WSRC-TR-96-0088

Final Report for TTP# SR-16-PL-42 (Formerly SR-141019) – Bioremediation of Aqueous Pollutants Using Biomass Embedded in Hydrophilic Foam (U)

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Table of Contents

1.0 Abstract....3

2.0 Introduction....4

3.0 Identification, Characterization and Selection of Metal Contaminated Waste Sites and Waste Streams at SRS....7

4.0 Selection and Culturing of Algal Strains....17

5.0 Characteristics of the Foam Used for Embedding....25

6.0 Bioremoval Capabilities of Foam-Embedded Microbes....27

7.0 Polyurethane Foam Immobilization of TCE-Degrading Bacteria: Entrapment Effectiveness and Influence on Metabolic Activity....35

8.0 Degradation of Trichloroethylene and Benzene by Embedded Bacteria....61

9.0 Conclusions....80

10.0 Recommendations for future work....82

11.0 Acknowledgments....83

12.0 Literature Cited....84

Appendices....A1

1.0 ABSTRACT

The major objective of this project was to examine the potential of a novel hydrophilic polyurethane foam as an immobilization medium for algal, bacteria, and other types of biomass, and to test the resulting foam/biomass aggregates for their use in cleaning up waters contaminated with heavy metals, radionuclides and toxic organic compounds. Initial investigations focused on the bioremoval of heavy metals from wastewaters at SRS using immobilized algal biomass. This effort met with limited success for reasons which included interference in the binding of biomass and target metals by various non-target constituents in the wastewater, lack of an appropriate wastewater at SRS for testing, and the unavailability of bioreactor systems capable of optimizing contact of target pollutants with sufficient biomass binding sites. Subsequent studies comparing algal, bacterial, fungal, and higher plant biomass demonstrated that other biomass sources were also ineffective for metal bioremoval under the test conditions. Radionuclide bioremoval using a Tc-99 source provided more promising results than the metal removal studies with the various types of biomass, and indicated that the alga *Cyanidium* was the best of the tested sources of biomass for this application. However, all of the biomass/foam aggregates tested were substantially inferior to a TEVA resin for removing Tc-99 in comparative testing.

We also explored the use of hydrophilic polyurethane foam to embed *Burkholderia cepacia*. *B. cepacia* is an efficient degrader of trichloroethylene (TCE), a contaminant of considerable concern at SRS and elsewhere. However, it does not adhere well to surfaces and hence is ill-adapted to use in bioreactors. We first optimized the conditions of foam manufacture so as to achieve a high degree of bacterial retention within the foam matrix. The type and concentration of surfactant and the biomass density used in the foaming process proved to be of crucial importance. Secondly, the general physiological status of the embedded bacteria was examined. The embedded population proved to be incapable of growth on nutrient media, but retained respiratory activity. Lastly, the degradative capabilities of embedded G4 were examined. Phenol- or benzene-induced bacteria retained the ability to degrade TCE; they were also capable of benzene degradation. Degradation of both compounds was inhibited in the presence of readily metabolizable carbon sources. Preliminary tests indicated that, once TCE-degrading enzyme activity was induced, it was of relatively short duration, potentially limiting shelf life of foam/bacterial aggregates. However, by appropriate manipulation of induction conditions, we were successful in inducing enzyme activity after the organisms had already been embedded. It may therefore be feasible to geographically and temporally separate the induction and use of the material (possibly for several consecutive cycles) from its growth and immobilization, greatly facilitating practical applications.

2.0 INTRODUCTION

The principal objective of this TTP was to evaluate, with the aid of an industrial partner, Frisby Technologies Inc., a new bioremediation scheme involving filter media consisting of biomass embedded in a hydrophilic foam matrix. Figure 2.1 depicts a simplified diagram of the process. Novel features of the conceptual process include: (1) the use of unique biomass strains, (2) use of a unique polymer to produce a biofilter medium consisting of unaltered biomass embedded within a hydrophilic matrix; and (3) use of a unique bioreactor system designed to optimize contact of bioagents with target pollutants. The utility of the foam embedded with biomass was investigated with actual and simulated wastewater contaminated with toxic heavy metals, radionuclides and toxic organic compounds, particularly TCE.

There is great potential for processes that utilize natural, biodegradable materials (e.g. microbial biomass) to remove toxicants (such as metals, radionuclides, and chlorinated hydrocarbons) from wastewaters. A major stumbling block to the development of such processes has been the lack of suitable containment or immobilization of the biomass to fully facilitate their bioremediation capabilities. This project was aimed at overcoming this liability, demonstrating the capability of clean-up of a variety of waste sites and waste streams at the SRS with biomass/foam combinations. The longer term goal is to broaden applicability to clean-up efforts at government and commercial facilities world-wide.

This project began in FY 1994. The principal focus of the project during the first year included the identification, characterization, prioritization and selection of SRS waste sites and waste streams potentially amenable to bioremediation; and the selection of and growth of algal strains well suited for the bioremediation process under development. During FY 1995, work was primarily directed toward evaluating the conceptual process using a novel laboratory test rig developed by Frisby, along with other laboratory apparati, for removal of metals from a coal pile runoff basin by the use of foam embedded with algal biomass. A wider scope of potential applications for the process was developed for work which occurred from late FY 1995 through the end of the project in March 1996. The wider scope was designed to give the foam a better evaluation, and enhance the possibility of developing a technology with commercial potential. The project was expanded in two directions. First, additional types of wastewater with additional contaminants were evaluated, and secondly, additional types of biomass were evaluated for their ability to remediate pollutants while embedded in foam. Thus, we expanded the technology evaluation from merely bioremoval of heavy metals by algae to both bioremoval and biodegradation of several types of pollutants by a variety of bioagents.

Wastewaters considered for the expanded program included a variety of groundwaters and process streams that contain TCE, toluene, and benzene in addition to heavy metals. Non-algal biomass tested for bioremoval included metal removing

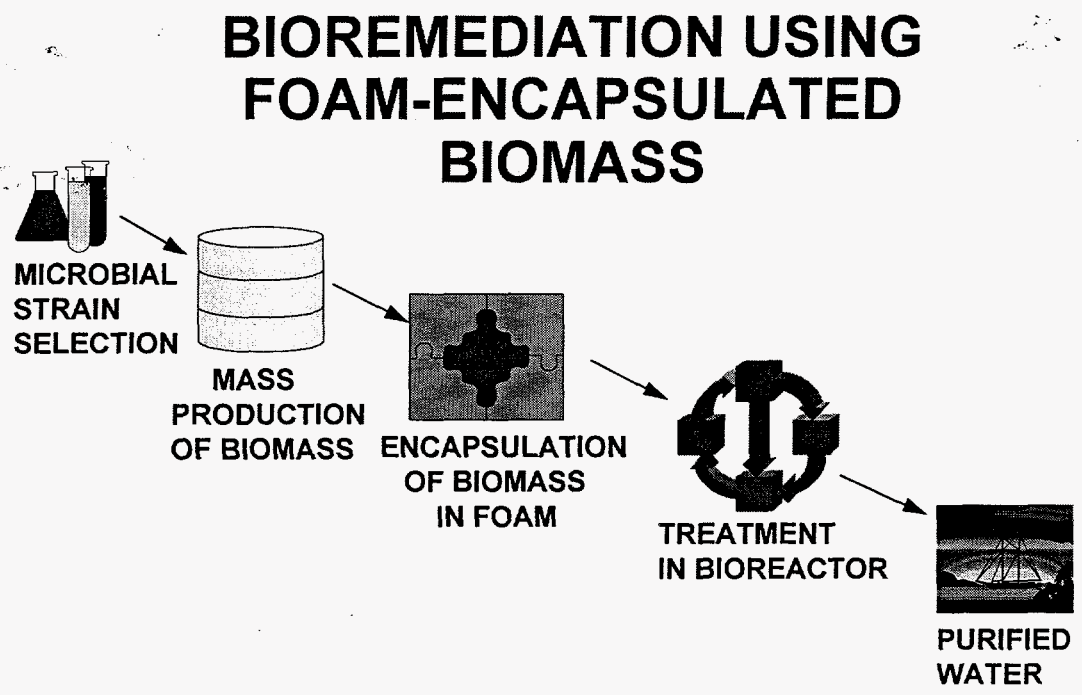

Figure 2.1 Simplified Diagram of Conceptual Process

bacteria (based on the literature), including a strain currently used by ORNL for U biosorption, and two fungal strains. A radionuclide uptake experiment included evaluation of the comparative uptake by algae, bacteria, fungi, higher plant biomass and an ion exchange resin. Biodegradation was evaluated by testing bacteria known to be high rate biodegraders containing oxygenases suitable for treating multiple hazardous organic wastes.

3.0 IDENTIFICATION, CHARACTERIZATION AND SELECTION OF METAL CONTAMINATED WASTE SITES AND WASTE STREAMS AT SRS

3.1 Introduction

An investigation was conducted to identify and prioritize heavy metal-containing waste waters at the Savannah river Site (SRS) in terms of their suitability for testing of and potential clean-up by bioremediation processes (Wilde and Radway, 1994).

The investigation included a review of information on surface and/or groundwater associated with all known SRS waste sites, as well as waters associated with all known SRS waste streams. Following the initial review, waste waters known or suspected to contain potentially problematic concentrations of one or more of the toxic metals (listed in Appendix Table A3.1) were given further consideration.

3.2 Waste Sites

Information on SRS waste sites was obtained by numerous discussions with Environmental Restoration (ER) personnel (see Appendix 3) and by reviewing published and unpublished documents provided by ER and other SRS personnel (e.g. the Savannah River Site Environmental Report for 1992, WSRC-TR-93-075). These reports provided a starting point for the process of investigating and selecting the most appropriate waste sites for future bioremediation.

The initial screening effort resulted in the identification of over 30 ER waste units that were reported to contain surface and/or groundwaters with heavy metals as pollutants, often in combination with radionuclides and/or toxic organic compounds (Appendix Table A3.2). The current ER contact person for each of these sites was identified and interviewed to obtain the most recent information about each site.

In addition to holding discussions with ER personnel, recent (1993) groundwater monitoring data were obtained and screened for measurements of toxic metal and radionuclides that exceeded regulatory limits. Sites with known or alleged metal contamination were also compared with groundwater monitoring data (summarized in WSRC-TR-93-075) to determine if drinking water standards were exceeded at these sites in 1992. This screening resulted in the selection of 25 RFI/RI waste sites that clearly have heavy metal polluted water. They are listed in Appendix Table A3.3 along with information used for evaluating the sites in terms of their suitability for incorporation into metal bioremoval studies.

A thorough review of WSRC-TR-93-075 also resulted in the identification of several other SRS facilities at which groundwater monitoring well samples contained heavy metal contamination. Appendix Table A.3.4 lists all sites displaying heavy metal

containing groundwaters along with the specific metal contaminant(s), the drinking water standard (DWS) for the metal, the highest concentration of the contaminant observed in 1992, the number of wells sampled, and the number of wells where drinking water standards were exceeded. As shown in the table, much of the groundwater contaminated with toxic metals is also contaminated with radionuclides and toxic organic compounds.

Following the data gathering process, the authors prioritized the ER waste units in terms of their potential for remediation by and compatibility with the heavy metal/radionuclide bioremediation process being developed. Since the studies were planned to be conducted in a non-radiation control area, the initial selection process was restricted to RFI/RI sites with non-radioactive, metal-containing waste waters that appear to require remediation and can be readily sampled by the researchers. The only SRS wastewaters fulfilling these criteria were the coal pile runoff basins and their associated contaminated groundwaters.

Although only non-radioactive sites were selected for the initiation of laboratory studies, it should be emphasized that radioactive metal-containing wastewaters may be more amenable to the bioremediation process being developed since it is expected that the process will be equally or more efficient at sequestering some radionuclides than non-radioactive heavy metals. Thus, the logistics of conducting the initial laboratory work was the principal criterion used in selecting the sites listed above and described in more detail below.

3.2.1 Coal Pile Runoff Basins (CPRBs)

It was concluded that the coal pile run-off basins were the best available ER waste units for the initial testing of a bioremoval process because they are non-radioactive, contaminated with a variety of heavy metals, readily available for sample collection (especially the surface waters) and believed to be in need of future remediation.

There are seven CPRBs at SRS located in A,C,D,F,H,K, and P-Areas. They provide receptacles for runoff from rainfall on coal piles located at these seven sites. The coal was used to fuel facilities producing steam and electricity for SRS. The facilities at A- and D-Areas are currently active, while the facilities in the other five areas have been shut down. Coal piles in C- and F-Areas were removed in 1985. Currently, rainwater runoff from the remaining coal piles (A,D,H,K, and P) flows into the CPRBs via gravity flow through ditches and sewers. The coal is generally of low sulfur content (1-2%) . Chemical and biological oxidation results in water that has a very low pH (due to the formation of sulfuric acid) and high concentrations of dissolved heavy metals. Contaminants leaching into the coal pile runoff basins during rainfall eventually contaminate underlying soil and groundwater. Principal toxic metal contaminants of concern include Al, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, and Se. All of these metals have been measured at levels above drinking water standards in samples collected from the basins. Appendix Table A.3.5 shows levels of metal contaminants in the basins from

three studies, including a recent one made by the authors (Wilde et. al., 1994, unpublished data). Maximum levels ranged from 107-11300% of the drinking water standards. Drinking water standards were exceeded by the largest margins for Al, Cd, Ni, Be, and Pb. The D-Area CPRB typically had the highest levels of metal contaminants and was thus our first choice as a source of wastewater for initial experimental work.

3.2.2 TNX Burying Ground

The TNX Burying Ground is located within the fence that surrounds TNX near the western border. This waste site was created in 1953 when an experimental evaporator containing 590 kg of uranyl nitrate exploded. Contaminated material included structural steel, tin, timber, drums, rags, and other items. The contaminated material was buried in four trenches, 6-8 feet below land surface. The waste trenches were rediscovered in 1980 during construction of buildings. Most of the contaminated material was removed in 1982 and 1983. However, an estimated 27 kg of uranyl nitrate along with other contaminants remain under buildings or in locations where the use of excavation equipment was restricted. This site contains Pb and Hg above DWS (WSRC, 1993). Recent (1993) groundwater monitoring data also revealed high levels of Al. This site also has substantial contamination by toxic organic compounds. The TNX Burying Grounds was considered a prime site for the metal bioremoval research program because of its proximity to and association with other bioremediation activities being conducted by the ESS Biotechnology Group based within the TNX complex.

3.2.3 Road A Chemical Basin

The Road A Chemical Basin is located about 0.5 mile southwest of the intersection of Highway 125 and SRS Road 6. This basin was 100 ft x 175 ft x 10 ft deep. It reportedly received miscellaneous radioactive and chemical aqueous waste for several years, but no records of the materials disposed of at the basin are available. The basin was closed and backfilled in 1973. It is currently part of the RFI/RI program. Recent data from groundwater monitoring wells below the basin reveal levels of Pb and Hg above drinking water standards. No other contaminants were observed above DWS during 1992 (WSRC, 1993). Thus, the site was considered for obtaining water samples from the groundwater monitoring sampling program and for testing of metal removal techniques in laboratories not set up for handling radionuclides or carcinogens.

3.2.4 Other Sites

Additional waste sites that appeared particularly well suited for bioremediation by the process being developed are listed below, followed by a brief description:

Burning Rubble Pits
D-Area Ash basins
Chemicals, Metals & Pesticide Pits (CMPs)
Miscellaneous Chemicals Basin/Metals Burning Pit
Retention Basin in H-Area
Seepage Basins

Silverton Road Waste Site
Burial Ground Complex
Acid/Caustic Basins
L-Area Oil & Chem. Basin

3.2.4.1 Burning Rubble Pits (BRPs)

There are numerous BRPs with heavy metal contaminated underlying ground water at SRS. The BRPs are primarily unlined pits that have received combustible wastes which were allowed to accumulate and periodically burned . These pits have subsequently been taken out of service, and backfilled with soil and sediments to grade level. Eight burning rubble pits were operated in A-, K-, P-, C-, L-, R-, and G-Areas for several years. Groundwater below these pits has been contaminated with heavy metals along with radionuclides and organics. Metals of concern include Pb, Cr, and Hg. Groundwater remediation is deemed necessary and remediation plans are being developed. Based on recent groundwater monitoring data (WSRC 1993), the BRPs in L-,N-,P-, and K-Areas have metal contaminated groundwater with no radioactive contaminants. Thus, water from these sites should be suitable for experimentation in a non-radiation controlled laboratories.

3.2.4.2 D-Area Ash Basin

The 488-D basin is located in the southwestern part of D-Area. It began operation in 1951 and was used to intercept, stabilize, and provide passive treatment of ash sluice water prior to discharge to local surface streams. The basin ceased receiving sluice water when two additional basins were constructed. The basin was subsequently used for placement of dry ash and coal crusher reject materials. Monitoring wells in the underlying ground water consistently show heavy metals and toxic organics above regulatory limits in the groundwater below this basin.

3.2.4.3 Chemicals, Metals & Pesticide Pits (CMPs)

The CMP Pits are located approximately one mile north of L-Area and one mile northeast of the 131-3L Rubble Disposal Area. This complex originally consisted of seven unlined pits which were designed to receive non-radioactive wastes, such as spent solvents, pesticides and toxic metals. The pits were used from 1971 until 1979. In 1984, the pits were excavated and waste materials were removed. Then the area was backfilled and capped with a geosynthetic material. Recent groundwater monitoring has demonstrated significant contamination by heavy metals. Remediation is deemed necessary and a formal remediation plan has not been developed.

3.2.4.4 Miscellaneous Chemicals Basin/Metals Burning Pit

This waste unit actually comprised two separate facilities in close proximity. Both are suspected to have polluted underlying ground waters. Contaminants of concern from the miscellaneous chemicals basin include Al (3483-7488 ppm), and Pb (2.65-10.5

ppm). Contaminants of concern from the metals burning pit include Al (range 1430-95,570 ppm) (Wilde and Radway, 1994).

3.2.4.5 H-Area Retention Basin

The old H-Area Retention basin (281-3H) is located just south of Road E near the intersection of Road E and Road 4. This basin was used for temporary emergency storage of cooling water containing radionuclides and trace quantities of other chemicals from the chemical separations process. Groundwater monitoring data show Al, Pb, and Sb to be among the contaminants exceeding DWS.

3.2.4.6 Seepage Basins

Several seepage basins at SRS are considered waste sites and have potential for clean-up using bioremediation. These include the 716-A Motor Shop Seepage Basin, the D-Area Oil Seepage Basin, the new TNX Seepage Basin, the old F-and H-Area seepage basins, the Ford Building seepage basin and seepage basins in all the reactor areas. Some of these basins still contain standing waters and all have underlying groundwater contaminated with metals and other pollutants, especially radionuclides.

3.2.4.7. Silverton Road Waste Site

The Silverton Road Waste Site is located about 1.5 miles west-southwest of A/M Area. This unit consists of an approximately 700 ft. x 300 ft. x 7 ft deep area that existed as an open pit prior to construction of SRS. During and after construction of SRS, the pit and surrounding area was used for the disposal of construction debris such as metal shavings, drums, and storage tanks. Operations at this location ceased in 1974, and the waste material is presently covered with soil and vegetation. Underlying groundwater contains several constituents exceeding DWS. These include Sb, Be, and Pb.

3.2.4.8. Burial Ground Complex (BGC)

The BGC occupies approximately 194 acres in the central part of SRS between the F and H Separations Areas. It consists of several adjacent facilities which were formerly, or are currently disposal sites for hazardous and radioactive wastes and spent solvents generated from plant processes. Groundwater below the BGC is contaminated with numerous toxic metals in addition to radionuclides and toxic organic compounds.

3.2.4.9 Acid/Caustic Basins

Acid/caustic basins are located in several areas (F,H,K,L,P, and R) of SRS. These basins are unlined earthen pits, approximately 50 ft x 50 ft x 7 ft deep, that received dilute sulfuric acid and sodium hydroxide solutions used to regenerate ion-exchange units in water purification processes at the reactor and separations areas. Other wastes discharged to the basins included water rinses from the ion-exchange units,

steam condensate, and runoff from the spill containment enclosures in the storage tanks. The basins allowed mixing and neutralization of the dilute solutions before their discharge to nearby streams. All of the basins were constructed between 1952 and 1954. They were taken out of service between 1964 and 1982. These basins are part of the RFI/RI program and closure, characterization and remediation plans are in various stages of development within the various areas. Basins in L- ands R- Areas are the farthest along in this process. However, all of the basins are expected to require remediation in the future.

3.2.4.10 L-Area Oil and Chemical Basin

The L-Area Oil and Chemical Basin is located in the southeastern portion of L-Area, just outside the L-Area perimeter fence. This 118 ft x 79 ft basin was put into operation in 1961 and continued to receive waste liquids until 1979. Contaminants of concern include Cd, Pb, Cr, and Hg, along with radionuclides and organics (Radway and Wilde, 1994). Groundwater monitoring data revealed that concentrations of Cd and Pb exceeded DWS in groundwater below the basin.

3.3 WASTE STREAMS

In contrast to the case with waste sites at SRS, documents comprehensively describing waste streams in various sectors of SRS could not be found. Thus, a slightly different approach was taken to identify and prioritize the waste streams in terms of their suitability for the bioremoval process. Key personnel throughout the site, such as environmental coordinators and site waste coordinators, were canvassed in an attempt to obtain information relevant to the selection process (Appendix Table A-3.8).

The SRS (Fig. 3.1) is subdivided into 18 principal areas. These are listed in Appendix Table A3.6, along with major activities previously and/or currently conducted at them. In compiling information about waste streams in these areas, we attempted to determine:(1) the general nature of the waste-generating process, (2) presence of radionuclides, (3) major metals present, (4) whether waste is currently generated, (5) volume stored and/or rate of generation, (6) availability of analytical data, (7) current method of treatment or disposal, and (8) need for further treatment; problems with treatment or disposal.

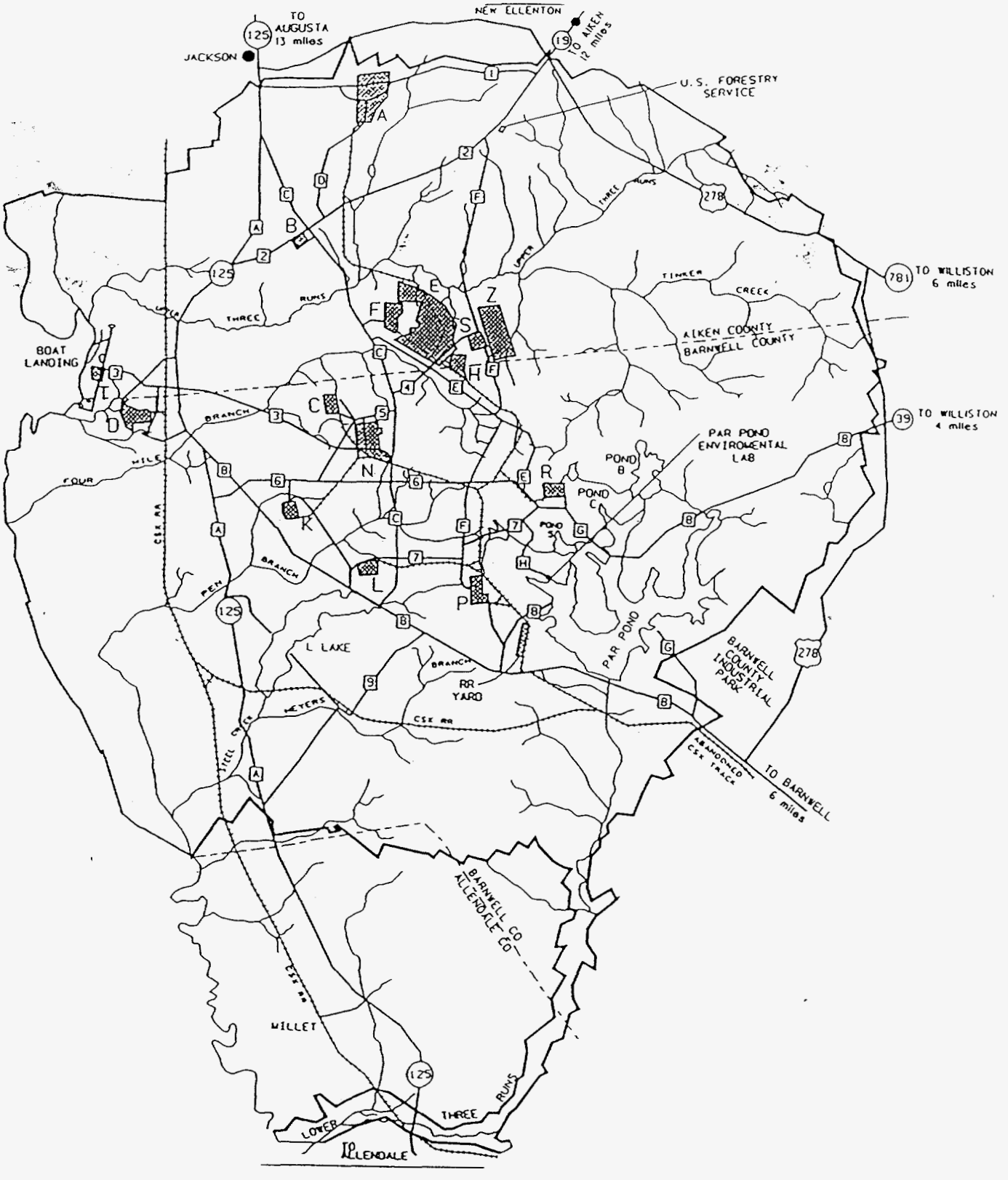

Figure 3.1. Map of SRS Showing Principal Site Areas

Information gained during the investigation is catalogued (according to site area) in Table A.3.7. Only metal-containing aqueous wastes are included. Those areas listed in Table A3.6 but not in Table A.3. 7 proved not to contain wastes of interest within the scope of our study.

Three major criteria were used in selecting those waste streams most amenable to bioremediation process development. First, there must be a need (or an anticipated need) for present or future treatment of the waste. Second, streams without radioisotopes are best suited to process development, with those containing low level contamination being less suitable and those with high level contamination being unsuited to experimental purposes. Third, the streams should be generated or stored in sufficient quantities to make the cleanup effort worthwhile.

In this manner, the streams catalogued in Table A.3.7 can rather quickly be reduced to a handful of candidates. Among these are the CIF (Consolidated Incineration Facility) blowdown water and a D Area lab waste containing Hg thiocyanate. These contain low level radiation, requiring the use of an RCA or of simulated wastes. Likewise, it is possible that algal biosorbents might provide a useful alternative or adjunct to the present ion exchange resin used to remove Hg from various lab wastes and Wastewater Neutralization Facility wastes. Future candidates are sanitary wastewater treatment facilities in which metals may eventually pose a sludge disposal problem. Bioremediation processes might also find application in the ongoing cleanup of reactor disassembly basins, but these wastes are not suitable for the initial development of such processes due to their high radiation levels.

The waste streams fell into several major groups. These are briefly discussed below:

3.3.1. Photographic wastes.
A number of on-site operations generate sizable amounts of spent fixer which can contain up to 4500 ppm silver. These wastes are currently being treated by an ion exchange process at a silver recovery unit in A-Area and one in N-Area. The treatment reduces Ag content to levels classified as non hazardous, allowing disposal via the sanitary sewer system.

3.3.2. Radioactive laboratory wastes (mixed wastes).
These include wastes collected via low level (high and low activity) drains in SRTC labs and stored in tanks in A Area, along with lab waste water generated during tests associated with the development of vitrification processes (S Area). The waste is periodically treated with Duolite GT-73 resin to remove mercury introduced by a contaminated drain, and will eventually be sent to the tank farm when tanks are full.

Two wastes containing Hg and heavy water are generated in D Area. The Water Quality Laboratory analyzes samples from reactor cores. Excess sample water (to which standard mercury solution has sometimes been added) is currently processed by the Heavy Water facility for recovery and recycling of its heavy water content. A second

lab waste, containing Hg thiocyanate, heavy water, permanganate, and a variety of organics, is no longer being generated. However, 100 drums (classified as hazardous) remain in storage at D Area, and 46 drums (classified as hazardous) are stored in N Area. These require cleanup before their heavy water can be recycled. The current treatment option consists of ion exchange using Duolite GT-73 resin, which reduces Hg content to acceptable levels. Resin performance is sometimes impaired due to clogging of the columns, and the system is currently undergoing modification to overcome this problem.

3.3.3. Non radioactive laboratory waste.

These include wastes generated by the M-Area Analytical and Metallurgy Laboratories and containing a variety of metals. They are currently treated at the Dilute Effluent Treatment Facility (DETF) prior to discharge. The treatment involves precipitation of metals followed by pressure filtration and is considered adequate. The laboratories are expected to move to an undecided location in the near future. Acidic wastes containing Cr and other metals are also generated by the A-Area Metallurgy Lab. They are currently being neutralized and stored pending the establishment of a Cr precipitation procedure which will allow their discharge via the sanitary sewer system.

3.3.4. Reactor wastes.

Although no reactors are operating at the present time, disassembly basins at C, K, L, P, and R Reactors contain water and sludge contaminated with radionuclides and metals (e.g. Cs, Pu, Al, Fe) . In K, L and P disassembly basins, a mixed bed ion exchange resin is used intermittently to reduce cation and anion levels in standing water. This is not done at C and R basins because little or no fuel is present and hence contamination levels are much lower. Resin is regenerated by a RBOF, (reactor basin for offsite fuel), facility in H Area and the eluted contaminants sent to a waste tank for storage. Spent resin is also stored pending the selection of a disposal method. An upgrade of the deionizing system is planned and may involve reverse osmosis carried out by an outside vendor. Sludge is periodically vacuumed from disassembly basins and is currently being stored in the absence of a disposal method.

3.3.5. Separations wastes.

High level radioactive wastes, containing Hg, Al, and various fission products, are generated (albeit currently at about 20% of previous levels) as a result of separations processes in F and H areas. During periods of ongoing separations activities, much larger amounts containing a variety of radionuclides and metals would be produced. Separations wastes undergo a series of treatments at the tank farms (H Area), ultimately leading to the discharge of purified water and shipment of contaminated solids and liquids to S and Z Areas respectively for immobilization and permanent storage.

3.3.6. Waste processing facilities

A large number of on-site facilities deal with the processing of wastes, and may themselves generate effluents which must be treated or disposed of. During full

operation, high level radioactive and mixed waste streams will be processed at the tank farms (F/H Areas) before being sent to S-Area or Z-Area for further processing into immobilized forms, while some liquid waste streams generated by the latter processes return to H Area for additional processing.

A Consolidated Incineration Facility (CIF) is slated for completion in 1996. It is expected to produce about 75,000 gal/yr of blowdown water, containing Pb, Hg, ^{90}Sr, ^{137}Cs, and possibly other metals. Evaluation of a treatment scheme, involving pH adjustment and coprecipitation of metals with iron followed by sulfide treatment to remove Hg, indicated that Cs and Sr were not affected by the treatment, and that the use of sulfide presented a disposal problem (Wilde and Radway, 1994). Current plans are to solidify the blowdown waste in the same manner as solid CIF wastes in order to minimize permitting requirements and avoid delays in startup of the facility. However, a cost-effective means of reducing the volume of blowdown waste requiring solidification and permanent storage would be of value.

Domestic waste water, which sometimes contains significant amounts of Pb, Zn, Cu, and/or Al, is processed by a system of sanitary waste water treatment plants located in various areas. The treated liquid meets water quality standards, but it has been noted that the sludge sometimes contains metals at levels approaching allowable limits for land application. It is conceivable that additional treatment might be needed if metal levels should rise or regulatory limits should change. Several waste water treatment units are expected to close soon because of the transition to a centralized facility, but the treatment method (and hence the sludge composition) is not expected to change.

3.3.7. Miscellaneous Wastes

Other wastes, such as those from the F Area cooling maintenance shop and cleanup activities in N Area, are generated as a result of miscellaneous activities. Most are generated infrequently, in small volumes, or one time only, and hence are not considered in detail here.

3.4 Selection Of Wastewaters for Study in the TTP

The purpose of this endeavor was to identify potential SRS waste waters that are amenable to clean-up by a novel bioremediation process being developed at SRS, and to select three sites for experimentation while the process is in the development stage. The three site selected for study in the short term were the CPRBs, the TNX Burying Grounds and the Road A Chemical Basin. These sites all have significant heavy metal contamination, they are not radioactive, and samples are readily obtainable. Furthermore, the CPRBs have standing water and all three waste water sources have monitoring wells where contaminated ground water samples can be obtained. All of these sites are in need of remediation and no formal remediation plan is available.

4.0 SELECTION AND CULTURING OF ALGAL STRAINS AT THE UNIVERSITY OF SOUTH CAROLINA

4.1 Introduction

Various microalgae have been shown to have potential for the bioremoval of pollutants such as toxic heavy metals from contaminated water sources (e.g. Wilde and Benemann, 1993). However, only a few of the tens of thousands of existing algal species and varieties have been tested for their effectiveness and specificity. Based on earlier work by SRTC (unpublished data), several algal strains were shown to be very effective at removing selected metal ions from simulated wastewaters. These strains included a strain of *Mastigocladus laminosus*, isolated from a reservoir receiving thermal effluent from a nuclear reactor, as well as strains of *Cyanidium caldarium* and *Chlorella pyrenoidosa*. The main objective of the work described in this section of the report was to obtain additional strains to evaluate in process development experiments. To this end, stock cultures of a variety of promising microalgal species representing several algal divisions were obtained to determine their growth characteristics in small and large volumes of culture media. The selection of the strains was based on the following criteria:

1. Absence of toxin production.
2. Ease of maintenance in a unialgal state.
3. Ability to grow in extreme environments (e.g. extremes of salinity, pH, or temperature).
4. Rapid growth in defined media.
5. Ability to achieve a high density in stationary phase.
6. Ease of harvesting (e.g. by means of filtration, phototropism, and/or flocculation).

Each strain was maintained at the University of South Carolina Algal Culture Collection and inocula of species showing promise were made available to scientists at SRS for further testing and in developing innovative approaches for maximizing bioremediation.

4.2 Materials and Methods

Eighteen freshwater and marine unialgal cultures obtained from various sources were transferred and maintained in 125 ml Erlenmeyer flasks containing approximately 75 ml of appropriate autoclaved liquid media (Table 4.1). Cultures were kept in environmental

Table 4.1 Algal species used, their sources and culture media.

Algal species	Algal division	Source	Strain. No.	Media
Chlorella capsulata	Chlorophyta	UTEX	2074	F/2
C. fusca var. *vacuolata*	"	UTEX	251	Bristol's + Proteose
C. stigmatophora	"	UTEX	993	F/2
Dunaliella salina	"	UTEX	1644	F/2
D. tertiolecta	"	UTEX	999	F/2
Scenedesmus acutiformis	"	UTEX	416	Bristol's
S. quadricauda	"	UTEX	614	Bristols
Cricosphaera carterae	Chrysophyta	UTEX	2167	F/2
Isochrysis galbana	"	UTEX		F/2
Amphiprora paludosa	Bacillariophyta	Groningen		F/2 + Silica
Chaetoceros gracilis	"	UTEX	2375	F/2 + Silica
Navicula pelliculosa	"	Bigelow		F/2 + Silica
Phaeodactylum tricornutum	"	UTEX	642	F/2
Surirella ovata	"	Groningen		F/2 + Silica
Peridinium trochoideum	Pyrrophyta	Bigelow		F/2
Rhodomonas sp.	Cryptophyta	UTEX	2419	F/2
Cyanidium caldarum	Rhodophyta	UTEX	2393	Cyanidium
Porphyridium cruentum	"	UTEX	161	F/2

chambers maintained at 20 °C and a 12 hr light:12 hr dark photoperiod with cool white fluorescent lighting at a photon fluence rate of approximately 100 μE m^{-2} sec^{-1}. Culture media are shown in Tables 4.1 and 4.2. Enriched seawater media such as F/2 (Guillard and Ryther, 1962) was prepared using 30 ‰ seawater collected from North Inlet Estuary, SC and filtered through Gelman GF/F glass fiber filters. Media preparation instructions and general maintenance procedures were taken from Starr and Zeikus (1993). Growth experiments were performed by transferring 5 ml of stock cultures to 75 ml of fresh media in 125 Erlenmeyer flasks and placing them into environmental chambers under the above temperature and light regime. All experiments were done in triplicate.

Additional experiments were run on some species to determine the effects of culture volume, salinity, pH, and growth media on their growth rates and maximum yields. Large volume experiments were conducted by inoculating *Chlorella capsulata* and *Phaeodactylum tricornutum* into 8 L of autoclaved, , F/2 media (30 ‰ salinity) contained in 10 L polycarbonate carboys kept at 25° C with aeration and constant cool-white fluorescent lighting at approximately 70 μE m^{-2} sec^{-1}.

To determine the effect of salinity on growth rate, cells of *Navicula pelliculosa* were inoculated into triplicate sets of 125 ml flasks containing quartz-glass-distilled water to 7.5, 15, 30, or 37 ‰ salinity and enriched with F/2 + silica stocks (Guillard and Ryther, 1962). Cultures were maintained at 25 °C and constant cool white fluorescent lighting at approximately 100 μE m^{-2} sec^{-1}.

The effect of pH and medium type on growth rate was tested simultaneously on *N. pelliculosa*, inoculated into either F/2 seawater media + silica, or F/2 + silica media made up using `Instant Ocean©' salts in distilled water, both at 30 ‰ and adjusted to a pH of either 6.5 using HCL or to 8.2 with NaOH and buffered with `Tris©'.

Population densities were determined by counting cells daily or other frequent intervals using a Model ZM Coulter Counter© until cultures had reached stationary phase (maximum yield). Maximum growth rates were calculated from cultures in log phase as doublings per day (μ_2) using the formula:

$$\mu_2 = 1/t \cdot \log_2 (N/N_0)$$

in which N_0 and N are cell concentrations at the initial stage and after the time period t respectively.

Table 4.2 Culture media used and their origins

MEDIUM	TYPE OF WATER	SOURCE
ASP-2	Artificial Seawater	Provasoli *et al* (1957)
Bristol's	Freshwater	Bold (1949)
Bristol's plus proteose	Freshwater	Starr and Zeikus (1993)
Cyanidium	Freshwater	Schlösser (1982)
F/2	Marine	Guillard and Ryther (1962)
F/2 with silica	Marine	Guillard and Ryther (1962)

4.3 Results

4.3.1 Initial Growth Experiment

Although each strain of microalgae was successfully transferred and grown in batch cultures, there was substantial variation in the growth rates and maximum densities among the species in the media and the growth conditions selected. Growth rates in the initial growth rate experiments varied from a low of 0.28 doublings d^{-1} in *Chlorella fusca* var. *vacuolata* to a high of 3.85 doublings d^{-1} in *Dunaliella tertiolecta*. Maximum densities also varied greatly from 5.00×10^4 cells ml^{-1} in *Peridinium trochoideum* to 3.33×10^7 cells ml^{-1} in *Navicula pelliculosa*. A summary of the results of the initial growth rate experiments can be seen in Table 4.3 and in Appendix Figures A.4.1-A.4.8.

4.3.2 Large Volume Experiment

Both *C. capsulata* and *Phaeodactylum tricornutum* reached maximum densities earlier than in the initial growth experiments, though their doubling rates and final densities were somewhat lower than those reached in the initial growth experiments (Appendix Figs A.4.9-A.4.10, Table 4.3). Maximum growth rates (μ_2) for the two species were 3.24 and 1.60 doublings d^{-1}. Cells remained suspended in the media during the growth cycle until about day 10 when they began to settle to the bottom. Aeration prevented settling but did not result in increases in cell density of either species.

4.3.3 Salinity Experiment

Cells of *N. pelliculosa* grew in all of the experimental salinities. Cells inoculated into the lower salinities (7.5 and 15 ‰) grew at higher rates than those in the higher salinities (30 and 37.5 ‰). Maximum densities were reached in 8 days at all salinities, with the highest density obtained at 7.5 ‰, approximately 3 time higher than that of cells grown at 30 ‰ in this and the initial growth experiments (Appendix Fig. A.4.11).

4.3.4 Growth Medium and pH Experiment

Cells of *N. pelliculosa* grew in both growth media and pH levels. Cells grown at a pH of 8.2 grew similarly in F/2 and `Instant Ocean' media, expressing virtually identical growth rates and maximum densities in five days. However, cells grown at a pH of 6.5 in `Instant Ocean' grew at a lower rate and reached a maximum density of only about a third that of the other cultures (Appendix Figs. A.4.12-A.4.13).

Table 4.3. Summary of initial growth rate experiments.

SPECIES	MAXIMUM GROWTH RATE (Doublings d^{-1})	MAXIMUM DENSITY (Cells ml^{-1})	GROWTH PROFILES
Chlorella capsulata	1.97	6.33×10^6	Fig. A.4.1
C. fusca var. *vacuolata*	0.28	1.41×10^6	Fig. A.4.2
C. stigmatophora	0.41	3.53×10^5	
Dunaliella salina	3.11	3.76×10^6	
D. tertiolecta	3.85	7.61×10^6	
Scenedesmus acutiformis	2.87	6.65×10^6	
S. quadricauda	3.13	9.49×10^6	
Cricosphaera carterae	2.89	4.56×10^6	
Isochrysis galbana	3.17	5.53×10^6	
Amphiprora paludosa	0.91	8.37×10^4	Fig. A.4.3
Chaetoceros gracilis	0.38	8.41×10^5	Fig. A.4.4
Navicula pelliculosa	3.13	3.33×10^7	Fig. A.4.5
Phaeodactylum tricornutum	1.23	5.43×10^6	Fig. A.4.6
Surirella ovata	1.02	9.51×10^4	Fig. A.4.7
Peridinium trochoideum	0.72	5.00×10^4	Fig. A.4.8
Rhodomonas sp.	2.77	5.73×10^6	
Cyanidium caldarum	1.11	7.65×10^5	
Porphyridium cruentum	2.67	5.33×10^6	

4.4 Discussion And Recommendations

Several algal culture collections have been developed for various objectives, such as a repository for teaching materials (e.g. Carolina Biological Supply Co.) or general basic research organisms (e.g. Starr and Zeikus, 1993), as well as for specific purposes such as those species selected and maintained for their ability to produce and store lipids for possible biofuel production (Barklay *et al*, 1986). The present small collection (Table 4.1) has been assembled to provide the basis of readily available cultures of species that may have desirable properties for successful bioremoval programs and that could be propagated and used in future screening studies.

Algal media and growth conditions have been developed which allow reasonably adequate growth of a large number of species. However, each species of algae is a genetic entity each with its unique optimum growth requirements. Thus, in the initial growth rate experiments, maximum rates and maximum yields expectedly varied greatly among the 18 species (Table 4.3). These results should not be taken as absolute values, as further experimentation might show other outcomes if adjustments were made in media formulation, culture volume, and/or light and temperature regimes . This can be seen when comparing the growth characteristics of *Chlorella capsulata* and *Phaeodactylum tricornutum* in the initial growth experiments with those in the large volume experiments. In large volume cultures (constant light) both species reached maximum density in less than half the time it took in smaller volumes (12 hr L:12 hr D photoperiod), but the yields were substantially higher in the smaller volumes (Compare Appendix Figs. A.4.1 and A.4.6 with A.4.9 and A.4.10). Without further experimentation it is not possible to determine whether it was the volume, the photoperiod, or both that resulted in the observed differences in growth characteristics.

Several of the species had high growth rates and high yields [e.g. *C. capsulata, Dunaliella salina, D. tertiolecta, Scenedesmus acutiformis, S. quadricauda, Cricosphaera carterae, Isochrysis galbana, Navicula pelliculosa, P. tricornutum, Rhodomonas* sp., and *Porphyridium cruentum* (Table 4.3)] and appear to be suitable candidate species for further testing.

Two of the species, *Chlorella fusca* var. *vacuolata* and *P. cruentum*, produced observable amounts of what looked like mucus. The former species is known to produce mucilaginous phytochelation complexes that are effective in sequestering heavy metals (Gekeler *et al.*, 1988). These two species in particular should be examined further to determine the optimum culture conditions for maximizing growth and the synthesis of chelators.

It is obvious that microalgae grown in large volumes would more efficiently produce biomass than when grown in smaller volumes. Numerous species are already routinely grown in large volumes to produce food for humans and hogs (e.g. *Spirulina*), food for rearing larval aquatic and marine invertebrates and fish (e.g. *Chlorella, Cyclotella, Isochrysis, Nitzschia, Prymnesium*, and *Thalassiosira*), and pharmaceuticals (e.g.

Dunaliella). It would be of value to optimize large scale cultivation of algal species that show promise at removing unwanted chemicals from water sources

Salinity has been shown to influence (sometimes dramatically) algal growth (e.g. Pringsheim, 1964). *Navicula pelliculosa* grew at all salinities tested, but grew best at the lower portion of the range (Appendix Fig. A.4.11). This species was originally described from freshwater (Joyce Lewin, pers. commun.), although the strain used in our experiments was isolated from a brackish pond (Robert Guillard, pers. commun.).

The composition of the culture medium provides adequate and balanced amounts of the essential macro- and micronutrients required for growth, and pH controls in part the availability of many nutrients (particularly CO_2 and heavy metals) to the algal cells. Thus, both the composition of the culture medium and the pH ought to profoundly affect growth of microalgae. In the case of *N. pelliculosa,* the choice of media had a greater effect on growth than did pH (Appendix Figs. A.4.12-A.4.13). There are also other indications that some media allow better growth than others. For example, ASP-2 media, an artificial seawater medium commonly used to grow a variety of marine algae was not a good choice for growing *Amphiprora paludosa, Chaetoceros gracilis, Surirella ovata* or *Peridinium trochoideum*, as their growth rates and yields were among the lowest of all the species tested (Table 4.3). Time did not allow us to grow these species in other media.

5.0 CHARACTERISTICS OF THE FOAM USED FOR EMBEDDING

5.1 Introduction

Studying the physical and chemical characteristics of the biomass-embedded foam was of interest for two reasons. First, it was important to know how biomass/foam aggregates would hold up when exposed to harsh conditions such as might be experienced during wastewater treatment. Secondly, we wanted to know what chemical treatment would be necessary should we want to dissolve the spent foam following bioremediation treatment for purposes of metal reclamation and/or waste disposal.

5.2 Methods

The chemical integrity of the foam filter media was investigated by exposing it to concentrated acid, base, and oxidizing solutions. Three experiments were conducted on the plain foam and foam impregnated with three different algae types(*Cyanidium*, *Phaeodactylum*, and *Chlorella)*. No heat was provided to enhance the reactions. The first experiment involved exposing the foam to a variety of acids, bases and oxidizing agents. In the second experiment, plain foam (no biomass) was exposed to 1:1 mixtures of acid or base with hydrogen peroxide. The third experiment involved exposing the three algae impregnated foams to a 1:1 concentrated mixture of H_2SO_4 and H_2O_2.

5.3 Results

Results of the experiments are shown in Tables 5.1a, 5.1b, and 5.1c. Table 5.1a shows the response of the foam and foam/biomass to the concentrated solutions. Concentrated sulfuric acid (94% H_2SO_4) dissolved the foam/biomass completely, but not immediately Sodium hypochlorite (5.6% NaOCl) was also a formidable dissolution agent for the foam. The mixture of 94% H_2SO_4 and 30% H_2O_2 (hydrogen peroxide) dissolved the foam in <5 minutes (Table 5.1b). The reaction generated heat, enhancing the dissolution of the foam. All foams dissolved in <5 minutes when exposed to a 1:1 concentrated mixture of H_2SO_4 and H_2O_2 (Table 5.1c). What seemed like sand particles remained in the solution containing *Phaeodactylum* and *Chlorella.*

5.4 Discussion

It appears that the foam is very resistant to degradation when exposed to hydrogen peroxide or various basic solutions. The foam can be dissolved when subjected to strong acids or hypochlorite solutions. The most effective chemical treatment for dissolving the foam was a combination of H_2SO_4 and H_2O_2 .

Table 5.1a Foam and Algae Impregnated Foam Response

Chemical	Cyanidium	Phaeodactylum	Chlorella	Plain Foam
30% H_2O_2	+1	+1	-	-
50% NaOH	-	-	-	-
28% NH_4OH	-	-	-	-
69% HNO_3	+2	+2	+2	+2
94% H_2SO_4	-	+2	+3	+3
38% HCL	-	+2	+2	+2
85% H_3PO_4	-	+2	+2	+2
5.6% NaOCl	+4	+4	+4	+4

(+) indicating some response. (-) indicating no response.

1. Some initial fizzling and bubbling upon addition of H_2O_2. No apparent degradation of Cyanidium algae-foam after two weeks of observation.
2. Initial degradation activity observed. After two weeks dissolution still incomplete.
3. Initial degradation activity observed. Foam/biomass completely dissolved after two week.
4. Foam/biomass begins to dissolve immediately. Solution becomes clear overnight. A fine white precipitate remains at the bottom of the container after two weeks.

Table 5.1b Response of Plain Foam to Mixture of HNO_3, H_2SO_4, NaOH with H_2O_2

	$HNO_3 + H_2O_2$	$H_2SO_4 + H_2O_2$	$NaOH + H_2O_2$
Plain Foam	+1	+2	-

1. Initial degradation activity observed. Most of foam dissolved overnight, only a few particles remain.
2. Complete dissolution in <5 minutes.

Table 5.1c Response of Algae Impregnated Foam to $H_2SO_4 + H_2O_2$

	Cyanidium	*Phaeodactylum*	*Chlorella*
$H_2SO_4 + H_2O_2$	+1	+2	+2

1. Dissolved in <5 minutes.
2. Dissolved in <5 minutes. A few sand-like particles remain in the bottom of the container.

6.0 BIOREMOVAL CAPABILITIES OF FOAM-EMBEDDED MICROBES

6.1 Introduction

It has been suggested by numerous authors (e.g. Wilde and Benemann, 1993; Gadd, 1990; Volesky, 1990; Macaskie, 1991) that there is considerable potential for development of a cost-effective and otherwise superior processes for sequestering toxic metals from wastewaters by the use of microorganisms, a process known as "bioremoval". Therefore, numerous experiments were conducted in attempt to develop such a process, at least on a bench scale. A process for metal and/or radionuclide removal using a novel biomass immobilization scheme, where the microbes are embedded in a hydrophilic foam, was a principal focus of the work of this TTP in FY 1994 and most of FY 1995. Despite an expansion of the scope of the project to include the biodegradation of toxic organic compounds (see Sections 7 and 8), process development for the bioremoval of metals and radionuclides remained a principal objective throughout the study until it was concluded at the end of March 1996.

6.2 Methods and Materials

This research involved an attempt to develop a novel process and thus involved a large number of unknowns. Initial attempts to develop a highly detailed experimental plan for the entire project (TTP) in an *a priori* manner proved to be of limited value. We found it necessary to make continuing modifications to the research plan and develop detailed step-by-step procedures experiment by experiment. Principal factors that went into the design of a given experiment included the results of previous experiments, the number of algae/foam samples to be prepared and the number of samples to be subjected to chemical analysis. These later two factors required consideration because they each represented specific costs that were built into the budgeted costs of work scheduled (BCWS) for the TTP and could not be exceeded without additional funding. A total of 14 bioremoval experiments were conducted during the study. These experiments and their results are described in detail in Appendix 6. An overview of the experimental procedures is provided here.

6.2.1 Biomass Types

Table 6.1 lists biomass types and sources as well as the experiments in which they were used. The blue-green algal strains *Mastigocladus* and *Nostoc* were grown in ND Media (Castenholz 1982). The green alga *Chlorella* was grown in Bold Basal Medium (Nichols and Bold 1965). The red alga *Cyanidium* was grown in Doemel's *Cyanidium* medium (Carolina Biological Supply, 1978), and the diatom *Phaeodactylum* was grown in F/2 medium (Guillard and Ryther, 1962). The bacteria , *Burkholderia* and *Pseudomonas*, as well as the two yeast strains, #14 and #42, were all grown in *Pseudomonas* medium (Atlas 1993). Antifoam A was added as required to minimize foam formation by bacteria and yeasts. All algal species were grown in aerated, 4-l

Table 6.1 Microbes used in Metal Removal Studies

Microbial Strain	Source	Experiments
Algae		
Mastigocladus laminosus #113	Isolated from SRS	2,4,5,9,10,11,12,13,14
Phaeodactylum tricornutum	Univ. SC (see Sect. 4)	3,5,8,9
Cyanidium caldarium	Carolina Biological Supply Co.	7,8,9,10,11,12,13,14.
Chlorella pyrenoidosa	same as above	8,9,10,11,12
Nostoc sp.	same as above	13,14
Bacteria		
Pseudomonas aeruginosa	ORNL (Dr. Faison)	13,14
Burkholderia cepacia G-4	Univ. W. Florida (Dr. Shields)	13,14
Fungi		
Yeast strain #R-14	Georgia State Univ. (Dr. Crow)	13,14
Yeast strain #R-42	same as above	13,14
Higher Plants		
Azolla Sp.	Frisby Technologies	13,14
Datura Sp.	same as above	13,14

bottles with 1% CO_2 enhancement, under continuous illumination of 300 $\mu E/sec/m^2$. Bacteria and yeast were grown similarly but without the lighting and CO_2. The thermophiles, *Mastigocladus* and *Cyanidium*, were grown at 45°C. All other microbial strains were grown near room temperature (20-25°C). All strains were harvested by centrifugation after they had reached stationary phase growth.

6.2.2 Embedding Procedures

Following harvesting from the culturing systems described above, the organisms were washed in deionized water (and/or other chemicals in the case of Experiment #11), and resuspended to a slurry concentration of approximately 10% dry weight of biomass per slurry. The slurry was analyzed with a moisture meter for solids concentration. Then, biomass slurry, surfactant, prepolymer and DI water were mixed in amounts calculated to obtain 10% biomass, by dry weight, in the completed foam product. Foam production was done at room temperature and the foam was ground and sieved prior to being used in metal removal experiments.

6.2.3 Laboratory Testing Apparatii

Most of the experiments (Exps.# 1,2,3,4,9,10,11,12,&14) were conducted using bioreactor systems developed by Frisby Technologies (see Appendix 6B) . However, a few experiment were conducted using shake flasks and/or Bio-rad columns in an attempt to elucidate the cause of poor results in some of the bioreactor experiments (Exps. # 5,6,7,8) or to evaluate radionuclide uptake(Exp.# 13).

6.2.4 Chemical analyses procedures

With the exception of Experiment 13, where Tc-99 concentrations were determined using a Packard-Tri-Carb 20-50A liquid scintillation spectrometer, concentrations of Fe, Al, Cr, Ni, and in some cases other toxic metals, were detected by inductively coupled plasma spectroscopy. Either ICP-ES or ICP-MS analyses were made Depending on the concentration of metal in the wastewater and the desired level of detection necessary to determine if significant bioremoval was occurring . For example, Fe and Al were analyzed by ICP-ES, while Cr and Ni were analyzed by ICP-MS.

6.3 Results

Despite the inherent positive factors in the conceptual design (e.g. we had shown in earlier unpublished work that algal biomass can typically remove >90% of toxic metal from mock waste solutions spiked with metal standards), our success in providing a bench scale demonstration was very limited. A brief summary of the results from all of the experiments appears in Table 6.2. Details of the 14 experiments, listed in

Table 6.2 Summary of Metal Removal Experiments

EXP. No.	TYPE OF EXPERIMENT	SUBJECT	OBJECTIVE	Results
1a	Use of Frisby laboratory test rig with D-Area coal pile run-off basin water	startup	Evaluate new lab test rig with wastewater but no filter media to evaluate the integrity of the test system in terms of: (1) leaching of metals from test system,(2) plating of metals on test system, and (3) uniformity of columns	No significant leaching or plating. Good uniformity among columns
1b	same as above	particle size (no algae)	Evaluate effect of foam particle size on metal uptake and flow dynamics (no algae)	No significant differences detected
2	same as above	particle size w/ algal species #1	Evaluate the effect of particle size on metal uptake and flow dynamics with an algal - foam aggregate containing 6% *Mastigocladus*	No significant metal removal with any particle size
3	same as above	particle size w/ algal species #2	Evaluate the effect of particle size on metal uptake and flow dynamics with an algal - foam aggregate containing 6% *Phaeodactylum*	No significant metal removal with any particle size
4	same as above	effect of algae/foam quantity	Determine effect of the quantity of algae/foam (0,2,8,12 &16 g/column of foam embedded with 6% Mastigocladus by dry wt.) on bioremoval efficiency	No significant metal removal by any amount tested
5	Incubation of algae/foam in shake flask with D-CPRB water	effect of algae/foam bioremoval in shake flasks	Determine bioremoval of foam plugs imbedded with Mastigocladus and Phaeodactylum in shake flask 1g foam per 50 ml D-CPRB water	No significant metal removal
6	Pretreatment of D-CPRB	Testing effect of pH adjustments and aeration on metal content of waste water	Evaluate the change in metal concentrations of D-CPRB water following pretreatments including aeration and pH adjustments to 3,4,5,& 6 from ambient pH of 2.5	Aeration was insignificant. Concentrations of metals greatly reduced with pH increases. Some metal still above regulatory limits at pH 6.
7	Mini-columns	Bioremoval of pretreated waste water and metal standards: Test #1	Evaluate the bioremoval efficiency of foam containing algae (6% Cyanidium) or no algae with foam granulated (8 mesh) or not granulated (plug) with D-CPRB water adjusted for pH 5 or ambient (pH 2.5) and with metal standard solutions containing 0.8 ppm Ni and 1.5 ppm Zn adjusted to pH 2.5 and 5.0	No significant removal at either pH from runoff. Enhanced Ni and Zn removal from test solutions at pH 5. Solid foam less effective than granular foam.

Table 6.2. Cont.

EXP. No.	TYPE OF EXPERIMENT	SUBJECT	OBJECTIVE	Results
8	Mini-columns	Bioremoval of pretreated waste water and metal standards: Test #2	Determine reasons for the poor performance of foam-algal aggregates with coal runoff compared to standard metal solutions in previous experiment. Compare pretreated D-CPRB water and a mixture of metal standards containing Al, Be, Cd, Co, Cr, Cu, Fe, Ni, and Zn at levels similar to that of the runoff. Also test single-metal solutions of Ni and Zn and water collected from a monitoring well below a CMP pit (CMP-13). Use granulated algae/foam aggregates and modify mini-columns for reduced flow rate to increase contact time. Algae were Phaeodactylum and Chlorella.	Phaeodactylum and Chlorella removed more metal from pH 5 runoff and Ni, Zn solutions than did Cyanidium, but Ni and Zn remained above water quality criteria. More removal from mixed metals than runoff, but less than from single metal solutions
9	Modified test rig	Bioremoval of contaminated ground water by various algal strains	Test metal removal efficiency by passing metal contaminated ground water through columns containing foam-algae aggregates comprised of four different algal strains (*Cyanidium*, *Mastigocladus, Phaeodactylum* and *Chlorella).* Measure Ni, Al, Cr, and Fe before and after treatment.	Significant removal of Al and Fe by all algae. Also significant removal of Cr by *Chlorella* and *Cyanidium.* No Ni removal by any alga.
10	Modified test rig	Bioremoval of contaminated ground water by various algal strains	Metal Removal of water from Well DC-4A using *Cyanidium*, *Mastigocladus,* and *Chlorella* in the modified Frisby test bed. (Repeat of Exp. #9, except *Phaeodactylum* deleted). Attempt to verify removal indicated in Exp. 9	No significant removal with any of the algal/foam aggregates, relative to controls.

Table 6.2 Cont.

EXP NO.	TYPE OF EXPERIMENT	SUBJECT	OBJECTIVE	RESULTS
11	Modified packed bed bioreactor	Bioremoval of metal - contaminated ground water by various algal strains subjected to various pretreatments	Metal Removal of water from Well DC-4A using three types of algae (*Cyanidium*, *Mastigocladus,* and *Chlorella)* and six biomass pretreatment schemes with the modified Frisby test bed.	Limited removal with any algae and pretreatment combination. Best results with acid pretreated *Cyanidium*
12	Modified packed bed bioreactor and static mixer bioreactor	Bioremoval of contaminated ground water by the use of two bioreactor configurations	Compare packed bed vs. static mixer bioreactors for removal of Cr, Ni, Fe, and Al by two algal species	Leaching of metals from pump of static mixer. No significant removal with packed bed
13	Mini-columns	Bioremoval of a radionuclide in spiked DI and river water samples	Compare Tc-99 removal by algae (2 spp), fungi (2 spp), bacteria (2 spp), higher plants (2 spp) and ion exchange resin	Best biomass results with *Cyanidium*, but far inferior to resin
14	Modified packed bed bioreactor	Bioremoval of contaminated ground water by the use various types of algal and non-algal biomass	Compare Ni, Cr, Al, and Fe removal by algae (2 spp), fungi (2 spp), bacteria (2 spp), and higher plants (2 spp).	Best bioremoval with algae and bacteria but less than 25% removal of any metal with any type of biomass

numerical and chronological order can be found in Appendix 6 along with descriptions of the test rigs provided by our industrial partner Frisby Technologies.

Metal removal experiments with algae provided inconsistent results with metal removal efficiency seldom exceeding 25% removal under any test conditions. Studies comparing algal, bacterial, fungal, and higher plant biomass demonstrated that other biomass sources were also ineffective for metal bioremoval under the test conditions. Radionuclide bioremoval using a Tc-99 source provided more promising results than the metal removal studies with the various types of biomass, and indicated that the alga *Cyanidium* (~35% removal) was the best of the tested sources of biomass for this application. However, all of the biomass/foam aggregates tested were substantially inferior to a TEVA resin (>95% removal) for removing Tc-99 in comparative testing.

6.4 Discussion

Three potential reasons for our lack of success in converting the conceptually designed process into an effective bench-scale process for metal removal included the following:

(1) interference in biomass-metal binding in "real" wastewater relative to "simulated" wastewaters,

(2) inappropriate wastewater for testing, and

(3) inadequate bioreactor systems

Numerous authors have described interference by one or more metals in the binding of others (Ting et al., 1989; Ting et al., 1991a,b; Harris and Ramelow, 1990. Non-metallic constituents can also interfere with metal binding and it was not surprising that we were able to obtain higher removal efficiencies using mixed metal standard solutions than with real wastewater solutions containing similar metal concentrations. In addition, we observed higher metal removal for a given metal using single metal solutions than with mixed metal solutions with the same amount of the given metal. Thus, as is usually if not always the case, simulated wastewater proved easier to clean up than real wastewater.

Despite an intensive search at SRS (see Section 3), we found a paucity of wastewater sources having: (1) toxic heavy metal concentrations in need of remediation, and (2) a lack of other contaminants that necessitate the use of special precautions for study (i.e. additional laboratory training and procedural controls). The coal pile runoff basins and their underlying groundwaters were the only waters that fit these two criteria. Unfortunately, these waters were far from ideal for biosorption due to very low pH (typically ca. 2.5) and extremely high iron and/or aluminum concentrations. Iron concentrations ranged from 79 to 1780 ppm in the D-CPRB basin water samples, but were < 1 ppm in the samples from monitoring well DCB-4A. Al concentrations in the raw wastewater ranged from 64-470 ppm in basin water samples and from 10-64 ppm in the well water (DCB-4A) samples. The only other wastewater included in the metal removal experiments was a sample from Monitoring Well CMP13, representing ground

water below a former chemical, metals, and pesticide (CMP) disposal site. This water, while relatively low in Fe and Al, was also only slightly above the very low drinking water limits (see Appendix Table A.3.10 for a few toxic metals including Be, Cd, and Cr. Thus, experiments designed to detect substantial metal removal, relative to controls, were virtually impossible with this source.

Another problem that cropped up during the course of the study was inadequacy of our bioreactor systems. These systems were developed on a fixed-cost subcontract basis, which for the most part, precluded modifications to the systems after experimentation had commenced. The first bioreactor (B.E.S.T.) (Appendix 6.B) proved to be sub-optimal for adequate contact between biomass and wastewater because the flow rates could not be maintained below 0.3 gpm, which appeared to be excessively high for good metal binding with the biomass. The size of the chambers holding the biomass also proved problematic in that our algal production capability for filling a chamber was taxed to provide sufficient biomass in a timely manner. Results improved somewhat when the bioreactor system was modified to accommodate lower flows and smaller columns for housing the foam/biomass aggregates. However, it is suspected that this modification resulted in a suboptimal ratio of biomass per unit wastewater. The maximum amount of biomass that could be packed in a column was ca. 0.4g dry weight biomass and the minimum amount of wastewater necessary to maintain system flow was about 1-liter. Thus, with this bioreactor system, we were not able to use the biomass to wastewater ratio of 1.6 mg/ml used in the earlier work where high(>90%) removal percentages were obtained with solutions spiked with metal standards (unpublished data). A static mixer bioreactor provided confounding results when it became apparent that metals such as Cr, Fe, and Ni. were being leached into the system by metallic components of the bioreactor pumping system .

In conclusion, future research in a quest for a bioremoval process using biomass embedded in foam should focus on the selection of appropriate wastewaters which would preferably be of near neutral pH and have higher concentrations of the target metals (those especially desired to be removed) than non-target (e.g. relatively innocuous metals of little value) constituents. It will also be necessary to develop bioreactor systems, containing all non-metallic components, that can maximize contact between the embedded biomass and the metal ions targeted for removal.

7.0 POLYURETHANE FOAM IMMOBILIZATION OF TCE-DEGRADING BACTERIA: ENTRAPMENT EFFECTIVENESS AND INFLUENCE ON METABOLIC ACTIVITY

7.1 Introduction

The immobilization of bacteria in artificial matrices as a means of exploiting their metabolic activities is receiving increasing attention by the biotechnology industry. One application of this technology relates to the production of chemicals in continuous systems or bioreactors. For example, immobilized fungal cells have been used for the production of cellulases and xylanases (Haapala et al., 1995), while immobilized yeast cells have been used to produce ethanol (Tanaka et al., 1986). The advantages of this approach over the classical fermentor/chemostat approach are the higher microbial densities and greater biomass retention attainable in immobilized systems.

Immobilized degradative bacteria are also potentially useful in the *ex situ* treatment of hazardous chemicals (Levinson et al., 1994). The transformation of phenol, p-nitrophenol, pentachlorophenol, p-cresol, and other compounds by immobilized microbial cells has been documented (e.g. Bettmann and Rehm, 1984; O'Reilly and Crawford, 1989a). Embedding media can protect immobilized cells against chemical toxicity by absorbing toxic compounds. For example, *Flavobacterium* sp. was capable of transforming pentachlorophenol (PCP) when immobilized in agarose beads but not as free cells (O'Reilly and Crawford, 1989b).

Entrapment or encapsulation appears particularly attractive in cases where the degradative bacteria are poorly adhesive and hence are not suited for use in bioreactors. One such organism is *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) G4, which degrades trichloroethylene (TCE) and several other compounds. Due to its poor attachment to surfaces, G4 is generally washed out from bioreactor systems. In one case, it was replaced by native microbes within days after a trickling filter reactor was opened to water from a contaminated aquifer (Berry, unpublished results). Partly because of this problem, G4 has not achieved widespread use for the *ex situ* treatment of TCE contaminated groundwaters.

Numerous techniques for immobilizing bacteria have been evaluated, most of which can be classified as either entrapment or absorption methods (Woodward, 1988). Immobilizing agents include polyacrilamide beads, agarose beads, alginate beads, carrageenan beads, clay, granular activated carbon, and polyurethane foams (Levinson et al., 1994). Although many factors will affect the success of an immobilization technique in a biotechnological application, the immobilization or entrapment efficiency and the material's effect on microbial activity are crucial. The ability to retain cells in the immobilization medium during exposure to flowing water will undoubtedly affect the effectiveness and functional lifetime of the material. In addition, the immobilization technique should not impair metabolic activities required for the desired application. Examination of these factors is therefore important when evaluating immobilization techniques.

In this study, we evaluated the use of a novel hydrophilic polyurethane based foam for the immobilization of *B. cepacia*. Previous workers have noted difficulties in achieving satisfactory entrapment of bacteria in polyurethane. Therefore, we first examined the effects of foam formulation (surfactant type and concentration, embedding temperature, presence of binding agents, and biomass density) on entrapment efficiency. Secondly, we explored the viability and physiological status of embedded cells, using colony counts, the most probable number (MPN) technique, respirometry, and 16S rRNA targeting oligonucleotide probes. Our objective was the identification of embedding conditions which allow effective entrapment of a metabolically active bacterial population.

7.2 Materials and Methods

7.2.1 Bacterial strains and growth conditions.

The strain *Pseudomonas* (*Burkholderia*) *cepacia* G4 and the constitutive mutant PR131 were kindly provided by M. Shields (University of West Florida). To assure culture purity, original stocks were kept at -70^{o}C. Prior to each experiment, stock material was spotted onto PTYG plates (Appendix 7.1) to provide inoculum for batch cultures. Axenic batch cultures were grown in *Pseudomonas* medium (Atlas, 1993) or a yeast-glucose medium (YGM) (Shields and Reagin, 1992), which consisted of basal salts medium (BSM)(Shields et al., 1989) plus 1 g/l glucose and 0.5 g/l yeast extract (Appendices 7.2, 7.3). Small-scale (100 - 250 ml) batch cultures were grown in shake flasks (200 rpm, 30^{o}C). Larger amounts of axenic biomass were grown by inoculating 20 ml of 1- 3 day old culture into 4000 ml polycarbonate bottles containing 3000 ml YGM or *Pseudomonas* medium. These cultures were maintained at 26 ± 2^{o}C and aerated through a sterile 0.2 μm filter to provide mixing and O_2. Cultures were routinely harvested for foam embedding after 3 days' growth, at which time biomass yield was about 0.5 g dry weight /l. For experiments requiring the induction of the toluene monooxygenase gene (Shields et al., 1989), G4 cells were exposed to 2 mM phenol for 2h prior to harvesting. Harvesting was by centrifugation (10,000 rpm, 10 min, 15 - 25^{o}C). Pellets were resuspended in BSM or *Pseudomonas* medium to a density of 2.2 - 17.7% dry weight. Aseptic technique was not used during the harvesting process.

7.2.2 Immobilization of bacterial cells.

Bacteria were routinely embedded in hydrophilic polyurethane foam within 24 h after slurry preparation. Colony counts indicated that storage of up to one week did not result in viability loss (data not shown). The foam samples were prepared by Frisby Technologies (Freeport, NY) at their Aiken, SC facility. Ingredients of the foam were: slurry, 20 g; prepolymer, 13.33 g; surfactant, 0.54 g. Thus, a 5% (dry wt) slurry would yield approximately 3 g dry wt bacteria per 100 g wet wt foam. Three surfactants were compared in the course of the study. These were HS-3 (lecithin-based), F-88 (a pluronic surfactant consisting of a mixture of ethylene oxide and propylene oxide), and

DC198 (a silicone-based surfactant). The prepolymer Bipol 6B (NCO=6) was routinely used, with three other prepolymers of lower NCO values (Bipol 3, #350, #802) being used for comparison purposes in viability studies. The addition of silane as a binding agent was tested in certain foam formulations. Prepolymers, surfactants, and silane were provided by Matrix, Inc.

