Response to reviewer comments

Reviewer #1:

First of all, we would like to thank you for your valuable time to review our manuscript and provide helpful suggestion. All advices are very useful to complete our paper. Please find our replies in blue sentences.

1. Introduction

- Please add more literature reviews about mechanical properties, antibacterial performance and biocide migration of silver and/or zeomic/biopolymer composites. For making introduction become more relevant to the article.

Thank you for your kind suggestion. We have added more literature reviews about the antibacterial performance of silver zeolite in the introduction part (page#2 lines#32-33 and page#3 line#1-5) in the introduction part. The literature reviews about biocide migration and the mechanical properties of silver zeolite/polymer composites have been added in page#3 line#7-18 and page#4 line# 21-30, respectively.

- Please fix this incorrect symbol (□), this may lead to misunderstanding. Page 4 Line 53-54, "low (Si/Al □ 1), intermediate (Si/Al □ 2-5)"

Thank you for your suggestion. We have fixed the incorrect symbol in the introduction section already (page#3 line#20 and 21).

2. Experimental

Materials

- Please give more details concerning the physical properties of silver nitrate; particle size, etc. Particle size is very important for morphology results in this article.

We greatly appreciate the reviewer suggestion. The physical properties of silver nitrate especially particle size has been added in the material section (page# 5 line# 13).

Characterization of AgZSM-5/PBS composite films

Antibacterial activity

- The authors should report initial bacteria cell number in term of CFU/mL or cell turbidity. In case of comparison, initial bacteria should be similar for making sure that there is no other factor affect the result.

We would like to thank you for your suggestion. The initial bacterial cell number used in this work, 1×10^6 CFU/ml, has been added in the revised manuscript in the antibacterial test of AgZSM-5/PBS composite films (page#8 line#20).

- Please add bacterial strain code (ATCC, CIP, NCIB, etc).

The bacterial strain codes for *E. coli* and *S. aureus* have been added in page#8 line#8. The bacterial strain codes of *E. coli* and *S. aureus* are ATCC 8739 *and* ATCC 6538 P, respectively.

- Please add the equation of antibacterial reduction (%).

We have included the equation of bacterial reduction (%) in the revised manuscript as shown in equation 2 (page# 8 line# 24-29).

3. Results and discussion
Chemical composition
- Please give more explanation and/or add some references of Na peak intensity disappearance. How can size and shape affected the chemical composition?

Thank you for your question and suggestion. The discussion as well as the references about the Na peak intensity disappearance has been added in the chemical composition discussion section (page#10 line 19-24) as shown in red sentences. The size and shape did not affect the chemical composition of zeolite. The amount of cation in zeolite depended on the Si/Al ratio of zeolite. Zeolite with low Si/Al ratio contained more cation content than zeolite with high Si/Al ratio. The explanation and references about the effect of size and shape on the chemical composition have been added in page#10 line#24-29.

Chemical composition

The elemental composition of materials was investigated by energy-dispersive x-ray spectroscopy (EDX). Figure 2 presents the EDX spectra of zeolite before and after silver incorporation. The results showed that the main compositions of zeolite were silicon (Si), aluminium (Al), oxygen (O), and sodium (Na). However, the peak intensity of Na in ZSM-5 zeolite was not observed. It could be explained that ZSM-5 zeolite used in this study was in the form of ammonium (NH_4^+) which contained small quantity of NH_4^+ due to the high Si/Al ratio of ZSM-5 zeolite.[8] The nitrogen elements were not presented in EDX spectrum because the low intensity of nitrogen peak was overlapped with carbon peak from carbon tape which in generally used as the sample holder for EDX analysis.[21] The chemical composition of zeolite was unaffected by the zeolite morphology, size, including framework type. For example, zeolite A and X with their different framework types had the same Si/Al ratio of 1, or zeolite X and Y having the same framework type contained different Si/Al ratios.[7] The cation content in zeolite was; however, affected by the Si/Al of zeolite. Zeolite with low Si/Al ratio provided more cation content than zeolite with high Si/Al ratio. The EDX peak of silver (Ag) was observed in the zeolite after exchanging with the $AgNO_3$ solution (Figure 2b, 2d, and 2f), which confirmed the successful loading of silver into zeolite. The presence of silver in zeolite structure was due to the larger difference of silver concentration between outside and inside zeolite. The silver ion outside zeolite diffused inside and exchanged with cations (Na^+) in zeolite structure. The ion exchange reaction of zeolite (NaZ) was showed as follow:

$$Ag^{+} + NaZ \rightarrow AgZ + Na^{+} \quad (3)$$

In case of ZSM-5 zeolite, the silver ion could be loaded into the zeolite structure by exchanging with NH_4^+ instead of Na^+.[8] In addition, the Si/Al intensity ratio of three zeolites before and after silver loading was not different. This result indicated that the incorporation of silver into zeolite did not affect the amount of Si and Al elements.

- Please adjust the X axis (keV) in Figure 2 to be similar scale for comparing the intensities correctly.

Thank you for your suggestion. The X axis (keV) in Figure 2 has been adjusted into similar scale according to your advice already.

Morphology and crystal structure

- Please give more explanation and discussion about the sentence "After loading with silver, the zeolite crystal size and morphology did not change". Is there any interaction between silver and zeolite?

We are thankful for your comment. More information about the morphology and crystal structure of silver zeolite have been added in order to clarify this part as shown in page#11 line#19-20. The revised sentences are presented in red sentences. The interaction between silver and zeolite is electrostatic interaction. The discussion about the interaction between silver and zeolite has been added in page#11 line#18-19.

