Adhesion of microorganisms to polymer membranes: a photobactericidal effect of surface treatment with TiO$_2$

V. Kochkodan*, S. Tsarenko, N. Potapchenko, V. Kosinova, V. Goncharuk

A.V. Dumansky Institute of Colloid Chemistry and Water Chemistry of National Academy of Sciences of Ukraine, 03680 Kyiv 142, Vernadsky Str. 42, Ukraine
Tel. +380-44 4247521; Fax +380-44 4238224; email: kochvik@iccwc.kiev.ua, vkochkodan@hotmail.com

Received 22 January 2007; accepted 30 January 2007

Abstract

The adhesion of *Escherichia coli*, *Pseudomonas putida* and *Acinetobacter calcoaceticus* cells to Microdyn-Nadir ultrafiltration membranes of various chemical nature: PS100 (polysulfone), P005 (polyethersulfone), C100 (regenerated cellulose) was studied. It was shown that an adhesiveness of the microorganisms to the membranes essentially depends on hydrophobic/hydrophilic properties of both the cells and membranes. In particular, it was found that the adhesion of relatively hydrophilic *E. coli* to membrane surfaces is essentially lower comparing with the adhesion of more hydrophobic *P. putida*, or *A. calcoaceticus* cells. In a turn the microorganisms attachment to more hydrophobic polyethersulfone and polysulfone membranes is higher than to hydrophilic cellulose one. It was shown that the volume fluxes of membranes with adhesive microorganisms dropped while samples were kept in contact with natural surface water due to increasing of cell number on membrane surface. In attempts to reduce membrane biofouling, TiO$_2$ particles were deposited on membrane surface with following ultraviolet (UV) irradiation at 365 nm. It was shown that due to photobactericidal effect the fluxes of surface modified membranes were 1.7–2.3 times higher comparing with those for control membrane samples (without TiO$_2$ deposition and UV treatment).

Keywords: Microorganisms adhesion; Membrane biofouling; Photobactericidal effect; TiO$_2$

1. Introduction

Membrane bio(fouling) is one of the main problems during an operation of membrane elements for water treatment [1,2]. Typical side effects of microbiological fouling are (i) a reduction of the performance of membranes due to formation of biofilm on their surfaces, (ii) the secondary pollution of the purified water by bacterial cells and products of their metabolism, (iii) an increase in the power consumption because of the higher pressure requirements to overcome the biofilm resistance and the flux decline.

In principle, thermal or chemical treatments are possible methods of disinfection of membrane surfaces. Thermal disinfection is not widely used

*Corresponding author.

Presented at the conference on Desalination and the Environment. Sponsored by the European Desalination Society and Center for Research and Technology Hellas (CERTH), Sani Resort, Halkidiki, Greece, April 22–25, 2007.
and is restricted to some types of MF and UF membranes because of low thermal resistance of most commercially available polymeric reverse-osmosis, ultra- and nanofiltration membranes. In turn, the effective liquid disinfectants are often very aggressive [3] so they can cause irreversible changes to the membrane selective layer.

A common approach to prevent the microbiological fouling of the membranes is an appropriate preliminary treatment of feed water. This enables to decrease the content of bacteria and nutrients consumed by the bacteria during their activity in water [4]. However, such a pretreatment is rather labor consuming and expensive.

Other attempts to reduce the biofouling problem consist of specific modification of membranes [5–10] aimed at alteration of the membrane surface chemistry to minimize the foulants adsorption and to increase the membrane permeability [5–7] or at introducing of potential biocide substances to inhibit the growth of microorganisms on the membrane surface [8–10].

Despite the efforts, a minimization of membrane biofouling is far from already solved problem therefore additional studies are needed on this matter. The attachment of bacterial cells to the surface of the membrane is the critical first step in membrane biofouling. The microbiological fouling may be even started with adhesion of a few bacteria capable of division on the membrane surface. In this paper the adhesion of different microorganisms to polymeric membranes of various chemical nature was evaluated. An influence of cells adhesion on membrane fluxes as well as a photobactericidal effect of membrane samples with deposited TiO$_2$ particles under black ultraviolet (UV) irradiation was investigated.

2. Materials and methods

*Escherichia coli* 1257 (*E. coli*), *Pseudomonas putida* (*P. putida*) or *Actinetobacter calcoaceticus* (*A. calcoaceticus*) microorganisms were used to evaluate the cells adhesion to polysulfone (PS100), polyethersulfone (P005), cellulose (C100) ultrafiltration membranes from MICRODYNNADIR GmbH (Wiesbaden, Germany).

For preparation of the bacterial suspensions the bacteria cultures grown on a meat–peptone agar were placed in 3 mL of meat–peptone broth (MPB) and cultivated for 24 h at 37°C for *E. coli* or at 28°C for *P. putida* or *A. calcoaceticus*. Then daily-aged cultures were transferred to 200 mL of MPB and were incubated at appropriate temperatures till starting of the stationary growth phase. After centrifugation at 2500 × g for 10 min (a K 26 D centrifuge) the broth cultures were washed three times with distilled sterile water and resuspended to a concentration of 10$^8$ colonies formed units (CFU) per mL determined by optical density (a KΦK-2 photocolorimeter, $\lambda = 540$ nm). Prior to filtration experiments the initial suspension was diluted to a corresponding concentration with distilled water.

