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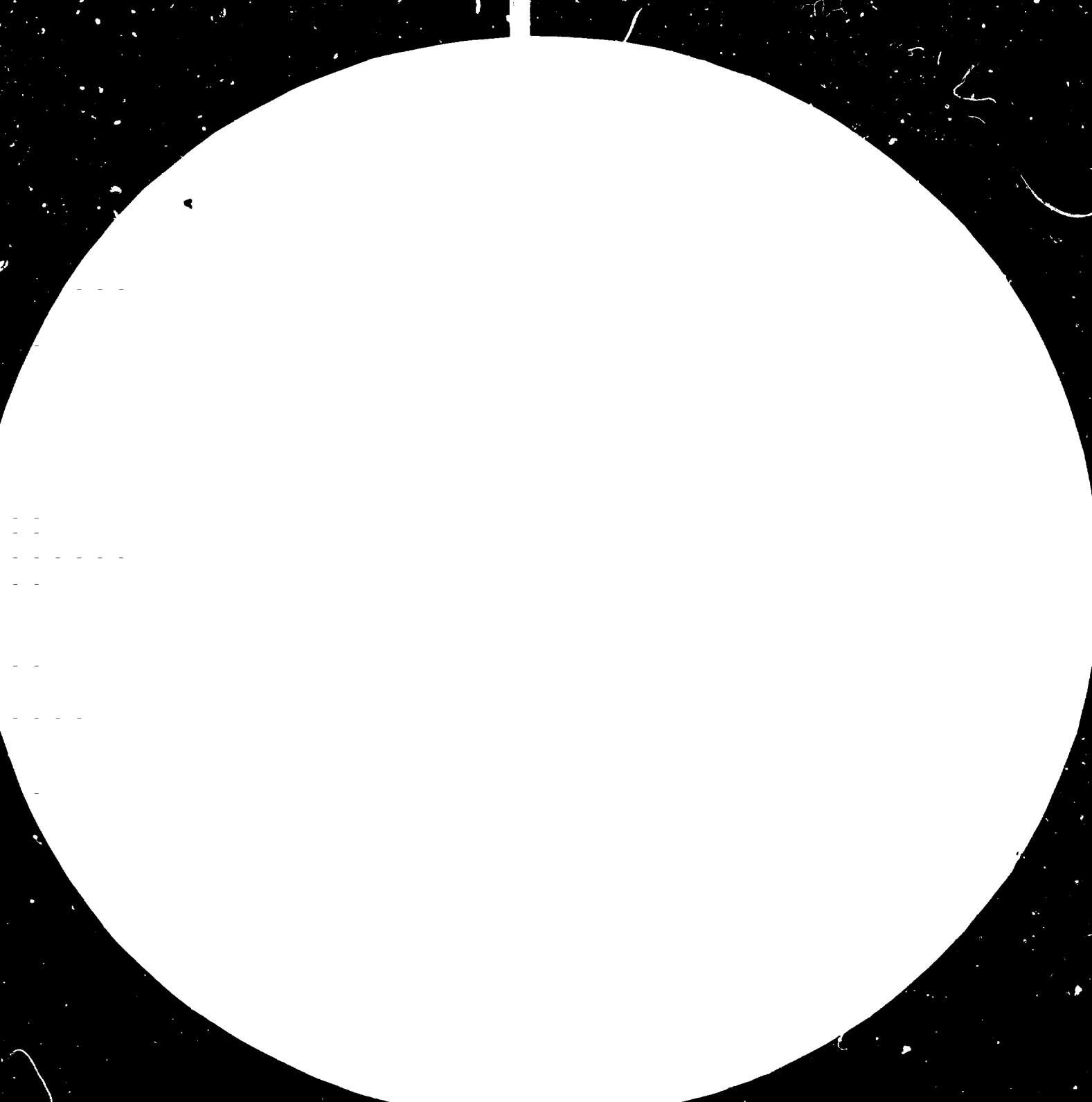

