Cadmium sorption and toxicity in autotrophic biofilms

Walter R. Hill, Angela T. Bednarek, and I. Lauren Larsen

Abstract: Autotrophic biofilms (periphyton) accumulate substantial quantities of metals from contaminated water. In this study, we measured the time course of biofilm cadmium sorption, examined the effects of current, biomass, and light on short-term cadmium sorption by biofilms, and tested the toxicity of cadmium to biofilm photosynthesis. The time course of cadmium sorption appeared to be a linear function of time over the 48-h measurement period. Biofilms in current ≥2 $cm \cdot s^{-1}$ sorbed three to five times more cadmium than biofilms in still water. Cadmium sorbed after 4 h was 75% greater in high-biomass biofilm (2.5 mg dry $mass \cdot cm^{-2}$) than in low-biomass biofilm (0.5 mg dry $mass \cdot cm^{-2}$), but only in moving water. Light enhanced the sorption of cadmium 40% in one biofilm type. Cadmium toxicity to photosynthesis was evident after 24 h in thin biofilms exposed to initial cadmium concentrations ≥10 $\mu g \cdot L^{-1}$; photosynthesis by thicker biofilms was not significantly impaired even at the highest concentration (100 $\mu g \cdot L^{-1}$). Variations in current, biofilm biomass, and light are likely to influence the movement of metals in flowing systems.

Résumé : Les films biologiques autotrophes (périphyton) accumulent des quantités importantes de métaux dans les eaux contaminées. Dans cette étude, nous avons mesuré la sorption du cadmium par des films biologiques en fonction du temps, examiné les effets du courant, de la biomasse et de la lumière sur la sorption du cadmium par ces films biologiques à court terme, et les effets toxiques du cadmium sur la photosynthèse dans ces films biologiques. Dans notre période de mesure de 48 h, la sorption du cadmium augmentait de façon linéaire en fonction du temps. Les films biologiques exposés à un courant de 2 $cm \cdot s^{-1}$ ou plus sorbaient trois à cinq fois plus de cadmium que les films qui se trouvaient en eau calme. Les films biologiques à forte biomasse (2,5 mg poids $sec \cdot cm^{-2}$) ont sorbé après 4 h 75% de plus de cadmium que les films à faible biomasse (0,5 mg poids $sec \cdot cm^{-2}$), mais seulement en eau vive. La lumière a accru la sorption du cadmium de 40% dans un type de film biologique. On a observé que le cadmium était toxique à l'égard de la photosynthèse après 24 h dans les films biologiques minces exposés à des concentrations initiales de cadmium ≥10 $\mu g \cdot L^{-1}$; la photosynthèse dans les films plus épais n'a pas été significativement affectée, même à la plus forte concentration (100 $\mu g \cdot L^{-1}$). Les variations du courant, de la biomasse des films biologiques et de la lumière peuvent probablement influer sur la circulation des métaux dans les systèmes fluviaux.

[Traduit par la Rédaction]

Introduction

Microalgae possess a tremendous capacity for accumulating dissolved metals. Phytoplankton and biofilms (periphyton) bioconcentrate cadmium and mercury more than four orders of magnitude above concentrations in the surrounding water (Hart and Scaife 1977; Hill et al. 1996), and microalgae in the laboratory can accumulate 1–10% of their dry mass as aluminum, tin, zinc, and manganese (Wong et al. 1984; Bender et al. 1994). The propensity of microalgae to bioconcentrate is important because many environmental toxicants that biomagnify to high concentrations in upper trophic levels first enter the food web at the autotroph level. Microalgae may also play an important role in the transport of metals through lotic ecosystems. Autotrophic biofilms cover virtually every exposed substrate in streams, presenting a potentially large sink for metals introduced into streams. The potential to remove dissolved metals from the water column makes autotrophic biofilms attractive for the development of biological systems to treat industrial and domestic waste water (e.g., Wilde and Benemann 1993).

Factors influencing the sorption of dissolved metals by autotrophic biofilms are not well researched. Effects of various water chemistry parameters (e.g., pH, phosphate) on metal sorption have been studied (Genter 1996), and efforts to describe the sorption dynamics of metals onto living and dead algal biomass have been made (Crist et al. 1988; Sloof et al. 1995). However, the effects of basic environmental parameters such as biofilm thickness, water flow, and light have received little research effort. These factors shape the architecture and ecophysiology of biofilms and are likely to influence biofilm metal sorption. Biofilm thickness affects the microenvironment and metabolic activity of cells composing the biofilm (Kuhl et al. 1996). It also affects the ratio of cells on the surface of the biofilm to cells in underlying layers. Rose and Cushing (1970) reported that zinc sorbed only to surface cells and suggested that metal sorption is a

