Enhanced Anti-fouling Properties of Carbohydrate Coated Polyethersulfone Membranes

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Abstract

Polyethersulfone membranes (PES) were modified with biologically active monosaccharides and disaccharides using aryldiazonium chemistry as a mild, one step, surface modification strategy. We previously proposed the modification of carbon, metals, and alloys with monosaccharides using the same method; herein we demonstrate modification of PES membranes and the effect of chemisorbed carbohydrate layers on their resistance to biofouling. Glycosylated PES surfaces were characterized using spectroscopic methods and tested against their ability to interact with specific carbohydrate-binding proteins. Galactose, mannose and lactose modified PES surfaces were exposed to Bovine Serum Albumin (BSA) solutions to assess unspecific protein adsorption in the laboratory and were found to adsorb significantly lower amounts of BSA compared to bare membranes. The ability of molecular carbohydrate layers to impart antifouling properties was further tested in the field via long term immersive tests at a wastewater treatment plant. A combination of ATP content assays, infrared spectroscopic characterization and He-ion microscopy (HIM) imaging were used to investigate biomass accumulation at membranes. We show that, beyond laboratory applications and in the case of complex aqueous environments that are rich in biomass such as wastewater effluent, we observe significantly lower biofouling at carbohydrate modified PES than at bare PES membrane surfaces.

Keywords: carbohydrates, biomimetic, membranes, polyethersulfone, antifouling, coatings.

1. Introduction

Polyarylsulfones such as bisphenol-A polysulfone (PSF), tetramethyl bisphenol-A polysulfone (TMPSF), and polyethersulfone (PES) are polymers with advantageous properties such as high thermal and chemical resistance, combined with excellent hydrolytic and mechanical stability in a range of environments. Polyarylsulfones are therefore excellent polymers for the fabrication of membranes suitable for liquid separation processes that require repeated cleaning with hot water or sterilization. Polyarylsulfones are widely used as materials for the fabrication of membranes in biomedical applications, water purification, wastewater treatment, and fractionation/concentration in the food, pharmaceutical and biotechnological industries.[1-3]

Despite possessing many desirable mechanical and chemical properties, polyarylsulfones are relatively hydrophobic, and membranes produced using these polymers can therefore be prone to fouling. Fouling is one of the most detrimental problems encountered in membrane associated processes, since it can result in higher energy demands, shorter membrane lifetime, unpredictable performance or product contamination.[4, 5] Because membrane fouling is mainly caused by the adsorption of nonpolar solutes, hydrophobic particles or proteinaceous material,[5-7] it is generally accepted that an increase in surface hydrophilicity offers improved resistance to fouling.[8-11] Within the polyarylsulfone family, PES is one of the polymers with highest water retention and hydrophilicity and is therefore often the polyarylsulfone of choice when fouling minimization in aqueous environments and/or low protein binding are required. However, even with the use of PES, fouling remains a major ongoing problem associated with the use of polyarylsulfone membranes. A number of strategies have been investigated in order to address the issue of membrane biofouling, generally the approach involves the design and testing of entirely new highly hydrophilic membrane polymers through blending, bulk or surface modification.[12] Interfacial polymerization or microemulsion coatings are some of the preferred methods for membrane modification. UV photografting, electron beam irradiation, plasma grafting and layer-by-layer methods have also been developed however, despite being effective for small scale development in labs their scale up has proved to be challenging. Nanomaterials and additives have also

been explored more recently, however further investigations are required due to safety and environmental concerns about these new materials.[13-17] An alternative approach consists of modifying the surface of polymers or membranes, such as PES or PES-based membranes with ultrathin molecular coatings in order to improve their antifouling properties, while at the same time preserving their desirable mechanical, thermal resistant, and chemical resistant properties. In this work, we describe how surface modification strategies can indeed be leveraged in order to reduce protein accumulation on PES membranes without compromising the mechanical integrity of the membrane.

In order to introduce new specific functionalities, and to inhibit early stages of the fouling cascade process, we have recently proposed a new, mild, one step surface modification strategy for the carbohydrate functionalization of various surfaces using aryldiazonium salts.[18] Carbohydrates are extremely important biomolecules that are involved in a diverse array of biological functions including fertilization, cell-cell communication and inflammatory responses.[19] Importantly, glycosylated surfaces have attracted much attention as platforms for investigating the mechanisms involved in nonspecific adsorption of proteins onto solid surfaces and as a biomimetic strategy for minimizing fouling in aqueous media.[20, 21] Surface coatings consisting of high molecular weight polysaccharides such as dextran have been shown to reduce protein or cell adhesion to surfaces,[22, 23] while modifications with short oligosaccharides or monosaccharides have also been demonstrated to have a pronounced effect on minimizing unspecific protein adsorption and biomass accumulation.[24-27]

Previous work from our group showed that Galactose-modified carbon surfaces obtained *via* spontaneous grafting of aryldiazonium salts display an increased resistance to Bovine Serum Albumin (BSA) adsorption, compared to bare carbon surfaces.[18] Such an effect was achieved, remarkably, via immobilization of only 1-3 ML of the monosaccharide. In this work we demonstrate that it is possible to apply this coating strategy beyond carbon and metal surfaces, to polymeric materials that find widespread application and are of great technological importance. We demonstrate that modification of PES membrane surfaces using mono- and di-saccharide coatings is possible *via* spontaneous aryldiazonium salt chemisorption reactions. Laboratory tests using single protein solutions indicate that

these coatings lead to a significant decrease in nonspecific protein adsorption at the membrane surface. The antifouling properties of carbohydrate-coated PES membranes were further investigated during immersive field tests at a wastewater treatment plant. Our results show that resistance to protein adsorption observed in the laboratory successfully translates into decreased adsorption of organic and proteinaceous material when carbohydrate modified membranes are exposed to complex, real world aqueous media that is rich in biomass.