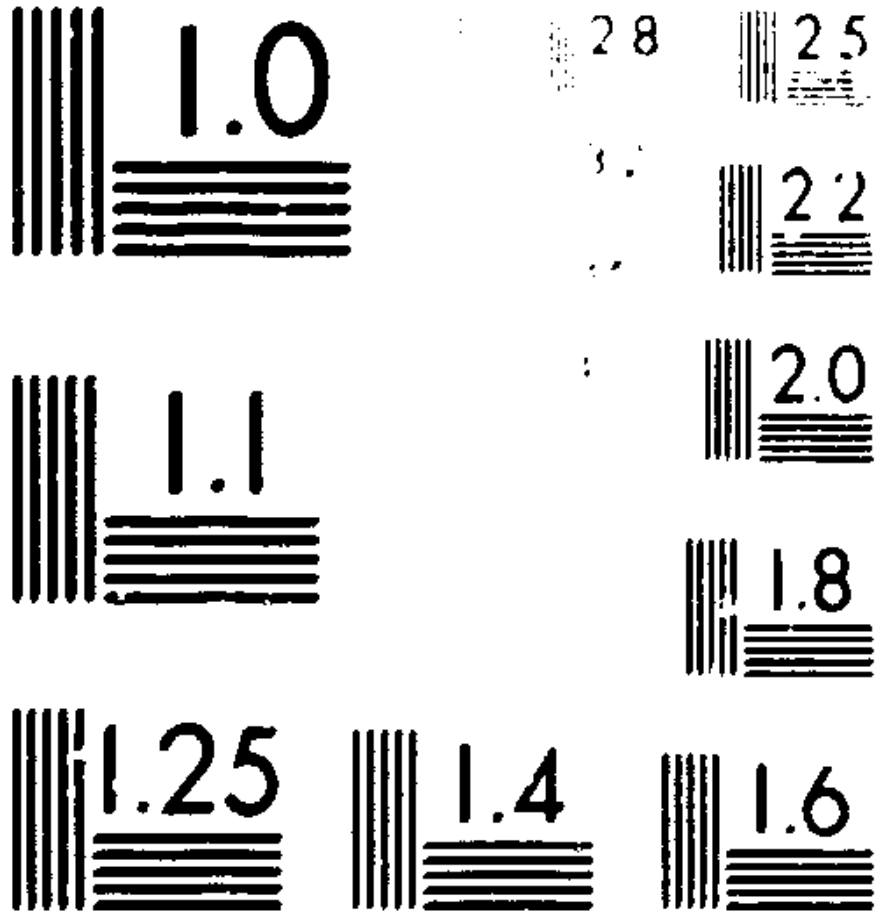
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HYDROCARBON MICROBIOLOGY WITH SPECIAL REFERENCE TO TERTIARY OIL RECOVERY FROM PETROLEUM WELLS*

prepared by

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Contents

A. BACKGROUND AND JUSTIFICATION

Microorganisms are well-known for their ability to attack both aliphatic and aromatic hydrocarbons present in crude oils (1,2). Pure or mixec bacterial cultures may bring about extensive changes in the composition of crude oil by total or partial digestion of hydrocarbon components by complete or co-oxidative metabolism (3). Such microbial growth usually leads to a change in the pH of the medium and an alteration in the physico-chemical properties of the oil.

It is widely recognized that the current technology of oil recovery allows recovery of 30 to 40 per cent of oil from most wells. The bulk of the oil is left behind embedded in the rocks because of its high viscosity. According to 1976 information, in the United States of America a total of 27×10^{10} barrels are known to exist, 6×10^{10} of which have been extracted by primary recovery methods, 3×10^{10} barrels are being recovered by enhanced recovery processes, and the remaining 18×10^{10} barrels remain underground as dead oil.

