OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

RESTRICTED

21193

DP/ID/SER.B/737
24 July 1995
ORIGINAL: ENGLISH

BIODEGRADATION OF ORGANIC POLLUTANTS ORIGINATING FROM PHARMACEUTICAL INDUSTRY

SI/JOR/94/802

THE HASHEMITE KINGDOM OF JORDAN

Terminal report*

Prepared for the Government of the Hashemite Kingdom of Jordan
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Drs. I. Balogh, F. Merchant and D. de Ford

Backstopping Officer: Dr. Zoltán Csizér,
Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

---

* This document has not been edited.

V.95 56172

TABLE OF CONTENTS

I. SUMMARY

Title of the project: Biodegradation of Organic Pollutants Originating from Pharmaceutical Industry

Number of the project: SI/JOR/94/802

This project was carried out jointly by three international consultants, namely, Dr. Istvan Balogh, Dr. Douglas DeFord, and Dr. Fahar Merchant (Team Leader)

The objectives of this mission were to demonstrate to the host laboratory (Environmental Research Centre ["ERC"] at the Royal Scientific Society of Jordan ["RSS"]), key technical approaches necessary for launching a successful project for biological treatment of organic pollutants. This involved, strain isolation and enrichment techniques, genetic improvement of existing strains, entrapment of bacterial cells, set-up and operation of bioreactor, and conceptual design of waste treatment bioreactors and a pilot scale facility.

Based on these activities and in combination with site visits to two pharmaceutical facilities (Dar-al-Dawa and Hikma), the Sahab Wastewater Treatment Plant, and the capabilities of the host organization, recommendations have been presented for continued development of the program at RSS and a proposal for longer term capability enhancement.

With the rapid growth in the pharmaceutical industry in Jordan and continued stress on water resources in the region, use of biotechnological approaches to water treatment will form the basis for sustained development in the area. The enthusiasm and desire of RSS and the local government to support and stimulate industry forms an important launching pad to take this project to prominence, not only in Jordan, but also in the Middle East.

By establishing a strong infrastructure in biological treatment of organic compounds in Jordan, we believe that RSS will be able to rapidly transfer this know-how and expertise to industry, as they have successfully done with other technical projects. This infrastructure will essentially be composed of additional manpower, upgraded laboratory facilities and a state of the art mobile pilot plant for biodegradation of waste streams. The spin-off benefits are expected to result in further technical enhancement in the area of fermentation technology and bioprocessing in general. It is therefore possible that such a capability enhancement will also stimulate the biotechnology industry in Jordan. More importantly, this capability would benefit soil and groundwater remediation programs.

II. INTRODUCTION

The pharmaceutical sector in Jordan is rapidly emerging as key sector of the Jordanian economy. As the largest exporter of pharmaceutical products in the Arab world, it is generating income and improving their of balance of trade. However, sustained development also necessitates concurrent development of appropriate infrastructure and capabilities to treat hazardous wastes generated by the pharmaceutical industry.

Biotechnology provides an opportunity for complete biodegradation of these wastes to CO_2 and H_2O. In recent years, biotechnology is moving into its third, and possibly the most important domain. After the biopharmaceutical and agri-food applications of biotechnology, the protection and restoration of the environment could become a priority goal of the life sciences. Biotechnology is certainly not the only technology to keep the environment clean, but it is an essential one, and its importance, in synergy with other tools, is growing rapidly.

The long tern market potential for environmental biotechnologies is vast. It is expected to grow from $40 billion in the early 1990's to approximately $75 billion by the year 2000 in the OECD countries alone. Therefore, its impact on industrial growth and employment could be significant.

The current opportunities for prevention, treatment, measurement and remediation of environmental damage can be attributed to the relative cost-efficiencies of biological clean up methods as compared to traditional chemical and physical methods. Several useful technologies have been developed in the industrial world and it is likely that these may be easily adapted in Jordan where ambient temperatures result in more rapid biological reaction rates.

In Jordan chlorophenols and other phenols have been found to common pollutants in effluent streams from pharmaceutical and other industries. These compounds are detrimental to aquatic and human life, and to the environment in general. The potential for environmental damage in arid countries such as Jordan can be even more severe, due to the lack of water as a diluting agent. At present such hazardous wastes are collected in barrels prior to disposal in liquid dump sites. Any attempt to develop novel bioprocesses for complete degradation of such hazardous organic compounds would fuel further economic growth in the region.

III. OBJECTIVES

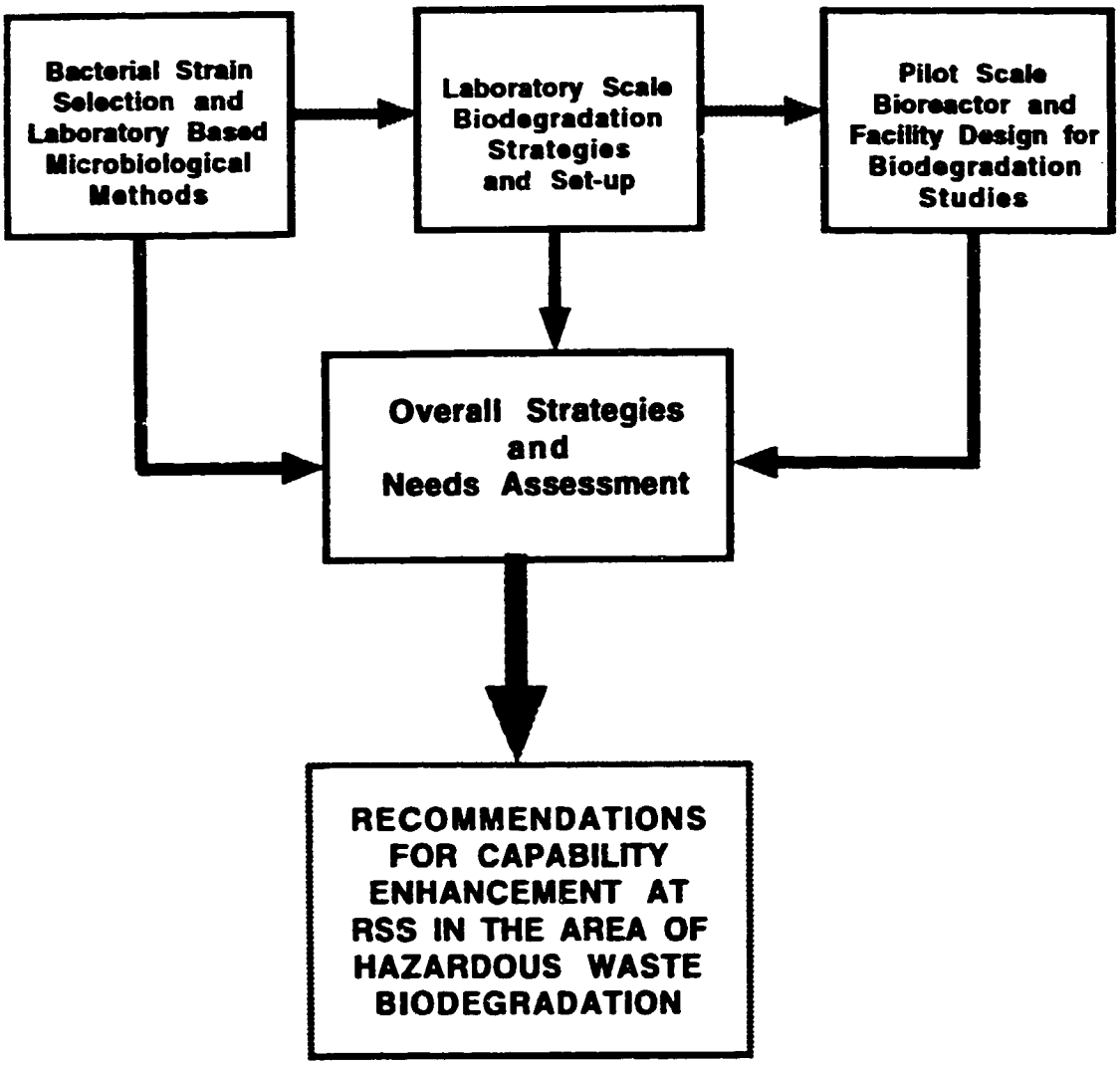
The Environmental Research Centre ("ERC") of the Royal Scientific Society ("RSS") is the premier research organization in Jordan that can potentially establish a local base of expertise to tackle growing environmental concerns in the country. RSS indicated its desire to assist the pharmaceutical sector in their waste treatment problems. In order to effectively do so, it requested the United Nations Industrial Development Organization ("UNIDO") to provide high level advice for the design of a pilot scale facility for the biodegradation of phenol and chlorophenols as representative organic pollutants from the pharmaceutical industry.

Following the meetings of the individual consultants with UNIDO and RSS responsible personnel, the original objectives of the project were modified to take into account the existing laboratory facilities available and the limited time available for field activities. Consequently, the revised objectives of this mission were as follows:

- Assess the pharmaceutical industry in Jordan and their environmental concerns
- Evaluate existing waste treatment capabilities and provide recommendations to address any deficiencies
- Select bacterial strain(s) for biodegradation of phenol and chlorophenols
- Advise on set up of a program for strain maintenance and improvement
- Provide laboratory scale strategies for performing biodegradation studies
- Assist in the design of a pilot scale facility for biodegradation studies
- Prepare a technical report on the work delivered
- Submit recommendations for future action in order to establish in-house capability in biodegradation of hazardous wastes.

The above objectives and deliverables are schematically represented in Figure 1.

Figure 1: Establishing a Pilot Scale Capability at RSS for Biodegradation of Hazardous Wastes



IV. SITUATION ANALYSIS

IV.1 THE PHARMACEUTICAL INDUSTRY IN JORDAN

The Jordanian Government aspires to establish a regional centre for industrial development and foreign investment in order to not only satisfy local needs but also to be able to compete with other industries in foreign markets. Jordan is centrally located among the Middle Eastern countries on the one side and is on the crossroads to Europe and the Far East, on the other.

However, due to lack of significant natural resources, Jordan's ability to set up heavy industries is limited. Industrial growth will therefore arise from significant growth in value added light industries such as computer software, electronics, and pharmaceuticals. The infrastructure to support such growth appears to be in place and is further enhanced by a well educated work force.

In order to stimulate manufacture of human and veterinary medicines, the companies are exempt from custom duties and taxes on:

- Raw materials used in the pharmaceutical manufacturing
- Capital equipment used for manufacture and filling of pharmaceuticals
- Supplies and materials used for packaging finished pharmaceuticals

Through government support and incentives, the pharmaceutical industry in Jordan has achieved the following:

- provided local consumers with affordable pharmaceuticals
- created employment opportunities for skilled personnel
- generated opportunities for technology transfer
- stimulated the supplies and service sector and other related industries, and
- provided the country with significant export revenues.

The industry has established a good reputation in the local and international markets. For instance, in 1992 alone, capital investment in the pharmaceutical sector exceeded $60 million and over 30 companies were known to be involved in the human and veterinary health care sector. These increases were largely attributed to increase in local health care expenditures, growth in population, and increased export demand.

From 1988 to 1992, the export revenues from sales of pharmaceutical products increased from $27 million to over $80 million of which 90% was due to human pharmaceuticals and the remainder for veterinary products. The majority of the exports are directed to other neighbouring Arab countries.

Increased growth and revenues in the pharmaceutical sector has therefore stimulated investment in research and development. For instance, local companies have initiated clinical trials, developed new drug formulations and established joint venture manufacturing projects with other countries such as, Portugal, Yemen, and the United States of America. Some of the major Jordanian exporters of human and veterinary pharmaceuticals are listed in the Table 1.

Table 1: Profile of major exporters of human and veterinary pharmaceuticals as of 1992

Company Name	Major Activity	Examples of Products	Number of Staff	Total Revenues in $US (%Export)
Arab Centre for Pharmaceuticals and Chemicals	Human medicines, gelatin capsules	analgesics, antibiotics, steroids, empty capsules, etc.	200	$4.8 million (94%)
Dar al Dawa Development and Investment Co. Ltd.	Human medicines	antibiotics, CNS, cardiovascular, diabetes drugs, antihistamines, etc.	400	$17.2 million (63%)
Hikma Pharmaceuticals	Human medicines	Antirheumatics, analgesics, muscle relaxants, steroids, vitamins, etc.	470	$24.6 million (82%)
The Arab Pharmaceutical Manufacturing Company	Human medicines & veterinary drugs	Anti-inflammatory, antacids, diuretics, antiasthmatics, enzymes, etc.	1,200	$33 million (81%)
The Jordanian Pharmaceutical Manufacturing Co. Ltd.	Human medicines	Antiulcers, ACE Inhibitors, antihelmintics, antibacterials, etc.	140	$5 million (60%)
The United Pharmaceutical Manufacturing Co. Ltd.	Human medicines	Cardiovascular, CNS, obstetric, GI drugs, lipid regulating agent	150	$17.2 million (10%)
Totals of Five Different Companies Specializing in Veterinary Products	Veterinary products	Vitamins, antibiotics, anticoccidals, feed additives, etc.	267	$17.5 million (69%)
Other Pharmaceutical Related Industries	Miscellaneous products	Diagnostic kits, syringes, gels, wound dressings, containers, etc.	404	$ 7.2 million (48%)
Combined Totals	**Human and Veterinary Medicines**	**Drugs, diagnostics, and supplies**	**3,291**	**$126.5 million (65%)**

IV.2 ENVIRONMENTAL CONCERNS FOR DEVELOPMENT OF A SUSTAINABLE PHARMACEUTICAL INDUSTRY IN JORDAN

In the face of increasing severity of environmental damage, seen during this century, there has been a growing awareness on the part of public and government organizations that actions are needed to maintain and restore environmental quality. While waste minimization and waste recycling programmes are being instituted in the pharmaceutical sector, new technologies are needed to aid in sustaining industrial development and environmental quality. This is especially true in arid regions of the world where water is a precious commodity. Jordan's desire to develop a thriving pharmaceutical industry will therefore also require parallel development of strategic capabilities in pharmaceutical waste treatment and management.

As shown in Table 1, the pharmaceutical industry in Jordan is not a homogeneous industry producing one type of product but a set of interconnecting industries. The basic sub-divisions are:

- Research and Development Sector
- Primary Manufacturing Sector
- Secondary Manufacturing Sector

Associated with each of these is a whole array of other manufacturers that produce solvents, raw materials, packaging materials, vials, needles, etc., which we shall call the "Support Industry".

Since the end products of the pharmaceutical industry are are often small in volume and weight, a vast majority of the materials of the industry ends up as waste. The three main sectors listed above can be placed in the order of "wastefulness" as represented by the approximate percentage of raw materials entering against the product leaving the factory gate as shown below:

- Research and Development ≈ 99% of raw materials ending up as waste
- Primary Manufacturing ≈ 60% of raw materials ending up as waste
- Secondary Manufacturing ≈ 30% of raw materials ending up as waste

Obviously, these figures are not accurate for all situations, but are presented here to rank the three sectors.

R&D does not have any real 'products' except ideas. However, R&D pilot plants do produce some products in the form of clinical trial material or small samples, but this is small in comparison to the overall quantity of material consumed. Generally, one can say that every raw material entering an R&D facility leaves as waste. The major waste from R&D activities is dirty effluent water resulting from a variety of laboratory operations. This in turn gets contaminated with solvents, chemicals, drug entities, and drug by-products.

The main purpose of primary manufacturing is the production of bulk active ingredients and these may be produced in tonnage quantities. These operations are usually associated with considerable use of organic solvents during chemical synthesis or water from fermentation processes. The disposal of waste solvent is largely dictated by its price and secondly, by the hazard that it may represent.

In secondary manufacturing, active components, excepients and packaging materials are converted to dosage form pharmaceuticals. The waste generated here includes off-spec product, contaminated packaging and lesser quantities of solvents and chemicals. In parenteral manufacturing, considerable quantities of water are used for washing and water for injection (WFI) which in turn produces large amounts of "waste water". Cleaning agents may also contaminate this water.

In Jordan, the majority of the pharmaceutical industry is based on secondary manufacturing and the support industry. More recently, however, pharmaceutical companies such as Dar-al-Dawa and Hikma, are also carrying out primary manufacturing activities and to a smaller extent, new drug or formulation development research. Due to the large scale of primary and secondary manufacturing operations, the quantities of waste water generated can therefore be quite significant.

In countries such as Jordan, water is a scarce material and therefore its efficient treatment, recovery or reuse is not an option but an essential requirement for sustained development of the pharmaceutical industry. One approach that is receiving increased attention by regulatory agencies, private sector, and government, is the use of novel biotechnologies for hazardous waste treatment. The Royal Scientific Society of Jordan is pro actively seeking assistance to establish this capability in Jordan, so that future growth of the young Jordanian pharmaceutical industry is not impeded by regulatory compliance or potential environmental problems in the future.

IV.3 THE ROYAL SCIENTIFIC SOCIETY

The Royal Scientific Society ("RSS") was established in 1970 as a research and development institution to work in the areas related to industrial growth in Jordan. The attractive campus which occupies 342,000 m^2 of land, incorporating 28,700 m^2 of laboratories and administration buildings, is adjacent to the University of Jordan. The budget of RSS is derived from contract revenues from technical services and consultations, research contracts, and an annual grant from the Government of Jordan. It also receives grants and donations from local institutions and technical assistance from industrialized countries as well as from regional and international organizations.

The objectives of RSS are as follows:

- Conduct scientific R&D work related to the development process in Jordan with special emphasis to the industrial sector
- Disseminate scientific awareness
- Provide technical consulting and services to the public and private sectors
- Develop scientific and technical corporation with similar institutions in the Arab world and internationally.

RSS consists of seven technical centres, namely:

- Environmental Research Centre ("ERC')
- Industrial Chemistry Centre
- Information and Computer Software Centre
- Building Research Centre
- Renewable Energy Research Centre
- Electronic Services and Training Centre
- Mechanical Design and Technology Centre

Further details on RSS including its structure, organization, collaborations and financial statement are included in Annex 1.

IV.3.1 The Environmental Research Centre ("ERC")

The ERC has a staff of 44 of which 30 are University graduates and 20 have higher degrees. The Director of ERC (Mr. Ayman Hassan) manages the operations of three divisions within ERC, namely:

- Water and Soil Division (Head: Mr. Raid Khashman)
- Ecology Division (Head: Dr. Ali El-Karmi)
- Air Pollution Division (Head: Dr. Jasseen Khayyat)

The ERC has conducted several studies on the quality of water in Jordan in collaboration with other government organizations. These studies identified bacterial contamination in water wells in remote areas. The Ecology Division recently completed a study to examine the impact, efficiency and advantages of using a solar disinfection method which uses natural UV-radiation to disinfect potable water. The results demonstrated the usefulness of this technology for producing disinfected drinking water and a large scale study is underway (in collaboration with a German Technical Agency) to evaluate the economic feasibility of UV-treatment.

This technology is also being applied to evaluate the treatment of pollutants commonly found in the effluents of pharmaceutical, paint and other industries. The abundance of solar energy in Jordan prompted the Ecology Division of ERC to investigate the possibility of using photocatalytic oxidation process which uses natural UV-radiation combined with titanium dioxide to treat organic pollutants. Titanium dioxide activated by light in the UV-range has the ability to oxidise organic solutes. Studies with this technology are at early stages of development and may be a useful strategy for pretreatment of hazardous wastes prior to biodegradation.

Activities are also in progress to develop an efficient process for biological treatment of olive oil-mill effluent streams using an Upflow Anaerobic Sludge Blanket ("UASB") reactor.

In addition to the above, ERC is involved in many environmental R&D projects. A list of these activities and other projects carried out by ERC are presented in Annex 2. The Ecology Division, under the leadership of Dr. ElKarmi, is essentially responsible for establishing capabilities in biodegradation of hazardous wastes. The research and technical personnel involved with this program are listed in Annex 3.

From the above information and our observations it is apparent that ERC is a premier research centre in Jordan actively engaged in development and commercialization of novel waste treatment technologies. Its reputation and stature in the Arab world would therefore make it an ideal location for developing a capability in biotechnological approaches for treatment of hazardous wastes generatred by the pharmaceutical and other industries in Jordan. The need to establish a strong capability in this area becomes apparent when reading some pages of a recent report on Industrial Pollution in Jordan (See Annex 4).

V. FIELD ACTIVITIES IN JORDAN

The field experts appointed by UNIDO were stationed at RSS in Amman and hosted by the Ecology Division of ERC for a period of approximately three weeks commencing October 8, 1994. The activities reported in this section were carried out in close consultation and collaboration with the staff of the Ecology Division. All laboratory activities, field trips and technical projects were performed as a result of their active participation. What follows in the subsequent sections is a summary of field activities accomplished during our brief stay in Amman. Laboratory and technical assistance was provided by Mr. Ali El Omari, Mrs. Lana Khamees, and Mr. Mohamed Abu Zaki. Additional technical and administrative support was provided by Dr. Ali ElKarmi and Mr. Hamed Ajarmeh

V.1 VISITS TO PHARMACEUTICAL POLLUTION SOURCES AND WASTE TREATMENT FACILITIES

As mentioned earlier, there are seven major pharmaceutical factories in Jordan. After inquiries and preliminary review of the different facilities, it was decided that the three largest factories should be toured in order to survey their current waste treatment capabilities, obtain information on their effluent streams and also to collect sludge samples. The factories identified for the tours were:

- Hikma Pharma Group
- Arab Drug Company
- Dar Al Dawa.

All tours were arranged and organized through personal connections of RSS researchers. Collection of the samples was coordinated through meetings with the management of the factories and waste water plants.

The Arab Drug Company did not allow us to tour their factory or even provide a sludge sample. Instead of the Arab Drug Company, arrangements were then made to tour the Sahab Wastewater Treatment Plant.

The quantities of waste generated by the above pharmaceutical companies are summarised in Table 2. The waste water analysis data for the Arab Drug Company and Dar-al-Dawa are presented in Table 3.

The managers at both factories that we visited (i.e. Hikma and Dar al Dawa) denied any pollution by chlorinated phenols (both factories deal also with chemical synthesis). There were some other contradictions in the information they provided. Overall we found that their small waste-water treatment plants were not of the best design, and the waste-water and sludge treatment seemed to be unsatisfactory.

Table 2: Quantities of Waste Generated in Select Jordanian Pharmaceutical Companies

Pharmaceutical Facility	Solid Waste	Wastewater (m3/day)
Dar-al-Dawa	All recycled	25
Hikma Pharma	Not available	36
Arab Drug Company	Not available	125

Table 3: Characteristics of Wastewater from Dar-al-Dawa and Arab Drug Company

Characteristics	Dar-al-Dawa	Arab Drug Company
pH	6	8.24
Conductivity	Not available	1,225 µs/cm
TDS	900 mg/L	1,044 mg/L
TSS	220 mg/L	294 mg/L
BOD	1,730 mg/L	658 mg/L
COD	4,870 mg/L	1,136 mg/L
Fe	Not available	2.4 mg/L
NH4	18 mg/L	Not available
ABS	32 mg/L	Not available

V.1.1 Hikma Pharma Group

The factory of Hikma Pharma Group is situated in the western part of Amman. It has been dealing with the production of human pharmaceuticals since 1965. The majority of the activity since then has been to carry out secondary manufacturing which essentially involved formulation, finishing and packaging of imported bulk drug substances. More recently (i.e. since early 1994) the company has been engaged in primary manufacturing to produce active compounds by chemical synthesis. At present, the Hikma Pharma Group exports 90 % of its products to Middle Eastern countries. Further details on Hikma's operations and wastewater characteristics are provided in Annex 5.

The factory generates two different liquid effluent streams. The first and smaller stream is generated from primary manufacturing activities associated with the chemical synthesis plant. The daily discharge rate from this source is approximately 5m^3.

This effluent stream has a high solvent content and is collected in two 10m^3 concrete pits. The major organic solvents and chemicals found in this effluent stream are as follows:

- Acetone (trace quantities)
- Dimethylformamide (up to 40%)
- Methylenechloride
- Acetonitrile
- Triethanolamine salts

According to the information provided by the technical management, the high solvent effluent is disposed off (somewhere) after combining it with solvents generated from various other laboratories in the factory. The management of this company realises that this is not an ideal way to handle their waste. It therefore plans to invest in a small solvent recovery plant in order to reduce the solvent contents of the effluent stream and also to reduce operating costs by recovery and reuse of the expensive solvents.

The second and much larger effluent stream (output of 36 m^3/day) is generated by secondary manufacturing activities at the formulating plants. The majority of this wastewater is treated in a 100 m^3 aerated concrete tank. The pit has four sections and a separate section serves as a clarifier pit. The plant has not instituted a pH control strategy, nor can any signs of sludge treatment be found. However, the size of the pit (providing a retention time of almost 3 days) and the aeration level of this system seems to be adequate.

It should be noted that the factory formulates modified cephalosporins such as cephalexine and cephadoxine. The effluent is likely to contain a lot of these antibiotics and the sludge could be a potential source of several cephalosporin resistant microbes. Therefore, the sludge should be treated and used with special care and preventive measures should be put in place in order to avoid leakage into the communal wastewater system.

Two samples were taken at Hikma Pharma Group; one from the effluent of the chemical synthesis plant and the other from a biological treatment pit.

V.1.2 Dar Al-Dawa

The Dar Al-Dawa factory is located at the South Western part of Amman. The factory carries out primary manufacturing activities associated with chemical synthesis of several medicines. A detailed list of these medicines was not made available.

According to the information provided by the technical leader of the plant, the effluent stream only contains small amounts of the following chemicals:

- Ethanol
- Methanol
- Chloroform, and
- Acetic acid

In view of the fact that the plant generates waste streams from both, chemical synthetic activities and from formulation lines, the claimed low levels of solvent generation (in terms of composition and quantity) seems to be unusually low.

Effluent is generated at a rate of 35 m^3/day which collects in a 200 m^3 covered container. It was not possible to withdraw samples from the deep covered container. However, a sample was later taken when the liquid was pumped out from the tank. The sludge was obtained by centrifugation of the sample (3000 rpm for 20 minutes). A wastewater analysis data sheet is provided in Annex 6.

The company was also in the construction phase of a biological treatment facility. This included four open 10 m^3 agitated and aerated carbon steel vessels which are planned to be used as aerobic bioreactors. For sludge thickening a large perforated stainless steel tray with a surface area of 10 m^2 will be installed. Based on the current stage of construction it seems that more than 80 % of the sludge water content will seep into the soil from the perforated sedimentation tray.

V.1.3 Sahab Wastewater Treatment Plant

The Sahab Wastewater Treatment Plant ("SWTP") , which is owned by the Jordan Industrial State Corporation, is situated in the Sahab industrial region which is approximately 10 km South of Amman's city limits. This region consists of a conglomerate of several industrial plants producing a total of 800-1000 m^3 of wastewater per day. The effluents are not treated at the point of discharge in any of the factories. Waste treatment is therefore left entirely up to the SWTP.

Considering the large diversity of the factories (e.g. detergents, paints, dyes, cosmetics, etc.) and their disregard for discharging polluted effluents, the SWTP encounters daily and dramatic changes in the quality and volume of the incoming effluent. Approximately 200 m^3/day of communal waste-water (which is treated together with the industrial effluents) probably helps in stabilizing this process.

The volume of the three aeration pits is 1000 m^3. Based on the quantity of waste water treated, these aeration pits provide a retention time of only one day, which is not sufficient and this in turn could lead to potential problems associated with overloading. The design of the aeration pits indicate that they may also function as clarifiers. If the 760 m^3 pretreatment pit, with its function of buffering and neutralization, could be used with proper aeration and mixing, it would help in coping with the usual problems of overloading.

The mixing is performed by two small blowers, but the pattern of the surface bubbles indicates that aeration is not sufficient. The performance of this pit could be improved by installing better air spargers which provide homogeneous air distribution along the bottom of the pit. Frequent and regular dissolved oxygen and pH measurements using portable equipment would be necessary for better monitoring and control of the process. In order to minimize deviations in pH associated with changes in the characteristics of wastewater being processed, installation of a pH control system is recommended. This could be accomplished by erecting $Ca(OH)_2$ sludge addition equipment which could be used for both, the pretreatment pits and the aeration pits.

The average COD value loaded is 2400 mg/L and the final effluent of the plant has a 150-180 mg/L COD level. Western standards require these levels to be less then 100 mg/L. The sludge is stored and thickened in large concrete pits. After being partially dried, the sludge is transported to dumping sites. The final effluent from the plant is used for irrigation. A sample of the thickened sludge was taken from the SWTP for further laboratory studies at RSS, as described later.

V.2 ENRICHMENT AND SELECTION OF MICROBES

V.2.1 Introduction

Isolation of indigenous microbial strains from local waste generation and treatment sites can be of great advantage. Having been in contact with the waste stream, surviving microbial populations may have adapted to the toxic compounds (such as chlorinated phenols and organic solvents) present in the wastewater. It is also likely, that the sludge of industrial effluents provides a good selection environment for microbial populations capable of metabolism and degradation of such waste products. Furthermore, mixed microbial strains thriving in the sludge of the contaminated wastewater usually have better degrading capabilities than a pure culture of a single strain. This is due to the fact that day to day changes in the composition of wastewater does occur and a mixed population of microbial species would be in a better position to adapt to variations in the quality of the waste stream.

Pure culture strains do provide excellent supplementary support for certain hazardous waste streams or well defined waste streams containing known contaminants with minimal deviation in the composition of the waste stream. However, in some instances these strains are difficult to obtain from research laboratories. Furthermore, these strains may not demonstrate the same level of activity under real conditions when compared to well designed and controlled conditions generally available in a laboratory setting. A recommended list of pure strains and their respective sources have been listed in Annex 7, and may be acquired by RSS for future research.

In this study, a strain selection program from sludge samples (collected from different waste sites discussed earlier) was therefore initiated at the RSS. It should also be mentioned, that with the exception of the Arab Drug company, sludge samples were made available by other organizations. Managers at Hikma and Dar al Dawa Pharmaceutical plants (which carry out manufacturing operations by chemical synthesis) indicated that chlorinated phenols were not used or generated in their facilities and was therefore not a component in their waste stream.

Evaluation of strain improvement methods was carried out using UV light as the the mutagen since the recommended mutagen, nitrosoguanidine (NTG), was not available at RSS. Nevertheless, selection using NTG was explained in detail including the hazardous nature of this compound and its safe handling procedures. The UV method could only be demonstrated by using a Pseudomonas aeruginosa strain which was available at ERC. The short duration of this mission did not provide us with adequate time to identify and obtain additional strains from the enrichment and selection experiments described in the next section.

Sample collection was coordinated through meetings with the management of the two pharmaceutical factories and the wastewater treatment plant. This part of the project was carried out under the supervision of Dr. Balogh.

V.2.2 Experimental Design and Results

As described below, a total of four sludge samples were used for the selection experiment:

Sample Source	Label
Hikma Pharma Group	H1, H2
Dar Al Dawa	D
Sahab Wastewater Treatment Plant	S

The sample originating from Dar Al Dawa was an effluent sample taken from the wastewater storage tank. This sample was centrifuged (3000 rpm, 20 minutes) in order to obtain sludge. The other samples were taken directly from wastewater sludge, which contained an enormously large variety of microbial species. The enrichment method described below is designed to select for chlorophenol ("CP") degrading microbes. Thus, this method would increase the probability of selecting for CP degrading bacteria. It does so by giving an advantage to the bacteria which have CP degrading characteristics (or are at least tolerant to CP) and repressing the growth of other strains.

V.2.2.1 Selection in Liquid Culture

From each sample, 0.2 g of sludge was added to pre-sterilized flasks containing 20 ml of Chlorophenol Enrichment (CPE) Media, labelled as CPE1, CPE2 and CPE3 (see Annex 8 for a description of media composition). The media composition was varied by adding different amounts of peptone and yeast extract. The reason for varying these compounds was that targeted microbes may have special requirements. In this particular experiment, no microbial growth could be observed in the absence of peptone and yeast extract. Each of the CPE1, CPE2 and CPE3 media were also supplemented with phenol and chlorinated phenols (after sterilization).

The compounds tested were as follows:

- phenol
- 2-chloro-5-methylphenol
- pentachlorophenol
- 4-chloro-2-methylphenol, and
- 2,4-dichlorophenol.

The final concentration of all these compounds was 106 mg/L. Prior to addition to the CPE1, CPE2 and CPE3 media, the above listed organic compounds were first dissolved in 1.0 ml of methanol. In many cases microbes have been known to require co-substrates during the degradation of highly toxic and stable compounds. In this case, phenol was used as a co-substrate. Methanol was used for dissolving crystals of the CP and phenol. The role of the $CaCO_3$ in the media was to prevent low pH conditions caused by generation of CO_2 and hydrochloric acid.

The inoculated flasks were shaken at approximately 250 RPM for four days by using small benchtop shakers at room temperature. The shakers were borrowed from the water laboratory and were stopped for 30 minutes, once every day in order to prevent the motors from burning out because of the heat generated. It was therefore not possible to incubate the shake flasks at the optimal 30 °C.

After four days of incubation, new flasks were started from the first set by inoculating them with 0.5 ml of broth. The new series of flasks contained the same CPE1, CPE2 and CPE3 media. The newly inoculated flasks were also shaken under similar conditions for four days.

Culture enrichment was determined by microscopic examination of methylene blue stained samples and also by measuring the optical density ("O.D.") at 600 nm against a distilled water blank after dilution with 0.2N HCl (in order to dissolve the $CaCO_3$ suspended in the media).

Table 4: Results of microscopic observation

Sample	CPE1 Medium	CPE2 Medium	CPE3 Medium
Hikma 1 (H1)	No growth	No growth	No growth
Hikma 2 (H2)	No growth	No growth	No growth
Dar al Dawa (D)	Poor growth	No growth	Poor growth
Sahab (S)	Dense growth	Poor growth	Dense growth

Table 5: Results of OD measurements at 600nm

Sample	CPE1 Medium	CPE2 Medium	CPE3 Medium
Hikma 1 (H1)	0.12	0.172	0.14
Hikma 2 (H2)	0.138	0.166	0.102
Dar al Dawa (D)	0.08	0.224	0.192
Sahab (S)	0.262	0.22	0.294

The above results demonstrate that this enrichment method can be used successfully to cultivate CP resistant species. This selection method could not be completed due to lack of time available for the project. Nevertheless, the proper methods were demonstrated to the staff in order for them to carry out further selection experiments with these and other future sludge samples.

V.2.2.2 Further Selection on Agar Plates

Based on OD measurements the five most promising flasks (D/CPE2, D/CPE3, S/CPE1, S/CPE2 and S/CPE3) were used for further selection experiments.

Agar plates were inoculated with 0.3 ml of each sample. The composition of the solid medium was identical to the CP3 medium, supplemented by the same amount of phenol, CP and 2% agar. The agar plates were incubated for four days in a 37°C incubator. After the incubation period all the plates were fully covered by microbes. However, the growth did not seem to be complete.

Plating was repeated with diluted samples of S/CPE1 and S/CPE3 flasks in order to obtain single colonies. The dilutions used were 1:100, 1:1000, 1:10000 and 1:100000. These plates were incubated for a longer time (one week) in order to obtain an adequate colony-size for subsequent inoculation of the nutrient slant agar tubes. In the case of insufficient growth, plating could be repeated using nutrient agar medium. Additional on-site activity with this phase of the project could not be completed due to time restrictions. What follows is a description of additional work recommended to the laboratory.

V.2.3 Additional Recommended Activities

The colonies that appear on the plates should be streaked onto slant nutrient agar media. The selected strains should be then maintained by restreaking them onto new slant nutrient agar tubes at a frequency of twice a year. These tubes should then be incubated at 30°C for three days. It is recommended that at least three (preferably more) slants from each distinguishing colony are inoculated. Two (or more) slants should be used for the purpose of strain maintenance (master culture). One (or more) slant(s) should be used as working cultures.

The working seed slants should be made using nutrient agar supplemented with 0.5% glucose. The presence of glucose provides a quick and intensive growth which is useful when using them to inoculate flask cultures. The master culture should be regularly validated at least once a year by streaking them on several agar plates containing different concentrations of CP.

After completion of the selection process using the above method, the selected strains should be identified by studying their morphological and biochemical characteristics.

The CP degrading capacity of the different isolates also should be checked by measuring the residual CP concentration after the shaking process. Following these standard inoculation and culturing process enables one to interpret the results more accurately and to validate the CP degrading abilities of the isolated strains.

V.2.4 Freeze Drying of CP Degrading Strains

Once RSS obtains a benchtop freeze dryer, the selected strains should be freeze dried in order to ensure their long term storage and safe maintenance.

For the purpose of freeze drying, cells should be harvested from slant agar test tubes after incubation for at least three days. In the case of spore forming microbes, incubation should be extended until sporulated cells are obtained. The rationale for using maturated cells for freeze drying is that active, "young", multiplying cells tend to be more sensitive and therefore their survival rate during the lyophilization tends to be low.

Cells from one slant agar can be harvested by resuspending them in 5 ml of sterile water followed by centrifugation at 3000 rpm for 30 minutes. The sedimented cells should then be resuspended in 2 ml of 25% diluted sterile milk. The suspension is then transferred into a lyophilization tube and connected to the freeze dryer. All steps prior to freeze drying should be carried out under sterile conditions. Thus, sterile pipettes, centrifuge tubes, and lyophilization tubes should be used, and all operations should be performed on a clean bench (laminar flow hood). After freeze drying is completed (based on the methods recommended by manufacturer) the tubes should be sealed and then stored in a refrigerator.

Sterile milk is usually employed to protect microbial cells from freeze damage. This is carried out by diluting 25 ml of milk to 100 ml with water. The diluted milk should then be maintained in a 100°C water bath for 30 minutes in order to kill vegetative cells. The treated milk is then stored for one day at room temperature. This period enables germination of all surviving spores. After this, a 10 minute autoclaving ensures sterility since all viable spores would have been in a vegetative state prior to autoclaving. Conventional sterilisation methods cause the milk to be burnt and is therefore undesirable.

For further details a short manual describing the principles and techniques of freeze drying is enclosed in Annex 9.

V.3 GENETIC IMPROVEMENT OF MICROBES

V.3.1 Introduction

Properties of a microbial species can always be altered or improved by genetic manipulation in combination with proper selection methods. In this case, the objective would be to increase CP degrading capabilities of the desired strain.

During the mission it was not possible to genetically improve the selected strains since the results from the microbial selection experiments were obtained towards the end of the working period. Therefore a demonstration on genetic improvement methods was carried out using an available strain of Pseudomonas aeruginosa (ATCC 9027). The demonstrated method can be applied later by the microbiology staff using any potentially useful strain.

V.3.2 Experimental Methods and Results

A freeze dried preparation of the P. aeruginosa strain was suspended in sterile water and 0.3 mL of this preparation was spread on nutrient agar (Oxoid, code: CM1, with 2 % of agar) plates. One dish was placed in a 37°C incubator without any treatment and this was used as the control. Other plates were were irradiated by UV light for different durations of exposure (i.e.: 1, 5, 30, 180, or 600 seconds).

Exposure was performed by using a UV lamp which was originally used for evaluation of thin-layer chromatograms in the Chemistry Department of RSS. The dark lamp cover was removed in order to increase the intensity of irradiation. The device had an output of 2x6 W and the plates were placed at a distance of 4 cm from the UV source. Irradiation was carried out in a temporary dark manipulation chamber, and the irradiated plates were then covered by aluminium foil in order to prevent photo-reactivation.

The UV-treated dishes were then incubated together with the control plate for four days at 37°C. Observation of the plates after the four day incubation period showed that the control dish contained round colonies, approximately 3 mm in diameter. The first irradiated dish (UV exposure of 1 second) contained 0.1 mm sized colonies which had not shown any further growth after the first day. These irradiated colonies were therefore lethal mutants which are able to survive for only some generations.The second irradiated plate (5 second exposure) had a similar response. However, all remaining dishes were clear.

One would have expected substantial growth on the surface of the control plate. But this was not the case indicating that the original freeze dried culture was not in good condition. Apparently, the freeze dried tube was opened on July 1994, and was thereafter stored by covering it with paraffin foil. It is likely that moisture was taken up by the lyophilized granules and slow damage of the living cells had occurred. New plates were therefore inoculated with a suspension made by using a new slant agar which had been previously started from the freeze dried tube.

In the second set of experiments, the intensity of the irradiation was decreased. This was achieved by increasing the irradiation distance (between the UV-source and plate) from 4 cm to 40 cm by installing a temporary stand for the UV lamp. Since there is a quadratic relationship between irradiation intensity and distance, a 10-fold increase in distance results in a 100-fold decrease in UV intensity. The duration of irradiation were the same as those in the first experiment.

The first and second dishes (1 and 5 second exposure, respectively) were covered completely. The third dish (30 second exposure) contained 50 colonies of normal size. The last two dishes (180 and 600 second exposure) contained three colonies each. On the basis of these results, a more suitable duration for irradiation is estimated to be 15 seconds.

Having optimized the irradiation conditions, it was decided that the property to be improved in this demonstration will be the cultures increased tolerance to phenol. Thus, the P. aeruginosa strain was inoculated by streaking on nutrient agar dishes supplemented with phenol. The final phenol concentration (in g/L) in the plates was 0.01, 0.05, 0.1, 0.5, 1.0, and 2.0. The purpose of this experiment was to determine the lowest non-tolerable concentration of phenol in the agar medium.

After three days of incubation at 37°C, the growth on the first two plates (phenol concentration of 0.01 and 0.05 g/l) was normal. The growth on the third plate (0.1 g/l) was repressed (the presence of the microbes at the traces of the streak could only just be observed). There was no growth detected on the higher concentrated dishes.

In order to select tolerable mutants, the remaining 0.01 g/l and 0.05 g/l phenol containing dishes were inoculated with a P. aeruginosa cell suspension, and UV-irradiated for 15 seconds at a distance of 40 cm. On the second day of incubation it was observed that there was no growth on the dishes containing 0.05 g/l phenol. There were two dishes made with 0.01 g/l phenol concentration. One of them contained a very vague, nearly continuous, blanket of microbes. The second one contained a couple of small colonies which had overgrown the slight blanket. This indicated that the colonies were new mutants with a higher phenol tolerance in comparison with the original strain.

V.3.3 Additional Recommended Activities

The above method was performed only for the purpose of demonstration and this simple procedure was chosen due to the short duration of the mission. The duration and distance of irradiation should be further optimized. Additionally, viable cell counts should be measured pre- and post-irradiation. Viable cell numbers should be determined by preparing decimal dilutions with sterile water and plating the diluted suspensions on agar dishes. In the case of irradiated samples the procedure should be carried out in a dark chamber, and the plates immediately covered by aluminium foil.

Irradiation can also be carried out using a 10 mL cell suspension in an empty plate. The cell suspension can be prepared by inoculating 20 ml of culture broth with a loopful of microbes from slant agar. The 20 ml of inoculated medium should be shaken (250 rpm, 30°C) overnight in a 250 ml flask. This method provides cells that are in an exponential growth phase and these cells tend to be very sensitive to mutagens. Exponentially growing cells have their DNA in an "unpacked" stage and consequently has a higher probability of partial damage caused by the mutagen. The cells should be separated by centrifugation (3000 rpm, 20 minutes), and resuspended in sterile water prior to mutagen treatment.

The irradiation conditions should be modified so that 99 to 99.9% lethality is achieved. In the case of UV treatment this high lethality rate was found to be necessary for obtaining a relatively high number of mutants among the survivors.

The preferred method for mutagenesis would be to use nitrosoguanidine ("NTG"), which is considered to be one of the most potent mutagens. Unfortunately NTG was not available at RSS and it was not possible to obtain it during the mission. In order to safely use this mutagen, a detailed explanation was provided to the staff and emphasis was placed on hazards associated with the use of NTG (see Annex 10).

A brief explanation was also given about the possibility of improving the facultative anaerobic bacteria being used to treat olive oil mill waste water using an Upflow Anaerobic Sludge Blanket (UASB) reactor. This waste stream is known to contain some toxic compounds which are not readily tolerated by the mixed culture of the reactor. In order to improve the performance of these bacteria, the mutation program described above may also be used with some degree of success. The procedures would have to be adapted to include the lowest intolerable concentration of the toxic component(s) present in the waste stream.

A short seminar on fermentation technology, biotechnology and waste treatment was presented at the ERC by Dr. Balogh. An abstract of this presentation, written by Dr. Balogh, is provided in Annex 11.

V.4 SETUP AND OPERATION OF A CONTINUOUS IMMOBILIZED CELL BIOREACTOR FOR TREATMENT OF PHENOLIC COMPOUNDS

V.4.1 Introduction

Upon arriving at RSS it was determined that the Ecology Laboratory did not posses any fermentation equipment in order to carry out controlled pure culture optimization studies as planned for this particular part of the project. Laboratory scale production of bacterial species for subsequent biodegradation studies of hazardous compounds is therefore not possible at present. This would therefore limit the Division's ability to gain experience in the area of fermentation technology which is absolutely essential if RSS is to pursue with pilot scale biodegradation studies. A basic manual describing the principles of fermenter set-up and operation has therefore been provided in Annex 12.

For the purpose of the visit, it was decided to demonstrate strategies for biodegradation of hazardous organic compounds. As an example, we therefore chose pentachlorophenol ("PCP") as the test compound for biodegradation and utilised the same Pseudomonas strain used in experiments described earlier. As a bioreactor, an approximately 8L plexiglass vessel was configured with an internal draft tube and a fritted glass aeration tube. When inoculated with immobilized bacteria, the reactor would then operate as a circulating bed bioreactor which is a hybrid of the fluidized bed and air-lift designs. Experimental design and approaches were therefore developed based on this reactor configuration and adapted according to available resources, supplies and equipment. There were no pH and dissolved oxygen meters and controllers for this study.

The purpose of immobilization is to ensure a continuous process without fear of culture washout. Culture washout in continuous processes especially, when dealing with feedstreams containing toxic compounds, can be a major problem. However, by immobilizing cells on support surfaces or entrapment in polymeric matrices, the cells have an opportunity to adjust to intermittent changes in the composition of the feed stream. Furthermore, immobilization ensures extremely high cell densities of the desired microbial population in the bioreactor and therefore increased biodegradation rates.

V.4.2 Experimental Methods and Results

i. Strain

A strain of Pseudomonas species used in earlier studies described above was used for these experiments.

ii. Biomass Growth

A loopful of the bacterial culture was inoculated in 100 mL of overnight growth medium (OGM). The inoculated cultures were incubated overnight at room temperature by shaking at approximately 200 RPM. The composition of OGM, was as follows:

Nutrient broth (see composition below)	13 g/L
Calcium Carbonate	1 g/L
Glucose	5 g/L

Nutrient broth was supplied by: NenTech Ltd., Brixworth, Northants, UK.

Nutrient broth composition:

Yeast Extract	1.5 g/L
Peptone	5.0 g/L
NaCl	5.0 g/L
Beef Extract	1.5 g/L

iii. Agar Immobilization Method

Autoclave 1.0L of 15 g/L agar and cool to 45°C. Mix 200mL of culture broth and pump the mixture through a pipette tip. Let the agar-cell slurry drop into a chilled water bath containing a magnetic stirrer. Adjust flow rate and height so that near spherical particles are formed. Let the beads harden for 30 minutes and wash the beads with cold tap water.

This procedure was attempted but did not work due to flow rates of the high throughput peristaltic pump. Lower flow rates necessary for forming single drops was not possible as a smaller sized pump was not available. Additionally, due to the low concentration of agar used in this experiment, gel formation was also not possible as the agar dissolved when added to the cool water.

In future, any attempt to entrap cells in polymeric gels will require a low flow pump or a large scale droplet generator if the same pump is used.

Due to difficulties associated with sphere agar bead generation, agar/cell slurry mixture (approximately 500mL) was poured on a tray and allowed to gel overnight. The gel was cut into approximately 1.0 cm squares (width approximately 0.5 cm) and placed in bench scale three phase fluidized bed bioreactor. The agar particles were suspended in 2L of non-sterile OGM overnight to enhance further bacterial growth in the agar gel.

The contents of the reactor were then drained using a sieve and washed with several volumes of water. The agar particles were reintroduced in the reactor and 5L of Biodegradation media ("BDG media") was added.

The composition of the BDG media was as follows:

KH_2PO_4	0.17 g/L
K_2HPO_4	0.65 g/L
$NaNO_3$	0.5 g/L
$MgSO_4.7H_2O$	0.1 g/L
$FeSO_4.7H_2O$	5.6 mg/L

(A 10x concentrated stock solution was prepared and diluted before use. Thus 500mL of conc BDG stock was made up to 5L using tap water.)

iv. Bioreactor Setup and Operation

Fill the bioreactor with 800 mL of the remaining fermentation broth (OGM), and add the agar beads. Make up to approximately 5L volume using concentrated BDG media and water. Do not add any PCP at this stage. Set up bioreactor with constant aeration and allow cells to grow overnight. The transparent gel should begin to look opaque and whiter as cells grow in the gel. Normally this would be best carried out with proper pH and dissolved oxygen control and under optimal temperature for bacterial growth.

Any future plexiglass reactors built at RSS should be placed in a controlled environment or be jacketed. After 24 to 48 hours of growth (depending on the bacteria used), the growth media may be drained and replaced with BDG media and PCP (initial concentration of 10 mg/L). A sample of the media should be taken after PCP addition and monitored at least once every day for residual PCP levels. Once, PCP concentration drops by 80% of its initial level, BDG media supplemented with 10 mg/L PCP can be fed on a continuous basis.

As the bioreactor shows improved adaptation and consumption rates of PCP, the concentration of PCP in the feed may be increased and the reactor operated at higher throughputs. The process may run smoothly for several months with the right bacterial strain.

V.4.3 Bioreactor Design

Use of a circulating bed or fluidized bed bioreactor provides some advantages especially if the waste stream contains particulate matter or the process has high oxygen requirements or excessive metabolic heat generation. Packed bed bioreactors would tend to plug with particulate matter causing high pressure drops and reactor shut down. Also oxygen transfer and heat removal efficiencies are quite low with packed bed bioreactors.

Furthermore, since fluidized reactors are generally well mixed, in continuous operations, the concentration of the toxic component "seen" by the microorganism is much lower then that in the feed stream. In packed bed and trickling bed reactors, due to the plug flow behaviour, the immobilized cells will experience higher concentrations of the toxic compound. Packed bed and trickle bed reactors have low energy requirements and much more easier to maintain.

V.4.4 Additional Recommended Activities

As discussed earlier, the agar method was not suitable for preparing small spherical immobilization beads. Preferred matrices for entrapment of bacteria in whole cells would be to use alginate or carrageenan as immobilization agents.

In the case of alginate, aqueous 4% solution of sodium alginate is prepared. This is then mixed with an equal volume of an overnight shake flask culture of the bacteria in growth media. The alginate-cell mixture is then extruded through a 22 gauge needle by using a peristaltic pump. Individual droplets are then allowed to fall in a chilled solution of calcium chloride (40g/L). Spherical calcium alginate beads will form immediately upon contact . Let gelation complete for an hour or so before charging the reactor with the spherical beads.

Unfortunately, neither sodium alginate or carrageenan were available in Amman during our stay. This material can be readily purchased from Sigma or other chemical supply companies. It would be a good idea for RSS to repeat the experiment using a better immobilization matrix and combine that with a long term biodegradation study as described above.

An alternate to polysaccharide gels is to use porous solid particles and immobilize the cells on the surface by hydrostatic interaction. More recently, material such as polyurethane foam has been used quite successfully. An outline of the various immobilization strategies and bioreactor design issues have been outlined in the attached articles (see Annex 14). Additional details on PCP degradation and immobilization techniques may be obtained from recommended literature and publications listed in the literature search and bibliographic list included in Annex 14.

V.5 DESIGN OF PILOT SCALE PRETREATMENT SYSTEM AND BIOREACTORS FOR DEGRADATION OF PHARMACEUTICAL WASTES

V.5.1 Design of Pretreatment System

There are several well known physico-chemical methods that may be used in combination with the biological process. Development of the most suitable combination of pretreatment technology and biodegradation will depend on the type and characteristics of the waste stream. It will therefore be essential for ERC to identify the optimal combination of technologies. As mentioned earlier, ERC has established the necessary expertise on the development of UV-irradiation as a means to disinfect water. It is likely that similar technology may be used to pretreat hazardous waste streams from the pharmaceutical industry, prior to the biodegradation step.

If this route is further pursued at ERC, then design features of a UV-irradiation system shown in Figure 2, may be used to construct a pilot scale facility at the RSS. This would have to be located in close proximity to the biodegradation pilot plant.

The UV-system shown in Figure 2 consists of a concave surface with a parabolic vertical section which concentrate large quantities of light (wide spectrum) & heat on a 100 mm diameter glass tubing. The concave parabolic reflection surface is divided into six sections, each one may be 1.5 m wide & 2.5 m long so the total length of the photolysis pretreatment pilot system is 15 m long. The six sections are set in a steel structure. Each concave parabolic mirror can be vertically adjusted as an independent unit and the set of six sections can be adjusted horizontally. This would provide two degrees of freedom in order to adjust for maximum light reflection on the glass tubing.

