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PROJECT PROPOSAL

1. Title: Research & Development Unit on microbial enhancement of oil recovery and bioremediation

2. Duration: Preparatory phase: 6 months

Project completion: 2 years

3. Estimated starting date: March 1991

4. Brief description: The immediate objective of the project is to increase national expertise and capabilities in technologies relating to microbial enhancement of oil recovery (MEOR) and oil cleanup (Bioremediation) through establishment of an R & D unit dealing with these areas.

One of the essential prerequisites for success in rapid industrialization of nations is the availability of adequate oil as energy source. The countries with some oil resources are constantly on the search for sustained and increased production of the oil. MEOR provides opportunities for boosting the secondary and tertiary oil output from the wells. The recent advances in genetic engineering offer promise in evolving microbes with enhanced ability in oil recovery.

The increased trade and transport of oil between nations is frequently posing environmental hazards due to accidental spillage. Certain microorganisms have been shown to degrade oil and thus aid in the cleanup. There is also a wide scope for evolving novel microorganisms that are environmentally compatible and have enhanced abilities of bioremediation.

The project is expected to contribute to R & D strengthening of the nations in oil service industries which is of economic, industrial and environmental importance.

5. Project background and justification:

5.1. Introduction:

Rapid industrial and economic development of a nation is very much dependent on the availability of oil. The increasing demands for energy from oil made most oil-exporting countries prosperous in a relatively short period. Therefore, attempts are being made by many nations to produce as much oil as possible to attain self sufficiency and also perhaps export the commodity to earn the much needed hard currency.

With the increasing demands for oil, the oil service industries are confronted with twin problems namely, to realize sustained and

increased oil recovery from the reservcirs and to prevent environmental pollution consequent to accidental spillage. Conventional non-biological technologies, although available to solve these issues, are too expensive for widespread application. The recent advances in biotechnology and genetic engineering provide potentially effective and relatively less expensive alternatives to tackle these problems. Several species of microorganisms with specialized functions have been identified which aid in boosting secondary and tertiary oil recovery (microbial enhancement of oil recovery, MEOR) from the wells and to effect rapid cleanup of the oil spillage (bioremediation). Also, genetic manipulation techniques are being increasingly employed in order to construct novel strains that are environmentally compatible and have enhanced abilities of these functions. Aerobic microorganisms belonging to different genera generate various metabolites, including surfactants and biopolymers which aid in enhanced oil recovery. Multi-plasmid strains are being constructed and examined for their ability to metabolize oil rapidly. The importance of research and development in attaining competence in areas of MEOR and bioremediation cannot be overemphasized.

5.2. Microbial enhancement of oil recovery:

The current technologies for primary oil recovery permit extraction of only 30-40 % of the oil from most wells. The bulk of the oil, therefore, is embedded in the rocks because of high viscosity and for other reasons. Furthermore, over a period of time, the quantity of oil

recoverable from oil reservoirs by primary methods diminishes, largely due to a progressive development of an obstructive "skin" in the reservoir matrix close to the well bore. The obstruction may result due to a variety of reasons such as the deposition of insoluble metallic scales resulting from the impact of mobile sand or clay particles and the presence of tarry residues from the crude itself and the accumulation of wax in the well bore and tubing. The chronic shortage of oil world-wide and the associated frequent price hikes necessitate enhancing efforts for secondary and tertiary oil recovery from the wells. There are several non-biological techniques for correcting some of the effects that retard oil recovery and for restoring the rate of fluid flow in the wells. These depend in general upon injection from the surface of water along with other materials such as surfactants, polymers, gases, steam etc. But these methods are too expensive for wide use and warrant search for alternatives. Among the newer techniques, the use of microbes seem to provide important opportunities to increase and extend oil production. All countries with some oil resources should be armed with such methods and build expertise in this area.

The concept of MEOR is more than 40 years old. Early proposals were poorly conceived and did not yield the expected results. More recently, the advances in microbial biotechnology resolved many specific problems in this field and resulted in evolving better tools. Basically, MEOR is brought about with the inoculation of large quantities of a consortium of microorganisms, usually mixtures of

aerobic and anaerobic strains along with nutrients such as molasses, inorganic compounds and water into an oil well. The well is then capped to allow fermentation and build-up of pressure resulting from the growth and metabolism of the bacteria and production of gases such as carbon dioxide, methane etc. Various solvents and organic acids are also formed in the process. The combination of all of these products together with the solubility of carbon dioxide in the oil strata, displacement of impacted particles and removal of accumulations of scale and hydrocarbons, facilitate oil mobility in the rocks leading to an increased recovery. Recent field trials conducted by several investigators reveal that a single treatment often may result in sustained increase in the net oil production of more than 40 %, which may continue for several years.

