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TRENDS IN BIOSEPARATIONS

TENDENCIAS EN BIOSEPARACIONES

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Abstract

The biotechnology industry is changing every day to improve the purity, yield and throughput of products, besides becoming more efficient while using fewer resources. In this sense, this short review aims to present a general overview of the trends in bioseparations that will be met in the bioprocessing area of biotechnology in order to continue overcoming these challenges. It is identified that these tendencies will impact the following stages of the bioseparation train: product sources, primary recovery and purification. New approaches involving process integration, intensification and automation, microscaling and greener practices are addressed. Biotechnological processes will continue to evolve becoming the spearhead of process development, targeting products that will benefit lifestyle and improve quality of life. Nonetheless, the aspects that are covered in this work are up to day the state of the art in this constant change and will undeniably have important repercussions in the future of this area.

Keywords: bioseparations, primary recovery, purification, microscale devices, green bioprocesses.

Resumen

La industria biotecnológica cambia todos los días para mejorar la pureza, el rendimiento y la recuperación de productos; además de hacerse más eficiente optimizando el uso de recursos. En este sentido, esta corta revisión busca presentar un panorama general de las tendencias en bioseparaciones que deben ocurrir en el área de bioprocesos para seguir alcanzando estos retos. Se ha identificado que las etapas del tren de bioseparaciones que serán impactadas son: las fuentes de materia prima, las etapas de recuperación primaria y la de purificación. Los nuevos enfoques que serán utilizados incluyen la integración, intensificación y automatización de procesos, el microescalamiento y prácticas amigables con el medio ambiente. Los procesos biotecnológicos seguirán evolucionando para convertirse en la punta de lanza del desarrollo de nuevos productos que mejorarán el estilo y la calidad de vida. No obstante, los aspectos que se cubren en este trabajo son hasta ahora el estado del arte de este cambio que sin duda alguna tendrán una repercusión importante en el futuro cercano del área.

Palabras clave: bioseparaciones, recuperación primaria, purificación, micro dispositivos, procesos verdes.

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1 Introduction

In the evolving field of biotechnology great efforts have been made in developing, optimizing and scaling processes for the recovery of high value products from a wide number of sources and with different degrees of complexities. Product recovery, as known, is not a trivial task, but it is a key element in the process chain. In most cases, the isolation of the products of interest require large separation trains with many unitary operations that might represent up to 80% of the operational costs. Moreover, it is well acknowledged that a high number of unit operations will directly impact the final yield of the process. These economical and operational bottlenecks push researchers to find new, alternative, time and cost-efficient methodologies to achieve better industrial conditions to meet product demands.

As a first approach, it is common to create multi-composite and highly specific culture mediums that increase product titers and overcome product losses in the following separation and purification stages. This of course is highly beneficial, but in many cases it comes with the cost of increasing the difficulty of the separation and purification operations and consequently lowering the final product yield. Besides this, each biotechnological product requires a tailored purification process making it almost impossible to create a standard protocol, as is sometimes done in other industries. Consequently, a more labor-intensive, time consuming and expensive process is needed when compared to a non-biological one.

In this sense, many achievements have been observed and knowledge has been generated from experiences in pharmaceutical processes. The high regulations associated to therapeutical products have pushed the creation of simplified separation trains that ensure product quality and safety. This understanding has been extrapolated in part to other biotechnological industries (*i.e.*, food, textile, fuels, etc.) with successful results (Cisneros-Ruiz and Rito-Palomares, 2005).

