# Comparative Studies of Free and Facilitated Pertraction of Erythromycin from *Streptomyces erythreus* Broths

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Original scientific paper
Received: February 12, 2009
Accepted: December 18, 2009

The paper presents the results obtained by analyzing the free and facilitated pertraction of Erythromycin from *S. erythreus* broths with cells concentration between 5 and 20 g $L^{-1}$ DM. using dichloromethane as liquid membrane and D2EHPA as carrier. The pertraction has been carried out in pseudosteady-state regime, using the U-shaped pertraction cell that allows obtaining and easily maintaining the free liquid membrane. The increase of the biomass concentration in the feed phase led to the significant reduction of the initial and final mass flows of antibiotic, the addition of carrier in the liquid membrane attenuating this effect. Compared with the pertraction from simulated broths having the same apparent viscosity, but without solid phase, the mass flows are reduced up to 5 times for free pertraction, respectively up to 3 times for facilitated pertraction. Only the membrane permeability was favorably influenced by the biomass presence and accumulation, but this effect did not lead to the real increase of process efficiency.

*Key words:*
Erythromycin, D2EHPA, liquid membrane, pertraction, mass flow, permeability factor

## Introduction

Erythromycin is a macrolide antibiotic biosynthesized by *Streptomyces erythreus* on glucose substrate, being very active against the infections produced by *staphylococcus*, gram-positive *bacterium*, etc. Generally, the pharmaceutical forms are as propionate or succinate salt.[1]

Erythromycin exhibits a significant effect of product inhibition, this leading to the diminution of microbial activity or cells lysis with the antibiotic accumulation in the broth. The phenomenon can be avoided by direct removal of antibiotic during the fermentation process.[2]

At industrial scale, the antibiotic separation from fermentation broths is carried out by physical extraction with butyl acetate, with or without preliminary filtration of biomass, followed by its reextraction with diluted solutions of hydrochloric acid.[1] The presence of aminic group in the antibiotic structure promotes its ionization in aqueous solution, according to the following equilibrium ($pK_b = 8.8$, at 25 °C):[1]

$$\text{Er-N(CH}_3)_2 + \text{H}_2\text{O} \rightleftharpoons \text{Er-N}^+\text{H(CH}_3)_2 + \text{HO}^-$$

Due to Erythromycin dissociation and to the low polarity of butyl acetate, the physical extraction is possible only in alkaline pH-domain, the maximum extraction yields being reached for the pH-value of aqueous phase greater than 9.[2] In these conditions, some other components of fermentation broths, which are non-dissociated at the extraction pH-value, can be supplementary extracted, the ulterior purification of the antibiotic becoming more difficult.

For the mentioned reason, a new separation method of Erythromycin by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) has been previously investigated.[3] This method has been developed and applied as extraction and transport through liquid membrane (pertraction), the addition of D2EHPA inside the liquid membrane (facilitated pertraction) allowing the increase of the antibiotic mass flow compared with the free pertraction without a carrier.[4,5] Thus, significant increase of separation efficiency can be reached at pH-values close to the optimum pH-domain for fermentation process, this method being recommended for direct removal of antibiotic during the fermentation.

These results correspond to the separation of Erythromycin from aqueous solution. In the case of

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solutions having rheological behavior and apparent viscosity similar to the *S. erythreus* broths (simulated fermentation broths), the efficiency of pertraction was strongly affected.[6] The increase of apparent viscosity of feed phase from $\eta = 1$ to 30 mN s m$^{-2}$ led to the maximum decrease of antibiotic mass flows by the factors of 42.5 and 7.5 for free and facilitated pertraction, respectively.

In continuation and development of the previous work concerning the mechanism and the influencing factors of the Erythromycin pertraction from simulated fermentation broths, the aim of this paper is to analyze comparatively the free and the facilitated pertraction of this antibiotic from non-filtered *S. erythreus* broths, with the purpose of quantifying the influence of the biomass on the separation performance. The possible influences of the other components of the broths will be analyzed in the future experiments.

## Materials and method

The experiments have been carried out using the pertraction equipment that allows for obtaining and easily maintaining the free liquid membrane.[7] The pertraction cell has been described in the previous papers and consists of a U-shaped glass pipe having an inner diameter of 45 mm and a total volume of 450 mL, the volume of each compartment being of 150 mL.[8,9]

The aqueous solutions and the solvent phase have been independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having a rotation speed of 500 rpm. The area of mass transfer surface, both for extraction and re-extraction, was $A = 15.9$ cm$^2$. The interfaces between the phases remained flat, and hence the interfacial area constant.

The experiments have been carried out in a pseudosteady-state regime, at the steady-state conditions related to the aqueous phases and unsteady-state mode related to the membrane phase. The aqueous solutions have been separately fed with a volumetric flow rate of $Q = 1.9$ L h$^{-1}$. Each experiment was carried out for about 20 min, the sampling starting 10 min from the beginning.

The liquid membrane phase consisted of a solution of $\gamma = 0$–100 g L$^{-1}$ (0–0.30 mol L$^{-1}$) D2EHPA (di-(2-ethylhexyl) phosphoric acid) (≥ 95 %, Sigma Chemie GmbH) as carrier dissolved in dichloromethane (≥ 99 %, Aldrich).

