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# Integration of Membranes and Bioreactors

*Katalin Belafi-Bako and Peter Bakonyi*

## Abstract

Combined application of bioreactors and membrane separations are considered as membrane bioreactors (MBRs). Examples for the application of MBRs are given in this chapter both for large scale (wastewater treatments) and in other areas in smaller scale. Wastewater treatments are the majority of the large-scale applications, where biological degradation is coupled with membrane filtration (microfiltration and ultrafiltration). Other types of MBRs include integration of biotransformations and bioconversions by microorganisms and enzymes with membrane separation processes, not only with filtration but also with pervaporation, electrodialysis, and gas separation. These MBRs provide significant advantages compared to the conventional batch bioprocesses. In this chapter, several examples are presented for both applications.

**Keywords:** ultrafiltration, pervaporation, electrodialysis, gas separation, biocatalysis

## 1. Introduction

"As per definition, the bioreactor is the designed space where biochemical reactions take place" [1]. If some compounds should be removed from the bioreactor, a separation step can be connected to the reactor. Among separation processes, membrane techniques are especially attractive since they operate under mild conditions, they are easy to combine and vary, the scale-up is simple due to the modular construction, and they do not need and produce hazardous materials (environmental-friendly processes) [2–4].

Application of membranes integrated in bioreactions is often considered as membrane bioreactors (MBRs). In the literature [5–8] the term *MBRs* itself is referred almost exclusively for various wastewater treatments. Concerning full-scale applications, it seems correct. In these technologies only pressure-driven membrane processes such as microfiltration (MF), ultrafiltration (MF), nanofiltration (NF), and reverse osmosis (RO) have been used, although there are numerous other membrane separation techniques that are available nowadays, which can be inserted into bioprocesses.

In this work the first section is going to summarize the "conventional" applications of MBRs, i.e., wastewater treatments, while in the second part, some other types of MBRs will be presented where—beyond pressure-driven processes—some more membrane separation methods, like pervaporation (PV), gas separation (GS), electrodialysis (ED), etc., will be presented.

## 2. Large-scale applications

The usage of membrane bioreactors means a well-established technology for several types of wastewater treatments. The recently worldwide opened (of will opened soon) large-scale—over 100,000 $m^3$/d capacity—MBR plants for municipal wastewater treatment are summarized in [8]. Other main application areas are as follows [6, 8]:

- Industrial wastewater treatment

- Landfill leachate treatment

- Sludge digestion

- Treatment of human excrement

In these applications MBRs are considered as complex systems integrating biological degradation of waste products with membrane filtration [6]. Thus an MBR is composed of two parts: a biological unit and a membrane module. According to the location of the membrane module (from the aspect of architecture), the MBRs can be classified into two groups: internal and external systems. The internal system (frequently called submerged MBR) of the membrane module is placed in the bioreactor [6]. Outer skin membranes are applied, and the permeate side is under suction (vacuum); moreover, aeration and mixing can be easily achieved, as well. In the external MBR, the membrane is located outside the bioreactor, and the treated wastewater is recirculated through the primary side of the membrane module. The driving force is provided by the pressure from the high cross flow volumetric rate along the membrane surface [6].

Regarding configuration there have been numerous types of modules (plate and frame, tubular, rotary disk, hollow fiber) and membranes (cellulose acetate, polyethylene, polysulfone, polyolefin, metallic, ceramic—mainly in ultrafiltration and microfiltration range) applied.

MBRs are widely considered as an effective technology in removing both organic and inorganic contaminants in wastewater, have a good control of biological activity—the effluent is generally free of bacteria and pathogens—need smaller plant size, and provide higher organic loading rates [5, 6]. Beyond the benefits, however, there are some serious drawbacks and issues which should be enhanced, like membrane fouling and energy consumption. To solve these problems and to widen the application opportunities, several special techniques and methods have been investigated and suggested, where unusual environments (e.g., varying the level of oxygen, flow pattern, airlift) and unique procedures (e.g., applying electric power, magnetic effects, illumination, vibration, osmosis) are added and/or applied for the MBR systems. Some of them are listed in **Table 1**, together with their abbreviations.

*Anaerobic* degradation of organic wastes [10, 11]—similar to the classical aerobic wastewater treatments—can be carried out by microbial consortia. The technology results in gas products ("biogas"). It consists usually of methane, CO2, and H2S mainly. To separate these compounds, membranes can be applied, as well, but these membranes have selectivity toward one of the compounds in the gaseous mixture [12, 15]. The process is called membrane gas separation, and its driving force is mainly the pressure difference, similar to UF and MF.

