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Acoustic field assisted enhanced demixing of aqueous two-phase systems

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Abstract

Aqueous two-phase extraction has been recognized as a versatile downstream processing technique for the recovery of biomolecules. A major deterrent to its industrial exploitation is the slow demixing of the two aqueous phases after extraction, due to their similar physical properties. A method to decrease the demixing times of these systems, employing a travelling acoustic wave field, is reported. The effects of phase composition and microbial cells on demixing in a polyethylene glycol/potassium phosphate two-phase system are studied in detail. As phase composition increased, demixing time decreased gradually. Phase volume ratio was found to have a significant effect on demixing time at low phase compositions. However, at intermediate and high phase compositions, only a small effect on demixing time was observed. The effect of phase composition and volume ratio on demixing behavior was explained based on the droplet size of the dispersed phase, which is the resultant effect of the physical properties of the phases. At all the phase compositions studied, the acoustically assisted process decreased the demixing time by 17–60% when compared to demixing under gravity alone. Increasing the cell concentration increased the demixing time markedly in case of yeast cells. However, it remained practically constant in the case of *Lactobacillus casei* cells. Application of an acoustic field reduced the demixing times up to 60% and 40% in the case of yeast and *L. casei* cells, respectively. Visual observations indicated that ultrasonication caused mild circulation currents in the phase dispersion enhancing droplet–droplet interaction, which in turn enhanced the rate of coalescence, eventually resulting in an enhanced demixing rate.

Keywords: Aqueous two-phase extraction; Coalescence; Demixing time; Microbial cell; Acoustics

1. Introduction

Aqueous two-phase extraction is a versatile downstream processing technique, which can be applied for the separation, characterization and study of biomaterials [1,2]. Major hindrances for its application in bio-industries are: high cost of the phase forming polymers and slow demixing rates [3,4]. The former problem is solved to a great extent by adapting temperature induced phase separation for recovery and recycling of the polymers [5,6], while the latter aspect is not addressed to the same extent. The slow demixing of the thoroughly mixed phases is due to a small difference in density between them, high viscosity of the individual phases and low interfacial tension [1,7].

Asenjo and coworkers [8] have studied in detail phase separation kinetics of polyethylene glycol (PEG, MW 4000)/potassium phosphate system under gravity. Their investigations were important for the design of large-scale aqueous two-phase separators. Albertsson [1] has described separation times for various systems at different volume ratios and observed a sudden decrease in time of phase separation as the volume ratio (top:bottom) was increased above a certain point.

Efforts have been directed at speeding up phase separation by methods alternative to the conventional gravity settling [7] and centrifugation [9] such as electrokinetic demixing [10–13] and magnetic field assisted demixing [14,15] – each having its own drawbacks [16]. From the economic point of view, centrifugation becomes prohibitively expensive on a large scale. Electrokinetic demixing requires fabrication of special equipment and chemical additions such as salts to the polymer/polymer systems [11,12] and this method is not applicable to PEG/salt systems. Magnetic field assisted processes require addition of micrometer sized iron particles or ferro-fluids to the system. The technique was not found to be useful when the PEG phase was dispersed [14].

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All these observations indicate that there is a need for newer methods to enhance the demixing rates in aqueous two-phase systems (ATPSs) in a simple and cost effective manner. Application of an acoustic field is one such potential method. Conventional wisdom indicates that application of an acoustic field causes mixing rather than demixing of the systems, since it imparts energy to the system in order to achieve dynamic agitation, shear, cavitation, heating, etc. This can be easily seen by the application of acoustics for cleaning of surfaces and disruption of microbial cell walls. However, we found that this is not true in the case of acoustic fields of higher frequency (MHz range). Our preliminary experiments showed that application of an acoustic field decreased the demixing time in PEG/salt systems by about 50%. Since the method was simple, easy to scale up and economical (the acoustic transducer being a high voltage and low current device), we carried out a detailed study of this method in PEG/potassium phosphate ATPS.

