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Theoretical and Experimental Studies of Enzyme-Catalyzed Isoamyl Acetate Synthesis with Ionic Liquid at the Microreactor Scale

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In this work, a lipase-catalyzed synthesis of isoamyl acetate, which is one of the most employed flavours in food industry, was studied in a microreactor. Synthesis of isoamyl acetate from acetic anhydride and isoamyl alcohol, and simultaneous extraction in organic phase was performed in microreactors of different geometries using 1-Butyl-3-methylpyridinium dicyanamide/*n*-heptane two-phase system. Experimental data, obtained at different process conditions have demonstrated good esterification yields in the chosen system.

1. Introduction

Enzymatic production of esters used in food, cosmetic and pharmaceutical industry is of high importance since such products can be labeled as “natural”. Isoamyl acetate is one of the most employed esters used in food, cosmetic and pharmaceutical industries due to its characteristic banana flavour. Lipases (EC 3.1.1.3.) of different origin, which can catalyze hydrolysis in aqueous environment, as well as esterifications in organic solvents, are generally employed in the production of flavours and fragrances (Krings and Berger, 1998, Hasan et al., 2006).

Ionic liquids (ILs) have received an increased attention as "green" solvents for organic synthesis in general and catalytic processes in particular. Recently, the use of ionic liquids as reaction media has been extended to enzymatic catalysis (Rantwijk et al., 2003) and whole cells biotransformations (Pfruender et al., 2004). Some ILs have been shown to be by far the best non-aqueous media for biocatalytic processes due to their positive influences on the enzyme stability and activity, as well as on the enantioselectivity of the reactions catalyzed by them. Just recently, a study on different ionic liquid-alcohol biphasic systems for the enzymatic production of isoamyl acetate was reported (Fehér et al., 2008). However, their use in industrial processes is restricted due to high prices at the market, so the development of the processes with these solvents at microscale is suggested here as a promising alternative.

Microtechnology has opened completely new scientific challenges and useful solutions in a broad range of areas, from electronic industry, medical technology, fuel production and processing, to biotechnology, chemical industry, environmental protection and process safety. Microstructured devices have demonstrated several advantages in (bio)chemical processes, due to the very large surface-area-to-volume ratio connected

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with very effective heat and mass transfer, easier control of process parameters and new production concepts (Ehrfeld et al., 2000). In recent years, a successful application of enzymatic microreactors has been reported, mainly in chemical analysis and kinetic studies. However, the potential of enzymatic microreactors for introduction into industrial-scale synthesis and in environmental protection is not yet exploited (Urban et al., 2006).

Here we report on the study of a lipase-catalyzed synthesis of isoamyl acetate and simultaneous extraction in a microreactor using 1-Butyl-3-methylpyridinium dicyanamide/*n*-heptane two-phase system. Different operating conditions within microchannels of various geometries were tested. The formation of droplets in the system was modeled using the level set method.

2. Materials and methods

- Lipase B (CALB L) from *Candida antarctica* (Novozymes, Bagsværd, Denmark)
- Isoamyl alcohol, acetic anhydride, isoamyl acetate, *n*-heptane, (Sigma-Aldrich ChemieGmbH, Steinheim, Germany)
- 1-Butyl-3-methylpyridinium dicyanamide (Solvent Innovation GmbH, Köln, Germany)
- Different microchannel systems (Micronit Microfluidics BV, Enschede, The Netherlands)
- PHD 4400 Syringe Pump Series (Harvard Apparatus, Holliston, USA)
- GC HP 6890 (Hewlett-Packard, Palo Alto, USA)
- Vibromix 204 EV (Tehtnica, Železniki, Slovenia)

