DOE/SR/18553--1

DOE Report No.: DOE/SR/18553-1

Research Report to Department of Energy

Savannah River Site

Grant Award No. DE-FG09-95SR18553

to Clark Atlanta University, Atlanta, GA 30314

Project Title: Bioremediation of Mixed Microbial Mats: System Development of Mixed Contaminants for Application at the Savannah River Site

Time Period: October 1, 1995 - September 30, 1996

Prepared by Judith Bender and Peter Phillips

September 24, 1996

DISCLAIMER

Research Report to Department of Energy Savannah River Site

Grant Award No. DE-FG09-95SR18553 to Clark Atlanta University, Atlanta, GA 30314

Project Title: Bioremediation of Mixed Microbial Mats: System Development of Mixed Contaminants for Application at the Savannah River Site

Prepared by Judith Bender and Peter Phillips

September 24, 1996

Contents:

I. Executive Summary:

Project Goals: The fundamental objective of this project is to develop and field test the mixed microbial mat bioremediation system for decontamination of target sites at SRS. Although microbial mats have performed well in several pilot projects in the past, atypical problems and site characteristics at SRS demand special field designs. In the interest of designing a pilot and locating it at an appropriate site, the project investigators have worked closely with the technical staff at the SREL. We have concluded that the diverse characteristics of contaminations at SRS may dictate testing several pilot designs during the course of this project.

Background on Microbial Mat Research and Development. Our bioremediation research group has developed a method for constructing microbial mats (Bender and Phillips, 1994) for potential low-cost bioremediation of mixed wastes (metals, radionuclides and organic compounds). These mats remove metals and metalloids from water, degrades organic pollutants such as oil, pesticides and polychlorinated biphenyls in water and soil, and can treat mixed waste by simultaneously degrading organic pollutants and removing metals from water (Figure 1).

The microbial mat can operate on-site, eliminating the need for costly transportation services.

The microbial mat is a community of natural heterotrophs and autotrophs dominated by blue-green algae (cyanobacteria). The diverse microbes organize into discrete microzones of oxic and anoxic character. These zones exist in close proximity and support aerobic/anaerobic communities simultaneously, thereby offering a unique array of biochemical mechanisms for the degradation of organic compounds and sequester of metals. Although photosynthetic, the microbial mat is known to utilize exogenous organic substrates under both light and dark conditions as a portion of the total carbon requirement for growth. The microbial mat exhibits high rates of biomass production in nutrient-depleted water and are highly competitive with other microbial communities under nutrient-rich conditions. Compared to other bioremediation systems, the microbial mat is especially unusual in that it can accumulate and tolerate high levels of toxic metals and metalloids.

Bioremediation Treatment Systems for Mixed Waste. Biological systems do not generally handle mixed waste materials. Generally, the heavy metal (or in some cases the radionuclide) is toxic to the enzymatic systems that function in biodegradation of the organic material. Bioremediation systems, designed for *in situ* treatment of these recalcitrant compounds should ideally present several characteristics which are found in microbial mats: (1) ability to deal with mixed wastes (2) contain a consortium of microbes which spontaneously generate heterogenous microzones (oxic/anoxic) in close proximity, thereby providing a variety of chemical environments (3) degrading microbes that can penetrate soils and sediments, and (4) mechanisms which transport contaminated materials from the sediment into the region of high microbial density.

Technological Development of Microbial Mat System The DOE is interested in fostering the development and evaluation of new risk reduction technologies in the areas of pollution prevention, end-of-pipe controls and remediation. This microbial mat system described in this proposal is in a technology transfer stage as a remediation technology (Phillips and Bender, 1995). In field pilot projects for the US Bureau of Mines (Colorado) and the Tennessee Valley Authority (Alabama), it has been used to remove manganese, zinc and other heavy metals (silver, chromium, cadmium, copper, lead, nickel and iron) from mine drainage. In another pilot project for Waste Management, Inc., microbial mats are being used to convert ammonia and remove metals from sanitary landfill leachate (Kentucky).

Expected results or benefits. Real world contamination is seldom comprised of one single organic compound or metal. Generally, contaminated water and soils/sediments contain mixtures of these. Numerous microbial-based biotreatment systems will adsorb, precipitate or otherwise transform metals. Likewise, the degradation, or even complete mineralization (complete breakdown to simple products such as carbon dioxide), of organic compounds is common among microorganisms, such as fungi, bacteria and blue-green algae. To achieve the two processes simultaneously, that of metal removal as well as organic degradation in one biological system, is unusual.

Bioremediation systems are generally composed of single microbial species selected or genetically manipulated to treat one type of contaminant. Although effective for specific applications, these single species systems often lack flexibility and can seldom be applied for simultaneous treatment of mixed contaminants. In addition, because they are generally heterotrophic (bacteria which consume nutrients provided by their surrounding environment rather than make their own through photosynthesis and nitrogen-fixation), they have inherent problems in terms of field maintenance and low-cost potential. The ideal bioremediation system must have field durability, be self-maintained under field conditions, have few growth requirements (low-cost maintenance) and demonstrate the ability to deal with several categories of contaminants simultaneously.

Because microbial mats have evolved under hostile conditions, similar to those expected in highly contaminated environments, survival adaptations of these ecosystems are directly applicable to remediation biotechnology.

First Year Objectives. The objectives of the first project year were to design and test several prototypes of possible SRS field pilots in our Clark Atlanta University laboratory.

Metal and Radionuclide Treatment

Canister experiment. Preliminary experiments with strontium, using mats immobilized on glass wool, contained in a canister showed good metal removal with deposit on mats and within the glass wool.

Reservoir/reactor. In these experiments the ranges of flow rates and hydraulic retention

times were determined, thereby suggesting specific characteristics for a field pilot. Results of these experiments show that high metal concentrations can be significantly reduced in a short period of time by recycling solutions over mats immobilized on various substrates. The following quantities of metals were removed from the water and deposited within the mat or at the bottom of the reactor (g/m^2 of mat filter): Cr^{+6} = 6, Pb = 5 and Cd = 4. These unusually high levels of metal removal were achieved in 0.5 h with hydraulic retention time of 20 min. Background research suggests that bottom deposits were mediated by bioflocculants synthesized and released into the metal-contaminated water by the cyanobacteria (blue-green algae).

In order to make maximum use of the mechanisms provided by microbial mats (sorption within the mat/mesh and flocculation at the bottom of a water reservoir), a second pilot design was tested. In this design water was flushed once through the microbial mats, then delivered to a reservoir for a 24 h retention time. Relatively high water volumes and low concentrations (ppb) of Mn, Co and Cs were used. After six batches of 20 gal (one batch/2-day period), the cumulative metal input (mg) was Mn: 101, Co: 63, and Cs: 449. Metal levels in the reservoir water column were Mn: 3%, Co: 2% and Cs: 20%. Metal retention was highest within the mat/mesh matrix for Mn and Co, whereas, for Cs most removal was accomplished by flocculation in the reservoir. In summary, this process was effective in removing 80 - 98% of the metals.

Plate and shelf reactor: This conventional reactor design was constructed with transparent PVC and layered with microbial mats immobilized on glass wool, coconut mesh and polyfiber. It is currently being tested with several SRS target metals.

Bag treatment: In an effort to design the simplest, low-cost pilot possible, a bag configuration was devised. In these experiments (still in progress) window screen fiberglass bags were constructed, filled with microbial mats immobilized on various substrates and floated in an outside pond. It was found that light was sufficient to maintain healthy mats and the bags could be easily designed for buoyancy at various levels in the water column or anchored at the bottom for sediment treatment.

Treatment of Organic/Inorganic Mixtures

In these experiments a model organic compound (ethylene glycol) and several metal contaminants (Cd, Co and Pb) were mixed and treated with microbial mats plus bacterial mixtures in a barrel reactor. Metals were effectively removed from the organic slurry at the same rates as they were from water (data above). The organic (ethylene glycol) was degraded during the process of metal removal.

Year two research plan: Results of the year one research showed promise for several field pilot designs. These will be placed in the field during the second year of the project and tested as pilots. The proposed sequence of events are as follows:

1. Submit pilot design (configuration, including flow rates and hydraulic retention times)

for DOE review.

2. With the assistance of SREL and DOE personnel, locate several appropriate sites.

3. Install pilots and take seasonal data.

4. Depending on data, i.e. flow rates and duration of metal removal, determine the size and configuration for full-scale treatment at one of the test sites. Submit plans for review.

Figure 1. Microbial mat cultured on coconut mesh for ease of field application.

II. Experimental Results.

1. High concentration strontium removal from water.

Test unit configurations: All units were acrylic plastic tanks measuring 30 x 9 cm and with 5 baffles (a piece of acrylic inserted at an angle to create a serpentine water flow through the tank). Glass wool, to a depth of 4 cm, was layered on the tank bottom and microbial mats were cultured on the glass wool. Each unit was covered with plastic film to retain moisture. See Figure 2 for a schematic diagram of this tank.

