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BIODEGRADATION OF ORGANIC POLLUTANTS ORIGINATING FROM PHARMACEUTICAL INDUSTRY

SI/JOR/94/802

THE HASHEMITE KINGDOM OF JORDAN

Technical report: Selection and improvement of bacterial strains with chlorinated phenolic compound degrading capacity*

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 Dr. István Balogh,
industrial microbiologist

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Vienna

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* This document has not been edited.

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ABSTRACT

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

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

Objective and duration of activity:

- Getting information about the polluted chemical compounds at the sources of the effluents
- Commencement of the selection of microbes and their genetic improvement
- Advise on maintenance and validation
- Giving a lecture on strain improvement, fermentation technology and bioreactors

The activity began on 7 October 1994 and lasted until 28 October 1994.

Main conclusions and recommendations:

The already-launched strain selection and improvement experiments provide a general selection and improvement method which can be used for obtaining and improving other degradation properties as well e.g. policyclic, poliphenolic, substituted and normal saturated hydrocarbons. The selected or improved bacteria can be used for the inoculation of waste-water biological treatment plants thereby increasing their efficiency; or for the removal of contaminants from polluted soils. The selected microbes should be identified by their morphological and biochemical characteristics.

Considering the special danger of the soil and groundwater contamination in Jordan, the project should be extended towards the setting up of a pilot scale fermentation plant. The plant should contain a versatile air-lift bioreactor for modelling the planned industrial waste-water treatment processes and an approximately 1000 l sterile fermenter for the purpose of inoculum production by using several types of microbes capable of degrading the contaminants in the soil. These microbes could be used for the inoculation of the industrial waste-water biological treatment units as well.

The pilot scale bioreactor should be capable of being converted to a trickling or packed bed reactor depending on its current task.

The biomass harvested from the 1000 l fermenter can be preserved by concentration (microfiltration, 0.2 micrometer pore size) and drying (spray- /or freeze-drying). According to the information gathered the small-scale production of the several chemical compound degrading bacteria would be profitable.

Prior to the realisation of the pilot plant the instalment of a large-scale shaker in a fixed temperature room, the investment of a simple benchtop laboratory fermenter and a laboratory scale air-lift fermenter with some basic instrumentation would be required.

There are no strict rules or regulations in Jordan concerning the quality of industrial firms' polluting effluents and there are no safe and controlled dumping places for hazardous wastes or incineration facilities. That is why the setup of an independent and strong authority for environmental protection is considered to be of crucial importance. This authority would need a reliable laboratory and scientific background. The Environmental Research Centre in the RSS has all the experience and facilities which are required for this purpose.

The organic solvent pollution of the pharmaceutical and chemical factories should be determined by qualitative analysis. The concentration of the organic solvents in the effluents should be regularly monitored by the RSS.

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

I. INTRODUCTION

The report is based on the work of Dr. I. Balogh (head of the L- lysine fermentation plant of Agroferm Ltd. Hungary).

The location of the mission was the Royal Scientific Society (P.O.BOX 925819 Amman, Jordan. Phone: +962-6-844701/9, Fax: +962-6-844806).

The activity began on 7 October 1994 and lasted until 28 October 1994. After 29 October a one-week home-based work has been started (selection of the most appropriate strains from the 1994 ATCC catalogue on the basis of information collected from up-to-date scientific publications/ preparation of the technical report).

The objectives of the mission are listed in the job description in the Annex-1 (page 21).

The original objectives were supplemented after all the information concerning the present stage of the project and its possibilities were surveyed (Annex-2, page 23).

A new modified longterm program (Annex-3, page 24) was suggested after consultation with Dr. Ali Elkarmi (head of the Ecology Division of RSS). According to information collected (including informal talks) there should be already contaminated areas which should be cleared independently of the realisation of the planned industrial biological treatment of the contaminated effluents. This question is particularly serious in Jordan because water resources are limited and there are no strict regulations and independent environmental protection authorities. Industrial firms always try to neglect the regulations. perhaps this behaviour is more succesful in the developing countries (Annex-4, page 25).

One excellent method for cleaning the contaminated soil is the inoculation of it with bacteria having high pollutant degradation capacity. This task can easily be solved by using an approximately 1000 l pilot fermenter. The activity could be performed like a small-scale industrial production. This type of venture is well-known and accepted within the framework of the RSS. Probably the demand for oil or organic solvent-degrading bacteria would be considerable in the neighbouring countries as well.

The isolation of bacteria from samples collected at the sites can be of great advantage. The bacteria that can be isolated from the sludge of the industrial effluents has already been in contact with the pollutant. that is chlorinated phenols or organic solvents. In other words. the environment on the site is a good selection agent itself. The mixed cultures living in the sludge of the contaminated waste-water usually have better degrading capacity than a sole

strain, and their adaptation ability vis-á-vis the day-to-day changes in waste-water composition is better as well. That is why the targets of the project can be reached by applying more than one strain. In addition to the above mentioned point of view, in some cases the strains which are published in scientific literature are not available, or the strains purchased from culture collections do not show the same level of capacity which was reported in the literature.

Therefore the selection of strains was begun in the RSS from sludge samples and, it was decided, some known strains from the literature should be purchased in parallel with this (Annex-5, page 29).

The collection of the samples was coordinated through meetings with the management of the factories and waste-water plant. The purpose of these visits was to get information about the recent levels of pollution, and also the waste-water treatment and handling practices.

It should also be mentioned, that the Arab Drug company refused both to provide sludge samples and to enter into a short consultation. The leaders of the two other (visited) companies denied the pollution of chlorinated phenols (both factories deal also with chemical synthesis). There were found to be some basic contradictions in the information they were prepared to supply, their small waste-water plants were not of the best designe, and the waste-water and sludge treatment seemed to be unsatisfactory.

The elaboration of the strain improvement methods were begun on the basis of UV mutation because the more effective NTG was not available. For all this, the NTG treatment method was explained in detail, emphasizing the danger of this mutagen compound. The UV improvement method could only be demonstrated by using a Pseudomonas aeruginosa strain which was available in the ERC. The limited duration of the mission did not allow us to wait for the resulting strains from the enrichment and selection process

After consultation about the experiments with the UASB reactor, UV treatment of the consortium of the anaerobic bacteria used for the process was also suggested. Provided the repression caused by some toxic compounds is the reason of the insufficient capacity of the system, the selection of resistant colonies would give a spectacular result.

