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## **Stabilization technology development for the liquid–liquid extraction process in a bioplant**



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#### a r t i c l e i n f o

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#### a b s t r a c t

In liquid–liquid extraction processes of bioplants, separation problems due to undesirable emulsifications between aqueous and organic layers are often observed. In this study, surfactants were applied to such a process to avoid separation problems and achieve stable liquid–liquid extraction operations. Homogenized yeast culture broth including a lipophilic physiological active substance was used as the aqueous phase and hexane was used as the extraction solvent. The results of lab-scale extraction tests indicated that adding a small amount of surfactant made of polyoxyethylene–polyoxypropylene block copolymers actualizes extractions with both of high yields and high separation stabilities. Liquid–liquid extraction processes with surfactants required higher power consumptions in order to require high power inputs to diminish droplets to sizes small enough to guarantee high mass transfer. The surfactant was also effective for continuous countercurrent extraction processes, in which it prolonged stable operations. In conclusion, application of a specific surfactant to liquid–liquid extraction processes was confirmed to be effective to achieve high stabilities and high extraction yields of two-layer separations in settlers which would lead to productivity increases and solvent recovery load reductions.

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#### **1. Introduction**

The conventional bioprocesses are consisted of fermentation and purification processes. The purpose of fermentation is to accumulate target substance in the fungus or the supernatant. In the purification processes, combinations of techniques are well used in order to eliminate fermentation-derived impurities. These combinations are often composed of separation techniques, such as extraction, filtration, fractionation processes, adsorption and crystallization processes. It also requires solvent recycle in terms of environment protection and energy saving.

Liquid–liquid solvent extraction process is one of the fundamental methods in the separation technology field and finds applications in the chemical and petroleum industries, biotechnology, nuclear technology, the food industry, waste

management, and other areas [\(Ban](#page--1-0) et [al.,](#page--1-0) [2000;](#page--1-0) [Birajdar](#page--1-0) et [al.,](#page--1-0) [2015;](#page--1-0) [Cheng](#page--1-0) et [al.,](#page--1-0) [2015;](#page--1-0) [Javanshir](#page--1-0) et [al.,](#page--1-0) [2012\).](#page--1-0) Liquid–liquid extractions in mixer–settlers are well used in bioplants for manufacturing products such as medicines, supplements, and functional foods. Continuous extraction processes such as countercurrent extraction process enables us to use small amounts of extraction solvents and smaller scale facilities compared to batch extraction system. However, in these plants, separation problems due to undesirable emulsifications of aqueous and organic layers are often observed ([Deshpande](#page--1-0) [and](#page--1-0) [Kumar,](#page--1-0) [2012;](#page--1-0) [Hadjiev](#page--1-0) [and](#page--1-0) [Paulo,](#page--1-0) [2005;](#page--1-0) [Reeve](#page--1-0) [and](#page--1-0) [Godfrey,](#page--1-0) [2002\).](#page--1-0) Decreases of the yields and the extraction solvent recovery rates due to such inefficient separations of the aqueous and organic layers often lead to significant problems in terms of production yields and operation efficiencies. To avoid separation problems, lower alcohols are often used

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as auxiliary solvents to obtain the suitable interfacial balance between the aqueous and the organic layers ([Kanaya](#page--1-0) et [al.,](#page--1-0) [2012\).](#page--1-0) This technique is known to promote mass transfer well by decreasing interfacial tensions and thus diminishing the dispersed droplets in sizes. It also has the effect of breaking emulsified liquids and separating the two layers faster in settlers. However, since lower alcohols in such multi-layer liquids increase affinities between organic and aqueous layers, they also promote blending organic solvents in aqueous layers, and thus would lead to separation problems ([Kanaya](#page--1-0) et [al.,](#page--1-0) [2015\).](#page--1-0) Such problems may easily occur only by operating slightly out of the normal operation range which is very narrow. Furthermore, lower alcohols require significant amounts of recycle energy, additional evaporators and distillation towers. Therefore, using auxiliary solvents would lead to complicated operations and high solvent recovery costs.

