Biochemical Engineering Journal 131 (2018) 77–83

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Microemulsion system for *Colletotrichum lini* ST-1 biotransformation of dehydroepiandrosterone to 7α,15α-diOH-DHEA

Xiaojuan Zhang[a], Yuying Liu[b], Hui Li[b], Lili Su[b], Longfei Zhou[b], Jun Peng[b], Rui Shen[b], Yi Zhang[b], Jinsong Shi[b], Zhenghong Xu[a,c,*]

[a] National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China, China
[b] School of Pharmaceutical Science, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China
[c] School of biotechnology, Jiangnan University,1800 Lihu Avenue, Wuxi 214122, China

ARTICLE INFO

Article history:
Received 24 July 2017
Received in revised form
16 November 2017
Accepted 20 December 2017
Available online 20 December 2017

Keywords:
Bioconversion
Microemulsion system
Repeated cycles
Cell sustainability

ABSTRACT

7α,15α-diOH-Dehydroepiandrosterone (7α,15α-diOH-DHEA) is an important intermediate in the synthesis of steroid pharmaceutical compounds, and can be bioconverted from DHEA by *Colletotrichum lini ST-1*. A microemulsion system can improve substrate bioavailability and may also minimize DHEA cellular toxicity as cells and substrate are retained in the water and oil phase respectively. In this study, several microemulsions with a different hydrophile lipophile balance were prepared and evaluated based on their stability and bioconversion efficacy. The yield of the best formula of 7α,15α-diOH-DHEA was 61.2%, which was 3.1 times that of the traditional water phase bioconversion system. This formula was further optimized using the Taguchi orthogonal experimental design, and the yield reached 73.3%. Due to less cellular toxicity of the substrate using the microemulsion system, the repeated-cycle bioconversion based on microemulsion using recycled cells was realized for the first time, and the overall 6-cycle production (41.5 g from 60 g DHEA) was 4.3 times that of the traditional method. This highly efficient microemulsion system, with improved cell sustainability as well as bioreactivity, may be expanded to the bioconversion of other insoluble and toxic substrates.

1. Introduction

Steroid pharmaceuticals are the second most required pharmaceuticals after antibodies [1,2]. One of the important tasks in drug development is to produce new steroidal ingredients [3]. Hydroxylation of steroid compounds is an important steroid modification/functionalization due to the higher biological activity of the hydroxylated form of the steroid [4]. Compared with traditional chemical synthesis, microbial steroid conversion is a powerful technique, which has advantages including stereo- and regio-specificity, capability of generating novel steroidal compounds, high efficiency, mild reaction conditions, and less pollution; therefore, has attracted increasing attention [3,5].

Many microorganisms, including *Colletotrichum lini* [6], *Gibberella zeae* [7] and *Fusarium oxysporum* [8], have been reported to be capable of converting Dehydroepiandrosterone (DHEA) to 7α,15α-diOH-DHEA, which is an important intermediate in the synthesis of steroid pharmaceutical compounds, such as the novel oral contraceptive Yasmin [9]. The inhibitory effect or toxicity to microbial cells due to steroid substrates, including DHEA, is a huge challenge in the application of microbial conversion technologies, especially in the case of high concentration substrates [3]. In addition, the insolubility of these substrates results in retained substrate and cell contact, which significantly limits the bioconversion efficacy [10]. Therefore, reducing substrate toxicity to retain cellular bioactivity while improving substrate solubility is the major bottleneck in DHEA bioconversion.

Previously, supplements with organic solvents, oil or liquid polymers were applied to enhance substrate solubility for biocatalyst processes, including soybean sterols to 17-Ketosteroids conversion [11], soy sterol to androstenedione [12], and DHEA to 7α,15α-diOH-DHEA using *Colletotrichum lini* [7]. Using these strategies, the bioconversion efficacy was improved due to enhanced substrate solubility. However, the substrate and the cells were trapped in different phases, and the opportunity for contact was limited to the water/oil interface area. It can be hypothesized that another advanced bioconversion system-emulsion system may be more suitable for steroid microbial conversion. An emulsion is a thermodynamically unstable, heterogeneous system contain-

* Corresponding author at: National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, 214122, China.
E-mail address: zhenghxu@jiangnan.edu.cn (Z. Xu).

