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STUDY ON BIODEGRADATION OF PERSISTENT ORGANIC POLLUTANTS

REPORT*

prepared by

Industrial Sectors and Environment Division

Based on the work of Messrs. I. Balogh, F. Merchant and D. de Ford, UNIDO consultants

* This document has not been edited.

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PREAMBLE

This study was prepared by the Chemical Industries Branch, Industrial Sectors and Environment Division of the United Nations Industrial Development Organization (UNIDO). The views expressed in this document are those of the authors and do not necessarily reflect the views of the Secretariat of UNIDO. Mention of firm names and commercial products does not imply the endorsement of UNIDO.

The study on Biodegradation of Persistent Organic Pollutants, more familiarly POPs has been based on the work of Messrs. István Balogh, industrial microbiologist, Fahar Merchant, biochemical engineer (team leader) and Douglas de Ford, chemical engineer, UNIDO consultants. The study has been based on an actual mission in a developing country. We have avoided all references to the country, institutions and names of various factories. This has been done throughout the text and drawings and therefore could allow the Secretariat of UNIDO to use it for general circulation. The content therefore essentially apply to any developing and transition economy nation. Where appropriate and for the sake of clarity, the names of countries, institutions have been replaced and/or other pseudonyms were used.

The study prepared by UNIDO is regarded as a contribution of our organization to the Inter-Organization Programme for the Sound Management of Chemicals (IOMC). By attempting this, the study shall be submitted for review to the participating organizations through the Inter-Organization Coordinating Committee (IOCC). All comments, remarks, suggestions for amendments and changes or even any major revision of the study is welcomed.

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I. SUMMARY

This report is a general study on typical pollution problems encountered as a result of increased industrial development in emerging nations. It is based on an actual case study carried out by three international consultants and their combined experiences.

The objectives of this report are to review the industry, its associated pollution problems and current methods used to treat the waste streams. Having established this background knowledge, we have evaluated the current technical expertise available in the country to handle hazardous wastes, especially as it relates to the use of the biodegradation route. Based on their existing resources, hands on technical solutions were then provided by the consultants in order to launch a successful project for biological treatment of organic pollutants. In this particular case, these activities included 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, a wastewater treatment plant, and the capabilities of the host organization, recommendations have been presented for continued development of the program and a proposal for longer term capability enhancement.

Due to the rapid growth of the pharmaceutical and chemical industry and continued stress on water resources, use of biotechnological approaches to water treatment will form the basis for sustained development in emerging nations. The enthusiasm and desire of the local government to support and stimulate industry forms an important launching pad to take any project to prominence.

However, by establishing a strong infrastructure in biological treatment of organic compounds, we believe that the local research centres 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. More importantly, this capability would benefit soil and groundwater remediation programs.

It is likely that each country will have different technical needs, varying areas of expertise, and unique environmental problems based on the local economy. The case study and its recommendations, which formed the basis of this report, provides a framework strategy for identifying pollution problems in other countries, determining local expertise in treating these waste streams, and developing a proposal for long term capability enhancement in the area of environmental biotechnology.

II. INTRODUCTION

The manufacture of chemicals and pharmaceuticals is rapidly emerging as a key sector of economic development in developing countries. In emerging nations, these industries are beginning to contribute significantly for 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 these industries.

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 the emerging nations where ambient temperatures result in more rapid biological reaction rates.

For example, chlorophenols and other phenols have been found to common pollutants in effluent streams from the chemical industries. These compounds are detrimental to aquatic and human life, and to the environment in general. The potential for environmental damage in arid countries 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 these countries and minimize the detrimental effects associated with persistent organic pollutants ("POP").

In this report, we provide strategies for assessing industrial pollution in a region or industrial sector (in this case the pharmaceutical industry), evaluation of their existing waste water treatment capabilities, review R&D capabilities and technical expertise, and propose strategies for developing a capability in the area of biodegradation of POPs. The report is essentially a case study that could form a useful model for other countries. Indeed the model presented here may not be ideal for any country and individual need assessment studies have to be carried out on a case by case basis.

III. OBJECTIVES

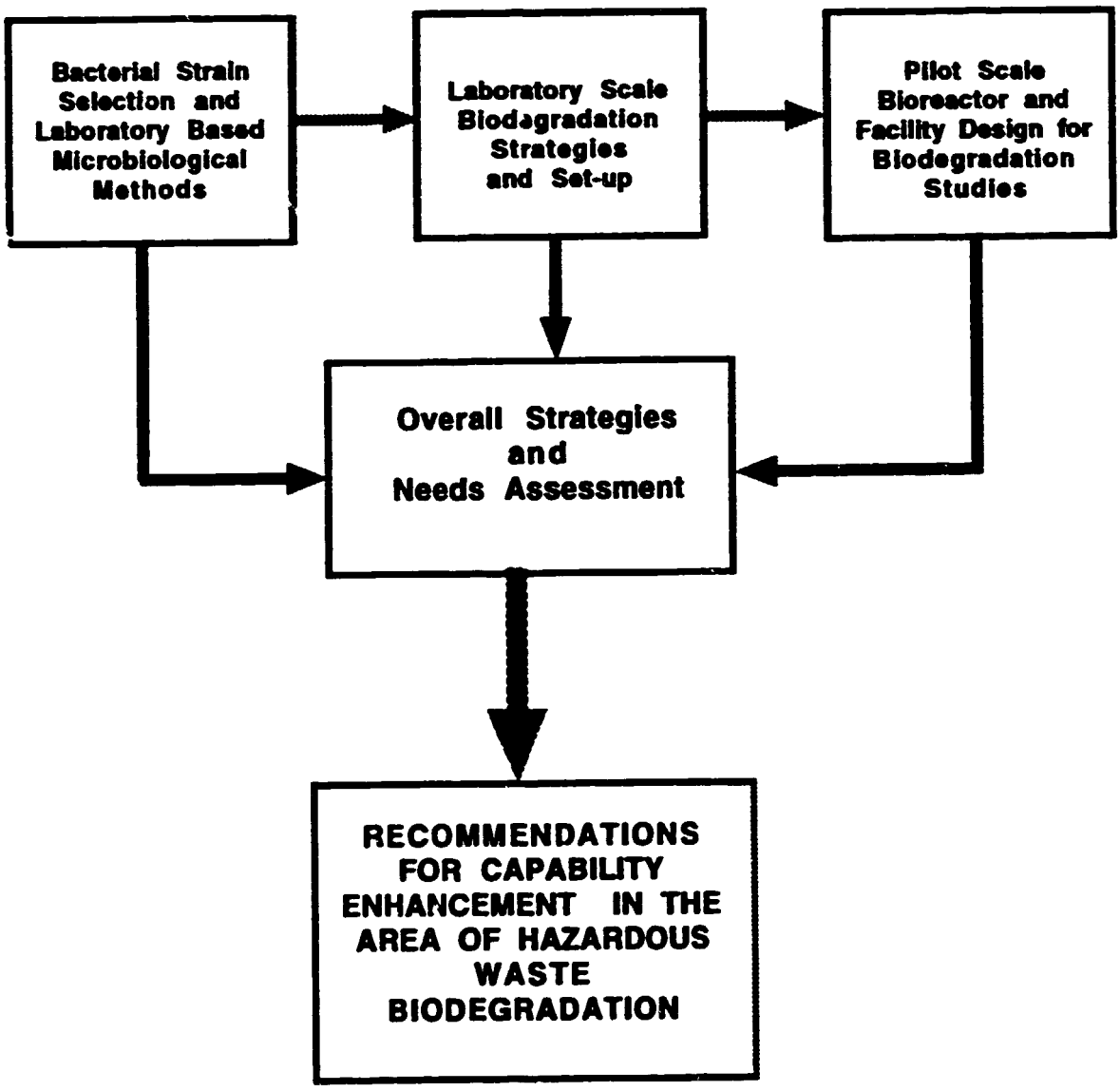
The Environmental Research Centre ("ERC") is the premier research organization in the country that can potentially establish a local base of expertise to tackle growing environmental concerns. ERC indicated its desire to assist the pharmaceutical sector in their waste treatment problems. In order to 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 ERC 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 pharmaceutical industry in the country and its 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 for Biodegradation of Hazardous Wastes



