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Development of Smart Membrane Filters for Microbial Sensing

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Abstract: Recent efforts aimed at minimizing membrane fouling have emphasized an increasing demand for on-line monitoring in an effort to accurately predict membrane performance. The development of an in-situ bacterial monitoring system that is integrated within the membrane can meet that need. Because the target is bacterial monitoring, organic matter fouling must be controlled to avoid interference/masking of bacterial sensing as well as to prevent permeability loses. Combining bacterial monitoring with a membrane designed for fouling control is a novel and unique concept. We have produced a fouling-resistant membrane by attaching a stimuli-responsive polymer film on the surface, which offers the potential to collapse or expand the polymer film. A temperature decrease can cause the film to expand into a hydrophilic state while a temperature increase causes a collapse into a hydrophobic state. By continuously triggering the phase transition, the non-equilibrium movement of the polymer film may offer better protection of the surface than at equilibrium. Increasing temperature to collapse the film and immediately decreasing temperature to expand it would create a sweeping motion at the molecular (nanometer) level along the surface. The surface of a cellulose acetate membrane was grafted with a thermally responsive hydroxypropyl cellulose (HPC) film layer. Aqueous solutions of HPC possess a lower critical solution temperature of approximately 40°C (while cross-linked structures had an LCST of 46°C): above this temperature the solution phase

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separates. When attached to the membrane surface, the film layer collapses upon increasing the temperature above the phase transition temperature and expands away from the surface when cooled. Biorecognition molecules targeting selected bacteria were covalently bound to specific moities originating from the polymer film for in situ detection. Typically, the biological recognition component consists of enzymes, receptors, nucleic acids, or antibodies specific to biological markers. In this proof-of-concept study, antibodies were used due to their simplicity, proven efficacy, and rapid response.

Keywords: antibodies, biorecognition, fouling, HPC, Membranes, stimuli-responsive polymers, ultrafiltration

INTRODUCTION

Membranes typically consist of a porous support layer, which provides mechanical strength and stability, and which is covered by a thin selective layer, responsible for providing the membrane with separation capabilities (44). Membranes are capable of separating species as a function of their physical and chemical properties when a driving force is applied (33), and they enable filtration for removal of colloids, cells, and molecules. This has stimulated polymer synthesis toward an improved design of membranes for an advanced level of performance. The fundamental understanding and technological improvement of membranes are major objectives in recent membrane science.

The thin selective layer, or skin, has surface functional groups such as carboxyl and amine that affect separations properties and can be used for further modification (7,10). Recent endeavors using PEG have been able to create a more hydrophilic surface to prevent biofouling by reducing hydrophobic interactions. (6) Membranes are manufactured from a variety of materials, such as cellulose acetate (CA), cellulose diacetate (CDA), cellulose triacetate, polyamide (PA), other aromatic polyamides, polyetheramides, polyetheramines, and polyetherurea; also, thin-film composite (TFC) membranes may be made from a variety of polymers consisting of several different materials for the substrate, the thin film and other functional layers.

Membrane replacement due to fouling is the single largest operating cost when membranes are used in water separation applications (48,11), and, thus, the greatest hindrance to the widespread use of membranes. Fouling (the irreversible (adhesive) macromolecular adsorption) refers to specific intermolecular interactions between macrosolutes present in the feed water and the membrane that occur even in the absence of filtration. These materials on the membrane surface, which cannot be

removed by cross-flow operation, backflushing, or backpulsing, result in permanent flux decline and lead to fouling. Many researchers agree that organic matter is considered a major contributor to abiotic membrane fouling in water separation applications (7,12,13,20,21,35,37,38,47).

The presence of microorganisms in feed water can further exacerbate fouling due to the accumulation of microorganisms onto the membrane surface and on the feed spacer between the envelopes, or biofouling. Microorganisms transported to the membrane element can attach to the feed side of the membrane and the spacer. Attachment depends on Van der Waals forces, hydrophobic interactions, and electrostatic interactions between the microorganisms and the surface. Biofouling control has been attempted via biocide additions; however, while a biocide may kill the biofilm organisms, it usually will not remove the biofouling layer (15), and may cause bacteria that survive disinfection to potentially become more resistant (2). Therefore, bacterial detection is essential in determining biofouling potential.

In situ detection of bacteria in membrane-based water treatment systems is critical since biofouling can significantly impact membrane efficiency. Moreover, there is a keen interest in tracking and eliminating potential pathogens in these systems. With very few exceptions, techniques for specific detection of bacteria in aqueous systems are based on membrane filtration followed by culturing and phylogenetic or functional analysis. Direct detection strategies, which eliminate the bias introduced in culture-based methods, are gaining in popularity. Biorecognition molecules have been designed and routinely used to label characteristic artifacts (e.g., exocellular proteins, fatty acids) and genomic material (e.g., nucleic acids). Methods based on the detection of antibodies against microbial specific exocellular proteins (antigens) are characterized by their simplicity, rapid response, and financial viability (25).

The work presented here produced a novel fouling-resistant membrane by attaching a stimuli-responsive polymer film (hydroxypropyl cellulose) on the surface, which offers the potential to collapse or expand the polymer film. The phase change arose from the existence of a lower critical solution temperature (LCST) such that the polymer precipitates from solution as the temperature was increased. This capability was exploited to control adsorption/desorption (28). A temperature decrease caused the film to expand into a hydrophilic state while a temperature increase caused a collapse into a hydrophobic state. Protein adsorption is reduced in the expanded, hydrophilic state relative to the collapsed, hydrophobic state. We then used the polymer film to act as the support medium for bacterial sensing (Fig. 1). To our knowledge, this is the first application of conjugated polymers attached to membranes for bacterial sensing.

