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# Lipase-catalyzed synthesis of sorbitol–fatty acid esters at extremely high substrate concentrations

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**Abstract**

Lipase-catalyzed synthesis of sorbitol–fatty acid esters was performed in eutectic media with extremely high substrate concentrations. Homogeneous eutectic melts of sorbitol and fatty acids of C6–C16 were prepared using an adjuvant mixture. Enhanced homogeneity of mixtures was confirmed by X-ray diffraction analysis. The substrate concentration was 3.63–6.67 M in the eutectic media, whereas in organic media the concentration was below 0.10 M. Esters were synthesized with an immobilized *Candida antarctica* lipase, and optimum conditions were analyzed. Compared to reactions in organic media, the initial reaction rate of ester synthesis and the overall productivity were significantly enhanced in eutectic media while the conversion yields were similar. Based on the kinetic analysis, highly viscous eutectic media were shown to influence the initial reaction rate and the apparent activation energy resulting in diffusion limitations.


*Keywords:* Eutectic media; High concentration; Enhanced homogeneity; Diffusion limitation

## 1. Introduction

The enzyme-catalyzed synthesis of biosurfactants has been reported during the past decade (Fiechter, 1992; Sarney and Vulfson, 1995). Among surfactants analyzed, the sugar–fatty acid esters are potentially useful in the pharmaceutical, cosmetic, and food industries as a non-ionic surfactant (Fregapane et al., 1994; Hill and Rhode, 1999; Hills, 2003). Because of hydrophobic and hydrophilic natures of substrates, heterogeneous immiscible mixtures were induced, which hindered enzymatic reactions. However, lipase-catalyzed synthesis of these esters has been successfully carried out in non-aqueous organic media using hydrophilic solvents such as acetone (Arcos et al., 1998a,b,c, 2001), 2-methyl 2-butanol (Flores and Halling, 2002; Salis et al., 2004), and *tert*-butanol (Cao et al., 1999; Degn et al., 1999; Moreau et al., 2004). Unfortunately, substrate concentrations in organic media were extremely low at several different mM levels, suggesting non-

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applicability for industrial uses even though high conversion yields of almost 100% can be attained.

Among non-conventional media that are used for biocatalysis, organic solvent media and solid–liquid two-phase systems have been widely used in preparing substrate mixtures (Vermuë and Tramper, 1995)–other non-conventional media such as supercritical fluids, ionic liquids, and reverse micelles were not dealt here. Organic solvent media formed a homogeneous liquid phase including a small quantity of substrate (Fig. 1A) owing to a low solubility (Arcos et al., 1998c; Plou et al., 2002). Bioreactions in organic solvent media have been widely used for many applications over the past two decades (Zaks and Klibanov, 1985; Dordick, 1988; Klibanov, 2001). The reaction yields in the organic media have been improved by enhancement of enzyme activity via immobilization, genetic or protein engineering, extractive biocatalysis via integration of reaction and separation, and novel enzyme screening (Vermuë and Tramper, 1995; Paiva and Malcata, 1997; Tsuzuki et al., 1999; Bornscheuer et al., 2002; Krishna, 2002; Reetz, 2002). However, concentrations are still extremely low, resulting in limited industrial applications.

The ‘solid phase media’ or ‘solid-to-solid system’ (Erbeldinger et al., 1998), which is a type of solid–liquid two-phase system, has a feature of liquid phase similar to that of organic solvent media (Fig. 1B). The solid phase media comprise a small volume of liquid phase and mostly solid substrate. However, since the substrate molecules existed in liquid phase can be only engaged in enzymatic reactions, reactants in liquid phase cannot greatly contribute to enhancement of overall productivity. According to a report of Cao et al. (1997), the initial reaction rate and overall productivity of reaction in solid phase media were enhanced approximately 10 times compared to those in organic solvent media. However, the substrate concentration in the liquid phase was only approximately 0.75 M (Cao et al., 1999) or lower (Yan et al., 1999). Reactions do not always proceed well in the solid-to-solid system, whose switch-like behavior was carefully investigated in several references (Ulijn et al., 2001, 2003; Ulijn and Halling, 2004).

Eutectic mixture formation is a well-known melting technique that lowers the melting point of a mixture below the melting point of each pure compound in the mixture (Gill and Vulfson, 1994). An adjuvant usually helps to decrease the melting point and the molten mixture can be maintained at room temperature or below (López-Fandiño et al., 1994a). The eutectic mixture, which exists as a state of homogeneous liquid

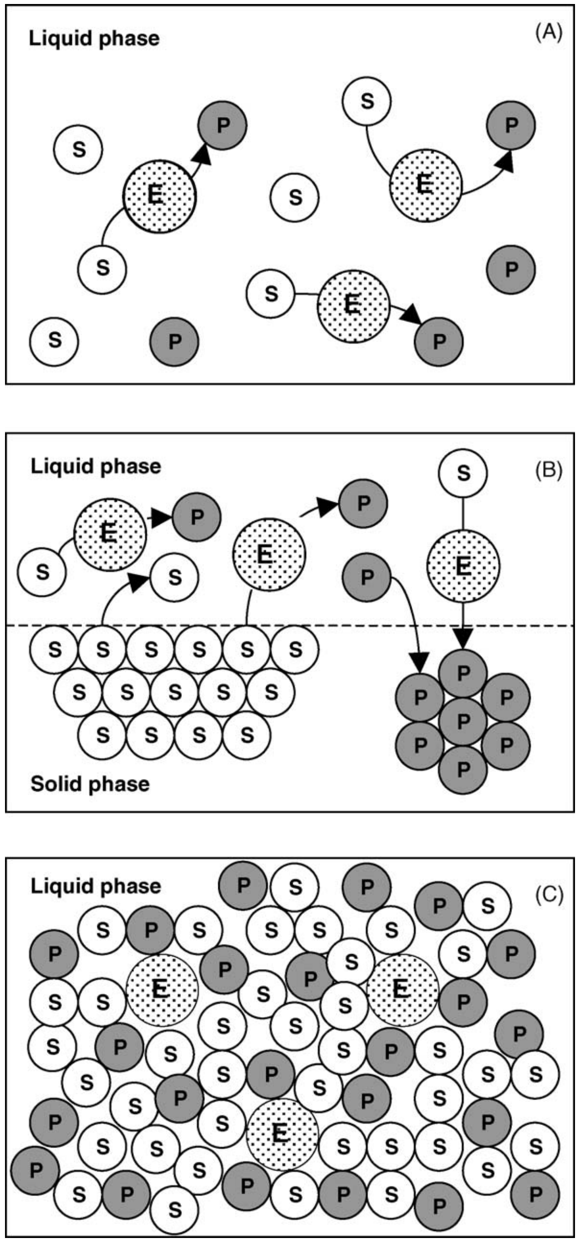


Fig. 1. Schemes of substrate mixtures in organic solvent media (A), solid phase media (B), and eutectic media (C); S: substrate; P: product; E: enzyme.