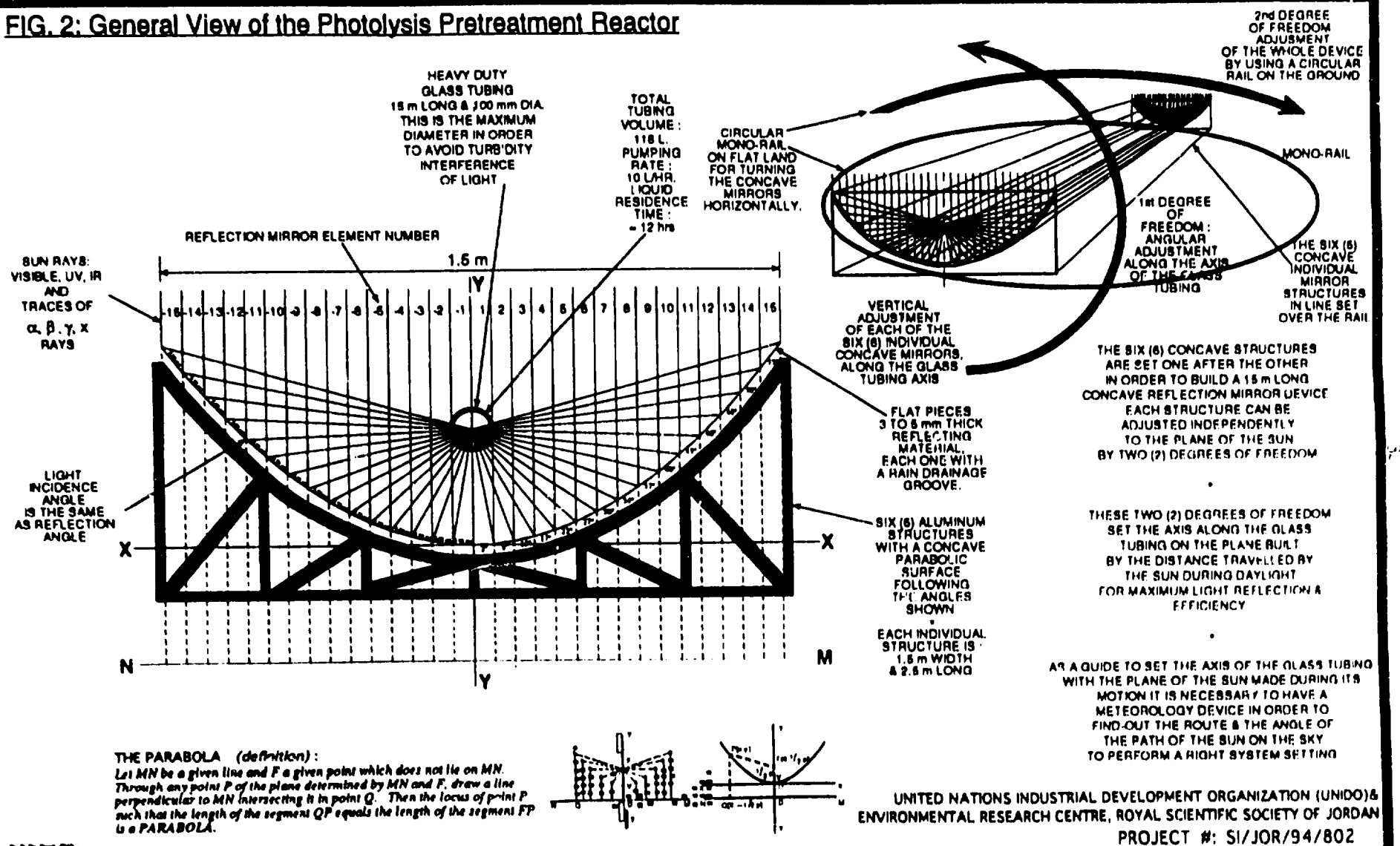
Capital expense for installing a photolysis pretreament unit is expected to be high. However, one would expect that with adequate pretreatment, the demand on microbial degradation will not be severe and complete biodegradation may be accomplished using smaller bioreactors.

V.5.2 Biodegradation Reactors and Pilot Plant

It is suggested that up to three different types of biodegradation systems be considered for ERC. This would include:

- A compact continuous fluidized bed bioreactor
- A continuous packed bed bioreactor
- A continuous trickling bed bioreactor.

FIG. 2: General View of the Photolysis Pretreatment Reactor



THE PARABOLA *(definition)* :

Let MN be a given line and F a given point which does not lie on MN. Through any point P of the plane determined by MN and F, draw a line perpendicular to MN intersecting it in point Q. Then the locus of point P such that the length of the segment QP equals the length of the segment FP is a PARABOLA.

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION (UNIDO)&
ENVIRONMENTAL RESEARCH CENTRE, ROYAL SCIENTIFIC SOCIETY OF JORDAN

PROJECT #: SI/JOR/94/802

A continuous high solids retention algal pond may also be considered if suitable strains and in-house expertise in algal culture and maintenance was established. For now we shall consider this to only be an option.

The packed bed and trickling bed could essentially be the same unit, but would only differ with respect to the type of packing material used and the direction of flow (flow by gravity for trickling bed bioreactor). In each of the three cases, the design is intended to allow easy transportation of the bioreactors to the source of waste stream. Once optimal conditions have been determined, the data could then be used for design of a commercial scale unit. For special waste streams and R&D activities, the bioreactors could be used at the ERC pilot plant which is currently used to store equipment and for occasional experimental work.

V.5.3 Fluidized Bed Bioreactor

The fluidized bed bioreactor (see Figure 3) should be mounted on an aluminium frame in order to save weight and ease transportation. The compiete system would include plastic feed and receiving tanks. The packing or immobilization matrix used in the fluidized bed could be sand (sieved from 0.3 to 0.9 mm), wood chips fragmented to 1 mm, styrofoam fragmented to 1 mm, plastic pieces graded to 1 mm or any other suitable material depending on the adsorbtion capacity of the support and the property of the microorganisms. Alternatively, bacterial entrapment in alginate gels may also be evaluated. The material of construction of the bioreactor could be plexiglass. RSS has well established capabilities to build such units in-house.

V.5.4 Continuous Packed Bed and Trickling Filter Bioreacto:

The packed bed bioreactor (Figure 4) may be filled with one of many inert supports for microbial immobilization. This would include coal, stones, or plastic "U" shaped pieces with an optimal size that would not result in significant pressure drops across the bed. Unlike the fluidized bed system, aerobic processes in packed bed reactors would require a separate aeration chamber to saturate the waste stream with oxygen prior to the biodegradation step.

Enhanced oxygen transfer occurs by recirculating the broth, with a centrifugal pump, through the oxygen transfer chamber. As above, the unit may be readily constructed at RSS using plexiglass. Dissolved oxygen, pH and foam controls should be performed in the oxygen transfer chamber. This bioreactor can also be transformed into a trickling filter bioreactor (Figure 5) by installing a diffuser plate on the top and changing the direction of the feed flow. Due to potential compression problems, immobilization of microbes in polysaccharide gels, such as alginate is not recommended when using the packed bed or trickling filter bioreactor configuration. All bioreactors should also be equipped with glass wool filters for inlet and exhaust air, as designed in Figure 6.

FIG. 3: General View and Set-up of a Continuous Pilot Scale Fluidized Bed Bioreactor for Treatment of Industrial Waste Water

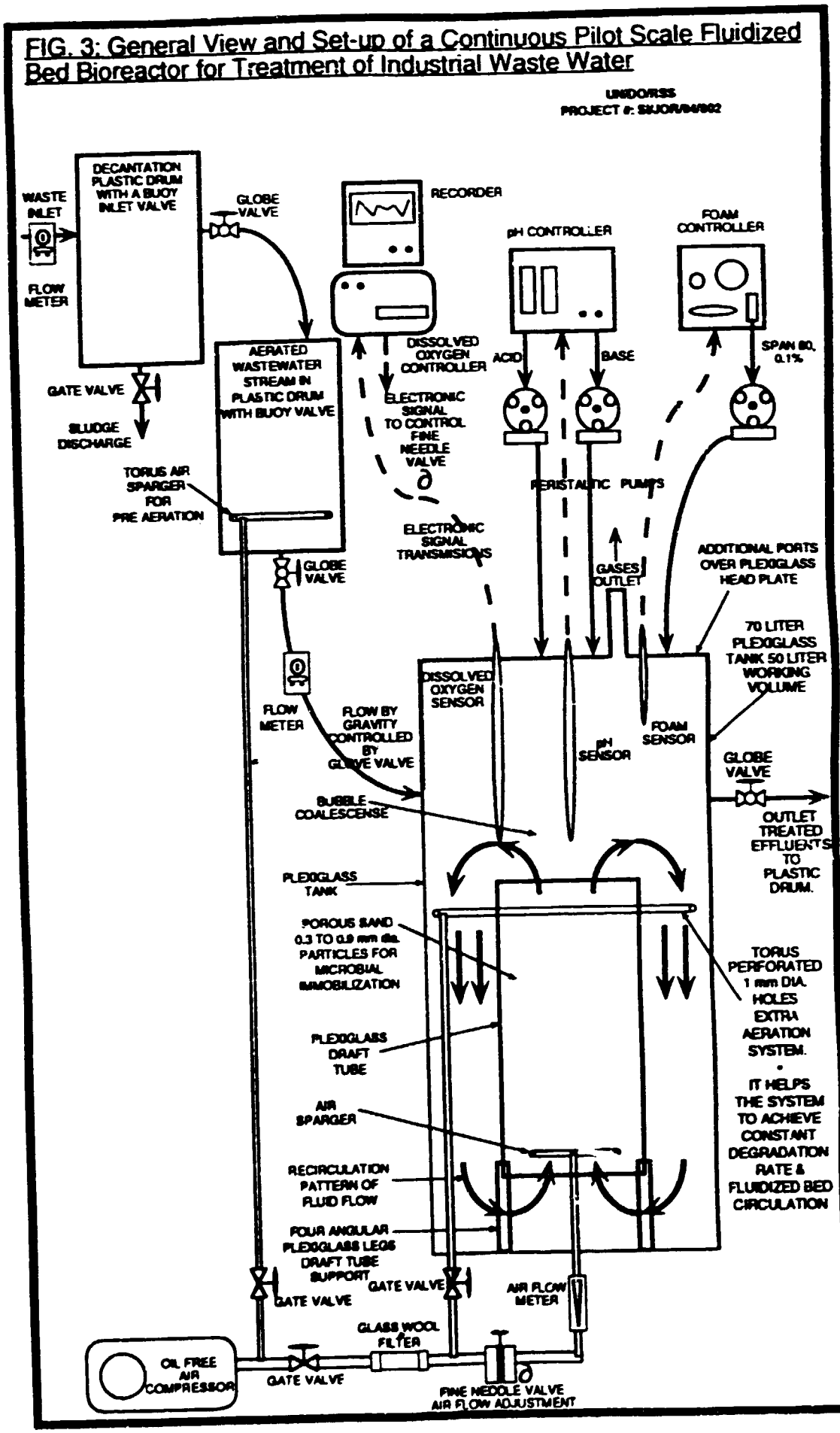


FIG. 4: General View of a Packed Bed Bioreactor

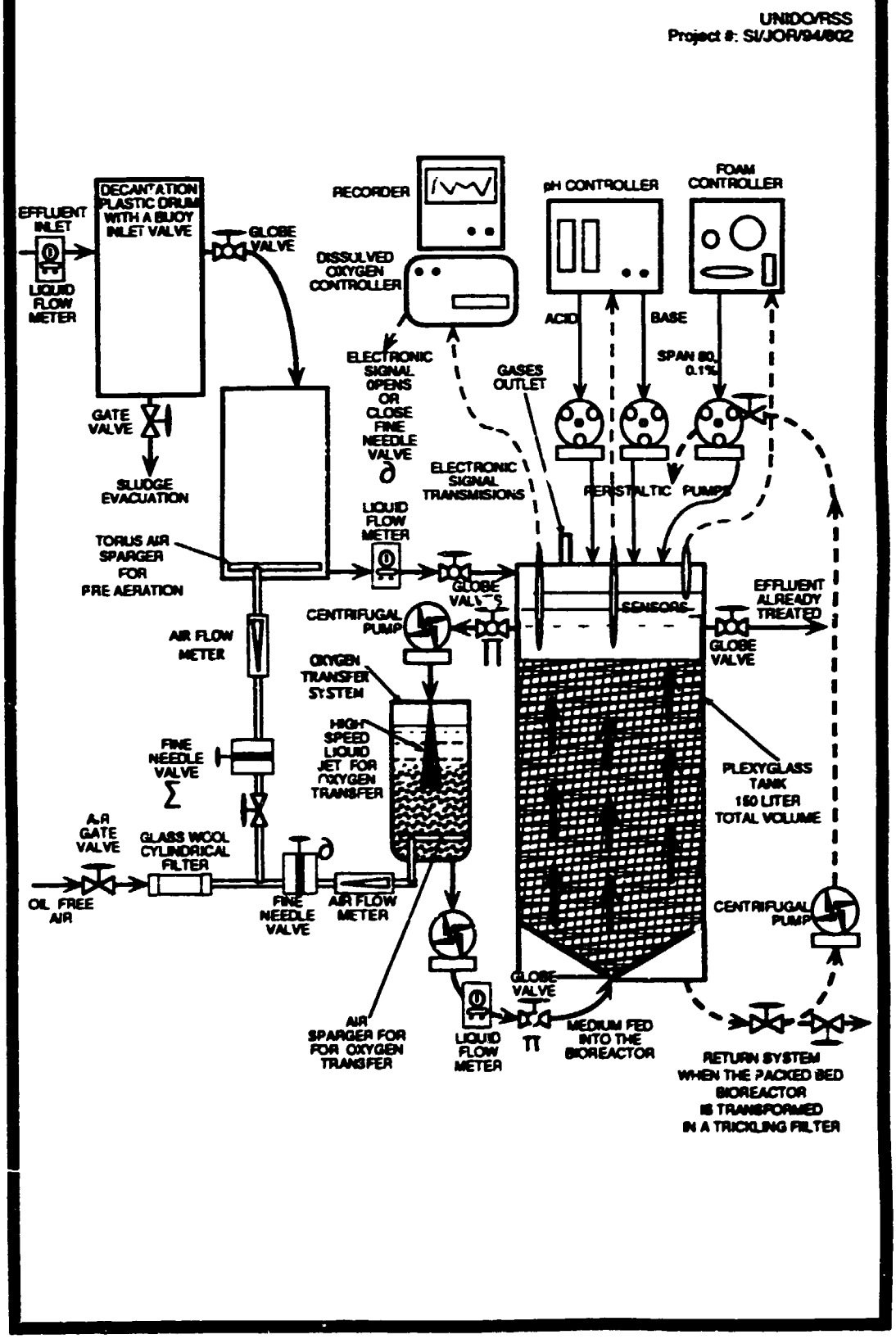


FIG. 5: GENERAL VIEW OF A CONTINUOUS TRICKLING FILTER BIOREACTOR

UNIDO/RSS
PROJECT #: SI/JOR/94/802

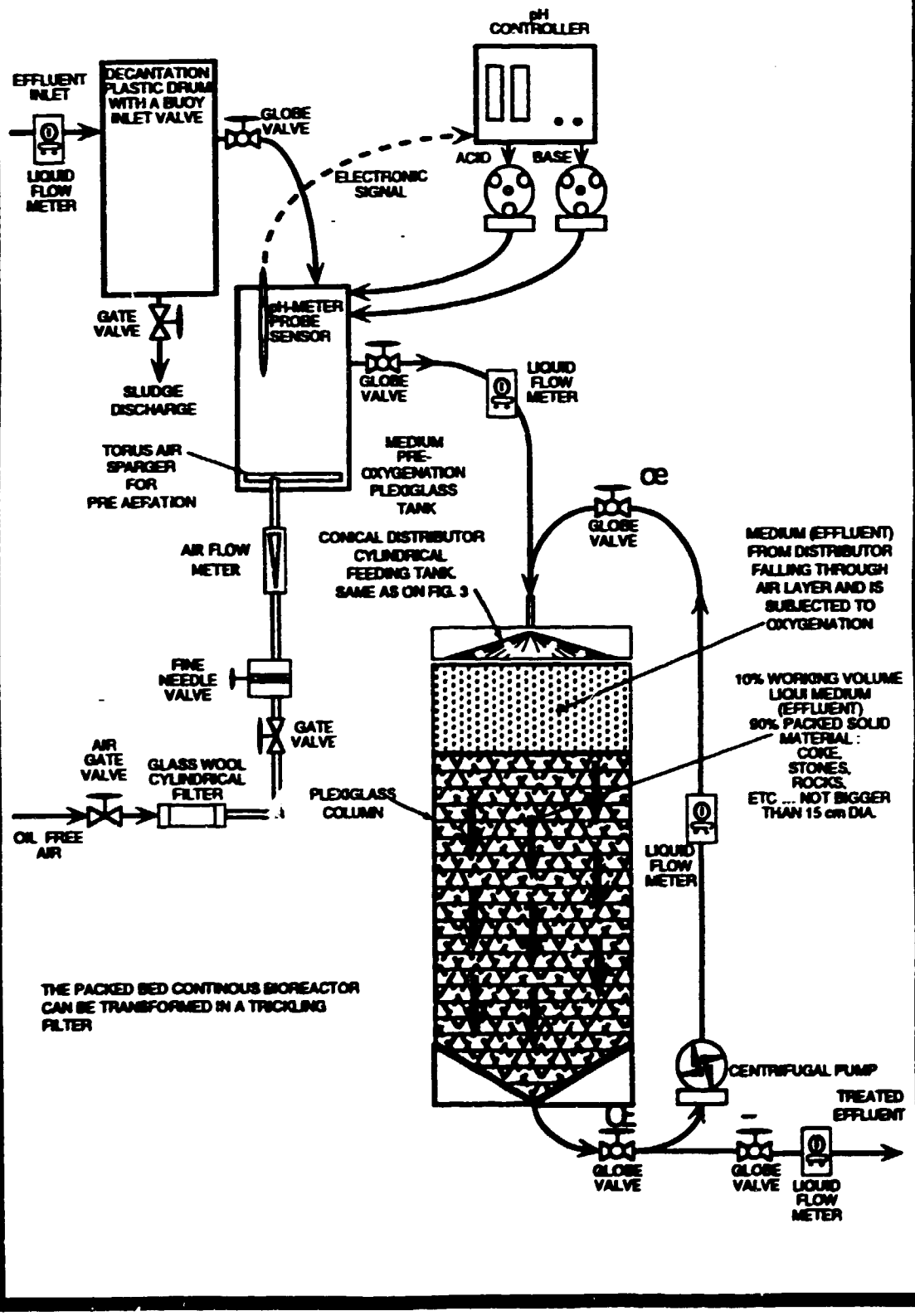
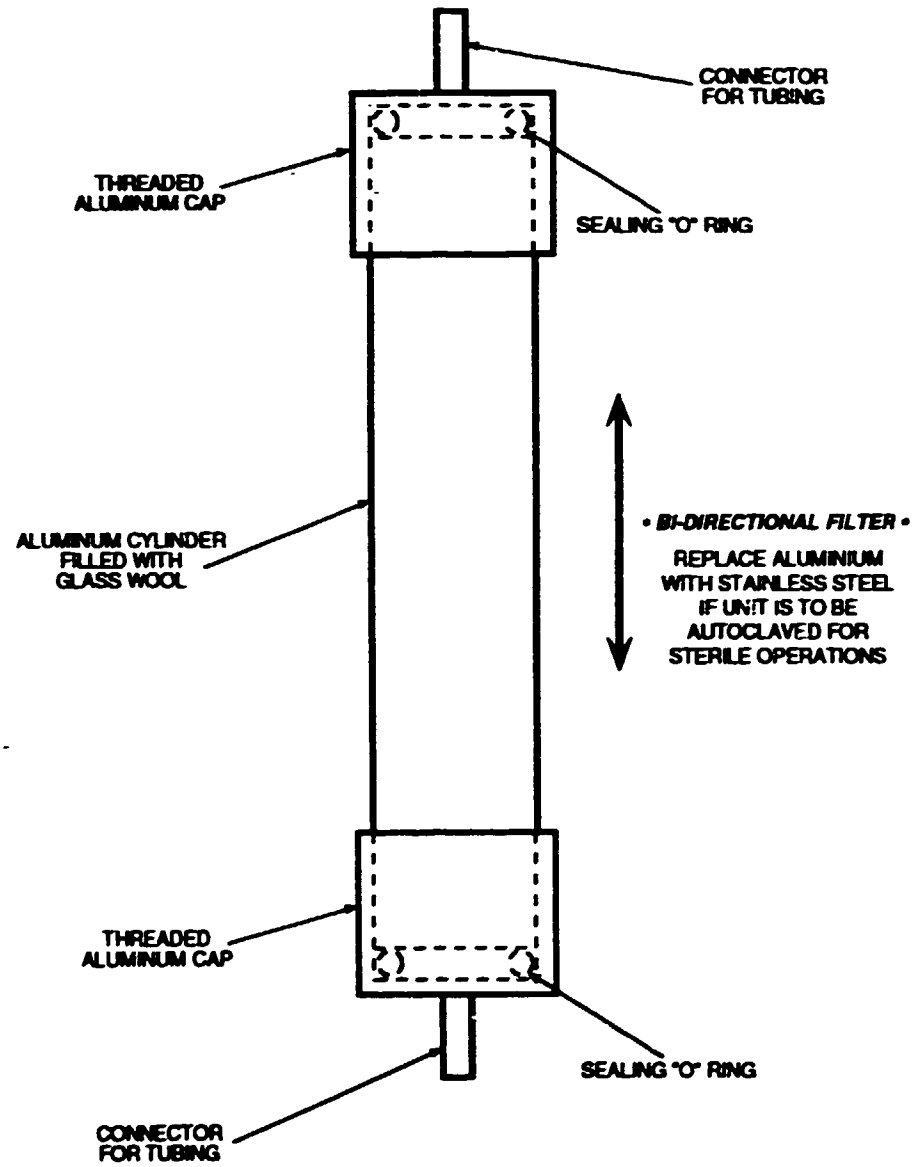


FIG. 6: GLASS WOOL AIR FILTERS

(Recommended for all Bioreactors)

UNIDO/RSS
PROJECT #: SI/JOR/84/802



V.5.5 Pilot Scale Continuous Algal Pond

The high retention time algal pond (Figure 7) could be 6 m long and 1.5 m wide, with a trapezoidal section and built with concrete. At 1 meter intervals a screen is installed with a mesh smaller than that of the algae. The bottom of the pond could measure 0.5 m wide and 0.5 m deep. On this basis, the pumping rate of the untreated wastewater in the pond should be 0.5 litre per minute in order to obtain a hydraulic retention time of 100 hrs (around 4 days). The design of the system is such that every section of the pond is an independent bioreactor which is inoculated with the desired algae.

The solids are retained by the screen where the biological degradation reaction takes place. The screens should be cleaned two times a day with a flat rubber wiper in order to prevent blocking-up of the screen. In the event an algal pond is established then it will be necessary to maintain an algae collection at ERC (with at least 15 species known to degrade organic compounds) and acquire appropriate skills for algal culture. For further details on design and operation of the algal pond, refer to Figures 7 and 8.

V.5.6 Inoculation of Bioreactors

It is necessary to immobilize the microorganisms over the surface of the solid materials, for the fluidized bed, packed bed, and the trickling filter bioreactors. For this operation it is necessary to prepare a liquid inoculum by using the appropriate microorganisms, depending on the type of wastewater effluent being treated. The microbial strain would be grown to optimal levels in the laboratory or pilot scale fermenter depending on the scale of operation.

The fluidized bed or packed bed bioreactor has to be inoculated with free microorganisms and with a seed of material already covered by microorganisms. At the beginning of this operation, the bioreactors have to be operational and without any microorganism so that it can be inoculated with mobile (free) microorganisms plus a small seed of support material already covered by microorganisms.

The method of inoculating the bioreactors is to apply a thin layer of seeded material ($\leq$1 cm) over a screen (see Figure 9). By recirculating a cell suspension for 24 to 48 through the bed material, cell attachment and growth occurs until most of the particle surface (sand or polyurethane foam) is covered by microorganisms.

For the high retention time algal continuous pilot pond, each sector of the pond should be inoculated with 10 litres of harvested algae previously grown in a fermenter using the wastewater effluent as the liquid medium. Then the pond can begin its continuous operation.

FIG. 7: GENERAL VIEW OF AHIGH RETENTION TIME ALGAE CONTINUOS PILOT POND FOR DEGRADATION OF INDUSTRIALWASTEWATER EFFLUENTS

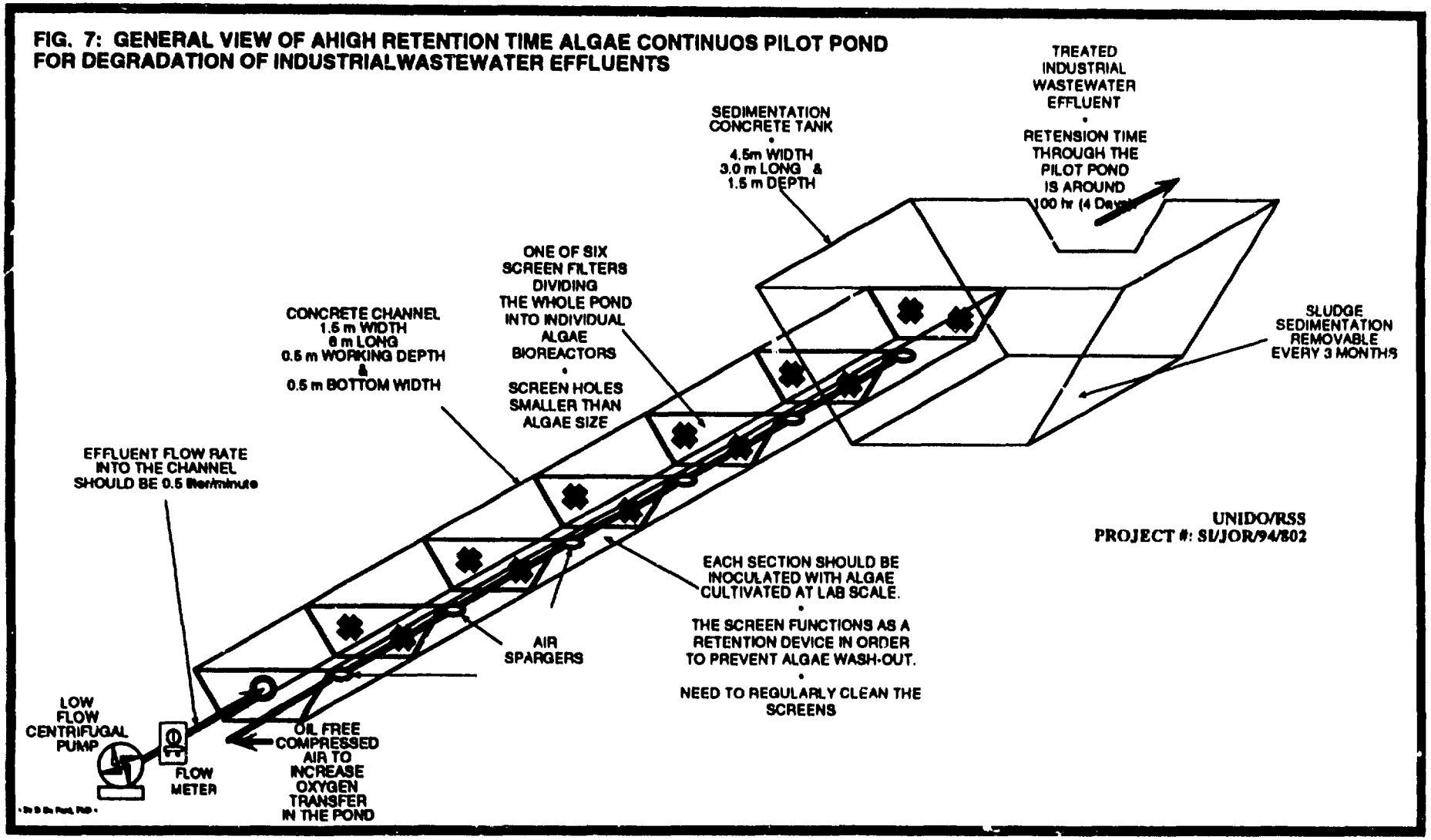
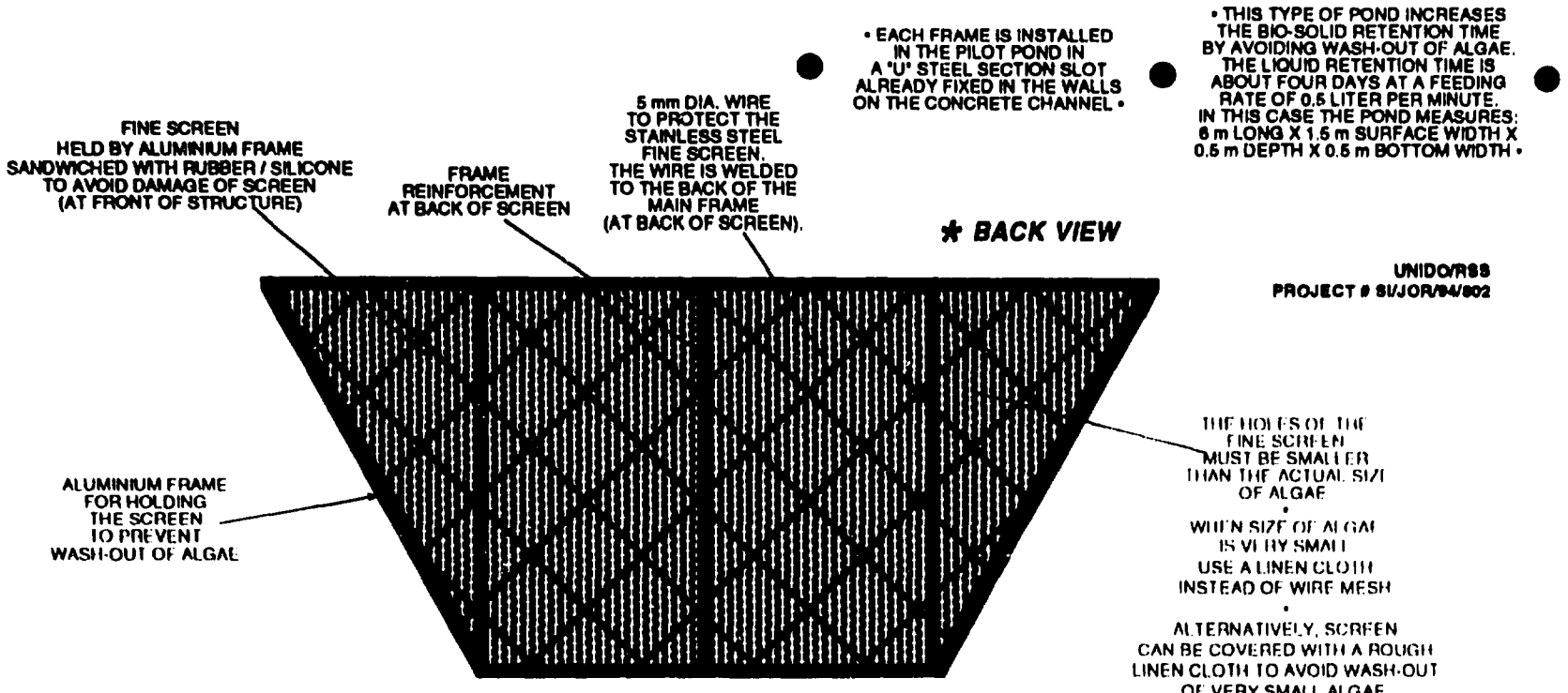


FIG. 8: Schematic Diagram Showing a Screen Filter (To Prevent Algal Washout) of a Continuous Flow Algal Pond



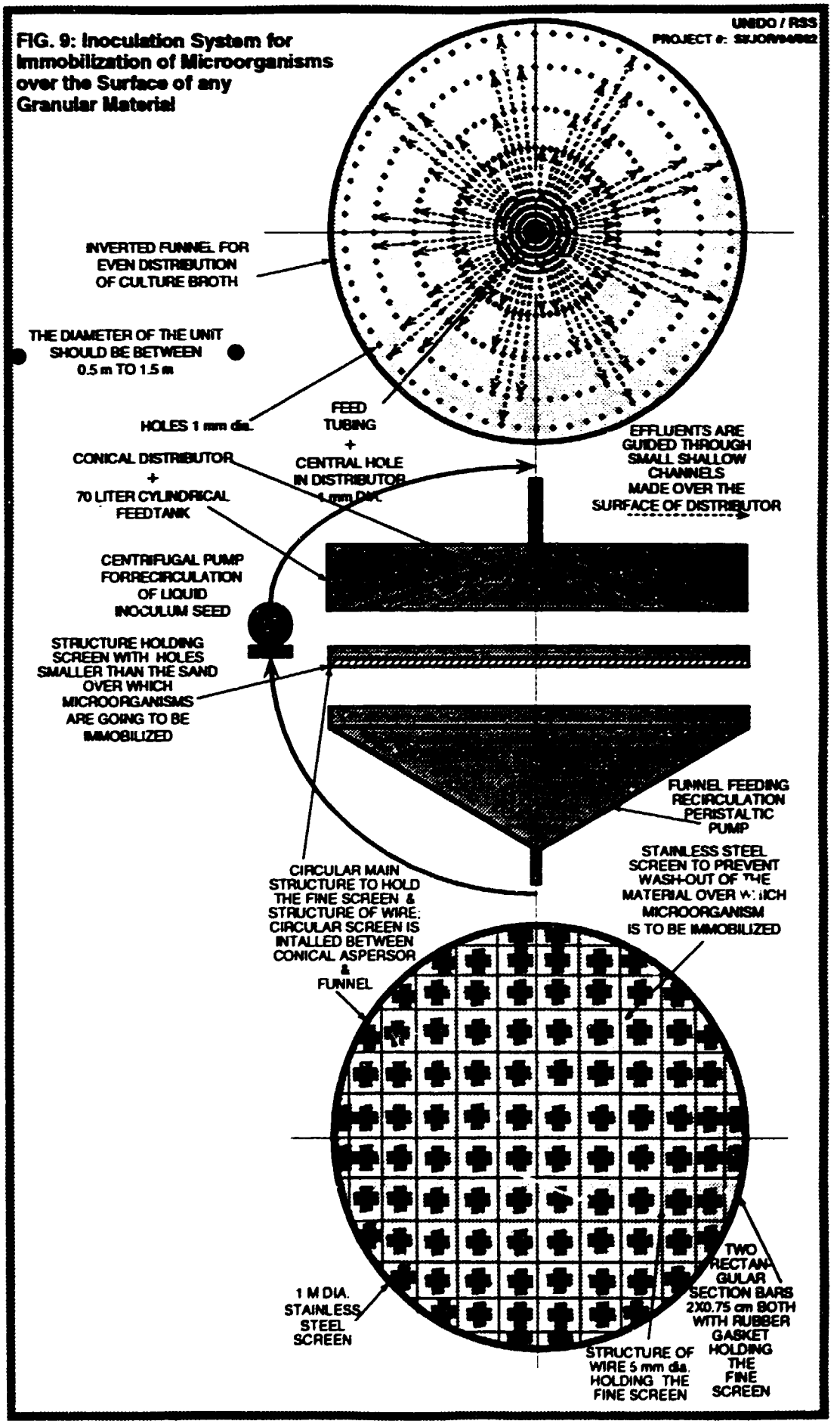


FIG. 9: Inoculation System for Immobilization of Microorganisms over the Surface of any Granular Material

V.5.7 Wastewater Treatment Pilot Plant for Processing Hazardous Waste

Adjacent to the ERC main building and across from the Ecology Laboratory, RSS also has a high head pilot plant building with basic utilities. This building is currently under used and we believe that it is ideal for establishing a state-of-the-art biotechnology pilot plant for fermentation and hazardous waste biodegradation work.

Figure 10 provides a conceptual layout for a pilot scale facility for batch production of pure cultures required for specific biodegradation activity and a couple of bioreactors to be used for treatment of hazardous wastes. The full design specifications of these units can only be provided once lab scale processes have been developed and kinetic parameters determined. At present we have assumed that only one half of the pilot plant will be available for biodegradation studies. An in-situ sterilizable 150L fermenter, a fluidized bed bioreactor, and a packed bed bioreactor (convertible to trickle bed configuration) are the key unit operations here. The pilot plant would be supported by appropriate utilities for steam, water for cooling, process water, compressed air, and electrical outlets. The bioreactors would be installed in such a way that they would be mobile and could be relocated adjacent to any pharmaceutical facility that may wish to evaluate the feasibility of the biological treatment process.

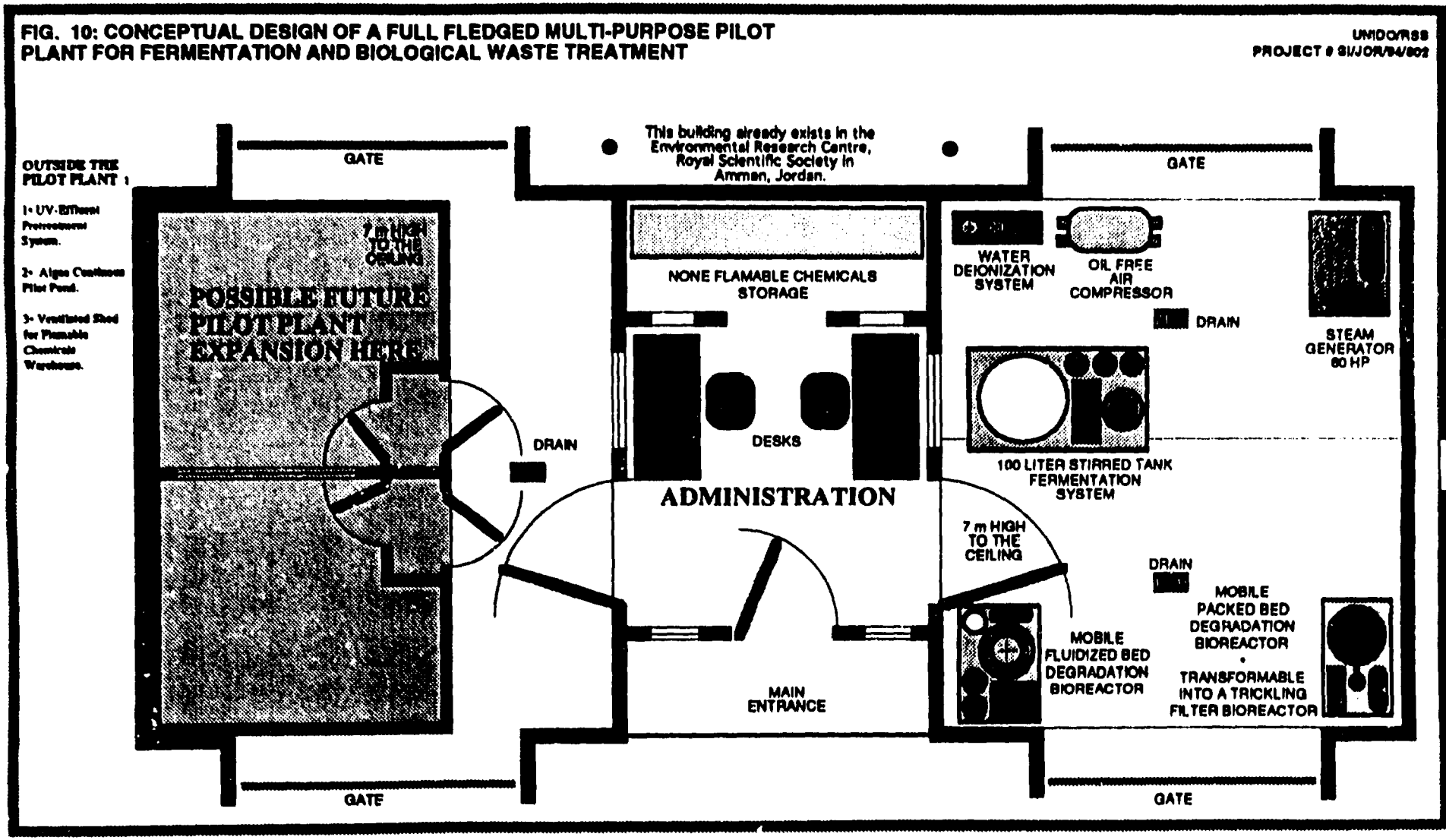
The data generated from these pilot studies would then enable potential end-users of the technology with realistic scale-up and engineering data for design of a commercial scale biodegradation facility.

The fermenter in the pilot plant could also be used to grow biomass for subsequent application in soil bioremediation studies. This would however require additional unit operations for biomass recovery (e.g continuous centrifuge) and a spray drier for long term biomass storage.

If additional space were made available at the pilot plant, these unit operation could be accommodated and perhaps an even larger scale fermenter installed. This would establish a semi-manufacturing capability whereby biomass produced at RSS could be used for subsequent sale to industry and/or export to other countries.

FIG. 10: CONCEPTUAL DESIGN OF A FULL FLEDGED MULTI-PURPOSE PILOT PLANT FOR FERMENTATION AND BIOLOGICAL WASTE TREATMENT

UNIDO/RSS
PROJECT # SI/JOR/94/802



VI. RECOMMENDATIONS

VI.1 RECOMMENDED EQUIPMENT LIST FOR IMMEDIATE PURCHASE TO ESTABLISH ESSENTIAL CAPABILITY

Following detailed evaluation of the laboratory facilities at ERC and discussions with the members of the Ecology Division, the items listed below were recommended for immediate purchase. The selection was limited by available funds up to a maximum of $15,000 US. This purchase will not by any means meet the long term needs of ERC. Therefore, a detailed proposal outlining the needs of ERC (including equipment needs) to establish a full fledged capability has been included as part of this report. Longer term capital needs have to be fulfilled if ERC is to establish a fully fledged capability as outlined in the next section.

The key items recommended for purchase and their estimated costs are listed below. Further details on price breakdown, including the names of suppliers, model numbers, specifications, and copies of brochures have been included in Annex 14.

1. Bench Scale Fermentor and Basic Controls

Applikon 2L fermentor (autoclavable) with top drive unit	$5,400.00
Antifoam, pH and dissolved oxygen sensors + cables (Ingold)	$1,665.00
Acid, base, and antifoam pumps + rotameter with solenoid for dissolved oxygen control	$1,215.00
Antifoam, pH and dissolved oxygen controllers	$1,800.00
TOTAL FERMENTOR PACKAGE ($US)	**$10,080.00**

2. Small Freeze Dryer

Edwards Micromodulyo Freeze Dryer	$2,100.00
Edwards (E2M2) Vacuum Pump	$1,300.00
Secondary Drying manifolds for up to 48 Ampules	$520.00
5 mL Ampules for Culture Drying and Storage (200/case)	$420.00
TOTAL FREEZE DRYER PACKAGE ($US)	**$4,340.00**

TOTAL EQUIPMENT PURCHASE ($US)	**$14,420.00**

The above equipment purchase will enable ERC staff to initiate purchase and/or isolation of suitable strains capable of phenol and chlorophenol degradation and subsequently store them for future use. Furthermore, purchase of a bench top fermentor and associated controls will enable them to develop their skills in fermentation technology, optimize conditions for microbial growth and biodegradation, and also to use the control units with their laboratory scale waste treatment bioreactor.

The above equipment will enable ERC to initiate a viable program, but it will not be able to sustain long term capability enhancement.

Please note that all of the above prices are in US currency. Quote from freeze dryer supplier (Edwards) was in Canadian currency and was converted to US$ using the current exchange rate. Fermentor quote is in US$ and includes a 10% discount offered by the manufacturer. Also note that the attached quotes provided by the suppliers includes several other optional accessories. Only items that are immediately needed have been included to initiate experimental work. These upgrades and optional equipment may be purchased at a later date. Both suppliers also have European distributors and representation in the Middle East. Quotes were also collected from several other manufacturers, but due to their higher price and lack of suitable features, they were not pursued further.

VI.2 LONG TERM CAPABILITY BUILDING

The long term strategy for establishing capability can be segmented into the following areas:

VI.2.1 Personnel

The work on degradation of hazardous wastes is being carried out by the Ecology Division of ERC under the headship of Dr Ali ElKamni. He is assisted by 5 in-line staff members, of which 4 are researchers with one technician (see Annex 3 for list of personnel involved in project and their background). It is anticipated that they will be seeking an additional researcher. Based on their individual backgrounds, the researchers have a good mix of engineering and microbiology expertise. In the event a pilot plant is established and lab-scale fermenter/bioreactor capability is in place. the group will need to add at least 3 additional technicians and pilot plant operators to assist the researchers

VI.2.2 Capital

The cost of capital items are estimates based on catalogue prices and recent phone quotes from various suppliers. A contingency factor of 20% has been added to include the additional cost of shipping, duties and taxes. The cost of local labour for installation and commission of large capital items has not been included. It is also recommended that the program, if implemented, include an annual cost of 10% of capital cost for establishing a parts inventory and equipment maintenance costs.

a) Laboratory Facilities

The existing laboratory is not adequately equipped for extensive R&D in the area of fermentation technology and biodegradation process optimization. Although the support services from other labs are excellent, these are largely in the areas of analytical chemistry. Table 6 provides a list of equipment and supplies that need to be purchased if this project is to be aggressively pursued. Reference has already been made for purchase of bench top fermentor and freeze dryer. Specific laboratory equipment and supplies have been identified in Table 6.

b) Pilot Plant

There is currently very little capital equipment at ERC for setting up a pilot plant. The building is well suited for the purpose and we believe that with some utility upgrades, the pilot plant could serve its purpose well. However, the biggest bottleneck here will be the purchase, installation and commission of the equipment. The capital items needed for the pilot plant are also listed in the Table 6.

We anticipate that plexiglass biodegradation reactors will be built on-site with extensive in-house expertise at RSS. The cost of establishing a UV-Pretreatment reactor and the algal pond has not been included. Similarly the additional cost of purchase of a vehicle for transporting the mobile bioreactor(s) to the intended site has not been included.

Table 6: Recommended Equipment and Supplies List at the Ecology Division of RSS for Capability Enhancement in Biodegradation of Hazardous Wastes

EQUIPMENT (Laboratory = L; Pilot Plant = P)	SPECIFICATIONS	QUANTITY	COST PER UNIT ($US)	TOTAL COST ($US)	SUPPLIES (S) OR CAPITAL (C)	PRIORITY: HIGH (H), MED (M), LOW (L)
Airflow meters (L)	0-10L/min	2	250	500	C	H
Immersion circulator for bench scale bioreactor (L)	22-95 C, analogue	1	1000	1000	C	M
Off-Gas Analyser (P)	CO2 analysis, portable	1	4000	4000	C	L
Weighing balance (P)	Industrial 0-150 Kg	1	1000	1000	C	M
Constant temperature shaker (L)	Bench top model with refrigeration unit	1	10000	10000	C	M
Plates (L)	Disposable			500	S	H
Test Tubes (L)	Disposable, various sizes			500	S	H
Pipettes (L)	Disposable			1000	S	H
Media bottles (L)	Glass, various sizes			1000	S	H
Centrifuge tubes and bottles (L)	disposable, various sizes			500	S	H
GC Analyser, supplies (L)	various columns			1000	S	L
Personal Computer (L & P)	Pentium or 486 DX-2, fully loaded	2	4000	8000	C	M
Computer scientific software (L/P)	Wordprocessing,spreadsheet, data acquisition, process control etc	2	2000	4000	S	M
Computer interface hardware (P)	Miscellaneous	1	2000	2000	C	M
Liquid dispensers (L)	various sizes	8	250	2000	S	M
Bench top fermentor (L)	2L size, autoclavable + agitator	1	6000	6000	C	H
pH meter with controller (L &P)	Chemcadet	3	500	1500	C	H
DO meter with controller (L & P)	Cole parmer	3	1000	3000	C	H
pH probes (L & P)	autoclavable + accesories	3	250	750	S	H
DO probes (L & P)	autoclavable + accesories	3	500	1500	S	H
Fermentor parts and supplies (L)	miscellaneous accesories	1	1000	1000	S	H
Foam sensor and controller (L & P)	Cole Parmer	3	500	1500	C	H
Acid, base and antifoam pumps for bench scale fermenter and pilot scale bioreactor (L & P)	Low flow rate peristaltic	9	500	4500	C	H
In-line air filter holders for inlet/exhaust gases for small scale fermenter (L & P)	Autoclavable	12	50	600	S	H
Inlet and exhaust gas filters for pilot scale bioreacors (P)	10 inch cartridge with holder	4	500	2000	S	H
Plastic Piping and fittings for pilot plant connections (P)	High density polyethylene or polypropylene			2000	S	H
Freeze dryer (L)	Lab-scale, bench top unit	1	4500	4500	C	L
Vacuum pump for freeze dryer (L)	Dual stage, belt driven	1		1200	C	L

Table 6: Recommended Equipment and Supplies List at the Ecology Division of RSS for Capability Enhancement in Biodegradation of Hazardous Wastes

EQUIPMENT (Laboratory = L; Pilot Plant = P)	SPECIFICATIONS	QUANTITY	COST PER UNIT ($US)	TOTAL COST ($US)	SUPPLIES (S) OR CAPITAL (C)	PRIORITY: HIGH (H), MED (M), LOW (L)
Fume hood for pilot plant (P)	Ductless or portable unit with	1	5000	5000	C	M
Sample bottles (L & P)	Various sizes			1000	S	H
Glass beakers (L)	Various sizes			500	S	H
Multihazard glove box (L)	Designed for handling potentially toxic compounds	1	15000	15000	C	L
Microcentrifuge (L)	Small 1 to 2 mL samples	1	3000	3000	C	M
Microcentrifue tubes (L)				300	S	M
Hot plates and magnetic stirrers (L)	bench scale	2	500	1000	C	M
UV Lamp with UV tube (L)		1	400	400	C	H
Fibre optic level sensors and controller (L)	For feed control in pilot scale bioreactors	2	1000	2000	C	M
Mixers (P)	Pilot scale, heavy duty, with variable speed drive	2	1200	2400	C	H
Ovens for dry weight determination and glass ware drying (L)	Table top	2	500	3000	C	M
Submersible housings for pH and DO probes (L)	plastic	4	100	400	C	H
Miscellaneous Plastic Ware and Supplies (L&P)	Autoclavable bags, beakers, jugs, bottles, carboys with and without tubulation, tubes, containers, funnels, cylinders, buckets, hand pumps, racks, ties, tension tool, vials, trays, etc			4000	S	H
Feed pumps (P)	Peristaltic, variable speed	2	1000	2000	C	H
Tubing (L & P)	Silicone and Tygon			2000	S	H
Safety apparel (gown, boots, hood, glasses, etc) [P]	For pilot plant operations			1000	S	H
Storage for hazardous materials (L)	lab use	1	600	600	C	H
Storage unit for hazardous materials (P)	plant use	1	2000	2000	C	H
Shower and Wash station (P)	Plant use	1	600	600	C	H
Reference books and resource materials (L & P)			2000	0	S	H
Autoclave (L)	Wall mounted	1	15000	15000	C	M
Steam Generator (L)	Lab autoclave	1	2000	2000	C	M
Steam Generator (P)	Pilot Plant	1	5000	5000	C	H
Plexiglass Bioreactors (P)	Pilot Plant, 100L scale	2	5000	10000	C	H
Spargers (P)	Aeration of Pilot Bioreactors	2	500	1000	C	H

Table 6: Recommended Equipment and Supplies List at the Ecology Division of RSS for Capability Enhancement in Biodegradation of Hazardous Wastes

EQUIPMENT (Laboratory = L; Pilot Plant = P)	SPECIFICATIONS	QUANTITY	COST PER UNIT ($US)	TOTAL COST ($US)	SUPPLIES (S) OR CAPITAL (C)	PRIORITY: HIGH (H), MED (M), LOW (L)
Laboratory drying rack (L)		1	100	100	C	M
Tachometer (P)	Optical	1	500	500	C	M
Mobile Cylindrical Feed tanks (P)	Pilot Plant, 200L	4	400	1600	C	H
Waste Storage Tank (P)	Pilot Plant wastes, 1000L	1	2700	2700	C	H
Mobile Skids (P)	For Mounting Bioreactors	2	2000	4000	C	H
Pilot Scale fermentor (P)	150L, fully equipped	1	150000	150000	C	M
UF System for Cell harvest (P)	Holow Fibre Cartridge System, Disposable	1	10000	10000	C	M
Spray Dryer (P)	Pilot Scale, Evaporation Rate of 20L/hr	1	30000	30000	C	L
Thermocouples and meters (P)	for bioreactors	2	300	600	C	H
Glassware Washer (L)	Undercounter domestic unit	1	600	600	C	M
Process chemicals (P)	Various nutrients			10000		H
Laboratory chemicals (L)	Nutrients and reagents			2000		H
Immobilization materials (L &P)	Alginate, foam, carrageenan, silica, etc			1000		H
Mutagens (L)	Various chemicals			200		H
Airflow meters (P)	10-100L/min	2	500	1000	C	H
Total				**360550**		
Add Contingency @ 20%				72110		
Spare parts and supplies for an additional year (@ 10% of Total)				36055		
GRAND TOTAL				**468715**		

VII. EXPLANATORY NOTES

RSS:	Royal Scientific Society
ERC:	Environmental Research Centre
UASB:	Up-flow anaerobic sludge-blanket (bioreactor)
CPE:	chlorinated phenol enrichment (media)
CP:	chlorinated phenol
ATCC:	American Type Culture Collection
NTG:	nitrosoguanidine (Mutagen compound)
UV:	ultraviolet
OD:	optical density
EC:	electric conductivity
BOD:	biological oxygen demand
COD:	chemical oxygen demand
TSS:	total suspended solid
TDS:	total dissolved solid
ABS:	alkyl benzene sulphonate
PCP:	pentachlorophenol
BDG:	biodegradation media
OGM:	overnight growth media

VIII. ANNEX

List and Page

ANNEX 1:Organizational Structure of the Royal Scientific Society

General Information

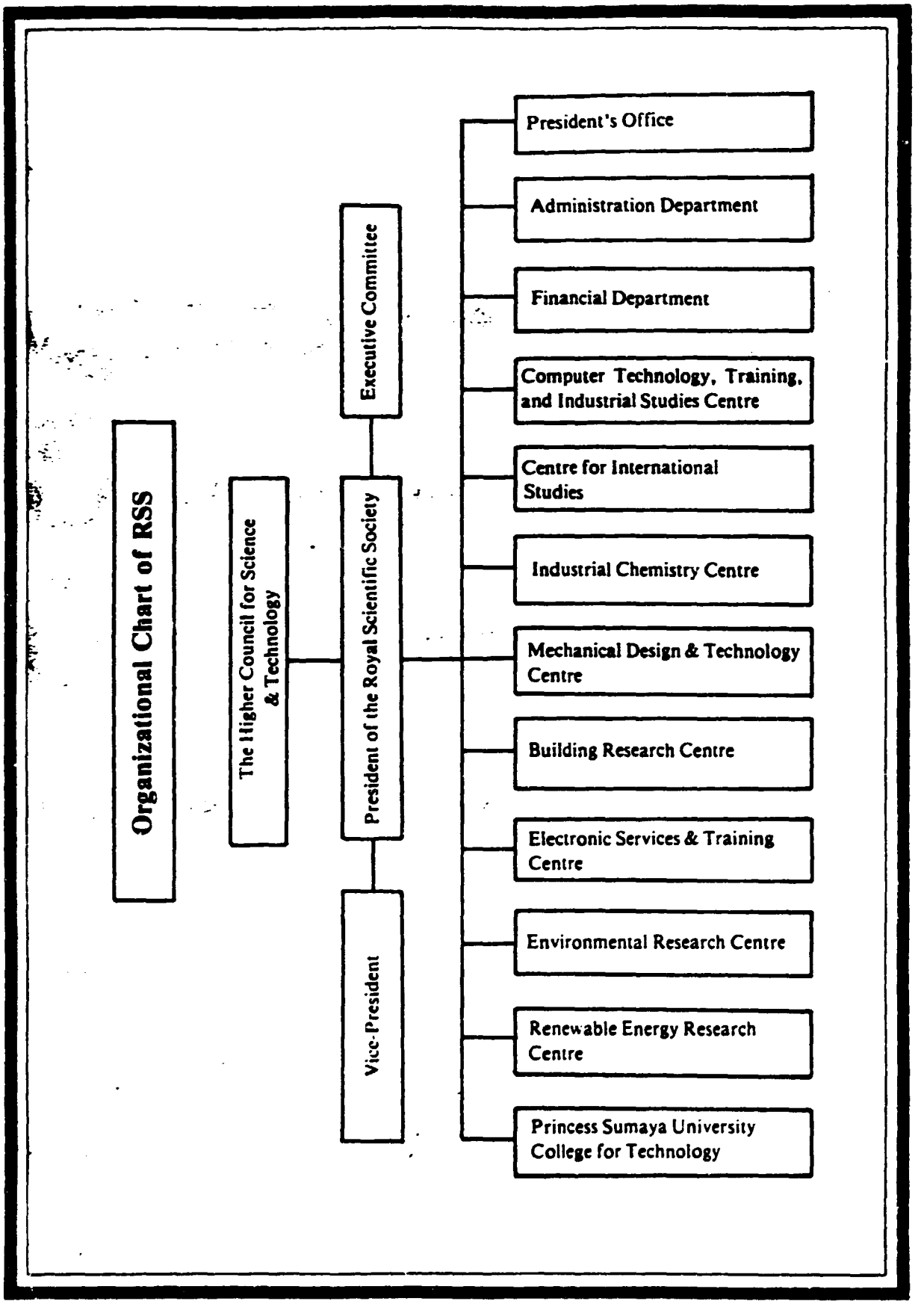
The Royal Scientific Society was established in 1970 as a research and development institution to work in fields related to the development process in Jordan.

