Production of functionalized particles with immobilized biocide for biofouling minimization in industrial water systems

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"Leaving her there. And just like that she was lost. Again. *Lost and Alone*."

TSOU, RC

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Abstract

Despite constant disinfection, water contamination is still a frequent topic, which raises the question of the effectiveness of the disinfection methods being used nowadays. Furthermore, even when water treatment is successful, biofilm might still prevail in the water disrupting the process and contaminating decontaminated water. Micro-nanotechnology has gained more prominence in the biofouling minimization and control; not just because it is more environmentally friendly, but because it can help increase the efficiency of the disinfection techniques already in use as well as develop new ones. The work carried out in this research project falls within the improvement of the pre-treatment of membrane filtration to prevent biofouling in their surface.

In this project four different particle cores (cationic resin, anionic resin, polypropylene (PP) and polyvinyl chloride (PVC)) were functionalized by the use of the layer-by-layer self-assembly method, using Polyethyleneimine (PEI) and poly(sodium 4-styrenesulfonate) (PSS), polyelectrolytes with opposite charges, and Benzyldimethyldodecylammonium (BDMDAC) as an antimicrobial agent. The goal was to develop functionalized particles that carry the antimicrobial agent in an environmentally friendly way, but effective nonetheless. The objective is to infer which combination of particle with immobilized biocide is the most efficient in killing *Escherichia coli* and therefore preventing biofilm formation.

Even though, four different types of cores were functionalized with different times of contact with BDMDAC, only the PP-PEI/PSS/BDMDAC-1-hour-of-contact showed antimicrobial effect, by successfully eliminating *E. coli* from the suspension. The bactericidal assays showed 40% survival ratio for the PP-PEI/PSS/BDMDAC-1 hour-of-contact.

Further studies were carried out to understand why these particles worked better than the others, such as kinetic assays, zeta potential and reutilization assays. The results showed that the PP-PEI/PSS/BDMDAC-1 hour-of-contact lost their bactericidal effect after the first use and subsequent washing steps. This might mean the particles are not stable enough for long term use or that the biocide is consumed during the process.

Finally, a packed bed reactor, containing PP-PEI/PSS/BDMAC-1-hour-of-contact particles, acting as the anitmicrobial agent, was simulated. The goal was to use the reactor

tested before the membrane treatment, in order to eradicate most of the planktonic microbial content, thus preventing biofilm formation in the membranes.

Despite the efficiency in suspension, the PP-PEI/PSS/BDMDAC-1-hour-of-contact particles were not successful in the packed bed reactor setting. Nevertheless, questions were raised such as if the particles had enough biocide or if the time of contact was not enough, since when the ratio volume of cells/volume of particles in the reactor was the same as in the suspension assays, a slight decrease in the survival ratio could be observed.

Key Words: micro-technology; water treatment; membrane filtration; biocide coated particles; benzyldimethyldodecyl ammonium chloride; antimicrobial effect; biofouling.

Resumo

Não obstante à assiduidade da descontaminação da água, a sua contaminação é um tópico frequente. Isto levanta a questão da eficiência dos métodos de desinfeção atualmente utilizados. Para além disso, mesmo quando o tratamento é eficaz, os biofilmes permanecem nas tubagens, membranas, etc, corrompendo o processo de descontaminação. Nas últimas décadas, a micro-nanotecnologia registou um aumento de notoriedade como uma possível alternativa para minimização e controlo de biofilmes. Não só porque são mais amigos do ambiente, mas também porque podem aumentar a eficiência de métodos atualmente já em utilização e, ao mesmo tempo, o desenvolvimento de novos métodos. O projeto desenvolvido está relacionado com um possível pré-tratamento para a filtração de membranas, a fim de prevenir a formação de biofilmes nas mesmas.

Neste projeto, a partir da técnica camada-por-camada (do inglês "layer-by-layer"), quarto núcleos (resina catiónica, resina aniónica, polipropileno e policloreto de vinila) foram funcionalizados utilizando polietilenoimina (PEI), poliestireno sulfonato de sódio (PSS) e o agente biocida benzildimetildodecil de amónia (BDMDAC). O objetivo principal consistiu no desenvolvimento de partículas funcionalizas com biocida, que o transportam para o ambiente, de uma forma amigável, mas também eficaz. A investigação visou inferir qual a combinação de núcleo com biocida imobilizado mais eficiente na morte da *Escherichia coli* em suspensão, e, consequentemente, prevenir a formação de biofilme.

Quatro núcleos foram funcionalizados com diferentes tempos de contacto com o BDMDAC, mas somente o PP-PEI/PSS/BDMDAC-1-hora-de-contacto mostrou efeito antimicrobiano relevante. Os ensaios bactericidas mostraram um decréscimo de 60% na percentagem de sobrevivência da *E. coli* em suspensão quando em contacto com as partículas PP-PEI/PSS/BDMDAC-1-hora-de-contacto.

Para se compreender a razão para as partículas PP-PEI/PSS/BDMDAC-1-hora-de-contacto funcionarem melhor que as restantes, ensaios de cinética, potencial zeta e reutilização foram feitos. Os resultados mostraram que as partículas PP-PEI/PSS/BDMDAC-1-hora-de-contacto, embora eficazes na primeira utilização, perderam o seu efeito bactericida após a utilização e lavagens. Isto pode significar que as partículas não são suficientemente estáveis para uso a longo prazo ou que o biocida é consumido na morte das células.

Por último, foi testado um protótipo de um reator de leito fixo, com as partículas PP-PEI/PSS/BDMDAC-1-hora-de-contacto no leito fixo a agir como agente antibacteriano. O propósito era utilizar o reator antes do tratamento por membranas, a fim de remover as células planctónicas e, assim, prevenir a formação de biofilmes nas membranas.

Apesar do potencial que demonstraram nos ensaios em suspensão, as partículas PP-PEI/PSS/BDMDAC-1-hora-de-contacto não foram bem-sucedidas no reator de leito fixo. No entanto, levantaram-se algumas questões tais como se as partículas possivelmente não tinham biocida suficiente imobilizado ou se o tempo de contacto entre as partículas e as células não foi suficiente, dado que quando a razão entre o volume das células e o volume das partículas foi o mesmo que nos ensaios em suspensão houve um pequeno decréscimo.

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Notation and Glossary

AOC – assimilable organic carbon

BDMDAC – benzyldimethyldodecyl ammonium chloride

BIT – 1,2-benzisothiazolinone

BBS – borate buffered solution

CMI – 5-chloro-2-methyl-3-isothiazolinone

DBDMH – 2,4-dibromo-5,5-dimethyl-donton

DBNPA – 2,2-dibromo-3-nitrilopropiomonide

EKA – eletrokinetic analyzer

EPS – extracellular polymeric substance

FDG – fluid dynamic Gaugin

HPLC – high performance liquid chromatography

LbL – layer-by-layer

MF – microfiltration

MI – 2-methyl-3-isothiazolinone

NF – nanofiltration

NZVI – nanoscale zerovalent iron

PDA – polydopamine

PEI – polyethyleneimine

PHMD – polymeric biguanidas

PP – polypropylene

PS – poly-(styrene)

PSf – polysulfone

PSS – sodium polystyrene sulfonate

PVC – polyvinyl chloride

QAC – quaternary ammonium compounds

RO – reverse osmoses

TiO_2 – titanium dioxide

UF – ultrafiltration

UV – ultra-violet

VCM – vinyl chloride monomer

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Chapter I – Work Outline

1.1 Background and Research Presentation

Water is an essential resource for life on earth. In particular, for animals and humans water quality is of major importance (Ali & Gupta 2006; Rozin et al. 2015). Yet, water contamination is a constant topic of investigation because waterborne diseases keep increasing in rate of occurrence and water is becoming a scarce resource (Shannon et al. 2008; Gupta et al. 2012). The search to find new and affordable ways to manage this resource keeps growing and expanding (Qu et al. 2013; Rai et al. 2009; Pendergast & Hoek 2011).

Industrial water systems have three major problems involved with the decrease in the efficiency of the process and increase in maintenance costs: corrosion, abiotic deposits and biofouling (results from the adhesion of microbial cells to the surfaces) (Demadis et al. 2007). One of the main points for biofouling in water systems are the membranes used during disinfection techniques (Nguyen et al. 2012). Preventing biofouling formation on the membranes is the problem this project will focus on. Although biofouling layers in real situations include abiotic material such as clay or silt particles and corrosion products, the present work is focused only on the microbial film component.

It is quite hard to control biofouling, since even when the vast majority of microorganisms are eradicated some might still remain in the systems, which is enough to potentiate the regrowth in a very short period of time (Demadis et al. 2007; Nguyen et al. 2012).

Many of the disinfection methods for microorganisms are based either on physical or chemicals techniques; the chemical techniques apply biocides, some of these biocides are effective but rather problematic because they react with other water components and create toxic by-products (Ferreira et al. 2010; Li et al. 2008; Rai et al. 2009), such as organochlorinated compounds. In order to avoid the decontrolled spread of the biocide in the water systems, researchers have been trying to implement micro-nanotechnology in water treatment, whilst being environmentally friendly (Li et al. 2008; Ferreira et al. 2013).

This project comes as a continuation of previous studies performed by researchers of the Biofilm Group of LEPABE. In this context, the development of functionalized particles carrying immobilized biocide in its surface is here presented, along with its possible application in a packed bed reactor to be a pre-treatment of membrane filtration.

1.2 Objectives

The main objective of this research project was to find an effective combination between different particle cores and immobilized biocide, to eliminate *Escherichia coli* and prevent biofilm formation in membrane filtration systems. Furthermore, this methodology seeks to reduce the free chemicals added to the water in its decontamination process.

The project also aimed at testing whether these functionalized particles were efficient in a fixed bed reactor, in order to study its applicability as pre-treatment to membrane filtration systems.

1.3 Thesis Organization

The present thesis is divided in six chapters.

The first is a work outline, which presents the objectives of the research project and its context and inspiration.

Chapter II presents the literature review with an overall view about biofilms in water systems and its physicochemical methods of disinfection, as well as new micro-nanotechnology approaches studied at the moment. Additionally, it also aims at describing the techniques in play to minimize biofouling in membrane filtration systems.

Chapter III describes the particles coating process and the layer-by-layer technique used. Moreover, the core particles and the antimicrobial agent are described in detail in this chapter.

Chapter IV comprises the trials to evaluate the particles efficiency against planktonic *E. coli*. Three different times of contact between the biocide and the core particles were tested in the cell suspension to infer which combination is the most efficient. Further assays, such as potential zeta, kinetic assays and assessment of bacteria membrane integrity were also carried out in this chapter to understand the functionalized particles.

Chapter V presents the packed bed reactor containing the most effective functionalized particles previously tested, in order to test a potential pretreatment for membrane filtration system. Two different set ups for the particles inside the reactor were used, inside a cage and inside a tissue netting; as well as three different conditions in the reactor: caged particles in batch mode; caged particles in continuous mode; net-trapped particles in batch mode, which allowed higher ratio particles/cells than the two previous steps.

Finally, chapter VI has the main conclusions of the research project and suggestions for future work based on the main issues raised during the work described in this thesis.

Chapter II – Literature Review

2.1 Biofilms in water systems and their problems

With the increase of worldwide population, water has become an even more important resource. However while the access to clean drinking water will affect poor and developing countries, the access to good sanitation will also affect developed countries (Pendergast & Hoek 2011). This is reflected by the presence of microorganisms in the water systems, because even when a biocide is applied their biofilm might persists (Melo & Bott 1997).

Biofilms comprise more than one type and species of microorganisms and this diversity gives them a higher protection and a higher tolerance towards disinfection and outside influences (Melo & Bott 1997; Nguyen et al. 2012). Thus, making microorganisms in suspension less resistant, when compared with their biofilm form (Melo & Bott 1997).

As is known, the microorganisms do not usually adhere directly to the surface, they adhere to the molecules present on the surface (Melo & Bott 1997). Therefore, the charge and the quantity of microorganisms and molecules are extremely important for the biofilm formation; they are crucial to determine whether the microorganisms and the molecules are attracted to each other or not (Melo & Bott 1997; Costerton et al. 1994).

Biofilm formation follows a development curve, presented by Melo & Bott (1997), and it is divided in eight crucial steps. The first is the formation of the conditioning layer; this consists in the mass transfer and adhesion of the suspended particles and organic molecules to the surface (Melo & Bott 1997; Al-Ahmad et al. 2000). The second and third steps are the microorganisms transport and, subsequently, adhesion to the conditioning film; these steps are influenced by the electrokinetic and hydrophobic interactions between the microorganisms and the molecules present in the conditioning layer (Melo & Bott 1997; Costerton et al. 1994). The fourth and fifth steps are related to the development curve. At this point some cells might detach, while others form tighter bonds with the molecules and matter present in the layer (Melo & Bott 1997; Ferreira, et al. 2010). The sixth step is the transfer of more nutrients and suspended particles to the biofilm, which leads to the seventh step, where the cells will multiply and grow, causing the biofilm to thicken with the extracellular polymeric substance (EPS) and the irreversible adhesion to occur (Melo & Bott 1997; Ferreira, et al. 2010; Branda et al. 2005). The eighth and final step happens once the biofilm has acquired a significant

thickness, after which a detachment process might occur (Melo & Bott 1997; Al-Ahmad et al. 2000).

Despite the generalized step-by-step formation, biofilm's morphology is quite different, since there are a number of external factors interfering, such as the microorganisms, the bulk composition, the type of surface, the temperature, pH, nutrients availability, flow rate and EPS (Nguyen et al. 2012). The nutrient availability is a rather important factor, because of its influence in the thickness of the biofilm and, consequently, on the diffusion of nutrients (Melo & Bott 1997). When a high concentration of nutrients is present, the biofilm tends to be less dense, which will allow an easier diffusion of the nutrients towards the microorganisms (Melo & Bott 1997). The types of particles/molecules that adhere to the surface are also relevant, since as mentioned above, they are the substratum in which the microorganisms will adhere. When the particles are inorganic there can be a number of influences; for example if the particles are inhibitors of other toxic substances, they can attach to the surface, allowing the microorganisms to grow without interference (Melo & Bott 1997; Nguyen et al. 2012).

2.2 Minimization techniques of biofouling in industrial water systems

Over the past decades, innumerous efforts have been made to develop techniques, that despite not totally eliminating biofouling, helped at least to control it (Pereira et al. 2009). Most techniques were designed with industrial settings in mind, however were first tested in smaller scales, and efforts are being made for the transition to larger scales to be made successfully (Storey et al. 2011). In order to investigate the best technique to prevent or control biofilms, most studies start by trying understand how the biofilm develops in a specific environment: the flow velocity, the temperature, the nutrient and the microorganisms present in the system (Storey et al. 2011). Nevertheless, in industrial water systems, three main types of techniques prevail, with new and enticing technologies entering in the mix in the past decades. These will be discussed in this section of the review.

2.2.1 Pre-treatments

Adsorption relies on the increase of the concentration of a contaminants at the surface to removed contaminants from water (Gupta et al. 2012). Powdered activated carbon (PAC)

is one of the most well-known adsorbents for water treatment, since it removes contaminants and reduces fouling in water systems (Gao et al. 2011; Ali 2012). In industrial water treatment, adsorption works because there are columns filled with the adsorbents, which will remove the pollutants (Gupta et al. 2012).

Coagulation is another pre-treatment method used in water and waste water treatment, responsible for removing colloidal, soluble organic materials and even some pathogens (Matilainen et al. 2010). This treatment reduces organic matter, minimizing biofouling formation, by destabilizing the sedimentation process (Volk et al. 2000; Gao et al. 2011). It has the disadvantage that sometimes clarification with adsorbents is needed (Volk et al. 2000), yet because of its low cost and easy operation it is widely used (Gao et al. 2011).

Flotation removes suspended solids, biological solids, greases, among other things, because these contaminants adhere to gas or air to form agglomerate that accumulate at the water surface (Gupta et al. 2012).

2.2.2 Physicochemical Techniques

Physicohemical methods are an incredibly vast and diverse mode of water disinfection and the choice depends on many factors, especially the microorganisms in question (Ferreira et al. 2010; Gogate 2007).

The EU Biocidal Products Directive 98/8/EC defines biocides as '*active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means'*. In short, biocides are chemical compounds with antimicrobial activity that will eradicate the microorganisms or prevent their growth in the water (Ferreira et al. 2011). However, the oxidant disinfectants are manly effective when the microorganisms are in suspension, as their penetration in the biofilms is limited, and therefore may not destroy the cells within the EPS effectively (Carpentier & Cerf 1993). Some examples of biocides are presented in the Table 1 as well as described below.

Chlorine (either in the gas form or as hypochlorite) is the most used and common oxidizing biocide used in water treatment in potable water, recreational waters and even cooling waters, for its cheap price and level of effectiveness (Melo & Bott 1997; Nguyen et al. 2012; WHO 2000; Kim et al. 2002). However it creates toxic by-products and stays

in the environment, which can be deleterious to animal and plant life (Nguyen et al. 2012; WHO 2000). Since it forms toxic by-products lower dosages are used, sometimes together with other less harmful disinfectants (Kim et al. 2002). Despite its common use, chlorine is more effective in waters with a pH below 7.6 (Kim et al. 2002). It can be found in water in two different forms, HOCl and OCl^-, and even though both are called free chlorine, HOCl is more biocidal than OCl^- (Kim et al. 2002).

Halogen releasing organics are organic compounds that release halogens when in water, which are the active agents in the disinfection (Kim et al. 2002). 2,4-dibromo-5,5-dimethylhy-dantoin (DBDMH) was shown to have the same efficiency as chlorine or bromine in cooling water disinfection (McCoy W.F. & Wireman J.W. 1989).

Quaternary ammonium compounds (QAC) have a broad spectrum of action, their antimicrobial activity is closely connected with their long chain with an alkyl group (Ferreira et al. 2011). These cationic surfactants can act as both a bactericidal or bacteriostatic; QACs affect the cell membranes permeability with their long alkyl chains (Wessels & Ingmer 2013). When the QACs come into contact with the cell membrane, they disrupt its charge, leading to the cell lyses after the degradation of the proteins (Wessels & Ingmer 2013).

