9
Membrane Bioreactors in Functional Food Ingredients Production

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9.1
Introduction

The present chapter focuses on the application of membrane reactors using catalysts of biological origin for food productions. An overview about the different membrane bioreactor types is reported, and their advantages together with the main drawbacks are discussed. The use of membrane bioreactors in the different food applications is described with more attention in the production of functional food.

9.2
Membrane Bioreactors and Functional Food

During food processing, flavor and odor are often lost, obtaining as final products lower food quality if compared with fresh ingredients. The development of better methods for delivering flavor is of high interest for the food industry to heighten user enjoyment, this is particular important if we consider continuing urbanization and increasing problems in transportation due to energy consumption.

An important sector that contributes in the food industry to this aim are separation techniques that can isolate flavor and odor chemicals early in the processing steps and resupply them to the processed foods. Functional food processes can recover small components active ingredients from by-product streams to be used as high-value additives.

Membrane bioreactors are able to integrate bioconversions with selective membrane separations leading to continuous clean, safe and low energy consumption production.

Although their potentialities have not been fulfilled yet, the current needs and challenges in satisfying the increasing consumer demand of safe goods and the limited resources availability will force the industry towards these selective and efficient techniques.

The use of biocatalysts in combination with membrane operations permits drawbacks to be overcome enabling biotransformation to be integrated into contin-

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uous production lines. These systems, being able to work at time-invariant conditions at steady state, permit a better control of reaction conditions with an increase of lifetime, productivity and economic viability of the process. In addition, the separation, purification and concentration of the obtained product can be obtained. Thanks to the biocatalyst and membrane selectivity the mass intensity can be very high, with no by-products formation, while producing high added value coproducts.

Membrane processes and in particular membrane bioreactors are regarded as particularly suitable for food applications because, in general they can operate under mild conditions of temperature, pressure and shear stress, therefore preserving the biological activity of the compounds to be recovered and the properties of the original media/matrix. In general, they do not require any extraction mass agent such as solvents, avoiding product contamination and the need for subsequent purification.

Based on the membrane role, bioreactors are divided into systems in which the membrane does not contribute to the reaction but only controls mass transport, and systems in which the membrane works as a catalytic/separation unit, a configuration in which the reaction also occurs at the membrane level. In this last case we talk about biocatalytic membrane reactors, BMR.

The first case represents the most commonly used, and due to the presence of different biocatalysts (enzyme or cells) of different molecular weight, in the literature several names are found to describe this. In this work, to indicate membrane bioreactor in which the membrane acts as separation unit, we will refer to free biocatalysts membrane bioreactors (MBR), in the other case we will refer to BMR. A schematic representation is reported in Figure 9.1. In biocatalytic membrane reactors the biocatalyst can be: entrapped, gelified, and bound to the membrane. Biocatalytic membrane reactors with biocatalyst bound to the membrane can result from ionic binding, cross-linking and covalent binding.

Membranes in a variety of configurations, including tubular, hollow fiber and spiral wound have been used in the food industry for many years. They can be applied within the production process, that is for clarification and concentration, as well as to treat the resulting wastewater prior to disposal or reuse. The main benefits of membrane technology/bioreactor are well documented. Examples of systems

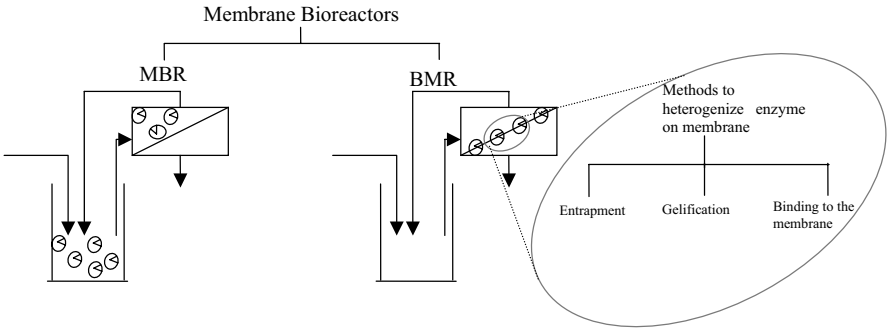

Figure 9.1 Schematic representation of membrane bioreactors.

developed at industrial production level have been recently reviewed [1]. However, more research effort needs to be invested in order to fully exploit their potentialities.

Their implementation falls primarily in two main objectives (a) improve production process (b) recovery of valuable products that previously would have been lost as wastes. A new trend in the development of membrane bioreactor is dictated from the strong need in food/feed to produce functional food.

Functional food or medicinal food is any fresh or processed food claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients.

To better explain the contribution of membrane bioreactors development in functional food production some definitions are outlined.

A *nutraceutical* is a part of functional food isolated and purified from foods that has physiological benefit or provides protection against chronic diseases. Bioactive compounds are examples of nutraceuticals.

Treated food with live cultures are considered as functional food with probiotic components, which is a viable microbial dietary supplement, that beneficially affects the host through its effects in the intestinal tract. In other words, probiotic foods are defined as those that contain a single or mixed culture of micro-organisms that affect beneficially the consumer's health by improving their intestinal microbial balance [2]. Another class of treated food is prebiotic. The term was first used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU). A prebiotic is a food ingredient that is not hydrolyzed by the human digestive enzymes in the upper gastrointestinal tract and beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health [3]. The fibers are included in this kind of compounds.

