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

Extraction Processes for Bioproduct Separation

J. Hartl[a]; R. Marr[a]
[a] Institut für Thermische Verfahrenstechnik und Umwelttechnik Technische Universität Graz Inffeldgasse, Graz, Austria

To cite this Article Hartl, J. and Marr, R.(1993) 'Extraction Processes for Bioproduct Separation', Separation Science and Technology, 28: 1, 805 — 819

To link to this Article: DOI: 10.1080/01496399308019522

URL: http://dx.doi.org/10.1080/01496399308019522

SEPARATION SCIENCE AND TECHNOLOGY, 28(1-3), pp. 805-819 (1993)

EXTRACTION PROCESSES FOR BIOPRODUCT SEPARATION

J. Hartl and R. Marr
Institut für Thermische Verfahrenstechnik und Umwelttechnik
Technische Universität Graz
Inffeldgasse 25
A-8010 Graz, Austria

ABSTRACT

The three-phase extraction process, a modification of reactive extraction, has been investigated for its applicability in the separation of organic acids from fermentation broth. It has been compared with reactive extraction, liquid membrane permeation, and supercritical fluid extraction.

These processes are based on the use of amine extractants, which have to be dissolved in nonpolar solvents, for the extraction of carboxylic acids, hydroxycarboxylic acids, and aminocarboxylic acids.

This paper considers the comparison of the above-mentioned processes. Furthermore, the extractability of acids from synthetic aqueous solutions and fermented broths has been compared. Principal consideration has been paid to the extraction of lactic acid, gluconic acid, citric acid, and L-leucine.

INTRODUCTION

The growing importance of biological production, expressed with new routes and increasing production rates, asks for adapted downstream processing for product separation. Extraction processes are favored, on account of the product selectivity and the high enrichment, which can be gained by optimizing process design. Extraction processes do not affect the thermal stability of bioproducts, and they require a low energy demand.

Reactive extraction has also proven its applicability in the separation of fermented products. The reactive substance, which is added to the organic solvent, enables the phase transfer of the polar acid from the aqueous phase to the nonpolar organic phase.

Compared with physical liquid-liquid extraction, the selectivity of separation is enhanced immensely, as amine-based extractants favorably separate acids. The by-products, impurities, and residues of the substrate shall be left in the spent fermentation broth.

The review on acid extraction, published by Kertes and King (1), distinguishes between the following three types of acid extractants:

- carbon-bonded oxygen-donor extractants,
- phosphorous-bonded oxygen donors, and
- ion-pair-forming, high-molecular-weight aliphatic amines.

The last group considers ionic interactions and shows high selectivity. Schügerl et al. (2,3) published a summary of the research done in this field.

The extraction processes for the separation of carboxylic acids, hydroxycarboxylic acids, and amino acids, described in this paper, have been conducted with amine extractants. The following processes have been investigated:

- reactive extraction,
- three-phase extraction,
- liquid membrane permeation, and
- supercritical fluid extraction.

The investigation of these processes is part of a national research program focusing on the optimization of extraction processes for the separation of acids from fermentation broth.

REACTIVE EXTRACTION

The application of reactive extraction for the separation of organic acids based on the use of amine extractants has been investigated intensely. It has been the subject of investigations carried out by Tamada, Kertes, and King (4) and Tamada and King (5,6). This recently published work covers the extraction behavior of the tertiary amine extractant Alamine 336 in the separation of malonic acid, maleic acid, fumaric acid, and succinic acid. The effects of polar and nonpolar diluents on extraction behavior in the separation of carboxylic acids with several functional groups were investigated. Conclusions were drawn up with regard to complex formation in the organic phase. Water coextraction and process design were examined with consideration to temperature and solvent swing effects.