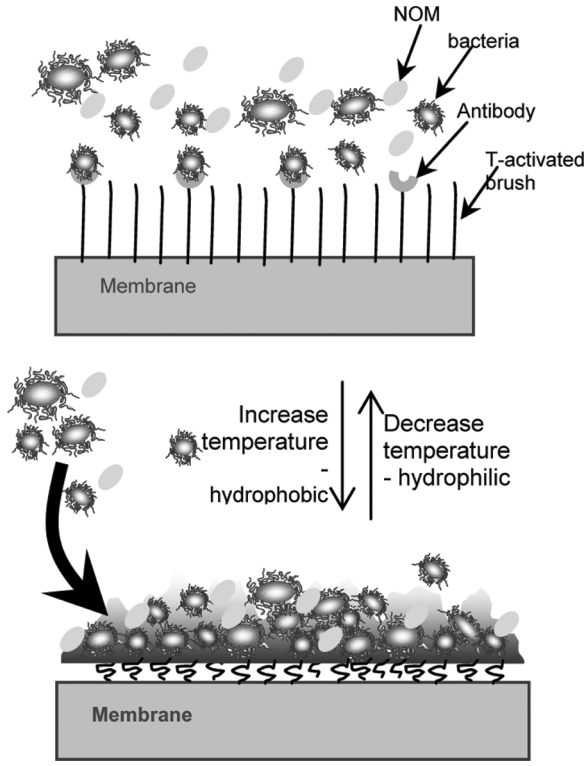


Figure 1. Stimuli-responsive polymer film acting as the support medium for bacterial sensing.

BACKGROUND

Membrane Post Synthesis Modifications

An intensive area of research to produce fouling-resistant membranes is through post-synthesis surface modifications, which include the modification of existing membranes. Modification methods that have been investigated include ion beam irradiation (18,30), plasma treatment or grafting (46,17), UV induced grafting (49,8,9,32), chemical sulfone enrichment (39), chemical dehydroflurination by alkaline solution (5), grafting with pH- and ionic strength-sensitive polymeric brushes (23,24), γ irradiation induced grafting (19), physical adsorption of water soluble polymers (26), formation of Langmuir-Blodgett films (29), and thermal grafting of a hydrophilic polymeric surface coating (40). Biocides such as silver nanoparticles may kill bacteria, but the remains of the bacteria are still present on the surface (41).

Biorecognition Molecule

In an effort to develop sensors that are rapid, portable, and sensitive, biosensors have frequently been applied for the detection of organic and biological molecules (25,42,36). 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, *r*RNA) are targeted. 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 (25,22). Species-specific antibodies specific to cell surface antigens are applied to environmental samples in conjunction with a fluorescent reporting system, resulting in a fluorescent signal from labeled cells. Antibody-based approaches are commonly used to detect Cryptosporidium oocysts and Giardia cysts in treated and untreated drinking water (46). Infectious rotaviruses have been detected in source waters by indirect immunofluorescence (1).

For application, 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 have included natural waters and sediments (34,3,31,14,16).

EXPERIMENTAL

Chemical and Membrane Information

While the overarching goal of this work was to develop fouling resistant layers with sensing capabilities for membrane systems, this work focused on detailed synthesis and characterization of individual components for a model membrane system (cellulose acetate). Experimentally, the surface of an ultrafiltration (UF) cellulose acetate (CA) membrane (molecular weight cutoff of 20,000 Da, manufactured by GE Osmonics, Minnetonka, MN) was grafted with a nano-sized thermally responsive film layer of HPC.

The divinyl sulfone (DVS, purity $\geq$98%), hydroxypropyl cellulose (HPC, MW of 100,000) and carbodiimide (commercial grade) were purchased from Sigma Aldrich, All other chemicals were purchased from Fisher Scientific.

Develop Methods to Systematically Modify the Membrane Surface with a Stimuli Responsive Film at Controlled Concentrations

Free standing crosslinking of HPC with Divinyl Sulfone (DVS) can be formed for LCST or general tests as needed. The results for free standing gels were 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 where the vinyl groups open up and the R groups connect to the carbon of the DVS via the oxygen atom. The hydrogen from the hydroxyl group transfers the the carbon connected to the sulfur (27,4):

$$R_{CA}^{-OH} + CH_2 = CHSO_2CH = CH_2 + OH - R_{HPC} \rightarrow R_{CA} - OCH_2CH_2SO_2CH_2CH_2 - O - R_{HPC}$$

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 to allow for modification of the cellulose acetate membranes as described below.

