# Advances in industrial biofilm control with micro-nanotechnology

**Ferreira C., Pereira A. M., Melo L. F, Simões M.***

LEPAE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
*To whom correspondence should be addressed: mvs@fe.up.pt

When attached to surfaces, aggregated microorganisms (predominantly bacteria) and their extracellular polymeric substances are commonly known as biofilms. They are ubiquitous in nature and its formation is part of a strategy that bacteria use in order to survive in hostile environments. Although biofilms play crucial roles in many processes (biodegradation of environmental pollutants or the microbial balance within the human body), they are often unwanted and cause serious problems in the food, biomedical, environmental and industrial fields. Biofilms can lead to increase in the costs of production and equipment maintenance, as well as to public health and environmental concerns and impacts.

Although various methods of biofilm control exist, these methods present limitations high cost and often fail to remove biofilms from surfaces, contributing to the dissemination of resistant microorganisms. The control of undesirable biofilms often includes the use of chemical products with antimicrobial properties such as biocides and surfactants. However, these products can be harmful to the environment and consequently they should be used in small quantities as possible. Moreover, and besides the fact that biocidal products used hitherto are not completely consumed by the interaction with the microorganisms, they can cause serious environmental and public health concerns. In the past few decades there has been a dilemma between effective disinfection and formation of harmful disinfection by-products.

Advances in the nanotechnology science led to the interest in its environmental and biological applications. Nanotechnology permits to create artificial systems with enormous potential for numerous applications. The aim here, in a biofilm control context is the development of nanostructures with functionalized surfaces that carry the antimicrobial agent. This will save significant amounts of the antimicrobial agent, prevent undesirable reactions with other components that can lead sometimes to the formation of potentially carcinogenic compounds (organochlorinated compounds), and finally will reduce the costs of wastewater treatment due to the presence of residual concentrations of biocides in the effluent. Several methodologies to develop functionalized micro and nanoparticles are available, such as the layer-by-layer method, which represent a significant advance, in terms of surface science applications, to the field of biofilm science and engineering.

## Biofilm formation and relevance

Biofilms can form on solid or liquid abiotic surfaces as well on soft tissue in living organisms and are typically resistant to conventional methods of disinfections (Huang et al. 2009) These biofilms are predominantly composed of bacteria cells enclosed within an extracellular polymeric (EPS) matrix which they produce (Davies et al. 1998; Decho 2010). The EPS matrix is the responsible for the cohesion (keeping the cells attached to one another) and the adhesion (to surfaces) of the biofilm and is composed essentially of polysaccharides and proteins (Simões et al. 2010). Biofilms are ubiquitous in nature and its formation is a strategy that bacteria use in order to survive in hostile environments.

Biofilms start to develop when bacteria or other microorganism attach to a surface. The polymers produced by the microbial cells play a determinant role in the adhesion process, sometimes creating a “polymer bridge” between the cells and the molecules adsorbed at the surface that strengthens the attached layer. The main processes involved in the biofilm build-up are: formation of a conditioning film; transport of the microrganisms from the bulk liquid to adhesion surface; initial adhesion of microorganisms to the surface; biofilm growth; biofilm maturation (equilibrium between the accumulation and detachment) (Melo 2003; Simões et al. 2010) (Fig. 1). The conditioning film is the first stage, preceding the formation of the bacterial film. The next stage is microbial first adhesion, at this stage nonspecific physicochemical force of interaction act between the molecules and microorganisms and the surface like: van der Waals forces, hydrophobic, electrostatic and London dispersion forces. Then irreversible adhesion begins when the cells becomes irreversibly attached to surface, and to each other they start to excrete the extracellular polymers which form the EPS matrix (Nikolaev and Plakunov 2007). Under favorable conditions biofilm growth continues for long time with loss of some cells and liberation of aggregates (detachment).

