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Process Intensification

Two-Phase (Bio)Catalytic Reactions in a Table-Top Centrifugal Contact Separator**

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Batch-wise production is the state-of-the art in the manufacture of fine chemicals, as it allows the multiple use of reactors for different processes. However, the use of batch reactors has some serious drawbacks: For the production of larger quantities, multiple batch runs have to be performed and this often leads to batch to batch variation in the product quality and performance. Furthermore, the productivity is often lower than for dedicated continuous reactors, and fixed costs are significant because it is labour intensive. Therefore, switching to continuous processes appears highly beneficial.[1] In addition, continuous production in small flow reactors is very advantageous for reactions in which highly toxic and/or explosive materials are used or produced.

Based on this analysis, many groups have started to work on concepts of process intensification aimed at the development of smaller reactors or the integration of the reactor with the separation.[2,3] The most visible activity in this field is undoubtedly the use of microreactors.[4–6] Poechlauer and coworkers have recently reported the use of a microstructured reactor for the ton-scale execution of a Ritter reaction.[7] Wakami and Yoshida have reported the pilot-scale production of a Grignard exchange reaction in a microreactor.[8] The use of enzymes as catalysts in microreactors has also been examined.[9] Ley and Baxendale published a series of papers that describe a cascade of reactions in microflow reactors where the reagents are present in an immobilized form.[10]

The principle of cascade catalysis in a sequence of continuous flow reactors is highly interesting.[11] To be effective, high conversions are required in each reactor of the sequence. However, not many reactions are fast enough to be used in microreactors, where residence times are typically on the order of seconds. In addition, these concepts are not always easily scaled up to ton amounts.

We decided to focus our research activities in this field on the use of a table-top-sized flow reactor for (bio-)catalytic reactions. The device is a centrifugal contact separator (CCS) that has been used for oil–water separation (for example, for cleaning up oil spills),[12] for the continuous extraction of fermentation products, such as penicillin[13] and phenylalanine,[14] and in the atomic waste industry for the extraction and purification of radioactive waste.[15,16] Figure 1 shows a schematic representation of a CCS. The device is in essence a centrifuge. The immiscible liquid phases are introduced in the small annular mixing zone between the outside of the rotor and the inside of the outer housing. Here, very efficient and fast mixing between the two phases occurs, which is highly conducive to a two-phase catalytic reaction. The dispersion is then sucked inside the centrifuge, where the two phases are gradually but very efficiently separated whilst moving upwards, after which they leave the device through separate exits.

To the best of our knowledge the use of CCSs as chemical reactors has not been reported to date. A centrifuge has been used to continuously remove the polymeric product (dextran) from the enzymatic conversion of sucrose in a batch mode.[17] It is potentially very attractive to use a CCS for biphasic

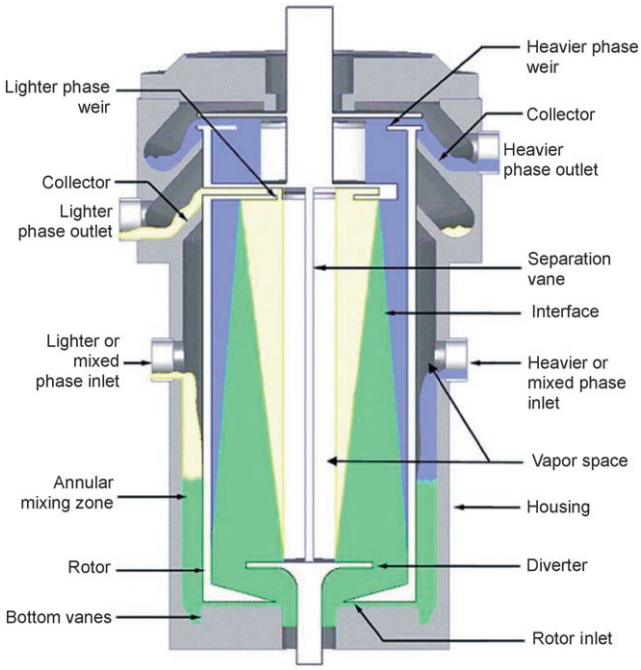

Figure 1. Schematic cross-section of a centrifugal contact separator (Courtesy of CINC-Solutions, The Netherlands).

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liquid–liquid (catalytic) reactions.[18] In this case, the annular zone acts as the reactor and the centrifuge as the liquid–liquid separator. Two-phase catalysis in flow devices has been reported by de Bellefon et al.[19] and by Claus et al.,[20] however, without integrated phase separation. Ryu and co-workers have reported a two-phase Heck reaction in a microreactor in which the phase containing the palladium catalyst was an ionic liquid. In this case the separation and recycling of the catalyst was fully integrated.[21] Cascade catalysis with different types of catalysts is in principle possible by connecting a number of these devices, as shown in Figure 2.

