Desalination and Water Treatment
www.deswater.com
5 (2009) 235–251
1944-3994/1944-3986

Cleaning kinetics and related mechanisms of *Bacillus cereus* spore removal during an alkaline cleaning of a tubular ceramic microfiltration membrane

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Received 7 July 2006; Accepted 25 August 2008

ABSTRACT

In membrane separation processes, biofouling of membranes is now well recognized as a major impediment to their efficient operation and overall performance. This study focuses on the first (attachment) phase of biofouling. We have investigated experimentally the cleanability of a ceramic microfiltration membrane fouled by *Bacillus cereus* spores in terms of both hydraulic and microbiological cleanliness and examined the interrelationship between the two types of cleanliness. Cleaning kinetics was described in terms of both the hydraulic membrane resistance changes during cleaning and the number of residual adhered spores per unit membrane surface area as a function of time. Hydraulic cleanliness was evaluated using three parameters: percent flux recovery (FR), percent irreversible removed fouling (RF) and a hydraulic cleanliness criterion (HCC, i.e. $(R_n - R_m)/R_m$ <0.05). Microbiological cleanliness was assessed by the measurement of the residual microbial population adhered to the membrane surface left after cleaning. A single-stage cleaning consisted of the recirculation in turbulent flow regime of a 0.5 wt% sodium hydroxide solution. The hydraulic membrane resistance changes during cleaning fitted a first order kinetics which predicted a useful cleaning duration close to 10 min above which membrane resistance was quasi-constant. The detrimental effect of time on cleaning efficiency was highlighted: the hydraulic membrane permeability could not be restored beyond a cleaning duration of 15 min due to the redeposition on the membrane surface of spore cells previously released in the cleaning solution. A simple model (first-order reaction) combining removal and deposition rates provided a fairly good fit of the variation with cleaning time of the adhered spore population ($r^2 = 0.97$, $p < 0.0001$, $n = 11$). The residual adhered population left after cleaning was positively correlated with the hydraulic cleanliness criterion, HCC (r=0.65, p <0.05, n=12). Nevertheless, residual contamination was not statistically correlated (p >0.05), neither with the standard percent flux recovery nor with the measured fouling resistance left after cleaning. Percent flux recovery was insufficient in indicating accurately the microbiological cleanliness.

Keywords: Cleaning-in-place; Microfiltration; *Bacillus cereus* spores; Cleaning kinetics; Sodium hydroxide; Removal kinetics

1. Introduction

In membrane separation processes [microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO)], biofouling involving attachment of microorganisms to the membrane surface and biofilm formation impairs the overall membrane performance making necessary frequent cleaning and sanitation procedures aimed at eliminating the biomass from the membrane material. Cleaning is a vital step in maintaining the permeability and selectivity of the membrane, and is necessary to minimize risks of bacteriological contami-

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nation and ensure a good hygiene level in the food product. The aim of cleaning and disinfection is to achieve a hygienic unobjectionable final state of the surface; the surface must be free of soil, pathogenic microorganisms, food spoiling microorganisms, and detergents and/or disinfectant agents. Three main cleanliness criteria have been defined for cleaning and disinfection procedures: (1) hydraulic cleanliness (restoration of membrane permeability or pure water flux recovery); (2) chemical cleanliness (membrane from which all foulants and impurities have been removed, including residues of the cleaning agents used); (3) microbiological cleanliness (membrane whose surface is free from living microorganisms). For microbiological cleanliness which is related to the level of membrane surface contamination, international standards have been widely accepted that have defined limits of acceptability (e.g. for an industrial dairy equipment, acceptability limit is equal to 2 microorganisms/cm² [1]). At the present time current industrial methods of cleaning-in-place (CIP) are generally automated but are derived empirically and are not optimized (most often cleaning does not allow to recover the pure water flux of the virgin membrane).

Biofouling, generically defined as the deleterious attachment of a mat of cells and extracellular polymeric substances (EPS) to a solid surface, is one of the most insidious forms of membrane fouling [2]. Bacteria can rapidly attach, grow onto the membrane surface and produce significant amounts of exopolymer substances that can stabilize attachment and thus facilitate the development of biofilms which exibit remarkable resistance to biocides. The steps involved in membrane biofouling are the following: (1) irreversible attachment of viable bacteria to the membrane surface, which is mediated by electrokinetic and hydrophobic interactions; (2) synthesis and extrusion of EPS, biopolymers which comprise mainly polysaccharides and proteins; and (3) biofilm development via cell growth and multiplication. One of the most important issues of membrane biofouling is the initial attachment of microorganisms since bacterial attachment is a necessary first step for membrane biofilm formation and represents the biofouling potential. Biofilm fouling (or "gel layer", called biofilm, which participates in the separation process as a secondary membrane) results in a variety of undesirable effects, including: (1) reduced permeate flux, (2) increased solute accumulation near the membrane surface (i.e. concentration polarization) leading to enhanced solute passage, (3) reduced membrane lifetime and selectivity, (4) increased module differential pressure resulting in higher energy consumption and (5) biodegradation of some polymeric membranes (especially cellulose acetate RO membranes [3]).

A number of studies provided useful qualitative information on biofouling of RO membranes. Flemming [4] observed that the biofilm adhesion process was strongly dependent on the number of cells in the suspension, and that different membranes had different biological affinities. The biological affinity of various membrane materials towards bacteria (*Pseudomonas*) was detected.

Ridgway et al. [5] found that the adhesion of a *Mycobacterium* strain (implicated in the initial stages of RO membrane biofouling) to a cellulose diacetate RO membrane occurred within 1–2 h and conformed closely to the Langmuir adsorption isotherm.

The initial cell attachment is largely controlled by physicochemical factors such as solution chemistry, membrane surface properties and hydrodynamic conditions. Using direct microscopic observation within a shear flow cell, the initial deposition rate of microbial cells on MF membranes was investigated and interpreted through an interaction force model [6]. Other studies concluded that solution chemistry and surface properties controlled microbial cell adhesion onto membranes [7–11].

The effectiveness of sanitation in terms of microorganism removal from a crossflow filtration system is generally assessed by the measurement of the microbial population which most often is comprised of bacteria, yeasts and molds. A number of microbiological techniques have been used for the enumeration of microorganisms (mainly bacteria) adhered onto membrane surfaces: (1) microscopic cell count (total direct cell counts) associated with staining using a particular fluorochrome (e.g. DNA-binding fluorochrome DAPI (4,6-diamidino-2-phenylindole) [12], or crystal violet coupled with visualization by bright field microscopy [7] or acridine orange as fluorochrome coupled with epifluorescence microscopy [11]); (2) heterotrophic plate counts on a nutrient medium, expressed as colony forming units (cfu) [13]; (3) incubating small sections (a few square centimeters) of membrane in a broth and calculation of the most probable microbial population [14]; (4) swabbing of membrane and module surfaces followed by dilution in sterile buffer and incubation [15,16]; the result is then expressed in colony forming units per unit surface area (log cfu/cm²); (5) use of radiolabeled bacteria, adherent bacteria being quantified by a liquid scintillation counter [8,9]. Direct scanning electron microscopy (SEM) examination can also be used, even though SEM cannot distinguish dead from viable cells [17].

One way to prevent biofouling consists in performing preventive/curative cleanings able to remove adhered microorganisms and EPS from the membrane surface [13]. Little information regarding the effect on the microbiological cleanliness of various cleaning procedures is available in the literature. In the dairy industry a few authors have evaluated the efficiency of a variety of cleaners and sanitizers for PS UF membranes fouled by skim milk or whey [14–16,18]. The levels of cleanliness

obtained were determined using suitable measurement procedures such as surface swabbing, SEM and permeate flux restoration. These authors have established microbiological criteria (under the form of a microorganism population threshold) for an UF equipment in the dairy industry which demands high cleanliness levels. Microbiological criteria consisted of an upper limit in the number of microorganisms per unit of surface area; for example membrane and stainless steel surfaces with less than 1 cfu/cm² were considered acceptable [2].

Smith and Bradley [16,18] have shown that sanitizers and sanitation procedures commonly used in the dairy industry were unable to kill microbial populations in UF membrane systems indicating potential safety problems. In the ultrafiltration of sweet whey on PS spiral-wound membranes, the number of microorganisms (in log cfu/cm²) which were present following soiling, cleaning and sanitizing were measured in order to assess the performances of four commercial enzyme-based cleaning solutions [18]. The four enzyme-based cleaners were unsatisfactory when microbiological criteria were considered. Loss of sanitizer strength and problems with yeast and especially mold growth over time indicated lack of effective cleaning.

The efficacy of three cleaners designed for use with UF systems (phosphoric acid pH 2.5, enzyme-based cleaner at pH 11.5 and a chlorinated alkaline cleaner at pH 11.5) has been determined in terms of both soil and microorganism removal by the same authors [16]. Cleaners did not ensure an efficient cleaning of the membrane fouled with sweet whey: SEM put forward the presence of viable microorganisms deposited on different membrane materials (membrane surface, retentate spacer, permeate mesh) on contact with cleaning agents. Subsequent to these studies, appropriate sanitizing procedures in the dairy industry using formulated disinfectants have enabled membranes free from viable microorganisms to be obtained [14,15]. The ability in controlling microbial populations in spiral-wound UF systems of chlorine dioxide (ClO_2) in an acidic solution has been demonstrated for an UF PS membrane fouled with cheese whey and skim milk and cleaned by an acid/caustic sequence [15]. The performance of sanitation procedures has been determined for cleaned spiral wound PS UF membranes after a fouling with cheese whey [14]. Chlorine-based disinfectants effectively sanitized the membrane. Dichloroisocyanurate-based and hypochlorite based sanitizer at 100 ppm, 54°C, and circulating for 15 min resulted in membranes free from viable microorganisms.

