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REVIEW PAPER

Biodeterioration of crude oil and oil derived products: a review

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Abstract Biodeterioration of crude oil and oil fuels is a serious economic and an environmental problem all over the world. It is impossible to prevent penetration of microorganisms in oil and fuels both stored in tanks or in oilfields after drilling. Both aerobic and anaerobic microorganisms tend to colonise oil pipelines and oil and fuel storage installations. Complex microbial communities consisting of both hydrocarbon oxidizing microorganisms and bacteria using the metabolites of the former form an ecological niche where they thrive. The accumulation of water at the bottom of storage tanks and in oil pipelines is a primary prerequisite for development of microorganisms in fuels and oil and their subsequent biological fouling. Ability of microorganisms to grow both in a water phase and on inter-phase of water/hydrocarbon as well as the generation of products of their metabolism worsen the physical and chemical properties of oils and fuels. This activity also increases the amount of suspended solids, leads to the formation of slimes and creates a variety of operational problems. Nowadays various test-systems are utilized for microbial monitoring in crude oils and fuels; thus allowing an express determination of both the species and the quantities of microorganisms present. To suppress microbial growth in oils and fuels, both physico-mechanical and chemical methods are applied. Among chemical methods, the preference is given to substances such as biocides, additives, the anti-freezing agents etc that do not deteriorate the quality of oil and fuels and are environmentally friendly. This review is devoted to the analysis of the present knowledge in the field of microbial fouling of crude oils and oil products. The methods utilized for monitoring of microbial contamination and prevention of their undesirable activities are also evaluated. The special focus is given to Russian scientific literature devoted to crude oil and oil products biodeterioration.

Keywords Biodeterioration · Biofouling · Crude oil · Oil derived products · Microbial contamination control · Biocide

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1 Introduction

Problems associated with the biodeterioration of crude oil and oil derived products have been of

immense interest to experts and scientists for a long time. Most of the research devoted to this phenomenon was carried out between the 50's and the 70's of the last century (Birshtekher 1957; Murzayev 1964; Hill 1967; Rozanova 1967; Vishnyakova et al. 1970; Odier 1976), when the comprehension of the dangers associated with this microbial activity was realized. However, the problem of hydrocarbon (HC) material biofouling is an urgent issue at the present time as well (Yang et al. 1992; Ferrari et al. 1998; Gaylarde et al. 1999; Chesneau 2000; Wilhelms et al. 2001; Watanabe et al. 2002; Roling et al. 2003; Allsopp et al. 2004), as it affects various aspects of society.

Crude oil deterioration was found, for example, upon its extraction by the flooding method. Moreover, the majority of applied microbiological methods of enhanced oil recovery also deteriorates oil and appears to be a source of microorganisms in natural reservoirs and oil pipelines (Vishnyakova et al. 1970). Interestingly, almost the same microorganisms are responsible for oil deterioration in natural reservoirs, storage tanks, oil pipelines, industrial systems of water cooling, systems of water preparation for pumping into oil fields as well as in the processes related to the biocorrosion of metal pipes and cement constructions (Chesneau 2000; Watanabe et al. 2002; Muthukumar et al. 2003). A long-term storage of oils and oil products in industrial tanks for strategic purposes still leads to its deterioration despite efforts such as the application of biocides undertaken to solve this problem (Vishnyakova et al. 1970; Chesneau 2000). Microbiological contamination of aviation fuel is a major concern as the deterioration of kerosene and rocket fuels often lead to accidents (Yang et al. 1992; Ferrari et al. 1998; Chesneau 2000).

Applications of various chemical compounds for crude oil and oil products disinfection often resulted in pollution of the environment due to the slow decomposition of these xenobiotics, many of which possess mutagenic and carcinogenic properties (Yang et al. 1992; Ferrari et al. 1998; Zhiglecova et al. 2000). Fifty years ago, methods for the determination of microbiological contamination of oil and oil products as well as monitoring of its disinfection were expensive and time-consuming. At the present time, methods and the test kits allowing a quick and reliable determination of microbial infection in fuels and crude oil are being developed. The identification and application of the most effective biocides and inhibitors of oil fouling are also being pursued (Bailey and May 1979; Girotti and Zanetti 1998; Gaylarde et al. 1999; Frundzhan and Ugarova 2000; Efremenko et al. 2002; Frundzhan et al. 2002; Bonch-Osmolovskaya et al. 2003).

The petroleum production and refining industry is one of the major industries in Russia, which is the third largest crude oil producer in the world. Therefore substantial efforts were and being made to solve specific problem such as microbial contamination of stored crude oil and petroleum products. The Russian scientists and engineers made a noticeable contribution to general knowledge about petroleum microbiology (Rozanova 1967, 1971; Kopteva et al. 2001; Miroshnichenko et al. 2001; Nazina et al. 2001; Zvyagintseva et al. 2001; Tarasov et al. 2002; Bonch-Osmolovskaya et al. 2003; Murygina et al. 2005) as well as to methods of monitoring and mitigation/suppression of petroleum contamination with microorganisms (Vishnyakova et al. 1970; Anderson and Effendizade 1989; Kuznetsov et al. 1997; Frundzhan et al. 1999; Nagornov et al. 2001; Gilvanova and Usanov 2003; Efremenko et al. 2005; Sirotkin et al. 2005).

The present review is devoted to the analysis of the current knowledge in the field of microbiological fouling of crude oil and petroleum products with focusing on the Russian literature that, due to language problems, is ordinarily fairly difficult to access for the most non-Russian reading scientists. The methods applied for detecting, monitoring, and preventing/suppressing the microbial contamination of these HCs are also evaluated.

2 Consequences of crude oil and petroleum products microbial contamination

Crude oil represents a mixture of a large variety (thousands) of organic substances, mainly HCs, with some admixture of oxygen-, nitrogen-, sulphur-containing organic compounds and some inorganic species (metals etc). HCs can be

straight and branched, saturated and unsaturated aliphatic, alicyclic, aromatic and polyaromatic compounds. Oil occurs in various beds in the layers often in association with water, and saturation of oil by macro- and microelements depends on the composition of these beds. It is postulated that water may range from 5 up to 20% and thus a significant amount of salts is also presented in crude oil. Generally, oil frequently comprises almost all the Mendeleyev periodical table of elements and often possesses radioactive contaminants (Allsopp et al. 2004). Usually the native oil occurs at depth of 2–3 and more thousands meters under the ground with temperatures of 60–90°C and above, and it is considered to be sterile, i.e., not contaminated by microorganisms (Wilhelms et al. 2001). However, as soon as the oil extraction starts and the layer is opened, microorganisms capable of oxidizing HCs and to use them as a source of carbon and energy start to grow.

When the self-flowing oil recovery stops, water flooding of a stratum is applied as an additional method of oil extraction. During such water flooding process, preliminary disinfection of water is usually not applied. For example, in Western Siberia, the major Russian oil extraction region, raw water from nearby streams, bogs and small rivers containing various microorganisms is usually used. Similarly, in the Pre-Caspian area, seawater with all microorganisms flourishing there is applied for water flooding. Thus, various microorganisms (mesophilic and thermophilic, aerobic, microaerophilic and anaerobic) find their way to the oil layer with the added water (Stuart 1994–1995). With regular water pumping into an oil layer, the temperature gradually decreases, especially in the working area of that layer. A cenosis of various microorganisms is formed where some species can oxidize HCs, while the others use oxidation products formed, thus finally transforming the HCs into water, carbon dioxide, methane, hydrogen sulphide, pitches and pyrobitumens. By this means, oil occurring in layers changes its initial structure and quality, i.e., becomes aged. It has been established that, from total oil losses due to microbiological deterioration, 12% is lost during extraction, 50%—during transportation and 38%—at oil refineries before its processing, i.e., during storage (Fukui et al. 1999).

The problem of microbiological ageing of crude oil and oil products in long-term storage industrial tanks is especially relevant because of sizeable allocation of reserves for strategic purposes (Loren et al. 2001). In large oil storage tanks, processes of oil degradation proceed even more intensively due to upper inflow of oxygen into the tanks and the presence of a water pillow at the bottom of these tanks. Crude oil or oil products can be classified as slightly or highly contaminated by the number of microorganisms presented in the water bottoms. About 10^5 bacteria/ml and 10^3–10^4 fungi/ml are characteristic for slight contamination whereas 10^6–10^8 bacteria/ml and 10^4–10^6 fungi/ml—for high contamination (Allsopp et al. 2004). The oil product considered as clean one usually contains less than 50 organisms per ml of product, meantime, the low quantities of associated water may carry high concentration of bacteria—till 10^4 organisms/ml water (Allsopp et al. 2004).

