ADDENDUM

BLASLAND & BOUCK ENGINEERS, P.C.

HOUSATONIC RIVER STUDY

135 Day Interim Report

ASSESSMENT OF REMEDIAL ALTERNATIVES

General Electric Company
Pittsfield, Massachusetts

September 1986

ADDENDUM

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135-DAY INTERIM REPORT
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GENERAL ELECTRIC COMPANY
PITTSFIELD, MASSACHUSETTS

SEPTEMBER, 1986

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ACKNOWLEDGMENT

This Report has been prepared for the General Electric Company by Blasland & Bouck Engineers, P.C., with the assistance of the Corporate Research & Development Group of General Electric Company, as well as Dravo Van Houten, Inc.

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SECTION 1 - GENERAL AND BACKGROUND

1.1 General Background Information

In accordance with the 1981 Consent Order with the USEPA and the State of Massachusetts, the General Electric Company has been performing extensive study efforts associated with PCB contamination of the Housatonic River. In May of 1985, a report on this subject entitled "135-Day Interim Report, Housatonic River Study, Assessment of Remedial Alternatives", was prepared by Blasland & Bouck Engineers, P.C. for the General Electric Company (Reference 1). This report culminated a series of previous reports by Blasland & Bouck (and others) which presented information on the various remedial alternatives for the PCB contamination associated with the Housatonic River. The recommendations of the 135-Day Report included continued research into the applicability of PCB biodegradation and implementation of the Flow and Sedimentation Control Pilot Study.

On May 23, 1986, the USEPA and DEQE provided concurrent conditional approval of the 135-Day Report with specific requests for additional information. Although some of the requested information has been provided previously (Reference 2), this report presents the specific information which the USEPA and DEQE requested to be provided within 90 days of issuance of their letter. A previous submittal on June 23, 1986 provided the USEPA and DEQE with information which had been requested within 30 days of issuance of their letter.

1.2 Purpose of this Report

As discussed above, this report presents information to supplement the 135-Day Report (as well as the previous reports) as requested by the USEPA and DEQE regarding the following subjects:

1. Additional assessment of "wet dredging" techniques;

2. Five-year plan for biodegradation research; and

3. Confirmatory sediment resampling plan.

The specific requests by the EPA and DEQE in their May 23, 1986 letter for additional information was as follows:

1. "Within ninety (90) days of receipt of this letter, General Electric shall submit to EPA for review and approval, a report which examines sediment removal techniques other than that described in the 135-day Report. At a minimum, the report shall include an analysis of hydraulic wet dredging of the Woods Pond area as well as alternative sediment transportation techniques including, but not limited to, railroads and pumping."

2. "Within ninety (90) days of receipt of this letter, General Electric shall submit to EPA for review and approval, a five year plan, including implementation schedule, for the study of the Biodegradation alternative. This plan shall list all relevant milestones and anticipated achievement dates, identify anticipated problem areas and provide for the submission of ninety day progress reports to EPA."

3. "Within ninety (90) days of receipt of this letter, General Electric shall submit to EPA for review and approval, a plan for selective, representative resampling of the Housatonic River sediments to confirm the present location, concentration, depth and volume of the majority of the PCB contamination."

Each of these subjects is discussed within the text of this report, including specific referenced work by others which is presented in the Appendices.

SECTION 2 - ADDITIONAL ASSESSMENT OF SEDIMENT REMOVAL TECHNIQUES

2.1 General

Several previous reports (References 1, 2 and 3) have included assessment of sediment dredging techniques for PCB-contaminated sediments. This section will address additional information requested by the EPA and DEQE on wet dredging techniques and their applicability for use in the Woods Pond area of the Housatonic River.

2.2 Summary of Previous Dredging Assessment

The reports referenced above have assessed the ability of various dredging techniques to remove contaminated sediments from the Housatonic River. Initially, all possible types of dredging equipment and procedures were assessed for potential application; however, during further development it became clear that each of the wet dredging techniques allowed for release of some quantity of sediments into the water column and therefore allowed for PCB transport downstream. Several of the wet dredging techniques included preventive steps to minimize concern over sediment transport; however, despite the control techniques used, each wet dredging technique allowed for some percentage of sediment loss downstream. As this condition was not considered acceptable, the focus of attention was placed on dry dredging, which could be performed with significantly greater control, both in terms of minimizing sediment re-suspension and with respect to accuracy of the dredging activity. The dry dredging techniques, however, present unfavorable environmental impacts of a different nature; specifically, those associated with the dewatering of the river system.

It should be noted that wet dredging techniques were never completely eliminated from consideration as it was not considered possible to dewater all areas of the river system for dry excavation (e.g. the main river channel itself). Likewise, it is not possible to dredge all areas using wet dredging techniques as some of the backwater areas are very shallow during portions of the year. In fact, some areas may even be dry during low water periods.

As noted in previous communications, implementation of the sediment removal alternative is less dependent upon sediment removal techniques than it is upon the selection of an ultimate sediment disposal location. As such, refinement of potential dredging apparatus is important but is not foremost in moving this alternative to a level of acceptance.

2.3 Summary of Additional Dredging Assessment

General Electric Company contracted with Dravo Van Houten, Inc. of New York, New York (Dravo) to provide additional assessment of the wet dredging technique. Dravo is an internationally-recognized expert in the area of sediment dredging and has previously provided expertise in this area for use in preparation of the 45-Day and 135-Day Reports. Dravo was asked to update their earlier assessment of dredging techniques and to provide an up-to-date assessment of the potential application of wet dredging techniques. Their report on this subject is presented in Appendix 1 and is summarized below.

As part of their effort, Dravo re-evaluated the potential application of mechanical dredging, hydraulic dredging, and of specialty dredges such as the Mudcat dredge. Each were evaluated with respect to experience of use in

similar situations, and capability with respect to use in the Housatonic River and to loss of sediments downstream. Dravo's conclusions regarding the potential application of mechanical and hydraulic dredges reinforces our previous understanding that use of this equipment is not appropriate due to significant sediment losses associated with the equipment. Dravo does conclude that several specialty dredges have seen limited use in similar situations with some degree of success; however, the experience base is very limited.

In addition, Dravo confirms that none of the dredging techniques are without problems, as all of the techniques allow for some degree of sediment loss downstream. For instance, studies have shown that turbidity at the Mudcat dredge head ranges between 100-200 mg/l and the plume dissipates approximately 6 meters from the head. Taking a conservative approach and utilizing the lower turbidity level of 100 mg/l, this translates to a minimum of 45,000 pounds of re-suspended sediments that would be available for transport during dredging activities in the Woods Pond area. The actual quantity of re-suspended material that will be carried downstream will depend primarily on the river currents, their velocity and direction at the time of actual work. The concern associated with this significant quantity of re-suspended sediment is that it results in the opportunity for PCBs to be transported downstream.

An additional concern noted by Dravo is the depth of water in which dredges can operate. Typical dredging projects have involved navigational harbors, lakes, or rivers of considerable depths, with the point of dredging being to remove accumulated sediments and prevent interference with the deep draft of large boats. In the case of the Housatonic River, over half the areas containing PCB-contaminated sediments have a water depth of less than

12 inches. In the Woods Pond area itself, approximately 15 percent of the area containing PCB-contaminated sediments have a water depth of less than 12 inches. Therefore, it is difficult to apply much of the existing experience data-base to this river system. A small number of shallow-depth dredging programs have been performed with specialty dredges on which additional buoyancy has been added. However, these projects have essentially been limited to industrial lagoons, or ponds in which sediment re-suspension and transport have not been studied.

A secondary concern related to the question of shallow water depth is the maneuverability of the dredging equipment. Deep water dredges are typically self-propelled or positioned using tug boats. Shallow water dredges are maneuvered from shore using a system of overhead cables and winches. Maneuverability of shallow dredges which have been modified to allow for increased buoyancy create additional problems. At a minimum, the shoreline on both sides of the river/backwater system in shallow areas will need to be disturbed to allow for access of cable/winch supports. This will probably require temporary roadways on each side of the shallow sections of the river system to allow for such access.

2.4 Additional Assessment of Sediment Transportation Techniques

Transportation of contaminated materials removed from the Housatonic River can be accomplished by one or a combination of the following techniques:

1. Trucks;
2. Railroads;
3. Barges; and
4. Pipelines.

Each transportation mode has advantages and disadvantages as a result of the removal technique utilized and the location of the ultimate disposal facility. It should be noted that a detailed assessment of the available transportation techniques cannot be conducted until the required remedial action for this site has been identified and the final disposal site(s) is selected. Therefore, a general discussion of each of the above-mentioned transportation modes (except truck transport which was addressed in the 45-Day Interim Report) is presented below.

Railroad

The existing railroad system (previously owned by Penn Central) that serves Pittsfield from the south is well situated for use if the selected disposal site is located along the river. This rail line could be used to transport sediments from various discharge stations along the river to the disposal site, provided a use agreement can be reached with Conrail.

Sediments transported in a "wet" condition would require the use of rail tankers to transport the sediments to the chosen disposal location for dewatering, while "dry" or dewatered sediments could be transported via hopper or box cars. The greatest restrictions associated with rail transport are the proximity to the chosen disposal site and scheduling use on the Conrail tracks. Should one of the four disposal sites identified in the 45-Day Interim Report be chosen as the disposal location, rail transport may provide a viable mode of transportation, providing an equitable agreement with Conrail can be reached.

Barges

Barges are commonly used to transport bulk materials in open waters such as harbors, lakes and river mouths. The Housatonic River is characterized by shallow backwaters and a relatively narrow main channel and therefore may not provide the needed depths for their use.

Due to the relatively shallow nature of the river, small barges with relatively limited capacities would have to be utilized. In addition, only a small number (perhaps as low as one to two) of barges could operate and maneuver in the main channel at a time. However, barges may be used to supplement another form of transportation to carry sediments to a disposal facility that is located along the river, such as the Woods Pond or Willow Creek Backwater sites previously identified.

Pipelines

The pumping system for delivering sediments from the dredge site to the disposal site would be dependent upon the disposal site selection. In this case, a liquid slurry of sediments and river water would be pumped from various points on the river through a pipeline or network of pipelines to the disposal site(s). Due to head loss considerations, booster pump stations may be needed to pump the slurry either to a higher elevation or a farther distance. However, there are practical limits to their use over great distances and this will most likely govern their applicability.

The pipelines would be conventionally located above ground (rather than underground) and would be supported by a temporary support system. Road, utility, and railroad crossings would require special treatment, possibly including placement of the pipelines either underground or overhead.

An alternative approach to the above discussion would be to pump the liquid sediment slurry from various points on the river to one or more dewatering facilities located in close proximity to the river. At these dewatering facilities, sediments would be allowed to drain of water, or would be mechanically "squeezed" to allow for the drying of the sediments. The outcome of this dewatering phase would be a drier sediment which could then be transported by rail or truck to the ultimate disposal location. Regardless of the transportation technique used, this sediment would have to be dewatered prior to placement in a disposal site, as EPA regulations restrict the placement of wet or "liquid" materials containing PCBs into landfills.

Finally, liquid sediment slurry could be pumped from the river directly into either tanker trucks or railroad tank cars for transportation to the ultimate disposal site. Since this method would result in transporting a greater quantity of river water rather than sediment, the number of tank trucks or railroad tankers needed to complete the project would be 3 to 10 times greater than transporting dried sediments.

In addition to identifying the various transportation alternatives available and their suitability to this project, a discussion of the probability of accident and leakage potential is warranted. These concerns should be incorporated into the detailed assessment of sediment transport alternatives once an appropriate sediment disposal site is identified. The potential for loss of sediment material from each mode of transportation is briefly reviewed below.

Pipelines, due to the nature of their construction, probably provide the greatest potential for leakage and loss of material. Every joint along the pipeline (normally every 10-20 feet, depending on the size of the line) is a potential route available for loss of material. Construction of the pipeline

above ground does provide for visual inspection of the line on a regular basis, but also provides the vulnerability to accidental damage or vandalism. Since the sediment materials are being pumped and the system is under pressure, if a break in the line were to occur, the material upgradient in the line would be free to "drain" from the pipe. Although protective measures (such as valving, etc.) would be taken to minimize pipeline leaks, it is most likely that some leaks will occur over the course of the dredging program. It should also be noted that leaks of contaminated sediment in a liquid slurry form would be much more difficult to recover than if this sediment were dewatered prior to transport.

Similar concerns noted above for leakage from pipelines would be associated with the transport of liquid sediment slurry in trucks or tankers. In this case, accidents involving tank trucks or railroad tankers could result in leakage and the release of the liquid slurry of PCB-contaminated sediments. Should this type of accident occur, the clean-up activities required would be much more extensive than if the sediments had been dewatered prior to transport.

The National Highway Traffic Safety Administration Statistics Department reports that individuals traveling in cars and trucks are involved in police reported accidents at an average rate of once every sixteen years. This corresponds to a probability of 6.25% on an annual basis that an individual will be involved in an accident.

Similar statistics are not available for rail accidents; however, for 1985 there were 39 rail accidents reported in the Commonwealth of Massachusetts. Nationally, 1,849 accidents were reported in 1984 for rail carriers transporting hazardous materials.

Since the vehicle used and the distance traveled for the transport of sediments varies based on the ultimate location of a disposal site, the potential number of accidents cannot be specifically calculated, but statistics indicate the likelihood that an accident may occur during the course of the project.

SECTION 3 - ADDITIONAL INFORMATION REGARDING BIODEGRADATION

3.1 General

As stated in the 135-Day Interim Report, considerable research in the area of PCB biodegradation was scheduled over a five-year period in parallel with the Flow and Sedimentation Control Pilot Study. During the past year, considerable effort by the General Electric Company, Research & Development Group, as well as independent research financed by General Electric, has been performed. A report on the status of this research was presented to the EPA and the State of Massachusetts in June, 1986. A copy of this report is presented in Appendix 2.

In response to the specific request for additional information by the USEPA, this Section has been prepared in an attempt to put forth the scope of the remaining efforts associated with the five-year plan.

Although it is very difficult to forecast the achievement of milestones in the area of biological research since submittal of the 135-Day Report, General Electric Company has performed a number of efforts which allow for some estimate of a five-year implementation schedule.

Research associated with the biodegradation alternative for PCB removal in the river system has been categorized into seven areas, namely:

I. Biochemistry of PCB Degradation;

II. Genetic Studies;

III. Reductive Dechlorination in Anaerobic Microbial Communities;

IV. Process Modeling: Aerobic Biodegradation;

V. Process Modeling: Surfactant Extraction from Soil;

VI. Development of a Biological Process; and

VII. Environmental Transformations.

The results of the past year's research activities in each of these areas and the status of this research are summarized below.

3.2 Summary and Status of Research Efforts

Biochemistry of PCB Degradation - The work conducted thus far has successfully identified three naturally-occurring bacterial species that are capable of degrading various forms of PCBs. Work is proceeding on identification of the biomechanisms or pathways by which this degradation takes place through identification of the enzymes involved. Work for this task is expected to require on the order of two to three additional years for completion.

Genetic Studies - Concurrent with the above work, the various bacterial genes responsible for degradation of PCBs are being isolated and identified. Such research can lead to improving the capability for bacteria to degrade more complex PCB formations in shorter periods of time. Work on this task is expected to extend for five years.

Reductive Dechlorination of PCBs in Anaerobic Microbial Communities - Work performed under this task is particularly appropriate in consideration of the quantity of sediments in the Housatonic River system that are likely to be in an anaerobic condition, as well as the general ability of anaerobic bacteria to degrade more complex PCBs. Concurrent with the above tasks, work has been initiated on the use of Hudson River sediments to demonstrate dechlorination in anaerobic lab cultures. Owing to the complexity of characterizing the biochemistry involved, it is anticipated that this work will require a period beyond five years to complete. Completion of intermediate goals should contribute to the success of this research.

Process Modeling: Aerobic Biodegradation of PCBs - Concurrent with the above work, the capability of bacteria to degrade soil-bound PCBs is being optimized. These tests are being conducted in the laboratory, under simulated field conditions. Such conditions are varied to reflect variable soil types and moisture conditions. This work is expected to require on the order of four years for completion.

Process Modeling: Surfactant Extraction of PCBs from Soil - This work is not specifically related to the in-situ degradation of PCBs, but rather the washing or removal of PCBs from soil after it has been removed from an aqueous environment. This research can be extremely complicated in that it represents the effect of physical, chemical and biological mechanisms. Work on this task is expected to require in excess of five years, although intermediate goals will contribute to further understanding this mechanism.

Development of a Biological Process for PCB Removal - This work involves the development of a biological reactor to assess all of the factors described above for the degradation of PCBs. The reactor will include aerobic and anaerobic degradation and will incorporate surfactant influence, if appropriate. The reactor will be used to simulate both a treatment unit for PCB degradation, as well as a means of representing mechanisms currently occurring in a river system.

Environmental Transformations - This task is essentially a summary of the work performed during this research effort, as well as research performed elsewhere. It represents an ongoing accumulation of knowledge to determine the various means of PCB transformation.

3.3 Implementation Schedule and Problem Areas

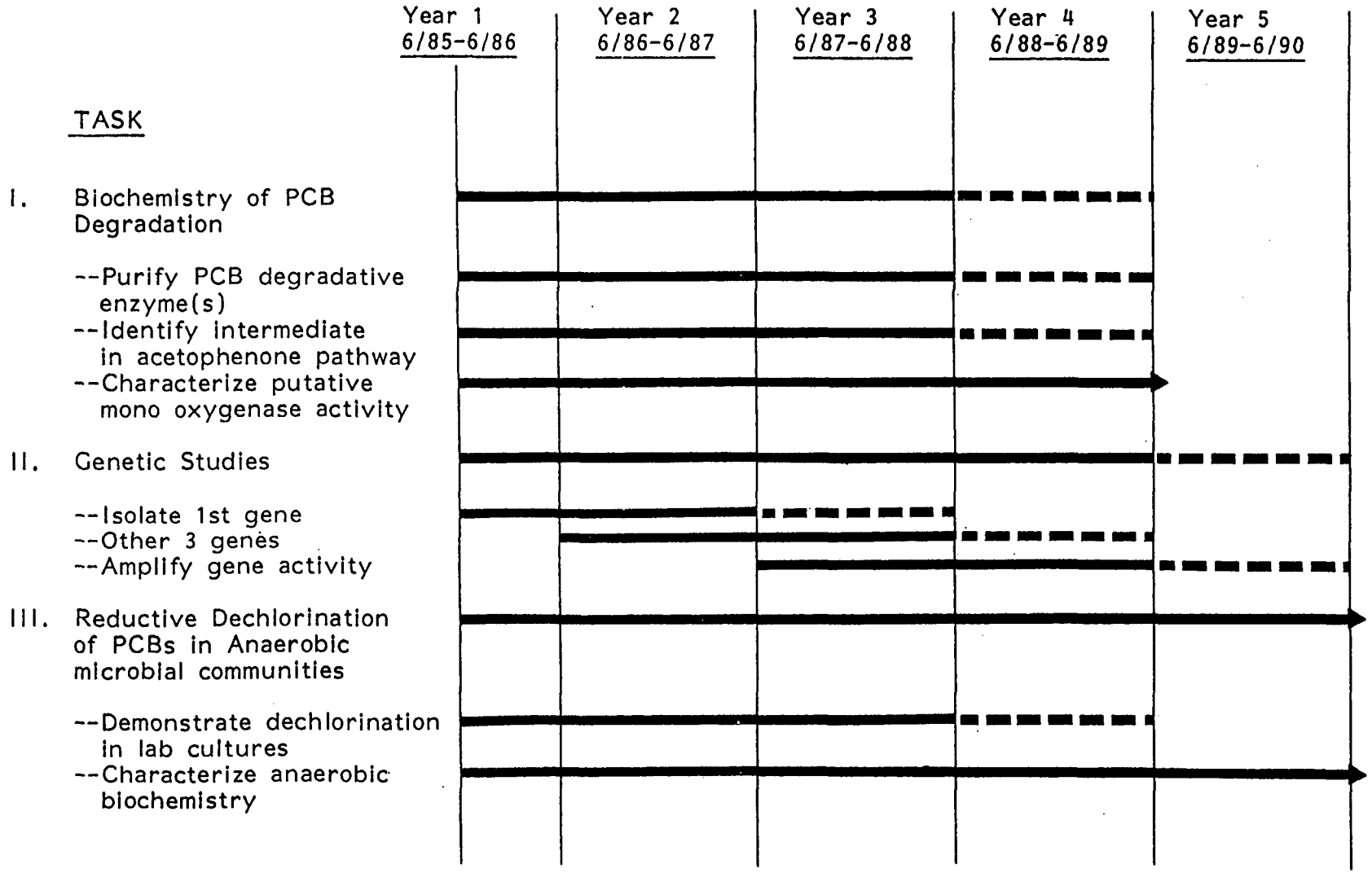
The attached table presents a summary of the scheduled research activities over the next four years. It must be stressed that since much of the research is original and without precedent, completion of many of the tasks are extremely difficult to predict and will be subject to change. As of this Report, progress has been encouraging. It should be noted that solid lines on the attached table represent optimistic time frames, while dotted lines represent more realistic timeframes. Arrows on the attached table indicate time requirements beyond five years.

Potential problem areas associated with the research activities can be classified into concerns related to anaerobic and aerobic processes. Anaerobic research is generally much more difficult owing to problems in growing and maintaining anaerobic bacteria. In addition, anaerobic degradation, while having greater capability with more complex compounds, may occur at a much slower rate than aerobic degradation. The area of aerobic research that may pose problems is in initiating genetic activity in laboratory strains to demonstrate more effective pathways of PCB degradation.

As noted above, it is difficult to predict the schedule of achieving milestones in the research activities. We have attempted to indicate milestones on the attached table; however, the schedule, as presented, is only the best current estimate, subject to change as research conditions change.

To keep the involved regulatory agencies up-to-date on the status of the research, General Electric Company proposes to continue with the annual submittal of formal reports, with project status meetings to be held every six months.

IMPLEMENTATION SCHEDULE
BIODEGRADATION ALTERNATIVE FOR
HOUSATONIC RIVER STUDY



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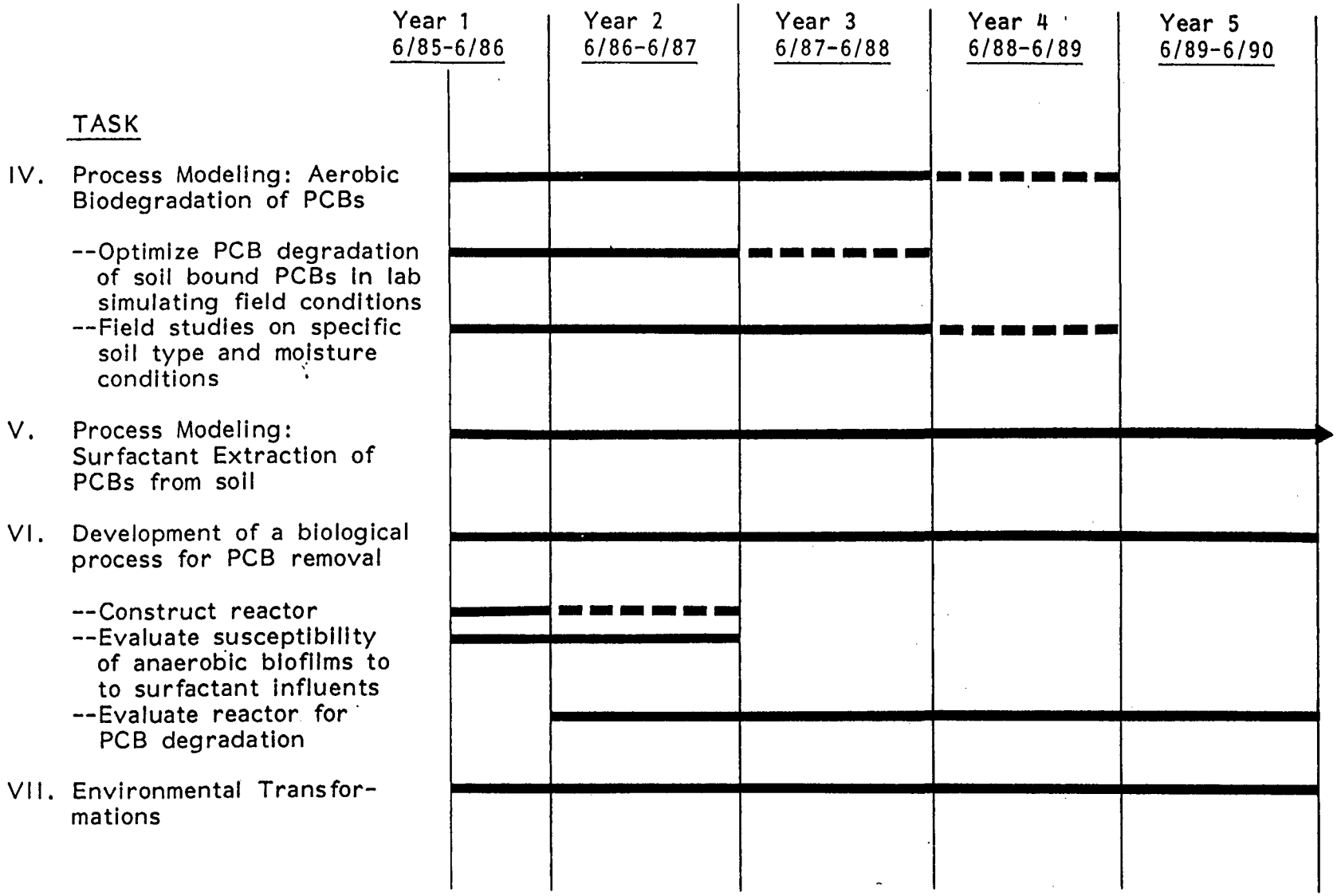

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SECTION 4 - PROPOSED RESAMPLING PLAN TO ASSESS PCB LOCATIONS

4.1 General

A significant amount of effort has been previously performed to identify the location, characteristics, and PCB concentration of sediments in the Housatonic River. This work was principally performed by Stewart Laboratories, Inc. under contract to the General Electric Company during 1980 and 1982. This work has documented the locations of PCB-contaminated sediments, which in turn has provided a basis for assessment of remedial alternatives. Since the sediment sampling and analysis work was performed four to six years ago, there has been concern raised as to whether the current PCB locations and characteristics represent the information provided earlier. For this reason, it has been proposed that a representative resampling program be performed to assure that the previous results are still applicable.

4.2 Summary of Previous Study

An extensive amount of PCB sampling analysis work had been performed during 1980 and 1982 by Stewart Laboratories, Inc. (Reference 4). This effort has documented the quantity, locations and concentrations of PCBs in sediments of the Housatonic River, as well as the PCB transport mechanisms present. During this two-year study, a total of approximately 900 samples were collected from the Housatonic River. The results of the sampling indicated that 90% of the PCBs present in the Housatonic River system were located above the Schweitzer Dam (at Woods Pond), and that the significant portion of these materials were located in either Woods Pond itself or in quiescent backwater areas to the Housatonic River. The relatively flat

meandering section of river between the New Lenox Road bridge and Woods Pond contained approximately 70% of the PCBs in the river system. The Stewart Report also documented the minimal PCB transport associated with sediment re-suspension in the river system due to the flow characteristics of the river system during normal and flood-stage levels.

This apparent lack of re-suspension and transport is further supported by a comparison of historic airphotos of this river section. Stewart compared the Housatonic River boundaries using historic airphotos from 1952 and 1981 and noted very little change. Since the boundaries were found to be essentially the same, this comparison supports the conclusion that minimal sediment, and therefore PCBs, is transported by the river waters during normal or flood flows.

It is our belief that these sediments are still present in the locations identified previously because of the quiescent nature of the river system in the areas which contain most of the PCB-contaminated sediments.

4.3 Proposed Resampling Plan

It should be noted that confirmation of the previously-identified locations and concentrations of PCBs is of minimal value until design and actual implementation of the selected remedial alternative occurs. For example, it would probably be necessary to confirm the exact location of PCB-contaminated sediments just prior to dredging, while it would not be necessary under the Flow and Sedimentation Control Alternative. At best, confirmation at this time can provide only assurance that the findings of the Stewart Study, with respect to sediment transport, are still applicable.

To assure that the current location and concentration of PCBs is similar to that during 1980 and 1982, and in response to a specific request by the EPA, a limited resampling plan is proposed. This plan consists of resampling a total of four stations which were previously sampled. These four stations have been selected on the basis that they represent significant quantities of PCB- contaminated material. This confirmation is not intended to replace or re-perform the Stewart Study, as that study was an extensive two-year effort.

Figure 1 presents the proposed resampling stations. The proposed stations have been highlighted on a figure from the Stewart Report to allow for direct comparison with that data-base. The sampling techniques to be utilized will be identical to those performed in 1980 and 1982 to allow for compatible procedures and therefore comparable results. The collected samples will be analyzed by depth for PCBs, as was performed earlier.

The proposed resampling work is scheduled to be performed during the Fall of 1986 with the analytical results available by approximately January 1, 1987. It should be noted that exact confirmation of the previous sampling data is not expected, as all environmental sampling programs contain variability factors associated with precise sampling location, sampling procedure, and analytical procedure. For the sampling locations selected, we would expect that this attempt to confirm the previous results will be reasonably successful. The four stations proposed for resampling were subject to a special study by Stewart Laboratories in 1982. These stations were sampled and analyzed in 2-inch depth increments, yielding a fairly good data base for use in comparison with the proposed sampling. These sampling locations were also sampled in duplicate and should provide a strong basis

for comparison with the proposed sampling. It should be noted that even the duplicate samples obtained in 1982 had reasonable variability, both in terms of depth of sediment obtained and PCB concentration.

REFERENCES

1. Blasland & Bouck Engineers, P.C., Housatonic River Study, 135-Day Interim Report; Assessment of Remedial Alternatives; May, 1985.

