Chapter 7
Process Intensification in Biotechnology Applications

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Abstract This chapter presents an overview on how process intensification has influenced biotechnology applications from a multidisciplinary perspective. Initially, the process intensification philosophy is contextualized into biotechnology due to the particular challenges of these processes. This leads to a conceptual map analyzing the disciplines' interaction to achieve bioprocesses intensification. Subsequently, intensification is explored mainly from transforming biomass into chemicals point of view as an integrated solution addressed within the biorefinery concept. The chapter focuses into revising and presenting representative examples from process engineering perspective. First, how to enhance raw materials utilization in fermentations and enzymatic systems is presented. Secondly, advances on in situ product removal/recovery in order to enhance the reaction environment are presented, emphasizing on membrane bioreactor technologies. Finally, some current and future challenges are assessed to achieve bioprocess intensification. We strongly believe that developing bioprocess intensification philosophy will bring new perspectives to increase the cost-effectiveness of industrial applications towards a more sustainable future.

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7.1 Introduction

It is evident how the growing world population stresses world climate, natural resources, and the environment. A trend to mitigate the adverse influence of humans is by developing an economy based on bioproducts from renewable resources, which can be transformed into food/feed, chemicals, materials or energy. As any other, bioprocesses are subject to continuous structural changes in order to achieve sustainable use of energy and resources. These structural changes can be accomplished through optimization of the design and operational conditions. However, it is expected that these processes achieve the top of their performance indexes as they reach maturity; then, a further improvement through an approach of intensification is appealing (as shown in Fig. 7.1).

Process intensification (PI) philosophy is essential to cope with sustainability challenges in the forthcoming years due to the expected radical innovation achieved by a design paradigm shift. Only by 'quantum leaps' in performance resulting from novel technologies, it would be possible to have the ability to deliver the grail of sustainability required for processes. Intensified processes can contribute significantly to the competitiveness of process industries worldwide by making them faster, more efficient and less adverse environmentally. From the chemical process intensification perspective, the quantum leaps are achieved by increasing several times performance indexes [1]. However, in bioprocesses is not always possible to see such changes in performance through technology innovations. Bioprocesses impose relevant challenges (i.e., lack of process understanding, complex dynamics, composition variability in renewable raw materials, microorganisms/enzyme sensibility, and monitoring difficulties, among other) that limit potential technological breakthroughs.

Bioprocess intensification represents one of the research focuses especially because approximately 50 % of the elements we use to decrease our dependency on fossil feedstock can be obtained from renewable sources. Then, there is a tremendous need to develop bioprocesses that further optimize biomass harnessing through reaction and advanced separation techniques relying on sustainable ideologies [2]. Additionally, bioprocesses face relevant challenges to be overcome in

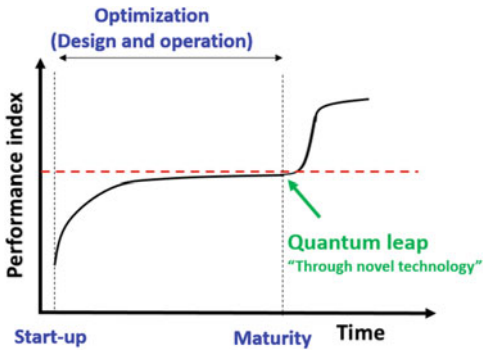

Fig. 7.1 Evolution on bioprocess improvement towards intensification

order to provide a sustainable future. Amongst the milestones identified within the international project Delft Skyline Debates for Process Intensification, the ones related to bioprocesses are [2]:

- Low-cost small-scale processing technologies for production applications in varying environments.
- Recycling of composite materials: design, engineering, and intensified production technologies.
- Towards perfect reactors: gaining full control of chemical transformations at the molecular level.
- Elemental sustainability: the total recovery of scarce elements.
- Production systems for personalized medicine.
- Bio-hybrid organs and tissues for patient therapy.
- Chemicals from biomass—integrated solution for chemistry and processing.

It is foreseen that achieving those milestones through bioprocess intensification, new breakthrough technologies will address not only processing issues but crucial societal problems, such as human health, the availability of water and food, energy and material resources, transport, and living standards.

Bioprocesses intensification cannot be seen as a single area, because this has been developed from different perspectives considering multidisciplinary and multiscale aspects, allowing improvements in production, purification and in the overall performance. Figure 7.2 illustrates an interpretation of how bioprocesses have been intensified in different areas (e.g., genetic engineering, biology,

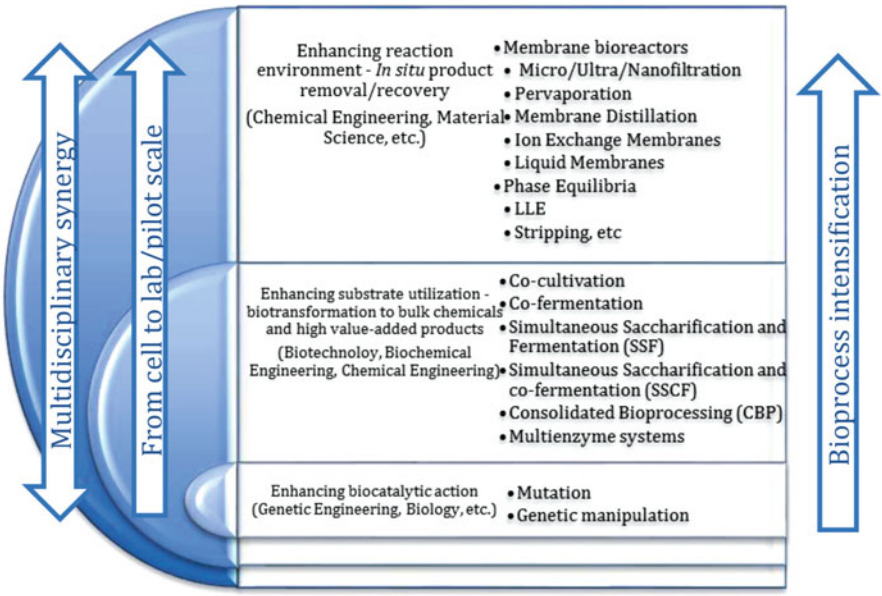

Fig. 7.2 Multidisciplinary interaction for achieving bioprocess intensification

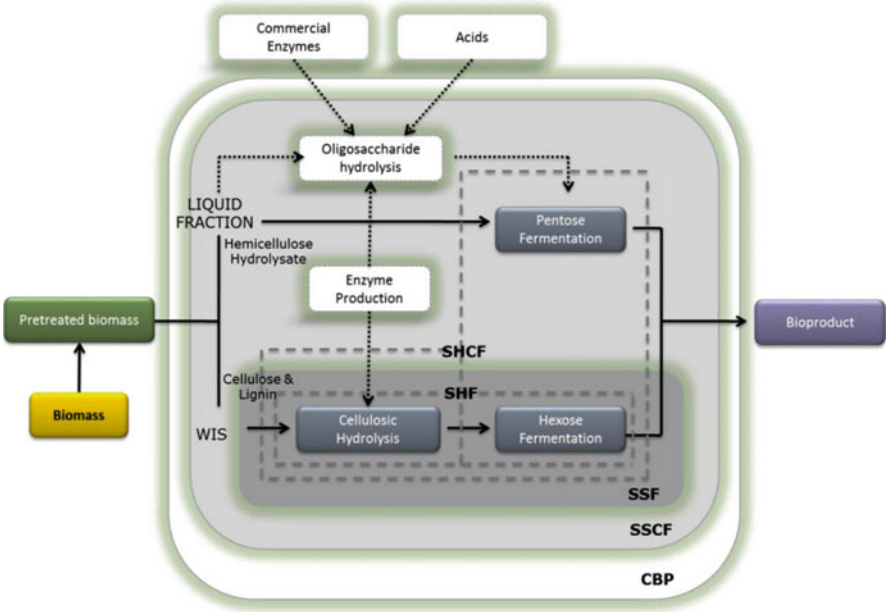

Fig. 7.3 Enhancing the water-insoluble solids (WIS) substrate utilization in one unit operation: simultaneous saccharification and fermentation (SSF) rather than separate hydrolysis and fermentation (SFC); simultaneous saccharification and co-fermentation (SSCF) instead of separate hydrolysis and co-fermentation (SHCF); consolidated bioprocessing (CBP). Adapted from Gírio et al. [3]

biotechnology, chemical engineering, material science), the synergy among them, and the analysis at different levels of abstraction by a multiscale approach (from a cell manipulation up to lab/pilot plant scale). In order to accomplish a bioprocess intensification, the different areas have to interact and also have a feedback for further improvements. For instance, the product yield in a biological system have been enhanced by increasing biocatalytic action (performed by genetic engineers and biologists), resulting in better substrate utilization (executed in the biotechnology and biochemical area) and have also been intensified by improving the reaction environment technologies (especially by chemical engineers).

Starting from the core, there is a trend in bioprocess intensification for metabolizing multiple carbon sources to enhance substrate utilization (such as sugars, alcohols, etc.) by the same microorganism. This issue has originated the exploration of new manners to boost the performance of the biological entity by internal manipulation (i.e., microorganism mutation and genetic manipulation to adapt part of the metabolic route from a different microorganism). Enhancing the biocatalytic action has also allowed getting more robust microorganisms towards inhibitors and reaction conditions, letting to intensify product yield and productivity.