Extraction of bioproducts is highly affected by the composition of the fermentation broth. Parameter sensitivity on the extraction yield is very high. It must be taken into account that impurities from the biocatalysts, which are left after mechanical separation of the acid solution, affect phase separation behavior (e.g., the formation of emulsions or phase disengagement in general), and, furthermore, they may diminish the extraction yield. Besides the extraction pH value, the ionic strength, and the temperature, process development must very carefully consider organic impurities. The necessity of additional process equipment (e.g., filtration units for the separation of solids from both phases) should also be investigated very carefully during process development.

Water coextraction increases with increasing polarity of the acid. When separating hydroxy acids or polyhydroxy acids from aqueous solutions, the solubility problems of the laden extractant determine the diluents. To enhance the solubility of polar acid-amine salts, modifiers (e.g., long-chain alcohols or esters) are added to the organic phase. This leads to an increased amount of water in the extractant phase, which is then coextracted. This water transfer diminishes the extraction efficiency and the purification effect of the extraction process for the separation of polyhydroxy acids.

Reactive Extraction from Synthetic Aqueous Solutions

The extraction behavior for the separation of lactic acid, gluconic acid, and L-leucine was investigated. Secondary, tertiary, and quaternary alkylamines were used for extraction.

The following substances were used:

- Amberlite LA-2 (di-dodecyl/tridecylamine),
- Hostarex A327 (tri-n-octyl/n-decylamine), and
- Aliquat 336 (trioctyl-methyl-ammoniumchloride).

Isodecanol was used for the solubility modification. Shellsol T (aliphatic hydrocarbons; boiling region 183-212°C) was used for dilution of the extractant and the modifier. The extraction of lactic acid was investigated initially. Although lactic acid is a strong acid, the extraction with the quaternary amine Aliquat 336 provided low distribution coefficients. The mechanism of extraction may be described in Eq. (1):

$$R_4N^+\,Cl^- + A^- \rightleftharpoons R_4N^+\,A^- + Cl^- . \qquad (1)$$

The maximum extraction ratio is achieved at pH values close to 14. The reason for the poor extraction efficiency at lower pH values derives from the dissociation constant of lactic acid compared to that of hydrochloric acid. The use of the tertiary amine Hostarex A327 results in higher distribution ratios. A direct relation between the extractant concentration in the organic phase and the separation efficiency was observed. Best yields of extraction are gained at a pH value of 4.5. The coextraction of water can be correlated with the acid transfer: increased acid extraction causes an increased polarity, resulting in a high water coextraction. Equation (2) shows the mechanism for the separation of lactic acid by tertiary amines:

$$R_3N + HA \rightleftharpoons R_3NH^+A^- . \qquad (2)$$

The secondary amine (Amberlite LA-2) yields in the highest extraction rates. At pH values of 4.5, which is higher than the dissociation value of lactic acid (pKa = 3.87), more than 90% separation of lactic acid from the aqueous phase is achieved. At low acid concentration, LA-2 shows better extraction yields than A327. At a high concentration of lactic acid, A327 gives better results pointing out that the extraction capacity of the tertiary amine for the separation of the nondissociated acid is also given. The mechanism of separation with LA-2 is shown in Eq. (3):

$$R_2NH + HA \rightleftharpoons R_2NH_2^+A^- . \qquad (3)$$

The mechanism may also explain the higher water coextraction, when using secondary amines. The solvation of the amine substituent of secondary amines at the interface is higher than the solvation of the tertiary amine.

When extracting gluconic acid, the extraction yield is much lower when compared with that of lactic acid. Best results were achieved by extraction with LA-2 with the highest extraction rate being 55%. An enormous water coextraction was observed: 55 mols of water/polyhydroxy acid. The high number of hydrogen-bridges with water endorses the water coextraction.