2. Erseco Reports, Report on Initial Screening of Housatonic River Remedial Alternatives, March 6, 1984, and Report on Proposed Engineering Evaluation of the Selected Housatonic Remedial Alternatives; May, 1984.

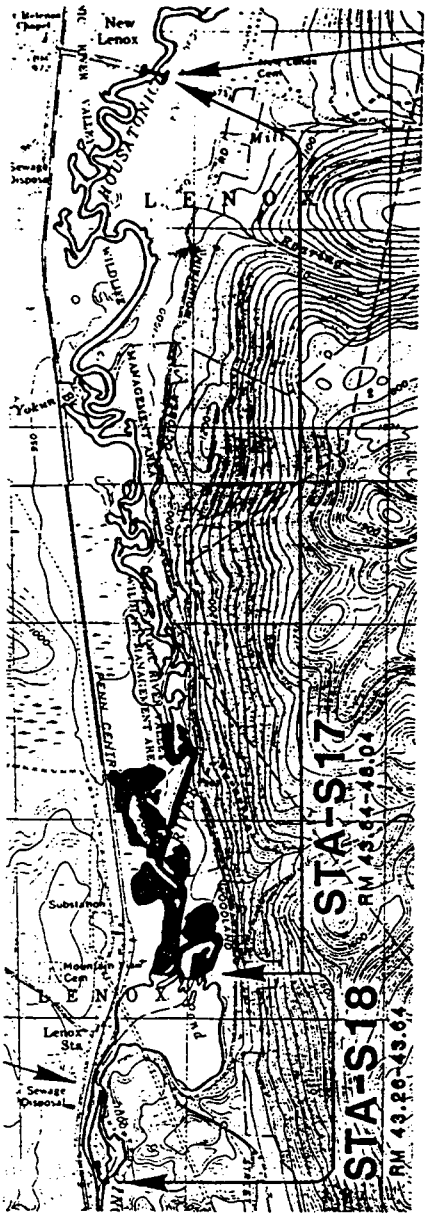
3. Blasland & Bouck Engineers, P.C., Housatonic River Study; 45-Day Interim Report; Remedial Alternatives Evaluation; Sediment Disposal Sites; October, 1984.

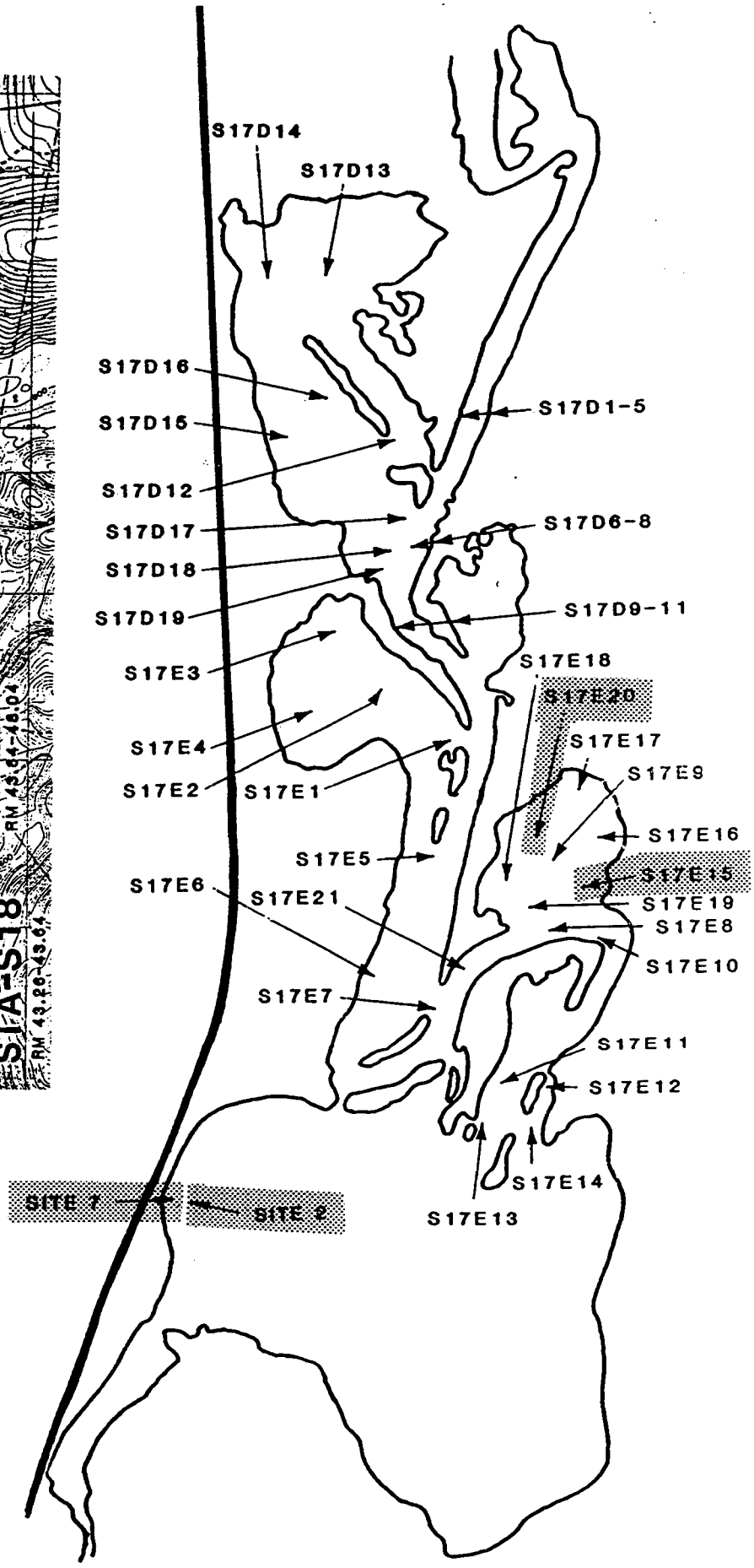
4. Stewart Laboratories, Inc., December, 1982 Housatonic River Study - 1980 and 1982 Investigations, Volume I and II prepared for General Electric Company.

Figures

BLASLAND & BOUCK ENGINEERS, P.C.

FIGURE 1





PROPOSED STATION

STA-17

Woods Pond Backwaters

REFERENCE: FIGURE TAKEN FROM 1982 STEWART REPORT

Appendix 1

BLASLAND & BOUCK ENGINEERS, P.C.

ASSESSMENT OF DREDGING TECHNIQUES

1.0 INTRODUCTION

The removal of soil and sediment from underwater, called dredging, has been done since antiquity. The body of knowledge about dredging is large but has not been systematic until very recently. In the past, a dredging technique was used in a particular soil because experience had shown that it worked, and this knowledge was passed from person to person. What research that did occur was aimed at removing more material faster from deeper water.

With the advent of the National Environmental Protection Act and the Federal Clean Water Act, the focus of concern was not so much on dredging as on the disposal of dredged material. The U.S. Army Corps of Engineers, the foremost dredging organization in the U.S., conducted a multi-million dollar study on productive use of dredged materials in the 1970's. In the 1980`s, the focus has shifted to ways of dredging contaminated materials with minimum release of the contaminants into the water column. The work is ongoing and the field is in a state of flux.

However, enough is known to comment on the appropriateness of certain dredging techniques for contaminated materials.

2.0 MECHANICAL DREDGES

Mechanical dredges such as grab bucket, dipper, bucket ladder and drag line dig the soil and carry it up through the water column for disposition into a nearby scow. These techniques cause a tremendous amount of turbidity. Mechanical dredging has been shown to raise the sediment in the water to four times the background level at a distance of 100 meters from the dredge. The turbidity plume around a typical operation extends 500 meters.

The Japanese, who have more experience than Americans in dredging contaminated materials, have developed a watertight bucket dredge. This uses rubber seals along the closing edges to keep sediment from leaking out as the bucket is lifted through the water column. It is reported that the sealed bucket reduces the turbidity from 30 to 70 percent when compared with a typical operation.

A characteristic of mechanical dredging is that it leaves a very uneven bottom. The operator being unable to see the bottom cannot exactly place the bucket. The advances in laser and computer technology that are being

used to control land based equipment have not yet been modified to work under water. When a mechanical dredge works in an area, it does not always remove all the sediment but will leave mounds unless overdredging is done.

3.0 HYDRAULIC DREDGES

Hydraulic dredges use suction to remove the sediment from the bottom and transport it to the surface through a pipeline. The main hydraulic dredges are trailing suction hopper, cutter head, plain suction and dustpan. The trailing suction hopper dredge is a full size ship and will not be discussed further because it cannot fit onto the upper Housatonic River. The dustpan dredge is similar to the plain suction and the two will be discussed together.

3.1 Cutter Head Dredges

The cutter head dredge is the most commonly used in the U.S. A rotating cutter at the end of the hydraulic suction pipe is used to loosen the sediment and draw it into the pipe. This type of dredge is well suited for use in hard packed sand and cohesive clays. The sediment is sucked to the surface as a slurry that contains 10 to 20 percent sediment.

Turbidity is generated by the rotating of the cutter head into the sediment. The level of turbidity is affected by the rate of rotation, depth of cut and material being dredged. For fine grained material being dredged by a competent operator, the turbidity level is approximately twice the background level within 20 meters of the cutter head. The turbidity plume can usually be traced 50 to 100 meters from the dredge. In absolute terms, the amount of sediment suspended close to the cutter head is 100 to 200 milligrams per liter (mg/l) and the plume, at its outer edge, is approximately 20 mg/l above the background.

Both the cutter head and plain suction dredges will leave a smooth bottom with all material being removed if the operator is competent. Electronic sensors placed on the end of the suction pipe precisely locate the head and keep the operator informed of the progress of the work.

The cutter head dredge has been used to remove sediments that are classified as contaminated by the protocols developed for the Federal Clean Water Act and the Marine Sanctuary Act, but not for the cleanup of a site. The cutter head is planned for use in the Hudson River PCB cleanup, however, no actual dredging has been done.

3.2 Plain Suction Dredges

The plain suction dredge is the simplest of the hydraulic dredges utilizing just a suction pipe. The dust pan dredge is very similar except there is a shroud surrounding the pipe. Certain dust pan dredges have water jets around the edge of the shroud to agitate the sediment. These dredges are only suitable for soft material. The slurry is generally less than 15 percent sediment.

There have been no studies on the amount of turbidity generated by a plain suction dredge. They have a low production rate and are not commonly used for commercial work. Visually only a small plume around the pipe has been observed. In free flowing material and without the use of water jets, the same holds true for the dust pan dredge.

A dust pan dredge was tried as part of the James River, Virginia attempt to cleanup Kepone. The dredge increased the turbidity to approximately 140 mg/l which was unacceptable to the Corps of Engineers. The causes of the turbidity were thought to be the unfavorable hydraulic radius of the shroud and the highly plastic clay sediment.

The New England Division of the Corps is under contract to the Environmental Protection Agency to investigate

dredging methods for removal of PCB contaminated sediments from the Acushnet River, New Bedford, Massachusetts. As part of this effort, the Waterways Experimentation Station of the Corps is developing a matchbox shroud to go onto a hydraulic dredge to minimize turbidity. A demonstration dredging trial was conducted in Indiana Harbor during the fall of 1985. The results are being analyzed and the final report is expected in September 1987.

4.0 SPECIAL DREDGES

There are a number of dredges that have been developed for special purposes or using unusual techniques. These dredges are not in widespread commercial use and a large body of knowledge about their characteristics and limitations has not been developed.

4.1 Pneumatic Dredges

These dredges use compressed air, rather than water as in a hydraulic system, to provide the suction to lift the sediments to the surface. The most commonly known of this type are the Pneuma pump and the Oozer pump. A vacuum is applied to the inlet chamber sucking up the sediment and compressed air is then used to drive the sediment to the surface. The manufacturers claim that the

slurry is 60 to 80 percent solids and field tests have shown that the solid content is approximately 55 to 75 percent.

During tests, the turbidity level was less than 50 mg/l at the head and no turbidity was found at 30 meters from the dredge. This test was conducted in loose fine sediments. Testing by the Corps' Waterways Experimentation Station has generally confirmed the manufacturers' findings.

The pneumatic dredges have been used to dredge contaminated soils at several sites in Japan. In the U.S., a pneumatic dredge was used to remove PCB contaminated sediment from the Duwamish Waterway in Seattle Harbor, Washington. The project was carried out under the direction of the Seattle District Corps of Engineers. The Seattle District has expressed satisfaction with the results.

4.2 Sludge Dredges

This class of dredges has been developed to remove sludge from industrial settling ponds and waste lagoons. The more commonly known dredges of this type include Waterless, Delta and National. Although they vary in technical details, they all use a knife or auger system to pull sediment towards a hydraulic suction pipe for transport

to the surface. All of them have specially designed shrouds to hold the turbidity to a minimum. Because the material that these machines are designed to remove will require treatment and disposal, the process uses very little water. The slurry is typically 50 percent solids.

No detailed studies have been conducted to determine the turbidity levels generated by these dredges. Visual observations show tht turbidity is usually very low. One test shows less than 10 mg/l suspended solids at 3 meters from the dredge. The type of sediment that was being dredged during the test was not known.

These dredges have been used in a large number of industrial ponds to remove contaminated materials. The Waterless dredge has been used to clean up an open water site which was contaminated with lead at Mill Pond, Connecticut.

4.3 Mudcat Dredge

The Mudcat is a specialized dredge for use in shallow open water ponds and streams. It uses a shrouded auger to draw the sediment to the suction pipe. The slurry is approximately 20 to 30 percent solids and the dredge can remove approximately 60 to 80 cubic yards of silt per hour.

The head can be controlled so that the material is removed precisely.

Studies have shown that the turbidity at the head ranges from 100 to 200 mg/l and the plume dissipates within 6 meters of the dredge.

The Mudcat has been used at many sites similar to Woods Pond and the Housatonic River to remove uncontaminated sediments. It has been used to remove some contaminated sediments at sheltered sites but not in a large area cleanup.

5.0 CONCLUSIONS

From a review of the equipment available and the limited studies, large scale commercial dredging techniques are not appropriate for use at Woods Pond and the Housatonic River. These include mechanical and cutterhead dredges. The development work that is being done by the Corps' Waterways Experimentation Station on matchbox shrouding for suction dredging could lead to an appropriate technique. This program will be monitored.

Some of the special dredges could be appropriate for use at Woods Pond. The Pneuma and the Waterless dredges

have been used at other open water sites for removal of contaminated sediments. The Mudcat has been used at a number of sites similar to Woods Pond. There is not a large body of information developed about these dredges and their limitations. What information is available, the conditions at sites where the dredges have been used and the results of the dredging will require careful examination to determine if any of these dredges are appropriate for Woods Pond, including the very shallow water depth in which some of the sediments are located. Dredges such as the Mudcat have a standard draft of approximately 21 inches. This could be modified to about a foot by using additional flotation devices, which in turn will effect dredge maneuverability. The speciality dredges typically are not self-propelled, but use winches and cables to deadmen to maneuver. This will have to be studied to determine if any of the dredges can work and maneuver in the shallow backwaters of Woods Pond and the Housatonic River.

An issue which has a direct bearing on the appropriateness of any of these dredges is the volume of sediment that can be released downstream. None of these dredges have zero turbidity; they all introduce some sediment into the water column during the dredging process. The use of silt curtains, coagulants and other techniques will reduce the sediment load going downstream, but no

technique will remove all the contaminated sediment from the water column. The allowable downstream load of suspended sediment under various flow conditions will be a major factor in deciding if a dredging technology can be used.

In the study, no attempt has been made to address the transportation mode of the sediments from the dredging location to the disposal site. The dredging technique will determine the characteristics of the sediment slurry which has an important effect on the transportation mode. However, an equally or more important factor in analyzing the transportation will be the location of the disposal site. Until a disposal site is chosen, it is not effective to analyze this aspect.

Appendix 2

BLASLAND & BOUCK ENGINEERS, P.C.

GENERAL ELECTRIC COMPANY
RESEARCH AND DEVELOPMENT PROGRAM
FOR THE DESTRUCTION OF PCBs

Fifth Progress Report

For the Period

June 1, 1985 through May 31, 1986

June 1986

Submitted by

Herman L. Finkbeiner and Stephen B. Hamilton

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EXECUTIVE SUMMARY

Our research this year has focused on four major areas: (1) bacterial oxidation of PCBs, (2) reductive dechlorination of PCBs in anaerobic communities, (3) analysis of environmental transformations of PCBs, and (4) process modeling for extraction and biodegradation of PCBs.

Bacterial oxidation of PCBs. We have focused on the genetics and biochemistry of PCB degradation in two of our most promising bacterial strains: *Pseudomonas putida* LB400 and *Alcaligenes eutrophus* H850. We have used conventional mutagenesis techniques to generate mutant strains of LB400 blocked at each of the four initial steps in the biphenyl 2,3-dioxygenase pathway and mutants of H850 blocked at the second and fourth steps of the pathway. These mutants have facilitated our biochemical studies of PCB degradation and have enabled us to establish that both LB400 and H850 have a complete biphenyl 2,3-dioxygenase pathway.

Our biochemical studies indicate that *A. eutrophus* H850 and *P. putida* LB400 may use several different mechanisms for PCB degradation. Both strains apparently use a 2,3-dioxygenase pathway to degrade some PCB congeners, but exhibit novel activities as well.

The 2,3-dioxygenase enzyme in H850 appears to have different congener specificity than previously described 2,3-dioxygenases. Additional mechanisms of PCB degradation in H850 appear to involve an enzyme capable of attacking at ring positions 3,4 and possibly an enzyme capable of attacking at a chlorinated position. A complete 3,4-dioxygenase pathway has not yet been demonstrated in this strain, nor is there any evidence for steps analogous to the second or third step of the 2,3-dioxygenase pathway. Instead, the 3,4-dihydrodiol metabolite of 2,5,2',5'-CB undergoes a second dioxygenase attack at position 3',4' of the other ring. We have demonstrated that ring-chlorinated acetophenones are formed during the metabolism of certain PCB congeners. The pathway leading to their formation is unclear and will be the focus of future studies.

Our results indicate that LB400 employs a monooxygenase to metabolize some PCB congeners via an epoxide intermediate. As a first step toward isolating and characterizing this and other PCB-degrading enzymes, we have developed a cell-free system that actively metabolizes PCBs. The enzymes that catalyze the third and fourth steps in the 2,3-dioxygenase pathway in LB400 have already been partially purified; the enzymes responsible for the first two steps of the pathway have not yet been separated. Experiments are in progress to determine whether this cell-free system also has monooxygenase or 3,4-dioxygenase activity.

Reductive dechlorination of PCBs in anaerobic communities. We are currently conducting experiments designed to determine whether the PCB dechlorination observed in various anaerobic freshwater sediments is biologically mediated. Sensitive assays have been developed to detect PCB dechlorination and are being used to assess the PCB-dechlorinating ability of several anaerobic cultures. The results from one- and four-week incubations of Hudson River sediments suggest that a very slow reductive dechlorination of PCBs may be occurring. This is particularly encouraging because anaerobic consortia frequently require months of acclimation before detectable dechlorination activity develops.

Environmental transformations of PCBs. Our characterization of environmental transformations of PCBs has been extended to include both physical and biological processes. We have demonstrated that evaporation can remove the more volatile PCB congeners, including some hexachlorobiphenyls, from soil surfaces. The evaporative loss of Aroclor 1242 at one New York site appears to have averaged about 6% per year. Water-extraction of PCBs accounts for a significant amount of downstream transport and is responsible for the appearance of PCBs in groundwater, but only the lower PCB congeners (fewer than four chlorines) are significantly mobilized.

In previous years we reported anaerobic dechlorination of PCBs in the Hudson River (New York) and Silver Lake (Massachusetts). Gas chromatograms submitted to us by other analysts provide evidence of PCB dechlorination in Waukegan Harbor (Illinois), Sheboygan Harbor (Wisconsin), and the Hoosic River (Massachusetts). The dechlorination process produces detoxified mixtures of lower congeners that are readily biodegradable by aerobic bacteria. We have found evidence for such aerobic biodegradation of PCBs in gas chromatograms of groundwater and river water.

Process modeling for extraction and biodegradation of PCBs. Our modeling studies have focused on three areas: (1) bacterial oxidation of PCBs bound to soil, (2) surfactant extraction of PCBs from soil, and (3) development of a combined anaerobic/aerobic process for PCB biodegradation.

Our studies with individual PCB congeners bound to moist soil have demonstrated that the oxidation steps observed in our aqueous resting cell assays also occur under conditions that more closely approximate field conditions. Under excess water conditions, we have demonstrated efficient oxidation of Aroclors 1242 and 1254 added to soil at 50 and 500 ppm, and of a modified Aroclor 1242 in an environmental soil sample (525 ppm). Both resting and actively growing cells have been shown to be capable of degrading PCBs on soil. Although lower rates of PCB degradation are anticipated at high PCB concentrations, we conclude from these studies that it should be possible to biologically degrade PCBs on contaminated soil in the environment with appropriate cell concentrations and moisture conditions.

We have modeled a mock biodegradation process to demonstrate how physical loss of PCBs might be mistaken for biodegradation. This experiment simulated an open air, aerated, stirring reactor, but was conducted anaerobically and without bacterial inoculation. The aeration (with argon gas) and stirring resulted in redistribution of the Aroclor 1260 from the soil to locations in the reactor that would not normally be assayed: glassware, stirrer, and coalesced droplets of PCB. Although PCB depletion was observed, it was not congener specific.

As part of our modeling studies for surfactant extraction of PCBs we have analyzed the soil types at Oakland and have selected appropriate model soils for further studies. Model studies using Aroclor 1260 on Oakland soil and a model clay have shown that both Triton X-100 and Surco 233 effect 35-100% removal of the PCB when used at greater than 1.0 wt%. However, our results indicate that a method for surfactant recovery must be included in the overall process in order to make the system economically practical.

We are attempting to integrate surfactant removal of PCBs and biodegradation in a combined anaerobic/aerobic process. Our studies have determined that Triton surfactants are toxic to anaerobic cultures at concentrations of 100 to 333 mg/L. Triton X-305 and Triton X-405, which are comparable to Triton X-100 in their ability to remove PCBs from soil [GE Reports, 1983, 1984, 1985], are considerably less toxic than Tri-

ton X-100 and may be better suited for such a process. These results indicate that surfactant PCB leachates may require dilution before biological treatment. Alternatively, it may be possible to remove the surfactant from the PCB leachate prior to biodegradation. A second area of this research involves the implementation of continuous flow experiments to study anaerobic degradation of PCBs. These experiments are now in progress.

Chapter 1

BIOCHEMISTRY OF PCB METABOLISM IN *ALCALIGENES EUTROPHUS* H850: OXIDATION OF BIPHENYL AND BIPHENYL DERIVATIVES

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INTRODUCTION

In last year's report we described the isolation and identification of *cis*-3,4-dihydroxy-3,4-dihydro-2,5,2',5'-tetrachlorobiphenyl (3,4-PCB-dihydrodiol) as a metabolite formed from 2,5,2',5'-tetrachlorobiphenyl by *Alcaligenes eutrophus,* strain H850. The 3,4-PCB-dihydrodiol was further metabolized to polar products by biphenyl-induced cells of *A. eutrophus* H850. There are several possible explanations for these observations: (1) Biphenyl-2,3-dioxygenase and *cis*-2,3-dihydroxy-2,3-dihydrobiphenyl dehydrogenase (enzymes involved in the biodegradation of biphenyl, see Figure 3-2) have relaxed substrate specificities which allow oxidation to occur at the unsubstituted 3,4 position of 2,5,2',5'-tetrachlorobiphenyl. (2) *Alcaligenes eutrophus* H850 has additional biphenyl dioxygenases and dehydrogenases which are specific for the unsubstituted 3,4-position of 2,5,2',5'-tetrachlorobiphenyl. (3) The 3,4-PCB-dihydrodiol is oxidized by a new ring-fission mechanism to give polar products.

This year's work has been directed primarily toward elucidation of the metabolism of 3,4-PCB-dihydrodiol. We have used two approaches: (1) the metabolism of biphenyl and 3,4-dihydroxybiphenyl by biphenyl-induced cells of *A. eutrophus* H850, and (2) the metabolism of 3,4-PCB-dihydrodiol by biphenyl-induced cells of *A. eutrophus* H850. Technical details of culture preparation, transformation procedures, and analytical methods were described previously [GE Report, 1985].

RESULTS

Oxidation of Biphenyl by *A. eutrophus* H850

The availability of a mutant strain of *A. eutrophus* H850, (strain FM803) isolated by Dr. F. Mondello at GE, has allowed us to study the initial reactions in biphenyl degradation more closely. The parent organism oxidizes biphenyl quite rapidly and the only intermediate that has been detected is 2,3-dihydroxybiphenyl. This compound appears transiently and was identified by its retention time when analyzed by high-pressure liquid chromatography. In contrast, the mutant strain FM803 accumulates *cis*-2,3-dihydroxy-2,3-dihydrobiphenyl (2,3-biphenyl-dihydrodiol). The identity of this metabolite was confirmed by its melting point (93 °C), NMR spectrum, mass spectrum, absorption spectrum, and dehydration to 2- and 3-dihydroxybiphenyl. All of these properties were identical to those of *cis*-2,3-biphenyl-dihydrodiol produced by a mutant strain of *Beijerinckia.*

Although the parent strain of H850 does not accumulate metabolic products during biphenyl degradation, the addition of 3-chlorocatechol to the reaction mixture causes the accumulation of 2,3-dihydroxybiphenyl. This metabolite was identified by its HPLC retention time and mass spectrometry. 3-Chlorocatechol apparently inhibits the

catechol dioxygenase which catalyzes the "meta" fission of 2,3-dihydroxybiphenyl (Figure 3-2) and thus causes catechol accumulation.

Biphenyl-induced cells oxidized *cis*-2,3-biphenyl-dihydrodiol and 2,3-dihydroxybiphenyl to a yellow ring-fission product which gave absorption spectra at acidic and basic pH values identical to those reported for the product formed by "meta" (1,2) cleavage of 2,3-dihydroxybiphenyl.

Polarographic studies with biphenyl-induced cells of *A. eutrophus* H850 and strain FM803 gave the results shown in Table 1-1.

Table 1-1

OXIDATION OF BIPHENYL AND METABOLITES BY *A. EUTROPHUS* STRAINS H850 AND FM803

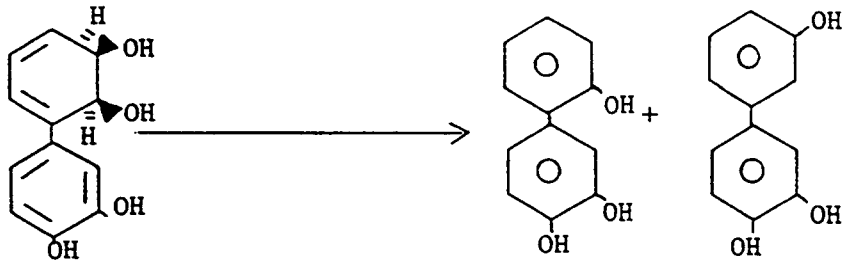
Substrate	Oxygen uptake (nmol O_2/mg protein)	
	H850	FM803
Biphenyl	0.19	0.01
cis-2,3-Biphenyl-dihydrodiol	0.44	0.00
2,3-Dihydroxybiphenyl	0.98	0.34

These results confirm previous data that indicate that biphenyl is oxidized by the previously reported meta fission pathway.

Oxidation of 3,4-Dihydroxybiphenyl by *A. eutrophus* H850

Although the main pathway for biphenyl degradation appears to be initiated by a 2,3-dioxygenase, we have confirmed our previous observations that biphenyl-grown cells of H850 oxidize 3,4-dihydroxybiphenyl. A polar product that accumulated during the oxidation of 3,4-dihydroxybiphenyl was isolated and identified as the methyl ester of 3,4-dihydroxybenzoic acid. Although the esterification reaction appears unusual, the formation of 3,4-dihydroxybenzoic acid could be explained by 2,3-dihydroxylation of the unsubstituted biphenyl ring followed by the well-established reactions of the meta fission pathway.

Preliminary studies have indicated that the mutant strain FM803 oxidizes 3,4-dihydroxybiphenyl to 2,3-dihydroxy-2,3-dihydro-3',4'-dihydroxybiphenyl. The evidence for the formation of this metabolite was obtained by GC/MS. Three compounds were detected with molecular weights of 220, 202, and 202, respectively. These results could be accounted for in terms of dihydrodiol formation on the unsubstituted ring followed by dehydration reactions which could occur during the chromatographic separation.



Oxidation of 2,5,2',5'-Tetrachlorobiphenyl by *A. eutrophus* H850

Previous studies have shown that *cis*-3,4-PCB-dihydrodiol is metabolized by biphenyl-induced cells of *A. eutrophus* H850 to polar products. Cell extracts, prepared from biphenyl-grown cells catalyzed an NAD^+-dependent oxidation of *cis*-2,3-biphenyl dihydrodiol to 2,3-dihydroxybiphenyl. However, no activity was observed with the 3,4-PCB-dihyrodiol formed from 2,5,2',5'-tetrachlorobiphenyl. These results indicate that the polar compounds formed from 3,4-PCB-dihydrodiol arise by a novel metabolic route. The polar metabolites formed from 3,4-PCB-dihydrodiol are shown in Figure 1-1.

