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Research article

Assessment of biocides and ultrasound treatment to avoid bacterial growth in diesel fuel

Luis Fernando Bautista [a,*], Carolina Vargas [a], Natalia González [b], María Carmen Molina [b], Raquel Simarro [b], Armando Salmerón [c], Yolanda Murillo [c]

[a] Department of Chemical and Energy Technology, Chemical and Environmental Technology, Mechanical Technology and Analytical Chemistry, ESCET, Universidad Rey Juan Carlos, C/Tulipán s/n, E-28933 Móstoles, Madrid, Spain
[b] Department of Biology and Geology, Physics and Inorganic Chemistry, ESCET, Universidad Rey Juan Carlos, C/Tulipán s/n, E-28933 Móstoles, Madrid, Spain
[c] Repsol Technology Centre, C/Agustín de Betancourt, s/n, E-28935 Móstoles, Madrid, Spain

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Prevention and avoiding growth of microorganisms inside fuel storage tanks is a major concern for the oil industry because the associated problems caused by corrosion, plugging and blockage in storage and dispensing facilities. To effectively control microbial growth in petroleum fractions, assessing new treatments and acquiring deepen knowledge about existing treatments must be performed. In the present work, we compare and evaluate the efficiency of several chemical and physical treatments on the growth of microorganisms found in real samples of diesel fuel from different storage tanks from petrol stations in Spain. The treatments include the use of ten organic biocides with different functional active groups, hydrogen peroxide and ultrasounds. The better results were obtained with water-soluble biocides, especially those with an oxazolidine group in the active compound, although the effectiveness of different biocides very much relies on biodiversity and physicochemical properties of the media.

1. Introduction

The use of petroleum fractions by some microorganisms as energy and carbon sources has been known for years. This property has been applied mainly for bioremediation of soils polluted with hydrocarbons. Nevertheless, this natural degradation of hydrocarbons mediated by microorganisms has become a serious problem for the oil industry, since microbial growth has been observed to occur inside fuel storage tanks. This fact is causing serious problems such as corrosion, filter plugging or blockage of pipes [1]. However, the presence of microorganisms in storage tanks causes no significant changes in the physicochemical properties and quality of fuels [2].

1.1. Fuel characteristics

Table 1 shows the main fuels derived from fractionation of petroleum. Properties of different types of fuels are controlled in terms of density, distillation, viscosity, lubricity, volatility, octane number, cetane number or stability, among others [3]. With this regard, several types of additives can be included in fuel formulations such as antiknock agents, corrosion inhibitors, lubricity modifiers, cetane number and cold flow diesel fuel improvers, detergents, dispersants, metal deactivators, antifoam or antioxidants for the elimination of free radicals [4]. The presence of some additives can be a nutrient resource for microorganisms promoting microbial growth in the amount of water and sludge formation.

Both diesel fuel (C16–C22) and kerosene (C9–C15) are prone to cause problems related to microbial growth [5]. It was found that C10–C18 is the optimum range for microbial metabolism while shorter-chain hydrocarbons inhibit microbial growth [4].

Moreover, worldwide increasing environmental restrictions in sulphur content in fuels contributes to microorganisms development [4].

1.2. Factors enhancing microbial growth

Underground fuel storage tanks are exposed to microbial contamination due to limitations in the design and maintenance. Moreover, serious pollution problems derived from fuel contamination can be generated because of corrosion and possible leakage from tanks. Therefore, buried tanks are being replaced by outdoor above-ground tanks in some areas, although higher fire risk is associated to this tank configuration.

Possible routes of entry of microorganisms into the tanks can be from the ground (buried tanks), through faulty seals or cracks in the tank; from the air, during loading operations and through the vents;

* Corresponding author.
E-mail address: fernando.bautista@urjc.es (L.F. Bautista).

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Table 1
Range of number of carbon atoms in main petroleum fractions [4].

Fraction	No of C atoms
Gas	1–4
Gasoline	5–8
Kerosene	9–15
Diesel	16–22

or introduced along with water during the washings of tanks [6]. Once the microorganisms are in the tank, they adhere to the walls or settle in the fuel-water interface at the bottom of the tank [6]. If the presence of water inside the tank is above 1% (v/v), microbial growth is developed [7]. The presence of water inside diesel storage tanks may be due to several causes such as the water content allowed by regulations, condensation of the air humidity that enters the tank through the vents or faults during the filling or draining of the tanks. Moreover, sometimes it is possible the entry of water in the tank during the operations needed for purging pipes as well as the formation of water as the end product of cellular respiration as a consequence of the metabolic activity of microorganisms [4].

The quantity of water that fuels contain in their composition is regulated. As an example, in the European Union, water content in automotive diesel fuel is limited to 200 mg/kg (EN 590 European Standard [8]). Moreover, water content should not exceed 500 mg/kg in biodiesel (FAME), which is regulated by the EN 14214 European Standard [9].

On the other hand, the presence of oxygen also affects the microbial growth. Although many of the species found inside the oil storage tanks are anaerobic or facultative anaerobic, the presence of oxygenated compounds facilitates their oxidation as they are more easily assimilated by the present microorganisms [4].

