Fuel 184 (2016) 701–707

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

Effect of different concentrations of *tert*-butylhydroquinone (TBHQ) on microbial growth and chemical stability of soybean biodiesel during simulated storage

Sabrina Anderson Beker [a,*], Yara Patricia da Silva [b], Francielle Bücker [a], Juciana Clarice Cazarolli [a], Patrícia Dörr de Quadros [a], Maria do Carmo Ruaro Peralba [b], Clarisse Maria Sartori Piatnicki [b], Fátima Menezes Bento [a]

[a] Fuels and Biofuels Biodeterioration Laboratory (LAB-BIO), Department of Microbiology, Immunology and Parasitology, Federal University of Rio Grande of Sul, Sarmento Leite Street # 500, Porto Alegre, RS, Brazil
[b] Department of Inorganic Chemistry, Federal University of Rio Grande of Sul, Bento Gonçalves Avenue # 9500, Porto Alegre, Brazil

HIGHLIGHTS

- First study on the effect of TBHQ on microbial growth in biodiesel.
- BHT did not present antimicrobial activity in the culture media.
- TBHQ appears to be neither promoter nor inhibitor of microbial growth in biodiesel under the conditions tested.
- Acid number and water content of biodiesel samples were out of specification for commercialization after 45 days of storage.

ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form 14 July 2016
Accepted 16 July 2016

Keywords:
Biodiesel
Antioxidants
Biomass
Oxidation stability
Water content

ABSTRACT

Biodiesel's susceptibility to oxidation and its hygroscopic characteristics can cause chemical oxidation and microbial growth during storage, leading to fuel biodeterioration. Although a wide variety of commercial antioxidants have been used to prevent biodiesel oxidation, little is known about the effects of these agents on microbial community. The minimum inhibitory concentration (MIC) of *tert*-butylhidroquinone (TBHQ) and butylated hydroxytoluene (BHT) against microorganisms isolated from fuel storage tanks was investigated, and only TBHQ presented antimicrobial activity. In laboratory scale, microcosm experiments with soybean biodiesel and different TBHQ concentrations (0; 50; 100; 200; 300; and 600 ppm) incubated for 45 days at 30 °C were performed. The microbial biomass formed at the oil-water interfaces was weighted, and DNA was isolated for DGGE analysis of 16S gene. The quality of commercial biodiesel was investigated by determining oxidation stability, acid number, viscosity, water content, and ester degradation. The results showed higher oxidation stability for the samples containing 100, 300, and 600 ppm TBHQ. The highest biomass formation occurred at TBHQ concentrations of 0, 300, and 600 ppm. The highest microbial diversity was observed in both the commercial samples and those containing 100 and 200 ppm TBHQ. The addition of antioxidant to the microcosms neither inhibited nor enhanced biomass production. An increase in viscosity, acid number, and water content was observed, thus the biodiesel was out of specification for commercialization. Degradation of C16, C18, C18:1, C18:2, and C18:3 esters was not observed in all samples.

1. Introduction

The large demand for energy and scarce energy resources has led to the exploitation of renewable energy sources with lower environmental impact. Biodiesel is a biofuel with great potential in this scenario, being an alternative to replace fossil fuels, or as an additive to petroleum-based fuels [1–5].

Despite the great demand of biodiesel in Brazil due to the National Biodiesel Program (Law 11097/2005), little is known about the chemical and microbiological quality parameters. The current biodiesel blend with diesel fuel in the Brazilian market is 7%, the so-called B7 [6].

* Corresponding author.
E-mail address: sabrinabeker@gmail.com (S.A. Beker).

http://dx.doi.org/10.1016/j.fuel.2016.07.067
0016-2361/

One of the most important quality parameters of biodiesel is water content, with maximum limits of 500 mg kg^{-1} and 200 mg kg^{-1}, in Europe and Brazil, respectively [7]. Water affects the chemical stability of biodiesel, leading to hydrolytic oxidation [8], changing its chemical characteristics over time along with the action of air, light, humidity, and temperature, thus compromising product's quality [9].

The presence of water in biodiesel storage tanks represents a concern for biodiesel quality, once it leads to microbial biomass accumulation and tank corrosion induced by acid-producing microorganisms. The microbial biomass formed in biodiesel tanks can clog pipes and filters, leading to engine failure and economic losses [10,11]. An effective housekeeping to prevent water accumulation is recommended for controlling microbial growth during fuel storage, including daily drainage of water from the bottom of the tanks [9,12]. Although antimicrobial compounds are added to the fuel to prevent contamination in some countries [12], this alternative may not always be feasible, and it is not regularized in Brazil.

Antioxidants (commercial additives) are widely used to prevent biodiesel oxidation in industry [13], including *tert*-butylhydroquinone (TBHQ), whose phenolic structure allows chemical stability during storage for a certain period of time [14].

The action of phenolic antioxidants depends on the number of hydroxyl/phenolic groups in *ortho* and *para* positions connected with the aromatic ring. In these compounds, the hydroxyl group provides active protons, which can inhibit the formation of free radicals, stopping the propagation reaction, thus slowing the biodiesel oxidation rate [15].