A lecture was given, according to the modified program on the topics of fermentation technology, strain improvement and bioreactors focussing mainly on the waste-water treating equipment.

All the objectives of the modified program were attained.

During the mission Mr. Ali El Omari and Mrs. Lana Hamis -both of whom are biologists- facilitated the work by laboratory assistance.

II. STRUCTURE AND ACTIVITY OF THE ENVIRONMENTAL RESEARCH CENTRE

The Environmental Research Centre is one of the institutes within the Royal Scientific Society.

Director: Mr. Ayman Hassan

Divisions: Water and Soil Division, head: Mr. Raid Khashman
Ecology Division, head: Dr. Ali Elkarmi
Air Pollution Division, head: Dr. Jasseen Khayyat

The Environmental Research Centre has not only research tasks and projects, but deals with studies, technical services and consultations ordered mainly by the Jordan government. One of the important tasks of the Enviromental Research Centre is the continuous monitoring of several water sources such as surface waters, ground waters and potable water samples including regular quality control of bottled mineral water. Also the ERC monitors the concentration of air pollutants in Jordan.

As sensitivity over limited water sources concerning any kind of pollution is very high in Jordan, the continuous checking of the several waste-water sources (industrial+public) is also an important task of the ERC.

In addition to the above mentioned public safety and environmental protection activities, the ERC is involved in many R+D projects and special studies connected with environment.

As the buildings of the Royal Scientific Society are adjacent to the Jordan University, there is an excellent opportunity to involve students in the R+D work.

According to the above mentioned scope of activites in the Environmental Research Centre, the projects are:

- Impact and risk assessment
- Monitoring the environmentally-sensitive Akaba region

- Monitoring and efficiency-increasing of the Samra waste-water plant, finding-out hazardous pollution sources regarding the effluent used for irrigation
- Monitoring the water quality of the King Talal water reservoir
- Monitoring the CO, SO2 and NOx concentrations in the air near to the industrial pollution sources (refinery, power station)
- Monitoring the total suspended particles (TSP) in the South-East area
- Anaerobic biological treatment of the effluents of olive oil mills by an UASB reactor
- Solar photocatalytic oxidation system for the elimination of organic wastes from effluents
- Solar water disinfection system for producing potable water

It should also be mentioned that the UASB reactor has a considerable COD eliminating capacity (approximatively 70 %), the effluent of which is treated in an aerobic reactor. The effluents of the oil-seed mills contain some compound(s) which are toxic to the bacterial consortium working in the UASP reactor. This repression seems to be the bottleneck of the process.

The solar water disinfection system uses the UV compound of solar light for disinfection. It has already been proved to be excellent in a pilot scale. This is why this project is very close to taht of commercial production with the collaboration of the German Technical Cooperation Agency.

The annual report of the RSS contains some more detailed information concerning the structure of it, the R+D cooperations, scientific relations and the activity of the ERC (Annex-6, page 30).

The laboratories of the ERC are equipped to a good useful level, the staff are young and capable.

III. VISITS AT POLLUTION SOURCES

There are seven pharmaceutical factories in Jordan. After surveying the possibilities, it was decided that the three main factories should be visited for the purposes of consultation and taking sludge samples: Hikma Pharma Group, Arab Drug Co. and Dar Al Daw. The Arab Drug Co. had not allow us to enter their factory. A waste-water analysis data sheet is shown in Annex-7 (page 36). Therefore instead of the Arab Drug Co. the Sahab Waste-Water Plant was visited.

The visits were organized through the personal connections of RSS researchers.

Hikma Pharma Group

The factory of the Hikma Pharma Group is situated in the Western part of Amman. It has been dealing with the production of medicines since 1965, and there have been active chemical synthesis production lines since early 1994. Previously the activity of the factory was exclusively reduced to the formulation of purchased raw materials. 90 % of the products are sold in Middle-Eastern countries. There are two separate streams of waste-water. The first type is the effluent of the synthesis plants, the daily amount of which is approximatelly 5 m3. The second type is that produced from the outlet of the formulating plants with a 36 m3/day flow rate. There is a plan to invest a small solvent-recovery plant for decreasing the high solvent content of the first effluent and to reduce the considerable expenses connected with the high amount of organic solvents.

The waste-water containing a high solvent concentration is collected in two concrete pits approx. 10 m3 each in volume. The organic solvents and the amounts of them to be found in this effluent are:

acetone	(traces)
Dimethyl-formamide	(40%)
Methylenechloride	(?)
Acetonitryle	(traces)
Triethanolamine /salt/	(?)
Xiloxenes /several type/	(?)

-according to the information given by the technical management. Obviously, the collected material is dumped somewhere: together with used solvents originating from the laboratories of the factory. Some more information concerning the structure of production (1993) and the quality of waste-water can be found in the Annex-8 (page 37).

The larger part of the waste-water is treated in an approximately 100 m3 aerated concrete tank. The pit has four sections and a separate part serves as a clarifier pit. Despite this there is no pH control, nor can signs of sludge treatment be found, although the size and the aeration level of this system seems adequate, giving nearly 3 days retention time. Nevertheless it should be considered that the factory formulates modified cephalosporines like cephalexine and cephadoxyne. The effluent surely contains a lot of these antibiotics, and therefore the sludge is a source of several cephalosporine resistant microbes. Therefore the sludge should be treated and used with special care avoiding leakage into the communal waste-water system. Two samples were taken at Hikma Pharma Group: one from the effluent of the synthesis lines and the second one from the biological treatment pit.

Dar Al Daw

The Dar Al Daw factory is to be found at the South-Western part of Amman. The factory deals with the chemical synthesis of several medicines which were not listed in detail. According to the information given by the technical leader of the plant, the effluent contains only small amounts of:

ethanol,
methanol,
chloroform and
acetic acid.

It should be mentioned that concerning the parallel existence of the chemical synthesis lines the limited solvent pollution (with regard to both quality and volume) seems to be unrealistic. The daily amount of waste-water is 35 m3. There is an approximately 200 m3 covered concrete container where the waste-water is collected. There are four pieces of 10 m3 agitated, aerated and opened carbon steel vessels under construction, which are planned to work as the aerobic reactors of the biological treatment system. For the thickening of the sludge a big perforated stainless steel tray with a surface of 10 m2 will be installed. The present stage of the construction work shows that the more than 80 % water content of the sludge will leak out from the tray into the soil. It was not possible to take samples from the deep covered container, therefore the sample were taken later when the liquid was pumped out from the tank. The sludge was obtained by centrifugation of the sample (3000 rpm for 20 minutes). A waste-water analysis data sheet is shown in the Annex-9 (page 41).

