**COURSE CODE:** MCB 406
**COURSE TITLE:** Petroleum Microbiology
**NUMBER OF UNITS:** **3** Units
**COURSE DURATION:** Three hours per week

## COURSE DETAILS:

**Course Co-ordinator:** Dr **S.**A. Balogun BSc, MSc, PhD(Ibadan)**.**
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**Office Location:** Room COLNAS
**Other lecturers:** Dr O.R. Afolabi and Dr A.K. Akintokun

## COURSE CONTENT:

- **What is Petroleum Microbiology?**
- **Genesis of fossil fuel /Crude oil /Petroleum hydrocarbon**
- **Microbes in oil prospecting**
- **History of Petroleum Exploration in Nigeria**
- **History of Oil spillages in Nigeria and other parts of the world.**
- **Causes of oil spillage**
- **Acid mine drainage**
- **Biodegradation of oil pollutant**
- **Pathway for the oxidation of alkanes**
- **Microbial oxidation of alicyclic hydrocarbon**
- **Microbial oxidation of aromatic ring structure hydrocarbon**

## COURSE REQUIREMENTS:

This is a compulsory course for all Microbiology major students. Prerequisite course is MCB 201. Seventy five percent attendance of courses and practicals are required by students to be eligible to write the final examination.

## READING LIST:

1. Wiley, J.M., Sherwood, L.M. and Woolverton, C.J. 2008. Prescott, Harley and Klein's Microbiology 7th Edition. McGraw-Hill International USA
2. Atlas, R.M. and Bartha, R. 1997. Microbial Ecology: Fundamentals and aplications. Addison Wesley Longman Inc.

# MCB 406 – PETROLEUM MICROBIOLOGY

**Dr. S.A. Balogun*, Dr A.K. Akintokun and Dr. O.R. Afolabi**

## Petroleum Hydrocarbon

Petroleum is a natural product which is a mixture of aliphatic and aromatic hydrocarbons and various heterocyclics. It includes oxygen, nitrogen and sulphur containing compounds. The aliphatic ones include methane, ethane, propane and butane besides longer chain non-gaseous ones. Petroleum hydrocarbon is a natural resource that has been of immense benefit to man in a lot of ways. It is a major source of power at homes, in automobiles and industries. They are feed stocks of the petrochemical and allied industries. Products from these industries include: cosmetics, paints, inks, drugs, fertilizers, electronic casings, among others.

## Origin of Petroleum Hydrocarbon

Petroleum hydrocarbons have been proved to originate from organic matter buried with sediments in sedimentary basins around the world. Petroleum accumulations are found in some folded, porous sedimentary rock strata, such as limestone or in other fractured rocks such as fissured shale. The age of reservoir rocks range from 500 million years to 1 – 13 million years. Very large petroleum reservoirs are found in rock of tertiary age (70 million years).

In offshore deposits, petroleum are mostly derived from plankton debris that was deposited on the floor of depression of shallow seas and ultimately buried under heavy layers of sediment, deposited perhaps by tidal currents over geologic time. The trapped organic matter become converted to petroleum and natural gas. The occurrence of these sediments indicate that most of them originated from organic carbon originally deposited with sediments that have since undergone extensive alteration. The stages of petroleum hydrocarbon formation within the sedimentary basins involve three major stages. The first stage is diagenesis, this involve biological, physical and chemical alteration of organic matter prior to a pronounced effect of temperature. Catagenesis, which is the next stage, is basically the thermal alteration of organic matter while the third stage metamorphism involves high temperature thermal alteration. The temperature ranges for these stages are $50^0 – 60^0C$; 175 – $200^0C$ and above $200^0C$ respectively.

Regionally, the Niger Delta, Nigeria is located on the Gulf of Guinea on the West Coast of Africa. It is built out into the Atlantic Ocean on the discharge mouth of the Niger-Benue river system during the tertiary. An area of catchment that encompasses more than 100,000$km^2$ of mostly savannah-covered lowland. The Delta is among the world largest petroleum provinces and has been rated as the sixth largest oil producer and twelfth giant hydrocarbon province. The Niger Delta covers a sub-aerial portion of about 75,000$km^2$ and extending more than 300km from the apex to the mouth and with a thickness of 12km in the central part.

