

Report as of FY2007 for 2006OH35B: "Development of a Novel Hydrogel-Based Sensor for the Detection of Biological Contaminants"

Publications

- Conference Proceedings:
 - Gruden, C.L., Escobar, I., Coleman, M., Gorey, C., and Mileyeva-Biebesheimer, O. 2007. Polymer brush based bacterial sensors for improved membrane performance in 33rd Regional Meeting of ACS. Covington, KY.
 - Gruden, C.L., Escobar, I., Coleman, M., Gorey, C., and Mileyeva-Biebesheimer, O. 2007. Development of fouling resistant water treatment membranes with microbial sensing capabilities in 34th National Meeting of ACS, Boston, MA.
- Other Publications:
 - Escobar, E., Gruden, C., and Coleman, M. 2007. Development of fouling resistant water treatment membranes with microbial sensing capabilities *Controlled Environments Magazine*. In Press.

Report Follows

PROJECT TITLE. Development of a Novel Hydrogel-Based Sensor for the Detection of Biological Contaminants

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PROBLEM. Coastal water quality is an important issue from a public health and an economic standpoint. A variety of diseases, including gastroenteritis, dysentery, and hepatitis, can be carried to coastal areas via contaminated waters. In addition, surface waters in the Great Lakes Region also act as source waters for surrounding communities. There is a keen interest in tracking and eliminating potential pathogens in these systems. Admittedly, much attention has been given to sensor development in the past several years, however, sensors that exhibit strong and selective binding for biological targets are still needed. Most rapid detection assays are affinity-based, where organism-specific biomolecules, such as artifacts (e.g., exocellular proteins, fatty acid composition) or genomic material (e.g., DNA, rRNA) are targeted. Once the target biomolecule is identified, the sensor must have the specificity to identify a target biomolecule in a complex system and the sensitivity to detect its presence, even at low concentration. Immunochemical assays, which rely on antibody (Ab) affinity to target analytes, are arguably the most frequently used biosensors due to their simplicity, rapid response, and financial viability (Ivnitski et al., 1999; Iqbal et al., 2000). For specific detection, antibodies (Ab) can be immobilized on surfaces for immunocapture of target bacterial species and subsequent separation of the target species from complex water samples (i.e. process water). Previously, support media for antibody-based sensors have included the surfaces of magnetic beads, microplates, and glass slides, and their applications include natural waters and sediments (Mazurek, 1996; Bard and Ward, 1997; Liu, et al, 2001; Favrin, et al, 2003; Furtado and Casper, 2000). In source water applications, biomolecule detection often requires both isolation and concentration of the target biomolecule to mitigate interference in complex water samples and cross-reactivity from competing analytes. Therefore, separations processes are needed with adjustable affinity properties specific to the analyte of interest.

Note: Initially, this work was to involve hydrogels as a support medium for biosensors as reflected in the project title. However, preliminary data indicated that this approach would not be viable since it was difficult to distribute biorecognition molecules evenly throughout the hydrogel matrix. In addition, the hydrogel pores became readily blocked in samples of moderate complexity. Therefore, the project was modified and combined with an ongoing project focused on development of a **novel** fouling-resistant membrane by attaching a polymer brush (hydroxypropyl cellulose) on the surface.

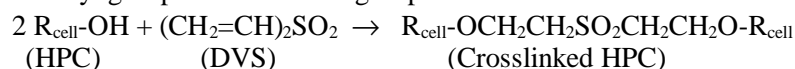
RESEARCH OBJECTIVES. *The goal of this proof-of-concept study was the synthesis, characterization, and performance evaluation of a novel membrane-based sensor for the specific detection of biomolecules.* The membranes were modified with a polymer brush (hydroxypropyl cellulose) on the surface, which acted as the support medium for bacterial sensing. To our knowledge, this is the first application of conjugated polymers attached to membranes for bacterial sensing. This technology can easily be translated to small membrane coupons or hand-held membrane based sensing devices. There were three main goals of this work: (i) develop chemistries to bind HPC to the membrane surface, (ii) demonstrate covalent binding of a model biorecognition molecule (antibody) to the brushes on membrane surface and (iii) demonstrate that the antibody-HPC modified membrane can immobilize the target organism.