Control (cell - free) foams were generated by substituting 20 g medium for bacterial slurry. Temperature was not controlled during the reaction, but did not exceed 42^{o}C. Foam samples were reduced to a particulate state by means of a Waring blender and stored at ca 4^{o}C prior to use.

7.2.3 Washout experiments.

Entrapment efficiency was measured using 10 ml Poly-Prep chromatography columns (Bio-Rad Laboratories) modified by the replacement of the stock fritted disk with 75-80 mg glass wool. Duplicate columns were loosely packed with 2.0 ± 0.01 g (wet wt) of each foam type. Cell retention by various foam types was routinely compared by passing 50 ml of autoclaved, 0.2 μm filtered deionized water through each column (gravity feed) and collecting the effluent. Effluents were preserved with 0.2 μm filtered formalin (3.7%) and their bacterial content was determined by direct counts. Certain foam formulations were retested using larger volumes of deionized water. In one experiment, 1000 ml water was passed through duplicate columns in 50-ml aliquots, with 50-ml samples of effluent being collected when cumulative water addition had reached 50, 150, 400, 550, 700, 850, and 1000 ml (intervening effluent aliquots were discarded). A second experiment involved passage of 2000 ml through duplicate columns, with the accumulated effluent being sampled when cumulative water addition totaled 50, 1000, 1500, and 2000 ml. Experimental parameters used in washout experiments are summarized in Appendix 7.4.

7.2.4 Viability, total cell numbers, and activity measurements.

To evaluate the effect of foam polymerization on the viability of immobilized cells, serial dilutions of immobilized and slurry cells were inoculated onto PTYG plates and incubated at 30^{o}C until stable colony numbers were obtained. Cells in the foam were released by vigorous vortexing (30 secs) prior to dilution and plating. In one experiment, we used the Most Probable Number (MPN) technique to measure culturability in liquid media. Percent viability was calculated from the ratio of CFU or MPN to total cell numbers, determined by a modification of the Acridine Orange direct count technique (Hobbie et al., 1977). Appropriate dilutions were spotted onto heavy teflon coated slides (Cel-Line Associates, Inc., Newfield, NJ), heat fixed, and stained with 0.01% of acridine orange for 2 min at room temperature. Excess stain was removed with 0.2 μm filtered nanopure water. Slides were allowed to air dry and observed under a Zeiss Axioskop epifluorescent microscope (filter set 09) using a 100X objective. Viable counts and direct microscopical counts were done in duplicates or triplicates, with at least 20 microscope fields being counted per sample. Experimental parameters used in viability tests are summarized in Appendix 7.4.

To determine the effect of immobilization on physiological activity of embedded cells, rates of CO_2 evolution and O_2 uptake were measured using a Micro-Oxymax v5.12 indirect closed circuit respirometer (Columbus Instruments, Columbus OH). Triplicate samples consisted of 8 g of foam-embedded bacteria or the equivalent number of cells from the bacterial slurry (5 ml). Cells were incubated at two different temperatures (20°C or 25°C) with or without agitation (130 rpm) to compare the effect of temperature and oxygen availability on the respiration rates of immobilized vs. slurry cells. Experimental parameters used in respirometry studies are listed in Appendix 7.5.

In one experiment, carbon sources (0.1% glucose, 0.05% yeast extract) were added and ribosomal probes utilized to elucidate the effect of embedding on metabolic activity. Immobilized and slurry cells were transferred to BSM, carbon sources were added, and the suspensions were incubated at room for 24h. Aliquots were taken after 2, 4, and 18 h, fixed with 3.7% formaldehyde (final concentration) and stored at 4°C for 24 h. Cells were centrifuged to remove formalin, resuspended on 0.2 μm filtered nanopure water, fixed onto slides and hybridized with a fluorescently labeled 16S rRNA targeting probe in an Autoblot hybridization oven (Bellco Glass, Inc., Vineland, NJ) following the procedure of Braun-Howland et at. (1992). The probe binds to all eubacteria and is complementary to the 342-360 region (essEscherichia coli asp). Fluorescing cells were observed by epifluorescence microscopy using a Zeiss Axioskop microscope and a Zeiss 15 filter set.

7.3 Results

7.3.1 Washout experiments.

A list of the different formulations evaluated in washout experiments is presented in Table 7.1. Foam 1 (made with 1% F-88) resembles the formulation used in previous chapters to immobilize algal cultures, while Foams 2 - 8 each vary from it in terms of one characteristic (bacterial density, surfactant type, surfactant concentration, embedding temperature, or presence of silane). As shown in Fig. 7.1, decreasing the concentration of F-88 from 1% to 0.5% or increasing it to 10% did not reduce bacterial washout (in terms of the percentage of total embedded bacteria removed by passing 50 ml deionized water through 2 g foam). In fact, increasing the concentration of surfactant considerably increased washout, possibly by increasing the number and size of interconnected, bacteria-containing pores in the matrix.

Table 7.1 Foam Formulation Used to Test Washout of *B. cepacia* PR131

Foam no.	% slurry	Type of surfactant	Surfactant concentration	Temperature of embedding[a]	Silane addition
1	8.10	E88	1%	RT	No
2	17.70	E88	1%	RT	No
3	8.10	DC198	1%	RT	No
4	8.10	HS-3	1%	RT	No
5	8.10	E88	0.5%	RT	No
6	8.10	E88	10%	RT	No
7	8.10	E88	1%	Cold	No
8	8.10	E88	1%	RT	Yes
10[b]	17.70	HS-3	1%	RT	No
11	8.10	HS-3	1%	RT	No
12	8.10	HS-3	1%	RT	Yes
13	17.70	HS-3	1%	RT	Yes

[a] Temperature at which the embedding process was performed. RT = room temperature (22±2); Cold = iced water bath.
[b] Foams 10, 11, 12, and 13 represent a separate experiment.

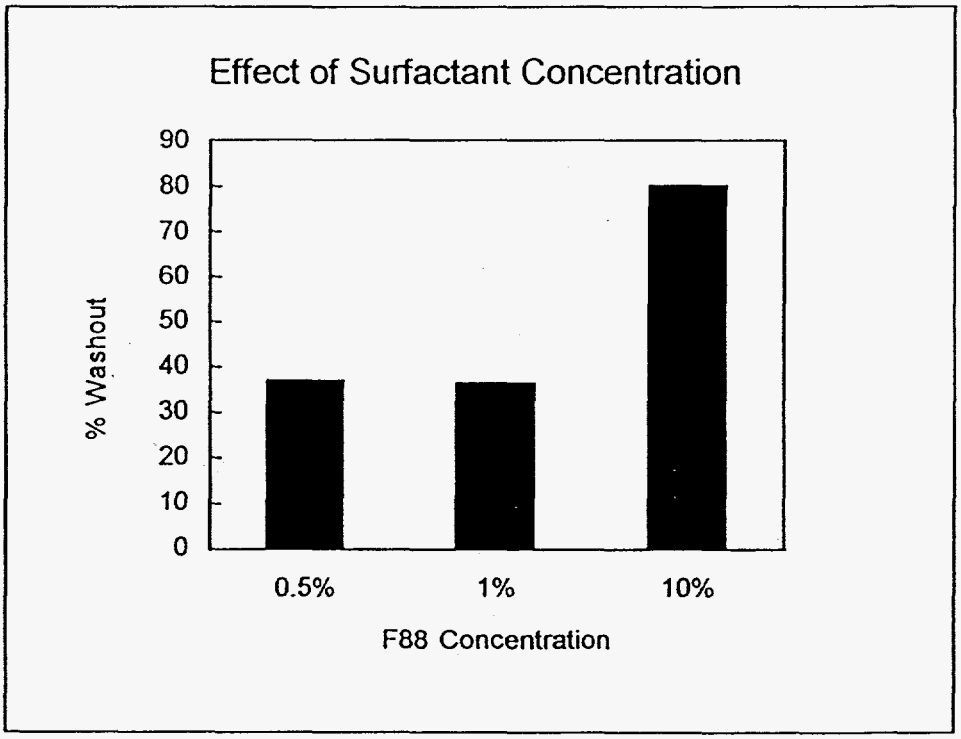

Fig. 7.1. Effect of surfactant concentration. Values represent mean percentages of total embedded PR131 cells removed from duplicate columns containing 2 g foam during passage of 50 ml water.

Bacterial washout declined somewhat (from 36% to 25%) when embedding was performed in the cold (Fig. 7.2). Adding silane to an F-88-containing foam also had a beneficial effect (Fig. 7.2) . However, even greater retention of bacteria (i.e., less than 8% washout) was obtained when the concentration of bacterial biomass in the slurry used for embedding was increased from 8.1% to 17.7% (Fig. 7.3).

Fig. 7.4 shows that surfactant type had a major effect on bacterial retention. Surfactant HS-3 was more effective than either DC198 or F-88, when equal levels of the three compounds were used. Based on results obtained with Foams 1-8, we selected HS-3 (1%) for use in all subsequent foam batches used in the study.

Using Foams 10-13 (Foam 9 was a cell-free control and is not included in this report), we retested the effects of biomass concentration and silane in foams made with HS-3, since both factors seemed to reduce washout from foams made with F88. Results are summarized in Fig. 7.5. Increasing bacterial biomass to approximately 18% reduced washout from nearly 6% (Foam 11) to less than 2% (Foam 10). This confirms our prior results with F88. Silane addition gave inconsistent results at the two biomass densities (Fig. 7.5). For this reason, silane was not used in subsequent foam batches.

Foams 10-13 were also used to examine washout during passage of larger volumes of water (Fig. 7.6, 7.7). In the experiment shown in Fig. 7.6, the first 50 ml of water liberated 0.2%, 4.5%, 5.5%, and 1.1% of the total embedded cells from Foams 10, 11, 12, and 13. These initially low values declined even further with subsequent aliquots of water. Similar results are shown in Figure 7.7. Foams 10, 11, 12, and 13 released 0.1%, 5.3%, 9.7%, and 0.6% of total embedded cells into the first 50 ml of water. Passage of 2000 ml water liberated 0.2%, 13.9%, 16.2%, and 6.0% of the total embedded cells. Results of these tests thus serve to validate our use of 50 ml volumes for routine comparisons between foams, since initial washout rates are highest and provide a reasonable means of predicting the comparative performance of foams subjected to larger volume washout tests.

7.3.2 Viability experiments.

Immobilization in polyurethane foam (made with 1% HS-3, no silane) caused *B. cepacia* PR131 to undergo a drastic decline in culturability on solid media (Fig. 7.8). The number of colony forming units dropped to 0.006 % of total cell numbers, a decrease of nearly five orders of magnitude. Dramatic decreases in culturability were also observed for other environmental isolates as well as for a mixed community previously enriched on chlorobenzene minimal salts media (Fig. 7.8). Colony counts performed using Foams 1-13 indicated that culturability was severely impaired by all the foam formulations shown in Table 7.1 (data not shown).

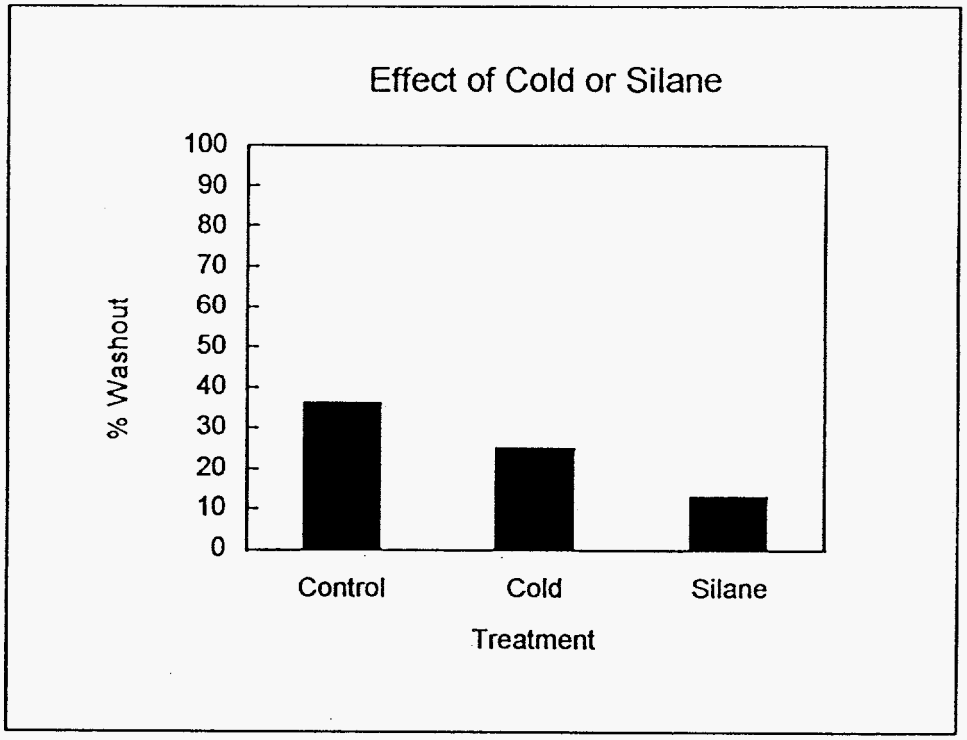

Fig. 7.2. Effect of cold or silane. All foams were made using an 8.1% slurry and 1% F-88 as a surfactant. Cold embedding was performed using an ice bath, while control and silane-containing foams were prepared without temperature control. Values represent mean percentages of total embedded PR131 cells removed from duplicate columns containing 2 g foam during passage of 50 ml water.

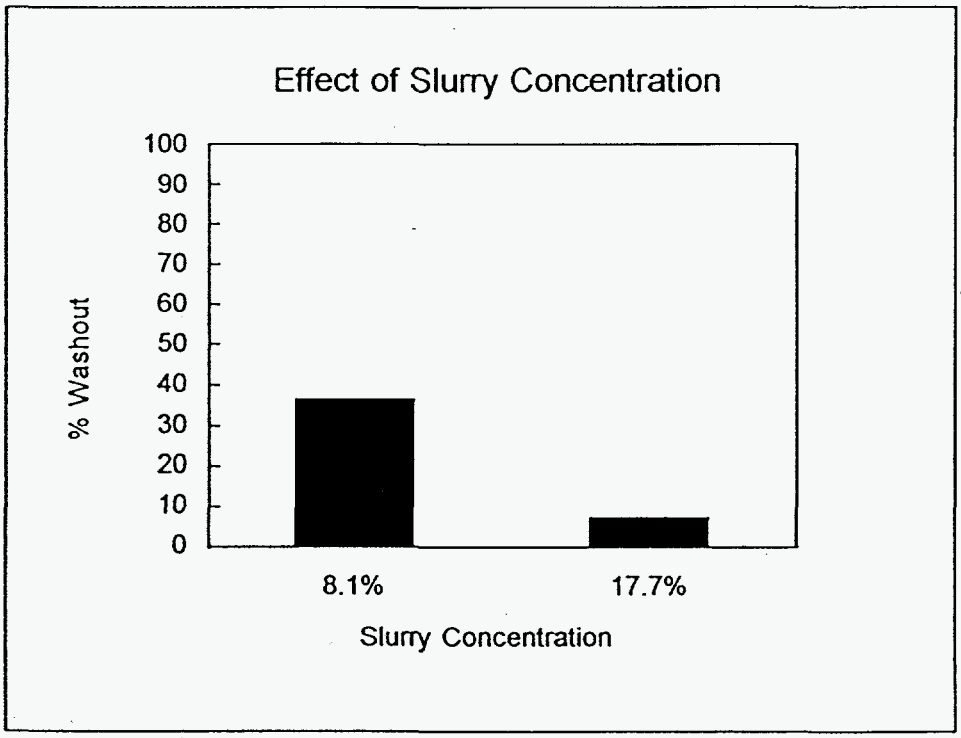

Fig. 7.3. Effect of slurry concentration. Foams were prepared using 1% F-88 as the surfactant and either an 8.1% (dry wt) PR131 slurry or an equal volume of 17.7% (dry wt) slurry. Values represent mean percentages of total embedded PR131 cells removed from duplicate columns containing 2 g foam during passage of 50 ml water.

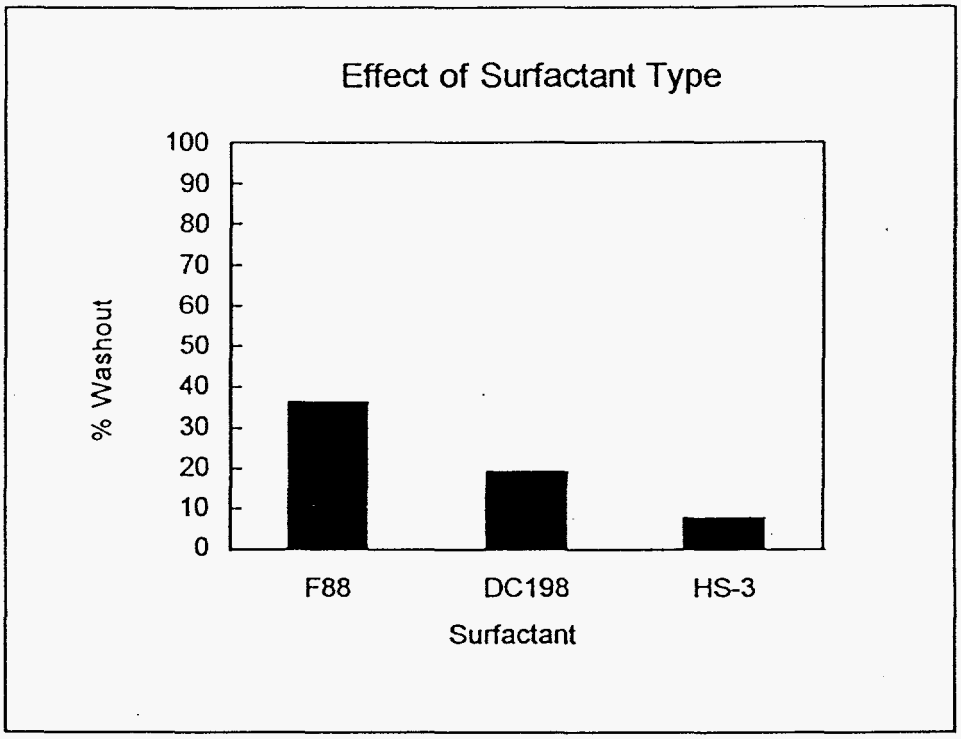

Fig. 7.4. Effect of surfactant type. Foams were prepared using an 8.1% slurry and equal volumes of a 1% solution of each surfactant. Values represent mean percentages of total embedded PR131 cells removed from duplicate columns containing 2 g foam during passage of 50 ml water.

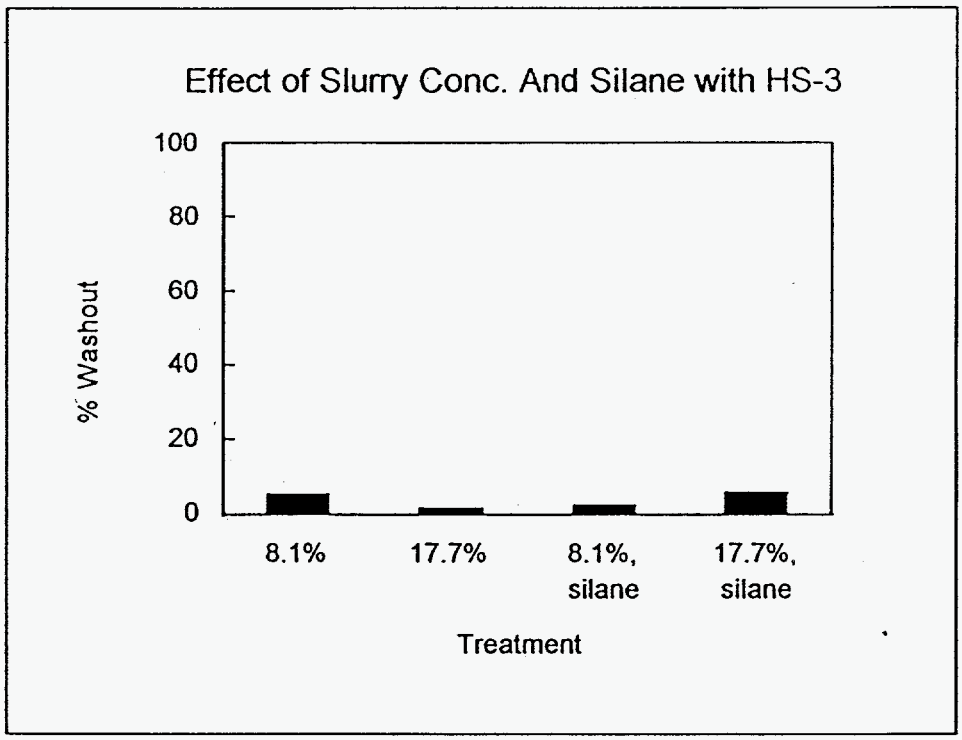

Fig. 7.5. Effect of slurry concentration and silane with HS-3. Foams were prepared using either an 8.1% or a 17.7% slurry, with or without added silane (binding agent). HS-3 (1%) was used as the surfactant. Values represent mean percentages of total embedded PR131 cells removed from duplicate columns containing 2 g foam during passage of 50 ml water.

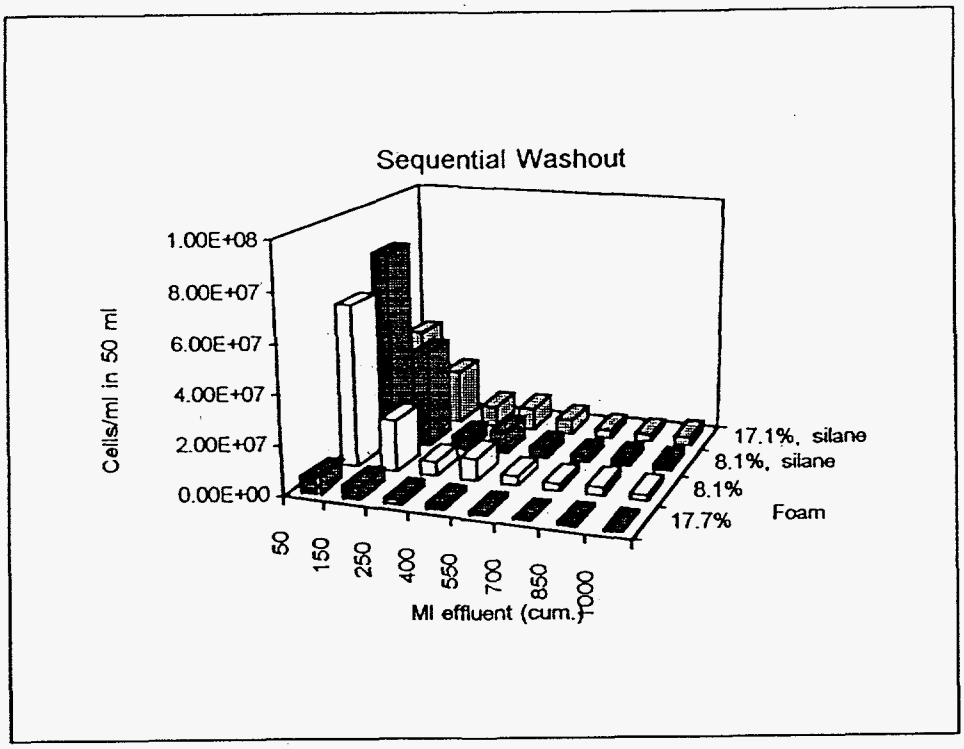

Fig. 7.6. Sequential washout (A). Foams were as described in Fig. 7.5. 1000 ml water was passed through duplicate columns in 50-ml aliquots. Total cell numbers were determined in 50-ml effluent samples collected at the points indicated. Cell content of other aliquots was not determined.

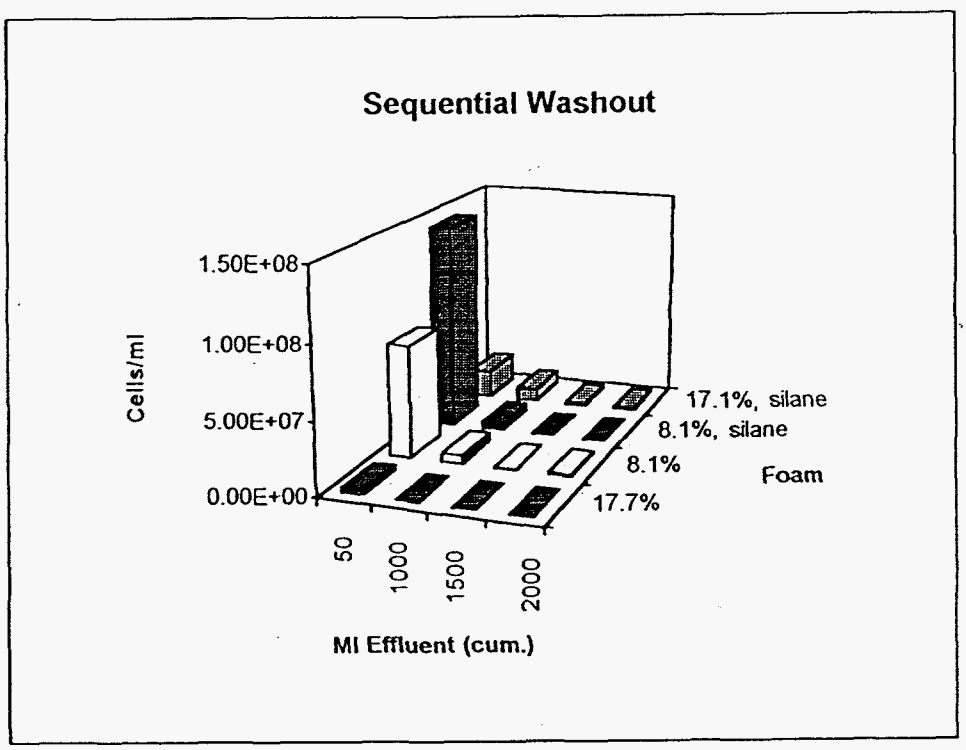

Fig. 7.7. Sequential washout (B). Foams were as described in Figs. 7.5 and 7.6. 2000 ml water was passed through duplicate columns. The first 50 ml effluent was collected, followed by samples containing 950 ml, 500 ml, and 500 ml. Values represent mean cell density (cells/ml) in each sample.

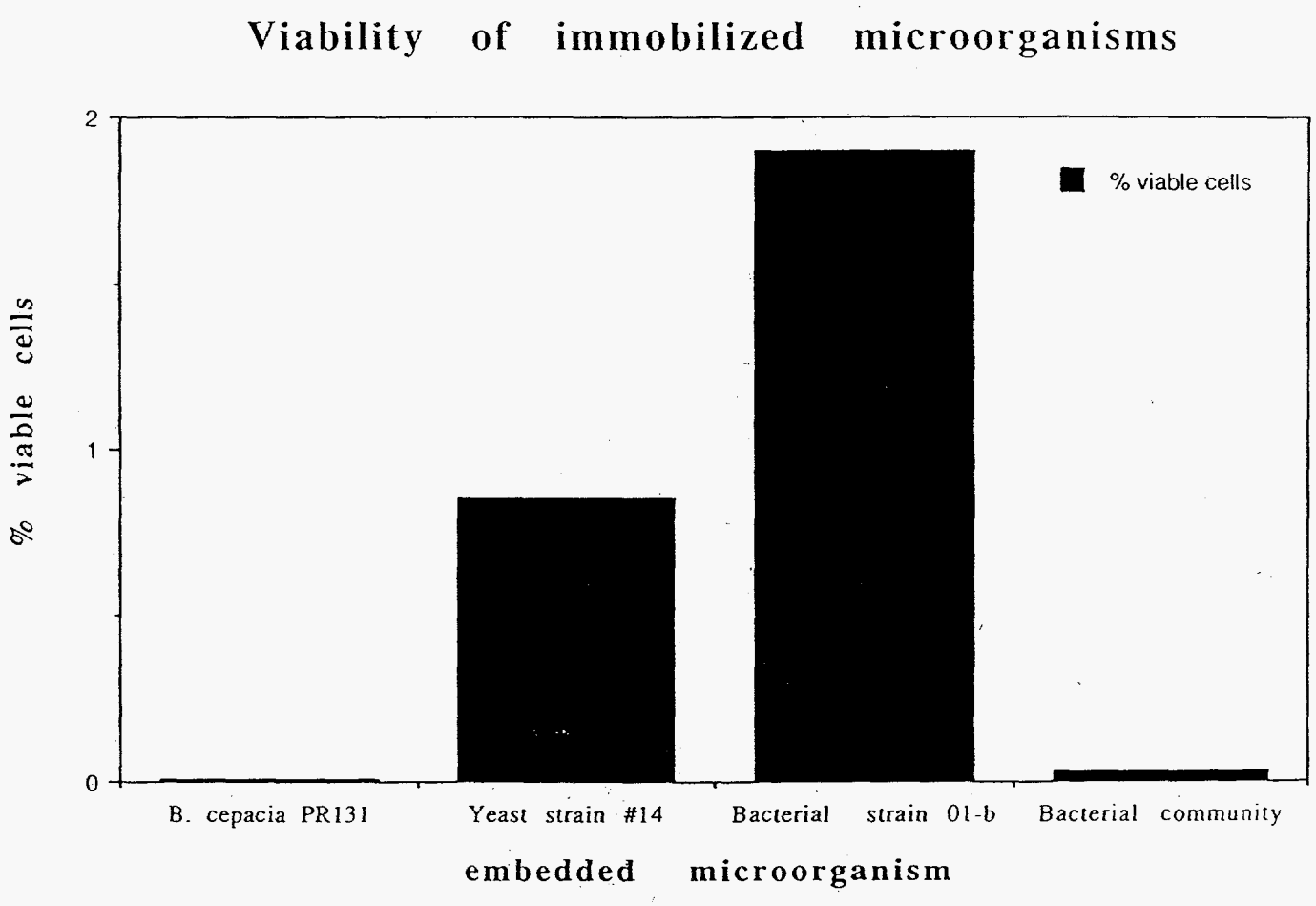

Fig. 7.8. Viability of immobilized microorganisms. Two bacterial strains, a yeast (*Candida* sp.), and a mixed bacterial community were embedded. Colony counts and total cell counts were performed on samples of cells removed from foam by vortexing. Values represent means of duplicate or triplicate samples.

During the polymerization process, initial pH of the reaction mixture is approximately 7; the pH then briefly drops to ca. 5 before returning to ca. pH 7 (P. Hermann, personal communication). We also observed a brief temperature increase to ≤42°C during foam production. To test whether these factors caused viability loss by embedded cells, we exposed PR131 slurries to heat shock (using a 42°C water bath) and a pH $7 \rightarrow 5 \rightarrow 7$ shift (accomplished by adding dilute HCl, then dilute NaOH). A control slurry was maintained at constant (room) temperature and pH 7. Total and culturable cell numbers were then determined for all three preparations . Fig. 7.9 shows that viability of the slurries underwent relatively little change as a result of temperature or pH shock. The effect of temperature on culturability was also tested by carrying out the polymerization reaction in an ice bath to prevent temperature increase (Fig. 7.10). Biomass concentration in slurries used for embedding was also varied to determine whether this factor affected viability. However, culturability of the embedded bacteria was extremely low under all conditions tested (Fig. 7.10).

To test whether embedding simply caused PR 131 to become unable to grow on agar-solidified media, we compared colony counts with results obtained using the Most Probable Number (MPN) technique. Both methods yielded exceedingly low viability estimates (data not shown), indicating that previously embedded cells were unable to grow on either liquid or solid media.

Toluene 2,4-diisocyanate (TDI) is used in the manufacture of polyurethane foams and is suspected to be a carcinogen. Therefore, we tested three additional prepolymers containing reduced TDI levels (ranging from 50% of the TDI content of Bipol 6B down to undetectable levels) in order to determine whether TDI was responsible for viability loss. However, culturability was again very low in all foam formulations tested (data not shown).

7.3.3 Respiration studies.

Since it is possible for bacteria to remain metabolically active but unable to form colonies on artificial media, we investigated the effect of embedding on respiration rates. CO_2 evolution and O_2 consumption rates of embedded *B. cepacia* G4 were compared with those of unembedded bacterial slurries. Surprisingly, embedded cells showed higher respiration activity than unembedded cells (Fig. 7.11). Respiration rates of unembedded cells increased when temperature was increased to 25°C (Fig. 7.12), and remained higher than those of embedded cells even after temperature was again reduced to ambient (20°C) (Fig. 7.13).

We also examined respiration rates of *B. cepacia* G4 which had been exposed to phenol (to induce the oxygenase enzyme responsible for TCE degradation) prior to immobilization. Immobilized cells again respired at higher rates than slurry cells when both populations were kept at 20°C (Fig. 7.14). When cells were incubated at 25°C with or without aeration by 140 rpm shaking, slurries were more active than immobilized cells (Fig. 7.15, Fig. 7.16). However, immobilized cells retained more than 50% of the

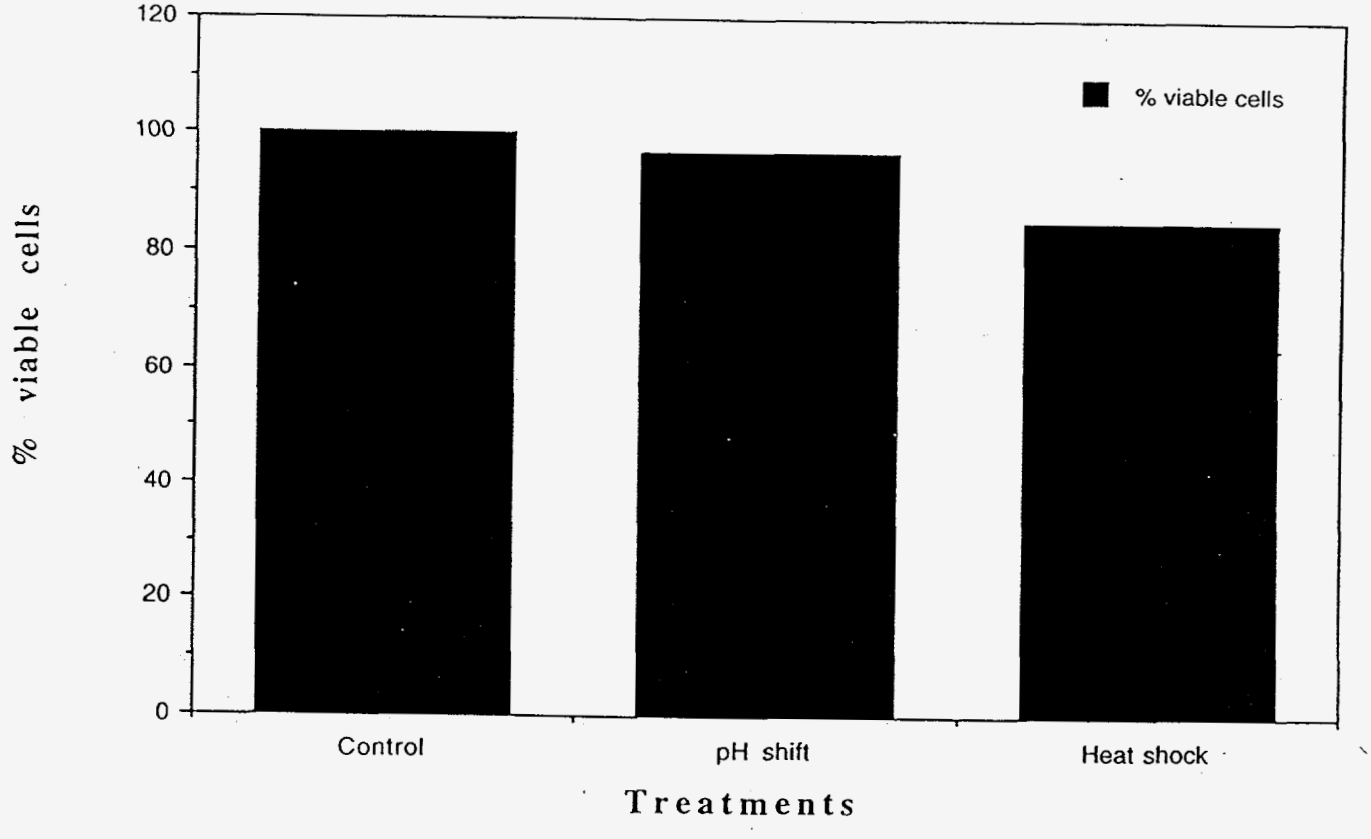

Fig. 7.9. Effect of temperature and pH shock on culturability. Samples of PR131 slurry were subjected to conditions simulating temperature increases and pH changes that occur during embedding. Colony counts and total cell counts were then performed on the slurries. Values represent means of duplicate or triplicate samples.

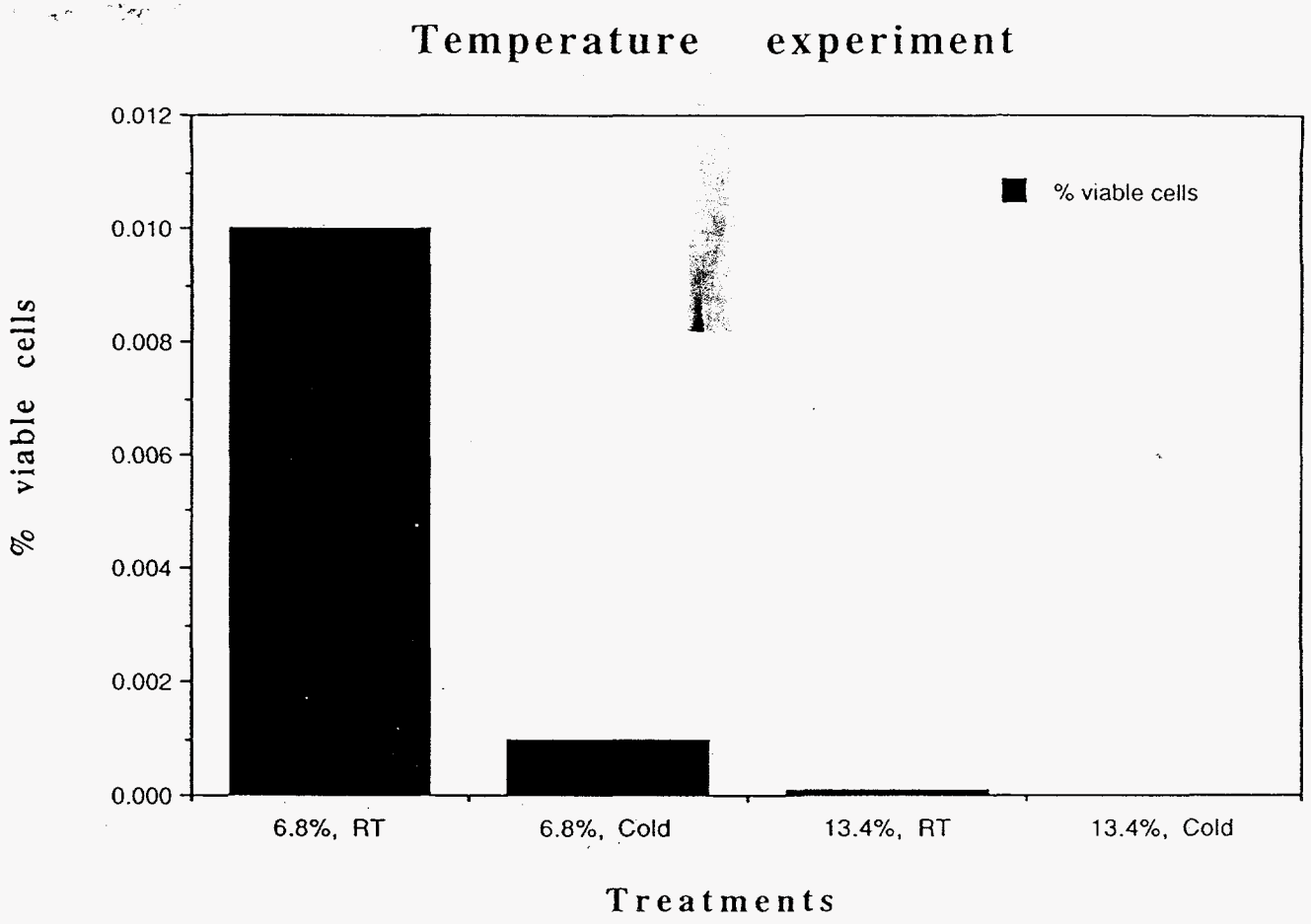

Fig. 7.10. Temperature experiment. Foams were prepared using either a 6.8% or a 13.4% PR131 slurry . Polymerizations were carried out both in an ice bath and without temperature control. Colony counts and total cell counts were performed on samples of cells removed from foam by vortexing. Values represent means of duplicate or triplicate samples.

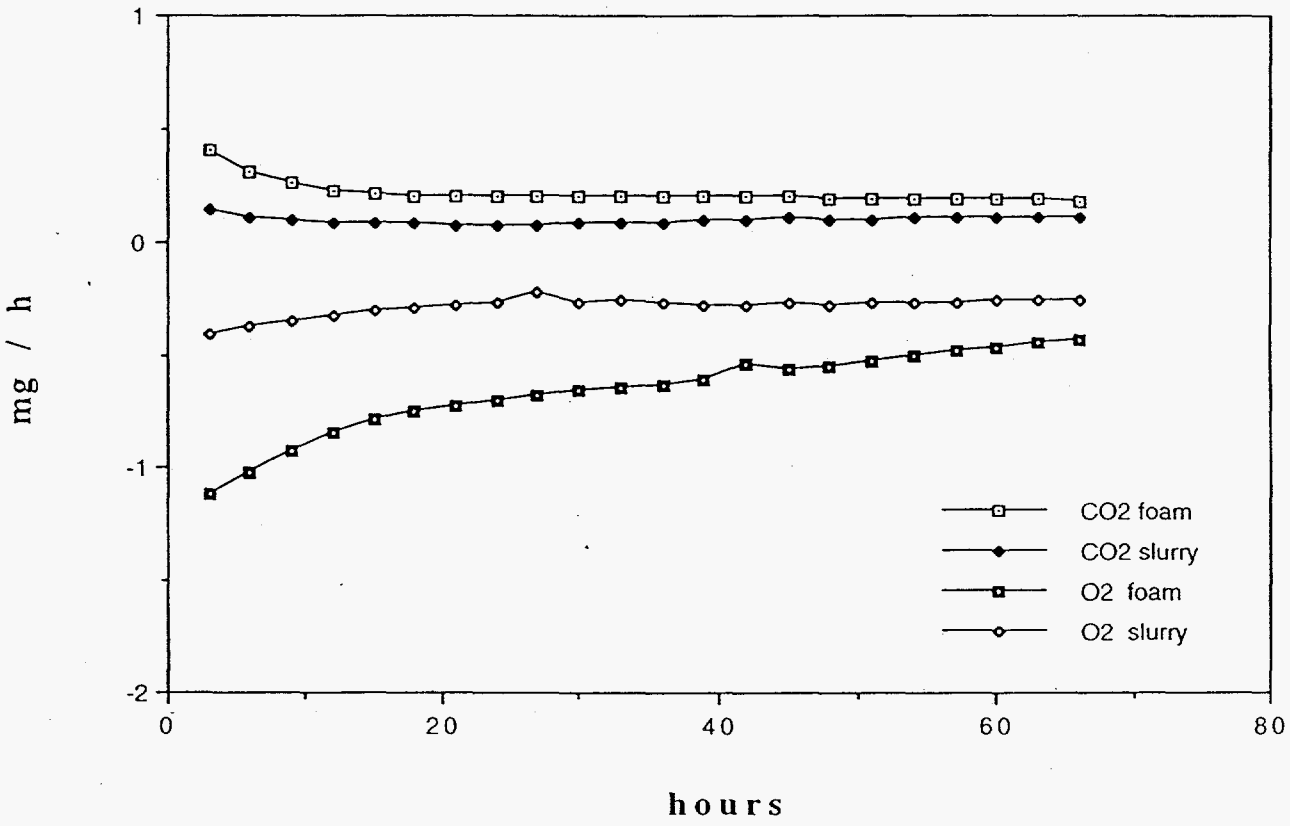

Fig. 7.11. CO_2 evolution and O_2 consumption rates of foams and bacterial slurries containing equal numbers of *B. cepacia*. Incubations were performed at room temperature (approx. 20°C). Measurements represent means of triplicate samples.

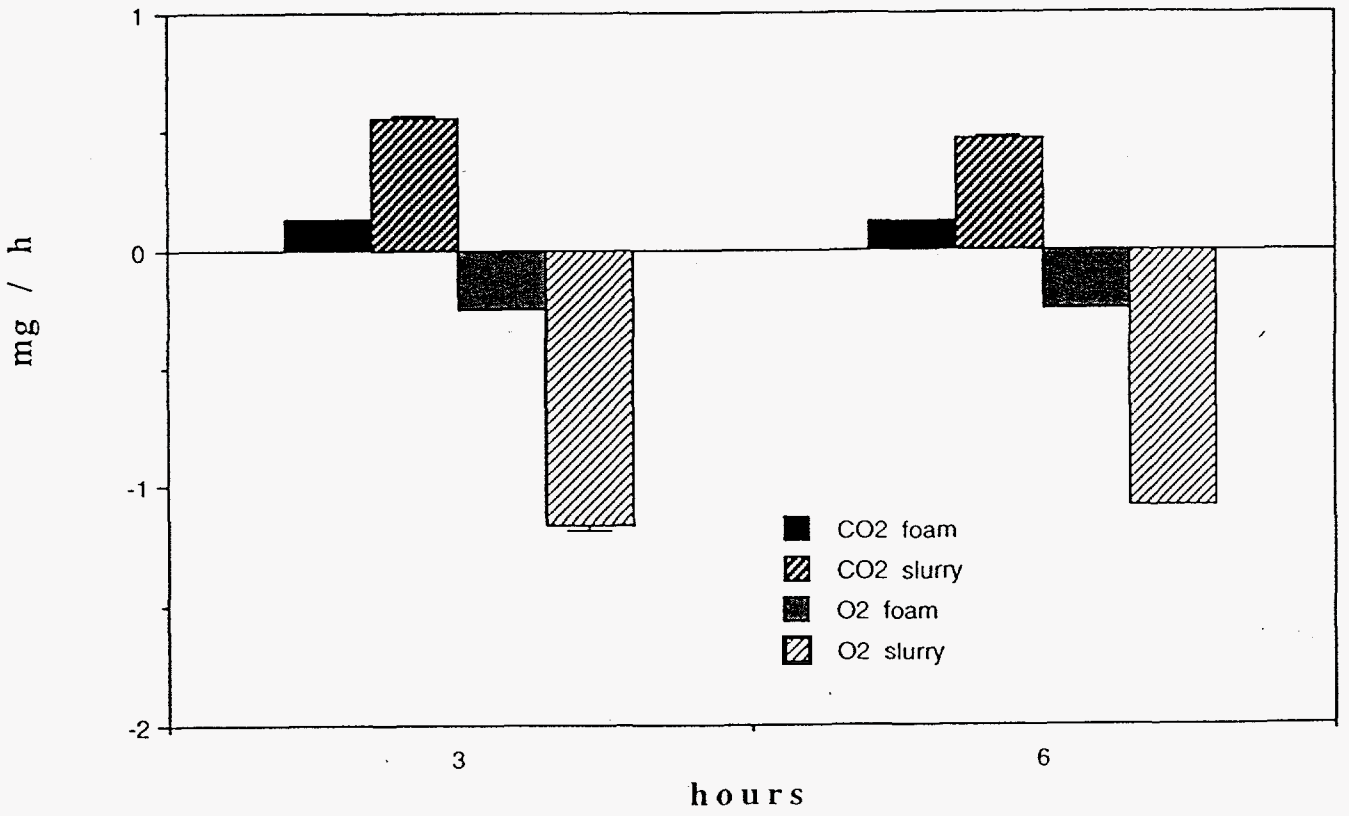

Fig. 7.12. Effect of temperature increase on respiration of *B. cepacia* G4 slurries. Foam and slurries from Fig. 7.11 were used in this experiment. Foams were kept at room temperature (20°C) while slurries were incubated at 25°C. Values represent means of triplicate samples.

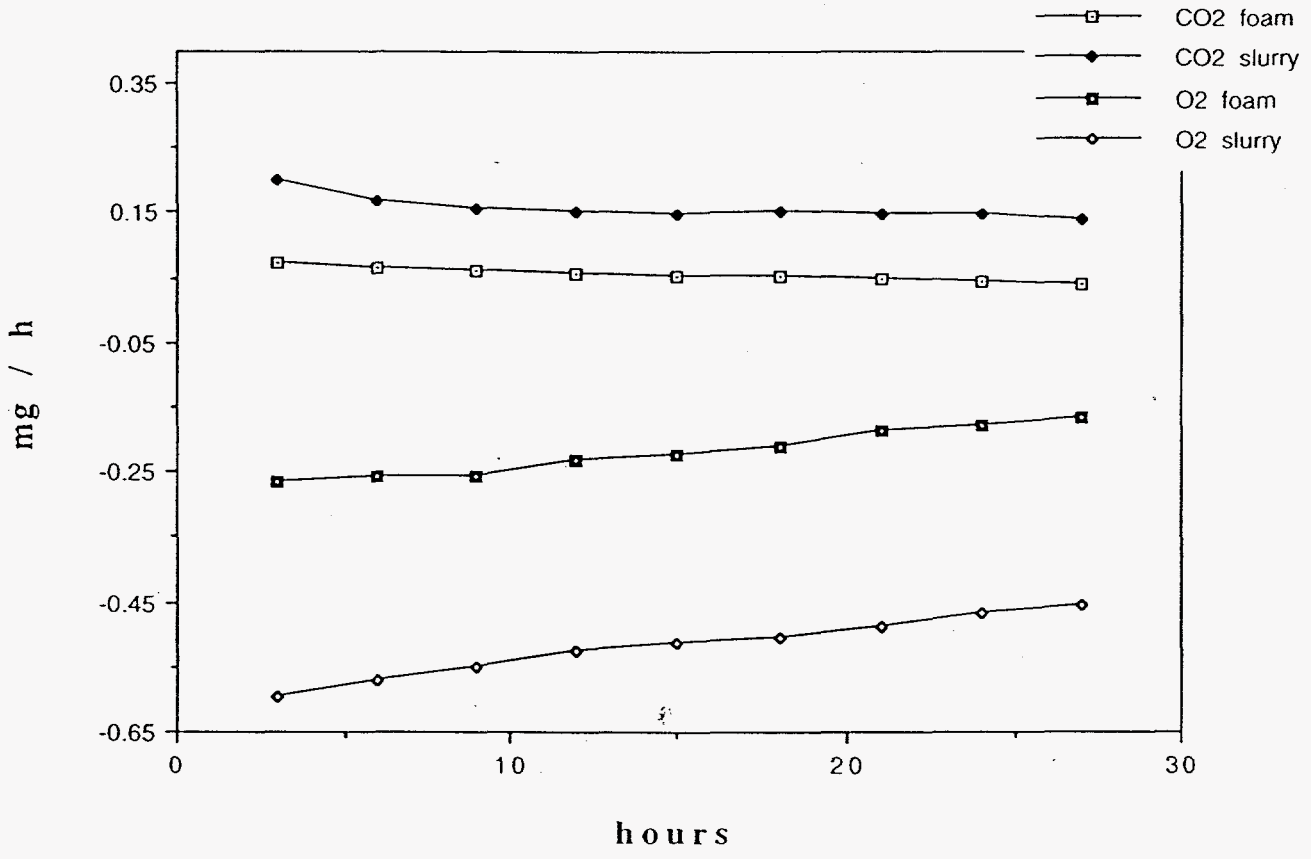

Fig. 7.13. Effect of temperature decrease on respiration of *B. cepacia* G4 slurries. Slurries from the experiment in Fig. 7.12 were returned to 20°C, after an 8 hr exposure to 25°C. Foam samples remained at 20°C. Values represent means of triplicate samples.

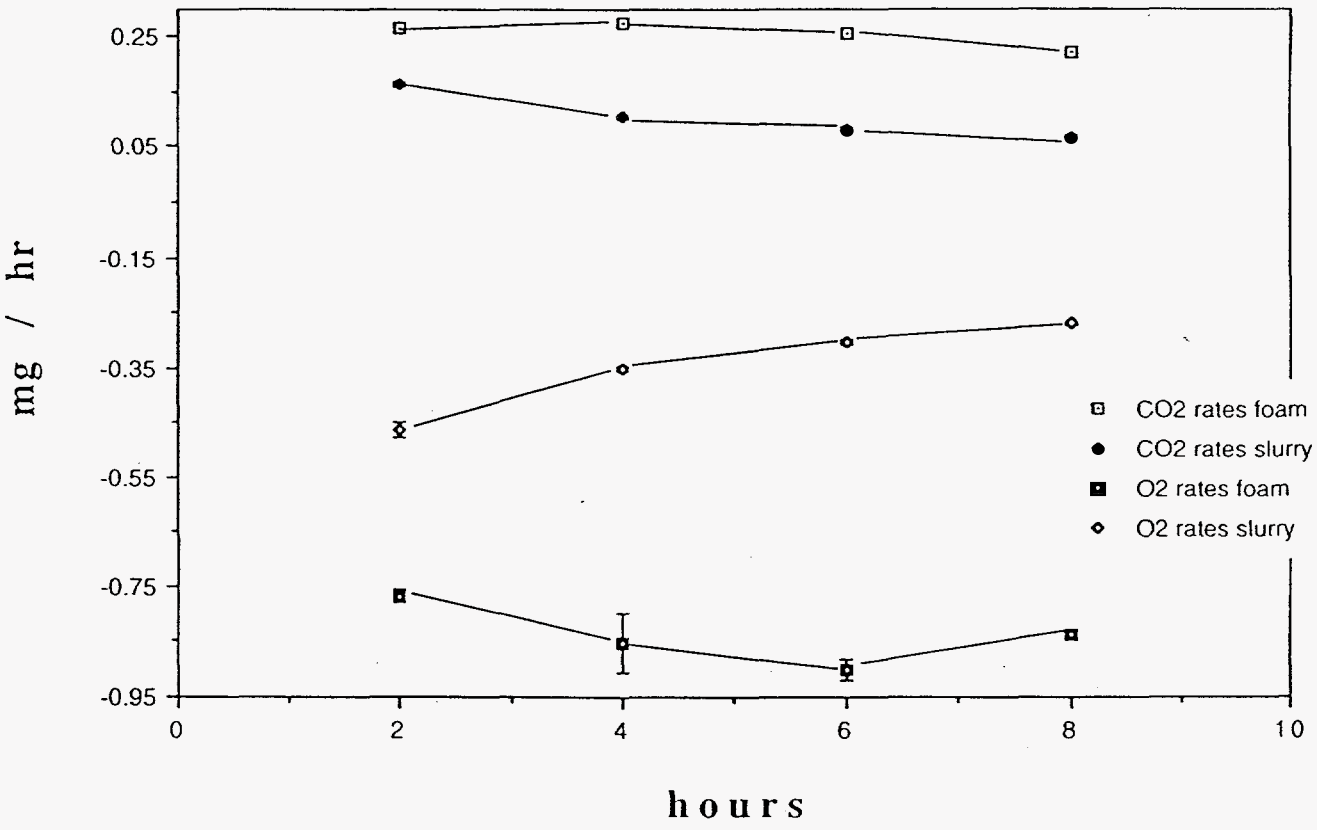

Fig. 7.14. Effect of phenol induction on respiration of *B. cepacia* G4. Bacterial cultures were exposed to 2 mM phenol for 2 hours prior to embedding. Foams and slurries were maintained at room temperature (20°C). Values represent means of triplicate samples.

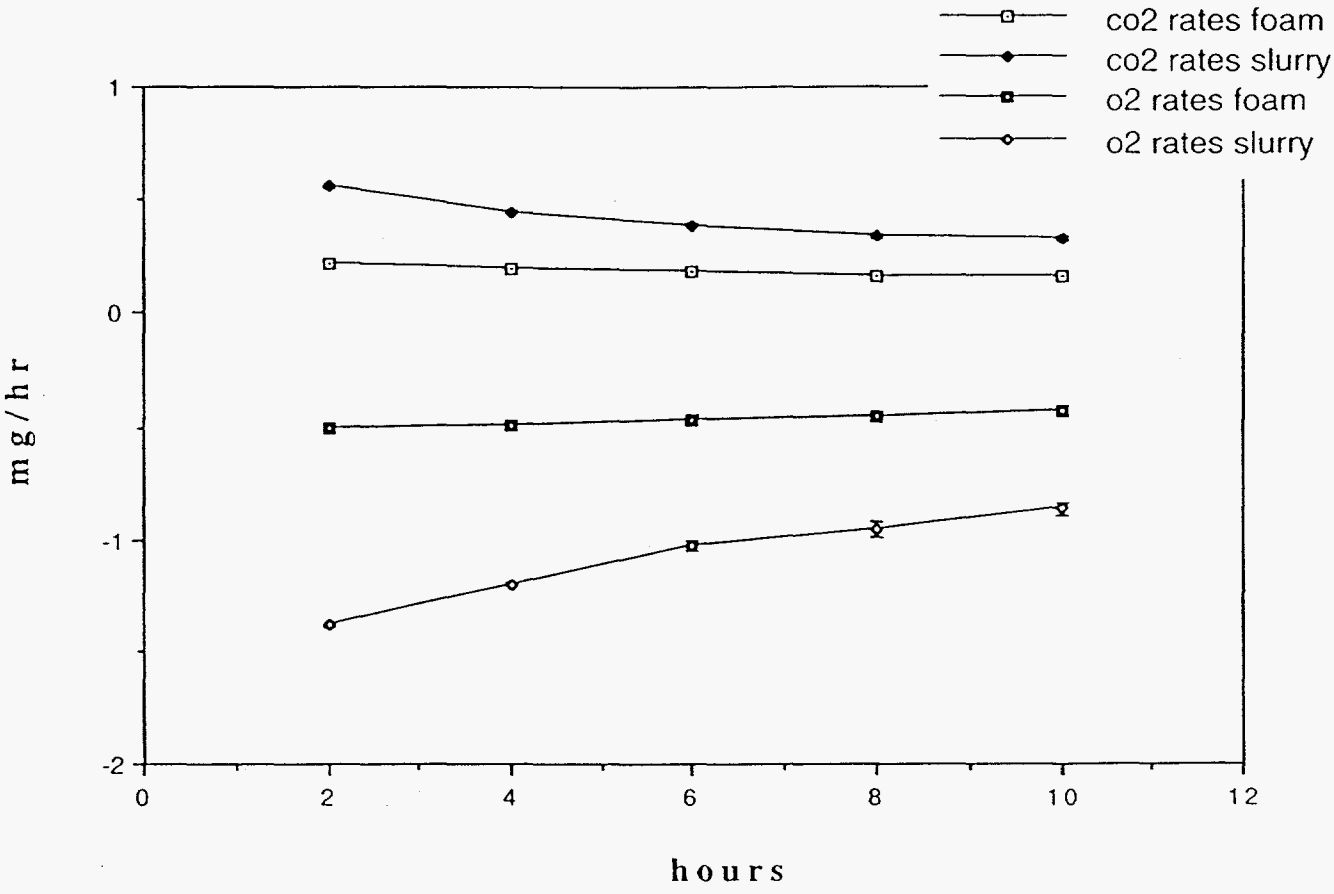

Fig. 7.15. Effect of aeration on respiration of phenol-induced *B. cepacia* G4. After collection of the data shown in Fig. 7.14, foams and slurries were incubated at 25°C with 130 rpm shaking. Values represent means of triplicate samples.

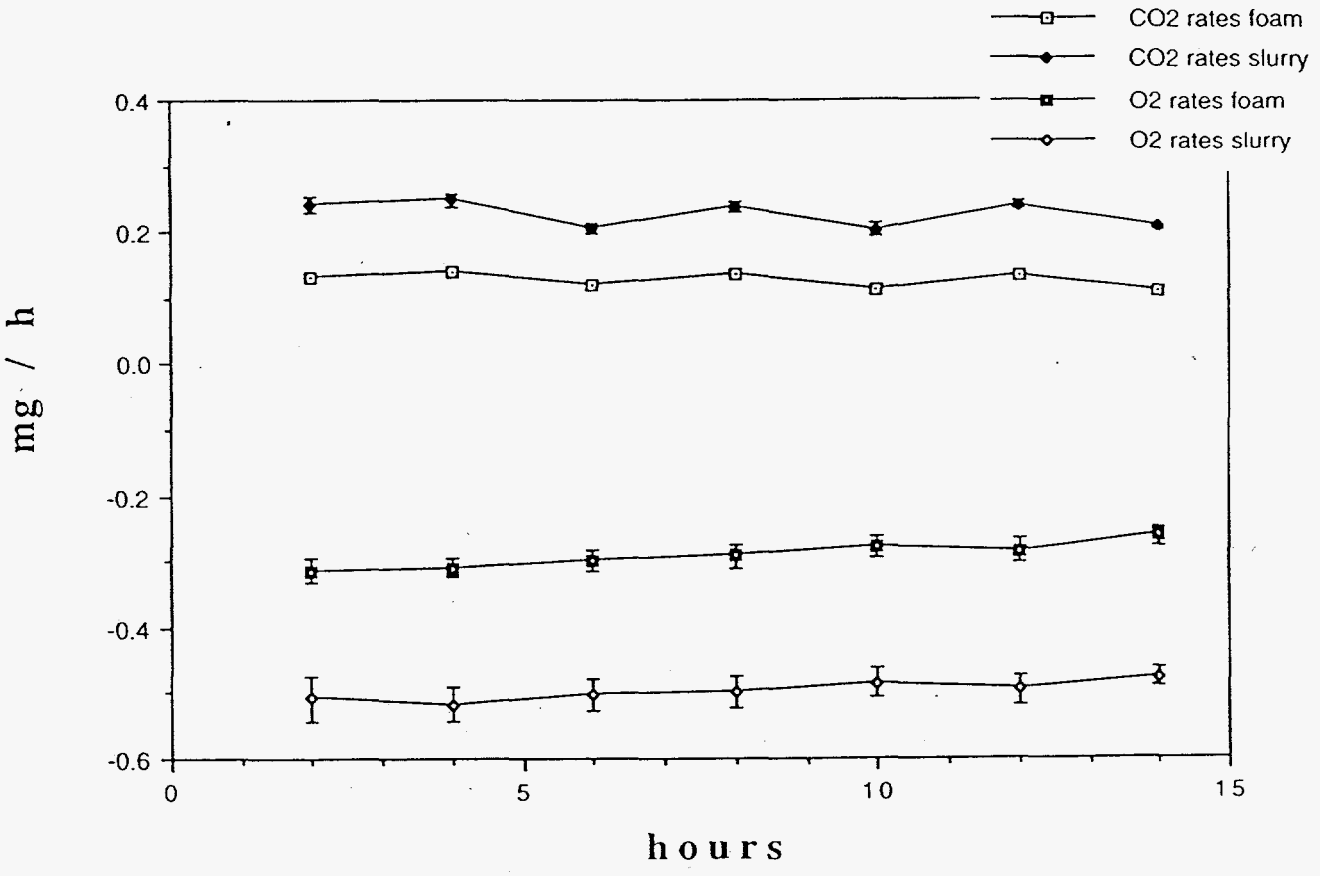

Fig. 7.16. Effect of decreased aeration on respiration of phenol-induced *B. cepacia* G4. The indicated respiration rates were measured after agitation of samples shown in Fig. 7.15 was discontinued. Temperature remained at 25°C. Values represent means of triplicate samples.

respiration activity shown by unembedded cells. This strongly suggests that physiological activity of *B. cepacia* is not as severely affected by the embedding process as the culturability results had indicated. Microscopic observations also indicated that immobilization did not cause any visible physical damage to the cells (data not shown).

Respiration rates of *B. cepacia* G4 and *B. cepacia* PR131 were compared to determine whether the two strains responded similarly to the embedding process (Fig. 7.17). No meaningful differences were observed. The slightly higher respiration rates of *B. cepacia* PR131 can probably be attributed to a small difference in slurry densities used for embedding (4.6% for PR131, 4.2% for G4).

7.3.4 Nutrient Amendments.

Both unembedded and previously embedded cells responded to the addition of carbon sources by significantly increasing in volume within 18 h (data not shown). A fraction of the previously embedded bacterial population responded by forming elongated cells. *In situ* hybridization of slurry and embedded cells with a fluorescently labeled rRNA-targeting probe revealed a simultaneous increase in ribosomal content as determined by an increase in fluorescent signal intensity.

7.4 Discussion

It is clear that immobilized degradative bacteria are potentially of great value for groundwater and wastewater treatment (Levinson et al, 1994). Several studies have shown that entrapment systems can deliver bacteria capable of transforming many pollutants (e.g. Weir et al., 1995; Xu et al., 1996). The use of immobilized bacteria might increase initial degradation rates of a compound by avoiding the lag time required for biofilm formation. Moreover, immobilized bacteria could be cost effective in bioremediation projects since they can potentially be used several times without significant lost activity (Rhee et al., 1996).

The aim of this study was to evaluate the use of polyurethane based formulations for the entrapment of degradative bacteria. Our main criterion was the ability of each formulation to prevent the release of cells. This criterion is of great importance in determining the functional longevity of this type of carrier system in bioremediation applications. Moreover, in applications involving genetically engineered bacteria, the ability to retain cells would seem critical in view of public concern about the release of such organisms into the environment. This aspect has received little attention by researchers evaluating immobilization agents, polyurethane foams in particular.

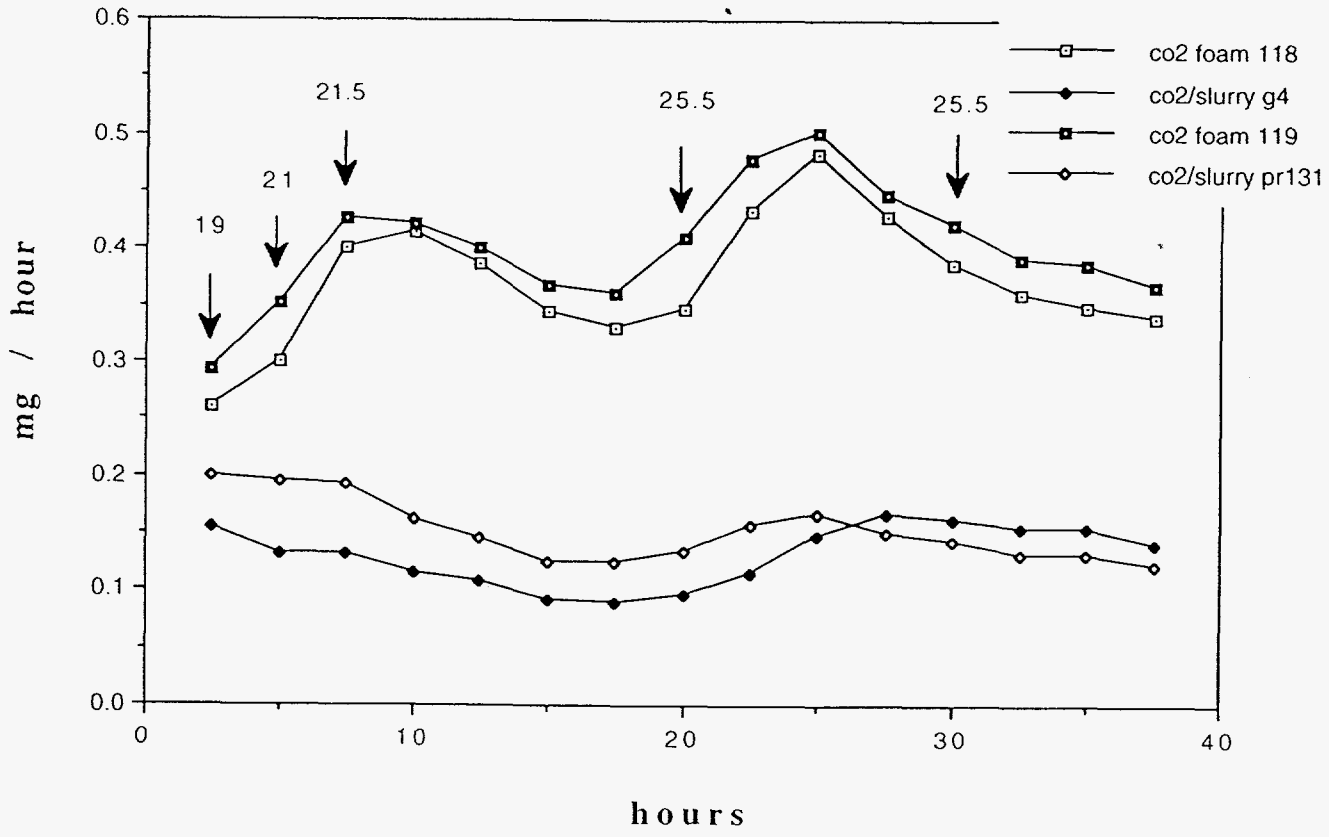

Fig. 7.17. Comparison of respiration by *B. cepacia* strains G4 and PR131. Increase in respiration rate coincides with periods of temperature increase (from 20°C to 25°C) while decreases in respiration rates can be attributed to decreases in temperature from 25°C to 20°C. Measurements represent averages of triplicates.

Our results show that biomass concentration and the type and concentration of surfactant are major determinants of cell retention. The effect of surfactants is undoubtedly related to the observation that large numbers of embedded cells are actually located in fluid-filled pores and hence are readily released after the foam is torn or cut (unpublished data). Increasing the surfactant concentration visibly increased total pore volume of the resulting foam and led to higher washout rates. The chemical composition of the surfactant also influences the size, total volume and interconnectedness of pores due to differences in surface tension, and hence will affect cell retention. The reason for the decrease in washout observed at higher biomass concentrations is unclear, but may be related to the lower water content of foams made with denser slurries. Based on our results, foam made with 1% HS-3 and an 18% dry weight bacterial slurry was most effective in retaining embedded *B. cepacia*. It remains to be seen whether this formulation is equally effective with other organisms.

The dramatic decrease in culturability as a result of immobilization suggested that viability was destroyed by the embedding process. However, we found no evidence that cells were killed by temperature or pH changes during embedding. Culturability was severely reduced in the presence of all three surfactants, and was not affected by the free TDI content of prepolymers used in foam manufacture. These findings suggested that some unknown factor inherent in the polymerization process was lethal to microorganisms, and led us to question the ability of polyurethane-based formulations to deliver functional bacterial cells.

However, respirometry data demonstrated that, although incapable of forming colonies, embedded *B. cepacia* remained metabolically active. Indeed, respiration by embedded bacteria compared favorably to that of free cells. This is consistent with the increase in volume and ribosomal content of embedded cells after nutrient addition. We conclude that embedding caused most cells to become viable but nonculturable (Roszak et al., 1984), and that culture techniques are not reliable indicators of the metabolic status of polyurethane-embedded cells. In light of these findings, it appears likely that embedded *B. cepacia* will retain its degradative capabilities and its potential for bioremediation.