During the manufacture of oil products, crude oil is exposed to thermal processing and products generated remain sterile. However, they loose sterility during warehousing and storage. For example, after oil refining, 3.2×10^4 cells/ml were found in oil products after pumping them to a factory tank, 7×10^4 cells/ml were encountered in oil products on a petroleum storage depot and 2.8×10^5 cells/ml were detected in oil products in distribution oil depot (Vishnyakova et al. 1970).

Similar measurements of the distribution of microorganisms in a tank with diesel fuel (Fig. 1) demonstrated that, at the bottom of the tank (in a water pillow), 8.2×10^7 cells/ml were found. On a level of an input–output of diesel fuel (above a water pillow), the microbial concentration was the highest (1.9×10^8 cells/ml). On a level of the bottom third of tank, the number of microbial cells decreased to 5.3×10^5 cells/ml. In the middle of the tank, only 117 cells/ml were found; whereas viable cells were not detected at all in the top part of the tank (Vishnyakova et al. 1970).

One of the enhancing factors of deterioration of crude oil and its products is the occurrence of microbial corrosion of pipelines and tanks caused by complex action of various bacteria inside the

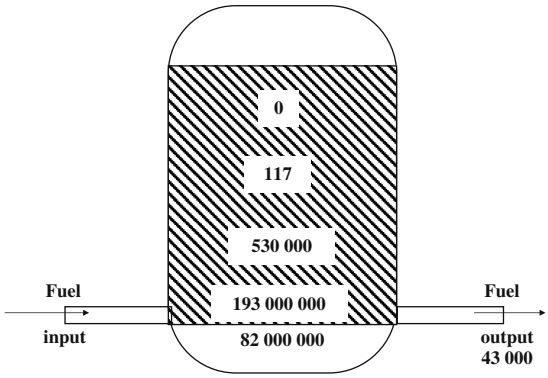


Fig. 1 Distribution of microorganisms in the diesel fuel tank (concentration per 1 ml), (Vishnyakova et al. 1970)

biofilm structure formed on the surface of the metals and concrete (Costerton and Lashen 1984; Chesneau 2000; Muthukumar 2003). It is believed that bacteria involved in a biological cycle of sulphur, especially sulphate-reducing bacteria (SRB), play a pivotal role in biocorrosion. As a result, the quality of crude oil decreases due to generation of hydrogen sulphide by SRB. The H_2S is known to deactivate the catalysts which are further used in the manufacture of oil products.

The presence of bacteria and fungi in fuel storage systems increases the content of water in fuels due to microbial degradation of HCs and other organic compounds. It should be also noted that microorganisms often excrete surfactants leading to fuel emulsification, and thus the probability of microbial penetration into a hydrophobic phase of fuels increases (Waires et al. 2001; Allsopp et al. 2004). The activity of aerobic bacteria and fungi leads to the formation of peroxides, pitches and acids in the fuels, as well as to an increase in viscosity and to a decrease of thermal stability and volatility of fuels (Vishnyakova et al. 1970; Chung et al. 2000; Chesneau 2000). Moreover, useful oil additives can be a frequent target of degrading activities of various microorganisms (Gaylarde et al. 1999; Allsopp et al. 2004; Lopes Ferreira et al. 2006). In addition, activity of microorganisms promotes an increase of suspended solids content in fuels in the form of sludge, corrosion debris and metal particles of pipelines and components of filters, such as glass fibre, paper or clap. Even very small quantities of solid particles (1 mg of particles in 100 ml of fuel) are sufficient to cause filtration problems (Gaylarde et al. 1999; Chung et al. 2000).

Consequently, crude oil and its manufactured products, imminently containing nutrients, represent a favourable environment for growth of various microorganisms. The latter not only consume HCs but also worsen operational and physico-chemical properties of petroleum products leading to breakages of the equipment and even accidents.

3 Factors influencing crude oil and oil products biodeterioration

The composition of crude oil and oil products, the presence of accessible forms of nitrogen, phosphorus, potassium, magnesium, other microelements and water as well as other environmental conditions such as temperature, pH and oxygen modulate microbial growth and thus biofouling processes. As a general rule, all the listed chemical species are present either in the oil or in the accompanying water phase including the water dissolved in the oil (Stuart 1994–1995; Gaylarde et al. 1999; Chesneau 2000; Allsopp et al. 2004; Olliver and Magot 2005).

3.1 Temperature and pH

Microorganisms promoting fouling of oil can live in a wide range of temperatures—from 4 up to 60°C and above (Chung et al. 2000), at pH value from 4 up to 9, however, they tend to prefer a neutral pH (Boszczyk-Maleszak et al. 2006). The species variety of HC-oxidizing (HCO) microorganisms is highest at temperatures between 25°C and 30°C (Stuart 1994–1995; Olliver and Magot 2005).

3.2 Water content

Microorganisms are capable of surviving at elevated temperatures and in presence of toxic substances, but are unable to live without water (Bailey and May 1979; Yang et al. 1992; Stuart 1994–1995; Gaylarde et al. 1999; Chesneau 2000). It is well known that 1% water is enough for

substantial microbial growth (Gaylarde et al. 1999; Allsopp et al. 2004; Olliver and Magot 2005), whereas spores of microorganisms can survive in the presence of 5–80 ppm of water in fuel system (Yang et al. 1992). It is interesting, that for a 1 μm size microorganism, 1 mm layer of water is comparable to a man standing next to 500 m of water (Chesneau 2000). Therefore, a fine film of water on tank surface is enough to allow microorganisms to start growing, and the cell metabolism, ones begun, causes accumulation of more water. Thus, the important factor limiting growth of microorganisms in oil is the availability of water. For example, anti-ice fuel additives such as glycols reduce the availability of water and thus inhibit growth of microorganisms (Neihof and Bailey 1978; Stuart 1994–1995).

Water penetrates into fuel systems with moist air condensing on cold metal and also with watered fuel, when water is pumped as ballast into ships (Bailey and May 1979; Gaylarde et al. 1999; Chesneau 2000; Chung 2000; Gardner & Stewart 2002). Water is heavier than HC-fuel and consequently it accumulates at the bottom where the biphasic system ''oil-water'' supports a growth of microorganisms which can use oil as a carbon source (Gaylarde et al. 1999; Chesneau 2000, Muthukumar 2003; Allsopp et al. 2004). In addition to the acceleration of oil biofouling, water, present in fuels, reduces their viscosity and renders pumps ineffective. Generally, it is extremely difficult to avoid the occurrence of water in tanks, as it is impossible to avoid the condensation phenomenon under conditions of changing temperatures.

3.3 Oxygen

Oxygen penetrates in storage tanks during fuel filling, ventilation of tanks, purification and processing of HC raw material. Oxygen can (photo)chemically reacts with HCs of oil and oil products with formation of coloured particles, pitches and water. Moreover, oxygen being a terminal electron acceptor for aerobic microorganisms directly contributes to microbial growth. A variety of microorganisms carrying out the decomposition of HCs, the formation of slime, biofilms and insoluble particles, are aerobic (Bailey and May 1979; Yang et al. 1992; Gaylarde et al. 1999; Waites et al. 2001). The rate of microbial HC oxidation obviously increases with an increase of aeration but, even at concentration of oxygen as low as 0.1 mg/l, conversion of HC still occurs (Chesneau 2000; Watanabe et al. 2002). Moreover, if it would be possible to create completely anaerobic storage conditions, oil and oil products are not protected against microbial degradation since many facultative aerobic and anaerobic microorganisms continue to thrive (Gaylarde et al. 1999; Watanabe et al. 2002; Olliver and Magot 2005). For example, during the storage of crude oil, SRB consuming HC and using available sulphate as electron acceptor actively grows there (Gaylarde et al. 1999).

3.4 Nutrients

The limiting factor of microbial growth is also an availability of mineral nutrients, for example, phosphates, which are usually present in fuels in concentrations as low as 1 mg/l (Gaylarde et al. 1999). On the contrary, significant growth of microorganisms has been observed in systems containing solution of mineral salts, for example, mineralized flooding water to enhance oil extraction or seawater which is pumped into tankers as a ballast (Stuart 1994–1995). The especial danger of these waters is related to abundant presence of sulphate which triggers the growth of SRB enhancing biodeterioration of oil as discussed above.

The corrosion caused by microorganisms in the presence of water promotes a destruction of tank walls and the influx of metal ions into oil and oil products (Chesneau 2000; Gardner and Stewart 2002; Muthukumar 2003). Thus, corrosion processes may supply metal ions which are required for the growth of microorganisms.