2. Materials and Methods

Chemicals and Materials. Phosphate buffered saline solution (PBS, pH 7.4, 0.010 M), acetone (HPLC grade), methanol (MeOH, semiconductor grade), sodium nitrite ($NaNO_2$), fluoroboric acid (HBF_4) and nitric acid (HNO_3) were purchased from Sigma Aldrich and were used without further purification. Lectins from Arachis hypogaea (peanut agglutinin, PNA) fluorescein isothiocyanate (FITC) conjugate, and Concanavalin A from Canavalia ensiformis (Jack bean, ConA) FITC conjugate, Type IV, were purchased as lyophilized powder from Sigma Aldrich. Bovine Serum Albumin FITC conjugate (FITC-BSA) and PES membranes (0.45 μm, 25 mm, Pall) were also purchased from Sigma Aldrich. Deionized water was used for all aqueous solutions; all glassware was cleaned with piranha solution before use.

Synthesis of carbohydrate-aryldiazonium salt precursors and surface modification. 4-aminophenol-β-D-galactopyranose (**1**) and 4-aminophenol-α-D-mannopyranose precursors (**2**), shown in Figure 1 were synthesized as previously reported.[18] The disaccharide lactose derivative, 4-aminophenol-β-D-lactopyranose (**3**) and its fluorinated derivative 4-amino-2-fluorophenol-β-D-lactopyranose (**3a**) (Figure 1) were synthesised using a similar synthetic approach. Commercially available D-Lactose was per-acetylated on treatment with sodium acetate and acetic anhydride. The product was converted to the glycosyl bromide on treatment with hydrogen bromide in acetic acid. Silver ion promoted glycosylation of the anomeric bromide, furnished the 4-nitrophenol lactose derivative which was subsequently deprotected under basic conditions, and the nitro group reduced to give the desired 4-aminophenol

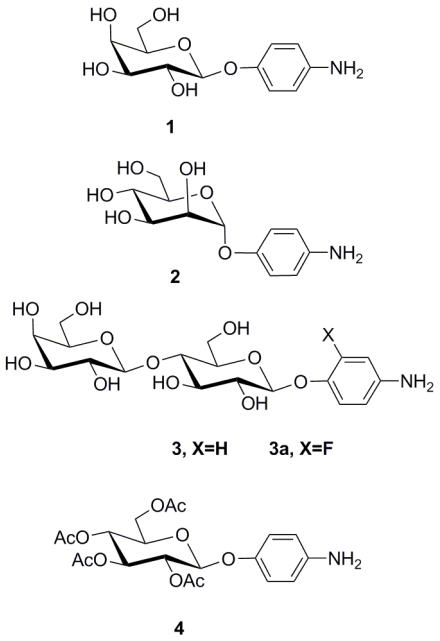


Figure 1: Compounds used for surface modification experiments: 4-aminophenol-β-D-galactopyranose (**1,** Gal-terminated) and 4-aminophenol-α-D-mannopyranose precursors (**2**, Man-terminated), 4-aminophenol-β-D-lactopyranose and its fluorinated derivative (**3** and **3a**, Lac-terminated) and per-acetylated 4-aminophenol-β-D-glucopyranose (**4**, Glc-terminated).

product **3** (see Supporting Information). Per-acetylated 4-aminophenol-β-D-glucopyranose (**4**) was synthesized as previously reported.[18]

Prior to modification, PES membranes were rinsed twice in deionized water, dried under vacuum and finally dried under nitrogen flow. Membranes were subsequently immersed for 1 h in the dark at room temperature in a 1.0 mM aqueous solution of the glycosyl-aryldiazonium cation; the cation was prepared *in situ* as described in our previous work.[18] Briefly, 4-aminophenyl glycosides (compounds **1-4**) were dissolved in 20.0 mL of 1.25 mM HBF_4, and kept in a beaker of ice. 5.0 mL of 5.0 mM $NaNO_2$ that was also kept in ice was then added dropwise over a period of 10 min to the precursor solution in order to generate the diazonium salt at a final concentration of 1.0 mM. Samples were then washed twice in deionized water and dried under nitrogen prior to further use.

Lectin-carbohydrate binding and protein adsorption studies. Lectin binding and protein adsorption studies were carried out on PES membranes coated with galactose (Gal-PES) mannose (Man-PES) and lactose (Lac-PES) units. Man-PES and Gal-PES surfaces were incubated for 2 h in

0.010 M PBS buffer at pH 7.4, with added 0.1 mM $CaCl_2$ and $MnCl_2$ and 0.5 mg/ml of FITC-ConA and FITC-PNA lectin fluorescent conjugates, respectively.[28, 29] Membranes were rinsed with PBS buffer in order to remove excess unbound protein prior to imaging. Protein adsorption studies were carried on Gal-PES and Lac-PES membranes; surfaces were incubated for 2 h with 200 μl of 0.2 mg/mL FITC-BSA solution in 0.010 M PBS at pH 7.4.[30-32] The surface was washed with the PBS solution to remove unbound protein prior to imaging. Fluorescence images were acquired with an Olympus BX51 inverted fluorescent microscope using a cellSense digital image processing software; only brightness and contrast were adjusted in all images presented. The images were acquired with a cube filter set having excitation filter at 470-495 nm, a dichroic filter at 505 nm and a barrier filter at 510-550 nm. Analysis of emission intensity was carried out using image analysis software (ImageJ) from 1600×1200 px^2 images, each covering a sample area of approximately 4 mm^2. Intensity values were calculated by defining a mask (314×263 px^2) in carbohydrate-modified and unmodified regions of the membranes. The mean emission intensities were ratioed in order to compare the amount of adsorbed fluorophore on both types of sample regions; standard deviations were calculated over three samples.