It is known that the kinetics of phase demixing strongly depends on the physical properties such as density, viscosity and interfacial tension of the system. These properties can be varied largely by changing the phase composition of the system. Hence, we studied the effect of phase composition on demixing times in a PEG/phosphate system both under gravity and in the presence of an acoustic field. From the practical point of view it is desirable to concentrate the target biomolecules/cells into small volumes of the phases. Hence, the effect of volume ratio was investigated. Large-scale processing involves large amounts of cells in the phase system. The presence of cells and cellular components in the system significantly increases the demixing time due to the formation of a stabilized emulsion [15]. Therefore, we also investigated the effect of microbial cells on the demixing times in a PEG/phosphate system in settling under gravity alone and under the influence of acoustic field.

2. Theoretical aspects of phase demixing

Some researchers have assumed that approximately Stoke's law (which was developed for a rigid sphere) can describe phase demixing under unit gravity;

$$V_s = \frac{D^2 \Delta\rho g}{18\mu_C} \quad (1)$$

where D is the droplet diameter, $\Delta\rho$ is the density difference between the phases, μ_C is the dynamic viscosity of the continuous phase, g is the acceleration due to gravity and V_s is the droplet rise/fall velocity.

Asenjo and coworkers [8] have rightly indicated that for a swarm of droplets considerable deviations from Stoke's law can be expected. As the droplets are not rigid the circulation inside them (induced by the drag of the continuous phase) has to be taken into account as given by the Hadamard–Rybzcynski equation

$$V_s = \frac{D^2 \Delta\rho g}{18\mu_C}\left(\frac{3\mu_D + 3\mu_C}{3\mu_D + 2\mu_C}\right) \quad (2)$$

where μ_C and μ_D are the viscosities of the continuous and dispersed phases respectively.

Phase demixing can be seen as the combined effect of droplet rise/fall and droplet coalescence. If a single droplet is considered then the two steps are clearly in series. The droplet has to rise/fall to the interface and there it coalesces with the interface [8]. In this situation, droplet migration will be the controlling step in the overall demixing process. In ATPSs this situation can be seen when the phase volume ratios are either very high or very low. The time required for the separation of the two phases in this situation can be represented by Eq. 2. However, this may not be the case generally. The presence of multiple droplets leads to considerable droplet–droplet interaction, which leads to coalescence as they rise/fall. This will increase the droplet size and this in turn alters their rise/fall velocities (proportional to the square of the droplet diameter). Hence, coalescence will be the controlling step, which is also the observation made by us in the present study. Sometimes streaming or fingering occurs during phase demixing when a swarm of droplets rapidly moves dragging the associated continuous phase along with them. Such streams do not behave according to Eqs. 1 or 2 but move much faster as the diameter of the stream is much larger than droplet diameters. We have not observed such a phenomenon in the present study of acoustic demixing. However, we have observed it in the case of electrokinetic demixing of ATPSs especially at high field strengths [11,12].

3. Materials and methods

3.1. Materials

PEG (batch No. T/823081) with a molecular weight of 6000 Da was obtained from Sisco Research Laboratories, Mumbay, India. Potassium dihydrogen phosphate and dipotassium hydrogen phosphates were obtained from Ranbaxy Chemicals, Punjab, India. Glucose and yeast extract were obtained from Difco Research Laboratories, Delhi, India. All other chemicals used were of analytical grade.

Yeast cells (*Saccharomyces cerevisiae*) in the form of cakes were purchased from a local market. Cell mass of *Lactobacillus casei* was obtained by growing them in a 2 l bioreactor at 37°C with medium composition as explained elsewhere [17]. Prior to use, both yeast cells and *L. casei* cells were thoroughly washed with distilled water and centrifuged at 5000 rpm for 30 min. The supernatants containing some of the soluble contaminants were discarded. The pellets were dried at 50°C for 2 h and then used for the experimentation.

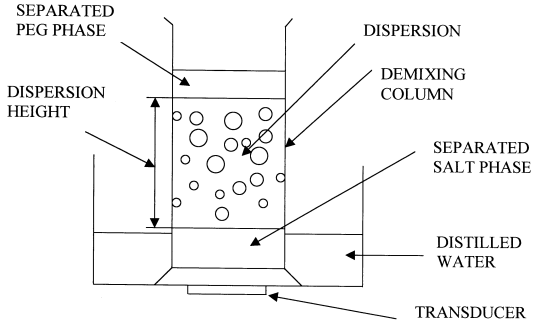

Fig. 1. Schematic diagram of the acoustically assisted demixing process.