Recently biogas plants are built in connection with wastewater plants to complete the degradation process and to obtain energy which may cover the energy demand of the process; moreover other types of wastes (slaughter wastes,

| Name | Abbr. | Ref |
|---|---|---|
| Anaerobic-anoxic-oxic membrane bioreactor | $A^2O$ MBR | [8] |
| Anaerobic fluidized bed membrane bioreactor | AFMBR | [9] |
| Anaerobic membrane bioreactor | AnMBR | [6–11] |
| Airlift oxidation ditch membrane bioreactor | AOXMBR | [8] |
| Bioelectrochemical membrane reactor | BEMR | [8] |
| Batch granulation membrane bioreactor | BG-MBR | [8] |
| Baffled membrane bioreactor | BMBR | [10] |
| Electrochemical membrane bioreactor | EMBR | [8] |
| Gas separation—membrane bioreactor | GS-MBR | [12] |
| Hybrid-growth membrane bioreactor | HG-MBR | [9] |
| Immersed hollow fiber membrane bioreactor | IHFMB | [13] |
| Membrane electro-bioreactor | MEBR | [8] |
| Membrane gradostat reactor | MGR | [14] |
| Magnetically induced membrane vibration membrane bioreactor | MMV-MBR | [8] |
| Membrane photobioreactor | MPBR | [8] |
| Osmotic membrane bioreactor | OMBR | [7] |
| Reciprocation membrane bioreactor | rMBR | [8] |
| Single fiber membrane gradostat reactor | SFMGR | [14] |

**Table 1.**
*Various unusual membrane bioreactors.*

agricultural wastes) can be degraded, as well. In these complex plants, MBRs can be applied in both aerobic (coupled with pressure-driven membrane techniques) and anaerobic (coupled with membrane gas separation) systems.

## 3. Novel applications in developing stage

### 3.1 Types of MBRs and classifications

Integrated systems where membranes are combined with the bioreactions—other than wastewater degradation—can be classified similarly: external and integral setups. However, it is difficult to connect certain types of membrane processes (e.g., pervaporation, electrodialysis) to the bioreactor *externally*; thus they are used as *internal* systems. **Figure 1** presents an example for internal MBR, where the configuration is illustrated. These systems have the advantage to handle (e.g., disconnect easily) the membrane module independently from the bioreactor.

On the other hand, there are successful examples for external (immersed membranes) MBR systems, which can be applied in special cases, e.g., for manufacture of pharmaceutical intermediaries. Loh and colleagues reported [13] that the biotransformation of indene to cis-indandiol was achieved in an IHFMB system resulting in higher effectiveness.

MBR systems can be distinguished according to the membrane process integrated [16, 17]. Beyond pressure-driven methods (microfiltration, ultrafiltration, nanofiltration), other techniques like pervaporation, electrodialysis, and gas

**Figure 1.**
*Outline of an internal (integrated) system.*
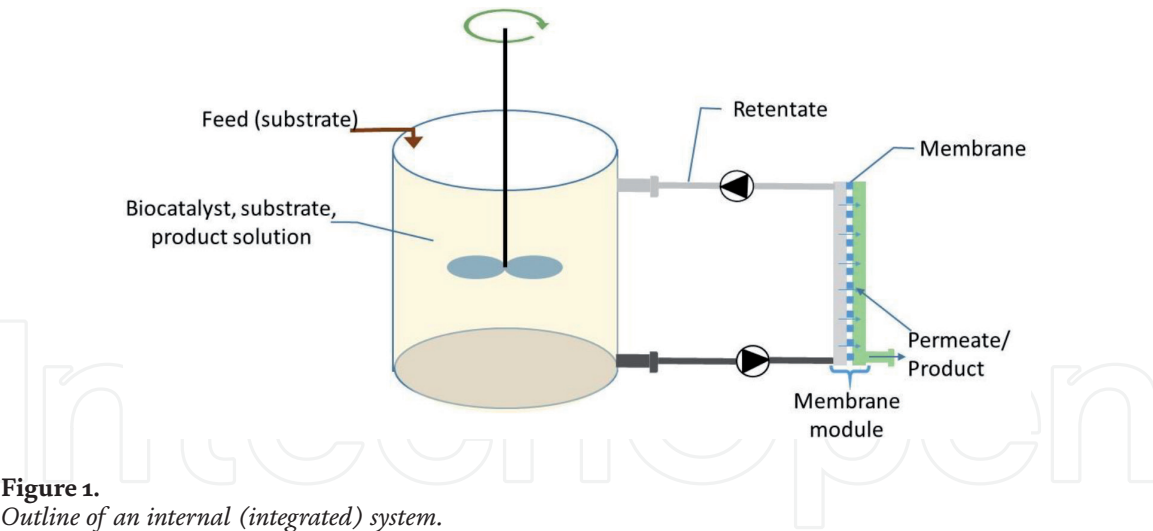
separation can be applied. To connect these membrane processes to the bioreaction, careful design and optimization should be accomplished before starting the operation of MBRs. Plate and frame, tubular, as well as hollow fiber modules can be used in the MBRs.