9.3
Membrane Bioreactor in Sugar and Starch Processing

The sugar and starch industries represent the competitive world sugar market, Table 9.1 summarizes major sugar productive countries and their production. Application of membrane technology in the sugar industry contributes to the sustainable development in the field. In particular, clarification of sugarcane juice, production of glucose or glycerol as well as are sugar-related products are aspects where membrane bioreactor can play a role.

In the confectionery and many food and beverage industries, sugar (present as starch, sucrose, fructose and glucose, etc.) is the main constituent in some of the process streams. Inevitably, it is also present in the effluent streams arising from these industries. There is, however, considerable interest among manufacturers to optimize process economics through product recovery, and to respond to environmental pressure to reduce the waste generation.

Enzymatic hydrolysis of starch is traditionally performed in large volume-batch reactors using soluble enzyme following a two-step procedure including the

Table 9.1 Major sugar-producing countries.

2007/08 est.	Production (million tons)	Exports (million tons)	Per capita consumption (kg)
Brazil	31.355	20.957 (1)	58
India	28.804	3.298 (4)	20
EU	17.567	1.400 (8)	34
China	14.674	—	11
Thailand	8.033	5.288 (2)	36
United States	7.701	—	29
Mexico	5.978	0.350 (15)	52
SADC	5.834	2.410 (5)	22
Australia	5.013	3.750 (3)	47
Pakistan	4.891	—	25

liquefaction and saccharification [4]. But nowadays it can be performed in a single step by using amylase enzyme termamyl that is able to produce dextrins [5, 6] using membrane reactor.

First, the starch being dissolved in water and partially hydrolyzed with an α-amylase to give maltodextrines and in the next step, saccharification enzymes transform liquefied starch into low molecular weight oligosaccharides such as glucose or maltose. The conventional batch reaction processes has a great number of disadvantages, such as incompatibility of enzyme recovery and reuse, high labor and purification cost, high capital investment and discrepancies in glucose syrup quality, low efficiency, batch to batch variations, and most of all, the high enzyme cost. But the application of membrane reactors make possible continuous operation in lower reactor volume, as well as in shorter reaction time [5–9] an increase the reactor's efficiency and finally to reuse enzymes in a continuous way.

The application of membrane reactors in the starch industry is particularly used in the production of smaller assimilable sugars. This reaction is carried out in systems in which the enzyme is not immobilized and the membrane works as a separation device (MBR). The enzymes used are amylolytic enzymes and debranching enzymes [10] or using liquefied starch as substrate [11]. The major problems in the application of the membrane reactor are a large decrease in permeate flux due to concentration polarization and fouling [5, 6, 12]. Different solutions are applied to decrease fouling phenomena such as the pretreatment of the raw starch solution. Other studies were devoted to the examination of factors that mainly affect membrane performance such as: molecular-weight cut-off, enzyme dosage, residence time, transmembrane pressure, carbohydrate composition, and retention factor [11].

Membrane bioreactors have been used for production of glucose, maltose, maltotetraose, and cyclodextrins [5–9, 13–16] for the food-grade industrial production [12] like puddings, jellies, and fruit desserts. In this system hydrolysis can be carried out simultaneously by separating syrups from enzymes and non-hydrolyzed starch [12]. An extra separator system to extract the product is not necessary but it is needed to

Table 9.2 Examples on the use of membrane bioreactors for sugar production.

Starch used	Enzyme used	Membrane used	Molecular weight cut-off (kDa)	Reactor type	Reference
Commercial potato starch	α-amylase (BAN 480L)	Tubular ceramic	50	MBR	[17]
Cassava	Termamyl	Carbosep M4	50	BMR	[18]
Cassava	Maltogenase and promozyme	Carbosep M4	50	BMR	[19]
Amylos	Amylolitic enzyme complex – from fermentation of whole wheat flour by Aspergillus awamori	Hydrophilic cellulose acetate	40	MBR/ BMR	[20]

concentrate the product by application of different advanced membrane operations (UF/NF).

Table 9.2 summarizes examples of membrane bioreactors used for sugar production.

Another field of membrane bioreactor application is the production of cyclodextrins or oligosaccarides. The development in this field was pushed from the high interest devoted to this compound in the last period, due to the fact that they have applications in several fields, including food, pharmaceutical, cosmetic, and plastic industries as emulsifiers, antioxidants and stabilizing agents. The production of cyclodextrins by membrane bioreactors was conducted using different starting sources including corn starch and soluble potato. A recent work reported their production also starting from tapioca starch [21].

The production of oligosaccarides to be used as functional food was also obtained by the immobilization of dextranase on polymeric matrix [22].

Cyclodextrins can be used as carriers for molecular encapsulation of flavors and other sensitive ingredients [23]. The molecular encapsulation of lypophilic food

Table 9.3 Examples of laccase immobilization on different membrane material.

Biocatalyst	Membrane material	Immobilization	Reference
Laccase from Aspergillus sp.	Nylon-66	Adsorption	[42, 44]
Laccase from Pyricularia oryzae	Polyethersulfone membranes	Adsorption	[45]
Laccase from Trametes versicolor	Hydrophilic PVDF microfiltration membrane	Covalent binding	[46]
Laccase from Trametes versicolor	Polyether sulfone membranes	Entrapment	[47]

ingredients with cyclodextrin improves the stability of flavors, vitamins, colorants and unsaturated fats, and so on.

Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, milk, honey. Oligosaccharides can also be used as functional food ingredients that have a great potential to improve the quality of many foods. In addition to providing useful modifications to physicochemical properties of foods – such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria, stimulation of mineral absorption, non- or anticariogenicity, and the improvement of both plasma cholesterol and blood-glucose level.

Basically oligosaccharides are short-chain sugars generally consisting of two to ten building block small sugars. It is used as a nutrition supplement in food ingredients and additives. Apart from direct extraction from plants the oligosaccharides can be processed by enzymatic synthesis using enzymes that possess hydrolytic or transglycosylation activity, in continuous membrane bioreactors. Both batch reactor with soluble enzymes and continuous systems with enzymes or whole cells immobilized have been used.

9.4 Membrane Bioreactor in Oil and Fat Processing Industry

The use of membrane bioreactors for the hydrolysis of oils and fats is intensively investigated. The biocatalysts used are mainly lipases and esterases and the processes in which they are involved for functional food production are ester synthesis to produce emulsifiers and aroma compounds and oil hydrolysis for free fatty acids, mono or diglycerides productions.

Monoglycerides, diglycerides, triglycerides, and glycerol are widely used in the food industry as emulsifiers for bakery products, margarines, dairy products, confectionery, and so on. In foods and beverages, glycerol serves as a humectant, solvent and sweetener, and may help preserve foods. It is also used as filler in commercially prepared low-fat foods (e.g., cookies), and as a thickening agent in liqueurs, although it has about the same food energy as table sugar. Glycerin has many uses, such as in the manufacture of food and in the production of pharmaceuticals too. The most commonly used products are glycerol monostearate, monooleate, and monoricinoleate [24].

The complex mixtures that contain 40–48% monoglycerides (MG), 30–40% diglycerides (DG), 5–10% triglycerides (TG), 0.2–9% free fatty acids (FFA), and 4–8% free glycerol are generally termed monoglycerides. These mixtures have applications in food fats (margarine, ice cream, sweets, etc.). Pure monoglycerides (90–97%), obtained by molecular distillation of the above mixtures, are also commercially available. The higher-purity monoglycerides are preferred for bakery uses because of their good amylase complexing ability. Most commercial MG are produced from edible, refined, hydrogenated animal fats (tallow, lard, etc.) or from hydrogenated vegetable oils (palm, soybean, corn, olive, peanut, etc.). High oleic vegetable oils can also be used as raw materials for the production of emulsifiers for liquid and low-fat margarines.

The monoglycerides can be produced on an industrial scale by glycerolysis of fats and oils by means of inorganic alkaline catalysts, such as sodium hydroxide or by enzymatic routes. Application of enzymes as catalysts for reactions in the oils and fats industry is being extensively studied in the literature. Enzymes are chosen since they show many advantages over traditional inorganic catalysts: they have large catalytic activity under mild operating conditions; they show large selectivity to the desired product with no significant side reactions, leading to products of high purity.

Various review papers about membrane bioreactors using lipase in vegetable oil and fat processing have been published during recent decades [25–27].

The influence of operating conditions of lipase immobilized in a two-separate phase membrane bioreactor has been reported [29–31]. In particular, the effect of immobilization method, amount of enzyme, hydrodynamic conditions, and micro-environment conditions (such as pH, temperature, membrane material) have been investigated [32]. Strategies to improve reaction performance as well as transport properties through the enzyme-loaded multiphase system have been exploited [33, 34]. Three different enzyme membrane reactors have been compared, as illustrated in the Figure 9.2. Lipase was used free in a stirred-tank reactor, and as immobilized in

