PREPARATION OF ANTIMICROBIAL PAPER BY MICROWAVE-ASSISTED TWO-POT *IN-SITU* DEPOSITION OF ZINC OXIDE ON FILTER PAPER

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Received June 20, 2020

We employed a microwave-assisted two-pot *in-situ* deposition technique to incorporate zinc oxide particulates in the structure of filter paper to produce antimicrobial paper. The process involved successive immersion of filter paper samples in $ZnSO_4$ (precursor solution) and NaOH (precipitating agent) to form $Zn(OH)_2$, which transformed into ZnO during microwave treatment. Successful deposition of ZnO particles on the filter paper was confirmed via X-ray diffraction and the corresponding morphologies were observed using field emission scanning electron microscopy. The ZnO-deposited papers were tested for antimicrobial activity and were found to be more effective against *Staphylococcus aureus* (gram-positive) than *Escherichia coli* (gram-negative). Bacterial populations were reduced by up to 92 ± 2% and 57 ± 4% for *S. aureus* and *E. coli*, respectively. Also, it was found that the samples prepared using higher concentrations of $ZnSO_4$ and NaOH exhibited better antimicrobial properties.

Keywords: filter paper, *in-situ* ZnO deposition, two-pot process, microwave treatment, antimicrobial activity

INTRODUCTION

An approach to minimizing the spread of communicable diseases is through incorporation of antimicrobial agents on different surfaces. The process of incorporating antimicrobial agents might be challenging for some surfaces, especially if the surfaces are inert and smooth. Alternatively, difficult-to-functionalize surfaces can be covered with antimicrobial paper. Having the inherent flexibility of paper, antimicrobial paper can be easily tailored to fit a wide variety of surfaces; it can be cut into different shapes and sizes, as well as folded and bended. Paper is a material composed mainly of cellulose, which is sensitive to bacterial decomposition. The incorporation of antimicrobial agents into the internal structure of paper not only gives rise to antimicrobial properties, it can also lengthen the decomposition time of paper.

A wide variety of organic and inorganic materials have been used to produce antimicrobial paper. Organic antimicrobial agents include alcohol (isopropyl alcohol) and those that have been derived from animals and plants (such as oil,[1-2] chitosan[3-4] and its derivatives[5]). Tissue papers with alcohol are already available on the market. They have been deemed effective in killing a wide range of microorganisms. However, alcohol is volatile and thus easily evaporates. These wet tissue papers need always to be kept in a sealed container for the organic solvent to remain on the paper. Animal and plant derived antimicrobial agents are used when the paper is intended to come in direct contact with our mouth or with food, such as food wrappings. Oils extracted from animals and plants also evaporate, thus reducing the lifetime of the antimicrobial

paper. Inorganic antimicrobial agents include metal and metal oxides.[6-8] Inorganic antimicrobial agents are more stable, compared to their organic counterparts, and thus can produce papers with longer antimicrobial lifetime. Silver is a known inorganic antimicrobial agent. Silver-incorporated papers are used as packaging for eating utensils. However, the raw materials needed to incorporate silver into paper are expensive. The raw materials for zinc oxide are a cheaper alternative. Zinc oxide is generally recognized as safe by the U.S. Food and Drug Administration (21CFR182.8991). It has been used in different health and wellness products.

ZnO-incorporated papers have been a topic of much research interest in recent years.[9-18] ZnO particles can be incorporated into the bulk of the paper product during the manufacturing process or embedded locally on the surface of pre-fabricated paper, where they can interact directly with the environment. The latter can be done either by direct assembly of synthesized ZnO particles or by *in-situ* assembly. In *in-situ* assembly methods, particles are synthesized as they are embedded into the paper. The synthesis and embedding of particles can be done by immersing the substrate into a pot containing the needed precursors. Using only one vessel, however, can be disadvantageous, as the desired particles may form not only on the substrate, but in the entire solution. This problem can be avoided by using two vessels. The first vessel contains a precursor, while the second vessel contains a solution that facilitates the required chemical reaction. In this two-pot method, the precursors trapped in the pores of the paper substrate serve as seed for growth and synthesis of the desired particles on and near the surface of the substrate.

