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Biofilm-based membrane reactors – selected aspects of the application and microbial layer control

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ABSTRACT

The membrane, a selective barrier, could serve also as a carrier for biofilm (a microorganism layer) immobilization. Then, it forms the so-called catalytic (active) membrane. The aim is to maintain a layer of controlled thickness and activity. The use of membrane as a biomass carrier is justified when the membrane performs an additional function. Such applications are discussed in the paper. Particular attention is given to the reactors in which microorganisms are immobilized on the membrane surface and the membrane simultaneously serves to provide substrate transport from a liquid and/or gas. The most interesting case is membrane oxygenation. The resulting different zones of aeration of biofilm allow simultaneous aerobic and anaerobic processes to occur in a single reactor. This integration can be applied in the treatment of any kind of municipal wastewater. A promising application of biofilms is their location on an ion-exchange membrane. In this case, catalytic membrane serves as a fuel cell. In the simplest solution, microbial fuel cells change the chemical energy collected in compounds (wastes, renewal biomass) into the energy necessary to support the biological functions of microorganisms and additionally create a supply of electrical energy. Keeping a stable thickness of the microbial layer is a key to provide the process at stable efficiency. Selected methods for *in situ* disposal of redundant biofilm are presented.

Keywords: Active (catalytic) membrane; Fuel cell; Membrane oxygenation; *In situ* biofilm control

1. Introduction

The concept of microbial membrane bioreactors (MBRs) is based on the integration of a bioreactor (most often a continuous stirred tank reactor—CSTR) containing suspended biomass with a micro/ultrafiltration process. The permeate stream should not contain microbial cells. They are recirculated into the bioreactor with the retentate stream. Therefore, biomass concentration enhancement in the reaction zone promotes increased process efficiency and/or decreased hydraulic residence time for a given reaction yield. MBRs are widely used for the biodegradation of industrial wastewater [1–4] and synthesis of various products [5,6].

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The biggest advantage of classical MBRs is easy process control through the outlet of an excess biomass. Maintaining a steady state is easy to implement. Unfortunately, not every process type could be carried out in MBRs. The reactions in which substances are poorly soluble in water or have strongly volatile properties are an example. In such case, a reactor with the biofilm (layer of biomass) located on the membrane surface near a dosing place (exactly on the other side of the membrane) is proposed. This type of reactors is known in the literature as MBfRs [7].

Biofilms can be inhabited by one strain of bacteria or, more commonly, by a consortium of different bacterial species, whose spatial organization in the biofilm is not accidental. This biofilm property enables its application in the complex processes of decomposition and synthesis [8,9].

A layer of microbial cells attached to the stable surface is surrounded by a protective structure produced by bacterial cells called glycocalyx. The beginning of glycocalyx formation was observed after few days of microbial layer presence on a surface. The glycocalyx has a gel-like structure and consists mainly of exopolysaccharides (EPS). This gel structure also traps other exogenous substances (nucleic acids, proteins, minerals, nutrients, cell wall material) and protects cells against drying [10,11]. Additionally, the glycocalyx stabilizes the microbial layer and attaches it to the surface. That is why detachment of the biofilm is not easy. Stronger shear forces result in denser biofilm structures caused by modified production of a glycocalyx composition.

Due to the simultaneous diffusion and metabolic processes, bacteria differentiate automatically with the biofilm thickness. This differentiation of cells, and generally higher resistance of bacteria when in higher concentrations, promotes MBfRs application in the biodegradation of particularly harmful or highly concentrated wastewater [12,13].

The processes of catalysis (degradation or synthesis) running inside the microbial layer integrated with reactant-selective mass transport and procedures to keep the process in steady state are described below.

2. Selected applications of MBfRs

2.1. Biodegradation of gaseous and volatile substances

The main limitation in supplying gaseous substances to CSTR is their generally low solubility in liquid environments. Similarly, in the case of volatile substances, there is a problem with their absorption into the air and then removal from the reactor.