Another factor to take into account for microbial growth is the presence of additives, devoted to improve fuel properties, containing chemical compounds or elements which are nutrients for microorganisms [4, 7]. Finally, both temperature and optimum pH for microbial growth are within usual the range of fuel storage tanks conditions, promoting again the proliferation of microorganisms [7].

1.3. Microorganisms inside the oil storage tanks

Most microorganisms are developed in the bottom of the tank where sediments and water are accumulated forming a sludge. Many species of microorganisms able to grow in the presence of hydrocarbons have been described and identified inside fuel storage tanks, some of them are shown in Table 2 [10–12].

Although water phase or water-organic interphase are the main environments where microorganisms are grown inside diesel fuel storage tanks, their corrosion effect is not limited to the submerged materials and dispensing infrastructure. The production of volatile and corrosive compounds, such as acetic acid, by bacteria of the family Acetobacteraceae can induce corrosion even in the headspace of the tanks [13,14].

In the group of sulfate-reducing bacteria (SRB) several species of bacteria are included, such as *Desulfovibrio* sp., *Desulfobacterium* sp. and *Desulfomicrobium escambium*, among others [15]. They use organic compounds by reducing the sulfate anion to hydrogen sulfide, which is highly corrosive and presents a significant risk human health. Davidova et al. [16] demonstrated that the reduction of sulfate to sulfide decreases in a half when high concentration of nitrates are present. The intermediates of nitrate reduction, nitric oxide and nitrous oxide, increase the temperature and inhibit the production of sulfides and therefore reduce SRB populations but encourage the growth of denitrifying bacteria.

The presence of microorganisms in the storage tanks of petroleum fractions is completely related to the corrosion problems present in both tanks and pipes [17,18]. There are products that are used to overcome this problem, but some microorganisms are able to degrade them [19]. In addition, another factor to take into account is that microorganisms can form flocs when they are washed from the tank producing problems such as obstruction in the filters [20].

1.4. Alternatives to prevent microbial growth

Several alternatives have been studied to avoid and prevent microbial growth in storage tanks of petroleum fractions. A possible alternative in order to solve this problem consists in the development of a controlled drainage system, with good maintenance practice and frequent cleaning [2]. Nevertheless, other methods under study to prevent microbial growth are ultraviolet radiation treatment, sonication, or chemical attacks with hydrogen peroxide and biocides.

Ultraviolet radiation has been used in many applications as a disinfectant of water or air as it produces photobiochemical reactions in microorganisms [21]. UV radiation causes alterations in DNA producing the bases intersection in the structure. Thymine and pyrimidine dimers are formed producing mutations that disable the cell replication leading to the microorganism death [22]. Studies of this effect on the bacterium *Escherichia coli*, reveal that the decrease in the population is proportional to the intensity of UV radiation received. This technique has some environmental advantages, such as the absence of chemicals without residual compounds reducing the environmental impact. Nevertheless, UV radiation treatment has some disadvantages such as an increase in the temperature of the medium and proliferation of more colonies of microorganisms. It is also known that microorganisms overshadow each other blocking UV and reducing the effectiveness of the technique [22].

Sonication is another treatment that has been studied in last decades to eliminate microorganisms as an alternative to conventional techniques [23,24]. It consists of applying ultrasonic waves in a liquid medium generating acoustic cavitation. Air bubbles are generated and they liberate a variable amount of energy when collapse, causing damage to the cell structure. Furthermore, highly reactive radicals, such as ·OH and ·H, are formed inside the bubbles are released to the medium, attacking the microorganism structure and causing cell death [25].

Hydrogen peroxide is a compound with a high oxidizing power. It produces free ·OH radicals that attack organic matter by destabilizing the cell wall and other structures such as DNA, causing cell death. This chemical disinfectant is more effective with anaerobic organisms because they lack of the enzyme catalase, which decomposes hydrogen peroxide into molecular oxygen and water. Although the disinfection capacity of ultrasonic and hydrogen peroxide treatment in aqueous environments is known, their use in a hydrocarbon medium could cause concern due to the flammability of diesel fuel and the oxidizing properties of hydrogen peroxide. However, recent literature [26–29] addresses the save use of ultrasonic treatment and addition of hydrogen peroxide

Table 2
Microorganisms identified in oil storage tanks.

Bacteria	Fungi	Yeast
Actinetobacter, *Bacillus sp.*, *Clostridium sporogenes*, *Flavofacterium diffusum*, *Micrococcus sp.*, *Pseudomonas sp.*, *Pseudomonas aeruginosa*, *SRB*[a], etc.	*Acremonium* sp., *Aspergillus* sp., *Aspergillus fumigatus*, *Cladosporium* sp., *Fusarium oxysporum*, *Penicillium* sp., *Penicillium citrinum*, *Penicillium funiculosm*, *Trichiderma* sp., etc.	*Candida* sp., *Candida famata*, *Candida lypolytica*, *Candida silvícola*, *Candida tropicalis*, *Rhodotorula* sp., *Saccharomyces* sp., etc.

[a] SRB: sulfate-reducing bacteria.