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REPEATED CADMIUM BIOSORPTION BY REGENERATED *ENTEROBACTER AEROGENES* BIOFILM ATTACHED TO ACTIVATED CARBON

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SUMMARY
The bacterium *Enterobacter aerogenes* has been used to develop a biofilm over activated carbon for biosorption from various strength cadmium solutions (25-500ppm). High bacterial resistance to metal poisoning allowed biofilm regeneration to raise the net loading of cadmium over the carbon by repeated biosorption runs.

INTRODUCTION
Removal of metal ions from aqueous solutions by biosorption with micro-organism generated biomass is a well proven phenomenon. Recovery of cadmium is of particular interest due to its extremely toxic nature. Species such as *Chlorella* (Ting *et al.*,1989), (Nakajima *et al.*,1979), *Citrobacter* (Macaskie and Dean,1982), *Arthrobacter*, *Pseudomonas* and *Enterobacter* (Scott and Palmer,1990) have all been demonstrated as efficient biosorbers of a range of metals including cadmium. However, the biomass has been typically dead, or if initially viable, not reused after exposure. The potential of micro-organisms as a self-regenerating adsorbent has not been therefore fully exploited.

A useful class of biosorbents that offer strong possibilities of regeneration are those that naturally excrete large quantities of polysaccharides. The polysaccharides, primarily in the form of a closely associated external biofilm which binds the cells, are significantly better metal accumulators than plain cellular biomass (Scott and Palmer,1988). The capsular coating also provides defense against toxic effects, such that after an exposure run, cells incorporated in the biofilm will successfully regenerate in fresh growth medium.

One such excreting species is the bacterium *Enterobacter aerogenes*. We have investigated use of its biofilm to cover activated carbon with the intention of exploiting the high biosorption capability to distribute metal over the carbon substrate. This provides both initial decontamination and also the possibility of re-use for further adsorption. It has been shown, for example at 25ppm cadmium concentrations, that the presence of these biofilms increases metal uptake per gram of carbon by a factor of at least six (Scott *et al.*,1992). Furthermore, the ability of the species to remain viable after exposure to cadmium has allowed regeneration for repeated biosorption cycles to further enhance metal loading.

MATERIAL AND METHODS

Development of a biofilm over the activated carbon

The bacteria used was the polysaccharide producer, *Enterobacter aerogenes* (University of Bath culture). Seeded 2dm^3 aerated, 25°C fermenters were used containing 1.0gl^{-1} "Lab-lemco" powder (OXOID), 2.0gl^{-1} yeast extract, 5.0gl^{-1} peptone and 5.0gl^{-1} sodium chloride. The medium pH was 7.4.

The activated carbon was charcoal with a particle diameter of 0.85-1.70mm. To develop an attached biofilm, the nutrient broth was circulated for 2 days through 15mm diameter glass columns containing 10g of fluidized particles. For subsequent biofilm regeneration after exposure to metal solutions, fresh sterile nutrient broth was circulated, again for 2 days.

Metal ion solutions and metal uptake determination

Solutions (0.5dm^3) of cadmium were prepared at metal ion concentrations of 25-500ppm. Columns containing carbon with an attached biofilm were disconnected from the broth circulator and reconnected to a circulator which re-fluidized them with the metal containing solution (25°C). A dropping mercury electrode polarograph using a 10gl^{-1} solution of sodium acetate electrolyte measured cadmium ion concentrations in solution (both initially and remaining after exposure to the biofilm/carbon). The presence of metal ions desorbing from the biofilm/carbon back into the fresh nutrient broth was also determined. This was achieved by treating the broth with hydrochloric acid to put the ions into solution, centrifuging for 30mins. at 4000rpm to remove cellular material and then raising the supernatant to pH7.4 for analysis by polarography. All cadmium uptake readings are an average of three runs with a difference better than ±3%.

