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Review Article

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Role of bacterial community in biodegradation of crude oil

Hina Upadhyay[1] and R. Sharma[2*]

[1]Department of Agriculture, Lovely Professional University, Phagwara-144411
[2]Department of Physics, Lovely Professional University, Phagwara-144411

ABSTRACT

Crude oil being a a dark sticky liquid is heterogeneous mixture of varying molecular weight from which various petroleum products are extracted. There are various technological processes which are used for refining it into consumer products like paraffin oils, gasoline, lubricants, vaseline, asphalt, domestic fuel oil and polymers. The biological, chemical, and geochemical transformations are responsible for formation of crude oil. Several microorganisms are associated with the crude oil formation. The type of substrate and microorganism(ie aerobic or anaerobic) are major parameters responsible for degradation of different petroleum products. For these microorganisms the main source of carbon in crude oil are hydrocarbons which are both aliphatic and aromatic, but also include organic compounds that are mostly the products of crude oil biodegradation. These micro organisms find application in enhancement of oil production using microbiological methods. The present review highlights the biodegradation of crude oil by bacterial community.

Keywords: Crude oil, microorganism, biodegradation, hydrocarbon.

INTRODUCTION

The German mineralogist Georg Bauer, in the year 1546 has used the term The word "Petroleum" which is a synonym to crude oil which was first used in the treatise "De Natura Fossilium" published in 1546[1]. Crude oil or petroleum is a natural environmental resource which is flammable liquid that occur as rock formation in the earth. It is a mixture of inorganic and organic compounds which include aliphatic and aromatic hydrocarbons. The non-hydrocarbon compounds including sulphur compounds (0.01–8 %), mostly as hydrogen sulfide (H_2S), mercaptans (compounds containing the –SH group), thiophenes, sulfides and disulfides as well as naphthothiophenes and benzothiophenes that exists in oil fractions . As these compounds are inauspicious due to their chemical recalcitrance, so their presence is taken into consideration for evaluating crude oil quality (Surygała, 2001)[2].

Microbial interaction with hydrocarbons has attracted the attention of several groups of workers looking for desirable as well as detrimental activities in the biodegradation of hydrocarbons.

Widdel and Rabus (2001) reported that the formation of crude oil includes the chemical, biological and geochemical transformations of organic matter, deposited in favorable locations. Geochemical studies proves that higher volume of hydrocarbon is present in immature form in crude oil with an odd number of carbon atoms, synthesized in plants [3].

According to the studies conducted by Surygała, 2001, the organic origin of crude oil is supplied by biomarkers, i.e. compounds whose carbon skeleton remain unchanged in geochemical processes which is formed by living organisms, e.g microorganisms like metalloporphyrines, terpenes and porphyrines [2].

The crude oil being a natural resource, but in few conditions its presence is unfavorable and causes destruction of the surroundings. The various autochthonous microorganisms are associated with the settings where crude oil and formation water in oil reservoirs with an extreme environment are present. The relationship between microorganisms and this extreme environment begins when crude oil is formed and it ends when these specialized microorganisms are applied for the bioremediation of the polluted environment by crude oil and oil-derived products.

Gałuszka and Migaszewski,(2007) has reported chemical structure of the most hydrocarbon existing in crude oil is responsible for their toxic effect. Both aliphatic and aromatic compounds, like polycyclic aromatic hydrocarbons (PAHs), come under these toxic hydrocarbons, whose toxicity increases in proportion to the number of carbon atoms present in the compound [4].

According to Dandie et al. 2004, the main sources of carbon for these microorganisms in crude oil are hydrocarbon which are both aliphatic and aromatic as well as organic compounds that are mostly the products of crude oil biodegradation. These organic compounds include: organic acids like , benzoic, butyric, acetic, formic, propanoic, and naphthenic acids reaching up to 100 mM. The electron donors may be H_2, and in the case of immature oil – asphalthenes and resins, whose metabolic availability is confirmed by the fact that anaerobic micro organisms may develop in cultures with crude oil without any modifications of its composition. There is sufficient evidence that shows the role of number of microbial community in crude oil formation [5].

