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A Closed Concept of Extractive Whole Cell Microbial Transformation of Benzaldehyde into L-Phenylacetylcarbinol by *Saccharomyces cerevisiae* in Novel Polyethylene-Glycol-Induced Cloud-Point System

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Abstract Extractive microbial transformation of benzaldehyde into L-phenylacetylcarbinol (L-PAC) by *Saccharomyces cerevisiae* (Baker's yeast) has been carried out in a novel polyethylene-glycol-induced cloud-point system (PEG-CPS). The extractive microbial transformation in the PEG-CPS and a downstream process for stripping of the product from the microbial transformation broth with microemulsion extraction are demonstrated. The results indicate that the PEG-CPS maintains the advantage of CPS for in situ extraction of polar product in the microbial transformation. At the same time, the utilization of hydrophilic nonionic surfactant in the PEG-CPS is favorable for stripping of product from the nonionic surfactant in the microbial transformation broth by Winsor I microemulsion extraction. Thus, a closed concept of in situ extraction of polar product in microbial transformation and its downstream process of product recovery are fulfilled at the same time.

Keywords Microbial transformation · Nonionic surfactant · Extraction · Cloud-point system · Microemulsion

Introduction

Extractive microbial transformation in two-phase systems, i.e., a microbial transformation carries out in an aqueous solution phase, whereas an immiscible auxiliary phase acts as a

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substrate reservoir and/or a product extractant, is a powerful tool to bioprocess engineering [1]. In principle, some obstacles of microbial transformation in aqueous solution, including substrate insolubility, substrate/product inhibition, product-further-degradation, and sometimes an adverse thermodynamic equilibrium, can be overcome by the extractive microbial transformation [2]. The auxiliary phase may be an organic solvent (water–organic solvent two-phase system) [3], a polymer solution (aqueous two-phase system) [4] or a nonionic surfactant aqueous solution (cloud-point system, CPS) [5].

CPS is an aqueous nonionic surfactant micelle solution at a temperature above its cloud point or in the presence of certain additives to form a two-phase system, where one phase is a dilute phase and the other is a surfactant-rich phase or coacervate phase. The CPS has been utilized as a separation method, which is known as cloud-point extraction, for extraction of metal ions, organic compounds, and bioactive materials in the analysis field [6, 7]. A large-scale extraction of protein [8] or noble metal [9] by cloud-point extraction has also been reported. Recently, extractive microbial transformation in CPS has also been exploited in our previous work [5, 10]. The main advantages of extractive microbial transformation in CPS include more biocompatible comparison to that of water–organic solvent two-phase system and more cheap in phase-forming components comparison to that of aqueous two-phase system [11, 12]. However, stripping of organic product from nonionic surfactant in a microbial transformation broth is very challengeable [13, 14].

Microemulsion is a ternary system consisting of water, organic solvent, and surfactant, occasionally with an alcohol as a co-surfactant, which is first described by Winsor [15] as shown in Fig. 1. The microemulsion conducts a transition from Winsor II to Winsor III and then to Winsor I with a decrease of temperature and salt concentration or an increase of surfactant hydrophile–lipophile balance (HLB) value [16]. Although extraction of organic compounds into the excess oil phase while remaining nonionic surfactant in the O_m phase by Winsor I microemulsion extraction [17] has been reported, the separation efficiency is limited by the low HLB value of nonionic surfactant [18]. Unfortunately, a nonionic surfactant with a relatively lower HLB value, which forms CPS at about room temperature, is prerequisite for extractive microbial transformations in CPS [5].

Microbial transformation of benzaldehyde into L-phenylacetylcarbinol (L-PAC) by *Saccharomyces cerevisiae* (Baker's yeast) as shown in Fig. 2 is inhibited severely by the

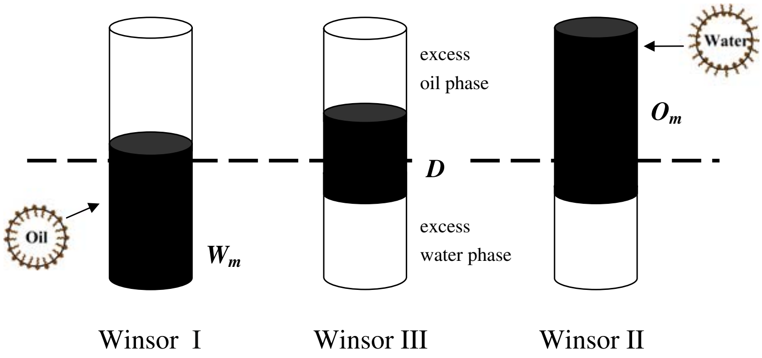

Fig. 1 Schematic representation of a ternary system consisting of water, organic solvent, and surfactant two-phase systems, called Winsor I and Winsor II, correspond to oil in water microemulsion (W_m) coexisting with an excess oil phase and water in oil microemulsion (O_m) coexisting with an excess water phase, respectively. A Winsor III system corresponds to concentrate most of surfactant into a bicontinuous phase (D) coexisting with excess oil phase and excess water phase. The symbol of *black area* represents containing rich surfactant; the *blank one* represents containing poor surfactant