A "grafting to" approach was used to modify the membranes (Fig. 2). Prior surface modifications were with "grafting from" which involves building the film from the membrane up, which makes more linear polymer structures. "Grafting to" involves making the polymer structures for the film first and then attaching it to the surface. This was done by making a loosely cross-linked solution of the polymer, which allows for "branch assembly" on the individual structures. The branched assemblies were then attached to the membrane surface via DVS linkages. This was done by attaching DVS to the CA surface and then exposing the surface to the loosely cross-linked solution. The modified membrane was allowed

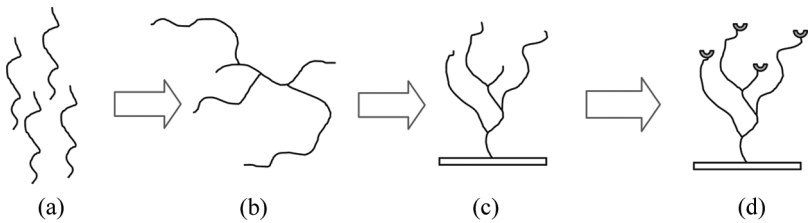


Figure 2. Membrane modification procedure: (a) Loose monomer in solution; (b) Moderately cross-linked polymer; (c) Film reacts to free radical sites on the membrane surface; and (d) Bacterial sensors attached to film.

to react for 48 hours and then soaked and rinsed repeatedly over several days to remove any unattached material. The long duration for reacting was because with this method, the high pH would make the membranes very brittle. Lower pH of 10 with longer reaction times offer the same results as with a pH of 12.

Characterize Chemical Structure, Microstructure and Thermal Responsiveness of HPC Membranes

- ATR-FTIR was used to probe the evolution in chemical structure of the covalently bound polymer film 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. Measurements were performed using a Digilab UMA 600 FT-IT microscope with a Pike HATR adapter and an Excalibur FTS 400 spectrometer. Scans with iterations of 256 were done in the range to $700\,cm^{-1}$ to $4000\,cm^{-1}$.
- Atomic force microscopy (AFM) was used to monitor the surface morphology as a function of temperature. 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 analysis approaches such as section, bearing, and roughness analysis. This device works due to the atomic force interactions that exist between the surface being scanned and the tip. The tip moves up and down with the changing topography, thereby flexing the cantilever it is attached to. A laser focused at the head of the cantilever has its light reflected into the data processing unit for the user to image on a screen and interact with. This particular cell that holds the tip has aqueous capabilities which provide the ability to complete AFM measurements in relatively cold and hot aqueous environments. AFM measurements were performed using a Nanoscope IIIa Scanning Probe Microscopy in the Department of Chemical Engineering at the University of Toledo.

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 (50). 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 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was chosen to attach the antibody to the surface bound HPC. The reaction chemistry is shown below:

$$\begin{aligned} &R_{CA}-OCH_2CH_2SO_2CH_2CH_2-O-R_{HPC} \\ &\quad -OH + \text{ carbodiimide } (CH_3CH_2N=C-NCH_2CH_2CH_2N(CH_3)_2 \\ &\quad +\text{antibody}(HOOC-R_{\text{antibody}}) \Longrightarrow HPC-\text{antibody} \end{aligned}$$

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

Mycobacteria species were targeted in this research as a potential biofouling agent. The recovery of target microorganisms (1×105 to 1×107 cells) on the smart membrane was evaluated using a modified protocol (50). The primary antibody (polyclonal antibody 9.7 mg/ml Biogenesis, USA) was chemically attached (1:1000 by volume) to the HPC-nanostructured membrane (8% by wt.), resulting in the smart membrane. The smart membranes (25 mm dia.) were manipulated in 6-well cell culture plates. Visual verification of primary antibody binding was achieved by adding fluorophore labeled secondary antibody (Leinco, USA) at varying dilutions (1:10 to 1:200) and incubated for 30 min at room temperature. Secondary antibody recovery, validated by fluorescence, suggested a primary antibody presence on the surface of the smart membrane. 2 uL of a DNA intercalating stain (Picogreen®, Invitrogen, Chicago, IL) was added and incubated for 5 min to detect bound bacteria. Samples were processed on a fluorescent microscope (1000X). Up to 20 fields (minimum 200 cells) were counted for each sample. Control samples for this experiment included: unmodified membrane, membrane modified with HPC only, and antibody-HPC modified membrane without

microorganisms. Non-specific binding of mycobacteria to the unmodified membrane and the membrane modified with HPC was negligible (less than 1 per field on average).

RESULTS AND DISCUSSION

The project goal was to determine the viability of the novel idea of combining a stimuli responsive film on a membrane for fouling resistance with a biosensor component to detect potential biofouling species. There were four main goals of this work:

1. develop chemistries to bind HPC to the membrane surface
2. determine if the lower solution critical temperature of the HPC was maintained
3. demonstrate covalent binding of a model biorecognition molecule (antibody) to the film moities on the membrane surface and
4. demonstrate that the antibody-HPC modified membrane can immobilize the target organism.

Formation of Stimuli Responsive Film 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 film, hydroxypropyl cellulose (HPC) via a divinylsulfone spacer (DVS). The first primary task of the project was to confirm this functionalization and verify if the HPC LCST would remain near 46°C when it is attached to the membrane. This differs from the aforementioned 40°C because that was dealing with free HPC monomer in solution. The 46°C comes from our LCST determinations of DVS cross-linked gels.

As an initial step, crosslinked gels of DVS and HPC were produced. While the DVS acts as a linker molecule between the membrane surface and the HPC film in the system of interest, it is used initially as a cross-linker to form bulk free standing gels of HPC. These were used to determine the LCST of the bulk cross-linked gels to analyze impact of DVS on LCST of the HPC. Figure 3 shows the weight loss of the gel in the ordinate and indicates that there is a distinct LCST at 46°C for the hydrogels irrespective of initial HPC concentration (shown both a 9% and a 10% HPC composition).