In MBfRs, it is possible to convert almost 100% of the supplied gaseous reactants in the biofilm, and thus, its concentration in the aqueous phase reaches a negligible value. In turn, volatile compounds dosed from a liquid medium are converted in the biofilm and have no possibility to be absorbed in gas. Like in the case presented in Fig. 1(a), different reactants could be supplied from one side of the biofilm in the liquid medium and from the other side in a gaseous stream. The porous membrane guarantees the extended interphase (transport) surface.

In the diffusive mass transport of gaseous components from the gas stream flowing on one side of the membrane, different resistances can be distinguished: a membrane resistance, a biofilm resistance, and a liquid resistance from the other side of the biofilm. The resistance of the layer on the gaseous side is normally negligibly small. Similarly, for volatile substances, the gas phase resistance can be omitted.

The concentration profile of these components depends on their particular mass transport resistances and their consumption rate by microorganisms inside the biofilm. Microorganisms that consume the components change the concentration profile and intensify mass transport through the membrane [14]. The existing liquid diffusion layer slows the transport of gaseous components into the liquid which, as a result, stay longer in the biofilm to be consumed by the microorganisms.

A significant problem in the MBfR application is the treatment of media with high solid content. Suspended solids (SS) can create an additional layer on the biofilm surface that substantially decreases the mass transfer of the reactants from the liquid to the biofilm. Thus, the SS layer should be controlled by removal during the process.

The membranes predominantly used in MBfRs are microporous and hydrophobic (e.g. made of polytetrafluoroethylene, polyethylene, polyvinylidene fluoride, polypropylene, or polysulfone). However, homogenous (lite) membranes, e.g. those made from poly(dimethylsiloxane) or composite membranes, have also been applied [12]. Both homogenous membranes and the separate layers of composite membranes should be as thin as possible.

Martin and Nerenberg [7] classified different MBfR configurations into six groups: (a) parallel flow, shell and tube type with free membrane ends, (b) parallel flow, shell and tube type with fixed membranes, (c) CSTR type with membrane bundles, (d) CSTR type with a wound membrane unit, (e) cross-flow type, and (f) spiral-wound type.

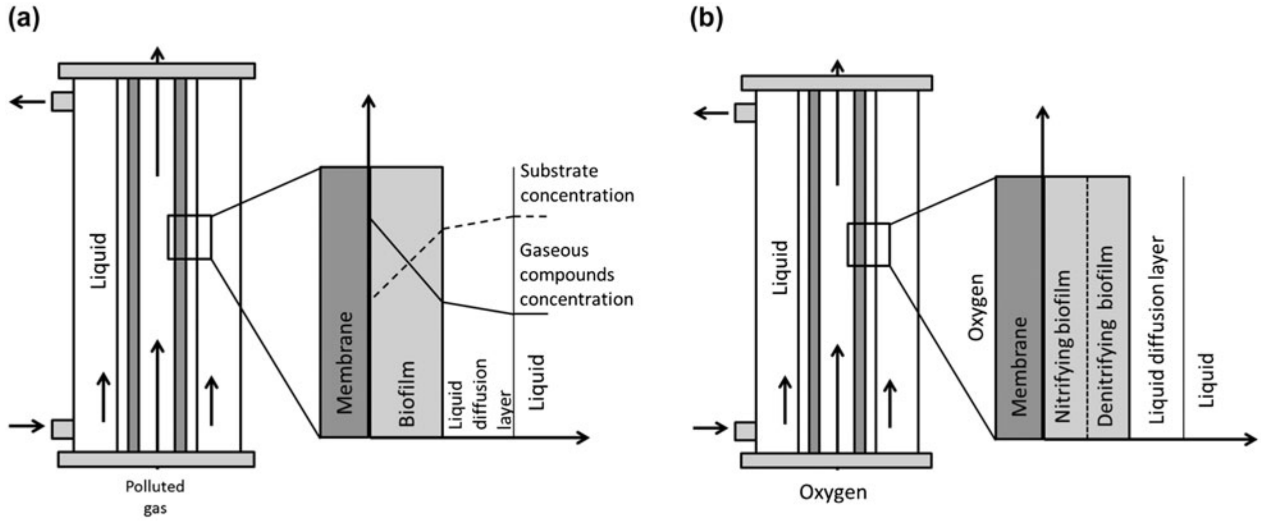

Fig. 1. Example of substrate concentration profiles in MBfR (a) and biofilm stratification (b).