The novel MBR systems can be further categorized regarding the biocatalyst used: enzymes as well as microbes (moreover microbial consortia) can be applied. The biocatalysts can be further classified concerning the state of the biocatalysts, as well (free or immobilized); moreover they can be immobilized onto the membrane (which is considered as *catalytic active membrane* [18]) or on other supports. Some examples are presented here for all the classes.

### 3.2 Enzymatic MBRs

Enzymatic MBRs have been used mainly in hydrolytic reactions where the substrates are, e.g., triacylglycerols and polysaccharides (starch, cellulose, pectin), but some other reactions occur, as well, like esterification. **Table 2** summarizes the important features of these systems.

Hydrolysis of triacylglycerols (fats and oils) results in glycerol and fatty acids (long chain carboxylic acids). The higher demand of fatty acids in various industrial sectors (e.g., production of cosmetics, detergents) made the hydrolysis an important process recently. The main difficulty of the process is that the two reactants, triacylglycerols and water, are not miscible. When the reaction is carried out by enzymatic catalysis [17], numerous advantages are provided: it takes place

| Reaction | Substrate | Enzyme | State | Membrane | Ref. |
|---|---|---|---|---|---|
| Hydrolysis | Triacylglycerol | Lipase | Immobilized | UF | [19–21] |
| Hydrolysis | Protein | Protease | Free and immobilized | UF | [18] |
| Hydrolysis | Starch | Amylases | Free | UF | [22, 23] |
| Hydrolysis | Cellulose | Cellulases | Immobilized | UF | [24] |
| Hydrolysis | Pectin | Pectinases | Free | UF ED | [25–27] [28–31] |
| Esterification | Acids and alcohols | Lipases | Immobilized | PV | [32–35] |

**Table 2.**
*Examples for enzymatic MBRs.*

under mild conditions, instead of high temperature and pressure, implying energy saving and better quality products. Accomplishing the hydrolysis in a MBR, further benefits are added to the process: the two phases remain separated during the reaction and there is no emulsion formed and no separation needed, since the products are separated by the membrane.

The enzyme suitable for the hydrolysis is called lipase. It can be immobilized onto the membrane. During the reaction, the pure triacylglycerol substrate (no solvent is present) and water are flown separately into the two sides of the membrane. Both hydrophilic and hydrophobic membranes can be applied [19]. In case of hydrophilic membrane, the substrate should be circulated on the enzyme side of the membrane, while water is on the other side. Thus fatty acids formed are remaining in the oily phase (and accumulated there), while glycerol is passing through the hydrophilic membrane into the water phase. Applying hydrophobic membrane the arrangement is the other way around: the substrate is flown on the nonenzyme side of the membrane. In our experiments lipase from *Candida rugosa* (Sigma) and hydrophilic cellulose acetate membrane (cut off 3000) was used [19] successfully for the hydrolysis of various oils and fats [20, 21].

In hydrolytic degradation of polymers (polysaccharides and proteins), the small-sized products (e.g., glucose) often have inhibitory effect, and thus their removal is beneficial for the reaction. MBRs provide a simple solution, since the membrane applied serves for rejection of the long polymer chains as well as the biocatalysts (enzymes or cells), while the product can easily pass through the membrane.

Hydrolysis of various proteins—e.g., from milk, whey, plasma—is an important step in industrial processes, and it is realized by protease enzymes. The MBRs provide significant advantages for the process [18] including retention and reuse of the biocatalysts, avoiding product inhibition—a possibility for continuous operation.

Regarding enzymatic hydrolysis of polysaccharides, starch, cellulose, and pectin are considered. Hydrolytic products of starch are utilized widely in food industry (e.g., maltodextrin, dextrose, and high fructose syrups). The reaction is catalyzed by amylase enzymes. When the hydrolysis is carried out in MBRs, higher effectivity can be achieved [22, 23]. In our work hollow fiber cellulose acetate (UF) membrane module (jacketed) was used, and the experimental results confirmed that not only purified amylase preparations but a crude fermentation filtrate, an enzyme complex solution (mainly glucoamylase) produced by *Aspergillus awamori*, was capable for degradation of starch effectively and continuously.

Cellulose is a long-chain polysaccharide containing glucose (monomer)—similarly to starch. Enzymatic hydrolysis of cellulose, however, is more difficult [24]. The reaction can be carried out in MBRs to overcome some of the problems. A special tubular membrane module was used to carry out the reaction, where a fine, hairy surface membrane was built in [24]. The hydrolysis was catalyzed by a Celluclast preparation (Novozymes), and it was found that the special MBR had a positive effect on the process and enhanced conversion and productivity of the enzyme reaction was accomplished.

