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Communication

Concept of an Enzymatic Reactive Extraction Centrifuge

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Abstract: Biocatalytic processes often provide an ecological alternative to many chemical processes. However, further improvements in terms of the economic efficiency are required. In order to achieve that, the concept of process integration is a promising option. Applying this within a biocatalytic process, a highly integrated apparatus working as a reactive extraction centrifuge was developed and operated. For this purpose, a commercially available extraction centrifuge was modified to implement a biocatalytic reaction. The novel apparatus was used within a multi-enzyme cascade for the production of a natural flavor and fragrance, namely cinnamic ester. The characterization of the reactive extraction centrifuge and the suitable operation conditions for the inlet streams and the rotational speed for a stable operation were determined. Furthermore, different initial substrate concentrations were applied to prove the reaction. The results provide a successful proof of concept for the novel reactive extraction centrifuge.

Keywords: process integration; biocatalysis; hybrid process; multi-enzyme cascade; process intensification

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1. Introduction

Biocatalytic and biotechnological processes show a good perspective in terms of sustainability [1]. They are especially characterized by mild reaction conditions and a high selectivity [2]. Additionally, multi-enzyme cascades provide further advantages for potential industrial application [3]. These are, for example, flexible reaction routes and no required separation of the intermediates or side products [4,5]. However, a big challenge remains economic competitiveness with well-established chemical processes [6]. This results in the so-called "valley of death" between academic research on the laboratory-scale and industrial application of novel processes [7,8]. To overcome the "valley of death", an implementation on a larger scale—for example a mini-plant and consideration of process intensification tools to improve the efficiency—is required [7,9,10]. One approach used to intensify biocatalytic processes is process integration [11,12]. It is also called the "hybrid process" and includes the combination of two or more tasks in one apparatus [13]. Although this offers huge advantages in terms of the equipment size and a more efficient technology, the operation can be challenging due to the limited operating window [14]. One example of a hybrid process is a reactive centrifuge [15,16]. It integrates a reaction and a phase separation in one process step. Recently, it has mainly been studied in the context of biodiesel production [17,18], and, in a particular study, also within a biocatalytic process [19]. Another example is an extractive centrifuge that combines extraction and phase separation. Recently, SEYFANG et al. [20] published an excellent review on the application of extractive centrifuges and evaluated it as a promising technology that requires further research. The extractive centrifuge offers a wide range of possible applications. It can be used especially in pharmaceutical [21,22] and biochemical contexts [23]. Our research group studied a biocatalytic process, or, more specifically, a multi-enzyme cascade, with an extraction centrifuge [24,25]. It was successfully operated and simulated and we found the

potential for process integration in the form of a reactive extraction centrifuge (REC) [26]. To the authors' best knowledge, the implementation of reaction, extraction and centrifugal separation into one apparatus has never been described before in the literature for a heterogeneous reaction. In order to assess the REC, an experimental set-up needs to be developed and implemented into a mini-plant scale. In order to determine the operational window, which is limited due to the process integration, the rotational speed as well as the residence time for the organic and aqueous phase are varied at a temperature of 35 °C. In a previous study, the same reaction system was operated with an extractive centrifuge [24]. This setup was modified within this project. For process application, the extractive centrifuge was modified to set up a reactive extraction centrifuge. Both constructional and operational modifications are required in order to allow for a combination of extraction, reaction and phase separation in one apparatus.

2. Materials and Methods

The biocatalytic process aims at the production of the natural flavor and fragrance cinnamyl cinnamate [27]. It was produced in a multi-enzyme cascade in a 2-phase system as shown in Figure 1. Three parallel and sequential reactions occurred in an aqueous phosphate buffer phase at pH 8.0 and an organic xylene phase. The first part of the reaction cascade took place in the aqueous phase. The substrate cinnamyl aldehyde was converted in the first step into cinnamyl alcohol. The reaction was catalyzed by an alcohol dehydrogenase (ADH) using the cofactor NADH. To regenerate the costly cofactor, a formate dehydrogenase (FDH) catalyzed reaction was used. Under the oxidation of formate to carbon dioxide, NAD^+ was reduced to NADH. The produced cinnamyl alcohol was transferred into the organic phase. In the organic phase, the cinnamic alcohol and cinnamic acid were esterified to cinnamyl cinnamate. The reaction was catalyzed by the commercially available, immobilized lipase Novozym 435. In a previous work, this reaction system was successfully operated at a miniplant scale [28] using an extractive centrifuge [24]. In that setup, different reactors for each phase were used. The mass transfer of the intermediate cinnamic alcohol from the aqueous phase into the organic phase takes place in the extractive centrifuge. Within this work, an integrative reactive extraction centrifuge was developed by integrating the lipase reactor into the extractive centrifuge. To gain a deeper understanding of the novel process, this study focuses on the esterification.