3.2. Methods

3.2.1. Preparation of the phase systems

Predetermined, weighed quantities of PEG and potassium phosphates (K_2HPO_4:KH_2PO_4 = 1.82:1) were added to a known quantity of distilled water, so as to make the total composition of the system 100% on a w/w basis. After dissolving the components, the system was mixed thoroughly for 30 min and allowed to separate overnight in a separating funnel. The equilibrated and separated phases were then collected and used as stock solutions for further experiments. In this way 300 g of systems were prepared at each phase composition.

3.2.2. Phase demixing experiments

All the demixing experiments were carried out in a 100 ml capacity measuring cylinder with a height to diameter ratio of 8.75. All the demixing studies were carried out at three volume ratios of the top to bottom phase, viz. 30/70, 50/50 and 70/30. The column was filled with a 100 ml freshly prepared thoroughly mixed (for 10 min) phase dispersion. The dispersion was subjected to ultrasonication at a frequency of 1.2 MHz and at a power level of 1.2 W/cm^2 using an ultrasonic transducer (model HM-460, Holmer Products Corp., Massachusetts, USA). The dispersion height is defined as the height of the unseparated cloudy/turbid region of the dispersion. The dispersion height was recorded as a function of time. For all the experiments, sonication was given from the bottom and with a travelling wave mode with the transducer at the bottom of the dispersion. A schematic diagram of the acoustically assisted process is shown in Fig. 1. Similar demixing experiments were carried out under gravity alone in the absence of ultrasonication. For all the experiments, the time for complete phase separation was taken as the time required for a clear horizontal interface to be formed. However, in the experiments with the cells, one or two droplets were found to be present at the interface even after the formation of the interface. For the experiments with the cells, predetermined, weighed quantities of cells were added to 100 ml of dispersion.

All the demixing experiments were carried out in triplicate and mean values are reported. The accuracy of the measurements was found to be well within ±5%.

3.2.3. Phase density and viscosity measurements

Density and viscosity of the individual phases were measured using specific gravity bottles and an Ostwald-U-tube viscometer of 10 ml capacity, respectively. All the measurements were carried out in duplicate at 27 ± 0.5°C and average values are reported.

4. Results

4.1. Demixing behavior with respect to phase composition

Systems selected to examine the effect of phase composition on demixing as well as their densities and viscosities are given in Table 1. Fig. 2 depicts the typical kinetics of phase demixing (dispersion height as a function of time) for an intermediate phase composition (15/11) both in the presence and in the absence of an acoustic field. Demixing times for the remaining phase compositions are given in Table 2. In order to bring out the positive effects of acoustics more clearly, demixing time is plotted against phase volume ratio with phase composition as a parameter in Fig. 3. It can be seen that as phase composition increased demixing time decreased gradually under gravity as well as in the acoustically assisted process. The acoustically assisted process resulted in an about 18, 43 and 55% reduction in demixing times at high (35/11), intermediate (15/11) and low phase compositions (7/11), respectively, as shown in Fig. 4. It may be noted that the efficacy of the acoustic field decreased with an increase in phase composition.

Table 1
Viscosity and density at various phase compositions of the PEG (6000)/potassium phosphate two-phase system

Sl. #	Phase composition (PEG/salt, % w/w)	Viscosity		Density	
		Top phase (mPa.s)	Bottom phase (mPa.s)	Top phase (kg m^{-3})	Bottom phase (kg m^{-3})
1	7/11	11.68	1.21	1121.9	1143.0
2	10/11	12.29	1.23	1130.9	1152.9
3	15/11	24.47	1.41	1135.2	1190.7
4	10/16	36.77	1.40	1138.2	1209.0
5	25/11	56.79	1.57	1142.6	1253.7
6	20/16	71.99	1.68	1144.4	1276.3
7	35/11	97.75	1.83	1152.0	1330.0