Field testing of PES membranes in wastewater effluents. Tests were carried out at Osberstown Wastewater Treatment plant in Co. Kildare (Ireland). PES membranes were mounted on flanged brass fittings via their compression nuts leaving an exposed geometrical area of 3.80 cm^2; fittings were mounted at regular intervals on a polycarbonate plate suspended at constant water depth by an aluminum frame placed inside a plastic water tank. For each test, four uncoated and four Lac-PES membranes were mounted on the plate; plates were immersed with the fittings facing the bottom of the tank and the membrane surface positioned at 15 cm from the bottom. Figure 2 shows a drawing of the testing frames used in our experiments. The tank was connected *via* a plastic hose to a wastewater effluent well; a pump (Model 410, Solinst) was programmed via a garden power switch to pump water for 10 min, five times a day, thus periodically exchanging the water in the tank with fresh effluent, while maintaining a constant water level in the tank. Tests were carried out over 1 month periods under ambient conditions of temperature and pressure. Air temperature varied between -0.1 and 14.4 °C (daily

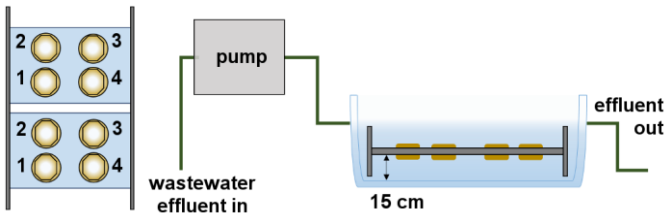
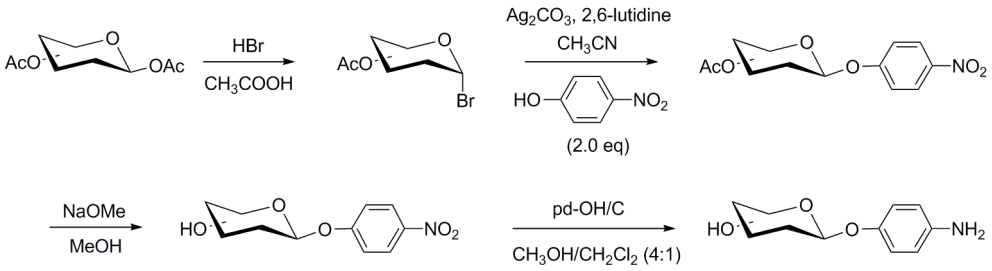


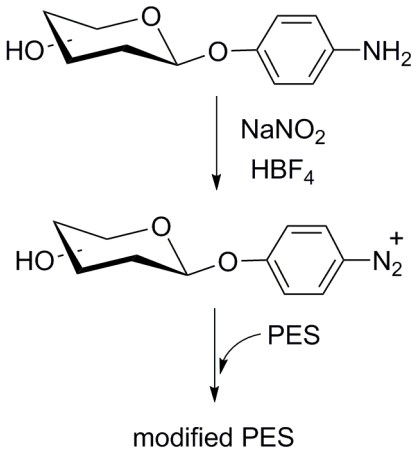
Figure 2: Experimental setup used for tests of membrane fouling in wastewater treatment effluents. Membranes were mounted at positions 1-4 as on the left panel, alternating controls and samples; all membranes were placed facing the bottom of the tank at a constant distance, as on the right.

average 6.4 °C), and between -3.3 and 14.8 °C (daily average 6.8 °C), whereas total rainfall was 113.2 and 58.5 mm for Test 1 and 2, respectively (Met Eireann, Casement Aerodrome station). Although temperature conditions were similar for both tests the higher rainfall over the duration of Test 1 resulted in significantly more fouling at end point.

Membrane characterization. Attenuated total internal reflectance infrared spectroscopy (ATR-FTIR) spectra were recorded using a Perkin Elmer diamond crystal accessory; 40 scans were collected at 4.0 cm^{-1} for each sample. All membranes were dried prior to ATR-FTIR characterization in order to minimize interference from water bands. X-ray photoelectron spectroscopy (XPS) was carried in a VG Scientific ESCAlab Mk II system equipped with a non-monochromatized Al Kα source, at 90° take-off angle. 15 scans were accumulated for each spectrum at 20 eV pass energy; analysis was performed using commercial software (CasaXPS) and Scofield relative sensitivity factors (F=4.43, C=1, N=1.8, S=1.68, O=2.93). He-ion microscopy (HIM) was carried out with a Zeiss Orion Plus (Peabody, MA) instrument equipped with an Everhart Thornley detector and a defocused electron flood gun for charge neutralisation, at 30 kV accelerating volatge and 1 pA beam current. Determination of Adenosine 5′-triphosphate (ATP) content in PES membranes was carried out using a commercial bioluminescence assay (Aquasnap Total kit and EnSURE luminometer, Hygiena) based on the ATP-dependent luciferase-luciferin reaction.[33, 34] After exposure to protein solution or wastewater effluent, modified and



Scheme 1: General synthetic strategy for the synthesis of 4-aminophenyl glycosides shown in Figure 1.



Scheme 2: Protocol for in situ modification of PES.

controlled membranes were lightly rinsed in water, cut to a size of 2.45 cm^2, placed into 15 ml of deionized water and sonicated for 20 min. The solution was then analyzed by dipping the tip of the test-tube, which is designed to sample 100 µl of solution; ATP values are reported as Relative Luminescence Units (RLU).