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simple function of total surface area, unrelated to biofilm mass. Flowing water reduces the thickness of the diffusive boundary layer overlying a biofilm, allowing faster replenishment of ions taken up from the boundary layer (Riber and Wetzel 1987). Flowing water may also cause advective currents to penetrate the boundary layer (Gunderson and Jorgensen 1990), increasing delivery rates of metals to biofilms. Light should stimulate metal sorption by biofilms through its effect on photosynthesis. Photosynthesis by autotrophic biofilms provides energy for active transport of metal ions into cells (Pawlik and Skowronski 1994), and it increases pH both in the diffusive boundary layer and within the biofilm matrix itself (Jorgensen et al. 1983; Kuenen et al. 1986; Hartley et al. 1996). Because sorption of metals is strongly influenced by pH (Genter 1996), photosynthetically induced changes in pH within or near the biofilm could have a signficant impact on metal sorption. Cadmium sorption by free-floating microalgae has been reported to be greater in the light (Ting et al. 1989; Pawlik and Skowronski 1994; Subramanian et al. 1994).

Biofilm thickness, water flow, and light vary substantially within and between streams. Understanding the potential influence of these factors on metal sorption is crucial to predicting the movement of metals through flowing-water ecosystems (Stewart et al. 1993). In this study, we examined the effects of biofilm thickness, water flow, and light on cadmium sorption by autotrophic biofilms in the laboratory. We first characterized the time course of cadmium sorption by biofilms and then performed two manipulative experiments that tested the effects of biofilm thickness, current, and light. Potential toxicity of cadmium was examined by measuring biofilm photosynthesis over a logarithmic gradient of cadmium concentrations.

Materials and methods

Sorption dynamics

The time course of cadmium sorption was measured in a flow-through laboratory stream. The stream (21 m long and 0.3 m wide) was supplied with water from an unpolluted section of upper First Creek on the Oak Ridge Reservation. Illumination was provided by metal halide lamps overhanging the stream; irradiances were 50–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The experimental stream was kept dry until the day before the sorption measurements began to keep biofilm development minimal. Discharge during the measurements was 0.25 $L \cdot s^{-1}$, resulting in an average velocity of approximately 6 $cm \cdot s^{-1}$ and a stream depth of approximately 1.5 cm. Temperature in the stream at the start of the experiment was 19°C, pH was 8.0, and alkalinity was 139 mg $CaCO_3 \cdot L^{-1}$.

Ceramic tiles (2.4 × 2.4 cm) with attached biofilm were transferred into the stream 2 h before the experiment began. The biofilm was grown on the tiles in an adjacent laboratory stream (supplied by the same water as the experimental stream) and was composed of diatoms (*Achnanthes*, *Synedra*, *Navicula*, and *Cymbella*) and a filamentous cyanophyte (*Lyngbya*). Standing dry mass of the biofilm was 4.85 ± 0.15 $mg \cdot cm^{-2}$ (mean ± SE). Cadmium chloride from a concentrated stock solution was added to the stream with a peristaltic pump positioned 3 m upstream of the biofilm-covered tiles. An array of small ceramic cylinders located between the pump and the tiles created a turbulent zone that mixed the stock solution with the stream water. The effectiveness of mixing was checked with dye before the experiment. Stock solution was added to the stream at a rate that resulted in a cadmium concentration of 1 $\mu g \cdot L^{-1}$ after dilution with stream water. The solution was spiked with ^{109}Cd as a radiotracer for cadmium; ^{109}Cd activity after dilution with stream water was 71 $Bq \cdot L^{-1}$. Stream water ^{109}Cd activity varied little during the experiment: the coefficient of variation of ^{109}Cd activity was 7% in water samples taken at 1, 2, 4, 8, 24, 32, and 48 h after the peristaltic pump was turned on.

Three sets of biofilm-covered tiles (each set consisted of four connected tiles) were removed 10 and 30 min and 1, 2, 4, 8, 24, 32, and 48 h after the peristaltic pump was turned on. The biofilm from each set of harvested tiles was dislodged with a stencil brush, rinsed with distilled water into an aluminum weighing pan, dried at 60°C, and weighed. The amount of ^{109}Cd in the dried biofilm in the pan was determined by gamma spectroscopy using a coaxial, high-purity, intrinsic germanium detector coupled to a Nuclear Data® microprocessor programmed to acquire data in 4096 channels. Full-width half-maximum was 2 keV for the 1332-keV ^{60}Co photon energy. Counting efficiency (8.3%) was determined by adding a known amount of ^{109}Cd to wet biofilm in an aluminum weighing pan, drying the biofilm, and counting the sample.

Biomass and flow effects

Cadmium sorption by different biomasses of autotrophic biofilm was measured in this experiment to determine if metal sorption is a simple function of surface area or a more complex function involving biofilm thickness. The experiment concurrently examined the effect of water movement on metal sorption. Three different biomass levels (0.5 ± 0.1 (mean ± SE), 1.1 ± 0.1, and 2.5 ± 0.3 mg dry $mass \cdot cm^{-2}$) of autotrophic biofilms were obtained by allowing pure cultures of *Stigeoclonium tenue* to grow for different periods of time on the ceramic tiles. This filamentous alga was obtained from the Culture Collection of Algae at the University of Texas at Austin and was grown in soil water amended with 1.8 mM concentrations of $NaHCO_3$, K_2HPO_4, $NaNO_3$, and Tris buffer (pH = 7.2). The three biomass levels corresponded to growing periods of 8, 10, and 12 weeks.

