Short communication

Biodegradation of biodiesel and microbiologically induced corrosion of 1018 steel by *Moniliella wahieum* Y12

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**A B S T R A C T**

A putatively novel basidiomycetous fungus termed *Moniliella wahieum* Y12T was isolated from a 20% biodiesel blend. The strain maximally degraded biodiesel at a rate of $3.56 \times 10^{-2}$ mg/h during log phase growth. Induction of metal corrosion by the strain in a mass loss procedure using 1018 metal coupons showed total mass reduction exceeded that in controls by 70% through 30 days. Enhanced corrosion was observed at the pellicle and due to medium acidification. This is the first investigation of a *Moniliella* sp. and its impact on biodiesel stability and 1018 steel corrosion. (*M. wahieum* Y12T = ATCC MYA-4962).

1. Introduction

Analysis, control and mitigation of microbial contamination of biofuels are important operational considerations. Such contamination may reduce fuel stability, enhance biofouling, and induce corrosion of fuel-related components. Biodiesel is also more likely to be contaminated by microbes than is petroleum diesel (Zhang et al., 1998; Lee et al., 2009; Achten et al., 2010; Stamper et al., 2011). Diverse *Fungi* and *Bacteria* have been implicated in biofuel contamination (Chao et al., 2010; Bückler et al., 2011), a phenomenon directly attributed to biodiesel’s hygroscopicity (Passman et al., 2009). Biodiesel’s water adsorption and subsequent phase separation promotes microbial growth. Moreover, biodiesel’s chemical and biological hydrolysis to fatty acids provides labile carbon for sustained growth, which in turn results in acidification and microbiologically influenced corrosion (MIC) (Passman, 2003; Leung et al., 2006; Corseuil et al., 2011; Passman, 2013). Enhanced oxidation rates through formation of anoxic environments and biofilms exacerbate MIC (Dzierzewicz et al., 1997; Rajasekar, 2010; Satoh et al., 2009; Stamper et al., 2011).

We describe here the isolation of a putatively novel basidiomycete, *Moniliella wahieum* Y12T from a 20% biodiesel blend (B20), and the strain’s impact on biodiesel stability, and 1018 steel corrosion (Active Standard ASTM A1018/A1018M).

2. Materials and methods

2.1. Sample description

One liter of biodiesel (B20) blend with visible water contamination was collected from an in use fuel storage tank by the City and County of Honolulu. The sample provided for the work described here has visibly separated into an upper fuel layer, an observable film at the interface, and lower opaque, brown water layer.

2.2. Isolation and identification of *M. wahieum* Y12T

The film at the interface of the fuel and aqueous layer was aseptically transferred to and serially diluted to $10^{-8}$ in YM broth
Biodiesel (B100) was obtained directly from a local manufacturer, Pacific Biodiesel (1003 Makepono St., Honolulu, Hawai’i). Pacific Biodiesel was then the only large-scale commercial supplier in Hawai’i, and also provided biodiesel to the City and County of Honolulu. The company’s biodiesel is produced from waste cooking oils. Blend B100 was initially profiled in a Scion Bruker 400-GC Series GC/MS with a 15 m × 0.25 BR-5 guard column; 60 m × 0.25 BR-1701 analytic column, source temperature set at 250 °C, and transfer temperature at 260 °C. Hydrocarbons were compared against a FAME reference standard (FAMQ-005) from AccuStandard Inc. (New Haven, CT, USA) (Fig. 1).