2.2. Biodegradation in MBfR integrated with membrane oxygenation

A special type of MBfR is an aerated membrane biofilm reactor (MABR) in which the membrane serves additionally to provide oxygenation. Considering oxygenation in MBRs, which generates up to 70% of the total energy cost [15,16], low oxygen losses resulting from two-sided membrane oxygenation (from one side of oxygenated liquid and from the other of air) significantly decrease these operational costs.

As a result of counter-diffusional transport of oxygen, particularly at thick layers microorganisms differentiate between living in the oxic and anoxic environments (Fig. 1(b)) [17]. Thus, it is possible to simultaneously perform aerobic and anaerobic processes in one bioreactor [18]. Thus, MABRs have been commonly applied in simultaneous processes of organic carbon biodegradation, nitrification and denitrification [14,19,20].

Biofilm colonization depends on the oxygen profile and other compounds concentration. Liu et al. [21] investigated the influence of different COD/N ratios on process efficiency and microorganism community structure. In the environments with low (<3) COD/N ratios, nitrifying species dominated in the biofilm. Meanwhile, at high (like 6) COD/N ratios, heterotrophic species inhibited nitrifying bacteria growth as a result of competition for oxygen. For the optimal value of COD/N equal to 5, it was possible to achieve effective and simultaneous chemical oxygen demand (COD) (85%) degradation, nitrification (93%) and denitrification (92%). Biodegradation efficiency, with reference to the COD and nitrification process, was comparable in activated sludge and in MBfR. Investigations have shown that better disposal of total nitrogen and phosphorous was obtained in MBfR [8].

The results obtained by Nisola et al. [22] indicate that the membrane material affects also the type of microorganisms present in the biofilm and the effectiveness of their actions. They investigated the influence of two hollow fibre membranes, an uncoated microporous polyvinylidene fluoride (PVDF) and a composite polyether-block-polyamide copolymer (PEBA)-coated PVDF membrane, on ammonium removal in MABR. The membranes were used as a support for the growth of ammonia-oxidizing bacteria. The hydrophobic PEBA membrane resulted in higher adhesion of the microorganisms, but the ammonium removal efficiency was better on the uncoated PVDF membrane. It means that the concentration profile obtained for this membrane was more favourable.

2.3. Biofilm application in energy production

In microbial fuel cells (MFCs), the chemical energy collected in substances (wastes, renewal biomass) is changed into the energy necessary to support the biological functions of "working" microorganisms and additionally creates a supply of electrical energy. An MFC tank contains electrodes usually made of carbon materials (e.g. graphite) submerged in liquids (anolyte and catholyte) separated by an ion-exchange membrane—Fig. 2.

In the anodic chamber, the organic substrates are degraded by microorganisms located on the anode and carbon dioxide is produced. As result of these

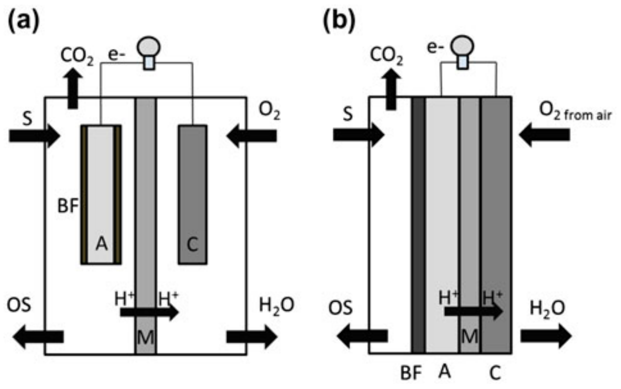

Fig. 2. Microbial fuel cell concept: two-chamber MFC (a) and single-chamber MFC with open air cathode (b); S—substrate, OS—oxidized substrate, M—ion-exchange membrane, A—anode, C—cathode).