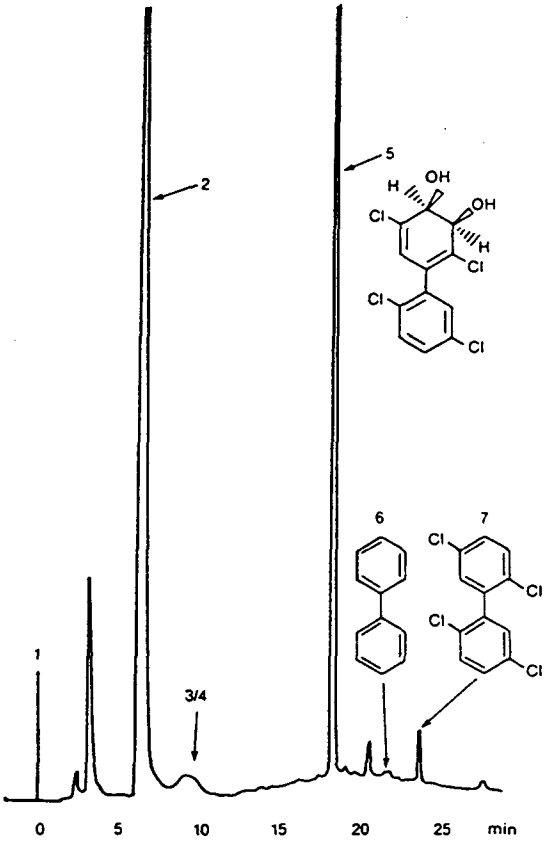


Figure 1-1. HPLC analysis of the transformation products of 2,5,2',5'-tetrachlorobiphenyl. Fifteen mg of 2,5,2'5'-CB were incubated with resting cells of H850 for 8 hours. Peaks 1-4 are polar products.

The major product was identified as *cis,cis*-3,4,3',4'-tetrahydroxy-3,4,3',4'-tetrahydro-2,5,2',5'-tetrachlorobiphenyl (*bis*-3,4-dihydrodiol). The mass spectrum of this compound is shown in Figure 1-2. The molecular ions at m/e 358, 340 and 322 are consistent with the proposed structure. In addition, the thermal dehydration products shown in Figure 1-3 were also separated by gas chromatography and identified by mass spectrometry.

The proton magnetic resonance spectrum of the *bis*-dihydrodiol gave signals at δ = 4.40, 4H (doublet of doublets) and δ = 6.0, 2H (broad doublet). Aromatic protons were not detected. The relative stereochemistry of the new dihydrodiol functional group was determined by the formation and isolation of an isopropylidene derivative. The mass spectrum of this compound is shown in Figure 1-4. These results confirm

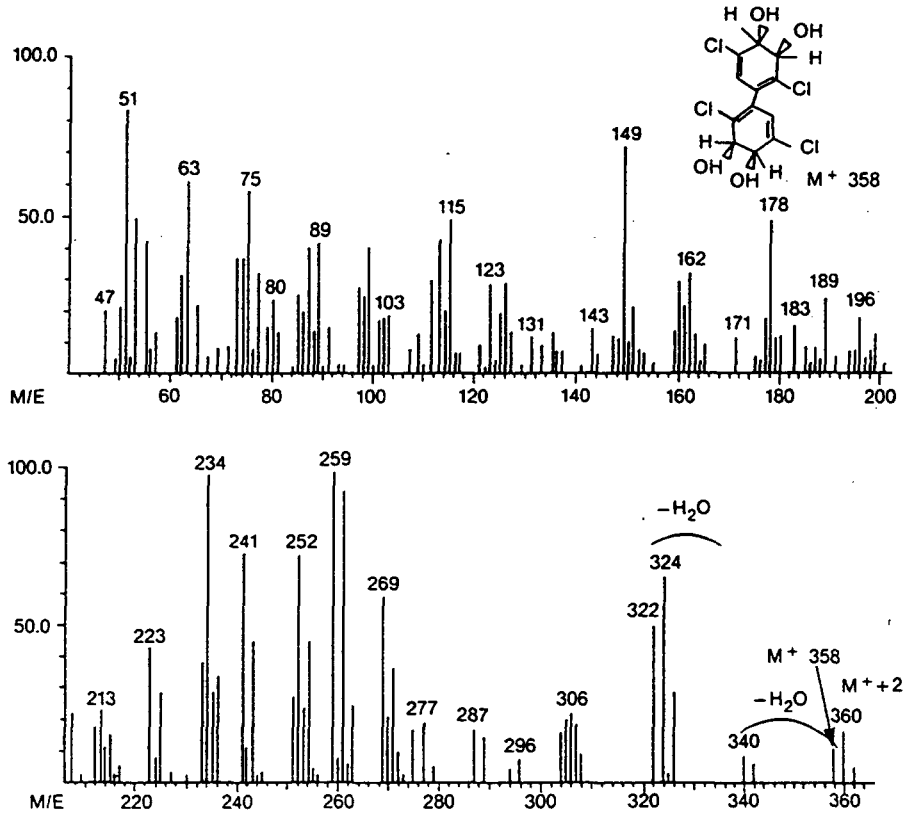


Figure 1-2. Mass spectrum of a metabolite of the *cis*-3,4-dihydrodiol of 2,5,2′,5′-CB. This compound, which eluted at scan number 450 of the GC analysis, has been identified as the *bis*-3,4-dihydrodiol of 2,5,2′,5′-CB.

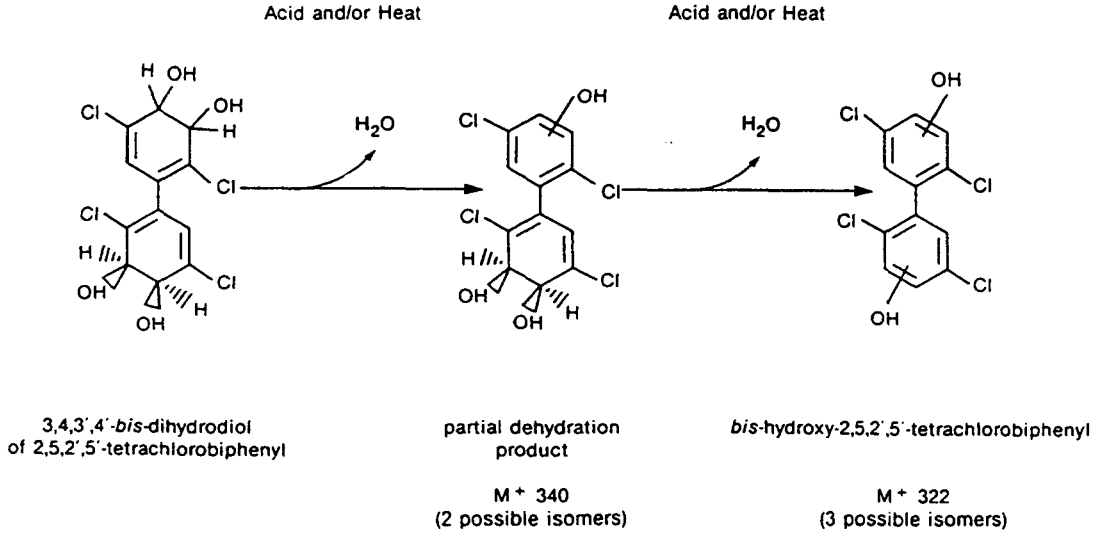


Figure 1-3. Proposed dehydration mechanism of the *bis*-3,4-dihydrodiol of 2,5,2′,5′-CB.

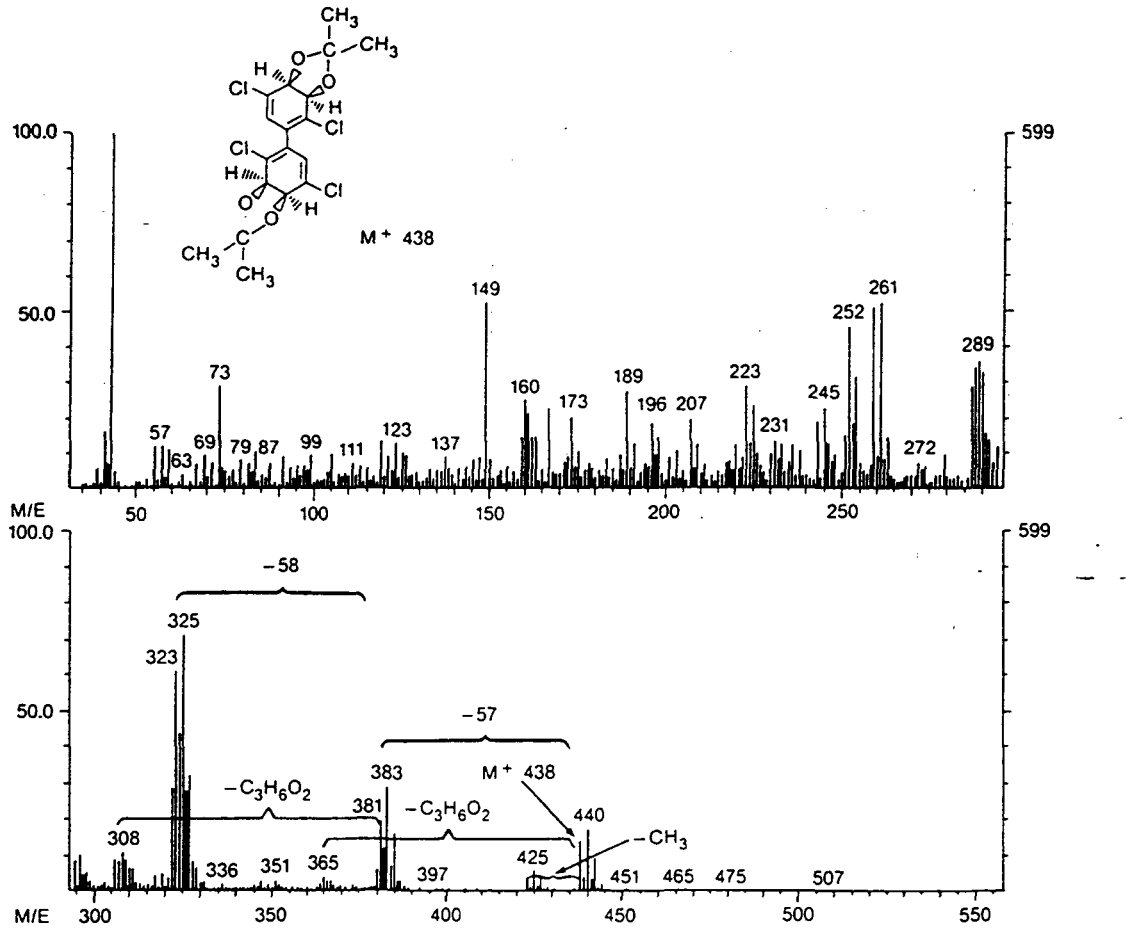


Figure 1-4. Mass spectrum of the isopropylidene derivative of the *bis*-3,4-dihydrodiol of 2,5,2′,5′-CB.

that *A. eutrophus* H850 oxidizes 2,5,2′,5′-tetrachlorobiphenyl to *cis,cis*-3,4,3′,4′-tetrahydroxy-3,4,3′,4′-tetrahydro-2,5,2′,5′-tetrachlorobiphenyl.

The *bis*-3,4-dihydrodiol was extremely stable. However, treatment of a methanolic solution of the dihydrodiol with concentrated sulfuric acid gave rise to three new compounds which were isolated by HPLC. The structure of these products was determined by derivatization with diazomethane and subsequent analysis by GC/MS which showed that they were the three dihydroxy products formed by acid catalyzed dehydration of the *bis*-3,4-dihydrodiol.

FUTURE PLANS

Dr. D.L. Bedard and her colleagues at GE have clearly shown that some PCB congeners are metabolized through chlorinated acetophenones (see Chapter 2). In a careful series of experiments it was shown that chloroacetophenones were formed when the chlorination pattern on the attacked ring was 3, or 2,5 or 2,4,5. We intend to confirm these results with 2,5′,2′-trichlorobiphenyl, 3,3′-dichlorobiphenyl and 2,4,5,2′,5′-pentachlorobiphenyl. The congener that gives the highest yield of chloroacetophenones will be studied in detail to determine the mechanism of formation of these unexpected metabolites.

Chapter 2

BIOCHEMISTRY OF PCB DEGRADATION IN *ALCALIGENES EUTROPHUS* H850: CONGENER SPECIFICITY AND PRODUCTION OF RING-CHLORINATED ACETOPHENONES

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INTRODUCTION

Alcaligenes eutrophus H850 degrades a wide variety of PCB congeners ranging from mono- to hexachlorobiphenyls, and is superior to all previously described PCB-degrading strains (except *P. putida* LB400) in its ability to degrade congeners with blocked 2,3 sites (such as 2,5,2',5'-CB) and even congeners that have no adjacent unchlorinated carbons (such as 2,4,5,2',4',5'-CB). This is particularly significant because the higher Aroclors contain many congeners that fall into these categories.

We have been trying to understand the biochemical basis of this broad and unusual degradative competence in H850. All previously described PCB-degrading bacteria, except *P. putida* LB400 and H850, are thought to use a biphenyl 2,3-dioxygenase pathway to metabolize PCBs [Furukawa, 1982]. Several years ago we postulated that H850 might exclusively use a 3,4-dioxygenase pathway to degrade PCBs [Bedard et al., 1984; GE Reports, 1984, 1985]. Drs. M.J. Schocken and D.T. Gibson have confirmed that H850 degrades 2,5,2',5'-CB via dioxygenase attack at position 3,4 [GE Report, 1985]. However, Gibson's laboratory has also unequivocally demonstrated that H850 has a complete biphenyl 2,3-dioxygenase pathway [GE Report, 1985; and Chapter 1].

Last year we reported evidence for a novel chloroacetophenone PCB-metabolite in H850, and evidence suggesting that the PCB-degradation system in H850 had greater affinity for those congeners chlorinated at positions 2,5. We also reported evidence that certain PCB congeners were constitutively degraded in succinate-grown cells, while others were degraded only by cells grown on biphenyl.

The goal of our research this year has been to gain a better understanding of how these findings relate to each other, with the ultimate goal of understanding the pathway(s) responsible for PCB degradation in H850. A key question is whether the observed 3,4-attack on 2,5,2',5'-CB is due to relaxed specificity of a 2,3-dioxygenase or, as we proposed earlier, to a novel enzyme (e.g., a 3,4-dioxygenase). We have approached this question by extending our examination of congener specificity, including studies to determine how chlorine substitution at positions 2,5 affects the degradation of PCBs. We were also interested in determining whether other congeners were degraded to chloroacetophenones, and whether these metabolites might be intermediates of a novel PCB-degradation pathway. Finally, we have extended our studies comparing PCB degradation in succinate- and biphenyl-grown cells.

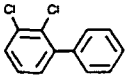
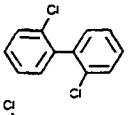
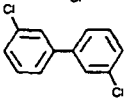
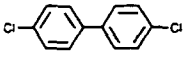
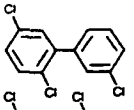
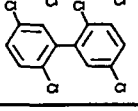
RESULTS AND DISCUSSION

Congener Specificity

Table 2-1 demonstrates the effect of chlorine substitution pattern on PCB degradation in H850. The individual congeners were incubated with resting cells of H850 in the presence of an internal standard (3,5,3′,5′-CB) that is not degraded by this strain. Each congener was tested at the highest concentration that could be completely degraded in a 72-h period; this permitted a comparison of the amount of each congener degraded within a 12-h period. As expected, 2,3-CB, which has an unchlorinated ring, was degraded most rapidly. There were substantial differences in the degradation of 2,2′-CB, 3,3′-CB, and 4,4′-CB; 2,2′-CB, 3,3′-CB, and 2,5,2′,5′-CB, respectively, were oxidized 337, 57, and 20 times more rapidly than 4,4′-CB (Table 2-1). In contrast, *Corynebacterium* sp. MB1, a bacterium that apparently degrades PCBs via a 2,3-dioxygenase, oxidized 4,4′-CB reasonably well (168 nmol in 12 h) but was unable to degrade 2,5,2′,5′-CB (data not shown). These results are entirely consistent with our previous observations.

Table 2-1

EFFECT OF CHLORINE SUBSTITUTION PATTERN ON PCB OXIDATION BY *ALCALIGENES EUTROPHUS* H850

STRUCTURE	CONGENER[a]	NANOMOLES DEPLETED IN 12 H[b]	RELATIVE RATE OF OXIDATION
	2,3	1750	437
	2-2	1350	337
	3-3	227	57
	4-4	4	1
	2,5-3	168	42
	2,5-2,5	83	21

[a] In the tables in this chapter congeners are written with a dash separating the chlorine substituents on each ring. The chlorination pattern common to a group of congeners is always shown on the right, even though this occasionally means that the chlorine substitution is not written according to convention. For example, in later tables we have written 2-2,5 and 3,4-2,5. According to our normal convention these would be written 2,5,2′ and 2,5,3′,4′.

[b] This column represents the nanomoles of PCB depleted by 1 $O.D._{615}$ of cells (approximately 10^9 cells) in 12 hours.

Effect of 2,5-Chlorophenyl Ring on Degradation of PCBs

Last year we noted that 2,6,2′,5′-CB and 2,4,6,2′,5′-CB were oxidized more rapidly than 2,6-CB and 2,4,6-CB, respectively. Because greater metabolism of the more highly chlorinated congeners was unexpected, we proposed that the PCB-degradative enzyme system in H850 might have a greater affinity for congeners with chlorines at the 2,5 positions. We have extended our study of this phenomenon by comparing

eight pairs of PCB congeners. Within each pair the chlorination pattern on the first ring was the same, while the second ring was either unchlorinated or chlorinated at the 2,5 positions. Figure 2-1 shows the results of these experiments. Congeners with an unchlorinated ring were metabolized more rapidly as long as the first ring was not chlorinated in both ortho positions (2,6). An example of this is illustrated in panel A. However, when the first ring was chlorinated in both ortho positions, the congener chlorinated at positions 2',5' was oxidized more rapidly (panels B through E).

These results suggest that congeners with an unsubstituted ring are degraded by 2,3-dioxygenase attack of that ring, provided that the other ring is not chlorinated in both ortho positions. Furthermore, these congeners are degraded more rapidly than the corresponding congeners with 2',5' chlorine substitution. Conversely, when the

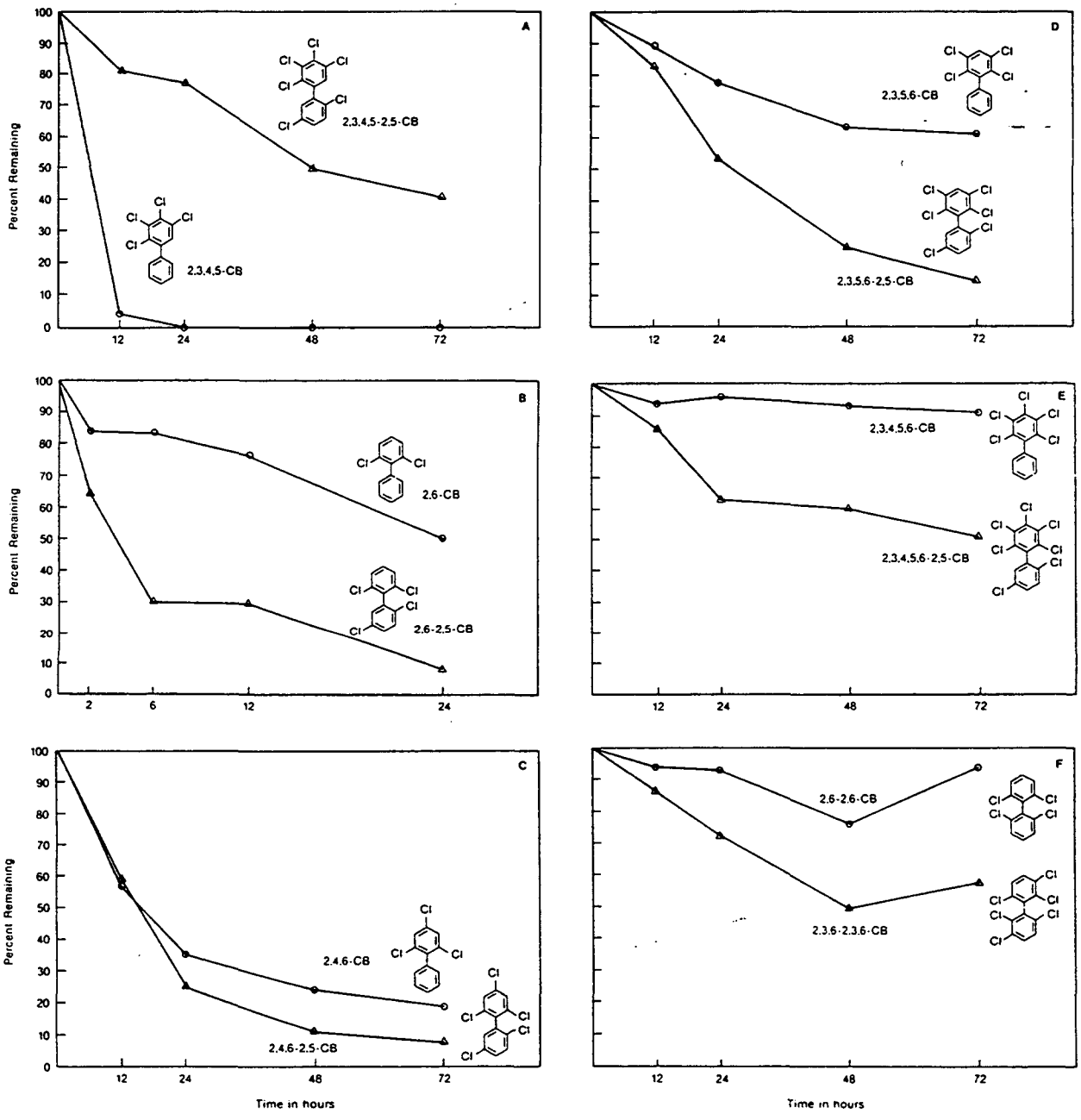


Figure 2-1. The effect of 2,5-chlorine substitution on PCB degradation in H850.

first ring is chlorinated in both ortho positions, these ortho chlorines may sterically interfere with the attack of a 2,3-dioxygenase. The more rapid degradation of diortho-substituted congeners that are chlorinated at positions 2',5' suggests that these congeners are degraded by a different enzyme (possibly a 3,4-dioxygenase), and that this enzyme has greater affinity for a chlorophenyl ring substituted at positions 2',5' than for an unsubstituted ring. Subsequent experiments showed that diortho-substituted congeners chlorinated at position 3' (which is equivalent to 5') were also degraded more rapidly than the corresponding congeners with unsubstituted rings. Thus, a single meta chlorine may be sufficient for attack by an alternative enzyme.

Novel Metabolites: Ring-chlorinated Acetophenones

Last year we observed a single-ring metabolite of 2,4,5,2',4',5'-CB and tentatively identified it as 2',4',5'-trichloroacetophenone (2',4',5'-CA). Since then we have synthesized 2',4',5'-CA for comparison with the metabolite. A comparison of the synthetic compound and the metabolite of 2,4,5,2',4',5'-CB by GC retention times on two different columns and by GC mass spectrophotometry has confirmed our identification.

More recently, we have determined that H850 produces ring-chlorinated acetophenones from many PCB congeners. Congeners that are known to be metabolized to chloroacetophenones are shown in Table 2-2; congeners from which chloroacetophenones have not been detected are listed in Table 2-3. It is particularly significant that in all cases chloroacetophenones were formed by attack on a chlorophenyl ring substituted at positions 3 or 2,5 or 2,4,5. The attacked ring was subsequently metabolized and broken open, generating the chloroacetophenone; e.g., 2',3',4'-CA was produced from 2,3,4,2',5'-CB. No chloroacetophenones were observed from the degradation of congeners with diortho substitution on the first ring (such as 2,6,2',5'-CB), possibly because of steric interference from the ortho chlorines. Furthermore, chloroacetophenones were not formed from attack on an unsubstituted ring or on chlorophenyl rings substituted at positions 2 or 4 or 2,3. This is further evidence that a unique enzyme or enzyme system is used to metabolize PCBs with chlorophenyl rings substituted at the 3 or 2,5 (or in this case, 2,4,5) positions.

We have measured the yield of chloroacetophenone for various congeners in an attempt to determine whether oxidation via acetophenones represents a major pathway in H850. The highest yield we have observed was for 3'-CA formed from the degradation of 3,3'-CB; in this case the 3'-CA accounted for 12% of the 3,3'-CB that had been oxidized. However, we have also determined that the chloroacetophenones do not accumulate, but are instead metabolized further, hence we currently have no way of measuring how much PCB is metabolized via chloroacetophenones.

We do not yet understand how PCBs are metabolized to chloroacetophenones because we have not yet been able to identify any of the earlier intermediates. However, we have determined that H850 is able to use acetophenone as a growth substrate. It is reasonable to assume that the enzymes involved in the oxidation of acetophenone are also involved in the metabolism of chloroacetophenones. If this is true, then a mutant that is unable to grow on acetophenone may accumulate chloroacetophenones formed from the degradation of PCBs, and possibly preceding intermediates as well. We plan to generate such mutants in the near future to aid in determining how much PCB is metabolized via acetophenones and how this metabolism occurs.

We have previously reported that H850 metabolizes some PCB congeners to chlorobenzoic acid [GE Report, 1985]. At least two congeners, 2,3,2',5'-CB and

Table 2-2

PCB CONGENERS THAT ARE METABOLIZED TO RING-CHLORINATED ACETOPHENONES BY *ALCALIGENES EUTROPHUS* H850

PCB CONGENER	CHLOROACETOPHENONE PRODUCED	CHLORINATION PATTERN ON ATTACKED RING
2-3	2′	3
3-3	3′	3
2,5-3	2′, 5′	3
3,4-3	3′, 4′	3
2-2,5	2′	2,5
4-2,5	4′	2,5
2,3-2,5	2′, 3′[a]	2,5
2,5-2,5	2′, 5′[a]	2,5
3,4-2,5	3′, 4′	2,5
2,3,4-2,5	2′, 3′, 4[a]	2,5
2,5-2,4,5	2′, 5′ and 2′, 4′, 5′[a]	2,5 and 2, 4, 5
2,4,5-2,4,5	2′, 4′, 5′[a]	2,4,5

[a]Verified by GC-MS

Table 2-3

PCB CONGENERS THAT ARE NOT OXIDIZED TO CHLOROACETOPHENONES

PCB CONGENER	CHLORINATION PATTERN ON POTENTIAL SITE OF ATTACK
2,3	-
2,3,4	-
2,4,5	-
2-2	2
4-2	2
3,4-2	2
2,3,4,5-2	2
2-4	4
4-4	4
2,3,4-4[a]	4
2,3,4,5-4[a]	4
2,3-2,3	2,3
2-3,4	3,4
3,4-3,4[a]	3,4
4-2,3,4[a]	2,3,4
2,3,4-2,3,4[a]	2,3,4

[a]These congeners were not significantly degraded

2,5,4′-CB, are degraded to both chlorobenzoic acid and chloroacetophenone via oxidation of the 2,5-chlorophenyl ring. We are trying to determine whether these metabolites arise by independent pathways or whether the chlorobenzoic acid might arise from the chloroacetophenone. The oxidation of 4-hydroxyacetophenone to 4-hydroxybenzoic acid has been demonstrated in an *Alcaligenes* species [Hopper and Elmorsi, 1984; Hopper et al., 1985]. In this case the methyl group of 4-hydroxyacetophenone was oxidized to methanol; subsequent cleavage between the keto group and the methanol substituent produced formic acid and 4-hydroxybenzoic acid. A second method by which acetophenone might be oxidized to benzoic acid is via oxygen insertion between the keto group and the methyl group and subsequent cleavage [Hopper and Elmorsi, 1985].

PCB Degradation by Succinate- and Biphenyl-grown Cells

Last year we reported that cells grown on succinate were virtually unable to degrade certain congeners, notably those substituted in both para positions (e.g., 4,4'). We offered two possible explanations. (1) Two distinct dioxygenases are responsible for PCB degradation in H850: a constitutive 3,4-dioxygenase, and a 2,3-dioxygenase that is inducible by biphenyl (and perhaps PCBs). (2) A single dioxygenase may be responsible for the degradation of all congeners, but may have much higher activity on some congeners than on others. This difference might be magnified in succinate-grown cells if the enzyme were present at lower levels in these cells. We have studied the rate of degradation of several PCB congeners in cells grown on either succinate (in the presence of biphenyl) or biphenyl. Cells grown on succinate degraded 3,3'-CB and 4,4'-CB much more slowly than biphenyl-grown cells, but we detected no such difference when we tested the oxidation of 2,2'-CB and 2,5,2',5'-CB (Figure 2-2). A difference was also seen for 2,3-CB (not shown), but only at very high concentrations (5 mM). The interpretation of these results is complicated by the fact that biphenyl was present in succinate-grown cells. However, we obtained similar results in several experiments on cells grown on succinate in the absence of biphenyl.

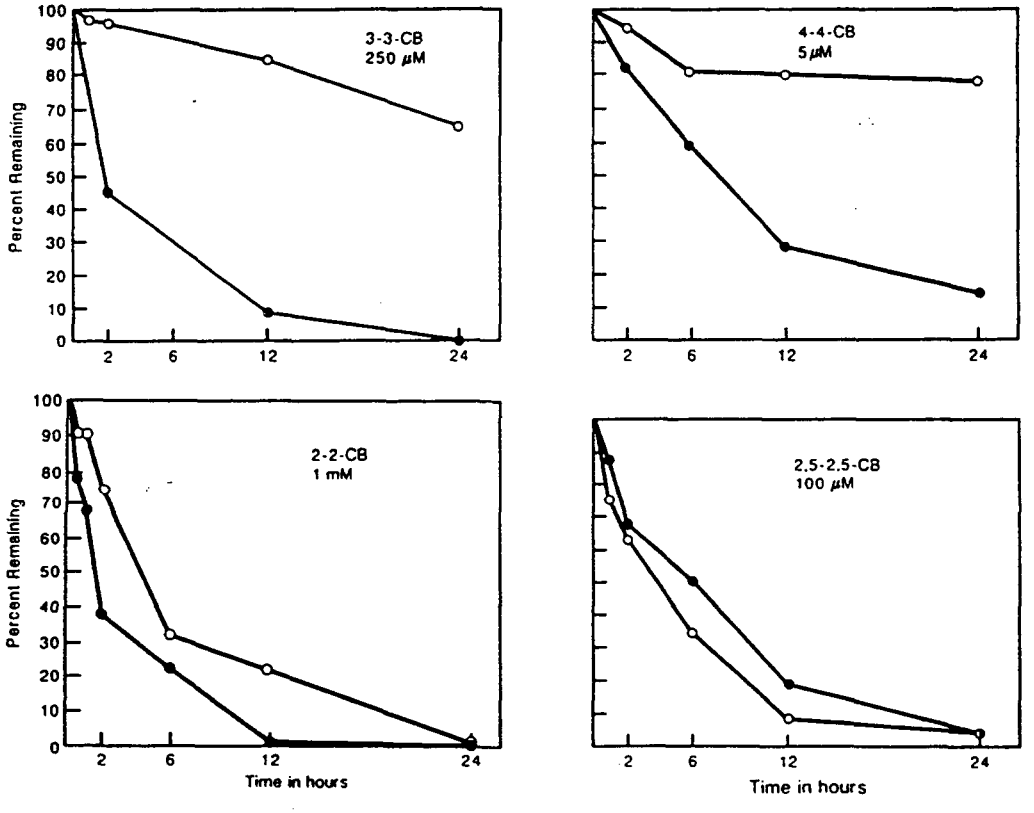


Figure 2-2. The effect of biphenyl-induction on the oxidation of selected PCB congeners; ○ - cells grown in biphenyl; ● - cells grown on succinate in the presence of biphenyl.