The *in-situ* assembly methods described above require intensive heat treatment. However, prolonged exposure to intense heat can potentially damage the paper substrates. Hence, the necessary chemical reactions must be carried out within a short span of time, in no more than a few minutes, such as in a microwave oven. Microwaves have the ability to transfer energy directly to the reactive components. This is not possible in the conventional heating methods.

In this study, we prepared antimicrobial active paper by *in-situ* generation and incorporation of ZnO particles on filter paper. In particular, we used a microwave-assisted two-pot *in-situ* deposition technique. The antimicrobial activity of the ZnO-deposited papers was tested against *Staphylococcus aureus* and *Escherichia coli*. The former represents gram-positive bacteria, while the latter – gram-negative bacteria. The effect of different preparation parameters (precursor concentration, precipitating agent concentration and microwave exposure time) on the antimicrobial properties of the functionalized papers was also examined.

EXPERIMENTAL

Materials

The substrates used were Whatman No. 1 filter papers. The precursor and precipitating agent used were zinc sulfate heptahydrate salts (AR, HiMedia Laboratories Pvt. Ltd.) and sodium hydroxide powder (AR, Macron Fine Chemicals), respectively.

Microwave-assisted two-pot *in-situ* deposition of zinc oxide on filter paper

Filter papers were dipped successively in the $ZnSO_4$ and NaOH solutions at different soaking times. Afterwards, the samples were subjected to microwave irradiation. During the treatment, the papers were immersed in water to avoid being burned. The microwave oven (General Electric model JEI2340WPSL) used had a pre-set power of 540 W.

Characterization

Successful deposition of ZnO particles on the filter papers was confirmed using X-ray diffraction (Shimadzu XRD-6100). The morphology of the samples was observed using a Field Emission Scanning Electron Microscope (FEI Helios Nanolab 600i FESEM). Mass differences of the samples prepared using different $ZnSO_4$ and NaOH concentrations, as well as soaking times, were noted.

Antimicrobial test

The antimicrobial properties of the samples were determined using the standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions (ASTM E2149-01). The test organisms used were *Staphylococcus aureus* (BIOTECH 1582) and *Escherichia coli* (BIOTECH 1634). In this method, the test culture was incubated in nutrient broth (Difco: 8 g/L), which in turn was diluted in a sterilized 0.3mM phosphate buffer. To obtain the working bacterial dilution of 1.5-3.0 x 10^5 colony forming units (CFU)/mL, subsequent dilution was made. The Mcfarland standard was used in the preparation of the test organisms.

The paper samples that were analyzed were ~0.2 g. The paper samples were placed in a 50 mL working bacterial dilution, and then incubated for 1 hour at 37 °C (with shaking at 120 rpm). Using a buffer solution, serial dilution was again done. After which, 1 mL was

plated onto Nutrient Agar. The plated agar was incubated at 37 °C for 24 hours. The antimicrobial property of the sample was expressed as the percent reduction of the test organism after one hour of contact with the paper sample, as compared to the number of organism cells after contact with the control.

RESULTS AND DISCUSSION

The microwave-assisted two-pot technique involved successive soaking of the filter papers into the precursor ($ZnSO_4$) and precipitating agent (NaOH) solutions. The precursor solution in the first pot contained zinc ions:

$$ZnSO_4 \text{ (aqueous solution)} \rightarrow Zn^{+2} + SO_4^{-2} \quad (1)$$

These ions are small enough to penetrate the pores of the paper. As the filter papers are soaked in the precursor, a considerable amount of $ZnSO_4$ adheres to the substrates. On the other hand, the precipitating agent in the second pot contained OH ions:

$$NaOH \text{ (aqueous solution)} \rightarrow Na^{+1} + OH^{-1} \quad (2)$$

When dipped in the second solution, Zn ions on the substrate react with the OH ions to form zinc hydroxide:

$$Zn^{+2} + OH^{-1} \rightarrow Zn(OH)_2 \quad (3)$$

Figure 1a shows the XRD pattern of a filter paper that has been immersed in the two solutions without undergoing microwave treatment. The peaks at 14.5, 16.1 and 22.5° (labeled 'x') correspond to the $(1\bar{1}0)$, (110) and (200) planes of cellulose, respectively. The peaks at 25.8 and 33.6° correspond to (011) and (211) planes of ε-$Zn(OH)_2$, while the peak at about 30.2° may be attributed to metastable β-$Zn(OH)_2$. These peaks associated to the presence of $Zn(OH)_2$ are labeled 'y'.

