Separation Science and Technology

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

Solvent Screening for Production of Lactic Acid by Extractive Fermentation

Yanping Tong; Makoto Hirata; Hirokazu Takanashi; Tadashi Hano; Michiaki Matsumoto; Shigenobu Miura

To cite this Article Tong, Yanping , Hirata, Makoto , Takanashi, Hirokazu , Hano, Tadashi , Matsumoto, Michiaki and Miura, Shigenobu(1998) 'Solvent Screening for Production of Lactic Acid by Extractive Fermentation', Separation Science and Technology, 33: 10, 1439 — 1453

To link to this Article: DOI: 10.1080/01496399808545059

URL: http://dx.doi.org/10.1080/01496399808545059

SEPARATION SCIENCE AND TECHNOLOGY, 33(10), pp. 1439–1453, 1998

Solvent Screening for Production of Lactic Acid by Extractive Fermentation

YANPING TONG, MAKOTO HIRATA, HIROKAZU TAKANASHI,
and TADASHI HANO*
DEPARTMENT OF APPLIED CHEMISTRY
OITA UNIVERSITY
OITA 870-1192, JAPAN

MICHIAKI MATSUMOTO
DEPARTMENT OF CHEMICAL ENGINEERING AND MATERIALS SCIENCE
DOSHISHA UNIVERSITY
KYOTO 610-0321, JAPAN

SHIGENOBU MIURA
MUSASHINO CHEMICAL LABORATORY LTD.
TOKYO 168, JAPAN

ABSTRACT

The extraction of lactic acid from aqueous solution by a number of extractants dissolved in different organic diluents was explored over a wide range of pH values. Tri-*n*-octylmethylammonium chloride (TOMAC or QCl), dissolved in oleyl alcohol, butyl acetate, and a mixture of hexane and oleyl alcohol, was found to be most appropriate in terms of high extraction capacity and simplicity of operation. Biocompatibility between the extraction reagents and lactic-acid-producing microbes was also examined. The results indicated that TOMAC was highly toxic, and both butyl acetate and hexane exhibited some detrimental effects. However, oleyl alcohol exhibited no toxicity and could, therefore, be considered the most suitable diluent. During extractions with TOMAC concentrations no greater than 0.1 kmol·m^{-3}, implementation of a cleaning column filled with oleyl alcohol seemed to effectively remove trace amounts of TOMAC dissolved in the fermented broth; extractive fermentation

---

* To whom correspondence should be addressed. Telephone/FAX: +81-975-54-7900. E-mail: thano@cc.oita-u.ac.jp

of lactic acid was accomplished, despite a little decline in lactic acid production relative to production obtained for a control experiment. However, higher TOMAC concentrations are desired for better extraction of accumulated lactic acid to reduce its own inhibitory effect. A cleaning column packed with the cation-exchange resin Amberlite IR-120B was more effective. As a result, satisfactory extractive fermentation was achieved, thereby proving the feasibility of efficiently producing lactic acid by extractive fermentation, with TOMAC dissolved in oleyl alcohol as the optimum extraction system.

Key Words. Lactic acid; Quaternary ammonium salt; Solvent extraction; Extractive fermentation; Biocompatibility

INTRODUCTION

Solvent extraction, accompanied by a reaction, has been recognized as a promising alternative to conventional calcium-salt precipitation and to nonreactive extraction processes for the separation and purification of fermented organic acids (1–3). The fermentation process involving in situ extraction, or extractive fermentation, has been receiving growing attention because it is capable of relieving end-product inhibition and bringing about high productivity; in situ recovery of fermented products is also attractive economically as a primary purification method (4–7). The extraction process can be carried out inside the fermentor or in an external extraction unit.

In a pioneering study of extractive fermentation, Finn attempted to reduce end-product inhibition of prodigiosin by extraction into kerosene, to no conclusive effect (8). Some success was achieved in extractive fermentations for such alcohols as ethanol and butanol (9–13).

Bar and Gainer attempted to develop a process for the production of lactic, citric, and acetic acids, but were hampered by poor extractions and highly toxic extraction solvents (14). Yabannavar and Wang managed to apply extractive fermentation to the production of lactic acid at pH 4.2, using 15% Alamine 336 dissolved in oleyl alcohol. To overcome toxicity of Alamine 336, they immobilized the lactic acid-producing bacteria *Lactobacillus delbrueckii* in *k*-carrageenan, and later added soybean oil to the *k*-carrageenan matrix and achieved higher productivity relative to a control run. However, since the immobilized cells were to be brought into direct contact with the extractant, relatively low extractant concentrations were necessary (4). Ye et al. implemented multiple extraction stages in the production of lactic acid with 40% Alamine 336 dissolved in oleyl alcohol. They used a hollow fiber module to remove cells from the fermented broth prior to extraction in order to keep cells from directly contacting the extractant (15). It is noteworthy that in the latter two cases the process had to be operated at pH 4.2, a compromise value between the optimum extraction pH of Alamine 336 in oleyl alcohol (ca. pH 3.0), and the optimum fermentation pH of 5.0–6.0.

