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Mixed reverse micellar extraction and effect of surfactant chain length on extraction efficiency

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ABSTRACT

This study investigated the effect of surfactant chain length on mixed reverse micellar extraction of cellulase. The mixed reverse micelle systems were formed by biosurfactant rhamnolipid and a nonionic surfactant tween (tween-20, tween-40, tween-60, or tween-80). Electron spin resonance (ESR) method was used in order to find the suitable chain length of tween and the best ratio of rhamnolipid/tween mixture. The key parameters such as pH and temperature in forward and backward extraction were studied, and the results showed that mixed reverse micelle with different surfactant chain length had different pH sensitivities. Rhamnolipid/tween-80 system was more sensitive to pH than other systems, and rhamnolipid/tween-20 system was the least sensitive to pH among four systems. The experimental results also showed that chain length had limited sensitivity to temperature. Rotation correlation time of cellulase solutions (original and extraction) were investigated, which proved the superiority of longer chain length system (rhamnolipid/tween-80 system).

1. Introduction

Lignocellulase has received growing attention for its role in lignin degradation and potential applications in degradation of the highly stubborn environmental pollutants [1,2]. Thus there is a trend of extracting and purifying lignocellulase in order to obtain enhanced application. Cellulase, as one representative of lignocellulase, acts as a significant role in solid waste disposal industry. For example, cellulase can break down the cellulose molecule into monosaccharides such as beta-glucose, or shorter polysaccharides and oligosaccharides. Meanwhile, cellulase is one of the lignocellulase most successfully used in industry, such as the pulp and paper industry [3–5], textile wet processing [6], bioethanol industry [7–9], wine industry [10], food processing industry [11,12], and animal feed industry [13]. As a result, the scaling-up of productive methods for commercial cellulase production has become a decisive issue [14,15].

Although many methods about extracting and purifying lignocellulase have been studied, reverse micellar extraction is still a type of novel technology worth exploring. Reverse micelles are the nanometer-sized "water in oil" structures of surfactant-molecules-based apolar organic solvents surrounding inner cores of water. These surfactant aggregation structures are known to be thermodynamically stable and have been shown to be capable of solubilizing the bioactive compounds such as enzymes, protein molecule, or amino acids [16–18]. The application of biosurfactant in reverse micellar extraction has brought a new concept of extraction. Biosurfactant-based reverse micellar extraction has tremendous advantages compared with other methods, such as low interfacial tension, no loss of native activity, and potential for continuous operation [19]. Rhamnolipid can be produced by *Pseudomonas aeruginosa*, which frequently behaved as the best characterized of the bacterial surfactants. Reverse micelle formed by rhamnolipid showed good properties in lignocellulase extraction [20].

It was reported that the addition of nonionic surfactant into ionic surfactant could modify the interface and produce considerable change in the elastic rigidity of the interface [21]. Mixed reverse micelle formed by ionic and nonionic surfactants is the typical mixed system, for the system is more flexible in the physicochemical properties compared with single-surfactant reverse micelles. Also, mixed reverse micelles possess better synergistic performance [22,23]. In our previous work, biosurfactant was added into nonionic surfactant in order to form mixed reverse micelle system, and the mixed system was found to possess more

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flexible physicochemical properties in terms of detergency, solubilization, and so on [24]. From literatures, differences in chain length of surfactant resulted in the change of reverse micelle structure and abilities [25]. Although these studies gave a wealth of important information on mixed reverse micelle systems, it is not clear at this stage to what extent the effect is due to the differences of surfactant chain length on reverse micellar extraction efficiency, especially in mixed reverse micellar extraction. The contributions of increasing or decreasing the chain length in the mixed reverse micelle system have yet to be fully deconvoluted. So it is of significance to investigate the effect of surfactant chain length on mixed reverse micellar extraction.

In this work, mixed reverse micellar extraction has been applied to extract cellulase. Biosurfactant rhamnolipid and a nonionic surfactant including tween-20, tween-40, tween-60, or tween-80 were used to form mixed systems, aiming at studying the effect of chain length of surfactant on extraction efficiency. Electron spin resonance (ESR) was used to optimize the ratio of biosurfactant and nonionic surfactants. Meanwhile, the key parameters like pH, temperature, and alcohol (in backward extraction) were investigated during the extraction process.