IV. SITUATION ANALYSIS

IV.1 OVERVIEW OF THE LOCAL PHARMACEUTICAL INDUSTRY

The 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.

However, due to lack of significant natural resources, its 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 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 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 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)
Company 1	Human medicines, gelatin capsules	analgesics, antibiotics, steroids, empty capsules, etc.	260	$4.8 million (94%)
Company 2	Human medicines	antibiotics, CNS, cardiovascular, diabetes drugs, antihistamines, etc.	400	$17.2 million (63%)
Company 3	Human medicines	Antirheumatics, analgesics, muscle relaxants, steroids, vitamins, etc.	470	$24.6 million (82%)
Company 4	Human medicines & veterinary drugs	Anti-inflammatory, antacids, diuretics, antiasthmatics, enzymes, etc.	1,200	$33 million (81%)
Company 5	Human medicines	Antiulcers, ACE Inhibitors, antihelmintics, antibacterials, etc.	140	$5 million (60%)
Company 6	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 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. The country'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 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.

Research and Development ("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 this country, the majority of the pharmaceutical industry is based on secondary manufacturing and the support industry. More recently, however, the major players in the pharmaceutical sector 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 where water is a scarce raw material, 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 ERC is proactively seeking assistance to establish this capability in the country, so that future growth of the young pharmaceutical industry is not impeded by regulatory compliance or potential environmental problems in the future.

IV.3 THE NATIONAL SCIENTIFIC COUNCIL

The National Scientific Council ("NSC") was established in 1970 as a research and development institution to work in the areas related to industrial growth in the country. 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. The budget of NSC is derived from contract revenues from technical services and consultations, research contracts, and an annual grant from the Government. 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 NSC are as follows:

- Conduct scientific R&D work related to the development process 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 neighbouring countries and internationally.

NSC 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

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 ERC has conducted several studies on the quality of water in the country in collaboration with other government organizations. These studies identified bacterial contamination in water wells in remote areas. ERC 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 has prompted 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 program to establish capabilities in the area of biodegradation of hazardous wastes has also been recently established.

From the above information and our observations it is apparent that ERC is a premier research centre in the region and actively engaged in development and commercialization of novel waste treatment technologies. Its reputation and stature in neighbouring countries 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 the region. The need to establish a strong capability in this area becomes apparent when reading some pages of a recent report on industrial pollution in the region.

V. FIELD ACTIVITIES

The field experts appointed by UNIDO were stationed at the headquarters of the NSC and hosted by the 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 ERC. 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 at NSC. Administrative, laboratory and technical assistance was provided by senior management of the ERC.

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

As mentioned earlier, there are several major pharmaceutical factories in the capital city. 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. For confidentiality reasons, the factory's names have been omitted form this report and are identified as follows:

- Factory A
- Factory B
- Factory C

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

Factory C did not allow us to tour their factory or even provide a sludge sample. Instead of Factory C, arrangements were then made to tour the Local Wastewater Treatment Plant.

The quantities of waste generated by the above pharmaceutical companies are summarised in Table 2. The waste water analysis data for Factory A and C are presented in Table 3.

The managers at both factories that we visited (i.e. Factory A and B) 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 Selected Pharmaceutical Companies

Pharmaceutical Facility	Solid Waste	Wastewater (m3/day)
Factory A	All recycled	25
Factory B	Not available	36
Factory C	Not available	125

Table 3: Characteristics of Wastewater from Factory A and Factory C

Characteristics	Factory A	Factory C
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 Factory A

Factory A is situated in the western part of the city. 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, this factory exports 90 % of its products to neighbouring countries.

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 from Factory A; one from the effluent of the chemical synthesis plant and the other from a biological treatment pit.

V.1.2 Factory B

Factory B is located at the South Western part of the city. This 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).

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 Local Wastewater Treatment Plant

The Local Wastewater Treatment Plant ("LWTP") , which is owned by the Industrial State Corporation, is situated in the industrial region which is approximately 10 km South of 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 LWTP.

Considering the large diversity of the factories (e.g. detergents, paints, dyes, cosmetics, etc.) and their disregard for discharging polluted effluents, the LWTP 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 LWTP for further laboratory studies at NSC, as described later.