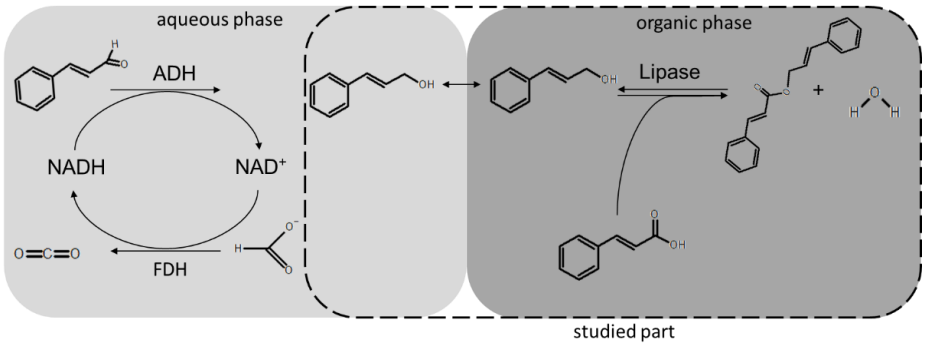

Figure 1. Reaction scheme of the multi-enzyme cascade for the production of cinnamyl cinnamate in a two-phase system.

Within this work, a reactive extraction centrifuge was developed by integrating the lipase reactor into the extractive centrifuge. To gain a deeper understanding of the novel process, the first part of the reaction cascade, the production of the intermediate cinnamyl alcohol in the aqueous phase, was not considered. This is marked in Figure 1 as the "studied part". Cinnamyl alcohol and cinnamic acid were directly supplied into the organic phase. The studied process was realized in a miniplant setup. The organic and aqueous phase were stored in different tanks and pumped into the REC. Within the REC, the aqueous and organic phases were brought into contact and the mass transfer between both phases took place. The immobilized lipase was placed inside the centrifuge and it catalyzed the esterification. Subsequently, the organic and aqueous phases were separated in the centrifuge, analyzed and led back to the tanks.

3. Results and Discussion

In order to develop an REC, an extractive centrifuge was modified in the first place. Subsequently, operation conditions for the centrifuge for the extraction and separation, as well as initial substrate concentrations for the reaction, were determined. In the following part the constructional set-up of the REC and the operational results are presented.

3.1. Construction

The REC is based on the extraction centrifuge V02 by the company CINC. It has a liquid hold-up of 140 mL and allows for a rotational speed of up to 6000 rpm. In order to enable a reaction, immobilized lipase that catalyzes the esterification reaction was placed inside the mixing zone of the centrifuge. ILMI et al. [29] used the same immobilized lipase for the biodiesel production within a reactive centrifuge. First, an analogue setup was used for the reaction system. This setup led to a destruction of the enzyme carriers and therefore did not enable a successful operation. In comparison to that, ILMI et al. [29] used the same immobilized lipase for the biodiesel production within a reactive centrifuge and did not observe a destruction of the immobilized enzymes. These contradicting results could be explained by the different solvents. The influence of the utilized solvent on the immobilized lipase can be observed by comparing the results of AJMAL et al. [30] and WIERSCHEM et al. [31]. Both studied the influence of ultrasound on the activity of immobilized enzymes. Ajmal et al. [30] observed a destruction of the enzymes. In contrast to that, Wierschem et al. [31] observed an increased enzyme activity in a similar frequency range depending on the different solvents.

