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Reactor Concept for Lipase-Catalyzed Solvent-Free Conversion of Highly Viscous Reactants Forming Two-Phase Systems

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Abstract:

To overcome limitations due to the high viscosity in the solvent free esterification of polyglycerol-3 and related polyols, such as poly(ethylene glycol)s, an alternative reactor concept was developed. The new reactor comprises a bubble column that prevents mechanical erosion of Novozym 435 (lipase B from *Candida antarctica*) found by mechanical stirring of the mixture. That way polyglycerol-3 laurate was synthesized at a space time yield (sty) of 3042 g/L/d and PEG-55-propylene glycol dioleate at a sty of 738 g/L/d. To proof the broad application range of this system, low-viscosity myristyl myristate was synthesized at a sty of 6731 g/L/d, thus outperforming conventional methods such as stirred tank or fixed bed. The newly developed reactor concept is universally applicable to esterification reactions and can be advantageously applied in the synthesis of a broad range of high quality surfactants.

1. Introduction

The production of bulk chemicals via biocatalysis in contrast to classical synthesis is only successful when economic advantages are given. Additionally, ecological benefits such as reduced carbon footprint can increase the market potential of enzymatic products, dependent on the actual pressure exerted by the respective governments and societies. In addition, biocatalyzed synthesis can be an alternative in cases where classical organic synthesis fails.[1] Biotransformations often apply milder reaction conditions[2] and are more selective.[3] This means fewer purification steps, a lower amount of side products, and lower energy consumption because of lower reaction temperatures.[4] These are also the arguments for the usage of enzymes in industrial synthesis of bulk chemicals such as surfactants for cosmetic applications.[5] Here, high standards regarding color, odor, and purity have to be reached. Subsidiary enzymatic conversions in solvent-free systems instead of using organic solvents are advantageous because of high initial rates and a simpler downstream processing. Additionally, the volumetric production is increased, costs for organic solvents are minimized,[6] and the above-mentioned environmental benefits could be proven by a Life Cycle Assessment.[7]

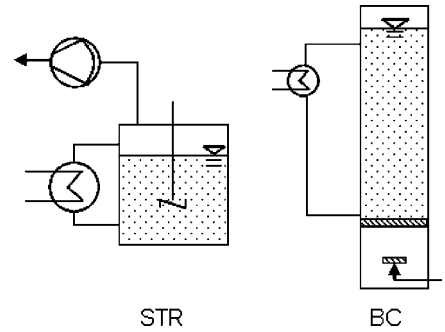

Figure 1. **Reactor setups for esterification of high-viscosity reactants: stirred tank reactor (STR) and bubble column reactor (BC).**

To further exploit the potential of biotechnology-produced surface active ingredients from renewable resources a biotechnological production platform has to be designed, outperforming classical syntheses. These originally are carried out using heavy metal catalysts or strong acids at temperatures of above 200−250 °C to produce ester oils as one example for surfactants.[8] One of the disadvantages of these reaction conditions is the low purity of the raw products due to the formation of side products. Many purification steps are necessary to reach the minimal requirements of product quality in cosmetic applications regarding color and odor. New processes based on more selective heterogeneous catalysts utilizing mesostructured materials might enable more efficient processes for large-scale synthesis of polyol esters.[9] Nevertheless, enzymes also offer a big potential in the selective synthesis of surfactants. This is the reason why recently the synthesis of emollient esters, such as cetyl ricinoleate or myristyl myristate on industrial scale was established by means of biocatalysis.[5] The enzymatic production process can be carried out in a fixed bed reactor with

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(1) Rao, N. N.; Lutz, S.; Seelbach, K.; Liese, A. In *Industrial Biotransformations*; Liese, A. ; Seelbach, K. ; Wandrey, C., Eds.; Wiley-VCH: Weinheim, 2006, pp 115−145.

(2) Lorenz, P.; Zinke, H. *Trends Biotechnol.* **2005**, *23*, 570.

(3) (a) Hilterhaus, L.; Liese, A. In *White Biotechnology*; Ulber, R. ; Sell, D., Eds.; *Adv. Biochem. Eng. Biotechnol.* **2007**, *105*, 133. (b) Hilterhaus, L.; Minow, B.; Muller, J.; Berheide, M.; Quitmann, H.; Katzer, M.; Thum, O.; Antranikian, G.; Zeng, A. P.; Liese, A. *Bioprocess Biosyst. Eng.* **2008**, *31*, 163.

(4) Schoemaker, H. E.; Mink, D.; Wubbolts, M. G. *Science* **2003**, *299*, 1694.

(5) Thum, O. *Tenside, Surfactants, Deterg.* **2004**, *41*, 287.

(6) Sandoval, G.; Condoret, J. S.; Monsan, P.; Marty, A. *Biotechnol. Bioeng.* **2002**, *78*, 313.

(7) Thum, O.; Oxenbøll, K. M. *SOFW J.* **2008**, *134*, 44.

(8) Kosswig, K. *In Ullmann's Encyclopedia of Industrial Chemistry*, Bohnet, Ed.;2002.

(9) Marquez-Alvarez, C.; Sastre, E.; Perez-Pariente, J. *Top. Catal.* **2004**, *27*, 105.