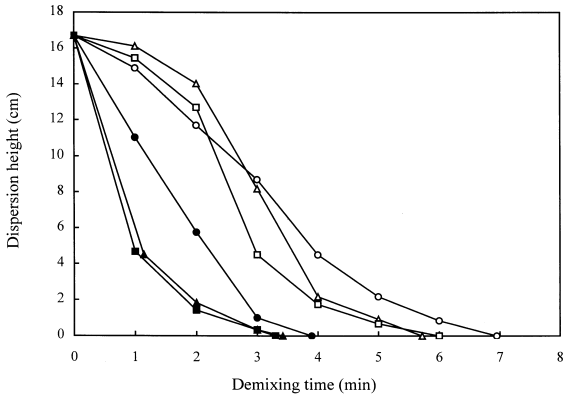

Fig. 2. Kinetics of phase demixing at 15% PEG and 11% potassium phosphate two-phase system (dispersion height as a function of time). Open symbols: demixing under gravity alone, closed symbols: demixing under ultrasonication, □: volume ratio 70/30, △: volume ratio 50/50, ○: volume ratio 30/70.

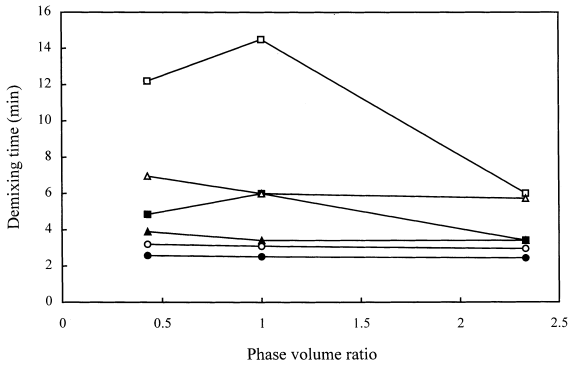

Fig. 3. Effect of phase volume ratio on demixing time in the PEG/potassium phosphate two-phase system. Open symbols: demixing under gravity alone, closed symbols: demixing under ultrasonication, □: phase composition 7/11, △: phase composition 15/11, ○: phase composition 35/11.

4.2. Demixing behavior with respect to phase volume ratio

It can be noted from Fig. 3 that volume ratio has a significant effect on demixing time at low phase composition and the effect decreases gradually as phase composition increases. As a general trend, demixing time decreased with an increase in volume ratio. An exception to this trend was at the 50/50 volume ratio of low phase composition where the demixing time was higher than that at the 30/70 volume ratio.

4.3. Phase demixing in the presence of microbial cells

Addition of microbial cells to the PEG/phosphate system significantly increased the demixing time. Table 3 shows the experimental results of the effect of yeast and *L. casei* cells on the demixing behavior in the PEG/phosphate system at 1 and 8% cell concentrations. Experiments were carried out at an intermediate composition of PEG 15% and potassium phosphate 11% because of the suitability of this phase composition for large-scale applications. In both cases, almost all cells partitioned to the bottom phase forming a band between the interface and the bottom phase. Demixing time in the case of yeast cells was found to be much higher when compared to *L. casei* cells. It was observed that in both cases the volume ratio had little effect on demixing time, at intermediate phase composition. Hence, the effect of cell concentration (2–7%) was studied at the 70/30 volume ratio only.

As cell concentration was increased, at a constant volume ratio, demixing time increased significantly in the case of yeast cells. However, a very marginal increase in demixing time was observed in the case of *L. casei* cells as indicated in Fig. 5. The acoustically assisted process was found to decrease the demixing time significantly in these systems having cells. In the case of *L. casei* cells up to 40% and in the case of yeast cells up to 60% decreases in demixing times were observed (Table 3 and Fig. 5). Addition of microbial cells did not significantly affect the physical properties such as density and viscosity of the systems (Tables 4 and 5).

5. Discussion

5.1. Demixing behavior with respect to phase composition

Droplet coalescence is the critical factor for phase demixing in ATPSs. Ultrasonication generates mild circulation currents in the phase dispersion thereby increasing the droplet–droplet interaction (collision), as the binary collision frequency is proportional to the shear [18,19]. An increase in collision frequency enhances the probability of coalescence, which in turn hastens phase demixing due to increased migration velocity of the larger droplets.