V.2 ENRICHMENT AND SELECTION OF MICROBES

V.2.1 Introduct :

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 pos ..on 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 was provided to the scientists, and may be acquired by ERC 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 NSC. It should also be mentioned, that with the exception of Factory C, sludge samples were made available by other organizations. Managers at Factory A and Factory B (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 NSC. 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
Factory A	A1, A2
Factory B	B
Local Wastewater Treatment Plant	L

The sample originating from Factory A 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. 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 CPE, 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
A1	No growth	No growth	No growth
A2	No growth	No growth	No growth
B1	Poor growth	No growth	Poor growth
L	Dense growth	Poor growth	Dense growth

Table 5: Results of OD measurements at 600nm

Sample	CPE1 Medium	CPE2 Medium	CPE3 Medium
A1	0.12	0.172	0.14
A2	0.138	0.166	0.102
B	0.08	0.224	0.192
L	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 (B/CPE2, B/CPE3, L/CPE1, L/CPE2 and L/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 L/CPE1 and L/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 ERC 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.

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 NSC. 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 NSC 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.

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.

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

V.4.1 Introduction

Upon arriving at NSC it was determined that the ERC 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 ERC's ability to gain experience in the area of fermentation technology which is absolutely essential if ERC is to pursue with pilot scale biodegradation studies.

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 c[illegible] on support surfaces or entrapment in polymeric matrices, the cells have an opportunity to adjust to intermittent changes in the composition of the feed str[illegible]am. Furthermore, immobilization ensures extremely high cell densities of the d[illegible]ed microbial population in the bioreactor and therefore increased biodegradation r[illegible]s.

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 ERC 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 during our stay. This material can be readily purchased from Sigma or other chemical supply companies. It would be a good idea for ERC 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. Relevant research papers outlining methods for immobilization and bioreactor design issues were provided as part of the report submitted. A bibliography with additional details on PCP degradation and immobilization techniques was also included in the report submitted.

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 ERC. 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.

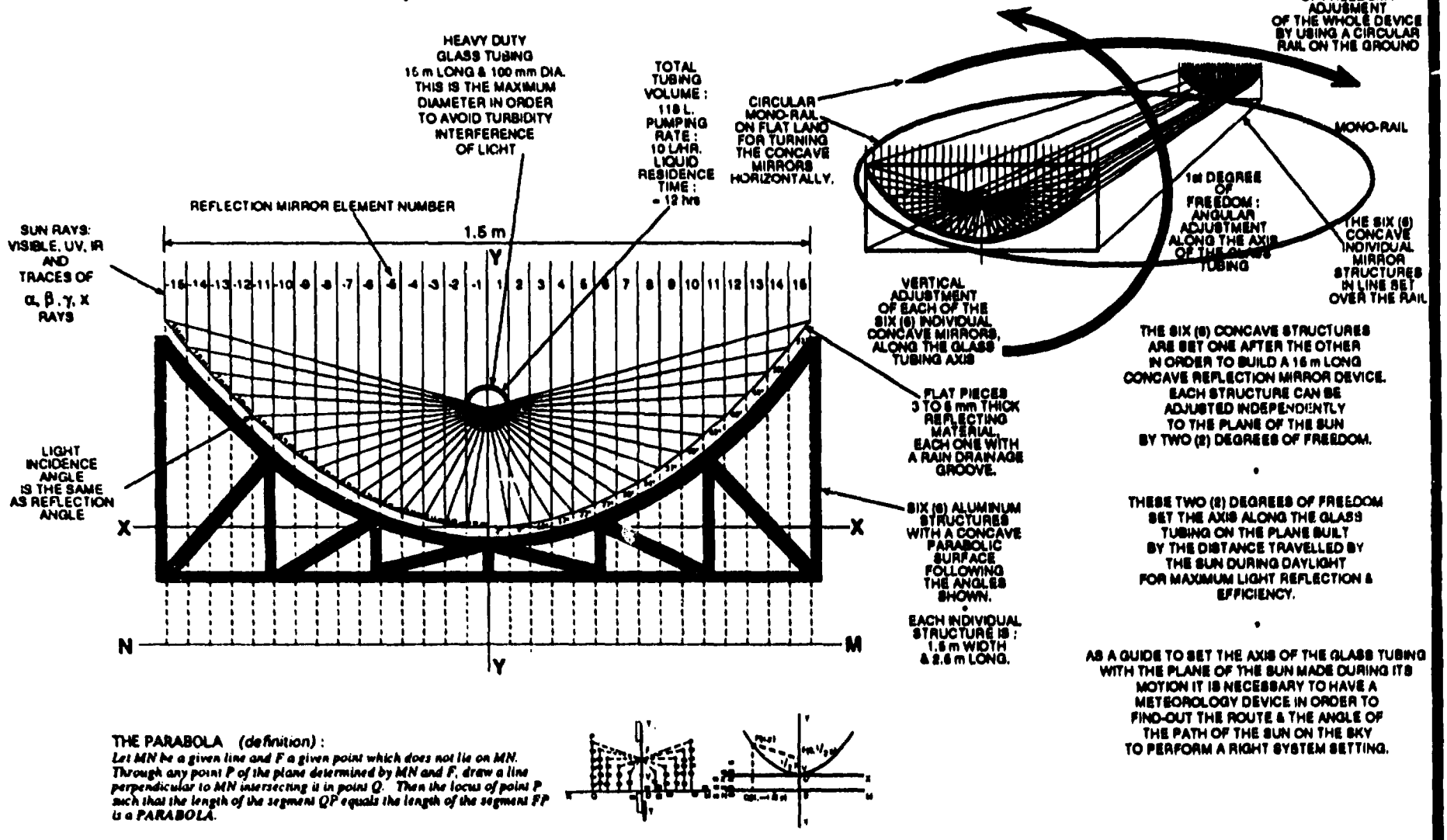
Capital expense for installing a photolysis pretreament unit is expected to be high. However, one would expect that with adequate pretreatment, the demand on microbial degradation will not be severe and complete biodegradation may be accomplished using smaller bioreactors.

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



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 complete 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. NSC has well established capabilities to build such units in-house.