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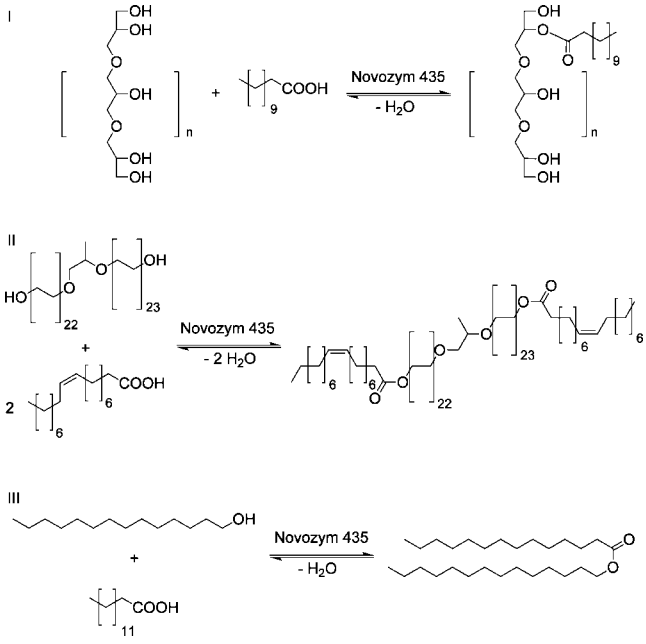
Scheme 1. **Reaction systems carried out within the developed reactor: polyglycerol-3/lauric acid (I); poly(ethylene glycol)-propylene glycol/olein (II); myristic alcohol/myristic acid (III)**



immobilized biocatalysts applying organic solvents[10] or in a solvent-free manner.[11] However, due to the maximum possible pressure drop over the column length, it is limited to organic solutions or low-viscosity reactants and products[12] as well as to one-phase systems.

The solvent-free synthesis of surfactants from high-viscosity or high-melting reactants, as for example sorbitol,[13] glucose,[14] fructose,[15] xylitol,[16] diglycerol,[17] polyglycerol, or other polyols such as α-butylglucoside,[18] is not possible by the existent fixed bed technology. Production in conventional stirred tank reactors is also prohibited due to the mechanical stress caused by the stirrer that usually destroys the used biocatalysts and thereby reduces its half-life time significantly. Therefore, the aim of the work reported here is to enable conversion of high-viscosity polyols and fatty acids from renewable resources to ester-based surfactants by developing a new synthesis platform.[19] As a model system the esterification of technical polyglycerol-3 with lauric acid catalyzed by lipase B from *Candida antarctica* (Novozym 435) was studied in bubble column reactors. The feasibility was demonstrated up to pilot-scale synthesis.

In the bubble columns (BC) that are used up until now within industrial production processes mostly reactions in gas/liquid two-phase systems are carried out.[20] In some cases an additional solid material, e.g. a catalyst, is suspended in the liquid phase. Only rarely are gas/liquid/liquid/solid multiphase systems described.[21] In the newly designed reactor system esterification of polyglycerol-3 with lauric acid starts as a four-phase system. The reaction is catalyzed by the heterogeneous biocatalyst Novozym 435, whereby the formed reaction water is removed by aeration with pressurized air. Here, the gas phase serves two functions, for one it is used to shift the thermodynamic equilibrium instant removal of formed reaction water. Second, it mixes the two liquid phases with only low impact on the mechanical stability of the used enzyme immobilisates. Regarding this coupled application only very few investigations have been made.[22] The development of a four-phase bubble column[23] for the hydrogenation of protected amino acids is one recent example. The transfer from our setup (Figure 1) to a second high-viscosity system (PEG-55-propylene glycol dioleate) or a simple low-viscosity homogeneous mixture (myristic alcohol/myristic acid) (Scheme 1) is demonstrating the broad applicability and the easy scalability.

2. Theoretical Basis

Bubble columns are reactors that are mainly applied in those cases where mass transfer limitations between a gas and a liquid phase are given. They facilitate the contact and/or reaction of a liquid and a gaseous phase. The gas can also interact with a substance dissolved in the liquid phase or with a component suspended in the liquid phase. Catalytic processes where the catalyst is suspended dominate the number of applications.[20]

In multiphase systems preceding and succeeding the chemical reaction there are mass transfer steps, which can be the limiting steps of the whole process. Thus, high mass transfer rates have to be established by the formation of large phase interfaces. The reactants polyglycerol-3 and lauric acid under investigation in this study are forming a two-phase system. The esterification is catalyzed by the solid catalyst Novozym 435, and the formed reaction water is removed by pressurized air rising through the reactor. In consequence, the resulting limiting factors are shown in Table 1.

Regarding the reaction rate-limiting step of mixing reactants as well as removal of water is predominant. Mixing of reactants is essential for a fast reaction within the synthesis of polyglycerol-3 laurate, whereas the removal of water ranks first in the synthesis of myristyl myristate. In the case that reactant emulsion formation is the reaction rate-limiting step, the energy input

(10) Xu, J. H.; Kato, Y.; Asano, Y. *Biotechnol. Bioeng.* **2001**, *73*, 493.
(11) Muhlhaus, R.; Jupke, A.; Mehrwald, A. *Lipid* **1997**, *99*, 392.
(12) Kulkarni, M. G.; Sawant, S. B. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 387.
(13) (a) Kim, H. J.; Youn, S. H.; Shin, C. S. *J. Biotechnol.* **2006**, *123*, 174. (b) Arcos, J. A.; Bernabe, M.; Otero, C. *Biotechnol. Bioeng.* **1998**, *60*, 53. (c) Ducret, A.; Giroux, A.; Trani, M.; Lortie, R. *Biotechnol. Bioeng.* **1995**, *48*, 214.
(14) (a) Ganske, F.; Bornscheuer, U. T. *J. Mol. Catal. B: Enzym.* **2005**, *36*, 40. (b) Tsitsimpikou, C.; Stamatis, H.; Sereti, V.; Daflos, H.; Kolisis, F. N. *J. Chem. Technol. Biotechnol.* **1998**, *71*, 309.
(15) (a) Sabeder, S.; Habulin, M.; Knez, Z. *Ind. Eng. Chem. Res.* **2005**, *44*, 9631. (b) Sabeder, S.; Habulin, M.; Knez, Z. *J. Food Eng.* **2006**, *77*, 880.
(16) Castello, E.; Pezzetti, F. Navarro, A.; Lopez-Munguia, A. *J. Biotechnol.* **2003**, *102*, 251.
(17) Garcia, E.; Ferrari, F.; Garcia, T.; Martinez, M.; Aracil, J. *J. Surf. Deterg.* **2001**, *4*, 257.
(18) Bousquet, M. P.; Willemot, R. M.; Monsan, P.; Boures, E. *Biotechnol. Bioeng.* **1999**, *63*, 730.
(19) Otero, C.; Arcos, J. A.; Berrendero, M. A.; Torres, C. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 883.
(20) Kantarci, N.; Borak, F.; Ulgen, K. O. *Process Biochem.* **2005**, *40*, 2263.
(21) Deckwer, W. D. *Reaktionstechnik in Blasensäulen*; Otto Salle Verlag: Frankfurt/M, 1985.
(22) (a) Akita, K.; Yoshida, F. *Ind. Eng. Chem. Process Des. Dev.* **1974**, *13*, 84. (b) Kato, Y.; Kago, T.; Morooka, S.; Nishiwaki, A. *J. Chem. Eng. Jpn.* **1985**, *18*, 154. (c) Kato, Y.; Kago, T.; Morooka, S. *J. Chem. Eng. Jpn.* **1984**, *17*, 429.
(23) Yamada, H. Dissertation; Nagoya University: Japan, 1999.
(24) Deckwer, W. D.; Burckhart, R.; Zoll, G. *Chem. Eng. Sci.* **1974**, *29*, 2177.