3.5 Chemical composition

The chemical composition of crude oil and oil products also influences their susceptibility to biodegradation. The so called light oil, with mainly moderate chain aliphatic HCs and low content of aromatic HCs, is more quickly infected by microorganisms compared to high-aromatic

oils. For example, the light petroleum from Groznensky and Borislavsky oil fields (Caucasus) was heavily affected by microorganisms within 7–10 days whereas that from Anastasyevsky oilfield (Western Siberia) with an aromatic content up to 50% remained unaffected during 90 days of its storage under the same conditions. Moreover, during transport of light oils using pipelines, the intensive growth of microorganisms enhances sedimentation of paraffin on pipeline walls. Similarly, the so called sour oil having a high sulphur content is more susceptible to microbial infection and degradation compared to the so called sweet oil with low sulphur content (Vishnyakova et al. 1970). Probably, the absence of sulphur deficiency in the former oil promotes its biofouling.

Three most common motor fuels (gasoline, kerosene and diesel) are characterized by different chemical content, especially HC content, and this determines a specific composition of the microbial consortia affecting them (Vishnyakova et al. 1970; Gaylarde et al. 1999; Allsopp et al. 2004). This point can be readily illustrated with gasoline that consists mainly of C_5–C_{12} aliphatic HCs. Grades of gasoline differ in boiling temperature, volatility, octane number, stability and presence of various micro components such as sulphur compounds. Furthermore, gasoline compositions also include various additives such as antioxidants (aromatic amines and phenols preventing an occurrence of free radicals and formation of polymers), antiknock (antidetonation) additives (methyl tret-butyl ether, ethyl tret-butyl ether, etc), antifreeze additives (alcohols or surfactants), corrosion inhibitors, surfactants and sometimes dyes for identification purposes. Such a chemical content of gasoline seriously limits a specific composition of the deteriorating microflora because the low molecular weight HCs can dissolve cell membranes. In addition, antiknock additives or sulphur compounds act as weak inhibitors on microbial growth. On the other hand, some surfactants can serve as nutrient sources for microorganisms and stimulate their growth (Gaylarde et al. 1999; Allsopp et al. 2004).

Quality of fuel is extremely important in the case of kerosene (aviation fuel), which is exposed to microbial contamination mainly by fungi (Vishnyakova et al. 1970; Yang et al. 1992; Ferrari et al. 1998). Microbiological ageing of kerosene has been cited as the main reason of several recent airplane crashes (Yang et al. 1992; Ferrari et al. 1998). Kerosene should be ignited and soundly burned under any conditions without undesirable effects such as flashbacks or flameouts. Formation of solid particles is undesirable and steam pressure as well as the freezing point should be low. Kerosene usually includes C_{10}–C_{16} aliphatic HCs and anti-freezing additives, glycols and ethers, which can possess also biostatic properties (Neihof and Bailey 1978; Gaylarde et al. 1999). On the other hand, it has been shown that some microorganisms are capable of degrading antifreeze agents added to kerosene (Gaylarde et al. 1999).

Various grades of diesel contain mainly C_{15}–C_{22} aliphatic HCs. The decrease in the concentration of sulphur-containing compounds as well as the presence of various additives, such as stabilizing and chelating agents and surfactants serving as sources of nutrients, activate microbial growth (Lopes Ferreira et al. 2006). Products of microorganism metabolism in diesel lead to clogging of fuel nozzles (Gaylarde et al. 1999; Allsopp et al. 2004) and finally to the breakage of the engine.

Solid and liquid lubricants (technical vaselines, rope and gun oil) made of petroleum HCs are readily affected by fungi, mainly mould fungi, and bacteria during operation and storage in unprotected containers (Vishnyakova et al. 1970; Eisentraeger et al. 2002). The solid lubricants are usually contaminated by microorganisms only in a superficial film whereas the liquid ones are infected along the full layer.

Machine oils produced from petroleum of various oilfields have a different stability to microbial infection. A variation from 10% to 100% of biofouling was recorded during the same period of supervision (Vishnyakova et al. 1970). Biodegradation of various machine oils depends on HC structure, physical properties of processed oil and manufacture technology. A high concentration of aromatic and/or polar compounds increases the stability of machine oils. On the contrary, the machine oils produced from the sour oils are less biostable than those produced from

the sweet oils, by analogy with crude oils. Generally, the biodegradability of machine oils is an inverse function of kinematical viscosity and refractive index (Haus et al. 2001, 2003).

Cutting fluids including HC and water phases are easily exposed to microbial infection. Repeated reuse of cutting fluid promotes an active growth of microorganisms (Veillette et al. 2004). Growth of fungi and aerobic bacteria in cutting fluids leads to significant changes of its physical and chemical properties due to degradation of some components (Rossmoore and Rossmoore 1977).

In bitumens and asphalts consisting of C_{15}–C_{32} HCs as well as polyaromatic compounds, intensity of various bacterial and fungal growth is defined by their HC structure as well (Kopteva et al. 2001; Potter and Duval 2001; Robert et al. 2001). Some microorganisms use alkanes and alkenes of bitumen, others utilize alkyl-substituted aliphatic and aromatic HCs. For example, pseudomonades grow well in asphalts and bitumens with the high content of aliphatic HCs; however, an increase in the percentage of aromatic HCs inhibits their growth (Vishnyakova et al. 1970; Kopteva et al. 2001).

4 Microorganisms responsible for oil and oil products biodeterioration

As mentioned above, microorganisms get into contact with crude oil and oil products by various ways including ventilation and pumping systems. Generally it is very difficult to prevent microbial contamination of these products, because it is practically impossible to maintain sterile conditions during their transportation and storage.

Those microorganisms capable of utilizing oil and oil products as a sole source of carbon and energy occur practically everywhere: in air, water and soil. A large quantity of HCO microorganisms are present in soil polluted by oil, under asphalt, at the bottom of oil and fuel tanks, in oil pipelines, in sewage polluted by oil or its products (Olliver and Magot 2005). It is estimated that, in 1 g of unpolluted soil, there are only 100–1,000 cells of HCO microorganisms, whereas, in 1 g of soil polluted by oil, their number increases to 1×10^6–5×10^7 cells, especially if pollution occurred repeatedly and during a long time (Rosenberg et al. 1996).

At present time, hundreds of species responsible for deterioration of oil and oil products have been identified but only some of them are listed in Table 1. Among isolated strains, both HC-degraders and microorganisms utilizing metabolites of the latter have been found.

4.1 Aerobic microorganisms

Many species of aerobic bacteria, microscopic fungi and yeast can utilize HCs of crude oil and derived products. For example, bacterial species of *Pseudomonas*, *Acinetobacter*, *Mycococcus*, *Flavobacterium*, *Aeromonas*, *Micrococcus*, *Geobacillus* isolated from condensates of aviation fuel storage systems or from hot water reservoirs (thermophilic conditions) are responsible for deteriorating crude oil and its products both in natural conditions and in storage tanks (Odier 1976; Ferrari et al. 1998; Gaylarde et al. 1999; Bento and Gaylarde 2001; Norman et al. 2002; Rahman et al. 2002a, b; Bonch-Osmolovskaya et al. 2003; Olliver and Magot 2005). Representatives of isolated microfungi are *Cladosporium*, *Aspergillus*, *Penicillium*, *Hormoconis resinae*, *Paecilomyces*, while yeast *Candida*, *Rhodotorula glutinis* have also been identified from condensates of oil products storage systems (Odier 1976; Haggett and Morchat 1992; Yang et al. 1992; Lopes and Gaylarde 1996; Ferrari et al. 1998; Gaylarde et al. 1999; Bento and Gaylarde 2001; Olliver and Magot 2005).

Microbiological processes are stimulated by microaerophilic conditions often observed in storage tanks and lead to disparate transformation of oil and its products. This causes oil to get viscous and heavier while gaseous products tend to become dry. Bacterial infection is typical for crude oil (Head et al. 2003; Roling et al. 2003; Takahata et al. 2000) in comparison to aviation fuel (kerosene) that is usually contaminated by fungi (Neihof ans Bailey 1978; Yang et al. 1992; Ferrari et al. 1998). The examination of 350 aviation fuel samples has shown that practically all of the samples contained fungi, and 85% of them contained around 100 cells/l (Ferrari et al.