For NF and RO spiral-wound elements used to treat surface water, operational problems caused by biofouling (viz increased normalized pressure drop) were found to be difficult to solve by chemical cleaning [13,19]. Tests with cleanings of biofouled RO membranes showed that it was difficult to remove the biomass from the membrane [19]. For a biofouled RO membrane element treated with an industrial cleaning agent (pH 12), cleaning resulted in a clear reduction of both adenosinetriphosphate (ATP) of 94% and heterotrophic plate counts (HPC in cfu/cm²) of 99.8% but did not significantly reduce the total direct counts (TDC in cells/cm²), implying that nearly all cells were killed (inactivated) but not removed from the membrane surface [19].

It appears from the literature that although substantial research has been directed towards bacterial adhesion and the determination of physicochemical factors controlling the initial deposition rate of microbial cells onto microporous membranes, cleaning of membranes fouled with microorganisms has received much less attention. To our knowledge, no study has yet dealt with the removal mechanisms of microorganisms from the surface of a MF membrane during a cleaning-in-place (CIP) procedure and there is no quantitative data on the assessment of the microbiological cleanliness of MF membranes following cleaning. Little recent information is available regarding the assessment of the microbiological cleanliness of: (1) UF membranes [14–16,18], (2) RO membranes as determined from the biomass removal [13,19] and (3) spiral-wound NF membrane elements evaluated by the biomass concentration (expressed as ATP/cm²) [20]. Additionally the relationship (or correlation) between the hydraulic and microbiological cleanlinesses as well as the removal kinetics of adherent microorganisms under tangential flow conditions during chemical cleaning are still poorly understood. Understanding of the mechanisms and kinetics of the chemically enhanced hydrodynamic removal of microorganisms appears to be mandatory to optimize cleaning efficiency, increase membrane performance, ensure the microbial quality of the feed product and maintain a good hygienic status of the equipment (membrane, module). Knowledge of the cleaning kinetics is particularly necessary to establish the useful duration of a cleaning sequence and is an essential step in predicting cleaning efficiency.

The objective of this study was to model the removal kinetics of *Bacillus cereus* spores during an alkaline cleaning (using a simple sodium hydroxide solution) at constant crossflow velocity and transmembrane pressure, the fouling procedure proposed enabling a sufficiently high and reproducible contamination level on the MF membrane surface. Additionally we searched for significant relationships (linear regression) between hydraulic and microbiological cleanlinesses. This model microorganism-cleaning solution system associated with a ceramic MF membrane was selected in order to make easier the elucidation of the cleaning mechanisms.

2. Materials and methods

2.1. Materials

2.1.1. Experimental filtration rig

MF experiments were conducted using the crossflow filtration pilot plant represented in Fig. 1. The filtration rig is composed of three major parts: the feed unit, the crossflow filtration unit incorporating the membrane module and a CIP unit connected to the feed one by two three-way valves (numbered 1 and 2, Fig. 1). Crossflow filtration configuration corresponded to the so-called 'batch' mode. Tangential flow rate in the membrane channel was ensured by a volumetric pump (PCM Moineau, MR1L10, 0–1 m^3/h) for the fouling procedure or by a centrifugal pump (Someflu, maximum delivered flow rate of 0.8 m^3/h) for the rinsing and cleaning steps. Two differential pressure transducers (Schlumberger type D, ±0.25% of the full scale) were located at the inlet (DPe) and outlet (DPs) of the filtration module. Average transmembrane pressure (TMP) was defined as: $TMP = (DPe + DPs)/2$. TMP was adjusted by means of a control valve (VR2, Fig. 1) located downstream of the membrane for the fouling step and using a pressure regulation valve (VR1, Fig. 1) for the rinsing and cleaning steps. Crossflow velocity (v) in membrane channel was adjusted by means of the variable flow of the recirculation volumetric pump (PCR, Fig. 1). The convergent zone before the membrane entrance was designed to convert static pressure into purely dynamic pressure and to avoid friction loss due to flow contraction. The CIP circuit connected to the filtration rig was composed of three tanks: a 150-L clean water tank and two 100-L cleaning solution tanks whose temperature was regulated by a steam coil (±1 °C). The signals from the various sensors (electromagnetic flowmeters, platinum resistance probes and differential pressure transducers) were treated using a microcomputer through an analog/digital interface. Softened sterilized water (filtered through a 0.1 μm zeta+ dead-end cartridge, Pall Filtration) was used for the rinsing step and to make up the 80-L NaOH solution.

2.1.2. Membrane

The membrane selected was a tubular monochannel ceramic membrane with a nominal pore size of 0.45 μm (Kerasep, Novasep Process, France). It had a 6-mm internal channel diameter (d_h), a 10-mm external diameter and 0.4 m length for a filtration area of 0.0073 m^2. The selective layer was zirconium/titanium oxide bound on a ceramic support.

2.1.3. Bacterial strain and fouling suspension

Bacillus cereus spores, which are usual contaminants of closed or open surfaces found in the food industry such as the inner surfaces of dairy equipment, were chosen as test microorganisms for the assessment of membrane cleanability due to their high resistance to heat and chemicals and their strong adhesion to various materials [21]. The spore-forming strain used in this study, *Bacillus cereus* CUETM 98/4 (Collection Unité Ecotoxicologie, Villeneuve d'Ascq, France), was isolated from a food processing line. Spores were produced and harvested as described by Faille et al.[22] and stored at 4 °C until use. A total number of four spore batches was used throughout this study, each batch being utilized for up to 2 months to ensure constant surface properties. The fouling suspension was comprised of a 5-L phosphate buffer solution (10 mM, pH 7.2) in which spores were suspended at a concentration of 10^5–10^6 cfu/ml. The phosphate buffer solution (PBS) was composed of 0.079 wt% NaH_2PO_4 and 0.13 wt% Na_3PO_4,$12H_2O$ (pH 7.2, ionic strength 10 mM). The use of a retentate solution of constant pH and ionic strength enables both membrane and cell charges to be fixed during the fouling step. This appears to be necessary in obtaining a more reproducible initial population of adherent spores since pH and ionic strength significantly affect electric double layer interactions which may dramatically alter initial microbial deposition rates [6].

2.2. Methods

Each fouling and cleaning run consisted of five stages: initial pure water flux (conditioned membrane), fouling, first water rinse (or prerinse), NaOH cleaning and final rinse. The successive steps of one fouling and cleaning run together with the filtration operating conditions are described in Table 1. Before each experiment, the filtration rig was firstly disinfected by recycling a hydrogen peroxide and peracetic acid based sanitizer (0.3 wt% Oxygal, Penngar, France) for 30 min at 20 °C, followed by a 10 min rinse. The membrane was then conditioned by a NaOH-HNO_3 sequence according to operating conditions presented in Table 1. Membrane conditioning allowed for homogeneous membrane surface properties (especially the surface charge [23]).

2.2.1. Initial pure water flux

The water flux measurement allowed the determination of the initial membrane resistance at the beginning of the run (R_m). TMP was varied in an incremental manner between 0.5 and 1 bar so as to determine accurately R_m via the slope of the permeate flux J_{w0} vs. the TMP curve. R_m was given using Darcy's law: $J_{w0} = TMP/(\mu.R_m)$ where μ is the dynamic filtrate viscosity (Pa.s).

2.2.2. Fouling

The membrane fouling stage consisted of filtering the spore suspension at 20 °C, crossflow velocity v of 4 m/s

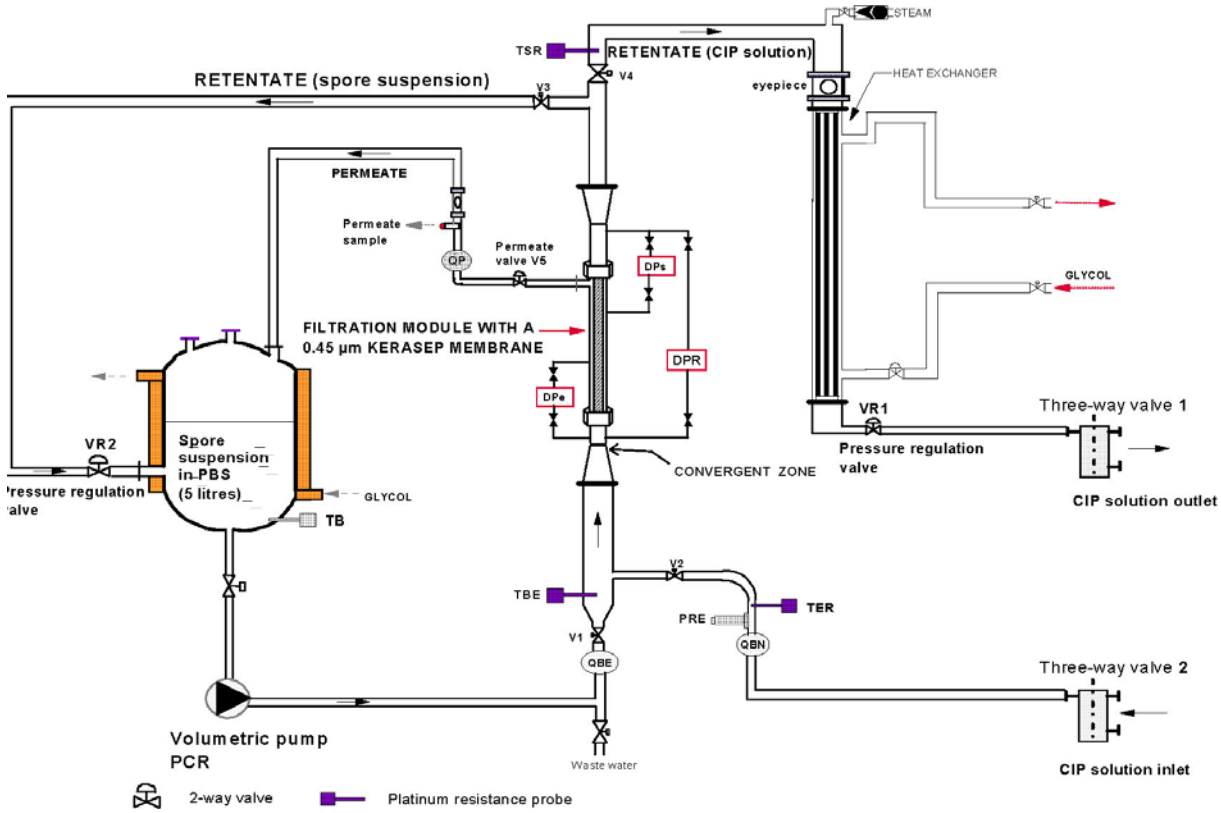

Fig. 1. Experimental set-up of the crossflow filtration unit. DPe, DPs: inlet, outlet transmembrane pressure; DPR: pressure drop in filtration module; PCR: feed pump; PRE: inlet retentate pressure; QBE: retentate loop flow rate; QBN: CIP solution flow rate; QP: permeate flow rate; TB: retentate tank temperature; TBE: inlet retentate temperature; TSR: outlet CIP solution temperature; TER: inlet CIP solution temperature.