3. Results and Discussion

3.1 Carbohydrate modification of PES membranes

Mono- and di-saccharide 4-aminophenyl glycosides shown in Figure 1 were prepared through the general synthetic pathway outlined in Scheme 1,[18] and used for the modification of PES membranes surfaces *via* immersion into their corresponding aryldiazonium cation solutions as shown in Scheme 2. We used Attenuated Total Internal Reflectance Infrared Spectroscopy (ATR-FTIR), XPS and binding

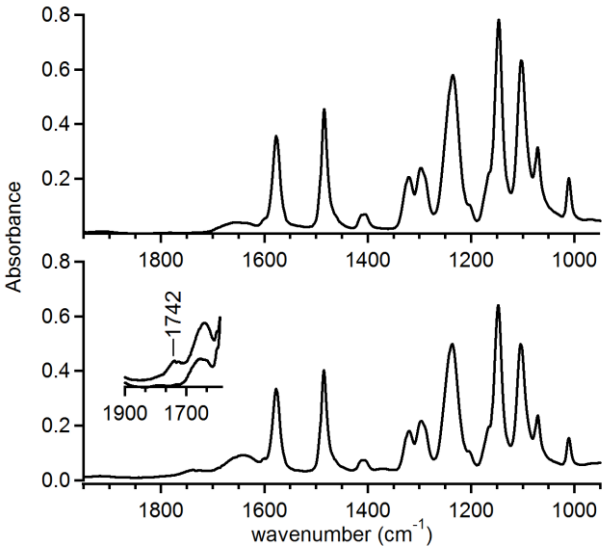


Figure 3: ATR-FTIR spectra of PES membranes unmodified (top) and modified with the aryldiazonium cation of compound **4**. The inset shows the difference between the two spectra in the carbonyl stretching region, where a C=O stretching peak is visible in the case of membranes modified with per-acetylated glucoside groups.

experiments in combination with fluorescence imaging in order to investigate the modification of PES surfaces.

Figure 3 shows ATR-FTIR spectra of a bare PES membrane (top) and of a PES membrane after modification in a 1.0 mM solution of the aryldiazonium cation of compound **4** (bottom)**,** in the fingerprint region. The spectrum of the bare PES membrane was found to be in good agreement with spectra reported in the literature.[35-37] Two peaks at 3096 cm^{-1} and 3070 cm^{-1} can be assigned to aromatic C–H stretching modes (see Supporting Information),[37, 38] whereas peaks at 1578, 1485 and 1408 cm^{-1} are characteristic of aromatic C=C stretching modes of aryl rings.[37, 39] Peaks at 1321 and 1297 cm^{-1} are assigned to S=O asymmetric stretchings in -SO_2-, while the 1146 cm^{-1} peak is assigned to the symmetric stretching mode.[39] The peak at 1236 cm^{-1} is characteristic of PES and is usually assigned to the asymmetric stretching of Ar-O-Ar ethers.[39] Broad peaks at 3400 cm^{-1} (see Supporting Information) and 1640 cm^{-1} can be attributed to stretching and bending modes of residual water within the membrane, respectively. A summary of infrared peaks and their assignments is reported in Table I. The spectrum of the PES membrane modified with per-acetylated glucopyranoside (**4**) displays a similar spectral profile

to that of bare PES with the exception of two additional peaks at 1742 cm^{-1} (inset) and 1370 cm^{-1} characteristic of C=O stretching modes of esters and of $–CH_3$ bending modes,[18, 38] respectively, which can be attributed to surface-bound acetyl groups. The presence of these peaks indicates that, after immersion of PES membranes in the aryldiazonium cation solution, their surface is modified with per-acetylated glucopyranoside moieties. ATR-FTIR spectra of PES membranes modified with de-acetylated 4-aminophenyl glycosides **1-3** did not reveal significant differences compared to that of a bare PES membrane due to spectral overlap in the mid-infrared between PES and phenyl glycosides.

Peak Position (cm^{-1})	**Assignment**
1011, 1070, 1103	-
1146	$\nu_s(SO_2)$
1236	ν_{as} (Ar-O-Ar) of aromatic ethers
1297, 1321	$\nu_{as}(SO_2)$
1370*	$\delta(CH_3)$
1408, 1485, 1578	ν(C=C) of aryl rings
1640	β(H-O-H)
1742*	ν(C=O) of esters

Table 1: Peaks and assignments of infrared spectra of membranes shown in Figure 3; peaks indicated with an asterisk (*) are present only on PES membranes modified with per-acetylated glucoside. ν = stretching; β = bending; δ = distorsion; *s* = symmetric; *as* = asymmetric.

XPS studies of PES functionalization were carried out using a fluoro-substituted derivative of the lactoside precursor, compound **3a** (Figure 1), as the presence of a F atom provides elemental contrast between the functional layer and the PES substrate. The XPS spectrum of bare PES membranes displays peaks for C, O, S and N (see Supporting Information); the O/C and S/C atomic ratios were found to be 23% and 7.9%, respectively, in good agreement with the expected stoichiometry of PES (O/C = 25% and S/C = 8.3%). Nitrogen is also present in the membranes at N/C = 4.4% content; the presence of

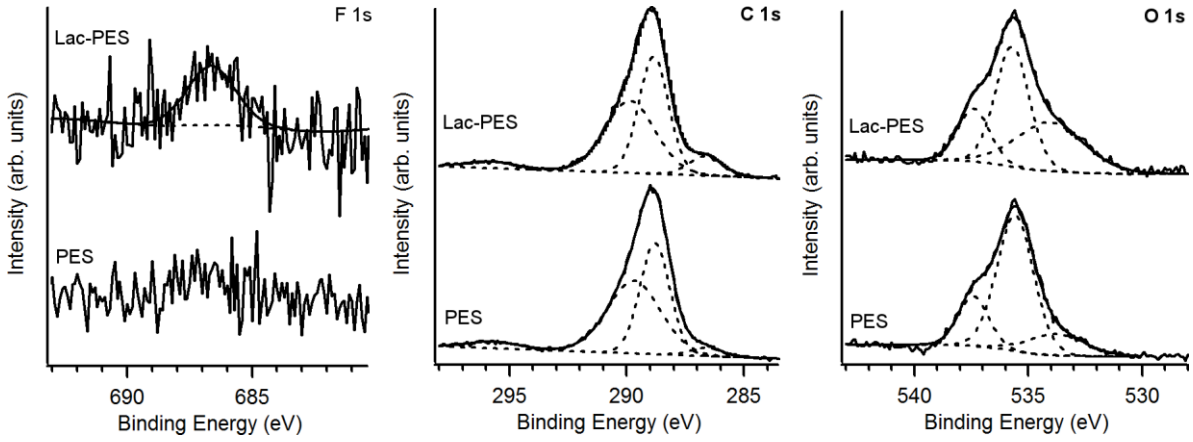


Figure 4: XPS spectra in the F 1s, C 1s and O 1s regions of bare PES membranes (bottom traces) and PES membranes functionalized with lactose units (top traces) via reaction with fluorinated compound **3a** in Figure 1. The figure also shows the deconvolution of peaks via fitting procedures.

