CHAPTER VI
PERMEABILITY AND ANTIBACTERIAL ACTIVITIES OF POLYPROPYLENE NANOCOMPOSITE FILMS

6.1 Abstract

Effect of OBEN-CuNP on barrier properties and antibacterial activity of polypropylene nanocomposite blown film was investigated. Water vapor permeability and oxygen permeability of blown film samples were studied in accordance to ASTM E398 and ASTM D3985, respectively. Antibacterial activity was studied by the agar diffusion test. Having ionic aggregates, the PP film was less water vapor permeate due to increasing in crystallinity. Organoclay caused flow path of water vapor to be more tacttoids and thus reduced water vapor flow rate compared to homogeneous PP matrix. Adding OBEN-CuNP into PP films increased water vapor permeability due to hydrophilicity of PVP coated on CuNP. In contrast, all PP films adding Surlyn or nanoparticles increased oxygen permeability because there were flow channels at the interfaces between Surlyn and PP matrix. From the agar diffusion tests, it is found that there were inhibition zones surrounding OBEN-CuNP pastes. However, the clear zone was not observed when using film samples which would be result from very low diffusivity of copper ion from the film samples into agar.

6.2 Introduction

The retail food market in Thailand is changing rapidly; the number of shops handling a narrow range of foods is decreasing, while supermarkets and large self service stores are increasing in number and size. There is considerable scope for expanding the market for fish through these modern outlets, provided a good quality product is attractively presented for sale in a suitable package. Some large stores are selling wet fish from a traditional slab, with a fishmonger in attendance, but this method has a number of disadvantages; a large space is needed, the slab has to be manned continuously, the products are inconvenient to handle and there may be some smell and possibly contamination. In contrast, properly packaged fish products can be easily handled by nonspecialist staff, examined by the shopper for type, quantity and price, purchased and carried home in the shopping basket with other foods. Prepacked chilled fish have been sold for many years from frozen food cabinets containing other foods. Prepacking is mainly a method of presentation, not of preservation; the shelf life of a wrapped wet fish product is virtually the same as that of an unwrapped one. There is sometimes a small increase in shelf life, but not enough to justify keeping prepacked fish longer in the shop. The most useful materials for making small packages of chilled fish are the thin flexible films produced mainly from plastics such as polyethylene, polypropylene, and polyvinyl chloride.

Chilled fresh fish requires the protection of a reasonably good barrier to water vapour to prevent it drying. Water vapour can pass through a film in two ways; the film may be porous so that vapour passes through holes in the material, or the film may be permeable, that is water vapour diffuses through it by dissolving in the material. Thin films are often porous, but porosity can be overcome by using a thicker film; however, a permeable film cannot be made impermeable in this way. Gases like oxygen or carbon dioxide for example are transmitted through a film in much the same way as water vapour.

Recent developments in polymer nanocomposites have attracted attention to the possibilities offered by this technology to enhance the barrier properties of inexpensive resins. There have been several studies demonstrated improvements in permeability reduction to gases, moisture, and organic vapors resulting from the addition of low concentrations of layered clay nanoparticles to various thermoplastic matrices. Preparation of PP nanocomposites due to PP high hydrophobicity should be performed in the presence of a compatibilizer to improve the affinity of the OMMT towards polymer matrix. Currently, PP-g-MA has been studied and widely used in many diverse applications. However, the presence of impermeable layers like clay in the polymer matrix can increase the tortuous path of the permeant and enhances the barrier properties. But the enhancement in these properties depends on the morphology and orientation of the clay platelets in the sample. Polypropylene nanocomposites are multiphase systems in which the coexistence of phases with different permeabilities can create some complex transport phenomena. One can assume that transport only occurs in the permeable phase, providing one phase is permeable to the penetrant, or shows a much higher permeability compared to the other phases. In semi-crystalline polymers, the crystalline regions are considered to be gas impermeable, therefore, by increasing the crystallinity, the decrease in solubility attributed to amorphous volume and diffusion reduction due to more tortuous path for the diffusing molecules.

Recent studies on the transport properties of PP/organoclay nanocomposites for various organic vapors have reported. Villaluenga *et al.* (2007) reported that an appreciable reduction in gas permeability with the addition of both the unmodified and modified nanoclay compared to the unfilled PP film was achieved. For helium, diffusion rather than solubility was responsible for the reduction in the permeability, in contrast, for nitrogen and oxygen, both diffusivity and solubility were reduced by the presence of fillers. This reduction may be attributed to the tortuous path for diffusing gas molecules and the reduced molecular mobility of polymer chains due to the presence of filler particles. In addition, for more condensable gases, the interactions along the clay-PP matrix interface may affect the gas sorption process in the polymer membrane. Mirzadeh *et al.* (2007) reported the decrease of oxygen permeability coefficient in PP/Clay nanocomposite films attributed to the quantity of organoclay and compatibilizer and their influence on morphology and orientation of silicate layers

In contrast, Dumont *et al.* (2007) reported that the unmodified PP exhibits fairly better barrier performance than its mixtures with PP-g-MA and/or OMMT for water vapor, toluene and methanol. The only improvement of barrier properties in their work was obtained for the helium barrier properties. They explained the negative effect on barrier properties of PP film by the interactions occurring between the diffusing molecules and the inherently hydrophilic clay or the quaternary ammonium salt used as a surfactant in the organically modified silicate layers.