Table 1
Operating conditions in the successive steps of fouling and cleaning experiments together with corresponding experimental hydraulic membrane resistances

Operation	Duration (min)	v ($m.s^{-1}$)	TMP (bar)	Temperature (°C)	Hydraulic membrane resistance (m^{-1})
Membrane conditioning:					
0.5 wt% NaOH	30	4	0.5	55	
Rinsing	10	4	0.5	20	
0.5 wt% HNO_3	30	4	0.5	55	
Pure water flux (initial conditioned membrane)	10	4	0.5–1	20	R_m
Fouling	120	4	0.75	15–20	R_t (=R_m + R_f)
First rinse (prerinse)	5	2[a]	0.5	20	R_r (=R_m + R_{if})
NaOH cleaning	5–30	4	0.3–0.4	55	$R_{n'}$
Final rinse (cleaned membrane)	5	2	0.5	20	R_n (=R_m + R_{rf})

[a]Some additional experiments were done at v_r =4 $m.s^{-1}$.

(Reynolds number $Re \approx 24{,}000$, wall shear stress $\tau_w = 39 \pm 8$ Pa) and TMP of 0.75 bar for 2 h. These conditions ensured a steady state flux (J_{ss}) at the end of the filtration. Total (reversible plus irreversible) fouling resistance at the end of the filtration, R_f, was defined by $R_f = TMP/\mu.J_{ss} - R_m$.

Collecting samples (50 ml) in the permeate side and enumerating them by plating on nutrient agar resulted in spore concentrations <1 cfu/ml, which means that *B. cereus* spores were completely rejected by the membrane and adhered only to the membrane surface in the absence

of pore plugging. Both permeate and retentate were returned to the feed tank in order to maintain constant inlet spore concentration.

2.2.3. Intermediate and final rinse

The fouling step was followed by a first rinse to remove unattached spores from the membrane surface. A final rinse was carried out to remove sodium hydroxide traces from the membrane material and calculate the cleaned membrane resistance (R_n). Both rinses were performed under the following conditions: v_r = 2 m/s (Re ≈ 12,000, $\tau_w = 5 \pm 1.7$ Pa), TMP = 0.5 bar, 20°C and a run time of 5 min. The water flux (J_{wr}) during the first rinse gives the total membrane resistance R_r: $R_r = TMP/\mu.J_{wr}$. The stabilized water flux (J_{wr}) in the first rinse gives the total irreversible fouling resistance (R_{if}): $R_{if} = TMP/\mu.J_{wr} - R_m$. In the same way the cleaned membrane resistance (R_n) was derived from the constant final water flux (J_{ww}), i.e. $R_n = TMP/\mu.J_{ww}$, whereas the residual fouling resistance left after cleaning (R_{rf}) was defined by $R_{rf} = TMP/(\mu.J_{ww}) - R_m$.

2.2.4. Cleaning

The operating conditions were as follows: v = 4 m/s, (Re ≈ 47,000, τ_w = 38 ± 4 Pa), TMP = 0.3–0.4 bar, temperature 55°C and a cleaning time (t_c) ranging from 5 to 30 min. A temperature of 55°C was chosen since a few authors reported that for MF membranes fouled with organic matter, the best cleaning efficiency was achieved at temperatures close to 50°C [24,25]. Sodium hydroxide at 0.5 wt% was chosen due to its well known efficiency in the removal of organic soils; a NaOH concentration of 0.5 wt% was found to be optimal for milk soil removal [24]. Also at 55°C, sodium hydroxide had no lethal action on spores [26]. Lowering TMP from 0.75 bar during fouling to 0.3–0.4 bar for cleaning is believed to result in an improved cleaning performance in terms of flux recovery as cleaning efficiency was found to be improved at low or zero TMP [25,27,28]. A low but non-zero TMP of 0.3–0.4 bar was thought to be convenient since it allowed the calculation of the hydraulic membrane resistance, $R_{n'}$ (from which cleaning kinetics is derived) with a sufficiently good accuracy. The cleaning flux, J_{wc}, was recorded to calculate $R_{n'}$ as $R_{n'} = TMP/\mu J_{wc}$.

2.2.5. Hydraulic cleanliness: evaluation of cleaning efficiency

The type of fouling which has to be removed here is the irreversible fouling left after the prerinse (R_{if}) involving spores that adhere strongly to the membrane surface. These strongly adherent spores need both the chemical action of sodium hydroxide and the mechanical action of the wall shear stress to be removed. Cleaning efficiency has been evaluated in terms of membrane permeability recovery using three parameters: the standard percent flux recovery (FR), the percent irreversible removed fouling (RF) and a hydraulic cleanliness criterion (HCC) representing the proportion of the hydraulic resistance of residual fouling left after cleaning, $R_n - R_m$, compared to the initial membrane resistance, R_m [23,29].

Percent flux recovery was defined as $FR = (R_m/R_n) \times 100$ (%). The percentage of irreversible removed fouling was given by $RF = [(R_{if} - (R_n - R_m)]/R_{if} \times 100$ (%), where R_{if} is the total irreversible fouling resistance left after the prerinse. Cleaning was considered to be efficient from a hydraulic point of view when $HCC = (R_n - R_m)/R_m < 0.05$, considering the sum of the relative errors for the membrane resistance measurement (i.e. $\Delta R/R \approx 0.05$).

2.2.6. Hydraulic cleanliness: evaluation of rinsing efficiency

The type of fouling involved corresponds to unattached (or loosely bound) spores at the membrane surface, i.e. reversible fouling. During prerinse, the cleaning effect is provided by the mechanical action of the turbulent flow and the solventing power of water. As for cleaning, rinsing efficiency has been evaluated using three parameters: percent flux recovery (RFR), percent reversible removed fouling (RRF) and a hydraulic rinsing cleanliness criterion (HRCC) representing the proportion of the hydraulic resistance of the irreversible residual fouling left after prerinse, $R_r - R_m$, compared to the initial membrane resistance, R_m. Percent flux recovery was defined according to $RFR = (R_m/R_r) \times 100$ (%).

The percentage of reversible removed fouling was expressed as follows :

$$RRF = \frac{R_f - (R_r - R_m)}{R_f} \times 100(\%)$$

where R_f is the total (reversible plus irreversible) fouling resistance at the end of fouling. Rinsing was considered to be efficient from a hydraulic point of view when HRCC = $(R_r - R_m)/R_m < 0.05$.

2.2.7. Microbiological cleanliness: enumeration of spores adhered to the membrane surface

The adhered spore population along the membrane path and remaining after the fouling or cleaning step was detached and evaluated by sonication of seven 5-cm long membrane pieces into a nonionic surfactant (Tween 80, ICN Biomedicals, USA) followed by enumeration on nutrient agar, and was expressed as the median of the seven $\log_{10}$cfu/cm² values.

Experimentally, following either the prerinse or the final rinse, the filtration module was disassembled and the membrane carefully removed. At the laboratory, the external membrane support of 10 mm external diameter

was surrounded by self-adhesive aluminium in order to prevent the redeposition throughout the sonication of detached spores (i.e. spores released in the nonionic surfactant solution) inside the coarse structure of the ceramic support. The 40-cm long membrane was cut into 7 membrane pieces of 5-cm long numbered lengthwise from A to G along the direction of the retentate fluid stream. The self-adhesive aluminium surface of the membrane pieces was disinfected with a swab previously immersed in a disinfectant (Deptyl' Ox 10%, Hypred, France) and rinsed gently in a sterile water beaker. Each membrane piece was immersed in a glass tube containing 20 ml of a 2 wt% Tween 80 solution. The tube was submitted to a first 2×2.5 min sonication (Ultrasonic bath, Detasonic, 40 kHz) with an intermediate 30 s vortexing. The membrane piece was taken out from the tube and immersed in a second glass tube containing 20 ml of a 2 wt% Tween 80 solution, which was sonicated as described above. The membrane piece was then removed from the tube and the two Tween 80 media were diluted in a logarithmic series, plated in duplicate on a nutrient agar composed of 13 g/l nutrient broth (Biorad, France) and 15 g/l bacteriological type E agar (Biokar Diagnostics, France). Enumeration was done after 24 h incubation at 30°C.

2.2.8. Microbiological cleanliness: measurement of the efficiency and reproducibility of the ultrasonication method in counting spores adhered to the membrane surface

The validity of the ultrasonication technique described above has been checked by measuring the population adhered along the membrane (i.e. on seven 5-cm long membrane pieces) following two standard fouling-prerinse experiments. For that the number of spores counted (noted y_i in cfu/cm²) as a function of the successive ith sonications (up to four) was measured for the 14 collected membrane pieces. Enumeration results together with the cumulative percent of count *B. cereus* spores (noted z_j, where j is the jth sonication) have been analyzed (data not shown). Data revealed that the cumulative percentage of spores counted at the second sonication is relatively constant and lies between 93% and 97% with a corresponding average log reduction value (LRV) close to 1.3. The proportion of spores counted after the second sonication represented less than 10% of the total number of spores counted. The third sonication usually yielded less than 5% of the total spore number. Two sonications can thus be reasonably considered as being sufficient and appropriate in practice to evaluate in a reproducible manner the total adhered spore population. Nevertheless, for each experiment the membrane pieces numbered 2, 4 and 6 were subjected to four successive sonications so as to ensure that the number of spores detached at the third and fourth sonication could be neglected.

