行政院國家科學委員會專題研究計畫成果報告

生物氣膠控制技術之評估

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執行期間：91 年 8 月 1 日至 92 年 7 月 31 日
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Evaluation of Bioaerosol Control Techniques

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一、中文摘要

近年來研究發現生物氣膠與呼吸道相關症狀具有高度相關性，而生物氣膠造成的傳染性疾病也是一大威脅；因此，如何在公共場所、醫院及居家生活環境中有效的控制生物氣膠乃是對抗傳染病、提升生活品質的第一要務。因此本研究選擇目前市面上新興的空氣清淨方式——光觸媒氧化作用為探討對象，評估市售光觸媒濾網進行實驗室定性、定量評估是否真正具有殺菌效果。

結果顯示生物氣膠經過二氧化鈦光觸媒處理後，其存活率為 0.32 到 1。由於市售之光觸媒濾紙常與靜電濾紙結合，因此我們也就此雙層的濾紙進行評估，結果顯示其穿透率可降低為 0.0008 到 0.3，而高流量降低其效果。此外，光觸媒濾紙的效果較靜電濾紙為差。

關鍵詞：光觸媒濾網、生物氣膠、殺菌

Abstract

Cleaner production has received considerable interest owing to global environment issues. Semiconductor photocatalysts such as and TiO2 illuminated with band-gap lift in aqueous suspension can oxidize organic solutes. Among the photocatalysts titanium dioxide (TiO2) is the well-known photocatalyst which includes the decomposition ability of many substances. It is widely used in HVAC and air cleaner applications. But we wonder the efficiency in the high-flow systems and the removal effectiveness in airborne viable microorganisms.

In this investigation, commercial TiO2 filters, microorganism species, relative humidity, and face velocity on on germicidal effectiveness of TiO2-coating filters with 365 nm UV-light irradiation were evaluated in a laboratory test chamber. A Collison nebulizer generated Escherichia Coli, Bacillus subtilis endospores, cells of C. famata var. flareri, spores of Penicillium *citrinum*, and nonbiological aerosols.

As a result, the survival fraction of bioaerosols on TiO2 filter were about 0.32 to 1. Because the commercial filters were complex with another filter such as electrical filter, we evaluated this kind of two layer filter and it had a low penetration about 0.0008 to 0.30. High airflow rate will reduce the removal efficiency. Evidently, the TiO2 photocatalyst effectiveness was poorer than electrical filter.

Keywords: TiO2 filter, *E. coli*, *B. subtilis*, yeast, *P. citrinum*, bioaerosol, control efficiency

二、緣由與目的

Recently, there are increasing numbers of people suffering from allergy, asthma, and bronchitis in Taiwan. Bioaerosols play an important role in these observed symptoms. In regard to bioaerosol reduction, the commonly used methods include filtration, ultraviolet germicidal irradiation, electrostatic precipitation, negative air ionization, and air ozonation. Currently,

there is a new trend for pollutant control by photocatalytic oxidation (PCO) using Titanium dioxide (TiO2). This process is referred to as heterogeneous photocatalysis or, more specifically, photocatalytic oxidation. The advantages of PCO are generally recognized as safe, less expensive with low power consumption, no consumption of oxidizing chemicals, and potentially long service life.

Regarding PCO, TiO2 is a semiconductor photocatalyst with band gap energy of 3.2 eV. When this material is irradiated with photons of less than 385 nm, the band gap energy is exceeded and an electron is promoted from the valence band to the conduction band. The resultant electron-hole pair has a lifetime in the space-charge region that enables its participation in chemical reactions. Hydroxyl radicals and super-oxide ions are highly reactive species that could oxidize air pollutants adsorbed on the catalyst surface (Jacoby, 1996). Particularly, the pollutants, VOCs, are preferentially adsorbed on the surface and oxidized to carbon dioxide. Therefore, rather than simply changing the phase and concentrating the contaminant, the absolute toxicity of the treated air stream is reduced, allowing the photocatalytic reactor to operate as a self-cleaning filter relative to organic material on the catalyst surface.

