Note

Function of Rayon Fibers with Metallophthalocyanine Derivatives: Potential of Low-Molecular Weight Polycyclic Aromatic Hydrocarbon Removal and *Bacillus* sp. Removal

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Polycyclic aromatic hydrocarbons (PAHs) are known as carcinogenic and/or mutagenic substances, and are present at high concentration in polluted environments. It has recently been reported that spore-forming bacteria (*e.g.*, *Bacillus* spp.) can be transported long distances alive in the atmosphere, which raises the possibility that some of the transported bacteria could have adverse effects on human health. There is thus a need for filters that can remove gaseous PAHs from the air that people breathe and that can inhibit bacterial growth on the filters. We focused on metallophthalocyanine derivatives (M-Pc) which are known to adsorb PAHs as well as to inhibit the growth of bacteria as a potential filtering agent. In this study, we developed different types of M-Pc-supported rayon fibers by changing central metals, functional groups, concentrations of M-Pc and rayon types, and evaluated their removal effects by measuring adsorption rates of 3- and 4-ring PAHs with a HPLC and growth curves of *Bacillus* sp. with a spectrophotometer. The results showed that both the effects depended on functional groups and concentrations of M-Pc, and rayon types. The most effective combination was observed in Fe-Pc with sulfo group supported on cationized rayon fiber at the concentration of 2 to 3.3 wt%. Central metal species of M-Pc were influenced only on the antibacterial properties. This fiber would be applicable to filtering agents and textiles.

Key words　phthalocyanine; polycyclic aromatic hydrocarbon; *Bacillus* sp.; adsorbent; antibacterial effect; fiber

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Several PAHs have been reported to be carcinogenic.[1] PAHs of lower molecular weights (2 or 3 rings) generally exist in the gas-phases,[2] and the phases of 4-ring PAHs are reversible depending on the temperature.[3] Although the adverse effects of 3- or 4-ring PAHs might be weak,[4] high concentrations of the gaseous PAHs[3,5–7] should be prevented as well as particulate PAHs.

Recently, it has been reported that spore-forming bacteria (*e.g. Bacillus* species) are transported long distances alive in the atmosphere.[8–12] *Bacillus* spp. include some pathogenic species and possibly cause opportunistic infection. Bacteria can be removed mechanically by filters, but they might grow on the filters during the use. Therefore, fibers used for filtering should have the potential inhibiting the growth of bacteria.

Metallophthalocyanine derivatives (M-Pc) have been used as an adsorbent of polyaromatic compounds The large planar structure of the M-Pc molecule generates π–π interaction with molecules having planar structures such as PAHs.[13–16] Moreover, M-Pc also exhibited effective enzyme-like catalytic activities.[17] They have several practical applications such as bactericidal systems. Indeed, it has been reported that some of M-Pc have cytocidal effects for bacteria such as *Staphylococcus aureus* or *Psuedomonas aeruginosa*.[17]

The aim of this study was to evaluate different types of M-Pc fibers that remove both of 3- and 4-ring PAHs and *Bucillus* sp.

Experimental

Test Fibers　Eighteen types of M-Pc-supported fibers were synthesized by Daiwabo Neu Co., Ltd. (Osaka, Japan) (see supplementary figure). The M-Pc fibers are denoted hereafter as

$$M\text{-}Pc\text{-}nR \, / \, X \, (Y \, wt\%) \qquad (1)$$

where M is the central metal (Fe(III), Co(II), or Cu(II)), R is the functional group (sulfonic acid (SO_3^-) or carbonic acid (COO^-)), and n is the number of R (1 for SO_3^-; 4 or 8 for COO^-). X is the type of cellulosic fiber: amorphous-rich rayon (AmR) or cationized rayon (CaR) which is crystal-induced regular rayon fibers cationized on its surface. AmR can covalently bond with M-Pc, CaR can form an ionic bond with M-Pc having an anion functional group. Y is weight percentage of the mass of the supported M-Pc per unit mass of the fiber (hereafter called concentration of M-Pc), and five different concentrations of M-Pc (0.2, 1, 2, 3.3 or 5 wt%) were prepared.

Chemicals　Pyrene (Pyr) and phenanthrene (Phe) were selected as representative 3- and 4-ring PAHs, respectively. These PAHs and their deuterated chemicals (pyrene-d_{10} and phenanthrene-d_{10}) were purchased as standards from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical-reagent grade.

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Water solutions of Pyr and Phe were prepared and finally adjusted to 5 nM and 50 nM, respectively, by using ultrapure water.

