Bacteria have successfully colonized virtually every environment on earth because they can rapidly adapt to changing conditions, and use a large and varied number of nutrients to generate energy. However, until recently, the environments of many petroleum reservoirs were considered too hostile for bacterial growth due to low availability of water, and high temperatures, pressures, and salinities (Bass and Lappin-Scott, 1997). The possibility of bacteria existing in larger, deep reservoir was largely ignored. The start of North Sea production in the 1960’s led to the realization that bacteria could produce hydrogen sulphide (H2S) as waste product and cause reservoir souring.

A reservoir can harbor good guys and bad guys of microbes. The bad guys are those groups of bacteria that use sulfur-based compounds present in sea-water and sometimes in formation water or aquifer water as a part of their energy chain, and use the simple carbon compounds that are present in formation as food. Waste from this growth includes H2S, which is poisonous to human, corrosive to tubular and top side tanks. Other detrimental microorganisms grow profusely around the well bore region-in mud filter cake and the formation-blocking rock pores and reducing permeability; still others break down and render ineffective chemicals that are added to facilitate production operations and increase wellbore life.

On the other hand, good microorganisms are those helpful bacteria which, during their growth, produce useful compounds for example, solvents, acids, gases, surfactants and biopolymers. It is these bacteria and their byproducts that can be used constructively in reservoirs.

To date, most petroleum microbiological work has centered on waterflooded reservoirs that offer a cooled, oxygen-free, saline environment, which meets the environmental requirements of many different groups of bacteria. Most bacteria have a natural tendency to grow attached to rock surfaces rather than free-floating in the liquid phase. In a petroleum reservoir, bacteria may attach to the
rock; start to grow and then produce exopolymers-sugars-that help them attach to each other and rock surfaces. Such growth is termed as biofilm and offers the advantages of protection from biocides while encouraging the bacteria to interact to best use nutrients and other resources (Bass and Lappin-Scott, 1997).

Microorganisms from different oil reservoirs of world have been studied viz. Alaska, Africa, Australia, France, Japan, USA, North Sea, Vietnam, Daquing, Mexico, Congo, Paris, and California, Russia.

Petroleum reservoirs constitute a group of unique terrestrial sites, because they present an usual combination of extreme environmental conditions including temperature, pressure, and salinity. Attention has been paid recently to the microbial ecology of petroleum reservoirs where anaerobes have always been considered as the dominant microorganisms. (Fardeau et al, 2004). Petroleum reservoirs harbor a rich and diverse community of microorganisms including (i) fermentative, (ii) sulfate-, thiosulfate, and sulfur-reducing, and (iii) methanogenic. (Salinas et al, 2004 a). Since the beginning of commercial oil production, almost 140 years ago, petroleum engineers have faced problems caused by microorganisms. Sulfate reducing bacteria (SRB) were rapidly recognized as responsible for the production of H2S, within reduced oil quality, corroded steel material, and threatened workers health due to its high toxicity (Cord-Ruwich et al, 1987). For instance, at concentration of 10 ppm and above, hydrogen sulfide causes conjunctival irritation, serious eye damage is caused by a concentration of 47ppm, at higher concentration above 150 ppm it has paralyzing effect on the olfactory perception, and at 268 ppm there is a risk of pulmonary oedema.

The possibility of living organisms to survive or thrive in oil field environments depends on the physical characteristics and chemical composition of the ecosystem. Temperature is the main limiting factor for microbial growth in oil reservoirs. Since temperature increases with depth at a mean rate of 3 °C per 100 m (but regional geothermal gradients may be significantly different), deep oil reservoirs which attain an in situ temperature exceeding 130-150 °C cannot sustain bacterial growth(Magot, 2005). This temperature range is considered the highest theoretical limit for growth due to the thermal instability of biological molecules (Stetter et al, 1993). Different types of data suggest the presence of indigenous bacteria in oil fields could be limited to a threshold temperature between 80-90 °C. Philippi noted that in situ oil degradation was never observed in reservoirs whose temperature exceeded 82 °C (Phillipi, 1977). Analysis of a set of 87 water samples
collected from North American oil reservoirs showed that fatty acid concentrations were maximum at a temperature of 80 °C in the reservoir (Bartha 1991). This indicated that maximum biodegradation occurs below 80 °C, and that thermal decarboxylation occurs above this temperature. In a microbiological study, hyperthermophilic bacteria could not be isolated from 100 oil field water samples whose reservoir temperature were higher than 82 °C (Bernard et al, 1992).

Hyperthermophilic microorganisms growing at temperatures as high as 103 °C have been isolated from some reservoirs, but the authors suggest that they represented exogenous bacteria resulting from sea water injections (Stetter et al, 1993).