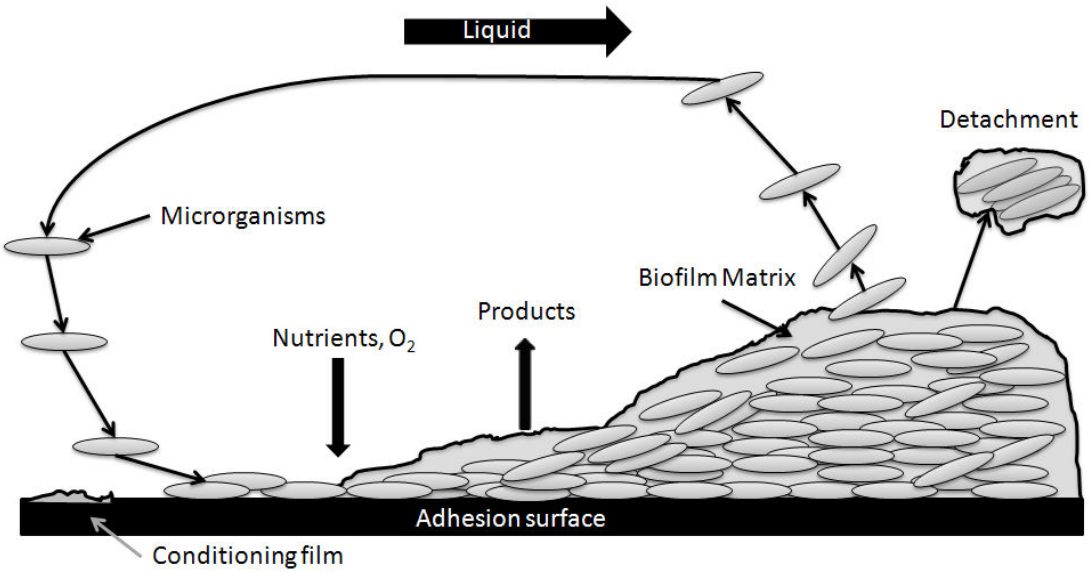


**Figure 1**: Schematic representation of biofilm formation (adapted from Melo 2003).

Some factors affect the formation and properties of biofilms, such as: characteristics of the microbial species and strains; composition and roughness of the surface material where the microorganisms attached; liquid composition, its pH, temperature and ionic strength; hydrodynamic of the fluid (velocity and turbulence) (Melo 2003).

Despite all the efforts and advances in science many health problems are still consequence of the lack of fresh and clean water. Microbial water pollution is a major problem both in industrialized and developing countries, with waterborne diseases (such as intestinal infections and diarrhea) remaining a significant cause of death (Li et al. 2008). Biofilms represent one major water contaminant as the majority of the microorganisms are comprised within these structures (Manuel et al. 2010). Biofouling also affects seriously energy and food production as well as the biomedical, environmental, industrial and economic fields (Shannon et al. 2008). From the economic and environmental point of view, three of the most important examples of industrial biofouling occur in membrane systems, cooling water tubes and heat exchanger channels (Ferreira et al. 2010; Melo and Flemming 2010). Biofilms can cause reduction of heat transfer, increased pressure drop, increased corrosion of metallic surfaces, contamination of the fluids flowing through the tubes and channels and of potable water produced in membrane systems, increased environmental costs associated to the need to treat wastewater containing the chemicals not consumed by the interaction with the microorganisms and increased costs of equipment cleaning and disinfection (Melo and Flemming 2010).

Although biofilms play crucial roles in many processes (like the biodegradation of environmental pollutants or the microbial balances within a body) they often are unwanted and cause serious problems in various areas, including the industrial and biomedical fields, which can lead to increase in the costs of production and maintenance, as well as to public health and environmental concerns and impacts. This leads to an increased use of antimicrobial chemicals, such as biocides, to control the unwanted formation of biofilms. In order to maximize the yield of action of biocides and to design efficient antimicrobials there is a growing need to comprehend not only the mechanisms of action of biocides, but also the mechanisms of resistance of bacterial cells (Russell 2002).

## Strategies for biofilm control

In industry, the operations of cleaning and disinfection are essential parts of the production process and the efficiency with which these operations are performed greatly affects the final product quality (Carpentier and Cerf 1993). Most cleaning regimes include removal of loose soil with cold or warm water followed by the application of chemical agents, rinsing, and disinfection. High temperatures can reduce the need for physical force (Carpentier and Cerf 1993; Maukonen et al. 2003). Chemical agents, usually surface active agents or alkali compounds, used as detergents, suspend and dissolve contaminant residues by decreasing surface tension, emulsifying fats, and denaturing proteins (Maukonen et al. 2003). These chemical agents are currently used in combination. Many situations require the occasional use of acid cleaners to clean surfaces soiled with precipitated minerals or having high mineral content. Mechanical action (water turbulence and scrubbing) are recognized as being highly effective in eliminating biofilms (Chmielewski and Frank 2003; Maukonen et al. 2003). An effective cleaning procedure must break up or dissolve the extracelular polymeric matrix associated with the biofilm so that disinfectants can gain access to the viable cells (Carpentier and Cerf 1993; Gibson et al. 1999). The cleaning process can remove 90 % or more of microorganisms associated with the

 

surface, but cannot be relied upon to kill them. Bacteria can redeposit at other locations and, given time, water and nutrients can form a biofilm. Therefore, disinfection must be implemented (Srinivasan et al. 1995; Gibson et al. 1999).

