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The involvement of lectins and lectin-like humic substances in biofilm formation on RO membranes - is TEP important?

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ABSTRACT

There are significant number of publications that state that the main cause of biofilm formation and organic fouling on reverse osmosis (RO) membranes is due to the presence of transparent exopolymer particles (TEP) and their precursors. Alcian Blue has been used to detect and quantify TEP and their precursors. However, Alcian Blue is not specific for TEP substances and interacts with other acidic substances, lectin-like humic substances which may also be important in biofilm formation and chemical conditioning of membrane surfaces.
Certain lectin-like humic substances may be present in RO feed waters. A new assay procedure which couples, ammonium sulfate protein precipitation, LC-OCD and Alcian Blue is presented which can detect and quantify these lectin-like humics in any water sample. It appears that bio-adhesion is dependent upon these lectin-like humics and they appear to control biofilm formation.
Based on results from a wastewater RO reclamation facility and examination of the literature, the presence and importance of TEP and their precursors in biofilm formation may be overstated and that biofilm and/or organic fouling on RO membranes appears to be initiated and controlled by the presence of certain lectin-like humics which may not be the dominant conditioning mechanisms in every RO desalination plant.

1. Introduction

Biofouling was first described in 1936 by ZoBell and Anderson [1] at the Scripps Oceanographic Institute in San Diego, California. In the mid 1940′s, it was noticed that any surface immersed in seawater would become populated with attached bacteria and that this surface-enhanced microbial growth was stimulated by concentrating organic nutrients that were present in the seawater [2]. Bacterial populations in seawater or any other water source would increase upon storage, because of an increase in the metabolic activity of the attached bacteria to the surface. Others showed that solid surfaces concentrated nutrients from natural water which promoted microbial activity [3–5]. The concentration of organics at the surface became more pronounced with the presence of colloidal and poorly soluble substances.

Biofilm formation on immersed surfaces occurs as a series of successive attached communities [6]. As the surface characteristic c, more complex organisms will appear on the surface, replacing simpler ones. The first and most important stage in biofilm formation of immersed surfaces in seawater or natural waters begins with the rapid adsorption of acidic exopolymeric substances (EPS), which can be acidic glycoproteins, acidic polysaccharides or humic-like material. It is believed that the presence of these acidic organics will adsorb to clean solid surfaces immediately after exposure to the seawater [7–9]. The development of the biofilm is dependent upon the affinity of the immersed surface to adsorb these organics. Chemical and biological fouling in seawater and in wastewater RO reclamation begins with a spontaneous deposition of this conditioning film. This process may take several days, but few bacteria will attach until this conditioning film is in place. The conditioning phase is a form of organic fouling preceding bacterial fouling.

The involvement of acidic in bacterial attachment has been well-documented for freshwater and marine bacteria [10–13]. It has been suggested that specific receptors on the bacterial surface interact with organic material adsorbed onto the membrane surface [14], thus promoting attachment and biofilm growth. The acidic EPS act like lectin-type substances, promoting bacterial attachment and aggregation.

Since 1993, it has been reported in the oceanographic and limnologic literature that there exists an abundance of previously undetected, acidic transparent exopolymer particles (TEP) in wastewater and seawater that could be visualized by staining with Alcian Blue [15–17]. TEP are synthesized from acidic polysaccharides and are one form of EPS that are abundant in most marine, brackish and non-saline surface waters and are operationally defined as being >0.4 μm that stain with Alcian Blue [18]. TEP are formed predominantly from algal exudates, bacterial mucus and particulate material from gelatinous envelopes surrounding

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phytoplankton. Exudate-rich diatom cultures and blooms are highly abundant in TEP and the aggregated TEP are disrupted by EDTA [19].

TEP have been shown to form abiotically and spontaneously from TEP precursors, <0.4 μm, which can pass through dialysis membranes with a nominal pore size of 8 kDa, suggesting that TEP-precursors are fibrillar and that these fibrillar precursors form large colloids and eventually TEP within hours [17,20]. TEP have been described as a major agent in the aggregation of particles in aquatic systems and has been described as a major agent in formation of marine snow which represents a pathway by which dissolved organic carbon (DOC) is removed from the euphotic zone [21,22].

Bar-Zeev et al. [19] have stated that TEP has 4 main key features which were as follows: (i) composed mainly of acidic polysaccharides, (ii) dispersed in the presence of different chelators such as EDTA, (iii) fractured to form fibrillary colloids that pass through 0.2 μm filters and (iv) spontaneously can reassemble from colloidal TEP precursors into TEP. TEP was found to burst apart when placed in 1 M EDTA, indicating the presence of cation bridges binding the subunits of the aggregates. Therefore, in the presence of EDTA, TEP will dis-assemble from being particulate (>0.4 μm) to colloidal (0.05 μm–0.4 μm).

Based on the ubiquitous and significant presence of TEP and its sticky nature in seawater and lake waters, a hypothesis has been put forth that TEP are the key component in the initiation and development of the biofilms on surfaces in aquatic environments and it has also been proposed that TEP play a major role in aquatic biofilm formation on membranes [18,19,23–28]. Berman and Holenberg [24] first stated that TEP may be the causative agent in biofilm formation on membranes. Bar Zeev et al. [19] then formulated a revised paradigm on how biofilms form on surfaces, which emphasized the importance of TEP, TEP protobiofilms, and TEP precursors in their model. Their revised paradigm implied that, in addition to the well-documented original model in which chemical conditioning of surfaces by organics and colloids facilitated the attachment of bacterial cells and aggregates that grow out, TEP and TEP pre-fabricated biofilms (protobiofilms) can immediately attach to surfaces and initiate biofilm formation. They emphasized that TEP precursors could adsorb to the membrane surface and act as a chemical conditioning layer, resulting in a thin, negatively charged surface. The term TEP precursors have been used to designate substances that are <0.4 μm and >0.05 μm which interact with Alcian Blue [19]. TEP precursors have been shown to form TEP by bubbling [29] and an increase in shear forces. Villacorte et al. [23] showed that in several RO plants, ultrafiltration proved to be the most effective method to remove TEP relative to other pretreatment methods; but the so-called TEP precursors were not completely removed, emphasizing the importance of measuring this component to better understand the role of TEP and acidic polysaccharides in RO membrane fouling.