The developments in the microorganism's level have allowed improving the substrate utilization at the lab and reactor level. Figure 7.3 illustrates the different

strategies for intensifying the reactor performance. This includes fermentation of one or more carbon sources with multiple microorganisms in the same bioreactor (the so-called co-cultivation), and the fermentation of two or more carbon sources by one microorganism (the so-called co-fermentation) instead of individual reactors for pentose and hexose in an ethanolic fermentation, for instance. The simultaneous saccharification and fermentation (SSF) has been another intensification approach for substrate conversion where the enzymatic hydrolysis and fermentation of one of the liberated carbon sources from the water-insoluble solids (WIS) is carried out simultaneously, instead of separate hydrolysis and fermentation (SHF). Another approach is the simultaneous saccharification and co-fermentation (SSCF) with the synchronized enzymatic hydrolysis and fermentation of two or more liberated carbon sources in the same unit, rather than separate hydrolysis and co-fermentation (SHCF). There is another manner to intensify the process known as consolidated bioprocessing (CBP) where the microorganism produce the necessary enzymes to liberate the carbon sources which are fermented by the same microorganism in the desired bioproduct [3].

Even though the previous bioprocesses intensification has been achieved specifically from the reaction point of view, there is a top layer approach that includes the modification of the reaction environment. This layer refers to the in situ removal of the products obtained in the biochemical reaction. For certain bioprocesses, some of the products can impair the microorganism(s), thus, decreasing the productivity and product yield. In situ product removal/recovery (ISPR) investigates how to adapt external or internal devices to bioprocesses that permits to in situ remove inhibitors from the reacting vessel (i.e., main or secondary products). There is a special interest on how to integrate bioreactors with membrane separation processes such as micro/ultra/nanofiltration, pervaporation, membrane distillation, ion exchange membranes, and liquid membranes. Beyond those, other approaches have also been considered as the use of the removal/separation based on phase equilibria, such as liquid–liquid equilibria, stripping removal, etc.

In this chapter, bioprocess intensification is explored mainly from the perspective of transforming biomass into chemicals as an integrated solution for bioprocessing. Then, bioprocess intensification is addressed within the biorefinery context. Examples are analyzed at the processing levels depicted as the multidisciplinary interaction of Fig. 7.2.

7.2 Biorefinery: Biomass to Chemicals

Considering that the chief component of oil is carbon, and that many products that currently drive our daily life proceed from crude oil, the interest on biomass harnessing, to begin substituting oil-based products, woke up in a natural way since plants are the most abundant source of renewable carbon.

Following a similar concept on the refining of crude oil, the chain or network of processing steps for obtaining several products from a certain biomass is called

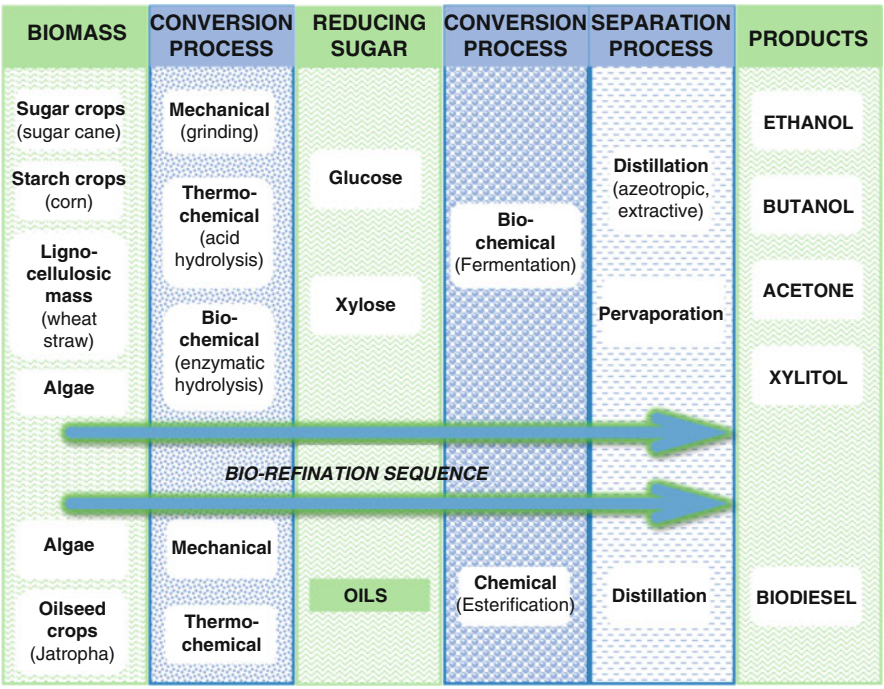

Fig. 7.4 Basic sequence on biomass refination

"biorefinery". The diverse forms of biomass are classified in three main groups: (1G) plants rich in either sugar, starch, or oil (e.g., sugarcane, corn, and soybean); (2G) lignocellulosic material with high content of cellulose but also of lignin and hemicellulose, coming from either agroindustrial waste or forest residues (e.g., wheat straw, grass, wood); and (3G) algae, rich in both oil and cellulose, which can be farmed at large scale. This biomaterial diversity lays a diversity of processing steps that, in turn, comes from the variety of reagents either chemical or biological that can be used, and from the diversity of equipment and process conditions that are implied (see Fig. 7.4). Diverse biorefineries arise depending on the raw biomaterial to be profited and the kind of conversion processes to be implemented (either chemical, biological, or thermochemical), and they are typically classified just according to the type of biomaterial in terms of "generations". First generation biorefinery uses biomaterial of the 1G group, second generation biorefinery harnesses 2G biomass and the third generation biorefinery processes algae. Moreover, it can be devised as different classifications of biorefineries; for example, one that distinguishes biorefineries based on converting reducing sugars from those based on vegetable oils transformation; or another that distinguishes biorefineries with a major part of pure biological conversion steps from those majorly based on chemical and thermochemical processes. In this sense, for example biodiesel is considered as a product of biological nature just because the raw material, but the corresponding processes are chemical and thermochemical.

Going upstream on the flowsheet of any biorefinery that mainly encloses biological processes for converting reducing sugars (see Fig. 7.4), two common steps appear: fermentation and purification. In general terms, through the growth of either yeast, fungi, or bacteria during fermentation, reducing sugars are converted into different products such as ethanol, butanol, acetone, xylitol, lactic acid, succinic acid, etc. [4]. Reducing sugars can also be used as carbon source to grow cells that secrete certain metabolites capable of, for example, working as reducing agents of metals in water treatment [5]. In any cell family, different metabolic pathways can be driven depending on the process conditions, yielding in turn, different products. For example, the growing of the *Saccharomyces cerevisiae*, the most famous of yeasts, must be carried out at anaerobic and warm conditions (~30 °C and pH in-between 4 and 7) to produce ethanol. But, if the process is carried out in aerobic conditions, the pathway to produce more yeast is favored. It is worthwhile to recall that *Saccharomyces cerevisiae*, by itself, has always gotten (and will have) a prominent place in the baking and beverage industry. Fermentation processes inherently imply diluted broths in such a way that purification processes are generally necessary. Typical separation systems are based on distillation but other alternatives as the use of membranes are also drawn on (as will be exposed in a following section); as in the case of bioethanol where either of mentioned processes can be applied. The purification processes become so important because they could account for more than 50 % of the transformation costs in a biorefinery [6].

As it can be noticed, the chief raw material for growing biomass and obtaining products corresponds to reducing sugars, and its source type provides the biorefinery type. Continuing upstream, in a 1G biorefinery of sugarcane, the feedstock is grinded up and passed by hot water or vapor to obtain syrups of reducing sugars of six carbons (fructose); meanwhile, in 1G-of-grains and 3G biorefineries, enzymatic hydrolysis processes to unfold starch up to glucose are involved, and even in 3G biorefineries an additional enzymatic hydrolysis could be included to depolymerize the cellulose contained in the algae wall. Through a more complex kinetics mechanism, in a 2G biorefinery, syrups of reducing sugars of six- and five-carbon sugars (e.g., glucose and xylose) are obtained through a process of enzymatic hydrolysis of cellulose and hemicellulose contained in corresponding pretreated feedstock. Since the complexity provided by the condition in which the starch, or cellulose and hemicellulose are available, the enzymatic reagent typically consists of a blend of specific enzymes. In the case of starch, the enzyme family of amylases is applied in such a way the starch passes through steps of gelatinization, liquefaction, and saccharification at temperatures around 100 and 60 °C. On the other hand, in the hydrolysis of pretreated lignocellulosic mass, the enzyme family of cellulases are applied at temperatures around 50 °C to unfold cellulose and hemicellulose in molecules of lower size up to glucose and five-carbon sugars, respectively.

In 1G-of-grains and 3G biorefineries, the pretreatment of feedstock consists on mechanical and thermal operations to soften the starch content and allow the working of enzymes. Meanwhile, lignocellulosic feedstock in 2G biorefineries must be pretreated more severely to break or eliminate the lignin wall that wraps

the cellulose and hemicellulose content in such a way that enzymes can work. There are many different ways to carry out a pretreatment in 2G biorefineries, whose variety comes from the different chemicals (and even biological reagents) that can be used [7]. For example, applying diluted sulfuric acid at high temperature (around 120 °C) is attractive because is cheaper than any other thermochemical pretreatment. In addition to the breaking or reduction of lignin wall, hemicellulose is hydrolyzed yielding five-carbon sugars. Another example is the thermal pretreatment called "autohydrolysis", that has become attractive because implies minimum amount of chemicals with a similar performance than sulfuric acid application.