Disinfection is the use of antimicrobials chemicals to destroy microorganisms. This is required, since wet surfaces provide favorable conditions for the growth of microorganisms (Maukonen et al. 2003). The aim of disinfection is to reduce the surface population of viable cells after cleaning and prevent microbial growth on surfaces before restart of production. Disinfectants do not penetrate the biofilm matrix left on a surface after an ineffective cleaning procedure, and thus do not destroy all the living cells in biofilms (Holah 1992; Carpentier and Cerf 1993). Disinfectants are more effective in the absence of organic material (fat, carbohydrates, and protein based materials). Interfering organic substances, pH, temperature, water hardness, chemical inhibitors, concentration and contact time generally control the efficacy of disinfectants (Mosteller and Bishop 1993; Cloete et al. 1998). The disinfectants must be effective, safe and easy to use, and easily rinsed off from surfaces, leaving no toxic residues that affect the sensory values of the product. Table 1 resumes the properties of disinfectants commonly used in industrial systems. The disinfectants to be used should be chosen based on the following statements (Wirtanen 1995; Wirtanen et al. 2000):

- is the disinfectant effective in the pH range used?
- is the disinfectant stable when diluted?
- is the disinfectant toxic, safe or irritating?
- what is the microbial spectrum of the disinfectant?
- how does the temperature affect the activity of the disinfectant?
- is the disinfectant corrosive at the surface?
- is the disinfectant surface active?
- is the disinfectant stable when reacting with organic material?

**Table 1** Chemical disinfectants commonly used in industry (based on Banner 1995; Wirtanen 1995)

| Disinfectant type | Applications |
|---|---|
| **Chlorine** | Neutral/alkaline conditions; stainless steel; food contact surfaces; floors/walls/air; clean-in-place (CIP), spray, soak, fog |
| **Chlorine dioxide** | Water treatment/slime/odour control; rinse for fruit/vegetables; acid form on food contact surfaces; stainless steel; CIP, spray, soak |
| **Iodine** | Acid conditions, < pH 3; stainless steel, plastics; food contact surfaces; floors/walls; CIP, spray, soak, manual; hand disinfectant; carbon dioxide atmosphere; helps dissolve mineral deposits |
| **Anionic surfactants at acid conditions** | Acid conditions, < pH 3; carbon dioxide atmosphere; stainless steel, plastics; foam on external surfaces; CIP, spray, soak, manual; carbon dioxide atmosphere; overnight disinfection; bilkstone/beerstone removal |
| **Peracetic acid** | Acid conditions; carbon dioxide atmosphere; stainless and mild steel, soft metals, plastic, rubber; food contact surfaces; CIP, spray, soak |
| **Quaternary ammonium compounds (cationic surfactants)** | Neutral/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas/residue can extend activity; mildew and odour control; water treatment; spray, soak, manual, circulation |
| **Amphoteric surfactants** | Neutral/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas; spray, manual soak; fog air; foam is suitable for external surface disinfection |
| **Polymeric biguanides** | Acid/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas; can/bottle warmers, water treatment; spray, soak, manual, circulation; fog air |
| **Glutaraldehyde** | Neutral/alkaline conditions; non-corrosive to all materials; water treatment/slime control in can/bottle warmers, tunnel pasteurizers; glycol and sweetwater systems in dairies; conveyor lines |
| **Isothiazolinones** | Acid, alkaline, neutral conditions; applicable to all materials; cooling water/towers, can/bottle warmers; long-term, continuous activity; conveyor lubricants |
| **Phenolics** | Lubricants for conveyor lines; water treatment |
| **Hydrogen peroxide** | Applicable to all materials; sporicide at high concentration at high temperature; aseptic packing of beverages |

Although the present chapter is particularly focused on the rational use of antimicrobial agents, a full strategy to control biofilms should include a toolbox of eco-friendly preventive methodologies that includes new antimicrobial

agent techniques, innovation in equipment and process design and development of efficient and reliable on-line monitoring techniques (Pereira and Melo 2009).

## Mechanisms of action of biocides

The word biocide is a general term to describe a chemical agent with antiseptic, disinfectant or preservative activity, that inactivate microorganisms (Russell 2003). Some are capable of destroying the microorganisms (e.g. bactericidal and fungicidal) whilst others can only prevent or inhibit their growth (e.g. bacteriostatic and fungistatic) (McDonnell and Russell 1999). Biocides have a broad spectrum of usage and differ from antibiotics in their lack of selective toxicity (Denyer and Stewart 1998; McDonnell and Russell 1999). In fact, they have multiple biochemical targets and have been used over the years in a diversity of areas (Simões et al. 2007). In medicine, biocides are used to prevent or limit microbial infections, preoperative skin disinfection or surface disinfection and in industrial systems they are used to prevent biofouling. But there are a lot more applications for these products. As examples, they are essential in food, pharmaceutical and cosmetic industries to prevent microbial contaminations (Russell 2003; Masaadeh and Jaran 2009). For their further design and development, it is vital to understand its mechanism of action as well as the mechanisms of resistance of bacteria.