per volume to emulsify the reactants has to be considered. In BC reactors the energy dissipation rate is given by the product of the gas flow rate and the pressure drop:

$$P = \dot{V}_G \rho_L g \varepsilon_L L \quad (1)$$

The volumetric flow rate of the gas $\dot{V}_G$ and the length L of the reactor are given by the experimental conditions and apparatus. For the laboratory-scale reactor used in the present study the minimal $\dot{V}_G$ is 0.6 L/min and the length L is 27 cm. The density of the liquid phase ρ_L is available by averaging the densities of the single components relative to their volume percentages which is for an equimass mixture 1.08 kg/L (ρ(polyglycerol-3) = 1.28 kg/L; ρ(lauric acid) = 0.88 kg/L). The density constant within the course of reaction as well as gravitation g need not to be considered. Interesting in term of energy input is the relative phase percentage of the liquid phase ε_L. It varies as a function of the volumetric flow rate of the gas phase $\dot{V}_G$ as well as the conversion and must be taken into consideration regarding the energy input P. The calculation of the hydrodynamic parameter ε_L and the mass transfer coefficient k_L as well as the specific interfacial area a is very complex. All these depend on material properties, type of gas distribution, and geometrical dimensions as well as process conditions. Simple BCs can be described by the proportionality:

$$k_L a = b u_G^{-n} \quad (2)$$

In this equation n and b are empirical constants.[21] Since the emulsification is the rate-limiting step, one can assume that the interfacial area and the mass transfer gas/liquid in the case of the applied high gas velocities u_G is not limiting anymore. Because of the complexity of the overall system under investigation in this study, the relative phase percentage of the gas was determined by analysis of different operating heights:

$$\varepsilon_G = \frac{L_A - L_0}{L_A} \quad (3)$$

L_0 is the height of the nonaerated liquid phase, whereas L_A is the resulting height while aerating the liquid phase. In the case of a flow rate of 0.6 L/min L_A is 26.7 cm, whereas in nonaerated mode L_0 is 22.6 cm. That leads to an ε_G of ~0.15. This calculation is an approximation because the gas and liquid content is dependent on time and height. The obtained parameters allow the determination of the energy input P/V for the system polyglycerol-3/lauric acid while applying pressurized air as aeration gas. In the laboratory-scale reactor P is 10 mW and V is 92.5 mL. During scale-up of the BC the ratio of P/V has to be kept constant:

$$\frac{P}{V} = \frac{\dot{V}_G \rho_L g \varepsilon_L L}{V} = const \quad (4)$$

Since the volume directly correlates to the reactor length, only the ratio of gas flow rate and radius is relevant and the equation is reduced to:

$$\frac{P}{V} = \frac{\dot{V}_G \rho_L g \varepsilon_L}{r^2 \pi} = const \quad (5)$$

Consequently, the ratio of gas throughput to column radius r has to be kept constant. The volume percentage of gas has to be kept constant, too. Only in this case comparable mass transfer rates are reached. The mass transfer liquid/gas is not rate limiting and therefore is also not limiting for the reaction itself. As a consequence the ratio of gas throughput to the column radius can be determined for a scaled-up BC reactor. These results allow the scale-up of the BC reactor for the reaction of polyglycerol-3 and lauric acid. Widening the radius r = 1.05 cm of the column reactor to r = 15 cm results in an increase of the volumetric flow rate from 0.6 L/min to 7 m^3/h. In case of the synthesis of myristyl myristate a minimal aeration rate of 0.15 L/min at a radius r = 1.05 cm was observed to establish suspension of Novozym 435 in the reactants (see below). In case of an upscaled reactor with a radius of 15 cm this leads to a minimal aeration rate of 1.8 m^3/h.

The minimal aeration rate calculated for efficient mixing has not necessarily to be the optimum with respect to the reaction rate, since the removal of water can also become a limiting factor. Therefore, the overall batch size, i.e. the amount of water formed in the vessel, has to be considered. For example an aeration rate of 1.8 m^3/h allows in the best case the removal of 64.8 mol water at 60 °C within 5 h, whereas an aeration rate of 13 m^3/h enables the removal of 468.2 mol of water in the same time. In conclusion, the minimal aeration rate for the synthesis to keep the catalyst suspended is given by the radius of the reactor, whereas the optimum aeration rate to completely remove the water is dependent on the batch size.

