Biodegradation of Sludge Containing Oil by using Bacterial Culture

Shital A.Nivle[1], Sachin J. Mane[2]
[1, 2] Civil Engineering Department
[1, 2] D.Y.Patil College of Engineering, Pune, India.

Abstract- *this paper presents the field of performance in oily sludge degradation by using bacterial culture. This system aims to identify biosurfactant producing bacteria and the efficiency of biosurfactant bacteria to degrade the oil from the sludge. Biosurfactants are surface active compound that reduce the interfacial tension between two liquids, or that between a liquid and a solid. Their unique property like nontoxic, easily biodegradable, eco-friendly and high stability, and wide variety of industrial application makes them highly useful group of chemical compound. Biosurfactants are produced from variety of microorganisms. The objective of this study is to isolate and characterize the biosurfactant producing bacteria from oil contaminated sludge analyse the oil degradation capacity of oily sludge by using biosurfactant bacterial culture, also find out the degradation efficiency of biosurfactant bacteria when cultivated during 10,20,30 days for different concentration of bacterial culture.*

This indicated that the bacterial culture has potential to be applied in bioremediation of oily sludge contaminated environments, favouring the reduction of environmental pollutants and increasing industrial productivity.

Keywords- Biodegradation, Biosurfactants, sludge containing oil, Gas Chromatography, spectrophotometer.

I. INTRODUCTION

Petroleum, lubricant oil manufacturing industries bring innumerous benefits to society but are globally recognized as an economic activity with great environmental impacts. Petrochemical industries and refineries and lubricant generate a large amount of solid waste sludge containing oil, classified as Hazardous Waste (residues that cannot be reutilized or recycled, and may present characteristics such as flammability, corrosivity, toxicity or pathogenicity).

The sludge containing oil is a recalcitrant residue characterized as an emulsion of water, oil, fats, solids, organic compounds and metals. Among the organic compounds, the most common are alkanes, cycloalkanes, benzene, toluene, xylenes, phenols and polycyclic aromatic hydrocarbons (HPAs). Many components of the oily sludge are listed as priority pollutants by the Environmental Protection Act due to their toxicity and their mutagenic and carcinogenic potential towards humans. Considering the pollution and toxicity of oil hydrocarbons, the development of an effective and environmentally friendly strategy to reduce it still remains a challenge. The increasing pressure by environmental regulatory agencies has motivated the companies to develop clean technologies, highlighting the bioremediation technology. Bioremediation can be defined as the use of microorganisms to remove environmental pollutants in water and soil, aiming the complete degradation of hydrocarbons into carbon dioxide and water. In order to increase the bioremediation process yield, the bioaugmentation strategy (addition of microorganisms exogenous or endogenous with effective degradation capacity in the contaminated local) has been proposed and evaluated.

The employment of a bacterial culture has demonstrated to be more advantageous in comparison with pure cultures due to synergistic interactions among members of the associations, which may lead to the complete degradation of the product . The utilization of microorganisms with proven degradation potential and survivability in the contaminated environment is crucial for a successful bioaugmentation. In view of this, the project aims to study the characterization of biosurfactant bacteria isolated from the soil and sludge itself also to find out the degradation capacity of that bacterial culture to degrade oil from the contaminated sludge.

II. MATERIAL AND METHOD

A) Material Requirement

1. Sample of sludge containing oil, soil sample.
2. Autoclave, Incubator, pipette, Petri-plates, test tubes, Air oven, Chemicals for different test.
3. Spectrophotometer and Shaker.

B) Methodology

1. Sample collection

The oily sludge and soil samples are collected from a Lubricant oil industry situated in the city of Bhosari, Pune.

2. Bacterial isolation

5g of soil sample and sludge is inoculated in 100 ml of Mineral Salt Medium (MSM) [composition in g/L: KH_2PO_4,1;$MgSO_4$,0.5;$FeSO_4$,0.01;$NaNO_3$,1.5;$CaCl_2$,0.002;$(NH_4)_2SO_4$,1.5;] with 3ml kerosene oil added to the conical flask having capacity of 250 ml, as the carbon sources for soil sample and then it was incubated for 72 hours at 30°C temperature. After then 1ml of incubated culture was spread on the petriplates. The samples then were serially diluted up to 10^{-2} dilution. 1 ml of 10^{-2} time dilution was transferred to nutrient agar for spread culture.

Figure 1. Bacterial isolation from soil sample b) Bacterial isolation from oily sludge sample

3. Bacterial identification of selected isolates

The bacterial isolates which shows the highest colony diameter was selected and identified based on physical properties of bacterial colonies.