(bio)chemical reactions, an electrochemical potential difference is created between the anode and cathode, and thus, an electron flow is induced through the outer electrical circuit to the cathode, and electrical energy is generated. Protons produced in the anodic chamber pass through the proton-exchange membrane to the cathodic chamber. The necessary oxygen in the cathodic chamber can be supplied by oxygenated water (Fig. 2(a)) or directly from the air (Fig. 2(b)).

Electrons formed in an MFC during the substrate oxidation process are directly or indirectly supplied to the anode (through mediators or electron transfer-supporting microorganisms). Different coulombic efficiencies of the energy transformation (chemical to electric) can be achieved depending on the microorganisms used in the MFC (exactly depending on their metabolic path and electron transfer mechanism) and the substrate consumed.

Pant et al. [23] presented potential substrates used in MFCs as divided basically into acetate, glucose, lignocellulose biomass, synthetic wastewater, brewery wastewater, starch-processing wastewater, dye wastewater, landfill leachates, cellulose and chitin, and nonorganic compounds. However, new opportunities are still being considered and mixtures of compounds contained in domestic sewage [24], agricultural wastewater [25,26], and petroleum wastewater [27] have been tested.

Bélafi-Bako et al. [28] investigated the influence of different substrates (fermentable and nonfermentable: glucose and acetate solution) added to the initial mesophilic anaerobic sludge on the performance of MFCs, with a focus on the stability and limitations of the MFC system. They observed that for a given pretreatment of anaerobic sludge (suppressing weaker methanogenic and acetogenic strains), MFC performed better with the use of fermentable glucose than non-fermentable acetate. This result is in contrast to the known fact that non-fermentable substrates serve better as an electron donor for power output and electron recovery [28].

The most promising reactor configurations utilize microorganisms that can transport electrons directly to the anode using cytochromes found on the outer membrane [29]. In this case, specialized groups of biofilm-creating bacteria (anode-respiring bacteria, e.g. *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Rhodoferax ferrireducens*) are used to transform energy collected from a broad spectrum of substrates and ultimately generate electrical energy. Therefore, it is possible to avoid the use of expensive, toxic electron mediators [30].

Different results regarding the effect of temperature on generated power density can be found in the literature. Aside from situations where higher temperatures led to the extinction of exoelectrogens and methanogens, some investigators observed a limited effect of temperature [31], some indicated mesophilic temperature [32], and some found lower temperatures to promote methanogen growth and give higher power densities [33]. Prasertsung and Ratanatamskul [34] observed that MFC worked well between mesophilic and thermophilic (up 45˚C) conditions and that increasing at the same time the organic loading rate (OLR) decreased the COD removal and power densities. Kim et al. [35] observed that the influent source, the OLR and the linear velocity significantly influenced MFC power generation, but they obtained opposite results to Prasertsung and Ratanatamskul [34]. According to Kim et al., as the OLRs increased, the maximum power density in MFCs with an identical influent source also increased.

3. *In situ* modern methods of biofilm thickness control

The biggest disadvantage of using biofilm-based processes is the uncontrolled growth of microorganisms resulting in process instability. It is essential to use *in situ* mechanisms to keep a constant thickness of the biofilm during the biodegradation processes. At stable biofilm located on the membrane surface reinforced by the presence of glycocalyx, the commonly used methods like backwashing or relaxation could be not sufficient. They should be integrated with other modern methods like vibrations.

3.1. Low- and high-frequency (ultrasound) vibrations

Vibrations have been used to support layer thickness in various MBR configurations [36,37]. The most frequent is the application of vibrations to submerged membranes working as a support for microbial layer or as a separated barrier. In the literature, there are the cases of applications of ultrasound vibrations [38–40] as well as low-frequency membrane oscillations (dozens to several dozens of Hz) [41]. Vibrations create high shear forces on the membrane surface, causing detachment of particles from the membrane surface and thus preventing the deposition of a thick, dense layer—Fig. 3. An example of a commercialized system is VSEP (vibratory shear enhanced process) technology. Depending on the duration and intensity of the forces, the membrane is renewed completely (which is the goal with a classic membrane process) or only partially (removal of excess biofilm).