V.5.4 Continuous Packed Bed and Trickling Filter Bioreactor

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 NSC using plexiglass. Dissolved oxygen, pH and foam controls should be performed in the oxygen transfer chamber. The 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

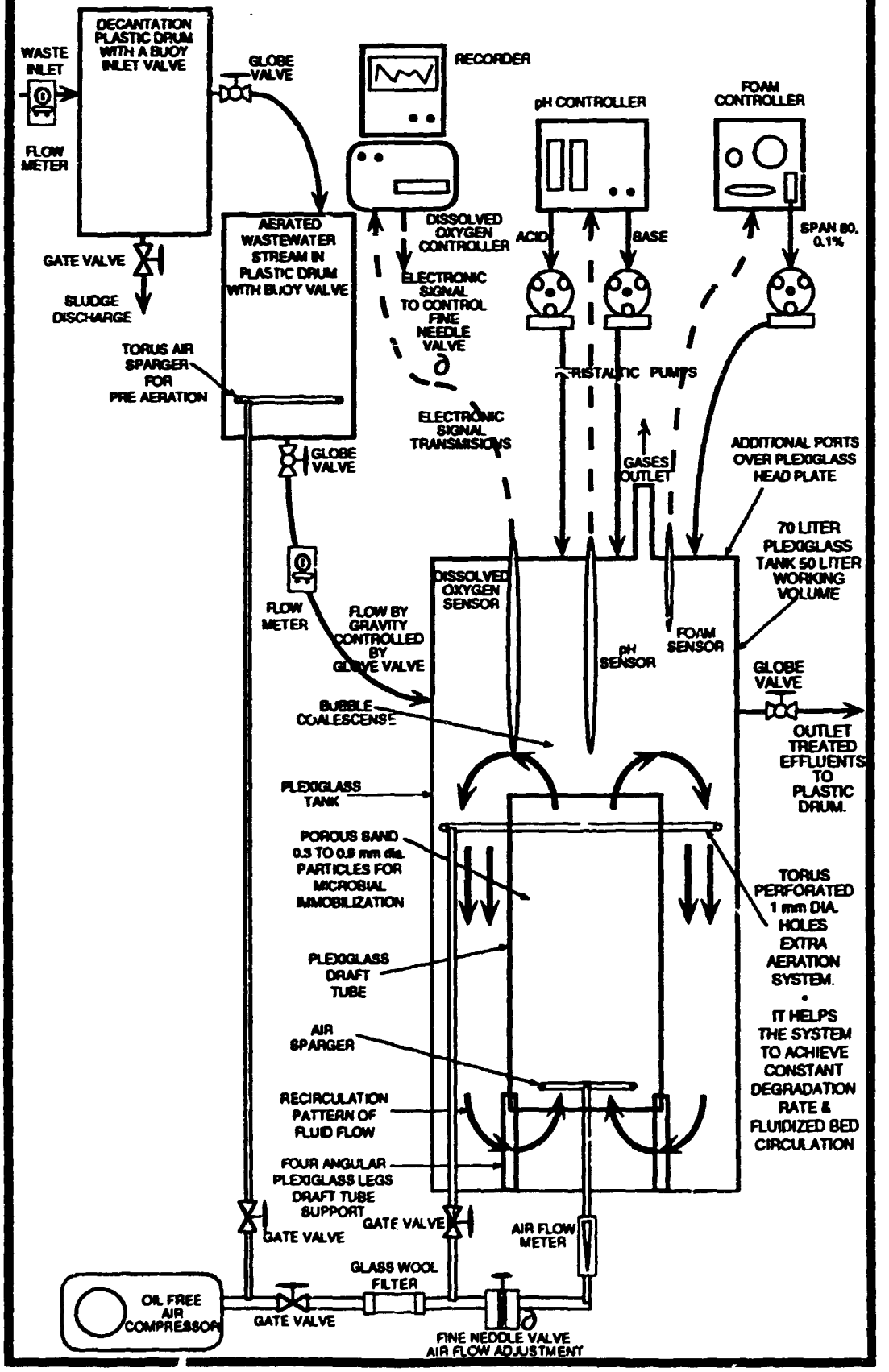


FIG. 4: General View of a Packed Bed Bioreactor

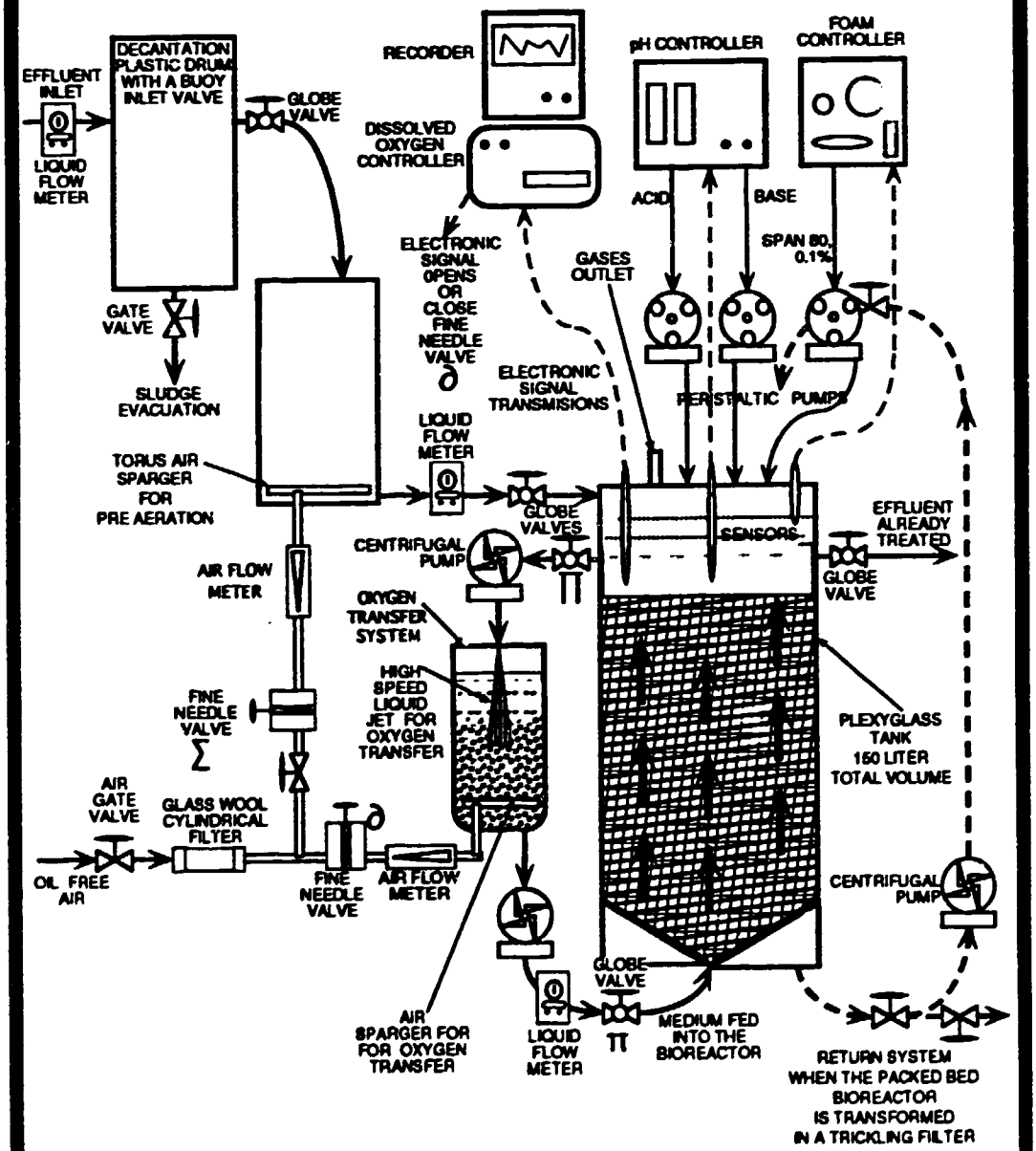


FIG. 5: GENERAL VIEW OF A CONTINUOUS TRICKLING FILTER BIOREACTOR

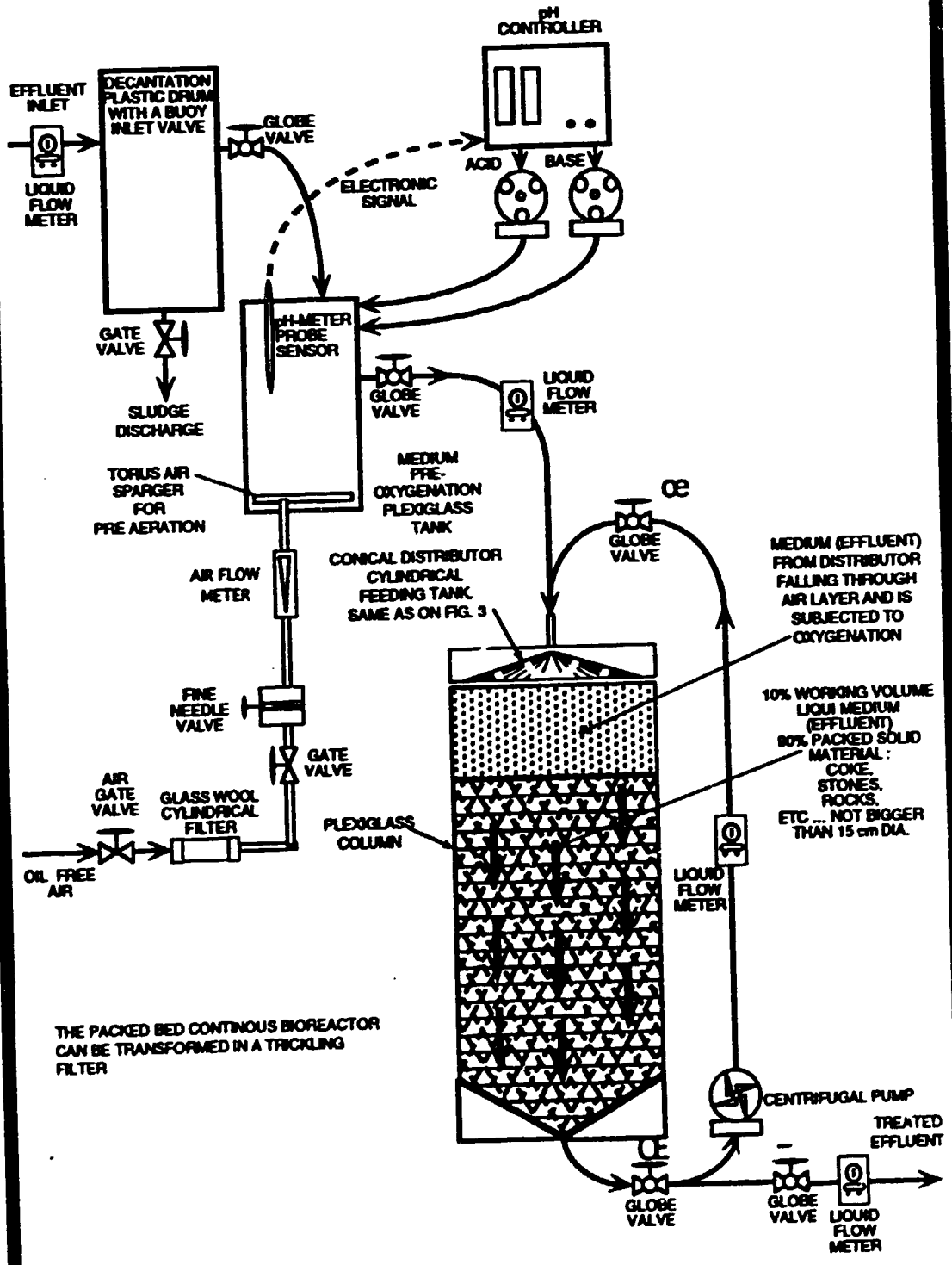
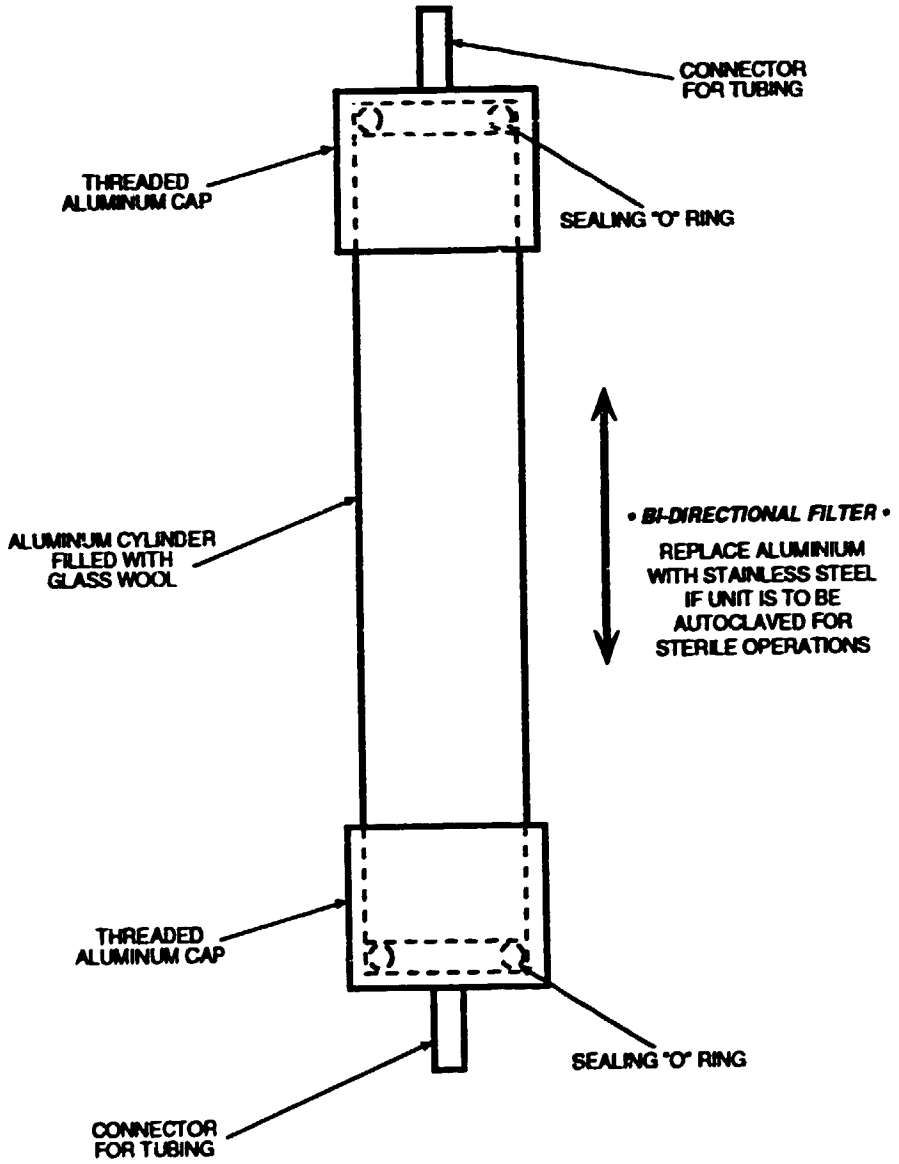