The feed phases were suspensions of *S. erythreus* with biomass concentration varying between 5 and 20 g L$^{-1}$ DM. The biomass was filtered from the fermentation broths, washed with distillated water and used for feed phase preparation. 1.83 g L$^{-1}$ Erythromycin (2.55 mmol L$^{-1}$) (≥ 99 %) (Merck) was dissolved in the feed phases. The pH-value of feed phases varied between 2 and 10.

The stripping phases consisted of aqueous solutions of hydrochloric acid or sodium hydroxide, the used pH-domain being of 2 to 10.

The pH adjustment of both aqueous phases was made with 5 % solutions of hydrochloric acid or sodium hydroxide, respectively. The pH-values of both aqueous phases were determined using a digital pH-meter of HI 213 (Hanna Instruments) type and recorded throughout each experiment.

The pertraction was analyzed by means of Erythromycin initial and final mass flows and permeability factors. The initial mass flow represents the solute mass flow from the feed phase to the liquid membrane, while the final (overall) mass flow represents the mass flow from the liquid membrane to the stripping phase. The permeability factor conveys the capacity of the solute transfer through liquid membrane, and has been defined as the ratio between the final mass flow and the initial mass flow of solute.[7,9,10] Because the experiments have been carried out in unsteady-state mode related to the membrane phase, the duration of experiments will affect the permeability factor value. As it was observed, this influence becomes important only for duration over minimum 45 min.

These parameters have been calculated by determining the antibiotic concentrations in the feed and stripping phases and by using the mass balance for the pertraction system. The antibiotic concentrations have been measured by the spectrophotometric technique, using a spectrophotometer of CAMSPEC M550 type. Therefore, the absorption of the aqueous solutions has been determined at $\lambda = 485$ nm.[1]

Each experiment was carried out three or four times under identical conditions. The average value of the measurements was used in the calculations. The maximum experimental error was ± 5.11 %.

## Results and discussion

The main condition for inducing the solute transfer from the feed phase to the stripping phase is to create and maintain the gradient of a certain property between the two phases. For most pertraction systems, this property is pH-value, the solute being transported against its concentration gradient as long as the pH-gradient between the two aqueous phases is maintained.[8]

In the case of Erythromycin pertraction from aqueous solutions, the previous experiments under-

lined a major influence of pH-gradient and carrier concentration on separation efficiency.[5] If the pertraction were carried out from simulated broths, their higher viscosity exhibited an important negative influence, by means of the resistance to the solute diffusion inside the feed phase.[6] The apparent viscosity induced a positive effect only on the permeability factor, due to the diminution of the initial mass flows.

Similar to the direct extraction of other biosynthetic compounds from the fermentation broths,[11–16] the presence of biomass could additionally affect the Erythromycin pertraction, owing to the following phenomena:

– appearance of supplementary resistance to the antibiotic transfer from the feed phase to the liquid membrane due to the physical barrier induced by the cell adsorption to the interface

– increase of the apparent viscosity of the feed phase, and, consequently, the amplification of antibiotic diffusional resistance

– mechanical lysis of cells, as the result of the shear stress promoted by the impellers, with the release of the cytoplasmatic compounds which can be co-extracted (amino acids) or can precipitate (proteins).

For quantifying the *S. erythreus* cells influence, the experiments have been carried out respecting the similar experimental conditions with those considered for pertraction from simulated broths without biomass, but having the same apparent viscosity.[6]

The study on Erythromycin pertraction from aqueous solutions or simulated broths indicated that the free pertraction is not possible for the pH-value of feed phase, $pH_f$, lower than 4, due to the pronounced antibiotic ionization.[3,6] By increasing the $pH_f$ above this level, both the initial and final mass flows are strongly increased, as the result of the increase of physical extraction efficiency. This dependence between the mass flows and the pH of feed phase is respected also in the case of Erythromycin free pertraction from *S. erythreus* suspensions (Fig. 1). But, from Fig. 1 it can be observed that the accumulation of biomass leads to the significant decrease of the initial mass flow (by increasing the biomass concentration from 0 to 20 g $L^{-1}$ DM, the initial mass flow has been reduced by about 7 times).

Independently of the biomass concentration in the feed phase, the permeability factor is continuously reduced by the increase of $pH_f$ over 4, this suggesting that $pH_f$ exhibits a more important influence on the initial mass flow than on the final one (Fig. 2). The magnitude of the effect of $pH_f$ is diminished with the biomass accumulation, because

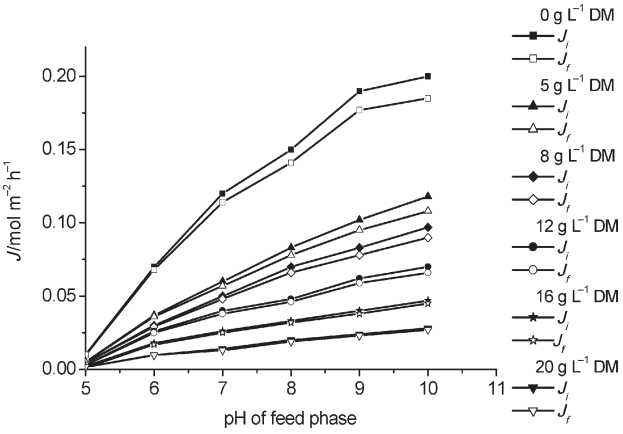



Fig. 1 – *Influence of pH-value of feed phase on mass fluxes of Erythromycin for free pertraction from S. erythreus broths (pH of stripping phase = 4, rotation speed 500 rpm)*