One tile of each biomass level (three tiles in total) were placed in each of six glass crystalizing dishes (12.5 cm in diameter, 6.5 cm high) that contained 250 mL of water from the laboratory stream (pH = 7.8, alkalinity = 96 mg $CaCO_3 \cdot L^{-1}$). Three of the dishes were stirred with a magnetic stir bar to create a current of approximately 6 $cm \cdot s^{-1}$ at the outer edge of the dish where the tiles were placed. The other three dishes were not stirred. The dishes were located in a large environmental chamber (80 cm wide, 120 cm high, 185 cm long) and were illuminated with a combination of incandescent and cool-white fluorescent bulbs at an irradiance of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature in the chamber was maintained at 21°C.

Cadmium chloride from a stock solution and ^{109}Cd were added to each dish in quantities to achieve an initial cadmium concentration of 10 $\mu g \cdot L^{-1}$ and an initial radioactivity of 40.4 $kBq \cdot L^{-1}$. Irradiance and temperature during the incubations were 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 21°C. After 4 h, the tiles with attached biofilms were removed from each dish, immersed for a few seconds in stream water to remove unsorbed ^{109}Cd, and dried at room temperature for 72 h. Gamma spectroscopy quantified the ^{109}Cd sorbed by the biofilm-covered tiles. Counting efficiency (11%) was determined placing a known amount of ^{109}Cd on tiles covered with biofilm, drying the biofilm, and counting the samples. Water samples (1 mL) were also taken from the dishes at the end of the experiment and analyzed with gamma spectroscopy to determine the concentration of cadmium remaining in the dishes. Counting efficiency (10%) for water samples was determined by adding a known amount of ^{109}Cd to a sample vial.

Current gradient and light

Six current velocities (0, 2, 4, 6, 10, and 18 $cm \cdot s^{-1}$) were estab-

lished in this experiment that simultaneously examined the effect of light on cadmium sorption. The six current velocities were created in 12 crystalizing dishes by varying the speed at which stir bars revolved in the dishes. Each current velocity was replicated in two dishes. Current velocity in each chamber was set by precalibrating the speed knob of the stirrer beneath each dish against current velocity that was quantified by timing the speed at which introduced particles moved around the outer edge of the dish (where tiles were placed). One dish of each current velocity was completely wrapped in aluminum foil to eliminate light.

Biofilms from two different sources were used in this experiment. Both biofilms were grown on ceramic tiles in streams on the Oak Ridge Reservation; biofilm on one set of tiles was grown in Walker Branch (for a description of this stream, see Mulholland et al. 1991), and the other biofilm was grown on tiles in East Fork Poplar Creek (EFPC) (for a description of this stream, see Hill et al. 1996). The biofilm from Walker Branch was dominated by *Stigeoclonium* basal cells and averaged 0.76 ± 0.08 mg dry mass·cm^{-2} (mean ± SE). The biofilm from EFPC was a mixed community of *Oscillatoria*, *Stigeoclonium*, and small diatoms (e.g., *Nitzschia*, *Surirella*, *Navicula*, *Gomphonema*); it averaged 4.24 ± 0.35 mg dry mass·cm^{-2}. One tile of each biofilm type was added to each dish, which contained 250 mL of laboratory stream water (pH = 8.2, alkalinity = 112 mg $CaCO_3 \cdot L^{-1}$). Cadmium chloride and ^{109}Cd were added in quantities to achieve an initial cadmium concentration of 10 μg·L^{-1} and an initial radioactivity of 99.2 kBq·L^{-1}. The biofilm-covered tiles were removed after 4 h, and ^{109}Cd sorbed by the tiles was measured as before. Water samples (1 mL) were taken from the dishes at the end of the experiment to determine the final concentration of cadmium in the water. The experiment was performed twice, on consecutive days.

Toxicity test

Potential toxic effects of cadmium on autotrophic biofilms were investigated in two experiments. Five cadmium concentrations were used in each of these experiments: 0, 0.1, 1, 10, and 100 μg·L^{-1}. These concentrations were chosen to include concentrations expected in both uncontaminated (<1 μg·L^{-1}) and contaminated (≥1 μg·L^{-1}) habitats. Each concentration was replicated three times in separate crystallizing dishes containing 250 mL of stream water (pH = 8.2, alkalinity = 137 mg $CaCO_3 \cdot L^{-1}$). The first experiment exposed biofilms to cadmium for 4 h; the second experiment exposed biofilms for 26 h. Three different biomasses of biofilms were used in both experiments: low-biomass biofilms grown in the laboratory streams, medium-biomass biofilms grown in EFPC, and high-biomass biofilms grown in EFPC. Differences between medium- and high-biomass biofilms were due to different development times in EFPC. The low-biomass biofilm was composed of *Audouinella*, *Stigeoclonium*, and diatoms (*Cocconeis*, *Fragilaria*). The medium- and high-biomass biofilms from EFPC were composed of *Stigeoclonium*, *Phormidium*, and diatoms (*Nitzschia*, *Achnanthidium*, *Fragilaria*, *Gomphonema*, *Navicula*). The dry masses of the low-, medium-, and high-biomass biofilms were 0.89 ± 0.05 (mean ± SE), 2.24 ± 0.11, and 2.98 ± 0.17 mg·cm^{-2}, respectively. One tile of each biofilm type was added to each crystalizing dish.

