Aqueous two phase extraction

Introduction

Aqueous two-phase extraction has applications in biochemical processes involving protein separation and purification. Aqueous two-phase extraction includes two different immiscible polymers or polymer and salt systems for protein recovery. To minimize costs, the protein must be recovered in highly purified form. In biotechnology industry one of the major challenges is large-scale purification on desired protein from fermentation broth which contains wide range of biomolecules. Aqueous two-phase system downstream process offers a solution for this challenge. In this group work we are introducing background of the process, theory to understand basis of the process and importance part which introduces biotechnology processes and applications which utilizes aqueous two-phase extraction.

1. Background

Since the late 19th century aqueous two phase extraction has been known. Nowadays, aqueous two phase can be formed with a wide variety of natural or synthetic water-soluble polymers. Aqueous two phase extraction is used for secure separation and purification of biomolecules, such as proteins, and extraction of enzymes. (Stanbury et al., 2016) Aqueous extraction has many advantages; it is biocompatible, it has high water content and low interfacial surface tension between phases, the process has an ability to integrate and a capability for intensification (Benavides et al., 2010; Goja et al., 2013). Also, the degree of degradation for biomolecules is low (Saravanan et al., 2007).

2. Theory

The basic principle of extraction is the same as usual; the goal is to separate the solute selectively from its carrier. Aqueous two phase system is formed by adding two uncompetitive water-soluble polymers (such as polyethylene glycol, PEG) or salt and one polymer into water above the critical concentration. These polymers react with the water and two immiscible aqueous phases are formed. Then soluble material, like proteins, are added to the system and it is distributed into two phases (Fig 1.). (Stanbury et al., 2016) The distribution of extracted component results from the interactions with the surrounding phase, such as Van der Waals, hydrophobic, hydrogen bond, and ionic interactions (Saravanan et al., 2007).
Stanbury et al. (2016) listed four different possible aqueous two phase systems. The first of these is to use two different nonionic polymers, such as polyethylene glycol and dextran, with water extraction. Another option is to use a polyelectrolyte, such as sodium carboxymethyl cellulose with a nonionic polymer, again using water extraction in separation. Third, two different polyelectrolytes may be used. The fourth option is to use a polymer and a low molecular weight compound, such as propyl alcohol. (Stanbury et al., 2016)

The ease of extraction is described by the distribution coefficient $K$:

$$K = \frac{\text{Concentration of solute in extract}}{\text{Concentration of solute in raffinate}}$$  \hspace{1cm} (1)

As equation 1 shows, the larger the value of $K$, the more stable the product which results in better separation. $K$ is also known as the partition coefficient. For better phase separation centrifugal and magnetic separators can be used. The distribution of solute between two phases can be influenced by temperature, pH, polymers and solutes molecular weight and size and by salt concentration. (Stanbury et al., 2016)

Extraction can be performed by batch or as a continuous operation. Commonly a continuous extraction process is more efficient and involves less processing time. (Goja et al., 2013)

However, there has been some difficulties in some industrial scale process using aqueous two phase extraction. Especially difficulties with PEG and salt extraction have been common, since the salts caused corrosion effects and precipitation of product. Furthermore, a high amount of waste salt formed is not environmentally sustainable and effective salt recycling is expensive. These are partially the reasons why still two polymer systems are more used than polymer and salt systems. Although mentioned polymer/salt extraction problems, it is still widely studied, because it is still less expensive than PEG/dextran extraction.

Nonetheless, aqueous two phase extraction is not widely used in large scale, because of high costs of the polymers (dextran) and difficulties in solvent recovery. For those reasons...
interest for developing new polymers for aqueous two phase extraction grows increasingly. (Goja et al., 2013)

3. Importance

Penicillin G and penicillin V have small use medically today, they are however the raw materials used in producing all semisynthetic penicillins (Marynova et al., 2014). Penicillin G is manufactured in multiple countries in a quantity of over 11 000 tonnes per year. Its large usage can be attributed to its lack of toxicity and irritancy. (Hossain & Dean, 2008) Traditionally penicillins are extracted from the fermentation broth with butyl acetate. However, many other techniques have been developed, including aqueous two-phase extraction. In aqueous two-phase system PEG is often used as polymer, while salts, such as sodium sulphate and ammonium sulfate, are introduced to the system in order for partition to take place. The process uses flotation by nitrogen bubbling, in order to get the PEG phase on the surface of the water solution. This sort of process is carried out at neutral pH, which is preferable to traditional extraction (pH=2), since penicillin quickly decays in low pH (half-life of 15 min at pH=2, 20°) (Standbury et al., 2016). Aqueous two-phase extraction with polymers allows for penicillin extraction from the whole broth. (Marynova et al., 2014)

Another potential use for aqueous two-phase extraction would be extraction of carotenoids. The interest in carotenoids has increased extensively due to their nutraceutical potential, with their estimated market value being 1000 million US$ by the end of the decade. Commercially carotenoids are produced with cyanobacteria. Traditionally their extraction involves cell disruption, cell debris removal and organic-aqueous fractionation. This however, involves large expenses and potential for environmental risks caused by the large amount of organic solvents used. As an alternative, aqueous two-phase extraction could be utilized to extract the product. In this sort of system, PEG-dextran could be used, in which the cyanobacteria would grow (upper phase). Lutein would continuously be extracted from this phase. If an additional cell-removal phase is added, such as centrifugation or microfiltration, β-carotene can be removed constantly from the upper phase. (Chavez-Santoscoy A. et al., 2010)

4. Conclusion

Aqueous two phase extraction is a suitable separation and purification technique for bioproducts in biotechnological field. Aqueous two phase extraction (ATPE) downstream processes are suitable for bioproducts by providing mild conditions that don’t harm bioproducts. In biotechnology ATPE processes are preferred instead of solvent extraction, because ATPE process provides lower surface tension. PEG/salt downstream processing technologies(DSP) offer efficient liquid-liquid extraction for a large variety of bioproducts including proteins and enzymes. However, using PEG/salt in the large scale may cause environmental problems due to the great amount of chemicals used during phase forming process and also high costs for effective recycling.
References