Table 1 Microorganisms contaminating various types of oil fuels during storage

Microorganism	Localization	Reference
Fungi		
Aspergillus, Penicillium	Kerosene, condensate from systems of fuel storage	Odier (1976); Haggett and Morchat (1992); Lopes and Gaylarde (1996); Ferrari et al. (1998); Gaylarde et al. (1999)
Cladosporium, Cephalosporium, Chaetomium Botrytis, Fusarium, Mucor	Kerosene, condensate from systems of fuel storage	Odier (1976); Yang et al. (1992); Ferrari et al. (1998); Gaylarde et al. (1999)
Ulocladium, Trichoderma, Trichosporon Phoma	Kerosene, condensate from systems of fuel storage	Odier 1976; Yang et al. 1992; Gaylarde et al. (1999)
Hormoconis resinae, *Rhinocladiella Rhizopus*	Condensate from systems of fuel storage	Haggett and Marchat (1992); Lopes and Gaylarde (1996); Gaylarde et al. (1999)
Paecilomyces, Acremonium, Alternaria	Condensate from systems of diesel storage	Gaylarde et al. (1999); Bento and Gaylarde (2001)
Yeast		
Candida, Saccharomyces, Hansenula	Condensate from systems of fuel storage	Odier (1976); Gaylarde et al. (1999); Bento and Gaylarde (2001)
Aureobasidium Rhodotorula	Condensate from systems of diesel storage	Gaylarde et al. (1999); Bento and Gaylarde (2001)
Aerobic bacteria		
Pseudomonas, Aeromonas Acinetobacter, Aerobacter, Enterobacter, Brevibacterium Rhodococcus	Condensate from systems of fuel storage, crude oil contaminated soil	Odier (1976); Ferrari et al. (1998); Gaylarde et al. (1999); Norman et al. (2002); Mishra et al. (2004); Murygina et al. (2005); Ouyang et al. (2005)
Bacillus	Condensate from systems of fuel storage	Odier (1976); Gaylarde et al. (1999)
Micrococcus, Serratia	Condensate from systems of fuel storage	Odier (1976); Gaylarde et al. (1999); Bento and Gaylarde (2001)
Flavobacterium, Corynebacterium, Actinomycetes	Condensate from systems of kerosene storage, crude oil contaminated soil	Ferrari et al. (1998); Gaylarde et al. (1999); Rahman et al. (2002a); Rahman et al. (2002b)
*Geobacillus**	Water dissolved in oil pool, thermophilic condition	Bonch-Osmolovskaya et al. (2003)
*Zoogloea**	Condensate from systems of crude oil storage	Watanabe et al. (2002)
Anaerobic bacteria		
Clostridium	Condensate from systems of crude oil storage	Gaylarde et al. (1999)
Thermotoga	Water dissolved in oil pool, thermophilic condition	Takahata et al. (2000); Bonch-Osmolovskaya et al. (2003); Head et al. (2003); Roling et al. (2003)
Thermoanaerobacter	Water dissolved in oil pool, thermophilic condition	Bonch-Osmolovskaya et al. (2003); Head et al. (003); Roling et al. (2003)
Thermosipho	Water dissolved in oil pool, thermophilic condition	Bonch-Osmolovskaya et al. (2003)
Petrotoga miotherma	Water dissolved in oil pool, thermophilic condition	Bonch-Osmolovskaya et al. (2003)
Fervidobacterium	Water dissolved in oil pool, thermophilic condition	Head et al. (2003), Roling et al. (2003)
Acetobacterium	Condensate from systems of crude oil storage	Watanabe et al. (2002)
Desulfotomaculum	Condensate from systems of crude oil storage	Watanabe et al. (2002)
Desulfovibrio	Condensate from systems of crude oil storage	Watanabe et al. (2002)
Desulfobacula	Condensate from systems of crude oil storage	Watanabe et al. (2002)

Table 1 continued

Microorganism	Localization	Reference
Desulfobacterium cetonicum	Condensate from systems of crude oil storage	Harms et al. (1999)
Desulfosarcina variabilis	Condensate from systems of crude oil storage	Harms et al. (1999)
Desulfococcus multivorans	Condensate from systems of crude oil storage	Harms et al. (1999)
Thiomicrospira denitrificans	Condensate from systems of crude oil storage	Gevertz et al. (2000); Watanabe et al. (2002); Kodama and Watanabe (2003)
Azoarcus	Condensate from systems of crude oil storage	Fukui et al. (1999)
Iron-reducing bacterium	Water dissolved in the oil	Rooney-Varga et al. (1999); Head et al. (2003); Roling et al. (2003)
Archae		
Thermococcus	Water dissolved in oil pool, hyperthermophilic condition	Head et al. (2003); Roling et al. (2003); Takahata, et al. (2000); Bonch-Osmolovskaya et al. (2003)
Archaeoglobus	Water dissolved in oil pool, hyperthermophilic condition	Head et al. (2003); Roling et al. (2003)
Methanogens	Water dissolved in oil	Bonch-Osmolovskaya et al. (2003); Head et al. (2003); Roling et al. (2003)

* Capable to grow in the denitrifying condition

1998). The predominant species were *Cladosporium* and *Aspergillus*. In samples from intermediate fuel storage tanks, water has been also found. It is interesting that, in the water collecting at the bottom of fuel tanks, bacteria were discovered. Concentration of aerobic bacteria in water samples varied from 100 to 8.8×10^7 cells/ml and 85% of samples contained high enough concentrations of these bacteria, typically from 10^4 to 10^7 cells/ml. In water, there was a predominance of *Pseudomonas*, though *Flavobacterium* and *Aeromonas* were also identified (Ferrari et al. 1998).

The main products of HCO microbial metabolism are carbon dioxide, water and in smaller quantities fatty acids and surfactants participating in stabilization of inverted water-oil emulsion (Odier 1976; Chesneau 2000; Olliver and Magot 2005). Furthermore, secretion of peptidoglycans, being the basis of biofilms, is characteristic for initial stages of aerobic HCO microorganism growth. Biofilm formation leads to slime or fungal mats causing filter contamination and malfunctioning of valves. They reduce the efficiency of combustion processes and lead to significant losses of engine capacity. On internal surfaces of plane fuel tanks, brown slime is formed from living and dead cells of aerobic microorganisms and their polymeric metabolites. The thickness of such a layer of slime can be up to 2 cm. Moreover, aerobic microorganisms create conditions for growth of anaerobic microorganisms, particularly SRB (Olliver and Magot 2005).

4.2 Anaerobic microorganisms

HCs were traditionally considered as persistent to anaerobic degradation because of absence of similar enzymes in anaerobic bacteria as in aerobic HC-degrading species. However, within last decades, it has been established that anaerobic microbes such as SRB and denitrifying bacteria (DNB) are also capable of growing on alkanes as a unique organic substrate. Although various DNB under anaerobic conditions decomposed aromatic HCs, for example, alkylbenzene (Heider et al. 1999) or alkenes containing one or two double bonds (Gilewicz et al. 1991; Harder and Probian 1995; Foss et al. 1998), it was only recently reported that pure DNB cultures can also oxidize linear unsubstituted alkanes (Ehrenreich et al. 2000).

Three mesophilic (Aeckersberg et al. 1991, 1998; So and Young 1999) and one thermophilic (Rueter et al. 1994) SRB mineralizing alkanes were isolated. Also, recently, it has been shown

that microorganisms are capable of oxidizing in anaerobic conditions even such chemically stable compounds as methane (Jorgensen et al. 2001; Thiel et al. 2001).

At the present time, the isolated anaerobic microorganisms capable of causing oil and oil products deterioration outnumber the aerobic ones (Table 1). These anaerobic species belong to various microbial genera and families including *Thermotoga*, *Thermoanaerobacter*, *Thermosipho*, *Petrotoga*, *Fervidobacterium*, *Acetobacterium*, *Desulfotomaculum*, *Desulfovibrio*, *Desulfobacula*, *Desulfobacterium*, *Desulfosarcina*, *Desulfococcus*, *Thiomicrospira*, *Azoarcus* etc. (Harms et al. 1999; Gevertz et al. 2000; Takahata et al. 2000; Gardner and Stewart 2002; Watanabe et al. 2002; Bonch-Osmolovskaya et al. 2003; Head et al. 2003; Kodama and Watanabe 2003; Roling et al. 2003; Olliver and Magot 2005).

Among all known anaerobic bacteria, SRB have attracted a special attention due to their major impact on deterioration processes. The optimal conditions for their active development are: absence of oxygen, presence of a carbon source, for example HCs or fatty acids, presence of sulphate, neutral or light alkaline environment, temperatures ranging from 30°C to 50°C or even higher. Such conditions, as a rule, are formed during the transportation and storage of crude oil and its products. SRB can also grow under high temperatures and high pressure (Rueter et al. 1994; Olliver and Magot 2005).

As mentioned above, SRB reduce sulphates present in the water phase to hydrogen sulphides and thus provide sulphur-oxidizing bacteria with a substrate (Watanabe et al. 2002; Kodama and Watanabe 2003). The latter are capable of growing in microaerophilic conditions. Japanese researchers (Kodama and Watanabe 2003) isolated the anaerobic chemolitotrophic bacterium *Thiomicrospira denitrificans* using sulphide, thiosulphate, elementary sulphur and hydrogen as electron donors and nitrate as an electron acceptor from a condensate accumulating at the bottom of an oil storage tank. The end-product of nitrate reduction by *T. denitrificans* is nitrite. This species is an example of microorganism which is unable to oxidize HC itself, but uses metabolites of other microorganisms in oil storages.