Besides the effects of the aeration rate a third factor has to be considered: the amount of enzyme necessary to facilitate the desired degree of conversion within a given time frame. This enzyme amount is dependent on the specific activity of the biocatalyst used. Additionally another limitation of enzymatic reactions is observed as the reaction rate v significantly decreases at low substrate concentration [S]. This means that the reaction rate is decreased as a function of the reaction progress, whereas the efficiency of the reaction is given by the reaction time needed to obtain a certain conversion. The mathematical correlation of substrate concentration and reaction rate is given by the Michaelis−Menten equation:

Table 1. **Limitations of the esterification of polyglycerol-3 and lauric acid catalyzed by lipase B from *Candida antarctica* (Novozym 435)**

challenge	mass transfer	limiting factor
emulsification of polyglycerol-3 and lauric acid	liquid/liquid	energy input per volume: P/V
suspending of the biocatalyst	solid/liquid	energy input per volume: P/V
reaction of polyglycerol-3 and lauric acid	diffusion/convection	porosity of the carrier $k_1\eta$ and thickness of film layer on outer shell
removal of reaction water	liquid/gas	volumetric oxygen transfer at gas/liquid interface: k_{La}

$$-\frac{d[S]}{dt} = v = k_{cat.} \cdot c_E \cdot \frac{[S]}{[S] + K_m} \quad (6)$$

Herein is c_E the enzyme concentration, K_m the Michaelis constant and k_{cat} is defined as the quotient v_{max}/c_E. Integration results in the theoretical minimal reaction time TR_n to reach a defined conversion n:

$$TR_n = \frac{[S]_0 - [S]}{v_{max}} + \frac{K_m}{v_{max}} \cdot \ln\left(\frac{[S]_0}{[S]}\right) \quad (7)$$

For the reaction of myristic acid with myristyl alcohol catalyzed by Novozym 435 a $k_{cat.}$ of 7000 μmol/mg/min and a K_m of 150 mmol/L were determined experimentally for the batch of enzyme used. Following, when using 0.4% (w/w) of Novozym 435 the theoretical minimal reaction time TR_{95} for a conversion of 95% is 93 min, TR_{98} for a conversion of 98% is 101 min, TR_{99} for a conversion of 99% is 106 min, and $TR_{99.6}$ for a conversion of 99.6% is 112 min, respectively. Comparing the observed effective reaction time ER_n with the theoretical minimal reaction time TR_n results in the efficiency quotient EQ_n for a conversion n:

$$EQ_n = \frac{ER_n}{TR_n} \quad (8)$$

By comparing the EQ_n values of different reaction setups their efficiency can be easily determined. However, the minimal requirement is that the reactions to be compared in the different reaction setups are carried out under the same reaction conditions.

3. Results

System Characterization. At first limiting factors for the conversion of polyglycerol-3 and lauric acid were investigated in stirred tank reactors (STR). One of these factors is the viscosity of the reactants.[25] First, the viscosities of the separate reactants, their mixture, and the product were analyzed as function of temperature (Figure 2). The temperature was varied in the range of 50−90 °C, where the enzyme is active and the fatty acid is liquid. It turned out that a significant dependence of viscosity on the temperature was found for polyglycerol-3 and the product polyglycerol-3 laurate. To establish a drastic reduction in viscosity the temperature has to be set far above 90 °C. Although Novozym 435 is active up to temperatures of 110 °C,[26] the stability of the biocatalyst at these high temperatures and under these reaction conditions is significantly reduced. However, the emulsion of both reactants shows the lowest viscosity. Thus, in the beginning of the reaction at 75 °C the viscosity is 30 mPa·s which will increase by a factor of >20 in the course of the reaction. Therefore, at the end of the reaction a limitation in mixing of reactants is caused by the raised viscosity of 700 mPa·s.

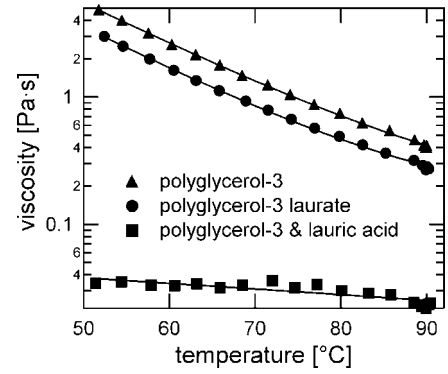


Figure 2. **Viscosities of the reactant polyglycerol-3, the mixture of polyglycerol-3 and lauric acid, and the product polyglycerol-3 laurate in the temperature range of 50−90 °C.**

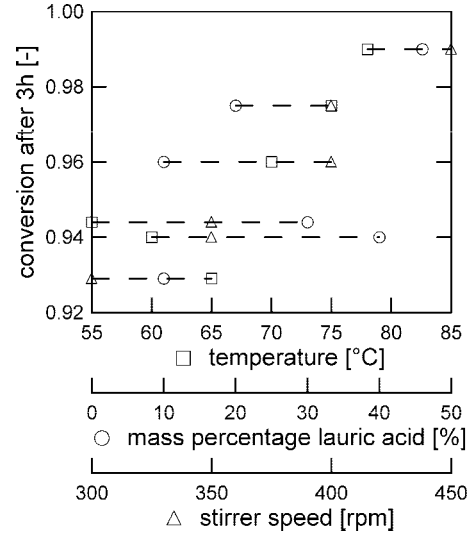


Figure 3. **Conversion after 3 h as a function of different sets of reaction parameters (stirrer speed, temperature, and reactant ratio). Reaction conditions: 10−36% (w/w) lauric acid; total mass 24 g; 5% (w/w) Novozym 435; 65−80 °C; STR 300−450 rpm and vacuum (<0.3 × 105 Pa).**

In the following the influence of mixing on the reaction was investigated.[15] The STR was operated at different revolutions per minute (rpm) under vacuum, and the resulting conversion after 3 h was analyzed. Mixing time in a specific reactor setup is dependent on the density and viscosity of both components, the volume ratio of both phases, and the difference in weight density $g \cdot \Delta\rho$. Therefore, mixing time in solvent-free processes is changing with reactant ratio, and optimization of this reaction parameter is needed. As a mathematical optimization procedure the Simplex method[27] was applied, aiming for maximum conversion. It turned out that a stirrer speed higher than 450 rpm leads to no enhancements in conversion (Figure 3). However, mechanical disintegration of the carrier of Novozym 435 was observed at this stirrer speed. This is a disadvantage for long-term use of the biocatalyst that is essential in the production of bulk chemicals. Hence, in the new reactor concept mechanical stirring has to be avoided.

