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THE DIESEL AND BIODIESEL FUEL BIODEGRADATION TESTING IN ORDER TO CONTROL SUPPLY CHAIN AND PRESERVE FUEL QUALITY

Abstract

The mayor microbial problem in the petroleum refining industry in the world is that microbial contamination of stored products leads to loss of product quality, sludge formation, deterioration of pipework and storage tank in the refinery due to bio corrosion processes, huge costs of equipment reparation and replacement, deposition and blockage of filters, pipes, valves, engine failures and finally loss of reputation (customers are talking about).

In this paper the results of biodegradability potential of autochthonous microbial culture isolated from hydrocarbon unpolluted soil are presented. Mixture of biodiesel/diesel B10 (10% biodiesel/diesel (v/v) and biodiesel B100 (FAME, fatty acid methyl ester) was tested. The results were compared to the results of reference fuel (euro diesel). From diesel and biodiesel fuel, mixed microbial cultures were isolated as well. The results showed that the mixed microbial population that has not previously been exposed to petroleum hydrocarbons in the laboratory degraded FAME completely after six days and pure diesel fuel but to a lower extent. The difference was observed in the growth rate depending on the source of carbon (FAME or pure eurodiesel) as well as in growth conditions, depending on the culture medium composition. From the study results, it can be concluded that in the entire fuel production, storage and supply chain after FAME addition there will be the enhanced microbial proliferation, which can, if "good housekeeping practice" will not be implemented, resulted with fuel quality reduction and biocorrosion.

The aim was to meet company authorities what kind of problems the company will face after the introduction of biodiesel (fatty acid methyl ester FAME) in the production process and to introduce the new fast and precise methods for fuel microbial infection detection. In addition, based on laboratory research result, company's fuel and distribution supply chain conditions and experience from petroleum industry in the world preventive and protective measures were proposed.

Key words: *fuels, biofuels, quality, biodegradation*

1. Introduction

Biodegradation or biodetrioration of fuels was primarily investigated for two reasons: first microbial spoilage of petroleum products has economic implications and the second; petroleum oil spills constitute a major source of contamination of the ecosystem. In the early forties of the last century, storage tank explosions were for the first time connected with microbial anaerobic production of explosive gases - methane, hydrogen and hydrogen sulphide. Until today in different industries many cases of tank explosion caused by microbial infections are known (1,2). The hazards associated with fuel microbial contamination was underscored until 1958 when a B-52 crash was directly attributed to the plugging of an in-line fuel filter (3). Besides the above phenomena, microbial activity became the major concern in world oil and gas production and transportation due to bio corrosion processes. Alaska's pipeline leak in the spring 2006 was the major cause of 4% cutting oil supply in the U.S. and oil prices rising, heightened attention to bio corrosion. As infrastructures are aging, biocorrosion is becoming more often a risk factor in many industrial operations (4). According to a recent survey, damage due to corrosion in the United States is estimated at 276 x 10^9 dollars per year. Similar surveys in the United Kingdom, Japan, Australia and Germany estimate the cost of corrosion to be 1-5% of the gross national product. Microbiologically influenced corrosion is reported to account for 20%-50% of the total cost of corrosion (5,6).

All materials are susceptible to biodegradation e.g. wood, metals, plastic, ceramic, glass and hydrocarbon fuels as well (7). Actually, the difference between bio-remediation of ecosystem and biodeterioration of products is purely commercial. On the outer side of the "fence", microbial activity is called bioremediation and on this side of the "fence" microbial activity is called biodetrioration when the product is losing commercial value due to microbial infections. Introduction of the fuel system icing inhibitor - ethylene glycol monomethyl ether (EGME), reduced the number of microorganisms in field fuel systems and many different additives developed since then have driven changes in the constitution of the fuel microbial communities. On the other side, low sulfur content and implementation of biodiesel blends increased the likelihood of microbial fuel infections and degradation.

Problems increased by introducing first generation biodiesel fuels due to more stringent regulation thorough Kyoto protocol 1997 and EU directive 2003/30/EG, 2003. Biodiesel (FAME) contributes to reduction of sulfur, carbon dioxide and particulate emissions. Despite benefits, biodiesel is more biodegradable than conventional fuels what will be presented in this paper. Absence of aromatic molecules, hygroscopic properties and availability of high-energy ester bonds contribute to biodiesel biodegradability that leads to the particulate formation (8). The presence of water in ethanol as second most wide used biofuel leads to the growth rate increase of fatigue crack of steel alloys. Bacteria known to accelerate corrosion have been identified in ethanol storage tanks (9).

Contamination of any kind can be introduced at different stages of production, storage and distribution (Fig. 1) (10).

