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REVIEW PAPER

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NEW EXTRACTION TECHNIQUES IN BIOSEPARATIONS 2. PERTRACTION, DIRECT EXTRACTION

The second part of this review presents our original results on the separation of some biosynthetic products (antibiotics, carboxylic acids, alcohols) by pertraction and direct extraction from broths without biomass filtration. For the analyzed systems, the experimental conditions required for reaching maximum separation efficiency and the mathematical models describing the process have been established. For all the studied cases, these extraction techniques simplify the technologies and reduce the overall cost of the product.

PERTRACTION

Extraction and transport through liquid membranes, called *pertraction or permeation through liquid membranes*, has been studied since the 1980s and is one of the most advantageous techniques of separation at the present, with important applications in biosynthetic products separation (antibiotics, amino acids, carboxylic acids), as well as in metals recovery from hydrometallurgical and nuclear industry wastes. This separation method consists of the transfer of a solute between two aqueous phases of different pH, which are separated by a solvent layer of various sizes.

Commonly, liquid membranes can be obtained either by emulsification (*liquid membrane extraction*) when its stability is poor, by including the solvent in a hydrophobic porous polymer matrix (*supported liquid membrane extraction*), or by using special equipment (*free liquid membrane*) [1–7]. Comparing extraction using liquid membranes with conventional liquid–liquid extraction, the advantages are as follows:

– the quantity of solvent used is small, because of its continuous regeneration

– the loss of solvent during extraction and transport process is reduced

– as long as a pH gradient between the two aqueous phases is maintained, there is the possibility of solute transport against its concentration gradient.

The pertraction efficiency could be significantly enhanced by adding a carrier in the liquid membrane, such as organophosphoric compounds, long chain amines or crown–ethers, the separation process being called *facilitated pertraction*.

Extraction and transport through liquid membranes have been applied for the separation of some biosynthetic products, namely: carboxylic acids (acetic acid, lactic acid, fumaric acid, citric acid, propionic acid, phenoxyacetic acid), amino acids and antibiotics (Penicillin G and Penicillin V) [1,5,8–16]. Transport through liquid membranes can facilitate some enzymatic reactions, for example the production of 6–aminopenicillanic acid using an integrated process of fermentation and enzymatic conversion of Penicillin G [17]. Moreover, extraction and transport through liquid membranes, integrated with fermentation, offer the advantage of the *in situ* removal of the biosynthetic products, thus avoiding product inhibition.

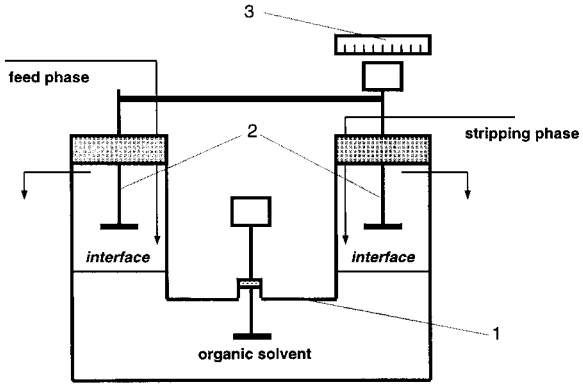

Figure 1. Experimental equipment for pertraction (1 – pertraction cell, 2 – blade stirrers, 3 – tachometer)

Our experiments were carried out in a continuous system using pertraction equipment of the U–shaped glass pipe type that allows the free liquid membrane to be obtained and easily maintainined (Figure 1).

As can be observed from Figure 2, the general mechanism of facilitated pertraction implies three successive stages:

1. solute reactive extraction at the interface between the feed phase and solvent phase (liquid membrane)

2. the diffusion of complex solute–carrier through the liquid membrane

3. the re–extraction of solute at the interface between the organic phase and the stripping phase.

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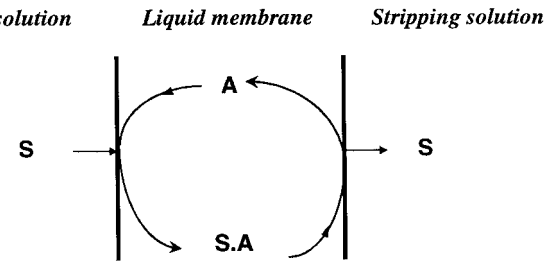

Figure 2. Mechanism of facilitated pertraction of solute S with carrier A

Among the factors which control the efficiency of permeation through liquid membranes (pH–gradient between the aqueous phases, carrier concentration, mixing intensity, physical and chemical characteristics of the system components), the pH–difference between the feed and stripping phase exhibits a significant influence. For transport systems through liquid membranes, the pH–values of the two aqueous phases will control the yields of the extraction and re–extraction processes, on the one hand, and the rate of solute transfer through the solvent layer, on the other hand.

The transport ability of these separation systems can be expressed by means of the solute *permeability factor*, P, defined as the ratio between the solute initial and total mass flows [10,12,17].

In original experiments we applied facilitated pertraction with Amberlite LA–2 solved in 1,2–dichloroethane for the selective separation of beta–lactamic antibiotics from their precursors and for the individual and selective separation of carboxylic acids obtained by citric fermentation.

Pertraction of antibiotics and biosynthesis precursors

As mentioned in previous papers, the extraction and re–extraction of **beta–lactamic antibiotics** are significantly influenced by the pH–value of the two aqueous solutions (the feed phase was an acidic solution, the stripping phase a sodium carbonate solution) [19–22]. The reduction of the feed phase pH–value and the increase of the stripping phase pH–value as suggested by Figure 3, therefore increase of the pH–difference between the two aqueous solutions, leads to the increase of the **Penicillin V** mass flow both from the feed solution to 1,2–dichloroethane (initial mass flow) and from the organic solvent to the stripping solution (total mass flow) [10,11].

The Amberlite LA–2 concentration in 1,2–dichloroethane also exhibits a decisive influence on the rate of the antibiotic mass transfer. Therefore, it can be see from Figure 4 that the Penicillin V mass flow both for extraction and re–extraction increases with increasing carrier concentration. The permeability factor has a particular evolution, it initially decreases to a minimum value corresponding to an Amberlite LA–2 concentration of 10 g/l, then increasing continuously with the carrier concentration.

This variation is possibly the result of the modification of the relative chemical reaction rates, which occur at both separation interfaces between the organic solvent and aqueous solutions. In the absence of carrier, the extraction and transport of Penicillin V is achieved by means of the physical processes of solubilization and diffusion, the limiting steps of the overall mass transfer process being of the diffusional type. By adding the carrier, a modification of the Penicillin V extraction and re–extraction processes is produced and, consequently, of the rates of these processes. Because of the chemical reactions between Penicillin G and Amberlite LA–2, at the first separation interface, and between the antibiotic–carrier complex and sodium carbonate, at the second separation interface, two additional limiting steps of the kinetic type appear.