Previously, there are a few investigations conducted to evaluate the germicidal ability of TiO2 photocatalytisis in drinking water and urban wastewaters. Matsunaga and his coworkers (1988) were the first to report antibacterial effects of powered semiconductor TiO2 on microorganisms in waters. It was found the E.coli cells were completely sterilized when 102 to 103 cells per ml were employed at a light intensity of 1,100 μE/s m2 for 30 min illuminated. In addition, it was also indicated that TiO2 concentration of 0.1% (w/v) had the most effective on Streptococcus cricetus and Actinomyces viscosus, but had no effect on Candida albicans and Streptococcus rattus BHT (Nagame et al., 1989). Using a photoreactor consists of a 300-400 nm lamp with coxially wrapped TiO2 coated fiberglass mesh, concentration reduction of total coliforms was found to be 99% with 18-min exposure time (Ireland et al., 1993). Moreover, it was also found that powered TiO2 had powerful killing effects on all serotypes of mutans streptococci (Streptococcus sobrinus AHT) (Saito et al., 1992), achieved two-log inactivation of coliform bacteria in 30 min (Watts et al., 1995), as well as performed bactericidal activity and decomposing activity for endotoxin (Sunada et al., 1998). Adding electric field (Butterfield et al., 1997), the germicidal effectivenesses of the photocatalytic system were observed to increase. It could be summarized that PCO could be highly effective in reducing microorganism levels in water phase at different TiO2 levels, exposure time and intensity, and microorganism species.

Regarding the mechanisms responsible for the PCO bactericidal germicide capacity, it was suggested that the decrease in the Coenzyme A content of cells could be the inactivation pathway. Moreover, oxidation of the cell wall or cell membrane may also be a possible mechanism, because of the diffusion-controlled oxidation reactions that begin outside the cell and probably do not have the opportunity to diffuse into the cell due to the short half-lives of the radicals (Matsunaga et al., 1988).

Until now, no data was available regarding germicidal effectiveness of TiO2 photocatalysts in airborne bioaerosols. In this current investigation, the influences of microorganism species, relative humidity, and face velocity on germicidal effectiveness of TiO2-coating filters with 365 nm UV-light irradiation were evaluated in a laboratory test chamber.

三、材料方法

Test microorganisms

The vegetative cells of *Escherichia coli*, endospores of *Bacillus subtilis* CCRC 12145 (Culture Collection & Research Center in

Taiwan), vegetative cells of *Candida famata* var. *flareri*, and spores (conidia) of *Penicillium citrinum* Thom CRCC 33168 are used in this evaluation. Cultures of *Escherichia coli* come from Graduate Institute of Microbiology, College of Medicine, National Taiwan University. The rod-shaped, 0.3 to 1.0 μm by 1 to 6μm, Gram-negative *E. coli* represent a sensitive bacterial strain, whereas the rod-shaped, 0.7 to 0.8μm by 1.5 to 1.8μm, Gram-positive *B. subtilis* spores are known to be very resistant to many adverse conditions (Sneath, 1986).

The cells of *Candida famata* var. *flareri* are used to represent yeasts in this study. This strain is common yeast isolate in Taiwan, and identified by Taiwan food industrial Research and Development Institute (FIRDI). It was found that geometric mean aerodynamic diameter (GAMD) is 2.44μm with geometric standard deviation (GSD) of 1.15.

Spores of *P. citrinum* Thom are subspheroidal to spheroidal, 2.0 to 3.6μm in diameter, and finely roughened (Tzean, 1994). The spores are used to represent the hardy fungal aerosols. In addition, this organism is found to be a common fungal isolate in Taiwan (Tzean, 1994).

Nonbiological test particles

Two monodisperse polystyrene latex sphere (PSL, Duke Scientific Corporation) aerosols are used to measure the physical aerosol penetration of test filters. One monodisperse PSL aerosol with a geometric diameter of 0.802 μm (standard deviation of 0.005 μm) is chosen to simulate the bacterial aerosols, another PSL aerosol with a geometric diameter of 2.013 μm (standard deviation of 0.005 μm) is chosen to simulate the fungal aerosols. Before nebulization, PSL spheres are diluted in deionized water to 0.05 ml/100ml. The aerosol concentrations are measure by an aerodynamic particle sizer (APS, model 3320, TSI Inc.).

Aerosol generation system

A Collison six-jet nebulizer (BGI Inc., Waltham, MA) is used to aerosolize pure cultures of four test microorganisms suspension at 3 L/min of dry, filtered, and compressed laboratory air, then passed through a Kr-85 particle charge neutralizer (model 3077, TSI). The aerosolized suspension is then diluted with filtered and compressed air at 47 L/min.

Relative humidity regulation system

The humidified gas stream is generated by passing a pure compressed air through a humidity saturator. The water vapor content (i.e. relative humidity) in the gas stream is adjusted by changing the flow rate ratio of humidified gas stream to dry gas stream, and finally determined with a hygrometer (Testo, Sekunden- Hygrometer 601), which is located in the sampling chamber. At dry condition (RH 35 %), humidified gas stream is 30 L/min and dry gas stream is 17 L/min. At medial condition (RH 55 %), humidified gas stream is 47 L/min without adding dry gas stream. At humid condition (RH 85 %), humidified gas stream of 47 L/min is heated to reach 85 % RH.

