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Microbiological aspects of biodiesel and biodiesel/diesel blends biodeterioration

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ABSTRACT

The present work consisted of evaluating and comparing, at bench scale, the biodeterioration processes of diesel oil, soy biodiesel (B100 soy), beef tallow biodiesel (B100 beef tallow) and the resulting blends containing 5% biodiesel and 95% diesel oil (B5), throughout 42 days storage. Fuels were stored with and without the inoculation of a microbial consortium from a water drainage tank, under conditions that simulated service station storage. Oxygen consumption and carbon dioxide production were more pronounced in the presence of microbial inoculum, especially for B100 and B5 beef tallow. Diesel oil instead was the least subject to chemical and/or biological oxidation. A trend of increase in acid number was also observed and for B100 soy and B5 beef tallow the regulatory limits were exceeded. Residues deposition was observed in all inoculated fuels, especially in B5 beef tallow. Communities' differentiation depended on biodiesel feedstock and occurred during storage time. Among all microcosms, 18 microbial genera (8 fungal and 10 bacterial) were identified by DNA sequencing, being 17 of them directly linked to: fuel microbial contamination and/or biodegradation (74%); biofuel production or related enzymes expression (26%); biofilm formation and/or exopolysaccharide production (26%), emulsifying properties (11%) and nitrogen fixation (5%). Finally, despite the biodeterioration events in all the fuels, such phenomenon had small magnitude, even in inoculated samples.

Introduction

Literature reports that biodiesel is more biodegradable than diesel oil and that the increase in the biodiesel content in blends tends to increase their biodegradability (Pasqualino et al., 2006; Mariano et al., 2008; Bücker et al., 2011; Cyplick et al., 2011; Sϕrensen et al., 2011; Passman, 2013). Biodegradation can be even more accentuated due to the hygroscopicity of biodiesel (Sϕrensen et al., 2011), which varies according to the composition of its feedstock (Oliveira et al., 2008). Therefore, the amount and the nature of the biodiesel applied in blends would be critical factors to determine the extent of fuel biodeterioration and its impact on fuel quality.

Besides the possibility of quality loss, microbial biomass produced during the biodeterioration process (Schleicher et al., 2009; Bücker et al., 2011; Sϕrensen et al., 2011) might potentially contribute to the obstruction and reduction of fuel filters life time (Passman, 2013), becoming a threat to operational activities. Thus, mechanisms involved in biodiesel/diesel blends (BX blends; $X = \%$ biodiesel in diesel) biodeterioration, especially under storage conditions, are matter of increasing interest (Leung et al., 2006;

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Schleicher et al., 2009; Bücker et al., 2011; Passman, 2013). Leung et al. (2006) demonstrated that increase in temperature and air exposure enhances biodiesel (from rapeseed oil) degradability at long-term storage (more than 10 weeks). On the other hand, Schleicher et al. (2009) verified that aerobic conditions were not favorable to microbial growth in pure rapeseed biodiesel, but in B5 and B20 blends microbial counts were higher just in the presence of O_2. These authors also reported that, in the blends, bacteria were the majority, and the highest bacteria proportion was found in B5, i.e. the one with the lowest biodiesel ratio. Besides, Bücker et al. (2011) demonstrated that the higher the proportion of soy biodiesel in blends, the higher the fungal growth. However, those authors have not discussed the effect of biodeterioration process on the fuel properties.

Thus, considering the reported evidences of increase in fuel biodegradability and susceptibility to microbial contamination in the presence of biodiesel, it becomes critical to evaluate the extent of microbial impact on the quality of biodiesel and biodiesel/diesel blends under storage conditions.

In this context, the aim of this study was to assess: (i), How susceptibility to biodeterioration varies according to biodiesel feedstock (soy and beef tallow); (ii), Microbial growth and activity impacts on the characteristics of the products tested; (iii), The occurrence and extent of microbial deposits formation; (iv), the main fungal and bacterial genera and/or species involved in the biodeterioration process. Special attention was given to soy and beef tallow biodiesel/diesel blends behavior under storage, due to their distinct level of saturation and importance in the Brazilian biodiesel market.

Materials and methods

A bench scale study was designed in order to comparatively evaluate the biodeterioration of petroleum diesel with 500 ppm sulphur (S500), soy- and beef tallow-based biodiesel and respective B5 blends. Experiments were carried out under critical storage conditions – presence of water and microorganisms able to use the fuels as carbon and energy sources. Such a proportion of biodiesel:petroleum diesel (5%: 95% v v^{-1}) was chosen because this is the ratio specified by Brazilian National Regulatory Agency (ANP, the acronym for *Agência Nacional de Petróleo, Gás Natural e Biocombustíveis*) for commercialization in Brazil (ANP, 2012). Additionally, since soybean and beef tallow oil are the most common feedstocks used to produce biodiesel in this country (ANP, 2014) and present quite different fatty acids profiles (Passman, 2013), they were selected for testing.