Summarizing, the processes of pretreatment, enzymatic hydrolysis, fermentation, and purification are identified as the basic ones to conform a biorefinery. Each of them can be carried out in different ways (considering the diversity on chemicals or biochemicals, equipment, and process conditions) that in turn yield different products. Therefore, many different configurations of biorefination can arise. The above description relates a conventional form of biomass harnessing. However, as it will be discussed in the next sections, each process step by itself is susceptible of performance enhancement, and even the bundling of two of them in just one process equipment aims to a major productivity; say, the Process Intensification on a framework of biorefination for biomass processing is discussed in the following sections.

7.3 Relevance of Process Intensification Within Biotransformations: Bulk Chemicals, Biofuels, and High Value-Added Products

7.3.1 Enzyme-Assisted Transformations: Fine Chemicals Application

The use of enzyme-assisted transformation, also known as biocatalysis, has grown enormously over the last decade and recently it has been extended to a wide range of applications at industrial level due to the exceptional selectivity of enzymatic reactions, combined with transformation under mild reaction conditions. Such characteristics drive the desired reactions to achieve cost-effective processes with the additional advantage of working under a friendly environment. While the current majority of biocatalytic reactions are implemented in the pharmaceutical industry, they are increasingly finding value at various points in the value chain.

In biorefinery, enzymes can be used in the early stages of the process in order to transform the low cost feedstock into a high cost feedstock (see Figs. 7.4 and 7.5). For example, the enzymes can be used for the saccharification of biomass in which a cocktail of different enzymes breaks down big carbohydrate molecules such as starch, hemicellulose, and cellulose into fermentable sugars. Likewise in the

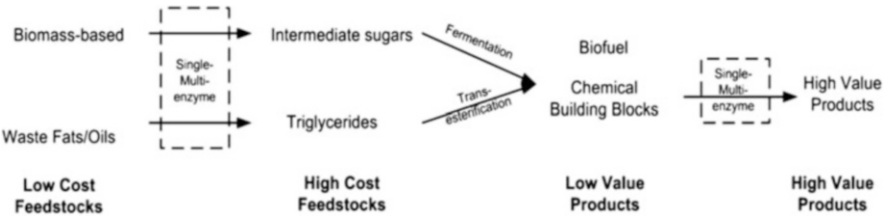

Fig. 7.5 Single or multienzyme steps applied in biorefinery

production of biodiesel, lipases enable unpurified triglycerides to be used as a starting point for the transesterification and thus the production of biodiesel and glycerol (as a co-product). The utilization of a low-cost feedstock drives to a lower costs for the process. Nevertheless, a real process sustainability can only be achieved when the production of biofuels is integrated in an efficient way with the production of other value-added products such as chemicals, fine chemicals, materials, food, feed, and pharmaceuticals. During the biorefinery steps, important building blocks are produced such as 1,4-Diacids, 2,5-Furan dicarboxylic acid, glycerol, sorbitol among others, and those are the main intermediates for the production of high-value products such as solvents, fibers, polymers, pharmaceuticals, food, and personal care. In these cases, another set of enzymatic reactions must be added to the chain in order to reach the desired product in an efficient manner [8].

Although enzymatic transformations can be found in many industrial sectors (e.g., food, fine chemicals, and pharmaceuticals), it remains the case that the vast majority of these processes use merely a single enzymatic step. Frequently the mild conditions of the enzyme reaction are in contrast to the other reactions preceding and following the enzymatic step. Hence, some changes to the process conditions may be required in order to adapt the optimal enzymatic operation within an existing process. However, this impact can be minimized when using two or more adjacent enzymatic steps in the process, since most enzymes operate under similar conditions. In the following section, such multienzymatic processes will be discussed as a superb example of PI in biotechnology.

7.3.2 Multienzyme Processes

A multienzyme process is characterized by the combination of two or more adjacent enzymes that react in a specific pathway to a given product of interest, via a cascade, parallel or network scheme (see Fig. 7.6). In nature, such pathways are characteristic of cells and are essential for the control of energy and redox inside microorganisms. For industrial application, the use of such pathways outside the cell is more useful since perfect control of each enzyme activity can be assured. Hence in vitro enzyme pathways studies help providing useful and controllable schemes to obtain certain products of interest.

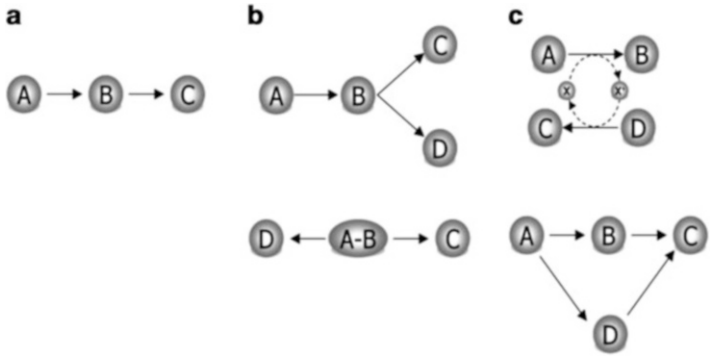

Fig. 7.6 Basic multienzyme reactions: (**a**) cascade, (**b**) parallel, and (**c**) network. Adapted from [9]

In the cell, such schemes are the way nature achieves process intensification and by analogy therefore in vitro applications also provide opportunities to develop PI technologies. Unfortunately, bioprocesses, via microorganisms or enzymatic, can experience physical, chemical, and/or thermodynamic limitations which can be caused by substrate/product inhibitions, unfavorable equilibrium constants, multiple phases, and co-factor dependency. The first most common way of overcoming these limitations is by applying controlled feeding processes, in situ product removal technologies, or/and protein engineering. Such process control strategies and biocatalyst modifications can give significant improvements to the processes in terms of higher yield, product concentration, and space–time yield at lower catalyst usage. The second way can be achieved via integration of two or more reaction steps into a single reaction unit. In this case, a PI is generated since the separation of intermediate products is no longer required. This combination of enzymes in a one-post reactor is perhaps the most common definition of a multienzyme process, but in all cases it represents an example of process intensification. Depending on the multienzyme arrangements, the reaction can be classified as cascade, parallel or network (see Fig. 7.6), and the spatial and temporal integration or separation can be envisaged depending upon the constraints in process integration. Two pharmaceutical applications are illustrated later in this section i.e., production of lactobionic acid and synthesis of iminosugar D-fagomine.

7.3.3 Implementation of Multienzyme-Based PI

The utilization of several enzymes gives the opportunity to use different reactor configurations. This depends on the characteristic of each individual enzyme and the most feasible and economical format (e.g., isolated, immobilized, or contained in a cell) in order to reach the required cost-effective process. Bioreactor can be designed applying the enzymes as crude or purified versions as well as soluble and

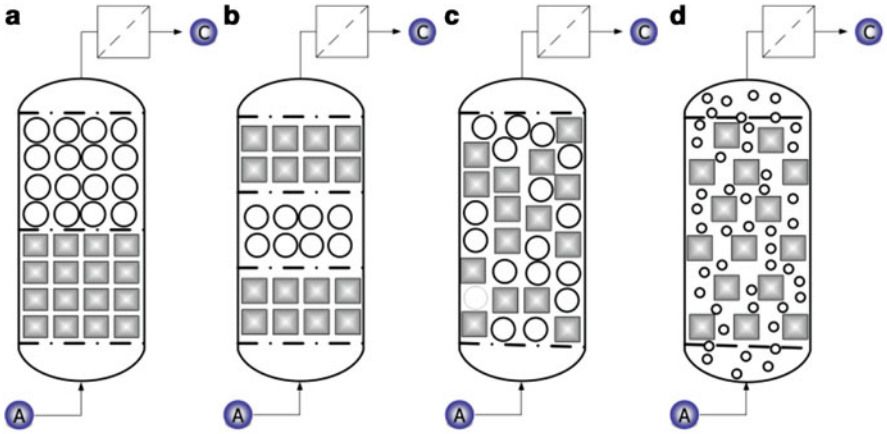

Fig. 7.7 Multienzyme process implementations using immobilized enzyme in a packed bed reactor. (**a**) Two sequential beds, (**b**) alternate beds, (**c**) mixed enzyme bed, and (**d**) one enzyme bed with a soluble enzyme. Adapted from [9]

immobilized (see Fig. 7.7). The use of immobilized enzymes can allow separation and compartmentalization, both of which can be useful concepts to exploit with multienzyme processes. Immobilization can also allow recycle of enzymes, hence improving biocatalyst yield (product obtained per amount of enzyme added). Where enzymes have matching stability, immobilization of multiple enzymes on a single support can also be achieved [10]. Aside from immobilization on particles (hydrophobic or hydrophilic), immobilization on membranes can also be used, where enzymes can be impregnated in the membrane or adsorbed on the surface.

The application of multienzyme processes has been mostly achieved in the production of higher value products such as pharmaceuticals and fine chemicals, where the specificity and region-selectivity of a chiral structure may play an important role in the final application. For example, an interesting case occurs during the synthesis of functionalized sugars from glycerol (a biodiesel co-product) that can be used as a raw material for the production of phosphate esters. These products are used as valuable intermediates in the synthesis of iminosugars which are monosaccharide-analogues capable of a specific inhibition of glycosidases, and are currently used therapeutically in several human disorders [11]. The synthesis of the iminosugar D-fagomine, from glycerol and a variety of aldehydes, has been proven by using a four-enzyme one-pot cascade reaction in which an acid phosphatase, glycerol-3-phosphate oxidase, catalase, and aldolase have been combined in a simple packed bed reactor configuration [12]. This work highlights the potential value of using enzymes in cascade reactions to selectively form complex products that by previous traditional organic chemistry could only be obtained via repeated isolation and purification of intermediates. Another multienzyme process that can be applied in biorefinery is the production of lactobionic acid

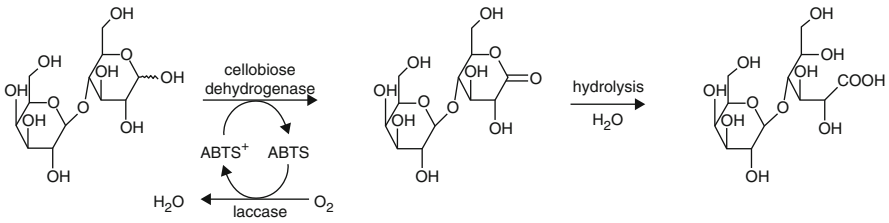

Fig. 7.8 Bi-enzymatic production of lactobionic acid from lactose

which is obtained by the enzymatic oxidation of lactose (see Fig. 7.8). Lactobionic acid is used as high-value product in pharmaceutical and food technology applications. The production of lactobionic acid has been achieved by the combination of two enzymes in one-pot in which cellobiose dehydrogenase (CDH) and laccase, in the presence of a redox mediator, has produced a successful multienzyme application [13].