Salinity and pH formation water can also limit bacterial activity. The salinity ranges from almost fresh water to salt saturated water and pH generally from 5-8. However, the pH measured at atmospheric pressure does not necessarily reflects the actual in situ pH, as it is influenced by dissolution of gasses under high pressure. The in situ pH is usually in the range 3-7. This physical characteristic has to be taken into consideration when designing culture media, or interpreting the potential indigenous nature of bacteria recovered from deep surface samples.

The availability of electron donors and acceptors governs the type of bacterial metabolic activities within oil field environments. The oil industry undertakes routine chemical analyses of oil environments chemical analyses of oil field hence a wide body of data are available; nevertheless critical data (e.g., nitrogen and phosphorous availability) necessary for understanding microbial metabolism is not routinely performed thereby thwarting our ability to understand microbial processes in situ. Since oil fields are deep subterranean environments and are generally absent: in particular oxygen, nitrate and ferric iron.

Stratal waters (water retained in the pores of rock) generally contain sulfate at various concentrations and carbonate, factors which have led to the assumption that the major metabolic processes occurring in such ecosystems are sulfate reduction, methanogenesis, acetogenesis, and fermentation. The potential electron donors include CO2, H2, of geochemical or bacterial, and numerous organic molecules, organic acids are present, but not in all oil reservoirs, and concentrations higher than 20 mM have been recorded (Bartha 1991). Connan showed that in vitro anaerobic biodegradation of crude oil by an indigenous bacterial community reproduced biodegradation effects which have been recorded under natural conditions (Connan et al, 1996). The presence of potential electron donors for anaerobic metabolism. Their presence could explain the observation that
diverse groups of strict anaerobes can grow with crude oil as a sole carbon and energy source without any modification of alkanes or light aromatic compounds.

**Thesis outline**

This study was conducted to study the diversity of microorganisms that could potentially produce hydrogen sulfide in oil reservoirs of India. To study the intraspecies diversity in strains of *Garciella nitratireducens*. Pilot scale microbiocide treatment was carried out to control sulfidogens in produced water at Kathloni oil field was carried out.

**Introduction**

This chapter explains the nature of problem that has been discussed in this study. The problem of hydrogen sulfide production in oil reservoirs has been addressed. Thereby, identifying the microorganisms responsible for the same and their control.

**Review of Literature**

The second chapter of this thesis briefly reviews the existing literature in the area of thermophilic sulfide production. The related topics that have been discussed are as follows:

- Petroleum reservoirs and their physiochemical characteristics, Reservoir temperature and indigenous microbial communities, Nutrient availability and metabolic processes in oil reservoirs and oil fields.

- Sulfate Reducing Bacteria recovered from subsurface oil field waters, Thermophilic SRBs, Fermentative, iron reducing and nitrate reducing microorganism, Thermophilic fermentative bacteria.

- Reservoir souring, Mechanisms of souring, Hydrogen sulfide scavenging in the reservoir, Sourcing control, sulfidogeneic bacteria, microbial prevention,
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- Biocide categories, Treatment categories, Conventional biofouling control measures, Underdosing of bactericide, Inappropriate dose regimens, Tolerance and resistance, Bactericide demand, System conditions, Inadequate testing and monitoring, Novel biofouling control programs, Nitrate, Molybdate, Sulfate removal, Pulse treatment

- Identification with 16s rDNA, Polymerase chain reaction based approaches as tool for genetic diversity studies, Study of ribosomal operon to elucidate phylogentic diversity, Organization of ribosomal RNA operon, Molecular processing of rRNA gene, Heterogeneity of rRNA gene, Polymorphism of bacterial rRNA operon, Causes of rRNA operon polymorphism, 16S rRNA signature nucleotide, 16S-23S rRNA internally transcribed spacer, Restriction analysis of the rRNA operon, Amplified ribosomal DNA restriction analysis, Restriction fragment length polymorphism 16S-23S DNA ITS, Bacterial community analysis with rRNA genes

Materials and Methods

This section mentions in detail the experimental procedures used in current study. The experimental regime was designed to meet the objectives. The various media composition that has been elaborated in Annexure were tried for the cultivation of microorganisms. The protocols for their identification have been described. Qualitative and quantitative estimation of the different fraction of total petroleum hydrocarbon in crude oil were estimated. The physical and chemical properties of the isolated microorganisms were studied. Parametric optimization of TERI SRB 1001 and TERI SRB 1010 were studied. The pilot scale microbiocide treatment of produced water to control H2S at Kathloni oil field was conducted.

Results

The results section describes the finding of the experiment done under the current objectives. The results of field trial have also been described in this section.
Discussion

In this section the results of the current study have been discussed and implication and interferences drawn.

Summary

This chapter summarizes the major finding of this study.