2.2.9. Enumeration of spores in retentate and permeate

Retentate suspension — After the 5-L phosphate buffer solution had been inoculated with the spore suspension and stirred by a magnetic stirring bar, a 10 ml sample was taken for enumeration on nutrient agar. This first counting was completed by a second one carried out on a 10 ml sample collected from the retentate loop in the course of the fouling step (generally after 1 h filtration). The differences of the two countings were always within the range of the experimental counting error implying that there was no cell aggregation in the bulk. The average value in cfu/ml was taken as the retentate spore concentration.

Permeate — The determination of spore counts (expressed in cfu/ml of permeate) was performed by collecting samples (50 ml) in the permeate side at filtration times of 1 and 2 h. A fixed volume of the sample (10 or 25 ml) was then filtered under vacuum through a sterilized 0.2 μm cellulose nitrate filter (Sartorius, Germany) which was deposited in a Petri dish containing a solid nutrient agar supplemented by 0.1‰ 2,3,5 triphenyltetrazolium chloride (TTC). This soluble non-coloured salt (which is an electron acceptor) is reduced in the bacterial cells to formazan which is non-soluble and red. *Bacillus* colonies appeared in red over the filter surface and could be easily counted. In this way permeate spore contents as low as 1 cfu/ml could be measured.

2.2.10. Spore surface characteristics

The partitioning method of microbial adhesion to solvents (MATS) originally described by Bellon-Fontaine et al. [30] was used to determine the hydrophobic and Lewis acid-base properties of the spores. This partitioning method is based on the comparison between microbial cell affinity to a monopolar solvent and an apolar one, and was slightly modified in our laboratory. The principle of the MATS method can be found in previous publications [31,32] and spore cells were prepared and tested according to the protocol described previously by Faille et al. [32].

Briefly, a spore suspension diluted in PBS with an optical density at 400 nm close to 0.8 was prepared; 2.4 ml of this spore suspension and 0.4 ml of solvent under investigation were vigorously shaken manually into a hemolysis tube during 45 s so as to form a fine emulsion. The mix was then allowed to stand for 15 min to ensure a complete separation of the two phases. The aqueous phase was then taken out with a Pasteur pipette and collected in a spectrophotometer vat. The percentage of cells bound to a given solvent was defined as:

$$(1 - A/A_0) \times 100$$

where A_0 is the optical density of the spore suspension (OD_{400} ~0.8) and A the optical density at 400 nm of the

aqueous phase after the water–solvent separation. Experiments were done in duplicate.

2.2.11. Statistical analysis

Statistical analyses were performed using SigmaStat 3.1 and SigmaPlot 9.0 software (Systat Software, Point Richmond, CA). The level of significance was $p_{value} < 0.05$.

3. Results and discussion

3.1. Characterizations of B. cereus *spores*

The particle size distribution of the spore suspension was measured using a laser diffraction technique (Mastersizer S, Malvern Instruments, UK) for each batch prepared. Spore suspension was resuspended in the phosphate buffer at circa 10^6 cfu/ml for measurement. For all batches used throughout this study, spore size distribution exhibited a main peak of the volume distribution, which was centered on 1.2 μm. Spore size distribution was narrow with percentile sizes at 10% and 90% of the cumulative undersize curve equal to 0.8 and 1.9 μm, respectively. Considering the average pore diameter of the membrane (0.45 μm), a total rejection of the cells can be expected so that only external (surface) fouling takes place.

Spore surface properties characterized by the affinity to apolar and monopolar solvents are presented in Table 2. A two-way analysis of variance (ANOVA) was performed to assess the spore batch effect on solvent affinities: it was found that there was not a statistically significant difference (p = 0.22) among the four batches used. Regardless of the solvent used, the affinity of *B. cereus* spores was very high (>80%, with the exception of batch 4 against hexadecane), indicating their marked hydrophobic character. It is noticed that there are little differences between affinity percentages for the basic (ethyl acetate) solvent and the corresponding apolar (decane) one, suggesting a poor electron-acceptor character. Spores exhibited a slight basic (electron-donor) character (affinity for chloroform is always superior to that for hexadecane, with least square means for chloroform and hexadecane equal to 96 ± 3.6 and 79 ± 3.6, respectively).

Table 2
Microbial adhesion to solvents (MATS). Percentage of *B. cereus* spores bound to apolar (decane and hexadecane) and polar (ethyl acetate and chloroform) solvents for the different batches

Solvent	Batch 1	Batch 2	Batch 3	Batch 4
Decane	91	90	90	80
Ethyl acetate	82	92	81	85
Hexadecane	90	80	87	60
Chloroform	99	100	91	96

3.2. Characterizations of the initial adhered spore population left after fouling

3.2.1. Type of fouling induced by spore adhesion and reproducibility

SEM examination (using a Hitachi S-3000N model) of the selective layer of the membrane was performed for a few fouling-prerinse experiments. Figs. 2a and 2b show SEM photographs of the selective layer surface following a fouling-prerinse run. SEM revealed isolated or scattered adhering spores onto the membrane surface as well as some clusters comprising several tens of cells, which were located between large areas without any cells. Fouling was thus heterogeneous along the membrane surface. A full monolayer was not observed; indeed a simple calculation shows that in the hypothetical case where the totality of spore cells contained in the 5-L retentate solution would adhere to the membrane, it would result in the build-up of up a full monolayer.

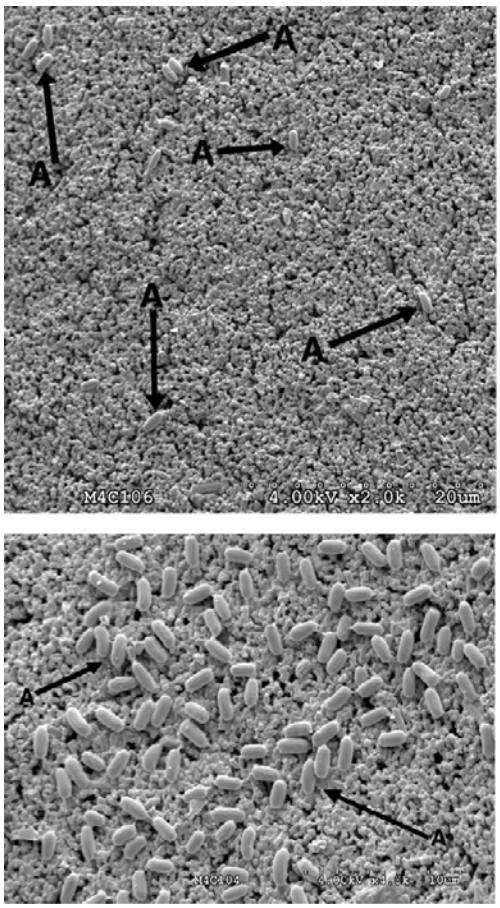

Fig. 2. SEM photographss showing *B. cereus* spores on membrane surface following a fouling-prerinse sequence. (a) bar = 20 μm; (b) bar = 10 μm. Arrow A: adhered *B. cereus* cells.

In addition to SEM examination the type of fouling was further characterized by applying the constant pressure blocking filtration laws that involve specifically an external (surface) fouling [33]. Experimental flux vs. time curves during the fouling step have been tested with the corresponding linearised forms of the blocking filtration laws [34,35]. It has been found that for all fouling runs, experimental J values supported the intermediate blocking filtration law (IBFL) at the initial stages of filtration (at a time t <13 min) during which filtration behaved as a dead-end filtration ($0.9 < r^2 < 0.99$). It is noticed that beyond the early stages of filtration, data always conformed to IBFL mediated by a back-transport of cells from the membrane surface into the bulk stream, as defined by Field et al. [36]. The applicability of IBFL clearly indicates that only external fouling takes place as evidenced by SEM visualization, and considering the physical meaning of IBFL [34,35], fouling was a micro-soiling which consisted of isolated or scattered adherent spores above the membrane surface. The knowledge of the nature and type of soiling layers to be removed (i.e. macro-soil or micro-soil) is of importance in the cleaning mechanisms involved in the removal of *B. cereus* spores from food processing equipment surfaces, as pointed out by Lelièvre et al. [26].

Fig. 3 shows a box plot graph of the initial adhered population along the membrane, N_0 (seven membrane pieces per experiment) expressed in $\log_{10}N_0$ cfu/cm² for the various fouling-prerinse runs. Median log values (the line within the box marks the median) exhibit quite a large variation (medians range from 3.3 to 6.8 log cfu/cm²). A one-way ANOVA was performed to assess the differences in the mean values of $\log N_0$ among the experimental groups; there was a statistically significant difference (p <0.001) that reflects the variability of the median log values. Such a variability of the number of adhering spores is similar to that reported for a soiling procedure under turbulent flow conditions with the same spore-forming strain adhered to stainless steel pipes [26]. It seems that this variability is inherent in a soiling procedure involving *B. cereus* spores under turbulent flow conditions [26]. It is noted that the fouling and prerinse procedures result in a sufficiently high level of contamination (>3 log) which is enough and convenient for the removal kinetic study.

The quite large variability of the initially adhered to population observed in Fig. 3 may be due to the unavoidable experimental change in the conditioned membrane resistance values ($10^{11} < R_m$ (m^{-1}) $< 10^{12}$), as evidenced by a descriptive statistics procedure (data not presented). As fouling was performed at a constant TMP, significantly different flux vs. time profiles were generated throughout this study. Consequently, the permeation drag (which is one of the predominant physicochemical factors in the initial cell deposition in crossflow MF [9]) can vary substantially from one run to another, which is believed to be responsible for the variability observed in Fig. 3.

