Environment Canada
Water Science and
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NANOSCALE INVESTIGATION OF FLOCS AND FOULED MEMBRANE FILTERS

K. Exall and G.G. Leppard

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Nanoscale investigations of flocs and fouled membrane filters

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Abstract

Membranes composed of various materials and possessing various pore sizes are increasingly used in drinking water and wastewater treatment to remove colloidal material, organic matter and microorganisms. Membrane fouling can limit the useful life of a membrane. Common foulants include inorganic colloidal material, organic matter, and biological growth; foulant layers often include a combination of these. Analysis of membrane surfaces after fouling can provide insight as to the mechanism of fouling. The current technical note reports on the initial steps in an examination of the effects of coagulants on fouling during ultrafiltration of drinking water and wastewater. Using various preparation techniques and transmission electron microscopy, three types of samples have been examined: 1) the character of flocs formed under various coagulation conditions in real and synthetic wastewaters; 2) the nature of the surfaces of clean regenerated cellulose and polyethersulfone membranes; and 3) membranes that have been fouled with filtered wastewater. Flocs formed in both synthetic and real wastewaters were shown to be composed of a variety of materials, likely including both clays and organic material. Real wastewater also contained high numbers of bacteria. Surface foulant layers have been clearly visualized on both types of membrane and mixed aggregated materials at the nanoscale can be detected. Images from TEM analysis suggested that both surface cake formation and pore blockage contributed to fouling under the conditions studied.

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1. Introduction

1.1 Membrane background

Membranes are increasingly used in drinking water and wastewater treatment for improved protection of human and environmental health. Membranes of various types may be used to remove colloidal material, organic matter including disinfection by-product precursors, and microorganisms. Removal of such contaminants generally occurs through physical separation processes, rather than chemical means.

The membranes used in drinking water and wastewater purification are defined according to pore sizes or molecular weight cut-offs (MWCOs), which determine the rejection or removal characteristics of the membranes. Microfiltration (MF) membranes remove materials of micrometer size, while ultrafiltration (UF) and nanofiltration (NF) membranes remove materials on the order of nanometres in size; the latter two are often defined by the MWCO of material removed by the membrane. Reverse osmosis (RO) membranes remove materials as small as ions. The following work will focus on UF processes. The ultrafiltration of aquatic colloids is reviewed in Guo and Santschi (2007).

For particles and macromolecules in the micrometer-to-nanometer size range (least diameter), their colloidal properties can complicate a physical separation process (Leppard and Buffle, 1998; Filella, 2007; Leppard, 2008). Reviews on filter structure and performance, in relation to complications arising from the colloidal properties of aquatic particles, are found in Leppard and Buffle (1998 and 2005) who use high resolution electron spectro-microscopy to do analyses on a "per colloidal particle" basis.

There is an accelerating interest in the lower end of the colloid size range, specifically the nanoparticles with a least dimension of 1-100 nm. Mechanical, electrical, thermodynamic and other types of properties of a given material can be strongly modified when the physical dimensions of that material enter the nanoscale (Hochella, Jr., 2002). Recently, it was estimated that the worldwide market for products with nanotechnology components would reach one trillion American dollars within a decade (Roco, 2005), with unknown environmental impacts. While the major focus of this urgent interest has been on atmospheric nanoparticles, a need has arisen to understand better the interactions of aquatic nanoparticles (both natural and manufactured) with membrane filters. Specifically, more research is needed on the roles of natural nanoparticles in fouling (to guide improvements in the cost-effectiveness of water treatment) and of effects on water treatment processes from an increasing array of "escaped" manufactured nanoparticles.

1.2 Fouling

Typical microfiltration plants operate with a flux near 100 L/(m^2·h), which is less than 5% of the hydraulic permeability observed in filtration of organic-free water through similar membranes in the laboratory (Howe and Clark, 2002). Fouling is a loss in productivity from an accumulation of foulants on membrane surfaces or within membrane pores. It may be reversible (i.e., remedied by backwashing, air scouring or cleaning measures) or irreversible. Particulate matter larger than membrane pores in MF or UF filters forms a cake at the membrane surface; colloidal or dissolved matter may form a surface gel layer or cake, or penetrate and adsorb within pores, reducing pore diameter.

Irreversible fouling may also occur as foulants strongly adsorb to the membrane surface or enter and block membrane pores. Fouling can be caused by inorganic colloidal material (clays and silts, precipitated metals or sparingly soluble salts, silica compounds), organic matter (polymers used in coagulation/flocculation or natural organic matter, NOM), or biological growth (a mixture of dead and living microorganisms that accumulate or actively colonize the membrane surface, and the extracellular polymeric substances (EPS) that they produce, yielding a biofilm). Each category will be further explained below. Most fouling occurs as a combination of some or all of the above, and depends on such variables as concentration and type of foulants, interactions between foulants, membrane type and material, operating pressure, and raw water characteristics (e.g., hardness, pH).

Colloidal fouling. Inorganic colloidal material, including clays and silts, precipitated metals or sparingly soluble salts, and silica compounds can build up at the surface of the membrane. Concentration, thickness and morphology of solids deposited in cakes are important for operation and can be partially controlled by varying crossflow velocity, transmembrane pressure, and membrane geometry. Critical membrane pressure corresponds to the force required to overcome repulsive energy barriers arising from diffuse layer interactions between particles (AWWA Membrane Technology Research Committee, 1998).

Specific effects of flow rate on the mechanisms for the artifactual aggregation of colloids are described in Leppard and Buffle (1998), who emphasize the concept of a "flow rate window" of minimum artifact. There is a considerable range of retention of colloids by a membrane filter according to flow rate, due to concentration polarization in a high flow rate domain and aggregation by bulk coagulation in a low flow rate domain. An intermediate flow rate window of minimum artifact can be determined experimentally for a given filtration system. When approaching membrane filter surfaces, both large colloids and nanoparticles can scavenge water for soluble pollutants and nutrients, thus preventing further movement of these "supposedly soluble" pollutants and nutrients through subsequent treatments. This scavenging activity is currently under intensive study (Lead and Wilkinson, 2006; Doucet *et al.*, 2007).

Organics, adsorption, and membrane fouling. Organic matter in drinking water and wastewater sources includes polymers used in coagulation/flocculation, natural organic matter (NOM) or effluent-derived organic matter (EfOM). A number of studies (e.g., Lin *et al.*, 2000; Yuan and Zydney, 2000; Fan *et al.*, 2001; Howe and Clark, 2002; AWWA Membrane Technology Research Committee, 2005) indicate that NOM is a major foulant in water separation. Both membrane surfaces and NOM are insufficiently characterized, although a substantial aquatic literature now exists on the major colloidal and macromolecular components of NOM, including nanoparticles (Leppard, 2008). Laîné *et al.* (2003) noted that in spite of the significant effort dedicated to elucidating the fouling mechanisms of polymeric membranes by NOM, no single model has yet been accepted and in fact, most of the existing literature is contradictory. The need for additional characterization (and the tendency of diverse aquatic nanoparticles to form mixed aggregates in water) complicate analysis of the interaction between membranes and organic foulants. While many studies (e.g., Lin *et al.*, 2000, Yuan and Zydney, 2000, Fan *et al.*, 2001) suggest that components with higher molecular weight cause the largest flux decline, Carroll *et al.* (2000) found more fouling with smaller molecular weight molecules and Howe and Clark (2002) determined that an intermediate-sized fraction was most important in fouling. There is therefore a need to better understand the role of such factors as molecular weight, composition, conformation, and chemistry of NOM in fouling.

One approach to better NOM characterization has been to employ high resolution spectro-microscopy on a "per particle" basis (Mavrocordatos *et al.*, 2007). Such an approach takes advantage of the fact that many macromolecules and small colloids in the NOM of natural waters and treatment tanks exist in the form of distinctive, well-defined nanoscale packets (Leppard *et al.*, 2004). An example is the almost ubiquitous polysaccharidic mucilage fibril (Leppard, 1997) which has recently been implicated in fouling (Liao *et al.*, 2004).

There has been an assumption that different kinds of NOM interact with a filter surface initially as separate particles/colloids/macromolecules, but certain common NOM colloids (such as the colloidal organic fibrils of ca 5 nm diameter and great length-to-width ratio) have been demonstrated to be accumulators of other kinds of colloids, including metal rich colloids (Filella *et al.*, 1993). Consequently, mixed aggregates with a diversity of functional groups at their surface are often what impacts on a filter surface, thus (sometimes) rendering meaningless physico-chemical data which predict that a certain NOM material will not adhere to a filter by virtue of lacking the requisite functional groups. The general mechanisms for the formation of natural mixed aggregates of humic substances, colloidal organic fibrils and mineral colloids (the most common aquatic colloids) have been elucidated in the three-colloidal component approach of Buffle *et al.* (1998). According to this idea, both the concentration of stable sub-micron particles and the formation of aggregates in natural waters depend on the proportions of three general classes of colloids. These classes are (i) compact inorganic colloids, (ii) large rigid biopolymers (such as mucilage fibrils) and (iii) fulvic acids (plus fulvic-like substances). The Buffle group (1998) analyzed the role of representative colloids from each colloid class with respect to both hetero- and homo-aggregation. They concluded that the fulvic compounds stabilized the inorganic colloids, whereas the rigid biopolymers destabilized them. These observations apply to colloids from a variety of natural waters and to all particle sizes, including nanoparticles. Many specific case studies of these interactions have been reviewed recently (Wilkinson and Reinhardt, 2005; Leppard, 2008).

Howe and Clark (2002) used a sequential filtration process, where the first filtration steps removed specific components (e.g., a glass fiber filter removed particulate matter; comparing flux decline with and without prefiltration indicates particulate contribution to fouling); later filtration steps evaluated membrane fouling by remaining components. Particulate matter (>0.45 μm) caused relatively little fouling compared with operationally-defined dissolved matter (<0.45 μm), contributing between 1 and 36% of fouling (average 13%). Samples were then fractionated by ultrafiltration. Very small colloids, 3-20 nm in diameter (nanoparticles), seem to be important foulants in various water sources, found by estimating pore size of ultrafilters with various MWCO values (YM3-YM100). This included both inorganic and organic matter, but the material was predominantly organic. When the colloidal OM was removed, 85-90% of total dissolved OM remained, but caused little fouling (Howe and Clark, 2002). However, particles in the nanoscale size range (1-100 nm least dimension) can behave operationally as solutes over short time scales (Buffle and Leppard, 1995; Stoll and Buffle, 1996) and such nanoparticles have become an issue of increasing importance for human and ecosystem health (Dunphy Guzman *et al.*, 2006; Leppard, 2008) and biogeochemical processes (Hochella, Jr., 2002; Leppard, 2008).

Biofouling. Biofouling results from an accumulation of microorganisms, their extracellular products and associated colloids at a membrane-water interface; the resultant biofoulant compromises system performance. Individual particles/colloids can be associated with a membrane filter merely by adhering to a surface (and piling up on that surface) or by virtue of microbial biofilm formation. A biofilm is a complex layer of dead and living microorganisms that grows or accumulates at the membrane surface, plus the extracellular polymeric substances that the microbes produce, with their EPS often acting so as to incorporate mineral particles and colloidal humic substances into the layer (Wingender *et al.*, 1999; Lewandowski and Beyenal, 2005). Rapid attachment of pioneering bacteria, promoted by suboptimal fluid dynamics, can lead to the formation and growth of a biofilm. Secondary attachment to, or entrainment within, the biofilm further develops the biofilm. Senescent biofilm bacteria during lysis can release materials (proteins, nucleic acids, lipopolysaccharides, and other macromolecules) that can sorb to and modify a membrane surface further (AWWA Membrane Technology Research Committee, 1998). Important variables in biofilm EPS roles in fouling are the biodegradability (Zhang and Bishop, 2003) and particle size distribution (Sophonsiri and Morgenroth, 2004) characteristics of EPS and EPS-rich particles, as well as effects of shear stress on EPS production and porosity (Ramasamy and Zhang, 2005); these variables in turn are subject to modification by dissolved nutrient levels in the water.