- RSS is a national institution enjoying financial and administrative independence.
- RSS became one of the scientific and technological centres of the Higher Council for Science and Technology, as from October 1987.
- RSS is administered by a president, a vice-president, and directors of centres and departments.
- RSS started its activities at offices of the Central Bank of Jordan. It then moved to a rented building in Amman before acquiring its present permanent site at Jubaiha, near Amman, which it occupied in February 1972.
- The area of the permanent site is 342,000 square metres.
- The buildings and laboratories cover a floor area of 28,700 square metres.
- The budget of RSS is derived from self-generated revenues from technical services and consultations, research contracts, an annual grant from the Government of Jordan, grants and donations from local institutions, and technical assistance from a number of industrial countries as well as from regional and international organizations.
- RSS consists of the following:

  . Computer Technology, Training, and Industrial Studies Centre
  Renewable Energy Research Centre
  . Centre for International Studies
  . Industrial Chemistry Research Centre
  . Mechanical Design and Technology Centre
  . Building Research Centre
  . Electronic Services and Training Centre
  . Environmental Research Centre
  . Administration Department

. Financial Department
. President's Office

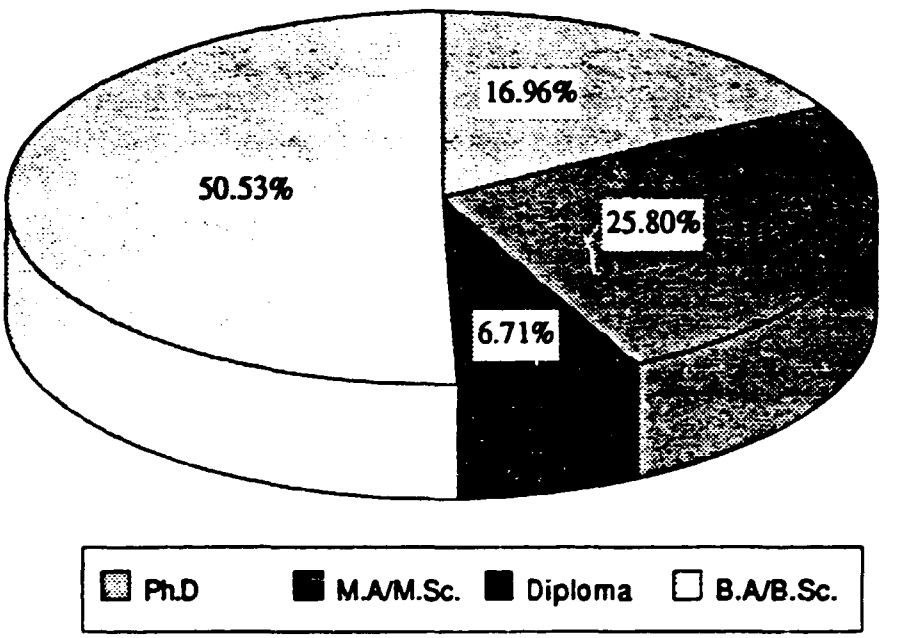
- RSS cooperates with a number of research institutions, universities, organizations, councils, centres and establishments at the Arab regional and international levels through agreements, memoranda of understanding and contract research and studies.
- RSS is a member of several Arab, regional and international unions, federations, councils, associations, organizations and societies.


Organizational Chart of RSS
The Higher Council for Science & Technology
Executive Committee
President of the Royal Scientific Society
Vice-President
President's Office
Administration Department
Financial Department
Computer Technology, Training, and Industrial Studies Centre
Centre for International Studies
Industrial Chemistry Centre
Mechanical Design & Technology Centre
Building Research Centre
Electronic Services & Training Centre
Environmental Research Centre
Renewable Energy Research Centre
Princess Sumaya University College for Technology

Employees, Their Specializations and Departments

No.	Department or Centre	Ph.D.	M.A.& M.Sc.	Diploma	B.Sc. & B.A.	Engineer Grad	Community College	One year after General Secondary	General Secondary	Below Secondary	Total
1.	President's Office	9	2	0	12	0	2	1	4	25	55
2.	Mechanical Design and Technology Centre	4	10	4	9	0	5	0	4	18	54
3.	Industrial Chemistry Centre	4	3	2	8	0	9	1	2	6	35
4.	Environmental Research Centre	6	14	0	10	0	5	2	2	5	44
5.	Building Research Centre	5	14	2	24	2	9	2	11	25	94
6.	Electronic Services and Training Centre	1	9	3	21	2	6	0	7	24	73
7.	Renewable Energy Research Centre	4	1	1	10	0	5	0	5	6	32
8.	Computer Technology, Training and Industrial Studies Centre	8	15	5	24	0	16	2	13	12	95
9.	Princess Sumaya University College for Technology	3	1	1	5	0	0	1	1	4	16
10.	Administrative Department	0	2	1	7	0	4	1	9	30	54
11.	Financial Department	0	0	0	9	0	2	2	3	4	20
12.	Centre for International Studies	4	2	0	4	0	0	2	0	2	14
	TOTAL	48	73	19	143	4	63	14	61	161	586

University Graduate Employees of RSS in 1993



Aims and Functions

Aims

The Royal Scientific Society aims at conducting scientific and technological research and development work related to the development process in Jordan with special attention to industrial research and services. It also aims at disseminating awareness in the scientific and technological fields and at providing specialized technical consultations and services to the public and private sectors. It seeks to develop scientific and technological cooperation with similar institutions within the Arab world and internationally.

Functions

1 . Carrying out studies and conducting applied scientific research related to industry in particular and to the various areas of development in general.
2 . Conducting economic and technical feasibility and analytical studies with regard to development projects which fall within the Society's scope of interest.
3 . Carrying out studies and research in the field of vocational and industrial education and producing books and publications in support of training and the industrialization process.
4 . Conducting research on a contract basis with institutions within Jordan and abroad.
5 . Carrying out joint research with scientific, production-oriented and service institutions at the national, Arab and international levels.
6 . Conducting research and development work leading to the production of prototypes for possible application in industry.
7 . Developing its laboratories, providing them with up-to-date equipment and orienting them towards serving the objectives of scientific and technological research and the needs of the public and private sectors.
8 . Carrying out tests and experimental work on materials as well as on finished and intermediate goods and providing related technical consultations to the users.

9 . Contributing to the solution of technical problems facing various organizations, particularly industrial establishments.
10. Cooperating with agencies concerned with the establishment of national technical standards and specifications and providing technical services which would facilitate their application and ensure proper quality control of goods and materials.
11. Attracting qualified Jordanian and Arab personnel and providing them with favourable working conditions.
12. Upgrading human capabilities and technical skills through the provision of distinctive training opportunities.
13. Producing books and other publications in the area of science and technology which contribute to the effective dissemination of scientific and technological concepts.
14. Preparing and servicing information systems in addition to processing, programming and implementing computer systems.
15. Contributing to the transfer and adaptation of technology and selecting appropriate technologies related to the Society's scope and field of expertise.
16. Cooperating in science and technology with local, Arab and other organizations for the purpose of exchanging information and expertise and conducting joint research.
17. Developing the instruments of scientific and technological management and the methods of setting up national science and technology policies and providing consultations in this regard at the national and Arab levels.
18. Contributing to the development of the Arab region through providing technical service and consultation and creating opportunities for specialized technical training.

Balance Sheet
31/12/1993
(Before Auditing)

ASSETS	December 31 1993 JD	December 31 1992 JD	LIABILITIES	December 31 1993 JD	December 31 1992 JD
Current Assets:			Current Liabilities:		
Cash on hand and at banks	925,747	797,622			
Accounts receivable	667,099	721,190	Accounts payable	360,870	226,657
Raw materials, spare parts and supplies	411,213	429,243	Accrued expenses and deposits	541,122	272,021
Other Assets	258,106	199,584	Unearned revenue	346,838	611,307
			Current portion of long-term loans	363,492	339,914
Total Current Assets	2,262,965	2,147,639	Provision for staff indemnities and vacation pay	1,205,468	1,087,580
Investment in Equity Securities-net of provision of JD 66,250 for the decline in market value for the year 1993 (21,250 for 1992)	239,131	229,693	Total Current Liabilities	2,817,790	2,537,479
			Long-term loans	1,413,234	1,723,094
Endowment fund at the Industrial Development Bank	2,411,810	2,411,810			
Fixed Assets:			ACCUMULATED FUND:		
Land and improvements	6,897,327	6,897,327	Accumulated Fund-beginning of year	14,294,874	11,983,343
Buildings and improvements	3,647,525	3,567,993	Net surplus for the year	664,077	2,311,531
Machinery, furniture and fixtures	11,141,907	10,238,197			
			Accumulated fund-end of year	14,938,951	14,294,874
	21,676,759	20,703,517			
Less: accumulated depreciation	7,430,690	6,937,212			
Net book value of Fixed Assets	14,256,069	13,766,305			
			TOTAL LIABILITIES AND ACCUMULATED FUND		
TOTAL ASSETS	19,169,975	18,555,447		19,169,975	18,555,447

RSS Cooperation Relations with Arab and International Organizations and Institutions

RSS is connected with a good number of Arab and international organizations and institutions through:

a. Agreements or protocols of cooperation with the following institutions:

- The National Institution for Scientific Research/Tunisia.
- Islamic Foundation for Science, Technology and Development (IFSTAD)/Kingdom of Saudi Arabia.
- Academy of Scientific Research and Technology/Egypt.
- King Abdul Aziz City for Science and Technology/Kingdom of Saudi Arabia.
- Renewable Energy Development Centre (Centre de Développement des Energies Renouvables)/ Morocco.
- Scientific and Technical Research Council of Turkey (TUBITAK)/ Turkey.
- The Academy of Sciences of the Russian Federation.
- National Technical Information Service/USA.
- Council of Scientific and Industrial Research/India.
- Council of Scientific and Industrial Research/Pakistan.
- Friedrich Ebert Stiftung/Germany.
- German Agency for Technical Cooperation (GTZ)/ Germany.
- Cambridge Applied Nutrition, Toxicology and Biosciences Group (CANTAB)/ United Kingdom.
- Polish Academy of Sciences/ Poland.
- Scottish Development Agency/ United Kingdom.
- Bahrain Centre for Research and Studies/ Bahrain.
- International Development Research Centre (IDRC)/ Canada.
- United Nations Development Programme (UNDP).
- Swiss Federal Laboratories for Materials Testing and Research (EMPA)/ Switzerland.

- Economic and Social Commission for Western Asia (ESCWA).
- Islamic Educational, Scientific and Cultural Organization (ISESCO).
- Centre for Caucasian Affairs Studies/ Grozny, Chechen Republic.
- The Institute for Social and Economic Policy in the Middle East, John F. Kennedy School of Government, Harvard University/ Cambridge/ Massachusetts, U.S.A.
- The Islamic Academy of Sciences/ Jordan.
- Arab Union of the Manufacturers of Pharmaceutical and Medical Appliances/ Amman.

b. Membership in the following organizations:

- Federation of Arab Scientific Research Councils/ Iraq.
- World Association of Industrial and Technological Research Organization (WAITRO)/ Denmark.
- International Council of Scientific Unions (ICSU)/ France.
- International Foundation of Science (IFS)/ Sweden.
- international Association for Housing Science/ USA.
- The International Federation of Institutes for Advanced Studies (IFIAS)/ Canada.
- International Measurement Confederation (IMEKO)/ Hungary.
- UNESCO/ Regional Office for Science and Technology for the Arab States (ROSTAS).
- UNESCO/ Intergovernmental Informatics Programme (IIP).
- Asian Energy Institute (AEI)/ New Delhi.
- Arab Union for Cement and Building Materials/Syria.

ANNEX 2: RECENT ACTIVITIES OF THE ENVIRONMENT RESEARCH CENTRE

Environment and Public Safety Sector

a. Research and Development and Studies

1 . Evaluation of the efficiency of utilizing solar radiation for the treatment of organic pollutants using a photocatalytic oxidation process (1992-1993).
2 . Monitoring of water quality in King Talal Dam (1980-1994).
3 . The national project for monitoring water quality in Jordan (1986-1993).
4 . Study of Assamra waste stabilization ponds project (1986-1994).
5 . Socio-economic and environmental study of King Talal Reservoir Region (1991-1994).
6 . Environmental and socio-economic study of olive oil mills waste treatment and disposal (1993-1996).
7 . Assessment of pollution by pesticides residues in Jordan.
8 . Industrial wastewater treatment plants projects and others (1991-1993).
9 . Industrial pollution control project (1992-1993).
10. Preliminary study to identify cement constituents in ambient dust at Fuheis area (1993-1994).
11. Air pollution monitoring at Rusaifa, Fuheis, Hashimiyeh and downtown Amman (1992-1993).

b. Technical Services and Consultations

As per the agreements which RSS concluded with the Aqaba Region Authority, RSS provided several specialized technical consultations on studies carried out by international experts on Aqaba coastal resources, environmental management, and Aqaba marine park. RSS also signed a number of agreements with private firms operating or due to operate in Aqaba region with the purpose of protecting the environment in this region.

In 1993, RSS provided technical consultations and prepared periodic reports vis-a-vis the performance of waste water treatment plants,

designing new plants, and control of the compatibility of water discharged from factories with the Jordanian standards. RSS carried out studies related to solving the problems of industrial waste water, and designing treatment plants suitable for a number of Jordanian industries. RSS rendered about (75) technical consultations for local and foreign parties, and conducted about (18000) variant laboratory tests for governmental departments, private companies and individuals.

- (1500) tests on quantitative and qualitative measurements or radionuclide on food,
- (4400) personal dosimetry tests for (1100) workers in various fields of application of ionizing radiation,
- (100) environmental dosimetry tests for 12 locations in Jordan,
- (50) tests on quantitative and qualitative radionuclides in water samples,
- (40) tests on quantitative and qualitative radionuclides in environmental samples,
- (6) calibrations of portable radiation measuring equipment,
- (1) agreement for personal radiation monitoring with Royal Medical Services,
- (2) technical consultations in the field of radiation protection.

RSS participated in the provision of technical consultations through the following committees:

- The Consultative Committee for Nuclear Energy,
- The Radiation Protection Commission,
- Permanent Licensing Committee in the Field of Ionizing Radiation,
- Food Hygiene and Safety Committee Regarding Radio Nuclide Contamination.
- Procurement of Radioactive Sources for Medical Radiotherapy Committee.

c. Standards and Specifications

RSS participated with other concerned sides in drafting Jordan Environment Act. RSS also contributed to putting instructions related to the safe handling of asbestos, took part in the formulation of proposed standards in several fields of environment, and proposes specifications for treated wastewater and its reuse.

d. Training

RSS carried out different training activities this year such as training of technicians from various institutions on conducting tests related to environment. RSS also participated in different seminars, conferences, and technical committees related to environment. RSS also dispatched a number of its employees on training courses abroad. A paper on "Wastewater Management and Sanitation Policies in Jordan" was submitted to a seminar held in Turkey and organized by the European Institute of Water.

RSS also participated in the following activities:

- One scientific visit in the field of industrial application of radiation and radioisotopes to Poland and India,
- Participaton in the coordination meeting on quality assurances and harmonization of analytical measurements in the Middle East and preparing a technical paper on Jordan's activities in this field,
- Participation in planning and preparedness for nuclear emergency and radiation accidents workshop, and preparing an emergency plan for Gamma Radiographic Accident,
- Lecturing on ionizing radiation and their benefits and risks on our daily life at Epascopal Cultural Centre.

ANNEX 3: PROJECT PERSONNEL AT THE ECOLOGY DIVISION OF RSS

Division Head: **Dr. Ali ElKarmi**
Ph.D. Bioengineering (1987)
Texas A&M University

Researcher: **Mr. Hamed Ajarmeh**
M.S. Environmental Engineering (1992)
Jordan University

Researcher: **Mrs. Mona Hindeya**
M.S. Microbiology (1989)
Jordan University

Researcher: **Mr. Ali Omari**
B.S. Biology/Microbiology (1984)
Yarmouk University (Jordan)

Researcher: **Mrs. Lana Khames**
B.S. Biology/Microbiology (1989)
Kuwait University

Technician: **Mr. Mohamed Abu Zaki**
Secondary School Certificate

Vacant Position for Researcher with a Ph.D in Microbiology.
This position was previously held by Dr. Bayan Abu Ghazateh (Ph.D. Microbiology, 1990; University of London).

Support Staff:

Additional support staff from Water Analysis Lab and Specialized Equipment Lab is also available upon request. The Ecology Division therefore has access to up to 14 additional technicians in these two support labs.

ANNEX 4: EXAMPLES OF INDUSTRIAL POLLUTION IN JORDAN

- The heavy metals concentration in the KTR effluents are within standard limits for irrigation use.

- Most heavy metals concentrations in the KTR sediments are similar to the content of metal in normal Jordanian soil.

6.8 Discharge of Effluents to the Wadis

The responsibility for managing the control of industrial wastes and effluents is not clearly defined today. Municipalities are involved if an accident happens or when citizens voice their concern about odours or nuisances, such as dumping of wastes or effluents. Intervention by the Public Safety Committee happens when accidents occur that result in injuries or loss of life. Some industries are located near populated areas, while others are located in open land where a dry wadi serves as a convenient means for wastewater disposal.

A number of industries in the study area are not served with sewer connection and others are located outside municipal boundaries. This situation makes it difficult for some industries to manage their effluents in an environmentally acceptable and cost-effective way.

In practice, however, several industries are allowed, by the respective municipality, to discharge untreated effluents onto a common wastewater disposal site in Wadi Sakkar, east of Ruseifa. Other industries are allowed to tanker their wastewater to sewage intake stations at Ain Gazal or Zarqa. Some discharge their wastewater at undisclosed locations without permission.

The uncontrolled siting of industry has resulted in some serious pollution problems. The following two examples illustrate this.

(1) The Pepsi Cola Pool

This is a large pool of water (estimated volume in 1993 was 200,000 m^3), which collects every winter. It is a result of the mining activity of the Jordan Phosphate Mining Company in Ruseifa. Large amounts of fine silt material were discharged into a small wadi in that area, and it blocked the natural flow of rain water every winter. The Pepsi Cola Company, which is located nearby, started to discharge wastewater into this wadi. It has been estimated that the Pepsi Cola Company discharged about 200 m^3 of wastewater every day. A large pool was created from the impoundment of effluents and rain water. A Palestinian refuges camp was in 1967 located close to the pool, and some blocks of houses were also built in the area.

The pool is known as the Pepsi Pool although the company was connected to the sewers in late 1990 and does not any longer discharge effluents to the pool. The water in the pool was stagnant and the local people used to dump all sorts of garbage into the pool. This resulted in the water in the pool becoming septic and dirty. Children from the refugee camps used to play in the area and some of them drowned in the pool.

The Amman Municipality decided to empty the pool in the summer of 1993 by pumping the water from the pool over the mound of earth to the downstream side of the wadi. All kinds of rubbish and odd objects started appearing as the bottom of the pool became exposed. Among these objects were two live Katuosha rockets, many rusted Kalashnikov automatic rifles (these are believed to have been dumped there after the 1970 civil war in Jordan), and hundreds of used car tires. The dirty water in the pool may have polluted the ground water in the area. The impact of the pool water on the environment, has not been studied by any agency.

(2) Wadi Sakkar Pond

This is a wadi to the southeast of Ruseifa. The wadi flow has been blocked by a large amount of earth disposed there by the Jordan Phosphate Mining Company. During winter, rain water is collected there every year. Industries, that are not allowed to connect to sewers, are given permission by the Amman Municipality to dispose their wastewater in the pond. This has resulted in large quantities of waste and polluted water being collected in the pond.

The pond is located behind mountains and is not accessible to the public. It is located in an area where the Phosphate Company has some ground water wells. It is possible that water may infiltrate from the pond to the water aquifers below and thus pollute the ground water. Nobody monitors the area for possible ground water pollution, and wastewater discharged into the pond is not monitored.

The cases of the Pepsi Pool and the Wadi Sakkar Pond are the result of industrial activities taking place in the area. The Jordan Phosphate Mining Co has been mining in this area for more than forty years, and has abandoned large exhausted quarry areas. This has resulted in many negative impacts on the landscape around Ruseifa and, in particular, it has disturbed the natural flow of rain and flood water. Also, the Amman Municipality has permitted various industries to discharge their polluted effluents into the excavations that resulted from the phosphate mines. No precautions have been taken to safeguard against pollution of surface or ground water in this area as a result of this type of uncontrolled wastewater disposal.

Industrial Wastewater Characteristics. Organic, Non-Toxic Pollution. Flow and Pollutant Load

No	Industry	Process wastewater*	Pre-treatment	Discharge	Analysis by	BOD	COD	TSS	TDS
		m3/day Aver.				kg/day	kg/day	kg/day	kg/day
1	Jordan Petroleum Refinery	3800	Mech.	Irrigation	JPCP	148	460	391	10252
					WAJ	148	532	236	12122
6	Jordan Paper and Cardboard	600	Biol.	Irrigation	JPCP	66	136	69	1148
					WAJ	124	287	110	1080
7	Eagle Distilleries Co.	70	Mech.	Sewer Samra	JPCP	89	153	4.4	119
					WAJ	126	229	8.1	194
8	Ain Ghazal Slaughter-house	630	No	Sewer Samra	JPCP	1485	2776	1309	-
					WAJ	4249	20340	1014	3974
9	Jordan Ice and Aerated Water Co.	950	No	Sewer Samra	JPCP	297	709	591	4160
					WAJ	463	537	219	2446
10	Arab Chemical Detergents	36	Chem.	Sewer Samra	JPCP	3.6	15	4.5	516
					WAJ	-	-	-	-
15	The ICA Co.	310	Biol.	Sewer Sahab	JPCP	3.1	8.1	6.2	397
					WAJ	-	-	-	-
18	Zeidans Refrigeration Co	17	No	Sewer Samra	JPCP	24	53	14	47
					WAJ	99	194	43	82
19	Arab Brewery Co	66	No	Sewer Samra	JPCP	1.8	4.8	1.5	89
					WAJ	13	26	9	107
21	Arabian Trade and Feed Co	33	No	Sewer Samra	JPCP	21	45	10	39
					WAJ	88	163	290	60
22	Jordan Brewery Co	110	No	Sewer Samra	JPCP	57	117	6.8	72
					WAJ	28	52	5.5	68
23	United Factories Co	6	Biol.	Sewer Samra	JPCP	3.7	8.9	1.4	10
					WAJ	1.8	3.7	0.37	6.5
24	Yeast Industries Co	530	No	Irrigation	JPCP	2862	4362	250	5610
					WAJ	3378	6141	597	3869
25	Jordan Dairy Co	80	Biol.	Sewer Samra	JPCP	2.0	11	5.3	307
					WAJ	2.0	6.2	35	82
26	Al Ab Ice-cream Co	18	Mech.	Sewer Samra	JPCP	71	125	46	-
					WAJ	-	-	-	-
27	Hikma Pharmaceuticals Jordan	16	No	Sewer Samra	JPCP	35	60	8.8	19
					WAJ	13	23	2.3	14
28	Danish-Jordanian Dairy Co	90	Biol.	Sewer Samra	JPCP	2.2	6.5	3.2	154
					WAJ	28	56	19	117
29	The Tanning Company	150	Biol. Chem	Sewer Samra	JPCP	13	111	69	1893
					WAJ	15	49	28	1501
30	Danish Food Industries Company	130	Mech.	Sewer Sahab	JPCP	115	187	51	143
					WAJ	-	-	-	-
Total		7641		19 comp.	JPCP	5300	9346	2941	24978
				15 comp.	WAJ	8764	28938	2614	25612
Total to be		2711		16 comp.	JPCP	2224	4389	2131	7967
				12 comp.	WAJ	5104	21978	1672	8741

* Working days

ANNEX 5: OPERATIONS AND WASTEWATER CHARACTERISTICS OF HIKMA PHARMA

35 Enterprise no. 27: Hikma Pharma Group

35.1 Production

Hikma Pharmaceuticals in Amman has a pharmaceutical as well as a chemical production. The two different factories are located in the same area, but this report only deals with the pharmaceutical production. There are appr 300 employees.

The pharmaceutical production includes 50 different products of which many exist in several varieties thus making the total number of products close to 200. The production can be divided into tablets, mixtures (syrups) and suppositories.

Production of tablets:

1. Mixing of raw materials (chemicals)
2. Wetting with water and/or alcohol
3. Drying (drying cupboard or fluid bed dryer)
4. Granulating of dry material
5. Mixing in V-shape mixer
6. Production of tablets in tablet-machines
7. Packing of tablets
8. Storage of packed tablets

Production of mixtures:

1. Mixing of raw materials (chemicals)
2. Filling of mixtures into bottles and containers
3. Packing
4. Storage of packed mixtures

Production of suppositories:

1. Melting of wax
2. Mixing of wax and raw materials (chemicals)
3. Filling
4. Cooling
5. Cutting
6. Packing
7. Storage of packed products

35.1.1 Consumption of Water

The consumption of water in the pharmaceutical production is difficult to estimate because the Company only has figures for the total consumption of water for the two factories. Total consumption is estimated, by the Company, as 36 m³/day. It is furthermore estimated that 10-15 m³/day is used as process water in the pharmaceutical plant. In the production, water is used for washing the machines, the production equipment and general cleaning of the buildings.

Most of the water is supplied by the municipal network, but 12 m³/day is supplied by tankers and stored in two underground tanks.

35.1.2 Consumption of Chemicals

More than 200 different chemicals are used for production of the pharmaceutical products. A computer programme is controlling the storage, and registering all movements to and from the storage. A list of the chemical consumption was not readily available and the Consultant therefore made the assumption that the consumption of chemical is reflected in the size of the storage. Emphasis was therefore given to those chemicals which were stored in large quantities. Below are listed all chemicals which where stored in quantities of more than 1,000 kg.

Ac-di-sol (cros carmellose)
Alcohol 95%
Alcohol, absolute
Alcohol, isopropyl
Ampicillin trihydrate powder
Aerosil 20D
Avicel PH 101
Cloxacillin
1206 FD&C Blue no.1
1311 FD&C Blue no.2
Sicopharm Brown Lake
1673 Erothrosin
1150 Dispersed Pink
Allura red FD&C red no.40
11959 Carmoisin
14031 Quinoline Yellow
14709 Tartrazine
19351 FD&C Yellow no.6
12116 FD&C Yellow no 6, lake
17248 Quinoline Yellow, lake
Dextrose Anhydrous
Explotab
Heparin sodium sheep origin
Lactose
Mannitol D

Penicillin V potassium
Paracetamol Mic
Propylene glycol
Potassium citrate
Sucralphate
Sodium chloride
Trisodium citrate dihydrate
Sodium hydroxide
Starch potato
Sugar
Talc fine powder
Trichloroethylene
Witepsol E75
Witepsol H12

The list clearly includes some main groups of chemicals. Different alcohols are used for wetting the mixed chemicals in the production of tablets and different carbohydrates are used for coating the tablets and for the mixtures. A variety of different colouring chemicals are used for final dressing of tablets.

35.2 Present Wastewater Disposal

35.2.1 Present Wastewater Practice

Process wastewater is primarily produced in the tablet production when the machines and auxiliary equipment are washed at the end of a batch production. Residual chemicals from the machines are collected in drums as is the first rinse water. These drums are disposed of as chemical waste. Further washing water is led to the sewer.

Tanks, pipes and auxiliary equipment from the production of mixtures and ointment are cleaned with water which is led directly to the sewer system afterwards.

35.2.2 Wastewater Control by IPCP and Others

In February, March and August 1993, the IPCP has sampled and analyzed the wastewater from the pharmaceutical production (August sample included heavy metals only). The tested wastewater is supposed to include both process and sanitary wastewater. No flow measurement was available.

A local consultancy firm has collected samples of the wastewater in September-October 1993. The results are presented below because they give a

valuable supplement for evaluating the wastewater problems at Hikma Pharmaceuticals.

Table 35.1 Results from IPCP wastewater control in February and March.

Locality	09.02.93	22.02.93	07.03.93	16.03.93	Discharge Limits
pH	6.11	5.94	5.67	5.00	5.5-9.5
COD, mg/l	1,380	5,550	2,840	5,210	2,100
BOD5, mg/l	440	2,890	1,860	3,620	800
TSS, mg/l	225	1,220	240	535	1,100
Turbidity, NTU	135	1,525	225	350	350
EC, µS/cm	440	1,000	735	935	-

IPCP has furthermore analyzed for heavy metals in one sample from August 1993, where only zinc is present in significant concentration (1.4 mg/l). Phosphorus and nitrogen were analyzed in the same sample, but the concentrations were below normal sanitary wastewater.

Table 35.2 Results from Jordanian control program in September-October 1993

Locality	COD, mg/l	BOD5, mg/l	TSS, mg/l	TDS, mg/l
No.1: To sewer	940-8,860	320-2,270	86-500	480-905
No.2: Lab	760-11,600	495-4,430	225-1,220	510-1,070
No.3: Syrup line	320-8,090	63-1,630	58-855	490-1,520
No.4: Wash etc.	1,640-46,000	810-5,300	105-710	440-12,600
No.5: Penicillin	230-3,980	135-830	49-170	490-1,530

No.1: Total wastewater going to the sewer
No.2: Wastewater from laboratory
No.3: Wastewater from syrup (mixture) lines, ovens and toilets
No.4: Wastewater from powder and coating room with washing activities
No.5: Wastewater from penicillin factory

The samples were collected from the total combined wastewater flow to sewer and from different man-holes with wastewater from various parts of the production. The samples were taken on 5 different days. There are no flow measurements, and the sampling period is not specified.

The results in table 35.1 and 35.2 show that most of the wastewater exceeds the discharge limits, and quite significantly so. High contents of organic matter result in high values of COD and BOD. The TSS is high but only periodically exceeds the limit. pH is low and sometimes below the

ANNEX 6: WASTEWATER ANALYSIS DATA SHEET FROM DAR-AL-DAWA

Chemical and Physical Characteristics of a Wastewater Sample from Dar Al-Dawa

Characteristic	Value
BOD	1730 mg/L
COD	4870 mg/L
TSS	220 mg/L
TDS	900 mg/L
NH_4	18 mg/L
ABS	32 mg/L
pH	6

ANNEX 7:RECOMMENDED LIST OF MICROBIAL STRAINS FOR FUTURE STUDIES

LIST OF STRAINS

(-recommended for the purpose of degradation of organic chemical compounds)

Sphingomonas paucimobilis	ATCC-29837
Nitrosomonas europaea	ATCC-25978
Pseudomonas putida	ATCC-11172
Pseudomonas cepacia	ATCC-25416
Alcaligenes eutropus	ATCC-17697
Flavobacterium sp.	ATCC-53874
Pseudomonas pickettii	ATCC-27511
Phanerochaete chrysosporium	ATCC-24725

ANNEX 8: COMPOSITION OF CHLOROPHENOL ENRICHMENT MEDIA

The composition of the CPE medium:

(g/l)

	CPE1	CPE2	CPE3
CaCO3	10	10	10
(NH4)2SO4	2	2	2
KH2PO4	0.05	0.05	0.05
CaCl2	0.1	0.1	0.1
MgSO4	0.1	0.1	0.1
FeSO4	0.01	0.01	0.01
ZnSO4	0.005	0.005	0.005
CuSO4	0.005	0.005	0.005
NiSO4	0.005	0.005	0.005
Piridoxine (B6	0.0005	0.0005	0.0005
Nicotinic acid	0.0005	0.0005	0.0005
Thiamine (B1)	0.0005	0.0005	0.0005
Yeast extract	0.005	0.05	0.5
Peptone (casein)	0.005	0.05	0.5

pH=7.0 adjusted by 10 % HCl

The trace elements and vitamins were dissolved separately in a 10x concentrated basic solution and this solution (in 10 %) was added to the main part of the medium prior to sterilisation.

ANNEX 9:FREEZE DRYING PRINCIPLES AND METHODS MANUAL

AN INTRODUCTION TO FERMENTATION METHODS AND EQUIPMENT

Fermentation is the intrinsic capability of a microorganism to perform complex chemical transformations upon organic compounds by means of metabolic activity and the action of enzymes. The process was used in the production of food and drink thousands of years before the existence of microorganisms was established. Today, fermentation refers to any controlled microbial action undertaken to produce commercially useful products.

Applications of fermentation have grown well beyond the traditional area such as the manufacture of alcoholic beverages, cheese, solvents, and pharmaceuticals. The need to fully understand the metabolism of living cells, coupled with spectacular results from the artificial manipulation of genetic material in bacteria, yeast and mammalian cells, have made fermentation the subject of intensive research in the last 10 years. To aid researchers in meeting the growing potential of fermentation technology, instrument manufacturers have developed modular fermentation systems that are compact, automated, and compatible with accessory monitoring and controlling devices.

BENCH-TOP FERMENTERS

A fermenter is a bioreactor designed to provide optimal conditions for the controlled growth of microorganisms. This is accomplished by regulating agitation, temperature, and aeration of the fermentation broth. Accessory process control sensors and instrumentation may be used to measure additional growth parameters such as pH and dissolved oxygen concentration, allowing the fermentation process to be monitored and controlled with greater precision.

The simplest type of research fermenter is a bench-top model. It is a closed vessel with a volume capacity ranging from 1-20 liters. To function as an effective research tool, the bench-top fermenter must provide reproducible data and ideally, feature design characteristics facilitating scale-up to larger volume production. Results obtained from self-styled or "homemade" fermenters are difficult to duplicate on a large scale, rendering most processes developed in such units of little if any, commercial worth. Shake flask technology, bearing little physical resemblance to pilot or production scale fermentation vessels, is limited by its inability to incorporate yield optimizing instrumentation.

The maintenance of aseptic operating conditions is an important criterion for judging the research value of a bench-top fermenter. As with larger units, bench-top bioreactors must be designed and operated to minimize opportunities for invasion by unwanted organisms. Most products sought through fermentation are synthesized in pure culture by a selected strain of species. When a contaminating organism invades the culture, it competes for available nutrients and can fatally interfere with the metabolic activity of the desired organism. It can also produce substances of its own that are difficult to separate from the product. In order to avoid contamination, all materials that come in contact with the fermentation broth should be noncorrosive, nontoxic, and able to withstand repeated autoclaving.

The noncorrosive materials commonly used in vessel construction are borosilicate glass and stainless steel. For research and educational purposes, glass vessels provide the advantage of allowing fermentation to be viewed in progress. The vessels of larger pilot-scale fermenters are generally constructed of stainless steel and are jacketed and pressure-coded so the equipment can be safely steam sterilized in-situ. Vessel headplates should also be made of stainless steel and incorporate positive-sealing penetrations that will maintain their integrity followed by repeated autoclaving and chemical attack. These penetrations facilitate air inlet and exhaust as well as allowing for liquid additions, aseptic sampling, and the installation of process measuring electrodes.

OPERATION

Agitation

Subsequent to the sterilization of the vessel assembly and nutrient medium, the fermenter is aseptically inoculated, usually through a resealable septum or addition port, with a measured quantity of the desired organism. Vessel contents are mixed by a centrally rotating shaft supporting several adjustable impellers. This motor-driven shaft can be magnetically or mechanically coupled, and either top or bottom mounted. In a mechanically coupled system, the shaft penetrates the vessel headplate, creating the need for a contamination preventing mechanical seal. As such mechanical seals can fail unexpectedly if not properly maintained, they are frequently a source of contamination. A solution to this problem has been the development of high-torque, magnetically coupled agitation systems which eliminate the need for mechanical seals, as they do not penetrate the headplate. A motor-driven rotating magnet powers the magnet bearing agitation shaft, without a physical connection. Superior agitation systems incorporate advanced electronic feedback speed control technology, which enables maintenance of the desired rate, usually ranging to about 1250 RPM, despite viscosity changes.

The mixing action of the impellers makes nutrients and dissolved oxygen more uniformly available to the individual microbes, in addition to dispersing metabolic waste products. The impeller designs traditionally employed are the Rushton type six bladed turbine which typically supplies the best mixing and aerating characteristics, while marine blades produce significantly less shear stress, and are extensively used in mammalian and plant cell culturing applications.

The addition of baffles to the culture vessel increases the uniformity of turbulence, and adds to the efficiency of dissolved oxygen transfer in aerobic fermentation. Baffles extend inward from the sides of the vessel and act as stationary obstructions to the flow of its contents. They are often adjustable, allowing optimization of the turbulence patterns created, eliminating the inefficient aerating characteristics of vortex mixing.

Temperature Control

Microorganisms characteristically experience optimal growth within narrow temperature ranges. Therefore, the fermenter must provide an accurate, reproducible means of temperature control. In bench-top research units, temperature may be accurately controlled by proportional temperature control circuitry in conjunction with a cartridge heater, heat exchanger for cooling, and temperature sensing probe, with data registered on a digital display. In larger systems, temperature conditioned fluid (usually water) may be circulated through an external jacket enveloping the walls of the fermenter vessel. A temperature range of approximately 5° C above water supply temperature, to 60° C with an accuracy of +/-.25° C is generally sufficient for the culturing of most organisms. Higher wattage heaters or recirculating cooling devices can be adapted for applications requiring stricter conditions.

Aeration

Fermentations may be performed under either aerobic or anaerobic conditions, depending upon whether cells being cultured require the presence or absence of oxygen for growth. In aerobic fermentations, sterile air must be supplied to the culture vessel.

Sterilized air filters are employed to remove organisms and particulates greater than 0.2 micron in size, from the incoming air supply. An exhaust vent filter prevents back-in infection of the vessel contents, and the escape or organisms into the laboratory or production environment. This is especially important when working with pathogens or genetically engineered organisms. The volume of air entering the vessel is metered by a variable area flowmeter. The sterile air supply is introduced to the vessel contents through orifices in either nozzle type or sintered stainless steel spargers. Agitation impellers break up resultant air bubbles and distribute dissolved oxygen throughout the culture.

Anaerobic conditions may be established by eliminating or disconnecting the air supply and introducing a nitrogen gas overlay. Alternative gasses may also be introduced to the system.

PROCESS TYPES

Fermentation can be classified as either batch or continuous flow.

Batch Fermentation

Batch fermentation begins as a single starter-culture which is grown for a defined period of time and terminated when the concentration of the desired product reaches a comparatively high level. Vessel contents are then harvested, usually by filtration or centrifugation, and the desired product isolated, purified, and concentrated.

The advantages of batch fermentation are several:

- The system is relatively inexpensive.
- The equipment is less complicated because growth of the cells is a process separate from harvesting of the product.
- Moderate amounts of different products can be obtained using the same equipment (although cleaning and sterilization are required between applications).
- As the fermentation time is usually short, preventing contamination within the system is less difficult than with the longer times generally required for continuous-flow fermentations.

Continuous-Flow Fermentation

Continuous-flow fermentation is an invaluable method for growing large numbers of cells economically, and exploring microbial form, function and population dynamics. It required a steady-state environment in which sterile nutrient medium is fed to the culture at a continuous rate, while effluent containing the desired products is withdrawn and collected at a similar rate.

On the research scale, continuous culturing can be economically performed via a fermentation system featuring an overflow sidearm culture vessel (FIGURE 1). In this way, a peristaltic pump is required only for nutrient addition, while culture volume is maintained at a given level, with product containing fluid exiting the sidearm. Fresh nutrient medium introduced to the system should be added well below the level of the sidearm or product withdrawal tube, to prevent waste, and should be directed through a drip-tube providing a break in the medium flow path, to prevent growback of microorganisms into the feed line. Alternatively, peristaltic pumps may be employed for both nutrient addition and product harvesting (FIGURE 2).

The growth rate of cells cultured is dependent upon the medium formulation and the rate at which fresh medium is added. By manipulating these factors, the chemical composition of the cells can be altered. This makes continuous-flow fermentation useful in the study of cell physiology and in the determination of optimal conditions for a given process. The method is exacting, however, because of the necessity of (1) achieving and maintaining the steady-state conditions required for optimal growth and productivity, and (2) difficulty encountered in ensuring the continued sterility of the system during prolonged use.

Nonetheless, continuous-flow fermentation allows more efficient use of the fermenter than batch fermentation. Cleaning and sterilizing cycles are reduced in number, and harvesting takes place as a product is manufactured. Ideally, continuous fermentations should be conducted under conditions that inhibit further cell growth once optimum cell population density is reached, but still permit production of the desired product to continue. As a practical matter, growth is controlled commonly by limiting the availability of an essential nutrient.

PARAMETERS OF GROWTH AND THEIR MONITORING

The Growth Curve

Fermentations typically follow the growth curve shown in Figure 3. Mathematically, such a graph is known as a sigmoid or S-shaped curve. At the beginning, there is a "lag" phase permitting the cells to recover from sudden dilution in the fermentation vessel. This is followed by a "log" phase characterized by the doubling of cells at a rate determined by each cell type (this is a general rule for both bacterial and mammalian cells). However, because cells growing in a common environment have inhibitory effects on one another, the log phase of growth slows to a stationary phase. The cycle concludes with a log "death" phase.

Growth Goals

The maintenance of stationary phase is the goal of many fermentations. In others, however, maximum harvesting of the desired product requires maintenance of the log phase. The latter is typical in continuous-flow fermentations; the former may be characteristic of either batch or continuous-flow fermentations. If a cell culture is not diluted or does not have growth medium continually replaced, the final log "death" phase will be entered.

Measuring Growth

To measure growth, the fermenter must be designed to facilitate aseptic sampling of the culture during any phase of growth. The efficiency of agitation, the location of the sampling port, and the method of sample withdrawal will determine how representative each sample is. Cell density can be measured either by counting the number of cells in each sample or by centrifuging the sample and measuring the size of the resultant pellet of material. As an alternative, the supernatant can be analyzed for the amount of desired product, the decrease of a particular nutrient or raw material, or even the change in pH, dissolved oxygen, or turbidity.

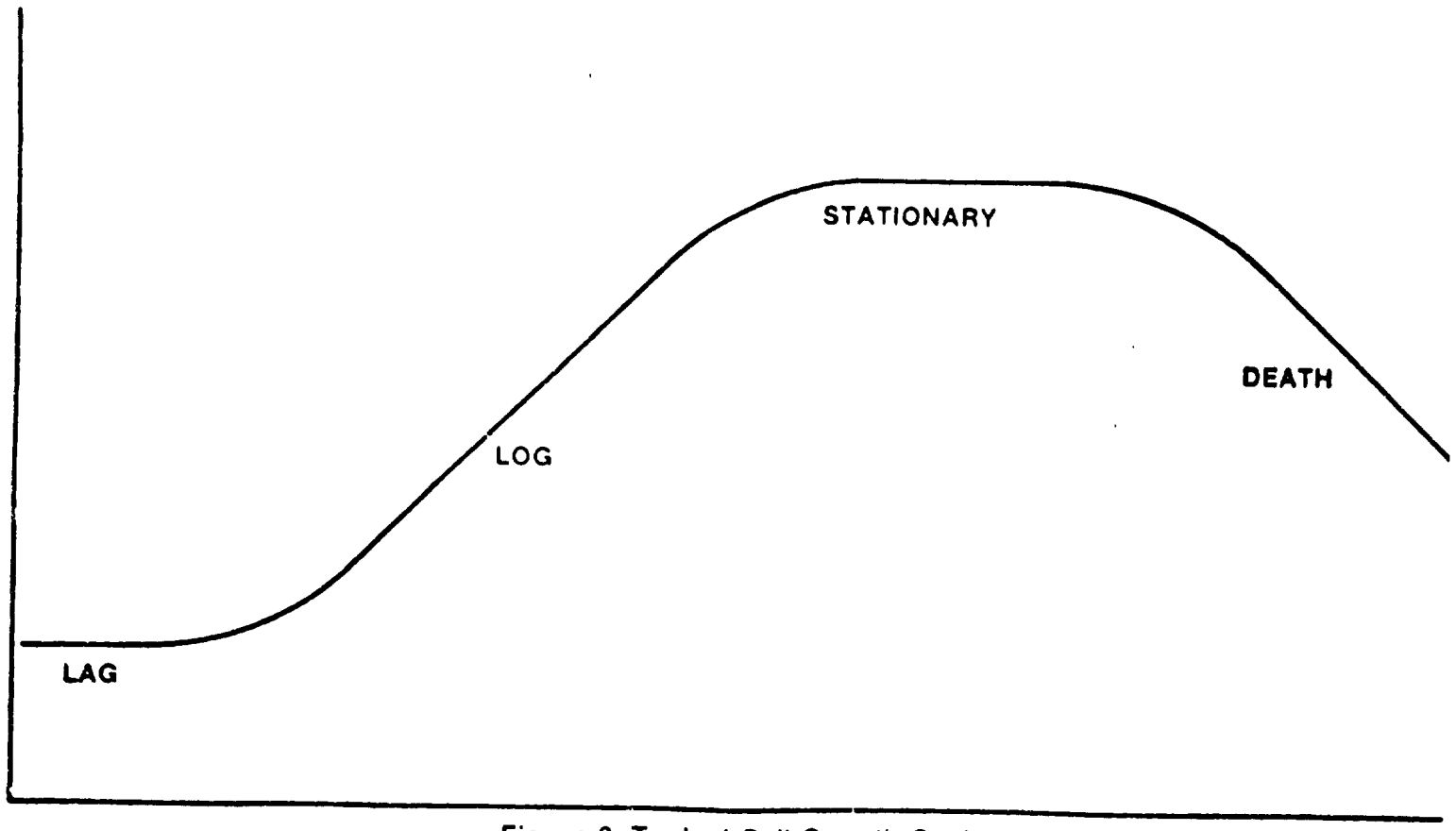


Figure 3. Typical Cell Growth Cycle

Process Control

To maximize productivity during a fermentation, the physical and chemical environment inside the reaction vessel must be controlled within precise limits. In addition to the regulation of the primary process growth parameters (agitation, temperature, aeration), in order to generate reproducible results and data that can be reliably extrapolated into scale-up values, accessory process control instrumentation may be used to measure additional growth parameters, allowing the fermentation process to be monitored and analyzed with greater precision. Commonly, these parameters include pH and redox potential, foaming and dissolved oxygen concentration.

pH and Redox Potential

Any change in pH concentration, which can be altered by metabolizing nutrients, must be corrected to prevent organisms from terminating themselves and /or disrupting fermentation kinetics. Automatic pH control instrumentation is available to maintain selected optimal pH values through appropriate additions of corrective acid or base reagents. Sterilizable combination reference and measuring electrodes, selectively permeable to hydrogen ions, are routinely used in monitoring Ph values. These values are converted by the probe into electrical signals via electrolytic soiution, which are compared to pre-selected high and low setpoints, in the control unit. Sterile corrective reagents are introduced to the fermentation by means of associated peristaltic metering pumps which are automatically activated upon violation of a setpoint limit. Timers facilitate the adjustment of pump dosages and delay intervals, enabling the conservation of reagents, and minimizing overshoot. A continuous strip chart recording provides useful data pertaining to process characteristics.

The oxidation-reduction potential (redox) may be determined with electrodes and instrumentation similar to those used to measure pH. Typically, a sterilizable platinum combination electrode is used, and measurement is in millivolts. The redox potential of a fermentation broth can be controlled by adding chemical agents such as ascorbic acid, sodium thioglycolate and other reducing agents, or by sparging with nitrogen gas.

Dissolved Oxygen

A determination of dissolved oxygen concentration is useful in understanding mass transfer mechanisms in a fermentation process. In an aerobic fermentation, if the biochemical rate of oxygen utilization is great, the dissolved-oxygen level will tend toward zero and oxygen will become the rate-limiting factor for the fermentation. Therefore, it is essential that sufficient oxygen be supplied in an amount characteristic to the specific process.

Dissolved oxygen monitoring equipment is available, consisting of a sensing electrode which converts the level of oxygen in the sample into a proportional electrical signal, and an amplifier containing conditioning circuitry. Oxygen which is present in the sample diffuses through a membrane in the sensor, where it is reduced. This membrane is permeable to oxygen, but impermeable to proteins and ions which can interfere with measurement of oxygen. Commercially available oxygen sensors function either on galvanic or polarographic principals of operation

In a polarographic electrode, a cathodic electrode of a noble metal, usually platinum, is charged with a fixed polarization voltage with repsect to a silver reference anode in an appropriate electrolytic solution. The galvanic electrode does not require external voltage for the reduction of oxygen, as it generates its own electrochemical voltage, sufficient for spontaneous reduction.

A dissolved oxygen control module may be obtained which interfaces with the aeration and agitation systems of the basic fermenter. Control is provided upon either one or both of these parameters in conjunction, to maintain desired concentration of dissolved oxygen.

Foaming

During fermentation, excessive foaming can develop as a result of agitation and aeration rates, and the chemical nature of media constituents. If unchecked, this foaming can lead to infection of the culture through backflow and wetting of the air filtering material. In addition, the presence of a foam layer is a barrier to effective oxygen transfer. Foaming can be controlled chemically through the action of nontoxic antifoam reagents (usually silicone-based), that reduce the surface tension at the air-liquid interface. Such chemical antifoams can be automatically introduced into the fermentation vessel by means of a chemical antifoam control device, incorporating an adjustable foam sensing probe and an associated peristaltic metering pump. Upon contact with foam, the probe generates an electrical signal which activates the peristaltic pump to dispense a suitable chemical foam suppressing reagent into the vessel. When the foam level subsides and falls beneath the probe tip, the circuit is broken and pump operation ceases. Adjustable, electronic process timers enable the determination of the length of the antifoam additional cycle, and the interval of delay between doses. Probe sensitivity is also usually adjustable to eliminate responses due to erroneous signals generated from occasional splashing. The level of foam can also be controlled mechanically by high-speed impellers or centrifugal devices that physically break up the foam by dispersing it against the walls of the fermenter.

Scale-Up Considerations

In general, fermentation equipment may be categorized on the basis of volume capacity as Laboratory (1-20 liter), Pilot (40-250 liter) and Production (500 liter and larger). To be effective as a research or educational tool, it is a key requirement for bench-top laboratory fermentation apparatus to approximate the physical characteristics of larger systems to facilitate scale-up to increased volume levels. Scale-up is essentially defined as the combination of techniques used in transferring a fermentation process developed on the lab scale to these larger volumes. The research fermenter should be designed to reflect generally accepted fermentation engineering standards with respect to vessel assembly and component configuration and relative proportionality, which may effect control parameters. Scale-up involves the study and exploration of the effects of scale on the various fermentation parameters, both physical and biological, such as mass transfer, mixing, heat transfer and the metabolic responses of microorganisms. In many cases scale-up requires going back down to a smaller scale to obtain additional data. The number of volume stages incorporated within the scale-up procedures is generally dependent upon time constraints, capitol invested and operating cost limitations, therefore it is usually necessary to assess the effects of scale through extensive experimentation in a short period of time. These requirements are often met by incorporating a series of instrumented bench-top units in parallel, and later a fully instrumented pilot plant study.

ANNEX 10: USE OF NITROSOGUANIDINE AS A MUTAGEN

MUTAGEN TREATMENT WITH NTG

The effectiveness of NTG is much higher than UV treatment or treatment by using other types of mutagens. Usually, even in the case of very low lethalities the 30-40 % of the survivants are mutants. The preparation of cell suspension is the same as described in the case of UV treatment. The cell suspension is to be made by inoculating 20 ml of bouillon with a loopful of microbes from slant agar. The 20 ml of inoculated medium should be shaken (250 rpm, 30 oC) overnight in a 250 ml flask. The cells should be separated prior to treatment by centrifugation (3000 rpm, 20 minutes). The centrifuged cells are to be suspended directly in the NTG solution (instead of the sterile water).

The NTG solution should be made by measuring the weight of one NTG crystal in a small glass centrifuge tube. The solution is made by adding a 0.01 phosphate buffer (pH=6.0). The volume of the buffer depends upon the weight of the crystal, and the solution should have a concentration between 300 - 500 microgram/ ml. The NTG is not an easily dissolving compound, which is why the stopped centifuge tube should be shaken very carefully until complete dissolution.

The cells suspended with the NTG solution should be incubated for 30 minutes at 30 oC. After this incubation period has passed, the suspension should be cleaned from the NTG by centrifugation and resuspension by using sterile water. The suspended cells can be transferred to any kind of selective agar medium.

In this case the lethality should be adjusted to approximately 50 %, in order to obtain a high number of mutants. In the case of NTG the higher lethality is followed by the generation of multiplied mutants among the surviving cells, and the accumulation of these genetic mistakes can cause several difficulties during the application of such strains.

The handling of the NTG should be careful, forexample avoid contact with skin, inhalation or swallowing because of its strong carcinogenic properties. The compound does not sublimate into the air but it can explode if exposed to high temperatures.

