Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Phase Separation of Lactan Gum from Fermentation Broths by Addition of Water-Miscible Organic Solvents

A. Foufopoulos[a]; M. R. Etzel[a]
[a] DEPARTMENTS OF FOOD SCIENCE AND CHEMICAL ENGINEERING, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

To cite this Article Foufopoulos, A. and Etzel, M. R.(1996) 'Phase Separation of Lactan Gum from Fermentation Broths by Addition of Water-Miscible Organic Solvents', Separation Science and Technology, 31: 6, 757 — 768
To link to this Article: DOI: 10.1080/01496399608001322
URL: http://dx.doi.org/10.1080/01496399608001322

SEPARATION SCIENCE AND TECHNOLOGY, 31(6), pp. 757–768, 1996

Phase Separation of Lactan Gum from Fermentation Broths by Addition of Water-Miscible Organic Solvents

A. FOUFOPOULOS and M. R. ETZEL
DEPARTMENTS OF FOOD SCIENCE AND CHEMICAL ENGINEERING
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN 53706

ABSTRACT

A microbial polysaccharide (lactan gum) produced by bacterium ATCC 55046 was precipitated from fermentation broths by the addition of ethanol, acetone, isopropanol, or *tert*-butanol. Compositions of the precipitate and supernatant phases were determined as a function of organic solvent concentration and used to construct binodal solubility curves. Lactan did not precipitate at bulk–mixture organic solvent concentrations below 35% (wt) ethanol, 35% acetone, 33% isopropanol, or 25% *tert*-butanol. At organic solvent concentrations just exceeding the solubility transition point, the precipitates were soft, moist, and sponge-like in texture, with low lactan concentrations. At higher organic solvent concentrations the precipitates were compact and dense. The maximum lactan concentration in the precipitate was 25–37%, depending on the organic solvent type and concentration. Increasing the organic solvent concentration beyond 50% for ethanol, or 70% for acetone, decreased the lactan concentration in the precipitate. No such decrease occurred for isopropanol and *tert*-butanol. Thus, organic solvent usage, from greatest to least, was in the order ethanol, acetone, isopropanol, and *tert*-butanol, but the maximum lactan concentration in the precipitate, from greatest to least, was in the order acetone, isopropanol, ethanol, and *tert*-butanol.

Key Words. Lactan gum; Precipitation; Phase separation; Fermentation broth; Binodal solubility curve; Organic solvent; Ethanol; Acetone; Isopropanol; *tert*-Butanol

INTRODUCTION

Microbial polysaccharide gums are widely used in industry to modify the rheology of aqueous solutions. Lactan gum is a newly discovered microbial polysaccharide produced by the facultative anaerobe Gram-negative bacterium ATCC 55046 (1, 2). It is an anionic galactomannan polysaccharide with a weight-average molecular weight of approximately 7000 kDa. The viscosity of aqueous solutions of lactan gum is stable over a wide range of pH, particularly in alkaline environments. The solutions exhibit elastic flow and shear thinning behavior, obeying the power law model. These characteristics are useful for food and nonfood products requiring a modest degree of thickening.

Because microbial polysaccharides are totally miscible in fermentation broths and other aqueous solutions, isolation and recovery is traditionally accomplished by either evaporation, resulting in a product of inferior quality, or by techniques which lower the solubility of the gum (3). Examples are the addition of a water-miscible organic solvent (OS) or large amounts of salt or acids (4, 5). Precipitation through the addition of an OS such as isopropanol (IPA) is widely practiced for several reasons, in part because the alcohol can be purified simultaneously with the final product and can be recycled (6). Studies characterizing the phase separation of microbial polysaccharides from fermentation broths are of general interest because the results are fundamentally applicable to the recovery of other industrially important hydrophilic macromolecular colloids from aqueous solution (7).

The goal of this work was to investigate the effect of OS type and concentration on the precipitation of lactan gum from fermentation broths. The methodology of the current research parallels that of Flahive et al. (8) on the alcohol precipitation of xanthan gum from fermentation broths. Binodal solubility curves were constructed that link the compositions of the precipitate and supernatant phases formed by the addition of either ethanol (EtOH), acetone (ACE), IPA, or *tert*-butanol (tBA) to the fermentation broths. These phase diagrams characterize the nature of the separation process and are useful in the scale-up and operation of technologies for the recovery of microbial polysaccharide gums.