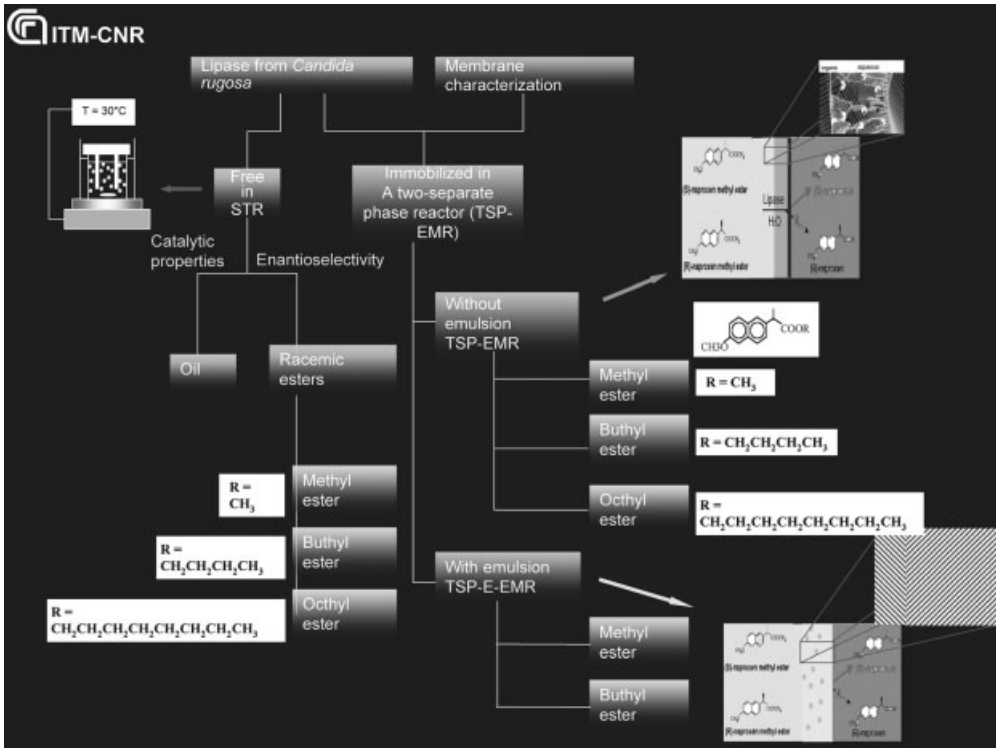

Figure 9.2 Schematic representation of bioreactors studied [29–31]using lipase as biocatalyst: free stirred-tank reactor, two-separate phase enzyme membrane reactor and two-separate phase enzyme membrane reactor with emulsions.

a membrane in the absence and in the presence of oil/water droplets. The use of oil droplets immobilized together with the enzyme significantly improved the performance of the system thanks to the positive effect of the o/w interface uniformly distributed through the membrane on the enzyme activity as well as on the substrate transport.

Lipase has been immobilized on polymer membranes with hydrophilic [35] hydrophobic [36] properties, as well as on inorganic membranes [37].

Another application of membrane bioreactors is production of specific structured lipids in an enzymatic route from rapeseed oil and capric acid [38]. Production of ω3-polyunsaturated fatty acid (ω3-PUFA) concentrates from fish liver oils (which have been claimed to provide beneficial health effects via prevention of coronary heart diseases) for use as nutropharmaceutical food supplements is another application of lipase in membrane bioreactor, and sequential lipase-catalyzed chemical incorporation in triglycerides. Lipase from *Candida rugosa* was also immobilized on Cuprophane membrane [39] in a hollow-fiber module.

9.5
Membrane Bioreactors in Hard Drink Industry and Liquid Beverages

9.5.1
Wine

Membrane bioreactors are developing in the wine field for the production of aromatic compounds and flavor by the use of glucosidases, the production of additives from pectinase hydrolysis, and the production of preservatives molecule such as lactic acid by the use of malolactic bacteria.

The production of wine in terms of cropped surfaces and product yield fluctuates in a significant way over the years. At the end of the 1990s the production tended to decrease, but a significant increase was achieved at the end of 2004/2005 going back toward another decrease in 2005/2006, where the production was 4% less. Nevertheless, the production of European countries (27 countries) in 2007 was about 174 449.170 (Wine, production – 1000 hl). With respect to the total worldwide production, Europe represents the higher producer of wine (http://news.reseau-concept.net/images/oiv/client/STATISTIQUE__Verone_2008_EN_definitif_41dia-pos.pps#1) having leading countries like France, Italy and Spain. The United States is another important producer followed by Argentina and China, while the economy of other countries like Germany, South Africa and Chile, is growing in the last three years.

Thanks to the action of different yeasts, both *Saccaromyces* and non-*Saccaromyces* type, in the first part of wine making there is the conversion of glucose in ethanol CO_2 and other products. The presence of the yeast is fundamental in must fermentation due to the production of particular enzymes that help the fermentation process.

The use of these type of enzymes or directly the yeasts with this enzymatic activity, immobilized with membrane or on other support for wine fermentation is of high interest.

Some examples of coupling the enzymes useful in wine making and membrane reactors are reported in Table 9.4.

β-glucosidase is an important enzyme in wine making, the enzyme is employed in different applications like production of rosé wine from red grapes, for the hydrolysis of antocianines, and for the hydrolysis of terpenglucosides and so on [40]. The immobilization of this enzyme, or bacteria and yeast showing that enzymatic activity is of high interest in beverages production with enhanced aroma. In the literature are reported some examples [40] about the immobilization of β-glucosidase on different support (Cellulose PEI (Baker), alpha-aluminia CT 2000 (Alcoa Chemie), gamma-aluminia (Akzo), chitosan (Chitobios) and polymeric) applying adsorption, covalent bonding by glutaraldeide and cross-linking immobilization techniques.

Some immobilized glucosidase enzymes has also been proved on a pilot scale. They were used on a continuous-flow stirred-tank membrane reactor in a model system and also during wine making [41]. In this system the enzyme was immobilized on chitosan pellets and to simulate the natural process, the medium was also supplemented with chemicals present in the wines (fructose, ethanol, nerol, linanol, geraniol). Fructose did not decrease biocatalyst stability, while alcohol affected the enzyme half-life from 2586 h at 3% (w:v) ethanol to 1378 h at 12% (w:v).

Enzyme stability was not dependent on substrate concentration and was considered satisfactory for an industrial process (a half-life of 1.2 years).

Many precursors of the aromatic components of wine are monoterpenes (geraniol, nerol, citronellol, linalool, a-terpineol, etc.) in di-glycosidic form, that contain b-D-glucopyranose bound directly to aglycon and/or other sugars among which are a-L-rhamnopyranose and a-L-arabinofuranose.