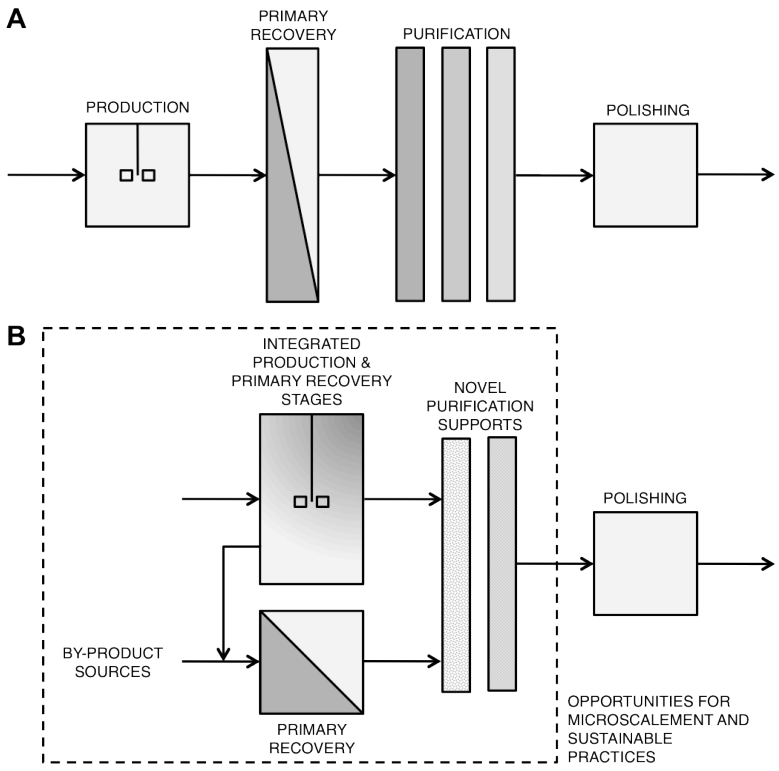

Fig. 1. Schematic process representation of a traditional biotechnological process (A) and a process designed incorporating the novel trends seen in the area (B). These trends include integrated production and primary recovery stages, the use of novel purification support matrixes where by-products can be recovered from alternative or recycled sources and opportunities for microscalement and sustainable practices (B).

However, pharmaceutical processes by themselves face new challenges that come from new products that are rising as potential therapeutical agents like monoclonal antibodies, secondary metabolites, modified proteins, viruses or virus-like particles and stem cells. The same is observed in the rest of the biotechnological industry where novel engineered biomolecules are being designed to meet market demands making the installation of appropriate purification strategies necessary.

Nonetheless, several tendencies can be foreseen in the bioseparation field. For instance, recovery of high-valued products from what is normally considered industrial wastes is starting to arise as a common practice since these by-product sources are cheap and generated in great amounts. Primary recovery and purification strategies are evolving with the integration and intensification of several unitary operations. Also, great efforts are being made to implement micro-scaling purification processes and to promote green practices to meet global sustainability goals. A schematic representation of a process incorporating these trends compared to a traditional one is presented in Fig. 1. The aim of this short review is to present the state of the art in these areas and to describe the importance and repercussion of these advances in bioseparations.

2 By-product sources

During years biotechnological processes have been mainly focused in the recovery of high-value products from fermentation broths. Nowadays, the use and recycling of waste sources, especially from food, has gained the attention of many industries. Manufacturers worldwide are required to dispose their wastes in a safe manner that usually involves an expense that must be considered in the manufacturing costs. The potential of biological waste streams has been widely overseen while in fact these by-product sources represent a cheap, extensive and safe source of high-value products. In some cases, the processing of these sources can be optimized for the recovery of part of the prime goods used in the production process. This of course, could represent an attractive alternative in saving the costs associated in waste disposal and some of the prime goods required or even making a profit out of what normally is considered rubbish. In this sense, proteins, phenolic compounds, polysaccharides, fibers, vitamins, lipids, and natural dyes had been recovered from waste sources (Cahú *et al*., 2012; Galanakis, 2012; Mayolo-Deloisa *et al*., 2009; Murthy and Madhava Naidu, 2012; Trejo-Hernandez *et al*., 2001). Moreover, it is possible to obtain a high number of different products from this type of sources, thus enhancing their potential. For example, protein hydrolysate, chitin, chitosan, carotenoids, and sulfated glycosaminoglycans can be recovered from shrimp head wastes (Cahú *et al*., 2012).

