

Calcium Carbonate Biofouling in The Presence of Heavy Metals

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ABSTRACT

The presence of heavy metals in wastewater is significant to the extent they are involved in biofouling. Microalgae and other microorganisms play a significant role in the transport of heavy metal ions in the environment while at the same time they are closely related to the formation of minerals in aquatic systems, mainly with the formation of calcium carbonate. Metabolic byproducts of microalgae act as sequestrants of metal ions, thus contributing to the transport of heavy metals on mineral deposits. In this study, the effect of zinc and cadmium in the precipitation of calcium carbonate induced by microalgae was investigated at 25°C, pH 8.5. Living and dead microalgae colonies were used to inoculate calcium carbonate supersaturated solutions. The presence of trace metals resulted in the inhibition of calcium carbonate precipitation, contrary to the accelerating role of microalgae to the crystal growth rate. Also, the presence of metals resulted at the inhibition of microalgae growth and more specifically of photosynthesis, as suggested by the reduction of total chlorophyll measured. The extracellular components of the test microalgae play important role on the nucleation and the subsequent rate of precipitation of calcium carbonate and determine the ability of microalgae for the uptake of heavy metals.

Keywords: calcium carbonate, scale, zinc, cadmium, nickel, copper, microalgae, kinetics

INTRODUCTION

Heavy metals are released into water due to various anthropogenic activities. Their treatment is of special importance, since heavy metals are nonbiodegradable and persist in the environment as a threat to the biota due to their toxic nature. Some of the heavy metals, such as copper, nickel, and zinc, are, at very low traces, essential for life because they are important in metabolic processes of living cells.¹ However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Metals in the aquatic environment occur in different chemical forms such as ions dissolved in water and salts or minerals. They can also be bound in organic or inorganic molecules forming complexes.^{2,3} The chemical form of a metal in the environment is constantly changing because of the broad range of biochemical processes which influenced by biotic (interactions with living organisms, such as microorganisms, plants, and animals) and abiotic factors (temperature, pH, organic matter, and ionic strength).⁴ As these metals cannot be degraded, they are major toxic contaminants, causing both environmental and health problems. Research for efficient, cost-effective technologies for detoxification of metal contaminated sites is needed. Microalgae are often used as biological sensors for detecting potential toxic effects of heavy metals.⁵

Specifically, they can accommodate relatively large amounts of heavy metals, a process involving the uptake (absorption) and accumulation of the metal ions inside microalgae cells, in which heavy metals enter via micronutrient transporters. Heavy metal detoxification may be achieved through their binding to specific intracellular compounds.⁶ The entire biosorption process of heavy metals by microalgae is generally a biphasic process where surface adsorption and internal diffusion are involved in the uptake of metals by algae.^{7,8}

Different physicochemical methods including electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption for heavy metal removal from waste streams have been developed for the remediation of heavy metal contamination of aquatic environment.⁹ In the present work the tolerance of the microalgae species *Acutodesmus obliquus* with respect to heavy metals was examined in association with the formation of calcium carbonate mineral deposits. The biosorption of heavy metal ions like zinc, cadmium, nickel and copper as a function of the contact time with living algae or inactivated substrates.

EXPERIMENTAL PROCEDURE

Algae culture development

The microalgae species used was *Acutodesmus obliquus*. Cultures of algae were grown in specific culturing medium. Following the preparation of the culturing Basal medium (BBM),¹⁰ the microalgae population was grown in 250ml (batch cultures) conical bottles. Air was pumped in the culture environment, while the light conditions favored the growth of microorganisms. Specifically, cool white 3500lx lamps were used. Cultures were grown at $25\pm 2^\circ\text{C}$ and the pH value was adjusted to 6.5.

Adsorption experiments

Sorption experiments were carried out on calcite crystals for various concentrations of each metal. Calcite particles suspensions were prepared by slowly mixing equal volumes of 0.2M CaCl_2 and 0.2M NaHCO_3 solutions, over a period of ca. two hours. The precipitate was aged in the mother liquor for 24h at room temperature, under continuous stirring with magnetic stirrer and a Teflon coated stirring bar. Next, the crystals were separated by filtration, washed thoroughly on the filters and dried at room temperature. The BET specific surface area of the precipitates was $0.33 \text{ m}^2/\text{g}$. X-ray diffraction analysis confirmed the presence of calcite exclusively. Saturated calcite solutions were prepared by suspending 100 mg of calcite in sodium chloride aqueous solutions 0.1M or 0.15M and the pH was adjusted to 8.5. After an equilibration time of 1hour, accurately measured amounts of the corresponding metal were added from stock solution to achieve the desired initial concentrations in the calcite suspensions. The solutions were allowed to reach equilibrium under continuous stirring at constant temperature 25°C . After three or five days, samples were withdrawn, filtered and the filtrates were analyzed for calcium and total metals concentration.