Several test methods have been developed to determine the efficacy of antimicrobial film packaging. The tests to evaluate the antibacterial properties generally fall into two categories: agar diffusion test (qualitative method) and dynamic shake test (quantitative method). The bacterial species Escherichia coli (Gram negative), Staphylococcus aureus (Gram positive) and Klebsiella pneumoniae (Gram negative) are used in most test methods. The agar diffusion tests are only qualitative, but are simple to perform and are most suitable when a large number of samples have to be screened for the presence of antimicrobial activity. In this method, the agar surface is inoculated by making a parallel streak, and then the sample is pressed onto the inoculated plate. The method is used for obtaining an estimate of activity, in that the growth of the inoculum organism decreases from one end of each streak to the other and from one streak to the next resulting in increasing degrees of sensitivity. After incubation at 37 ± 2 °C for 18–24 hours, a clear area of interrupted growth (clear zone) underneath and along the sides of the test material indicates antibacterial activity of the specimen. The difference in zones of inhibition does not necessarily mean that a specimen is more biocidal or less. The zone of inhibition depends on the migratory property of the antibacterial agent to diffuse into the agar; hence, it does not depend only on the strength of the biocidal agent.

In this research, Bentonite organoclay and copper nanoparticles were added into polypropylene films for barrier property and antimicrobial activity. Fabrication of nanocomposite films was performed via a water-cooled blown film extrusion typically used to produce clear films for food packaging. Effect of CuNP content (5, 10, 15 and 20 wt% of total filler) on barrier properties and antibacterial activity of PP nanocomposite blown films were investigated ad reported in this chapter.

6.3 Experimental

6.3.1 Materials

Polypropylene homopolymer (PP 1102K) pellets with MFI of 4 g/10 min (190 °C, 2.16 kg) was purchased from IRPC Co. Ltd., Thailand. Additives such as slip and antiblock agents were not added. Bentonite organoclay (OBTN) was prepared in our laboratory by using sodium activated bentonite (kindly supplied by Thai Nippon Co., Ltd., Thailand) and distearoylethyl hydroxyethylmonium methosulfate and cetearyl alcohol. Surlyn® P350, sodium neutralized ethylene-methacrylic acid ionomer, with MFI of 4.5 g/10min (190 °C, 2.16 kg) was purchased from Dupont™, USA. Polyvinylpyrrolidone (PVP, MW 40000) and copper (II) nitrate (Cu(II)NO_3.$5H_2O$) was purchased from Sigma-Aldrich, Germany. L-ascorbic acid was purchased from Ajax Finechem, Australia.

6.3.2 Preparation of OBEN/CuNP Masterbatch

The masterbatch of OBEN/CuNP was prepared by mixing dried OBEN/CuNP mixture with Surlyn® P350 in a ratio of 1:2 by weight, in a Haake Rheomex PTW-16 co-rotating twin-screw extruder with D = 16 mm and L/D = 25. The operating temperature of extruder was set at 180 °C with a screw speed of 70 rpm. Extrudate was pelletized for further mixed with PP pellets in a ratio of PP:OBEN/CuNP = 99:1 wt%.

6.3.3 Preparation of Neat PP and PP Nanocomposite Films

Neat PP and PP nanocomposite blown film samples were fabricated using a water-quenched blown film extruder (PP50, Thailand). Temperature profiles were 210, 220, 220, and 210 °C from feed zone to die. Screw speed was kept constant at 80 rpm. Bubble forming ring of 23 cm in water bath was used, which final width of blown film was about 22-23 cm. Nip-roll speed and pull-out speed were adjusted to produce blown films with final thickness of 40-50 μm.

6.3.4 Permeability of Neat PP and PP Nanocomposite Films

Vapor Permeation Tester Model L80-4000, LYSSY was used to determine water vapor permeability of neat PP and PP nanocomposite blown films. Water vapor permeation experiments were investigated following procedure described in ASTM E398. The test was performed at 38°C with water vapor pressure of 49.7 mmHg. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over test area.