A more efficient extractive fermentation process for organic acids should be able to exploit both optimum pH values for the extraction and fermentation processes (16, 17). Furthermore, many properties are required of potential extraction reagents; for example, water-immiscibility, simplicity of operation, and no or removable toxicity toward microbes. So far, the absence of an adequate extraction system continues to be a major hurdle in the development of efficient extractive fermentation processes for organic acids.

In this study the extraction behavior of lactic acid from aqueous solution was investigated at various pH values and by using different extractants and organic diluents, with the aim of harmonizing extraction pH with fermentation pH. Lactic acid was selected because it is a typical fermentation-derived organic acid. It is widely used in food processing and chemical industries. Moreover, it is a feedstock for the production of biodegradable polymers and environmentally "green" solvents, and its market is expected to expand as a result of increasing concern about the environment (18–20).

MATERIALS AND METHODS

Chemicals, Strain and Culture Conditions

The extractants used were tri-*n*-octylmethylammonium chloride (TOMAC) whose purity was 80.3%, dioctylamine (DOA), tri-*n*-octylamine (TOA), tri-*n*-octylphosphineoxide (TOPO), and tri-*n*-butylphosphate (TBP). The organic diluents used were oleyl alcohol (OleylOH), hexane, and butyl acetate (BuAc). All chemicals except TOMAC were of AR grade and were used as received except lactic acid which was heat-treated prior to extraction; it usually self-esterifies or dimerizes in aqueous solution concentrations over 20 wt%. The aqueous phase was prepared by dissolving the monomerized lactic acid in deionized water, and the pH was adjusted using sulfuric acid or aqueous ammonia solution. The organic phase was prepared by dissolving the extractants into the organic diluents.

Lactobacillus rhamnosus IFO3863 was used to produce L(+)-lactic acid from glucose. The composition (in $kg{\cdot}m^{-3}$) of the medium was as follows: glucose, 100 and 225; KH_2PO_4, 0.5; NaCl, 0.1; $MgSO_4{\cdot}7H_2O$, 2.0; yeast extract, 10.

Experimental Methods

Some extractants, like TOMAC, have been reported to be highly toxic to lactic acid-producing bacteria. Initially, therefore, a bioassay was used to test all extraction reagents for relative toxicity toward *L. rhamnosus*. The toxicity assays were carried out by placing 1.0×10^{-6} m^3 of a sample of extraction reagent on the nutrient agar gel inoculated with lactic acid bacteria in a test tube of 1.0×10^{-2} m diameter. After incubation for 48 hours at 37°C, the toxicity was evaluated by measuring the length of clear zone, i.e., the part of agar gel in which bacterial colonies could not be observed (21). Generally, the length of clear zone corresponds to the toxicity of the reagent against the microbes, i.e., the longer the length, the higher the toxicity. The maximum concentration of TOMAC guaranteeing appreciable growth of microbes (or *minimum inhibitory concentration*) was determined by measuring the OD_{660} with a spectrophotometer (Jasco Ubest-35, Japan Spectroscopic Co. Ltd., Japan) following fermentations in culture media containing different concentrations of TOMAC.

Removal of trace concentrations of toxic substances was also attempted by passing the extracted broth through a column filled with organic solvent, or packed with ion-exchange resin, before recycling back to the fermentor.

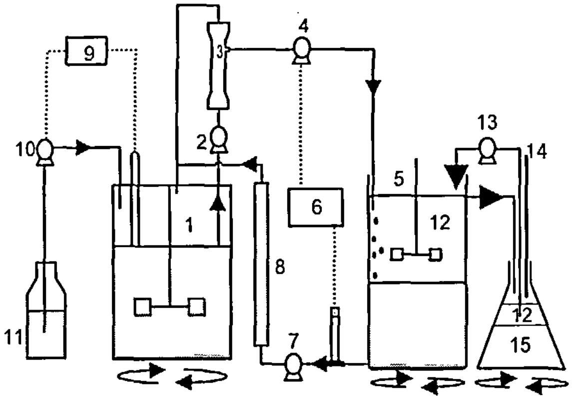
Equilibrium investigations were then carried out by varying the concentrations of the composition of both the aqueous and the organic phases. An aqueous solution of known concentration was well mixed with an equal volume of organic phase in a glass vial at 25°C for 24 hours. Then, as equilibrium was attained, the lower aqueous phase was carefully pipetted out and analyzed for residual lactic acid concentration. The extraction equilibrium constant could be determined when sufficient data had been acquired.