5 Mechanisms of oil and oil products biodegradation

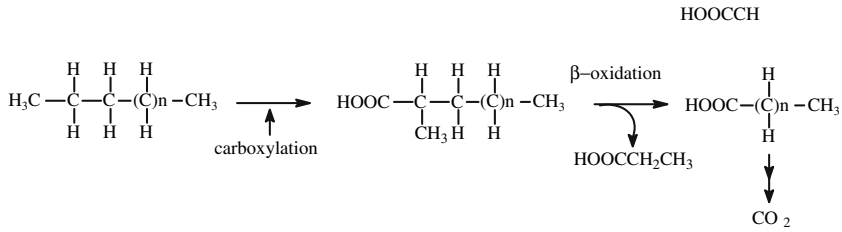
As it is seen from Table 1, numerous microorganisms use HCs as a source of carbon and energy, however, aerobic and anaerobic species have different enzymatic systems and metabolic pathways for degradation of HCs. It is important to note that, for the majority of microorganisms, the rate of biodegradation decreases in this order: *n*-alkanes, simple aromatic HCs (benzene, toluene, etc.), branched alkanes, cycloalkanes, isoprenes and the condensed polyaromatic HCs. (Heath et al. 1997). The recent review of Van Hamme et al (2003) gives a comprehensive picture of the latest advances in petroleum microbiology. Below we will point out only major mechanisms of biodegradation of various HCs.

In most cases, degradation of aliphatic HCs begins with an oxidation of extraterminal methyl groups to primary alcohol groups, though intraterminal oxidation has been also described (Gottshalk 1982). The primary alcohols are then oxidized to aldehydes, which, in turn, under action of NAD-dependent dehydrogenase are oxidized to corresponding fatty acids. These are degraded by β-oxidation or are used by the cells as a building material (Gottshalk 1982; Sharma and Pant 2000).

Anaerobic microorganisms can also degrade aliphatic HCs. Mechanism of *n*-alkanes biotransformation by SRB is presented on Fig. 2. The principal step of anaerobic alkane degradation, as well as anaerobic degradation of aromatic HCs, is the carboxylation of substrate molecules (Vasu et al. 1977; So and Young 1999). Various substances can act as carbon donors, which is included in carboxylic group formed, namely: fumarate (when degrading toluene, xylene and alkanes), bicarbonate (when degrading naphthalene and alkanes) etc. (Young and Phelps 2005). Further decomposition proceeds via the known β-oxidation pathway (Gottshalk 1982).

It is interesting also to have a brief look on mechanism of cycloalkanes biodegradation because the strains capable of utilizing these substrates (Gordonia, Xanthobacter) have specific enzymatic systems differing from those used by microorganisms for acyclic alkane oxidation. The pathway of cycloalkane degradation is well

Fig. 2 The mechanism of anaerobic degradation of alkanes (So and Young 1999; Young and Phelps 2005)



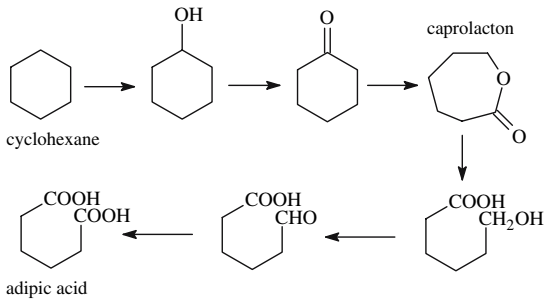


Fig. 3 The mechanism of bacterial degradation of cycloalkanes in aerobic conditions (Cerniglia and Yang 1984)

studied and presented in Fig. 3 (Cerniglia and Yang 1984; Tadashi et al. 2004).

There are two basic strategies utilized by microorganisms to degrade aromatic compounds. The first strategy is used by aerobic microorganisms and involves the oxidation of the aromatic ring into dihydroxyaromatic compounds (catechol or hydroquinone intermediates) with subsequent oxidizing cleavage of the aromatic ring (Fig. 4). Oxidative ring cleavage of the catechol intermediate can occur in two ways: intradiol (or *ortho*) cleavage to give a muconic acid or extradiol (or *meta*) cleavage to give a hydroxymuconaldehydic acid derivatives (Bugg and Winfield 1998). Long aliphatic substitute of aromatic compounds are decomposed by *β*-oxidation to shorter ones and the formed intermediates undergo degradation of aromatic ring by one of mechanisms outlined previously.

The second strategy is used by anaerobic microorganisms and involves the reduction of the aromatic ring with the subsequent defragmentation of formed cycloalkane derivatives (Neidle et al. 1989; Mason and Cammack 1992; Asturias and Timmis, 1993; Nakatsu and Wyndham 1993; Massey 1994; Haak et al. 1995; van der Meer 1997). The principal stage of aromatic HCs

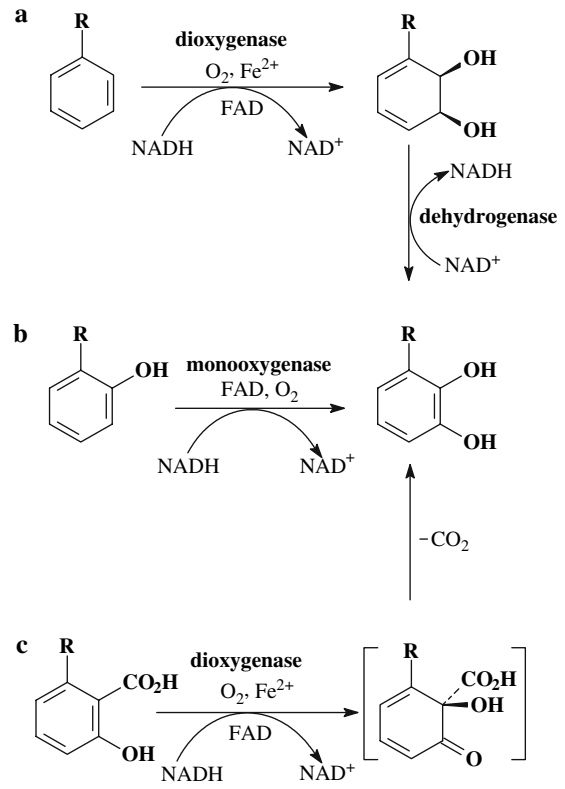


Fig. 4 The principal pathways of aromatic structure fission by aerobic microorganism

destruction in anaerobic conditions is also formation of carboxylic derivative of substrate. Again various substances can act as carbon donors, which is included in carboxylic group formed, namely: fumarate (when degrading toluol, xylol), bicarbonate (when degrading ethyl- and propylbenzene, polyaromatic CHs) etc. (Young and Phelps 2005). Then reduction of aromatic ring with further fragmentation of cyclohexane derivatives occur. Anaerobic biodegradation of aromatic structure is presented on Fig. 5, where Fig. 5a demonstrates ethylbenzene degradation using bicarbonate as carbon donor whereas

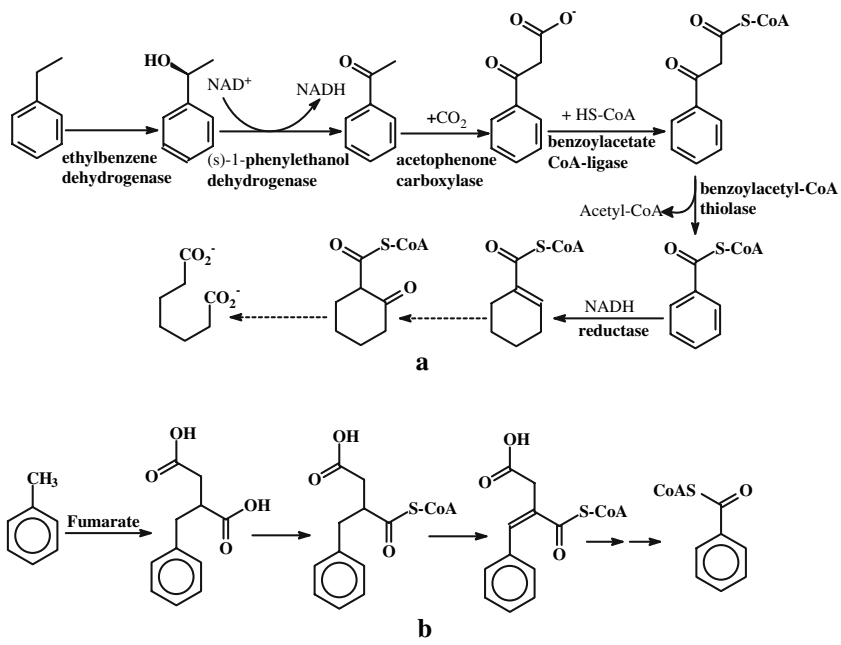


Fig. 5 The pathway of anaerobic aromatic hydrocarbons degradation (Rabus et al. 2002)

Fig. 5b demonstrates toluene degradation using fumarate as carbon donor for carboxylic group formation. For example, some denitrifying strains transform aromatic substrates to benzoic acid and then to benzoyl-CoA. The latter is further reduced to cyclohexenyl-CoA derivatives which then are hydrolytically decomposed, and the formed products undergo β-oxidation (Rabus et al. 2002; Young and Phelps 2005).