According to these results, a different approach for the placement of the immobilized enzymes in the centrifuge has to be applied. In order to reduce the mechanical stress and thus prevent a destruction, the rotor part of the centrifuge was chosen for the placement of the enzymes. As the place inside the rotor is limited by the separation vane in the original centrifuge by CINC, a new rotor and separation vane were designed and integrated. They consist of a reactive and separation part as shown in Figure 2. In the reactive part, the immobilized enzymes are placed in a metal mesh, including 11 g of enzyme particles. Thus, the mechanical stress on the immobilized enzymes is reduced further.

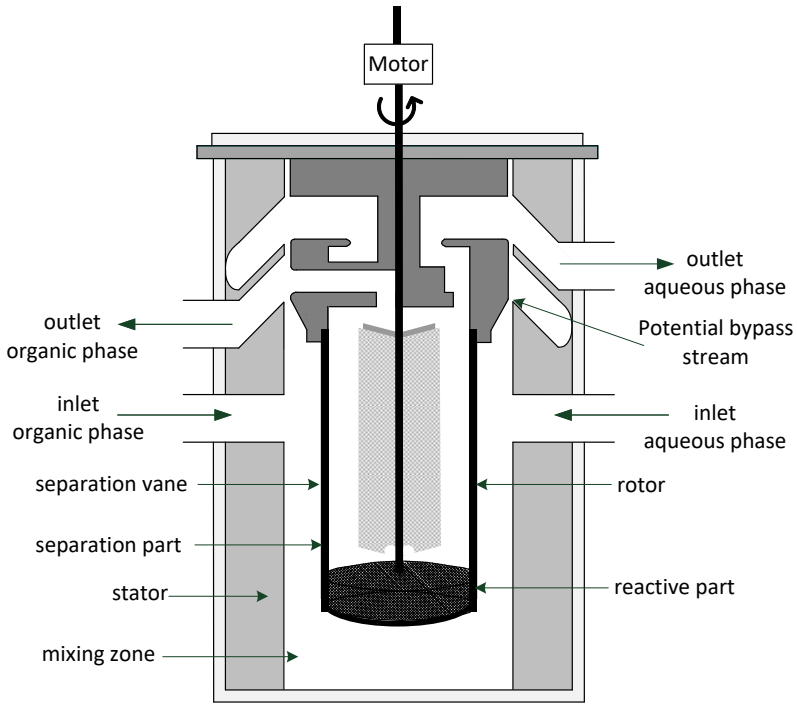

Figure 2. Scheme of a reactive extraction centrifuge (REC).

3.2. Operation

Compared to the extractive centrifuge the REC offers new challenges for the operation. Due to the reactive zone in the rotor, the pressure drop within the centrifuge is increased. This leads to a limitation of the operation window to avoid bypass streams from the inlet to the outlet. Furthermore, the reaction and the extraction between both phases is strongly influenced by the operation conditions. This applies especially for the residence time, which is defined by the volume flow into the centrifuge. Therefore, three main parameters were assessed to describe the operation window: the organic volume flow into the REC, the aqueous volume flow into the REC and the rotational speed of the centrifuge. The pumps allow for a maximum volume flow of 800 mL/min for the organic and aqueous phase, respectively.

Figure 3 shows the operation conditions that enable the reaction, extraction and separation in one apparatus. The rotational speed was varied between 7.5 and 13.5% of the maximum rotational speed of 6000 rpm. Higher rotational speeds lead to an insufficient phase separation as both phases exit the centrifuge through the aqueous phase outlet. Similarly, a rotational speed below that leads to an outlet of both phases through the organic phase outlet. An organic volume flow between 200 and 500 mL/min was investigated. Examples for the influence of the aqueous volume flow on the operating window of 180 and 250 mL/min were considered. The operation window that allows for a stable operation was constricted by different influences of the process. The upper boundaries of both volume flows were limited by the pressure drop in the rotor. A too high pressure drop leads to the aforementioned bypass stream from the inlets directly to the outlet. Therefore, the medium does not enter the rotor with the reactive and separation part and does not allow for a reaction or separation. The lower boundary is defined by the ratio between both phases. If one of the volume flows is significantly lower than the other one, a backflow through the inlet occurs. A rotational speed above the marked area leads to a non-persistent phase separation and an accumulation in the aqueous tank. For a rotational speed below the marked area, the centrifugal force is not sufficient to ensure a phase separation. Therefore, a bypass stream from the inlets to the outlet of the organic phase occurs.