Therefore, to develop the aromatic potential of a wine to the full, together to ramnopyranose (Rha), it is also necessary to utilize the other glycosidases: a-L-arabinofuranosidase (Ara, EC 3.2.1.55), and first b-D-glucopyranosidase (bG, EC 3.2.1.21) and a-L-rhamnopyranosidase (Rha, EC 3.2.1.40).

An important reaction that occurs in wines and in particular in white wine and in rosé is the development of madeirized flavor. The process is mainly due to polyphenols, that can have also beneficial health effect because of their antioxidant properties. Oxidative enzymes like laccase coming from fungi are used to improve the process. Several studies were performed on the use of Laccase in phenol-removal processes for must and wine stabilization [42]. Laccase was immobilized on different membrane materials by applying different immobilization techniques from different sources (Table 9.3).

Cantarelli and Giovanelli [43] carried out assays in order to determine if the enzymatic preparations could be used in white-wine production for polyphenols reduction in musts (and consequent stabilization of the wine color) instead of oxidation. The results demonstrated that the enzymatic treatment coupled with filtration with polyvinylpolypyrrolodone (PVPP) reduced the quantity of oxidized polyphenols.

Other important enzymatic activities in wine making and in particular in wine-clarification processes are pectinases, which are usually used to improve

Table 9.4 Examples of membrane bioreactors for pectins hydrolysis.

Biocatalyst	Membrane	Bioreactor configuration	Application	Reference
Pectin lyase from *Penicillum italicum*	Ultrafiltration membrane	MBR	Production of pectic oligosaccarides	[48]
Polygalacturonase from *A. niger*	30-kDa flat regenerated cellulose membrane	MBR	Production of D-Galacturonic acid	[49]
Polygalacturonase and pectin lyase from *A. niger*	Spiral-wound polysulfone membrane (10 kDa)	MBR	Wine clarification	[50]
Endo-polygalacturonase from *Aspergillus pulverulentus*	Amicon 10 kDa	MBR	Production of pectic oligosaccarides	[51]
Polygalacturonase from *A. niger*	Titania microfiltration	BMR	Production of pectic oligosaccarides	[52]
Rapidase liquid plus	Polyvinilidene fluoride tubular, polysulfone spiral wound	BMR	Apple-juice clarification	[53]
Amylase and pectinase	Polysulfone single-hollow fiber	BMR	Fruit-juice processing	[54]
Commercial pectinaase	Hollow-fiber ultrafiltration	BMR	Fruit-juice processing	[55]
Endopectidase from *A. niger*	10-kDa spiral-wound polysulfone	MBR	Apple-pectin hydrolysis	[50]

processability and to produce additives. The main membrane bioreactor configuration used in the wine industry using pectinases action is a free enzyme membrane reactor (BMR). The soluble enzyme is confined in the retentate side of the membranes where it is in contact with the substrate. In Table 9.4 some examples and membrane material used for the pectines hydrolysis are reported. These applications are referred both to the wine and fruit-juice treatment.

Together with protein immobilization, the alternative strategy for wine making, is cell immobilization. Although this application is rapidly expanding in the research area, development at the industrial scale is still limited. Takaya *et al*. [56] studied the efficiency of two membrane bioreactor systems for continuous dry wine making. The first configuration was a single-vessel bioreactor, while the second configuration included two vessels; one operated as a continuous stirred-tank reactor and the other was a membrane bioreactor. The double vessel resulted in 28 times more productive that the single one.

Cell immobilization is a rapidly expanding research area. The purpose of this technique is to improve alcohol production and overall product aroma, taste and quality. Many support are used for cell immobilization in this field divided into inorganic, organic and natural materials. Some examples of different supports and its main application are reported in Table 9.5.

Malolactic fermentation is a secondary process that occurs in wines during the maturation period. Lactic bacteria predominately of the genera *Oenococcus, Lactobacillus* and *Pediococcus* are responsible of this process, where L-malic acid is converted to lactic acid, an important food preservative, and carbon dioxide. As a consequence of this reaction the total acidity of the wine decreases. *Oenococcus oeni* can carry out this process in one step, without the production of piruvic acid. Other by-product produced during this fermentation can affect wine flavor. Also some yeast as *Saccaromyces* can convert malic acid through maloethanolic fermentation [65].

The immobilization technology is important also in this field, where the cell compartimentalization can help to (i) increase the tolerance towards malolactic

Table 9.5 Materials used for cell immobilization.

	Immobilized cell	Application	Reference
Inorganic material			
Mineral kissis	Saccaromices	Aroma improvement	[57]
γ-aluminia	Saccaromices	Aroma improvement	[58]
Organic support			
Cellulose covered with Ca-alginate	Saccaromices and Candida	Enhance glycerol formation in wine	[59, 60]
Ca-alginate beds	Saccaromices	Must fermentation	[61]
Natural support			
Delignified cellulose	Saccaromices	Fermentation	[62–64]
Gluten pellets		Production of wine with less alcohol content	

fermentation bacteria, (ii) develop the desired flavor selecting the appropriate cultures, (iii) accelerate the process increasing cell densities, (iv) reuse of the cell.