EXPERIMENTAL

Lactan Gum Fermentation

The bacterium *Rahnella aquatilis* sp. ATCC 55046 (American Type Culture Collection, Rockville, MD) was grown in a 5-L mechanically agitated laboratory fermenter (BioFlo III, New Brunswick Scientific, New Bruns-

wick, NJ) containing 4 L of sterile sweet whey permeate (9). A 7.5% (v/v) inoculum, grown at 26°C for 24 hours on sterile sweet whey permeate in 1 L corner-baffled shake flasks operated at 175 rpm, was aseptically combined with the culture medium in the fermenter. The fermentation cultures were grown at 26°C and maintained at pH 7 by addition of 4 N NaOH. An antifoaming agent (Mazu DF 60P, Mazer Chemicals, Gurnee, IL) was added. Sterile air was supplied at a rate of 1 VVM. The agitation rate was first held at 800 rpm, and then toward the end of the fermentation it was increased to 1000 rpm to compensate for the increased viscosity of the broth.

The broth was transferred to a 5-L airtight Pyrex bottle and pasteurized, unstirred, at 90 to 95°C for 1 hour in a water bath to inactivate cells and enzymes. The broth was cooled to room temperature overnight and stored at 4°C prior to use in recovery experiments.

Gum Precipitation

Each OS was greater than 99% in purity: EtOH (Absolute U.S.P. Punctilious grade, Quantum Chemical Co., Tuscola, IL), IPA (Certified A.C.S. grade, Fisher Scientific, Pittsburgh, PA), tBA (Certified grade, Fisher Scientific, Pittsburgh, PA), and ACE (Certified A.C.S. grade, Fisher Scientific, Pittsburgh, PA). The fermentation broth and one of these OS were mixed according to the procedure outlined by Flahive et al. (8). Briefly, the broth and OS were combined in the desired ratios in preweighed 250 mL centrifuge bottles to a total solution mass of approximately 100 g per bottle. The bottles were sealed tightly, shaken vigorously by hand for 1 minute, and placed in a shaking water bath at 120 rpm and 15°C for 24 ± 2 hours to reach equilibrium. The bottles were then centrifuged at 15,000*g* and 15°C for 30 minutes (model RC-5 Superspeed Refrigeration Centrifuge with GSA rotor, DuPont Instruments, Newton, CT). The supernatant was poured from the bottle immediately after removal from the centrifuge, and 50 mL of it was saved in an airtight tube for later analysis. Supernatant that remained after pouring was removed carefully with a Pasteur pipet. The wet precipitate was weighed in the bottle and then immediately analyzed.

Analytical Methods

Cell concentration in the broth was measured by diluting a known mass of broth 1:20 with deionized water to decrease the viscosity, and then centrifuging at 15,000*g* and 5°C for 30 minutes to isolate the cells. The resulting cell pellet was washed with 125 mL of distilled water and centrifuged again using the same conditions. The washed pellet was resuspended

and dried to constant mass in a tared aluminum pan at 70°C for 12 hours. Cell concentration was calculated by dividing the dry cell mass by the initial mass of the broth.

Lactan concentration of the precipitate and the broth was measured by dialyzing a known mass for 3 days in 50 kDa MWCO dialysis tubing (Spectra/Por 6 Molecularporous Dialysis Membrane, Spectrum, Houston, TX). The tubes were dialyzed against 3 to 4 L of distilled water, which was changed twice a day. Dialyzed broth was dried in tared aluminum pans to constant mass for 12 to 24 hours at 70°C. Lactan concentration was calculated by first dividing the dry, dialyzed mass by the initial wet mass, then subtracting the cell concentration.

The total solids concentration in the precipitate and broth samples was measured in duplicate by drying a known mass of the wet precipitate in a tared aluminum pan to constant mass at 70°C. Total solids were calculated by dividing the dry solids mass by the wet precipitate mass.

Lactose concentrations were measured enzymatically using a food analysis kit (Lactose/D-Galactose Food Analysis Kit, Boehringer Mannheim GMBH, Mannheim, Germany).