Types of microorganisms

1) ***Fermentation bacteria***

Till date various species of fermentation bacteria are found in crude oil as reported by Nazina et. al, (2007).The bacterial strains which are competent for reduction of thiosulfate (S_2O_3) and elemental sulfur (S^o) are also discovered. These microorganisms posses electron donors like proteins, hydrogen, carbon dioxide, sugars and hydrocarbons. The products of metabolic reactions like organic acids and gases, eg H_2 and CO_2 , may increase the reservoir pressure. These microorganisms find application in the enhancement of oil production using microbiological method[6].

The thermophilic fermentation bacteria are more abundant than mesophilic ones as reported by Nazina *et al*. (2007).

[6]. During carbohydrate fermentation, acetate or ethanol are produced by the activity of first group of haloanaerobes like *Haloanaerobium acetoethylicum*, *H. congolense*, and *H. salsugo*. The parameters like type of substrates used and their tolerance to salt content (up to 10 %) mainly marked the difference among these microorganisms.As an example, *Spirochaeta smaragdinae* isolated from a Congo oil field prefers salt contents of up to to 5 %. *Dethiosulfovibrio peptidovarans* with specific metabolism is also isolated from the same source. These bacteria are also responsible for biodegradation of protein extracts and the organic acids like acetic, isobutyric, isovaleric, and 2-methylbutyric acids are produced as a result of their metabolism [6].

Magot *et al*. (2000) has reported that in crude oil reservoir, their exists various group of microorganisms along with water. For example, the formation water of the Tatarstan and western Siberia reservoirs existence of *Acetoanaerobium romaskovii* which uses amino acids, acetates, hydrogen, carbondioxide and sugars as sources of energy and carbon are reported. Thermostable enzymes that are present in thermophilic micro organisms like *Thermotoga: T. elfii*, *T. sub terranean* and *T. hypogea* are capable of resisting temperatures exceeding even 100^o C and posses ability of reducing thiosulfate to sulfides. The bacteria similar to *Thermotoga* can reduce elemental sulfur and exists at low salt content of 2.4% of NaCl and during glucose degradation, they even can produce acetic acid and L-alanine [7].

2) Sulfate reducing bacteria (SRB)

According to report of Rabus et al., (2000), one among the primitive microorganism on earth belongs to the group of sulfate reducing bacteria (SRB). As per their history of development and activity, they resemble the organism of the

Proterozoic Era. The first study was conducted in the year 1864 on the biology and metabolism of these microorganisms [8].

Voordouw, (1992) has reported that the metabolic pathway of dissimilation of sulfate reduction is one that did not show horizontal gene transfer and mutations [9]. According to the report of Baker *et al*.,(2003) there are evidence that proof that the gene coding the enzyme catalyzing the first stage of dissimilative reduction is conserved evolutionarily and remains unchanged from the date of its formation [10]. The sulfur isotopic studies done by Kopp *et al*., (2005) reveals that bacterial sulfate reduction would have developed earlier than oxygen photosynthesis [11].

The biogenic origin of hydrogen sulfides in marine sediments was first observed by Meyer (1864) and Cohn (1867)[12,13]. Acoording to Bastin (1926) , the SRB exists in the area of crude oil exploitation and first sporing thermophilic SRB was reported. These reports, however shows the role of microorganisms in the corrosion of drilling equipment. For understanding the metabolic processes conducted by SRB, first attempts were reported in the year 1950s and 1960s [14].

According to Postgate, (1984) and Gibson, (1990) SRB are heterotrophic organisms and absolute anaerobes that use sulfates as well as other oxygenated sulfur compounds (sulfites, thiosulfites, trithionate, tetrathionate, and elemental sulfur) as final electron acceptors in respiration processes [15,16]. Except the species of *Desulfonema*, all SRB are gram negative. This group of bacteria exhibits diversity and various types of bacteria based on the soil and water composition, can be found within this group such as psychro, meso, thermophilic, halo and barophilic. The spore developing ability is possessed by few species of SRB like *Desulfosporosinus orientis* , *Desulfotomaculum halophilum* sp. Nov [17] and *Desulfosporosinus meridiei* sp. nov. [18] The activity of these microorganisms decreases the permeability of reservoir rocks caused by the precipitation of insoluble sulfides, as well as carbonates as reported by Magot et al., (2000) and Nemati et al., (2001). Postgate has shown that SRB always accompany crude oil and therefore for long time were considered as indicator organisms when searching for new reservoirs [15].