A loop of Y12 was transferred from YM agar to 3 ml of YM broth and incubated with shaking for 24 h at 30 °C. Cells were then harvested by centrifugation at 5000 g for 1 min. Labile carbon was removed by washing the cells three times in a modified M9 minimal medium (MgSO₄ (1 M, 2 ml) and CaCl₂ (1 M, 100 µl) in 780 ml distilled water, 200 ml (5X) M9 salts (comprising Na₂HPO₄ · 2H₂O, 64 g; KH₂PO₄, 15 g; NaCl 2.5 g; NH₄Cl 5.0 g) for 1 min. Labile carbon was removed by triple washing in a modified M9 minimal medium, as described above. Cells were resuspended in modified M9 medium and used to inoculate replicates, including non-inoculated controls. Of the suspension of washed cells, 1 ml was used to inoculate each experimental jar. Controls were inoculated with 1 ml of sterile M9 medium. Aerobic conditions were maintained in the jars through a 0.22 µm filter inserted into a hole in the cap. Jars were shaken at 50 rpm at 30 °C.

Mass loss was determined after incubation as described above for 30 and 60 days. Metal coupons were removed and submerged in a solution of diammonium citrate (200 g in 1 L distilled water) at 80 °C for 20 min (International Organization for Standardization, method ISO 8407:1991 designation C3.4). Coupons were air dried before weighing and mass loss calculation.

3. Results

3.1. Isolation and identification

A filamentous fungus was cultivated from a film observed at the interface of the fuel and aqueous layers in contaminated biodiesel (B20) obtained from the City and County of Honolulu. A BLAST comparison of a 565 nt fragment of the D1/D2 region of the 26S rRNA gene showed the strain shared 98% sequence identity with its nearest described relative, Moniliella suaveolens var. nigra CBS 542.78T (AF335524) (Altschul et al., 1997). Strain Y12 has been tentatively assigned to the genus Moniliella as the type strain of M. wahieum Y12T (ATCC MYA-4962) (Fig. 2).

3.2. Biodiesel degradation

Biodiesel degradation during cultivation of Y12 was determined through loss of chromatographic peaks from B100 biodiesel obtained from a local manufacturer (Pacific Biodiesel, 1003 Makepono St., Honolulu, Hawaii). Three dominant chromatograph peaks determined by GC/FID were used in the loss determination and plotted against cell density (Fig. 3A, B).

According to standard peak comparisons, elution times in GC/FID correlated with the GC/MS chromatogram as follows: 14.194 min — hexadecanoic acid methyl ester; 16.060 min — co-elution of 9,12- octadecenoic acid methyl ester and 9-octadecenoic methyl ester; 16.126 min — co-elution of methyl stearate and 9,12, 15- octadecatrienoic acid methyl ester.
steatorate and 9, 12, 15-octadecatrienoic acid methyl ester (Fig. 3). This biodiesel was degraded at a maximum rate of 3.56 \times 10^{-2} \, \text{mg/h} during log phase growth. By 70 h, biodiesel in solution was completely utilized and logarithmic cell growth ceased. Degradation rates for all fatty acid methyl esters in the biodiesel sample were not determined. However, dominant esters in this fuel sample correlated with those comprising 17 and 19 carbon atoms, with varying degrees of saturation.

3.3. Oxidation of 1018 metal coupons

Composition and carbon content of 1018 steel coupons used here approximately matches that of material used to build many fuel containment vessels. Our data show this carbon steel is oxidized at an elevated rate during biodiesel degradation in the presence of *M. wahieum* Y12\textsuperscript{T} (Fig. 4). Metal coupons in the presence of only biodiesel and medium showed an average mass reduction of 0.49 ± 0.22 mg after 30 d, and 0.85 ± 0.07 mg after 60 days. Mass loss from coupons in the same medium in the presence of Y12\textsuperscript{T} exceeded that in controls by 70% after 30 days, and 50% after 60 days (mass reduction of 0.85 ± 0.06 mg, and 1.30 ± 0.14 mg after 60 days). The pH of the negative control remained the same (pH 7), whereas that of the B100 had fallen to 5.