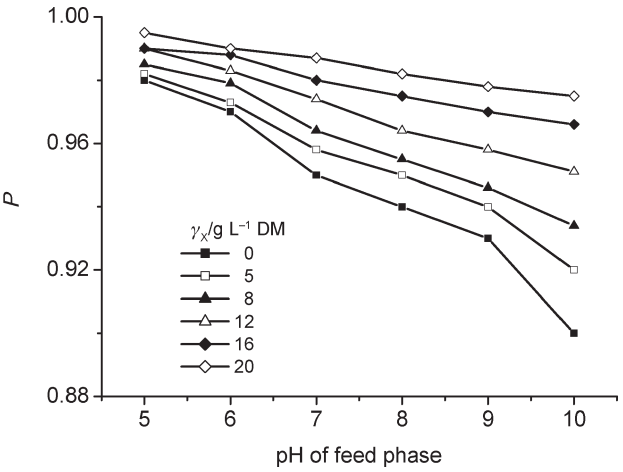
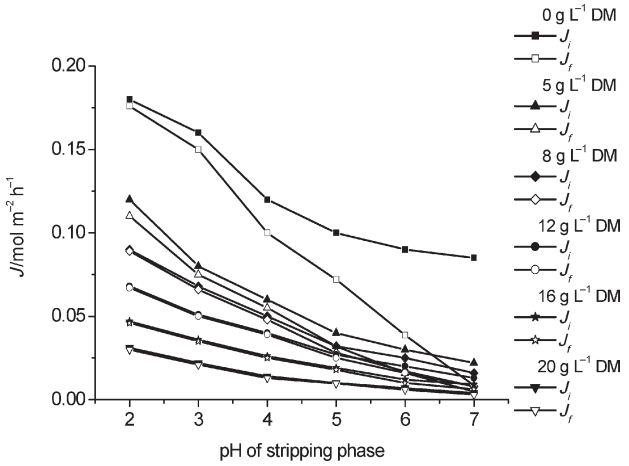


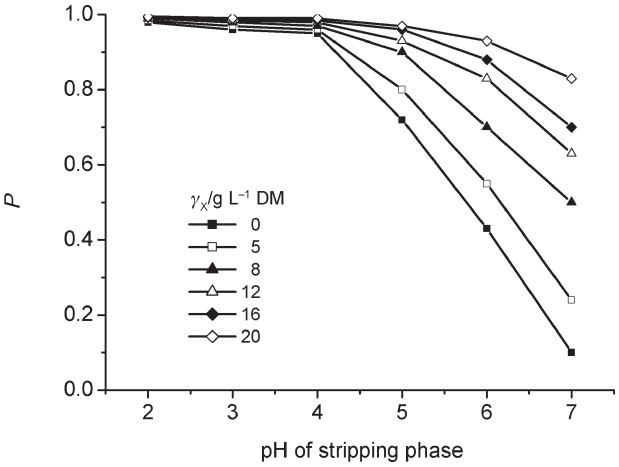

Fig. 2 – *Influence of pH-value of feed phase on permeability factor for free pertraction from S. erythreus broths (pH of stripping phase = 4, rotation speed 500 rpm)*

the amount of antibiotic extracted in the liquid membrane becomes lower, thus facilitating its almost complete reextraction in the stripping phase. For this reason, for *S. erythreus* concentration of 20 g $L^{-1}$ DM, the permeability factor varied slowly between 0.995 and 0.975 for the considered $pH_f$ domain. Therefore, compared with the antibiotic free pertraction from simulated broths, the presence of solid phase diminished the differences between the initial and final mass flows, due to its negative effect on the solute transfer from the feed phase to the liquid membrane.

The increase of the stripping phase pH-value, $pH_s$, leads to significant reduction in the antibiotic initial mass flow, this effect being more pronounced with the microorganism accumulation in the feed phase (Fig. 3). In this case, the negative influence of the biomass is amplified by increasing $pH_s$, as the result of the supplementary effect of the neutral domain of $pH_s$ (by increasing the biomass con-




F i g . 3 – *Influence of pH-value of stripping phase on mass fluxes of Erythromycin for free pertraction from S. erythreus broths (pH of feed phase = 7, rotation speed 500 rpm)*




F i g . 4 – *Influence of pH-value of stripping phase on permeability factor for free pertraction from S. erythreus broths (pH of feed phase = 7, rotation speed 500 rpm)*

centration from 0 to 20 g $L^{-1}$ DM, the initial mass flow of Erythromycin decreasing for about 5.8 and 19.2 times at $pH_s$ of 2 and 7, respectively.)

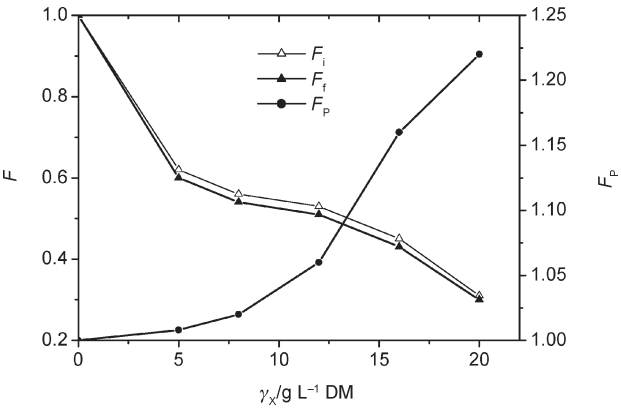
The decreasing of the final mass flow is more important, this parameter reaching the value 0 for $pH_s$ over 7 (at this value of $pH_s$ the pH-gradient between the feed and stripping phases becomes 0). In accordance with the conclusions drawn from the previous studies,[5,6] in these conditions the system tends towards the stationary state, and, implicitly, the antibiotic extraction and reextraction to the two aqueous boundary layers formed at the interfaces between the aqueous phases and liquid membrane tend to become equal. As mentioned above, the presence of biomass diminishes the difference between the initial and the final mass flows of Erythromycin.

