Analysis of Phase Composition in Aqueous Two-Phase Systems Using a Two-Column Chromatographic Method: Application to Lactic Acid Production by Extractive Fermentation

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Abstract: A new chromatographic system for the simultaneous analysis of polyethylene glycol, dextran, sugars, and low-molecular-weight fatty acids was developed. The system is based on a gel exclusion column which allows a first separation between high- and low-molecular-weight compounds, and a cationic exchange column used to further separate the low-molecular-weight compounds. Two applications of the system were demonstrated: (i) after optimizing eluent conditions the gel exclusion column was used to determine the influence of lactic acid, phosphate buffer, and lactic acid bacteria on the ethylene oxide propylene oxide–dextran T40 phase diagram by HPLC; (ii) the ion exchange column was coupled in series with the gel exclusion column and the concentration of polyethylene glycol, dextran, glucose, lactate, acetate, and formate was determined in samples from the fermentative production of lactic acid in a polyethylene glycol 8000–dextran T40 aqueous two-phase system. The fermentation was operated without pH control in a repeated extractive batch mode, where the cell-free top phase was replaced four times, whereas the cell-containing bottom phase was reused repeatedly. The yield was 1.1 mol of lactic acid formed per mole of glucose added and the productivity was 4.7 $mM \cdot h^{-1}$. The polymeric composition of the fermentation system was monitored during the five repeated extractive batches, and it showed a progressive depletion in polyethylene glycol and a progressive enrichment in dextran. © 1997 John Wiley & Sons, Inc. *Biotechnol Bioeng* **54**: 303–311, 1997.
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INTRODUCTION

Aqueous two-phase systems (ATPS) can be used for liquid–liquid extraction, and for bioconversion of many different substances (Andersson and Hahn-Hägerdal, 1990; Hustedt et al., 1985). Bioconversions in ATPS have been reported for systems involving enzymes, cell organelles, and living cells. The use of ATPS as fermentation systems has been described for the production of bulk chemicals (Drouin and Cooper, Jarzebski et al., 1992; Tomáska et al., 1995), hormones (Kaul and Matthiasson, 1986), enzymes (Andersson et al., 1985; Persson et al., 1991), and antibiotics (Kuboi et al., 1994).

Fermentation systems based on ATPS imply the partition of cells to one of the phases, allowing the removal of the product from the cell-free phase. Thus, the cell-containing phase is being reused, whereas the other phase is repeatedly renewed. This principle has been applied in so-called repeated extractive batch fermentations (Andersson and Hahn-Hägerdal, 1990; Chen and Lee, 1995; Planas et al., 1996). In polymer–polymer ATPS, each phase is composed of a mixture containing mainly one of the two phase forming polymers, and a lesser amount of the other polymer. Thus, the top phase always contains a certain amount of the bottom phase forming polymer and vice versa. When the cell-free phase is replaced, some of the cell-rich phase forming polymer is removed. Because the concentration of polymers in both phases is known from the phase diagram (Albertsson and Tjerneld, 1994), the system can be kept at a set point when one of the phases is being replaced by adding the necessary amount of each polymer. However, the ATPS may change when the cell-free phase is replaced if the phase diagram changes because of the changing conditions in the system, or if small deviations of the initial point lead to a phase composition different from the calculated one. This problem limits the application of ATPS in extractive bioconversions including both enzymatic and fermentative processes. This has been demonstrated in a continuous fermentation with lactic acid bacteria (LAB) and recycling of polymers where the system had already broken down after 103 h (Katzbauer et al, 1995).

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Many factors affect the phase diagram, so that for a given polymer composition the composition of top and bottom phases differs with different temperatures (Albertsson and Tjerneld, 1994) and with different salt concentrations (Zaslavsky et al., 1988). Similarly, the critical point moves down at increasing biomass concentrations (Köhler et al., 1989). In addition, changes in the phase diagram will modify the partitioning of cells, macromolecules (Albertsson and Tjerneld, 1994) and small molecules such as divalent salts (Johansson, 1970). The combined effects of salts and cells, on the other hand, are not fully characterized. This is due to the difficulty of generating phase diagrams by the traditional methods when compounds other than the phase-forming polymers are present in the system.

Several methods alternative to the classical refractive index and polarimetry methods (Albertsson, 1986) have been used to analyze the phase composition in ATPS. King et al. (1988) used three gel exclusion HPLC columns, coupled in series, to study the effect of several salts on the phase diagrams of polyethylene glycol (PEG)–dextran systems. Ion exchange HPLC has been used to determine the concentration of PEG and phosphate in a PEG–phosphate ATPS (Sebastião et al., 1995). In the present study, gel exclusion HPLC was used to determine the influence of lactic acid, phosphate, and the lactic acid bacteria *Lactococcus lactis ssp. lactis* ATCC 19435 on the ethylene oxide/propylene oxide (EO/PO)–dextran T40 (DEX) phase diagrams. ATPS containing EO/PO were studied because of the possibility of removing lactic acid product by temperature-induced phase separation in systems containing this polymer (Alred et al., 1994; Harris et al., 1991). Moreover, the system was also used to monitor a repeated extractive batch fermentation in a PEG–DEX system where the top phase was replaced four times based on phase compositions calculated from a phase diagram reported in the literature.