nitrogen in prisitine membranes has previously been reported in the literature at very similar concentrations and it has been attributed to membrane additives.[40] Figure 4 shows high resolution spectra of bare PES and Lac-PES prepared using the fluorinated compound **3a** in the F 1s, C 1s and O 1s region. After functionalization the F 1s region shows the appearance of a peak that can be assigned to the F atom in ortho to the lactoside group in compound **3a**; this result indicates that after functionalization the aryl group is bound to the PES surface (F/C = 0.6%). The C 1s and O 1s spectra are significantly shifted to higher binding energies with respect to those typical of organic C and O atoms in organic molecules due to substrate charging. Qualitative and quantitative analysis indicates that functionalization leads to the appearance of significant contributions at 286.6 eV and 534.0 eV in the C 1s and O 1s regions, respectively, indicating the presence of C- and O-containing groups different from those found in the PES substrate. We attribute these two contributions to the presence of C and O atoms from the lactoside units and the aryl ring of **3a**. No significant changes were observed in the N 1s region (see Supporting Information), thus suggesting that functionalization via aryldiazonium occurs through nitrogen elimination and C—C bond formation between the aryl ring and the PES substrate, as is the case with aryldiazonium functionalization on other substrates such as carbon.[41]

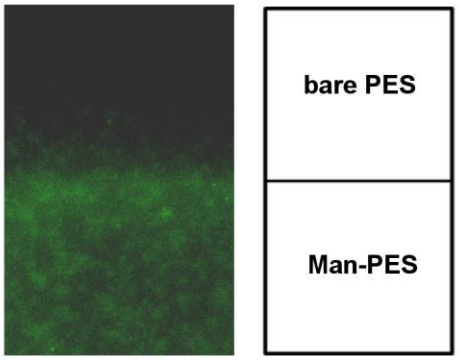


Figure 5: Fluorescence image of PES membranes modified with mannose (Man-PES) over selected regions, after incubation in FITC-ConA solutions (image width = 550 μm). The region that was not functionalized with mannose appears darker in the image.

Finally, we investigated whether simple saccharides were immobilized at PES surfaces by carrying out surface binding experiments using fluorescently labeled lectins *via* fluorescence microscopy. PES surfaces were immersed in solutions of the aryldiazonium cation of 4-aminophenyl mannoside (**2**) as described above. Surfaces were then incubated for 2 h in a 0. 5 mg mL^{-1} solution of FITC-ConA in PBS in the presence of Mn^{2+} and Ca^{2+} salts. ConA is known to display specific binding interactions towards Man units in solution[42] and has previously been used to probe surface modifications with Man groups and their availability to binding.[18, 42, 43] Figure 5 shows a fluorescence microscopy image of a PES membrane, where only the lower half of the surface is modified with α-mannose (Man-PES). After rinsing in PBS, the Man-PES area displays stronger FITC emission than bare PES regions, thus indicating that ConA preferentially adsorbs onto Man-PES compared to PES surfaces. Therefore, Figure 5 strongly suggests that PES surfaces can be modified with monosaccharides *via* spontaneous reaction with the aryldiazonium salts of 4-aminophenyl glycosides.

ATR-FTIR and fluorescence imaging results both indicate that aryldiazonium cations can be used for the modification of PES membranes in a single step *via* immersion into solutions of cations prepared *in situ*. Aryldiazonium salts bearing small organic groups had previously been used for the modification of carbon,[41] metals[44] and oxides.[45] However, there have been only limited reports on their use for the surface modification of polymers; recently, Barrière and co-workers demonstrated that aryldiazonium

salts could be used to modify polymers with nitro, nitrile and carboxyl groups *via* addition of a reductant in solution.[46] Our results suggest that in the case of PES, aryldiazonium chemistry can be used to modify the polymer surface in a single step via immersion of the surface into the coating solution in the absence of reductants. Although the mechanism of the reaction with PES surfaces has not been studied in detail in this manuscript, aryldiazonium cations are highly reactive species that are known to cross-couple to aryl rings in solution. It is likely that the aryl rings present at PES surfaces offer reactive sites for aryldiazonium cross-coupling; potential mechanistic routes are discussed in the Supporting Information.

3.2 Interaction of PES modified surfaces with biomolecules

Previous work from our group on the modification of carbon surfaces with aryldiazonium salts of 4-aminophenyl glycosides, had shown that surface modification with monosaccharide units resulted in a reduction of the amount of adsorbed proteins in aqueous solution.[18] In order to investigate the potential of carbohydrate layers as protein antifouling coatings for PES surfaces, we carried out protein adsorption experiments using Bovine Serum Albumin (BSA) solutions.

Modified and unmodified PES membranes were incubated for 2 h in a 0.2 mg mL^{-1} solution of FITC-BSA in PBS at pH 7.4;[30, 31] the membranes were then washed with the PBS solution in order to remove unbound protein prior to imaging. Figure 6a shows a fluorescence microscopy image of a PES coated membrane, where only the lower half of the area shown was modified with β-galactose using compound **1** as a precursor. A comparison of the emission intensity levels between the two sample regions clearly shows that the Gal-PES area of the sample displays lower FITC emission than the unmodified area, thus indicating that modification with Gal layers significantly reduces BSA adsorption from solution.