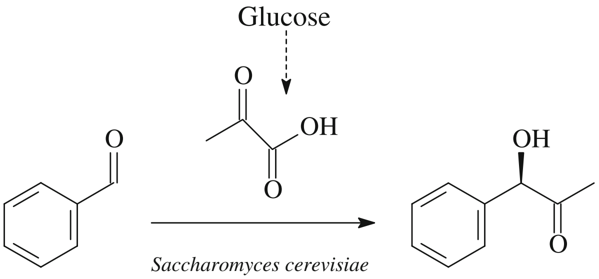

Fig. 2 Microbial transformation of benzaldehyde into L-PAC by *Saccharomyces cerevisiae*

toxic substrate and product [19]. Even more, the product has a relatively higher polarity with log *P* 0.6 (*P* is defined as the partitioning coefficient of an organic compound between octanol and water). The relatively higher polar product L-PAC is inaccessible to water–organic solvent two-phase system for extractive microbial transformation [20] as the biocompatibility and in situ removal of polar product fails to fulfill at the same time. Extractive microbial transformation in a novel polyethylene-glycol-induced cloud-point system (PEG-CPS) has been developed [10, 21] and a separation of nonvolatile organic compounds from high HLB value nonionic surfactant in a PEG-CPS by Winsor I microemulsion extraction has also been carried out in our recent work [22]. In the present work, a hydrophilic nonionic surfactant with a relatively higher HLB value, which was defined as H-Triton X-100, was obtained from the commercial nonionic surfactant Triton X-100 by Winsor I microemulsion extraction. Then, phase diagrams of PEG-CPS containing H-Triton X-100 and PEG 20,000 were determined. A closed concept of extractive microbial transformation of benzaldehyde into L-phenylacetylcarbinol (L-PAC) by *Saccharomyces cerevisiae* (Baker's yeast) in the PEG-CPS and the downstream process for stripping of the product from the microbial transformation broth with microemulsion extraction were demonstrated.

Experimental

Materials

Dried *Saccharomyces cerevisiae* (baker's yeast) was purchased from Angel Yeast Co. Ltd. (Wuhan, China). It was isolated and purified as detailed in our previous work [23]. Nonionic surfactants Triton X-100 (*t*-octylphenoxy polyoxethylene ethyl ethers with an average number of ethylene unit about 10, cloud point 68 °C and HLB value 14) was purchased from Fluka and PEG 20,000 (with average molecular weight of 20,000, the fraction of molecular weight from 18,000 to 22,000 above 95%) was purchased from Shanghai Chemical Agent Co. Ltd. (Shanghai, China). The other reagents were of reagent grade or better quality.

Fraction of Hydrophilic Nonionic Surfactant

A certain volume of 10% (*w*/*v*) nonionic surfactant Triton X-100 aqueous solution was mixed completely with equal volume of organic solvent *n*-butyl acetate. Then, it was sealed with stopper and incubated into a 25°C water bath for enough time until complete phase separation. Thus, a Winsor I microemulsion was formed. The volumes of the W_m phase and the excess oil phase were recorded and the nonionic surfactant concentration in each phase was determined by HPLC analysis. The excess oil phase and the W_m phase were fetched out and the surfactant in each phase was recovered by rotated evaporation under 85°C and 0.08-MPa vacuum. The fraction of nonionic surfactant was calculated by the amount of nonionic surfactant in each phase to the total amount of nonionic surfactant in the Winsor I microemulsion system. The residual surfactant of each phase was washed with a certain volume of distilled water to get a 1-g/100-ml nonionic surfactant aqueous solution. The nonionic surfactant aqueous solution was subjected to cloud-point determination. A recovery of the hydrophilic nonionic surfactant in the W_m phase was called as H-Triton X-100 and was used in the following experiments.

Determination of Cloud Point

Cloud point was determined by visually observed the turbidity of an aqueous nonionic surfactant solution [24]. Every sample of nonionic surfactant aqueous solutions was put into a tube with a cap to prevent it from evaporation, which was heated or cooled with a temperature step of 0.5°C in every time and then stabilization of 2 min for thermoequilibrium. The nonionic surfactant aqueous solution became cloudy with the increase of temperature and the cloudy became clear again with the decrease of temperature. The average temperature of appearance and disappearance of the cloudy was taken as cloud point.