7.4 Co-cultivation of Carbon Sources

The synthesis of bioproducts via single fermentation processes consists of the conversion of one or more renewable carbon sources into specific products by the action of one microorganism. The search for improving the production of biobased chemicals have opened new opportunities such as the co-cultivation, where two or more different microorganisms as a consortia are employed during fermentation. The intensification by the co-culture fermentation occurs by the synergistic exploitation of the metabolic pathways of the involved strains. Here the cell growth of a given strain may be enhanced or inhibited by the activities of other microorganisms present in the medium. Enhanced growth rate can also be observed when the enzymatic activity of one strain in the co-culture supplies the substrate required by another strain. Another positive interaction is the reduction of the available oxygen by aerobic microbes, creating an anaerobic environment that promotes the growth of anaerobic strains. Similar phenomena appear with the formation of primary and secondary metabolites [14].

7.4.1 Use of Co-cultivation for Different Products

The co-cultivation have been applied in different areas, for example, pharmaceutical products manufacture [15], biofuels, food additives, enzymes production, bulk chemical, fine chemicals, microbial fuel cells, among others. In the following, the most famous applications are described.

7.4.1.1 Ethanol Production

The production of bioethanol from lignocellulosic material involves various steps where the implementation of co-cultivation could enhance the process performance. He et al. [16] proposed a co-cultivation employing a *Clostridium thermocellum* and *Thermoanaerobacter* spp. and found an improvement by 194–440 % in the production by combining different microorganisms rather than using only a *C. thermocellum*. The combination of co-cultivation and enhanced operating policies has also shown larger improvements in the production of ethanol by at least two orders of magnitude in the concentration. The advantage of using different microorganisms from the frequently used (e.g., *Saccharomyces cerevisiae*, *Zymomonas mobilis*, etc.) for ethanol production is that more extreme conditions can be used. For example, higher temperatures at the fermentation process, which facilitates in situ product removal and recovery, reduction in cooling costs and least chance of contamination [17]. A recent study has evaluated the combination of as *S. cerevisiae* and *Z. mobilis* ATCC31825 [18] varying the ratio of microorganisms concentration in order to metabolize pentose and hexose molecules from pretreated sweet sorghum bagasse; the *Z. mobilis* to *C. cerevisiae* ratio of 5:10 g/L allowed to reach a yield of 0.5 g-ethanol/g-reducing sugars. Ethanol production has also been carried out by using *S. cerevisiae* ITV-01 and *Scheffersomyces stipitis* NRRL Y-7124 strains, by fermenting hydrolyzed bagasse residues and sugarcane molasses from cane sugar production process [19]; the yield in the co-cultivation reach up to 0.41 g-ethanol/g-sugars, compared to 0.38 and 0.37 g-ethanol/g-sugars when only using *S. stipitis* NRRR Y-7124 and *S. cerevisiae* ITV-01 strains, respectively.

7.4.1.2 Acetone–Butanol–Ethanol Production

The ABE process (acetone, butanol, and ethanol production) have once again become important, capturing attention from the research community due to the variety of products obtained in one single fermentation pot; specially for the butanol production which has several advantages as a biofuel [20, 21]. The aims for improving the yields, conversion of intermediate products, and selectivity for specific products have also made possible to explore the co-culture fermentation for ABE process. The *Clostridium* strains are the most employed microorganisms to produce acetone, butanol, and ethanol, and the combination with different strains to improve sugars conversion has also been evaluated. The co-culture fermentation employing *Clostridium beijerinckii* and *Clostridium cellulovorans* was tested using alkali-extracted deshelled corn cobs as substrate. It was possible to determine the mechanism of cooperation and competition between the two strains to increase the ABE production, thereby opening a pathway to optimize the artificial symbiosis between the strains [22]. Li et al. [23] proposed the butanol production employing *C. beijirinckii* and *Clostridium tyrobutyricum* in a free cell and immobilized cell

reactors, observing that the co-culture fermentation enhanced significantly the butanol production over the fermentation in a single culture. Yao and Nokes [24] presented the co-cultivation and strategy for intermittent flushing of the fermentation media for the cellulolytic and/or solventogenic phases using *Clostridium thermocellum* and *C. beijerinckii*; from the operating point of view, this study has tested five operating strategies where it was observed that cycling through the cellulolytic phase with or without the re-inoculation of *C. thermocellum* improved glucose availability for the following solventogenic phase, increasing the solvent accumulation.

7.4.1.3 Hydrogen Production

Hydrogen has become another promising biofuel. Biobased hydrogen is naturally produced through a dark fermentation, which is a series of biochemical reaction employing a microorganism consortia in the absence of light. Thereby co-culture fermentation is the most common way to carry out the hydrogen production. This process comprises the hydrolysis of the polysaccharides, acidogenesis to break down the molecules into acetic acid, hydrogen and carbon dioxide, and finally the acetogenesis which also produces hydrogen and carbon dioxide. One of the main challenges during hydrogen production is avoiding methane production, thus the use of a suitable co-culture media during the fermentation has a paramount importance. Anaerobic microbes employed in the several steps during hydrogen production are diverse including *Clostridium*, *Enterobacter,* and *Escherichia* [14]. The combination of such microorganisms has been tested for example by the use of *Clostridium butyricum* and *Escherichia coli*, showing a more efficient utilization of glucose than with each single microorganism [25]. Another approach using *Clostridium thermocellum* and *Clostridium themosaccharolyticum* was evaluated by Li and Lui [26]. The authors tried different operating scenarios by changing the culture feeding policy, operating under continuous or batch conditions, and comparing fermentation performance when using each single culture against the co-culture. Under the best condition co-culture fermentation showed 94 % higher hydrogen production. Other authors have evaluated the interaction of various strains. For instance Masset et al. [27] used four different *Clostridium* cultures and three different co-culture systems in order to determine the best conditions for hydrogen production. The system with *C. butyricum* CWBI1009 and *Clostridium pasteurianum* DSM525 showed the highest hydrogen production among the three synergies.

Another interesting raw material for hydrogen production is the glycerol obtained from biodiesel production. In an interesting review, Sarma et al. [28] mentioned several studies employing different co-culture systems to produce hydrogen from glycerol via fermentation process.

7.4.1.4 Other Important Products in the Market

There are other bioproducts with relevant importance as a bulk or fine chemicals. The lactic acid is one of those important compounds, which has been produced using co-culture fermentation. Some studies have employed the sugars derived from lignocellulosic raw material, finding the advantage of using the intensified fermentation by the use of two cultures rather than a single one [29]. Even that one of the obstacles is the carbon catabolite repression existing using renewable resources as raw material [30], the results have shown in general an intensification in the lactic acid yield using co-cultivation. Enzymes production has also been accomplished via co-cultivation, in order to increase the productivity and performance in certain biological systems. For instance, the β-glucosidase is an important enzyme in the production chain of lignocellulosic derivatives, and the co-culture have been also applied to this end [31]. Xylanases and laccases enzymes are other enzymes with high importance in the industry, for example, in the paper production and potentially in the biofuels industry since are useful for lignin and xylan degradation; those enzymes have similarly been produced using co-culture fermentation in order to improve the production [32]. Citric acid from lignocellulosic residues have likewise been also produced using co-cultures (Y*arrowia lipolytica* SWJ-1b and Immobilized *Trichoderma reesei* Mycelium), and this has resulted in an increased production of product by 91 % [33].

The scope of the co-culture implementation is wide and it is still necessary to analyse the combination of other microorganisms, relying on the previous analysis of the metabolic pathways and possible drawbacks by the present of certain inhibitors of cultures. This could be achieved with the creation of one multidisciplinary team including specialist from the biotechnology, genetic and engineering areas.

7.5 Co-fermentation of Renewable Carbon Sources

The main objective of fermentation and one of the core sections in a bioprocessing system, is the transformation of a carbon source into a specific product (such as alcohols, organic acids, gases, etc.) by the use of a microorganism. The well-known process of metabolizing glucose to produce ethanol and carbon dioxide by using baker's yeast *Saccharomyces cerevisiae* is an example of such approach. However, some microorganisms (including the mentioned *S. cerevisiae*) are not able to ferment other types of sugars (e.g., xylose), reducing the opportunity of increasing ethanol productivity when combining different types of sugars in the same unit. This bottleneck was identified many years ago and since the beginning of the 80s, scientists have been working on manipulating and developing a genetically engineered *Saccharomyces* yeast capable of metabolizing xylose. In 1993 some researchers achieved the development of genetically engineered *Saccharomyces* yeasts, which can ferment both glucose and xylose to ethanol [34]. This was carefully accomplished by redesigning the yeast metabolic pathway, cloning three

xylose-metabolizing genes pathways for fermenting xylose to ethanol. Thereby, it was possible obtaining a microorganism capable of metabolizing a compound that was impossible to transform before. During the genetic manipulation techniques, the metabolic flux is altered by blocking undesirable pathways, typically via homologous recombination-mediated "gene knockout" and/or by the overexpression of genes associated with desirable pathways [35]. The co-fermentation of glucose and xylose by the same microorganisms was a breakthrough from the biotechnology point of view, which permitted the intensification of the ethanol production.