Sahab Waste-Water Plant

The Sahab Waste-Water Plant (owned by the Jordan Industrial State Corporation) is situated in the Sahab industrial region at approximately 10 km from the South border of Amman. This region is a conglomerate of several industrial plants which are producing an overall daily amount of 800-1000 m3 waste-water. The effluents are not treated at all in the factories, and this would be the task of the Sahab Waste-Water Plant. Considering the large diversity of the factories (e.g. sanitary detergents, paints, dyes, paper and cosmetics) and the lack of interest in the quality of the polluted effluents, the Sahab Waste-Water Plant encounters dramatic changes in the quality and volume of the incoming waste-water day-to-day. Probably the approximately 200 m3/day communal waste-water (which is treated together with the industrial effluents) helps in stabilizing this process.

The volume of the three aeration pits is 1000 m3 which is not sufficient for the amount of waste water processed, giving only a one-day retention time. The aeration pits are round in shape so perhaps they function as clarifiers too. Seemingly, if the 760 m3 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 appearing bubbles shows that the dispergation is not sufficient. The performance of this pit would be increased by applying proper air spargers and the air distribution should be more homogenous along the bottom of the pit. Frequent and regular dissolved oxygen and pH measurement by using portable equipment would be necessary for the proper and safe handling of the process. In the case of pH deviations caused by changes in the waste-water being processed, the installment of a pH regulation system by erecting a Ca(OH)2 sludge addition equipment would be advisable. The sludge should not be used only for pretreatment but also with regard to the aeration pits as well.

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 prescribe 100 mg/l or less. for example in Hungary 100 mg/l.)

The sludge is stored and thickened in large concrete pits. After being partially dried, the sludge is transported to dumping places.

The final effluent of the plant is used for irrigation.

One sample was taken in the Sahab Waste-Water Plant from the thickened sludge.

IV. ENRICHMENT AND SELECTION OF MICROBES

There were four samples used for the selection:

Source	Mark
Hikma Pharma Group	H1, H2
Dar Al Daw	D
Sahab Waste-Water Plant	S

The sample originating from Dar Al Daw was an effluent sample taken from the waste-water storage tank. This sample was centrifuged (3000 rpm, 20 minutes) in order to obtain sludge. The other samples were taken from waste-water sludge, which contains an enormously high number of several types of bacteria, and that is why (for the purpose of selecting CP degrading microbes) an enrichment method was introduced. This method increases the probability of the succesful selection by giving an advantage to the bacteria which have the CP degrading characteristic (or are at least tolerant to the presence of CP) and repressing the growth of the other types.

From each sample 0.2 g of sludge was added to the previously sterilized flasks containing 20 ml of the CPE1, CPE2 and CPE3 media (Annex-10, page 42). The media contained different amounts of pepton and yeast extract. The reason for varying these compounds was that targeted microbes can have special requirements but (in this cas) no growth could be observed in the lack of these nutrients. The CPE1, CPE2 and CPE3 media were supplemented with phenol and chlorinated phenols (after sterilisisation).

The compounds were:

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

The concentration of all the compounds was 106 mg/l. (The overall concentration was 530 mg/l.) Prior to the addition to the media the compounds were dissolved in 1.0 ml of methanol. In many cases in the literature it is mentioned that the microbes require cosubstrates during the degradation of highly toxic and stable compounds. In our case the phenol is a cosubstrate. The methanol was used for dissolving the crystals of the CP-s and the phenol. There was only 0.1 g of each CP available for this work, and that is why the distribution of the chemicals between the three types of medium could be made only in liquid form - after the solution. The measurement of a smaller amount of sticky and sublimating crystals can not be carried out accurately. The role of the $CaCO_3$ in the media was the prevention of the acidification caused by the generation of CO_2 and hydrochloric acid.

The inoculated flasks were shaken for four days by using small benchtop shakers (approximately 250 rpm) at room temperature. The shakers were borrowed from the water laboratory and once a day were stopped for 30 minutes by the staff of the laboratory. The reason of this was to prevent the motors from burning out because of the heat generated. There was no possibility of incubating the shaken flasks at the optimal 30 oC.

On the fourth day of shaking, new flasks were started from the first series 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 for four days.

The result of the enrichment was judged by the observation of microscopic preparates stained by methyleneblue, and by measuring the optical density. The optical density was measured at 600 nm against distilled water after a dilution with 0.2 n HCl in order to dissolve the $CaCO_3$ suspended in the media.

Result of the microscopic observation:

	CPE1	CPE2	CPE3
H1	-	-	-
H2	-	-	-
D	+	-	+
S	++	+	++

- : no growth
+ : poor growth
++ : dense culture

Result of the OD measurement:

	CPE1	CPE2	CPE3
H1	0.120	0.172	0.140
H2	0.138	0.166	0.102
D	0.080	0.224	0.192
S	0.262	0.220	0.294

The most promising five flasks (D/CPE2, D/CPE3, S/CPE1, S/CPE2 and S/CPE3) were used for the next selection step.

The results demonstrate that the enrichment was succesful.

The selection step has not been completed because of a shortage of time. Nevertheless the proper methods were presented for the accomplishment of the selection.

Agar plates were inoculated with 0.3 ml of each sample. The composition of the solid medium was equal to the CP3 medium, supplemented by the same amounts of phenol, CP-s and 2% of agar. The agar plates were incubated for four days in a 37 oC incubator. After the incubation period it was obvious, that the plates were fully covered by microbes. However, the growth seemed not to be complete. The plating was repeated with diluted samples in order to obtain single colonies. The plating was repeated with the S/CPE1 and S/CPE3 flasks. The dilutions applied were 1/100, 1/1000, 1/10000 and 1/100000. It was decided to incubate these plates for a longer time (one week) for obtaining a proper colony-size which is enough for the inoculation of the nutrient slant agar tubes. In the case of insufficient growth, the plating whould be repeated by using a nutrient agar medium.