Fermentation was carried out at 42°C in a standard stirred-jar fermentor (KMJ-2B, Mitsuwa Rikagaku Co. Ltd., Japan) with a working volume of 1×10^{-3} m^3 and stirred at 110 rpm. During fermentation, pH was maintained at 6.0 by the automatic addition of 25 vol% aqueous ammonia solution. To ensure anaerobic conditions, a small amount of nitrogen was sparged into the fermentor. The complete extractive fermentation system is schematically illustrated in Fig. 1. The broth was filtered by a hollow fiber module (Cultureflo L, L2042L, Asahi Medical Co. Ltd., Japan, see Table 1 for the specifications of the hydrophilic polyolefin hollow fiber membrane) which was washed in ethanol and then in hot sterile water immediately before fermentation was initiated.

After the fermentation had proceeded for a while, some broth was withdrawn from the fermentor by a pump at a fixed rate and passed through the

TABLE 1
Specifications of the Microporous Hollow Fiber Membrane

ID (μm)	OD (μm)	Thickness (μm)	Pore size (μm)	Porosity (—)
350	430	40	0.5	0.7



1. Fermentor 2. Circulation pump for fermented broth 3. Hollow fiber module 4. Circulation pump for filtrated broth 5. Extractor 6, 14. Level controllers 7. Circulation pump for extracted broth 8. Cleaning column 9. pH controller 10. Feed pump for ammonia solution 11. 10wt% ammonia solution 12. Organic solvent 13. Circulation pump for organic solvent 15. Stripping reagent

FIG. 1 Schematic diagram of extractive fermentation system.

hollow fiber module. The cells separated from broth were recycled to the fermentor, and the cell-free filtrate was pumped into the extractor. The extracted broth, after passing through the cleaning column, or by-passing it in the case of control fermentation, was recycled to the fermentor. A level controller regulated the flow of fermented broth into the extractor. Concurrently, the organic phase was circulated between the extraction unit and the stripping unit in which lactic acid was recovered with 4 kmol·m^{-3} sodium hydroxide solution. The liquid level in the stripping unit was controlled by using a glass tube.

Analytical Method

HPLC (LC-6A, Shimadzu, Co. Ltd., Japan) equipped with a refractometric detector (Jasco 830-RI, Japan) and a UV detector (SPD-6A, Shimazu Co. Ltd., Japan, wavelength set at 210 nm) was used to measure glucose and/or lactic acid concentrations, the former for samples taken from fermented broth, and the latter for samples of the raffinate after extraction and from the stripping phase, respectively. A TSK-Gel column (ODS-80Ts, Tosoh. Co. Ltd., Japan) was utilized with the mobile phase of aqueous ammonium dihydrogen phosphate solution (2.5 mol·m^{-3}, adjusted to pH 2.0 with orthophosphoric acid) flowing at a rate of 1.0 × 10^{-6} m^{3}·min^{-1}.

RESULTS AND DISCUSSION

Effect of pH on Extraction of Lactic Acid

To enable smooth implementation of extractive fermentation, it is necessary to find an extraction system whose optimum pH corresponds to the fermentation pH. Therefore, initial efforts were directed at elucidating the pH effect on lactic acid extraction by a variety of extractants and diluents.

Before using the extractants, extraction of lactic acid (LA) with oleyl alcohol and butyl acetate was attempted. Neither of the two diluents gave good extraction, which could be attributable to the fact that they could only extract lactic acid by interacting with it through hydrogen bonding and other weak electrostatic forces.

Reactive Extraction with Extractants Dissolved in Oleyl Alcohol

Figure 2 illustrates the effect of pH on extraction with TOMAC, DOA, TOA, TOPO, and TBP diluted in oleyl alcohol. Apparently the degrees of extraction with TOPO and TBP were very low around the optimum fermentation pH 6.0; the fact that extraction improved with falling pH shows that they are solvation extractants (22). As for TOA, its extraction power was considerably high at about pH 4.5 but fell before pH 6.0. DOA exhibited its highest extraction power around pH 6.0, followed by TOMAC. Here, the high extraction capacity of TOMAC at high pH value is consistent with the fact that it is an anion-exchange extractant. Therefore, with oleyl alcohol as the diluent, DOA and TOMAC proved to be appropriate extractant candidates.

