Chemical Geology 451 (2017) 17–24

Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

Measurements of bacterial mat metal binding capacity in alkaline and carbonate-rich systems

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ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 22 December 2016
Accepted 4 January 2017
Available online 6 January 2017

Keywords:
Bacterial mats
Reactivity
Metal adsorption
Alkaline
Carbonates
Geochemistry
Biogeochemistry

ABSTRACT

Measuring the metal binding potential and reactivity of bacterial mats is challenging in alkaline and carbonate-rich systems. Traditional methods used to measure these parameters, such as potentiometric titrations and metal adsorption pH edges, are difficult to implement due to the presence of the carbonate minerals that buffer pH and prevent assessment of mat surface reactivity. Additionally, under alkaline conditions metals may form hydroxide and/or carbonate precipitates. In this study we examined the metal binding capacity of four distinct bacterial mats collected from Fairmont Hot Springs, BC, Canada. To prevent metal precipitation, the bacterial mat concentration was varied under a constant initial cadmium (Cd) concentration of 8.89 μM and at pH 8. In addition to the intact bacterial mats, a carbonate mineral sample and two bacterial mats in which the carbonate mineral was removed via acid-treatment, were used as end-members to assess the mechanisms of reactivity in the whole system. Freundlich adsorption isotherms were used to fit metal adsorption data and directly compare surface reactivity among intact mats and mat components. Two of the intact mats exhibited a higher affinity for Cd compared to the mineral at metal equilibrium concentrations above 2.5 μM, while the other two intact mats had lower affinities under all experimental conditions. Generally, we found the acid-treated mats had higher Cd adsorption capacities than the carbonate mineral. When compared to their equivalent intact mats, only one acid-treated mat had a higher affinity for Cd. Further, we modeled whether metal adsorption in the intact mats, containing microbes and carbonate mineral, could be explained by a linear combination of the observed metal uptake by the organic and inorganic components through end-member experiments. Metal adsorption additivity results were mixed. Metal uptake by one intact mat was found to be additive, while for the other mat the additive model significantly underestimated the observed Cd accumulation. Our study demonstrates the potential, as well as the limitations, of using modified metal adsorption edges to determine the metal binding affinity and surface reactivity of bacterial mats in alkaline and carbonate-rich systems.

1. Introduction

Bacteria are found in nearly every environment on Earth, and because of their ubiquity, they play an integral part in the cycling of most elements (see Konhauser, 2007 for details). The initial step in many biogeochemical processes is the adsorption of metal cations to the negatively-charged cellular surfaces (Beveridge and Murray, 1976). The adsorbed cations subsequently facilitate numerous chemical processes including metal reduction (e.g., Newman et al., 1997), oxidation (e.g., Nealson et al., 1988), interacellular accumulation (Southam and Beveridge, 1994), and in many cases, biomineralization (e.g., Ferris et al., 1986).

Significant work has been undertaken to determine the mechanisms by which metal cations bind to bacteria (Mullen et al., 1989; Fein et al., 1997; Lalonde et al., 2007; Alessi and Fein, 2010). Bacterial cell envelopes contain multiple surface functional groups that have distinct proton and metal binding capacities (Beveridge and Murray, 1980; Fein et al., 1997). Typically, potentiometric titrations of bacterial cells combined with data from bulk metal adsorption experiments to the same bacteria have been used to develop protonation models and metal surface complexation models (SCM), respectively (Cox et al., 1999; Fowle and Fein, 1999; Ngwenya et al., 2003). For the SCM approach, the proton reactivity of a suspension of bacteria is tested by potentiometric titration; that is, by adding precisely-measured aliquots of acid or base to the suspension, and measuring the pH change due to the addition of that aliquot after equilibrium has been achieved, across a wide pH range. The buffering capacity measured may then be ascribed to a

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http://dx.doi.org/10.1016/j.chemgeo.2017.01.002
0009-2541/

number of surface functional groups with discrete binding constants (pKa) and site concentrations. Once the surface functional group concentrations and pKa value(s) have been determined, bulk metal sorption experimental data from either metal adsorption pH edges (fixed metal concentration with pH varied) or metal adsorption isotherms (fixed pH with metal concentration varied) are fitted to determine the metal binding (equilibrium) constants onto the proton-active functional groups calculated from the potentiometric titration data (Fein et al., 1997; Cox et al., 1999; Haas et al., 2001; Ngwenya et al., 2003).

While this body of work demonstrates the potential of bacteria to bind and sequester metal cations, most of these studies have focused on pure cultures of bacteria grown as suspensions under carefully controlled laboratory conditions (e.g., Teitzel and Parsek, 2003). However, most bacteria found in natural environments grow in dynamic multispecies communities, referred to as either biofilms, or their thicker versions, bacterial mats (Kolter and Greenberg, 2006; Elias and Banin, 2012). Mats are also composed of dead cells and exopolymeric substances (EPS) which have been shown increase the number surface reactive sites, measured as mmol/g, compared to the intact bacterium itself (Baker et al., 2009). The secreted EPS have metal binding properties distinct from the bacterial cells and, therefore, they also impact the adsorption of metal cations from solution by bacterial mats. For example, Spath et al. (1998) measured the distribution of adsorbed cadmium (Cd^{2+}) and zinc (Zn^{2+}) on mats collected from wastewater treatment sequencing batch biofilm reactors. The EPS was found to retain 20% of the total sorbed metal cations. This indicates that there is a substantial difference between in the behavior of bacterial mats in nature, and the individual cells that make up the mats.