Compared with physical extraction, the formation of the Penicillin V–Amberlite LA–2 complex increases the extraction degree. However, because Penicillin V reacts in the complex form with sodium carbonate, not in the

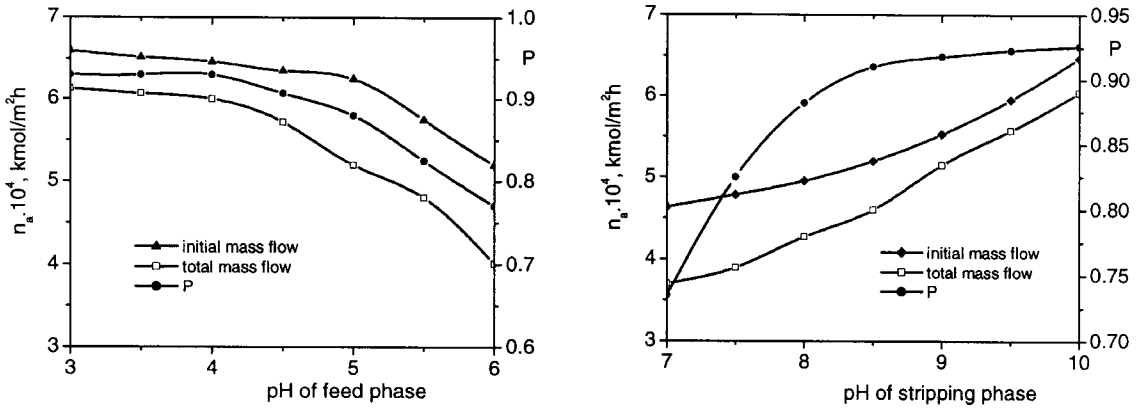

Figure 3. Variation of the Penicillin V mass flows and permeability factor as a function of the pH of the feed and stripping phases (carrier concentration 80 g/l, rotation speed 500 rpm)

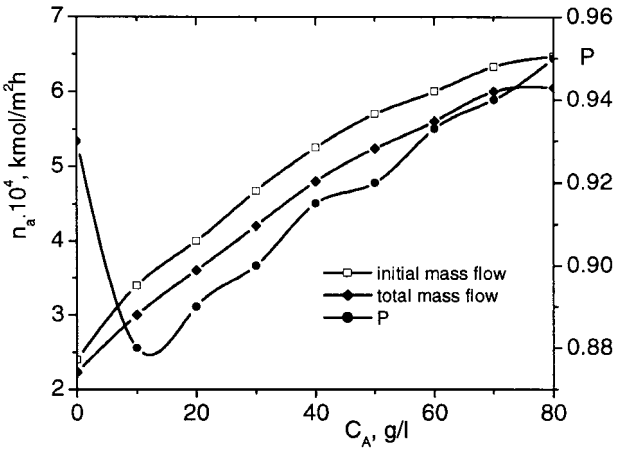

Figure 4. Variation of the Penicillin V mass flows and permeability factor as a function of the Amberlite LA–2 concentration (pH =3.5 for the feed phase and pH=10 for the stripping phase, rotation speed 500 rpm)

free acid form, the formation rate of the Penicillin V sodium salt increases more slowly with carrier concentration. Thus, compared with physical extraction, the permeability factor will initially reach a lower value.

The increase of the mixing intensity of the aqueous phases determines the increase of the Penicillin V mass flows for extraction and re–extraction, due to the diminution of the resistance caused by antibiotic diffusion towards the interface, the permeability factor having a similar evolution.

The cumulated effect of the carrier concentration and rotation speed on the total mass flow of Penicillin V through the liquid membrane is depicted in Figure 5.

Although the obtained curves are similar for the whole Amberlite LA–2 concentration interval, the increase of this parameter value from 0 to 80 g/l induces a two–fold increase of the total mass flow of the

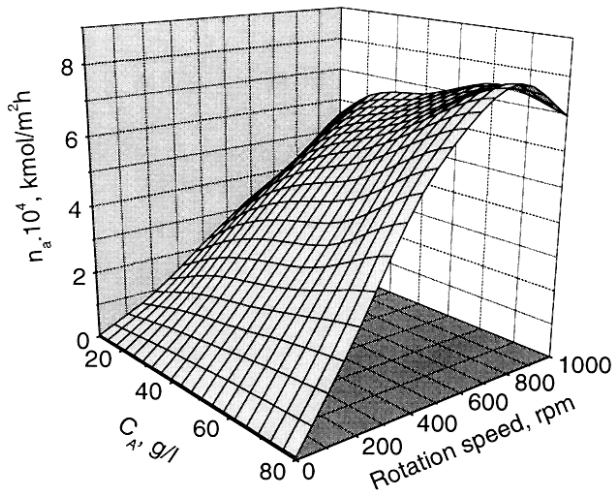

Figure 5. Cumulative effects of the carrier concentration and mixing intensity on the Penicillin V total mass flow (pH =3.5 for the feed phase and pH=10 for the stripping phase)

antibiotic. A mathematical correlation which describes the effects of the carrier concentration and mixing intensity on the total mass flow of Penicillin V was established using the experimental data for the feed solution pH–value of 3.5, stripping solution pH–value of 10 and Re less than 540 [11]:

$$n_a = e^{6\cdot10^{-2}\cdot C_A - 15.65} \cdot Re^{1.30 - 8.57\cdot10^{-3}\cdot C_A} \quad (1)$$

Compared with the experimental data, this equation has an average deviation of ± 9.02%.

Selective separation with the help of liquid membranes of beta–lactamic antibiotics from the precursors used in their biosynthesis was also studied [5,12,23–26]. In this context, we analyzed the selective extraction conditions of **Penicillin V** from **phenoxyacetic acid** using liquid membranes consisting of 1,2–dichloroethane and Amberlite LA–2 as the carrier.

Due to its toxicity, phenoxyacetic acid was added in portions during the fermentation, its concentration being maintained at a constant level. Thus, the final acid concentration in the fermentation broth varied between 0.2 and 0.5 g/l, depending on the strain and biosynthesis conditions. For this reason, selective separation is required for obtaining an antibiotic with high purity. This operation is difficult because of the similarities in the physical and chemical characteristics of the antibiotic and precursor. In order to establish the optimum conditions for an efficient selective separation, the influences of the main parameters (the pH value for both aqueous phases, the concentration of the carrier in the organic layer, the mixing intensity) on the mass flows of Penicillin V and phenoxyacetic acid through liquid membranes were studied.

The efficiency of selective separation was described by means of the *selectivity factor*, which represents the ratio between the total mass flows of Penicillin V and phenoxyacetic acid, respectively, through the liquid membrane.

Figure 6 demonstrates the major role of pH on the performance of the selective separation of these compounds. It could be observed that the maximum values of the selectivity factor were obtained in the case of the minimum difference between the pH–values of the aqueous phases. Thus, at a constant level of the stripping phase pH of 10 and for a pH value of 6 for the feed phase a value of S = 80.4 was obtained. At a maintained pH value of the feed phase of 3, S = 24.2 for a pH value of the stripping phase of 7.

The mass flows of antibiotic and precursor are intensified with increasing carrier concentration, but the permeability factors of the two compounds evolve differently, [10,12,24]. Thus, they initially decrease from a value corresponding to the absence of Amberlite LA–2 in the organic solvent to a minimum value for a concentration of 10 g/L Amberlite LA–2, and finally, they increase concomitantly with the carrier concentration (Figure 7). This variation could be the result of the

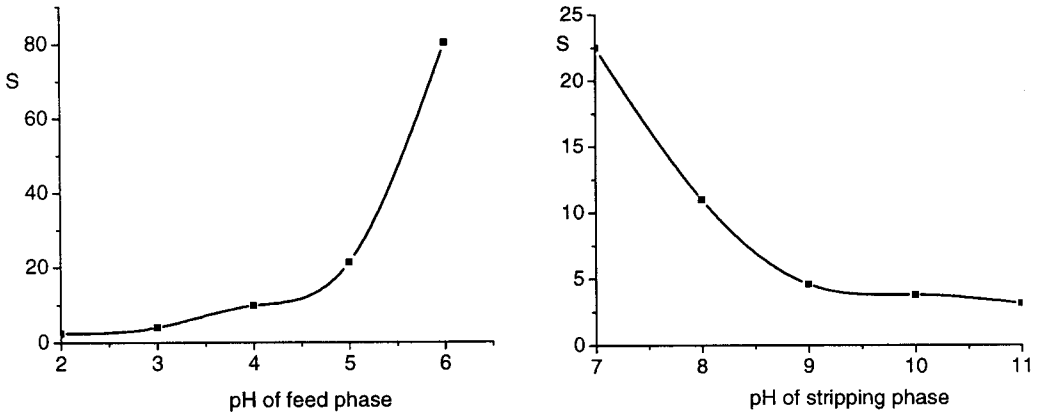

Figure 6. Effect of the feed phase and the stripping phase pH on the selectivity factor (rotation speed = 500 rpm, carrier concentration = 80 g/l)

changes in the relative rate of the interfacial chemical reactions, as mentioned above.

