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Review on the Extraction of Biomolecules by Biosurfactant Reverse Micelles

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Abstract

Surfactant reverse micelles have been widely applied in biotechnology areas for decades. Due to its unique criteria in protecting the bioactivies of biomolecules, the attempts have typically been done for recovery, separation and purification of many biological products such as proteins, enzymes, and antibiotics. Surfactant is a key role in stabilizing the transfer process into reverse micelles phase. Its application in reverse micelles extraction has so far been confined to chemical surfactant which synthetically derived from petroleum. By replacing synthetic surfactant with natural biosurfactant in reverse micelles, it may contribute generating products into a green application as well as helping the future to diminish the side effect of using a toxic surfactant to the environment. Biosurfactant which synthesized by microorganism will be a potential candidate as an environmental-friendly stabilizer due to its environmental acceptability. Production of bioactive molecules will be greener as well as meets the worldwide biotechnology needs.

Keywords: Extraction, Mixing, Optimization, Affinity, Reverse Micelles, Antibiotics

1. Introduction

The biotechnological process has grown since early 1980s and becoming more important nowadays because it offers numerous advantages over to chemical synthesis. In separating the biological products, the constraint is the maintenance of the producing environments which are susceptible to chemical and physical

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changes with respect to time. These diluted complex molecules and multicomponent mixtures are also prone to be sensitive to temperature, pH, and concentration throughout the process [1]. Liquid-liquid extraction is a versatile technology and very useful in the separation of compounds such as biomolecules, chemicals and nanoparticles. The process transfer is motivated by the differences in polarity and hydrophobic or hydrophilic character of the feed components [2]. Liquid-liquid extraction by reverse micelles has successfully been demonstrated to be capable of selectively solubilise the biomolecules such as proteins [3], amino acids [4] and enzymes [5]. It greatly received attention in recent years due to its ability to solubilise the biomolecules while retaining their bioactivities and native structures in the organic environment [6]. The distribution of biomolecules between the organic media and aqueous phase in reverse micelles system is largely driven by the operational parameters such as water content, aqueous pH, ionic strength, type of salt, type of solvent, concentration and type of surfactant [7]. Surfactant applications in reverse micelles extraction have so far been confined to chemical surfactants which synthetically derived from petroleum, thus may causing the environmentally and toxically problems. Therefore, biosurfactant which synthesized by variety of microorganism will be a potential candidate as environmental-friendly development in modern bioseparation technology. In this short review, we disscuss the potential applications of biosurfactant in bioseparation as an alternative to the synthetic surfactant. With its ability as stabilizer in reverse micelles system, we can look forward to biosurfactant as green molecules of the future.

2. Biomolecules Separation

The typical production of biomolecules mainly involves two main steps, i.e. fermentation section, and subsequently the downstream process. The manufacturing of biomolecules initiated with a highly specialized fermentation process containing very dilute and complex mixtures which end up with low products concentration [8]. Then, the products quality depends strongly throughout the downstream processing which comprises a large, energy-intensive separation and purification steps.

2.1. Reverse Micelles System

Reverse micelles consist of spheroidal aggregates of surfactant molecules which spontaneously formed in the organic solvent media [9]. As shown in Fig. 1, the system is forming when the polar head groups of surfactant molecules are attracted by aqueous core and oriented towards inside, and a non-polar part of hydrocarbon chain is attracted by organic phase and point towards outside [10]. This is due to the aggregates generally constituted in ternary surfactant-water-oil mixtures which can be referred as a phase region of Winsor II system [11]. The inner core of reverse micelles essentially is a nano-sized droplet of water act as a host of solubilised molecules. Water and hydrophilic molecules such as biological active compounds can be solubilised inside the cores, which stabilized by surfactant layer shielding the water phase from the oil continuum [9].

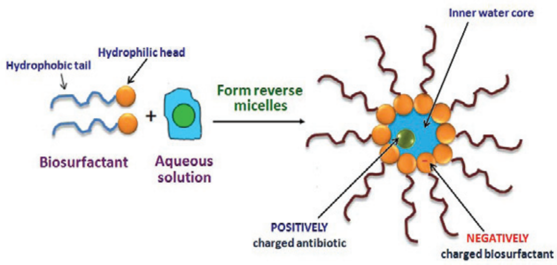

Fig. 1. A typical structure of reverse micelle

2.2. Reverse Micelles Properties and Benefits

The reverse micelles extraction has a great potential to be implemented in the recovery, separation and purification of the targeted biomolecules due to it can be implemented only under moderate conditions. The main advantage is the selective solubilisation of biomolecules inside reversed micelles which provides a logical aqueous environment protecting the bioactivities of the biomolecules [12]. Reverse micelles have successfully been carried out by many researches due to its system has a lower viscosity and shorter phase separation time [13]. Furthermore, reverse micelles are thermodynamically stable, optically transparent, low costs, energy savings, and can easily be operated continuously at steady state, which are beneficial both for large-scale production and for quality control [13].