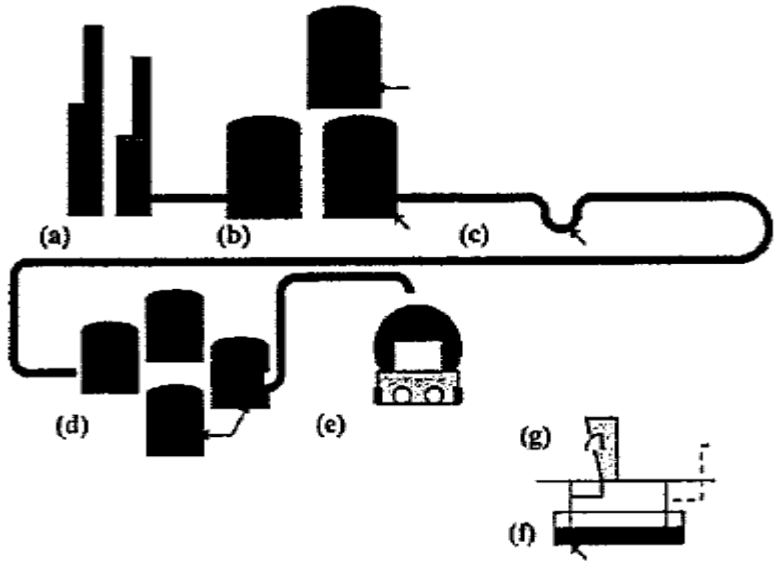

Figure 1: Fuel distribution systems (10). Arrows show where water and microorganisms tend to accumulate: a) production, b) refinery tanks, c) pipeline, d) warehouse tanks, e) transportation, f) underground tanks, g) distribution

Clean fuel contains no solid particles or liquid contaminants, microbial biomass, microbial metabolic products such as bio surfactants, gases (hydrogen sulphide, methane) or acids. Sources of microbial infections are water (even present in fuel in micro amounts) and air that is entering tanks thorough vents. The main consequence are higher amount of solids from corrosion (biological and chemical), microbial particles, sludge on the tank bottoms and biofilm on the tank walls.

The modern diesel engines injectors are operating in substantially higher pressures and bigger number of orifices, which are discharging the fuel into combustion chamber at extremely high pressures. However, the tolerances on the injection components are much smaller and if higher demands on the fuel cleanliness (e.g. particulate of 2 μm in diameter) and quality are not met, the engine will not perform, as designed and premature failure will result (11). The engine manufacturers (www.acea.be) defines incoming fuel cleanliness levels which for new HPCR engines (High Pressure Common Rail) require diesel fuel cleanliness levels as low as 12/9/6 per ISO 4406.

For this reason, diesel fuel cleanliness control must be managed with advanced particulate and water filtration and fuel polishing technologies from the point of delivery, thorough storage to the dispenser nozzle.

The aim of this study was to determine the degree of susceptibility to biodegradation of three types of fuel (diesel, B10, B100) and compare biodegradation rate under different conditions. In addition, the aim was to isolate microorganisms from the fuel stored for a long time to prove their presence in the product.

2. Experimental part

Materials and methods

Microbial mixed culture was isolated from soil unpolluted by oil hydrocarbons or fatty acid methyl esters in order to prove that even unadapted microbial consortia from the environment can degrade tested fuels. One gram of the soil was inoculated to 100 ml culture medium. Source of carbon was diesel fuel (5% v/v). In the exponential growth phase, 10 ml of inoculum was added to 90 ml of two different mineral media (N4 and D1) for microorganisms' cultivation and with three different source of carbon (diesel, B10, B100) separately. The growth of mixed microbial cultures in D1 mineral media with different sources of carbon (diesel, B10, B100) was monitored by optical density (OD) measurements for 12 days and pH adjustment (Fig. 6). Fuel biodegradation degree was monitored by gas chromatography (Figs. 7-10). All assays were conducted in Erlenmayer flasks vol 500 ml and 100 ml media for submerged cultivation in the shaker with controlled temperature (pH 7,0±0,2, 25 °C i 130 rpm). All assays were conducted in duplicate and with control flasks where microorganisms were not inoculated. After 6 to 12 days of cultivation oil was extracted with tetra chloromethane (CCl_4) and in the samples biodegradation rate was analyzed by gas chromatography on Varian 3100 CX and Varian CP 3800 after extraction. Isolation of microorganisms from stored fuels in the laboratory vessels was performed by filtration and culture method (ASTM D6974-09) (Figs. 2-4). Fuel was sampled with sterile 50 ml pipette from the bottom of the vessel where water and microorganisms are settled during fuel stilling.

Medium for inoculum cultivation:

100 ml of minimal Davis Broth D1 without dextrose and citrate in 500 ml Erlenmeyer flasks with diesel fuel 5% (v/v) as only source of carbon.

