are an inexpensive, abundant, and renewable raw material to produce high quality and homogenous chitosan $(C_8H_{13}NO_5)_n$.
- The addition of chitosan into a geopolymer network results in a hybrid composite with high flexural strength.
- Chitosan was found to be an effective material to inhibit the growth of bacteria or fungi on the surface of the geopolymer.

- The geopolymer-chitosan composite is a material which is suitable for applications at temperature up to 700 °C without any structural damage.

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