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KWIC index; immobilization, biomass, polyurethane, alginate, adsorptive filter, Hg.

Introduction

We describe the development of immobilized, processed algal biomass for use as an adsorptive filter in the removal of toxic metals from waste water. To fabricate an adsorptive filter from processed biomass several crucial criteria must be met, including: i) high metal binding capacity, ii) long term stability (both mechanical and chemical), iii) selectivity for metals of concern (with regard to ionic competition), iv) acceptable flow capacity (to handle large volumes in short time frames), v) stripping/regeneration (to recycle the adsorptive filter and concentrate the toxic metals to manageable volumes). This report documents experiments with processed algal biomass (*Spirulina platensis* and *Spirulina maxima*) immobilized in either alginate gel or preformed polyurethane foam. The adsorptive characteristics of these filters were assessed with regard to the criteria listed above.

Methods

Spirulina platensis and *Spirulina maxima* were grown as reported by Aiba and Ogawa (1972), either as liquid cultures or in preformed 0.5 cc polyurethane cubes by the method of Brouers *et al* (1989). The impregnated cubes were removed and washed with distilled water then packed into Bio-Rad disposable chromatography columns to create an adsorptive filter prior to treatment. Filters were 5 ml bed volume, run with gravity feed and a pressure head of 15-20 cm. Flow rates were restricted to 0.5 ml/min. Immobilization of biomass in Ca cross-linked alginate was as described by Brouers *et al* (1989).

Biomass was determined by dry weight analysis or calculated from the chlorophyll content (assuming a chlorophyll content of 1% of the dry weight). Chlorophyll was determined by the method of Mackinney (1941).

Hg was detected by the method of Snell and Snell (1948) using nitrosobenzene and potassium ferrocyanide. The complex gave an absorption at 528 nm, and was sensitive for Hg down to 10μg/l. For lower levels of Hg, a Perkin Elmer 403 flameless atomic absorption spectrophotometer with a sensitivity down to 1μg/l was employed.

Results

Polyurethane foam filters

Untreated (living algae) filters lost cellular material upon washing with various buffers and salt treatment, which resulted in clogging of the column. Moderately packed filters (where the foam was compressed to 20% of its initial volume) did not help this

situation, and flow rates (gravity only) were minimal (< 5 ml/hr.). Although Hg binding to the unprocessed filters was initially encouraging (1mg/l reduced to 2-3ug/l), prolonged exposure resulted in cell degradation (observed by the release of blue phycobiliproteins) and release of Hg. Filters which were heat treated did not exhibit any of the cellular release and clogging phenomena. These filters could be either boiled for 10 min. or (better) autoclaved for 20 min. at 120°C. Denaturation of the cell filaments by the heat treatment probably caused them to become immobilized within the foam pores. Autoclaved (sterile) filters have been stored for up to two months (so far) without deleterious effects on the binding capacity. Subsequent washing removed some soluble material released by the heat processing, but loss of large cell fragments was not observed. Flow rates through the processed material were excellent (1-2 ml per min. for a 5ml bed volume filter) and were not a limiting factor for laboratory-scale Hg removal from aqueous solutions (10 min processing time). The quantity of immobilized biomass was estimated as 7 mg dry wt. cells per 1cc foam cube. Because of the compressibility of the foam and its excellent flow characteristics, biomass concentrations of the order of 70 mg dry wt. per 1cc of filter are presently attainable.

Binding and stripping of Hg

The removal of Hg by processed biomass proved to be highly modulated by the acidity of test waters. Decreasing the pH from 7 to 5 enhanced the capacity of processed algal biomass to sequester Hg by two orders of magnitude (Fig 1). The Hg binding capacity of processed *Spirulina maxima* biomass was determined to be 3μg Hg per mg dry weight of biomass at pH 5. The filter was loaded with 1ml 50μM (10mg/l) $HgCl_2$ and eluted sequentially with 2M NaCl, pH 3.5, and pH 1 buffers. The column removed all detectable Hg from the test solution (residual Hg $\leq$ 1μg/l). The Hg was subsequently stripped from the filter by the 2M NaCl wash with a recovery of 93%. The column was

put through three such cycles with no loss of binding capacity and elution performance. Low ionic strength (NaCl < 500mM) or divalent cations (Ca = 10mM) had no effect on the Hg binding.

Alginate immobilized biomass filters

Processed *Spirulina maxima* was immobilized in alginate gel beads and packed onto a column to give a 5 ml bed volume. The alginate filter was run identically to the polyurethane filters described above. The filter was loaded at pH 5 with 2ml of 1mg/l Hg. 85 % of the added Hg was retained by the filter, although only 5% of the theoretical Hg binding capacity was reached. This would suggest that the flow rate is more critical when using alginate beads, since there is a large void volume around the spheres and the water to be processed must permeate into the gel. Elution with various salt and acid solutions failed to strip the Hg from the filter. Experiments with alginate alone suggested that the Hg was exchanging with the divalent cation cross linkers in the alginate matrix rather than binding to the processed biomass and could not be displaced by moderate salt treatments. Prolonged incubation of the alginate in water with low divalent cation content (< 100μM) caused destabilization of the alginate beads, with release of the immobilized biomass and Hg. Moreover, anions that competed with the alginate for the cross-linking divalent cations (such as phosphate) rapidly destabilized the alginate matrix.