It would seem that neither of our proposed explanations is entirely correct. *Alcaligenes eutrophus* FM803 is a mutant of H850 that accumulates 2,3-biphenyl-dihydrodiol, and that is grown on succinate because it is unable to grow on biphenyl (Chapter 4). This mutant exhibits the same kinetics on all of these congeners as succinate-grown H850, and appears to be using a 2,3-dioxygenase for the degradation of 2,3-CB and

2,2'-CB since it accumulates a metabolite from each of these congeners. On the other hand, FM803 behaves similarly to H850 in its metabolism of 3,3'-CB and 2,5,2',5'-CB and produces chloroacetophenone metabolites from each of these congeners.

One possibility is that there are at least two separate dioxygenase activities: (1) an inducible 2,3-dioxygenase that is responsible for the degradation of 2,3-CB, 4,4'-CB, the majority of 3,3'-CB, and at least a portion of 2,2'-CB, and (2) a separate non-inducible enzyme activity that is responsible for the degradation of 2,5,2',5'-CB, a small portion of 3,3'-CB, and possibly 2,2'-CB. Both of these activities are present in succinate-grown cells, but the 2,3-dioxygenase activity is induced to higher levels in biphenyl-grown cells.

SUMMARY AND CONCLUSIONS

There appear to be two different mechanisms involved in PCB degradation in *Alcaligenes eutrophus* H850. A 2,3-dioxygenase pathway is probably responsible for the degradation of congeners with an unsubstituted phenyl ring or a monochlorophenyl ring. Our data suggest that the specificity of this enzyme is quite different from that of MB1 since H850 metabolizes 2,2'-CB at a much higher rate than 4,4'-CB and the reverse is true for MB1.

An enzyme capable of oxidation at positions 3,4 appears to be involved in the degradation of congeners with chlorophenyl rings substituted at positions 2,5 and 2,4,5 (and to a lesser extent, 3- and 2,3,6-). It is possible that it is this enzyme that initiates the pathway leading to the formation of chloroacetophenones. Alternatively, an enzyme capable of attacking at a chlorinated carbon might be involved in the formation of chloroacetophenones. We do not yet know how much PCB is oxidized via chloroacetophenones, or how they are metabolized. We plan to address these issues in future experiments.

FUTURE PLANS

Our primary goals for next year are to determine how much PCB is metabolized via chloroacetophenones, to identify intermediates in the pathway leading to chloroacetophenone production, and to determine how chloroacetophenone is metabolized. We will continue to use resting cell assays for these experiments, but will also use fractionated cell-free extracts in order to separate the various enzyme activities. We also intend to generate mutants that are incapable of growth on acetophenone as an aid in our studies of chloroacetophenone production.

Chapter 3

BIOCHEMISTRY OF PCB DEGRADATION IN *PSEUDOMONAS PUTIDA* LB400

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INTRODUCTION

Pseudomonas putida strain LB400 can degrade PCB congeners falling into several structural classes, including those chlorinated at both para positions with at least one unsubstituted 2,3 position (e.g., 4,4'-CB), those at least partially chlorinated at all 2,3 positions but having one or more unsubstituted 3,4 position (e.g., 2,5,2',5'-CB), those having at least one 2,3 position and one 3,4 position unsubstituted (e.g., 2,5,4'-CB), and those lacking vicinal unchlorinated carbon atoms (e.g., 2,4,5,2',4',5'-CB) [Oakland Report, 1985; Bopp, 1986; Bedard et al., 1986]. We have gained some information about the catabolic pathways involved in the metabolism of PCBs in LB400 by the isolation and structural characterization of several metabolites [Oakland Report, 1985]. However, it has become evident that a complete understanding of PCB degradation by this strain will require detailed study of the various pathways in an unambiguous fashion.

Two approaches have been used. The first is a genetic approach; we have generated mutants blocked at various steps in the PCB-degradative pathways. These will be used in a coordinated strategy with recombinant DNA techniques to isolate, identify, and characterize the various genes responsible for PCB degradation (see Chapter 4). The second is a classical biochemical approach. We have developed cell-free systems which contain many of the enzymatic activities involved in PCB degradation. These cell-free preparations are being fractionated and the enzymes of interest purified and characterized. The techniques involved in isolation, purification, and characterization of PCB metabolites continue to be an important part of this research, since definitive structural analyses of PCB metabolites is crucial to an unambiguous understanding of the biochemistry.

RESULTS AND DISCUSSION

PCB Epoxides

Previous evidence indicated that in LB400 the PCB degradation products of 2,4,5,2',5'-CB, included an epoxide [GE Report, 1985]. However, we had no standard of a PCB epoxide for comparison, and we were unable to isolate a sufficient quantity of the putative epoxide metabolite for definitive structural analysis by proton NMR. Recently, however, Dr. Hans Reich at the University of Wisconsin generously provided us with a synthetic PCB epoxide, the 3,4-epoxide of 2,5,2',5'-tetrachlorobiphenyl.

We have analyzed this synthetic epoxide by mass spectrometry and proton NMR and have confirmed its structure. Comparison of the electron impact mass spectrum of this compound with the putative epoxide metabolite of 2,4,5,2',5'-CB confirms that they are identical except for a difference of 35 in molecular weight (one has five chlorines, the other four). This and other data demonstrate that LB400 converts a portion of this PCB congener to the epoxide and subsequently to the isomeric 3-hydroxy- and 4-hydroxy-pentachlorobiphenyls.

Monooxygenase attack of PCBs yielding an epoxide appears to be a minor degradative pathway in LB400, and does not yield sufficient product for NMR analysis. However, the potential exists to isolate and purify this monooxygenase using the methods described below. If that goal can be achieved, a genetic probe can be synthesized which will allow the isolation of the monooxygenase gene. This may then be used to overexpress the enzyme and produce larger quantities of the epoxide for structural analysis, and to study the relatedness of the gene to monooxygenase genes in other organisms. Interestingly, epoxides are commonly formed in eukaryotic metabolism of aromatic compounds [Jerina et al., 1971] but are unknown in bacteria except in the hydroxylation of phenylalanine [Bowman et al., 1973]. Isolation of this unusual gene may help us to understand the evolution of the novel biodegradative pathways present in LB400.

Cell-free Systems

A cell-free extract was prepared from LB400 and fractionated as shown in Figure 3-1. PCB-degradative activity was assayed at each step by following disappearance of PCB, and by looking for conversion of 2,3-dihydroxybiphenyl to its yellow ring-fission product. Both the crude cell-free extract (10,000 × g supernatant) and the membrane-free extract (110,000 × g supernatant) oxidized 2,3-CB, 2,4,4'-CB, and 2,5,2',5'-CB with or without the addition of NADH. In the case of 2,3-CB, 2,3-dichlorobenzoic acid was seen as a product, indicating that the 110,000 × g supernatant contained the first four enzymes involved in the degradation of this congener (Figure 3-2).

The 110,000 × g supernatant was further fractionated on an anionic exchange column using fast protein liquid chromatograhy (FPLC). Twenty-six fractions were collected and assayed for 2,3-dihydroxybiphenyl catechol dioxygenase activity and, using the yellow ring-fission product from that reaction as substrate, for hydrase activity (see Figure 3-2). Biphenyl 2,3-dioxygenase activity was also assayed by using 2,3-CB as a substrate and using gas chromatography to follow both the disappearance of substrate and production of products. Using these analytical techniques, the catechol dioxygenase and hydrase have been partially purified. Biphenyl 2,3-dioxygenase activity has been found in a single column fraction, but has not yet been separated from biphenyl dihydrodiol dehydrogenase activity. Biphenyl 2,3-dioxygenase activity is NADH-dependent, but neither the catechol dioxygenase nor the hydrase require cofactors. Figure 3-3 shows the enzymatic activities associated with the anion exchange column fractions referred to above, while Figure 3-2 illustrates the first four steps of the pathway.

The development of cell-free preparations that actively metabolize PCBs has allowed us to begin to separate and purify individual PCB catabolic enzymes. This will greatly facilitate our attempts to gain a thorough understanding of PCB degradation by LB400, since it allows us to avoid the ambiguities that inevitably arise when working with whole cells that possess several different catabolic pathways for the same substrate.

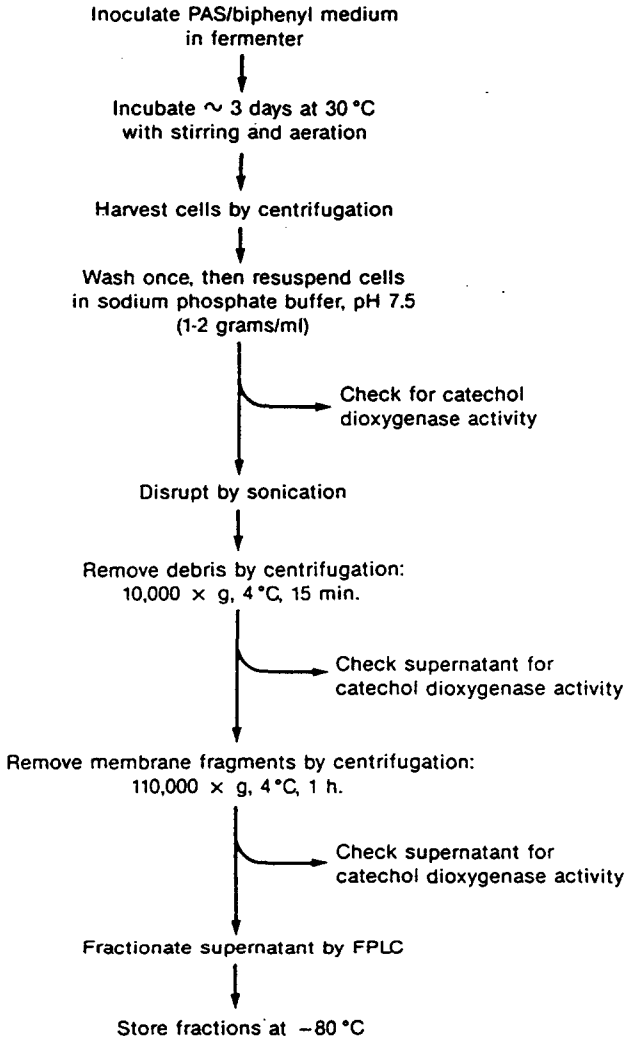

Figure 3-1. Flow chart for the preparation of cell-free fractions with PCB-degrading activity.

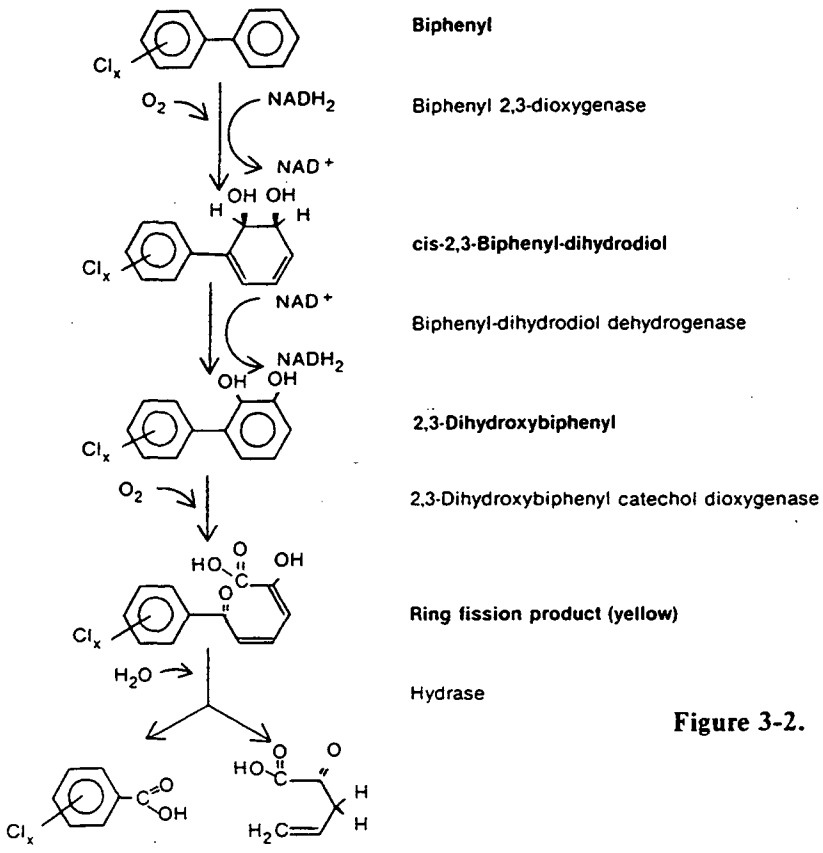

Figure 3-2. Degradation of biphenyl and PCBs by the biphenyl 2,3-dioxygenase pathway.

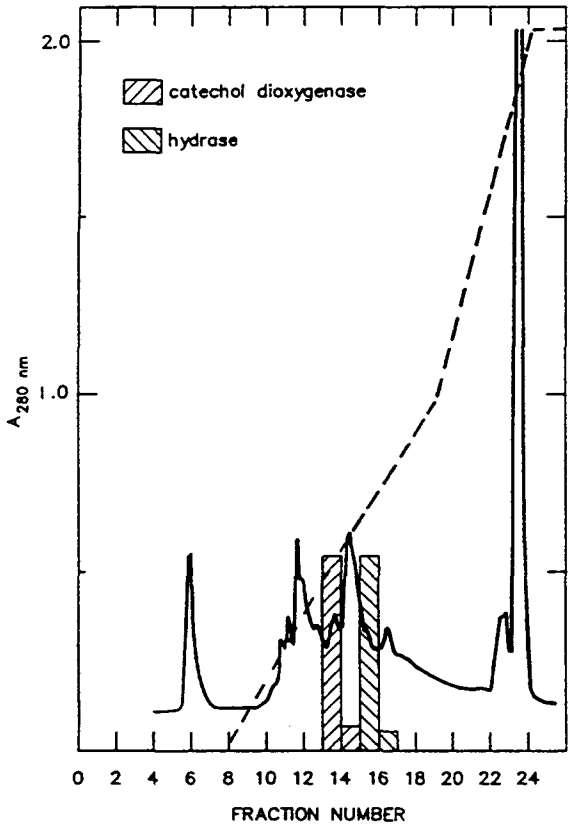

Figure 3-3. FPLC fractionation of the 110,000 × g supernatant of crude enzyme extract containing PCB-degrading activity.

FUTURE PLANS

We are currently examining the substrate specificities and kinetics of the various enzymes in the biphenyl 2,3-dioxygenase pathway. We hope to purify some of these enzymes to near homogeneity in order to use sequence information gained from them to assist in isolating the genes that specify their synthesis.

Crude enzyme preparations are also being used to generate intermediates in the PCB-degradative pathways, some of which are especially difficult to synthesize chemically or to produce with mutant cultures. Both 2,3-dihydroxy-2',3'-dichlorobiphenyl and the yellow ring-fission product of 2,3-dihydroxy biphenyl are being produced in this way. These will then be purified and used as substrates in further enzymatic studies.

A major goal of our research with cell-free studies is to gain a clear understanding of the basis of the 2,3- and 3,4-dioxygenase activities believed to be responsible for the attack of different structural classes of PCB congeners in LB400 (e.g., 4,4'- and 2,5,2',5'-CB) so that the type of pathway illustrated in Figure 3-2 can be unambiguously completed for these two structural classes of PCBs as well as for biphenyl. We are particularly interested in learning whether these activities are associated with a single enzyme or with two different enzymes, and in elucidating their substrate specificities and their mode(s) of regulation. It is also our intention to try to understand at the enzymatic level the mechanisms by which LB400 can degrade PCB congeners lacking vicinal hydrogen atoms and form epoxides from some PCBs.

Finally, this biochemical approach will be used in conjunction with the molecular genetics described in Chapter 4 to explore in detail the parameters necessary to maximize the PCB-degradative ability of LB400. This may involve, for example, isolating the genes involved in PCB degradation and cloning them in high expression vectors so that larger amounts of the enzymes can be produced than could otherwise be obtained.

It is hoped that this integrated approach can be extended to other organisms as well. It should then be possible to investigate problems that cannot be readily addressed using whole-cell systems, and to begin to understand how the various mechanisms for PCB degradation in these organisms are related.

Chapter 4

GENETIC STUDIES OF PCB METABOLISM BY *ALCALIGENES EUTROPHUS* H850 AND *PSEUDOMONAS PUTIDA* LB400

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INTRODUCTION

We have recently begun to apply the techniques of genetics and molecular biology to the study of bacterial PCB catabolism. One of our major goals is the cloning and isolation of the genes encoding biphenyl/PCB degradation. This will enable us to examine the structural and controlling elements of the pathway(s), and may eventually allow us to increase gene expression and the PCB metabolic competence of our organisms.

The isolation and characterization of strains containing mutations in the biphenyl/PCB metabolic pathway is a crucial step toward gene isolation. Such strains aid in the identification of recombinant plasmids containing the genes for PCB degradation. In addition, they facilitate the isolation and identification of metabolites formed from PCB degradation, and thereby aid in determining the pathway(s) involved in PCB catabolism.

Previous studies have indicated a close relationship between the pathways used for metabolizing biphenyl and PCBs [Furukawa, 1979; Furukawa and Miyazaki, 1986]. For example, all PCB-degrading bacteria thus far examined are also able to metabolize biphenyl, and, in fact, growth on biphenyl is frequently used as a selective enrichment in the isolation of PCB-degrading organisms [GE Reports 1983, 1984, 1985; Bedard et al., 1986; and reviewed in Furukawa and Miyazaki, 1986]. The genes encoding the biphenyl 2,3-dioxygenase pathway of *Pseudomonas pseudoalcaligenes* have recently been cloned. These genes were also found to be responsible for the degradation of several PCB congeners [Furukawa and Miyazaki, 1986]. It is therefore possible that the catabolic pathways for biphenyl and PCBs in our organisms may be wholly or partially composed of the same enzymes. If this is true, the isolation of mutant strains unable to grow using biphenyl should provide a convenient method of obtaining strains defective in PCB metabolism. Such studies will also be of great use in establishing the existence of a separate 3,4-dioxygenase enzyme (see Chapter 2).

Mutations can be introduced in a bacterial genome using either chemical or genetic methods. The treatment of a population of cells with N-methyl-N'-nitro-N-nitrosoguanidine (NTG), results in alkylation and depurination of the DNA primarily at the site of replication [Freifelder, 1983].

Mutations can also be created genetically through the use of transposons. Transposons are specific DNA segments that contain the genetic information for a selectable marker (e.g., antibiotic resistance), and that are able to insert themselves randomly

into nearly any site in a bacterial genome. Insertion of a transposon into a gene results in the inactivation of that gene due to interruption of the normal coding sequence [Freifelder, 1986]. An important advantage of this technique is that the inserted transposon provides a genetic "tag" that can be used to isolate the gene via DNA:DNA hybridization.

Selection of Bph$^-$ Mutants

After treatment of the bacterial population with the mutagenic agent, it is necessary to identify those cells containing the desired mutations. We have successfully used a selective/differential medium that enables us to screen thousands of cells simultaneously for those unable to grow using biphenyl as the sole source of carbon and energy (Bph$^-$) [Finette et al., 1984]. When plated onto this medium, wild-type cells with the ability to grow on biphenyl (Bph$^+$), appear as large red colonies, while strains that are unable to grow on biphenyl form small, colorless colonies. After the initial selection, the Bph$^-$ mutants are purified and retested before further characterization.

Rapid Enzyme Screening

The degradation of biphenyl/PCBs via the biphenyl 2,3-dioxygenase pathway is shown in Figure 3-2. We have applied a series of rapid enzyme assays to determine which steps in this pathway are inactivated in Bph$^-$ mutants.

The presence of an active biphenyl dioxygenase, which catalyzes the first step in the pathway, can be determined using the ethereal spray technique developed by Kiyohara et al. [1982]. The method involves spraying an ether solution of biphenyl onto the surface of an agar plate containing the colonies to be tested. This results in deposition of a thin film of biphenyl on the plate surface. Colonies of cells with an active biphenyl dioxygenase form a clear zone in this film upon incubation while colonies lacking this enzyme do not (see Figure 4-1).

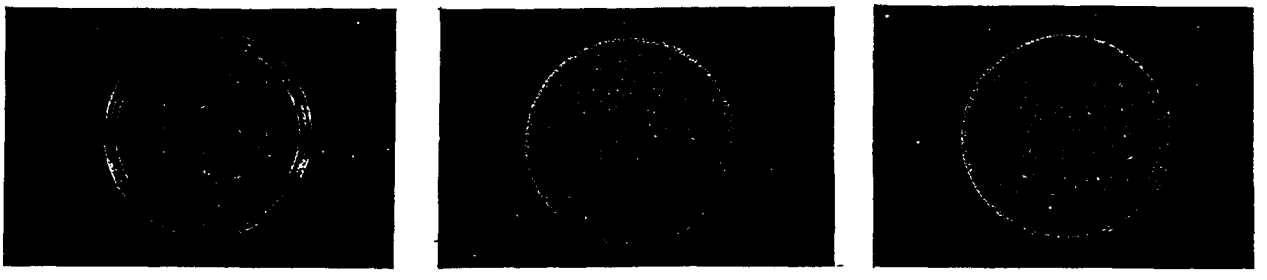

Figure 4-1. Clearing assay for biphenyl dioxygenase activity. Left, Agar plate containing patches of *P. putida* strains LB400 and FM202, a biphenyl dioxygenase mutant; center, appearance of plate immediately after spraying with an ether solution containing 1% biphenyl; right, appearance after 24 h incubation at 30 ° C. The patch lacking a cleared zone indicates the position of the dioxygenase mutant.

The third and fourth steps of the pathway involve, respectively, the production and metabolism of the ring-fission product of 2,3-dihyroxybiphenyl. The activity of 2,3-dihydroxybiphenyl catechol dioxygenase was tested colorimetrically as described previously [GE Report, 1985]. Hydrase mutants (unable to metabolize the yellow ring-fission product) were identified by the visible accumulation of this metabolite during growth of the organism in the presence of biphenyl (see Figure 4-2).

Figure 4-2. Accumulation of the yellow ring-fission product of 2,3-dihydroxybiphenyl by Bph⁻ mutants lacking hydrase activity. Cells of *P. putida* strains: (A) wild-type LB400, and the Bph⁻ mutants (B) FM205 and (C) FM206, after 8 h exposure to biphenyl.

Strains that were Bph⁻ but were found to be active in steps 1, 3, and 4 of the pathway were suspected of containing a mutation in biphenyl dihydrodiol dehydrogenase, which catalyzes the second step in the pathway. Such cells can be identified by their accumulation of *cis*-2,3-biphenyl dihydrodiol when grown in the presence of biphenyl. This metabolite can be detected spectrophotometrically by its characteristic absorbance maximum at 303 nm [Gibson et al., 1973].

RESULTS AND DISCUSSION

Characterization of Mutants Blocked in the Biphenyl 2,3-Dioxygenase Pathway

Our studies have focused on two bacterial strains found to have outstanding PCB-degradative competence: *Alcaligenes eutrophus* H850, and *Pseudomonas putida* LB400 [GE Reports, 1983, 1984, 1985]. Both types of mutagenic techniques have been applied to each of these organisms in attempts to generate strains unable to grow using biphenyl as the sole source of carbon and energy.

We have been successful in using transposon mutagenesis to generate and isolate a variety of Bph⁻ mutants of *P. putida* LB400. The characterization of these strains indicates that we have achieved our goal of obtaining a series of mutants blocked in each of the four initial steps of the biphenyl pathway. As expected, cells with inactivated enzymes for steps 2, 3, and 4 of the biphenyl pathway accumulate the metabolites 2,3-biphenyl-dihydrodiol, 2,3-dihydroxybiphenyl, and the ring-fission product of 2,3-dihydroxybiphenyl, respectively (see Figure 4-2).

Thus far we have been unable to produce transposon mutants of *A. eutrophus* H850. This may be due to poor mobilization of the plasmid containing the transposon into H850, or to an inherent inability of the transposons Tn*5* or Tn*7* to function in this bacterium. Further attempts will be made to mutagenize this organism with other transposable elements. NTG mutagenesis of *A. eutrophus* H850 has been more successful. In this organism, two types of Bph^- mutants that accumulate metabolites from biphenyl oxidation have been isolated. Strain FM803 accumulates large quantities of biphenyl dihyrodiol, indicative of the inactivation of biphenyl dihydrodiol dehydrogenase. The second mutant strain, FM888, appears to contain a defective hydrase because growth in the presence of biphenyl results in the accumulation of the yellow ring-fission product.

Effect of Biphenyl Pathway Mutations on PCB-degradative Competence

If the enzymes involved in biphenyl metabolism also function in the degradation of PCBs, then Bph^- mutants should also be defective in PCB metabolism. This may be detected either as a buildup of metabolites not normally accumulated during catabolism, or by the inability to degrade a congener in a depletion assay. We have recently begun to address this question by comparing the abilities of the wild-type and mutant cells to degrade certain PCB congeners. The biphenyl dihydrodiol dehydrogenase mutant FM803 of *A. eutrophus* H850 has been found to accumulate a dichlorobiphenyl-dihydrodiol metabolite (presumably *cis*-2,3-dihydrodiol-2′,3′-dichlorobiphenyl) from the degradation of 2,3-dichlorobiphenyl. This metabolite is not detected during the incubation of 2,3-CB with the wild-type strain. These results indicate that the biphenyl dihydrodiol dehydrogenase enzyme from the biphenyl 2,3-dioxygenase pathway is used in the degradation of this PCB congener.

We have also found evidence for the existence of PCB-specific enzymes. Certain PCB congeners (for example, 2,5,2′,5′-CB) are metabolized normally by cells that contain an inactive biphenyl dihydrodiol dehydrogenase (FM803). This dehydrogenase is therefore not involved in the metabolism of 2,5,2′,5′-CB. Conversely, the enzyme responsible for metabolizing the *cis*-3,4-dihydrodiol of 2,5,2′,5′-CB is not active on *cis*-2,3-biphenyl dihydrodiol, since the latter compound is accumulated by FM803.

FUTURE PLANS

We are continuing our investigation of how mutations in the biphenyl 2,3-dioxygenase affect the ability to degrade the various structural classes of PCBs. In addition, we are preparing to clone, into an *Escherichia coli* host, gene libraries for both *P. putida* LB400 and *A. eutrophus* H850. Once constructed, we will use our enzyme-screening techniques to identify the recombinant clones containing genes for biphenyl/PCB metabolism.

Chapter 5

REDUCTIVE DECHLORINATION OF PCBs IN ANAEROBIC MICROBIAL COMMUNITIES

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INTRODUCTION

The polychlorinated biphenyl (PCB) congener distribution patterns for anaerobic sediment samples from the upper Hudson River [Brown et al., 1984] and Waukegan Harbor [Stalling, 1982; J.F. Brown, Jr., personal communication] suggest that the preferential reductive dechlorination of some of the more highly chlorinated congeners may be occurring in those environments. It is believed that a biological process must be involved because congener selectivity is observed and no known nonbiological agents having sufficient reducing power can exist in this sediment [Brown et al., 1984]. Others have suggested that the loss of higher chlorinated congeners may be explained by selective partitioning of these congeners. Thus independent experiments that directly demonstrate biological dechlorination are important to clarify the mechanism and to make sound judgments on PCB cleanup schemes.

We have demonstrated microbially mediated anaerobic dechlorination of a variety of aromatic compounds including chlorobenzoates [Suflita et al., 1983; Shelton and Tiedje, 1984], chlorophenols [Boyd et al., 1983; Boyd and Shelton, 1984], chlorobenzenes [Tiedje et al., in press], pentachlorophenol [Mikesell and Boyd, 1985], 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) [Mikesell and Boyd, 1985; Suflita et al., 1984], and 2,4-D [2,4-dichlorophenoxyacetic acid, [Mikesell and Boyd, 1985]. Here we report our progress in conducting experiments to demonstrate biological, anaerobic dechlorination of selected PCB congeners and our plans for future work in this area.

Our basic experimental procedure consists of adding a mixture of five PCB congeners (see Table 5-1) in 50 μl of acetone to 50 ml of anaerobic Hudson River sediment or sewage sludge in tightly stoppered serum bottles. The sludge used was collected from the sewage treatment plant in Jackson, Michigan and was used immediately ("fresh") or after acclimation to each of the (ortho-, meta-, and para-) monochlorophenols. The PCB mixture was added to yield a final concentration of 1 ppm of each congener. Samples are taken periodically (zero time and after 1, 4, 8, 16 and 32 weeks) and analyzed for a decrease in the concentration of the congeners added and/or the appearance of dechlorinated products. The samples are extracted with 15% methylene chloride in hexane and analyzed by gas chromatography using a capillary column.

