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Removal and transformation of high molecular weight polycyclic aromatic hydrocarbons in water by live and dead microalgae

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ABSTRACT

The removal and transformation of seven high molecular weight polycyclic aromatic hydrocarbons (PAHs), namely benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene, by a freshwater microalga *Selenastrum capricornutum* under gold and white light irradiation was studied. The two light sources did not result in significant differences in the biodegradation of the selected PAHs in live algal cells, but white light was more effective in promoting photodegradation than was gold light in dead cells. The removal efficiency of seven PAHs, as well as the difference between live and dead microalgal cells, was PAH compound-dependent. Benz[a]anthracene and benzo[a]pyrene were highly transformed in live and dead algal cells, and dead cells displayed greater transformation levels than live cells. Further investigation comparing the transformation of single PAH compound, benzo[a]pyrene, by *S. capricornutum* and another green microalgal species, *Chlorella* sp., demonstrated that the transformation in dead cells was similar, indicating the process was algal-species independent. Dead algal cells most likely acted as a photosensitizer and accelerated the photodegradation of PAHs.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously environmental contaminants found in air, soil and aquatic environments [1,2]. PAHs have been demonstrated to be mutagenic, teratogenic and carcinogenic, and their toxicity increases with their molecular weights. PAHs with high molecular weights (HMW) are greatly lipophilic and chemically stable. The half-lives of certain HMW PAHs, including fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF) and dibenzo[a,h]anthracene (DA), are in the range of 1000–3000 h in aquatic environments [3]. The presence of persistent HMW PAHs in water poses risks to aquatic organisms and human health, and their high stability also results in the bioaccumulation and biomagnification of the toxicants along aquatic food chains [4].

Studies on the removal and transformation of organic pollutants have mainly focused on bacteria and fungi [5,6], with less attention on microalgae, which are found in nearly all parts of the world and in all types of habitats [7]. On the other hand, microalgae have been applied in various wastewater treatment processes, and their capacity to remove toxic organic pollutants has been demonstrated [8–10]. However, the removal and transformation of PAHs by microalgae have mainly focused on 2–4 ring PAHs, and there have been relatively few studies of HMW PAHs [8,11,12]. Furthermore, the studies of HMW PAHs has been limited to one or two PAH compounds, with little research on mixed HMW PAHs despite the fact that it is common to find mixtures of PAHs in wastewater [2].

In addition to live microalgae, dead algal cells have also been used to remove pollutants in wastewater and have achieved the same or even higher removal efficiency [13–15]. Blue-green algae photosensitized the reactions of several pesticides more rapidly when the cells were denatured by boiling [16]. The photodegradation rates of aniline in the presence of dead algae (*Nitzschia hantzschiana*, *Chlorella vulgaris*, *Chlamydomonas sajao* and *Anabaena cylindrica*) were higher than live cells of the same species [17]. The transformation of aniline was larger when the cells of *Chlamydomonas* sp. were heat-killed at 50 °C for 30 min [18]. Zhang et al.

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[19] reported that the algal cells heated for 15 min had higher bisphenol A degradation than those not heated.

These studies have demonstrated that both live and dead microalgal cells can be used to remove hazardous contaminants, although it would be difficult to maintain a viable biomass for live cells during environmental remediation. It is also more advantageous to use dead microbial cells for wastewater treatment because dead organisms are not affected by toxic wastes, and do not need any continuous supply of nutrients [20]. Furthermore, there are a large number of dead algae in the natural environment, which might contribute to the contaminants removal. It is a potential pathway to get rid of organic pollutant from nature environment with dead organism. However, no research has been reported on the removal of HMW PAHs using dead algal cells in water.

Being photoactive and photosensitive, PAHs can be activated by various types of radiation. White light is a broad-spectrum source that resembles the solar spectrum and has a wide range of wavelengths between 310 and 750 nm, whereas gold light wavelengths are from 400 to 780 nm with a reduction in the intensity of illumination in the near-UV region. The light source has significant impacts on the effectiveness of the bioremediation of organic contaminants through its influences on photodegradation and microalgal growth. Cody et al. [21] found that BaP completely inhibited the growth of *S. capricornutum* at a concentration of 160 $\mu g\,L^{-1}$ when illuminated with white light, but BaP, even at a very high concentration (1200 $\mu g\,L^{-1}$), did not produce any inhibition under gold fluorescent light. The toxicity under white light irradiation could be related to the formation of quinones and other toxic products of PAHs by photodegradation [22].