The next step was to functionalize the CA membrane with HPC via the DVS spacer and characterize the HPC-modified membrane using

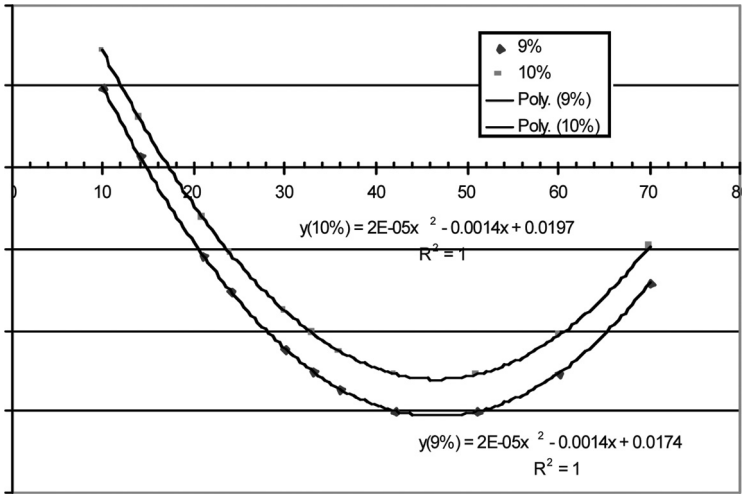


Figure 3. Distinct LCST at 46°C for the hydrogels irrespective of initial HPC concentration (shown both a 9% and a 10% HPC composition).

FTIR (Fig. 4a). 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 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. Figure 4b shows the qualitative differences in peak heights of the major peaks between the virgin membrane and the HPC modified membrane. The peaks associated with DVS on the spectrum indicate that what was anchored to the membrane was a crosslinked gel of HPC-DVS. Thus, an increase in the peak intensities indicated qualitatively that some of the gel material was deposited.

The subsequent stage was to show that the film bound to the membrane was activated using temperature. To this end, atomic force microscopy (AFM) was used to determine the roughness of the membranes in presence of solutions of varying temperatures. As shown in Fig. 5, the unmodified membrane displayed a roughness of 2.242 nm and 4.245 nm at 25°C and 60°C, respectively. The negligible difference between the cold and hot temperature measurements indicates that temperature had no effect on the unmodified membrane. On the other hand, the HPC modified membrane displayed a roughness of 8.398 nm at 25°C and 0.915 nm at 60°C. The difference in roughness demonstrated that the HPC-modified membrane could be activated by temperature. The increase in roughness at the cold temperature was attributed to the extension of the surface

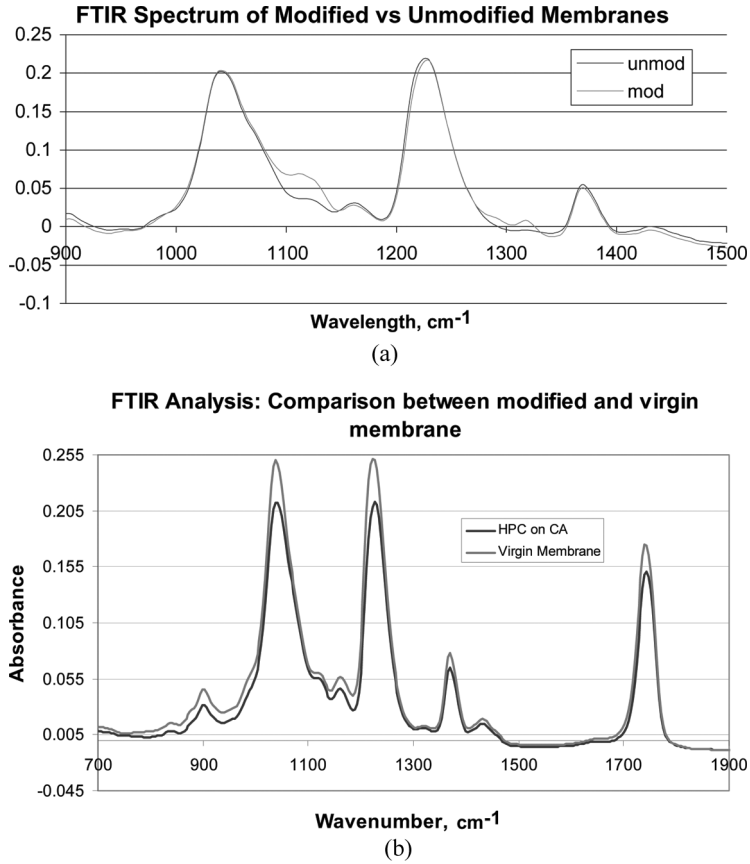


Figure 4. (a) Characterization of the HPC-modified cellulose acetate membrane using FTIR; (b) Comparison of peaks between virgin membrane and HPC modified membrane.

attached film. The significant decrease in roughness at 60°C shows that the film collapses at high temperatures (i.e. in its hydrophobic stage). The images were taken on the same locations for the different membranes at the high and low temperatures. Scan rates may vary because depending on certain conditions, one would obtain clearer images with slower scan rates.