Reactive Extraction with Extractants Dissolved in Hexane with and without Phase Modifier

Figure 3 presents the results of extraction with nonpolar hexane as a diluent. It is clear that each extractant showed poor extraction at pH 6.0, indicating that hexane does not qualify in this instance as a suitable diluent. This result corresponds to other findings that nonpolar diluents are less favorable than polar diluents, as shown in previous studies on the extraction of citric (3) and lactic acids (23). On the other hand, TOPO appeared to extract very well in the vicinity of pH 2.0, suggesting that the combination of TOPO with hexane would be suitable for extractions in fermentations of low optimum pH.

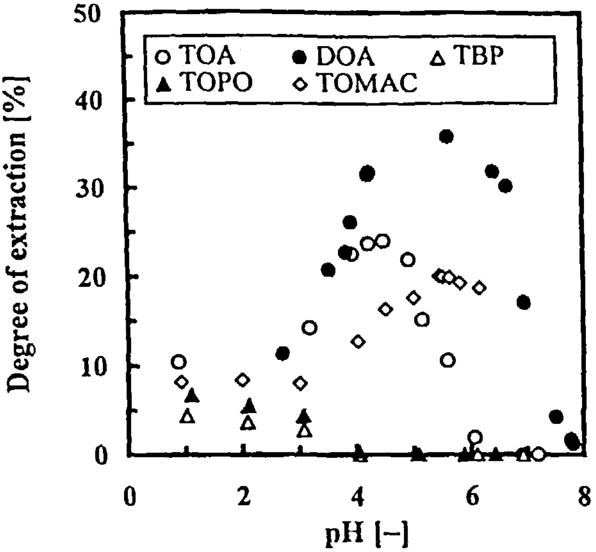


FIG. 2 Effect of pH on extraction with extractants dissolved in oleyl alcohol ($C^0_{\mathrm{org,ext}}$ = 0.3 kmol·m^{-3}; $C^0_{\mathrm{aq,LA}}$ = 0.3 kmol·m^{-3}).

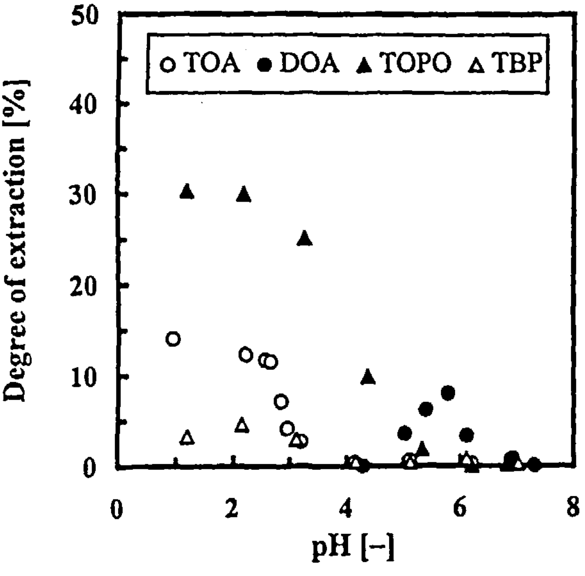


FIG. 3 Effect of pH on extraction with extractants dissolved in hexane ($C^0_{\mathrm{org,ext}}$ = 0.3 kmol·m^{-3}; $C^0_{\mathrm{aq,LA}}$ = 0.3 kmol·m^{-3}).

Extractions with TOMAC in hexane were not performed since the solubility of TOMAC in hexane is low. The weak extractions of DOA can also be attributed to its low solubility in hexane. However, low solubilities could be overcome by adding a small amount of oleyl alcohol as a modifier to hexane. Extraction of lactic acid with TOMAC in a mixture consisting of 92.5 vol% hexane and 7.5 vol% oleyl alcohol was attempted by varying concentrations of the organic phase at pH 6.0. The results are presented in Fig. 4; they are interesting because the degree of extraction is comparable to that for TOMAC in oleyl alcohol or in butyl acetate.

Reactive Extraction with Extractants Dissolved in Butyl Acetate

In order to further explore the influence of the diluent's polarity on extraction, butyl acetate was employed for its strong polarity. As shown in Fig. 5, the results followed patterns similar to those for oleyl alcohol, i.e., DOA and TOMAC achieved the best extractions around pH 6.0, at degrees comparable to those for oleyl alcohol. Hence, polar diluents can also give satisfactory extractions.