The increase of *S. erythreus* concentration induces the supplementary decrease of antibiotic final mass flow. Thus, comparatively with the pertraction from water, at 20 g $L^{-1}$ DM *S. erythreus* the final mass flow of Erythromycin is reduced by about 2.8–5.8 times, this effect being amplified at lower values of $pH_s$.

Owing to the more important reduction of the final mass flow, the permeability factor decreases strongly with the increase of $pH_s$. This variation, plotted in Fig. 4, suggests that at higher values of stripping phase pH the antibiotic accumulation rate inside the liquid membrane exceeds that of its reextraction. But, as the result of the diffusional resistance to the extraction in the membrane phase, the accumulation of *S. erythreus* in the feed phase leads to the equalization of the initial and final mass flows. Consequently, the negative influence of the stripping phase pH is attenuated at $pH_s$ 7 (at this value of $pH_s$ the permeability factors increased for 8.3 times with the biomass increase from 0 to 20 g $L^{-1}$ DM).

As presented above, the additional resistance to the antibiotic transfer from the feed phase to liquid membrane due to the physical barrier created by the cells adsorption to the interface diminishes the mass flows compared with the Erythromycin pertraction from simulated broths having the same viscosities but without biomass. For quantifying this effect, the *reduction factor*, F, has been defined as the ratio between the initial mass flows corresponding to the pertraction from *S. erythreus* broths and simulated broths, $F_i$, respectively between the final mass flows recorded for the same two cases, $F_f$.

The influence of the biomass, separately from that of the apparent viscosity, is clearly underlined in the Fig. 5, which indicates the reduction of over 3 times of the factors $F_i$ and $F_f$ with the accumulation of biomass to 20 g $L^{-1}$ DM. This effect is stronger for *S. erythreus* concentration increase up to 5 g $L^{-1}$ DM, thus emphasizing the important role of solid phase in hindering the pertraction.




F i g . 5 – *Influence of biomass concentration on factors F and $F_P$ for free pertraction from S. erythreus broths (pH of feed phase = 7, pH of stripping phase = 7, rotation speed 500 rpm)*

The analysis of the biomass effect on the permeability through liquid membrane has been made by means of the factor $F_P$, calculated as the ratio between the permeability factors obtained for pertraction from *S. erythreus* broths and simulated broths respectively, in the similar experimental conditions. As can be seen from Fig. 5, the dependence between the factor $F_P$ and biomass concentration is opposite to those discussed above. Because the antibiotic concentration at the interface between the feed phase and liquid membrane is more diminished by *S. erythreus* cells accumulation than by increasing the apparent viscosity, the final mass flow tends to become equal to the initial one at higher concentration of biomass. Therefore, the permeability factors are higher for the free pertraction from *S. erythreus* broths, the factor $F_P$ increasing continuously in the considered domain of biomass concentration.

The addition of the carrier, D2EHPA, in the liquid membrane offers the possibility to carry out the pertraction also at pH-values lower than 4, due to the modification of the mechanism of Erythromycin extraction in the dichloromethane phase. Thus, the previously proposed and verified mechanism of antibiotic reactive extraction with D2EHPA occurs by means of an interfacial reaction of ionic exchange type controlled by the pH of aqueous phase:[3]

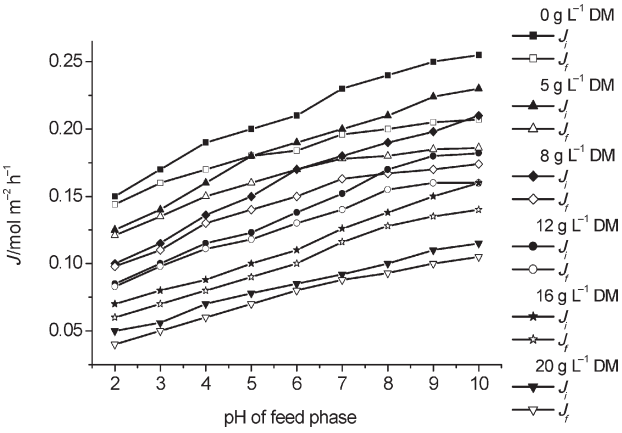
$$\text{Er-N}^+\text{H(CH}_3)_{2\ (aq)} + \text{HP}_{(o)} \rightleftharpoons \rightleftharpoons \text{Er-N}^+\text{H(CH}_3)_2\text{P}^-_{(o)} + \text{H}^+_{(aq)}$$

where HP is the carrier.

According to the extraction mechanism, the carrier reacts only if Erythromycin exists in aqueous solution in its cationic forms, therefore at an acidic pH-value of the solution ($pH_f < 4$).

By increasing the $pH_f$-value, the physical extraction of Erythromycin becomes more important, this leading to the favorable effect on its initial mass flow (Fig. 6). The final mass flow is also accelerated with the $pH_f$ increase, this influence being less pronounced for $pH_f > 7$. The maximum level of reextraction rate, which is recorded only for facilitated pertraction, suggests that a higher amount of antibiotic is quickly extracted in the membrane phase, its complete reextraction in the stripping phase being impossible.