Despite the final effect of biocides, they all undergo a sequenced series of step: uptake of biocide by cell, biocide partition to targets and its concentration at those targets and finally the damage of the targets (Denyer 1995). The mechanism of action of these products can be divided into four wide categories: the oxidants (that act via radical-mediated reactions eliciting the oxidation of organic material), the electrophilics (that will covalently react with cellular nucleophiles thus inactivating some enzymes), the weak acids (that will interfere with the cell membrane ability to maintain the pH balance leading to the failure of the cell metabolism as a consequence of the acidification of the interior of the cell) and the cationic membrane active biocides (that weaken membranes as far as cell lysis) (Chapman 2003).

Within bacterial cells, the biocides principal targets are the cell wall, cytoplasmic membrane and cytoplasm (Denyer 1990). The main target is arguably the cytoplasmic membrane. Cell wall (composed by a network of peptidoglycan with a lipopolysaccharide overlayer in Gram-negative bacteria) (Fig. 2) little more provides other than structural integrity and although there are a large number of molecules that allow cells to grow and multiply themselves (such as ribosomes, enzymes and other proteins) this molecules are probably not primary targets since biocides would have to penetrate inside the cell to reach them (Denyer 1990; Maillard 2002). Thus, the cytoplasmic membrane is considered the main target of biocides. This membrane is composed by phospholipids forming a bilayer and some proteins embedded this bilayer sheet, and it regulates the passage of solutes and metabolites in and out the cytoplasm (Maillard 2002). Interaction of the biocide with the cytoplasmic membrane often causes changes both in membrane structure and function manifested by phenomena like disruption of the membrane, dissipation of the proton motive force, inhibition of the respiration reactions and membrane-associated enzymes activity and loss of membrane integrity with consequent leakage of essential intracellular constituents (Denyer 1990; Denyer and Stewart 1998; Maillard 2002) , to name a few.

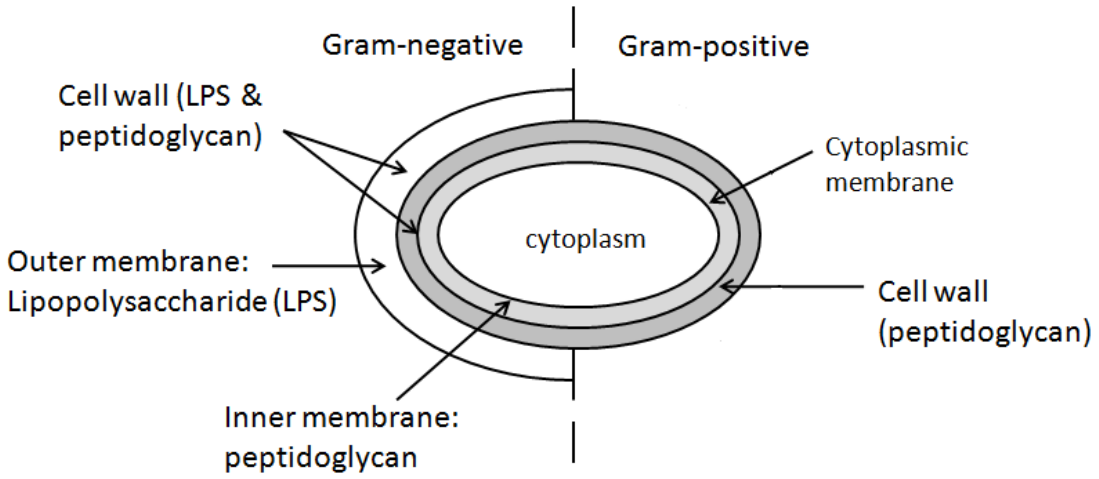


**Figure 2** – Bacterial cell wall composition (adapted from Denyer 1995).

## Bacterial mechanism of resistance

Biofilm resistance to biocides is increasing thus becoming a serious problem. Often resistant bacteria are difficult to eradicate or even to treat. This brings a huge economic and environmental implication. In medicine, resistant bacteria are becoming frequent in hospitals and other healthcare institutions which can have serious consequences in patients,