The colonies that appear should be streaked onto slant nutrient agar media. The selected strains should be maintained by transferring them onto new slant nutrient agar tubes twice a year. Incubation should be performed at 30 oC for three days. Three parallel slants are to be inoculated. One is for the purpose of strain maintenance, the second is a spare one and the third should be the source of the working seeds - if required. The working seed slants should be made by using nutrient agar supplemented with 0.5 % of glucose. The presence of glucose provides a quick and intensive growth which is beneficial in the case of using them for inoculation. The maintained strains should be regularly validated once a year by streaking them on CP - containing agar. The proper concentration of the CP-s and phenol should be adjusted by streaking the strains onto plates with several CP concentrations.

After completion of the selection, the selected strains should be identified by their morphology and biochemical characteristics.

The CP degrading capacity should be checked by measuring the residual CP concentration after the shaking process. A standard inoculation and shaking process enables us to use the results for a more accurate validation.

After obtaining benchtop freeze-drying equipment, the selected strains should be freeze-dried in order to ensure their longterm, safe maintenance.

For the purpose of freeze-drying, the cells should be harvested from slant agar test tubes after the incubation period of at least three days. In the case of spore-forming microbes, incubation should be extended until sporulated cells are obtained. The reason for using maturated "old" culture for the freeze-drying is that the active, "young", multiplicating cells are more sensitive and the survival rate during the liophilisation therefore tends to be low. The cells from one slant agar are to be harvested by washing them out with 5 ml of

sterile water and should be centrifuged at 3000 rpm for 30 minutes. The sedimented cells are to be suspended with 2 ml of 25 % diluted sterile milk. The suspension is to be poured in to a liophilisation tube and connected to the freeze dryer. All the steps prior to the freeze-drying should be made in sterile conditions using sterile pipettes and centrifuge tubes, and all operations should be done in a clean bench. Of course, the tubes for the liophilisation should also be sterile. The freeze-drying lasts for 24 hours at -4 oC. The tubes should be stopped and separated by melting and should be stored in a refrigerator.

The role of the sterile milk is in freeze protection. For the proper preparation, 25 ml of milk should be diluted to 100 ml with water and the liquid should be maintained in a 100 oC water bath for 30 minutes in order to kill the vegetative cells. The treated milk is to be stored for one day at room temperature. This period enables the germination of all surviving spores. After this a 10 minute autoclaving leads to a 100 % sterility because all the living spores had already turned to a vegetative form. Normal sterilisation conditions result in the milk being burned.

V. GENETICAL IMPROVEMENT OF MICROBES

An existing property of a microbe can allways be improved by genetic methods combined with proper selection. In the present case the CP degrading capacity should be increased.

During the mission it was not possible to improve the selected microbes because the results of the selection were gained at the end of the working period. Therefore the demonstration of the improvement know-how was presented by using an available Pseudomonas aeruginosa (ATCC 9027) strain. The demonstrated method can be applied later on by the staff of the microbiology laboratory in the case of any strain.

The freeze-dried P. aeruginosa strain was suspended by dilution with sterile water and was inoculated on nutrient agar (Oxoid, code: CM1, with 2 % of agar) plates by using 0.3 ml of the suspension. One dish as a control was placed in the 37oC incubator without any treatment, the others were irradiated by UV for several periods (1; 5; 30; 180 and 600 seconds). The exposure was performed by using a UV lamp which was originally used for the evaluation of thin-layer chromatograms in the laboratory of the Chemical Department at the RSS. The dark cover of the lamp was removed for obtaining a higher intensity. The device had an output of 2x6 W, the distance applied was 4 cm. The irradiation was made in a temporary dark manipulation

chamber, and the irradiated plates were covered by aluminium foil in order to prevent photoreactivation.
The dishes were incubated together with the control plate for four days in a 37 oC thermostate. After the incubation period, the control dish contained round colonies of approximately 3 mm diameter. The first (1 second) irradiated dish contained 0.1 mm size colonies which had not shown any growth after the first. day. The irradiated colonies were judged to be lethal mutants which are able to live only for some periods of multiplication. The next (5 seconds) irradiated plate showed a similar picture, but all the other dishes were clear.

Based on the results of the first irradiation, the circumstances were changed. The control plate contained only single colonies instead of being covered completely by bacteria, which showed that the living cell number was very low in the suspension. The freeze-dried tube was opened in July 1994, and was stored by stopping it with a paraffin foil. Obviously some moisture has been taken up by the liophilised granules and slow damage of the living cells has started. Therefore the new plates were inoculated with a suspension made by using a new slant agar which had been started previously from the freeze dried tube. The intensity of the irradiation was decreased. The irradiation distance vas increased to 40 cm by applying a temporary stand for the UV lamp. As there is a quadratic connection between the irradiation intensity and the distance the 10x magnification results in a 100x decrease in intensity. The irradiation times were the same as in the previous experiment. The results were satisfactory in this case: the first and second dishes (1 and 5 seconds) were covered completely, the third (30 seconds) contained 50 colonies of normal size, and the last two dishes (180 and 600 seconds) contained three colonies each. On the basis of these results the proper irradiation time was determined as 15 seconds for this demonstration.

After obtaining the proper irradiation conditions it was decided that the property to be improved in this demonstration will be the tolerance against phenol. Therefore the Pseudomonas aeruginosa strain was inoculated by streaking on nutrient agar dishes supplemented with phenol in the range of concentrations (g/l):
0.1; 0.05; 0.1; 0.5; 1.0 and 2.0. The purpose of the experiment was to choose the smallest non-tolerable concentration of phenol in the agar medium.

After three days of incubation at 37oC the plates were evaluated. The growth on the first two (0.01 and 0.05 g/l) was normal. The growth on the next 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.

For the sake of selecting tolerable mutants the remaining 0.1 g/l and 0.5 g/l phenol containing dishes were inoculated by P. aeruginosa cell suspension, and were irradiated by UV for 15 seconds at a distance of 40 cm. On the second day of the 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. These colonies were new mutants with a higher phenol tolerance in comparison with the original strain.

The above method was made only for the purpose of demonstration. The reason for applying such a simple method was the short duration of the mission. The time and distance of the irradiation should be adjusted more accurately. The living cell number should be measured prior to the irradiation and afterwards as well. The cell number should be measured 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 made in a dark chamber, and the plates should be duly covered by aluminium foil.

The irradiation should be made by using cell suspension in an empty plate. The volume of the suspension should be standard (10 ml). 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. This method provides cells existing in the exponential growth phase which are very sensitive to mutagen agents having their DNA in an "unpacked" stage - with a high probability of partial damage caused by the mutagen agent. The cells should be separated prior to the treatment by centrifugation (3000 rpm, 20 minutes). After centrifugation the cells should be resuspended with sterile water. (If the same volume is used the resulting cell density will be the same as in the case of the overnight culture.)