RESULTS

Variation due to sampling, extraction, and detector response mean that looking for reduction in parent compound concentration is a relatively insensitive method for detecting dechlorination. A far more sensitive method is to look for the appearance (or increase in size) of peaks associated with potential dechlorination products. Because the more highly chlorinated congeners are most likely to be dechlorinated, we

Table 5-1

LIST OF SELECTED CONGENER NUMBERS[a]

Congener number	Chlorine substitution pattern	Retention time (min)
Congeners added:		
66	2,4,3',4'	14.668
116	2,3,4,5,6	16.882
155	2,4,6,2',4',6'	15.299
183	2,3,4,6,2',4',5'	21.729
194	2,3,4,5,2',3',4',5'	30.376
Potential dechlorination products from PCB congeners 183 and 194:		
85	2,3,4,2',4'	17.080
91	2,3,6,2',4'	14.981
97	2,4,5,2',3'	16.658
99	2,4,5,2',4'	15.591
105	2,3,4,3',4'	19.526[b]
118	2,4,5,3',4'	18.499
128	2,3,4,2',3',4'	21.506
129	2,3,4,5,2',3'	21.118
138	2,3,4,2',4',5'	20.735[b]
141	2,3,4,5,2',5'	20.100
146	2,3,5,2',4',5'	19.260
153	2,4,5,2',4',5'	19.653
156	2,3,4,5,3',4'	23.068[b]
167	2,4,5,3',4',5'(?)	21.965
170	2,3,4,5,2',3',4'	25.655
180	2,3,4,5,2',4',5'	23.968

[a]Ballschmiter and Zell, 1980

[b]Congeners which coelute at these retention times are 132 (2,3,4,2',3',6'-CB); 163 (2,3,5,6,3',4'-CB), and 171 (2,3,4,6,2',3',4'-CB), respectively.

looked for dechlorination products of the 2,3,4,5,2',3',4',5',-octachlorobiphenyl and 2,3,4,6,2',4',5' heptachlorobiphenyl. The potential dechlorination products of these congeners and their retention times are listed in Table 5-1.

Data analysis has been completed only for incubations of one and four weeks, because we have saved the later samples for analysis as one batch. There was no evidence for PCB dechlorination in any of the sludge samples in this time period. The chromatograms had a relatively low background, indicating that a simple visual inspection of the chromatograms would be sufficient to detect the appearance of peaks associated with dechlorination products. However, none were observed.

Because the Hudson River sediments contain PCBs, dechlorination products must be detected by looking for increases in their relative peak areas with time. To facilitate comparisons, ratios were formed by dividing the area of the potential product by that of the parent congener and multiplying by 10,000. Comparisons were made only between samples from the same bottle in order to alleviate any problem of variation in PCB concentration.

Several dechlorination products may be indicated in the Hudson River sediment treatments. Results are equivocal, as there were differences between the two experimental replicates (denoted E1 and E2). There were also changes over time for the autoclaved controls (denoted C1 and C2), although generally to a lesser extent than for the experimental treatments.

The largest changes were observed in the relative peak areas for the potential dechlorination products of the octachlorobiphenyl (Table 5-2). After one week of incubation with the PCB mixture, relative peak areas for E1 had increased more than for controls for congeners 129, 105 (and 132), 138 (and 163), 141, 146, 153, and 167. By the second week, all relative peak areas except 141 had decreased to near zero time values. In E2 the relative peak areas for congeners 105 (and 132) and 153 increased during the first week and did not change appreciably thereafter. The relative peak area for congener 156 (and 171) had increased slightly more than controls by the fourth week.

Table 5-2

CHANGE IN RELATIVE PEAK AREAS FOR POTENTIAL DECHLORINATION PRODUCTS OF 2,3,4,5,2',3',4',5'- OCTACHLOROBIPHENYL

Congener number	Change from zero time (%)							
	E1		E2		C1		C2	
	1 wk	4 wk	1 wk	4 wk	1 wk	4 wk	1 wk	4 wk
85	8	2	15	−4	105	128	118	139
105(+132)	56	2	91	118	11	20	1	14
118	28	6	−4	−2	5	28	11	20
128	39	0	−40	2	5	24	37	48
129	145	8	−39	−63	3	23	70	72
138(+163)	29	3	13	2	8	20	14	17
141	278	60	−27	3	22	13	10	−9
146	153	1	−3	14	60	17	4	58
153	55	5	132	138	9	17	8	16
156(+171)	69	0	−11	87	12	21	66	85
167	186	3	0	157	7	15	146	173
170	16	14	−13	−15	−6	16	−13	−9
180	−20	−26	−11	35	11	20	14	18

There was even less consistency between replicates when comparing relative peak areas for potential dechlorination products of the heptachlorobiphenyl (Table 5-3). For E1, increases were greater than controls for peaks 118 and 138 (and 163) after one week. Congener 138 could also be formed from the octachlorobiphenyl. For E2, increases were greater than controls for peaks 91, 97, and 99. By the second week, relative peak areas for E1 and E2 were closer to the zero time values than for the controls. The relative peak area for 85 more than doubled in both controls, but did not change appreciably in the experimental treatments.

Table 5-3

PERCENT CHANGE IN RELATIVE PEAK AREAS FOR POTENTIAL DECHLORINATION PRODUCTS OF 2,3,4,6,2',4',5'- HEPTACHLOROBIPHENYL

Congener number	Change from zero time (%)							
	E1		E2		C1		C2	
	1 wk	4 wk	1 wk	4 wk	1 wk	4 wk	1 wk	4 wk
85	6	−1	37	11	107	121	119	130
91	2	0	80	10	11	14	24	18
97	12	8	64	23	13	18	6	25
99	5	2	70	13	10	17	12	16
118	26	4	14	13	12	19	8	15
138(+163)	27	1	3	12	9	16	15	13

SUMMARY AND CONCLUSIONS

As a whole, the data suggest that dechlorination may be occurring at a very slow rate (less than 1% of the octa- and heptachlorobiphenyl added in four weeks). The evidence at this time is weak due to the difficulty in detecting such small increases against the PCB background present in the Hudson River sediment and the inherent variation in peak areas near the threshold of detection.

FUTURE PLANS

Our future experiments will be carried out using, in addition to the Hudson River sediments, highly enriched chlorobenzoate and chlorophenol cultures, and biphenol enrichments from sludge and sediments.

There are two basic problems to be overcome in future studies: maintaining biological activity during long term incubations, and detecting the accumulation of dechlorination products against the background level of PCBs present in the Hudson River sediment. We intend to try several approaches to solving each problem.

While it is evident that detection of PCB dechlorination will require incubation times of at least several months, in similar studies we have noticed that biological activity, as indicated by methane production, decreases drastically after three to four weeks. Thus it will be necessary to feed the culture periodically during long term incubations, but not with substrates that inhibit dechlorination.

We intend to try several different classes of substrates to feed the cultures, with the emphasis on those substrates that are least likely to limit dechlorination. These studies have already been initiated but the results are not yet available. Substrates to be tried include:

(1) Substrates to provide electrons for reducing power, such as ethanol and H_2. The dechlorination mechanism obviously requires reducing power since the products are more reduced, and show net H_2 consumption.

(2) Analogous substrates; such as methoxy- and hydroxybiphenyls. Such substrates may induce enzymatic pathways that allow microorganisms to cometabolize PCBs.

(3) Substrates to support microbial scavengers, such as rumen fluid and sludge extract. Such scavengers must have diverse metabolic capabilities that may include dechlorination. The pure culture (DCB-1) that dechlorinates chlorobenzoates has characteristics typical of microbial scavengers and is stimulated by rumen fluid [Linkfield, 1985].

(4) Yeast extract. We have previously observed that small quantities of yeast extract may support dechlorination. It may provide necessary cofactors or enrich for unusual organisms.

[^{36}Cl]-labeled hexachlorobenzene (HCB) will be used in similar experiments to learn more about which substrates better support dechlorination. We have already detected HCB dechlorination products by gas chromatography and have developed a sensitive assay procedure for detecting $^{36}Cl^-$ released by dechlorination.

There are several ways to alleviate the problem of detecting PCB dechlorination products against high background PCB levels. We plan to eventually use [^{36}Cl]-PCB congeners and look for the $^{36}Cl^-$ as the product rather than PCB daughter products. Alternatively, we will use sediments with lower PCB levels or will culture Hudson River sediment microorganisms in a sediment-free system. The obvious risk in the last approach is that we may inadvertently select for the wrong organism. A significant advantage is that with no sediments to bind the added PCBs, which may be a factor limiting dechlorination, their bioavailability may be increased.

If dechlorination can be demonstrated (or preferably enhanced) even if at a slow rate, cost-effective schemes can be envisioned in which the dechlorination could be allowed to proceed in situ, while isolated from other fates and impacts of PCBs.

Chapter 6

ENVIRONMENTAL TRANSFORMATIONS OF PCBs

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INTRODUCTION

Two years ago, we discovered a method for characterizing the transformations of PCBs in environmental deposits. This method is based on a special peculiarity of PCB composition. This is, that the commercial PCB products (e.g., Aroclors) were not individual chemical compounds, but instead were complex mixtures of isomers and homologs (congeners) that were formed in fixed relative proportions by the electrophilic chlorination process used in their manufacture. Since every chemical, physical, or biological process removes these PCB congeners according to a different set of relative rates, the congener distribution in the PCB residue from an environmental deposit will show a pattern of alteration that is characteristic for those agents that have acted on the PCB. During the past year we have identified new PCB alteration patterns that differ from those previously reported [GE Reports, 1984; 1985; Brown et al., 1984], and have expanded our examination of sites at which the various types of PCB transformation might be occurring.

RESULTS

Additional Transformation Patterns Characterized

Aroclor Evaporation Products. Aroclor 1242 samples were allowed to evaporate at 25 °C over periods of up to 6 weeks, and then analyzed for congener distribution and weight loss. It was noted that specimens evaporated to 30-50% weight loss were virtually indistinguishable from Aroclor 1248 by DB-1 capillary GC. After higher (>90%) weight loss, the chromatograms showed mainly the strong peaks of mono-ortho tetra- and pentachlorobiphenyls.

An unmistakable Aroclor 1260 evaporation pattern showed up in one soil surface sample: the hepta- and octachlorobiphenyl peaks were in the same relative proportions as in 1260, but those of the hexachlorobiphenyls were reduced by half, and those of the pentachlorobiphenyls were nearly absent.

Aroclor Extraction Patterns. A gas chromatogram of PCBs recovered from an aqueous extract of Aroclor 1242 resembled that previously reported for an extract of Aroclor 1016 [Mieure et al., 1976]. Due to the greater solubility of lower congeners, there was an eightfold enhancement of monochlorobiphenyls relative to trichlorobiphenyls and an eightfold depression of pentachlorobiphenyls.

Pattern X and X'. The capillary GC patterns of several sediment specimens and water samples from the upper Hudson showed marked enhancement of the peaks given by 2,6,X'-trichlorobiphenyls relative to those of the di- and other trichlorobiphenyls.

A related pattern, Pattern X', often seen in the chromatograms of Aroclor 1242-contaminated soils, showed the packed column peaks at relative retention times (RRT) 21, 28, 35, and 40 depressed relative to those at 24, 32, 37, and 47, indicating a similar pattern of selective congener removal. This selective congener removal is most likely the result of aerobic biodegradation.

Pattern W. A review of the reported PCB GC patterns [Stalling, 1982] and congener distributions in Waukegan Harbor sediments and fish provided evidence for another meta-, para- (m,p-) dechlorination system, characterized by Apolane C-87 capillary GC Pattern W. It is believed that the corresponding DB-1 capillary pattern (which we have not yet obtained) would be similar to upper Hudson Pattern B [GE Reports 1984, 1985, Brown et al., 1984], although with less persistence of 2,5,2',5'- and 2,4,2',5'-chlorobiphenyls.

Survey of Environmental PCB Deposits

Airborne PCBs. In order to determine the contribution of airborne PCBs to those found in soils and sediments, three specimens of arctic-alpine zone peat were collected from the summit of Mt. Algonquin (5114 ft elevation, Essex County, N.Y.), the second highest peak in the Adirondacks. Observed levels of PCBs (primarily Aroclor 1242 plus some 1254/1260) were 46 to 87 ppb; of DDT and its derivatives, 118 to 432 ppb.

PCBs on Soils. Several dozen archival packed column chromatograms and a few new DB-1 capillary chromatograms from three Aroclor 1242 spill sites were reviewed. These sites were (1) the Suisman and Blumenthal Scrap Metal Company in Hartford, Conn.; (2) the now-remediated Caputo landfill near Fort Edward, N.Y.; and (3) the dragstrip return road at South Glens Falls, N.Y., which had been oiled with scrap Aroclor 1242 in the 1960s. Most samples from each site showed patterns resembling evaporated 1242 or evaporated Pattern X'.

PCBs in Water. Two samples of water from wells in Fort Edward, N.Y., that were down-gradient from Aroclor 1242-containing landfills prior to remediation showed similar gas chromatograms, exhibiting Pattern X. Detailed analysis of the composition of one of these, which contained 11.8 ppb total PCB, suggested that it arose by water extraction of Aroclor 1242, followed by biodegradation of the more easily degradable congeners, which constituted about 75% of the total extract. This is believed to be the first observation of PCB biodegradation in groundwater.

The GC of filtered water from the upper Hudson River collected during the spring runoff in April 1983 [Bopp et al., 1985] gave a pattern that showed greater than 98% removal of the mono-ortho dichlorobiphenyls 2,3'- and 2,4'-CB. The PCB could not have been in the river water more than a few hours at the time of sampling; however, the samples were not filtered immediately, so the observed biodegradation may have taken several days.

PCBs in Sediments. Gas chromatograms submitted to us by other analysts showed ortho-, meta-, para-dechlorination patterns like F or G in the sediments of Sheboygan Harbor (Wis.), the Hoosic River (Mass.), and in a few of those from the Hudson River below Stillwater, N.Y. Meta-, para-dechlorination Pattern W was seen to varying extents in all those from Waukegan Harbor (Ill.), and Pattern X in some from New Bedford Harbor (Mass.) and a few of those from the upper Hudson River. The "Aroclor 1016" pattern previously reported for many 1977 sediments of the lower Hudson [Bopp et al., 1984] was found to be similar to the early developmental stages of meta-, para-dechlorination Pattern B.

SUMMARY AND CONCLUSIONS

The foregoing observations and previous reports delineate five types of transformations that may affect PCBs in appropriate environmental niches:

Evaporation. This physical process is evidently capable of removing the more volatile PCB congeners, up through at least the hexachlorobiphenyls, from the surface of soils or sediments. For Aroclor 1242 at a northern New York State dragstrip, the evaporation loss rate appears to have averaged about 6% per year.

Extraction into Water. This physical process has been found to account for a sizeable part of the downstream PCB transport in the Hudson River at times of low water flow [Bopp et al., 1985], the remainder of the transported PCB being particle-borne. It must also account for the totality of any PCB found in groundwater more than a few meters from the point of deposit. Only the lower PCBs are subject to significant extraction; water extracts of Aroclor 1242 contain only 1% congeners with more than four chlorines per biphenyl, versus nearly 10% in the original Aroclor.

Degradation by Sunlight. This photochemical process probably induces several forms of chemical alteration, but predominantly reductive dechlorination, particularly of the more heavily chlorinated PCB congeners. Modeling studies indicate that these may be removed from the upper layers of large lakes or oceans with half-times of 1 to 2 years [Bunce et al., 1978].

Dechlorination in Anaerobic Sediments. This presumably microbiological process, first described in earlier reports of this series [GE Report 1984; 1985] and subsequently elsewhere [Brown et al., 1984; 1985] accomplishes the dechlorination of those more heavily chlorinated PCB congeners that have accumulated in inland sediments rather than in the off-shore water column. It has now been observed in the sediments of the Hudson River in New York, Silver Lake in Pittsfield, Mass., the Hoosic River (Mass.), Sheboygan Harbor (Wis.) and Waukegan Harbor (Ill.). The process produces detoxicated mixtures of lower PCB congeners that are readily biodegradable by aerobic bacteria [Brown et al., 1984]. In upper Hudson sediments, the more reactive congeners (e.g., the 3,4,2′-; 2,3,4′-; 2,3,2′,5′-; 2,3,2′,4′-; 2,4,5,4′-; 2,5,3′,4′-; 2,4,3′,4′-; 2,3,4,4′-; and 2,3,3′,4′-chlorobiphenyls) are being dechlorinated with half-times of about 3 to 4 years.

Oxidative Microbial Biodegradation. This microbiological process, which can be effected by a wide variety of environmental bacteria in the laboratory [Bedard et al., 1986; Bopp, 1986] has now been detected via congener depletion patterns in soil, sediment, fish, and water samples from several sites. The most rapidly removed PCB congeners include the mono- and dichlorobiphenyls and certain of the trichlorobiphenyls. Additional tri- and tetrachlorobiphenyls and some pentachlorobiphenyls are known to be biodegradable in the laboratory [Bedard et al, 1986; Bopp, 1986] and to be generally missing from environmental PCB samples collected at locations remote from point sources [Nisbet, 1972]; hence, they are probably also being biodegraded in the environment, but more slowly.

FUTURE PLANS

During the next year we plan to define the kinetics of PCB dechlorination in Hudson River sediments, and characterize the transformations experienced by the PCBs resident in groundwater, Hudson River fish, and other environmental accumulations.

Chapter 7

PROCESS MODELING: AEROBIC BIODEGRADATION OF PCBs

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INTRODUCTION

Last year we expanded our studies of PCB biodegradation in an effort to explore the possibility of using one or more of our new bacterial isolates for the decontamination of PCB-laden soil. *Corynebacterium* sp. MB1, *Alcaligenes eutrophus* H850, and *Pseudomonas putida* LB400 had all been shown to have excellent PCB-degrading capabilities under laboratory assay conditions; however, little was known about their ability to oxidize PCBs adsorbed to soil. Our initial studies [GE Report, 1985] using sand as a model demonstrated that PCBs were biodegradable even when bound to solid substrates.

More recently we began model biodegradation studies using PCB-laden soil containing either Aroclors or pure PCB congeners. Experimentally, our approach is identical to our previously described resting cell assays, except that the PCBs are bound to soil when mixed with the bacteria. For these studies the cultures were grown on PAS [GE Reports, 1982 through 1985] with biphenyl (BP) as the sole carbon source, then harvested, washed, and resuspended in buffer at a concentration of approximately 10^9 cells/ml. These washed cells were then incubated with one of several PCB/soil formulations: 50 ppm of Aroclor 1242 spiked onto clean (i.e., non-PCB containing) soil; 500 ppm of Aroclor 1242 on clean soil; 50 ppm of Aroclor 1254 on clean soil; an environmental sample (South Glens Falls, N.Y., dragstrip site 43C), which contains 525 ppm PCB (very similar in composition to Aroclor 1248); and various concentrations of pure PCB congeners spiked onto clean soil. In addition, some assay variations were conducted using unwashed cells, Luria-grown cultures, cultures actively growing on biphenyl, unstirred samples, and limited amounts of water.

RESULTS AND DISCUSSION

Biodegradation of Soil-bound Aroclors

The results to date have been very promising. Our first experiments with soil containing 50 ppm of Aroclor 1242 demonstrated extensive biodegradation of this Aroclor by all three bacterial strains assayed (MB1, H850, LB400). MB1 and LB400 degraded better than 95% of the PCBs in as little as one day. A parallel assay was also conducted with MB1 grown on Luria broth. The Luria-grown cells exhibited only moderate PCB-degrading activity: less than 25% of the PCBs were degraded in one day. This is consistent with our previous observations using standard non-soil assays, which showed that BP-grown cells have much better PCB-degradative competence [GE Re-

port, 1985]. In a second parallel assay using BP-grown MB1 the rate of degradation of PCBs bound to soil was the same as that for free PCBs in a non-soil assay. This result was surprising, yet encouraging. One might have expected the PCBs to be irreversibly sequestered in soil particles, thereby decreasing the extent of degradation. This was not the case for any of the time points assayed (2 hours, 1 day, 3 days). This may be because these soil assays were conducted under water-excess conditions (1.0 ml aqueous culture with 0.1 g soil). Using field conditions (much less water) we may observe a difference between free and bound-PCB biodegradation. Such studies are currently under way.

Several other PCB-degrading cultures (H201, Pi704, and H430) were also assayed for their ability to degrade PCBs on soil (50 ppm of Aroclor 1242). These moderately active strains (as defined by our previous in vitro studies) [GE Report, 1985] are also only moderately active toward soil-bound PCBs.

As we observed for free PCBs, each of our strains also exhibits congener-specific degradation of soil-bound PCBs. That is, MB1 is better able to degrade soil-bound double-para-substituted PCBs (e.g., 4,4'-CB), and H850 and LB400 are better able to degrade soil-bound congeners with blocked 2,3 positions, such as 2,5,2',5'-CB. This specificity is clearly evident in Figure 7-1. LB400 more readily degrades peak 1 and the center peak of the triplet designated by three dots. On the other hand, MB1 more readily degrades peaks 3,4 and the first and third peaks of the triplet. Because the specificities of MB1 and LB400 complement each other, it seemed reasonable to try a biodegradation using a mixed culture consisting of both strains. Figure 7-1 (third panel) shows the results of such an assay and demonstrates the additive effect of using a mixed culture. This confirms the proposition we put forth several years ago that a greater degree of biodegradation could be obtained by exploiting the two different biological activities harbored by MB1 (2,3-dioxygenase activity) and H850 or LB400 (3,4-dioxygenase activity).

We have extended our Aroclor biodegradation studies to higher concentrations and more difficult Aroclors. LB400 was able to degrade greater than 85% of the PCB in a 500 ppm Aroclor 1242 assay incubated for two days. In a 50 ppm Aroclor 1254 assay, LB400 degraded greater than 65% of the PCB in one day. Studies are currently under way with Aroclors at 500 and 5000 ppm on soil.

In addition to the resting cell assays described above, we are interested in modeling an in situ biodegradation using actively growing cultures. For these studies we have introduced a 1.0% LB400 inoculum into an assay flask containing soil-laden PCB, PAS growth medium, and solid biphenyl as carbon and energy source. The assay was monitored for both cell count and PCB biodegradation. In our first experiment LB400 grew well (five cell doublings in one day), and the actively growing cells degraded the PCB. However, the extent of Aroclor 1242 degradation (40% in one day) was significantly less than with resting cells. By the next time point (3 days) the viable cell count was down more than 10,000-fold; i.e., the culture had died sometime after 24 hours. (This instability in LB400 has been observed previously with Luria-grown LB400 cultures and is under investigation). The degree of PCB degradation for the 3-day and 6-day (also not viable) samples was essentially the same as for the 1-day samples. It thus appears that when the culture died, PCB degradation ceased. There are several possible explanations for the lower amount of degradation seen for growing cells as compared to resting cells. One of these is that the biphenyl, which is in vast excess over the PCB, competes with the PCB for a limited amount of enzyme. To address this question we have tried several resting cell assays in the presence of BP. For

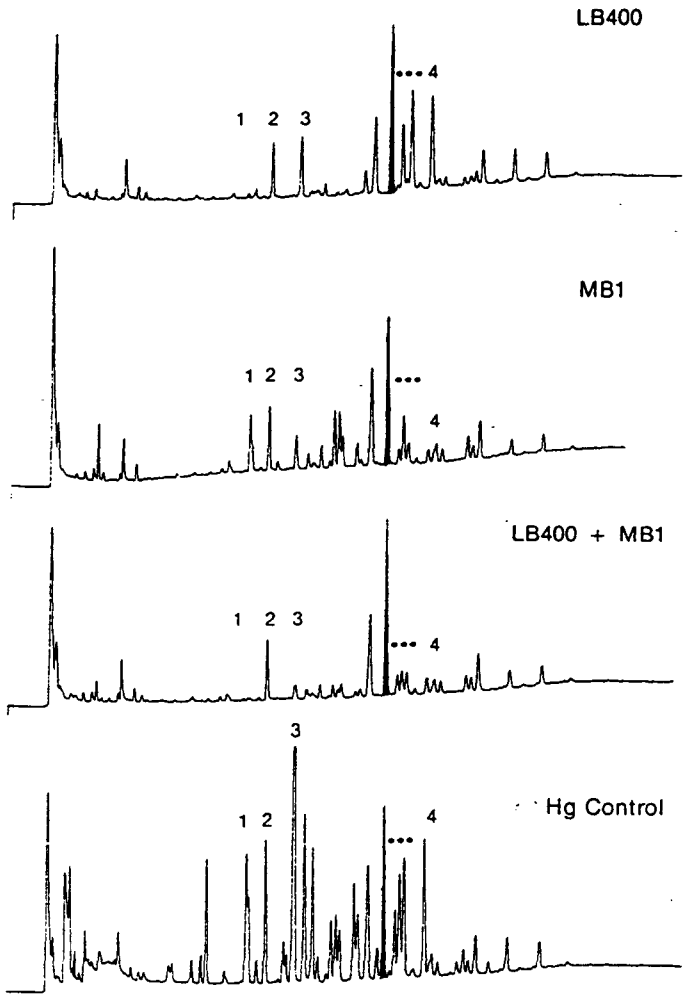


Figure 7-1. Biodegradation of soil-bound Aroclor 1242 by MB1 and LB400 applied separately or as a mixture. Soil spiked with 50 ppm of Aroclor 1242 was incubated with resting cells for 3 days. Cells (2 ml at approximately 10^9 cells/ml) were incubated with 0.4 g of soil. The shaded peak is a nondegradable PCB congener used as an internal standard.

example, MB1 showed little or no difference in the degree of PCB degradation (50 ppm 1242 on soil) when assayed with and without 0.1% BP present. These results indicate that the presence of biphenyl is not a problem; however, further work needs to be done.

We have recently begun studies with an actual PCB-contaminated soil from New York State. This environmental sample is from the site of a former racing dragstrip where PCB oils were used for dust control. The site contains Aroclor 1242 at concentrations up to 7000 ppm. The sample we obtained contains 525 ppm of a transformed Aroclor 1242 that is depleted in the di- and trichlorobiphenyls, and therefore appears similar in composition to Aroclor 1248. We have been unable to determine whether this transformation is evaporative and/or biological; however, we have been able to duplicate an almost exact congener depletion profile by biodegrading soil-bound Aroclor 1242 using a higher soil:water ratio, which limited the biodegradation to lower chlorinated congeners.

Resting cell biodegradation studies using the dragstrip soil have shown substantial PCB biodegradation. As seen in Figure 7-2, LB400 degraded 15% of the PCBs in one day and 51% in three days. This compares with 85% degradation of Aroclor 1242 (500 ppm) on laboratory soil. MB1 and H850 also biodegraded the dragstrip PCBs, but to a somewhat lesser extent. Our current studies are focusing on conditions for maximizing the rate and extent of PCB degradation in this environmental sample.

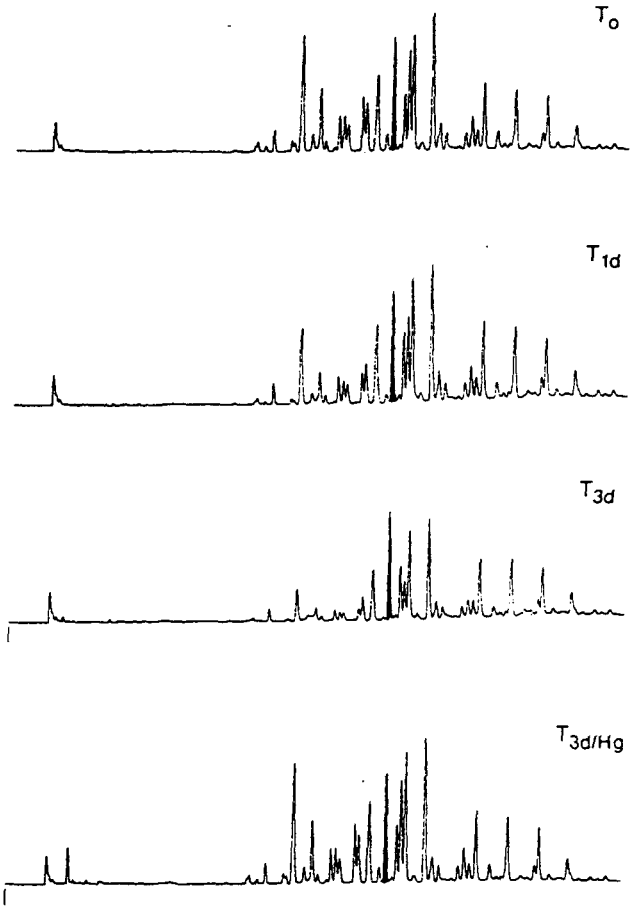


Figure 7-2. Biodegradation of PCBs in an environmental sample by *Pseudomonas putida* LB400. Cells (2 ml at approximately 10^9 cells/ml) were incubated with 0.2 g of soil from dragstrip site 43C (described in the text). The shaded peak is a nondegradable PCB congener used as an internal standard.

Biodegradation of Soil-bound Pure PCB Congeners

In addition to our Aroclor studies we have also been conducting biodegradation assays using soil-bound pure PCB congeners. Experiments have been initiated to define the rates of degradation of selected PCB congeners in unstirred, moist soil, (soil:water ratio = 2) incubated at 22 °C in open containers. The rates of metabolite formation have also been examined.

Initially, we conducted experiments to determine the effect of cell concentration on the initial rate of PCB degradation. This was done to establish a convenient inoculum level for observing reasonable degradation rates without excessively high cell doses.

The cell densities used were 0.13, 0.5, and 1.0×10^9 cells/ml. As expected, we observed the highest initial rate of degradation at 1.0×10^9 cells/ml. Subsequent experiments were conducted at this cell density.