Bioaerosol sampling and counting

An Andersen one-stage viable sampler (Andersen Samplers, Inc., Atlanta, GA) is used as the test sampler. Colony forming unit (CFU) counting is done on plates containing between 30 and 300 colonies (Lembke, 1981; Thorne, 1992). The lower limit (30 colonies) is to obtain sufficient statistical power for comparison purposes. The limit (300 colonies) is the maximum range in which one could easily count and differentiate colonies (Jensen, 1992). There was only one sampling port, and samples were taken in sequence first without and then with test filters fastened. In addition, the sampling times ranged from 30 sec to 60 sec, and from 60 sec to 10 min for without and with filter fastened, respectively. After the colonies were counted, a positive-hole correction factor was applied, as recommended by the manufacturer, to take account of multiple hits. Bacteria were collected on TSA and incubated at 37 oC for 24 hr. Fungal spores were collected by MEA and incubated at 25 oC for 72 hr.

Moreover, the concentrations of the generated bioaerosols during each evaluated experiments were found to be stable (coefficient of concentration variation less than 5 %). In addition, the natural decay rates of the suspended solutions were found to be insignificant (data not shown).

Photoreactors

In this investigation, commercial TiO_2 filters (DAIKIN, Air filter No. 1119589, Janpan) are selected as photorcatalyst. The TiO_2 catalyst can be excited by photons with wavelengths shorter than 385 nm. The photon sources in this investigation are an 8-W and a 36-W fluorescent black lights (Philip lamp) with a spectral maximum of 365 nm. The light intensity are 7.4 and 31.8 mW/m^2, respectively. The commercial filters are combinated with one layer electret filter (pore size is about 20 μm) and one layer TiO_2 filters (pore size is about 500 μm), so we total use three types of filters: complex filter, electret filter, and TiO_2 filter. The evaluated parameter, face velocity(0.33, 0.1302, 0.0833 m/s), is flow rate (50 lpm) divides filter area (25, 64, and 100 cm^2). The evaluated relative humidity values included 35% (dry condition), 55% (medial condition), and 85% (humid condition). At least triplicate tests were performed for each set with different filter type, face velocity, relative humidity and test microorganisms. The test system was located in a chemical hood so that the exhausted gas was vented outside.

Experimental set-up and procedures

The experimental set-up is illustrated in figure 1. First, we generated bioaerosols in nebulizer. Then passed them through diffusion dryer and neutralizer. When flow had continued for at least 10 min to provide time for the bioaerosol to stabilize with respect to temperature and humidity, the first "C_{in}" samples were then taken by the Andersen sampler. Then we fasten the test filter in the reactor and take the "C_{out}-UV off" samples downstream the filter. After that the UV lamp was turned on for at least 5 min more for stabilization, the "C_{out}-UV on" samples were then taken.

Calculation

The survival fraction (S) of bioaerosols is calculated by the ratio of the bioaerosol concentration without passing through filter (C_{in}), and the bioaerosol concentration passing through filter (C_{out}):

$$S = C_{in}/C_{out}$$

四、結果

Survival fraction of bioaerosols on electret filter

The electret filters are high efficiency and have low airflow resistance filters. The filters operate by employing pre-charged electrets – a plastic material with a permanent static charge. As the moving air flows past the filter material, the oppositely charged particles in the airstream cling to the filter's fibers. The survival fractions of bacterial and fungal aerosols are illustrated in figure 2. It is found that survival fraction of E. coli and B. subtilis are ranged from 0.12 to 0.34 0.03 to 0.27, respectively. Survival fraction of yeast and P. citrinum are ranged from 0.01 to 0.06 and 0.005 to 0.07, respectively. Thus, this electret filter has excellent collection efficiency for bioaerosols especially for fungal aerosols. When the face velocity increased, the penetration of bioaerosols also increased. In the results of relative humidity, different bioaerosols have different effects and no definitive general relationship can be established.

Survival fraction of bioaerosols on TiO2 filter

There are two conditions here: one is TiO2 filter illuminated under 365 nm UV light, the other is TiO2 filter without UV light as control experiments. The survival fractions of bacteria and fungi are illustrated in figure 3 and 4. It is found that survival fraction of E. coli are ranged from 0.72 to 1 when UV lamp off, ranged from 0.57 to 1 when UV lamp on. Survival fraction of B. subtilis are ranged from 0.81 to 1 when UV lamp off, ranged from 0.79 to 0.96 when UV lamp on. Survival fraction of yeast are ranged from 0.35 to 0.98 when UV lamp off, ranged from 0.32 to 0.88 when UV lamp on.