A significant feature is that the degree of extraction with TOMAC was insensitive to pH until around pH 2.0. This implies that the extraction mechanism at low pH differs from that in a relatively high pH range. A further point is that, unlike TOMAC, the pH after extraction with DOA could rise as high as 3 because DOA is a fairly strong basic extractant. A change of such magnitude is likely to result in poor extractions if the final pH value should exceed 7.0.

Thus far, it can be concluded that TOMAC dissolved in oleyl alcohol or butyl acetate, or a mixture of hexane and alcohol, are suitable for extractive fermentation.

Toxicity of Extraction Reagents toward Microbes and Its Countermeasures

Toxicity of extraction reagents to microbes was investigated and evaluated as described above. However, it is noteworthy that the length of the clear zone functions only as a rough measure of toxicity since it is actually an overall outcome of the intrinsic toxicity, solubility, and diffusivity of the reagent. From the results presented in Fig. 6, TOMAC and DOA appear to be the most toxic. Butyl acetate and hexane exhibit a degree of toxicity. Oleyl alcohol does not, and this may be partially accounted for by its low solubility in the fermented broth and simply by its lower toxicity as a compound. Consequently, oleyl alcohol was chosen as the most suitable diluent.

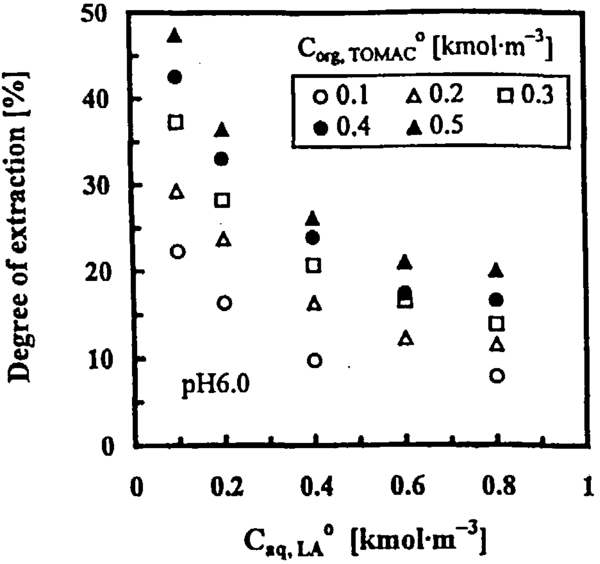


FIG. 4 Degree of extraction as a function of lactate and TOMAC concentrations (with a mixture of hexane and oleyl alcohol as diluent).

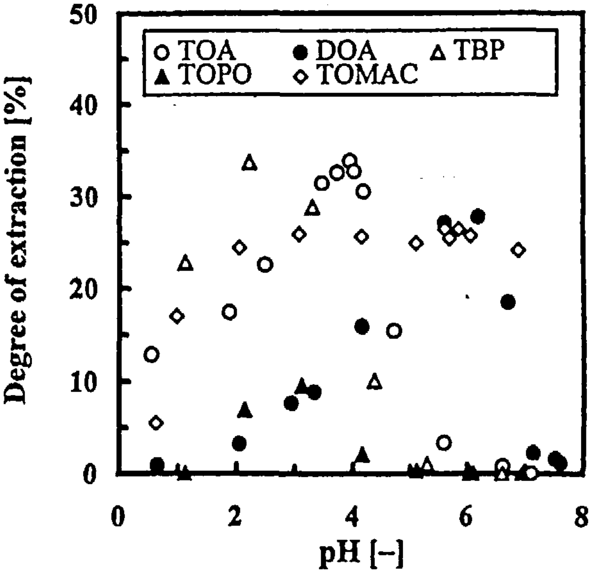


FIG. 5 Effect of pH on extraction with extractants dissolved in butyl acetate ($C_{org,ext}^0$ = 0.3 kmol·m^{-3}; $C_{aq,LA}^0$ = 0.3 kmol·m^{-3}).