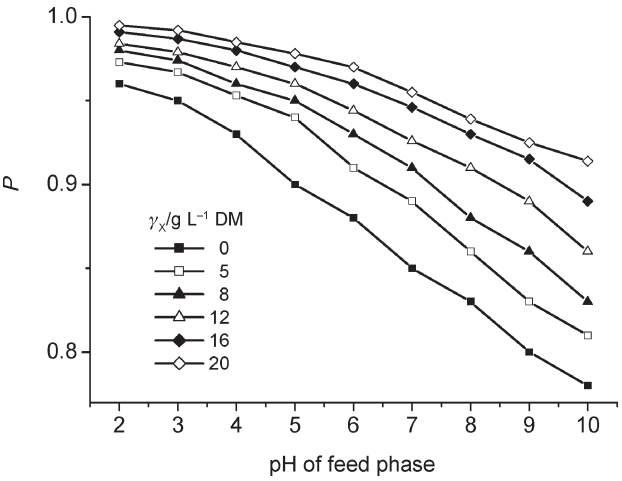
Although the biomass accumulation in the feed phase does not modify the above-discussed variations of the antibiotic mass flows, it reduces their values obtained for a certain $pH_f$. From Fig. 6 it can be observed that by increasing the concentration of *S. erythreus* in the considered domain, the initial and final mass flows of Erythromycin decreased



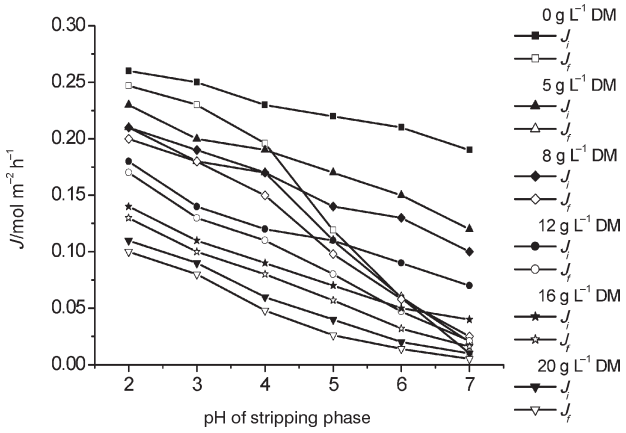

F i g . 6 – *Influence of pH-value of feed phase on mass fluxes of Erythromycin for facilitated pertraction from S. erythreus broths (pH of stripping phase = 2, carrier concentration $\gamma = 40$ g L$^{-1}$, rotation speed 500 rpm)*

about 1.6–3.6 times, this reduction being more important at lower values of $pH_f$. However, due to the presence of carrier and, implicitly, the antibiotic transfer from the feed phase to liquid membrane by reactive extraction, the negative effect of the increased diffusional resistance induced by the viscosity and biomass is attenuated. Therefore, the decreasing of the mass flows has been less pronounced than in the case of free pertraction.

According to the variation plotted in Fig. 7, the increase of the $pH_f$ leads to the decrease of the permeability factor. Due to reaching the maximum level of reextraction rate, this influence is stronger than that observed for the free pertraction of Erythromycin. The amplitude of this effect is diminished with the accumulation of biomass in the feed phase, due to the more pronounced reduction




F i g . 7 – *Influence of pH-value of feed phase permeability factor for facilitated pertraction from S. erythreus broths (pH of stripping phase = 2, carrier concentration $\gamma = 40$ g L$^{-1}$, rotation speed 500 rpm)*

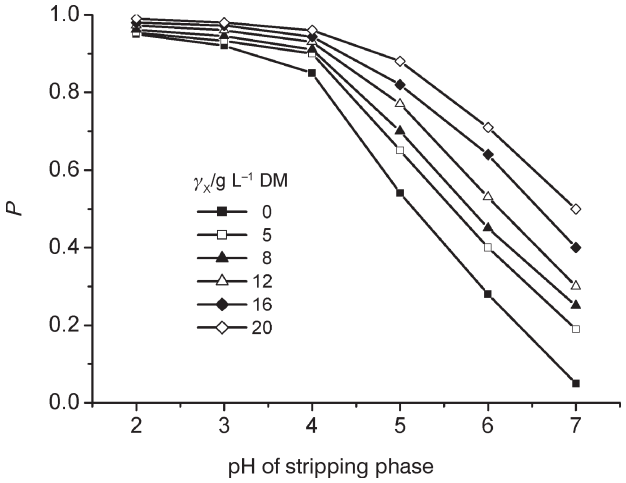



F i g . 8 – *Influence of pH-value of stripping phase on mass fluxes of Erythromycin for facilitated pertraction from S. erythreus broths (pH of feed phase = 4, carrier concentration* $\gamma = 40$ *g* $L^{-1}$*, rotation speed 500 rpm)*

of the initial mass flow compared with the final one. For this reason, the permeability factor increases for 1.03–1.20 times with the *S. erythreus* concentration increase to 20 g $L^{-1}$ DM, the highest value being recorded for the $pH_f$-value corresponding to the dominant contribution of free pertraction ($pH_f$ 10).

Moreover, compared with the antibiotic free pertraction, the addition of the carrier in the liquid membrane diminishes the negative influence of biomass on the permeability factor.