MATERIALS AND METHODS

Chemicals

All water was from Millipore (Waters, Millipore Corp., Milford, MA). Glucose 99.9% pure (BDH, Poole, UK), lactate lithium salt 98% pure (Sigma Chemical Co., St. Louis, MO), and pro-analysis grade formic and acetic acids (Merck, Darmstadt, Germany) were used to prepare the chromatographic standards. Ethanol 95% (Kemetyl, Stockholm, Sweden) was used to prepare the chromatographic eluent. Phosphate buffer (Merck) and lactic acid (Svenska Hoeschst AB, Göteborg, Sweden) were used to determine the influence of solutes on the phase diagram. DEX (dextran T40, Lot 102391) was from (Pharmacia Biotech Norden AB, Sollentuna, Sweden); PEG (polyethylene glycol 8000) from Sigma; and UCON 50-HB-5100 (ethylene oxide [EO] propylene oxide [PO] random copolymer, EO:PO ratio 1:1, M_r 4000) from Union Carbide (New York).

Chromatography

Eluent flow was provided by an LC pump (Model LC 6A, Shimazu Corp., Kyoto, Japan). Samples were injected in the flow line by an autosampler (Marathon model, Spark Holland BV, Emmen, The Netherlands). DEX and EO/PO were separated at 70°C on a prepacked gel exclusion chromatography column (Ultrahydrogel Linear, 300 mm length × 7.8 mm internal diameter, Waters, Millipore Corp.) and detected with a differential refractometer (Model RID 6A, Shimadzu Corp.). The mobile phase for the simultaneous analysis of DEX and EO/PO with the gel exclusion column was 20% (v/v) aqueous ethanol solution. These conditions were used to determine the effect of lactic acid, phosphate buffer, and LAB on the EO/PO–DEX phase diagram.

Simultaneous analysis of DEX, PEG, glucose, lactate, formate, and acetate in samples from the extractive fermentation was performed after separation on the gel exclusion column coupled in series with a prepacked cation exchange column (Aminex HPX 87-H, 300 mm length × 7.8 mm internal diameter, Bio-Rad laboratories, Richmond, CA). After injection, the sample first passed through the gel exclusion column, and then through the ion exchange column, in the same flow line, prior to detection by differential refractivity. Separation occurred at 60°C, and the mobile phase was 0.005 M H_2SO_4 in a 5% (v/v) aqueous ethanol solution. The pH of the eluent was between 2 and 3 yielding the optimal conditions for the interaction of lactate, formate, and acetate with the ion exchange column.

The flow rate was 0.5 mL/min in all cases. Standards were injected separately before the samples. Quantification was done by computer integration of the area under each chromatographic peak using the EZChrom chromatographic data system software package (Scientific Software, Inc., San Ramon, CA).

Phase Diagrams

Phase diagrams were determined by measuring the polymer concentrations of the top and bottom phases of systems with a known total content of polymers. The systems were prepared from stock solutions of the polymers in water, 60% (w/w) EO/PO, and 30% (w/w) DEX. The polymer solutions were weighed out and mixed in test tubes with an aliquot of concentrated solution of additive: lactic acid; phosphate buffer; or phosphate buffer plus LAB. After adjusting the weight to 10 g with water, the tubes were capped and gently shaken. The phase separation occurred at 30°C until a clear interface was obtained. Samples from the top and bottom phases were withdrawn and analyzed. The polymeric composition was analyzed by gel exclusion chromatography for all phase diagrams except for the reference phase diagram where EO/PO was analyzed by refractometry (Carl Zeiss Refractometer, Zeiss Svenska AB, Lund, Sweden), and DEX by polarimetry (Model AA-10, Optical Activity Ltd., UK) as described elsewhere (Albertsson and Tjerneld, 1994).

Lactococcus lactis sp. *lactis* ATCC 19435 (*L. lactis*) from the American Type Culture Collection (Rockville, MD) was grown overnight in M17 medium (Merck), centrifuged, and resuspended in phosphate buffer so that the final suspension was 30 times more concentrated than the original. One hundred microliters of this suspension was added to each system giving a final concentration of about 10^8 CFU/mL.

Repeated Extractive Batch Fermentations

L. lactis was precultured overnight at 30°C in a growth medium containing (per liter): tryptone, 5 g; yeast extract, 5 g; casamino acids, 1 g; K_2HPO_4, 2.5 g; KH_2PO_4, 2.5 g; and $MgSO_4 \cdot 7H_2O$, 0.5 g; supplemented with 10 g/L (55.5 m*M*) of glucose, pH 6.8. Repeated extractive batch fermentations were performed in 1-L Erlenmeyer flasks with 200 g of ATPS containing growth medium holding 10 g/L (55.5 m*M*) of glucose. The initial polymer composition was calculated from the concentrations of stock solutions measured by refractivity, giving a system with 4.1% (w/w) PEG–7.8% (w/w) DEX. Cultures were maintained under orbital agitation (200 rpm) at 30°C.

Eight hours after inoculation the ATPS was transferred to a settling flask where the phases were allowed to separate for 45 min at room temperature (about 22°C). The bottom phase was then transferred back to the fermentation flask, whereas the top phase was replaced with a fresh top phase. After 3 h the top phase was again replaced, and this was repeated three more times. The fresh top phases were supplied with nutrients assuming that the bacteria had consumed half of the nutrients initially present in the ATPS. Glucose was added to the fresh top phases to compensate for the glucose removed. The amount of glucose added to the system at each step was based on HPLC analysis performed immediately after sampling. At low glucose concentrations, glucose was added to maintain a 10 m*M* concentration in the medium. The polymer content of the fresh top phase was based on the composition of the top phase at 22°C, 5.5% (w/w) PEG–5.2% (w/w) DEX (Diamond and Hsu, 1989).

Analyses

All samples for chromatography were filtered through 0.2-μm cellulose acetate filters (Sartorius AG, Göttingen, Germany). Sterile samples were taken from the well-mixed ATPS fermentation system, an aliquot was then diluted at least ten times so that phase separation no longer occurred, the rest of the sample was allowed to separate for 45 min, and finally sterile samples from the top and the bottom were withdrawn. Absorbance was measured at 620 nm in a Schimazu spectrophotometer (Model UV-120-02). Viable counts were determined using M17 broth (Merck) and 1.5% agar (Difco, Detroit, MI) plates incubated for 48 h at 30°C.