Oxygen Permeation Analyzer Model 8000, Illinois Instrument Inc., was used to determine oxygen permeability of neat PP and PP nanocomposite blown films. Gas permeation experiments were investigated following procedure described in ASTM D3985. The test was carried out at 23°C with oxygen flow rate of 40 cm^2/min. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured by using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over test area.

6.3.5 Antimicrobial Activity of PP and PP Nanocomposite Films

Bacterial sensitivity to antibiotics was carried out using the agar diffusion test. Prior to mixing, sample powder and distilled water were autoclaved at 120 °C. Paste of OBEN and OBEN-CuNP were prepared by mixing 0.2 gram of sample powder in 50 μl distilled water. The Escherichia coli (E.coli) suspension (100 μl of $10^4 - 10^5$ CFU ml^{-1}) was applied uniformly on the surface of nutrient agar plate, and then five holes were cut on the agar plate for placing the powder paste. Distilled water was put into the center hole as the control reference. Each sample (OBEN, OBEN-CuNP5, OBEN-CuNP10, OBEN-CuNP15, and OBEN-CuNP20) were put into two holes in the plates. The plates were incubated at 35 °C for 24 h, after which photo of the inhibition zone (clear zone) surrounding the paste was taken for comparison. The same procedure was also performed using small pieces of neat PP and PP composite blown films as the antibiotics.

The modified test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions was also carried out in order to investigate antimicrobial activity in PP nanocomposite films. Two grams of PP nanocomposite films were cut into small pieces and put into a 250-ml Erlenmeyer flask having 100 ml of nutrient broths which was referred as "Test flask". Another flask with the same amount of nutrient broths was used as the "Control flask". Both test and control flasks were autoclaved at 120 °C for 1 hour. After cooling down to room temperature, a trace of E.coli was added into both flasks and shaken using a flask shaker for 18 hour contact time at ambient temperature. A series of glass tubes having 9 ml of nutrient broths was prepared for dilution. The bacterial suspension was then serially diluted 10 fold, and 10 drops of 10 μl-diluted suspension were spotted on autoclaved agar plates. The plates were incubated at 35 °C for 24 h, after which numbers of E.coli colonies in each plate were counted and calculated the numbers of colonies on the plate.

6.4 Results and Discussion

6.4.1 Water Vapor Permeability and Oxygen Permeability of Neat PP and PP Nanocomposite Films

Table 6.1 presents water vapor permeability (WVP) of neat PP and PP nanocomposite blown films and graphically presented in Fig.6.1. Water vapor permeability of neat PP film is 5.44004 x 10^{-13} g/m.s.Pa. Although PP is hydrophobic in nature that has good barrier property against water, the water vapor would penetrate and diffuse into polymer matrix through free volume in amorphous region. There are three main factors that determine the permeability of a polymeric material: the degree of crystallization, the structure compactness, and the polarity. Adding ionic aggregates from Surlyn into non-polar PP matrix decreased water vapor permeability slightly. This could be attributed from increasing in crystallinity of PP matrix having Surlyn to be the nucleating sites as already discussed in the previous chapter (DSC results). Although the SEM results (Fig.5.9) show immiscible blend between PP matrix and ionic aggregates providing flow channel for water vapor to pass through their interfaces, the polar water molecules could not adsorb well into the relatively nonpolar PP matrix to diffuse through the existing channels.

In PP/OBEN blown film, the water vapor permeability increased slightly compared to PP/Surlyn film but it was still lower than those of neat PP. This could be result from relatively increasing in crystallinity of PP matrix when OBEN nanoclay was dispersed with the help of Surlyn (confirmed by DSC and XRD). It is reported (Villaluenga *et al.*, 2007) that organoclay caused flow path of water vapor to be more tacttoids and thus reduced water vapor flow rate compared to homogeneous polymer matrix. In PP/OBEN-CuNP blown film, the increasing trend of water vapor permeability with respect to CuNP content was observed. Although the crystallinity of PP matrix was increased with the nucleating effect of CuNP, the hydrophilicity of PVP coated on CuNP would provide better adsorption of water vapor and diffuse through the existing flow channels between the interface of PP matrix, ionic aggregates, and nanoparticles. Also, the SEM micrographs (Fig.5.9 and 5.10) show there were voids present between the interface of PP matrix and dispersed phases prompting for water vapor to diffuse into these available channels.