The usefulness of microorganisms in the recovery of secondary oil cannot be overemphasized. In the United States of America until the mid-seventies, the price of oil has been regulated and maintained at an appreciably lower rate than in most other parts of the world. There was no economic incentive to obtain more oil than could be extracted readily by the available technical means. Since then, there has been a continuing shortage of oil worldwide, resulting in a drastic price increase. Because of the shortage of oil, the price of secondary oil has recently been deregulated and the necessity for increased production of crude oil has become apparent.

Although the microbiological means of recovery of secondary oil has not been taken seriously in the United States of America, some of the concepts of the technology certainly evolved in this country between the forties and sixties (4,56). In contrast, microbial recovery of secondary oil has been subject to field trials in East European countries, particularly in the Union of Soviet Socialist Republics (USSR), Hungary, Poland and Czechoslovakia. Thus, Soviet workers (7) have reported

successful release of oil from reservoirs containing highly viscous oil with a permeability of 1000 md. by injecting mixed cultures of bacterial strains. Extensive field trials in Hungary have demonstrated the applicability of microbial recovery of crude oil (8). In a typical trial, 20 cubic metres of a mixture consisting of 120 m^3 of well water, 40 tons of molasses, 120 kg KNO_3 and 100 kg of sugar was injected into a well along with 100 litres of a mixed bacterial culture comprised of Pseudomonas, Clostridium and Desulfovibrio (9). The well was closed for three months, at the end of which the following results were obtained (Table 1).

Table 1

Changes in reservoir composition after injection of microorganisms in an oil well

Parameter measured	Conditions	
	in original well	after 3 months incubation
Oil production	unspecified	60 per cent higher than original
Oil viscosity	42 centistokes	18 cSt at 40°C
CO_2 gas	0	40 m^3/day
Dissolved CO_2	0	370 mg/litre
pH of reservoir water	9	6

Similar reduction in the viscosity of oil as well as the pH of the reservoir water was noted in other field trials. In another typical field experiment, Karaskiewicz (10) has reported changes of viscosity from 10.67 centistokes to 4.37 cSt with a simultaneous drop in specific gravity from 0.8516 to 0.8320. Microorganisms capable of digesting the heavy fractions of the crude oil producing light fractions and gases are believed to be responsible for such reduction of viscosity (11).

Extensive field trials in Poland have been reported by Karaskiewicz (10). In a typical trial, a well in the Carpathian region produced 4460 kg oil, 16610 kg water and 3780 m^3 of gas per month. The porosity of the

reservoir rock was 13 per cent and permeability of 120 md. The well was injected with mixed bacterial cultures and nutrients, and sealed for three months. At the end of this period, oil production was increased by 360 per cent and the increased production (260 per cent on the average) persisted for six months before declining to the previous level. The pH of the reservoir water fell from 8.7 to 6.7 during this period.

Field trials for microbial recovery of oil in the United States have been few (6) but one report (12) points out that an average increase in oil production in excess of 350 per cent may be expected at a cost varying from approximately 15 ¢ to 50 ¢ per additional barrel of oil. In addition, recent investigations by Petrogen Inc. (personal communication from Dr. A.G. Swan, Research Director of Petrogen) during a recent 100 day test around the end of 1981 on four low-producing oil wells treated with their specially-adapted microorganisms (Biopackage I) demonstrated recovery of oil worth about $24,800. Immediately previous to the treatment with Biopackage I, the four oil wells produced the amount of crude worth about $4,132 during the 100 day test period. Thus microbial treatment of the oil wells has been reported to result in a six fold increase in oil recovery in this particular instance.

Characteristics of Microorganisms

The field trial experiments appear to demonstrate the practical feasibility of microbial recovery of secondary oil from abandoned oil wells. It should be emphasized that the increased production of oil due to injection of microbial cultures has presumably been minimal, since no special precaution was taken to use microorganisms especially designed for this purpose. It appears that in almost all cases, the increased production of oil was basically due to two reasons:

(a) production of large amounts of CO_2 due to bacterial metabolism in the reservoir. Since CO_2 is partially miscible with oil, to swell and become less viscous; and

(b) production of large amounts of acetic, propionic and other organic acids which react with reservoir alkaline carbonate in the formation, leading to increased porosity of the rocks as well as production of more CO_2. Excess of CO_2 and other gases also exert pressure to drive the oil out of the pockets of sand and rock.