A relevant microorganism survival characteristic is the ability to undergo rapid biochemical and genetic adaptation in response to changing nutritional, hydrodynamic and other conditions. The microbial consortia of biofilms can even change the overall physical attributes of large portions of biofilm (density, thickness, hydrophobicity and surface charge at external surfaces, channel formation) in response to external events (Liss, 2002; Leppard, 2008). Cost-effective, ecologically-sound technologies for biofilm control are needed; such technologies should address the great capacity of microbes to adapt, and experimental manipulations of those biofilm architectural changes which facilitate adaptation. A biofouling tendency depends on membrane polymer type; how and why this happens are poorly understood. Identified research needs are: (1) the structure of microbial communities in membrane biofilms; (2) the specific genetic or biochemical traits of specific microbes; (3) the analysis of adhesion processes at the molecular level to understand fundamental physicochemical forces involved in cell attachment; (4) the relationship of molecular conditioning films to bacterial attachment; (5) the discovery of critical events in early biofilm formation which are manipulable (such as the incorporation of a biocidal agent into the membrane surface); (6) the interaction of bacterial exopolymers with synthetic membrane materials during cell attachment and biofilm development; (7) the structural integrity of the biofilm itself (which depends on polymer-polymer intermolecular forces of incompletely known nature and magnitude, associated with mutual interactions of neighbouring EPS); (8) the physiological ecology of membrane biofilms, noting that there is a suggested biochemical signalling among the attached bacteria involved (AWWA Membrane Technology Research Committee, 1998).

Analysis of fouled membranes. Analysis of membrane surfaces after fouling ("membrane autopsy") can provide insight as to the mechanism of fouling. Various techniques have been used to study deposited foulants, including pyrolysis-gas chromatography-mass spectrometry, pyrolysis and attenuated total reflectance-Fourier transform infrared spectroscopy, electron microscopy, secondary ion mass spectrometry, and x-ray photoelectron spectroscopy, and combinations of spectroscopic analyses (Howe and Clark, 2002; AWWA Membrane Technology Research Committee, 2005).

1.3 Membrane cleaning and pre-treatment

Fouling may be remedied by membrane cleaning, which can be done through backwashing, where the flow of permeate is reversed to dislodge loosely held foulants from the membrane surface. Chemicals are often added to the backwash water to dissolve and oxidize foulants and inactivate biological growth (known as chemically enhanced backwash). Alternatively, chemical cleaning or cleaning-in-place can be used, which employs the use of appropriate oxidant, acid, caustic, or detergent solutions to loosen and dissolve foulants without reacting with the membrane itself. In the case of cleaning-in-place, solutions are tank-batched and circulate directly through the membrane. The appropriate choice of cleaning agent requires knowledge of the predominant foulants in source water (AWWA Membrane Technology Research Committee, 1992; AWWA Residuals Management Research Committee, 2003).

Fouling may alternatively be controlled by pre-treatment of the feed water. Chemical or physical pre-treatment techniques include coagulation, activated carbon adsorption, and oxidation. The pre-treatment may slow fouling, typically by removing one category of foulant (e.g., particulate material by pre-filtration, organic matter by activated carbon adsorption, or fractions of colloidal and organic material by coagulation). The effects of many pre-treatment techniques on membrane performance are not well understood, and some methods may result in a transition to another dominant fouling mechanism (AWWA Membrane Technology Research Committee, 1992). The current report will focus on coagulation pre-treatment.

Coagulation in water treatment uses chemical processes to accelerate the kinetics of destabilization (coagulation) and aggregation (flocculation) of colloidal and particulate material. Coagulation involves the addition of a chemical agent to destabilize colloids in the water and requires intensive mixing to rapidly disperse the coagulant. Flocculation, on the other hand, is the formation of aggregates, or flocs, of the destabilized colloids and requires gentle mixing to allow interparticle collisions to occur to form flocs, without shearing the aggregates. Common coagulants include both metal salts and charged polymers (polyelectrolytes), while flocculants are typically composed of high molecular mass polymers and are otherwise known as coagulant aids (Exall, 2005). Coagulation has long been utilized as an aid to sedimentation in water and wastewater treatment and may be used to accelerate removal of solids in stormwater or combined sewer overflow treatment facilities (Exall *et al.*, 2005; Droppo *et al.* 2008). Separation of the flocs and other particulate matter from the water may then occur by sedimentation, dissolved air flotation, or filtration (Exall, 2005). Coagulation pre-treatment for membrane filtration has commonly been used to remove particulate matter, reduce organic foulant and disinfectant by-product precursor concentrations, and improve removal of arsenic and other contaminants.

The current work is part of an examination of the effects of coagulants and flocculants (coagulant aids) on fouling during ultrafiltration of drinking water and wastewater. Using transmission electron microscopy, three types of samples were examined:

(1) the character of flocs formed under various coagulation conditions in real and synthetic wastewaters;
(2) the nature of the surfaces of clean regenerated cellulose (RC) and polyethersulfone (PES) membranes;
(3) RC and PES membranes that were fouled with filtered wastewater;

Wastewater was chosen for its high foulant concentration and therefore its ability to rapidly foul membrane surfaces. The current report discusses results that have been obtained to date with the three types of samples; future work will explore membrane surfaces that were fouled with pre-treated (coagulated) wastewater.

2. Methods

2.1 Flocs

Sample sites and characteristics. Transmission electron microscopy (TEM) images were taken of flocs produced in both raw wastewater (obtained from the Niagara Falls wastewater treatment plant, Niagara Falls, Ontario) and synthetic combined sewer overflow (CSO) samples, treated with alum (supplied by Eaglebrook, Inc., Brantford, Ontario) or alum with a polyacrylamide flocculant aid (PAM, supplied by CIBA Specialty Chemicals, Mississauga, Ontario). The synthetic CSO contained $NaHCO_3$ to provide an alkalinity of 50 mg/L (as $CaCO_3$), tannic acid to provide a dissolved organic carbon (DOC) concentration of 25 mg/L and kaolinite to produce an initial suspended solids concentration of 300 mg/L. The suspended solids and DOC concentrations were chosen to represent concentrations typically observed in CSO and wastewater samples. Appropriate coagulant dosages for both sample types were chosen with the use of a jar test using a Phipps and Bird six-place paddle stirrer, consisting of the following stages: coagulant addition, rapid mixing at 100 rpm for 1 minute for coagulant dispersal, slow mixing at 30 rpm for floc growth, and sedimentation.

Fixation of flocs. Flocs for TEM analysis were produced using the same procedure as in the jar test, but eliminating the sedimentation stage; that is, floc samples were collected directly from the beaker during mixing. After floc formation and growth, samples were collected using a disposable wide-bore Pasteur pipette. The flocs were fixed and preserved in one of three solutions: a hydrophilic melamine embedding resin with fixative properties (Frosch and Westphal, 1989), and either glutaraldehyde or glutaraldehyde with ruthenium red (GRR), followed by a secondary fixation in an osmium tetroxide-based solution, prior to embedding in a hydrophobic resin (Pazin *et al.*, 2005). Fixative solutions are designed to preserve as faithfully as possible the ultrastructure of a biological sample, exactly as that ultrastructure was, in its natural setting, at the instant before cells were killed by the fixative. There is no universal fixative for preserving all kinds of samples equally well, with regard to intracellular contents and extracellular structures, but the strengths and weaknesses for a given fixative are known. The detection, assessment and minimization of artifacts are described in a monograph by Mavrocordatos *et al.* (2007). The appropriateness of the fixatives and resins selected here is described in Liss *et al.* (1996), while an application to the analysis of biofoulants is found in Liao *et al.* (2004).

Rationale for fixative selection. To summarize briefly our use of the three selected preservation procedures, the images resulting from each procedure could be analyzed correlatively to ascertain specific artifacts inherent to each one when used independently. Thus we were able to construct an overall image by mentally combining the three related images. For examinations of biofoulant composition, in terms of the colloid types present, the principal artifacts of concern were excessive extraction, shrinkage of hydrated structures and internal damage to cells. The hydrophilic resin permitted one to omit the solvent dehydration step, thus minimizing extraction and shrinkage while having the defect of yielding minimal ultrastructural details of cell contents. Glutaraldehyde alone yielded excellent ultrastructural details of cell contents, while permitting considerable extraction of extracellular materials and consequent shrinkage of the EPS. Glutaraldehyde plus ruthenium red minimized all three problems of concern, but presented a slightly grainy image which interfered with high resolution analyses.

Sectioning and the proxy preparation. Nanoscale observations were made on ultrathin sections (ca. 50-70 nm thickness) of flocs and membranes that had been prepared by the protocols of Pazin *et al.* (2005), which had been derived from the detailed protocols of Liss *et al.* (1996) and Leppard *et al.* (2003). As per the highly detailed protocol descriptions of Liss *et al.* (1996), a research focus was placed on one preparatory protocol which was found to produce representative images with low but known artifact content (see Liss *et al.*, 1996, for scientific rationale). This proxy preparation consisted of sample immersion (1:1) at room temperature in a solution of 0.1 M sodium cacodylate-buffered (pH 7.1) primary fixative containing both (freshly mixed) 3 % glutaraldehyde and 0.1 % ruthenium red (ammoniated ruthenium oxychloride). The wash steps and the secondary fixation (on ice, in a freshly mixed solution of cacodylate-buffered 1 % osmium tetroxide and 0.05 % ruthenium red), followed by washes and a solvent exchange series, leading to infiltration into and embedding by the hydrophobic epoxy resin of Spurr (1969), exactly as described in Liss *et al.* (1996). After polymerization of the resin in a mould, all samples were sectioned identically with a diamond knife mounted in an ultramicrotome (Leica Ultracut UCT ultramicrotome; Leica Mikrosysteme, Wien, Austria). All ultrathin sections were mounted on formvar-covered copper (electron microscope) grids. To maximize specimen contrast in the TEM, the epoxy resin sections were counterstained with a drop of concentrated uranyl acetate in 50 % ethanol for 10 minutes in the dark, and then rinsed with distilled water. The grids were then stained again for 5 minutes in Reynolds' lead citrate solution, in a carbon dioxide-free environment, rinsed again and allowed to dry. The counterstaining was carried out exactly as in Pazin *et al.* (2005). This combination of counterstains produces a high differential contrast for the documentation of nanoscale structures by TEM at high resolution (Lewis and Knight, 1977). For a thorough treatment of artifacts and their correction, with regard to ultrathin sections, consult Hayat (1970). For a spectromicroscopical mineralogical analysis of inorganic colloids whose native electron opacity is sufficiently great, there is an option; the staining can be omitted and one can proceed directly to both EDS analyses and selected area electron diffraction, on a "per colloid" basis (Chanudet and Filella, 2006)

Documentation. Viewing and documentation by TEM were done with a JEOL JEM 1200 EX TEMSCAN scanning transmission electron microscope (JEOL, Peabody, MA, USA), operated in transmission mode at 80 kV. The practical resolution for the epoxy-embedded sections was ca. 3 nm. The searches of TEM views of flocs, to select representative images of floc (and membrane) ultrastructural features, were done systematically according to the protocol of Leppard *et al.* (2003). Representative ultrathin sections were searched initially using both low (4,000 x) and medium (15,000 x) primary magnifications to get an overview of the relative abundance and associations of the most common ultrastructural components, as defined by morphology and size. Observations and documentations were then made in greater detail at a primary magnification of 50,000 x. For examining the smallest nanoparticles, much higher magnifications (to 250,000 x) were used. To establish representativity, the common kinds of particles and their aggregates were defined, based on their relative abundances (Jackson and Leppard, 2002) following extensive observation.