Estimation of Phase Diagram

A series of aqueous solution (20 ml) containing a certain concentration of nonionic surfactant and PEG 20,000 or NaCl were mixed completely. Then the aqueous solutions were sealed with stopper and incubated into a 25°C water bath for enough time until complete phase separation. The phases were collected separately by a syringe and the volume of each phase was recorded. The nonionic surfactant concentration in every phase was determined by HPLC analysis. The total weight of nonionic surfactant and PEG or NaCl in every phase was determined by evaporation to removal of water with rotated evaporation at 45°C and 0.08-MPa vacuum. The PEG or NaCl weight in every phase was obtained by a subtraction of the nonionic surfactant weight from the total weight of nonionic surfactant and PEG or NaCl in the corresponding phase, respectively. In all determinations, the recovery of nonionic surfactant and PEG/NaCl with a relative standard deviation less than 5% was accepted in our experimental error ranges.

Microbial Transformation

The microorganism *Saccharomyces cerevisiae* was maintained on an agar slant with 15 g agar per liter of nutrient medium. The nutrient medium consisted of 20 g glucose, 20 g peptone, and 10 g yeast extract per liter of tap water. Inocula were grown in 25 ml of the nutrient medium in a 250-ml flask at 200 rpm, 30°C for 12 h. Then, 1 ml of the inocula was added aseptically into 25 ml of the nutrient medium for growing culture in a 250-ml flask at 200 rpm, 30°C for 1 day. The culture broth was subjected to centrifuge and the biomass was harvested for microbial transformations.

The microbial transformation medium consisted of 20 g peptone, 25 g glucose, 10 g yeast extract, 35 g $Na_2HPO_4 \cdot 12H_2O$, 10.7 g citric acid, 1 g $MgSO_4 \cdot 7H_2O$ and 0.05 g

$CaCl_2 \cdot 2H_2O$ per liter of tap water. The CPS consisted of the microbial transformation medium with a certain concentration of PEG 20,000 and nonionic surfactant H-Triton X-100. A microbial transformation procedure was the same as evaluation in our previous work [21], i.e., every 100 ml of CPS combining with 0.6 ml acetaldehyde (35% volume content in aqueous solution) and 7 g wet baker's yeast cell as biocatalyst were filled into a 500-ml flask for microbial transformation at 200 rpm, 30°C for 8 h. The concentrations of PEG, nonionic surfactant and substrate benzaldehyde were detailed in the legend of the figures. After the microbial transformation, the CPS was subjected to centrifuge to removal of biomass. The clear supernatant was used in the following downstream process.

Downstream Process Procedure

Two kinds of downstream process procedures were applied for separation of the product from the clear supernatant of microbial transformation broth. One was a cascade of Winsor II microemulsion extraction and Winsor I microemulsion extraction with organic solvent *n*-butyl acetate. The other was a direct Winsor I microemulsion extraction with organic solvent ethyl ether.

In the cascade of Winsor II microemulsion extraction and Winsor I microemulsion extraction, the product, byproduct, and substrate along with the nonionic surfactant in the supernatant of the microbial transformation broth were firstly extracted into the O_m phase with Winsor II microemulsion extraction by an addition of equal volume of *n*-butyl acetate at 60°C. The hydrophilic components, such as PEG, were left in the excess water phase. Then, the O_m phase of the Winsor II microemulsion extraction was subjected to Winsor I microemulsion extraction by addition of equal volume of water at 10°C. The product, byproduct, and substrate remained in the excess oil phase and the nonionic surfactant was extracted into the W_m phase. Thus, the nonionic surfactant and the product were separated out.

In the direct Winsor I microemulsion extraction, an equal volume of organic solvent ethyl ether was added into the supernatant of a microbial transformation broth and kept at 10°C to form Winsor I microemulsion. The product, byproduct, and substrate were extracted into the excess oil phase while the nonionic surfactant and PEG remained in the W_m phase. Thus, the nonionic surfactant and the product were separated out.

Analysis Methods

The high stereoselectivity of various species of yeasts makes the microbial transformation of benzaldehyde get high chiral purity product L-PAC [25]. Thus, substrate, along with product L-PAC contents was usually determined by a conventional HPLC procedure [26]. A basic procedure was performed on Water series with Hypersil C_{18} column (5 μm, 250× 4.6 mm) and Waters 2996 photodiode array detector as our previous work [21]. One milliliter sample from mixture supernatant or phases of microemulsion was diluted with 2 ml of ethanol. The substrate and product L-PAC were detected with UV detection at 283 nm with acetonitrile:water (70:30) as the mobile phase (1.0 ml/min) at a retention time of 9.1 and 19.2 min, respectively. The byproduct benzylalcohol was detected at 254 nm with a retention time of 8 min. The nonionic surfactant concentration analysis was also carried out with HPLC procedure. The sample was diluted with methanol from ten to 100-fold according to the sample concentration. The nonionic surfactant was detected at 276 nm with a retention time 5.9 min with methanol:water (95:5) as the mobile phase (1.0 ml/min). The concentration of surfactant, product, byproduct, and substrate was estimated with its corresponding peak area, respectively.