8.0 DEGRADATION OF TRICHLOROETHYLENE AND BENZENE BY EMBEDDED BACTERIA

8.1 Introduction

Due to its widespread use as a degreaser and solvent, trichloroethylene (TCE) is a common contaminant of groundwater at hazardous waste sites, and represents a threat to many aquifers. At the Savannah River Site, TCE concentrations well above regulatory limits have been reported from numerous monitoring wells at waste sites (WSRC, 1995). Consequently, there is strong interest on-site and elsewhere in technologies for reducing levels of this compound. Currently, the most promising technologies involve bioremediation, either by anaerobic or aerobic microorganisms. However, anaerobic TCE degradative processes are relatively slow and frequently result in the production of other hazardous compounds, including vinyl chloride. Aerobic processes are more rapid and result in complete mineralization, but usually require the presence of a primary substrate to induce oxygenase activity.

The aerobic bacterium *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) strain G4 has recently received much attention (e.g. Folsom et al., 1990; Folsom and Chapman, 1991; Shields and Reagin, 1992; Luu et al., 1995) due to its oxidative TCE degradation capabilities. Although G4 requires a primary substrate to induce enzyme activity (phenol being the most commonly used, but benzene or toluene also act as inducers), constitutive expression of toluene monooxygenase has been reported in a mutant strain (PR1) developed by Dr. Malcolm Shields of the University of West Florida (Shields and Reagin, 1992; Luu et al., 1995).

The use of G4 and the constitutive mutant in bioreactors has hitherto been limited in part by their poor adhesion capabilities. This hinders their use in nonsterile wastewaters. In the previous section (Section 7.0), we have described the optimization of techniques for embedding the constitutive mutant PR131 in hydrophilic polyurethane foam in such a manner that cells are effectively entrapped and retain metabolic activity, as evidenced by ribosomal probes and measurement of respiration rates. However, loss of culturability by the embedded bacteria indicated that some cellular activities were impaired during the entrapment process. In this section, we compare the ability of immobilized and free cells of G4 and PR131 to degrade TCE. Our objective is a preliminary assessment of the potential of polyurethane-embedded *B. cepacia* for the remediation of TCE-containing groundwaters.

8.2 Materials and Methods

8.2.1 Bacterial strains, culture media, and growth conditions.

Burkholderia cepacia strains PR131 and G4 were kindly provided by Dr. Malcolm Shields. Strain G4 requires induction of toluene monooxegenase activity for TCE degradation, while the mutant strain PR131 has been reported (Shields and Reagin, 1992) to produce the enzyme constitutively.

To assure culture purity, original stocks were kept at - 70°C in 20% glycerol. Prior to each experiment, stock material was spotted onto PTYG plates (Appendix 7.1) to provide inoculum for batch cultures. Axenic batch cultures were grown in a yeast-glucose medium (YGM) (Shields and Reagin, 1992), which consisted of basal salts medium (BSM; Shields et al., 1989) plus 1 g/l glucose and 0.5 g/l yeast extract (Appendix 7.3). *Pseudomonas* medium (Atlas, 1993) was used for comparison purposes in one experiment (Appendix 7.2). Small-scale (100 - 250 ml) batch cultures were grown in shake flasks (200 rpm, 30°C). Larger amounts of axenic biomass were grown by inoculating 20 ml of 1- 3 day old culture into 4000 ml polycarbonate bottles containing 3000 ml YGM. These cultures were maintained at 26 ± 2°C and aerated through a sterile 0.2 μm filter to provide mixing and O_2. Cultures were routinely harvested for foam embedding after 3 days' growth, at which time biomass yield was about 0.5 g dry weight /l. Harvesting was by centrifugation (10,000 rpm, 10 min, 15 - 25°C). Pellets were resuspended in BSM medium to a density of 2.2 - 8.9% (typically ca. 5%) dry weight. Aseptic technique was not used during the harvesting process.

8.2.1 Preparation of hydrophilic polyurethane foam.

Bacteria were routinely embedded in hydrophilic polyurethane foam within 2 h after slurry preparation. In two experiments involving the induction of enzyme activity subsequent to slurry preparation, slurries were stored overnight at 4°C prior to induction and embedding. Colony counts indicated that storage of up to one week did not result in viability loss (data not shown). The foam samples were prepared by Frisby Technologies (Freeport, NY) at their Aiken, SC facility. Ingredients of the foam were: slurry, 20 g; prepolymer, 13.33 g; surfactant, 0.54 g. Thus, a 5% (dry wt) slurry would yield approximately 3 g dry wt bacteria per 100 g wet wt foam. A 1% solution of the lecithin-based compound HS-3 (Amisol) was used as the surfactant, based on our previous results indicating that this material enhanced retention of cells within the foam. The prepolymer Bipol 6B (Matrix, Inc) was selected on the basis of availability and our previous respirometry data. Control (cell-free) foams were generated by substituting 20 g BSM medium for bacterial slurry. Temperature was not controlled during the reaction, but did not exceed 42°C. Foam samples were reduced to a particulate state by means of a Waring blender, stored at ca 4°C, and used for experiments within 2-4 h unless otherwise stated.

8.2.2 Induction of enzyme activity.

Toluene monooxygenase activity in G4 was induced in one of several ways. Unless stated otherwise, 2 mM phenol was added to cultures 2 h before the commencement of harvesting. Benzene (2 mM) was sometimes used as an inducer for comparison purposes. In one experiment measuring the kinetics of TCE disappearance, 2 mM phenol was added to the 5.6% (dry wt) bacterial slurry 2 h prior to centrifugation and resuspension of the cells in BSM, after which the slurry was embedded in foam. In attempts to induce enzyme activity in pre-embedded G4, 0.1 g quantities of G4/foam aggregate were placed in quadruplicate 22 ml headspace vials (Hewlett-Packard) to

which 10 ml BSM and 2 µl phenol or 2 µl benzene were added. Quadruplicate control (uninduced) vials received G4/foam and medium only. Vials were sealed and shaken (200 rpm) for 2, 4, or 21 h. After induction, excess liquid was pipetted from the vials and replaced with 10 ml fresh BSM. For comparison purposes, 0.295 ml of G4 slurry (equivalent to the biomass content of 0.5 g G4/foam) was placed in each of triplicate bottles containing 50 ml BSM medium and 10 µl phenol, 10 µl benzene, or no inducing agent. Bottles were sealed and shaken in the same manner as foam samples, after which cells were pelleted (8000 rpm, 15 min, 15 - 25^{o}C) and the supernatant decanted. Pellets were resuspended in 50 ml fresh BSM and 10 ml of each suspension was placed in quadruplicate headspace vials for use in TCE degradation assays.

8.2.3 Degradation assays.
Assays for TCE biodegradative capability were typically conducted using quadruplicate 22 ml headspace vials (Hewlett-Packard) containing 10 ml BSM and 0.059 ml bacterial slurry or 0.1 g foam/bacterial aggregate (these amounts of slurry and foam were calculated to contain equivalent biomass). TCE (3 ppm) was added as a 1 ppt methanol solution, after which vials were immediately sealed with crimp caps and Teflon-coated septa, incubated 3 - 5 (typically 3) days at 30^{o}C, and subjected to headspace analysis by gas chromatography. Results were interpreted with the aid of a standard curve generated from TCE-inoculated BSM samples, which had been incubated under the same conditions as experimental samples. Benzene consumption was measured similarly, using 2 ppm benzene added as a ca. 800 ppm methanol solution.

In experiments with unembedded PR131, 10 ml aliquots of YGM-grown cultures were placed directly in headspace vials, and TCE or benzene was added as described above. Unembedded G4 cultures were tested after induction with phenol (2 mM, 2 h), centrifugation (15 min, 8000 rpm, 15 - 25^{o}C), and resuspension in BSM equivalent to the original culture volume. 10 ml aliquots of this suspension were then dispensed into headspace vials for testing.

8.2.4 Groundwater sampling.
Several groundwater monitoring wells at SRS were sampled for use in the study. Well # 1TC-SZP-9D is located at the B-Area Sanitary Landfill, while Wells MSB 25A, MSB 34A, and MSB 75B are located in M-Area. Chemical characteristics of the various groundwater sources are described in Appendix 8.1. All groundwater samples were taken after purging ca. 3 well volumes of groundwater, and were collected in headspace vials that were immediately sealed with Teflon-coated septa and crimp caps. Only vials containing no air bubbles or bubbles of less than ca. 2 mm diameter were used to provide water for experiments. Samples were used within 6h after collection. Samples containing bubbles of less than 6 mm diameter are considered adequate for quantitative analysis of volatile organics (U.S. EPA, 1986).

8.2.5 Analyses.

TCE was measured on a Hewlett-Packard 5890A gas chromatograph equipped with a Hewlett-Packard 19395A automated gas headspace analyzer, an electron capture detector, and a 60-m Vocol (Supelco) column. Column temperature was held at 35°C for 7 min, increased 5°C/min to 55°C, and then increased 15°C/min to 90°C. Samples were equilibrated at 75°C prior to analysis. Detection limits for TCE and benzene were 1.0 and 20.0 ppb respectively.

Dry weight of bacterial suspensions was measured in duplicate after centrifugation (10000 rpm, 10 min, 15 - 25°C), washing once with an equal volume of deionized water, recentrifugation, resuspension in deionized water, drying to constant weight at 104°C, and cooling in a desiccator.

8.2.6 Data interpretation and statistical analyses.

Data were stored magnetically and interpreted through linear regression analysis of standard curves generated during each experiment. The nonlinearity of the standard curves required the fitting of 3 - 4 lines to various portions of the concentration range. Regression analysis, t-tests, and calculations of sample standard deviations were performed using Microsoft Excel. Error bars in all figures represent standard deviations of triplicate or quadruplicate samples. Dates and major experimental parameters for all biodegradation experiments are summarized in Appendix 7.4.

8.3 Results

8.3.1 TCE degradation.

Initial experiments with PR131 cultures showed little or no significant TCE degradation under our experimental conditions. Although a 36% removal efficiency can be calculated from data in Table 8.1, this value is dubious due to variability in the standard curve used to interpret the results. A one-tailed t-test indicates no significant difference ($P = 0.19$) between raw TCE peak areas of PR131-containing samples and controls receiving the same TCE addition (1.043 ppm). A second PR131 stock culture was obtained from the University of West Florida, but gave similar results. However, a G4 culture removed substantial amounts of TCE from the solutions after 2 h induction with phenol (Table 8.1) The difference between TCE peak areas of G4-treated samples exposed to ca. 1 ppm TCE and controls exposed to ca. 0.5 ppm TCE was highly significant ($P = 0.0008$), and calculated removal was 81%.

TCE degradation by PR131 has been demonstrated in other laboratories, and no obvious reason for its absence in our experiments immediately presents itself. In the interests of expediting our investigation of the effects of polyurethane

Table 8.1. Comparison of TCE Removal from 1.043 ppm Solutions by PR131 and G4 Cultures.

Sample or Standard	Peak Area (X 10^7)	TCE, ppm[a]
Std 0.522 ppm	4.852	
Std 0.522 ppm	4.915	
Std 0.782 ppm	5.576	
Std 0.782 ppm	5.987	
Std 1.043 ppm	6.477	
Std 1.043 ppm	5.440	
Std 1.043 ppm	5.470	
Std 1.303 ppm	6.009	
Std 1.303 ppm	6.164	
Std 1.563 ppm	6.303	
Std 1.563 ppm	5.727	
G4	2.097	0.224
G4	1.458	0.156
G4	1.480	0.158
G4	2.577	0.275
PR131	5.243	0.626
PR131	5.440	0.678
PR131	5.635	0.729
PR131	5.320	0.646

[a] Calculated from the following regression lines: ppm = 1.069×10^8 A (for A < 4.9×10^7), ppm = 2.61×10^8 A - 7.42×10^7 (for A + 4.9×10^7 - 5.8×10^7) where A = TCE peak area.

foam embedding on biodegradation, we therefore decided to direct our attention toward the more cooperative G4 strain.

To select an appropriate TCE spike concentration for further experimentation, we added 1, 2, or 3 ppm TCE to BSM suspensions of G4 (grown in YGM). A BSM suspension of a culture grown in *Pseudomonas* medium was inoculated with 2 ppm TCE as a comparison. Fig 8.1 shows that TCE removal by YGM-grown cells was linear over the 1 - 3 ppm range, with 85 - 87% of the compound being degraded. No conclusion can be drawn from the lower mean TCE removal by cells grown in *Pseudomonas* medium, since no attempt was made to standardize biomass content of the two cultures. However, cells grown in *Pseudomonas* medium were much more variable in their response to 2 ppm TCE, as shown by the very large standard deviation in Fig. 8.1. Therefore, all subsequent experiments were performed with YGM-grown cells. A 3 ppm TCE addition was routinely used for subsequent work, since this value gave adequate results and is similar to that of many groundwater monitoring wells in TCE-contaminated areas of SRS.

After TCE degradation had been demonstrated in G4 cultures, an experiment was conducted to test whether it could occur after foam embedding (Fig. 8.2). Foam was made using slurries (2.2 - 2.4 % dry wt) suspended in BSM and YGM medium in order to test the effects of primary carbon sources on the degradation process. Aliquots of unembedded slurry were tested as a control. Embedded and unembedded G4 removed similar amounts of TCE (Fig. 8.2). The BSM-suspended cells removed 95 - 97.5% of the substrate. However, the presence of primary carbon sources (1 g/l glucose, 0.5 g/l yeast extract) dramatically decreased TCE removal.

Although 3-day incubations with TCE were routinely used in our experiments, the use of embedded G4 in a bioremediation process would require information on the kinetics of TCE degradation over various time periods. A preliminary examination of degradation over a 2-day period is shown in Fig. 8.3. A 4 - 8 h lag period occurred before TCE levels dropped below those of controls, and most degradation of the compound occurred in the first 24 h. Small amounts of TCE disappeared over a 48-h incubation from samples containing uninduced G4/foam aggregates, cell-free foam, and uninduced G4 slurry. This phenomenon might be due to sorptive processes.

TCE degradation efficiencies in Fig. 8.3 did not approach those seen in previous experiments. Low degradation levels were seen in both foam and slurry samples, indicating that the embedding process was not responsible for the poor results. One explanation is that phenol induction of toluene monooxygenase was carried out after cells had been concentrated to a 5.5 - 5.6% (dry wt) slurry, rather than prior to centrifugation as in other experiments. The total amount of phenol available per cell was hence much smaller than previously, and induction may have been incomplete. A second possibility is that high bacterial densities in the foam could impair TCE removal. The foam used to generate data in Fig 8.2 contained about half as much biomass as that used for Fig. 8.3. To test this idea, we conducted an experiment with foam of two

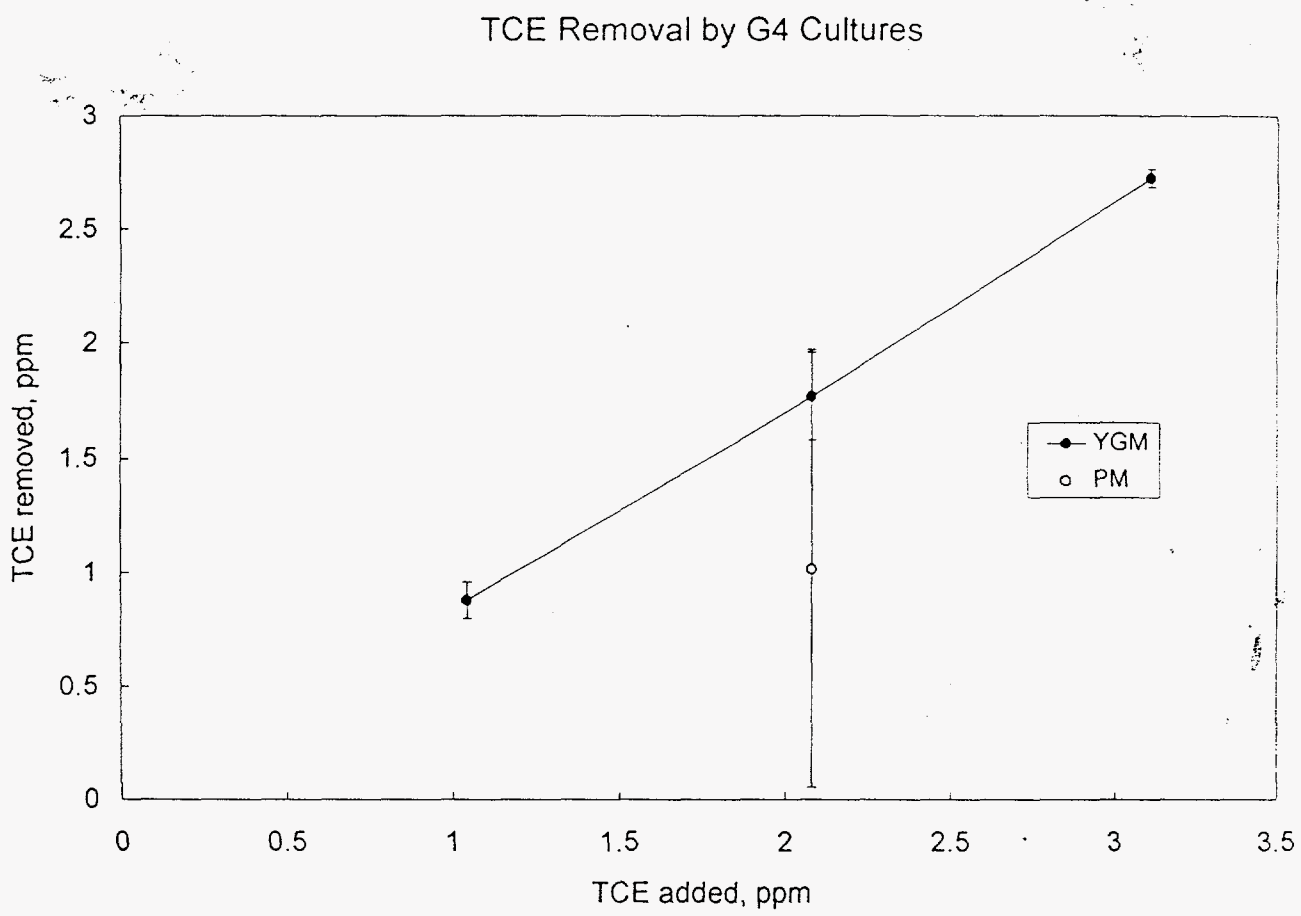

Fig. 8.1. TCE removal by G4 cultures. TCE degradation is shown as a function of the concentration originally present.

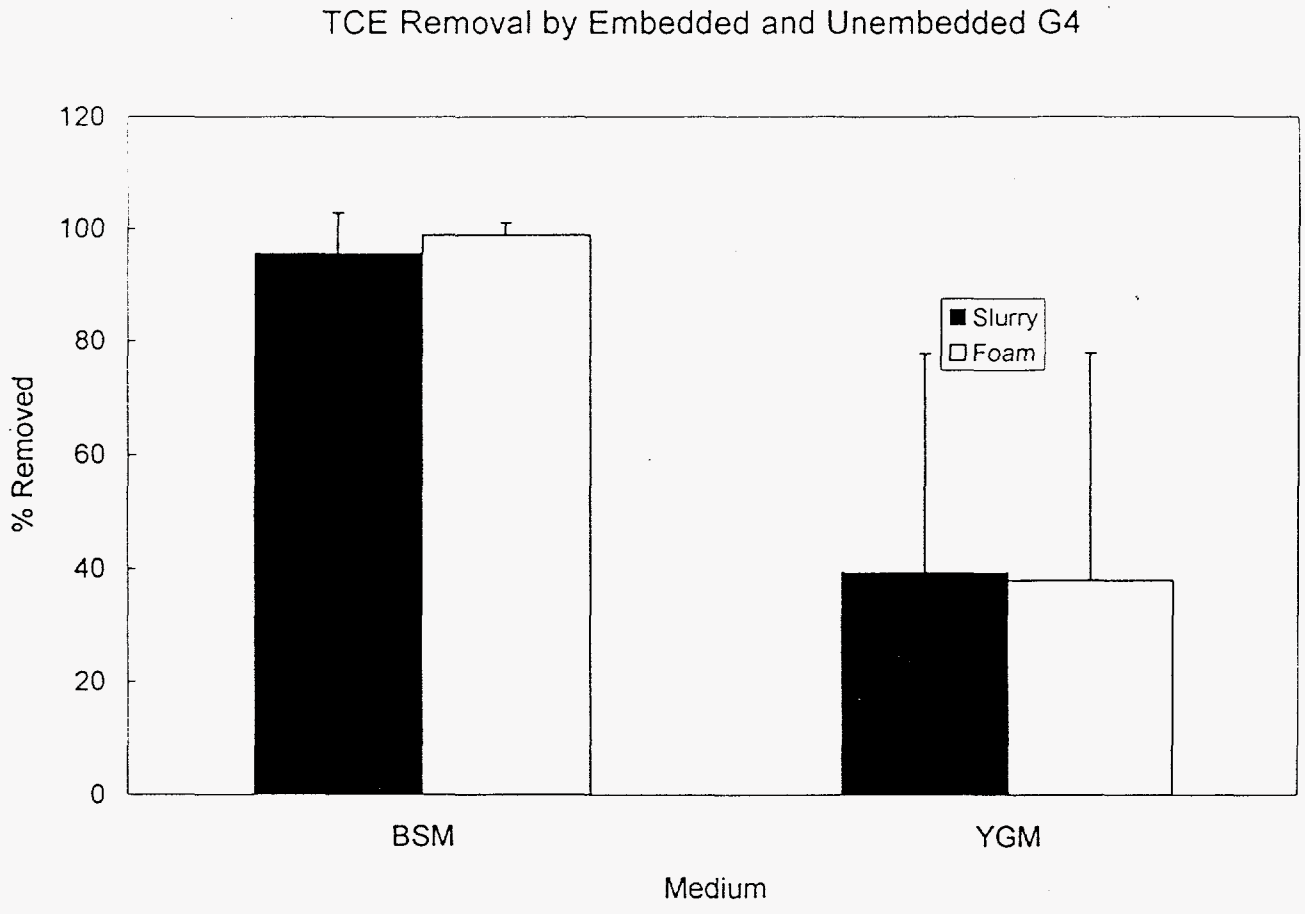

Fig. 8.2. TCE removal by embedded and unembedded G4. Quadruplicate foam and slurry samples containing 1.3 - 1.4 mg dry wt bacteria were exposed to 3.1 ppm TCE in 10 ml BSM or YGM. Induction was performed prior to slurry preparation.

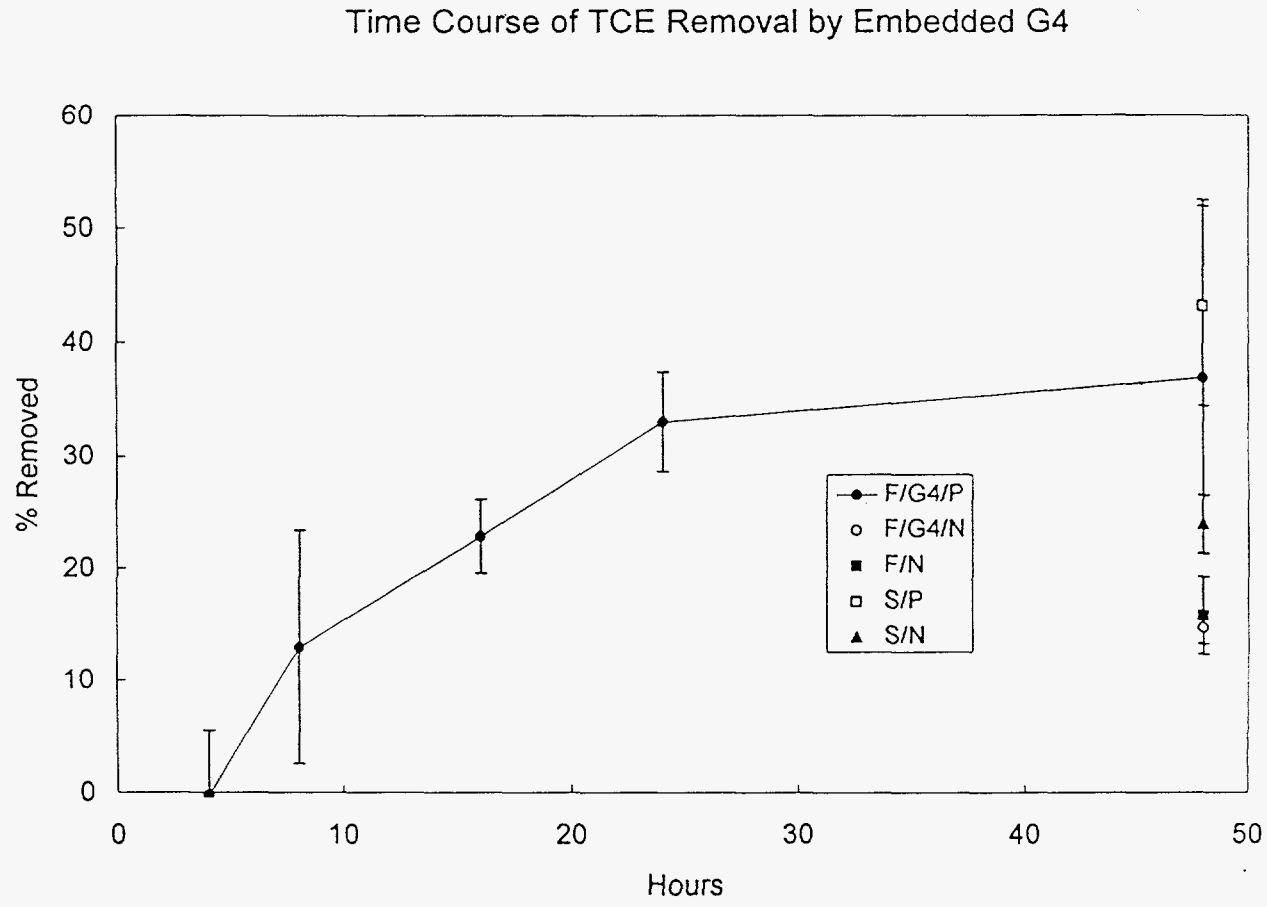

Fig. 8.3 Time course of TCE removal by embedded G4. Quadruplicate foam and slurry samples, each containing 3.2 - 3.3 mg dry wt bacteria, were exposed to 3.0 ppm TCE in 10 ml BSM. Controls were incubated for 48 h only, while phenol-induced G4-foam aggregates were tested for various time periods. Induction was performed on slurry preparations prior to foam manufacture. Legend: F/G4/P, foam containing phenol-induced G4; F/G4/N, foam with uninduced G4; F/N, uninduced cell-free foam; S/P, phenol-induced slurry; S/N, uninduced slurry.

biomass densities (Fig. 8.4). Phenol induction was carried out immediately before harvesting and slurry preparation. A separate set of foam and slurry samples received 0.1 g/l glucose and 0.05g/l yeast extract to validate our previous finding that the presence of primary carbon sources impaired TCE degradation. Fig. 8.4 shows that both a 5.1% slurry and the resulting foam degraded more TCE than a 2.5% slurry and associated foam. The high degradation efficiency (compared with Fig. 8.3) of the 5.1% slurry and resulting foam suggests that phenol induction of growing cultures was superior to induction of concentrated slurries. However, foam made from 2.5% slurry showed poor TCE removal, despite the cells having been grown and induced in the same manner and concentrated to a similar density as those seen in Fig 8.2. We speculate that some factor involved in polymer handling, the polymerization reaction, or subsequent foam processing may vary between individual foam samples and affect TCE removal.

Very little TCE degradation occurred in samples containing glucose and yeast extract (Fig. 8.4). This suggests that toluene monooxygenase activity is repressed in the presence of readily metabolizable primary substrates, and confirms the results shown in Fig. 8.2.

Since G4 requires induction of toluene monooxygenase for TCE degradation to occur, its utility for groundwater treatment will in part depend on the duration of enzyme activity, once induced. In a preliminary exploration of its useful lifetime, unused foam from the previous experiment was stored in a sealed container at 4^{o}C and retested after 3 days (primary carbon sources were not used in the second test). Results are shown in Fig. 8.5, with data from Fig 8.4 (for samples without primary carbon sources) being repeated (as "3 h samples") for comparison purposes. TCE degradation dropped markedly in both foams and both slurries during storage. The useful lifetime of the product would therefore appear to be considerable less than 3 days.

For a G4-based process to be practical, it would therefore be desirable to induce enzyme activity on-site. This would be most conveniently done using cells already embedded in foam. To test the feasibility of inducing pre-embedded G4, we exposed foam/G4 aggregates to 2 mM phenol or 2 mM benzene for various time periods (Fig. 8.6). A 2 h induction (such as was used in previous experiments) had some effect on slurry preparations, but had no effect on foam. TCE degradation by slurries was maximal after a 4 h induction, but activity of phenol-induced and possibly benzene-induced foam preparations was higher after a 21 h induction. It is not known whether longer induction periods would have further increased TCE removal. The experiment demonstrates that, assuming retention of overall metabolic activity in embedded G4, the induction and use of the product could be geographically and to some extent temporally separated from its manufacture. It also raises the possibility that enzyme activity could be induced repeatedly, extending the useful lifetime of the product. Lastly, the data suggest that TCE removal efficiencies may be improved by longer induction periods than those routinely used in our experiments.

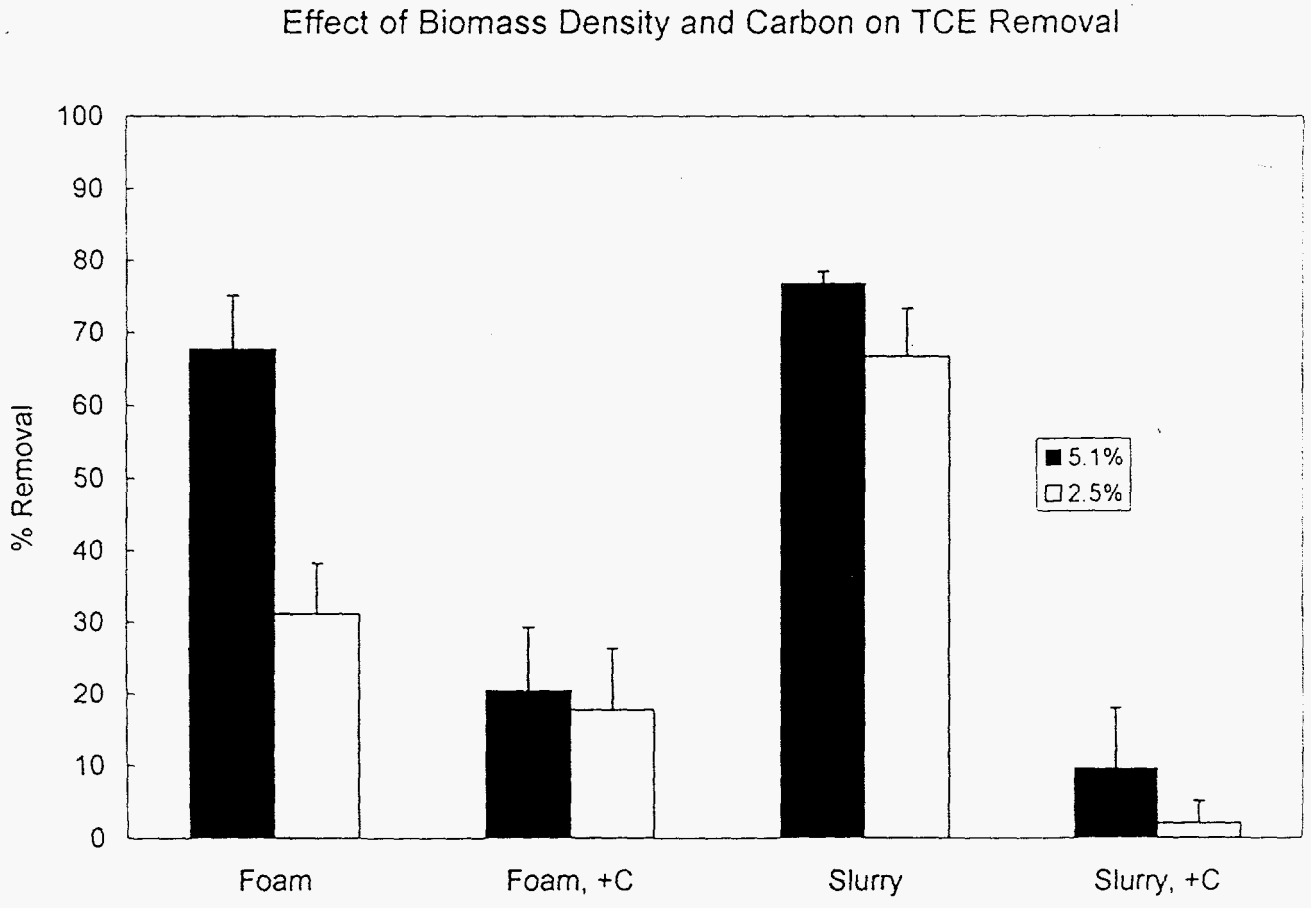

Fig. 8.4. Effect of biomass density and carbon on TCE removal. Quadruplicate foam and slurry samples containing 1.5 mg or 3.0 mg dry wt bacteria (corresponding to 2.5% or 5.1% slurry density) were exposed to 3 ppm TCE in 10 ml BSM with or without added carbon (0.1 g/l glucose and 0.05 g/l yeast extract). Induction was performed prior to slurry preparation.

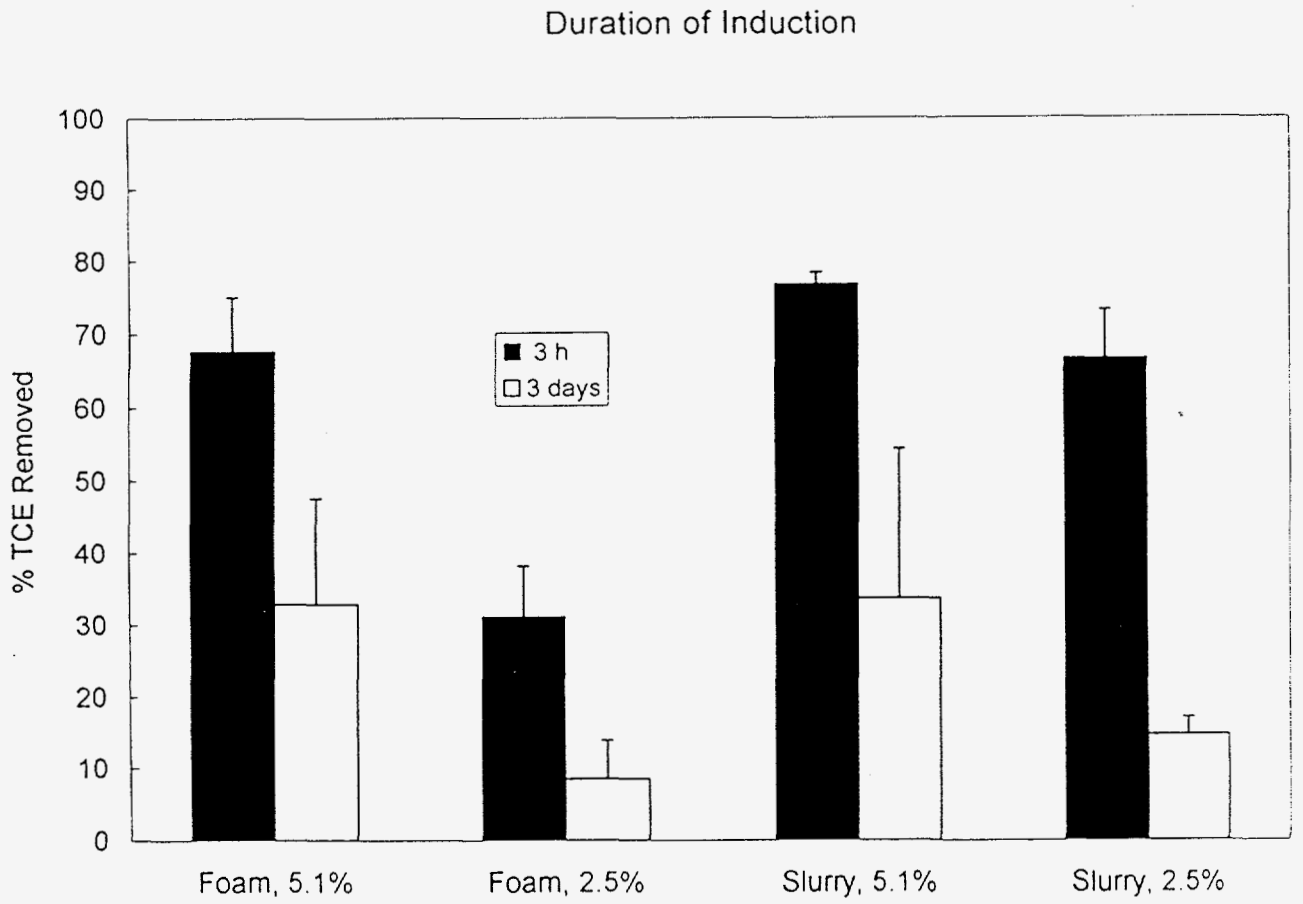

Fig. 8.5. Duration of induction. Foams and slurries from batches used in Fig. 4 were exposed to 3 ppm TCE in 10 ml BSM after 3 days' storage at 4°C. 3 hour data is repeated from Fig. 8.4 for comparison.

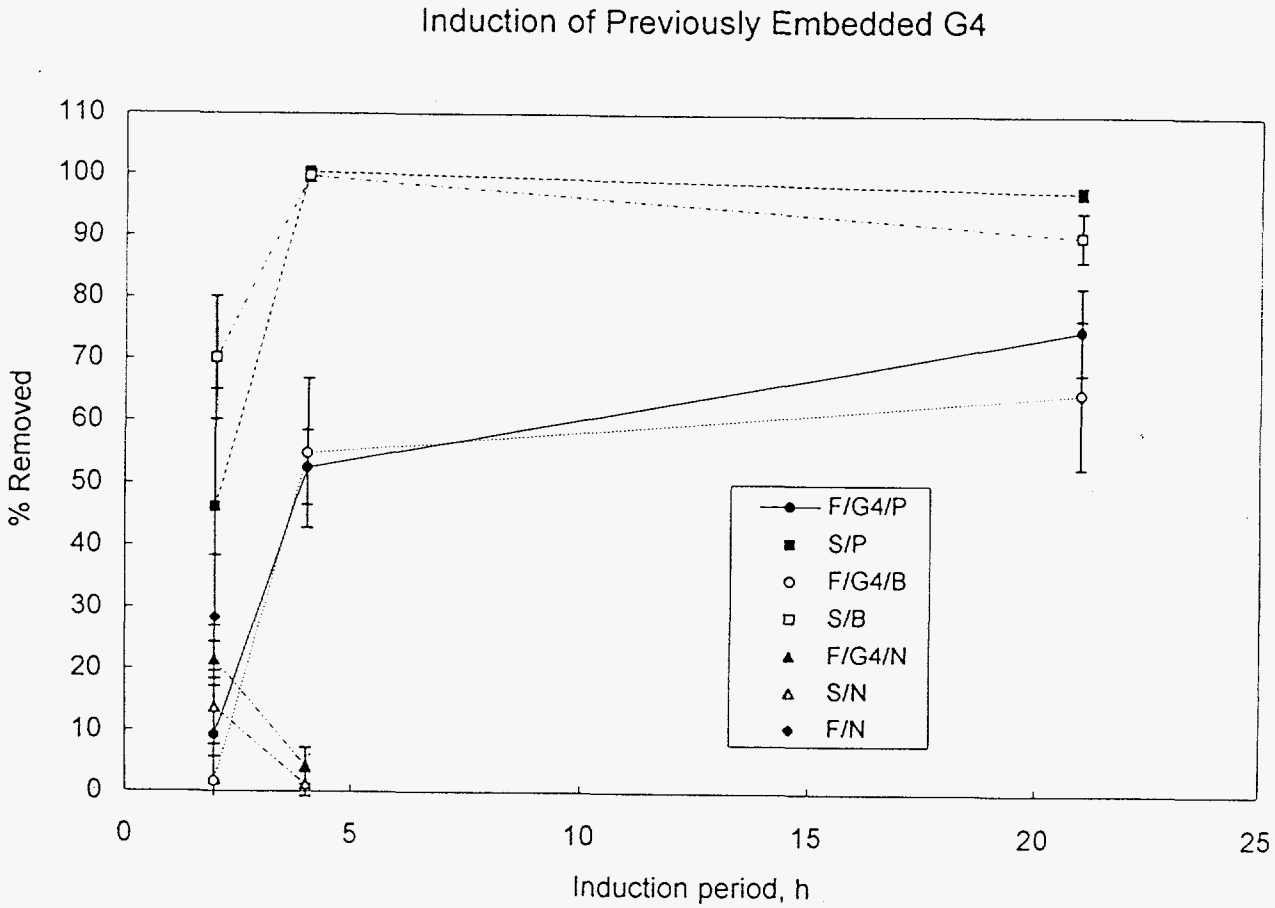

Fig. 8.6. Induction of previously embedded G4. Quadruplicate foam and slurry samples containing 3.2 - 3.4 mg dry wt bacteria were exposed to 3 ppm TCE in 10 ml BSM. Induction was performed with phenol or benzene (2 mM) on foam or slurry samples for 2, 4, or 21 h prior to TCE exposure. Uninduced controls were held for 2h or 4 h prior to testing. Legend: F/G4/P, phenol-induced foam-G4 aggregate; F/G4/B, benzene-induced foam-G4 aggregate; S/P, phenol-induced slurry; S/B, benzene-induced slurry; F/G4/N, uninduced Foam-G4 aggregate; S/N, uninduced slurry; F/N, uninduced cell-free foam.

It is expected that TCE removal from actual groundwaters may differ from that in test solutions, due to the presence of indigenous microorganisms, competing organics, and other factors. Two experiments were conducted using water samples collected from SRS monitoring wells. The first batch of water was taken from well # ITC-SZP-9D at the B Area Sanitary Landfill Site. This water contained TCE in quantities below regulatory limits and was therefore spiked with 0.1, 1.0, or 3.0 (nominal) TCE to simulate a more heavily contaminated source. Slurry samples removed 94 - 100% of the compound at all TCE levels tested, while equivalent foam samples removed 86% at the two lower levels and only 50% at the highest level tested (Fig. 8.7). Control samples of sterile BSM containing 3 ppm TCE and treated with foam or slurry gave results similar to 3 ppm-spiked groundwater. This suggests that TCE removal from the groundwater was indeed accomplished by G4 and not by indigenous microorganisms.

In another experiment, groundwaters were sampled from the M-Area wells 25A (1.99 ppm TCE), 34A (2.71 ppm), and 75B (1.09 ppm). These were exposed to G4 slurries and foam without additional TCE spiking. Slurries removed 88 - 100% of TCE from all groundwater samples (Fig. 8.8). However, foam-embedded G4 yielded variable results, ranging from 12% removal in 34A samples to almost 80% in 25A samples. These differences are not correlated with the original TCE content of the samples. Since all samples were treated with foam from a single batch, the data suggest that groundwater composition strongly influences TCE removal by embedded G4, but has much less influence on unembedded bacteria.

8.3.2 Benzene degradation by G4.

Embedded G4 (2.95 mg dry wt in 100 mg wet wt foam) degraded 90% of the benzene content of 2.07 ppm solutions (Fig. 8.9). This approached the degradation efficiency (97%) achieved by the equivalent amount of unembedded slurry. A 29% benzene loss was observed in controls containing cell-free foam. This suggests that the foam material itself binds some benzene. We do not at present know whether benzene absorbed in this manner is still available for bacterial degradation.

An experiment comparing removal of a 2 ppm benzene solution from BSM medium with that from YGM medium demonstrated that benzene removal was more variable and was (on average) impaired in the presence of glucose and/or yeast extract (Fig. 8.10). Thus, it appears that one or more of the organic compounds in YGM medium was used preferentially as a carbon source. Comparison of the Fig. 8.9 (experiment performed immediately after embedding) with Fig. 8.10 (experiment performed 2 days after embedding) indicates that benzene degradation activity was unimpaired by 48 h storage at $4^{o}C$.

8.4 Discussion

In this study, we attempted to evaluate the practicality of using hydrophilic polyurethane foam as a means of immobilizing G4 for use in bioremediation processes. Our results indicate that although foam-entrapped cells can potentially degrade TCE at levels

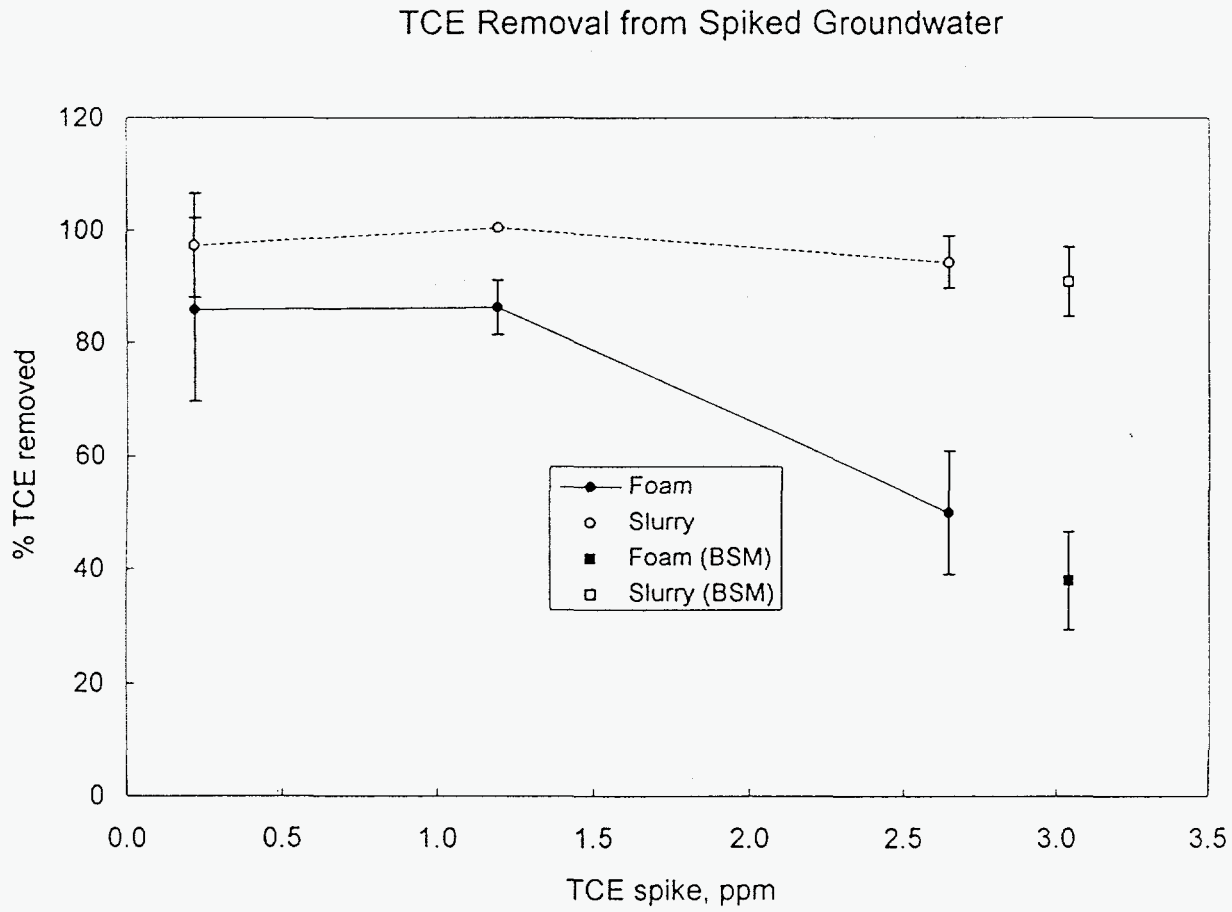

Fig. 8.7. TCE removal from spiked groundwater. Quadruplicate foam and slurry samples containing 3.0 mg dry wt bacteria were exposed to 10 ml sanitary landfill water amended with various TCE levels. Induction was performed prior to slurry preparation.

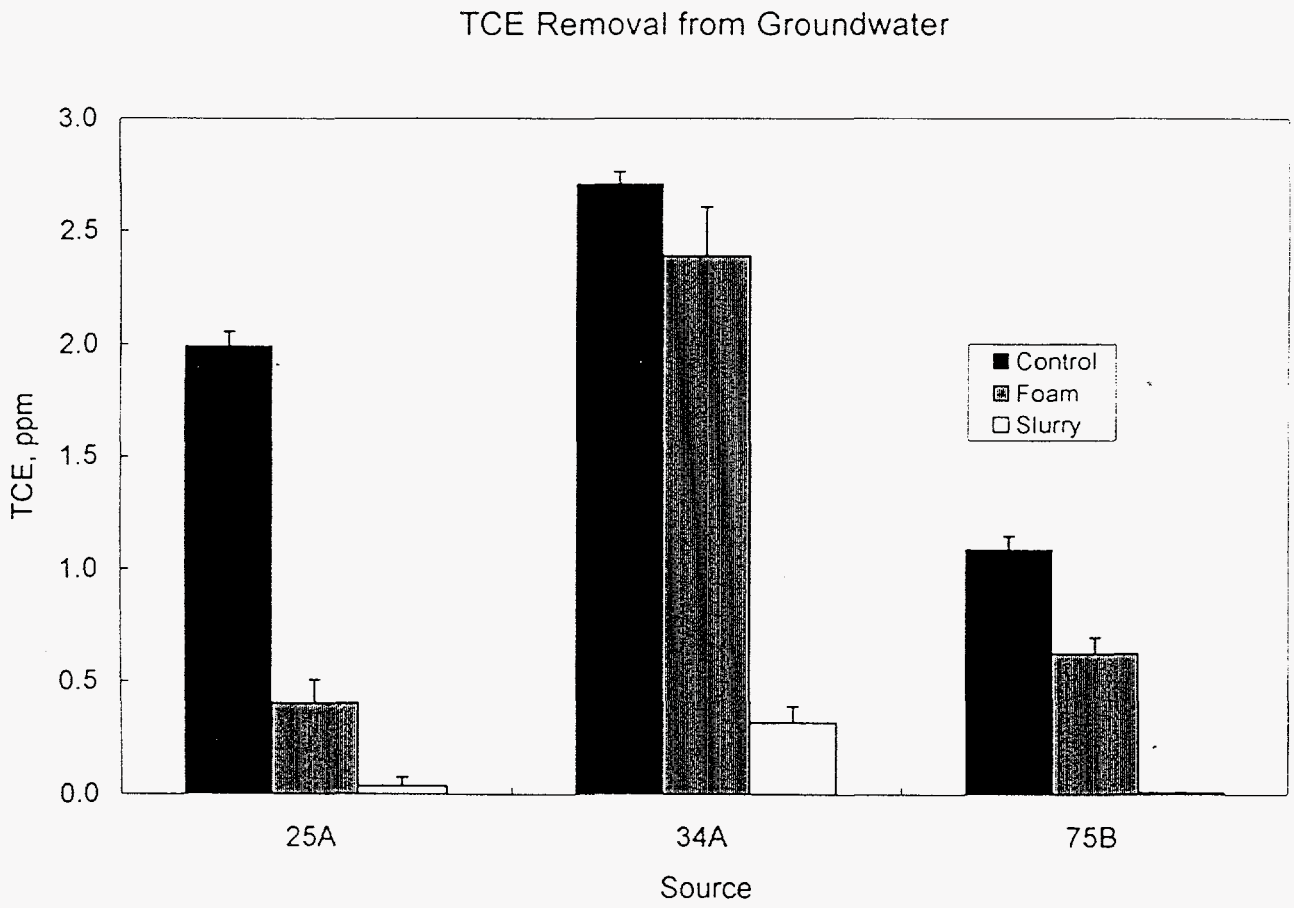

Fig 8.8. TCE removal from groundwater. Quadruplicate foam and slurry samples containing 3.1 mg dry wt bacteria were exposed to 10 ml of three M Area groundwaters containing various TCE levels. Induction was performed prior to slurry preparation.

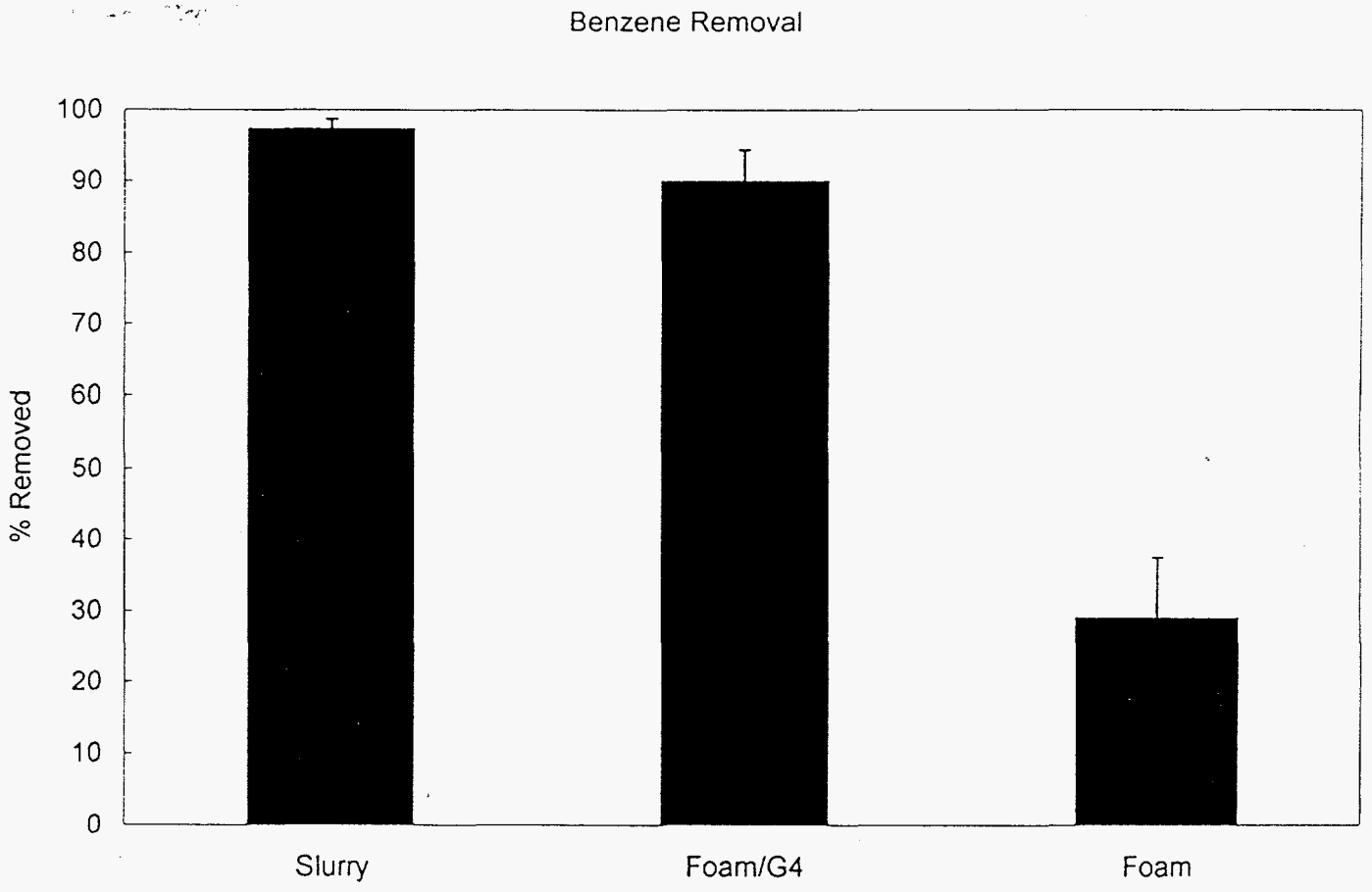

Fig. 8.9. Benzene removal. Quadruplicate foam and slurry samples containing 2.95 mg dry wt bacteria were exposed to 2 ppm benzene in 10 ml BSM. No induction was performed. Cell-free foam was used as an additional control.

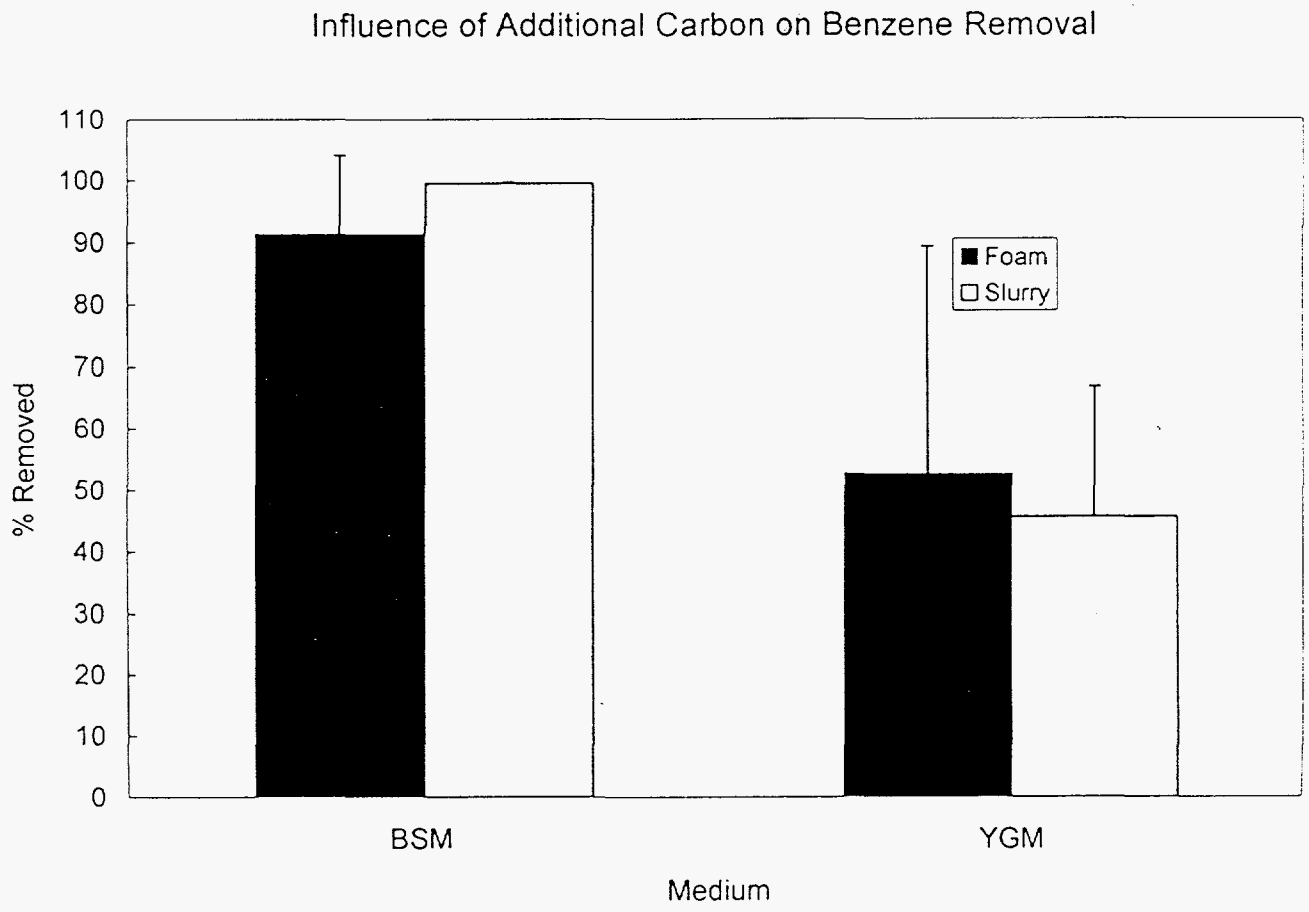

Fig. 8.10. Influence of additional carbon on benzene removal. Quadruplicate foam and slurry samples (identical to those shown in Fig. 8.2) containing 1.3 - 1.4 mg dry wt phenol-induced bacteria were stored for 2 days at $4^{O}C$ and then exposed to 2 ppm benzene in 10 ml BSM or YGM.

similar to those of free cells, they apparently lose the ability to divide. Their applicability to use in groundwater treatment would therefore depend in part upon microbial densities already present in the groundwater and upon the susceptibility of the foam material to colonization by indigenous microbes.

Our findings suggest several additional considerations that will be of importance in designing a groundwater treatment process using G4. In particular, the fact that the presence of readily metabolizable carbon sources inhibits the degradation of TCE would affect process design. Also, the relatively short duration of toluene monooxygenase activity, once induced, presents an obstacle. However, our success in inducing the enzyme in previously embedded cells raises the possibility that foam/bacterial aggregates could be prepared and stored for later use, with induction being performed at the bioremediation site (perhaps during several consecutive induction/TCE degradation cycles). This would greatly increase the functional longevity of the material and its convenience for the end user.

Implementation of a G4/foam based process would require considerable work beyond the scope of the current preliminary study. In particular, additional work is needed to achieve consistently high TCE degradation rates. We obtained varying results with identically grown G4 cultures embedded at similar densities and tested under the same conditions. The source of variability is therefore likely to be found in the embedding process itself. The same proportions of prepolymer, surfactant, and slurry were used throughout the study and the polymerization reaction was carried out under consistent conditions. We surmise, however, that aging of the prepolymer and variability in procedures used to bring it to a suitable temperature for foam manufacture may have affected the resulting G4/foam aggregates.

Although many questions remain to be answered before a G4/hydrophilic foam-based process can be devised, the present study demonstrates that the potential exists for the development of such a process. We conclude that this possibility is deserving of continued investigation.

9.0 CONCLUSIONS

The SRS has numerous waste sites and waste streams contaminated with toxic heavy metals, radionuclides and toxic organic compounds. Yet, few wastewaters at SRS have significant heavy metal contamination with an absence of additional radiological and/or carcinogenic pollutants. A notable exception is the coal pile runoff basins and their associated underlying groundwaters. However, these waters typically have extremely low pH and high levels of iron and aluminum that make them particularly difficult to remediate in terms of the adsorption of toxic metals.

Numerous algal strains with desirable characteristics for bioremediation can be cultured in defined media with rapid growth and the ability to achieve high densities when the cultures are in stationary phase. Furthermore, algal-foam aggregates can be prepared with sufficient structural integrity when subjected to a range of chemicals typically encountered in wastewaters. In addition, the foam can be converted back to a liquid rather easily with the addition of chemicals if desired. This adds flexibility to aspects of the process relating to disposal and recovery of pollutants from the spent foam.

However, results from experiments designed to assess and optimize toxic metal removal from actual SRS wastewaters in bioreactor systems packed with foam/biomass aggregates were generally disappointing. An experiment with radionuclide uptake by the foam/biomass aggregates provided slightly more encouraging results, with the alga *Cyanidium caldarium* proving to be the best of several types of biomass tested. However, the performance of *Cyanidium* (~ 35% removal) was far inferior to that of a TEVA resin (>95%) in comparative tests. Thus, these results did not offer encouraging prospects for scale-up and commercialization. Lack of success in this area was partially attributable to the nature of the wastewater available for study and the lack of appropriate bioreactor systems for contacting optimization.

More encouraging results were obtained evaluating the foam/biomass aggregate concept for use in biodegrading toxic organic compounds. The TCE-degrading bacterium *Burkholdia cepacia* was used for this part of the study. This organism is not ordinarily suitable for use in bioreactors due to its poor adhesion capabilities. However, we demonstrated that it is possible to embed *B. cepacia* so that passing 50 ml water through 2 g particulate foam washes out only 0.1% of the embedded cells, and 2000 ml water washes out only 0.2% of the cells. Surfactant type, surfactant concentration, and biomass density were most important in determining bacterial retention.

Although incapable of growth (cell division) in culture media, foam-embedded *B. cepacia* G4 cells are metabolically active. The nonculturability of embedded cells is not due to free TDI content of prepolymers or to temperature or pH changes during the embedding process.

Foam containing phenol-induced *B. cepacia* G4 removed substantial amounts of TCE from test solutions. TCE removal by embedded cells sometimes equaled that by free cells. Substantial benzene degradation by embedded G4 was also seen. However, variable results obtained with actual groundwaters suggest that groundwater composition affects TCE removal by embedded G4.

TCE degradation activity by embedded G4 can be induced by benzene as well as phenol. Once TCE-degrading activity is induced, it is of limited duration. However, G4 can be induced after it has been embedded. Growth and immobilization can thus be separated from induction and use of the product. This may allow repeated induction and reuse, extending the useful lifetime of the product.

10.0 RECOMMENDATIONS FOR FUTURE WORK

Continued research to develop a metal or radionuclide removal/reclamation process utilizing selected microbes embedded in foam has some merit because of the following features:

(1) demonstrated ability to culture algal strains with severe environmental requirements (for ease in maintaining a unialgal condition),
(2) an affinity of certain algae for certain metals and radionuclides, and
(3) the successful demonstration of the foam's ability to maintain structural integrity under harsh conditions, house viable organisms with minimal washout, and be readily dissolved with defined chemical treatment.

Although foam-embedded biomass might prove to be an effective means of metal removal in some applications, we do not feel that it holds enough potential for the remediation of SRS coal pile runoff water (basin or groundwater) to merit continued investigation. Any further work along these lines would hinge on the identification of a wastewater more amenable to treatment. Such a water source would preferably contain a single toxic and/or valuable metal of concern. It should also be of appropriate pH, should not have excessive iron levels, and should not be contaminated with other types of pollutants (e.g. hazardous organics or radionuclides) requiring additional limitations to laboratory studies. In view of our previous extensive survey of on-site wastewaters, identification of such a wastewater at SRS is unlikely.

Continued work with TCE-degrading bacteria would appear potentially rewarding. Any further research should be ultimately directed toward achieving control over the TCE degradation process, maintaining it in a bioreactor system, and assessing the efficiency and economic competitiveness of the technology.

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APPENDICES

APPENDIX 3

IDENTIFICATION, CHARACTERIZATION AND SELECTION OF METAL CONTAMINATED WASTE SITES AND WASTE STREAMS AT SRS

Table A.3.1. Toxic Metals And Regulatory Limitations

Metal	Priority Pollutant [a]	National Drinking Water Std.[b] mg/l	Aquatic Life Freshwater Std. [c] ug/l	EPA Quality Criteria Freshwater ug/l	Water Quality Criteria Human Health ug/l	Maximum Contaminant Concentrations mg/l [e]	Waste Std., Regulatory Conc. Units ppm [f]
Aluminum	no	(.05)	0.174	-	-	-	-
Antimony	yes	0.005	-	1600	4308	1	-
Arsenic	yes	0.05	360	190	1.4	5	5
Barium	no	2	-	-	-	200	100
Beryllium	yes	0.004	-	5.3	1.17	0.1	-
Cadmium	yes	0.005	1.79	0.66 [d]	10	0.5	1
Chromium (III)	yes	0.1	984	120 [d]	673077	10	5
Copper	yes	1.3	9.2	6.5 [d]	-	-	-
Lead	yes	0.015	34	1.3 [d]	50	1.5	5
Mercury	yes	0.002	2.40	0.012 [d]	0.153	0.2	0.2
Nickel	yes	0.1	789	56 [d]	4584	10	-
Selenium	yes	0.05	20	35 [d]	10	5	1
Silver	yes	(.09)	1.23	1.2 [d]	50	20	5
Zinc	yes	-	65	59 [d]	-	700	-

[a] Classified by the Clean Water Act, Section 307

[b] Secondary DW standards in parentheses

[c] S.C. Aquatic Life Standard, criterion maximum concentration

[d] At hardness 50

[e] 40 CFR, 1, pg374, Section 261.24 (7/1/86)

[f] Federal Register 1992

Table A.3.2 ER Waste Units With Potential for Bioremediation of Toxic Metals and/or Radionuclides Showing Information Contacts

Section #[a]	Waste Unit Name	Building #	SRS Contact
C-5	C-Area Burning/Rubble Pit	131-C	R. Plunkett (4-6797)
C-9	CMP pits (7)	080-17g,-17.1g,-18g,18	R. Soucha (4-6908)
C-10	D-Area Burning/Rubble Pits (2)	431-D, -1D	J. Hammock (4-1801)
C-11	F-Area Burning/Rubble Pits (3)	231-F, 1F,-2F	J. Hammock(4-1801)
C-12	K-Area Burning/Rubble Pit	131-K	J. Hammock (4-1801)
C-13	K-Area Rubble Pile	631-20G	J. Hammock (4-1801)
C-14	L-Area Burning/Rubble Pit	131-L	J. Hammock (4-1801)
C-15	L-Area Rubble Pit 131-1L	131-1L	J. Hammock (4-1801)
C-16	L-Area Rubble Disposal Pile 131-3L	131-3L	J. Hammock (4-1801)
C-17	Misc. Chem Basin/Metals Burn Pit	731-4A,-5A	J. Hammock (4-1801)
C-18	P-Area Burning/Rubble Pit	131-P	J. Hammock (4-1801)
C-19	R-Area Burning/Rubble Pits (2)	131-R, 131-1R	J. Hammock (4-1801)
D-2	488-D Ash Basin	488-D	K. Ward (4-6941)
D-3	716-A Motor Shop Seep. Basin	904-101G	R. Plunkett (4-6796)
D-4	Coal Pile Runoff Basins A,C,D,F,H,K,& P	189-C,-K,-P, 788-3A, 489-D, 289-F,-H	K. Ward (4-6941)
D-5	D-Area Oil Seepage Basin	631-G	R. Plunkett (4-6797)
D-6	F- and H-Area Retention Basins	281-3H, 281-3F	K. Kuelske (4-6659)
D-8	K-Area Reactor Seepage Basin	904-65G	G. Blount (4-6775)
D-9	L-Area Oil/Chem Basin	904-83G	G. Blount (4-6775)
D-10	L- and R-Area Acid/Caustic Basins	904-77G, 904-79G	G. Blount (4-6775)
D-12	New TNX Seepage Basin	904-102G	R. Soucha (4-6908)
D-13	Old F-Area Seepage Basin	904-49G	K. Kuelske (4-6659)
D-14	Old TNX Seepage Basin	904-76G	K. Kuelske (4-6659)
D-15	R-Reactor Seepage Basins	904-57G,-58G, 59G, 60G,-103G,-104G	K. Wise (4-1819)
D-16	Road A Chemical Basin	904-111G	R. Soucha (4-6908)
D-17	SRL Seepage Basins (4)	904-53G1,-53G2, 54G,-55G	K. Jerome (4-6786)
E-1	Tank 16	241-H	T. Gaughan (4-6773)
E-4	Gunsite 218	631-23G	H. Hickey (4-1802)
E-6	Burial Ground Complex	643-E, 643-7E	K. Lewis (4-6750)
E-7	Central Shops Sludge Lagoon	080-24G	R. Plunkett (-4-6797)
E-10	Ford Building SeepageBasin	904-91G	G. Blount (4-6775)
E-19	Silverton Road Waste Site	731-3A	K. Ward (4-6941)
E-20	TNX Burying Grounds	643-5T	K. Kuelske (4-6659)

[a] - Section numbers referenced in RCRA Facility Investigation/CERCLA Remedial Investigation Workplan Summaries

Table A.3.3 ER Waste Units With Best Potential for Bioremediation of Toxic Metals and/or Radionuclides Showing Selection Criteria

Sect. #[a]	Waste Unit Name	Building	Type Water[b]	Char. Data Avail.?	Metal Contam.[c]	Rad. Contam.?	Remed. Req'd?
C-9	CMP Pits (7)	080-17g,-17.1g,-18g,	GW	YES	Pb	NO	YES
C-11	F-Area Burning/Rubble Pits (3)	231-F, 1F,-2F	GW	YES	Al,Sr	YES	YES
C-12	K-Area Burning/Rubble Pit	131-K	GW	YES	Al,Pb	NO	YES
C-14	L-Area Burning/Rubble Pit	131-L	GW	YES	Pb	NO	YES
C-15	L-Area Rubble Pit 131-1L	131-1L	GW	YES	Pb	NO	YES
C-17	Misc. Chem Basin/Metals Burn Pit	731-4A,-5A	GW	YES	Pb	YES	YES
C-18	P-Area Burning/Rubble Pit	131-P	GW	YES	Pb,Al	NO	YES
D-2	488-D Ash Basin	488-D	SW & GW	YES	Cd,Cr, As, Al,C	NONE	YES
D-3	716-A Motor Shop Seep. Basin	904-101G	GW	YES	Sb	YES	YES
D-4	Coal Pile Runoff Basins A,C,D,F,H,K,	189-C,-K,-P, 788-3A, 489-D, 289-F,-H	SW & GW	YES	Cd,Cr, As, Al,C	GW ONLY	YES
D-5	D-Area Oil Seepage Basin	631-G	GW	YES	Al,Pb	YES	YES
D-6	H-Area Retention Basin	281-3H	GW SW	YES	Al,Pb,Sb	YES	YES
D-8	K-Area Reactor Seepage BasSin	904-65G	GW?	YES	Al,Pb	YES	YES
D-9	L-Area Oil/Chem Basin	904-83G	GW	YES	Cd,Pb,Al	YES	YES
D-10	L- and R-Area Acid/Caustic bAsins	904-77G, 904-79G	GW	YES	Al,Pb	NO	YES
D-12	New TNX Seepage Basin	904-102G	GW&SW	YES	Al	NO	YES
D-13	Old F-Area Seepage Basin	904-49G	GW&SW?	YES	Al,Cd	YES	YES
D-14	Old TNX Seepage basin	904-76G	GW	YES	Pb, Hg,Al	NO	YES
D-15	R-Reactor Seepage Basins	904-57G,-58G, 59G, 60G,-103G,-104G	GW	YES	Al,Cd,Pb,Hg	YES	YES
D-16	Road A Chemical Basin	904-111G	GW	YES	Hg, Pb	NO	YES
E-1	Tank 16	241-H	GW	NO	Al,Cd,Pb	YES	YES
E-6	Burial Ground Complex	643-E, 643-7E	GW	YES	Cd,Pb,Hg,Sb,Ni	YES	YES
E-10	Ford Building Seepage Basin	904-91G	GW&SW	YES	Pb,Al	YES	?
E-19	Silverton Road Waste Site	731-3A	GW	YES	AL,Pb,Be,Sb	NO	YES
E-20	TNX Burying Ground	643-5T	GW	YES	Hg,Pb,Al	YES?	YES

[a] - Section numbers referenced in RCRA Facility Investigation/CERCLA Remedial Investigation Workplan Summaries

[b] - GW - Ground Water
SW - Surface Water

[c] - Based on exceedances reported in 1992/1993 SRS Monitoring Reports

Table A.3.4 SRS Waste Sites where heavy metal concentrations in underlying ground waters exceed drinking water standards (based on data from the SRS Environmental Report for 1992, WSRC-TR-93-075).