Even if the effect of carrier concentration is quite similar for the two components of the mixture, the initial decrease of the permeability factor of phenoxyacetic acid is more significant. When the Amberlite LA–2 concentration inside the liquid membrane, is increase the values of the permeability factors of the two compounds approach one another. This phenomenon, indicated in Figure 7 by the ratio of permeability factor evolution, suggests that at low concentrations the carrier will preferentially react with the compound of higher acidity, namely Penicillin V. At high Amberlite LA–2 concentrations, additional amounts of carrier will exist near the interface, which means that the carrier will react even with the weaker acid, namely phenoxyacetic acid.

These results were obtained when the selectivity factor was varied, which under the considered experimental conditions, had a maximum of 6.5 for 10 g/L Amberlite LA–2 in the membrane phase.

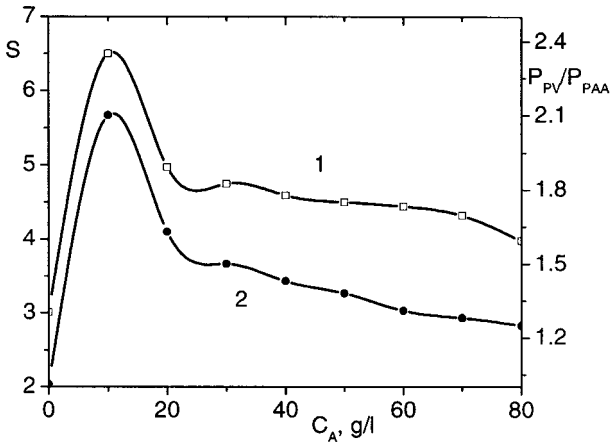

Figure 7. Effect of the rotation speed on the ratio between the permeability factors of Penicillin V and phenoxyacetic acid and on the selectivity factor (pH of the feed phase = 3, pH of the stripping phase = 10, carrier concentration = 80 g/l; 1 – P_{PV}/P_{PAA}, 2 – selectivity factor)

The mixing intensification of the two aqueous phases determined the transfer acceleration of the initial solution components through the liquid membrane, this effect being more significant for Penicillin V. The selectivity factor attanined a maximum value of 4.2 for 1000 rpm, these data suggesting that, among the factors influencing the selectivity of pertraction, the mixing intensity had the smallest influence.

Pertraction of carboxylic acids

Citric acid is one of the important carboxylic acids, having multiple applications in the chemical, pharmaceutical, food and cosmetic industries. This compound is mainly obtained by biosynthesis by *Aspergillus niger* cultivated on molasses [27,28]. As presented in the first part of this review, due to the presence of other carboxylic acids in the final broth, especially **maleic** and **succinic acids** as secondary metabolic products, the separation and purification technology of citric acid becomes more compliciated [29]. On an industrial scale, the separation and purification of citric acid needs a high amount of raw materials and energy consumption, without leading to the high purity of citric acid.

The separation of citric acid by facilitated pertraction is based on the reactive extraction process. As stated in previous papers, the reactive extraction of polycarboxylic acids with Amberlite LA–2 occurs by means of an interfacial chemical reaction. The structures of the formed hydrophobic complexes depend on the molar ratio between the extraction system components [29–32]. The reactive extraction mechanism can generally be expressed by the following interface equilibrium:

$$m\,R(COOH)_{n\,(aq)} + p\,A_{(o)} \overleftrightarrows \check{S}R(COOH)_n]_m.A_{p(o)}$$

where n = 2 for maleic and succinic acids and n = 3 for citric acid (A is a carrier of the amine type).

For a constant value of the carrier concentration, the structure of the formed complex was determined by the level of the carboxylic acid concentration, as follows:

a. for the molar ratio m:p less man 1, the interfacial reaction product is $R(COOH)_n.A_n$

b. for a molar ratio m:p nearly 1, the extraction system components react in equimolecular proportion forming: $R(COOH)_n.A$

c. at high initial concentration of the organic acids, a third phase with high acidic complex concentration can appear in non-polar diluents. In this case, the structure of the acidic complex is $[R(COOH)_n]_m.A$.

These mechanisms are valid for maleic and succinic acids. The reactive extraction of citric acid with Amberlite LA-2 occurs only by the interfacial reaction type described by mechanism **b**. Modification of the citric acid extraction mechanism compared with those for maleic and succinic acids could be the result of citric acid size, which generates steric hindrances between the molecules constituting the carrier-acid complex [14,29,30]. Thus, the facilitated pertraction of citric acid with Amberlite LA-2 is based on $R(COOH)_3.A$ formation in the organic layer.

For the pertraction of these carboxylic acids the pH gradient between the feed and stripping phases results in a significant influence both on the yields of extraction

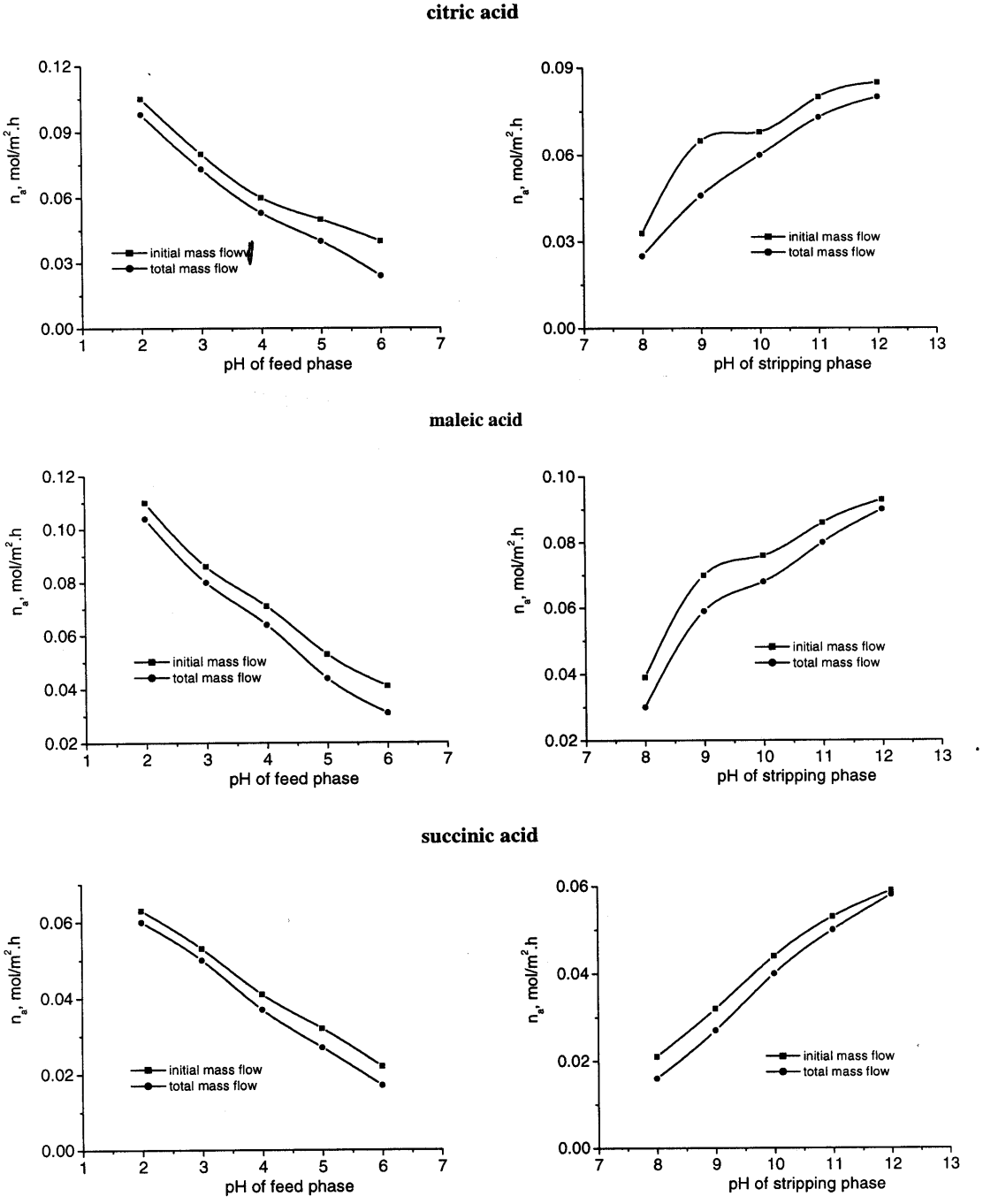

Figure 8. Influence of the pH of the feed and stripping phases on the citric, maleic and succinic acid mass flows (carrier concentration = 0.3 M, rotation speed = 500 rpm)