RESULTS

Chromatography

To develop a method for the analysis of ATPS where compounds other than the phase forming polymers are present, a chromatographic method based on size exclusion was developed. Mixtures of EO/PO and DEX were analyzed on a gel exclusion column (Fig. 1). The first peak corresponded to DEX followed by an EO/PO peak. Linearity was obtained between 0.1% (w/w) and 5.0% (w/w) with a correlation coefficient higher than 0.9980 both for EO/PO and DEX.

When an ion exchange column was placed in series with the gel exclusion column a chromatogram with five peaks corresponding to DEX, PEG, glucose, lactate, formate, and acetate was obtained for standards (Fig. 2). The polymers were resolved and separated from the low-molecular-weight compounds in the gel exclusion column. Downstream of the gel exclusion column low-molecular-weight compounds were resolved in the Aminex column, which did not affect the polymer peaks. The resulting chromatogram first showed the peaks of the polymers (peaks 1 and 2 in Fig. 2), and then the peaks of glucose, lactate, formate, and acetate, which were retained in the Aminex column. Linearity was achieved between 0.1% (w/w) and 5% (w/w) for both PEG and DEX, and between 0.1 g/L and 1 g/L for glucose, lactate, formate, and acetate with correlation coefficients higher than 0.990. When analyzing fermentation samples an additional peak corresponding to phosphate buffer appeared after the PEG peak and it only interfered with the negative peak between PEG and glucose peaks (Fig. 2).

This analytical setup could not be used for samples containing EO/PO because the Aminex column does not tolerate elution conditions for the EO/PO involving 20% (v/v) ethanol in water.

Phase Diagrams

To study whether the new chromatographic method was comparable with the traditional refractive index–polarimetry (RI/Pol) method (Albertsson and Tjerneld, 1994) to determine phase diagrams, an EO/PO–DEX phase diagram determined by chromatography was compared with a phase diagram determined by RI/Pol (Fig. 3). Both the binodial and the tie line slopes were in good agreement in the region near the critical point. However, further away from the critical point, the binodial obtained by chromatography was shifted toward the axes, and the tie lines showed a tendency to have a higher slope compared with the results obtained by RI/Pol.

The influence of phosphate buffer, lactic acid, and cells of *L. lactis* on the EO/PO–DEX phase diagrams was studied by gel exclusion chromatography (Fig. 4). The phase diagram of the ATPS containing only polymers (Fig. 4A) was taken as reference. When lactate was added to a final concentration of 0.6 *M* at pH 6.4 (Fig. 4B) the critical point was lowered from 4.65% (w/w) EO/PO–6.65% (w/w) DEX to