Survival fraction of P. citrinum are ranged from 0.43 to 0.83 when UV lamp off, ranged from 0.40 to 0.73 when UV lamp on. There are little differences of survival fraction between UV lamp off and UV lamp on, so this TiO2 catalyst filter does not serve a good germicidal capacity. Compare with electret filter, the survival fraction of TiO2 filter are much higher.

Survival fraction of bioaerosols on complex filter

This complex is combined with electret filter and TiO2 filter. There are also two conditions here: one is complex filter illuminated under 365 nm UV light, the other is complex filter without UV light as control experiments. The survival fractions of bacteria and fungi are illustrated in figure 5 and 6. It is found that survival fraction of E. coli are ranged from 0.04 to 0.29 when UV lamp off, ranged from 0.22 to 0.30 when UV lamp on. Survival fraction of B. subtilis are ranged from 0.03 to 0.38 when UV lamp off, ranged from 0.03 to 0.18 when UV lamp on. Survival fraction of yeast are ranged from 0.01 to 0.06 when UV lamp off, ranged from 0.01 to 0.06 when UV lamp on. Survival fraction of P. citrinum are ranged from 0.005 to 0.07 when UV lamp off, ranged from 0.0008 to 0.073 when UV lamp on. The results of these two conditions does not show any significant difference, and the survival fraction of complex filter is similar to electert filter. In other words, the germicidal capacity of TiO2 photocatalyst is almost negligible.

Penetration of nonbiological particles on three types of filters

The percentage penetration of PSL aerosols on three types of filters are illustrated in figure 7. The eletret filter has a maximum penetration of about 22.3 % at particle size 2.0 μm, and about 42.7 % at particle size 0.8 μm. The TiO2 filter has a maximum penetration of about 95.9 % at particle size 2.0 μm, and about 100 % at particle size 0.8 μm. The complex filter has a maximum penetration of about 13.2 % at particle size 2.0 μm, and about 26.8 % at particle size 0.8 μm. As seen, the penetration of 0.8 μm PSL is large than 2.0 μm. When the face velocity increases, the penetration fraction increases, too. These results are confidently with bioaerosols. Moreover, the penetrations of PSL aerosols are large then bioaerosols in our study. Willeke et al. (1996) had found that rod-shaped bacteria penetrate less. The penetration difference between the spherical and rod-shaped bacteria depends on the aspect ratio (length to width) of the bacteria. For an aspect ratio of 4, the penetration of rod-shaped bacteria is about half that of spherical ones.

五、討論

The germicide and filtration efficacy of TiO_2 photocatalyst are influence with several factors. From previous water treatments investigations, the photoreactor systems were mostly formed with titanium dioxide aqueous suspensions with titanium dioxide concentration of 0.25 g/L (Watts et al., 1995), 0.05 % weight fraction (Vidal et al., 1999), 0.1~8 g/L (optimum of 2 g/L by Li et al., 1996), 0.01~10 g/L (optimum of 1 g/L by Satio et al., 1992), 0.05 %~0.5 % (w/v) (optimum of 0.1 % by Nagame et al., 1989), 0.25 g/L (Herrera Melian et al., 2000), 0.1~1 g/L (optimum of 1 g/L by Wei et al., 1994), and 0.1~5 g/L (optimum of 1 g/L by Bekbolet, 1997). If the concentrations of TiO_2 aqueous suspensions are more than optimum concentration, it may failure of light to reach all surfaces of the TiO_2 particles and to initiate the mechanism of the antibacterial effect. However, the method that suspends TiO_2 powders in the water as a catalyst will be difficult to retrieved from the disinfected water. To overcome this shortcoming Matsunaga et al. (1988) immobilized TiO_2 particles on an acetylcellulose membrane, Ireland et al. (1993) coated TiO_2 on a fiberglass mesh, and Butterfield et al. (1997) produced an 100 mm diameter with 1 mm thick sol-gel TiO_2 film reactor. Here we use in this study is a filter coated with TiO_2 power but we don't know the concentration

that coated, so we don't know whether the concentration is suit for our case.