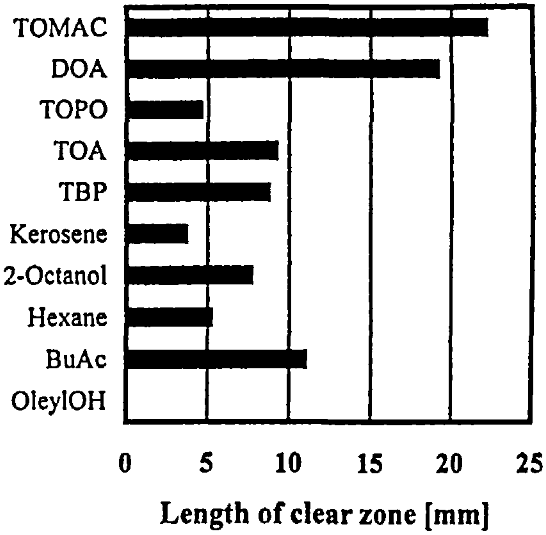


FIG. 6 Toxicity of extraction reagents toward *Lactobacillus rhamnosus*.

Since oleyl alcohol is biocompatible with the lactic acid-producing bacteria, and solubility of TOMAC in it is high, it was investigated as a rinsing solvent for fermentation broths, following extraction and prior to recycling into the fermentor, to relieve the toxicity of TOMAC. The results presented in Fig. 7, were evidently quite successful relative to those without implementing the cleaning column. Production was a little bit lower than that in control fermentation without extraction, and can be ascribed to the toxicity of traces of TOMAC remaining in the fermented broth. However, simultaneous recovery of lactic acid was achieved in extractive fermentation, which is its advantage over fermentation without extraction. It is noteworthy that this column had a limited effect when the concentrations of TOMAC employed were greater than 0.2 kmol·m^{-3}. Higher concentrations of TOMAC extracted more lactic acid and better relieved product inhibition. The concentration limit of TOMAC for appreciable growth of microbes was found to be 1×10^{-3} mol·m^{-3} from separate experiments specifically on the toxicity of TOMAC, as shown in Table 2. This limit is obviously beyond the cleaning capability of oleyl alcohol based on simple distribution. Experiments with a cleaning column packed with the cation-exchange resin Amberlite IR-120B were successful, as shown in Fig. 8. Lactic acid was produced smoothly and continuously with 0.2 kmol·m^{-3} TOMAC. Simultaneous removal of nutrients in the

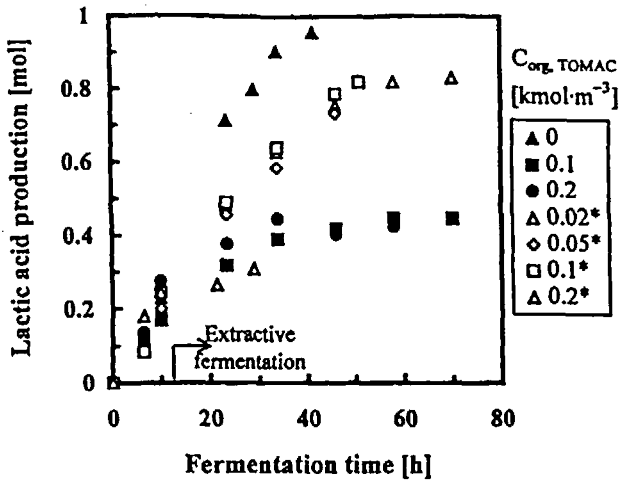


FIG. 7 Comparison of extractive fermentations with oleyl alcohol treatment (marked with an asterisk) against those without.

medium was assumed to be negligible since no decline in growth rate was observed.

The high viscosity of TOMAC in oleyl alcohol hinders the achievement of fast extraction rates. TOMAC can dissolve in a mixture of 92.5 vol% hexane and 7.5 vol% oleyl alcohol, as mentioned earlier. Since hexane is of low viscosity and volatile, an extraction system of TOMAC in hexane with oleyl alcohol as a phase modifier may, in this case, be a better candidate, although hexane is rather toxic. Therefore, further work needs to be done to optimize the system.

TABLE 2

Toxicity of TOMAC on *Lactobacillus rhamnosus*

Concentration (kmol·m^{-3})	Growth[a]
0	+ + +
2×10^{-7}	+ + +
6×10^{-7}	+ + +
1×10^{-6}	+ +
2×10^{-6}	+
6×10^{-6}	−

[a] + + + = average growth; + + = less satisfactory growth; + = slight growth; − = no growth.