Figure 6 shows a comparison of the flux declines for the unmodified and modified membranes. It is important to note that the higher flux values observed at the high temperature experiments were due to the decrease in water viscosity as a function of temperature. The feed solution filtered was a high fouling solution as it contained hydrophobic

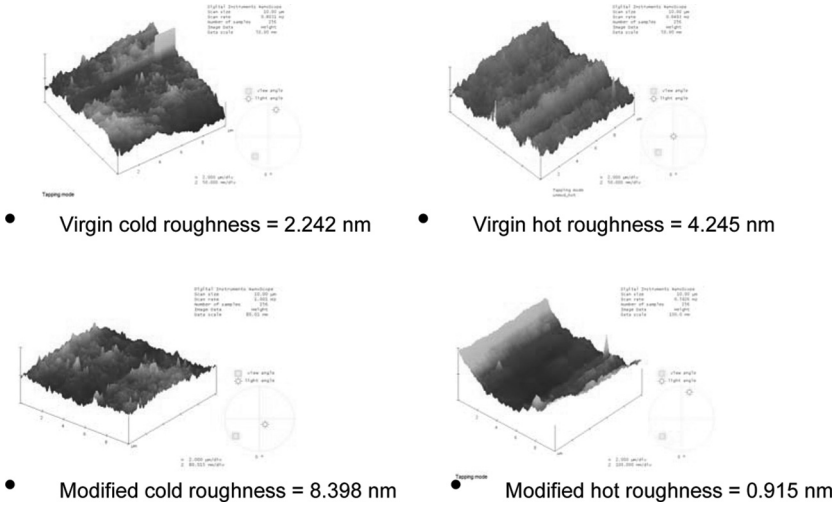


Figure 5. AFM images used to determine the roughness of the unmodified and modified membranes.

organic matter (in the form of humic and fulvic acids). Therefore, the solution was expected to foul the membrane more, as observed by a decline in flux. Under constant cold operation, the initial and final fluxes for the unmodified membrane were 119.42 L/m^2-h and 100.43 L/m^2-h

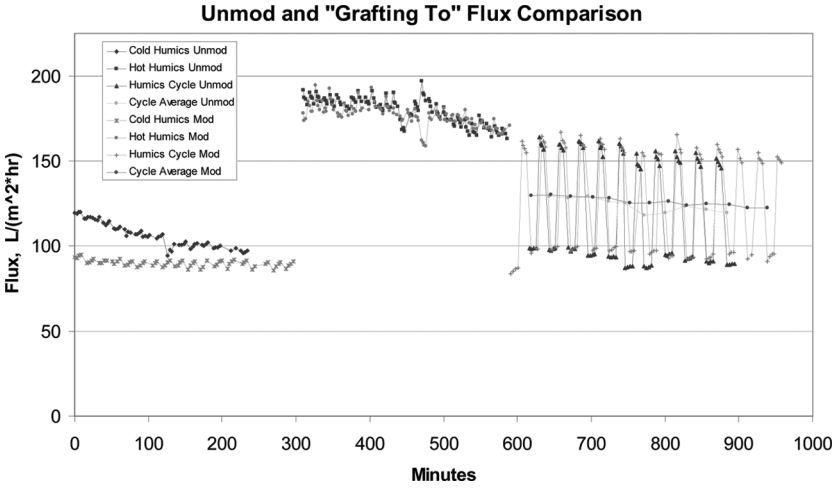


Figure 6. Flux comparison of Virgin membrane and modified membrane with a humic solution to create fouling.

(a 15.9% decrease), while for the modified membrane, the initial and final fluxes were 93.39 L/m^2-h and 90.80 L/m^2-h (a 2.78% decrease). Under constant hot operation, the initial and final fluxes for the unmodified membrane were 184.70 L/m^2-h and 163.20 L/m^2-h, which was a 11.64% decrease. On the other hand, for the modified membrane, hot operation led to initial and final fluxes of 179.43 L/m^2-h and 170.60 L/m^2-h (a 4.92% decrease). Lastly, under cold/hot oscillations, the initial and final fluxes for the unmodified membrane were 129.30 L/m^2-h and 119.68 L/m^2-h, or a 7.44% decrease in flux, while for the modified membrane, these were 129.47 L/m^2-h and 122.32 L/m^2-h (a 5.52% decrease). Therefore, under all conditions, the modified membrane showed a lower flux decline than the unmodified membrane. Further, the advantage of the cycling of hot/cold was essentially that higher flux values were maintained for the modified membrane.

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 was chosen. Fourier transform infrared spectroscopy was used to monitor the membrane

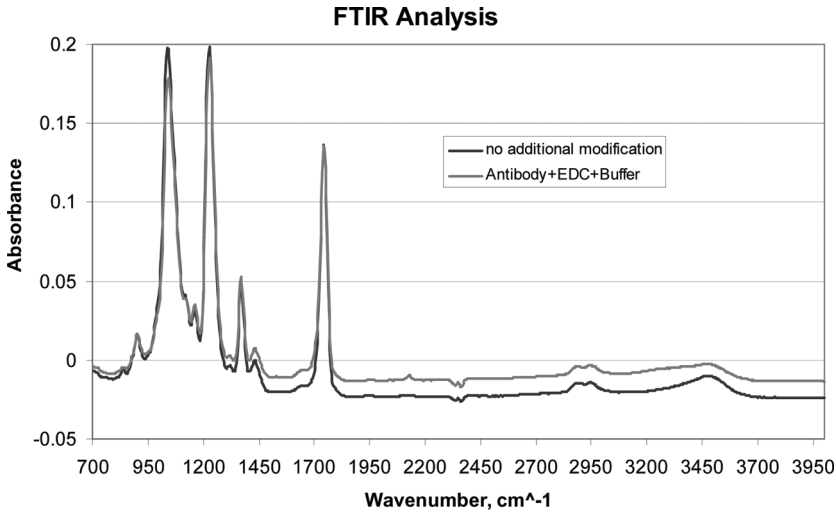
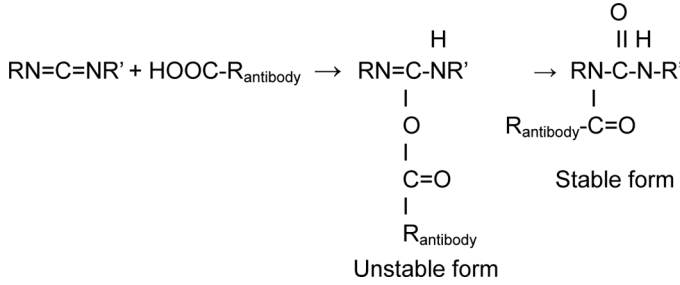