A kinetic analysis was carried out using three different immobilization techniques of malic enzyme for the development of a membrane bioreactor: (1) polymeric membranes [66] and cross-linking reaction, (2) within polyurethane foams, and within a gel-like membrane formed on active side of ultrafiltration polymeric membranes [67].

Enzymatic cell-free reactors, did not allow to efficient, complete and rapid consumption of the L-malic acid to be achieved [68, 69].

9.5.2
Beer

Beer is the second most consumed beverage in the world behind tea, and it continues to be a popular drink. The brewing industry has an ancient tradition and is still a dynamic sector open to modern technology and scientific progress. Brewers are very concerned that the finishing techniques they use are the best in terms of product quality and cost effectiveness [70].

Beer production requires about seven days of fermentation and large-scale fermentation and storage capacity. The main field in which membrane bioreactors can be developed in beer are the alchohol-free beers and in the maturation and aroma control.

In the first process, the two main approaches currently used are the removal of the alcohol from product and limited fermentation. In the case of limited fermentation the system is most efficient where the fermentation cells are immobilized. The yeasts commonly used for this process are *S. cerevisiae*.

Different kinds of support are used to immobilize the yeasts in brewing, they can be divided in inorganic, organic and natural. The prevalent organic support are: polyethylene, PVC, polysaccarides, DEAE-cellulose; the inorganic porous ceramic and silicon and the natural support are delignified cellulose and gluten pellets [71].

9.5.3
Ethanol Production

The requirement of ethanol in the beverage industries as an additive has been steadily increasing and so is the pursuit of immobilized microbial cell systems for ethanol fermentation. Research on alcohol production usually focuses on volatile by-product formation, because these constituents are critical parameters for distillates and alcoholic beverage quality. For ethanol production different yeast strains are used such as: *S. cerevisiae, S. diastaticus, K. marxianus and Candida sp.*, and different bacteria like *Zymomonas mobilis*. The requirement for food-grade purity is not essential due to the employment of a distillation step. A membrane distillation bioreactor was developed for ethanol production [72, 73], where the batch fermentation was coupled with a membrane distillation process. The porous capillary polypropylene membranes were used for the separation of volatile compounds from

the feed. The elimination of these compounds allows an increase in ethanol productivity and rate. In this case the yeast used was *S. cerevisiae*.

A membrane bioreactor for the production of ethanol was developed in a pilot plant [74]. This system integrated ceramic microfiltration membranes with a stirred-tank bioreactor.

9.6
Membrane Bioreactor in Other Liquid Beverages

The main applications of membrane bioreactors in other drink industries are: reducing the viscosity of juices by hydrolyzing pectins, reducing the lactose content in milk and whey by its conversion into digestible sugar.

9.6.1
Fruit-Juices Production

The production of fruit juices is divided into six major steps: crushing, pressing, clarification, centrifugation or filtration, concentration, pasteurization. During the fruit crushing there is the solubilization of pectins, these compounds can usually affect the processability, creating turbidity and cloud forming.

Pectinase, the pectolitic enzyme responsible for pectins hydrolysis are commonly used in the fruit-juice industry, in two steps: pressing and clarifications.

During pectin hydrolysis the monomer of pectin, D-galacturonic acid is also produced, which is an important compound, as a raw material in the food, pharmaceutical and cosmetic industries to manufacture, for example vitamin C, or acidifying, tensioactive agents.

Oligosaccarides derived from pectin hydrolysis can also have some important applications as repressors of liver lipid accumulation in rats [75], as antifungal phytoalexin-elicitors in plants [76], inducers of flowering and antibacterial agents [115].

Traditionally, enzymatic hydrolysis of pectins has been conducted in batch systems. Unfortunately, after each cycle of operation the enzyme could not be recovered for further use and immobilized enzyme could suffer from steric hindrance effects and losses in enzyme activity as a result of immobilization. The use of membrane bioreactor is the alternative efficient strategy, in which the enzyme is retained or compartmentalized, thus increasing enzyme utilization. One of the membrane bioreactor configurations commonly used is with the enzyme compartmentalized in the retentate side of the membrane together with the substrate, while the product is separated in the permeate.

Different works were carried out for pectin hydrolysis in membrane bioreactor systems using a free enzyme membrane reactor. Alkorta *et al.* [77] studied the reduction in viscosity of pectins catalyzed from pectin lyase from *Penicillum italicum* in a membrane reactor. This enzyme results as the only pectinase enzyme capable of hydrolyzing α-1,4 glycosidic bond of highly esterified pectins, without altering the

volatile compounds responsible for the aroma of various fruits [77, 78] the reduction in viscosity was demonstrated with high efficiency towards different fruit juices: grape, peach, melon, apple and pear, showing a little decrease in the case of apple and pear juice.

Another biocatalyst used frequently in pectin hydrolysis was polygalactorunase from *A. niger*. A. niger pectinases are most widely used in industry because this strain possesses GRAS (generally regarded as safe) status, so the metabolites coming from its production can be directly used without further treatment [79]. The pectinases produced from this strain are: polymethylgalacturonase (PMG), polygalacturonase (PG) and pectinesterase. However, particular pectinases are used for specific purpose, for example only polygalacturonnase is used for baby-food products [79].