MEOR, however is not based on application of a single methodology. The value of microorganisms for oil production lies in their ability to generate a wide variety of chemical products that help mobilize trapped oil in the rocky milieu of the oil wells. The major advantage of MEOR is that the technology is relatively inexpensive and is technically feasible. However, considerable research needs to be conducted in order to select the most appropriate conditions for maximum oil recovery. The technologies and the conditions applied depend on the type of the oil well and its location. Among the various factors that need to be considered to evolve at optimal conditions, the following need particular attention.

Characteristics of the microorganisms: The microbial systems have to be adapted to specific problems of oil recovery from a chosen target reservoir. Microorganisms selected should have the desirable characteristics. They should be able to produce acids in situ which help in dissolution of carbonate rock structures, increase the porosity of the rocks and facilitate oil migration. The strains should be able to produce certain biopolymers and surfactants to decrease the surface tension of the oil-water interface resulting in decreased viscosity and an easier flow of the oil. Certain microorganisms that selectively remove the waxy and other highly viscous components of the oil and those that utilize sulphur-containing contaminants help in recovery of high quality oil. The organisms should possess thermophilicity and grow and disperse at a wide range of temperatures and low oxygen tensions occurring in the deep-seated oil wells. They should have simple nutritional requirements and grow on inexpensive substrates. The organisms should be preferably tolerant to salinity, extreme pH, and be non-pathogenic and ecologically compatible. In this context, appropriate genetically-engineered microorganisms may need to be constructed to fulfil some of these conditions.

Types of oil well: The lithology of the sites surrounding the oil well and the complex nature of the oil reservoirs need to be studied and understood. Shallow wells having predominantly alkaline rock structures need different treatments from those near silicate rocks. High pressure in some oil wells require special attention. Certain other operational problems may arise in some reservoirs such as

development of fractures, fissures or high permeability beds. Under such conditions, plugging by generation of a local seal may be facilitated by selection of an appropriate polymer-producing bacterium.

5.3. Bioremediation:

The increasing production and of export-import of oil in large quantities bring in their wake the problem of oil spills and the associated environmental pollution. As recently as 1989, EXXON oil tanker's spill in the Alaskan region resulted in large scale contamination of the sea and the beaches with the consequent death of marine animals and threatening of human health. In the early 1990, the supertanker Mega Borg caught fire and spilled nearly 4 million gallons of crude oil into the Gulf of Mexico causing untold harm to the marine life. With the increasing trade and transportation facilities, such accidents are likely to happen more often and there is an urgent need to undertake remedial measures. Among the various methods adopted to tackle this important problem is bioremediation which has been proving to be an effective and relatively less expensive way of cleaning the environment from oil pollution. It is vital for both the oil exporting and importing countries to be at the cutting edge of these developments by supporting active research in this area.

Although microbes are widely used in classical fermentation biotechnology, their use is limited in oil and chemical industries. Of

the many different types of naturally occurring microorganisms, aerobes mostly are known to degrade oil and other hydrocarbons. The bacteria are capable of producing fatty acids from oil, a process which leaves the remaining oil products more water soluble. The largely insoluble acids may serve as food for plankton and other organisms.

Besides the naturally occurring organisms, genetic improvements can be brought about in the microorganisms to evolve strains that have ravenous petro appetites. An understanding of the genetic organization and regulation of hydrocarbon degradative pathways may provide interesting opportunities for genetic manipulation and in constructing strains that potentially use hydrocarbons as substrate for growth. The hydrocarbon-degrading microorganisms also produce surface-active compounds that are useful for tanker cleanup and oil-slick dispersions. These surfactants compare favourably with synthetic ones because they are easy to produce, cheap, highly effective at low concentrations and are biodegradable.

Some of the microbial strains recently developed at the University of Texas are reported to metabolize wide variety of crude oils and have interesting characteristics in that they are averse to water. Consequently, they live as long as the oily substrate is available and then die thereafter thus obviating any persisting contamination. Such organisms are particularly relevant as their use does not lead to any disturbance of ocean ecology, a factor one must consider in using non-

indigenous bacteria. Yet another development is using fertilizers like Inipol, developed by a French petroleum company, over the oil spill regions. Researches on the development of such fertilizers started as early as in late 1960s and aimed at stimulating the growth of naturally occurring bacteria that metabolize oil and thus help in rapid cleanup. Most researchers use nitrogen and phosphate fertilizers. The bacteria grown that way do not seem to have significant toxic effects on marine life. However, these fertilizers are not always effective, for example of oil contaminations on rocky portions when these do not stick. Dispersion of oil across the surface of the site is needed for the fertilizers to be effective. Also, in cold regions this technique will not be very effective due to the inhibitory effects on the bacterial growth.

Thus, the developments in bioremediation area, although very promising, warrant renewed researches for finding suitable fertilizers and to better understand the nature of the shoreline microorganisms which also may lead to construction of effective genetically-engineered ones in order to develop more advanced bioremediation methods for dealing with oil spills on land and on the sea.

6. Project:

Based on the above considerations, a project has been proposed for the establishment of a research and development unit on MEOR and bioremediation in order to build national capabilities in these

technologies.

