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Rapid communication

The ability of dissolved organic matter (DOM) to influence benzo[a]pyrene bioavailability increases with DOM biodegradation

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Abstract

The biodegradation of two substrates and the ability of dissolved organic matter (DOM) to influence benzo[a]pyrene bioavailability as DOM biodegradation progressed were monitored in reactors. Substrates were composed of algae extracts and an artificial substrate that mimics raw wastewater, which were considered to be autochthonous and anthropogenic allochthonous models for DOM, respectively. The soluble microbial products formed during biomass activity were also studied. The aromaticity of DOM was investigated with specific ultraviolet absorbance. Partitioning coefficients between DOM and benzo[a]pyrene, K_{DOC}(biol), were biologically determined by means of 4-h bioaccumulation experiments on *Daphnia magna*. Parent and degraded substrates always significantly reduced the bioaccumulation of benzo[a]pyrene at environmental DOM concentrations. Soluble microbial products also significantly affected the benzo[a]pyrene bioaccumulation. K_{DOC}(biol) ranged between 2×10^4 and 4×10^5 L/kg. As the artificial wastewater biodegraded, DOM aromaticity increased, as did K_{DOC}(biol). During the biodegradation of algae extract DOM, K_{DOC}(biol) increased, whereas their aromaticity slightly decreased.

Keywords: Dissolved organic matter; Biodegradation; Bioavailability; Partitioning coefficient; K_{DOC}; Aromaticity; Benzo[a]pyrene; *Daphnia magna*; Bioconcentration; Soluble microbial products

1. Introduction

Organic matter plays a dominant role in many aspects of the aquatic environment. Its availability controls the bacterial loop in aquatic ecosystems and consequently affects most living organisms. It also affects the fate and the bioavailability of microcontaminants (Stumm and Morgan, 1981). In the water column, a large fraction of hydrophobic persistent organic pollutants (POPs) may bind to dissolved organic matter (DOM), which modifies their bioavailability to aquatic organisms (see review in Haitzer et al., 1998). Most studies report that the presence of DOM in the exposure media reduces the bioaccumulation of POPs in pelagic crustaceans (Leversee et al., 1983; Kukkonen and Oikari, 1991; Haitzer et al., 2001), in fish (McCarthy and Jimenez, 1985a; Freidig et al., 1998), or in benthic organisms (Landrum et al., 1985; Haitzer et al., 1999a). The generally accepted assumption is that only the unbound fraction of contaminants (also referred to as the free fraction) is bioavailable to these organisms (Landrum et al., 1985).

The magnitude of the sorption of contaminants depends on the characteristics of DOM. Because of their high hydrophobicity, humic substances have a great ability to bind POPs (Kukkonen et al., 1990).

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Most published studies focus on the effect of humic substances or humic-rich natural waters on the bioavailability of POPs (Haitzer et al., 1998). However, recent studies showed that nonhumic natural riverine DOM (with low aromaticity and low carboxylic content) could also affect the bioavailability of POPs (Akkanen et al., 2001) and that highly biodegradable DOM was able to reduce the bioavailability of some polycyclic aromatic hydrocarbons (PAH) (Gourlay et al., 2003).

Water bodies under anthropogenic influence contain high levels of nonhumic allochthonous organic matter of urban origin from industrial or domestic effluents (Namour and Müller, 1998). Moreover, this organic matter may be subject to further changes in the medium. Tusseau-Vuillemin and Le Reveillé (2001) found that 25–61% of DOM from wastewater treatment plant effluents was biodegradable. In the highly anthropized river Seine (France) area, 80% of DOM in combined sewer overflows and 40% of DOM in river water are biodegradable (Seidl et al., 1998). Since anthropogenic nutrient discharges may also enhance eutrophication, anthropized waters may also contain high levels of autochthonous biodegradable DOM of algal origin. Mean and Kirchmann (2001) determined that 68% of DOM produced during a phytoplanktonic bloom was biodegraded within 18 days. Not only can DOM impact the bioavailability of POPs, but the progressive biodegradation of DOM is likely to modify the bioavailability of POPs again. However, no comprehensive study on the effect of the DOM present in anthropized waters on the bioavailability of POPs is available, although they carry the highest concentrations of microcontaminants.

This study aimed to evaluate the ability of two types of DOM to influence the bioavailability of benzo[a]pyrene (BaP) and the changes in this influence as DOM is biodegraded. Two types of DOM were studied. Soluble microbial products (SMP), i.e., DOM formed by the bacterial activity during the substrate biodegradation, were also analyzed for their relative importance in refractory DOM composition and their influence on the bioavailability of BaP.

2. Materials and methods

2.1. Water, organic matter, chemicals, and solvents

A solution of 1 mg/L BaP in methanol was prepared in the laboratory from solid PAH powder (purity 98%; Aldrich, Steinhein, France). A mixture of high-purity dichloromethane and methanol (4:1 v/v) (LiChroSolv; Merk Eurolab, Fontenay-sous-Bois, France) was used for the extraction of BaP in organisms.

Mineral water (Evian, Evian, France; pH 7.2, dissolved solids 309 mg/L, conductivity 567 μS/cm, organic carbon <0.3 mg C/L) was used both for experiments and for daphnid culture. Its composition is stable, with a neutral pH and a moderate mineralization.

Raw wastewater and activated sludge were sampled at the Noisy-Le-Grand (France) combined sewer treatment plant. Wastewater was filtered on a precombusted 0.7-μm glassfiber filter (GF/F Whatmann; Kent, UK) and used within 4 h after sampling. Activated sludge was kept under constant aeration until use.

Wastewater and river waters may have highly variable compositions and may contain some toxic compounds that render the biological experiments difficult. To obtain reproducible results, model substrates were used to evaluate the influence of DOM on BaP bioavailability. A commercially available mixture composed of meat and vegetal extracts and sugars (Viandox, Rueil Malmaison, France) was chosen for that purpose as a model for allochthonous anthropogenic DOM. It contains 42 g/L of carbohydrates, 173 g/L of proteins, and 0.77 mg/L of fats (Pernelle et al., 2001). So-called artificial wastewater was prepared by diluting Viandox to 1% in mineral water. As a model for autochthonous DOM, a solution of algae extracts was prepared as follows: *Selenastrum capricornutum* were cultured, concentrated by decanting, and autoclaved for 90 min at 120 °C to accelerate cell lysis. The solution was filtered on a precombusted GF/F filter to preserve only the dissolved fraction. Both substrates were prepared the day of the experiment.

2.2. Biodegradation experiments

The biodegradation of each model substrate was monitored in a 6-L cylindrical, open reactor equipped with a mechanical stirrer. The temperature was controlled at 15 °C. We put 4.5 L of the initial DOM stock solution in the reactor, with a 4.5-mL activated sludge inoculum. The solution was regularly aerated to retain oxic conditions. The biodegradation process was monitored for approximately 15 days. We particularly focused on the first days, when biodegradation was the most rapid. Degrading solutions were regularly sampled and filtered on precombusted GF/F Whatmann filters. For each sample, the dissolved organic carbon (DOC) concentration and the particulate organic carbon (POC) concentration were measured using a carbon analyzer (O.I. Analytical, College Station, TX, USA). The absorbance at 254 nm (A_{254}) of the filtrate was measured using a spectrophotometer (Lambda 11; Perkin-Elmer, Courtabeuf, France) and the specific ultraviolet absorbance (SUVA = A_{254}/DOC in $cm^{-1}g^{-1}L$) was calculated. Other aliquots were periodically sampled, filtered, and used to test the influence of DOM on the bioaccumulation of BaP by daphnids, as described below.