During the microwave treatment, the filter papers were kept in a water bath to prevent them from burning. Two possible reactions may have occurred in the process:

$$Zn(OH)_2 \rightarrow ZnO + H_2O \quad (4)$$

$$Zn(OH)_2 + O_2 \rightarrow ZnO + H_2O + O_2 \quad (5)$$

In both cases, $Zn(OH)_2$ changed into ZnO. The XRD pattern of a sample subjected to microwave treatment for 8 minutes is shown in Figure 1b. We observe the same peaks corresponding to cellulose. We also find new peaks (labeled 'z') corresponding to the (100), (002) and (101) planes of zinc oxide. These peaks located at 31.7, 34.5 and 36.1° confirm successful deposition of ZnO on the substrates.

Soaking times

First, we examined the effect of varying $ZnSO_4$ soaking times on the functionalized papers by noting changes in the mass of the filter papers before and after deposition (Fig. 2). In sample preparation, 350 mM $ZnSO_4$ and 700 mM NaOH solutions were used. The substrates were immersed in $ZnSO_4$ at pre-determined times, soaked in NaOH for one minute, and subjected to microwave treatment for 10 minutes.

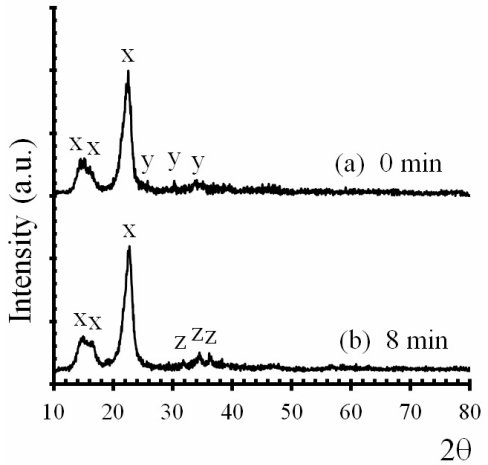


Figure 1: X-ray diffraction pattern of samples prepared with (a) 0 min and (b) 8 min microwave treatment. Peaks labeled 'x', 'y', and 'z' are indicative of cellulose, $Zn(OH)_2$, and ZnO, respectively (preparation conditions: 350 mM $ZnSO_4$, 700 mM NaOH, 1 min soaking time, and eight min microwave exposure time)

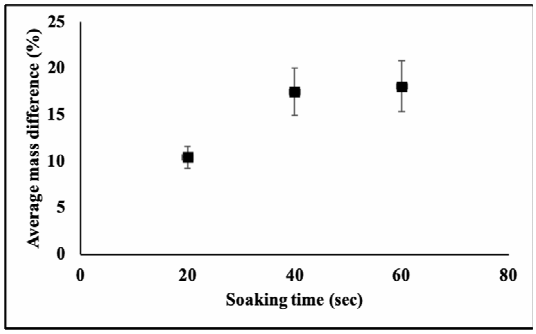


Figure 2: Average mass differences of functionalized papers prepared using different $ZnSO_4$ soaking times under the following conditions: 350 mM $ZnSO_4$, 700 mM NaOH, 1 min NaOH soaking time, and 10 min microwave exposure time

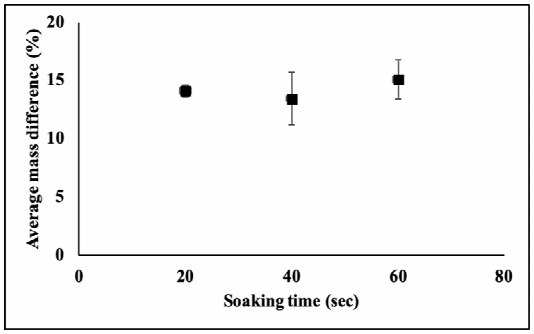


Figure 3: Average mass differences of functionalized papers prepared using different NaOH soaking times under the following conditions: 350 mM $ZnSO_4$, 700 mM NaOH, 1 min $ZnSO_4$ soaking time, and 10 min microwave exposure time

On the average, the mass of the filter papers dipped in the precursor solution for 20 seconds increased by 10%. When the $ZnSO_4$ soaking time was doubled, the samples registered a mass difference of about 15-20%. A larger positive mass difference could mean that more ZnO particles have been embedded on the filter papers. However, prolonged immersion in the precursor solution does not necessarily imply greater deposition of ZnO particles. There is no significant difference in the observed mass gain of the substrates soaked in $ZnSO_4$ for 40 and 60 seconds.

