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Journal of Molecular Catalysis B: Enzymatic 5 (1998) 355–359

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Biocatalysis in microstructured lyotropic liquid crystals

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Received 26 September 1997; accepted 20 November 1997

Abstract

Biphasic liquid crystal systems consisting of organic solvent, water and surfactant are interesting media for biocatalysis in a non-aqueous environment. The application of such systems for the (*S*)-hydroxynitrile lyase catalyzed synthesis of (*S*)-mandelonitrile is demonstrated. Screening a favourable liquid crystal system is the first step. Experimental results of the influence of temperature, enzyme and substrate concentration on the kinetics are presented. Interactions of the three-dimensional liquid crystalline immobilization matrix and the mass transfer and biochemical reaction kinetics are shown.

Keywords: (*S*)-hydroxynitrile lyase; Liquid crystal; Organic solvent

1. Introduction

Liquid/liquid or liquid/liquid/solid systems containing water and water-immiscible organic solvents have enjoyed increasing popularity as media for enzymatic reactions [1,2]. In such biphasic systems, high concentrations of poorly water-soluble substrates or products are possible, reaction equilibria may be shifted favourably, substrate or product inhibition may be reduced and recovery of products may be facilitated.

But a major drawback of using an organic solvent in biocatalysis is the lack of enzyme stability because of denaturation. Introducing surfactant into the two-phase system leads to different, very interesting structures for the field of applied biocatalysis. Reversed micelles, which are globular microstructures of surfactant coating a water-pool, were applied first in biocatalysis [3,4]. The enzymatic stability in such systems is quite good, but the separation of product and recovery of reverse micelles is often a problem.

A promising, current trend in the field of biocatalysis is the immobilization of enzymes and whole cells in a biphasic system consisting of lyotropic liquid crystal (LC) and organic solvent. Lyotropic liquid crystals are surfactant aggregates formed by certain amphiphilic molecules when they are dissolved in organic solvent/water mixtures. Substructures of the LC are reverse or regular micelles which ag-

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1381-1177/98/$19.00
PII: S1381-1177(98)00049-6

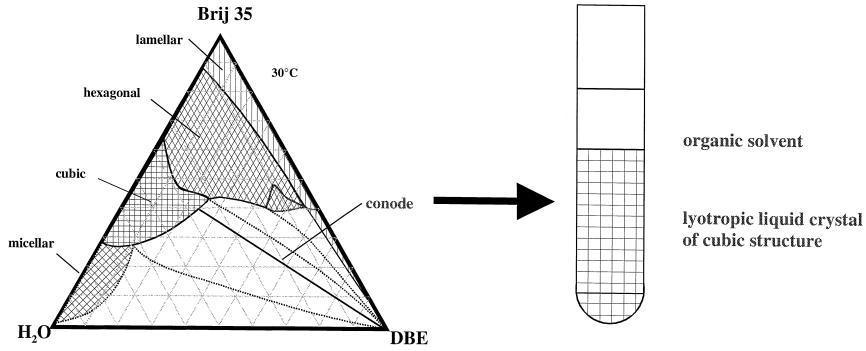

Fig. 1. Biphasic liquid crystalline system resulting in accordance to a conode in the ternary system.

glomerate to a three-dimensional structural network of lamellar, cubic or hexagonal order. Because of its high viscosity, it is of little practical interest to use the solid LC phase alone, but the situation changes if a biphasic system consisting of the LC and an organic solvent phase is used. Such a system is illustrated in Fig. 1. Because of the thermodynamic equilibrium between the organic and LC phase, it is possible to work in such systems with any amount of organic solvent over the LC phase. Poorly water-soluble substrates can be dissolved in the organic phase and products can be extracted out of the LC phase. Additionally, the liquid crystalline matrix has protective effects on the enzymes to decrease the denaturation drastically. A long-term stability can be reached. Moreover, the enzymatic activity increases [5,6]. In contrast to reverse micellar systems, the use of the biphasic organic solvent/LC system combines the protective surrounding with the immobilization of the enzyme. Due to the immobilization, extractive, continuous processing is possible.

2. Experimental

Liquid crystalline systems were prepared using Brij® 35 (Merck), 10 mM sodiumphosphate buffer, pH 7, and dibutylether (Merck, p.a.), *tert*-butyl-methylether (Merck, p.a.) or decamethylcyclopentasiloxane (Volasil™ 245, BDH Laboratory Supplies, p.a.) as organic solvent. For *tert*-butyl-methylether the organic solvent:buffer:surfactant ratio was varied from 40:30:30 (BME 1), 50:20:30 (BME 2), 50:30:20 (BME 3), 60:20:20 (BME 4), 50:40:10 (BME 5) to 70:20:10 (BME 6). We examined also decamethylcyclopentasiloxane systems consisting of 50:30:20 (DMCP 1), 60/20/20 (DMCP 2), 70:10:20 (DMCP 3) and 60:10:30 (DMCP 4) weight ratio. Finally, we investigated a dibutylether based system with 86:8:6 (DBE 1), 86:9:5 (DBE 2) and 86:10:4 (DBE 3) wt.% organic solvent, buffer and surfactant, respectively. To study the effect of process parameters on the overall kinetics, a system consisting of 86 wt.% dibutylether, 9 wt.% sodium glutamate buffer, pH 3.5, and 5 wt.% Brij® 35 was chosen.