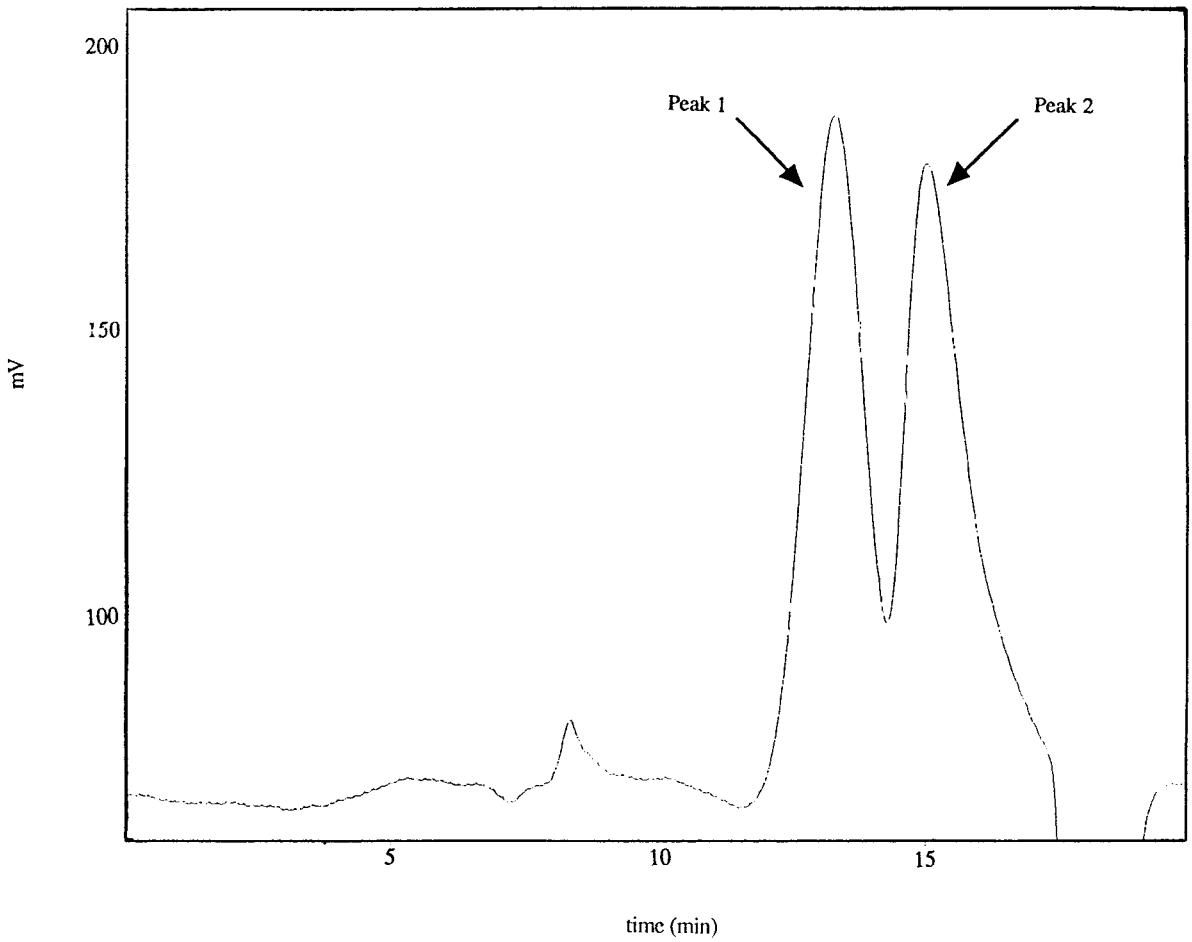

Figure 1. Chromatogram obtained by separation on a gel exclusion column (Ultrahydrogel Linear) of a mixture of dextran T40 3% (w/w) (peak 1) and EO/PO 3% (w/w) (peak 2).

4.5% (w/w) EO/PO–6.5% (w/w) DEX. Further away from the critical point the binodial was shifted toward the EO/PO axis, and away from the DEX axis, compared with the reference. Furthermore, the tie lines had a lower average slope of 0.55 (absolute value) compared with 0.64 in the reference. The average of the tie line slopes was calculated without taking into account the tie lines closer to the critical point, which often have different slope compared with those far from the critical point.

Because phosphate buffer is often used to provide the correct osmotic conditions in fermentations a phase diagram was determined for a system containing 32.2 m*M* of phosphate buffer, pH 6.4 (Fig. 4C). The binodial was shifted to the EO/PO axis and away from the DEX axis. The tie-line slope changed from 0.64 in the reference diagram, to 0.54 in the diagram for the system containing phosphate buffer. When *L. lactis* was added to the system containing phosphate buffer, both the shift of the binodial toward the EO/PO axis and the shift away from the DEX axis increased further (Fig. 4D). However, the tie-line slopes (0.61) were closer to those of the reference tie lines (0.64). Assuming that the effects are additive, and subtracting the effect of the phosphate buffer, the effect of the bacteria alone on tie-line slope would be an increase of the slope to 0.68 compared with 0.64 in the reference.

Lactic Acid Production in a Repeated Extractive Batch Fermentation

Lactic acid production was performed as five repeated extractive batch fermentations. Polymer concentrations, glucose consumption, lactic acid formation, and the formation of other organic acids, were determined with the chromatographic system composed of an ion exchange column in series with a gel exclusion column (Fig. 5). The optical density (OD) of the culture reached 1.5 absorbance units (AU) after 8 h of cultivation at the end of the first batch. After each phase replacement the OD value was at about 1.5 and increased up to values of about 2. The lactic acid concentration decreased momentarily after each top phase replacement, but increased to values progressively higher with each batch to reach a final concentration of 54.6 m*M*. Other organic acids, if produced, were below the detection limit of the chromatographic system. The glucose concen-