In the bioseparation context, the fractionation, recovery and purification steps highly depend on the source of the original matrix, the final purity required and/or of the final application of the product. The use of by-product sources generates more sustainable processes, but can also complicate the downstream stages. Therefore, the processing of these sources should by all means be as simple as possible resulting in an attractive challenge for researchers, since several high value products can be recovered from the same process. Frequently, mechanical pressing, centrifugation, ultrafiltration, and chromatography steps have been utilized for the recovery of by-products. The key in these processes is to generate "clean" stages with high recovery levels that are economically feasible. The potential of other techniques such as emerging technologies (*i.e*., pulsed electric fields), liquid-liquid extractions, membrane chromatography and monoliths should be investigated and applied. The attractiveness of recovering products from waste sources is vast, and a greater use of these sources should be seen in the coming years.

3 Primary recovery

It can be said that the first step in any bioseparation train involves the isolation and concentration of the products of interest preferably from the vast majority of the contaminants, suspended solids, whole cells and cell debris that might come from the fermentation and cell disruption upstream stages. It is also desired to procure low product losses and preserve product integrity and functionality. Furthermore, all these goals need to be accomplished while reducing the number of stages required in the purification process, lowering operational costs and achieving the highest product yield possible.

Depending on the complexity of the stream where the products come from, several approaches can be used to achieve this. Techniques like centrifugation, filtration, ultrafiltration, isoelectric precipitation, flocculation, solid-phase adsorption,

and liquid-liquid extraction have been widely used; each with their own advantages and disadvantages, but always with important improvements in product yields. For instance, Singh and collaborators have recently reported the use of high capacity depth filters to clarify high-density mammalian cell culture streams by optimizing the pore size distribution of the filter media with a significant improvement in filtration efficiency (Singh *et al*., 2013). Nonetheless, one of the major tendencies observed nowadays is the intensification and integration of the fermentation stage with the primary recovery steps in a same unitary operation making the production and recovery of a wider range or amount of products possible with the use of fewer unitary operations thus reducing equipment size, energy consumption and waste production.

In situ product recovery or extractive fermentation has been used under different conditions to separate products early on the production stages. Gas stripping, for instance, is used for the recovery of volatile products; addition of solvents or liquid polymers and the addition of solid adsorbents into the fermenter have also been reported (Phillips *et al*., 2013). In this context, aqueous two-phase systems (ATPS) have been exploited as means of integrating production and separation of products in a single stage. Reactive ATPS have the capacity of producing a chemical or biochemical reaction while separating the reactants and products, preferably to different phases. Bioconversion in these biphasic systems has been mainly described with the use of enzymes, organelles, live cells or the simultaneous use of cells and enzymes, where the product usually partitions towards the top phase (Andersson and Hahn-Hägerdal, 1990).

When extractive fermentation approaches are not easily implemented, ATPS strategies can be also used and optimized for the primary recovery of the products as a standalone operation. In fact, recent developments in the use and optimization of this technique are helping to promote their widespread use. For instance, molecular dynamic simulations are now being used with successful results in predicting phase formation and protein partitioning in polyethylene glycol (PEG) - phosphate systems (Dismer *et al*., 2013). Flowsheet optimization models have been also developed to predict protein purification minimizing operational costs with constrains on product yield, purity and phase settling rates (Ahmad and Przybycien, 2012). Furthermore, the use of novel phase forming chemicals like pH and temperature responsive polymers, known as smart polymers, is being studied with successful results. This includes their advantages in polymer recycling and easy removal from the purified stream, besides the benefits reported in the recovery of proteins and stem cells (Al-Hamouz and Ali, 2013; González-González and Rito-Palomares, 2013).

In general, the main focus on commonly used primary recovery strategies in bioprocessing is the optimization of the different unitary operations. This is being achieved mainly by the development of better engineering designs supported by mathematical modeling and/or the use of novel materials in the operation. Either way the integration of several primary recovery stages or the optimization of a standalone operation translates directly to increments in product yields and process robustness that undoubtedly benefits the following steps in the downstream train.