Spectro-microscopy. Spectro-microscopy was employed to confirm the identity of individual distinctive colloids. The scanning mode of the TEMSCAN instrument was used to generate a microprobe beam of electrons for EDS; spectra were obtained using a Tracor Northern X-ray detector (Noran, Madison, WI, USA) and EDS 2004 microanalysis software (IXRF Systems, Inc., Houston, TX, USA). On a "per colloid" basis, using a selection of individual colloids seen in a field of view, EDS was used to identify elements (Chandler, 1977). The relative abundances of pertinent elements ($Z>10$) were ascertained according to the approach of Jackson *et al.* (1999). The minimum detectable mass fraction (in the beam "spot", which is the volume of section irradiated by the beam) of elements of interest was in the range of 0.1%-0.5%. A minor constraint on measuring the relative abundances of elements in the ultrathin sections had to be taken into account, using the "P" value as defined by Russ (1972). This value differs for different elements and is essentially the relative intensity of X-ray emission of each element from the same concentration. Anticipated elements and their "P" values considered at the K-alpha line were Al (58%), Cl (87%), Na (34%), Ru (32%), S (82%) and Si (67%). Anticipated elements and their "P" values considered at the L-alpha line were Os (52%), Pb (46%), Ru (53%) and U (35%).

Optical microscopy. Light microscope images were employed to give an overall view of large portions of membrane structure. This "low-resolution but large-volume" approach complements the "high-resolution but small-volume" approach of TEM. The light microscope sections (approximately 1 µm thick) were cut from samples embedded in Spurr's epoxy resin (Spurr, 1969) after processing through the standard GRR procedure. Sections were made with glass knives on the Leica UCT ultramicrotome and stained with Toluidine Blue O (Lillie, 1969). They were documented on an Axioplan Universal microscope (Zeiss, Oberkochen, Germany) with a 20X objective in bright field mode, for PES samples and in DIC (differential interference contrast) mode for RC samples. Images were acquired using a Photometrics Coolsnap HQ CCD camera (Roper Scientific, Tucson, AZ).

2.2 Membranes

Two types of membrane materials with a nominal MWCO of 10kD were examined, a regenerated cellulose (RC) membrane, sold as Ultracel YM10, and a polyethersulfone (PES) membrane, Biomax PBGC (both from Millipore Corporation, Bedford, Massachusetts). The RC membrane has a hydrophilic, tight microstructure, which is meant to assure high retention with the low adsorption of protein or other macromolecules. The PES membrane has an open microstructure, which provides faster separation capability. Millipore's standard definition is that an ultrafiltration membrane with a stated nominal MWCO will retain, or reject, at least 90% of a globular solute of that molecular weight. Because ultrafiltration membranes contain a distribution of pore diameters, solute retention is not absolute. Solutes with molecular weights close to a membrane's cut-off are neither completely transmitted nor completely retained (Millipore Corporation, no date).

Pre-cleaning was performed by soaking membranes for 30 minutes in distilled, deionized water, then permeating 200 mL of distilled water, followed by 100 mL of distilled, deionized water. After the membranes had been washed, a small section (approx. 3 mm x 20 mm) was cut from the centre of the membrane with clean scissors for the clean membrane sample. As with the flocs, the membrane sections were preserved in melamine resin, glutaraldehyde or glutaraldehyde with ruthenium red (GRR).

Fouling of the membrane samples was achieved by filtering through a cleaned membrane wastewater that was obtained at the Woodward Wastewater Treatment Plant in Hamilton (Ontario), then filtered through a 0.45 micrometre pore size polyethersulfone membrane. The pre-filtration was conducted to remove particulate matter, leaving only "dissolved" foulants. In order to observe the materials responsible for irreversible fouling, filtered wastewater samples were alternated with manufacturer-recommended cleaning steps (soaking in a solution of 0.1 M NaOH/1ppm $NaOCl_3$ for 30 min, followed by rinsing with distilled, deionized water). Approximately 1 – 1.5L of micro-filtered wastewater were filtered, followed by the cleaning step and storage in a 10% ethanol solution until the next filtration. After ~4L of micro-filtered sewage had passed through the membranes, it was no longer possible to remove the coloured foulant layer from the surface of the membrane with the soaking and rinsing procedure; the membranes were then considered to be adequately fouled for electron microscopic analysis.

3. Results and Discussion

3.1 Flocs

Representative images of flocs obtained under each condition, then fixed in GRR fixative and embedded in the hydrophobic resin, are provided in the following figures. Similar images were obtained using the glutaraldehyde fixative followed by embedding in the hydrophobic resin and by using the hydrophilic melamine resin without a prior chemical fixative. The melamine resin has a fixative action of its own (Frosch and Westphal, 1989). The differences in micrograph information content (between TEM preparations) were minimal, meaning that extensive use of the GRR proxy was appropriate.

By comparing the images produced using all three preparation techniques, one can see that common colloid-sized particles include clay micelles, bacteria, granules and fibrils. Observed frequently were fractal aggregates of granules arranged as a 3-D gel. Many of the bacteria were pleiomorphic and many possessed internal storage granules. Bacteria with EPS fibrils were scarce in some samples but not in others. The fibrils had multi-micrometre length but a nanoscale thickness. Flocs of various sizes could be seen, although in general they appeared small; in ultrathin sections, flocs almost always appear smaller than their real size, simply because sections taken through their maximum dimension occur only rarely. The formation of these agglomerates, in the absence of alum or polymers of organic and clay materials, may be facilitated by the calcium ions added as calcium bicarbonate (to provide alkalinity).

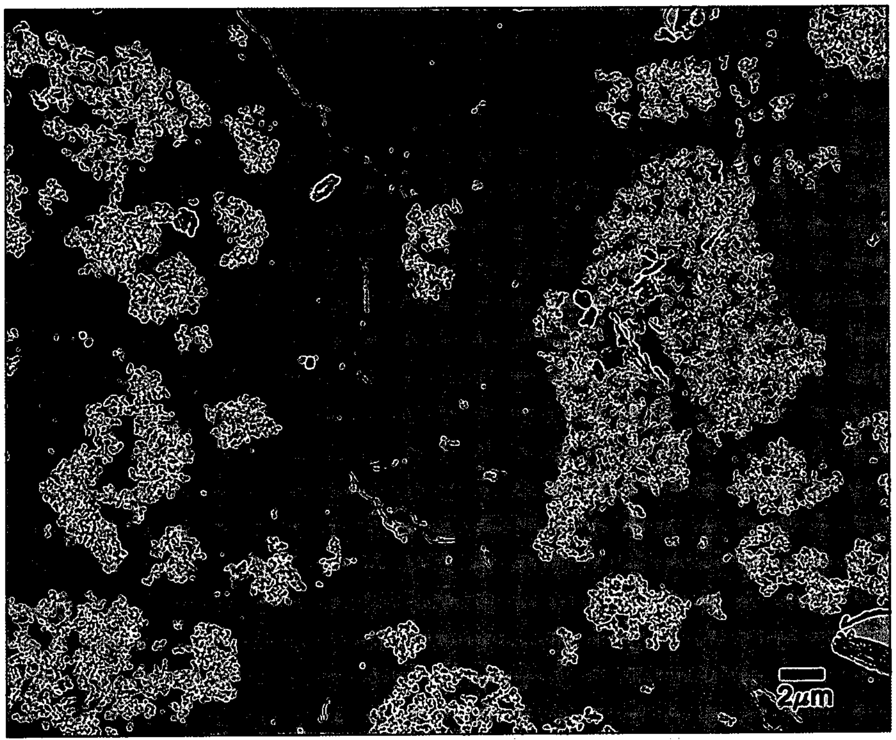

Figure 3-1: Synthetic CSO fixed by GRR.

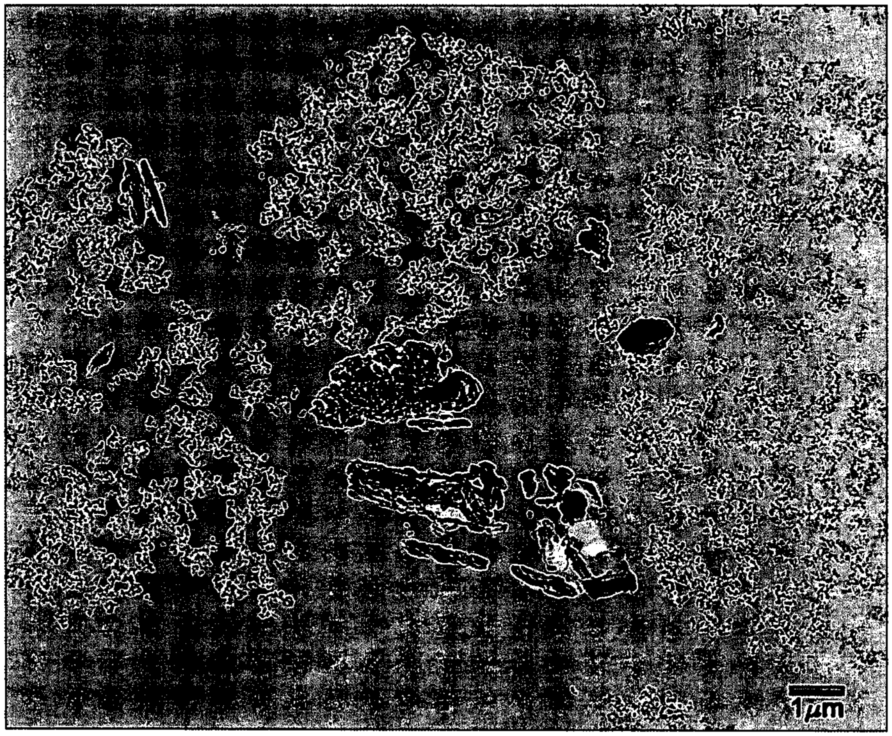

Figure 3-2: Synthetic CSO fixed by GRR.

In the images of synthetic CSO, fixed by GRR (above; Figures 3-1 and 3-2), fairly large kaolinite clay particles are apparent as dark, dense angular material. When the synthetic CSO was treated with alum (Figures 3-3 and 3-4), some kaolinite is still visible, but the images contain fewer small flocs, with independent particles appearing as part of a gel matrix. This conclusion comes from an analysis of a series of sections, which permits a systematic selection of representative images. For synthetic CSO, the distinction between adjacent small organic-rich flocs and porous 3-D gels (in a single ultrathin section) becomes blurred when one considers that the small organic-rich flocs are themselves porous 3-D gels. A quantitative floc size distribution analysis per synthetic CSO sample type should be undertaken; unfortunately such an analysis by TEM is cost-prohibitive. Results for synthetic CSO treated with both alum and PAM (Figure 3-5) were similar to those when alum was used alone.

EDS analysis of synthetic CSO samples (Figure 3-6) indicated that abundant electron-translucent (medium-grey) nanoscale colloids were rich in Al, Si and Cl. This presence of a composite material, which is heterogeneously mixed down to the nanoscale (see arrow), has important implications for floc interactions with membrane filters. Carbon compounds were evidenced by the presence of the "marker" for some classes of organics, the Os of osmium tetroxide, which selectively marks unsaturated lipids, lipoproteins and proteins rich in certain amino acids (Hayat, 1970). Mineral particles (see double arrow) could be distinguished by their high electron-opacity (blackness) and peaks for known minerals (in this case an aluminosilicate). Note that Cu is detected due to its presence in the sample support grid, but is not in the sample itself.