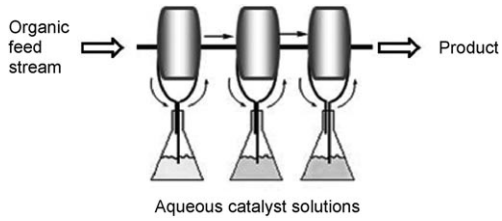


Figure 2. Cascade two-phase catalysis using CCSs in series.

We decided to test the efficiency of the CCS for the continuous production of biodiesel from sunflower oil.[22] The reaction is a typical example of a catalytic liquid–liquid reaction. It was performed on sunflower oil with a sixfold molar excess of methanol at elevated temperatures (60 °C) and using a basic catalyst (NaOMe, 1 % w/w with respect to sunflower oil). The CCS was equipped with a heating jacket to ensure isothermal conditions. The sunflower oil was pre-heated to 60 °C and was pumped at 12.6 mL min^{-1} into one entrance of the CCS. A solution of NaOMe in MeOH was then introduced through the other entrance at a flow rate of 3.1 mL min^{-1}. After about 40 minutes, the system reached a steady state and the fatty acid methyl esters (FAME) containing some residual sunflower oil came out as the light phase, whereas the heavy phase consisted of a solution of glycerol in MeOH. Depending on the rate of the centrifuge, a maximum yield of 96 % of FAME was reached under these conditions (Figure 3).[22]

The conversion reaches a maximum at 30 Hz. At a higher rate of rotation the increased separatory power of the centrifuge leads to a reduction in the volume of the mixed phase in which the reaction takes place (Figure 4). The mixing process becomes less efficient at reduced rotational speeds of the centrifuge which results in larger average drop sizes in the dispersed phase and thus to reduced rates of mass transfer and conversion.

By using the established optimum conditions, biodiesel was produced at a rate of 61 kg biodiesel m^{-3} min^{-1}, which compares well with the 42 kg m^{-3} min^{-1} reported for typical batch processes.[23] In addition, the current process is much more efficient, since there is no distinct separation step, and cleaning of the reactor between batches can be omitted.[24]

Next, the potential of the CCS to perform enzymatic conversions was investigated. Most enzymes function optimally in an aqueous environment, and as such are ideal

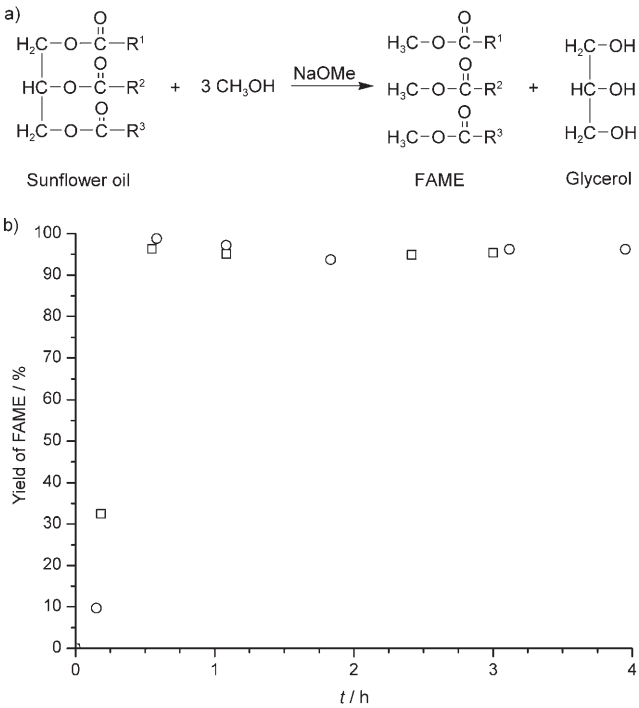


Figure 3. Continuous conversion (in duplicate) of sunflower oil into FAME in a CCS at 30 Hz.

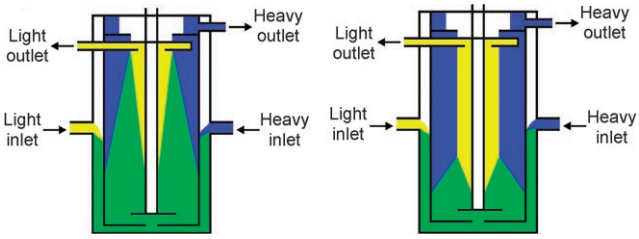
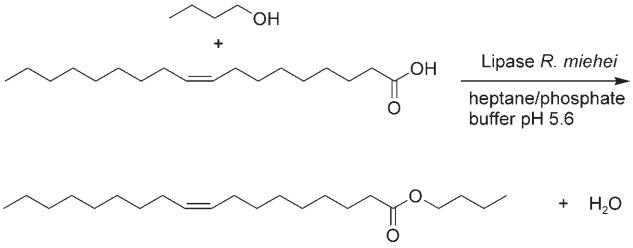


