

DIOXYGENASES AND BIOSURFACTANTS CARRIED OUT IN CRUDE OIL EXERTED BY DEGRADING SOIL BACTERIA WITH WATER TREATMENT AND ISOLATION AND CHARACTERIZATION.

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Abstract:

Crude oil demand in day by day .This study focused on the isolation and characterization of bacterial strains with superior oil degrading potential from crude-oil contaminated soil. The specific activity of catechol 1,2 dioxygenase (C12O) and catechol 2,3dioxygenase (C23O) was determined in these three strains wherein the activity of C12O was more than that of C23O. Oil spills incidence all over the world is also occasionally occurring. The remediation of oil contaminated soils and waters has become a crucial problem to be solved in several oil producing countries. It is eco-friendly, cost-effective, and efficient for the abatement and decontamination of hydrocarbon pollution and many other pollutants, but might take more duration than the conventional technique because of its natural process. Oil polluted soil phytoremediation involves several process namely crude oil uptake from soil.

Bacteria degrading from soil :

The recovery of spilled crude oil could be achieved by adopting physical and chemical methods but they can take care of only 10–15% of oil spillage. On the other hand, bioremediation by exploiting complex microbial communities can act as a self-driven, economical and eco-friendly method. Many bacterial species including *Pseudomonas aeruginosa*, *Bacillus subtilis*,

Bacillus megaterium, *Corynebacterium kutscheri*, have been reported to possess oil degrading potential. The biochemical process of crude oil degradation by microbes involves the action of several enzymes including oxygenases, dehydrogenases and hydroxylases that fragment aromatic and aliphatic hydrocarbons. A major constraint in bio-degradation of oil is its hydrophobicity but biosurfactants produced by oil degrading bacteria facilitate uptake of hydro-carbons by the bacterial cells. those bacterial strains that possess the ability to produce biosurfactants along with enhanced oil degrading capacity are widely recommended for use in order to achieve fast degradation of crude oil. among the 12 bacterial isolates from oil contaminated soil samples, three strains which had the isolate designation of PA, PM and BM were selected owing to their superior oil degrading activity. The extent of degradation of aliphatic and aromatic compounds of total petroleum hydrocarbon (TPH) in crude oil by these strains was also analyzed.

Physical and Chemical Properties of Crude Oil and Oil Products

1- Density, Specific Gravity, and API Gravity

Density is defined as mass per unit volume of a fluid. Density is a state function and for a pure compound depends on both temperature and pressure and is shown by ρ .

$$SG = \frac{\text{density of liquid at temperature } T}{\text{density of water at temperature } T}$$

Since the standard conditions adopted by the petroleum industry are 60°F (15.5°C) and 1 atm,

specific gravities of liquid hydrocarbons are normally reported at these conditions. Water density at 60°F is 0.999 or almost 1 g/cm³, thus

Crude Oils API = 10 – 50, crude oils can generally be classified according to API as shown

Crude Category	Gravity
Light crudes	API > 38
Medium crudes	38 > API > 29
Heavy crudes	29 > API > 8.5
Very heavy crudes	API < 8.5

Viscosity

The viscosity of oil is a measure of its resistance to internal flow and an indication of its

oiliness in the lubrication of surfaces. There are two types of viscosity: dynamic and kinematics viscosity.

$$\text{Kinematic viscosity } (\nu) = \frac{\text{dynamic viscosity } (\mu)}{\text{density } (\rho)}$$