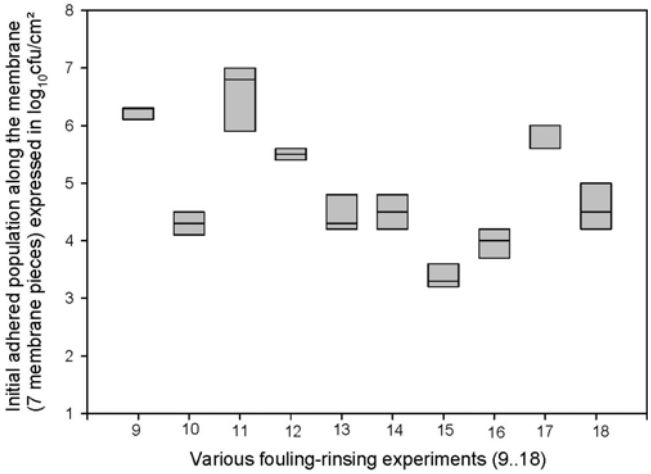

Fig. 3. Box plot graph of the initial population adhered along the membrane (seven membrane pieces per experiment) for the various fouling-prerinse experiments, expressed in log cfu/cm². Line = median, bottom boundary of the box = 25th percentile and upper boundary of the box = 75th percentile.

In addition the initial adhered population must be even along the membrane. A two-way ANOVA with run and membrane location as factors was performed to assess the membrane location effect (the corresponding levels were the seven membrane pieces numbered lengthwise from A to G): there was not a statistically significant difference among the levels of membrane location (p >0.05), which proves that the initial contamination left after fouling was uniform along the membrane.

3.2.2. Relationship between hydraulic and microbiological cleanliness

It is generally accepted that cleaning is complete when the water permeate flux is restored to its initial value, i.e. FR = 100%. For PS UF membranes fouled with whey or skim milk, restoring permeate flux to initial values did not indicate that the system was effectively cleaned and permeate flux alone could not indicate sanitary conditions [15]. The relationship between flux decline and microbial fouling is not completely understood [37]. We have thus sought statistically significant correlations between the adhered spore population (expressed as the median of $\log N_0$ (cfu/cm²) values) and both the measured fouling resistances (R_f, R_{if}, R_{rf}) and the variables of rinsing efficiency assessment (RFR, RRF, HRCC). It was of interest to determine whether the extent of fouling had a measurable influence on the initial adhered population. An analysis by linear regression showed that the correlation between median $\log N_0$ values (at v_r = 2 m/s) and the total (reversible plus irreversible) fouling resistance at the end

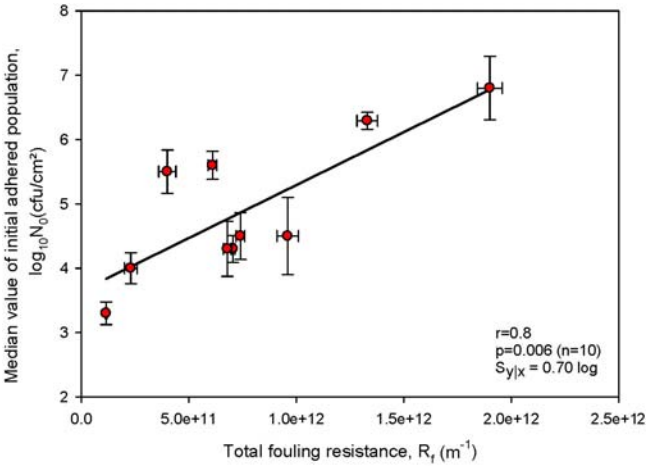

Fig. 4. Variation of the median of the initially adhered to population left after prerinse ($\log N_0$ cfu/cm²) with total fouling resistance at end of fouling (R_f) for various fouling–prerinse experiments. Prerinse operating conditions: v_r = 2 m/s, TMP = 0.5 bar, 20°C, 5 min duration.

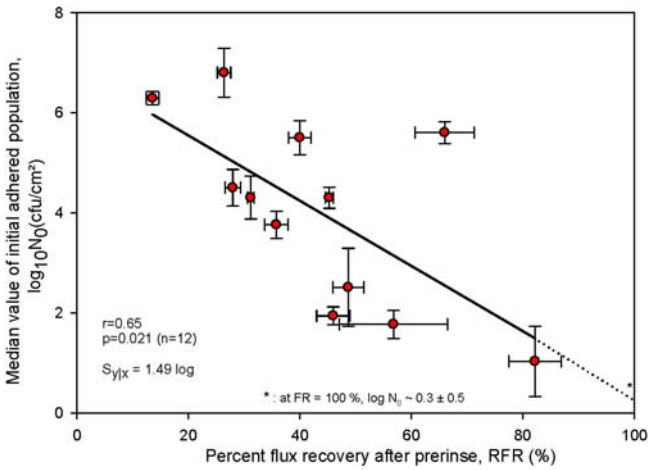

Fig. 5. Variation of the median of the initially adhered to population left after prerinse ($\log N_0$ cfu/cm²) with the percent flux recovery after prerinse (RFR) for the various fouling–prerinse experiments. Prerinse operating conditions: v_r = 2 and 4 m/s, TMP = 0.5 bar, 20°C, 5 min duration.

of fouling (R_f) was statistically significant at the 99% (p <0.01) confidence level (see Fig. 4).

After the first and final rinse, the residual fouling resistance (R_{if} and R_{rf}, respectively) arises from the irreversibly attached spores which lower the effective filtering membrane surface area and contribute to surface spore blocking according to IBFL [33] (see Section 3.2.1). Residual fouling resistance (R_{if}, R_{rf}) was found to be a poor indicator of the adhered spore population: a plot of the median values of the adhered population left after the prerinse ($\log N_0$) or after the final rinse ($\log N$) vs. R_{if} or R_{rf}, respectively, showed that the correlation was not statistically significant (r^2 =0.17, p >0.05, n = 16). In the same way, plotting median $\log N_0$,N values vs. logarithm values of R_{if} and R_{rf} did not improve the correlation (r^2 =0.16, p >0.05, n=16). Residual fouling resistance was thus unable to predict the adhered spore population which means that complex interactions (possibly nonlinear effects) take place between hydraulic and microbiological cleanings.

A Pearson product moment correlation was used to determine the strength of the association between median $\log N_0$ values and the variables of rinsing efficiency assessment (RFR, RRF, HRCC) at v_r = 2 and 4 m/s. Percent flux recovery (RFR) was found to be the sole variable capable of correlating the initial contamination left after the prerinse with a p_{value} less than 0.05 (r = −0.65, p = 0.021, n = 12, see Fig. 5). It is noticed in Fig. 5 that by extrapolating the regression curve at FR = 100%, $\log N_0$ = 0.3 and $N_0 \approx$ 2 cfu/cm²; this contamination level corresponds precisely to the limit of acceptability of the microbiological contamination for an industrial dairy equipment (2 microorganisms/cm² [1]). So for the rinsing operation following the fouling stage, percent flux recovery proved to be sufficiently precise to predict a membrane surface free of microorganisms (2 cfu/cm²) when hydraulic cleanliness is achieved (RFR=100%).

3.3. Sodium hydroxide cleaning

3.3.1. Variation of cleaning efficiency with time

Figs. 6a and 6b present the variation of the percent flux recovery (FR) and the hydraulic cleanliness criterion (HCC) against cleaning time t_c. FR and HCC are both affected by cleaning time which has a detrimental effect on the hydraulic cleanliness; HCC increases linearly with t_c and becomes superior to 0.05 at t_c = 15 min. Therefore, in order to achieve hydraulic cleanliness, cleaning duration must not exceed 10 min where the adhered to log population was found to be minimal (2.7 log cfu/cm²; see Fig. 9).

It can be seen in Fig. 6a that FR is higher than 100% for some runs at t_c <10 min (or HCC <0.05 in Fig. 6b). This observation may be attributed to the adsorption on the membrane material of phosphate ions used to make up the phosphate buffer solution in the fouling stage; indeed the hydraulic resistance of the membrane in contact with the spore suspension in the early stages of filtration was sometimes less than that of the initial conditioned membrane (R_m).

3.3.2. Relationship between hydraulic and microbiological cleanliness

In order to assess the correlation between hydraulic and microbiological cleanlinesses, a Pearson product moment correlation was carried out to measure the strength of the association between the median log$N$ cfu/cm²

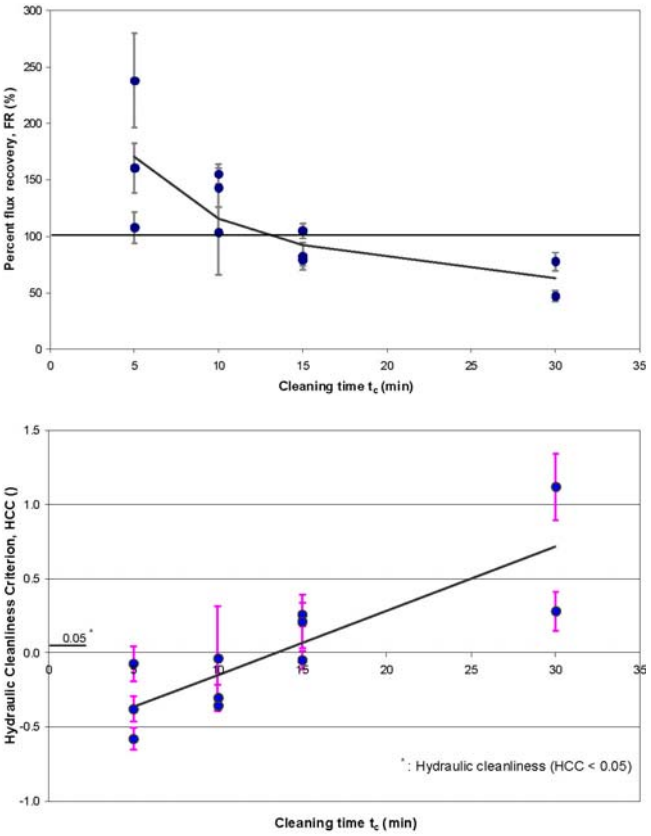

Fig. 6. Variation of the hydraulic cleanliness criterion, HCC (a) and the percent flux recovery, FR (b) with cleaning time (t_c) for the various fouling–cleaning experiments. Operating conditions: 0.5 wt% NaOH, 55°C, v = 4 m/s, TMP = 0.3–0.4 bar.

values and the variables of cleaning efficiency assessment (FR, RF, HCC). The percent irreversible removed fouling (RF) was found to be inappropriate to cleaning efficiency calculation because R_{if} and $R_n - R_m$ were sometimes inferior to zero so that RF had no physical meaning. HCC was the sole variable able to correlate the median log$N$ values with a p_{value} less than 0.05 (r = +0.65, p = 0.02, n = 12; see Fig. 7). Standard percent flux recovery alone was insufficient in indicating the membrane cleanliness (p >0.05).