2. Materials and methods

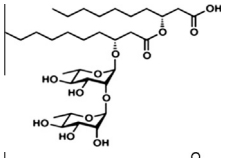
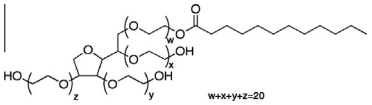
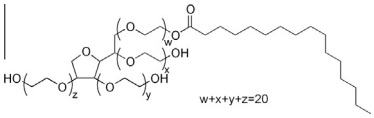
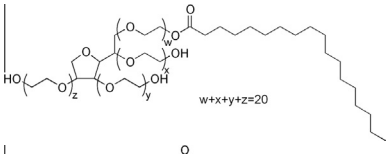
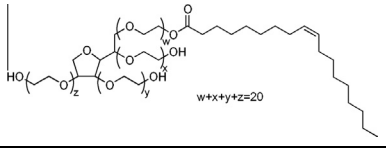
2.1. Chemicals

Rhamnolipid, tween-20, tween-40, tween-60, tween-80, and 16-doxyl stearic acid (16-DSA) were obtained from Sigma. Detailed information of surfactants in the mixed reverse micelle system are listed in Table 1. Cellulase was purchased from Shanghai Beta Biological Products Co., Ltd. All chemicals were of analytical grade. Distilled water was used throughout the experiments.

2.2. Forward and backward extraction processes

The procedure of forward extraction is as follow: weighting desired amount of surfactant (r, r is the molecular ratio of rhamnolipid and tween) and dissolving them in organic solvent (equal volume isooctane with hexanol). Meanwhile, 10 ml aqueous cellulase solution was prepared. And then the organic phase and aqueous phase (volume ratio 1:1) were mixed. The mixture was mixed thoroughly for at least 1 h using magnetic stirrer and then separated by a centrifuge at 10,000 rpm for 10 min. The two phases were separated after standing still for 20 min. The backward extraction was carried out by mixing the organic phase obtained from the forward extraction with an equal volume of stripping phase. During both the forward and backward extraction, the contents were mixed thoroughly for 40 min using magnetic stirrer and separated by a centrifuge at 10,000 rpm for 10 min. All the experiments were conducted with the best parameters. The extraction procedures were conducted in triplicate.

Table 1
Detailed information of surfactants in the reverse micelle system.

Surfactant	Chemical formula	Structure
Rhamnolipid	$C_{32}H_{58}O_{13}$	
Tween-20	$C_{58}H_{114}O_{26}$	
Tween-40	$C_{62}H_{122}O_{26}$	
Tween-60	$C_{64}H_{126}O_{26}$	
Tween-80	$C_{64}H_{124}O_{26}$	

2.3. Estimation of cellulase activity and protein content

The activity and protein content of cellulase was measured by 3,5-dinitrosalicylic acid method and Bradford method. For activity study, the absorbance was measured at 550 nm. Protein analyses were conducted against the respective blank solutions, and the absorbance was measured at 595 nm [26]. Each sample was triply analyzed and the standard deviations of all analyses were less than 5%. The activity recovery and protein recovery equations are as follow:

$$\text{Activity recovery} = \text{activity after extraction}/\text{activity before extraction} \quad (1)$$

$$\text{Protein recovery} = \text{protein after extraction}/\text{protein before extraction} \quad (2)$$

3. Results and discussion

3.1. ESR study for surfactant ratios

Surfactant ratio is a key parameter in extraction. Fig. 1 shows the effect of surfactant ratios on ESR intensity, indicating the sensitivity of ESR signal. From the result, the intensity changed with the surfactant ratio accordingly. There were peaks at 0.4, 0.5, 0.5, and 0.6 for tween-20, tween-40, tween-60, and tween-80.

In reverse micelle system, spherical cavity of water is the most typical shape which surrounded by a shell of surfactant molecules. These surfactant molecules locate between the polar cavity and nonpolar medium, functioning as an interface. The surfactant molecules dissolved in organic phase can orient themselves with

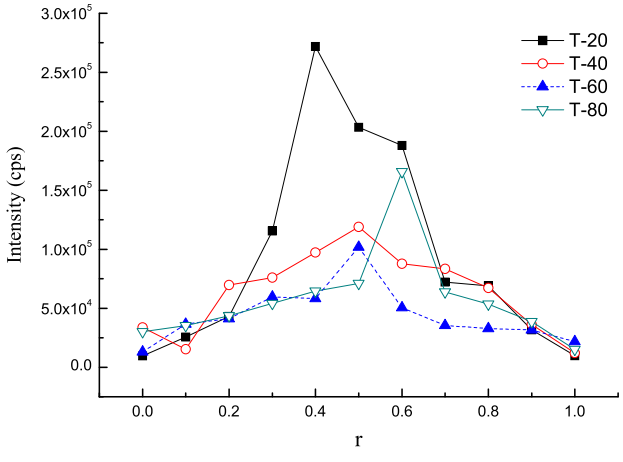


Fig. 1. Effects of r (molecule ratio of tween and rhamnolipid) on ESR intensity.