Mineralization Experiments

All solutions were prepared from stock solutions which were prepared from the corresponding crystalline reagents dissolved in deionized, triply distilled water and filtered through $0.22\mu\text{m}$ polycarbonate membrane filters. For the mineralization experiments, solutions supersaturated with respect to calcium carbonate were prepared in a double walled, Pyrex® glass water jacketed reactor (500 ml). The temperature was kept constant at $25^\circ\pm 0.2^\circ\text{C}$. This parameter was the only one which was controlled (free drift calcification experiments). Different concentration of nutrient components, which were important for the algae growing, were also added to the supersaturated solutions. Next the active microalgae or the dried algae substrates ($24\text{h}/25^\circ\text{C}$) were added to the reactor. The solution kept under continuous stirring and illumination, if it was necessary. A few days later, the required amount of freshly prepared sodium carbonate solution, was added in the reactor to make up the desired supersaturation and the calcification experiment started. The pH of the supersaturated solutions was monitored throughout the experiment, and calcium and heavy

metal concentrations were measured by sampling with time. Moreover, the optical density and total chlorophyll of the growing algae were measured to estimate the status of the algae cultivation. The precipitated solids separated by filtration of the samples withdrawn were examined with respect to their morphological features by optical microscopy and by X-ray diffraction (XRD).

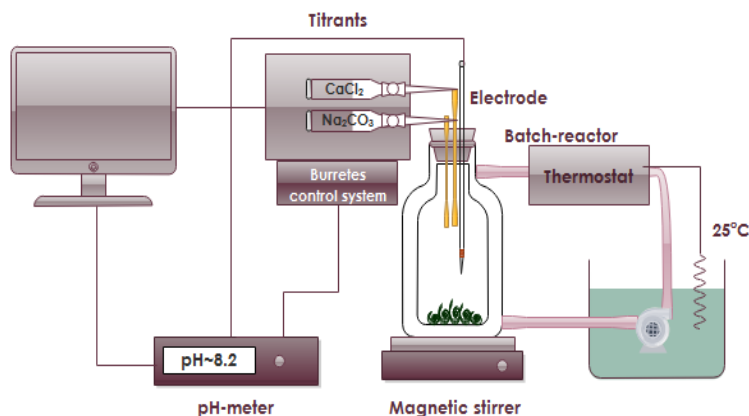


Figure 1: Experimental setup for mineralization experiments on algae substrates in the absence and in the presence of heavy metals.

RESULTS AND DISCUSSION

The interaction of the metals at the solid/electrolyte solution interface, under conditions in which the corresponding metal solutions were stable, was investigated by adsorption measurements and plotting the corresponding adsorption isotherms for cadmium, nickel, copper and zinc.

Adsorption of heavy metals on calcite Zinc (Zn)

Zn (II) sorption were measured on calcite. Saturated solutions were prepared by equilibration of 100 mg of calcite in 0.1M NaCl at initial pH 8.5. Past equilibration of one hour, accurately measured quantities of stock solution 0.7 mM $\text{Zn}(\text{NO}_3)_2$ were added to adjust the concentration of the test metal in the series of suspensions at the desired level. The initial conditions of the suspensions used in the adsorption study are summarized in Table 1. The solutions were stirred magnetically at constant temperature 25°C. Past three days, the solids were separated from the aqueous phase. The filtrates were analyzed for zinc by spectrophotometry at 620 nm⁻¹ 11. The equilibrium concentrations are summarized in Table 2. The calculation of the metal uptake on the mass w of the calcite crystals, was done according to Equation (1):

$$\Gamma = \frac{V \times (C_0 - C_{eq})}{W \times SSA} \quad (1)$$

Where V is the volume of the solution, C_0 : the initial concentration of the sorbent and C_{eq} the corresponding equilibrium concentration. The surface concentration, $\Gamma(\text{mol} \cdot \text{m}^{-2})$ – equilibrium concentration data were fitted to the Langmuir linear isotherm (Equation 2). As may be seen in in Figure 2, the data gave excellent fit.

$$\frac{C_{eq}}{\Gamma} = \frac{1}{K_L \times \Gamma_{max}} + \frac{C_{eq}}{\Gamma_{max}} \quad (2)$$

Where K_L is the Langmuir constant, Γ_{max} : the concentration of the sorbent needed for complete monolayer coverage.

Table 1
Initial Total Zn concentration, C_o , Equilibrium Concentration, C_{eq} and Surface Concentration, Γ , of Zn on Calcite; 25°C, 0.1M NaCl, pH (Initial) 8.50.

C_o / $\times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$	C_{eq} / $\times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$	Γ / $\times 10^{-5} \text{ mol} \cdot \text{m}^{-2}$
4.02	0.12	5.98
2.24	1.42	1.26
5.04	3.51	2.34
4.57	3.35	1.87
5.65	4.12	2.34
6.11	4.73	2.11

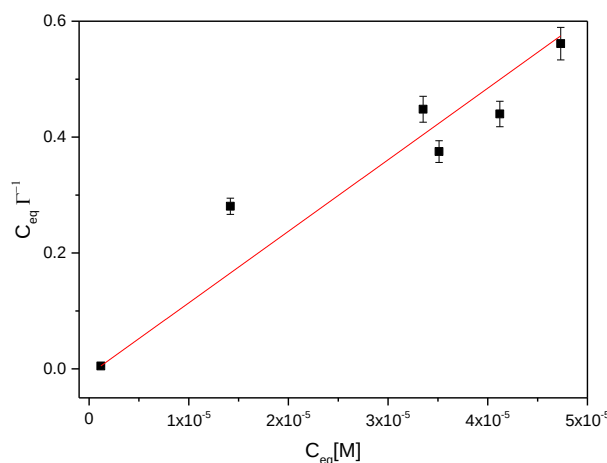


Figure 2: Zn sorption on calcite: Linear form Langmuir Isotherm; 25°C, 0.1M NaCl, pH (initial) 8.50.