In the precipitation experiments, the water concentration in the supernatant was measured by Karl Fischer titration (model 701 KF Titrino, Metrohm AG, Herisau, Switzerland). For most experiments duplicate measurements were sufficiently equivalent. For experiments having low reproducibility, which was the case for low OS concentration samples which contained a small amount of soluble lactan, triplicate or quadruplicate measurements per sample were necessary.

The concentrations of the remaining components in the supernatant and precipitate were obtained through mass balances (9).

RESULTS

Fermentation Kinetics

Figure 1 contains the time course of the viscosity and concentrations of cells, gum, and lactose. Cell growth was characterized by no detectable lag time, with growth primarily occurring during the first third of the fermentation (up to 14 hours). The dry cell mass asymptotically approached 0.6 g/L. Lactan gum was produced throughout the fermentation with no dependence on active cell proliferation. Its production rate declined at the late fermentation stages. There was no correlation between cell growth and lactose consumption. The gum yield on lactose was a constant value of 0.65 g/g during the entire fermentation, in agreement with the report of Flatt et al. (2). Approximately 15 g/L lactan were produced at an average

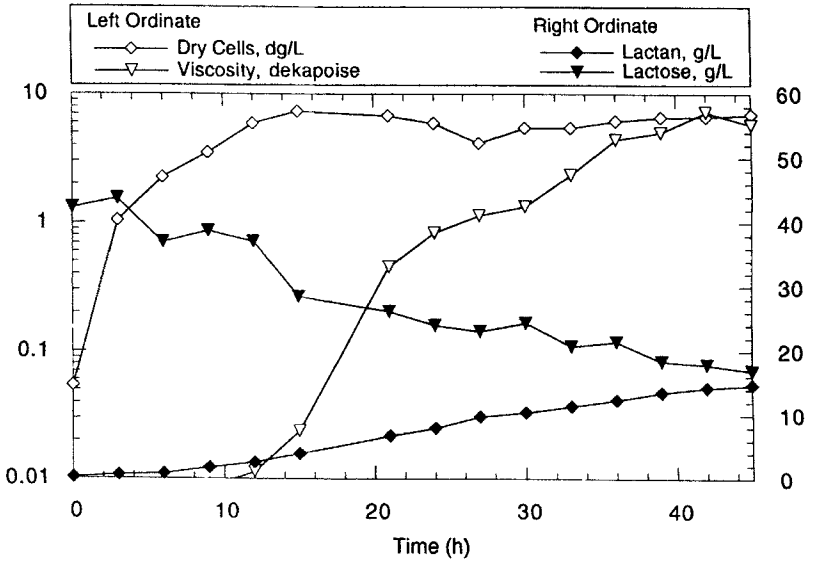

FIG. 1 The dry cell, lactan, and lactose concentrations as well as the viscosity of the broth given as a function of fermentation time.

volumetric productivity of 0.34 g/(L·h). Fermentation was terminated after 45 hours, at which point lactose utilization was 60%.

Effect of Organic Solvent Type and Concentration

No precipitation was visible at OS concentrations below 25% (Fig. 2). However, between 25 and 35% OS (depending on the OS), there was a solubility transition resulting in the formation of a precipitate. For OS concentrations up to 50%, the precipitates were soft, moist, and pliable, with a sponge-like texture, and no coloration. In this region it was difficult to completely separate the precipitate from the supernatant. The precipitates were heavy, weighing 5 to 35 times the initial mass of lactan, contained mostly volatile components (OS and water), and had a low lactan content.

At intermediate OS concentrations (50 to 80%, depending on the OS type), the precipitate mass ratio dropped to a broad minimum of 5 to 6. The precipitates at these OS concentrations were compact and dense, allowing complete separation of solid and liquid phases. At high OS con-