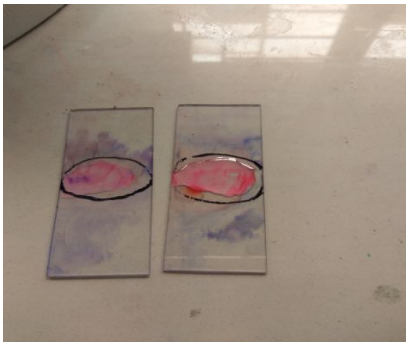

Figure 2. Bacterial identification slides

4. Screening of Biosurfactant bacteria

After identification of o biosurfactant bacteria gram staining test shows the gram positive results, which shows the Bacillus species Bacteria. These bacteria screened in Agharkar research institute by biochemical characterization of that species shows the geobacillus thermodenitrificans having biosurfactant property.

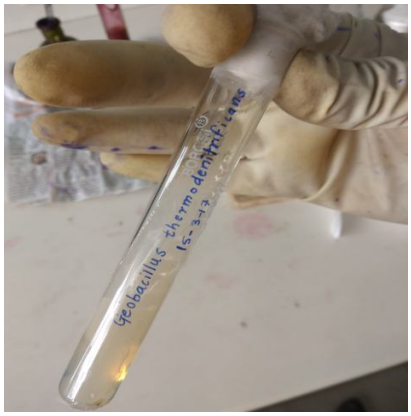

Figure 3. Biosurfactant bacillus species culture

5. Growth kinetics

Growth study of isolated bacteria from sludge sample done by using spectrophotometer. The standard oil degradation conducted by using toluene further compare it with degradation by bacterial isolates by changing time and concentration. Find out the percentage Transmission.

6. Extraction of Oil

We did not use the oily sludge sample in gas chromatography directly for this we should add pure oil separated from sludge. Before testing to the gas chromatography extraction of oil from sludge sample is necessary, Standard extraction process is followed to separate oil and organic sludge sample. After completion of desired reaction period, reaction mixture was extracted by n-hexane (3X10ml) The organic layer wash with brine ,dried over anhydrous Na2So4 evaporated under reduced pressure kept in rotaevaporator to evaporate liquid mass. From this evaporation the separation of oil and organic sludge sample gets separated and oil sample can use directly for gas chromatography.

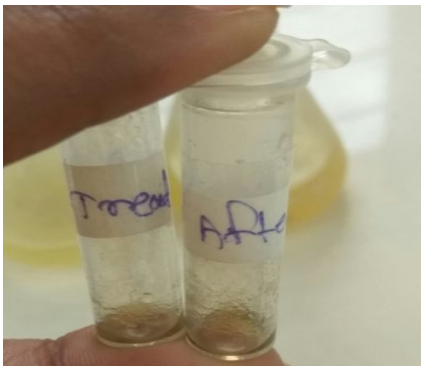

Figure 4. After treatment Extracted oil from sludge

7. Biodegradation rate of sludge containing oil in liquid medium by using Gas Chromatography

The oily sludge biodegradation capacity by bacterial isolates was evaluated by using gas chromatography. The initial and final concentration of oil find out by its peak difference individually. After biodegradation of oil by varying time 10, 20, 30 days. Gas chromatography used in for separating and analysing compounds that can be vaporized. In this method mobile phase is a carrier gas nitrogen and H2 is used as a fuel column AT-19091J-413; HP-5 30m x0.32mm ID,0.25um used with Flame Ionization Detector(FID) having wavelength 220nm. Extracted oil sample from sludge is kept in 80^{o}-280^{o}C then hold for 10 min shows peak by changing time.

The objectives of the study are as follows:

- The objective of this study is to isolate and characterize the biosurfactant producing bacteria from sludge containing oil and soil sample.
- To find out degradation capacity of oil by Biosurfactant Bacterial culture.
- Addition of different concentration of bacterial culture to degrade the oil by varying the time period.
- Experimental work
- Sample collection (sludge containing oil collected from end of wastewater treatment plant)
- Isolation of biosurfactant bacteria by using spread plate method.
- Isolated colonies of bacteria were taken and tested for the confirmation of biosurfactant bacteria that comprises following test

1. Oil spreading technique
2. Emulsification assay
3. Foaming activity

- Physical characterization by gram staining.
- Identification of percentage of fractions of oil by using gas chromatography before and after bacterial inoculation.

III. RESULTS & DISCUSSION

A. Bacterial Identification

Physical characterization of Bacteria is done by gram staining procedure which shows gram positive bacillus species bacteria from this bacillus species the geobacillus thermodenirificans are used for the further biodegradation.