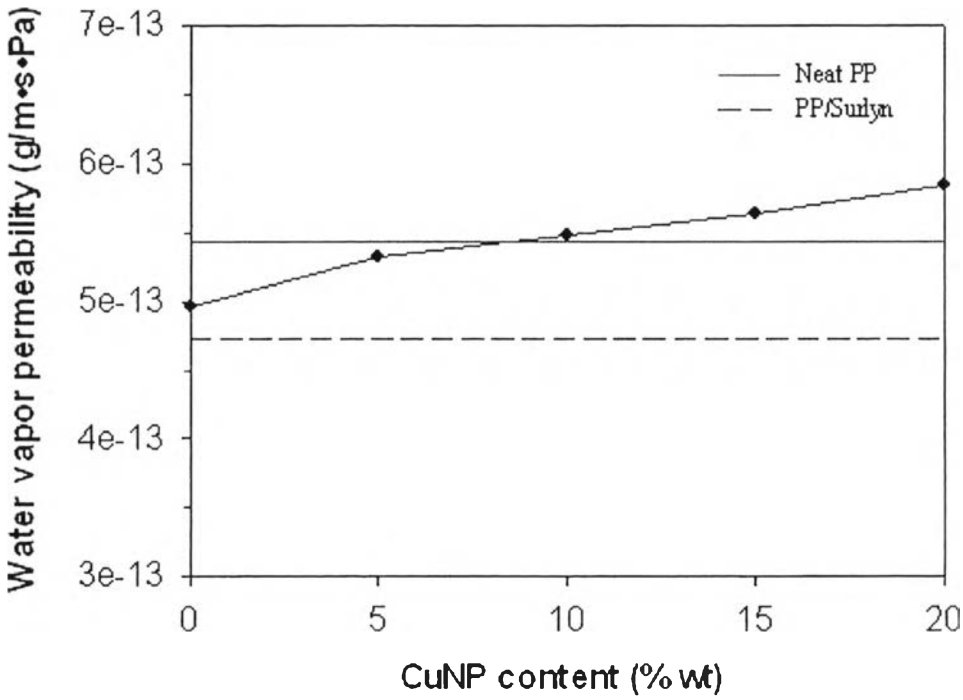

Figure 6.1 Water vapor permeability of neat PP and PP nanocomposite films.

Table 6.2 presents oxygen permeability of neat PP and PP nanocomposite blown films and graphically presented in Fig.6.2. In contrast to water vapor permeability (WVP), oxygen permeability of PP/Surlyn blown film was higher than neat PP. This result is correlated to the incompatibility between PP matrix and Surlyn ionomer dispersed phases which Dumont *et al.* (2007) argued that the lack of interactions between PP chains and inorganic tactoids might lead to the formation of voids in the structure that boost gas diffusivity. The main reason for this behavior in the PP nanocomposite film is the presence of Surlyn. As it is known the better dispersion of nano-particles can be achieved by adding more compatibilizer but this sort of compatibilizer has also a reverse effect on permeability. It increases the diffusion coefficient of the nanocomposite due to its low molecular weight. (Mirabella, 2009).

The contradiction between water vapor and oxygen permeability in the PP nanocomposite films could be explained based on the difference of molecular sizes and polarity between water molecules and oxygen molecules. Molecular size of non-polar oxygen molecule is about 0.292 A^{0}, while polar water molecule is about ten times bigger (2.75 A^{0}). Since oxygen is much smaller, their flow path through PP/Surlyn and OBEN-CuNP blown films would occur in either the exiting flow channels along the interface between PP matrix and Surlyn dispersed phases or the amorphous region of PP matrix. Although crystallinity of PP was increased with respect to CuNP content, the relatively lesser amount of clay aggregates and individual platelets dispersed in the PP matrix provided less tortuosity for oxygen molecules to pass through the PP matrix. Therefore, these combined actions lead to an overall decrease in the oxygen barrier properties of the PP nanocomposite blown films.