FIG. 6: GLASS WOOL AIR FILTERS

(Recommended for all Bioreactors)



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

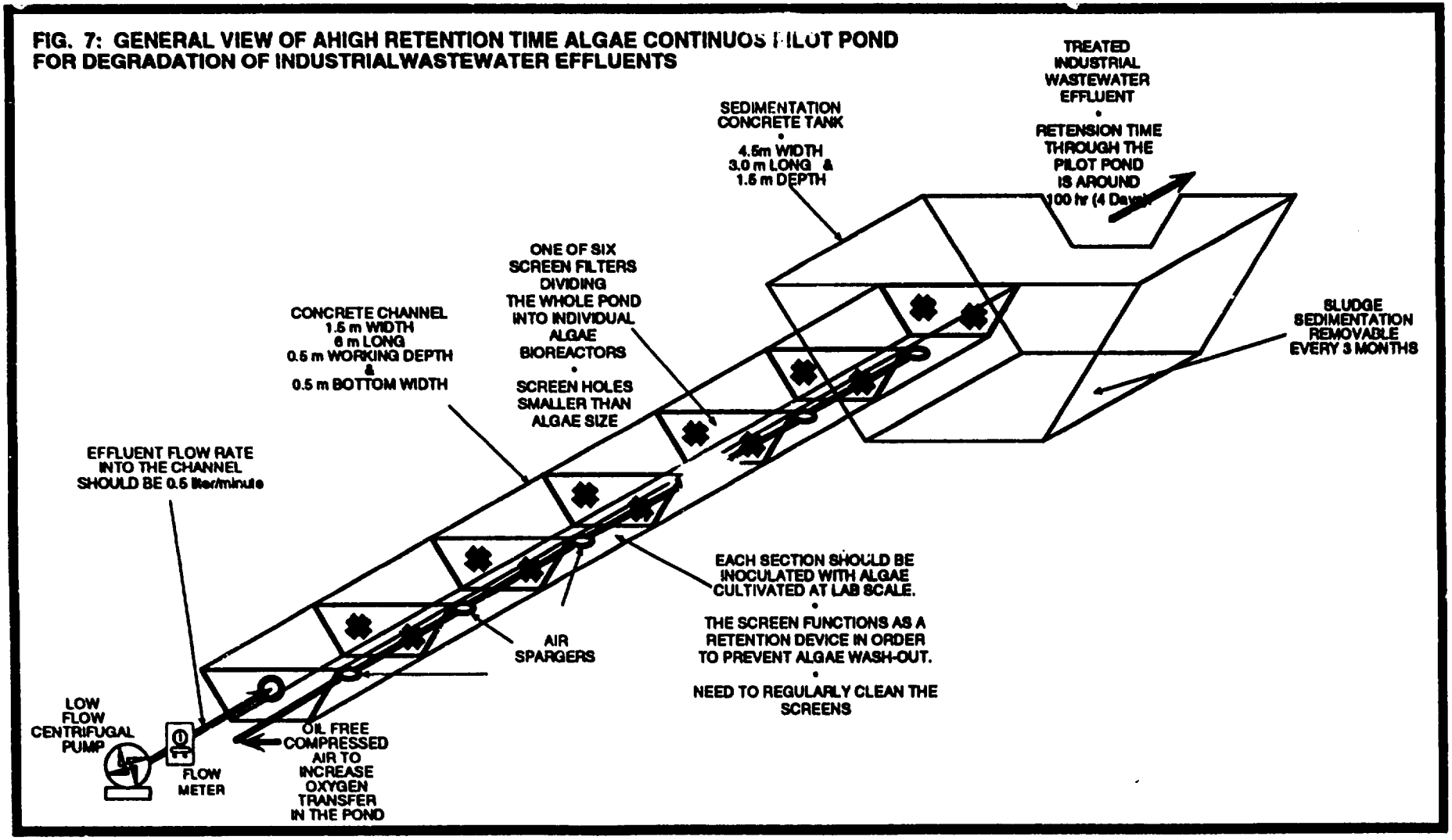


FIG. 8: Schematic Diagram Showing a Screen Filter (To Prevent Algal Washout) of a Continuous Flow Algal Pond