Photosynthesis was the response variable in both experiments. It was measured by ^{14}C uptake. In the 4-h experiment, ^{14}C was added at the beginning of the experiment. In the 26-h experiment, ^{14}C was added 24 h after cadmium had been added. Each crytallizing dish received 148 kBq of $NaH^{14}CO_3$. At the end of each experiment, tiles with attached biofilms were removed from the crystallizing dishes, rinsed briefly in stream water to remove unincorporated ^{14}C, and placed in extraction jars containing dimethyl sulfoxide for the extraction of chlorophyll *a* and ^{14}C-labeled photosynthate (Palumbo et al. 1987). Extracted chlorophyll *a* was measured spectrophotometrically, and the ^{14}C in the labeled photosynthate was assayed via liquid scintillation. A more detailed description of the ^{14}C methodology is given in Hill and Boston (1991).

Results

Cadmium sorption dynamics

Cadmium sorbed by biofilm increased steadily over the 48-h period of sampling (Fig. 1). Linear regression fit the data well (r^2 = 0.99), even at early sampling times. Sorption may have been nonlinear before 10 min, but the amount of cadmium sorbed in this period was very small (<10% of the amount sorbed over 2 h, <1% of the amount sorbed over 48 h).

Biomass and flow effects

Cadmium sorption increased with both biofilm biomass and water movement (Fig. 2). The effect of biomass appeared to depend on flow: cadmium sorption in moving water increased approximately 75% as biofilm biomass increased from 0.5 and 2.5 mg dry mass·cm^{-2}, whereas the sorption increase with biomass was quite small in still water. Regression through the sorption data from the containers with moving water resulted in a slope significantly greater than zero (P = 0.002), whereas regression through the data from the containers with still water resulted in a slope estimate that did not differ significantly from zero (P = 0.30). The effects of current were strong for all biomasses: cadmium sorption was three to five times greater in moving water than it was in still water. Cadmium concentrations at the end of the experiment were 5.0 ± 0.1 μg·L^{-1} (mean ± SE) in the dishes with still water and 3.1 ± 0.1 μg·L^{-1} in the dishes with moving water (initial cadmium = 10 μg·L^{-1}). Although depletion of cadmium to 3–5 μg·L^{-1} may have constrained uptake to some extent by the end of the experiment, it would have constrained all biomass treatments equally because they were all represented in each container.

Current gradient and light

Cadmium sorption was again strongly influenced by current. Sorption in both dark and illuminated chambers increased dramatically as current velocity increased from 0 to 2 cm·s^{-1} (Fig. 3). Velocities >2 cm·s^{-1} did not appear to further increase cadmium sorption by Walker Branch biofilm. Mean cadmium sorbed after 4 h by Walker Branch biofilm was 14.1 ± 2.9 ng·cm^{-2} (mean ± SE) at zero current and 59.0 ± 6.0 ng·cm^{-2} at current ≥2 cm·s^{-1}. Sorption by EFPC biofilm increased slightly as velocity increased above 2 cm·s^{-1}. Mean cadmium sorbed by EFPC biofilm after 4 h was 13.2 ± 2.2 ng·cm^{-2} (mean ± SE) at zero current and 67.5 ± 6.5 ng·cm^{-2} at current ≥ 2 cm·s^{-1}. Cadmium concentrations at the end of the experiment were 7.7 ± 0.2 μg·L^{-1} (mean ± SE) in dishes with still water and 3.3 ± 0.3 μg·L^{-1} in dishes with moving water (current velocities ≥ 2 cm·s^{-1}).

Light effects depended on biofilm type. Cadmium sorption by Walker Branch biofilm was consistently higher in illuminated chambers than in darkened chambers at all current velocities. The average increase in sorption caused by light in this biofilm was approximately 40%. Differences between light and dark treatments were generally smaller and less consistent for EFPC biofilm.

Fig. 1. Time course of cadmium sorption by autotrophic biofilms. Sorption data are expressed as the quantity of cadmium absorbed per unit of substrate area. Each point is the mean concentration of cadmium sorbed by three replicated sets of tiles. Inset illustrates sorption over the first 2 h. Error bars represent ±1 SE; some error bars are covered by the mean points. The equation for the regression line is $y = 0.99x + 0.24$ ($r^2 = 0.99$, $P < 0.001$).