Extraction of PAHs from Fiber Fifty milligram of each fiber was shaken with 50 mL of the Pyr or Phe solution in a conical flask for 1 h at 37°C. Three samples were tested for each experiment.

The fiber was washed by distilled water, and then evaporated to dryness for 5 min. To the dried fibers, the internal standards, 20 μL of 1.25 μM Pyr-d_{10} or 50 μL of 50 μM Phe-d_{10}, and 20 mL of the mixture of methanol and concentrated ammonia (25%) at 50:1 (v/v) were added.[16] The flask was supersonicated for 15 min. This extraction of PAHs using methanol/ammonia solution was repeated twice, and the two extracted solutions were combined.

The extracted solutions from fiber were concentrated to 3 to 5 mL by a rotary evaporator in a water-bath at 60°C. The extracts were filtered by a membrane filter (0.45 μm, HLC-DISK 13, Kanto Chemical Co., Inc.). After adding 100 μL of dimethyl sulfoxide (DMSO), the extracts were concentrated to 100 μL with N_2 gas. Finally, 400 μL of acetonitrile was added to the extracts and mixed. Then an aliquot of the solution was injected into HPLC.

Analysis of PAHs by HPLC-FL Pyr and Phe were quantified by a HPLC system (LC-10A, Shimadzu Co., Ltd.). The system consists of a mobile phase pump, a degasser, a column oven (40°C), a fluorescence detector, and an injector with a 20 μL loop. A separator column was Cosmosil 5C$_{18}$-AR-II, 4.6 i.d.×250 mm (Nacalai Tesque, Inc.). The mobile phase was methanol 80% at a flow rate of 1.0 mL/min. The fluorescence detector was set at the optimum excitation and emission wavelengths for each PAH.

Bacteria *Bacillus* sp. BASZUB0801 (Accession no. AB523858) isolated by separated culture from the aerosols during Asian dust event over the Noto peninsula, Japan,[11] was used as a bacteria to evaluate antibacterial effect. The species is one of the representative bioaerosols associated with Asian dust.[12]

Analysis of Bacterial Growth For preculture process, two loopfuls of *Bacillus* sp. BASZUB0801 cells were moved into a test tubes with 10 mL of Yeast extract-Peptone-Dextrose (YPD) liquid culture medium which was consisted of 5 g/L yeast extract (Becton, Dickinson and Company), 10 g/L polypeptone (Nihon Pharmaceutical Co., Ltd.), and 10 g/L D(+)-glucose (Wako Pure Chemical Industries, Ltd.). The tubes were shaken for about 20 h under 30°C with a reciprocal shaker and then 1 mL of the liquid was transferred to a conical flask containing autoclaved 50 mg test fibers and 100 mL YPD liquid medium. Main culture was made at 30°C in the rotary shaker (BR-300L, TAITEC Co., Ltd.) at 100 rpm.

Aliquots (about 1 mL) of the culture were sampled at every 4 h for 24 h, and they were used for measurement of cell concentration. The cell concentrations were determined by measuring optical density of cells (OD) at 600 nm using a spectrophotometer (UV mini-1200, Shimadzu Co., Ltd.). Three samples were tested for each experiment. The blank test that is obtained through the incubation without test fibers was also simultaneously carried out for each experiment. To evaluate antibacterial efficiencies of the test fibers, the OD ratios are defined as below;

$$\text{OD ratio} = \text{OD}_{\text{fiber}} / \text{OD}_{\text{control}} \qquad (2)$$

where OD_{fiber} is the OD obtained by the experiments incubated with the test fibers and $\text{OD}_{\text{control}}$ is the OD obtained by the blank test without the test fibers. The lower value of OD ratio means that bacterial growth was inhibited by the test fibers.

Results and Discussion

Three- and Four-Ring PAH Adsorption Figure 1 shows Pyr and Phe adsorption rates on Fe-Pc-SO$_3^-$/CaR with five different concentrations of Fe-Pc. Additionally, regular rayon without M-Pc was tested to compare with the M-Pc fibers. The adsorption activity of regular rayon was very weak for Pyr and was not observed for Phe. The effective concentrations of Fe-Pc-SO$_3^-$/CaR were 1 to 3.3 wt% for both Pyr and Phe.