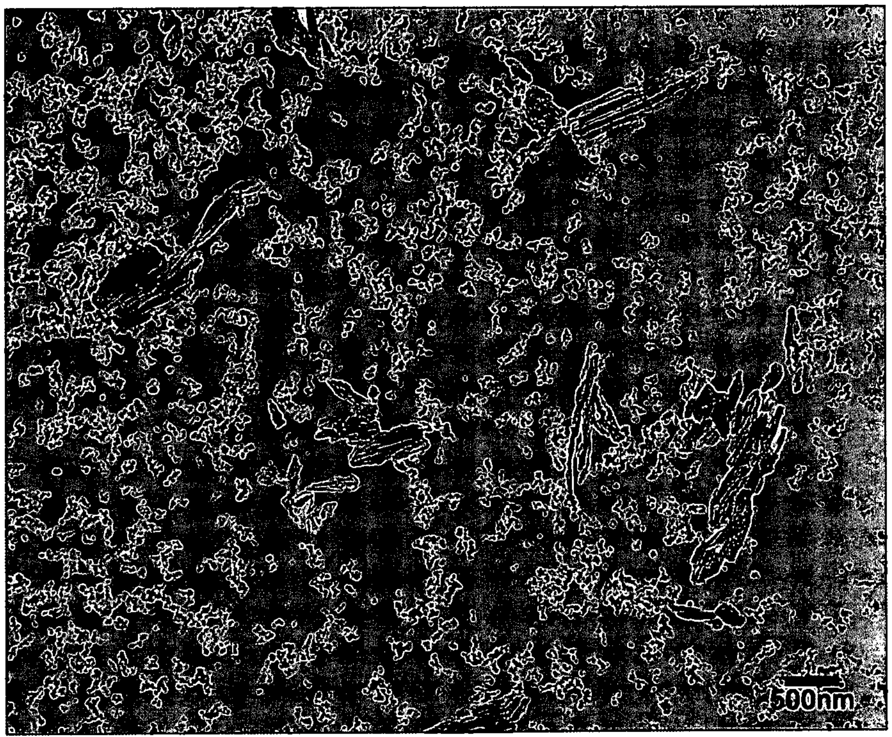

Figure 3-3: Synthetic CSO treated with alum, fixed by GRR.

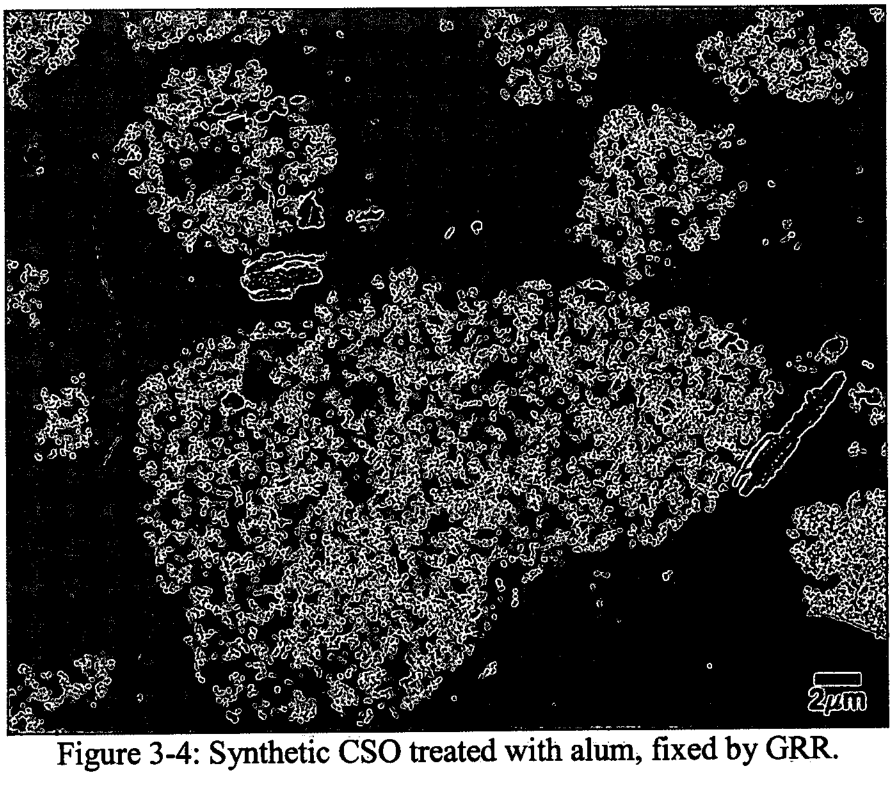

Figure 3-4: Synthetic CSO treated with alum, fixed by GRR.

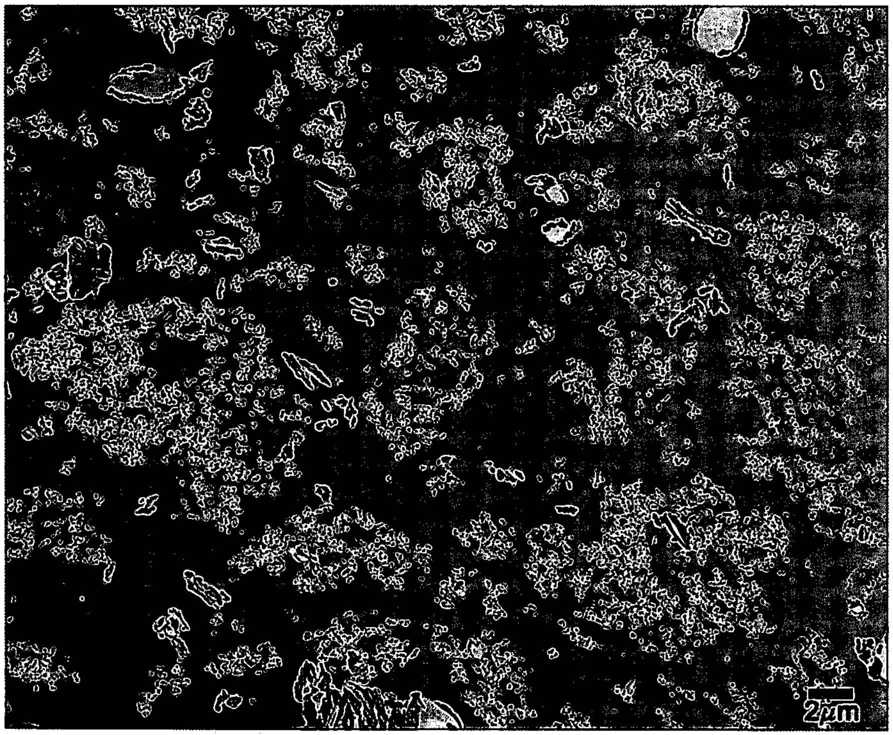

Figure 3-5: Synthetic CSO treated with alum + PAM, fixed by GRR.

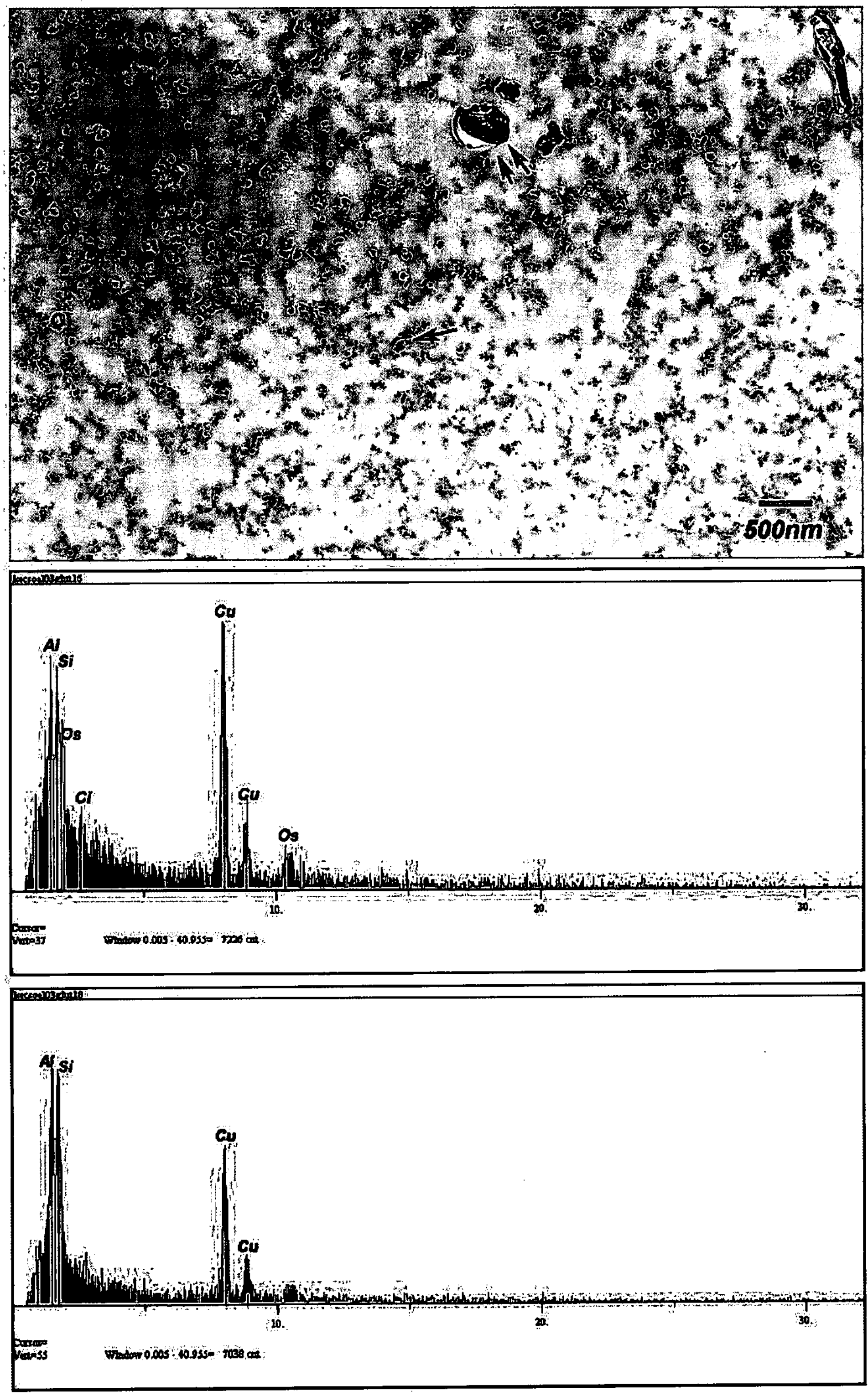

Figure 3-6. EDS spectra from selected colloids in synthetic CSO, treated with alum (as seen in the TEM image above). The upper spectrum was taken from the nanoparticle cluster indicated

by an arrow. The lower spectrum was taken from the electron-opaque (black) colloid indicated by a double arrow. The copper peak came from a nearby grid bar.

Real sewage (Figure 3-7) has many more features evident in the TEM images, particularly biological material. Some flocs are apparent in untreated sewage, as well as a number of biological cells. Many bacterial cells appear to be surrounded by extracellular polymeric material, which may act as polymer bridges between cells, or between bacteria and inorganic material, to form flocs. Figure 3-7 has features in common with flocs from a wastewater treatment system described earlier by Liss *et al.* (1996), namely numerous bacterial cells and a low frequency of mineral particles.

Effects of alum (Figure 3-8) and alum + polyacrylamide (Figure 3-9) on flocs formed in real sewage were similar to the effects in synthetic water, in that the images contain fewer small flocs, with independent particles appearing as part of an extended gel matrix. The connectedness of large floc parts to one another, via bridges, is not obvious until one has examined a series of sections through a floc. As was the case for synthetic CSO samples, a quantitative floc size distribution analysis by TEM is cost-prohibitive; however, size distribution analyses should be undertaken using new technology being developed as a cost-effective aid to TEM (Leppard, 2009). The relatively strong peaks exhibited by Os in the EDS spectrum (see Figure 3-8 lower part, which is a representative spectrum of electron-translucent biologicals) reveal a higher proportion of microbial cells and their extracellular products in the flocs formed from real sewage, relative to what one discerns in synthetic CSO.