ANNEX 11: ABSTRACT OF LECTURE PRESENTED BY DR. BALOGH

SUMMARY OF LECTURE PRESENTED BY DR. ISTVAN BALOGH

TITLE: FERMENTATION TECHNOLOGY, STRAIN IMPROVEMENT AND BIOREACTORS

The RSS has no tradition in the field of biotechnology. Therefore the aim of giving this lecture is to provide basic information about the production systems connected with microbiology. It was emphasized during the lecture that the main principles of the waste degradation technology are the same.

For the purpose of introduction, the meaning of the word "fermentation" was explained, mentioning the two implemented part of it as the fermentation software (strain, technology) and the fermentation hardware (equipment). The strains and technologies are patented but the processes are usually kept in secret. The equipment (including the bioreactors, piping arrangements, accessories, and downstream facilities) is a crucial part of the know-how. In wider terms the operation know-how is also an important part of the technology insofar as it has a fundamental effect on the fermentation results.

The traditional fermentation processes were listed that is, the production of several fermented foods and drinks, like the sour dairies' cheese, yoghourt, kefir, koumis, soybean sauce, koji, beer, arak, brandy, wine, sake, sour cabbage and cucumber, sour fish etc.

The main principles of pharmaceutical fermentation technology were shown. The several fermentation products were listed with a brief explanation: antibiotics, vitamins, amino acids, organic acids, citric acid, products created by means of "genetic surgery", solvents, biodegradable polymers, polysaccharide and products made by bioconversions.

The rough connection between the amount of the marketed product and the market price was demonstrated using the examples of citric acid and daunomycin. The possibility of reaching extra profit was explained by mentioning the R+D work concerning the hyaluronic acid production by fermentation technology. There are not only technical problems with this because of the extremely high viscosity of the accumulated product but with the licensing as well (due to the fact that the production strain is the Streptococcus zooepidemious which has hemolytic properties). The strain was obtained from bovine mucous membranes.

The productive industrial strain is the basic component of the fermentation software. The mutation and selection methods were explained in detail by introducing the genealogy of a Corynebacterium glutamicum strain having approximately 140 g/l l-lysine production capacity.

The basic principles of the fermentation technology were explained (the composition of the fermentation media, the temperature, the pH and oxygen supply). The fermentation media should follow the chemical composition of the bred biomass together with the product generated during the process. The chemical composition of the microbes were shown and the main nutrient sources used in industry were introduced. The oxygen is one of the nutrients of main importance in the case of aerobic fermentations. The majority of the scaling-up and engineering tasks is connected with reaching the required level of oxygen mass transfer rate required by the process in the large scale bioreactor. The importance of medium optimisation was emphasized, and the roles of pH and temperature were explained in connection with the effects of pH and temperature on the enzymatic reactions.

A short explanation was given on the basic principles of batch technology, the feed-batch, and continuous fermentation technology. The role of catabolite repression was demonstrated by showing the effect of different carbon source concentrations on batch fermentation technology. It was shown that the relevance of the catabolite repression is also valid in the case of biodegradation processes.

Additionally the operational know-how was introduced by mentioning some examples and showing the crucial importance of it.

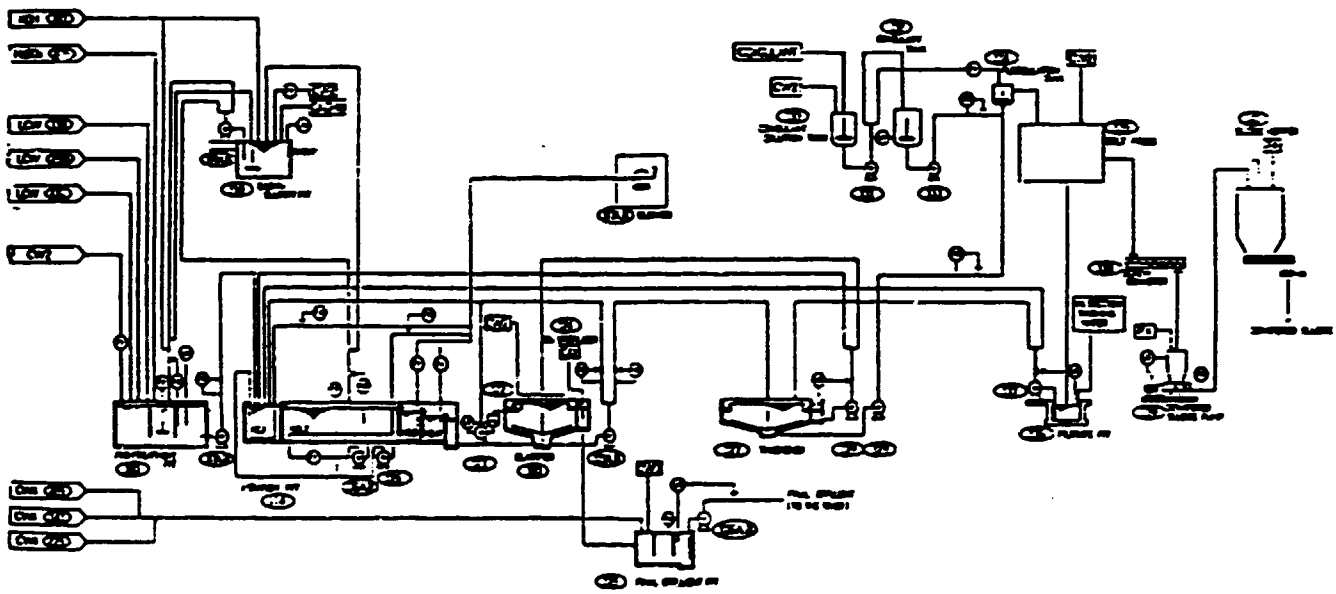
The basic part of the fermentation hardware is the bioreactor. The main parts of the fermentor were introduced by explaining their functions. The requirements of sterile processes were explained, and the special accessories responsible for the maintenance of sterility were described. It was shown that the arrangement of piping has a great effect on performance and levels of sterility.

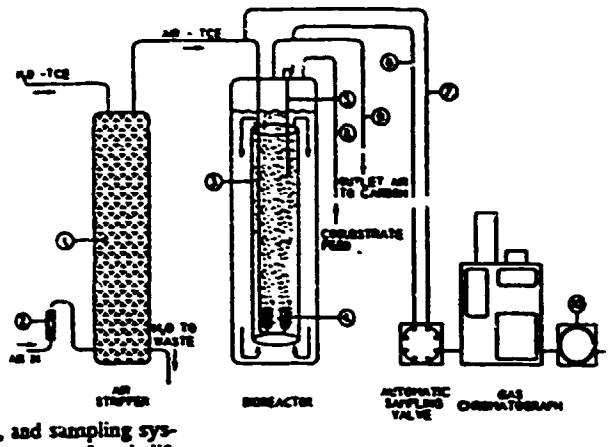
It was mentioned that, except in recent decades waste-water treatment plants were designed by civil engineers. The equipment was made of concrete; the usual arrangement being a flat type pit with an (at least) 1:5 height-width ratio. The traditional waste-water treatment basins are insufficient in terms of both their aeration level, and mixing capacities. The design is usually made by neglecting nearly all the relevant perspectives of industrial microbiology.

The principles of the traditional waste-water treatment plant were demonstrated by introducing a well designed 1500 m3/day plant (Annex-12, page 44). The volume of the pit segments are 500 m3, 2000 m3, 350 m3 and 150 m3. The internal recycling volume of the aeration pit is 24-36000 m3/day, the recycling volume from the second to the first pit is 4-7000 m3/day. The amount of returned sludge from the clarifier is 930 m3/day. The aeration rate of the aeration pit is 3500-3750 Nm3/h. The COD content of the final effluent is 60, the NH3- nitrogen content is 6.7 mg/l. (The loaded COD is 2900 mg/l, and the NH3-nitrogen concentration is 700 mg/l.) The purpose of the extremely high liquid recycling rates is to compensate for the insufficient mixing capacities of the system.

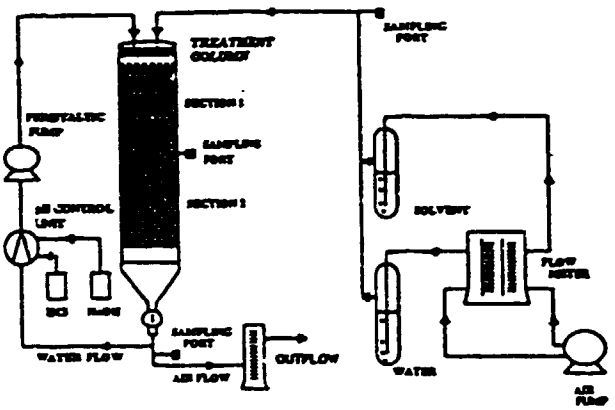
Some advanced industrial biodegradation equipment was shown like the "UNOX" system, the "BIOHOCH" at BAYER Ag. (Germany) and other bioreactor systems produced also by the German "UHDE" company at SANDOZ (Switzerland) and at ZIMPRO ENVIRONMENTAL Inc. (USA) and at NALCO CHEMICAL Ag. (Germany). Generally these reactors were designed by calculating the requirements of the biological processes. The additional novelty of the UHDE equipment is the application of a fluid-bed bioreactor packed with activated carbon. The role of the activated carbon is the fixation of microbes and the chemical absorption of the chemicals to be degraded. It should be mentioned that the first such bioreactor was originally designed for chemical absorption and for batchwise regeneration of the activated carbon. The operation personnel observed that in the case of a postponing of the regeneration period the "absorption" capacity was not decreased. This means that the settled/mixed culture of microbes had started to decompose the organic wastes.

THE STRUCTURE OF A TRADITIONAL BIOLOGICAL TREATMENT PLANT





Schematic of air stripper, bioreactor, and sampling system. 1, glass wool packing; 2, rotameter; 3, downcomer for air lift reactor; 4, sintered steel sparging stones; 5, sampling-and-addition tube; 6, reactor air outlet sampling line; 7, reactor air inlet sampling line; 8, cosubstrate feed tube; 9, reactor air outlet; 10, vacuum pump for sampling system.



Schematic diagram of a trickling air biofilter with pH control and water recirculation. For details, see the text.

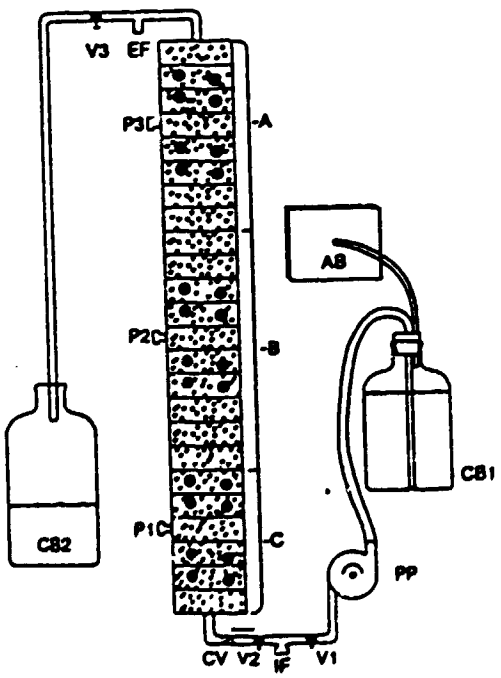


Diagram of the column. Section A contained composite sediments from the saturated zone; section B and C sediments were from unsaturated zones. Side ports were for sampling sediments and pore waters. V1 to -3, valves; CB1, column feed water carboy; CB2, collection carboy; AB, Teflon gas bag; CV, check valve; P1 to -3, pore water sampling ports; IF and EF, influent and effluent sampling ports, respectively; PP, peristaltic pump. Large solid circles, sediment sampling ports.

BIBLIOGRAPHY

1. Chowdhury J.:Fouhy K.: Biology boosts waste treatment. Chemical Engineering, 101, 2. , 30-37., 1994

2. Michael V. E.: Flynn P. et al. : Reductive dechlorination of trichloroethylene and tetrachloroethylene under aerobic conditions in a sediment column. Appl. Env. Microb. June 1994. p.2200-2204

3. Ensley B. D.: Kurisko P. R.: A gas lift bioreactor for removal of contaminants from the vapor phase. Appl. Env. Microb. January 1994. p. 285-290

4. Young Sook Oh; Bartha R.: Design and performance of a trickling air biofilter for chlorobenzene and o-dichlorobenzene vapors, Appl. Env. Microb. August 1994. p. 2717-2722

5. Armenante P. M.: Pal N.: Lewandowsky G.: Role of mycelium and extracellular protein in the biodegradation of 2.4.6,-trichlorophenol by Phanerochaete chrysosporium. Appl. Env. Microb. June 1994. p. 1711-1718

6. Der Yi Mu: Scow K. M.: Effect of trichloroethylene (TCE) and toluene cocentrations on TCE and toluene biodegradation and the population density of TCE and toluene degraders in soil. Appl. Env. Microb. July 1994. p. 2661-2665

7. Nadeau L. J.:Fu-Min Menn: Brenn A.: Aerobic degradation of 1.1.1-trichloro-2.2-bis(4-chlorophenil)ethane (DDT) by Alcaligenes eutropus A5. Appl. Env. Microb. January 1994. p. 51-55

8. Winkler M. A.: Thomas A.: Biological treatment of aqueous wastes. in: Wiesemann A. (ed.): Topics in enzyme and fermentation biotechnology, Ellis Horwood. Chichester. 1978.. p. 200-279

9. Chakrabarty A.: US patent 3.913.315. (1974)

10. Grasmer L. L.: Microorganisms for waste treatment. p. 211-222. in: Peppler H. J.: Perlman D. (eds.): Microbial technology. vol. II. Academic Press. New York

ANNEX 12:FERMENTATION PRINCIPLES AND METHODS MANUAL

INTRODUCTION TO THE FREEZE DRYING METHOD

The method of drying biologicals by sublimation of ice in a vacuum has been known for over fifty years, when Shackell (1909) applied vacuum pumps to his experiments to accelerate the process, but it wasn't until shortly before the Second World War that primitive designs of laboratory freeze dryers were made commercially available. During this war, much attention was given to the development of equipment and techniques for the purpose of supplying enormous quantities of dried blood plasma and penicillin to the Armed Forces. By the end of the war, the technique had become accepted as one of the most perfect methods of preserving biological materials.

What is Freeze Drying?

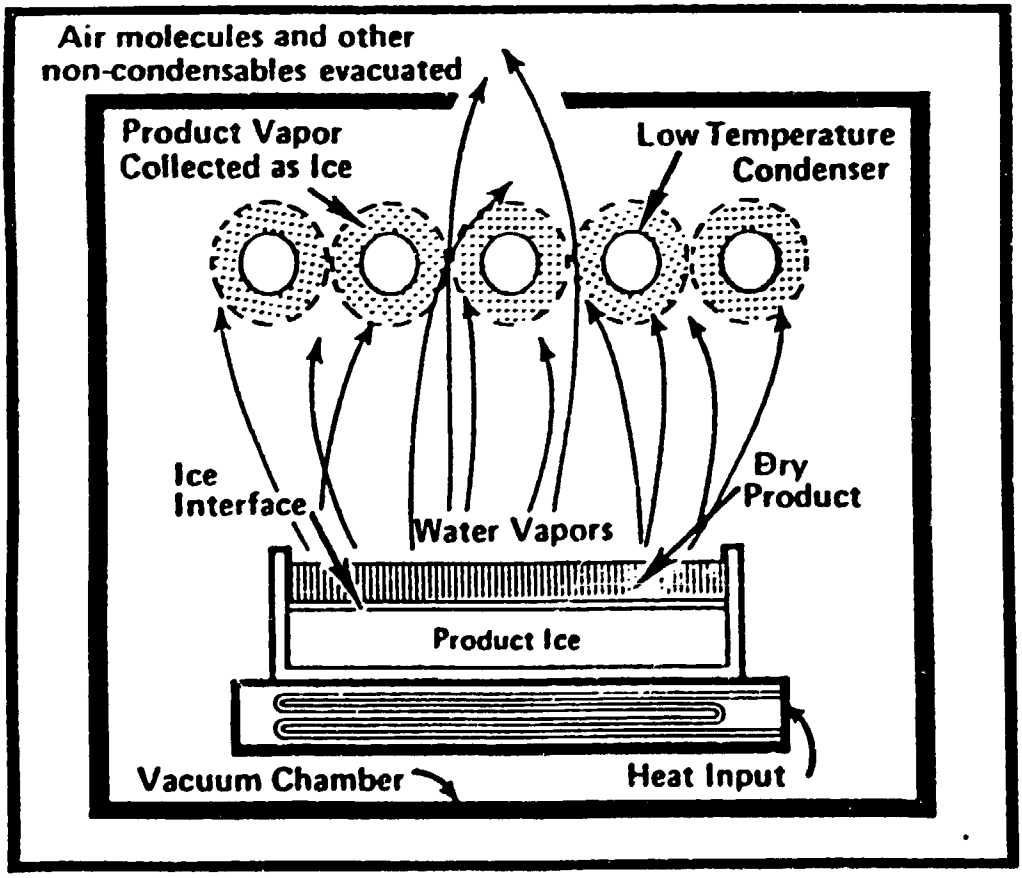
The freeze drying technique itself is similar to ordinary vacuum distillation, but with one very essential difference: the material to be dried must be solidly frozen before being subjected to a very low absolute pressure (high vacuum) and a controlled heat input. Under these conditions the water content (in the form of an ice matrix) is selectively removed via sublimation, i.e. — ice transforms directly to vapor, by-passing the intermediary liquid phase. The key to understanding the excellent retention of the essential characteristics of a freeze dried material lies in the fact that the solid particles of the materials are locked into this matrix during the entire dehydration and cannot interact. Often the only requirement for long term storage of such a material is the ability of the container to prevent the re-entry of moisture into the sample.

The Freeze Drying System is a Kinetic System.

From beginning to end, a constantly changing state of unbalance must exist between the product ice and the system pressure/temperature conditions. The migration of water vapor from the product ice interface occurs only if this state of unbalance exists and the product ice is at a higher energy level than the rest of the system. Freeze drying equipment is designed to present an isolated set of controlled conditions effecting and maintaining the optimum temperature pressure differences for a given product and thereby drying the product in the least amount of time. The limit of unbalance is determined by the maximum amount of heat which can be applied to the product without causing a change from the solid to the liquid state, or "melt back". This may occur even though the chamber pressure is low since the product dries from the surface closest to the area of lowest pressure. This surface is called the ice interface. The arrangement of the dry, solid particles above this interface offers resistance to the vapors released from below raising the product pressure/temperature. To avoid "melt back", heat energy applied to the product must not exceed the rate at which water vapor leaves the product. Another limit is the rate at which heat energy applied to the product ice (and carried away by the

migrating vapors) is removed by the condenser refreigeration system. Only by maintaining a low condenser temperature can one hope to trap the vapors as ice particles and effectively remove them from the system, greatly reducing and simplifying the vacuum pumping requirement. Air, other non-condensable molecules within the chamber, as well as mechanical restrictions located between the product ice and the condenser, will offer additional resistance to the movement of vapors migrating toward the condenser.

Four Conditions are Essential for Freeze Drying.
Information on the application of this kinetic principle for a variety of materials tells us that although each product may demand different handling techniques, there are four conditions which are essential to practical freeze drying technique. It is also necessary that these conditions are met in the following order: (1) The product must be solidly frozen below its eutectic point. (2) A condensing surface must be provided lower than -40° C. (3) The system must be capable of evacuation to an absolute pressure of between 5 and 25 microns of Hg. (4) A source of heat input to the product, controlled between -40° C and +65° C, must be employed to drive the water from the solid to the vapor state (Heat of Sublimation).



The physical arrangement of equipment designed to satisfy these conditions varies widely from small laboratory bench set ups with a

drying capacity of one liter or less to the full scale production equipment necessary for pharmaceutical or food freeze drying operations.

TEMPERATURE Vs VAPOR PRESSURE

°C	MM (Hg)	
0	4.579	
−2	3.880	
−4	3.280	
−6	2.765	
−8	2.326	Typical
−10	1.950	Product
−12	1.632	Temperatures
−14	1.361	
−16	1.132	
−18	0.939	
−20	0.776	
−22	0.640	
−24	0.526	
−26	0.430	
−28	0.351	
−30	0.2859	
−32	0.2318	
−34	0.1873	
−36	0.1507	
−40	0.0966	
−44	0.0609	
−48	0.0378	Typical
−50	0.0269	Condenser
−52	0.0230	Temperatures
−56	0.01380	
−60	0.00808	
−64	0.00464	
−68	0.00261	
−70	0.00194	
−72	0.00143	
−76	0.00077	
−80	0.00040	
−84	0.00020	
−88	0.00010	
−92	0.000048	
−96	0.000022	
−98	0.000015	

Table 1 Vapor Pressure/Temperature Chart

(1) Product Pre-freezing.

The basic reason for pre-freezing a product is to lock its solid particles firmly into position, so that moisture can be sublimed, and physical and chemical reactions cannot take place. Samples resulting from the combination of several organic or inorganic compounds in the liquid state generally exhibit more than one eutectic as freezing occurs. Each product considered for freeze drying should, therefore, be examined for its lowest eutectic point, that is, the minimum freezing point for the total product. If the lowest eutectic point is not determined, the product may appear to be frozen, when in fact a small percentage may yet be in the liquid state. This small volume of liquid will cause the solids in suspension to react, spoiling the results of the dehydration. On the other hand freezing the product too far below its lowest eutectic is unnecessary

and poor practice economically. Efficient rates of sublimation are obtained by the maintenance of a vapor pressure differential between product ice and condenser ice: Table 1 shows that the pressure in mm of Hg for ice collecting on a condenser with a temperature of -40° C is .096 mm (96 microns). If a sample with its lowest eutectic at -4° C is frozen to a temperature of -30° C, the initial vapor pressure of the sample ice would be .286 mm Hg (286 microns). The resulting vapor pressure differential would be 286 less 96 or 190 microns. If this same product were frozen to -10° C, the differential pressure would be 1853 microns, greatly increasing the initial sublimation rate.

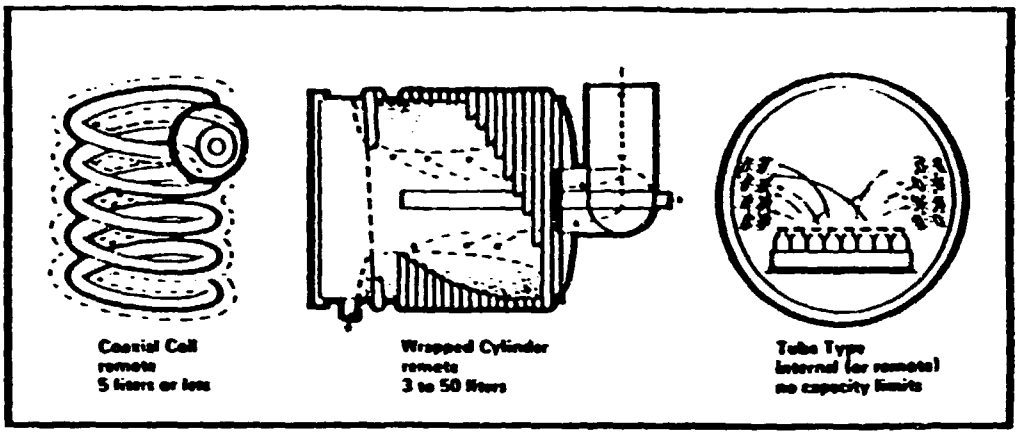
(2) The Condenser.

It is important that the condenser be placed in the direct path of migrating vapors. As vapor molecules leave the product ice, they migrate toward the low pressure areas in the system; first the area just above the product ice, then to the localized low pressure area surrounding the condenser. On contacting the condenser, migrating vapors give up their heat energy, turn to ice, and are effectively removed from the system. Air and other non-condensable molecules emitted from the product pass the condenser and are evacuated by the vacuum pump.

When selecting equipment, consider the work to be done and the range of eutectic points likely to be encountered. Most products can be dried efficiently with condenser temperatures between -40° C and -60° C.

Looking again at Table 1, you can see that vapor pressure drops rapidly with decreasing temperatures below 0° C, but very small changes in pressure result after -60° C. Unless working regularly with extremely low product eutectics, it is unnecessary in terms of equipment complexity, cost and energy expenditure to specify refrigeration systems providing condensing temperatures far in excess of the -60° C range, since neither drying speed nor final dryness will be materially influenced.

There are, however, certain solvents and acids that cannot be trapped at normal condenser temperatures. If these are encountered in large quantities a cascaded refrigeration system providing temperatures in the range of -90° C may be necessary. If the amounts are small, a secondary trap (condenser) can be placed in the vacuum line. Liquid nitrogen secondary traps provide condenser temperatures to -195° C, dry ice to -78° C. These small secondary traps do their trapping during the initial portion of the run. They can then be valved out of the system as the main condenser collects the remaining ice.



Equally important as low temperature is the way the condenser is designed, its mechanical configuration, what it's made of, its surface area, and how it is placed in the system.

Condenser designs fall into two categories, internal or remote, both offering special advantages to the particular application. Two variations of remote condensers and one variation of an internal condenser are shown in the illustration. Remote condensers are housed in a vacuum chamber separate from the product chamber, and may be isolated from the product by means of a high vacuum valve. The condenser can thus be defrosted while the product chamber is re-loaded and the next product batch frozen. Internal condensers are housed in the product chamber. They eliminate the cost of a second vacuum chamber and provide the least possible mechanical restriction (pressure drop) between product and condenser. Therefore, they are unmatched for efficiency and cost effectiveness.

Small research dryers can use inexpensive and efficient coaxial coil condensers. Refrigerant runs through a capillary tube in the center, then returns inside the outer wall of the coil. These are convenient in units under 5 liters and may be quickly defrosted using warm water or by allowing a natural room temperature defrost.

The wrapped cylinder becomes more efficient for mid-size dryers. The smooth sided cylinder is easily cleaned, and allows the ice "plug" to slide out as soon as it breaks free using a hot gas defrost cycle.

The tube type condenser offers outstanding design flexibility and presents a 360° cold surface along its entire length. It can be placed easily and efficiently in either a remote or internal chamber to provide generous condensing ability, and has excellent characteristics and flexibility with regard to internal refrigerant flow.

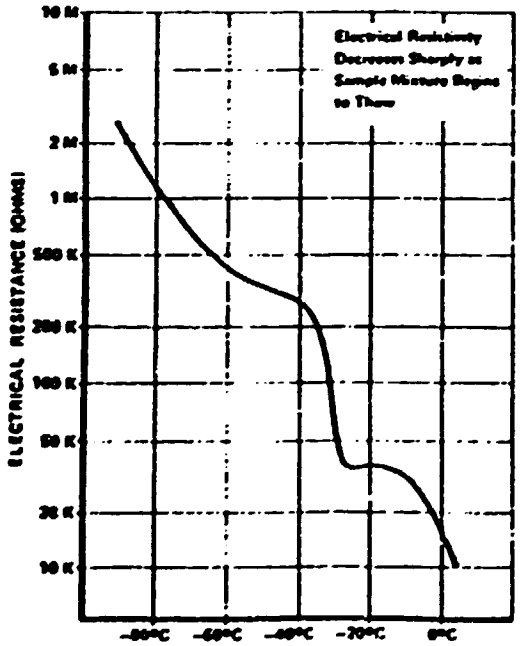
Whether remote or internal, condensers should be constructed of stainless steel, since acids trapped during drying are concentrated

and extremely corrosive. Plastic coatings have been tried but they have been found to soften, chip, or peel away. Stainless is hard, corrosion resistant, can be sterilized with gas, washed with steam, and even scrubbed down with stainless steel wool if necessary. For the same reasons, stainless is preferred for drying chambers and shelves, burnished smooth to discourage lodging of particulate matter.

(3) High Vacuum.

The purpose of the vacuum system is to evacuate non-condensable gasses from the chamber, creating the vacuum necessary for efficient sublimation. This effectively reduces the resistance to the flow of water vapors migrating from product to the condenser. The absence of air in the system also prevents oxidation during drying. The pump should be capable of dropping the pressure within an unloaded drying chamber from atmosphere to 25 microns or less. Ultimate vacuum on an unloaded system should be at least 5 microns.

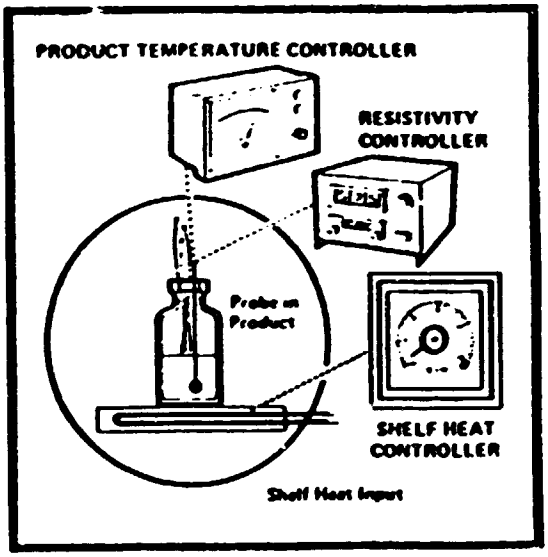
When on-the-shelf pre-freezing is provided in the dryer, a pump capable of evacuating the system to a low pressure of 5 to 50 microns within 15 to 30 minutes is sufficient, since the product cannot thaw on the cold shelves. Considerably larger pumping capacities will be necessary if no cooling is provided. In this case, pump-down time should not exceed 5 minutes. Substantial economies are realized with smaller vacuum pumps used in conjunction with dryers equipped with refrigerated shelves.



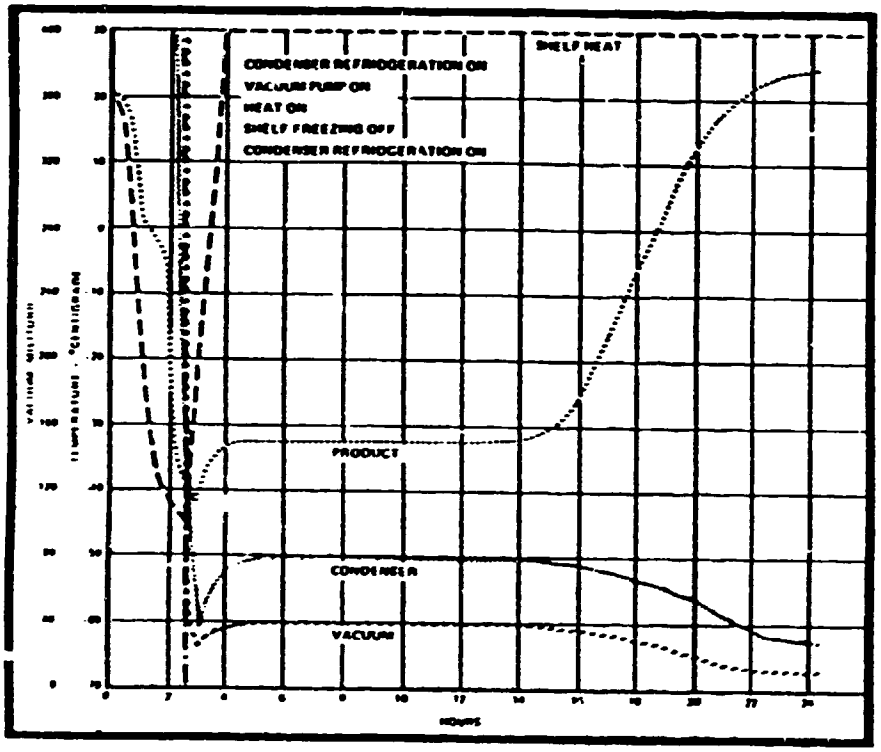
A TYPICAL RESISTANCE/TEMPERATURE FUNCTION

(4) Controlled Heat to Product.
Heat is applied to the product ice to initiate and accelerate the migration of water vapors from the product toward the condenser. The application of heat to the product supplies the necessary energy to drive off these vapors raising the temperature of the product (an amount corresponding to the increase in vapor pressure at the ice interface). In the beginning of the cycle, the total volume of the product is frozen and will accept a high heat setting. Later when a portion of the product is dry and offering resistance to the vapors released from below, a lower heat setting is desirable to avoid "melt back". In the final portion of the drying cycle, a higher setting may again be desired to drive off any remaining bound molecules. Therefore, it is necessary that heat supplied to the product be controlled. For very sensitive materials, a system that can alternately apply both heat and refrigeration may be required. In order to dry a wide variety of products, the range of control should be between -40° C and +65° C.

Since the most important variable during the freeze drying process is product temperature, it should be continuously indicated, recorded, and directly or indirectly controlled: (1) Direct control — sensing product temperature and adding heat below the control point and refrigerating above the control point — has limitations due to the nature of real heat transfer systems where the thermal lag or overshoot can cause excessive cycling. A single point is sensed throughout the entire product. (2) Indirect control — shelf temperature sensing and control utilizing time temperature programs to produce the desired product temperature — is the most practical control procedure. While the method presupposes experience with the product, it is an averaging rather than single point approach. The high degree of run-to-run uniformity obtained is paramount to the production unit.



Resistivity recording and control, another form of indirect control, provides complete information on product eutectic temperatures. Based on that information, heat is supplied above a safe resistivity set point (and refrigeration provided below the set point) to maintain settings just as with direct product temperature control. While the disadvantages of discreet point-sensing and thermal overshoot are present, the information gained can be of great value in determining optimum drying programs.



TYPICAL DRYING CYCLE

The curves plotted show a typical sublimator drying cycle. In this case milk was chosen as a representative product. Values for vacuum, shelf temperature, product temperature, and condenser temperature are plotted as they reflect equipment performance. The first part of the curve (up to hour 2½) indicated product and shelf temperature as the product load was frozen on the drying chamber shelves. Actual freeze drying was not initiated until the product temperature had reached -40° C.

After the drying cycle began and the shelf heating system reached its control setting of +30° C, the refrigerated condenser stabilized at a temperature at which it could absorb the thermal loading of the condensing water vapor. Correspondingly, system vacuum also rose slightly and stabilized. The straight line nature of product, condenser and vacuum recordings (hours 4 through 14) indicates a product with relatively unrestricted vapor passage above the ice interface as the interface recedes.

As the product finished drying (hours 14 through 24) the product ice sublimated and the product began to experience sensible heat gain due to continued shelf heat input. Since the condenser was condensing a decreasing amount of water vapor it began to drop in temperature seeking a new thermal balance. The system vacuum followed as the refrigerated vapor condenser "pumped" to lower pressure. Primary drying of the product was indicated as the product temperature paralleled shelf temperature (hours 23 to 24).

With many products a secondary phase of drying would now begin, lasting usually from 2 to 24 hours, to remove additional bound water molecules from the product. The extent to which secondary drying is necessary varies widely due to individual product properties and particular end point moisture requirements.

REFERENCES

Freeze Drying and Advanced Food Technology. Edited by S.A. Goldbith, L. Reynold, W.W. Rothmayr. Academic Press, 1975.

Advances in Freeze-drying. Edited by L. Rey. 1966 Hermann. 115 Boulevard Saint-Germain, Paris VI.

Freeze Drying of Foods. C Judson King. CRC Press, 1971.

Biological Applications of Freezing and Drying. Edited by R.J.C Harris. Academic Press, 1954.

ANNEX 13: EQUIPMENT QUOTATIONS AND SPECIFICATIONS

applikon®
DEPENDABLE INSTRUMENTS

FAX MESSAGE FROM APPLIKON, INC.

TO: Fermentagen FAX #:403-430-0106

ATTN: Dr. F. Merchant

FROM: Fritz Kleppinger

DATE: March 13, 1995 PAGES:1

PLEASE REPLY TO OUR FAX # (415) 578-8836 OR CALL US AT (415) 578-1396 IF YOU SHOULD REQUIRE ANY ASSISTANCE.

FAX MESSAGE # F-5F0024

SUBJECT: 2 Liter Fermentor System

Dear Dr. Merchant,

Below are ball park prices for a 2L fermentor, as specified. The system includes all components necessary for operation.

- ✓ 2 Liter fermentor, basic unit - $ 6,000
- ✓ AF, pH, DO sensors and cables (Ingold) - $ 1850
- ✓ 3 pumps, Rotameter w. solenoid valve - $ 1350

- ✓ AF, pH & DO controllers (B&C) - $ 2000
- ✗ Biocontroller 1030 (AF, pH, DO) - $ 8750

We will give you a 10% discount if you decide to purchase a system.
Please give me a call if you have further questions.

Sincerely,

F. Kleppinger

EDWARDS

Edwards High Vacuum
A division of BOC Canada Limited
3375 North Service Rd, Units B2,B3
Burlington, Ont. L7N 3G2
Telephone : 905-336-9119
1-800-387-4075
Facsimile : 905-845-4824

QUOTATION No. 4862-7 **DATE:** 21-Mar-95

erchant
stagen
nton, Alberta
G1

YOUR REF:

PHONE NO: 403-436-0574
FAX NO: 403-430-0106

Qty.	Description	Amount	Total
	We are pleased to provide you with the following quotation:		
1	Edwards Micromodulyo Freeze Dryer Features • High Capacity-1.5kg • Small Footprint • Ultimate low temperature of -50° C • Full function protection via ready/overload indicator • High quality standards to meet all European and U.S. Standards • Ontario Hydro electrical approval Operation at 220-240V/1 phase/60Hz C/W vacuum pump connector kit Part Number F105-04-000	$2,963.00	$2,963.00
1	Edwards Model E2M2, direct drive, rotary vane, two-stage vacuum pump. Displacement 2 cfm (57 l/m), Ultimate Vacuum (partial pressure) 10 -4 mbar. Features • Advanced oil lubrication - ensures reliable running even at high gas loads • Vacuum systems protected from oil and air suck-back • Easy-to-fit accessories protect the environment and the pump • Low noise levels and minimum vibration • Direct drive for compactness and excellent operator protection • Easy maintenance with convenient service kits and international customer support C/W NW25 inlet flange, NW25 centering ring and o'ring, (extra 15mm o.d. nozzle is supplied for inlet), 15mm o.d. exhaust nozzle, initial oil charge, and 1/3 h.p. motor for operation at 115/230v, 1ph, 50/60Hz. Part Number A360-01-981	$1,845.00	$1,845.00
1	Spin Freezer 96 that Utilizes the evaporative technique for freezing material in ampoules. A low power motor with an independant power supply drives carrier plates which are interchangeable depending upon ampoule size. The vacuum chamber is made from transparent acrylic material. A programmable timer prevents spinner overrun after freezing. Motor: 110V,1ph,50/60Hz; Speed: 760 rev/min (approx) Ampoules/(ml capacity): 96 (0.5), 80 (1.0), 24 (2.5), 36 (4.0) 12 (5.0) Part Number F056-38-000	$2,935.00	$2,935.00

SUBJECT TO TERMS AND CONDITIONS

Terms: Cash - 30 Days
Sales Tax - Extra
Freight - F.O.B. Burlington

Brian Duncan R.T.- Inside Technical Sales

EDWARDS

Edwards High Vacuum
A division of BOC Canada Limited
3375 North Service Rd, Units B2,B3
Burlington, Ont. L7N 3G2
Telephone : 905-336-9119
1-800-387-4076
Facsimile : 905-846-4824

QUOTATION No. 4862-7 DATE: 21-Mar-95

[illegible]
[illegible]
[illegible], Alberta
G1

YOUR REF:

PHONE NO: 403-436-0574
FAX NO: 403-4[illegible]-0106

Qty.	Description	Amount	Total
1	Secondary Drying Manifold. A 48 ampoule capacity for secondary drying and sealing of ampoules normally pre-frozen and dried in the spin freezer (F056-37-000). Two dessicant trays are also supplied. Part Number F029-64-000	$731.00	$731.00
1	Ampoules, 2.5ml. (500/lot) Part Number H014-30-092	$1,071.00	$1,071.00
1	Ampoules, 5.0ml (200/lot) Part Number H-014-30-083	$592.00	$592.00
	Optional Accessories		
1	EMF10 Oil Mist Filter for trapping exhaust vapours from rotary vane vacuum pump. C/W oil filter element, odour filter element, 3/4 inch BSP to NW25 adapter, NW25 centering ring with o'ring and NW25 clamp. Part Number A462-26-000	$307.00	$307.00
	We trust this is the information you require. If we can be of any further assistance please do not hesitate to contact our office.		
	Co. Ed Kole Technical Sales-Western Region		
	Please Note: Delivery, 2-3 weeks, subject to confirmation.		

SUBJECT TO TERMS AND CONDITIONS

Terms: Cash - 30 Days
Sales Tax - Extra
Freight - F.O.B. Burlington

Brian Duncan R.T.- Inside Technical Sales

ANNEX 14: BIBLIOGRAPHY, LITERATURE SEARCH AND RESEARCH ARTICLES

A COMPILATION OF RELEVANT REFERENCES IN THE AREA OF STRAIN SELECTION, BIOREACTOR DESIGN AND PROCESS DEVELOPMENT FOR BIODEGRADATION OF PHENOLS, CHLOROPHENOLS AND OTHER PERSISTENT ORGANIC POLLUTANTS

Chemical Engineering Abstract

Concept 1:BIODEGRAD? AND CHLOROPHENOL?

Regulation of chloro- and methylphenol degradation in Comamonas testosteroni JH5.
AUTHOR: Hopp, J.; Hollender, J.; Dott, W.
CORPORATE SOURCE: Tech. Univ. BerlinFachgebiet Hygiene13353 BerlinGermany
Applied and Environmental Microbiology, Volume: 60, Issue: 7, Page(s): 2330-2338
ABSTRACT: C. testosteroni JH5 was isolated from a mixed bacterial culture enriched on different chloro- and methylphenols. The strain completely mineralized a mixture consisting of 4-chlorophenol (4-CP) and 4-methylphenol (4-MP). Mineralization of 4-CP and that of 4-MP occurred successively and were accompanied by diauxic growth, whereas 4-CP and 2-methylphenol were mineralized simultaneously. Neither a reversible enzyme inhibition nor potential toxic intermediates caused the observed diauxie. It is suggested that the successive degradation of 4-CP and 4-MP was regulated at the level of transcription. C. testosteroni JH5 contained a meta-cleaving enzyme when pregrown on 4-CP and the isomeric monomethylphenols. Inactivation of this enzyme in the presence of 3-chlorocatechol was observed.

Isolation and characterization of a pentachlorophenol- degrading bacterium.
AUTHOR: Gestel, Y. P. C. M. van; Breure, A. M.; Andel, J. G. van
CORPORATE SOURCE: Natl. Inst. Public Health Environ. ProtectionLab. Waste Materials Emissions3720 BA BilthovenNetherlands
BIOTECHNOLOGY '94: ICHEME SYMPOSIUM, , Page(s): 119-121
ABSTRACT: A versatile chlorophenol-degrading strain was isolated from aerobic enrichment cultures degrading pentachlorophenol (PCP). The isolated, strain P5, was tentatively placed in the genus Flavobacterium and was capable of degrading a broad range of polychlorinated phenols: PCP, all isomers of tetrachlorophenol, 5 isomers of trichlorophenol, and some dichlorophenols. Some of these chlorophenols can be utilized as source of carbon and energy. Strain P5 was able to mineralize PCP up to concentrations of 1 mM. [Conference: Biotechnology '94: Second International Symposium on Environmental Biotechnology (ISEB2), Brighton, UK, 4-6 Jul 1994].

Role of mycelium and extracellular protein in the biodegradation of 2,4,6-trichlorophenol by Phanerochaete chrysosporium.
AUTHOR: Armentante, P. M.; Pal, N.; Lewandowski, G.
CORPORATE SOURCE: New Jersey Inst. Technol.Dep. Chem. Eng. Chem. Environ. Sci. Newark, NJ 07102-1982USA
Applied and Environmental Microbiology,Volume: 60, Issue: 6,: pp: 1711-1718
ABSTRACT: In experiments with P. chrysosporium mycelial suspension,the degradation of 2,4,6-trichlorophenol (2,4,6-TCP) occurred in the absence of ligninase. Chloride ion was recovered in nearly stochiometric amounts at the end of the process. The microorganism did not retain its degradation ability for more than 6 days under substrate-deficient conditions. Neither the mycelium nor the extracellular protein alone could degrade 2,4,6-TCP; both were required for complete degradation to occur. In experiments in which 2,4,6-TCP was exposed to the culture supernatant separated from its mycelium, negligible degradation was obtained and no chloride ion recovered. In experiments performed with washed mycelium separated from its supernatant, no degradation took place until the mycelium release additional extracellular protein 5 to 6 h into the incubation. Additions of washed mycelium to active cultures produced an increase in the rate of degradation in correspondence with the protein release. Additions of culture supernatants containing a high concentration of extracellular protein to active cultures produced an increase in the rate of 2,4,6-degradation.

Immobilization of Phanerochaete chrysosporium on porous polyurethane particles with application to biodegradation of 2-chlorophenol.
AUTHOR: Wang, X.; Ruckenstein, E. State Univ. New YorkBuffalo NYUSA
BIOTECHNOLOGY TECHNIQUES, Volume: 8, Issue: 5, Page(s): 339-344
ABSTRACT: The authors describe the immobilization of the white rot fungus Phanerochaete chrysosporium on porous polyurethane carriers. The immobilized organism showed high lignin peroxidase activity and enhanced 2-chlorophenol degradation, compared to free pellets or fungus immobilized on other supports.

Production of lignin peroxidase by Phanerochaete chrysosporium immobilized on porous poly(styrene-divinylbenzene) carrier, and its application to the degrading of 2-chlorophenol.
AUTHOR: Ruckenstein, E.; Wang, X.-B., State Univ. of New YorkBuffalo NYUSA
BIOTECHNOLOGY AND BIOENGINEERING, Volume: 44, Issue: 1, Page(s):79-86
ABSTRACT: The white rot fungus Phanerochaete chrysosporum was immobilized on a new carrier, porous poly(styrene-divinylbenzene), prepared by the concentrated emulsion polymerization method. High lignin peroxidase activity was obtained in both batch and repeated batch shake cultures. Better results were obtained with spore inoculation for immobilization than with small mycellial pellets.When used as a biocatalyst for the degradation of 2-chlorophenol, the activity of immobilized spores was higher than of free and immobilized pellets.

Adsorption and biodegradation of pentachlorophenol by polyurethane-immobilized Flavobacterium.
AUTHOR: Hu, Z.-C.; Korus, R.A.; Levinson, W.E.; Crawford, R.L., Idaho Univ.Moscow IDUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY, Volume: 28, Issue: 3, Page(s):491-496
ABSTRACT: Actively growing Flavobacterium sp. was important in avoiding lag times, and pentachlorophenol (PCP) removal was enhanced by the presence of utilizable compounds such as glucose and sodium glutamate. Adsorption of PCP on the polyurethane foam (PUF) matrix was a simple procedure, and effectively removes PCP from contaminated water and reduces PCP toxicity. The dynamic bed reactor was shown to be appropriate for this process.

Pentachlorophenol degradation by Pseudomonas aeruginosa.
AUTHOR: Rajakumar, S. S.; Premalatha, A.
CORPORATE SOURCE: Central Leather Res. Inst.Bacteriology Lab.Madras-600 020 India
WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY,Vol:10,(3), pp334-337
ABSTRACT: Five Pseudomonas species were tested for ability to degrade pentachlorophenol (PCP). P. aeruginosa completely degraded PCP up to 800 mg/L in 6 d with glucose as cosubstrate. With 1000 mg PCP/l, 53% was degraded. Ammonium salts were better at enhancing degradation than organic nitrogen sources and shake-cultures promoted PCP degradation compared with surface cultures. Degradation was maximal at pH 7.6-8.0 and at 30-37.degree.C. Only PCP induced enzymes that degraded PCP and chloramphenicol inhibited this process.

Solid-phase treatment of a pentachlorophenol-contaminated soil using lignin-degrading fungi.
AUTHOR: Lamar, R.T.; Evans, J.W.; Glaser, J.A.
CORPORATE SOURCE: US Dep. of AgricultureMadison WIUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY,Volume:27,Issue:12, pp:2566-2571
ABSTRACT: Starting with a soil containing 672 microg of PCP/g of soil, and 4017 microg of creosote, an inoculum of 10 per cent of Phanerochaete sordida resulted in an 89 per cent decrease in PCP over 8 weeks. The PCP decreases with the other two lignin-degrading fungi used were 67-72 percent with P.chrysosporium, and 55 per cent with Trametes hirsuta. (To be continued).

A rapid and simple screening technique for potential crude oil degrading microorganisms.
AUTHOR: Hanson, K.G.; Desai, J.D.; Desai, A.J. M. S. Univ. of BarodaBarodaIndia
BIOTECHNOLOGY TECHNIQUES, Volume: 7, Issue: 10, Page(s): 745-748
ABSTRACT: A technique for semi-quantitative screening of organisms for crude oil degrading capability is decribed. The method uses the redox indicator 2,6-dichlorophenol indophenol in Bushnell and Haas medium with crude oil in a microtitre plate. The medium is turned colourless within 24 hours by bacteria possessing crude oil degrading capability and the relative capabilities of different cultures can be assessed according to the time taken for decolourisation.

Activation of an indigenous microbial consortium for bioaugmentation of pentachlorophenol/creosote contaminated soils.
Otte, M.-P.; Gagnon, J.; Comeau, Y.; Matte, N.; Greer, C.; Samson, R., NRC, CanadaMontreal/PQCanada
Applied Microbiology and Biotechnology, Volume: 40, Issue: 6, Page(s): 926-932
ABSTRACT: The paper describes the isolation from contaminated soil and characterization of a microbial consortium able to degrade pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAH). The presence of soil as a support or source of nutrients was essential in order to obtain an active consortium in a fed-batch bioreactor. The consortium tolerated a PCP concentration of 400 mg/l in batch experiments. Over a period of 35 days, production of a PCP-degrading consortium in a fed-batch slurry bioreactor enhanced the activity of PCP biodegradation by a factor often. Over the same period PAH biodegradation increased by a factor of 30 for phenanthrene and 81 for pyrene. Results indicate that the activated soil process could be useful for restoring contaminated soils.

Growth and enrichment of pentachlorophenol-degrading microorganisms in the nutristat, a substrate concentration- controlled continuous culture.
Rutgers, M.; Bogte, J. J.; Breure, A. M.; Andel, J. G. van Natl. Inst. Public Health Environ. ProtectionLab. Waste Materials Emissions3720 BA BilthovenNetherlands
Applied and Environmental Microbiology, Volume: 59, Issue: 10, Page(s): 3373-3377
ABSTRACT: The nutristat, a substrate concentration-controlled continuous culture, was used to grow pentachlorophenol (PCP)-degrading microorganisms. With PCP concentrations between 45 and 77 .mu.M, a stable situation was established in the nutristat, with an average dilution rate of 0.035 .+-. 0.003/h. Growth rates were higher than in fed-batch or chemostat cultures, and PCP accumulation to inhibitory levels in the culture was prevented.

Strategy using bioreactors and specially selected microorganisms for bioremediation of groundwater contaminated with creosote and pentachlorophenol.
Mueller, J.G.; Lantz, S.E.; Ross, D.; Colvin, R.J.; Middaugh, D.P.; Pritchard, P.H.,SBP TechnologiesGulf Breeze FLUSA U.S. EPA Gulf Breeze FL USA
ENVIRONMENTAL SCIENCE & TECHNOLOGY, Volume: 27, Issue: 4, Page(s):691-698
ABSTRACT: Using two-stage cotinuous flow and batch mode sequential treatments,bench-scale and pilot-scale studies were conducted with groundwater from an abandoned wood-preservative factory. Performance was monitored for 30-40 creosote constituents in influent and effluent as was the toxicity.Pilot scale operation reduced the creosote constituents by more than 99 per cent but the overall efficiency of the system appeared less than other treatment systems.However the study points out the importance of the abiotic factors of partitioning to biomass and physical adsorption in accounting for contaminant removal

Fungal degradation of pentachlorophenol by micromycetes.
Seigle-Murandi, F.; Steiman, R.; Benoit-Guyod, J.-L.; Guiraud, P., Univ. J. Fourier Groupe l'Etude Devenir Xenobiotiques l'Environ. (GEDEXE)38243 MeylanFrance
Journal of Biotechnology, Volume: 30, Issue: 1, Page(s): 27-35
ABSTRACT: A wide range of fungi was screened for their activities towards pentachlorophenol, with focus on the possible role of phenoloxidases.

Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete Phanerochaete chrysosporium.
AUTHOR: Joshi, D. K.; Gold, M. H.
CORPORATE SOURCE: Oregon Graduate Inst. Sci. Technol.Dep. Chem. Biol. Sci. Beaverton, OR 97006-1999USA
Applied and Environmental Microbiology, Volume: 59, Issue: 6, Page(s): 1779-1785
ABSTRACT: Under secondary metabolic conditions the white rot Phanerochaete chrysosporium rapidly mineralizes 2,4, 5-trichlorophenol. The pathway for degradation of 2,4, 5-trichlorophenol was elucidated by the characterization of fungal metabolites and oxidation products generated by purified lignin peroxidase (LiP) and manganese peroxidase (MnP). The multistep pathway involves cycles of peroxidase-catalyzed oxidative dechlorination reactions followed by quinone reduction reactions to yield the key intermediate 1,2,4,5-tetrahydroxybenzene, which is presumably ring cleaved. The quinone intermediate is recycled by a reduction reaction to regenerate an intermediate which is again a substrate for peroxidase-catalyzed oxidative dechlorination. This pathway apparently results in the removal of all three chlorine atoms before ring cleavage occurs.

Chlorophenol toxicity removal and monitoring in aerobic treatment:recovery from process upsets.
AUTHOR: Makinen, P.M.; Theno, T.J.; Ferguson, J.F.; Ongerth, J.E.; Puhakka,J.A.
CORPORATE SOURCE: Univ. of WashingtonSeattle, WAUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY, Volume:27,Issue:7,Page(s):1434-1439
ABSTRACT: A laboratory study of the bioremediation of simulated groundwater by an enriched biomass in a fluidized-bed reactor using pure oxygen.Steady state operation at a chlorophenyl loading rate of 445 mg per litre per day,with a hydraulic retention time of 5 hours achieved chlorophenol removal at over 99.7 per cent. Interrupting the oxygen supplies for 1 or 2 days gave rise to recovery times of 10-40 days and the Microtox assay employed (luminescent bacteria) was deemed a reliable, rapid and sensitive toxicity indicator.