Table 2
Demixing times (min) at different volume ratios of various phase compositions in the PEG(6000)/potassium phosphate two-phase system

Phase composition (PEG/salt, % w/w)	70/30 volume ratio		50/50 volume ratio		30/70 volume ratio	
	Gravity	Acoustics	Gravity	Acoustics	Gravity	Acoustics
7/11	6.00	3.45	14.40	6.00	12.20	4.90
10/11	5.80	3.35	11.96	5.83	10.92	4.80
10/16	4.83	3.14	5.28	3.35	6.16	3.82
25/11	4.10	2.82	4.20	2.86	4.40	2.94
20/16	3.5	2.5	3.70	2.6	3.87	2.65
35/11	2.95	2.40	3.15	2.53	3.25	2.60

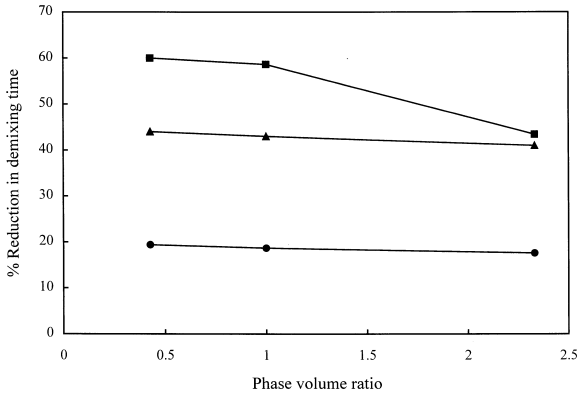

Fig. 4. Efficacy of acoustics on demixing behavior in the PEG/potassium phosphate two-phase system. Closed symbols: percent reduction in demixing time, ■: phase composition 7/11, ▲: phase composition 15/11, ●: phase composition 35/11.

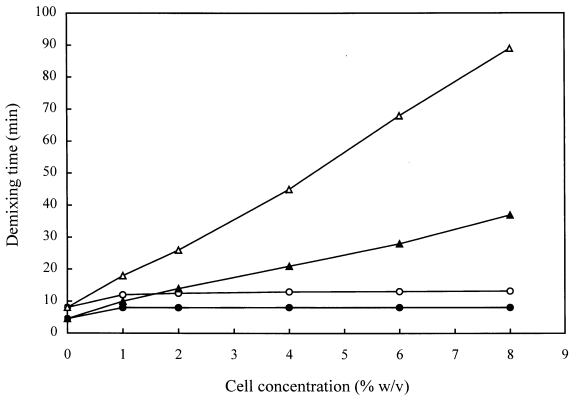

Fig. 5. Effect of microbial cell concentration (1–8%) on demixing time at a 70/30 volume ratio of 15% PEG and 11% potassium phosphate two-phase system. Open symbols: demixing under gravity alone, closed symbols: demixing under ultrasonication, △: yeast cells, ○: *L. casei* cells.

The enhanced rates could be observed by the increased slopes in Fig. 2. This is how the acoustically assisted demixing process has resulted in a decrease in demixing time (Fig. 3). The observed decrease in efficacy of acoustics with an increase in phase composition is due to attenuation of ultrasound intensity with an increase in phase viscosity. For similar reasons attenuation of ultrasound intensity was also observed when the top viscous phase was forming the continuous phase at any given composition (Fig. 4).

Kula and coworkers [20] have suggested that in PEG/salt systems, the fastest separation could be expected at intermediate compositions especially when the volume of the higher viscous phase is smaller than that of the lower viscous phase. However, we did not observe the fastest separation at the intermediate phase composition. Instead, as phase composition increased, demixing time gradually decreased (Fig. 3 and Table 2). Further, at all the studied phase compositions, the demixing time was higher when the volume of the higher viscous (PEG rich) phase was smaller than that of the lower viscous (salt rich) phase (30/70 volume ratio) as shown in Table 2 and Fig. 3. The observed decrease in demixing time with an increase in phase composition mentioned above (Fig. 3) is mainly due to the increase in droplet size of the dispersed phase with an increase in phase composition.