4 Purification

Chromatography is and will continue being the most used technique for secondary recovery and purification steps in bioseparations. The challenges in this area are the optimization of processes and the design of new adsorbents (*i.e*., reversed phase, ion exchange, hydrophobic interaction and affinity) with higher selectivity and capacity (mass loadability) (Mayolo-Deloisa *et al*., 2012). Mathematical models have been proposed as an alternative of optimization. Operational conditions such as flow rate, ionic strength gradient and operational time can be selected using models in order to find the minimum cost achieving the best separation performance (Asenjo and Andrews, 2008). However the potential of these tools has not been sufficiently explored and applied to industrial processes.

In the same line, new adsorbents must be designed and others such as organic monoliths, chromatographic membranes, mixed matrix membranes (MMM) and PEGylated (polyethylene glycol modified) resins should be explored. Monolithic columns seem to be a good alternative for high-efficient separations due to their high permeabilities and low backpressures (Núñez *et al*., 2012). Membrane chromatography can significantly reduce downstream bioprocessing costs due to its high binding capacities and improved mass transfer properties (Orr *et al*., 2013). MMM has been recently reported to produce cation exchange and hydrophobic interaction membranes to recover proteins (Saufi and

Fee, 2011, 2013). PEGylation of resins is mainly being explored to improve the affinity chromatography technique, wherein protein A is used for the recovery of antibodies reducing non-specific binding interactions and in other techniques to better separate similar chemical modified proteins.

Alternatively, one of the most recent trends in chromatographic purification is the use of mixed-mode supports which incorporate at least two separation principles in conjunction. Mixed-mode chromatography (MMC) can be implemented by using different kinds of sorbents in a single column or connecting different columns in series (Gilar *et al*., 2008). MMC has been successfully implemented but has mainly been used in the separation of peptides, nucleic acids and other small molecules (Strege *et al*., 2000; Gilar *et al*., 2008; Zhang *et al*., 2010). The potential of using different sorbents in mixed-modes still needs exploration since method development and mathematical modeling is indeed more complicated and less understood.

Due to its versatility, chromatography has a high potential and would not be replaced in the near future. This technique in all its forms represents the golden standard in purification technologies. As mentioned, better understandment of the physicochemical properties involved in the mass transfer and final purification of products is needed but can be achieved via mathematical modeling and a better theoretical description of these phenomena. Without doubt, current research efforts are in the right path of achieving this giving chromatography even more uses and advantages over other purification procedures (Mayolo-Deloisa *et al*., 2012).

5 Microscale bioprocessing

A clear tendency for the miniaturization of instrumentation and analysis devices has lately been seen in the fields of biology, chemistry, biotechnology, medical sciences, drug discovery, and tissue engineering. This phenomenon is mainly motivated by the advantages implied in microscale operations that include: reduction in human manipulation, time, equipment, reagents, sample, and wastes. Moreover, a greater number of variables can be tested simultaneously or in shorter periods of time. Process integration and automation is also possible, as well as running parallel systems which offer advantages over full scale processes that usually are run in series. With these considerations, microscale devices result in a portable, flexible, high-throughput, cost-effective, robust, and greener methodology that improves overall process understanding, accuracy and reproducibility.

In the biotechnology sector, microscale devices have been used in both upstream and downstream bioprocesses. For example, in the upstream stages, microbioreactors and fermenters have been widely implemented to optimize the process parameters in a faster and cost-effective manner. Likewise, they have been exploited to determine the optimum medium for cell culture. In the downstream area, these devices are utilized to trap, manipulate, separate, treat, concentrate, and analyze the product of interest, including living cells as leucocytes (SooHoo and Walker, 2009; Tsukamoto *et al*., 2009) and stem cells (Wang *et al*., 2000).