Figure 7.9 shows details of the two different pathways available in nature for the conversion of pentose: (1) oxidoreductase-based pathways (type I pathways) found in most fungi, and (2) isomerase-based pathways (the type II pathway) found in most bacteria. In major enzymes, the genes encoding the pentose pyrophosphate pathway are XR (xylose reductase), XDH (NAD+-dependent xylitol dehydrogenase), and XK (xylulose kinase). However, these genes are expressed at such a low level that the xylose utilization by *S. cerevisiae* is not allowed, while XK is the rate-limiting step of the pentose phosphate pathway. By using DNA technologies, the genes linked to the pentose metabolism from bacteria and other fungi have been inserted into the genome of *S. cerevisiae* for the utilization of sugars from hemicellulose hydrolysates [36].

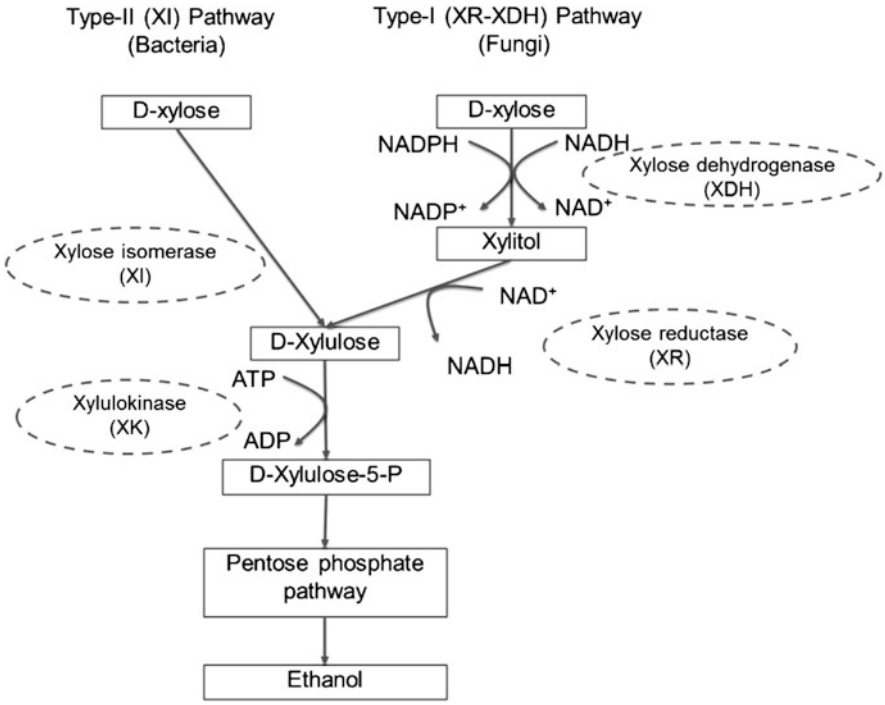

Fig. 7.9 Outline of the xylose metabolism pathway in fungi and bacteria as previously reported. Adapted from Laluce et al. [36]

The demand of some specific products, such as biofuels or other high value-added products has triggered out the search of new manners to produce the same product using different raw materials simultaneously in the same pot. The second generation bioprocesses employing lignocellulosic residues release a list of several compounds at the pretreatment section, for example, arabinose, xylose, galactose, lignin, among others; the mixture of available sugars exhibits a huge opportunity for improving in the most "simple manner", the production of certain compound metabolizing two (or more) different sugars by a genetically engineered microorganism under an intensified process scheme in a co-fermentation.

7.5.1 Co-fermentation Applied for Ethanol Production

The production of ethanol in a biobased process has captured the attention of researchers in the intensification area. Many *Saccharomyces* strains have been subject to genetic manipulation techniques, essentially for the simultaneous glucose and xylose fermentation, working with hypothetical mixtures and also with different lignocellulosic residues. The identification of glucose and xylose as raw materials relies on the high concentration of these sugars after the pretreatment or enzymatic hydrolysis. The glucose and xylose compounds could theoretically reach in average up to 650 g/kg before entering to the fermenter, rather than only glucose concentration (up to 415 g/kg) for a simple fermentation. Thus, the amount of carbon source is 1.6 times higher thereby ethanol production as well. The last permits to visualize the potential improvement using a co-fermentation technology.

The cellobiose molecule is an intermediate bioproduct at the pretreatment and enzymatic hydrolysis stage [37]. Cellobiose is converted into glucose by the β-glucosidase enzyme but due to the variety of factors during the enzymatic hydrolysis reaction (e.g., enzyme adsorption, product inhibition, etc.), cellobiose is not completely converted. The efforts to overcome this issue have also promoted the genetically engineered modification of certain microorganisms. For example some strains of *Escherichia coli* have been modified to metabolize glucose and cellobiose [38]. Also *S. cerevisiae* strains were genetically modified to convert xylose and cellobiose into ethanol in an intensified process [39].

7.5.2 Co-fermentation for Other Biofuels and High Value-Added Products

Besides ethanol, there are several products that are also produced by using co-fermentation such as hydrogen, lactic acid, lipids, food preservatives, basic biochemical products, etc.

The production of hydrogen through a biobased process relies on a dark fermentation, where the most common substrates are sugars that could also be obtained from lignocellulosic residue, carbon sources from livestock waste, and organic fraction of municipal waste. Even though the research for enhancing biohydrogen production relies mostly on the bioreactor operation, the search for the appropriate combination of carbon sources and the use of diverse microorganisms individually or as a consortia have been studied [40]. Different pure cultures that have been intensively investigated include strict anaerobic genera (*Clostridia*, metanogenic bacteria, archaea), facultative anaerobic genera (*E. coli*, *Enterobacter*, *Citrobacter*), and also aerobic genera (*Alcaligenes*, *Bacillus*) [41]. Biohydrogen production intensification has also included the combination of several of these cultures, aiming to transform combined substrates or sugars in a co-fermentation.

Lipids production from sugars by using microorganism has become important, since triglycerides can be subsequently used as raw material for biofuels production. Unfortunately, most of the microorganisms are not naturally able to convert pentose and hexose simultaneously, thus, the search for improving the performance of microorganisms has been directed towards their bioengineering manipulation [42]. Glucose and xylose are the most used carbon sources to produce lipids when employing genetically engineered microorganisms, such as, *E. coli* [43], *S. cerevisiae* [44], *Rhodococcus opacus* [42], among others.

Other bioproducts with high relevance in the market are starting to be produced employing intensified processes with genetically modified microorganisms. Lactic acid, which is used for biobased polymers production, is usually obtained by glucose fermentation; however recent investigations have focused on the fermentation of different sugars such as xylose [45]. The new studies, also including modified microorganisms, aim to carry out co-fermentation of different feedstocks containing hexoses and pentoses [46]. Other bioproducts obtained by co-fermentation process that have been recently studied include propionic acid from glucose and glycerol [47], butanodiol from glucose and xylose [48], and ε-poly-L-lysine from glucose and glycerol [49].

7.5.3 Glucose, Xylose, and Arabinose as Raw Material in Co-fermentation

Due to the low concentration of other liberated sugars such as mannose, galactose, etc. during hydrolysis of polysaccharides, few studies on the exploitation of such sugars with modified microorganisms have been reported. The simultaneous fermentation of xylose and glucose has been carried out with arabinose that could be considered as the third sugar with the higher concentration after the pretreatment. The intensified conversion of arabinose + glucose + xylose into ethanol has been achieved by modification of *Zymomonas mobilis* [50], and *P. stipitis* [51]. Hydrogen production has been accomplished by simultaneous conversion of

arabinose and glucose using a thermophilic anaerobic mixed culture [52], and by conversion of glucose, xylose, and arabinose using a consortium of *E. coli* strains [53].

Beside the advantage of co-fermentation, the intensification in the performance of the microorganisms should not only cover the modification of the metabolic pathways to ferment new compounds. The inhibition effect is another bottleneck that should be tackled in order to improve the performance of a microorganism. The robustness of the microorganism to support higher temperatures, resistance to toxic compounds concentrations that inhibit and reduce the capability of the microorganism to work properly.

7.6 Simultaneous Saccharification and Co-fermentation

The SSCF consists of one unit operation where the liberation of carbon sources such as hexose and pentose sugars is performed by the action of specific enzymes, and the subsequent sugars fermentation is carried out at the same time by microorganisms. Figure 7.10 represents a SSCF unit where the process is performed in one reactor rather than two, reducing the reacting volume and the operating costs, and also improving the yield of desired products.

The SSCF have been mostly analyzed for the ethanol production from glucose and xylose employing mainly genetically engineered microorganisms able to perform a co-fermentation process. The modified microorganisms to produce ethanol have allowed to use diverse lignocellulosic sources such as hardwoods [55], sugarcane bagasse [56], Kraft mill sludge [57], corn stover [58], wheat straw [59], among others. A validated mathematical model to describe the SSCF process was already presented by Morales-Rodriguez et al. [54], which allowed to perform a process design, operation, and control [60, 61].

