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Bacteria transfer by deformation through microfiltration membrane

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ABSTRACT

Living particles such as bacteria are able to transfer through membrane pores that are smaller than cell size due to the specific stiffness of this type of microorganism. This phenomenon can lead to a significant loss of selectivity in the filtration process, which is a major cause of concern in the sterilizing filtration step. This study investigates the retention of three bacteria strains: *Escherichia coli* CIP 54124, *Pseudomonas aeruginosa* CIP 103467 and *Staphylococcus aureus* CIP 53154 by model porous membranes for various operating conditions (transmembrane pressure, feed concentration and the physicochemical composition of filtered media with antibacterial agent added at sublethal concentration). The first part of this study is dedicated to defining the size and the nanomechanical properties of the envelope of the studied bacteria by microscopic techniques (Transmission electron microscopy & Atomic-force microscopy), in order to then explore the role of these quantifiable characteristics on the cell transfer through the pores by deformation mechanisms. Our results lead to the development of a numerical model to connect the observed retention efficiency of the filtration experiment and the microscopic information about individual particles.

1. Introduction

The production of drinking water or sterile fluid uses membrane processes to remove potentially pathogenic microorganisms, such as bacteria, present in suspension. With that aim, Ultrafiltration and Microfiltration are considered as efficient processes and it is accepted that membranes with pore sizes smaller than 0.22 μm can be used for clearance of biological particles contamination [1].

However, recent studies demonstrated that bacterial transfer through the membrane structure takes place during filtration operations, even if the pore size is significantly smaller than the bacteria cell size at rest in suspension [2–8]. Several reasons were put forward to explain these bacteria leaks through the membrane: firstly, the presence of defects in the membrane structure which appear during the membrane manufacture or ageing [9]; secondly, the biological nature of the filtered particles confers them the fitness to override the size-based selectivity mechanism. Living unicellular particles such as bacteria, yeasts, algae or red blood cells exhibit, in environmental conditions, a large variability of individual particle size, shape and envelope flexibility. Effectively, in the specific case of bacteria filtration, the cell size of the microorganisms is not the only parameter which determines the transfer through the membrane [10]. Recent works demonstrated that the shape of the bacteria cell [3] and the Gram type [7,8], which characterize the cell wall composition, are also important parameters that play a key role in the particle retention. The main part of bacteria species is classified as Gram-positive or Gram-negative according to their cell wall structure. The cell envelope of Gram-positive bacteria corresponds to a thick layer of peptidoglycan (cross-linked polymer) exposed outside of the cell membrane, whereas Gram-negative bacteria present a thin layer of peptidoglycan located between the cell membrane and the outer membrane (description available in a dedicated study [11]). This difference in structure, composition and organization of the cell wall and more specifically, the thickness of the peptidoglycan layer can lead to different retention rates during filtration tests: Gram-positive bacteria exhibiting a thicker peptidoglycan layer, are less deformable, and as a consequence, present higher retention rates [7,8].

Furthermore, it has to be noted that the physicochemical properties of the suspension used as feed in the filtration process, such as osmotic pressure [12], pH [13], concentration of nutrients [14] and exposure to antibacterial agents at sublethal concentrations [15], can result in changes in bacteria cell size, morphology or metabolic activity and can lead to a modification of the structure and the composition of the cell wall thus separation efficiency. Moreover, some antimicrobial molecules able to be present at low level in environment, are known for inducing alterations of the bacteria envelope and the mechanical

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properties such as elasticity of the cell wall, at concentrations without a loss of cell viability [16,17]. The impact of bacterial exposure to chemical agents on filtration retention efficiency has only been studied in a few cases of literature. For example, chemical treatment with molecules that impact the cell properties, such as fixation with glutaraldehyde [3] or cell wall alteration with antibiotics like beta-lactams [7], demonstrate a significant impact on the filtration selectivity. This can be due to, as supposed by the authors, the modification of the flexibility of the bacteria cell wall. Mycoplasma, bacteria without cell wall (with dimensions in the range of 0.2–1 μm) have been earlier demonstrated capable of penetrating 0.2 μm membrane filters, and even some 0.1 μm rated filters [18,19].

Direct observation of the deformation of the biological cell during the transfer through a membrane pore is only possible on a large scale and the study of blood cell constriction allows the production of numerical simulations of the dynamics of one individual particle transfer [20–24].

In the case of particles at a smaller scale, such as bacteria (with dimensions under 1–10 μm), there is still a need for better understanding of the key roles played by morphological properties of the individual particles on the selectivity mechanism. This is in order to describe the global behavior of a suspension during the filtration process.

The objective of this study is to evaluate the influence of several operating parameters on the transfer of bacteria that are in direct contact with the membrane interface, and to establish the correlation between bacteria cell morphology/mechanical properties and membrane retention efficiency. In addition, this work aimed to investigate the impact of antibiotics (at low concentration in the filtered medium) on the filtration selectivity, in order to explore the major concern of trace contaminants found in water resources and encountered in treatment processes.