In this sense, microscale devices can be divided in two main categories: (1) multi-microwell plates (μwells) and (2) lab-on-a-chip microfluidic devices (μdevices) or BioMEMS. The μwells are mainly made from polymers and can be available in the format of 24, 48, 96 and 384 wells per plate, allowing a high-throughput screening of process parameters. Another advantage is that they are compatible with other specialized equipment as the spectrophotometers, facilitating data analysis. Microwell plates are mostly used as microbioreactors or cell culture systems (Barrett *et al*., 2010) to speed up the optimization stage, as they permit parallel investigation of a wide range of variables. On their part, μdevices are predominantly made from polymers, silicon and glass through photolithography, laser ablation, microinjection molding or surface treatment. They are composed of reservoirs, microchannels, mixers and outlets embedded in a flat chip and are employed for continuous analysis.

A great advantage of microfluidic devices is that laminar flow is easily and stably within the microchannels and the mass and heat transfer is very fast. Furthermore, the process can be easily scaled-up by operating multiple systems in parallel. Also, they are very flexible and allow the application of different mechanisms like dielectrophoresis (DEP), electrophoresis (EP), electroosmotic flow (EOF), hydrodynamics, and field flow fractionation (FFF) to govern the process. As well, other separating technologies can be implemented in microdevices, highlighting aqueous two-phase systems (Nam *et al*., 2005; SooHoo and Walker, 2009; Tsukamoto *et al*., 2009). ATPS is also suitable for the recovery of biological products in microdevices due to the mild condition supplied by the aqueous environment.

Moreover, the advantages of microdevices are enhanced with the benefits of ATPS, maximizing the technology (González-González *et al*., 2012; Sósol-Fernández *et al*., 2012).

More recently, the use of disposable microfluidic devices has gained the attention of researchers due to the multiple advantages they offer. For example, microfluidic devices made from recyclable and low cost materials that are easily sterilized are attractive for rapid evaluations, as they eliminate the cleaning and maintenance steps. Furthermore, if this technology is equipped with non-invasive sensors (e.g. temperature, pressure, pH, dissolved oxygen and flow); the screening, optimization and validation steps could be done in a smoother path, with low material input and enabling *in situ* measurement of critical process parameters.

6 Green(er) bioprocessing

One of the major, if not the most important global goal, is the achievement of sustainability in all aspects of human life. International pressure and regulations are pushing the industry to find better production and purification processes that imply lower emissions, better primary good utilization, enhanced product environmental performance and durability, to have a positive impact in the economical and social sectors. During the last decades, it has been seen that industries have considered for the first time the use of biological sciences to impact their business and have explored the use of biotechnology in beneficial ways (Gavrilescu and Chisti, 2005). Biotechnology, in this sense, has been of great use in many ways. However the question that arises is if biotechnology, and more specifically, bioprocessing can be designed and optimized to increase sustainability levels.

Ultimate "Green" bioprocessing can be achieved by using the minimum of resources with the aim of using exclusively renewable and recyclable feedstock. Therefore any process where mass transfer is intensified, equipment size is reduced and energy is saved is in the path of reaching such goal (Liu *et al*., 2010). To further increase sustainability, selection of the processing materials should shift to bio-based chemicals, which are those materials produced through a biomass origin and/or a bioprocessing route. Among these we find platform chemicals (e.g. propane, butanediols, isoprene, and ethanol), polymers and industrial enzymes (Philp *et al*., 2013). The use of tunable solvents for instance, can be used to optimize yield, selectivity and conversions; offering process flexibility and a cost-effective alternative to other medium for separations and reactions (Eckert *et al*., 2000). On the other hand, wide available bio-materials such as cellulose are also important in the production of certain products and in the generation of better matrixes for the purification of products (Klemm *et al*., 2005).

It would result impossible to describe all of the approaches that could be implemented to reach better sustainable standards in bioprocessing, but it should be noted that in any process there are many areas of opportunity that could be optimized to reach this goal. However, all the different trends described in this work, including the use of disposable materials for the extraction of high value products, process intensification and integration to achieve better product primary recoveries, optimization of purification strategies and the final scaling down of bioprocessing stages, present sustainable (and attractive) alternatives to current bioprocessing strategies.