An increase in phase composition increases the interfacial tension, density difference and the phase viscosities. The combined effect of these properties is that the average drop size and the drop velocity increase with an increase in the system composition [21,22]. Visual observations have also indicated an increase in droplet size with an increase in phase composition at all volume ratios. The higher the droplet size, the faster its rise/fall thereby decreasing the demixing time. As a result the demixing time decreased with an increase in phase composition. In polymer/polymer type ATPSs, it was reported that at high phase compositions the demixing rate becomes very slow owing to a very high viscosity of the individual phases [1], which drastically reduces the migration of the phase droplets. However, such an effect of viscosity in reducing the migration of droplets was not observed in the PEG/salt systems employed in our studies.

5.2. Demixing behavior with respect to phase volume ratio

At a 70/30 volume ratio, salt is the dispersed phase and the more viscous PEG phase forms the continuous phase. Owing to the higher viscosity of the continuous phase, relatively less of the total energy given to form the dispersion will be transferred to the dispersed phase when compared to the situation where the PEG phase is dispersed and the salt phase of much lesser viscosity forms the continuous phase (50/50 and 30/70 volume ratios). Hence, the droplet size will be larger at a 70/30 volume ratio when

Table 3
Demixing times in the 15% PEG and 11% potassium phosphate two-phase system in the presence of yeast and *L. casei* cells

Cell concentration (% w/v)	Volume ratio (top/bottom)	*L. casei* cells demixing time (min)		Yeast cells demixing time (min)	
		Gravity	Acoustics	Gravity	Acoustics
1	70/30	12.00	8.00	18.00	10.00
	50/50	12.20	8.10	19.00	10.30
	30/70	12.10	8.00	18.40	10.00
8	70/30	13.20	8.10	89.00	37.00
	50/50	13.40	8.20	94.00	40.00
	30/70	13.20	8.10	92.00	40.00

Table 4
Viscosity and density of the 15% PEG and 11% potassium phosphate two-phase system in the presence of *L. casei* cells

Cell concentration (% w/v)	Volume ratio (top/bottom)	Viscosity (mPa.s)		Density (kg m^{-3})	
		Top phase	Bottom phase	Top phase	Bottom phase
0	70/30	17.01	1.46	1134.1	1166.9
1	70/30	17.23	1.46	1134.2	1166.4
	50/50	17.21	1.46	1132.1	1167.3
	30/70	17.19	1.46	1131.0	1171.3
2	70/30	16.45	1.46	1134.5	1163.8
4	70/30	15.75	1.46	1133.3	1165.8
6	70/30	15.42	1.46	1133.8	1167.5
8	70/30	14.98	1.46	1134.6	1168.3

compared to 50/50 and 30/70, resulting in a lower demixing time at this volume ratio at any given phase composition (Fig. 3 and Table 2).

At 50/50 and 30/70 volume ratios, the dispersed phase being the same (PEG phase), the lower demixing time at the 50/50 volume ratio than that at the 30/70 volume ratio at intermediate and high phase compositions (Fig. 3) is mainly due to the higher droplet size at the 50/50 volume ratio (visual observations). This higher droplet size at 50/50 is the result of relatively lower energy availability (energy/unit volume) to form the dispersion owing to a higher volume of the dispersed phase when compared to that of the 30/70 volume ratio. However, as an exception to this trend, at the 50/50 volume ratio of low phase composition, although the droplet size was observed to be higher, the demixing time was higher than that at the 30/70 volume ratio as shown in Fig. 3. Visual observation indicated that at this volume ratio, after reaching the interface, the droplets assembled near the interface forming a densely packed zone and took longer time to coalesce with the already formed PEG layer. The formation of a densely packed zone was due to the lower rate of droplet coalescence with the interface when compared to the rate of migration of the droplets. The droplet packing was also observed at the 30/70 volume ratio of low phase composition. However, due to a lower concentration of the dispersed phase droplets the density of packing was lower than that at the 50/50 volume ratio. This kind of accumulation was observed only at this phase composition.

It can be noted that the extent of the decrease in demixing time when the volume ratio was increased from 30/70 to 70/30 reduced with an increase in phase composition (Figs. 3 and 4). For instance in gravity demixing, the extent of decrease in demixing was about 51, 18 and 8% at 7/11, 15/11 and 35/11 phase compositions, respectively (Fig. 3). This is due to the synergistic effect of the increase in droplet size with an increase in phase composition as well as phase volume ratio.