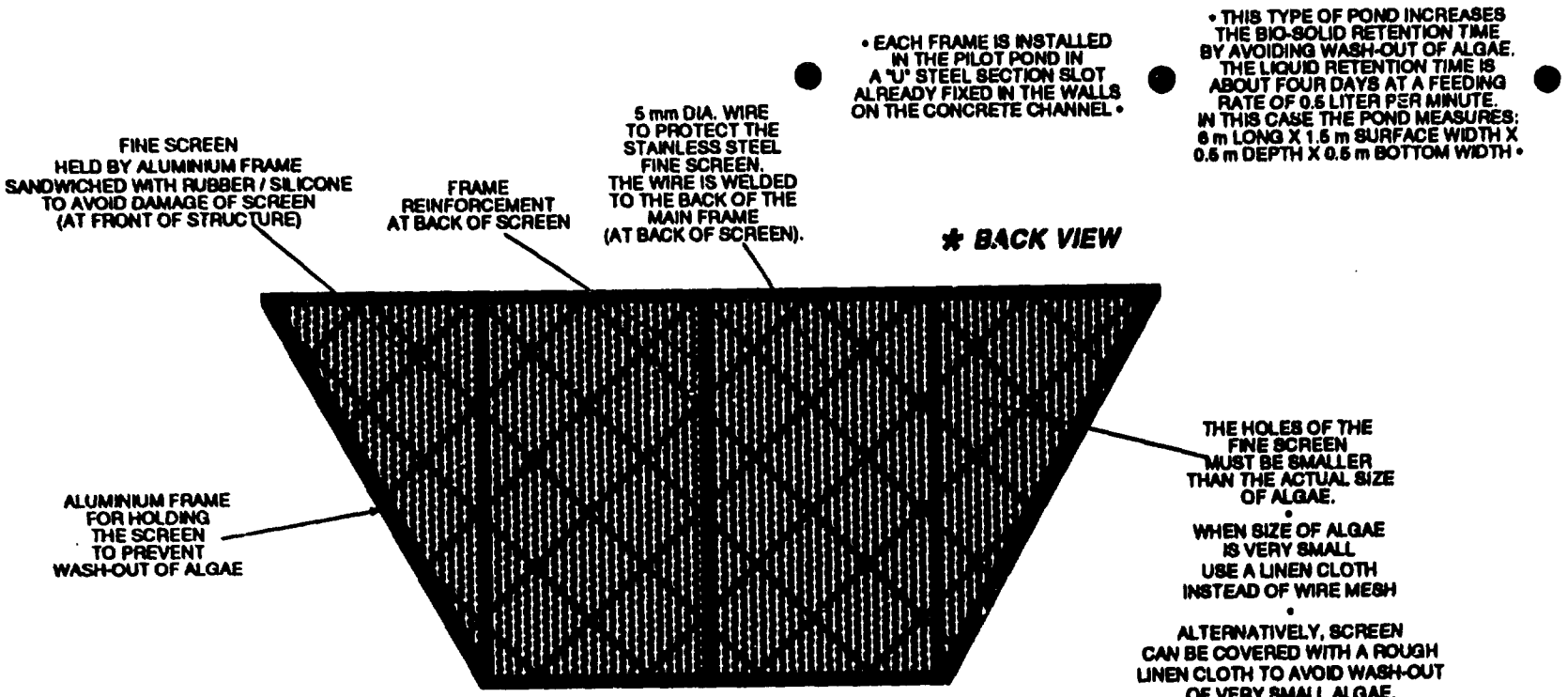
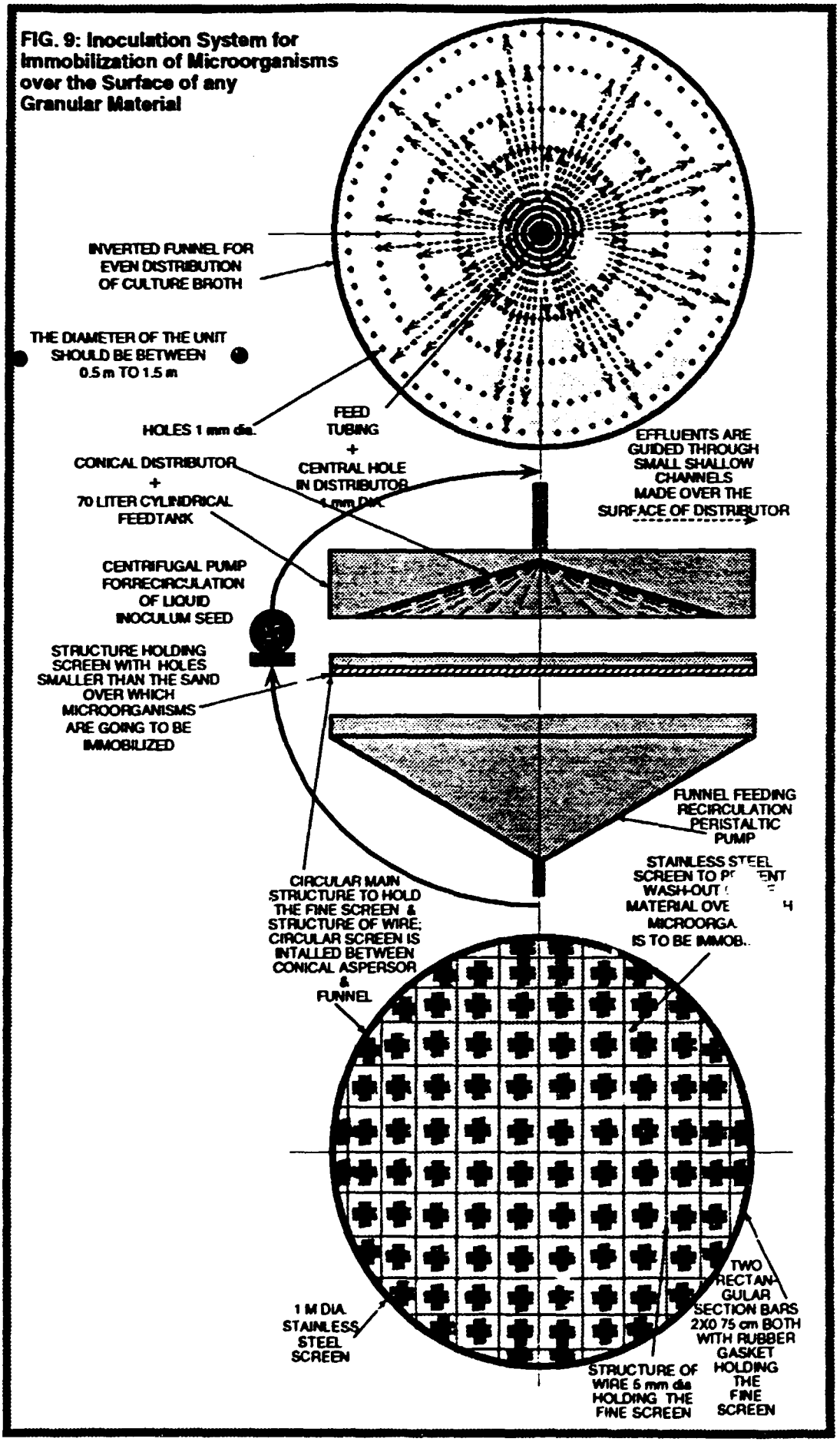


FIG. 9: Inoculation System for Immobilization of Microorganisms over the Surface of any Granular Material



V.5.7 Wastewater Treatment Pilot Plant for Processing Hazardous Waste

Adjacent to the ERC main building, NSC 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.