Polyaromatic HCs are most recalcitrant compounds of all the oil constituents (Heath et al. 1997). There is a fundamental distinction in mechanisms of polyaromatic molecules fission by microorganisms. Bacteria and some green algae oxidize polyaromatic hydrocarbons (PAHs) using both atoms of the oxygen molecule (reaction catalyzed by dioxygenases), thus cis-dihydrodiols are obtained which then are transformed to catechols by dehydrogenation. Some fungi oxidize PAHs by means of cytochrome P-450 monooxygenase by incorporating only one atom of the oxygen molecule into PAH or by the action of peroxidase, which in presence of H_2O_2 transforms PAH to aryl radical undergoing further oxidation to form quinone. However, these fungi are most likely not involved in the degradation of oil or fuels (Steffen et al. 2002). Generally, the mechanism of bacterial and fungal biodegradation of complex PAH is well studied (Heitkamp et al. 1988; Samanta et al. 1999; Kanaly and Harayama 2000; Pinyakong et al. 2000; Dean-Ross et al. 2001; Steffen et al. 2002; Habe and Omori 2003) and is depicted in Fig. 6.

Thus, there are essential differences in mechanisms of HC degradation by aerobic and anaerobic microorganisms. Aerobic degradation begins with the oxidation of varying groups of atoms of a substrate molecule by different enzyme systems. Unlike aerobic processes, the mechanism of anaerobic HC degradation starts with attachment of some groups of atoms to a substrate molecule, for example, often there is an attachment to carboxyl groups.

6 Methods of crude oil and oil products microbial contamination monitoring

The majority of microbial contamination of crude oil and oil products is presented in water phase or on the interphase between water and HC. Thus, the primary attention during oil and fuel biofouling monitoring should be put on water phase investigation. About 30 years ago, classical microbiological methods were used for these purposes (Rozanova 1971). All these methods are very

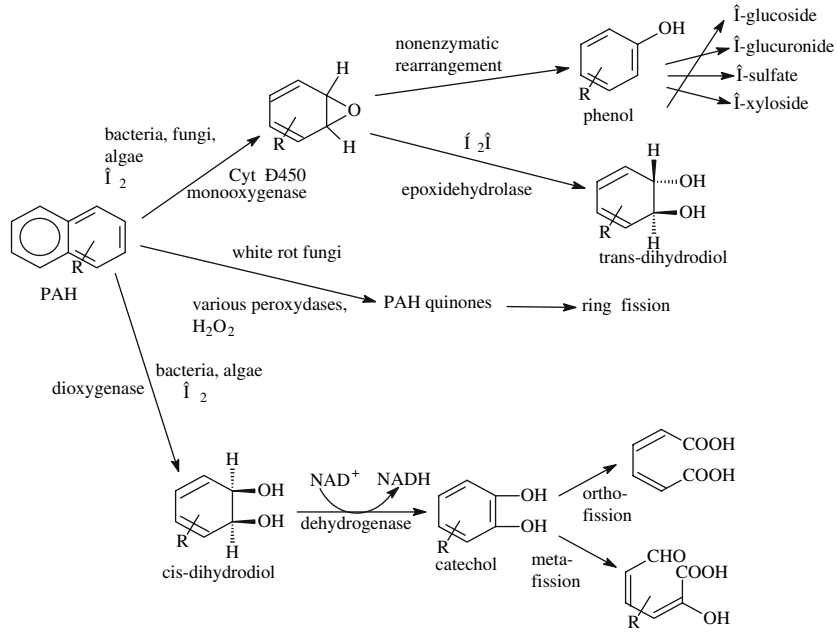


Fig. 6 The major pathway in the microbial metabolism of PAHs

laborious, time-consuming (for several days) and not very precise, so, we just list them below:

- execution of passages on solid media (agar) followed by calculation of colonies-forming units (CFU) or in liquid selective media from ten-fold dilutions of samples (MPN-method);
- use a microscope to stain and visualize microbial cells (by Gram-staining method);
- concentration of bacterial cells on membrane filters with subsequent quantification using a microscope with or without staining;
- determination of wet or dry biomass of microorganisms etc.

It should be noted that some microbiological methods are impossible to apply for crude oil or oil products biofouling monitoring. For example, ''indirect'' methods for measuring turbidity (turbidimetry) or dispersion of light (nephelometry) caused by microbial cells suspensions are not used at all due to high background signal of the biphasic system HC-water. Methods of total nitrogen (micro-Kjeldal and micro diffusive method of ammonium determination) and total carbon content (by van Slaik-Foulch) determination developed for the measurement of cell protein concentrations or modification of biuret procedure and other colorimetric methods are hardly practiced now because of difficulties associated with sample preparation.

Methods of bacterial contamination determination as well as efficacy of biocides and additives estimation may also be based on simple direct measurement of substances related to growth of microorganisms: consumption of O_2, formation of CO_2 or organic acids and surfactants (Gaylarde et al. 1999; Allsopp et al. 2004). These methods can help to indicate the potential problem, but linear dependence between metabolic activity and exact cells number is difficult to be obtained, especially in high-density cell suspensions.

Among contemporary and rapid methods of microbial contamination of oil and oil products monitoring, immunological technique, test-kits, PCR-analysis and bioluminescence method could be marked out. Usage of immunological methods allows speeding up the enumeration of microorganisms (Lopes and Gaylarde 1996; Gaylarde et al. 1999). The application of the antibodies with various markers, e.g., fluorescent ones, underlies the technique. However, this technique does not show a total number of all kind microorganisms because of impossibility to have antibodies for all numerous species presented.

More than 15 years ago, a range of different test-kits were developed for monitoring of the state of biological fouling in both laboratory mesocosms, which simulated plugging water wells, and real objects such as water wells, surge tanks, process lines, heat exchangers, where slimes, plugging, corrosion, discoloured water and outbreaks of infections can occur. When the sample is added to the test tube under sterile conditions, the coloration or precipitation is observed under optimal temperature in several days. It allows not only a generic definition of microbes but also their quantification. These kits manufactured by a number of companies (BART^{TM} Dryocon Bioconcepts Inc., Canada; MicrobMonitor BP International Limited, UK; B-F^{TM} Indicator Troy Corporation, USA etc) are also suitable for testing and examination of efficient biocides and suppressors of corrosion and biofouling of oil and fuels (Bailey and May 1979; Girotti and Zanetti 1998; Gaylarde et al. 1999; Bonch-Osmolovskaya et al. 2003).

Recently molecular biology methods, in particular the PCR-analysis, have been widely applied all over the world for determination of both number and the species of the microorganisms isolated from oils and fuel storage tanks, soils, deposits, sludges and also the water surface as well as ground-water polluted by various HCs, pesticides, herbicides, radio-nuclei and others pollutants. Methods of DNA extraction from tested objects are well processed, probes of 16s RNA have been produced for many microbial species (but not all) and libraries of 16s RNA have been created. However, it is necessary to recognize that methods of molecular biology are still quite expensive and require an advanced infrastructure, which is frequently not available at oil processing units (e.g., in Russia).

During the last two decades, a tendency to apply bioluminescent methods for detecting microbial contamination of various objects has been exhibited, for example, in food industry and medicine where a strict control of microbiological purity of products is required (Belyaeva et al. 1983; Frundzhan et al. 1997, 1999). Such an approach is relatively new for the petroleum contaminated samples due to their multi-phase (oil-water-solid) nature. However, the feasibility of the bioluminescent method by determination of intracellular ATP with luciferase for quantification of various HCO bacteria such as *Rhodococcus* sp., *Pseudomonas* sp. in the oil contaminated soil (Efremenko et al. 2002, 2005) or mixed cultures of microscopic fungi (*Aspergilluis niger*, *Fusarium solani*, *Penicillium chrysogenum*, *Seopulariopsis, Phialophora fastigiated)* and bacteria (*Bacillus brevicaulis)* in the lubricants (Egorov et al. 1985) has been demonstrated. It should be noted that the high profile for application of this method has a pre-treatment of oil sample using dimethylsulfoxide (DMSO) and chloroform to release intracellular ATP because of frequent losses or under-extraction of the latter. Generally, the attractiveness of bioluminescent ATP-method for monitoring of oil biofouling is related to its high sensitivity (10^{-13}–10^{-18} M ATP) and quickness (only 1–2 min per analysis), while the main disadvantage is a high sensitivity of luciferase to inhibitors and ATP hydrolyzing and synthesizing enzymes which may be present in the testing samples. The proper sample preparation and ATP extraction enables the prevention such effects as stressed above. In any case, the difficulty associated with ATP has to be taken into account when this technique is utilized.