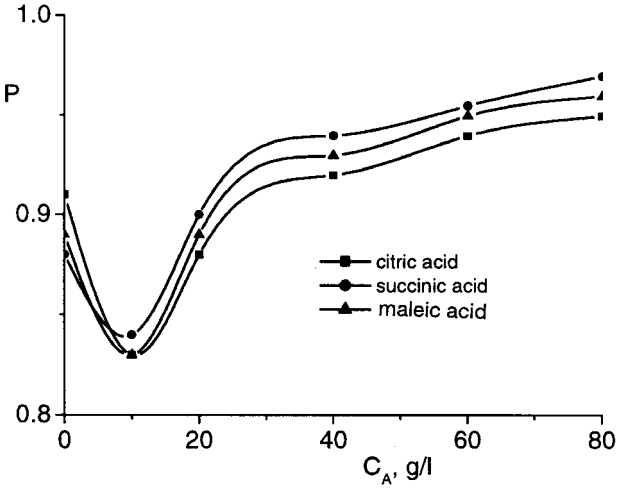

Figure 9. Influence of the carrier concentration on the citric, maleic and succinic acid permeability factors for individual pertraction (rotation speed = 500 rpm, pH of the feed phase = 3, pH of the stripping phase = 11)

and re-extraction and on the transport rate through the solvent layer. As shown in Figure 8, the reduction of the feed phase pH and the increase of the stripping phase pH, therefore, the increase of the pH gradient, leads to the increase of acids mass flows [13–15].

The permeability factors for all the studied acids tend to 1 with increasing pH gradient, thus indicating the approach between the acid extraction and re-extraction yields.

Another important factor is the concentration of Amberlite LA–2 inside the liquid membrane. The mass flows of citric, maleic and succinic acids are intensified with increasing carrier concentration, but the permeability factors evolve differently (Figure 9). Thus, they initially decrease from a value corresponding to the absence of Amberlite LA–2 in the organic solvent to a minimum value for a concentration of 10 g/L Amberlite LA–2 and, finally, increase concomitantly with the carrier concentration.

Similarly to the pertraction of Penicillin V analyzed above, this variation could be the result of the changes in the relative rate of the interfacial chemical reactions [13,14,17]. Under identical experimental conditions, the order of the increase of the pertraction efficiency with carrier concentration was as follows:

succinic acid < citric acid < maleic acid

due to the different acidity and hydrophobicity of these compounds.

The mixing intensification of the two aqueous phases determined the transfer acceleration of the acids, as a result of the diminution of resistance to the diffusion through the boundary layers at the separation interfaces, evolution that is also similar for the permeability factor. The dependence of the acid mass flows on the rotation speed suggests that the overall separation process can be controlled by diffusion or chemical reactions. Therefore, the increasing domain of mass flow indicates that pertraction occurs in the diffusional regime below a rotation speed of 700 rpm for citric and maleic acids, and below 600 rpm for succinic acid. At higher values the chemical reaction becomes the limiting step. The increase of the permeability factors with rotation speed intensification indicates the stronger influence of mixing on the final mass flow, due to the more accentuated resistance to diffusion through the stripping phase, as a result of the larger size of sodium salt molecules compared with free acid molecules from the feed phase [14,15].

For the individual pertraction of citric, maleic and succinic acids, some mathematical models that quantify the studied influences of the pH–value of the feed phase, carrier concentration and mixing intensity (Reynolds number) were established by statistical analysis, using a second order factorial experiment. Hence, the obtained regression equations were:

a. citric acid

$$P = 0.663 - 8.8 \cdot 10^{-2} \cdot x_1 - 1 \cdot 10^{-2} \cdot x_2 + 0.133 \cdot x_3 + \\ + 3.75 \cdot 10^{-3} \cdot x_1 \cdot x_2 + +1.875 \cdot 10^{-2} \cdot x_1 \cdot x_3 - \\ - 6.25 \cdot 10^{-3} \cdot x_2 \cdot x_3 - 8.1 \cdot 10^{-2} \cdot x_1^2 + 0.177 \cdot x_2^2 - \\ - 1.2 \cdot 10^{-2} \cdot x_3^2 \quad (2)$$

b. maleic acid

$$P = 0.667 - 8.6 \cdot 10^{-2} \cdot x_1 - 8 \cdot 10^{-3} \cdot x_2 + \\ + 0.139 \cdot x_3 + 2.5 \cdot 10^{-3} \cdot x_1 \cdot x_2 + 1.5 \cdot 10^{-2} \cdot x_1 \cdot x_3 - \\ - 2.5 \cdot 10^{-3} \cdot x_2 \cdot x_3 - 1.4 \cdot 10^{-2} \cdot x_1^2 + 5.8 \cdot 10^{2} \cdot x_2^2 - \\ - 6 \cdot 10^{-3} \cdot x_3^2 \quad (3)$$

c. succinic acid

$$P = 0.687 - 8.8 \cdot 10^{-2} \cdot x_1 - 7 \cdot 10^{-3} \cdot x_2 + 0.133 \cdot x_3 + \\ + 2.5 \cdot 10^{-3} \cdot x_1 \cdot x_2 + +1.5 \cdot 10^{-2} \cdot x_1 \cdot x_3 - 2.5 \cdot 10^{-3} \cdot \\ \cdot x_2 \cdot x_3 - 3.8 \cdot 10^{-2} \cdot x_1^2 + 7.4 \cdot 10^{2} \cdot x_2^2 - 7 \cdot 10^{-3} \cdot x_3^2 \quad (4)$$

where x_1, x_2 and x_3 designated the pH of the feed phase, carrier concentration and Re number, respectively.

The determination coefficients, which represent the square of the correlation coefficients, indicate that the considered variables influence the efficiency of carboxylic acid pertraction, described by the permeability factor, to more than 99% extent, the mixing intensity being the most important factor.

The previous results indicated that due to the differences between the pertraction mechanisms, the acidity of these carboxylic acids and the hydrophobicity of the compounds formed with the carrier, the selective separation of citric acid from the maleic and succinic acids by facilitated pertraction with Amberlite LA–2 becomes possible. Therefore, we studied the influences of the pH–gradient between the aqueous phases, the carrier concentration and mixing intensity on the

pertraction selectivity for citric, maleic and succinic acid separation from mixture obtained by citric fermentation [16,17].

For the petraction of carboxylic acids obtained by citric fermentation, the pH gradient between the feed and stripping phases induces a significant influence both on the efficiency of extraction and re-extraction and on the transport rate through the solvent layer. It was observed that the order of pertraction efficiency increases as follows:

succinic acid < citric acid < maleic acid.

In the case of pertraction from a mixture, the dependence of the mass flows of these compounds on the pH gradient must be correlated with their acidity, because the acidity controls the rate of interfacial reactions between the solute and the carrier. Thus, the obtained order is the result of the higher acidity of citric and maleic acids, on the one hand, and of the superior hydrophobicity of the maleic acid–Amberlite LA–2 complex.

Moreover, the values of the permeability factors suggest an inverse proportionality between the transport capacity of the liquid membrane and the acidity of the transferred solute, the order of permeability factor decrease being:

succinic acid > maleic acid > citric acid.

This order could be explained by the similar variation of the rate of the interfacial reaction between the acid–carrier compound and sodium hydroxide, the increase of acidity leading to the appearance of a kinetic resistance to the re-extraction process.