Use of a sequencing batch reactor to study the biodegradation of 4-chlorophenol in soil.
AUTHOR: Kiilerich, O.; Buitron, G.; Capdeville, B.
CORPORATE SOURCE: Institut National des Sciences AppliqueesToulouseFrance
BIOTECHNOLOGY TECHNIQUES, Volume: 7, Issue: 2, Page(s): 149-154
ABSTRACT: A sequencing batch reactor was used to study the biodegradation of 4-chlorophenol (4CP) added to a soil slurry. The carbon dioxide evolution rate (CER) in the gas phase was a reliable indicator of 4CP degradation, the CER reaching a maximum when the 4CP concentration was zero. The biodegradation rate was increased from 3.2 to 67 mg. 4CP/l.h after 13 cycles. This method is applicable to the study of other xenobiotics in soil.

Performance of anaerobic granules for degradation of pentachlorophenol.
AUTHOR: Wu, Wei-Min; Bhatnagar, L.; Zeikus, J. G.
CORPORATE SOURCE: Michigan Biotechnol. Inst.Lansing, MI 48909USA
Applied and Environmental Microbiology, Volume: 59, Issue: 2, Page(s): 389-397
ABSTRACT: Anaerobic granules degrading pentachlorophenol (PCP) with specific PCP removal activity up to 14.6 mg/g of volatile suspended solids per d were developed in a laboratory-scale anaerobic upflow sludge blanket. The reactor was able to treat synthetic wastewater containing 40-60 mg of PCP/L at a volumetric loading rate of up to 90 mg/L of reactor volume per d, with a hydraulic retention time of 10.8-15 h. PCP removal of more than 99% was achieved. Results of adsorption of PCP by granular biomass indicated that the PCP removal by the granules was due to biodegradation rather than adsorption. The PCP-degrading granules also exhibited a higher tolerance to the inhibition caused by PCP for methane production and degradation of acetate, propionate, and butyrate, compared with anaerobic granules unadapted to PCP.

Degradation of 2,4,6-TCP and a mixture of isomeric chlorophenols by immobilized Streptomyces rochei 303.
Golovleva, L.A.; Zaborina, O.E.; Arinbasarova, A.Y. Russian Acad of SciencesPushchinoRussia
Applied Microbiology and Biotechnology, Volume: 38, Issue: 6, Page(s): 815-819
ABSTRACT: The degradation of 2,4,6-trichlorophenol (2,4,6-TCP) and other chlorophenols (CPs) by immobilized cells of Streptomyces rochei 303 was investigated. Among carriers tested, polycaproamide (PCA) fibre was found to be optimal for cell immobilization. During continuous fermentation in a column reactor with constant flow of the substrate solution, S. rochei cells immobilized on PCA fibres could degrade high concentrations of individual mono- to pentachlorophenols (CPs), including the most toxic and persistent derivatives, and their mixtures. At 2,4,6-TCP-concentrations of 1 g/l, the system was stable for 40 days, and at lower toxicant concentration the system could be operated for 11 months without any loss of activity. S. rochei cells immobilized on PCA fibres may be used for the bioremediation of waste-waters containing CPs.

Biodegradation of chlorophenol mixtures by Pseudomonas putida.
AUTHOR: Dapaah, S.Y.; Hill, G.A.
CORPORATE SOURCE: University of SaskatchewanSaskatoonCanada
BIOTECHNOLOGY AND BIOENGINEERING, Volume: 40, Issue:11, Page(s):1353-1358
ABSTRACT: A new, simplified model of lag-phase behavior of Pseudomonas putida is developed which is based on empirical observations on the death and growth characteristics of this microorganism when growing on phenolics. The model involves continuous biomass death, inhibited lag-phase growth, and a switch to log-phase metabolism. It accurately predicts the dynamic behavior of these cultures right from inoculum until growth is fully completed.

Environmental factors influencing the biodegradation of pentachlorophenol in contaminated soils by inoculated Rhodococcus chlorophenolicus PCP-1.
AUTHOR: Briglia, M.; Middeldorp, P.J.M.; Salkinoja-Salonen, M.S.
CORPORATE SOURCE: Univ.HelsinkiFinland Agric. Univ. Wageningen Netherlands
PUBLISHER: DECHEMA, Frankfurt, D, Page(s): 109-115
Soil Decontamination Using Biological Processes., 6-9 December 1992, Karlsruhe, D
ABSTRACT: For laboratory testing forest soils are contaminated by 30 to 600 mg PCP and inoculated with immobilized R. chlorophenolicus. Mineralization rate depends on soil type and less on moisture content. A microbial density of 10(exp 8)/g soil is sufficient. R. chlorophenolicus proved effective with strong polluted soils.

The application of immobilized microorganisms in soil decontamination.
AUTHOR: Rehm, H.-J.
CORPORATE SOURCE: Univ.MuensterFed. Rep. Germany
PUBLISHER: DECHEMA, Frankfurt, D, Page(s): 69-78
Soil Decontamination Using Biological Processes., 6-9 December 1992,Karlsruhe, D
ABSTRACT: Microorganisms for soil decontamination are immobilized by adsorption to various supports or entrapment using preferably natural polymers. Biodegradation of 4-chlorophenol with Alcaligenes on clay, dichloroacetic acid with Xanthobacter autotrophicus on lecaton, and diesel fuel with a mixed culture on lava is investigated. The microorganisms proved effective to digest recalcitrants and xenobiotics and are resistant to the soil microflora.

Removal of chlorinated aromatics in presence of sophorose biosurfactants.
AUTHOR: Kosaric, N.; Lu, G. Univ. West. OntarioLondonCanada
PUBLISHER: DECHEMA, Frankfurt, D, Page(s): 44-51
Soil Decontamination Using Biological Processes., 6-9 December 1992, Karlsruhe, D
ABSTRACT: Sophorose lipids are applied to farm land soil to investigate the decomposition of the herbicide metolachlor and 2,4-dichlorophenol. A sharp decline in metolachlor is due to a metolachlor-sophorose-microbial complex. Chlorophenol degradation is enhanced.

Degradations of xenobiotics by fungi.
Fritsche, W. Univ.JenaFed. Rep. Germany
PUBLISHER: DECHEMA, Frankfurt, D, Page(s): 31-36
Soil Decontamination Using Biological Processes., 6-9 December 1992, Karlsruhe, D
ABSTRACT: Soil fungi and white rot fungi and their enzyme systems are investigated for oxidation processes of hydrocarbons and derivatives, PAH, pentachlorophenol, PCBs and TNT. Enzymes for lignin degradation and phytoalexin detoxification are active in decontamination. Aromatic compounds are incorporated in humus.

Photodegradation, biodegradation and chemical fixation of pentachlorophenol (PCP).
Shukla, S. S.; Nguyen, A.; Shukla, A. Lamar Univ., Chem Dep.PO Box 10058, Beaumont, TX 77710USA
JOURNAL OF HAZARDOUS MATERIALS, Volume: 28, Issue: 1-2, Page(s): 222
ABSTRACT: A number of methods were examined for the disposal and degradation of PCP and other toxic compounds. PCP is degraded photochemically in homogeneous solution to give a complex mixture of products, whereas in microheterogenous media degradation to carbon dioxide and water occurred. PCP was biodegraded to lower chlorinated phenols by naturally occurring bacteria. About 97% of PCP can be stabilized in a cement matrix.

Biodegradation of multiple substrates stripped from contaminated soils.
Ralph, A.; McCreary, E.; Autenrieth, R.; Bonner, J. Texas A&M Univ. Dep. of Civil Eng. College Station, TX77843-3136USA
JOURNAL OF HAZARDOUS MATERIALS, Volume: 28, Issue: 1-2, Page(s): 195-196
ABSTRACT: The kinetics were examined of multiple substrate feeds with acclimatized microorganisms in pulse-fed batch reactors and chemostats. The feasibility of air stripping of organic hazardous substances from the solid and introducing them to bioreactors through the air stream was also investigated. Synergistic and antagonistic interactions of multiple substrate degradation were studied to determine if they could be combined to optimize the degradation of selected compounds. Long-term studies on batch-fed reactors revealed preferential substrate degradation in the order glucose, phenol, pentachlorophenol (PCP). The presence of glucose improved the rate of phenol degradation. Phenol enhanced PCP degradation, but glucose and PCP were degraded separately.

Characterization of a novel Pseudomonas species that mineralizes high concentrations of pentachlorophenol.
AUTHOR: Radehaus, P. M.; Schmidt, S. K.
CORPORATE SOURCE: Univ. ColoradoDep. Environ.Boulder, CO 80309-0334USA
Applied and Environmental Microbiology, Volume: 58, Issue: 9, Page(s): 2879-2885
ABSTRACT: A pentachlorophenol (PCP)-mineralizing bacterium was isolated from polluted soil and identified as Pseudomonas species strain RA2. In batch cultures, Pseudomonas species strain RA2 used PCP as its sole source of carbon and energy and was capable of completely degrading this compound. Pseudomonas species strain RA2 was able to mineralize a higher concentration of PCP (160 mg/l) than any previously reported PCP-degrading pseudomonad. At a PCP concentration of 200 mg/l, cell growth was completely inhibited and PCP was not degraded, although an active population of Pseudomonas species RA2 was still present in these cultures after 2 weeks. The inhibitory effect of PCP was partially attributable to its effect on the growth rate of Pseudomonas species strain RA2. The highest specific growth rate was reached at a PCP concentration of 40 mg/l but decreased at higher or lower PCP concentrations, with the lowest .mu.m (0.05 h-1) occurring at 150 mg/l. Despite this reduction in growth rate, total biomass production was proportional to PCP concentration at all PCP concentrations degraded by Pseudomonas species RA2. In addition to its effect as an uncoupler of oxidative phosphorylation, PCP may also inhibit cell division in Pseudomonas species strain RA2. PCP and glucose were simultaneously mineralized by Pseudomonas species strain RA2, but glucose had no effect on the rate of PCP mineralization. PCP, on the other hand, significantly enhanced the metabolism of glucose by Pseudomonas species strain RA2.

Degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of Alcaligenes sp. A7-2.
Menke, B.; Rehm, H.-J. Univ. MuensterFed. Rep. Germany
Applied Microbiology and Biotechnology, Volume: 37, Issue: 5, Page(s): 655-661
ABSTRACT: The biodegradation of the monochlorophenol isomers 2- (2CP), 3- (3-CP) and 4-chlorophenol (4-CP) and phenol by Alcaligenes sp. A7-7 was studied. Experiments were conducted with free cells in batch culture and with cells immobilized by adsorption on lava granules in continuous culture. Both free and immobilized cells expressed similar regulation of chlorophenol and phenol degradation. In batch culture, 3-CP was only metabolized in combination with 2-CP or phenol. In substrate mixtures phenol accelerated the degradation of 2-CP in batch and continuous culture. Degradation of phenol was incomplete in batch culture, as indicated by catechol accumulation in the culture fluid. Biphasic growth of free cells was observed with the mixture of 2-CP and 4-CP. Immobilized cells built up a biofilm on the lava, thereby establishing a high cell load in the reactor.

Metabolism and kinetics of PCP transformation in anaerobic granular sludge.
Hendriksen, H.V.; Ahring, B.K. Tech. Univ.LyngbyDenmark
Applied Microbiology and Biotechnology, Volume: 37, Issue: 5, Page(s): 662-666
ABSTRACT: The metabolism and kinetics of pentachlorophenol (PCP) dechlorination was studied in a granular sludge originating from an glucose-amended upflow anaerobic sludge blanket reactor. Under methanogenic conditions, PCP was dechlorinated to lower chlorinated phenols. The initial dechlorination reaction and removal of the intermediate 3,5-dichlorophenol seemed to be rate-limiting. Nitrate was strongly inhibitory for the transformation of PCP, whereas addition of sulphate was inhibitory only in the presence of glucose. Among various carbon sources only glucose had a stimulating effect on the dechlorination rate. Addition of specific inhibitors to the consortium resulted in reduced transformtion rates. A low number of dechlorinating organisms was found in disintegrated granular sludge as compared to the number of glucose degraders and methanogens.

Biodegradation of phenol and chlorophenols by an immobilized mixed culture in soil
AUTHOR: Balfanz, J.; Rehm, H.-J.
PUBLISHER: DECHEMA, Frankfurt a.M., DVolume: 4, , Page(s): 319-324
World Congress of Chemical Engineering
ABSTRACT: Lecture given at Fourth World Congress of Chemical Engineering, Karlsruhe, D, 16.-21.6.1991. The degradation of many problematic chemical substances such as phenol and chlorinated phenols by microorganisms in water and soil is becoming increasingly important. It is possible to isolate specialized cultures whose ability for degradation of these substances is much better than that of microorganism present in the natural environment. The used materials and experimental methods were described. The results showed a different efficiency for two used microorganisms and for different phenols and chlorophenols. The results were discussed.

Biodegradation and -transformation of polychlorinated phenols in soil
AUTHOR: Middeldorp, P.; Briglia, M.; Kitunen, V.; Valo, R.; Salkinoja-Salonen, M.
CORPORATE SOURCE: Univ. HelsinkiFinland Alko Biotechn. RajamaekiFinland
PUBLISHER: DECHEMA, FrankfurtVolume: 9, , Page(s): 360-363
JOURNAL: DECHEMA-Fachgespraeche Umweltschutz
ABSTRACT: Lab experiments showed Rhodococcus chlorophenolicus immobilized on PUR foam to enhance mineralization of pentachlorphenol in soil up to 260 mg/kg in 4 month. R. chlorophenolicus counteracts the toxic pentachloroanisol formed by O-methylating bacteria. The threshold amount of inoculation cells depends on the type of soil and varies from 500 to 10(exp 5) cells g/soil.

Pentachlorophenol as carbon and energy source for aerobic enrichment cultures
AUTHOR: Rutgers, M.; Bogte, J.J.; Breure, A.M.; Andel, J.G.van
CORPORATE SOURCE: RIVMBilthovenNetherlands
PUBLISHER: DECHEMA, FrankfurtVolume: 9, , Page(s): 218-222
JOURNAL: DECHEMA-Fachgespraeche Umweltschutz
ABSTRACT: Dutch soil and suldge samples were inoculated with PCP degrading cultures. After 100 to 120 days the increase in Cl-ions indicated complete mineralization. Biomass and CO2 are main carbon compounds. Degradation is inhibited by a pulse of 10 microM PCP and ceases after 100 microM PCP. Tri- and tetrachlorophenols are biodegraded as well.

Biodegradation of phenolic wastes.
AUTHOR: Autenrieth, R. L.; Bonner, J. S.; Akgerman, A.; Okaygun, M.; McCreary,E. M.
CORPORATE SOURCE: Texas A&M Univ.Civil Eng. Dep.TX 77843-3136USA
JOURNAL OF HAZARDOUS MATERIALS , Volume: 28, Issue: 1-2, Page(s): 29-53
ABSTRACT: Phenolic biodegradation kinetics were determined in bioreactors with large solids retention times. Long term kinetic experiments were conducted in pulse-fed batch reactors for single substrate (phenol) and multiple substrates (combinations of glucose, phenol and pentachlorophenol). Short term initial rate experiments were conducted on the single and multiple substrate reactors. Results indicate that phenol is metabolized at a maximum rate of 0.55/h with a half saturation coefficient of 10 mg/L. Phenol concentrations in excess of 50 mg/L inhibit the biodegradation rate. Results also indicate that pentachlorophenol is cometabolized in the presence of phenol. Biodegradation of phenolic waste is a viable treatment option because the organisms, through their metabolic processes, reduce the waste concentrations below detection limits.

Effect of adsorbents on degradation of toxic organic compounds by coimmobilized systems
AUTHOR: Siapush, A.R.; Jian-Er Lin; Wang, H.Y., The University of MichiganAnn ArborUS.
BIOTECHNOLOGY AND BIOENGINEERING, Volume: 39, Issue: 6, Page(s):619-628
ABSTRACT: The effects of adsorbent content, solution pH, and surfactant concentration on adsorption and biodegradation pentachlorophenol (PCP) by Arthrobacter ATCC 33790 coimmobilized with powdered activated carbon within calcium alginate capsules are studied. Additionally, a mathematical model is derived to describe the diffusion, adsorption, and degradation of PCP in batch aqueous cultures. The results demonstrate that PCP biodegradation strongly depends on variations in adsorbent capacity and affinity.

Bacteria that degrade p-chlorophenol isolated from a continuous culture system.
AUTHOR: Kramer, C. M.; Kory, M. M., Univ. AkronDep. Biol.OH 44325-3908USA
Canadian Journal of Microbiology, Volume: 38, Issue: 1, Page(s): 34-37
ABSTRACT: Two gram-positive coryneform bacteria that degraded p-chlorophenol isolated from a continuous culture system are characterized. Isolate B (probably an Arthrobacter species) completely removed the p-chlorophenol from a medium with a concomitant increase in cell density within 16 h. Isolate F similarly removed the p-chlorophenol within 28 h but without an increase in cell density. Isolates B and F also removed the p-chlorophenol from a medium with p-chlorophenol as the sole carbon source within 32 and 48 h, respectively. The optimal temperature for degradation by both organisms was 25-30.degree.C and the optimal pH range was pH 7-9 for isolate B and pH 8-9 for isolate F.

Isolation of Pseudomonas pickettii strains that degrade 2,4,6-trichlorophenol and their dechlorination of chlorophenols.
Kiyohara, Hohzoh; Hatta, Takashi; Ogawa, Yuhzoh; Kakuda, Tetsuro; ETAL. Okayama Univ. Sci.Biotechnol. Lab.Okayama 700Japan
Applied and Environmental Microbiology, Volume: 58, Issue: 4, Page(s): 1276-1283
ABSTRACT: Three strains of Pseudomonas pickettii that can grow with 2,4,6-trichlorophenol (2,4,6-TCP) as the sole source of carbon and energy were isolated from different mixed cultures of soil bacterial populations that had been acclimatized to 2,4,6-TCP. These strains released 3 mol of chloride ion from 1 mol of 2,4,6-TCP during the complete degradation of the TCP. Of these strains, P. pickettii DTP0602 in high-cell-density suspension cultures dechlorinated various chlorophenols (CPs). Cells that were preincubated with 2,4,6-TCP converted isomers of 4-CP to the corresponding chloro-p-hydroquinones. The ability of DTP0602 to dechlorinate 2,4,6-TCP was induced by 2,6-dichlorophenol, 2,3,6- and 2,4,6-TCP, and 2,3,4, 6-tetrachlorophenol and was regressed in the presence of succinate or glucose.

EPA SITE demonstration of the BioTrol soil washing process
Stinson, M.K.; Skovronek, H.S.; Ellis, W.D.
U.S. Environmental Protection AgencyEdisonUSA Science Applications ParamusUSA
JOURNAL OF THE AIR POLLUTION CONTROL ASSOC.,Vol:42,Issue:1, pp:96-103
ABSTRACT: The BioTrol soil washing process was demonstrated on soil contaminated by wood treating waste, primarily pentachlorophenol (PCP) and creosote-derived polynuclear aromatic hydrocarbons (PAHs). Water was used to separate contaminated soil fractions from the bulk of the soil. The washed soil retained 10% of the feed soil contamination, while 90% were contained in the within the woody residues, the fine particles and process water. The soil washer achieved up to 89% removal for PCP and 88% for PAHs. The BioTrol biological water treatment process degraded up to 94% of PCP in the process water from soil washing. It appeared that both PCP and PAHs in the slurry of contaminated fines from soil washing can be biodegraded by the BioTrol biological slurry treatment process once steady-state operation of the bioreactor is achieved.

Degradation of pentachlorophenol by non-immobilized, immobilized and coimmobilized Arthrobacter cells.
Lin, Jian-Er; Wang, H. Y.Univ. MichiganDep. Chem. Eng.Ann Arbor, MI 48109USA
Journal of Fermentation and Bioengineering, Volume: 72, Issue: 4, Page(s): 311-314
ABSTRACT: Non-immobilized, immobilized and coimmobilized Arthrobacter (ATCC 33790) cells were examined for their ability to degrade pentachlorophenol (PCP) in a mineral medium. Non-immobilized cells could completely remove PCP from the aqueous phase and mineralize 77% of the added PCP within 135 h. Alginate-encapsulated cells mineralized 86% of the PCP with a similar profile as free cells. Use of coimmobilized cells resulted in rapid removal of PCP from the aqueous phase and extensive PCP mineralization.

A 7-2 in soil
AUTHOR: Balfanz, J.; Rehm, H.-J. Univ.MuensterFed. Rep. Germany
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (BERLIN), Volume: 35, Issue:5, Page(s): 662-668
ABSTRACT: Alcaligenes sp. A 7-2 immobilized on granular clay was applied in a percolator to degrade 4-chlorophenol in sandy soil. Good adsorption rates were achieved using high cell concentrations and media at pH 8.0. The influence of various parameters, such as aeration rate, pH, temperature,concentration of 4-chlorophenol and inoculum size, on the biodegradation rate was investigated. All degradation kinetics for 4-chlorophenol could be divided into a lag phase and a degradation phase. During fed-batch fermentations under optimal culture conditions, concentrations up to 160 mg x l(exp -1) 4-chlorophenol could be degraded. Semi-continuous cultivations dembnstrated that the degradation potential in soil was enhanced by the addition of immobilized bacteria. Continuous fermentations were performed with varying 4-chlorophenol concentrations in the feed.

A 7-2 in soil
AUTHOR: Balfanz, J.; Rehm, H.-J.
CORPORATE SOURCE: Universitaet MuensterFed. Rep. Germany
PUBLISHER: VCH, Weinheim; Deerfield BeachVolume: 4, , Page(s): 623-626
JOURNAL: DECHEMA Biotechnology Conferences
IABSTRACT: The biodegradation of this chlorinated compound (sandy oil) in a percolator by clay immobilized Alcaligenes is reported. To this end the effects of aeration rate, pH, temperature, inoculum size, and substrate concentration are studied. Fed-batch and continuous cultures are investigated. The results show a maximum degradation rate of 1.64 gLd.

Treating of bleaching effluents in aerobicanaerobic fluidized biofilm systems
AUTHOR: Fahmy, M.; Heinzle, E.; Kut, O.M.
CORPORATE SOURCE: Eidgenoessische Technische HochschuleZuerichSwitzerland
PUBLISHER: VCH, Weinheim; Deerfield BeachVolume: 4, , Page(s): 547-550
JOURNAL: DECHEMA Biotechnology Conferences
ABSTRACT: The possible biodegradation of the waste waters coming from chlorination and extraction stages of the bleaching process of cellulose is examined with special attraction to the degradation of chlorophenolic compounds. Adapted biofilms in fluidized sand bed reactors are used. The results show an overall CODAOX removal between 20 and 30% in the effluents of an aerobic fluidized bed, of anaerobic-aerobic reactors in series, and of an anaerobic-aerobic recycle reactor.

Use of coimmobilized biological systems to degrade toxic organic compounds
AUTHOR: Jian-Er Lin; Wang, H.Y.; Hickey, R.F.
CORPORATE SOURCE: The University of MichiganAnn ArborUSA Michigan Biotechnology Institute LansingUSA
BIOTECHNOLOGY AND BIOENGINEERING, Volume: 38, Issue: 3, Page(s):273-279
ABSTRACT: The coimmobilization of cells andor enzymes together with adsorbent, in a hydrogel matrix for biodegradation of toxic organic compounds is introduced. The proposed technique is evaluated using the biodegradation of pentachlorophenol (PCP) by a system of activated carbon and Phanerochaete chrysosporium. Results are compared with those from nonimmobilized systems. Architecture of the carbon-fungus capsules, PCP adsorption and removal, effects of retaining solid cosubstrates in capsules, effects of isolating degrading agents from interrupting microflora by the capsule membrane, and model studies on simulated contaminated soil extract and sand are described. It can be shown that the novel coimmobilized systems are superior to nonimmobilized ones.

Bench-scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol- and creosote-conatminated materials: slurry-phase bioremediation
AUTHOR: Mueller, J.G.; Lantz, S.E.; Blattmann, B.O.; Chapman, P.J.
CORPORATE SOURCE: Southern Bio ProductsInc., Technical Resources, Inc., U.S. EPA Res. Lab., Gulf BreezeUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY,Volume:25, Issue:6,Page(s):1055-1061
ABSTRACT: Slurry-phase bioremediation of pentachlorophenol- (PCP)- and creosote-contaminated soils and sediments was investigated at a bench-scale level. Aqueous slurries were prepared from sediment and surface soil, and the extent and rate of biodegradation of PCP and 42 targeted creosote constituents were monitored. Microbial activity of contaminated surface soils was rather slow and generally confined to the more readily biodegradable lower molecular weight compounds. By contrast, slurry-phase bioremediation of contaminated sediments adjusted to pH 7.1 resulted in rapid and extensive biodegradation. Abiotic losses of monitored constituents were significant, particularly of the higher molecular weight compounds. When compared to conventional solid-phase bioremediation, slurry treatment offered significant advantages in terms of time and efficiency.

Bench-scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol- and creosote-contaminated materials: solid-phase bioremediation
AUTHOR: Mueller, J.G.; Lantz, S.E.; Blattmann, B.O.; Chapman, P.J.
CORPORATE SOURCE: Southern Bio ProductsInc., Technical Resources, Inc., U.S. EPA Res. Lab., Gulf BreezeUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY,Volume:25,Issue:6, Page(s):1045-1055
ABSTRACT. The biodegradation of pentachlorophenol (PCP)- and creosote from contaminated soils and sediments by indigenous microorganisms was investigated. The effects of nutrient amendment on the rate and extent of biodegradation of PCP and 42 targeted creosote constituents were monitored. In general, solid-phase bioremediation resulted in slow losses of targeted pollutants. Lower molecular creosote constituents were more readily biodegradable than higher molecular weight contaminants, and the more recalcitrant pollutants such as PCP tended to persist. Abiotic losses were greater with sediment than with surface soil. During soil bioremediation, the number of phenathrene degraders was significantly greater in nutrient-amended soils than in unamended soils. The performance data suggested, that solid-phase bioremediation strategies are of limited usefulness for full-scale site bioremediation.

EPA site demonstration of BioTrol aqueous treatment system
AUTHOR: Stinson, M.K.; Skrovronek, H.S.; Chresand, T.J.
CORPORATE SOURCE: U.S. Environmental Protection AgencyEdison, USAUSA Science Applications Int. Corp. Science Applications Int. Corp., ParamusUSA
JOURNAL OF THE AIR POLLUTION CONTROL ASSOCIATION, Volume: 41, Issue: 2, Page(s): 228-233
ABSTRACT: The mobile BioTrol fixed-film biological aqueous treatment system was evaluated for its effectiveness in removing pentachlorophenol (PCP) from groundwater. The system employs indigenous microorganisms amended with a PCP-degrading Flavobacterium species. Groundwater provided from a well at a wood preserving site was fed to the system with minor pretreatment. At a flowrate of 5 gpm the system was capable of achieving about 96% removal of PCP. At lower flow rates (1 and 3 gpm) removals were even higher. Biodegradation appeared to be the predominant mechanism for PCP removal. A completely nontoxic effluent was obtained. The results were consistent with complete mineralization of PCP. The process is assumed to be also effective on other hydrocarbons and appears to be a cost-effective treatment for contaminated wastewaters requiring minimal operation attention once acclimated.

Effect of glutamate on the degradation of pentachlorophenol by Flavobacterium sp.
AUTHOR: Gonzalez, J.F.; Hu, W.-S.
CORPORATE SOURCE: Univ.MinneapolisUSA
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (BERLIN), Volume: 35, Issue:1, Page(s): 100-104
ABSTRACT: The effects of pentachlorophenol (PCP) concentration and the presence of glutamate on viability and PCP-degrading activity of a Flavobacterium sp. were investigated by inoculating induced cells into cultures containing either PCP alone or PCP and glutamate as carbon sources. Supplying PCP alone, the degradation activity increased with PCP concentration. However, a lag phase was observed,which was more pronounced at higher PCP concentrations. This lag in PCP degradation was reduced in the presence of glutamate, and depletion of glutamate caused a lag in PCP degradation. The PCP degradation rate increased with increasing glutamate concentration. The lag in PCP degradation was suggested to be a result of viability loss in the presence of PCP and in the absence of glutamate.

Biodegradation of pentachlorophenol in soil: the response to physical, chemical, and biological treatments.

AUTHOR: Seech, A. G.; Trevors, J. T.; Bulman, T. L.
CORPORATE SOURCE: Dearborn Environ. Consulting Group Ltd.Mississauga, ON L5A 3T5Canada

Canadian Journal of Microbiology, Volume: 37, Issue: 6, Page(s): 440-444

ABSTRACT: The effects of physical, chemical, and biological treatments on biodegradation of pentachlorophenol (PCP) were studied in a silt-loam soil contaminated with 175 mg PCPkg and uniformly 14C-labelled PCP. Biodegradation of 14C-labelled PCP and technical-grade PCP were monitored over 210 days incubation. Mineralization of labelled PCP was significantly (p = 0.05) influenced by soil treatments. Negligible biodegradation occurred in either the sterile control soil or the uninoculated control soil, with less than 1% of added 14C recovered as 14CO2. Inoculation of unamended soil with a strain of Flavobacterium (ATCC 39723) known to degrade PCP increased biodegradation of PCP; approximately 60% of the [14C]PCP was recovered as 14CO2. Increased soil water content enhanced biodegradation, while increased chloride ion concentration and anoxic conditions were inhibitory. Residual soil PCP concentrations were also influenced by various treatments. In the sterile control soil and noninoculated control, after 210 days incubation, concentrations of PCP were 143 and 123 mgkg, respectively, while the PCP concentration in the inoculated soil was 21 mgkg. When soil organic matter was increased by adding finely ground red clover leaf and stem material, the residual PCP concentration was reduced to 6 mgkg after 210 days. Increased soil water content resulted in a residual PCP concentration of 5 mgkg. Biodegradation of PCP in soil was significantly influenced by various soil amendments.

Potential for thermophilic (50.degree.C) anaerobic dechlorination of pentachlorophenol in different ecosystems.

Larsen, S.; Kendriksen, H. V.; Ahring, B. K., Tech. Univ.Dep. Biotechnol.DK-2800 LyngbyDenmark

Applied and Environmental Microbiology, Volume: 57, Issue: 7, Page(s): 2085-2090

ABSTRACT: Thermophilic (50.degree.C) anaerobic biodegradation of pentachlorophenol (PCP) was investigated by using different inocula from natural ecosystems and anaerobic digesters. Inocula tested were three freshwater sediments,four anaerobic sewage sludge samples from digesters treating sludge from wastewater plants with various industial inputs, and digested manure from an anaerobic reactor. Only one digested-sludge sample and the manure sample were from thermophilic environments. Initial PCP concentration was 7.5. or 37.5 .mu.M. After 8 months, PCP had disappeared from the sediment samples and various, less chlorinated intermediates were present. Additions of extra PCP were degraded within 4 weeks, and a maximal observed dechlorination rate of 1.61 .mu.molLday in the vials with addition of 7.5 .mu.M PCP and 7.50 .mu.molLday in the vials with addition of 37.5 .mu.M PCP were measured for a freshwater sediment. In contrast, only 2.8 to 17.5% of the initial PCP added had disappeared from the sludge samples after 8 months of incubation. The complex pattern of intermediates formed indicated that the dechlorination of PCP processed via different pathways, involved at least two different populations in the dechlorination processes.

Biodegradation of trace concentrations of substituted phenols ingranular activated carbon columns

AUTHOR: Speitel, G.E.jr.; Lu, C.-J.; Turakhia, M.; Zhu, X.-J. Univ. of HoustonUSA

ENVIRONMENTAL SCIENCE & TECHNOLOGY, Volume: 23, Issue: 1, Page(s):68-74

ABSTRACT. P-nitrophenol and 2,4-dichlorophenol are readily biodegradable in GAC columns, even at trace concentrations. Pentachlorophenol also is biodegradable but at a slower rate. Significant biodegradation of sorbed substrate occured only with p-nitrophenol. A substantial fraction of the 2,4-dichlorophenol appears to be irreversibly sorbed and biodegradation of the sorbed chemical is limited by slow desorption kinetics. Biodegradation of sorbed pentachlorophenol is limited by its slow microbial degradation rate.

Degradation of 2,4,6-trichlorophenol by Azotobacter species strain CP1.
Li, Deng-Yu; Eberspaecher, J.; Wagner, B.; Kuntzer, J.; Lingens, F. Univ. HohenheimInst. Mikrobiol.D-7000 Stuttgart 70Germany
JOURNAL: Applied and Environmental Microbiology,Volume:57,Issue:7,pp 1920-1928
ABSTRACT: A bacterium which utilizes 2.4,6-trichlorophenol (TCP) as a sole source of carbon and energy was isolated from soil. The bacterium, designated strain CP1, was identified as an Azotobacter species. TCP was the only chlorinated phenol which supported the growth of the bacterium. Resting cells transformed monochlorophenols, 2,6-dichlorophenol, and 2,3,6-trichlorophenol. Phenol and a number of phenolic compounds, including 4-methylphenol, all of the monohydroxybenzoates, and several dihydroxybenzoates, were very good carbon sources for Azotobacter species strain GP1. The organism utilized up to 800 mg of TCP per L; the lag phase and time for degradation, however, were severly prolonged at TCP concentrations above 500 mgL. Repeated additions of 200 mg of TCP per L led to accelerated degradation, with an optimum value of 100 mg of TCP per L per hour. TCP degradation was significantly faster in shaken than in nonshaken cultures. Optimum temperature for degradation was 25-30.degree.C. Induction studies, including treatment of cells with chloramphenicol prior to TCP or phenol addition,revealed that TCP induced TCP degradation but not phenol degradation and that phenol induced only its own utilization. Per mol of TCP, 3 mol of cl- was released. 2,6-Dichloro-p-benzoquinone was detected in the resting-cell medium of Azotobacter species strain GP1. By chemical mutagenesis, mutants blocked in either TCP degradation or phenol degradation were obtained. No mutant defective in the degradation of both phenols was found, indicating separate pathways for the dissimilation of the compounds. In some of the phenol-deficient mutants,pyrocatechol accumulated, and in some of TCP-deficient mutants, 2,6-dichlorohydroquinone accumulated.

Biodegradation of 4-chlorophenol by adsorptive immobilized Alcaligenes A7-2 in soil.
AUTHOR: Balfanz, J.; Rehm, H.-J.Univ. MuensterInst. Mikrobiol.W-4400 MuensterGermany
Applied Microbiology and Biotechnology, Volume: 35, Issue: 5, Page(s): 662-668
ABSTRACT: Alcaligenes species A7-2 immobilized on granular clay was applied in a percolator to degrade 4-chlorophenol in sandy soil. Good adsorption rates on granular clay were achieved using cell suspensions with high titres and media at pH 8.0. The influence of various parameters such as aeration rate, pH, temperature, concentration of 4-chlorophenol and size of inoculum on the degradation rate were investigated. During fed-batch fermentations under optimal culture conditions, concentrations of 4-chlorophenol up to 160 mgl could be degraded. The degradation potential in soil could be well established and enhanced by the addition of immobilized bacteria. Continuous fermentation was performed with varying 4-chlorophenol concentrations in the feed and different input levels. The maximum degradation rate was 1.64 gl.day.

Characterization of chlorophenol and chloromethoxybenzene biodegradation during anaerobic treatment
AUTHOR: Woods, S.L.; Ferguson, J.F.; Benjamin, M.M.
CORPORATE SOURCE: Oregon State Univ. CorvallisUSA Univ. of Washington SeattleUSA
ENVIRONMENTAL SCIENCE & TECHNOLOGY, Volume: 23, Issue: 1, Page(s): 62-68
ABSTRACT: The removal of chlorinated phenols and chlorinated methoxybenzenes during continous anaerobic treatment of a complex, concentrated wastewater is investigated. The chlorinated compounds are converted to lesser chlorinated compounds by biologically mediated dechlorination reactions. Mineralization is not observed and no evidence is found for dechlorination of monochlorophenols.

Treatment technologies for hazardous wastes: part I A review of treatment alternatives for dioxin wastes

AUTHOR: Freeman, H.M.; Olexsey, R.A. U.S. Environmental Protection AgencyCincinnatiUSA

JOURNAL OF THE AIR POLLUTION CONTROL ASSOC, Volume:36,Issue:1, pp: 67-75

ABSTRACT: Several treatment processes for dioxin wastes that have been proposed to be used instead of land disposal for disposing of solid and liquid wastes containing dioxins are discussed. Dioxin wastes cover wastes from the production of certain chlorophenols and chlorophenoxy pesticides, manufacturing use of tetra-, penta- and hexachlorobenzene under alkaline conditions, as well as discarded unused formulations containing tri-, tetra- and pentachlorophenols and their derivatives. There are several thermal and nonthermal alternative treatment technologies for dioxin wastes. Processes which have been used to treat dioxin wastes, including a mobile rotary kiln incinerator, the advanced Electric Reactor, the Shirco infrared system, liquid injection incinerators and dioxin photolysis, are described. Processes which have potential to treat dioxin wastes include rotary kiln incinerators, circulating bed combustion, chemical dechlorination, the use of supercritical fluids and biodegradation by the white rot fungus. While many of these processes described may now appear to be expensive or exotic, as EPA moves to implement landfill restrictions,these processes may assume more practically. Most certainly, the adaption of the advanced technologies described will improve the quality of the environment.

Biodegradation of creosote and pentachlorophenol in contaminated groundwater: chemical and biological assessment.

AUTHOR: Mueller, J. G.; Middaugh, D. P.; Lantz, S. E.; Chapman, P. J.

CORPORATE SOURCE: Southern Bio Products Inc.Gulf Breeze, FL 32561-3999USA

Applied and Environmental Microbiology, Volume: 57, Issue: 5, Page(s): 1277-1285

ABSTRACT:Shake flask studies examined the rate and extent of biodegradation of pentachlorophenol (PCP) and 42 components of coal-tar creosote present in contaminated groundwater. The ability of indigenous soil microorganisms to remove these contaminants from aqueous solutions was determined by gas chromatographic analysis of organic extracts of biotreated groundwater. Changes in potential environmental and human health hazards associated with the biodegradation of this material were determined at intervals by Microtox assays and fish toxicity and teratogenicity tests. After 14 days of incubation at 30 degC, indigenous microorganisms effectively removed 100, 99, 94, 88, and 87% of measured phenolic and lower-molecular-weight polycyclic aromatic hydrocarbons (PAHs) and S-heterocyclic, N-heterocyclic,and O-heterocyclic constituents of creosote, respectively. However, only 53% of the higher-molecular-weight PAHs were degraded; PCP was not removed. Despite the removal of a majority of the organic contaminants through biotreatment, only a slight decrease in the toxicity and teratogenicity of biotreated groundwater was observed. Data suggest that toxicity and teratogenicity are associated with compounds difficult to treat biologically and that one may not necessarily rely on indigenous microorganisms to effectively remove these compounds in a reasonable time span; to this end, alternative or supplemental approaches may be necessary. Similar measures of the toxicity and teratogenicity of treated material may offer a simple, yet important, guide to bioremediation effectiveness.

Sequential degradation of chlorophenols by photolytic and microbial treatment

AUTHOR: Miller, R.M.; Singer, G.M.; Rosen, J.D.; Bartha, R.

CORPORATE SOURCE: Rutgers Univ.New BrunswickUSA

Environmental Science and Technology, Volume: 22, Issue: 10 , Page(s): 1215-1219

ABSTRACT: Disruption of carbon-halogen bonds by photolysis followed by traditional effluent treatment may possibly offer an alternative to activated charcoal treatment. 2, 4-Dichlorophenol and 2,4,5-trichlorophenol are photolyzed (300nm) with and without added H2O2. Biodegradation of intact and photolyzed compounds to CO2 and to polar respective bound metabolites is compared.

Degradability of polychlorinated phenols by bacterial populations in soil.
AUTHOR: Kiyohara, H.; Takizawa, N.; Uchiyama, T.; Ikarugi, H.; Nagao, K.
CORPORATE SOURCE: Okayama Univ. Sci.Biotechnol. Lab.1-1 Ridai-cho, Okayama 700 Japan
J. Fermn. Bioeng., Volume: 67, Issue: 05, Page(s): 339-344
ABSTRACT: The biodegradabilities of polychlorinated phenols including 5 isomers of trichlorophenols, 3 isomers of tetrachlorophenols and pentachlorophenol, were tested with 170 samples of soil collected from various environments. After the samples were inoculated into a succinate-containing mineral medium and incubated, the cultures were acclimatized to phenol concentrations from 10 to 100 ppm. Twenty six samples (15%) were observed to degrade 2,4,6-trichlorophenol and a mixed sample of soil degraded 2,3,4, 6-tetrachlorophenol, but no degradation was seen with other chlorophenols. All of the mixed cultures acclimatized to and degrading 2,4,8-trichlorophenol also degraded phenol. For the degradation of 2,4, 6-trichlorophenol nitrate, the nitrate-ion was preferred to the ammonium +ion as a nitrogen source. At concentrations below 500 ppm, 2,4,6-trichlorophenol was degraded completely within 8 days and the chloride ion was detected in the culture broth at an amount corresponding to that of the chlorinated phenol, although cell growth was inhibited at a 2,4, 6-trichlorophenol concentration of 1,000 ppm. No possible intermediate products were detected in the cultures.

Biodegradation of pentachlorophenol
AUTHOR: Crawford, R. L.
CORPORATE SOURCE: Minnesota, University ofUSA
PATENT NUMBER: US 4713340
PUBLICATION DATE: 15 Dec 1987 (871215) LANGUAGE: English
PRIORITY PATENT APPLICATION(S) & DATE(S): US 620231 (840613)
ABSTRACT: A method of detoxifying pentachlorophenol is provided. The method comprises culturing a strain of bacteria in an aqueous medium containing dissolved pentachlorophenol. The bacterial strain is of the genus Flavobacterium and is capable of sustained growth in medium concentrations of pentachlorophenol between 250 mg/l and 400 mg/l as its sole source of carbon and energy.

Biodegradation of toxic chemicals using commercial preparations
AUTHOR: Lewandowski, G.; Salerno, S.; u.a.
Environmental Progress, Volume: 5, Issue: 3, Page(s): 212-217
ABSTRACT: Three commercial microbial preparations were tested for their ability to degrade phenol, 2-chlorophenol, and 2, 4-dichlorophenoxyacetic acid (2,4-D), in an aerated biological batch reactor at room temperature (about 26 Cel). A municipal mixed liquor was used as the control. The municipal population performed better than any of the commercial preparations alone. However, there was an improvement in performance (for the phenolic compounds only) when the commercial preparation cultures were added to the municipal mixed liquor in a 1:20 ratio based on MLSS. Nevertheless, such a high rate of addition would be economically prohibitive. Aqueous concentrations of the individual substrates were determined by direct injection into a gas chromatograph. Changes in the microbial populations were observed using standard plating techniques and light microscopy.

Biodegradation of 4-chlorophenol by entrapped Alcaligenes sp. A 7-2.
AUTHOR: Westmeier, F.; Rehm, H. J. Univ. MuensterInst. Mikrobiol.D-4400 MuensterW. Germany
Appl. Microbiol. Biotechnol., Volume: 22, Issue: 5, Page(s):301-305
ABSTRACT: The degradation of 4-chlorophenol by free and by Ca-alginate-immobilized cells of Alcaligenes sp. A 7-2 has been studied. Increasing concentrations of 4-chlorophenol (0.4-0.55mM) were better tolerated and more quickly degraded by the immobilized organisms than by free cells. The capability for haloarene-degradation is inducible. In semicontinuous fermentation at pH 7 a minimal degradation time of 5 hours for degrading 0.2mM 4-chlorophenol was reached. Fermentation temperature was important for inducing the degradation capability, but less important for the degradation rate by induced organisms.

HAZARDOUS WASTE & HAZARDOUS MATERIALS
Volume 11, Number 3, 1994
Mary Ann Liebert, Inc., Publishers

Degradation of Pentachlorophenol in Bench Scale Bioreactors Using the White Rot Fungus *Phanerochaete chrysosporium*

GUYOUNG KANG and DAVID K. STEVENS

Utah Water Research Laboratory
Department of Civil and Environmental Engineering
Utah State University
Logan, UT 84322-4110

ABSTRACT

Biodegradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium* was investigated in three bench scale bioreactors: mechanically mixed suspended bioreactor, upflow fixed-film bioreactor, and fluidized bed bioreactor. PCP disappearance was enhanced by increased ligninase activity in a mechanically mixed suspended bioreactor after initial adsorption onto the mycelium. Live fungal cultures were able to degrade PCP in the sorbed phase. PCP degradation data in the upflow fixed-film bioreactor was rapid and adequately explained with a quasi-first order steady state model with a rate constant of 0.071 L/g biomass-hr.In the fluidized bed bioreactor, effluent PCP concentration varied slightly at hydraulic residence times of 5 to 90 minutes. The steady state PCP degradation first order rate constant was 0.1 L/g biomass-hr. Effluent PCP concentration was related to its influent concentration at a 5 minute hydraulic retention time. The PCP removal efficiency was 37 to 72% and improved by increasing hydraulic retention time and decreasing influent PCP concentration. The fluidized bed bioreactor using *P. chrysosporium* shows considerable promise for the degradation of PCP. Degradation was effective under conditions of short hydraulic residence time and the process was stable in the face of large variations in influent conditions.

INTRODUCTION

Pentachlorophenol (PCP) is the most heavily used wood preservative, fungicide, and insecticide in the United States [1]. The most significant source of PCP containing wastewaters and ground water is the wood preserving industry. Extensive use of PCP has led to the contamination of aquatic ecosystems world-wide. It is also possible that large numbers of

The literature contains few reports concerning the aerobic biodegradation of PCP, and most deal with its fate in soil [3,4,5]. Even though numerous authors have found that PCP undergoes biodegradation it is often slow [1,2,6].

Much research has tried to directly effect biodegradation of toxic compounds in the environment. The white rot fungus *Phanerochaete chrysosporium* has been shown to degrade a wide variety of environmentally persistent organopollutants, including PCBs, PCP, DDT, and PAHs [7,8,9,10,11]. Several researchers reported that the purified ligninase or the crude extracellular enzymes produced by the white-rot fungus *Phanerochaete chrysosporium* convert PCP into an oxidation product, 2,3,5,6-tetrachloro-2,5-cyclohexadiene-1,4-dione (TCHD) [11,12]. This result provides evidence that the extracellular ligninases are able to catalyze the initial oxidation of the PCP degradation process.

Current interest in using the white-rot fungus *P. chrysosporium* in waste and ground water treatment systems is based on the fact that this microorganism is able to degrade compounds that are often refractory to biodegradation in other biological wastewater treatment systems such as the activated sludge system. Although Lin et al. [12] have studied kinetics of pentachlorophenol degradation by ligninase, they used crude enzyme at extremely high ligninase activities, which are never produced in a natural *Phanerochaete chrysosporium* culture. However, they found that PCP was degraded by both extracellular enzymes and cell mass. It was not clear that PCP degraded in a suspended bioreactor system with a natural *Phanerochaete chrysosporium* culture.

Although there is much information in the literature concerning the growth patterns and biochemistry of *P. chrysosporium*, there is very little information concerning xenobiotic degradation suitable for an engineered reactor design. Among the important external factors affecting the ligninase activity of the microorganism are temperature, pH, dissolved oxygen concentration and fixed nitrogen concentration [13,14,15]. The optimum temperature for growth of the fungus has been established as 39°C, and the optimum pH as 4.0-4.5. Under these conditions, the ligninase activity rapidly peaks after 5 days but drops rapidly after reaching maximum activity. Ligninase activity normally is present for a total of 7 to 8 days. A important challenge is to apply the lignin degrading system under conditions considered non-optimal for the growth of *P. chrysosporium* and ligninase production, including low temperature, and dynamic influent concentrations and low growth substrate concentration as would likely be found in a full scale application.

For development of a bioremediation process for the treatment of hazardous wastes, PCP, a priority organopollutant, was chosen as a model compound for this study since it has been reported to be mineralized by *P. chrysosporium* under ligninolytic conditions [11,12]. The objectives of this study were to investigate, using laboratory experiments, behavior of degradation of PCP, removal rate of PCP, and ultimately to develop a fluidized bed bioreactor to degrade this environmentally persistent chemical.

MATERIALS AND METHODS

Experimental Design

These experiments were carried out to investigate degradation of PCP by *P. chrysosporium* in bench scale bioreactors at room temperature (25 ± 2°C). The bench scale bioreactors used here are 1) mechanically mixed suspended growth bioreactor (MMSGB), 2) upflow fixed-film bioreactor (UFB) with nylon web media, and 3) fluidized bed bioreactor (FBBR) with silica sand. In this study, the nylon web and silica sand were used as support media for immobilizing the fungus. The silica sand support medium in FBBR was chosen due to the very low adsorption capacity for PCP [16]. These bioreactors were used in this study

from the aqueous phase and in *P. chrysosporium* mycelia using the MMSGB. Since the most of reports on degradation by *P. chrysosporium* are based on determination of $^{14}CO_2$ release in batch stationary system, it is difficult to distinguish the precise functions of cell mass and extracellular enzymes in the biodegradation process [7,8,9]. Secondly, kinetic parameters for PCP degradation were determined using the UFB in continuous mode at steady state since the UFB eliminated adsorption of PCP into the mycelium and nonporous nylon web support medium at steady state. Finally, fluidized bed bioreactors have been successfully used for 20 years for treatment conventional and industrial wastewaters using mixed cultures of bacteria. Due it its large surface area for growth and immobilization of contaminants and, possibly, extracellular enzymes (such as ligninase), the fluidized bed was chosen for this research.

Microorganism and Culture Conditions

P. chrysosporium (BKM-F-1767) was obtained from the Biotechnology Center at Utah State University, and was originally obtained from U.S. Department of Agriculture, Forest Products Laboratory, Madison, Wisconsin. The fungus was maintained on 2% malt agar slants at 25 ± 2°C and was subcultured every 40 to 60 days.

Mycelium pellets of *P. chrysosporium* were grown on a shaker in a nitrogen-limited culture medium similar to that used by Tien and Kirk [15] except that a 10 mM Na-acetate buffer, pH 4.5, was used instead of a 2,2-dimethylsuccinate (DMS) buffer. The composition of the nitrogen-limited culture medium is shown in Table 1.

Cultures were prepared for growth by adding a spore suspension of *P. chrysosporium* to 250 mL of culture medium so that the optical density ($OD_{650\ nm}$) was approximately 0.5 in a 1 mL disposable cuvette with a 1 cm path length. This suspension was then mixed with the 2.25

Table 1. The composition of nitrogen-limited culture medium and continuous reactor feed in 10 mM Na-acetate buffer (pH=4.5)

Chemical	Composition (g/L)	
	Fungal growth and MMSGB	UFB and FBBR
KH_2PO_4	2.0	2.0
$MgSO_4 \cdot H_2O$	0.5	0.5
$CaCl_2 \cdot 2H_2O$	0.1	0.1
Thiamine	0.001	0.001
Tween 80	0.05[a]	0.0
Veratryl alcohol	0.252	0.252
Glucose	10.0	1.0
Ammonium tartrate	0.221	0.0221

[a]MMSGB experiment only used
MMSGB: Mechanically mixed suspended growth bioreactor
UFB: Upflow fixed-film bioreactor

L of culture medium. One hundred mL of the diluted spore suspension was inoculated in a 500 mL Erlenmeyer flask, and grown for 8 days at 25°C on a shaker table at 160 rpm. The cultures were harvested after 8 days agitation. The flasks were closed with cotton plugs to allow sufficient air transfer while preventing contamination [16]. The surfactant Tween 80 was only used in the MMSGB while it was not added in the other systems (UFB and FBBR). Since Tween 80 is believed to provide protection for enzyme against mechanical deactivation and holds the enzyme in the aqueous phase [16]. It was found that without Tween 80, ligninase activity could not be detected and it was assumed that the enzyme sorbs onto the fungal hyphae.

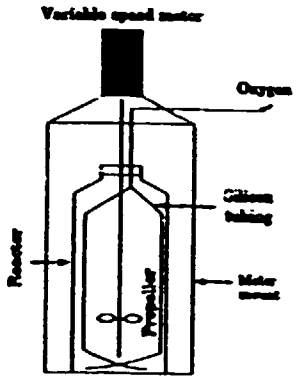


Figure 1. Mechanically mixed suspended growth bioreactor (MMSGB) diagram.

Mechanically Mixed Suspended Growth Bioreactor (MMSGB).

The MMSGB was fabricated from a 3 L total volume of glass bottle with a propeller impeller at 200 rpm for mixing as shown in Figure 1. The impeller diameter was 5 cm and mounted 5 cm above the bottom in the total two liter working volume bioreactor.

An aeration system was installed comprising 65 cm of 0.64 cm ID silicon tubing (Cole-Parmer Co., Niles, IL), which is oxygen permeable at a rate of 0.003 mg/min/cm at 133 kPa gage pressure. This system considerably reduced the turbulence level in the MMSGB compared to sparged aeration and allowed dissolved oxygen levels averaging 12 (± 3, standard deviation, σ) mg/L [16].

Table 2. Experimental conditions for the degradation of PCP in the MMSGB

Run	Volume (L)	Silicon tubing	Dry biomass (g/L.)		Initial LiP[b] (Unit/L)
			Mean	SD[a]	
1	2	No	0.0	0.0	0.0[c]
2	2	Yes	0.0	0.0	0.0
3	2	Yes	2.5	0.31	0.0
4	1	No	3.4	0.28	0.0
5	2	Yes	2.7	0.54	210
6	2	Yes	3.8	0.61	216

[a]Standard deviation
[b]Ligninase activity
[c]No fungus or fungus killed by autoclaving

For the MMSGB, the harvested cultures were placed in the reactor and continuously grown for 3 days before spiking with 1 mL stock PCP (10 mg PCP/mL in ethanol) in 2 L working volume. The behavior of PCP degradation was assessed through the disappearance of PCP in the aqueous and mycelia phases in the MMSGB after spiked PCP, in order to distinguish the precise functions of the cell mass and extracellular enzymes in the biodegradation process using *P. chrysosporium*. The experimental conditions are presented in Table 2. The residual aqueous phase PCP concentrations were measured over time for each run. Three control runs (Runs 1, 2, and 4) were investigated to determine the influence of the silicon tubing or killed (autoclaved) mycelium as shown in Table 2. After each run was terminated, the remaining PCP in the mycelium was analyzed.