Liquid mineral media for mixed culture cultivation:

- Liquid mineral media D1: Minimal Davis Broth without dextrose and citrate, Sigma-Aldrich, pH 7, 0±0, 2

- Liquid mineral media N4: KH_2PO_4 2,81g/L; NH_4Cl 3,82 g/L; $MgSO_4x7H_2O$ 1,0 g/L; $CaCl_2xH_2O$ 0,38g/L; NaCl 0,1 g/L: pH 7, 0±0, 2

Carbon source: INA euro diesel (diesel); FAME - fatty acid methyl ester (B100); 10% FAME in euro diesel (v/v) (B10).

Extraction procedure for GC analyses:

100 ml of sample from Erlenmeyer flask in 250 ml separation funnel with 50 ml of tetra chloromethane (CCl_4) shaken for 40 minutes on laboratory shaker. From tetra-chloromethane, layer aliquot is transferred for analyses. In the case of emulsion formation, the samples were centrifuged (10000 rpm, 10 min).

Gas chromatography analyses: Varian 3100 CX, Varian CP 3800

Solid culture media for isolation of microorganisms from fuel on Petri dishes:

- for yeast and mold cultivation: MEA Malt extract agar pH 5.2±0.2, Merck, Germany, for suppression of bacteria growth 1 ml 1.0% solution of tetracycline hydrochloride (Calbiochem, USA) added.
- for bacteria cultivation: TSA Tryptone soya agar pH 7.3 ± 0.3 (Oxoid, England)

3. Results and discussion

Direct consequences of fuel microbial contamination are: metabolic attack on hydro-carbon and fuel additive molecules, metabolic production of surfactants, organic acid and sulfide production, increasing of biomass fraction and biofilm formation. As further consequences in the fuel production, storage and distribution processes are microbially induced corrosion; filter plugging, engine wear, corrosive deposits on engine parts, reduced heat of combustion, fuel property changes and loss of additive. To prevent all these manifestations good knowledge of very specific microbial physiology of insoluble oily substrates is of great importance.

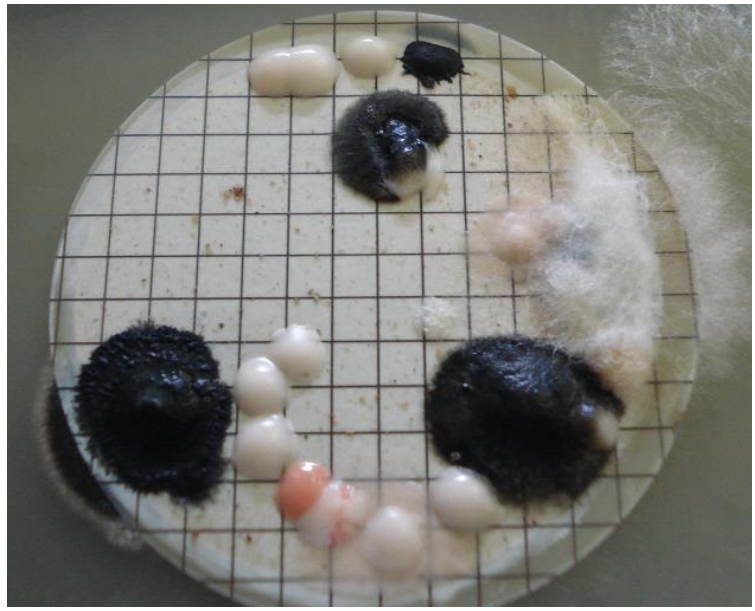

Figure 2: Mixed microbial culture isolated on MEA from the bottom of laboratory FAME tank after 2 month of storage

The introduction of fast and accurate methods for determining the cleanliness of supply chain and fuels should be based on good knowledge of microbial consortium physiology in a medium such as hydrocarbons and biofuels primarily due to their characteristics of insolubility in water. The composition of microbial populations in the facilities is very different and changes fast depending on the conditions in which microbes grow (Figs. 2-4).

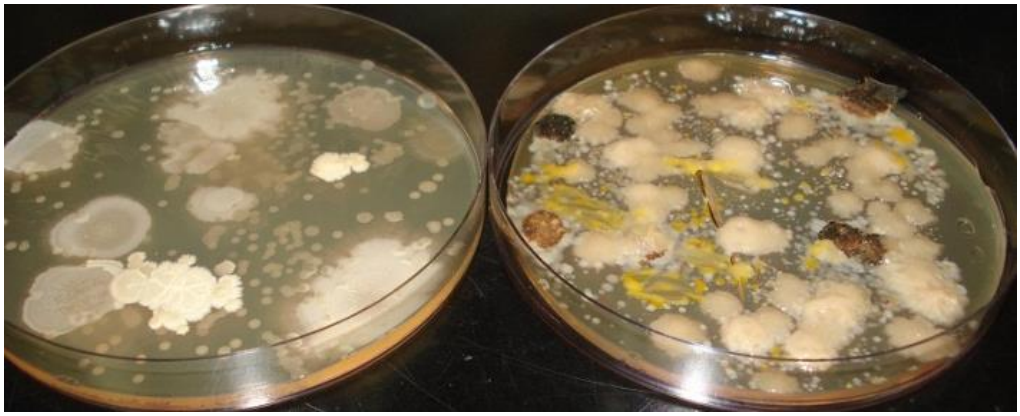

Figure 3: Mixed microbial culture isolated in laboratory conditions from water layer on the bottom of the laboratory vessel with B7 on two different growth media TSA, MEA

