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REVIEW PAPER

BIOMOLECULE APPLICATIONS FOR MEMBRANE-BASED PHASE CONTACTING SYSTEMS Distribution, Separation and Reaction—a First State of the Art

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This paper proposes a first review of potential applications of biomolecules for membrane contactor and reactor design. More exactly, it is shown how by depositing proteins, lipids, chitosan etc. at the surface of a macroporous membrane material, appropriate surface modifications may be induced thus leading to valuable properties as regards various phase contacting, separation or reaction purposes: emulsification, evaporation, enzymatic reaction and so on. This kind of process is particularly valuable where low temperature operations are considered as it is often the case in food industries or biotechnologies. Biocompatibility is improved and easy regeneration of support material at the end of operation may be envisaged with the help of classical cleaning methods. Easy know-how protection and multiuse plants are other arguments which preach in favour of this new approach.

Keywords: membrane; contactor; reactor; protein; lipid; chitosan.

INTRODUCTION

The literature concerning the mechanisms of transmission and the rate of filtration during the crossflow filtration of protein solutions has been extensively studied and reviewed by Marshall *et al.* (1993). As a rule, the rate of filtration of solutions decreases with time at constant applied pressure while macromolecule rejection increases. The amplitude of phenomena is depending on numerous parameters such as fluid velocity, solute concentration, temperature or even pH which may change the electric charge of either macromolecules or amphoteric membrane material. This has been explained in terms of deposition of protein on the front face of the membrane through various complex mechanisms: molecules adsorption, aggregation and/or denaturation of proteins... It has been also proposed that fouling can switch from adsorption into the pores to deposition on the external active surface.

In the same way, the treatment of oily solutions with membrane filtration has been verified to be an effective way and applied widely to industry (Marchese *et al.*, 2000). But a serious problem in such membrane operation is flux reduction due to adsorption of oil droplets on/in membranes. Oil affinity of membrane is an important characteristic of membranes. Many attempts have been made to investigate the adsorptive fouling of membrane, particularly in relation between the contact angles. However it has appeared that characterizing affinity of membrane by using only contact angle may lead to great uncertainty. Therefore some researchers (Jonsson and Jonsson, 1995) suggested that the measurement have to be supplemented with measurements of other parameters such as the roughness and porosity of membrane. The application of membrane treatment to food oil treatment is a much more recent initiative which raises the same questions. The majority of these studies attest of the efforts made by the authors to improve the rate of filtration operations. Another approach could consist in trying to draw advantage from the natural interactions which develop between the biomolecules and the material constituting the barrier to get original separation or reaction conditions. Indeed one can easily imagine using the natural or modified protein deposit here abovementioned as a dynamic membrane with specific properties, or to use natural hydrophobicity obtained by putting the barrier in contact with lipids. The idea of using biomolecules for the design and development of membrane systems is attractive with many regards. Indeed there is a huge amount of biopolymers in nature—proteins (gelatine, β-lactoglobuline, gluten, etc.), polysaccharides (chitin, chitosan, etc.) or lipids (triglycerides)—and they are ordinarily inexpensive. These molecules are biodegradable, biocompatible and not poisoning.

They constitute the basis of all foods in the world since decades. A new tendency is observed which aims to

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revalorize agricultural or marine sources not directly aimed to food production. As an example, due to their very attractive film and gel forming properties, it has been proposed to use these molecules as substrates for the preparation of biodegradable packaging (Petersen *et al.*, 1999) and edible films (Wu *et al.*, 2002; Han *et al.*, 2004). These films are able to prevent moisture migration between food and environment or between compartments of different water activities (a_w). They also permit to extend the shelf life of products by protecting them from microbial attack (Han *et al.*, 2004).

In addition, the use of biomolecules is also of a main interest for medical applications. Gelatin or chitosan have been employed as artificial skin to accelerate wound and ulcer healing has been reported (Wang *et al.*, 2003; Lee *et al.*, 2003). Thanks to their surface charge properties, biopolymer layers are very good support for cell culture, particularly for the regeneration of brain cells (Cheng *et al.*, 2003). In another field, due to its attractive biocompatible and adsorption properties, chitosan constitutes as a very good vehicle for sustained and/or controlled release of drugs (Sinha *et al.*, 2004). Other applications of lipids—preparation of films for drug encapsulation, inclusion of active molecules in cosmetics or manufacturing of biosensor layers—have been also reported (Kochev and Karabaliev, 2004).