Time courses for the degradation of several PCB congeners and for the formation of metabolites in moist soil are shown in Figure 7-3. Even at 500 ppm, 3-CB is degraded in 3 days under simulated field conditions. Furthermore, its metabolite, 3-chlorobenzoic acid (3-Cba) is rapidly produced and then degraded, suggesting that this congener may be completely mineralized.

Time courses for the degradation of 2,3-CB (80 ppm) and 2,5,2'-CB (86 ppm) are also shown (Figure 7-3). In both cases an initial rapid reaction is followed by a slower rate of reaction. The rate of degradation of 2,3-CB is closely correlated with the rate of production of its metabolite, 2,3-dichlorobenzoic acid (2,3-Cba). This single metabolite accumulates because LB400 is unable to degrade it. The rate of degradation of 2,5,2'-CB is rapid, yet slower than that observed for 2,3-CB. This is consistent with previous observations in non-soil assays. Rapid production of the 2,5-Cba metabolite correlates well with the depletion of 2,5,2'-CB; however, this metabolite is subsequently oxidized.

These results demonstrate that all of the steps of oxidation that we have observed in our aqueous resting cell assays also occur under conditions that more closely approximate field conditions. In soil experiments using excess water, both 2,5,2'-CB and its metabolite 2,5-Cba are oxidized more rapidly. One possible explanation is that the higher effective concentration of 2,5-Cba (or other metabolites) in moist soil inhibits the degradation of 2,5,2'-CB. Alternatively, the substrate may be less available, or the cells may be less active in moist soil.

As expected, the degradation of 2,4,5,2',5'-CB (85 ppm) is considerably slower than that of the lower chlorinated congeners (Figure 7-3). Even so, the continued production of metabolite indicates that metabolism continues for at least four days. The constraint on 2,4,5,2',5'-CB degradation may be entirely due to the position and number of chlorine substituents on the ring. It is also possible that the accumulation of hydroxylated intermediates inhibits degradation. The latter possibility will be investigated in the coming year.

Modeling a Mock Biodegradation Process

As part of our biodegradation process modeling research, we have evaluated bacterial soil-decontamination processes reported by other laboratories and companies. One pitfall that we (and other investigators) must be concerned with is congener depletion in a "biodegradation" process that results from physical loss of the PCB and not from true biological degradation. With Aroclor studies these alternatives can be easily distinguished because biodegradation processes result in depletion of specific congeners, yielding GC profiles that are distinctly different from those of Aroclors. Physical depletion, on the other hand, results in uniform depletion of all congeners (e.g., adsorptive loss) or a depletion of lower chlorinated congeners due to their higher volatility (e.g., evaporative loss). Of course, the production of PCB metabolites is another unequivocal method for demonstrating the biological basis of PCB depletion.

In order to better evaluate and understand results from open air, aerated, stirring reactors, we have attempted to model a typical process that appears to be biologically mediated, but is not. A sample of Oakland, Calif., soil contaminated with Aroclor 1260 was air-dried at room temperature, homogenized with a glass mortar and pestle, and then sieved. Argon was bubbled through a water/soil mixture (approximately

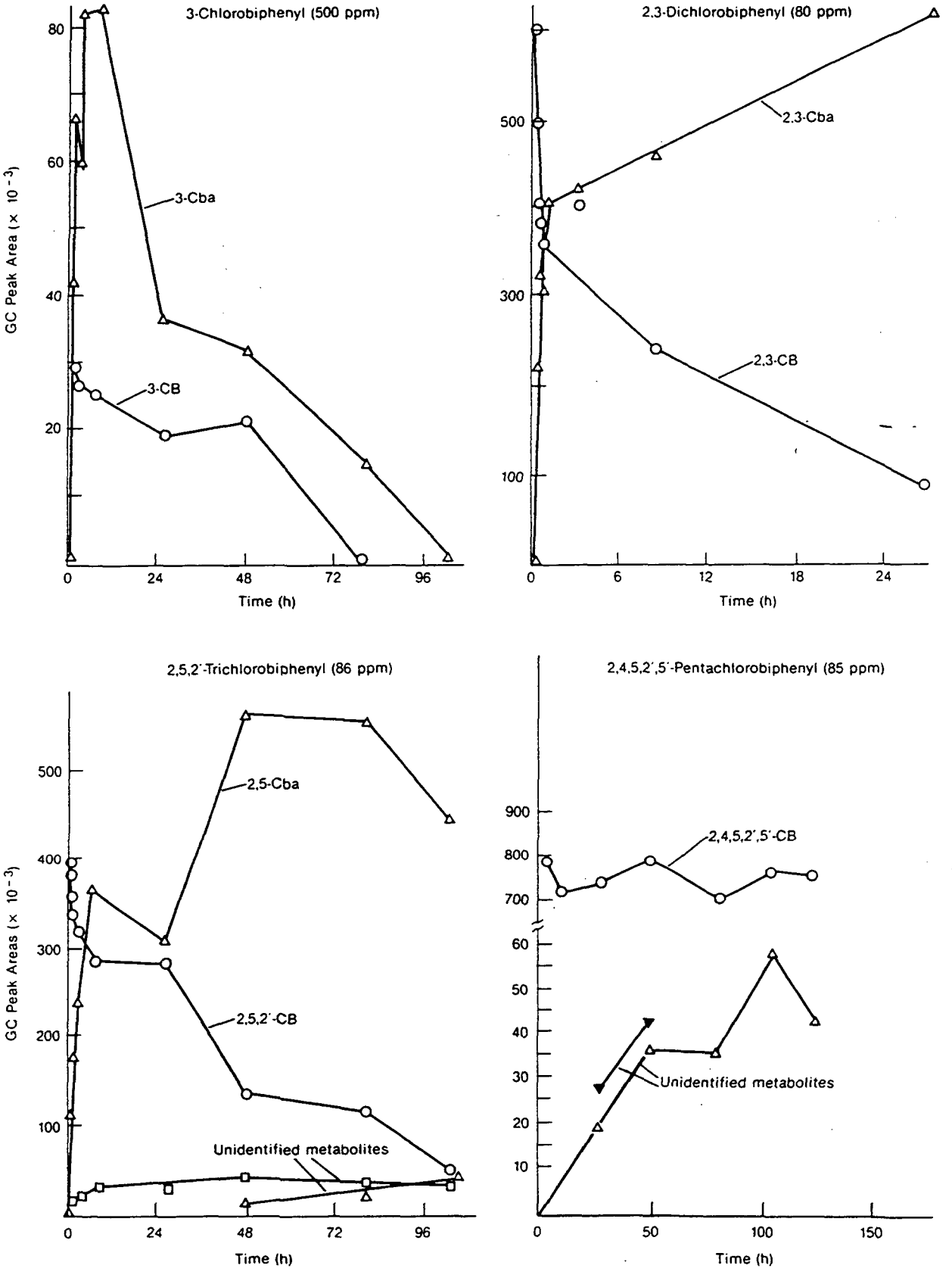


Figure 7-3. Time course of PCB degradation and metabolite production in moist soil inoculated with resting cells of *P. putida* LB400.

125 ml) in a 250-ml round-bottom flask at a flow of approximately 200 ml/min. A florosil sample tube was attached to trap PCBs in the argon as it exited the vessel. Samples (20 ml) were taken periodically while mixing to ensure a homogeneous sample. The volume in the vessel was maintained by adding 20 ml of distilled water after each sampling, and the florosil sample tube was replaced each time a sample was taken. The soil was mixed and purged with argon at room temperature for 19 days, after which the vessel was disassembled.

Each soil sample and florosil tube was extracted for GC analysis. The remaining soil and water were removed and pooled for GC analysis. The entire vessel was washed several times with hexane/acetone (1:1) to remove any PCBs bound to the vessel. These extracts were also pooled for GC analysis. Upon disassembly we observed a tar-like substance sticking to the Teflon stirrer. This was removed and added to the soil and water fraction before the hexane/acetone extractions.

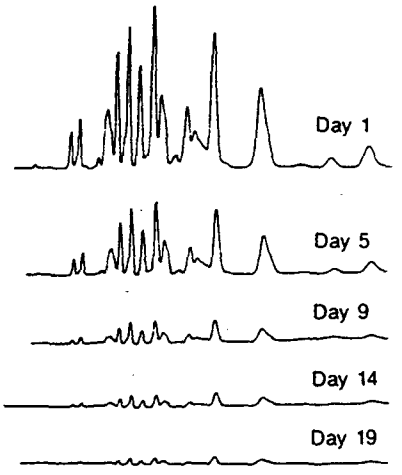
Neither oxygen nor bacterial inoculum was introduced in this mock process, yet the analytical results (Figure 7-4) might be mistaken for biodegradation. Although the time course analysis indicated greater than 90% PCB depletion, it is clear from the mass balance calculations (Figure 7-4) that the aeration and stirring of the soil resulted in the redistribution of PCBs from the soil to unassayed locations in the reactor (glassware, stirrer, coalesced droplets of PCB). The GC profiles also demonstrate that the observed depletion was not due to a biological process, since all GC peaks are depleted proportionally. Therefore, experiments that purport to show biodegradation of PCBs by quantifying GC peak areas should be carefully evaluated. It is for this reason that we include nonbiodegradable PCB internal standards wherever possible. If such standards or dead-cell controls cannot be included, then one must rely on differential congener depletion as evidence for the biological basis of PCB "biodegradation" processes.

SUMMARY AND CONCLUSIONS

Our studies have demonstrated the biodegradation of various soil-bound PCBs under a variety of conditions, using both pure and mixed cultures of MB1, H850, and LB400. We conclude from our studies that it should be possible to biologically degrade PCBs on contaminated soil in the environment with appropriate cell concentrations and moisture conditions. Lower rates of PCB degradation are to be anticipated at high PCB concentration or low levels of PCB-degrading bacteria. The former effect may also be attributable at least in part to the accumulation of metabolites, including chlorobenzoic acids. Effective strategies for enhancing the rate of degradation depend on understanding and/or circumventing the inhibitory effects of metabolites, as well as on optimizing the protocol for applying and maintaining active cell populations in the soil.

FUTURE PLANS

For the coming year our soil research will be expanded to include additional experimental conditions. These include further studies of biodegradation, using cultures actively growing on biphenyl or Luria broth, and additional pure congener studies. We will expand our Aroclor studies to include additional Aroclors and higher concentrations. We will investigate various soil:water ratios, and will conduct Aroclor biodegradation assays in unstirred soil. We also intend to conduct a laboratory scale-up of a possible in situ process and, if feasible, an in situ environmental test.



Day	Sample wt.	ppm	mg
1	2.75 g	7800	21
2	2.84 g	5300	15
5	2.75 g	3400	9.4
6	2.06 g	2900	6.0
7	2.16 g	2100	4.5
9	1.73 g	1500	2.5
12	1.57 g	990	1.6
14	1.69 g	830	1.4
16	1.84 g	640	1.2
19	1.53 g	620	0.9

Starting material = 35 g Oakland soil

Total starting PCBs = 273 mg

PCBs Recovered:

Sampling total (for 20.9 g soil) =	64	mg
Glassware =	62	mg
Florosil sample tubes total =	0.4	mg
Final soil + water =	41	mg
Tar balls (in final soil) =	68	mg
Total PCBs recovered =	235	mg (86% recovery)

Figure 7-4. Depletion of Aroclor 1260 in a mock biodegradation experiment.

Chapter 8

PROCESS MODELING: SURFACTANT EXTRACTION OF PCBs FROM SOIL

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INTRODUCTION

The basic feasibility of extracting PCBs from contaminated soil by using an aqueous surfactant solution was demonstrated in earlier phases of the GE research program [GE Reports, 1982; 1983; 1984; 1985]. One year ago, chemical engineering studies were initiated to address the technical issues surrounding the design and bench-scale demonstration of a practical soil extraction process. The work plan for engineering studies was divided into two phases. Phase I consists of the gathering of basic materials, properties, and process data needed for engineering design; progress on this phase is summarized in the current report. Phase II, which will be started in the coming months, will include the definition of process steps, equipment specification, and bench-scale demonstration of the process.

As part of Phase I, all available Oakland site survey information on PCB levels and distributions was reviewed to determine the potential scale of processing necessary. Samples of the principal soil types (near-surface mixed fill material, subfill layer clay matrix, and sand/gravel lens material) were analyzed for the physical and chemical properties most likely to affect processing. This background information was then used to select model soil systems for subsequent process development studies. Model soils have the advantage of being homogeneous, well-defined systems that can be loaded to known PCB levels and thus aid in the laboratory process development.

Recent studies have focused on quantifying the interactions between the surfactant solutions, the model soils, and PCBs. The surfactants used were Triton X-100 and Surco 233, since screening studies showed these to be the surfactants of choice [GE Reports, 1982; 1983; 1984]. The model soils investigated to date comprise a near-surface, PCB-free soil from the Oakland site and an unrefined kaolin clay. Aroclor 1260, the commercial PCB preparation found at the Oakland site, was used in the studies. The interactions under study are the maximum uptake of PCBs into the surfactant solutions, the equilibrium adsorption of the surfactants on the soils, and the distribution of the PCBs between the soils and surfactant solutions.

RESULTS

Soil Characterization/Model Development

Soil samples from the Oakland site (5 to 30 feet depths) were characterized in an effort to select representative model soils. Typical analyses carried out for the core samples and the candidate model soils were particle size distribution, bulk density, particle density, specific surface area (BET), water content, organic content, soluble salt concentrations, pH, clay minerology, and PCB levels (see Table 8-1). The high sodium content found in the Oakland soil samples (Table 8-1) was likely due to the site's

Table 8-1

SOIL ANALYSIS RESULTS

	Oakland Fill	Oakland Matrix (30 ft)	Foundry Hill Creme Clay	Gravel/Sand Lens (20 ft)
Water content (%)	7.2	17.3	1.2	12.8
pH	6.5	7.6	4.6	7.8
Bulk density (g/cm^3)	1.23	2.0	0.6	2.1
Particle density (g/cm^3)	2.74	2.7	2.7	2.6
Surface area (m^2/g)	12.0	40.6	18.3	7.7
Organic content (%)	0.5	0.1	0.4	0.3
Soluble salts (ppm):				
Na^+	31.8	52.0	11.9	71.2
K^+	4.0	9.9	4.6	2.6
Ca^{2+}	7.4	9.1	19.8	3.8
Mg^{2+}	9.8	24.0	9.9	3.8
SO_4^{2-} (as S)	13.8	6.4	-	13.3
Percent finer than:				
1000 μm	98.5	99.5	100.0	56.5
500 μm	97.0	99.5	100.0	51.5
100 μm	85.5	91.0	100.0	28.5
50 μm	58.8	73.0	94.2	21.5
10 μm	28.9	49.0	42.2	12.0
5 μm	19.8	39.5	36.0	8.5
1 μm	2.2	7.0	6.7	0

proximity to San Francisco Bay. The fill layer was mostly silt and sand with little clay content. All the soils studied had very low organic content (<0.5%).

From these results and existing site survey information [GE Report, 1981] two model soils were initially selected for this study, one to represent the fill layer (0 to 5 feet) and another to represent the matrix clay. Further studies with models of sand/gravel lens materials were judged to be unnecessary due to the relative ease of cleaning and processing of these materials. The model selected for the fill layer was PCB-free fill soil from the Oakland site. The matrix clay was modelled by Foundry Hill Creme Clay (H.C. Spinks Clay Company), an unrefined kaolin clay.

Recent X-ray diffraction analyses have indicated that the Oakland matrix clay is a montmorillonite, rather than kaolin clay. Since these two clays have different structures, and consequently different properties (e.g., the montmorillonite has much greater specific area per unit mass), a montmorillonite clay has been selected for additional model studies.

Surfactant Sorption Isotherms

A series of experiments were performed to determine the adsorption/desorption isotherms for the candidate surfactants (Triton X-100 and Surco 233) with the model soils. Surco 233 (anionic) is approximately 50% active ingredients, the bulk of which is sodium dodecylbenzene sulfonate. Triton X-100 (nonionic) is 100% octyl phenoxy polyethoxy ethanol.

Figure 8-1 is a typical sorption isotherm. The adsorption/desorption results showed no hysteresis (i.e. the sorption appeared to be reversible). The "break" in the

sorption isotherm corresponded approximately to the critical micelle concentration (CMC). The adsorption maxima were roughly proportional to the surface areas found for the model soil (see Table 8-2). The maximum adsorption for Triton X-100 was almost five times that of Surco 233.

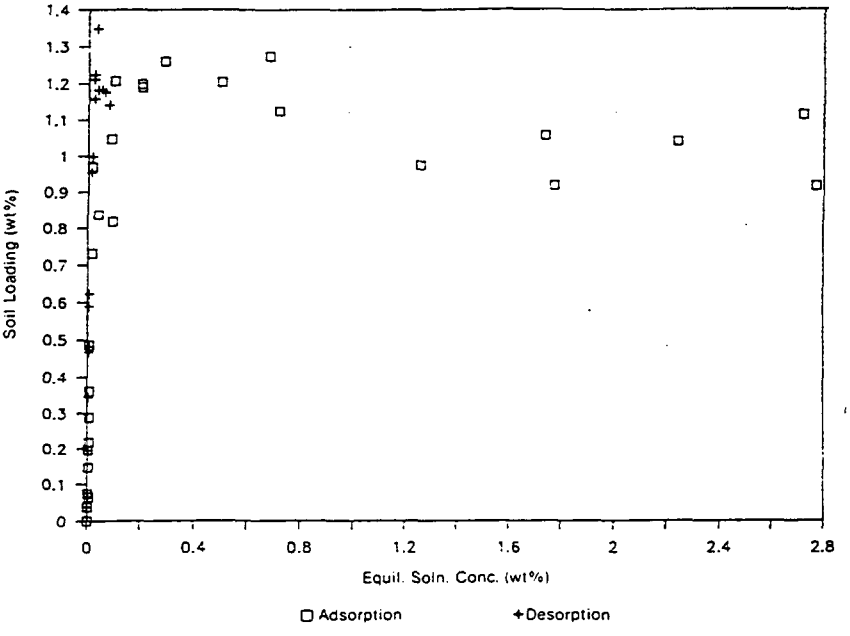

Figure 8-1. Sorption isotherms for Triton X-100 on Foundry Hill Creme Clay.

Table 8-2

MAXIMUM SURFACTANT LOADINGS AS WT% OF MODEL SOILS

	Triton X-100	Surco 233[a]
Foundry Hill Creme Clay	1.15	0.24
PCB-free Oakland Soil	0.85	0.18

[a]These values reflect adsorption of active ingredients

PCB Solubility

The solubility of Aroclor 1260 in various concentrations of each surfactant was studied. Emulsions of PCBs were formed in many of the samples. Consequently, the samples were centrifuged to remove the emulsion for the true solubility reading.

The first batch of Aroclor 1260 used in the tests proved to be only 80% pure. The 20% diluent was not trichlorobenzene; further tests are under way to determine its identity. Early results with a second batch of Aroclor 1260 (pure) indicated that the diluent had no effect on the solubility of the PCBs in Triton X-100; however, the diluent appeared to enhance the solubility of the PCBs in Surco 233.

Solubilization appeared to be linear with surfactant concentration (Figures 8-2 and 8-3). Triton X-100 is much better at solubilizing Aroclor 1260 than Surco 233; also

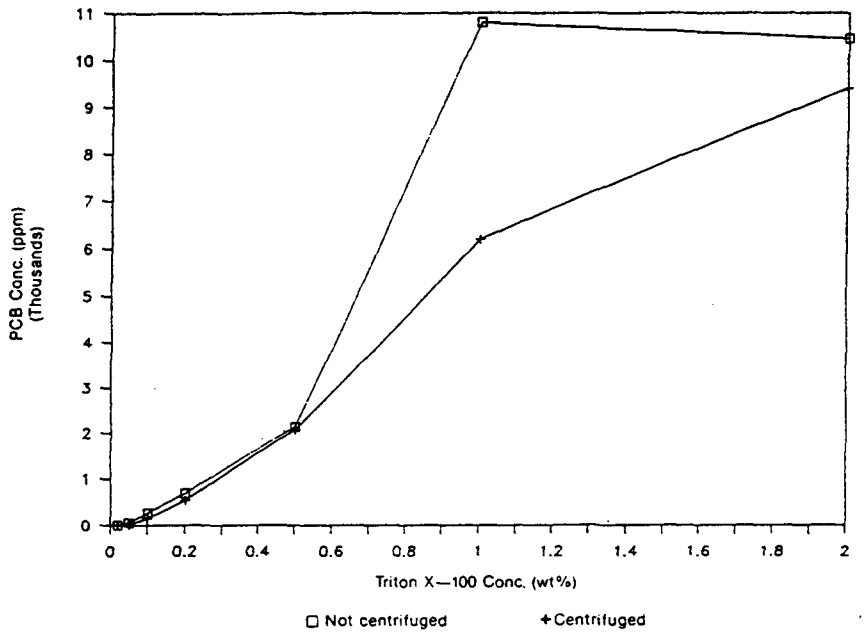

Figure 8-2. Solubility of Aroclor 1260 in Triton X-100.

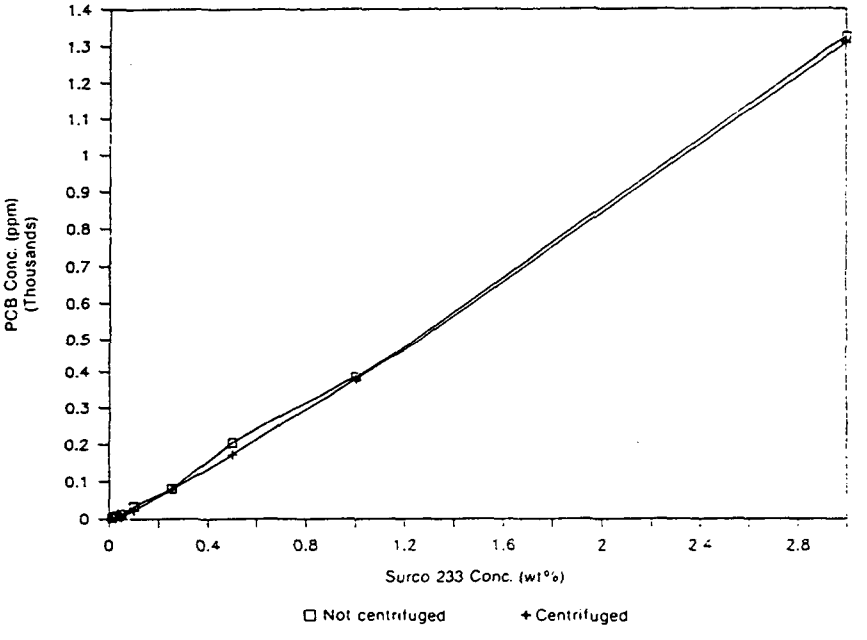

Figure 8-3. Solubility of Aroclor 1260 in Surco 233.

Triton X-100 tended to form emulsions of Aroclor 1260 which resulted in the mobilization of more PCBs. Triton X-100 showed no congener selectivity, while Surco 233 tended to favor the lower chlorinated congeners.

The solubility of Aroclor 1260 in deionized water is 2 to 3 ppb, so a few weight percent surfactant greatly enhances the solubility of Aroclor 1260 in the aqueous phase.

PCB Extraction

Each of the model soils was spiked with Aroclor 1260 to a level of 4800 ppm. These resulting soils were contacted with various concentrations of Triton X-100 and Surco 233 in a liquid to soil ratio of 4.

Both surfactants performed very well (Tables 8-3 and 8-4). As expected, the kaolin clay proved to be the more difficult case. When in contact with soil, Surco 233 was able to exceed the uptake observed in the earlier solubility study; this was likely due to salts in the soils. The addition of salts was previously shown to improve the solubility of Aroclor 1260 in Surco 233 [GE Report, 1985]. Neither surfactant showed congener selectivity in the extraction studies.

Table 8-3

AROCLOR 1260 EXTRACTION FROM FOUNDRY HILL CREME CLAY

Triton X-100 (wt%)	PCB Removal (%)	Surco 233[a] (wt%)	PCB Removal (%)
3.0	77	3.0	57
2.0	42	2.0	72
1.0	35	1.0	49
0.5	0	0.5	15
0.2	0	0.25	0

[a]Values given are weight percent active ingredients

Table 8-4

AROCLOR 1260 EXTRACTION FROM PCB-FREE OAKLAND SOIL

Triton X-100 (wt%)	PCB Removal (%)	Surco 233[a] (wt%)	PCB Removal (%)
3.0	100[b]	3.0	92
2.0	86	2.0	79
1.0	82	1.0	75
0.5	61	0.5	52
0.2	2	0.25	30

[a]Values given are weight percent active ingredients
[b]This value was in excess of 100% reflecting experimental uncertainty

SUMMARY AND CONCLUSIONS

The following is a summary of the major results of this investigation.

1. The Oakland site contains three principal soil types: a fill layer composed of mainly sand and silt, a subsurface clay matrix, and sand/gravel lenses contained within the clay matrix. Model soils for further studies have been selected for the first two soil types.
2. It is more difficult to extract PCBs from clays than from other soil components.
3. Surfactants readily adsorb onto soils. The amount of surfactant adsorbed can be significantly large, particularly for Triton X-100 (soil loading >1 wt%).

4. Surfactants increase the solubility of Aroclor 1260 in water by up to seven orders of magnitude.
5. The relative proportions of PCB congeners solubilized is a function of surfactant type and extraction environment.
6. Solutions with 1 wt% surfactant or greater effected 35 to 100% removal of Aroclor 1260 from the model soils.

Based on these encouraging findings, extraction with surfactant solutions remains an attractive potential method for the removal of PCBs from soil. The required surfactant-to-soil ratios, however, indicate that a method for surfactant recovery must be included in the overall process in order to develop an economically practical system.

FUTURE PLANS

More data is needed for the development and design of a process utilizing surfactant solutions for the removal of PCBs from soils. The sorption behavior of a model montmorillonite clay will be studied in an analogous manner to the Foundry Hill Creme Clay described here. All of the studies to date have been involved in the determination of equilibrium data. Rate measurements will be made for the extraction of PCBs from soils and for the separation of soils from the aqueous phase. Both of these rates will have a strong influence on the size and throughput capacity of the process equipment.

Process criteria should be established for the levels of surfactant and water remaining in the soil to determine what, if any, post-treatment is necessary. Process steps to separate and recycle surfactants should also be developed.

The culmination of this work (Phase I) will be the identification and scaling of process equipment, followed by the construction of a laboratory scale unit to demonstrate the capabilities of this process with model and Oakland soils (Phase II). The long range goal is to link this extraction technology with a biodegradation process for the removal and destruction of soil-contaminating PCBs.

Chapter 9

DEVELOPMENT OF A BIOLOGICAL PROCESS FOR PCB REMOVAL

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INTRODUCTION

The purpose of this portion of the research is to examine the factors affecting the biodegradation of chlorobiphenyls in aerobic and anaerobic environments and to develop a combined anaerobic/aerobic process to encourage PCB biodegradation. Thus the primary objective of this project is to develop a fundamental understanding of the biodegradation of PCBs by mixed cultures such that their removal in wastewater treatment or in the environment can be enhanced.

The overall experimental program involves the use of batch and continuous processes to evaluate toxicity and biodegradation of chlorobiphenyls and surfactants. Surfactants are being tested for their toxicity to bacteria because this information will aid in the selection of a surfactant for use in soil decontamination and in subsequent continuous flow reactor studies.

RESULTS AND DISCUSSION

Batch Anaerobic Toxicity Assays

Batch toxicity assays were performed for several chlorobiphenyls and surfactants using the procedure described by Owen et al. [1979]. In this technique, a prereduced nutrient media, bacterial inocula, and toxicants are anaerobically transferred to serum bottles and incubated at 35 °C. The media contains resazurin (a redox indicator), sodium sulfide (a reducing agent), sodium bicarbonate (to buffer pH), nitrogen (as ammonia), phosphorus, a wide range of trace inorganic nutrients, and vitamins. Gas production and composition are measured to perform a mass balance on the degradable organic compounds present in the serum bottles. The rate of gas production from a readily degradable spike is measured with and without the presence of a potential toxicant. Inhibition is defined as a decrease in the rate of acetate and methanol degradation compared to a blank containing no chlorobiphenyls or surfactants. Overall gas production was used as a measure of biodegradation.

Anaerobic toxicity assays (ATAs) were performed for biphenyl, 4-chlorobiphenyl, 2,4,5- and 2,4,6-trichlorobiphenyls, and 2,3,4,6-tetrachlorobiphenyl, as well as several Triton surfactants. Individual toxicants were added at increasing concentrations.

Representative data from two series of ATAs are shown in Figures 9-1 and 9-2. Each curve represents the mean of triplicate serum bottle analyses. 2,4,6-trichlorobiphenyl and 2,3,4,6-tetrachlorobiphenyl were tested at initial concentrations between 50 and 1500 μg per liter. Equilibrium soluble PCB concentrations were not measured. Based on the similarity in the rates of gas production between the control (containing an acetate and methanol spike) and the serum bottles (containing the spike and increasing concentrations of a chlorobiphenyl), these PCB congeners did not significantly decrease the rate of gas production.

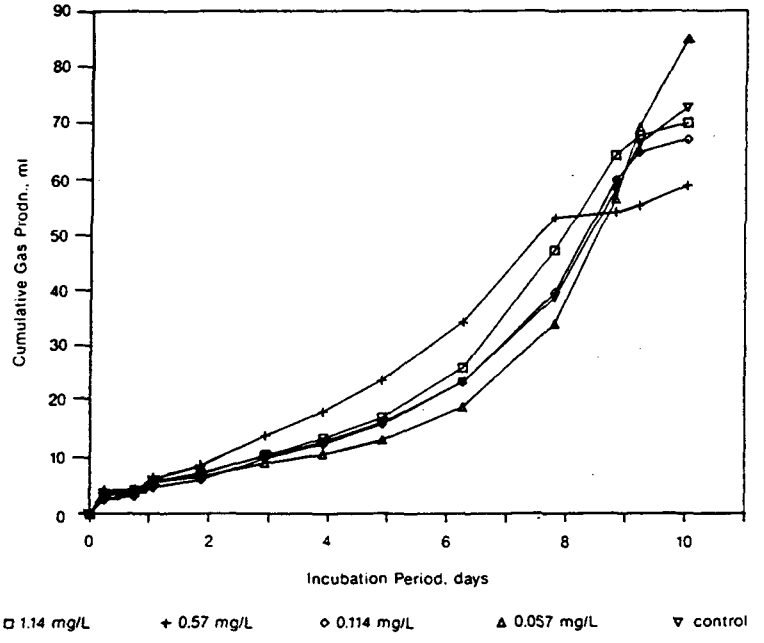

Figure 9-1. Anaerobic toxicity assay results for 2,4,6-trichlorobiphenyl.