In addition to being composed of bacteria and EPS, mats often incorporate detrital and authigenic or allogenic minerals into their structure (e.g., Konhauser et al., 1998; Jones et al., 2004). These minerals have their own reactivity, which can alter the overall reactivity of the mats (Konhauser and Urrutia, 1999). For instance, Lalonde et al. (2007) applied a SCM approach to model the surface reactivity of intact mats from an alkaline hydrothermal spring in Yellowstone National Park. To determine the mat reactivity they performed acid titrations from pH 10 to 4, on both intact and acid treated mats. They found that the surface reactivity of the intact mats was dominated by a single surface site with a modeled pKa of approximately 7, which they attributed to the presence of incorporated carbonate precipitates. With the majority of carbonate precipitates removed, the acid treated mats exhibited an order of magnitude less buffering capacity (measured as mmol protons consumed per g mat) distributed across the tested pH range of 4 to 10. While that study was able to determine the relative buffering capacity of the mats, the authors never removed all of the carbonate minerals, meaning that even in their acid treated mat the carbonate minerals likely made a substantial contribution to the overall buffering capacity measured. Additionally, since titrations were only preformed in a single direction, the reversibility of the system was undetermined. If carbonate dissolution occurred during acid titration, a reverse titration (with base) would have shown hysteresis in the buffering capacity between the forward and reverse titration curves due to this loss of carbonate mineral. The study by Lalonde et al. (2007) highlights the difficulties in determining reactivity in alkaline and carbonate systems and our inability to use potentiometric titrations in the presence of carbonate precipitates or other pH sensitive precipitates.

In this study, we quantify the reactivity of four different bacterial mats collected from the outflow of an alkaline hot spring located at Fairmont Hot Springs, British Columbia. Because of the reasons described above, traditional methods to probe reactivity, such as potentiometric titrations, adsorption isotherms, and adsorption pH edges, are likely to be incompatible due to potential interferences caused by the rich abundance of carbonate minerals and the alkaline conditions of Fairmont Hot Springs (Rainey and Jones, 2009). Based on the results of Lalonde et al. (2007), the dissolution of carbonate minerals intertwined with the bacterial component of the intact mats during acid titrations would likely dominate the proton buffering capacity and thereby obscure direct measurement of the proton reactivity of the bacteria in the mat (Warchola et al., in review), which is ultimately key in quantifying the contribution of mat bacteria to overall observed metal removal from solution. Additionally, during metal adsorption pH edge experiments, the presence of buffering carbonates would limit the pH range able to be tested. Methods such as metal sorption edges, which are conducted under alkaline conditions, often create conditions in which formation and precipitation of carbonate and hydroxide minerals are favorable. For example, in a system in equilibrium with otavite ($CdCO_3$), the total Cd in solution decreases exponentially as a function of increased pH (Fig. 1). In this case Cd and otavite are used as proxies for divalent metals and their carbonate precipitates, and clearly illustrate that under increasingly alkaline conditions greater divalent cation precipitation can be expected. Accordingly, this precludes the investigation of metal adsorption using traditional metal adsorption isotherms for alkaline conditions.

As metal adsorption and the reactivity of bacterial mats are understudied in carbonate rich and alkaline systems, in this study, we propose a method to bypass the issues posed by traditional methods. To address these issues, modified metal adsorption experiments (in which Cd was used as a proxy for environmentally relevant divalent metals) were used to quantify the reactivity of the bacterial mats from a carbonate-rich system under metabolically inactive conditions. To minimize Cd precipitation, the concentration of sorbent (the bacterial mat or mineral) was varied with a constant initial Cd concentration in lieu of varying the metal concentration. Adsorption is thought to be a fast process occurring on a time scale of seconds to minutes (Xue et al., 1988; Kuyucak and Volesky, 1989; Matheickal et al., 1999), whereas precipitation of mineral phases is the slower step on a time scale of hours to days (Sadiq, 1992). Therefore, the length of Cd exposure was limited to 4 h to allow for adsorption to reach equilibrium and to exclude significant precipitation of Cd as carbonates or hydroxides. In addition to determining the reactivity of intact bacterial mats, reactivity was also determined for travertine (a lithified carbonate sediment associated with microbial mats in hot springs) and two of the bacterial mats that were acid treated to remove the incorporated carbonate minerals. The reactivity of the intact bacterial mats, endmember mineral component, and pure bacterial components (acid treated mats) was quantified through the Cd sequestration potential. By quantifying the potential of not just the intact mats but each endmember component, the contribution of each endmember to the overall observed Cd sequestration in intact mat samples was assessed.

2. Methods

2.1. Handling and collection of samples

Bacterial mats and travertine samples were collected from an alkaline hot spring located at Fairmont Hot Springs, BC, Canada on

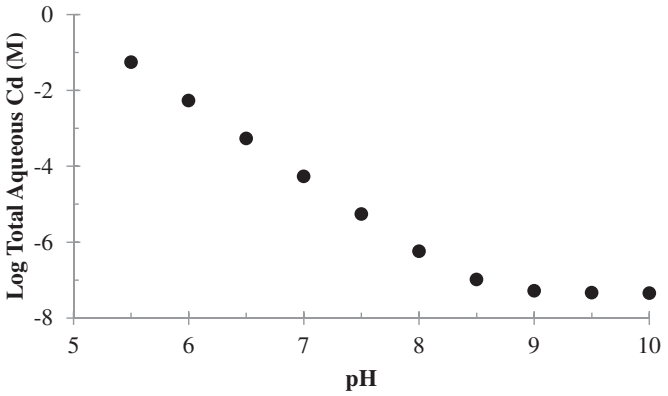

Fig. 1. Total Cd in solution in equilibrium with otavite as a function of pH.