Figure 4. Liquid hold-up in the CCS at low- (left) and high-speed (right); blue = heavy phase, yellow = light phase, green = mixed phase.

catalysts to test in the CCS. As enzymes can be easily damaged by shear forces, we used a CCS with a low-mix bottom plate. This bottom plate is connected to a protective cylinder around the centrifuge to avoid direct contact of the entering liquids with the rotating centrifuge. As a model reaction, the esterification of oleic acid with 1-butanol catalyzed by a *Rhizomucor miehei* lipase was investigated (Scheme 1).



Scheme 1. Lipase-catalyzed esterification of oleic acid with 1-butanol.

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The lipase-catalyzed reaction between oleic acid and ethanol was already known, but we found that replacement of ethanol by 1-butanol led to much higher conversions.[25] The esterification of oleic acid with butanol using a crude extract of *penicillium coryophilum* in a micellar system has also been described.[26] In batch mode, this reaction goes to full conversion despite the large excess of water present. Presumably, the reaction is driven by the lipophilicity of the reactants. In a first series of experiments an organic phase consisting of a mixture of oleic acid (0.6 mol L^{-1}) and 1-butanol (0.9 mol L^{-1}) in heptane was used. The aqueous phase consisted of a solution of *R. miehei* lipase (1 g L^{-1}) in a phosphate buffer at pH 5.6. We first examined the effect of the flow rates of both phases and the rotational speed of the centrifuge on the conversion (Figure 5). Under these conditions, the highest

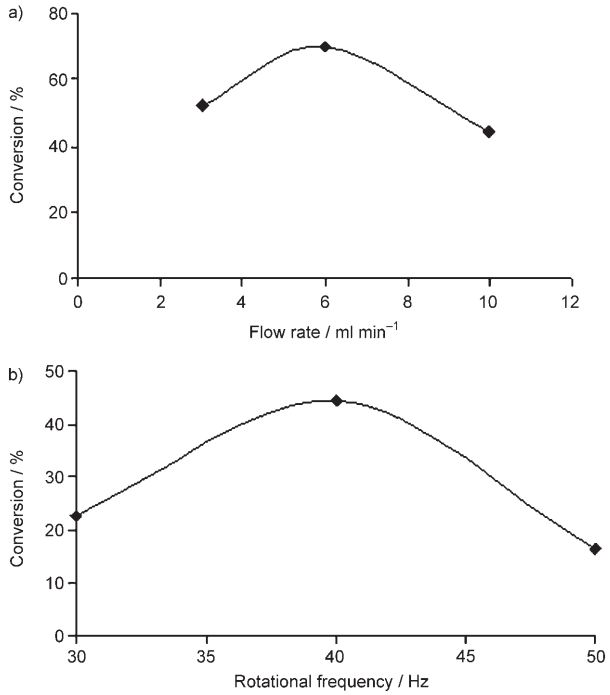

Figure 5. Effect of flow rate (a) and rotational speed (b) on the enzymatic esterification ([oleic acid] = 0.6 M, [BuOH] = 0.9 M, [lipase] = 1 g L^{-1} T = 50 °C; a) rotational speed = 40 Hz, $\Phi_{org} = \Phi_{aq}$; b) $\Phi_{org} = \Phi_{aq}$ = 10 mL min^{-1}).

steady-state conversion (70 %) was found at a rotational speed of 40 Hz, and a flow rate of both phases of 6 mL min^{-1}. The conversion shows a clear maximum with respect to the flow rate of each phase. At lower flow rates, phase separation in the CCS takes place more efficiently at the expense of the mixed phase, and is comparable to the effect of high spinning rates. At higher flow rates, the residence time in the CCS is too short, and also leads to lower conversions. In this particular case, the optimum flow rate was 6 mL min^{-1} for each phase. Similar to the biodiesel case, the rotational speed of the centrifuge has a profound effect on the conversion of oleic acid, and an optimum value was found to be 40 Hz.

Using the optimum settings determined above, a lipase-catalyzed esterification reaction at a higher enzyme loading (3.0 instead of 1.0 g L^{-1}) was performed (Figure 6). After about 2 h, the conversion became reasonably steady—fluctuating between 78 and 87 %, with an average of 82 % over the experiment. Repeat runs showed good reproducibility.