Static batch experiment: Initial water volumes, containing strontium, were 300 mL. Initial Sr concentrations were 12.6 mg/L. Three experimental and three control units were used. See attached Table 1 and Figure 3 for results. At 4 h, the experimental triplicate mean Sr level was 1.972 mg/L, representing an 84.3% decrease. At 96 h, the mean Sr level was 1.45 mg/L, representing an 88.5% decrease. Control mean levels showed a 18.7% decrease (to 10.243 mg/L) and a 26.4% decrease (to 9.273 mg/L), respectively.

Flow-through experiment: Initial water volumes, containing strontium, were 300 mL. Initial Sr concentrations were 11 mg/L. Three experimental and three control units were used. The entire 300 mL was added to a tank unit, drained, collected and run through the tank again. Each flow-through episode lasted 15 min. This was repeated 12 times/tank. See attached Table 2 and Figure 4 for results. There was an approximate linear decrease in Sr concentration per flow. After 12 "runs" the triplicate mean Sr level in the experimental units was 0.277 mg/L, representing a 97.5% decrease, whereas in the control units the Sr level was 9.467 mg/L, representing a 13.9% decrease.

Results of strontium removal in a prototype bioreactor: The capacity of the canister-type bioreactor was 28 gallons (Figure 5). For Experiment 1, on day 0, 28 gallons of Sr solution (41.4 mg/L) were added to the reactor. Samples were taken every 15 minutes for the first 90 minutes and then every 24 hours from the starting point until 72 hours. The bioreactor was allowed to rest and recover from day 4 to day 26 when we did a second experiment. The initial Sr concentration for Experiment 2 was 22.5 mg/L. Samples were taken as described for Experiment 1 for the first 90 minutes and on days 1, 2 and 5. The samples were hydrolyzed and analyzed using the atomic absorption. For Experiment 1, the average removal in the first 90 minutes was 18% and removal peaked at 24% on day 3. With Experiment 2, the average removal in the first 90 minutes was 26%, peaking at 68% by day 5. A black precipitate was observed to be present on the mat after Experiment 2 (Table 3 and Figure 6). We are presently analyzing this precipitate and suspect that it might be Sr.

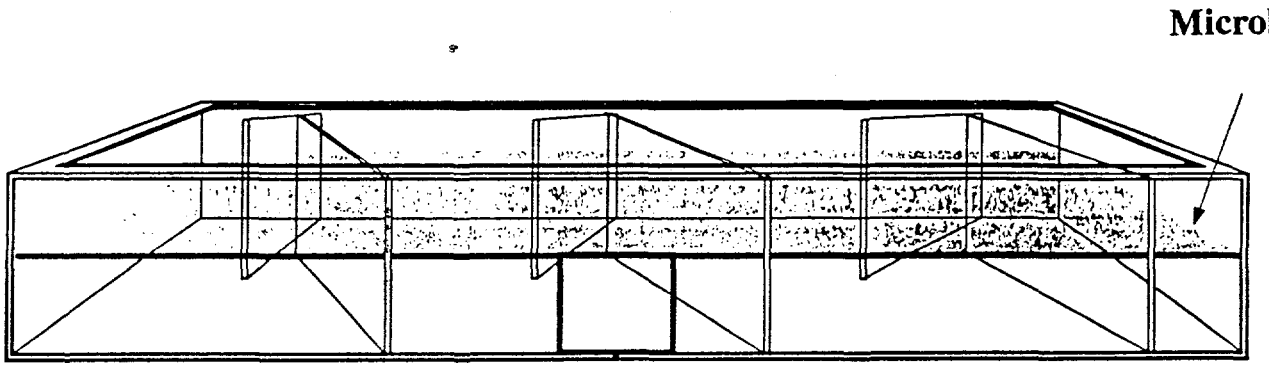

Figure 2. Schematic diagram of a baffled tank for strontium removal from water.

Figure 3. Strontium removal from water in a static batch experiment in a baffled tank.

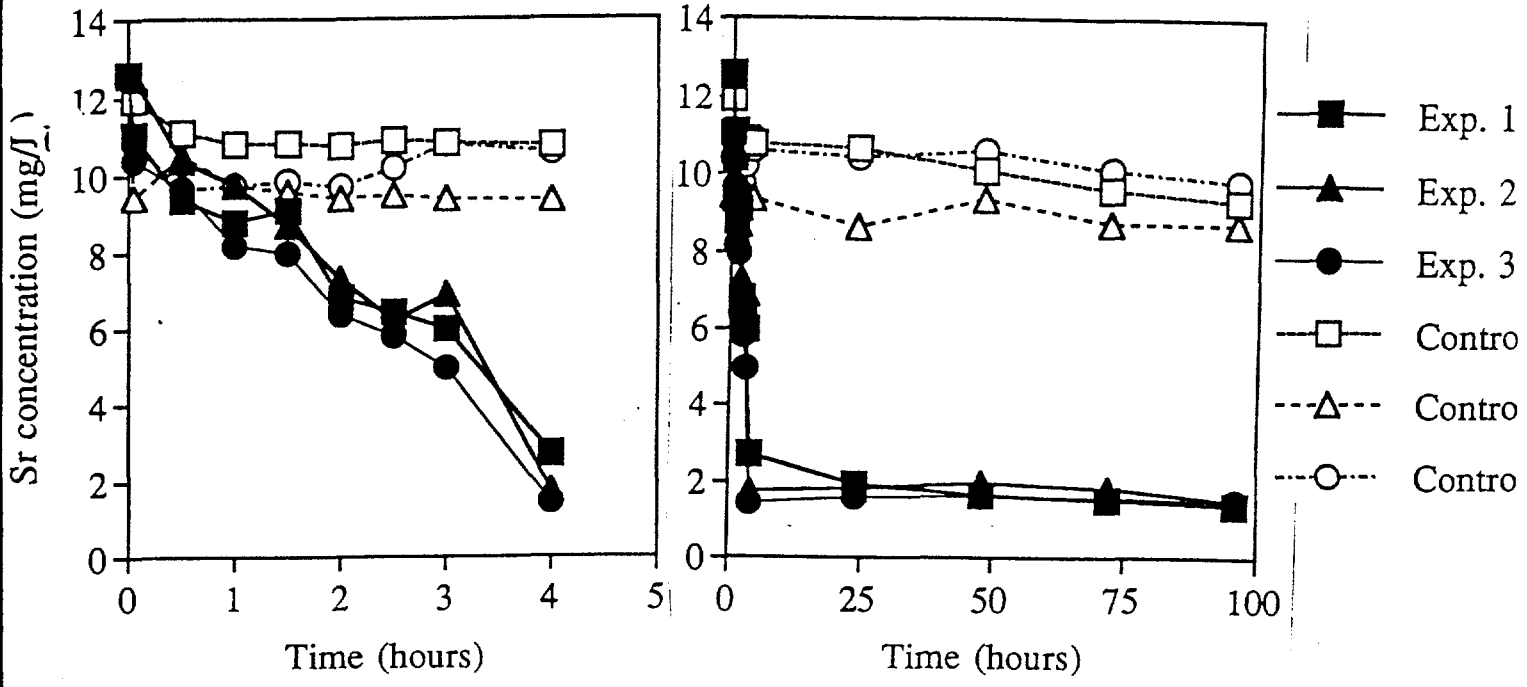

Table 1. Raw data on strontium removal from water in a static batch experiment in a baffled tank.

	1	2	3	4	5	6	7
	Time (hr)	Baffle 1	2	3	4	5	6
1	0	12.6	12.6	12.6	12.6	12.6	12.6
2	0.05	11.053	12.52	10.395	11.945	9.439	10.855
3	0.5	9.36	10.38	9.67	11.1	10.5	9.65
4	1	8.79	9.76	8.17	10.8	9.75	9.73
5	1.5	9.1	8.7	7.97	10.82	9.57	9.85
6	2	6.812	7.3	6.36	10.76	9.41	9.71
7	2.5	6.434	6.2	5.81	10.9	9.49	10.2
8	3	5.98	6.9	4.94	10.83	9.39	10.8
9	4	2.718	1.754	1.444	10.773	9.36	10.595
10	24	1.937	1.822	1.591	10.666	8.66	10.435
11	48	1.639	1.97	1.62	10.1	9.38	10.6
12	72	1.52	1.82	1.57	9.6	8.74	10.1
13	96	1.38	1.49	1.48	9.3	8.72	9.8

Figure 4 Strontium removal from water in a flow-through experiment in baffled tanks.