Benzaldehyde (Aldrich, 99 + %) was distilled before use. Purified racemic mandelonitrile with less than 0.5% benzoic acid was a gift from DSM Chemie Linz. To study the influence of process parameters on (*S*)-mandelonitrile synthesis we added waterfree hydrogen cyanide as second substrate. The enzyme used was a recombinant (*S*)-hydroxynitrile lyase from *Hevea brasiliensis* (gift from H. Schwab, Institute of Biotechnology, TU Graz). After cell disruption, the resulting broth was lyophilized. We achieved an average specific activity of 23 IU/mg.

To determine the distribution coefficients of benzaldehyde and mandelonitrile in different LC systems, we added 0.18 cm^3 LC to 5 ml 100 mM substrate solution. After storage of 48 h to achieve equilibrium, samples were taken from the supernatant, acetylated and analyzed on the GC (Chrompack Chirasil-Dex, N_2 carrier, Init. Temp.: 80°C, 4 min; Rate: 5°C/min, Final Temp.: 145°C, Final Time: 1 min; Ret. Time: benzaldehyde: 5.6 min, (*R*)-mandelonitrile: 14.8 min, (*S*)-mandelonitrile: 16.1 min).

Batch experiments were performed to study the influence of process parameters on the reaction rate. The enzyme was immobilized in 5.5 cm^3 LC which was applied in a 0.2-cm thin layer at the bottom of the reactor. The substrates benzaldehyde and hydrogen cyanide were added by syringes to 130 cm^3 supernatant. Samples were taken by syringes and analyzed as described before.

A description of the simultaneous small and wide-angle X-ray scattering (SWAX) measurements of the liquid crystalline phases in respect to their use as biocatalysts immobilization matrices can be found in Ref. [7].

3. Results and discussion

The aim of the presented work is to demonstrate the application of microstructured lyotropic liquid crystals as reaction media for biocatalytic reactions with very sensitive enzymes. The development of such a process is a multi-step approach. Screening a favourable LC system, finding out the different mechanisms of mass transfer and the intrinsic biochemical reaction kinetics and determining the key model parameters are important steps followed by proposing a mathematical model for scale-up and process optimization purposes.

The synthesis of enantiomeric pure (*S*)-mandelonitrile by (*S*)-hydroxynitrile lyase was chosen as model reaction. A chemical reaction competes with the enantioselective enzymatic reaction and lowers the enantiomeric excess.

The distribution coefficients of mandelonitrile and benzaldehyde in different LC systems and subsystems are presented in Fig. 2. It is obvious that the distribution coefficients of benzaldehyde and mandelonitrile depend on the chosen organic solvent. The organic

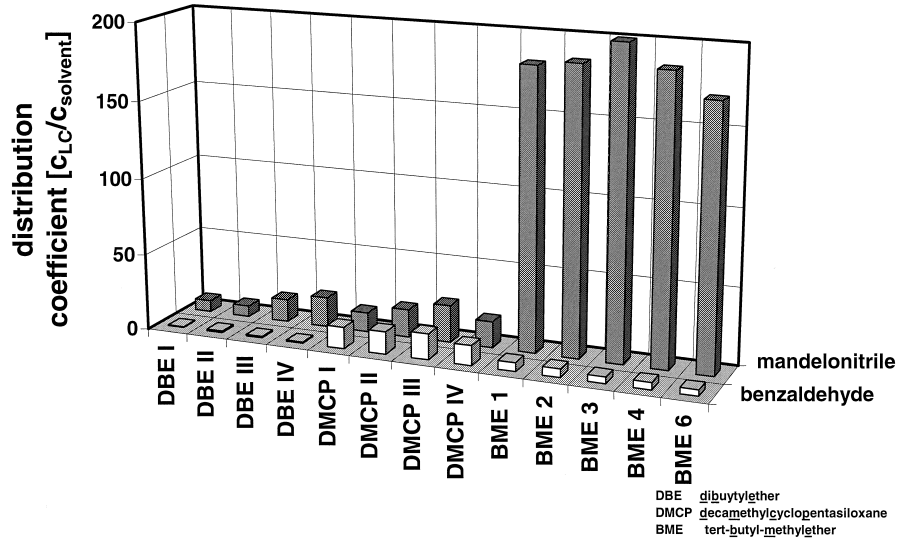

Fig. 2. Distribution coefficients of mandelonitrile and benzaldehyde in different liquid crystal systems and subsystems.

solvent/buffer/surfactant ratio plays a minor role. It is favourable to choose a system which offers a high distribution coefficient for the substrate benzaldehyde and a low distribution coefficient for the product mandelonitrile. For this reason, we excluded *tert*-butyl-methylether as organic solvent. Comparing decamethylcyclopentasiloxane and dibutylether, the first one seemed to be more suitable because of a higher distribution coefficient for the substrate benzaldehyde. Preliminary experiments indicated a higher mechanical stability of the dibutylether system which might be interesting for continuous processing. For this reason, further experiments were performed with dibutylether.