Also, magnetically induced membrane vibration (MMV) applied in the MBR is described in the literature [42]. It was mentioned that a membrane configuration (distance between hollow fibre membranes) in this system had a relevant impact on the system performance. In paper [43], MMV approach was applied in the bioethanol production bioreactor using lignocellulose hydrolysates as substrates. For a high-viscosity feed with higher solid concentration, filterability for the vibrated system was slightly better than for the system without vibrations. A strong impact of vibration-induced shear was observed with increased dilution of the filtrated medium. For diluted systems, the results indicated strong fouling in the first phase of filtration followed by vibration-related fouling control in the next phase. A very strong influence of vibration amplitude on permeation was also observed. This result was connected with the viscosity of the medium, which in general decreased at higher shear rates and limited the build-up of filtration cake [43].

Kola et al. [41] investigated the influence of rotationally oscillating fluid and transverse vibration in submerged MBR on filtration characteristics. When vibration was used, fouling by both particulates and macromolecules was limited. The application of transverse vibration improved the critical flux and slowed the increase in transmembrane pressure during long-term filtration. It was mentioned that an optimal oscillation value could be found that depends on different permeate fluxes and feed solution properties, such as concentration. Further increase in vibration settings above the optimal value had no additional effect on filtration characteristics [41]. By combining very low-frequency vibrations (4.2 Hz) with other methods such as backwashing or relaxation, the best results were achieved for the mode of vibration and relaxation. When the frequency was increased to 20 Hz, better results were obtained for the mode of vibration and backwashing [44].

3.2. Membrane oxygenation

In part 2.2, we have described MABRs in which membrane serves *inter alia* to provide oxygenation. An oxygenation efficiency affects the differentiation of microorganisms inside the biofilm layer. Simultaneously,

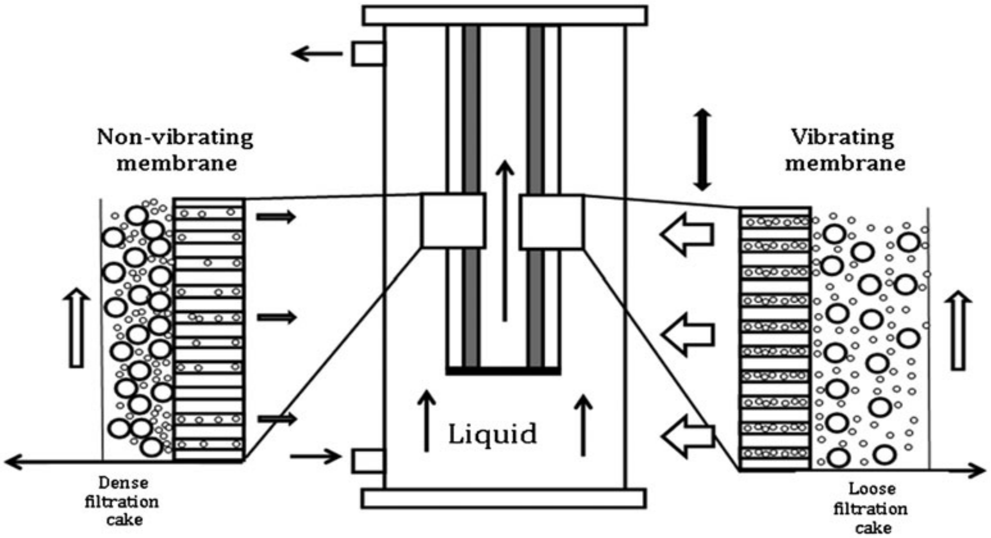

Fig. 3. Membrane regeneration (biofilm thickness control) with the application of vibrating membrane.

the oxygenation efficiency could influence a microbial layer stability.

Bilad et al. [45] and Mezohegyi et al. [46] compared the integration of membrane filtration with aeration and vibration. The vibration-supported system performed better than aeration, but the investigators observed that the rapid and irreversible fouling during the initial phase of filtration could not have been mitigated by either filtration system.