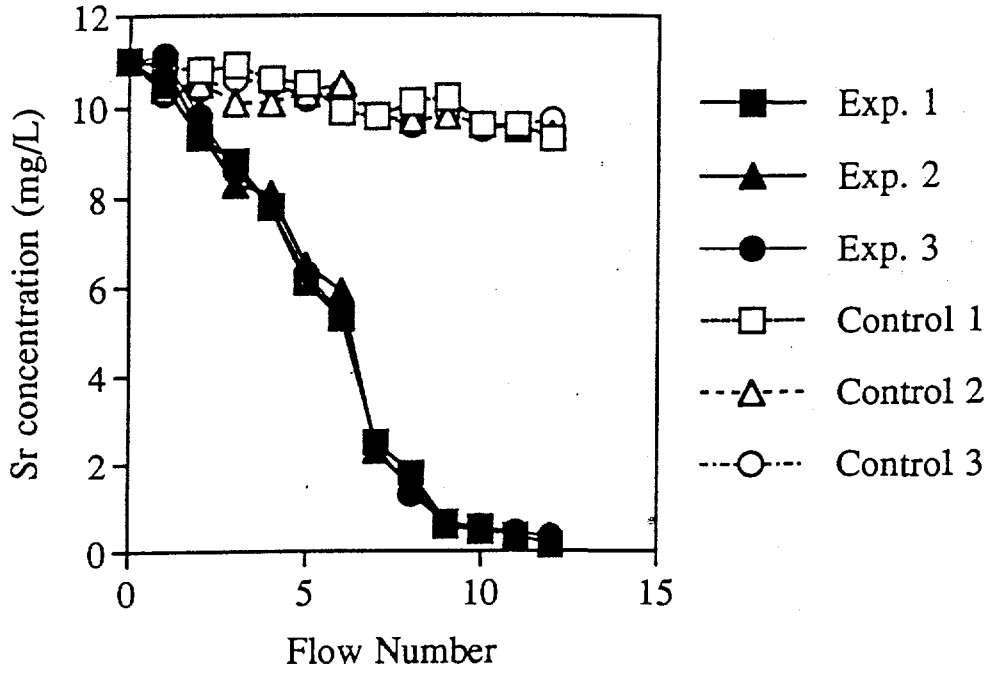

Table 2. Raw data on strontium removal from water in a flow-through experiment in baffled tanks.

	1	2	3	4	5	6	7
	Flow #	Baffle 1	2	3	4	5	6
1	0	11	11	11	11	11	11
2	1	10.5	10.9	11.1	10.9	10.4	10.3
3	2	9.35	9.5	9.8	10.8	10.5	10.4
4	3	8.8	8.3	8.6	10.9	10.1	10.6
5	4	7.8	8.1	7.8	10.6	10.1	10.5
6	5	6.1	6.5	6.3	10.5	10.3	10.2
7	6	5.3	5.9	5.4	9.9	10.5	10.3
8	7	2.5	2.3	2.5	9.8	9.8	9.8
9	8	1.8	1.5	1.3	10.1	9.7	9.6
10	9	0.65	0.58	0.63	10.2	9.8	9.9
11	10	0.54	0.45	0.55	9.6	9.6	9.5
12	11	0.36	0.39	0.43	9.6	9.5	9.6
13	12	0.15	0.36	0.32	9.3	9.4	9.7

Figure 5. Schematic diagram of a recycling bioreactor for strontium removal from water.

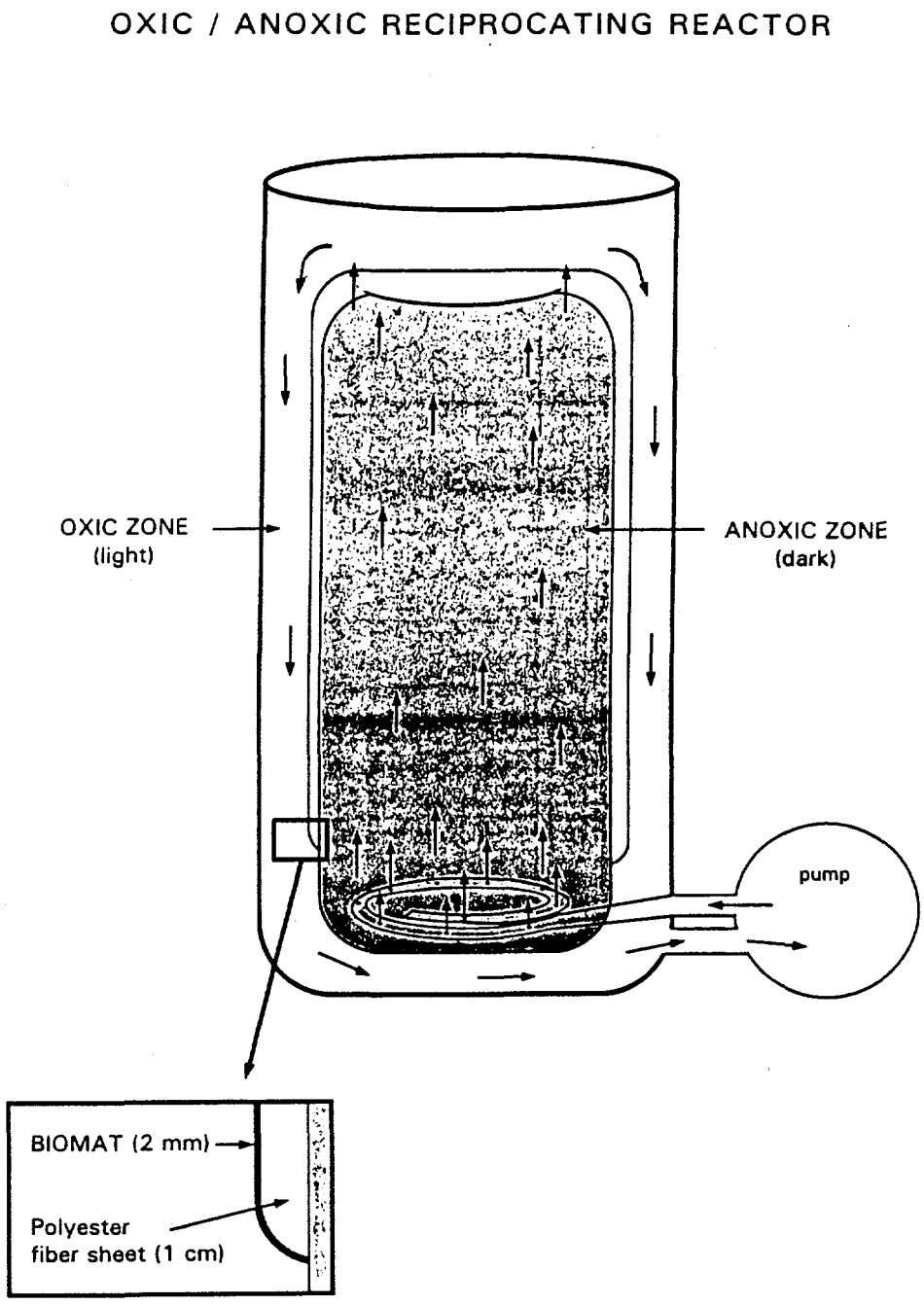

Figure 6. Strontium removal from water contained in a recycling bioreactor.

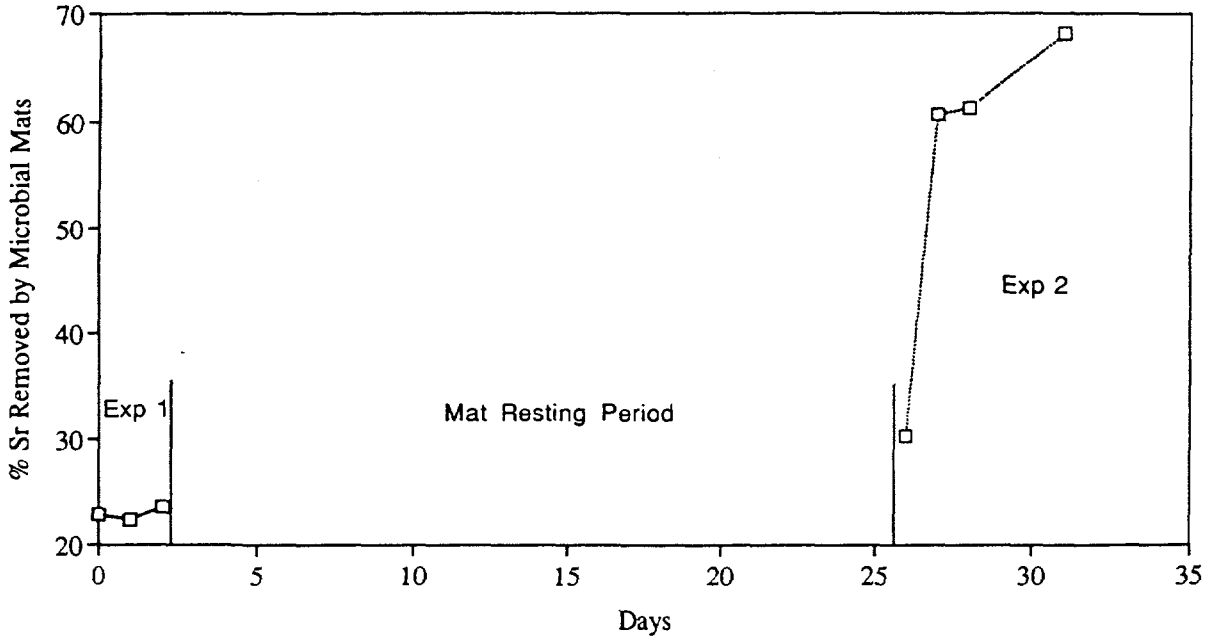

Table 3. Raw data on strontium removal from water contained in a recycling bioreactor.