Figure 2 shows the adsorption rates of Pyr on M-Pc-SO$_3^-$/X fibers with different central metals and different cellulose types. For the cellulose types of CaR, the profiles of the adsorption rates were very similar to each other in spite of the different metal, and the highest adsorption rates were observed when the concentrations of M-Pc-SO$_3^-$/CaR were in the
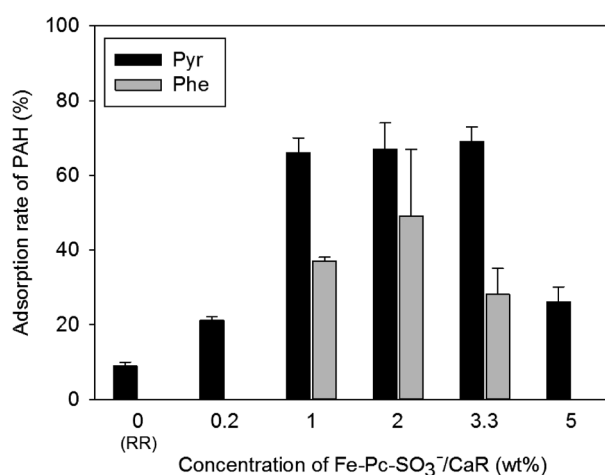

Fig. 1. Adsorption Rates of Pyr and Phe on Fe-Pc-SO$_3^-$/CaR (0.2 to 5 wt%) and Regular Rayon (RR) with the Different Pc Concentrations in the Test Fibers
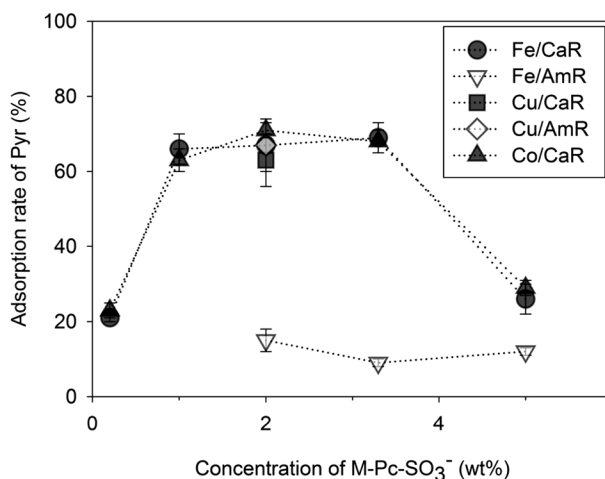

Fig. 2. Adsorption Rates of Pyr on M-Pc-SO$_3^-$ X Fibers with Different Central Metals and Cellulose Types

range from 1 to 3.3%.

In the case of Cu-Pc-SO$_3^-$/Y (2 wt%), the adsorption rates were about 70% for the both fibers of CaR and AmR (Fig. 2). The efficiency was similar regardless of the different cellulose type. Contrastingly, when the central metal was Fe, the efficiency of AmR was 15% or less, which was much lower than that of CaR (*ca.* 70%) and comparable to the result of M-Pc-unbound fibers. The variations of adsorption rates to the M-Pc concentrations were very different between Fe/AmR and Fe/CaR.

M-Pc molecules connected to AmR would stack onto the fiber surface, and the stacking reduce the PAH adsorption site. On the other hand, M-Pc molecules connected to CaR would efficiently distribute through the connection one by one between the cation sites on the rayon fiber and the anion sites of the M-Pc functional group, which causes the increase in the number of M-Pc molecules on the fibers. Consequently, the distributed monomolecular M-Pc increases the number of PAH adsorption sites. Thus the M-Pc/CaR showed the high adsorption rates.

M-Pc/CaR at the high concentration (5 wt%) reduced the adsorption rates (Fig. 2), being probably due to the stacking of M-Pc molecules even if the M-Pc is supported on CaR. M-Pc at the low concentration (0.2 wt%) also did not efficiently trap PAHs, because of the reduction in the number of adsorption sites. These results suggested that M-Pc concentration is an important factor to remove PAHs effectively, and the optimum value was in the range from 1 to 3.3 wt%.

When the Fe-Pc-X/CaR (2 wt%) had different functional groups, the adsorption rate of Pyr in the case of 8COO$^-$ was 60%, which was slightly lower than that of SO$_3^-$ (67%) but more efficient comparing with that of 4COO$^-$ (43%).

With the increase in the number of carboxyl groups, the electron density of the interlayer increases. Consequently, the stacking of M-Pc molecules is suppressed due to the electrostatic repulsion of negatively charged carboxylate ions, and the concentration of monomeric M-Pc molecules increases. As the result, the PAH adsorption might efficiency increase. This hypothesis was supported by the experimental results

that the 8COO$^-$ showed higher adsorption rates for Pyr than 4COO$^-$. The catalytic activity to decompose odor substances was higher by Fe-Pc with 8COO$^-$ than that with 4COO$^-$ as the functional group, suggesting that the difference is due to the aggregation and spin state of M-Pc.[18]

The molecular size of sulfo group is larger than that of carboxyl group. Therefore, increase in the number of SO$_3^-$ might cause preventing the invasion of PAHs into the interlayer between M-Pc molecules and the PAH adsorption might be reduced, although the difference of the number was not compared in this study. These functional groups affect the steric effects of M-Pc, changing the area of the adsorption site.