The reported success of the microbial recovery of secondary oil, though sporadic, and the continuing increase in the price of oil (presently at about $34 per barrel) have aroused considerable interest in exploring this technology as a viable means of increased oil production, particularly since the secondary oil so produced would be deregulated and draw a premium price. In a Symposium organized by the United States Department of Energy (13), the consensus was reached that microbial recovery of oil is a technically achievable goal and the process can perhaps be considerably enhanced if the microorganisms would have some desirable characteristics. The following characteristics have been deemed particularly desirable:

(a) Production of acids

In addition to producing CO_2 from metabolism, microorganisms should also be able to produce acids in situ. Such acids may then help in the dissolution of carbonate rock structures, increasing the porosity of the rocks to facilitate oil migration and also allowing release of additional CO_2 to push the oil from the embedded rocks. The acids should be strong mineral acids such as HCl or H_2SO_4 or organic acids such as acetic, propionic, succinic, etc.

(b) Production of biopolymers or surfactants

Biopolymers and surfactants are routinely used in flood water to decrease the surface tension of the oil water interface, resulting in decreased viscosity and an easier flow of oil. Ideally, they should be produced in situ by microorganisms, ensuring a continuous supply of such materials in difficult to reach areas of oil-embedded rocks. This also eliminates the problem of transportation, storage and delivery of such materials during water flooding.

(c) Consumption of highly viscous components of the oil

The decreased mobility of secondary oil is primarily due to the high viscosity of the oil. If microorganisms can selectively remove the waxy and other highly viscous components of the oil, resulting in a decrease of the viscosity, then such oil may be recovered easily by water flooding and pumping. Additionally, any loss of sulphur-containing components at this time will result in the recovery of low-sulphur crudes, which usually draw a premium price.

(d) Growth and tolerance

Microorganisms that are deemed appropriate for use in secondary oil recovery must have the ability to grow at a wide range of temperature (35°C to 45°C or beyond), have simple nutritional requirements, preferably with the capability of using cheap substrates (and/or highly viscous components of the oil itself) as sole sources of carbon and using nitrate or fixing N_2 for its source of cell nitrogen. They should be non-pathogenic, ecologically compatible, easy to produce in the laboratory in large quantities and easy to monitor by incorporation of a visible genetic marker of some unique growth-related property.

Advantages and Disadvantages of the Microbial Oil Recovery System:

(a) The major advantage of a microbial system is that it is cheap and simple. Injection of a few hundred gallons of mixed culture and nutrient mixture in the well is relatively simple and straightforward;

(b) The technology for recovery of oil is well-known and the processing does not involve any major technical innovation;

(c) The process may be long-term as bacteria continually digest highly viscous oil fractions releasing acids and light fractions. The acids may continually react with rocks producing more CO_2 and increasing its porosity, thereby releasing more oil

The major disadvantage of the process is the extreme environment in most oil wells that is not conducive to sustained microbial growth. The following points are particularly important:

(a) Temperature: Most oil wells that have been highly productive are quite deep and the temperature may vary from 70 to 95°C in general. Thus, mesophilic and moderately thermophilic microorganisms would not be able to proliferate under such conditions.

(b) Pressure: Although pressure is normally not a factor, higher pressure at some oil wells (50 atm) may reduce biological activity in an otherwise unfavourable environment.

(c) Nature of sands and rocks: Although carbonate rocks can be gradually corroded by the microbially produced acids, the silicate rocks are quite resistant under such conditions.

It would therefore seem that microbial recovery of oil, at least initially, must be confined to shallow wells having predominantly alkaline carbonate rock structures. If the process allows enhanced oil production, thermophilic bacteria having desirable characteristics may be designed based on the characteristics of individual oil wells. Given the vast amount of oil trapped within the rocks, the shortage and spiralling price of crude oil, and the inexpensive nature of the microbial process, it is imperative that microbial oil recovery process should be attempted in a number of oil wells to test the effectiveness of the process.