3.3.3. Kinetics of sodium hydroxide cleaning

The modelling of cleaning kinetics is an essential step in predicting cleaning efficiency and determining the useful cleaning duration. Throughout the cleaning, the total membrane resistance, R, varied from the resistance at the end of the prerinse (R_r, at t_c = 0) to that at the end of cleaning ($R_{n'}$). All R vs. t_c curves exhibited the same characteristic profile: a sharp decline within the first few minutes, followed by a more gradual decrease, then R was constant up to the end of cleaning ($R = R_{n'}$). Cleaning kinetics was modelled here as reduction of the total membrane resistance with time. The rate of membrane resistance reduction associated with the detachment of

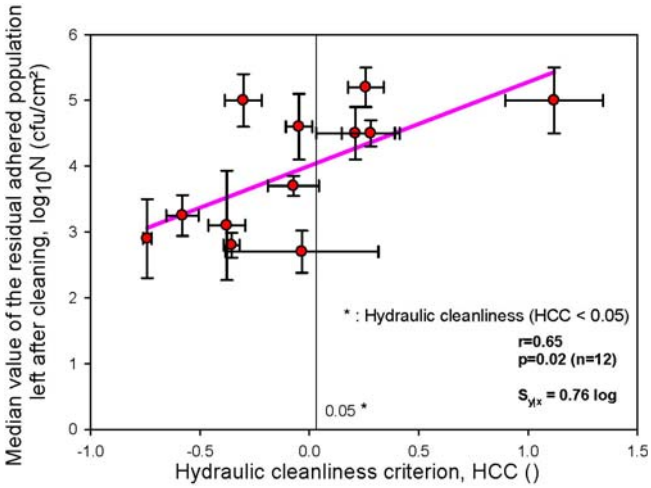

Fig. 7. Variation of the median value of the residually adhered to population left after cleaning (log$N$ cfu/cm^2) with the hydraulic cleanliness criterion (HCC) for the various fouling–cleaning experiments. Operating conditions: 0.5 wt% NaOH, 55°C, v = 4 m/s, TMP = 0.3–0.4 bar.

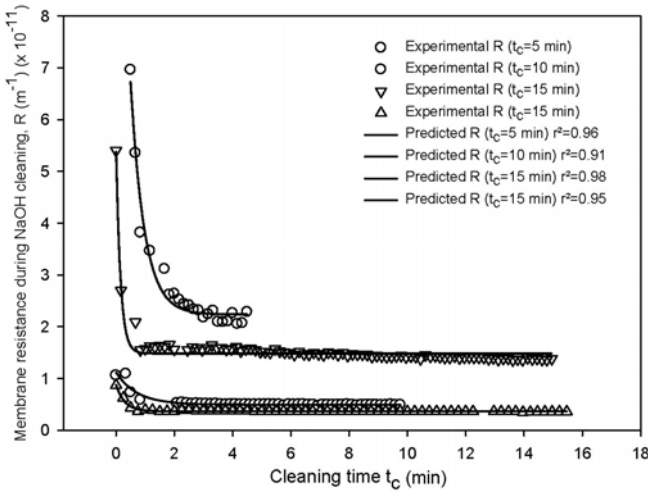

Fig. 8. Examples of variation of experimental and predicted [derived from Eq. (2)] hydraulic membrane resistance (R) as a function of cleaning time (t_c). Operating conditions: 0.5 wt% NaOH, 55°C, v = 4 m/s, TMP = 0.3–0.4 bar.

B. cereus spores was found to be described by a first order equation:

$$\frac{dR}{dt} = -k(R - R_{n'}) \quad (1)$$

where k is a first-order rate constant (min^{-1}). Integrating Eq. (1) between 0 and t, $R(t)$ was defined by

$$R(t) = R_{n'} + (R_r - R_{n'})\, e^{-k.t} \quad (2)$$

At t = 0, $R = R_r$ and when t tends towards infinity, R

Table 3
Cleaning kinetic data [derived from Eq. (2)] for NaOH cleaning: fitting of experimental time-dependent membrane hydraulic resistance during cleaning, $R(t_c)$. Experimental conditions: 0.5 wt% NaOH, 55°C, v_c = 4 m/s, TMP = 0.3–0.4 bar, 5 < t_c (min) < 30

Cleaning time t_c (min)	Coefficient of determination r^2 [Eq. (2)]	Rate constant k (min^{-1})	Standard error on k (min^{-1})	Initial membrane resistance (left after prerinse), R_r (m^{-1})	Membrane resistance left after cleaning at t_c, R_n' (m^{-1})	t_c[a] (min)
5	0.96	2.1	0.25	1.52×10^{12}	2.24×10^{11}	2.2
10	0.91	0.92	0.09	1.07×10^{12}	5.70×10^{11}	3.2
10	0.77	0.8	0.2	7.84×10^{11}	5.70×10^{11}	2.5
10	0.91	1.4	0.2	1.12×10^{11}	4.90×10^{10}	2.3
15	0.83	0.17	0.03	1.51×10^{11}	1.29×10^{11}	7.1
15	0.98[a]	3.4	0.15	8.80×10^{10}	3.64×10^{10}	1.0
15	0.95[a]	6.5	0.5	5.40×10^{11}	1.48×10^{11}	0.6
15	0.90[a]	5.2	0.7	6.80×10^{11}	2.25×10^{11}	0.7
30	0.75[a]	0.39	0.04	5.64×10^{11}	2.56×10^{11}	8.0

[a]Experiment for which Eq. (3) of higher order provides a statistically better fit of $R(t_c)$, i.e., p <0.05.

approaches R_n'. The above first-order equation was previously proposed to model the rinsing kinetics of a ceramic UF membrane fouled with whey proteins [38]. Fig. 8 shows that good agreement was obtained between experimental and predicted membrane resistance profiles (for all experiments, 0.75 < r^2 <0.98, Table 3). The first-order rate constant k was not affected by cleaning time: k oscillates around an average value (<k> = 2.3 ± 2 min^{-1}) with no noticeable trend. The useful cleaning duration was assessed from the calculation of a cleaning time, t_c*, for which $R = 1.05 \times R_n'$; t_c* ranged from 0.6 to 8 min, indicating that membrane permeability was fully restored at a cleaning time close to 10 min.

It should also be stated that for four cleaning experiments at t_c = 15 and 30 min (see Table 3), another first-order equation of higher order [composed of four parameters compared to three for Eq. (2)] provided a statistically better fit and also a better agreement was obtained between experimental and predicted membrane resistance profiles during cleaning. The total membrane resistance variation with time is represented by the following equation of higher order:

$$R(t) = \alpha R_r e^{-k_1 t} + (1-\alpha) R_r e^{-k_2 t} \quad (3)$$

where α is the fraction of species 1 in the initial fouling deposit and k_1 and k_2 are the removal rate constants of species 1 and species 2, respectively. Eq. (3) involves a simultaneous two-species removal with each deposit species (here a spore population attached to the membrane surface) displaying a first-order resistance decrease with time. This model was previously reported to provide a good fit to fouling resistance changes in the course of the chemical cleaning of both a 0.1 μm polyethersulphone membrane fouled with yeast cells [39] and a 0.1 μm ceramic membrane fouled with a whey protein concentrate [23]. Eq. (3) was tested for all cleaning experiments (using SigmaPlot 9.0 software) and then a F-test mathematical procedure was used to determine a possible statistical improvement in regressions using Eqs. (2) and (3). Eq. (3) provided a better fit of R vs. t_c curves just for four cleaning runs with a long cleaning duration (t_c = 15, 30 min; see Table 3). It is noticed that for these cleaning experiments, unlike the others where R rapidly becomes constant up to the end of cleaning as shown by the short t_c* values in Table 3, following the sharp decline of the membrane resistance in the initial stages of cleaning, R decreases slightly in a linear manner and then is constant a few minutes before cleaning ceases. The better agreement obtained between experimental and predicted membrane resistance changes during cleaning using Eq. (3) can be related to the spore redeposition process that occuring beyond 10 min of cleaning. A possible explanation is that at a cleaning duration superior to 10 min, the redeposition on the membrane surface of spores previously released in the cleaning solution implies a cleaning mechanism combining simultaneaous removal and deposition rates, which results in more complex membrane resistance profiles that are not taken into account by Eq. (2). The rate of membrane resistance reduction for species 1 is far greater than that for species 2 (k_1 is two orders of magnitude higher than k_2), indicating that each fouling species has different removal characteristics. Modelling of membrane resistance changes during cleaning using Eq. (3) and thus suggests the existence, at a cleaning duration higher than 10 min, of two fouling species (i.e. two spore populations adhered to the membrane surface) which exhibit a different ease of removal. Individual curve fittings showed an initial composition in species 1 (α) in the range 50–70% for optimal k_1 and k_2 values.