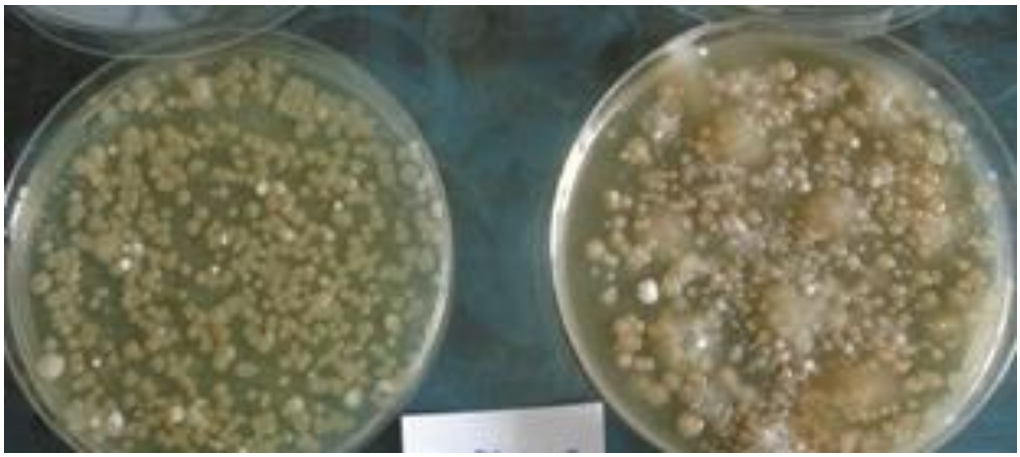

Figure 4: Mixed microbial culture isolated on two different growth media (TSA, MEA) from laboratory vessel bottom with euro diesel after 3 month of storage

Oil has to pass cell membrane to be assimilated by microorganisms, which are clustering around small hydrocarbon spheres (Fig. 5) (12). The ability of microorganisms to accumulate hydrocarbon substrates intracellularly that, in turn, indicates transport across the cell membrane was determined by electron micrograph, gas chromatography and x-ray fluorescence (13). To pass thorough cell membrane the volume of oil droplets should be reduced by secretion of extracellular surfactants (13). That is common characteristic of many oil-growing microorganisms (14,15). As the first consequence of surfactants production, in fuel chain production and delivery the appearance of emulsion is not rare even in the upstream processes. The second consequence is that bio surfactant can change physical characteristics of fuel.

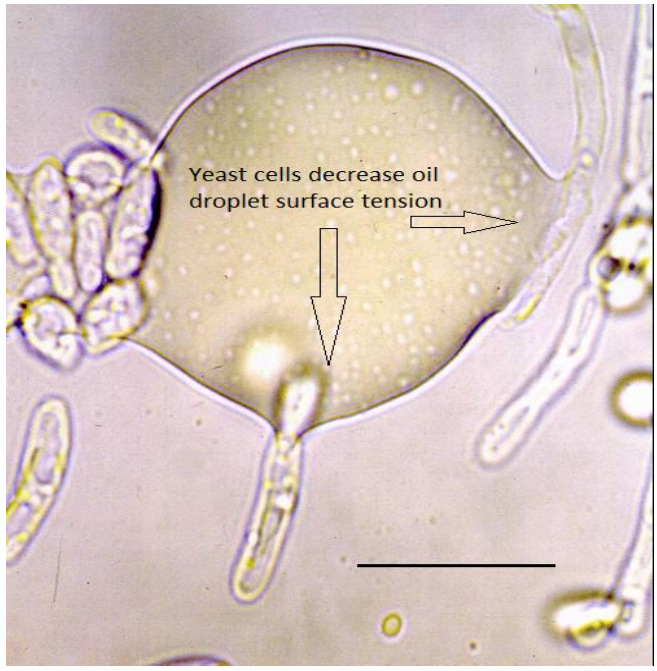

Figure 5: Yeast cells isolated from northern Adriatic Sea on Angola crude oil droplet in the artificial sea medium (12), bar 10 μm; (Photo: Authors)

In this work the growth microorganisms on different carbon sources from oil as single carbon source was investigated: diesel fuel, B10 and pure FAME. The origin of fuel microorganisms is environment – air, dust, moisture and water. The results of growth on different carbon sources presented on Fig. 6 show that microbial mixed culture during growth on B10 and pure FAME had shorter lag phase and longer log phase than microorganisms that had diesel as single carbon source. This corresponds to the results of the biodegradation rate between pure diesel and B10 (Fig. 7).