7 Methods of oil and oil products biodeterioration suppression

As it has been shown above, biodeterioration of oil and, especially, fuels is a still big concern today, as it was fifty years ago. So far there is no economically reasonable approach to prevent it completely; however, various methods have been proposed to suppress/mitigate this dangerous phenomenon. These can be divided into two major groups—physico-mechanical and chemical ones.

7.1 Physico-mechanical methods

The physico-mechanical methods of fuel biofouling suppression include, first of all, regular and careful cleaning of tanks for fuel storage from sediments, an effective filtration of fuel, excluding

superficial microbial contamination, more frequent dewatering of fuels, ultra-violet and electromagnetic fuel irradiation, heating, ultrasonic processing etc (Vishnyakova et al. 1970; Stuart 1994–1995).

As water is of critical importance for microbiological processes and is always present in oil and fuels, one obvious way of stabilizing such systems is to try to remove water. Water solubility in oil and oil products is proportional to storage temperature in the range from 0°C to 60°C. As it has been stated before, spores of microorganisms in the infected fuel can survive at concentration of water from 5 ppm to 80 ppm. Higher water content of fuels does not promote the survival of spores. Various static and dynamic methods of water removal using molecular sieves can reduce the concentration of water in oil to less than 5 ppm. However, under conditions of increased humidity, such fuel becomes very hygroscopic and is quickly saturated by water (Yang et al. 1992). Nevertheless, careful removal of the water in the bottom of storage systems is a primary way for preventing crude oil and its products from microbial contamination (Stuart 1994–1995; Gaylarde et al. 1999).

Since the average size of microorganisms is several microns (some of them are less than 1 micron), they easily pass through regular filters. Therefore, a conventional filtration of fuels for removing mechanical impurities does not make them free from microorganisms. An effective filtration of fuel contaminated with microorganisms can be achieved by using a complex filtration system with several filters (Stuart 1994–1995) including antibacterial filters. Cotton, glass fibre, synthetic rubber treated with silver nitrate possessing bactericidal properties etc are used in such filters (Vishnyakova et al. 1970).

Heating is considered as one of traditional methods against microorganisms. Potentially bacteria are capable of surviving at temperatures up to 150°C (under elevated pressures). However, most effectively microbiological degradation of oil HCs takes place at temperature below 80°C. Nevertheless, there are natural oilfields with temperature below 80°C in which biological degradation of native oil is not observed. Wilhelms et al. (2001) suggested that, in such ''cold'' oilfields, oil layers have been raised from deeper and hot areas of the Earth, where they have already undergone sterilization. When there was no repeated contamination of such ''sterile'' deposits with HCO bacteria, degradation of oil did not occur even at low temperatures.

The temperature and duration of heating has a great value for suppression of microbial growth. For example, heating of crude oil at 70°C within 20 s or at 80°C within 10 s essentially reduces microbial populations (Stuart 1994–1995). However, it is difficult to reach such temperatures for the entire volume of big storage tanks. As a result, currently high-temperature processing is applied only for sterilization of small tanks for storage of mainly fuels (Stuart 1994–1995; Chesneau 2000). It should be also noted that the thermal processing usually results in some fuel deterioration.

Electromagnetic radiation of various frequencies was proposed to suppress microbial growth (Vishnyakova et al. 1970). It was supposed that cellular walls of microorganisms collapse and perish or division of microorganisms stops during the passage through a magnetic field, and thus fuel disinfecting is carried out. However, there is no unequivocal proof of killing microorganisms by the magnetic field by direct microbiological investigations.

The interesting method implying pulse fields of the superhigh frequency (SHF) together with UV-radiation for sterilization of various kinds of oil fuels has been patented (Sirotkin et al. 2005). During the corresponding treatments of diesel and other fuels, the temperature of fuel remained constant; the chemical content did not change while microbial destruction was 100%. Nevertheless, the combined treatment of the SHF and UV-radiation has not received a wide application so far because of its labour input, high cost and potential danger to the service personnel.

Besides problems of the physico-mechanical methods mentioned above, their other major deficiency is the short time impact, i.e., re-infection after treatment. Therefore, chemical protection of oil and oil products, i.e. use of various chemical compounds, which operate during long periods of time, represents a more effective strategy to combat biodeterioration of oil.

7.2 Chemical methods

One of the main causes of microbiological ageing of oil, microbial corrosion and other problems arising during processing and usage of oil and its products is the activity of SRB. Though the SRB growth remains negligible when oil is in a layer of oilfields without water flooding the deposit, it becomes considerable during the pumping of oil into the storage (Davidova et al. 2001). One conventional method for controlling SRB activity is the use of nitrates to enhance the activity of competing DNB (Davidova et al. 2001; Myhr et al. 2002). Indeed, nitrate addition from 0.5 mM (Myhr et al. 2002) up to 10 mM (Davidova et al. 2001) to the water phase inevitably present in systems of oil and oil products storage has led to a full inhibition of SRB activity and an increase of DNB population. However, the SRB community restored their activity within 5 months in case of subsequent nitrate absence; at the same time, the periodical addition of nitrate during 3.5–5.5 months has led to a full suppression of SRB population (Myhr et al. 2002).

Various fuel additives, for example, antiknock, antioxidant and also corrosion suppressors with antistatic and anti-icing additives are used to improve aviation fuel quality. Meantime, these additives usually possess biocidal (biostatic) properties as well. For example, 2-methoxyethanol added to kerosene in concentration of 0.1–0.15% for prevention of ice formation efficiently suppressed the growth of microorganisms in the water accumulated at the bottom of fuel tanks (Neihof and Bailey 1978).

Nevertheless the major stream of industrial prevention and control of microbial growth is a use of special biocide-active substances that can kill numerous microorganisms (bacteria, fungi, yeasts, microalgae etc.). Biocides efficiency strongly differs in relation to various genera of microorganisms as well as to various species and even strains within one genus. For example, gram-negative bacteria followed by fungi have more sensitivity to biocides (Maillard 2002), while *Mycobacterium* strains show the greatest stability to biocides among non spore-forming microorganisms. It is also known that the oil products themselves are more toxic to microorganisms than the crude oil, and various microorganisms have different sensitivity to long contact with oil products, for example, *Penicillium* sp. survive longer in comparison with *Cladosporium* sp.

Biocides have a diversified chemical structure, and its action is effective if they interact with a definite microbial target. A majority of biocides (but not all) tend to penetrate the bacterial cell and subsequently exert their toxicity. Mechanisms of biocide action may be classified according to the cell components they affect. Maillard (2002) and Novikov (2001) describe three important modes of biocide activity:

(a) interaction with external cellular structures (e.g., ethylenediaminetetraacetic acid, glutaraldehyde, phenols etc.);
(b) interaction with cellular membranes (e.g., ethanol, i-propanol, surfactants, copper, silver and arsenic compounds etc.);
(c) interaction with cytoplasm structures (e.g., arylmethan and acridine dyes, alkylating agents, prophlavine, aldehydes etc.).

It should be noted that significant research all over the world is constantly being done in an attempt to develop new and more effective biocides for crude oil and oil products preservation. For example, more than 500 million dollars are spent annually in the Western Europe for this purpose (Neale 2003; Knight and Cooke 2002). Also in Russia (the thirds world oil producer), biocides and corrosion suppressors possessing anti-bacterial properties are intensively developed, approved and put into practice.

According to the current policy in the oil-producing and oil-refining field, the developed biocides should meet the following requirements (Rossmoore et al. 1988; Gaylarde et al. 1999; Neale 2003):

- High biocidal activity and wide spectrum of action;
- Long term action;
- Good solubility in water and HCs;
- Effective activity at low concentration (1–100 ppm) without fuel deterioration;
- Chemical and thermal stability;
- Corrosion-inertness with regard to the equipment used;

– Ecological safety;
– Compatible with components of borehole solutions without changing their physical and chemical properties;
– Not causing damage of catalysts at oil refining factories.
– Made from cheap and accessible raw material;
– Cost effectiveness.