The aim of this study was to evaluate the antimicrobial activity of two commercial antioxidants, butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), as well as the relationship between TBHQ concentration, microbial growth, bacterial diversity and chemical stability of soybean biodiesel during simulated storage.

2. Materials and methods

2.1. ***PHASE 1:*** *Preliminary study: Antimicrobial activity of butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ)*

2.1.1. Antioxidants

The commercial synthetic antioxidants butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) both purchased from Fluka $\geqslant$ 98% were used.

2.1.2. Microorganisms

Oil-deteriorating microorganisms were isolated from diesel and biodiesel storage tanks and used in the experiments. The filamentous fungi *Paecilomyces variotii* and *Pseudallescheria boydii*; the yeast-like fungus *Candida guilliermondii,* and the bacterium *Bacillus pumilus* from the collection of the Laboratory of Fuel and Biofuel Biodeterioration UFRGS [16–19] were used.

2.1.3. Preparation of inoculum from the isolated microorganisms

The inoculum of the filamentous fungus *Paecilomyces variotii* and *Pseudallescheria boydii* was prepared from 7-day-old cultures on agar-malt agar in inclined tubes incubated at 29 ± 1 °C, with addition of 2 mL sterile saline (0.85%), and 2 mL of 0.01% surfactant (Tween 80). Spores were counted in a Neubauer chamber, and the suspension had a final concentration of 10^5 spores mL^{-1}. The inoculum of the yeast-like fungus *Candida guilliermondii* was obtained from yeast grown in agar-malt in slanted tubes, with addition of 2 mL of sterile saline (0.85%). The final microcosm concentration was 10^5 cells mL^{-1}. Inoculum of *B. pumilus* was prepared from petri culture containing Luria-Bertani agar after 24 h of incubation at 29 ± 1 °C. The final microcosm concentration was 10^5 cells mL^{-1}.

2.1.4. Preparation of uncharacterized inoculum

Uncharacterized inoculum was prepared according to ASTM E1259 [20]. Briefly, 2% of B10 mixture (previously filter-sterilized) were added to 100 mL Bushnell-Haas mineral medium in an Erlenmeyer flask, and inoculated with 5 mL microbiological sludge from a contaminated tank. The flask was incubated in an orbital shaker at 29 ± 1 °C, 200 rpm for 7 days. The final cell concentration in the flasks was 10^5 CFU mL^{-1}.

2.1.5. Evaluation of minimum inhibitory concentration (MIC) of BHT and TBHQ

MIC was determined using the broth dilution method, with turbidity detected by naked eye. Successive dilutions from 0 ppm to 1000 ppm were prepared. Then, 4 mL nutrient medium were added to 15 mL sterile vials, according to each type of microorganism. Malt broth was used for the yeast-like *Candida guilliermondii* and the filamentous fungus *Paecilomyces variotii* and *Pseudallescheria boydii;* Luria-Bertani broth was used for *Bacillus pumillus*, and Bushnell-Haas mineral medium was used for the uncharacterized inoculum. All assays were performed in triplicate.

2.2. ***PHASE 2:*** *Laboratory scale of biodiesel storage using different TBHQ concentrations*

2.2.1. Oil phase

The biodiesel used in this study was obtained from soybean oil and purchased in a company at Passo Fundo (RS/Brazil). Two types of biodiesel were used: (i) biodiesel without addition of antioxidant (0 ppm), and (ii) biodiesel with addition of antioxidant (commercial biodiesel).

2.2.2. Water phase

Bushnell and Haas minimum medium [21] at pH 7.2 was used.

2.2.3. Assembling of microcosms

TBHQ was selected for this step, based on the results of Phase 1, as previously described. Six TBHQ concentrations (0, 50, 100, 200, 300, and 600 ppm), and an unknown concentration (routine use in biodiesel industry) were investigated. The microcosms were produced in sterilized 250 mL glass bottles containing 100 mL biodiesel (oil phase) and 5 mL Bushnell-Haas mineral medium (aqueous phase). The vials were wrapped with aluminum foil, and incubated at 30 °C for 45 days. All assays were performed in triplicate.

2.3. Analyses of aqueous phase and oil-water interface

2.3.1. Biomass formation

After 45 days, the interfacial biomass was filtered through previously weighed filter paper, which was rinsed with 3 mL hexane to remove the fuel adhered from the biomass. Then, the filters were incubated at 30 °C for 48 h, and transferred to a dehydrating chamber for 24 h to remove water. The dry weight was recorded, and biomass was calculated as final weight minus initial weight (in milligrams). The experiments were performed in triplicate.

2.3.2. DNA extraction

Both the biomass formed at the oil-water interface after 45 days, and the water phase from three replicates (destructive microcosms) were used for DNA extraction, using the PowerSoil DNA Isolation Kit (MOBIO, Inc., Laboratories, USA), according to the manufacturer's instructions.