The irradiation conditions should be modified until reaching 99-99.9 % lethality. 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 original idea about mutation treatment was the application of NTG, which is considered to be one of the mutagens with the highest efficiency. Unfortunately the compound was not available and there was no possibility of obtaining it during the mission.

In order to be able to use this mutagen a detailed explanation was given about its application which emphasized the serious danger of the compound (Annex-11, page 43).

A brief explanation was given about the possibility of improving the facultative anaerobic bacteria working in the UASB reactor. According to the information the treated olive-oil mill waste-water contains some toxic compounds which are tolerated with difficulty by the mixed culture of the reactor. The working program for its improvement should be the same, that is the treatment conditions should be adjusted, and the agar media for selection should be chosen by obtaining the smallest intolerable concentration of waste in the solid medium. After these steps, the mutagen treatment should be followed by the selection process, and hopefully by repeating this procedure some improved strain can be isolated in the near future.

VI. LECTURE ON 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 insofor 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, yoghurt, kefir, koumis, soybean soyce, 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, aminoacids, organic acids, citric acid, products created by means of "genetic surgery", solvents, biodegradable polymers, polysaccharids 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 licencing 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 bach technology, the feed-batch, and continuous fermentation technology. The role of catabolit repression was demonstrated by showing the effect of different carbon source concentrations on batch fermentation technology. It was shown that the relevance of the catabolit 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 fermenter were introduced by explaining their functions. The requirements of sterile processes were explained, and the special accessories responsible for the maintenence 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.

There were some new results published (Annex-14, page 46, - 2, 3 and 4) in 1994 in the Journal of Applied and Environmental Microbiology. A laboratory system with a trickling air bioreactor, an air lift reactor and a composite sediment column reactor were introduced (Annex-13, page 45). Each reactor was applied to the biological degradation of chlorinated compounds.

VII. RECOMMENDATIONS

Investments and activity for the present tasks

For the purposes of succesful strain selection and improvement work some investment is necessary.

A larger shaker should be purchased and erected in a temperature-controlled room. The capacity should be at least 50 X 500 ml flask.

For the proper strain maintenance and improvement work some hundreds of good quality test tubes and other glassware should be purchased.

For the purpose of UV treatment an adequate UV lamp should be invested in and a proper size dark chamber should be built, which can be placed into the clean bench.

At the present time, the nitrosoguanidine is thought to be the most effective mutagen used in strain improvement, therefore the mutagen treatment should be performed using this compound.

For the improvement method at least 20 g of each chlorinated compound would be required. In the event of the continuation of the program these compounds should be purchased.

The improvement and selection methods can be used for the selection and improvement of other properties as well, therefore the Environmental Research Centre should select a set of microbes capable of degrading several contaminant compounds.

The selected microbes should be identified by their morphology and their biochemical characteristics.

The selected strains should be characterized by their CP degrading capacity that is by measuring the CP concentration after the shaking process.

For the proper characterization of the new strains a benchtop laboratory fermenter should be set up in the microbiology laboratory. This fermenter would be applied to the inoculation of the planned pilot scale sterile fermenter.

There are several strains known from the literature capable of decomposing chlorinated products. The purchase of eight strains is recommendedin order to provide a good basis for strain improvement, that is the chlorinated phenols are not the only contaminants to be considered: in short the diverse purposes would require several kind of microbes. The suggested strains are:

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 and
Phanerochaete	chrysosporium	ATCC-24725.

The identification of isolated new strains can be made by using Bergey's Manual of Systematic Bacteriology, the purchase of this book is therefore recommended. Some other books are also recommended for being able to get more familiar with biotechnology as the "Molecular Biotechnology" by Bernard R. Glick and J. Pasternak, ASM Press, 1325 Massachusetts Ave. NW, Washington DC 20005 USA, April 1994, ISBN 1-55581-071-3LF, and the "Manual of Industrial Microbiology and Biotechnology" by Arnold L. Demain and Nadine A. Solomon, 1986, ISBN 0-914826-72-7LF. It would also be beneficial to subscribe the Journal of Applied and Environmental Microbiology of the American Society for Microbiology.

For proper and safe strain maintenence a laboratory scale benchtop freeze-dryer should be purchased.

Investment in a laboratory-scale versatile bioreactor with basic instrumentation is also recommended in order to study the degradation processes and to provide a design basis for the scaling up.

Routine work in the microbiology laboratory should be based on the activity of technicians and laboratory assistants. The employment of at least two assistants is recommended. If this would be realised the graduate researchers would be able to deal more efficiently with R+D work.

Investments and activity for the extended project program

Considering the lack of serious regulations with regard to the quality of the polluted effluents of industrial plants, the evaluation of regulations and the setting up of an independent and strong environmental protection authority should be promoted.

The organic solvent content of the effluents polluted by factories should be determined and regularly monitored.

For the purpose of increasing the efficiency of the individual industrial waste-water plants, and for the sake of obtaining a basis for soil bioremediation, the extension of the present project is suggested (Annex-3, page 24). The erection of a pilot plant is

recommended with a portable versatile bioreactor which would be able to model the biological treatment of industrial waste-water. This bioreactor could be taken to the polluting factories in order to treat their wastes. Technology can be elaborated on a pilot scale and together with the staff of the factory in parallel with consultations and demonstrations of the system\processes.

The pilot plant should contain a 1000 l sterile fermenter (with basic instrumentation), for the purpose of growing microbe biomass which can be used for soil remediation. The sterile fermenter can also be used for the inoculation of biological treatment plants by using microbes with increased degrading capacity. The biomass harvested in the fermentation broth can be dispatched to the contaminated site or biological treatment plant by tank cars. If the distance is bigger the difficulties of transportation can be solved by cooled tanks.

If there is a requirement that the biomass be preserved, it can be dryed by spray- or fluid-bed drying equippment.

Prior to the drying processes the fermentation broth should be concentrated. Considering the sensitivity of the biomass high-speed separation or the microfiltration is recommended for this purpose.

For the sake of a succesful continuation of the project, it would be beneficial if the biologists of the microbiology laboratory would be sent to training courses or study tours in developed countries to obtain further practical experience in the field of biotechnology. There would be an excellent opportunity for gathering information by participation in the Third International Symposium "In Situ and On-Site Bioreclamation" which will deal with bioremediation as a main topic (April 24-27, 1995 San Diego, California, registration: Bioreclamation Symposium, The Conference Group, 1919 West Fifth Avenue, Suite 5, Columbus, Ohio 43212-1912 USA).