Beside the composition of the system, batch experiments were performed to study the influence of temperature, enzyme and benzaldehyde concentrations and hydrogen cyanide excess on the kinetics. The enantiomeric excess as a function of temperature and enzyme concentration is presented in Fig. 3. Based on a statistical analysis of the results, we found no significant effect of benzaldehyde concentration and HCN excess. In general, low temperatures and high enzyme concentrations are favourable. Low temperatures suppress the chemical reaction. For aqueous systems, it has been shown that higher temperatures favour the chemical reaction which finally leads to a decrease in enantiomeric excess. X-ray scattering experiments of the LC phases indicate a decreasing hydration of the surfactant polyoxyethylene chains with rising temperature. Free water is necessary for the chemical reaction; consequently, the structural change of the LC phase with temperature may have an additional negative effect on the enantiomeric excess. The amount of free water may be an explanation for an optimum enzyme concentration of about 1000 $U/g_{liquid\ crystal}$, as well. At higher enzyme loading, the hexagonal structure of the used LC changes to isotropic, leading to a higher amount of accessible free water. Below 1000 $U/g_{liquid\ crystal}$, the three-dimensional structure of the LC was found to be independent of the enzyme concentration.

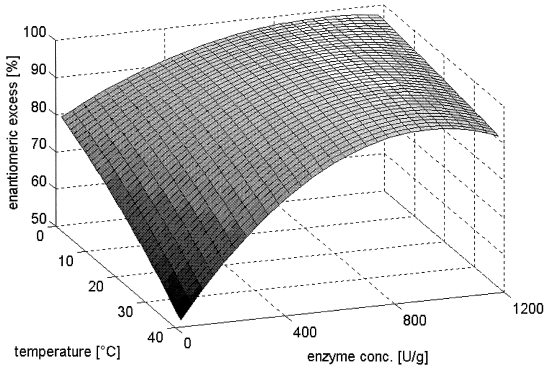

Fig. 3. Enantiomeric excess of (*S*)-mandelonitrile production using (*S*)-hydroxynitrile lyase as function of temperature and enzyme concentration (52 mM benzaldehyde; 286 mM hydrogen cyanide).

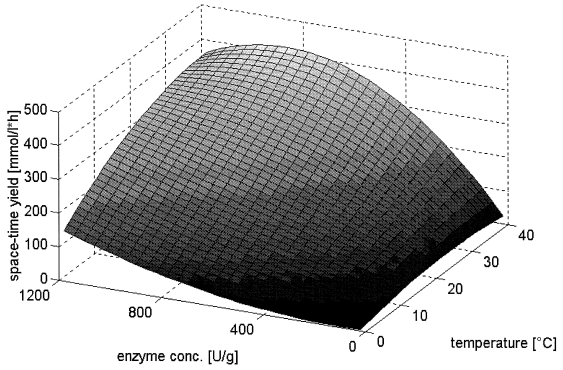

Fig. 4. Space–time yield of (*S*)-mandelonitrile production using (*S*)-hydroxynitrile lyase as function of temperature and enzyme concentration (52 mM benzaldehyde; 286 mM hydrogen cyanide).

Fig. 4 shows the space-time yield of (*S*)-mandelonitrile production as a function of temperature and enzyme concentration. Again, an optimum enzyme concentration of approximately 1000 $U/g_{liquid\ crystal}$ could be found. In contrast to the enantiomeric excess, a high temperature is favourable. Using the liquid crystalline system for the (*S*)-mandelonitrile production a compromise between enantiomeric excess and space–time yield has to be found. Based on the statistical analysis of the results, HCN excess was without any effect, again. The benzaldehyde concentration was increased to 100 mM, which leads to a growing space–time yield.

4. Conclusions

Lyotropic liquid crystals are highly flexible reaction media for biocatalytic purposes. Varying the organic solvent allows to develop biphasic systems with favourable distribution coefficients of substrates and products. Key parameters to optimize the system are enzyme and substrate concentrations, temperature and the structure of the liquid crystalline immobilization matrix. These parameters interact in a non-linear manner with mass transport and biochemical reaction kinetics. Therefore, it is necessary to develop a mathematical model for further process optimization and scale-up purposes.

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