Ridgway et al. [11,12] modified technique was used to study the adhesion of microorganisms cells to membrane surface. Membrane samples were immersed in 50 mL of bacterial suspension and were shaken for 1–4 h at 20°C using an ABU-2 shaker (Mashprom Corp, Russia). Then membranes were removed from suspension and rinsed twice with 50 mL of distilled sterile water for 15 min. The rinsed membranes were placed in test tubes with 10 mL of distilled sterile water. One set of membranes with adhesive cells was treated in an ultrasound bath (UM-4) for 15 min whereas another one was shaken for 15 min. A quantity of cells realized from membrane surface was calculated as a CFU number under incubating on Endo medium at 37°C for *E. coli*, or on a nutrient agar at 28°C for 24–48 h for *P. putida* or *A. calcoaceticus*. The number of cells adhered to membrane bacteria was calculated from the difference between the numbers of the cells detached from membrane surface at ultrasound treatment comparing with one at shaking.

*P. putida* was used as a test culture to evaluate an alteration in membrane flux vs. the time
of membrane exposure to natural surface water (Nivka River, Kyiv). The main characteristics of this water were: color — 42–45 units; phosphates — 8–13 mg/L; ammonium — 0.2–0.3 mg/L. The preliminary thermally sterilized surface water infected with \textit{P. putida} cells was filtered through membranes samples using FM-10 dead-end filtration cell (Hust, Ukraine) with the membrane area of 4.9 cm$^2$. Thereafter the membranes were placed in Petri dishes filled with sterilized surface water. After the determined time samples were taken out from the solution and the membrane flux $J$ (L/h) was measured as $J = V/t$, where $V$ (L) is a volume of permeate that passed through the membrane during time $t$ (h) at operating pressure of $\Delta P$ (bar).

In parallel, the number of \textit{P. putida} cells on the membrane surface was evaluated. To shake down the deposited bacteria from the membrane surface the samples were treated in ultrasound bath for 15 min. Additionally, the control experiments were performed using both the membrane samples without deposited cells and the membranes through those a UV-treated suspension of \textit{P. putida} cells was filtered. A high pressure mercury UV-lamp of SVD-120A type (a $\lambda$ of radiation in a range of 200–400 nm) was used for the suspension treatment for 20 min. The intensity of UV-irradiation measured by an UV-meter (NPO Lambit-Entis, Ukraine) was 1.89 W/cm$^2$.

To produce the membranes with potential photobactericidal effect the samples were immersed in TiO$_2$ suspension (Sigma–Aldrich, 99.9%, $<5$ µm) with concentration of 2 g/L for 10 min. To evaluate an antimicrobial effect of the obtained membranes a suspension of \textit{P. putida} with concentration of $1.6 \times 10^4$ CFU/mL was filtered both through the membranes with deposited TiO$_2$ particles and through the control samples (without TiO$_2$). Thereafter the membranes were illuminated with a black UV-lamp (SVD-120A) equipped with optical filters UFS-6 and BS-7. The UV-light intensity was 153 $\mu$W/cm$^2$ at 365 nm. After UV-illumination the number of \textit{P. putida} colonies on the surface of modified and control membranes was determined.

While studying an alteration in volume flux with time of membrane exposure to river water, the membrane samples were irradiated with black UV-light for 4 h per day. The fluxes were measured each 24 h.

3. Results and discussion

As can be seen in Fig. 1, the adhesiveness of the used test-microorganisms to C100 cellulose membrane slightly increases in the following order: \textit{E. coli} < \textit{A. calcoaceticus} < \textit{P. putida}. It should be noted that adhesion of \textit{A. calcoaceticus} or \textit{P. putida} to P005 polyethersulfone membrane is nearly identical, while \textit{P. putida} cells are somewhat less adhesive to PS100 polysulfone membrane than \textit{A. calcoaceticus}. Unequivocally, the adhesion capability of \textit{E. coli} to attachment to the membrane surfaces is the lowest among the test-microorganisms being studied. Obviously, this finding may results from the different hydrophobic/hydrophilic properties of the used microorganisms. The contact angle of wetting for \textit{P. putida} cell is 38.5° while for \textit{E. coli} (various

![Fig. 1. Adhesion of the test-microorganisms (1 — \textit{E. coli}; 2 — \textit{A. calcoaceticus}; 3 — \textit{P. putida}) to various membranes. The density of suspension is $1 \times 10^8$ CFU/mL, time of membrane contact with suspension is 4 h.](image-url)
strains) balances within a range of 16.7–24.7° [13]. The values of another parameter of cell’s hydrophobicity measured from adsorption of bacteria on a surface of hydrophobic liquid (hexadecane) for E. coli K-12 and A. calcoaceticus are 0 and 91.1%, respectively [13]. These data indicate that E. coli cells are more hydrophilic comparing with A. calcoaceticus or P. putida. On the other hand, polyethersulfone and polysulfone membranes are more hydrophobic than cellulose. This is confirmed by higher contact angle of wetting: 92° ± 2° and 82° ± 2° for polyethersulfone and polysulfone membranes, respectively, compared to 59° ± 3° for cellulose one [14]. Thus, it can be concluded that hydrophobic interactions between the bacterial cell and the membrane surface play an important role in adhesion of microorganisms to membranes.