Considering the fact that aromatic compounds having 1 or 2 rings are not efficiently adsorbed by blue cotton,[13] the adsorption efficiency to PAHs by M-Pc would become high with increase in the number of the rings of PAHs. This explains well that the adsorption rates of Pyr were predictably higher than Phe (Fig. 1).
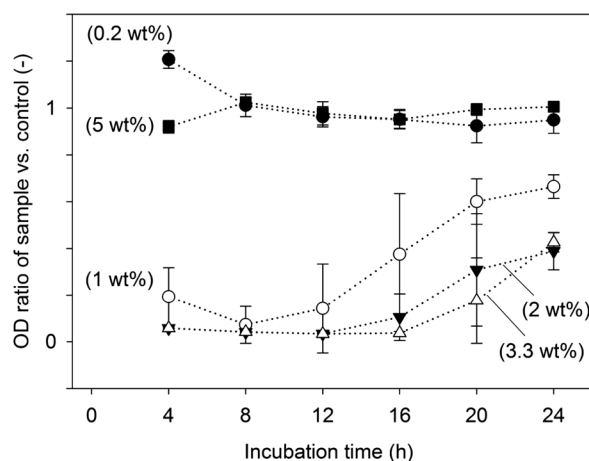

Fig. 3. Time Courses of Antibacterial Activities of Fe-Pc-SO$_3^-$/CaR (0.2 to 5 wt%)

The values in parentheses indicate the concentrations of Fe-Pc-SO$_3^-$ on the test fiber.
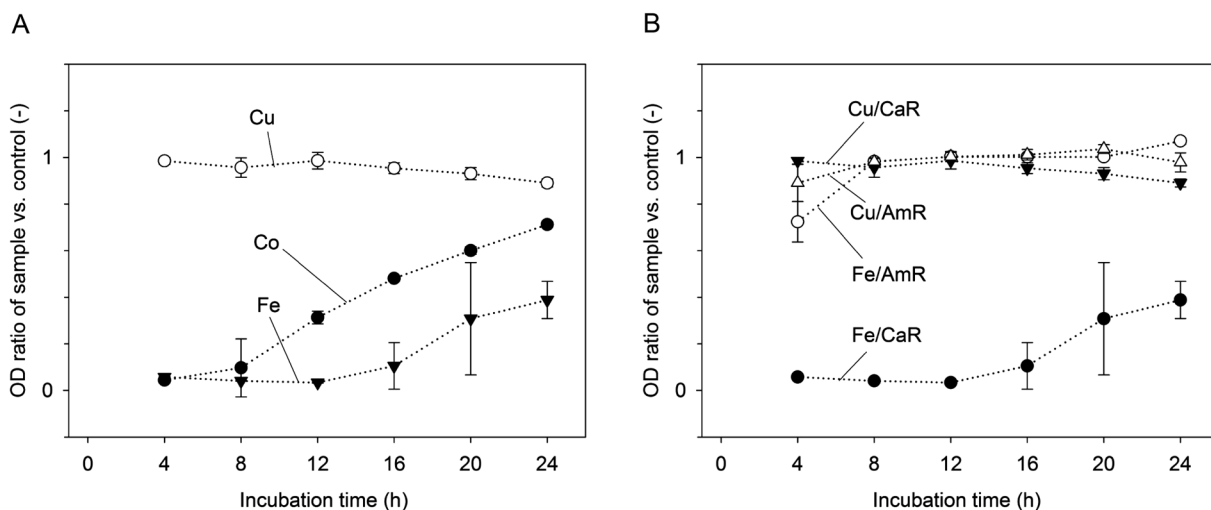

Fig. 4. Time Courses of Antibacterial Activities to the *Bacillus* sp.