EXPERIMENTAL

This work focused on detailed synthesis and characterization of individual components for a model membrane system (cellulose acetate).

Objective 1: Develop Methods to Modify the Membrane Surface with Polymer Brushes

Free standing crosslinking of HPC with DVS can be formed. The results for free standing gels was compared to those for the modified membranes. The reaction with either CA or HPC occurs by addition of vinyl group of DVS to OH group of cellulose as shown in the following reaction (Kabra, 1993):



When used to crosslink HPC and other cellulose ethers in isotropic aqueous solutions, DVS forms gels at pH 12 in less than an hour. These results suggested that it is possible to crosslink HPC with DVS fast enough by keeping the pH of the mixture high. This chemistry was modified for modified to allow for modification of the cellulose acetate membranes as described below.

The membranes were modified using a method sequential coating method. The membranes were coated with a (0.5 – 1.5) weight-percent DVS solution, washed thoroughly with ethanol and coated with a 7-10 weight percent HPC (MW=100,000) solution. While a high MW HPC chain was used to ensure good extension from the membrane surface at low temperatures, the length of the HPC can also be varied to modify surface properties. The DVS reaction time was varied from 30 minutes to 6 hours to modify the surface density. The coating was allowed to dry for 48 hours and after drying, the membranes were kept overnight in DI before running.

Objective 2: Membrane Characterization

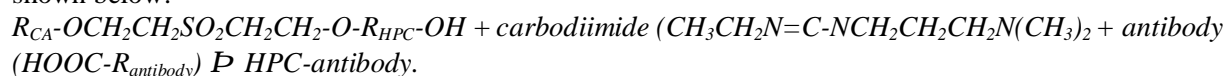
- ATR-FTIR was used to probe the evolution in chemical structure of the covalently bound polymer brush and CA surface that constitutes the surface of the membrane. FTIR uses measurements of vibrational spectra to identify the chemical structure of materials. The ATR attachment allowed measurement on the top surface layer of membrane. Measurement was performed using a Digilab UMA 600 FT-IT microscope with a Pike HATR adapter and an Excalibur FTS 400 spectrometer.
- AFM provides an easy and fast method to observe the surface structure of a wide range of materials. AFM allows acquiring 3D topographic data with a high vertical resolution. Accurate and quantitative data about surface morphology are provided over a wide range of magnifications and can be used in several quantitative analyses approaches such as section, bearing and roughness analysis. AFM measurement was performed using a Nanoscope IIIa Scanning Probe Microscopy in the Department of Chemical Engineering at the University of Toledo.

Objective 3: Sensor Development and Testing

Bacterial culture *Mycobacterium parafortuitum* (#19688) was ordered from American Type Culture Collection (ATCC). The culture was grown at 37°C on Lowenstein-Jenson solid media (BD 220908, BD) and in MiddleBrook 7H9 broth (#R061346, Remel Inc, KS).

A model biorecognition molecule (i.e. antibody) was selected for attachment to the membrane and subsequent immobilization of mycobacteria for detection. The primary polyclonal antibody (rabbit anti-mycobacterium IgG #6398-0006, Biogenesis, NH) was selected for this work (Yi et al., 1998). Fragments of goat anti-rabbit IgG (H+L) antibodies with a fluorescent tag (Alexa Fluor[®] 594) were used as secondary antibody for verification of primary Ab attachment to the modified membrane surface. Secondary antibodies (Ab) were obtained from Invitrogen[™] (#A11072, Invitrogen Corporation, IL).

A carbodiimide (CDI) was chosen to attach the antibody the surface bound HP. The reaction chemistry is shown below:



Specifically, the CDI is a zero-length linker that facilitates the reaction between hydroxyl groups (i.e. the brush) and carboxyl groups (i.e. the antibody). For this reaction, the CDI was dissolved in buffer solution, applied to the membrane and placed in the antibody (Ab) solution (concentration?). FTIR with ATR was used to monitor the membrane following each reaction step.