(25) Fernandez-Perez, M.; Otero, C. *Enzyme Microb. Technol.* **2003**, *33*, 650.

(26) Tufvesson, P.; Annerling, A.; Hatti-Kaul, R.; Adlercreutz, D. *Biotechnol. Bioeng.* **2007**, *97*, 447.

(27) Borgwardt, K. H. *The simplex method*; Springer: Berlin, 1987.

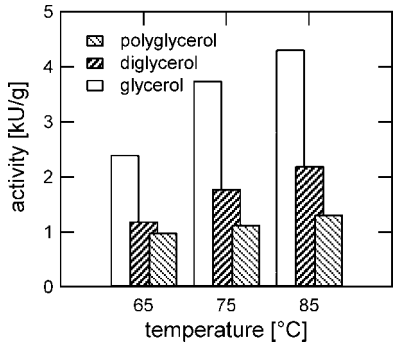


Figure 4. **Activity of Novozym 435 within the esterification reaction of the reactants glycerol, diglycerol, and polyglycerol-3 with lauric acid; STR; 400 rpm; vacuum.**

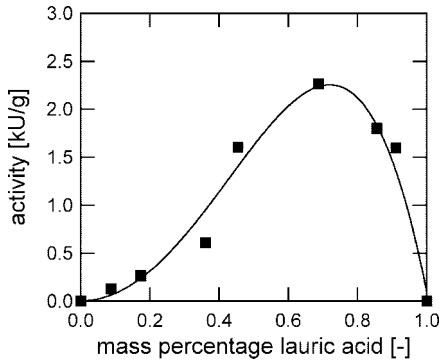


Figure 5. **Variation of the ratio of reactants and its effect on the initial esterification rate; equimass amounts of reactants, 5% (w/w) Novozym 435; 75 °C; 400 rpm; vacuum.**

Adequate mixing and efficient removal of reaction water are prerequisites for total conversion of polyglycerol-3 and lauric acid catalyzed by Novozym 435. Sufficient mixing of the reactants is a key parameter for enhancing the sty. In part this is possible by increasing the temperature as mentioned above and thus reducing the velocity. The elevated temperature is a compromise between facilitating mixing and increasing enzyme activity, while deactivating the enzyme due to thermal effects.[28]

The used polyglycerol-3 is of technical grade, meaning respective batches vary slightly in composition, containing different oligomers and isomers.[29] Thus, the composition of the reactant polyglycerol-3 might have an influence on the reaction rate. In order to investigate this influence, the esterification rates of glycerol, diglycerol, and polyglycerol-3 (principal components, triglycerol and diglycerol) were analyzed. The results show that the initial reaction rates of the different oligomers decrease with increasing chain length of the used oligomer (Figure 4) and increase with rising temperature.

Further on, the reaction rate is influenced by the ratio of the starting materials[6] polyglycerol-3 and lauric acid (Figure 5). Total conversion of the fatty acid can be reached within a certain range, since the reactant polyglycerol-3 consists of multiple hydroxyl functions. Thus, at the start of the reaction the viscosity can be reduced by the ratio of the reactants, whereas the esterification rate is not hindered by a missing alcohol component. The esterification reaction shows a maximum in reaction rate at a percentage of 70% (w/w) lauric acid. In a range of 10 percentage points to lower and higher fatty acid content, no drastic decrease of the reaction rate is observed. In all further experiments a ratio of 50:50 (w/w) was chosen. Hereby, a large amount of monoester can be attained, which has the highest value regarding the final application in cosmetics.

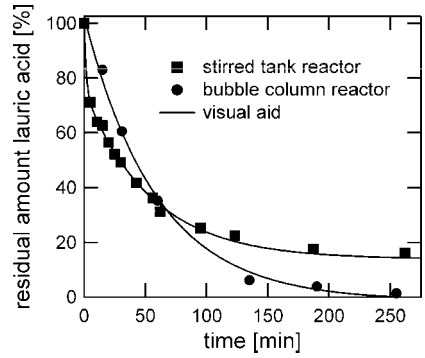


Figure 6. **Comparison of the initial rates of the conversion of polyglycerol-3 and lauric acid while water is removed by vacuum (STR) or by aeration (BC).**

Evaluation of the Bubble Column Reactor. Comparing the reaction courses of the conversion in the STR and in the BC (Scheme 1), the initial reaction rate is comparable in both cases (Figure 6). During the reaction one can recognize the increasing viscosity of the reaction medium by the formation of polyglycerol-3 laurate. The lower power input due to the rising bubbles in the BC in comparison to that of the stirrer in the STR becomes obvious. However, the big advantage of the conversion in the BC is the lower mechanical erosion of the carrier of Novozym 435.[30] The distinct lower mechanical shear forces prevailing in the BC allow a considerable longer usage of the biocatalyst in this type of reactor.

Further on, the synthesis of polyglycerol-3 laurate was carried out in the bubble column in a solvent-free manner at a temperature of 95 °C catalyzed by Novozym 435. The conversion was successful and led to initial rates of 1.24 kU/g regarding the conversion of lauric acid. Novozym 435 showed a half-life time of 9 h within the repetitive use, meaning this biocatalyst has a reasonable stability at elevated temperatures. Nevertheless, after reusing the enzyme four times, complete conversion of the lauric acid could no longer be reached (data not shown). In order to enhance the sty regarding the biocatalyst the reaction temperature was decreased to 75 °C. These experiments (Figure 7) show the reusability of Novozym 435 within the chosen type of reactor up to nine times. The observed initial reaction rates of around 1.2 kU/g are marginally below that of the reaction at 95 °C. Thus, half-life time was increased up to 19.9 h by reducing the temperature.

Important for a successful industrial application is the stability and reusability of the biocatalyst. Even though the mechanical stress on the used biocatalyst is significantly reduced in the new reactor setup, the observed half-life time still needs to be increased. Taking into account that the lipase B in the Novozym 435 is noncovalently adsorbed on the carrier and that the formed polyglycerol-3 esters are strong surfactants, the

(28) (a) Tornvall, U.; Orellana-Coca, C.; Hatti-Kaul, R.; Adlercreutz, D. *Enzyme Microb. Technol.* **2007**, *40*, 447. (b) Guo, Z.; Sun, Y. *Food Chem.* **2007**, *100*, 1076.

