Anti-biofouling efficiency of electrospun polylactic acid membranes doped with silver and copper nanoparticles supported on sepiolite

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Membrane technologies have been in use to meet the increasingly stringent regulatory requirements for drinking water production. Microfiltration and ultrafiltration membranes can achieve a high level of pathogen removal without using chemical treatments or allowing a reduced disinfectant dosage. Membranes are also a choice for wastewater treatment particularly in case of restrictive discharge limits such as those affecting nitrogen and phosphorus. Despite the advantages of membrane processes, their application requires an improved control of biofouling, which is due to the accumulation of biomass by growth or deposition to such a level that it causes flux reduction combined with increased pressure drops.

Generally, the surface properties of membranes determine their performance under fouling or biofouling conditions. It has been observed that hydrophilic membrane surfaces are less prone to the microbial adhesion triggering biofouling [1]. As most commercial polymeric membranes are hydrophobic, considerable interest has been shown in developing new membranes or modifying existing ones to increase their hydrophilicity [2]. Surface charge determines microbial adhesion because cell membranes possess large negatively charged domains, which are repelled by surfaces bearing negative charges such as those obtained by graft polymerization of acrylic acid [3]. Nevertheless, it has also been reported that positively charged surfaces result in strong electrostatic attractive forces that hinder microbial replication [4]. Another approach consists of incorporating anti-biofouling substances to prevent microbial growth. For example, the incorporation of silver ions in hydrophilic membranes enhances their charge-driven anti-adhesive properties and provides a specific anti-microbial function. This acts as a safeguard in case some bacteria become attached to membrane surface [5].

In the current study, PLA and sepiolite were chosen for the preparation of reinforced porous membranes. PLA shows higher natural hydrophilicity than conventional thermoplastic polymers. This is due to the access of water molecules to polar oxygen linkages in PLA, which can help in improving fluxes and reducing the biofouling tendency of the membrane [6]. Sepiolite particles used in this work have an average length of 1 to 2 μm, width ~10 nm, and the dimensions of open channels (along the axis of a particle) ~3.6 Å x 10.6 Å. The arrangement of these particles results in loosely packed and porous aggregates with an extensive capillary network. In addition, the high density of silanol groups (-SiOH) on the surface enhances its hydrophilicity. To address the problems associated with biofouling, we used silver and copper functionalized sepiolite. The extent of fouling was assessed by measuring permeate flow and by performing membrane autopsy.

Electrospinning is a process which relies on electrical forces to form fibres with a minimal diameter and large surface to mass ratio. In this work, we prepared non-woven electrospun mats using polylactic acid (PLA) as matrix. Biocidal nanoparticles were prepared from sepiolite particles with a surface area of 250 m^2/g. Silver (Ag, 26 wt%) and copper (Cu, 26 wt%) functionalized sepiolite was prepared according to a proprietary methodology. Sepiolite particles were negatively charged in all cases, with zeta potential in the (-15)-(-37) mV range and higher negative charge at higher pH. NANON-01A electrospinning unit (MECC Co. Ltd. Japan) was used to process PLA and sepiolite or functionalized sepiolite which represented a 5 wt. % of the whole composite membrane.

The experimental device for filtration experiments was a cross-flow filtration device for 90 mm membranes supported on a wired mesh support base. As model biofoulants we used cultures of *Saccharomyces cerevisiae* (pH 4.5) and *Pseudomonas putida* (pH 7.5) pumped across the membrane during 24/48 h from a thermostated culture. The assessment of microbial growth was performed by measuring the concentration of adenosine-5'-triphosphate (ATP) in used membranes. A Molecular Probes' ATP Determination was used which is based on the quantitative reaction of ATP with luciferase/luciferin system. ATP is a direct measure of active biomass and was expressed as ATP membrane/culture ratio (ATP_m/ATP_c). ATP_m was measured as μmol m^{-2} by computing the surface of membrane digested with the standard reaction solution prescribed by the manufacturer. The results show the absence of biocidal effect of sepiolite loaded membranes, for which the biofouling ratios were similar to those obtained by unloaded PLA mats. There is, however, a significant decrease of the amount of surface ATP for PLA doped with siver and copper functionalized sepiolite. The effect was particularly intense for silver-sepiolite in contact with *S. cerevisiae*, for which the reduction accounted to 85% with respect to a raw PLA control. Copper-sepiolite also reduced 20-40% the ATP load of fouled membranes. The effect was in both cases greater for *P. putida* (pH 7.5) than for *S. cerevisiae* (pH 4.5). Silver-sepiolite yielded a 35% biofouling decrease in contact with *P. putida* and a remarkable 85% for *S. cerevisiae*. We observed a significant variation in ATP results for different fragments of the same biofouled membrane. Typical differences ranged from 0.3 to 0.7 expressed as coefficient of variation (CV), which was defined as the ratio of standard deviation to the mean. This was a consequence of the flow pattern, which induced preferential biofouling of certain parts of the membrane. ATP results were calculated using the sum of all fragments from the same membrane and were not affected by this heterogeneity.

Considering the low probability of electrostatic interaction of microorganisms with the negatively charged sepiolite and metal-loaded sepiolite included in the membrane, the speciation of copper and silver as a function of pH was the sole factor determining the species reaching the solution and affecting microorganisms. It has been shown that the predominant form of copper at pH < 6.5 is Cu^{2+}, whereas at pH 7.5 aqueous $Cu(CO_3)_2$ accounts for almost all dissolved copper [7]. In the presence of chloride, it has been shown that silver chloride is the predominant complex with a minor contribution of Ag^+ and $AgCl_2^-$. The speciation does not depend on pH or the content of silver ions, Ag^+, in solution which decreases with chloride concentration. Based on these results, free Cu^{2+} ions would be the biocidal species for cultures of *S. cerevisiae*, which could partially explain the better results obtained with respect to *P. putida*. The chloride content of the culture medium could also be responsible for silver complexation reducing silver bioavailability. This effect could be responsible for the results obtained for Ag/Sep membranes where much lower biofouling was observed when using *S. cerevisae* as model organisms.

It has been shown that nanocopper or nanosilver produce toxicity in aquatic organisms, which is largely due to effects of particles as opposed to the release of dissolved ions. Griffitt et al. encountered different gene expression associated with the exposure of *Danio rerio* to nanometals and soluble metals, suggesting that each exposure produces toxicity by a different mechanism [8]. On the other hand, the cause of nanocopper toxicity has been associated with soluble Cu^{2+} in a number of studies [9]. Kasemets et al., however, attributed only 50% of the nanocopper oxide toxicity to *S. cerevisiae* to the soluble form of Cu^{2+}, the rest being associated with certain kinds of nanotoxicity [10]. Navarro et al. also showed that the toxicity of nanosilver particles was much higher than that of ionic Ag^+, so that dissolved silver could not fully explain the observed toxicity to the freshwater alga *Chlamydomonas reinhardtii* [11]. Our results cannot be explained by the internalization of nanoparticles as they remained firmly attached to sepiolite fibers. The biological effect was most probably associated with the release of soluble metal ions into the water environment and the observed differences are probably the consequence of the differences in ion availability. The direct contact between microorganisms and nanosilver or nanocopper is also hindered by the negative charge of sepiolite and functionalized sepiolite.

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