Fig. 4 shows data obtained in our experiments. We monitored changes of the biomass concentration in broth and permeate stream density during membrane oxygenation.

After 200 s of membrane oxygenation 41% of the biomass located on the membrane surface and created stable layer before the membrane oxygenation was detached. At this time, the permeate stream density increased to 21% of initial value. The biofilm remained active on the membrane. We have shown that at membrane oxygenation it is possible to keep the stable both the biofilm thickness and consequently the permeate stream. The oxygen stream, process duration and time-out are the parameters that should be optimized.

3.3. Membrane rotation

Energy consumption of aerated membrane system was compared to a rotating membrane system. It was shown [47] that the use of rotation to mitigate membrane fouling rate was much more efficient.

Rotary filters have been successfully applied to a variety of systems, presumably by reducing biofilm layer build-up, membrane additional fouling and concentration polarization. Among the investigated five variables influencing the process stability, i.e. mixed liquor SS, bound extracellular polymeric substances, rotary speed, mean particle size, and aeration rate the strongest impact on fouling mitigation was exerted by the rotary speed. A similar effect of the rotary speed was obtained in an anaerobic MBR with rotary disks [48]. An effect of combined use of rotary disk and membrane materials was additionally investigated.

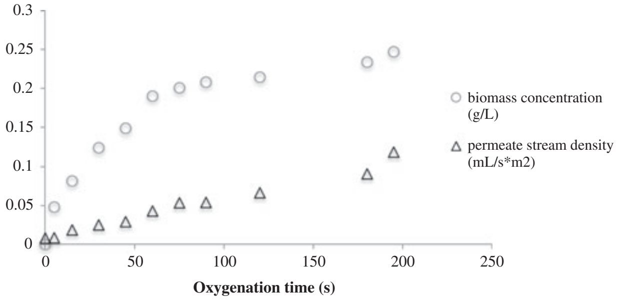

Fig. 4. Biomass detachment and permeate stream increase during membrane oxygenation (TAMI ceramic membrane at 0.14 μm pores diameter and internal surface $A = 0.0045\ \mathrm{m}^2$, $\Delta P = 0.2$ MPa, oxygen supply from the permeate side by a stream equal to 0.7 vvm).

It was concluded that the application of rotary disk rotation and the membrane made of low-cost polyurethane sponges provided great reduction in membrane fouling and showed an advantage on the operational electricity reduction in MBR.

4. Conclusions

Based on the broad applications of microbial biofilms, there are many different research and development topics regarding biofilm-based bioreactors, such as the MBfR, MABR, and MFC. They serve first of all for the degradation of strongly harmful compounds or/and for the treatment of highly concentrated wastewater. The most attractive applications of membrane biofilms related to fuel cells are limited by the cost of ion exchange membranes. Therefore, the challenges regarding MFCs include the design and manufacturing of biocompatible anode materials with high electrical conductivity, chemical stability and expanded specific surface area. Their pollutant insensibility and corrosion resistance should ensure long-term usage, reducing the cost of their application.

Biofilm thickness control is necessary to keep the biofilm working near its highest activity during long work periods. The development of biofilm thickness control should integrate chemical, physical and biochemical/biological methods to clean the membrane before the process and mechanical forces based on relaxation, backwashing vibrations or membrane rotation to regulate the layer thickness during the process. In the case of oxygenated reactors, membrane oxygenation can contribute also to the control of microbial layer thickness.

Controlling biofilm thickness and maintaining its high activity are directly related to the mass transport of substrates and products (the latter ones when they have a negative influence (inhibitors) on processes occurring in the biofilm). Multifunctional biofilms require a particular profile of reagents, most frequently transported on both sides of the biofilms (from liquid and/or gas) that contribute to the phenomena of co-diffusion, counter-current diffusion, and back-diffusion. In most cases, the process runs in unsteady conditions because of the growth of the biofilm. The description and projection of the processes conducted in biofilm-based reactors are the real challenge for chemical engineering.

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