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STUDY ON ETHANOL FERMENTATION INTEGRATED WITH SIMULTANEOUS SOLVENT EXTRACTION AND ENZYMATIC REACTION

E. CSÁNYI, K. BÉLAFI-BAKÓ,* N. NEMESTÓTHY and L. GUBICZA

Research Institute of Chemical and Process Engineering, H-8200 Veszprém, Egyetem u. 2. Hungary

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Ethanol recovery from aqueous fermentation broth by extraction using oleic acid with simultaneous esterification by lipase enzyme was studied. To determine the optimal conditions for the complex process, the ternary system was characterised; binodal curves and tie lines of (ethanol+oleic acid+water) system were determined. Enzymatic esterification of ethanol and oleic acid was carried out and resulted in higher than 50% conversion with simultaneous reduction of ethanol content in the broth. Finally, the effect of the ester product (ethyl oleate) on the distribution of ethanol was determined.

Keywords: lipase, extractive fermentation, oleic acid, ethanol, enzymatic esterification, ternary system

Ethanol is one of the most important fermented products in the food industry, having a significant role in the energy sector, as well (RÉCZEY, 1998), although it should be dehydrated for using as a biofuel (ATRA et al., 1999). Ethanol can be produced by fermentation using yeast strains (e.g. *Saccharomyces cerevisiae*). The product itself has a strong inhibition effect on the microbial conversion: ethanol beyond 10% concentration stops the fermentation (MURTAGH, 1999). To avoid product inhibition, ethanol should be removed from the fermentation broth. One of the possibilities is the extraction of ethanol with organic solvents. In case of on-line ethanol recovery, however, the solvent has to be selected very carefully. It must meet several requirements:

– it should be completely miscible with ethanol,
– it should not be harmful for the microbe,
– it should not be too volatile.

Fatty acids seemed to be proper solvents for in situ ethanol extraction, as it was presented earlier (BÉLAFI-BAKÓ et al., 1995). Among fatty acids, oleic acid was found to be the most selective for ethanol extraction (AIRES BARROS et al., 1987).

Since ethanol to be extracted is present in an aqueous solution, ternary system is formed during the extraction with oleic acid, which – in certain concentrations – is a

* To whom correspondence should be addressed.
Fax: +36-88-424 424; e-mail: bako@mukki.richem.hu

two-phase system. Ethanol is distributed between the aqueous and the fatty acid phases. Thus, the mixture of oleic acid+ethanol+water belongs to the classical group where two compounds with limited solubility are present (oleic acid and water), while the two other pairs (ethanol+water and oleic acid+water) are completely miscible. So, the behaviour of the particular system can be presented by using the classical ternary phase diagram.

The description of the ternary phase diagram for the oleic acid+ethanol+water system has not been found in literature, therefore in this project one of our purposes was to determine the liquid-liquid equilibrium experimentally on the one hand, and to predict the conditions by using a modelling program, on the other hand. The temperature of the determination was 37 °C, since this is the optimal temperature for lipase enzyme used for the esterification (GUBICZA et al., 2000).

In this work not only the equilibrium conditions of the oleic acid+ethanol+water system were planned to be described, but the extract obtained was planned to be processed further on to manufacture ethyl ester of oleic acid – an important bio-lubricant – by using lipase enzyme. Then the changes in the system's behaviour caused by the ethyl oleate produced from the esterification reaction were studied, as well. The detailed description of the system helps us to adjust the reaction parameters to an optimal level to enhance the effectiveness of the whole, two-step process.

1. Materials and methods

In the experimental determination and the prediction of thermodynamic data, analytical methods and the so-called JUSTEMIX program were used, respectively.

In the experiments 9-octadecenoic acid (oleic acid, molecular weight 282) and ethanol (molecular weight 46) were purchased from Reanal Ltd (Hungary), while ethyl oleate (molecular weight 310) was supplied by Aldrich (USA). All the compounds used were of analytical grade. Bi-distilled water was used for the measurements. In the esterification experiments lipase enzyme preparation from *Mucor miehei* SP 225 (liquid form, activity 600 U ml^{-1}, Novo Nordisk, Denmark) was used.