The major objective of the programme is to enhance the recovery of oil from wells already drilled and from which the bulk of the oil has been pumped out. This programme therefore falls under the category of the secondary and tertiary recovery of oil. Given the fact that there are innumerable oil wells in various developing countries that produce less than one barrel per day, an increase in oil output by microbial technology would help alleviate some of the energy problems in these countries. Another objective is to facilitate the storage and transportation

of crude oil by developing genetically-engineered bacteria that should be able to utilize the waxy components of crude oil, thereby greatly reducing its viscosity. Such low-viscous free-flowing oil not only attracts a premium price, but is less likely to form solid mass during transportation in winter months, thereby greatly increasing the cost of pumping. Free-flowing oil is also much easier to store in underground caverns than the highly viscous oil. Still another objective is to develop microorganisms that would be able to utilize the common sulphur-containing compounds present in crude oil. Desulphurization of crude oil can therefore be accomplished through use of such microorganisms. Along with sulphur-containing compounds organics, many crude oil varieties also contain trace quantitires of metals such as vanadium, nickel, molybdanum, etc. that lower the quality of the oil. Microorganisms capable of selectively removing these metals will also be developed. Lastly, oil spill, either through deliberate washing off of residual oil from oil tankers of accidental due to collision or grounding of oil tankers, can be managed through application of genetically-engineered microorganisms or through use of bacterially-produced emulsifying agents. Such emulsifying agents may also find wide application in cleaning up of residual oil and oily wastes present in drums, tank cars or trucks and oil tankers. Field tests for such micro-organisms or emulsifying agents would be conducted with oil samples taken from oil wells of various developing countries.

B. ACTIVITIES

Since the Oil Recovery Programme would address several other features in addition to tertiary recovery of oil i.e., dewaxing and desulphurization of oil, oil spill clean up and removal of metals, it is clear that several different kinds of activities would be associated with the programme. The major component would be the research and development component, where search in all the above areas would be conducted under a group leader associated with the ICGEB. About three group leaders, each with one post doctoral fellow and one technician will be working in all the five projects. It is anticipated that five trainees from developing countries, preferably from oil producing countries, would be trained in all the above research

areas i.e., one in each of dewaxing, desulphurization and metal ion removal and two in the development of aerobic and anaerobic cultures capable of releasing oil from abandoned oil wells. For countries rich in shale oil or tar sands (such as Canada), some research activities in this area may also be initiated. Yearly workshops will be conducted by this group, in collaboration with petroleum microbiology groups of other institutions in developing and developed countries, on topics such as the role of microorganisms in the degradation of hydrocarbons present in crude and spilled oil, limiting factors (i.e., nature of oil, pH, temperature, O_2 tension, etc.) in such biodegradation, methods for stimulating natural biodegradation, genetic improvements in the biodegradation of hydrocarbons of various chain lengths, etc. In addition, the group would be guided by an advisory committee composed of geneticists, microbiologists, ecologists, geologists and reservoir engineers to advise on the applications of any constructed microorganisms in the open environment, either for oil spill clean up, dewaxing and desulphurization, or in the use of such microorganisms in oil wells for enhanced release of oil.

Extensive pilot plant facilities for successful field trials with the microorganisms will be needed at the ICGEB. Hopefully, there will be centralized facilities for this; otherwise, several fermenter with capacities of 10 to 1000 gallons will be needed. One or more technicians, knowledgable in running fermenters and harvesting cells, should be recruited for this purpose. In absence of a centralized facility, additional 2000 sq. ft. area will be needed to house the fermentation facilities. The cost for the fermenters would be about $100,000.

For actual field trials, the fermenter grown microorganisms along with the nutrients must be transported near the oil well site and the microorganisms, along with the nutrients, need to be pumped in the oil well. A couple of trucks fitted with pumping gear would be needed for this operation. Hopefully, this would be provided by the participating oil well operators or owners.