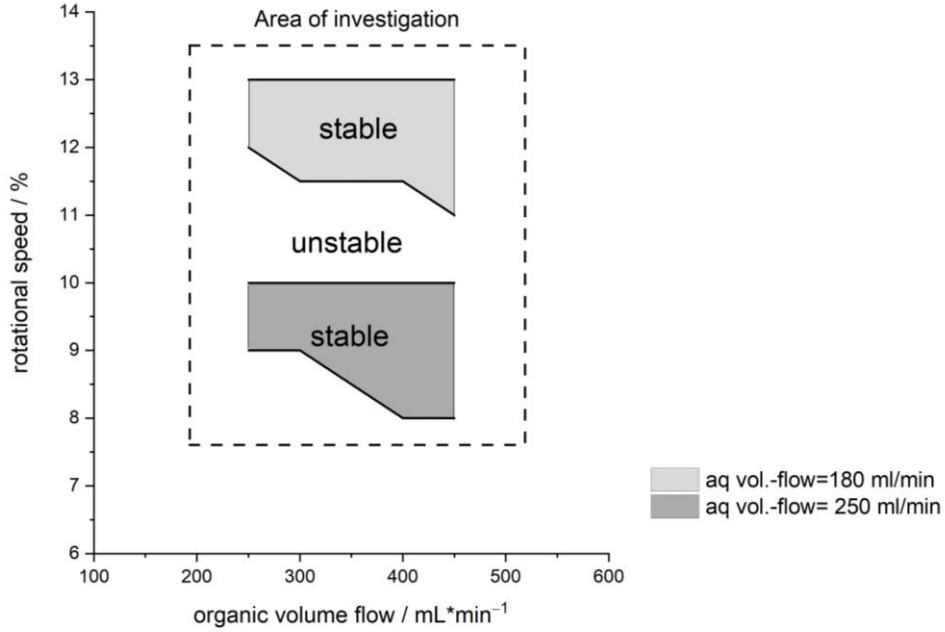

Figure 3. Operation window depending on the rotational speed of the rotor and the volume flow of the organic and aqueous phase.

Beside the novel operation conditions in terms of the volume flow and rotational speed, the most significant change compared to the extractive centrifuge is the implementation of

the reactive part inside the REC. It provides a volume of 65 mL. The initial concentrations, as well as the temperature, are the main parameters that influence the reaction. The upper limit of the temperature is defined by the thermal stability of the enzyme, as well as the flammability of the xylene. In contrast to that, a lower reaction rate leads to a decreased reaction rate. A temperature of 35 °C provides a good trade-off. For the cinnamic acid, an initial concentration of 50 mmol/L was chosen. The cinnamyl alcohol was provided from the prior reaction step. Initial concentrations of cinnamyl alcohol of 5, 25 and 50 mmol/L were studied. As the cinnamic acid was supplied externally within the cascade, a high concentration was chosen.

Figure 4 shows the results of the reaction in the REC. The error bars represent the statistical error in the operation of the process and the deviation within the mass balance. The statistical error in the operation was determined, with a fivefold repetition of an experiment under the same initial and operational conditions, to be 12.7%. The deviation from the mass balance was calculated for each experiment separately and was 15.0%, 5.0% and 3.6% in descending order of the initial alcohol concentration. With an initial alcohol concentration of 5 mmol/L, the product concentration shows a nearly linear increase over the observed time span of 6.5 h. In contrast to that, for initial alcohol concentrations of 25 and 50 mmol/L, the product concentration shows a steep increase in the first 2 h. After that, only a slight increase can be observed. This course of the concentration is typical for a reversible and equilibrium-based reaction. Because of the increasing product concentration, the reaction rate reduces over time until equilibrium is reached. Within the equilibrium, the observed reaction rate is zero due to the equality of the forward and backward reaction. As expected, the final concentration of the product increases with an increasing initial substrate concentration because the reaction is equilibrium-based. Within the operation duration of six hours space-time yields of 0.72 mmol/(l·h), 0.52 mmol/(l·h) and 0.058 mmol/(l·h), respectively, are achieved. The non-integrated whole process (including the dehydrogenase reactions) shows a space-time yield of up to 0.03 mmol/(l·h) [26]. Therefore, the studied integration ends up in intensification. Compared to the minimum space-time yield of 1 mmol/(l·h) [32] for the industrial application of biotechnological processes, these results are not yet sufficient. Within the first hour of operation, space-time yields of 3.2 mmol/(l·h), 1.8 mmol/(l·h) and 0.12 mmol/(l·h) are achieved, which is comparable to the space-time yield of a mathematical optimized whole process [32]. This proves that the process can provide a sufficient space-time yield for a possible industrial application.

