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Graphene oxide-based nanofilters efficiently remove bacteria from fuel

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ABSTRACT

This study examined the applicability of graphene oxide (GO) nanomaterial as an efficient filtration medium for removing bacteria from fuel. It was shown that GO columns efficiently trapped bacteria and allowed fuel to flow freely. Scanning electron microscopy and fluorescent microscopy confirmed that bacterial cells were trapped within the GO filter matrix and binding strength tests showed that cells were strongly bound to the GO matrix. Additionally, silver-decorated GO (Ag-GO) in the form of free-standing films and coatings were shown to be antimicrobial against Gram positive and Gram negative bacteria. Ag-GO filter columns maintained the same filtration efficiency as undecorated GO, although some Ag-GO leached into the fuel. However, the positive results observed with Ag-GO present the possibility of combining GO with Ag-GO to increase the service life of the nanofilter by killing bacteria on contact. These findings significantly increase our understanding of the properties of GO and provide a new bioengineering application for the purification of fuel and non-polar solvents.

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Introduction

Microorganisms are known to colonize and biodeteriorate hydrocarbon fuels. Multiple species of bacteria have been isolated from fuel storage tanks, pipelines, and aircraft wing tanks (Edmonds and Cooney, 1967; Jung et al., 2002; Rauch et al., 2006; Brown et al., 2010; Korenblum et al., 2010; White et al., 2011). Some of the problems associated with microbial growth in aviation fuels include wing and storage tank corrosion, fuel pump failures, filter plugging, injector fouling, topcoat peeling, engine damage, and deterioration of fuel chemical properties and quality (Passman et al., 2001; Rodriguez-Rodriguez et al., 2009; Aktas et al., 2012; Lee et al., 2012; Stamper et al., 2012; Suflita et al., 2012; Gunasekera et al., 2013; Striebich et al., 2014). Rampant microbial growth and biofilm formation can lead to costly damage to fuel systems and vehicle hardware (Passman, 2012).

Currently, prevention of fuel biodeterioration relies on housekeeping practices which include removing water from fuel by using fuel–water coalescers and filtration of coarse particulates. Filtration is the most important method used to remove coarse impurities, but even the best fuel filters are incapable of completely filtering out particles smaller than 10 μm in diameter. Microorganisms can range from less than a micrometer to several micrometers in size. Due to their small pore size, 0.22–0.45 μm, filters that can trap bacteria are difficult to use in fuel applications because they present a strong barrier to fuel flow. Another effective way of preventing biofouling is to remove free water from the fuel because microorganisms require water to grow. Military jet fuel is also treated with diethylene glycol mono-methyl ether (DiEGME), a fuel anti-icing inhibitor (FSII) additive which can also help reduce microbial growth and formation of biofilms (Passman, 2012). The use of this additive, however, can be expensive and may present some adverse effects including tank topcoat peeling (Zabarnick et al., 2010) and environmental toxicity (Mushrush et al., 1999). Filtering out microorganisms at different stages during fuel transportation and storage would be an efficient way to reduce biodeterioration. It is now more pressing than ever to develop new methods of preventing fuel biodeterioration given the ubiquitous use of biofuels with greater biodegradation potential. Biodiesel

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(100% (v/v) fatty acid methyl esters) and ethanol, for example, have been shown to be readily affected by microbial growth (Alvarez and Hunt, 2002; Demirbaş, 2008).

A previous study (Ruiz et al., 2011) demonstrated that under certain conditions, bacteria strongly attached to materials containing graphene oxide (GO). It was observed that while aqueous solutions strongly interacted with GO-containing matrices, other organic solvents, including jet fuel, tend to flow efficiently through GO without much interaction. The decision to test the applicability GO as an effective filter matrix for purification and sterilization of fuel was based on these observations and the need for an efficient filtration system to remove microorganisms from fuel.

Graphene oxide (GO), a sheet nanomaterial formed by monolayers of carbon atoms arranged in thick honeycomb arrays, has been proposed as an excellent material for bioengineering and biomedical applications (Novoselov et al., 2004; Szabo et al., 2006; Geim and Novoselov, 2007; Sabourin et al., 2009). The presence of reactive hydroxyl, epoxyl, carbonyl and carboxyl functional groups on the surface of GO provides interesting physical properties that include high solubility in polar solvents, good colloidal properties, low production costs, low toxicity, and a large surface area that can be decorated with antimicrobial agents, including nanosilver (Bao et al., 2011; Das et al., 2011; Ruiz et al., 2011; Zhang et al., 2011). The solubility of GO in water is highly dependent on the degree of surface functionalization imparted during oxidation. Furthermore, GO presents low or no cytotoxicity to human and animal cells (Chen et al., 2008; Agarwal et al., 2010; Hu et al., 2010; Park et al., 2010; Chang et al., 2011; Ruiz et al., 2011; Wang et al., 2011a,b, 2012; Some et al., 2012). These characteristics make GO a great nanomaterial for new biotechnological applications.