The major output of the programme would be the construction of genetically engineered strains capable of dewaxing and desulphurizing crude oil, capable of utilizing spilled oil rapidly, capable of removing certain undesirable metals from such oil and finally capable of releasing oil from rock-embedded high-viscosity oil that cannot normally be pumped out by the present day technology. Some of these goals may not be met within the five-year lifetime of the programme. Even if the genetically engineered strains can be constructed, the effectiveness of such strains in an open environment remains to be tested. Recently, another genetically engineered strain capable of digesting a known persistent toxic chemical such as 2,4,5-T has been demonstrated to be quite efficient in removing this pollutant from the environment (14). In this sense, the constructed strains for oil release or dewaxing/desulphurization of crude oil are expected to be effective in their respective areas of application.

Construction of Strains for Tertiary Oil Recovery

Many of the parameters of the microbial enhanced oil recovery have recently been evaluated by a series of studies sponsored by the United States Department of Energy and a major conclusion that has emerged is that about 30 to 40 per cent of the oil wells in Oklahoma, Texas, Louisiana, Kansas, etc. are suitable for microbial enhanced oil recovery processes if the proper microbes and processes can be developed (see report on the United States DOE contract No. DE-AC10-80BC10169, 1981). Assuming that such microorganisms can be developed by the ICGEB and that such microorganisms would be able to allow recovery of 10 to 20 per cent of the residual oil, the tremendous impact of such a technology in the availability of crude oil throughout the world can easily be envisaged.

There would be two fundamental research approaches that the ICGEB should take in constructing appropriate microorganisms. One would be the route taken by the United States Department of Energy, which is to isolate thermophilic, salt-tolerant, anaerobic cultures either from sewage treatment plants or from oil well reservoir cores. Mutational or other genetic improvements can then be attempted to improve the performance of the cultures with respect to their tolerance to extreme pH, salinity,

pressure, temperature, etc., and to improve their ability to convert waxy materials to liquid oil (see reports on United States DOE contracts DE-AT19-78BC30201, DE-AS19-80BC10302, DE-AS19-81BC10507, DE-AC19-80BCk0300, etc.).

It should be emphasized that anaerobes are not particularly suitable for optimum oil release programmes. Anaerobes are not known to biodegrade hydrocarbons at an appreciate rate, so that easily assimilable carbon sources must be supplied in the oil well for their proliferation. Secondly, genetic studies with anaerobes are at their infancy, so that no appreciable genetic improvement can realistically be made at this time. Nevertheless, recent experiments with anaerobic cultures in several oil wells in Texas and Oklahoma by a firm named Petrogen Inc. have demonstrated that out of 24 oil wells injected in six different formations at depths ranging from 300 to 4,600 feet by specially acclimated anaerobic cultures, four showed doubling of pre-injection production levels over a six month period while another 12 demonstrated increases of 50 per cent over pre-injection production levels for a three month period (Dr. A.G. Swan, Research Director, Petrogen Inc., Personal Communications).

The second route to enhanced microbial oil recovery programme to be undertaken by the ICGEB would be to genetically manipulate aerobic cultures, particularly Pseudomonas type, for this purpose. Bacteria belonging to the genus Pseudomonas are well-known for their ability to utilize hydrocarbons, including the C20 and other waxy types. They grow rapidly at temperatures up to 44°C, are tolerant to high pressure, fair amounts of salinity (marine Pseudomonas sp. are tolerant up to high salinity), pH variations, etc. Pseudomonas species are also known to produce polysaccharide biopolymers and can release large amounts of strong acids such as HCl from chlorinated compounds. Genetic tools for improving Pseudomonas species are fairly well advanced. The only drawback with the use of Pseudomonas species is the fact that such bacteria are aerobic, although some species can grow quite well under anaerobic conditions with nitrate as an electron acceptor. In addition to the use of nitrate, it is possible to use the Recombinant DNA techniques to introduce foreign genes into Pseudomonas species that will allow them to produce oxygen in situ in the well. Thus the construction of a Pseudomonas bacterium capable of digesting highly-

