Biosorption of Copper by a Bacterial Biofilm on a Flexible Polyvinyl Chloride Conduit

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Inexpensive technologies with less-than-optimal efficiencies as a strategy for countering economic restraints to pollution control have been evaluated by using a laboratory-scale biotreatment process for copper-containing effluent. Economizing measures include the use of polyvinyl chloride (PVC) cylinders fashioned from commercially available flexible PVC conduit to support a biofilm that was cultured in an inexpensive medium prepared in wastewater. The biofilm was challenged by aqueous copper solution in a bioreactor and subsequently analyzed under a scanning electron microscope with energy-dispersive X-ray microanalysis.

Removal of metals from industrial wastewater has conventionally been accomplished mainly by precipitation, ion exchange, and electrolytic technologies (2). More recently, biosorption of metals by immobilized cell systems has been used effectively for removal of metals from industrial effluent (7). This technology exploits the natural tendency of cells to accumulate elements or their innate ability to degrade recalcitrant organic compounds. Cells with such abilities are immobilized either as entrapped biomass or as a biofilm to form a system for treating wastewater known as a bioreactor.

Entrapment techniques make use of various porous gels, resins, or polymers to physically entrap (or rather embed) cells, whereas biofilms are produced by coating a support surface with a thin film of cells. Traditionally a very diverse range of materials—such as rocks, sands, plastics, latex, paper, and steel, etc.—have been used as biofilm supports. Polystyrene sheets, needle-punched polyester, and polyvinyl chloride (PVC) foils in various geometries have lately been in use with growing popularity (3, 6, 9). Although commercially available effluent treatment systems work with high efficiency, their high cost often prohibits financially poor countries from treating their effluent before discharging it into the environment.

In this regard, two high-priority areas were identified that could improve the prospects of implementation of pollution control measures, namely, the use of indigenous resources and the use of inexpensive materials to reduce the running cost. This study demonstrates (i) the use of PVC supports that were fashioned from locally available flexible conduit material used commonly in civil electrical wiring, (ii) the ability of sodium acetate to sustain bacterial growth for biofilm production, (iii) the use of wastewater modeled after mining effluent in culture medium as a replacement for distilled water, and (iv) the evaluation of thus-produced biofilm to filter Cu from defined (model) wastewater. Copper is found in effluents from various industries, including tanning, mining, metal processing and finishing, electroplating, the automobile industry, and the pharmaceutical industry.

Filter matrix production: biofilm. There are a number of criteria that characterize a good biofilm support: large surface-to-volume ratio, surface characteristics suitable for bacterial attachment, and good porosity to permit unhindered flow of wastewater. Glass materials have not been found to be good supports, as bacteria have difficulty in attaching themselves to the smooth inert surface (16). Although PVC is much better than glass, it is superseded by fired clay and punched polyester (17). PVC is more commonly used in treatment of wastewater by biological means, and a variety of PVC supports have been developed specifically for this purpose and are available commercially. Some of the purpose-built PVC supports have been designed with grated surfaces to increase surface roughness, which facilitates cell attachment (11). Considering the economic restraints in Third World countries, the biofilm support studied here was fashioned from flexible PVC conduit, used commonly for civil electrical wiring, etc. Hollow cylinders (approximately 13 mm by 12.7 mm [diameter]) were cut from conduit and used as a support for immobilizing the bacterial biofilm (Fig. 1A). The cylinders satisfied all of the criteria cited above, in having a large specific surface area ($13,000 \text{ m}^2 \text{ m}^{-3}$), good porosity (96%), and suitable surface topology. The gross surface of PVC conduit was provided with grooves for flexibility (Fig. 1A), a characteristic that was expected to favor bacterial attachment. Electron microscopy (see below for methodology) of the surface topology revealed a rippled surface texture (Fig. 1B). The ripples resulted in pits of approximately 1 to 1.5 μm by 0.5 to 0.75 μm , which approximately matches the dimensions of the average bacillus cell. Gjeltema et al. (8) have clearly demonstrated that biofilm formation depends mainly on hydrodynamic conditions and particle collisions in airlift reactors and also that increased surface roughness promotes biofilm accumulation on suspended supports.