Results

Phase Diagram of PEG-CPS

A 10% (*w*/*v*) of commercial nonionic surfactant Triton X-100 aqueous solution was subjected to Winsor I microemulsion extraction by addition of equal volume of *n*-butyl acetate at 25 °C. The nonionic surfactant fraction and the corresponding cloud point of the nonionic surfactant in each phase were determined as shown in Fig. 3. The cloud point of the commercial nonionic surfactant Triton X-100 was 66 °C, which was higher than that of the excess oil phase and lower than that of the W_m phase. The nonionic surfactant in the W_m phase was the hydrophilic fraction of Triton X-100 and had a relatively higher HLB value by deduced from the change of the cloud point. It was defined as H-Triton X-100 and used in this text.

The phase diagrams of nonionic surfactant aqueous solution induced by PEG 20,000 or NaCl at 25 °C were determined as shown in Fig. 4. The nonionic surfactant and PEG (salt) aqueous system was designated by a binodal curve which separated the two-phase area from the single phase zone. The binodal curve gave the exact compositions of each phase. The systems consisted of a surfactant-rich phase in equilibrium with a polymer or salt rich phase, where both phases had a high water fraction. It was similar to that of aqueous two-phase system. The phase separation of the hydrophilic nonionic surfactant aqueous solution at about room temperature was ascribed to the decrease of the cloud point of nonionic surfactant aqueous solution by addition of polymer PEG (salt) [27]. The binodal line shifted up and the two-phase area decreased with the increasing nonionic surfactant HLB value as shown in Fig. 4a and b. It was ascribed to the high HLB nonionic surfactant with a high cloud point.

Microbial Transformation in PEG-CPS

Microbial transformations in PEG-CPS with different concentration of PEG and H-Triton X-100 were determined as shown in Fig. 5. To a certain PEG-CPS, the byproduct formation was inhibited at high substrate concentrations. The product concentration increased and

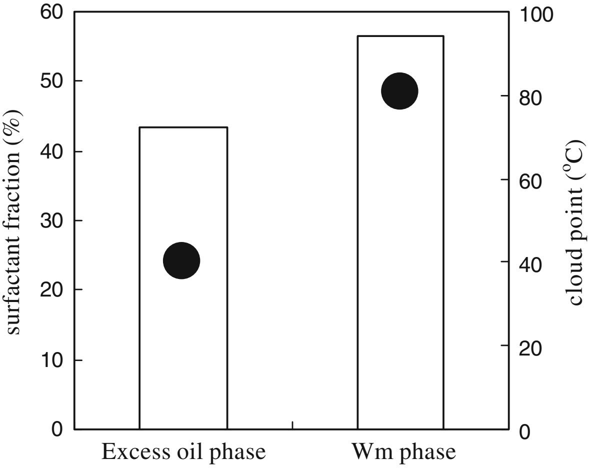

Fig. 3 Fraction of hydrophilic part of the commercial nonionic surfactant Triton X-100 with Winsor I microemulsion extraction. The *blank bar* was the surfactant fraction of each phase and the filled dot was the corresponding cloud point of nonionic surfactant aqueous solution with concentration 1% (*w*/*v*), respectively