Figure 7. Characterization of the HPC-modified cellulose acetate membrane with the attached antibody using FTIR.

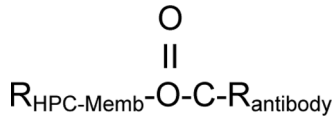
following each reaction step. The only peak that was affected was at $2129\,cm^{-1}$ (Fig. 7) and is the C=N=C peak. Other peaks from the carbodiimide were not detectable because of peaks already present from the membrane. Since the carbodiimide is acting as a zero-length linker, we hypothesize that the appearance of the peak at $2129\,cm^{-1}$ is due to the film binding to the antibody. This is the known method of attachment:

Modified Membrane + carbodiimide + antibody:

1. antibody + carbodiimide ($CH_3CH_2N{=}C{=}NCH_2CH_2CH_2N(CH_3)_2$ or $RN{=}C{=}NR'$) → activated antibody



2. activated unstable antibody + modified membrane (R_{CA}–OCH_2CH_2-$SO_2CH_2CH_2$–O–R_{HPC}–OH or $R_{HPC-Memb}$-OH):



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 (n = 9 membranes) with a range of initial mycobacteria concentrations (1×10^5 to 1×10^7/mL), 5–15% were recovered on the antibody-HPC modified membrane surface (Fig. 8). The recovery efficiency was not influenced by incubation time or intitial concentration. However, it is anticipated that an increase in

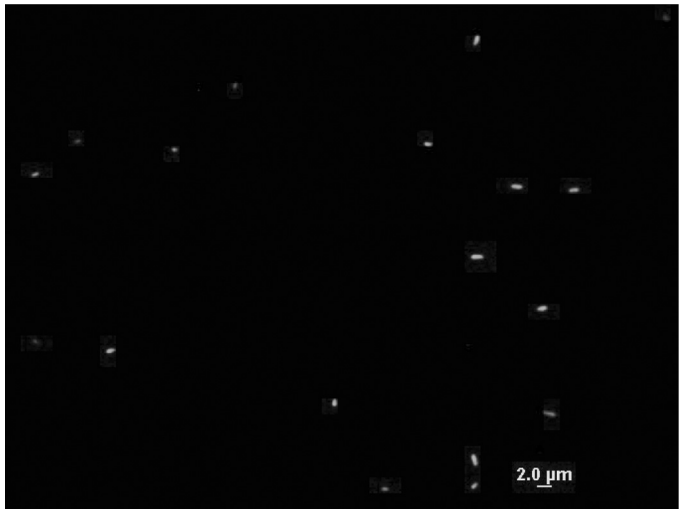


Figure 8. *Mycobacteria* were captured on the surface of antibody-HPC modified membrane (1000X).

the amount of primary antibody bound to the membrane surface will result in increased recovery efficiency.

CONCLUSIONS

The project produced a fouling-resistant membrane by attaching a stimuli-responsive polymer film on the surface. A temperature decrease caused the film to expand into a hydrophilic state while a temperature increase caused a collapse into a hydrophobic state. By continuously triggering the phase transition, the non-equilibrium movement of the polymer film may offer better protection of the surface than at equilibrium. The surface of a cellulose acetate membrane was grafted with a thermally responsive hydroxypropyl cellulose (HPC) film layer. Aqueous solutions of HPC possessed a lower critical solution temperature of approximately 40°C (while cross-linked structures had an LCST of 46°C): above this temperature the solution phase separated. Biorecognition molecules targeting selected bacteria were covalently bounded to the end of polymer film for in situ detection.

The 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 paper addresses detection of bio-

logical contaminants in water systems, the sensor developed from this work can be adapted to address a range of national and international environmental concerns.

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REFERENCES

1. Abad, F.X.; Pinto, R.M.; Bosch, A. (1998) Flow cytometry detection of infectious rotaviruses in environmental and clinical samples. *Applied and Environmental Microbiology*, *64* (7): 2392–2396.
2. Baker, J.S.; Dudley, L.Y. (1998) Biofouling in membrane systems–A review. *Desalination*, *118*: 81–90.
3. Bard, D.G.; Ward, B.B. (1997) A species-specific bacterial productivity method using immunomagnetic separation and rediotracer experiment. *Journal of Microbiological Methods*, *28*: 207–219.
4. Bordawekar, M.; Escobar, I.; Lipscomb, G. (2002) "Application of a Temperature-Sensitive Polymer Brush to Reduce Fouling on Membranes," Ohio Water Environment Association Annual Conference, Columbus, OH, July 25–27, 2002.
5. Bottino, A.; Capannelli, G.; Monticelli, O.; Piaggio, P. (2000) Poly(vinylidene fluoride) with improved functionalization for membrane production. *Journal of Membrane Science*, *166* (1): 23–29.
6. Bloisi, F.; Vicari, L.; Papa, R.; Califano, V.; Pedrazzani, R.; Bontempi, E.; Depero, L.E. (2007) Biomaterial thin film depositionand characterization by means of maple technique. *Materials Science and Engineering C*, *27*: 1185–1190.
7. Childress, A.; Elimelech, M. (1996) Effect of solution chemistry on the surface charge of polymeric reverse osmosis and nanofiltration membranes. *Journal of Membrane Science*, *119*: 253–268.
8. Davis, R.H.; Bowman, C.N. (1999a) Microfiltration with rapid backpulsing and surface-modified membranes. US Department of The Interior–Bureau of Reclamation, Water Treatment Technology Program Report No. 28, September 1999.