7 Trends and challenges

As depicted in Fig. 2, bioseparation procedures used to be designed from two main but different perspectives. The first of them consisting in optimizing the production train to lower the amount of contaminants to be removed and increasing product titers; and the second one in reducing and optimizing the downstream processing stages to obtain the highest possible product yields and purity. With the advances seen today, it can be said that both perspectives need to be taken into consideration when possible. For instance, as it has been previously reviewed, the production of recombinant proteins is now fully optimized and efficient with protein yields and concentrations hundreds of times higher than in the last two decades reducing the number of purification steps to one or two with therapeutic grade purities (Asenjo and Andrews, 2008). Therefore, the main challenge consists in designing jointly a highly efficient production procedure and a compact and robust purification train that complement each other considering in the way Quality by Design goals. All this is of course assisted by research focused in developing better mathematical models and the theoretical aspects behind them.

In this context, each of the trends presented in this short review face challenges of their own.

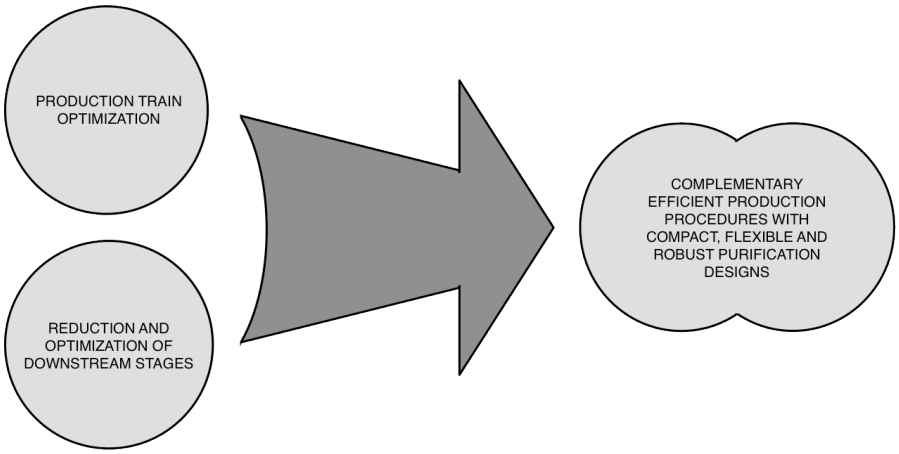

Fig. 2. Evolution of the bioseparation design perspectives.

For example, the use of waste streams to obtain high-value products needs the use of compact and cheap bioseparation strategies because the least desired thing would be to invest large amounts of money in procedures for by-products where an economical benefit is desired. Primary recovery and purification strategies require further optimization and development especially in those cases where integration of several unitary operations is searched (*i.e*., extractive fermentation and MMC). In fact, since most of these technologies are still under development research is needed to describe, model and transfer these procedures to industrial levels. On the other hand, better designs and description of the multiple uses and advantages of microscale devices are also needed to spread their use in industrial applications.

Everyday new opportunities arise in developing better procedures to improve product yields and lower manufacturing costs. In general, from the recovery of high-value products from wastes, the integration, intensification and microscaling of traditional unitary operations and the design of novel materials to be used as adsorbents in secondary purification stages, bioprocessing is evolving to achieve more compact and efficient production trains.

Conclusions

Recent innovations in bioseparations have been driven by the compelling necessity to lower the operational costs and increase the over-all yield of the process. Thus, it is important to change this and anticipate the challenges instead of reacting to them. From this short review, it is clear that the current technologies in the bioseparation area are not adequate *per se* for the biotechnological industry of tomorrow. Thus, novel integrated techniques are needed to be implemented to further optimize yields and minimize the costs. In this sense, the trends described in this work are only a glimpse of some of the things that will be observed in a near future in the bioseparations field. Thus, research in this area enhanced with interdisciplinary efforts plays a key role in helping this dynamic field evolve into a more robust platform in the coming years. Better and more efficient separation stages translate directly in savings that benefit the manufacturer and consequently the consumers. If besides this, everything is framed under sustainable practices bioprocessing should set an example to other industrial sectors.

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