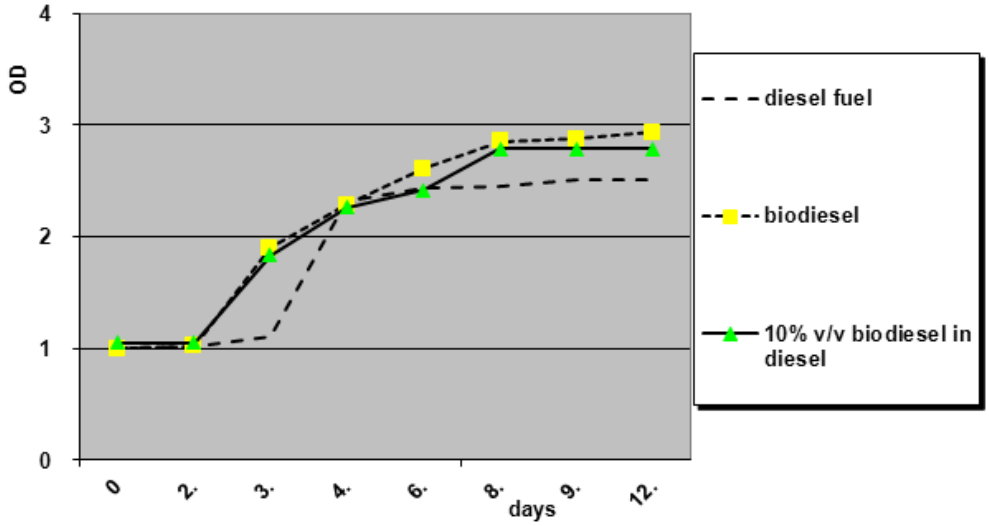

Figure 6: Microbial culture growth in liquid media (D1, 25 °C, 130 rpm) on different carbon sources during 12 days determined by optical density increase (OD 460 μm)

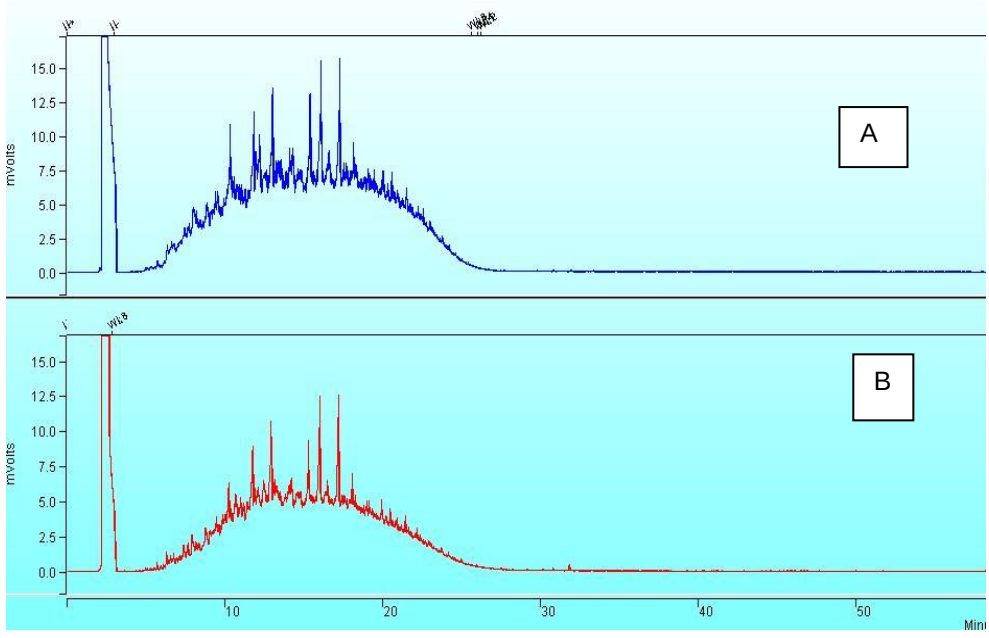

Figure 7: Biodegradation of two different carbon sources on the same mineral media (N4); A) diesel fuel, B) B100

By comparing the chromatogram of diesel and B10 relating to the biodegradation of the same nutrient medium (N4), the degradation of both samples is observed, with a substantial deterioration of the sample using a B10 as substrate. The different biodegradation results of substrates in different media (N4 and D1) shows the impact of different environmental conditions on the growth and biodegradation rate (Figs. 8 and 9).