2,2-dibromo-3-nitrilopropionamide (DBNPA) is a non-oxidant biocide that acts as an oxidant biocide, compatible with most water systems, and it has been increasingly more applied in water treatment plants, especially in recirculating cooling systems (Endall et al. 1996; Bertheas et al. 2015; Ullah 2011; Enkrott 2015). It is used in a discontinuous way, and eradicates both aerobic and anaerobic bacteria (Bertheas et al. 2015). When compared with other biocides, it is not only rather quick in its action, but it also has a fast degradation rate, which makes it less dangerous than most (Endall et al. 1996; Bertheas et al. 2015). DBNPA's mode of action is related with the thiol amino acids oxidation, preventing the disulfide species from forming, which will lead to the corruption of the membrane components (Ullah 2011). Consequently, metabolites transport will be compromised, and the cell's survival rate is diminished as its essential functions do not happen (Ullah 2011).

Isothiazolones are used as preservatives in personal care products, due to their antimicrobial characteristics, but they are also used as biocides in cooling water circuits and paper industries (Rafoth et al. 2007). 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-methyl-4-isothiazolin-3-one (MIT) are the most frequently isothiazolone used in a

ratio of 3:1, nevertheless 2-methyl-3-isothiazolone (MI) or 5-chloro-2-methyl-3-Isothiazolone (CMI) and 1,2-BenzIsothiazolone (BIT) are also well known isothiazolinones (Rafoth et al. 2007; Williams 2007). Isothiazolone acts in two different stages, the first is the growth inhibition and metabolism (disruption of metabolic pathways with dehydrogenase enzymes involved), followed by cell lyses within hours (because of protein thiols being destroyed and formation of toxic radicals) (Williams 2007; Rafoth et al. 2007). As with most biocides, when found in water can have some environmental risks that need to be taken into consideration, especially because it is used in high doses (Kim et al. 2002; Rafoth et al. 2007). It has been reported that BIT is less dangerous to the aquatic ecosystem than the CMI/MI mixture (Madsen et al. 2001).

Polymeric biguanides (PHMB) were recognized as superior antibacterial when compared with other cationic biocides, nevertheless, because it is so hard to be defined chemically, it is not as frequently used (Gilbert & Moore 2005). Despite not being well-known, PHMBs have a wide spectrum of applications, especially in swimming pools sanitizers (Gilbert & Moore 2005). PHMB mode of action is not different from the QACs', it binds itself to the membrane and its lipopolysaccharide and peptidoglycan components, leading to their loss of function and, therefore, loss of transport, biosynthetic and catabolic proprieties, leading to the cell lyses and death (Gilbert & Moore 2005).

Glutaraldehyde is used as both a disinfectant and a sterilizer, in particular for low temperature disinfection and sterilization; it has a wide spectrum of action against microbial activity from bacteria and fungi to viruses (McDonnell & Russell 1999; Leung 2001). Its mode of action involves cross-linking with proteins and macromolecules of the outer-membrane, leading to the corruption of the cell membrane (McDonnell & Russell 1999). Environmentally it has many risks, when not in high temperatures and low pH (stable conditions) (Leung 2001). It can be harmful to aquatic and human life, yet when it is at concentrations lower than 5 mg/L, it degrades at a relatively fast rate (Leung 2001).

Ozone seemed to be promising in disinfecting water from all types of microorganisms, yet its reactivity proved to also be a downside, since it produces assimilable organic carbon (AOC) which will promote bacteria growth (Hammes et al. 2007). Furthermore, it also needs to be produced on site because of how unstable it is (Richardson 2003). It is still used with other techniques to increase their efficiency and lower their environmental risks (WHO 2000).

Ultra-violet (UV) irradiation is a technique used in the small wastewater treatment plants and neutralizes or kills bacteria and viruses by producing hydroxyl radicals (Parrota & Bekdash 1998; Lehtola et al. 2003). This treatment has many benefits, such as not having to add chemicals to the water, being simple and not needing a lot of space, nevertheless has a high price and is a non-optimized process (Smith 2002). Moreover, both the ozone and UV are point-of-contact treatments, meaning that even though they are effective where they are being applied, the rest of the system is unprotected.

Mechanical methods to minimize biofouling in water systems are probably the more diverse, and are divided in three main techniques: physical removal of biofilm from surface both on and offline; circulation of rubber sponge balls through the system in the water; and surface alteration with antimicrobial polymers (Melo & Bott 1997; Li et al. 2008). These techniques are extremely efficient in removing EPS from the surface (Ferreira et al. 2010). Yet, their application is still depending on the ratio between need and cost (Ferreira et al. 2010).

Membrane filtration is another physical technology used to remove unwanted compounds from the water. The pressure driven membranes have different classification based on their removal goal: microfiltration (MF) is commercially available to remove suspended bacteria and protozoa, while ultrafiltration (UF) is for virus and colloid removal, nanofiltration (NF) filters heavy metal and dissolved organic matter and reverse osmosis (RO) allows for desalination, water reuse and ultrapure water production (Malaeb & Ayoub 2011; Guo et al. 2010; Huang & Schwab 2009; Gao et al. 2011; Pendergast & Hoek 2011). Further discussion on membrane filtration is presented in the section 2.3.

The micro-technology discussed in the present work aims to be a pre-treatment before the membranes to remove bacteria that tends to form biofilms on the membrane.

Table 1 - Chemical disinfectants commonly used in water treatment; their mode of action, typical dosage (mg/L), contact time, behavior in environment and legislation.

Disinfectants	Mode of action	Typical Dosage (mg/L)	Contact time	Environment behavior	Legislation (EC)	Reference
Chlorine	Oxidizing agent, used in excess. Used in gaseous or hypochlorite form.	0.5 – 1	minutes to hours	Dissipates in side reactions rather quickly; forms toxic by-products with nearly all compounds present in water.	231-959-5	(WHO 2000; Enkrott 2015; Kim et al. 2002)
Chlorine dioxide	Oxidizing agent, soluble in water and can decompose to chlorite.	0.5 – 2	minutes to hours	Creates less harmful by-products than chlorine.	N.A.[1]	(WHO 2000; Kim et al. 2002)
Chloramines	Oxidizing agent with high CT value[2]. Poor primary disinfectant, but used as a secondary disinfectant for maintenance.	1 -50	hours to days	When used with other disinfectant methods can decrease by-products formation.	204-847-9	(WHO 2000; Kim et al. 2002; WHO 2004)
Bromine	Oxidizing agent.	0.1 – 10	minutes to hours	When in swimming pools can cause bad dermatologic reactions.	N.A.	(Kim et al. 2002; Rycroft & Penny 1983)
Hydrogen peroxide and potassium permanganate	Oxidizing agent. Poor primary disinfectant compared with chlorine	1 – 10	minutes to hours	Creates toxic by-products.	231-765-0	(Kim et al. 2002)

[1] N.A. – Not Allocated
[2] CT value is the product of the disinfectant concentration in mg/L and the contact time in minutes required to inactivate 99% of the microorganisms

Quaternary ammonium compounds	Cationic surfactants with long alkyl chains, which affect membrane permeability.	10 - 500	hours to days	Not environmentally dangerous at the used concentrations. Deactivated with anionic surfactants.	273-545-7	(Ferreira et al. 2011; Wessels & Ingmer 2013)
Isothiazolone	Inhibits cellular metabolism and leads to cell lyse.	1 - 100	hours to days	Involves high doses, which can lead to environmental risk.	420-590-7	(Rafoth et al. 2007; Kim et al. 2002; Williams 2007)
2,2-dibromo-3-nitrilopropionamide	Non-oxidizing agent. prevents the disulfide species from forming, leading lead to the corruption of the membrane	1 - 100	hours to days	Degrades in water by two pathways, both take a matter of hours and occur naturally in the presence of natural organic compounds. The by-products can further degrade in organic acids but slower.	233-539-7	(Kim et al. 2002)
Gluteraldehyde	Cross-linking of proteins and macromolecules of membrane, leading to its corruption	10 - 500	hours to days	Acutely toxic for aquatic life. But its degradation is quick if below 5 mg/L.	N.A.	(Kim et al. 2002; McDonnell & Russell 1999; Leung 2001)
Polymeric biguanides	Binds itself to cell membrane and its components, leading to less mobility and lyse.	1 - 100	hours to days	Low toxicity in the environment.	Polymer	(Kim et al. 2002; Gilbert & Moore 2005)

2.2.3 Micro-Nanotechnology Approaches

The minimization techniques discussed in the section 2.2.2 are efficient in eliminating pathogens, however most techniques involve chemicals that can react with other water components and create undesirable by-products (Krasner et al. 2006; Li et al. 2008). These by-products are a problem for public health, since some might be carcinogenic (Krasner et al. 2006).

Therefore the design and development of new and alternative approaches to that treatment have gained relevance in the past decade (Li et al. 2008; Ferreira et al. 2010; Nir & Reches 2016). Micro-nanotechnology is an area rising in interest for biofouling control, and the particles are not just being thought of as coatings, but also as cores for coated particles (Li et al. 2008; Ferreira et al. 2010; Nir & Reches 2016).

One approach is the use of surface materials which are naturally antifouling, since they are based on natural systems, and are, often, more resistant towards biofouling (Nir & Reches 2016).

Nanoparticles can be used to control biofilm in water systems, especially in decentralized or in specific points of use along the water systems (Dankovich & Gray 2011). These particles show greater efficiency and robustness in a smaller scale, which might be beneficial in places where the water runs slowly (Li et al. 2008). Nanomaterials have certain characteristics that make them good options for surface coatings. Since nanomaterials have a big specific area and high reactivity they are good adsorbents, catalysts, and sensors (Li et al. 2008) and their mode of action is different from the common oxidants. Therefore, the problem of undesirable by-product formation is also solved, because they are non-oxidant in water (Li et al. 2008).

Micro-nanotechnology truly has potential to be the next-generation of treatments for water systems (Qu et al. 2013). In fact, many are not necessarily new treatments, just better and improvements of treatments already in use (Qu et al. 2013).

Coating Particles

One of the first attempts to use antifouling materials was the coating, of already used materials, with chemically active compounds (Nir & Reches 2016).

Sometimes, these coatings can be biocides, nanoparticles or different antibiotics, which will be released or come into contact with the cells, eradicating them or preventing their settlement and growth on the surface (Nir & Reches 2016). When nanoparticles are

applied as antimicrobial particles or antimicrobial coatings they can interact with microorganisms in the following ways: interrupt electrons transport through the membrane; corrupt the membrane; oxidate the cell's components; or produce of secondary products which cause damage, as seen in Figure 1 (Li et al. 2008).

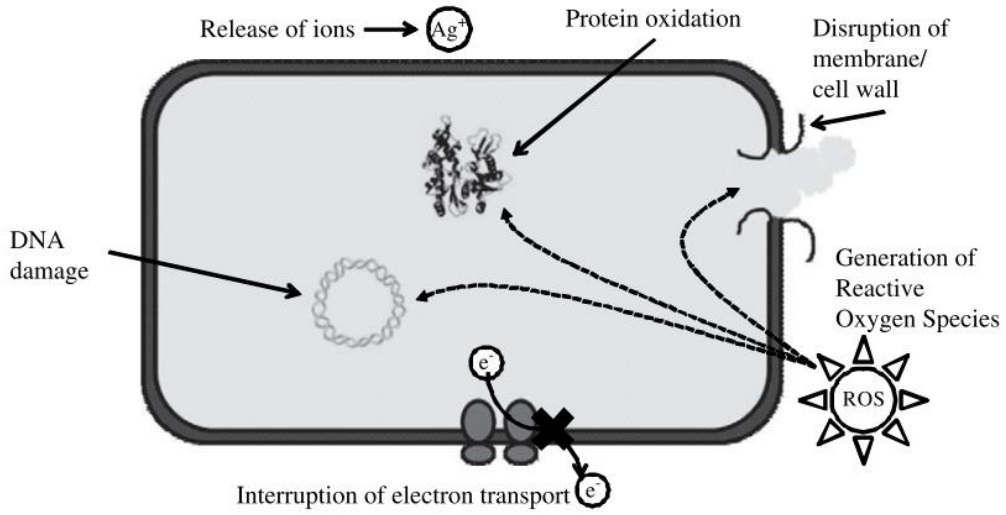

Figure 1 - Nanomaterials' various antimicrobial mechanisms (Li et al. 2008)

Silver nanoparticles are the most famous nanoparticles, since they have been widely used, despite their price (Silvestry-Rodriguez et al. 2008). Even though they have been reported to be good at inactivating bacteria when in suspension, silver nanoparticles are not very good at destroying already matured biofilms, therefore they are used as a biofilm retardant and not a disinfectant (Dror-Ehre et al. 2010). Gogoi et al. (2006) showed that silver nanoparticles are more toxic for *E. coli* when their size is smaller than 10 nm. The silver nanoparticles share most of the same antimicrobials characteristics as the other nanomaterials, but they have the particularity of helping the UV inactivation of the microorganisms when in ion form (Li et al. 2008; Kim et al. 2008).

Titanium dioxide (TiO_2) nanoparticles are rarely used by themselves, usually they involve UV radiation or silver nanoparticles, making them much more toxic for bacteria (Li et al. 2008; Page et al. 2006; Du et al. 2016). They have several advantages, like being non-toxic for animals and humans, not needing electricity - only solar radiation - or control and being cheap, making it a good option for developing countries in remote places (Li et al. 2008; Chong et al. 2010). Nevertheless, they also have disadvantages, such as their aggregation rate when added to the water as just particles (Li et al. 2008).

Chitosan has been relatively known for many decades as an antimicrobial, yet only in the past decade has it been used for nanoparticles applications (Ye et al. 2006; Qi et al. 2004; Tayel et al. 2016). It eradicates a wide range of microorganisms, yet it is more efficient for viruses and fungus (Rabea et al. 2003; Tayel et al. 2016). Qi et al. (2004) stated that chitosan causes an increase in cell membrane permeability, leading to its disruption, this having been confirmed by Tayel et al. (2016) in their study. Rabea et al. (2003), on the other hand, stated that chitosan causes enzyme inhibition in bacteria and inhibit RNA synthesis by binding to the DNA in fungi. Unlike the silver nanoparticles, it is relatively cheap, which might make it a good option for developing countries, similarly to titanium dioxide (Li et al. 2008). Tayel et al. (2016) suggested chitosan be applied as a filter in water systems to remove microbial and heavy metals contamination.

Mcguffie et al. (2015) showed that zinc oxide nanoparticles were capable of preventing bacteria growth. Besides being more selective in terms of which bacteria they react with, the zinc oxide nanoparticles prepared in this study had a positive zeta potential, which gave them an innate capacity to interact with the negative membrane of the bacteria (Mcguffie et al. 2015). Unlike the usual consensus that the smaller the particles, the more effective they are, Mcguffie et al. (2015) stated that the pyramid particles, bigger than the others, showed an efficiency equal and sometimes better than the smaller ones. When applied to biofilms, zinc oxide nanoparticles were good at reducing gram-positive bacteria biofilm but not gram-negative bacteria biofilm (Mcguffie et al. 2015).

Nanoscale zerovalent iron (NZVI) was tested as an antimicrobial against *Escherichia coli* by Li et al. (2010). NZVI has been tested for water treatment, to remove heavy metals and organic solvent contaminants for nearly two decades (Crane & Scott 2012; Li et al. 2010), yet only recently has it been thought as a good option as a bactericidal (Li et al. 2010). NZVI surface owns strong reducing proprieties, which leads to the disruption of protein functional groups in the cell membrane; additionally, they can also cause damage by an oxidative stress induced by oxygenation of reduced Fe species, caused by Fenton's chemistry (Auffan et al. 2008; Lee et al. 2008).

Particles as cores

Another option presented in the last decade is particles with immobilized biocide, these particles are reactive and because their ratio between area and volume is high, they are capable of carrying a high dose of biocide (Ferreira et al. 2013). Ferreira et al. (2010)

used poly-(styrene) (PS) core particles treated with polyethyleneimine (PEI) and sodium polystyrene sulfonate (PSS) carrying Benzyldimethyldodecylammonium chloride (BDMDAC) to kill *Pseudomonas fluorescens*' planktonic cells and biofilm and were successful. Ferreira et al. (2013) compared two different core particles, PS and calcium carbonate ($CaCO_3$), prepared with the same layer-by-layer technique and biocide. The conclusion was that $CaCO_3$ particles were better and did not disintegrate or aggregate, but both were able to idle at least 90% of the viable cells and biofilm formed by *P. fluorescens* (Ferreira et al. 2013).

2.3 Membrane Filtration biofouling minimization

Worldwide there seems to be a lack of clean, fresh water; with the increase of the world population this problem is only going to aggravate itself (Shannon et al. 2008; Gupta et al. 2012). A proposed solution is the reutilization of water from non-traditional sources, however before that same water can be used again it needs to be rid of contaminants, pathogens and organic matter (Shannon et al. 2008; Ali & Gupta 2006).

Membrane filtration is a technology used to help in this process. UF, MF, NF and RO membranes are an important pressure-driven step in water treatment, yet they are also significantly affected by of fouling. (Nguyen et al. 2012; Huang & Schwab 2009; Malaeb & Ayoub 2011).

Membranes are prone to fouling when feed water passes through, by losing permeability with the accumulation of impurities (Gao et al. 2011). There are three major types of fouling happening in the membranes, based on their foulants (Gao et al. 2011; Malaeb & Ayoub 2011):

- Organic fouling, coming from the natural organic matter and dissolved organic matter that after a certain concentration, adsorbs to the surface leading to a cake layer deposition;
- Colloidal and particle fouling happens when larger particles accumulate on the membrane surface or smaller one within the pores, leading to a cake formation and, consequently, decreasing the membrane permeability;
- Biofouling comes from the microbial attachment to the membrane surfaces, leading to the EPS and biofilm formation.

Biofouling contributes to more than 45% of the fouling in the membrane, making it one of the most important problems to solve (Komlenic 2010). As mentioned previously, during biofilm formation there is also accumulation of EPS (Nguyen et al. 2012). EPS is an important factor in membrane biofouling, because EPS forms a gel layer when cross-linking with the membrane material, this gel increases in strength with time and accumulates into the membrane pores (Nguyen et al. 2012; Meng et al. 2009). EPS can also interfere with membrane cleaning, since it retards the flow and the penetration of the antimicrobial agents to the biofilm (Meng et al. 2009; Nguyen et al. 2012).