On the basis of findings obtained during visits at pollution sources, the Hikma Pharma Group could be the best industrial model of the project. This model would serve as a good example for the other pharmaceutical factories and for the environmental authorities in Jordan.

VIII. ANNEXES

Annex-1

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

JOB DESCRIPTION

SI/JOR/94/XXX/11-51

Post title	Industrial Microbiologist
Duration	1 month
Date required	asap
Duty station	Amman, Jordan.
Duties	In co-operation with the international consultants, the industrial microbiologist shall carry out the following duties:

1. Identify bacterial strain of Pseudomonas to be purchased for biodegradation of phenol and chlorophenols.
2. Advise on bacterial strain maintenance.
3. Advise and set a programme for strain improvement.
4. Advise on establishment of a seed lot system.
5. Advise on validation of master and working seeds.
6. Prepare a technical report on the above work.

Qualifications	Industrial microbiologist with personal experience of strain improvement. Working experience in developing countries would be an asset.
Language	English

Background information:

The pharmaceutical industry is one of the most important industrial subsectors of the Jordanian economy since it is among those few subsectors that are income generating and improve the balance of trade. Jordan is the number one exporter of pharmaceuticals among the Arab countries.

The pharmaceutical industry is generating significant amounts of chemical pollutants, mostly in the form of effluents. Since cleaner technologies are important elements of the sustainable industrial development, therefore, existing regulations are not the only ones to be considered but to aim at the most optimal technological solutions that are economically feasible.

A specific distinction between a Jordanian and a European pharmaceutical company lies in the fact that in Jordan neither rivers are available to dilute effluents nor the regular disposal of the wastes either by incineration or dumping may be feasible.

Organic pollutants such as chlorophenols and other phenolic compounds commonly found in the effluents of pharmaceutical and other industries are detrimental to both human and aquatic life, as well as to the environment as a whole, if left without effective treatment.

The Royal Scientific Society requested UNIDO's assistance to provide high level advice for design of a pilot fermentation facility for biodegradation of organic pollutants such as those mentioned above.

Annex-2

Dr. Ali Elkarmi
Head of Ecology Division
RSS

12 October 1994

Modified working program

1. Sampling of the industrial waste water at sites (Consultation about the quality, concentration and amount of the effluents)

2. Start-up of the selection of microbes (Elaboration of the medium for enrichment: the selection medium; inoculating the appearing monocultures on slant agar medium; advise on strain maintenance: elaboration of selection medium for strain improvement)

3. Advise on purchasing strains from culture collections

4. Elaboration of the genetical improvement method for the strains by using the available Pseudomonas aeruginosa ATCC 9027 strain (NTG and UV treatment method) in order to reach the proper lethality, and finding out the adequate dilution rate for plating

5. Advise on genetical improvement of the facultative anaerob microbes used in the UASB reactor

6. Advise on validation, maintenance and seed-lot system

7.Giving a lecture (On strain improvement, fermentation processes and bioreactors: fermenters/ bioreactors for waste water treatment)

8. Preparation of the technical report

Dr. I. Balogh

Annex-3

Dr. Ali Elkarmi
Head of Ecology Division
RSS

Program plan suggested in connection with the project SI/JOR/94/802

12 October 1994

After having been acquainted with the present situation two main goals can be declared:

A.

To clean the already contaminated territories, dumping places, wadis etc. to prevent ground water contamination
Method: inoculating the soil with special microbes cultivated in pilot scale fermenters having a high organic solvent degrading capacity

B.

To prevent the pollution of factories
Method: setting up special bioreactors with mixed culture in the factories for the biological treatment of the effluents

Steps of elaboration:

1.(A+B) Collecting information about the pollution sources, types of pollutants, concentration and volume to find out the contaminated territories and the rate of their contamination
2.(A) Selecting the proper strains of microbes from site samples and from culture collections, and improvement of the selected microbes
2.(B) design of a lab-scale mixed culture bioreactor, and biodegradation technology
3.(A) Investment in a benchtop laboratory fermenter, and elaboration of the inoculation technology
3.(B) Scaling-up of the laboratory-scale bioreactor for microbiological degradation to industrial scale
4.(A) Investment in a 1000 l sterile pilot fermenter for the preparation of microbial inoculations in contaminated territories

The above activity (in the case of realisation) could be performed within the framework of a venture

Dr. I. Balogh

- 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* m3/day Aver.	Pre-treatment	Discharge	Analysis by	BOD kg/day	COD kg/day	TSS kg/day	TDS kg/day
1	Jordan Petroleum Refinery	3800	Mech.	Irrigation	IPCP	148	460	391	10252
					WAJ	148	532	236	12122
6	Jordan Paper and Cardboard	600	Biol.	Irrigation	IPCP	66	136	69	1148
					WAJ	124	287	110	1080
7	Eagle Distilleries Co.	70	Mech.	Sewer Samra	IPCP	89	153	4.4	119
					WAJ	126	229	8.1	194
8	Ain Ghazal Slaughter-house	630	No	Sewer Samra	IPCP	1485	2776	1309	-
					WAJ	4249	20340	1014	3974
9	Jordan Ice and Aerated Water Co.	950	No	Sewer Samra	IPCP	297	709	591	4160
					WAJ	443	837	219	2446
10	Arab Chemical Detergents	36	Chem.	Sewer Samra	IPCP	3.6	15	4.5	516
					WAJ	-	-	-	-
15	The ICA Co.	310	Biol.	Sewer Samra	IPCP	3.1	8.1	6.2	397
					WAJ	-	-	-	-
18	Zeidans Refrigeration Co.	17	No	Sewer Samra	IPCP	24	55	14	47
					WAJ	99	194	43	82
19	Arab Brewery Co.	66	No	Sewer Samra	IPCP	1.8	4.8	1.5	89
					WAJ	13	26	9	107
21	Arabian Trade and Food Co	33	No	Sewer Samra	IPCP	21	45	10	39
					WAJ	88	163	290	60
22	Jordan Brewery Co.	110	No	Sewer Samra	IPCP	57	117	6.8	72
					WAJ	28	52	5.5	68
23	United Factories Co	6	Biol.	Sewer Samra	IPCP	3.7	8.9	1.4	10
					WAJ	1.8	3.7	0.37	6.5
24	Yeast Industries Co.	530	No	Irrigation	IPCP	2862	4362	350	5610
					WAJ	3378	6141	597	3869
25	Jordan Dairy Co	80	Biol.	Sewer Samra	IPCP	2.0	11	2.5	50
					WAJ	2.0	6.2	3.5	82
26	Ala Ali Ice-cream Co.	18	Mech.	Sewer Samra	IPCP	71	125	46	-
					WAJ	-	-	-	-
27	Hikma Pharmaceuticals Jordan	16	No	Sewer Samra	IPCP	35	60	8.8	19
					WAJ	13	23	2.3	14
28	Danish-Jordanian Dairy Co	90	Biol.	Sewer Samra	IPCP	2.2	6.5	3.2	154
					WAJ	28	56	19	117
29	The Tanning Company	150	Biol Chem	Sewer Samra	IPCP	13	111	69	1895
					WAJ	15	49	28	1560
30	Danish Food Industries Company	130	Mech.	Sewer Sahab	IPCP	115	187	51	143
					WAJ	-	-	-	-
Total		7641		19 comp.	IPCP	5300	9346	2941	24978
				15 comp.	WAJ	4784	26938	2614	25812
Total to sewer		2711		16 comp.	IPCP	2224	4389	2131	7967
				12 comp.	WAJ	5104	21978	1672	8741