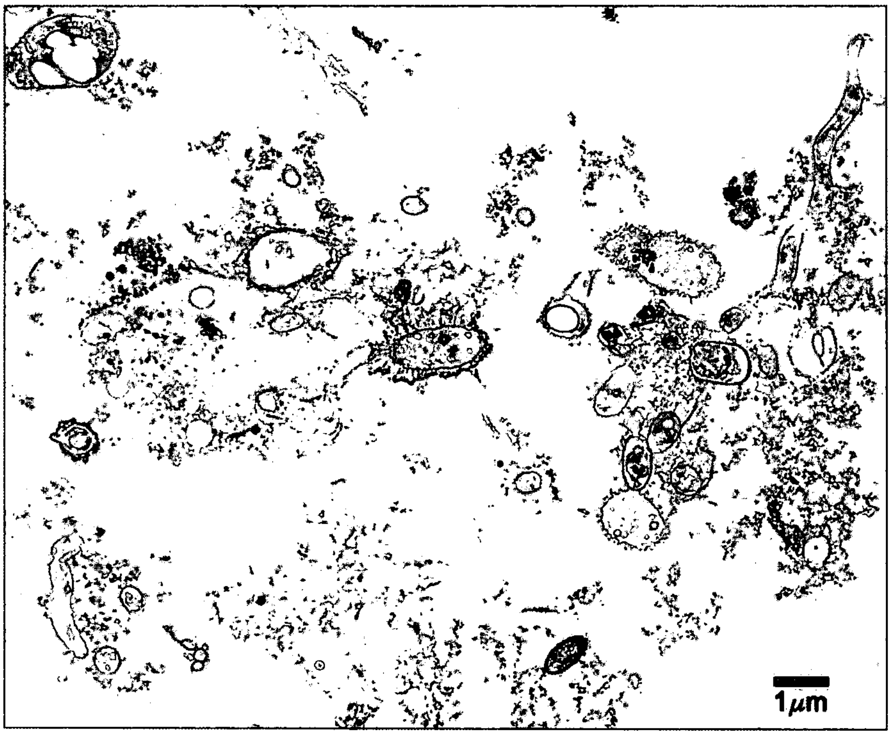

Figure 3-7: Niagara Falls sewage, fixed by GRR.

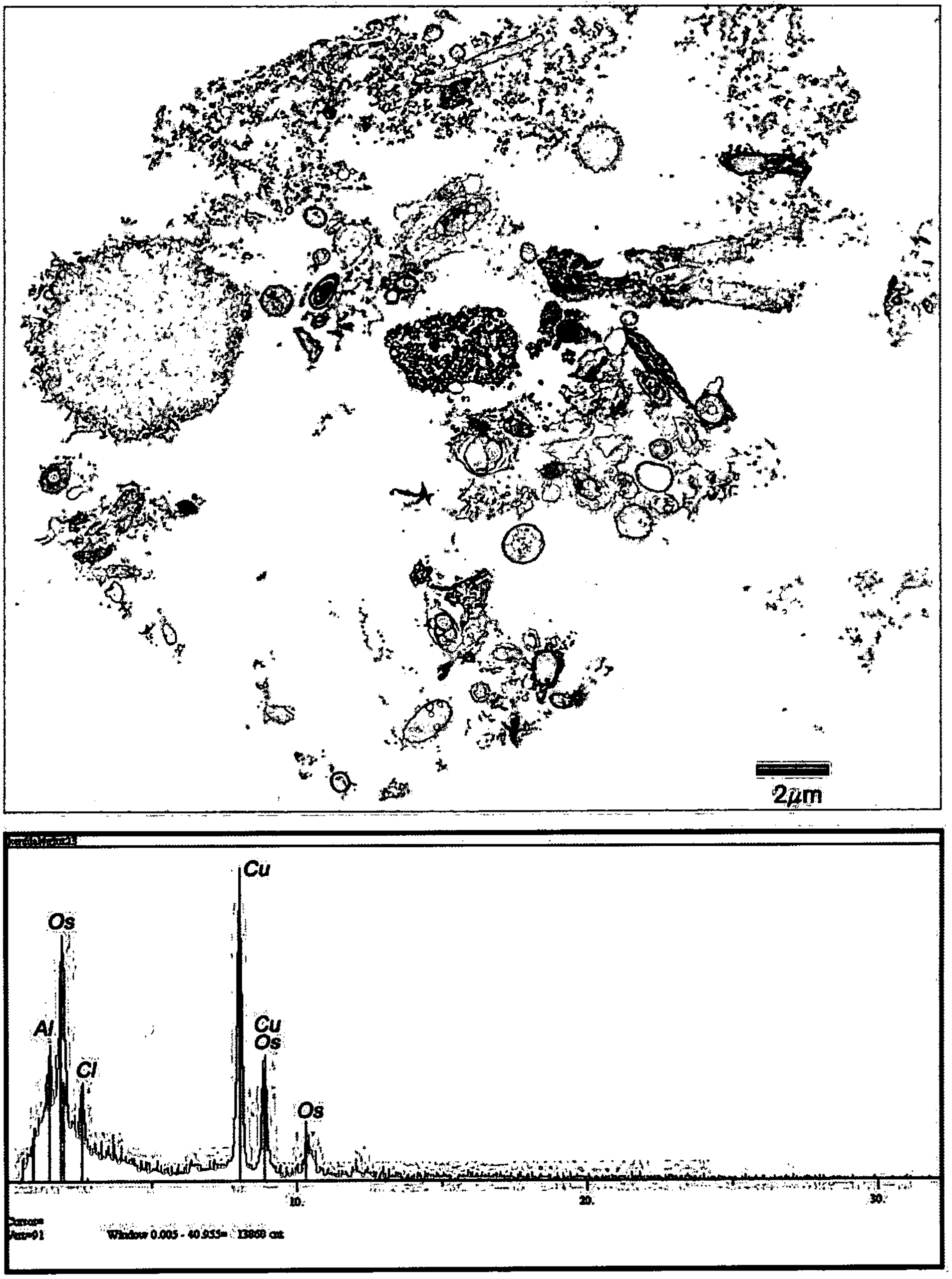
2µm
Cu
Os
Al
Cl
Cu
Os
Os
10.
20.
30.
Window 0.005 - 40.955 = 13868 cnt

Figure 3-8: A micrograph of Niagara Falls sewage treated with alum, then fixed by GRR. The representative EDS spectrum (lower portion) was taken from electron-translucent colloids located near microbial cells (Niagara Falls sewage, treated with alum).

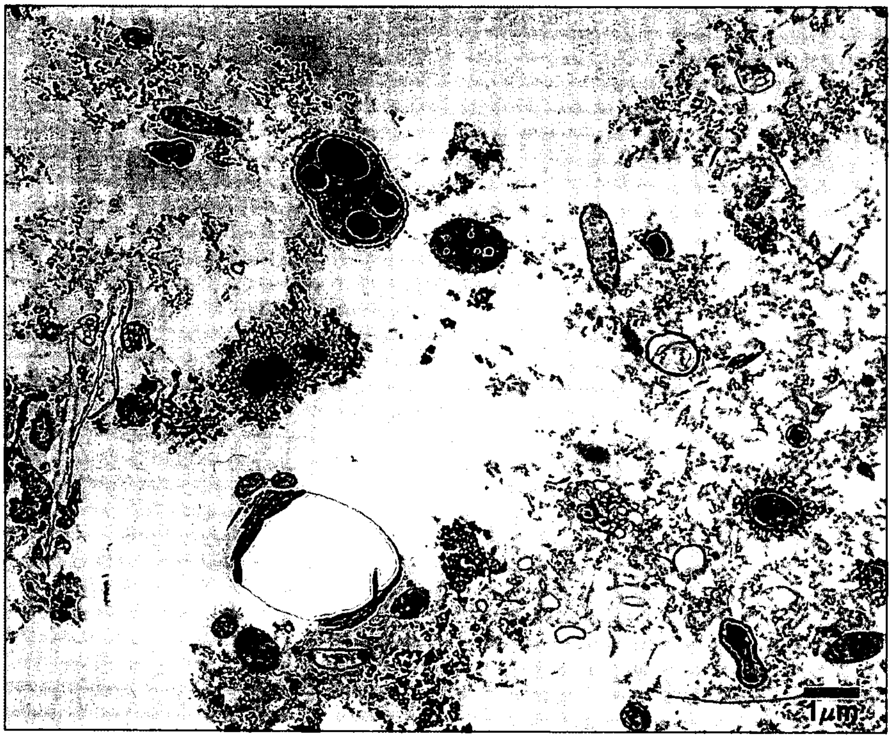

Figure 3-9: Niagara Falls sewage treated with alum + PAM, fixed by GRR.

3.2 Clean membranes

Images of the regenerated cellulose (RC) and polyethersulfone (PES) membranes can be seen in Figures 3-10 to 3-14.

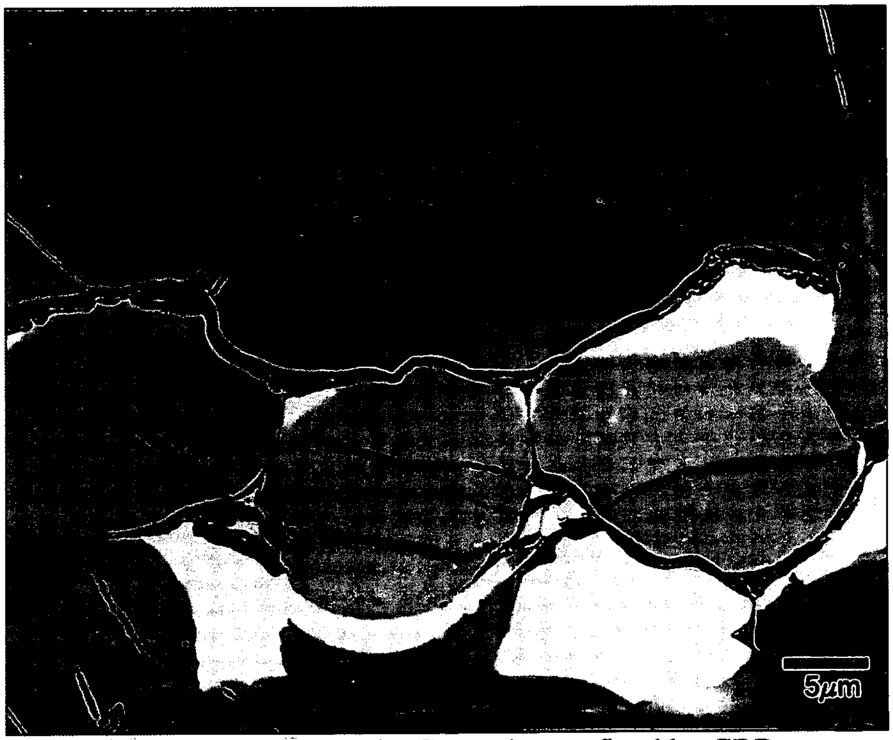

Figure 3-10: Cleaned RC membrane, fixed by GRR.

Note that the membrane in Figure 3-10 was sometimes poorly retained in Spurr's epoxy resin, leading to portions falling out on sectioning. In Figures 3-11 and 3-12, the basic structure of the RC membrane can be seen; Figure 3-11 reveals a thin surface layer on top of a thick porous support layer, while Figure 3-12 reveals nanoparticles within the porous support layer. Anisotropic membranes such as the RC and PES membranes used in this study generally consist of a thin, selective layer supported on a thicker, highly permeable microporous substrate. The thinness of the selective layer allows higher fluxes, while the microporous substrate provides strength (Baker, 2004). In spite of the cleaning procedure, impurities are evident both on the surface and within the pores of the RC membrane. The PES membrane (longisection in Figure 3-13 and glancing section in Figure 3-14) appears to have a different structure, with a thin surface layer above large dead-end voids. According to supplier specifications, the RC membrane is designed with a hydrophilic, tight microstructure to allow high retention with low adsorption of macromolecules, while the PES membrane has an open microstructure which provides high flow and rapid separation capability.