(A) M-Pc-SO$_3^-$/CaR (2 wt%) using Fe, Cu, or Co as the central metal, and (B) M-Pc-SO$_3^-$/X (2 wt%) using different central metal (M=Fe or Cu) and cellulose type (X=CaR or AmR).
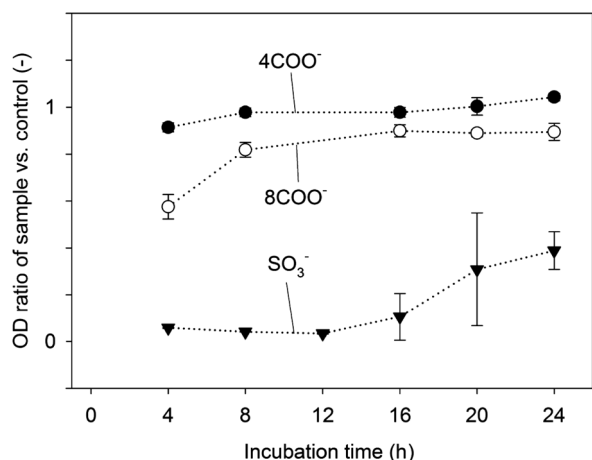
Fig. 5. Time Courses of Antibacterial Activities to *Bacillus* sp. of M-Pc-SO$_3^-$/X (2 wt%) Using Different Central Metal (M=Fe or Cu) and Cellulose Type (X=CaR or AmR)

***Bacillus* sp. Growth Inhibition** The time courses of antibacterial activities of Fe-Pc-SO$_3^-$/CaR (0.2 or 5 wt%) fibers showed the similar tendencies to the control (Fig. 3). In contrast, Fe-Pc-SO$_3^-$/CaR (1 to 3.3 wt%) showed low OD ratios. Especially, Fe-Pc-SO$_3^-$/CaR (2 and 3.3 wt%) remained OD values less than 0.1 for about 16 h after the beginning of incubation (Fig. 3). These results suggested that the effect to inhibit the *Bacillus* sp. growth depended on the concentration of Fe-Pc. As in the case of PAH adsorption (Fig. 1), the interlayer area between M-Pc molecules was a key factor for inhibition of the *Bacillus* sp.

Figure 4 shows the time courses of antibacterial activities of M-Pc-SO$_3^-$/X (2 wt%) fibers with different central metals and cellulose types. The OD ratios of Fe- or Co-Pc/CaR were clearly low. In contrast, when the central metal was Cu or cellulose type was AmR, the OD ratios were close to 1 over 24 h. As the result, only Fe-Pc-SO$_3^-$/CaR effectively inhibited the growth of the *Bacillus* sp. for about 16 h. These results suggested that the central metals and the cellulose types were important to produce the antibacterial effects.

Figure 5 shows the OD ratios of the *Bacillus* sp. incubated with Fe-Pc-nR/CaR (2 wt%) at different functional groups. The OD ratio of SO$_3^-$ was less than 0.1 for 12 h, which showed the lowest OD ratio among the functional groups. The OD ratio of the 8COO$^-$ was approximately 0.6 at 4 h which was significantly lower than that of the value of 4COO$^-$. This comparison indicated that the functional group influenced the inhibition of *Bacillus* sp. growth.

The species of central metals were important for the inhibition of the bacterial growth (Fig. 4A), in spite of the result that it was not a key factor for PAH adsorption (Fig. 2). The different results suggested that other mechanisms are necessary for the antibacterial effects, although the PAH adsorption was simply explained by the π–π stacking described above.

The antibacterial activities were high when Fe or Co was used as the central metal, but not Cu was used. The similar tendency was reported in the effect of M-Pc to remove allergenic proteins or antihistamine.[19] These results suggest that central metal might play an important role to inhibit bacterial growth and other bioactivities.

M-Pc has the molecular structure similar to heme that is the

reaction center of hemoprotein enzyme. It has been reported that alkyl peroxyl radicals (ROO·) are generated by heme-iron catalyzed decomposition in the presence of organic peroxides, suggesting the some products by the enzymatic reaction cause bactericidal activity.[20] The bactericidal activity observed in Fe-Pc-8COO$^-$ is similar to the reaction by heme.[17] The present study showed that the most effective functional group was SO$_3^-$ to the *Bacillus* sp. The differences in steric structures related to the functional groups of M-Pc would cause the differences in the antibacterial capability. The antibacterial effect by M-Pc might be due to alkyl peroxyl radicals which attack the cells as a possible mechanism as described above. This study suggests that antibacterial effect is related to adsorptive property and active-species generation capacity as the complex catalyst by M-Pc fibers.

Conclusion

Different types of M-Pc-supported fibers were prepared by changing the central metals, functional groups, concentrations of M-Pc, and cellulose types. The removal of low molecular weight PAHs (Phe and Pyr) and the inhibition of the *Bacillus* sp. (BASZUB0801) growth were evaluated in aqueous solution on the basis of laboratory tests using the M-Pc fibers. As a consequence, Fe-Pc-SO$_3^-$/CaR (2 to 3.3 wt%) was the most effective to remove both the PAHs and the *Bacillus* sp.

Supplementary Materials The online version of this article contains supplementary materials.

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