This biofouling can cause significant operational problems on the membrane systems such as decrease of membrane permeability, increase of differential and feed pressure, salt passage and energy consumption and membrane degradation (Nguyen et al. 2012; Vrouwenvelder & van der Kooij 2003).

MF is usually used in membrane bioreactors to replace gravity sedimentation and clarify the water (Shannon et al. 2008). It removes particles up to 1 μm and the filters are usually of polypropylene, fluorated hydrocarbon polymers, fiberglass cellulose among others (Gupta et al. 2012).

UF is considered a low-pressured process, ideal for drinking water treatment, because of its small pores, easy mechanisation and high removal of turbidity and microbial contaminants (Gao et al. 2011).

NF is a low pressure technology that allows the control and removal of organic, inorganic and microbial contaminants, with the membranes made with polymeric films (Hong & Elimelech 1997). NF has characteristics of both UF and RO, depending on its cut off, Table 2, making it versatile for water treatment (Hong & Elimelech 1997).

RO membranes are semi-permeable membranes which based on size, charge and physical-chemical interactions reject dissolved elements of the feed water (Malaeb & Ayoub 2011). In reverse osmoses membranes, it exists a spacer in order to create turbulence and to allow the water to flow, yet some areas in which the flow is not so strong also are created and these promote biofouling formation (Nguyen et al. 2012; Malaeb & Ayoub 2011). RO membranes are an important biofouling case, since their cleaning is rather expensive (Bertheas et al. 2015). It is thought to have a future in a large scale application in desalination of sea water, because it can remove microbial content, organic matter and dissolved solids up to 99% (Gupta et al. 2012). It is highly used for

ultra-pure water used in pharmacy and medicine for its cost and efficiency (Gupta et al. 2012).

Table 2 - Molecular weight cut-off for microfiltration, ultrafiltration, nanofiltration and reverse osmosis (The Dow Chemical Company 2014)

Membrane	Microfiltration	Ultrafiltration	Nanofiltration	Reverse Osmosis
Molecular weight cut-off (MWCO) (kDa)	≥ 100	$1 \geq MWCO \leq 300$	$0.1 \geq MWCO \leq 10$	≤ 1

There is an overall consensus that membrane filtration has a lot of potential, nevertheless, the biofilms are a concern to the operation of such systems (Nguyen et al. 2012; Gao et al. 2011). Thus, membranes with improved productivity, selectivity, fouling resistance, while maintaining cost are required, which also required new and improved technology (Pendergast & Hoek 2011). Additionally, water pretreatment upstream the membranes is also a crucial way to effectively remove some chemical and microbial contaminants, modifying the feed water, and improving the performance of the membranes (Huang & Schwab 2009).

The project here presented merges both thoughts into one by producing functionalized particles with BDMDAC, a QAC, which would be implemented in a reactor to precede membrane filtration to prevent biofilm formation in the membrane surface by eradicating the microbial cells whilst in their planktonic form.

2.3.1 Pre-treatments

Membrane biofouling is the biggest limitation to the use of membrane filtration, leading to the pre-treatment necessity to prolong membrane life and prevent microbial and organic agglomeration on their surfaces (Malaeb & Ayoub 2011; Gao et al. 2011).

Coagulation is frequently used in water treatment, it changes the particle characteristics, which in membranes can improve permeation rates and its quality (Volk et al. 2000; Matilainen et al. 2010). Aluminium and iron-based coagulants can help membrane pre-treatment by removing soluble EPS (Song et al. 2008; Gao et al. 2011). However, despite the effectiveness of the coagulation treatment, the coagulant residuals can have potential negative effects on the membranes (Gabelich et al. 2002; Bereschenko et al. 2011).

Moreover, the coagulation effectiveness is still dependant on the type of membrane, the type of feed water and the coagulant used (Gabelich et al. 2002; Bereschenko et al. 2011; Gao et al. 2011). For example, for UF, the adsorbent PAC showed to be effective and membrane friendly in water decontamination by binding to the impurities (Gao et al. 2011).

There are some oxidizing agents used as disinfectants in water treatment, however when membrane filtration is in the system they cannot come into contact because of their oxidative effect on their surface (Kim et al. 2002; Nguyen et al. 2012). Therefore, they are used in a pre-treatment lines - pipes, stagnant flow areas, among others (Saad 1992).

2.3.2 Physicochemical Treatments

The most frequent biofouling treatments are based on the use of biocides; these treatments efficiency varies depending on the biocide, the maturity of the biofilm, the membrane material and the dosages used (Gogate 2007). Moreover, biocides have a tendency to only eradicate microbial cells, and cannot reduce AOC levels, leading to the necessity of the constant application of biocides in the water systems (Nguyen et al. 2012; Hu et al. 1999).

As mentioned before, chlorine either in gas or hypochlorite form is the most used disinfectant, however it cannot be used in membrane treatment due to the membranes polymeric materials not being tolerant to this biocide (Saad 1992; Nguyen et al. 2012; Malaeb & Ayoub 2011). Chloramines, mentioned in Table 1, are usually used as a secondary disinfectant, since it is a weak oxidizing agent (WHO 2000; Kim et al. 2002; WHO 2004). In membrane treatment they can be used as an alternative for chlorine and its derivatives, as they do not form toxic by-products (WHO 2000; Kim et al. 2002; Applegate & Erkenbrecher Carl W. 1987; Malaeb & Ayoub 2011).

Another disinfectant extensively applied in water treatment is potassium permanganate, Liang et al. (2008) stated that permanganate could be used with UF membrane. Nevertheless, more studies need to be done to optimize the process and to come into contact with all the possible outcomes of the treatment (Gao et al. 2011).

Some non-oxidant biocides used are not compatible with the industrial membranes, because they damage the thin polyamide layer in them (Bertheas et al. 2015). DBNPA, however, it is, especially for the RO membranes (Bertheas et al. 2015). In the particular case of the RO systems, DBNPA can applied continuously causing an increase in the energy savings and decrease of fouling; furthermore it has the advantage of allowing the

plant to work while the biocide is being applied, decreasing the downtime of production breaks and its costs (Bertheas et al. 2015).

Minimization of biofilm formation can start with the most common cleaning techniques such as backwashing/back pulsing, pneumatic cleaning application of ultrasounds or electrical fields (Gao et al. 2011; Nguyen et al. 2012). These techniques disrupt the interaction between the microbial cells and the membrane surface, leading to the detachment of some microbial matter (Gao et al. 2011; Nguyen et al. 2012). To insure treatment was fully successful, some apply chemical disinfectants, but in smaller doses (Nguyen et al. 2012).

Ozone is a strong oxidant, making it an effective treatment for many kind of microorganisms, such as bacteria, protozoa, viruses, among others (Hammes et al. 2007). It is used frequently along with membrane filtration to increase their performance and help prevent microbial attachment (Schlichter et al. 2004). Nevertheless, when with UF membranes, ozone has critical side effects with the formation of bromate, which are not only toxic for the environment but are also negative to the membrane permeability (Gao et al. 2011).

UV irradiation has been used for water treatment, because not only does it not create harmful by-products, it is not a chemical and can act in different microorganisms like ozone, as described in the 2.2.2 section (Parrota & Bekdash 1998; Lehtola et al. 2003). Despite, UV irradiation being also used to optimize TiO_2 treatments (Li et al. 2008), it cannot be used to control biofouling within membrane modules (Nguyen et al. 2012); hence the need for a combination of techniques (Li et al. 2008).

Even though, coagulation, adsorption and oxidation have proved to be effective pre-treatments for low-pressure membranes, novel technologies are being developed to try and control biofilm (Huang & Schwab 2009). Some will be discussed in the next section of this review.

2.3.3 Micro-Nanotechnology Approaches

Water treatment relies heavily on chemicals to prevent biofilm formation, however membranes are relatively sensitive to most biocides (Nguyen et al. 2012). As said in 2.2.3, micro-nanotechnology raised in prominence because it is a reliable alternative approach

to the traditional treatments (Dankovich & Gray 2011; Li et al. 2008; Pendergast & Hoek 2011).

For minimization of biofilm formation in membrane systems, the most typical area where micro-nanotechnology helps is the modification of the membrane surfaces (Nguyen et al. 2012; Pendergast & Hoek 2011). When coating the membranes, one must make sure the membrane characteristics, except roughness, surface charge and hydrophobicity, do not change to guarantee the improvement of the membrane resistance towards biofilm formation (Nguyen et al. 2012).

Silver nanoparticles have previously demonstrated antimicrobial activity (Li et al. 2008). Some studies have showed its application coating membranes to minimize biofilm formation, however, these particles seem to be rather incompatible with polymeric membranes and after a while end up leached out (Li et al. 2008; Kim & Van Der Bruggen 2010; Huang et al. 2016). Thus, recently, studies have been trying to find a way to improve membranes proprieties in order for the silver nanoparticles to not leach out or interfere with the permeability (Huang et al. 2016; Andrade et al. 2015). Andrade et al. (2015) investigated how the incorporation of the silver nanoparticles into the polysulfone (PSf) membrane affected its antimicrobial effect. They tested an *ex situ* technique in which they incorporated already synthetized silver nanoparticles in the polymer solution, and another technique where the silver nanoparticles were synthetized *in situ* (Andrade et al. 2015). They found that the way silver nanoparticles are incorporated into the membrane can make a difference, since the *in situ* method had the nanoparticles more uniformly distributed (pores, top and bottom surfaces), while the *ex situ* only showed nanoparticles within the pores (Andrade et al. 2015). Huang et al. (2016) also studied the immobilization of silver nanoparticles on PSf UF membranes but via polydopamine (PDA). Their premise was that PDA is a "bio-inspired" polymer with adhesive characteristics, which would allow the silver nanoparticles to adhere to the PDA layer more efficiently, without interfering with the membranes pores (Huang et al. 2016). Their coated membrane showed an increase in the water flow, along with antibacterial and biofouling mitigating proprieties against *E. coli* and *B. subtilis* (Huang et al. 2016).

TiO_2 nanoparticles are usually used in combination with other treatment methods, like UV radiation (Li et al. 2008; Page et al. 2006; Du et al. 2016). They are already applied to enhance UV water treatment in wastewater treatment plants as a coating in membranes, since it is a highly efficient photocatalyst disinfectant (Li et al. 2008; Du et al. 2016;

Chong et al. 2010). Nonetheless, the organic matter and inorganic particles formed during the oxidation processes can also lead to membrane fouling, which can also reduce the membranes performance (Du et al. 2016; Chong et al. 2010).

Chitosan and QACs are antimicrobials additives mostly used as a coating in membranes or tanks, for its low toxicity to humans and animals (Li et al. 2008; Liu et al. 2010). Liu et al. (2010) studied how the coating the membranes with chitosan and QACs or heparin would affect the biofouling. They found *E. coli* adhered less to membranes coated with chitosan and QACs when compared to membranes just coated with chitosan, because of the difference in hydrophilicity (Liu et al. 2010). However, these membranes were not the most effective for *E. coli*, in fact, membranes coated with chitosan and heparin showed less bacteria adhered because they had a negative zeta-potential (-10 mV), repelling the *E. coli* (-25.75 mV), unlike the chitosan and QACs membranes (positive zeta potential of +12 mV) (Liu et al. 2010).

Another approach, it is the employment of naturally antifouling materials as the membranes materials or incorporation of nanomaterials into the manufacturing process, preventing from the beginning the adhesion of microbial cells to the surface (Nir & Reches 2016; Kim & Van Der Bruggen 2010). Incorporation of nanomaterials in the membranes material can be accomplished by assembling them into the pores or blending them with the polymeric film of the membrane (Kim & Van Der Bruggen 2010; Andrade et al. 2015). These seem to be the most promising, since coating membranes does not appear to have a long term efficiency (Nguyen et al. 2012; Kim & Van Der Bruggen 2010). Moreover, the membrane change can lead to pore size change and permeability change which can also decrease the membranes flow and efficiency (Mansouri et al. 2010).

Thus, despite the promise of the micro-nanotechnology approach, more research and investigation needs to be done for it to be costly-efficient for mitigating membrane biofouling (Kim & Van Der Bruggen 2010; Ayadi et al. 2016).

Chapter III – Particles Development

3.1 Introduction

Since the beginning of water treatment, physical and chemical methods have been the primary source of pathways to remove and kill microorganisms from the surfaces and from the water (Melo & Bott 1997). With the passing years chemical methods became more prominent; these methods use biocides, which have been effective but also problematic (Shannon et al. 2008; Ferreira, Pereira, et al. 2010).

Biocides have antimicrobial activity, allowing them to help control microbial content and activity in the water, nonetheless some of these biocides can create harmful by-products when they come into contact with other feed water components (Ferreira et al. 2010; Li et al. 2008). These by-products can be toxic and endanger not only human health but also aquatic life. (Li et al. 2008; Ferreira et al. 2010) This chain reaction is something that in the past decade scientists have been trying to avoid; one of the possible solutions is the application of micro-nanotechnology (Li et al. 2008; Ferreira et al. 2013).

Micro-nanotechnology has been more frequently applied in membrane systems, in order to minimize biofouling in their surface and improve its operational characteristics (Dankovich & Gray 2011; Pendergast & Hoek 2011; Nguyen et al. 2012).

In this project four different cores were functionalized by the use of the layer-by-layer self-assembly method, using two polyelectrolytes with opposite charges and a QAC as an antimicrobial agent. The goal was to develop functionalized particles that carry the antimicrobial agent in environmentally friendly way, but effective nonetheless.

3.2 Particles Cores

3.2.1 Anionic Resin

Anionic Resin has a polymeric structure, polystyrene crosslinked with divinylbeneze, functionalized with ammonium quaternary groups, this gives the particles a positive charge, Figure 2 (Cullex 2000a). The particles remove all anionic components and silica from the water, as well as complex organic materials, like fulvic and humic acids (Cullex 2000a). It is a homogenous material and the size varies from 0,60 to 0,85 mm (Cullex 2000a). A real representation of the core is presented in Figure 5.

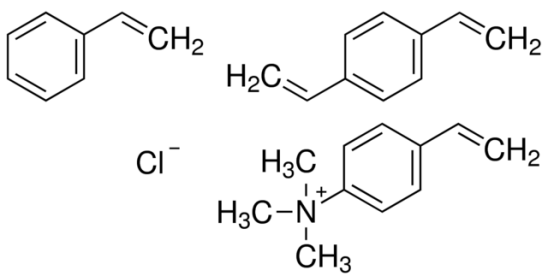

Figure 2 - Chemical structure of Anionic Resin (SIGNMA-ALDRICH 2016)

3.2.2 Cationic Resin

Cationic Resin has a polymeric structure, polystyrene cross-linked with divinylbeneze, functionalized with sulphonyl groups, giving the particles a negative charge (Cullex 2000b). The negative charge allows the particles to remove positive charged particles and suspended matter present in the water (Cullex 2000b). It is an homogenous material and the size varies from 0,65 to 0,85 mm (Cullex 2000b). Since it is already used in industrial water, it has been approved by U.S. Food and Drugs Code of Federal Regulations section 21, paragraph 173.25 (Cullex 2000b). A real representation of the core is presented in Figure 5.

3.2.3 Polyvinyl chloride

Polyvinyl chloride (PVC) is the third most produced polymer in the world (Allsopp & Vianello 2012). It is prepared from vinyl chloride monomer (VCM), Figure 3, and it is quite cheap and has multiple applications, making it widely versatile (Allsopp & Vianello 2012). Each manufacturer makes PVC varying in morphology and in molecular mass based on the use of the material, having in mind PVC is never used alone (Allsopp & Vianello 2012).

Based on the manufacturer's information this PVC is rigid and inert (Purolite 2016b), which was one of the reasons why it was chosen for this project. It has the shape of a cube with a length that ranges between 3 and 5 mm (Purolite 2016b). A real representation of the core is presented in Figure 5.

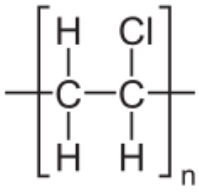

Figure 3 - Chemical structure of PVC monomer

3.2.4 Polypropylene

Polypropylene (PP) is a widely used polyolefin polymer, Figure 4, invented nearly sixty years ago (Hasegawa et al. 1998; Karger-Kocsis 1995). One of its main attractions is its ability to be moulded for the different types of applications, and since it is a relatively cheap material, many new applications have been developed (Karger-Kocsis 1995). It is used in MF membranes for water treatment (Gupta et al. 2012).

These PP particles were chosen because of their advantageous proprieties, both chemical and mechanical, but also because the manufacturer designed them for use in packed or partially packed and fluidised bed reactor, which operate in the up-flow counter-flow mode (Karger-Kocsis 1995; Purolite 2016a). These applications are important later on in the project. The particles are shaped as cylinder, with a diameter varying from 1.1 to 1.5 mm and a length varying from 0.8 to 1.6 mm (Purolite 2016a). A real representation of the core is presented in Figure 5.

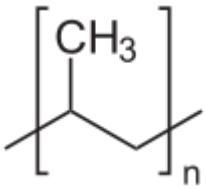

Figure 4 - Chemical structure of PP monomer

Figure 5 - Particles Cores: a) Anionic Resin; b) Cationic Resin; c) Polyvinyl chloride; d) Polypropylene

3.3 Antimicrobial Agent

Benzyldimethyldodecylammonium chloride (BDMDAC) was the antimicrobial agent used in the coating process of the cores.

BDMDAC is a QAC with a chain of twelve carbons, which gives the biocide hydrophobic characteristics (Ferreira et al. 2011). This biocide has a low toxicity and an active surface, this makes it the perfect biocide to be used in both public, household water systems and personal care products (Ferreira et al. 2011; Ferk et al. 2007). Even though it has been known for a few decades, QACs affect the cytoplasmic membrane (McDonnell & Russell 1999), and, according to Ferreira et al. (2011), BDMDAC affects bacteria membranes by making them hydrophobic and damaged, leading to the cell's death.

BDMDAC was a good biocide for this project because not only it is frequently used, but also has a positive charge necessary for the coating process of the cores, Figure 6.