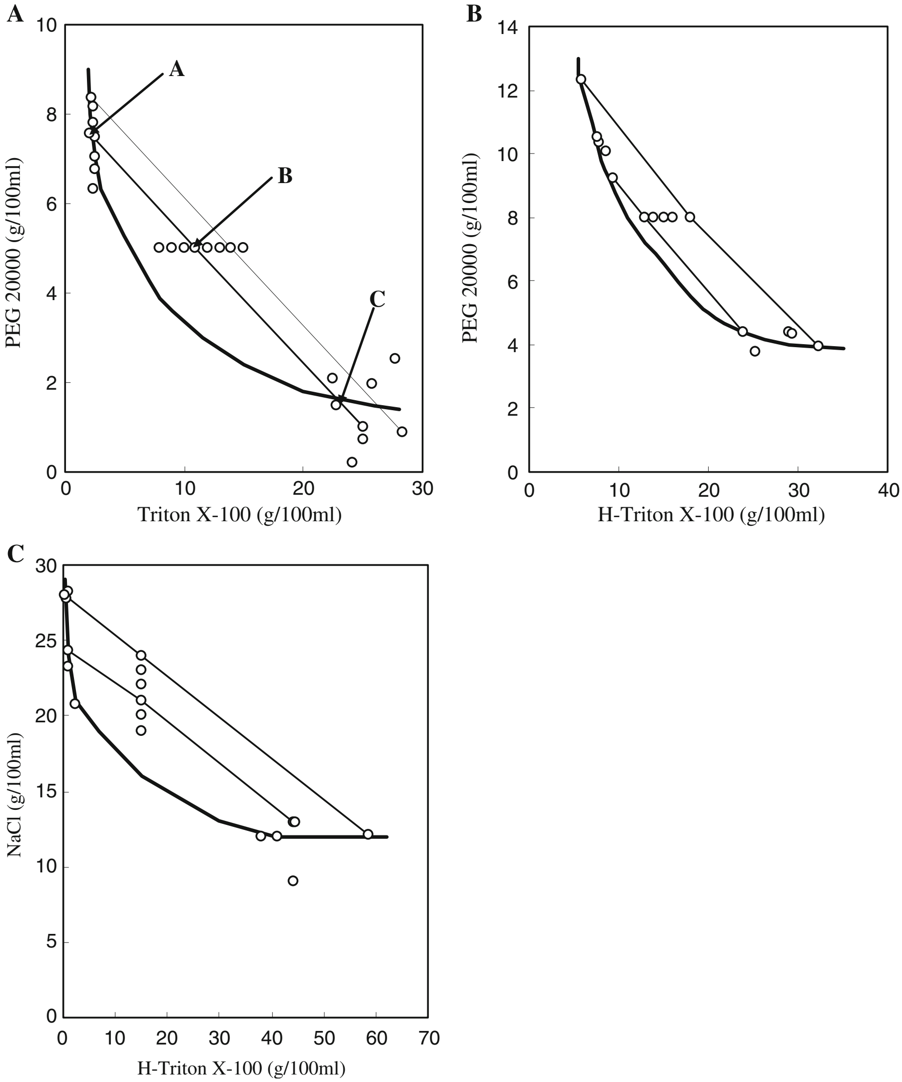

Fig. 4 Phase diagram of PEG (salt)-CPS at 25°C **a** Triton X-100 and PEG 20,000; **b** H-Triton X-100 and PEG 20,000; **c** H-Triton X-100 and NaCl. A point in the two-phase area (such as *B* in the **a**) represents the compositions of a two-phase system. The line-joining points (such as *A* and *C* in the **a**) in the binodal line is called tie-line

then decreased down with increasing substrate concentration, i.e., there was an optimal substrate concentration and its corresponding maximum product concentration. A similar result has also been reported in literatures [28]. For different PEG-CPS, corresponding to different tie-line length as shown in Fig. 4b, the maximum product concentration increased and then decreased down again with increasing tie-line length. A similar result has also been recorded in a microbial transformation of sterols in a CPS [5]. The fraction of substrate/product solubilization into the surfactant micelle increases with increasing nonionic surfactant concentration. On the one hand, the solubilization of substrate into

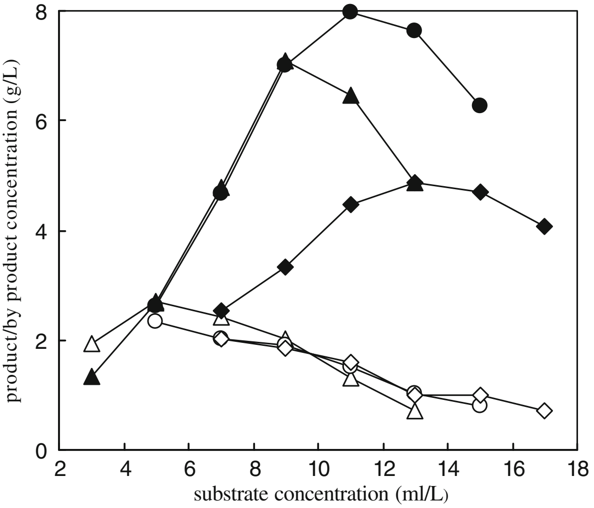

Fig. 5 Microbial transformations in PEG-CPS with different tie-line length. Triangle was 3 g PEG 20,000, 10 g H-Triton X-100; Circle was 6.4 g PEG 20,000, 15 g H-Triton X-100 and diamond was 9 g PEG 20,000, 20 g H-Triton X-100 in every 100 ml of microbial transformation medium, respectively. The *filled symbols* were product L-PAC concentrations; the open ones were byproduct benzylalcohol concentrations

the surfactant micelle eliminates the toxicity of substrate/product in extractive microbial transformation in PEG-CPS [10]. On the other hand, an excess solubilization of the substrate into the surfactant micelle at high surfactant concentration also decreases the available substrate concentration by the microorganisms [29], which leads to the low product concentration. Even more, the high PEG concentration in the PEG-CPS may also lead to cell aggregation, change of cell-surface hydrophobicity and insufficient dissolved oxygen [30], which are important factors affect the production of product L-PAC [25]. The PEG-CPS consisting of 6.4 g PEG 20,000 and 15 g H-Triton X-100 in every 100 ml of microbial transformation medium had a maximum product concentration at the corresponding optimal substrate concentration, which was chosen in the following study.