	1	2
	DAYS	% Sr Removed
1	0	22.9
2	1	22.4
3	2	23.6
4	3	
5	4	
6	5	
7	6	
8	7	
9	8	
10	9	
11	10	
12	11	
13	12	
14	13	
15	14	
16	15	
17	16	
18	17	
19	18	
20	19	
21	20	
22	21	
23	22	
24	23	
25	24	
26	25	
27	26	30.3
28	27	60.8
29	28	61.4
30	29	
31	30	
32	31	68.2

Exp 1 Original Sr conc. = 51.8 ppm

Reactor drained, AA salts with 2X phosphate added

Recovery period

Exp 2 Original Sr conc = 22.5 ppm

II. Experiment Results.

2. High concentration mixed metals removal from water.

Results of mixed heavy metal removal in a batch-style refluxing bioreactor: Experiments were conducted using a tub and sink style bioreactor (Figure 7). The tub held a submersible pump which continuously refluxed the metal-contaminated media. The media was dripped over a 0.166 m^2 piece of glass wool on which was cultured a microbial mat. The reflux rate of the media averaged 5 L/min. Chromium, cadmium and lead, in mixture, at approximately 10 mg/L each, were examined for their removal rate from the liquid media, and for metal packing rate over time. Results of the final set of experiments are summarized graphically (Figure 8) and in tabular form.

Bioreactor #1: Conditions were:

- Allen-Arnon media (Allen and Arnon, 1955) with the addition of 5 mL of 1 M $NaNO_3$/liter.
- 12 light:12 dark cycle.
- One layer of microbial mat on glass wool, which was previously used in a metal sequester experiment.
- Total volume of media used was 100 liters.

% removal of each metal in three-metal mixture:

Time	30 min	1 hour	24 hours
Cr	81.3	84.4	99.4
Cd	56.5	58.3	98.7
Pb	75.2	77.6	99.6

Grams of metal removed per meter^2:

Time	30 min	1 hour	24 hours
Cr	5.926	6.152	7.245
Cd	4.016	4.144	7.016
Pb	5.083	5.245	6.732

Total grams of metal removed:

Time	30 min	1 hour	24 hours
	15.025 g	15.541 g	20.993 g

Bioreactor #2: Conditions were:

- Filtered tap water.
- Darken conditions.
- Two layers of fresh microbial mat on glass wool.
- Total volume of media used was 100 liters.

% removal of each metal in three-metal mixture:

Time	30 min	1 hour	24 hours
Cr	78.7	85.8	99.6
Cd	75.0	82.6	98.7
Pb	77.1	85.3	99.6

Grams removed per meter^2:

Time	30 min	1 hour	24 hours
Cr	6.301	6.869	7.974
Cd	5.535	6.096	7.284
Pb	5.234	5.791	6.762

Total grams removed:

Time	30 min	1 hour	24 hours
	17.07 g	18.756 g	22.02 g

Bioreactor #3: Conditions were:

- Filtered tap water.
- 12 light:12 dark cycle.
- Two layers of fresh microbial mat on glass wool.
- Total volume of media used was 100 liters.

% removal of each metal in three-metal mixture:

Time	30 min	1 hour	24 hours
Cr	9.6	-0.03	100.0
Cd	61.6	76.0	97.8
Pb	65.9	80.0	99.7

Grams removed per meter^2:

Time	30 min	1 hour	24 hours
Cr	1.090	0	11.355
Cd	4.483	5.531	7.117
Pb	4.315	5.239	6.528

Total grams removed:

Time	30 min	1 hour	24 hours
	9.888g	10.77 g	25.0 g

The removal efficiency of the microbial mat immobilized on glass wool was extremely high under all three scenarios. The bioreactor held under dark conditions performed the best (not tested statistically yet), especially for cadmium removal. A possible explanation for this is that under darken conditions, the anoxic zones within the microbial mat and glass wool matrix was greater. If so, this would likely provide a greater pool of sulfide for cadmium precipitation as a sulfide. It is unclear why chromium removal was slowed in Bioreactor #3. At 24 hours, all three metals were nearly completely eliminated from the 100 liters of media.

Figure 7. Tub and sink style bioreactor. The tub held a submersible pump which continuously refluxed chromium, cadmium and lead, in mixture. The solution was dripped over a 0.166 m^2 piece of glass wool on which was cultured a microbial mat. The reflux rate of the media averaged 5 L/min. at approximately 10 mg/L of each metal.

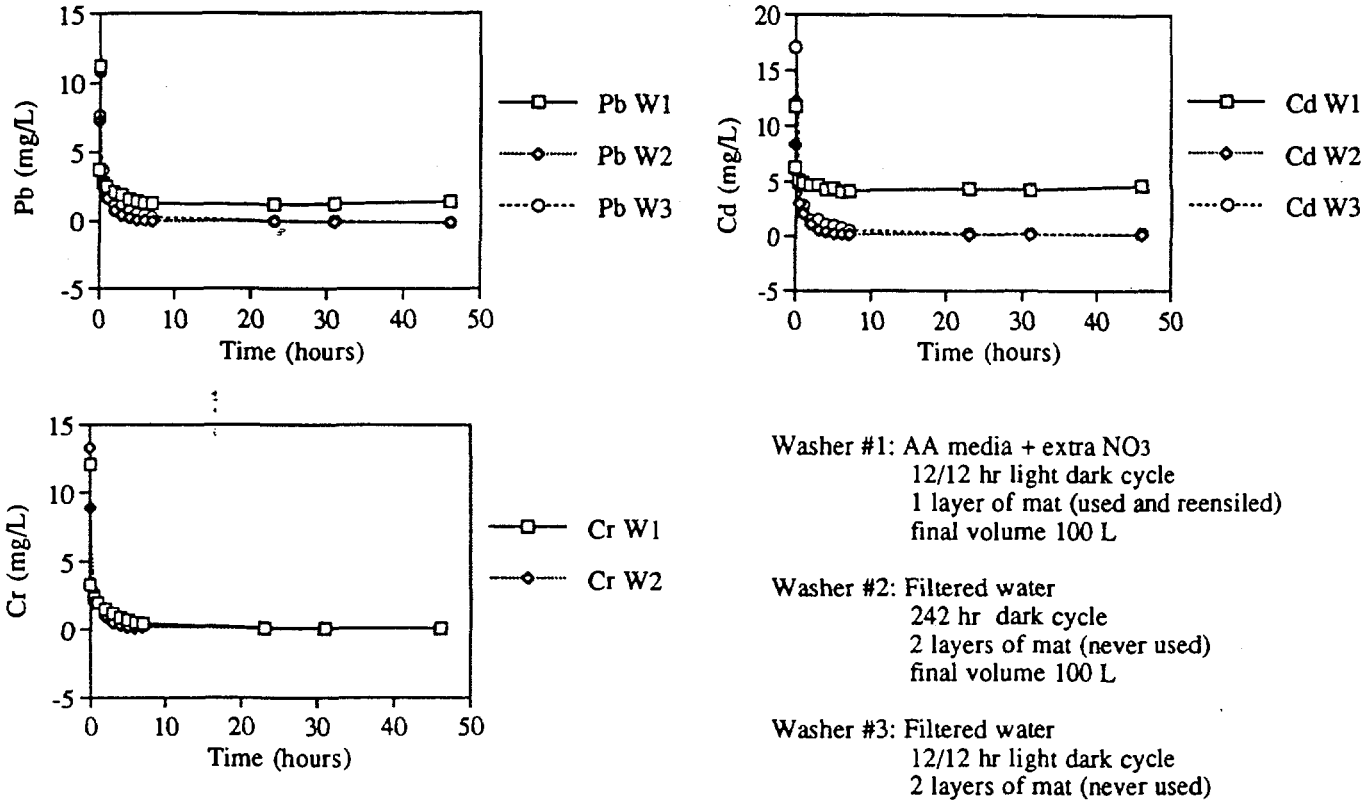

Figure 8. Chromium, cadmium and lead removal from the tub and sink style bioreactor. Each metal had an initial concentration of approximately 10 mg/L.

II. Experiment Results.

3. Low level mixed metals removal from water.

Objectives: 1) To investigate the metal removal from high volume, low concentration treatments; 2) To determine which treatment factors remove most metal: the microbial mat filter or the bioflocculent residing in the reservoir.

Methods: Upper recycling tank, six batches of 20 gallons each. Total cumulative additions, after five batches, was 2,236 mg each of manganese, cobalt and cesium. Total volume added was 100 gallons. This could be all accumulated in the lower reservoir tank.

Five additions of metal solution were added and recycled to 24 h (sometimes these times were extended to 72 or 96 h). Then the upper recycling tank was drained into the lower reservoir tank.

Sampling: Samples were taken from the upper recycling tank faucet before any drainage occurred into the lower reservoir tank (equivalent to t-24), after 10 gallons of the tank's 20 gallons were drained ("a" in Figure 9, referred to as mid-outflow) and as the tank was nearly empty ("b" in Figure 9, referred to as emptying). This three-point sampling was to assess whether metals were being flocculated to the bottom of the upper tank or trapped in the filter. "a" samples were taken at t-0 (immediately after draining the upper tank into the lower tank) and t-24 (or some variation of settling times), representing the settling time before the next drainage.

