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Impact of biodiesel on biodeterioration of stored Brazilian diesel oil

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ABSTRACT

Alternative fuels are receiving considerable attention, especially biodiesel, which is recognized for its environmental benefits. One advantage is its biodegradability. However, biodegradability may allow the fuel to be more susceptible to microbial contamination, especially during storage. The susceptibility to biodeterioration of biodiesel, diesel, and diesel containing 5, 10, and 20% biodiesel was evaluated using fungi isolated from contaminated oil systems. *Paecilomyces* sp. produced the highest biomass in 20% and 100% biodiesel, while *Aspergillus fumigatus* grew best in pure biodiesel. Yeasts had the highest rates of degradation, especially *Candida silvicola*, with 100% degradation of all esters. *Rhodotorula* sp. showed greatest activity for C18:3 (linolenic acid), at 39.4%, followed by C18:1 (oleic acid) and C16 (palmitic acid), at 21% and 15%, respectively, after 7 days of incubation. The results are relevant for the resolution of the decade-long debate on the increase in diesel biodegradability due to the addition of biodiesel.

1. Introduction

The use of biodiesel has been widely encouraged in many countries, and global projections indicate increasing substitution of fossil fuels by biofuels. In addition to the recognized environmental benefits of biodiesel, such as lower emissions of particulate matter and greenhouse-effect gases, and no release of sulfur and volatile aromatic compounds into the atmosphere, biodiesel also has lower toxicity and is more easily biodegraded in the environment (Marchetti et al., 2007; Mariano et al., 2008; Sharma et al., 2008; Murugesan et al., 2009a,b; Demirbas, 2009). Diesel oil is a complex mixture of normal, branched, and cyclic alkanes and aromatic compounds. Biodiesel is defined as a mixture of mono-alkyl esters of long-chain fatty acids (FAME) derived from the transesterification of animal fats and vegetable oils, and its physical and chemical characteristics allow it to be added to diesel (Demirbas, 2009). The chain length and degree of unsaturation can vary in animal fats and vegetable oils for many reasons (DeMello et al., 2007). Soybean oil is the primary feedstock used in Brazil for the production of biodiesel. In EU countries, especially Germany, the mixture reaches 5%, and sometimes biodiesel may even be used in its pure form (Schleicher et al., 2008). In Brazil, its application as an alternative fuel became mandatory from January 2008, when 2% biodiesel was added to diesel according to regulations of the National Petroleum Agency (ANP) (Junior et al., 2009). In 2009 the percentage was raised to 4% and in 2010 to 5%. During storage, chemical and physical changes in the properties of biodiesel and binary mixtures may occur as a result of degradation processes, leading to increase in acidity, potential corrosion, and formation of sediment (Passman and Dobranick, 2005). Despite its many benefits, biodiesel has some vulnerabilities: Due to its chemical structure, it is more susceptible to oxidative, thermal and hydrolytic degradation (Dunn, 2005; Leung et al., 2006; DeMello et al., 2007; Knothe, 2007; Mariano et al., 2008; Junior et al., 2009).

The oxidation stability of various biodiesel esters from different feedstocks may be caused by multiple factors: (1) Molecular structure of the fatty esters: Biodiesel is a blend of fatty acid esters having different molecular structures with varying chain lengths, levels of unsaturation, and conformation. (2) Presence of antioxidants: Antioxidants can be naturally present in the feedstock or can be added during or after processing. These compounds usually prevent radical formation in oils by oxygen or light and subsequent degradation. (3) Presence of impurities and degradation products: Some

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impurities can catalyze the formation of radicals in oils, while other impurities can catalyze the degradation pathways of oils once radicals are formed (Waynick, 2005).

In addition, some of its features increase the biodegradability of the fuel, such as the absence of hydrocarbons, the presence of esters of fatty acids, and its greater affinity for water. According to Passman (2003), its biodegradability is a bonus from the perspective of environmental protection, but it is a problem when it occurs in the fuel systems. An increased biodegradability of diesel/biodiesel blends has been demonstrated in many investigations (Bento et al., 2006; Pasqualino et al., 2006; Mariano et al., 2008; Junior et al., 2009). On the other hand, in some studies (DeMello et al., 2007; Mariano et al., 2008; Owsianiak et al., 2009) the biodegradation of hydrocarbons was not accelerated by the presence of biodiesel. It has been postulated that this observation might be related to the catabolic abilities of the chosen microorganisms toward petroleum hydrocarbons (Junior et al., 2009). The microbial contamination of stored fuels, mainly diesel oil, is a major problem in refineries and distribution systems (Bento and Gaylarde, 1996, 2001; Bento et al., 2004). Many factors, such as the presence of water in the bottom of the tanks during storage, have been cited as increasing microbial growth in the systems and can lead to blocking of pipelines and filters, affecting the final quality of the fuel and corrosion of the tanks (Bento and Gaylarde, 2001; Bento et al., 2004). Good housekeeping, monitoring, and proper usage of an effective biocide are crucial measures for an anti-microbial strategy (Gaylarde et al., 1999; Passman, 2003; Passman and Dobranick, 2005; Siegert, 2009). A concentration of only 1% water in a storage system is sufficient for the growth of microorganisms, bacteria, and yeasts, as well as for the development of fungal biomass at the oil/water interface (Gaylarde et al., 1999; Chesneau, 2000; Bento et al., 2004). Examples of moulds and yeasts isolated from fuels include members of the genera *Candida*, *Rhodotorula*, *Aspergillus*, *Paecilomyces*, *Fusarium*, *Hormoconis*, *Penicillium*, and *Alternaria* (Bento and Gaylarde, 1996, 2001; Gaylarde et al., 1999). Many authors have reported the biodegradability of biodiesel blends in environments such as soil (Mariano et al., 2008; Junior et al., 2009) and water (Zhang et al., 1998; DeMello et al., 2007). Zhang et al. (1998) found that the rate of diesel biodegradation in water can be three times greater in the presence of biodiesel. According to Mariano et al. (2008), although biodiesel is more easily and rapidly biodegraded than diesel oil, among the diesel/biodiesel blends evaluated (2%, 5%, and 20%), only those with the highest proportions of biodiesel were significantly degraded more efficiently than pure diesel in soil. Junior et al. (2009) stated that it has been debated for a decade whether the addition of biodiesel facilitates diesel biodegradability. In addition, the influence of varying the proportions of biodiesel in biodiesel/diesel blends on fungal biodeterioration in tanks has not been investigated. The aim of this work was to assess in the laboratory the effect of adding various proportion of soy-derived biodiesel to diesel oil, on the growth of deteriogenic fungi (moulds and yeasts). The production of biosurfactants and the degradation of esters assessed by chromatography were also investigated.