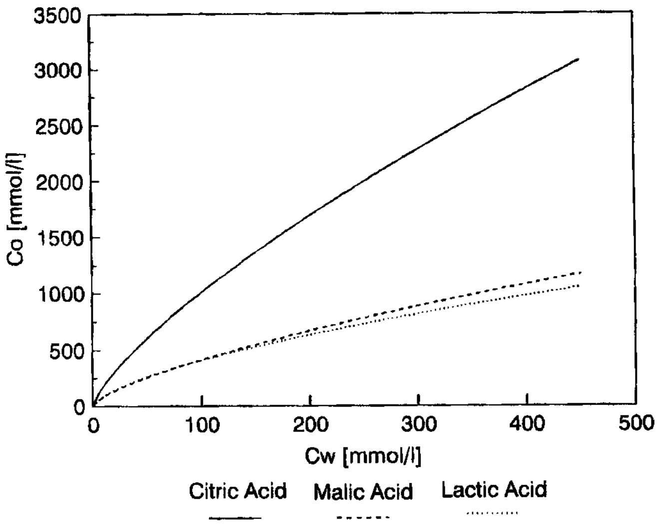

FIGURE 1. Extraction of carboxylic acids with LA-2.

When investigating the aminocarboxylic acid L-leucine, the above-mentioned extraction mechanism is no longer valid. Based on their chemical nature, the aminocarboxylic acids can act both as a cation and an anion. At low pH values, an aminocarboxylic acid acts as a cation and might possibly be extracted by a cation exchanger. At high pH values, the anionic nature determines the extraction by amines. The extraction yield of L-leucine with amines is about 30%, with the best results being achieved with the use of the quaternary amine.

When summarizing the extraction behavior of the different low-molecular-weight carboxylic acids, with and without hydroxy- and aminosubstituents, it has been found that the secondary amine LA-2 extracts these acids with better yields than does the tertiary amine A327. Hydroxy- and aminosubstituents decrease the extraction rate significantly. Some extraction results with synthetic solutions are given in Figure 1 (7).

Reactive Extraction from Fermentation Broth

The extraction behavior in the separation of lactic acid and gluconic acid from fermentation broth was investigated. Gluconic acid was produced by *Gluconobacter* *suboxidans* from glucose, and lactic acid was produced by *Streptococcus cremoris*. Both fermentation broths were stabilized during fermentation, at a pH value of 6.5 to 7.

From synthetic solutions it was known that at this pH value, the separation efficiency is poor. Therefore, sulfuric acid was added to obtain a pH of 4 to 4.5. The yield of acid extraction was about 50% less than that noted in the separation from synthetic solutions. As already mentioned, the loss in separation efficiency derives from several ingredients of the fermentation broth.

The crucial step of the process is the reextraction step. Sulfuric acid, used for the stabilization of the pH at 4.5, is coextracted. When using NaOH for reextraction, the coextracted sulfuric acid causes low reextraction yields. About 80% of the originally fed acid can be regained. Now the extraction/reextraction process for the purification of the acid is possible, but enhancement in concentration is poor. To achieve better results, further reextraction methods need to be investigated.

LIQUID MEMBRANE PERMEATION

Liquid emulsion membranes (LEMs) are formed as described (see Figure 2): the inner aqueous phase (phase I = receiving = interior phase) is emulsified in an organic phase (phase II = membrane phase) by stirring the emulsion at a high velocity. Phase II contains a surfactant which stabilizes the micronic droplets of phase I in the membrane phase. The emulsion is then poured into the fermentation broth (phase III = delivering = outer phase), while stirring at a low velocity, to form globules of several hundred micrometers in diameter. At this stage, a multiple emulsion of very tiny droplets from phase I, in small globules of phase II, is formed in phase III.

When separating fermented carboxylic acids such as lactic acid, phase I would contain sodium carbonate. Phase II contains 2% of the surfactant (ECA 11522) and 5% of a tertiary amine (or 2% of the secondary amine Amberlite LA-2). Kerosene is used for dilution. The fermentation broth (held constant at a pH level of 4.5 by adding sulfuric acid), from which the acid shall be separated, forms the third phase.