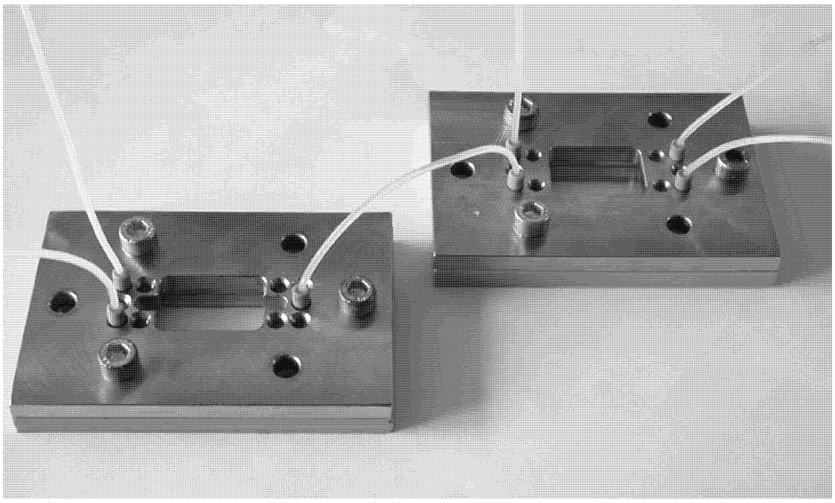

Figure 1. The experimental setup.

2.1 Batch experiment

Batch experiments were performed in a vigorously mixed test tube containing 2 mL of the reaction mixture and 2 mL of *n*-heptane. Different concentrations of enzyme solutions in ionic liquid ranging from 5 to 20 % (v/v) were used, while initial concentrations of acetic anhydride and isoamyl alcohol in ionic liquid were kept 0.5 M and 1.5 M, respectively. The concentration of the synthesized isoamyl acetate in heptane phase was analyzed with a gas chromatograph and plotted against time.

2.2 Simultaneous isoamyl acetate synthesis and extraction in the microreactor

The microreactor used for the experiments had two inlet channels and one outlet channel. The dimensions of the channels were: width 220 μm, depth 100 μm, and length 1328 mm. One inlet channel contained the ionic liquid, enzyme, isoamyl acetate and acetic anhydride, while *n*-heptane was pumped through the second inlet and was used for continuous extraction of the product. The concentration of acetic anhydride was 0.5 M and the concentration of isoamyl alcohol was 1.5 M. The enzyme concentration was 20 % by volume.

2.3 Isoamyl acetate analysis

Preliminary experiments on isoamyl acetate, isoamyl alcohol and acetic anhydride batch extraction from ionic liquid revealed that only isoamyl acetate was efficiently extracted into heptane, with extraction yields ranging between 70 and 80 %, while both reactants had very low partitioning coefficients in this system. Product content in the heptane phase was determined by a gas chromatograph HP 6890 (Hewlett-Packard, Palo Alto, USA) equipped with a hydrogen flame ionization detector and a HP-INNOWAX column (30 m × 0.25 mm i.d. × 0.25 μm). Nitrogen was used as a carrier gas at a flow rate of 29 ml/min. The temperature of the oven at the injection was 100 ºC and was kept constant for 1 min. The linear increase in temperature to 200 ºC was set by 35 º/min, and was kept at 200 ºC till the end of the analysis. Injector and detector temperatures were set at 250 ºC. The retention time for isoamyl acetate was 2.5 min.

3. Results

3.1 Batch experiments

Initial studies were done in a batch experiment, where the suitability of the chosen ionic liquid was tested. After 100 minutes a yield of over 100 % in regard to the initial acetic anhydride concentration was obtained due to the secondary reaction with acetic acid (Fig. 2). 1-Butyl-3-methylpyridinium dicyanamide was found to be a very successful medium for this particular reaction. In the case of 5 % enzyme concentration, the conversion was only 60 %. Higher enzyme concentrations gave higher yields and increased the rate of reaction.