TEP deposition factors and specific deposition rates, based on Alcian Blue assays, indicated that TEP accumulation occurred at many RO facilities [19]. These observations were verified by autopsy of RO membranes where Alcian Blue staining deposits were found present on the RO membrane. At the Ashkelon seawater RO plant with conventional media filtration, pretreatment removed only 28% of TEP in the feedwater which may play an important factor in biofilm development, even though Ashkelon has shown to be a successfully operating seawater RO plant [30]. Other studies have confirmed that TEP concentrations are not significantly reduced by conventional pretreatment in desalination and wastewater treatment plants [19].

Bar-Zeev et al. [19] introduced the term “TEP protobiofilms” as being large TEP or TEP prefabricated biofilms which were heavily colonized by bacteria and other organisms and were hypothesized to play a critical role in the initial stages of biofilm formation and could accelerate the rate of biofilm formation. It has also been reported that TEP can be colonized by bacteria [16] and these bacteria can be brought to solid surfaces through attachment of TEP due to their high stickiness. It was suggested that TEP protobiofilms may have applied importance for the water industry in which fouling of membranes is a major concern. The presence of TEP protobiofilms was detected with the use of Alcian Blue. Whether TEP protobiofilms are in reality bacterial aggregates held together and embedded in acidic polysaccharides is still unknown. TEP protobiofilms may be considered one form of free-floating biofilms, which are common in both anthropogenic and natural aquatic environments. The formation of free-floating biofilms involves a self-produced mucopolysaccharide matrix [31,32] which may involve TEP or have no TEP involvement. Alcian Blue has been used to determine the presence of these mucopolysaccharides which may be TEP or Alcian Blue stained material that may not be TEP.

Alcian Blue (copper-phtalocyanin with four methylene-tetramethylcisothiouronium chloride sidechains) can stain sulfated and carboxylated polysaccharides and glycoproteins [19,33,34]. Any substance that interacts with Alcian Blue has been classified as TEP, if >0.4 μm, and TEP precursors, if <0.4 μm. However, many substances, other than TEP and TEP precursors can also interact with Alcian Blue and thus, Alcian Blue is not specific for TEP [18]. Alcian Blue can complex with many different algal acidic polysaccharides containing anionic carboxyl and half-ester sulfate groups that are not involved in TEP formation. Scott and Willet [35] were the first to suggest that Alcian Blue could be used in quantification for any polyanion, not just TEP. Alcian Blue has been shown to interact with acidic glycoproteins [36] and with non-TEP capsular polysaccharides [37]. Acidic polysaccharides such as agar, carrageenan, and pectin all react with Alcian Blue [34]. Certain biofilm EPS which were not TEP have been quantified using Alcian Blue [38]. Alcian Blue has been shown to contain carbohydrate-binding properties which interact with surface glycans of GP120 glycoprotein of the HIV virus [39]. Thornton and Visser [40] demonstrated that acidic polysaccharides which were extracted from salt marsh sediments and interacted with Alcian Blue may not be related to TEP. As Thornton [41] pointed out, acidic polysaccharides (APS) found in the marine environment can be present as surface coatings from microorganisms, which are dissolved in the water column and may not be related to TEP. Alcian Blue, acting lectin-like, can also react with carbohydrate conjugated proteins such as acidic glycoproteins and proteoglycans [42]. Alcian Blue has been shown to possess lectin-like activity and can detect other lectin-like substances based on its ability to bind to specific sugar groups and its ability to agglutinate cells [39]. Floating biofilms have also been visualized with Alcian Blue [32]. Therefore, before assigning the term TEP or TEP precursor to any material that interacts with Alcian Blue that material must be shown to either be affected by the chelating agent EDTA which can disrupt TEP into its precursor form or can form spontaneously particulate TEP from its precursor colloidal origin. The statement that TEP are specifically stained by Alcian Blue [19], therefore, may be misleading. The presence of TEP and TEP precursors in feedwater at RO facilities, as identified in the literature, should be re-visited to determine if the particulate TEP were actually Alcian Blue stained material, such as floating biofilms and if the TEP precursors were actually Alcian Blue stained material such as lectins or lectin-like humic substances.

Lectins are carbohydrate-binding protein macromolecules of non-immunological origin that are highly specific for certain monosaccharide molecules which can agglutinate cells. Lectins were originally named because of its ability to agglutinate red blood cells. The term “lectin-like” has been used to refer to substances that have characteristics similar to lectins [43] in binding to specific sugars and agglutinating cells, but may not be proteins. Lectins and lectin-like substances may mediate attachment of bacteria to their intended target and may cause agglutination of bacterial cells as shown by Liljemark et al. [44].

The carbohydrate component of lectin-like humics may interact with specific protein component of other lectin and lectin-like substances present on bacterial cells and enhance bacterial aggregation and biofilm formation. Corpe [45] has shown that several marine periphytic bacteria secreted EPS which were lectin-like substances and which interacted with Alcian Blue. *Pseudomonas aeruginosa PAO1*, a model organism for biofilm formation studies, produces 2 lectins, LecA and LecB, that are involved in biofilm formation [46,47] and exhibit