In the UFB, mycelium pellets were separated using their culture medium using cheese cloth after 8 days growth on a shaker for immobilization of the fungus on the support medium. The mycelia were then homogenized in a blender for 30 seconds (10 g of wet mycelium plus 100 mL of remaining culture supernatant). The homogenate was added to the bioreactors as inoculum with supernatant and immobilized on the support medium for 3 days using a batch recirculating mode. PCP and the culture medium were fed continuously after the fungi were immobilized on the support medium. The feed stream consisted of approximately 5 mg/L PCP with other nutrients as shown in Table 1. The concentration of PCP was measured continuously until the run was terminated: the samples were collected at periodic intervals.

Upflow Fixed-film Bioreactor (UFB)

A schematic of the fixed-film bioreactor is shown in Figure 2. Three glass cylinders (2" ID x 6" length) and one glass cylinder (2" ID x 4" length) were sealed by a mechanical coupling. This system had oxygen supplied by air through the porous diffuser for maintaining the dissolved oxygen above 5 mg/L. The fungus was immobilized on the nylon web medium as described above. Two reactors were installed for this study. Reactor 1 was operated for 37 days at five different hydraulic retention times while reactor 2 was operated for 22 days at two different hydraulic retention times. The net reactor volumes were 720 mL for reactor 1, and 730 mL for reactor 2. The net reactor volume was that occupied by the aqueous phase only, subtracting air volume and nylon-web support medium volume.

Air was supplied at between 150 to 200 mL/min through a 0.2 μm air filter to prevent contamination. The reactors were operated in batch mode for 3 days to establish the fungus on the nylon web prior to the onset of continuous feed at various hydraulic residence times.

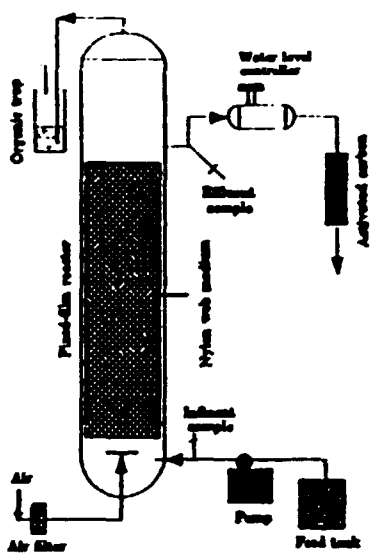


Figure 2. Upflow fixed-film bioreactor (UFB) diagram.

Fluidized Bed Bioreactor (FBBR)

The FBBR was fabricated from a 2.01 cm internal diameter glass tube with a 33.3 mL empty bed working volume. The fluidized bed bioreactor was run in continuous mode with recycle. The reactor configurations for each mode are shown in Figure 3.

In the fluidized bed experiments for PCP degradation, silica sand was used as a support medium, since it provides a favorable environment for growth of microorganisms [17]. In addition, the capacity of the sand to adsorb PCP was negligible, eliminating adsorption as a competing process [16]. Culture immobilization and feed composition were as described above for the UFB.

During the experiments, the excess mycelium was frequently manually removed by stirring with a stirring rod, resulting in few clogging problems and maintenance of a stable expanded bed depth. The sloughed mycelium floated to the water surface and washed out into the sand separator. This mycelium was removed manually.

Pentachlorophenol Assay

Reagent grade PCP was obtained from Sigma Chemical Co. (St. Louis, MO). The amounts of PCP remaining in aqueous and biomass phases were assayed by HPLC [11]. At the time of sampling, 1 μM of sodium azide was added to the 10 mL culture sample to inhibit enzyme activity [11]. Extraction of the aqueous sample was performed two consecutive times, each time using fresh 50 mL aliquots of HPLC grade methylene chloride. The extracts were

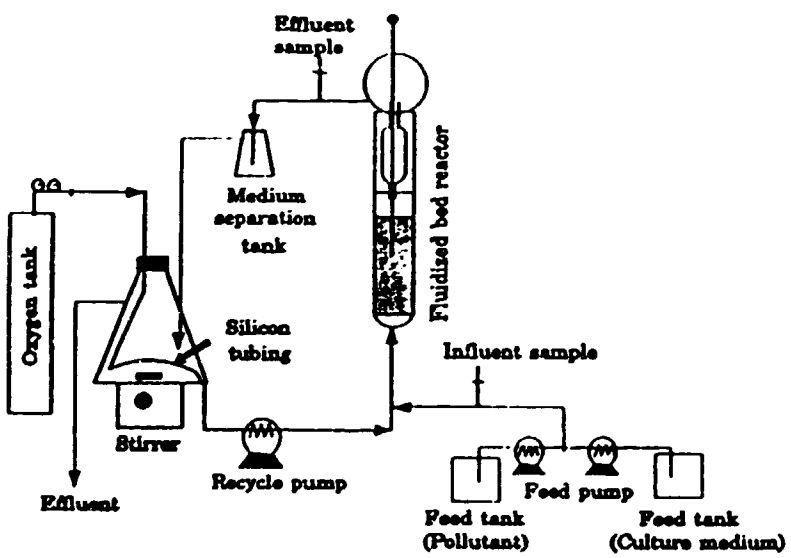


Figure 3. Fluidized Bed Bioreactor (FBBR) Containing Silica Sand as Support Medium with Recycle

combined, dried through a column of anhydrous sodium sulfate, and concentrated to 2 to 3 mL in a Kuderna-Danish (KD) concentrator. The sample was then placed into a 12 mL amber bottle with a teflon lined cap. Methylene chloride was completely evaporated using nitrogen blowdown evaporation and the residual was redissolved using the desired volume of acetonitrile for injection into the HPLC (Beckman System Gold, Beckman Instruments, Inc., San Ramon, CA). The limit of quantitation for the HPLC method was 100 µg/L.

In MMSGB, the remaining PCP in the biomass was measured after termination of the run. The remaining mycelium was separated using Whitman No 1 filter paper and weight. The mycelium was then homogenized in a Potter-Elvejem homogenizer equipped with a Teflon pestle. The homogenized material was then extracted with two 50 mL portions of methylene chloride, which were then pooled. Following the methylene chloride extraction, the analytical sample was prepared as described above.

Ligninase Activity

Oxidation of veratryl alcohol to veratryl aldehyde by ligninase, a standard assay for the ligninase activity [15], was monitored spectrophotometrically at 310 nm. Reaction mixtures with 0.5 mL aqueous sample contained 0.1 mM sodium tartrate buffer, pH 2.5, 500 µM H_2O_2, and 1.5 mM veratryl alcohol in the total 1 mL volume. The reaction is initiated by addition of hydrogen peroxide. One unit of ligninase activity oxidizes 1 µM of veratryl alcohol per minute at 25°C. The change absorbance is recorded with UV 160U spectrophotometer (UV 160U, UV-Visible Recording Spectrophotometer, Shimadzu, Japan).

Ligninase activity was only measured in the MMSGB, since Tween 80 was contained in the culture medium. Kang [16] found that without Tween 80, ligninase activity could not detected.

Quality Assurance and Quality Control (QA/QC)

The majority of analytical data collected during the studies were used to estimate the equilibrium for the process and to characterize the affinity of *P. chrysosporium* for PCP. The quality of data was determined based on precision and accuracy estimates. In general, analytical measurements must have had adequate precision to produce a pooled coefficient of variation less than 10% in replicate determinations. Accuracy criteria required that PCP recovery from the sample matrix should not vary more that 10% from the true value. When PCP compound extraction from the aqueous phase was required, extraction efficiency was not less than 75% of the "spiked" value. The extraction efficiency was also to have a coefficient of variation less than 10%.

RESULTS AND DISCUSSION

Using the extraction method described above (PCP assay), the extraction efficiency of PCP in two sample matrices, sodium acetate buffer and culture medium at pH = 4.5, were 95.4 ± 7.0 and 97.5 ± 4.9%, respectively. Using a probability level of 0.05, percent recoveries of the sodium acetate buffer solution and culture medium were found not to be significantly different than 100%. The overall average extraction recovery and pooled standard deviation were 96.7%, and 5.6%, respectively. Based on these results, extraction efficiency was not used to correct PCP concentrations determined by this extraction method.

Mechanically Mixed Suspended Growth Bioreactor

The residual aqueous phase PCP concentrations for the suspended growth bioreactor are plotted versus time in Figure 4. The disappearance of initial and final are plotted versus time in A, and B, respectively, by expanding the time and concentration scales in Figure 4. The culture medium control (no-fungus, and no silicon tubing) PCP concentration decreased slightly over the 60 hour period, from 4.6 mg/L to 4.2 mg/L, likely due to adsorption onto the container walls (Figure 4a). The PCP concentration in the silicon tubing-only control dropped rapidly within the first 10 hr of the experiment to 1 (± 0.04 mg/L, σ) (75% decrease) and then more slowly until 70 hr. The remaining runs showed similar behaviur, although the controls (Runs 3 and 4) reached equilibrium after 10 hours while the runs with active fungal cultures (Runs 5 and 6) showed a significant, although still slow, drift downward in PCP concentration, until hour 70 (Figure 4c).

In all treatments with fungus and/or silicon tubing PCP disappeared initially by adsorption (Figure 4b). Although in the controls without silicon tubing, the dissolved oxygen was lower than in those with the tubing, since the fungal culture was autoclaved prior to addition, the impact of oxygen concentration is felt to be negligible.

Table 3 shows that a major fraction of original PCP was degraded and not simply accumulated in the biomass in live fungus. In the killed (autoclaved) fungus without silicon tubing control, 85% of the initial mass of PCP was extracted from the mycelium, and for the killed fungus with silicon tubing, 78.2% of the initial mass of PCP was recovered (See dashed box in Table 3). In runs with live fungus, with 210 and 216 U/L of initial ligninase activity, only 0.96 and 0.75% of the initial mass of PCP was recovered from the mycelium. Clearly, although little difference is seen in the aqueous PCP disappearance behavior, properly maintained fungal

Table 3. Summary of percentage of PCP mass remaining in the MMSGB after 70 hr

Run[a]	Remaining PCP mass, %	
	Liquid	Total cell
1	91.3	NA[b]
2	9.5	NA
3	9.2	78.2
4	12.0	85.4
5	4.9	0.96
6	3.9	0.75

[a]Run number for each experimental condition
1: Na-acetate buffer only
2: Silicon tubing in buffer solution only
3: Killed fungus with silicon tubing
4: Killed fungus without silicon tubing
5: Initial ligninase activity = 209 U/L
6: Initial ligninase activity = 216 U/L
[b]Not available
[c]Non detected
[d]Dashed box: system operated with fungus

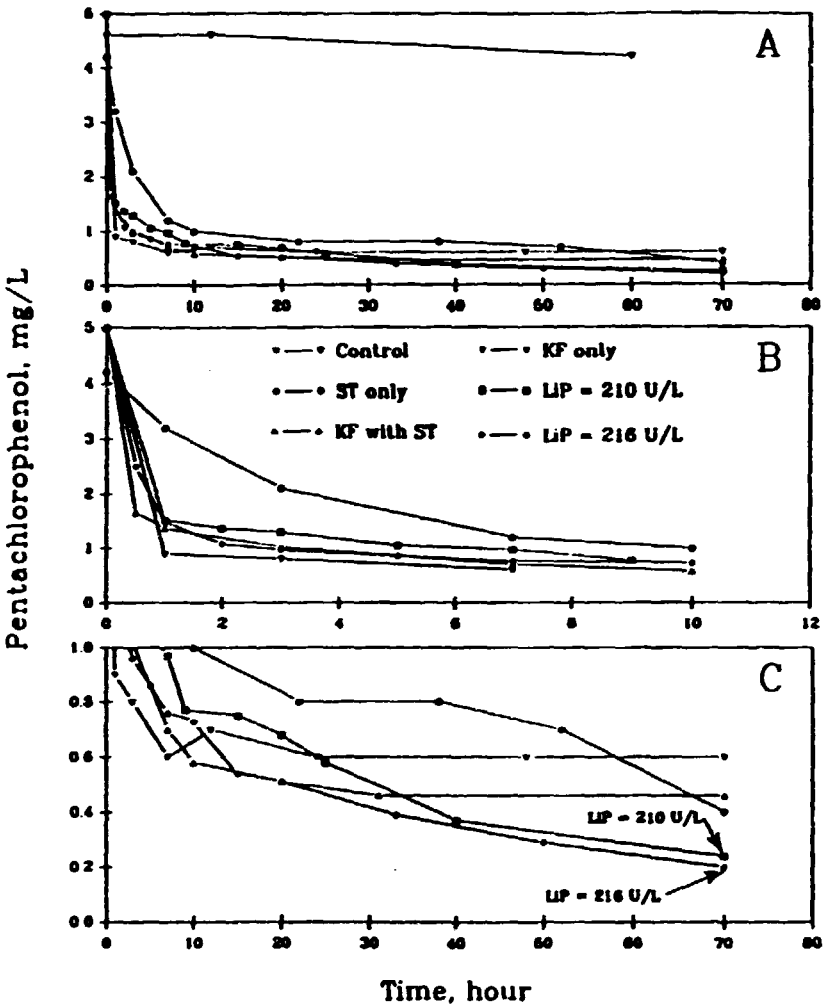


Figure 4. PCP disappearance at different experiment conditions in the MMSGB. ST = silicon tubing, KF = killed fungus, LiP = initial ligninase activity.

cultures are clearly able to degrade the PCP in the sorbed phase. This result shows that biodegradation of PCP in a MMSGB is possible since a difference between the PCP remaining in a killed fungus vs. live fungus was observed and PCP degradation by *P. chrysosporium* has been conclusively demonstrated in the literature [11,12]. The high degree of disappearance of PCP suggests that either the ligninase enzyme was present and active in the live fungus treatment in the MMSGB, or the adsorbed PCP was transported across the cell membrane and broken down by intracellular enzymes.

Upflow Fixed-film Bioreactor

The upflow fixed-film bioreactors were operated at an influent PCP concentration of approximately 4.5 mg/L starting 3 days after the nylon web medium was inoculated. Steady state with regard to effluent PCP concentration was reached after 6 days (> 100 hydraulic residence times) at several different HRTs. The steady state results are summarized in Table 4 and the residual PCP is plotted vs. hydraulic retention time in Figure 5.

A kinetic model was proposed for the upflow fixed-film bioreactor (UFB) under the following assumptions: (1) elimination of adsorption of PCP into the mycelium since PCP should be saturated with respect to the mycelium at steady state, (2) steady state,plug flow reactor hydraulics, and (3) quasi-first-order disappearance of the PCP model compound assuming the biomass is ligninolytic and produces a constant amount of ligninase activity. Under these assumptions, the plug flow model is shown in Equation 1.

$$V\frac{dC}{dz} = -kXC \qquad (1)$$

where $\frac{dC}{dz}$ = change in concentration of pollutant in reactor length, mg/L-cm, V = water velocity, L/T, X = dry biomass, g/L, and C = pollutant concentration, mg/L, and k is the quasi-first-order PCP degradation rate constant (L/g biomass-hr),. Equation 1 is integrated from C_0 to C_e and 0 to Z (reactor length) yielding

$$C_e = C_o e^{-kX\tau} \qquad (2)$$

where C_o and C_e are the influent and effluent organopollutant concentrations (mg/L), and τ is the hydraulic residence time (hr).

The data were correlated to estimate an "overall" organo-pollutant degradation rate coefficient. Using the steady state results in Table 4, the value of the quasi-first order rate

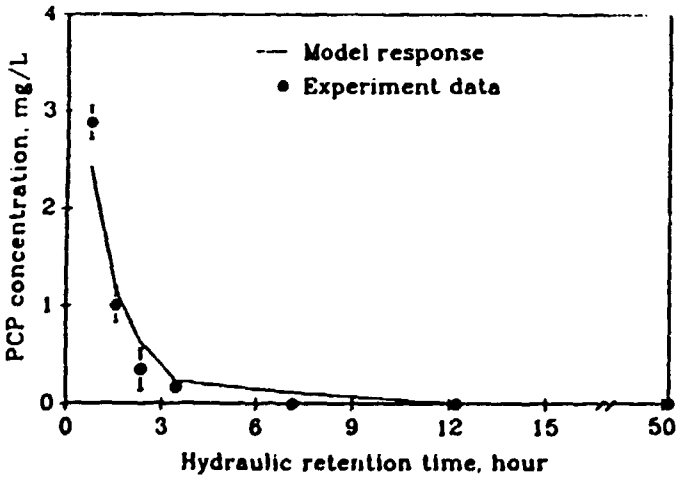


Figure 5. PCP concentration vs. hydraulic retention time in the fixed-film bioreactor.

Table 4. Summary of operation conditions and results for PCP degradation in the fluidized bed bioreactor

Run	Influent flow (ml/min)		HRT[b] (min)	Influent PCP (mg/L)		Effluent PCP (mg/L)		Removal efficiency (%)
	mean	SD[a]		mean	SD	mean	SD	
1	1.70	0.02	19.59	4.77	0.32	2.17	0.04	55
2	6.07	0.03	5.49	4.60	0.11	2.90	0.07	37
3	6.08	0.02	5.48	2.63	0.08	1.50	0.00	43
4	0.37	0.01	90.00	4.40	0.08	1.23	0.22	72

[a]Standard deviation
[b]Hydraulic retention time based on the influent flow rate

constant was estimated by fitting Eq. 2 to the steady state data using non-linear least squares regression. The predicted PCP concentrations are compared with the experimental results in Figure 5. The value of the rate constant was estimated to be 0.071 (± 0.015, 95% confidence interval (CI)) L/g biomass-hr. These results suggest that these PCP degradation data can be explained adequately with a quasi-first order model. Although the rate constant cannot be compared with others reported due to the different temperature and system type used, the value of 0.071 L/g biomass-hr compares well with rate constant of 0.048 L/g biomass-hr at 39°C using varied crude extracellular enzyme and cell mass concentrations in an agitated cultures reported by Lin et al. [12]. It is probable that the fungus is highly active in the fixed-film bioreactor, and the production of ligninase may be protected from shearing effects found in mixed systems by the presence of the support medium.

Fluidized Bed Bioreactor

The FBBR was operated under continuous mode with recycle for PCP degradation. Run 1 was carried out to steady state while the remaining Runs 2 through 4 were conducted under dynamic conditions by changing influent flow rate and/or influent PCP concentration. Operating conditions and experimental data are shown in Table 5.

Figure 6 shows the influent flow, and the influent and effluent PCP concentrations in the fluidized bed bioreactor for 4 runs over 27 days. At an influent PCP concentration of 4.77 (± 0.32 mg/L, standard deviation, σ based to replicate samples taken at steady state), the effluent rose rapidly from 0 to 4.0 mg/L at about 1 day before stabilizing at 2.17 (± 0.04 mg/L, σ) after 12 days of operation. The specific PCP degradation rate at steady state (Run 1) was 0.33 (± 0.008, 95% CI) mg/g biomass-hr. This yields a quasi-first order rate constant of 0.10 (± 0.0052, 95% CI) L/g biomass-hr based on the 24.1 g/L dry biomass at the end of the run, and influent flow rate.

The PCP degradation rate in the FBBR was 1.4 times higher than that in the upflow fixed-film bioreactor mention above. This is due, in part, to the fact that fluidized bed reactors typically provide an extremely large surface area for biological growth, which usually results in the development of large biomass concentrations greater than those typically attainable in fixed-bed or most other treatment systems. A large surface area would promote thinner biofilm and reduce mass transfer resistance to degradation. In this system, the particle surface area is estimated to be 7.08 m^2/L reactor volume.

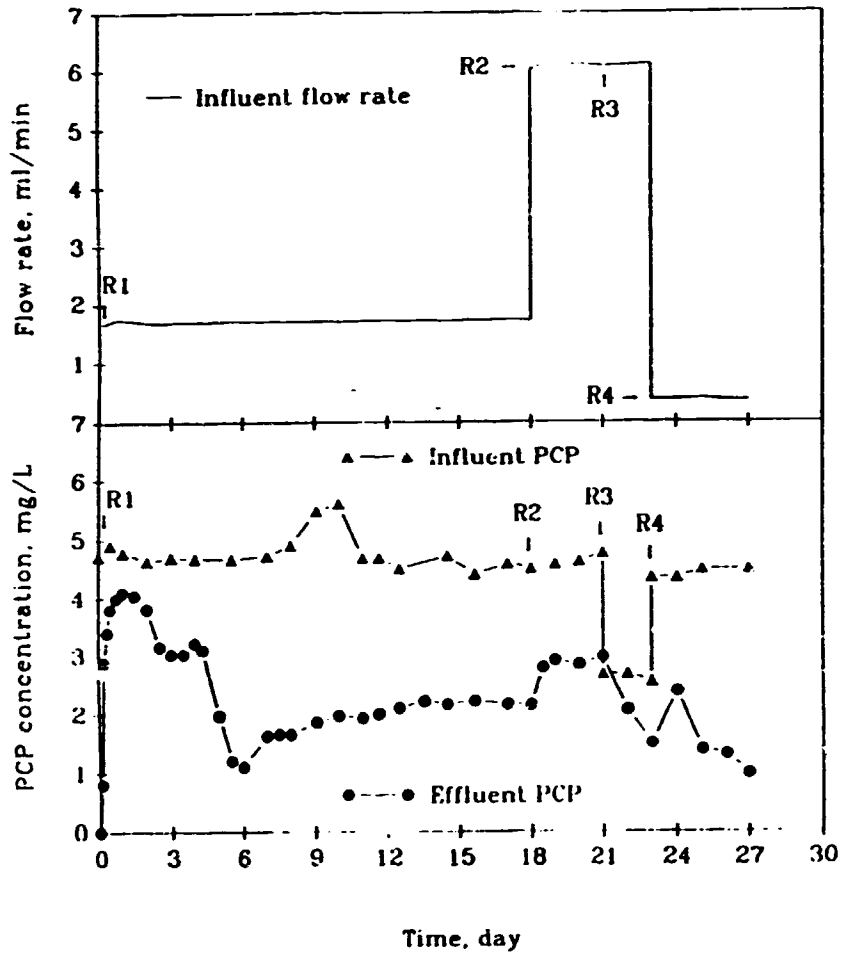


Figure 6. Influent flow rate, and PCP concentration vs. time in the fluidized bed bioreactor at 59 mL/min recycle flow rate: R1 = Run 1, R2 = Run 2, R3 = Run 3, and R4 = Run 4.

After 18 days of steady state operation, dynamic behavior was initiated by varying the influent flow rate and concentration of PCP. The effluent PCP concentration varied only slightly (± 0.73 mg/L, σ) at hydraulic residence times from about 5 to 90 minutes. However, decreasing the influent PCP concentration from 4.60 (± 0.11, σ) mg/L to 2.63 (± 0.08, σ) mg/L at a 5.4 minute hydraulic retention time resulted in a rapid drop in effluent PCP.

PCP removal efficiency was enhanced at increased hydraulic retention time or decrease influent flow rate. These results indicate that the PCP removal efficiency showed a significant dependence on influent flow rate (Runs 1, 2, and 4) and the influent PCP concentration (Run 3). Effluent PCP concentration is controlled by hydraulic retention time. In all of the

Ligninase Enzyme

The ligninase enzyme of *P. chrysosporium* has been implicated in the degradation of a variety of contaminants in batch cultures. A number of standard assays exist for determining lignin peroxidase enzyme activity, the most common of which is to monitor the production of veratryl aldehyde, an oxidation product of veratryl alcohol, which plays an important role in the ligninase system. In batch cultures a common component of the culture medium is the surfactant Tween 80 which is believed to provide protection for the enzyme against mechanical deactivation and holds the enzyme in the aqueous phase [16]. It was found that without Tween 80, enzyme activity could not be detected and it was assumed that the enzyme sorbs onto the fungal hyphae. Tween 80 was not a component of the FBBR feed for either contaminant and ligninase activity was not monitored in the experiments reported. However, the high degree of disappearance of PCP suggests that the ligninase enzyme was present and active in the fixed-film systems (UFB and FBBR) and was very likely sorbed onto the fungal biomass.

SUMMARY AND CONCLUSIONS

For the MMSGB using silicon tubing in batch mode, increasing initial ligninase activity increased the rate and extent of PCP disappearance. PCP disappeared initially by adsorption and did not accumulate in the biomass when operated with live fungus. Although little difference was seen in the aqueous PCP disappearance behavior due to adsorption, live properly maintained fungal cultures were clearly able to degrade PCP in the sorbed phase. This result is consistent with the expectation that PCP is degraded by *P. chrysosporium* via the ligninolytic system.

The quasi-first order kinetic model can be used for degradation of PCP in the fixed-film continuous system. The quasi-first order degradation rate constant was 0.071 L/g biomass-hr for PCP. An understanding of the kinetics of PCP degradation provides a foundation for process analysis and design for the optimum removal of the compound in treatment systems. Results indicated that efficient removal of PCP could be obtained at short hydraulic retention times.

A fluidized bed bioreactor using silica sand for support of attached fungal growth was developed for degradation of PCP for which the specific degradation rate at steady state was 0.33 mg/g biomass-hr (0.1 L/g biomass-hr based on the quasi-first order model) based on the influent flow rate. The disappearance of PCP can be directly ascribed to degradation by *P. chrysosporium*. Under dynamic conditions, PCP removal efficiency was significantly dependent on influent flow rate and influent PCP concentration.

On the basis of these experiments, the fluidized bed bioreactor with recycle mode using the white rot fungus *P. chrysosporium* shows considerable promise for degradation of PCP. Degradation was effective under conditions of short hydraulic retention time and the process was stable in the face of large variations in influent conditions.

ACKNOWLEDGMENTS

This research was supported by United States Geological Survey (USGS) Grant 14-08-0001-G1733. Very special thanks are extended to Dr. Steven D. Aust and Dr. Thomas A. Grover for their support in using analytical instruments in the Biotechnology Center.

REFERENCES

2. Rao, K. R. Pentachlorophenol:chemistry, pharmacology, and environmental toxicology. Plenum Press, New York and London. 1978.

3. Murthy, N. B. K., D. D. Kaufman, and G. F. Fries. Journal of Environmental Science and Health B14(1):1 (1979).

4. Lamar, R. T., J. A. Glaser, and T. K. Kirk. Soil Biology Biochemistry 22(4):433 (1990).

5. Lamar, R. T. and D. M. Dietrich. Applied and Environmental Microbiology 56(10):3093 (1990).

6. Moos, L. P., E. J. Kirsch, R. F. Wukasch, and C. P. L. Grady, Jr. Water Research 17(11):1575 (1983).

7. Bumpus, J. A. Applied and Environmental Microbiology 55(1):154 (1989).

8. Bumpus, J. A. and S. D. Aust. BioEssays 6:166 (1986).

9. Haemmerli, S. D., M. S. A. Leisola, D. Sanglard and A. Fiechter. The J. of Biological Chemistry 261(15):6900 (1986).

10. Hammel, K. E., B. Kalyanaraman, and T. K. Kirk. The Journal of Biological Chemistry 261(36):16948 (1986).

11. Mileski, G. J., J. A. Bumpus, M. A. Jurek and S. D. Aust. Applied and Environmental Microbiology 54(12):2885 (1988).

12. Lin, J., H. Y. Wang, and R. F. Hickey. Biotechnology and Bioengineering 35:1125 (1990).

13. Kirk, T. K., F. Schultz, W. J. Connors, L. F. Lorenz and J. G. Zeikus. Archives of Microbiology 117:277 (1978).

14. Leisola, M. S. A., D. C. Ulmer and A. Fiechter. Archives of Microbiology 137:171 (1984).

15. Tien, M. and T. K. Kirk. Methods in Enzymology 161:238 (1988).

16. Kang, G. Biodegradation of organopollutants in bench scale bioreactors using the white rot fungus *Phanerochaete chrysosporium*. Ph.D. Dissertation, Utah State University, Logan, Utah. 1993.

17. Cario, P.R., J. McElhaney, I.H. Suffet. J. American Water Works Association. 71(11):660 (1979).

Request reprints from
David K. Stevens, Assoc. Professor
UMC 4110
Utah State University
Logan, UT 84322-4110
stevens@quito.cee.usu.edu

HAZARDOUS WASTE & HAZARDOUS MATERIALS
Volume 11, Number 3, 1994
Mary Ann Liebert, Inc., Publishers

Biodegradation of Pentachlorophenol in Soil Amended with the White Rot Fungus *Phanerochaete chrysosporium*

RONGHUI LIANG and MICHAEL J. McFARLAND

Utah Water Research Laboratory
Department of Civil and Environmental Engineering
Utah State University
Logan, UT 84322-8200

ABSTRACT

The effects of bioaugmentation of pentachlorophenol (PCP)-contaminated soil with the fungus *Phanerochaete chrysosporium* was evaluated using a chemical mass balance approach. While there was no observed PCP removal for the unamended soil, 93% of the initial mass of PCP was removed within 56 days in soil amended with *P. chrysosporium*-inoculated corncobs. Within the same incubation period, approximately 58% of the initial mass of PCP was removed from soil amended with uninoculated corncobs. PCP removal followed first order kinetics with rate constants of 0.04 ± 0.017 day^{-1}, 0.06 ± 0.015 day^{-1}, and 0.018 ± 0.006 day^{-1} for PCP contaminated soils amended with fungus-inoculated sterile corncobs, fungus-inoculated nonsterile corncobs, and uninoculated nonsterile corncobs, respectively. Comparison of the nonsterile systems indicated that the presence of the fungus increased the first order removal rate constant by more than 200% compared to the uninoculated system. The extent of PCP mineralization after 56 days of incubation was 7%, 17%, and 8% for PCP contaminated soil amended with fungus-inoculated sterile corncobs, fungus-inoculated nonsterile corncobs, and uninoculated nonsterile corncobs, respectively. The extent of bound residue formation was 13%, 19%, and 27% for PCP contaminated soil amended with fungus-inoculated sterile corncobs, fungus-inoculated nonsterile corncobs, and uninoculated nonsterile corncobs, respectively. Mass balance analyses indicated that soil aeration may enhance removal of PCP and/or its chemical intermediates through a process of facilitated transport and/or volatilization.

INTRODUCTION

Pentachlorophenol (PCP) is used primarily as a wood preservative and pesticide [1]. As PCP is toxic to most life forms, its widespread use and release to the environment has caused concern to the U.S. Environmental Protection Agency (U.S. EPA). Direct releases by accidental spillage from the timber, energy, textile, and agricultural industries are, by far, the most important sources of PCP in the environment. The majority of PCP released to the environment is associated with soil [2].

Several treatment approaches have been proposed for the remediation of PCP-contaminated soil. One of the most promising is bioremediation using the white rot fungus *Phanerochaete chrysosporium*. This fungus has been extensively studied and it has been reported to be effective in degrading PCP in both liquid and soil systems [3, 4, 5].

Due to their modified chemical structure, PCP intermediates produced by *P. chrysosporium* may be more easily metabolized by indigenous soil microorganisms than PCP itself. Consequently, *P. chrysosporium* may establish synergistic relationships with other microbial populations in the PCP

of *P. chrysosporium* in a microbially active, oil tar-contaminated soil was synergistic in [illegible] mineralization.

The goal of the present study was to evaluate the effect of bioaugmentation in the remediation of PCP-contaminated soil using the white rot fungus *P. chrysosporium*.

MATERIALS AND METHODS

Soil

The soil used in this study was Kidman sandy loam. The soil characteristics are reported in Table 1.

Chemicals

Pentachlorophenol (PCP, 99% purity), 2,4,6-tribromophenol (TBP, 99% purity), and uniformly ^{14}C-labeled pentachlorophenol (specific activity 5.8 mCi/mmol, radiochemical purity > 98%) were purchased from Sigma Chemical Co. (St. Louis, MO).

Fungus

Phanerochaete chrysosporium strain BKM-F1767 was obtained from the Biotechnology Center, Utah State University, Logan, UT. The fungus was grown and stored on a 2% malt extract agar slant at 4°C before use in this study.

TABLE 1
Characteristics of Kidman Sandy Loam Soil[a]

Characteristics	Classification	Typic Haplustoll
Texture		Sandy loam
	Sand	52%
	Silt	32%
	Clay	16%
Physical		
	Bulk density	1.5 g/cm^3
	Moisture (@ 1/3 bar)	12.44%
Chemical		
	pH	7.9
	Cation exchange capacity	10.1 meq/100g
	Organic carbon	0.68%
	Iron	9.0 ppm
Biological		
	Bacteria	6.7 x 10^6 cell/gm
	Fungi	1.9 x 10^4 cell/gm

[a]Analyzed by the Soil, Plant and Water Analysis Laboratory at Utah State University [illegible]

Preparation of Fungus Inoculum

To prepare the fungal inoculum, fungal spores were first harvested and mixed with double-deionized water (DDW). The fungal spores were then added to growth media (fifty milliliters of 2% malt extract) which had been previously autoclaved at 121°C for 15 minutes in 125 ml Erlenmeyer flasks. One milliliter of the aqueous fungal solution was added to each flask and mixed with the liquid medium. The flasks were shaken at 270 rotations per minute (Lab-Line, junior orbit shaker) at room temperature for seven days. The fungal solution was then used to inoculate both sterile and nonsterile corn cobs. The sterile corncobs (Mt. Pulaski Products, Inc., Mt. Pulaski, IL) were prepared aseptically by autoclaving at 121°C for one hour over three successive days.

Experimental Design and Set-up

A description of each soil treatment is given in Table 2. Treatment 1 was used as a control to evaluate the ability of indigenous soil microorganisms to remove PCP. In Treatment 2, PCP contaminated soil was amended with fungal inoculated sterilized corn cobs. In Treatment 3, PCP contaminated soil was amended with fungal inoculated nonsterile corn cobs. In Treatment 4, nonsterile uninoculated corn cobs were added to PCP contaminated soil. Finally, in Treatment 5, nonsterile corn cobs were added to clean (i.e., uncontaminated) soil.

TABLE 2
Inoculation and Substrates Introduced into the Nonsterile Kidman Soil

Treatment	Fungus	Corncob	PCP (mg/kg)
1	NA[a]	NA	100
2	*P. chrysosporium*	S[b]	100
3	*P. chrysosporium*	NS[c]	100
4	NA	NS	100
5	NA	NS	NA

[a]Not applicable
[b]Sterile
[c]Nonsterile

Erlenmeyer flasks (125 ml) were used as microcosms in the experimental program. Microcosms were sealed with rubber stoppers equipped with two Teflon® tubes with two-way Luer-Lok® stopcocks which served as inlet and outlet valves. To each microcosm, 10g of soil (dry weight) were added. Except for blank microcosms (Treatment 5), 100 µl a of PCP stock solution were added to each microcosm and thoroughly mixed with a glass stirring rod. The initial PCP concentration in soil was 100 mg/kg. To monitor PCP mineralization and to conduct a mass balance evaluation, an initial radioactivity (^{14}C-PCP) loading of 40,000 disintegration per minute (DPM) was added to each microcosm (Treatments 1 through 4). The mass of radiolabeled PCP represented less than 1% of the total PCP added to the microcosms.

The water content of each microcosm was adjusted to 60% of field capacity by adding sterile double-deionized water (DDW). For microcosms with corncob amendments, five grams (dry weight) of ground corncobs were added to soil using a vortex mixer. Before being added to microcosms, the moisture content of the corn cobs was measured. If the moisture content were less than 60%, the corncob moisture was adjusted to approximately 60% with sterile DDW. The microcosms were periodically weighed, with moisture added, if necessary, to maintain the proper water content (i.e., 60%).

Microcosms were connected by a Nalgene™ tube manifold system which allowed the oxygen gas to evenly evacuate a number of microcosms at the same time. The microcosms were purged with

To evaluate PCP removal kinetics, [illegible] microcosm replicates were prepared for Treatments 1 through 4. Eight microcosm replicates were prepared for Treatment 5. Each set of microcosms included triplicate samples for Treatments 1 through 4 and duplicate samples for Treatment 5. The sacrificed microcosms were chosen randomly using a random digits table. Soil sampling occurred on days 0, 7, 14, 28, and 56. On each sampling day, one set of microcosms was removed from the incubator and extracted for residual PCP and radioactivity. All microcosms were incubated in the dark at 28°C.

Prior results showed that during fungal amended soil treatment, insignificant ^{14}C-labeled volatile organics were evolved. Therefore, only the volatile CO_2 trapping impingers were used in the present study. The CO_2 trapping solution was a mixture of monoethanolamine, methanol, and Ready Gel™ scintillation cocktail solution (1:4:5 by volume) [8]. Each impinger contained 15 ml of trapping solution. Fresh trapping solution was used for each oxygen gas purging event. After purging, the CO_2 trapping solution was counted by liquid scintillation (Beckman, model LS 6000SE).

Soil Extraction and PCP Analysis

Soil samples were extracted for total PCP remaining in the microcosm according to procedures modified from Middeldorp, Briglia, and Salkinoja-Salonen [9]. At the appropriate sampling day, the soil, with or without corncobs, was transferred to a 125 ml I-Chem jar (I-Chem, New Castle, DE). Approximately 0.5 ml of a 50% analytical quality sulfuric acid and 50 ml analytical quality acetone solution were added. The mixture was sonicated for 5 minutes (Heat Systems, model XL2020™ ultrasonic liquid processor, equipped with a standard probe) after which it was mixed (Lab Line, junior orbit shaker) at 275 rpm for 20 minutes. The mixture was then centrifuged at 2000 rpm (480 x g) for 10 minutes (Beckman, model J2-21 centrifuge). A portion of the supernatant was transferred to a 10 ml volumetric flask for dilution. An internal standard (2,4,6-tribromophenol) was added to account for variations in instrument response.

PCP was quantified using a Hewlett-Packard 5880A gas chromatograph equipped with a Restek RTX-1 fused silica capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film) and a ^{63}Ni electron capture detector. The average PCP extraction recovery efficiency was 98.70±10.14%. Statistical analysis showed that this recovery efficiency is not significantly below 100%.

Mass Balance Analysis

Samples for mass balance evaluation were extracted according to the soil extraction procedures previously described. After centrifugation of the soil slurry, the supernatant was decanted into a 100 ml volumetric flask. The extraction procedure was repeated using an additional 40 ml of acetone as extraction solvent. The supernatant from this slurry was also decanted into the volumetric flask. Acetone was then added to bring the volume to 100 ml.

One milliliter of the extract was mixed with 1 ml of methanol and 4 ml of Ready Gel™ scintillation cocktail solution. The radioactivity of the mixture was counted using liquid scintillation.

The residual soil was air dried overnight at room temperature (22°C) then homogenized using a ceramic pulverizer. One gram of the pulverized material was combusted using a biological oxidizer (R.J. Harvey, model OX600). The $^{14}CO_2$ evolved from the oxidizer was collected and counted by liquid scintillation.

The total radioactivity recovery was calculated by summing the radioactivity in the acetone extract, the radioactivity in the CO_2 trapping solution, the radioactivity adsorbed by the flask and the radioactivity bound in the soil matrix.

Statistical Analysis

Two-way analysis of variance for PCP removal and mineralization was conducted using the SAS® GLM procedure [10]. The experiment was designed to have three observations for each treatment and time combination. However, some of the combinations had only two observations because several data points were rejected or missing. The effects model was established and the type III sums of squares hypotheses were tested. The means analysis and contrast analysis were also

RESULTS

PCP Removal

PCP removal was determined by measuring PCP concentrations in solvent extracts over time. Extraction of clean soil (Treatment 5) indicated no PCP in any of the samples analyzed. Therefore, only results from the first four treatments are presented (Figure 1).

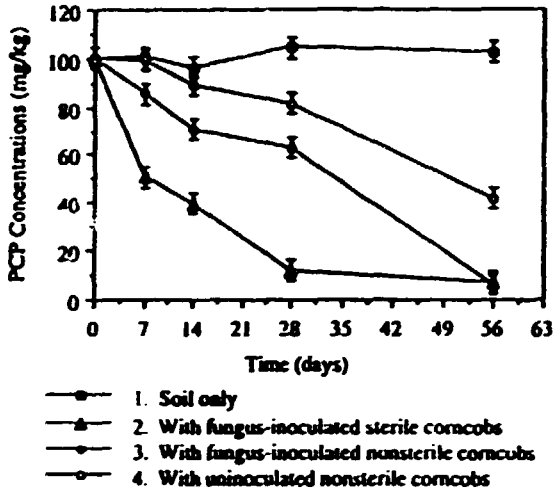


FIGURE 1
Removal of Pentachlorophenol in Soil with Various Treatments. Error Bars Denote Least Significant Differences, LSD = 8.38 mg/kg.

The two-way analysis of variance with contrast for PCP percent removal was conducted. Time, treatment, and the interaction of time and treatment had a significant effect on PCP removal ($P = 0.0001$). The contrast analysis indicated that PCP removal amongst the four treatments was significantly different from each other ($P = 0.0001$).

For Treatment 1, no PCP degradation was observed over the entire treatment period. For Treatment 2, 49.0±4.6% (s.d.) and 88.1±4.2% (s.d.) of PCP was removed after 7 days and 30 days, respectively. However, only 4.8% of PCP was removed between days 30 and 56. This may have been due to nutrient limitations or reduced bioavailability of PCP [11]. These data were similar to those published by Lamar, Glaser, and Kirk [4] in which it was reported that the majority of PCP was removed in the first week of treatment.

For Treatment 3, 37.1±21.4% of PCP was removed after 28 days of incubation. Approximately 10% of initial PCP loading occurred in the first week of treatment. The reason for this is unclear but it is possible that indigenous non-PCP degrading microorganisms may have predominated during the first month of incubation. Previous work with *P. chrysosporium* amended Kidman soil revealed the presence of various indigenous fungi including, *Penicillum*, *Aspergillus*, *Trichoderma*, and various species of bacteria [11]. These fungi and other indigenous microbes may have limited the growth of Phanerochaete chrysosporium by out competing for scarce nutrients in the early stages of incubation. The difference in rates and extent of PCP removal can be attributed to microbial competition. In Treatment 2, the concentration of Phanerochaete chrysosporium cells was

grow quickly resulting in rapid removal of PCP. In Treatment 3, it is assumed that there were significant numbers of other microbes associated with corn cobs. These other microbes limited the growth of Phanerochaete chrysosporium in the early stages of incubation together with the rate and extent of PCP removal. However, by day 56, Phanerochaete chrysosporium had become predominant which is reflected in the enhanced PCP removal.

For Treatment 4, 57.9±13.2% of PCP was removed within 56 days. The PCP removal was significantly enhanced by the introduction of the nonsterile corncobs. Since corncobs had not been autoclaved, microorganisms associated with corn cobs may have contributed to PCP removal.

Reaction Kinetics and PCP Half-life Estimates

Except for Treatment 1, PCP removal data fit the first order reaction model. The estimated half-life of PCP supported the observation that the addition of ground corncobs, either with or without *P. chrysosporium* inoculation, enhanced PCP biodegradation. The PCP first order reaction rate constants were estimated using the SAS® REG procedure [12]. Based on the estimated rate constants, PCP half-lives were calculated. Table 3 summarizes the estimated reaction rate constants and PCP half-lives for each of the treatments.

TABLE 3
First Order Reaction Kinetics and Half-Life for Pentachlorophenol in Soil[a]

	Rate Constant k (day^{-1})		Half-life $t_{1/2}$ (day)			
Treatment[b]	Estimates	95% CI[c]	Estimates	95% CI	R^2	*P*
1	0.000949	0.00329	726.24	1859.2	0.0504	0.5328
2	0.04084*	0.01685	16.97	7.24	0.7897	0.0006
3	0.05594*	0.01542	12.39	3.48	0.8644	0.0001
4	0.01809*	0.00629	38.32	13.67	0.8000	0.0001

*Significantly different from zero at the 95% confidence level.
[a]Based on the overall PCP removal data (8 weeks).
[b]Treatment:
1: Soil only;
2: With fungus-inoculated sterile corncobs;
3: With fungus-inoculated nonsterile corncobs;
4: With uninoculated nonsterile corncobs.
[c]95% Confidence Interval.

Comparison of Treatments 3 and 4 (Table 3) provide an estimate of the effects of fungal inoculation on the rate of contaminated removal. The first order PCP removal rate constant estimated for soil amended with nonsterile corn cobs was 0.01809 day^{-1} which was significantly lower than the rate constant of 0.05594 day^{-1} recorded for soil amended with fungal inoculated organic amendment. These data suggest that over the 56 day treatment period, the fungal inoculation increased the removal rate constant by over 200%. The enhanced removal rate constants are also reflected in the chemical half life calculations in which it was found that fungal inoculation reduced the PCP chemical half life from 38.32 days (estimated with the addition of uninoculated organic amendment) to 12.39 days.

PCP Mineralization

time and treatment all had a significant effect on PCP removal ($P = 0.0001$). The least significant difference (LSD) was 1.14 %. The contrast analysis indicated that Treatments 2, 3, and 4 were significantly different from Treatment 1 ($P = 0.0001$, 0.0001, and 0.0002, respectively). Treatment 3 was also significantly different from Treatments 2 and 4 ($P = 0.0001$). However, Treatments 2 and Treatment 4 were not significantly different ($P = 0.1523$) (Figure 2).

Throughout the 56 days of incubation, no significant PCP mineralization was detected in Treatment 1. PCP mineralization from Treatment 2 was observed only after 7 days of incubation. The average PCP mineralization was 4.35±0.51% after 28 days of incubation, but only 6.80±1.53% after 56 days of incubation. For Treatment 3, a 2-week lag phase occurred after which the extent of PCP mineralization was 5.73±2.05% on day 28 and 17.25±3.64% on day 56. In Treatment 4, more than 3 weeks were required to produce detectable levels of radioactive carbon dioxide. The average extent of PCP mineralization was 1.33±0.33% on day 28 and 7.94±3.43% on day 56.

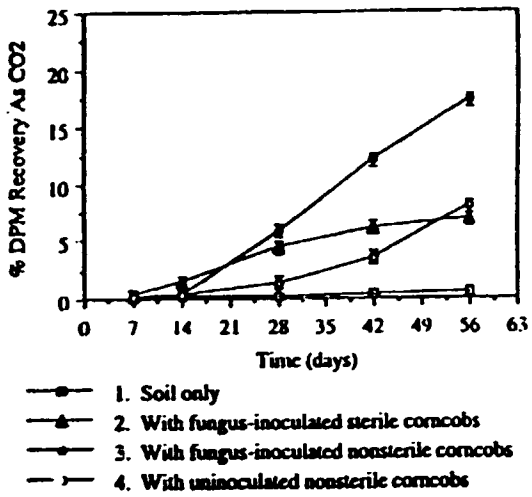


FIGURE 2
Mineralization of Pentachlorophenol in Soil with Various Treatments. Error Bars Denote Least Significant Differences, LSD = 1.14%

Mass Balance Analysis

The radioactivity recoveries after 56 days of incubation are summarized in Table 4. The total mass balance after the 56 days incubation was 91.28±1.65%, 54.38±3.32%, 75.78±0.11%, and 82.80±4.93% for Treatments 1, 2, 3, and 4, respectively. There was concern over the poor mass balance achieved for Treatment 2. A possible explanation for this loss is the volatilization of PCP or its chemical intermediates. Before evaluating the extent of organic compound volatilization, the percentage of PCP derived intermediates was estimated.

Comparison of both the PCP and radioactivity recovered in the acetone extracts after 56 days of incubation is shown in Figure 3. From Figure 3, all of the radioactivity recovered in Treatment 1 was from the activity of ^{14}C-PCP. For Treatments 2, 3, and 4, the percent radioactivity recoveries were higher than the percent PCP recoveries. The differences between these two measurements, which ranged from 2% to 30%, would be an estimate of the PCP chemical intermediate concentrations. The

TABLE 4
Mass Balance of Pentachlorophenol After 56 Days of Incubation

Treatment[a]	Radioactivity Recovery (%)				
	Acetone Extract	Mineralization	Soil Combustion	Flask Adsorption	Total Balance
1	83.71 (2.75)[b]	0.49 (0.07)	6.53 (1.28)	0.55 (0.33)	91.28 (1.65)
2	31.19 (2.76)	6.80 (1.53)	12.63 (2.75)	3.78 (1.51)	54.38 (3.32)
3	37.27 (2.21)	17.25 (3.64)	19.03 (1.61)	2.23 (0.08)	75.78 (0.11)
4	46.74 (7.40)	7.94 (3.43)	26.67 (1.72)	1.45 (1.37)	82.80 (4.93)

[a]Treatment:
1: Soil only;
2. With fungus-inoculated sterile corncobs;
3. With fungus-inoculated nonsterile corncobs.
4: With uninoculated nonsterile corncobs.

[b]Values in parentheses denote standard deviations

Organic Compound Volatilization

To evaluate contaminant volatilization, the PCP mineralization and mass balance experiments were repeated for Treatments 2 and 3. The incubation period was 21 days. The experimental set-up and procedures used were the same as those described earlier. However, a piece of polyurethane foam was placed at the outlet of the microcosm to trap volatile organics. At the end of the incubation period,

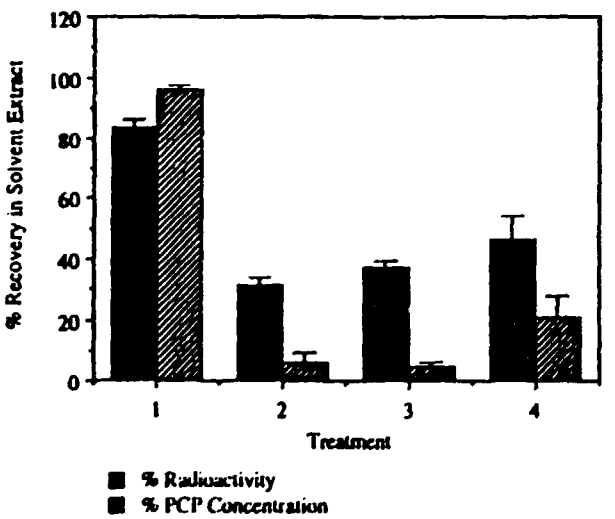


FIGURE 3

the polyurethane foam was extracted with a mixture of acetone and methylene chloride (1:1 by volume) using a Soxhlet extractor. Table 5 summarizes the radioactivity recovery.

The radioactivity recovery from the polyurethane foam extract was 6.01±2.55% for Treatment 2, and 4.21±0.34% for Treatment 3. The results suggest that a portion of the radioactivity lost in the microcosms of Treatments 2 and 3 of the previous experiment may have been volatilized.

Factors contributing to radioactivity loss during the initial soil treatment study may include: 1) inadvertent removal of particulate matter (e.g., fungal spores, soil, etc.) to which PCP or its intermediates may have been adsorbed during soil aeration (i.e., facilitated transport), or 2) enhanced radioactivity removal through PCP partitioning into the gas phase (it should be noted that the PCP vapor pressure increased from 1.7×10^{-4} mm Hg at room temperature 22°C to approximately 6.8×10^{-4} mm Hg at the incubation temperature 28°C [13]).

TABLE 5

^{14}C Recovery After 21 Days of Incubation with a Polyurethane Foam Volatile Organic Trap

Treatment[a]	Radioactivity Recovery (%)					
	Acetone Extract	Foam Extract	Mineralization	Soil Combustion	Flask Adsorption	Total Balance
2	61.32(6.05)[b]	6.01(2.55)	2.22(0.08)	12.28(0.96)	4.81(0.23)	86.64(4.66)
3	53.00(2.20)	4.21(0.34)	2.18(0.35)	14.78(0.37)	4.40(0.29)	78.57(2.07)

[a]Treatment:
2: With fungus-inoculated sterile corncobs;
3: With fungus-inoculated nonsterile corncobs.

[b]Values in parentheses denote standard deviations.

DISCUSSION AND CONCLUSIONS

The present laboratory study demonstrated statistically that the bioremediation of pentachlorophenol (PCP) contaminated soils may be significantly enhanced by employing a bioaugmentation approach using the white rot fungus *Phanerochaete chrysosporium*. PCP removal rates were enhanced by more than an order of magnitude using bioaugmentation under suboptimal fungal growth temperatures and without the addition of inorganic nutrients other than those contained in the organic amendment.

The contaminant half life was reduced from 730 days (no amendment) to 12 days with the addition of fungal inoculated corn cobs. Addition of uninoculated organic matter also led to enhanced PCP removal rates with an estimated contaminant half life of 38 days observed in soils amended with nonsterile corn cobs.

Mass balance analyses revealed that the major mechanism for PCP removal from soil was biological conversion to nonvolatile chemical intermediates. Approximately 50% of these chemical intermediates were solvent (*e.g.*, acetone) extractable while the remainder were nonextractable (*i.e.*, soil bound) residue. Fungal mediated bound residue formation has been previously observed to be a major removal mechanism for organic contaminants in soils [14]. Although PCP adsorption on the corn cobs may account for some loss of the compound, at the pH of the soil (i.e., 7.9), over 99% of the PCP would have been in its ionized pentachlorophenolate form.

Although transformation to chemical intermediates was the predominant removal mechanism, mineralization (*i.e.*, conversion to CO_2) was found to be significant in soils amended with fungus inoculated nonsterile corn cobs. With the addition of fungal amended organic matter, over 17% of the initial radioactivity could be accounted for as radioactive carbon dioxide. For all other treatments, the extent of PCP mineralization was significantly less than 10%.

PCP removal rates in soil reported in the present study are in general agreement with work

the present laboratory study. Moreover, like the present study, nonvolatile chemical intermediate production was found to be the predominant PCP removal mechanism.

Although loss of PCP through volatilization was previously found to be negligible, loss of PCP or its chemical intermediates during the present study may have been enhanced during the soil aeration process through the mechanism of "facilitated transport". This mechanism involves the adsorption of PCP or its chemical intermediates to soil particles which then may be removed in the gas phase during microcosm head space evaluation. This contaminant removal process would be a concern only under conditions where the soil aeration rate is sufficient to cause air borne mobilization of soil particulate matter.

ACKNOWLEDGMENTS

The authors would like to thank the Electric Power Research Institute (EPRI) and Dr. Ishwar P. Murarka (Program Manager) for their support of this research program under Contract No. RP2879-9. The authors would also like to express their appreciation to Dr. Ronald C. Sims and Dr. Darwin L. Sorensen for their technical evaluation of this effort. The authors would also like to thank Ms. Ivonne Cardona Harris for the preparation of this manuscript.

REFERENCES

1. Crosby, D.G. Environmental Chemistry of Pentachlorophenol. A Special Report on Pentachlorophenol in the Environment. Pure and Applied Chemistry 53:1051-1080 (1991).

2. Wild, S.R., Harrad, S.J. and Jones, K.C. Pentachlorophenol in the UK Environment, I: A Budget and Source Inventory. Chemosphere 24:833-845 (1992).

3. Mileski, G.J., Bumpus, J.A., Jurek, M.A. and Aust, S.D. Biodegradation of Pentachlorophenol by the White Rot Fungus *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 54:2885-2889 (1988).