The present study aims to study the removal and transformation of HMW PAHs under dark, gold and white light irradiation in live and dead cells of the freshwater unicellular green microalga, *Selenastrum capricornutum*. This microalga was selected as a model species because of its ubiquitous occurrence and easy cultivation. This species has also been used frequently in toxicity studies [23] and has been reported to be one of the most effective microalgae in degrading single and binary HMW PAHs, such as Fla, Pyr and BaP from aqueous solutions [24,25]. The mixed PAHs included one 4-ring PAH (BaA), four 5-ring PAHs [benzo[b]fluoranthene (BbF), BkF, BaP and DA] and two 6-ring PAHs [benzo[g,h,i]perylene (BghiP) and indeno[c,d]pyrene (IP)]. All seven PAHs used in the present study are commonly found in the contaminated water and sediments [26,27]. Owing to their low water solubility and high octanol–water partition coefficient (K_{ow}), the concentrations of these PAHs in natural water were in ng L^{-1} level, but as high as 3500 ng g^{-1} in suspended particulate matters (SPMs) [28,29]. The present study chose the PAHs concentration of 100 $\mu g\,L^{-1}$ to evaluate the potential of this reported method.

As BaP is known to be the most toxic compound among the PAHs, it had extremely high transformation efficiency in the present study. In order to confirm the high transformation of BaP by dead algal cells and whether the capability of dead algal cells to transform BaP was correlated with the algae species, the transformation of this particular HMW PAH by *S. capricornutum* and *Chlorella* sp. under white light was compared. *Chlorella* sp. was a local isolate enriched from influent collected from a sewage treatment plant in Hong Kong and was demonstrated by our preliminary study to have the ability to degrade PAHs (unpublished result).

2. Materials and methods

2.1. Chemicals

Standards of BaA (99%), BbF (99.5%), BkF (99%), DA (98.8%), BghiP (98.8%) and IP (98.3%) were purchased from Chem. Service (USA). BaP (98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The structures and physicochemical properties of the selected PAHs are listed in Table 1. M-Terphenyl (99%), hydrochloric acid (HCl, 36%) and sodium hydroxide (NaOH, 98%) were also obtained from Sigma–Aldrich (St. Louis, MO, USA). Ethyl acetate (99.8%), ethanol (99.8%) and acetone (99.5%) were purchased from LabScan Asia Company Limited (Thailand). Sodium chloride and anhydrous sodium sulfate were purchased from Farce Chemical Supplies (China).

2.2. Microalgal species and culture conditions

S. capricornutum was purchased from Carolina Biological Supply Company, Burlington, NC, USA and cultured in soil extract (SE) medium. *Chlorella* sp. was cultured in Bristol medium. Both microalgae were grown under axenic conditions in an environmental chamber at 22 ± 2 °C, illuminated with cool white fluorescent tubes at a light intensity of 50 μmol photons $s^{-1}\,m^{-2}$ with a light–dark duration 16:8 h. Cultures were continuously aerated with 0.2 μm filtered air through a mechanical air pump. Cells reaching the mid to late exponential growth phase (5–7 days) were harvested by centrifugation at 9000 × *g* for 10 min at 4 °C and washed with sterile deionized water. The cells were counted using a hemocytometer with Neubauer improved rulings (Boeco, Germany).

2.3. Removal and transformation of mixed PAHs by live and dead cells of S. capricornutum

Cells of *S. capricornutum* were washed twice with sterile deionized water and re-suspended in the culture medium. A series of 250 mL conical flasks were prepared and divided into three treatments: live cells, dead cells and abiotic controls. For live cells, 100 mL cultures with a cell density of 3.5×10^6 cell mL^{-1} were added into each flask. For the dead-cell treatment, cells with the same density as the live-cell treatment were autoclaved at 121 °C for 10 min prior to inoculation. After autoclaving, cell viability was checked under a fluorescence microscope (Axioskop, Zeiss, Oberkochen, Germany) at ×400 magnification. Viable cells are autofluorescent and give red color at 450 nm. The flasks without any cells were used as the abiotic controls for monitoring any abiotic loss of the seven PAHs. All flasks were spiked with mixed PAH solution consisting of seven PAHs (BaA, BbF, BkF, BaP, DA, IP and BghiP), each at a concentration of 100 $\mu g\,L^{-1}$. The flasks of the same treatment were subdivided into three groups and illuminated under two light sources (gold light and white light) and non-illuminated (dark). Gold light was provided by incandescent lamps (Osram Indonesia, b778, 100 W/230 V), and white light was provided by cool white fluorescent lamps (Philips essential TL5 14 W/840). The light intensity of both two light sources was the same (50 μmol photons $s^{-1}\,m^{-2}$).

Each flask was covered with a cotton wool plug stopper wrapped with aluminum foil. The stopper helped to minimize the abiotic loss of PAHs but allowed sufficient gas exchange during the experiment. The flasks were then shaken on a rotary shaker at 160 rpm in an environmental chamber at 22 ± 2 °C with a 16:8 h light/dark cycle. Triplicate flasks from each group were retrieved after 1, 2, 3 and 4 days of exposure, and the residual amounts of PAHs in the media and the algal cells were determined.

2.4. Removal and transformation of BaP by live and dead cells of S. capricornutum and Chlorella sp.

The experimental setup was similar to that described in the section above, except for the following: (i) two microalgal species, namely *S. capricornutum* and *Chlorella* sp., were examined; (ii) only