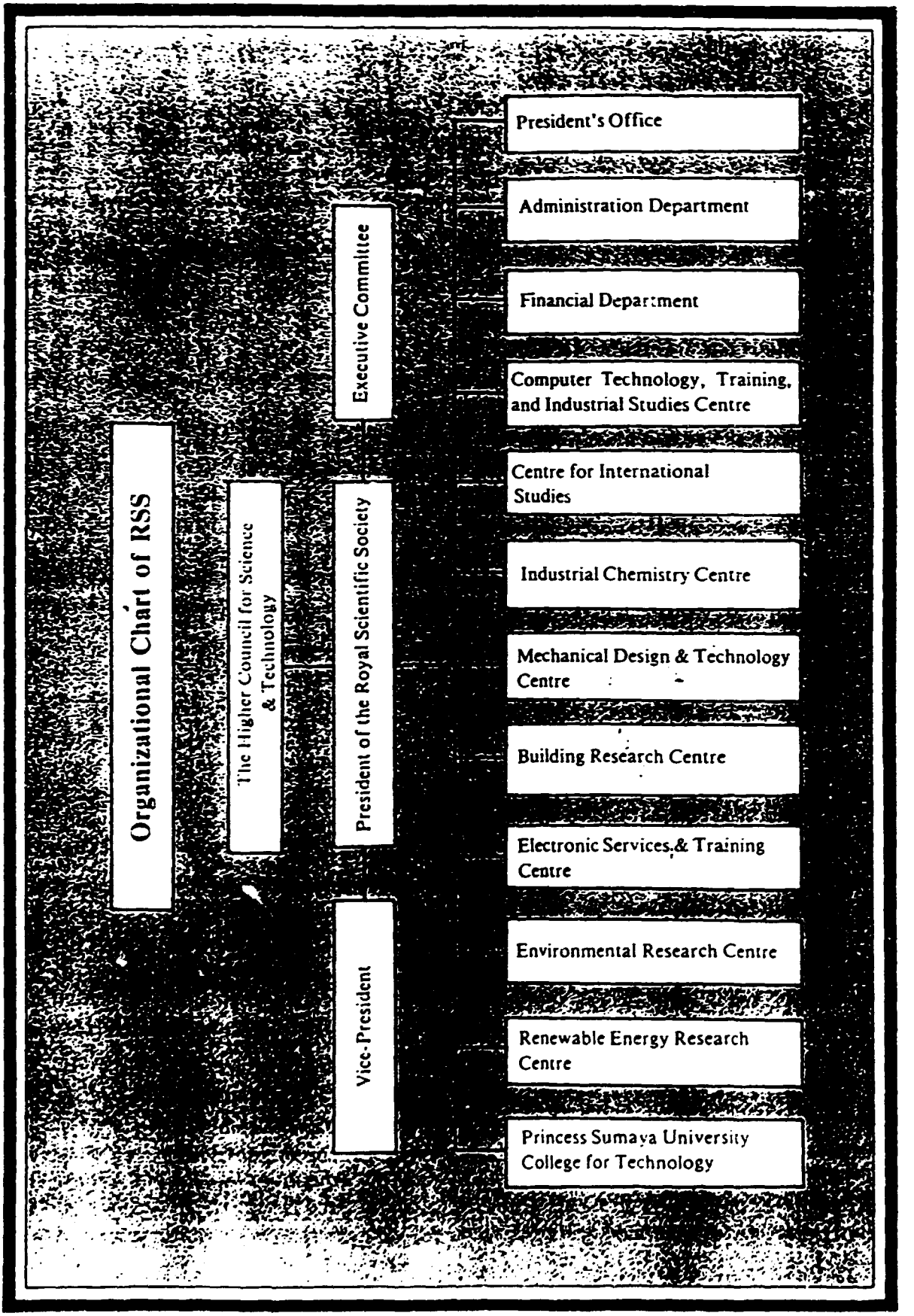
* Working days

Annex-5

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


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

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.

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-7

WASTE WATER ANALYSIS DATA- ARAB DRUG Co.

فيما يلي نتائج تحاليل عينة مياه عادمة تابعة لمصنع البحيرة تم جمعها وإرسالها من قبلكم بتاريخ ١٩٩٤/٢/٢:-

الفحص	الوحدة	النتيجة
pH	SU	8.24
EC	us/cm	1225
TDS	mg/L	1044
TSS	mg/L	294
BOD5	mg/L	658
COD	mg/L	1136
Fe	mg/L	2.4

* * * * * * * * * * *

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
Clozolin
1206 FD&C Blue no.1
1311 FD&C Blue no.2
Sicopharm Brown Lake
1675 Erothrosin
1150 Dispersed Pink
Allura red FD&C red no.40
11959 Carmosin
14031 Quincline Yellow
1409 Tartrazine
1351 FD&C Yellow no.6
12116 FD&C Yellow no 6. lake
19248 Quinoline Yellow, lake
Dextrose Anhydrous
Explotab
Heparin sodium sheep origo
Lactose
Mannitol D

Penicillin V potassium
Paracetamol Mic
Propylene glycol
Potassium citrate
Sucraiphate
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	07.03.92	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-9

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_3	18 mg/L
ABS	32 mg/L
pH	6

Annex-10

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-11

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.