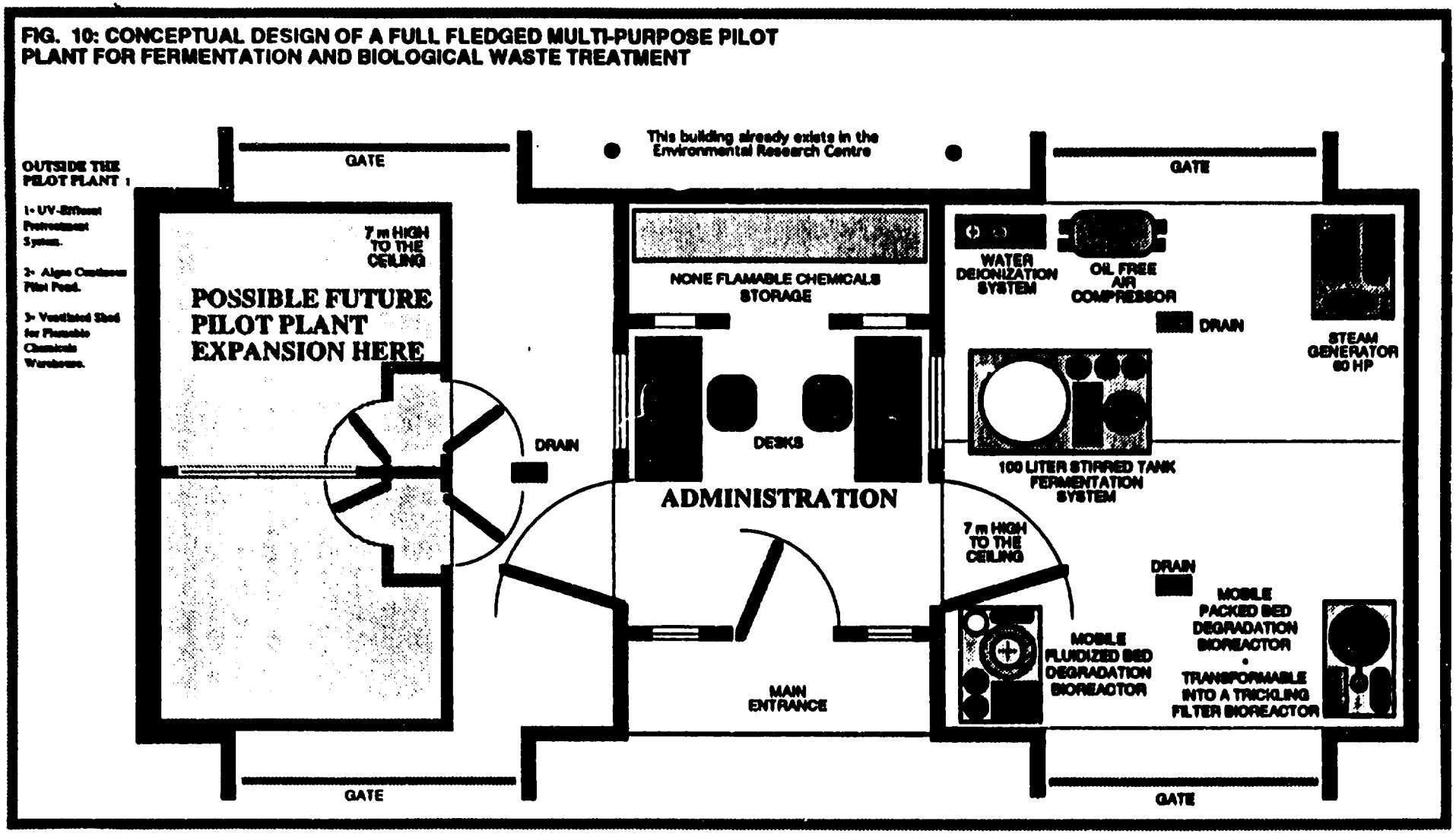
Figure 10 provides a conceptual layout for a pilot scale facility for batch production of pure cultures required for specific biodegradation activity and a couple of bioreactors to be used for treatment of hazardous wastes. The full design specifications of these units can only be provided once lab scale 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 NSC 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



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 staff members, 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, additional recommendations outlining the longer term needs of ERC (including equipment needs) to establish a full fledged capability has been presented in this Chapter of the report.

The key items recommended for purchase and their estimated costs are listed below.

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.

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 ElKarmi. He is assisted by 5 in-line staff members, of which 4 are researchers with one technician . 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.

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. The major components would include:

- A 150L bioreactor for production of pure microbial cultures
- Plexiglass fluidized and packed bed bioreactors
- Associated feed and effluent collection tanks and transfer pumps
- Support instrumentation and controls
- Utilities for steam, chilled water, air, etc,
- Biomass harvest and spray drying with plant expansion

We anticipate that plexiglass biodegradation reactors will be built on-site with extensive in-house expertise at NSC. 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. Pilot plant operational issues regarding explosion proof design, biosafety/safety guidelines and waste disposal practices, need to be implemented.

c) Supplies

Table 6 also lists key supplies and disposables needed on an on-going basis. A stock of chemicals such as NTG, chlorophenols, immobilization compounds, etc (not listed here) should also be established. Large quantities would be required especially when pilot scale activities are initiated.

A microbial collection of the following strains is also recommended:

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

Other Issues:

- Establish an authoritative Environmental Regulatory Agency
- Extend the program to include soil bioremediation
- Monitor quality of waste streams and determine levels of POPs being discharged in to the water streams. Lack of such data severely restrict development of rationale national programs for addressing pollution problems.

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 for Capability Enhancement in Biodegradation of Persitent Organic Pollutants

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	1500	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, comtainers, 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	[illegible]	C	H
Waste Storage Tank (P)	Pilot Plant wastes, 1000L	1	2700	[illegible]	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

NSC:	National Scientific Council
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