In the field of microfluidic channels techniques, several examples of liquid–liquid extractions with surfactants to enhance mass transfer have been reported [\(Kralj](#page--1-0) et [al.,](#page--1-0) [2005;](#page--1-0) [Okubo](#page--1-0) et [al.,](#page--1-0) [2008\).](#page--1-0) However, since surfactants also have the effect of preventing separations of two layers, these techniques need emulsion breaking devices, such as electric fields. In fact, practical applications of liquid–liquid solvent extractions using surfactants without particular devices have not been reported so far.

Therefore, with a background like the emulsification problem, we propose a unique liquid–liquid extraction method using a specific surfactant instead of auxiliary solvents in order to actualize extractions with both of high yields and high separation stabilities. In this study, at first, the phenomenon which produces emulsifications in mixer–settlers was clarified by measurements of liquid–liquid extraction processes. Based on this clarification, surfactant screening tests were performed to develop an operationally stable liquid–liquid extraction process without using auxiliary solvents or devices. In particular, surfactant screening tests played a large role in this study. The influence of agitation intensities on extraction yields was also investigated. Furthermore, the proposed extraction method using a surfactant was applied to the continuous countercurrent extraction and evaluated on its performances.

#### **2. Experimental**

#### *2.1. Experimental materials*

Homogenized yeast culture broth including lipophilic physiological active substance was used as the aqueous phase. The solid content of the broth was 13mass%. The viscosity and the density of the broth were <sup>∼</sup>0.5 Pa <sup>s</sup> and <sup>∼</sup><sup>1030</sup> kg/m3 at 293 K, respectively. In this study, ergosterol in the yeast cell



**Fig. 1 – Schematic diagram of the batch extraction apparatus.**

was regarded as the extraction target substance. Ergosterol is one of the lipophilic substances constituting cell membranes, and known as a precursor of vitamin  $D_2$  ([Margalith,](#page--1-0) [1989\).](#page--1-0) Hexane was used as the extraction solvent. 2-Propanol was used as the auxiliary solvent when needed.

Screened surfactants for extractions without auxiliary solvents are as follows: polyoxyethylene–polyoxypropylene block copolymer surfactant (Pluronic L-62, Pluronic L-64, ADEKA Co., Ltd.), polyvinyl alcohol (PVA, Wako Pure Chemical Industries Co., Ltd.), distilled monoglyceride (DMG, Riken Vitamin Co., Ltd.), tetraglycerol condensed ricinoleate (TGCR, Riken Vitamin Co., Ltd.), soybean lecithin (SL, Wako Pure Chemical Industries Co., Ltd.), and sucrose palmitate (SP, Mitsubishi-Kagaku Foods Co., Ltd.). All of the surfactants used in this study have already been applied to foods and healthcare applications.

#### *2.2. Apparatus and procedures*

#### *2.2.1. Electric conductivity measurement*

In order to clarify what phenomenon produces emulsifications in mixer–settlers, electric conductivities during liquid–liquid extraction experiments were measured. Fig. 1 shows a schematic diagram of the experimental apparatus. Into a glass-made cylindrical vessel with a diameter of 110mm, the broth and the extraction solvent were poured to a depth of 90mm. The vessel was equipped with four equally spaced 11 mm wide baffles. A six-bladed Rushton turbine impeller with a diameter of 50mm was located at the midpoint between the liquid surface and the torispherical bottom of the vessel. The impeller and the shaft were both made of stainless-steel. The conductance meter (CM-117, Kyoto Electronics Manufacturing Co., Ltd.) was placed in the liquid–liquid dispersion. The cylindrical vessel was set in a water bath to control the temperature.

The electric conductivities during liquid–liquid extractions were measured as follows. First, the aqueous layer and the extraction solvents were prepared as shown in Table 1. The volume ratios of the extraction solvents to the aqueous layer



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