Pour Point

The pour point is defined as the lowest temperature at which the sample will flow and is a

rough indicator of the relative paraffinic and aromaticity of the crude

$$T_p = 130.47[SG^{2.970566}] \times [M^{(0.61235-0.47357SG)}] \times [\nu_{38(100)}^{(0.310331-0.32834SG)}]$$

where T_p is the pour point (ASTM D 97) in kelvin, M is the molecular weight, and $\nu_{38(100)}$ is the kinematic viscosity at 37.8°C (100F) in cSt. This equation was developed with data on pour points of more than 300 petroleum fractions with molecular weights

ranging from 140 to 800 and API gravities from 13 to 50

Physicochemical and biochemical characterization of biosurfactants

To characterize the biosurfactants produced by PA, PM and BM, the bacterial strains were inoculated individually in to MSM broth containing 1% crude oil and incubated at 30°C for 5 days in a shaker (200 rpm). The cell free supernatant was collected by centrifugation at 10,000 rpm for 20 min and was subjected to physicochemical and biochemical characterization. The emulsification index (E₂₄) and the oil displacement capacity of the cell free supernatant was determined. Qualitative analysis to detect glycolipids and lipoproteins in the cell free supernatants of PA, PM and BM was carried out respectively by Phenol sulfuric acid¹⁸ and Bradford Method. The sugar content was quantitatively determined by orcinol assay.

SOIL TEST

Sampling and isolation of crude oil degrading bacterial strains

Crude oil and soil samples from crude oil contaminated sites were collected from Shanmuganathi buried place, palani, Tamilnadu, India. For the isolation of crude oil degrading bacteria, 1 g of soil sample was inoculated into 100 mL of mineral salt medium (MSM pH 6.8 ± 0.2) supplemented with 1% crude oil and incubated at room temperature for 1-week.

Diesel oil and mineral oil contaminated soils (real samples) were used. Uncontaminated soils samples from the same regions were used as the reference samples. Sawdust, paper mill waste, straw, heater, trades cantia and agate leaves samples were used as the source of biogenic hydrocarbons. Biogenic materials used in composting of oil and biological sludges (straw, bark, lop), initial biological and oil sludges, and samples prepared mixing

these materials at starting and final composting status were used as model mixtures. Six composting variants have been studied:

Variation I (Specific gravity = 2.36) (Water content = 0.11%)

Variation II (Specific gravity= 2.47) (Water content = 0.11%)

Variation III (Specific gravity= 2.19) (Water content = 0.11%)

Variation IV (Water content = 0.15%)

Variation V (Specific gravity = 2.45) (Water content =0.15%)

Diesel oil, mineral oils (No R953 and No R932) (Slovnaft Ltd.) were used as the reference materials of petroleum fractions. C30-17 β (H)21 α (H)-hopane (Chiron AS, Trondheim, Norway) was used as the standard reference material for the determination of C30-17 α (H)21 β (H)-hopane



Dried samples (1-10 g) were extracted using 50 ml distilled acetone thoroughly shaking for 15 minutes and subsequently a 100 ml distilled isohexane was added and shaken for next 15 minutes. Resulting slurry was left for 3 days while the equilibrium was achieved and then passed through a filter paper. Obtained extractants were divided into two equal portions. The first part was concentrated to the final volume of 1 ml using vacuum rotary evaporator and extractable compounds (TSEM) and naphthalene homologues were measured. The rest portion was brought to almost dryness using vacuum rotary

evaporator, a 1 ml isohexane was added and quantitatively transferred onto the silica gel column using an additional 4 ml isohexane to complete the transfer. A chromatographic column was plugged with glass wool at the bottom and rinsed with isohexane. The column was packed with 4 g of activated silica gel (180 °C, 6 hours) with tapping to settle the silica gel. 50 ml hexane was used to elute the non polar extractable compounds out of the column. The hexane fraction was concentrated to the volume of 1 ml using vacuum rotary evaporator and used for analysis of non polar extractable compounds (TPH) and hopanes, respectively.

PREPARATION OF CRUDE OIL

Physical Separation Processes

Physical division procedures isolate a blend, for example, a raw petroleum without changing the compound attributes of the segments. The partition depends on contrasts of certain physical properties of the constituents, for example, the bubbling and liquefying focuses, adsorption affinities on a specific strong, and dispersion through specific layers. The imperative physical detachment forms, examined here, are refining, retention, adsorption, and dissolvable extraction.