2.3. Factors Affecting Biomolecules Transfer

The partitioning of biomolecules between the micellar phase and an aqueous phase is dependent upon the environments of both bulk aqueous phase and organic phase. Factors that affect the phase transfer include water content, pH values, ionic strength, temperature, surfactant type and concentration, and type of solvent [14]. The fundamental studies of the factors concluding selective separation of biomolecules are essential to establish correlations between physicochemical properties of the biomolecules and shows below:

1) Water content is described by the water to the surfactant molar ratio ($[H_2O]/[\text{surfactant}]$) renowned as w_o or water in oil [15]. This parameter indicates the structure and size of the micelles, and the number of surfactant molecules per micelle with the assumption of that all the surfactant is located in the micellar wall. Reverse micelles is formed when the surfactant aggregates contain a small amount of water, i.e. once w_o is below 15 [10].

2) The water phase pH of the solution determines the net charge of biomolecules. The pH must be at a level that generates a biomolecule net charge opposite in sign to the surfactant headgroup, so that there is an attraction between the biomolecule surface and the polar headgroups on the internal surface of the reversed micelle. Studies on the pH value effect on protein extraction by the reversed micelle process indicate that the difference between the pH and the isoelectric point (pI) of the protein must oscillate between 1 and 2 points for higher extraction efficiency [16].

3) In reverse micelles structures, repulsion occurs between the surfactant molecules which caused by the surfactant charge and ions of the opposite charge present in the micellar water phase. These ions control the repulsion force of the surfactant headgroups and consequently the micellar sizes. Increases in the ionic strength of the biomolecules feed solution can be expected to reduce the interaction between the biomolecule and surfactant head groups, hence decreasing the biomolecules solubilization. This is due the screening effect, that is, the formation of an electrostatic shield that reduces the intensity of the electrostatic interaction between biomolecule and surfactant as explained by Andrews [17].

4) Changes in temperature have drastic effects on the physicochemical properties of the reverse micellar system. Marcozzi [18] observed a significant increase in α-chymotrypsin recovery when carrying out backward extraction at 38^{o}C, and when Glatz [19] conducted the backward extraction of glucoamylase at 35^{o}C, the recovery of enzymatic activity increased from 40% to 90%.

5) Solvent has known to influence the biomolecules transfer between aqueous phase and organic phase. The solvents that commonly be used in reverse micelles are organic and immiscible in water [15, 20]. They included isooctane, n-octane, heptanes, cyclohexane, benzene, kerosene, and chloroform. However, Ruth and his researchers [21] reported the possibility of creating the reverse micellar environment with addition of

several different polar but does not support for large micellar size.

6) Surfactant type and concentration is the most important variables for biomolecules solubilisation by reverse micelle. Biomolecule solubilisation in the organic phase is increased by increasing the surfactant concentration [22]. Choosing an appropriate surfactant concentration in the organic phase can change the selectivity of the reverse micellar phase for a targeted biomolecule [23].

3. Biosurfactant Potential Applications

Biosurfactant is a natural microbial surfactant which abundantly produced from natural resources by variety of microorganism [24]. It is a surface-active amphiphatic molecule of biological origin which omnipresent in biological systems and possess similar mechanisms as synthetic surfactant. Biosurfactant contains hydrophobic and hydrophilic moities that grant the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and respecitively [25]. Biosurfactant have gained considerable interest recently in a wide range of industrial applications, especially in food and pharmaceutical sector. The reason for their popularity as high value microbial products is primarily because of their specific action, lower toxicity, higher biodegradability, stable at extremes of temperature, pH, salinity and widespread applicability, and their unique structures which provide new properties that classical surfactants may lack [26].

As proclaimed by Goto [27], it is important to insure the chosen biosurfactant has no negative impact on the stability or activity of the product since biosurfactant may bind to proteins and other bioactive molecules. Thus, from the classification properties, the most known biosurfactant found out are glycolipids, which consist of long-chain aliphatic acids or hydroxyaliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids [28]. For instance, sophorolipids (SLs) is one of the most important members of glycolipids biosurfactant is produced by yeast and derived from plant. SLs consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxy fatty acid, and have a mixture of acidic and lactonic forms with at least 6 to 9 different hydrophobic sophorosides. Schippers [29] revealed that CMC and the solubilization ratio of the SLs were found to be in a good range compared to synthetic surfactant.

4. Conclusion

Since the toxicity of chemically synthesized surfactant may causing the serious health and environmental problems, replacement by alternative biosurfactant in reverse micelles extraction is a green movement in biotechnology application. Sophorolipids is the potential candidate of biosurfactant which produced by yeast, i.e. non-pathogenic molecule. With good range of critical micelle concentration and solubilization ratio, it should be further emphasized for biomolecules extraction in downstream processing. Consequently, this will contribute to diminish the side effects of releasing toxically chemical surfactant to the environment as well as promoting and commercializing the development of new green technology in bioseparation industry to the country and worldwide.

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