Downstream Process

The effect of temperature on H-Triton X-100 partitioning in an oil–water–surfactant ternary system was determined as shown in Fig. 6. With an increase of temperature, the oil–water–surfactant ternary system transferred from two phases to three phases and then to two phases again. The oil-phase volume and the surfactant fraction in the oil phase increased with increasing temperature as shown in Fig. 6a and b, respectively. It indicated that the ternary system had been transferred from Winsor I to Winsor III and then to Winsor II microemulsion as shown in Fig. 1. Different from most of ionic surfactant in oil–water–surfactant ternary system, the phase behavior of nonionic surfactant in oil–water–surfactant ternary system was very sensitive to temperature. Above 55% surfactant in the W_m phase at 10°C had transferred to above 95% in the O_m phase at 60°C as shown in Fig. 6b. It provokes us to propose a cascade of Winsor II and Winsor I microemulsion extraction downstream processes for separation of the product from the nonionic surfactant in the microbial transformation broth. A further purification of product to get crude product [28] and reactive purification of crude product [31] had been reported in the literature, which we did not examine in our text.

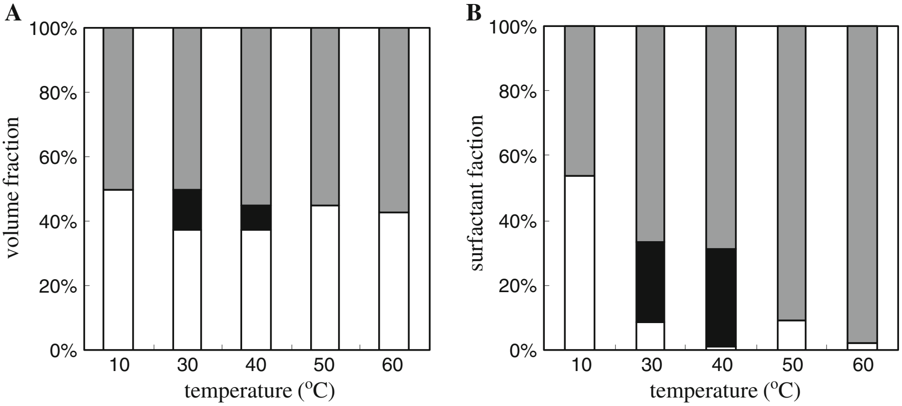

Fig. 6 Effect of temperature on the partitioning of nonionic surfactant in oil–water–surfactant ternary system **a** volume fraction; **b** surfactant fraction. The volume ratio of the supernatant of a microbial transformation broth to organic solvent *n*-butyl acetate was 1:1. The *gray bars* were organic solvent phases (*top phase*); the *black ones* were bicontinuous phases (*middle phase*); the *blank ones* were the water phases (*bottom phase*)

The clear supernatant of a microbial transformation broth was subjected to Winsor II microemulsion extraction by addition of equal volume of *n*-butyl acetate at 60 °C. A recovery ratio of product, substrate, and surfactant into the O_m phase were about 94, 98, and 94 %. It was failed to separate the product from the nonionic surfactant in this process. However, the hydrophilic compounds, such as PEG, were left in the excess water phase. The elimination of PEG is favorable for separation of organic compounds from nonionic surfactants aqueous solution by Winsor I microemulsion extraction [22].

The aforementioned O_m phase was subjected to a Winsor I microemulsion extraction by addition of equal volume of water at 10 °C as shown in Fig. 7. After the first-time extraction, the excess oil phase was transferred and subjected to extraction with equal volume of water again, and so on. Ethyl ether is usually utilized to extract the product L-PAC from the microbial transformation broth [28]. However, the low boiling point of ethyl ether excludes its application in the cascade of Winsor II and Winsor I microemulsion extraction procedure. A direct extraction of the clear supernatant of the microbial transformation broth by Winsor I microemulsion extraction with ethyl ether at 10 °C was also determined as shown in Fig. 7.