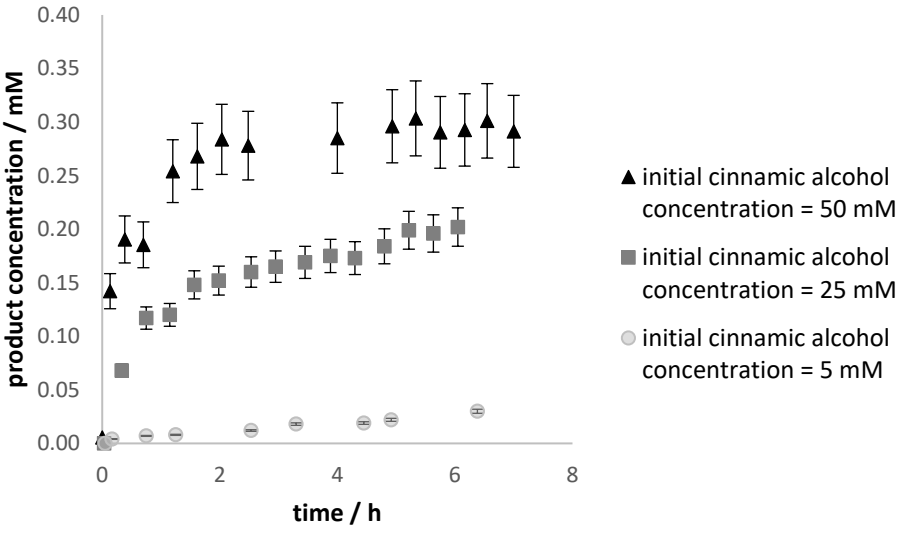

Figure 4. Operational results for the production of cinnamyl cinnamate depending on the time for three different initial cinnamyl alcohol concentrations (V_{orga} = 0.6 L, V_{aq} = 0.6 L, m_{Lipase} = 8 g, $c_{int,\,acid}$ = 50 mmol/L).

4. Conclusions and Outlook

Within this study, a reaction, extraction and centrifugal separation were integrated for the first time into one novel apparatus: the reactive extraction centrifuge. In order to study the REC, an extraction centrifuge was successfully modified to integrate a reaction part into the centrifuge. To ensure the mechanical stability of the immobilized enzymes, the reactive part was implemented into the rotor. Operational conditions in terms of volume flows of the organic and aqueous phase into the centrifuge and rotational speed were determined in order to enable a sufficient extraction and phase separation. Additionally, the reaction in the REC was investigated with three different initial substrate concentrations. Within all of these experiments, an increase in the product over a time span of 6.5 h was observed. Therefore, a clear proof the operability of the REC within a biocatalytic process is given.

For future research, further studies concerning the reaction part are necessary. In particular, the influence of the temperature and a more detailed study on the initial substrate concentrations as well as the equilibrium are important to characterize in order to gain a deeper understanding of the process. Besides that, the operation of the whole cascade with the REC is targeted. Besides the experimental development, a model-based description of the process will be carried out, which is an important step towards the industrial application of the apparatus [33]. For this, an existing model for a similar process using an extractive centrifuge can be extended and validated to describe the novel REC. This model can be used for further case studies and a model-based optimization of the process.

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