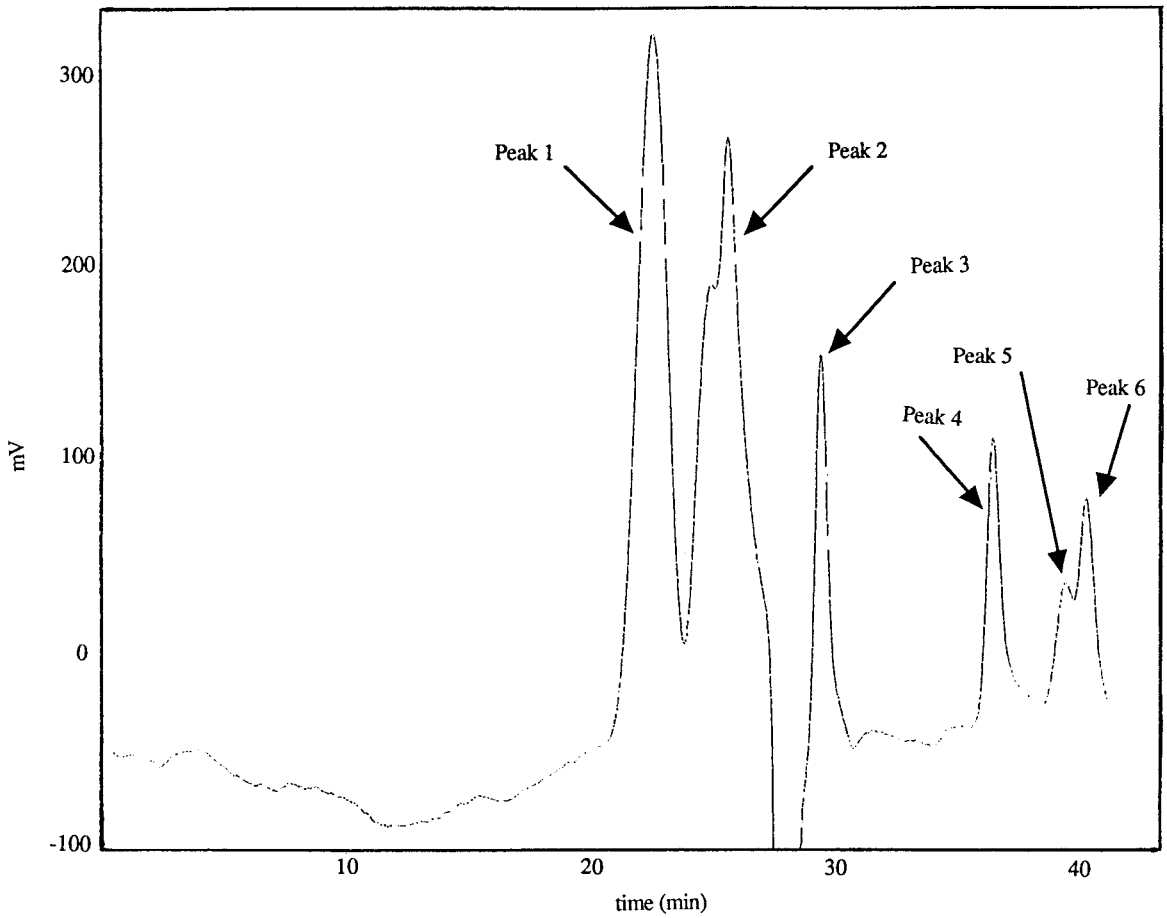

Figure 2. Chromatogram obtained by separation on an ion exchange column (Aminex HPX 87 H) coupled in series with a gel exclusion column (Ultrahydrogel Linear) of a mixture of dextran T40 5% (w/w) (peak 1), PEG 8000 5% (w/w) (peak 2), glucose 1 g/L (peak 3), lactate 1 g/L (peak 4), formate 0.6 g/L (peak 5), and acetate 0.6 g/L (peak 6).

tration decreased from an initial value of 61 m*M* to 10.6 m*M* at the end of the third batch, and it was maintained between 5 and 10 m*M*, by addition of glucose with the fresh top phases, for two more batches until the fermentation was stopped. The yield was 1.1 mol of lactic acid formed per mole of glucose (mol/mol), with 2 mol/mol being the theoretical yield. The productivity was 4.7 m$M \cdot h^{-1}$, but taking into account only the four first batches and including the time (45 min) for each phase separation.

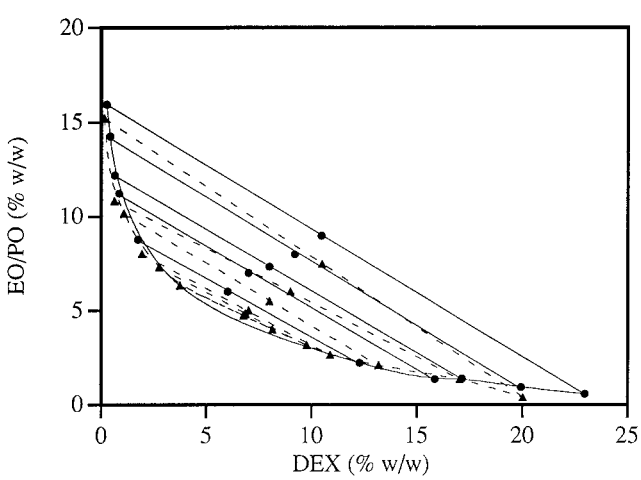

Figure 3. Phase diagrams for EO/PO–dextran T40 at pH 6.4 and 30°C, obtained by refractive index and polarimetry (–●—), is compared with the phase diagram obtained by gel exclusion chromatography (- - ▲ - -).

Samples from the top and bottom phases were also analyzed separately to determine the partitioning of the bacteria and of the different compounds. Measurements of the bacteria viability were performed from both phases (Fig. 6). Even though most of the cells were in the bottom phase and the interface, some cells were also found in the top phase. After the first batch, and until the fermentation was completed, the number of cells in the top phase was around 3×10^6 CFU/mL, and 5×10^8 CFU/mL in the bottom phase.

In samples taken at the beginning of the second, third, fourth, and fifth batches, the lactate concentration was, on average, 10 m*M* higher in the bottom phase than in the top phase (Fig. 7A). In the samples from the end of the batches, the lactic acid concentration was, on average, only 4 m*M* higher in the bottom phase than in the top phase. Glucose showed an even distribution between the two phases throughout all the five repeated extractive batch fermentations (Fig. 7B).