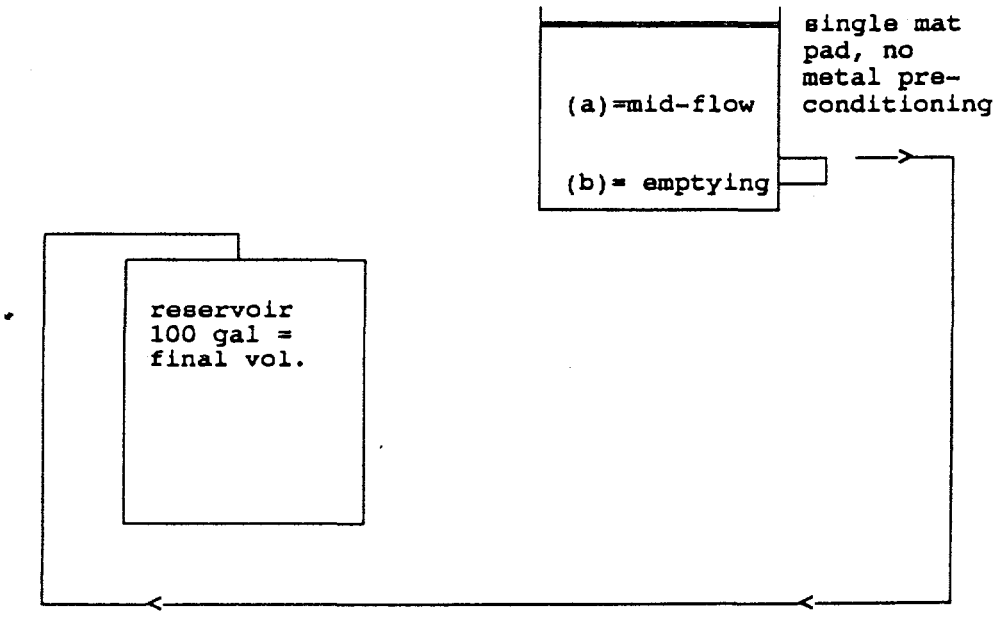

Figure 9. Scheme for low concentration mixed metal removal by microbial mat and/or bioflocculant produced by microbial mat.

Data reporting and analysis: The most important summary of data is:

- A total of 2,236 mg of each metal (Mn, Co, Cs) was added to the upper recycling tank as the cumulative amounts of the five 10-gallon batches. After 11 days, the final quantities remaining in the lower reservoir tank water column[1] were:
  - Mn = 101 mg
  - Co = 63 mg
  - Cs = 449 mg

Of these amounts the following divisions between upper recycling tank and lower reservoir tank deposit were calculated and given in Table 4.

[1]This was calculated by averaging the fourth and fifth batches at t-26 and t-24.

Table 4. Cumulative mixed metal removal in recycling tank which was periodically drained, new mixed metal solution was added (five batches) and the drainage was accumulated in a reservoir tank.

Metal	% Removed in Recycling Tank	% Removed in Reservoir Tank	% Remaining in Reservoir Water Column
Mn	63	34	3
Co	76	22	2
Cs	~0	80	20

Sample calculation:

Mn, total input = 2,236 mg
Recycling tank outflow = 833.8 mg
Therefore, 63% was removed in the recycling tank.

833.8 mg was delivered to the reservoir tank and after 11 days, 75.8 % remained. Therefore, 833.8 - 75.8 = 758 mg removed, equal to 34%. This left 3% in the water column. Table 5 contains the raw data.

Observations:

1. Comparison of upper recycling tank at t-0 to t-24 shows good reduction of Mn and Co, not Cs. Most Cs is removed in reservoir.

2. Comparison of "mid-outflow" to "emptying" concentrations indicates that some of the metals are in the process of flocculating out in the recycling tank over time and some are being entrapped in the microbial mat (unclear as to how these two locations compare as to amounts of metals deposited.

3. More time in the recycling tank may improve the settling of metals.

4. Pre-treating microbial mats with metals may improve the production of bioflocculants.

5. If reductases and oxygenases change the oxidation states of the metals to make precipitation more likely (probably only an issue with Mn), then the presence of anions is important and additions of minimal media may improve metal removal.

7. The mechanisms may be metal attachment to amorphous poly-polar organic molecules. These may be, in fact, bioflocculants. Forces are a little stronger than van der Waals or water/hydrogen bonding. Since they have many sites, one molecule can bind many metal ions.

Table 5. Raw data for the low concentration metal experiment.

Reactor/Reservoir data Skidaway — Fri, Aug 30, 1996 12:49 PM Pa

		2	3 *	4	5 *	6	7	8	9	10
	reactor	Mn ppb	% metal wshr	Co ppb	% metal wshr	Cs ppb	reservoir	Mn ppb	Co ppb	Cs ppb
1	1st t0	1043		961		990	1st t0	14	218	914
2	1st t24	6	5.0%	188	19.0%	904	1st t1	8	196	833
3	st MidoutFlo	7		183		884	1st t3	8	199	856
4	1st Emptying	26		203		878	1st t6	10	219	939
5	2nd t0	850		805		959	1st t24	1	143	664
6	2nd t72	80	9.8%	79	10.0%	1041	2nd t0	22	110	814
7	2nd Emptying	105		102		1045	2nd t1	23	116	812
8	3rd t0	896		828		960	2nd t3	31	118	870
9	3rd t24	325	36.0%	149	18.0%	927	3rd t0	120	117	830
10	rd midoutflo	331		155		966	3rd t1	121	117	833
11	3rd Emptying	349		168		940	3rd t3	118	119	835
12	4th t0	989		983		934	3rd t7	114	112	855
13	4th t24	387	39.0%	189	19.0%	915	4th t0	180	124	893
14	th midoutflo	406		195		957	4th t1	177	125	858
15	4th Emptying	457		235		940	4th t6	174	120	882
16	5th t0	1091		1070		1074	4th t26	172	120	927
17	5th t26	510	47.0%	256	24.0%	1066	5th t0	222	134	923
18	th midoutflo	485		246		988	5th t1	232	148	909
19	5th Emptying	763		494		1021	5th t3	237	149	928
20	6th t0	1084		1079		1105	5th t24	250	143	945
21	6th t96	394	36.0%	176	16.0%	1165				
22	th midoutflo	395		176		1150				
23	6th Emptying	509		213		1234				
24										
25	TOTAL ng/ml	2209	1415.0%	6101	TOTAL ng/ml	211	131.5	936		
26	#Total mg	833.8	530.6%	2233	##Ave mg	101.3	63.1	449.0	ave 4th & 5th	
27	Total vol	each batch	= 20 gal		Total vol in	reservoir =	~120 gal =	480 liters		
28	Total cumult.	adds.(init.) to	washer=223	mg ea/metal						
29	# Total mg=	washer after	11days of	treatment						
30	## Ave mg=	4th & 5th	batches in	the reservoir		"Midoutflow"	sample taken	from nozzle	after 10gal	drained into
31	Reservoir t0	= after	emptying into	reservoir		"Emptying"	sample was	the last 10gal	on the bottom	of washe
32	Resrvoir t24	setting time	before next d	rain from W		Total vol= 6	flows X 20gal	/flow=120ga	=480 L in	one washer

* % metal in washer: first measured time div by amt at T-0: ex= 6 div by 1043.
It is assumed that the remaining % metal was drained into ...

II. Experiment Results.

4. Uranium and mixed metal removal from water (parallel study conducted at ORNL).

This data was not supported by Grant Award No. DE-FG09-95SR18553, but is useful to this report as supporting data for the success of microbial mats for metal and radionuclide removal. The entire study, which compared 12 sorbents, showed that the microbial mat (identified here at MATS) was only one of two sorbents that hold promise for treatment of these contaminants are Oak Ridge National Laboratories. Complete results are available on the World Wide Web at http://128.219.18.198/bcv/.

Procedure for uranium study: 0.25 g of microbial mat was combined with 50 mL of groundwater and 550 μl of a 101-mg uranium/l solution. The groundwater contained Al (3 ppm), Ca (56 ppm), Cl (30 ppm), Fe (2.5 ppm), Mg (6.5 ppm), K (5.5 ppm), Si (10 ppm), Na (14.5 ppm) and sulfate (12 ppm). This solution in a tube was shaken for 24 h. Upon completion, the tube was centrifuged at 1000 rpm for 5 min. The supernatant was transferred to a new centrifuge tube before 100 μl of concentrated nitric acid was added as a preservative.

The samples were analyzed for uranium following the EPA method SW846-6010A for ICP analyses. For each sample, three burns were performed on a ICP-ES and the average of the three burns was used in determining results. The samples were filtered before analysis.

Results: The microbial mat removed about 50% of the spiked uranium (Figure 10).