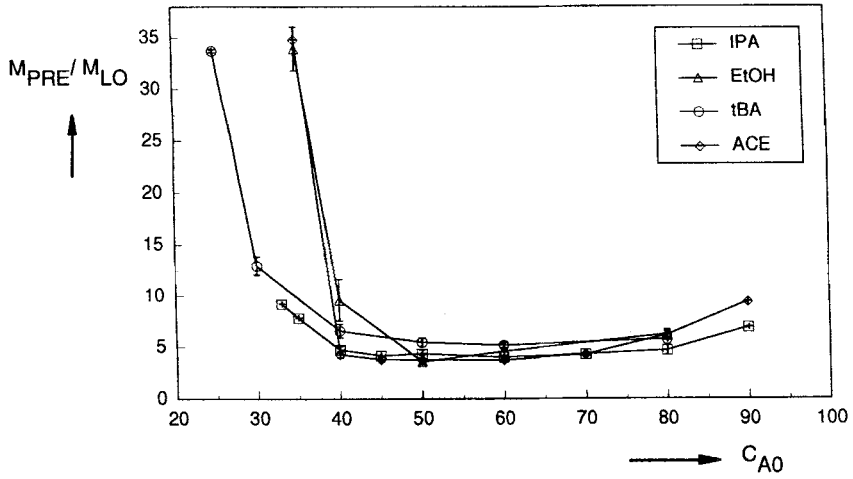

FIG. 2 The lactan precipitate mass ratio (M_{PRE}/M_{LO}) vs the weight percent ethanol (EtOH), acetone (ACE), isopropanol (IPA), or *tert*-butanol (tBA) in the bulk mixture (C_{A0}). To account for differences in the mass of lactan in the initial bulk mixtures, the precipitate mass (M_{PRE}) was normalized by dividing it by the initial mass of lactan gum in the bulk mixture (M_{LO}). Concentrations of organic solvents are given as weight percent (impurities-free basis) in the initial bulk mixtures. Error bars indicate a 95% confidence interval.

centrations (>80%), the precipitate mass ratio increased slightly, and the precipitates were dense and compact in consistency.

Binodal Solubility Curves

Figures 3, 4, 5, and 6 contain the results of the experiments in which lactan gum was recovered from fermentation broths with EtOH, ACE, IPA, and tBA, respectively. The weight percent of lactan in the precipitate, bulk solution, and supernatant phases are plotted against the weight percent OS in the corresponding phases. In each figure the initial bulk–mixture compositions are connected by tie lines to the corresponding equilibrium precipitate and supernatant compositions. Curves are drawn to indicate the border between the two-phase region and the region of total miscibility. Any bulk–mixture composition which falls underneath the binodal solubility curve should separate into two phases with compositions given by the tie line which passes through the bulk–mixture composition point. The position of the bulk–mixture composition point on the tie line determines the relative amounts of the supernatant and precipitate

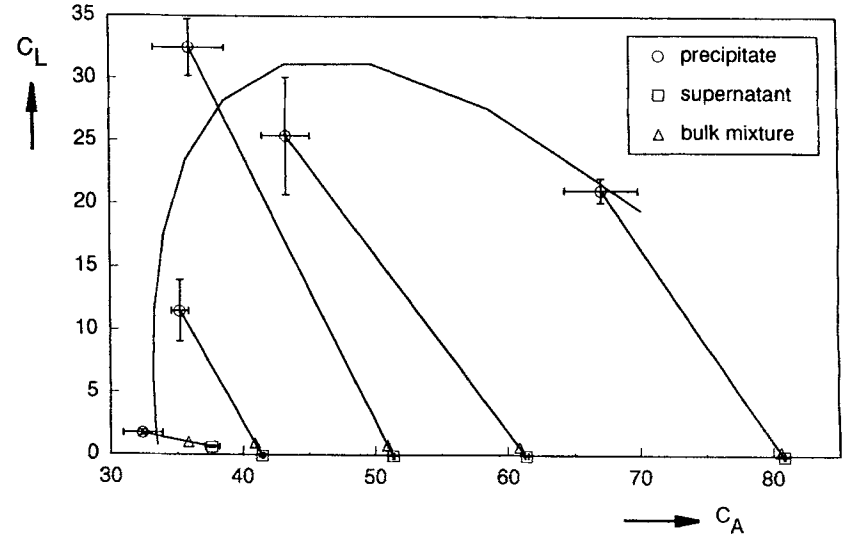

FIG. 3 The weight percent lactan (C_L) vs the weight percent ethanol (C_A). Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points.