The differences in structure likely relate to the manufacturing processes used for each membrane type. Manufacturing processes are typically proprietary but can include phase separation (including the Loeb-Sourirajan water precipitation technique, described in Baker, 2004), interfacial polymerization, and solution-coating. The procedures used affect such membrane properties as porosity, pore size, selective layer thickness, and substrate structure (Baker, 2004).

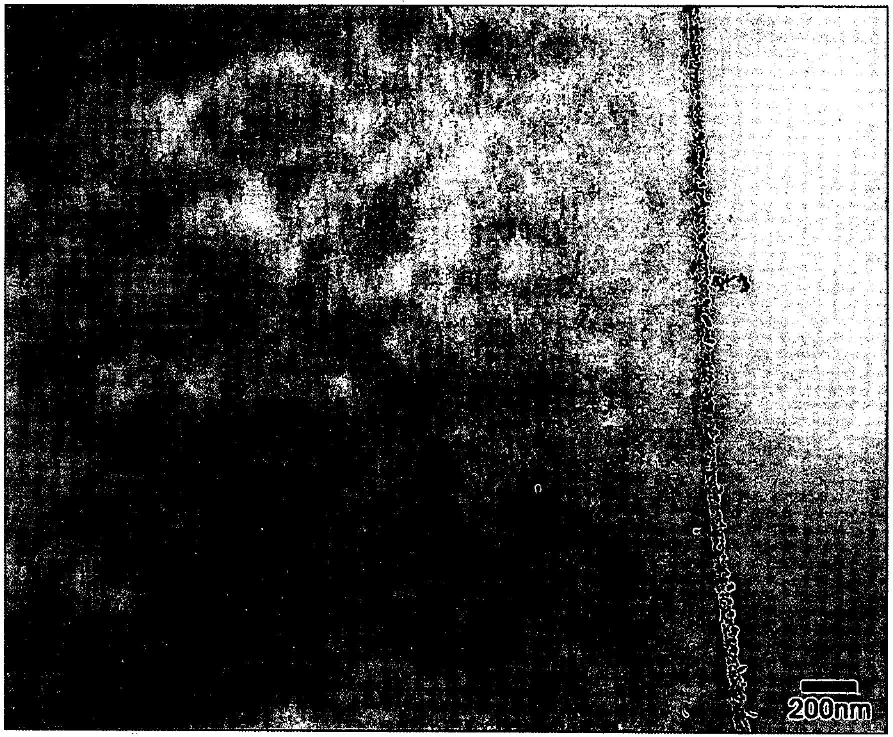

Figure 3-11: Cleaned RC membrane, fixed by GRR

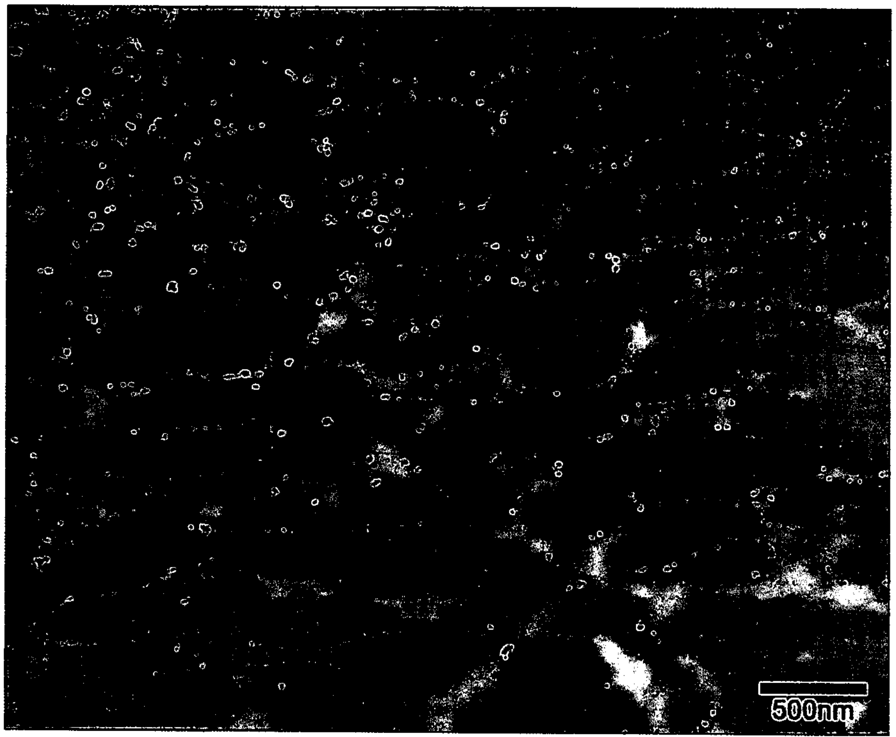

Figure 3-12: Cleaned RC membrane, fixed by GRR

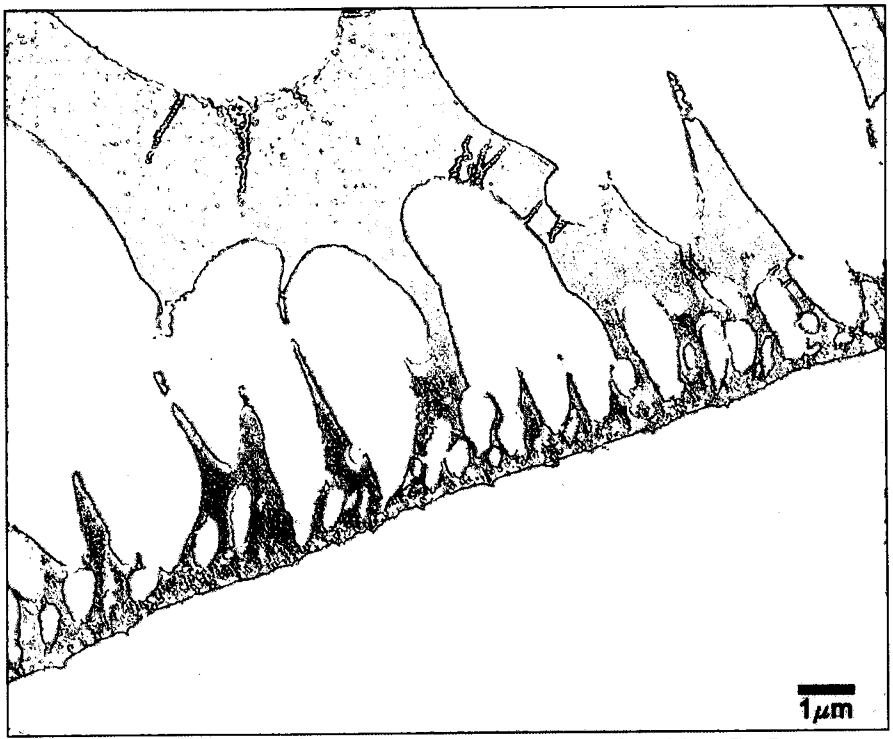

Figure 3-13: Cleaned PES membrane, fixed by GRR

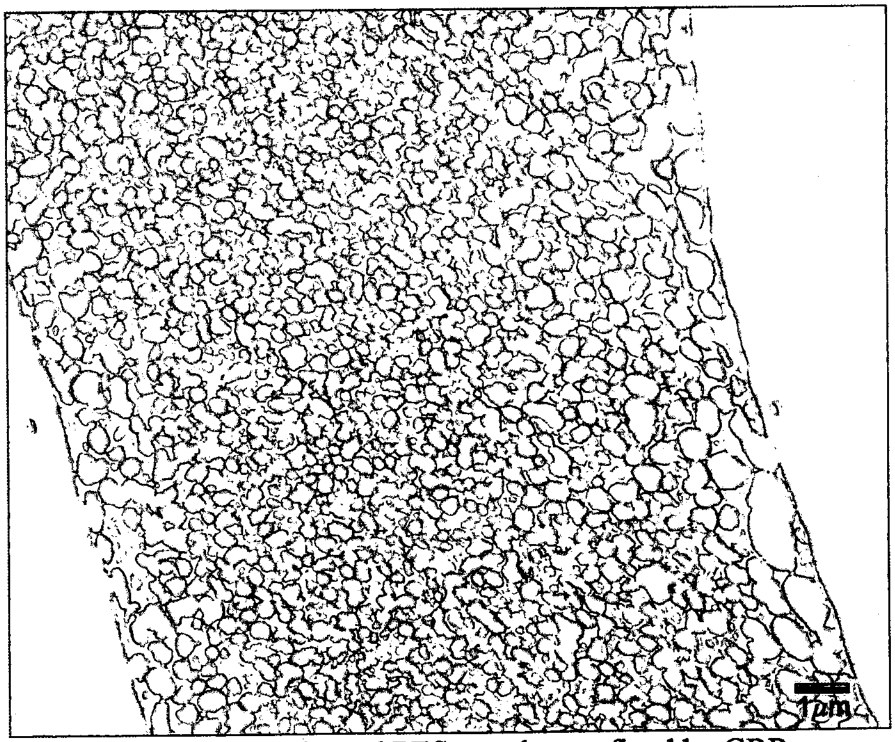

Figure 3-14: Cleaned PES membrane, fixed by GRR

3.3 Membranes fouled with filtered wastewater

Membranes were examined after multiple fouling and cleaning processes, as described above. In Figures 3.15 to 3.18, the foulant can be clearly seen on the surface of the RC membranes. Additionally, solid material can be observed spread throughout the membrane support structure in all of the TEM images, suggesting that fouling occurs by a number of mechanisms, including formation of surface cake and pore constriction or blockage. Taniguchi *et al.* (2003) also suggested that organic matter caused both surface cake formation and pore blockage, where the extent of each mode depends on the molecular weight of the OM and the degree of fouling. Low molecular weight organics may penetrate the pore structure, causing irreversible fouling by pore blockage, while larger molecular weight organics cause more reversible fouling through surface cake formation. The mechanism may also evolve as fouling progresses, as the presence of a cake alters the surface of the membrane (Taniguchi *et al.*, 2003).

Similar to the three-colloidal approach of aggregate formation of Buffle *et al.* (1998) described in section 1.2, Jermann *et al.* (2008) found a synergistic effect during combined particle-NOM fouling, which was greater than the sum of particle and organic fouling alone. In both cases, specific components of NOM may stabilize inorganic colloids, changing the nature and porosity of a fouling cake. Laîné *et al.* (2003) also discussed the formation of a fouling cake based on clay bound by a NOM-based gel, and suggested that the degree and irreversibility of fouling was dependent on the nature of the organic matter as well as the nature of the membrane material. The foulants observed here were responsible for irreversible fouling, as chemical cleaning (NaOH / NaOCl solution) did not remove them from the membrane.