In Table 2, some biocides widely used on the Russian domestic oilfields are listed. Quaternary ammonium bases, isothiazolines, glutaric aldehyde, HC components with length of a carbon chains from C_{10} to C_{12}, chlorine-, iodine- or bromine-organic compounds, poly-non-saturated nitrogenous–heterocyclic and aromatic amines, amines of thiopicolinic acid and some metals (copper, nickel, cadmium, arsenic etc) are the most common ones (Anderson and Effendizade 1989). Some biocides trademark, such as domestically produced ''Biocide C'' and ''Ucarcide 142'' of world-wide company, imply similar substances or mixture (Table 2).

In laboratory simulation of biocide testing for protection of oil fuel in storage tank, an isothiazoline mixture and quaternary ammonium compounds were shown to be the most effective, whereas glutaraldehyde and a formaldehyde-realizing agent were active only in high concentration, if not at all. Concentration of effective biocides used for slightly contaminated oil products is 0.1 ppm and for highly contaminated oil products is 10 ppm (Allsopp et al. 2004).

Usually biocides are applied as complex mixtures, some examples of those and also some other specificity for processing oil and its products are discussed below in more details.

The biocide additive to oil and oil products was offered on a basis of poly-alkylene-guanidine or its salts which additionally contain a low-molecular polyethyleneglycol or its implant copolymer as well as a quaternary ammonium compound (Kuznetzov et al. 1997). There is also a patented additive recommended for the preservation of light oil products in steel tanks. It is a 2,2′-dihydroxy-azo-compound which increases the chemical and physical stability of mixing fuel in storage, reduces the rate of corrosion and the amount of sediment formation (Nagornov et al. 2001).

Morpeth (1994) introduced a composition consisting of halomethylglutaronitrile and polymethylenethyasoline which is effective against both bacteria and fungi. Complex compounds of metals (zinc) with thiohydroxamic acid or dithiocarbamate, that are soluble in water and active against fungi, have also been utilized (Austin and Morpeh 1994). A synergetic effect of various biocides is also often used (Hsu 1991, 1988). For example, the combined application of 2 biocides (tri-butyltin acetate and Katon or tri-butyltin acetate and Baccide) has shown to exhibit a synergetic inhibition action on micromycetes *Penicillium* sp., *Actinomyces* sp. and *Cladosporum* sp. (Zhygletzova et al. 2000). A combination of boric acid together with amines soluble in oil or HC fuel has been suggested to possess biocide properties; however, this mixture tends to raise the boiling point of fuel (Amick 1974). Generally, the development of methods enhancing biocide efficiency including its use in various combinations is an important task, as biocide application in high concentrations reliably guaranteeing full suppression of microbial growth has an adverse effect on the environment (see below).

The main drawback of water-soluble biocides usage is the necessity of repeated treatments of tanks together with newly added fuel in order to prevent microbial infection. Moreover, water-soluble biocides used for the treatment of tank bottoms for oil products or fuel storage can inadvertently suppress the activity of microorganisms in subsequent biological wastewater treatment plants. To avoid this situation it is recommended to use petrol-soluble waterproof biocides that retain their properties during fuel pumping. One more common disadvantage of biocide application to the systems already contaminated with microorganisms is additional blocking of filters and distributive pumps by dead microorganisms (cell debris). These residues form sludge at the bottom of tanks and their subsequent treatment, e.g., by biological methods, can be problematic due to accumulated toxic compounds.

Thus, a rationale usage of biocides for crude oil and oil products preservation is based, first of all, on knowledge of their activity, mechanism of action, their effective concentration etc. Many

Table 2 Some biocides currently used in the Russian oil and petrochemical industry

No	Chemical compound	Commodity mark	The manufacturer	Dosage
Domestic				
1	Derivatives of hexamethylentetraamine (urotropin) and light fractions of chlorine-HCs	''Bactericide LPE-11V	Joint-stock company NPO ''Technologist '' on base of PO ''Kaustik'', Sterlitomak	0.2 kg/m^3
2	Poly-demethyl-deallil-ammonium chloride	Water-soluble polyelectrolyte cathionic marks VPK-402	PO ''Kaustik'', Sterlitamak	0.5–3.0%
3	Quarternary ammonium salt alkyl-pyridineum-bromide	Suppressor-biocide SNPX-1003	NPO ''Sojus-neftepromchem'' Kazan, Joint-Stock Company ''Pressure'', Kazan	50 g/m^3
4	Product of interaction between dealkil-phosphoric acids and supreme amine	Bactericide-suppressor of corrosion SNPH-1004 SNPH-1004A SNPH-1004P	NPO ''Sojus-neftepromchem'', joint-stock company AO Chemeprom'', Novocherkassk	100–200 ppm
5	Product of interaction of phenolic pitch with sodium-caustic in organic solvent	Bactericide SNPH-1–2 (marks A and B)	NPO ''Sojus-neftepromchem'' Kazan, Joint-Stock Company ''Pressure'', Kazan	1.5–3.0 kg/m^3
6	–	Biocide R-2	Scientific research institute ''Reactive''	50 ppm
7	–	BiocideSNPH-1050	OAO NIINefte-promchem'', Kazan	150–350 ppm
8	Cationic surfactant in complex solvent	Bactericide-suppressor of corrosion GIPH-6b	GIPH, Saint -Petersburg	15–25 ppm
9	Glutaric aldehyde with quaternary ammonium salt	Biocide-C	Sofex Co, Moscow	20–2,500 ppm
Foreign				
10	Antimicrobial com-ponent, surfactant, glycols	Inkracept-28 (L and X)	Byelorussia, BelNIPINeft	20 ppm
11	42.5% glutaraldehyde, 7.5% alkyl dimethyl benzyl ammonium chloride	Ucarcide 142	''Dow Chemical'', USA	–
12	1,2-benzisothiazolin-3-one	Proxel	''Arch Biocides'', UK	0.075–0.5%
13	2-Bromo-2-nitropropane-1,3-diol	Myacide AS	''Basf'', Germany	12–3,200 ppm
14	Fat aliphatic amines	Bactiram 607	''Ceca'', France	50–100 ppm

– No data

methods using test-microorganisms were developed for estimation of biocides efficiency. Efficiency testing is usually based on microbial number or microbial activity determination. In the first case, concentration of biocides that half-decrease microbial number (Gilvanova and Usanov 2003) or common logarithm of microbial number (Selvaraiu et al. 2005) is in the centre of attention. Other methods are related to suppression of luminescence of reductive activity of test-microorganisms (Kholodenko et al. 2001).

Finally, it should be stressed that the key aspect of prolonged oil and fuel storage without deterioration of their quality is the prevention of microbial contamination instead of the subsequent treatment which can be much more expensive (Chesneau 2000).

8 Concluding remarks

The current review shows that deterioration of oil and fuels or its ''ageing'' due to microbial activity and the protection against this undesirable phenomenon remains until now a very serious economic and global environmental problem. It is impossible to prevent penetration of microorganisms in storage tanks of oil and fuels or into an oilfield after drilling. Furthermore, existing methods of enhanced oil recovery by water flooding

without preliminary sterilization of the water used or the application of microbiological methods for these purposes promote processes of oil ageing and increase the contamination by various microorganisms. Modern methods like express test-systems, bioluminescent ATP-method and PCR-methods allow the quick and reliable monitoring of oil and oil products microbial contamination.

Hence, the development of protocols for correct storage of oil and fuels in order to prevent their microbial contamination (instead of subsequent treatment, which, as a rule, is more expensive) is of supreme importance. Preventive measures of microbiological contamination for oil and oil products should include a monitoring system of storage conditions and strict storage and maintenance rules which can be summarized as follows.

1. Removal of bottom water from tanks.
2. Careful treatment of tanks with steam or/and detergents.
3. Application of biocides.
4. Control of water and microorganisms at the bottom of storage tanks.

For a long-term storage of strategic stocks of oil and fuels in industrial tanks, biocides (which are less labour-consuming and provide a long-term fuel protection against microbial infection) are widely applied. Biocides are capable to influence external cellular structures, cellular membranes and cytoplasmic structures causing death of microorganisms. The most widely applied biocides are based on quaternary ammonium bases, glutaric aldehyde, HCic components with length of a carbon chain from C_{10} up to C_{12}, chlorine, iodide- and bromine-organic compounds, poly-unsaturated aromatic amines as well as some metals (copper, nickel, cadmium, arsenic etc).

However, application of various chemical compounds for protection of oil and fuels frequently leads to environmental pollution due to the slow degradation of these compounds, many of which possess mutagenic and carcinogenic properties. A search for effective biocides, which would combine various useful properties and would operate in low concentration with no negative environmental impact, is being pursued.

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