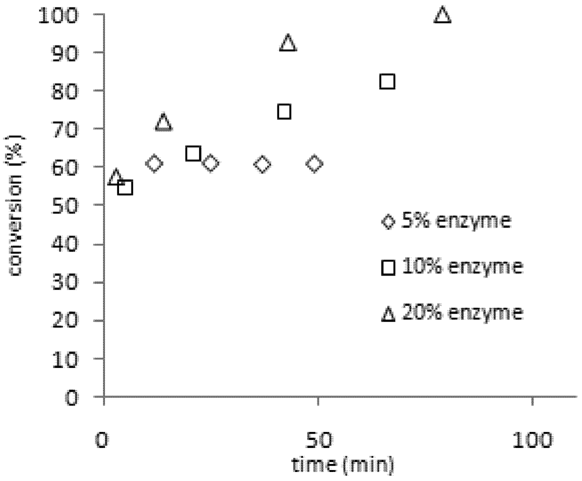

Figure 2. The results of batch experiments. Experiment were done at room temperature with 0.5 M acetic anhydride, 1.5 M isoamyl alcohol and varying enzyme concentrations.

3.2 Simultaneous isoamyl acetate synthesis and extraction in the microreactor

Optimum conditions derived from the former experiments were also proven to work best for the synthesis of isoamyl acetate in the microreactor. The ionic liquid flows were set to 5 µL/min each, and the flow of heptane to 10 µL/min. The yield of isoamyl acetate achieved was 62 % at the residence time of 88 seconds, which is twice as fast as in the batch experiment. Slug flow (Fig. 3) and further intense emulsification took place (Fig. 4), which insured effective mixing of reactants and a large interfacial area for the reaction and product extraction, which was also observed by Burns and Ramshaw (2001) and Žnidaršič-Plazl and Plazl (2007). Although lower flow rates gave larger residence times, the mixing was not as effective, and the lowering of flow rate by half gave a significantly lower yield of only 21 %. Higher yields can be achieved by increasing the length of the microchannel.

3.3 Droplet formation

The problem of a moving boundary was addressed in the simulation in which the geometry's topology changes with time. The fluid-fluid interface is tracked by a level set method. A momentum transport equation, a continuity equation and a level set equation was solved using the finite elements method. The model presumes a wetted wall boundary condition, takes into account the differences in densities and viscosities as well as the effect of surface tension. The contact angle between the fluid interface and the solid wall was measured and incorporated in the equation system. The modeling was done using Comsol Multiphysics.

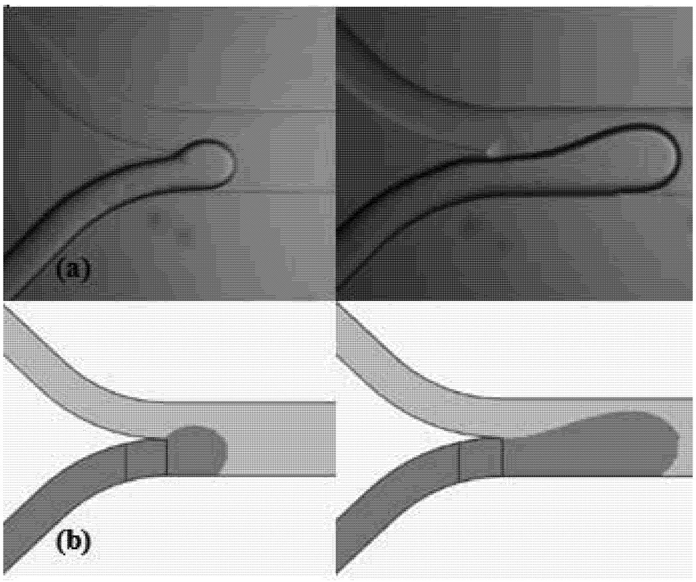

Figure 3. Microreactor inlet. (a) shows photos taken from the experiment while (b) shows the droplet formation simulation using the level set method. Heptane flows in the lower channel while the other channel contains the ionic liquid, the enzyme and the reactants.