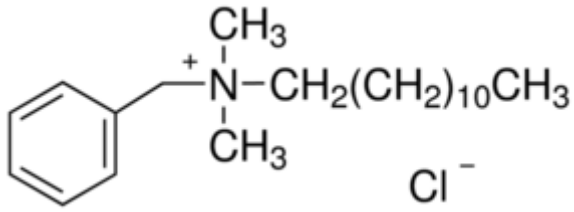

Figure 6 - BDMDAC chemical structure (SIGMA-ALDRICH 2016).

3.4 Layer-by-layer self-assembly technique

The layer-by-layer (LbL) assembly technique was first introduced in the world of science by Iler (1966), and since then many applications have been studied and developed, because it allows an inexpensive and easy manipulations of materials' thickness and characteristics (Richardson et al. 2015; Sukhorukov et al. 1998; Ariga et al. 2007).

Even though in the past two decades, many new technologies were based in the LbL technique, there is still a lot to understand concerning coating particles, where comparisons are limited to form a definitive opinion (Richardson et al. 2015).

There are a number of ways in which the polyelectrolytes come into contact with the materials, yet the main driving force for a stable assembly is the irreversible electrostatic attraction (Richardson et al. 2015; Sukhorukov et al. 1998). According to Ariga et al. (2007), the method relies on the presence of high concentration of polyelectrolytes with an opposite charge from the particle, leading to an adsorption of the polyelectrolytes, and consequentially charge reversal or neutralization, Figure 7.

However, one cannot always be sure the material is interacting with the polyelectrolyte or with the right polyelectrolyte, since it in some cases the addition of a new polyelectrolyte to the solution may cause the release of the previous one (Sukhorukov et al. 1998; Bolto & Gregory 2007).

Thus, when applying this technique a number of parameters should be taken into consideration, like the core material and the type of interactions might have with the polyelectrolytes, what type of polyelectrolytes to choose, their chains and their ionic strength at a specific pH (Ariga et al. 2007; Richardson et al. 2015). One must also take into consideration how polyelectrolytes interact with each other, to make sure they do not form agglomerates (Sukhorukov et al. 1998; Ariga et al. 2007).

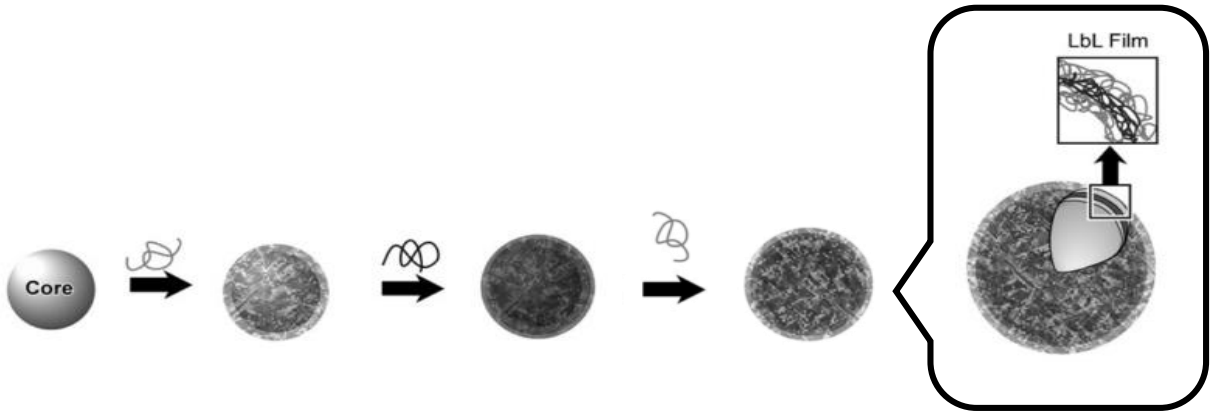

Figure 7 - Schematic representation of LbL technique. Adapted from (Ariga et al. 2007).

3.5 Coating Process

Polyethyleneimine (PEI – molecular weight 750 000 g mol^{-1}) 50% in water, poly(sodium 4-styrenesulfonate) (PSS – molecular weight 70 000 g mol^{-1}) and boric acid were purchased from Sigma-Aldrich (Portugal). BDMDAC (molecular weight 339.9 g mol^{-1}) was purchased from Fluka (Portugal).

The coating of the core particles was done using the layer-by-layer self-assembly (LBL) technique, which relies on the electrostatic attraction and the complex formation between polyanions and polycations (Ferreira et al. 2013).

The borate buffer solution (BBS) (0.1 M) was chosen throughout the whole process for its ionic strength. The pH 9 was used because it promotes the right superficial charge for the different molecules for the layer-by-layer process (Ferreira et al. 2013).

3.5.1 Strong Anionic Resin

Strong anionic resin was obtained from Culligan International.

The strong anionic resin coating process was divided in 2 steps. The first is the 20 minutes' interaction between the PSS solution (1 mg ml^{-1} in borate buffer solution) and the positively charged core. The second step is the interaction among the negatively charged particle and the BDMDAC (1 mg ml^{-1} in borate buffer solution); in the second step three different times of contact were tested (20 minutes, 60 minutes and 24 hours). After each contact period, a washing procedure with 0.1 M borate buffer, pH 9, was applied to remove excesses of the polymers and biocide (this procedure was repeated five times each). The interactions happened while the Erlenmeyer flasks were in a roller mixer (SRT6D), so that the contact between the polymers/biocide and the core was homogeneous.

3.5.2 Strong Cationic Resin

Strong cationic resin core particles were obtained from Culligan International.

The strong cationic resin coating process was divided in 3 steps. The first is the 20 minutes' interaction between the PEI solution (1 mg ml^{-1} in borate buffer solution) and the core. The second step is another 20 minutes' interaction, but between the PSS solution (1 mg ml^{-1} in borate buffer solution) and the now positively charged core. The third step is the interaction among the negatively charged core and the BDMDAC (1 mg ml^{-1} in borate buffer solution); in the third step three different times of contact were tested (20 minutes, 60 minutes and 24 hours). After each contact period, a washing procedure with 0.1 M borate buffer, pH 9, was applied to remove excesses of the polymers and biocide (this procedure was repeated five times each). The interactions happened while the Erlenmeyer flasks were in a roller mixer (SRT6D), so that the contact between the polymers/biocide and the core was homogeneous.

3.5.3 Polypropylene and Polyvinyl chloride

PP and PVC core particles were obtained from The DOW Chemical Company.

The PP and PVC coating process was divided in 3 steps. The first is the 20 minutes' interaction between the PEI solution (1 mg ml^{-1} in borate buffer solution) and the core. The second step is another 20 minutes' interaction, but between the PSS solution (1 mg ml^{-1} in borate buffer solution) and the now positively charged core. The third step is the

interaction among the negatively charged core and the BDMDAC (1 mg ml^{-1} in borate buffer solution); in the third step three different times of contact were tested (20 minutes, 60 minutes and 24 hours). After each contact period, a washing procedure with 0.1 M borate buffer, pH 9, was applied to remove excesses of the polymers and biocide (this procedure was repeated five times each). The interactions happened while the Erlenmeyer flasks were in a roller mixer (SRT6D), so that the contact between the polymers/biocide and the core was homogeneous.

Chapter IV – Particles efficiency against planktonic cells

4.1 Introduction

Chemical treatment is by far the most common practice when it comes to antimicrobial disinfection in water treatment in industrial systems. However, most of the chemicals thrown into the water for treatment are not properly treated and removed, which leads to environmental problems, such as tolerance towards these disinfectant and toxicity in the water for animals and plants (Chaves Simões & Simões 2013; Berry et al. 2006).

More and more, scientist have been trying to achieve new and controlled ways of eradicating pathogens from the water, without causing much harm towards the environment (Ferreira et al. 2010; Li et al. 2008), not just because the laws are getting tighter on the issue, but also because the price is increasing and the efficiency is not. Biofilms are one of the main reasons why it is becoming increasingly harder to guarantee quality decontamination. Biofilms form in most water systems, particularly, when the flow velocity is low or there is a hydrodynamic "death" space such as spacers in RO membranes. In these systems, the cells have a better chance to adhere to the surface, such is the case of many membranes (Nguyen et al. 2012).

When a microorganism is dispersed in suspension, it is less resistant, compared with the microorganisms in the biofilm (Melo & Bott 1997). Biofilms compromise more than one type and species of microorganisms, their polymeric matrix giving them a higher protection and a higher tolerance towards disinfection and other external influences (Melo & Bott 1997; Nguyen et al. 2012).

Thus, one of the main strategies to eradicate microbial contamination from the water should be preventive, i.e., try to eradicate as much planktonic cells as possible before they start to adhere to the surface and mature into biofouling.

Escherichia coli, Figure 8, was chosen for this project, as it is a bacterium universally utilized as an indicative of fecal contamination and of the possible presence of other pathogens it is also responsible for some waterborne diseases (Edberg et al. 2000; Heidarpour et al. 2010; Williams & Braun-Howland 2003).

As mentioned previously, micro-nanotechnology has gained more prominence in the biofouling minimization and control; the work carried out in this chapter falls within this research dynamic. The purpose of the present study was to determine the efficiency of the different functionalized particles, presented in Chapter 3, against planktonic *E. coli*.

The particles effectiveness was compared with the action of the non-functionalized particles and no biocide in the solution using the same concentrations and environmental conditions.

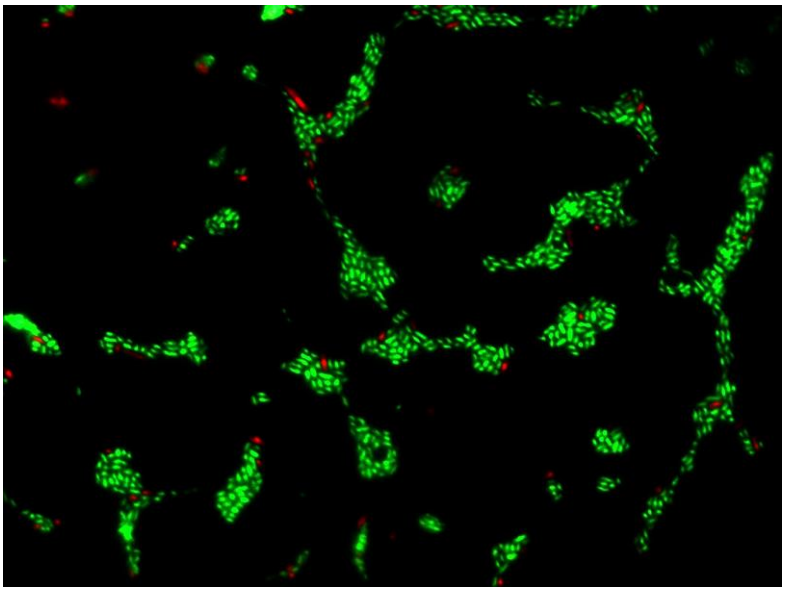

Figure 8 - Epifluorescence photomicrographs of *E. coli* x100, stained with SYTO 9TM and PI, under 480-500 nm excitation filter in combination with a 485 nm emission filter 8Chroma 61000-V2 DAPI/FITC/TRITC in a LEICA DMLB2 with a mercury lamp HBO/100W/3.

4.2 Materials and Methods

4.2.1 Microorganism and culture conditions

E. coli ATCC 25922 (Leibniz-Institut DSMZ) was cryopreserved at -80 ºC, in a mixture of nutrient broth and 30% glicerol. Bacterial cells were distributed evenly over the surface of Plate Count Agar (PCA) and incubated at 25±3 ºC for 24 hours. The culture method was adapted from (Gomes et al. 2013). An overnight culture was obtained by inoculation of *E. coli* in Nutrient Broth (Liofilchem, s.r.l.) at 30 ºC, with orbital agitation (120 rpm) (Icubator Shaker Series I26, New Brunswick Scientific).

The nutrient broth contains 5.0 g/l of peptone, 1.0 g/l beef extract, 5.0 g/l sodium chloride and 2.0 g/l of yeast extract, with a final pH 6.8 ± 0.2 (Berkowitz & Laforce 2009).

4.2.2 Bactericidal Assay

To test the bactericidal effect of the BDMDAC-coated particles on *E. coli*, planktonic cells from liquid medium were harvested by centrifugation (3202 *g* for 15 min) and appropriate dilutions in sterile saline solution (0.85% NaCl, Prolab) were done to obtain an inoculum with 10^8 cells ml^{-1} (Gomes et al. 2013).

The bactericidal assay is an adaptation from (Ferreira et al. 2010). An aliquot of 2.5 ml was collected from the inoculum and an aliquot of 2.5 ml of the particles were used to test the AMB effect of the coated particles during 60 minutes of incubation at room temperature under agitation in a multipositions plate (RO 15 POWER, IKA). Control experiments were executed with the aliquot in saline solution and in contact with non-coated particles.

Afterwards, each suspension was serially diluted to 10^{-5} and the track drop plate method was used to spread a 10 µl drop of sample on PCA plates (direct dilution and triplicates were done for each condition). The PCA plates were incubated at 30 ºC for 24 hours and then the CFUs were counted.

4.2.3 Zeta-potential

The zeta potential of the four cores was determined from streaming potential measurements with a commercial electrokinetic analyzer (EKA) (Anton Paar GmbH, Austria) using a special powder cell adapted inside a cylindrical cell, used for particles, at i3S (Instituto de Investigação e Inovação em Saúde da Universidade do Porto).

The solid particles were mounted inside the powder cell occupying a volume of 48,75 mm^3, thus maintaining an overall constant height of sample for all the measurements. Streaming potential was measured using Ag/AgCl electrodes installed at both ends of the streaming channel – the cylindrical cell. The electrolyte used was 1 mM KCl. Experiments were performed around 23 °C. The conductivity of the electrolyte solution was measured during the assay. The streaming potential was measured while applying an electrolyte flow in alternating directions and pressure ramps from 0 to 200 mbar. For each, six pressure ramps were performed (three in each flow direction in order to cope with the asymmetric potential fluctuations).

4.2.4 Kinetic Assay

To study the velocity of how the biocide killed *E. coli*, time points every fifteen minutes were taken for one hour. This assay was undertaken for two different conditions: free biocide and 1 hour of contact polypropylene particles, after the AMB assays proved them to be the most efficient. Each time point was serially diluted to 10^{-5} and the track drop plate method was used to spread a 10 µl drop of sample on PCA plates (direct dilution and triplicates were done for each time point). The PCA plates were incubated at 30 ºC for 24 hours and then the CFUs were counted.

4.2.5 Assessment of *E. coli* membrane integrity due to propidium iodide uptake

In order to evaluate the *E. coli* membrane's integrity, the method used was adapted from (Borges et al. 2013) and applied after a bactericidal assay to a control without the particles and to an assay of particles of with one hour of contact with the BDMDAC.

The Live/Dead *Bac*light™ kit (Invitrogen) evaluates membrane integrity by how the two different stains react with the cells. The method was applied in order to infer the difference between viable cells and total counts of bacteria, as well as corroborate the bactericidal assay results.

*Bac*light comes with two different nuclei acid-binding stains; SYTO 9™ penetrates all cells membranes, staining them green, while PI only penetrates damages membranes, binding to single and double-stranded nucleic acid, staining only unviable cells. The combination of the two stains generates a red fluorescing colour in the cells.

After the bactericidal assay, samples were transferred to a saline solution in order to be diluted 1:10. 500 microliters of each diluted sample was filtered through a Nucleopore® (Whatman, UK) black polycarbonate membrane (pore size 0.22 μm); then 200 and 50 microliters of diluted SYTO 9™ and 50 microliters of diluted PI were used to stain the sample for seven minutes in the dark at room temperature. The membrane was then transferred to the mounting oil, as described in the manufactures instructions.

The microscope used for the samples was a LEICA DMLB2 with a mercury lamp HBO/100W/3, incorporating a CCD camera to acquire images using LAS V4.2 software (LEICA) and an a x100 oil immersion fluorescence objective. The optical filter combination for optimal viewing of stained mounts consisted of 480-500 nm excitation filter in combination with a 485 nm emission filter 8Chroma 61000-V2 DAPI/FITC/TRITC. The total number of cells and the number of damaged cells was estimated from counts of ≥ 20 images of each sample. Two independent experiments were performed for each condition tested.

4.2.6 Reutilization Assay

With the objective of assessing whether the particles could be used more than one time, a test of reutilization was done.

After a bactericidal assay, the suspension, including the functionalized particles, was transferred to a 15 mL flask, in which the liquid solution was removed. The particles were washed up to five times with sterile saline (0.85 NaCl) and then kept in BBS pH 9.

To test the reutilization another bactericidal assay was done, following the same method as the other but with the particles previously used and washed. As control, never-used functionalized particles were also tested and no particles or biocide.

4.2.7 Statistical analysis

Data was analyzed using the statistical program SAS University Edition (Statistical Analysis System). The mean and SD within samples were calculated for all conditions; to compare the different conditions with the control condition the t-test student was done with a confidence level of equal or higher 95 % ($p < 0.05$ was considered statically significant).

4.3 Results and Discussion

Chemical treatment has always been the default way to remove and control microbial content in the water. However, the applied biocides have environmental risks, since they are not only applied in high doses, but they are still not entirely treated from the water, which can lead to the products of toxic by-products (WHO 2000). In the recent years, new technologies have been studied, where micro-particles are used as carries of the biocide, so that the biocide is not delivered into the environment in a decontrolled way (Ferreira, et al. 2010; 2013). The results and conclusions presented in this chapter are an evaluation of the functionalized particles with BDMDAC against planktonic *E. coli*, in order to infer which of the particles was the most efficient. Further studies to understand those particles were also carried on.

4.3.1 Particles Efficiency Evaluation

The effect of the BDMDAC functionalized particles in the different assays was investigated on planktonic *E. coli* cells and the results are presented as survival ratio between the CFU count for the bactericidal assay with the particles and the CFU count in saline solution (Figure 9). In this, N.F. represents the non-functionalized particles.

The PP particles which were in contact with the BDMDAC for 1 hour were the only functionalized particles that showed a significant decrease in the survival ratio. This was expected since Heidarpour et al. (2010) used this material as a core for their nano-silver coated filter to test *E. coli* removal and found that the filters were 100% effective, after 5 hours of filtration, proving PP is a good material for these kind of coating processes.