Atmospheric Distillation

Air refining isolates the unrefined petroleum complex blend into various parts with moderately contract bubbling reaches. By and large, detachment of a blend into parts is construct fundamentally with respect to the distinction in the breaking points of the segments. In air refining units, one or additionally fractionating segments are utilized. Refining an unrefined petroleum

begins by preheating the food by trade with the hot item streams. The food is further warmed to around 320°C as it goes through the warmer channel (pipe still radiator).

The hot food enters the fractionator, which ordinarily contains 30-50 fractionation plates. Steam is presented at the base of the fractionator to take off light segments. The proficiency of partition is a component of the quantity of hypothetical plates of the fractionating tower and the reflux proportion. Reflux is given by gathering part of the tower overhead vapours. Reflux proportion is the proportion of vapours consolidating back to the still to vapours gathering out of the still (distillate). The higher the reflux proportion, the better the partition of the blend

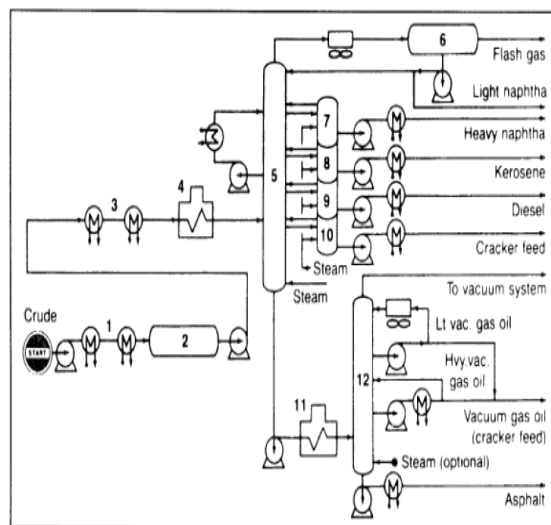
Conversion Processes

Upgrade lower-esteem materials, for example, substantial deposits to more important items, for example, naphtha and LPG. Naphtha is mostly used to supplement the gas pool, while LPG is utilized as a fuel or as a petrochemical feedstock.

- Improve the attributes of a fuel. For instance, a lower octane naphtha part is changed to a higher octane reformat item. The reformat is essentially mixed with naphtha for fuel detailing or extricated for acquiring aromatics required for petrochemicals creation.
- Reduce hurtful contaminations in petroleum divisions and deposits to control contamination and to abstain from harming certain handling impetuses. For instance, hydro treatment of naphtha sustains to synergist reformers is key since sulfur and nitrogen polluting influences harm the impetus

Transformation procedures are either warm, where just warmth is utilized to impact the required change, or synergist, where an

impetus brings down the response actuation vitality. The impetus additionally coordinates the response toward a craved item or items (particular impetus).



(1,3) heat exchangers

(2) desalter, (3,4) heater;

(5) distillation column,

(6) overhead condenser,

(7-10) pump around streams,

(11) vacuum distillation heater;

(12) Vacuum tower.

Thermal Conversion Processes

Thermal splitting was the primary procedure used to build gas generation. After the advancement of synergist splitting, which enhanced yields and item quality, Thermal breaking was given different parts in refinery

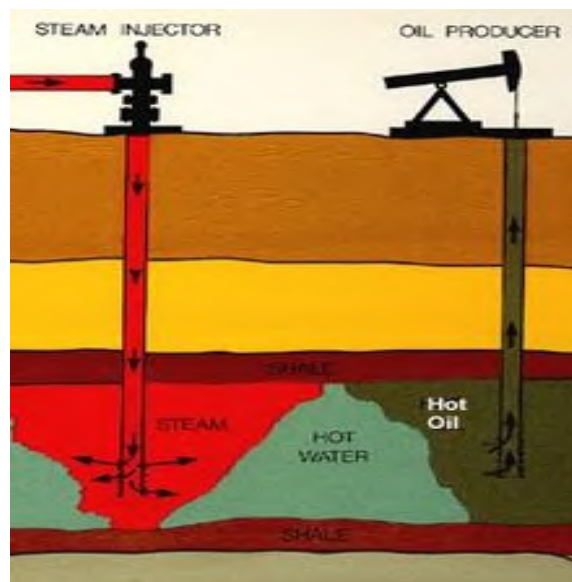
operations . The three vital warm splitting methods are coking, consistency breaking, and steam breaking. Steam breaking is of unique significance as a noteworthy procedure planned particularly to produce light olefins. It is talked about independently later in this part.