Extraction Equilibrium of Lactic Acid with TOMAC/Oleyl Alcohol

Equilibrium studies were carried out at 25°C by varying the concentrations of both the organic and aqueous phases; the pH of the latter was adjusted to 6.0 with aqueous ammonia solution. In this case, lactic acid can be assumed to completely disassociate into L^-. If it is assumed the extraction of lactic acid with TOMAC proceeds in the following manner:

$$L^-_{(aq)} + Q^+Cl^-_{(org)} \rightleftarrows Q^+L^-_{(org)} + Cl^-_{(aq)} \qquad (1)$$

then the extraction equilibrium constant can be given as

$$K_{ex} = C^*_{org,QL} \cdot C^*_{aq,Cl^-} / C^*_{aq,L^-} \cdot C^*_{org,QCl} \qquad (2)$$

and thus

$$\log C^*_{org,QL}/C^*_{aq,L^-} = \log C^*_{org,QCl}/C^*_{aq,Cl^-} + \log K_{ex} \qquad (3)$$

Based on Eq. (3), the experimentally measured equilibrium data were plotted as Fig. 9; a straight line of unit slope was obtained. This result provides evidence for the above assumption about the stoichiometry of lactic acid extraction with TOMAC. From the intercept, the equilibrium constant of extraction K_{ex} at 25°C for TOMAC/oleyl alcohol was determined to be 0.073.

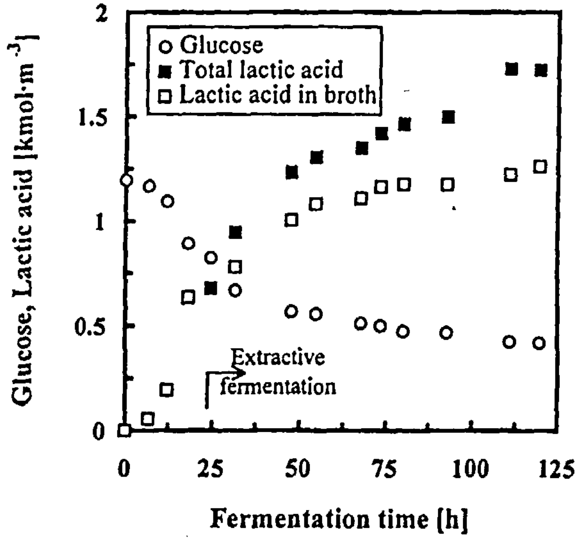


FIG. 8 Extractive fermentation with cation-exchange resin treatment ($C_{org,TOMAC}$ = 0.2 kmol·m^{-3}).

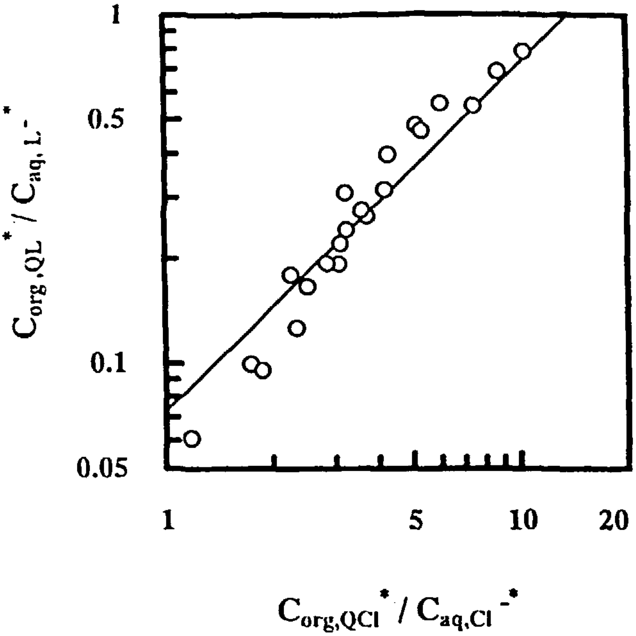


FIG. 9 Double logarithmic plot to obtain extraction equilibrium constant.

CONCLUSIONS

The nonreactive extraction of lactic acid with pure organic solvents was extremely poor. The effect of pH on extraction with assorted extractants was elucidated, indicating that TOMAC in oleyl alcohol, butyl acetate, or a mixture of the two diluents was most appropriate because of high extraction capacity and simplicity of operation.

However, TOMAC was found to be highly toxic to lactic acid-producing bacteria and butyl acetate was also fairly toxic, whereas oleyl alcohol was not. Implementation of a cleaning column filled with oleyl alcohol was effective in removing some trace amount of TOMAC entrained in the fermented broth during extraction with as low as 0.1 kmol·m^{-3} concentration of TOMAC. Extractive fermentation of lactic acid was accomplished in spite of a small decline in production in comparison with the control run. At higher TOMAC concentrations, a cleaning column packed with the cation-exchange resin Amberlite IR-120B was more adequate, giving a satisfactory extractive fermentation of lactic acid. Consequently, TOMAC dissolved in oleyl alcohol was considered to satisfactorily meet the criteria for lactic acid extractive fermentation. The equilibrium constant of extraction with TOMAC in oleyl alcohol was determined to be 0.073 at 25°C. Recovery of lactic acid from the organic phase was found to be possible with aqueous solutions of such inorganic salts as sodium chloride and will be reported in a later paper.