Morphology and crystal structure

The SEM micrographs of zeolite with and without silver are exhibited in Figure 3. The cubic, hexagonal, and irregular shape were found in ZA, ZY, and ZSM-5, respectively. The average particle size of zeolite was measured by ImageJ program. It was found that the particle size of three zeolites was different. The average particle size of ZA, ZY, and ZSM-5 was around 2.62, 0.90, and 0.20 μm, respectively. After loading with silver, the silver exchanged with cation in zeolite and attached to zeolite structure by electrostatic interaction. Incorporation of silver did not modify the zeolite morphology. The silver mapping of three silver zeolites is exhibited in Figure 3. The white points of silver atom indicated the presence and homogeneous distribution of silver in silver loaded zeolites. Figure 4 displays the XRD patterns of zeolite before and after silver loading at various $AgNO_3$ concentrations. The XRD patterns of pure zeolite (ZA, ZY, and ZSM-5) matched with the database of zeolite patterns from International Zeolite Association (IZA).[15] The diffractograms of zeolite after silver loading did not change compared with those of the original zeolites, only slight decrease in peak intensity when increasing

$AgNO_3$ concentration was noticed. The decreasing in XRD intensity might result from changes in the electron intensity distribution in unit cell of zeolite when cations in zeolite were exchanged with silver ions, causing the alteration of intensity in XRD patterns.[22] The results of SEM micrographs and XRD patterns revealed that the incorporation of silver did not influence on the zeolite structure modification. This finding was in good agreement with other reports.[1, 4]

- Please use the same magnifying SEM micrographs in Figure 3. Moreover, please point and define the area of Ag and zeolite in SEM micrograph.

The SEM micrographs in Figure 3 have been adjusted into the same magnification. The area of Ag cannot be pointed out or identified from these SEM images at this magnification because the amount of silver loading is very small, and the size of Ag is much smaller when compared with zeolite particle. However, we have added the EDX mapping of Ag in silver zeolite in Figure 3. This technique can identify the area of silver and investigate the homogeneous distribution of Ag in silver zeolite samples. The discussion about Ag mapping result has been added in page#11 line#20-21.

Antibacterial activity

- Figure 7 and 8, in case of comparison the colour and density of the same bacteria should be similar. Please clarify, why the colour of testing bacteria on agar dishes of figure a-c and d-f are entirely different?

Thank you for your comment and appreciated for your question. The color of testing bacteria of figure a-c and d-f are different because photos of control and silver zeolite sample plates were taken on slightly different backgrounds which led to the different color tone of picture. We have effortly adjusted the color tone of these photos into similar color tone as showed in Figure 7 and 8.

- Figure 9, the legend should not overlay across the columns. Moreover, please give more explanation about antibacterial performance of neat PBS (without AgZSM-5). How can neat PBS reduce S. aureus growth (25%) and reduce S. aureus higher than E. coli?

We are appreciated your suggestions. The position of the legend in Figure 9 has been adjusted. The explanation about the antibacterial performance of neat PBS against *S. aureus* and the discussion why neat PBS reduced *S. aureus* higher than *E. coli* have been added in the revised manuscript as showed in red sentences on page#18 line#14-24.

Antibacterial activity

The antibacterial behavior of PBS composite films containing different AgZSM-5 concentrations was performed against *E. coli* and *S aureus*. The colonies of bacterial strains before and after 24 h of incubation with films were counted. The percentage of bacterial reduction is exhibited in Figure 9. In the case of neat PBS film, there was no antibacterial activity for *E. coli* but surprisingly it can reduce *S. aureus* growth (25%). These results are different from other previous works that neat polymer films without antibacterial agent had no antibacterial activity.[1, 5, 33] The ability to reduce the *S. aureus* growth of the neat PBS film might be from the small amount of succinic acid, with antibacterial properties, which did not completely react in the production process of PBS polymer.[34] Neat PBS films inhibited the growth of *S. aureus* higher than *E. coli* because *S. aureus* (gram-positive bacteria) does not contain outer membrane layer; therefore, thick peptidoglycan layer of bacterial cell wall can easily absorb the succinic acid, leading to the cell destruction. However, *E. coli* (gram-negative bacteria) contains outer membrane layer, covering thin peptidoglycan layer of bacterial cell wall, which can interrupt the penetration of succinic acid through the bacterial cell.[35] For the AgZSM-5 loaded PBS composite films, 99.9 % of bacterial reduction could be observed after 24 h of incubation due to the bactericidal effect by silver ion from PBS samples. The PBS composite films containing just only 0.5 %wt of AgZSM-5 could show high antibacterial performance.

Mechanical properties

- Figure 11, b-f, the authors should point out that aggregation of zeolite particles lead to poor mechanical properties.

Thank you for your suggestion. We have added the arrow into each SEM micrograph in order to point out the aggregation of zeolite particles in Figure 11 b-f already.

4. Conclusions

- The discussion should not be in conclusions section because the authors have already discussed in results and discussion section.

We have revised the conclusion section by deleting the discussion already, as showed in red sentences in conclusion section (page#22).

- How did authors know about the optimum content of AgZSM-5 in PBS films was 0.5%wt? The authors haven't varied the concentration of silver less than 0.5 %wt.

We are highly appreciated your kind recommendation. We have adjusted the explanation (page#22 line#10-12) in order to give better understanding in this point. Actually, the optimum content of AgZSM-5 in PBS films that we mentioned was solely based on the scope of this research and also following the EFSA regulation. Accordingly, base on this work, PBS films at 0.5%wt of AgZSM-5 loading should be the appropriate condition because the silver migration from 0.5AgZSM-5/PBS films was less than EFSA regulation. If the amount of AgZSM-5 content was less than 0.5%wt, the amount of silver migrated from PBS films would be low, leading to the short-term or less effective antibacterial activity of material when compared with PBS composite films with 0.5%wt of AgZSM-5 loading.