When TiO_2 concentration is controlled within certain ranges, incident light intensity and exposure time become the most important parameters for this photocatalystic reaction. The kinetic model can be expressed as follows (Li et al., 1996 and Vidal et al., 1999);

$$C_t=C_0e^{-KIt}$$

Where C_t is the number of microorganisms remaining at time t, C_0 the number of microorganisms at time zero, K the rate constant characteristic of the type of microorganisms, I the incident light intensity and t is the exposure time. For example, when I is 25 W/m^2, K is 0.25 /min for E. coli.

The oxidation time occur by hydroxyl radical is very short (less than one second) and the shift area is only 1 nm when titanium dioxide is irradiated. On the other hand, disinfection by photocatalytically generated hydroxyl radicals may be limited by mass transfer through the cell membrane. Photocatalytically inducd surface oxidations of the cell wall, which may results in altered permeability and leakage of the cytoplasm, are documented as disinfection mechanisms (Watts et al., 1995). The heterogeneous photocatalytic disinfection needs more than 30 mins reaction time to achieve effective microbial inactivation in many studies. In our study or in the application of photocatalytic air cleaner, the bioaerosols penetrate the TiO_2 filter in a short time so it is too late to inactivate the microorganisms. Moreover, a large scale of particles are collected by the electret filter, from the study of Qian et al. (1997) no reaerosolization was detected at RH more than 35 %, so the opportunity is rarely for particles to penetrate to TiO_2 TiO_2 filter. In addition, Wang et al. (1999) described neither of the test bacteria was able to grow on the filters even under optimal nutrition and incubation conditions. There are no new borne microorganisms that can reaerosolize from electret filter.

六、結論

In conclusion, electret filter has excellent collection efficiency for bioaerosols especially for fungal aerosols, and the survival fraction of complex filter is similar to electert filter. When the face velocity increased, the penetration of bioaerosols also increased. In the results of relative humidity, different bioaerosols have different effects and no definitive general relationship can be established. There are little differences of survival fraction between UV lamp off and UV lamp on, so this TiO2 catalyst filter does not serve a good germicidal capacity. Compare with electret filter, the survival fraction of TiO2 filter are much higher. In other words, the germicidal capacity of TiO2 photocatalyst is almost negligible.

七、計畫成果自評

本計畫已在實驗室中建立光觸媒對生物氣膠控制技術的評估系統，並已運用此系統評估光觸媒對兩種細菌與兩種真菌之控制效能，此成果可運用於環境中生物氣膠控制技術的選擇，並提供更完整的生物氣膠控制評估之技術。

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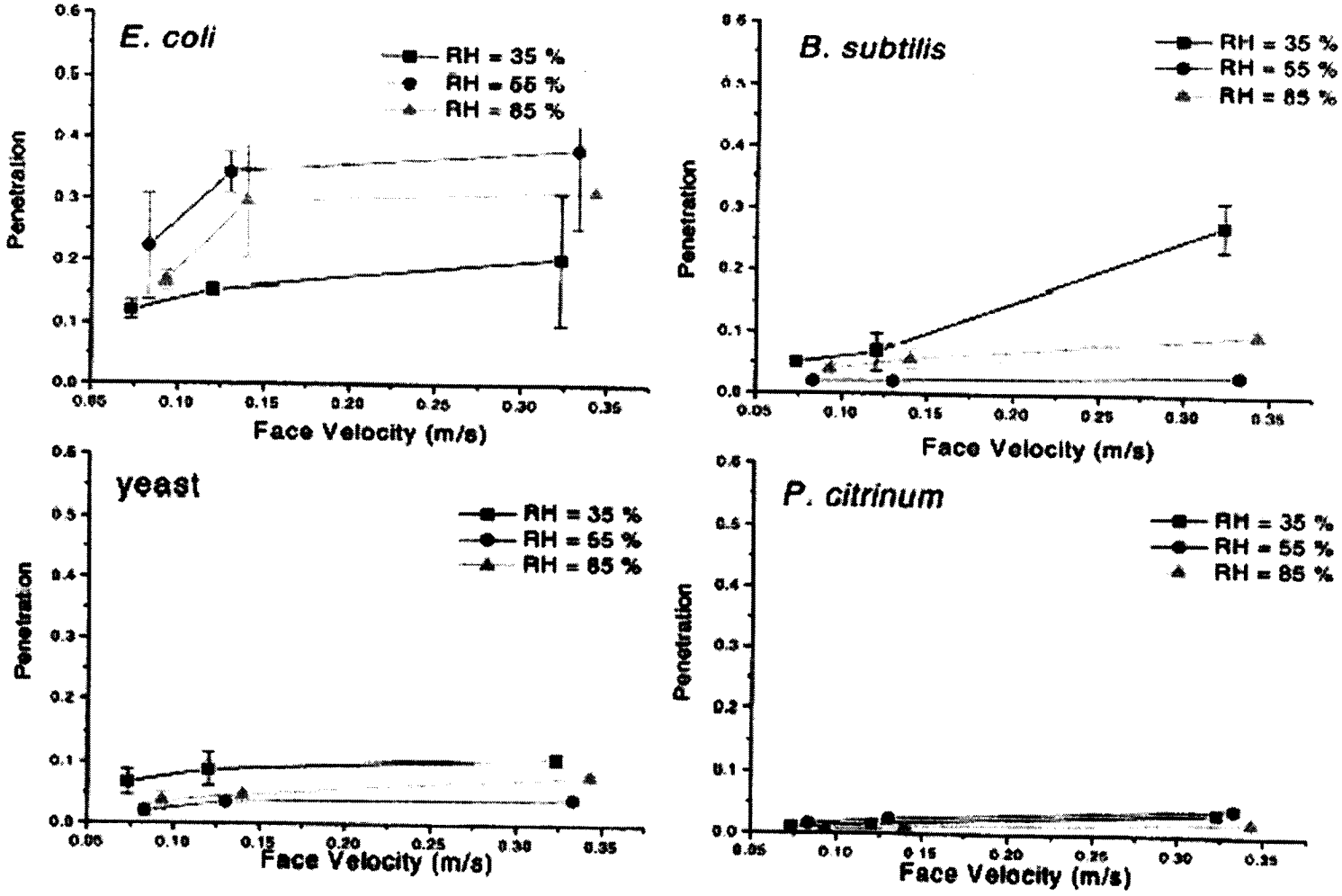

