**Integrated Separation in a Complex 2-Phase Multienzymatic Cascade Reaction System**

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**Highlights**

* A complex 2-phase multienzymatic cascade reaction system is introduced.
* A pilot plant is successfully set up for a continuous process.
* Extractive centrifugation for *in-situ* intermediate recovery is implemented.
* Influence of the extraction temperature on the total production efficiency is evaluated.

**1. Introduction**

In the field of white biotechnology the sustainable production of flavors and fragrances is increasingly gaining attention [1]. Enzymatic reactions allow for high selectivity and purities under mild reaction conditions, less by-products, and therefore enabling an overall sustainable production process [2]. In this contribution, a novel *in-vitro* multienzymatic cascade reaction system for the continuous and sustainable production of cinnamyl cinnamate from cinnamyl aldehyde in a 2-phase system is introduced. Two co-factor coupled dehydrogenases are used in a water-based buffer system, thus ensuring co-factor regeneration within the reaction pathway. An extractive centrifugation allows for a reaction integrated intermediate separation with an organic phase, consequently enhancing the productivities of the dehydrogenases. In the organic phase, a lipase is used to catalyze an esterification reaction of the intermediates resulting in cinnamyl cinnamate formation. During extractive centrifugation the organic phase is saturated with water, thereby decreasing the lipase activity in the last reaction step. In this contribution, the pilot plant set up, as well as experimental results are presented. Reaction kinetic data of all enzymes were determined. A computer model for the simulation of the cascade reaction is introduced. Experiments in pilot scale were performed, and process parameters were analyzed. Possible solutions to counter the challenge of product saturation in the organic phase are presented and evaluated.

**2. Methods**

The kinetic data of the two-substrate red-ox reactions catalyzed by the dehydrogenases was determined in the buffer system saturated with the organic solvent. Using ACM© from AspenTech a mathematical model was implemented. Experiments of the co-factor coupled red-ox reactions were performed to validate the model.

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| **Figure 1.** Pilot scale reactor set up with integrated downstream processing. |

Experiments of the lipase catalyzed esterification reaction of cinnamyl alcohol and cinnamyl acid to cinnamyl cinnamate were performed in a pilot scale reactor (Figure 1) at the temperature optimum of the lipase (60 °C) to evaluate possible solutions to increase lipase activity. Firstly, the temperature during integrated extraction was varied. Secondly, an integrated adsorption unit operation to dry the organic phase was analyzed.

**3. Results and discussion**

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| **Figure 2.**  Lipase activity for different extraction temperatures. |

For the dehydrogenases the maximum activities are 11.3 U/mg and 0.1 U/mg respectively, however, the solubilities of the substrates limit these activities during operation. Exemplary for the results of the pilot scale experiments, Figure 2 shows the lipase activity for extraction temperatures of 15 °C, 30 °C and 60 °C. In all cases, the esterification reaction itself was carried out at a temperature of 60 °C. It can be shown that a temperature decrease of 75 % in the extraction step leads to an activity increase of over 300 %. The activity increase can be

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| **Figure 3.**  Temperature dependent water solubility in the organic phase. |

explained by analysing the water saturation concentration of the organic phase exiting the centrifuge (Figure 3). A temperature decrease of 75 % during extraction results in a 75 % lower water saturation concentration, thereby decreasing the side product concentration prior to the esterification reaction. These results show that further drying of the organic phase is desirable. Hereby, promising results were achieved using an additional integrated adsorption step.

**4. Conclusions**

A novel 2-phase multienzymatic cascade reaction system for a continuous and sustainable production of cinnamyl cinnamate is introduced. The kinetic data of the dehydrogenases was determined. A mathematical model was implemented. Additionally, a pilot scale reactor set up for a continuous production of cinnamyl cinnamate is presented. Exemplary for the analyzed process parameters in pilot scale operation the influence of the extraction temperature on the lipase activity was evaluated. Lipase activity was increased by over 300 % by decreasing the extraction temperature from 60 °C to 15 °C. These results can now be compared to an adsorption step prior to the lipase reaction. They are also helpful for a further development of the mathematical model.

**References**

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2. Abu, R., Woodley, J.M. 2015. Application of Enzyme Coupling Reactions to Shift Thermodynamically Limited Biocatalytic Reactions. ChemCatChem 7, 19, 3094-3105.