6.1. Objectives: To create in-house capabilities in MEOR and bioremediation technologies through establishment of an R & D unit.

The unit will carry out the state-of-the-art research in identification and isolation of microorganisms that are of value for MEOR and bioremediation. To achieve these goals, in addition to the screening of internally isolated organisms, externally referred species will also be examined. Further, novel strains with the requisite properties and characteristics will be evolved by construction and genetic manipulation. On completion of the project, a full-fledged R & D unit will be available to the nation manned by high calibre scientists that cater to the needs of the oil service industries.

6.2. Outputs

6.2.1. Establishment of a research and development laboratory

6.2.2. Manning the unit with trained personnel

6.2.3. Organization of training activities

6.2.4. Pilot plant studies and field trials

6.3. Activities

6.3.1. Establishment of a R & D unit: The unit will be established with some 27 well qualified scientific and supportive staff. The unit will have adequate office and laboratory space, relevant facilities such as furniture, running water, electricity, vacuum and high-pressure line, gas connections, and chemical hoods, biological containment systems and computer facilities.

In the interests of economy, the R & D unit will be located within the campus of a university/ institute / R & D of an industry with established department s of life sciences. Such an arrangement will not only provide the unit an intellectually stimulating atmosphere but also will enable the staff of the unit to share some of the common infrastructural facilities such as physical, equipment, library, canteen etc.

6.3.2. Equipment: The following equipment will be made available to the R & D unit.

Cold rooms

Hot rooms

Deep Freezers

Fermenters

Sterilizers, Laminar flows

Centrifuges

Spectrophotometers

GLC - Mass Speck

HPLC & FPLC systems

Radioactivity counters

Chromatography systems

Electrophoretic units

Fraction collectors

Lyophilizers

French Pressure cells & Sonicators

Shakers & incubators

6.4. Inputs:

6.4.1. Buildings and infrastructure. An 800 Sq.mt. space will be provided to establish the R & D unit. The unit will be equipped with offices and laboratory furniture with appropriate fittings.

6.4.2. Personnel: The unit will be manned by well-qualified scientists specialized in petroleum engineering, molecular biology, microbiology, biochemistry and geology with an economist on the staff. There will be emphasis on imparting a scientific and techno-economic character to the unit.

Category	Number	Specialization
Project Director	1	Microbiology

Principal investigators	2	Petroleum engineering
		Microbiology
Senior scientists	4	Petroleum engineering
		Microbiology
		Molecular biology
		Economics
Junior scientists	8	Microbiology - 2
		Molecular biology - 2
		Biochemistry - 2
		Physical chemistry - 1
		Geology - 1
Post-doctoral fellows	4	Microbiology
		Molecular biology
		Biochemistry
		Physical chemistry
Technicians	4	
Other supportive staff	4	
Total	27	

International experts	3	Petroleum engineer - 1
		Microbiologist - 2
National experts	4	Petroleum engineer - 2
		Microbiologist - 2

6.4.3. Training activities: Adequate training facilities will be provided to the staff of the unit through invited national and international experts, conducting seminars (2) and workshops (2) and through international exchange programme.

6.4.4. Pilot plant studies and field trials: Pilot plant studies will be done under simulating conditions constructed in the laboratory and field trials will be conducted in collaboration with oil service industries and oil well operators.

7. Budget

7.1. National currency component:

Space and infrastructural structural facilities

Scientific staff:

Category	Distribution of the budget over the years		
	1991	1992	1993
	m/m	m/m	m/m
Project Director	6		
Principal Investigators	8	24	16
Senior Scientists	16	48	32
Junior Scientists	32	96	64
Post-doctoral Fellows	16	48	32
Technicians	16	48	32
	94	264	176
National Experts	6	18	18

7.2. Foreign Exchange component

		$ U.S. thousands		$ U.S. thousands		$ U.S. thousands
	m/m		m/m		m/m	
International Experts	12	120	12	120	12	120
Training						
Seminars 2				20		20
Workshops 2				20		20
Scientific Exchange (10)				50		50
Equipment		100		75		25
Consumables		50		35		15
Computers		15		10		
Library		15		7		3
Miscellaneous		10		15		5
Total		310		352		258

	U.S. $ in Thousands		
Support Costs, 13 %	41	45	34
Grand Total, $	351	397	292

8. Project monitoring, reporting and evaluation:

The project will be monitored by national and international experts in association with the Project Director and the Principal Investigators. A six monthly progress report will be prepared which will be evaluated by a panel of scientific advisors which include national and international experts.

9. Risks:

The risks that may be envisaged in the satisfactory completion of the project are difficulties in identification, isolation and construction of appropriate microorganisms that are efficient in MEOR and oil cleanup. The cloning of genetically engineered microcrganisms may lead to a concern of environmental contamination that require special attention. By taking adequate precautions, the investigators can take proper steps to contain the non-indigenous bacteria for the purposes intended.