Figure 1. Penetrations of *E. coli*, *B. subtilis*, yeast, and *P. citrinum* for the electrical filter.

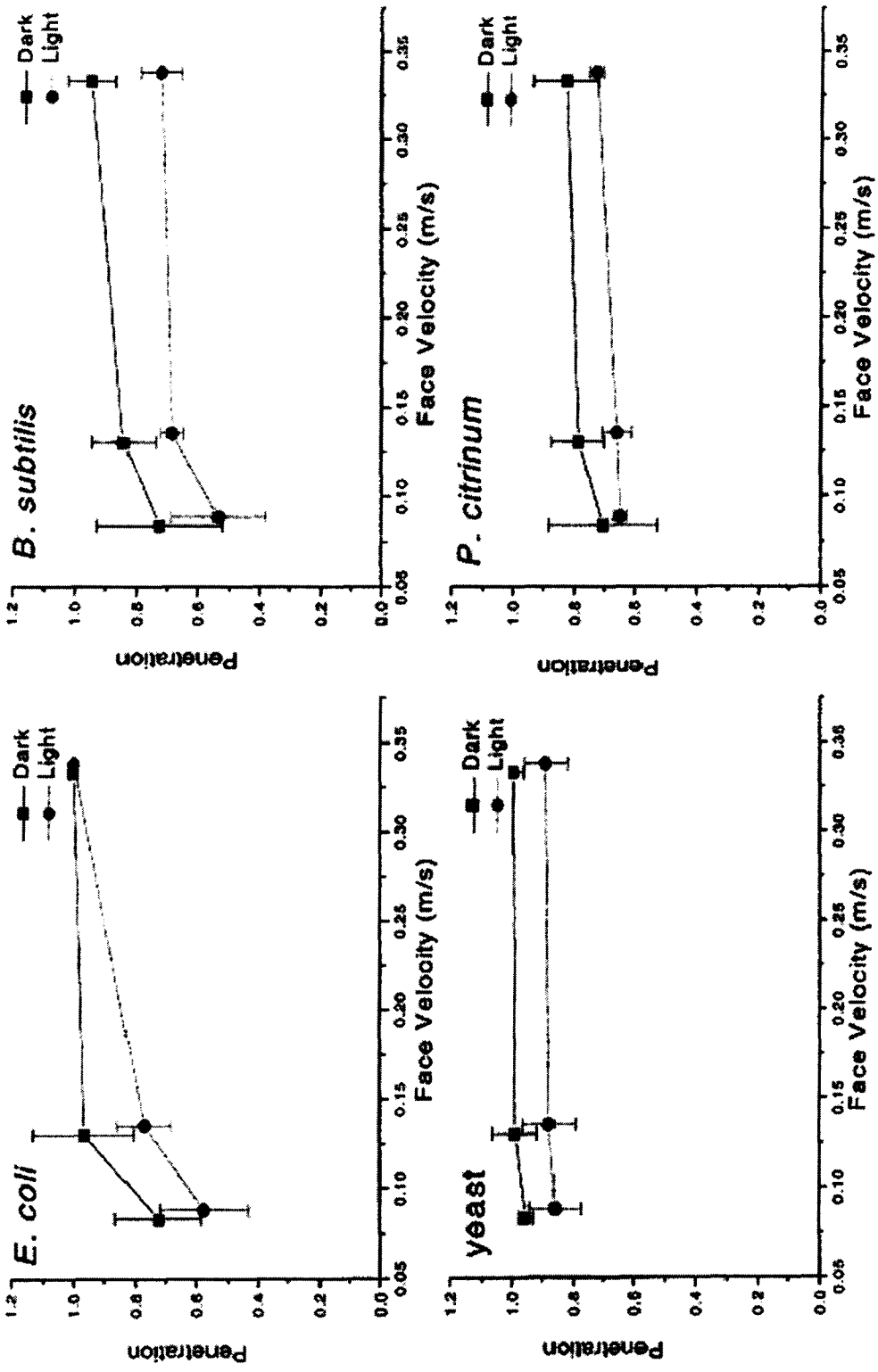

Figure 2. Penetrations of *E. coli*, *B. subtilis*, yeast, and *P. citrinum* for the TiO_2 filter with and without 8 W blacklight irradiation at RH 55%.