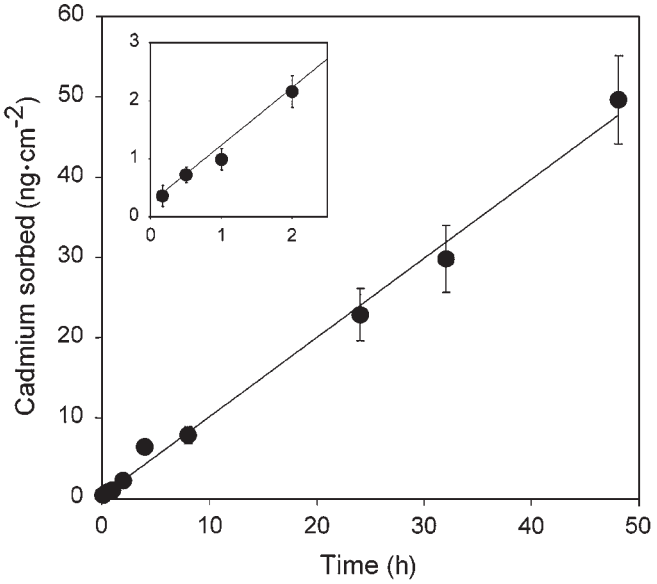
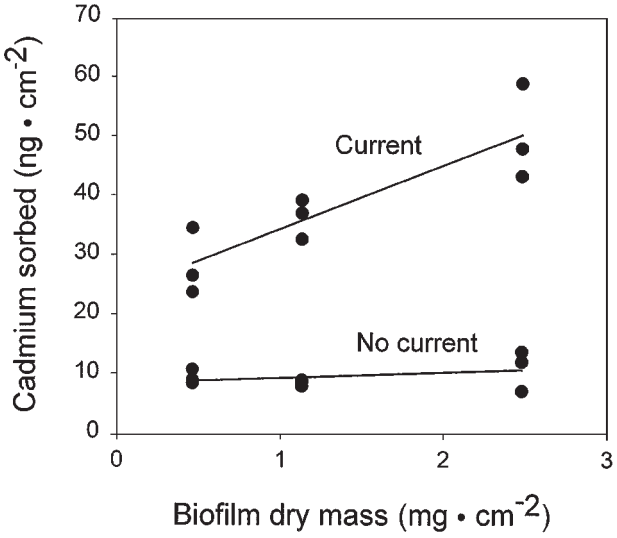


Fig. 2. Biomass and flow effects on the sorption of cadmium by autotrophic biofilms. Data represent the mass of cadmium accumulated per unit of substrate area after 4 h. Current = 6 cm·s^{-1}. The regression line through current data is $y = 11x + 24$ ($r^2 = 0.74$, $P = 0.002$); the regression line through the no current data is $y = 0.93x + 8.3$ ($r^2 = 0.15$, $P = 0.3$).



Cadmium toxicity

The autotrophic biofilms were relatively resistant to cadmium toxicity. After 4 h of exposure, photosynthesis was not significantly impaired at any cadmium concentration, although photosynthesis by the low-biomass biofilm may have been verging on impairment at 100 μg·L^{-1} (Fig. 4). After 26 h, photosynthesis by the high-biomass biofilm remained unaffected by cadmium, photosynthesis by the medium-biomass biofilm was slightly (but not significantly) diminished by increasing cadmium concentration, and photosynthesis by the low-biomass biofilm declined steadily at concentrations ≥1 μg·L^{-1} (Fig. 4).