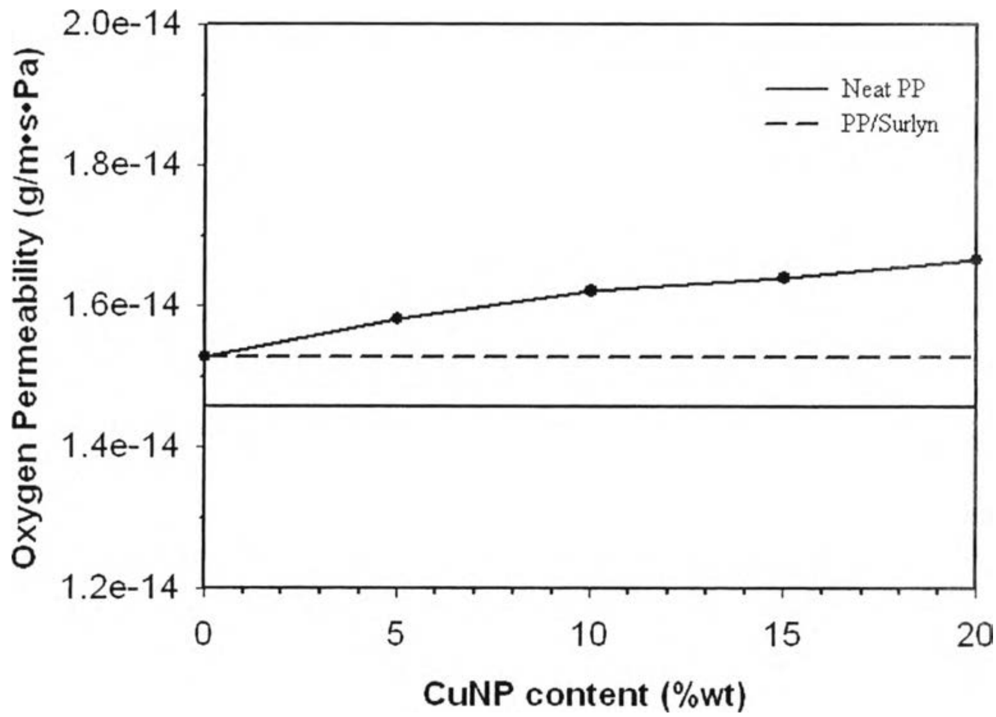

Figure 6.2 Oxygen permeability of neat PP and PP nanocomposite films.

Table 6.1 Water vapor transmission rate and water vapor permeability of neat PP and PP nanocomposite films

Abbreviations	Film thickness (μm)	Film thickness (m)	Water Vapor Transmission rate ($g/m^2 \cdot day$)			Water Vapor Permeance ($g/m^2 \cdot s \cdot Pa$)	Water Vapor Permeability ($g/m \cdot s \cdot Pa$)
			1	2	Average		
Neat PP	50	$50\times10^{(-6)}$	5.60	5.61	5.61	1.0880×10^{-8}	5.44004×10^{-13}
PP/Surlyn	38	$38\times10^{(-6)}$	6.39	6.43	6.41	1.2443×10^{-8}	4.72823×10^{-13}
PP/OBEN	33	$33\times10^{(-6)}$	7.81	7.69	7.75	1.5044×10^{-8}	4.96447×10^{-13}
PP/OBEN-Cu5	47	$47\times10^{(-6)}$	5.83	5.83	5.83	1.1317×10^{-8}	5.31892×10^{-13}
PP/OBEN-Cu10	43	$43\times10^{(-6)}$	6.57	6.55	6.56	1.2734×10^{-8}	5.47557×10^{-13}
PP/OBEN-Cu15	40	$40\times10^{(-6)}$	7.24	7.28	7.26	1.4093×10^{-8}	5.63707×10^{-13}
PP/OBEN-Cu20	46	$46\times10^{(-6)}$	6.52	6.56	6.54	1.2695×10^{-8}	5.83973×10^{-13}

Table 6.2 Oxygen transmission rate and oxygen permeability of neat PP and PP nanocomposite films

Abbreviations	Film thickness (μm)	Film thickness (m)	Oxygen Transmission rate (cc/m^2•day)			O_2 Permeance (mol/m^2•s•Pa)	O_2 Permeability (g/m•s•Pa)
			1	2	Average		
Neat PP	52	$52\times10^{(-6)}$	1728	1706	1717	8.7534 x 10^{-12}	1.45657 x 10^{-14}
PP/Surlyn	49	$49\times10^{(-6)}$	1917	1903	1910	9.7373 x 10^{-12}	1.52681 x 10^{-14}
PP/OBEN	34	$34\times10^{(-6)}$	2733	2775	2754	14.040 x 10^{-12}	1.52756 x 10^{-14}
PP/OBEN-Cu5	59	$59\times10^{(-6)}$	1623	1663	1643	8.3762 x 10^{-12}	1.58142 x 10^{-14}
PP/OBEN-Cu10	46	$46\times10^{(-6)}$	2156	2167	2162	11.020 x 10^{-12}	1.62207 x 10^{-14}
PP/OBEN-Cu15	56	$56\times10^{(-6)}$	1783	1808	1796	9.154 x 10^{-12}	1.64033 x 10^{-14}
PP/OBEN-Cu20	56	$56\times10^{(-6)}$	1804	1844	1824	9.299 x 10^{-12}	1.66636 x 10^{-14}

6.4.2 Antimicrobial Activity of Neat PP and PP Nanocomposite Films

The antibacterial activity of OBEN and OBEN-CuNPx pastes for E. coli was measured by the agar diffusion method which results are presented in Figure 6.3 and 6.4. Colonies of E.coli could not be viewed in the clear zone (inhibition zone) around the holes having OBEN-CuNP pastes, whereas such colonies were formed all over the control holes (water) and just OBEN. The microbial inhibition indicates that there was antibiotic released from the pastes and diffused into the agar layer, retarding the development of microbial cells in the agar. This clearly demonstrates that the antimicrobial activity is only due to copper nanoparticles impregnated into OBEN organoclay. Ruparelia *et al.* (2008) reported that the bactericidal effects observed in their study were impacted by the release of Ag^{+}/Cu^{2+} ions in solution. They stated that the presence of nanoparticles in suspension would ensure continuous release of ions into the nutrient media. Silver or copper ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death. However, the exact mechanism behind bactericidal effect of copper nanoparticles has not been reported yet and needs to be studied further.