Site	contaminant	unit	standard	maximum	#wells sa	#wells
A-Met Burn PIT	Pb	mg/l	0.015	0.029	6	1
M-Area HWMF	Sb	mg/l	0.005	0.015	41	2
	Pb	mg/l	0.015	0.073	42	5
	Hg	mg/l	0.0020	0.0024	42	1
	U	mg/l	0.0020	0.029	41	2
	toxic organics				42	40
	radionuclides				42	8
Misc. Chem Basi	Pb	mg/l	0.015	0.036	7	2
	radionuclides				7	1
	toxic organics				7	6
Motor Shop Oil B	Sb	mg/l	0.005	0.0085	7	2
	radionuclides				7	1
	toxic organics				7	6
Plume def. wells	Cd	mg/l	0/005	0.0081	203	2
	Pb	mg/l	0.015	0.14	204	12
	Hg	mg/l	0.002	0.0034	209	1
	radionuclides				208	20
	Toxic organics				210	111
Silverton Rd. Wa	Sb	mg/l	0.0050	0.0097	29	3
	Be	mg/l	0.0010	0.0043	29	1
	Pb	mg/l	0.015	0.040	29	5
	Radionuclides				29	1
	Toxic organics				29	5
C-Dis. Basin	Pb	mg/l	0.015	0.16	2	2
	radionuclides				2	2
C-Seep. Basin	Pb	mg/l	0.015	0.044	4	1
	tritium				4	4
K-Acid/Caustic B	Pb	mg/l	0.015	0.022	9	2
K- BR Pit	Pb	mg/l	0.015	0.033	4	2
K-Dis. Basin	Pb	mg/l	0.015	0.090	3	3
	radionuclides				3	3
K-Ret Bas.	Pb	mg/l	0.015	0.017	4	1
	radionuclides				4	4

Table A.3.4. (Cont'd)

Site	contaminant	unit	standard	maximum	#wells sa	#wells
L-acid/caustic &	Cd	mg/l	0.005	0.068	4	1
	Pb	mg/l	0.015	0.047	4	1
	Pb	mg/l	0.015	0.033	4	1
	Cd	mg/l	0.005	0.0059	4	1
	Te-99				4	1
	radionuclides				4	2
	toxic organics				4	2
L-BR pit	Pb	mg/l	0.015	0.069	4	3
L-Dis. Bas.	Pb	mg/l	0.015	0.074	2	2
L-RX Seep. bas.	Pb	mg/l	0.015	0.046	4	1
	tritium				4	3
P-BR Pit	Pb	mg/l	0.015	0.049	4	2
	toxic organics				4	1
P-CPRB	Pb	mg/l	0.015	0.034	4	1
P-Dis. Bas.	Pb	mg/l	0.015	0.093	2	1
	tritium				2	2
P-Seep Bas.	Pb	mg/l	0.015	0.047	7	5
	tritium				7	7
	toxic organics				7	1
R-Acid/Caustic b	Pb	mg/l	0.015	0.022	4	1
	radionuclides				4	1
R-Dis. bas.	Pb	mg/l	0.015	0.023	3	2
R-Seep Bas.	Cd	mg/l	0.005	0.096	21	16
	Pb	mg/l	0/015	0.020	21	1
	Hg	mg/l	0.0020	0.0080	21	6
	Sr-90				21	4
	radionuclides				16	50
	Sb	mg/l	0.005	0.0069	4	1
E-Area Haz. wast	Pb	mg/l	0.015	0.042	4	3
	tritium				4	1
Old Burial grnd	Cd	mg/l	0.0050	0.028	37	8
	Pb	mg/l	0.015	0.23	37	13
	Hg	mg/l	0.002	0.004	37	5
	radionuclides				48	35
	toxic organics				30	6

Table A.3.4. (Cont'd)

Site	contaminant	unit	standard	maximum	#wells sa	#wells
Radioactive wast	Pb	mg/l	0.015	0.033	45	2
	Hg	mg/l	0.002	0.0026	45	1
	Ni	mg/l	0.10	0.11	45	1
	radionuclides				45	33
	toxic organics				45	17
	more Pb &Sb					
F-Acid/Caustic B	Pb	mg/l	0.015	0.097	6	1
	radionuclides				6	2
F-Burma Rd. Rub	Pb	mg/l	0.015	0.025	5	4
	radionuclides				5	3
F-BRPits &RP	no metals					
F-canyon etc.	Pb	mg/l	0.015	0.86	10	5
	Sr-90				9	2
	Cs-137				3	1
	Toxic organics				10	6
	Radionuclides				10	5
F-CPRB	Pb	mg/l	0.015	0.15	5	3
	Radionuclides				5	1
	toxic organics				5	1
F-Process Sewer	Pb	mg/l	0.015	0.020	3	1
(inactive)	radionuclides				3	2
	toxic organics				3	2
F-Seep basin	Sb	mg/l	0.005	0.012	67	3
	Cd	mg/l	0.005	0.037	67	19
	Pb	mg/l	0.015	0.13	67	19
	Hg	mg/l	0.002	0.012	67	6
	Ni	mg/l	0.10	0.38	67	3
	U	mg/l	0.02	5.4	67	27
	radionuclides				73	58
	toxic organics				27	2
	NO2+NO3-N	mg/l	10	885	67	45
	As	mg/l	0.050	0.096	18	1
F-sludge land Ap	Pb	mg/l	0.015	0.15	4	3
	Hg	mg/l	0.002	0.0058	4	1
	radionuclides				4	2

Table A.3.4. (Cont'd)

Site	contaminant	unit	standard	maximum	#wells sa	#wells
Old F seep bas.	Sr-90				4	2
	U	mg/l	0.02	0.077	4	1
	radionuclides				4	3
	toxic organics				4	2

H-Canyon Bldg.	Pb	mg/l	0.015	0.063	4	2
	radionuclides				4	4
	toxic organics				4	2
H-CPRB	Pb	mg/l	0.015	0.036	4	2
	radionuclides				4	4
H-inactive Proces	Pb	mg/l	0.015	0.020	7	1
	tritium				7	7
H-Ret. basins	Sb	mg/l	0.005	0.012	5	2
	Pb	mg/l	0.015	0.024	5	2
	radionuclides				5	4
H-Seep bas.	Sb	mg/l	0.005	0.013	108	13
	As	mg/l	0.050	0.10	108	2
	Cd	mg/l	0.005	0.0098	108	1
	Co-60				40	10
	Pb	mg/l	0.015	0.071	108	13
	Hg	mg/l	0.002	0.0079	108	15
	NO2+NO3-N	mg/l	10	90	108	49
	radionuclides				113	88
	toxic organics				26	5
H-area tank farm	Cd	mg/l	0.005	0.34	25	9
	Pb	mg/l	0.015	1.0	32	19
	Hg	mg/l	0.0020	0.0039	32	8
	radionuclides				32	18
Z-Area Saltstone	Sb	mg/l	0.005	0.008	3	1
N-BR Pits	Pb	mg/l	0.015	0.054	4	1
N-Diesel Spill	Pb	mg/l	0.015	0.021	9	1
	toxic organics				5	3
Ford Bldg Seep	Pb	mg/l	0.015	0.022	5	1
	radionuclides				5	1
Hydrofluoric Aci	Pb	mg/l	0.015	0.040	4	1
D-CPRB & Ash b	Cd	mg/l	0.005	0.030	14	2
	Cr	mg/l	0.10	0.82	14	3
	radionuclides				14	4
	toxic organics				14	4

Table A.3.4. (Cont'd)

Site	contaminant	unit	standard	maximum	#wells sa	#wells
TNX Burying Gro	Pb	mg/l	0.015	0.016	5	1
	Hg	mg/l	0.002	0.0029	5	1
	radionuclides				5	2
	toxic organics				5	2
G-Area CMP Pits	Pb	mg/l	0.015	0.073	19	5
	toxic organics				19	3
	radionuclides				19	1
IWT sites	Pb	mg/l	0.015	0.026	2	1

Par sludge land a	Pb	mg/l	0.015	0.015	4	1
NPR site	Cd	mg/l	0.005	0.008	14	1
	Pb	mg/l	0.015	0.044	14	2
	radionuclides				14	1
Road A Chem. Ba	Pb	mg/l	0.015	0.054	5	3
	Hg	mg/l	0.002	0.0027	5	1
Sanitary Landfill	Sb	mg/l	0.005	0.012	30	21
	Cd	mg/l	0.005	0.031	42	2
	Pb	mg/l	0.015	0.021	42	1
	Hg	mg/l	0.002	0.0029	42	1
	radionuclides				56	4
	toxic organics				56	15

Table A.3.5. Maximum concentrations (mg/l) of selected heavy metals based on samples collected from Coal Pile Run Off Basins (CPRBs) at SRS during three studies. Area of CPRB shown in parenthesis.

Metal	DW Std.[a]	Study#1[b]	Study#2[c]	Study#3[d]	% DW Std.
Al	0.050	264 (D)	NS[e]	56.8 (D)	528000
As	0.050	0.077 (D)	0.10 (D)	0.086 (D)	200
Be	0.004	0.0274 (D)	NS	0.014 (D)	685
Cd	0.005	0.047 (D)	0.056 (D)	0.024 (D)	11200
Cr(III)	0.100	0.222 (D)	0.42 (D)	0.035 (D)	420
Cu	1.3	1.395 (D)	NS	0.296 (A)	107
Hg	0.002	0.00036 (K)	0.005 (D)	NS	250
Ni	0.100	4.7 (D)	NS	0.657 (D)	4700
Pb	0.015	0.0149 (C)	0.09 (H)	0.049 (D)	600
Se	0.05	0.018 (H)	0.05 (D)	0.200 (D)	400

[a] National drinking water standard. All are primary except Al which is secondary.

[b] O'Brien and Gere 1987.

[c] Corbley, A. L. 1992.

[d] Wilde, et. al., 1994 (unpublished data)

[e] Not Sampled

Table A.3.6. Past and Present Activities Within Principal Areas of SRS

Area	R	S	FF	RW	SW	L
A	-	-	-	X	X	X
B	-	-	-	-	X	X
C	X	-	-	-	X	-
D	-	-	-	X	X	X
E	-	-	-	X	-	-
F	-	X	-	X	X	X
G*	-	-	-	X	X	X
H	-	X	XT	X	X	X
K	X	-	-	-	X	-
L	X	-	-	-	X	-
M	-	-	XR	X	X	X
N	-	-	-	X	X	-
P	X	-	-	-	X	-
R	X	-	-	-	-	-
S	-	-	-	X	X	X
TNX	-	-	-	-	X	X
Y	-	-	-	-	-	-
Z	-	-	-	X	-	-

Activities Code:

R	Reactor
S	Separations
FF	Fuel Fabrication (R=reactor fuels; T=tritium)
RW	Radioactive or mixed waste management
SW	Sanitary waste treatment
L	Laboratory activities

*Facilities not confined to any of the other specific areas are collectively considered to exist in G-Area.

Table A.3.7 Metal-Containing Wastes at SRS

Area	Description	Radioactive?	Metals	Volume	Currently Generated?	Analysis Available?	Current Treatment	Additional Treatment Needed?
A	Photo lab waste	No	Ag	1200-1600 gal/yr	Yes	Sampled post-treatment	Ion exchange (A-Area Ag Recovery Unit)	No
A	Medical Dept. photo waste	No	Ag	350 gal/yr	Yes	Sampled post-treatment	Ion exchange (N-Area Ag Recovery Unit)	No
A	SRTC lab wastes	Yes (low level)	Hg, Cr, U, Pu	52,000 gal on hand	Yes	Yes	Ion exchange, storage	Eventual disposal via LLW (haz.) and landfill (nonhaz.)
A	Wastewater Neutralization Facility	No	Hg	5000 gal on hand	No	Yes	Ion exchange upon demand	No
A	Met Lab wastes	No	Ni, Cr, Fe, Al, Cu, others?	4 gal/yr now, up to 20 gal/mo future	Yes	In progress	Neutralization, storage	Procedure being estab. to ppt. Cr, allowing discharge to sanitary sewer
A, B, C, D, F, G, H, K, L, P, S, TNX	Sanitary wastewater treatment plants	No	Pb, Zn, Cu, Al	Up to 4,000,000 gal/day (H Area)	Yes, but some will shut down in March '95	Yes	Conventional wastewater treatment , land application	Not at present. Metal levels occas. near land application limits
C	Disassembly basin	Yes	Cs, Pu, Al, Fe, others	3,550,000 gal present	No	Partial	None	Yes

Table A.3.7 (Cont'd).

Area	Description	Radioactive?	Metals	Volume	Currently Generated?	Analysis Available?	Current Treatment	Additional Treatment Needed?
D, N	Hg thiocyanate lab waste	Yes	Hg	146 drums on hand	No	Yes	Ion exchange (Duolite GT-73)	Columns tend to plug
D	Water Quality Lab waste	Yes	Hg	?	yes	yes	Heavy water recovery	No
F	Separations wastes	Yes	Similar to H-Area separations waste	Current: 5000 gal/mo. Future: 20,000 gal/mo.	On small scale	Yes	ITP, ETF, immobilization	No
F	Separations-FB-Line Waste (from chillers)	Yes	Pu, Cr	27 gal. on hand	Intermittently	In prep.-NMPSB 0930009	Recycled or sent to Mixed Waste Storage Facility	No
F	Cooling maintenance shop	No	Pb	<1 drum/yr	Yes	In prep.	Recycling & reuse	No
F	Separations-Evaporator overhead	Yes	Similar to other separations wastes, but lower conc.	formerly 160,000 gal/d, very little currently	Yes	Yes	ETF	No
H	CIF blowdown water	Yes	Pb, Sr, Cs, Hg, poss. others	75,000 gal/yr projected	Projected for 1996	Predicted, WSRC TR 93623	Solidification @ Saltstone	Volume reduction desirable

Table A.3.7 (Cont'd).

Area	Description	Radioactive?	Metals	Volume	Currently Generated?	Analysis Available?	Current Treatment	Additional Treatment Needed?
H	Separations wastes	Yes	Current: Pu, Fe. During normal operation: Pu, Sr, Cs, Hg, Cr, U, Fe, Al, Ag, Ba, Tc, Pm, Ni, Th	Current: 5000 gal/mo. Previous : 30,000 gal/mo.	On small scale	Partial	ITP, ETF, DWPF (vitrification),Salt stone (concrete)	No
H	Separations-Filter backwash water, resin regeneration water	Yes	Al, Zn, Cs, Se, Co	29,000-45,000 gal/mo.	Yes	Yes	pH adjustment, sent to tank farm (hi level) or GP evaporator (lower level)	No
K	Disassembly basin	Yes	Cs, Pu, Al, Fe, others	3,400,000 gal present	Yes (contam. continues due to presence of fuel)	Yes	ion exchange sludge vacuumed	Disposal of eluted contaminants, resin, sludge
L	Disassembly basin	Yes	U, Th (sludge), Cs, Zn (water)	3,400,000 gal present	Yes (contam continues due to presence of fuel)	Yes SRTC-ADS-93-0411	ion exchange, sludge vacuumed	Disposal of eluted contaminants, resin, sludge
M	Analytical and Metallurgy Lab effluents	No	As, Ba, Cu, Ag, Pb	260 gal/wk.	Yes, but will move soon	Yes	DETF (metal ppt. then pressure filt.	No

Table A.3.7 (Cont'd).

Area	Description	Radioactive?	Metals	Volume	Currently Generated?	Analysis Available?	Current Treatment	Additional Treatment Needed?
N	Photographic wastes: EBASCO Svcs., Medical Dept., Paint Shop, Document Ctrl.	No	Ag	350 gal on hand	Yes	Sampled post-treatment	Ion exchange (N Area Ag Recovery Unit)	No
N	Wash water-bubble tower and gas tank cleanup	No	Hg, Cr	4 drums on hand	No	Yes	offsite vendor	No
P	Disassembly basin	Yes	Cs, Pu, Al, Fe, others	4,800,000 gal present	Yes (contam. continues due to presence of fuel)	Yes	Ion exchange, sludge vacuumed	Disposal of eluted contaminants, resin, sludge
R	Disassembly basin	Yes	Cs, Pu, Al, Fe, others	4,500,000 gal. present	No	Yes	None	Yes
S	Lab & process wastewater (from waste immobilization activities)	Yes	Hg (current), various radionuclides (future)	200,000 gal present	Cold runs only	Yes- DHEC permit proposal, CPES document	Vitrification/storage. Steam stripping/reduction of Hg	No
TNX	Lab waste from IDMS sample analyses	No	Hg	2320 gal/yr	Yes	Yes	Offsite vendor	No

Table A.3.7 (Cont'd)

Area	Description	Radioactive?	Metals	Volume	Currently Generated?	Analysis Available?	Current Treatment	Additional Treatment Needed?
Z	Saltstone facility wastes (from waste immobilization activities)	Yes (low leve/mixed waste)	Cs^{137}, misc. beta/gamma, Cr(VI)	Current: 15,000 gal/mo, future: 3-6 X 10^6 gal/yr	Yes	WSRCTR94-0364 (in prep)	Immobilization/ storage	No

TABLE A.3.8 ASSISTING PERSONNEL FOR WASTE SITE AND WASTE STREAM IDENTIFICATION AND CHARACTERIZATION

Numerous SRS personnel contributed information during our investigation to identify metal contaminated wastewaters on the site. Many of them are listed below:

Name	Organization
R. Aylward	ER
J. Baker	Reactor Engineering RBOF
D. K. Beasley	Solid Waste
R. Beck	HLW/WWPF
C. Bennett	EPD
N.N. Bhatt	Water Quality Laboratory
J. P. Bibler	T IWT
G. Blount	ER
D. Boring	HP
B. Boulineau	Photographic Services
D. Bowman	ROD
E. Brass	ST
P. Brooks	Heavy Water
D.B. Burns	CIF
B. Bush	HB-Line
J. Chen	Saltstone
D. Clark	ER
A.L Corbly	ESS
D. Costner	Separations
B. Culligan	T LS
W. Daugherty	MTS
R.W. Deible	RE
E.L. Dunbar	HLW F Tank Farm
G. Froidl	Central Shops
S. Fuller	CS
T. Gaughan	ER
G.K. Georgeton	HLW Engineering
A. Gibbs	RT
J. Gladden	SRTC/ESS
M.D.D. Goodman	HLWE H-ETF
N. Halverson	T LS
J. Hammock	ER
W. F. Harlow	Separations
L. Haselow	ER
C.R. Hayes, Jr.	EPD
H. Hickey	ER

J. Howell	SRTC
R. Huffines	PE
M.S. Jackson, Jr.	HLW/DWPF
R.W. Jackson	Separations H-Canyon
K. Jerome	ER
W. Johnson	ER
W.H. Jones, Jr.	Bechtel Construction
C. Knapp	ER
K.Kuelske	ER
C. Langton	SRTC
K. Lewis	ER
B.B. Looney	SRTC/ESS
H.L. Martin	RME
J. Mayer	EPD
H. Moore	HLW/DWPF
B. Myers	Reactor Engineering
M. Newman	SREL
R. Nichols	SRTC/ESS
T.O. Oliver	Sep RBOF
W.L. Payne	EPD
C. Pickett	F/H Tech Support
J. Pickett	RME&T
R. Plunkett	ER
L.K. Pressley	Separations Maintenance
J.R. Price	DWPF
O.D. Rosier	Reactors
G. Rucker	ER
H. Schultz	RM
B.D. Silas	Environ. & Chem. Systems
D. Simmons	T LS
W.R. Sims	ER
D.K. Singer	Site Services
D.P. Skiff	Reactors
S.E. Smith	Environment & Water
R. Soucha	ER
S.M. Spearman	SRTC
W. Specht	T ES
P.J. Spitzer	Separations
S.O. Stallings	Reactor Materials
C.A. Stanford	DWPF
K. Steeg	FB-Line
C. Strogan	SREL
G. Swisstack	ST
D. Thompson	Z/Saltstone
J. Travis	SSE

L. Turner	Separations F-Canyon
K. Ward	ER
F.A. Washburn	Environ. Restoration
R .W. Weigel	EPD
M. Whitaker	SRTC/ADS
K. Wise	ER
D. M. Wittry	RME

APPENDIX 4

SELECTION AND CULTURING OF ALGAL STRAINS

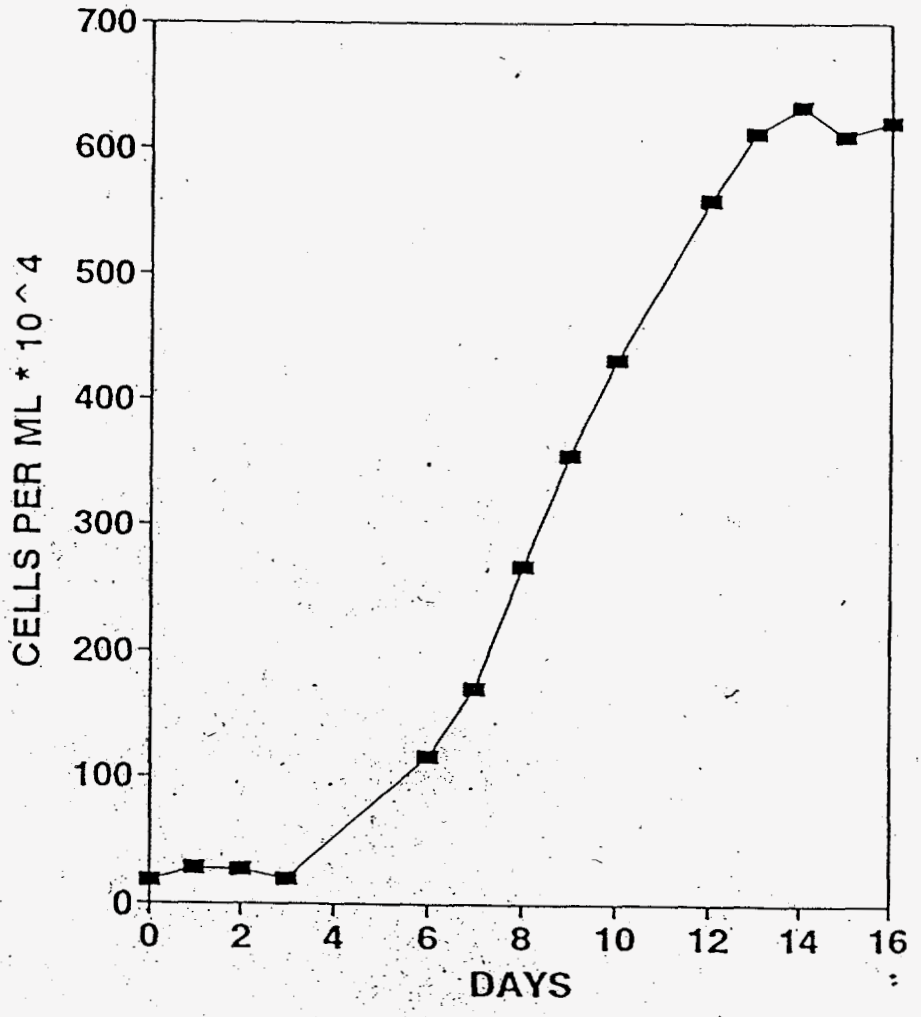


Figure A.4.1 Initial growth curve for *Chlorella capsulata*

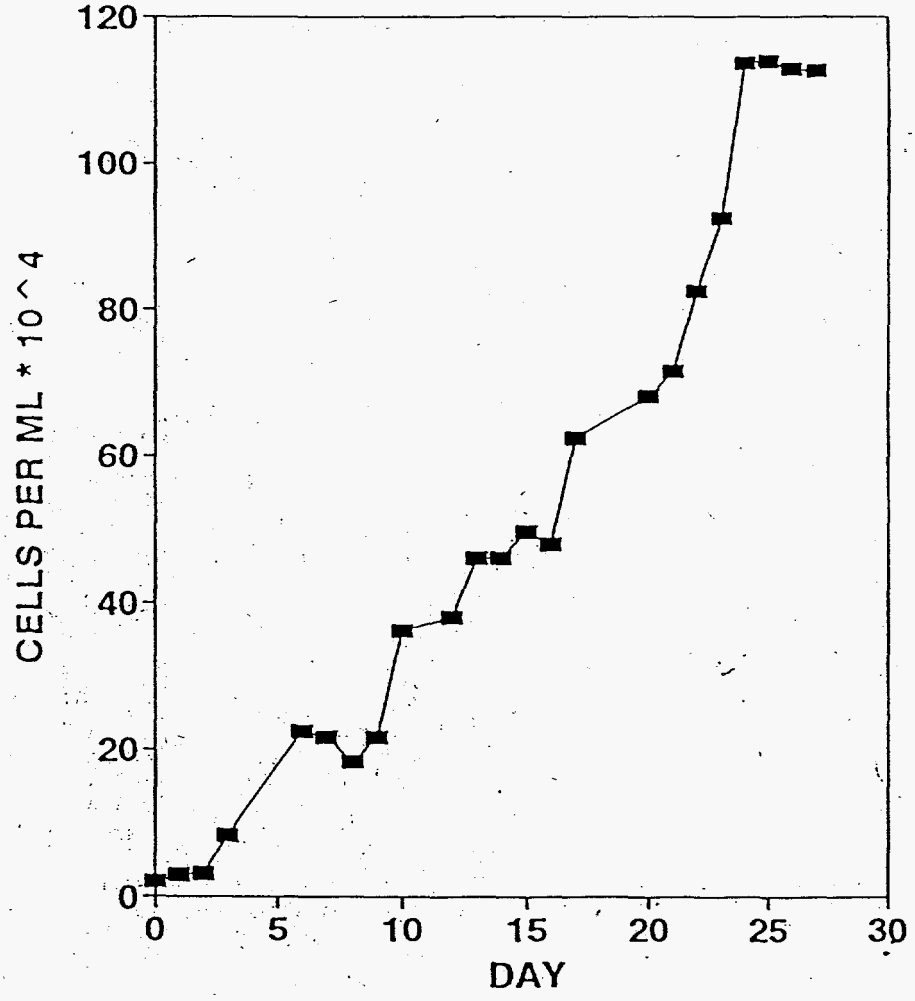


Figure A.4.2 Initial growth curve for *Chlorella fusca* var. *vacuolata*

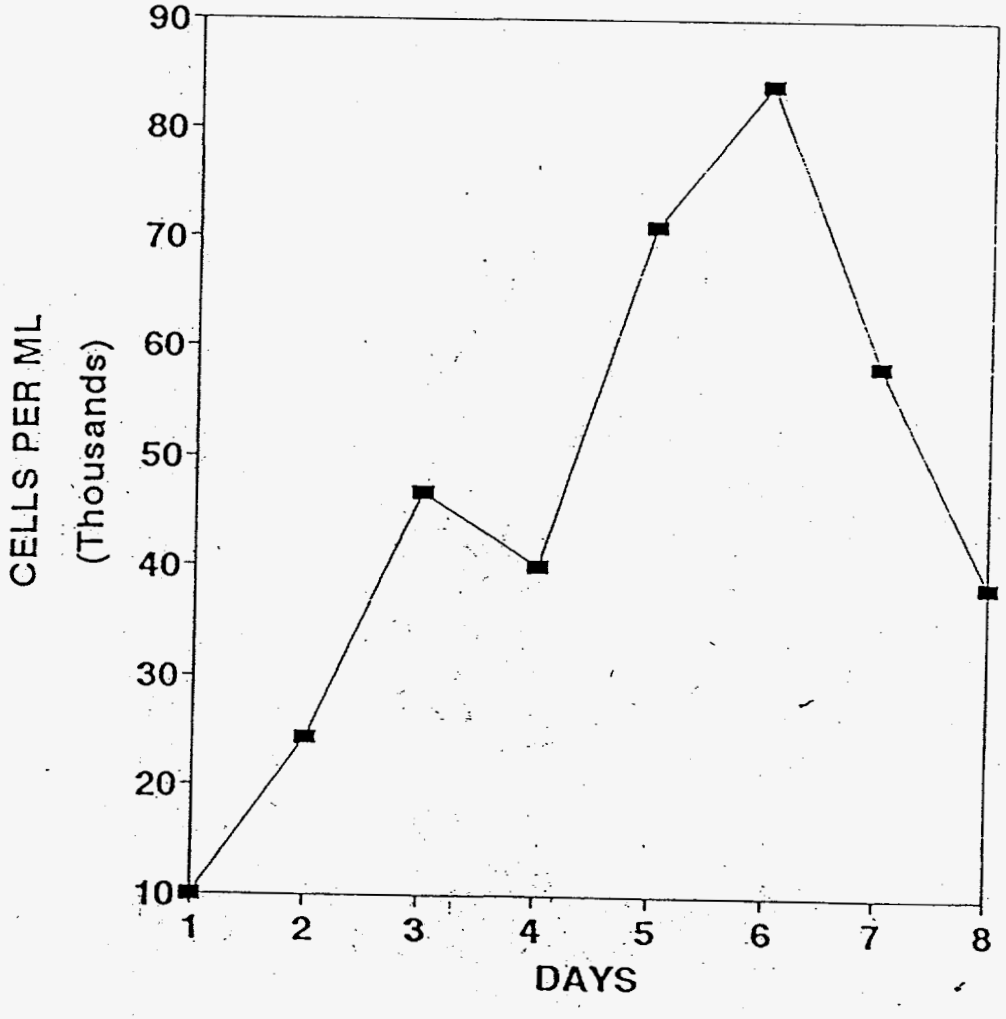


Figure A.4.3 Initial growth curve for *Amphiprora paludosa*

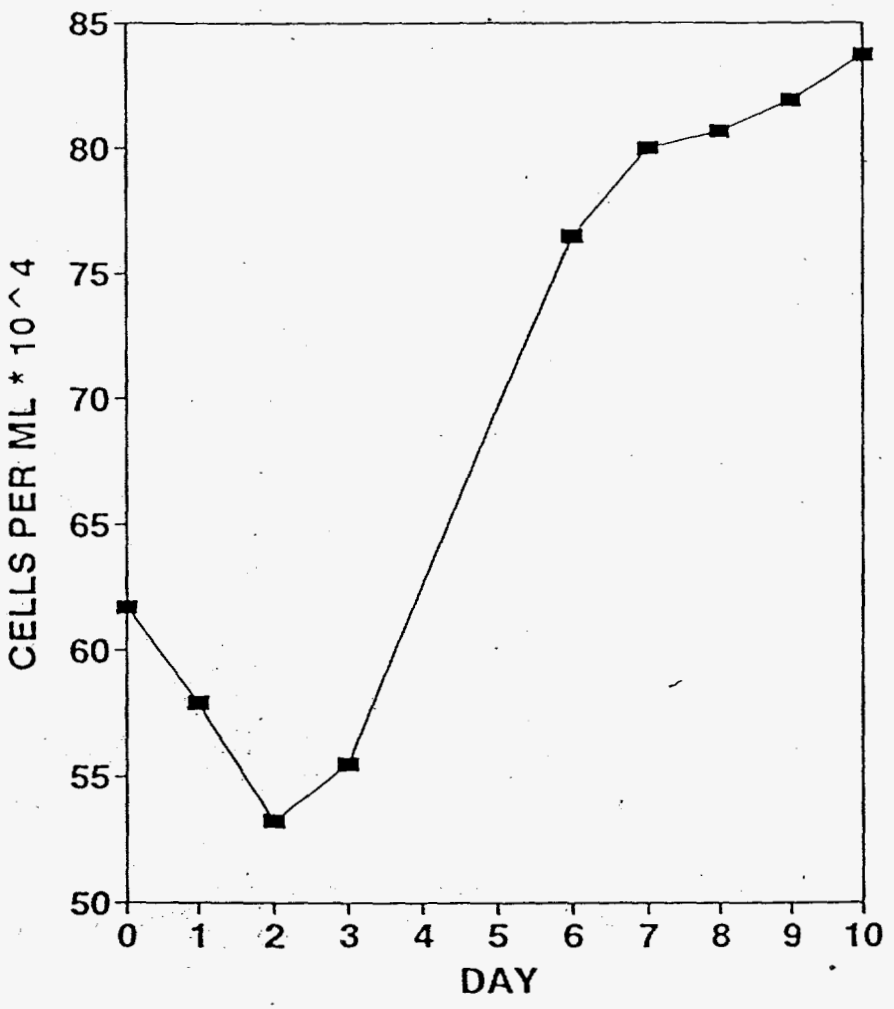


Figure A.4.4 Initial growth curve for *Chaetoceros gracilis*.

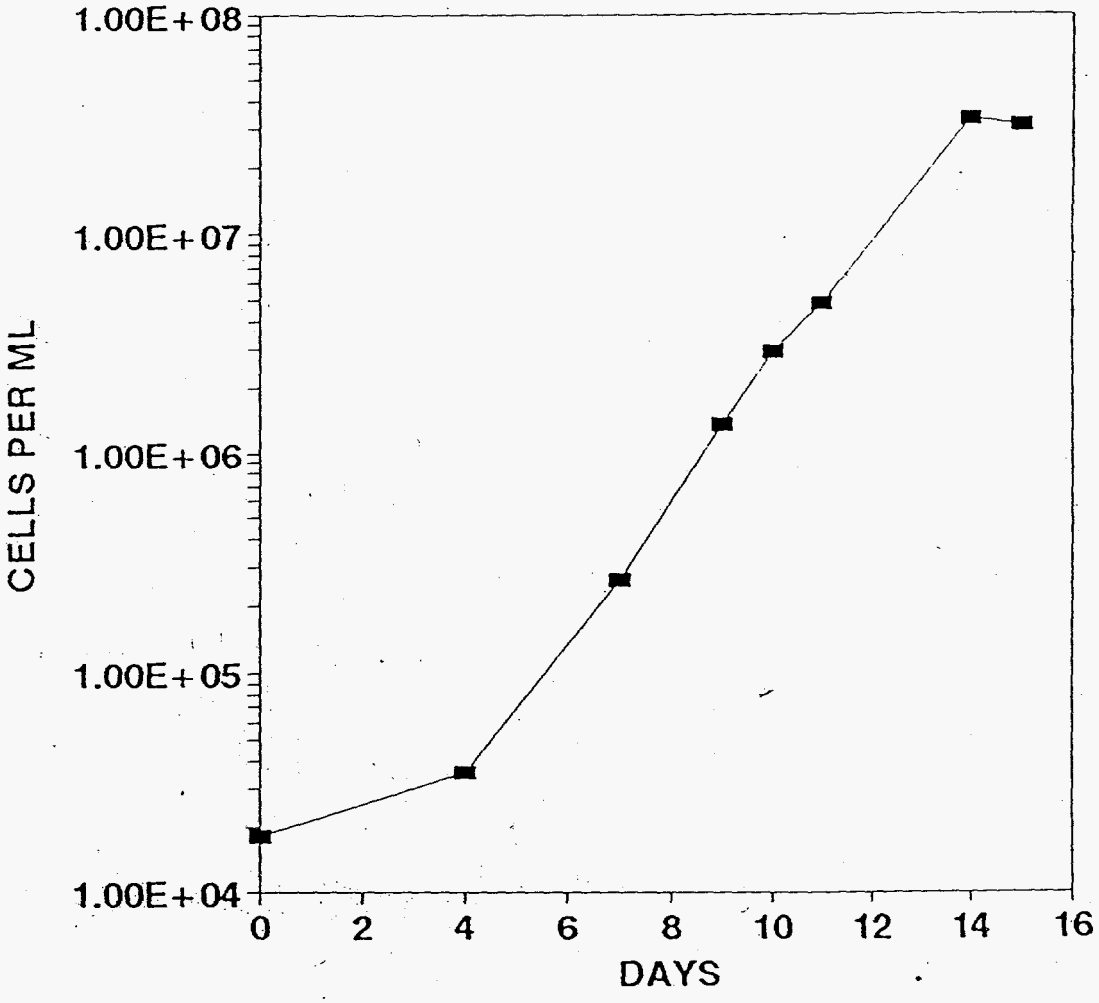


Figure A.4.5 Initial growth curve for *Navicula pelliculosa*.

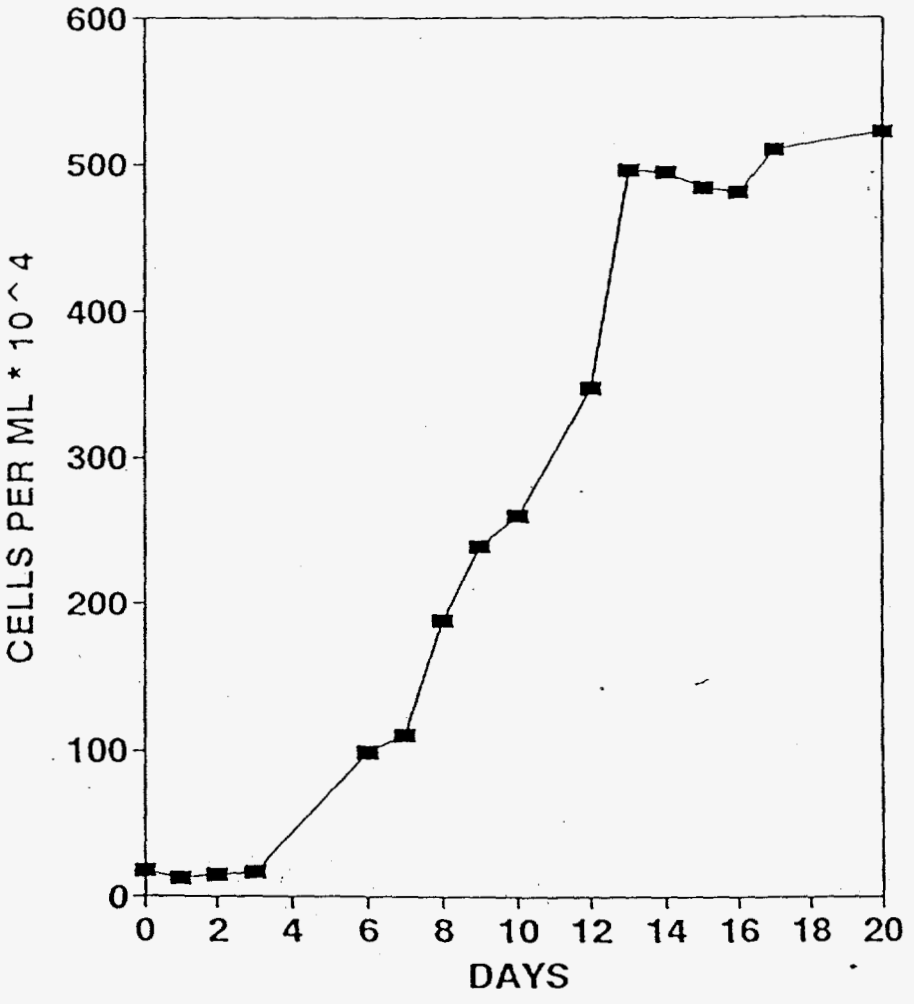


Figure A.4.6 Initial growth curve for *Phaeodactylum tricornutum.*

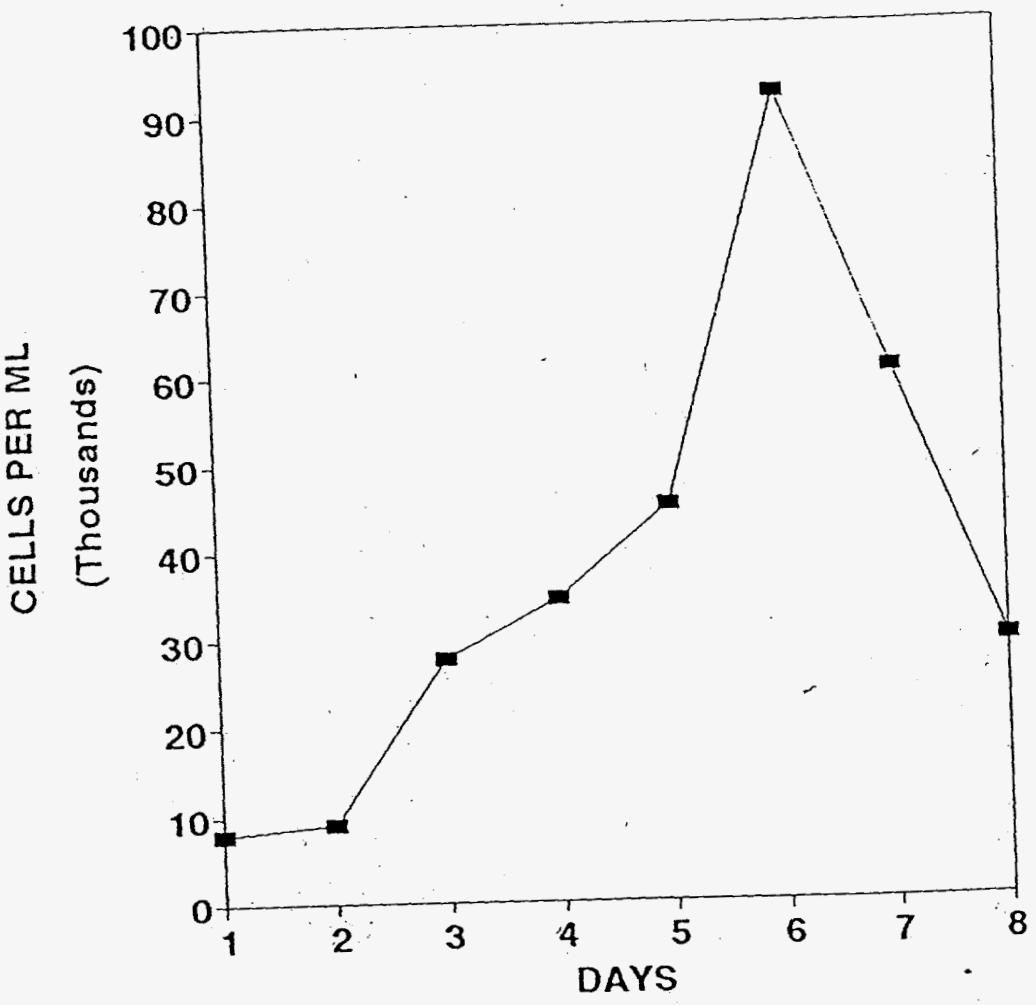


Figure A.4.7 Initial growth curve for *Surirella ovata*.

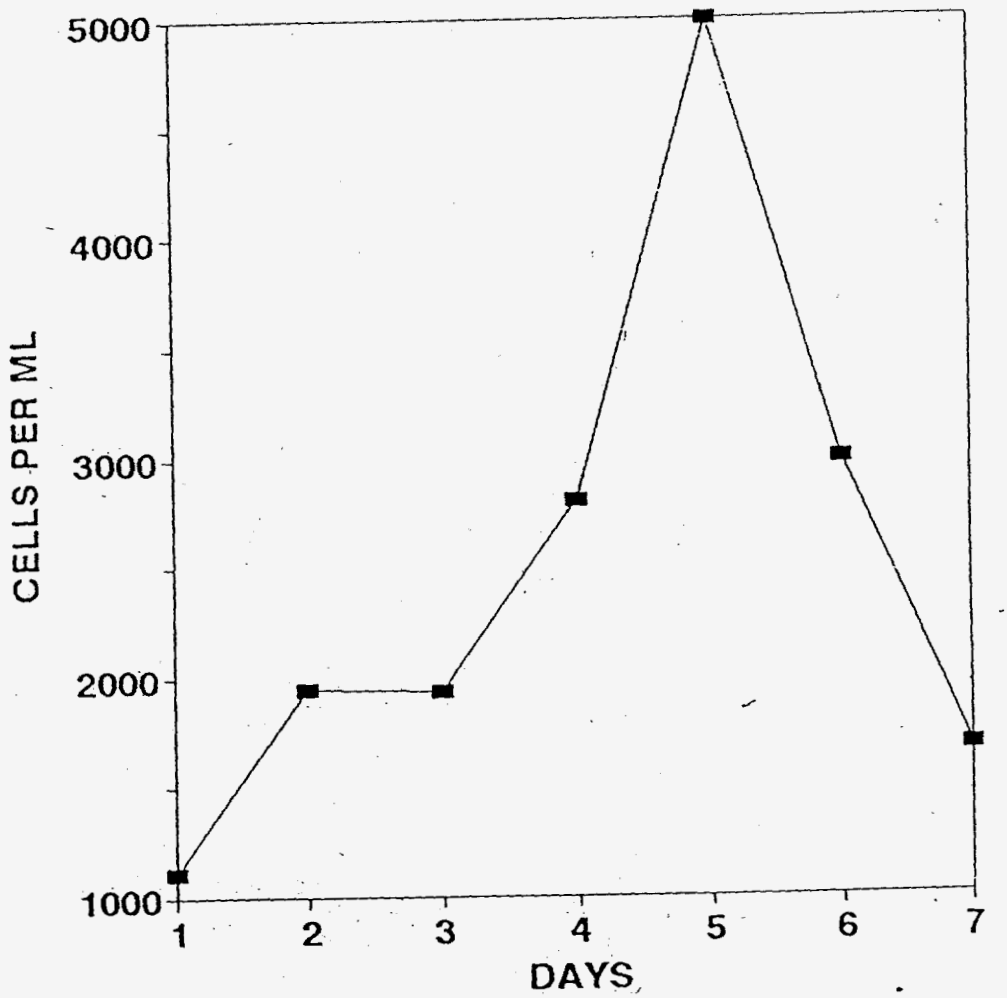


Figure A.4.8 Initial growth curve for *Peridinium trochoideum*.

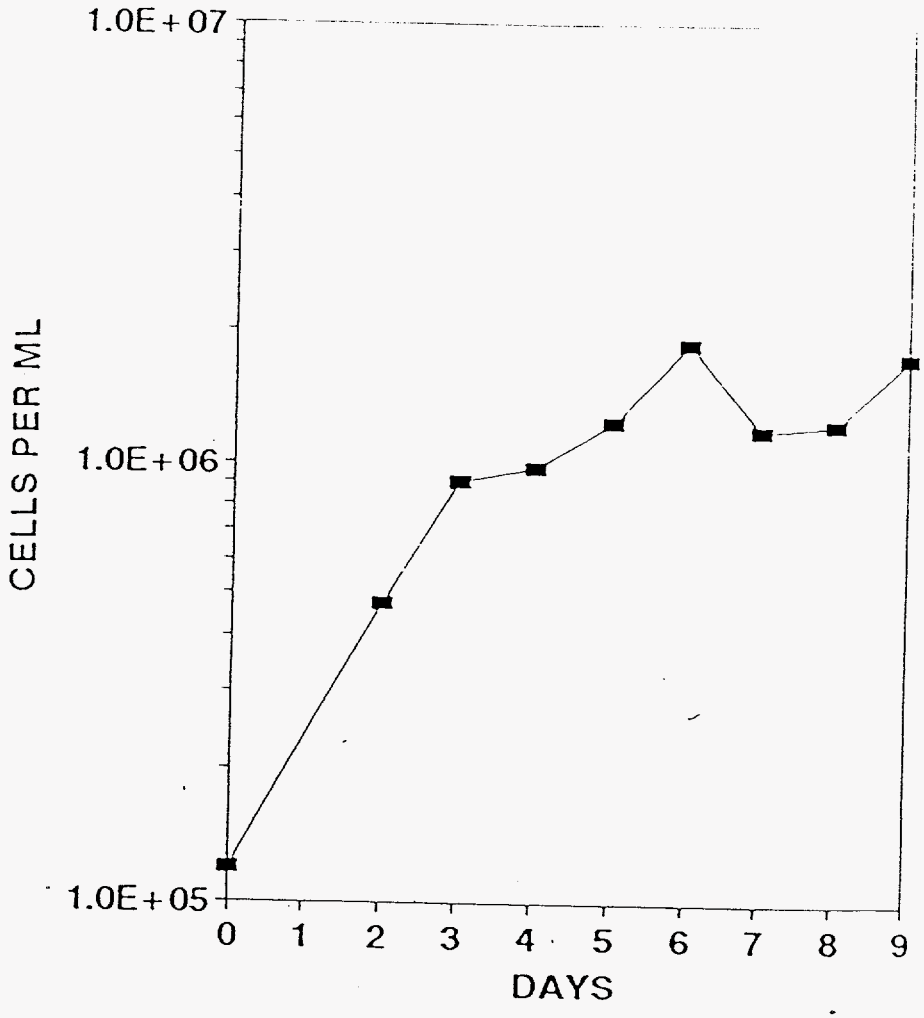
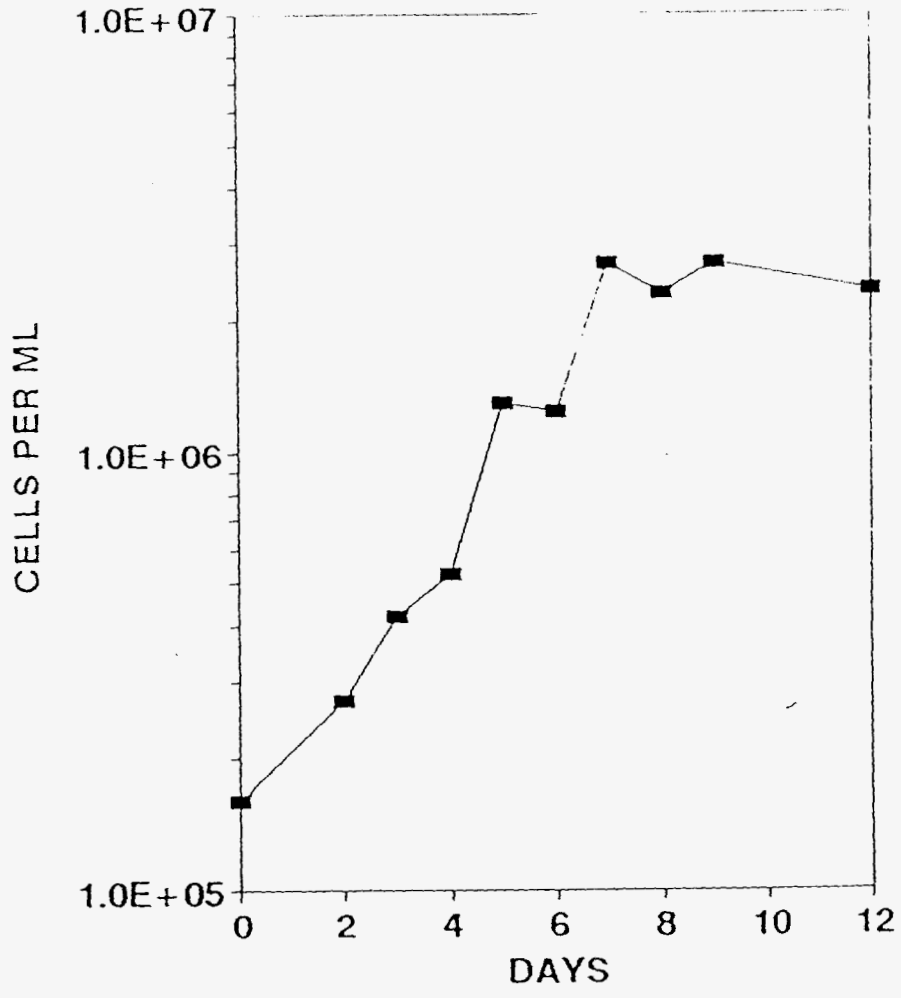


Figure A.4.9 Large volume growth curve for *Chlorella capsulata*.



FigureA.4.10 Large volume growth curve for *Phaeodactylum tricornutum*.

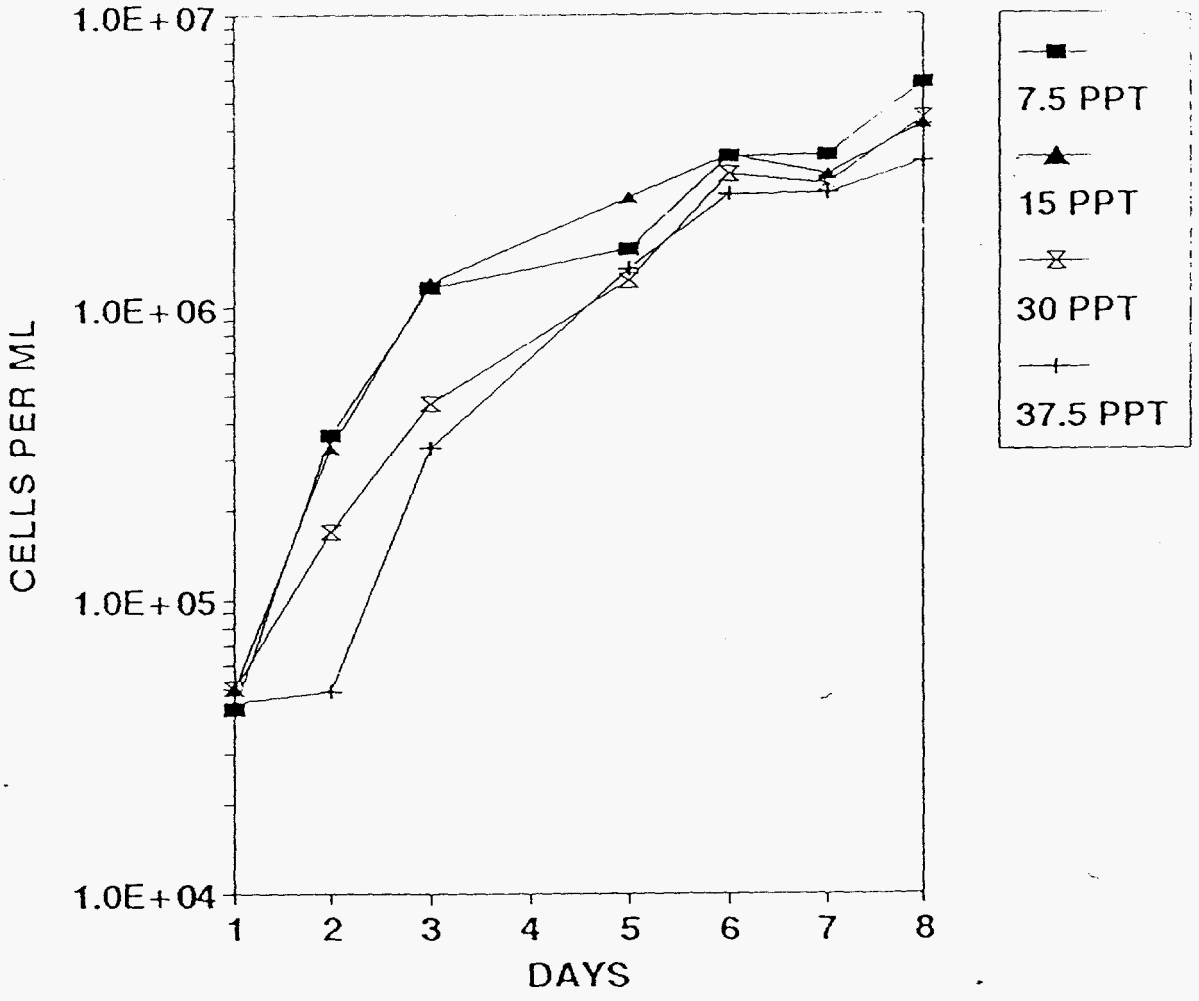


Figure A.4.11 Effect of salinity on the growth curve of *Navicula pelliculosa*.

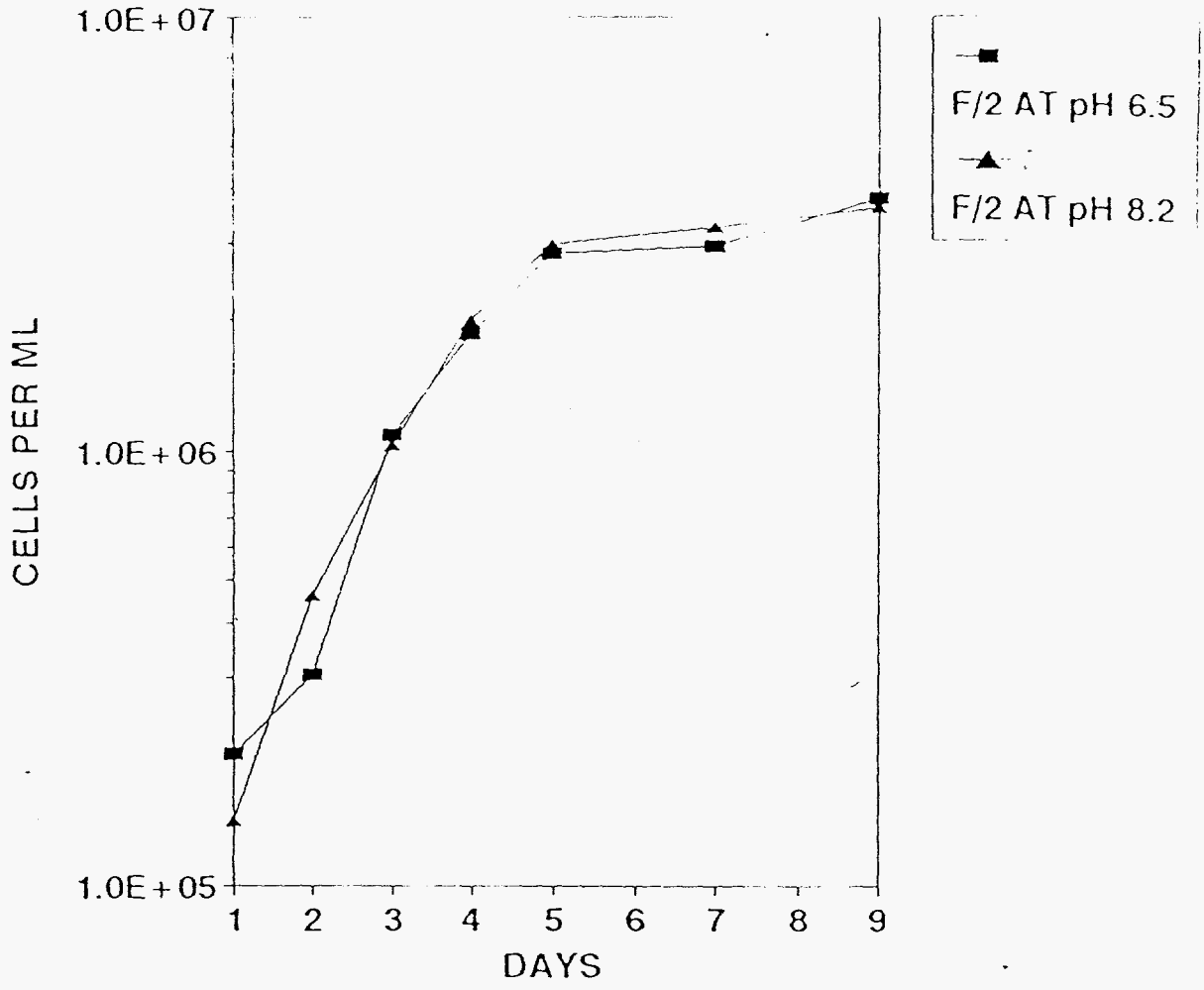


Figure A.4.12 Effect of pH on the growth curve of *Navicula pelliculosa* grown in F/2 media.

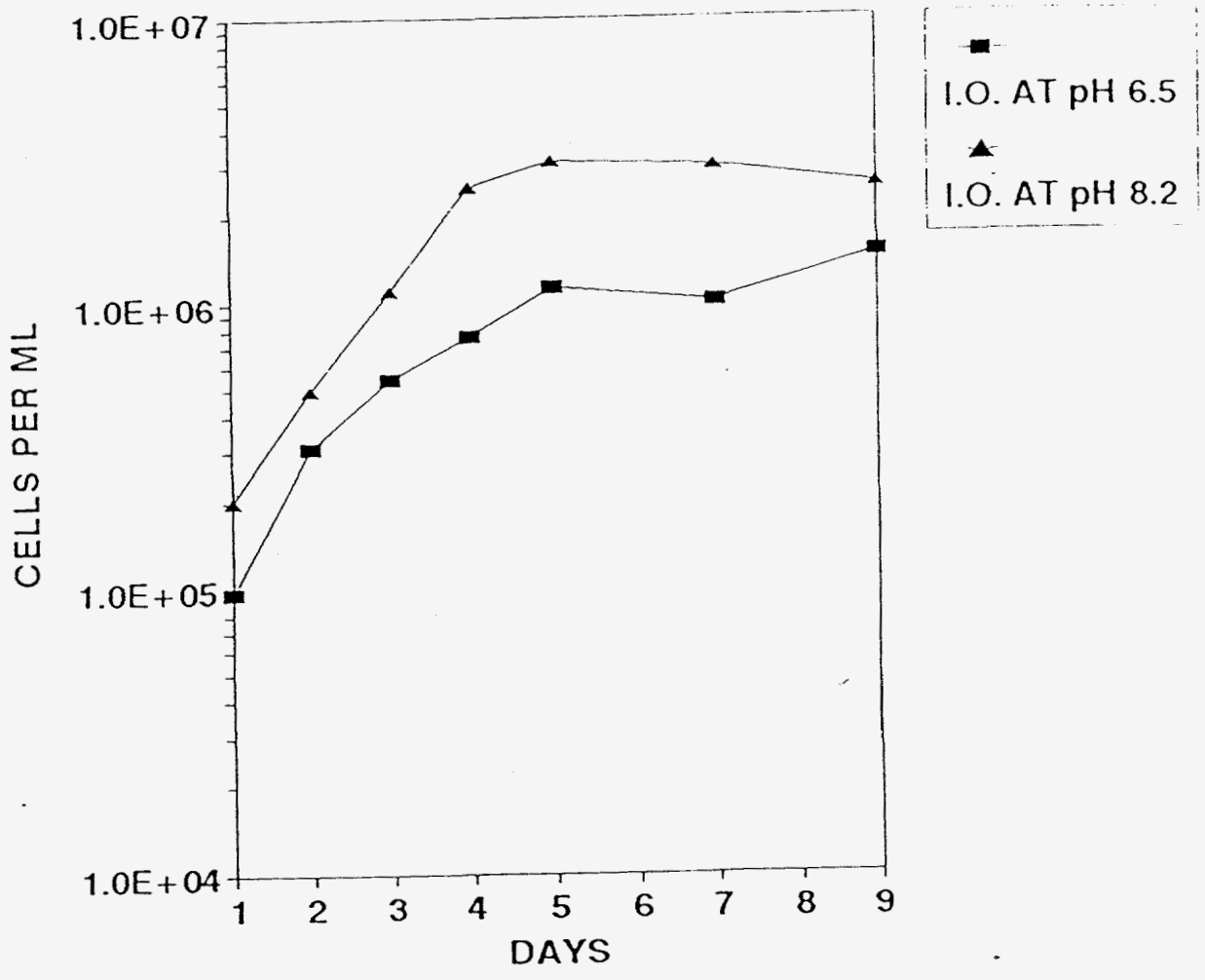


Figure A.4.13 Effect of pH on the growth curve of *Navicula pelliculosa* grown in F/2 enrichment salts added to "Instant Ocean" solution.

APPENDIX 6

BIOREMOVAL CAPABILITIES OF FOAM- EMBEDDED MICROBES

APPENDIX 6A METHODS AND RESULTS FROM 14 EXPERIMENTS

Table A.6.1 Experimental Procedures and results for Experiment 1

Test Plan for Experiment 1 11/8/94

TESTING WITH EIGHT CHAMBERED TEST ASSEMBLY CONSTRUCTED BY FRISBY USING WASTEWATER FROM THE D- AREA COAL PILE RUNOFF BASIN

Experiment 1A

General Experimental Protocol

The first experiments (1a and 1b) with the Frisby Test rig will be conducted using waste water from the D-Area coal pile runoff basin (D-CPRB). There are 11 metals of concern in D-CPRB water: Al, Be, Co, Ni, Zn Cd, Cr, As, Cu, Pb, and Se. All of these will be measured in triplicate by SRTC/ADS following the passage of selected wastewater volumes through the test assembly using various formulations of filter media to assess metal removal efficacy. Flow rates, pressure drop and total accumulated flow volumes through each chamber will be monitored by pressure meters and flow/totalizer meters attached to each chamber. Collections of treated wastewater for metal analyses will be extracted from each chamber effluent line at four time intervals (nominally 10, 30, 60, & 120 minutes). Untreated water samples will be used as controls.

Part

Objective: Test system with wastewater but no filter media to evaluate the integrity of the test system in terms of:

leaching of metals from test system,
plating of metals on test system, and
comparability between cartridges (chambers)

Experimental Procedure:

1. Select sampling location at the D-CPRB.

2. Collect about 34 gallons of D-CPRB water by filling eight 6-gallon carboys to the 4-gallon mark and one carboy to about the 2-gallon mark.

3. Measure pH, and other water quality parameters at the time of collection using a Horiba Water Quality Checker.

4. Record collection data in lab notebook and return to lab.

5. Place two 50 ml aliquots of the raw waste water into labeled bottles (to be used as controls)

6. Calibrate and flush test assembly with DI water and adjust flow to 0.03 gpm through each chamber. Discard DI water used to flush system.

7. Add 4 gallons of wastewater to each of eight feed tanks. Start pumps and feed wastewater at a rate of 0.03 gpm through the eight test chambers in a recycling mode. Collect 50 ml samples from the downstream side of each of the eight test chambers after 10, 30, 60, and 120 min time intervals (representing approximately 0.3, 0.9, 1.8, and 3.6 gallons) and place in labeled bottles. Maintain flow at 0.03 gpm throughout run (by adjusting needle valves if necessary) and record pressure gage readings at 10 minute intervals. Also record accumulative flow at each sample collection time as stated above.

8. Preserve the 34 samples with acid HNO_3 (0.1ml concentrated acid per sample bottle) for future ADS for analyses of the 11 metals described above.

9. Shut down test rig and re-zero flow/totalizer meters making sure all data are recorded in lab notebook.

Experiment 1b

Objective: Select an initial particle size configuration for subsequent experiments using foam and determine metal uptake by plain foam w/ no algae.

Experimental Procedure:

1. Have Frisby prepare two disks 1.27 cm (0.5 in) thick and 5.08 cm (2.0 in) in diameter using a "standard" density. Prepare another batch of foam of the same density. Grind and sieve this batch of material to get three additional pairs of samples having three different particle size configurations and weighing the same as the two disks. Thus, the end result should be four pairs of samples all weighing the same, all made from chemically identical foam prepared to the same density, and representing four different particle size configurations.

2. Pack the foam samples from step #1 into the eight test cartridges (one of each pair of duplicates samples on opposing sides of the test assembly).

3. Use same D-Area CPRB water as in Experiment #1. Restart system and maintain flow at 0.03 gpm while monitoring pressure drop and flow accumulations (volumes) through each test chamber for two hours while collecting chamber effluent samples at intervals of 10, 30, 60, and 120 minutes.

4. Preserve the 32 50-ml samples along with two more controls from collection jug with original controls and take all samples to ADS for analyses.

Table A..6.1 (Cont.)

Exp. 1- 1st run with test rig, 11-8-94

PARAMETERS

Effluent	D coal runoff	*Turb*	-10
Date coll.	11/8/94	*DO*	8.3
pH	2.4	*Temp*	17.7
Cond.	3.18 mS/cm	*Sal*	0.15%

Run 1 (To determine uniformity of columns)

Biosorbent	None
Duration	30 min
Mode	Recirc
Flow rate	0.30 gal/min.