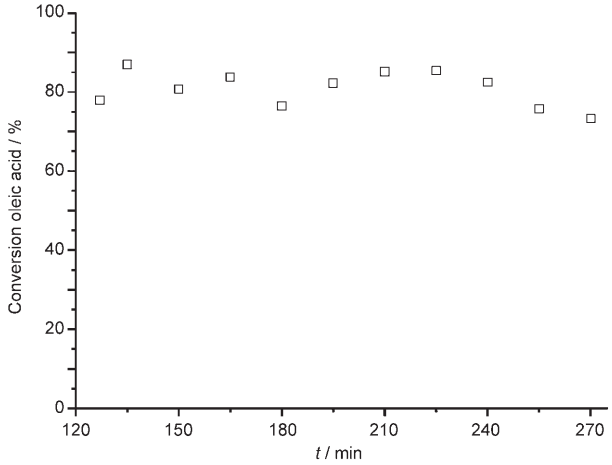

Figure 6. Lipase-catalyzed esterification of oleic acid with *n*BuOH in a CCS ([oleic acid] = 0.6 M, [*n*BuOH] = 0.9 M, [lipase] = 3.0 g L^{-1}, $\Phi_{org} = \Phi_{aq}$ = 6 mL min^{-1}, spinning rate = 40 Hz, T = 50 °C).

In the previous experiments, the enzyme solution was used in a once-through mode. To boost the turnover number of the enzyme, an experiment was performed in which the enzymatic solution was continuously recycled in combination with a partial recycling of the organic phase. With a 90 % recycling of the organic phase, a close to 80 % conversion of oleic acid into butyl oleate was achieved (Figure 7). The reactor was run in this mode for a period of 13 h, which led to a TON of 486 g butyl oleate per gram of enzyme. Although there is some erosion of conversion over time, the stability of the enzyme during this period can be considered remarkable in view of the high speed of the centrifuge. The stability of the

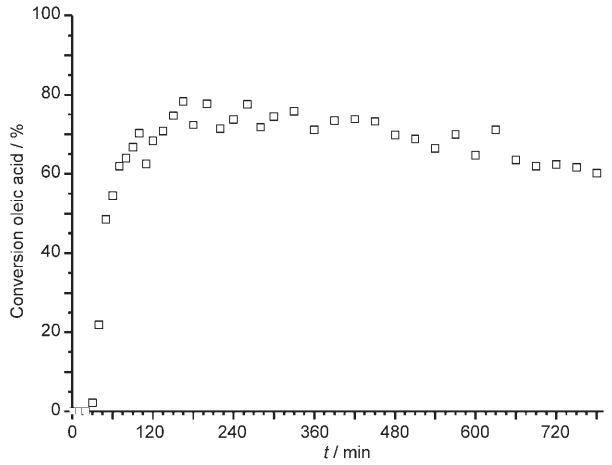

Figure 7. Lipase-catalyzed esterification of oleic acid with *n*BuOH in a CCS with full recycling of the water phase and 90 % recycling of the heptane phase. ([oleic acid] = 0.6 M, [*n*BuOH] = 0.9 M, [Lipase] = 6.0 g L^{-1}, $\Phi_{org} = \Phi_{aq}$ = 6.2 mL min^{-1}, spinning rate = 40 Hz, T = 50 °C).

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enzyme remains a key issue for future developments. The deactivation of the catalyst observed in Figure 7 may have a number of different causes: The most likely hypothesis is that an organic component which acts as an enzyme inhibitor accumulates in the aqueous phase. This hypothesis is currently under investigation.

In conclusion, we have shown that it is possible and highly advantageous to perform chemo- and biocatalytic conversions continuously in a table-top centrifugal contactor separator. Even in the current low-cost equipment, which can be situated in a fume cupboard, it is already possible to produce 100 kg amounts of chemicals in a matter of days. With larger volume CCSs commercially available, the way is opened for the continuous production of fine chemicals on a ton scale using two-phase catalysis.

Experimental Section

Typical experimental procedure for the enzymatic esterification of oleic acid with butanol: The experiments were performed in a CINC V-02 separator (also known as CS-50).[27] Two Verder VL 500 control peristaltic tube pumps equipped with a double pump head ($3.2 \times 1.6 \times 8$R) were used to feed the CCS. In the case of the enzymatic reaction, the low-mix bottom plate was used. To operate the reactor at a desired temperature, it was equipped with a jacket which was connected to a temperature-controlled water bath that had an accuracy of ± 0.01 °C. The CCS was fed with pure heptane and pure water, both with a flow rate of 6 mL min^{-1}. The centrifuge was then started (40 Hz, which corresponds to 2400 rpm) and the set-up was allowed to equilibrate for a period of 1 h. At this point, the heptane feed stream was replaced by the organic feed stream (oleic acid (0.6 M) and 1-butanol (0.9 M) in heptane). After equilibration for 10 min, the reaction in the CINC was started by replacing the water stream with the aqueous feed stream (0.1 M phosphate buffer, pH 5.6, containing 1 g L^{-1} of the lipase form *Rhizomucor miehei*). Samples were taken at regular intervals and analyzed by GC.

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