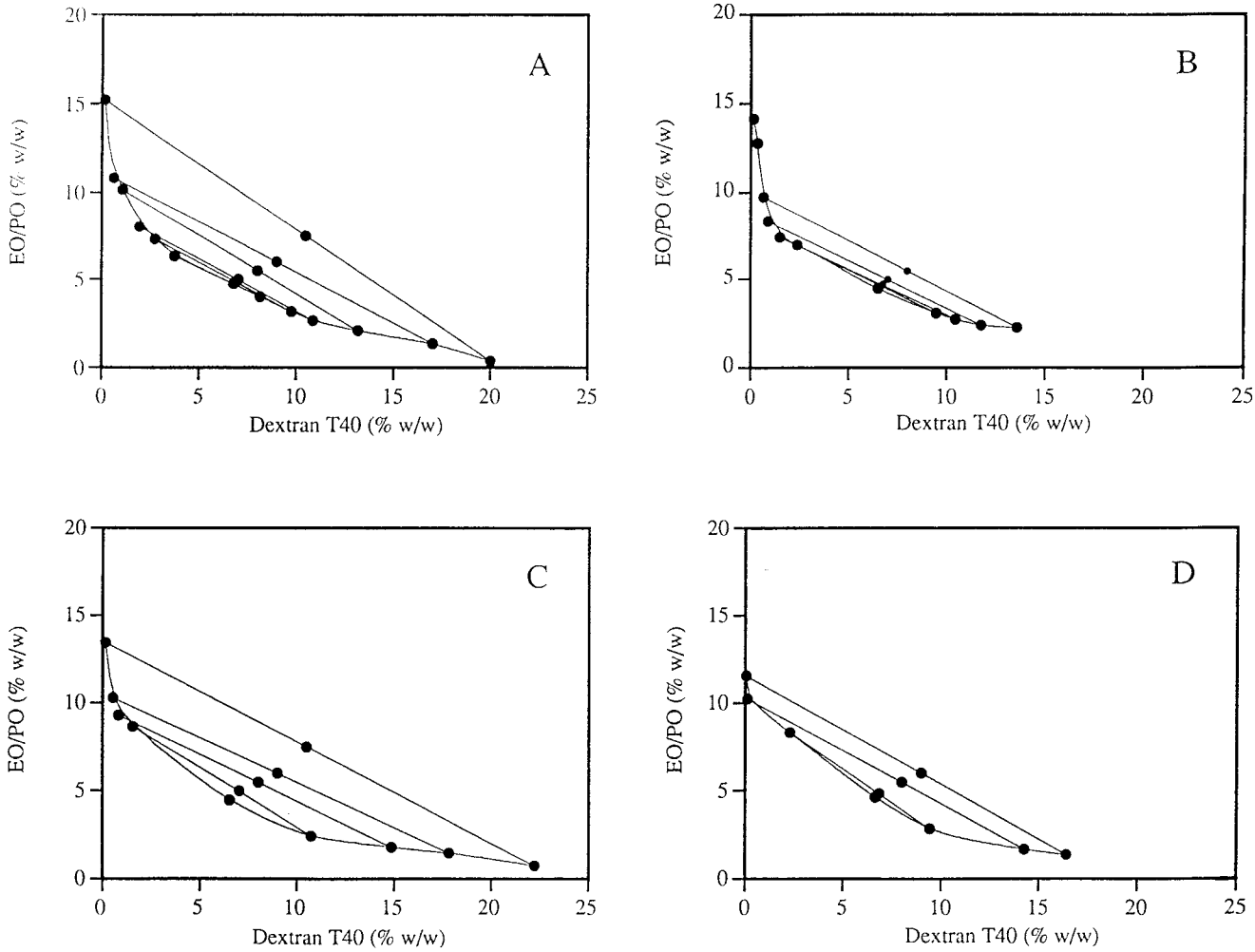

Figure 4. Phase diagrams determined with gel exclusion chromatography for EO/PO–dextran T40 at pH 6.4 and 30°C in the presence of different compounds present in lactic acid fermentations. (A) Reference diagram without any additive. (B) System containing 50 g/L of lactate. (C) System containing 32.2 m*M* of phosphate buffer. (D) system containing 32.2 m*M* of phosphate buffer and about 10^8 CFU/mL of *Lactococcus lactis* sp. *lactis* ATCC 19435.

Analysis of Polymers During Fermentation

The composition of the ATPS was measured throughout the repeated extractive batch fermentation (Fig. 8) to study the phase system stability and determine any deviation from the calculated values. The initially measured composition of the system was 6.6% (w/w) PEG–10.0% (w/w) DEX, whereas the precalculated values were 4.1% (w/w) PEG–7.8% (w/w) DEX. The volume ratio (volume of the top phase over volume of the bottom phase) of each sample was recorded, and a decrease from an initial volume ratio of 1.3 to 0.6 after the fifth batch was observed (Fig. 8A). The total polymer concentration in the system was obtained by analyzing the composition of well-mixed samples, before phase separation. A progressive decrease in PEG concentration and a simultaneous increase in DEX concentration were noticed (Fig. 8B). The difference in concentration from the beginning of the first batch to the end of the fifth batch was a reduction of 1.6% (w/w) for PEG and an increase of 4.6% (w/w) for DEX. The top phase polymer concentration evolved from initial values of 10.4% (w/w) PEG and 1.1% (w/w) DEX, to 12.7% (w/w) PEG and 0.2% (w/w) DEX (Fig. 8C), whereas the bottom phase became more concentrated in DEX (from 19.4% to 22.2%) (Fig. 8D).

DISCUSSION

The phase diagram obtained with the new chromatographic system showed good agreement with that obtained by traditional methods (Albertsson and Tjerneld, 1994). Furthermore, the new chromatographic system made it possible to determine phase diagrams in the presence of other compounds such as ions and bacteria. Previously, the determination of the phase composition of ATPS containing compounds which partition unequally between the phases required as many methods as there were components. A chromatographic method based on three gel exclusion columns placed in series have been used to study the effect of salts in PEG–dextran ATPS (King et al., 1988). Gel exclusion chromatography has also been used to analyze the partition of modified PEG in PEG–dextran systems (Schluck et

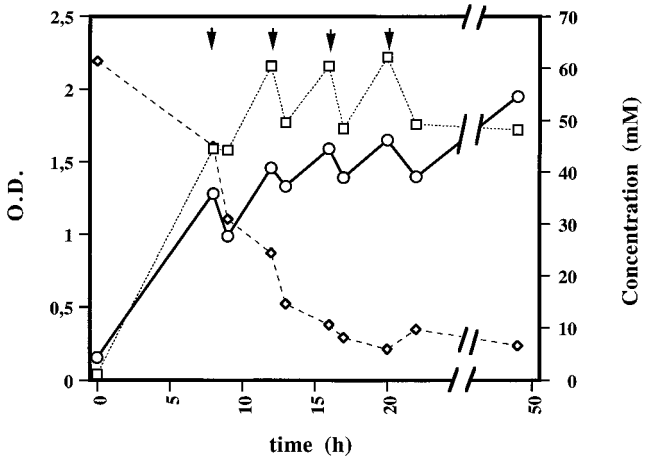

Figure 5. Time course of repeated batch fermentations in an ATPS composed of PEG–DEX. OD (□), lactate (○), and glucose (◇). The exchange of top phases is indicated by the vertical arrows.