3.3.4. Removal kinetics of B. cereus *spores during sodium hydroxide cleaning*

As a fouled membrane control was not available for each cleaning run, it was necessary to verify that the initially adhered to population for the cleaning runs was close to that of the fouling runs. Predicted $\log N_0$ (cfu/cm^2) values were fitted using a linear best subsets regression procedure followed by a multiple linear regression (SigmaStat 3.1., Systat Software) (data not shown). Briefly, the independent variables were the fouling parameters derived from IBFL applied to the fouling stage [35] plus a relevant calculated fouling parameter (noted F_y), and the dependent variable was the experimental median log N_0 values. A statistically significant correlation (r = 0.93, p = 0.003, n = 9) was found which involved both the fouling parameter associated with IBFL (σ in m^{-1}) and F_y, expressed in kg.m.s^{-2}. Predicted $\log N_0$ values derived from the fouling stage of the cleaning runs were then compared with those of the fouling runs. The average value of the predicted $\log N_0$ data was found to be very close to that of the fouling runs (4.9 ± 0.2 log compared to 5.09 ± 0.3 log, respectively). An unpaired t-test performed on $\log N_0$ values for the fouling and cleaning runs gives a p_{value} equal to 0.7 for the two groups; there was not a significant difference in the initial contamination level between the fouling and cleaning runs.

Fig. 9 represents a box plot graph of the adhered log population along the membrane as a function of cleaning time t_c. The adhered log population varies in a nonlinear manner with t_c: it decreases linearly between 0 and 10 min where the contamination is minimal (2.7 log cfu/cm^2), then increases and reaches a plateau value close to 4.6 log cfu/cm^2 between 15 and 30 min. A redeposition process on the membrane surface of spores previously released in solution is clearly observed beyond 10 min. In the 0.5 wt% NaOH solution, ionic strength is high (0.125 M) so that repulsive electrostatic interactions between spores and the membrane (both negatively charged) are reduced due to a charge screening effect; *B. cereus* spores having a pronounced hydrophobic character, hydrophobic interactions are likely to play a predominant role in the redeposition process. Fig. 9 suggests an optimal cleaning time of 10 min from a microbiological point of view, which is consistent with the useful cleaning duration (t_c*) derived from the membrane resistance vs. cleaning time curves (see Table 3) for which membrane resistance was quasi-constant above 10 min of cleaning. At t_c = 10 min, the contamination level is close to 600 cfu/cm^2, i.e. two orders of magnitude larger than the acceptability limit for industrial dairy equipment (2 microorganisms/cm^2 [1]): microbiological cleanliness was not achieved. For a cleaning time inferior to 10 min, microbiological cleanliness was not achieved whereas the membrane could be considered as being clean hydraulically (FR = 100% and HCC <0.05, Figs. 6a and 6b); flux

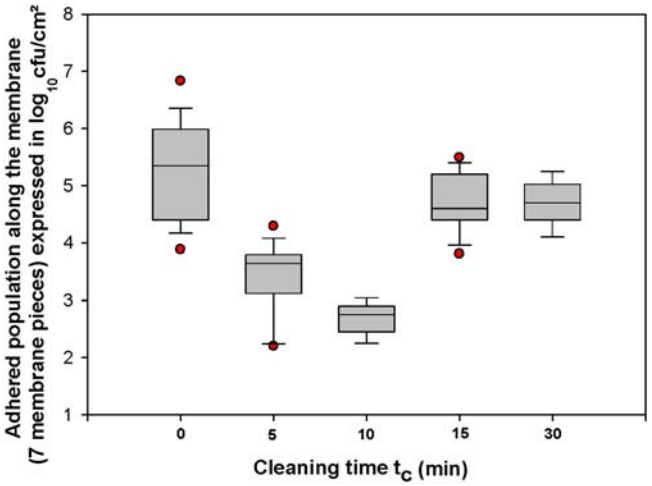

Fig. 9. Box plot graph of the initially ($\log N_0$ cfu/cm^2) and residually ($\log N$ cfu/cm^2) adhered population along the membrane (seven membrane pieces per experiment) for the various fouling–prerinse and fouling–cleaning experiments, respectively. Line = median, bottom boundary of the box = 25th percentile and upper boundary of the box = 75th percentile.

recovery data alone are therefore inappropriate in assessing membrane microbiological cleanliness.

The contribution of the wall shear stress alone on the removal of *B. cereus* spores was assessed by performing some additional fouling experiments at a crossflow velocity in the prerinse stage of v_r = 4 m/s (τ_w =30 ± 2 Pa). Median $\log N_0$ values were compared for two groups (one group at v_r = 2 m/s, τ_w = 5 ± 1.7 Pa, one group at v_r = 4 m/s) using an unpaired t-test. A statistically significant difference between the two groups was found (p <0.001) with a mean $\log N_0$ value equal to 4.9 at v_r = 2 m/s and 2.2 at v_r = 4 m/s, which means that an increased wall shear stress during prerinse substantially reduces the remaining adhered population. Cleaning therefore implies the combined action of both sodium hydroxide (chemical action) that lowers the adhesion strength of spores on the membrane surface and the wall shear stress that acts mechanically on spore removal (spore cells attached to the membrane experience hydrodynamic drag, torque at the center of the cell and lift [40]); this was previously reported for the cleaning of stainless steel pipes soiled by *B. cereus* spores [26].

As for the initial contamination left after fouling, it was of interest to verify if the residual contamination left after cleaning was uniform or not along the membrane. Unlike the fouling experiments, a statistically significant difference among the levels of the membrane location (i.e. membrane pieces numbered A to G) was calculated for the median $\log N$ values (p <0.05). Moreover, using a pairwise multiple comparison procedure (Holm–Sidak method), the difference was only significant for the inlet (A) and outlet (G) of the membrane, which is likely to be due to the

effect of pressure drops along the membrane (on average 0.13 bar for the fouling step and 0.1 bar for the cleaning step). Large pressure drops in a membrane module are thus liable to result in changes in membrane cleanability along the membrane path.

The removal kinetics of *B. cereus* spores during cleaning was modelled using the variation with t_c of the number of adhered spores per unit membrane surface area (N in cfu/cm^2), which represents a potential risk for further feed contamination. As for most of the investigations dealing with the modelling of the removal kinetics of macro-soiling and micro-soiling, the removal kinetics of *B. cereus* spores from the membrane surface is based in this study on a process combining removal and deposition as a function of time [41]. Such a modelling is consistent with the experimental shape of the adhered log population vs. the t_c curve, which exhibits a nonlinear variation (see Fig. 9); a redeposition phenomenon onto the membrane surface of spores previously released in the sodium hydroxide solution is clearly seen beyond t_c = 10 min. Consequently, the hypothesis of a cleaning mechanism combining removal and deposition was justified from these experimental results. The removal part can be defined using a first-order reaction:

$$dN/dt = -k_3.N \quad (4)$$

where k_3 is the effective removal rate constant. The extent of spore deposition is related to the bulk population that is linked to the number of spores previously released in the filtration loop. At time t, the amount of removed spores is the difference between the initial amount N_0 and $N(t)$. The deposition part can thus be expressed as

$$dN/dt = k_4.(N_0 - N) \quad (5)$$

where N_0 is the initial spore surface density (cfu/cm^2) at t_c = 0 and k_4 an apparent deposition rate constant; k_4 depends on the concentration of removed spores in solution, namely, involves the ratio of the membrane surface area S (0.0073 m^2) to the detergent solution volume V (0.08 m^3). The removal and deposition processes are simultaneous and independent and Eqs. (4) and (5) can be associated to yield:

$$dN/dt = k_4.(N_0 - N) - k_3.N \quad (6)$$

Integrating Eq. (6) between 0 and t, $N(t)$ is defined by

$$N = \frac{k_3 N_0}{k_3 + k_4} + \frac{k_4 N_0}{k_3 + k_4} e^{-(k_3 + k_4)t} \quad (7)$$

When t = 0, $N = N_0$ and when t tends towards infinity, N approaches $N_\infty = k_3.N_0/(k_3 + k_4)$. Eq. (7) was tested (using SigmaPlot 9.0 software) and was found to provide a good fit to experimental data (r^2 = 0.97, p <0.0001, see Fig. 10a). In addition, the plot of the standardized residuals (Fig. 10b) indicated that no bias was induced by the model. After overall optimisation, the values of k_4 and k_3 were 0.51 min^{-1} and 12.7 min^{-1}, respectively. As shown in Fig. 10a, much of the spore removal occurs within the first minute of cleaning ($k_3/k_4 \approx 25$) and N tends to N_∞ = 4.3×10^4 cfu/cm^2 thereafter. The strength of the model is its simplicity and a direct linkage to observed phenomena. Nevertheless, although good agreement was obtained between the experimental and predicted adhered spore population as a function of cleaning time [Eq. (7), r^2 = 0.97, n=11], the model combining first-order removal and deposition rates does not exactly describe the complexity of the experimental residually adhered population profile. The model, which is under the form of a three-parameter single-exponential decay, does not take into account the substantial increase (from 2.7 to 4.7 log cfu/cm^2; see Fig. 9)