Column	Sample	Al	As	Be	Cd	Co	Cr	Cu	Fe	Ni	Pb	Se	Zn	Flow gal.
Raw	1-0A	82.17	<0.1	0.0261	0.0172	0.3327	0.0449	0.2258	471.3	0.9301	<0.05	<0.1	1.968	
water	1-0B	83.09	<0.1	0.0261	0.0157	0.3201	0.0473	0.2278	475.7	0.9235	<0.05	<0.1	1.917	
	Mean	**82.63**	**<0.1**	**0.0261**	**0.01645**	**0.3264**	**0.0461**	**0.2268**	**473.5**	**0.9268**	**<0.05**	**<0.1**	**1.9425**	
1	1-1A	78.39	<0.1	0.0249	0.0161	0.5787	0.0452	0.2255	456.8	0.8845	<0.05	<0.1	2.603	
	1-1B	77.99	<0.1	0.0247	0.0151	0.5666	0.0471	0.2249	453.4	0.8854	<0.05	<0.1	2.562	
	Mean	**78.19**	**<0.1**	**0.0248**	**0.0156**	**0.57265**	**0.04615**	**0.2252**	**455.1**	**0.88495**	**<0.05**	**<0.1**	**2.5825**	**9.38**
2	1-2A	80.93	<0.1	0.0257	0.0151	0.3906	0.0477	0.2335	474.4	0.9114	<0.05	<0.1	1.914	
	1-2B	80.84	<0.1	0.0309	0.0156	0.3782	0.0581	0.2304	477.4	0.9188	<0.05	<0.1	1.9	
	Mean	**80.885**	**<0.1**	**0.0283**	**0.01535**	**0.3844**	**0.0529**	**0.23195**	**475.9**	**0.9151**	**<0.05**	**<0.1**	**1.907**	**9.91**
3	1-3A	80.13	<0.1	0.0263	0.016	0.3408	0.048	0.2239	472.8	0.9198	<0.05	<0.1	3.827	
	1-3B	81.11	<0.1	0.0258	0.0155	0.351	0.0578	0.2256	473.7	0.918	<0.05	<0.1	3.863	
	Mean	**80.62**	**<0.1**	**0.02605**	**0.01575**	**0.3459**	**0.0529**	**0.22475**	**473.25**	**0.9189**	**<0.05**	**<0.1**	**3.845**	**10.22**
4	1-4A	80.64	<0.1	0.0257	0.0158	0.3512	0.0481	0.2231	473.1	0.9179	<0.05	<0.1	1.922	
	1-4B	80.04	<0.1	0.0257	0.0151	0.3583	0.0517	0.2254	472.7	0.9255	<0.05	<0.1	1.897	
	Mean	**80.34**	**<0.1**	**0.0257**	**0.01545**	**0.35475**	**0.0499**	**0.22425**	**472.9**	**0.9217**	**<0.05**	**<0.1**	**1.9095**	**10.16**
5	1-5A	80.62	<0.1	0.0226	0.0177	0.3482	0.0407	0.1931	475	0.9051	<0.05	<0.1	1.842	
	1-5B	80.76	<0.1	0.021	0.0154	0.3399	0.0492	0.1909	471.8	0.9146	<0.05	<0.1	1.862	
	Mean	**80.69**	**<0.1**	**0.0218**	**0.01655**	**0.34405**	**0.04495**	**0.192**	**473.4**	**0.90985**	**<0.05**	**<0.1**	**1.852**	**8.42**
6	1-6A	80.86	<0.1	0.0206	0.0154	0.3464	0.0392	0.1964	472.2	0.9105	<0.05	<0.1	1.836	
	1-6B	80.65	<0.1	0.0204	0.0153	0.349	0.0501	0.1976	471.1	0.9118	<0.05	<0.1	1.812	
	Mean	**80.755**	**<0.1**	**0.0205**	**0.01535**	**0.3477**	**0.04465**	**0.197**	**471.65**	**0.91115**	**<0.05**	**<0.1**	**1.824**	**8.63**
7	1-7A	79.87	<0.1	0.0202	0.0159	0.3517	0.0384	0.1885	470.5	0.9005	<0.05	<0.1	1.814	
	1-7B	80.28	<0.1	0.0201	0.0159	0.3484	0.0421	0.1886	470	0.9121	<0.05	<0.1	1.794	
	Mean	**80.075**	**<0.1**	**0.02015**	**0.0159**	**0.35005**	**0.04025**	**0.18855**	**470.25**	**0.9063**	**<0.05**	**<0.1**	**1.804**	**9.53**
8	1-8A	80.23	<0.1	0.0203	0.0161	0.3517	0.0426	0.1917	469.7	0.9064	<0.05	<0.1	1.803	
	1-8B	79.84	<0.1	0	0.0154	0.361	0.001	0.1917	469.8	0.9059	<0.05	<0.1	1.779	
	Mean		**<0.1**	**0.01015**	**0.01575**	**0.35635**	**0.0218**	**0.1917**	**469.75**	**0.90615**	**<0.05**	**<0.1**	**1.791**	**9.49**

Table A..6.1 (Cont.)

Run 2 (To determine effect of foam particle size)

Biosorbent	Foam only, no algae. Mesh sizes 8, 10, 12, unground cylinder. 4.44 g/column
Duration	30 min
Mode	Recirc
Flow rate	0.30 gal/min.

Column	*Sample*	*Al*	*As*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Pb*	*Se*	*Zn*	*Flow, g*
1-cyl	1-1A	79.93	<0.1	0.0188	0.016	0.4353	0.0406	0.2165	467.7	0.9015	<0.05	<0.1	1.82	
	1-1B	80.06	<0.1	0.0189	0.0157	0.9179	0.0422	0.1989	472	0.9103	<0.05	<0.1	1.796	
	Mean	**79.995**	**<0.1**	**0.01885**	**0.01585**	**0.6766**	**0.0414**	**0.2077**	**469.85**	**0.9059**	**<0.05**	**<0.1**	**1.808**	**8.46**
5-cyl	1-5A	78.97	<0.1	0.0254	0.0168	0.5258	0.0493	0.1965	477.4	0.904	<0.05	<0.1	1.817	
	1-5B	79.15	<0.1	0.0255	0.0166	0.515	0.0446	0.1973	476.5	0.9119	<0.05	<0.1	1.833	
	Mean	**79.06**	**<0.1**	**0.02545**	**0.0167**	**0.5204**	**0.04695**	**0.1969**	**476.95**	**0.90795**	**<0.05**	**<0.1**	**1.825**	**7.17**
Mean cylinder		**79.53**	**<0.1**	**0.0222**	**0.0163**	**0.5985**	**0.0442**	**0.2023**	**473.4**	**0.9069**	**<0.05**	**<0.1**	**1.817**	**7.815**
2-12 mes	1-2A	80.02	<0.1	0.0297	0.015	0.4525	0.0525	0.2085	473.6	0.9142	<0.05	<0.1	1.852	
	1-2B	79.63	<0.1	0.0262	0.0168	1.084	0.0434	0.2036	479.6	0.917	<0.05	<0.1	1.87	
	Mean	**79.825**	**<0.1**	**0.02795**	**0.0159**	**0.76825**	**0.04795**	**0.20605**	**476.6**	**0.9156**	**<0.05**	**<0.1**	**1.861**	**8.36**
6-12 mes	1-6A	77.77	<0.1	0.0247	0.016	1.231	0.0472	0.2015	472.5	0.8965	<0.05	<0.1	1.826	
	1-6B	77.73	<0.1	0.0249	0.0152	1.2	0.0442	0.2021	472.2	0.9032	<0.05	<0.1	1.782	
	Mean	**77.75**	**<0.1**	**0.0248**	**0.0156**	**1.2155**	**0.0457**	**0.2018**	**472.35**	**0.89985**	**<0.05**	**<0.1**	**1.804**	**7.82**
Mean 12 mesh		**78.79**	**<0.1**	**0.0264**	**0.0158**	**0.9919**	**0.0468**	**0.2039**	**474.5**	**0.9077**	**<0.05**	**<0.1**	**1.833**	**8.09**
3-10 mes	1-3A	79.22	<0.1	0.0257	0.0154	0.401	0.0476	0.2178	474.9	0.9112	<0.05	<0.1	3.803	
	1-3B	79.09	<0.1	0.0255	0.0183	0.8021	0.0456	0.1986	477.3	0.9108	<0.05	<0.1	3.769	
	Mean	**79.155**	**<0.1**	**0.0256**	**0.01685**	**0.60155**	**0.0466**	**0.2082**	**476.1**	**0.911**	**<0.05**	**<0.1**	**3.786**	**8.12**
7-10 mes	1-7A	80.02	<0.1	0.024	0.0151	0.9773	0.0409	0.2216	473.2	0.9113	<0.05	<0.1	1.896	
	1-7B	80.53	<0.1	0.0213	0.0161	0.9392	0.0481	0.2146	467.8	0.9034	<0.05	<0.1	1.873	
	Mean	**80.275**	**<0.1**	**0.02265**	**0.0156**	**0.95825**	**0.0445**	**0.2181**	**470.5**	**0.90735**	**<0.05**	**<0.1**	**1.8845**	**7.78**
Mean 10 mesh		**79.72**	**<0.1**	**0.0241**	**0.0162**	**0.7799**	**0.0456**	**0.2132**	**473.3**	**0.9092**	**<0.05**	**<0.1**	**2.835**	**7.95**
4-8 mesh	1-4A	79.27	<0.1	0.0253	0.0173	0.935	0.0448	0.2037	477	0.9123	<0.05	<0.1	1.841	
	1-4B	78.48	<0.1	0.0253	0.0165	0.9192	0.0457	0.2028	473.2	0.9025	<0.05	<0.1	1.818	
	Mean	**78.875**	**<0.1**	**0.0253**	**0.0169**	**0.9271**	**0.04525**	**0.20325**	**475.1**	**0.9074**	**<0.05**	**<0.1**	**1.8295**	**9.14**
8-8 mesh	1-8A	80.44	<0.1	0.0206	0.016	1.431	0.0418	0.2127	470.1	0.9084	<0.05	<0.1	1.869	
	1-8B	80.65	<0.1	0.0203	0.0163	1.411	0.0435	0.2104	470.3	0.9102	<0.05	<0.1	1.865	
	Mean	**80.545**	**<0.1**	**0.02045**	**0.01615**	**1.421**	**0.04265**	**0.21155**	**470.2**	**0.9093**	**<0.05**	**<0.1**	**1.867**	**7**
Mean 8 mesh		**79.71**	**<0.1**	**0.0229**	**0.0165**	**1.1741**	**0.044**	**0.2074**	**472.7**	**0.9084**	**<0.05**	**<0.1**	**1.848**	**8.07**

Table A.6.2 Experimental procedures and results for Experiments #2

Experiments #2: 11/29/94

I. PURPOSE

Determine Effect of Particle Size on Bioremoval by *Mastigocladus*

II. Alga

11-day-old *Mastigocladus laminosus* culture

III. FOAM:

6% algae by dry weight air dried and seived
Samples obtained for 8,10, and 12 mesh

IV. EFFLUENTS: D-Area coal pile runoff basin - near outfall

V. TEST APPARATUS:

Frisby Test rig

VI. PROCEDURE:

Fill reservoirs w/ 4 liters of wastewater
Fill columns with 4.4g of foam
Run at 0.3gpm for 30 minutes
Collect samples and acidify (0.5ml conc. HNO3) prior to taking samples to ADS for chemical analyses

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS:

Label	*Description*
2-1a&b	8mesh Mast.
2.2a&b	"
2.3a&b	10mesh Mast.
2.4a&b	"
2,5a&b	12mesh Mast.
2.6a&b	"
2.7a&b	#8mesh blank foam
2.8a&b	"
2.0a&b	controls

Table A.6.2 (Cont.)

Exp. 2- Effect of particle size on bioremoval by 6% Mastigocladus - 11-29-94

PARAMETERS

Effluent	D coal runoff	24
Date coll.	11/28/94	8.4
pH	2.4	19
Cond.	5.88 mS/cm	0.30%

Biosorbent	Mastigocladus, 31, 6 % by dry wt, embedded 11-28, 4.44 g/column
Duration	30 min
Mode	Recirc
Flow rate	0.30 gal/min.

Column	*Mesh*	*Sample*	*Flow, ga*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
											2.912	
											2.923	
Raw	*None*	2-0A		250	0.0541	0.045	0.9387	0.2931	0.6127	1796	**2.9175**	5.817
water		2-0B		246.2	0.0538	0.045	0.9447	0.291	0.6147	1767		5.848
Mean raw water				**248.1**	**0.054**	**0.045**	**0.9417**	**0.2921**	**0.614**	**1781.5**	2.297	**5.8325**
											2.815	
1	Control	2-1A		203.6	0.0375	0.0326	0.8679	0.2276	0.4843	1536	**2.556**	4.537
		2-1B		258	0.0487	0.0433	1.055	0.2786	0.5993	1924		5.596
		Mean	9.28	**230.8**	**0.0431**	**0.03795**	**0.96145**	**0.2531**	**0.5418**	**1730**	2.729	**5.0665**
											2.82	
2	Control	2-2A		246.4	0.047	0.0427	1	0.2724	0.5786	1864	**2.7745**	5.402
		2-2B		263.4	0.0491	0.0445	1.17	0.2787	0.6055	1973	**2.6653**	5.61
		Mean	9.63	**254.9**	**0.04805**	**0.0436**	**1.085**	**0.27555**	**0.59205**	**1918.5**		**5.506**
Mean control (8 mesh)			**9.455**	**242.85**	**0.0456**	**0.0408**	**1.0232**	**0.2643**	**0.567**	**1824.3**	2.403	**5.2863**
											2.818	
3	8 mesh	2-3A		222	0.0395	0.0371	0.8825	0.2404	0.5109	1677	**2.6105**	4.739
		2-3B		262.4	0.0494	0.0445	1.274	0.2806	0.6061	1968		5.674
		Mean	8.34	**242.2**	**0.04445**	**0.0408**	**1.07825**	**0.2605**	**0.5585**	**1822.5**	2.366	**5.2065**
											2.825	
4	8 mesh	2-4A		220.4	0.0383	0.0351	0.8603	0.2336	0.504	1654	**2.5955**	4.658
		2-4B		8.045 ?	0.0489	0.0442	1.415	0.284	0.6073	683.6	**2.603**	5.557
		Mean	10.37	**220.4**	**0.0436**	**0.03965**	**1.13765**	**0.2588**	**0.55565**	**1168.8**		**5.1075**
Mean 8 mesh			**9.355**	**231.3**	**0.044**	**0.0402**	**1.108**	**0.2597**	**0.557**	**1495.7**	2.599	**5.157**
											2.743	
5	10 mesh	2-5A		242.3	0.0425	0.0378	0.9493	0.2577	0.5584	1820	**2.671**	5.047
		2-5B		260.6	0.0465	0.0411	1.208	0.2747	0.5981	1936		5.416
		Mean	8.68	**251.45**	**0.0445**	**0.03945**	**1.07865**	**0.2662**	**0.57825**	**1878**	2.3	**5.2315**
											2.694	
6	10 mesh	2-6A		196.6	0.0605	0.0534	0.8004	0.2284	0.4999	1443	**2.497**	4.573
		2-6B		237.3	0.1256	0.1267	0.9757	0.2736	0.5892	1733	**2.584**	5.267
		Mean	10.23	**216.95**	**0.09305**	**0.09005**	**0.88805**	**0.251**	**0.54455**	**1588**		**4.92**
Mean 10 mesh			**9.455**	**234.2**	**0.0688**	**0.0648**	**0.9834**	**0.2586**	**0.561**	**1733**	2.378	**5.0758**
											2.788	
7	12 mesh	2-7A		206.7	0.0777	0.0812	0.8606	0.2376	0.5008	1510	**2.583**	4.629
		2-7B		243.2	0.0669	0.0631	1.002	0.2767	0.5916	1770		5.44
		Mean	8.56	**224.95**	**0.0723**	**0.07215**	**0.9313**	**0.25715**	**0.5462**	**1640**	2.239	**5.0345**
											2.825	
8	12 mesh	2-8A		189.1	0.0435	0.0407	0.7663	0.2263	0.4669	1377	**2.532**	4.402
		2-8B		242.1	0.053	0.0449	0.9681	0.2793	0.5914	1752	**2.5575**	5.512
		Mean	8.66	**215.6**	**0.04825**	**0.0428**	**0.8672**	**0.2528**	**0.52915**	**1564.5**		**4.957**
Mean 12 mesh			**8.61**	**220.28**	**0.0603**	**0.0575**	**0.8993**	**0.255**	**0.538**	**1602.3**		**4.9958**

Table A.6.2 (Cont.)

SUMMARY

Column Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean raw water	248.1	0.05395	0.045	0.9417	0.29205	0.6137	1781.5	2.9175	5.8325
Mean control (8 me	242.85	0.045575	0.040775	1.023225	0.264325	0.566925	1824.25	2.66525	5.28625
Mean 8 mesh	231.3	0.044025	0.040225	1.10795	0.25965	0.557075	1495.65	2.603	5.157
Mean 10 mesh	234.2	0.068775	0.06475	0.98335	0.2586	0.5614	1733	2.584	5.07575
Mean 12 mesh	220.275	0.060275	0.057475	0.89925	0.254975	0.537675	1602.25	2.5575	4.99575

Percent Removal (% 8 mesh control)

Column Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean 8 mesh	4.756022	3.400987	1.348866	-8.28019	1.768656	1.737443	18.01288	2.33562	2.445022
Mean 10 mesh	3.561869	-50.9051	-58.7983	3.896992	2.165894	0.974556	5.002056	3.04849	3.982029
Mean 12 mesh	9.295862	-32.2545	-40.9565	12.1161	3.537312	5.159413	12.16938	4.04277	5.495389

Percent Removal (% raw water)

Column Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean control (8 me	2.116082	15.52363	9.388889	-8.65722	9.493237	7.621802	-2.39966	8.6461	9.365624
Mean 8 mesh	6.771463	18.39666	10.61111	-17.6542	11.09399	9.226821	16.04547	10.7798	11.58165
Mean 10 mesh	5.60258	-27.4791	-43.8889	-4.42285	11.45352	8.522079	2.722425	11.431	12.97471
Mean 12 mesh	11.21524	-11.7238	-27.7222	4.507805	12.69474	12.38797	10.06175	12.3393	14.34634

Table A.6.3 Experimental procedures and results for Experiments #3

Experiments #3: 11/29/94

I. PURPOSE

Determine Effect of Particle Size on Bioremoval by *Phaeodactylum*

II. Alga

11-day-old *Phaeodactylum tricornutum* culture

III. FOAM:

6% algae by dry weight air dried and seived
Samples obtained for 8,10, and 12 mesh

IV. EFFLUENTS: D-Area coal pile runoff basin - near outfall

V. TEST APPARATUS:

Frisby Test rig

VI. PROCEDURE:

Fill reservoirs w/ 4 liters of wastewater
Fill columns with 4.4g of foam
Run at 0.3gpm for 30 minutes
Collect samples and acidify (0.5ml conc. HNO3) prior to taking samples to ADS for chemical analyses.

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS:

Label	*Description*
3-1a&b	8mesh Phaeo.
3.2a&b	"
3.3a&b	10mesh phaeo.
34a&b	"
3,5a&b	12mesh Phaeo.
3.6a&b	"
3.7a&b	#8mesh blank foam
38a&b	"

Table A.6.3 (Cont.)

Exp.3 - Effect of particle size on bioremoval by 6% Phaeodactylum - 11-29-94

PARAMETERS												
Effluent		D coal runoff		24								
Date coll.		11/28/94		8.4								
pH		2.4		19								
Cond.		5.88 mS/cm		0.30%								
Biosorbent		Phaeodactylum, GR10_31, 6 % by dry wt, embedded 11-28, 4.44 g/column										
Duration		30 min										
Mode		Recirc										
Flow rate		0.30 gal/min.										
Column	Mesh	Sample	Flow, ga	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Raw	None	2-0A		250	0.0541	0.045	0.9387	0.2931	0.6127	1796	2.912	5.817
water		2-0B		246.2	0.0538	0.045	0.9447	0.291	0.6147	1767	2.923	5.848
Mean raw water				**248.1**	**0.054**	**0.045**	**0.9417**	**0.2921**	**0.614**	**1781.5**	**2.9175**	**5.8325**
1	Control	3-1A		173.5	0.0343	0.0306	0.7144	0.2132	0.4466	1252	2.095	4.218
		3-1B		226.1	0.0485	0.0413	0.9738	0.2708	0.5799	1611	2.705	5.452
		Mean	8.72	199.8	0.0414	0.03595	0.8441	0.242	0.51325	1431.5	2.4	4.835
2	Control	3-2A		188	0.0384	0.0355	0.7399	0.2162	0.4604	1404	2.172	4.373
		3-2B		231.3	0.0488	0.0419	1.166	0.2639	0.5704	1729	2.651	5.402
		Mean	9.55	209.65	0.0436	0.0387	0.95295	0.24005	0.5154	1566.5	2.4115	4.8875
Mean control (8 mesh)			**9.135**	**204.73**	**0.0425**	**0.0373**	**0.8985**	**0.241**	**0.514**	**1499**	**2.4058**	**4.8613**
3	8 mesh	3-3A		177.4	0.0342	0.0305	0.6995	0.2073	0.4439	1325	2.058	4.143
		3-3B		237.2	0.0494	0.0415	1.198	0.2699	0.581	1749	2.675	5.464
		Mean	9.8	207.3	0.0418	0.036	0.94875	0.2386	0.51245	1537	2.3665	4.8035
4	8 mesh	3-4A		180	0.0345	0.0297	0.7146	0.2078	0.4424	1320	2.067	4.131
		3-4B		237.6	0.0489	0.0421	1.328	0.2684	0.5837	1743	2.677	5.468
		Mean	8.81	208.8	0.0417	0.0359	1.0213	0.2381	0.51305	1531.5	2.372	4.7995
Mean 8 mesh			**9.305**	**208.05**	**0.0418**	**0.036**	**0.985**	**0.2384**	**0.513**	**1534.3**	**2.3693**	**4.8015**
5	10 mesh	3-5A		207.6	0.0423	0.0356	0.8091	0.237	0.5148	1534	2.351	4.834
		3-5B		237.8	0.0501	0.0437	1.277	0.2719	0.589	1738	2.704	5.528
		Mean	9.28	222.7	0.0462	0.03965	1.04305	0.25445	0.5519	1636	2.5275	5.181
6	10 mesh	3-6A		179.4	0.0345	0.0313	0.6978	0.206	0.4442	1312	2.071	4.169
		3-6B		235.1	0.0495	0.0444	1.009	0.2685	0.5882	1715	2.687	5.494
		Mean	7.82	207.25	0.042	0.03785	0.8534	0.23725	0.5162	1513.5	2.379	4.8315
Mean 10 mesh			**8.55**	**214.98**	**0.0441**	**0.0388**	**0.9482**	**0.2459**	**0.534**	**1574.8**	**2.4533**	**5.0063**
7	12 mesh	3-7A		164.1	0.0318	0.0288	0.6611	0.1919	0.3953	1222	1.929	3.875
		3-7B		227.6	0.048	0.0423	1.023	0.2568	0.5406	1710	2.576	5.297
		Mean	10.18	195.85	0.0399	0.03555	0.84205	0.22435	0.46795	1466	2.2525	4.586
8	12 mesh	3-8A		184.6	0.036	0.0329	0.7138	0.2102	0.4399	1364	2.095	4.284
		3-8B		224.7	0.0469	0.0418	1.083	0.2496	0.5326	1650	2.508	5.213
		Mean	8.82	204.65	0.04145	0.03735	0.8984	0.2299	0.48625	1507	2.3015	4.7485
Mean 12 mesh			**9.5**	**200.25**	**0.0407**	**0.0365**	**0.8702**	**0.2271**	**0.477**	**1486.5**	**2.277**	**4.6673**

Table A.6.3 (Cont.)

SUMMARY

Column	Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean raw water		248.1	0.05395	0.045	0.9417	0.29205	0.6137	1781.5	2.9175	5.8325
Mean control (8 me		204.725	0.0425	0.037325	0.898525	0.241025	0.514325	1499	2.40575	4.86125
Mean 8 mesh		208.05	0.04175	0.03595	0.985025	0.23835	0.51275	1534.25	2.36925	4.8015
Mean 10 mesh		214.975	0.0441	0.03875	0.948225	0.24585	0.53405	1574.75	2.45325	5.00625
Mean 12 mesh		200.25	0.040675	0.03645	0.870225	0.227125	0.4771	1486.5	2.277	4.66725

Percent Removal (% 8 mesh control)

Column	Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean 8 mesh		-1.62413	1.764706	3.683858	-9.62689	1.109843	0.306227	-2.35157	1.5172	1.229108
Mean 10 mesh		-5.00672	-3.76471	-3.81782	-5.53129	-2.00187	-3.83512	-5.05337	-1.9744	-2.98277
Mean 12 mesh		2.185859	4.294118	2.344273	3.149606	5.767037	7.237642	0.833889	5.35176	3.990743

Percent Removal (% raw water)

Column	Mesh	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
Mean control (8 me		17.48287	21.22335	17.05556	4.584793	17.47132	16.19277	15.85742	17.5407	16.65238
Mean 8 mesh		16.14268	22.61353	20.11111	-4.60072	18.38726	16.44941	13.87875	18.7918	17.67681
Mean 10 mesh		13.35147	18.25765	13.88889	-0.6929	15.81921	12.97865	11.60539	15.9126	14.16631
Mean 12 mesh		19.28658	24.60612	19	7.589997	22.23078	22.25843	16.55908	21.9537	19.97857

Table A.6.4 Experimental procedures and results for Experiment #4

Experiments #4: 11/29/94

I. PURPOSE

Determine effect of various biomass (*Mastigocladus*) amounts on bioremoval

II. Alga

11-day-old *Mastigocladus laminosus* culture

III. FOAM:

6% algae by dry weight air dried and 8 mesh

IV. EFFLUENTS: D-Area coal pile runoff basin - near outfall

V. TEST APPARATUS:

Frisby Test rig

VI. PROCEDURE:

Fill reservoirs w/ 4 liters of wastewater
Fill columns with the following amounts of foam:

Cols. 1&2 - 2g
Cols. 3&4 - 8g
Cols. 5&6 - 12g
Cols. 7&8 - 16g

Run at 0.3gpm for 30 minutes
Collect duplicate ca 50ml samples from each column
Centtifuge 10 min @ 10,000rpm
Acidify w/ 50 µl HNO3
Take to ADS for chemical analyses

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS

Label	***Description***
4-1a&b	2g foam
4-2a&b	"
4-3a&b	8g foam
4-4a&b	"
4-5a&b	12g foam
4-6a&b	"
4-7a&b	16g foam
4-8a&b	"

Table A.6.4 (Cont.)

Exp. 4 - Effect of foam amount on bioremoval by 6% Mastigocladus - 11-29-94

PARAMETERS			
Effluent	D coal runoff	24	
Date coll.	11/28/94	8.4	
pH	2.4	19	
Cond.	5.88 mS/cm	0.30%	
Biosorbent	Mastigocladus 6 % by dry wt, embedded 11-28		
Duration	30 min		
Mode	Recirc		
Flow rate	0.30 gal/min.		

NOTE- USED LEFTOVER WATER FROM EXP. 3- SEE DATA FOR ZERO TIME VALUES FOR EACH COLUMN

Column	*g foam*	*Sample*	*Flow* gal.	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
1		4-1A		239.1	0.0497	0.0447	0.9468	0.2655	0.5573	1792	2.647	5.491
		4-1B		239	0.0492	0.0432	1.049	0.264	0.563	1792	2.65	5.501
	2.0006	Mean	9.43	239.05	0.04945	0.04395	0.9979	0.26475	0.56015	1792	2.6485	5.496
2		4-2A		237.9	0.0493	0.045	0.9864	0.2617	0.558	1788	2.631	5.47
		4-2B		239.2	0.0491	0.0453	0.9674	0.2616	0.5567	1779	2.635	5.46
	2.0061	Mean	9.29	238.55	0.0492	0.04515	0.9769	0.26165	0.55735	1783.5	2.633	5.465
	2g	Mean	9.36	238.8	0.0493	0.0446	0.9874	0.2632	0.559	1787.8	2.6408	5.4805
3		4-3A		242.7	0.0493	0.0455	0.9841	0.2733	0.5785	1818	2.688	5.494
		4-3B		241	0.0498	0.0442	0.9876	0.2687	0.5805	1795	2.675	5.51
	8.0013	Mean	8.81	241.85	0.04955	0.04485	0.98585	0.271	0.5795	1806.5	2.6815	5.502
4		4-4A		235.9	0.0516	0.0525	0.9883	0.2675	0.6133	1749	2.739	5.43
		4-4B		236.2	0.0496	0.0511	1.612	0.2708	0.6102	1741	2.738	5.381
	8.0058	Mean	9.53	236.05	0.0506	0.0518	1.30015	0.26915	0.61175	1745	2.7385	5.4055
	8g	Mean	9.17	238.95	0.0501	0.0483	1.143	0.2701	0.596	1775.8	2.71	5.4538
5		4-5A		239.3	0.0493	0.0505	0.9569	0.2717	0.62	1767	2.73	5.404
		4-5B		239.4	0.049	0.0499	1.437	0.2707	0.6221	1755	2.741	5.396
	12.0096	Mean	8.99	239.35	0.04915	0.0502	1.19695	0.2712	0.62105	1761	2.7355	5.4
6		4-6A		238.2	0.0481	0.0491	0.9149	0.267	0.6116	1744	2.714	5.345
		4-6B		234	0.0482	0.0489	1.082	0.2696	0.6119	1720	2.727	5.368
	12.0078	Mean	9.22	236.1	0.04815	0.049	0.99845	0.2683	0.61175	1732	2.7205	5.3565
	12g	Mean	9.105	237.73	0.0487	0.0496	1.0977	0.2698	0.616	1746.5	2.728	5.3783
7		4-7A		226.2	0.0454	0.0477	0.9162	0.2571	0.5647	1645	2.579	5.131
		4-7B		223.8	0.0453	0.0467	1.188	0.2543	0.5713	1625	2.616	5.084
	16.0089	Mean	9.47	225	0.04535	0.0472	1.0521	0.2557	0.568	1635	2.5975	5.1075
8		4-8A		222	0.0445	0.0459	0.8734	0.2553	0.5666	1588	2.553	5.058
		4-8B		219.8	0.0444	0.0469	1.16	0.2551	0.5681	1566	2.572	5.033
	16.0064	Mean	9.02	220.9	0.04445	0.0464	1.0167	0.2552	0.56735	1577	2.5625	5.0455
	16g	Mean	9.245	222.95	0.0449	0.0468	1.0344	0.2555	0.568	1606	2.58	5.0765

Table A.6.4 (Cont.)

SUMMARY

Column	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
2 g, mean	238.8	0.049325	0.04455	0.9874	0.2632	0.55875	1787.75	2.64075	5.4805
Previous	204.725	0.0425	0.037325	0.898525	0.241025	0.514325	1499	2.40575	4.86125
% Removal	-16.6443	-16.0588	-19.357	-9.89121	-9.20029	-8.63753	-19.2628	-9.76826	-12.738
8 g, mean	238.95	0.050075	0.048325	1.143	0.270075	0.595625	1775.75	2.71	5.45375
Previous	208.05	0.04175	0.03595	0.985025	0.23835	0.51275	1534.25	2.36925	4.8015
% Removal	-14.8522	-19.9401	-34.4228	-16.0377	-13.3103	-16.1628	-15.7406	-14.3822	-13.584
12 g, mean	237.725	0.04865	0.0496	1.0977	0.26975	0.6164	1746.5	2.728	5.37825
Previous	214.975	0.0441	0.03875	0.948225	0.24585	0.53405	1574.75	2.45325	5.00625
% Removal	-10.5826	-10.3175	-28	-15.7637	-9.72137	-15.4199	-10.9065	-11.1994	-7.4307
16 g, mean	222.95	0.0449	0.0468	1.0344	0.25545	0.567675	1606	2.58	5.0765
Previous	200.25	0.040675	0.03645	0.870225	0.227125	0.4771	1486.5	2.277	4.66725
% Removal	-11.3358	-10.3872	-28.3951	-18.8658	-12.4711	-18.9845	-8.03902	-13.307	-8.7685

Table A.6.5 Experimental procedures and results for Experiment #5

Experiments #5: 11/29/94

I. PURPOSE

Determine bioremoval by foam embedded with *Mastigocladus* and *Phaeodactylum* after mixing in shake fflasks with waste water for 30 minutes.

II. Alga

11-day-old *Mastigocladus laminosus, and Phaeodactylum tricornutum* cultures

III. FOAM:

6% algae by dry weight air dried and 8 mesh

IV. EFFLUENTS: D-Area coal pile runoff basin - near outfall

V. TEST APPARATUS:

Shake flasks in Pschrotherm incubator 20°C 150rpm 30min.

VI. PROCEDURE:

Add 50 ml wastewater to 6 125ml Erlenmeyer flasks
Add 1g foam to each flask as described below:
Flasks 1&2 = plain foam, 8 mesh
Flasks 3&4 = Mastigocladus, 8 mesh
Flask 5&6 = Phaeodactylum, 8 mesh
Incubate for 30 minutes at 20°C with shaker set for 120rpm
Remove flasks from Pschrotherm
Centrifuge liquid for 10 minutes at 10,000rpm
Acidify w/ 50 µl HNO_3
Take to ADS for chemical analyses

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS

Label	***Description***
5-1a&b	Plain foam
5-2a&b	*Mastigocladus,* 8 mesh
5-3a&b	Phaeodactylum, 8 mesh

Table A.6.5 (Cont.)

Exp. 5 - 11-29-94. Incubation of Mastigocladus & Phaeodactylum + foam in shake flasks of runoff											
PARAMETERS											
Effluent		D coal runof	24								
Date coll.		11/28/94	8.4								
pH		2.4	19								
Cond.		5.88 mS/cm	0.30%								
Biosorbent		Mastigocladus and Phaeodactylum, 6 % by dry wt, embedded 11-28, 1 g/50 ml runoff.									
Duration		30 min									
Mode		Shake flasks, 150 rpm, 20 C									
Flask	*Alga*	*Sample*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
Raw	*None*	2-0A	250	0.0541	0.045	0.9387	0.2931	0.6127	1796	2.912	5.817
water		2-0B	246.2	0.0538	0.045	0.9447	0.291	0.6147	1767	2.923	5.848
Mean raw water			248.1	0.054	0.045	0.9417	0.2921	0.614	1781.5	2.9175	5.8325
1	Foam	5-1A	245.7	0.0543	0.0537	0.981	0.3071	0.7148	1827	3.013	5.72
		5-1B	256.7	0.052	0.0485	0.9777	0.3014	0.6818	1897	2.973	5.728
		Mean	251.2	0.05315	0.0511	0.97935	0.30425	0.6983	1862	2.993	5.724
2	Mast.	5-2A	256.8	0.0506	0.0491	0.9653	0.2997	0.6643	1909	2.951	5.622
		5-2B	258.8	0.0502	0.0479	0.943	0.3005	0.6585	1924	2.93	5.6
		Mean	257.8	0.0504	0.0485	0.95415	0.3001	0.6614	1916.5	2.9405	5.611
3	Phaeo	5-3A	259.9	0.0492	0.0464	0.9251	0.3023	0.8146	1931	2.897	5.639
		5-3B	259.9	0.0495	0.0487	0.9249	0.2963	0.8409	1914	2.879	5.632
		Mean	259.9	0.04935	0.04755	0.925	0.2993	0.82775	1922.5	2.888	5.6355

Experiments #6 1/9/95

I. PURPOSE

Determine effect of metal reduction in coal pile runoff following aeration and pH adjustment.

II. Alga

none

III. FOAM:

none

IV. EFFLUENTS: D-Area coal pile runoff basin - near outfall

V. TEST APPARATUS:

Bench top experiment w/ standard laboratory glassware

VI. PROCEDURE:

Collect sample from D-CPRB and split into 6 250ml fractions
Treat samples as follows:

1. no treatment
2. aertate overnight
3. adjust pH to pH 3 with NaOH and aerate overnight
4. adjust pH to 4 with NaOH and aerate overnight
5. adjust to pH 5 with NaOH and aerate overnight
6. adjust pH to 6 with NaOh and aerate overnight

Centrifuge samples (10,000rpm for 10 min.)
Collect two 30 ml samples of the supernatant from each treatment and acidify with 0.6ml conc. HNO3.

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS

Label	*Description*
6-1a&b	Untreated control, pH 2.5
6-2a&b	Aerated, pH 2.5
6-3a&b	Aerated and pH adjusted to 3.0
6-4a&b	Aerated and pH adjusted to 4.0
6-5a&b	Aerated and pH adjusted to 5.0
6-6a7b	Aerated and pH adjusted to 6.0

Table A.6.6 (Cont.)

Exp. 6- Effect of pH increase and aeration on metal content of runoff - 1-9-95

Effluent D coal runoff (pH 5)

Date coll. 1/9/95

Method

250 ml samples of fresh runoff were subjected to the following treatments:

1) no treatment
2) overnight aeration
3)adjustment to pH 3 with 18 drops NaOh, overnight aeration
4)adjustment to pH 4 with 11 drops NaOh, overnight aeration
5)adjustment to pH 5 with 33 drops NaOh, overnight aeration
6)adjustment to pH 6 with 19 drops NaOh, overnight aeration

Following 16 h aeration, samples were centrifuged (10 min, 10000 rpm).
Two 30 ml samples of each supernatant were acidified with 0.6 ml conc. HNO3.

pH	*Aerated?*	*Sample #*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
2.5	No	6-1A	65.92	0.0179	0.0152	0.2567	0.0319	0.1926	396.8	0.8018	1.551
2.5	No	6-1B	66.46	0.0174	0.0154	0.2537	0.0319	0.1916	398.8	0.8079	1.551
		Mean	**66.19**	**0.01765**	**0.0153**	**0.2552**	**0.0319**	**0.1921**	**397.8**	**0.80485**	**1.551**
2.5	Yes	6-2A	66.68	0.0165	0.0151	0.2573	0.0282	0.1911	396.1	0.8021	1.554
2.5	Yes	6-2B	66.41	0.0166	0.0148	0.256	0.0299	0.193	396.5	0.8086	1.554
		Mean	**66.545**	**0.01655**	**0.01495**	**0.25665**	**0.02905**	**0.19205**	**396.3**	**0.80535**	**1.554**
3	Yes	6-3A	63.16	0.0164	0.0107	0.2554	0.0285	0.1847	197.1	0.7609	1.54
3	Yes	6-3B	63.82	0.0165	0.0105	0.2561	0.03	0.1839	195.8	0.7569	1.535
		Mean	**63.49**	**0.01645**	**0.0106**	**0.25575**	**0.02925**	**0.1843**	**196.45**	**0.7589**	**1.5375**
4	Yes	6-4A	49.61	0.0163	0.0074	0.2442	0.0036	0.1725	23.68	0.6902	1.517
4	Yes	6-4B	49.87	0.0158	0.0061	0.2416	0.0022	0.1714	23.16	0.6921	1.505
		Mean	**49.74**	**0.01605**	**0.00675**	**0.2429**	**0.0029**	**0.17195**	**23.42**	**0.69115**	**1.511**
5	Yes	6-5A	4.755	0.0025	0.0055	0.2319	-0.0004	0.0868	9.94	0.6364	1.439
5	Yes	6-5B	4.726	0.0025	0.0045	0.2314	-0.001	0.0866	9.951	0.6372	1.434
		Mean	**4.765**	**0.0025**	**0.005**	**0.23165**	**-0.0007**	**0.0867**	**9.9455**	**0.6368**	**1.4365**
6	Yes	6-6A	0.0857	-0.0014	0.0035	0.1983	-0.002	0.0133	0.2673	0.4921	0.7102
6	Yes	6-6B	0.1023	0.0202	0.0034	0.1953	0.001	0.0234	0.3746	0.5048	0.7103
		Mean	**0.094**	**0.0094**	**0.00345**	**0.1968**	**-0.0005**	**0.01835**	**0.32095**	**0.49845**	**0.71025**

SUMMARY

Aer	pH	Al	Be	Cd	Co	Cr	Cu	Fe	Ni	Zn
no	2.5	66.19	0.01765	0.0153	0.2552	0.0319	0.1921	397.8	0.80485	1.551
yes	2.5	66.545	0.01655	0.01495	0.25665	0.02905	0.19205	396.3	0.80535	1.554
yes	3	63.49	0.01645	0.0106	0.25575	0.02925	0.1843	196.45	0.7589	1.5375
yes	4	49.74	0.01605	0.00675	0.2429	0.0029	0.17195	23.42	0.69115	1.511
yes	5	4.755	0.0025	0.005	0.23165	-0.0007	0.0867	9.9455	0.6368	1.4365
yes	6	0.094	0.0094	0.00345	0.1968	-0.0005	0.01835	0.32095	0.49845	0.71025

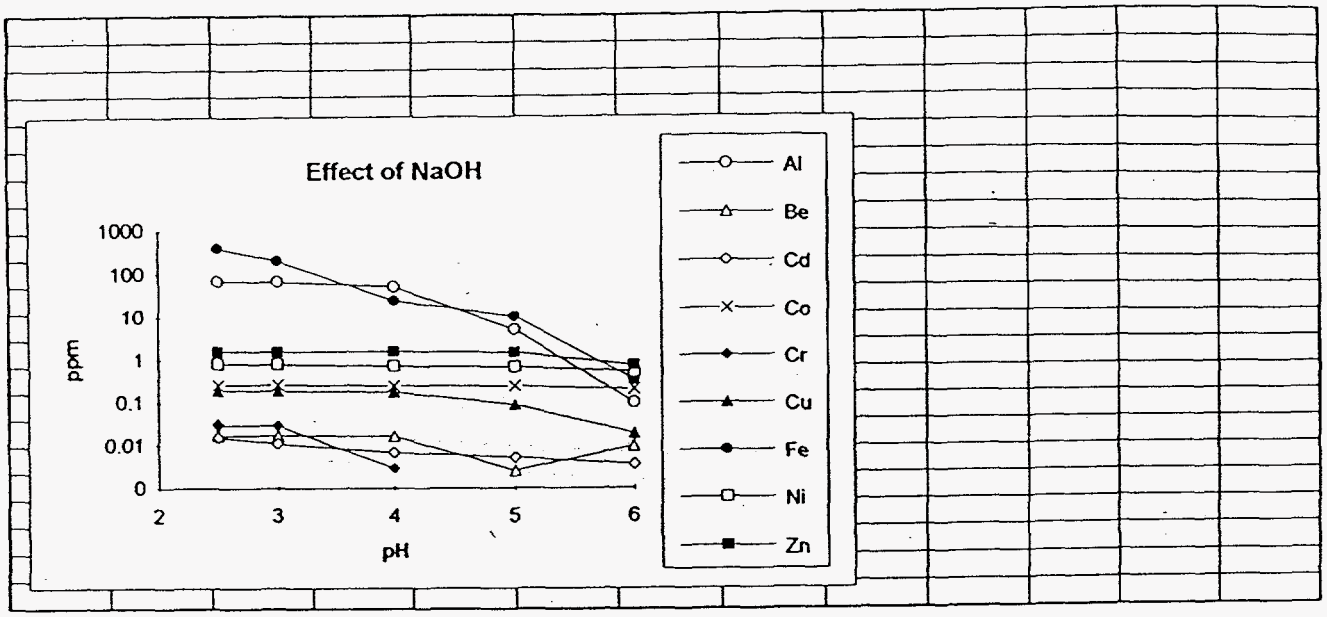

Effect of NaOH
1000
100
10
1
0.1
0.01
0
ppm
2
3
4
5
6
pH
Al
Be
Cd
Co
Cr
Cu
Fe
Ni
Zn

Table A.6.7 Experimental procedures and results forExperiment #7

Experiment #7: Methods and Materials

Metal Removal using *Cyanidium* in Mini-Columns

I. PURPOSE:

To attempt to maximize metal removal, we will 1) use 10 ml Bio-Rad mini-columns packed with foam to allow slower flow and a greater algae/wastewater ratio than is achievable using the test bed. 2) test coal basin runoff at both the natural pH and after adjustment to pH 5 to remove most iron and aluminum, which may be competing with the other metals. 3) compare metal removal from actual runoff with Zn and Ni standards at similar concentrations and pH values to further elucidate effects of pH and competing ions. Zn and Ni were chosen because they exceed water quality standards and remain in solution at pH 5. 4) compare foam particles with solid plugs to determine which gives the best exposure of metals to the algae.

II. ALGA:

13-day old thermophilic *Cyanidium caldarium* (GR 1_6) Harvested by centrifugation, washed once with DI, resuspended at 3% by dry wt in DI. Embedded 1-23-95 at 6% in foam.

III. FOAM:

8 mesh versus solid plugs (made directly in columns) About 2 g/column.

IV. EFFLUENTS:

D area coal basin runoff, collected from basin
0.8 ppm Ni standard
1.5 ppm Zn standard

V. TEST APPARATUS (GENERAL DESCRIPTION):

10 ml BioRad columns, 50 ml funnels

VI. PROCEDURE:

A. Preparation of effluent

1. Collect surface water from D area coal pile runoff basin (1 carboy). Measure pH.

2. Upon returning to lab, prepare 2 flasks containing 1.5 liters each of runoff.

3. Adjust pH of one flask to 5 using NaOH. Record initial and final pH values and volume of NaOH added (in drops; 1 drop = 0.042 ml).

4. Centrifuge both batches of runoff to remove precipitates (10 min, 10000 rpm). Decant or pipet off supernatant so as not to disturb pellet.

5. Recheck and record pH values of each batch of supernatant.

6. Take two 30 ml samples of each, preserve (0.6 ml conc. HNO3) for analysis.

B. Preparation of standards

1. Nickel, 100 ppm standard. Place 10 ml of a 1000 ug/ml NIST Ni standard (in 2% HNO3) in a 100 ml volumetric flask. Fill to mark with deionized water .

2. Nickel working standard, 0.8 ppm. Place 8 ml of 100 ppm standard in a 1000 ml volumetric, dilute to 1 liter. Do twice.

3. Check the pH of each batch of standard. Adjust one to the pH of the non-adjusted effluent used in the experiment (probably about 2.5), the other to pH 5.0. Use NaOH and/or HNO3 for these adjustments. Record initial and final pH as well as drops of acid or base added.

4. Centrifuge both batches of standard and decant supernatant in the same manner as for effluent.

5. Take two 30-ml samples from each batch of effluent, preserve and send for analysis.

6. Zinc, 100 ppm standard. Place 100 ml of 1000 ug/ml NIST standard (in 2% HNO3) into a 100 ml volumetric. Fill to mark with deionized water.

7. Zinc working standard, 1.5 ppm. Place 15 ml of 100 ppm standard into a 1000 ml volumetric, dilute to 1 liter. Do twice.

8. Check pH, adjust to effluent pH and to pH 5, centrifuge, decant, and sample in the same manner as for Ni standards (Steps 3-5).

C. **Preparation of columns**

1. Columns containing solid plugs of foam or foam+algae are prepared by Frisby Technologies.

2. Pack 12 columns with 2 g of particulate foam + algae (8 mesh) each.

3. Pack 12 columns with 2 g plain particulate foam (8 mesh) each

D. **Contacting biosorbent with effluent**

1. Set up 12 columns containing ground foam + Cyanidium. Place funnels on top and acid-washed 125 ml flasks underneath. Number columns GC1-12.

2. Place 50 ml standard or effluent in each funnel as follows:

   Columns GC 1, 2 - Coal runoff (unmodified pH)
   Columns GC 3, 4 - Coal runoff, pH 5
   Columns GC 5, 6 - Ni standard, low pH
   Columns GC 7, 8 - Ni standard, pH 7
   Columns GC 9, 10 - Zn standard, low pH
   Columns GC 11, 12 - Zn standard, pH 5

3. Wait 30 min or until liquid stops coming out of columns (whichever is longer). Remove 35 ml from each flask to a labeled centrifuge tube.

4. Centrifuge 10 min, 10000 rpm

5. Pipet off 30 ml from each tube to a labeled sample bottle. Preserve for analysis.

6. Set up 12 more columns containing plain ground foam (no algae). Label them G 1-12. Repeat Steps 2-5.

7. Set up 12 columns with plugs of algae + foam. Label them PC 1-12. Repeat Steps 2-5.

8. Set up 12 columns with plain foam plugs, labeled P 1-12. Repeat Steps 2-5.

9. Weigh leftover plugs and record weight of foam.

VII. APPARATUS

Coal basin runoff, at least 3 liters
1000 ppm Ni standard
1000 ppm Zn standard
HNO3, 1 N
HNO3, 10 N
NaOH, 1 N
NaOH, 10 N
HNO3, ca. 7 N for acid washing
Deionized water

Cyanidium
Foam as described in methods
Bio-Rad columns and funnels, about 30
Carboy for effluent

The following glass and plasticware should be acid-washed.

50 125 ml flasks
50 centrifuge tubes
at least 60 sample bottles
16 250 ml centrifuge bottles (may have to rewash halfway through)
2 100 ml volumetrics
4 1000 ml volumetrics
2 2 liter flasks
8 1 liter flasks
5 ml pipet tips, lots

VII. SAMPLE LABELS

The following labels will be used on samples sent to Analytical.

Label	*Description*
7-1	Control - Effluent, unmodified pH
7-2	"
7-3	Control - Effluent, pH 5
7-4	"
7-5	Control - Ni standard, low pH

7-6	"
7-7	Control - Ni standard, pH 5
7-8	"
7-9	Control - Zn standard, low pH
7-10	"
7-11	Control - Zn standard, pH 5
7-12	"
7-13	Sample from GC 1
7-14	Sample from GC 2
7-15	Sample from GC 3
7-16	" " GC 4
7-17	" " GC 5
7-18	" " GC 6
7-19	" " GC 7
7-20	" " GC 8
7-21	" " GC 9
7-22	" " GC 10
7-23	" " GC 11
7-24	" " GC 12
7-25	" " G 1
7-26	" " G 2
7-27	" " G 3
7-28	" " G 4
7-29	" " G 5
7-30	" " G 6
7-31	" " G 7
7-32	" " G 8
7-33	" " G 9
7-34	" " G 10
7-35	" " G 11
7-36	" " G 12
7-37	" " PC 1
7-38	" " PC 2
7-39	" " PC 3
7-40	" " PC 4
7-41	" " PC 5
7-42	" " PC 6
7-43	" " PC 7
7-44	" " PC 8
7-45	" " PC 9
7-46	" " PC 10
7-47	" " PC 11
7-48	" " PC 12
7-49	" " P 1
7-50	" " P 2

Table A.6.7 (Cont.)

7-51	"	"	P 3
7-52	"	"	P 4
7-53	"	"	P 5
7-54	"	"	P 6
7-55	"	"	P 7
7-56	"	"	P 8
7-57	"	"	P 9
7-58	"	"	P 10
7-59	"	"	P 11
7-60	"	"	P 12

Table A.6.7 (Cont.)

Exp. 7- Mini-columns - 1-26-95

Effluent	D coal runoff (pH 2.5 and adjusted to 5.0)
	cond = 2.9, turb = -1, DO = 11.2, temp 11.2, sal 0.1
	0.8 ppm Ni standards, pH 2.5 and 5.0
	1.5 ppm Zn standards, pH 2.5 and 5.0
Date coll.	1/26/95
Foam	Four foam types (made 1-24)were used:
	8 mesh with Cyanidium caldarium (high temp; GR1_6.XLS) 2.00 +-0.01 g/column
	8 mesh, no algae. 2.00 +- 0.01 g/column
	Solid plug with Cyanidium (avg wt of 3 was 2.35 g)
	Solid plug, no algae (avg wt of 2 was 1.49 g)
Method	EXP7PRO.DOC
Notes	Adjusted runoff was actiually 4.76 after centrifugation, unmodified was 2.48
	Ni 2.5 req/d 3.8 ml/l for pH adjustment
	Ni 5.0 took 0.2 ml
	Zn 2.5 took 3.3 ml
	Zn 5.0 took 0.2 ml
	Runoff 5.0 took5.0 ml
Results	

Sample	*Eff.*	*pH*	*Foam*	*Algae?*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
7_1	Runoff	2.5	Control		63.76	0.0191	0.0205	0.261	0.032	0.2	380.4	0.8345	1.571
7_2	Runoff	2.5	Control		64.38	0.0192	0.0211	0.2613	0.0347	0.2	381.2	0.8285	1.566
Mean					**64.07**	**0.0192**	**0.0208**	**0.26115**	**0.0334**	**0.2**	**380.8**	**0.8315**	**1.5685**
7_3	Runoff	5	Control		5.184	0.0063	0.0087	0.2325	0.0001	0.068	9.964	0.626	1.414
7_4	Runoff	5	Control		5.158	0.0049	0.0066	0.2317	0.0042	0.066	9.694	0.623	1.388
Mean					**5.171**	**0.0056**	**0.00765**	**0.2321**	**0.0022**	**0.067**	**9.829**	**0.6245**	**1.401**
7_5	Ni	2.5	Control		0.0123	0.0003	0.0006	nd	0.0005	0.008	0.0168	0.7707	0.0104
7_6	Ni	2.5	Control		0.0334	0	0.0007	nd	0.0003	0.008	0.0464	0.7704	0.0089
Mean					**0.02285**	**0.0002**	**0.00065**	**nd**	**0.0004**	**0.008**	**0.0316**	**0.7706**	**0.00965**
7_7	Ni	5	Control		nd	0.0001	nd	0.001	0.0004	0.009	0.0292	0.7705	0.0104
7_8	Ni	5	Control		nd	0	0.0002	nd	nd	0.009	0.0296	0.7691	0.0117
Mean					**nd**	**0.0001**	**0.0002**	**0.001**	**0.0004**	**0.009**	**0.0294**	**0.7698**	**0.01105**
7_9	Zn	2.5	Control		nd	nd	0.001	0.001	nd	nd	nd	0.01	1.293
7_10	Zn	2.5	Control		nd	nd	0.0001	0.0025	0.0017	6E-04	0.0108	0.0039	1.287
Mean					**nd**	**0**	**0.00055**	**0.00175**	**0.0017**	**6E-04**	**0.0108**	**0.007**	**1.29**
7_11	Zn	5	Control		0.4272	nd	0.0003	0.0015	0.0014	0.002	nd	0.0089	1.382
7_12	Zn	5	Control		0.4277	nd	0.0004	0.0009	0.0013	0.001	nd	0.0087	1.382
Mean					**0.42745**	**nd**	**0.00035**	**0.0012**	**0.0014**	**0.002**	**nd**	**0.0088**	**1.382**
7_13	Runoff	2.5	8 mesh	Yes	68.62	0.018	0.0195	0.255	0.0345	0.33	388.2	0.7975	1.583
7_14	Runoff	2.5	8 mesh	Yes	69.42	0.0179	0.0191	0.2557	0.0334	0.327	387.6	0.7999	1.562
Mean					**69.02**	**0.018**	**0.0193**	**0.25535**	**0.034**	**0.328**	**387.9**	**0.7987**	**1.5725**
7_15	Runoff	5	8 mesh	Yes	3.868	0.0038	0.0055	0.2282	0.001	0.06	10.62	0.5857	1.364
7_16	Runoff	5	8 mesh	Yes	3.797	0.0036	0.006	0.2242	0.002	0.065	10.3	0.5751	1.358

Mean					3.8325	0.0037	0.00575	0.2262	0.0015	0.063	10.46	0.5804	1.361
7_17	Ni	2.5	8 mesh	Yes	0.3545	nd	nd	0.0026	0.0001	0.074	0.0158	0.5658	0.0668
7_18	Ni	2.5	8 mesh	Yes	0.3752	nd	0.0001	0.0034	nd	0.08	0.0094	0.5397	0.0665
Mean					0.36485	nd	0.0001	0.003	0.0001	0.077	0.0126	0.5528	0.06665
7_19	Ni	5	8 mesh	Yes	0.4395	nd	nd	0.0039	nd	0.031	0.0031	0.2114	0.0313
7_20	Ni	5	8 mesh	Yes	0.1016	nd	nd	0.0027	0	0.036	nd	0.2112	0.0323
Mean					0.27055	nd	nd	0.0033	0	0.034	0.0031	0.2113	0.0318
7_21	Zn	2.5	8 mesh	Yes	0.3567	0.0005	0.0002	0.0053	0.0032	0.069	0.0024	0.0185	1.028
7_22	Zn	2.5	8 mesh	Yes	0.248	0.0001	nd	0.0039	0.0001	0.072	nd	0.0167	1.059
Mean					0.30235	0.0003	0.0002	0.0046	0.0017	0.07	0.0024	0.0176	1.0435
7_23	Zn	5	8 mesh	Yes	0.4188	0	nd	0.0011	nd	0.03	nd	0.0134	0.2821
7_24	Zn	5	8 mesh	Yes	0.2774	0.0001	nd	0.0026	nd	0.029	nd	0.015	0.3974
Mean					0.3481	5E-05	nd	0.00185	nd	0.03	nd	0.0142	0.33975
7_25	Runoff	2.5	8 mesh	No	72.44	0.0181	0.0184	0.2613	0.0298	1.078	435.7	0.8069	1.575
7_26	Runoff	2.5	8 mesh	No	72.15	0.0179	0.0188	0.2655	0.0313	1.089	435	0.8046	1.552
Mean					72.295	0.018	0.0186	0.2634	0.0306	1.084	435.35	0.8058	1.5635
7_27	Runoff	5	8 mesh	No	5.779	0.0041	0.0056	0.2398	0.0005	0.217	11.07	0.599	1.375
7_28	Runoff	5	8 mesh	No	5.682	0.0042	0.0068	0.2373	0.0011	0.195	10.8	0.5909	1.366
Mean					5.7305	0.0042	0.0062	0.23855	0.0008	0.206	10.935	0.595	1.3705
7_29	Ni	2.5	8 mesh	No	0.356	nd	0.0001	0.0011	0.0003	0.166	0.0041	0.7318	0.0414
7_30	Ni	2.5	8 mesh	No	0.3986	nd	0.0002	0.0005	0	0.213	0.0009	0.7267	0.0441
Mean					0.3773	nd	0.00015	0.0008	0.0002	0.19	0.0025	0.7293	0.04275
7_31	Ni	5	8 mesh	No	0.2559	0.0003	0.0006	nd	nd	0.102	0.0042	0.7254	0.0356
7_32	Ni	5	8 mesh	No	0.2334	0.0006	nd	nd	0.0009	0.101	0.0087	0.7191	0.0314
Mean					0.24465	0.0005	0.0006	nd	0.0009	0.101	0.0065	0.7223	0.0335
7_33	Zn	2.5	8 mesh	No	0.275	0	0.0841	nd	0.0003	0.2	0.0053	0.0242	1.406
7_34	Zn	2.5	8 mesh	No	0.2571	nd	0.0004	nd	0.0009	0.177	nd	0.0189	1.363
Mean					0.26605	0	0.04225	nd	0.0006	0.189	0.0053	0.0216	1.3845
7_35	Zn	5	8 mesh	No	0.2208	nd	nd	nd	0.0018	0.091	nd	0.0156	1.294
7_36	Zn	5	8 mesh	No	0.1733	0.0016	0.0011	0.0007	0.0019	0.092	nd	0.019	1.318
Mean					0.19705	0.0016	0.0011	0.0007	0.0019	0.091	nd	0.0173	1.306
7_37	Runoff	2.5	Plug	Yes	66.99	0.0178	0.019	0.2572	0.0279	0.179	402	0.7847	1.493
7_38	Runoff	2.5	Plug	Yes	67.36	0.0177	0.0192	0.2503	0.033	0.182	402.5	0.7916	1.471
Mean					67.175	0.0178	0.0191	0.25375	0.0305	0.181	402.25	0.7882	1.482
7_39	Runoff	5	Plug	Yes	5.423	0.0043	0.0062	0.235	0.0007	0.053	10.45	0.5841	1.332
7_40	Runoff	5	Plug	Yes	5.535	0.0043	0.0063	0.2312	0.0009	0.057	10.46	0.5935	1.356
Mean					5.479	0.0043	0.00625	0.2331	0.0008	0.055	10.455	0.5888	1.344
7_41	Ni	2.5	Plug	Yes	0.3158	0.0207	0.0193	0.0207	0.0197	0.022	nd	0.7283	0.0209
7_42	Ni	2.5	Plug	Yes	0.3052	0.008	0.0083	0.0141	0.0098	0.016	nd	0.6932	0.0219
Mean					0.3105	0.0144	0.0138	0.0174	0.0148	0.019	nd	0.7108	0.0214
7_43	Ni	5	Plug	Yes	0.1838	0.0021	0.0014	0.0021	0.0016	0.01	0.0167	0.6889	0.0235
7_44	Ni	5	Plug	Yes	0.1027	0.0007	0.0003	0.0028	0.001	0.006	nd	0.7059	0.0116
Mean					0.14325	0.0014	0.00085	0.00245	0.0013	0.008	0.0167	0.6974	0.01755
7_45	Zn	2.5	Plug	Yes	0.2005	0.0001	nd	0.0019	0.0012	0.003	nd	0.0161	1.274
7_46	Zn	2.5	Plug	Yes	0.1618	0	nd	nd	nd	0.003	nd	0.0169	1.275
Mean					0.18115	5E-05	nd	0.0019	0.0012	0.003	nd	0.0165	1.2745

7_47	Zn	5	Plug	Yes	0.1717	0	nd	0.0015	nd	0.002	nd	0.0172	1.241
7_48	Zn	5	Plug	Yes	0.1393	nd	nd	0.0003	nd	0.003	nd	0.0165	1.252
Mean					0.1555	0	nd	0.0009	nd	0.003	nd	0.0169	1.2465
7_49	Runoff	2.5	Plug	No	69.95	0.0184	0.0169	0.2766	0.0309	0.189	419.4	0.8	1.56
7_50	Runoff	2.5	Plug	No	69.23	0.0181	0.0198	0.2716	0.0297	0.183	414.5	0.7984	1.539
Mean					69.59	0.0183	0.01835	0.2741	0.0303	0.186	416.95	0.7992	1.5495
7_51	Runoff	5	Plug	No	5.428	0.048	0.0581	0.3095	0.0752	0.104	10.39	0.6617	1.465
7_52	Runoff	5	Plug	No	5.496	0.0275	0.0355	0.2845	0.0364	0.083	10.6	0.6342	1.447
Mean					5.462	0.0378	0.0468	0.297	0.0558	0.093	10.495	0.648	1.456
7_53	Ni	2.5	Plug	No	0.1193	0.0062	0.0072	0.0152	0.0094	0.015	0.0009	0.7431	0.0361
7_54	Ni	2.5	Plug	No	0.1338	0.0008	0.002	nd	0.0006	0.008	nd	0.7254	0.023
Mean					0.12655	0.0035	0.0046	0.0152	0.005	0.011	0.0009	0.7343	0.02955
7_55	Ni	5	Plug	No	0.1291	0.0004	0	nd	0.0004	0.008	nd	0.7256	0.0109
7_56	Ni	5	Plug	No	0.0783	0.0001	nd	nd	nd	0.005	nd	0.737	0.0094
Mean					0.1037	0.0003	0	nd	0.0004	0.006	nd	0.7313	0.01015
7_57	Zn	2.5	Plug	No	0.1279	0.0002	nd	nd	0.0011	0.004	nd	0.031	1.297
7_58	Zn	2.5	Plug	No	0.0194	0	nd	nd	0.001	0.002	nd	0.0202	1.329
Mean					0.07365	0.0001	nd	nd	0.0011	0.003	nd	0.0256	1.313

7_59	Zn	5	Plug	No	0.1067	0.0001	nd	0.0013	0.0016	0.001	nd	0.0186	1.342
7_60	Zn	5	Plug	No	0.0197	nd	nd	0.0006	0.003	0.002	nd	0.0187	1.343
Mean					0.0632	0.0001	nd	0.00095	0.0023	0.002	nd	0.0187	1.3425
Summary - Means													
Effluent	*pH*	*Foam*	*Algae?*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*	
Runoff	2.5	Control		64.07	0.01915	0.0208	0.26115	0.03335	0.1997	380.8	0.8315	1.5685	
Runoff	5	Control		5.171	0.0056	0.0077	0.2321	0.00215	0.0667	9.829	0.6245	1.401	
Ni	2.5	Control		0.023	0.00015	0.0007	nd	0.0004	0.0082	0.032	0.7706	0.0097	
Ni	5	Control		nd	0.0001	0.0002	0.001	0.0004	0.0088	0.029	0.7698	0.0111	
Zn	2.5	Control		nd	0	0.0006	0.00175	0.0017	0.0006	0.011	0.007	1.29	
Zn	5	Control		0.427	nd	0.0004	0.0012	0.00135	0.0016	nd	0.0088	1.382	
Runoff	2.5	8 mesh	Yes	69.02	0.01795	0.0193	0.25535	0.03395	0.3281	387.9	0.7987	1.5725	
Runoff	5	8 mesh	Yes	3.833	0.0037	0.0058	0.2262	0.0015	0.0628	10.46	0.5804	1.361	
Ni	2.5	8 mesh	Yes	0.365	nd	0.0001	0.003	0.0001	0.0769	0.013	0.5528	0.0667	
Ni	5	8 mesh	Yes	0.271	nd	nd	0.0033	0	0.0335	0.003	0.2113	0.0318	
Zn	2.5	8 mesh	Yes	0.302	0.0003	0.0002	0.0046	0.00165	0.0705	0.002	0.0176	1.0435	
Zn	5	8 mesh	Yes	0.348	0.00005	nd	0.00185	nd	0.0296	nd	0.0142	0.3398	
Runoff	2.5	8 mesh	No	72.3	0.018	0.0186	0.2634	0.03055	1.0835	435.4	0.8058	1.5635	
Runoff	5	8 mesh	No	5.731	0.00415	0.0062	0.23855	0.0008	0.206	10.94	0.595	1.3705	
Ni	2.5	8 mesh	No	0.377	nd	0.0002	0.0008	0.00015	0.1897	0.003	0.7293	0.0428	
Ni	5	8 mesh	No	0.245	0.00045	0.0006	nd	0.0009	0.1013	0.006	0.7223	0.0335	
Zn	2.5	8 mesh	No	0.266	0	0.0423	nd	0.0006	0.1887	0.005	0.0216	1.3845	
Zn	5	8 mesh	No	0.197	0.0016	0.0011	0.0007	0.00185	0.0911	nd	0.0173	1.306	
Runoff	2.5	Plug	Yes	67.18	0.01775	0.0191	0.25375	0.03045	0.1805	402.3	0.7882	1.482	
Runoff	5	Plug	Yes	5.479	0.0043	0.0063	0.2331	0.0008	0.055	10.46	0.5888	1.344	
Ni	2.5	Plug	Yes	0.311	0.01435	0.0138	0.0174	0.01475	0.0191	nd	0.7108	0.0214	
Ni	5	Plug	Yes	0.143	0.0014	0.0009	0.00245	0.0013	0.0082	0.017	0.6974	0.0176	
Zn	2.5	Plug	Yes	0.181	0.00005	nd	0.0019	0.0012	0.0029	nd	0.0165	1.2745	
Zn	5	Plug	Yes	0.156	0	nd	0.0009	nd	0.0027	nd	0.0169	1.2465	
Runoff	2.5	Plug	No	69.59	0.01825	0.0184	0.2741	0.0303	0.1857	417	0.7992	1.5495	
Runoff	5	Plug	No	5.462	0.03775	0.0468	0.297	0.0558	0.0933	10.5	0.648	1.456	
Ni	2.5	Plug	No	0.127	0.0035	0.0046	0.0152	0.005	0.0115	9E-04	0.7343	0.0296	

Ni	5	Plug	No	0.104	0.00025	0	nd	0.0004	0.0061	nd	0.7313	0.0102	
Zn	2.5	Plug	No	0.074	0.0001	nd	nd	0.00105	0.0032	nd	0.0256	1.313	
Zn	5	Plug	No	0.063	0.0001	nd	0.00095	0.0023	0.0017	nd	0.0187	1.3425	

Summary - Means minus original metal levels in effluents and standards

Effluent	*pH*	*Foam*	*Algae?*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
Runoff	2.5	8 mesh	Yes	69.02	0.01795	0.0193	0.25535	0.03395	0.3281	387.9	0.7987	1.5725
ppm Removed				-4.95	0.0012	0.0015	0.0058	-0.0006	-0.128	-7.1	0.0328	-0.004
% removed				-7.73	6.26632	7.2115	2.22095	-1.7991	-64.27	-1.86	3.9447	-0.255
Runoff	5	8 mesh	Yes	3.833	0.0037	0.0058	0.2262	0.0015	0.0628	10.46	0.5804	1.361
ppm Removed				1.339	0.0019	0.0019	0.0059	0.00065	0.0039	-0.63	0.0441	0.04
% removed				25.88	33.9286	24.837	2.54201	30.2326	5.7764	-6.42	7.0616	2.8551
Ni	2.5	8 mesh	Yes	0.365	nd	0.0001	0.003	0.0001	0.0769	0.013	0.5528	0.0667
ppm Removed				-0.34	#VALUE!	0.0006	#VALUE!	0.0003	-0.069	0.019	0.2178	-0.057
% removed				-1497	#VALUE!	84.615	#VALUE!	75	-837.2	60.13	28.266	-590.7
Ni	5	8 mesh	Yes	0.271	nd	nd	0.0033	0	0.0335	0.003	0.2113	0.0318
ppm Removed				nd	#VALUE!	######	-0.0023	0.0004	-0.025	0.026	0.5585	-0.021
% removed				nd	nd	nd	-230	100	-280.7	89.31	72.551	-186.5
Zn	2.5	8 mesh	Yes	0.302	0.0003	0.0002	0.0046	0.00165	0.0705	0.002	0.0176	1.0435
ppm Removed				nd	-0.0003	0.0004	-0.0029	5E-05	-0.07	0.009	-0.011	0.2465
% removed				nd	#DIV/0!	66.667	-162.86	2.94118	-11642	78.18	-151.4	19.109
Zn	5	8 mesh	Yes	0.348	0.00005	nd	0.00185	nd	0.0296	nd	0.0142	0.3398
ppm Removed				0.079	#VALUE!	######	-0.0007	#VALUE!	-0.028	#####	-0.005	1.0423
% removed				18.56	#VALUE!	######	-54.167	#VALUE!	-1810	#####	-61.36	75.416
Runoff	2.5	8 mesh	No	72.3	0.018	0.0186	0.2634	0.03055	1.0835	435.4	0.8058	1.5635
ppm Removed				-8.23	0.00115	0.0022	-0.0022	0.0028	-0.884	-54.6	0.0258	0.005
% removed				-12.8	6.00522	10.577	-0.8616	8.3958	-442.6	-14.3	3.0968	0.3188
Runoff	5	8 mesh	No	5.731	0.00415	0.0062	0.23855	0.0008	0.206	10.94	0.595	1.3705
ppm Removed				-0.56	0.00145	0.0015	-0.0065	0.00135	-0.139	-1.11	0.0296	0.0305
% removed				-10.8	25.8929	18.954	-2.779	62.7907	-209	-11.3	4.7318	2.177
Ni	2.5	8 mesh	No	0.377	nd	0.0002	0.0008	0.00015	0.1897	0.003	0.7293	0.0428
ppm Removed				-0.35	#VALUE!	0.0005	#VALUE!	0.00025	-0.182	0.029	0.0413	-0.033
% removed				-1551	#VALUE!	76.923	#VALUE!	62.5	-2213	92.09	5.3598	-343
Ni	5	8 mesh	No	0.245	0.00045	0.0006	nd	0.0009	0.1013	0.006	0.7223	0.0335
ppm Removed				nd	-0.0004	-4E-04	#VALUE!	-0.0005	-0.092	0.023	0.0476	-0.022
% removed				nd	-350	-200	#VALUE!	-125	-1051	77.76	6.1769	-201.8
Zn	2.5	8 mesh	No	0.266	0	0.0423	nd	0.0006	0.1887	0.005	0.0216	1.3845
ppm Removed				nd	0	-0.042	#VALUE!	0.0011	-0.188	0.006	-0.015	-0.095
% removed				nd	#DIV/0!	-6942	#VALUE!	64.7059	-31342	51.82	-207.9	-7.326
Zn	5	8 mesh	No	0.197	0.0016	0.0011	0.0007	0.00185	0.0911	nd	0.0173	1.306
ppm Removed				0.23	#VALUE!	-8E-04	0.0005	-0.0005	-0.09	#####	-0.009	0.076
% removed				53.9	#VALUE!	-214.3	41.6667	-37.037	-5774	#####	-96.59	5.4993
Runoff	2.5	Plug	Yes	67.18	0.01775	0.0191	0.25375	0.03045	0.1805	402.3	0.7882	1.482
ppm Removed				-3.11	0.0014	0.0017	0.0074	0.0029	0.0192	-21.5	0.0434	0.0865

% removed				-4.85	7.3107	8.1731	2.83362	8.69565	9.6144	-5.63	5.2135	5.5148	
Runoff	5	Plug	Yes	5.479	0.0043	0.0063	0.2331	0.0008	0.055	10.46	0.5888	1.344	
ppm Removed				-0.31	0.0013	0.0014	-0.001	0.00135	0.0117	-0.63	0.0357	0.057	
% removed				-5.96	23.2143	18.301	-0.4308	62.7907	17.554	-6.37	5.7166	4.0685	
Ni	2.5	Plug	Yes	0.311	0.01435	0.0138	0.0174	0.01475	0.0191	nd	0.7108	0.0214	
ppm Removed				-0.29	-0.0142	-0.013	#VALUE!	-0.0144	-0.011	#####	0.0598	-0.012	
% removed				-1259	-9466.7	-2023	#VALUE!	-3587.5	-132.9	#####	7.7607	-121.8	
Ni	5	Plug	Yes	0.143	0.0014	0.0009	0.00245	0.0013	0.0082	0.017	0.6974	0.0176	
ppm Removed				nd	-0.0013	-7E-04	-0.0015	-0.0009	0.0006	0.012	0.0724	-0.006	
% removed				nd	-1300	-325	-145	-225	6.8182	42.41	9.405	-58.11	
Zn	2.5	Plug	Yes	0.181	0.00005	nd	0.0019	0.0012	0.0029	nd	0.0165	1.2745	
ppm Removed				nd	-5E-05	######	-0.0002	0.0005	-0.002	#####	-0.01	0.0155	
% removed				nd	#DIV/0!	######	-8.5714	29.4118	-383.3	#####	-135.7	1.2016	
Zn	5	Plug	Yes	0.156	0	nd	0.0009	nd	0.0027	nd	0.0169	1.2465	
ppm Removed				0.272	#VALUE!	######	0.0003	#VALUE!	-0.001	#####	-0.008	0.1355	
% removed				63.62	#VALUE!	######	25	#VALUE!	-70.97	#####	-91.48	9.8046	
Runoff	2.5	Plug	No	69.59	0.01825	0.0184	0.2741	0.0303	0.1857	417	0.7992	1.5495	
ppm Removed				-5.52	0.0009	0.0025	-0.013	0.00305	0.0141	-36.2	0.0323	0.019	
% removed				-8.62	4.69974	11.779	-4.9588	9.14543	7.0356	-9.49	3.8845	1.2113	
Runoff	5	Plug	No	5.462	0.03775	0.0468	0.297	0.0558	0.0933	10.5	0.648	1.456	
ppm Removed				-0.29	-0.0322	-0.039	-0.0649	-0.0537	-0.027	-0.67	-0.023	-0.055	
% removed				-5.63	-574.11	-511.8	-27.962	-2495.3	-39.98	-6.78	-3.755	-3.926	
Ni	2.5	Plug	No	0.127	0.0035	0.0046	0.0152	0.005	0.0115	9E-04	0.7343	0.0296	
ppm Removed				-0.1	-0.0034	-0.004	#VALUE!	-0.0046	-0.003	0.031	0.0363	-0.02	
% removed				-454	-2233.3	-607.7	#VALUE!	-1150	-39.63	97.15	4.7109	-206.2	
Ni	5	Plug	No	0.104	0.00025	0	nd	0.0004	0.0061	nd	0.7313	0.0102	
ppm Removed				nd	-0.0002	0.0002	#VALUE!	0	0.0027	#####	0.0385	0.001	
% removed				nd	-150	100	#VALUE!	0	30.682	#####	5.0013	8.5586	
Zn	2.5	Plug	No	0.074	0.0001	nd	nd	0.00105	0.0032	nd	0.0256	1.313	
ppm Removed				nd	-0.0001	######	#VALUE!	0.00065	-0.003	#####	-0.019	-0.023	
% removed				nd	#DIV/0!	######	#VALUE!	38.2353	-425	#####	-265.7	-1.783	
Zn	5	Plug	No	0.063	0.0001	nd	0.00095	0.0023	0.0017	nd	0.0187	1.3425	

ppm Removed			0.364	#VALUE!	######	0.00025	-0.001	-2E-04	#####	-0.01	0.0395
% removed			85.21	#VALUE!	######	20.8333	-70.37	-9.677	#####	-111.9	2.8582
Percentage Removal from pH 2.5 Runoff											
	Al	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*		
8 +, algae	-7.7259	6.266319	7.212	2.22095	-1.799	-64.271	-1.8645	3.9447	-0.26		
8, no algae	-12.838	6.005222	10.58	-0.8616	8.3958	-442.56	-14.325	3.0968	0.319		
Plug + algae	-4.8463	7.310705	8.173	2.83362	8.6957	9.61442	-5.6329	5.2135	5.515		
Plug, no al	-8.6156	4.699739	11.78	-4.9588	9.1454	7.03555	-9.4932	3.8845	1.211		
Percentage Removal from pH 5 Runoff											
	Al	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*		
8 + algae	25.8847	33.92857	24.84	2.54201	30.233	5.77644	-6.4198	7.0616	2.855		
8, no algae	-10.82	25.89286	18.95	-2.779	62.791	-209	-11.252	4.7318	2.177		
Plug + algae	-5.9563	23.21429	18.3	-0.4308	62.791	17.5544	-6.3689	5.7166	4.069		
Plug, no al	-5.6275	-574.107	-512	-27.962	-2495	-39.985	-6.7759	-3.755	-3.93		
Percent Nickel Removal from pH 2.5 Ni standard											
	Ni										
8 + algae	28.2655										
8, no algae	5.35981										
Plug + algae	7.76069										
Plug, no al	4.71092										
Percentage Nickel Removal from pH 5 Ni standardf											
	Ni										
8 + algae	72.551										
8, no algae	6.1769										
Plug + algae	9.408										
Plug, no al	5.0013										
Percentage Zinc Removal from pH 2. 5 Zn standard											
	Zn										
8 + algae	19.109										
8, no algae	-7.326										
Plug + algae	1.2016										
Plug, no al	-1.783										
Percentage Zinc Removal from pH 5 Zn standard											
	Zn										

8 + algae	75.4161												
8, no algae	5.49928												
Plug + algae	9.80463												
Plug, no al	2.85818												

Table A.6.8 Experimental procedures and results forExperiment #8

EXPERIMENT 8 Metal Removal using *Cyanidium* in Mini-Columns

I. PURPOSE:

To investigate reasons for the poor performance of foam-Cyanidium aggregates with coal runoff compared to standard metal solutions, we will attempt to treat both runoff (pretreated to reduce iron and Al content) and a mixture of metal standards containing Al, Be, Cd, Co, Cr, Cu, Fe, Ni, and Zn at levels similar to that of the runoff. Single-metal solutions of Ni and Zn will also be tested as a comparison to the mixtures and to the previous experiment. The experiment is expected to show whether the problem relates to competition between metal ions or to some feature of the chemical environment (such as organic content) specific to the actual runoff. Only pH 5 will be used this time, since metal removal is clearly superior at this pH. Similarly, based on the previous experiment we have selected pulverized foam rather than plugs. The mini-columns will be modified to reduce the initial rapid flow rate observed previously, increasing average contact time of metal ions with the algal biomass. This is expected to improve metal removal percentages.