This study describes the development, characterization, and use of a novel graphene oxide-based nanofilter for removing bacteria from jet fuel. Jet-A fuel samples were inoculated with bacteria and then subjected to nanofiltration. Multiple biomolecular approaches including quantitative real-time PCR (qPCR), cell-based colony counting, scanning electron microscopy (SEM), and fluorescent microscopy were applied to demonstrate the filtration efficiency, functionality and mode of action of the GO nanofilter. The GO-based nanofilters removed bacteria from fuel efficiently while allowing rapid fuel flow and low resistance. Further, silver-decorated GO (Ag-GO) efficiently killed bacteria, which presents the possibility of combining GO and Ag-GO to produce filter matrices with longer durability.

Materials and methods

Preparation of graphene oxides (GOs)

GO was prepared by following the Hummers method with minor modification (Hummers and Offeman, 1958). Briefly, concentrated H_2SO_4 (10 mL) in a 500 mL flask was heated to 80 °C, to which $(NH_4)_2S_2O_8$ (0.9 g) and P_2O_5 (0.9 g) were added. The mixture was stirred until the reagents were completely dissolved. The graphite sample (1 g) was added, and the resulting mixture was heated at 80 °C for 4.5 h. The sample was cooled to room temperature and the reaction mixture diluted with water (250 mL) and kept for ~12 h. Then, the GO preparation was filtrated and washed repeatedly with water, followed by drying in a vacuum oven. The solid sample was added to concentrated H_2SO_4 (40 mL) in a 500 mL flask cooled in an ice bath. The mixture was added slowly to $KMnO_4$ (5 g over 40 min) and the temperature maintained below 10 °C. When the reaction mixture changed color from black to greenish brown it was heated at 35 °C for 2 h, followed by dilution with 85 mL of water (*Caution*: kept the temperature below <35 °C throughout the process) and stirred for 2 h. The reaction mixture was poured into a large beaker, to which 250 mL of water and 10 mL of aqueous 30% H_2O_2 were added. Bubbles from the aqueous mixture and a color change to brilliant yellow were observed. After the mixture was allowed to settle for approximately 12 h, the clear supernatant was decanted, and the precipitate was washed repeatedly, first with an aqueous solution of H_2SO_4 (5 wt %)-H_2O_2 (0.5 wt %) and then a 10% HCl solution and finally washed repeatedly with water until no layer separation was observed after centrifuging. The sample was then dialyzed (MWCO ~ 3500) against water for 7 days to yield a clean aqueous dispersion of GO. The water was removed using a rotary evaporator and the GO was recovered as a black flaky powder that was stored in glass vials at room temperature for up to one year. Suspensions in ultrapure water containing 250 μg/ml GO were produced for experimentation.

Ag-GO synthesis

Ag-GO was prepared by sonochemical method by first combining 50 mg of GO, 25 mg of silver acetate, and 15 mL of dimethyl formamide (DMF) in a three-arm sonochemical flask (Sonics Inc., Suslick flask). The mixture was sonicated at 37% amplitude and 20 kHz for 20 min using a pulsed (1 s on, 1 s off) procedure. After the sonication procedure, the Ag-GO solution turned black and was stable for a few hours without any noticeable precipitation. To recover the Ag-GO, the mixture was transferred to a round-bottom flask and DMF was removed using a rotary evaporator. The remaining solid Ag-GO material was transferred to a centrifuge tube and washed five times with deionized water and ethanol. The ethanol was removed by drying by blowing nitrogen across the surface of solution. The Ag-GO dry powder was recovered and stored in glass vials at room temperature for up to one year. Two preparations of Ag-GO were produced, one with 15% (w/w) silver loading and another with 33% silver loading. The 33% loading Ag-GO was used in all tests with the exception of the Ag-GO filter column tests, where both 15% and 33% loading Ag-GO were used.

Characterization of GO and Ag-GO by XRD and TEM

The crystal structure of GO and Ag-GO samples was analyzed by x-ray powder diffraction (XRD) using a Bruker D8-Advanced equipped with a Cu α source, monochromator, and a Sol-X detector. Observed XRD patterns were identified by comparison with the ICDD crystallographic database. A Hitachi H-7600 operated at 100 kV was used to record transmission electron microscopy (TEM) images. Samples were prepared by diluting a small amount of powdered sample in isopropyl alcohol and drip-spotting onto 400 mesh copper grids.

Preparation of GO and Ag-GO columns

Small GO columns used for filtration testing were developed by packing 32 mg of GO and 32 mg of Ag-GO into a 500 μL Eppendorf plastic syringe casing with 0.1375 inches in interior diameter (Fig. 1a). The larger GO columns were produced by packing 260 mg of GO into a 2.5 mL Eppendorf plastic syringe casing with an interior diameter of 0.3125 inches (Fig. 1b). Glass wool (GW) columns containing an amount of glass wool equivalent to the volume covered by the GO in the GO columns were used as filtration controls. A 1 mL mechanical pipette was used to provide the flow pressure required to filter fuel through the 32 mg GO columns. The 260 mg GO columns were tested as part of an apparatus (later described) that allowed us to use the laboratory vacuum system (50 kPa).