https://doi.org/10.1016/j.bej.2017.12.014
1369-703X/

ing at least one immiscible liquid phase intimately dispersed in another in the form of droplets [13]. The types of emulsions are oil in water (O/W) and water in oil (W/O) depending on the composition and preparation method. Emulsions basically have three components: water phase, oil phase, and surfactants. The physical and chemical properties of these components can affect emulsion behavior, including droplet size, size distribution and stability of the emulsion. The thermodynamic conditions as well as preparation methods determine the above-mentioned properties [14]. In an O/W emulsion bioconversion system, the organic solvent phase, which is a small droplet-shaped reservoir with dissolved hydrophobic substrate, is well dispersed in the water phase, where the cells are located. Similar to the other two phase reaction systems, use of the O/W emulsion system can improve substrate solubility and bioavailability, and the substrate/product inhibition during microbial transformation can be significantly reduced as the substrate and microbe are retained in separate phases [14]. It is worth to note that a unique merit of the emulsion compared with other two-phase systems is the significantly larger interfacial area between the organic and water phases, which is beneficial for bioconversion due to enhanced mass transfer between the two phases [15]. There are reports on the use of microemulsion systems for biotransformation. Smolders *et al.* [16] applied microemulsion for 1,2-dehydrogenation of 16-methyl-Reichstein's compound S-21-acetate (16MRSA) in high concentration, and optimized several parameters which affected the system. Prichanont et al. [17] developed a microemulsion system that can use *Mycobacterium sp*. for chiral epoxide production. Zheng *et al.* [18] developed an emulsion system for DDT degradation by the white rot fungus *Phanerochaete chrysosporium*. In this study, microemulsion system for *Colletotrichum lini ST-1* biotransformation of dehydroepiandrosterone to 7α,15α-diOH-DHEA was established, giving the considerations to the principles for developing stable emulsion systems, as well as the essential factors for bioconversions, including activity of microorganism, substrate toxicity and substrate availability etc. The high efficacy of emulsion system for DHEA bioconversion was confirmed, and the mechanism underpin the high bioconversion efficacy using microemulsion system was discussed. The information obtained in this study is useful for further improvement of emulsion system or the development of other new format of bioconversion system.

2. Materials and methods

2.1. Materials

DHEA (99% purity), 7α,15α-diOH-DHEA (98.4% purity), and progesterone (99.6% purity) were obtained from Xianju Pharmaceutical Company (Zhejiang, China). Tween-65, Span-80, Triton X-100, acetonitrile and ethyl acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were used without further purification and were of chromatographic or analytical grade. Colleseed oil (food grade) was purchased from Yihai Kerry Investment Company Ltd. (Shanghai, China).

2.2. Microorganism and medium

The microorganism used in this study was *Colletotrichum lini* ST-1. This strain was deposited in the China General Microbiological Culture Collection Center with the depositing No. of 6051; Beijing, China). *C. lini* ST-1 cells were cultured on slant medium containing (in g/L) glucose 30, $FeSO_4$ 0.01, $NaNO_3$ 3, K_2HPO_4 1, KCl 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, and agar 20 at pH 7.0 and 30 °C. The seed culture was prepared in medium containing (in g/L) glucose 15, yeast extract 15, corn steep liquor 3, and bean cake powder 10 at pH 7.0, and grown in 500 mL flasks with 100 mL of seed medium on a rotary shaker at 220 rpm and 30 °C for 24 h. The fermentation medium was inoculated with 10% seed culture. The medium composition and culture conditions were the same as for the seed culture. *C. lini* ST-1 cells cultured for 24 h and at the end of logarithmic growth phase, were harvested via filtration using eight-layers of sterile gauze and washed twice with 20 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 6.5) for further use.

Table 1
The composition and HLB value of each formula and their stability.

Formula #	Composition (%)				HLB value	Stability score
	Canola oil	Tween65	Span80	Triton100		
1	40	8	2	0	9.3	68
2	40	4	2	2	10.0	92
3	40	9	2	0	9.4	57
4	40	6	2	1	9.6	78
5	40	11	4	0	8.8	37
6	40	8	4	3	9.6	83
7	20	7	1	0	9.7	78
8	20	4	2	2	10.0	100
9	20	9	3	0	9.0	100
10	20	8	6	0	7.8	80
11	20	8	4	4	10.0	100
12	20	5	3	0	8.2	68

*Determined as mass ratio.

Table 2
The arrangement of factors and levels.

Level (g/L)		Factor			
		A	B	C	D
		oil	Span 80	Triton 100	Tween 65
Design 1	1	100	20	10	20
	2	200	30	20	40
	3	300	40	30	60
Design 2	1	200	15	15	30
	2	250	20	20	40
	3	300	25	25	50

2.3. Construction of the oil-in-water microemulsion

Each emulsion sample contained 20%–40% of colleseed oil, and DHEA was pre-dissolved in colleseed oil at 60 °C at a concentration of 2.5% and 5% respectively, to achieve 1% DHEA concentration in the final microemulsion system. Different amount of surfactants, including Span-80, Tween-65, and Triton X-100, were added to the oil to reach the desired hydrophile lipophile balance (HLB) value, which was calculated using the weight fraction of each surfactant as shown in Eqs. (1) and (2):

$$HLB(mixture) = HLB_A \times 0.01 \times A + HLB_B \times 0.01 \times B \quad (1)$$

$$A\% + B\% = 100\% \quad (2)$$

All percentile mentioned in this paper is in mass ratio, and The specific values are listed in Tables 1–3. PBS buffer was then added to the mixture to reach the final sample size of 30 g. The mixture was heated to 90 °C, and vortexed for 1.5 min to form a microemulsion.

2.4. Bioconversion process

When the microemulsion had cooled to room temperature, 4.5 g freshly collected *C. lini* ST-1 cells were added to the 30 g emulsion system. Biotransformation of DHEA to 7α,15α-diOH-DHEA took place in a 250 mL shake flask on a rotary shaker at 220 rpm and 30 °C for 48 h. All experiments were performed in triplicates.