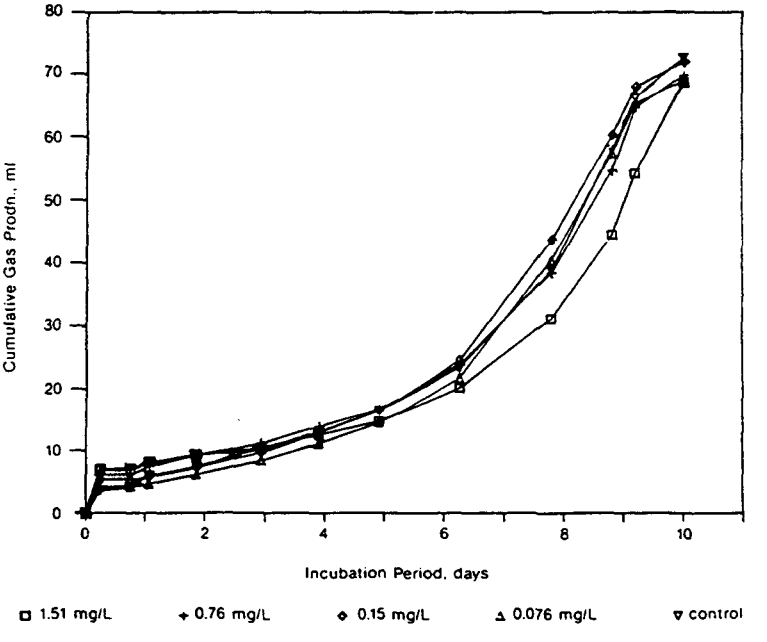

Figure 9-2. Anaerobic toxicity assay results for 2,3,4,6-tetrachlorobiphenyl.

Similar results were obtained for biphenyl, 4-chlorobiphenyl and 2,4,5-trichlorobiphenyl. None of the biphenyls tested were toxic under the test conditions. Thus the PCBs would not be expected to be toxic in biological treatment at these concentrations. Similar experiments were performed with several surfactants in the Triton series. Surfactants were tested individually as a function of concentration. The surfactants chosen for study included octylphenol and nonylphenol Tritons with the total number of ethoxy (EO) units on the hydrophilic side chain varying between nine (Triton X-100) and 40 (Triton X-405).

The rate of gas production from an acetate spike decreased with increasing surfactant concentration for each surfactant tested. Results for Triton X-100 (with nine EO units) are shown in Figure 9-3 and are representative of each of the surfactants tested.

Most surfactants had little effect on acetate biodegradation at concentrations less than 33 mg per liter. However, at 100 and 333 mg per liter the rate of gas production decreased significantly for several of the surfactants (Figures 9-4 and 9-5). The rate of gas production was greatest for Triton X-305 and X-405 (with 30 and 40 EO units, respectively). Gas production decreased with the number of EO units. Thus treatment of surfactant/PCB leachates will require selection of an appropriate surfactant (with 30 to 40 EO units) and may require dilution of the surfactant/PCB leachate prior to treatment.

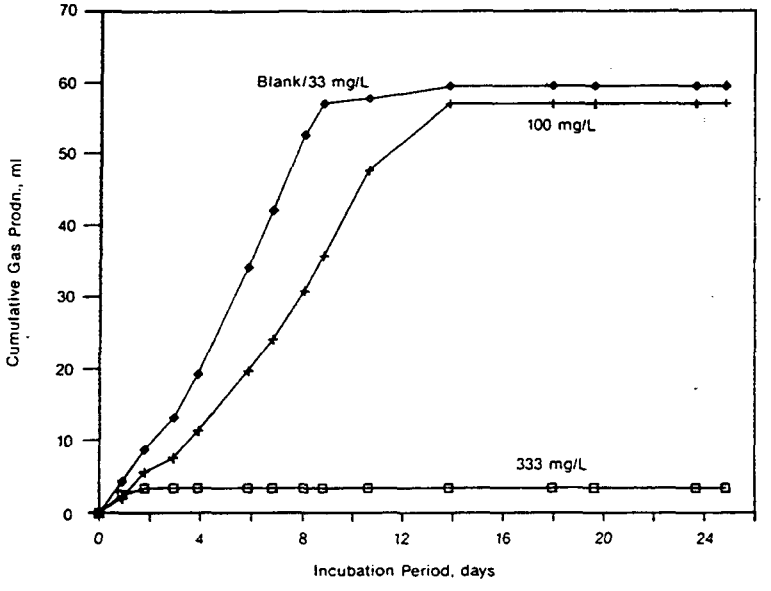

Figure 9-3. The effect of Triton X-100 on the anaerobic biodegradation of acetate.

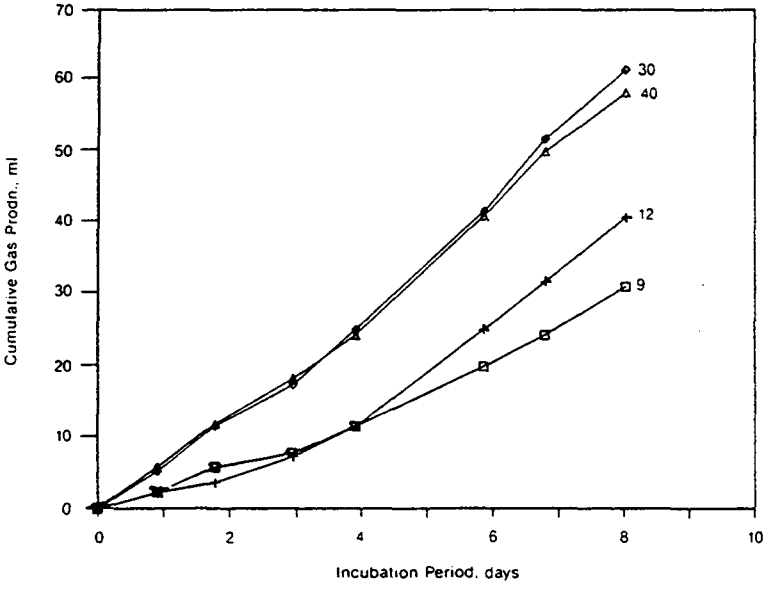

Figure 9-4. The effect of octylphenol Triton surfactants (100 mg/liter) on the anaerobic biodegradation of acetate.

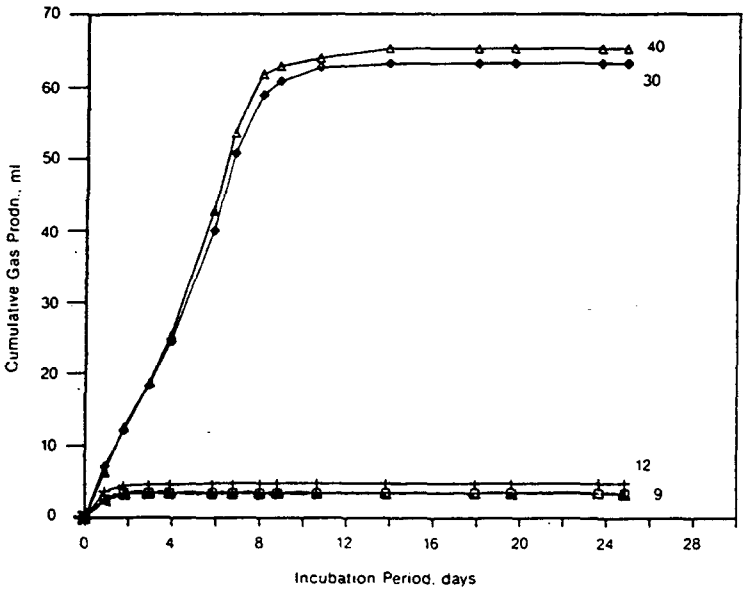

Figure 9-5. The effect of octylphenol Triton surfactants (333 mg/liter) on the anaerobic biodegradation of acetate.

Continuous Flow Studies

Continuous-flow experiments are currently being conducted to evaluate the anaerobic degradation of chlorobiphenyls. Continuous flow studies are being conducted using a 3.4-liter upflow anaerobic sludge blanket reactor with recycle. The reactor is in a walk-in environmental chamber held at 27 °C. The body of the reactor is constructed of Kimax-beaded process pipe with Teflon-flanged fittings. Feed solution is held in glass reservoirs located outside the environmental chamber and is pumped into the chamber by a positive displacement pump using Teflon tubing for connections.

The reactor is currently operating at a hydraulic retention time of 16.3 hours. Reactor effluent samples are collected at a port on the effluent line. The basic feed solution for this reactor consists of acetate, methanol, and glucose, with adequate inorganic nutrients and vitamins. A methanol solution containing an individual chlorobiphenyl or a suite of chlorobiphenyls is added to the basic feed solution each day. Concentrations of the parent chlorobiphenyls and possible metabolites in the reactor effluent are monitored daily.

After four weeks of treating 2,4,5-trichlorobiphenyl and monitoring the effluent by GC/ECD, several compounds are beginning to appear in the chromatograms from the reactor effluent. However, these compounds have not been identified and are present at very low levels. Further work is currently being conducted to identify these compounds and to complete a mass balance on the parent chlorobiphenyl.

FUTURE PLANS

Anaerobic Biodegradation of PCBs

During the next year, we will continue to characterize the anaerobic biodegradation of chlorobiphenyls. Individual chlorobiphenyls or a suite of chlorobiphenyls will be continuously fed to the anaerobic sludge blanket reactor, removal will be characterized, and anaerobic degradation pathways will be determined.

Aerobic/Anaerobic Biofilm for PCB Biodegradation

We will also continue to evaluate a combined aerobic/anaerobic biofilm for the degradation of chlorobiphenyls. A 2-liter biofilm reactor will be used to test the feasibility of treating PCBs under conditions in which the growth of both aerobic and anaerobic bacteria is encouraged. The biofilm is grown on a goretex membrane which allows transfer of gas to the bacteria at the base of the biofilm (growing next to the goretex support). Oxygen is provided to the goretex, thus supporting the growth of aerobes. The biofilm is operated such that the oxygen provided is used within the biofilm, leading to the growth of anaerobes at the surface of the biofilm and in the bulk liquid. Proper operation of this biofilm reactor should result in the development of a consortia of bacteria with the capability to dechlorinate PCBs under anaerobic conditions as well as organisms with the ability to hydroxylate and cleave the biphenyl molecule under the aerobic conditions at the surface of the goretex.

Physical-Chemical Process of PCB Removal

Additional studies are being conducted to evaluate a physical-chemical process for the removal of PCBs from contaminated PCB-surfactant leachates from soil washing. Preliminary studies conducted in our laboratories have shown that a simple sublation procedure is effective in concentrating PCBs and surfactants in a solvent. Air is bubbled through a solution of a surfactant and chlorobiphenyls. The PCB associates with the hydrophobic portion of the surfactant molecule. The sublation process takes advantage of the surface active properties of the surfactant to remove the surfactant/PCB complex from solution. As air is bubbled through the solution, the surfactant and associated PCB is transported to the top of the water column, where it concentrates in the solvent. The solvent can then be collected and evaporated, resulting in a small quantity of solids for disposal. A reactor is currently being constructed to evaluate this process under varying operating conditions, to determine kinetic coefficients for the binding of the PCB to the surfactant, and to characterize the transport of the surfactant to the solvent layer.

RECOMMENDATIONS

Our research for the next year will emphasize five areas: (1) the genetics and biochemistry of bacterial oxidation of PCBs, (2) reductive dechlorination of PCBs by anaerobic communities, (3) analysis of environmental transformations of PCBs, (4) development of laboratory scale models for evaluating physical-chemical processes for soil decontamination, and (5) development of laboratory scale units for evaluating both aerobic and anaerobic processes for biodegrading PCBs.

Genetics and biochemistry of bacterial oxidation of PCBs. Our results indicate that both *Alcaligenes eutrophus* H850 and *Pseudomonas putida* LB400 have diverse mechanisms for PCB degradation. A thorough understanding of the genetic and biochemical basis for PCB degradation in these organisms offers us the potential to improve or amplify their PCB-degradative competence. Alternatively, we may be able to use isolated PCB-degradative enzymes in a biological treatment process. In the coming year we intend to clone and isolate the genes responsible for PCB degradation in LB400 and H850, and to assay the enzymes encoded by these recombinant DNA organisms against the spectrum of PCB congeners metabolized by LB400 and H850. Attempts will be made to subclone the genes encoding the initial oxygenases in a high expression vector. This will enable us to produce large amounts of enzyme for mechanistic studies of the different types of PCB-degradative enzymes. In a related effort, fractionated cell-free systems will be used to separate and characterize these enzymes from both organisms.

We also plan to elucidate the mechanism responsible for the formation of ring-chlorinated acetophenones from PCBs in H850. These studies will include a determination of the amount of PCB that is metabolized by this route, the isolation and characterization of intermediate metabolites, and characterization of the subsequent metabolism of chloroacetophenones. We will use mutagenic techniques to generate strains of H850 that are unable to grow on acetophenone for use in these studies. Our approach will involve the use of both whole cell and cell-free assays.

Reductive dechlorination of PCBs by anaerobic communities. We will continue to evaluate the PCB-dechlorinating ability of the anaerobic communities described in this report. In addition, we will attempt to demonstrate PCB dechlorination in highly enriched anaerobic cultures that are known to dechlorinate chlorobenzoates and chlorophenols. Because the maintenance of biological activity during long-term incubations requires the addition of growth substrates, we have initiated studies to determine which substrates are suitable. The current gas chromatographic evaluation of PCB dechlorination will be supplemented with a sensitive assay that measures the release of $^{36}Cl^-$ from [^{36}Cl]-labeled PCBs.

Environmental transformations of PCBs. Our plans include continued analysis of PCB transformations occurring in groundwater, fish, and other environmental repositories of PCBs, and a determination of the kinetics of PCB dechlorination in Hudson River sediments.

Physical-chemical processes for soil decontamination. The surfactant mobilization studies will be expanded to include the behavior of surfactants on model montmorillonite clay that closely resembles clay at the Oakland site. We will develop process steps to recover and recycle surfactants, and based on these results will identify and scale process equipment. A laboratory scale unit will be constructed to demonstrate

PCB mobilization capabilities with model and Oakland soils. Our long range goal is to link this process with a biodegradation process for removal and destruction of soil-contaminating PCBs.

A related study will involve a physical-chemical process for the removal of PCBs from PCB-surfactant leachates. Preliminary studies have shown that sublation effectively concentrates both PCBs and surfactants in an organic solvent. Evaporation of this solvent would yield highly concentrated solids, containing the surfactant and PCB, for disposal. A reactor to evaluate this process is currently being constructed.

Aerobic and anaerobic processes for biodegradation of PCBs. Our in situ oxidative degradation studies will be expanded to more closely approximate field conditions. Experimental conditions will include additional Aroclors, higher concentrations of Aroclors, and moist unstirred soil. We plan to conduct a laboratory scale-up of an in situ process and, if feasible, and in situ test at an environmental site.

We will continue to evaluate anaerobic biodegradation of PCBs in a surfactant leachate. These studies will include a determination of the toxicity of Surco 233 on anaerobic cultures and determination of concentrations of PCBs that can be effectively degraded.

We also plan to evaluate a combined aerobic/anaerobic process for degradation of PCBs in a 2-liter biofilm reactor. These experiments will determine the feasibility of biodegradation of PCBs under conditions that encourage the growth of aerobic bacteria at the base of the biofilm and anaerobic bacteria at its surface.

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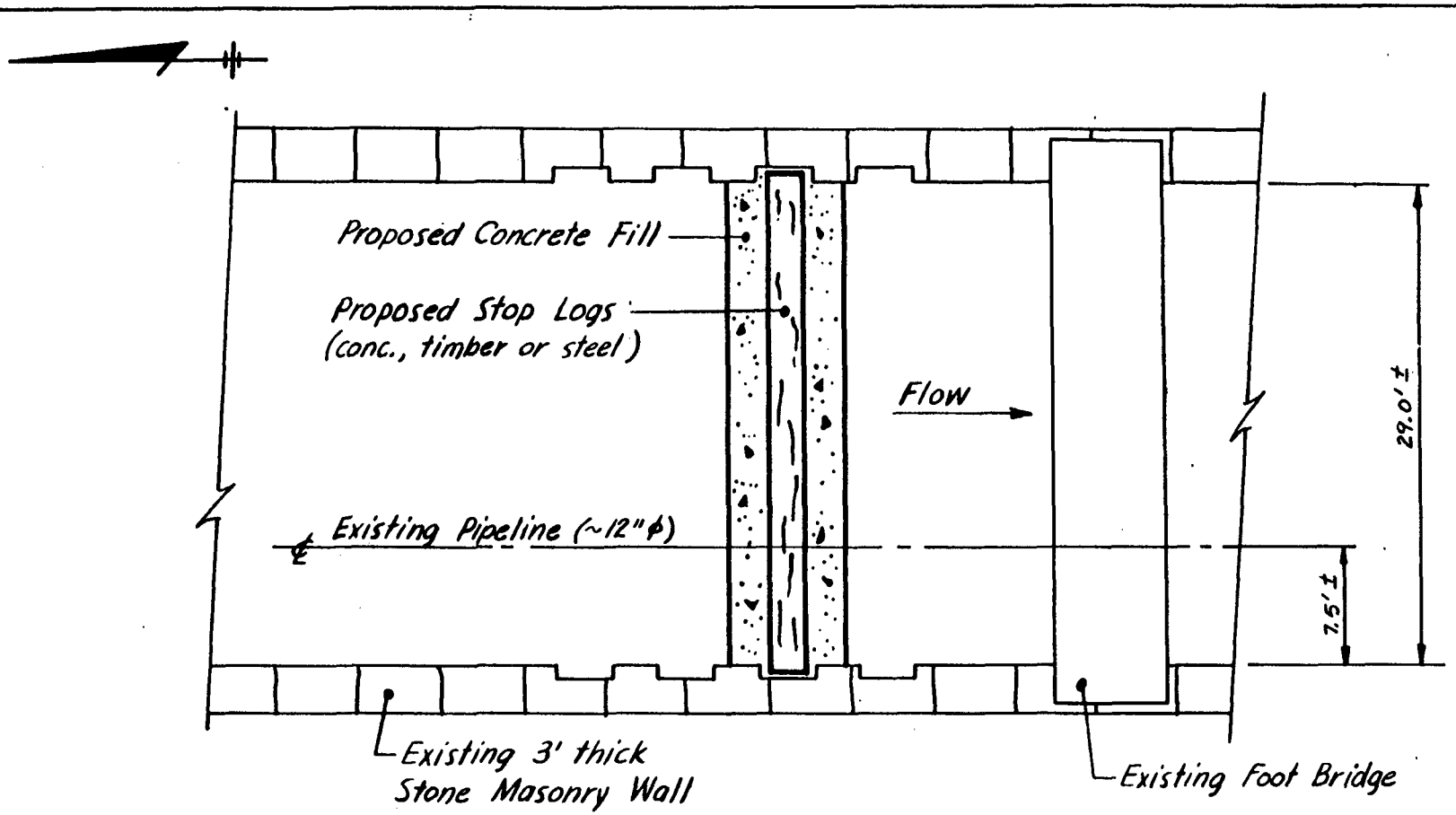
Figures

BLASLAND & BOUCK ENGINEERS, P.C.

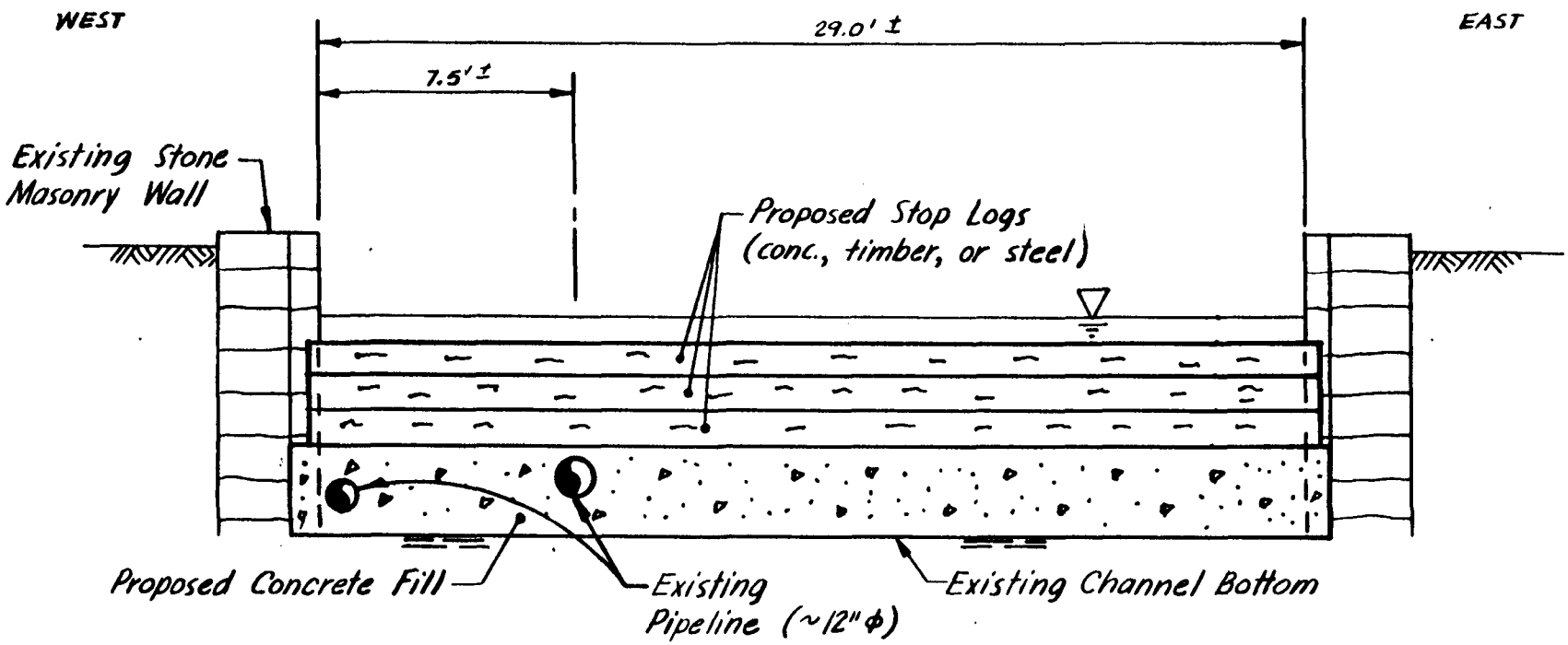


PLAN VIEW

SCHWEITZER DAM RACEWAY - TEMPORARY BAFFLE

FIGURE 3

BINNELAND & SOUCK ENGINEERS, P.C.

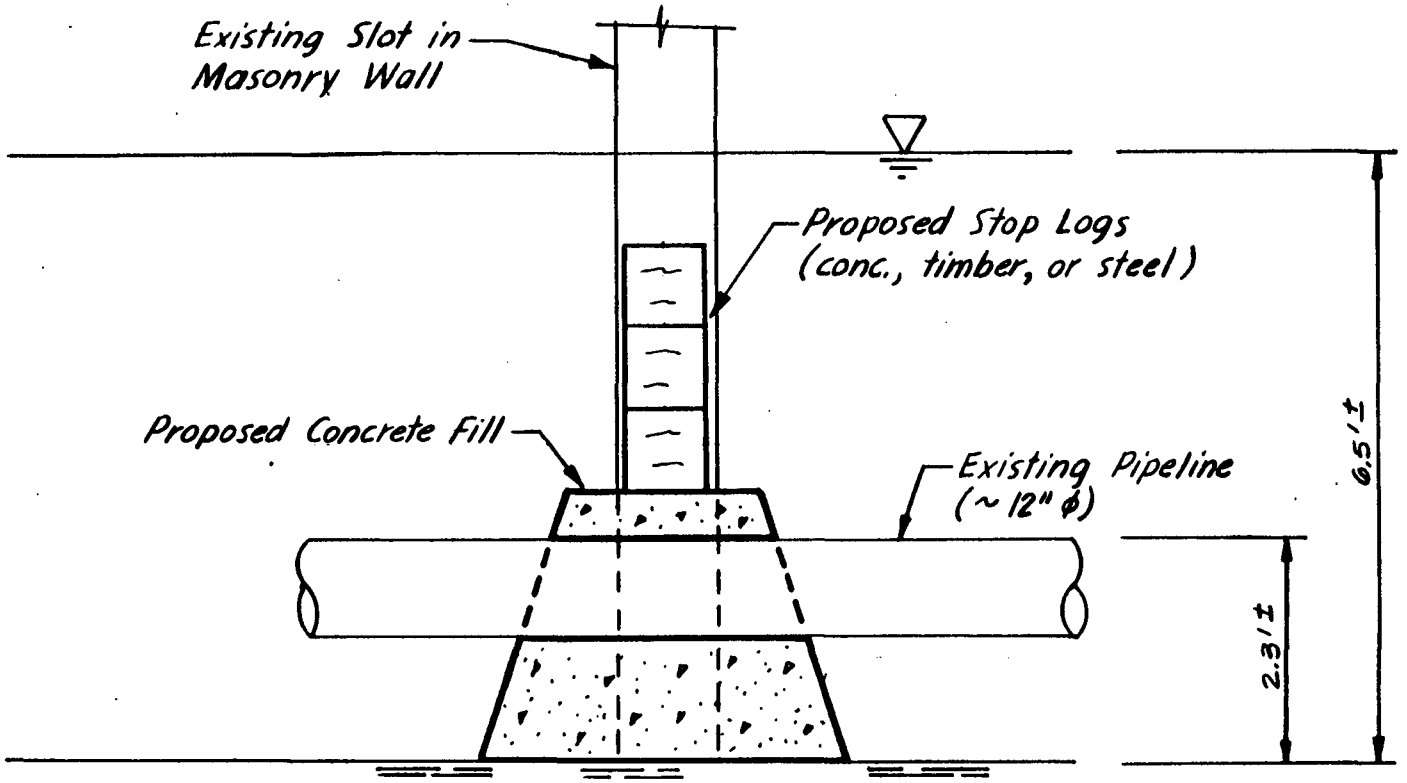


ELEVATION

SCHWEITZER DAM RACEWAY - TEMPORARY BAFFLE

FIGURE 4

BLASLAND & BOUCK ENGINEERS, P.C.



SECTION

SCHWEITZER DAM RACEWAY - TEMPORARY BAFFLE

FIGURE 5

BLASLAND & BOUCK ENGINEERS, P.C.

Appendix

BLASLAND & BOUCK ENGINEERS, P.C.

APPENDIX A

DRAWDOWN CALCULATIONS

General

This section presents flow computations for the Schweitzer Dam/Raceway Channel area. In addition, previous work efforts by Stewart Laboratories Inc. regarding flow and suspended solids content are presented.

Summary of Pertinent Data

1. Drainage area for the Housatonic River at the Schweitzer Bridge: 101 square miles (Stewart Report).

2. Annual storm event represents a flow of 2,600 cfs at the outlet to Woods Pond (Berkshire Co. Water Quality Management Plan, 9/78).

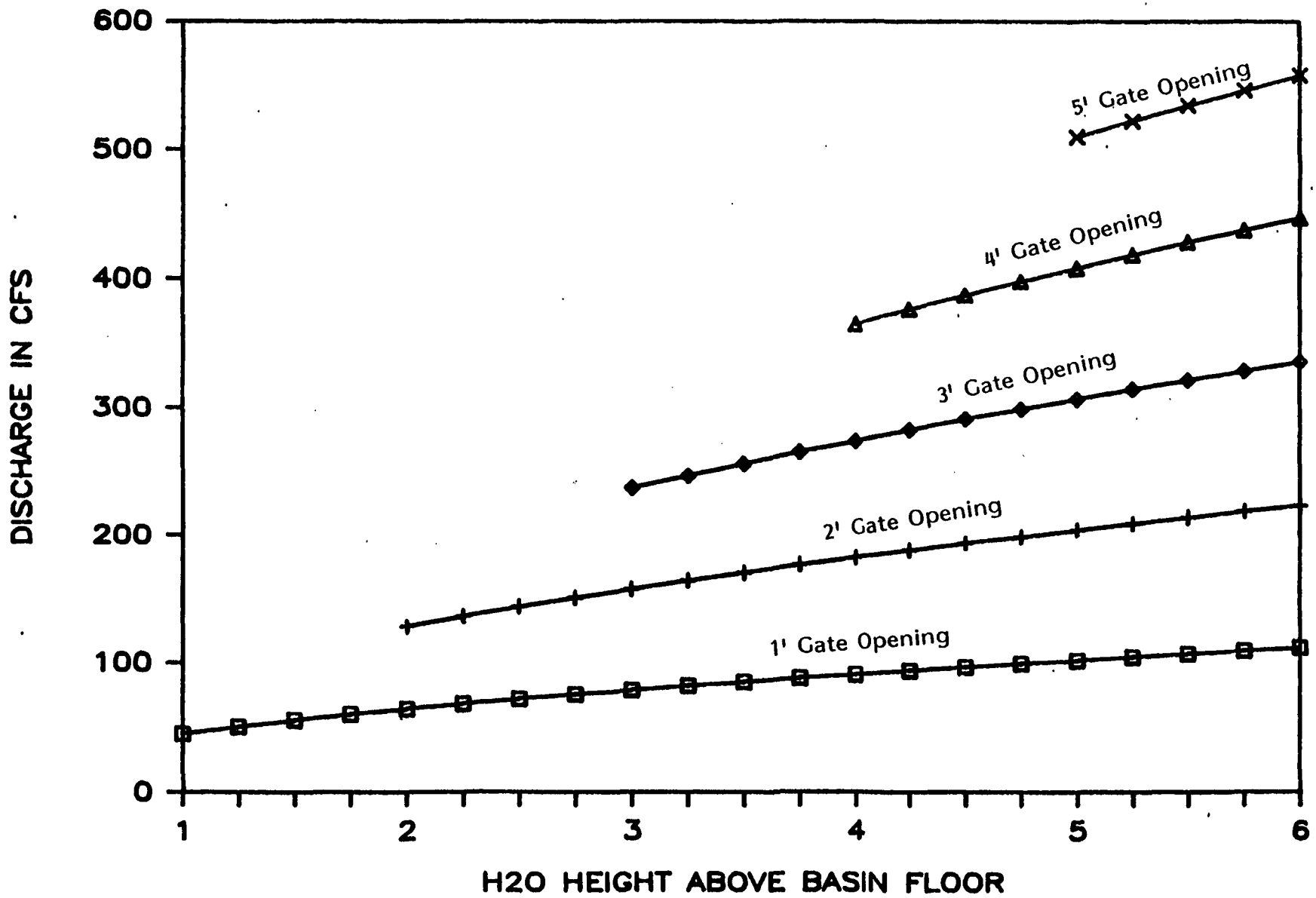
3. Crest elevation of Schweitzer Dam: 948.6 ft. National Geodetic Vertical Datum (NGVD) of 1929 as surveyed by Blasland & Bouck Engineers, P.C.