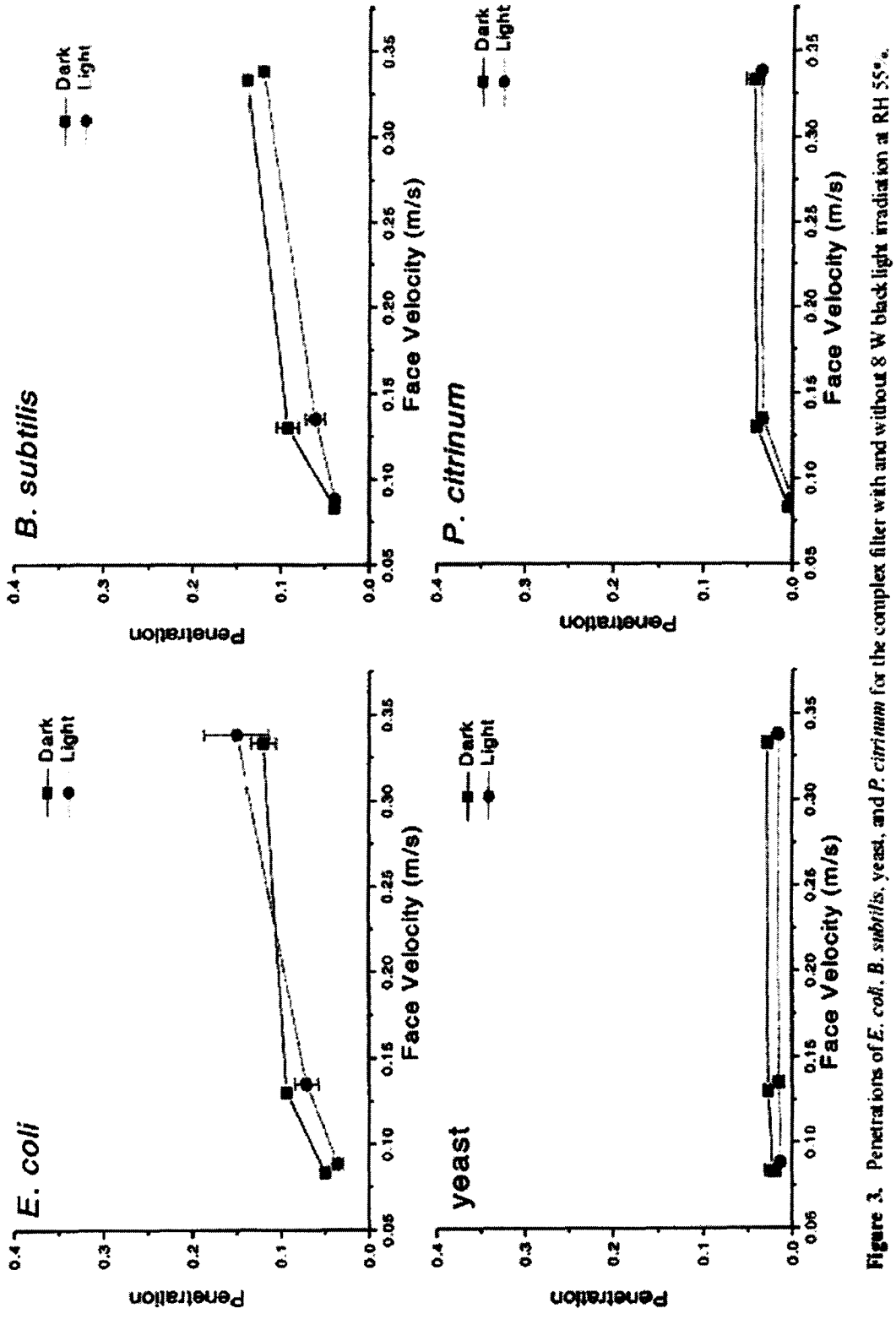

Figure 3. Penetrations of *E. coli*, *B. subtilis*, yeast, and *P. citrinum* for the complex filter with and without 