The activation of the amine occurs during the first separation step. At a pH value of 4.5, the H^+ concentration in phase III is high enough for the protonation of the amine. This protonation occurs at the interphase between phases II and III. The

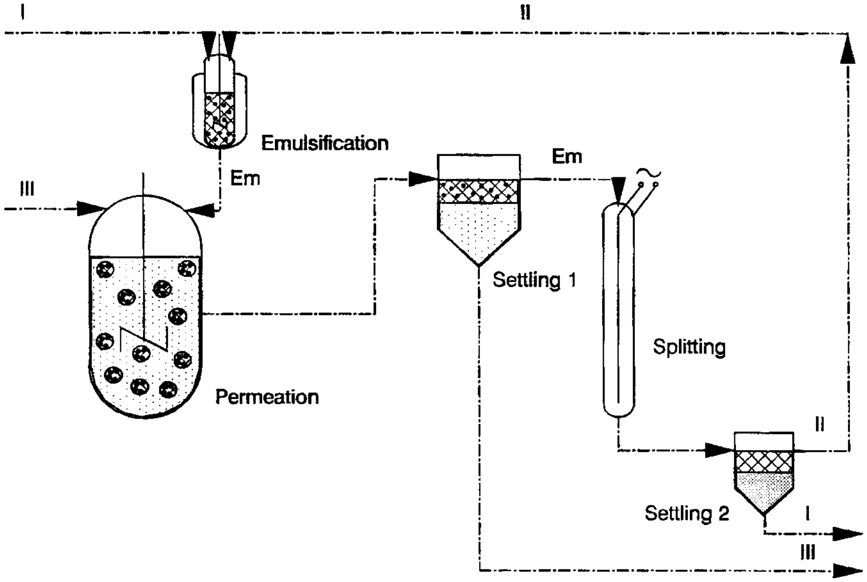

FIGURE 2. Flow sheet of a continuous LEM-permeation. (I, II, III = Phases I, II, III, Em = Emulsion.)

protonated amine is able to catch a lactic acid anion to form an acid-amine complex, which is soluble in the organic phase II. This complex can then diffuse through phase II. At the interphase between phase II and I, the amine is deprotonized because the H^+ concentration is kept very low in the inner phase, by sodium carbonate. Because the lactic anion cannot react with the deprotonized amine again, it remains in phase I.

Thus, lactic acid is transported from phase III to phase I as long as there is a considerable difference in pH between the inner and the outer aqueous phases. The amine can only react with acids; the transport is selective. The lactic acid is separated from the by-products (proteins, color) of the substrate. To a certain extent, water and sulfuric acid are copermeated.

In phase I, neutral salts, instead of caustic substances, can be used as well. The driving force for the separation of lactic acid is now determined not by copermeation, but by an anion counter transport. Lactic acid diffuses from phase III to phase I, and, simultaneously, the counter ion diffuses from phase I to phase III. The counter transport mechanism does not result in comparably good separation efficiency, as demonstrated by the proton cotransport mechanism.

After finishing the mass transfer step, the different phases are separated by settling and by electrocoalescing in a pulsed electromagnetic field. Phase I contains the enriched, purified, and concentrated lactic acid. Phase II can be used again for further separation.

Under optimal conditions, during liquid membrane permeation, lactic acid can be separated from fermentation broth with an efficiency of 90%, concentrated up to three times and purified from most of its by-products. About 1% of glucose and amino acids are permeated with the lactic acid.

Comparing the permeation rate of different carboxylic acids, the lower the pKa value of the acid is and the fewer polar substituents it has, the more efficiently it can be separated. When separating dicarboxylic acids, mass transfer is retarded by the second carboxyl group. Separation of the acid decreases as the distance between the two carboxyl groups decreases.

The mutual effect is not very strong when several different organic acids need to be separated from the same fermentation broth. This rule applies when the concentration of the acids is about 0.1 mol/L. Sulfuric acid heavily affects permeation when added to the fermentation broth to keep the pH at a constant level of 4.5, which is necessary to optimize permeation. Results of the permeation are given in Figure 3 (8).