Table 1. Morphological & Physiological analysis of Bacteria isolated from soil and lubricant oil sludge

Characteristic	Stenotrophom-onas acidaminiphilia	Pseudomonas aeruginosa	Bacillus cibi	Geobacillus thermodenitrif icans
Gram staining test	Gram negative	Gram negative	Gram positive	Gram positive
Morphology	Rod shape	Rods	Rods	Rods
Endospore	-	-	Central	Central
Mackonkey agar growth	Positive	Positive	-	-
Anaerobic Growth	Negative	Negative	Facultative	Facultative

B. Growth Kinetics and pH measurements

Growth kinetics of Bacteria identified by spectrophotometer which shows as time increases the percentage transmission decreases which show the biomass of bacteria in the oily sludge sample increases as time increases.

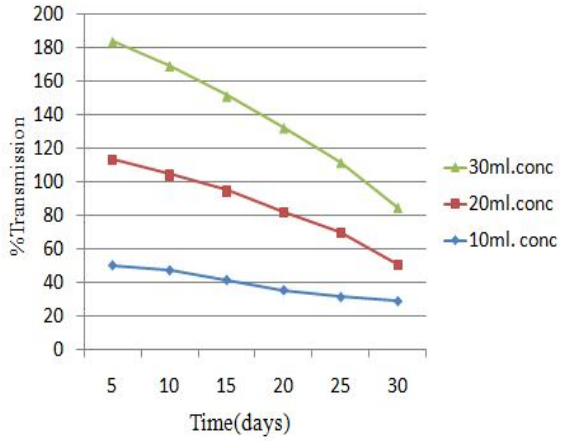

Figure 5. Growth kinetics

The pH of the initial sludge sample is high, after treated it ith bacterial culture it shows the pH increases which shows acidic sludge sample with culture after teatment changes into basic.

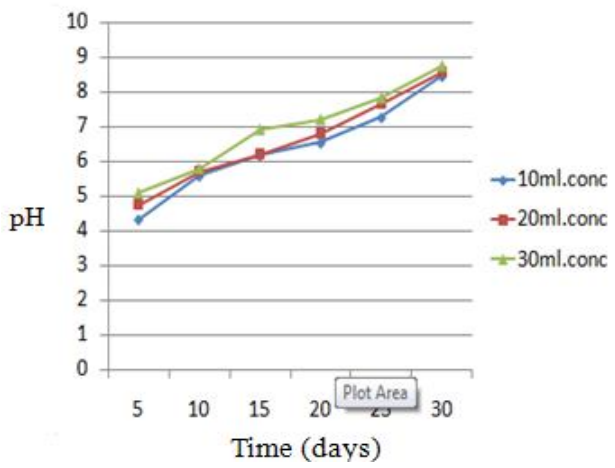

Figure 6. Microbial conc. Shows pH concentration

C. Peak of oil sample by Gas Chromatography

After placing the extracted oil sample for gas chromatography Based on degradation capacity of bacteria is determined by using gas chromatography After testing in Gas chromatography shows the peak difference.

$$\text{Percentage Degradation} = \frac{\text{Initial Area of Peak} - \text{Final Area}}{\text{Initial Area under Peak}}$$

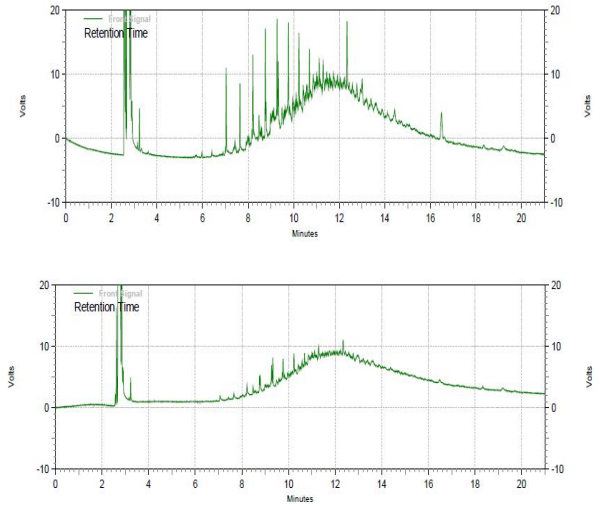

Figure 7. GC spectra of a) lubricant oil in broth without culture b) Lubricant oil in broth inoculated with culture

IV. CONCLUSION

This study showed that amongst all bacterial isolates geobacillus thermodenitrificans shows oil emulsification and degradation ability. Therefore, this microbes suitable to be applied in bioremediation process in sites contaminated with sludge containing oil. This microbe competent degrader of hydrocarbons is an interesting ecofriendly strategy since it contributes to the minimization of process time and environmental impacts.

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