The oil-producing states in Nigeria are: Akwa-Ibom, Bayelsa, Delta, Edo, Rivers, Ondo, Cross-River and Abia states.

**Tutorial question**

**List some of the oil producing communities within the oil-producing states.**

**The History of Oil Exploitation and Production (E & P) in Nigeria**

Petroleum hydrocarbons have been found seeping into the environments naturally (National Research Council, 1985).The actual Exploration and Production (E and P) of petroleum hydrocarbon did not commence until 1859 in Pennsylvania, USA. Colonel Drake and his men dug the first wild cat well from which crude oil was produced. This marked the beginning of the petroleum industry. The E and P of oil in Nigeria commenced in 1908 when a German firm, Nigeria Bitumen Company commenced operation in the Western flank of the Niger Delta. Exploration activities stopped due to the First World War. Later in 1937, Shell D’Arcy Company (Forerunner of Shell-BP) commenced reconnaissance geological work in a concession covering nearly 1km$^2$ of the Niger Delta basin. Exploration activities also stopped for 6 years as a result of the Second World War. After the period of inactivity, many other exploration companies arrived. The first commercial discovery was made by Shell at Oloibiri in 1956 and the first giant field Bomu, was discovered in 1958.

After Oloibiri and Bomu, Nigeria moved into the exclusive club of the worlds producers of oil. When production reached 6,000bpd in February, 1958, export of Nigerian crude started. At that time most of the oil are found on-shore in areas such as Greater – Port-Harcourt area, Afam, Ebubu, Imo River, Umuechein, Apara and Koroko in Ogoni division, the greater Ughelli area in what is now Delta State and the greater Non-River area in the heart of the delta swamps.

Shell-BP has been actively involved in oil E&P activity in Nigeria for about two decades. She enjoyed the advantage of choosing locations, which had the best geological indications for crude oil formulations. Shell-BP production had averaged 350,000bpd from 135 millions in 15 oil fields in the then Eastern Nigeria. Then she accounts for 85% of the total crude oil production in the whole country.

Apart from Shell-BP, other foreign companies also played pioneering role in the Nigerian oil industry. They include Mobil Producing Nigeria (an affiliate of Socony-Mobil Oil Company) was granted an Exploration License (EPL) covering the whole of the former Northern Region, including parts of the then Northern Cameroons under British adminsitration. In the same year, 1957, the company obtained another EPL covering an area of 4,500 sq miles in Lagos State. As a result of Shell-BP relegation of some area it considered less lucrative, other compares like Tennesse Nig Ltd came in. It obtained prospecting concessions in April, 1960.

**Tutorial question:**

**List 10 oil companies currently operating in the country.**

**Microbes in Oil Prospecting**

The presence of hydrocarbon-utilizing microorganisms has been proposed for prospecting for petroleum. The basis for this approach is the detection of microseepage of petroleum or some of its constituents, especially the volatile components, in the ground overlying a deposit using the presence of the hydrocarbon-utilizing microorganisms as indicators. It involves enriching the soil, sediment and water samples from a suspected seepage area for microbes that can metabolize gaseous hydrocarbons and demonstrating hydrocarbon consumption. The enrichment for these sets of microorganisms will be composed of mineral salts solution with added volatile hydrocarbon (ethane, propane, butane, isobutane).

Methane-oxidizing bacteria are poor indicators in petroleum prospecting because $CH_4$ can occur in the absence of petroleum deposits and also some methane-oxidizing bacteria are unable to oxidize other aliphatic hydrocarbons. Presumptive evidence for a hydrocarbon seepage and petroleum reservoir can be detected by the presence of bacteria that can oxidize ethane and longer chain hydrocarbons.