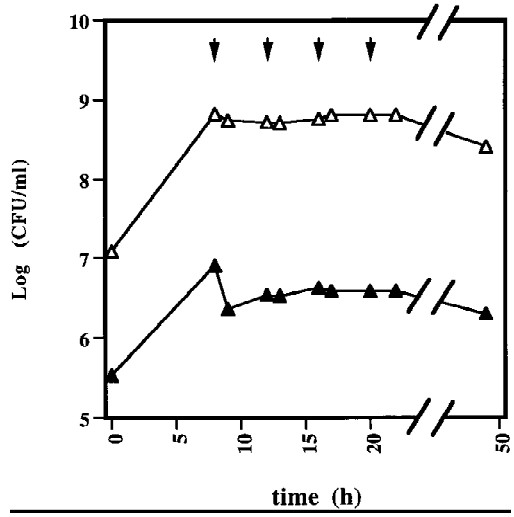

Figure 6. Concentration of *Lactococcus lactis* sp. *lactis* ATCC 19435 in top (filled symbol) and bottom (open symbol) phases during repeated extractive batch fermentations. The exchange of top phases is indicated by vertical arrows.

al., 1995). In the present study, the elution conditions for the EO/PO were defined so that this compound could be analyzed by gel exclusion HPLC. The ease of using HPLC techniques together with the broad range of chromatographic supports commercially available makes the system developed in this work a suitable and adaptable technique for composition determinations in ATPS.

The chromatographic system presently developed was used to study the effects of lactic acid, phosphate, and LAB in an EO/PO ATPS. Systems containing nonionic polymers should hardly be affected by the addition of low concentrations of salts (Albertsson, 1986), which was later confirmed (King et al., 1988). The present study, however, showed that phosphate and lactate modify the phase diagram of EO/PO–DEX ATPS at the concentrations used. Thus, both lactate and phosphate decreased tie-line slope by a similar amount. The effect on the binodial shape was more pronounced in the ATPS containing lactate, probably because the concentration of lactate (555.0 m*M*) was higher than that of phosphate (32.2 m*M*). High concentrations of salts induce phase separation in aqueous solutions of PEG (Albertsson, 1986). Lactate at high concentrations will, thus, act as a phase forming component relative to PEG, which results in a change in binodial shape.

It has been stated previously that variations in the quantity of cells, in a phase system, do not alter their distribution behavior (Albertsson, 1986). The β-galactosidase recovery, and the volume ratio of the phases were affected by the

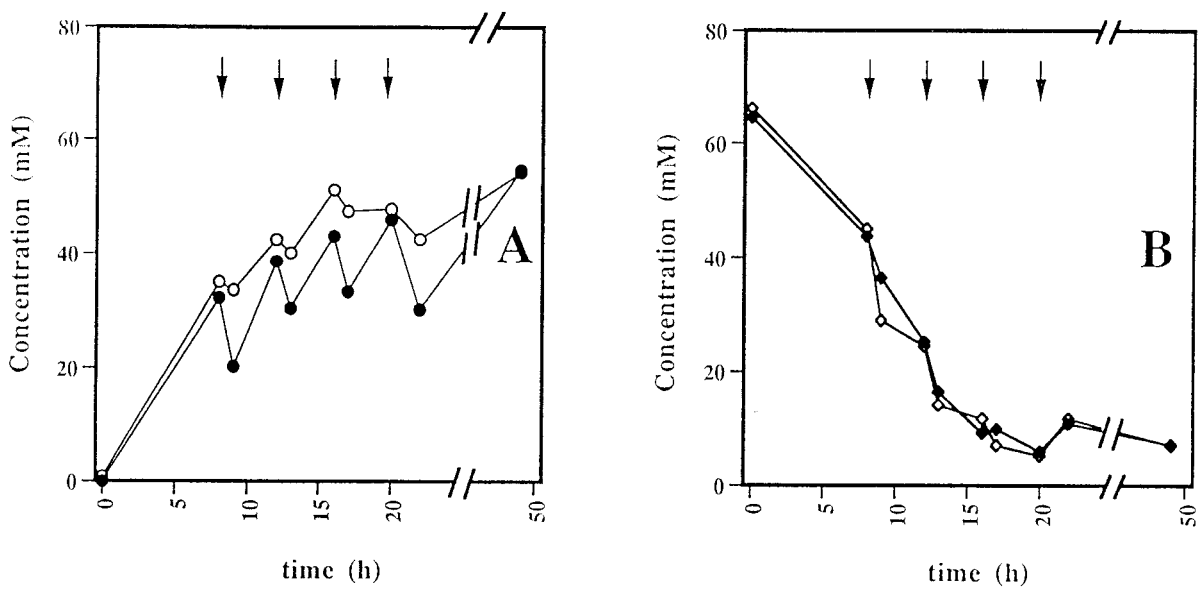

Figure 7. Concentration of lactate (A), and glucose (B) in top (filled symbol) and bottom (open symbol) phases, during repeated extractive batch fermentations. The exchange of top phases is indicated by vertical arrows.

percentage of disintegrated cells of *E. coli* (Veide et al., 1983). Similarly, a displacement of the binodial caused by disintegrated cells of *E. coli* has been reported (Köhler et al., 1989). In the present study, the chromatographic method showed that cells of *L. lactis* changed the binodial shape of EO/PO–DEX ATPS, affecting both the region near the critical point which became flatter, and the regions far away from the critical point.

Current industrial processes for lactate production achieve yields close to the theoretical, and productivities from 7 to 18 m$M \cdot h^{-1}$, depending on the pH of the fermentation. The yield (1.1 mol/mol) and productivity (4.7 m$M \cdot h^{-1}$, including phase separation time) obtained in the present investigation reflect the fact that pH was not controlled, but allowed to drop to 4.5. Similarly, in a regular batch fermentation without pH control, the yield was 0.74 mol/mol, and the productivity 2.5 m$M \cdot h^{-1}$ (Planas et al., 1996). The higher productivity obtained in the present study was because fermentation was carried out in ATPS allowing part of the lactic acid to be intermittently removed with the top phase.