The binodal curve was determined by the cloud-point method in a thermostatic cell at 37 °C. In the experiments for the determination of tie lines, the ethanol+water+oleic acid or ethyl oleate system, having a certain composition, was mixed vigorously in a shaking incubator for 2 h and was left to separate for 24 h. In the phases the concentrations of the compounds were analysed. The amount of oleic acid was measured by titration with 0.1 M alcoholic KOH solution using phenolphtalein indicator. Concentrations of ethyl oleate and ethanol were determined by gas chromatography (Hewlett Packard 5890 equipment, FFAP column [30 m×0.53 mm] with 1 μm film thickness (HP), oven temperature 120 °C, detector temperature 150 °C, flame ionisation detector). The FID signal was monitored by a HP 3396 integrator.

The enzymatic esterification reactions were carried out in a shaking incubator (New Brunswick, G24) at 150 r.p.m. and 37 °C temperature, using the soluble lipase preparation (10 U for each experiment) isolated from *Mucor miehei* species.

The program used for the prediction of data was the Joback-Unifac Estimator for Equilibrium Properties of Mixtures (JUSTEMIX) based on the Unifac group-contribution method, which is suitable to provide information on the binodal curve and tie lines of the ternary system. The program was successfully applied for the estimation of phase equilibrium data on similar types of ternary mixtures earlier (RATKOVICS et al., 1991).

2. Results

2.1. Ternary phase diagram

The data on the experimental saturation isotherm and tie lines of the ternary mixture at 37 °C are listed in Tables 1 and 2, where x_i is the mole fraction of component i. Experimental as well as predicted data (dotted line) on the ternary phase equilibrium are presented in Fig. 1.

Table 1. Data on experimental binodal curve for
[x_1 C_2H_5OH+x_2 $CH_3(CH_2)_7CH{:}CH(CH_2)_7CO_2H$+(1–$x_1$–$x_2$) H_2O]
ternary mixture at 37 °C (error in the measurements within ±8%)

x_1	x_2
0.000	0.0002
0.091	0.002
0.203	0.004
0.352	0.006
0.448	0.009
0.591	0.010
0.661	0.040
0.709	0.052
0.690	0.141
0.648	0.220
0.580	0.304
0.461	0.452
0.323	0.591
0.251	0.689
0.157	0.792
0.103	0.804
0.062	0.921
0.000	0.962

In the experimental isotherm the initial points on the water–oleic acid line (the mutual solubilities) were found as x_2=0.0002 and x_2=0.962 at the "water" and "oleic acid" vertices, respectively (where x_2 is the molar fraction of oleic acid). The left part of the experimental isotherm fits smoothly to the line of the triangle's left side. At the "highest" point of the experimental binodal curve the ethanol concentration (x_1 C_2H_5OH) was found to be 0.709. As it can be seen from the figure, the experimental

data and the predicted values (dotted line) obtained by the JUSTEMIX program have shown quite good correlation, though the prediction provided higher area for the two-phase system by almost 10%.

Table 2. Experimental tie lines for the [x_1 ethanol + x_2 oleic acid + (1−x_1−x_2) water] ternary mixture at 37 °C and values of D distribution coefficient (calculated from the ratio of the ethanol concentrations – M – in the organic and the aqueous phase)

Aqueous phase		Organic phase		D
x_1	x_2	x_1	x_2	
0.401	0.006	0.517	0.379	0.272
0.213	0.004	0.321	0.589	0.188
0.133	0.003	0.204	0.740	0.167
0.083	0.001	0.160	0.791	0.155
0.039	0.001	0.096	0.868	0.149

Beyond the tie lines data, distribution coefficients D (calculated from the ratio of the ethanol concentrations; M in the organic and the aqueous phase) are listed in Table 2, as well.