 

especially critically ill ones (Tenover 2006). But the extend of resistant bacteria to other areas can have adverse implications in processes like cooling water systems, drinking-water distribution and food processing (Cloete 2003). Some explanations for bacterial resistance include the limited penetration of antimicrobial compounds due to the presence of the EPS matrix, the imposition of nutrient deficiency in deeper biofilm bacteria, the transformation of the antimicrobial products into a non-toxic form by enzymes localized in the biofilm matrix, the expulsion of biocide by the biofilm physical barrier to penetration preventing antimicrobials to reach its target(s) by size exclusion. But due to the charged nature of the matrix, diffusion of antimicrobial though the biofilm can be limited by chemical (ionic) interactions between biofilm and the matrix components, that will act as an ion-exchange resin (Cloete 2003; Gilbert et al. 2003; Simões et al. 2010). Within biofilms not all the cells receive the same amount of nutrients. Deep-lying cells are exposed to lower concentrations of substrates than outlying ones. This nutrient limitation will reduce the growth rates. Therefore biofilm cells will exist in a slow-growing state and in different metabolic states, making them less susceptible to antimicrobials (Costerton et al. 1999; Gilbert et al. 2002).

As mentioned before, bacterial genetic adaptation is another form of biofilm resistance to biocide. Bacteria may become resistant to antimicrobials by spontaneous mutation or by acquiring the genetic information for resistance from other bacteria. Mutations in the genetic information can translate in modification of the antimicrobial product target protein, production of specific enzymes that will inactivate the biocide, alteration of outer membrane protein channel required for the antimicrobial entry in cells or expression of efflux pumps (Tenover 2006) that will extrude toxic substances for the bacteria (including therapeutic drugs) out of the cell. DNA and genetic technology allowed the identification of many genes in bacteria that confer resistance to biocides (Webber and Piddock 2003).

Overall, Gram-negative bacteria (such as *Escherichia coli* and *Pseudomonas fluorescens*) are more resistant to biocides than the Gram-positive ones and this difference in sensibility has been attributed to the fact that the Gram-negative bacteria possess an additional outer membrane (OM) that acts as an extra permeability barrier (Morton et al. 1998; McDonnell and Russell 1999; Russell 1999). The presence of lipopolysaccharide (LPS) molecules in the outer leaflet prevents the access of hydrophilic molecules (including antibiotics and biocides) to the cell interior (Morton et al. 1998; McDonnell and Russell 1999). Moreover, in this OM there are a wide number of proteins (outer membrane proteins (OMP)) that, although are responsible for the entrance of hydrophobic antimicrobials to the cell, are associated to bacterial resistance given that development of resistance to this products is related not only to loss of some OMP but also to the over-expression of OMPs (resistance OMPs) (Masuda et al. 1995; Winder et al. 2000).

## Micro and nanotechnology in biofilm control

The conventional methods hitherto applied for water disinfection and decontamination have been effective in the control of microbial pathogens. However, new problems are being associated to them. Besides requiring a considerable economic effort and expensive infrastructures (Shannon et al. 2008), the chemical disinfectants are responsible for the production of harmful disinfection by-products (DBP). Chemicals such as free chorine, chloramines and ozone can react with diverse natural water constituents thus forming DBPs, many of which are toxic and/or carcinogenic (Li et al. 2008; Shannon et al. 2008). For these reasons, and in order to successfully control waterborne pathogens in water, it is imperative the development of new biofilm control strategies. Advances in the micro-nanotechnology field promoted significant interest in its environmental and biological applications.

Nanotechnology can be defined as the engineering and utilization of material, structures, devices and systems at the atomic, molecular and macromolecular scale. Nanomaterials and nanostructures have nanoscale dimensions roughly between 1 and 100 nm and frequently exhibit novel and significantly physical, chemical and biological changed proprieties and functions resulting from their small structures (Roco 2003; Theron et al. 2008). They are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity (Li et al. 2008). Some of these nanomaterials are engineered to perform specific tasks, being able even to respond to outside signals by changing their structure and properties. For this, they are label as “smart” materials (Ratner and Ratner 2003).

The field that applies this technology and particles to understand and transform biosystems can be defined as nanobiotechnology (Roco 2003). Nanotechnology can present a unique alternative as detection methods of bacterial targets, in particularly nanosensors that could directly detect the presence of a particular pathogen agent or indirectly detect them through the assessment of the pathogens metabolic activity (e.g. by monitoring the nutrients consumption rate) (Kaittanis et al. 2008). Several engineered and natural nanomaterials have shown strong antimicrobial properties, including titanium dioxide, silver nanoparticles and chitosan. Chitosan derives from chitin (a natural polysaccharide abundant in arthropod sells) and has been recently engineered into nanoparticles (Qi et al. 2004). One of the antimicrobial mechanisms proposed for chitosan involves the interaction of positively charged chitosan molecules with negatively charged cell membranes, leading to an increased of membrane permeability and ultimately cell membrane rupture with consequent linkage of intracellular constituents (Li et al. 2008). Metal nanoparticles such as palladium, platinum, silver and gold nanoparticles are very attractive due to their unique physical and chemical properties. In fact, gold nanoparticles have been widely used to construct biosensors because of their ability to immobilize biomolecules (Du et al. 2007). Nanoparticles present other advantages like high reactivity, unique interactions with biological systems, small size and large surface to volume ratio optimized for mass loading and carrying of antimicrobials (Weir et