Conclusion

Of the two types of immobilizing matrices examined, the preformed polyurethane foam outperformed the Ca cross-linked alginate gels in most respects, particularly with respect to stability and recycling performance. From the binding capacity data obtained with the polyurethane foam filters (70 mg biomass per 1cc of filter and 3μg Hg bound

per mg biomass), we can calculate the binding capacity of a laboratory-scale adsorptive filter. A 1 liter filter would have the capacity to bind 210,000 μg Hg, which is equivalent to processing 21,000 liters of water contaminated with 10μg/l Hg down to a level below 1 μg/l.

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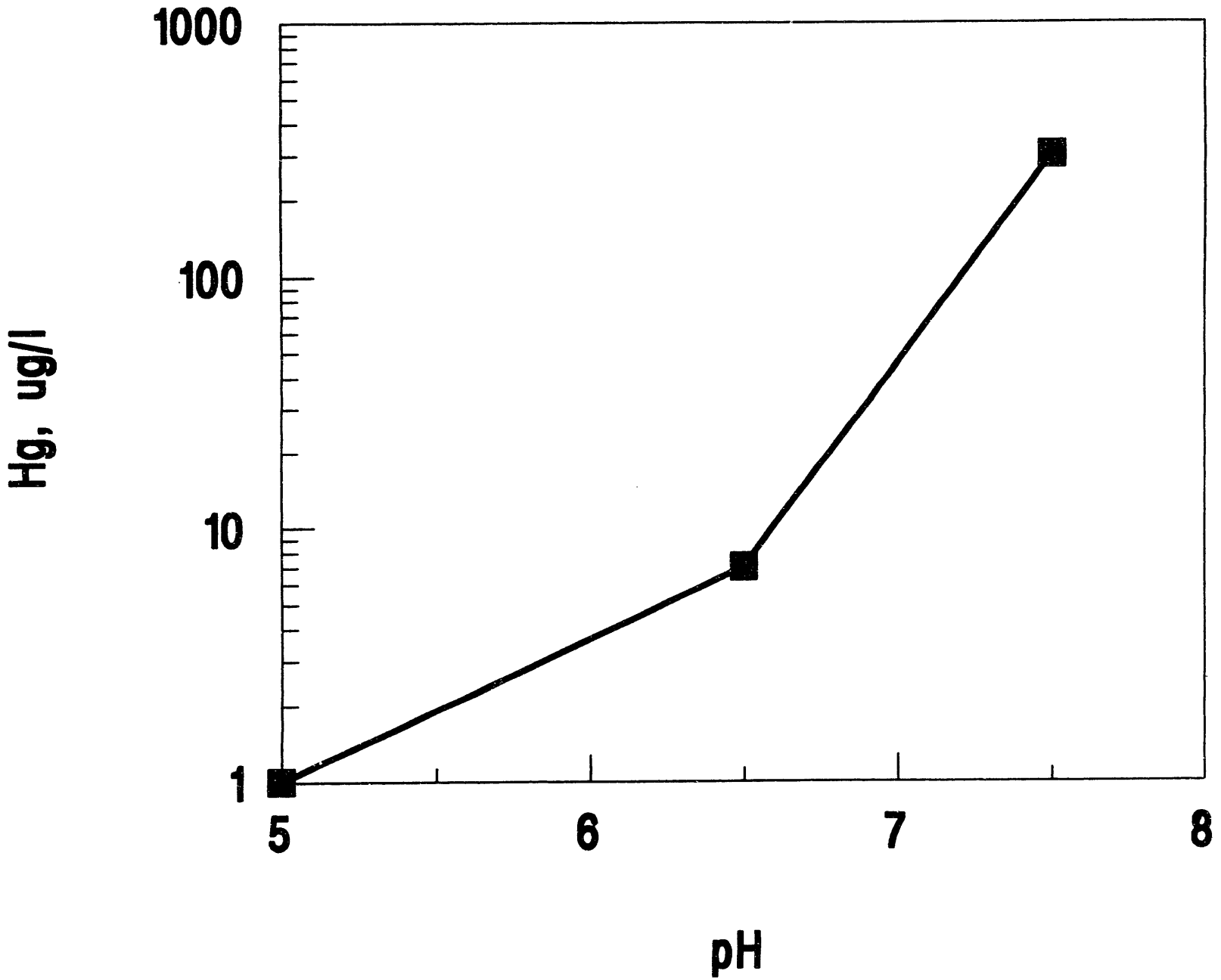
Figure legend

Figure 1. Mercury levels in synthetic test waters of variouse pH after passage through a processed biomass/polyurethane filter. Initial Hg level 1mg/l.


1000
100
10
1
Hg, ug/l
5
6
7
8
pH

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