THREE-PHASE EXTRACTION

Three-phase extraction is another technical possibility for the reactive extraction of organic acids. Product enrichment, in connection with high selectivity, should be obtained by an additional phenomenon of reactive extraction.

Extraction of carboxylic acids with alkylamines results in the formation of salts, which are preferably dissolved in the organic phase. At high acid loading in inert diluents, molecular clusters of these salts are formed. Depending on the solutant properties of the amine, the extracted acid, and the organic solvent, these ionic clusters separate from the organic phase by forming an intermediate layer.

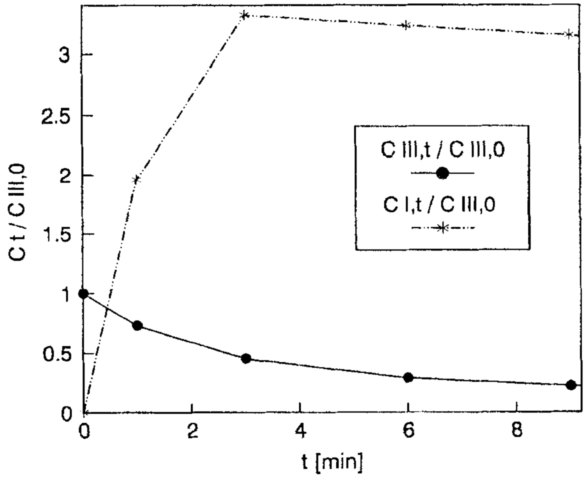

FIGURE 3. Lactic acid separation by LEMs. Concentration profile in phase III (C III, t) and phase I (C I, t) during batch permeation from a fermentation broth (related to the initial concentration C III, 0).

The "third phase" contains the highly concentrated acid and ordinarily has a high viscosity. Its density enables the formation of the third phase at the water-solvent interphase.

The main condition for the application of this type of extraction is the formation of the third phase during extraction. Therefore, different amine extractants with an experimentally evaluated concentration (50%) were tested. Shellsol T (SST) was used as a diluent. The following amine-extractants were tested: tri-n-butylamine, tri-n-hexylamine, tri-n-octylamine, tri-2-ethylhexylamine tri-iso-octylamine (Adogen 381), tri-n-octyl/decylamine (Hostarex A327, trilaurylamine (Alamine 304), di-dodecyl/tridecylamine (Amberlite LA-2), and di-iso-tridecylamine (HOE F 2562).

The tests showed that tri-n-butylamine is salted-in in water because of the high mutual solubility of the alkylammoniumlactate and water. Tri-n-hexylamine performed well during this test, but it is a poor selection because of the high solubility losses of the extractant in the aqueous phase. Tri-n-octylamine had nearly the same extraction efficiency when compared with Hostarex A327. The latter proved to be better,

TABLE 1. THIRD-PHASE FORMATION FOR ACID EXTRACTIONS

Extractant \ Acid	HLac	HForm	HAc	HProp	HCl
tributylamine	-	---	---	---	-
trihexylamine	+	+	+	+	+
tri-n-octylamine	+	+	+	+	+
tri-2-ethylhexylamine	-	+	-	-	+
tri-iso-octylamine	+	+	+	-	+
tri-n-octyl/decylamine	+	+	-	-	+
trilaurylamine	-	-	-	---	---
di-dodecyl/tridecylamine	+	+	-	-	-
di-iso-tridecylamine	+	-	-	-	-

Extractions were done with 50 vol % extractant in SST.
The aqueous feed concentration was 2 mol/L.
+ third-phase formation; --- not investigated;
- no third phase was formed.

considering the extraction yield in connection with phase disengagement and third-phase formation.

In Table 1, third-phase formation is qualitatively shown for the extraction of lactic acid (HLac), formic acid (HForm), acetic acid (HAc), propionic acid (HProp), and hydrochloric acid (HCl). During these experiments, the aqueous feed concentration was about 2 mol/L. Generally, the performed experiments showed that n-alkylamines achieve better results in three-phase extraction than do iso-alkylamines. Furthermore, long alkyl chains cause emulsification. Phase disengagement using secondary alkylamines was worse than that using tertiary amines.