A recent work reports the use of polygalactorunase from *A. niger* in a flat-sheet membrane reactor, which shows excellent stability for more than 50 h. In this case, the membrane used was a 30-kDa regenerated cellulose membrane [49]. The same biocatalyst was used in a free enzyme membrane reactor where the membrane used was a spiral-wound polysulfone membrane (10-kDa MWCO), attaining a conversion of 83% and a stability for a long period (15 day) [50].

The performance of pectin hydrolysis was also tested by immobilizing directly the enzyme on the membrane and conducting the reaction in a biocatalytic membrane reactor [77]. The use of pectinases immobilized on ultrafiltration membrane hydrolyze the pectin to lower molecular weight species, permitting an extension of membrane operation without cleaning [55].

Pectinase was also immobilized by physical immobilization on a titania microfiltration membrane [52] and on a polysulfone hollow-fiber membrane [55], and coimmobilized with amylase on a polymeric hollow-fiber membrane to hydrolyze simultaneously starch and pectins. The coimmobilization showed an improvement of flux of an additional 35% [54].

An integrated membrane process for producing apple-juice and apple-juice aroma concentrates was proposed by Álvarez *et al.* [80]. The efficient system involves the following operations: an integrated membrane reactor to clarify the raw juice; reverse osmosis to preconcentrate the juice, pervaporation to recover and concentrate the aroma compounds, and final an evaporation step to concentrate apple juice. These operations were tested in laboratory and pilot-plant units, giving promising results both on the yield of product and also for economical aspects.

Some examples of immobilized pectic enzyme are present at the industrial scale [29, 30, 48]

9.6.1.1 Functional Food Production in the Milk and Whey Field by Membrane Bioreactor

The first application on a large scale of a membrane bioreactor was the hydrolysis of lactose by immobilized β-galactosidase on a cellulose fiber for the production of milk with low lactose content [81].

Lactose, together with high molecular weight proteins, are allergenic compounds present in both milk and whey.

Intolerance to milk comes from the fact that some subjects cannot digest proteins, contained in milk and whey, with a molecular weight higher than 5 kDa.

9.6.1.1.1 **Lactose Hydrolysis** Lactose is the dominant carbohydrate in milks and it is also contained in whey. A large number of people do not digest lactose properly due to the lack or inactivity of the intestinal β-galactosidase and they suffer from intestinal dysfunction. In addition, lactose is a sugar with a high BOD, low sweetness, and low solubility and has a strong tendency to adsorb flavors and odors compared to its hydrolysis products; glucose and galactose. Lactose hydrolysis is an important food process, not only to produce lactose-free milk, but also to improve processes for the production of refrigerated diary products, because some technological difficulties occurs associated with lactose crystallization [82]. Another important application of lactose hydrolysis is the production of additives, like lactic acid, glucose and galactose that can be used in the human diet [83].

For the industrial applications of enzymes to the productions of large quantities of product, the enzymes should be immobilized to be used in continuous reactors. Several procedures for β-galactosidase have been studied: entrapment, adsorption, ionic interaction, affinity, complex formation with metal, and covalent bonds [83].

Several reactors were also tested using different membrane reactors configuration and different starting sources. In Table 9.6 some examples showing support material application are reported. The main enzyme used in membrane bioreactors for lactose hydrolysis are from *Kluyveromyces* yeast and *Aspergillus* fungi, micro-organisms considered safe (GRAS). In particular the enzymes from fungi can be used in acid wheys since their optimum pH is 3.5–4.5, while the enzymes from yeasts can be used in milk and sweet wheys since their optimum pH is between 6.5–7 [84].

As previously mentioned, the other application of membrane bioreactors in the lactose hydrolysis is the production of lactic acid. Lactic acid is one of the value-added product produced from processing cheese whey. The food and drug administration have approved lactic acid and its salts to be GRAS. The bacteria usually used for the production of lactic acid by fermentation process from cheese whey are *Lactobacillus helveticus* [91–93] and *Lactobacillus casei*, while *Bifidobacterium longum* converts lactose into lactic acid and produces antibacterial compounds [94]. The main configuration of a membrane bioreactor for the production of lactic acid is a fermentation reactor with a membrane unit as reported in Table 9.7. in this kind of configuration cell, protein and lactose are separated by a filtration unit and returned to the fermentor while lactic acid is separated in the permeate. Some examples of biocatalytic membrane reactors are also present in the literature. *L helveticus* cell were immobilized in a polymeric membrane reaching a lactose conversion of 79% and a lactic acid yield of 0.84 g of lactic acid/g of lactose utilized [97].

A two-stage continuous fermentation with membrane recycle has been studied that enhances lactic acid productivity from 21.6 $g\,dm^{-3}\,h^{-1}$ in a single stage to 57 $g/dm^{-3}\,h^{-1}$ in two stages [95].

9.6.1.1.2 **Protein Hydrolysis in Milk and Whey by MBR** The hydrolysis of high molecular weight proteins into small polypeptides is an alternative approach to produce low allergenic (β-lactoglobulin) fresh milk.

The possibility to hydrolyze high molecular weight proteins by membrane bioreactors provides a rich source of peptides that are latent until released and

Table 9.6 Examples of membrane bioreactor used to hydrolyze lactose.