**Tutorial questions**

**i) Enumerate 5 Methane-oxidizing microorganisms.**

**ii) List 10 hydrocarbon-utilizing microorganisms**

**Environmental Pollution by Petroleum Hydrocarbon**

Pollution has been defined as an undesirable change in physical, chemical and biological characteristics of air, water and land. The sudden input of large amount of hydrocarbon ($HCO_{(s)}$) associated with spillages stresses the environment in a way not imposed by natural hydrocarbons. Oil spillage as it is referred to have deleterious impact on flora, fauna and microbiota of the ecosystem.

The economic life of the populace in the affected area is disrupted and the fragile ecobalance is usually disturbed. Farm lands, navigational activities, availability of clean potable water and fishing resorts are badly affected. Spill incidence remains amongst the various mean by which petroleum HCO pollute the environment. Crude oil spillage can occur at different stages of production and transportation either for export or refining processes. The spillages can be categorized depending on the barrels of oil spilled. It was categorized into (i) Minor (ii) Medium (iii) Major according to the NNPC Inspectorate division.

Minor Impact: < 25 bbl in inland water or 250 bbl on land that does not pose a threat to public health.

Medium Impact: Discharge of oil 25 – 250 bbl on inland water or 250 – 2,500 bbl on land offshore and coastal waters.

Major Impact: Any discharge over > 250 bbl in inland water or > 2,500 on land offshore and coastal water. Any uncontrolled well blow out, pipe rupture or storage tank failure.

**Tanker Wreckages**

It results from the wreck of oil tankers whose sizes and capacity have increased tremendously in recent times with advance in technology. In 1957, a tanker wrecked off Baja, California spilling about 9,000 tonnes of diesel fuel containing a high proportion of Aromatics. Argea Prima in July, 1962 spilled about 5,000 tonnes of oil in the port of Guatemalla, Puerto Rico.

**Well blow out**

Well blow out does not occur frequently, however it results in the largest volume loss per time if not brought under control on time. The Ixtoc oil well blow out in 1979 remain a case study in history with about 500,000 tonnes of oil loss. Ekofisk, in the North Sea recorded 200,000 tonnes of oil losses. In Nigeria, 400,000 tonnes of oil was spilled in the 'Funiwa 5' oil well blow out of 1980. Also, Shell 2, Sefrap (Elf), and Obagi 21 blow out of 1972 respectively.

**Causes of Oil Pollution incidence in Nigeria**

Causes of oil pollution incidences in Nigeria can be attributed to:

i. Burst/Rupture/Corrosion of flow-line/Pipelines

ii. Over pressure failure/Overflow of process equipment components.

iii. Sabotage of well heads and flowlines.

iv. Hose failure during tanker loading.

v. Failure along pump discharge manifolds.

vi. Blow outs.

vii. Sabotage

viii. Illegal bunkering

**Oil spills incidences in Nigeria (1976 – 1986)**

| Year | Net Vol. Spilled (BBLS) |
|---|---|
| 1976 | 26,157 |
| 1977 | 32,879 |
| 1978 | 489,294 |
| 1979 | 694,117 |
| 1980 | 600,511 |
| 1981 | 42,723 |
| 1982 | 42,841 |
| 1983 | 48,351 |
| 1984 | 40,209 |
| 1985 | 11,876 |
| 1986 | 2,038,710 |

Selected oil spill incidences around the world

| Vessel | Date | Quantity spilled (tonnes) | Location |
|---|---|---|---|
| Arrow | 1970 | 12,000 | Canada |
| Bohlen | 1976 | 10,000 | Brittany |
| Chryss | 1970 | 31,000 | N.E Bernuda |
| Ennerdole | 1971 | 42,000 | Seychelles Island |
| Gino | 1979 | 40,000 | Brittany |
| Poly Commander | 1970 | 13,000 | Spain |
| Urquiola | 1976 | 107,000 | Spain |
| Amoco Cadiz | 1978 | 230,000 | Brittany |