The concentration of Amberlite LA–2 inside the liquid membrane induces a different influence on the pertraction efficiency of the carboxylic acids. As stated, for the reactive extraction or individual pertraction of citric, maleic and succinic acids with Amberlite LA–2, the difference in the carrier influence is due to the difference in acid mechanisms extraction, as well as to the difference in solute acidity and hydrophobicity of the extracted compounds [13–17,30]. It can be observed from Figure 10 that by increasing the carrier concentration the maleic acid, succinic acid and citric acid are successively pertracted.

The succinic acid was extracted after the Amberlite LA–2 concentration exceeded the value stoichiometrically needed for the interfacial reaction with maleic acid, respectively, after it exceeded the molar ratio between the carrier and maleic acid of 1 (the molar ratios were calculated as the ratio between the molar concentrations of the acids in the feed phase and the Amberlite LA–2 molar concentration in the membrane phase). The citric acid was extracted for a carrier concentration level higher than that corresponding to an equimolecular ratio with maleic and succinic acids. Below carrier concentrations that allow the reactive extraction of succinic and citric acids, their pertraction was possible only by physical solubilization in 1,2–dichloroethane, but the acid mass flows were very low. These results underlined the major influence of the Amberlite LA–2 concentration inside the liquid membrane on pertraction selectivity.

The acid permeability factors had a different evolution. Thus, they initially decreased from a value corresponding to the absence of Amberlite LA–2 in the organic solvent to a minimum value for a concentration of 0.05 M Amberlite LA–2 and, finally, increase concomitantly with the carrier concentration (Figure 11).

The dependence of acids mass flows on the rotation speed suggested that the overall separation process could be controlled by diffusion or chemical

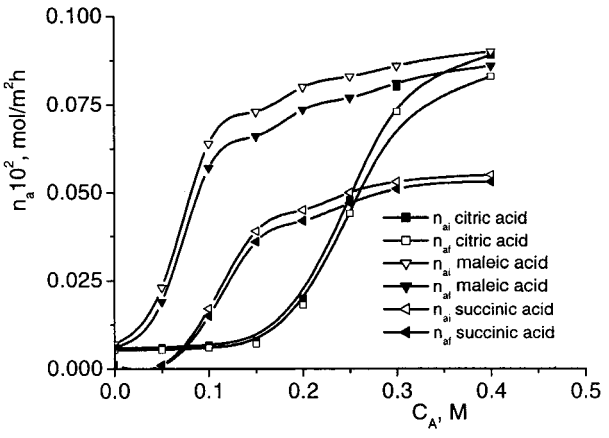

Figure 10. Influence of the carrier concentration on citric, maleic and succinic acid mass flows (citric acid concentration in the feed phase = 7.8 10^{-2} M, maleic acid concentration in the feed phase = 7.8 10^{-2} M, succinic acid concentration in the feed phase = 7.8 10^{-2} M, rotation speed = 500 rpm, pH of the feed phase = 3, pH of the stripping phase = 11)

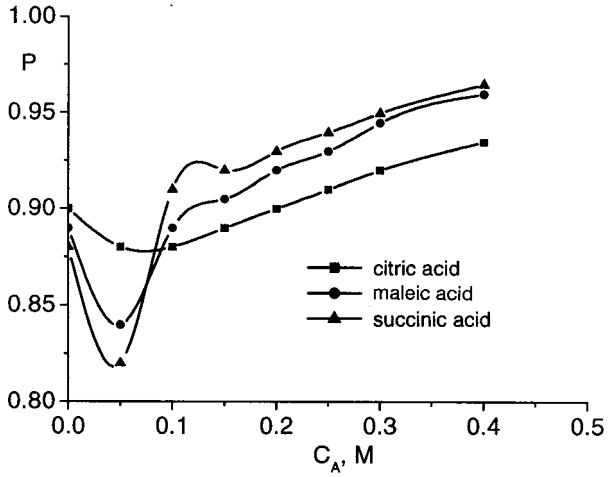

Figure 11. Influence of the carrier concentration on the citric, maleic and succinic acid permeability factors (citric acid concentration in the feed phase = 7.8 10^{-2} M, maleic acid concentration in the feed phase = 7.8 10^{-2} M, succinic acid concentration in feed the phase = 7.8 10^{-2} M, rotation speed = 500 rpm, pH of the feed phase = 3, pH of the stripping phase = 11)

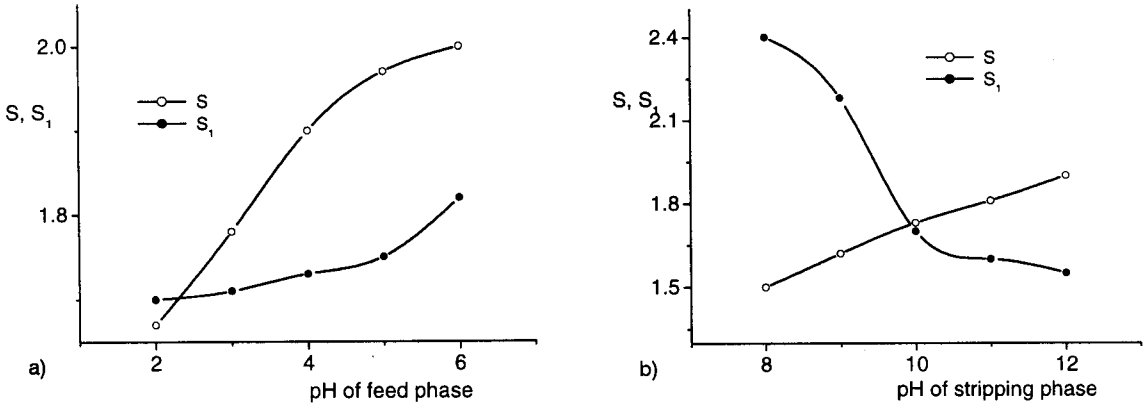

Figure 12. Influence of the pH of the feed and stripping phases on the selectivity factors (citric acid concentration in the feed phase = 7.8 10^{-2} M, maleic acid concentration in the feed phase = 7.8 10^{-2} M, succinic acid concentration in the feed phase = 7.8 10^{-2} M, carrier concentration = 0.3 M, rotation speed = 500 rpm; a – pH of the stripping phase = 11, b – pH of the feed phase = 3)

reactions. The increasing domain of mass flows indicates that the pertraction occurs in the diffusional regime, the extent of this domain being correlated with solute acidity. Therefore, the pertraction was diffusionally limited at rotation speeds below 700 rpm for citric and maleic acids and below 600 rpm for succinic acid. At higher values the chemical reactions become the limiting step.

These results suggested the possibility of the selective pertraction of maleic and succinic acids, the citric acid remaining in the raffinate phase. The maleic acid can, furthermore, be selectively separated from the concentrated phase. To confirm this hypothesis and establish the required conditions to reach a high selectivity of separation, the influences of the pH gradient between the aqueous phases, the carrier concentration and mixing intensity on the pertraction selectivity were studied.

The selectivity of pertraction was described by means of the *selectivity factor*, defined for the separation of maleic and succinic acids from citric acid as [16,17]:

$$S = \frac{n_{a_{f,maleic.acid}} + n_{a_{f,succinic.acid}}}{n_{a_{f,citric.acid}}} \quad (5)$$

and for the separation of maleic acid from succinic acid as:

$$S_1 = \frac{n_{a_{f,maleic.acid}}}{n_{a_{f,succinic.acid}}} \quad (6)$$

As can be observed from Figure 12, reduction of the pH gradient leads to an increase of the selectivity factors S and S_1, but the magnitude of this effect is rather different. Modification of the pH value of the feed phase induces a stronger effect on separation selectivity of secondary carboxylic acids from citric acid, while modification of the stripping phase pH exhibits a more pronounced effect on the separation selectivity of maleic acid from succinic acid.