A biofilm of *Pseudomonas aeruginosa* strain CMG156 was produced aerobically in 1 liter of stirred-tank chemostat during continuous culture at a dilution rate (D) of 0.05 h^{-1} (μ_{max} , 0.32) under carbon-limiting conditions (CH_3COONa) for 430 h at room temperature ($\approx 30^\circ\text{C}$). The culture medium was prepared in two parts, autoclaved separately (at 121°C for 15 min) and merged together when at room temperature. Part 1 consisted of 1.0 g of NH_4Cl , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of

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FIG. 1. Biofilm support. (A) Hollow cylinders (13 mm by 12.7 mm [diameter]) cut from flexible PVC conduit. A cross-section through the wall of the cylinder can be seen as a spirillum-like object near the bottom of the panel. Length was measured by stretching a longitudinal section of cylinder wall under a press to eliminate the corrugation while the diameter was averaged from a large population of measurements from one cutting edge to another. Bar, 1 cm. (B) The support surface appears rippled under very-high magnification. Bar, 1 μm .

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5.0 g of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, and 0.5 g of yeast extract in 990 ml of synthetic wastewater (with the pH adjusted to 7.0 with 2 N NaOH). Part 2 consisted of 0.5 g of K_2HPO_4 in 10 ml of distilled water and with the pH adjusted to 7.0. The synthetic wastewater used for medium preparation was modeled after wastewater of metal mining operations and included (in parts per million) As (0.045), Br^- (<0.01), Cd (0.01), Cl^- (1,650), Co (1.2), Cr (<0.01), Fe (<0.01), Na (2,050), Ni (0.88), SO_4^{2-} (1,880), and Zn (0.1). Industrial applications requiring biomass production require water as a major component in culture media along with ancillary services such as heating, cooling, cleaning, and rinsing (15). The resultant biofilm demonstrates the scientific feasibility of such economizing measures as use of wastewater and of acetate as an economical carbon source in culture medium.

With an average thickness of 190 μm , the biofilm varied markedly in depth and was found more frequently in grooves on both outer and inner surfaces of PVC cylinders (Fig. 2A). Lack of biofilm on ridges may be attributed to the liquid shearing in chemostat. High shear rates have been shown to result in low biofilm growth rates since the detachment rate can be equivalent to or higher than the biofilm growth rate (1, 4, 13, 14). The maximum thickness measured was 730 μm (from a number of scanning electron micrographs; data not shown here) whereas biofilms produced artificially (12) or in natural environments (5) can range from 100 μm to 3 mm; therefore, significant improvements might be possible from optimization of the biofilm production process.

High-magnification scanning electron microscopy (SEM) (Fig. 2B) showed an intercellular connective network that appears like an extracellular matrix (see below for methodology). Wherever biofilm was found as a thick crust, it appeared to be totally covered by extracellular polysaccharide. The role of extracellular matrix in cell aggregation is well known (18). Microchannels seen in Fig. 2B not only serve as a means of communication to provide nutrients to the inner depths of biofilm but also permit the deeper regions of the biofilm to accumulate metals that it encounters in the liquid effluent. For

this reason the effective surface area of a biofilm becomes much greater than that of the bare support.

Bioreactor and copper biofiltration. The biofilm was cultured in two temporally spaced replications, pooled together, and packed into Pyrex glass columns (40 cm by 5 cm [diameter]) capped by rubber stoppers fitted with glass tubes to provide inlet and outlet ports for the bioreactors. Unbuffered aqueous solution of CuSO_4 (6.39 mg liter $^{-1}$) was fed into the reactors by peristaltic pumps at 33 ml h $^{-1}$. Outflow from the bioreactors was measured periodically for Cu concentration. A cell-free control column was similarly analyzed in parallel. Each column had about a bed volume of 528 ml and a fluid capacity of about 415 ml, with 150 cylinders present in the bed. Copper was quantitatively measured by a spectrophotometric method based on that of L. E. Macaskie (10). The assay, scaled to be carried out directly in 1.5-ml disposable cuvettes, was modified to increase the sensitivity of the reaction (1 ml) from a copper concentration from 7.98 mg liter $^{-1}$ down to 0.25 mg liter $^{-1}$ by replacing the water in Macaskie's method with a very dilute aqueous sample. To 100 μl of borate buffer [26.9 g of $\text{B}(\text{OH})_3$, 2.6 g of NaOH in 900 ml of distilled water adjusted to pH 8.1 with 2 M NaOH and made up to 1 liter] 20 μl of reagent (0.5 g of *bis*-cyclohexanone-oxalyldihydrazone in 100 ml of 1:1 [vol/vol] water-ethanol, dissolved by heating and cooled and filtered before use) was added, followed by addition of 880 μl of aqueous sample, and the solution was mixed thoroughly. The colored complex thus formed was measured at 595 nm against a standard copper calibration curve prepared in a similar manner. The modified assay was found to generate a linear curve in the range tested, i.e., 0.25 to 15 mg liter $^{-1}$. The biofilm column was found to possess 85% removal efficiency (Fig. 3); on the whole the biofilm outperformed the control with twofold efficiency.