Pectin is a polysaccharide occurring in the cell walls of certain plants, mainly in the fruits like apple, orange, some berried, etc. It is important to use pectolytic enzymes in processing of fruits (production of fruit juices) [25, 26] to enhance yields and improve liquefaction and clarification. As a result of the hydrolytic degradation of pectin, galacturonic acid (monomer) is formed. It was assumed that the hydrolysis was inhibited by the monomer; therefore MBRs seemed a promising reactor type to realize the process effectively. Regenerated cellulose UF membrane (30 kD) and polygalacturonase enzyme preparation (Sigma) from *Aspergillus niger* were used in the experiments, and it was found that the MBR worked reliably with

excellent stability for long and higher volumetric productivity was achieved than the batch system. To enhance the effectiveness of the process, vacuum was applied in the secondary side of the membrane [27].

The reaction between acids and alcohol results in esters and water. It can be catalyzed by lipase enzymes. The reaction is reversible and product removal enhances the yield. Both products can be separated from the reaction mixture by membranes. If the substrates are low molecular weight acids and alcohols, flavor esters are formed, which are volatile compounds. Thus pervaporation (PV) can be applied for their recovery that is quite easy to connect online to the reaction, forming an integrated MBR system. Ethyl acetate [32] and other flavor esters [33] were manufactured effectively by a lipase preparation (*Candida antarctica* immobilized onto a resin, from Novozymes) and separated online by PV using hydrophobic membranes, and the permeate was obtained by using vacuum traps cooled by dry ice.

PV is not suitable for the separation of higher molecular weight ester products, but it is possible to apply it for removal of water formed during the esterification. Ester type biolubricant from oleic acid and alcohols [34] and ester of 2-choloropropionic acid and 1-butanol [35] were manufactured by using integrated PV for water removal.

Electrodialysis also can be combined to enzymatic processes, where charged compounds are formed and they can be separated online. This kind of MBR was applied for continuous recovery of galacturonic acid obtained from the hydrolysis of pectin by a pectinase preparation [28]. Both monopolar and bipolar membranes were successfully used [29, 30].

Summarizing the application possibilities of MBRs in enzymatic processes, it can be concluded that they provided real advantages for the bioprocesses listed above and—beyond process intensification [31, 36]—these systems can be operated in continuous mode of operation: constant uptake of substrate (and biocatalyst, if necessary) and release of product can be achieved.

### 3.3 Microbial MBRs

In microbial MBRs a single microorganisms can work, but sterile conditions should be provided. One of the examples given here is the biohydrogen production, where gas separation is connected and the other one is manufacture of organic acids, where electrodialysis is used for in-situ separation of the products.

Biohydrogen can be produced by fermentation using various microbes. Some of them need light for the operation (photofermentation), while others do not (dark fermentation). *Escherichia coli* belong to the dark fermentative group and is able to form biogas containing mainly hydrogen and $CO_2$ in the headspace of the bioreactor. When the bioreactor is integrated by a membrane gas separation unit (MBR), higher productivity was possible to achieve, as proven in Veszprem [37–42]. Polyimide hollow fiber membranes and *E. coli* XL-1 Blue strain were used for the experiments, which resulted in a feasible concept for the integrated production and purification of the promising energy carrier, hydrogen gas [43–46]. Additionally, hydrogen fermenters could be attached to microbial electrochemical technologies, thus giving opportunity for adequate treatment of the effluents containing residual organic matter [47–50].

Transformation of fumaric acid into L-malic acid—which is the second most popular food acid—is an equilibrium reaction; thus, product removal could increase the yield. The reaction is catalyzed by immobilized cells of *Leuconostoc* and *Brevibacterium* species containing fumarase enzyme, and the acid (in salt form) can be separated by electrodialysis [51] integrated to the reaction.

Malic acid is not the only acid that can be produced in integrated (MBR) system, other organic acids—manufactured by fermentation—can be online separated by monopolar or bipolar electrodialysis systems.

A relatively novel area of MBR applications is the immobilization and cultivating of microbes; the system is called membrane gradostat reactor (MGR) [15]. The biofilm growing (and attached) on the surface has a significant effect on the permeability. The behavior of these systems was studied in case of, e.g., *Streptomyces coelicolor*, in a vertically oriented capillary MGR.

## 4. Conclusions

Bioprocesses integrated with membrane separations (MBRs) are able to provide more effective, successful bioconversions and bioreactions. The MBRs have been used in large scale worldwide in wastewater treatments, on one hand, while there are other, special applications in smaller scale (and sometimes only in a developing stage), on the other hand. Examples for both areas were given in this chapter.

## Acknowledgements

## Abbreviations

| | |
|------|------|
| ED | Electrodialysis |
| GS | Gas separation |
| MBRs | Membrane bioreactors |
| MF | Microfiltration |
| NF | Nanofiltration |
| PV | Pervaporation |
| RO | Reverse osmosis |
| UF | Ultrafiltration |

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