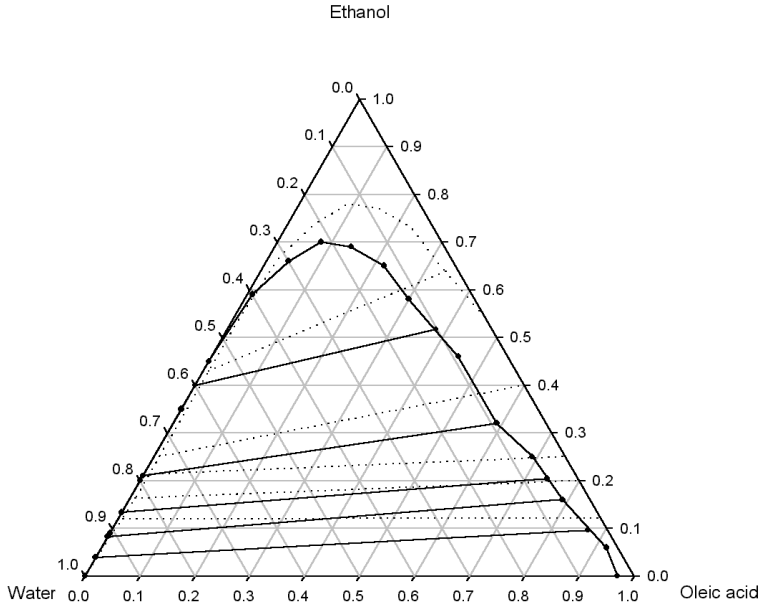

Fig. 1. Saturation isotherm and tie lines of oleic acid+ethanol+water system (dotted line: predicted values)

The data obtained are similar to those reported earlier (0.171 at temperature 30 °C) in the paper of AIRES BARROS and co-workers (1987). The values plotted as a function of the ethanol concentrations in the aqueous phase are shown in Fig. 2. It can be seen that the distribution coefficients increased slightly at higher ethanol concentrations.

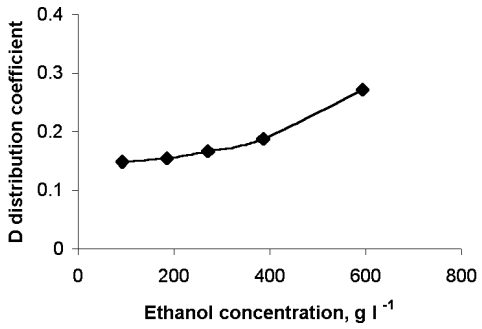

Fig. 2. Values of the distribution coefficient D as a function of ethanol concentration

2.2. Esterification by lipase enzyme

Experiments in shaking flasks were carried out to manufacture ester compounds from the alcohol and acid substrates (both are present in the extract) by adding *Mucor miehei* lipase enzyme to the solution at 37 °C, 180 r.p.m. The time courses of the esterifications using 5 and 10%, v/v (since 10%, v/v is the highest achievable ethanol concentration in the fermentation process) initial ethanol concentrations are presented in Fig. 3. More than 50% conversion was obtained during the reactions after 30 h, which is rather high taking into account the fact that diluted aqueous solutions were used initially.

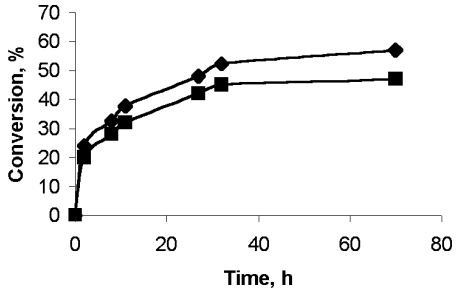

Fig. 3. Conversion of esterification versus time in 5 and 10% initial ethanol concentration (acid-alcohol molar ratio 1:1, 37 °C). –◆–: 10%; –■–: 5%

The acid–alcohol initial molar ratio was selected firstly as 1:1 (the ratio in weight is more than 6:1!). Excess acid seems advantageous from the extraction as well as the reaction (used both as a solvent and as a reactant, substrate) points of view. Therefore, further experiments were conducted to find out the effects of the acid-alcohol ratio on the conversion rate. Figure 4 presents the experimental data obtained with 1:1, 1.2:1 and 1.5:1 acid-alcohol molar ratios (6.2:1, 7.5:1 and 9.3:1 weight ratios, respectively). It can be seen that higher amount of acid applied had an advantageous effect on the reaction, resulting in higher conversions (calculated on the amount of ethanol converted). However, it is not possible to increase the amount of acid too much, considering the conditions of the ethanol fermentation system.