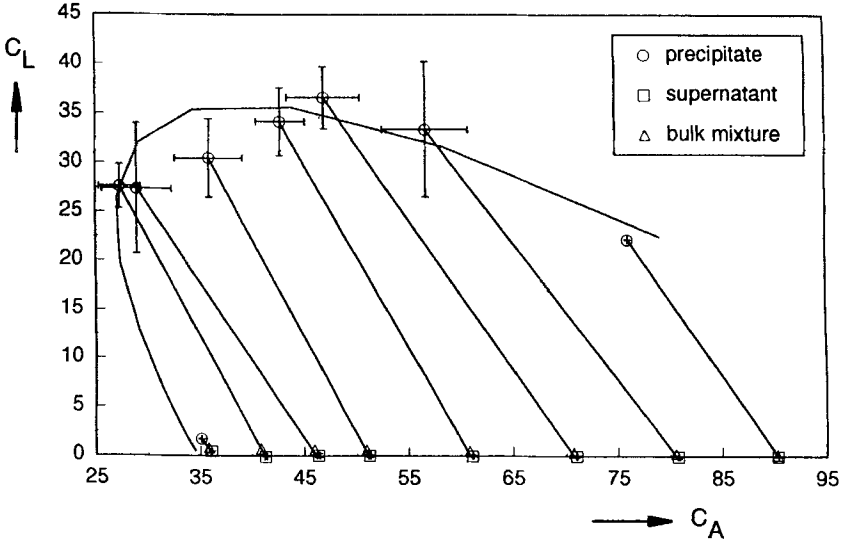

FIG. 4 The weight percent lactan (C_L) vs the weight percent acetone (C_A). Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points.

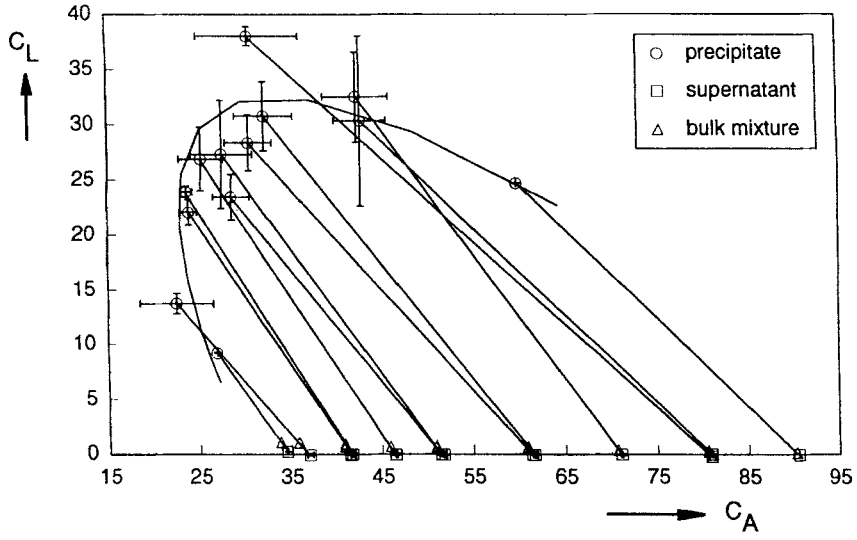

FIG. 5 The weight percent lactan (C_L) vs the weight percent isopropanol (C_A). Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points.