(29) (a) Behrens, H.; Mieth, G. *Food* **1984**, *28*, 815. (b) Schütze, I. *Food* **1977**, *21*, 405.

(30) Fu, C. C.; Wu, W. T.; Lu, S. Y. *Enzyme Microb. Technol.* **2003**, *33*, 332.

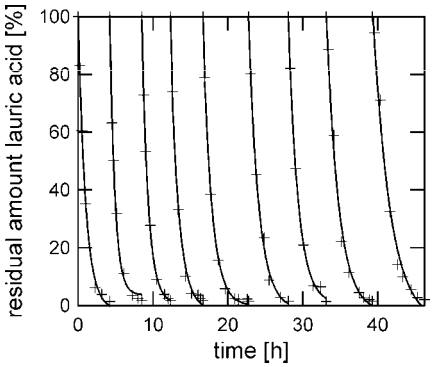


Figure 7. **Repetitive batch reaction of polyglycerol-3 and lauric acid catalyzed by Novozym 435 at 75 °C; equimass amounts of reactants; 5% (w/w) Novozym 435; 2.2 L/min pressurized air.**

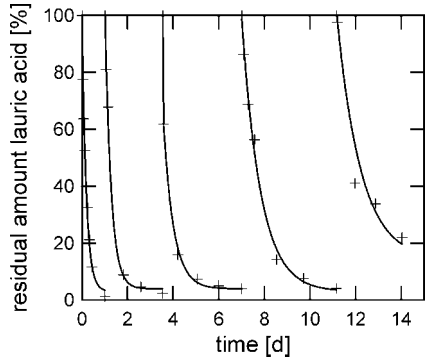


Figure 8. **Repetitive batch at 75 °C applying a cross-linked carrier-bound lipase B preparation within the reaction of equimass amounts of polyglycerol-3 and lauric acid in BC.**

observed deactivation might be due to leaching. Therefore, the results found for Novozym 435 were compared to those found by using a more leaching-stable version of immobilized lipase B (glutardialdehyde cross-linked after immobilization on carrier). The activity (1.06 kU/g) was slightly lower than that of Novozym 435, but as expected, this preparation showed a much higher stability (half-life time $\tau_{1/2} = 68$ h), thus proving the leaching sensitivity of Novozym 435 (Figure 8). Altogether we could show that the reactor setup allows very good enzyme stabilities even at elevated temperatures, as long as all other parameters and reaction conditions do not deactivate the biocatalyst.

To illustrate the broad applicability of BCs within the solvent-free conversion of fatty acids and polyols, two further systems were investigated, first the synthesis of poly(ethylene glycol)-propylene glycol dioleate as another example of a high-viscosity product and second the synthesis of myristyl myristate, a simple low-viscosity emollient ester. Both syntheses lead to industrially important surfactants and have been successfully conducted in our BC reactor. Initial rates of 0.2 kU/g in the case of PEG-propylene glycol dioleate and 7.0 kU/g in the case of myristyl myristate were reached.

The synthesis of myristyl myristate was further optimized in view of economic realization. Therefore, the stepwise reduction of the aeration rate and enzyme content was carried out at laboratory scale. Concerning the enzyme content, it was shown that the synthesis can be conducted in a range of 0.15−1.0% Novozym 435 (w/w). A further increase of biocatalyst concentration did not lead to an increase in the reaction rate (data not shown), probably due to the fact that the water removal is the time-limiting step at those enzyme concentrations (see below). In all cases the total conversion (>98%) of myristic acid after 24 h was reached. The aeration rate is the parameter which enables mixing of the reactants as well as water removal. Improved mixing will enable higher initial rates of esterification but also increases the costs for the production of myristyl myristate. These costs are caused by the supply of the pressurized air itself. Conditions using the least possible gas input without significantly decreasing the sty were identified, and the lower limit for successful conversion of the reactants at laboratory scale was determined to be 0.15 L/min. Thereafter, the dispersion of the biocatalyst particle is no longer possible.

As described above, based on the determined minimal gas input of 0.15 mL at laboratory scale, calculations for an upscaled reactor with a radius of 15 cm led to a required minimal aeration rate of 1.8 m^3/h for catalyst suspension. Nevertheless, those calculations only yielded a rough estimation of the theoretically minimum aeration for full water removal. For example an aeration rate of 5 m^3/h allows in best case the removal of 250 mol water at 60 °C within 7 h. To investigate the effect of the aeration rate on the conversion, a set of experiments were performed, using a 250 mol batch of myristyl myristate synthesis with two aeration rates: 5 m^3/h and 13 m^3/h, in a BC with $r =$ 15 cm. As the amount of enzyme used (0.4 wt % of NZ435) should allow theoretically (see Table 2) a turnover of 99.6% in about 2 h, the efficient water removal is the rate-limiting step. As can be seen in Figure 9 the increase of aeration rate has indeed a significant effect on the observed reaction time, thus shortening it from about 8.5 h, which fits quite well to the calculated minimum of 7 h, to about 6 h, thereby increasing the sty by more than 40% (see Table 3).

To compare the efficiency of the BC reactor system with that of commonly used reactor types, the synthesis of myristyl myristate was performed in a pilot plant BC reactor ($r = 15$ cm, batch size: 22 kg (about 49 mol), aeration: 5 m^3/h pressurized air), in a stirred flask reactor (batch size: 1015 g) and in a conventional fixed bed reactor (batch size: 1015 g, flow rate: 60 mL/min). In all three reactions 0.4% (w/w) of Novozym 435 was used as catalyst and the reaction temperature was set to 60 °C (Figure 10). In case of the stirred flask reactor and of the fixed bed reactor vacuum (10 mbar) was applied for water removal.