4. Lamar, R.T., Glaser, J.A. and Kirk, T.K. Fate of Pentachlorophenol (PCP) in Sterile Soils Inoculated with the White-rot Basidiomycete *Phanerochaete chrysosporium*: Mineralization, Volatilization and Depletion of PCP. Soil Biology and Biochemistry 22:433-440 (1990).

5. Lamar, R.T. and Dietrich, D.M. In Situ Depletion of Pentachlorophenol From Contaminated Soil by *Phanerochaete* spp. Applied and Environmental Microbiology 56:3093-3100 (1990).

6. Ruttimann, C., Vicuna, R., Mozuch, M.D. and Kirk, T.K. Limited Bacterial Mineralization of Fungal Degradation Intermediates from Synthetic Lignin. Applied and Environmental Microbiology 57:3652-3655 (1991).

7. Brodkorb T.S. and Legge, R.L. Enhanced biodegradation of phenanthrene in oil tar-contaminated soils supplemented with *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 58:3117-3121 (1992).

8. Abbott, C.K., Sorensen, D.L. and Sims, R.C. Use and efficiency of ethylene glycol monomethyl ether and monoethanolamine to trap volatilized [7-^{14}C]naphthalene and ^{14}CO2. Environmental Toxicology and Chemistry 11:181-185 (1992).

9. Middeldorp, P.J., Briglia, M. and Salkinoja-Salonen, M.S. Biodegradation of Pentachlorophenol in Natural Soil by Inoculated *Rhodococcus chlorophenolicus*. Journal of Microbial Ecology 20:123-139 (1990).

10. SAS. The SAS System, Release 6.07. SAS Institute Inc., Cary, North Carolina, (1989).

11. Qiu, X.J. Benzo(a)pyrene Transformation in Soil Composting Process Using White Rot Fungus, *Phanerochaete chrysosporium*. Unpublished MS thesis. Merrill Library, Utah State

12. SAS. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, North Carolina. 956 p. (1985).

13. Carswell, T.S. and Nason, H.K. Properties and Uses of Pentachlorophenol. Journal of Industrial Engineering Chemistry 30:622-626 (1938).

14. Qiu, X.J. and McFarland, M.J. Bound Residue Formation in PAH Contaminated Soil Composing Using *Phanerochaete chrysosporium*. Journal of Hazardous Wastes and Hazardous Materials 8(2):115-126 (1991).

Address Reprint Requests to

Dr. Michael J. McFarland
Utah State University
Utah Water Research Laboratory
Logan, UT 84322-8200

Adsorption and Biodegradation of Pentachlorophenol by Polyurethane-Immobilized *Flavobacterium*

Zhong-Cheng Hu,[†] Roger A. Korus,*[,†] William E. Levinson,[‡] and Ron L. Crawford[‡]

Department of Chemical Engineering and Department of Bacteriology and Biochemistry, The Center for Hazardous Waste Remediation Research, University of Idaho, Moscow, Idaho 83843

Flavobacterium cells immobilized in polyurethane foam were used to degrade pentachlorophenol (PCP). Adsorption of PCP by the polyurethane immobilization matrix played an important role in reducing the toxicity of PCP. The adsorption followed the Langmuir model with q_m = 21.3 mg of PCP/g of dry foam and K_d = 28.7 mg/L for cell-free foams. Cell growth contributed significantly to PCP removal rates especially when the degradation medium was supplemented with a utilizable carbon source. A substrate inhibition growth model described PCP consumption by *Flavobacterium* with model parameters $\mu_m = 0.005\ min^{-1}$, $K_s = 25$ mg/L, $K_i = 1.5$ mg/L, $Y_{X/S} = 0.12$ g of cell/g of PCP, and $k_d = 3.33 \times 10^{-5}\ min^{-1}$. PCP removal was enhanced by the presence of utilizable compounds such as sodium glutamate and glucose and was inhibited by non-utilizable *p*-cresol. For process applications, a dynamic bed reactor, which created convective flow through the immobilization matrix, was designed to enhance the mass transfer rates of PCP into foam and CO_2 elution from the foam.

Introduction

Many bacteria, including *Rhodococcus*, *Mycobacterium*, *Flavobacterium*, *Arthrobacter*, and *Alcaligenes*, degrade chlorophenols (*1*). *Flavobacterium* sp. ATCC 39723 is especially efficient at the degradation of pentachlorophenol (PCP). PCP is an EPA priority pollutant and has been widely used as a wood preservative (*2*). PCP can be completely mineralized into CO_2 and Cl^- by *Flavobacterium* sp. ATCC 39723 (*3, 4*). The maximum concentration of PCP tolerated by free *Flavobacterium* sp. ATCC 39723 is 150–200 mg/L. In order to increase cell viability at high PCP concentrations and for the retention of biomass in a continuous reactor, *Flavobacterium* sp. ATCC 39723 cells were immobilized in calcium alginate beads and polyurethane foam (PUF) in our lab (*5–7*). PUF strongly adsorbed PCP (*7*). The adsorption–biodegradation with the PUF-immobilized cell system was superior to traditional adsorption–regeneration by physical/chemical processes because regeneration of the PUF adsorbent matrix was achieved by PCP degradation and mineralization. However, adsorption data for the PUF–PCP system and the effects of adsorption on PCP biodegradation have not been reported other than limited data by O'Reilly and Crawford (*7*).

This work examined the adsorption and biodegradation of PCP using PUF-immobilized *Flavobacterium* sp. ATCC 39723. PUF-immobilized cell reactors were compared with calcium alginate immobilized cell reactors. Both free- and immobilized-cell degradation kinetics were modeled. Strategies for PCP removal rate enhancement through physiological, mass-transfer, and toxicological considerations were investigated.

Materials and Methods

Chemicals. Pentachlorophenol (99+% pure, chemical grade) and [U-^{14}C]pentachlorophenol were purchased from Sigma Chemical Co., St. Louis, MO. Biosafe II scintillation cocktail was from Research Products International Corp., Mount Prospect, IL. Polyurethane prepolymer HYPOL FHP3000 was obtained from W. R. Grace Co., Lexington, MA. HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] and all other chemicals used were from Sigma Chemical Co., St. Louis, MO.

Microorganism and Cell Cultivation. The bacterium used in this study was a PCP-degrading *Flavobacterium* sp. ATCC 39723 (*3*). The strain was routinely subcultured from frozen stocks (2 mL, containing 20%, vol/vol, DMSO as a cryoprotectant) by addition to a 250-mL flask containing 100 mL of defined minimal medium (3.6 mM K_2HPO_4, 1.4 mM KH_2PO_4, 5.9 mM $NaNO_3$, 0.4 mM $MgSO_4$, 4 g/L sodium glutamate, pH 7.3). Iron in the form of $FeSO_4$ was added to the sterilized medium to a final concentration of 50 μM from a filter-sterilized 50 mM $FeSO_4$ aqueous solution. Cultures were grown at 200 rpm in flasks on an orbital shaker at 25–30 °C. Growth was monitored with a UV/visible spectrophotometer by measuring absorbance at 560 nm. When absorbance at 560 nm reached 0.5, the 100 mL cultures were transferred into 1 L of sterilized culture medium in 2-L flasks. Transferred *Flavobacterium* sp. ATCC 39723 cultures were grown again on sodium L-glutamate and induced for PCP-degrading activity at mid-log phase (absorbance about 0.5) by the addition of 50 mg/L PCP from a 10 000 mg/L stock solution prepared in 0.25 N NaOH. Degradation of PCP was monitored spectrophotometrically by measuring culture supernatant solution absorbance at 320 nm. When approximately 80–90% of the PCP was degraded and absorbance at 560 nm reached 1.0–1.5, cultures were centrifuged at 10 000 rpm and 4 °C for 15 min. Cell slurries thus obtained were mixed well with cold, sterile HEPES immobilization buffer (HIB: 50 mM HEPES, 1 mM $MgSO_4$, 0.5 g/L sodium glutamate, 20 μM $FeSO_4$, pH 7.3) to give 10–50% (cell wet weight/liquid volume) cell–HIB suspensions and then stored at 4 °C.

Analytical Methods for PCP Degradation. When the disappearance of PCP in liquid medium was due only to biodegradation by the *Flavobacterium* sp. ATCC 39723, degradation was monitored at 320 nm using a UV visible spectrophotometer equipped with a thermoelectric flow cell (Bausch & Lomb Spectronic 2000). In the presence of adsorption, degradation was monitored based on the release of either Cl^- or $^{14}CO_2$. An ion-selective electrode (model 94-17; Orion Research, Inc., Cambridge, MA) was used to measure the release of chloride ions. The electrode was calibrated giving a linear relationship between ln[Cl^-]

[†] Department of Chemical Engineering.
[‡] Department of Bacteriology and Biochemistry.

and mV using 0–1000 mg/L NaCl in an experimental buffer as standards. [U-^{14}C]PCP was used for some experiments to determine the mineralization rate to $^{14}CO_2$. Reactors for radioactive experiments were 250-mL flasks. Each flask was sealed with a rubber stopper from which a small glass cup was suspended. The cup contained 0.75 mL of 1 N NaOH in which the released $^{14}CO_2$ was trapped. When sampling, the NaOH in the cup, including 1.5 mL rinsing water, was transferred into a 20-mL scintillation vial. A sum of 17 mL of Biosafe II scintillation cocktail was added. After being shaken, vials were held overnight before counting to allow the quenching of autofluorescence. Counts per minute for the trapped $^{14}CO_2$ were measured in a Beckman LS 7000 liquid scintillation counter.

Degradation Kinetics. Unless otherwise stated, 1 mL of fresh cell–HIB suspensions was used for degrading PCP in 100 mL of HIB medium with 0.5 g/L sodium glutamate. Degradation media were contained in 250-mL flasks shaken on a rotary shaker at 200 rpm at 25 °C. The following models were used to fit PCP degradation data for free cells:

$$-\frac{dS}{dt} = \frac{\mu_m X}{Y_{X/S}}\left(\frac{S}{K_s + S + S^2/K_i}\right) \quad (1)$$

$$\frac{dX}{dt} = \mu_m X\left(\frac{S}{K_s + S + S^2/K_i}\right) - k_d X \quad (2)$$

These two coupled nonlinear first order differential equations were solved by a fourth-order Runge–Kutta routine with self-adjusting step size using TK Solver software (Universal Technical Systems, Inc., Rockford, IL), and model parameters were adjusted to fit the experimental data. Normalized standard error (NSE %) used to compare experimental data with model calculations was defined as

$$\text{NSE \%} = \left[\frac{\sum_{i=1}^{n}\left(\frac{S_{Model,i} - S_{Expt,i}}{S_{Expt,i}}\right)^2}{(n-1)}\right]^{1/2} \times 100\% \quad (3)$$

In other experiments, the effects of supplementary carbon (0, 0.5, 3, 5 g/L sodium glutamate and 3 g/L glucose) and a non-utilizable compound (50 mg/L *p*-cresol) on PCP degradation were investigated. All experiments were in 100 mL of HIB with 50 mg/L PCP plus 1 mL of fresh cell suspension at 25 °C in 200 rpm shaking flasks.

Cell Immobilization in PUF and in Calcium Alginate Beads. One gram of polyurethane prepolymer was cooled on ice, and 1 mL of HIB was added. The mixture was stirred well for 1 min. Then 1 mL of cell-HIB suspension was added and mixing continued for 1 additional min. Cell-free foam was made for control or adsorption studies by substituting HIB for the cell–HIB suspensions. The cylindrical reaction vessel for polymerization was kept on ice overnight while the PUF hardened. The foam with entrapped cells was rinsed with buffer three times before use. To prepare alginate-entrapped cells, 5 mL of cell–HIB suspension containing 2.5 g of cells was mixed with 5 mL of cold (4 °C), sterile, 4% sodium alginate (Sigma type VII). Cold 2% sodium alginate was added to bring the mixture to a final volume of 50 mL. The cell–alginate mixture was added dropwise to cold 50 mM $CaCl_2$ aqueous solution using a peristaltic pump and sterile silicone tubing fitted with the end of a standard 200-μL pipet tip. Each drop hardened into a bead (approximately 3 mm in diameter) containing entrapped cells. The beads were allowed to solidify further in stirred 50 mM $CaCl_2$ for 30 min, and then they were collected by filtration. Beads were stored at 4 °C in HIB containing 1 mM $CaCl_2$.

PCP Removal by PUF Adsorption and by Immobilized Cells. Cell-free foam was cut into 0.5-cm cubes for adsorption equilibrium and dynamic studies. The effects of buffer medium (50 mM HIB and sodium phosphate), pH, and surfactant (Tween 80) on PCP adsorption were investigated. For all experiments 100 mL of media in 250-mL flasks were shaken at 25 °C and 200 rpm in a dark room for about 1 week until adsorption equilibrium was reached. The Langmuir isotherm model was used to fit the equilibrium data:

$$q = \frac{q_m S_e}{K_d + S_e} \quad (4)$$

For the comparison of PCP removal rates by adsorption and degradation, 100 mL of 50 mg/L PCP medium (with 3 g/L sodium glutamate) at 25 °C was used in five reactors: free-cell stirred reactor, stirred reactor or fixed-bed column (100 mL/min) with calcium alginated bead entrapped cells, and stirred reactor or dynamic bed reactor (DBR) with PUF-immobilized cells. PCP removal rates from the liquid phase were monitored at 320 nm. A sum of 2.5 mL of 50% (w/v) cell–HIB suspension was used for free-cell degradation experiments or for cell immobilization. In the DBR as previously described (*8*), polyurethane foams (with entrapped cells) were packed as the bed in a cylindrical tube and squeezed periodically by a piston. PCP medium was pumped through the dynamic bed and recirculated into a batch reservior. The bed compression ratio was 0.5, the residence time of PCP medium in the bed was 5 s, and the average recirculation flow rate through the bed was approximately 100 mL/min.

To determine net degradation rates for the DBR and for the stirred reactor with PUF-immobilized cell particles, voltage changes due to Cl^- release during PCP degradation were measured for both reactors.

Modeling of PCP Adsorption–Degradation with PUF-Immobilized Cells. A model describing the effect of adsorption, mass transfer, and cell properties on PCP degradation was derived under the following assumptions: (i) Immobilized cell particles are spherical. (ii) Each particle with radius R has a homogeneous pore structure, and bacterial cells are uniformly distributed inside initially. (iii) Most bacteria are present as suspended cells in the entrapped pore liquid, and attachment as microcolonies to the pore membrane surface are rare (*6*). (iv) Cells are able to grow by consuming PCP. (v) Under aerobic conditions, the oxygen level is not a limiting factor to cell growth and degradation kinetics (*5*). (vi) Interfacial mass-transfer resistance can be ignored when the external solution is mixed. (vii) There is no convective flow in the liquid-filled pores of a particle. Intraparticle mass transfer is assumed to occur only through liquid in the pores, and the effective diffusion coefficient of PCP is independent of concentration. (viii) Adsorption reaches equilibrium quickly relative to the diffusion rate. (ix) The equilibrium isotherm follows the Langmuir model. (x) The growth and PCP degradation kinetic properties of immobilized cells are similar to that of free cells. (xi) The cell leakage out of the particle can be ignored once cells are immobilized and prepared for PCP degradation.

When immobilized cell PUF particles are placed in a reservoir containing PCP contaminated water, the general

material balance for component i in the pore liquid of a particle is (9)

$$\epsilon_p \frac{\partial C_i}{\partial t} + \rho \frac{\partial q}{\partial t} = \left(\frac{D_{p,i}\epsilon_p}{\tau}\right)\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial C_i}{\partial r}\right) + \frac{D_{s_i}}{r^2}\rho \frac{\partial}{\partial_i}\left(r^2 \frac{\partial q_i}{\partial r}\right) + \epsilon_p R_{p_i} + \rho R_{s_i} \quad (5)$$

This general expression (eq 5) can be simplified based on the above assumptions. By substitution of eqs 1 and 4 into eq 5, the material balance for PCP becomes

$$\left(\epsilon_p + \rho \frac{q_m K_d}{(K_d + S)^2}\right)\frac{\partial S}{\partial t} = D_e\left(\frac{\partial^2 S}{\partial r^2} + \frac{2}{r}\frac{\partial S}{\partial r}\right) - \frac{\mu_m}{Y_{X/S}} X\left(\frac{S}{K_s + S + S^2/K_i}\right) \quad (6)$$

The overall material balance on PCP in the reservoir is

$$V\frac{dS_b}{dt} = -D_e A\left(\frac{\partial S}{\partial r}\right)_{r=R} \quad (7)$$

Equation 7 is a boundary condition for eq 6.

The initial and other boundary conditions are

$$\begin{aligned} t = 0; \quad & S = 0, X = X_0 \quad (\text{for } 0 \le r \le R) \\ & S_b = S_{b_0} \quad (\text{for } r > R) \\ r = 0; \quad & \frac{\partial S}{\partial r} = 0 \quad (\text{for } t \ge 0) \\ r = R; \quad & S = S_b \quad (\text{for } t > 0) \end{aligned} \quad (8)$$

The above partial differential equation (eq 6) was converted into ordinary differential equations using the method of lines and solved simultaneously with eqs 2, 7, and 8 using the fourth-order Runge–Kutta routine in the TK Solver software.

Effect of PCP Adsorption on Biodegradation. In one experiment, different amounts of adsorbent (cell-free PUF cut in 0.5-cm cubes) were added to flasks containing 100 mg/L PCP in HIB with 0.5 g/L sodium glutamate. After reaching equilibrium overnight, 1 mL of cell–HIB suspension was added to each flask, and Cl^- concentrations in the supernatant of the degradation medium were measured. In another experiment, PUF-immobilized cells were used for degrading 25 mL of radiolabeled PCP-contaminated water with high concentrations (>200 mg/L) in 50-mL flasks shaken on rotary shaker at 200 rpm and 25 °C, and $^{14}CO_2$ elution from the mineralization of PCP was monitored with time.

Results

Typical PCP concentration versus time data (Figure 1) show complete PCP degradation in flasks containing up to 150 mg/L PCP and partial degradation in flasks containing higher PCP concentrations. Negligible degradation was observed for PCP concentrations higher than 200 mg/L since cell death rate was higher than the growth rate based on the viable cells count after PCP was added to cell suspensions in buffered nutrient media. Increases in the PCP degradation rate with time (i.e., the downward curvature of degradation curves in Figure 1) were due to cell growth and reduction in PCP inhibition with the consumption of PCP. Cell growth during degradation and PCP inhibition to cell growth were observed (data not shown). The substrate inhibition model of eqs 1 and 2 predicted the degradation kinetics with normalized standard error NSE % from 5% to 10%. Model parameters used for fitting the experimental data in Figure 1 were as follows: $\mu_m = 0.005\ min^{-1}$, $K_s = 25$ mg/L, $K_i = 1.5$ mg/L, $Y_{X/C} = 0.12$ g of cell/g of PCP, $k_d = 3.33 \times 10^{-5}\ min^{-1}$. X_0 was used as an adjustable parameter in fitting degradation curves. The values of X_0 corresponding to curves from bottom to top were 0.055, 0.141, 0.263, and 0.395 mg/mL, respectively. As shown in Figure 1, with freshly prepared cells, no lag times were observed preceeding PCP degradation. However, for resting cells stored in the refrigerator, there was a lag up to 5 h.

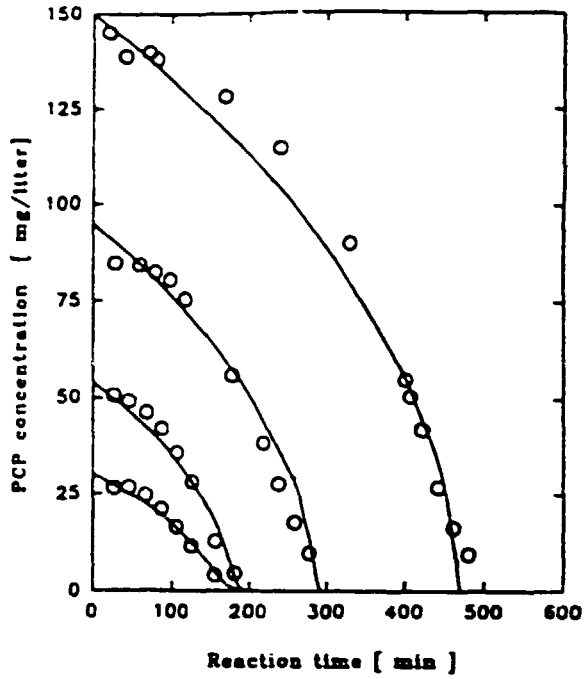


Figure 1. Degradation kinetics of free cells at different initial PCP concentrations. Curves were generated from eqs 1 and 2.

Table 1. Langmuir Model Parameters for PCP Adsorption in PUF

pH	buffer	Tween 80 (%)	q_m (mg/g)	K_d (mg/L)	R^2
7.3	HIB	0	21.3	29.7	0.986
7.3	phosphate	0	76	47	0.993
8.5	phosphate	0	23	86	0.992
7.3	phosphate	0.5	65	345	0.939
7.3	phosphate	1.0	30	370	0.805

The effects of several supplementary compounds (sodium glutamate, glucose, and *p*-cresol) on the PCP degradation kinetics are shown in Figure 2. The presence of the utilizable compounds, sodium glutamate and glucose, always enhanced degradation rates. The optimum concentration used for sodium glutamate was 3 g/L. However, the non-utilizable toxic compound *p*-cresol inhibited PCP degradation.

Table 1 shows Langmuir adsorption parameters for the PCP–PUF system using cell-free foams. With decreasing pH or increasing surfactant (Tween 80) concentration, adsorption decreased (i.e., q_m decreased and K_d increased). Also, adsorption varied with buffer type. The adsorption capacity was lower with PUF than with activated carbon powder ($q_m = 210$ mg/g, $K_d = 8.3$ mg/L), and the adsorption was reversible. The adsorption and desorption dynamics increased with decreasing pH or surfactant concentration (data not shown).

Calcium alginate immobilized cells were used for comparison to PUF-immobilized cells for PCP removal from the simulated contaminated water. Immobilization in calcium alginate beads (3–4 mm in diameter) was mild,

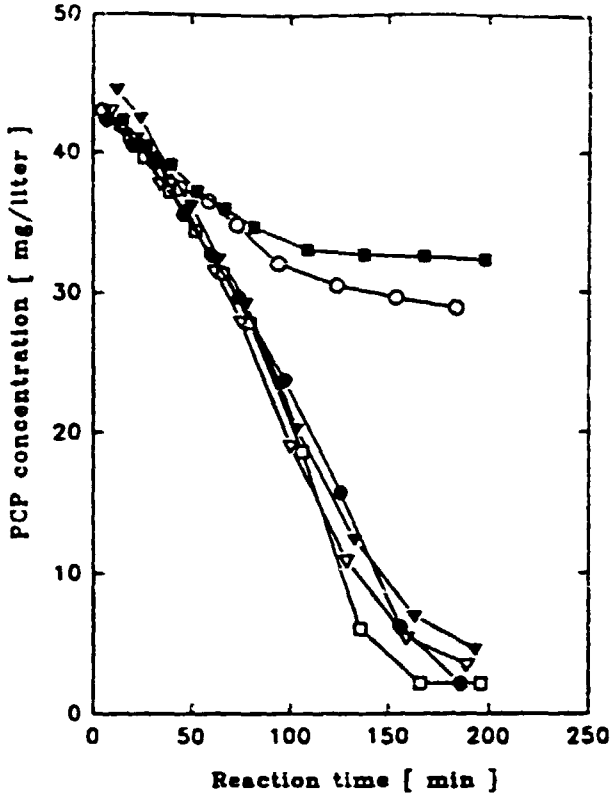


Figure 2. Effects of supplementary substrates on degradation of PCP by free cells. Supplementary substrate: (○) none; (●) 0.5 g/L sodium glutamate; (▽) 3 g/L sodium glutamate; (▼) 5 g/L sodium glutamate; (□) 3 g/L glucose; (■) 50 mg/L *p*-cresol.

while more cells were inactivated or killed during immobilization in PUF based on activity measurements with similar size (3–4 mm) particles of PUF-immobilized cells. Calcium alginate immobilized *Flavobacterium* cells were unable to degrade PCP at higher than 250 mg/L although some authors have reported a protection effect of the calcium alginate matrix from toxic compounds (*10*). PCP was not adsorbed to calcium alginate but adsorbed strongly to PUF. Also, calcium alginate beads were observed to be easily swollen and broken during operation in stirred reactors and fixed-bed columns.

Figure 3 shows PCP removal from external solutions in five immobilized cell reactors in which the same amount of cell biomass was used for immobilization or added as free cells. The activity of cells immobilized in calcium alginate was lower than that of free cells and showed no difference between fixed-bed column and stirred reactor.

It is difficult to compare the PCP removal rates in PUF reactors with the degradation rates by either free cells or calcium alginate immobilized cells. In addition to microbial degradation, the initial removal rates for PUF-immobilized cells were enhanced by PCP adsorption to PUF. The solid curves in Figure 3 were generated from eqs 2, 6, 7, and 8 with the following model parameters: $\epsilon_p = 0.93$, $\rho = 0.07$ g/cm^3, $q_m = 21.3$ mg/g, $K_d = 0.0287$ mg/cm^3, $X_0 = 0.0037$ mg/cm^3, $\mu_m = 8.33 \times 10^{-5}$ s^{-1}, $Y_{X/S} = 0.12$ g/g, $K_s = 0.025$ mg/cm^3, $K_i = 1.5 \times 10^{-3}$ mg/cm^3, $k_d = 5.56 \times 10^{-7}$ s^{-1}. The fitting parameter D_e was 5.0×10^{-6} cm^2/s and 1.0×10^{-4} cm^2/s for foam particles operated in the stirred reactor and in the DBR, respectively. For foam particles in stirred solutions, the intraparticle PCP concentration distribution from the model calculations indicated that PCP was not transported very far into the foam particle and was distributed near the outer surface

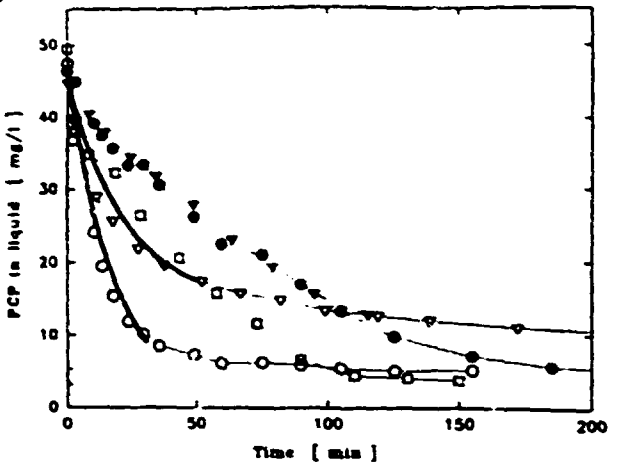


Figure 3. PCP removal in immobilized cell bioreactors. Solid curves: model fitting using eqs 5–7. (○) PUF-immobilized cells in DBR; (▽) PUF-immobilized cells in stirred reactor; (●) calcium alginate bead entrapped cells in fixed-bed column; (▼) calcium alginate entrapped cells in stirred reactor; (□) free cells in stirred reactor.

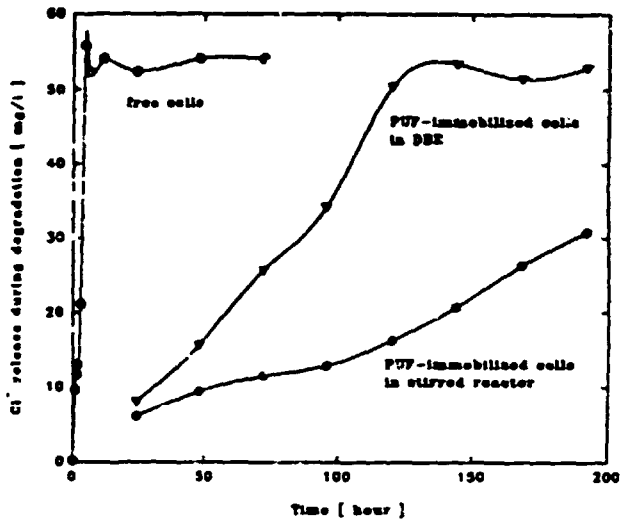


Figure 4. Enhancement of PCP degradation rate by forced-convective flow within the PUF immobilization matrix. Degradation condition: 1 mL of 50% cell–HIB suspensions were used for degrading 100 mL of 100 mg/L PCP at 25 °C. One immobilized cell foam cylinder (22-mm diameter and 40-mm length) were used in both the DBR and the stirred bioreactor.

of the particle (data not shown). The DBR enhanced the PCP removal rate relative to the stirred reactor.

Rather than the disappearance of PCP, Cl^- release was monitored to determine the effect of convective flow in the dynamic matrix bed on the PCP biodegradation rate (Figure 4). The degradation rate based on the chloride release measurment was enhanced in the DBR as compared to that in the stirred reactor but was still low relative to the free-cell degradation rate. Apart from diffusional effects, this low activity of PUF-immobilized cells may be due to the inactivation of active cells during immobilization.

Experiments with free cells incubated with cell-free PUF (Figure 5) indicated that the addition of adsorbent lowered PCP concentration (at equilibrium: 43.6, 58.5, 68.9, 73.0, and 100 mg/L corresponding to curves from top to bottom) and thus reduced the toxicity of PCP to cells. Calculations based on the adsorption isotherms predicted that the PUF-immobilized cells could degrade highly contaminated waters (as high as 700 mg/L) if 150 mg/L was chosen as

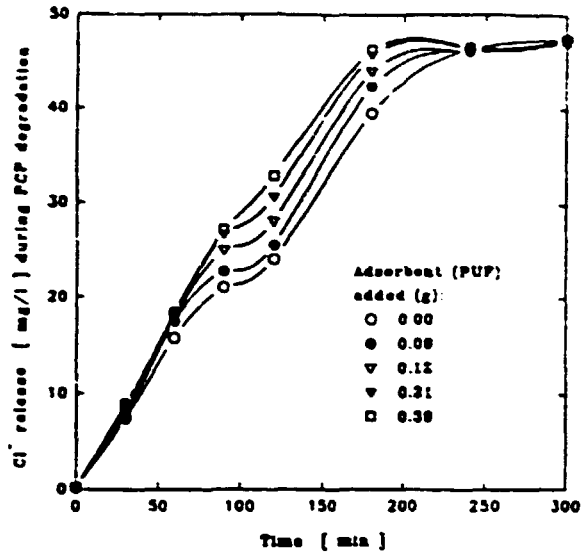


Figure 5. Effect of adsorption on PCP degradation. Degradation condition: 100 mL of 100 mg/L PCP in HIB with 0.5 g/L sodium glutamate at 25 °C at 200 rpm; 1 mL of 20% cell–HIB suspension was used.

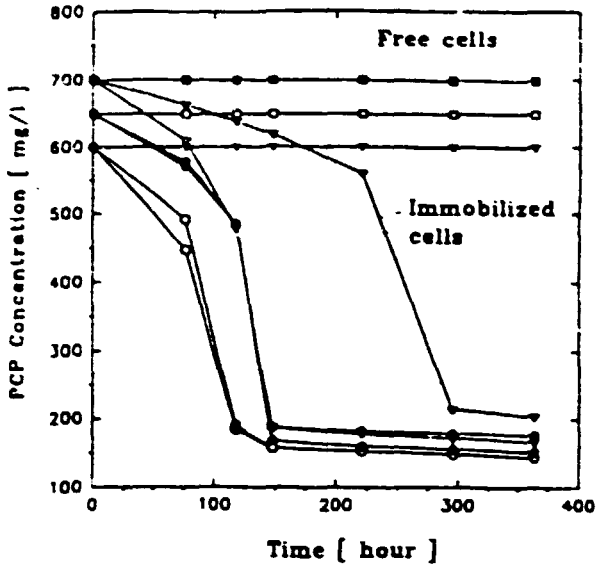


Figure 6. Degradation of PCP by PUF-immobilized and free *Flavobacterium* cells. Data was converted from percent of ^{14}C added and trapped as $^{14}CO_2$ to mg/L. At least 70% of the total radioactivity added was trapped from each experimental flask. Less than 0.4% was trapped from control flasks.

the maximum PCP concentration tolerated by free cells. Experiments designed to test this possibility (Figure 6) show that PUF-immobilized cells have much higher degrading capacity and survivability than free cells and, thus, have potential for treatment of highly contaminated water.

Discussion

Although PCP degradation by PUF-immobilized *Flavobacterium* has been reported (*5, 7*), detailed kinetic data have not been analyzed. The efficient operation of biological treatment systems is largely dependent on the kinetic properties of the microbial population, and the determination of kinetic parameters is essential to the development of operational strategies for the effective removal of pentachlorophenol during wastewater treatment (*11*). *Flavobacterium* sp. ATCC 39723 showed similar substrate metabolism and growth inhibition as the *Arthrobacter* sp. isolated by Edgehill and Finn (*12*) and the continuously enriched culture of PCP-degrading bacteria of Klecka and Maier (*11*). Cell growth during PCP degradation, one of the factors responsible for the downward curvature of the degradation curves in Figure 1, was considered in the kinetic analysis of this work. Previous work (*5, 7*) showed similar curvature for free-cell degradation kinetics which can be similarly modeled by the substrate inhibition model of eqs 1 and 2. To get the best fit, Klecka and Maier (*11*) used both μ_m and X_0 as adjustable parameters. In our work, we only adjusted X_0 to fit the data. In addition, the initial lag preceding PCP degradation has not been considered in our model.

Parameters in the immobilized cell model (ϵ_p, q_m, K_d, μ_m, K_s, K_i, D_e, R, and X_0) may be varied to affect PCP removal rates. The PCP degradation kinetics of free cells (μ_m, K_s and K_i) are affected by physiological factors such as oxygen level, nutrients and energy source, temperature, and pH (*13*). For *Flavobacterium* sp. ATCC 39723, oxygen level did not significantly affect PCP degradation as long as aerobic conditions were maintained (*6*). The addition of utilizable compounds such as sodium glutamate and glucose to the degradation medium enhanced the PCP degradation rate by increasing cell growth rate, biomass adaptation to the toxic environment, and maintenance ability (*14*). The same optimum sodium glutamate concentration (3.0 g/L) for stimulating the PCP degradation rate was obtained in this work as was previously reported by Topp et al. (*14*). With concentrations of sodium glutamate higher than 5 g/L, the PCP degradation was significantly slowed. This may be explained by competition between PCP consumption and sodium glutamate utilization to support cell growth. For the analysis of PCP degradation data when a supplementary compound was added, a modified form of eqs 1 and 2 may be used as described previously (*15*). Also, culture conditions for cell production may affect cell viability and PCP degradation kinetics. Topp and Hanson (*16*) reported that cells grown under phosphate, glucose, and glucose + PCP limitation were more sensitive to PCP and degraded PCP at lower rates than cells grown under ammonium, sulfate, and PCP limitation.

In a previous report (*17*), we concluded that the effective mass-transfer coefficient D_e for PCP diffusing through the liquid-filled PUF matrix was enhanced at least 10 times due to the adsorption of PCP molecules onto pore membranes and the migration of the adsorbed molecules on solid pore surfaces. With convective flow through the pores of a foam particle matrix, the foam was impregnated uniformly with PCP medium, and thus the adsorption capacity was enhanced. Furthermore, CO_2 produced during PCP degradation may accumulate inside the PUF matrix producing gas-filled pores and restricting liquid-phase mass transport. This may be the major reason for the lower degradation rate for the stirred reactor relative to the DBR (Figure 4).

PCP removal by fresh PUF-immobilized cells can be separated into two stages: First, adsorption at a low active cell population after the immobilization and then degradation of adsorbed PCP after a lag time for cell adaption and growth. The degradation of adsorbed PCP in PUF particles may be modeled in a manner similar to the remediation process for a contaminated soil aggregate (*9*). Our simulation results (data not shown) based on eqs 2, 6, 7, and 8 indicated that if the initial active cell population

in the PUF matrix (X_0) is sufficiently large, the adsorption and the mineralization of adsorbed PCP may be coupled over the same time range, unlike the two-stage case described above. The coupled adsorption–biodegradation process can be more efficient than the biphasic process in removing PCP from contaminated water. After immobilization, PUF-immobilized cells should be incubated in nutrient media to allow the survived cells to grow to an optimum level before they are used for degradation. Thus the lag times shown in Figures 4 and 6 may be shortened or avoided and the degradation rate enhanced.

As compared to free cells, alginate-immobilized cells can degrade PCP at a slightly higher level (200–250 mg/L) because of a protective diffusion barrier (*10*). However, PUF-immobilized cells showed the ability to degrade PCP at concentrations as high as 700 mg/L in this work. As previously reported by O'Reilly and Crawford (*7*), the adsorption of PCP to foams reduced the toxicity of PCP. The PUF immobilization system should have all the advantages of the co-immobilized biological systems proposed by Lin et al. (*18*), who co-immobilized activated carbon powder and *Phanerochaete chrysosporium* cells in a hydrogel matrix for the degradation of PCP. Also, the roles of activated carbon (*19*) and bark chips (*20*) have been examined as detoxifying media for protecting microorganisms due to their adsorption properties.

Summary and Conclusions

A population of actively growing *Flavobacterium* sp. ATCC 39723 cells was important in minimizing or avoiding the lag before the initiation of PCP degradation. The substrate inhibition model (eqs 1 and 2) predicted the free-cell degradation kinetics well as cell growth was closely coupled with PCP degradation. Supplemental addition of utilizable compounds such as sodium glutamate and glucose was an effective means of increasing overall PCP degradation rates. PUF immobilization was simple and produced a matrix with excellent strength. The Langmuir model was adequate in describing PCP adsorption in PUF. The DBR was shown to be a good reactor design for enhancing the degradation rate. Adsorption of PCP to the PUF matrix reduced the toxicity of PCP and effectively removed PCP from highly contaminated water.

Acknowledgments

This work was funded by a grant from the University of Idaho Center for Hazardous Waste Remediation Research. We thank Keith Stormo and Thomas Carleson for their help.

Nomenclature

A	external surface area of particles in the reservoir
C_l	concentration in the pore liquid
D_e	effective pore diffusivity, $D_p\epsilon_p\tau$
D_p	pore diffusivity
D_s	surface diffusivity
k_d	endogenous cell decay coefficient
K_d	dissociation constant
K_i	inhibition constant
K_s	Monod constant
n	number of data points
q	amount of PCP adsorbed in PUF matrix
q_l	adsorbed concentration on the solid phase
q_m	maximum capacity of the adsorbent for PCP
R_p	reaction rate in the pore liquid
R_s	reaction rate on the pore surface
S	PCP concentration in the cell suspension
S_b	PCP concentration in the bulk solution in the reservoir
S_{b0}	initial PCP concentration in the bulk solution
S_e	PCP concentration in the bulk solution at equilibrium
V	volume of bulk solution (excluding pore liquid)
X	active biomass concentration
X_0	initial active cell concentration
$Y_{X/S}$	cell yield coefficient

Greek Letters

μ_m	maximum specific growth rate coefficient
ϵ_p	volume fraction of liquid in the PUF particle
τ	tortuosity of the PUF matrix
ρ	bulk density of the PUF particle

Literature Cited

(1) Valo, R. J.; Häggblom, M. M.; Salkinoja-Salonen, M. S. *Water Res.* **1990**, *24*, 253–258.
(2) Crosby, D. G. *Pure Appl. Chem.* **1981**, *53*, 1051–1080.
(3) Saber, D. L.; Crawford, R. L. *Appl. Environ. Microbiol.* **1985**, *50*, 1512–1518.
(4) Steiert, J. G.; Crawford, R. L. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 825–830.
(5) O'Reilly, K. T.; Kadakia, R; Korus, R. A.; Crawford, R. L. *Water Sci. Technol.* **1988**, *20*, 95–100.
(6) O'Reilly, K. T. Ph.D. Dissertation, University of Idaho, 1989.
(7) O'Reilly, K. T.; Crawford, R. L. *Appl. Environ. Microbiol.* **1989**, *55*, 2113–2118.
(8) Hu, Z. C.; Korus, R. A.; Stormo, K. E. *Appl. Microbiol Biotechnol.* **1993**, *39*, 289–295.
(9) Dhawan, S.; Fan, L. T.; Erickson, L. E.; Tuitemwong, P. *Environ. Prog.* **1991**, *10*, 251–260.
(10) Keweloh, H.; Heipieper, H.-J.; Rehm, H. J. *Appl. Microbiol Biotechnol.* **1989**, *31*, 383–389.
(11) Klečka, G. M.; Maier, W. J. *Appl. Environ. Microbiol.* **1985**, *49*, 46–53.
(12) Edgehill, R. U.; Finn, R. K. *Eur. J. Appl. Microbiol Biotechnol.* **1982**, *16*, 179–184.
(13) Valo, R. J.; Apajalahti, J. H. A.; Salkinoja-Salonen, M. S. *Appl. Microbiol. Biotechnol.* **1985**, *21*, 313–319.
(14) Topp, E.; Crawford, R. L.; Hanson, R. S. *Appl. Environ. Microbiol.* **1988**, *54*, 2452–2459.
(15) Klečka, G. M.; Maier, W. J. *Biotechnol. Bioeng.* **1988**, *31*, 328–335.
(16) Topp, E.; Hanson, R. S. *Appl. Environ. Microbiol.* **1990**, *56*, 541–544.
(17) Gu, Y.-X.; Hu, Z. C.; Korus, R. A. *Chem. Eng. J.* **1993**, in press.
(18) Lin, J.-E.; Wang, H. Y.; Hickey, R. F. *Biotechnol. Bioeng.* **1991**, *38*, 273–279.
(19) Ehrhardt, H. M.; Rhem, H. J. *Appl. Microbiol. Biotechnol.* **1985**, *21*, 32–36.
(20) Apajalahti, J. H. A.; Salkinoja-Salonen, M. S. *Microb. Ecol.* **1984**, *10*, 359–367.

Received for review July 15, 1993. Revised manuscript received November 23, 1993. Accepted November 24, 1993.[•]

[•] Abstract published in *Advance ACS Abstracts*, January 1, 1994.

Appl Microbiol Biotechnol (1991) 35: 100–104
0175759891000?X

Applied Microbiology Biotechnology

Effect of glutamate on the degradation of pentachlorophenol by *Flavobacterium* sp.

Jorge F. Gonzalez* and Wei-Shou Hu

Department of Chemical Engineering and Materials Science, 421 Washington Avenue SE, University of Minnesota, Minneapolis, MN 55455, USA

Received 12 June 1990/Accepted 8 November 1990

Summary. The degradation of pentachlorophenol (PCP) by a *Flavobacterium* sp. was investigated by inoculating induced cells into cultures containing PCP alone or PCP and glutamate as carbon sources. Using PCP as the sole carbon source, the degradation activity increased with PCP concentration. However, a lag phase was observed and this lag was more pronounced at higher PCP concentrations. Exposure of cells to higher PCP concentrations during induction did not reduce the lag. The presence of glutamate reduced the lag in PCP degradation. Such an elimination of the lag phase appears to be due to maintaining cell viability with the presence of glutamate.

Introduction

Pentachlorophenol (PCP) is widely used as a wood preservative, insecticide and herbicide (Hoos 1978; Cirelli 1978). Serious soil contamination has been found in the soil surrounding several wood treatment plants and saw mills (Ranga Rao 1978; Valo et al. 1985). The contaminants present in these soils are often transported into freshwater streams, lakes and groundwater by soil run-off. PCP has been found to be toxic to aquatic animals at a concentration as low as 0.5 μg/l (Borthwick and Schimmel 1978). Because of this toxicity, there is a need to decontaminate the PCP-laden soils. A particularly attractive way to eliminate PCP is by biodegradation to convert it to essentially harmless CO_2, H_2O and Cl^-.

The biodegradation of PCP has been reported to be carried out by undefined mixed cultures (Hakulinen et al. 1981; Moos et al. 1983) or by pure cultures of a *Pseudomonas* sp. (Watanabe 1977), a *Flavobacterium* sp. (Martinson et al. 1986), an *Arthrobacter* sp. (Stanlake and Finn 1982), and a *Rhodococcus* sp. (Apajalahti et al. 1986). Given the existence of several PCP degraders, the persistence of PCP in the environment appears to be a consequence of the environmental conditions in situ or the lack of a sufficiently high concentration of PCP degraders. Understanding these issues would facilitate their use in the decontamination process. These studies considered *Flavobacterium* sp. (ATCC 39723), which was first isolated from a PCP contaminated soil and shown to utilize glutamate, glucose and cellobiose as carbon sources (Pignatello et al. 1983; Saber and Crawford 1985). This organism was also capable of removing PCP in situ (Crawford and Mohn 1985). The purpose of these experiments was to study the effect of PCP concentration and the presence of glutamate on the viability and PCP degrading activity of a *Flavobacterium* sp.

Materials and methods

Organism and medium. *Flavobacterium* sp. (ATCC 39723) was obtained from the Gray Freshwater Biological Institute, University of Minnesota, Navarre, Minn., USA. The medium was a modification of the minimal salts (MS) medium originally reported by Watanabe (1973) supplemented with glutamate or PCP as a carbon source. The flasks were incubated in a rotary shaker at 200 rpm and 30°C. The composition was as follows: KH_2PO_4, 0.13 g/l; K_2HPO_4, 0.65 g/l; $NaNO_3$, 0.5 g/l; $MgSO_4$, 0.1 g/l; $FeSO_4 \cdot 7H_2O$, 5.6 mg/l. The PCP concentrations varied when used as a carbon source. Stock cultures were maintained at −80°C in a 20% glycerol solution. Cells from the stock culture were transferred to Luria agar slants (tryptone, 10 g/l; yeast extract, 5 g/l; NaCl, 2.5 g/l; glucose, 2 g/l; agar, 15 g/l) for 1 week before they were used for inoculation.

Cell preparation. The inoculum for batch cultures was prepared in 300 ml erlenmeyer flasks with 100 ml medium using glutamate as the carbon source. When a large amount of inoculum was needed, the seed culture was prepared in a 2 l Multigen fermentor (New Brunswick Scientific, Edison, N.J., USA) with a 1.0 l working volume. The medium used was the same as that for seed cultures prepared in flasks. The agitation rate was initially 400 rpm and was later increased to 600 rpm to maintain a dissolved oxygen level above 15% of saturation with air. Growth was monitored by measuring the turbidity of the culture at 600 nm using a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Md., USA). One absorbance unit was equivalent to a cell concentration of 3×10^9/ml. No difference in growth rate was observed in erlenmeyer flask cultures and fermentor cultures. When the fermentor was used, the pH was controlled at 7.40 ± 0.05 by the addition of 1.0 M NaOH or 0.50 M H_2SO_4 using a Chemcadet pH controller (Cole Parmer, Chicago, Ill., USA). In the late exponential phase 50 mg/l PCP was added to induce the cells for PCP degradation activity (Crawford and Mohn 1985). Decrease in PCP concentration was taken as the indication of successful induction. After the PCP concentration decreased to below 10 mg/l, it was replenished to about 50 mg/l. Two replenishments were performed for normal induction. After induction, cells were centrifuged, washed and resuspended in 5.0 mM potassium phosphate buffer (pH 7.4) before they were used for inoculation.

PCP degradation experiments. Unless otherwise specified, the experiments were carried out in 300 ml erlenmeyer flasks containing 100 ml medium. For experiments of longer duration, 500-ml flasks with a working volume of 300 ml were used. The medium used was MS medium supplemented with various concentrations of PCP. The induced *Flavobacterium* cells were inoculated at approximately 0.1 absorbance units or 3×10^8 cells/ml and the cultures were incubated in a shaker at 200 rpm and 30°C. The cell concentration was measured by turbidity at 600 nm as described above. When the viable cell concentration was measured, culture samples were diluted with 5.0 mM phosphate buffer (pH 7.4) before they were plated on nutrient agar plates.

In both the seed culture and the PCP degradation culture, the glutamate concentrations were measured by an enzymatic method (Boehringer Mannheim Biochemicals, Indianapolis, Ind., USA). PCP concentrations were measured by spectrophotometry at 320 nm (Chalmorovsky and Vacek 1971).

Results and discussion

Induced cells were washed and inoculated into erlenmeyer flasks containing MS medium supplemented with PCP at 10, 20, 40, 80 and 200 mg/l, respectively. No higher PCP concentrations were tested because no cell growth occurred at PCP concentration higher than 300 mg/l (data not shown). PCP was measured periodically and replenished to its original concentration using a solution of 0.187 M NaOH and 37.5 mM PCP. The 5:1 molar ratio of NaOH to PCP was to keep the pH constant by neutralizing the 5 mol HCl produced for each mole of PCP degraded. We have observed that the PCP degradation rate was drastically reduced below pH 6.9. By using the NaOH/PCP solution to replenish PCP, the PCP degradation rate was independent of the small pH fluctuation. A possible drawback of this approach is that the added salt concentration may affect either cell growth or PCP degradation rate. We examined the growth kinetics at various NaCl concentrations. The negative effect of NaCl on cell growth was not seen until the concentration was higher than 86 mM, which was much higher than the final concentration of NaCl reached in our study. Thus, increased salt concentration at high PCP concentrations is unlikely to have a biased effect in our study.

Shown in Fig. 1a are the kinetics of cell growth as measured by turbidity. Higher PCP concentrations resulted in higher final cell concentrations. Cumulative PCP consumption is shown in Fig. 1b. The total

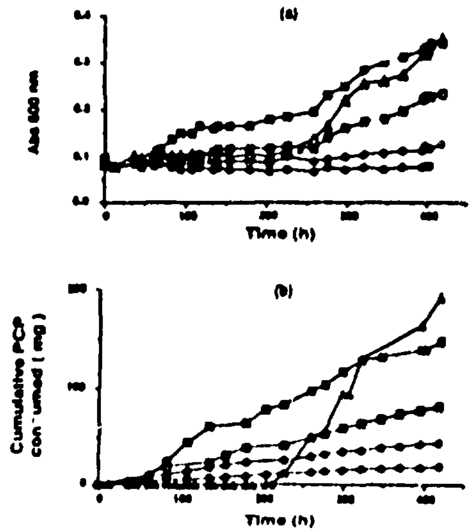


Fig. 1. Growth kinetics (a) and cumulative pentachlorophenol (PCP) consumption (b) by *Flavobacterium* sp. at PCP concentrations of 10 mg/l (○), 20 mg/l (●), 40 mg/l (□), 80 mg/l (■) and 200 mg/l (▲). Abs, absorption

amount of PCP degraded increased from about 25 mg PCP in the 10 mg/l culture to 200 mg in the 200 mg/l culture over a 420 h period. A lag phase in PCP degradation was observed in these cultures, during which no reduction in PCP concentration was observed and no replenishment of PCP was necessary. The end of the lag phase coincided with the onset of cell growth, which was more evident with 80 and 200 mg PCP/l. The duration of the lag phase was 10 h at 10 mg PCP/l and increased steadily to 30 h for 20 mg/l, 55 h for 40 mg/l, 80 h for 80 mg/l, and 200 h for 200 mg/l. This lag phase thus appeared to increase linearly with PCP concentration. A similar linear relationship between length of the lag phase and PCP concentration was also reported when an *Arthrobacter* strain was used (Stanlake and Finn 1982).

The specific degradation rate (q) of PCP was calculated by dividing the slope of the cumulative PCP degradation curve by the cell concentration at the time point of interest. The results are shown in Fig. 2, where the curves are plotted from the time at which the degradation was first observed. At higher concentrations the specific activity was initially higher, but it decreased more rapidly than at lower concentrations. A similar decrease in activity was observed with immobilized *Flavobacterium* sp. cells (O'Reilly and Crawford 1989).

The effect of PCP concentration on the lag phase and specific PCP degradation activity is reproducible in

---

* *Present address:* Departamento de Ingeniería Química, Facultad de Ingeniería, Juan B Justo 4302, 7600 Mar del Plata, Argentina

Offprint requests to: W. S. Hu

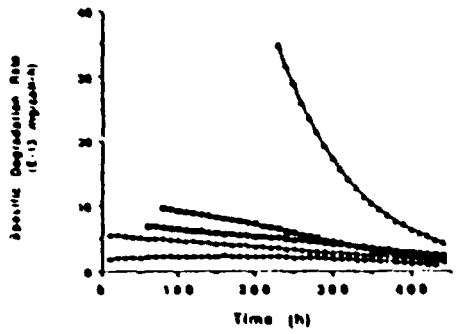


Fig. 2. Specific PCP degradation rate of *Flavobacterium* sp. at PCP concentrations of 10 mg/l (○), 20 mg/l (●), 40 mg/l (□), 80 mg/l (■) and 200 mg/l (▲). E-11, 10⁻¹¹

repeated experiments. In all those experiments, the cells were induced at 50 mg PCP/l and subsequently exposed to various PCP concentrations in biodegradation. It is possible that the longer lag phase observed at high PCP concentrations was due to the low concentration of PCP used in the induction. It has been reported that resistance to toxic substrates is affected by the concentration of those substrates to which these cells had been previously exposed (Wild and Hinshelwood 1955, Dijkuinen and Harder 1975, Typler and Finn 1974). It is conceivable that if a higher concentration of PCP was used in the induction, the lag phase could be shortened or eliminated.

Flavobacterium cells were exposed to 150 mg PCP/l during induction. The induced cells were centrifuged, washed and resuspended in 50 mM phosphate buffer before they were inoculated into 300 ml erlenmeyer flasks containing MS medium and PCP at concentrations of 50 and 100 mg/l. The residual PCP concentration was measured daily and replenished to the original level, if necessary. The results for the two cultures were similar to those obtained in the previous experiments: at the higher PCP concentration of 100 mg/l the specific PCP degradation rate was higher, however, it also decreased with time (Fig. 3). The lag for the culture at 100 mg/l was about twice that of a 50 mg PCP/l culture. These results indicate that under the experimental conditions used, a lower level of PCP concentration during induction was not the cause of the lag phase.

We then speculated that the interruption of PCP exposure during harvesting and washing of the cells might allow a turn over of the PCP degrading enzymes, thus rendering the cells vulnerable to PCP. A similar suggestion was made by Topp et al. (1988). To test this possibility, PCP depletion was avoided by transferring the cells directly from the induction culture into the experimental erlenmeyer flasks without the centrifugation and washing steps. The cells were induced by the addition of 50 mg PCP/l. To minimize the carry-over of glutamate, the incubation time of the seed culture in the fermentor was prolonged. As the PCP concentration decreased to approximately 10 mg/l, it was replenished. After two to three replenishments, PCP addition was coupled to pH control by feeding an alkaline solution with a 5 to 1 molar ratio of NaOH to PCP. As PCP was degraded, the production of H^+ resulted in a decrease in pH from 7.4, triggering the addition of NaOH/PCP solution (Fig. 4).