The antibody-HPC modified membrane was tested for its ability to detect *mycobacteria*. The protocol for determining the recovery of target microorganisms using an immunoassay was modified from Yi et al. (1998). The membranes (25mm diameter) were manipulated in 6-well cell culture plates. 1mL of concentrated bacteria ($7.7 \times 10^5/\text{mL}$) in laboratory grade water was added to the surface of the membrane and incubated for up to 90min covered on a shaker table. After incubation, the antibody-HPC modified membrane was rinsed 3X with and resuspended in 1mL of laboratory grade water. 2uL of a DNA intercalating stain (Picogreen[®], Invitrogen, Chicago, IL) was added and incubated for 5min to detect bound bacteria. In replicate samples, fluorophore labeled secondary antibody (Leinco, USA) was added at varying dilutions (1:10 to 1:200) and incubated for 30min at room temperature to verify the presence of the antibody on the membrane surface. Samples were processed on a fluorescent microscope. Control samples for this experiment included: unmodified membrane, membrane modified with HPC only, antibody-HPC modified membrane. Non-specific binding of mycobacteria to the unmodified membrane and the membrane modified with HPC only was negligible.

RESULTS AND DISCUSSION

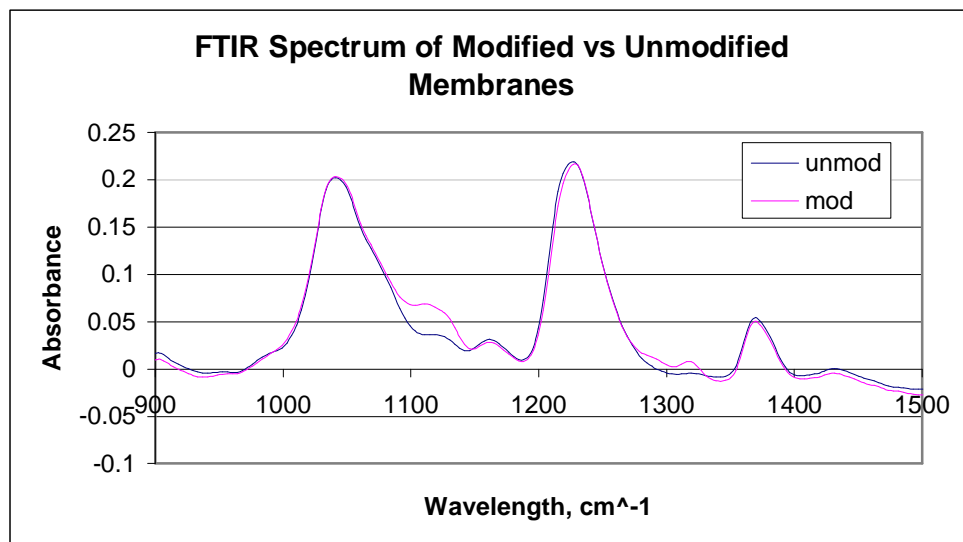
The project goal was to determine the viability of the *novel* idea of combining a brush on membrane for fouling resistance with a biosensor component to detect potential biofouling species.

Formation of Brushes on Membrane Surface

The membrane used for the preliminary work was a hydrophilic cellulose acetate (CA) ultrafiltration membrane with a molecular weight cutoff (MWCO) of 20,000 Daltons. The membrane surface was modified with the polymer brush, hydroxypropyl cellulose (HPC) via a divinylsulfone spacer (DVS). As an initial step, crosslinked gels of DVS and HPC were produced using standard chemistry reported in literature (Kabra 1993). While the DVS acts as a linker molecule between the membrane surface and the

HPC brushes in the system of interest, it is used initially as a crosslinker to form bulk free standing gels of HPC. The next step was to functionalize the CA membrane with HPC via the DVS spacer and characterize the HPC-modified membrane using FTIR. Since the

structure of HPC and the CA are very similar, modification was verified using FTIR analysis of the peak for the sulfonic bond of DVS. The adjacent FTIR spectra shows the onset of peaks for SO_2 bond in two locations at 1130 and 1315 cm^{-1} following exposure of CA membrane to DVS in NAOH solution for one hour followed by exposure to HPC.