Next, we modified the length of time the samples were immersed in the precipitating agent solution, while fixing the $ZnSO_4$ soaking time to one minute (or 60 seconds). Likewise, 350 mM $ZnSO_4$ and 700 mM NaOH solutions were used in the preparation samples and microwave exposure time was set to 10 minutes. Data showed no significant change in the amount of mass gained by the substrates with increasing NaOH soaking time (Fig. 3). After immersion in the precursor solution, the substrates could have been close to saturation and could no longer absorb more NaOH, even if soaking times were extended.

$ZnSO_4$ concentration

We varied the concentration of the precursor solution, while keeping all the other preparation parameters constant. Results showed that increasing the $ZnSO_4$ concentration has no direct effect on the amount of additional mass acquired by the substrates during the embedding process (Fig. 4). The solubility of $ZnSO_4$ may have been too high (at 100 mg/1 mL or ~348 mM zinc sulfate solution) to allow distinct detectable difference in the amount of deposited mass. However, in terms of antimicrobial activity against *S. aureus* and *E. coli*, the samples prepared using higher concentrations of $ZnSO_4$ were more effective in reducing bacterial populations (Table 1). After one hour of contact, *S. aureus* and *E. coli* populations decreased by up to 92 ± 2% and 57 ± 4%, respectively. The antimicrobial properties observed for the samples prepared without $ZnSO_4$ may be attributed entirely to sodium hydroxide, which is a known disinfectant.

NaOH concentration

In a similar way, we prepared samples using different NaOH concentrations. The mass gain of the functionalized papers, due to embedded particles, was greater at higher NaOH concentrations (Fig. 5). This may be attributed to the presence of more hydroxyl ions (OH-), which triggered the formation of $Zn(OH)_2$, which is necessary to produce ZnO.

The effects of varying NaOH concentrations on the antimicrobial activity of the samples are summarized in Table 2. The samples that were not soaked in NaOH solution, but were immersed in $ZnSO_4$ solution and subjected to microwave treatment, were able to reduce the population of *S. aureus* and *E. coli* by 32 ± 6% and 17 ± 2%, respectively, after one hour of contact. This may be due to residual Zn-based materials that have accumulated on the substrates during the treatment. Data also indicate better antimicrobial activity in samples produced using higher concentrations of NaOH. The papers

functionalized using 700 mM NaOH precipitating agent were able to reduce *S. aureus* and *E. coli* populations by 89 ± 2% and 43 ± 3%, respectively.

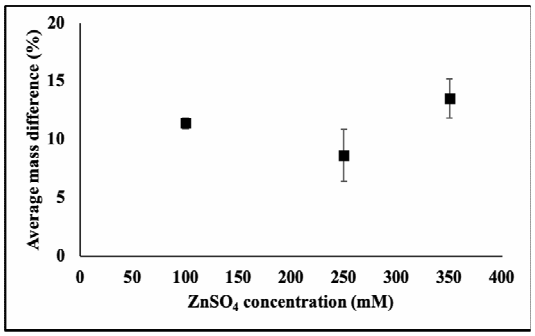


Figure 4: Average mass differences of functionalized papers prepared using different concentrations of $ZnSO_4$ under the following conditions: 700 mM NaOH, 1 min soaking time, and 10 min microwave exposure time

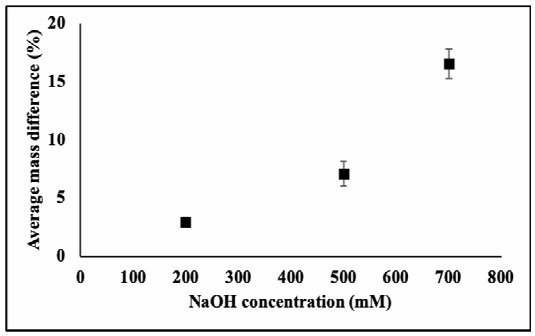


Figure 5: Average mass differences of functionalized papers prepared using different concentrations of NaOH under the following preparation conditions: 350 mM $ZnSO_4$, 1 min soaking time, and 10 min microwave exposure time