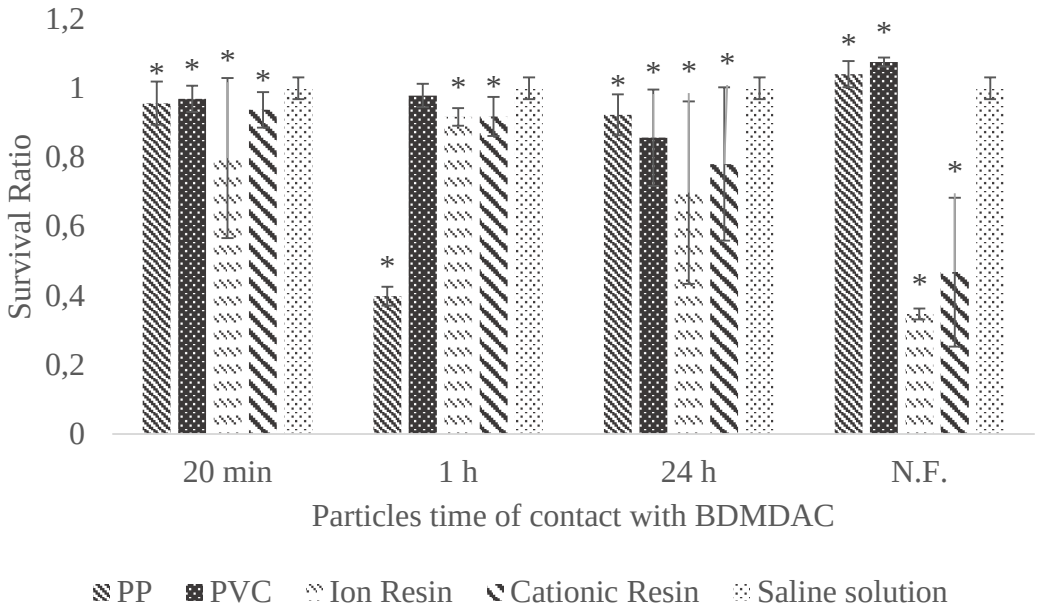

Figure 9 - Survival ratio of planktonic *E. coli* exposed to control conditions (saline solution and N.F. particles) and to BDMDAC-functionalized particles of PP, PVC, Anionic Resin, Cationic Resin prepared with different times of contact with the BDMDAC 20 minutes, 1 hour and 24 hours. The values are means ± SDs of nine independent measurements.

Also, the anionic resin particles in contact for 24 hours showed some decrease, yet because the results were so inconsistent, one cannot be sure of their real efficiency.

Considering the saline solution as the survival ratio of 1, the results obtained from the majority of the functionalized particles proved that these particles, both the functionalized and the N.F., regardless of the time of contact with the BDMDAC, do not have significant antimicrobial effects, since the numbers of bacterial cells are the same as for the saline solution (survival ratios close between 0.8 and 1).

Regarding the functionalized particles, despite the fact that the survival ratios are not low enough to make the particles efficient, the differences observed were statistically significant ($p < 0.05$) for all the particles, except PVC-PEI/PSS/BDMDAC-1-hour-of -contact; this might be justified by the size of the sample. However, in my opinion, even though statistically speaking the results show that the particles have antimicrobial effect, in the microbiology and water treatment analysis, if a treatment allows 80% of survival of microorganisms, it is not an effective treatment.

The results for the functionalized particles were unexpected given the efficiency of the method in similar studies of Ferreira et al. (2010). In this study the polystyrene (PS) core particles when coated with PEI/PSS/BDMDAC presented a clear antimicrobial effect (Ferreira et al. 2010). One possible explanation for the different results is the different

size of the particles, while Ferreira et al. (2010) worked with particles with 4.37 ± 0.07 μm, the smaller particles tested in this study were around 72.5 μm.

Mcguffie et al. (2015) stated that the particles surface's material might interfere with the way the particle interacts with the cells, thus N.F. assays were carried out to insure that the particles material did not influence the results.

An additional possible theory for the resins inadequacy came to surface based on the results of the resins-core N.F. particles. These N.F. particles are used in the industry in packing beds to removed certain ions from the water, yet they do not have a antimicrobial effect known (Cullex 2000a; Cullex 2000b). This lead to question whether the bacteria were really dying because of the particles, or if they were simply getting attracted to the resins charge, like it was speculated by Mcguffie et al. (2015) and, actually, seen in transmission electron microscopy by Auffan et al. (2008) for their particles with gram-negative bacteria.

Table 3 - pH results from the bactericidal assay

Particles		**pH**
cationic resin	20 min	12
	1 h	12
	24 h	12
	N.F.	3
anionic resin	20 min	12
	1 h	12
	24 h	12
	N.F.	3
PP	20 min	6
	1 h	6
	24 h	6
	N.F.	6
PVC	20 min	6
	1 h	6
	24 h	7
	N.F.	7
Buffer		9
Positive Control		6

To evaluate the non-coated resins were stable in the solution, a pH test was done. As seen in Table 3, the resins particles cause a big variation in the solution pH, thus meaning *E. coli* might be dying, because of the extreme acid pH, and not because the core particles have antimicrobial characteristics.

The pH assay raised the question of why both resins, when not functionalized, had an acidic pH; one should be acidic and the other should be alkaline. Therefore, a Zeta Potential assay was done to confirm the particles charges; the results are presented in the Table 4.

Table 4 - Zeta Potential (mV) results of the core particles before being functionalized. The values are means ± SDs of six independent measurements

Zeta Potential (mV)			
Cationic Resin	Anionic Resin	PP	PVC
[-1.30;3.61]	[-9.23;3.96]	- 18.67 ± 0. 42	- 17.96 ± 0. 22

The results went against the manufacturer's information, however agreed with the theory to why the method was not working. Since the quantification of the biocide uptake in the particles surface was not performed (High Performance Liquid Chromatography not available at the moment), one has to go from the CFUs and the Zeta Potential results and infer that perhaps the method did not work, i.e., the coating process did not occur as it should. Therefore, the particles were not truly coated by BDMDAC and the *E. coli*, with a negatively charged Zeta Potential (Liu et al. 2010; Mcguffie et al. 2015), felt attracted towards the resin, but it was not, in fact, dying. Further studies needed to be done to infer in which step did the method stop working and a scanning electron microscopy (SEM) should be performed to infer whether the *E. coli* was getting attached to the non-coated particles. Nonetheless, the zeta potential of the PP untreated particles, along with the materials characteristics, might shed some light on why they worked.

4.3.2 PP-PEI/PSS/BDMDAC-1 hour-of-contact particles

Nevertheless, as mentioned previously, the PP-PEI/PSS/BDMDAC-1-hour-of-contact particles showed the highest biocidal activity (a reduction of 4 in the logarithmic scale), with 39.8% of survival ratio. Therefore, further studies were done to try to understand the system: three different times of contact with BDMDAC were tested; an assessment of the membrane integrity due to propidium iodide uptake was made; a kinetic assay and reutilization assays of the particles was performed.

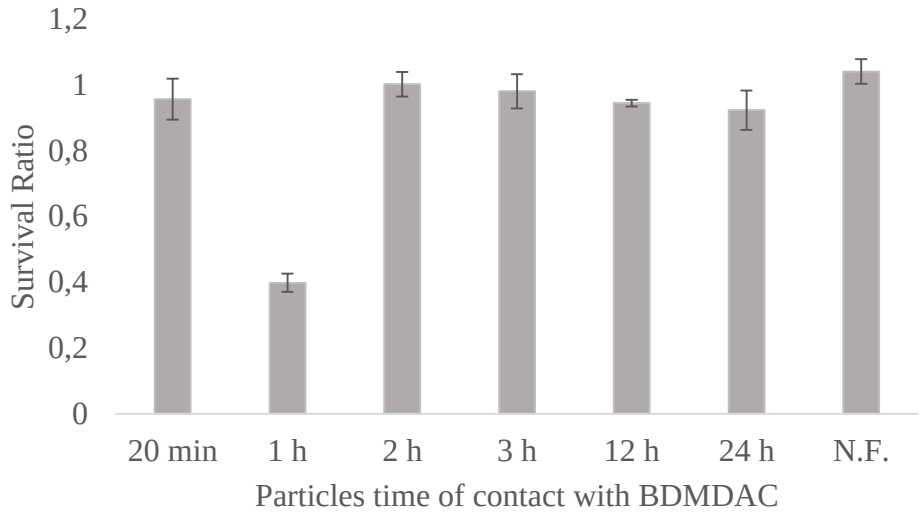

Figure 10 - Survival ratio of planktonic *E. coli* exposed to control conditions (saline solution and N.F. particles) and to BDMDAC-functionalized particles of PP prepared at different times of contact with the BDMDAC 20 minutes, 1, 2, 3 12 and 24 hours. The values are means ± SDs of nine (20 min, 1 h, 24h N.F.) and three (2, 3, 12 h) independent measurements.

In addition to, the 20 minutes, 1 hour and 24 hours already tested, another three different times of contact (2, 3 and 12 hours) were tested in order to see if 20 minutes were too little time and 24 hours was too much. However, as it can be seen in Figure 10, only the contact time of 1 hour was efficient.

Once again, the measurement of the amount of BDMDAC adsorbed by the particles could not be quantified (High Performance Liquid Chromatography not available at the moment), yet the results can be justified based on the present data. 20 minutes of contact between the PP-PEI/PSS particles might not have been enough for the BDMDAC to adhere properly to the particles, while 2 hours and beyond might have been too long. BDMDAC is a surfactant, and it has a tendency to create micelles (Freem 2015), thus explaining why after the 1 hour mark the particles did not adsorb enough biocide to be effective.

Moreover, Sukhorukov et al. (1998) stated that when in solution some polyelectrolytes might create agglomerates and that the combination between the material and the polyelectrolytes has to be very well studied in order to prevent this; i.e., the attraction between the material and the polyelectrolytes needs to be bigger than the attraction of the polyelectrolytes, for example. Mcguffie et al. (2015) also agreed that polyelectrolytes, capping agents and surfactants used in standard coating processes of LbL might influence the results.

Since it has been documented that the quantification of CFU has numerous drawbacks (Banning et al. 2002; Simões et al. 2005), an assessment of the membrane integrity was done to verify the results. The Live/Dead *Bac*Light viability kit permits the distinction between viable and dead cells in a bacteria population, making it a viable option to study membrane integrity. Cells stained with fluorescing green are considered viable cells, not having any damaged to their membrane, while cells stained with fluorescing red (PI stain) are considered dead cells, with their membrane damaged; examples of the images obtained can be found in Appendix A.2, Figure 20.

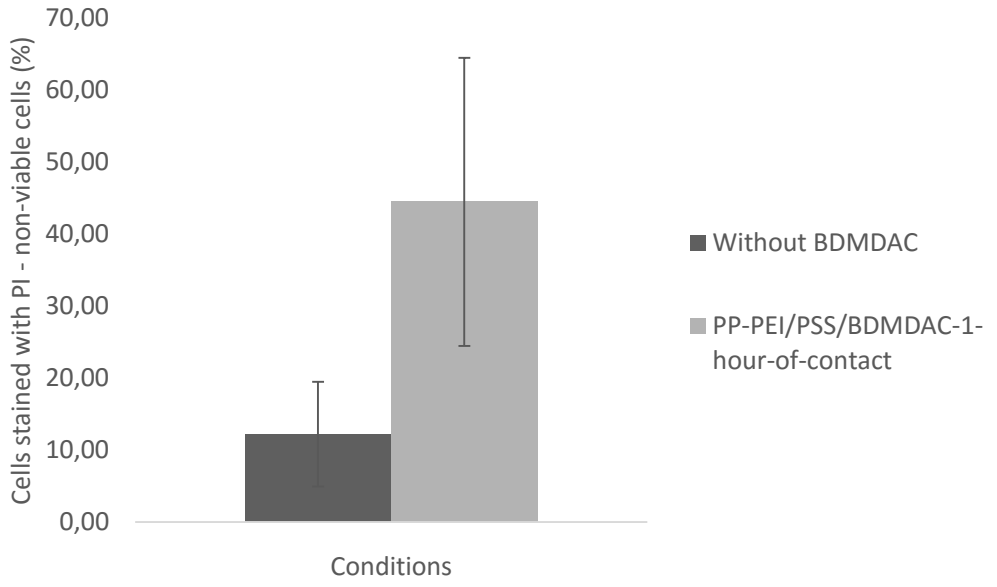

Figure 11 - Permeability of *E. coli* to PI after treatment with PP 1 hour of contact with BDMDAC and control without particles or BDMDAC. The percentage of cells non-stained with PI corresponded to the fraction of viable cells. The means ± SD for two replicates are illustrated.

The PI uptake results corroborates the survival ratio results, proving the PP-PEI/PSS/BDMDAC-1-hour-contact particles functionalized with BDMDAC are compromising the integrity of the membrane, Figure 11. As it can be seen, the percentage of cells stained with PI in the control without particles were about 12.2 %, while when in contact with the treatment of PP particles coated with BDMDAC, the number of stained cells increases to 44.5 %.

Ferreira et al. (2011) studied the quantity of free BDMDAC needed in a solution to eradicate *Pseudomonas fluorescens* namely by quantifying the viable and non-viable cells stained with PI treatment. They found 2.5 mg/L of BDMDAC was needed in the solution for 40% of *Pseudomonas fluorescens* population to be stained with PI after one hour of treatment with BDMDAC (Ferreira et al. 2011). Assuming *E. coli* has the same reaction

with BDMDAC as *P. fluorescens*, one can speculate that in these bactericidal assays there was 2.5 mg/L of BDMDAC in the solution; however, this theory must be verified with High Performance Liquid Chromatography (HPLC).

Before the particles were applied in a fix-bed reactor and in order to further study their behavior, a kinetic assay was done to determine the inactivation constant (Kd) in the suspended bacteria assays, Figure 12.

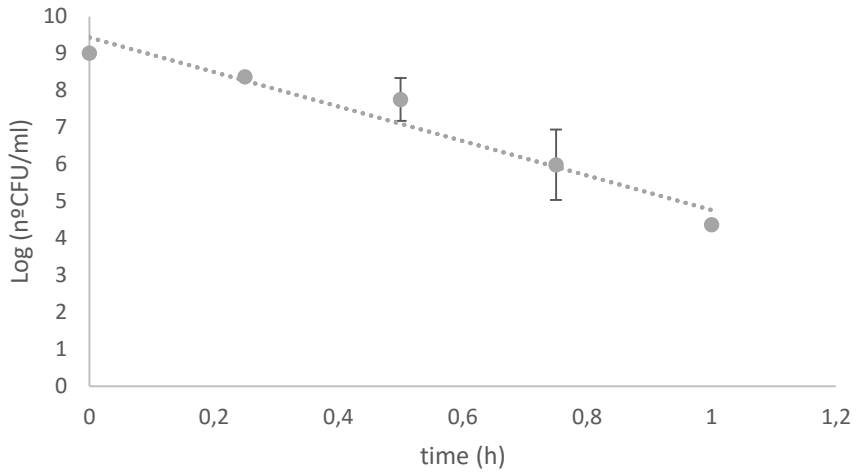

Figure 12 - Logarithm of the number of cells per mL of planktonic *E. coli* cells exposed to PP-PEI/PSS/BDMDAC 1 hour particles during 60 minutes. Each point indicates the mean ± SD of three independent experiments.

Kd was obtained by linear approximation of the curve presented in Figure 12, assuming a first rate equation. The Kd for the PP-PEI/PSS/BDMDAC-1 hour-of-contact particles was 4.67 h^{-1}, Table 5, meaning it took around 13 minutes of contact between the particles and *E. coli* to decrease the number of cells by 1 in the logarithm scale.

Table 5 - Inactivation constant (Kd) and correlation coefficients (r^2) obtained from linear approximation of the curve presented in Figure 12. The presented values are means ± SD of three independent experiments.

	Sample	**Kd (h^{-1})**	**Correlation coeficiente (r^2)**
60 min	PP-PEI/PSS/BDMDAC-1 h	4.67 ± 2.34	0.95 ± 1.43

A reutilization assay was done to test the particles efficiency over time. Even though, the 1 hour of contact between functionalized particles and *E.coli* cells showed great promise in the first use, when applied for the second time they completely lost their ability to idle the cells, Figure 13. These results go once again against what was expected based on the literature. In preliminary tests with reused BDMDAC coated micro-particles developed

under the same method showed great potential, even after three times reprocessing (Ferreira et al. 2013). So, even though, it was previously suggested that when $CaCO_3$ core particles coated with PEI/PSS/BDMDAC the biological agent was not consumed during the contact with the cells (Ferreira et al. 2013), in the PP particles this does not seem to be the case. This confirms what was previously mentioned that further analysis regarding what happened once the PP-PEI/PSS/BDMDAC-1-hour-of-contact come into contact with the *E. coli* is needed.

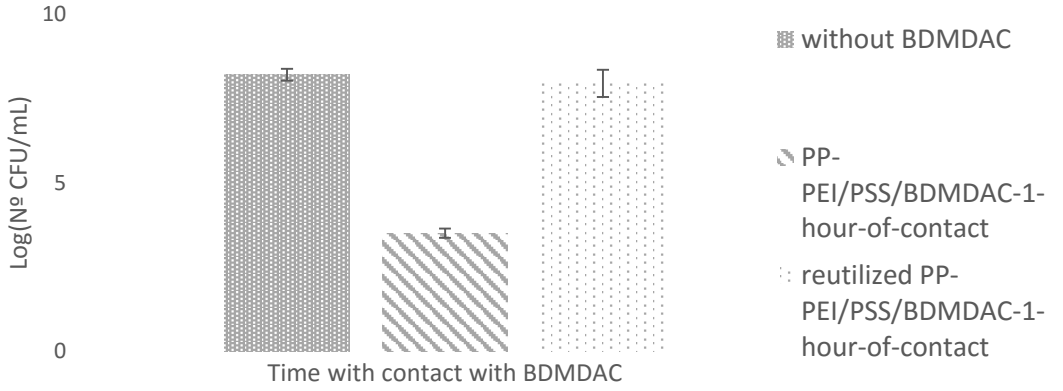

Figure 13 - Logarithm of the number of cells per ml of planktonic *E. coli* exposed to control condition without biocide, PP 1 hour of contact with BDMDAC and reutilized particles of 1 hour of contact with BDMDAC. The values are means ± SDs of nine independent measurements.