Different ratios of the amine and diluent (SST) were used for equilibrium curve estimation. The dependence of the extraction efficiency on different feed concentrations of the acids was measured. Lactic acid separation increases with decreasing pH and shows an optimum separation efficiency at a low pH value of about 2. Then the volume of the third phase and the coextraction of hydrochloric

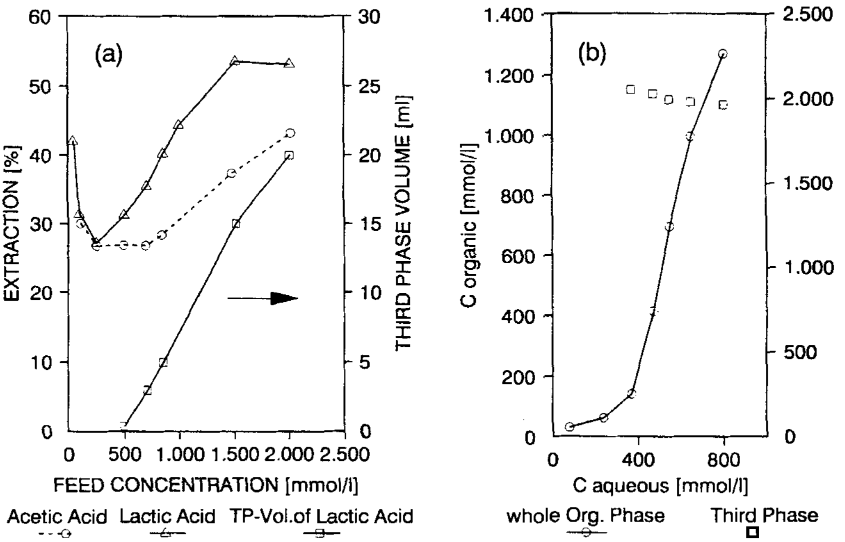

FIGURE 4. a) Extraction and third-phase volume of lactic acid compared to the extraction of acetic acid with Hostarex A327 (50 vol %) in SST.
b) Equilibrium distribution of lactic acid with tri-n-octylamine (50 vol %) in SST.

acid, which was used for pH adjustment, increase as well. Therefore, an optimum pH value (at 2.5) has to be found to enable an unaffected extraction.

The dependence of the extraction rate on different feed concentrations of acetic acid and lactic acid is shown in Figure 4a (9). It also shows the volume of the third phase when extracting lactic acid. The organic phase volume was 30 mL (50 vol % A327 in SST) and the phase ratio was 1.

It can be observed that both the decreasing pH value with increasing acid concentration in the aqueous phase and the growth in polarity of the organic phase due to the acid-amine salt formation enhances the extraction efficiency.

When using Hostarex A327, the extraction of acetic acid does not result in the formation of an intermediate layer. This leads to the assumption that coextraction of acetic acid should be selective as the extractant phases split. Only a slight enhancement in selectivity (10% at maximum) was observed after separation of the two organic layers.

The content of the extracted acid in the third phase was about the same for different feed concentrations, though it strongly varied with the species of the extractant. When using the extractant Hostarex A327, a highly concentrated third phase is obtained with acid contents of 1 to 2 mol/L independent of the partition coefficient (see Figure 4b). When using the extractant tri-n-hexylamine, the third phase was concentrated up to 3 mol/L for lactic acid and up to 4 mol/L for acetic acid. As already mentioned, extreme solubility losses are a serious reason for objecting to the use of the latter. Further purification steps (recovery) may help overcome this limitation.

SUPERCRITICAL FLUID EXTRACTION

The main advantages of supercritical fluid extraction (SFE) processes, affected selectivity of the solvent and the separation of low-volatility compounds, give incentive for the use of these extraction techniques in bioseparation engineering.