2. Materials and methods

2.1. Microorganisms

The fungi *Aspergillus fumigatus* and *Candida silvicola* were isolated from the interfacial biomass of diesel storage systems in Brazil (Bento and Gaylarde, 2001); *Paecilomyces* sp. and *Rhodotorula* sp. were isolated from the sediments of biodiesel sampled in a Brazilian bus company. The sediment was plated on malt agar and incubated at 28 °C. After purification by repeated subculture, moulds were identified by macroscopic examination of colonies on malt agar slant tubes and microscopic examination (slide culture technique). Yeasts were identified according to carbohydrate assimilation patterns, using the API 20C 190 AUX (bioMérieux) galleries, which were used according to the manufacturer's recommendations.

2.2. Fuels

The fuels used were diesel oil (0.2% sulfur, metropolitan grade) and biodiesel (from soybean oil), both supplied by the Brazilian Petroleum Company, Ipiranga. Soybean oil is the primary feedstock used in Brazil for biodiesel production. Biodiesel blends were prepared in the laboratory at the following volume percentage compositions of biodiesel/diesel: 0/100, 5/95, 10/90, 20/80, and100/0 – respectively denominated B0, B5, B10, B20, and B100. The fuels and the blends were sterilized by vacuum filtration, using membranes of pore size 0.22 μm, and placed in previously sterilized glass bottles, which were covered with aluminum foil to prevent photo-oxidation of the fuel.

2.3. Growth assays

The mineral medium used (Richard and Vogel, 1999) contained (g L^{-1}): 0.7 KCl, 2.0 KH_2PO_4, 3.0 Na_2HPO_4, 1.0 NH_4NO_3, 4.0 $MgSO_4$, 0.2 $FeSO_4$, 0.2 $MnCl_2$, and 0.2 $CaCl_2$. The sole carbon source was diesel, biodiesel, or the blends described in Section 2.2.

2.3.1. Moulds

Moulds inocula were grown on malt agar at 28 °C for 7 days, and yeasts on GYMP broth (glucose, 20 g l^{-1}; malt extract, 20 g l^{-1}; yeast extract, 5 g l^{-1}; monobasic sodium phosphate, 2 g l^{-1}) for 48 h at the same temperature. After growth, suspensions of mould spores and yeast cells were prepared in distilled water, and counted in a Neubauer chamber. The final concentration used was 10^7 spores ml^{-1} for the moulds, and 10^4 cells ml^{-1} for the yeasts. Mould growth tests were carried out in 150-mL flasks containing 25 ml minimal medium and 25 ml diesel oil (B0), biodiesel (B100), or the blends (B5, B10, B20) as the only carbon source. After 7, 14, 21, 28, 35, 42, and 60 days the biomass of moulds formed at the oil–water interface was filtered (total volume). Ten milliliters of hexane was utilized to remove the residual oil, the filter paper with biomass was dried to a constant weight, and the final weight was recorded.

2.3.2. Ability of yeast to grow in fuel phase (preliminary tests)

Yeasts' ability to grow in diesel oil and blends was evaluated according to Hanson et al. (1993). This method consists of incorporating into the medium an electron acceptor, 2,6-dicholorophenol–indophenol (DCPIP), thus testing the ability of the microorganisms to utilize the hydrocarbon substrate, which is observed when the color of DCPIP changes from blue (oxidized) to colorless (reduced). Each microtitre-plate well was filled with 250 μl of mineral medium (Richard and Vogel, 1999), 10 μl of fuel (diesel oil, B0; pure biodiesel, B100; diesel blends: B5, B10, and B20) and 25 μl of each microbial suspension standardized at 10^4 cells ml^{-1}. All plates were incubated at 28 °C, and fuel concentration was determined according to the change in the color of the culture medium containing DCPIP after 12 h of incubation (Miranda et al., 2007; Junior et al., 2009). Yeast curves were performed in 250-ml flasks containing 160 ml of mineral medium and 1% of biodiesel (B100), or 1% of a mixture of 20% biodiesel in diesel (B20). All replicates were incubated at 28 °C and 120 rpm. The yeasts were serially diluted in distilled water every 24 h (for 186 h, about 7 days) and enumerated as colony-forming units (CFU) on GYMP agar. Five replicates were set up for each mould and three for each yeast and uninoculated controls.