5.3. Phase demixing in the presence of microbial cells

The presence of cell surface associated biomolecules and their interaction with the phase system will affect the charge, size and hydrophobicity of the dispersed phase droplet and thus alter its surface properties, which in turn discourages droplet coalescence. This could be responsible for the increase in demixing time (Table 3). It was reported that the presence of cellular components and other biomolecules in ATPSs can form stabilized emulsions that will considerably increase the phase separation time [15].

It is known that yeast cell wall contains mannoproteins and lipopolysaccharides [23], which stabilize the dispersion through steric stabilization by adsorbing at the interface. As the extent of this adsorption increases with an increase in yeast cell concentration, the demixing time increased consistently with an increase in cell concentration (Fig. 5). However, the *L. casei* cell wall is not known to contain any such materials having emulsifying properties, which appears to be the reason for the insignificant increase in demixing time with an increase in cell concentration.

The acoustically assisted process decreased the demixing

Table 5
Viscosity and density of the 15% PEG and 11% potassium phosphate two-phase system in the presence of yeast cells

Cell concentration (% w/v)	Volume ratio (top/bottom)	Viscosity (mPa.s)		Density (kg m^{-3})	
		Top phase	Bottom phase	Top phase	Bottom phase
0	70/30	17.01	1.46	1134.1	1166.9
1	70/30	17.51	1.46	1137.1	1166.8
	50/50	17.19	1.46	1138.1	1166.7
	30/70	17.33	1.46	1140.0	1165.8
2	70/30	18.21	1.46	1138.0	1166.2
4	70/30	19.98	1.46	1141.6	1166.0
6	70/30	21.24	1.46	1142.0	1166.2
8	70/30	22.64	1.46	1143.3	1166.3

times up to 40% in the case of *L. casei* cells and up to 60% in the case of yeast cells (Table 3 and Fig. 5). This decrease is again due to the mild circulation current caused by the ultrasonic waves in the phase dispersion as explained earlier. In addition to the advantages already mentioned, another attractive feature of this acoustic method is that it provides gentle treatment to the system, thereby not causing any damage to the cells. This is quite in contrast to the classical application of acoustic fields for disruption of cells to release intracellular compounds. Further, the rise in temperature of the system due to acoustic field application was found to be less when compared to an electrokinetic demixing process. This is more true in the case of PEG/salt systems in which the application period of the acoustic field is short.

6. Conclusions

Acoustic demixing, a new method for enhancing the demixing rates of ATPSs, is reported. The effect of phase composition and volume ratio on demixing behavior was explained based on droplet size, which is determined by the resultant effect of the physical properties of the system. The present work suggests that the acoustic demixing of two-phase systems would be useful in enhancing the demixing rates of the phases especially at low and intermediate phase compositions irrespective of phase volume ratio and even at high microbial cell concentration. The process is simple, economical, easy to scale up and readily available ultrasonic transducers could be employed.