The SSCF have also been implemented to produce lactic acid mostly employing modified microorganisms of *Bacillus*, *Aspergillus*, and *Lactobacillus* [62–

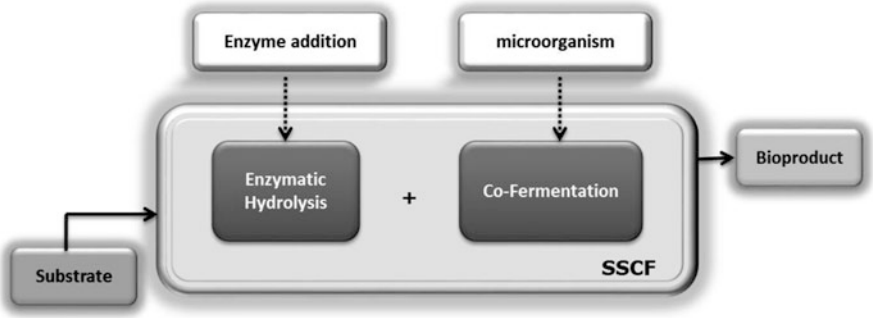

Fig. 7.10 Simultaneous saccharification and co-fermentation process. Adapted from Morales-Rodriguez et al. [54]

64]. The production of lactic acid via SSCF is still on development, thus, it is necessary to try implementing the SSCF for other feedstock, process conditions, other manipulated strains, etc.

The application of SSCF has not achieved maturity for other systems as it has been the case for SSF or bioethanol production. There are still potential processes that can be subject of intensification by performing saccharification and fermentation in the same unit, for example, in the production of acetone–butanol–ethanol, xylitol, citric acid, etc. It is important to highlight that the use of SSCF requires microorganisms capable of metabolizing the two or more carbon sources released by the enzymes.

7.7 Consolidated Bioprocessing

The CBP has been applied specially in biofuels production from lignocellulosic biomass. The CBP have been conceived as the unit operation, where the microorganism(s) is able to produce, in situ, the required enzymes for the lignocellulose hydrolysis, in which, the sugars are obtained and fermented by the same microorganism(s). These are all the required steps to get a bioproduct performed in one pot (Fig. 7.11). By avoiding enzyme addition, utility consumptions are reduced (water and cooling water) allowing reduction in the operating cost. Most likely this is the ultimate configuration for low-cost hydrolysis and fermentation of lignocellulosic biomass [65].

Olson et al. [66] argued that CBP-enabling microbes must be able to both solubilize a practical biomass substrate, and produce desired products at high yield under industrial conditions. Unfortunately, microorganisms with those characteristics have not been found in nature and it is necessary to employ genetic engineering. For example, the Trichoderma reesei Rut-C30 strain is able to produce xylanases and cellulases simultaneously from plasma pretreated wheat straw, which could liberate xylose and cellulose from the xylan and cellulose of the lignocellulosic material, but the fungi would not be able to convert those sugars into a specific bioproduct [67].

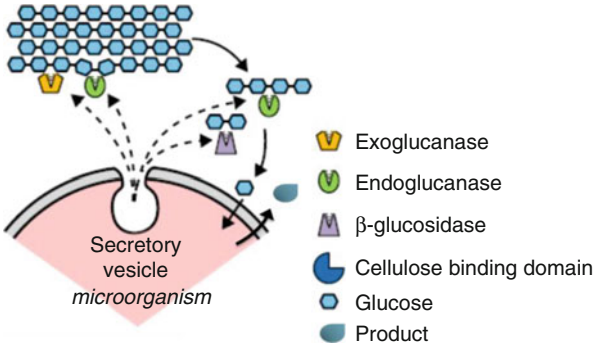

Fig. 7.11 CBP by production of secreted enzymes that allow random free access to insoluble cellulosic material. Adapted from [68]

den Haan et al. [68] have classified the approaches to achieve CBP in two categories relying on the capability of the microorganism: (1) cellulases production by naturally fermentative microorganisms (e.g., yeasts and bacteria), and (2) engineering cellulases producers to produce ethanol or other desirable products (e.g., fungi). Regarding the first category, the genetic modification for constructing *Saccharomyces cerevisiae* strains include three strategies related with the mechanisms in the CPB: (a) production of secreted enzymes that allow random free access to insoluble cellulosic material (see Fig. 7.11), (b) binding of cellulases to the cell wall and (c) tethering of cellulases to the cell wall in mini-cellulosomes; the final end of the genetic modification includes increasing the production of the necessary total enzymes by the host microorganism improving both total expression and specific activity of the enzyme system. The genetic engineering application has not been exclusive of *S. cerevisiae*, because some non-cellulolytic bacteria (such as, *Clostridium thermocellum*, *Clostridium cellulolyticum*, *Zymomonas mobilis*, *Escherichia coli*, and *Bacillus subtilis*) have also been modified in order to include cellulases production. Regarding the second category, there have been some assessments using modified fungi to achieve a CBP approach, for example, the use of *Fusarium oxysporum* [69], *Aspergillus*, *Trichoderma*, and *Rhizopus strains* [70].

The use of CBP has been employed for ethanol production, and also there are some studies that have focused on butanol production employing model substrates (for example, avicel, filter paper, cellulose MN 301, etc.) as well as lignocellulosic biomass (such as, hardwood, grass, rice straw, corn stover, etc.) [66].

The current state of the art illustrates some reached goals, but in order to succeed at industrial scale employing CBP, there are still challenges to overcome. First of all, from the microorganism's point of view, it is however necessary to improve the tailor-made genetic modification of the organisms, for instance, it is still required to improve the proportional enzyme production and carbon source consumption by the microorganism aiming to obtain higher product yield and titer, promote the fermentation of more than one substrate, enhance the behavior of the microorganisms for recombinant fermentation, among others. Another opportunity for process intensification that has not been yet considered is the inclusion of simultaneous removal/recovery of the substances during the fermentation, in order to accomplish a better environment for the microorganism and increasing yield of the desired products.

7.8 Simultaneous Reaction and Purification processes: In Situ Product Removal/Recovery

It has been recognized that downstream processing is both technical and economically challenging, especially in bioprocesses (e.g., complex mixtures, dilute solutions, sensible microorganisms and enzymes, time variant systems, among others). In

order to improve the bioprocesses cost-effectiveness especially in batch operation, process intensification framework proposes to integrate the reaction and separation stages in the same processing unit [71]. This technological trend has been called ISPR. There is a small difference between product removal and product recovery. Product removal is usually referred to any product that is removed from the reactor (i.e., main or secondary components that can have negative effect such as inhibition, degradation, or transformation into unwanted substances). On the other hand, product recovery is used when the main product is removed.

ISPR has been extensively investigated since early 1990s, and several hundreds of publications have been referenced in relevant reviews [71–74]. The processes have been classified from the technology and, more recently, from the product point of view. The ISPR technologies include a diverse gamma of hybrid reactive processes with extraction, adsorption, ion-exchange, high-gradient magnetic field fishing, membrane-based, among other units. Recently, there is an increasing interest in hybrid membrane bioreactors mainly for biofuels and organic acids production; therefore, recent advances on those topics are analyzed.

7.8.1 Hybrid Membrane Bioreactors

Membrane separation processes are not new technologies, they have been under development since XVII century. Nevertheless, it took many years to scale-up from laboratory to industrial level due to high capital and operating costs, limited selectivity, low productivity, and the poor reliability of the membranes. It was only until 1960s when real commercial applications drove substantial improvements in membrane technology, mainly with pressure and electrically driven separation processes [75]. Relevant breakthroughs in membranes manufacture, process design, and system operation have boosted the application of other membrane-based technologies at industrial level, and lately within the process intensification framework. Taking into account the membrane technology development trend, it can be said that these separation processes are still evolving where others have reached their technological limits.

An overview of the membrane technology applications in biotechnology has been summarized in Fig. 7.12. It can be seen that pressure driven membrane separation processes are applied in broad spectra of industrial biotransformations at different stages. Notice that specific applications have not been included such as dialysis, membrane chromatography, gas separation, liquid membranes, and electrophoresis.

In most of the revised membrane technology applications, they have shown formidable performance at laboratory and pilot plant scale in terms of some of the following indexes: selectivity, products purity, increasing productivity, minimizing/eliminating chemical usage, working at more friendly conditions for microorganism, reducing plant and carbon footprint, enabling continuous operation, reducing energy consumption, favoring process safety, among others [76–78].

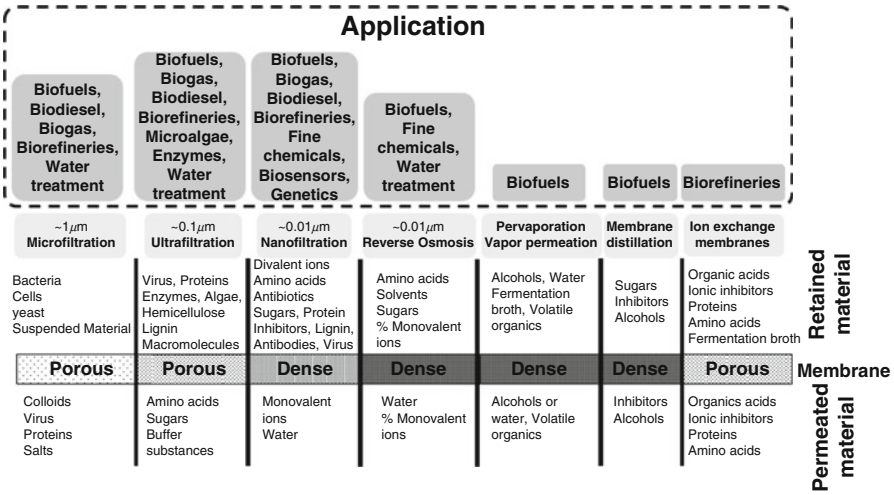

Fig. 7.12 Overview of membrane-based separation technology applications in bioprocesses

However, not all the applications stated in Fig. 7.12 can be considered as intensified processes. The most relevant applications of membrane-based process intensification lie within the so-called hybrid membrane bioreactors. Hybrid technologies can combine membrane-based in situ product removal and cultures/enzymes retention, if required, in order to: increase productivities, improve product yield, ensure sterility, enable continuous operation at higher dilution rates, favors the control of the cultivation parameters, decrease the energy requirements, enhance the product separation, and ease scalability [79].