9. Davis, R.H.; Bowman, C.N. (1999b) Microfiltration with Rapid Backpulsing and Surface-Modified Membranes. US Department of The Interior – Bureau of Reclamation, Water Treatment Technology Program Report No. 58, December 1999.
10. Elimelech, M.; Chen, W.H.; Waypa, J.J. (1994). Measuring the zeta (electrokinetic) potential of reverse osmosis by a streaming potential analyzer. *Desalination*, *95*: 269–286.
11. Escobar, I.; Hoek, E.; Gabelich, C.; DiGiano, F.; Le Gouellec, Y.; Berube, P.; Howe, K.; Allen, J.; Atasi, K.; Benjamin, M.; Brandhuber, P.; Brant, J.; Chang, Y.; Chapman, M.; Childress, A.; Conlon, W.; Cooke, T.; Crossley, I.; Crozes, G.; Huck, P.; Kommineni, S.; Jacangelo, J.; Karimi, A.; Kim, J.; Lawler, D.; Li, Q.; Schideman, L.; Sethi, S.; Tobiason, J.; Tseng, T.; Veerapaneni, S.; Zander, A. (2005) American water works association membrane technology research committee report: Membrane fouling–recent advances and research needs. *Journal American Water Works Association*, *97* (8): 79–89.
12. Escobar, I.; Randall, A.; Hong, S.; Taylor, J.S. (2002) Effect of solution chemistry on assimilable organic carbon removal by nanofiltration: Full and bench scale evaluation. *Journal of Water Supply: Aqua*, *51* (2): 67–76.
13. Escobar, I.C.; Hong, S.; Randall, A.A. (2000) Removal of assimilable organic carbon and biodegradable dissolved organic carbon by reverse osmosis and nanofiltration membranes. *Journal of Membrane Science*, *175*: 1–17.
14. Favrin, S.J.; Jassim, S.A.; Griffiths, M.W. (2003) Application of a novel immunomagnetic separation-bacteriophage assay for the detection of *Salmonella enteritidis* and *Exchrichia coli* O157:H7 in food. *International Journal of Food Microbiology*, *85*: 63–71.
15. Flemming, H.C.; Schaule, G.; Griebe, T.; Schmitt, J.; Tamachkiarowa, A. (1997) Biofouling–the achilles heel of membrane processes. *Desalination*, *113*: 215–225.
16. Furtado, A.L.D.; Casper, P. (2000) Different methods for extracting bacteria from freshwater sediment and a simple method to measure bacterial production in sediment samples. *Journal of Microbiological Methods*, *41*: 249–257.
17. Gancarz, I.; Pozniak, G.; Bryjak, M. (1999) Modification of polysulfone membranes 1. CO2 plasma treatment. *European Polymer Journal*, *35* (8): 1419–1428.
18. Good, K.; Escobar, I.; Xu, X.; Coleman, M.; Ponting, M. (2002) Effects of ion beam irradiation on the transport properties and fouling control of commercial membranes. *Desalination*, *146*: 259–264.
19. Hegazy, E.A.; Elrehim, H.A.A.; Khalifa, N.A.; Atwa, S.M.; Shawky, H.A. (1997) Anionic/cationic membranes obtained by a radiation grafting method for use in waste water treatment. *Polymer International*, *43* (4): 321–332.
20. Hong, S.; Escobar, I.; Hershey, J.; Hobbs, C.; Cho, J. (2005) Biostability characterization in a full-scale hybrid NF/RO treatment system. *Journal American Water Works Association*, *97* (5): 101–110.
21. Hong, S.; Elimelech, M. (1997) Chemical and physical aspects of natural organic carbon (NOM) fouling of nanofiltration membranes. *Journal of Membrane Science*, *132*: 159–181.