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References

[1] P.A. Albertsson, Partitioning of Cell Particles and Macromolecules, 3rd edn., Wiley, New York, 1986.
[2] A.D. Diamond, J.T. Hsu, Aqueous two-phase systems for biomolecule separation, Adv. Biochem. Eng./Biotechnol. 47 (1992) 89–133.
[3] K.S.M.S. Raghavarao, N.K. Rastogi, N.G. Karanth, M.K. Gowthaman, Aqueous two-phase extraction for downstream processing of enzymes/proteins, Adv. Appl. Microbiol. 41 (1995) 97–171.
[4] K.S.M.S. Raghavarao, M.R. Guinn, P. Todd, Recent developments in aqueous two-phase extraction in bioprocessing, Separ. Purif. Methods 27 (1998) 1–50.
[5] I. Galaev, B. Mattiasson, Thermoreactive water-solube polymers, nonionic surfactants and hydrogels as reagents in biotechnology, Enzyme Microb. Technol. 15 (1993) 354–366.
[6] H.-O. Johansson, G. Cundh, G. Karstorm, F. Tjerneld, Temperature induced phase partitioning of peptides in water solutions of ethylene oxide and propylene oxide random co-polymers in water, Biochim. Biophys. Acta 1335 (1997) 315–325.
[7] H. Hustedt, K.H. Kroner, M.R. Kula, Applications of phase partitioning in biotechnology, in: H. Walter, D.E. Brooks, D. Fisher (Eds.), Partitioning in Aqueous Two-phase Systems: Theory, Methods, Uses, and Applications to Biotechnology, Acsdemic Press, Orlando, FL, 1985, pp. 529–587.
[8] A. Kaul, R.A.M. Pereira, J.A. Asenjo, J.C. Merchuk, Kinetics of phase separation for polyethylene glycol-phosphate two-phase systems, Biotechnol. Bioeng. 48 (1995) 246–256.
[9] H. Hustedt, K.-H. Kroner, N. Papamichael, Continuous crosscurrent extraction of proteins in process scale, in: D. Fisher, I.A. Sutherland (Eds.), Separations Using Aqueous Phase Systems. Applications in Cell Biology and Biotechnology, Plenum Press, New York, 1989.
[10] D.E. Brooks, K.A. Sharp, S. Bamberger, C.H. Tamblyn, G.V.F. Seaman, H. Walter, Electrostatic and electrokinetic potentials in two polymer aqueous phase systems, J. Colloid Interface Sci. 102 (1984) 1–13.
[11] K.S.M.S. Raghavarao, R.M. Stewart, P. Todd, Electrokinetic demixing of aqueous two-phase systems. I: Separation rates of poly(ethylene glycol)-dextran mixtures, Separ. Sci. Technol. 25 (1990) 985–996.
[12] K.S.M.S. Raghavarao, R.M. Stewart, P. Todd, Electrokinetic demixing of aqueous two-phase systems. II: Separation rates of poly(ethylene glycol)-maltodextrin mixtures, Separ. Sci. Technol. 26 (1991) 257–267.
[13] K.S.M.S. Raghavarao, R.M. Stewart, S.R. Rudge, P. Todd, Electrokinetic demixing of aqueous two-phase systems. III: Drop electrophoretic mobilities and demixing rates, Biotechnol. Prog. 14 (1998) 922–930.
[14] P. Vikrostrom, S. Flygare, A. Grondalen, P.O. Larsson, Magnetic aqueous two-phase separation: a new technique to increase rate of phase-separation, using dextran-ferrofluid or larger iron oxide particles, Anal. Biochem. 167 (1987) 331–339.
[15] P.-O. Larsson, Magnetically enhanced phase separation, Methods Enzymol. 228 (1994) 112–117.
[16] S.K. Sikdar, K.D. Cole, R.M. Stewart, D.C. Szlag, P. Todd, H.J. Cabezas, Aqueous two-phase extraction in bioseparations: An assessment, Bio/Technology 9 (1991) 252–254.
[17] A. Demirci, L.A. Pometto III, B. Lee, N.P. Hinz, Media evaluation of lactic acid repeated-batch fermentation with *Lactobacillus plantarum* and *Lactobacillus casei* subsp. *rhamnosus*, J. Agric. Food Chem. 46 (1998) 4771–4774.
[18] A.K. Chesters, The modeling of coalescence process in fluid liquid dispersions, Trans. I Chem. E 69 (1991) 259–270.
[19] P.A. Arp, S.G. Mason, Orthokinetic collisions of hard spheres in simple shear flow, Can. J. Chem. 54 (1976) 3769–3774.
[20] M.R. Kula, K.H. Kroner, H. Hustedt, Purification of enzymes by liquid-liquid extraction, Adv. Biochem. Eng./Biotechnol. 37 (1982) 73–118.
[21] R. Clift, J.R. Grace, W.E. Weber, Bubbles, Drops and Particles, Academic Press, New York, 1978.
[22] K.R. Jafarabad, S.B. Sawant, J.B. Joshi, S.K. Sikdar, Enzyme and protein mass transfer coefficient in aqueous two-phase systems – I. Spray extraction columns, Chem. Eng. J. 47 (1992) 57–68.
[23] E. Valetin, E. Herrero, F.I.J. Pastor, R. Sentadrue, Solubilization and analysis of mannoprotein molecules from the cell wall of *Saccharomyces cerevisiae*, J. Gen. Microbiol. 130 (1984) 1419–1428.