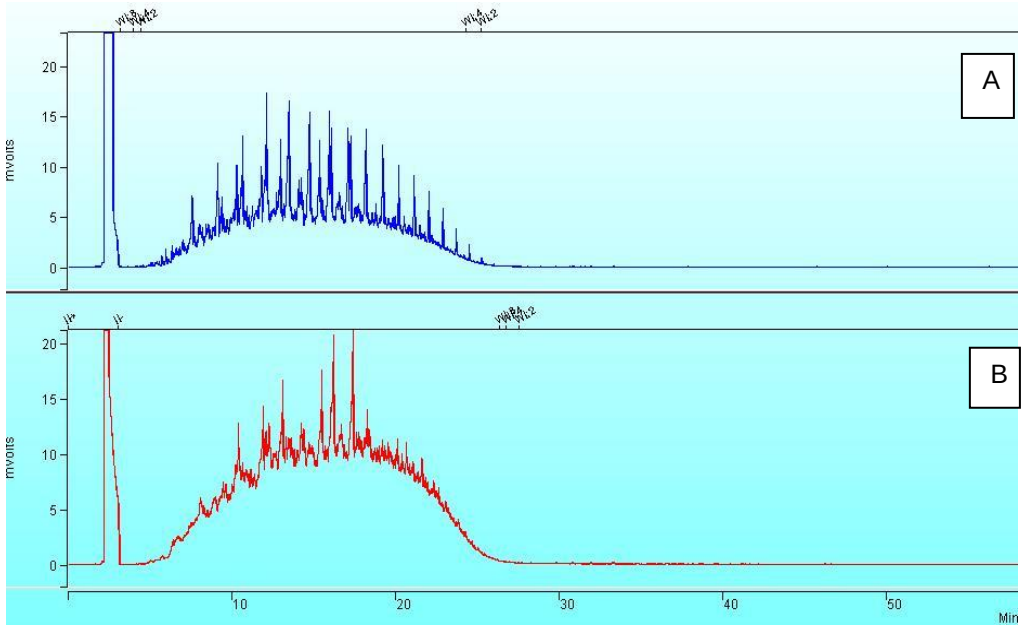

Figure 8: The difference of the diesel fuel biodegradation rate depending on growth media; A) diesel fuel biodegradation on growth media D1, B) diesel fuel biodegradation on the growth media N4;

Gas chromatography analysis results of the diesel biodegradation in the nutrient medium N4 indicates the degradation of all hydrocarbon compounds, with a more pronounced degree of degradation of hydrocarbons with higher boiling point. It means that along the production, supply chain conditions are changing, and as the consequence, different degree and type of infection will be present. In the laboratory conditions biodegradation of B10 after six days was complete (Fig. 10). Peaks of hydrocarbons with a pronounced peak of fatty acid methyl esters characterize chromatogram B10 of abiotic control after 12 days. Because of biodegradation of the samples after 6 days the significant absence of hydrocarbon peaks is observed and the complete absence of the of fatty acid methyl esters peak. Shorter lag phase growth and longer log phase growth on B10 as a single carbon source (Fig. 6) and complete biodegradation in six days in the laboratory conditions (Fig. 10) indicate

that microbial infections of fuel chain with FAME added will be of the larger scale than with no FAME in diesel. It explains the large increase of numerous gas stations clogging in the world after FAME implementation and in most cases when in the underground storage tanks (UST) water and microbial population was already present.

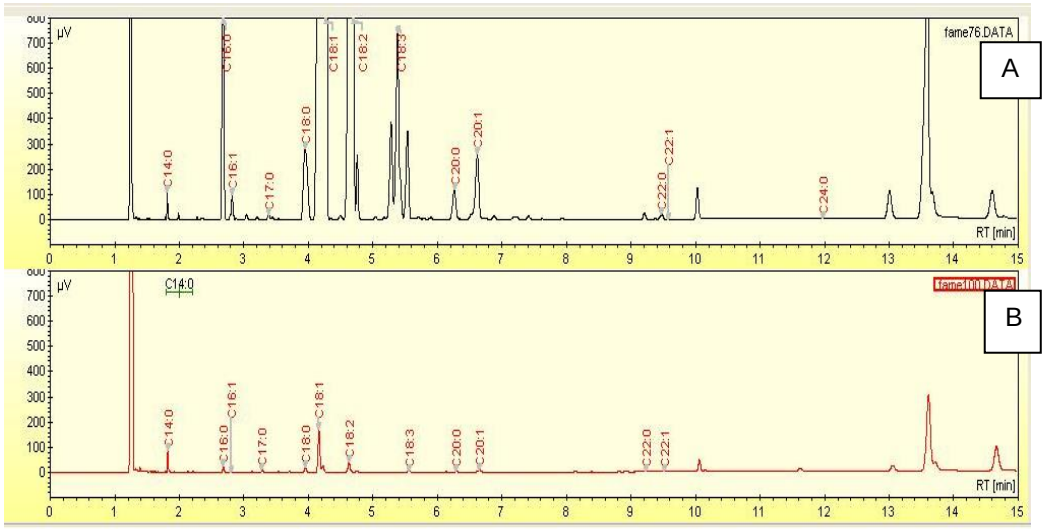

Figure 9: Biodegradation of FAME as sole carbon source on two different growth media; A) D1, B) N4