4. Discussion

Biological contamination of fuels has been known for some time (cf. Gaylarde et al. 1999). The fundamental interaction in such
contamination is the ability of a microorganism to utilize a chemical component as a growth substrate. Transfer of carbon through the production of metabolites can subsequently support other metabolisms, and also chemically impact the local environment (Passman, 2013). Here, we investigated the ability of \textit{M. wahieum} Y12\textsuperscript{T} to utilize FAMEs as sole carbon sources, and the subsequent impact on corrosion. Many microorganisms thrive on biodiesel as their sole carbon source (Zhang et al., 1998). Of the three isolates purified from our sample described here, \textit{M. wahieum} Y12\textsuperscript{T} was the only one considered potentially novel on the basis of the rRNA gene nucleotide sequence. Since to our knowledge no \textit{Moniliella} sp. has been characterized as a fuel contaminant, we chose to focus our investigation of microbiologically induced corrosion on this organism. Moreover, due to the complexity of interactions that occur in a natural community we chose also to study this process in a pure culture of \textit{M. wahieum} Y12\textsuperscript{T}. The degree and rate of biodiesel degradation are dependent on the component hydrocarbons’ saturation and fatty acid chain length (Knothe, 2005). \textit{M. wahieum} Y12\textsuperscript{T} did not appear to prefer a specific hydrocarbon, and complete utilization of both saturated and unsaturated FAMEs was observed at nearly identical rates (Fig. 3B). Differences between biodiesel biodegradability could also be explained by differences in their physicochemical properties, mainly viscosity. Higher viscosity results in decreased bioavailability and slower biodegradation (Corseuil et al. 2011). Consistent with this observation, degradation rates we determined were also limited by bioavailability. Available
carbon occurs only at the phase interface through increased surface area due to emulsion formation, or smaller droplet sizes due to mechanical disruption in aqueous media. While all of our experiments were performed in a shaker incubator, the degradation rates depended on how well the biodiesel dispersed. Our isolates were obtained only in aerobic conditions. Oxygenic conditions also have an impact on biodiesel degradation rates and has been shown to also occur at high rates during anaerobic respiration (Aktas et al. 2010). Oxidation and acidification are typically observed during diesel fuel degradation (Prankl and Schindlbauer, 1998); biofilm formation is also recognized as a contributory factor in metal corrosion (Achten et al., 2010; Damon, 1941). A reduction in pH was observed during Y12T growth here, with medium pH falling from 7.0 to 5.3 after 30 days; this may arise through products of beta-oxidation including acetic acid, smaller fatty acids and ultimately CO2. We also observed film formation at the fuel/water interface on the metal coupon, and on inner surfaces of the jar (Fig. 4). Further studies will determine the nature of this film.

5. Conclusion

The capacity to degrade biodiesel and corrodie 1018 steel coupons was investigated in M. wahieum Y12T cultivated from a contaminated biodiesel (B20) blend. Biodiesel can be utilized as the sole carbon in a minimal medium in aerobic conditions, resulting in acidification of the medium. Although many isolates are capable of thriving on biodiesel as their sole carbon source. Moniliella spp. have not thus far been described as being involved in fuel contamination. Indeed, few environmental isolates have been used to simultaneously investigate biological corrosion and degradation rates.

The presence of M. wahieum Y12T in retail fuel system components or underground storage tanks would be detrimental through direct degradation of fuel and the development of biofilms that result in biofouling and metal corrosion. It is important to consider that metabolite production by M. wahieum may also provide bioavailable carbon to species unable to directly metabolize biodiesel. Such transfer of carbon could support additional biological processes with the potential to facilitate sustained corrosion, enhanced degradation and biofouling. While analysis of natural communities is important, primary metabolism in the sequence of carbon transfer is supported by the ability to first utilize biodiesel as bioavailable carbon. Investigating this metabolism will determine how we can reduce the negative impacts of this community. Studying the whole genome of Moniliella and differential expression of its proteome should provide insights that will facilitate rapid identification and prevention of contamination. Few isolates have been specifically described in terms of their ability to degrade biodiesel, so M wahieum Y12T has been deposited in the ATCC as MYA-4962 with such investigations in mind.

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References