The first part of this study focuses on the measurements of reliable quantitative information regarding the size, shape and the stiffness of challenged bacteria cells, with and without antibiotics, through microscopic observations. In that section, we used Atomic Force Microscopy (AFM) to analyze, at the nanoscale, living cell characteristics, such as morphological and nanomechanical properties of the cell envelope [25,26]. In the second part of this study, bacteria filtration was conducted with different operating conditions: various transmembrane pressures and bacterial cell concentrations in the feed (estimated in colony-forming units per mL: CFU/mL). To complete the analysis of bacteria transfer through membrane pore, a simple numerical approach will be proposed linking all the collected data (from bacterial cell morphology to behavior during filtration). This easy-to-use model aimed to anticipate the impact of the operative parameters on the filtration selectivity towards bacteria suspension and to provide a tool for membrane processes implementation that allows to select the optimal conditions to avoid downstream contamination.

The challenge operating conditions were chosen to allow the quantification of transfer magnitude of different bacteria strains, treated or not by non-lethal concentration of antibiotic, through membrane pores smaller than the expected dimensions of the bacteria. The selected membrane should have specific intrinsic characteristics such as calibrated pore size and geometry. For this reason, we have selected isopore track-etched membranes [27]. Performed for low filtered volumes, short filtration runs will provide direct measurements of bacteria transfer in conditions without relevant fouling in order to avoid enhancement of the retention by the deposit on the membrane surface.

2. Materials and methods

2.1. Microorganisms, culture and enumeration conditions

Three different bacterial strains were used for this experimental study: *Escherichia coli* (CIP 54124), *Pseudomonas aeruginosa* (CIP 103467) and *Staphylococcus aureus* (CIP 53154) from the Pasteur Institute Collection (Paris, France). These bacteria have been selected in order to cover species with different morphological and structural characteristics and for their implication in waterborne diseases and/or nosocomial infections.

For the preparation of the suspension used for this work, bacteria were grown aerobically on tryptone soy agar (Biomérieux, Crapone, France) plates at 37 °C for 24 h. This pre-culture was transferred on tryptone soy agar plate for another 24 h culture. Colonies from the second culture were suspended in sterile NaCl aqueous solution at 9 g/L (noted here as physiological solution) in order to obtain a concentration of around 10^8 CFU/mL controlled by optical density at 640 nm. Then, tenfold dilution series were realized to adjust the feed concentration to 10^4, 10^6 and 10^7 CFU/mL.

In order to evaluate the bacteria concentration in samples (feed and permeate samples collected during filtration experiments), tenfold dilution series were performed after homogenization and 1 mL of each dilution was deposited in a sterile Petri dish before adding 12–15 mL of melted tryptone soy agar medium previously cooled to 45 ± 1 °C.

Colony Forming Units (CFU) were enumerated after overnight incubation of the plates at 37 °C, considering only dilutions with counts between 30 and 300 CFU. For each sample collected in this study, bacteria enumeration was performed in duplicate.

2.2. Antibiotic treatment

Part of this project was to explore the impact of chemical agents, such as pharmaceuticals that can be found in the environment, on filtration selectivity towards bacteria. Specific molecules which induce morphological alteration, like antibiotics, have been selected with that aim. The bacteria suspensions were then treated with beta-lactam antibiotics: amoxicillin (Sigma Aldrich-A8523) for *E. coli* and *S. aureus*, or ticarcillin (Sigma Aldrich-T5639) for *P. aeruginosa*. This class of antibiotics avoids cross-linkages between the peptidoglycan polymer chains (the network responsible for the mechanical properties of the bacteria cell wall) and was used in this study at sub-inhibitory and sub-lethal concentrations to affect the stiffness of the bacterial cell wall without altering the viability and cultivability of the microorganisms.

In first instance, we aimed to determine the antibiotic concentration that will be added in the bacteria suspension without impairment of cell viability. Minimal Inhibitory and Bactericidal Concentrations (MIC and MBC) of the selected molecules were determined against the tested strains using a Mueller-Hinton broth (Biomérieux) in microtiter plates with agar dilution method [28], followed by a subculture on Mueller-Hinton agar (Biomérieux), as previously described [7]. The viability and the cultivability of the bacteria in suspension were then checked by numeration on trypcase soy agar after 180 min of contact with the selected antibiotic at MIC/2 concentration (amoxicillin: 1 μg/mL for *E. coli* and 0.05 μg/mL for *S. aureus*, or ticarcillin: 15 μg/mL for *P. aeruginosa*). In these conditions, no decrease of CFU counts was observed. Finally, for bacteria characterization steps or filtration run, the bacterial feed suspension at challenged concentrations was kept in contact or not (control) with the selected antibiotic at MIC/2 for 90 min in a physiological solution before the experiment.

2.3. Membrane

Polycarbonate microfiltration membranes used for this study are provided by Nuclepore – Whatman. According to manufacturer specification, they exhibit a narrow controlled pore size distribution with a nominal pore diameter of 0.4 μm, with porosity in the range of 4–20% and a membrane thickness of 20 μm. These membranes have been used as model material because of their well-defined pore geometry (cylindrical). In our study, the effective area of the micro-

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