As shown in Fig. 7, the utilization of *n*-butyl acetate for Winsor I microemulsion extraction, its best separation efficiency with surfactant recovery ratio 78% was achieved by the first-time Winsor I microemulsion extraction. The nonionic surfactant in the excess oil phase was recovered and redissolved to get 1% (*w*/*v*) nonionic surfactant concentration aqueous solution with a cloud point 35 °C, which indicated that the residual nonionic surfactant in the excess oil phase of the first-time Winsor I microemulsion extraction was a hydrophobic fraction of the nonionic surfactant H-Triton X-100. However, by utilization of ethyl ether as organic solvent for direct Winsor I microemulsion extraction, a marked increase of the accumulative surfactant recovery ratio in the second run of Winsor I microemulsion extraction was found. A further increase of extraction recycles led to a limited increasing accumulative surfactant recovery ratio. At the same time, the residual product/substrate recovery ratio decreased, and the residual product recovery ratio decreased more rapidly, by further Winsor I microemulsion extraction as shown in Fig. 7. A replacement of organic solvent *n*-butyl acetate with ethyl ether in the Winsor I

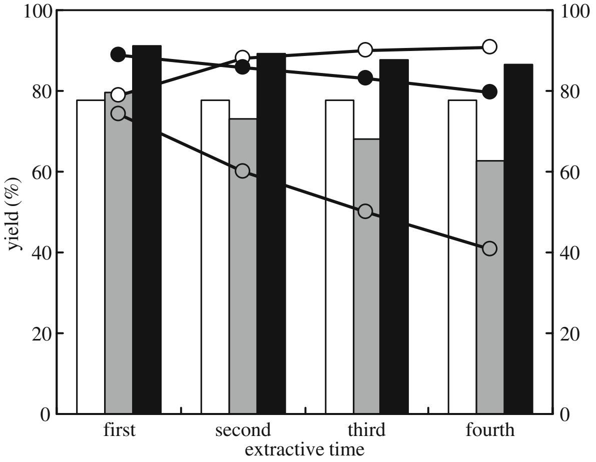

Fig. 7 Winsor I microemulsion extraction The result of Winsor I microemulsion extraction of the O_m phase with *n*-butyl acetate was represented by the *bars*. The result of direct Winsor I microemulsion extraction of the clear supernatant of the microbial transformation broth with ethyl ether was represented by the *circles*. The accumulative surfactant recovery yield in the W_m phase (*blank ones*) was defined as the ratio of the combined amount of surfactant in the W_m phase to that of the O_m phase of Winsor II microemulsion extraction or the clear supernatant of the microbial transformation broth. However, the residual substrate (*black ones*)/product (*gray ones*) recovery ratio was defined as the amount of substrate/product in the excess oil to that of the O_m phase of Winsor II microemulsion extraction or the clear supernatant of the microbial transformation broth, respectively

microemulsion extraction had also improved the maximum accumulative surfactant recovery ratio from 78% to 92%.

Discussion

The polarity of phase-forming components of water–organic solvent two-phase system, aqueous two-phase system, CPS and PEG-CPS are schematically represented in Fig. 8. All those two-phase systems for extractive microbial transformation have the same aims, i.e., increase of substrate insolubility, elimination of substrate/product inhibition, prevent product from further degradation, and sometimes shift adverse thermodynamic equilibrium [1, 3]. Water–organic solvent two-phase system has a wide polar spectrum. Organic solvents with polarity between 1 and 4 are usually demanded for extraction of moderate polar organic products in extractive microbial transformation. Unfortunately, those ranges of organic solvents are toxic for most of microorganisms [2]. Although aqueous two-phase system has a narrow polar spectrum, the relatively higher polarity of phase-forming components and high water content make aqueous two-phase system usually utilize in microbial transformation for extraction of relatively higher polar products, even protein products [32]. The expensive phase-forming components and the inconvenient downstream process of aqueous two-phase system are the main obstacles to its industrial application [17, 30]. CPS combines the advantage of water–organic solvent two-phase system (inexpensive phase-forming components) and the advantage of aqueous two-phase system (biocompatibility at relatively higher polarity) [11, 12]. However, a strip of nonvolatile organic compounds from the nonionic surfactant in the downstream process is very challengeable [14]. PEG-CPS introduces PEG to induce the phase separation of high HLB value nonionic surfactant aqueous solution at about room temperature, which is a precondition for extractive microbial