SEM and X-ray microanalysis. Biofilm-laden and control (i.e., without biofilm) PVC cylinders were cut into sections (2 by 3 mm), air dried, fixed in glutaraldehyde for 1 h, dehydrated through alcohol, critical point dried, mounted on stubs, sputter coated with gold, and observed under an SEM. The Cu-exposed biofilm and unexposed control biofilm were sub-

FIG. 2. Scanning electron micrographs of biofilm on PVC cylinders. (A) Cross section through the wall of PVC support through the groove region, seen here as a U-shaped object (against background of the specimen mounting stub) colonized by biofilm, which appears as crusts on the inner surface of the U shape. The U-shaped object is a magnified region of the cross-sectional view shown in Fig. 1A. Bar, 1,000 μm . (B) This close-up view of biofilm near the apex of a ridge on the surface of PVC support shows the extracellular matrix, microchannels, and the slime layer. The surface of the PVC can also be seen (in the lower right half of the panel). Bar, 10 μm .

jected to total elemental analysis by using energy-dispersive X-ray microanalysis, which confirmed accumulation of Cu in the biofilm and revealed hitherto-unknown coaccumulation of Fe and Zn (Fig. 4). Since Fe and Zn were not present in bioreactor influent and since they were part of wastewater that was used to prepare the culture medium, they were therefore taken up from the culture medium. This demonstrates the multipurpose potential of the biofilm.

Uptake of metals from wastewater medium is disadvantageous since it may prematurely saturate the biofilm during production. On the other hand biofilms produced in the presence of metals may be more resistant to higher metal concentrations. The latter factor may be of importance in situations in which the organisms must be maintained in a viable state to perform their intended function. However, since the metal accumulation mechanisms exploited in this study do not depend on the viability of cells, the use of wastewater is therefore judged to be of no advantage other than that of reducing the requirement of distilled water for biofilm production. However, the presaturation problem may be overcome by desorbing metals from a fresh biofilm by means of dilute acids before using the biofilm in filters.

Conclusion. Inexpensive materials and cost-effective techniques can be used to produce metal-removing biological fil-

ters that function with reasonable efficiency. Although such methods may not be the most optimized, they still might provide enough efficiency to be of practical use to poor countries where pollution generators are not able to afford the cost of conventional or high-performance treatment facilities.

FIG. 3. Effluent Cu after treatment with *P. aeruginosa* CMG156 biofilm immobilized on PVC cylinders. The bioreactor columns were challenged with aqueous Cu solution ($6.39 \text{ mg liter}^{-1}$) at a flow rate of 33 ml h^{-1} . Symbols: —, influent Cu; ×, effluent; □, biofilm.

FIG. 4 Coaccumulation of Cu, Fe, and Zn was determined by energy-dispersive X-ray microanalysis of the biofilm exposed to Cu (top) compared to no accumulation in unexposed biofilm (bottom). Peaks of Au and Pd are a result of gold coating of the specimen.

Widespread small-scale on-site applications of biological filters might provide a practical solution to environmental problems in poor countries. Pollution, localized or widespread, eventually has global consequences and is already present in unmanageable proportions; therefore, control of pollution closest to its source is the strongest countermeasure against its spread.

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