al. 2008; Taylor and Webster 2009). Antimicrobials can be loaded into nanoparticles by physical encapsulation, adsorption or chemical conjugation and this can present several advantages such as significantly improve the activity of the antimicrobial, in contrast to the free product, and release of antimicrobial at a sustained and controlled manner (Zhang et al. 2008; Zhang et al. 2010). From several types of nanoparticles for antimicrobial delivery application, polymeric nanoparticles present the advantages of being structurally stable and of having on their surfaces functional groups that can easily be chemically modified with both antimicrobials and targeting ligands (Davis et al. 2008; Zhang et al. 2010).

Nanoscience and its application are very recent fields and fundamental properties of nanoparticles are being discovered every day. Further studies and investigation are still needed but the ability of nanoparticles to penetrate the biofilm, enter the cells and affect their biochemical functions makes them potential tools in biofilm control.

## Layer-by-layer technique

The layer-by-layer (LbL) self-assembly of oppositely charged polyelectrolytes onto colloidal particles has been used to create novel nano- and microparticles with well controlled size and shape, finely tuned wall thickness and variable wall compositions (Decher 1997; Caruso et al. 1998; Donath et al. 1998; Cordeiro et al. 2004). The original method was introduced in 1991 by Decher and co-workers for the construction of pure polymer multilayer films on planar supports (Caruso 2001).

This technique uses electrostatic attraction and complex formation between polyanions and polycations to form supramolecular multilayer assemblies of polyelectrolytes. The first stage of shell fabrication involves step-wise deposition of polyelectrolytes from aqueous solutions. The polyelectrolyte multilayer film is formed by the alternate adsorption of oppositely charged layers on to the particle. After each adsorption step, the non adsorbed polyelectrolyte in solution is removed by repeated centrifugation or filtration and washing (Fig. 3) (Donath et al. 1998).