Table 1
Antimicrobial activity of samples prepared using different concentrations of $ZnSO_4$ under the following conditions: 700 mM NaOH, 1 min soaking time and 10 min microwave exposure time

$ZnSO_4$ concentration (mM)	Percent reduction (%)	
	S. aureus	*E. coli*
0	22 ± 2	13 ± 3
100	76 ± 2	37 ± 2
250	92 ± 2	57 ± 4
350	90 ± 2	49 ± 3

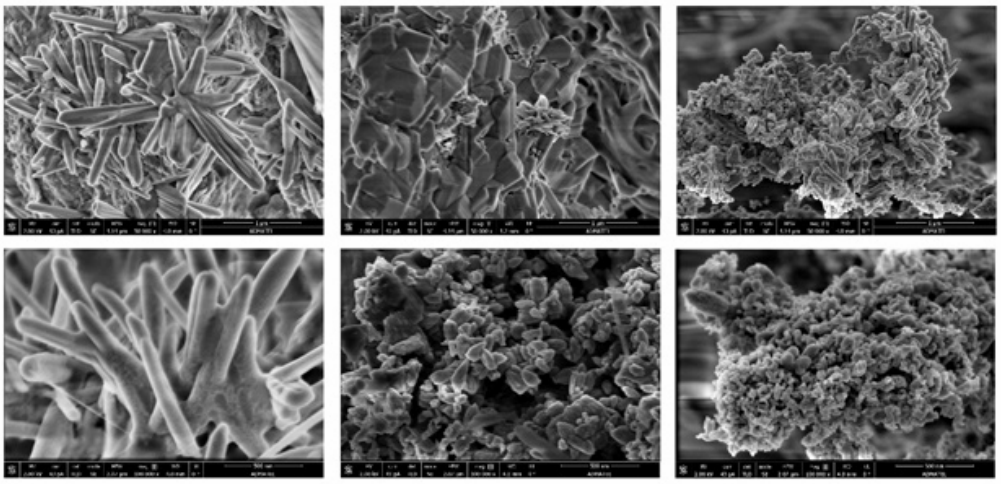
Table 2
Antimicrobial activity of samples prepared using different concentrations of NaOH under the following conditions: 350 mM $ZnSO_4$, 1 min soaking time and 10 min microwave exposure time

NaOH concentration (mM)	Percent reduction (%)	
	S. aureus	*E. coli*
0	32 ± 6	17 ± 2
200	73 ± 4	31 ± 2
500	88 ± 4	38 ± 2
700	89 ± 2	43 ± 3

Microwave treatment

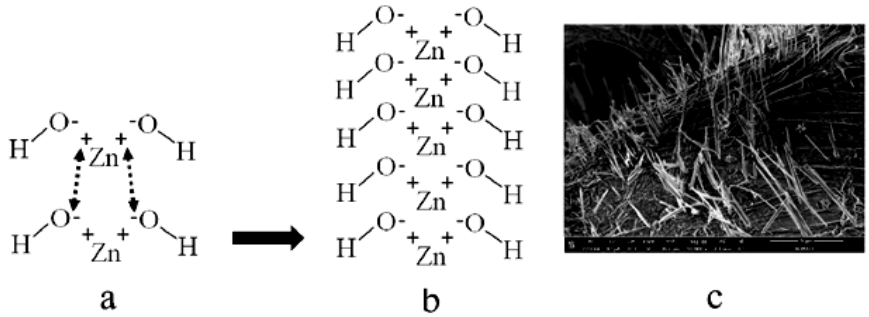
Figure 6 presents scanning electron micrographs of samples in different stages of microwave treatment, illustrating how $Zn(OH)_2$ is changed to ZnO. $Zn(OH)_2$ and ZnO have different microstructures. The structures of $Zn(OH)_2$ are rod-like (Fig. 6a), excessively elongated in one direction. This rod-like structure of $Zn(OH)_2$ is attributed to the large probability that the other $Zn(OH)_2$ particles orient along the Zn and O atoms due to electrostatic considerations (Fig. 7). Meanwhile, the structures of ZnO are particle-like (Fig. 6b, 6c), which have no excessive elongation in any of the crystallographic directions.