Under these conditions, the synthesis of myristyl myristate in a stirred tank reactor takes about 24 h to reach a conversion of 99.6%; whereas in the fixed bed reactor only 14 h were needed. In contrast, in the BC reactor this conversion is already obtained after 5.5 h. Comparing these observed effective reaction times ER_n with the theoretical reaction times TR_n and calculating the efficiency quotients EQ_n revealed the high efficiency of the new reactor system for enzymatic esterifications. The efficiency quotients $EQ_{99.6}$ of 14.2 using the stirred tank reactor and 8.3 when using the fixed bed reactor are significantly reduced to 3.3 when applying the developed BC reactor.

4. Discussion:

An alternative reactor concept was developed overcoming limitations by viscosity in solvent free conversions and mini-

Table 2. **Comparison of different reactor setups for the synthesis of myristyl myristate**

conversion n	95%		98%		99%		99.6%	
TR_n [min]:	93		101		106		112	
	ER [min]	EQ_{95}	ER [min]	EQ_{98}	ER [min]	EQ_{99}	ER [min]	$EQ_{99.6}$
stirred flask	350	3.75	640	6.33	802	7.94	1440	14.25
fixed bed	280	3.00	410	4.06	530	5.24	840	8.31
bubble column	150	1.61	215	2.13	250	2.47	330	3.27

mizing mechanical erosion of Novozym 435. The enzymatic synthesis of mono-, di-, and triacyglycerols from (poly)unsaturated fatty acids was carried out as a solvent-free reaction in a packed bed reactor by Arcos et al.[31] However, a fixed bed reactor is restricted to raw materials and products having a viscosity which is low enough to allow its pumping through the bed of immobilized enzyme.[5]

In the case of the solvent free conversion of long chain fatty acids and fatty alcohols in first instance, a stirred tank reactor is used. For example, Laudani et al. investigate the synthesis of octyl oleate in a stirred batch reactor.[32] A high stirring rate of 500 rpm was applied and led to 84% conversion (63 mmol of each substrate) after 5 h applying 5.45% (w/w) Lipozyme

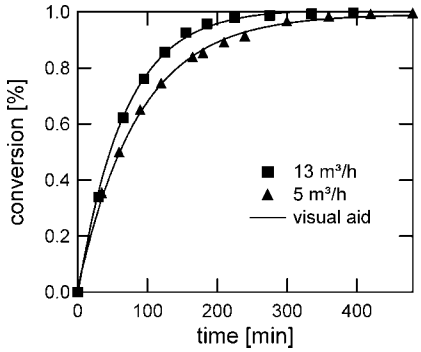


Figure 9. **Analysis of the aeration rate in the BC in the case of myristyl myristate synthesis.**

Table 3. **Space time yield and reaction rate of products under investigation**

product	sty [g L^{-1} d^{-1}]	reaction rate [kU g^{-1}]
polyglycerol-3 laurate	3042	1.2
PEG-propylene glycol dioleate	738	0.2
myristyl myristate	6731	7.0

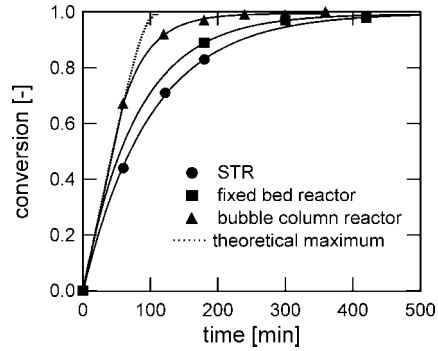


Figure 10. **Comparison of different reactor setups for the synthesis of myristyl myristate with the theoretically calculated reaction time. All reactions with 0.4% (w/w) of Novozym 435 at 60 °C.**

RM and reaching an initial reaction rate of 0.04 mmol/g/min. Beforehand, Yong et al. reached 77% conversion after 3 h at 30 °C also applying Lipozyme from *Rhizomucor miehei*[33] without giving information regarding stirring. Dianóczki et al. checked the reusability of Novozym 435 in a solvent free medium.[34] They applied 8.9% (w/w) Novozym 435 at 65 °C under vacuum for 2 h per batch and could reuse the biocatalyst 10 times without deactivation. However, in these entire reactor setups high enzyme loading and fast stirring is essential for sufficient conversion, thus leading to high enzyme costs and reduced reusability, respectively.

An alternative is the removal of reaction water using a dry gas stream. Such a process was developed by Petersson et al.[35] for the production of wax esters in a solvent free manner by passing a stream of dry air through the reactor. At a temperature of 65 − 93 °C they reached 95−99% conversion to the low-viscosity esters cetyl palmitate, behenyl behenate, dibehenyl adipate and dibehenyl sebacate after ∼24 h and initial reaction rates of 1.4−3.4 mmol/min/g. Their calculations led to an aeration rate in a 100 L reactor of 963 L/min. The application of vacuum driven nitrogen-stirring is reported by Guo et al. for the solvent-free production of 1,3-diglycerides.[28] They used 55.4 g/L Novozym 435 (61% (w/w)) to convert 0.138 mol/L glycerol and 0.277 mol/L linoleic acid at 50 °C. After 6 h they reached total conversion of the fatty acid. Nevertheless, the amount of nitrogen used was not quantified. Ten repetitive batches in the synthesis of 1,3-diglyceride were conducted, illustrating the reusability of Novozym 435 and the possible productivity of 146 kg of product per 1 kg of Novozym 435. In comparison the productivity reached by the myristyl myristate process described here is much higher: 250 kg per 1 kg of Novozym 435 can be reached easily in a single use. Additionally, reusability has been proven for at least 10 times (data not shown), thus increasing the productivity to at least 2500 kg per 1 kg of Novozym 435, thus outperforming all processes cited above. Further on, within these studies two effects are not highlighted: the quantification of the amount of gas which is needed to sufficiently carry out the reaction and the effect which arises from the availability of the formed surface-active product.