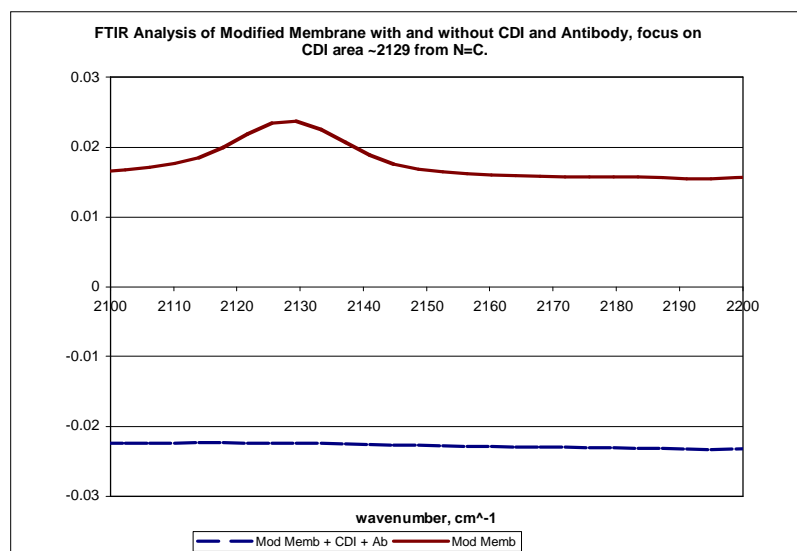


Attachment of Model Biorecognition Molecule

A model biorecognition molecule (i.e. antibody) was attached to the membrane and to verify the ability of the membrane-based sensor to detect bacteria. While a number of chemistries are available to attach the antibody the surface bound HPC, a carbodiimide (CDI) was chosen. Fourier transform infrared spectroscopy was used to monitor the membrane following each reaction step.

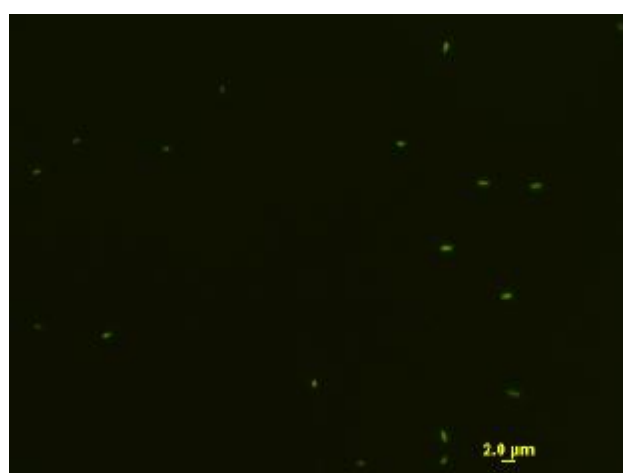
The only peak that was affected was at 2129 cm^{-1} . Since the CDI is acting as a zero-length linker, we hypothesize that the appearance of the peak at 2129 cm^{-1} is due to the brush binding to the antibody.

In addition, fluorescently-labeled secondary antibody was added to the antibody-HPC modified membrane and an unmodified membrane for comparison. It was determined that primary antibodies were immobilized on the antibody-HPC modified membrane and were available for binding since approximately 4×10^5 fluorescently-labeled secondary antibodies remained on the membrane.



Mycobacteria Detection with Antibody-HPC Modified Membrane

The antibody-HPC modified membrane was tested for its ability to bind *mycobacteria*. After multiple experiments with a range of initial mycobacteria concentrations (1×10^5 to 1×10^7 /mL) approximately 10% were recovered on the antibody-HPC modified membrane surface.



***mycobacteria* were captured on the surface of antibody-HPC modified membrane (1000X)**

SIGNIFICANCE AND IMPACTS OF RESEARCH ACTIVITIES

The project produced a fouling-resistant membrane by attaching a polymer brush on the surface. Biorecognition molecules targeting selected bacteria were covalently bounded to the end of polymer brushes for in situ detection. Typically, the biological recognition component consists of enzymes, receptors, nucleic acids, or antibodies specific to biological markers.

Our proposed membrane modification/sensing technique developed stronger membranes capable of withstanding harsh raw water quality conditions without losing their reliability in producing a high quality safe product. Although the research outlined in this proposal addresses detection of biological contaminants in water treatment systems, the sensor developed from this work can be adapted to address a range of national and international environmental concerns.

ACKNOWLEDGEMENTS

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