Discussion

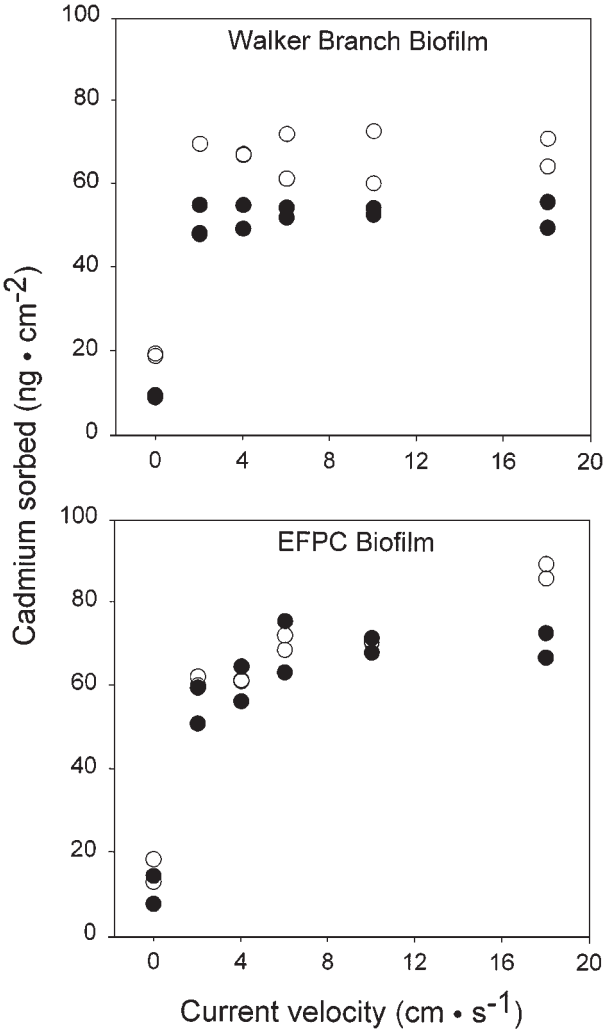
Sorption dynamics

Metal sorption by microalgae is often characterized by an initial phase of rapid sorption followed by a second phase of slower uptake that gradually approaches an asymptote after a few to many hours (e.g., Genter et al. 1988; Reinfelder and Chang 1999). In the biphasic model of metal uptake that describes these dynamics, metal ions bind to and saturate negatively charged sites (e.g., carboxylic groups) on the exterior of cells in the first phase and are then transported to the interior of the cell via energy-dependent processes in the second phase (Xue et al. 1988; Ting et al. 1989). The time course of cadmium sorption by our biofilm exhibited little evidence of a significant first phase, suggesting that internal uptake was the most important component of cadmium accumulation. The linear sorption dynamics that we observed may be due to a comparatively low, but environmentally realistic, cadmium concentration that allowed active transport to keep pace with adsorption. Biphasic sorption of cadmium by phytoplanktonic diatoms became more linear as cadmium concentration decreased (Conway and Williams 1979), and accumulation of cadmium at <0.1 μg·L^{-1} by lake periphyton did not exhibit a significant rapid uptake phase (Stephenson and Turner 1993).

Current

Current had a large stimulatory effect on cadmium sorption by biofilms in our study, increasing sorption three to five times over that in still water. Several processes may account for the effect of current. First, current decreases the thickness of the diffusive boundary layer above biofilms. Metal concentrations in the diffusive boundary layer should be lower than those in the overlying bulk water because of metal uptake by the biofilm. Uptake should create a vertical zone of ion depletion through which new ions must diffuse from the bulk water to reach the biofilm. Reduction in the thickness of this layer could directly increase the rate at which cadmium ions move to the biofilm. Second, current may increase ion delivery to biofilms by increasing the frequency of advective currents that penetrate the boundary layer and biofilm (Gunderson and Jorgensen 1990). Third, heterogeneities in biofilm architecture may allow current to penetrate into the biofilm matrix (deBeer et al. 1994), allowing delivery of metal ions to subsurface cells. Fourth, current may increase cadmium sorption indirectly by stimulating biofilm metabolism. Metabolic processes such as photosynthesis, respiration, and nutrient uptake all occur at higher rates in moving water because metabolic substrates move to the biofilm faster and metabolic byproducts are moved away faster (Riber and Wetzel 1987). However, increases in biofilm photosynthesis cannot explain the stimulatory effect of water flow on cadmium sorption in our experiments because

Fig. 3. Flow and light effects on the sorption of cadmium by autotrophic biofilms. Data represent the mass of cadmium accumulated per unit of substrate area after 4 h. Data points are from individual sorption chambers. Solid circles, dark chambers; open circles, light chambers.



sorption by biofilms in the dark also increased with water flow.

Increases in current beyond 2 $cm \cdot s^{-1}$ had little additional effect on cadmium sorption. This apparent saturation at low current velocity may have been in part due to depletion of dissolved cadmium in the stirred containers. Cadmium concentrations in dishes with moving water were reduced from 10 to <4 $\mu g \cdot L^{-1}$ by the end of the experiment. These lower concentrations may have slowed sorption rates in these containers and caused a flattening in our sorption versus current response. No quantitative analysis of the effect of current on metal sorption has been published in the literature, but the effect of current on biofilm metabolism plateaus at velocities ranging from 5.4 to 50 $cm \cdot s^{-1}$ (Whitford 1956; Lock and John 1979; Horner et al. 1990). The specific relationship between sorption and current velocity may depend on the total mass or density of biofilms, particularly if horizontal flow through the biofilm is an important vector of ions to interior sorption sites. Regardless of the precise shape of the sorption versus current curve, small current velocities clearly had a large influence on cadmium uptake by our biofilms.