viscous waxy material from the oil well, producing polysaccharide polymers and HCl from chlorinated compounds and thriving in the anaerobic environment with in situ production of oxygen can be accomplished at the ICGEB. The construction of such specialized strains and a study of their effectiveness in enhanced recovery of oil from abandoned oil wells will therefore be a major part of the overall programme projects at the ICGEB. Forcing air into the oil wells and allowing genetically manipulated Pseudomonas strains to thrive for oil release will also be a part of the ICGEB field trials.

Dewaxing and Desulphurization of Crude Oil

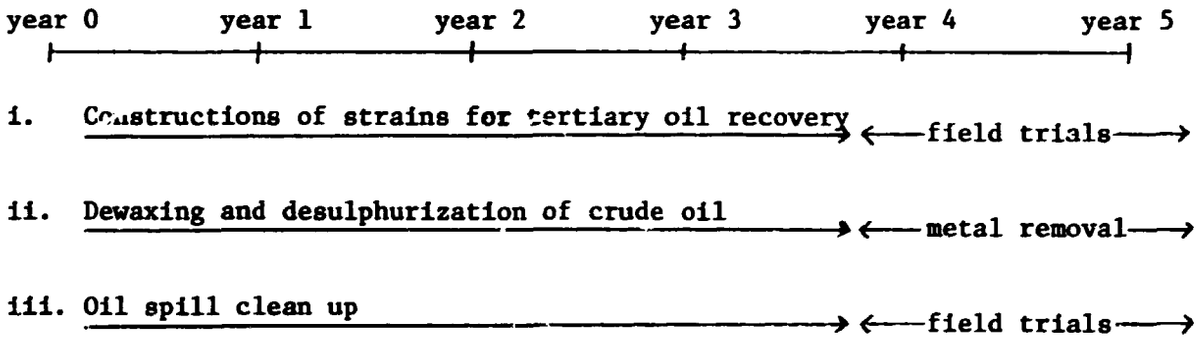
Recent genetic studies with Pseudomonas species have demonstrated that it is possible to specially breed in the laboratory strains that can utilize a known, persistent compound such as the herbicide 2,4,5-T (15). Since there are no naturally occuring microorganisms that can utilize 2,4,5-T as a sole source of carbon, the laboratory-breeding of such a culture raises hope that similar strains capable of utilizing other recalcitrant compounds such as dibenzothiophenes or furans, known to occur in crude, can be developed. The use of bacterial cultures capable of dissimilating dibenzothiophenes to a water soluble product in removing 90 per cent of the sulphur from crude oil has now been demonstrated. Since the presence of wax or sulphur-compounds in crude leads to transportation and pollution problems, development of cultures capable of dewaxing or desulphurizing the crude would be very useful. In addition, as mentioned in the Enchanced Recovery section, such characteristics imparted in the strains to be used for EOR would not only release the oil from low-producing wells but the oil would demand a premium price because of the dewaxing and desulphurization in situ. Such microorganisms would be particularly useful for crudes obtained in the People's Republic of China, Indonesia, Nigeria, Venezuela, etc. Removal of some of the metals present in crude oil in certain countries will also be attempted through the use of genetically manipulated bacteria.

Oil Spill Clean-Up

Another project that the ICGEB should be interested in is the construction of multi-plasmid strains capable of utilizing spilled oil rapidly. Although the general patent on construction of such strains

and their use in the area of the spill is owned by the General Electric Company, there are numerous other improvements that can be made over such strains. The details of the construction and genetic manipulation of such strains have been described (16). Similar approaches could be taken at the ICGEB to construct a more efficient strain. The usefulness of such a strain in removing spilled oil would be tested over a variety of areas, such as fresh water lakes and rivers, coastal and far-off seas, etc., to determine the effectiveness of the strain. If necessary, separate strains would be constructed, depending upon the nature of the spilled oil, the ecological status of the body of water (i.e., fresh water lakes versus seas), etc.