4.4 Conclusion

Four different types of cores were tested for the LbL technique, Cationic Resin, Anionic Resin, PP and PVC. Only the PP-PEI/PSS/BDMDAC-1-hour-of-contact showed antimicrobial effect, by successfully eliminating *E. coli* from the suspension.

Zeta-Potential measurements showed that a possible reason for this factor was the misleading information about the particles charge, which lead to the wrong treatment and therefore the unsuccessful results. Furthermore, the results on the N.F. resin particles showed that these cores, even though cheap, were unsuitable for this study, as the change in pH was killing the bacteria and not the particles antimicrobial effect.

Further investigation into the PP-PEI/PSS/BDMDAC-1 hour-of-contact particles showed that they lose their bactericidal effect after the first use. However, an HPLC assay needed to be done, in order to infer whether the particles lost the BDMDAC after the first use and continuous washes.

Chapter V – Particles immobilized in packed bed reactor for inactivation of suspended cells

5.1 Introduction

Membrane biofouling is a problem not just because it decreases the membranes ability to filtrate properly, but it increases costs of maintenance (Nguyen et al. 2012; Vrouwenvelder & van der Kooij 2003). It has been reported that biofouling is a major problem in RO and NF membranes all over the world, leading to a clear need to detect and diagnose the problem before it worsens (Vrouwenvelder & van der Kooij 2003; Vrouwenvelder et al. 2008).

So far, the most used way to control and eradicate biofilms in these systems is to use chemical products with antimicrobial properties or surfactants to disperse them (Nguyen et al. 2012). However, these methods are not 100% efficient, and are also harmful to the environment and the membranes, since the biocides are not eliminated from the water and can be deleterious to the ecosystems, especially when they react with other chemicals (including natural products) and create toxic by-products, such as organochlorinated compounds (Li et al. 2008; Nguyen et al. 2012; Ferreira et al. 2010). Furthermore, for example, even though most bacteria have a low resistance towards chlorine (Chaves Simões & Simões 2013), membrane systems are not compatible with chlorine (Saad 1992; Malaeb & Ayoub 2011), as mentioned in the section 2.3.2.

Reports indicate that the presence of *E. coli* and other pathogens in biofilms disrupts the water treatment industry by misleading their results, i.e., when the bacterial counts do not show contamination, they only mean there is no planktonic contamination (Williams & Braun-Howland 2003; Camper et al. 1991). Another very important problem with biofilms in water systems is the fact that from time to time, they detach part of their structure, causing a contamination even in previously disinfected waters (Williams & Braun-Howland 2003; Berry et al. 2006; Ferreira et al. 2013) .

Over the past decade, a new approach for fighting biofilms has been introduced, using micro-nanotechnology. Many studies have been focused on applying micro-nanotechnology in water treatment as a way to decrease the consumption and spread of harmful chemicals and/or enhance the traditional water treatment (Ferreira et al. 2010; 2013; Dror-Ehre et al. 2010; Mcguffie et al. 2015; Li et al. 2008). Moreover, this new approach has also been applied in minimizing biofouling in the membranes systems, with

coating membranes' surfaces or altering their base proprieties with nanomaterials to enhance their anti-biofouling capabilities (Nguyen et al. 2012; Pendergast & Hoek 2011).

The aim of the work presented in this chapter was to develop a packed bed reactor, with the PP-PEI/PSS/BDMAC-1-hour-of-contact particles (preparation: 1 hour of contact with the biocide, Chapter 3 & Chapter 4) acting as the anitmicrobial agent. This reactor will preceed the membrane treatment and eradicate most of the planktonic microbial content, thus preventing biofilm formation in the membranes. The particles effectiveness in the system was compared with the system without particles using the same concentrations and conditions (but not biocide).

5.2 Materials and Methods

5.2.1 Fixing Particles

Two different types of methods to fix the particles inside the reactor were applied after the particles were functionalized as described in Chapter 3. Once the PP-PEI/PSS/BDMDAC-1 hour-of-contact particles were determined to be the most efficient, and their behavior tested, Chapter 4, they were the ones used in the present chapter.

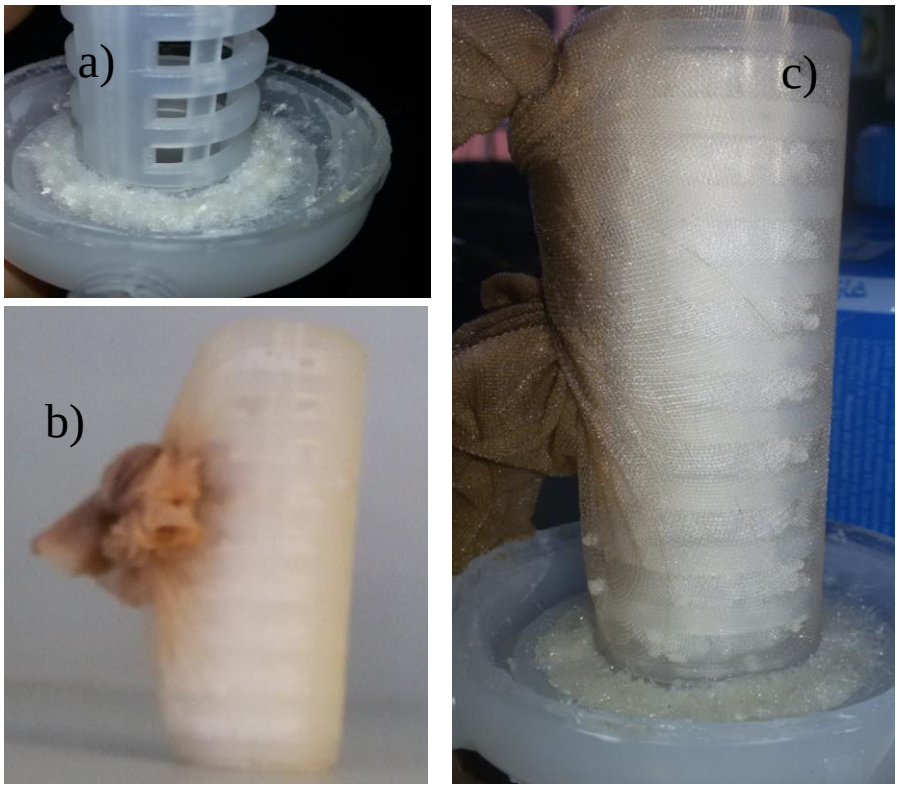

Figure 14 - Images of the fixed particles set up. a) grid structure assembled in the reactor without any particles; b) PP-PEI/PSS/BDMDAC-1 hour-of-contact particles inside the grid structure covered the tissue netting ("caged particles"); c) grid structure with the PP-PEI/PSS/BDMDAC-1 hour-of-contact particles inside a tissue netting in the reactor.

In the first method, the functionalized particles are transferred to a grid structure, as seen in Figure 14, a). To prevent the particles from getting out a thin tissue netting was placed around the grid structure, so that way the liquid moves through it, but the particles can not, Figure 14, b) & c). This method is here entitled “caged particles".

In the second method, the functionalized particles were in equal ratio between the volume of particles and the volume of cells, as in the suspended assays of Chapter 4, and kept inside the reactor in a tissue netting, without the grid structure, as seen in Figure 15. This method is entitled “net-trapped particles”.

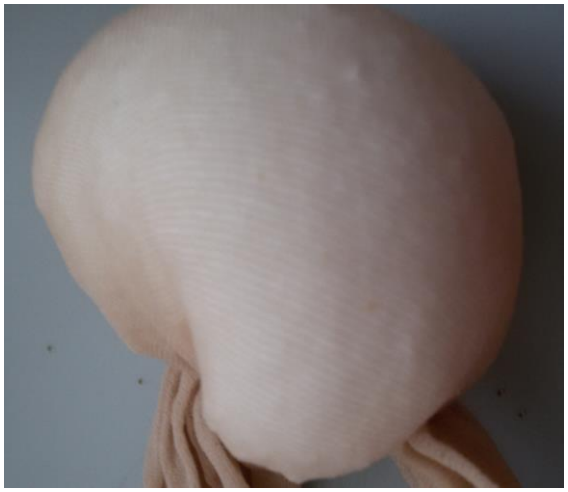

Figure 15 - Image of the second particles set up.: PP-PEI/PSS/BDMDAC-1-hour-of-contact particles inside the tissue netting (“net-trapped particles”).

5.2.2 Microorganism and culture conditions

The *Escherichia coli* strain chosen and the culture conditions for this work were the same as described in 4.2.1

5.2.3 Packed Bed Reactor set-up

To test the bactericidal effect of the PP-PEI/PSS/BDMDAC-1-hour-of-contact particles on *E. coli*, planktonic cells from liquid medium were harvested by centrifugation (3202 *g* for 15 min) and appropriate dilutions in sterile saline solution (0.85% NaCl, Prolab) were performed to obtain an inoculum with 10^8 cells ml^{-1} (Gomes et al. 2013). This inoculum was then used as the feed to the reactor.

The reactor was obtained from ENKROTT, S.A., the main goal being to simulate a packed bed reactor, in which the particles filled part of the hollow interior. Three different reactor conditions were tested, two under the first method described in the section 5.2.1; and one under the second method.

5.2.3.1 Caged Particles Batch Mode

In the first set up, the inoculum was fed to the reactor, with caged particles (grid structure), Figure 16, at a constant rate (1.2 Lh^{-1}), for two hours. The feed entered from below after a peristaltic pump and came into contact with the functionalized particles at the top of the reactor, where the particles were caged, Figure 16. The feed was recirculated in order to allow the inoculum to enter the reactor pass through the particles several times. In the first condition, the reactor operates in batch mode.

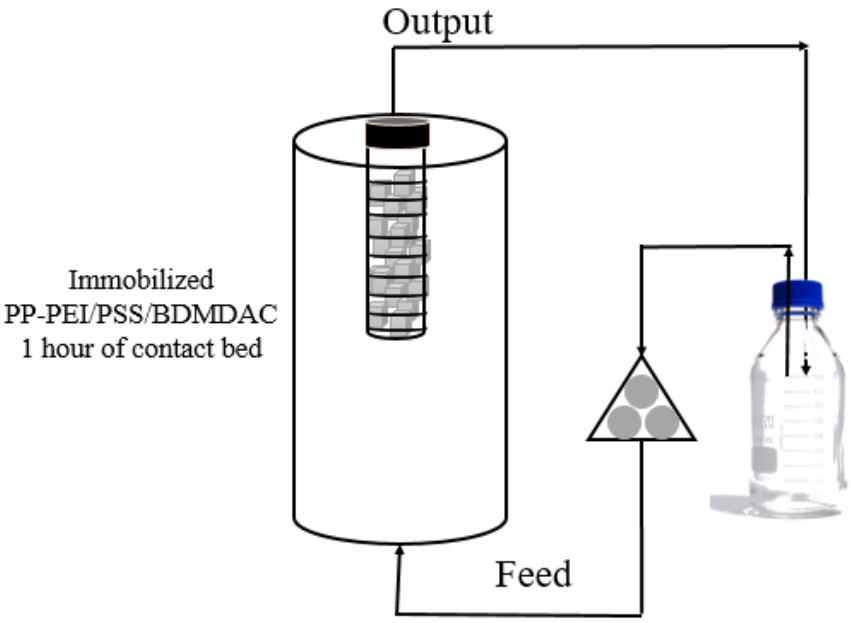

Figure 16 - Schematic representation of the batch-mode packed bed reactor system; with the particles inside a grid structure.

5.2.3.2 Caged Particles Continuous Mode

The second condition, equal to the first one except the system operates as a continuous one, with a recycle circuit, was implemented with the goal of increasing the time of contact between the cells and the particles, Figure 17. The feed entered from below after a pump and came into contact with the functionalized particles at the top of the reactor, where the particles were caged, Figure 17, open circuit. The recycle stream relied on a peristaltic pump to allow the output to reenter the circuit and come into contact with the functionalized particles again. The open circuit fed the reactor for two hours with a constant flow rate of 0.6 Lh^{-1}, while the recycle circuit (seen in Figure 17, as recycle stream) worked at 1.5 Lh^{-1} for the same two hours.

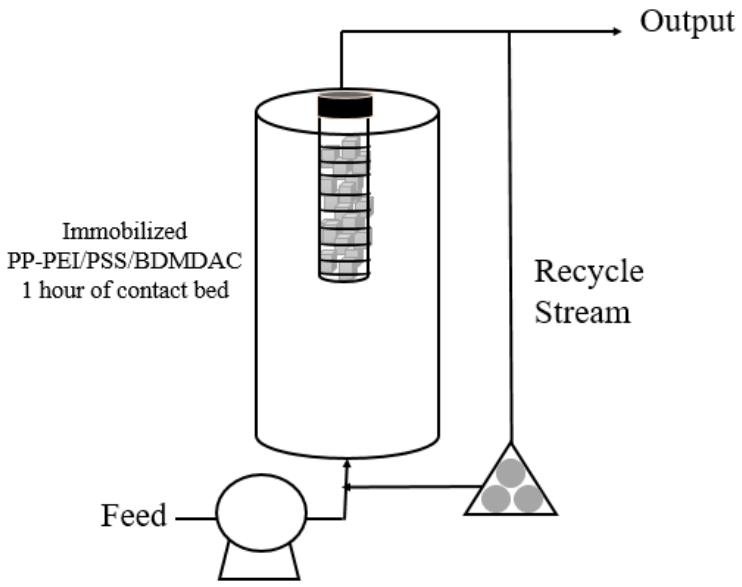

Figure 17 - Schematic representation of the continuous-mode packed bed reactor system; with the particles inside a grid structure.

5.2.3.3 Net-trapped particles in batch mode

The third set up, Figure 18, worked in batch operation, and there were more particles in the system, i.e., there was the same mass ratio of particles-cells in the system as there were in the suspended assays of Chapter 4. However, these particles were not caged, but trapped inside a tissue netting, as described for the second method in section 5.2.1. The feed entered from below after a peristaltic pump and came into contact with the functionalized particles at the top of the reactor, where the particles were once the reactor was full, Figure 18. This condition aimed at properly comparing the results of the packed bed reactor with the results in Chapter 4. The reactor was uninterruptedly fed for two hours with 1.2 Lh^{-1}.

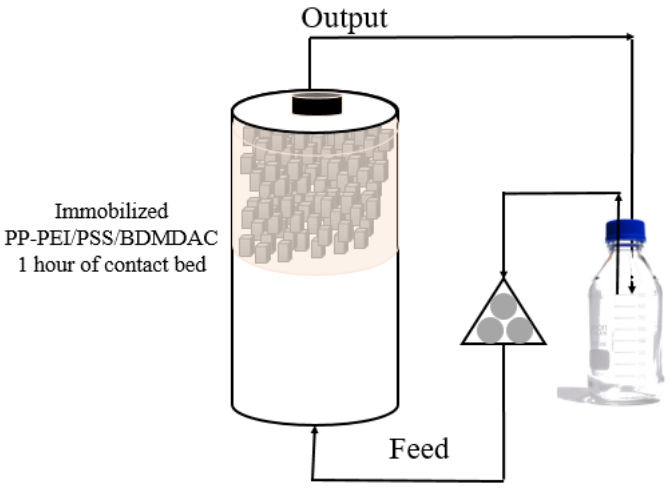

Figure 18 - Schematic representation of the batch operation with particles trapped inside a tissue netting (ratio of PP-PEI/PSS/BDMDAC-1 hour-of-contact particles to cells to the suspended cells assays).

A control experiment with just saline solution was also performed to guarantee that the system without particles did not have antimicrobial effect. The inoculum was fed to the free of particles reactor with 1.2 Lh^{-1}.

The dilution and flow rate of each condition were calculated based on the volume of liquid that passed through the pump for one minute and the reactor's volume (200 mL).

Table 6 - Packed bed reactor conditions, dilution rate and feeding flow rate for the three different tested conditions caged particles in batch operation; caged particles in continuous operation and net-trapped particles in batch particles (equal ratio between the cells and the particles as in Chapter 4). A control test was performed without particles.

		Caged particles (batch mode)	**Caged particles (continuous mode)**	**Net-trapped particles (batch mode)**	**Control**
Open Circuit	dilution rate (h^{-1})	6	3	6	6
	flow rate (Lh^{-1})	1.2	0.6	1.2	1.2
Recycle Circuit	dilution rate (h^{-1})	-	7.7	-	-
	flow rate (Lh^{-1})	-	1.5	-	-

5.2.4 Bactericidal Assay

Three time points were taken: at time zero, at 60 minutes and at 120 minutes. Each sample was serially diluted to 10^{-5} and the track drop plate method was used to spread a 10 µl drop of sample on PCA plates (direct dilution and triplicates were done for each condition). The PCA plates were incubated at 30 ºC for 24 hours and then the CFUs were counted.

5.2.5 Statistical analysis

Data was analyzed using the statistical program SAS University Edition (Statistical Analysis System). The mean and SD within samples were calculated for all conditions; to compare the different conditions with the control condition the t-test student was done with a confidence level of equal or higher 95 % ($p < 0.05$ was considered statically significant).

5.3 Results and Discussion

Biofouling in water treatment membranes has become a problem many have tried to solve, and micro-nanotechnology has increased in prominence as a possible solution (Kim & Van Der Bruggen 2010; Pendergast & Hoek 2011). Yet, most of the solutions being studied aim at coating the membranes' surface or combining membrane filtration with another technique like ozone (Nguyen et al. 2012; Pendergast & Hoek 2011). The

simulated packed bed reactor in this chapter containing the PP-PEI/PSS/BDMDAC-1-hour-of-contact particles was thought as a possible pretreatment preceding the membrane filtration, in order to prevent biofilm formation in the membranes.

The PP-PEI/PSS/BDMDAC-1-hour-of-contact particles were once again investigated for eradication of *E. coli* planktonic cells, but this time the particles were immobilized in the reactor, and the results are presented as survival ratio between the CFU count for the bactericidal assay with the particles in the outlet stream and the CFU count in feed solution (Figure 19).