Light

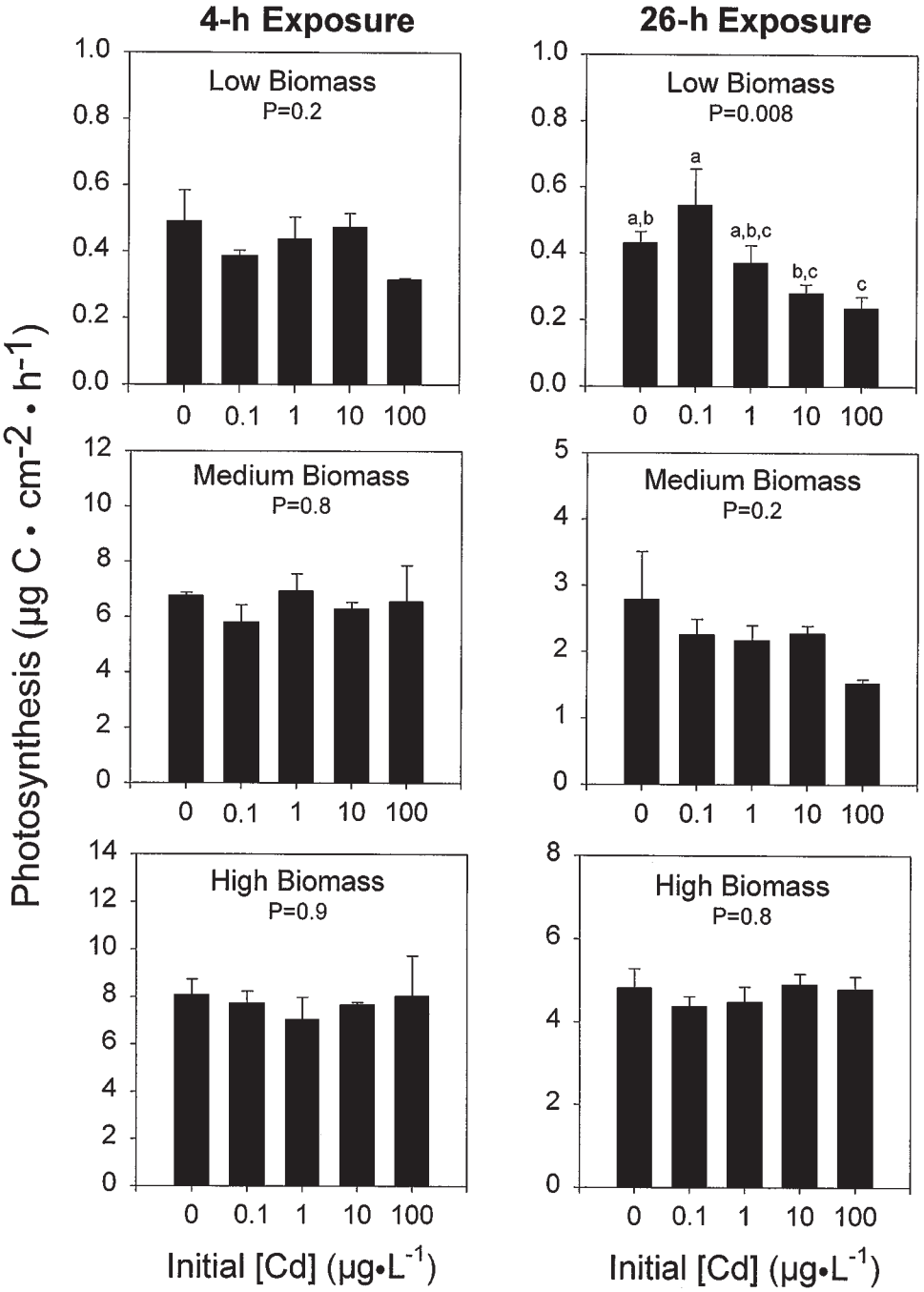
The effect of light on cadmium sorption varied between biofilm types. Light increased sorption 40% by Walker Branch biofilm, but its effect on EFPC biofilm was inconsistent. Other studies report strong and positive effects of light on the sorption of metals by phytoplankton (Ting et al. 1989; Pawlik and Skowronski 1994; Skowronski et al. 1998) and autotrophic biofilms (Gray and Hill 1995), so the inconsistent response to light by EFPC biofilm was somewhat surprising. It is unclear if this response is an anomaly unique to the specific EFPC biofilms used in this study or if these biofilms represent a more general subset of biofilms that do not respond to light.

When light does increase metal uptake by microalgae, its effect is presumed to be mediated through photosynthesis. Darkness and photosynthetic inhibitors such as 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea similarly reduce cadmium sorption by the cyanobacteriium *Synechocystis aquatilis* (Pawlik and Skowronski 1994). Biofilm sorption of nickel over a gradient of light intensities closely matches carbon uptake rates over the same gradient, suggesting a quantitative link to photosynthesis (Gray and Hill 1995). Pawlik and Skowronski (1994) and Skowronski et al. (1998) argued that photosynthesis increases cadmium sorption because it provides the metabolic energy required for the metal's active transport into algal cells. Light/photosynthetic effects may also operate external to algal cells in biofilms. Photosynthesis can raise pH several units in the water immediately surrounding microautotrophs, making protons relatively scarce in the diffusive boundary layer and within the biofilm itself. Positively charged metals such as cadmium compete with protons for negatively charged binding sites (Crist et al. 1988), so adsorption of cadmium at these sites increases with increasing pH (e.g., Skowronski 1986; Sampedro et al. 1995). Increases in pH due to photosynthesis can also cause the precipitation of calcite ($CaCO_3$) at the biofilm surface or within the biofilm (Hartley et al. 1996), where pH can exceed 9 (Jorgensen et al. 1983; Kuenen et al. 1986). Cadmium binds strongly to precipitating calcite particles (Cicerone et al. 1999).