8 W black light irradiation at RH 55%.

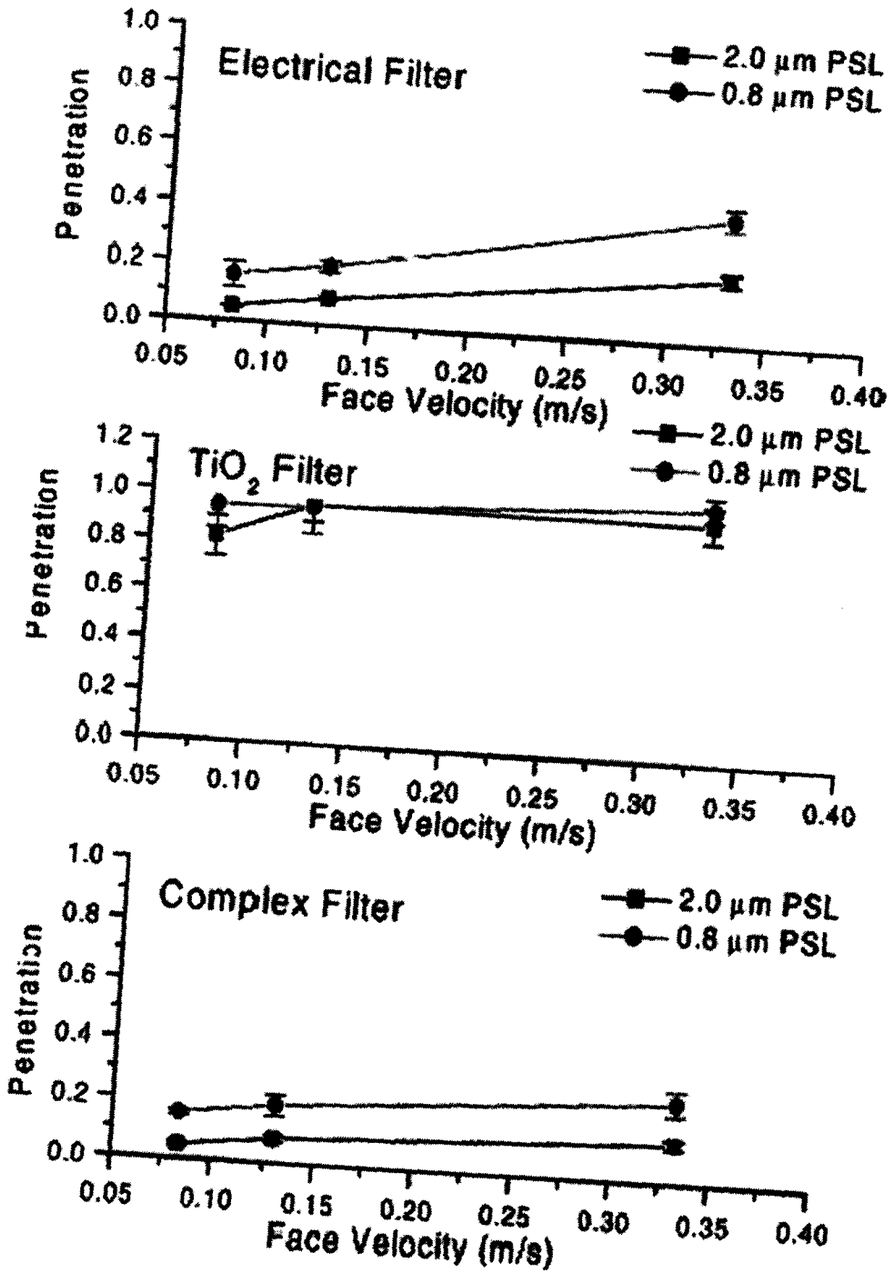

Figure 4. Penetrations of nonbiological PSL particles for 3 types of filters.