The PP-PEI/PSS/BDMDAC-1-hour-of-contact particles showed a higher antimicrobial effect in Chapter 4 (suspended particles) than when they were applied in the packed bed reactor setting they did not succeed in reducing the bacteria content. All three different conditions showed survival ratio around 1, similar to the control experiment without the biocidal particles present, as in can be seen in Figure 19. The number of bacteria in the presence of the particles was approximately the same as in the control with only saline solution, the results were all statistically significant ($p<0.05$).

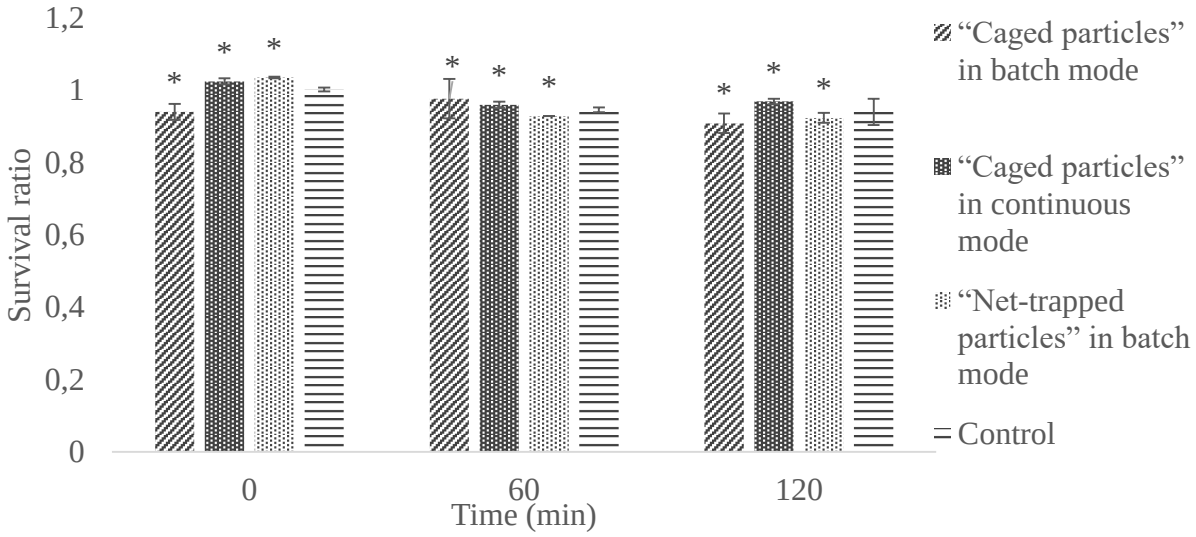

Figure 19 - Survival ratio of planktonic *E. coli* exposed to control conditions (saline solution) and to PP-PEI/PSS/BDMDAC-1 hour-of-contact particles in three different reactor conditions: caged particles in batch mode operation; caged particles in continuous mode operation; and net-trapped particles in batch mode operation (equal ratio between particles and cells as Chapter 4). The values are means ± SDs of three independent measurements.

A continuous reactor with recycling was tested, in order to increase the time of contact between the cells and the particles, without increasing the number of particles. The flow rate of the recycle flow was much higher than the main circuit, Table 6, to increase the contact time (residence time). However, this was also not effective, since there is no significant difference between the survival ratio at time 0 and after two hours of treatment.

Table 7 - Volume relations between E. coli cells and PP-PEI/PSS/BDMDAC-1 hour-of-contact particles in different tests: chapter 4, suspended particles assays; caged particles in batch packed bed reactor and continuous packed bed reactor with recycling; met-trapped particles in batch packed reactor using the same ratio between cells vs particles as in Chapter 4 (suspended particles).

Conditions	Volume of cells in saline solution (mL)	Volume of particles (mL)	Volume ratio cells / particles
Biocidal particles in suspension, Chapter 4	10	2	0.2
"Caged particles" in batch mode packed bed reactor and in continuous mode packed bed reactor	400	22.5	0.06
"Net-trapped particles" in batch mode packed bed reactor (with the same ratio of cells vs particles as in Chapter 4)	400	80	0.2

With this in mind, another condition was tested where the number of particles was increased to the same relation between volume of cells and volume of particles as used in Chapter 4, Table 7. As can be seen in Figure 19, this new approach, unlike in the previous ones, showed a slight decrease in the survival ratio. A decrease to 0.92 of survival ratio, which might indicate that a time factor came into play. In other words, if the treatment had been carried out for longer than two hours in the reactors (higher residence time), perhaps the treatment would have been more effective. Moreover, it also had higher ratio volume of cells/volume of particles which can also influence the decrease in the survival ratio for this set-up.

5.4 Conclusion

Despite showing great promise in the suspended particles assays in Chapter 4, the PP-PEI/PSS/BDMDAC-1-hour-of-contact particles were not successful in decreasing the number of *E. coli* in suspension in the packed bed reactor settings. A possible explanation for this is that perhaps the particles do not have enough BDMDAC to be effective as an antimicrobial agent or that they were not in contact with the particles enough time for them to scientifically decrease the bacteria count.

In the three different reactor settings tested, the survival ratios were nearly 1, indicating that the cells were not being affected by the biocidal particles present in the reactor.

Nevertheless, the last set-up, where the ratio volume of cells vs. volume was the same as in the assays in Chapter 4, caused a slight decrease in the survival ratio after two hours of contact. This raises the question of whether the particles would have decreased the CFU count even more given more time.

Chapter VI – General conclusions and Future Work

6.1 Conclusion remarks

The main goal of this work was to develop functionalized particles, which would carry the biocide in a controlled and safe way in the industrial water systems. These functionalized particles, now with antimicrobial capabilities, would help the disinfection of the water systems and help prevent biofilm formation along the pipes and membranes, without wasting non-consumed biocide in the bulk water.

The LbL self-assembly technique has been widely used for the production of drug delivery in pharmaceutical and medical fields. However, few have tried to implement this technique in the water industry, despite the fact that micro-nanotechnology has been gaining momentum in this field.

Four different cores, Cationic Resin, Anionic Resin, PP and PVC, were coated with this technique using two different widely use polyelectrolytes, PEI and PSS, along with the antimicrobial agent BDMDAC. For the coating process, three different times of contact with the biocide were used. These functionalized particles were tested against planktonic *E. coli* cells for 1 hour, after which only the PP-PEI/PSS/BDMDAC-1-hour-of-contact showed significant antimicrobial effect. Further studies were carried out to understand why these particles worked better than the others. Nonetheless few conclusions could be taken other than the core is made of polypropylene, which allows such coatings to be successful, as proved by other studies, while the other core were not suitable for the technique here applied. Furthermore, reutilization assays were done, and the results showed that the PP-PEI/PSS/BDMDAC-1-hour-of-contact lost their bactericidal effect after their first use and consequent washing steps. This might mean the particles are not stable enough for long term use or that the biocide is consumed in the killing process.

Moreover, once the PP-PEI/PSS/BDMDAC-1-hour-of-contact were determined as the most effective ones, they were tested in a packed bed reactor, where three different set ups were tested. Yet, none of the three different set ups was effective, raising the questions of whether the biocidal particles needed more time in contact with the cells for their antimicrobial effect to prevail or if there was not enough biocide in the system.

6.2 Future Work

Even though the novel technique shows great promise (see results in Chapter 4), the reality is that there is still a lot of unknown factors in play before they can actually be applied in an industrial setting.

Firstly, and foremost, a clear and thorough characterization of the core particles must be undertaken before any further work. Despite the literature and the manufactures' information, one can only be sure of the characteristics of the materials once the proper tests are carried out in the research laboratories.

Secondly, there is a need to re-evaluate the coating process, in order to know, for sure, the amount of biocide being carried by the particles. Additionally, a better understanding of whether or not they are stable once they come into contact with the cells, or if they lose the biocide, is required. This can be accomplished with chemical analysis tools such as HPLC, which will allow the quantification of the BDMDAC in the supernatant resulting from the coating process and after the assays.

Another interesting thing to investigate is what makes the 1 hour of contact with BDMDAC work, while the 20 minutes and 2 hours do not. A better understanding about what happens in the coating process might help clarify the doubts in this step, not just for the PP particles, but for all the cores.

Once these are complete, further assays in the reactor should also be done with more thought out repercussion and less time limitations, in order to see if the antimicrobial effect increases with the time of contact between the particles and the cells, whilst making sure the cells are dying because of the biocide and not because they do not have any nutrients.

Finally, and because this project was only a small part of a larger research line, different microorganisms should be studied with different cores and possibly different biocides. This technique has great potential to minimize the impact of the disinfection methods in the environment and it should be further explored.

References

Al-Ahmad, M. et al., 2000. Biofuoling in RO membrane systems Part 1: Fundamentals and control. *Desalination*, 132(October), pp.173–179.

Ali, I., 2012. New generation adsorbents for water treatment. *Chemical Reviews*, 112(10), pp.5073–5091.

Ali, I. & Gupta, V.K., 2006. Advances in water treatment by adsorption technology. *Nature protocols*, 1(6), pp.2661–2667.

Allsopp, M.W. & Vianello, G., 2012. Polystyrene and Styrene Copolymers. *Ullmann's Encyclopedia of Industrial Chemistry*, 29, pp.6.5–621. Available at: http://doi.wiley.com/10.1002/14356007.a21_615.pub2.

Andrade, P.F. et al., 2015. Improved antibacterial activity of nanofiltration polysulfone membranes modified with silver nanoparticles. *Water Research*, 81, pp.333–342. Available at: http://www.sciencedirect.com/science/article/pii/S0043135415002857.

Applegate, L.E. & Erkenbrecher Carl W., J., 1987. Monitoring and control of biological activity in Permasep(R) seawater RO plants. *Desalination*, 65, pp.331–359. Available at: http://www.sciencedirect.com/science/article/B6TFX-44C99NP-1J/2/f6f20ab875a29fbd1afd2bf9a2d0a532.

Ariga, K., Hill, J.P. & Ji, Q., 2007. Layer-by-layer assembly as a versatile bottom-up nanofabrication technique for exploratory research and realistic application. *Phys. Chem. Chem. Phys.*, 9(19), pp.2319–2340. Available at: http://pubs.rsc.org/en/content/articlepdf/2007/cp/b700410a.

Auffan, M. et al., 2008. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward Escherichia coli. *Environmental Science and Technology*, 42(17), pp.6730–6735.

Ayadi, S. et al., 2016. Preparation and characterization of carbon microfiltration membrane applied to the treatment of textile industry effluents. *Separation Science and Technology*, 51(6), pp.1022–1029. Available at: http://www.tandfonline.com/doi/full/10.1080/01496395.2016.1140201.

Banning, N., Toze, S. & Mee, B.J., 2002. Escherichia coli survival in groundwater and effluent measured using a combination of propidium iodide and the green fluorescent protein. *Journal of Applied Microbiology*, 93(1), pp.69–76.

Bereschenko, L.A. et al., 2011. Effect of conventional chemical treatment on the microbial population in a biofouling layer of reverse osmosis systems. *Water Research*, 45(2), pp.405–416. Available at: http://dx.doi.org/10.1016/j.watres.2010.07.058.

Berkowitz, V. & Laforce, F.M., 2009. Liofilchem s.r.l . , pp.11–12.

Berry, D., Xi, C. & Raskin, L., 2006. Microbial ecology of drinking water distribution systems. *Current Opinion in Biotechnology*, 17(3), pp.297–302.

Bertheas, U. et al., 2015. Use of DBNPA to control biofouling in RO systems Use of DBNPA to control biofouling in RO systems. , 3994(November), pp.1–5.

Bolto, B. & Gregory, J., 2007. Organic polyelectrolytes in water treatment. *Water Research*, 41(11), pp.2301–2324.

Borges, A. et al., 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial drug resistance (Larchmont, N.Y.)*, 19(4), pp.256–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23480526.

Branda, S.S. et al., 2005. Biofilms: The matrix revisited. *Trends in Microbiology*, 13(1), pp.20–26.

Camper, A.K. et al., 1991. Growth kinetics of coliform bacteria under conditions relevant to drinking water distribution systems. *Applied and Environmental Microbiology*, 57(8), pp.2233–2239.

Carpentier, B. & Cerf, O., 1993. Biofilms and their consequences, with particular reference to hygiene in the food industry. *The Journal of Antimicrobial Chemotherapy*, 75(6), pp.499–511.

Chaves Simões, L. & Simões, M., 2013. Biofilms in drinking water: problems and solutions. *RSC Advances*, 3(8), p.2520. Available at: http://xlink.rsc.org/?DOI=c2ra22243d.

Chong, M.N. et al., 2010. Recent developments in photocatalytic water treatment technology: A review. *Water Research*, 44(10), pp.2997–3027. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0043135410001739.

Costerton, J.W. et al., 1994. Biofilms, the customized microniche. *Journal of Bacteriology*, 176(8), pp.2137–2142.

Crane, R.A. & Scott, T.B., 2012. Nanoscale zero-valent iron: Future prospects for an emerging water treatment technology. *Journal of Hazardous Materials*, 211-212, pp.112–125. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0304389411014531.

Cullex, C., 2000a. Technical Sheet for Strong Anionic. , (3), pp.601–602.

Cullex, C., 2000b. Technical Sheet for Strong Cation. , pp.601–602.

Dankovich, T. a & Gray, D.G., 2011. Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environmental science & technology*, 45(5), pp.1992–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21314116.

Demadis, K.D. et al., 2007. Industrial water systems: problems, challenges and solutions for the process industries. *Desalination*, 213(1-3), pp.38–46. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0011916407003001.

Dror-Ehre, A. et al., 2010. Control of biofilm formation in water using molecularly capped silver nanoparticles. *Water Research*, 44(8), pp.2601–2609. Available at: http://dx.doi.org/10.1016/j.watres.2010.01.016.

Du, X. et al., 2016. Cake properties in ultrafiltration of TiO2 fine particles combined with HA: in situ measurement of cake thickness by fluid dynamic gauging and CFD calculation of imposed shear stress for cake controlling. *Environmental Science and Pollution Research*, 23(9), pp.8806–8818. Available at: http://link.springer.com/10.1007/s11356-015-5984-3.

Edberg, S.C. et al., 2000. Escherichia coli: the best biological drinking water indicator for public health protection. *Symposium series (Society for Applied Microbiology)*, (29), p.106S–116S.

Endall, R.O.J.K., Mith, E.R.E.S. & Olomon, K.E.R.S., 1996. An ecological risk assessment for the use of the biocide, dibromonitrilopropionamide (DBNPA), in industrial cooling systems. , 15(1), pp.21–30.

Enkrott - Gestão e Tratamento de Águas, S.A., 2015. Ficha de dados de segurança EQ BNX 425 - Gestão e Tratamento de Águas, S.A.

Enkrott, S.A., 2015. Estratégias de Bom-Senso para a Minimização do Desenvolvimento de Legionella em Redes e Sistemas de Água.

Ferk, F. et al., 2007. Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations. *Mutagenesis*, 22(6), pp.363–370.

Ferreira, C., Pereira, A.M., et al., 2010. Advances in industrial biofilm control with micro-nanotechnology. , pp.845–854.

Ferreira, C. et al., 2013. Biofilm Control with New Microparticles with Immobilized Biocide. *Heat Transfer Engineering*, 34(8-9), pp.174–179.

Ferreira, C., Rosmaninho, R., Simo, M., et al., 2010. Biofouling control using microparticles carrying a biocide. , 26(2), pp.205–212.

Ferreira, C., Rosmaninho, R., Simoes, M., et al., 2010. Biofouling control using microparticles carrying a biocide. *Biofouling*, 26(2), pp.205–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19937490.

Ferreira, C. et al., 2011. Physiological changes induced by the quaternary ammonium compound benzyldimethyldodecylammonium chloride on Pseudomonas fluorescens. , pp.1–8.

Freem, M.L., 2015. iMedPub Journals Effects of Surfactant Aggregation and Adsorption on Steel Corrosion Inhibition in Salt Solution Abstract I ntroduction. , pp.1–8.

Gabelich, C.J. et al., 2002. Effects of aluminum sulfate and ferric chloride coagulant residuals on polyamide membrane performance. *Desalination*, 150(1), pp.15–30.

Gao, W. et al., 2011. Membrane fouling control in ultrafiltration technology for drinking water production: A review. *Desalination*, 272(1-3), pp.1–8. Available at: http://dx.doi.org/10.1016/j.desal.2011.01.051.

Gilbert, P. & Moore, L.E., 2005. Cationic antiseptics: Diversity of action under a common epithet. *Journal of Applied Microbiology*, 99(4), pp.703–715.

Gogate, P.R., 2007. Application of cavitational reactors for water disinfection: Current status and path forward. *Journal of Environmental Management*, 85(4), pp.801–815.

Gogoi, S.K. et al., 2006. Green Fluorescent Protein-Expressing Escherichia c oli as a Model System for Investigating the Antimicrobial Activities of Silver Nanoparticles. *Langmuir*, 22(22), pp.9322–9328. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17042548.

Gomes, L.C. et al., 2013. Macroscale versus microscale methods for physiological analysis of biofilms formed in 96-well microtiter plates. *Journal of Microbiological Methods*, 95(3), pp.342–349. Available at: http://dx.doi.org/10.1016/j.mimet.2013.10.002.

Guo, H. et al., 2010. Low-pressure membrane integrity tests for drinking water treatment: A review. *Water Research*, 44(1), pp.41–57. Available at: http://dx.doi.org/10.1016/j.watres.2009.09.032.

Gupta, V.K. et al., 2012. Chemical treatment technologies for waste-water recycling—an overview. *RSC Advances*, 2(16), p.6380.

Hammes, F. et al., 2007. Formation of assimilable organic carbon (AOC) and specific natural organic matter (NOM) fractions during ozonation of phytoplankton. *Water Research*, 41(7), pp.1447–1454.

Hasegawa, N. et al., 1998. Preparation and Mechanical Properties of Polypropylene – Clay Hybrids Using a Maleic Anhydride-Modified Polypropylene Oligomer. *Journal of Applied Polymer Science*, 67(96), pp.87–92.

Heidarpour, F., Ghani, W. & Ahmadun, F., 2010. Nano silver-coated polypropylene water filter: II. Evaluation of antimicrobial efficiency. *Dig J Nanomater Biostruct*, 5(3), pp.797–804. Available at: http://www.chalcogen.ro/797_Heidarpour-mss2.pdf.