RESULTS AND DISCUSSION

The physical nature of the polysaccharide excretions produced by *E.aerogenes* is illustrated by the scanning electron micrograph (SEM) in Figure 1. The net-like appearance is typical of this type of coating and can provide an extensive and cohesive covering over the activated carbon surface.

Figure 2 shows metal ion uptake over an exposure period of 60mins, where a rapid first stage indicates physical adsorption, followed by slower uptake indicative of at least in part active metabolically sponsored accumulation. The initial uptake is due to metal ion sorption onto the external surfaces of the biofilm. For active uptake cadmium ions are transported through the cell membrane and into the cytoplasm where precipitation, often associated with sulphides, can occur (Scott and Palmer, 1990).

The time taken to reach metal uptake equilibrium is related to the initial metal ion concentration. Not surprisingly, as the concentration increases, the time decreases as sites available for direct cadmium adsorption are rapidly taken up. At 25ppm cadmium, equilibrium took around 120mins, compared to 30mins at concentrations of 100ppm and greater.

Figure 1 SEM of excreted bacterial polysaccharide biofilm over activated carbon particles (1000x)

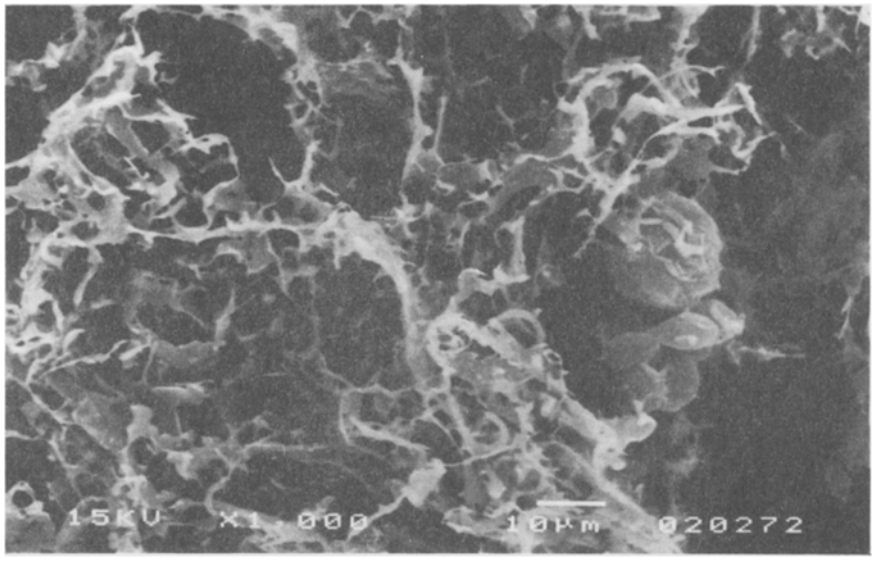


Figure 2 Cadmium uptake from different solutions

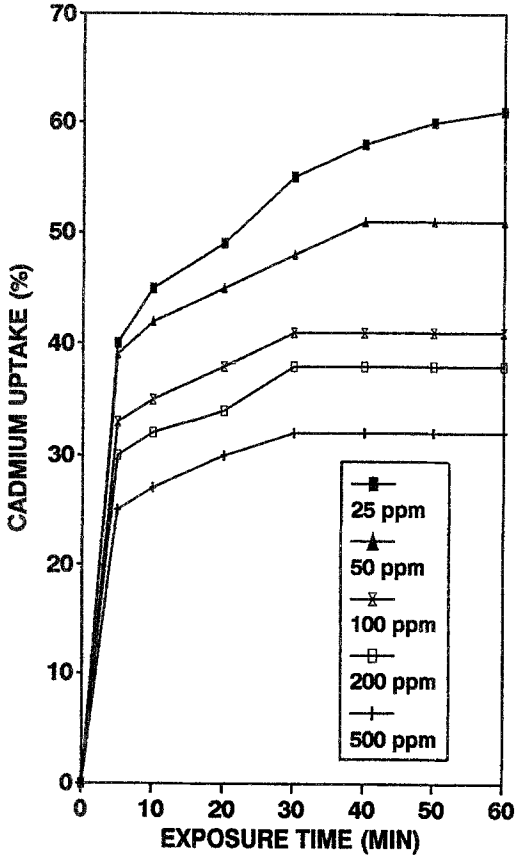
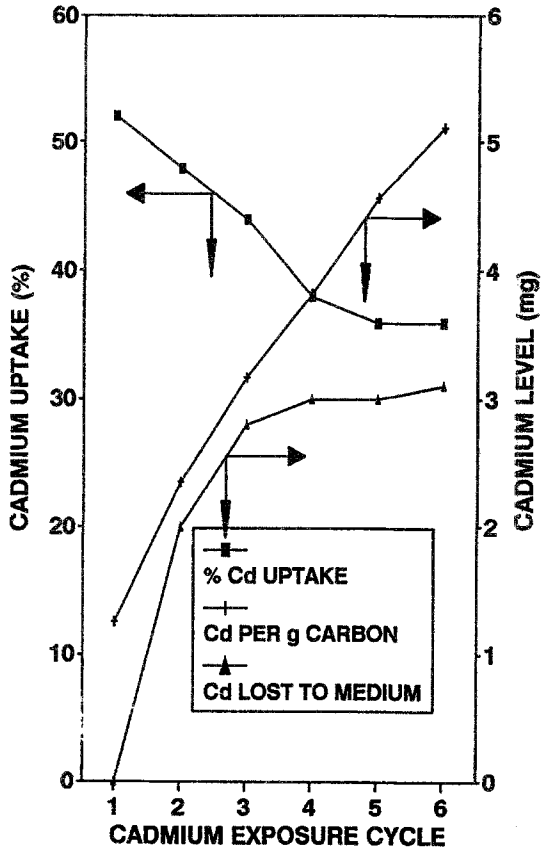


Figure 3 Cadmium uptake by regenerated biofilms



E.aerogenes has shown an ability to remain viable and be capable of self regeneration after exposure to cadmium concentrations up to at least 500ppm (unpublished data). This resistance has been utilised for progressive accumulation of cadmium from 50ppm solutions by using regenerated biofilm/carbon columns. Columns after 60mins exposure to cadmium were drained, flushed gently with Ringers solution and reconnected to a sterile (metal free) supply of fresh medium for 2 days. After this they were reintroduced to a metal solution. This cycle was repeated six times over 12 days.