These variations are due to the different ionization of the carboxylic acids due to modification of the aqueous solution pH, the efficiency of extraction and

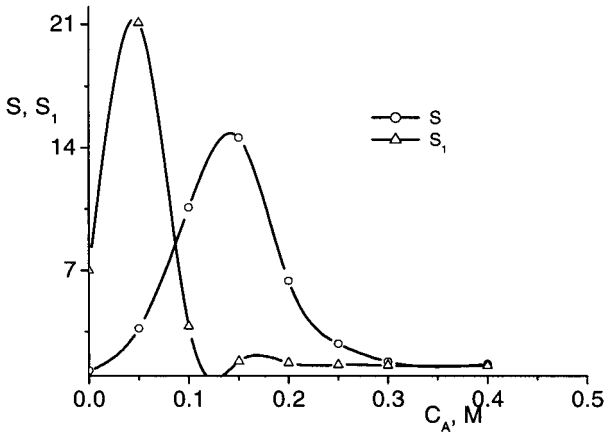

Figure 13. Influence of the carrier concentration on the selectivity factors (citric acid concentration in the feed phase = 7.8 10^{-2} M, maleic acid concentration in the feed phase = 7.8 10^{-2} M, succinic acid concentration in the feed phase = 7.8 10^{-2} M, rotation speed = 500 rpm, pH of the feed phase = 3, pH of the stripping phase = 11)

transport of the corresponding ionic species through the liquid membrane thus being different. It can be concluded that the selectivity of separation of maleic and succinic acids from citric acid could be enhanced by increasing the pH values of both aqueous phases and that the separation of maleic acid from succinic acid could be carried out by pertraction in a neutral pH domain.

The decisive influence of the carrier concentration on the pertraction selectivity is underlined by the dependence between the selectivity factors and this parameter (Figure 13).

Similarly to the variation of acid mass flows with carrier concentration, the experimental data indicate that the maximum selectivity for both the separation of secondary carboxylic acids from citric acid and for the separation of maleic acid from succinic acid is reached for an equimolecular ratio between Amberlite LA–2 and the extracted acids. Furthermore, an apporoximately

sevenfold increase in the selectivity factors could be achieved by optimizing the carrier concentration compared to optimizing by modification of the aqueous phase pH.

The effect of the mixing intensity on the selectivity factors S and S_1 is different. The selectivity of the separation of maleic and succinic acids from citric acid is not influenced by the rotation speed. However, the selectivity of the separation of maleic acid from succinic acid is amplified by mixing intensification. These variations confirm the previous results that indicated a diffusional resistance more accentuated in the case of maleic acid pertraction compared with that of succinic acid.

Therefore, although the separation of maleic and succinic acids from citric acid is not influenced by the mixing intensity, increase of the rotation speed leads to an increase of the acid mass flows through the liquid membrane and to the enhancement of the separation selectivity of maleic acid from succinic acid.

In order to verify these conclusions, the selective pertraction of maleic and succinic acids from a mixture similar to that obtained by citric fermentation (50 g/l of citric acid, 2.5 g/l of maleic acid, respectively 2.5 g/l of succinic acid) was performed, using the separation conditions that offer maximum selectivity and a high rate of transport through the liquid membrane (a carrier concentration of 0.04 M, a rotation speed of 500 rpm, a pH of the feed phase of 4 and a pH of the stripping phase of 11). The obtained results are given in Table 1.

It was observed that, by combining the favorable effects of the pertraction parameters, superior values of the selectivity factors were obtained. Consequently, the facilitated pertraction of carboxylic acids obtained by citric fermentation enables a high selectivity of separation and constitutes an advantageous alternative to techniques presently applied for the separation of citric acid from fermentation broths.

Using the experimental data, two mathematical models that describe the influence of the feed or stripping phase pH, the carrier concentration and impeller rotation speed on the selective pertraction of maleic and succinic acids from citric acid, respectively. The selective pertraction of maleic acid from succinic acid were established by statistical analysis. For this purpose, two second order factorial experiments were used.

Thus, the correlations between the selectivity factors S and S_1 and the considered parameters that influence the process can be written as follows [16]:

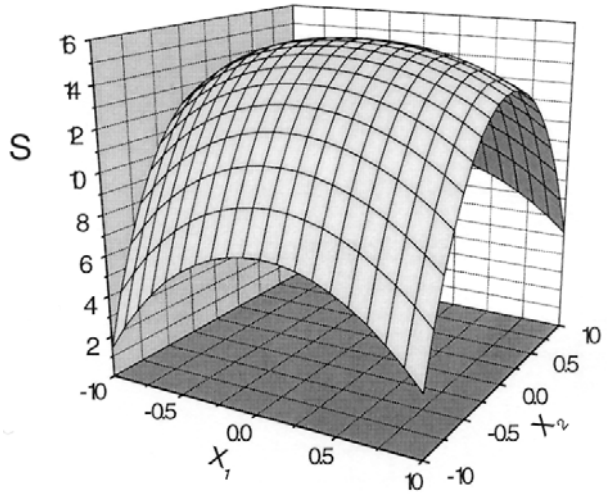

Figure 14. Cumulative effects of the pH of the feed phase (code x_1) and carrier concentration (code x_2) on the selectivity factor S (pH of the feed phase between 2 and 6, carrier concentration between 0 and 0.3 M)

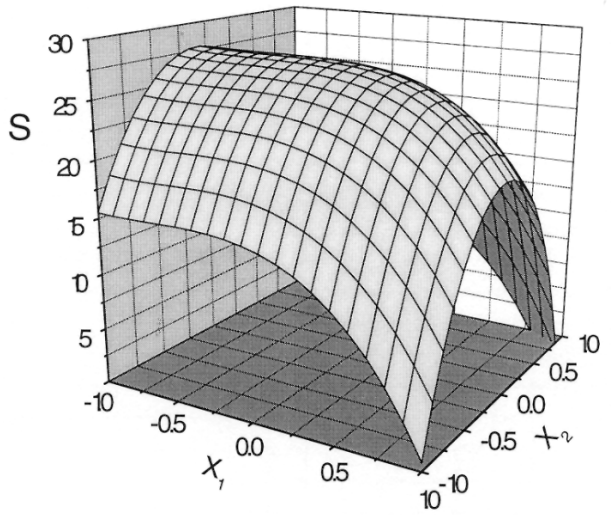

Figure 15. Cumulative effects of the pH of the stripping phase (code x_1) and carrier concentration (code x_2) on the selectivity factor S_1 (pH of the stripping phase between 6 and 10, carrier concentration between 0 and 0.15 M)

$$S = 15.61 + 0.46 \cdot x_1 + 0.64 \cdot x_2 + 0.04 \cdot x_3 + 0.027 \cdot \cdot x_1 \cdot x_2 + 0.0075 \cdot x_1 \cdot x_3 + 0.01 \cdot x_2 \cdot x_3 - 2.28 \cdot x_1^2 - - 7.70 \cdot x_2^2 - 3.24 \cdot x_3^2 \quad (7)$$

$$S_1 = 27.35 - 4.30 \cdot x_1 - 2.18 \cdot x_2 + 2.41 \cdot x_3 + 1.31 \cdot \cdot x_1 \cdot x_2 - 1.12 \cdot x_1 \cdot x_3 + 0.24 \cdot x_2 \cdot x_3 - 3.92 \cdot x_1^2 - - 14.67 \cdot x_2^2 - 3.04 \cdot x_3^2 \quad (8)$$

Table 1. Selective separation of maleic and succinic acids from citric acid.

Citric acid		Maleic acid		Succinic acid		Selectivity factor, S
$n_{ai}\,10^2$, mol/m^{2}h	$n_{af}\,10^2$, mol/m^{2}h	$n_{ai}\,10^2$, mol/m^{2}h	$n_{af}\,10^2$, mol/m^{2}h	$n_{ai}\,10^2$, mol/m^{2}h	$n_{af}\,10^2$, mol/m^{2}h	
0.45	0.29	5.8	4.8	2.7	2.3	24.5

where x_1, x_2 and x_3 designate the pH of the feed phase (for the calculation of S) or of the stripping phase (for the calculation of S_1), the carrier concentration and rotation speed, respectively.