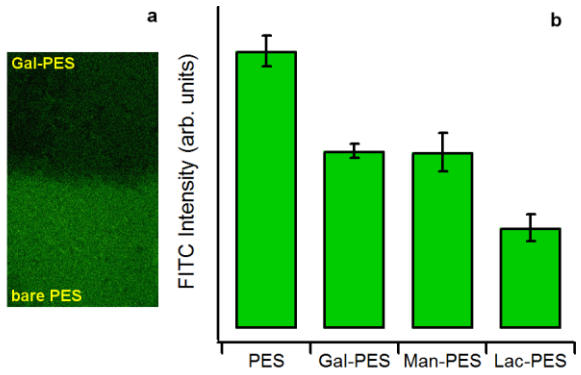


Figure 6: (a) Fluorescence images of PES membranes modified with galactose (Gal-PES) over selected regions, after incubation in FITC-BSA solutions (image width = 550 μm). The region that was not functionalized with galactose appears brighter in the image, thus indicating greater BSA adsorption. **(b)** Comparison of average intensity measured at membranes modified with galactose (Gal-PES), mannose (Man-PES) and lactose (Lac-PES) compared to that observed on bare PES membranes.

In order to compare the effectiveness at minimizing BSA adsorption of mono- and a di-saccharides, we carried out fluorescence imaging of Gal-PES, Man-PES, Lac-PES and bare PES membranes. Modified and unmodified PES membranes were incubated for 2 h in 0.5 mg mL^{-1} solutions of FITC-BSA in PBS at pH 7.4, rinsed in PBS buffer and then imaged via fluorescence microscopy under identical conditions. Figure 6b shows a summary of the FITC emission intensity measured at bare PES, Gal-PES, Man-PES and Lac-PES membranes, after incubation in FITC-BSA. The emission is the highest for bare membranes and is reduced by ~35%% for mono-saccharide modified membranes and by 63% for Lac-PES under the experimental conditions. Interestingly, Lac-PES display smaller FITC-BSA emission than either Gal- or Man-PES surfaces thus suggesting that lactose layers are better at minimizing protein adsorption than monosaccharide layers. Our findings on modified PES surfaces are consistent with results obtained on gold using self-assembled alkylthiol layers (SAMs). Ederth et al.[27] found that galactoside-terminated SAMs reduce protein adsorption on gold, while Ostuni and Whitesides[26] observed reduced protein adsorption for Gal- and Lac-terminated SAMs on gold, but greater reductions with the latter. In the case of monosaccharide SAMs on gold, it has been suggested that fouling resistance arises at least in part from the presence of hydrophilic groups and from a

modulation of hydrogen bonding accepting/donating properties at the interface.[27] In the case of modified PES membranes we speculate that similar mechanisms are at play as the aryldiazonium carbohydrate layers prepared via spontaneous reactions share many of the properties of carbohydrate SAMs such as surface density in the 1-3 ML range[18] and smooth conformal coverage (see Supporting Information).

3.3 Studies of biomass accumulation on PES membranes in wastewater

BSA adsorption studies carried out in the laboratory indicated that modification of PES surfaces with simple carbohydrates leads to a reduction in the amount of unspecifically adsorbed protein. This result is significant because of the great interest that exists in developing new technologies to control biofouling. Surface fouling is a complex multistep process that is proposed to begin with the adsorption of proteins and other small molecules; initial protein adsorption creates a conditioning layer that later supports colonization by cells and larger organisms. In principle, coatings that can minimize protein adsorption hold the potential to reduce biofouling by delaying the initial binding steps of the fouling cascade.

We were interested in investigating whether resistance to protein adsorption observed in the laboratory could indeed translate into an advantage in field applications, where membranes are typically exposed to complex environments containing multiple components, cells and organisms. Microfiltration PES membranes, such as those used in this work, find practical applications in environmental water sensors, where the membrane is positioned at the sampling port in order to protect active parts and remove unwanted solids prior to analysis.[47, 48] Biofouling at the sampling port can affect water flux and, importantly, analyte readings; therefore fouling control is critical for long term sensor stability and performance. Hence, to investigate the ability of carbohydrate coatings to provide an advantage in field applications, we tested the effect of PES surface modification on the adsorption of biomass from wastewater effluents.

Lac-PES surfaces were used in all tests while unmodified PES membranes were used as controls. The setup shown in Figure 2 and described in the Experimental Section was used to carry out field tests: two 1-month long tests were carried out at Osberstown Wastewater Treatment plant in Co. Kildare

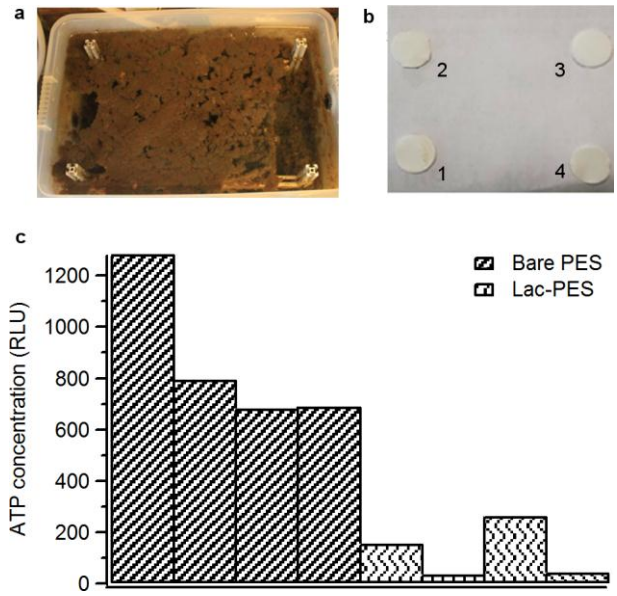


Figure 7: (a) Wastewater tank in which experiments with PES membranes were carried out; the image shows the wastewater after the December 2013 test-run. **(b)** Four membranes exposed to the water in (a), after light rinsing and removal from the fittings; the image shows two Lac-PES (2 and 4) and two control membranes (1 and 3). **(c)** Results of ATP tests for Lac-PES and PES membranes.

during the months of December 2013 and February 2014. Wastewater effluent conditions were different during both test runs due to differences in rainfall between the two months; Figure 7a shows the water in the tank at the end of the first test run. At the end of the test, the aluminum frame was removed from the tank and all fittings were lightly rinsed to remove loosely bound solids. Membranes were removed from the fittings and inspected visually at first. Figure 7b shows four membranes after the first test run; little difference among membranes was observed by visual inspection, except for a slight yellow coloration observed on unmodified membranes.