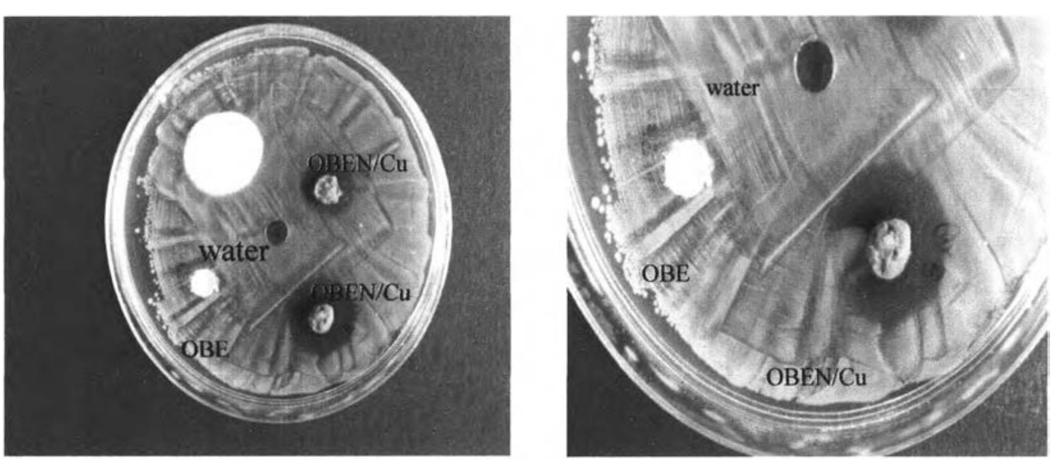

Figure 6.1 Agar diffusion test showing clear zone around OBEN/Cu5 pastes compared to OBEN powder and water (reference).

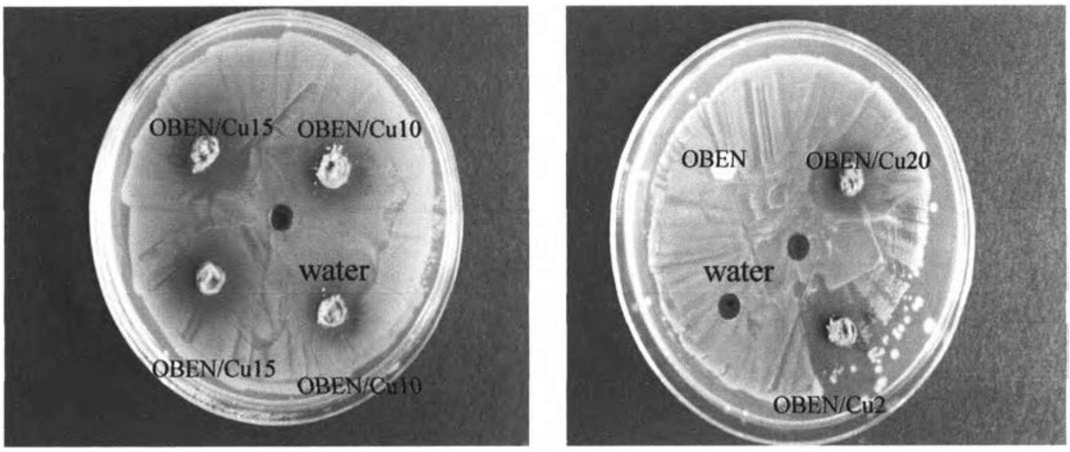

Figure 6.2 Agar diffusion test showing clear zone around OBEN/Cu10, OBEN/Cu15, and OBEN-Cu20 pastes compared to OBEN and water (reference).

Figure 6.3 Agar diffusion tests of neat PP and PP nanocomposite blown films.

Figure 6.5 presents the agar diffusion test of neat PP and PP nanocomposite blown films. In contrast to the agar diffusion test of OBEN-CuNP pastes, there was no clear zone surrounding any PP nanocomposite films while there was clear zone around the control reference (antibiotic agent). This would demonstrate the low diffusivity of copper ion from the film samples into agar to inhibit the grown of E.coli. Polypropylene is hydrophobic in nature, so there is rarely solubility of water into polymer matrix to cause oxidation of copper nanoparticles and diffuse into surrounding agar.