Prior to the microwave treatment, the samples showed antimicrobial activities against *S. aureus* and *E. coli* by reducing the respective bacterial populations by 38 ± 5% and 23 ± 2% after one hour of contact (Table 3). This is due to $Zn(OH)_2$ deposited on the substrates. The transformation of $Zn(OH)_2$ into ZnO by the microwave treatment significantly enhanced the antimicrobial properties of the paper. The populations of *S. aureus* and *E. coli* decreased by as much as 86 ± 3% and 42 ± 2%, respectively.



(a) 0 min (b) 8 min (c) 10 min

Figure 6: Scanning electron micrographs of samples at (a) 0 min, (b) 8 min and (c) 10 min microwave exposure times, at magnifications of 50,000x (top) and 100,000x (bottom); preparation conditions: 350 mM $ZnSO_4$, 700 mM NaOH and 1 min soaking time



a b c

Figure 7: Probable mode of interaction of (a) hydroxide and zinc ions to form (b) $Zn(OH)_2$ rods, as shown in (c) scanning electron micrograph of $Zn(OH)_2$ on paper at 10,000x magnification

Table 3
Antimicrobial activity of samples using different microwave exposure times under the following conditions: 350 mM $ZnSO_4$, 700 mM NaOH, and 1 min soaking time

Microwave exposure time (min)	Percent reduction (%)	
	S. aureus	*E. coli*
0	38 ± 5	23 ± 2
6	80 ± 3	39 ± 5
8	86 ± 3	37 ± 3
10	84 ± 4	42 ± 2

Antimicrobial property

In general, the ZnO-deposited papers exhibited greater antimicrobial activity towards *S. aureus* than *E. coli.* Higher antimicrobial activities of some materials against *S. aureus*, compared to *E. coli*, have also been observed and reported earlier.[19-22] These antimicrobial activities may have arisen from zinc ions released slowly from the zinc oxide and absorbed by the cell.[23] Within the intracellular compartment of the cell, metal ions may interact directly with functional groups of proteins and nucleic acids, inhibiting the growth of the microorganism, by interfering with normal processes and changing the structure of the cell. *E. coli* is a gram-negative bacterium. Its cell wall is composed of a thin layer of peptidoglycans, surrounded by an outer membrane containing lipoproteins, phospholipids,

and lipid polysaccharides (LPS). This barrier allows the entry of only certain macromolecules. *S. aureus*, on the other hand, is a gram-positive bacterium. Although it is surrounded by layers of peptidoglycans, which are many times thicker than that found in gram-negative bacteria, it lacks an outer membrane. Furthermore, gram-positive bacteria have an abundance of pores, allowing the penetration of foreign materials.

CONCLUSION

We have successfully prepared antibacterial paper using microwave-assisted two-pot *in-situ* deposition of zinc oxide on filter paper. In this technique, $ZnSO_4$ was used as precursor, while NaOH served as precipitating agent. Sequential dipping in $ZnSO_4$ and NaOH allowed the formation and deposition of $Zn(OH)_2$ in the substrates. Afterwards, the samples were subjected to microwave treatment to facilitate the transformation of $Zn(OH)_2$ to ZnO. Of the different preparation parameters, only the variations in NaOH concentration resulted in distinguishable changes in the masses of the samples and, by inference, the amount of deposited ZnO particles. Compared to the one-pot technique, this two-pot technique localizes the *in-situ* deposition of zinc oxide on or near the surface of the paper, instead of in the entire solution, thus avoiding wastage of raw materials and minimizing formation of by-products. The two-pot technique may be employed in the deposition of other metal oxides on paper or on other cellulose-based materials.

The zinc oxide-deposited papers were found to be effective against *S. aureus* and *E. coli.* The samples prepared using higher concentrations of $ZnSO_4$ and NaOH showed better antimicrobial properties. The ZnO-deposited papers were able to reduce bacterial populations by as much as 92 ± 2% for *S. aureus* and 57 ± 4% for *E. coli* after one hour of contact. In the future, the zinc oxide-deposited papers can be tested against other species of bacteria and fungi. In general, antimicrobial paper can be tailored to become surface cover, patches, linings, curtains, mats and many others, which can potentially minimize the spread of pathogens.

ACKNOWLEDGEMENT: This research is funded by Philippine Council for Industry, Energy, and Emerging Technology Research and Development (PCIEERD) Grant-In-Aid Program with project number 04013.

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