The amount of biomass accumulated at the membrane surfaces was examined using a combination of ATP content analysis, ATR-FTIR spectroscopy and HIM imaging of the PES surface. Total ATP is an indicator of microbial biomass content[49] and can be used to assess biomass accumulation at membrane surfaces.[50] PES membrane sections of the same size were immersed into identical volumes of deionized water and sonicated, in order to extract the accumulated biomass; a commercial bioluminescence assay was used in order to compare the ATP content extracted from control and Lac-PES membranes. All

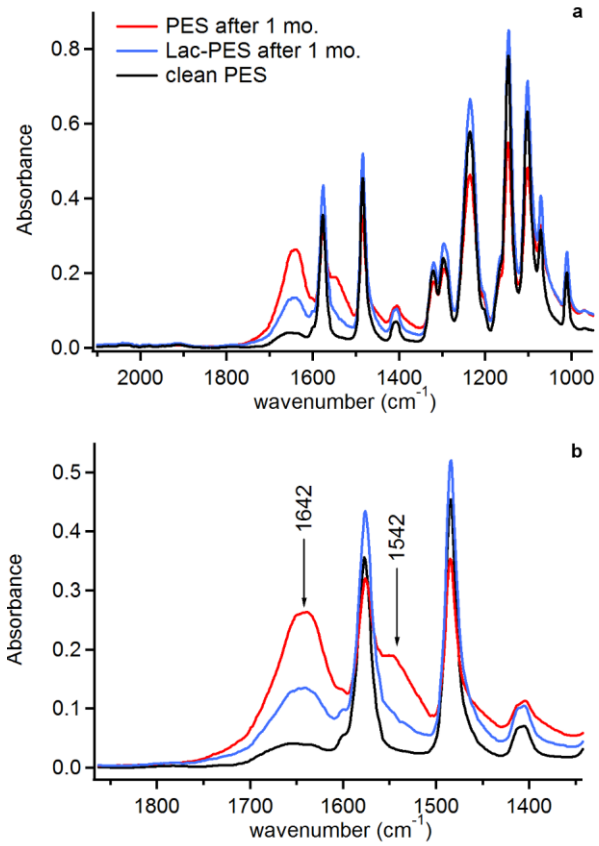


Figure 8: ATR-FTIR spectra of PES membranes exposed to wastewater effluent for 1 month under ambient conditions. The PES membrane modified with Lactose groups (Lac-PES, blue) has a lower intensity than that of bare PES membranes (red) in the region of the Amide I and Amide II bands (indicated with arrows). The spectrum of a clean PES membrane (black) is shown for comparison.

RLU values measured were found to be within the linear dynamic range of the assay[34] and therefore were considered to be proportional to ATP concentrations in the extract. Figure 7c shows differences in ATP content between Lac-PES and bare PES membranes measured in RLU. Our results from the first test indicate that Lac-PES membranes accumulated on average only 14% of the biomass accumulated on bare PES membranes. A significant reduction in biomass accumulation was also found after the second test, although the reduction was smaller (36% reduction), likely due to differences in the wastewater biomass content.

ATR-FTIR analysis was carried out on Lac-PES and bare PES (control) membranes after one-month test runs. Figures 8a and 8b show ATR-FTIR spectra of Lac-PES (blue) and bare PES (red) membranes after one-month exposure to wastewater; the spectrum of a clean PES membrane (black) is also reported

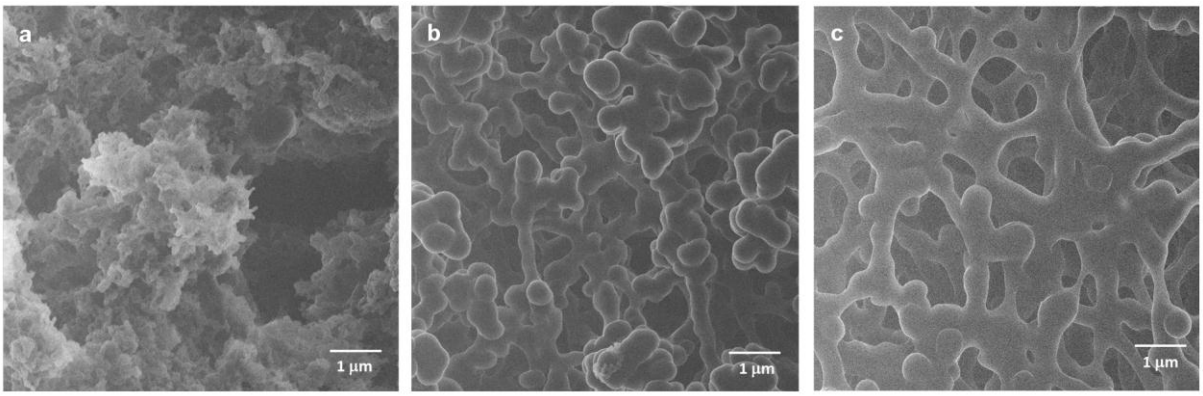


Figure 9: He-Ion microscopy (HIM) images of **(a)** uncoated PES membrane and **(b)** lactose-coated (Lac-PES) membrane after a one-month long exposure to wastewater effluent; **(c)** a bare, clean PES membrane is reported for comparison. HIM images show greater biomass accumulation on uncoated PES membranes, whereas Lac-PES membranes preserve a similar appearance to that of pristine PES membranes.

for comparison. After exposure to wastewater, spectra show increased absorption at 1650 and 1540 cm^{-1} (see expanded spectra in Figure 8b), the wavenumbers corresponding to amide I and amide II modes, respectively, which are characteristic of proteins.[38] Amide I and II peaks of Lac-PES samples are smaller than those observed on unmodified PES, thus indicating that smaller amounts of proteinaceous material accumulate on lactose coated samples than on bare PES samples.