When ATPS are used in repeated extractive batch bioconversions, such as fermentation systems, where one or both phases are recirculated, changes in the phase composition will ultimately affect the performance of the process. The recovery of the product (Persson et al., 1991; Veide et al., 1983), and the performance of the separation step (Katzbauer et al., 1995) are affected by changes in the system. In the present study, the composition of the system was analyzed throughout five repeated extractive batch fermentations. A difference between the initially measured polymer composition and the precalculated values was observed. This phenomenon is evidenced by the difficulties encountered while weighting out all the components in the laminar flow chamber prior to inoculation of the ATPS. The volume ratio shift was correlated with alterations in polymer composition. The whole system became progressively enriched in DEX, and depleted in PEG. Changes in volume

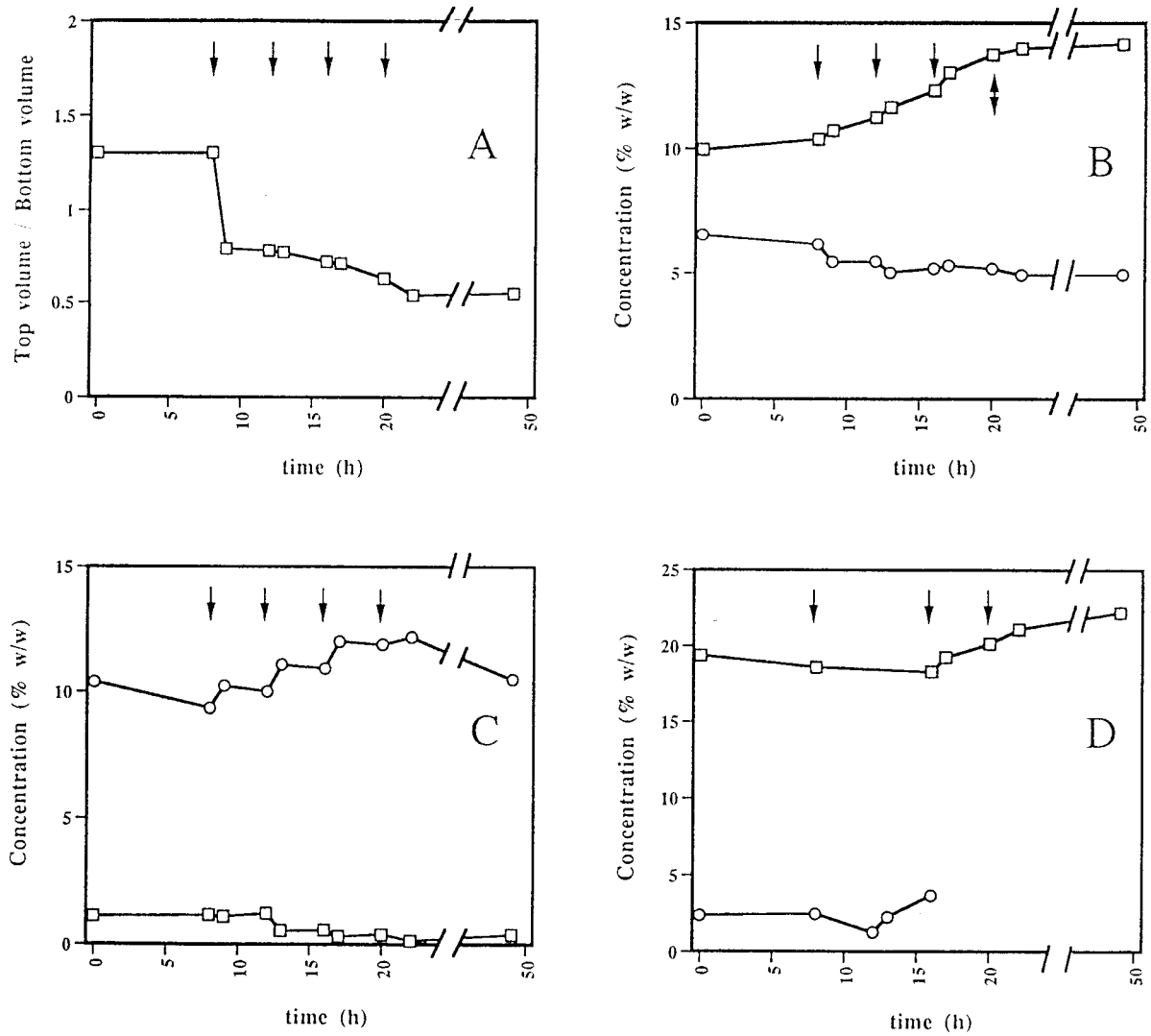

Figure 8. Volume ratios (A) and polymer concentrations in PEG 8000 (○)–dextran 500 (□) ATPS during repeated batch fermentations: concentrations for the whole system (B); top phase (C); and bottom phase (D).

ratio and in total polymer composition do not necessarily imply changes in the composition of the phases as long as the system moves along a tie line. However, the present study demonstrated that the composition of the phases also changed during the repeated extractive batch fermentations (Fig. 8C and D).

The long-term stability of an ATPS as either an extraction system or a system for extractive bioconversions will depend on its capacity to maintain the polymer concentrations in a range which minimizes volume ratio changes and changes in the composition of the phases. The present study showed that the top phase replacements, based on calculations using predetermined phase diagrams, led to unpredictable changes of volume ratio and composition of the system. The chromatographic system involving two HPLC columns provided rapid and reliable measurements of polymer concentrations as well as fermentation components, and may be used to control the composition of ATPS in extractive bioconversions.

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