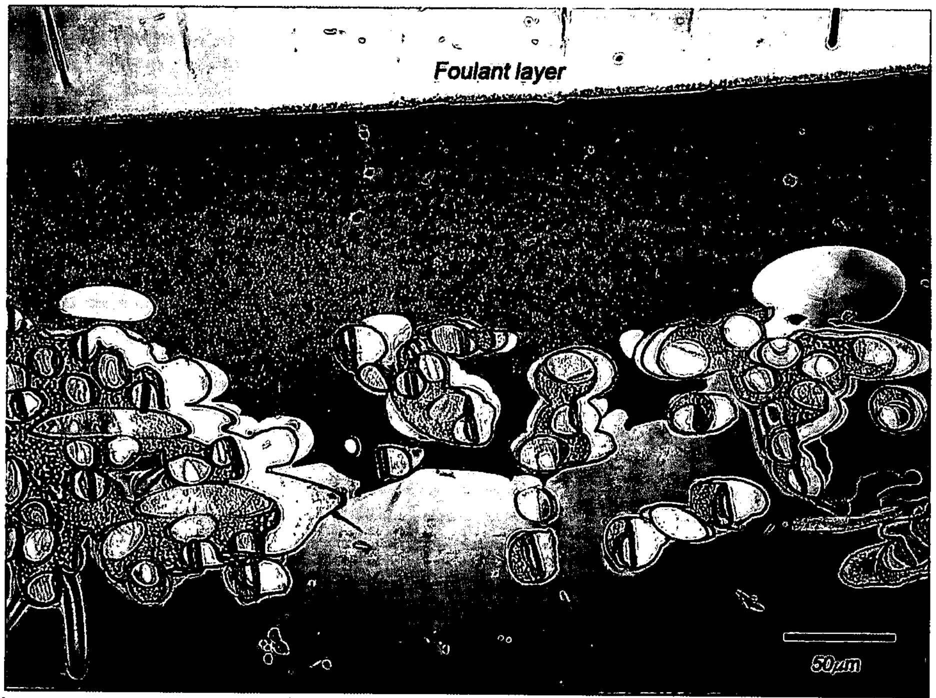

Figure 3-15: Fouled RC membrane orientation image (optical microscope).

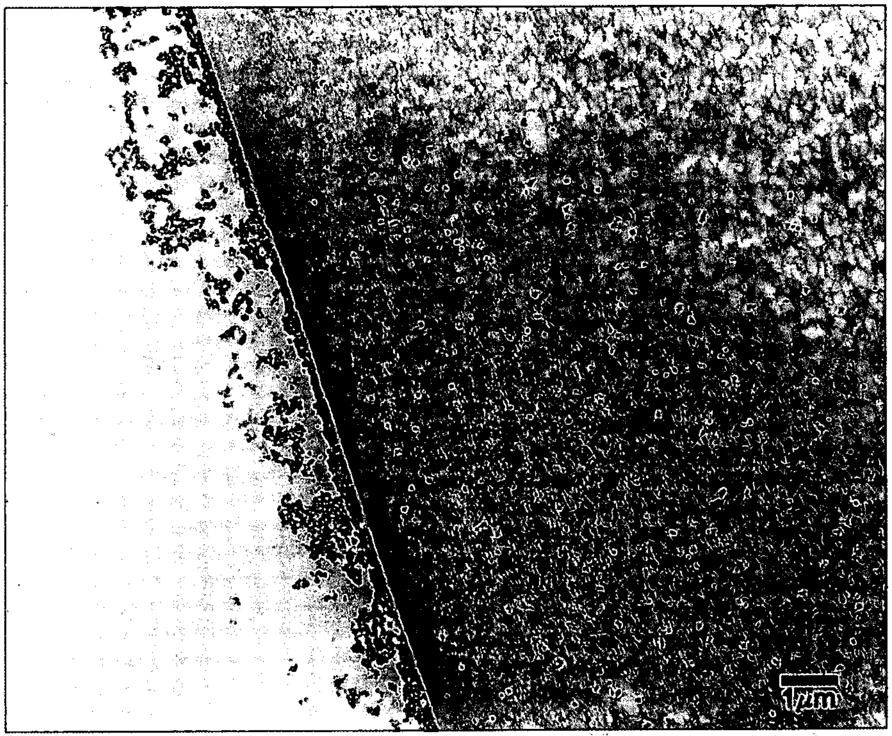

Figure 3-16: Fouled RC membrane, fixed by glutaraldehyde

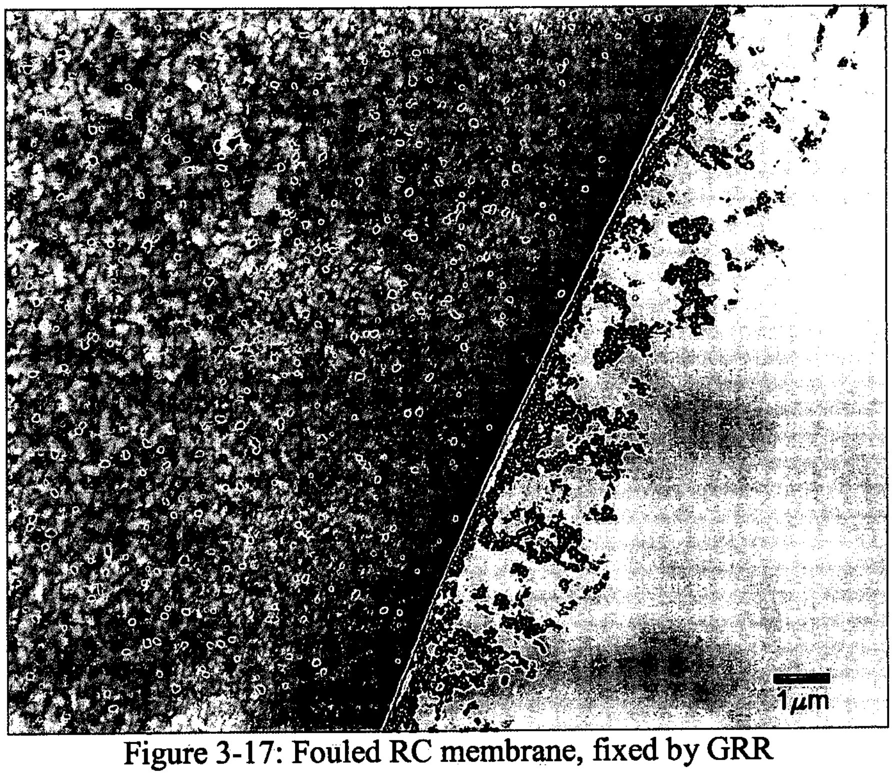

Figure 3-17: Fouled RC membrane, fixed by GRR

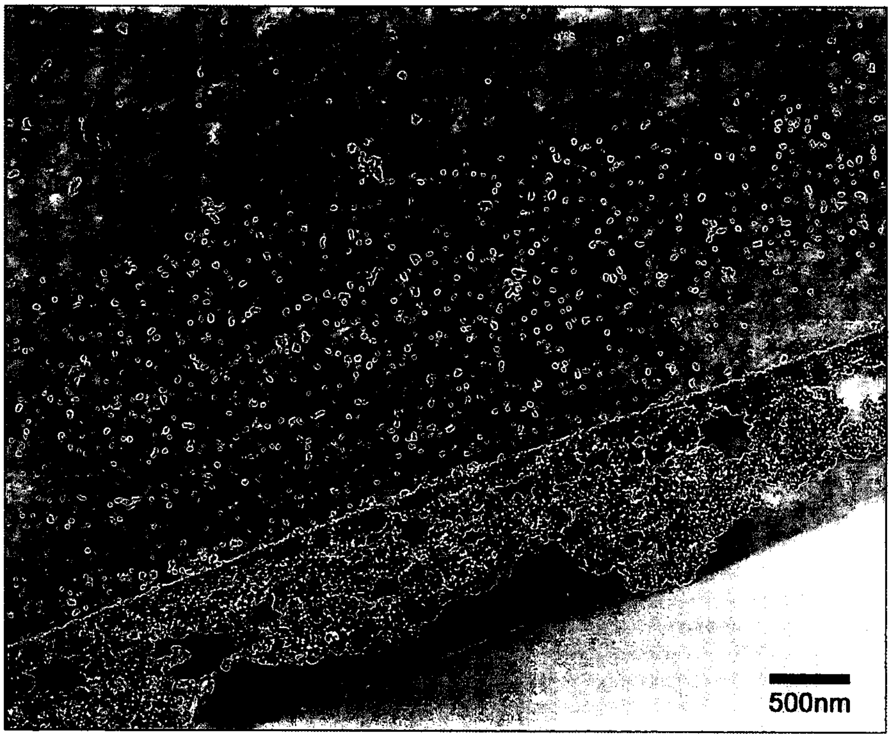

Figure 3-18: Fouled RC membrane, embedded by Nanoplast resin

In Figs. 3-19 to 3-23, the foulant can be seen on the surface of the PES membranes. However, little solid material can be observed within the pores of the membrane support structure in all of the TEM images, indicating that fouling is primarily in the form of a strongly-bound surface cake that was not removed by chemical cleaning. Note that the dark lines in Fig. 3-19 are folds in the thin section and not a feature of the membrane.

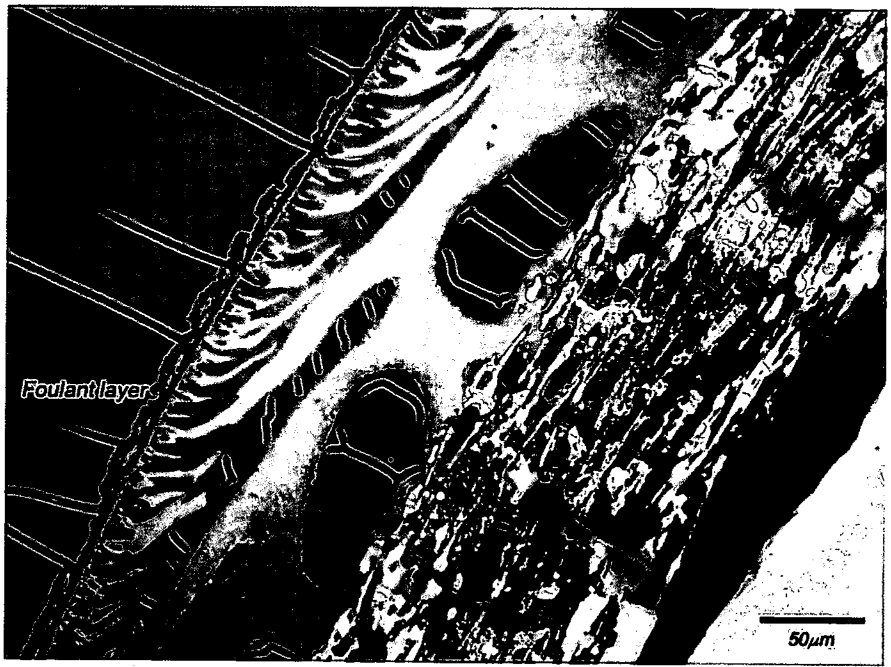

Figure 3-19: Fouled PES membrane orientation image (optical microscope).