One could also imagine deriving benefit from these properties to generate uniform films onto the surface of porous supports. In that way, a few attempts to modify the surface of membranes using biopolymers deserve to be mentioned. Authors took advantage of the fact that proteins or lipids are constituted of both hydrophobic and hydrophilic chemical groups, to get a particular arrangement of molecules that would lead to deposits having properties quite different from those of the supporting material (Tsapiuk, 1996; Baba *et al.*, 1994). Moreover, biomolecules own functional groups such as hydroxyl and/or amine which make them reactive. Thus layers could be easily functionalized by grafting enzymes (Edwards *et al.*, 1999) or ligands (Zeng and Ruckenstein, 1998; Yılmaz *et al.*, 2004) as an example. Finally the sensitivity as regards chemical treatments of these molecules is a main advantage for whom wishes to regenerate the porous support at the end of operation.

In what follows, we have focused on new applications of membrane-based contacting systems for low temperature operations encountered in food processing and biotechnology. Different types of biomolecules (proteins, lipids, chitosan and chitin) likely to be used to modify membrane surface and to get specific properties and/or functions, have been considered successively.

USE OF PROTEINS

Enzymatic Reactions

During the last decade, enzyme processes have been commonly used in the production of food, pharmaceuticals and other biological products. In order to overcome the well-known disadvantages of batch reactors (especially the problems with inactivating and separating enzymes after each batch), various attempts to operate enzymatic reactions in a continuous way and reuse enzymes have been made. In that way enzymatic membrane reactors involving free enzymes have been proposed (Prazeres and Cabral, 1994; Paolucci-Jeanjean *et al.*, 1999). Basically, membrane cut off is chosen so as to reject enzyme and substrate while letting pass products in the permeate. This solution is particularly attractive when the molecular weight of products is lower than the size of substrates and enzyme. Another alternative reactor design with enzyme immobilization has been proposed by Balcão *et al.* (1996). Such a device offers a great efficiency and allows a complete integration of separation and reaction. Three main methods have been reported to fix the enzyme onto the membrane (Giorno and Drioli, 2000): (i) biocatalyst entrapped within membrane pores; (ii) biocatalyst gelified on membrane; (iii) biocatalyst bound to membrane by cross linking or support binding (physical adsorption, ionic binding or covalent binding). The last method which prevents from biocatalyst leaching, demands the activation of membrane if there is no active groups on the surface. Indeed, for the sake of covalently immobilizing enzyme onto ceramic membrane it is necessary to previously functionalize the support, for example thanks to a functional layer.

In last decade, Negrel *et al.* (1996, 1997) reported the preparation of an hybrid membrane based on the dynamic gel layer formed during the tangential ultrafiltration of a gelatin solution onto an α-alumina macroporous ceramic support. The deposited layer was then hardened and tightened by a chemical (tanning) and physical (thermal drying) treatment. More recently Bullon *et al.* (2000) optimized this manufacturing procedure, by lowering the total time of preparation of five to less than two hours and by succeeding in reducing the pore size which was estimated around 3 nm (Bullon *et al.*, 2001). These membranes have the advantage of being biocompatible, not clogging, of an easy preparation and regeneration. Moreover, insofar as they can be used either in aqueous or in organic solvents without any leaching of the coated layer and insofar as they present many reactive amino groups from lysine or arginine residues, these hybrid membranes appear to be good candidates for enzyme immobilization via a cross-linking agent (Lozano *et al.*, 2000a). In addition, in order to enhance the interest of the membrane, gelatine solution can be replaced by a gelatin-polyethylene imine (PEI) mixture 1:1 w/w thus increasing the amount of available amino groups (Lozano *et al.*, 2002).

In that view, Lozano *et al.* (2000a) proposed an original way how to immobilize enzyme which mainly involves three steps: the formation of a dynamic biopolymer layer on the membrane surface its activation with a cross-linking agent (glutaraldehyde) and then enzyme attachment. The optimized procedure for enzymatic membrane preparation is illustrated in Figure 1. Detailed method and successful reproducibility tests are proposed in a previous article (Magnan *et al.*, 2004). It is worth noting that, in addition to the advantages of the formed-in-place membrane, the hydrophilic character of the ultra-thin layer provides an adequate micro-environment for enzyme. Particularly water molecules present in the micro-environment preserve enzyme activity even in nonaqueous media (organic solvent, supercritical fluids). This new membrane was placed in a classical enzymatic membrane reactor (EMR) (see Figure 2) for performing different reactions. Feed is pumped towards the membrane module in which the