Cadmium (Cd)

For these measurements, a stock standard cadmium solution, of concentration $1.8 \times 10^{-4} \text{ M}$ was prepared from crystalline $\text{CdNO}_3 \cdot 4\text{H}_2\text{O}$. The substrates on which cadmium uptake was measured included: **(I)** CaCO_3 crystals (BET specific surface $0.33 \text{ m}^2/\text{g}$), **(II)** algae dried at 25°C for 24h and **(III)** grown algae suspension directly from the culture. Cadmium uptake was measured in suspensions of 100 mg calcite, 100mg dry algae substrate, and 10ml grown algae suspension directly from the culture (10ml), over a series of initial total Cd concentrations, summarized in Table 2.

Saturated calcium carbonate solutions were prepared, volume totaling 50ml, ionic strength either 0.1M or 0.15M NaCl. In these solutions, the study substrates (calcite seeds or dry algae or specific amount from the initial culture) were suspended by stirring and the initial pH value was adjusted to 8.5 by the addition of alkali as needed. The measurements were done at constant temperature 25°C. The solutions were stirred for 5 days with magnetic stirrers. Throughout this period, samples were withdrawn filtered and total cadmium concentration of the filtrates was measured by atomic absorption spectrometry. The results of the measurements of total cadmium concentration as a function of equilibration time are shown in Figure 3.

Table2
Total Initial Cd Concentration in the Calcite Suspensions in 0.1M NaCl Solutions;
25°C, pH 8.50 (Initial).

# Experiment	$C_{Cd}/\times 10^{-5} \text{ M}$	$C_{Cd} \text{ (ppm)}$
1	0.2	0.23
2	0.4	0.45
3	0.6	0.68
4	0.8	0.90
5	1.0	1.12
6	1.2	1.35

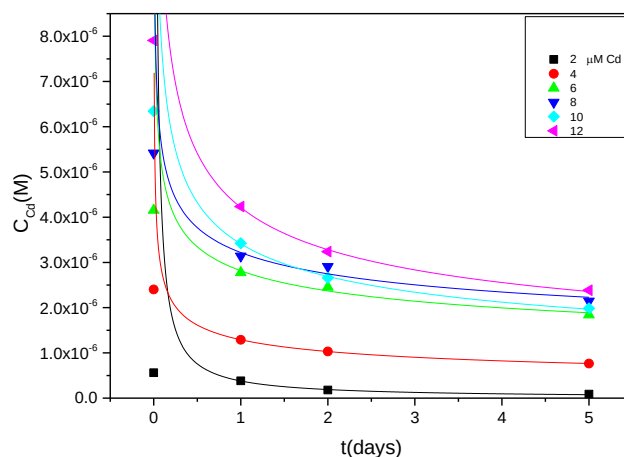


Figure 3: Cadmium concentration as a function of time in calcite suspensions; Ionic strength 0.1M NaCl, 25°C, initial pH 8.5.

As may be in Figure 3, cadmium concentration in the suspensions decreased dramatically as a function of time. The adsorption seemed to be complete past 48 hours, with a slight increase up to 5 days, suggesting that the substrate porosity may also play some role in the uptake process.

Based on Figure 4, the maximum cadmium concentration for full monolayer coverage was calculated, $\Gamma_{\max} = 2.96 \times 10^{-6} \text{ mol} \cdot \text{m}^{-2}$.

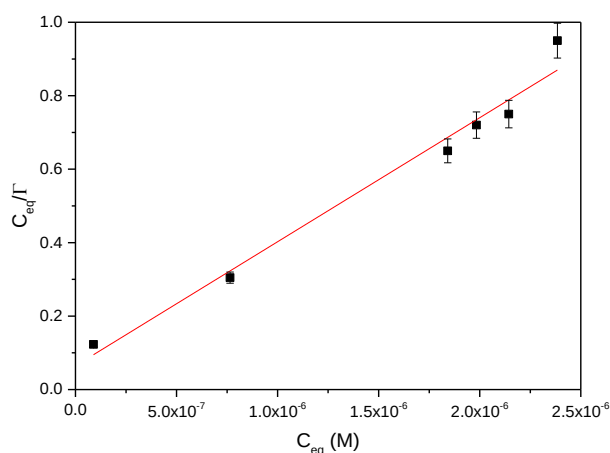


Figure 4: Cd sorption on calcite: Linear form Langmuir isotherm; 25°C, 0.1M NaCl, pH (initial) 8.50.