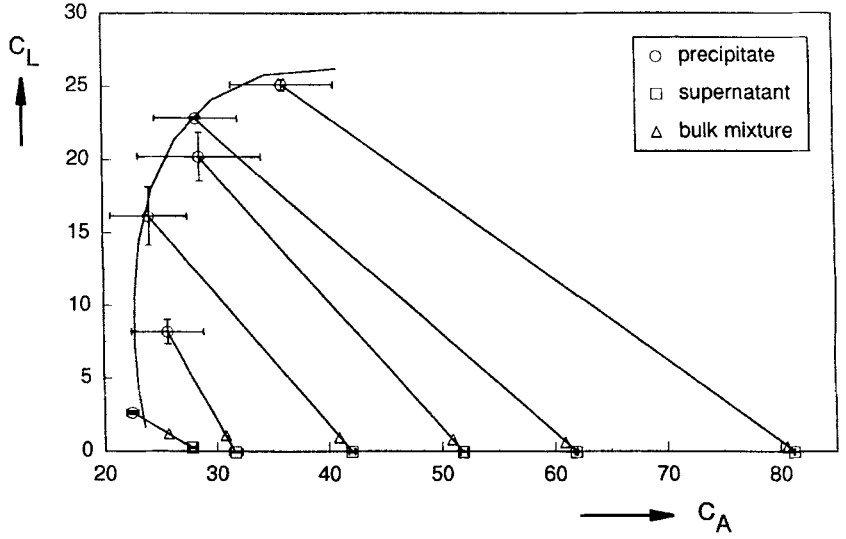

FIG. 6 The weight percent lactan (C_L) vs the weight percent *tert*-butanol (C_A). Error bars, where used, indicate a 95% confidence interval. Open circles without error bars are for single data points.

phases according to the lever rule. Except in the solubility transition, the concentration of lactan in the supernatant was essentially zero, and all of the lactan was recovered in the precipitate.

In the EtOH recovery experiments (Fig. 3), the maximum lactan gum concentration in the precipitate was 32% when the initial bulk mixture contained 51% OS. For the ACE recovery experiments (Fig. 4), the maximum concentration was 37% lactan at 71% OS. Using IPA (Fig. 5), the highest lactan concentration in the precipitate was 33% at 71% OS in the initial bulk mixture. For the tBA recovery experiments (Fig. 6), the maximum lactan concentration in the precipitate was 25% at 81% OS.

DISCUSSION

Lactan gum is a large and highly charged polyanionic polysaccharide (1). Addition of OS to the aqueous lactan gum solutions lowers the solubility sufficiently to cause aggregation and precipitation, probably by decreasing attractive interactions between the solvent and hydrophilic moieties on the polymer, and by increasing the affinity between cations in solution and the anionic polymer (7). Binding of cations to the anionic polymer conceivably causes charge neutralization and shielding of repulsive polyanion–polyanion electrostatic interactions, promoting precipitation of the polymer. Furthermore, addition of OS may decrease the extent of solvation of the polymer by water by forming OS–water hydrogen bonds at the expense of polymer–water hydrogen bonds. Eventually, attractive polymer–polymer interactions are greater than polymer–solvent interactions, and the polymer coil shrinks and expels entrapped solvent.

In general, the dielectric constant (DC) represents the effectiveness of a solvent in decreasing the force between charges. Lowering the mixture DC decreases electrostatic repulsion between anionic charges on the polymer, leaving an attractive force between the polymer chains due to van der Waals–London forces (7).

For lactan gum, insolubility occurred at different concentrations (25 to 35%, depending on the OS type), but insolubility occurred in a narrow DC range of 58 ± 2 (Table 1). For example, as EtOH was added to aqueous lactan gum solutions, insolubility occurred as the DC fell below a critical value of 60. For comparison, Gonzales et al. (10) found that as EtOH was added to xanthan gum solutions, insolubility occurred when the DC fell below 63. In agreement, Flahive (11) reported that xanthan insolubility occurred at a DC of 61 for EtOH. For lactan and xanthan gum, the most nonpolar alcohol (tBA) was the most efficient at lowering the bulk–mixture DC and had the lowest concentration at the phase transition point; EtOH was the least efficient, and was required in the highest concentration

TABLE I
The Minimum Organic Solvent Concentrations Causing Insolubility of Lactan Gum and Corresponding Dielectric Constants. Values of the DC Were Estimated Using Equations from Åkerlöf (12)

Water-miscible organic solvent[a]	Concentration for insolubility[b]	Dielectric constant
EtOH	35	60
ACE	35	57
IPA	33	56
tBA	25	60

[a] Abbreviations: EtOH = ethanol, ACE = acetone, IPA = isopropanol, tBA = *tert*-butanol.
[b] Weight percent in the initial bulk mixture.

for precipitation. The DC may be a useful tool in searching for new, more efficacious OS for precipitation.

Tie lines for phase separation of lactan gum tended to slope backwards, that is, the OS concentration was always lower in the precipitate than in the supernatant. Gonzales et al. (10) and Flahive et al. (8) reported similar behavior for xanthan gum. The preferential migration of the OS to the supernatant has an important practical consequence because it is more easily recovered in this phase by distillation.