Within this study we could show that there is a strong influence of mixing by the aeration rate and the stirrer speed

(31) Arcos, J. A.; Garcia, H. S.; Hill, C. G. *Biotechnol. Bioeng.* **2000**, *68*, 563.
(32) Laudani, C. G.; Habulin, M.; Primoziz, M.; Knez, Z.; Della Porta, G.; Reverchon, E. *Bioprocess Biosyst. Eng.* **2006**, *29*, 119.
(33) Yong, Y. P.; AlDuri, B. *J. Chem. Technol. Biotechnol.* **1996**, *65*, 239.
(34) Dianoczki, C.; Recseg, K.; Kovari, K.; Poppe, L. *J. Mol. Catal. B: Enzym.* **2007**, *45*, 45.
(35) Petersson, A. E. V.; Gustafsson, L. M.; Nordblad, M.; Borjesson, P.; Mattiasson, B.; Adlercreutz, P. *Green Chem.* **2005**, *7*, 837.
(36) Thum, O.; Hilterhaus, L.; Liese, A. DE102008004726, 2008.

on the conversion rate. Nevertheless, from an industrial point of view, the air flow should be as low as possible. Due to the costs generated by high air flow, the flow was reduced until no dispersion of the biocatalyst could be ensured. This flow rate also enables the reaction of the alcohol and the acid, the flow rate still being high enough for the removal of the reaction water. Thus, the synthesis of myristyl myristate was optimized to an aeration rate 0.15 L/min on laboratory scale. Additionally, the enzyme content was optimized, so that in comparison to the works mentioned above, only 0.15% (w/w) Novozym 435 is necessary to carry out the reaction. Based on the data provided within this study, the optimal parameters regarding column geometry, aeration rate, and amount of enzyme used can be calculated when upscaling to an industrial-scale process in order to minimized production costs by optimizing the balance of maximized sty and of minimized enzyme costs.

5. Conclusions

The system characterization for polyglycerol-3 and lauric acid showed that, during the reaction, viscosity will increase by a factor of 20. Therefore, emulsifying the reactants by efficient mixing is essential. The mechanical stability of Novozym 435 is not given at the necessary stirrer speed. Therefore, an alternative mixing process was established by applying a BC. Herein the synthesis of polyglycerol-3 laurate was possible, and the setup was also used for the synthesis of PEG-propylene glycol dioleate and myristyl myristate (Tab. 3).

Further on, the setup was successfully scaled up to pilot-plant scale where its advantages over conventional reactor concepts for enzymatic esterification could be demonstrated for low-viscosity as well as for high-viscosity reaction mixtures, especially when aiming for high degrees of conversion.[3,6]

6. Experimental Section

Chemicals. All chemicals used were purchased from Fluka and Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Solvents were from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Polyglycerol-3 was from Daicel, Japan. Myristic acid and myristic alcohol (all technical grades) were from Cognis, Monheim, Germany; Novozym 435 was from Novozymes A/S, Bagsvaerd, Denmark.

Determination of Acid Number. Acid number of ester oils was determined by direct titration with a 0.1 M solution of sodium hydroxide in EtOH. Phenolphthalein was used as indicator; 0.5 g of the respective sample was dissolved in 30 mL ethanol, indicator (10 μL; 1% (w/v)) was added, and the titration was started until neutralization was reached.

Apparatus. Laboratory-scale reactions were carried out in a glass bubble column (Figure 1, BC). The reactor vessel was cylindrical (2.1 cm diameter) with a thermostatted jacket. The carrier of the biocatalyst was retained by a stainless-steel sieve (mesh 50 μm) installed at the bottom of the bioreactor, ensuring a good dispersion of gas bubbles at the same time. Gas flow was controlled and adjusted manually during the course of reaction, and constant flow was ensured. Circulation of reactants was enabled by a flexible-tube pump (Verder VRX 200, Haan, Germany).

Pilot-scale reactions were carried out in a similar system, having a diameter of 30 cm and a bubble length of up to 250 cm.

Determination of Polyglycerol-3 Content. Analyses were carried out by means of an analytical HPLC (Agilent 1100) with a column oven, autosampler, vacuum solvent degassing module, diode-array detector and evaporative light scattering detector (Polymer Laboratories 2100). The column used was a reverse-phase C-18 column: LiChrospher RP-18, 5 μm, 250 mm $\times$ 4.6 mm (Merck, Germany). Flow rate was 1.0 mL/min and column temperature 50 °C. As eluent acetonitrile/water, 60:40 (v/v), was applied. Quantification of reaction products was carried out by evaporative light-scattering detection (ELSD; evaporator 60 °C, nebulizer 60 °C, 1.0 mL/min nitrogen flow). The sample injection volume was 5 μL. A gradient of acetonitrile (ramped up to 100% acetonitrile in 5 min) was used to wash off the nonreacted lauric acid. Typical retention times were polyglycerol-3 2 min, lauric acid 11 min, polyglycerol-3 laurate 6−8 min.

Viscometry of Reactants and Products. Kinematic viscosity was measured using a Cannon-Fenske capillary viscometer (State College, PA). The viscometer was calibrated with standard fluids supplied by the manufacturer. Samples were thermally equilibrated for at least 30 min before each determination, ranging from 50 to 90 °C.

General Procedure for Batch Experiments. Equimass amounts of reactants were mixed for 15 min at a given temperature. In the BC mixing was enabled by an air stream of 0.15 L/min (laboratory scale) up to 13 m^3/h (pilot scale), in stirred tank reactor by stirring at 400 rpm with an overhead plate stirrer. The reaction was started by addition of a certain amount of biocatalyst. Samples were withdrawn after specific time intervals and analyzed.

General Procedure for Repetitive Batch Experiments. Equimass amounts of reactants were mixed for 15 min at a given temperature. The reaction was started by addition of a certain amount of biocatalyst. After reaching the abortion criterion of the experiment, the product was filtered of, and the biocatalyst was retained in the BC by the stainless steel sieve. In the case of a repetitive batch experiment new reactants were added. This procedure was repeated until a certain loss of biocatalyst activity was reached.

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