Extraction with compressed gases is useful for the separation of nonpolar substances. When extracting polar species with low solubility, entrainers have to be added to the extraction gas. Normally, these are substances like water, methanol, ethanol, or acetone, the volatility of which is between that of the gas and that of the product.

Substances that can hardly be extracted with pure compressed gas from aqueous solutions are, for example, organic acids, such as acetic or lactic. Within this program, an attempt was made to investigate the reactive extraction of carboxylic acids with supercritical carbon dioxide. The reactive substances added to the extraction gas were secondary and tertiary amines.

The investigated process is illustrated in Figure 5 (10). Liquid CO_2 from the storage vessel is compressed to the extraction pressure and preheated, and the entrainer (amine) is dissolved in a mixing chamber. In the extractor, the dissolved amine reacts with the acid. The acid-amine salt is precipitated in the separator, and the carbon dioxide is reused. The acid has to be stripped from the amine in the same way as it is done in solvent extraction.

Three amines were tested for their solubility in carbon dioxide. Hostarex A327 showed too low a solubility in liquid, as well as in supercritical carbon dioxide.

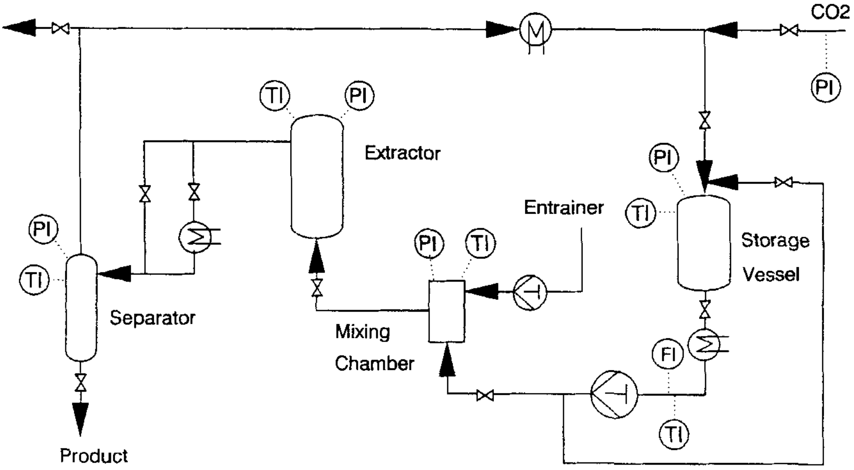

FIGURE 5. SFE: Flow sheet of the discontinuous plant.

This amine solidified under increased pressure (formation of amine-CO_2-salt?). Trihexylamine is highly soluble at 25 to 40° C. The secondary long-chain amine LA-2, a di-iso-C_{12}/C_{13} amine, led to loadings of 4-5 wt % at maximum.

All the batch-extraction experiments were performed at 40°C and 130 bar. The acid contents were within 1-2 wt % during the extraction of the synthetic solutions. LA-2 showed fairly good results for the separation of lactic acid. Extraction with the entrainer tri-n-hexylamine was very weak in lactic acid; qualitatively, the tests with acetic acid gave better results. Applying the tertiary amine, the partition coefficients were three times greater when using acetic acid compared with using lactic acid. Figure 6 shows results for the separation of acetic acid and lactic acid by SFE.

Extraction yields of the fermentation broths were as high as those of the synthetic solutions. Amine loadings of the CO_2 during these experiments were within 0.5 to 5 wt %. The mass flow of the gas was 10-15 kg/h at that time.

Experiments in a continuous multistage plant, with a packed column, have already been run. Both acids mentioned gave separation yields of 95-100%. Further experimental work is planned to test other amine entrainers under different pressure and temperature levels.