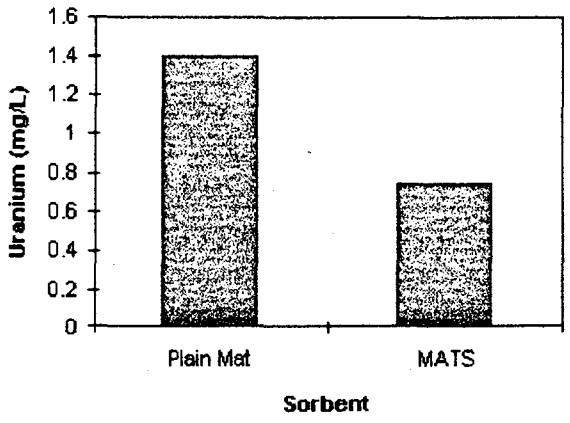

Figure 10. Screening study conducted at ORNL for uranium removal by microbial mat. The "Plain Mat" blank was glass wool.

Procedure for mixed metals study: 0.25 g of microbial mat was combined with 50 mL of groundwater. The groundwater contained Al (81 ppm), Ba (20 ppm), Ca (>1000 ppm), Cl (180 ppm), Fe (37 ppm), Mg (224 ppm), Mn (>100 ppm), Ni (3 ppm), nitrate (8500 ppm), K (48 ppm), Na (379 ppm) and sulfate (12 ppm). This solution in a tube was shaken for 24 h. Upon completion, the tube was centrifuged at 1000 rpm for 5 min. The supernatant was transferred to a new centrifuge tube before 100 μl of concentrated nitric acid was added as a preservative.

The samples were analyzed for uranium following the EPA method SW846-6010A for ICP analyses. For each sample, three burns were performed on a ICP-ES and the average of the three burns was used in determining results. The samples were filtered before analysis.

Results: In Figure 11, the left bars corresponds to the concentration of metals in the groundwater, the right bars correspond to concentration of metals present in the solution after being in contact with the microbial mat for 24 h. The high concentrations of Ca (>1000 ppm) and Na (379 ppm) likely inhibited the removal of the metals.

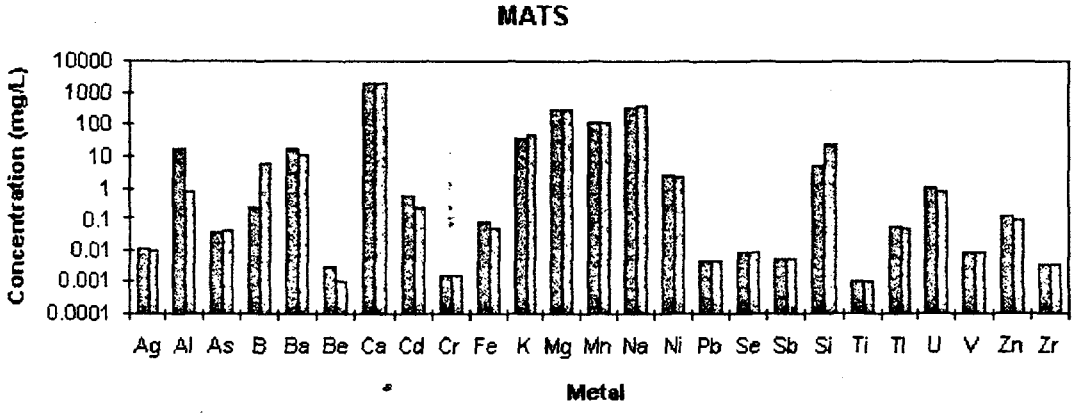

Figure 11. Screening study conducted at ORNL for mixed metal removal by microbial mat. The left bars corresponds to the concentration of metals in the groundwater, the right bars correspond to concentration of metals present in the solution after being in contact with the microbial mat for 24 h.

II. Experiment Results.

5. Toxic characteristic leaching procedure (TCLP).

A TCLP test was conducted on two samples of microbial mat and/or coconut mesh (substrate used for microbial mat attachment). The following results show extremely high metal retention by microbial mat and coconut mesh.

Table 6. TCLP results for microbial mat and coconut mesh substrate.

Sample 1:

Metal	Metal Accumulation in mg/kg	TCLP Analysis in mg/L	% Retained by Microbial Mat/Coconut Mesh
Al	2,022	1.72	99.9
Cd	67.6	0.752	98.9
Cu	887	3.68	99.6
Pb	280	<DL	100
Mn	13,870	14.9	99.9
Zn	10,980	186	98.3

Sample 2:

Metal	Metal Accumulation in mg/kg	TCLP Analysis in mg/L	% Retained by Coconut Mesh
Al	2,281	13.9	99.4
Cd	28.9	0.40	100
Cu	531	4.8	99.1
Pb	171	0.59	99.7
Mn	4,547	7.9	99.8
Zn	8,239	147	98.2

Sample 3:

Metal	RCRA Analysis Average in mg/kg	TCLP Analysis Average in mg/L	% Retained by Microbial Mat
Iron	1,305.75	0.71	99.9
Barium	292.8	2.6	99.1

II. Experimental Results.

6. Simultaneous organic contaminant biodegradation and metal removal.

These experiments on mixed heavy metal removal in the presence of organic pollutants were conducted in a batch-style refluxing bioreactor (see Figure 7). The organic compound chosen was used as a model compound and is not necessarily a contaminant at the Savannah River Site.

Methods for metal removal in the presence of ethylene glycol solution: Experiments were conducted using three tub and sink style bioreactors (see Figure 7). Each tub held a submersible pump which continuously refluxed the contaminated media. Media was dripped over a 0.166 m^2 piece of glass wool on which was cultured a microbial mat. The reflux rate of the media averaged 5 L/min.

In these experiments, the contaminant was a mixed organic compound and metal waste. Chromium, cadmium and lead, in mixture, at approximately 10 mg/L each, were examined for their removal rate from the liquid media. The organic waste was ethylene glycol.

In the first bioreactor (Experimental #1), two layers of microbial mat grown on glass wool were placed on top of a third layer of glass wool that was previously inoculated with a mixed aerobic/anaerobic bacterial culture. The inoculation procedure was to hold the glass wool overnight in under dark conditions. Additionally, 200 mL of a sulfate-reducing bacterial culture were added in between the two layers of wool containing microbial mat. This reactor was kept on a 12L:12D cycle.

In the second bioreactor (Experimental #2), no microbial mat was used. All other conditions were identical to those of the first bioreactor. This reactor was kept on a 24D cycle.

In the third bioreactor (Control), neither microbial mat nor any bacterial culture was used. Only three clean layers of glass wool was applied. No lights were attached to this reactor, nor was it kept in total darkness.

All reactors had two air lines installed and the liquid was continuously recirculated.

After noting that there was no increase in turbidity (indication of high bacterial populations), 0.5 liters of anaerobes and 2 liters of aerobes were added to the first two bioreactors. Because still no increase in turbidity was observed, the media was supplemented with $NaNO_3$ and $NaHPO_4$ to a final concentration of 150 g/L and 35 g/L, respectively.

Results: Table 7 gives results of the experiment. Metal content in the media of all three bioreactors decreased at a similar rate (no statistically significant difference among the three). The control glass wool removed metals equally well. In order to determine the actual removal capacity of the different bioreactors, it would be necessary to periodically spike the media with additional metal. Previous research (Bender et al., 1995) showed that in a baffled-tank style bioreactor, eventually the available sites on the glass wool for

metal sorption become saturated and metals are not removed (especially chromium) in the glass wool control, whereas microbial mat on glass wool continues to precipitate metal.

Table 7. Metal removal by microbial mat in the presence of ethylene glycol. The experiment was conducted in a recycling bioreactor. Results in mg/L.

Pb

Sample Time	Exp. #1 (MAT)	Exp. #2 (Microb.)	Control
TO	8.68	7.29	7.15
3 HR	2.59	4.39	5.03
24 HR	0.49	0.87	1.71
48 HR	0.55	0.89	1.35
72 HR	0.47	0.96	1.26
168 HR (7 DAYS)	0.73	0.96	1.68
168 HR AFT ADD	0.77	1.07	1.68
264 HR(11 DAYS)	1.72	0.98	2.04

Cr

Sample Time	Exp. #1 (MAT)	Exp. #2 (Microb.)	Control
TO	10.07	8.24	7.94
3 HR	1.41	3.39	4.31
24 HR	0.05	0.06	0.52
48 HR	0.05	0.04	0.07
72 HR	0.04	0.08	0.04
168 HR (7 DAYS)	0.07	0.05	0.07
168 HR AFT ADD	0.06	0.06	0.05
264 HR(11 DAYS)	0.34	0.09	0.06

Cd

Sample Time	Exp. #1 (MAT)	Exp. #2 (Microb.)	Control
TO	9.93	7.76	7.88
3 HR	4.83	5.15	5.45
24 HR	4.69	4.04	4.34
48 HR	5.38	4.21	4.69
72 HR	5.23	4.52	4.70
168 HR (7 DAYS)	7.83	5.99	5.73
168 HR AFT ADD	8.41	5.61	5.79
264 HR(11 DAYS)	23.00	6.79	8.51

III. Proposed designs for testing microbial mats in SRS field pilots, related to Year 1 research.

1. Baffled tank. The baffled tank configuration can be used to interrupt a stream flow and forcing it to pass in a serpentine fashion through microbial mats cultured on a substrate, such as glass wool (Figure 12). This configuration is proposed for testing at the ORNL project site.

