

## Cutinase activity in supercritical and organic media: water activity, solvation and acid–base effects

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### Abstract

We performed a comparative study on the activity of *Fusarium solani pisi* cutinase immobilized on zeolites NaA and NaY, in *n*-hexane, acetonitrile, supercritical ethane (sc-ethane) and sc-CO<sub>2</sub>, at two different water activity ( $a_w$ ) values set by salt hydrate pairs in situ and at acid–base conditions fixed with solid-state buffers of aqueous  $pK_a$  between 4.3 and 10.6. The reaction studied was the transesterification of vinyl butyrate by (*R,S*)-2-phenyl-1-propanol. The transesterification activity of cutinase was highest and similar in sc-ethane and in *n*-hexane, about one order of magnitude lower in acetonitrile and even lower in sc-CO<sub>2</sub>. Activity coefficients ( $\gamma$ ) generated for the two substrates indicated that they were better solvated in acetonitrile and thus less available for binding at the active site than in the other three solvents.  $\gamma$  data also suggested higher reaction rates in sc-ethane than in *n*-hexane, as observed, and provided evidence for a direct negative effect of sc-CO<sub>2</sub> on enzyme activity. Manipulation of the acid–base conditions of the media did not afford any improvement of the initial rates of transesterification relative to the blanks (no added acid–base buffer, only salt hydrate pair), except in the case of cutinase immobilized on zeolite NaA in sc-ethane at  $a_w = 0.7$ . The poor performance of the blank in this case and the great improvement observed in the presence of a basic buffer suggest a deleterious acidic effect in the medium which, an experiment without additives confirmed, was not due to the known acidic character of the salt hydrate pair used to set  $a_w = 0.7$ . In acetonitrile, increasing  $a_w$  was accompanied by a decrease in initial rates of transesterification, unlike in the other solvents. There was considerable hydrolysis in acetonitrile, where initial rates of hydrolysis increased about 20-fold from  $a_w = 0.2$  to 0.7. Hydrolysis was less pronounced in sc-ethane and in *n*-hexane, and only at  $a_w = 0.7$ , and in sc-CO<sub>2</sub> butyric acid was detected only at very long reaction times, in agreement with a generally low catalytic activity. Cutinase enantio-selectivity towards the alcohol substrate was low and unaffected by any manipulation of medium conditions.

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### 1. Introduction

Environmental concerns are an important driving force towards green chemistry today and the use of supercritical fluids (sc-fluids) is a good strategy to meet those concerns [1,2]. One of the areas where the use of sc-fluids holds promise is that of biocatalysis [3]. It is now firmly established that

in nonaqueous media enzymes are able to catalyze reactions that are difficult or impossible to carry out in water, become more stable and can exhibit altered selectivity [4], and some applications of nonaqueous enzymology are already commercialized [4,5]. In these media, the preservation of enzyme function is due to the fact that the enzyme essentially retains its native structure, as revealed by X-ray studies [6–8]. The limited availability of water ensures that the enzyme remains kinetically trapped in its native conformation [9]. Water acts as a molecular lubricant and although a certain level of hy-

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