Two different configurations for membrane bioreactors are reported in literature: submerged membranes and external loop systems. Those have been nicely reviewed recently [74]. Representation of both configurations is shown in Fig. 7.13. Submerged membranes are advantageous since they minimize the induced shear stress, reduce energy consumption, facilitate sterilization, and reduce the risk of contamination. On the other hand, external membranes provide a better fouling control at the expense of extra equipment volume and energy requirements, and they are also easy to replace. When using recirculation loops, cells as subject to extra shear stress that might inhibit metabolite production and cell viability, which is particularly critical in animal cells. However, it has been pointed out that membrane bioreactors provide such as protective environment for cell growth that compensates the induced stress [80].

Several efforts had been made in order to reduce the adverse influence of the membrane fouling, especially in submerged membranes. Mechanisms have been proposed as backflushing, backshoking, agitation, aeration, membrane vibration, cross-flow operation, rotating membranes, turbulence enhancers, and ultrasonic waves. Depending on the application, submerged or external loop membranes are appropriate. Submerged membranes have great potential be used for high-value

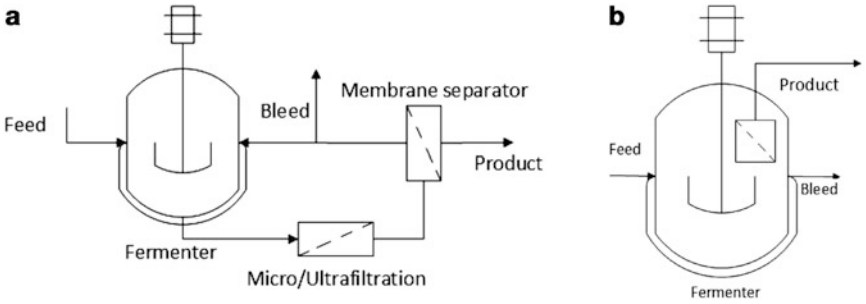

Fig. 7.13 Scheme of external loop and submerged membrane configuration for hybrid membrane bioreactors. (**a**) External loop, (**b**) submerged membrane

products such as antibodies or enzyme production. On the other hand, external loop membranes are advantageous for commodities production due to the large membrane area required. Specific applications impose challenges to overcome in order to allow hybrid membrane bioreactors to be used extensively in the future. Some relevant challenges are discussed in the following sections.

7.8.2 Hybrid Membrane Bioreactor for Cell/Enzyme Retention and Proteins/Enzymes Separation

Micro/Ultrafiltration membranes have been used within hybrid membrane bioreactors for different purposes. Filtration membranes have the ability to retain components depending on their size, affinity with the membrane material, and operational conditions. Microfiltration (MF) membranes have been used to retain microorganisms such as cells, bacteria, and yeast in the bioreactor and/or to clarify the fermentation broth before another separation stage (see Fig. 7.13a). Clarification of the fermentation broth considerably reduces the fouling potential of a subsequent membrane module. Simultaneously, cell retention allows operation at higher cell densities, which promotes a better substrate utilization. Cell retention is particularly interesting during continuous operation since it allows operating at dilution rates higher than the specific growth rate. However, cell retention using membranes does not necessarily imply an improved culture viability. For aerobic cultivations, it is more difficult to achieve high cell-density operation since the oxygen transport rate might become the limiting growth factor. If that is the case, the bioreactor design must account for the limitation and enhance oxygen transport rate, for instance, through jet diffusors and static mixers [81, 82]. Additionally, a purge or bleeding must be done in order to avoid accumulation of unviable cells in the reactor. This task is not necessarily straightforward from the monitoring and control point of view [83]. Despite those limitations, most of the reviewed contributions show an improved performance compared to conventional fermentation [74].

For cell retention in bioreactors, ceramic, polymeric, glass and metallic membranes have been studied. Among the investigated materials, ceramic membranes are attractive due to its superior performance, thermal/mechanical/chemical stability and well distributed pore sizes (i.e., compared to polymeric membranes). However, ceramic membranes are still expensive compared to polymeric. Membrane fluxes up to 1–70 LMH ($L/m^2/h$) have been achieved with microfiltration submerged membranes for *Saccharomyces* retention. An external loop had achieved up to 30–80 LMH in comparable systems and concentrations [74].

Ultrafiltration (UF) membranes can retain suspended material and colloids. This ability makes them interesting to apply for enzyme and protein retention in bioreactors. UF membranes are used to retain free enzymes or, more conveniently, be the support for the enzyme immobilization [10]. When membranes are utilized to retain free enzymes, those tend to adsorb on the membrane surface due to affinity with the material. As a consequence, the adsorption can lead to the enzyme activity reduction. The operational conditions need to be carefully selected in order to minimize membrane fouling and enzyme activity decay [84].

Due to this limitation, enzyme immobilization has been recently investigated. The available commercial membranes require modifications to make them suitable for enzyme immobilization [85]. Results have shown the benefits of using ultrafiltration membranes as support, including total retention of the enzymes and a trade-off between activity lost and system stability after the immobilization process [86–88]. This compromise suggests that more research is required to find the appropriate immobilization mechanisms in order to improve the biocatalytic performance before this technology can be scaled up.

From the modelling perspective, efforts have been focused on membrane bioreactors for wastewater treatment using semi-empirical fouling models within resistance in series approach. Due to the models nature, they have reduced predictive power and their application is limited [89]. Therefore, pilot plant experiments are mandatory in order to scale-up the processes [16].

7.8.3 Hybrid Membrane Bioreactors for Biofuels Production

Besides the fermentation challenges during the biofuels production, another big limitation is the separation stage to recover and purify the key component from the dilute mixture, since it is one of the most energy consuming stages. Conventional bioethanol production using fermentation and distillation is energetically deficient because 5.99 kJ are invested to produce 4.19 kJ contained in the alcohol [78]. The high temperatures required to separate ethanol from the fermentation broth using distillation enable a deeper integration between reaction and separation processes. Therefore, the untransformed substrates are not reused. The product inhibition and the separation of the biofuel issues are addressed from a process intensification perspective within the in situ product removal framework. Integrated bioreactors with alternative separation processes can selectively remove in situ, the

components that impair the microorganisms, i.e., alcohols, phenols (flavors), butanol, and acetone, among others. Besides, it has been shown that lower product concentrations reduce the fermentation broth osmotic pressure, then the undesired glycerol production during the fermentation decreases and there is an increase in the number and viability of cells. Combining these factors, there is a substantial improvement of the bioprocess productivity using hybrid technologies [90, 91]. Two similar systems have been investigated:

7.8.3.1 Hybrid Fermentation–Pervaporation System

For few decades, pervaporation (PV) has been considered as the most promising process for azeotropic mixtures separation due to its effectiveness, reduced energy demand and it is environmental friendly. As result, for 2006 there were more than 100 pervaporation plants worldwide operating for alcohols dehydration [92]. More recently, the hybrid fermentation and pervaporation process have been investigated in order to intensify the biofuels production, mainly bioethanol and the ABE process (Acetone, Butanol, and Ethanol).

Pervaporation is a chemical potential-driven membrane separation process, where the transport mechanism is solution-diffusion. The driven force is induced by a pressure difference across the membrane generated by a vacuum pump, rapid condensation, or a sweeping gas (N_2). In pervaporation, dense inorganic, polymeric or composite membranes are used. These materials can be hydrophilic or hydrophobic depending on the key component to separate, i.e., water or organics (VOC), respectively [75, 93].

A conventional integrated pervaporation bioreactor system uses hydrophobic membranes in order to have continuous separate inhibitors from the fermentation broth [78, 94]. Two configurations have been investigated, submerged membranes (SM) or external loop membranes (EL) [74]. In external loop membrane configurations, there are possibilities to control fouling issues at the expense of induced cells stress and potential fermentation fluctuations. This can be done by using an intermediate micro/ultrafiltration stage. On the other hand, submerged membranes do not stress biomass but are subject to substantial membrane fouling, as previously discussed.

The most used material as selective layer to remove ethanol and butanol is Polydimethylsiloxane (PDMS). This material is stable at the fermentation conditions showing high transport rates (up to, 8000 g/m^2/h for ethanol and 300 g/m^2/h for butanol) [94, 95]. Hybrid pervaporation and fermentation systems have shown an improved performance compared to conventional bioethanol cultivations increasing productivity up to three times [78]. Still, research is required to improve selectivity, performance, and stability and reducing the membranes cost, especially for the biobutanol case.

