Decyl acetate synthesis by enzyme catalysis in *sc*CO2

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The growing interest in industrial biocatalysis and in the research concerning enzymatic catalysis opened a wide range of applications in the field of pharmaceuticals, (fine-)chemicals and intermediates, leading to an increasing number of industrial biotransformations (Rozzell, 1999). Simultaneously, increased concern for the environment led to awareness for environmental friendly production methods. In this field, supercritical fluids may play a major role as they can be used for the reduction of organic waste, with the additional benefit that separation after reaction is relatively simple, which offers several process advantages. Among the four most extensively studied supercritical, *sc*, fluids (CO_2, ethane, ethene and water), there is a wide interest in the use of *sc*CO_2 since it has several advantages like non-toxicity, non-flammability, availability in high purity and solubilities of solutes can be tuned by changing temperature and pressure. As *sc*CO2 has the GRAS (generally regarded as safe) status, it can be used in food and pharmaceutical processes without major regulatory issues. The possibility to combine two sustainable technologies – biocatalysis and supercritical technology using green/natural solvents – allows to establish processes without environmental costs, and whose products are considered natural, resulting in a significant increase of their market value due to the present trends of such products.

In this work, the production of decyl acetate was studied. It is an ester with applications in the industry of fragrances where its floral odour is greatly appreciated. Since it mainly appears in nature in expensive oils, enzyme catalysis in a green solvent, such as CO2, can be a valuable process for production of natural decyl acetate. The reaction studied was the transesterification of vinyl acetate with decanol, obtaining decyl acetate as the main product. Lipase B of *Candida antarctica* (CALB), immobilized in the macroporous resin Lewatit B (Novozym 435®), was chosen as catalyst.

The synthesis of decyl acetate was studied in a high-pressure experimental set-up, equipped with a variable volume batch reactor, operating isotermally at 35 ºC and 100 bar, using as solvent CO2 in supercritical conditions.

The enzymatic content was determined for each particle size of the catalyst. It was shown that the smallest particles have a larger specific amount of enzyme, with an inversely proportional relation between the enzymatic content and the particle size (Table 1). It seems that the enzyme is located in an external shell of the particle, following an “egg-shell” model type, with a thickness of 60 μm (assuming a homogeneous distribution), independent of the particle size.

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Table 1: Enzymatic content for each particle size.

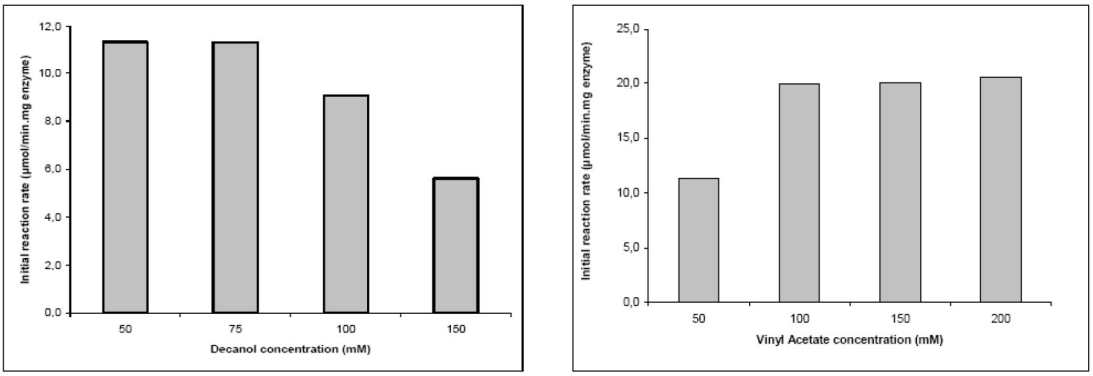
Particle diameter (μm)	Enzymatic content (mg enzyme/g particle)
>598	54,09
425-500	61,94
297-350	84,13
250-297	89,18

The mass transfer resistances were evaluated, both external and internal. For the first ones, the reactor stirring speed was varied, keeping all the other conditions constant (temperature, pressure, amount of enzyme, particle size, initial substrate concentrations). The experimental results showed that external mass transfer resistances are eliminated using an agitation of 950 r.p.m..

To access internal mass transfer resistances, several reactions were performed using different particle sizes, but with the same amount of enzyme, the same initial substrate concentrations, the same stirring speed, temperature and pressure. The results show that the size of the catalyst particles does not have a significant effect on the initial reaction rate. Therefore, the internal diffusional limitations can be considered as negligible.

The effect of the feed concentration of substrates on the initial reaction rate was also studied. It was observed that, from a certain concentration of decanol in excess relatively to vinyl acetate, the reaction is inhibited by the alcohol (Figure 1a). On the contrary, the reaction is favored when the reactor is fed with excess of vinyl acetate, with a significant enhancement of its initial rate (Figure 1b). These results are consistent with a Ping-pong bi-bi type mechanism with competitive inhibition by the alcohol, commonly used in the description of enzymatic reactions of esterification/transesterification (Yadav and Devi, 2004).

Intrinsic kinetic parameters will be determined using an activity based Ping-pong bi-bi kinetic equation to account for the thermodynamic non-ideality of the reaction mixture.



(a) (b)

Figure 1: Effect of the feed concentration of substrates on the initial reaction rate: (a) excess of decanol; (b) excess of vinyl acetate.

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