Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–9, 2018

www.elsevier.com/locate/jbiosc

REVIEW

Recovery of biotechnological products using aqueous two phase systems

Win Nee Phong,[1] Pau Loke Show,[2] Yin Hui Chow,[3] and Tau Chuan Ling[1,*]

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia,[1] Bioseparation Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia,[2] and Faculty of Built Environment, Engineering, Technology and Design, Taylor's University, No. 1 Jalan Taylor's, 47500 Subang Jaya, Selangor, Malaysia[3]

Received 30 November 2017; accepted 10 March 2018
Available online xxx

Aqueous two-phase system (ATPS) has been suggested as a promising separation tool in the biotechnological industry. This liquid-liquid extraction technique represents an interesting advance in downstream processing due to several advantages such as simplicity, rapid separation, efficiency, economy, flexibility and biocompatibility. Up to date, a range of biotechnological products have been successfully recovered from different sources with high yield using ATPS-based strategy. In view of the important potential contribution of the ATPS in downstream processing, this review article aims to provide latest information about the application of ATPS in the recovery of various biotechnological products in the past 7 years (2010–2017). Apart from that, the challenges as well as the possible future work and outlook of the ATPS-based recovery method have also been presented in this review article.

[**Key words:** Aqueous two-phase system; Biotechnological products; Downstream processing; Recovery; Liquid-liquid extraction technique]

Aqueous two-phase system (ATPS) separation technique was first developed by a Swedish biochemist, P. A. Albertsson in 1986 (1). Since then, this liquid-liquid extraction technique has become a powerful bioseparation tool (1,2) and has been extensively exploited to process a range of biotechnological materials such as proteins, enzymes, phytochemicals, nucleic acids, and pigments (3,4). In ATPS, two immiscible phases can be formed by mixing two water miscible solutes beyond critical concentrations (5). Typical ATPS mixture consists of two different polymers [ethylene oxide propylene oxide co-polymer (EOPO), polyethylene glycol (PEG), polyacrylates, dextran] or a combination of polymer/a low molecular weight alcohol and kosmotropic salt (phosphate, citrate, and sulfate).

ATPS is renowned as an efficient, economical and versatile emerging technique for the bioprocessing of biotechnological products. Over the last two decades, ATPS has gained increasing interest due to its potential in circumventing some of the technical limitations exist in the downstream processing. This system demonstrates a good ability in eliminating most of the contaminants in the early steps of recovery process. It has been reported extensively that intracellular compounds can be separated from cell debris (5) or from the mixture of other soluble components (6,7) effectively using ATPS. Another author also agreed that ATPS has more versatility over the conventional solvent extraction methods in the downstream processing of biomolecules (3). For instance, this well-established method is not only able to extract target compound by partitioning them into one of the phases, but also capable of concentrating the target compound by partitioning them into the smaller volume of the extraction phase (8). A schematic illustration of product recovery using ATPS is presented in Fig. 1.

Overall, ATPS represents an interesting advance in bioseparation process and has been proposed as a promising alternative technique to recover diverse biomolecules from various sources. In this review article, we first describe the latest information about the recovery of various types of biotechnological products based on ATPS strategies in the past 7 years (2010–2017), then we study the challenges and future perspective of the ATPS-based recovery method.

ADVANTAGES OF ATPS

The use of conventional extraction method particularly chromatography-based method involves complex scale-up, batch operation, low capability in process integration, laborious processing cycles, require high energy input and high cost (9). On the other hand, the removal of cell debris by filtration or centrifugation may be challenging when processing high biomass load due to heterogeneous distribution of particle size and high viscosity (10). On top of that, a higher number of operational steps would incur higher overall cost owing to the loss of some amount of target compound in each processing stage.

In response to the increasing market demand of biotechnological products, a more versatile and economical bioseparation process which can offer higher process throughput, shorter processing time and scalability is necessary to cope with the current rate-limiting downstream processes (11). However, it should be noted that the selection of an appropriate separation method is crucial in the downstream processing. Otherwise, inappropriate extraction technique could negatively affect the biofunctionality of target

* Corresponding author. Tel.: +60 3 79674104; fax: +60 3 79674178.
E-mail addresses: tauchuan.ling@gmail.com, tcling@um.edu.my (T.C. Ling).

