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Mini-bioreactors with immobilized microalgae for the removal of benzo(a)anthracene and benzo(a)pyrene from water

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), especially those having four or more rings, are abundant and toxic environmental contaminants that are highly persistent and difficult to remove from contaminated ecosystems. Bioreactors with microalgae have been used to remove some low-molecular-weight PAHs. Immobilization is a good choice for the recovery of biomass from reactors once its function of removing contaminants is fulfilled. Alginate is a natural material that is transparent, is permeable and acts as a protective barrier against other microorganisms and harmful particulates suspended in water. Therefore, in this study, we designed and evaluated four mini-bioreactors packed with alginate beads that contain two microalgal species, *Selenastrum capricornutum* and *Scenedesmus acutus* which are capable of removing the high-molecular-weight PAHs benzo(a)anthracene and benzo(a)pyrene. One of the bioreactors was a batch reactor with a continuous magnetic stirring tank, and the other bioreactors were enclosed tubular bioreactors operating in semi-continuous mode with two cycles. The influence of parameters such as the reactor shape, stirring speed, flow, number of alginate beads, number of cells and cell species ratio, as well as the time of contact and the use of two operation cycles were studied. The amounts of the remaining PAHs after exposure tests with microalgae were determined. It was found that the shape and the mode of operation of the bioreactor markedly influenced the efficiency of removal. The removal capacity of these mini-bioreactors and the advantages and the disadvantages of using these mini-bioreactors were analyzed.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds with two or more fused aromatic rings, and they are persistent and widely distributed in the environment because they are formed from the incomplete combustion of organic materials from both natural and human activities. There are 16 PAHs, listed by the US Environmental Protection Agency (US EPA) that are considered priority pollutants because they are toxic, carcinogenic and mutagenic (Cerniglia, 1992; Brinkhuis, 2001). Benzo(a)anthracene (BaA), with four rings, and benzo(a)pyrene (BaP), with five rings, are included in this group.

Due to their ubiquity and danger, great attention is focused on the remediation of natural habitats contaminated by PAHs. In the environment, these compounds can be removed by adsorption, volatilization, photolysis, and chemical and microbial degradation; the latter is the major degradation process (Haritash and Kaushik, 2009). Compared to bacteria and fungi, relatively little attention has been paid to the biodegradation of PAHs by microalgae, although these microorganisms are one of the major primary producers in aquatic ecosystems and play vital roles in the fate of PAHs in those environments (Ghosal et al., 2016). In fact, microalgae are capable of removing toxic organic contaminants from water by bioaccumulation or degradation (Semple et al., 1999); for this reason, they have a high potential for use in bioremediation. Some microalgal species are capable of removing PAHs such as fluoranthene, phenanthrene and pyrene from media via biosorption and biotransformation. The removal mechanisms of the green microalgae *Selenastrum capricornutum* for these compounds, includes initial adsorption onto the cell walls and subsequent degradation of the adsorbed PAHs

Abbreviations: PAH, polycyclic aromatic hydrocarbon; BaA, benzo(a)anthracene; BaP, benzo(a)pyrene; SPE, solid phase extraction; HPLC, high performance liquid chromatography; UV, ultraviolet; SCAP, *Selenastrum capricornutum*; SAC, *Scenedesmus acutus*; USEPA, United States Environmental Protection Agency; UNAM, National Autonomous University of Mexico.

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(Chan et al., 2006; Lei et al., 2007). As in previous cases, most of the studies about the ability of microalgae to biotransform PAHs have been directed at low-molecular-weight compounds (two or three rings), and few studies have demonstrated the ability of algae to degrade high-molecular-weight compounds with four or more rings (Ghasemi et al., 2011).

BaP is the most studied high- molecular-weight PAH for microbial degradation (Juhasz and Naidu, 2000). This compound can be removed from aqueous medium by sorption and degradation by the microalgae *Selenastrum capricornutum* and *Scenedesmus acutus*, producing dihydrodiol metabolites (García de Llasera et al., 2016; Hernández-Blanco and García de Llasera, 2016; Olmos-Espejel et al., 2012; Warshawsky et al., 1995). Recently, it has been shown that *S. capricornutum* has the same behavior with respect to benzo(a)anthracene (García de Llasera and García Cicourel, 2017).