Water is the main source for microbial growth. Newly refined fuel cools, dissolved water start to condense and tends to accumulate in tank bottom and in pipeline low points. This process continues during transport downstream and depends on initial water content, settling time and position of suction line. Dust and moisture enter also thorough not protected tank breathers. Regular control and water removal is the main activity that can lower microbial growth. It could be difficult due to tank construction and even more problematic in underground storage tanks on gas stations where water accumulates on the low point. Even 1 mm of water is sufficient to start microbial proliferation and even the greater problem is fuel system fouling where biomass accumulation restricts fuel flow. The most common symptom is filter plugging, malfunction of valves, pumps or other moving parts (16, 17).

The fouling is the process of attaching microbes on the surfaces of tanks and pipelines where biofilm microbes are embedded in a complex, heterogeneous extracellular polysaccharides matrix (18) under which process of microbial corrosion (MIC) is present (19).

The creating of chemical and electro potential (Galvanic cell) gradients between biofilm covered surfaces and surfaces that are exposed to bulk fluid starts with aerobic bacteria which scavenge oxygen and create anoxic environment for sulfate reducing bacteria or iron oxidizing bacteria. The metabolite production and microbial activity in the biofilm facilitate pitting, consumption of corrosion inhibitors and degradation of protective coatings (20). Black iron sulphide formation show sulfate reducing bacteria activity on the fuel tank bottom (Fig. 11) (21). The consequence of this process is corrosion particles formation (Fig. 12) (36).

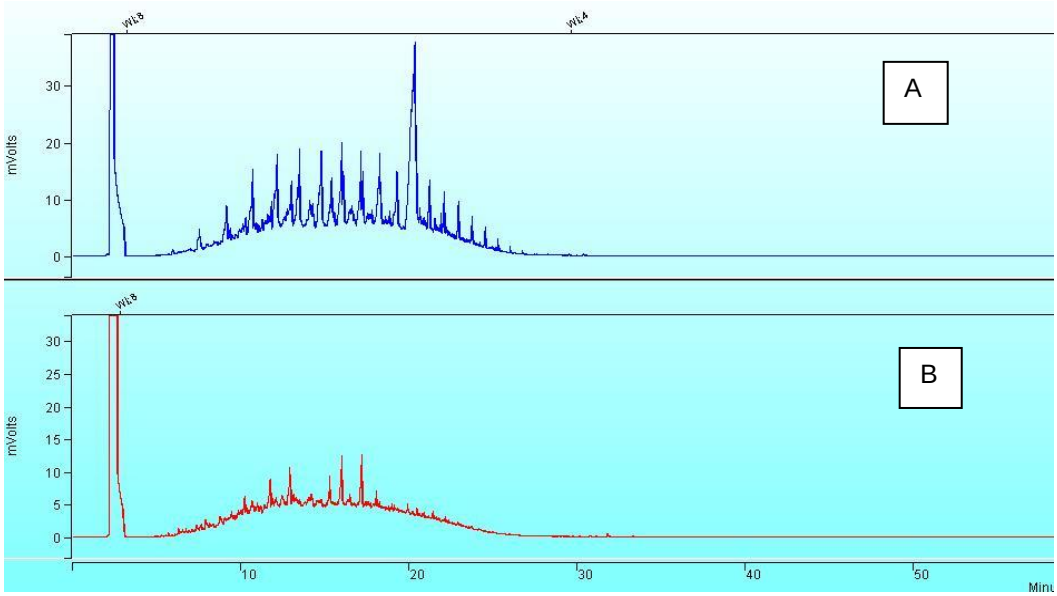

Figure 10: The biodegradation of 10% w/v biodiesel in diesel after 6 days of mixed microbial culture submerged growth (N4); A) 10% of biodiesel in diesel, abiotic control, 12 days, B) 10% of biodiesel in diesel, 6 days

As presented in this work microbial activity is higher in B100 and in FAME blends where could be even higher than in B100 (22). Biodeterioration is very complex biological process dependent of many physiological and environmental factors one of is presented in this work as biodegradation on two different growth media (Figs. 8 and 9). Apart of water drainage, biocide application could be one of preventative measures but it does not prevent the introduction of water and cannot replace good housekeeping practice. Moreover incorrect biocide application may even increase the resistance of microorganisms to treatment. Therefore, specialist should execute biocide treatment who can also advise on the selection of the most appropriate biocide for the type of the microbial infection (23).