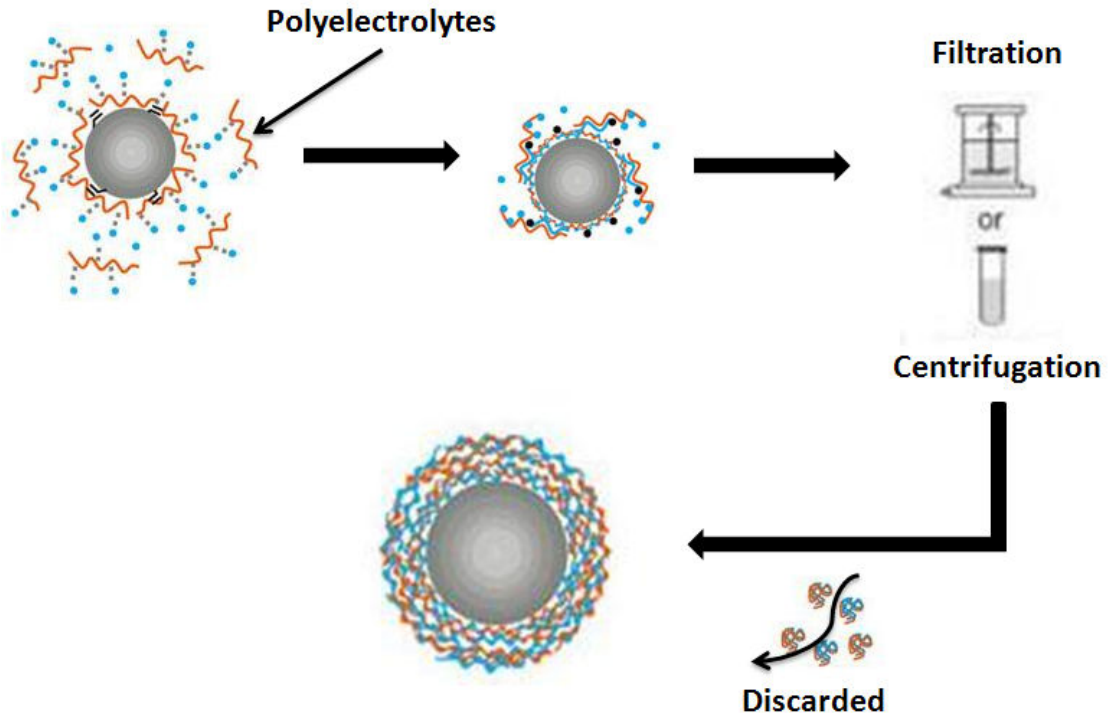


**Figure 3** – Schematic representation of LbL technique. Polyelectrolyte added into a system adsorbs onto the template leading to the charge reversion. After removal of polyelectrolyte excess (by washing of flat substrate or filtration or centrifugation of colloidal cores), oppositely charged polyelectrolyte is added. The cycle is repeated to obtain a multilayer film or shell (adapted from Feng et al. 2007).

At present, there are two general approaches to encapsulate macromolecules into polyelectrolyte capsules using the LbL technique. The first method consists of formation of particles out of molecules subjected to encapsulation. Dyes and drug nanocrystals were used to template LbL assembly leading to encapsulation. The second approach for encapsulation of macromolecules exploits preformed hollow capsules and incorporates the macromolecules from the surrounding medium by switching the permeability of the hollow capsule shell (Volodkin et al. 2004).

 

Recently our group developed microparticles with biocidal properties. The methodology proposed was based on the use of microparticles with functionalized surfaces that act as carriers for antimicrobial molecules. The biocide used was the quaternarium ammonium compound (QAC) benzildimethyldodecyl ammonium chloride (BDMDAC). An advantage of the proposed methodology is that the retention of the antimicrobial on the particle surfaces makes it easier to recover and reuse the non-spent product, therefore substantially reducing the environmental load in the discharged water.

This microparticles was prepared using a LbL technique. The oppositely charged electrolytes, polyethyleneimine (PEI), sodium polystyrene sulfonate (PSS) and benzyldimethyldodecyl ammonium chloride (BDMDAC) were assembled on PS (polystyrene) cores, in a process that comprises 3 steps (Fig. 4). PS particles were allowed to interact with the PEI solution (1 mg/mL in borate buffer solution) for 20 min, and then washed in 0.1 M borate buffer solution pH 9 to remove the excess polymer. After this procedure, the core positively charged was used for the deposition of the polyanion PSS, followed by the BDMDAC, both solutions at 1 mg/mL in borate buffer pH 9. The adsorption steps were carried by adding the polymer solution to the PS cores for 20 min, centrifuging at 2880 g for 4 min and resuspending them in borate buffer pH 9. This step was repeated twice (Ferreira et al. 2010).

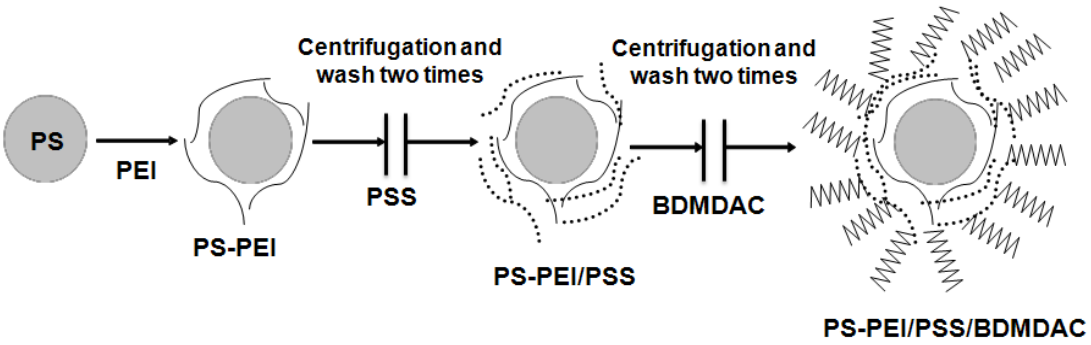


**Figure 4** – Schematic representation of the particles production process. (PEI-polyethyleneimine, PSS- sodium polystyrene sulfonate and BDMDAC- benzyldimethyldodecyl ammonium chloride).

In order to assess the effect of the BDMDAC in coated particles three concentrations were test (0.87, 6.33, 11.75 mg/L) for 60 minutes against biofilm of the *Pseudomons fluorescens* (Fig. 5). *Pseudomonas* spp. are known to be good biofilm producers and major microorganisms found in industry (Simões et al. 2005; Simões et al. 2008). The biofilm control effects of the BDMDAC coated particles (PS-PEI/PSS/BDMDAC) were tested by comparison with the effect of the non-coated particles and the effect of free BDMDAC. Biofilms were developed in a well stirred continuous reactor (Simões et al. 2003).