Additionally, the entire experiment will be duplicated using Phaeodactylum and Chlorella as a comparison with Cyanidium.

II. ALGAE:

10 - 14 - day old *Phaeodactylum* and thermophilic *Cyanidium caldarium* and *Chlorella vulgaris* Harvested by centrifugation, washed once with DI, resuspended at 3% by dry wt in DI. Embedded at 6% in foam.

III. FOAM:

8 mesh, 2 g/column.

IV. EFFLUENTS:

D area coal basin runoff, collected from basin, adjusted to pH 5 and centrifuged
0.8 ppm Ni standard, pH 5, centrifuged
1.5 ppm Zn standard, pH 5, centrifuged
Mixture of the following (pH 5, centrifuged)
- Al standard, 64 ppm
- Be standard, 0.02 ppm
- Cd standard, 0.02 ppm
- Co standard, 0.26 ppm
- Cr standard, 0.03 ppm
- Cu standard, 0.2 ppm
- Fe standard, 380 ppm

Ni standard, 0.8 ppm
Zn standard, 1.5 ppm

V. TEST APPARATUS (GENERAL DESCRIPTION):

10 ml BioRad columns, 50 ml funnels. Modify to standardize and reduce flow rates. Acid-washed.

VI. PROCEDURE:

A. Preparation of effluent

1. Collect surface water from D area coal pile runoff basin (1 carboy). Measure pH.

2. Upon returning to lab, prepare a flask containing 1.5 liters of runoff.

3. Adjust pH to 5 using NaOH. Record initial and final pH values and volume of NaOH added (in drops; 1 drop = 0.042 ml).

4. Centrifuge to remove precipitates (10 min, 10000 rpm). Decant or pipet off supernatant so as not to disturb pellet.

5. Recheck and record pH value of supernatant.

6. Take two 30 ml samples of each, preserve (0.6 ml conc. HNO3) for analysis.

B. Preparation of standards

1. Nickel, 100 ppm standard. Place 10 ml of a 1000 ug/ml NIST Ni standard (in 2% HNO3) in a 100 ml volumetric flask. Fill to mark with deionized water .

2. Nickel working standard, 0.8 ppm. Place 8 ml of 100 ppm standard in a 1000 ml volumetric, dilute to 1 liter.

3. Check the pH of working standard. Adjust to pH 5.0. Use NaOH and/or HNO3 for these adjustments. Record initial and final pH as well as drops of acid or base added.

4. Centrifuge standard and decant supernatant in the same manner as for effluent.

5. Take two 30-ml samples , preserve and send for analysis.

6. Zinc, 100 ppm standard. Place 10 ml of 1000 ug/ml NIST standard (in 2% HNO3) into a 100 ml volumetric. Fill to mark with deionized water.

7. Zinc working standard, 1.5 ppm. Place 15 ml of 100 ppm standard into a 1000 ml volumetric, dilute to 1 liter.

8. Check pH, adjust to pH 5, centrifuge, decant, and sample in the same manner as for Ni standards (Steps 3-5).

9. Be, 100 ppm standard. Place 1 ml pf 10 mg/ml NIST standard into a 100 ml volumetric. Fill to mark with deionized water.

10. Cd, 100 ppm standard. Dilute 10 ml of 1000 ug/ml NIST standard using a 100 ml volumetric.

11. Co, 100 ppm standard. Dilute 1 ml of 10 mg/ml NIST standard using a 100 ml volumetric.

12. Cr, 100 ppm standard. Dilute 10 ml of 1000 ug/ml NIST standard using a 100 ml volumetric.

13. Cu, 100 ppm standard. Dilute 10 ml of 1000 ug/ml NIST standard using a 100 ml volumetric.

14. Mixed metal solutions. Prepare a mixture of the following in a 1000 ml volumetric:

Al - 64 ml of 1000 ug/ml NIST standard
Be - 200 ul of 100 ppm standard.
Cd - 200 ul of 100 ppm standard
Co - 2.6 ml of 100 ppm standard
Cr - 300 ul of 100 ppm standard
Cu - 2 ml of 100 ppm standard
Fe - 380 ml of 1000 ug/ml NIST standard
Ni - 8 ml of 100 ppm standard
Zn - 15 ml of 100 ppm standard

Adjust pH to 5, centrifuge and decant.
Take 2 X 30 ml samples, preserve as before.

C. Preparation of columns

1. Pack 8 columns with 2 g of particulate foam + Cyanidium (8 mesh) each.

2. Pack 8 columns with 2 g particulate foam + Phaeodactylum (8 mesh) each.

3. Pack 8 columns with 2 g plain particulate foam (8 mesh) each

D. **Contacting biosorbent with effluent**

1. Set up 8 columns containing ground foam + Cyanidium. Place funnels on top and acid-washed 125 ml flasks underneath. Number columns GC1-8.

2. Place 50 ml standard or effluent in each funnel as follows:

Columns GC 1, 2 - Coal runoff, pH 5
Columns GC 3, 4 - Ni standard, pH 5
Columns GC 5, 6 - Zn standard, pH 5
Columns GC 7, 8 - Mixed metal standards, pH 5

3. Wait until liquid stops coming out of columns. Remove 35 ml from each flask to a labeled centrifuge tube.

4. Centrifuge 10 min, 10000 rpm

5. Pipet off 30 ml from each tube to a labeled sample bottle. Preserve for analysis.

6. Set up 8 more columns containing ground foam + Phaeodactylum. Label them GP 1-8. Repeat Steps 2-5.

7. Set up 8 columns with plain ground foam (no algae). Label them G1-8. Repeat Steps 2-5.

VII. APPARATUS

Coal basin runoff, at least 3 liters
1000 ppm Ni standard
1000 ppm Zn standard
1000 ppm Al standard

10000 ppm Be standard
1000 ppm Cd standard
10000 ppm Co standard
1000 ppm Cr standard
1000 ppm Cu standard
1000 ppm Fe standard
HNO3, 1 N
HNO3, 10 N
NaOH, 1 N
NaOH, 10 N
HNO3, ca. 7 N for acid washing
HNO3, ca 3 N for rinsing pH electrode
Deionized water
Cyanidium
Phaeodactylum
Foam as described in methods

Carboy for effluent

The following glass and plasticware should be acid-washed.

Bio-Rad columns and funnels, about 30 acid-washed
24 125 ml flasks
32 centrifuge tubes
at least 32 sample bottles
16 250 ml centrifuge bottles (may have to rewash halfway through)
7 100 ml volumetrics
4 1000 ml volumetrics
4 2 liter flasks
8 1 liter flasks
5 ml pipet tips, lots

VII. SAMPLE LABELS

The following labels will be used on samples sent to Analytical.

Label	*Description*
8-1	Control - Effluent, pH 5
8-2	"
8-3	Control - Ni standard, pH 5
8-4	"
8-5	Control - Zn standard, pH 5
8-6	"
8-7	Mixed metal standard, pH 5

8-8	"
8-9	Sample from GC 1
8-10	Sample from GC 2
8-11	Sample from GC 3
8-12	" " GC 4
8-13	" " GC 5
8-14	" " GC 6
8-15	" " GC 7
8-16	" " GC 8
8-17	" " GP 1
8-18	" " GP 2
8-19	" " GP 3
8-20	" " GP 4
8-21	" " GP 5
8-22	" " GP 6
8-23	" " GP 7
8-24	" " GP 8
8-25	" " G 1
8-26	" " G 2
8-27	" " G 3
8-28	" " G 4
8-29	" " G 5
8-30	" " G 6
8-31	" " G 7
8-32	" " G 8

Table A.6.8 (Cont.)

Exp. 8- Mini-columns - 3-14-95

Effluent	D coal runoff (pH 2.5 and adjusted to 5.0), collected 3-14-95
	cond = 2.760, turb = -1, DO = 8.36, temp 17.4, sal 0.13
	CMP13 pit water 3-13-95, bottle 1252, pH 5.521
	0.8 ppm Ni standards, pH 5.0
	1.5 ppm Zn standards, pH 5.0
	Mixed metal standard as described in Exp8pro.doc
Foam	Four foam types (made 3-11, ground up 3-13)were used:
	Ground but unsieved, 11.03% Cyanidium. Used 1.7 g/column (.1875 g dw algae)
	Ground but unsieved, 9.46% Phaeodactylum. Used 2 g/col (.1892 g dw algae)
	Ground but unsieved, 9.76% Chlorella. Used 1.9 g/col (.1854 g dw algae)
	Ground but unsieved, no algae
Method	EXP8PRO.DOC
Notes	Adjusted runoff was actiually 4.95 after centrifugation, unmodified was 2.524
	Ni 5.0 took 0.42ml/l to adj. pH
	Zn 5.0 took 0.59 ml/l
	Runoff 5.0 took 3.36 ml
	Mixed metals took 5.29 ml/500 ml

Results

Sample	*Effluent*	*pH*	*Foam*	*Algae?*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
8_1	Coal	5	None	None	3.173	0.0245	0.0377	0.2562	0.0267	0.078	3.675	0.591	1.271
8_2	Coal	5	None	None	2.708	0.0077	0.0161	0.2332	0.0041	0.0529	4.922	0.5696	1.262
Mean					2.9405	0.0161	0.0269	0.2447	0.0154	0.0655	4.2985	0.5803	1.2665
8_3	Ni	5	None	None	0.2003	nd	0.0005	0.0017	nd	nd	0.2652	0.77	0.0225
8_4	Ni	5	None	None	nd	0.064	0.0686	0.0623	0.0567	0.0686	0.1473	0.834	0.0877
Mean					0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.2063	0.802	0.0551
8_5	Zn	5	None	None	nd	0.0259	0.0292	0.0262	0.0375	0.0304	0.0409	0.0345	1.44
8_6	Zn	5	None	None	0.0462	0.0096	0.0106	0.015	0.0098	0.0041	nd	0.01	1.432
Mean					0.0231	0.0178	0.0199	0.0206	0.0237	0.0173	0.0205	0.0223	1.436
8_7	Metal mix	5	None	None	17.19	0.0109	0.0531	0.238	0.0454	0.0942	79.9	0.5887	1.216
8_8	Metal mix	5	None	None	17.31	0.0185	0.0169	0.2092	0.0075	0.1066	81.42	0.576	1.179
Mean					17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975
8_9	DI-H2O	5	None	None	0.2243	0.0059	0.0054	0.0018	nd	nd	2.046	0.0027	0.0275
8_10	DI-H2O	5	None	None	nd	0.0437	0.0403	0.0474	0.0525	0.0463	1.143	0.0356	0.0347
Mean					0.11215	0.0248	0.02285	0.0246	0.0263	0.0232	1.5945	0.0192	0.0311
8_11	CMP water	5.52	None	None	0.0855	0.0681	0.0628	0.0641	0.0666	0.103	nd	0.0585	0.0977
8_12	CMP water	5.52	None	None	nd	0.029	0.0327	0.0374	0.0375	0.0568	nd	0.03	0.0638
Mean					0.04275	0.0486	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.08075
8_13	Coal	2.5	None	None	65.17	0.0268	0.0262	0.2638	0.0259	0.2075	392.9	0.7366	1.474
8_14	Coal	2.5	None	None	65.73	0.0159	0.0128	0.244	0.0219	0.1955	398.1	0.7332	1.489
Mean					65.45	0.0214	0.0195	0.2539	0.0239	0.2015	395.5	0.7349	1.4815
8_15	Coal	5	Yes	Cyanidium	2.1	nd	nd	0.2103	nd	0.0216	4.19	0.5086	1.232
8_16	Coal	5	Yes	Cyanidium	2.269	nd	nd	0.2117	nd	0.0202	2.682	0.5199	1.227
Mean					2.1845	0	0	0.211	0	0.0209	3.436	0.5143	1.2295

8_17	Ni	5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	0.6324	0.0097
8_18	Ni	5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	0.6534	0.0109
Mean					0	0	0	0	0	0	0	0.6429	0.0103
8_19	Zn	5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	nd	1.205
8_20	Zn	5	Yes	Cyanidium	0.0223	nd	nd	nd	nd	nd	nd	nd	1.235
Mean					0.01115	0	0	0	0	0	0	0	1.22
8_21	Metal mix	5	Yes	Cyanidium	3.952	nd	0.0035	0.1616	nd	0.0234	12.61	0.4349	0.9601
8_22	Metal mix	5	Yes	Cyanidium	3.776	nd	0.0023	0.1594	nd	0.0263	9.665	0.4264	0.9465
Mean					3.864	0	0.0029	0.1605	0	0.0249	11.138	0.4307	0.9533
8_23	DI H2O	5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	nd	0.0064
8_24	DI H2O	5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	nd	0.0019
Mean					0	0	0	0	0	0	0	0	0.00415
8_25	CMP	5.5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	nd	0.0213
8_26	CMP	5.5	Yes	Cyanidium	nd	nd	nd	nd	nd	nd	nd	nd	0.0203
Mean					0	0	0	0	0	0	0	0	0.0208
8_27	Coal	5	Yes	Phaeodac	1.26	nd	nd	0.1814	nd	0.0128	3.393	0.4262	0.9766
8_28	Coal	5	Yes	Phaeodac	1.377	nd	nd	0.1789	nd	0.0158	3.376	0.4189	0.9707
Mean					1.3185	0	0	0.18015	0	0.0143	3.3845	0.4226	0.97365
8_29	Ni	5	Yes	Phaeodac	nd	nd	nd	nd	nd	nd	0.9306	0.3177	0.0055
8_30	Ni	5	Yes	Phaeodac	0.0299	nd	nd	nd	nd	nd	0.9459	0.2845	0.0031
Mean					0.01495	0	0	0	0	0	0.9383	0.3011	0.0043
8_31	Zn	5	Yes	Phaeodac	nd	0	0.0012	0.0005	0.0046	0.0116	0.9866	0.0059	0.4417
8_32	Zn	5	Yes	Phaeodac	nd	nd	0.0006	nd	nd	0.0123	0.9744	0.0091	0.3438
Mean					0	0	0.0009	0.00025	0.0023	0.012	0.9805	0.0075	0.39275
8_33	Metal mix	5	Yes	Phaeodac	0.739	0.0014	0.0109	0.1654	0.0029	0.0336	0.3474	0.41	0.7871
8_34	Metal mix	5	Yes	Phaeodac	0.9005	0.0015	0.011	0.1686	0.0026	0.0322	0.4192	0.4057	0.7901
Mean					0.81975	0.0015	0.01095	0.167	0.0028	0.0329	0.3833	0.4079	0.7886
8_35	DI H2O	5	Yes	Phaeodac	0.0816	nd	0.0012	0.0013	0.0025	0.011	0.896	0.0043	0.0171
8_36	DI H2O	5	Yes	Phaeodac	0.0964	nd	0.0007	0.004	0.0029	0.0089	0.996	0.0015	0.01454
Mean					0.089	0	0.00095	0.00265	0.0027	0.01	0.946	0.0029	0.01582
8_37	CMP	5.5	Yes	Phaeodac	nd	nd	nd	nd	0.0029	0.0191	1.004	0.0088	0.0225
8_38	CMP	5.5	Yes	Phaeodac	0.0122	nd	0.0006	0.0021	0.0038	0.0189	0.9552	0.0033	0.0199
Mean					0.0061	0	0.0003	0.00105	0.0034	0.019	0.9796	0.0061	0.0212
8_39	Coal	5	Yes	Chlorella	1.55	0.0025	0.005	0.2028	0.0011	0.0307	2.288	0.4657	1.07
8_40	Coal	5	Yes	Chlorella	1.396	0.0025	0.0051	0.1948	0.0012	0.0297	2.25	0.4584	1.059
Mean					1.473	0.0025	0.00505	0.1988	0.0012	0.0302	2.269	0.4621	1.0645
8_41	Ni	5	Yes	Chlorella	0.099	0.0028	0.004	nd	0.0082	0.0144	0.0746	0.1226	0.0134
8_42	Ni	5	Yes	Chlorella	nd	0.0002	0.0017	nd	nd	0.0069	0.0251	0.1285	0.0087
Mean					0.0495	0.0015	0.00285	0	0.0041	0.0107	0.0499	0.1256	0.01105
8_43	Zn	5	Yes	Chlorella	0.0576	0.0002	0.0015	nd	0.0024	0.0016	0.0387	nd	0.2385
8_44	Zn	5	Yes	Chlorella	0.1331	0	0.0006	nd	0.0016	nd	0.0277	nd	0.3601
Mean					0.09535	0.0001	0.00105	0	0.002	0.0008	0.0332	0	0.2993
8_45	Metal mix	5	Yes	Chlorella	2.063	0.0023	0.0099	0.1347	nd	0.0303	5.053	0.3948	0.7634
8_46	Metal mix	5	Yes	Chlorella	1.094	0.002	0.0091	0.139	0.0021	0.0262	1.51	0.3863	0.7602
Mean					1.5785	0.0022	0.0095	0.13685	0.0011	0.0283	3.2815	0.3906	0.7618

8_47	DI H2O	5	Yes	Chlorella	nd	0	0.0005	nd	nd	nd	0.0256	nd	0.0071
8_48	DI H2O	5	Yes	Chlorella	0.2719	nd	0.0009	nd	0.0028	nd	0.0215	nd	0.0097
Mean					0.13595	0	0.0007	0	0.0014	0	0.0236	0	0.0084
8_49	CMP	5.5	Yes	Chlorella	nd	nd	nd	nd	0.0036	0.0121	0.0274	nd	0.013
8_50	CMP	5.5	Yes	Chlorella	nd	nd	0.0002	nd	0.0009	0.0076	0.0181	nd	0.0157
Mean					0	0	0.0001	0	0.0023	0.0099	0.0228	0	0.01435
8_51	Coal	5	Yes	None	2.388	0.0032	0.0068	0.2095	nd	0.0478	2.723	0.5295	1.188
8_52	Coal	5	Yes	None	2.211	0.0032	0.007	0.207	nd	0.0508	3.221	0.5208	1.178
Mean					2.2995	0.0032	0.0069	0.20825	0	0.0493	2.972	0.5252	1.183
8_53	Ni	5	Yes	None	0.1367	nd	0.0008	nd	nd	0.0084	nd	0.6724	0.0164
8_54	Ni	5	Yes	None	nd	nd	0.0015	nd	nd	0.0076	nd	0.6669	0.0139
Mean					0.06835	0	0.00115	0	0	0.008	0	0.6697	0.01515
8_55	Zn	5	Yes	None	nd	nd	0.0005	nd	0.0034	0.01	nd	0.0002	1.252
8_56	Zn	5	Yes	None	nd	nd	0.0009	nd	0.0034	0.0105	nd	nd	1.245
Mean					0	0	0.0007	0	0.0034	0.0103	0	0.0001	1.2485
8_57	Metal mix	5	Yes	None	12.33	0.0057	0.0163	0.1754	0.0046	0.0838	54.17	0.5037	1.064
8_58	Metal mix	5	Yes	None	13.17	0.0058	0.0167	0.1731	0.0049	0.0835	56.62	0.4985	1.054
Mean					12.75	0.0058	0.0165	0.17425	0.0048	0.0837	55.395	0.5011	1.059
8_59	DI H2O	5	Yes	None	0.0397	nd	0.0011	nd	0.0003	0.0112	nd	0.0014	0.0117
8_60	DI H2O	5	Yes	None	nd	nd	0.0004	nd	nd	0.009	nd	nd	0.0054
Mean					0.01985	0	0.00075	0	0.0002	0.0101	0	0.0007	0.00855
8_61	CMP	5.5	Yes	None	nd	0.0005	0.0007	nd	nd	0.0261	nd	0.0038	0.0273
8_62	CMP	5.5	Yes	None	nd	0.0001	0.0011	nd	nd	0.0265	nd	0.0043	0.0371
Mean					0	0.0003	0.0009	0	0	0.0263	0	0.0041	0.0322

8_63	Metal mix	5	None	None	12.77	0.0066	0.0172	0.1902	0.0032	0.085	55.27	0.5478	1.179
	centrif twice like column samples												
Summary - Means													
	Effluent	*pH*	*Foam*	*Algae?*	*Al*	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*
	Coal	5	None	None	2.9405	0.0161	0.0269	0.2447	0.0154	0.0655	4.2985	0.5803	1.2665
	Ni	5	None	None	0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.2063	0.802	0.0551
	Zn	5	None	None	0.0231	0.0178	0.0199	0.0206	0.0237	0.0173	0.0205	0.0223	1.436
	Metal mix	5	None	None	17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975
	DI H2O	5	None	None	0.11215	0.0248	0.02285	0.0246	0.0263	0.0232	1.5945	0.0192	0.0311
	CMP water	5.52	None	None	0.04275	0.0486	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.08075
	Coal	2.5	None	None	65.45	0.0214	0.0195	0.2539	0.0239	0.2015	395.5	0.7349	1.4815
	Coal	5	Yes	Cyanidium	2.1845	0	0	0.211	0	0.0209	3.436	0.5143	1.2295
	Ni	5	Yes	Cyanidium	0	0	0	0	0	0	0	0.6429	0.0103
	Zn	5	Yes	Cyanidium	0.01115	0	0	0	0	0	0	0	1.22
	Metal mix	5	Yes	Cyanidium	3.864	0	0.0029	0.1605	0	0.0249	11.138	0.4307	0.9533
	DI H2O	5	Yes	Cyanidium	0	0	0	0	0	0	0	0	0.00415
	CMP	5.5	Yes	Cyanidium	0	0	0	0	0	0	0	0	0.0208
	Coal	5	Yes	Phaeodac	1.3185	0	0	0.18015	0	0.0143	3.3845	0.4226	0.97365
	Ni	5	Yes	Phaeodac	0.01495	0	0	0	0	0	0.9383	0.3011	0.0043
	Zn	5	Yes	Phaeodac	0	0	0.0009	0.00025	0.0023	0.012	0.9805	0.0075	0.39275
	Metal mix	5	Yes	Phaeodac	0.81975	0.0015	0.01095	0.167	0.0028	0.0329	0.3833	0.4079	0.7886
	DI H2O	5	Yes	Phaeodac	0.089	0	0.00095	0.00265	0.0027	0.01	0.946	0.0029	0.01582
	CMP	5.5	Yes	Phaeodac	0.0061	0	0.0003	0.00105	0.0034	0.019	0.9796	0.0061	0.0212
	Coal	5	Yes	Chlorella	1.473	0.0025	0.00505	0.1988	0.0012	0.0302	2.269	0.4621	1.0645
	Ni	5	Yes	Chlorella	0.0495	0.0015	0.00285	0	0.0041	0.0107	0.0499	0.1256	0.01105
	Zn	5	Yes	Chlorella	0.09535	0.0001	0.00105	0	0.002	0.0008	0.0332	0	0.2993
	Metal mix	5	Yes	Chlorella	1.5785	0.0022	0.0095	0.13685	0.0011	0.0283	3.2815	0.3906	0.7618
	DI H2O	5	Yes	Chlorella	0.13595	0	0.0007	0	0.0014	0	0.0236	0	0.0084
	CMP	5.5	Yes	Chlorella	0	0	0.0001	0	0.0023	0.0099	0.0228	0	0.01435
	Coal	5	Yes	None	2.2995	0.0032	0.0069	0.20825	0	0.0493	2.972	0.5252	1.183
	Ni	5	Yes	None	0.06835	0	0.00115	0	0	0.008	0	0.6697	0.01515

	Zn	5	Yes	None	0	0	0.0007	0	0.0034	0.0103	0	0.0001	1.2485
	Metal mix	5	Yes	None	12.75	0.0058	0.0165	0.17425	0.0048	0.0837	55.395	0.5011	1.059
	DI H2O	5	Yes	None	0.01985	0	0.00075	0	0.0002	0.0101	0	0.0007	0.00855
	CMP	5.5	Yes	None	0	0.0003	0.0009	0	0	0.0263	0	0.0041	0.0322

	Metal mix	5	None	None	12.77	0.0066	0.0172	0.1902	0.0032	0.085	55.27	0.5478	1.179
	centrif twice like column samples												
Metal Removal Compared to Controls (not passed through foam)													
CYANIDIUM (.1875 g algae, 1.7 g foam/column)													
		Al	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*			
Runoff		2.9405	0.0161	0.0269	0.2447	0.0154	0.06545	4.2985	0.5803	1.2665			
ppm Removed		0.756	0.0161	0.0269	0.0337	0.0154	0.04455	0.8625	0.066	0.037			
% removed		25.7099	100	100	13.772	100	68.0672	20.0651	11.382	2.9214			
mg/g algae		0.2016	0.004293	0.0071733	0.00899	0.0041	0.01188	0.23	0.0176	0.0099			
mg/g foam		0.02224	0.000474	0.0007912	0.00099	0.0005	0.00131	0.02537	0.0019	0.0011			
Ni		0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.802	0.0551			
ppm Removed		0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.1591	0.0448			
% removed		100	100	100	100	100	100	100	19.838	81.307			
mg/g algae		0.02671	0.008533	0.0092133	0.00853	0.0076	0.00915	0.055	0.0424	0.0119			
mg/g foam		0.00295	0.000941	0.0010162	0.00094	0.0008	0.00101	0.00607	0.0047	0.0013			
Zn		0.0231	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	1.436			
ppm Removed		0.01195	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	0.216			
% removed		51.7316	100	100	100	100	100	100	100	15.042			
mg/g algae		0.00319	0.004733	0.0053067	0.00549	0.0063	0.0046	0.00545	0.0059	0.0576			
mg/g foam		0.00035	0.000522	0.0005853	0.00061	0.0007	0.00051	0.0006	0.0007	0.0064			
Mixed metals		17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975			
ppm Removed		13.386	0.0147	0.0321	0.0631	0.0265	0.07555	69.5225	0.1517	0.2442			
% removed		77.6	100	91.714286	28.22	100	75.249	86.192	26.05	20.392			
mg/g algae		3.5696	0.00392	0.00856	0.01683	0.0071	0.02015	18.5393	0.0405	0.0651			
mg/g foam		0.39371	0.000432	0.0009441	0.00186	0.0008	0.00222	2.04478	0.0045	0.0072			
DI		0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.0311			
ppm Removed		0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.027			
% removed		100	100	100	100	100	100	100	100	86.656			
mg/g algae		0.02991	0.006613	0.0060933	0.00656	0.007	0.00617	0.4252	0.0051	0.0072			
mg/g foam		0.0033	0.000729	0.0006721	0.00072	0.0008	0.00068	0.0469	0.0006	0.0008			
CMP water		0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.0808			
ppm Removed		0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.06			
% removed		100	100	100	100	100	100	#DIV/0!	100	74.241			
mg/g algae		0.0114	0.012947	0.0127333	0.01353	0.0139	0.02131	0	0.0118	0.016			
mg/g foam		0.00126	0.001428	0.0014044	0.00149	0.0015	0.00235	0	0.0013	0.0018			
PHAEODACTYLUM (.1892 g algae, 2 g foam/column)													
Runoff		2.9405	0.0161	0.0269	0.2447	0.0154	0.06545	4.2985	0.5803	1.2665			
ppm Removed		1.622	0.0161	0.0269	0.06455	0.0154	0.05115	0.914	0.1578	0.2929			
% removed		55.1607	100	100	26.3792	100	78.1513	21.2632	27.184	23.123			
mg/g algae		0.42865	0.004255	0.0071089	0.01706	0.0041	0.01352	0.24154	0.0417	0.0774			
mg/g foam		0.04055	0.000403	0.0006725	0.00161	0.0004	0.00128	0.02285	0.0039	0.0073			
Ni		0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.802	0.0551			
ppm Removed		0.0852	0.032	0.03455	0.032	0.0284	0.0343	-0.732	0.5009	0.0508			
% removed		85.0724	100	100	100	100	100	-354.91	62.456	92.196			
mg/g algae		0.02252	0.008457	0.0091305	0.00846	0.0075	0.00906	-0.1934	0.1324	0.0134			
mg/g foam		0.00213	0.0008	0.0008638	0.0008	0.0007	0.00086	-0.0183	0.0125	0.0013			
Zn		0.0231	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	1.436			
ppm Removed		0.0231	0.01775	0.019	0.02035	0.0214	0.0053	-0.9601	0.0148	1.0433			

% removed	100	100	95.477387	98.7864	90.275	30.7246	-4694.6	66.292	72.65			
mg/g algae	0.0061	0.004691	0.0050211	0.00538	0.0056	0.0014	-0.2537	0.0039	0.2757			
mg/g foam	0.00058	0.000444	0.000475	0.00051	0.0005	0.00013	-0.024	0.0004	0.0261			
Mixed metals	17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975			
ppm Removed	16.4303	0.013235	0.02405	0.0566	0.0237	0.0675	80.2767	0.1745	0.4089			
% removed	95.2478	90.03401	68.714286	25.3131	89.603	67.2311	99.5248	29.965	34.146			
mg/g algae	4.34203	0.003498	0.0063557	0.01496	0.0063	0.01784	21.2148	0.0461	0.1081			
mg/g foam	0.41076	0.000331	0.0006013	0.00142	0.0006	0.00169	2.00692	0.0044	0.0102			
DI	0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.0311			
ppm Removed	0.02315	0.0248	0.0219	0.02195	0.0236	0.0132	0.6485	0.0163	0.0153			
% removed	20.642	100	95.842451	89.2276	89.714	57.0194	40.6711	84.856	49.132			
mg/g algae	0.00612	0.006554	0.0057875	0.0058	0.0062	0.00349	0.17138	0.0043	0.004			
mg/g foam	0.00058	0.00062	0.0005475	0.00055	0.0006	0.00033	0.01621	0.0004	0.0004			
CMP water	0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.0808			
ppm Removed	0.03665	0.04855	0.04745	0.0497	0.0487	0.0609	-0.9796	0.0382	0.0596			
% removed	85.731	100	99.371728	97.931	93.564	76.2203	#DIV/0!	86.328	73.746			
mg/g algae	0.00969	0.01283	0.0125396	0.01313	0.0129	0.01609	-0.2589	0.0101	0.0157			
mg/g foam	0.00092	0.001214	0.0011863	0.00124	0.0012	0.00152	-0.0245	0.001	0.0015			
CHLORELLA (.1854 g algae, 1.9 g foam/column)												
Runoff	2.9405	0.0161	0.0269	0.2447	0.0154	0.06545	4.2985	0.5803	1.2665			
ppm Removed	1.4675	0.0136	0.02185	0.0459	0.0143	0.03525	2.0295	0.1183	0.202			
% removed	49.9065	84.47205	81.226766	18.7577	92.532	53.8579	47.2141	20.377	15.949			
mg/g algae	0.39577	0.003668	0.0058927	0.01238	0.0038	0.00951	0.54733	0.0319	0.0545			
mg/g foam	0.03862	0.000358	0.000575	0.00121	0.0004	0.00093	0.05341	0.0031	0.0053			
Ni	0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.802	0.0551			
ppm Removed	0.05065	0.0305	0.0317	0.032	0.0243	0.02365	0.1564	0.6765	0.0441			
% removed	50.5741	95.3125	91.751085	100	85.538	68.9504	75.8303	84.345	79.946			
mg/g algae	0.01366	0.008225	0.0085491	0.00863	0.0065	0.00638	0.04218	0.1824	0.0119			
mg/g foam	0.00133	0.000803	0.0008342	0.00084	0.0006	0.00062	0.00412	0.0178	0.0012			
Zn	0.0231	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	1.436			
ppm Removed	-0.0723	0.01765	0.01885	0.0206	0.0217	0.01645	-0.0128	0.0223	1.1367			
% removed	-312.77	99.43662	94.723618	100	91.543	95.3623	-62.347	100	79.157			
mg/g algae	-0.0195	0.00476	0.0050836	0.00556	0.0058	0.00444	-0.0034	0.006	0.3066			
mg/g foam	-0.0019	0.000464	0.0004961	0.00054	0.0006	0.00043	-0.0003	0.0006	0.0299			
Mixed metals	17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975			
ppm Removed	15.6715	0.01255	0.0255	0.08675	0.0254	0.07215	77.3785	0.1918	0.4357			
% removed	90.8493	85.37415	72.857143	38.797	96.03	71.8625	95.9317	32.936	36.384			
mg/g algae	4.2264	0.003385	0.006877	0.0234	0.0069	0.01946	20.868	0.0517	0.1175			
mg/g foam	0.41241	0.00033	0.0006711	0.00228	0.0007	0.0019	2.03628	0.005	0.0115			
DI	0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.0311			
ppm Removed	-0.0238	0.0248	0.02215	0.0246	0.0249	0.02315	1.57095	0.0192	0.0227			
% removed	-21.222	100	96.936543	100	94.667	100	98.523	100	72.99			
mg/g algae	-0.0064	0.006688	0.0059736	0.00663	0.0067	0.00624	0.42367	0.0052	0.0061			
mg/g foam	-0.0006	0.000653	0.0005829	0.00065	0.0007	0.00061	0.04134	0.0005	0.0006			
CMP water	0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.0808			
ppm Removed	0.04275	0.04855	0.04765	0.05075	0.0498	0.07005	-0.0228	0.0443	0.0664			
% removed	100	100	99.790576	100	95.677	87.6721	#DIV/0!	100	82.229			
mg/g algae	0.01153	0.013093	0.0128506	0.01369	0.0134	0.01889	-0.0061	0.0119	0.0179			
mg/g foam	0.00113	0.001278	0.0012539	0.00134	0.0013	0.00184	-0.0006	0.0012	0.0017			

FOAM ONLY, 2 g/column												
Runoff	2.9405	0.0161	0.0269	0.2447	0.0154	0.06545	4.2985	0.5803	1.2665			
ppm Removed	0.641	0.0129	0.02	0.03645	0.0154	0.01615	1.3265	0.0552	0.0835			
% removed	21.799	80.12422	74.349442	14.8958	100	24.6753	30.8596	9.5037	6.593			
mg/g foam	0.01603	0.000323	0.0005	0.00091	0.0004	0.0004	0.03316	0.0014	0.0021			
Ni	0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.802	0.0551			
ppm Removed	0.0318	0.032	0.0334	0.032	0.0284	0.0263	0.20625	0.1324	0.04			
% removed	31.7524	100	96.671491	100	100	76.6764	100	16.502	72.505			
mg/g foam	0.0008	0.0008	0.000835	0.0008	0.0007	0.00066	0.00516	0.0033	0.001			
Zn	0.0231	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	1.436			
ppm Removed	0.0231	0.01775	0.0192	0.0206	0.0203	0.007	0.02045	0.0222	0.1875			
% removed	100	100	96.482412	100	85.624	40.5797	100	99.551	13.057			
mg/g foam	0.00058	0.000444	0.00048	0.00052	0.0005	0.00018	0.00051	0.0006	0.0047			
Mixed metals	17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975			
ppm Removed	4.5	0.00895	0.0185	0.04935	0.0217	0.01675	25.265	0.0812	0.1385			
% removed	26.087	60.88435	52.857143	22.0707	82.042	16.6833	31.3228	13.952	11.566			
mg/g foam	0.1125	0.000224	0.0004625	0.00123	0.0005	0.00042	0.63163	0.002	0.0035			
DI	0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.0311			
ppm Removed	0.0923	0.0248	0.0221	0.0246	0.0261	0.01305	1.5945	0.0185	0.0226			
% removed	82.3005	100	96.717724	100	99.429	56.3715	100	96.345	72.508			
mg/g foam	0.00231	0.00062	0.0005525	0.00062	0.0007	0.00033	0.03986	0.0005	0.0006			
CMP water	0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.0808			
ppm Removed	0.04275	0.04825	0.04685	0.05075	0.0521	0.0536	0	0.0402	0.0486			

% removed	100	99.38208	98.115183	100	100	67.0839	#DIV/0!	90.847	60.124			
mg/g foam	0.00107	0.001206	0.0011713	0.00127	0.0013	0.00134	0	0.001	0.0012			
Percent Removal From Runoff												
	Al	*Be*	*Cd*	*Co*	*Cr*	*Cu*	*Fe*	*Ni*	*Zn*			
Original ppm	2.9405	0.0161	0.0269	0.2447	0.0154	0.06545	4.2985	0.5803	1.2665			
% Removal by:												
Cyanidium	25.7099	100	100	13.772	100	68.0672	20.0651	11.382	2.9214			
Phaeodactylum	55.1607	100	100	26.3792	100	78.1513	21.2632	27.184	23.123			
Chlorella	49.9065	84.47205	81.226766	18.7577	92.532	53.8579	47.2141	20.377	15.949			
Foam only	21.799	80.12422	74.349442	14.8958	100	24.6753	30.8596	9.5037	6.593			
Percent removal from Ni solution												
Original ppm	0.10015	0.032	0.03455	0.032	0.0284	0.0343	0.20625	0.802	0.0551			
% Removal by:												
Cyanidium	100	100	100	100	100	100	100	19.838	81.30672			
Phaeodactylum	85.072391	100	100	100	100	100	-354.9091	62.456	92.19601			
Chlorella	50.574139	95.3125	91.75108538	100	-85.53792	68.950437	75.830303	84.345	79.94555			
Foam only	31.752371	100	96.67149059	100	100	76.676385	100	16.502	72.50454			
Percent removal from Zn solution												
Original ppm	0.0231	0.01775	0.0199	0.0206	0.0237	0.01725	0.02045	0.0223	1.436			
% Removal by:												
Cyanidium	51.731602	100	100	100	100	100	100	100	15.042			
Phaeodactylum	100	100	95.477387	98.7864	90.275	30.7246	-4694.6	66.292	72.65			
Chlorella	-312.7706	99.4366197	94.72361809	100	91.54334	95.362319	-62.34719	100	79.157			
Foam only	100	100	96.48241206	100	85.62368	40.57971	100	99.55056	13.057			
Percent Removal From Metal Mix												
Original ppm	17.25	0.0147	0.035	0.2236	0.0265	0.1004	80.66	0.5824	1.1975			
% Removal by:												
Cyanidium	77.6	100	91.714286	28.22	100	75.249	86.192	26.05	20.392			
Phaeodactylum	95.2478	90.03401	68.714286	25.3131	89.603	67.2311	99.5248	29.965	34.146			
Chlorella	90.8493	85.37415	72.857143	38.797	96.03	71.8625	95.9317	32.936	36.384			
Foam only	26.087	60.88435	52.857143	22.0707	82.042	16.6833	31.3228	13.952	11.566			
2ndcentrifugation	25.971	55.10204	50.857143	14.9374	87.902	15.3386	31.4778	5.9329	1.5449			
Percent removal from DI												
Original ppm	0.11215	0.0248	0.02285	0.0246	0.0263	0.02315	1.5945	0.0192	0.0311			
% Removal by:												
Cyanidium	100	100	100	100	100	100	100	100	86.656			
Phaeodactylum	20.642	100	95.842451	89.2276	89.714	57.0194	40.6711	84.856	49.132			
Chlorella	-21.222	100	96.936543	100	94.667	100	98.523	100	72.99			
Foam only	82.3005	100	96.717724	100	99.429	56.3715	100	96.345	72.508			
Percent removal from CMP water												
Original ppm	0.04275	0.04855	0.04775	0.05075	0.0521	0.0799	0	0.0443	0.0808			
% Removal by:												
Cyanidium	100	100	100	100	100	100	#DIV/0!	100	74.241			
Phaeodactylum	85.731	100	99.371728	97.931	93.564	76.2203	#DIV/0!	86.328	73.746			
Chlorella	100	100	99.790576	100	95.677	87.6721	#DIV/0!	100	82.229			
Foam only	100	99.38208	98.115183	100	100	67.0839	#DIV/0!	90.847	60.124			

Table A.6.9 Experimental procedures and results forExperiment #9

EXPERIMENT #9 Comparing Metal Removal from well water by foam embedded with algae packed in the DCB-4A water using in the modified Frisby test bed. 11/7/95

I. PURPOSE:

Compare bioremoval efficacy of four algal species by passing metal contaminated ground water through columns containing foam-algae aggregates. Metals to be measured will include Al, Cr, Fe, & Ni,

II. ALGAE:

Cyanidium (33 days old)
Mastigocladus (33 days old)
Phaeodactylum (21 days old)
Chlorella (21 days old)

All harvested by centrifugation.

III. FOAM:

8 mesh containing 10% algae by dry weight

IV. Effluent:

Monitoring Well DCB-4A

V. PROCEDURE:

A. Preparation of algae

Set up water baths for the growth of 16 4-l bottles of algae. (8 for thermophiles and 8 for non-thermophiles). Make media for growing 4 bottles of of each of the following 4 species: *Cyanidium caldarium, Mastigocladus laminosus, Phaeodactylum tricomutum, and Chlorella vulgaris*. Fill each bottle with 2 liters of media and autoclave. Set bath apparatus for light conditions and 0.1% CO2 bubbling as before. Inoculate each bottle with with a 25ml aliquot taken from the most recent culture transfer, following homogenization. Measure dry wt. of each bottle after 5 days and 10 days. Harvest by centrifugation (10 min, 10000 rpm). Wash cells with DI water and resuspend at 5% by dry wt. with DI.

B. Preparation of foam/algae aggregates

Take algal suspensions to Frisby for embedding. Have them use the best combination of surfactant, prepolymer and biomass as determined for minimal "washout" in G-4 experiments. Following embedding, the samples should be ground and sieved to 8 mesh. Plan to use about 2 g of granulated foam per column per test run.

C. Preparation of effluent

1. Collect water from D area monitoring well DCB-4A Measure pH and other parameters with a Horiba ASAP after collection.

D. Preparation of columns in Test Bed

1. Pack 2 columns with 2 g of particulate foam +algae for each of the four species

E. Contacting biosorbent with effluent in Test Bed

1. flush apparatus with DI water and drain
2. Fill Reservoirs with 1 liter of untreated runoff each
3. start pumps and adjust flow rates to 2.5gal/hr
4. Run for 8 hrs = 53 bed volumes
5. collect samples after 8 hrs by opening effluent valves and collecting 30 +ml of treated water in acid-washed 60ml vials
6. preserve with 0.5 ml conc. HNO3

VI. Supplies needed

Carboy for Coal pile runoff basin water
68 acid cleaned 60 ml vials for samples to be taken to ADS
HNO3 concentrated for preserving samples
HNO3, 1N (for adjusting pH)
HNO3, 10 N "
NaOH, 1 N "
NaOH, 10 N "
HNO3, ca 3 N for rinsing pH electrode
Deionized water

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS:

Label	*Description*
9-1 a&b	Chlorella
9-2 a&b	Cyanidium
9-3 a&b	Mastigocladus
9-4 a&b	Phaeodactylum
9-5 a&b	Chlorella
9-6 a&b	Cyanidium
9-7 a&b	Mastigocladus
9-8 a&b	Phaeodactylum

Table A.6.9 Bioremoval in Modified Test Rig #1(Cont.)

Experiment 9 -Modified Frisby Packed Bed Rig 11-7-95								
Effluent		Well DCB-4A						
		cond = 0.290 mS/cm , turb = 1, DO = 7.4 mg/l, temp = 20.4 , sal = 0.01%						
Foam		Four algae, Chlorella, Cyanidium, Mastigocladus, and Phaeodactylum were						
		embedded in foam. All had 10 % algae by dry weight in the final foam.						
Method		EXP9PRO.DOC						
Notes	Percent removal by the algae calculated relative ot metal concentrations in untreated wastewater							
Results								
Algae	*Al*	*Fe*	*Cr*	*Ni*	*% Al Rem.*	*% Fe Rem.*	*% Cr Rem.*	*% Ni Rem.*
Cont/6	10.54	0.27	2.5	67				
Cont/6	10.59	0.26	2	63				
Cont/6	10.57	0.27	2.25	65	1.376896149	-11.57894737	-8.43373494	-1.5625
Cont/7	10.91	0.21	1.9	64				
Cont/7	10.81	0.21	1.9	62				
Cont/7	10.86	0.21	1.9	63	-1.376896149	11.57894737	8.43373494	1.5625
Chlor.	4.60	0.11	0.4	63				
Chlor.	4.67	0.12	0.3	61				
Chlor.	4.64	0.12	0.35	62	56.7327888	51.57894737	83.13253012	3.125
Chlor.	3.22	0.12	0.8	71				
Chlor.	3.23	0.12	0.6	65				
Chlor.	3.23	0.12	0.7	68	69.8949825	49.47368421	66.26506024	-6.25
Cyan.	3.54	0.14	0.5	66				
Cyan.	3.36	0.13	0.5	67				
Cyan.	3.45	0.14	0.5	66.5	67.79463244	43.15789474	75.90361446	-3.90625
Cyan.	3.21	0.18	0.6	67				
Cyan.	3.25	0.17	0.6	68				
Cyan.	3.23	0.18	0.6	67.5	69.84830805	26.31578947	71.08433735	-5.46875
Mast.	2.94	0.16	2	74				
Mast.	3.12	0.16	1.8	72				
Mast.	3.03	0.16	1.9	73	71.71528588	32.63157895	8.43373494	-14.0625
Mast.	5.76	0.17	2.4	68				
Mast.	5.81	0.18	2.4	68				
Mast.	5.79	0.18	2.4	68	45.99766628	26.31578947	-15.6626506	-6.25
Phaeo.	4.32	0.17	1.8	69				
Phaeo.	4.46	0.17	1.7	68				
Phaeo.	4.39	0.17	1.75	68.5	59.01983664	28.42105263	15.6626506	-7.03125
Phaeo.	5.91	0.17	2.2	67				
Phaeo.	6.02	0.17	2.5	69				
Phaeo.	5.97	0.17	2.35	68	44.31738623	28.42105263	-13.25301205	-6.25

Table A.6.10 Experimental procedures and results forExperiment #10

EXPERIMENT #10 Follow-up comparison of metal Removal from well water by foam embedded with algae packed in the DCB-4A water using in the modified Frisby test bed. 11/28&29/1995

I. PURPOSE:

Repeat conditions of Experiment 9, except add controls to replace Phaeodactylum which did the poorest in previous test. Compare bioremoval efficacy of three algal species by passing metal contaminated ground water through columns containing foam-algae aggregates. Metals to be measured will include Al, Cr, Fe, & Ni,

II. ALGAE:

Cyanidium (33 days old at harvest)
Mastigocladus (33 days old)
Chlorella (21 days old)

All harvested by centrifugation.

III. FOAM:

8 mesh containing 10% algae by dry weight

IV. Effluent:

Monitoring Well DCB-4A

V. PROCEDURE:

1. Use same foam/algae aggregates as in Experiment 9

2. For preparation of effluent, collect water from D area monitoring well DCB-4A. Measure pH and other parameters with a Horiba ASAP after collection.

3. Prepare columns in Test Bed by packing 2 columns with 2 g of particulate foam +algae for each of the three algae. Pack the other two Columns with plain foam and no foam, respectively.

4. To contact biosorbent with effluent in test bed:

    1. flush apparatus with DI water and drain
    2. Fill Reservoirs with 1 literof untreated runoff each
    3. start pumps and adjust flow rates to 2.5gal/hr
    4. Run for 8 hrs = 53 bed volumes

5. collect samples after 8 hrs by opening effluent valves and collecting 30 +ml of treated water in acid-washed 60ml vials
6. preserve with 0.5 ml conc. HNO3

VI. Supplies needed

Carboy for wastewater
18 acid cleaned 60 ml vials for samples to be taken to ADS
HNO3 concentrated for preserving samples

VII. SAMPLE LABELS

The following labels will be used on samples sent to ADS:

Label	*Description*
10-1 a&b	Chlorella
10-2 a&b	Cyanidium
10-3 a&b	Mastigocladus
10-4 a&b	wastewater only - no foam
10-5 a&b	Chlorella
10-6 a&b	Cyanidium
10-7 a&b	Mastigocladus
10-8 a&b	plain foam

Table A.6.10 (Cont.)

Experiment 10 - Bioremoval using the Modified Frisby Packed Bed Rig								
Effluent		Well DCB-4A						
		cond = 1.14 mS/cm , turb = 0, DO =7.12 mg/l , temp = 20.7 , sal = 0.05						
Date	11/28/95							
Algae = Chlorella , Cyanidium, Mastigocladus,								
Foam	10%algae by dry weight in final foam							
Method		EXP10PRO.DOC						
Results								
Algae	***Al***	***Fe***	***Cr***	***Ni***	***% Al Rem.***	***% Fe Rem.***	***% Cr Rem.***	***% Ni Rem.***
Control/1	52.15	0.11	1.50	260				
Control/1	51.09	0.09	1.50	260				
Control/1	**51.62**	**0.10**	**1.50**	**260**	**0.09**	**-8.97**	**1.64**	**1.42**
Control/2	51.98	0.08	1.50	260				
Control/2	51.44	0.09	1.60	275				
Control/2	**51.71**	**0.08**	**1.55**	**268**	**-0.09**	**8.97**	**-1.64**	**-1.42**
Chlor	44.48	0.11	1.70	260				
Chlor	45.39	0.09	1.60	255				
Chlor	**44.94**	**0.10**	**1.65**	**258**	**13.03**	**-11.37**	**-8.20**	**2.37**
Chlor	48.94	0.09	0.60	245				
Chlor	50.33	0.11	0.60	235				
Chlor	**49.64**	**0.10**	**0.60**	**240**	**3.93**	**-13.33**	**60.66**	**9.00**
Cyan.	49.66	0.11	0.40	230				
Cyan.	48.63	0.10	0.40	230				
Cyan.	**49.15**	**0.11**	**0.40**	**230**	**4.88**	**-21.82**	**73.77**	**12.80**
Cyan.	49.89	0.08	0.30	230				
Cyan.	49.67	0.05	0.30	230				
Cyan.	**49.78**	**0.06**	**0.30**	**230**	**3.65**	**27.97**	**80.33**	**12.80**
Mast.	49.75	0.08	0.50	230				
Mast.	49.36	0.07	0.50	220				
Mast.	**49.56**	**0.08**	**0.50**	**225**	**4.08**	**15.67**	**67.21**	**14.69**
Mast.	49.27	0.13	0.60	220				
Mast.	49.14	0.12	1.10	250				
Mast.	**49.21**	**0.12**	**0.85**	**235**	**4.76**	**-36.91**	**44.26**	**10.90**
No Foam	52.75	0.06	0.80	225				
No Foam	52.20	0.03	0.70	225				
No Foam	**52.48**	**0.05**	**0.75**	**225**	**-1.67**	**47.92**	**50.82**	**14.69**
Plain Foam	47.71	0.10	0.50	225				
Plain Foam	47.53	0.05	0.50	220				
Plain Foam	**47.62**	**0.08**	**0.50**	**223**	**7.83**	**14.33**	**67.21**	**15.64**

Table A.6.11 Experimental Procedures and Results for Experiment #11

EXPERIMENT 11(2/8/96-2/15/96)

Metal Removal using foam embedded with *Cyanidium*, *Chlorella* and *Mastigocladus* following various pretreatments

I. PURPOSE:

Evaluate effects of pretreatment of biomass prior to embedding in foam for three species of algae on metal removal

II. ALGAE:

33-day old cultures of:
Cyanidium caldarium
Chlorella sp.
Mastigocladus laminosus

All Harvested by centrifugation,

III. PRETREATMENTS:

Acid wash (0.2N HCL)
Organic solvent wash (O.2N Acetone)
Heat killing (110°C for 4hrs)
salt shock (0.2N NaCl)
NaOH (0.2N)
DI wash

IV. FOAM:

8 mesh containing 10% algae by dry weight

V. EFFLUENT:

Monitoring well DCB-4A

V1 TEST APPARATUS:

Modified Frisby packed bed bioreactor

VII. PROCEDURE:

A. Preparation of effluent

1. Collect water from pump at well near D-Area coal pile runoff basin
2. Upon returning to lab measure Horiba parameters(pH, etc.)

B. Preparation and Use of Bioreactor

1. Flush test bed with several bed volumes of DI water

2. Make three 8-hr runs with the test rig (one for each alga)

3. Fill columns with 2 g of foam using the following strategy:
   Column 1 - HCL
   Column 2 - NaOH
   Column 3 - NaCl
   Column 4 - Heat
   Column 5 - Acetone
   Column 6 - DI
   Column 7 - Plain Foam
   Column 8 - no foam (raw wastewater)

Also prepare samples of untreated (not run through test bed) waste water for controls

4. fill test rig with 1liter of wastewater for each column assembly and run in recirculating mode for 8 hrs at a flow rate of 2.5 gal/hr

5. Collect 30ml samples for chemical analyses in EPA-certified acid cleaned bottles containing 0.5 ml concentrated HNO_3.

6. Take samples to ADS for the following chemical analyses:
   Cr & Ni by ICP-MS
   Fe & Al by ICP-ES

VIII. SAMPLE LABELS

The following labels were used on samples sent to ADS:

Label	*Description*
11CY -1A&B	Cyanidium, HCL
11CY -2A&B	Cyanidium, NaOH
11CY -3 A&B	Cyanidium, NaCl
11CY -4A&B	Cyanidium , Heat killed
11CY -5A&B	Cyanidium, acetone
11CY -6A&B	Cyanidium, DI wash
11CY-7A&B	plain foam
11CY -8A&B	treated control - no foam or algae
11Cont 2/8 - A&B	untreated control
11CH -1A&B	Chlorella, HCL
11CH -2A&B	Chorella, NaOH
11CH -3 A&B	Chlorella, NaCl
11CH -4A&B	Chlorella, Heat killed
11CH -5A&B	Chlorella, acetone
11CH -6A&B	Chlorella, DI wash
11CH-7A&B	plain foam
11CH -8A&B	treated control - no foam or algae
11Cont 2/13 - A&B	untreated control
11M -1A&B	Mastigocladus, NaCl
11M -2A&B	Mastigocladus, heat killed
11M -3 A&B	Mastigocladus, acetone
11M -4A&B	no foam
11M -5A&B	DI wash
11M -6A&B	No foam
11M -7A&B	plain foam
11M -8A&B	no foam
11Cont 2/15 - A&B	untreated control

Table A.6.11 (Cont.)

Experiment 11 Bioremoval by three algae using six pretreatments										
Date	2/8, 2/13, & 2/15/96									
Wastewater		Well DCB-4A								
WQ	pH- 3.96, Cond. - 1.05ms/cm, Turb -0, Temp. 17.6, DO 6.56, Sal. 0.04									
Rig	Modified packed Bed	2.5gal/hr -for 8hr. in recirc. mode								
							Percent removal *			
Alga	Treatment	Al3	Fe2	Cr	Ni		Al	Fe	Cr	Ni
Cyan.	HCl	49.85	0.1114	3.4	320					
Cyan.	HCl	49.89	0.0643	3.4	330					
Cyan.	HCl	49.87	0.08785	3.4	325		5.12	16.85	37.04	15.58
Cyan.	NaOH	48.84	0.1105	3.8	330					
Cyan.	NaOH	49.13	0.0828	4	330					
Cyan.	NaOH	48.985	0.09665	3.9	330		6.80	8.52	27.78	14.29
Cyan.	NaCl	48.61	0.0777	9	350					
Cyan.	NaCl	48.84	0.0886	3.6	330					
Cyan.	NaCl	48.725	0.08315	6.3	340		7.30	21.30	-16.67	11.69
Cyan.	Heat	48.26	0.1183	4.6	340					
Cyan.	Heat	49.91	0.0945	5	340					
Cyan.	Heat	49.085	0.1064	4.8	340		6.61	-0.71	11.11	11.69
Cyan.	Acetone	49.89	0.1046	4.2	350					
Cyan.	Acetone	49.27	0.1576	4.4	360					
Cyan.	Acetone	49.58	0.1311	4.3	355		5.67	-24.09	20.37	7.79
Cyan.	DI	49.77	0.0575	4.6	360					
Cyan.	DI	50.26	0.1218	4.6	360					
Cyan.	DI	50.015	0.08965	4.6	360		4.84	15.14	14.81	6.49
Cyan.	Plain Foam	50.18	0.1669	6.8	370					
Cyan.	Plain Foam	50.19	0.1003	5.4	370					
Cyan.	Plain Foam	50.185	0.1336	6.1	370		4.52	-26.46	-12.96	3.90
Cyan.	No Foam	51.86	0.0861	5	370					
Cyan.	No Foam	51.48	0.0736	4.8	370					
Cyan.	No Foam	51.67	0.07985	4.9	370		1.69	24.42	9.26	3.90
Cyan.	Control 2/8	52.71	0.1243	5.4	390					
Cyan.	Control 2/8	52.41	0.087	5.4	380					
Cyan.	Control 2/8	52.56	0.10565	5.4	385		0.00	0.00	0.00	0.00
Chlor.	HCl	47.94	0.0958	4.8	340					
Chlor.	HCl	47.9	0.1143	4.6	330					
Chlor.	HCl	47.92	0.10505	4.7	335		7.84	-28.42	6.93	6.94
Chlor.	NaOH	49.38	0.1042	8.8	352					
Chlor.	NaOH	48.6	0.249	4.8	340					
Chlor.	NaOH	48.99	0.1766	6.8	346		5.78	-115.89	-34.65	3.89
Chlor.	NaCl	48.09	0.1032	4.8	340					
Chlor.	NaCl	49.17	0.1098	4.8	340					
Chlor.	NaCl	48.63	0.1065	4.8	340		6.47	-30.20	4.95	5.56
Chlor.	Heat	47.09	0.0774	5.4	340					
Chlor.	Heat	49.02	0.06	5.2	340					

Chlor.	Heat	48.055	0.0687	5.3	340		7.58	16.01	-4.95	5.56
Chlor.	Acetone	50.05	0.0998	4.7	330					
Chlor.	Acetone	49.6	0.1101	4.6	340					
Chlor.	Acetone	49.825	0.10495	4.65	335		4.17	-28.30	7.92	6.94
Chlor.	DI	49.84	0.084	5.1	340					
Chlor.	DI	48.79	0.0928	5.4	350					
Chlor.	DI	49.315	0.0884	5.25	345		5.15	-8.07	-3.96	4.17
Chlor.	Plain Foam	51.25	0.1605	4.9	350					
Chlor.	Plain Foam	49.4	0.1016	4.9	350					
Chlor.	Plain Foam	50.325	0.13105	4.9	350		3.21	-60.21	2.97	2.78
Chlor.	No Foam	50.07	0.1789	4.6	350					
Chlor.	No Foam	51.25	0.0771	4.9	350					
Chlor.	No Foam	50.66	0.128	4.75	350		2.57	-56.48	5.94	2.78
Chlor.	Control 2/13	52.36	0.0794	5	360					
Chlor.	Control 2/13	51.63	0.0842	5.1	360					
Chlor.	Control 2/13	51.995	0.0818	5.05	360		0.00	0.00	0.00	0.00
Mast.	NaCl	50.6	0.337	4.8	370					
Mast.	NaCl	51.47	0.1419	4.8	350					
Mast.	NaCl	51.035	0.23945	4.8	360		1.93	-102.15	5.88	5.26
Mast.	Heat	50.98	0.1716	5	380					
Mast.	Heat	51.44	0.0867	5.1	380					
Mast.	Heat	51.21	0.12915	5.05	380		1.59	-9.03	0.98	0.00
Mast.	Acetone	50.84	0.0888	5.1	370					
Mast.	Acetone	51.03	0.0512	5.4	380					
Mast.	Acetone	50.935	0.07	5.25	375		2.12	40.90	-2.94	1.32
Mast.	No Foam	52.12	0.133	5.3	380					
Mast.	No Foam	52.4	0.0964	5.1	370					
Mast.	No Foam	52.26	0.1147	5.2	375		-0.42	3.17	-1.96	1.32
Mast.	DI	51.1	0.0831	5.1	380					
Mast.	DI	49.78	0.0576	4.9	380					
Mast.	DI	50.44	0.07035	5	380		3.07	40.61	1.96	0.00
Mast.	No Foam	51.39	0.1406	5	380					
Mast.	No Foam	52.3	0.0826	5.1	380					
Mast.	No Foam	51.845	0.1116	5.05	380		0.37	5.78	0.98	0.00
Mast.	Plain Foam	50.31	0.1243	5.3	380					
Mast.	Plain Foam	47.4	0.0915	5.3	380					
Mast.	Plain Foam	48.855	0.1079	5.3	380		6.12	8.91	-3.92	0.00
Mast.	No Foam	51.45	0.1073	5.1	370					
Mast.	No Foam	52.35	0.1148	5	370					
Mast.	No Foam	51.9	0.11105	5.05	370		0.27	6.25	0.98	2.63
Mast.	Control 2/15	51.87	0.1441	5.1	380					
Mast.	Control 2/15	52.21	0.0928	5.1	380					
Mast.	Control 2/15	52.04	0.11845	5.1	380		0.00	0.00	0.00	0.00
NT. = No treatment										
NF = No Foam										
PF = Plain foam										

Table A.6.12 Experimental Procedures and Results for Experiment #12

EXPERIMENT 12 (3/13&15/96)

Metal Removal using foam embedded with *Cyanidium* and *Mastigocladus* using packed bed and static mixer bioreactors

I. PURPOSE:

Evaluate effects of treatment with two different bioreactor types using two species of algae for metal removal

II. ALGAE:

35-day-old cultures of:
Cyanidium caldarium
Mastigocladus laminosu
Chlorella pyrenoidosa

All Harvested by centrifugation,

III. PRETREATMENTS:

DI wash

IV. FOAM:

8 mesh containing 10% algae by dry weight

V. EFFLUENT:

Monitoring well DCB-4A

V1 TEST APPARATUS:

Modified Frisby packed bed bioreactor and Static Mixer bioreactor

VII. PROCEDURE:

A. **Preparation of effluent**

1. Collect water from pump at well near D-Area coal pile runoff basin
2. Upon returning to lab measure Horiba parameters(pH, etc.)