Independently of the microorganism concentration in the feed phase, the dependence between the mass flows and pH of stripping phase is similar to that recorded for the free pertraction. However, as shown in Fig. 9, the influence of $pH_s$ on Erythromycin initial mass flow is less important, owing to the interfacial reaction which




F i g . 9 – *Influence of pH-value of stripping phase on permeability factor for facilitated pertraction from S. erythreus broths (pH of feed phase = 4, carrier concentration* $\gamma = 40$ *g* $L^{-1}$*, rotation speed 500 rpm)*

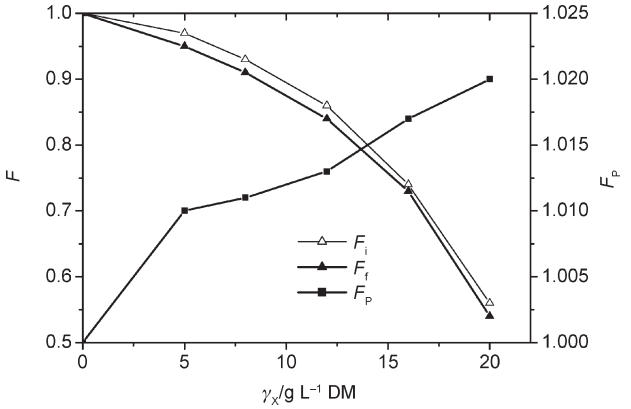
moves the chemical equilibrium towards the formation of Erythromycin di-(2-ethylhexyl) phosphate salt in the liquid membrane. Therefore, the extraction rate is mainly controlled by the reduction of the free carrier concentration in dichloromethane, due to the interfacial reaction with Erythromycin.

The increase of biomass concentration from 0 to 20 g $L^{-1}$ DM generates the decrease of the initial mass flow from 2.5 times at $pH_s$ 2 to 17 times at $pH_f$ 7, the magnitude of the effect being inferior to that corresponding to the free pertraction, as the result of D2EHPA presence. But, the influence of the stripping phase pH on the final mass flow is more significant, especially for $pH_s > 4$, due to the strong reduction of the pH-gradient between the aqueous phases. The negative influence of the biomass on the final mass flow is also attenuated by carrier addition (the transfer rate of Erythromycin from the membrane phase to the stripping phase is only 2 times lower at the most concentrated suspension of *S. erythreus* compared with 0 g $L^{-1}$ DM biomass in the feed phase).

Similar to the free pertraction, the permeability factor decreases strongly with the $pH_s$ increase, mainly for pH values over 4. Although this variation has been recorded for the entire experimental domain of *S. erythreus* concentration, the increase of biomass concentration exhibited a positive effect on the liquid membrane permeability, for the reasons discussed above. However, the importance of this influence was lower than for free pertraction, due to the supplementary amount of antibiotic reactively extracted in the membrane phase (the permeability factor increased for about 1.04–10 times with the $pH_s$ variation between 2 and 7).

By means of these results, it can be concluded that the increase of the pH-gradient between the feed and stripping phases, by using neutral or alkaline values for $pH_f$ simultaneously with acidic values for $pH_s$, induces the acceleration of Erythromycin mass flows, both for free and facilitated pertraction, this influence being not affected by the microorganism accumulation in the feed phase. But, the transport capacity of the liquid membrane reaches the maximum level in the acidic domain of pH of both aqueous phases.

The influence of the solid phase can be analyzed for the facilitated pertraction also by means of the reduction factors. From Fig. 10 it can be seen that by the increase in biomass concentration from 0 to 20 g $L^{-1}$ DM the factors $F_i$ and $F_f$ are reduced about 1.8 times. Comparatively with the free pertraction, the magnitude of this effect is attenu-




F i g . 1 0 – *Influence of biomass concentration on factors F and $F_P$ for facilitated pertraction from S. erythreus broths (pH of feed phase = 4, pH of stripping phase = 2, carrier concentration $\gamma$ = 40 g L$^{-1}$, rotation speed 500 rpm)*

ated by D2EHPA addition, which increases the initial mass flows of Erythromycin.

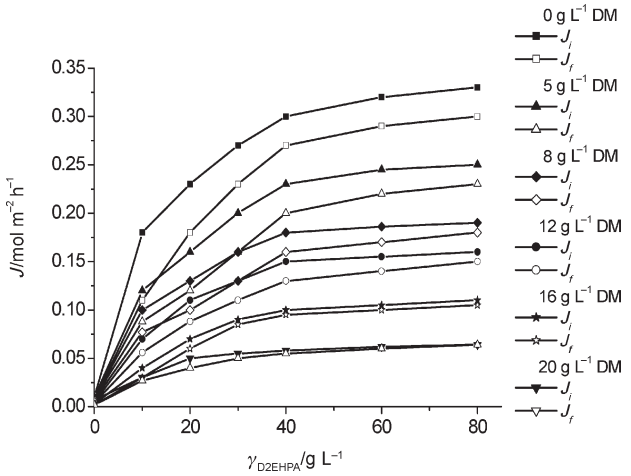
The dependence between the factor $F_P$ and biomass concentration is opposite to those describing the variation of mass flows ratios. According with those discussed in the case of free pertraction, the accumulation of *S. erythreus* induces the equalization of the final and initial mass flows. For this reason, the permeability factors are greater for the facilitated pertraction from *S. erythreus* broths than those for the facilitated pertraction from simulated broths, thus leading to the increase of the factor $F_P$ with the biomass concentration.