C. WORK PLAN



D. CO-OPERATION WITH OTHER INSTITUTIONS

Co-operation with petroleum institutes and other relevant institutions will be secured in the implementation of the programme.

Co-operation with national institutes and agencies for carrying out effective field trials will be a significant component of the work programme.

E. PREREQUISITES

A major prerequisite of the programme is the appropriate academic qualifications of the trainees from developing nations. Such trainees must have very good background in microbial physiology and genetics with considerable knowledge in analytical techniques. Preliminary screening for trainees should also take into account the type of institution the trainees will go back to, that is, if the trainee is employed in a petroleum research institute rather than a medical institute or a university department of life sciences, then the possibility of the trainee using his skills to pursue research in microbial recovery of oil or clean-up of oily waters is much higher in the petroleum research institute than in the other two. The same rationale applies to trainees from oil producing countries than from countries without any oil, in as much as trainees from the former countries will have ample opportunity to practice their skills than if the trainees were recruited from the latter.

The cost of the field trials could be shared between the ICGEB and the oil well operators, particularly if the developed microorganisms are found to be effective in enhancing recovery of oil by several folds. Since growth of microorganisms or supply of nutrients are not expensive items, the cost for each injection in the oil well should not exceed $5000. In some cases, this cost may be recouped by the earnings from the recovered oil. In general, the total cost of field trials per year should not exceed $100,000.

F. FINANCIAL REQUIREMENTS

The following staff will be required for implementing this work programme:

- two senior scientists
- four junior scientists
- three post doctoral fellows
- four technicians

15 trainees are expected to participate in the development of the programme.

Five-Year Budget

STAFF		(US$ thousands)
(first year 40 per cent, second year 60 per cent of full operation)		
Senior scientist	8 man year	600
Junior Scientist	16 man year	720
Post doctoral scientist	12 man year	288
Technicians	16 man year	272
Subtotal		1,880
Management of the Centre and Supporting Personnel		489
Total Staff		2,369
OPERATIONAL ACTIVITIES		
Visiting Scientists	20 man months	160
Expert group meetings	2 man months	50
Advisory services	15 man months	150
Training	30 man year	675
Information material		15
Purchase of chemicals etc.	56 man unit year	560
Associateship		75
Miscellaneous		72
Total Operational Activities		1,757
Total Work Programme		4,126

ANNEX I

EQUIPMENT REQUIREMENTS

As mentioned previously, this programme would involve about two to four ICGEB staff and five to six trainees from developing countries. This staff does not include the personnel necessary for conducting the field trials. Since the field trials will be conducted in collaboration with oil well operators and their engineering personnel, the need for having such people at the ICGEB is not obvious.

The space requirement for this programme would be about 5,000 sq. ft. of a laboratory with working benches, chemical hoods and biological containment systems. This does not include the office spaces for the personnel. It would be advisable to separate the genetic manipulation part of the laboratory from the microbial physiology laboratory to avoid excessive contamination problems. The laboratory should be equipped wigh modern preparative and analytical equipments such as:

- ultracentrifuges (Beckman L8 or DuPont OTD65)
- recording spectrophotometers (i.e. Gilford 2600 or Beckman 35)
- RC-2B and RC-5 centrifuges
- gas chromatograph (Varian 3700)
- high pressure liquid chromatography (Perkin Elmer series 2)
- liquid scintillation spectrometers (Packard Tri Carb or Beckman L57000)
- Gilson Oxygraphs and differential respirometers
- electrophoresis and TLC densitometers
- lyphilizers
- French Pressure cells and sonicators
- shakers and incubators

In addition, the research group must have access to electron microscopes, combined gas chromatography - mass spectrometry, IR, NMR and ESR analytical equipments. The cost of such equipments could go as much as $200,000.

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