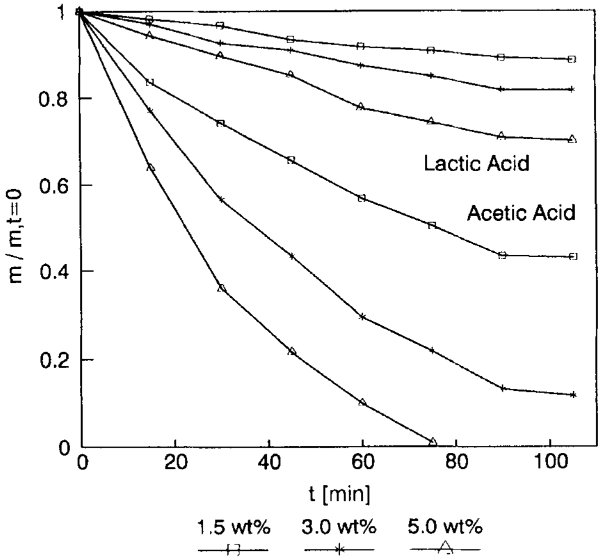

FIGURE 6. Separation of lactic acid and acetic acid by SFE using tri-n-hexylamine (1.5, 3.0, and 5.0 wt %) as entrainer in supercritical carbon dioxide.

CONCLUSIONS

Reactive extraction has already proven its applicability in biological downstream processing. Liquid membrane permeation is a technology for the separation and enrichment of different substances from low-concentration feed solutions. It does not show advantages over extraction when dealing with fermentation broth with high acid concentrations. The three-phase extraction process, based on the formation of a second organic layer during extraction, gains high enrichment for most of the investigated acids. This technique shows limited extraction rates. Supercritical fluid extraction provides good extraction rates for acetic acid separation. It has shown that it is not very suitable for lactic acid extraction. In general, this technology needs further investigation.

ACKNOWLEDGMENTS

This work was supported by the Austrian "Fonds zur Förderung der wissenschaftlichen Forschung" (FWF).

REFERENCES

1. A. S. Kertes and C. J. King, Extraction Chemistry of Fermentation Product Carboxylic Acids, Biotechnol. Bioeng. 28, 269 (1986).

2. K. Schügerl, Physical and Reactive Extraction of Carboxylic Acids, Proc., Sep. Tech., Eng. Found. Conf., 336 (1987).

3. K. Schügerl und W. Degener, Gewinnung niedermolekularer organischer Verbindungen aus komplexen wäßrigen Gemischen durch Extraktion, Chem.-Ing.-Tech. 61, 796 (1989).

4. J. A. Tamada, A. S. Kertes, and C. J. King, Extraction of Carboxylic Acids with Amine Extractants. 1. Equilibria and Law of Mass Action Modeling, Ind. Eng. Chem. Res. 29, 1319 (1990).

5. J. A. Tamada and C. J. King, Extraction of Carboxylic Acids with Amine Extractants. 2. Chemical Interactions and Interpretation of Data, Ind. Eng. Chem. Res. 29, 1327 (1990).

6. J. A. Tamada and C. J. King, Extraction of Carboxylic Acids with Amine Extractants. 3. Effect of Temperature, Water Coextraction, and Process Considerations, Ind. Eng. Chem. Res. 29, 1333 (1990).

7. E. Hauer und R. Marr, Extraktive Stofftrennverfahren in der Biotechnologie, Chem.-Ing.-Tech. 63, 809 (1991).

8. H. Reisinger, R. Marr, and W. Preitschopf, Liquid-Membrane-Permeation in the Fields of Biotechnology, Proc., ICOM'90, Chicago, Illinois, U.S.A. (1990).

9. J. Hartl and R. Marr, Utilization of Third-Phase Formation in Solvent Extraction Systems Employing Amine Extractants, Proc., ISEC'90, Kyoto, Japan (1990).

10. F. Fröschl and R. Marr, Recovery of Organic Acids by SFE, Proc. 2nd Int. Symp. on High Pressure Chem. Eng., GVC, Erlangen, Germany (1990).