The tie lines can be used to extend the specific results plotted in Figs. 3, 4, 5, and 6 to other bulk–mixture compositions. According to the lever rule, the ratio of the lengths of the line segments is equal to the ratio of the masses of the precipitate and supernatant. The compositions of these phases are given by the tie line, which must pass through the bulk–mixture composition point. By increasing the feed lactan concentration, the precipitate should maintain the same composition, but much less OS should be required for precipitation. Methods to increase the gum concentration in the fermentation broth prior to precipitation using OS include hollow fiber ultrafiltration, as reported by Lo et al. (13).

CONCLUSIONS

The focus of much of the research on microbial polysaccharides since the mid-1960s has been on the fermentation process. However, overall process costs are greatly influenced not only by the fermentation process but also by the OS precipitation recovery step. In this research, binodal solubility curves were reported for the microbial polysaccharide lactan

gum. These curves define the two phase envelope, and specify the composition of the supernatant and precipitate phases for any initial bulk–mixture composition. These curves are useful in the design and operation of efficient recovery processes.

Recovery efficiency for lactan gum could be improved significantly by simply changing the type of OS used. The balance between forming a precipitate high in lactan concentration while using a small amount of OS was best for IPA and ACE, and worst for EtOH and tBA. In all cases the OS migrated preferentially to the supernatant phase, where it is more easily recovered by distillation. The phase behavior and phase separation of lactan gum, like that for xanthan gum, could be correlated well by the DC of the solution.

ACKNOWLEDGMENT

This work was funded in part by a grant from the State of Wisconsin Industrial and Economic Development Research Fund.

NOMENCLATURE

C_A	weight percent of nonsolvent
C_{A0}	weight percent of nonsolvent in bulk mixture
C_L	weight percent of lactan
M_{PRE}	mass of wet lactan precipitate (g)
M_{L0}	initial mass of lactan in bulk mixture (g)

REFERENCES

1. J. H. Flatt, T. A. Cooper, J. M. Gonzalez, D. E. Dogger, E. N. Lightfoot, and D. C. Cameron, *Biotechnol. Prog.*, *8*, 327–334 (1992).
2. J. H. Flatt, T. A. Cooper, J. M. Gonzalez, D. E. Dogger, E. N. Lightfoot, and D. C. Cameron, *Ibid.*, *8*, 335–339 (1992).
3. J. F. Kennedy and I. J. Bradshaw, *Prog. Ind. Microbiol.*, *19*, 319–371 (1984).
4. A. Margaritis and G. W. Pace, in *Comprehensive Biotechnology* (M. Moo-Young, Ed.), Pergamon, Oxford, 1985, pp. 1005–1044.
5. R. Gonzales, M. R. Johns, P. F. Greenfield, and G. W. Pace, *Process Biochem.*, *24*, 200–203 (1989).
6. M. R. Johns and E. Noor, *Aust. J. Biotechnol.*, *5*, 73–77 (1991).
7. I. H. Smith and G. W. Pace, *J. Chem. Tech. Biotechnol.*, *32*, 119–129 (1982).
8. J. J. Flahive III, A. Foufopoulos, and M. R. Etzel, *Sep. Sci. Technol.*, *29*, 1673–1687 (1994).
9. A. Foufopoulos, "Alcohol Precipitation of Lactan Gum from Fermentation Broths," M.S. Thesis, University of Wisconsin, Madison, 1994.
10. R. Gonzales, M. R. Johns, P. F. Greenfield, and G. W. Pace, *Carbohydr. Polym.*, *13*, 317–333 (1990).

11. J. J. Flahive III, "Alcohol Precipitation of Xanthan Gum from Pure Solutions and Fermentation Broths," M.S. Thesis, University of Wisconsin, Madison, 1993.
12. G. Åkerlöf, *J. Am. Chem. Soc.*, *54*, 4125 (1932).
13. Y.-M. Lo, S.-T. Yang, and D. B. Min, "Recovery and Separation of Xanthan Gum from Fermentation Broth Using a Hollow Fiber Ultrafiltration Process," *Proc. Am. Inst. Chem. Eng. Conf. Food Eng.*, November 2–3, 1995, Chicago, IL.

Received by editor July 19, 1995