B. **Preparation and Use of Bioreactors**

- Flush each with several bed volumes of DI water

1. Static Mixer:
   Flow rates = 2.5 gal/hr for Mastigocladus,
   19.6gal/hr for Cyanidium
   algae+foam/wastewater ratio = 160g/8liters wastewater
   Run for 8 hours pulling samples each hour

2. Packed Bed
   run as before

- Make one 8-hr run along with the 1st static mixer run.
- Also prepare samples of untreated (not run through test bed) wastewater for controls
- fill test rig with 1liter of wastewater for each column assembly and run in recirculating mode for 8 hrs at a flow rate of 2.5 gal/hr
- Collect 30ml samples for chemical analyses in EPA-certified acid cleaned bottles containing 0.5 ml concentrated HNO_3.
- Take samples to ADS for the following chemical analyses:
  Cr & Ni by ICP-MS
  Fe & Al by ICP-ES

VIII. SAMPLE LABELS

The following labels were used on samples sent to ADS:

Label	*Description*
12PB -1A&B	Mastigocladus packed bed
12PB -2A&B	Cyanidium "
12PB -3 A&B	Chlorella "
12PB -4A&B	Plain foam "
12PB -5A&B	Mastigocladus "
12PB -6A&B	Cyanidium "
12PB-7A&B	Chlorella "
12PB -8A&B	treated control - no foam or algae
12Cont - A&B	untreated wastewater control
12SM -1A&B-M	Mastigocladus 1hr
12SM -2A&B -M	Mastigocladus 2hr
12SM -3 A&B-M	Mastigocladus 3hr
12SM -4A&B -M	Mastigocladus 4hr
12SM -5A&B-M	Mastigocladus 5hr
12SM -6A&B-M	Mastigocladus 6hr

12SM-7A&B-M	Mastigocladus 7hr
12SM -8A&B-M	Mastigocladus 8hr
12SM -1A&B -CY	Cyanidium 1hr
12SM -2A&B- CY	Cyanidium 2hr
12SM -3 A&B- CY	Cyanidium 3hr
12SM -4A&B -CY	Cyanidium 4hr
12SM -5A&B -CY	Cyanidium 5hr
12SM -6A&B-CY	Cyanidium 6hr
12SM-7A&B- CY	Cyanidium 7hr
12SM -8A&B -CY	Cyanidium 8hr
Cont. .A&B	untreated wastewater controls

Table A.6.12 Cont.)

Experiment 12 Bioremoval by two algae using two test rigs

Date 3/13/96 (run #1) 3/15/96 (run #2)

Effluent Well DCB-4A

Water Q pH 3.79, Cond. 1.11ms/cm, DO 7.27, Temp 21.3, Sal 0.05%

Test Rig Modified packed bed bioreactor and static mixer bioreactor

		Metal concentrations					Percent removal •			
Alga	Rig/Col.	Time	Al(ppm)	Fe(ppm)	Cr(ppb)	Ni(ppb)	Al	Fe	Cr	Ni
Cyan.	PB/2	8 Hr	62.027	0.126	2.7	360				
Cyan.	PB/2	8 Hr	62.121	0.123	2.9	390				
Cyan.	PB/2	8 Hr	62.074	0.1245	2.8	375	-0.959	-8.261	6.6667	8.5366
Cyan.	PB/6	8 Hr	61.591	0.125	3.6	420				
Cyan.	PB/6	8 Hr	61.51	0.117	3.2	390				
Cyan.	PB/6	8 Hr	61.5505	0.121	3.4	405	-0.107	-5.217	-13.333	1.2195
Cyan.	SM	0 Hr	64.947	0.053	4.7	449				
Cyan.	SM	0 Hr	64.813	0.054	4.6	480				
Cyan.	SM	0 Hr	64.88	0.0535	4.65	464.5	0	0	0	0
Cyan.	SM	1 Hr	57.08	0.191	40.5	365				
Cyan.	SM	1 Hr	57.2	0.189	42	369				
Cyan.	SM	1 Hr	57.14	0.19	41.25	367	11.93	-255.1	-787.1	20.99
Cyan.	SM	2 Hr	59.264	0.368	99	426				
Cyan.	SM	2 Hr	59.177	0.36	87.7	378				
Cyan.	SM	2 Hr	59.2205	0.364	93.35	402	8.723	-580.4	-1907.5	13.455
Cyan.	SM	3 Hr	59.192	0.51	133	431				
Cyan.	SM	3 Hr	59.089	0.513	127.2	408				
Cyan.	SM	3 Hr	59.1405	0.5115	130.1	419.5	8.846	-856.1	-2697.8	9.6878
Cyan.	SM	4 Hr	59.312	0.656	147.8	402				
Cyan.	SM	4 Hr	59.15	0.641	155.9	430				
Cyan.	SM	4 Hr	59.231	0.6485	151.85	416	8.707	-1112	-3165.6	10.441
Cyan.	SM	5 Hr	59.142	0.795	204	487				
Cyan.	SM	5 Hr	59.183	0.791	209.9	496				
Cyan.	SM	5 Hr	59.1625	0.793	206.95	491.5	8.812	-1382	-4350.5	-5.813
Cyan.	SM	6 Hr	58.568	0.974	242.3	537				
Cyan.	SM	6 Hr	59.123	0.908	224.6	503				
Cyan.	SM	6 Hr	58.8455	0.941	233.45	520	9.301	-1659	-4920.4	-11.95
Cyan.	SM	7 Hr	59.242	1.005	257	531				
Cyan.	SM	7 Hr	59.107	0.997	256.3	537				
Cyan.	SM	7 Hr	59.1745	1.001	256.65	534	8.794	-1771	-5419.4	-14.96
Cyan.	SM	8 Hr	58.458	1.048	292.7	581				
Cyan.	SM	8 Hr	58.366	1.048	289.8	580				
Cyan.	SM	8 Hr	58.412	1.048	291.25	580.5	9.969	-1859	-6163.4	-24.97
Mast.	PB/1	8 Hr	61.198	0.153	3	380				

Mast.	PB/1	8 Hr	61.293	0.119	3	380					
Mast.	PB/1	8 Hr	61.2455	0.136	3	380		0.389	-18.26	0	7.3171
Mast.	PB/5	8 Hr	61.224	0.102	2.8	390					
Mast.	PB/5	8 Hr	61.205	0.118	2.9	400					
Mast.	PB/5	8 Hr	61.2145	0.11	2.85	395		0.439	4.348	5	3.6585
Mast.	SM	0 Hr	61.358	0.101	3	410					
Mast.	SM	0 Hr	61.611	0.129	3	410					
Mast.	SM	0 Hr	61.4845	0.115	3	410		0	0	0	0
Mast.	SM	1 Hr	57.122	0.375	47	490					
Mast.	SM	1 Hr	57.183	0.365	47	500					
Mast.	SM	1 Hr	57.1525	0.37	47	495		7.046	-221.7	-1466.7	-20.73
Mast.	SM	2 Hr	56.846	0.363	56	500					
Mast.	SM	2 Hr	56.56	0.373	55	490					
Mast.	SM	2 Hr	56.703	0.368	55.5	495		7.777	-220	-1750	-20.73
Mast.	SM	3 Hr	56.745	0.398	64	500					
Mast.	SM	3 Hr	56.751	0.384	64	520					
Mast.	SM	3 Hr	56.748	0.391	64	510		7.704	-240	-2033.3	-24.39
Mast.	SM	4 Hr	56.014	0.409	72	520					
Mast.	SM	4 Hr	56.521	0.391	73	520					
Mast.	SM	4 Hr	56.2675	0.4	72.5	520		8.485	-247.8	-2316.7	-26.83
Mast.	SM	5 Hr	56.245	0.44	88	530					
Mast.	SM	5 Hr	56.625	0.443	89	540					
Mast.	SM	5 Hr	56.435	0.4415	88.5	535		8.213	-283.9	-2850	-30.49
Mast.	SM	6 Hr	56.92	0.44	98	540					
Mast.	SM	6 Hr	56.784	0.469	99	550					
Mast.	SM	6 Hr	56.852	0.4545	98.5	545		7.534	-295.2	-3183.3	-32.93
Mast.	SM	7 Hr	56.608	0.52	100	550					
Mast.	SM	7 Hr	57.261	0.559	110	550					
Mast.	SM	7 Hr	56.9345	0.5395	105	550		7.4	-369.1	-3400	-34.15
Mast.	SM	8 Hr	56.907	0.558	110	560					
Mast.	SM	8 Hr	57.096	0.566	110	560					
Mast.	SM	8 Hr	57.0015	0.562	110	560		7.291	-388.7	-3566.7	-36.59
Chlor.	PB/3	8 Hr	61.208	0.11	2.9	380					
Chlor.	PB/3	8 Hr	61.744	0.114	3	390					
Chlor.	PB/3	8 Hr	61.476	0.112	2.95	385		0.014	2.609	1.6667	6.0976
Chlor.	PB/7	8 Hr	61.293	0.139	*	*					
Chlor.	PB/7	8 Hr	61.332	0.128	3.3	400					
Chlor.	PB/7	8 Hr	61.3125	0.1335	3.3	400		0.28	-16.09	-10	2.439
P F	PB/4	8 Hr	62.153	0.116	2.9	390					
P F	PB/4	8 Hr	61.936	0.113	3.1	400					
P F	PB/4	8 Hr	62.0445	0.1145	3	395		-0.911	0.435	0	3.6585
N F	PB/8	8 Hr	61.585	0.129	3.1	400					
N F	PB/8	8 Hr	61.312	0.112	3.1	410					
N F	PB/8	8 Hr	61.4485	0.1205	3.1	405		0.059	-4.783	-3.3333	1.2195
* No data											

Table A.6.13 (Cont.) Experimental Procedures and Results for Experiment #13

EXPERIMENT 13 (3/25-29/96)

Bioremoval of Tc-99 using foam embedded with nine types of biomass compared to removal by ion exchange resin

I. PURPOSE:

Evaluate algal and non-algal biomass for radionuclide removal. Use methodology routinely used by Donna Beales for Tc-99 spike experiments, i.e. 1g filter media packed in bio-rad columns , 500 ml spiked solution (river water and DI), gravity flow, etc. to comparatively evaluate removal efficacy of biomass/foam aggregates and ion exchange resins.

II. Biomass:

Alga#1 = Mastigocladus laminosus
Alga#2 = Cyanidium caldarium
Alga#3 = Nostoc Sp.
Bacteria#1 = Strain G-4
Bacteria#2 = Pseudomonas aeruginosa
Fungus#1 = Yeast strain #R14
Fungus#2 = Yeast strin R-42
Plant#1 = *Datura* (Gypsum weed)
Plant#2 = *Azolla*
Ion exchange resin = TEVA resin

III. BIOMASS PRETREATMENTS:

DI wash

IV. FOAM:

8 mesh containing 10% biomass by dry weight

V. EFFLUENT:

Tc-99 spiked DI water and Tc-99 spiked DI water

V1 TEST APPARATUS:

Bio-Rad Columns

VII. PROCEDURE:

A. Preparation of effluent

1. Add 16nCi Tc-99 to 500 ml of water (DI or River)

B. Preparation and Use of Bio-rad Poly prep column

Make two runs , one w/ Tc-99-spiked DI water, and one w/ Tc-99 spiked river water. Set up duplicate columns for each adsorbent material for each run as follows:

Column arrangements for Run #1 DI water and Run #2 River water

Col.1 and 12 - algae 1 (Mast.)
Col.2 and 13 - algae 2 (Cyan)
Col.3 and 14 - Bact 1 (G-4)
Col.4 and 15- Bact. 2 (P. aerug.)
Col.5 and 16 - Fungi 1 (yeast #14)
Col.6 and 17 -Fungi 2 (Yeast #42)
Col.7 and 18 - Plant seed 1 (Azolla)
Col.8 and 19 - plant seed 2 (Datura)
Col 9 and 20 - ion exchange resin
Col 10 and 21 - plain foam control
Col 11 and 22 - Control - no foam or resin

Total number of columns used = 44 (22 columns per run X 2 runs)
Total number of samples = 88 (duplicate samples taken from each column)

2. Add 1.00-1.05 gram of material to each column.

Table A.6.13 Cont.)

Experiment #13 Tc-99 extraction using foam embedded with nine types of biomass/resin

Date 3/25-28/96

Test apparatus/technique: Bio-Rad polyprep columns and overnight batch extraction

Percent of Tc-99 extracted from solution

		Deionized water		Unfiltered river water		Deionized water/batch	
Biomass	Amount	Test 1	Test 2	Test 1	Test 2	Aliquot 1	Aliquot 2
Mast.	0-250ml	2.1	4.5	5.6	2.9	3.4	3.2
Mast.	250-500ml	0	0.1	0	0		
Cyan.	0-250ml	4.8	25.1	16.4	18.3	33.8	37
Cyan.	250-500ml	0.8	9.3	0.7	0		
G-4	0-250ml	3.6	10.1	5	3.5	3.4	3
G-4	250-500ml	*	3.2	0	0		
P. aerug.	0-250ml	0.9	0	0	0.3	1.3	0
P. aerug.	250-500ml	0.5	0	0	0		
Yeast #14	0-250ml	4.9	0.3	0.9	0	0	1.5
Yeast #14	250-500ml	0.5	0	1.1	**		
Yeast #28	0-250ml	6.5	6.9	12	1.7	5.6	5.9
Yeast #28	250-500ml	1.1	0	0	0		
Azolla	0-250ml	3.4	0.8	1.3	0	0	0
Azolla	250-500ml	0	0	0	0		
Datura	0-250ml	3.1	24.1	0	0	0.6	0
Datura	250-500ml	0.5	**	0	0		
TEVA resin	0-250ml	99.8	99.8	84.6	96.7	95.5	97.1
TEVA resin	250-500ml	99.8	100.3	95.9	97.6		
Plain foam	0-250ml	5.5	11.2	6.7	13.4	17.4	21.2
Plain foam	250-500ml	0.3	2.5	0	0.3		
No foam	0-250ml	0.3	0	0	0.6	0	0
No foam	250-500ml	0.3	0	2.4	0		

* sample lost

** Column plugged, did not pass whole sample

Table A.6.14 Experimental Procedures and Results for Experiment #14

EXPERIMENT 14 (3/26-28/96)

Bioremoval of metals using foam embedded with nine types of biomass using the packed bed bioreactor

I. PURPOSE:

Evaluate algal and non-algal biomass for metal removal

II. Biomass:

Alga #1 = *Mastigocladus laminosus*
Alga #2 = Cyanidium caldarium
Alga #3 = Nostoc Sp.
Bacteria #1 = Strain G-4
Bacteria #2 = Pseudomonas aeruginosa
Fungus #1 = Yeast strain #R14
Fungus #2 = Yeast strain R-42
Plant #1 = *Datura* (Gypsum weed)
Plant #2 = *Azolla*

III. PRETREATMENTS:

DI wash

IV. FOAM:

8 mesh containing 10% biomass by dry weight

V. EFFLUENT:

Monitoring well DCB-4A

V1 TEST APPARATUS:

Modified Frisby packed bed bioreactor

VII. PROCEDURE:

A. Preparation of effluent

1. Collect water from pump at well near D-Area coal pile runoff basin
2. Upon returning to lab measure Horiba parameters(pH, etc.)

B. Preparation and Use of Bioreactor

1. Flush test bed with several bed volumes of DI water

2. Make two 8-hr runs with the test rig

3. Fill columns with 2 g of foam using the following strategy:

Run#1

Column 1 Mastigocladus
Column 2 -Cyanidium
Column 3 - G-4
Column 4 - P. aeruginosa
Column 5 -R-14 (yeast)
Column 6 - R-42 (yeast)
Column 7 - Plain Foam
Column 8 - no foam (raw wastewater)

Also prepare samples of untreated (not run through test bed) waste water for controls

Run#2

Column 1 Datura
Column 2 -Azolla
Column 3 - Nostoc-D
Column 4 - Nostoc - ND
Column 5 -not used
Column 6 - not used
Column 7 - Plain Foam
Column 8 - no foam (raw wastewater)

4. fill test rig with 1liter of wastewater for each column assembly and run in recirculating mode for 8 hrs at a flow rate of 2.5 gal/hr

5. Collect 30ml samples for chemical analyses in EPA-certified acid cleaned bottles containing 0.5 ml concentrated HNO_3.

6. Take samples to ADS for the following chemical analyses:
Cr & Ni by ICP-MS
Fe & Al by ICP-ES

VIII. SAMPLE LABELS

The following labels were used on samples sent to ADS:

Label	*Description*
14 -1A&B	Mastigocladus
14 -2A&B	Cyanidium
14 -3 A&B	G-4
14 -4A&B	P. aeruginosa
14 -5A&B	R-14
14 -6A&B	R-42
14-7A&B	plain foam
14 -8A&B	treated control - no foam or algae
14 Cont.	untreated control
14 -1C&D	Datura
14 -2C&D	Azolla
14 -3 C&D	Nostoc-D
14 -4C&D	Nostoc-ND
14-7C&D	plain foam
14 -8C&D	treated control - no foam or algae
14Cont. C&D	untreated control

Table A.6.14 (Cont.)

Bioremoval of metals using foam embedded with nine types of biomass using the packed bed bioreactor									
Date	3/26/96 (run #1) 3/28/96 (run #2)								
Effluent	Well DCB-4A (same water as Exper. #12)								
Water Qual.	pH 3.79, Cond. 1.11ms/cm, DO 7.27, Temp 21.3, Sal 0.05%								
Test Rigs	Modified packed bed bioreactor								
		Metal concentrations				Percent removal *			
Biomass	**Column**	**Al(ppm)**	**Fe(ppm)**	**Cr(ppb)**	**Ni(ppb)**	**Al**	**Fe**	**Cr**	**Ni**
Mast.	1	64.122	0.09	3	440				
Mast.	1	64.689	0.064	2.8	440				
Mast.	**1**	**64.4055**	**0.077**	**2.9**	**440**	**-0.32166**	**-20.31**	**10.77**	**22.807**
Cyan.	2	63.87	0.064	2.9	460				
Cyan.	2	63.948	0.064	3.2	480				
Cyan.	**2**	**63.909**	**0.064**	**3.05**	**470**	**0.45172**	**0**	**6.154**	**17.544**
G-4	3	63.494	0.064	3	480				
G-4	3	63.219	0.064	3.1	480				
G-4	**3**	**63.3565**	**0.064**	**3.05**	**480**	**1.31233**	**0**	**6.154**	**15.789**
P. aer.	4	63.515	0.064	3.1	490				
P. aer.	4	63.135	0.064	3.5	510				
P. aer.	**4**	**63.325**	**0.064**	**3.3**	**500**	**1.36139**	**0**	**-1.538**	**12.281**
#14	5	62.685	0.064	3.1	510				
#14	5	62.444	0.064	3.8	530				
#14	**5**	**62.5645**	**0.064**	**3.45**	**520**	**2.54599**	**0**	**-6.154**	**8.7719**
#42	6	61.776	0.064	4.2	580				
#42	6	61.977	0.064	4	580				
#42	**6**	**61.8765**	**0.064**	**4.1**	**580**	**3.61766**	**0**	**-26.15**	**-1.7544**
Plain Foam 3/26	7	63.098	0.064	3.9	550				
Plain Foam 3/26	7	63.364	0.064	3.8	550				
Plain Foam 3/26	**7**	**63.231**	**0.064**	**3.85**	**550**	**1.50781**	**0**	**-18.46**	**3.5088**
No Foam 3/26	8	63.165	0.064	3.6	550				
No Foam 3/26	8	63.256	0.064	3.5	550				
No Foam 3/26	**8**	**63.2105**	**0.064**	**3.55**	**550**	**1.53974**	**0**	**-9.231**	**3.5088**
Control 3/26		64.397	0.064	3.2	560				
Control 3/26		64.001	0.064	3.3	580				
Control 3/26		**64.199**	**0.064**	**3.25**	**570**	**0**	**0**	**0**	**0**
Gypsum Weed	1	62.356	0.125	2.1	380				
Gypsum Weed	1	62.504	0.112	2.2	390				
Gypsum Weed	**1**	**62.43**	**0.1185**	**2.15**	**385**	**1.90055**	**-85.16**	**4.444**	**8.3333**
Azolla	2	61.404	0.115	2.6	400				
Azolla	2	61.493	0.107	2.5	400				
Azolla	**2**	**61.4485**	**0.111**	**2.55**	**400**	**3.44283**	**-73.44**	**-13.33**	**4.7619**
Nostoc - D	3	62.784	0.064	2.4	400				
Nostoc - D	3	62.814	0.064	2.3	390				
Nostoc - D	**3**	**62.799**	**0.064**	**2.35**	**395**	**1.32072**	**0**	**-4.444**	**5.9524**

Nostoc - ND	4	62.758	0.064	2.3	410					
Nostoc - ND	4	62.359	0.064	2.2	400					
Nostoc - ND	**4**	**62.5585**	**0.064**	**2.25**	**405**		**1.69863**	**0**	**0**	**3.5714**
Plain Foam 3/28	7	62.633	0.064	2.5	410					
Plain Foam 3/28	7	62.6	0.064	2.5	400					
Plain Foam 3/28	**7**	**62.6165**	**0.064**	**2.5**	**405**		**1.60749**	**0**	**-11.11**	**3.5714**
No Foam 3/28	8	62.122	0.064	2.4	410					
No Foam 3/28	8	62.481	0.064	2.2	410					
No Foam 3/28	**8**	**62.3015**	**0.064**	**2.3**	**410**		**2.10247**	**0**	**-2.222**	**2.381**
Control 3/28		63.852	0.064	2.2	420					
Control 3/28		63.427	0.064	2.3	420					
Control 3/28		**63.6395**	**0.064**	**2.25**	**420**		**0**	**0**	**0**	**0**

APPENDIX 6B

BIOREACTOR SYSTEMS DEVELOPED FOR USE IN EVALUATING BIOREMOVAL CAPABILITIES OF FOAM-EMBEDDED MICROBES

Bioreactor System #1
Bioremoval Evaluation System Test stand (B.E.S.T)
(pages 110-115)

Bioreactor System #2
Bioremoval Evaluation System Test stand (B.E.S.T)
REV. 1 (pages 116-134)

Bioreactor System #3
Static Mixer Contracter
(pages 135-143)

Bioreactor System #4
Biofiltration Equipment for Test and Research (B.E.T.R.)
(pages 144-157)

OPERATING INSTRUCTIONS

FOR

THE FRISBY TECHNOLOGIES

BIOREMOVAL EVALUATION SYSTEM TESTBED

DELIVERED UNDER

CONTRACT NO. AA07217N

PREPARED FOR:

WESTINGHOUSE SAVANNAH RIVER COMPANY

SAVANNAH RIVER SITE, AIKEN, SC

NOVEMBER 29,1994

Prepared By: _____
Michael Browning, Test Engineer

Approved By: _____
Paul Hermann, Principal Investigator

Approved By: _____
Douglas McCrosson, Program Manager

Frisby Technologies, Inc
3635 Whiskey Road
Aiken, SC

TO FILL BIOREMOVAL EVALUATION SYSTEM TESTBED

1. Locate three-way valve at the bottom of panel. Turn the handle to fill. (The arrow should be facing straight up.) This shuts the flow off.

2. Locate air bleed valve at the top of the filter. Turn the handle to open. (The handle should be vertical.) This allows the trapped air to bleed into the reservoir while the filter is being filled.

3. Remove the cover of the reservoir. Fill reservoir with the fluid being tested. Replace cover and make sure that the air bleed and return lines are secured in the tank cover.

4. Turn the pump on by the switch located at the top of the testbed. There may be a surging sound coming from the pump. This is caused by air in the feed line. The switch may have to be turned off, wait a few seconds, then turned back on. If this does not clear the line the pump may have to be lifted so the pump head is higher than the reservoir to release the air pocket created. Replace pump and try again.

5. Set the Batch Meter to zero, make sure the meter is on. Hold down the DISPLAY button for three seconds until zeros appear. Release the button.

6. After the filter fills with liquid, allow the trapped air to circulate back into reservoir.

7. Close the air bleed valve (handle horizontal), locate the flow control valve at the top of the panel and close. Open the three way valve to either DRAIN or RUN, depending on the test being run.

8. Slowly open the flow control valve to desired flow rate.

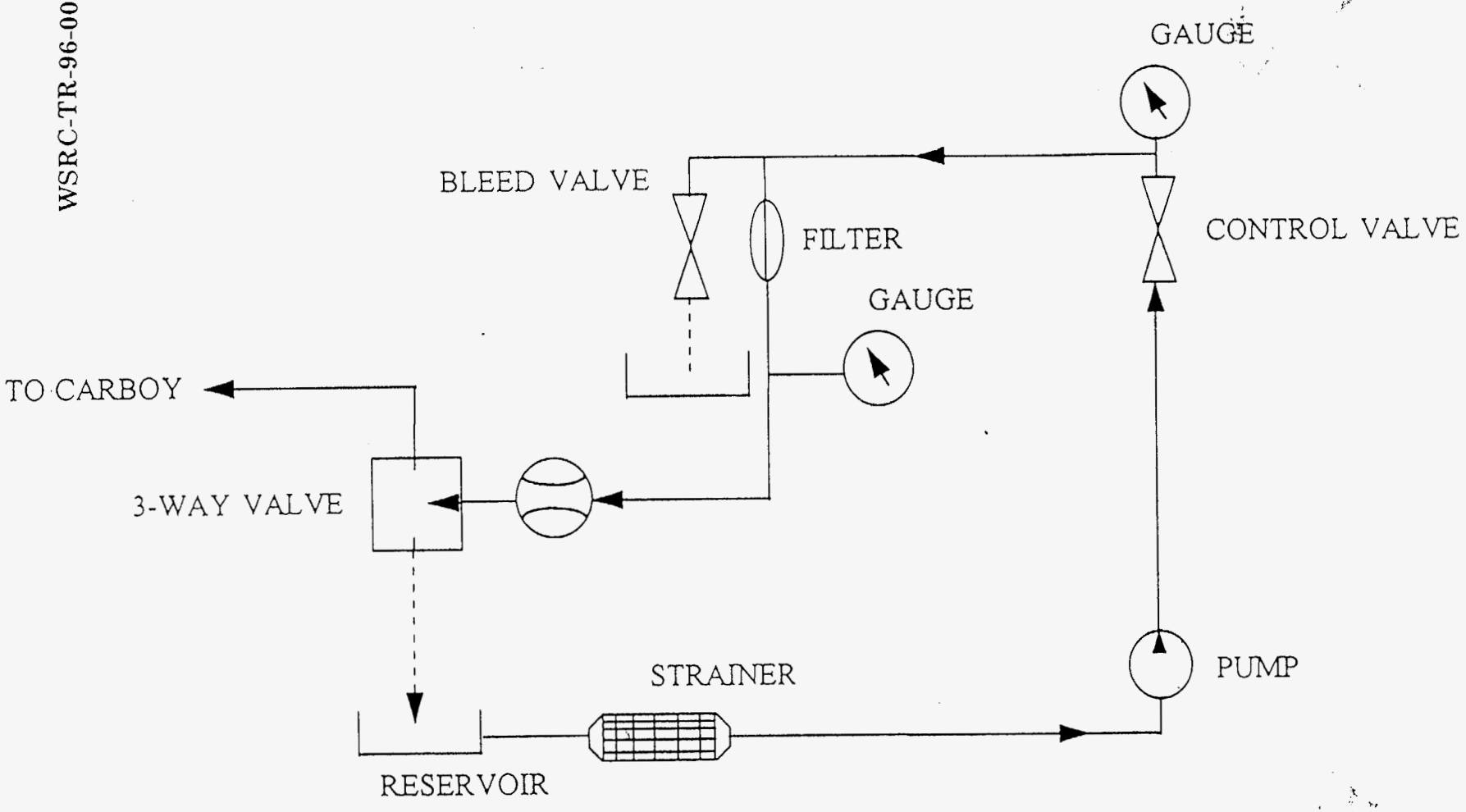
TO DRAIN FILTER

1. Turn off the pump by the switch located at the top of the testbed.
2. Open the air bleed valve, pull the air bleed line out of the fluid in the reservoir, and allow the filter to drain. If the filter does not completely drain, switch the 3-way valve to drain and drain remaining fluid.

TO CHANGE FILTER MEDIA

1. Make sure the filter is empty.
2. Loosen the top and bottom unions.
3. Remove the filter, cover the inlet (a #3 rubber stopper, found in chemistry labs, will work), and turn upside down.
4. With the tool supplied, loosen the screen housing of the filter. Remove the screen housing, and remove the existing media.
5. Clean the stainless steel screen, fill with new media and replace screen housing.
6. Tighten the screen housing, and reinstall the filter, being careful to only tighten the unions hand tight

PLUMBING SCHEMATIC

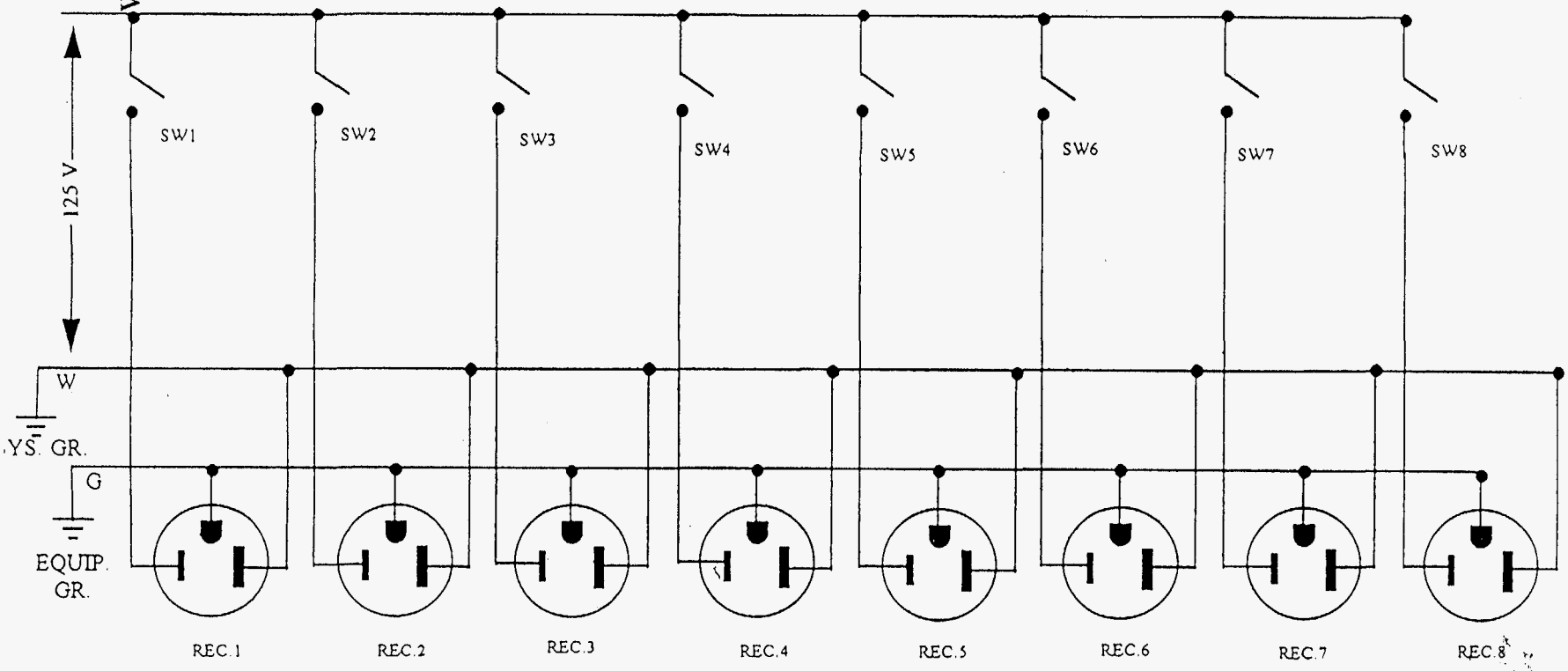


TECHNOLOGIES

FOR EACH INDIVIDUAL UNIT

ELECTRICAL SCHEMATIC

WSRC-TR-96-0088



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SAVANNAH

OPERATING INSTRUCTIONS FOR THE FRISBY TECHNOLOGIES

B.E.S.T

REV. 1

PREPARED BY MICHAEL B. BROWNING

TO FILL BIOREMOVAL EVALUATION SYSTEM TESTBED

Rev. 1

Flow Rates of .04 GPM to .36 GPM

Valve positions

Low flow shut off (black handle) open

Control valve (orange handle) open

Bleed air valve (gray handle) open

Three way valve (white handle) close

Drain valve (located in back of testbed) close

1. Fill the reservoir with water.
2. After the pump has filled with water, locate the control valve at the top of the testbed (orange handle) and close. This will close the high flow rate circuit off. Locate the low flow shut off valve (black handle) also at the top of testbed and close.
3. Turn the pump on by the switch located at the top of testbed.
4. Slowly open the low flow shut off valve (black handle) so the fluid will not "hammer" the rotameter and damage the float.
5. As the rotameter and filter fill with water there will be air bubbles present. As the air passes through the rotameter it will cause some surging.
6. After the air bubbles dissipate, adjust the flow by the black knurled knob located at the base of the rotameter.

   * ***This valve is a high turn metering valve and caution should be taken to never fully seat the valve.***

TO CHANGE FILTER MEDIA

1. Make sure the filter is empty of water.
2. Loosen the top and bottom unions.
3. Remove the filter, and turn upside down and remove media.
4. Clean the stainless steel screen, by flushing with water.
5. Refill with new media, and reinstall the filter, being careful to only tighten the unions hand tight.

TO DRAIN SYSTEM

1. With the supplied male disconnect insert into the mating disconnect located at the inlet of the pump. Drain remaining fluid into a suitable container.
2. Open the drain valve located in the back of the test stand. Drain remaining fluid into a suitable container.
3. Because of the small lines and the high turn metering valve this part of the system will drain slowly. Take the top of the small filter and lay over the front of the test bed. Open the control valve (orange handle) and this will introduce more air into the lines allowing the fluid to drain faster.

TO FILL BIOREMOVAL EVALUATION SYSTEM TESTBED

Rev. 1

Flow Rates of .3 GPM to 1.8 GPM

Valve positions check list:

Low flow shut off (black handle) closed

Control valve (orange handle) open

Bleed air valve (gray handle) open

Three way valve (white handle) close

Drain valve (located in back of testbed) close

1. Locate three-way valve (white handle) at the bottom of panel. Turn the handle to fill. (The arrow should be facing straight up.). This shuts the flow off.

2. Locate air bleed valve (gray handle) at the top of the filter. Turn the handle to open. (The handle should be vertical.) This allows the trapped air to bleed into the reservoir while the filter is being filled.

3. Locate low flow ball valve (black handle) at the top of the panel above the control valve (orange handle) and turn to the off position (vertical). This shuts off the water flow to the low flow rate circuit.

4. Remove the cover of the reservoir. Fill reservoir with the fluid being tested. Replace cover and make sure that the air bleed and return lines are secured in the tank cover.

5. To set the Batch Meter to zero, make sure the meter is on. Hold down the DISPLAY button for three seconds until zeros appear. Release the button.

6. Turn the pump on by the switch located at the top of the testbed.

7. After the filter fills with liquid, allow the trapped air to circulate back into reservoir.

8. Close the air bleed valve (handle horizontal), locate the flow control valve (orange handle) at the top of the panel and close. Open the three way valve to either DRAIN or RUN, depending on the test being run.

9. Slowly open the flow control valve (orange handle) to desired flow rate.

TO DRAIN FILTER

1. Turn the three way valve (white handle) to drain
2. When the flow meter is reading zero ,turn off the pump by the switch located at the top of the testbed.
3. Open the air bleed valve, and allow the filter to drain. The totalizer has already taken this amount into effect.

TO CHANGE FILTER MEDIA

1. Make sure the filter is empty.
2. Loosen the top and bottom unions.
3. Remove the filter, cover the inlet (a #3 rubber stopper, found in chemistry labs, will work), and turn upside down.
4. With the tool supplied, loosen the screen housing of the filter. Remove the screen housing, and remove the existing media.
5. Clean the stainless steel screen, fill with new media and replace screen housing.
6. Tighten the screen housing, and reinstall the filter, being careful to only tighten the unions hand tight.

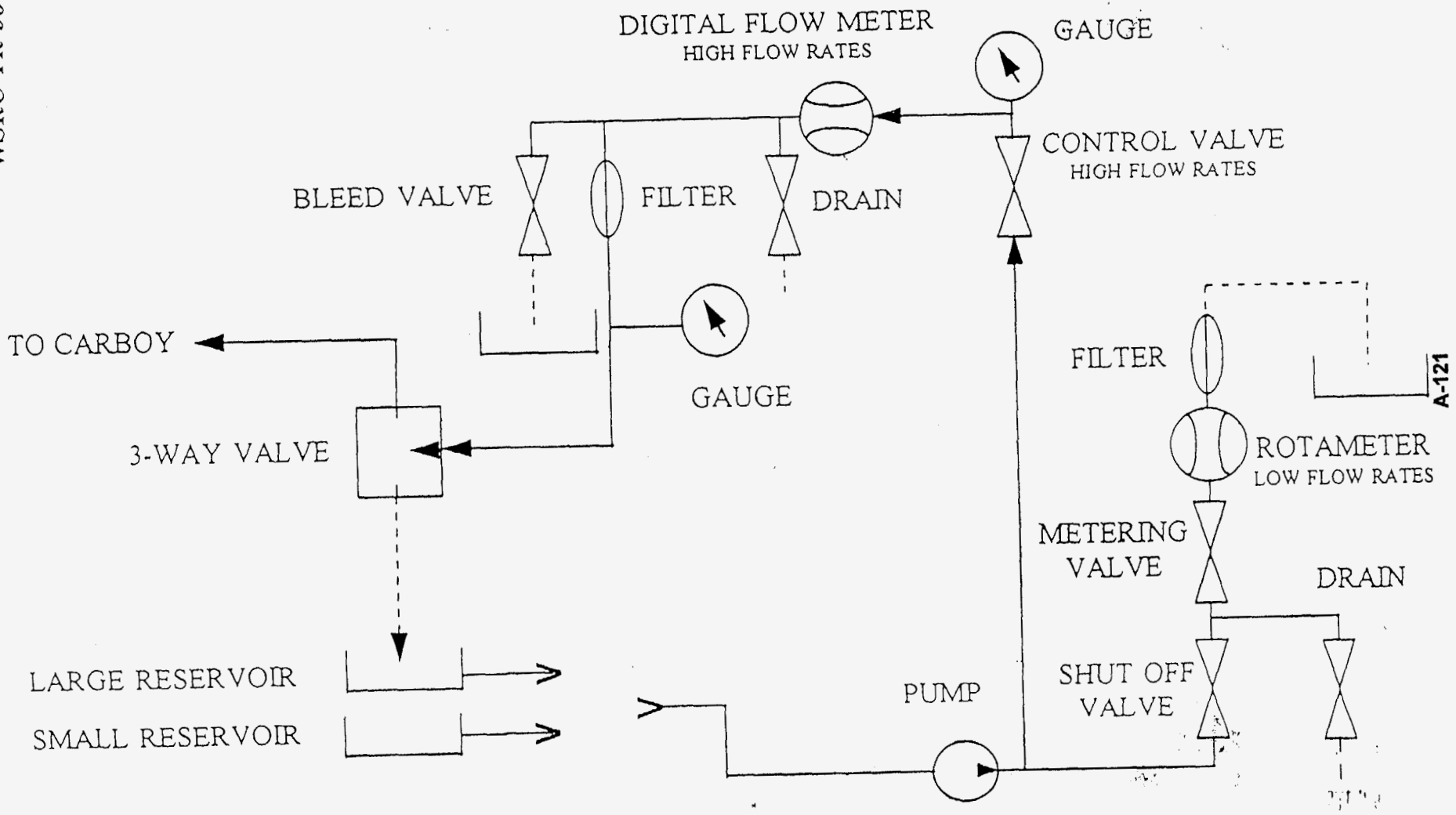
TO DRAIN SYSTEM

1. With the supplied male disconnect insert into the mating disconnect located at the inlet of the pump. Drain remaining fluid into a suitable container.

2. Open the drain valve located in the back of the test stand. Drain remaining fluid into a suitable container.

3. To drain the remaining fluid from the return line going to the reservoir, turn the three way valve to run wait a few seconds and then turn back to drain.

PLUMBING SCHEMATIC



FRIGBY TECHNOLOGIES

FOR EACH INDIVIDUAL UNIT

PARTS LIST FOR B.E.S.T TEST STAND

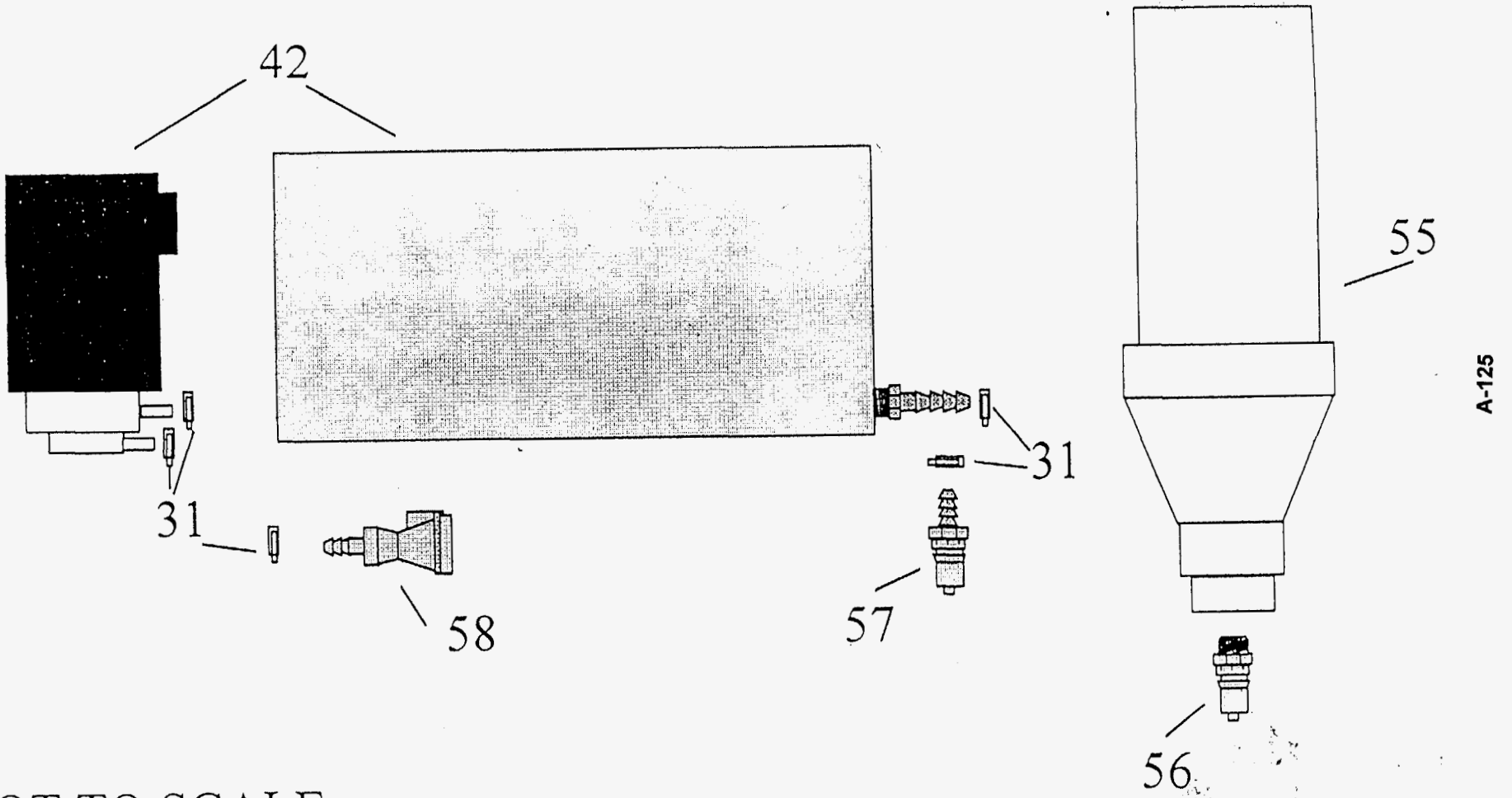
ITEM #	DESCRIPTION	QUANTITY
2	3/4 x1/2 HEX BUSHING SCH 80 PVC	16
3	1/2 x C NIPPLE SCH 80 PVC	144
4	1/2x 3 NIPPLE SCH 80 PVC	8
5	1/2 NPT TEE SCH 80 PVC	40
6	1/2 NPT BULKHEAD SCH 80 PVC	56
7	1" DIA. SS TYPE 304 30 MESH SCREEN	8
8	1/2 x 1/4 SS HEX BUSHING	32
9	2" PVC CLEAR TUBE CUT TO 6" LONG	8
10	3/4 DOUBLE UNION PVC BALL VALVE BODY CUT IN HALF & MACHINE PER SKETCHES	8
12	1/4 PVC LAB COCK	24
13	3- WAY 1/2 NPT BALL VALVE SCH 80 PVC	8
14	1/2 NPT 1/2 PLASTIC HOSE NIPPLE	56
15	1/2 NPT ELBOW SCH 80 PVC	56
16	DIGITAL TOTALIZER	8
17	1 x 1/2 HEX BUSHING SCH 80 PVC	16

ITEM #	DESCRIPTION	QUANTITY
20	3/8 OD TUBE x 1/4MPT PARFLEX ADAPTER	24
21	3/8 OD TUBE x 1/2 MPT PARFLEX ADAPTER	8
29	1/2 Y-STYLE STRAINER SCH 80 PVC	8
31	316 SS UNPERFORATED WORM DRIVE HOSE CLAMP	88
37	1/2 TUBE OD 1/2 NPT MALE ELBOW POLYPROPYLENE	32
41	0-30 PSI 316 SS 1/4 MPT GAUGE	16
42	LITTLE GIANT 5 GALLON TANK & PUMP ASSEMBLY	8
43	CHEMTROL 1/2 TRU-UNION BALL VALVE	8
44	FABRICATED STAND (THERMCRAFT)	2
50	5/8 OD STAINLESS STEEL WORM GEAR CLAMPS	8
51	3/8 OD TUBE x 1/4MPT PARFLEX TEE	8
52	1/4 ID TUBE x 1/4MPT BARBED HOSE FITTING	8
53	1/4 ID TUBE x 1/2MPT BARBED HOSE FITTING	16
54	SMALL FILTER ASSEMBLY	8
55	SMALL RESERVOIR	8
56	1/2 MPT COUPLING INSERT	8
57	1/2 TUBING COUPLING INSERT	8

ITEM #	DESCRIPTION	QUANTITY
58	1/2 TUBING COUPLING BODY	8
59	1/4 FPT 2 WAY SS PANEL MOUNT BALL VALVE	8
60	22 GPH ROTAMETERS	8
	3/16ID x 1/2 OD POLYETHLYNE TUBING	16
	3/8 VINYL CLEAR TUBING	22
	1/4 VINYL CLEAR TUBING	24
	1/2 ID 3/4 0D VINYL CLEAR TUBING	76
	1/4ID x 3/8 OD POLYETHLYNE TUBING	24

PUMP ASSEMBLY AND RESERVOIRS

WSRC-TR-96-0088



A-125

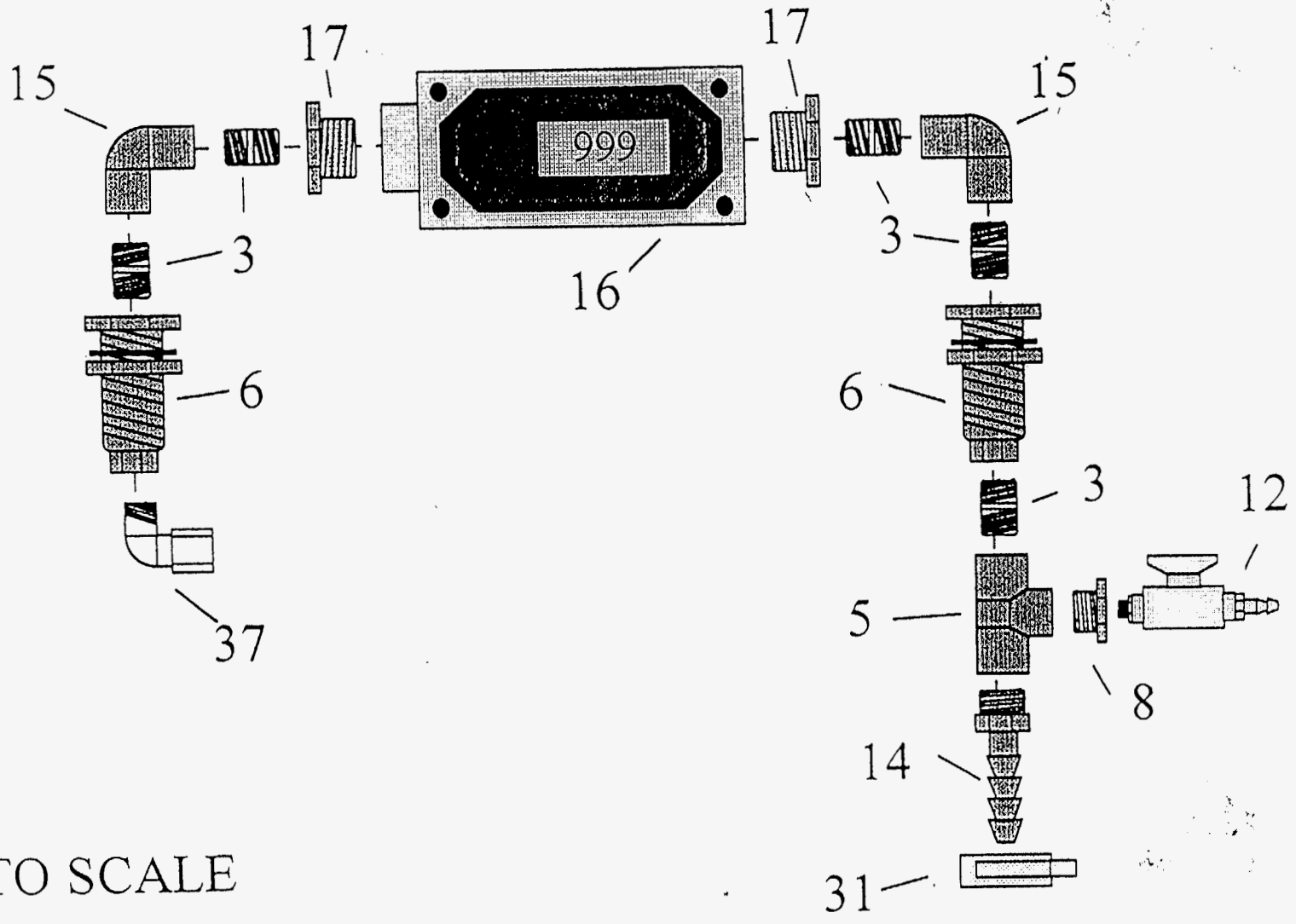
NOT TO SCALE

FRIGBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

WSRC-TR-96-0088

FLOW / TOTALIZER



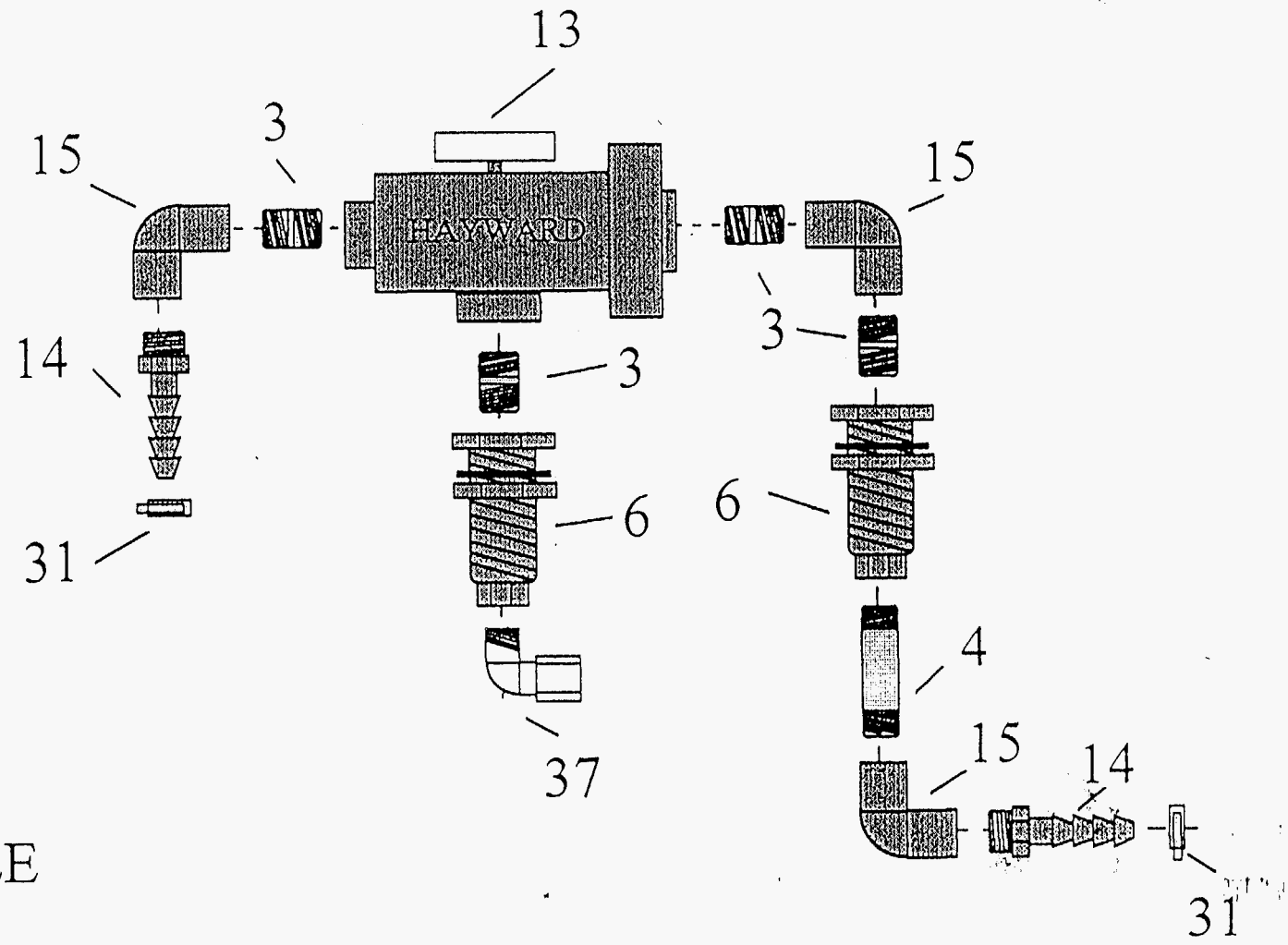
A-126

NOT TO SCALE

FRIGIDY

8 ASSEMBLIES NEEDED

DRAIN / RUN VALVE

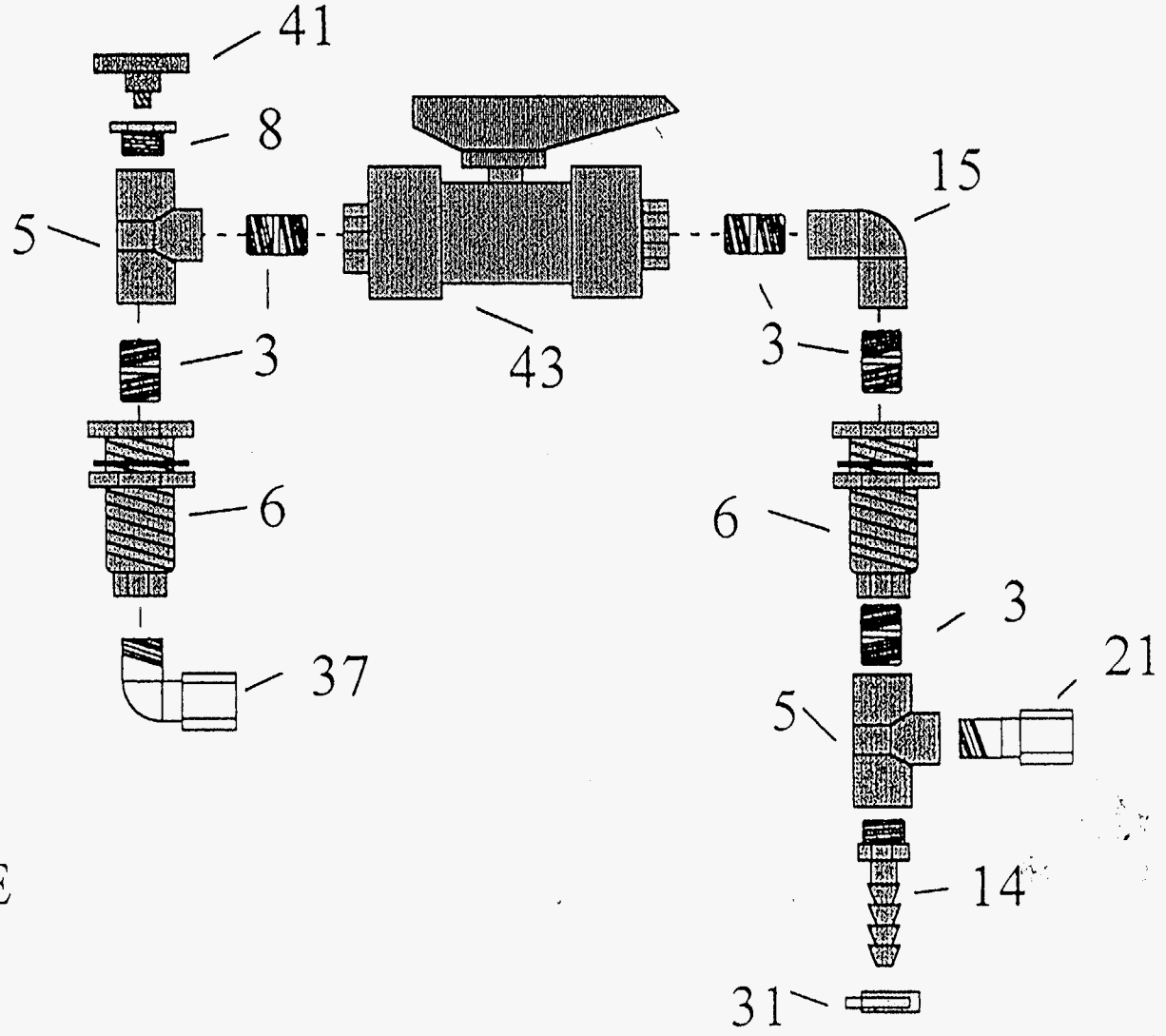


NOT TO SCALE

FRIGBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

FLOW CONTROL



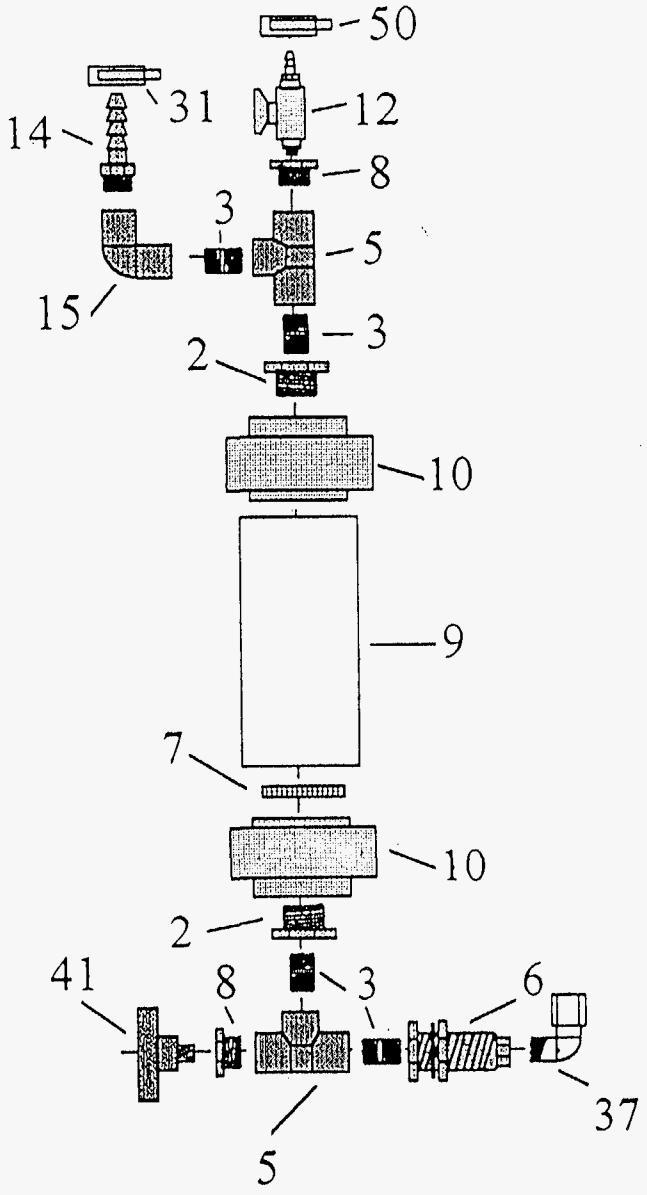
NOT TO SCALE

FRISBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

WSRC-TR-96-0088

LARGE FILTER ASSEMBLY



A-129

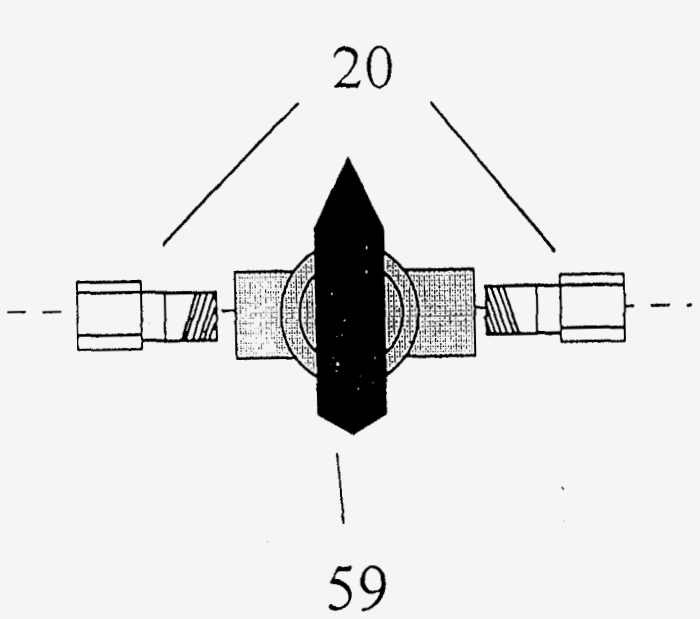
NOT TO SCALE

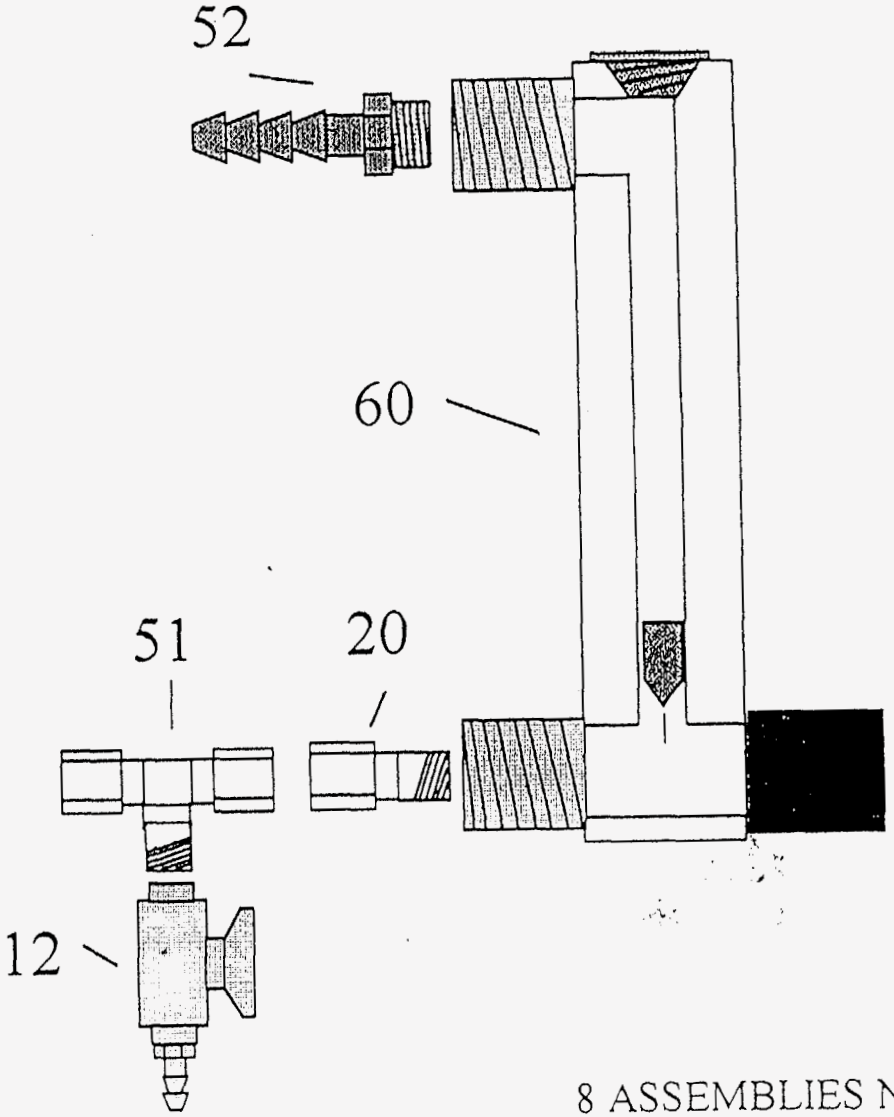
FRIGBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

WSRC-TR-96-0088

LOW FLOW SHUT OFF AND FLOWMETER





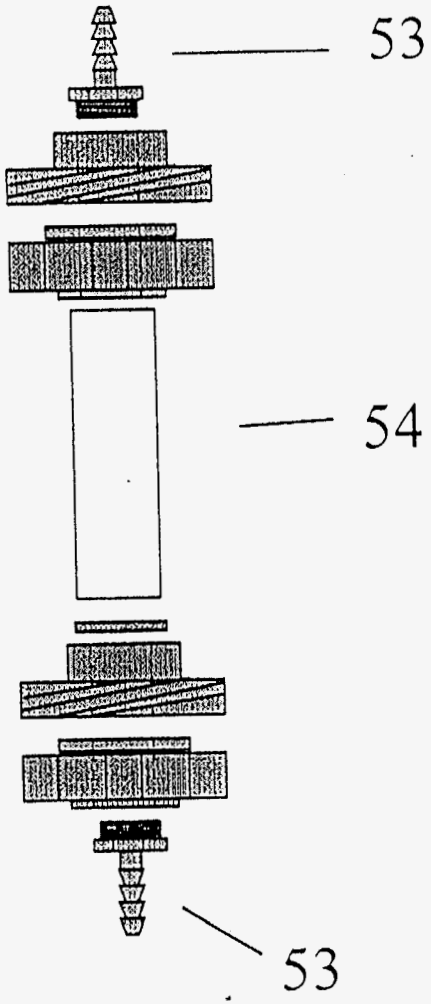
A-130

NOT TO SCALE

FRIGBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

SMALL FILTER ASSEMBLY



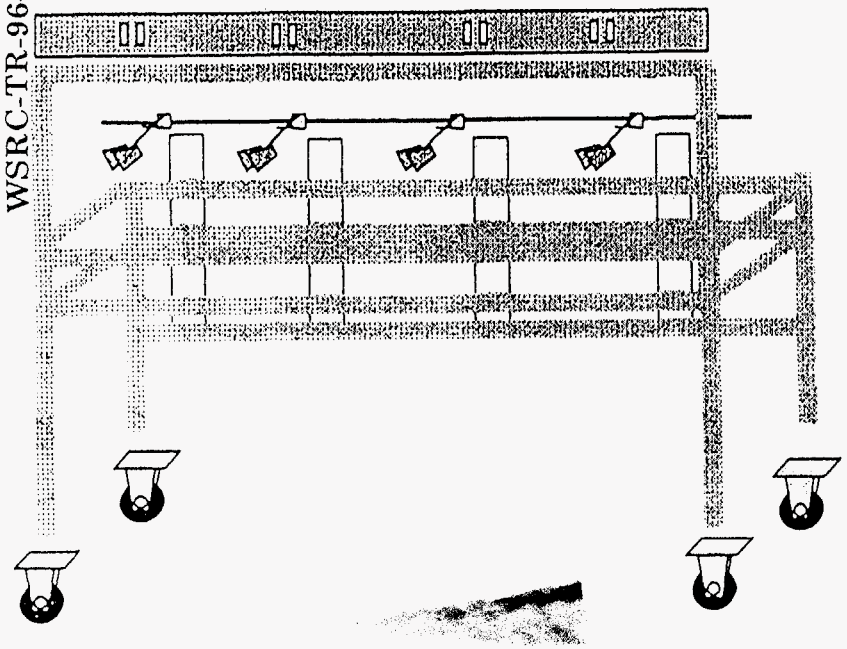
NOT TO SCALE

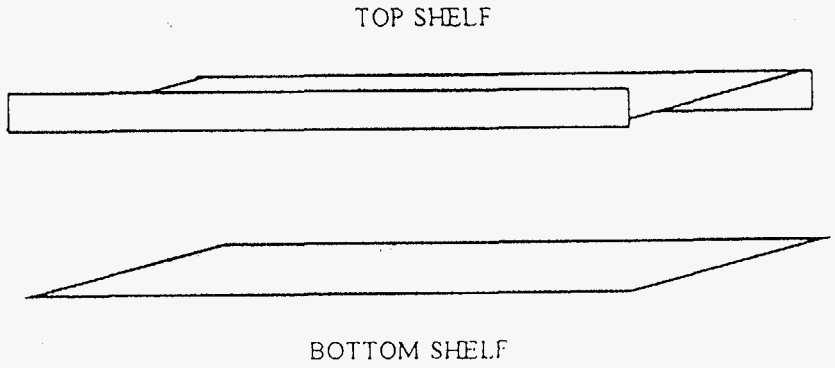
FRIGBY TECHNOLOGIES

8 ASSEMBLIES NEEDED

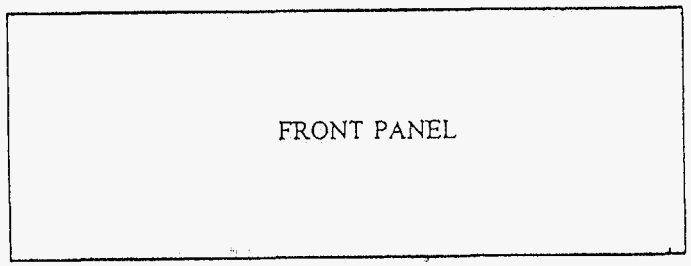
STAND

WSRC-TR-96-0088





A-132

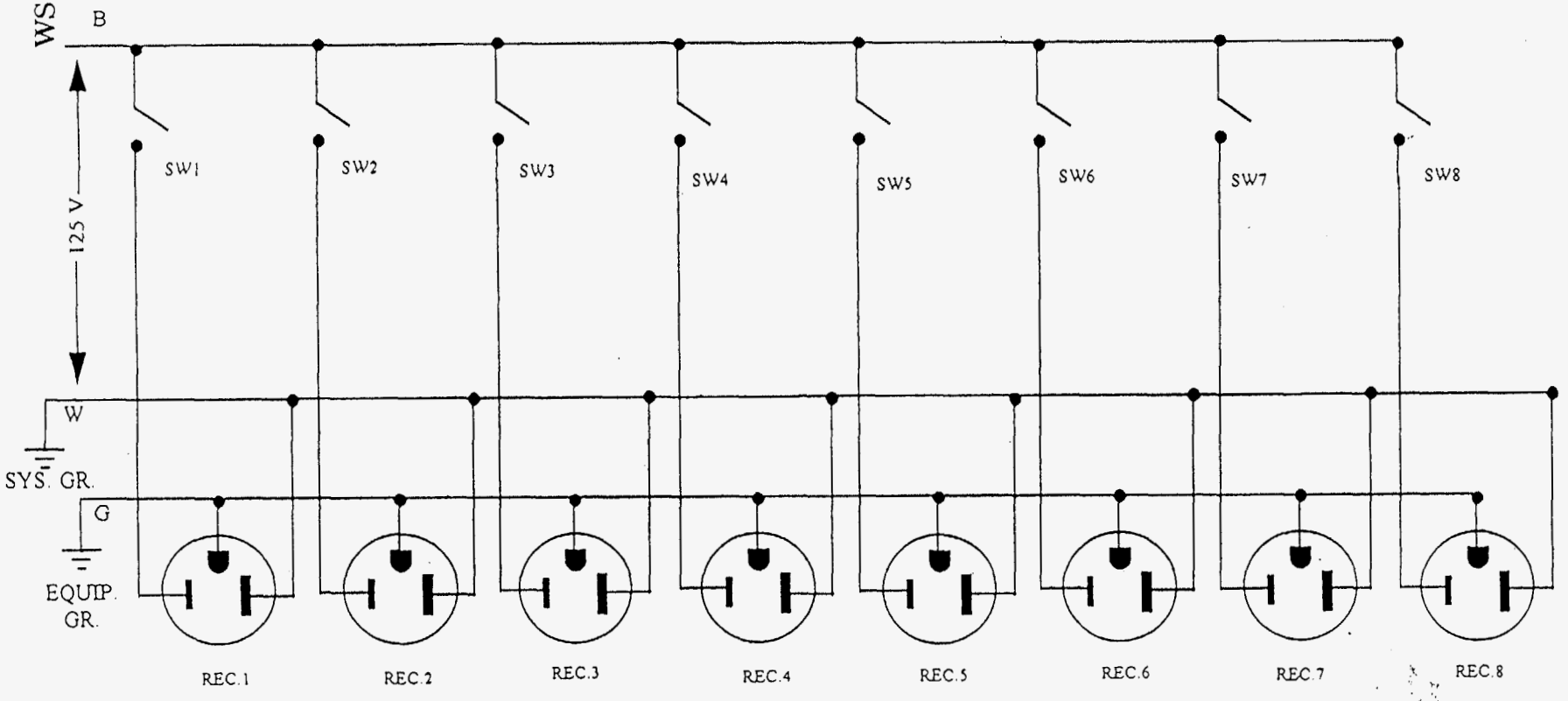


NOT TO SCALE

FRIGBY TECHNOLOGIES

2 ASSEMBLIES NEEDED

ELECTRICAL SCHEMATIC



PER STAND

INSTRUCTION MANUAL

STATIC MIXER CONTACTER

FRISBY TECHNOLOGIES

3635 WHISKEY ROAD • AIKEN, SC 29803
803 642-0296 • FAX: 803 642-1128
e-mail: frisbytb@aol.com

CORPORATE HEADQUARTERS
417 SOUTH MAIN STREET • FREEPORT, NY 11520
516 378-0162 • FAX: 516 378-0262

MATRIX R & D CORPORATION
21 Fieldstone Drive
Dover, NH 03820

(1)

STATIC MIXER CONTACTER

The unit consists of two separate flow circuits as shown on the schematic 'A' and 'B'. In both circuits the effluent in reservoir gravity feeds to the input of pump and is pumped through 'A' mixer circuit or 'B' filter circuit.