Biocatalyst	Source	Material and reactor configuration	Application	Reference
B. circulans	Skimmed milk	MBR	High-quality milk	[85]
K. lactis, A. oryzae	Lactose	MBR with ceramic membrane	Production of galactosil-oligosaccarides	[86]
β-glycosidases from the archaea *Sulfolobus solfataricus* (Ss β Gly) and *Pyrococcus furiosus* (CelB)	Lactose	MBR with an ultrafiltration unit	Production of oligosaccarides	[87]
β -galactosidase from *Kluyveromyces lactis*	Lactose	BMR	Galactose and glucose production	[88]
β-galactosidase commercial enzyme	Lactose	MBR	Production of oligosaccarides	[89]
A. orza, K. lactis	Lactose	MBR	Production of Galactosyl-oligosaccharides	[90]

Table 9.7 Examples of membrane bioreactors in the production of lactic acid.

Biocatalyst	Source	Membrane-reactor configuration	Reference
L. ramnosus	Glucose	MBR	[96]
L helveticus	Whey	MBR	[97, 98]
L. casei	Lactose	MBR	[99]
L. ramnosus	Lactose	MBR	[97, 98]

activated, for example, during gastrointestinal digestion or milk fermentation. Once activated, these peptides are potential modulators of many regulatory process.

Milk-derived bioactive peptides can have physiological functionality on cardiovascular, (antihypertensive, antioxidative, antithrombotic, hypocholesterolemic), nervous (agonistic, anthagonistic oppioid activity), gastrointestinal (antiappetizing, antimicrobial) and immune (antimicrobial, immunomodulatory, citomodulatory effect) systems [100]. The active peptides can be produced by the hydrolysis of digestive enzymes, through proteolytic micro-organism and through the action of proteolytic enzymes derived from micro-organisms or plant.

Some examples are reported in Table 9.8.

Commercial production of bioactive compounds from milk proteins is limited. The use of enzymatic membrane reactors for continuous production of specified peptide sequences was introduced during 1990. Nowadays, it has been widely studied, in the literature, for total conversion of food proteins of various origins with improved nutritional and/or functional properties. Continuous extraction of bioactive peptides in membrane reactors has been mainly applied to milk proteins using different membrane material and different membrane reactor configuration (See Table 9.9).

Table 9.8 Examples of biocatalyst used to produce active peptides from protein source.

Biocatalyst	Protein source	Active peptides produced	Reference
Pepsin	Casein	(ACE) inhibitory peptides	[100]
Trypsin	Casein	(ACE) inhibitory calcium-binding phosphopeptides	[101, 102]
Protease N	Whey protein	Different peptides	[103]
Lactococcus lactis	Casein, milk	(ACE) inhibitory peptides	[100]
Lactococcus helveticus	Casein, whey proteins	(ACE) inhibitory peptides	[104]
Lactobacillus delbruecki ssp. vulgaris	Casein	(ACE) inhibitory peptides	[105]

Table 9.9 Examples of production of bioactive peptides using MBR.

Biocatalyst	Substrate	Membrane reactor configuration	Application	Reference
Alcalase	Casein	MBR	Production of peptides	[106]
Trypsin	Caseinomacropeptides	MBR with ultrafiltration unit	Recovery of antithrombotic peptides	[107]
Pepsin	Goat whey	MBR	Production of α-lactorphin	[108]
Trypsin	Milk protein	BMR using polyacrilamide membranes	Production of phosphopeptides	[109]
Trypsin, chymotrypsin	Whey protein concentrate (WPC) and heat treated WPC	MBR with ultrafiltration unit	Production of polypeptides and rich fraction of small peptides	[110]
Pepsin, trypsin, chymotrypsin, pancreatin, elastase, carboxypeptidase	α-lactalbumin and β-lactoglobulin	MBR with two step ultrafiltration system (30 and 1 kDa)	Production of ACE-inhibitory peptides	[111]
Protex 6 L from bacillus licheniformis	Whey protein	MBR	Production of whey-protein hydrolyzates	[112]
Hydrolytic enzymes	Whey protein hydrolisates	MBR with ultrafiltration unit	Production of emulsifying peptides	[113]

During protein hydrolysis by a membrane bioreactor it has to be considered that an excessive hydrolysis should be avoided because a high content of free aminoacids involves negative effects like bad sensory properties and high osmolarity [114].

This means that to develop the system on an industrial scale, the hydrolytic reaction has to be strictly controlled.

Different works were focused on the optimization of process parameters for a continuous production of whey-protein hydrolysates.

Guadix *et al*. [114] developed a MBR with an ultrafiltration unit (polyethersulfone) where no effects on enzyme activity, due to mechanical shear stress, adsorption to the membrane or enzyme leakage were observed.

The effect of temperature on the performance of a batch reactor with an ultrafiltration unit made of polysulfone material of 8 kDa was analyzed in the hydrolysis of whey-protein hydrolyzates [112]. The experimental data perfectly fit a mechanistic model also proposed in the same article.

9.7 Conclusions

In this chapter the main application of membrane bioreactor and biocatalytic membrane reactor in food with emphasis on the production of functional food is reported. The main aspects were outlined to understand the recent development of the technology and its potential future applications in the field.

Research efforts are needed to improve aspects such as reproducibility on the large scale, enzyme life-time and immobilized enzyme stability during membrane-cleaning procedures. Technological strategies able to control these parameters are expected to fuel the further development of the biocatalytic membrane reactor on a large scale.

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