22. Iqbal, S.S.; Mayo, M.W.; Bruno, J.G.; Bronk, B.V.; Batt, C.A.; Chambers, J.P. (2000) A review of molecular recognition technologies for detection of biological threat agents. *Biosensors and Bioelectronics*, *15*: 549–578.
23. Ito, Y.; Ochiai, Y.; Park, Y.; Imanishi, Y. (1997) pH-sensitive gating by conformational change of a polypeptide brush grafted onto a porous polymer membrane. *J. Am. Chem. Soc.*, *119*: 1619–1623.
24. Ito, Y.; Park, Y.S. (2000) Signal-responsive gating of porous membranes by polymer brushes. *Polymers for Advanced Technologies*, *11*: 136–144.
25. Ivnitski, D.; Abdel-Hamid, I.; Atanasov, P.; Wilkins, E. (1999) Biosensors for detection of pathogenic bacteria. *Biosensors and Bioelectronics*, *14*: 599–624.
26. Jönsson, A.; Jönsson, B. (1991) The influence of nonionic surfactants on hydrophobic ultrafiltration membranes. *Journal of Membrane Science*, *56*: 49–76.
27. Kabra, B.P.G.P. (1993) "Synthesis, structure and swelling of microporous gels", Doctoral Dissertation, University of Cincinnati, Cincinnati, OH.
28. Kikuchi, A.; Okano, T. (1998) Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Progress in Polymer Science*, *27*: 1165–1193.
29. Kim, K.J.; Fane, A.G.; Fell, C.J.D. (1989). The effect of langmuir-blodgett layer pretreatment on the performance of ultrafiltration membranes. *Journal of Membrane Science*, *43*: 187–204.
30. King, S., Escobar, I.; Xu, X. (2004) Ion beam irradiation modifications of a polyether sulfone commercial water treatment membrane. *Environmental Chemistry*, *1* (1): 55–59.
31. Liu, Y.; Che, Y.; Li, Y. (2001) Rapid detection of salmonella typhimurium using immunomagnetic saparation and immuno-optical sensing method. *Sensors and Actuators B*, *72*: 21–218.
32. Ma, H.; Bowman, C.N.; Davis, R.H. (2000) Membrane fouling reduction by backpulsing and surface modification. *Journal of Membrane Science*, *173*: 191–200.
33. Mallevialle, J.; Suffet, I.H.; Chan, U.S. (1992) *Influence and removal of organics in drinking water*; Lewis Publishers: Boca Raton.
34. Mazurek, G.H.; Reddy, V.; Murphy, D.; Ansari, T. (1996) Detection of *Mycobacterium tuberculosis* in cerebrospinal fluids following immunomagnetic enrichment. *Journal of Mycobacterium Tuberculosis*, *34*: 450–453.
35. Nilson, J.A.; DiGiano, F.A. (1996) Influence of NOM composition on nanofiltration. *Journal of American Water Works Association*, *88*: 53–66.
36. Paitan, Y.; Biran, D.; Biran, I.; Shechter, N.; Babai, R.; Rishpon, J.; Ron, E.Z. (2003) On-line and in situ biosensors for monitoring environmental pollution. *Biotechnology Advances*, *22*: 27–33.
37. Peng, W.; Escobar, I. (2003) Rejection efficiency of water quality parameters by reverse osmosis (RO) and nanofiltration (NF) membranes. *Environmental Science and Technology*, *37* (19): 4435–4441.
38. Peng, W.; Escobar, I.; White, D. (2004) Effects of water quality and membrane properties on performance and fouling–A model development study. *Journal of Membrane Science*, *238* (1–2): 33–46.

39. Pozniak, G.; Krakewska, B.; Trochimczuk, W. (1995) Urease immobilized on modified polysulfone membrane–preparation and properties. *Biomaterials*, *16* (2): 129–134.
40. Stengaard, F.F. (1988) Characteristics and performance of new types of ultrafiltration membranes with chemically modified surfaces. *Desalination*, *70*: 207–224.
41. Lee, Seung Yun; Kim, Hee Jin; Patel, Rajkumar; Im, Se Joon; Kim, Jong Hak; Min, Byoung Ryul. (2007) Silver nanoparticles immobilized on thin film composite polyamide membrane: Characterization, nanofiltration, antifouling properties. *Polymers for Advanced Technologies*, *18*: 562–568.
42. Suzuki, M.T.; Taylor, L.T.; Delong, E.F. (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5-Nuclease Assays. *Applied and Environmental Microbiology*, *66*: 4605–4614.
43. Szleifer, I.; Carignano, M.A. (2000) Tethered polymer layers: phase transitions and reduction of protein adsorption. *Macromolecular Rapid Communications*, *21*: 423–448.
44. Tomaschke, J.E. (2000) Interfacial composite membranes. *III/Membrane Preparation*, 3319–3330.
45. Ulbricht, M.; Belfort, G. (1996) Surface modification of ultrafiltration membranes by low temperature plasma 2. Graft polymerization onto polyacrylonitrile and polysulfone. *Journal of Membrane Science*, *111* (2): 193–215.
46. Vesey, G.; Hutton, P.; Champion, A.; Ashbolt, N.; Williams, K.L.; Warton, A.; Veal, D. (1994) Application of flow cytometric methods for the routine detection of *Cryptospordium* and *Giardia* in water. *Cytometry*, *16*: 1–6.
47. Vrijenhoek, E.M.; Hong, S.; Elimelech, M. (2001) Influence of membrane surface properties on initial rate of colloidal fouling of reverse osmosis and nanofiltration membranes. *Journal of Membrane Science*, *18*: 115–128.
48. Wiesner, M.R.; Hackey, J.; Sandeep, S.; Jacangelo, J.G.; Laine, J.M. (1994) Cost estimates for membrane filtration and conventional treatment. *Journal of American Water Works Association*, *86*: 33–41.
49. Yan, W.; Yang, P.; Wang, Y. (1988) UV-radiation grafting of acrylamide onto cellulose acetate reverse osmosis membrane. *Shuichuli Jishu*, *14*: 213–222.
50. Yi, W.-C.; Hsiao, S.; Liu, J.-H.; Soo, P.-C.; Horng, Y.-T.; Chung, W.-C. (1998) Use of fluorescein labeled antibody and fluorescence activated cell sorter for rapid identification of mycobacterium species. *Biochemical and Biophysical Research Communications*, *250* (2): 403–408.