4. Invert elevation of existing sluice gates in raceway channel: 943 ft. National Geodetic Vertical Datum (NGVD) of 1929 as estimated by Blasland & Bouck Engineers, P.C.

5. Flood stage elevations at the Schweitzer Dam (Flood Insurance Study, Town of Lenox, MA, 1/5/82).

50 year:	953.2 NGVD
100 year:	954.5 NGVD
100 year:	955.0 NGVD
500 year:	956.0 NGVD

Discharge Calculations For Existing Sluice Gates



Notes

1. "Basin Floor" refers to bottom of raceway channel.

4/17/85

DRAWDOWN TIME

ORIGINAL SURFACE ELEVATION OF 6 FT

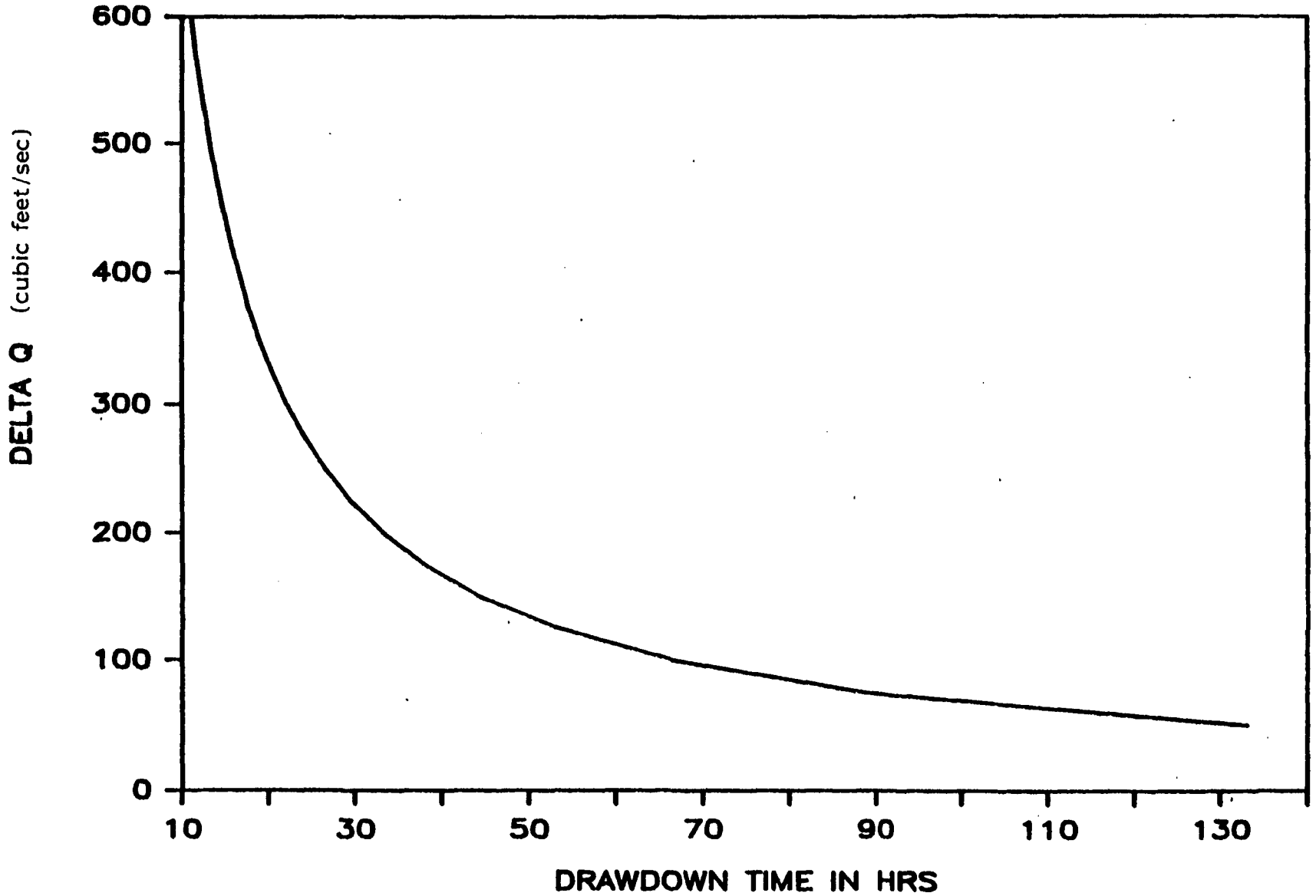
TIME TO DRAWDOWN (hours)

DELTA Q	1 FT	2 FT	3 FT	4 FT	5 FT
600	11.09	20.17	27.23	32.27	38.82
575	11.57	21.04	28.41	33.67	40.51
550	12.10	22.00	29.70	35.20	42.35
525	12.68	23.05	31.11	36.88	44.37
500	13.31	24.20	32.67	38.72	46.59
475	14.01	25.47	34.39	40.76	49.04
450	14.79	26.89	36.30	43.02	51.76
425	15.66	28.47	38.44	45.55	54.81
400	16.64	30.25	40.84	48.40	58.23
375	17.75	32.27	43.56	51.63	62.11
350	19.01	34.57	46.67	55.31	66.55
325	20.48	37.23	50.26	59.57	71.67
300	22.18	40.33	54.45	64.53	77.64
275	24.20	44.00	59.40	70.40	84.70
250	26.62	48.40	65.34	77.44	93.17
225	29.58	53.78	72.60	86.04	103.52
200	33.28	60.50	81.68	96.80	116.46
175	38.03	69.14	93.34	110.63	133.10
150	44.37	80.67	108.90	129.07	155.28
125	53.24	96.80	130.68	154.88	186.34
100	66.55	121.00	163.35	193.60	232.93
75	88.73	161.33	217.80	258.13	310.57
50	133.10	242.00	326.70	387.20	465.85

NOTES

1. Delta Q is outflow minus inflow to basin in cubic feet per second.

Drawdown Curve For 1 Foot Drop In Elevation



Assumptions: Initial Area = 600 acres
Final Area = 500 acres

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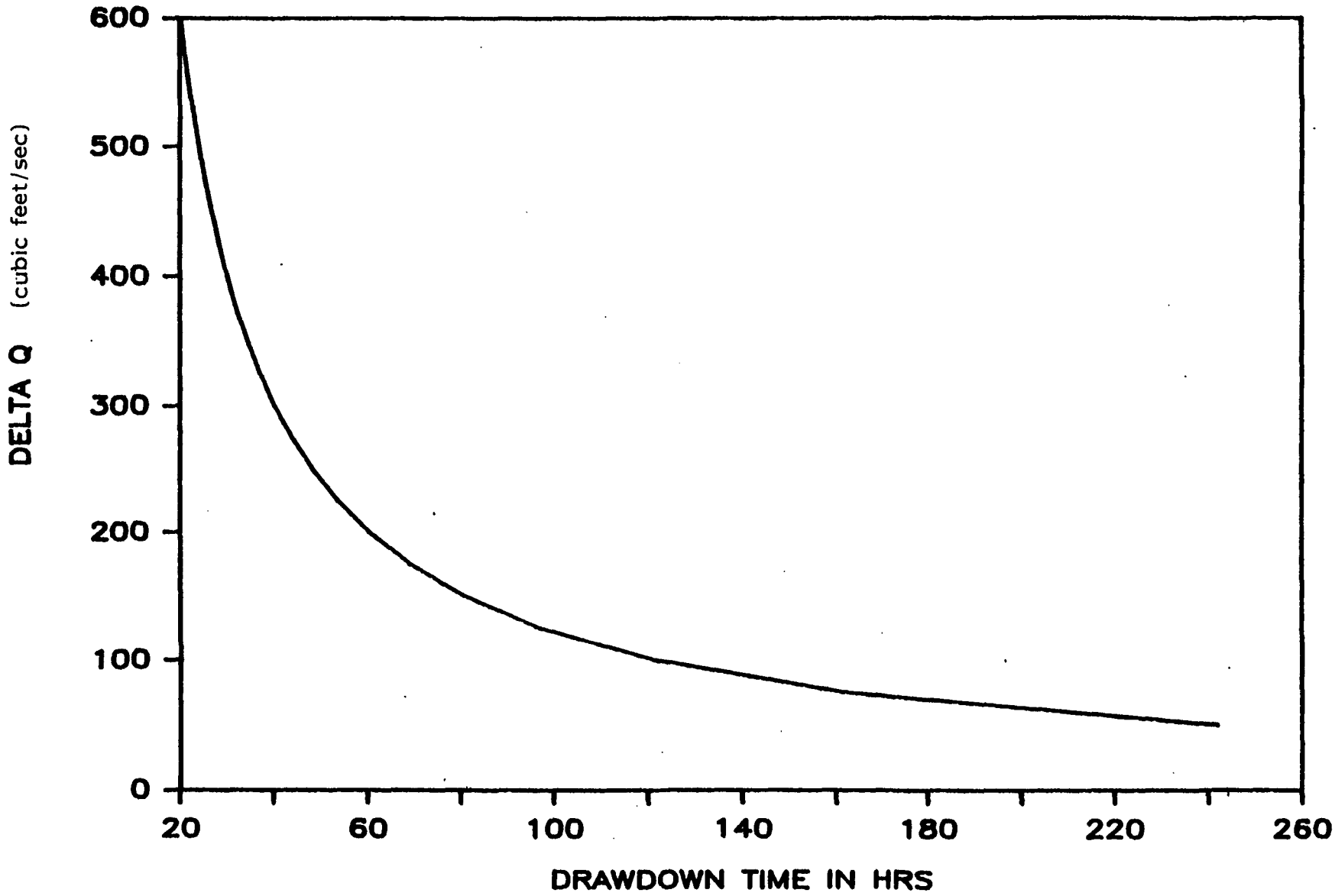
Drawdown Curve For 2 Foot Drop In Elevation



Assumptions: Initial Area = 600 acres
Final Area = 400 acres

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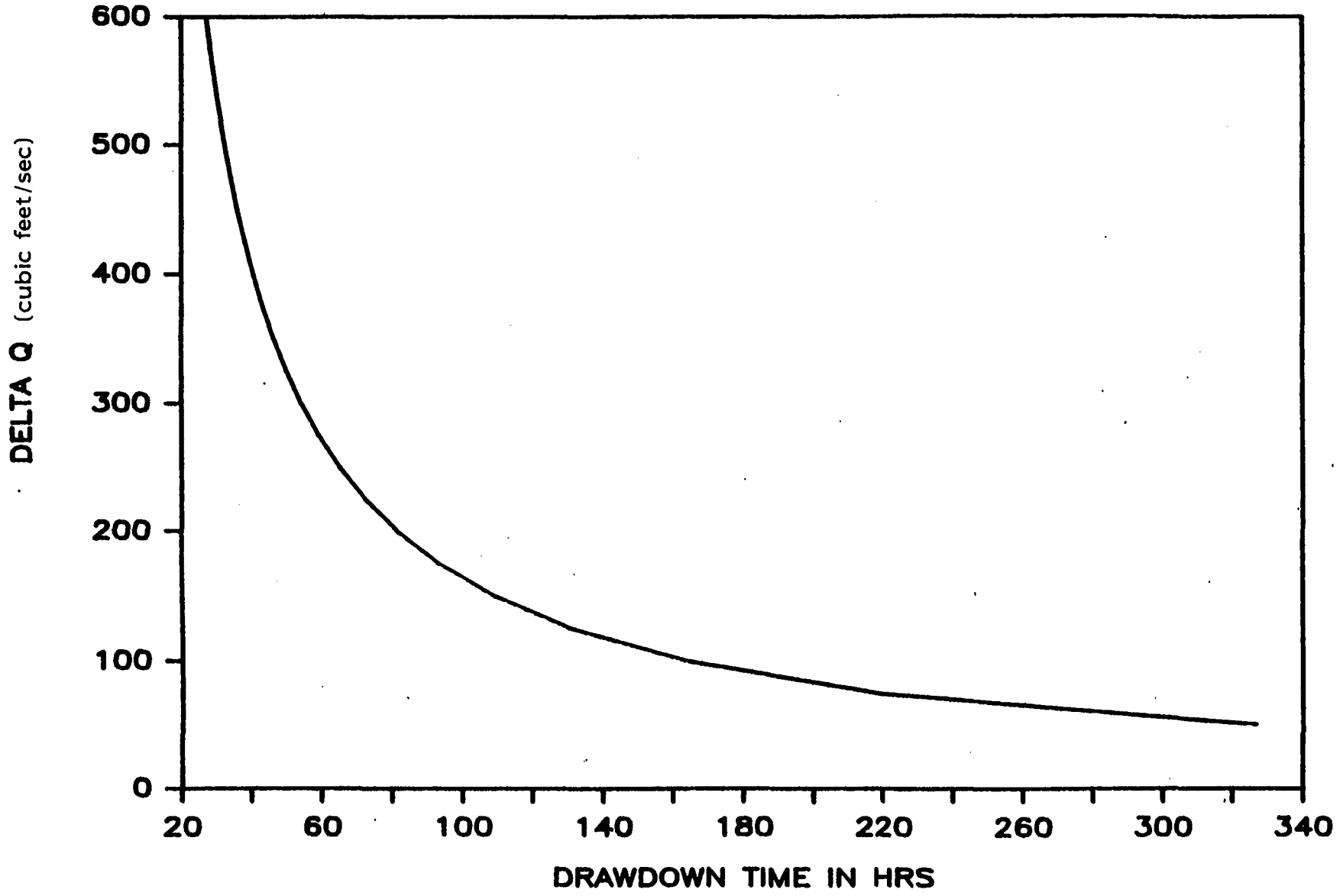
Drawdown Curve For 3 Foot Drop In Elevation



Assumptions: Initial Area = 600 acres
Final Area = 300 acres

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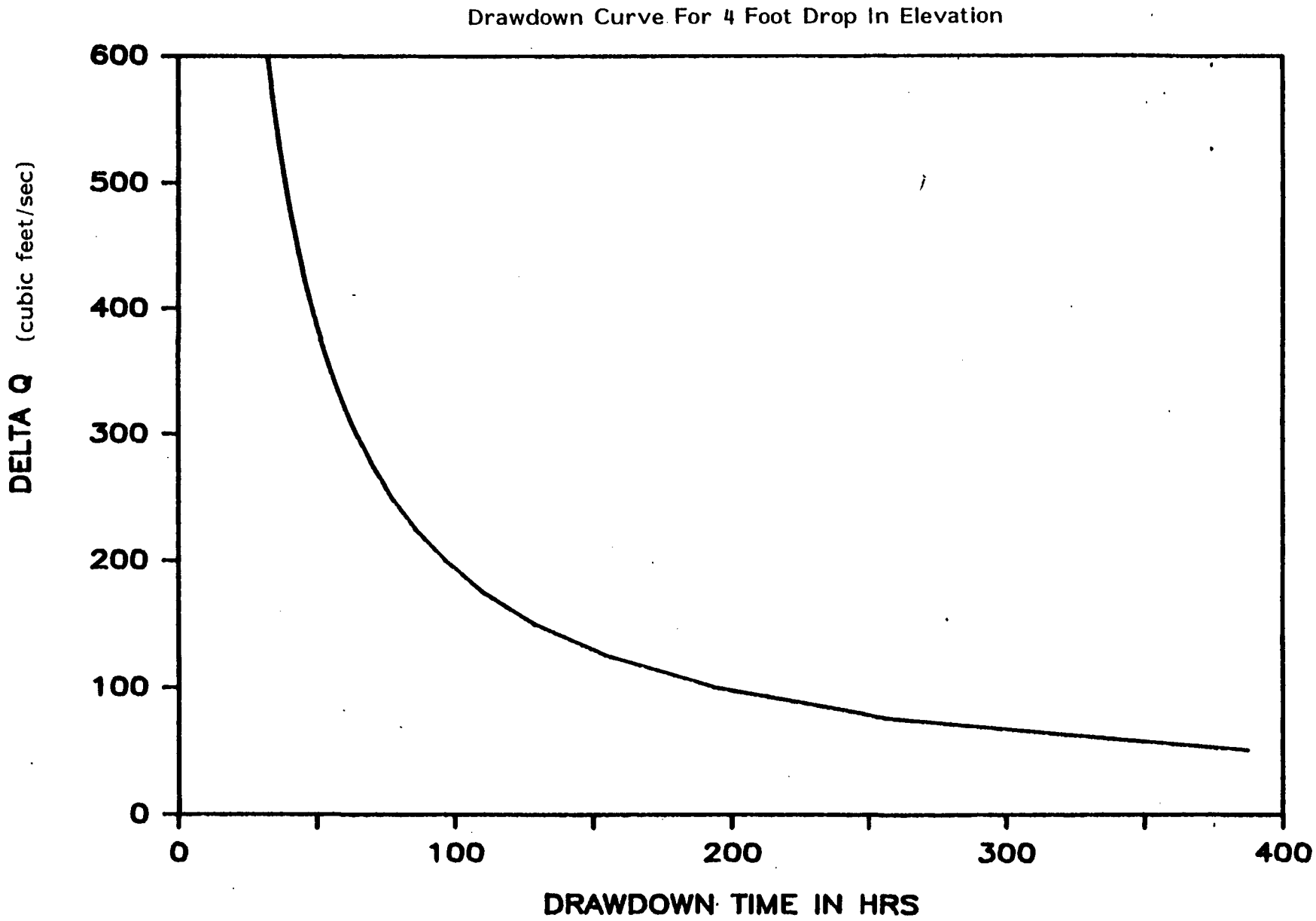
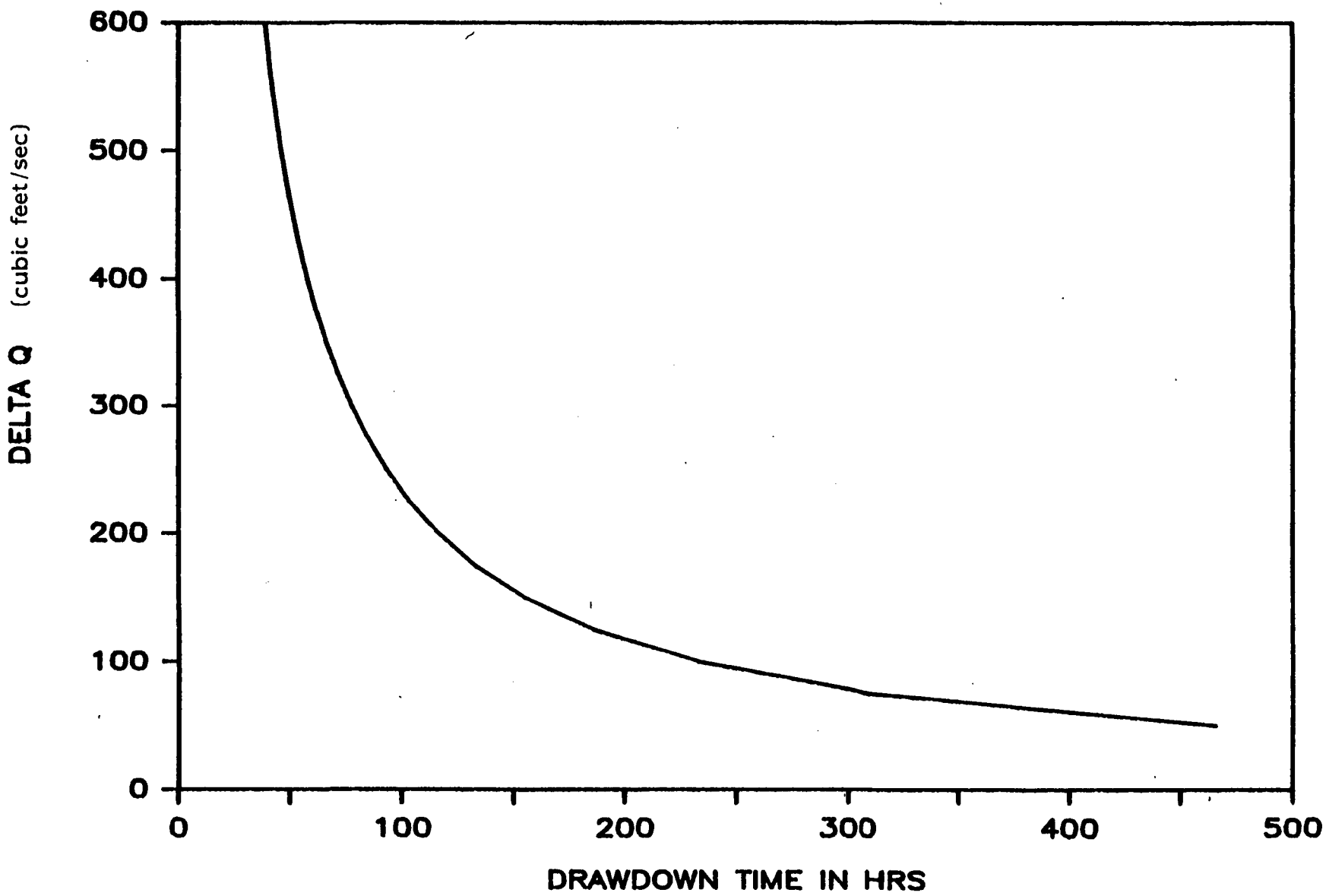


Assumptions: Initial Area = 600 acres
Final Area = 200 acres

4/17/85

Drawdown Curve For 5 Foot Drop in Elevation



Assumptions: Initial Area = 600 acres
Final Area = 50 acres

4/17/85

Assumptions for Basin Discharge Calculations

For the family of discharge curves, the discharge calculation is based on:

$$Q = Cd * A0 * Sqrt(2 * G * H)$$

where A0 = area of discharge gate
G = 32 ft/sec^2
Cd = coefficient for flow resistance = .71
H = height of the water surface above the basin floor

The discharge curves represent the discharge under conditions which range from a maximum water depth of 6 ft down to the depth of the water at the height of the gate. If the water surface falls below the height of the gate the basin discharge will be open channel flow.

==========

Assumptions for Drawdown Time Calculations

The calculations for drawdown times are based on two assumptions:

1. The relationship between flow into the basin and discharge from the basin is constant, delta Q is constant

2. The relationship between basin area at some H0 and the basin area at some Hi is linear.

$$A(h) = mh + b$$

With these two assumptions the drawdown time can be calculated based on the the following relationship between discharge and basin area.

$$(Q_{out} - Q_{in})dT = A(h)dh$$

if $Q_{in} - Q_{out} = \text{delta } Q = \text{constant}$

then $T = (1 / \text{delta } Q) * \int A(h)\, dh$

Schweitzer Bridge Site - Transport Data Summary

Date and Time	Instanteous Discharge (ft^3/sec)	Nonfilterable Suspended Solids (ppm)
February Winter Background		
2/24/82 10:00 a.m.	234	1.0
2/24/82 2:30 p.m.	405[1]	3.8
2/27/82 8:15 a.m.	273	1.4
March Snow Melt		
3/13/82 11:00 a.m.	358	8.2
3/14/82 10:30 a.m.	427	9.0
3/15/82 11:20 a.m.	457	6.4
3/15/82 6:15 p.m.	457	4.4
3/16/82 5:40 p.m.	550[1]	8.6
April Storm Event		
4/20/82 4:00 a.m.	2320	7.5
4/20/82 7:00 a.m.	2080	8.9
4/20/82 5:00 p.m.	1740	7.8
4/21/82 12 Noon	1520	5.7
4/22/82 9:00 a.m.	1310	6.6
4/22/82 9:00 p.m.	1115	9.4
4/23/82 10:00 a.m.	830	6.8
4/24/82 12 Noon	705	3.3

[1]By-pass gates open.

(Reference: Housatonic River Study, 1980 and 1982 Investigations, Stewart Laboratories, Inc. 12/82)

Schweitzer By-pass Canal - Transport Data Summary

Date and Time	Instanteous Discharge (ft^3/sec)	Nonfilterable Suspended Solids (ppm)
3/16/82 4:30 p.m.	206[1]	4.0
3/16/82 6:15 p.m.	372[2]	4.0
4/23/82 6:15 p.m.	423[2]	2.8

[1]Gates one-half open.

[2]Gates full open.

(Reference: Housatonic River Study, 1980 and 1982 Investigations, Stewart Laboratories, Inc. 12/82)

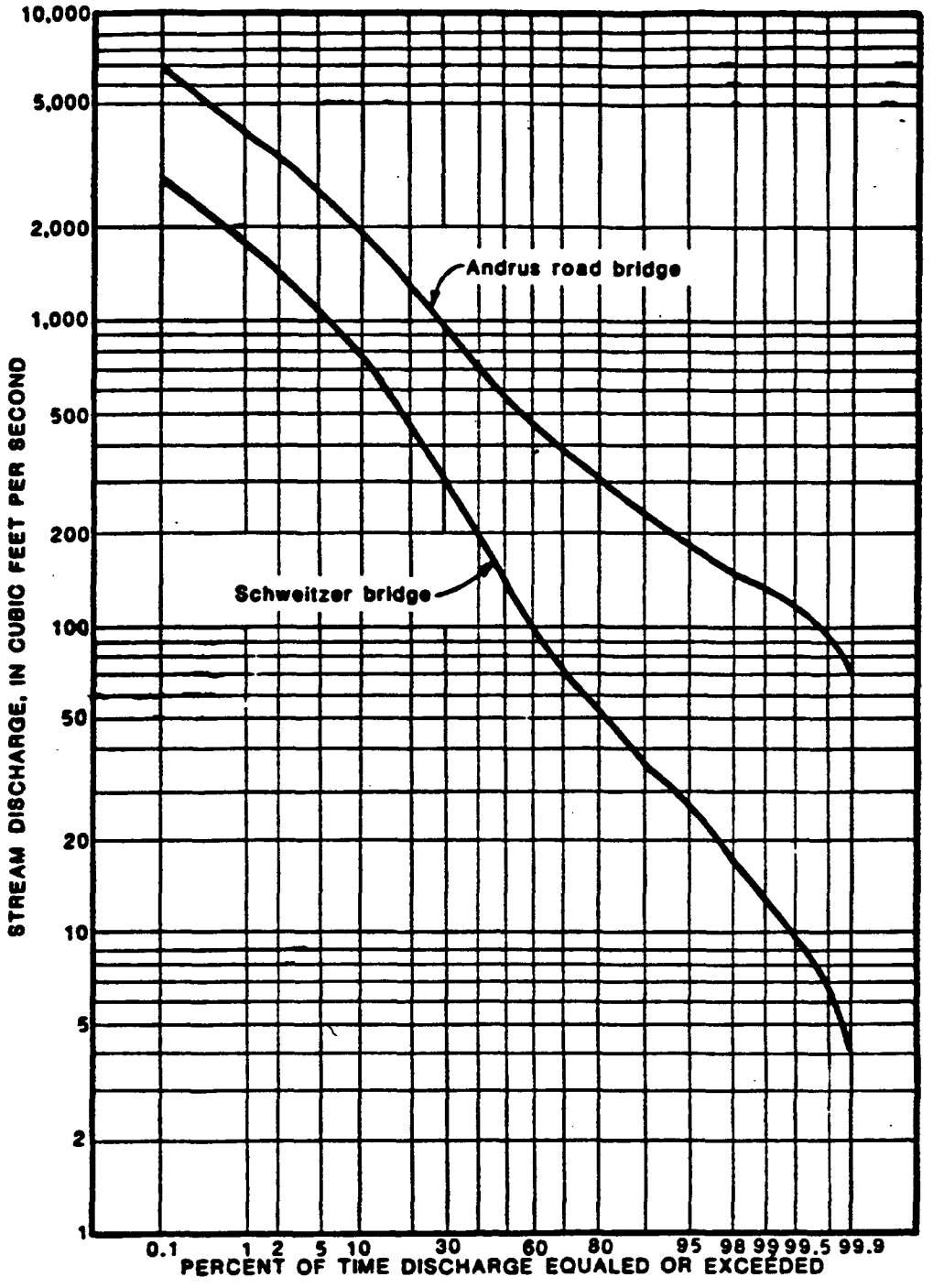


Figure 4-5

Duration Curves of Stream Discharge for the Housatonic River at Schweitzer Bridge and Andrus Road Bridge

Reference: Housatonic River Study, 1980 and 1982 Investigations, Stewart Laboratories Inc. 12/82