Biomass

Increasing cadmium sorption with increasing biofilm biomass implies a significant contribution of subsurface cells to metal sorption. These cells may contribute directly to sorption by providing additional sorption sites, or they may contribute indirectly by providing additional photosynthetic units that raise pH. The implication of a three-dimensional sorption process is that streams or water treatment channels containing thick biofilms will be more effective at removing metals than streams or channels with thin biofilms. However, the effectiveness of additional biofilm biomass in cadmium sorption may be limited to flowing water, since the effect was evident only in chambers that were stirred in our experiment. In the absence of current, diffusion of cadmium into cells deep in the biofilm matrix may be so slow that effects of biomass can only be detected over periods longer than 4 h.

Fig. 4. Cadmium effects on photosynthesis by low-, medium-, and high-biomass biofilms. Letters above bars in the top right graph indicate results of Fisher's protected least significant difference multiple comparison procedure; bars with the same letter are not significantly different ($P > 0.05$).



Toxicity of cadmium to biofilms

The biofilms used in our experiments appeared to be relatively resistant to the toxicity of cadmium. Photosynthetic impairment was detected only in the 26-h exposure, and only in the low-biomass biofilm. Toxicity has been observed at cadmium concentrations <100 $\mu g \cdot L^{-1}$ in a variety of microalgae (Peterson et al. 1984; Lawrence et al. 1989; Leborans and Novillo 1996) and at concentrations as low as 2 $\mu g \cdot L^{-1}$ in natural phytoplankton (Conway 1978; Nalewajko 1995). These effects were generally observed after exposures lasting for several days. In our study, photosynthetic impairment was statistically significant only in the longer of the two toxicity experiments, suggesting a significant lag between sorption and toxicity. This lag may result from the time required for cadmium ions to accumulate to toxic levels inside the cell, where metabolic disruption occurs. Some of the lag may also be due to slow diffusion of cadmium ions through the entire three-dimensional biofilm matrix.

The greater sensitivity of the thin biofilms to cadmium suggests that additional biomass in thicker biofilms mitigates the toxicity of cadmium in some way. Additional biomass sorbs more cadmium, effectively reducing the concentration

of cadmium in the water and reducing cadmium available per cell in the biofilm. The diluting effect of biomass should be effective in reducing toxicity as long as the sorption capacity of the biofilm is not reached. Biomass may not mitigate toxicity in contaminated streams where a steady supply of new cadmium ions is constantly transported to attached biofilms.

Implications

Our experiments have important implications for the transport and fate of metals in flowing-water systems. Extrapolation of our results to natural streams suggests that natural variability in biofilm biomass, light, and current will affect in the movement of metals through these ecosystems. Streams or stream sections with higher standing crops of biofilms should retain a higher proportion of dissolved metals near the metals' point of entry. In terms of solute spiralling, this means that the average "uptake distance" for metals will be reduced in streams where biofilm thickness is greater due to higher biofilm productivity or lower losses from grazing or scour (e.g., Mulholland et al. 1983). Flowing water obviously transports metals downstream, but this downstream movement should be offset at least partially by the very large increase in sorption rate stimulated by current. Metal sorption by biofilms may be significantly slower in pools where current velocities are nearly zero. It should also be slower in biofilms growing in the shade of terrestrial vegetation and at depths where light has been attenuated by the overlying water column.

Our results can also be applied to the design of biofilm-based water treatment systems. Artificial stream channels constructed to treat industrial or domestic waste water contaminated with metals should allow the development of at least moderate thicknesses of autotrophic biofilm. The time course of cadmium sorption indicates that moderately thick biofilms can be used to remove dilute concentrations of contaminants for at least 48 h without reaching saturation. This biofilm should be illuminated during waste water treatment to encourage photosynthetically related sorption, and current should be provided to increase the efficiency of metal sorption. Biofilms in engineered systems should be relatively tolerant of cadmium concentrations up to 100 $\mu g \cdot L^{-1}$ for at least 4 h, unless cadmium-sensitive microalgal species dominate the biofilm.

Autotrophic biofilms are expected to be important reservoirs for contaminants because they cover virtually every exposed surface in aquatic ecosystems. Biofilm sorption processes should be especially important in streams where the ratio of streambed area to water volume is relatively large. Because of the ubiquitous distribution, sorptive nature, and trophic contributions of autotrophic biofilms, variations in the environmental factors that affect the biofilm sorptive properties could significantly impact the movement of contaminants in the water column and through the food web.

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