Despite the lack of systematic surveys about the amount of water usually found in service station tanks just before drainage operations, 1% (v v^{-1}) water content in such situations was assumed to be a possible scenario and, therefore, it was applied in this study. It is worth to mention that Brazilian regulation determines that the amount of water in either biodiesel or blends with S500 cannot exceed 500 mg Kg^{-1} (0.05% v v^{-1}) (ANP, 2012, 2013), the same requirement established by international standards (e.g. ASTM D975-14 – diesel oil, ASTM D6751-12 or EN14214:2012 – biodiesel).

Fuels

Diesel distributed in Brazil by the time of this study was a blend of biodiesel (5%) and petroleum diesel (95%) as specified by ANP (2012). Also according to this Agency, the biodiesel feedstock most frequently used to compose the blends in Brazil is soy biodiesel (70–80%), followed by beef tallow biodiesel (15%) (ANP, 2014). Based on these considerations, biodeterioration assays were performed with: petroleum diesel oil with 500 ppm sulphur (100% diesel; "S500"), soy biodiesel (100% soy biodiesel; "B100 soy"), beef tallow biodiesel (85% beef tallow biodiesel + 15% soy biodiesel v v^{-1}; "B100 beef tallow") and their respective blends containing 5% biodiesel and 95% diesel oil S500 (B5) – "B5 soy" and "B5 beef tallow". Fuels were utilized as received, without sterilization and did not receive any additive in their formulation (i.e. antioxidants). Nevertheless, all fuels were analyzed in terms of microbial contamination (total population and fungi) prior to test execution. Excepting for B100 soy (which did not present any cultivable microorganism), all the investigated fuels presented populations lower than 10^{-1} MPN mL^{-1} (total population) and 10^{-1} CFU mL^{-1} (fungi).

Microbial inoculum source, preservation and reactivation

The inoculum used was obtained from a liquid sample collected from a drainage tank of light fuels (i.e. gasoline, diesel and biodiesel) in a storage terminal, as suggested on ASTM E1259-10. The sample was enriched in Büshnell Haas medium containing B5 soy as carbon source. Ten milliliters of sample was added to 90 mL medium (Büshnell Haas medium with 3% B5 soy) in a 500 mL Erlenmeyer flask, incubated at 30 °C and agitated at 150 rpm for 72 h. This procedure was repeated three times. At the last time, inoculum growth was monitored until medium optical density stabilization (at 600 nm in portable Hach DR 2010 spectrophotometer) and it was later centrifuged, re-suspended and cryopreserved in glycerol solution at 10% v v^{-1}.

In B5 soy and B100 soy tests, inoculum reactivation was performed with B5 soy substrate, 72 h prior to the beginning of the tests. Similarly, B5 beef tallow was used to reactivate the inoculum applied in the B100 beef tallow and B5 beef tallow assays. In both cases the mineral medium, carbon source amount and growth conditions were the same used for enrichment. The same standardized inoculum (10^5 MPN.mL^{-1}) was added to all fuels, being mainly composed by *Bacillus amyloliquefaciens*, *Bacillus* sp., *Micrococcus* sp., *Candida* sp., *Candida dubliniensis*, *Candida viswamathii* and *Pichia anomala*.

Experimental conditions

The experimental conditions simulated those of fuel storage at service stations. Special care was taken to establish storage time and temperature, water presence and air exposure, items reported in the literature (Leung et al., 2006) as the main ones to affect biofuels stability.

By sampling weekly along 42 days, different storage periods could be simulated. Another noteworthy aspect is that the resupply of fuel storage tanks in service stations is supposed to allow oxygen to enter the system from time to time. Therefore, at least minimum microaerophilic conditions were guaranteed in the biodeterioration tests.

Fuels biodeterioration was evaluated based on microbial growth and activity, microbial community analysis and determination of chemical and physicochemical parameters susceptible to microbial influence. The assays were established in 500 mL amber flasks with 99 mL of fuel + 1 mL of either inoculum or sterile deionized water (control). Besides, 10 mL flasks with 5 mL (4.95 mL of fuel + 0.05 mL of either inoculum or sterile deionized water) were also used specifically to determine CO_2 production. To simulate a real storage condition, it was adopted 1% (v v^{-1}) of water phase composed of either deionized water (control) or 0.85% m v^{-1} saline suspension inoculum with ca. 10^7 MPN mL^{-1} (test). Thus, at the beginning of assays, the total microbial population in all inoculated samples was