394d Effect of Fermentation Co-Solutes on the Crystallization of Levodione

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ABSTRACT Direct product crystallization from fermentation media is becoming important in biocatalytic processes and is already applied. With a few exceptions, the crystallization kinetics of the target product has not been studied, and neither the influence of co-solutes (impurities) present in the medium. In our previous studies [1-2], whole-cells have been employed as catalyst and the vital addition of nutrients and salts in the reaction medium caused an increase in the bulk of dissolved co-solutes, which included the unreacted substrate and a by-product.

In this work, the influence of co-solutes present in the medium to the crystallization kinetics of 6R-dihydro-oxoisophorone (DOIP), also known as levodione, is studied. Levodione is a key intermediate in the synthesis of some carotenoids and flavors, and synthesized via the enantioselective reduction of 4-oxoisophorone by Saccharomyces cerevisiae (baker's yeast), where in situ removal of the product in an external crystallization loop was employed [1,2]. Figure 1 shows the synthesis reaction involved. As this is a promising process option for a biocatalyzed reaction system, it is important to elucidate the effects of fermentation co-solutes during product crystallization.

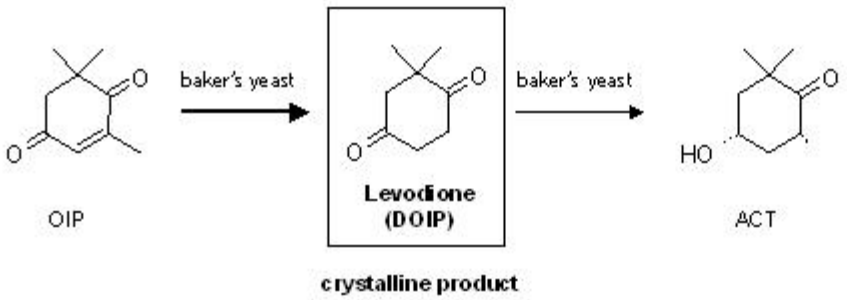
The crystallization kinetics of levodione are described by determining nucleation and crystal growth kinetic parameters simultaneously using the experimental data on the desupersaturation curves at different initial supersaturation ratios (So) employing cooling crystallization at a temperature Tc = 278 K; and the crystal size distribution profiles using image analysis. Low values of initial supersaturation ratios (So) are used as these are typical for in situ crystallization processes. Model validation is performed for a seeded batch crystallization experiment conducted in three solvents/media, namely: water, phosphate buffer employed in [1], and culture medium employed in [2].

Figure 1. Synthesis of DOIP from 4-oxoisophorone (OIP) by baker's yeast. By-product formed is actinol (ACT). The desired product (levodione) is recovered from the reactor by in situ crystallization to avoid degradation.

Results showed that the solubility of DOIP slightly decreases with increasing quantity of co-solutes present in the medium. However, there are no significant differences in the kinetic parameters during crystallization in these three media. The nucleation and growth rates are dependent on the supersaturation ratio S and can be described by the following relations: J = 2.0x105 exp [-0.023/(ln S)2] #.m-3.s-1 and G = 1x10-8 (S-1)0.86 m.s-1, respectively. The co-solutes in the medium increase the crystallization rate of DOIP as a particular DOIP concentration leads to a higher supersaturation ratio in the presence of co-solutes, due to lower DOIP solubilities. This is a favorable situation for an integrated reaction-crystallization process carried out involving cell cultivation. The interfacial tension (g) is 0.0092 J.m-2, indicating a primary heterogeneous nucleation mechanism. From the process-engineering point of view, crystal growth is the most significant sub-process to consider during crystallization as □99% of the mass of crystals is accounted for by (the growth of) large crystals.

Word count: 466 (maximum 1500)

References [1] Buque-Taboada EM, Straathof AJJ, Heijnen JJ, van der Wielen LAM. 2004. In situ product removal using a crystallization loop in asymmetric reduction of 4-oxoisophorone by Saccharomyces cerevisiae. Biotechnol Bioeng 86, 795-800. [2] Buque-Taboada EM, Straathof AJJ, Heijnen JJ, van der Wielen LAM. 2005. Microbial reduction and in situ product crystallization coupled with biocatalyst cultivation during the synthesis of 6R-dihydro-oxoisophorone. Adv Synth Catal, in press.



crystalline product