The filter media to be tested will be added to reservoir and must be continuously mixed by stirrer supplied by client.

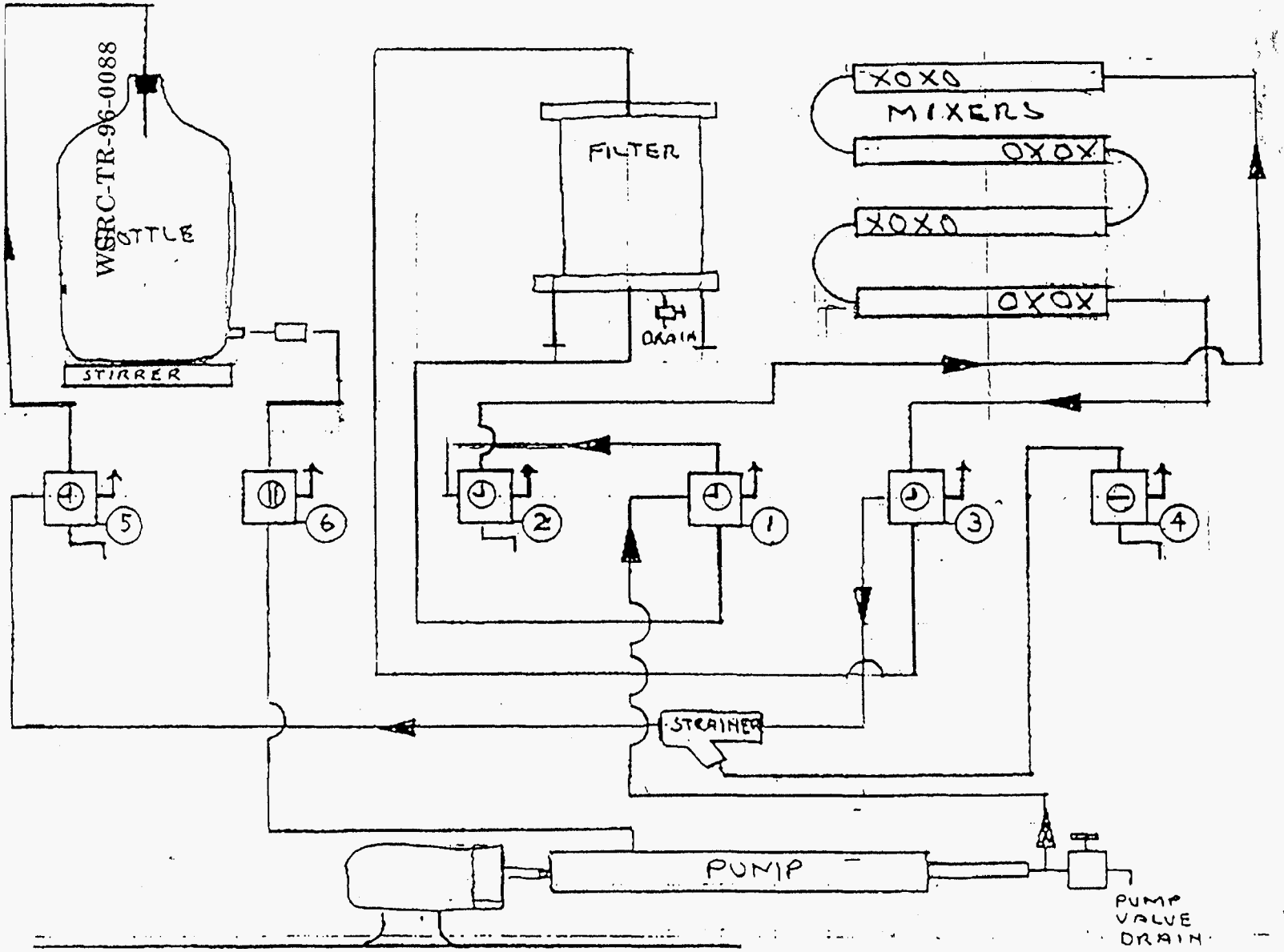
CIRCUIT 'A' STATIC MIXERS

The static mixers ensure complete contacting and plug flow conditions which will maximize contact of effluent and media. Each molecule of effluent will be in contact with media at some point along the length of mixer tubes.

CIRCUIT 'B' FILTER STAND

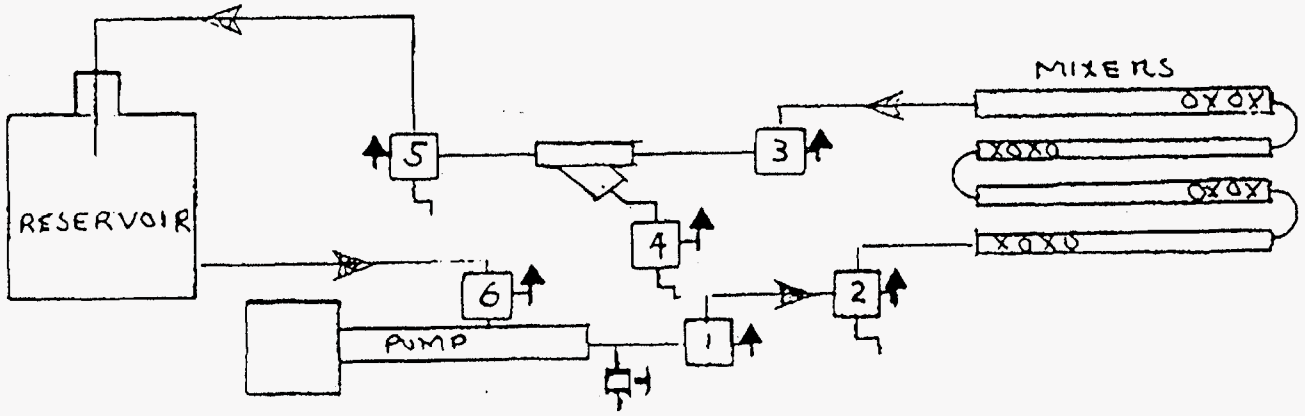
The filter stand is provided to collect and separate media from effluent after it has been determined that the media has been in contact with effluent for its desired length of time. The filter stand contains a flat disc filter in top plate to filter media from effluent as flow in from bottom progresses through unit.

STATIC MIXER CONTACTER



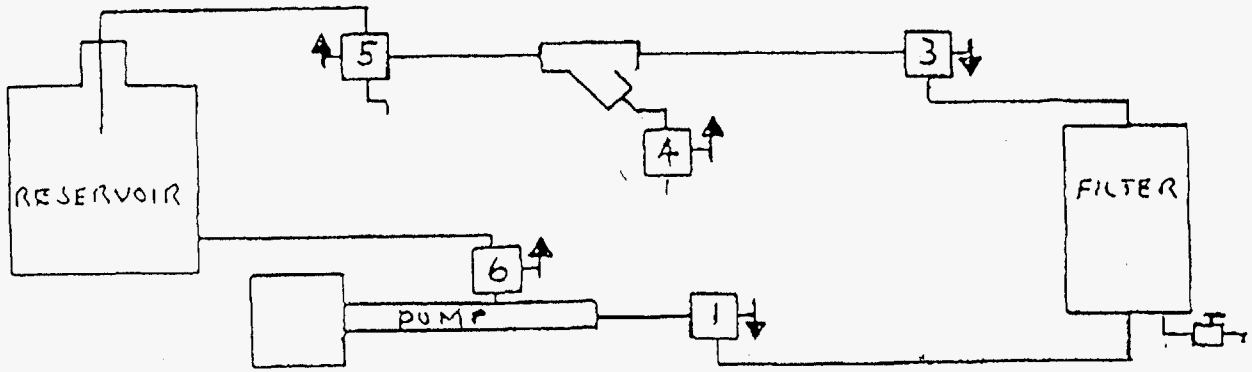
MATRIX R & D CORPORATION
21 Fieldstone Drive
Dover, NH 03820

STATIC MIXER CONTACTOR



VALVES 1-6 UP POSITION

CIRCUIT 'A'
THRU MIXERS



VALVES 1+3 DOWN POSITION
2,4,5,+6 UP POSITION

CIRCUIT 'B'
THRU FILTER

(2.1)

(3)

SAMPLING PORTS

Sampling ports are provided by means of valves 2, 4 and 5. The individual valve functions are as follows:

CIRCUIT A

POSITION	VALVE NO.	FUNCTION
Front Exit Tube	2 down position	Empty Reservoir or Sample prior to mixer
Right Side Exit Tube	4 pointing left	Sample of Effluent without media after mixer
Left Side Exit Tube	5 down position	Sample of Effluent and media after mixer or purge of entire system

CIRCUIT B

POSITION	VALVE NO	FUNCTION
Right Side Exit Tube	4 pointing left	Sample of effluent without media after filter

STATIC MIXER CONTACTER

(4)

VALVE POSITIONING PER FUNCTIONS

FLOW DIRECTION	INST MANUAL STEP	VALVE 5	VALVE 6	VALVE 2	VALVE 1	VALVE 3	VALVE 4
THRU MIXER OUT LEFTSIDE PURGE TUBE	5	⇩	⇧	⇧	⇧	⇧	⇧
THRU FILTER OUT LEFT SIDE PURGE TUBE	8	⇩	⇧	⇧	⇩	⇩	⇧
THRU FILTER OUT RIGHT SIDE PURGE TUBE	9	⇨	⇧	⇧	⇩	⇩	⇦
THRU PUMP OUT FRONT PURGE TUBE	10	⇧	⇧	⇩	⇧	⇧	⇧
AIR PURGE THRU STOPPER OUT FRONT PURGE TUBE	12	⇧	⇧	⇧	⇧	⇧	⇧
AIR PURGE THRU STOPPER OUT VALVE ON FILTER BOTTOM	13	⇧	⇧	⇧	⇧	⇩	⇧

(5)

SET UP AND RUN

Prior to any test the reservoir should be rinsed with a compatible cleaning solution and the system should be flushed. To accomplish a flush, proceed as follows:

1. Remove reservoir and filter
2. Empty any residual material and clean both. Refill reservoir with appropriate cleaner.

3. Replace reservoir on stirrer, place valve 6 in off position. Connect Quick disconnect and install stopper.

4. Reinstall filter and connect quick disconnects.

5. Reset position of valving:

NOTE: DO NOT START MOTOR AT ANY TIME WITHOUT CHECKING VALVE POSITIONS

VALVE	5	6	2	1	3	4
	down	up	up	up	up	up

6. Place container under SS tube exiting stand on left side of unit to catch flow of cleaning solution

7. Turn speed on potentiometer to zero. Apply AC power to unit (swiches on). Slowly advance speed potentiometer towards maximum. Flow will start and reverse flush system through mixers and out left hand purge tube.

8. Stop motor by turning off power switch. Swap valves 1 and 3 from up position to down position. Restart power and flow will flush through filter out left side purge tube.

(6)

9. Stop power. Swap *Valve 5* to center off position. Swap *Valve 4* to center sample position. Place container under SS tube exiting right side stand
Start power and clean *Valve 4* and strainer.

9.1 During flush of *Valve 4*, remove stopper from reservoir. Place tube into waste container, CENTER momentarily swap *Valve 5* from **down** position to **up** position to flush leg from *Valve 5* through stopper.

10. Stop power. Place all Valves in **UP** position with exception of *Valve 2* which should point down. Place container under front purge tube.

11. Start power and empty reservoir through purge tube on front of machine.

NOTE: Steps 1-11 may be repeated with distilled water to remove cleaner from system. In any case the system will remain full of liquid cleaner or water. To remove the residual liquid proceed as follows:

AIR PURGE

BLOW CASE

12. Using low pressure air line (30) psi or lower. Insure **all valves** are in **up** position and drain valve on pump outlet is open. Catch flow out of 1/4 line exiting pump outlet valve. Gently blow air into tube entering stopper to air flush back through mixer circuit. Momentarily swap *Valve 4* to **down** position and clean strainer and *Valve 4*. Stop air flow and close pump outlet drain valve.

13. Reset Valve 3 to down position and open valve on bottom of filter. Gently blow air into (stipper) tube and catch fluid out of valve on bottom of filter. STOPPER

NOTE: The filter may contain full volume of liquid. Leg from reservoir outlet to pump input will still contain water.

(7)

RUN MACHINE

To run machine the reservoir must be filled with effluent and media mix of choice. The unit is provided with quick disconnect on outlet of glass reservoir and stopper, so as to be able to remove reservoir for filling or cleaning. The media and effluent must be stirred and a magnetic stirrer is suggested to be provided by client which will keep media in suspension. Media should be 20-8 mesh (.033-.093) in size to prevent plugging.

As shown on schematic CIRCUIT A through mixers, requires all valves in **up** position pointer towards black lettering.

CIRCUIT B through filter requires Valves 3 and 1, pointing **down** to red lettering.

NOTE: Shut off power to swap valves and check valve positioning before restarting pump. In A or B runs, sequence valves as above and start pump.

90° ELBOW
SCREW BOTTLE TOP

SAVANNAH RIVER SITE

OPERATING INSTRUCTIONS AND PARTS MANUAL FOR THE FRISBY TECHNOLOGIES

B.E.T.R

BIOFILTRATION EQUIPMENT for TEST and RESEARCH

PREPARED BY MICHAEL B. BROWNING

OPERATING INSTRUCTIONS

FOR THE

BIOFILTRATION EQUIPMENT for TEST and RESEARCH

B.E.T.R

CAUTION Before operating the testbed read all owners manuals !! Make sure that the air supply regulator is set to the correct pressures.(Not to exceed 30 PSI) Failure to do so could result in serious injury and damage to the equipment.

TO FILL FILTER

1. Close the ball valves to the inlet of the air tanks. These valves (yellow handles) are located on each tank at the air supply inlet.
2. Turn the **effluent** and **nutrient** valves to the **off** position.
3. Separate the quick disconnect assemblies located at the filter. There are three sets of disconnects for each filter.
4. Disassemble the filter by removing only the white nylon wing nuts located at the top of the filter assembly
5. Remove the aluminum top and top gasket . Fill with media to be tested.
6. Place the gasket on top of the glass making sure the glass and gasket align , then place the aluminum top on filter assembly, tighten wing nuts finger tight only.

TO RUN SYSTEM

1. Turn the **effluent** and **nutrient** valves to the **off** position.
2. Turn the **recirculate / carboy** valve to **fill**.
3. Open the filter bleed valve (located on top of the filter assembly).
4. Open the air supply valves (located at the inlet of each tank).
5. Turn the **effluent** and **nutrient** valves to the **run** position.
6. Adjust the flow rate by the black knob (marked with an L) located at the bottom of the flow meters. To read the flow meters center the float on the scale reading. The scale reading correlates to a calibration sheet furnished by the manufacturer. It is important that the right flow meter is matched with the right calibration.
7. When the filter assembly is full, close filter bleed valve, turn the **recirculate / carboy** valve to the operation to be performed.

TO RECIRCULATE FLUID

1. Turn the **effluent** and **nutrient** valves to the **off** position.
2. Turn the **recirculate / carboy** valve to **recirculate**.
3. Set the peristaltic pumps to desired flow rate. (see operating manual)

TO SEND FLUID TO CARBOY

1. Turn the **effluent** and **nutrient** valves to the **run** position
2. Turn the **recirculate / carboy** valve to **carboy**. The fluid will now flow into the carboy.

TO SAMPLE FLUID

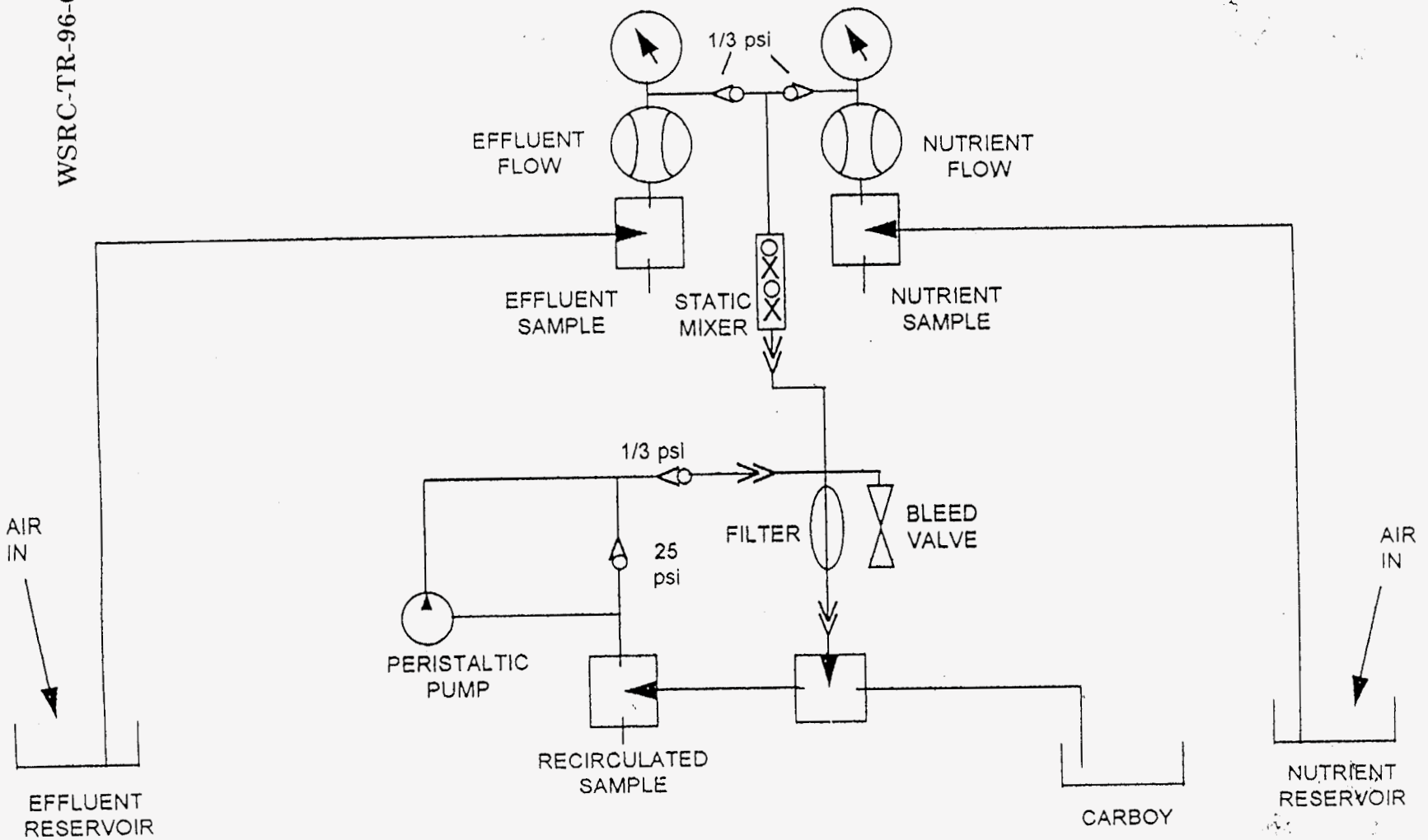
1. To sample any of the fluids turn the corresponding valve to **sample**.

TO PURGE SYSTEM

Since there are check valves through out the system, purging the system with air is the only method of draining.

1. Turn the air supply off to the tanks by closing ball valves located at the inlet of each tank.
2. Bleed air out of the pressure tanks by opening the tank bleed valves.
3. Remove any existing fluid.
4. Reseal tanks, close tank bleed valves.
5. Turn the **effluent** and **nutrient** valves to the **run** position
6. Turn the **recirculate / carboy** valve to **carboy**.
7. Open air supply valve and purge remaining fluid into carboys or suitable containers.

PLUMBING SCHEMATIC



FRIGBY TECHNOLOGIES

PLUMBING IS IDENTICAL FOR LEFT AND RIGHT SIDE OF TESTBED.
BOTH SIDES SHARE THE EFFLUENT & NUTRIENT RESERVOIR.

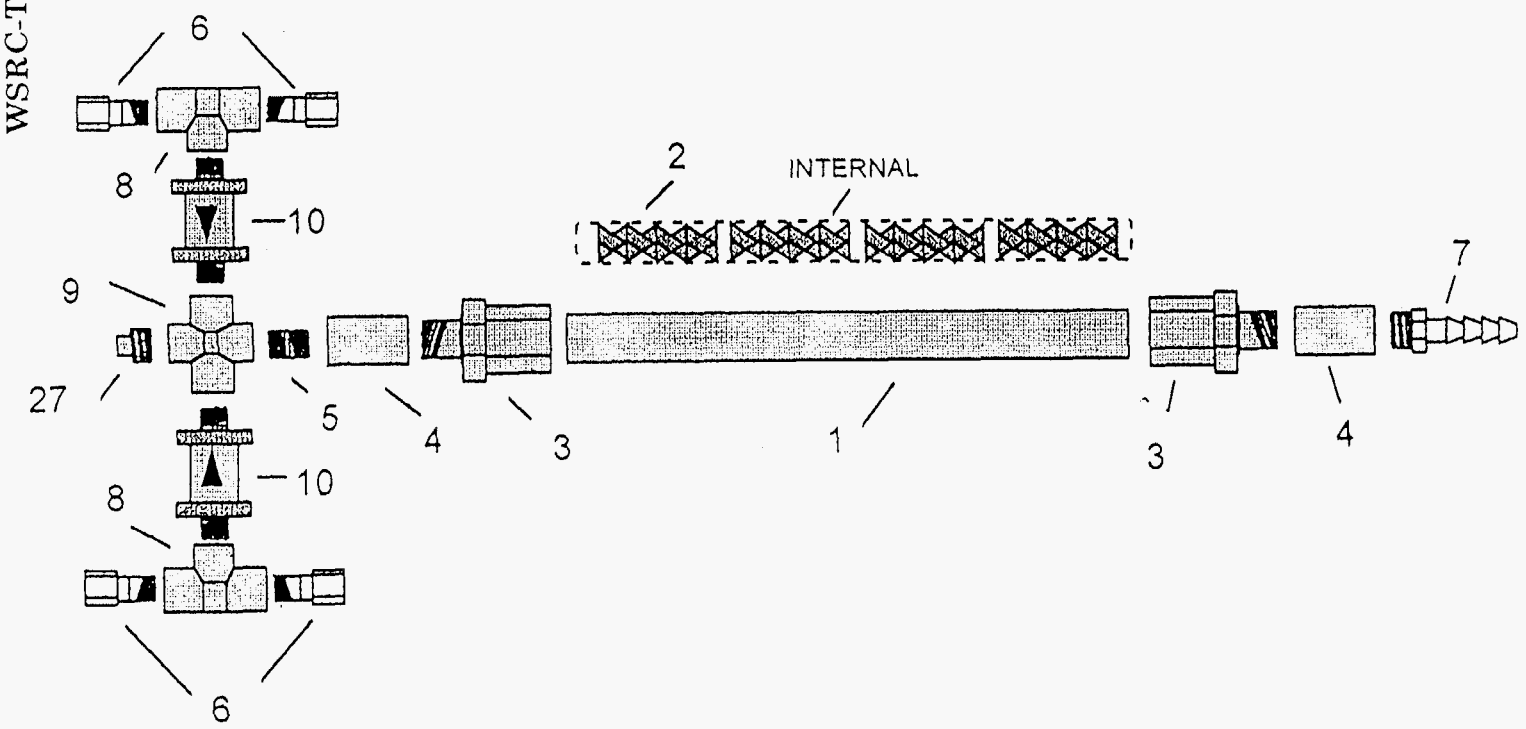
PARTS LIST FOR B.E.T.R TEST STAND

ITEM #	DESCRIPTION	QUANTITY
1	DASH 8 STAINLESS STEEL TUBING	2
2	STATIC MIXERS	8
3	DASH 8 to 1/4 MPT ADAPTER	4
4	1/4 x 1/8 FPT SS COUPLING	8
5	1/8 x CLOSE SS NIPPLE	4
6	1/8 MPT x1/4OD TUBE ADAPTER	38
7	1/8 MPT x1/4 ID TUBE BARBED FITTING	26
8	1/8 MPT SS TEE	8
9	1/8 MPT SS CROSS	2
10	CHECK VALVE 1/3 PSI 1/8 MPT	6
11	1/8 FPT SS COUPLING	4
12	CHECK VALVE 25 PSI 1/8 MPT	2
13	4 1/2" 30PSI SS GAUGE	4
14	ROTOMETER	4
15	1/8 3WAY SS VALVE	8
16	1/4 TUBING COUPLING BODY	6
17	1/4 TUBING COUPLING INSERT	6
18	GLASS COLUMN	2
19	TFE GASKET	4
20	ALUMINUM PLATES	2
21	5/16-18 THREADED ROD	8
22	5/16-18 NYLON WING NUT	8
23	5/16-18 SS LOCK NUT	8

PARTS LIST FOR THE
B.E.T.R.

ITEM #	DESCRIPTION	QUANTITY
24	5/16-18 SS NUT	8
25	1/8 FPT SS STREET ELL	2
26	1/8 NPT SS PLUG	2
27	1/8 2WAY SS BALL VALVE	2
28	1/4NPT 2WAY BRASS BALL VALVE	2
29	REGULATOR	2
30	BACK PRESSURE REGULATOR	2
31	1/4 x CLOSE SS NIPPLE	5
32	1/4 NPT SS STREET ELL	2
33	2" 160 PSI GAUGE	2
34	2 1/2" 30 PSI GAUGE	2
35	MANUAL RELIEF VALVE	2
36	1/4 MPT x1/4OD TUBE TEE	2
37	5 GAL SS PRESSURE VESSEL	1
38	1/4 SS PLUG	2
39	1/4 x 3 SS NIPPLE	1
40	1 GAL SS PRESSURE VESSEL	1
41	PERISTALTIC PUMP AND DRIVER	2
42	#12 RUBBER STOPPER	2
43	CARBOY	2

STATIC MIXER ASSEMBLY

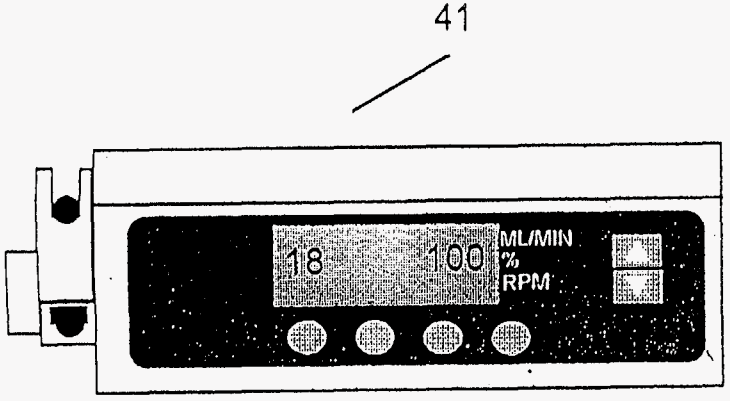


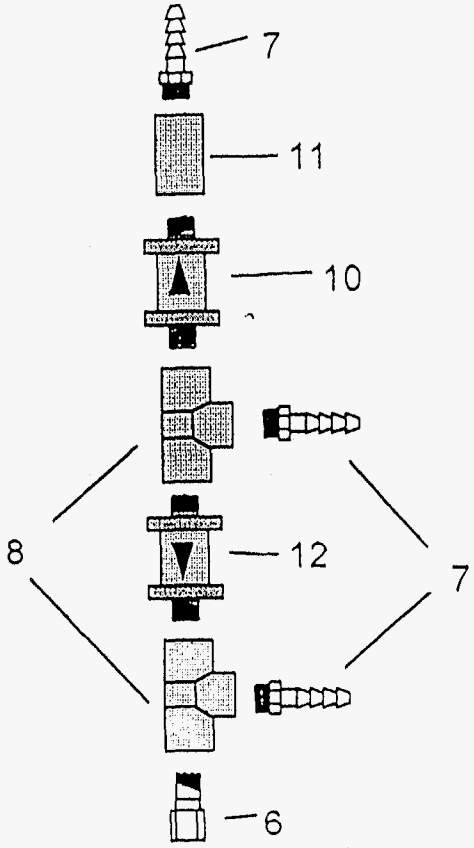
NOT TO SCALE

FRISBY TECHNOLOGIES

2 ASSEMBLIES NEEDED

RECIRCULATE ASSEMBLY



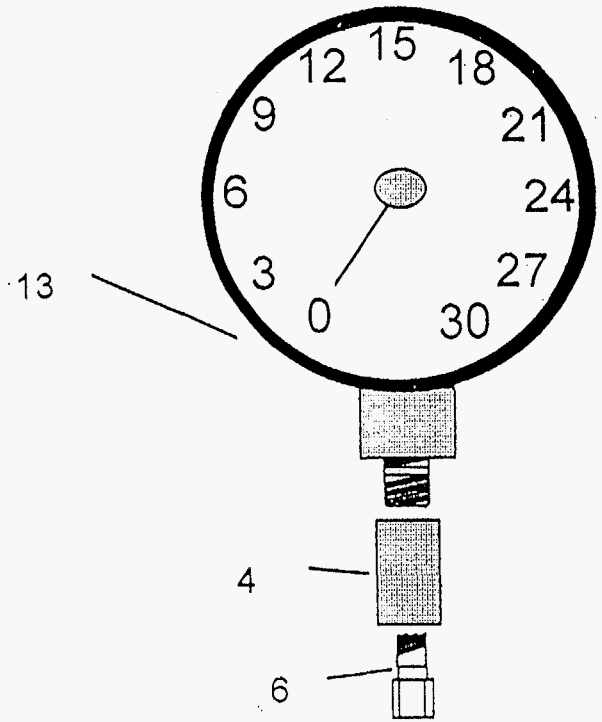


NOT TO SCALE

TRICOR TECHNOLOGIES

2 ASSEMBLIES NEEDED

GAGE ASSEMBLY

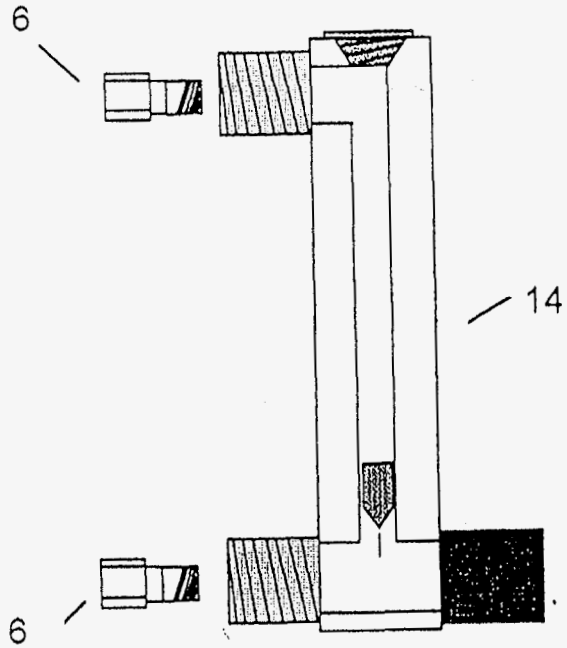


NOT TO SCALE

FRIGBY TECHNOLOGIES

4 ASSEMBLIES NEEDED

FLOW METER ASSEMBLY

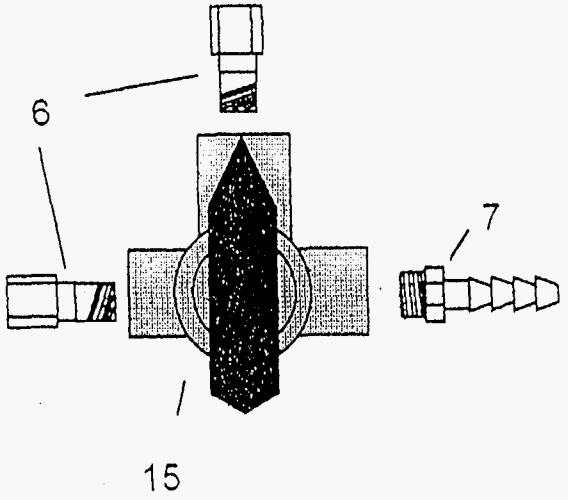


NOT TO SCALE

FRIGBY TECHNOLOGIES

4 ASSEMBLIES NEEDED

VALVE ASSEMBLY

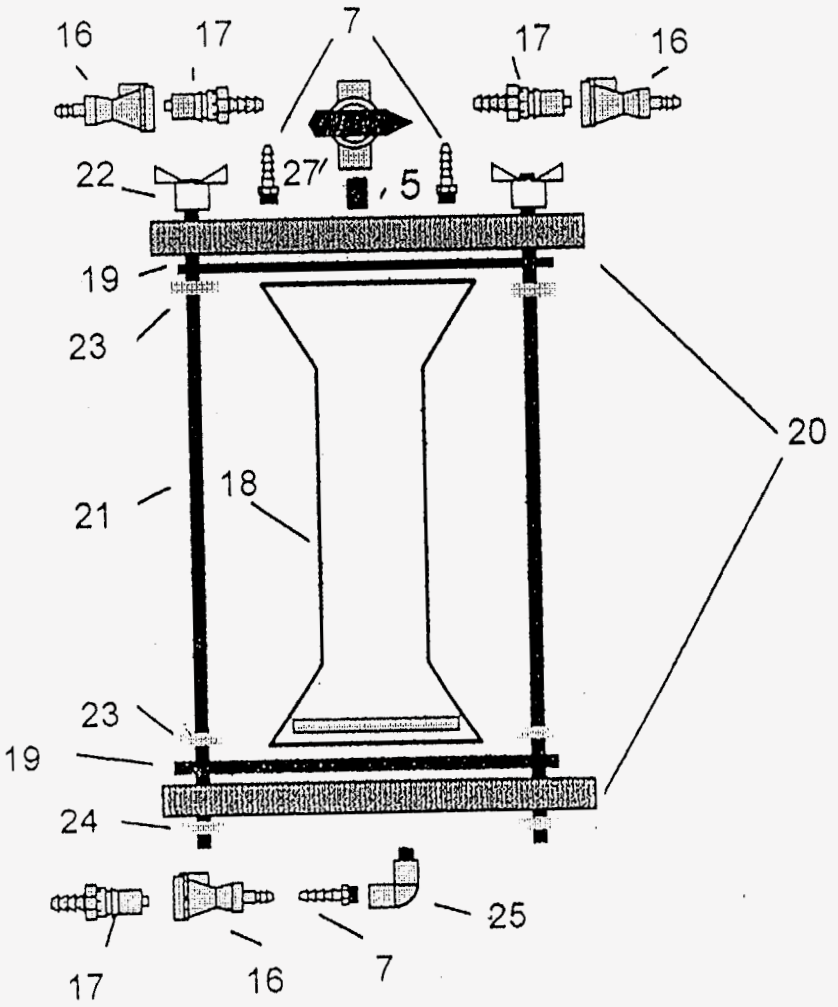


NOT TO SCALE

FRISBY

8 ASSEMBLIES NEEDED

FILTER ASSEMBLY

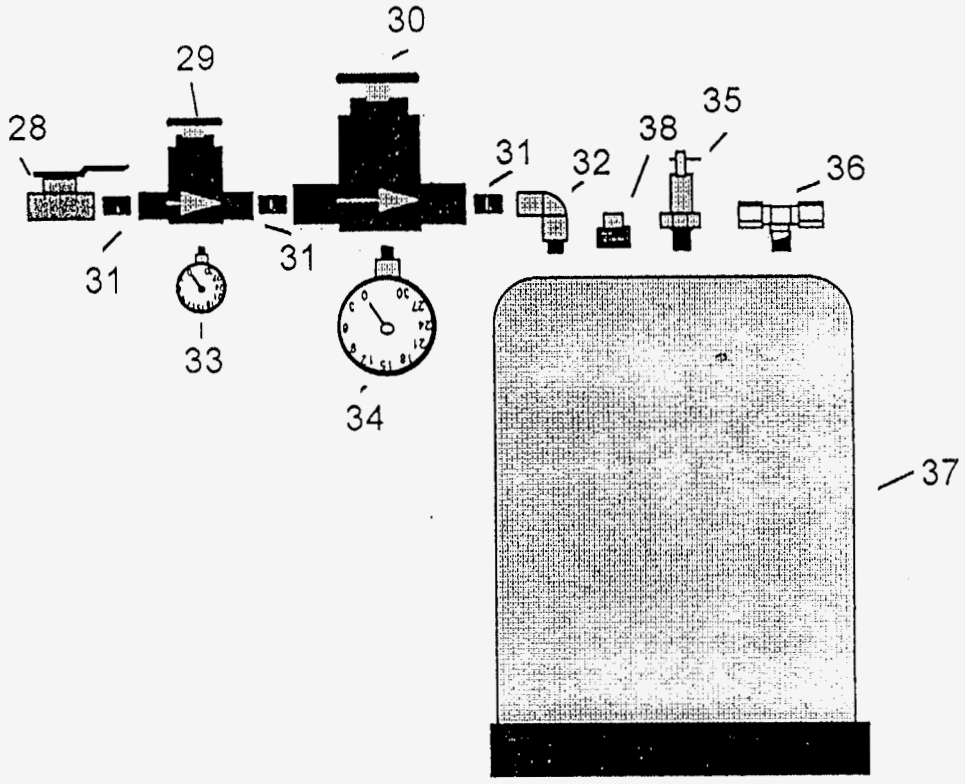


NOT TO SCALE

FRISBY TECHNOLOGIES

2 ASSEMBLIES NEEDED

EFFLUENT TANK ASSEMBLY

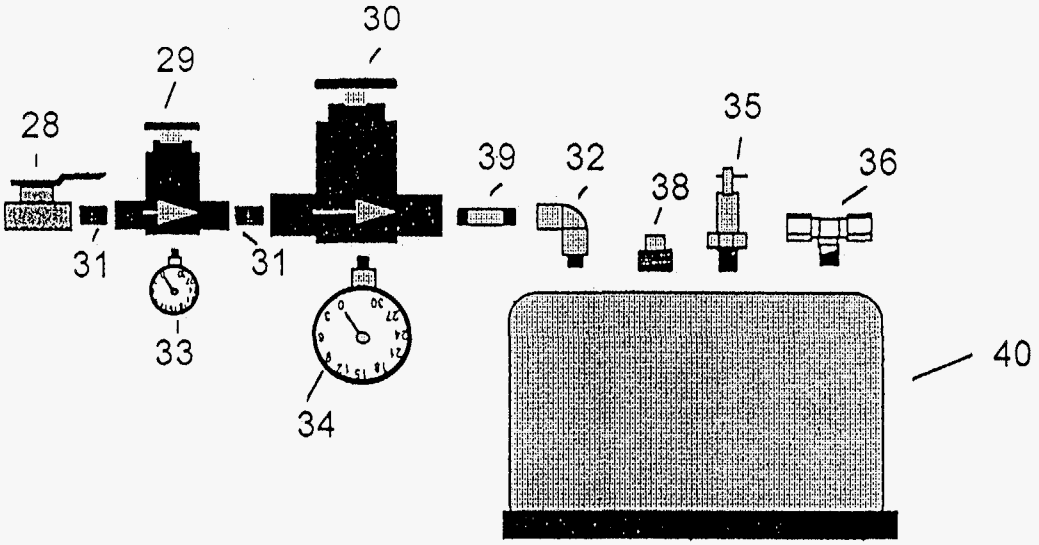


NOT TO SCALE

TRIGBY TECHNOLOGIES

1 ASSEMBLY NEEDED

NUTRIENT TANK ASSEMBLY

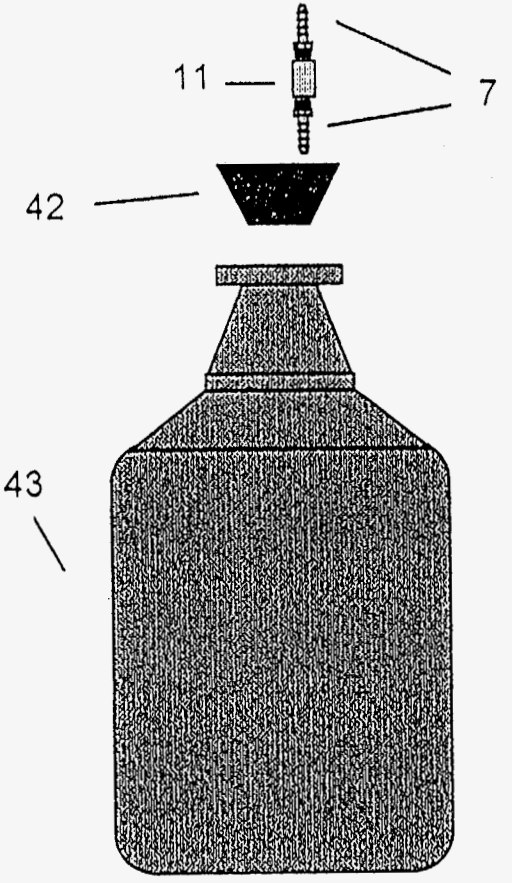


NOT TO SCALE

FRIGIDY TECHNOLOGIES

1 ASSEMBLY NEEDED

CARBOY ASSEMBLY



NOT TO SCALE

FRIGBY TECHNOLOGIES

2 ASSEMBLIES NEEDED

APPENDIX 7

POLYURETHANE FOAM IMMOBILIZATION OF TCE-DEGRADING BACTERIA: ENTRAPMENT EFFECTIVENESS AND INFLUENCE ON METABOLIC ACTIVITY

Appendix 7.1

Peptone Tripticase Yeast Glucose (PTYG) Agar

Deionized water	1 liter
Glucose (dextrose)	10 g
Yeast extract	10 g
Tripticase (tryptone)	5 g
$MgSO_4 \cdot 7\ H_2O$	0.60 g
$CaCl_2 \cdot 2\ H_2O$	0.070 g
Agar, purified	15 g

Heat to boiling while stirring
Autoclave
Cool to 50°C
Pour plates such that 1 l makes 35 - 40 plates.

Appendix 7.2

Pseudomonas Medium

Solution 1

Deionized water	895 ml
K_2HPO_4	12.5 g
KH_2PO_4	7.2 g
$(NH_4)_2SO_4$	1.0 g
$MgSO_4 \cdot 7H_2O$	0.1 g
Trace elements	5.0 ml

pH to 7.2
Heat to boiling
Autoclave
Let cool, then add glucose (100 ml/l)

Solution 2. Trace Elements

H_3BO_3	0.232 g
$ZnSO_4 \cdot 7H_2O$	0.174 g
$FeSO_4 \cdot 7H_2O$	0.082 g
$CoCl_2 \cdot 6H_2O$	0.069 g
$Na_2MoO_4 \cdot 2H_2O$	0.004 g
$CuSO_4 \cdot 5H_2O$	0.008 g
$MnSO_4 \cdot 4H_2O$	0.008 g

Solution 3. Glucose

Deionized water	100 ml
Glucose (dextrose)	14.4 g

Sterile filter

Appendix 7.3

Basal Salts Medium (BSM) and Yeast-Glucose Medium (YGM)

Soln. 1. BSM Stock (20X)	*2000 ml*	*4000 ml*
K_2HPO_4	170 g	340 g
$NaH_2PO_4 \cdot H_2O$	40 g	80 g
NH_4Cl	80 g	160 g

pH to 7.2 before adjusting to final volume
Use 50 ml/lliter final volume in DI to make BSM or YGM

Soln. 2. Trace Metals (20X)		
Nitrilotriacetic acid, trisodium salt	5.377 g	10.754
$MgSO_4 \cdot 7H_2O$	8.0 g	16.0 g
$FeSO_4 \cdot 7H_2O$	0.48 g	0.96 g
$ZnSO_4 \cdot 7H_2O$	0.12 g	0.24 g
$MnSO_4 \cdot H_2O$	0.12 g	0.24 g

Store cold
Use 50 ml/lliter final volume in DI to make BSM or YGM

Soln. 3 Glucose-Yeast Extract (for YGM only)	
Deionized water	100 ml
Glucose (dextrose)	10 g
Yeast Extract	5 g

Sterile filter
Add to BSM at 100 ml/liter final volume to make YGM

Appendix 7.4

Summary of Washout, Viability, and GC Experiments

Experiment Date:	6/29/95
Embedding Date:	N/A
Organism(s):	PR131
Growth Medium:	NB vs *Pseudomonas* medium
Type of Experiment:	Growth Yield vs Glucose Content and Medium Type
Slurry Description:	N/A
Foam #, Description:	N/A
Notes:	Pseud medium better than NB, 20 g/l glucose enough.
Filenames:	NA

Experiment Date:	7/13/95 - 7/14/95
Embedding Date:	7/13/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Washout and viability
Slurry Description:	8.1% and 17.7% in Pseud medium
Foam #, Description:	#1-13. Orig. prepolymer (Bipol 6B; NCO 6). Varied foam formulation as described in file.
Notes:	Surfactant type & amount and slurry density were most important. This exp. was 50 ml DI through 2 g foam in duplicate. 10% formalin, AODC. Viability very low.
Filenames:	WASHOUT.XLS, WASHOUT.PPT

Experiment Date:	7/20/95
Embedding Date:	7/13/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Larger volume washout of Foams 10-13
Slurry Description:	8.1% and 17.7% in Pseud medium
Foam #, Description:	#10-13. Bipol 6B. Varied foam formulation as described in file.
Notes:	This exp. was 1000 ml DI through 2 g foam in duplicate. Collected 50 ml at 50, 150, 250, 400, 550, 700, 850, 1000 ml
Filenames:	WASHOUTB.XLS, WASHOUT.PPT

Experiment Date:	7/20/95
Embedding Date:	7/13/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Larger volume washout of Foams 10-13
Slurry Description:	8.1% and 17.7% in Pseud medium
Foam #, Description:	#10-13. Bipol 6B. Varied foam formulation as described in file.
Notes:	This exp. was 2000 ml DI through 2 g foam in duplicate. Collected 1st 50 ml, then 950, then 1500 ml
Filenames:	WASHOUTB.XLS, WASHOUT.PPT

Experiment Date:	8/16/95
Embedding Date:	8/15/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Viability and Washout (Bipol 3; NCO 3), effect cold embedding
Slurry Description:	6.8% and 13.4% in Pseud medium
Foam #, Description:	#14-18. New prepolymer (81195 = Bipol 3; NCO 3) vs original.
Notes:	50 ml washout in duplicate, 2 g foam. Viability low again. Washout still low.
Filenames:	NEWPOLVB.XLS, WASHOUT.PPT

Experiment Date:	8/31/95
Embedding Date:	8/31/95
Organism(s):	PR131, #14, Chlorobenzene degraders, 01-b
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Viability (prepolymer 350), effect heat & pH shift on viability
Slurry Description:	6.8% PR131, 5.4% #14 in Pseud medium
Foam #, Description:	#21-25. Prepolymer 350; NCO unknown but thought < Bipol 3
Notes:	Did plates and MPN's
Filenames:	NEWPOL3.XLS, WASHOUT.PPT

Experiment Date:	9/18/95
Embedding Date:	9/17/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Viability (plate & MPN)
Slurry Description:	6.4% PR131 in Pseud medium
Foam #, Description:	#26 -31 Prepolymer 350
Notes:	Did plates and MPN's
Filenames:	POL3EM2.XLS

Experiment Date:	10/3/95 - 10/4/95
Embedding Date:	10/3/95
Organism(s):	PR131
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Viability 10/3 and TCE 10/4, prepolymer 350
Slurry Description:	6.4% PR131 in Pseud medium
Foam #, Description:	#32 - 33 controls, 34-37 PR131, prepol 350
Notes:	Plate counts, TCE on foam. MSB medium for TCE exp.
Filenames:	? Have printout, no TCE removal. POL3EM3.XLS.

Experiment Date:	10/18/95
Embedding Date:	10/18/95
Organism(s):	PR131, P. aeruginosa
Growth Medium:	*Pseudomonas* medium
Type of Experiment:	Viability

Slurry Description:	6.9% PR131, 0.4% P. aer in Pseud medium
Foam #, Description:	#38 Polymer 350 control, #39 Polymer 802 (NCO unkhown, thought < prepol 350) control, #40 350 PR131, #41 802 PR131, #42 802 P. aer.
Notes:	Plate counts, compare old & new plates.
Filenames:	POL3_4.XLS

Experiment Date:	11-1-95
Embedding Date:	N/A
Organism(s):	18d, 2d-b, 01-b, G4
Growth Medium:	YGM
Type of Experiment:	Benzene degradation by cultures
Slurry Description:	N/A
Foam #, Description:	N/A
Notes:	Not worth plotting
Filenames:	110895JR.ALL, EXE, SUM or variants

Experiment Date:	11/3/95
Embedding Date:	N/A
Organism(s):	PR131, G4 (phenol)
Growth Medium:	YGM
Type of Experiment:	TCE removal by cultures
Slurry Description:	N/A
Foam #, Description:	N/A
Notes:	Worked!
Filenames:	110695JR.ALL, EXE, SUM or variants

Experiment Date:	11/9/95
Embedding Date:	N/A
Organism(s):	G4(phenol)
Growth Medium:	YGM
Type of Experiment:	TCE removal, cultures, PM vs YGM, vary TCE level
Slurry Description:	N/A
Foam #, Description:	N/A
Notes:	
Filenames:	111395JR.ALL, EXE, SUM or variants. VIDEO.PPT

Experiment Date:	11/14/95
Embedding Date:	11/14/95
Organism(s):	G4 (phenol)
Growth Medium:	YGM
Type of Experiment:	TCE removal by foam & slurry, induced in culture. BSM vs YGM
Slurry Description:	G4 2.4% in BSM, 2.2% in YGM
Foam #, Description:	71 BSM, 72 YGM, 73 G4/BSM, 74 G4/YGM. Orig. prepolymer (Bipol 6B) from now on.
Notes:	Worked! BSM better than YGM. Remade standards 11/25/95

Filenames:	111795JR, 112795.ALL, EXE, SUM or variants. VIDEP.PPT

Experiment Date:	11/16/95
Embedding Date:	11/14/95
Organism(s):	G4 (phenol)
Growth Medium:	YGM
Type of Experiment:	Benzene removal by foam & slurry. BSM vs YGM
Slurry Description:	G4 2.4% in BSM, 2.2% in YGM
Foam #, Description:	73 G4/BSM, 74 G4/YGM
Notes:	
Filenames:	112095JR.ALL, EXE, SUM or variants

Experiment Date:	12/5/95 - 12/6/95
Embedding Date:	12/5/95
Organism(s):	G4 (phenol)
Growth Medium:	YGM
Type of Experiment:	TCE removal by foam & slurry. Induced w/ phenol, benzene after embedding (foam) or slurry prep (slurry). Uninduced controls. TCE, viability 12/5/95. Washout 12/6/95.
Slurry Description:	G4 5.8% in BSM
Foam #, Description:	75 BSM, 76 G4
Notes:	Contaminated. Don't use.
Filenames:	120795JR.ALL, EXE, SUM or variants.

Experiment Date:	12/13/95
Embedding Date:	12/13/95
Organism(s):	G4 (phenol or benzene)
Growth Medium:	YGM
Type of Experiment:	Repeat previous exp't
Slurry Description:	G4 8.9% in BSM
Foam #, Description:	77 BSM, 78 G4 (cups mislabeled 96, 97)
Notes:	Slurry made previous day.
Filenames:	121595JR.ALL, EXE, SUM or variants. VIDEO.PPT, VIG41213.XLS

Experiment Date:	12/19/95
Embedding Date:	12/19/95
Organism(s):	G4 (phenol added to slurry)
Growth Medium:	YGM
Type of Experiment:	Time course TCE removal
Slurry Description:	G4 in BSM, 5.6% induced, 5.5% uninduced
Foam #, Description:	79 BSM, 80 induced G4, 81 uninduced G4
Notes:	Induced in slurry form. Uninduced controls. Slurry made previous day.
Filenames:	122095JR.ALL, EXE, SUM or variants.

Experiment Date:	1/5/96
Embedding Date:	1/5/96
Organism(s):	G4 (phenol added to cultures)
Growth Medium:	YGM
Type of Experiment:	Ribosomal probes/growth stimulation
Slurry Description:	5.5% G4 in BSM
Foam #, Description:	82 BSM, 83 G4
Notes:	
Filenames:	NA

Experiment Date:	1/11/95 - 1/12/96
Embedding Date:	1/11/96
Organism(s):	G4 (uninduced)
Growth Medium:	YGM
Type of Experiment:	Benzene removal by foam & slurry 1/11/95 Viability 1/12/96
Slurry Description:	G4 5.0% in BSM
Foam #, Description:	84 BSM control, 85 G4
Notes:	
Filenames:	011696B.SUM, ALL, EXE, VIDEO.PPT

Experiment Date:	1/18/95
Embedding Date:	1/18/96
Organism(s):	G4 (induced in culture)
Growth Medium:	YGM
Type of Experiment:	TCE removal, spiked landfill water
Slurry Description:	G4 5.1% in BSM
Foam #, Description:	86 BSM control, 87 G4
Notes:	3 TCE levels, spiked BSM controls
Filenames:	012196JR.SUM, ALL, EXE. VIDEO.PPT

Experiment Date:	1/25/95
Embedding Date:	1/25/96
Organism(s):	G4 (induced in culture)
Growth Medium:	YGM
Type of Experiment:	TCE removal, M area water
Slurry Description:	G4 5.2% in BSM
Foam #, Description:	CHECK NUMBERS
Notes:	3 wells, spiked BSM controls
Filenames:	012896JR.SUM, ALL, EXE. VIDEO.PPT

Experiment Date:	2/6/96
Embedding Date:	2/6/96
Organism(s):	G4 (uninduced), PR131
Growth Medium:	YGM
Type of Experiment:	Viability, respirometry
Slurry Description:	4.2% G4, 4.6% PR131 in BSM
Foam #, Description:	117 BSM, 118 G4, 119 PR131
Notes:	
Filenames:	VIAB2_4.XLS (wrong date in name)

Experiment Date:	2/16/96
Embedding Date:	2/16/96
Organism(s):	G4 (phenol-induced in culture)
Growth Medium:	YGM
Type of Experiment:	Eff. slurry density, C source on TCE removal
Slurry Description:	5.1% and 2.5% (nominal) G4 in BSM
Foam #, Description:	120 BSM, 121 G4 (hi), 122 G4 (lo)
Notes:	Compared BSM with BSM + 0.1 g/l glucose, 0.05 g/l YE
Filenames:	0220SUMB.XLS

Experiment Date:	2/19/96
Embedding Date:	2/19/96
Organism(s):	G4 (phenol-induced in culture) Old material from 2/16.
Growth Medium:	YGM
Type of Experiment:	Duration of induction (used same foam as last time)
Slurry Description:	5.1% and 2.5% (nominal) G4 in BSM
Foam #, Description:	120 BSM, 121 G4 (hi), 122 G4 (lo)
Notes:	Compared BSM with BSM + 0.1 g/l glucose, 0.05 g/l YE
Filenames:	022696J.ALL, EXE, SUM, variants

Experiment Date:	2/23/96
Embedding Date:	2/23/96
Organism(s):	G4 (induced after embedding)
Growth Medium:	YGM
Type of Experiment:	Induction after embedding
Slurry Description:	5.5% G4 in BSM
Foam #, Description:	123 BSM, 124 G4
Notes:	Overnight vs 4 h induction
Filenames:	022796J.ALL, EXE, SUM, variants

APPENDIX 8

DEGRADATION OF TRICHLOROETHYLENE AND BENZENE BY EMBEDDED BACTERIA

Appendix 8.1 Characteristics of M Area Groundwaters

WELL MSB 25A

MEASUREMENTS CONDUCTED IN THE FIELD

Sample date: 01/27/95
Depth to water: 148.41 ft (45.24 m) below TOC
Water elevation: 217.99 ft (66.44 m) msl
Sp. conductance: 23 µS/cm
Turbidity: 1 NTU
Water evacuated before sampling: 140 gal

Time: 14:14
pH: 5.4
Alkalinity: 1 mg/L
Water temperature: 19.7 °C
Air temperature: 13.1°C

LABORATORY ANALYSES

F	Analyte	Result	Moc	Unit	Lab
0	Aluminum, total recoverable	<33		µg/L	GE
0	Arsenic, total recoverable	<3.3		µg/L	GE
0	Barium, total recoverable	5.6		µg/L	GE
0	Benzene	<84	/O	µg/L	GE
0	Bromodichloromethane	<84	/O	µg/L	GE
0	Bromoform	<84	/O	µg/L	GE
0	Bromomethane	<84	/O	µg/L	GE
0	Cadmium, total recoverable	<3.3		µg/L	GE
0	Carbon tetrachloride	<84	/O	µg/L	GE
0	Chlorobenzene	<84	/O	µg/L	GE
0	Chloroethane	<84	/O	µg/L	GE
0	Chloroethene (Vinyl chloride)	<84	/O	µg/L	GE
0	2-Chloroethyl vinyl ether	<84	/O	µg/L	GE
0	Chloroform	<84	/O	µg/L	GE
0	Chloromethane	<84	/O	µg/L	GE
0	Chromium, total recoverable	<6.7		µg/L	GE
0	Cyanide	<8.3		µg/L	GE
0	Cyanide	<8.3	J/O/L	µg/L	GE
0	Dibromochloromethane	<84	/O	µg/L	GE
0	1,1-Dichloroethane	<84	/O	µg/L	GE
0	1,2-Dichloroethane	<84	/O	µg/L	GE
0	1,1-Dichloroethylene	<84	/O	µg/L	GE
0	trans-1,2-Dichloroethylene	<84	/O	µg/L	GE
0	Dichloromethane	<84	/O	µg/L	GE
0	1,2-Dichloropropane	<84	/O	µg/L	GE
0	cis-1,3-Dichloropropene	<84	/O	µg/L	GE
0	trans-1,3-Dichloropropene	<84		µg/L	GE
0	Ethylbenzene	<84	/O	µg/L	GE
0	Iron, total recoverable	4.3	J/E	µg/L	GE
0	Lead, total recoverable	<5.0		µg/L	GE
0	Lithium, total recoverable	<8.3		µg/L	GE
0	Manganese, total recoverable	<3.3		µg/L	GE
0	Mercury, total recoverable	<0.33		µg/L	GE
0	Nickel, total recoverable	<6.7		µg/L	GE
0	Nitrate-nitrite as nitrogen	1,600		µg/L	GE
0	Selenium, total recoverable	<3.3		µg/L	GE
0	Silver, total recoverable	<3.3	/I	µg/L	GE
0	Sulfate	1,270	J/E	µg/L	GE
0	1,1,2,2-Tetrachloroethane	<84	/O	µg/L	GE
2	Tetrachloroethylene	205	J/O/H	µg/L	GE
0	Toluene	<84	/O	µg/L	GE
0	Total organic carbon	<1,670		µg/L	GE
2	Total organic halogens	68		µg/L	GE
0	1,1,1-Trichloroethane	<84	/O	µg/L	GE
0	1,1,2-Trichloroethane	<84	/O	µg/L	GE
2	Trichloroethylene	1,720	J/O/H	µg/L	GE
0	Trichlorofluoromethane	<84	/O	µg/L	GE
0	Zinc, total recoverable	<3.3		µg/L	GE
0	Gross alpha	1.1E-09±5.4E-10		µCi/mL	GP
0	Nonvolatile beta	9.8E-10±6.8E-10	UI	µCi/mL	GP
0	Radium, total alpha-emitting	4.0E-10±4.0E-10		µCi/mL	GP
0	Tritium	1.3E-06±4.2E-07		µCi/mL	GP

WELL MSB 75B

MEASUREMENTS CONDUCTED IN THE FIELD

Sample date: 01/20/95
Depth to water: 115.79 ft (35.29 m) below TOC
Water elevation: 210.91 ft (64.29 m) msl
Sp. conductance: 61 µS/cm
Turbidity: 7 NTU
Water evacuated before sampling: 110 gal

Time: 14:17
pH: 6.2
Alkalinity: 11 mg/L
Water temperature: 18.6°C
Air temperature: 9.1°C

LABORATORY ANALYSES

F	Analyte	Result	Mod	Unit	Lab
1	Aluminum, total recoverable	44		µg/L	GE
0	Arsenic, total recoverable	<3.3		µg/L	GE
0	Barium, total recoverable	24		µg/L	GE
0	Benzene	<8.4		µg/L	GE
0	Bromodichloromethane	<8.4		µg/L	GE
0	Bromoform	<8.4		µg/L	GE
0	Bromomethane	<8.4		µg/L	GE
0	Cadmium, total recoverable	<3.3		µg/L	GE
0	Carbon tetrachloride	<8.4		µg/L	GE
0	Chlorobenzene	<8.4		µg/L	GE
0	Chloroethane	<8.4		µg/L	GE
0	Chloroethene (Vinyl chloride)	<8.4		µg/L	GE
0	2-Chloroethyl vinyl ether	<8.4		µg/L	GE
0	Chloroform	<8.4		µg/L	GE
0	Chloromethane	<8.4		µg/L	GE
0	Chromium, total recoverable	<6.7		µg/L	GE
0	Cyanide	<8.3	J/O/L	µg/L	GE
0	Dibromochloromethane	<8.4		µg/L	GE
0	1,1-Dichloroethane	<8.4		µg/L	GE
0	1,2-Dichloroethane	<8.4		µg/L	GE
0	1,1-Dichloroethylene	<8.4		µg/L	GE
0	trans-1,2-Dichloroethylene	<8.4		µg/L	GE
0	Dichloromethane	<8.4		µg/L	GE
0	1,2-Dichloropropane	<8.4		µg/L	GE
0	cis-1,3-Dichloropropene	<8.4		µg/L	GE
0	trans-1,3-Dichloropropene	<8.4		µg/L	GE
0	Ethylbenzene	<8.4		µg/L	GE
0	Iron, total recoverable	49		µg/L	GE
0	Lead, total recoverable	<5.0		µg/L	GE
0	Lithium, total recoverable	<8.3		µg/L	GE
1	Manganese, total recoverable	27		µg/L	GE
0	Mercury, total recoverable	<0.33		µg/L	GE
0	Nickel, total recoverable	<6.7		µg/L	GE
0	Nitrate-nitrite as nitrogen	3,420		µg/L	GE
0	Phenols	<8.3		µg/L	GE
0	Selenium, total recoverable	<3.3		µg/L	GE
0	Silver, total recoverable	<3.3		µg/L	GE
0	Sulfate	3,000		µg/L	GE
0	1,1,2,2-Tetrachloroethane	<8.4		µg/L	GE
0	Tetrachloroethylene	<8.4		µg/L	GE
0	Toluene	<8.4		µg/L	GE
0	Total organic carbon	<1,670		µg/L	GE
2	Total organic halogens	62	/I	µg/L	GE
0	1,1,1-Trichloroethane	<8.4		µg/L	GE
0	1,1,2-Trichloroethane	<8.4		µg/L	GE
2	Trichloroethylene	1,110		µg/L	GE
0	Trichlorofluoromethane	<8.4		µg/L	GE
0	Zinc, total recoverable	3.0	J/E	µg/L	GE
0	Gross alpha	1.5E-09±7.3E-10		µCi/mL	GP
0	Nonvolatile beta	2.4E-09±9.3E-10		µCi/mL	GP
0	Radium, total alpha-emitting	1.1E-09±6.0E-10		µCi/mL	GP
0	Tritium	1.2E-07±3.5E-07	UI	µCi/mL	GP

WELL MSB 34A

MEASUREMENTS CONDUCTED IN THE FIELD

Sample date: 01/20/95
Depth to water: 164.18 ft (50.04 m) below TOC
Water elevation: 219.82 ft (67.00 m) msl
Sp. conductance: 20 µS/cm
Turbidity: 1 NTU
Water evacuated before sampling: 182 gal

Time: 9:41
pH: 5.6
Alkalinity: 1 mg/L
Water temperature: 19.2°C
Air temperature: 7.1°C

LABORATORY ANALYSES

F	Analyte	Result	Mod	Unit	Lab
0	Aluminum, total recoverable	23	J/E	µg/L	GE
0	Arsenic, total recoverable	<3.3		µg/L	GE
0	Barium, total recoverable	5.9		µg/L	GE
0	Benzene	<167		µg/L	GE
0	Bromodichloromethane	<167		µg/L	GE
0	Bromoform	<167		µg/L	GE
0	Bromomethane	<167		µg/L	GE
0	Cadmium, total recoverable	<3.3		µg/L	GE
0	Carbon tetrachloride	<167		µg/L	GE
0	Chlorobenzene	<167		µg/L	GE
0	Chloroethane	<167		µg/L	GE
0	Chloroethene (Vinyl chloride)	<167		µg/L	GE
0	2-Chloroethyl vinyl ether	<167		µg/L	GE
0	Chloroform	<167		µg/L	GE
0	Chloromethane	<167		µg/L	GE
0	Chromium, total recoverable	<6.7		µg/L	GE
0	Cyanide	<8.3	J/QL	µg/L	GE
0	Dibromochloromethane	<167		µg/L	GE
0	1,1-Dichloroethane	<167		µg/L	GE
0	1,2-Dichloroethane	<167		µg/L	GE
0	1,1-Dichloroethylene	<167		µg/L	GE
0	trans-1,2-Dichloroethylene	<167		µg/L	GE
0	Dichloromethane	<167		µg/L	GE
0	1,2-Dichloropropane	<167		µg/L	GE
0	cis-1,3-Dichloropropene	<167		µg/L	GE
0	trans-1,3-Dichloropropene	<167		µg/L	GE
0	Ethylbenzene	<167		µg/L	GE
0	Iron, total recoverable	8.2		µg/L	GE
0	Lead, total recoverable	<5.0		µg/L	GE
0	Lithium, total recoverable	<8.3		µg/L	GE
0	Manganese, total recoverable	2.8	J/E	µg/L	GE
0	Mercury, total recoverable	<0.33		µg/L	GE
0	Nickel, total recoverable	<6.7		µg/L	GE
0	Nitrate-nitrite as nitrogen	917		µg/L	GE
0	Phenols	<8.3		µg/L	GE
0	Selenium, total recoverable	<3.3		µg/L	GE
0	Silver, total recoverable	<3.3		µg/L	GE
0	Sulfate	1,390	J/E	µg/L	GE
0	1,1,2,2-Tetrachloroethane	<167		µg/L	GE
0	Tetrachloroethylene	<167		µg/L	GE
0	Toluene	<167		µg/L	GE
0	Total organic carbon	<1,670		µg/L	GE
0	Total organic carbon	<1,670		µg/L	GE
2	Total organic halogens	537	/I	µg/L	GE
0	1,1,1-Trichloroethane	<167		µg/L	GE
0	1,1,2-Trichloroethane	<167		µg/L	GE
2	Trichloroethylene	2,720		µg/L	GE
0	Trichlorofluoromethane	<167		µg/L	GE
0	Zinc, total recoverable	64		µg/L	GE
0	Gross alpha	7.7E-10 ± 5.3E-10		µCi/mL	GP
0	Nonvolatile beta	-1.4E-10 ± 6.7E-10	UI	µCi/mL	GP
0	Radium, total alpha-emitting	7.0E-10 ± 5.0E-10		µCi/mL	GP
0	Tritium	-1.1E-07 ± 3.4E-07	UI	µCi/mL	GP