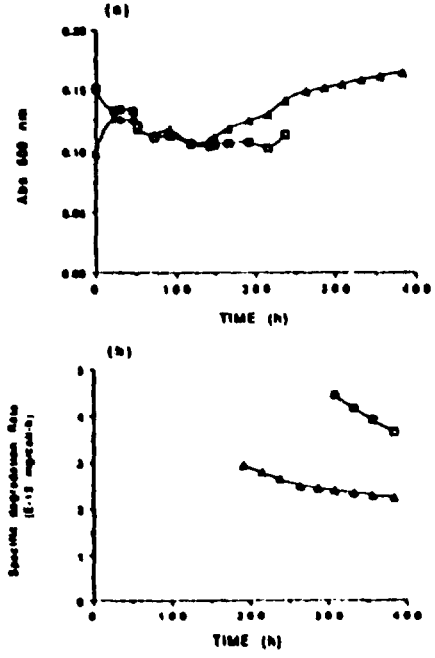


Fig. 3. Growth kinetics (a) and specific PCP degradation rate (b) of *Flavobacterium* sp. induced at 150 mg/l of PCP. PCP concentration in the culture was 50 mg/l (▲) and 100 mg/l (□). E-12, 10^{-11}

Cells thus induced were then inoculated directly into the erlenmeyer flasks for PCP degradation experiments. Even though care was taken to minimize the glutamate concentration, some glutamate was still transferred together with the cells. In one set of such cultures, glutamate was present at 30 mg/l and samples were taken every 20 to 30 min for measurements of PCP and cell concentration. In all cases, no lag in PCP degradation was observed. Using the initial PCP degradation rate, the specific rate was calculated and is shown in Fig. 5. The specific PCP degradation rates were fitted to a inhibition equation by non-linear regression using the Levenberg-Marquardt technique:

$$q = \frac{q_{max} \cdot [P]}{K_s + [P] + [P]^2/K_i} \qquad (1)$$

where P is the PCP concentration, K_s is the saturation constant, K_i is the inhibition constant, and q_{max} is the maximum specific degradation rate. The K_i value obtained was 3.09 mg PCP/l and q_{max} was 2.79×10^{-11} mg/cell per hour. Similar experiments were performed using cells induced at lower PCP concentrations of 30, 50 and 80 mg/l. As with cells induced at 150 mg/l, no lag in degradation was observed (data not shown).

Since a small amount of glutamate was present in the experiment described above, it was not clear if elimination of the lag phase was due to the continuous exposure of cells to PCP or to the presence of glutamate. In one of the fermentor runs to prepare inoculum, cell induction was allowed to continue until glutamate was depleted after a portion of the cells was used for inoculation. When the specific PCP degradation rate was calculated, it was observed that the specific activity increased steadily when glutamate was still present but decrease rapidly after glutamate depletion (Fig. 4). This

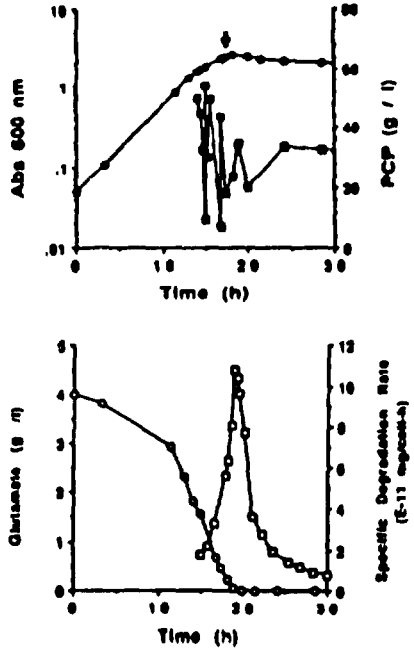


Fig. 4. Growth kinetics of *Flavobacterium* sp. in glutamate-containing medium and induction of PCP degradation activity. The arrow indicates the initiation of PCP feeding by using pH change as an indicator: ●, cell concentration; ■, PCP concentration; ○, glutamate concentration; □, specific PCP degradation rate. E-11, 10^{-11}

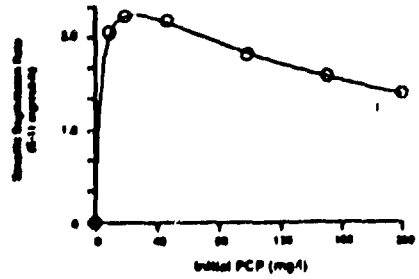


Fig. 5. Effect of PCP concentration on specific PCP degradation rate. Cells were induced at 150 mg PCP/l. E-11, 10^{-11}

indicates that the presence of glutamate may play a role in maintaining PCP degradation activity. To examine that possibility, induced cells from a seed culture in which glutamate was depleted were inoculated into erlenmeyer flasks containing MS medium supplemented with the same concentration of PCP but varying amounts of glutamate ranging from 0 to 200 mg/l. The concentration profiles of PCP in those cultures are shown in Fig. 6. Without glutamate, a lag phase of over 40 h was observed, while in the presence of glutamate the degradation occurred immediately after inoculation. The PCP degradation rate increased with increasing glutamate concentration. To ensure that such an effect of glutamate was not due to an artifact caused by the prolonged induction period in the seed culture, another experiment was carried out using cells induced under normal conditions. Again no lag phase was observed and the PCP degradation rate increased with increasing glutamate concentration (data not shown). The results thus suggest that the depletion of glutamate in the culture caused a lag phase in PCP degradation.

In the experiment described above the turbidity remained relatively constant throughout the experiment. It has been suggested that supplementing carbon besides PCP may prevent the viability loss of *Flavobacterium* (Topp et al. 1988). We thus measured the viability of three of these cultures in the biodegradation experiment. The concentration of viable cells varied under different conditions. The results are shown in Fig. 6. In the PCP degradation culture containing 50 mg/l of PCP, cell viability remained high in the presence of 91 mg/l of glutamate, whereas in the absence of glutamate, the viable cell concentration decreased rapidly to less than 10% in 22 h. The viable cell concentration eventually increased again; such an increase in viable cell concentration appeared to coincide with the onset of PCP degradation. In the control without either glutamate or PCP, the viable cell concentration remained high for over 40 h before it began to decrease. The results thus suggest that the lag in PCP degradation is a result of viability loss in the presence of PCP and in the absence of glutamate.

We observed no viability loss during cell harvesting and washing before inoculation into the PCP degrada-

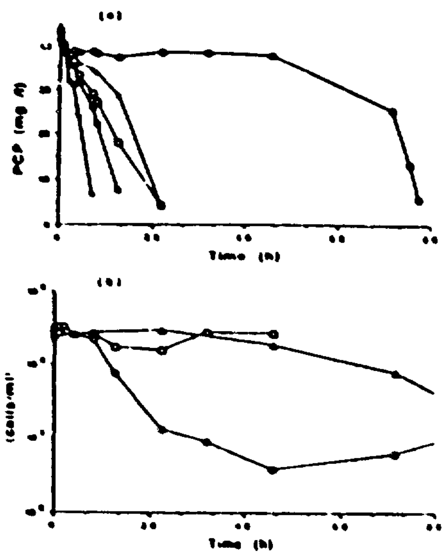


Fig. 6. Effect of glutamate on PCP degradation and viability. A, no PCP, no glutamate. All others included 50 mg PCP/l and glutamate at 0 mg/l (O), 46 mg/l (△), 91 mg/l (□), 141 mg/l (●) and 201 mg/l (■)

tion culture. This is consistent with the observation that cells remain viable for a long time in the absence of PCP (Fig. 6). It appears that the loss of viability was affected by the presence of PCP and such a loss of viability can be prevented by the addition of glutamate. Whether other carbon sources that are also utilized by *Flavobacterium* sp., such as glucose and cellobiose, also exert a similar effect as glutamate is not known and needs to be investigated.

Acknowledgements. This study was supported in part by grants from BioTrol, Inc. (Chaska, Minn., USA), Ecolab (St. Paul, Minn., USA), and the National Science Foundation (ECE 83 52630). JFG was an external fellow of Consejo Nacional de Investigaciones Científicas Técnicas of the Republic of Argentina.

References

Apajalahti JH, Karpanoja P, Salkinoja Salonen MS (1986) *Rhodococcus chlorophenolicus* sp. nov., a chlorophenol mineralizing actinomycete. Int J Syst Bacteriol 36: 246–251
Buikema PW, Schimmel SC (1978) Toxicity of pentachlorophenol and related compounds to early life stages of selected estuarine animals. In: Ranga Rao K (ed) Pentachlorophenol, chemistry, pharmacology and environmental toxicology. Plenum Press, New York, pp 141–146
Cirelli DP (1978) Patterns of pentachlorophenol usage in the United States of America – an overview. In: Ranga Rao K (ed) Pentachlorophenol, chemistry, pharmacology and environmental toxicology. Plenum Press, New York, pp 13–18
Crawford RL, Mohn WW (1985) Microbiological removal of pentachlorophenol from soil using a *Flavobacterium*. Enzyme Microb Technol 7: 617–620
Dijkhuizen L, Harder W (1975) Substrate inhibition in *Pseudomonas oxalaticus* OX1: a kinetic study of growth inhibition by oxalate and formate using extended cultures. Antonie van Leeuwenhoek 41: 135–146
Drahonovsky J, Vacek Z (1971) Dissoziationskonstanten und Austauschchromatographie der Chlorphenole. Collect Czech Chem Commun 36: 3441–3450
Hakulinen R, Salkinoja-Salonen M, Saxelin M-L (1981) Purification of kraft bleach effluent by an anaerobic fluidized bed reactor and aerobic trickling filter at semitechnical scale. In: Proceeding Technical Association of Pulp Paper Industry Environmental Conference, 1981, TAPPI Press, Atlanta, pp 197–201
Hoos RAW (1978) Patterns of pentachlorophenol usage in Canada – an overview. In: Ranga Rao K (ed) Pentachlorophenol, chemistry, pharmacology and environmental toxicology. Plenum Press, New York, pp 3–11
Martinson MM, Steiert JG, Saber DL, Crawford RL (1986) Microbiological decontamination of pentachlorophenol contaminated natural waters. Biodeterioration 6: 529–534
Moos LP, Kirsch EJ, Wukasch RF, Grady CLP (1983) Pentachlorophenol biodegradation I. Aerobic. Water Res 17: 1575–1584
O'Reilly KT, Crawford RL (1989) Degradation of pentachlorophenol by polyurethane immobilized *Flavobacterium* cells. Appl Environ Microbiol 55: 2113–2118
Pignatello JJ, Martinson MM, Steiert JG, Carlson RE, Crawford RL (1983) Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. Appl Environ Microbiol 46: 1024–1031
Ranga Rao K (ed) (1978) Pentachlorophenol, chemistry, pharmacology and environmental toxicology. Plenum Press, New York
Saber DL, Crawford RL (1985) Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. Appl Environ Microbiol 46: 1024–1031
Stanlake GJ, Finn RK (1982) Isolation and characterization of a pentachlorophenol-degrading bacterium. Appl Environ Microbiol 44: 1421–1427
Topp E, Crawford RL, Hanson RS (1988) Influence of readily metabolizable carbon on pentachlorophenol metabolism by a pentachlorophenol degrading *Flavobacterium* sp. Appl Environ Microbiol 54: 2452–2459
Tyler JE, Finn RK (1974) Growth rates of a pseudomonad on 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol. Appl Microbiol 28: 181–184
Valo R, Kitunen V, Salkinoja-Salonen MS, Räisänen S (1985) Chlorinated phenols and their derivatives in soil and ground water around wood preserving facilities in Finland. Water Sci Technol 17: 1381–1384
Watanabe I (1973) Isolation of pentachlorophenol decomposing bacteria from soil. Soil Sci Plant Nutr 19: 109–116
Wild DG, Hinshelwood C (1955) The development of drug resistance in strains of *Saccharomyces cerevisiae*: resistance to 2,4-dinitrophenol and to brilliant green. Proc R Soc Lond (Biol) 144: 287–297

Appl Microbiol Biotechnol (1991) 35: 105–109
0175759891000960

Applied Microbiology and Biotechnology
© Springer-Verlag 1991

Degradation of chlorinated lignin compounds in a leach plant effluent by the white-rot fungus *Trametes ve* *lor*

Matthias Bergbauer, Claudia Eggert, and Gunda Kraepelin

Institut für Biochemie und Molekulare Biologie, Abteilung Botanik und Mikrobiologische Chemie, Technische Universität Berlin, D-1000 Berlin 10, Franklinstrasse 29 OE 5/1, Federal Republic of Germany

Received 21 May 1990/Accepted 3 January 1991

Summary. Chlorinated lignin derivatives in a combined bleach plant effluent from sulphite pulping were degraded by several white-rot fungi among which *Trametes versicolor* (*Coriolus versicolor*) strains were the most efficient. With glucose as co-substrate, about 90% colour reduction was achieved within 3 days. Simultaneously, the concentration of chloro-organic compounds measured as adsorbable organic halogens decreased by about 45%. As shown by gel chromatography, the high-molecular-weight fraction in the effluent was completely depolymerized while over 50% of total aromatic compounds were degraded. The presence of a co-substrate was necessary for all these activities of the fungus. The residue obtained after degradation was extremely recalcitrant and not further degradable.

Introduction

Pulp-mill effluents from bleaching procedures are dark brown due to their content of chromophoric, polymeric lignin derivatives. Moreover, chlorinated organic components have toxic and mutagenic properties (Rogers 1973; Ander et al. 1977; Eriksson et al. 1979; Kringstad et al. 1981) and may accumulate in aquatic organisms (Landner 1979). The fate of such chlorolignins in ecosystems has to be studied in more detail because aquatic pollution by discharging large amounts of these effluents into streams and oceans may result in serious environmental problems.

Whereas conventional methods of waste-water treatment such as activated sludge or aerated lagoons are rather ineffective for the biodegradation of waste-water lignins, wood-degrading terrestrial fungi seem to be the most promising organisms for this particular purpose. Most studies in this field have been done with kraft bleach plant effluents and efficient decolorization has already been obtained with various white-rot species (Marton et al. 1969; Fukuzumi et al. 1977). Degradation has been particularly studied with *Phanerochaete chrysosporium* (Eaton et al. 1980; Sundman et al. 1981) and optimization of colour removal was achieved by immobilized mycelia in the MyCoR process (Eaton et al. 1982). Besides decolorization, some dechlorination of chloro-organic compounds has also been observed (Huynh et al. 1985). Addition of a co-substrate proved to be essential for effective degradation in all cases. Moreover, decolorization by *P. chrysosporium* strongly depended upon the strain used (Augusta et al. 1986). The origin of the bleach plant effluent is also important for decolorization efficiency and generally effluents from sulphite pulping seem to be more recalcitrant than those from sulphate pulping (Farcher et al. 1985).

One of the white-rot fungi known to decolorize kraft mill effluents from sulphate pulping is *Trametes versicolor*. Efficient colour removal from such effluents was obtained with calcium-alginate-immobilized mycelium in batch cultures (Livernoche et al. 1981, 1983) and in a continuous process (Royer et al. 1983). Interestingly, this fungus is also able to brighten hardwood kraft pulp (Kirkpatrick et al. 1989).

In a previous paper we described for the first time the degradation of bleach plant chlorolignins from sulphite pulping by *T. versicolor* (Bergbauer et al. 1989). Here we report the culture conditions and their optimization for *T. versicolor* strains regarding decolorization, adsorbable organic halogen (AOX) reduction, depolymerization and degradation of chlorolignins derived from sulphite pulping.

Materials and methods

Organisms. All fungi used in this study are listed in Table 1. *T. versicolor* (*Coriolus versicolor*) CBS 297.33 and *Schizophyllum commune* CBS 266.60 were obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. All other strains are own isolates. Stock cultures of the fungi were stored on malt agar at 10°C and periodically subcultured.

Offprint requests to: M. Bergbauer

much as 85%. The use of RO and evaporation were recommended to meet the sponsors objective for the demonstration of innovative wastewater treatment technology. This technique would be applied to the two remaining major sources of chromium contamination (the gun line rinse and the bright-dip rinse). Pilot-scale RO tests indicated that the gun line rinse waters could be successfully treated by RO using a 1 to 5 mo prefilter. Pretreatment of the bright-dip rinse water proved to be ineffective, however, cleaning the membrane with a 1% solution of EDTA restored the permeate flux to 93% of its initial value. This allowed the use of RO treatment of the bright-dip rinse by oversizing the RO unit to take into account the expected loss in permeate flux. The full-scale treatment unit was built, transported to the industrial facility, and started up using wastewater stored at the facility. The rejection of chromium exceeded 99%, which was typical of the chromium rejections in the pilot studies. During this short-term test, the permeate flux decreased with time. The decrease in permeate flux with time was less than the decrease obtained during the pilot operation when the pilot unit was started up on a new membrane. The RO system was not run long enough to completely evaluate the loss in permeate flux with time. The lost permeate flux was restored by cleaning with a mixture of phosphoric acid.

LITERATURE CITED

1. Walker, J. F., Jr., *et al.*, "A Plan for Implementation of Innovative Hazardous Waste Minimization Techniques at an Eastern U.S. Naval Plating Shop," *Proc. 1985 40th Annual Water Pollution Control Federation Conference*, 129-141, Oct. 4-8, 1987, Philadelphia, PA.

Biodegradation of Chlorinated Hydrocarbons in an Immobilized Bed Reactor

Gary P. Miller, Ralph J. Portier, and Darryl G. Hoover
Institute For Environmental Studies, Louisiana State University, Baton Rouge, LA 70803

David D. Friday
Environmental Remediation, Inc., Baton Rouge, LA

Jerry L. Sicard
Ethyl Corporation Research and Development, Baton Rouge, LA

A 75 liter immobilized microbe biological reactor with a bed retention time of 20.5 hours was used in a continuous flow mode to remediate contaminated groundwater containing ethylene dichloride (EDC), tetrachloroethylene, and trichloroethylene, with EDC being the predominant contaminant. The reactor was initially seeded with Xanthobacter autotrophicus, *a demonstrated halogenated aliphatic substrate utilizer. The reactor was operated for forty-two days. Material balance determinations for primary volatile aliphatics of concern indicated an average of 90.2% mineralization of EDC, 81.7% of the trichlorethylene (TCE) and 64.0% of the tetrachlorethylene (TeCE). In addition to* Xanthobacter autotrophicus, *four indigenous bacterial species from the groundwater had successfully acclimated to the reactor bed.*

INTRODUCTION

Biological treatment using an immobilized microbial consortia has been studied by several researchers. Portier *et al.* [1] used an immobilized substrate to enhance PCB degradation, Chibata [2] studied immobilization to enhance aspartase deactivation, and Welsh *et al.* [3] tested eight different immobilization supports for effectiveness in biological treatment. There has also been an interest in the ability of bacteria to degrade halogenated hydrocarbons (Vogel *et al.* [4], Livesey and Anders [5], Friday and Portier [6]), and the species ethylene dichloride in particular (Stucki *et al.* [7], Bouwer and McCarty [8], Relay and Daniels [9], and Janssen *et al.* [10]). The use of biological reactors is a low maintenance, cost effective method of removing hazardous components from groundwater or wastewater streams. An advantage of biological treatment over physical separation techniques is that the hazardous material is chemically altered to nontoxic material or mineralized to CO_2 and biomass. With physical separation the hazardous material is recovered, but still requires disposal.

In this study, a biological reactor was used to remediate groundwater contaminated with chlorinated hydrocarbons. The major contaminants were ethylene dichloride (EDC), 1,2-$C_2H_4Cl_2$, trichloroethylene (TCE), C_2HCl_3, and tetrachloroethylene (TeCE), C_2Cl_4. The rationale of these continuous flow tests was to identify operating parameters and related materials handling needs to successfully design and operate such a system as a permanent remediation facility. Additionally, the evaluation of mineralization rates for minor halogenated aliphatic contaminants in the ground water was of interest both from a microbial physiological perspective as well as a need to include these data to refine the design and process make up considerations.

...esign

...ation

...ic schematic of the 75 liter reactor used to remediate the groundwater is shown in Figure 1. Contaminated groundwater with minimal nutrient addition for biomass growth optimization was fed into the reactor at 1.5 L/h. The feed entered the recycle line on the suction side of the recycle pump. The recycle stream was then aerated using 300 standard cc/min of air at 27 psi in a co-mingling jet configuration. The air and liquid stream are in contact through the aerator, where oxygen dissolves into the liquid stream. The liquid leaves the aerator with a dissolved oxygen (DO) level of 2.5 mg/L. The feed stream then entered a heated reactor maintained at 30°C. Some of the liquid was discharged out the reactor discharge line to maintain a constant liquid level in the reactor. The remaining aerated liquid stream enters into the packed bed section of the reactor and flows down through the bed.

The bed was packed with spherical diatomaceous earth beads (Manville #630 carrier). After leaving the bed the treated stream enters the recycle line. The recycle flow rate was 1.5 gallons per minute (gpm). Having a high recycle flow rate to feed flow rate ratio provided for good mixing in the reactor and allowed the reactor feed to maintain a high DO content. In this manner, the immobilized microbial bioreactor operated as a continuously stirred tank reactor (CSTR), wherein chlorinated aliphatic levels in the reactor approached levels in the reactor discharge line.

EXPERIMENTAL APPROACH

In order to develop a healthy biomass with which to treat the groundwater, the reactor was inoculated with 3 liters of a harvested broth containing *Xanthobacter autotrophicus* and 1.5 liters of nutrient solution. The reactor was then operated on a batch mode to develop the bacterial biomass density and to allow for complete colonization and subsequent immobilization on the carrier bed.

After the biomass had become established in the bed, the reactor was fed the contaminated groundwater for 42 days at a feed rate of 1.5 liters/min. This initial 42-day period allowed the reactor bed to be acclimated to chlorinated aliphatics in the groundwater and also allowed the indigenous bacterial species in the groundwater to develop a secondary biomass niche in the packed bed. The intent of the original experiment was to have only *Xanthobacter autotrophicus* as the active bioremediation species in the bed. However, due to the nature of the bacteria in the groundwater, it was impossible to keep all indigenous microbiota out of the reactor bed with its ideal growing conditions. The resulting layered bacterial mix was used in the bed for the reactor experiments.

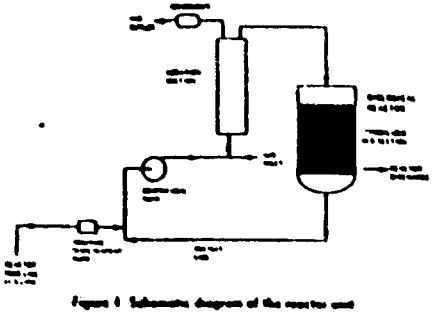


Figure 1. Schematic diagram of the reactor unit.

The reactor was operated for a second 42-day period during which material balance data were collected. Composition was determined by gas chromatography (FID detector, Carbopak B packed capillary column). Three chemical species were targeted to determine how effective the reactor would be in removing them from the feed stream. These were ethylene dichloride (EDC), 1,2-$C_2H_4Cl_2$; trichloroethylene (TCE), C_2HCl_3; and tetrachloroethylene (TeCE), C_2Cl_4. The feed rate was 1.5 liters/h of groundwater and the average contaminant loading was 226 mg/L for EDC, 10.9 mg/L for TCE, and 2.5 mg/L for TeCE.

RESULTS

Operating conditions for the 42-day test period are shown in Figure 2. Figure 2(a) presents the dissolved oxygen in the reactor, 2(b) shows the reactor pH, and 2(c) gives the temperature. The pH and temperature were held constant throughout the test, with pH ranging from 6.5 to 7.5 and the temperature from 85°C to 102°C. The dissolved oxygen content in the reactor varied over a wide range, from 1.2 mg/L to 3.3 mg/L.

Reactor efficiency in degrading EDC is shown in Figure 3. The average EDC reduction was 98.8% of the EDC in the feed stream. A calculation of the air stripping rate

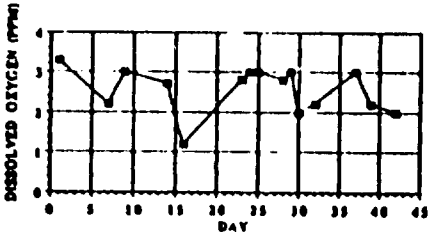


Figure 2a. Dissolved oxygen levels in the reactor.

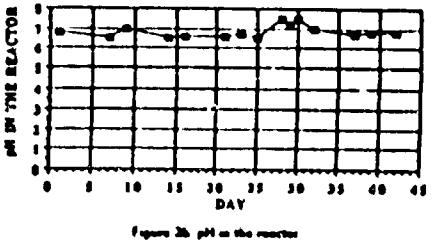


Figure 2b. pH in the reactor.

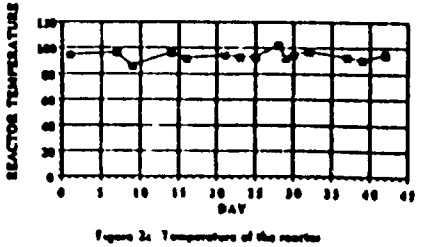


Figure 2c. Temperature of the reactor.

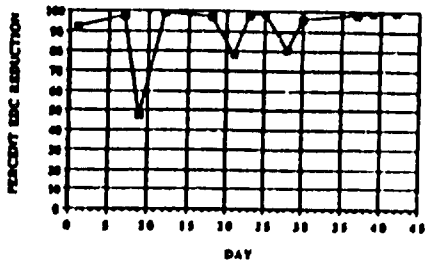


Figure 3. Percent removal of ethylene dichloride (EDC) from the reactor feed stream.

in the reactor showed that only 0.4% of the EDC was removed in the air stream by stripping. The Henry's law constants used in the stripping calculations are given in Table 1. The EDC feed rate fluctuated dramatically, as seen in Figure 4. The concentration of EDC in the feed stream ranged from 0.8 mg/L to 428 mg/L, with an average of 226 mg/L. The removal efficiency was above 75% for all measurements except one. This was a 47% efficiency on day nine. The highest daily removal was 99.6% on day twenty-five.

TeCE and TCE levels in the feed stream were found at much lower concentrations than EDC, but showed reaction removal efficiencies near those of EDC. The average reactor efficiency in removing TCE was 73.6% and for TeCE was 77.1%. The calculated stripping rates accounted for 9.4% of TCE removal. Mineralization was presumed to be the only other removal pathway, giving the reactor a biological reduction efficiency of 81.7% for C_2HCl_3. Biological reduction efficiency for each species, i, was calculated from the mass flow rate of species i in the feed stream, the effluent stream and the air stream as:

$$\text{BIOLOGICAL REDUCTION EFFICIENCY} = \frac{\dot{m}_{i,\text{feed}} - \dot{m}_{i,\text{effluent}} - \dot{m}_{i,\text{air}}}{\dot{m}_{i,\text{feed}} - \dot{m}_{i,\text{air}}} \times 100 \quad (1)$$

The reduction efficiency for C_2HCl_3 is given in Figure 5. The best single day TCE removal was 100% on day seven and the poorest daily removal efficiency was 46.6% on day twenty-one. The TCE loading is shown in Figure 6. The average concentration of C_2HCl_3 in the feed was 10.9 mg/L, with the highest loading being 19.1 mg/L and the lowest 2.7.

For TeCE the average reduction was 77.1%. The biological reduction efficiency for TeCE was 64.0% and the stripping rate was calculated to be 19.9%. TeCE removal

Table 1. Henry's Law Constants Used in the Stripping Calculations

Chemical Species	Henry's Law Constant (mm Hg)
EDC	10,392
TCE	141,851
TeCE	173,917

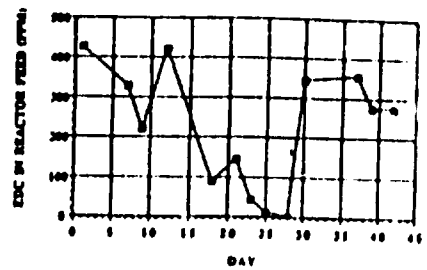


Figure 4. Ethylene dichloride concentration in the reactor feed stream.

was consistent at 80% after day ten, but varied significantly before that. On day nine the efficiency was 18% dropping to zero on day nine. The TeCE feed rate was an order of magnitude lower than the EDC feed rate. The average TeCE fraction in the feed was 2.5 mg/L, with a maximum of 5.3 mg/L and a minimum of zero.

SPECIES IDENTIFICATION

Bacteria were isolated from both the inlet and outlet reactor streams. Though the reactor was initially inoculated with only one species, *Xanthobacter autotrophicus*, four bacterial species were isolated from the effluent stream. Five isolates were found in the groundwater.

Of the five effluent isolates only two have been identified. These are *Xanthobacter autotrophicus* and *Comamonas acidovorans*. *Comamonas acidovorans* was also identified as one of the influent isolates. The three unidentified species from the reactor discharge are thought to have originated in the groundwater.

DISCUSSION AND CONCLUSIONS

An immobilized bed biological reactor was found to effectively remove chlorinated hydrocarbons from contaminated groundwater. The reactor was operated for forty-two consecutive days, treating contaminated groundwater containing three chlorinated hydrocarbons. The reactor showed a very high removal efficiency for ethylene dichloride, the major contaminant in the groundwater. For EDC the average removal efficiency due to biological activity was 90.2% and the average fraction of EDC in the feed was 226 mg/L.

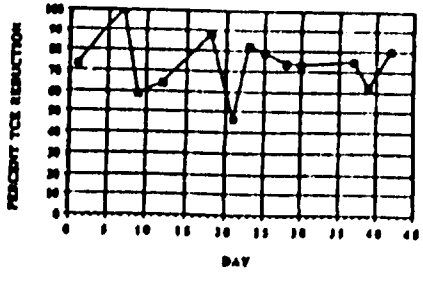


Figure 5. Percent removal of trichloroethylene (TCE) in the reactor.

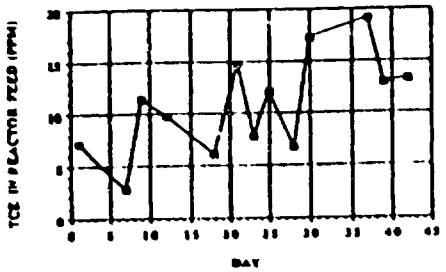


Figure 6. Trichloroethylene (TCE) concentration in the reactor feed stream

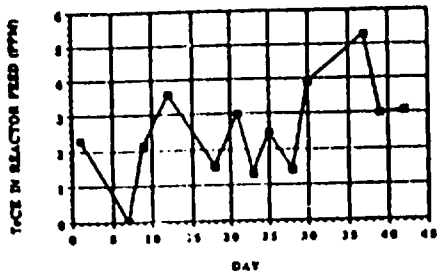


Figure 8. Tetrachloroethylene (TeCE) concentration in the reactor feed stream

The reactor also demonstrated a good removal efficiency for trichloroethylene and tetrachloroethylene. The average mineralization of TCE in the reactor was 81.7% and the mineralization of TeCE was 64.0%. The average feed of TCE was 10.9 mg/L of the total feed rate, and for TeCE this was 5.8 mg/L (see Figures 7 and 8).

The granular substrate in the reactor bed provided an excellent support for bacterial growth. The bed biomass was stable and effective throughout the test, and the bed proved capable of supporting the large bacterial populations required for effective treatment of the groundwater.

The reactor was initially inoculated with only one bacterial species, *Xanthobacter autotrophicus*. However, after initial operation of the reactor it was found that four species from the groundwater were successfully growing in the reactor. The only species that has been identified is *Commamonas acidovorans*.

ACKNOWLEDGMENTS

Institutional research funds from Louisiana State University to support field pilot evaluative studies were provided by the State of Louisiana Board Of Regents Education Trust Fund Matching corporate support for engineering services and analytical determinations of chlorinated aliphatics was provided by Ethyl Corporation. The daily help and cooperative interest and input by employees of Ethyl Corporation at the North Baton Rouge facility is not only readily acknowledged but deeply appreciated.

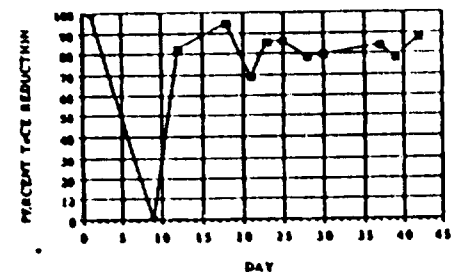


Figure 7. Percent removal of tetrachloroethylene (TeCE) from the reactor feed stream

LITERATURE CITED

1. Portier, R. J. and A. Fujioka, "Enhanced Biotransformation and Biodegradation of Polychlorinated Biphenyls in the Presence of Aminopolycarboxylates," Aquatic Toxicology and Hazard Assessment: 10th Volume, ASTM STP 971, Editors, W. J. Adams, G. A. Chapman, and W. G. Landis, American Society for Testing and Material, Philadelphia, pp 517-527 (1988).
2. Chihata, T., (editor), *Immobilized Enzymes*, p. 140, Kodansha Ltd., Tokyo (1978).
3. Welsh, F. W., R. E. Williams, and J. A. Veltby, "Solid Carriers for a *Clostridium acetobutylicum* That Produces Acetone and Butanol," Environ. Metals Technol., Vol. 9, pp. 500-502 (August 1987).
4. Vogel, T. M., C. S. Criddle, and P. L. McCarty, "Transformations of Halogenated Aliphatic Compounds," Environmental Science and Technology, Vol. 21, No. 8, pp. 722-736 (1987).
5. Lovesey, J. C. and M. W. Anders, Drug Metab., Dispos., Vol. 7, pp. 199-213 (1979).
6. Fraley, David and R. J. Portier, "Evaluation of a Packed Bed Immobilized Microbe Reactor for the Continuous Biodegradation of Haloaromatic Contaminated Groundwaters," Proceedings of the AIChE/AWMA/EPA International Symposium, 1988.
7. Stucki, G., U. Krieger, and T. Leisinger, "Bacterial Growth on 1,2 Dichloroethane," Experientia, Vol. 38, pp. 1371-1373 (1983).
8. Bouwer, E. J. and P. L. McCarty, "Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds Under Methanogenic Conditions," Appl. and Environ. Microbiol., pp. 1286-1294, April, 1983.
9. Belay, N. and L. Daniels, "Production of Ethane, Ethylene, and Acetylene from Halogenated Hydrocarbons by Methanogenic Bacteria," Appl. Environ. Microbiol., (47), pp. 1604-1610 (1987).
10. Jansen, D. B., A. Schipper, L. Dijkhuizen, and B. Witholt, "Degradation of Halogenated Aliphatic Compounds by *Xanthobacter autotrophicus* GJ10," Appl. Environ. Microbiol., (49), pp. 673-677, 1985.

Cleanup of Contaminated Soils by Pyrolysis in an Indirectly Heated Rotary Kiln

Dietmar Schneider
Deutsche Babcock Anlagen AG, Krefeld, Federal Republic of Germany

and

Blake D. Beckstrom
Ford, Bacon & Davis Utah, Inc., Salt Lake City, UT

The destruction of hazardous waste and cleanup of contaminated soils by pyrolysis in an indirectly heated rotary kiln system, followed by combustion of the volatiles in a secondary combustion chamber, has been tested in a pilot plant and demonstrated in an industrial scale plant. Deutsche Babcock Anlagen AG conducted comprehensive tests in a pilot plant with throughput rates of 0.5 to 1 ton/hour. Following these tests, Deutsche Babcock designed and constructed the industrial scale demonstration plant with a throughput rate of 7 ton/hour for Bergbau AG Westfalen near Dortmund, West Germany.

INTRODUCTION

Soils and other materials to be treated are heated in the kiln to 500°C to 650°C, with a maximum temperature of 750°C. The residence time of solids in the kiln is approximately 1 hour. Data from the tests, including destruction efficiencies for various polycyclic aromatic compounds and percent organics remaining in the ash, are presented. In general, the destruction efficiency exceeds 99%.

Operation costs of the system, including maintenance and amortization of capital costs, are between 130 and 160 Deutsche Marks per ton of soil (equivalent. $65-$80 per ton of soil).

Advantages of the system include:

1. Due to the slow warming-up of the material and good mixing, no crust formation occurs during drying. Thus, volatilization of organic constituents is improved.
2. The supply of heat to the indirectly heated kiln can be controlled by heating in sections, giving a higher degree of material temperature control.
3. Lower heat input is required.
4. The gas cleaning equipment can be down-sized.
5. The physical characteristics of the soil are unchanged.
6. No refractory liner is required in pyrolysis kiln.

The solid residue from the indirectly heated kiln is purified to the point where it can be backfilled on the same site.

PILOT-PLANT TEST

Planning for a pilot-plant test of the concept began in 1984 and the tests were completed in 1986. Deutsche Babcock conducted the comprehensive tests in a pilot-plant constructed at the Babcock Krauss-Maffei Industrial Equipment Center in Garching, near Munich.

The pilot plant indirectly heated kiln has a length of 7.20 meters and a diameter of 0.8 meters. Throughput rates near 0.5 ton/hour were tested. Soils and other materials to be treated were heated in the kiln to 500°C to 650°C, with a maximum potential of 750°C. This temperature, combined with the soil residence time of one hour in the kiln, was sufficient to remove 90 to 99+ percent of the highly volatile as well as the most thermally stable substances, such as polycyclic aromatic hydrocarbons.

As may be expected, the destruction efficiency for all compounds tested intensified with increasing treatment temperature and decreasing residence time in the kiln.

Operating experience shows destruction efficiencies are consistently above 99 percent. In fact, at a temperature of 650°C (measured by the internal kiln wall), the destruction efficiency was at least 99.9 percent for all compounds tested. And still further increases in destruction efficiency can be achieved by increasing the temperatures up to 750°C, which is possible in the indirectly heated kiln, and/or increasing the residence time of the soil in the rotary kiln.

The high destruction efficiency for an indirectly heated rotary kiln in the decontamination of contaminated soil from the coking plant is demonstrated by the sample results presented in Table 1.

TABLE 1. POLYCYCLIC AROMATIC HYDROCARBONS REMAINING IN THE ASH AFTER TREATMENT IN THE PILOT PYROLYSIS PLANT

Chemical Compound	Rotary Kiln Wall Temperature			
	550°C	650°C	550°C	650°C
Methyl Naphthalene	7.8	0.9	n.d.	n.d.
Phenanthrene	9.2	0.9	0.2	n.d.
Benzoflouranthene	6	1	n.d.	n.d.
Benzopyrene	2.2	0.6	n.d.	n.d.

BIOTECHNOLOGY TECHNIQUES
Volume 8 No.5 (May 1994) pp.339-344
Received as revised 15th March

Immobilization of *Phanerochaete Chrysosporium* on Porous Polyurethane Particles with Application to Biodegradation of 2-Chlorophenol

X. Wang and E. Ruckenstein*

Department of Chemical Engineering
State University of New York at Buffalo
Buffalo, New York 14260-4200, USA

ABSTRACT

Porous polyurethane particles were prepared and used for the immobilization of white rot fungus *Phanerochaete chrysosporium*. The immobilized cells were employed for the production of lignin peroxidase. Polyurethane immobilized spores, or mycelial pellets of *Phanerochaete chrysosporium* as well as freely suspended mycelial pellets of fungus were used as biocatalyst for the degradation of 2-chlorophenol. The polyurethane carriers appear to be superior to the other carriers already used for the immobilization of fungus.

INTRODUCTION

White rot fungus *Phanerochaete chrysosporium* excretes a highly effective extracellular oxidative enzyme, ligninase, which being nonspecific can break down not only lignin and methoxylated lignin but also a wide variety of compounds, such as chlorocarbons and polycyclic aromatics (Arjmand and Sandermann, 1985; Bumpus and Aust, 1987; Bumpus et al., 1985; Eaton, 1985; Huynh et al., 1985; Sanglard et al., 1986).

The production of lignin peroxidase by fungus in shaken cultures using veratryl alcohol for enhancement (Leisola et al., 1985), as well as its immobilization on various carriers was investigated in numerous papers. Some of them are: Bonnarme and Jeffries, 1990; Chen et al., 1991; Kirkpatrick and Palmer, 1987; Linko, 1988; Linko et al., 1987; Linko and Zhong, 1987; Linko and Zhong, 1991; Ma et al., 1990; Polymen et al., 1991; Ruckenstein and Wang, 1994. In this article a novel carrier for cell immobilization, namely, porous polyurethane particles, is suggested. The immobilized cells have been used to investigate the degradation of 2-chlorophenol. A similar carrier was previously prepared by us for the immobilization of lipase and used in the hydrolysis of triacylglycerides (Wang and Ruckenstein, 1993). The carrier was prepared as previously described (Wang and Ruckenstein, 1993). The porous polyurethane particles possess a specific surface area of 4.8 m^2/g and excellent mechanical properties.

Organism and culture conditions

Phanerochaete chrysosporium ATCC 24725, maintained on 3% malt agar slants, was grown aerobically on a carbon limited medium with the composition: 2 g/L glucose, 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4 . 7H_2O$, 0.12 g/L NH_4Cl, 0.1 g/L $CaCl_2 . 2H_2O$, and 0.001 g/L thiamine. The medium was kept at pH 4.5 by buffering with a 20 mM sodium tartrate solution. Pellets have been generated by inoculating spores (1 mL, containing about 1.5×10^7 spores/mL) into 75 mL of medium placed in 250 mL Erlenmeyer flasks shaken at 100 rev/min, at 39°C, for one day (after which they were used for immobilization), or two days to obtain free suspended pellets.

Immobilization

Particles of porous polyurethane (2.5 g) of about 0.1 cm size were placed in 250 mL Erlenmeyer flasks containing either free suspended spores or one day old pellets of fungus cells in 75 mL of medium. Then, the system was shaken at 100 rev/min, at 39°C for two days.

Enzyme production

After two days of growth period, the volume of the medium was reduced to 25 mL for freely suspended pellets and, for practical experimental reasons, only to 40 mL for immobilized fungus. The enzyme production was enhanced by adding veratryl alcohol to obtain a concentration of 1.5 mM, and carried out under 100% oxygen atmosphere, at 39°C, with shaking at 30 rev/min.

Lignin peroxidase activity

The activity of lignin peroxidase was measured at room temperature, by determining the initial oxidation rate of veratryl alcohol to veratraldehyde (Tien and Kirk, 1984), using a Beckman DU-70 UV spectrophotometer, at 310 nm. To 0.5 mL of supernatant containing 1.5 mM veratryl alcohol, 0.47 mL sodium tartrate buffer of pH 3.0 and 0.03 mL of 30 wt% hydrogen peroxide were added to obtain concentrations of 0.1 M and 0.3 mM, respectively. The concentration of veratraldehyde was measured after one minute. The activities are reported in U/L, where one unit of U is defined as one micromole of veratryl alcohol oxidized in one minute.

Repeated batch production with immobilized fungus

Spores immobilized on 2.5 g of porous polyurethane carrier were allowed to grow at 39°C, in 75 mL of carbon limited medium, containing 1 g/L glucose, placed in 250 mL Erlenmeyer flasks with shaking at 100 rev/mon until the entire carbon was consumed. To enhance the enzyme production, the volume of the liquid was reduced to 40 mL and veratryl alcohol was added to obtain a concentration of 2.0 mM. The production of lignin peroxidase was carried out under 100% oxygen atmosphere, at 39°C, with shaking at 30 rev/min. Since the addition of trace element and vitamin solutions improves the production of lignin peroxidase, 0.2 mL of trace element solution (containing 3.0 mg/L $ZnSO_4 . 7H_2O$, 1.5 mg/L Na-nitrilotriacetate, 1.0 mg/L $CoCl_2 . 6H_2O$, 1.0 mg/L $MnSO_4 . H_2O$, 0.01 mg/L $CuSO_4 . 5H_2O$, 0.01 mg/L

$AlK(SO_4)_2 \cdot 12H_2O$), 0.01 mg/L Na_2MoO_4 and 0.01 mg/L H_3BO_3), and 0.2 mL of vitamin solution (containing 100 mg/L thiamine-HCl, 20 mg/L pyridoxine-HCl, 10 mg/L 4-aminobenzoic acid, 10 mg/L thioctic acid, 10 mg/L riboflavin, 4 mg/L folic acid, 4 mg/L biotin and 0.2 mg/L cyanocobalamin) were introduced into each batch of the repeated batch production. The time interval for each batch was 48 h. At the end of a batch, the culture supernatant was decanted off and 40 mL of fresh carbon limited medium containing 1 g/L glucose, 2.0 mM veratryl alcohol, 0.2 mL of trace element solution, and 0.2 mL of vitamin solution were added to the Erlenmeyer flask. When the flasks were opened, either for sampling or for changing the medium, they were flushed with 100% oxygen for 10 minutes.

Degradation of 2-chlorophenol with free pellets

Spores (1 mL, containing about 1.5×10^7 spores/mL) were inoculated into 75 mL of medium placed in a 250 mL Erlenmeyer flask and shaken at 100 rev/min, at 39°C, for two days. The culture containing the formed suspended mycelial pellets was then introduced into the bioreactor, a 2-L New Brunswick Scientific Microferm MF 114 batch fermentor, containing 1350 mL of medium. The suspended pellets formed via the agglomeration of the spores were allowed to grow at 39°C, with stirring at 100 rpm, and an aeration rate of 0.3 L/min. The enzyme production was enhanced by adding veratryl alcohol to the system to obtain a concentration of 1.0 mM, after the glucose was consumed (about 2 days). After five days, 0.675 g of 2-chlorophenol was introduced into the fermentor along with 0.5 g/L of the surfactant Tween 80.

Degradation of 2-chlorophenol with immobilized systems

For the immobilization of mycelial inocula, suspended two day old pellets were introduced into the bioreactor that contained 2.5 g particles of porous polyurethane carriers (about 0.1 cm in size) and 1350 mL of medium. The immobilization and the growth of the cells as well as other processes were carried out as described above. Experiments have been also carried out by introducing the polyurethane carrier and suspended spores into 75 mL of medium from the beginning, the other conditions remaining the same as above.

Analytical assay of 2-chlorophenol

The concentration of 2-chlorophenol was determined with a HPLC (ISCO-2350 pump/ISCO V_4 detector at 254 nm, 15 cm × 4.6 mm Hypersil® Green ENV column using a solution of 1 vol% acetic acid in methanol as the mobile phase.

The stripping rate of 2-chlorophenol during aeration was measured in the presence of dead fungus in the fermentor. The off-gas was passed through three flasks in series, each containing 500 mL of water, in order to absorb 2-chlorophenol. Samples from the fermentor and gas traps were analyzed. A material balance was used to evaluate the amount of biodegraded 2-chlorophenol.

RESULTS AND DISCUSSION

Enzyme production

The dependence of lignin peroxidase activity in batch production on time, for free pellets, polyurethane immobilized spores and immobilized pellets is presented in Figure 1. The lignin peroxidase activity is markedly improved by immobilization, the immobilization of spores providing a higher activity than the immobilization of one day old pellets. Figure 1 shows that the higher activity of immobilized spores is due to both the immobilization and the physiological difference between spores and pellets. The immobilized spores provide an activity of 357 U/L 2 days after the enhancement of enzyme production by veratryl alcohol, and a maximum activity of 697 U/L after about 7 days. In contrast, the maximum activity of 322 U/L is obtained after 14 days with the free pellets. (Since the volume of the medium was reduced to 25 mL for free pellets and 40 mL for the immobilized systems, a few experiments have been carried out in which the volume was reduced to only 40 mL for free pellets. The differences in mean activities have been negligible). The volumetric productivities calculated on the basis of the maximum activities obtained with free pellets and with immobilized spores are 0.96 and 4.15 U/L h, respectively. The highest productivity obtained before was 3.4 with spores immobilized on an agarose carrier (Linko and Zhong, 1987).

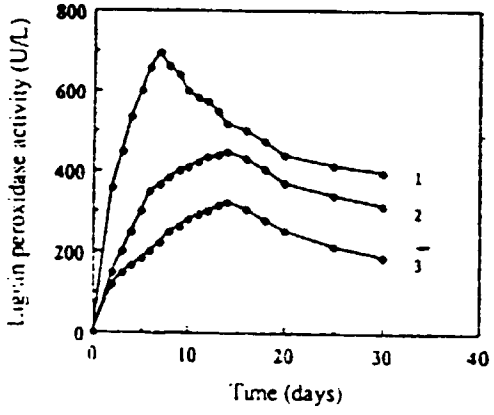


Fig. 1.
Lignin peroxidase activity (U/L) with free pellets (3), immobilized pellets (2), and immobilized spores (1). The time is measured from the moment of addition of veratryl alcohol.

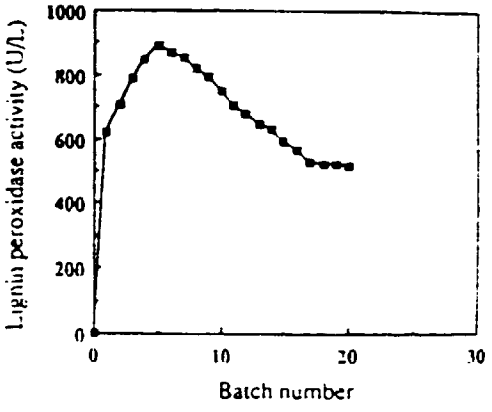


Fig. 2.
Lignin peroxidase activity (U/L) produced in 48 h by each batch, in repeated batch series with immobilized spores.

To investigate the stability of the immobilized biocatalyst, repeated batch production of lignin peroxidase with polyurethane immobilized spores was carried out. Figure 2 presents the lignin peroxidase activity produced in 20 successive batches lasting 48 h each. One can note that the activity was always higher than 500 U/L.

Degradation of 2-chlorophenol by biocatalysts

The results for a feed concentration of 500 ppm (g/g) 2-chlorophenol are presented in Figure 3, which shows, as expected, that, for the same amount of original spores, the immobilized spores provide much higher activities than the immobilized pellets and free pellets in the degradation of 2-chlorophenol. The degradation activity is expected to become much higher by increasing the amount of immobilized spores and the aeration rate. Indeed, because of the conditions employed, the production of lignin peroxidase in the bioreactor have been much smaller (324 U/L for immobilized spores) than those in the flask experiments (448 U/L for immobilized spores).

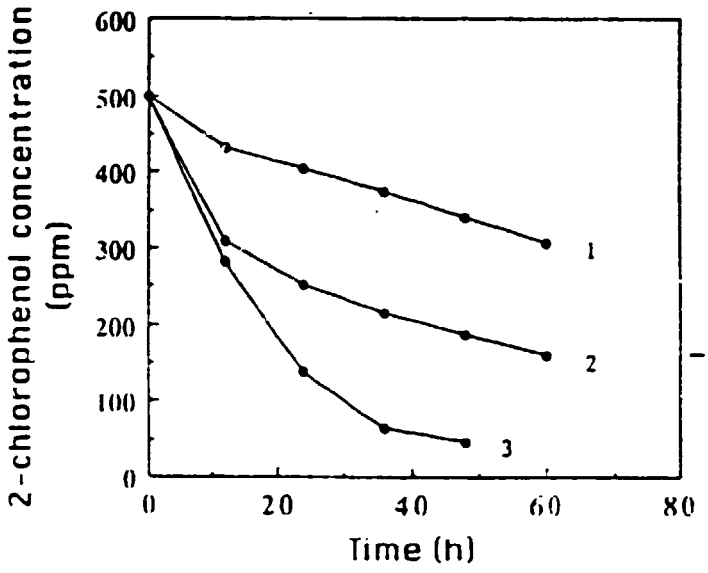


Fig. 3. Degradation of 2-chlorophenol

(1) by free pellets;
(2) by polyurethane immobilized pellets;
(3) by polyurethane immobilized spores.

The concentration of 2-chlorophenol is expressed in (g/g) x 10^6.

CONCLUSIONS

1. Porous polyurethane carriers have been prepared and used for the immobilization of white rot fungus *Phanerochaete chrysosporium*.

2. Polyurethane immobilized *Phanerochaete chrysosporium* exhibits a very high lignin peroxidase productivity and stability in both batch shake cultures and repeated batch shake cultures. Their productivity is much higher than that of the free pellets, particularly when spores are immobilized.

3. Polyurethane immobilized spores exhibit a higher activity in the degradation of 2-chlorophenol than the immobilized and the free pellets.

REFERENCES

Arjmand, M. and Sandermann, H. (1985), Agric. Fd. Chem., 33, 1055.
Bonnarme, P. and Jeffries, T. W., (1990), J. Ferm. Bioeng., 70, 158.
Bumpus, J. A. and Aust, S. D., (1987), Appl. Envir. Microbiol., 53, 2001.
Bumpus, J. A., Tien, M., Wright, D. and Aust, S. D., (1985), Science, 228, 1434.
Chen, A. H. C., Dosoretz, C. G., Grethlein, H. E., (1991), Enzyme Microb. Technol., 13, 404.
Eaton, D. C., (1985), Enzyme Microb. Technol., 7, 194.
Huynh, V. B., Chang, H., Joyce, T. W., Kirk, T. K., (1985), TAPPI, 68, 98.
Kirkpatrick, N. and Palmer, J. M., (1987), Appl. Microbiol. Biotechnol., 27, 129.
Leisola, M. S. A. and Fiechter, A., (1985), FEMS Microbial. Lett., 29, 33.
Linko, S., (1988), Enzyme Mocrob. Technol., 10, 410.
Linko, S. and Zhong, L-C., (1991), Bioprocess Engineering, 6, 43.
Linko, S. and Zhong, L-C., (1987), Biotechnol. Techn., 1, 251.
Linko, S., Zhong, L-C., Leisola, M., Linko, Y.-Y., Fiechter, A., Linko, P., (1987), In Lignin Enzymic and Microbial Degradation (Odier, E., ed.), INRA Publications, Versailles, p. 209.
Ma, D., Hattori, T., Shimada, M., Higuchi, T., (1990), Wood Res., 77, 35.
Polvinen, K., Lehtonen, P., Leisola, M., Visuri, K., (1991), ACS Symp. Ser., 460, 225.
Ruckenstein, E and Wang, X., Biotechnol. Bioeng. (in press).
Sanglard, D., Leisola, M. Fiechter, A., (1986), Enzyme Microb. Technol., 8, 209.
Tien, M. and Kirk, T. K., (1985), Proc. Natl. Acad. Sci., USA, 81, 2280.
Wang, X. and E. Ruckenstein, E., (1993), Biotechnol. Prog., 9, 661.