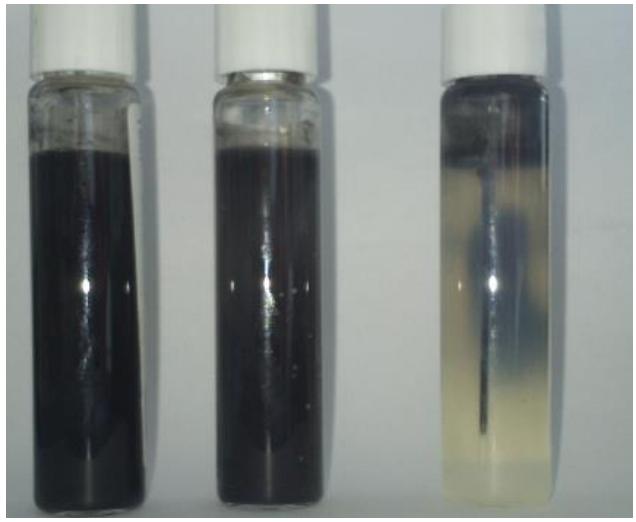

Figure 11: Iron sulphide formation in the fuel tank bottom sample (Easicult test for anaerobic sulphide generating bacteria (21)

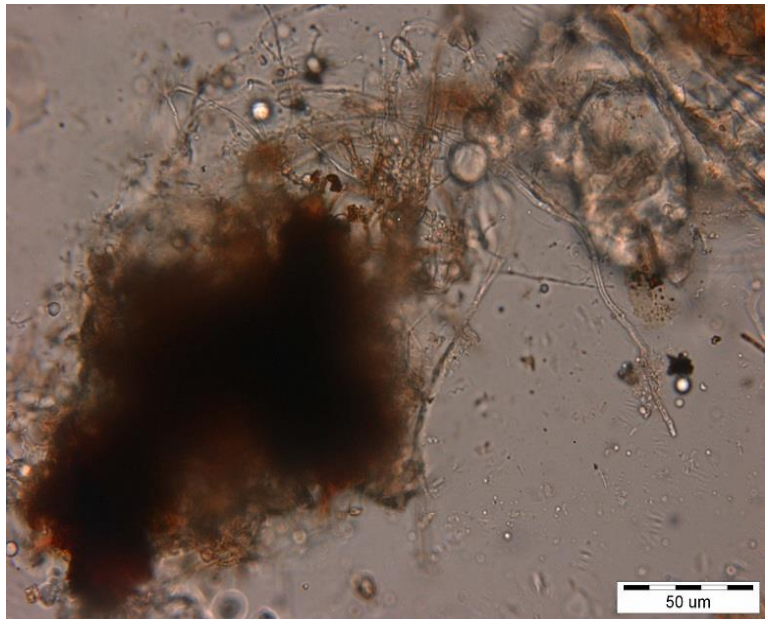

Figure 12: Micrograph of the corrosion particle (dark) embedded in the biomass in the sample from the diesel fuel metallic tank (filamentous artifacts);
(Photo: Authors) (36)

4. Conclusion

In previous works biodegradation capability of different oil hydrocarbons by autochthonous microbial population from the north part of Adriatic Sea was investigated as part of the project "*Microbial research of ecological risks at sea"* (12,14,24-29). "*Soil bioremediation and groundwater protection from accidental oil spills"* project was proposed in order to implement cost benefit methods for cleaning of hydrocarbon polluted soil and methods for cleaning and biodegradation of the company own hazardous waste before disposal (reduction in waste disposal costs) (30-34). Although company management give up of these projects, that was the excellent base for acquiring a lot of specific knowledge about hydrocarbon microbiology, laboratory practice and experience in the procedures for determining the rate of insoluble substrates biodegradation under different conditions.

Laboratory results showed that in the entire fuel production, storage and supply chain after FAME addition there would be enhanced microbial proliferation, which can, if "good housekeeping practice" will not be implemented, result with fuel quality reduction and biocorrosion.

Based on acquired knowledge and extensive field practice the cleanliness of entire fuel chain is determined. New microbiological techniques and analysis were introduced, and that was the basis for the introduction of the methods for microbial control estimation and control in fuel chain, preparation of different measures of fuel chain maintenance and finally, formulation of *Good housekeeping guide* for the whole fuel production and distribution chain (35-38). To preserve quality of stored products from refinery to distributor and to prevent microbial contamination a good housekeeping is the main approach. The control of all maintenance procedures, periodic microbial testing, cleaning procedures and biocide application should be good documented in order of cost reduction and customer satisfaction.

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