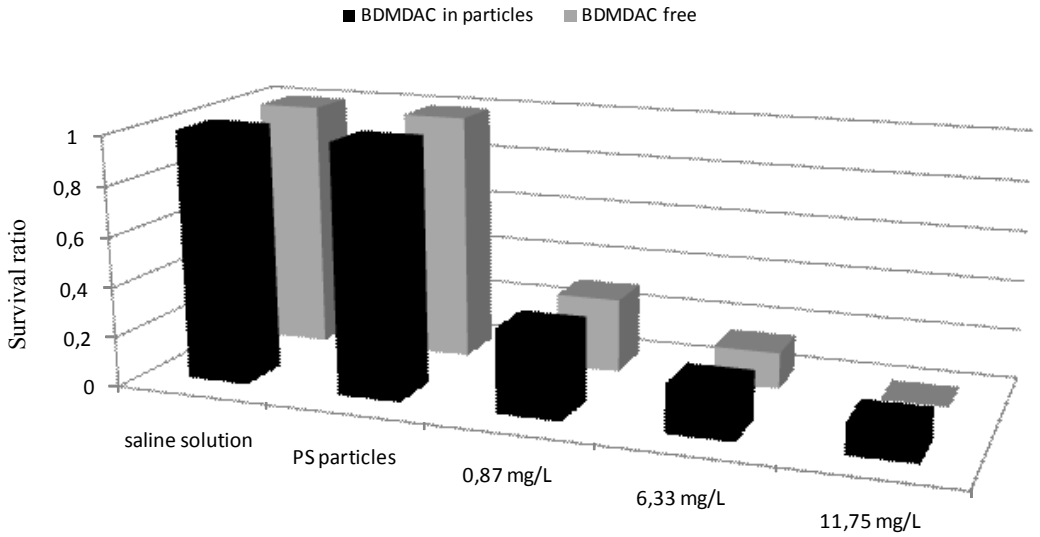


**Figure 5** – Survival ratio of *P. fluorescens* biofilms exposed to control conditions (saline solution and PS particles) and to BDMDAC coated particles and free BDMDAC for a 60 min exposure period.

The biofilm exposure to the biocidal particles with a very low concentration of BDMDAC (0.87 mg/L) resulted in a viability decrease 66.5 % of the total biofilm population for 60 min exposure time the free BDMDAC at the same concentration have a decrease of 70.3%. For the concentrations of 6.33 and 11.7 mg/L of BDMDAC in coated particles decreased the number of biofilm viable cells in 80.6 % (6.33 mg/L) and 87.2 % (11.7 mg/L) while the free QAC decreased the viable cells in 85.6 % (6.33 mg/L) and 100 % (11.7 mg/L). The differences in the decrease of cell viability by the application of free BDMDAC or BDMDAC coated particles were not significant. The moderately higher antimicrobial effect of the free QAC could be related with diffusion limitations associated with the particle size. There are evidences that the particle size and shape can influence antibacterial activity (Sanders et al. 2000; Fatin-Rouge et al. 2004; Pal et al. 2007). It is conceivable that reducing the particle size or increasing the antimicrobial concentration to the levels applied in industrial systems (Block 1983; McCoy 1983; Baker and Christensen 1988) will increase the antimicrobial effects.

The environmental aspects of the current use of antimicrobials are of severe concern because of their residual presence in surface and ground waters and the consequent propagation to the food-chain, with risks to the public health. The controlled application and reuse of antimicrobials based on highly efficient strategies might avoid the dissemination of antimicrobial resistance. The prolonged exposure of microorganisms to sub-lethal concentrations promotes antimicrobial resistance and cross-resistance events (McDonnell and Russell 1999; White and McDermott 2001). There are several reports indicating the ability of bacteria to acquire resistance to QAC`s (Méchin et al. 1999; Ishikawa et al. 2002). Many studies have demonstrated that bacteria are capable of adapting to disinfectants used in industrial settings after prolonged exposure to sublethal concentrations (Aase et al. 2000; To et al. 2002). (Loughlin et al. 2002) reported the ability of *P. aeruginosa* to adapt to increasing concentrations of benzalkonium chloride and verified the co-resistance to other membrane-active biocides. Also, the economical costs associated with the continuous application of antimicrobial chemicals should be considered. The LBL strategy used to control biofilm cells allows the rational use and reuse of antimicrobials. After 18 months in borate buffer pH 9, the particles coated with BDMDAC released only 15 % of the QAC. The results obtained in this study clearly demonstrate that this novel biofilm control strategy may have potential public health, environmental and economical benefits by effectively limiting the levels of biocides used in cleaning and disinfection practices.

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