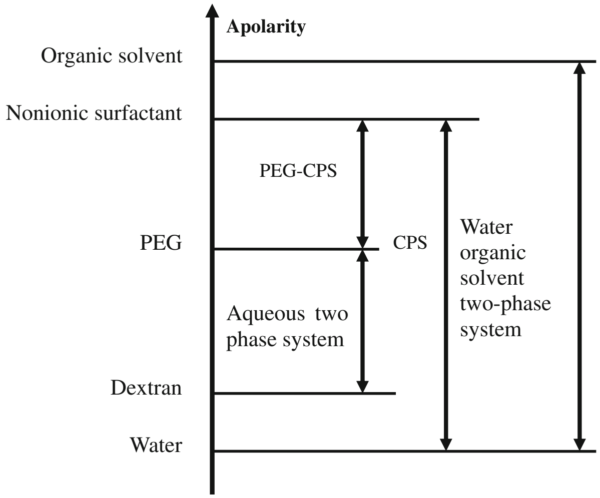

Fig. 8 Polarity spectrum of water-organic solvent two-phase system, aqueous two-phase system, CPS, and PEG-CPS

transformation [10]. The hydrophilic nonionic surfactant in a PEG-CPS is also favorable for recovery of nonvolatile organic compounds by Winsor I microemulsion extraction [22].

The commercial nonionic surfactant Triton X-100 is mixture of *t*-octylphenoxy polyoxethylene ethyl ethers with different number of ethylene unit. A Winsor I microemulsion extraction divides the commercial nonionic surfactant into a hydrophilic part with cloud point 82°C and a hydrophobic part with cloud point 24°C as shown in Fig. 3. The hydrophilic nonionic surfactant aqueous solution has been induced to form two-phase system by addition of PEG/salt as shown in Fig. 4b/c, respectively. The phase separation temperature of the hydrophilic nonionic surfactant aqueous solution has been decreased to about room temperature, which is favorable for extractive microbial transformation. PEG-CPS as shown in Fig. 4b is chosen for whole cell microbial transformation as shown in Fig. 5.

A general strategy with a cascade of Winsor II and Winsor I microemulsion extraction with organic solvent *n*-butyl acetate is proposed to stripping of the organic product from the hydrophilic nonionic surfactant in a microbial transformation broth. At the same time, a direct Winsor I microemulsion extraction of microbial transformation broth with organic solvent ethyl ether by omitting Winsor II microemulsion extraction is also demonstrated. A comparison of the results as shown in Fig. 7 indicates that an addition of Winsor II microemulsion extraction before the Winsor I microemulsion extraction has excluded the interference of the components (such as PEG) in the microbial transformation broth and the maximum separation efficiency has been achieved in the first-time extraction. However, with direct Winsor I microemulsion extraction by organic solvent ethyl ether, the second extraction is necessary to reach its maximum surfactant recovery. Those results are consisted with our previous report that the presence of PEG affects the separation of nonionic surfactant and organic compound by Winsor I microemulsion extraction [22]. The result of Fig. 7 also shows that nonionic surfactant, solvents, and extractive solutes are important factors to affect the separation efficiency. With the same solvent, the more apolar substrate has a high residual recovery ratio comparison to that of product, which is ascribed to the relatively higher solubility of the polar product in aqueous solution. A replacement of *n*-butyl acetate with ethyl ether, the accumulative surfactant recovery ratio has also improved from 78% to 92%.

More important, the utilization of hydrophilic nonionic surfactant H-Triton X-100 (cloud point 82 °C) has achieved surfactant recovery ratio 92% by Winsor I microemulsion extraction with ethyl ether as organic solvent. However, the same Winsor I microemulsion extraction with ethyl ether, even with a countercurrent extraction operation mode, only 67% surfactant recovery ratio has been achieved for Triton X-100 [18]. Those results are consisted with the microemulsion types are affected by organic solvent and nonionic surfactant [16, 33].

The cloud point of nonionic surfactant in the excess oil phase of Winsor I microemulsion with *n*-butyl acetate as organic solvent (35 °C) is lower than that of H-Triton X-100 as shown in Fig. 3 (82 °C), which indicates that an incomplete division of the commercial nonionic surfactant Triton X-100 into a hydrophilic part (H-Triton X-100) and hydrophobic part by a single stage of Winsor I microemulsion extraction. The hydrophobic nonionic surfactant in the excess oil phase is difficult to strip into the W_m phase by further Winsor I microemulsion extraction has also been reported in our previous work [18]. It leads to an incomplete separation of the product from the nonionic surfactant as shown in Fig. 7.

In general, the exploitation of PEG-CPS leads a hydrophilic nonionic surfactant aqueous solution to form two-phase system at about room temperature. The novel PEG-CPS maintains the advantages of CPS for in situ extraction of polar product in a microbial transformation. The utilization of hydrophilic nonionic surfactant in the PEG-CPS is favorable for stripping of product from the nonionic surfactant by Winsor I microemulsion extraction. Thus, a closed concept of extractive microbial transformation in PEG-CPS and stripping of product from the microbial transformation broth can be realized by selection of a relatively higher hydrophilic nonionic surfactant, suitable organic solvent, and operation parameters.

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