1389-1723/$ – see front matter © 2018, The Society for Biotechnology, Japan. All rights reserved.
https://doi.org/10.1016/j.jbiosc.2018.03.005

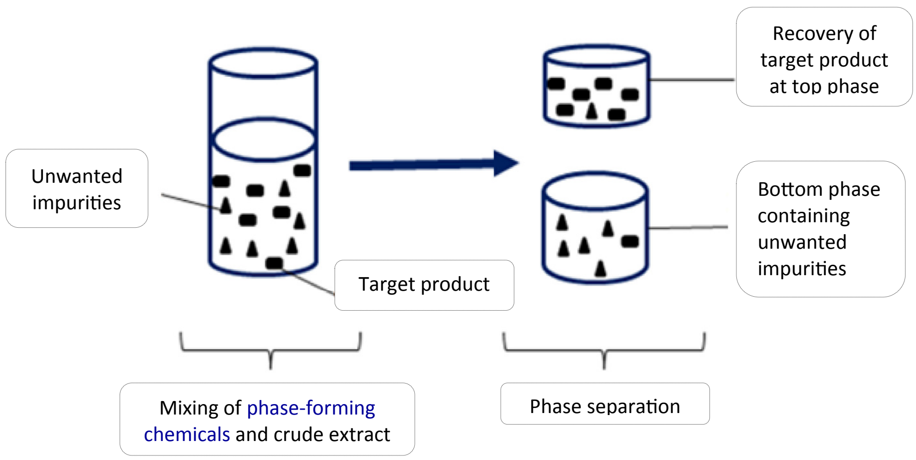

FIG. 1. Schematic view of recovery of target product based on ATPS concept.

molecules and would therefore limit their application to a great extent (12).

Alternatively, ATPS exhibits application potential with great technical and economic advantages in downstream processing. Several discrete stages which involve separation, concentration into smaller volume and purification can be reduced and substituted by ATPS, allowing the overall recovery process become more energy-efficient and cost-effective (3). Compared to the conventional method, the use of ATPS for bioseparation offers many advantages (2). These include simple process operation, rapid separation, high selectivity, low energy consumption and low cost (3,8,13–16). ATPS has also been able to offer a biocompatible environment for the isolation of biotechnological products due to the presence of high water content in both phases (1,17). The extremely low interfacial tension of this system (between 0.0001 and 0.1 dyne/cm) creates high interfacial contact area of the dispersed phases, which in turn, enhances the efficiency of the mass transfer (1).

Chemical cost is considered one of the dominant cost factors for large-scale bioseparation process. The use of inexpensive phase components in ATPS would make the whole downstream processing more economical. Problem of downstream pollution may also be avoided by recycling the ATPS phase components (2). Extraction using ATPS is relatively rapid and the processing capacity of this technological simple technique is quite high. Due to the simplicity and reliability of scaling-up approach, the extent of ATPS extraction to industrial scale application is feasible and practical (8,11,13).

Influence of parameters on the partitioning behavior of ATPS The selective distribution of the compounds to be separated between the two phases serves as the basis of partitioning in ATPS. In ATPS, the partition profile of the solutes depends on different physicochemical interactions between the biomaterial and the phase forming chemicals (18). Interactions such as van der Waals' forces, hydrogen bond, electrostatic interactions, steric effects, hydrophobicity, biospecific affinity interactions as well as conformational effects between the phase components and the substances contribute to the partitioning of the particular substance (1).

To achieve an effective bioseparation process, the partitioning behaviour of biomolecule in ATPS can be influenced by changing some of the dominating factors such as molecular weights and size of polymers, type and composition of phase component, type of ions in the system, the addition of neutral salts like NaCl, tie line length (TLL), system temperature and pH (3,5,13).

ATPF integration process Downstream processing represents the key economic constraint to the large production of biotechnological products at lower cost (19). As part of the downstream processing, isolation and extraction continue to be a significant challenge towards the commercial production of biotechnological products. When developing an economically practical recovery process, aspects such as recovery, operational cost, throughput, biocompatibility, recyclability, upscaling need to be considered to favour the economic feasibility of the recovery process (20,21). For environmental benefits and long-term sustainability, all the processing stages should be easily adopted and simplified without the involvement of extensive energy input.

The concept of implementing process integration into the downstream processing of biotechnological products at commercial level offers considerable potential benefits and is of great economic and practical interest (22–24). Through process integration, several downstream processes such as separation, concentration and extraction can be integrated into one single step and this could reduce waste and processing time, lessen energy consumption and consequently cut the overall production cost (6–8,11,13).

Owing to its flexibility and biocompatibility, ATPS can be combined with other separation methods or processes to overcome commonly observed issues in biotechnological processes like low productivity, expensive operation and long processing time. Application of ATPS in extractive bioconversion, extractive fermentation or extractive disruption is one of the elegant emerging examples of process integration.

Incorporating ATPS into extractive bioconversion or extraction fermentation could improve the efficiency of biotechnological processes. In general, both strategies involve the continuous removal of bioproduct from its site of production via bioconversion or fermentation to the opposite phase simultaneously during processing (10,25).

On the other hand, the direct incorporation of cell disruption treatment with recovery process can be achieved in the ATPS extractive disruption. The flow diagrams of conventional discrete process and integrated process (extractive disruption) are illustrated in Fig. 2.

TYPES OF BIOPRODUCTS

Protein There is an increasing request for a large number of new proteins due to their versatile applicability in various sectors like food, medical, pharmaceutical and chemical industries.

Please cite this article in press as: Phong, W. N., et al., Recovery of biotechnological products using aqueous two phase systems, J. Biosci. Bioeng., (2018), https://doi.org/10.1016/j.jbiosc.2018.03.005