**Effect of Oil pollution**

**Marine ecosystem** Is the ultimate recipient of surface run-off from soil and rivers which finally go into lagoons. Devastating oil spills occur more often in the oceans. e.g Gulf of Mexico. The response of microbes to spillages in various ecosystems varies but usually there is increase in number of hydrocarbon degraders. Algal diversity and consumption will shift as a result of pollution. The species diversity in the coral reefs is badly affected. Invertebrates such as zooplanktons, crustaceans as well as vertebrates are adversely affected. The larval stages of shrimps are very susceptible to oil spill. Studies on the toxicity of oil to mangroves reveals that *Avicenia* sp will die within a week while it is mild on *Rhizophora* sp.

**Freshwater ecosystem**

It has been reported that there are drastic changes in the levels of microbial populations and the ability of indigenous microorganisms to degrade the hydrocarbon spilled/polluting the environment. Nitrogen fixation is reduced to about 8% after exposure to HCO. It has been reported, that some hydrocarbons not originally present in the crude were found present, after exposure to the estuarine community in the water body.

**Soil ecosystem**

Contamination of the terrestrial ecosystem affects not only the microbiota of the soil but also the resident microcommunity. It has negative effects on the pH community both by contact toxicity and indirect deleterious effects. Among the various effects are oxygen deprivation of roots and generation of phytotoxic compounds such as hydrogen sulphides. Herbaceous vegetation is quickly killed.

**Tutorial question**

**Highlight the impact of oil spillage or pollution on the Niger Delta communities.**

**Major Pathways of Petroleum hydrocarbon degradation**

Susceptibility to degradation varies with the type and size of the hydrocarbon molecule. N-alkane of intermediate chain length ($C_{10}$ – $C_{24}$) is degraded most rapidly. Short chains are toxic to many microorganisms, but generally evaporate from oil slick, rapidly. Very long chain alkanes become increasingly resistant to biodegradation. As the chain length increases and the alkanes exceed a molecular weight of 500, the alkanes do not serve as carbon sources. Branching, in general reduces the rate of biodegradation.

Aromatic compounds, especially of the condensed polynuclear type are degraded more slowly then Alkanes. Alicyclic compounds are frequently unable to serve as the sole carbon source for microbial growth unless they have a sufficiently long aliphatic side chain, but they can be degraded via cometabolism by two or more co-operating microbial strains with complementary metabolic capabilities.

**List of some hydrocarbon-utilizing microorganisms**

*Pseudomonas aeruginosa*
*P. fluoresens*
*P. putida*
*Alcanivorax borkumensis*
*Corynebacterium*
*Micrococcus*
*Serratia marscense*
*Norcadia sp*
*Mycobacterium*
*Rhodococcus erythropolis*
*Saccharomyces sp*
*Rhodotorula sp*

**Alkanes**

The preliminary attack on alkanes occurs by enzymes that have a strict requirement for molecular oxygen, that is monooxygenases or dioxygenases. One atom of $0_2$ is incorporated into the alkane, yielding a primary alcohol.

**Pathway of degradation of Aliphatic (straight chain) hydrocarbons by Microorganisms**

1. (a) $R-CH_2-CH_3+O_2+NADPH \rightarrow R-CH_2-CH_2-OH+NAD+H_2O$

   OR

   (b) $R-CH_2-CH_3+O_2 \rightarrow R-CH_2CH_2-OOH$ (Diterminal oxidation)

2. $R-CH_2-COOH-OOH+NADPH_2 \rightarrow R-CH_2-CH_2-OH+NADP+H_20$

3. $R-CH_2-CH_2-CH_2-COOH \rightarrow C_0A$

4. $R-CH_2-CH_2-CH_2-\overset{\overset{O}{\parallel}}{C}-CoA \rightarrow -2H^+$

5. $R-CH_2-CH=CH-\overset{\overset{O}{\parallel}}{C}-CoA \rightarrow H_2O$

6. $R-CH_2-\underset{\underset{OH}{|}}{C}H-CH_2-\overset{\overset{O}{\parallel}}{C}-CoA \rightarrow +2H^+$