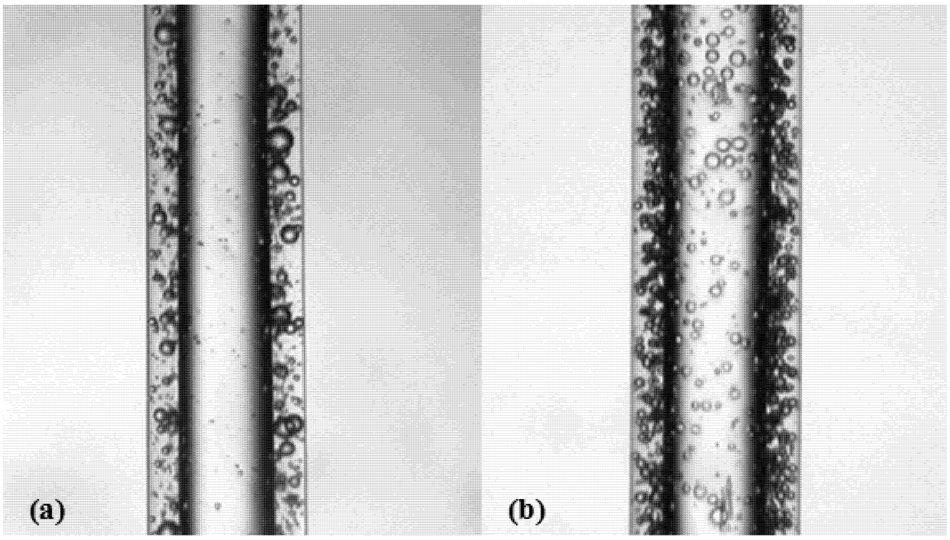

Figure 4. At higher flow rates, emulsion was formed within the microchannel: a) the emulsion at the flow rate of 10 μL/min; b) a much more intense emulsion at the flow rate of 20 μL/min. Heptane is positioned in the center.

4. Conclusions

- Enzymatic synthesis of isoamyl acetate in the selected ionic liquid was found to be very successful.
- Effective mixing is crucial for an efficient reaction and extraction.
- Although phase separation was not achieved inside the microreactor, a successive microreactor with an embedded membrane would separate the two phases.
- For a higher product yield, a longer channel is expected to give the desired result.

5. References

Burns J.R. and Ramshaw C., 2001, The intensification of rapid reactions in multiphase systems using slug flow in capillaries, Lab Chip 1, 10-15.

Ehrfeld W., Hessel V. and Löwe H, 2000, Microreactors: New Technology for Modern Chemistry, Wiley-VCH, Weinheim.

Fehér E., Illeová V., Kelemen-Horváth I., Bélafi-Bakó K., Polakovič M. and Gubicza L., 2008, Enzymatic production of isoamyl acetate in an ionic liquid-alcohol biphasic system, J. Mol Cat B Enzym 50, 28-32.

Hasan F., Ali Shah A. and Hameed A., 2006, Industrial applications of microbial lipases, Enzyme Microb. Technol. 39, 235-251.

Krings U. and Berger R.G., 1998, Biotechnological production of flavours and fragrances, Appl. Microbiol Biotechnol 49, 1-8.

Pfruender H., Amidjojo M., Kragl U. and Weuster-Bothz D., 2004, Efficient whole-cell biotransformation in a biphasic ionic liquid/water system, Angew Chem Int Ed 43, 4529-4531.

Urban P.L., Goodall D.M. and Bruce N.C., 2006, Enzymatic microreactors in chemical analysis and kinetic studies, Biotech Adv 24, 42-57.

Van Rantwijk F., Madeira Lau R. and Sheldon R.A., 2003, Biocatalytic transformations in ionic liquids, Trends Biotechnol. 21, 131-138.

Žnidaršič-Plazl P. and Plazl I., 2007, Steroid extraction in a microchannel system-mathematical modelling and experiments, Lab Chip 7, 883-889.