The carrier presence and concentration in the liquid membrane exhibits a significant influence on Erythromycin pertraction, independently on the biomass concentration in the feed phase. Fig. 11 indicates the strong increase in the initial mass with the increase of the carrier concentration to a certain value, owing to the acceleration of the interfacial reaction rate. Over a certain value of carrier concentration, the increase in antibiotic initial mass flow becomes slower, this indicating the maximum level of the reextraction rate that can be reached in the considered experimental conditions. Because the solid phase hinders the diffusion of Erythromycin in the feed phase towards the interface, the carrier concentration related to the maximum level of extraction rate is lower and reached easier than for the facilitated pertraction from simulated broths without biomass. Therefore, by increasing the *S. erythreus* concentration in the feed phase, the value of carrier concentration corresponding to the relative constant level of initial mass flow decreases from 30 to 10 g L$^{-1}$.

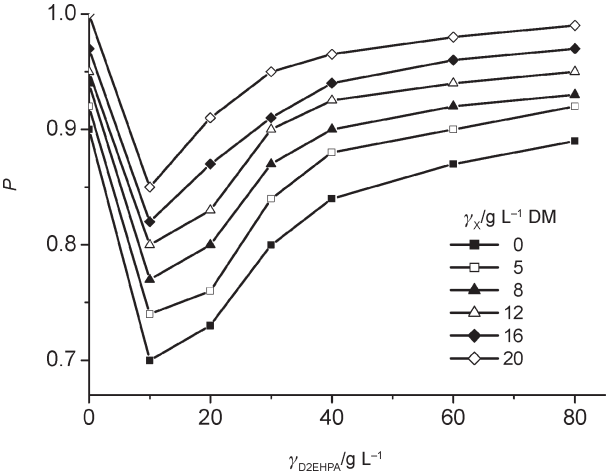
The variation of final mass flow with carrier concentration is similar, the acceleration of final mass flow being the result of the increase of antibiotic concentration in the liquid membrane.

Besides this effect, the accumulation of biomass from 5 to 20 g L$^{-1}$ DM determines the diminution of the mass flows of Erythromycin for about 3.5–4 times, the lowest values being reached for low carrier concentration.

Confirming the previous results,[5,6] the analysis of the permeability factor indicated a particular dependence on D2EHPA concentration (Fig. 12). Thus, regardless of the biomass concentration, the permeability factor initially decreases from a value corresponding to the absence of carrier in the organic solvent to a minimum level for a concentration of 10 g L$^{-1}$ D2EHPA, and finally increases concomitantly with the carrier concentration increase. This variation is the result of the changes in the relative rate of the chemical reactions at the two




F i g . 1 1 – *Influence of carrier concentration on mass fluxes of Erythromycin for facilitated pertraction from S. erythreus broths (pH of stripping phase = 4, pH of stripping phase = 2, rotation speed 500 rpm)*




F i g . 1 2 – *Influence of carrier concentration on permeability factor for facilitated pertraction from S. erythreus broths (pH of stripping phase = 4, pH of stripping phase = 2, rotation speed 500 rpm)*

interfaces. In the absence of the carrier (free pertraction), the extraction and transport of Erythromycin through the liquid membrane occur by physical processes of solubilization, the limiting steps of the overall separation process being only of diffusional type. The addition of carrier in dichloromethane leads to the change of pertraction mechanism. Due to the chemical reaction between solute and carrier at the feed phase-liquid membrane interface, as well to the chemical reaction between solute-carrier compound and hydrochloric acid at the liquid membrane-stripping phase interface, the reextraction rate is diminished and, consequently, additional limiting step of kinetic type appears. Consequently, although the formation of the hydrophobic compound of solute-carrier type enhances the solute extraction from the feed phase comparing with the free pertraction, the permeability factor will be initially smaller in the case of facilitated pertraction.

The increase of the biomass concentration in the feed phase reduces the diffusion rate of antibiotic towards the interface and, implicitly, the initial mass flow. In this circumstance, the permeability factor is 1.2 times greater for 20 g $L^{-1}$ DM *S. erythreus* concentration than that obtained for facilitated pertraction from water.

Indifferent of the carrier concentration, the presence of *S. erythreus* cells induces the reduction of the factors $F_i$ and $F_f$. Therefore, the accumulation of biomass from 5 to 20 g $L^{-1}$ DM leads to the decrease of these factors by 1.5–2.2 times, the lower decreasing degree being obtained for higher D2EHPA concentration (Fig. 13).

The permeability factors recorded for the facilitated pertraction from *S. erythreus* broths are superior to those of the facilitated pertraction from simulated broths, this difference being amplified by the increase of the biomass concentration, as it can be observed from Fig. 14. However, the value of factor $F_P$ is reduced at higher carrier concentration, due to the amplification of the antibiotic transfer from the feed phase to the liquid membrane and, consequently, to the diminution of the membrane permeability.