In order to investigate the antibacterial activity of the nanocomposite films, the shake flask test was performed. Figure 6.6 presents the antibacterial activity of PP/OBEN-Cu20 nanocomposite blown films compared with the control sample. It is seen that the number of colonies in the PP-OBEN-Cu20 culture sample was less than the control sample. This clearly indicates that the copper nanoparticles in the nanocomposite films could inhibit the growth of E.coli in the nutrient broths. Figure 6.7 presents agar plates from the shake flask test of all PP/OBEN-Cu nanocomposite blown films compared with the control sample. Also, the numbers of E.coli colonies were counted and presented in Table 6.3. It is found that the numbers of E.coli colonies were reduced after the nanocomposite films were added into the nutrient broths.

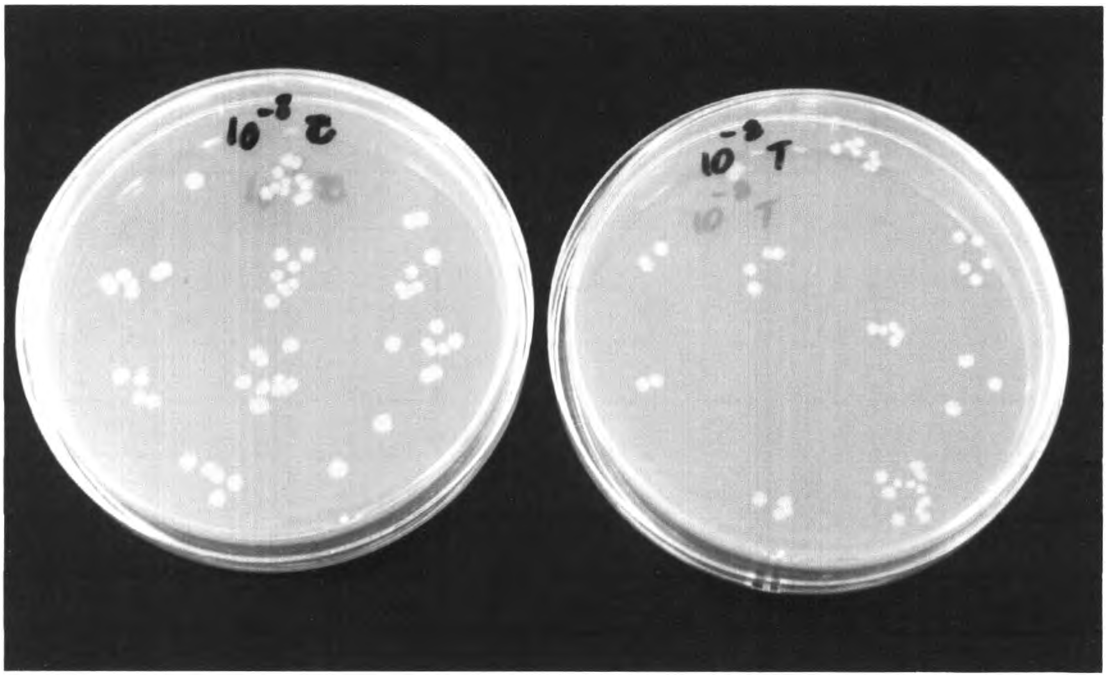

Figure 6.4 Agar plates of PP/OBEN-Cu20 nanocomposite films showing the numbers of E-coli colonies after incubated for 24 hours. The left agar plate was the control culture sample and the right plate was the PP/OBEN-Cu20 culture sample.