3. Methanogenic archaea

Nazina *et al*., (2007) has reported the existence of next important group in crude oil reservoir ie. Methanogenic archaea bacteria. Methane is produced due to their activity which in turn is measured by their production rate [6]. These bacteria are found in diverse settings. The chemical and physical factors like pH, salt content and temperature effect their development and activity. However, methanogenes are mostly mesophilic, but some extremophiles are also found like M*ethanopyrus kandleri*, occurring at 110^o C [19]. However few reports are available in literature on psychrophilic methanogenic archaea. The salt content of environment and anaerobic conditions are two major parameter necessary for the activity and development of methanogenic archaea. Elias et al., (1999) has reported that at very low concentrations of oxygen (even in several ppm range), they are very sensitive [20].

According to Zeikus and Wolfe, (1972), prototrophic species of methanogenic bacteria require only mineral salts, hydrogen and carbon dioxide for their growth. *Methanobacterium thermoautotrophicum* of this group is one among them. Most methanogenes usually needs electron donor as H_2 and CO_2 as their electron acceptor. Methane is produced as the final product in this process. The methanogenesis is the process of formation of biogenic methane, which is a type of anaerobic respiration with low energy yield. At very low reduction potential and anaerobic environment, these methanogenic archaea degrade organic matter in their final stage [21].

The varying salt content are favourable for occurrence of mesophilic and thermophilic methanogenic archaea If high temperatures and salt concentrations are present simultaneously, they may restrict the microbial activity of methanogenic archaea. Among methanogenic caryopsis, *Methanococcoides (Methanohalophilus) euhalobius* was detected which uses methylamines as found in samples of oil well during their drilling.

The hydrogen oxidizing species of methanogenic archaea, are most prevailing group in formation water of low salt content. The disc-shaped *Methanococcus termolitotrophicus*, *Methanoplanus petrolearius*, and *Methanocalculus halotolerans*; and the rod-like *Methanobacterium thermoautotrophicum*, *M. bryantii*, and *M. ivanovii* are included in the group. According to Ollivier et al., (1998), during methanogenesis the oxidation of hydrogen may take place below 9 percent NaCl concentrations. As an example, *Methanocalculus halotolerans can* carry out this metabolic activity up to 12 percent salt content [22].

4. Iron III reducing bacteria

Grenne *et al*., (1997) has reported that the iron reducing bacteria like *Shewanella putrefaciens* posses potential for reducing elemental sulfur, sulfites, and thiosulfates to sulfides. This bacteria can survive in the adverse environment of oil wells.Hydrogen or formate may act as electron donor and iron oxide and hydroxide as acceptors. A bacterium named *Deferribacter thermophilus* that not only reduces iron but also capable of reducing nitrates and manganese using peptone or yeast extract.The hydrogen and numerous organic acids serves as source of energy. Due to lack of information regarding iron and manganese content, their exist doubt on occurrence of such type of metabolism in such type of conditions [23].

CONCLUSION

Biodegraded oil resulting from the action of microorganisms that destroy hydrocarbons and other oil components have been a problem for the petroleum industry. So, this paper highlights an overview of the presence as well as the involvement of microbial community in degradation of hydrocarbon and formation of crude oil in such type of environment. These microorganisms posses potential for degrading hydrocarbons and include a wide distribution of bacteria for example fermentation bacteria, sulphate reducing bacteria (SRB), methanogenic archea and iron (III) reducing bacteria respectively. The concentration of these microorganisms is affected by physical, chemical and biological factors. Stetter & Huber, (1999) reported that the temperature is one of the important physical factor affecting microorganism activity [24]. Magot et al., (2000) has reported that at temperatures above 130^o to 150^o C, the living organisms does not survive theoretically as biological compounds become unstable. These conditions prevail at the depth from 4030 to 4700 m in deep oil reservoirs. We thus conclude that bacteria are the most active agents that play a key role in the formation of crude oil through microbial degradation [7].

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