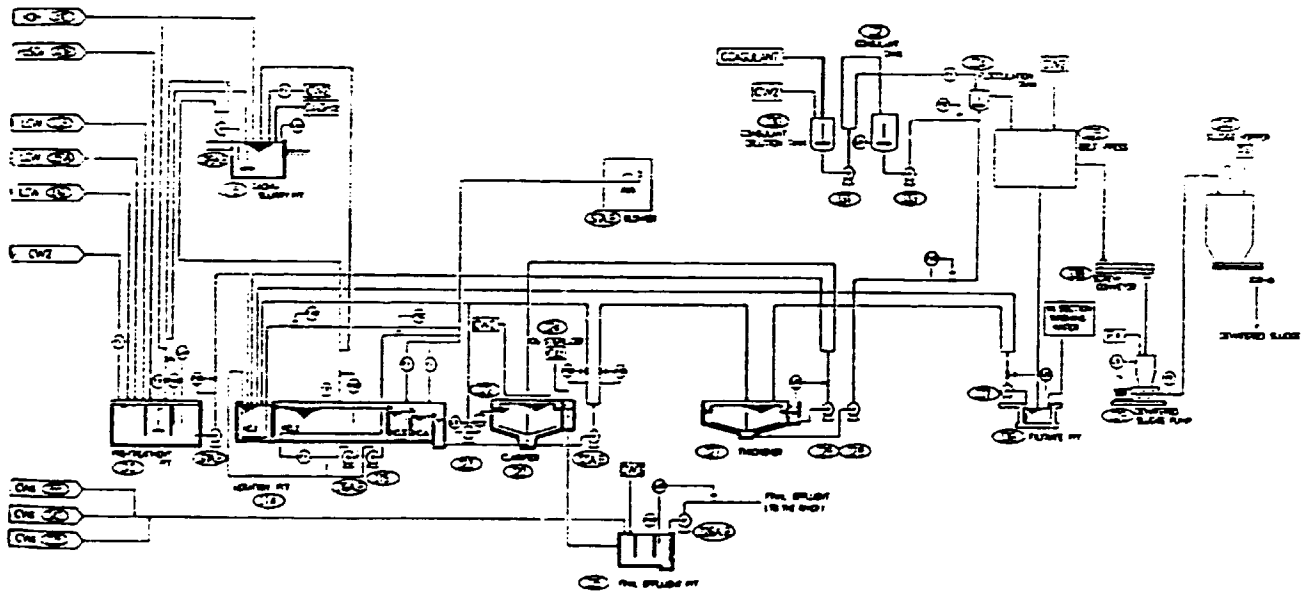
The cells suspended with the NTG solution should be incubated for 30 minutes at 30 oC. After this incubation period has passed, the suspension should be cleaned from the NTG by centrifugation and resuspension by using sterile water. The suspended cells can be transferred to any kind of selective agar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

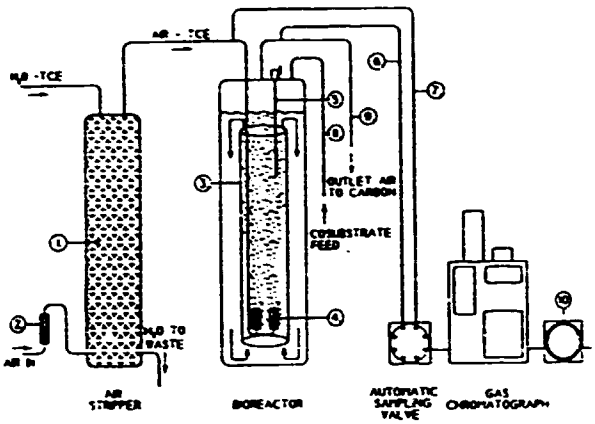
In this case the lethality should be adjusted to approximately 50 %, in order to obtain a high number of mutants. In the case of NTG the higher lethality is followed by the generation of multiplied mutants among the surviving cells, and the accumulation of these genetic mistakes can cause several difficulties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

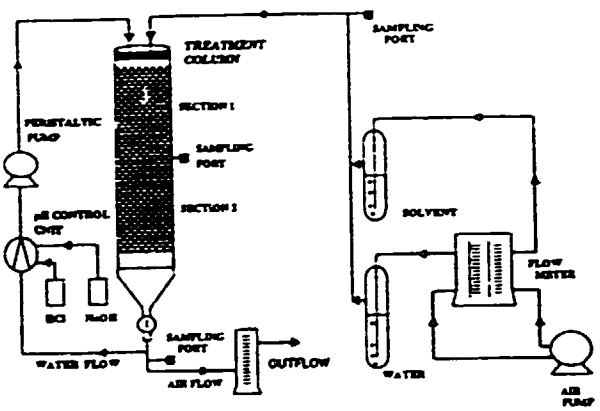
Annex-12

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.

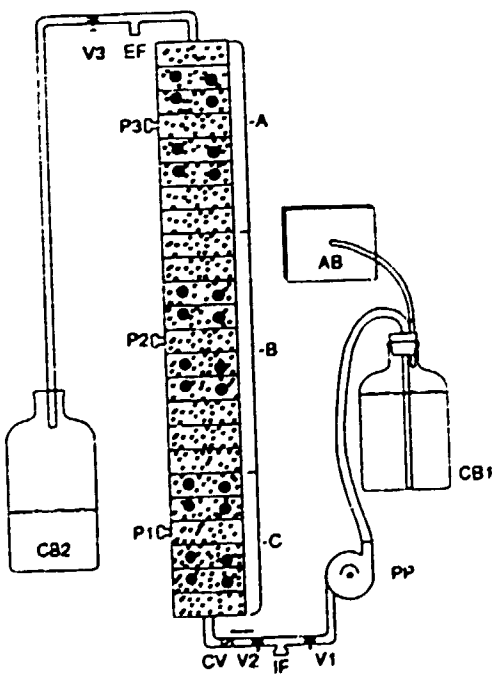


Diagram of the column. Section A contained composite sediments from the saturated zone; section B and C sediments were from unsaturated zones. Side ports were for sampling sediments and pore waters. V1 to -3, valves; CB1, column feed water carboy; CB2, collection carboy; AB, Teflon gas bag; CV, check 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.

Annex-14

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