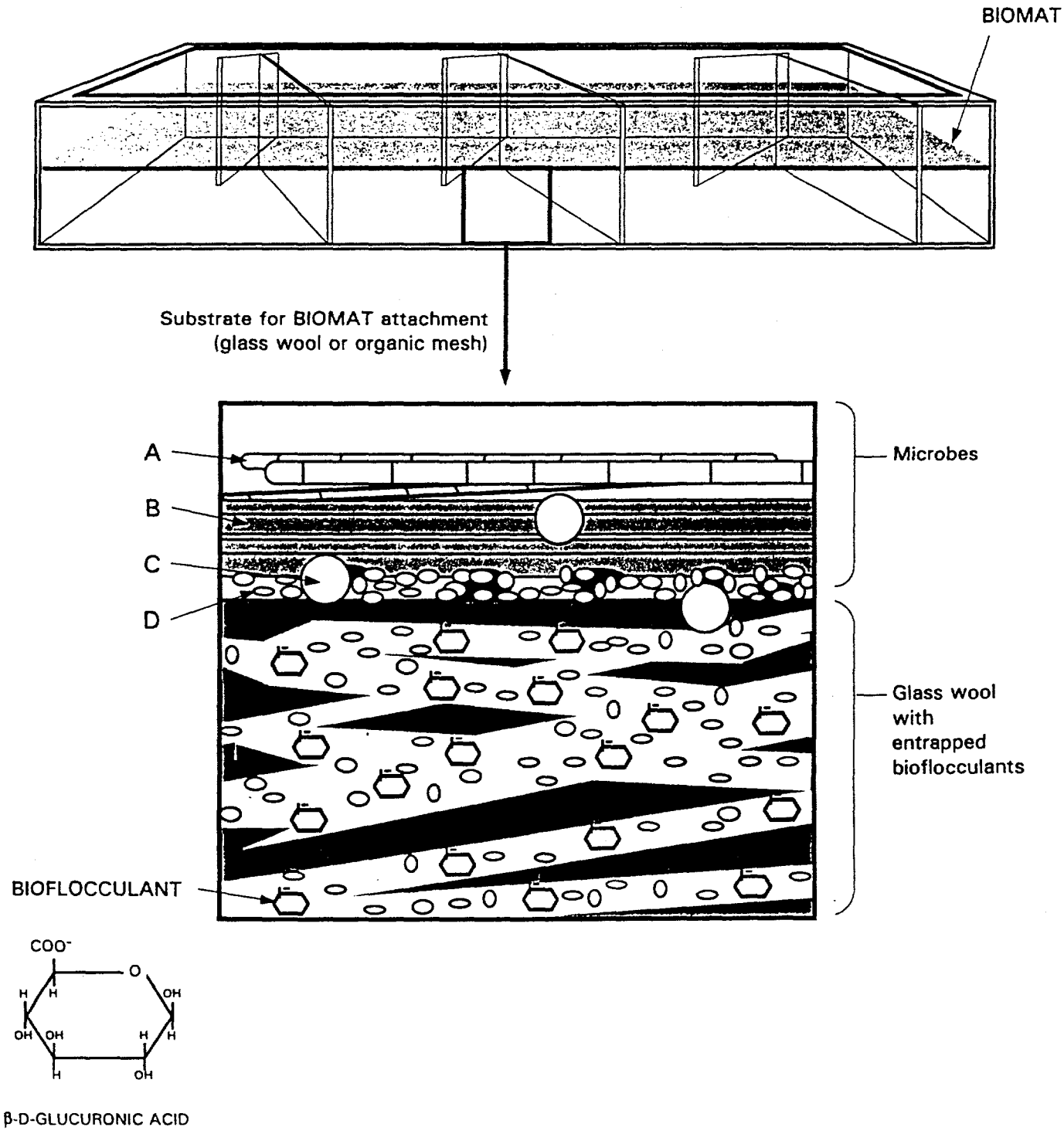

Figure 12. Diagram of a baffled tank and an expanded cross-section of microbial mat.

III. Proposed designs for testing microbial mats in SRS field pilots, related to Year 1 research.

2. Plate and shelf reactor for slow flow and low contaminant concentration. This reactor is currently being tested for microbial mat stability and for metal removal (Figure 13, two pages).

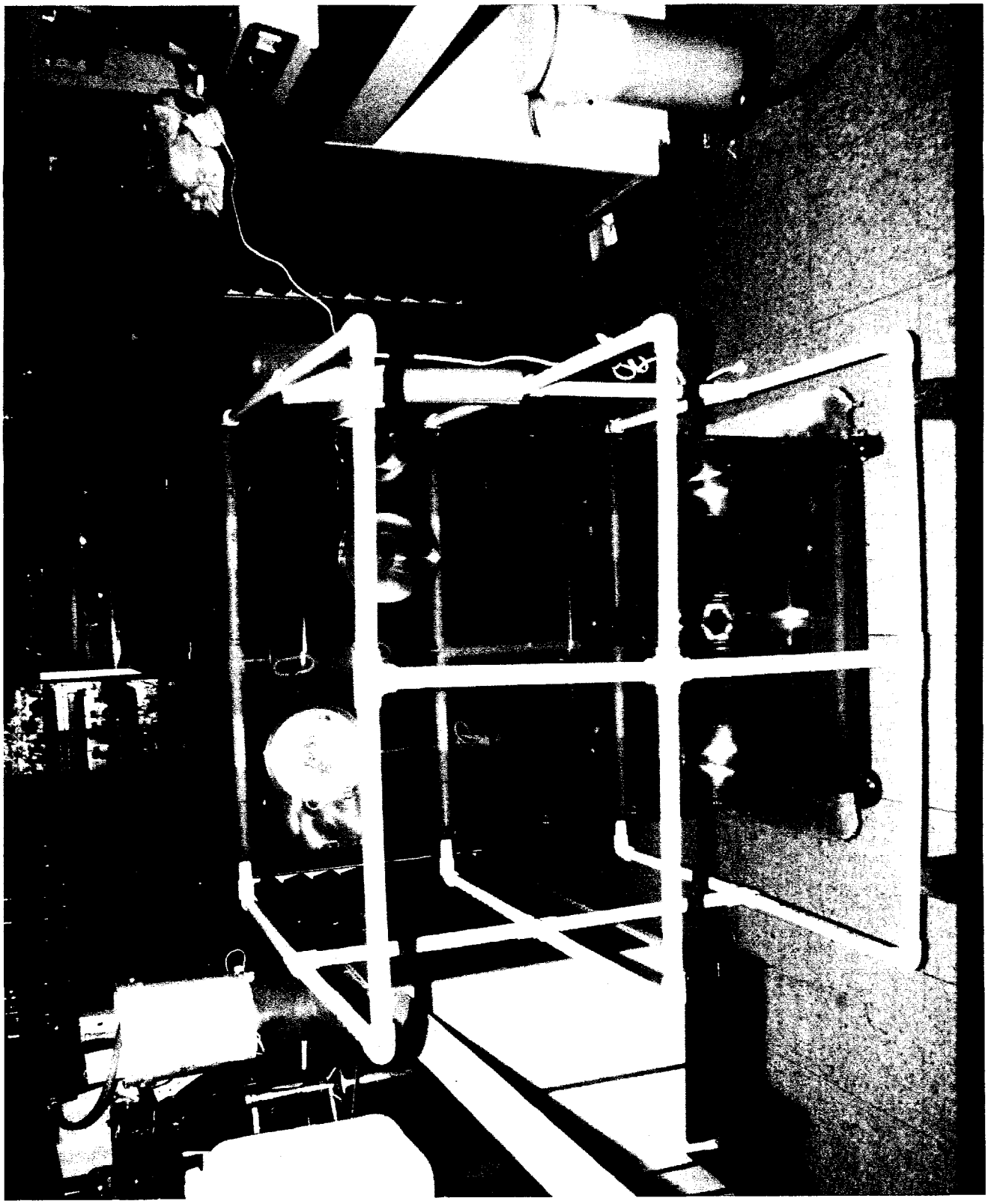

Figure 13 (page 1 of 2). Plate and shelf reactor for slow flow and low contaminant concentration.