Hong, S. & Elimelech, M., 1997. Chemical and physical aspects of natural organic matter (NOM) fouling of nanofiltration membranes. *Journal of Membrane Science*, 132(2), pp.159–181.

Hu, J.Y. et al., 1999. The effect of water treatment processes on the biological stability of potable water. *Water Research*, 33(11), pp.2587–2592.

Huang, H. & Schwab, K., 2009. Critical Review Pretreatment for Low Pressure Membranes in Water Treatment : A Review. *Environmental science & technology*, 43(9), pp.3011–3019.

Huang, L. et al., 2016. In situ immobilization of silver nanoparticles for improving permeability, antifouling and anti-bacterial properties of ultrafiltration membrane. *Journal of Membrane Science*, 499, pp.269–281. Available at: http://dx.doi.org/10.1016/j.memsci.2015.10.055.

Iler, R.K., 1966. Multilayers of colloidal particles. *Journal of Colloid and Interface Science*, 21, pp.569–594.

Karger-Kocsis, J. ed., 1995. *Polypropylene Structure, blends and Composites: Volume 3* 1st Editio., Dordrecht: Springer Netherlands. Available at: https://books.google.pt/books?hl=pt-PT&lr=&id=IYP1CAAAQBAJ&oi=fnd&pg=PP9&dq=Polypropylene+&ots=B3VU69ujB2&sig=MmiJCtoxNXmMrT8g135_qwjNzm8&redir_esc=y#v=onepage&q=Polypropylene&f=false.

Kim, B.R. et al., 2002. Literature review - Efficacy of various disinfectants against Legionella in water systems. *Water Research*, 36(18), pp.4433–4444.

Kim, J. & Van Der Bruggen, B., 2010. The use of nanoparticles in polymeric and ceramic membrane structures: Review of manufacturing procedures and performance

improvement for water treatment. *Environmental Pollution*, 158(7), pp.2335–2349. Available at: http://dx.doi.org/10.1016/j.envpol.2010.03.024.

Kim, J.Y. et al., 2008. Enhanced inactivation of E. coli and MS-2 phage by silver ions combined with UV-A and visible light irradiation. *Water Research*, 42(1-2), pp.356–362.

Komlenic, R., 2010. Rethinking the causes of membrane biofouling. *Filtration and Separation*, 47(5), pp.26–28. Available at: http://dx.doi.org/10.1016/S0015-1882(10)70211-1.

Krasner, S.W. et al., 2006. The Occurrence of a New Generation of Disinfection By-Products S1 S2. *Environmental science & technology*, 40(23), pp.7175–7185.

Lee, C. et al., 2008. Bactericidal Effect of Zero-Valent Iron Nanoparticles on Escherichia coli. *Environ Sci Technol.*, 47(43), pp.4927–4933.

Lehtola, M.J. et al., 2003. Impact of UV disinfection on microbially available phosphorus, organic carbon, and microbial growth in drinking water. *Water Research*, 37(5), pp.1064–1070.

Leung, H.W., 2001. Ecotoxicology of glutaraldehyde: review of environmental fate and effects studies. *Ecotoxicology and environmental safety*, 49, pp.26–39.

Li, Q. et al., 2008. Antimicrobial nanomaterials for water disinfection and microbial control : Potential applications and implications. *Water Research*, 42(18), pp.4591–4602. Available at: http://dx.doi.org/10.1016/j.watres.2008.08.015.

Li, Z. et al., 2010. Adsorbed polymer and NOM limits adhesion and toxicity of nano scale zerovalent iron to E. coli. *Environmental Science and Technology*, 44(9), pp.3462–3467.

Liang, H., Gong, W. & Li, G., 2008. Performance evaluation of water treatment ultrafiltration pilot plants treating algae-rich reservoir water. *Desalination*, 221(1-3), pp.345–350.

Liu, C.X. et al., 2010. Modification of membrane surface for anti-biofouling performance: Effect of anti-adhesion and anti-bacteria approaches. *Journal of Membrane Science*, 346(1), pp.121–130.

Madsen, T., Boyd, H. & Nylén, D., 2001. Environmental and health assessment of substances in household detergents and cosmetic detergent products. , (615), p.240. Available at: http://www.mst.dk/udgiv/Publications/2001/87-7944-596-9/pdf/87-7944-597-7.pdf.

Malaeb, L. & Ayoub, G.M., 2011. Reverse osmosis technology for water treatment: State of the art review. *Desalination*, 267(1), pp.1–8. Available at: http://dx.doi.org/10.1016/j.desal.2010.09.001.

Mansouri, J., Harrisson, S. & Chen, V., 2010. Strategies for controlling biofouling in membrane filtration systems: challenges and opportunities. *Journal of Materials Chemistry*, 20(22), p.4567.

Matilainen, A., Vepsalainen, M. & Sillanpaa, M., 2010. Natural organic matter removal by coagulation during drinking water treatment: A review. *Advances in Colloid and Interface Science*, 159(2), pp.189–197. Available at:

http://dx.doi.org/10.1016/j.cis.2010.06.007.

McCoy W.F. & Wireman J.W., 1989. Efficacy of bromochlorodimethylhydantoin againt Legionella pneumophila in industrial cooling water. *Journal of Industrial Microbiology*, 4, pp.403–408. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed2&AN=1989275943.

McDonnell, G. & Russell, A.D., 1999. Antiseptics and Disinfectants : Activity , Action , and Resistance. *Clinical Microbiology Reviews*, 12(1), pp.147–179.

Mcguffie, M.J. et al., 2015. Zinc Oxide Nanoparticle Suspensions and Layer-by-Layer Coatings Inhibit Staphylococcal Growth. *Nanomedicine: Nanotechnology, Biology and Medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S1549963415001926.

Melo, L.F. & Bott, T.R., 1997. Biofouling in water systems. *Experimental Thermal and Fluid Science*, 14(96), pp.375–381.

Meng, F. et al., 2009. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research*, 43(6), pp.1489–1512. Available at: http://dx.doi.org/10.1016/j.watres.2008.12.044.

Nguyen, T., Roddick, F. & Fan, L., 2012. Biofouling of Water Treatment Membranes: A Review of the Underlying Causes, Monitoring Techniques and Control Measures. *Membranes*, 2(4), pp.804–840. Available at: http://www.mdpi.com/2077-0375/2/4/804/.

Nir, S. & Reches, M., 2016. Bio-inspired antifouling approaches: the quest towards non-toxic and non-biocidal materials. *Current opinion in biotechnology*, 39(Figure 2), pp.48–55. Available at: http://www.sciencedirect.com/science/article/pii/S0958166915001755.

Page, K. et al., 2006. Titania and silver–titania composite films on glass—potent antimicrobial coatings. *The Royal Society of Chemistry*, 17, pp.95–104.

Parrota, M.J. & Bekdash, F., 1998. UV Disinfection of small groundwater supplies. *American Water Works Association*, 90, pp.71–81.

Pendergast, M.M. & Hoek, E.M.V., 2011. A review of water treatment membrane nanotechnologies. *Energy & Environmental Science*, 4(6), p.1946.

Pereira, A. et al., 2009. Fouling and cleaning monitoring using the MSS - Industrial perspective. *Heat Exhanger Fouling and Cleaning VIII*.

Purolite, 2016a. IP4 PP. , p.229334.

Purolite, 2016b. IP9 PVC. , p.229334.

Qi, L. et al., 2004. Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydrate Research*, 339(16), pp.2693–2700.

Qu, X. et al., 2013. Nanotechnology for a safe and sustainable water supply: Enabling integrated water treatment and reuse. *Accounts of Chemical Research*, 46(3), pp.834–843.

Rabea, E.I. et al., 2003. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules*, 4(6), pp.1457–1465.

Rafoth, A. et al., 2007. Analysis of isothiazolinones in environmental waters by gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1164(1-2), pp.74–81.

Rai, M., Yadav, A. & Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), pp.76–83. Available at: http://dx.doi.org/10.1016/j.biotechadv.2008.09.002.

Richardson, J.J., Bjornmalm, M. & Caruso, F., 2015. Technology-driven layer-by-layer assembly of nanofilms. *Science*, 348(6233), pp.aaa2491–aaa2491. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.aaa2491.

Richardson, S.D., 2003. Disinfection by-products and other emerging contaminants in drinking water. *TrAC - Trends in Analytical Chemistry*, 22(10), pp.666–684.

Rozin, P. et al., 2015. Psychological aspects of the rejection of recycled water: Contamination, purification and disgust. *Judgment and Decision Making*, 10(1), pp.50–63. Available at: http://search.proquest.com/openview/2f74b7e3d679ef0ee97c402651acf6c6/1?pq-origsite=gscholar\npapers3://publication/uuid/D0839BBA-27D2-472F-BCEF-13EFA50B8774.

Rycroft, R.J.G. & Penny, P.T., 1983. Swimming pool wheezing Dermatoses associated with brominated swimming pools glucose concentrations in healthy. *Medical Journal*, 287(August), pp.461–462.

Saad, M.A., 1992. Biofouling prevention in RO polymeric membrane systems. *Desalination*, 88(1-3), pp.85–105.

Schlichter, B., Mavrov, V. & Chmiel, H., 2004. Study of a hybrid process combining ozonation and microfiltration/ultrafiltration for drinking water production from surface water. *Desalination*, 168(1-3), pp.307–317.

Shannon, M.A. et al., 2008. Science and technology for water purification in the coming decades. *Nature*, 452(7185), pp.301–310. Available at: http://www.nature.com/doifinder/10.1038/nature06599.

SIGMA-ALDRICH, 2016. CAS 139-07-1. , pp.97–99.

SIGNMA-ALDRICH, 2016. CAS 69011-19-4.

Silvestry-Rodriguez, N. et al., 2008. Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. *Applied and Environmental Microbiology*, 74(5), pp.1639–1641.

Simões, M., Pereira, M.O. & Vieira, M.J., 2005. Validation of respirometry as a short-term method to assess the efficacy of biocides. *Biofouling*, 21(1), pp.9–17.

Smith, D.W., 2002. Advanced technologies in water and wastewater treatment. *Journal of Environmental Engineering and Science*, 1(4), pp.247–264.

Song, K.G., Kim, Y. & Ahn, K.H., 2008. Effect of coagulant addition on membrane fouling and nutrient removal in a submerged membrane bioreactor. *Desalination*,

221(1-3), pp.467–474.

Storey, M. V., van der Gaag, B. & Burns, B.P., 2011. Advances in on-line drinking water quality monitoring and early warning systems. *Water Research*, 45(2), pp.741–747. Available at: http://dx.doi.org/10.1016/j.watres.2010.08.049.

Sukhorukov, G.B. et al., 1998. Layer-by-layer self assembly of polyelectrolytes on colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 137(1-3), pp.253–266.

Tayel, A.A., El-Tras, W.F. & Elguindy, N.M., 2016. The potentiality of cross-linked fungal chitosan to control water contamination through bioactive filtration. *International Journal of Biological Macromolecules*, 88, pp.59–65. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0141813016302422.

The Dow Chemical Company, ., 2014. FILMTEC Membrane Description Technical Sheet. , (ID 490).

Ullah, S., 2011. *Biocides in Papermaking Chemistry*. University of Jyvaskyla.

Volk, C. et al., 2000. Impact of enhanced and optimized coagulation on removal of organic matter and its biodegradable fraction in drinking water. *Water Research*, 34(12), pp.3247–3257.

Vrouwenvelder, J.S. et al., 2008. Quantitative biofouling diagnosis in full scale nanofiltration and reverse osmosis installations. *Water Research*, 42(19), pp.4856–4868. Available at: http://dx.doi.org/10.1016/j.watres.2008.09.002.

Vrouwenvelder, J.S. & van der Kooij, D., 2003. Diagnosis of fouling problems of NF and RO membrane installations by a quick scan. *Desalination*, 153(1-3), pp.121–124.

Wessels, S. & Ingmer, H., 2013. Modes of action of three disinfectant active substances: A review. *Regulatory Toxicology and Pharmacology*, 67(3), pp.456–467. Available at: http://dx.doi.org/10.1016/j.yrtph.2013.09.006.

WHO, 2000. *Disinfectants and disinfectant by-products*, Available at: www.who.int/ipcs/publications/ehc/ehc_216/en/.

WHO, 2004. Factors Affecting Disinfection.

Williams, M.M. & Braun-Howland, E.B., 2003. Growth of Escherichia coli in Model Distribution System Biofilms Exposed to Hypochlorous Acid or Monochloramine Growth of Escherichia coli in Model Distribution System Biofilms Exposed to Hypochlorous Acid or Monochloramine. *Applied and environmental microbiology*, 69(9), pp.5463–5471.

Williams, T.M., 2007. The Mechanism of Action of Isothiazolone Biocides. *PPChem*, 9(1), pp.14–22.

Ye, W. et al., 2006. Durable antibacterial finish on cotton fabric by using chitosan-based polymeric core-shell particles. *Journal of Applied Polymer Science*, 102(2), pp.1787–1793.

Appendix

A.1. Estimation of the number of particles in the stock solution

A.1.1 Cationic Resin

Cationic Resin is a spherical particle with a medium diameter of 0.0725 cm, the volume of each particle was determined based on the Equation 1:

$$V = \frac{4}{3} \times \pi \times r^3 \quad \text{Eq. 1}$$

According with the manufacture, the particles density is 1.08 g/cm^3, and using the Equation 2, it was determined the mass of a single particle as 2.15 x 10^{-4} g (Cullex 2000b).

$$\rho = \frac{m}{V} \quad \text{Eq. 2}$$

A.1.2. Anionic Resin

Anionic Resin is also spherical particle with a medium diameter of 0.075 cm, the volume of each particle was determined based on the Equation 1. And using the information presented by the manufacture along with the Equation 2, the mass of a single particle was determined as 2.85 x 10^{-4} g (Cullex 2000a).

A.1.3. Polypropylene

The Polypropylene particles were described by the manufacture as cylindrical particles and with a medium diameter of 0.13 cm and a height of 0.12 cm, therefore their volume was determined based on the Equation 3:

$$V = \pi \times r^2 \times h \quad \text{Eq. 3}$$

Taking into account the information in the data sheet of the product and cconsidering the water density as 1.00 g/cm^3, the particles density was determined as 0.85 g/cm^{3}along with the mass of a single particle as 1.35 x 10^{-3} g (Purolite 2016a).

A1.4. Polyvinyl Chloride

The PVC particles were described by the manufacture as cubic particles and with a medium diameter of 0.4 cm, thus their volume was determined based on the Equation 4:

$$V = r^3 \qquad \text{Eq. 4}$$

Taking into account the information in the data sheet of the product and cconsidering the water density as 1.00 g/cm^3, the particles density was determined as 1.525 g/cm^{3}along with the mass of a single particle as 9.76 x 10^{-2} g (Purolite 2016b).

A.2. Assessment of *E. coli* membrane integrity due to propidium iodite uptake pictures

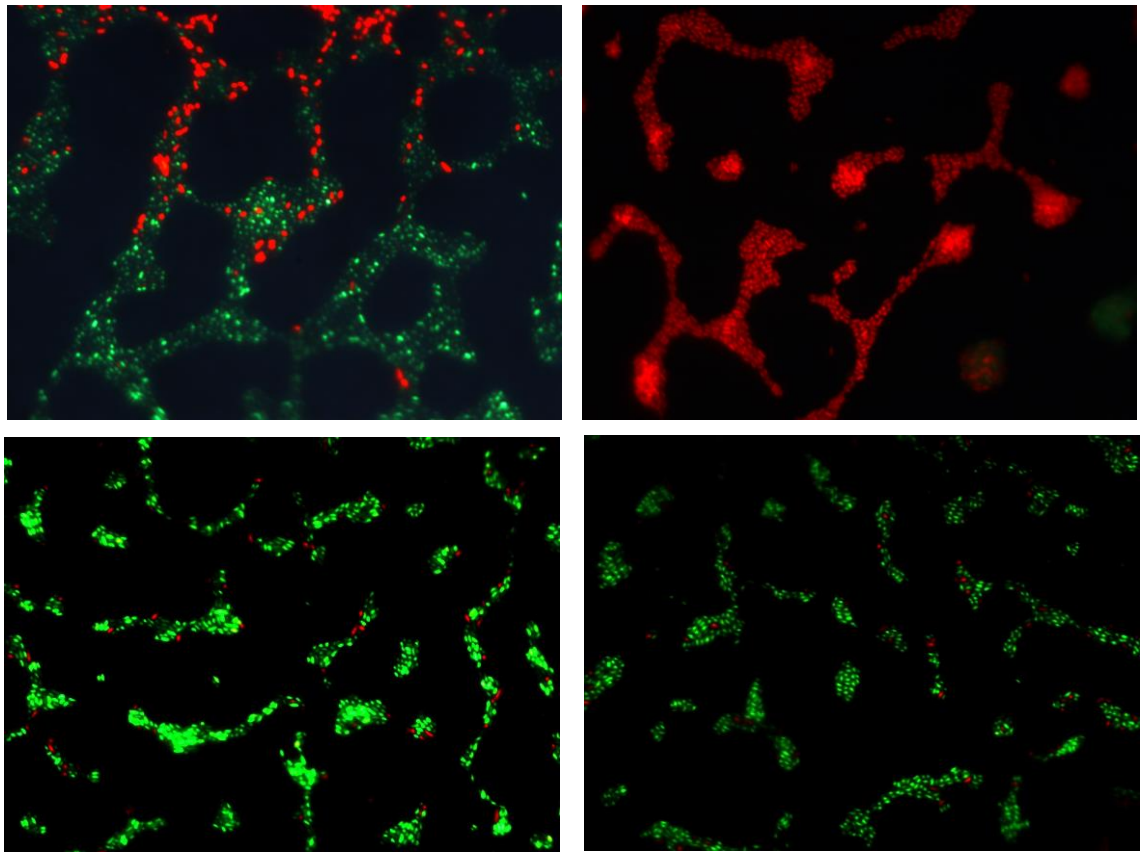

Figure 20 - Epifluorescence photomicrographs of *E. coli* x100. a) and b) with PP-PEI/PSS/BDMDAC-1-hour-of-contact treatment; c) and d) control without particles or BDMDAC.