These results are supported also by characterization of membranes via microscopy. Figure 9 shows HIM images of bare PES (Fig. 9a) and Lac-PES (Fig. 9b) membranes after exposure to wastewater for one-month, and of a bare and clean PES membrane for comparison (Fig. 9c). In Lac-PES images it is still possible to clearly discern the pore structure of the membrane, even after one month of exposure to wastewater: there is no significant difference between its surface and that of a clean, PES membrane. Bare PES membranes, on the contrary, undergo significant deterioration after exposure to wastewater: HIM images indicate that biomass accumulates at bare PES surfaces to the point that the original pore structure is obscured. In summary, ATP, ATR-FTIR and HIM results are in agreement and all indicate that lactose coating of PES membranes results in lower biomass accumulation when membranes are exposed to wastewater effluents.

4. Conclusions

In conclusion, we demonstrate that aryldiazonium chemistry offers an effective strategy for the modification of polyethersulfone membranes with carbohydrates. We have shown that carbohydrates can be spontaneously chemisorbed from solution onto PES membranes, while retaining their characteristic lectin recognition properties. Our modification approach results in a reduction of nonspecific protein binding at PES membranes and the results obtained in the laboratory suggest that these carbohydrate coatings might offer an effective biomimetic strategy for preventing PES biofouling in aqueous environments. We tested this hypothesis using Lactose-modified PES membranes exposed to effluents from a wastewater treatment plant and our tests confirmed that biomass accumulation on modified membranes is significantly lower than that observed at bare PES membranes.

These results are extremely promising because they indicate that it is possible to modify polymeric membrane surfaces with molecular layers via aryldiazonium grafting in order to enhance their performance in real field applications. The use of molecular layers is attractive because it does not compromise pore structure or mechanical properties; furthermore, it offers a potentially simpler route to tailoring of membrane properties for specific applications, compared to strategies that involve redesigning membrane polymeric chains. The use of extremely short saccharides was found to minimize unwanted biomass accumulation not only in the laboratory but also in "real-world" biomass-rich aqueous environments, thus opening the door to relatively simple non-toxic, biomimetic approaches to fouling control in polymeric membranes. The results of this study strongly suggest that coating of polymeric materials with carbohydrates via aryldiazonium chemistry may have far reaching implications for addressing challenging biofouling environments both in research and in industrial settings.

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Supporting Information Available. Detailed synthesis, purification and characterization of glycosides, NMR data of precursor compounds, infrared spectra and proposed chemisorption mechanism scheme. This material is available free of charge via the Internet at http://pubs.acs.org.

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Peak Position (cm^{-1})	Assignment
1011, 1070, 1103	-
1146	$\nu_s(SO_2)$
1236	ν_{as} (Ar-O-Ar) of aromatic ethers
1297, 1321	$\nu_{as}(SO_2)$
1370*	$\delta(CH_3)$
1408, 1485, 1578	ν(C=C) of aryl rings
1640	β(H-O-H)
1742*	ν(C=O) of esters

Table 1: Peaks and assignments of infrared spectra of membranes shown in Figure 3; peaks indicated with an asterisk (*) are present only on PES membranes modified with per-acetylated glucoside. ν = stretching; β = bending; δ = distorsion; *s* = symmetric; *as* = asymmetric.

Figure 1: Compounds used for surface modification experiments: 4-aminophenol-β-D-galactopyranose (**1,** Gal-terminated) and 4-aminophenol-α-D-mannopyranose precursors (**2**, Man-terminated), 4-aminophenol-β-D-lactopyranose and its fluorinated derivative (**3** and **3a**, Lac-terminated) and per-acetylated 4-aminophenol-β-D-glucopyranose (**4**, Glc-terminated).

Scheme 1: General synthetic strategy for the synthesis of 4-aminophenyl glycosides shown in Figure 1.

Scheme 2: Protocol for in situ modification of PES.

Figure 2: Experimental setup used for tests of membrane fouling in wastewater treatment effluents. Membranes were mounted at positions 1-4 as on the left panel, alternating controls and samples; all membranes were placed facing the bottom of the tank at a constant distance, as on the right.

Figure 3: ATR-FTIR spectra of PES membranes unmodified (top) and modified with the aryldiazonium cation of compound **4**. The inset shows the difference between the two spectra in the carbonyl stretching region, where a C=O stretching peak is visible in the case of membranes modified with per-acetylated glucoside groups.

Figure 4: XPS spectra in the F 1s, C 1s and O 1s regions of bare PES membranes (bottom traces) and PES membranes functionalized with lactose units (top traces) via reaction with fluorinated compound **3a** in Figure 1. The figure also shows the deconvolution of peaks via fitting procedures.

Figure 5: Fluorescence image of PES membranes modified with mannose (Man-PES) over selected regions, after incubation in FITC-ConA solutions (image width = 550 μm). The region that was not functionalized with mannose appears darker in the image.

Figure 6: (a) Fluorescence images of PES membranes modified with galactose (Gal-PES) over selected regions, after incubation in FITC-BSA solutions (image width = 550 μm). The region that was not functionalized with galactose appears brighter in the image, thus indicating greater BSA adsorption. **(b)**

Comparison of average intensity measured at membranes modified with galactose (Gal-PES), mannose (Man-PES) and lactose (Lac-PES) compared to that observed on bare PES membranes.

Figure 7: (a) Wastewater tank in which experiments with PES membranes were carried out; the image shows the wastewater after the December 2013 test-run. **(b)** Four membranes exposed to the water in (a), after light rinsing and removal from the fittings; the image shows two Lac-PES (2 and 4) and two control membranes (1 and 3). **(c)** Results of ATP tests for Lac-PES and PES membranes.

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