Whilst the quantity of metal taken up during subsequent exposure runs is reduced, it does nevertheless tend to level off to a new equilibrium. Therefore, although there is after six cycles a 30% decline in the level of cadmium uptake, a significant increase in the net loading of cadmium over the activated carbon is achieved by repeated exposure (i.e. from 1.2 to 5.2mg Cd/g carbon).

Loss of cadmium back into the growth medium (by desorption and/or attrition of metal laden biomass) during biofilm regeneration was also measured. It can be seen from Figure 3 that it was relatively low, increasing to around 3mg (in the $2dm^3$ of growth medium) after three cycles. With the last cycle, this represents a loss from the columns of approximately 33% of the cadmium taken up after 60mins exposure to 50ppm cadmium (i.e. initially there was 36% uptake (9mg) from the 25mg of cadmium present in the circulated metal solution).

CONCLUSIONS

The regenerative capacity of the polysaccharide producers greatly increases their efficient use as a biosorbent. In addition, combining the use of particulate activated carbon as a support substrate with recycling, introduces the possibility of developing this route as a means of producing an enhanced adsorption surface. For example, we have looked at the use of heat-treatment to fix the metal laden biofilm onto the carbon (Scott and O'Reilly, 1991), and are currently utilising these particles for organic pesticide removal. To advance this work we are addressing several factors, such as dealing with the loss of metal back into the growth medium and the best means of permanent fixing of the metal/biofilm to the carbon.

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