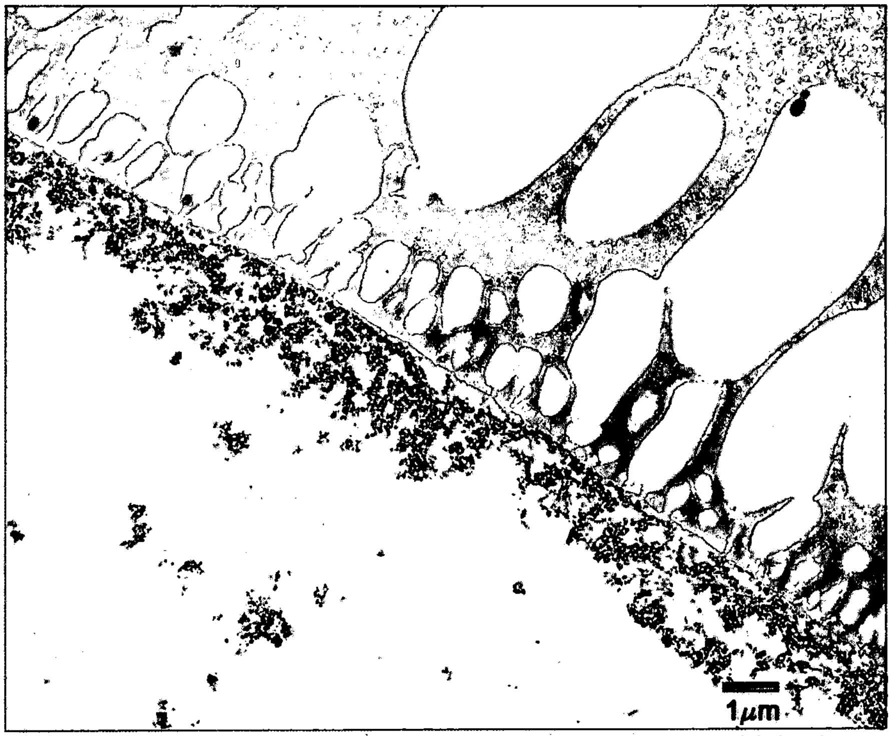

Figure 3-20: Fouled PES membrane, fixed by glutaraldehyde

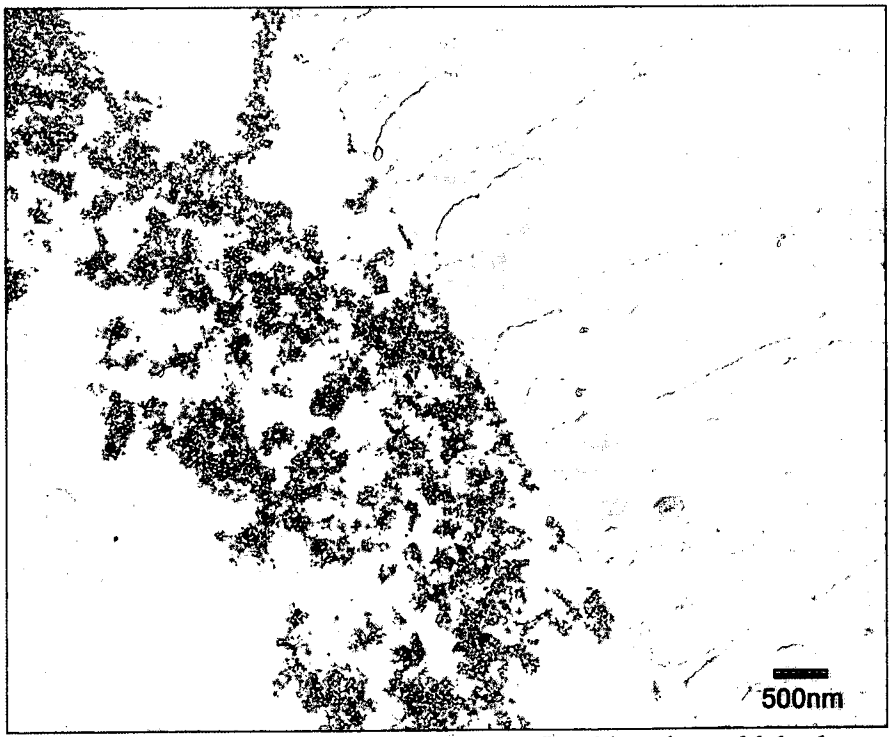

Figure 3-21: Fouled PES membrane, fixed by glutaraldehyde

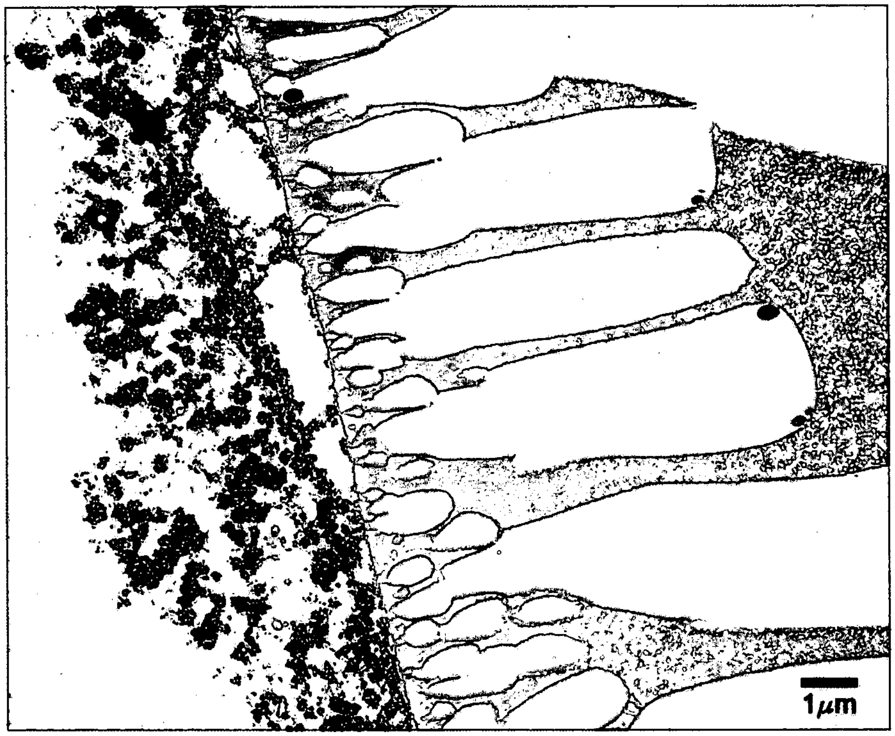

Figure 3-22: Fouled PES membrane, fixed by GRR

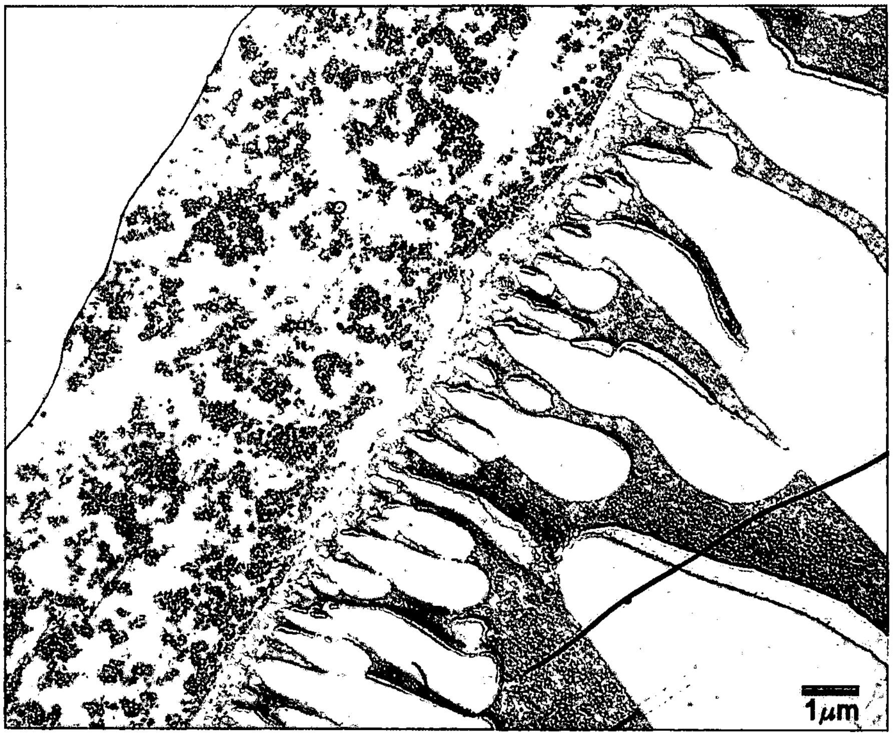

Figure 3-23: Fouled PES membrane, embedded in Nanoplast resin

4. Conclusions

Electron microscopy-based investigation of flocs and fouled membrane filters, embedded in resins, allows close examination of structural characteristics at the nanoscale. Ultrastructural characteristics (size, shape, internal differentiation, native associations, degree of electron opacity) permit identification of some particles in the colloidal size range, with a confirmation by spectro-microscopy in some cases. Observations on the nanoscale heterogeneity of foulants suggest promise for an improved understanding of foulant/membrane interactions.

Surface foulant layers can be clearly visualized (down to 1-3 nm resolution) using various complementary TEM preparations. Mixed aggregated materials at the nanoscale can be detected. This heterogeneity phenomenon might set a constraint on a specific filter with regard to biofouling. A colloidal aggregate of mixed nanoparticles might have positive, negative and uncharged patches at its surface as well as having both hydrophobic and hydrophilic patches. Consequently, a material which ordinarily wouldn't bind to a certain kind of filter will adhere simply because it is bound to something else which can bind to the membrane. We have reached a point in the application of TEM for nanoscale examinations whereby focussed experiments can begin on greater manipulation of experimental variables, for the purpose of minimizing fouling by particles in the colloid size range. Nanoparticles in that range can be visualized clearly and routinely (on membrane surfaces) and large ones can be assessed for element abundances. For the larger colloids, individual particle heterogeneities can be detected and assessed, while those with distinctive morphologies can often be identified by the criteria of Leppard (1992).

Future TEM-based research on flocs should be complemented by particle size distribution analyses on each sample type, for both flocs and suspended individual colloids. The approach of Droppo *et al.* (1996) has been used successfully with TEM analyses of flocs for more than a decade. To extend the particle size distributions into the colloidal size range, a good start might be to adapt the colloid size distribution approach of Walther *et al.* (2006), which extends down to 15 nm.

5. Recommendations

The sample preparation protocols for TEM-based spectro-microscopy are complex, time-consuming, costly, and require extreme levels of skill and fastidiousness on the part of the technician. The upside for this technology is that one can obtain information not possible by other means. A simplified approach to cost-effective protocol selection is as follows:

(1) use the TEM preparatory protocols as detailed explicitly in Pazin *et al.* (2005);

(2) use the GRR proxy to obtain the major portion of the nanoscale information, employing the other two preparations to check for artifacts;

(3) for element analysis, start with EDS to assess element abundances per particle, then whenever feasible, do a re-analysis of the same particle by selected area electron diffraction or electron energy-loss spectroscopy for mineralogical information (Mavrocordatos *et al.*, 2007).

During the course of these nanoscale investigations, several technological advances became available which were not utilized. Some new technologies which would be valuable in extending these nanoscale investigations are as follows: (1) the multi-method approach of Schafer *et al.* (2007) for the spectro-microscopy of aquatic organic colloids; (2) the multi-method approach of Chanudet and Filella (2006) for the spectro-microscopy of aquatic mineral colloids;

(3) correlative spectro-microscopical analyses of colloids by "in tandem" use of electron microscopy and X-ray microscopy, as has been accomplished for mixed-material aquatic colloids by Chan *et al.* (2004) and for biofilm colloids (Lawrence *et al.*, 2003; Reid *et al.*, 2005); (4) the use of atomic force microscopy (Lead *et al.*, 2005); the use of specialized ultra-high resolution microscopes which can examine "thick" sections (300 nm and up), thus providing much more ultrastructural information per examination than the 50 nm sections required by standard TEM instruments (such specialized equipment is available in the Canadian Centre for Electron Microscopy on the McMaster University campus in Hamilton, ON).

6. Acknowledgments

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7. Glossary

CIP – cleaning-in-place
CSO – combined sewer overflow
DOC – dissolved organic carbon
EPS – extracellular polymeric substances
EDS – energy dispersive spectroscopy
FTIR – Fourier transform infrared spectroscopy
GRR – glutaraldehyde + ruthenium red
MF – microfiltration
MWCO – molecular weight cut-off
NF – nanofiltration
NOM – natural organic matter
OM – organic matter
PAM – polyacrylamide
PES – polyethersulfone
RC – regenerated cellulose
RO – reverse osmosis
TEM – transmission electron microscopy
UF – ultrafiltration

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AWWA Membrane Technology Research Committee. 2005. Committee report: Recent advances and research needs in membrane fouling. *Journal AWWA*, 97(8): 79-89.

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