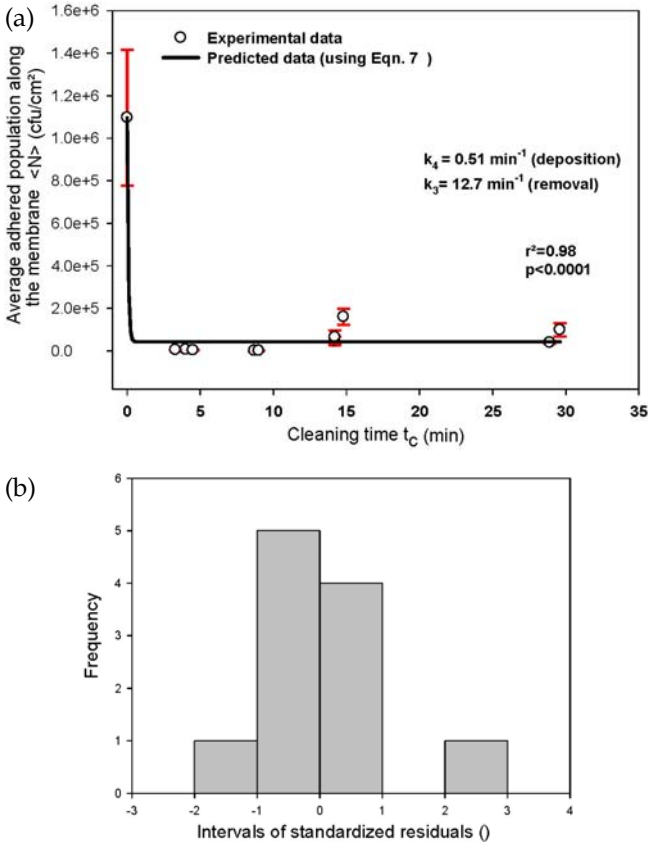

Fig. 10. Removal kinetics of *B. cereus* spores. (a) Variation of experimental and predicted [Eq. (7)] values of average adhered population along the membrane (<N> cfu/cm^2) as a function of cleaning time (t_c). (b) Plot of standardized residuals between predicted and experimental data. Operating conditions: 0.5 wt% NaOH, 55°C, v = 4 m/s, TMP = 0.3–0.4 bar.

in the adhered population between 10 and 15 min of cleaning due to the redeposition process; this results in an observable underestimation of the experimental residual membrane contamination beyond 10 min of cleaning (see Fig. 10a).

A similar kinetic model [26] was used for the removal of the same spore-forming strain from stainless steel pipes under similar cleaning conditions (0.5 wt% NaOH, 60°C, $0.5 < \tau_w < 70$ Pa). The extrapolation of k_3 at $\tau_w = 40$ Pa in the cleaning stage here gives $k_3 \approx 0.2$ min^{-1} [26], that is, two orders of magnitude lower than k_3 found in this study (12.7 min^{-1}). Thus the ceramic membrane induces a greater ease of removal compared to stainless steel (304 L), which may be caused by the membrane surface porosity, different physicochemical properties of the material and differences in spore-substratum adhesion strength. The redeposition process observed beyond 10 min of cleaning (contrary to what Lelièvre et al. [26] reported using 0.5 wt% NaOH with $k_4 = 0$ for stainless steel pipes) is likely to be enhanced by the permeate flux at a positive TMP of 0.3–0.4 bar: the normal permeation drag force acting on spore cells enhances the adhesion of spores previously released in the cleaning solution. This highlights the complexity of cleaning mechanisms in crossflow MF compared to those occuring with non porous materials (stainless steel pipes) for which spore removal kinetics reduced to a first-order equation without deposition rate, i.e. $N=N_0e^{-k3.t}$ [26].

Further work is required to elucidate the role played by the transmembrane pressure during cleaning (TMP = 0 or TMP >0) on the extent of the deposition rate (k_4) which has to be as low as possible to limit or prevent spore redeposition. A possible way to adjust the relative importance of removal and deposition rates would be to use formulated detergents consisting of a mixture of sodium hydroxide and a surfactant (e.g. a non ionic surfactant like Tween 80), as previously shown by Lelièvre et al. [42].

4. Conclusions

A simple model (first-order reaction) combining removal and deposition rates was shown to give a good fit ($r^2 = 0.97$) to the shape of the residual adhered population changes measured experimentally. The removal kinetics of *B. cereus* spores from the surface of a ceramic MF membrane was found to be more complex than that encountered with nonporous materials (stainless steel pipes [26]). The model especially needs to be improved so as to successfully fit the spore redeposition process occuring beyond 10 min of cleaning.

An optimum cleaning duration close to 10 min has been put forward beyond which membrane resistance was quasi-constant but did not decrease further. Additionally, the adhered log population was minimal at t_c = 10 min. In spite of a residual spore population left after cleaning that ranged from 2.7 to 5.2 log cfu/cm^2, hydraulic cleanliness was achieved at cleaning times less than 15 min (HCC <0.05). Unlike hydraulic cleanliness, microbiological cleanliness was not achieved with the cleaning procedure used. The spore redeposition process observed at t_c greater than 10 min must be avoided if microbiological cleanliness is required. Controlling spore redeposition during cleaning with sodium hydroxide may be achieved by performing a cleaning at higher wall shear stresses (i.e. at an increased tangential drag force acting on deposited cells) or at a zero TMP for which there is no permeation drag applied to cells.

This study examined the relationship between hydraulic and microbiological cleanings to gain knowledge relevant to the relationship between flux decline and microbial fouling left after a chemical cleaning. Results indicated that complex interactions (possibly nonlinear effects) take place between hydraulic and microbiological cleanlinesses. In particular the spore redeposition process occuring beyond 10 min of cleaning did not result in a parallel increase in the hydraulic membrane resistance.

The fundamental knowledge of mechanisms of *B. cereus* spore removal from the surface of a ceramic MF membrane and the development of cleaning models require further research. It appears notably necessary to achieve alkaline cleanings at a varying wall shear stress and transmembrane pressure, removal rate constant (k_3) and deposition rate constant (k_4) being liable to be affected by these two operating parameters, as shown in the alkaline cleaning of pipes soiled by *B. cereus* spores where the removal rate constant was significantly influenced by the wall shear stress applied during cleaning [26].

5. Symbols

d_h — Internal membrane channel diameter, m

F_y — Calculated fouling parameter equal to the integral sum over the fouling stage of the flux-dependent instantaneous permeation drag force at time t minus the permeation drag force at the quasi-steady state flux (J_{ss}), kg.m.s^{-2} or N

FR — Percent flux recovery, $=R_m/R_n \times 100$, %

HCC — Hydraulic cleanliness criterion, $=(R_n - R_m)/R_m$

$HRCC$ — Hydraulic rinsing cleanliness criterion, $=(R_r - R_m)/R_m$

J — Instantaneous permeate flux, $= TMP/\mu.R$, m.s^{-1}

J_{ss} — Steady-state flux at end of fouling, $= TMP/\mu.R_t$, m.s^{-1}

J_{w0} — Initial pure water flux, $= TMP/\mu R_m$, m.s^{-1}

J_{wc} — Permeate flux during sodium hydroxide cleaning, m.s^{-1}

J_{wr} — Permeate flux during prerinse, = $TMP/\mu R_r$, $m.s^{-1}$
J_{ww} — Permeate flux during final rinse, = $TMP/\mu R_n$, $m.s^{-1}$
k — First-order rate constant, Eq. (1), min^{-1}
k_1 — Removal rate constant for species 1, Eq. (3), min^{-1}
k_2 — Removal rate constant for species 2, Eq. (3), min^{-1}
k_3 — Effective removal rate constant, Eq.(4), min^{-1}
k_4 — Apparent deposition rate constant, Eq. (5), min^{-1}
LRV — Log reduction value at the second sonication, = $-\log_{10}(1-z_2/100)$
N — Number of residually irreversibly adhered spore cells per unit membrane surface area following cleaning–final rinse sequence, cfu/cm^2
N_0 — Number of irreversibly adhered spore cells per unit membrane surface area following fouling–prerinse sequence, cfu/cm^2
OD_{400} — Optical density (or absorbance) at 400 nm
r — Correlation coefficient
R — Hydraulic membrane resistance, m^{-1}
r^2 — Coefficient of determination
Re — Reynolds number, = $\rho.v.d_h/\mu$
R_f — Total fouling resistance at the end of fouling, m^{-1}
R_{if} — Total irreversible fouling resistance following the prerinse, m^{-1}
R_m — Initial (conditioned) membrane resistance, m^{-1}
R_n — Cleaned membrane resistance during final rinse, m^{-1}
$R_{n'}$ — Hydraulic membrane resistance at the end of cleaning, m^{-1}
R_r — Hydraulic membrane resistance at end of prerinse, m^{-1}
R_{rf} — Residual fouling resistance following final rinse, m^{-1}
R_t — Hydraulic membrane resistance at end of fouling, m^{-1}
RF — Percentage of irreversible removed fouling, $=[R_{if}-(R_n-R_m)]/R_{if}\times 100$, %
RFR — Percent flux recovery for rinsing efficiency, $=R_n/R_r\times 100$, %
RRF — Percentage of reversible removed fouling for rinsing, $=[R_f-(R_r-R_m)]/R_f\times 100$, %
S — Membrane surface area, m^2
$S_{y|x}$ — Standard error of the estimate
t — Time, s
t_c — Cleaning time, s
t_c^* — Cleaning time for which $R = 1.05\times R_{n'}$, s
TMP — Average transmembrane pressure, Pa
v — Crossflow velocity in membrane channel, $m.s^{-1}$
v_r — Crossflow velocity in membrane channel in first rinse (prerinse) step, $m.s^{-1}$
y_i — Number of *B. cereus* spores counted at the *i*th sonication, cfu/cm^2
z_j — Cumulative percent of counted spores at the *j*th sonication, $= \frac{\sum_{1}^{j} y}{\sum_{i=1}^{4} y_i}\times 100$, %

Greek

α — Fraction of species 1 in the initial fouling deposit [Eq. (3)]
μ — Dynamic permeate viscosity, $kg.m^{-1}.s^{-1}$
ρ — Specific mass of solution, $kg.m^{-3}$
σ — Blocked area per unit filtrate volume, m^{-1}
τ_w — Wall shear stress, $kg.m^{-1}.s^{-2}$ or Pa

Acknowledgements

The authors wish to thank Laurence Ficot for technical assistance.

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