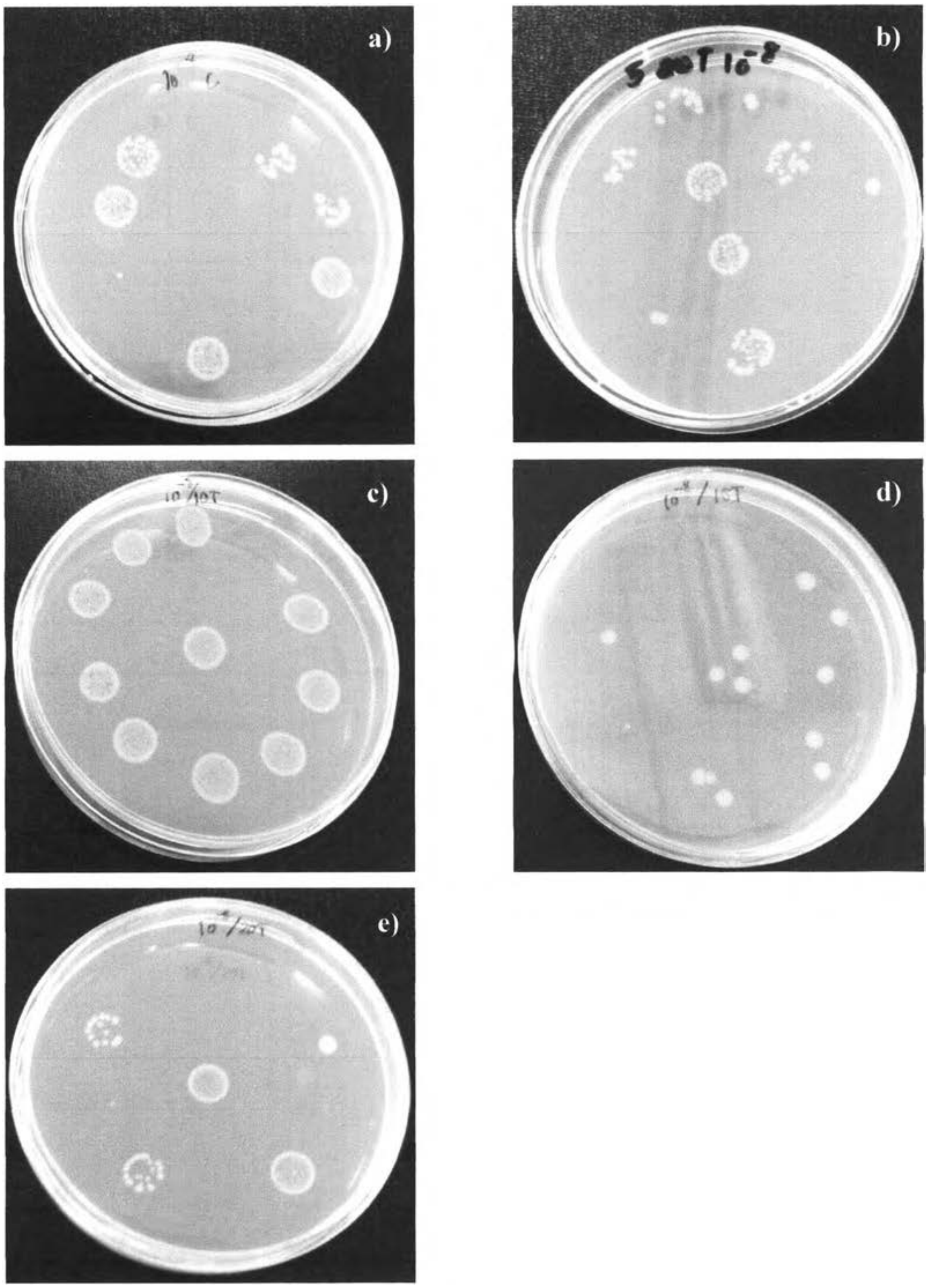

Figure 6.5 Agar plates showing numbers of E-coli colonies after incubated for 24 hours after the shake flask test of a) the control sample, b) the PP-OBEN-Cu5 sample, c) PP-OBEN-Cu10 sample, d) PP-OBEN-Cu15 sample, and e) PP-OEN-Cu20 sample.

Table 6.3 Antimicrobial activity values (colony-forming units per ml, CFU ml^{-1}) of the inoculation and control sample at a control time of 18 h, used to evaluate antimicrobial activity of PP-OBEN-Cu blown films against Escherichia coli

Sample	Number of colonies (CFU/ml)
Broth Control	335×10^9
Broth adding OBEN-Cu5 film	219×10^9
Broth adding OBEN-Cu10 film	N/A
Broth adding OBEN-Cu15 film	7.40×10^9
Broth adding OBEN-Cu20 film	50×10^9

6.5 Conclusions

Having ionic aggregates, the PP film was less water vapor permeate due to increasing in crystallinity. Organoclay caused flow path of water vapor to be more tacttoids and thus reduced water vapor flow rate compared to homogeneous PP matrix. The decrease in water vapor permeability in PP nanocomposite films is favor for the prepacked chilled fish since it would prevent it drying. However, adding OBEN-CuNP with CuNP more than 10 wt% into PP films increased water vapor permeability slightly due to hydrophilicity of PVP coated on CuNP. In contrast, all PP films adding Surlyn or nanoparticles increased oxygen permeability because there were flow channels at the interfaces between Surlyn and PP matrix. This would allow oxygen to flow in and out from the prepacked chilled fish packages easier and prevent bad odor trapped in the packages.

From the agar diffusion tests, it is found that there were inhibition zones surrounding OBEN-CuNP pastes. This indicates that OBEN-CuNP has antibacterial activity against the bacteria. However, the clear zone was not observed when using film samples which would be result from very low diffusivity of copper ion from the film samples into agar. This is favor for the prepacked chilled fish packages since the films would prevent the growth of bacteria that might come with the flow-in of oxygen that might bring bacteria from outer atmosphere to cause spoilage of the fresh fish.

6.6 References

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