7. $R-CH_2-\underset{\underset{O}{\parallel}}{C}-CH_2-\overset{\overset{O}{\parallel}}{C}-CoA \rightarrow +C_OA$

8. $R-CH_2-\underset{\underset{O}{\parallel}}{C}-C_OA+CH_3-\overset{\overset{0}{\parallel}}{C}-CoA$

9. $R-CH_2-CH_3 \rightarrow -2H^+$

10. $R-CH=CH_2 \rightarrow +H_2O$

11. $R-\underset{\underset{OH}{|}}{C}H-CH_2 \rightarrow -2H^+$

12. $R-CH_2-CHO \rightarrow 2H^+,+H_2O$

13. $R-CH_2-COOH$

Though not in all cases, the initial attack is targeted at the **terminal – $CH_3$** (Methyl group) thereby forming a **primary alcohol**. This is in turn oxidized to an aldehyde and fatty acid. Occasionally both terminal-$CH_3$ are oxidized in this manner, resulting in the formation of a **dicarboxylic acid**. This variation is referred to as **diterminal or ω-oxidation**. This is one of the several ways to bypass a block to β-oxidation due to branding of the carbon chain. Once a fatty acid is formed, further catabolism occurs by the **β-oxidation sequence**. The long-chain fatty acid is converted to its acyl coenzyme-A form and is acted upon by a series of enzymes with result that an acetyl CoA group is cleared off and the fatty acid is shortened by a two-carbon unit. This sequence is then repeated. The acetyl CoA units are converted to $CO_2$ through the TCA cycle. Thus the end products of hydrocarbon mineralization are $CO_2$ and $H_2O$.

**Microbial Desulfurization**

Sulfur is usually the third most abundant element in crude oil, normally accounting for 0.05 to 5%, but up to 14% in heavier oils. Most of the sulfur in crude oil is organically bound, mainly in the form of condensed thiophenes, and refiners use expensive physicochemical methods, including hydrodesulfurization to remove sulfur from crude oil. These high costs are driving the search for more efficient desulfurization methods, including biodesulfurization. In developing a lower cost biologically based desulfurization alternative, promoting selective metabolism of the sulfur component (attacking the C-S bonds) without simultaneously degrading the nonsulfur (C-C bonds) fuel components in organic sulfur will be the most important consideration.

Aerobically grown strains, such as *Rhodococcus erythropolis* and related species, remove the sulfur from compounds such as dibenzothiophene (DBT) without degrading the carbon ring structure. These strains can use sulfur from DBT as a sole source of sulfur, which facilitates a strategy for isolation of desulfurizing organisms. Other aerobic selective desulfurizing microbes include *Nocardia* spp., *Agrobacterium* sp. Strain MC501, *Mycobacterium* spp. *Gordona* sp. Strain CYKS1, *Klebsiella* spp, *Xanthomonas* spp, and the thermophile *Paenibacillus* .

*Rhodococcus* sp. strain was isolated from a mixed culture obtained from a sulfur-limited continous-culture system capable of using organically bound sulfur. Strain IGTS8 converts DBT to dibenzothiophene-5-oxide (DBTO), then to dibenzene-5,5-dioxide (DBTO2), then to 2-(2-hydroxybiphenyl)-benzenesulfinate (HPBS), and finally to 2-hydroxybiphenyl (HBP) to release inorganic sulfur in a pathway involving two monooxygenases and a desulfinase. This enzyme system also transforms alkyl- and arylsubstituted DBT. Since the HBP product partitions into the oil phase, its fuel value is not lost. The flammability and explosive risks from the above oxygen-requiring process have led to consideration of cloning the desulfurization genes into anaerobic hosts, which would hyperproduce the enzymes for addition to the crude oil. Desulfurization rates for non-engineered *Rhodococcus* spp. are 1 to 5 mg of HBP per g of dry cells per h, with 55 to 75% of the DBT being released as HBP.