Graphical presentations of the obtained regression equations are given in Figures 14 and 15 for a constant value of the rotation speed (x_3).

Consequently, the facilitated pertraction of carboxylic acids obtained by citric fermentation enables a high selectivity of separation and constitutes an advantageous alternative to techniques presently applied for the separation of citric acid from fermentation broths.

DIRECT EXTRACTION

Many biosynthesis processes are product inhibited, especially a batch system in which the final extracellular product concentration is high. The productivity of these fermentation processes can be significantly increased by direct recovery of the biosynthetic products from the fermentation broths during their formation.

Generally, two techniques can be used for direct separation from the fermentation broths [1,33]. The first employs an external separator, the fermentation broth being recirculated to the bioreactor (*ex–situ* separation), while the second achieves direct separation from the broth inside the bioreactor (*in–situ* separation). The methods usually applied for direct separation are: adsorption, distillation, precipitation, reverse osmosis, electrophoresis and liquid–liquid extraction.

The extraction of extracellular products directly from fermentation broths has been applied in a pilot plant for the separation of alcohols, carboxylic acids and some antibiotics, taking into account the compatibility between the microorganisms and the solvent:

– solvent toxicity

– high values of the distribution coefficient of the solute between the broth and the solvent

– the formation of emulsions with low stability

– easy recovery of the solvent after extraction.

Moreover, the fermentation broth characteristics are an important factor, especially the viscosity and rheological behaviour, which significantly influence the mass transfer rate.

In–situ extraction offers certain advantages over extraction in a certain type of equipment:

– in the second case, the product losses could be considerable

– if the cellular mass is filtered before the extraction, the product concentration in the liquid is reduced by washing the precipitate on the filter

– *in–situ* extraction avoids product inhibition

– extraction inside the bioreactor simplifies the biotechnological flux.

However, a major problem in direct extraction is the choice and design of adequate extraction equipment, because it is necessary to obtain a high mass transfer area by dispersing the solvent inside the fermentation broths which exhibit non–Newtonian behaviour and have high viscosities, taking into account the influence of shear stress on the microorganisms.

In our experiments, we studied the direct separation of beta–lactamic antibiotics by physical and reactive extraction and of butanol by physical extraction from simulated and real fermentation broths.

The direct extraction of beta–lactamic antibiotics

The separation of **Penicillin G** from fermentation broths is the main problem of the biotechnological process. Currently, physical extraction with butyl acetate from filtered or non–filtered broths is applied on an industrial scale. This technique requires an aqueous phase pH of 2– 2.5, thus leading to chemical inactivation of the antibiotic [27,34]. As stated in the previous paper, this drawback can be avoided by using reactive extraction with a high molecular weight amine, a method which offers the possibility to extend the pH interval required for the maximum separation yield to pH=4.5–5 [1,29].

Furthermore, another problem is product inhibition, especially for batch fermentations in which the final concentration of Penicillin G is high, a phenomenon that can be avoided by applying direct extraction of the antibiotic during the fermentation cycle. The performances of direct extraction are strongly influenced by the rheological characteristics of the broth (namely, the high apparent viscosity), as well as by the presence of some secondary compounds which can precipitate during the extraction process (proteins), or can be co–extracted (carboxylic acids, amino acids). The literature offers few information concerning Penicillin G *ex–situ* extraction using centrifugal extractors [1,35,36]. On a pilot scale, it was successfully achieved in an integrated process of Penicillin G fermentation and conversion to 6–aminopenicillanic acid by *penicillin G–amidase*, Penicillin G being extracted *in–situ* and the fermentation broth being recirculated [18,21].

In order to analyse the influence of the broth characteristics, biomass presence and separation conditions on the efficiency of Penicillin G direct separation by physical or reactive extraction, we carried out the experiments using extraction equipment of the Lewis cell type for simulated broths (solutions of the sodium salt of carboxymethylcelullose) and suspensions of *Penicillium chrysogenum* biomass. For all the studied systems, the influence of the considered parameters was included in some correlations describing the antibiotic mass flow or mass transfer coefficient [37–40].

In the case of direct separation from simulated fermentation broths by reactive extractied with Amberlite LA–2, the viscosity exhibited a significant influence on antibiotic diffusion from the aqueous phase to the interface, For example, as can be seen from Figure 16,

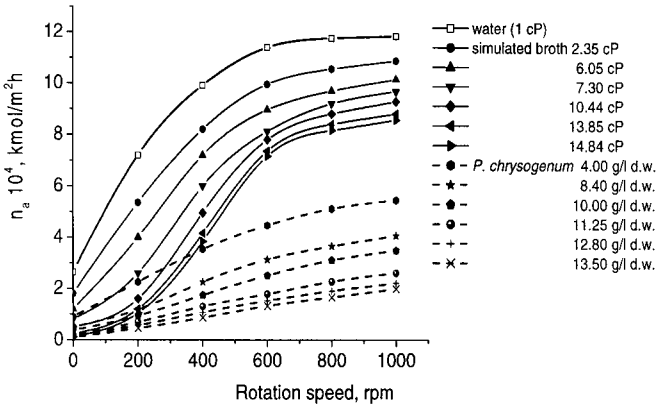

Figure 16. Influence of the mixing intensity on Penicillin G mass flow from simulated broths and P. chrysogenum suspensions to organic solvent (Amberlite LA–2 concentration 80 g/l)

for a rotation speed of 600 rpm and a viscosity range of 1–14.84 cP, the antibiotic mass flow decreased by about 40%.

The form of the curves also changed with increasing viscosity, too. Thus, for 1 cP and a rotation speed greater than 900 rpm, the specific mass flow remained constant, the interfacial chemical reaction between Penicillin G and Amberlite LA–2 becoming the limiting step [22]. But, even for rotation speeds greater than 900 rpm, a continuous increase of the mass flow was obtained by increasing the apparent viscosity of the aqueous solution. This phenomenon is explained by an increase of the importance of the resistance due to Penicillin G diffusion.

For direct extraction of the antibiotic from *P. chrysogenum* suspensions, the presence of biomass induces some drawbacks, namely:

– the appearance of a supplementary diffusional resistance to the overall mass transfer process,

– in the extraction equipment the biomass can be mechanically destroyed with the elimination of some secondary compounds into the fermentation broths. These compounds can be co–extracted, or can precipitate, the separation thus becoming more difficult,

– solvent loss due to its retention in the biomass.

As was observed in our experiments, for the same viscosity values, the presence of biomass in the aqueous phase induces a new resistance of the diffusional type. Thus, the presence of *P. chrysogenum* mycelia exhibits a significant negative influence on antibiotic diffusion from the aqueous phase to the interface. Compared to the extraction from pure aqueous solutions or simulated broths, the antibiotic mass transfer rate strongly decreases in the presence of biomass (Figure 16). For example, for a rotation speed of 600 rpm, the Penicillin G specific mass flow extracted from *P. chrysogenum* suspension with a biomass concentration of 13.5 g/l d.w. decreased by about 8.6 times compared to pure aqueous solution and by about 3 times compared with simulated broths without biomass, under identical experimental conditions. The obtained data indicated a significant increase of the resistance to mass transfer due to antibiotic diffusion towards the interface between the aqueous and organic phase. This phenomenon is the result of the cumulative effect of the apparent viscosity and biomass presence. Consequently, antibiotic diffusion into the aqueous phase controls the overall mass transfer for the whole impeller speed domain used, the resistance due to the interfacial reaction between Penicillin G and Amberlite LA–2 becoming insignificant.

Compared to reactive extraction from pure aqueous solutions, the values of the Penicillin G mass transfer coefficients obtained for reactive extraction from simulated and real broths were significantly reduced. For example, for a rotation speed of 600 rpm and a biomass concentration of 13.5 g/l d.w., the overall mass transfer coefficients for both phases decreased by about 27 times compared with that for pure aqueous solutions [40].