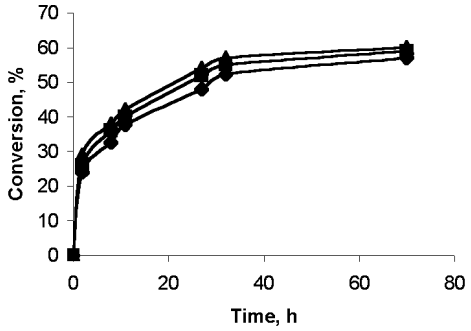

Fig. 4. Conversion data as a function of time in various acid-alcohol molar ratios (10%, v/v initial ethanol concentration, 37 °C). –◆–: 1:1 ratio; –■–: 1.2:1 ratio; –▲–: 1.5:1 ratio

It can be concluded from the results of the enzymatic esterifications that the enzyme works with high efficiency on the interfacial area of the phases. After the reactions, it was possible to reduce the ethanol concentration in the model fermentation broth considerably.

2.3. Effect of ethyl oleate

During the enzymatic esterification of ethanol and oleic acid, two products are obtained: ethyl oleate and water. Since water is present in excess (anyway) in the mixture, its effect can be neglected. The ester product, however, may have an effect on the phase equilibrium and the distribution coefficient. Therefore, experiments were carried out to determine it exactly, using mixtures of ethanol+water+oleic acid and ethyl oleate, where the simulated compositions were adjusted taking into account not only the increasing amount of product(s), but the proportionally decreasing (consumed) amounts of substrates, as well. The ethanol–water binary mixture contained 5 and 10%, v/v ethanol initially, which was (assumed to be) converted with oleic acid to ester enzymatically.

The distribution coefficient D as a function of the (bio)conversion rate is presented in Fig. 5. It can be seen that the oleic acid ethyl ester produced during the enzymatic

esterification had an unfavourable effect on the distribution, i.e. less amount of ethanol was dissolved into the organic phase as the bioconversion was in progress. However, the complete process (extraction with esterification) results in enhanced recovery of ethanol from the system, since ethanol is present not only dissolved in the acid but as part of the ester, as well.

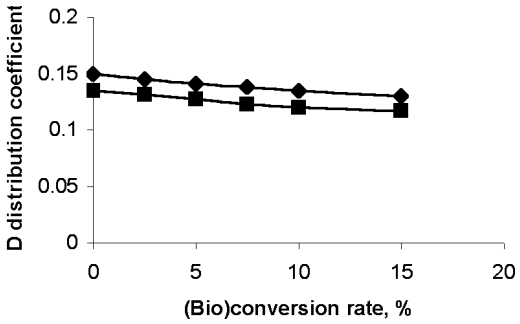

Fig. 5. Values of the distribution coefficient D as a function of the conversion rate in cases of 5 and 10%, v/v initial ethanol concentration. –◆–: 10%; –■–: 5%

3. Conclusions

In this study the purpose was to describe the ternary system formed in recovery of ethanol from fermentation broth by solvent extraction coupled with enzymatic esterification. From the extraction point of view, high amount of solvent, oleic acid is suggested to be used, since the highest ethanol concentration in the fermentation is only 10%, v/v, which is quite a diluted solution.

From the aspect of enzymatic esterification, the oleic acid excess – both as a solvent and as a reactant (substrate) – is advantageous, as well. The experiments for the esterification reactions by lipase resulted in unexpectedly high conversions (more than 50%) with the simultaneous reduction of the ethanol content in the (model) fermentation broth.

The esterification, however, would be most effective in a homogeneous system: in the one-phase area, beyond the saturation isotherm in the ternary phase diagram, where the ethanol concentration is extremely high. Nevertheless, due to the given initial conditions of the system (low ethanol content) we have to work in the two-phase area. Therefore, in the extraction coupled with esterification reaction a thoroughly mixed, carefully designed (batch) reactor with proper shaped impellers (resulting in high Reynolds number, high turbulence) is needed to realise the complete, multi-step process with enhanced effectiveness.

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