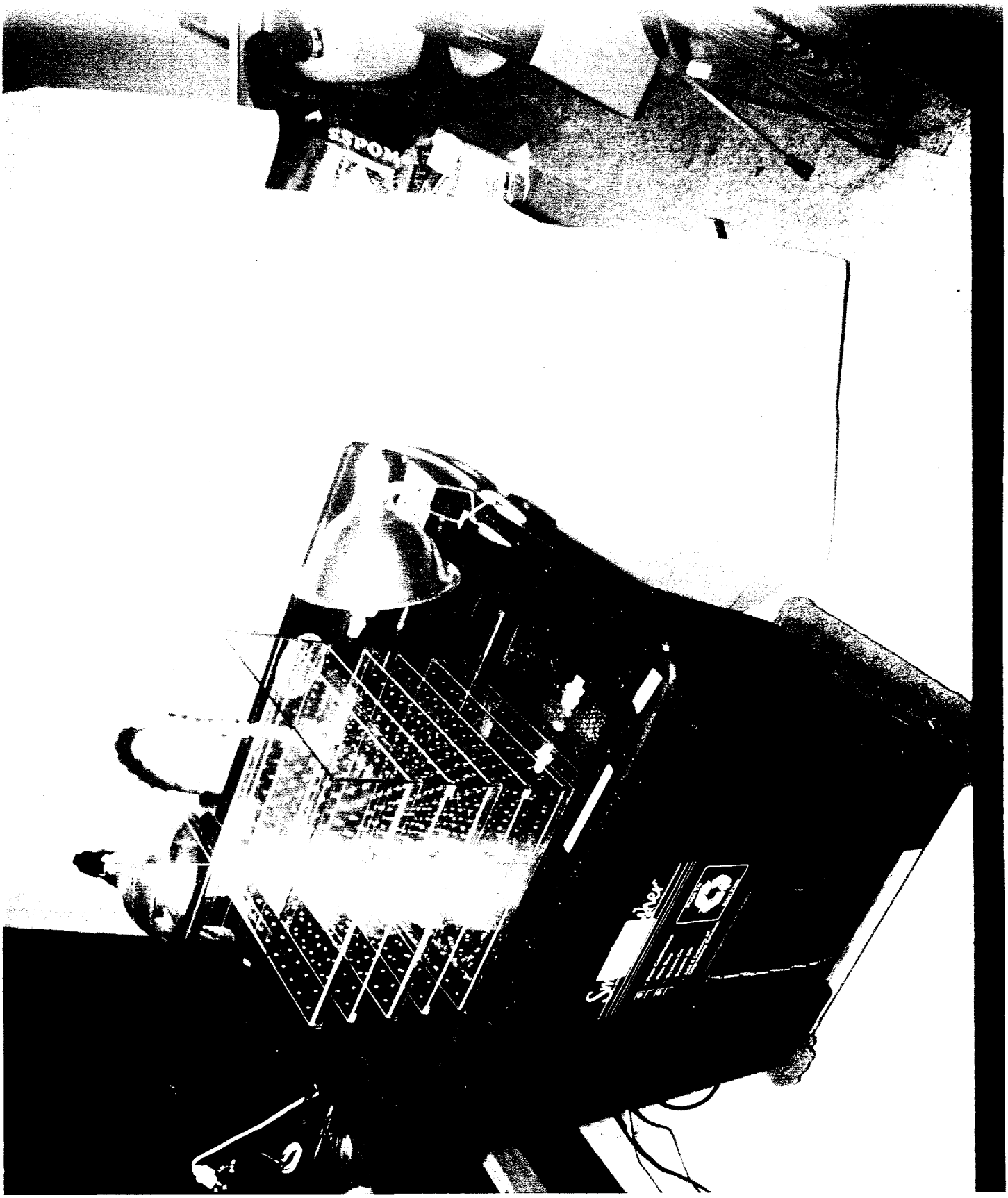

Figure 13 (page 2 of 2). Plate and shelf reactor for slow flow and low contaminant concentration.

III. Proposed designs for testing microbial mats in SRS field pilots, related to Year 1 research.

3. Pond incorporating bagged media. Based on previous research, we have proposed a microbial mat pond treatment system for metal-laden drainage (Figure 14). A modification of this system would incorporate "bagged" media containing microbial mats for bioflocculant production and protective substrate to protect microbial mats from abrasion and predation.(Figure 15). The bagged or floating media would be expected to bioaccumulated high concentrations of metals as seen in Figure 16.

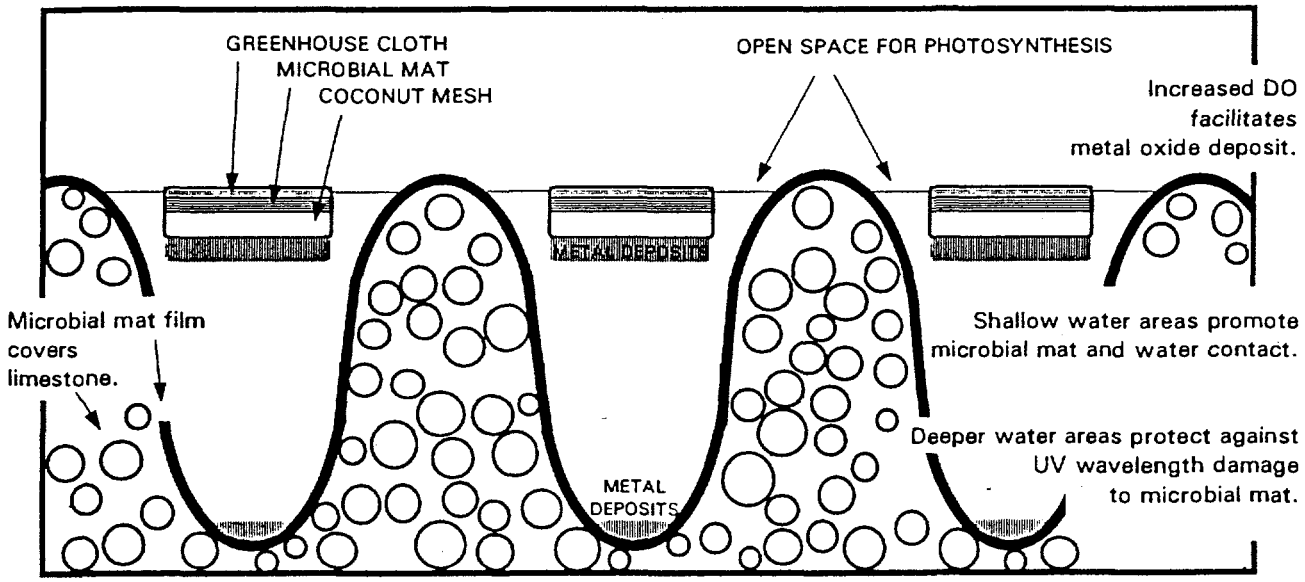

Figure 14. Proposed microbial mat pond treatment system for metal-laden drainage.

Figure 15. "Bagged" media containing microbial mats for bioflocculant production and protective substrate to protect microbial mats from abrasion and predation.

Figure 16. Photograph of a glass wool and microbial mat "floater" that had been placed in a cadmium contaminated solution. Cadmium bioconcentrated on the surface of the floater.

IV. Proposal for collaboration with the Savannah River Ecology Laboratory.

Department of Energy Office of Energy Research, Application for Program Notice 96-10, *A field test of the efficiency of a biotic system for remediating radionuclide and metal contamination in surface waters*. The biotic system to be tested is the Microbial Mat of Bender and Phillips. The principal investigator is Dr. Thomas Hinton of SREL.

Excerpted from the proposal:

The proposed research addresses three fundamental topics. Foremost, it is a field test of an aquatic bioremediation technique that removes radionuclides and metals from surface waters using microbial mats. The bioremediation research provides an opportunity to address contamination problems on DOE sites. Because the effectiveness of this remediation technology depends upon the sorption of radionuclides and metals from the water column, the second part of the experiment quantitatively compares the efficiency of the bioremediation technique to natural rates of contaminant sequestration and remobilization inherent in aquatic systems. The third component of the research allows us to test the accuracy of a computer model used to predict the transport and fate of radionuclides in aquatic systems.

We propose an experiment whereby we would add a suite of short-lived tracers to Pond 4, a 15 ha pond in the cooling water canal between P reactor and Pond C on the SRS. Candidate tracers include those of principal radioactive contaminants on the SRS (cesium, strontium, cobalt and mercury), heavy metals of concern to DOE (chromium and cadmium) and elements that illustrate fundamental oxidation/reduction processes occurring within lake ecosystem (iron and manganese).

The tracers would allow us to quantify the bioremediation efficiency of microbial mats for heavy metals and radionuclides under field conditions. The mats will be placed at the outfall of Pond 5 in a structured container through which all the water must pass. The tracers would be added to the upper reaches of Pond 4, flow through Pond 4 and Pond 5, and into the microbial mats at the outfall of Pond 5. Samples taken before and after the microbial mats would allow us to quantify the efficiency of the mats in removing the contaminants from the water column.

VI. References.

Allen, M.B. and D.I. Arnon. 1955. Studies on nitrogen-fixing blue-green algae 1. Growth and nitrogen fixation by *Anabaena cylindrica*. Lemm. Pl. Physiol. 30:366-372.

Bender, J., R.F. Lee and P. Phillips. 1995. Uptake and transformation of metals and metalloids by microbial mats and their use in bioremediation. Journal of Industrial Microbiology 14:113-118.

Bender, J., and P. Phillips. 1994. Implementation of microbial mats for bioremediation. In: Emerging Technology for Bioremediation of Metals (J. L. Means and R. E. Hinchee, eds.). Lewis Publishers, Boca Raton, FL. pp. 85-98.

Phillips, P. and J. Bender. 1995. Biological remediation of mixed waste by microbial mats. Federal Facilities Environmental Journal. Autumn 1995:77-85.