The mathematical description of the apparent viscosity or biomass effect on Penicillin G mass transfer was made by means of correlations for the mass flow or mass transfer coefficients [38–41]:

• reactive extraction from simulated fermentation broths:

for η_a = 2.35 – 7.30 cP

$$K_a = e^{-(6.65+0.39\cdot\eta_a)} \cdot N^{0.868+0.072\cdot\eta_a} \quad (9)$$

$$K_o = e^{-(9.61+0.197\cdot\eta_a)} \cdot N^{1.128+0.11\cdot\eta_a} \quad (10)$$

for η_a = 7.30 – 14.84 cP

$$K_a = e^{-(9.54+0.207\cdot\eta_a)} \cdot N^{1.528+0.020\cdot\eta_a} \quad (11)$$

$$K_o = e^{-(11.4+0.471\cdot\eta_a)} \cdot N^{10.452+0.128\cdot\eta_a} \quad (12)$$

• reactive extraction from *P. chrysogenum* suspensions (C_X bellow 13.5 g/l d.w.):

$$K_a = e^{-(7.20+0.170\cdot C_X)} \cdot N \quad (13)$$

$$K_o = e^{-(9.87+0.170\cdot C_X)} \cdot N \quad (14)$$

The direct extraction of butanol

The production of **acetone** and **butanol** by *Clostridium acetobutylicum* is impaired by butanol inhibition (acetone inhibition can be neglected compared to that of butanol). The literature indicated that the following solvents have been used for the *in–situ* extraction of butanol: dibutyl phthalate, oleyl alcohol and n–dodecanol [33,41–43]. In this context, we studied the direct extraction of butanol from simulated fermentation broths with 1,2–dichloroethane, the solvent being dispersed by a nozzle [44,45]. The aims of these experiments were to establish the influence of drop size on the solvent drop terminal velocities through the simulated broth and the dependence between the

butanol mass transfer coefficients, drop size and continuous phase viscosity.

The dependence between the solvent drop fall velocities and the drop size indicated a maximum corresponding to the drop pulsation appearance, this phenomenon reducing the drop velocity. By increasing the solution viscosity, the drop pulsations were reduced and the falling drop trajectory became linear, compared to the undulated drop fall in low viscosity solutions. Moreover, for the same diameter of the drops, the corresponding velocities were decreased by viscosity increase, reduction of the Reynolds number, thus affecting the mass transfer rate [44].

Furthermore, the coalescence rate of the solvent drops at the column bottom was hindered by the high viscosity. If the continuous phase viscosity is high, the solution between the drops cannot flow rapidly enough and remains as a dispersed phase included in the solvent phase. In real fermentation processes the increase of coalescence time, the and hindrance of emulsion breakage are both the result of the viscosity increase and of the adsorption of certain fermentation broth components (soluble proteins or cells) on the drop surface.

The experimental variation of butanol mass transfer coefficients with drop size was analogous to that of the terminal velocity, the maximum value corresponding to the maximum of the falling drop velocity or to the maximum of the Reynolds number, respectively (Figures 17).

By increasing the apparent viscosity of the simulated broth a decrease of the maximum value of the mass transfer coefficient was observed. For example, the maximum value of the butanol mass transfer coefficient for water was about 62 times greater than its maximum value for a solution with 44.6 cP viscosity [44]. Moreover, an increase of the drop diameter corresponding to the maximum value of the mass transfer rate was recorded with increasing apparent viscosity.

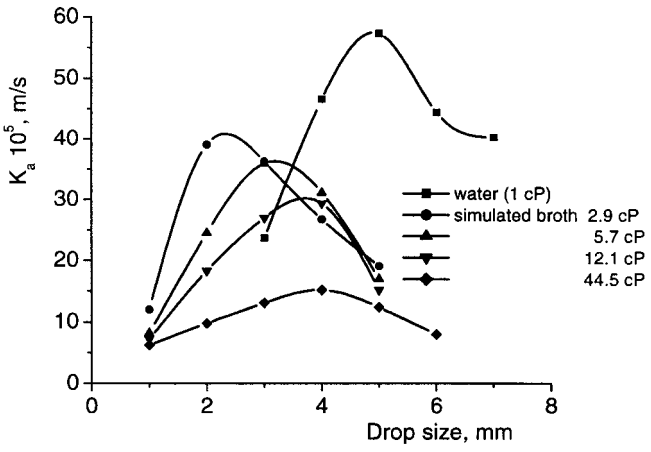

Figure 17. Influence of solvent drop size on the butanol mass transfer coefficient

As expected, for the same solvent drop size the mass transfer coefficients were reduced with increasing continuous phase viscosity, the mass transfer rate decrease being more significant for the largest drops. The observed phenomenon is explained by pulsations of the drops, which are important, especially for the largest drops in low viscosity solutions, because of the increase of the Reynolds number with the appearance of drop pulsations.

Criterial correlations for the Sherwood number were established using of the experimental data and according to the literature. These correlations can be used to calculate the butanol mass transfer rate from the aqueous continuous phase to solvent drops, being adequate for Re numbers lower than 650 and an apparent viscosity lover than 45 cP. The general expression of the proposed correlations is [45]:

$$Sh = 3 \cdot 10^{-7} \cdot Re^{\alpha} \cdot Sc^{1.667} \quad (15)$$

The significant influence of the viscosity and the non–Newtonian behaviour of the aqueous solution was described by the exponent α that decreased about 3 times from low viscous solutions to higher viscous ones (the exponent α decreased from 1.95 to 0.67 with an apparent viscosity increase from 5.7 to 45 cP).

Similarly to the case of the direct extraction of Penicillin G from *P. chrysogenum* suspensions, the values of the butanol mass transfer coefficients for a real fermentation could be lower than the values determined in these studies owing to the additional resistance to mass transfer due to the adsorption of soluble proteins or cells on the drop surface.

CONCLUSIONS

The reactive extraction, pertraction and direct extraction of biosynthetic products constitute advantageous alternatives to conventional separation methods because they reduce the number of stages required for an efficient separation and, therefore, the corresponding energy and material consumption. For these reasons, these extraction techniques have considerable potential, which is required for the further development of many biotechnologies, and represent very attractive research domains for biochemical engineers. The actual studies are focused to extending their area of application to other biosynthetic products which can be more efficiently separated and to their scaling–up to the industrial level.

NOTATIONS

C_A – carrier or extractant concentration in the organic phase
C_X – biomass concentration
K – overall mass transfer coefficient of the solute
N – stirrer rotation speed
n_a – mass flow of the solute from the aqueous to the organic phase
n_{ai} – initial mass flow of the solute from the feed phase to the membrane phase
n_{af} – total (final) mass flow of the solute from the feed phase through the membrane phase to the stripping phase
P – permeability factor
Re – Reynolds number

S, S_1 – selectivity factors
Sc – Schmidt number
Sh – Sherwood number

Subscript

aq – aqueous phase
o – organic phase

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IZVOD

NEW EXTRACTION TECHNIQUES ON BIOSEPARATIONS
2. PERTRACTION, DIRECT EXTRACTION

(Pregledni rad)

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U drugom delu ovog preglednog rada prikazuju se originalni rezultati separacije nekih proizvoda biosinteze (antibiotici, karboksilne kiseline, alkoholi) putem pertrakcije i direktne ekstrakcije iz fermentacione komine bez prethodne filtracije i odvajanja biomase. Utvrđeni su eksperimentalni uslovi na kojima je moguće ostvariti maksimalnu efikasnost separacije i razvijeni odgovarajući matematički modeli koji opisuju procese separacije. Za sve ispitivane sisteme koji se detaljno analiziraju u radu pokazano je da se ovim separacionim tehnikama znatno uprošćava tehnološki postupak sinteze odgovarajućih proizvoda i smanjuje njihova proizvodna cena.

Ključne reči: Pertrakcija • Nosač • Direktna ekstrakcija • Amberlit LA–2 • Beta–laktamski antibiotici • Prekursori • Karboksilne kiseline • Butanol • *P. chrysogenum* •

Key words: Pertraction • Carrier • Direct extraction • Amberlite LA–2 • Beta–lactamic antibiotics • Precursors • Carboxylic acids • Butanol • *P. chrysogenum* •