Therefore, bioremediation of PAHs can be performed in microalgae reactors. Indeed, the removal of nutrients, organic pollutants, and heavy metals from wastewater is performed in microalgae photobioreactors; simultaneously, their cultures produce high-value biochemical products and biogas (Dineshkumar et al., 2016; Doria et al., 2012; Hsieh and Wu, 2009; Judd et al., 2015; Nurra et al., 2014; Yadab and Sen, 2017). The most used microalgae in reactors belong to the genera *Chlorella* and *Scenedesmus,* but other types of microalgae or cyanobacteria can also be used (Queiroz et al., 2007). Microalgal-bacterial consortia have also been used for biomass production and nutrient removal (Cho et al., 2017), as well for the biodegradation of hazardous pollutants such as polycyclic aromatic hydrocarbons, phenols, organic solvents (Muñoz and Guieysse, 2006) and crude oil (Tang et al., 2010). Therefore, the use of reactors is very advantageous for the production of microalgae and the bioremediation of aqueous media. However, biomass harvesting is difficult and costly due to the small cell size and low culture density (Pires et al., 2013). Serious operational constraints for the treatment of industrial effluents appear when filters are clogged or when trying to recover the microorganisms from reactors. Hence, there is an interest in encapsulating cells in materials such as hydrogels to improve the size, strength, stiffness and porosity (Saed and Iqbal, 2006). Moreover, the confinement of microbial cells within a semipermeable polymeric matrix enables the physical isolation of cells from the external environment while maintaining a hospitable internal microenvironment (Rathore et al., 2013). Thus, the removal of nutrients (N and P), heavy metals and low-molecular-weight PAHs (phenanthrene, fluoranthene and pyrene) from wastewater by immobilized microalgae such as *Chlorella miniatis*, *C. vulgaris*, *C. kessleri*, *Scenedesmus quadricauda* and *Selenastrum capricornutum* has been managed in column reactors (Tam et al., 2009; Travieso et al., 1996). Immobilized microalgal-bacterial mixtures such as *C. vulgaris* and *Azospirillum brasilence* have been used for the treatment of wastewater in airlifting triangular reactors (Cruz et al., 2013), while a combination of *C. vulgaris* and *Pseudomonas putida* was used in a batch bioreactor (Praveen and Loh, 2015). Gel entrapment is the method usually employed for microalgae cell immobilization because light and substrates can easily reach the cells. Among the natural polymers used for immobilization, alginate is very excellent because its supplies a stable and porous system with good cellular viability (Vílchez et al., 1997). The success of encapsulation in alginate gel is primarily due to the gentle environment provided by the encapsulating material which also has properties such as high mechanical and chemical stability, porosity and low toxic substance content (Smidsrod and Skjäk-Braek, 1990).

The removal efficiency of encapsulated microalgae depends largely on the shape and operating conditions of the reactor. Photobioreactors can be designed as open or enclosed systems; the latter offer higher photosynthetic efficiencies and better control than the open systems but are more expensive and difficult to operate (Muñoz and Guieysse, 2006). Mixing is an important parameter to optimize in a photobioreactor; the increased turbulence enhances the exchange rates of nutrients, metabolites and other compounds between the cells and the growth medium (Groobelar, 2012).

The objective of this work was to design small bioreactors containing immobilized microalgae in alginate beads for the simultaneous removal of the two high-molecular weight PAHs, BaA and BaP, from drinking water. The efficiency of one open and three closed reactors assayed with different forms of agitation and operation modes was evaluated. The variations in the shape of the reactor, number of alginate beads, number of cells/bead and proportion of mixtures of microalgal species were also investigated. The efficiency of removal was monitored by analytical techniques, such as liquid chromatography coupled to a UV spectrophotometric detector, after the selective extraction of analytes from the water sample by solid-phase extraction. To the best of our knowledge, in literature the bioreactors have not been managed to remove these high-molecular-weight PAHs together. The results obtained in this work may be useful for scaling and future applications in the bioremediation of water ecosystems.

2. Materials and methods

2.1. Materials and reagents

All solvents (acetonitrile, methanol and isopropanol) were HPLC grade and obtained from J.T. Baker (Phillipsburg, NJ, USA). Water (18.2 M Ω cm^{-1} resistivity) was obtained from a Millipore Simplicity UV (Bedford, MA, USA) deionizer. Benzo(a)pyrene and benzo(a)anthracene with a certified purity >99% were purchased from Chem. Service (West Chester, PA, USA). Silica C18 Supelclean with an average particle size of 53.9 μm and average pore diameter of 73 Å (SUPELCO, Bellefonte, PA, USA) was used as a sorbent to perform solid-phase extraction. For immobilization, sodium alginate, calcium chloride ($CaCl_2$) and sodium citrate ($Na_3C_6H_5O_7$) were purchased from Sigma-Aldrich (MO, USA). The Bristol culture medium contained sodium nitrate ($NaNO_3$), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), dibasic sodium phosphate (Na_2HPO_4), monobasic sodium phosphate (NaH_2PO_4), sodium chloride (NaCl) (all from J.T Baker), and the proteose-peptone, which was acquired from MCD LAB (Tlanepantla, MEX, Mexico).

2.2. Sample treatment and HPLC analysis

To perform the extraction of PAHs from the drinking water samples incubated with immobilized microalgae in the alginate beads, the liquid medium was separated from the beads by decanting and was applied to the EFS cartridge packed with 300 mg of the C18 sorbent previously conditioned with 3 mL of acetonitrile and 5 mL of distilled water. The flasks or reactors containing the water sample and beads were rinsed with a volume of 10 mL of 20% isopropanol mixture in deionized water, and then, this mixture was applied to the EFS cartridge. The following sequence of elution solutions were then passed through this cartridge: 10 mL of 10:90 v/v acetonitrile-water mixture, 1.0 mL of 70:30 v/v of acetonitrile-water mixture and 3 mL of 45:55 v/v acetonitrile-water mixture. Finally, the analytes were eluted from the cartridge with 6 mL of acetonitrile. The extract was then evaporated to dryness at 70 °C in a Maria bath and reconstituted in 1 mL of acetonitrile. The evaporated extracts were analyzed by high-performance liquid chromatography with UV detection on a Smartline 1000 liquid chromatograph and a Smartline 2600 UV diode array detector Knauer (Berlin, Germany), using Eurochrom control and data processing software V. 3.05. This HPLC system was equipped with an injector switching valve with

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