7.8.3.2 Hybrid Fermentation–Membrane Distillation System

Membrane distillation (MD) has been proposed for desalination as a cost-effective alternative of reverse osmosis (RO) [96]. It is also an alternative separation process to remove ethanol and another inhibitory component from fermentation broths [97]. MD is a thermally driven separation process, similar to pervaporation. In this process, a microporous hydrophobic membrane separates vapor molecules. The driving force is generated by vapor pressure difference induced by the temperature gradient between both sides of the membrane. The separation is determined by the vapor–liquid equilibrium of the feed solution, which can lead to high rejection factors. In MD, the transport mechanisms are Knudsen diffusion, Poiseuille flow (viscous flow) and molecular diffusion, where the thermal boundary layer is considered to be the mass transfer-limiting factor. MD is economically competitive due to the high selectivity ethanol/water, high transport rate, high thermal efficiency, and reduced energy consumption [98].

Several results on integrated membrane bioreactors for bioethanol production have been reported. Volatile components (i.e., ethanol and other inhibitors) were removed from the fermentation broth increasing productivity and conversion rate. During batch fermentation, the ethanol yield was increased over 10 % and productivity was enhanced up to three times using an external loop bioreactor and membrane distillation system [99]. When using the intensified process, besides the transport limitations imposed by the thermal and concentration polarization, the yeast presence in the fermentation broth impaired severely the ethanol transport. Besides, it has been evidenced that the carbon dioxide in the mixture favors the transport of volatile components due to an increased turbulence [100]. Membrane fouling by biomass issue has been addressed including an intermediate microfiltration stage. Despite the advantages, it has been stated that membrane distillation is more difficult to apply on industrial scale due to limitations in the module design as well as heat loss during the process, which may lead to uncertain economic costs [101].

7.8.4 Hybrid Membrane Bioreactors for Organic Acid Bioproduction

Organic acids have already a place as chemical feedstock in chemical, food, cosmetic, and pharmaceutical industries. Among the organic acids obtained by fermentation, lactic acid has gained an increasing interest since it is the precursor for a sustainable production of Polylactic acid (PLA). PLA has been promoted due to its environmentally friendly characteristics such as: energy savings during production compared with traditional polymers, biodegradability, and suitability for PLA waste composting. Besides, lactic acid fermentation is relevant for the production of biomass as probiotic culture or starter culture for food industry [102]. Then, it

is expected that industrial applications of lactic acid overpass other organic acids in the future (e.g., citric, acetic) [103]. The bottleneck of most organic acid fermentations is that the microorganisms are normally impaired by product inhibition at a certain concentration level of the main metabolic product or one of the bi-products by disruption of cellular replication, disruption of sugar metabolism, or disruption of membrane integrity [104].

The inhibitory effect generated by the presence of organic anions can be potentially diminished by their continuous removal from the fermenter, resulting in a higher productivity and product yield. Additionally, continuous recycle of biomass will allow obtaining higher cell densities that minimizes the risk of a cell wash-out [83]. However, in situ separation of organic acids is challenging and costly. It has been estimated that the cost of recovery and concentration of lactate from the cultivation broth can be up to 80 % of the total production cost, and then the research has been focused on developing alternatives for downstream processing [105]. Processes such as solvent extraction, adsorption, direct distillation, and membrane separation processes have been extensively investigated. Among these processes, membrane-based separations are attractive since they can selectively remove lactate, are capable of operating aseptically, there are no by-products generation, and they allow biomass/substrates confinement.

7.8.4.1 Hybrid Fermentation–Solid Membrane Systems

The application of hybrid fermentation and solid membrane is well documented in literature; there in, pioneer developments are cited. Hybrid processes have used dialysis for lactic acid removal [106], Donnan dialysis carboxylic acid removal [107], electrodialysis for lactate removal [108, 109], electrodialysis with bipolar membranes for lactic acid recovery and concentration [110], ultrafiltration for cell-recycle [111, 112], nanofiltration, and reverse osmosis for lactic acid separation [113] and their combination to separate and concentrate the lactic acid [114–117]. Recent studies have been focused on electrically driven membrane separations such as electrodialysis, reverse electrodialysis, and reverse electro-enhanced electrodialysis - REED [118–121]. In electrically driven membrane separation processes, ion exchange membranes are used to selectively separate ions from dilute solutions. The main driving force is an electrical potential gradient across the membranes stack. However, concentration gradients play an important role in the transport mechanism [120]. The biggest obstacle using ion exchange membranes integrated to bioreactors is the membrane fouling. Fouling can be generated by bacterial attachment, extracellular protein adsorption, or colloidal particle deposition on the membrane surface. This process occurs because biomass, proteins, and colloids have local charged groups that are attracted by the ion exchange membranes. Additionally, certain multivalent ions such as calcium and magnesium contained in the feed solution are allowed to pass through cation exchange membranes, precipitating over the surface. These problems are minimized using REED technology since only anion exchange membranes are used and

the periodic inversion of the potential gradient [119]. The use of hybrid membrane bioreactors for lactic acid production has shown how productivity can be increased several times (four to ten times), achieving a better substrate utilization while reducing the operation time (for batch operation cases). However, the energy consumption to generate the external electrical potential gradient is still a concern.

Despite the promising results for lactic acid in situ recovery using membrane bioreactors, several design, and operability problems have been encountered. The main limitations are lack of system understanding, the dynamic nature of the processes (i.e., fermentation, membrane separation, or both), low predictive power of models if available (especially for the membrane) and the sequential strategy for process design and control [83]. Those issues might be the main constraints for scale-up of membrane bioreactors to industrial level. It is expected that further research within process intensification framework can supply the fundamental to address the mentioned limitations.

7.8.4.2 Hybrid Fermentation–Liquid Membrane Systems

A liquid membrane or carrier facilitated transport membrane is defined as an immiscible liquid barrier (membrane) between two liquid phases (donor and acceptor phases) that allows a selective transport of substances between them [75]. The liquid membrane systems have been referred to as perstraction. In order to have selective transport, the solute must be soluble in the membrane phase. The transport mechanisms are: passive diffusion due to a concentration gradient, facilitated transport generated by a chemical reaction between the carrier and the transported substance, and coupled transport that is the facilitated mechanism involving two counter transported species. The liquid membranes can be used as bulk liquid membranes, supported membranes or emulsified membranes. These membranes have been used to purify effluents and for separation of organic acids, polysaccharides, metals, and hydrocarbons [122]. Liquid membranes are interesting since they offer transport rates several orders of magnitude higher than solid membranes, since diffusion is faster in liquids than in solids [123]. The high permeation rate results in designs that are more compact, lower energy intensive, and with higher selectivity. However, liquid membranes hybrid systems are still under development since emulsion membranes need chemical additives to keep a stable emulsion. Besides, in supported systems there is a continuous membrane lost.

It has been shown through the previous sections the potential that hybrid membrane bioreactors have to be considered as process intensification within biotransformation industry. Most of the results analyzed here were obtained in laboratory or pilot plant scale. Still this technology faces relevant challenges to be overcome before can reach industrial scale.

7.9 Perspectives

There is no doubt that an increasing number of biocatalytic applications are found in industry, where the excellent selectivity and environmental profile of new or replacement processes offer a great potential for their further implementation. As expertise has been developed, the trend in research and development is from enhancing biocatalytic action to reaction/separation environment, representing an important development in biotechnology. Most interestingly, in the context of this chapter, the motivating driving force is process intensification achieved through removal of inherent constraints or alternatively through various degrees of integration in phenomena, space, and time. Many more of such examples will be reached in the near future, both, in the context of higher value products such as pharmaceuticals as well as lower value products such as biofuels and bulk chemical building blocks in biorefineries.

The biocatalytic improvements by using mutation or genetic manipulation of microorganisms have allowed increasing substrate utilization, by using diverse bioprocessing configurations, such as, co-cultivation, co-fermentation, SSF, SSCF, and CBP. Most of these studies have been performed employing a pragmatic point of view, which have permitted to visualize the opportunities on intensifying the production of bioproducts. In contrast, few works have developed and employed mathematical models in order to analyze and improve the performance of these intensified bioprocesses. Thus, there is an opportunity to improve bioprocessing production by employing mathematical modelling. This approach could be used to provide a screening tool to evaluate the performance of different intensified processes, which afterwards could potentially be experimentally implemented.

It is relevant to mention that the activities looking for the intensification of bioprocesses are not individual tasks, since include diverse expertise areas. Therefore, it is important to emphasize the conformation of multidisciplinary teams in order to improve the production of bioproducts using intensified technologies, that is, specialists on areas such as genetic engineering, biotechnology, process engineering, and purification processes.

Novel membrane bioreactors proposed within process intensification framework have a great potential to become a common technology at industrial level in the future, due to their versatility and performance. Membrane technology includes a broad gamma of possibilities for separation that have not reached their maximum capabilities. Continuous efforts are made to minimize the loss of the hybrid process performance due to fouling and thermal/concentration polarization. This can be achieved from design and operation perspectives as discussed previously. We believe there is still room for improvements in order to consolidate hybrid technologies. From the membranes manufacturing point of view, it is desired to produce more permeable and selective materials at lower cost. This is especially critical for the commodity chemicals production (biofuels and volatile organic components), since it is necessary that the membranes to be used are cost-effective. From operation perspective, research is focused on how to find the appropriate set of conditions

that enhance the process performance in a more systematic way. Then, there are opportunities in this field if more model-based approaches are used. Modelling is a powerful tool useful for system understanding, process design, process control, and optimization. However, it is understandable that model-based approaches are not popular due to the complexity of the hybrid biological-membrane systems. In several systems, process modelling is still based on semi-empirical approaches that provide little system understanding and have low predictive power. Then, the hybrid process design, optimization, operation, and scale-up require considerable experimental work.

We believe that hybrid technologies must be investigated as much as biotechnological processes in order to have a sustainable development. The promising results obtained at pilot scale constitute the driving force to scale-up hybrid technologies.

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