Production of Olefins

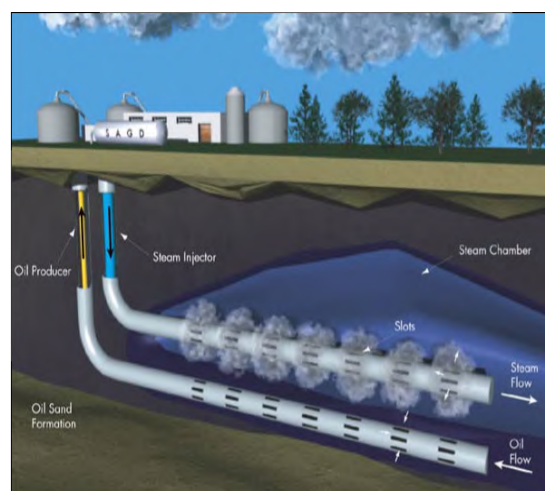
The most vital olefins and diolefins used to fabricate petrochemicals are ethylene, propylene, butylenes, and butadiene. Butadiene, a conjugated diolefin, is regularly coproduced with C2-C4 olefins from various splitting procedures. Partition of these olefins from reactant and warm breaking gas streams could be accomplished utilizing physical and compound division strategies. Be that as it may, the petrochemical interest for olefins is much more prominent than the sums these operations produce. Most olefins and butadienes are created by steam breaking hydrocarbons. Butadiene can be on the other hand delivered by other engineered courses examined with the union of isoprene, the second major diolefin for elastic generation.

EXTRACTION OF CRUDE OIL :

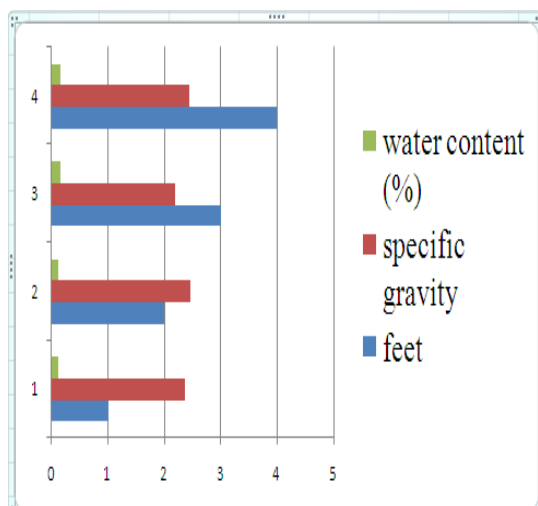
This is crude oil that's thicker, or more viscous, than "regular" (light) crude oil. That it's thicker it doesn't easily flow out of a well, requiring more work to extract. Typically, this means pumping steam underground to melt the viscous stuff. This costs lots of water and energy to convert that water to steam.



The resource use, water and the energy to make steam, is an environmental impact. According to the Wikipedia, this means "the extraction and refining of heavy oils and oil sands generates as much as three times the total CO2 emissions compared to conventional oil".



ANALYSIS :



Graph 1.1 Relationship Between (a)Water content (b) Specific gravity (c) Feet.

CONCLUSION :

From this ,I have been concluded about the fuel from the degradation of animals with the help of analysis report get the little bit sufficient fuel content of buried human bodies. So I wish to get the fuel from buried human bodies.

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