NOMENCLATURE

C	concentration ($\mathrm{kmol \cdot m^{-3}}$)
K_{ex}	equilibrium constant of extraction (—)
L^-	lactate anion
QCl	tri-octylmethylammonium chloride (TOMAC)
QL	lactate–TOMAC complex

Superscripts

0	initial
*	in equilibrium

Subscripts

aq	aqueous phase
ext	extractant
LA	lactic acid
org	organic phase

ACKNOWLEDGMENTS

The authors wish to thank Kouei Industry Co. Ltd. and Asahi Medical Co. Ltd. for kindly providing us with TOMAC and hollow fiber modules, respectively. Less would have been accomplished but for the contributions of Ms. M. Hironaka, Mr. S. Tsutsui, and Mr. C. Higuchi to the experimental portion of this work.

REFERENCES

1. A. S. Kertest and C. J. King, *Biotechnol. Bioeng.*, *28*, 269–282 (1986).
2. C. J. King, *Chemtech*, *22*, 285–291 (1992).
3. R. Wennersten, *Chem. Tech. Biotechnol.*, *33B*, 85–94 (1983).
4. V. M. Yabannavar and D. I. C. Wang, *Biotechnol. Bioeng.*, *37*, 1095–1100 (1991).
5. S. R. Roffler, Ph.D. Thesis, University of California, Berkeley, 1986.
6. H. Honda, Y. Toyama, H. Takahashi, T. Nakazeko, and T. Kobayashi, *J. Ferment. Bioeng.*, *79*(6), 589–593 (1995).
7. J. S. Seevaratnam, O. Holst, S. Hjorleifsdottir, and B. Mattison, *Bioprocess Eng.*, *6*, 35–41 (1991).

8. R. K. Fin, *J. Ferment. Technol.*, *44*, 305–310 (1966).
9. M. Minier and G. Goma, *Biotechnol. Bioeng.*, *24*, 1565–1579 (1982).
10. M. Minier and G. Goma, *Biotechnol. Lett.*, *3*, 405–408 (1981).
11. S. R. Roffler, H. W. Blanch, and C. R. Wilke, *Bioprocess Eng.*, *2*, 187–190 (1987).
12. S. R. Roffler, H. W. Blanch, and C. R. Wilke, *Ibid.*, *2*, 1–12 (1987).
13. D. W. Tedder, School of Chemical Engineering, Georgia Institute of Technology, Personal Communication, 1985.
14. R. Bar and J. L. Gainer, *Biotechnol. Prog.*, *3*(2), 109–114 (1987).
15. K.-M. Ye, S. Jin, and K. Shimizu, *J. Ferment. Bioeng.*, *81*(3), 240–246 (1996).
16. T. Hano, M. Matsumoto, T. Ohtake, and S. Miura, *Solvent Extraction in the Process Industries-2*, Elsevier Applied Science, London, 1993, pp. 1025–1031.
17. T. Hano, M. Matsumoto, M. Hirata, and S. Miura, *Value Adding through Solvent Extraction, Vol. 2*, University of Melbourne, Melbourne, 1996, pp. 1447–1452.
18. T. Hano, M. Matsumoto, S. Uenoyama, T. Ohtake, Y. Kawano, and S. Miura, *Bioseparation*, *3*, 321–326 (1993).
19. C. R. Soccol, B. Marin, Raimbault, M. Raimbault, and J. M.Lebeault, *Appl. Microbiol. Biotechnol.*, *41*, 286–290 (1994).
20. R. Datta, S.-P. Tsai, P. Bonsignore, S.-H. Moon, and J. R. Frank, *FEMS Microbiol. Rev.*, *16*, 221–231 (1995).
21. A. H. Linton, *Antibiotics: Assessment of Antimicrobial Activity and Resistance*, Academic Press, New York, NY, 1983, p. 19.
22. T. Hano, M. Matsumoto, T. Ohtake, K. Sasaki, F. Hori, and Y. Kawano, *J. Chem. Eng. Jpn.*, *23*(6), 734–738 (1990).
23. W. P. Ratchford, E. H. Harris, C. H. Fisher, and C. D. Willits, *Ind. Eng. Chem.*, *43*, 778–781 (1951).

Received by editor August 5, 1997
Revision received October 1997