As P. putida is more widely occurs in surface water comparing with A. calcoaceticus, this test-microorganism was used in the further experiments.

Membrane samples both with deposited native and UV-treated P. putida cells were used to evaluate the volume flux decline with time of membrane exposure to river water. Fig. 2 (curve 3) shows that the most essential drop of the membrane flux was observed for membrane sample with native P. putida cells. This may be explained both by the membrane pore plugging with deposited cells and by increasing in a number of microorganisms on the membrane surface in conjunction with the membrane fouling with various by-products of bacteria metabolism. The growth of cells on the membrane surface was confirmed by microbiological analysis: a number of bacteria on PS100 membrane after exposure to natural water for 7 days increased from 7.1 × 10⁵ up to 4.6 × 10⁷ CFU/membrane. The deposition of UV-treated cells on the membrane surface also results in flux decline (Fig. 2, curve 2) obviously due to the membrane pore plugging with the deposited cells, but the flux drop in this case is less pronounced comparing with one for the deposited native cells. This is because the UV-treated cells are incapable of multiplying on membrane surface. Flux decline for control membrane without P. putida bacteria (Fig. 2, curve 1) is apparently an evidence of possible colonization of the membrane surface by heterotrophic microorganisms which may be transferred to membrane from the surface of non-sterile filtration cell.

In attempts to minimize the membrane biofouling titanium dioxide particles were deposited on the surface of membrane samples followed by UV-irradiation as was described in Section 2. Antibacterial properties of the membranes thus prepared were estimated in the experiments with P. putida. Fig. 3 shows a number of P. putida cells on the surface of initial and TiO₂ modified membrane samples with and without UV-treatment. As can be seen, the cell numbers on the initial membrane in the dark, on the membrane with deposited TiO₂ in the dark and on the initial membrane treated by black UV-light are practically identical (Fig. 3, curves 1–3). On contrary, a
sharp reduction in a quantity of *P. putida* cells on the surface of membrane sample with deposited TiO$_2$ particles was found: the number of cells was dropped from $7.0 \times 10^4$ to $1.3 \times 10^2$ CFU/membrane under UV-irradiation for 6 h (Fig. 3, curve 4). The calculated bactericidal effect for this membrane samples in terms of CFU is about 98.1%. Thus, the antibacterial effect of this membrane sample was almost 100%. This means that membrane surface with deposited TiO$_2$ particles possess strong photobactericidal properties and may be potentially more resistant to biofouling.

The mechanism of the bactericidal action of TiO$_2$ under black UV-irradiation is based on formation of OH*, O$_2^*$, HO$_2^*$ radicals in aqueous systems [15]. Adhesion of bacterial cells to TiO$_2$ particles through the hydrophobic and charge interactions allows the active oxygen-containing species to reach and damage the outer bacterial membrane that results in cells death [16].

As can be seen in Fig. 4 (curves 1–3), water fluxes for (i) the initial membrane, (ii) the membrane treated by UV and (iii) the membrane with deposited TiO$_2$ particles but without UV-irradiation decreased gradually with time of samples exposure to surface water. On the contrary an essentially higher water flux was observed at the same conditions for TiO$_2$ deposited membrane treated with black UV-light (Fig. 4, curve 4). The water flux of this sample was 1.7–2.3 times higher comparing with those for the control samples. Obviously this may be explained by a significant reduction of a quantity of *P. putida* cells on the surface of membrane with deposited TiO$_2$ particles. Anti-bacterial species produced by this membrane under UV-treatment are capable to inhibit a growth of the microorganisms on the membrane surface thus reducing the membrane biofouling.

### 4. Conclusions

In this study the adhesion of *Escherichia coli*, *Pseudomonas putida*, *Acinetobacter calcoaceticus* cells to ultrafiltration membranes of various chemical nature: polysulfone, polyethersulfone, cellulose was studied. It was shown that the hydrophobic interactions between the membrane
and cells facilitate the adhesion of microorganisms to membrane surface. In particular, it was found that an adhesiveness of microorganisms to the surface of more hydrophobic polyethersulfone or polysulfone membrane is higher than to hydrophilic cellulose membrane while the adhesion of relatively hydrophilic \textit{E. coli} to membranes is essentially lower comparing with more hydrophobic \textit{P. putida} or \textit{A. calcoaceticus}.

It was showed that the fluxes of membranes with deposited native \textit{P. putida} microorganisms decreased with time of membrane exposure to natural surface water due to a growth of bacteria on the membrane surface. It was shown that membrane samples with deposited TiO\textsubscript{2} particles under black UV-irradiation possess a strong photobactericidal effect as a result the water fluxes of these samples were 1.7–2.3 times higher comparing with those for control membranes.

\textbf{References}