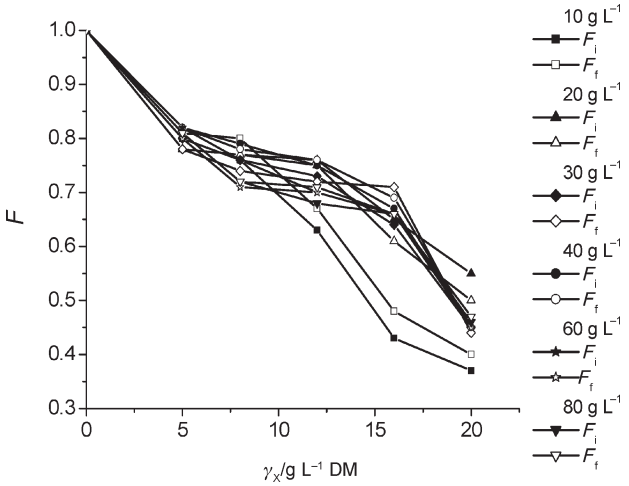



Fig. 13 – *Influence of biomass concentration and carrier concentration on factor F for facilitated pertraction from S. erythreus broths (pH of feed phase = 4, pH of stripping phase = 2, rotation speed 500 rpm)*

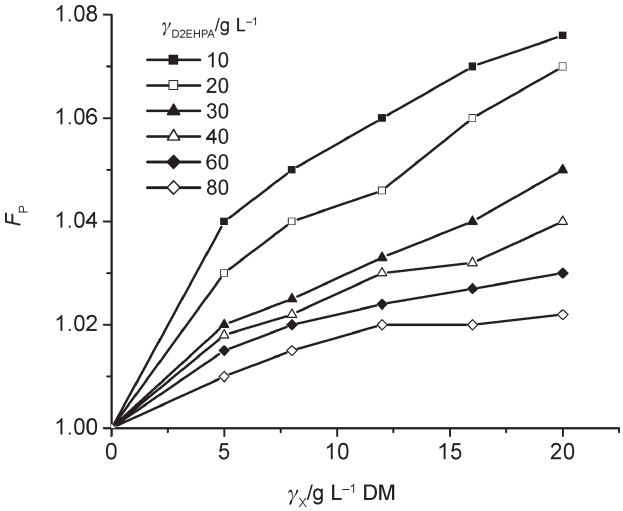



Fig. 14 – *Influence of biomass concentration and carrier concentration on factor $F_P$ for facilitated pertraction from S. erythreus broths (pH of feed phase = 4, pH of stripping phase = 2, rotation speed 500 rpm)*

## Conclusions

The comparative studies on free and facilitated pertraction of Erythromycin from *S. erythreus* broths with cells concentration between 5 and 20 g $L^{-1}$ DM underlined the major negative influence of biomass cumulated with that of apparent viscosity, by affecting the diffusion rate.

Therefore, both for free and facilitated pertraction with D2EHPA, the increase of the biomass concentration in the feed phase led to the significant decrease in the initial and final mass flows of the antibiotic. The addition of carrier in the liquid membrane attenuates this effect. Compared with the pertraction from simulated broths having the same apparent viscosity, without solid phase, the mass flows are reduced up to 5 times for free pertraction, respectively up to 3 times for facilitated pertraction.

The presence of solid phase exhibited a positive influence only on the membrane permeability, but this effect is due to the diminution of the initial mass flow and not to the real increase in process efficiency.

## Notations

$A$ – surface area, $cm^2$

DM – dry mass

$F_i$ – reduction factor related to the initial mass flows, –

$F_f$ – reduction factor related to the final mass flows, –

$F_P$ – reduction factor related to the permeability factors, –

$J_i$ – initial mass flux of Erythromycin, mol $m^2$ $h^{-1}$

$J_f$ – final (overall) mass flux of Erythromycin, mol $m^2$ $h^{-1}$

P – permeability factor, –

$pH_i$ – pH value of the feed phase, –

$pH_f$ – pH value of the stripping phase, –

$Q$ – volume flow rate, L $h^{-1}$

$\gamma_{D2EHPA}$ – carrier concentration, g $L^{-1}$

$\gamma_x$ – biomass concentration, g $L^{-1}$ DM

$\eta$ – dynamic viscosity, mN s $m^{-2}$

$\lambda$ – wavelenght, nm

## Subscript

aq – aqueous phase

o – organic phase

## References


1. *Galaction, A. I., Caşcaval, D.,* Secondary metabolites with pharmaceutical, cosmetic and food applications, Venus, Iaşi, 2006, pp. 109–111.
2. *Caşcaval, D., Oniscu, C., Galaction, A. I.,* Biochemical Engineering and Biotechnology. 3. Bioseparations, Performantica, Iasi, 2004, pp. 59–61.
3. *Caşcaval, D., Galaction, A. I.,* Chem. Ind. J. **58** (2004) 375.
4. *Kawasaki, J., Egashira, R., Kawai, T., Hara, H., Boyadzhiev, L.,* J. Membr. Sci. **112** (1996) 209.
5. *Caşcaval, D., Galaction, A. I., Nicuţă, N.,* Rev. Chim. **57** (2007) 297.
6. *Galaction, A. I., Caşcaval, D., Nicuţă, N.,* Roum. Biotechnol. Lett. **14** (2010) in press.
7. *Blaga, A. C., Galaction, A. I., Caşcaval, D.,* Chem. Biochem. Eng. Q. **22** (2008) 439.
8. *Galaction, A. I., Caşcaval, D., Nicuţă, N.,* Biochem. Eng. J. **42** (2008) 28.
9. *Caşcaval, D., Galaction, A. I., Turnea, M.,* J. Membr. Sci. **328** (2009) 228.
10. *Caşcaval, D., Oniscu, C., Caşcaval, C.,* Biochem. Eng. J. **5** (2000) 45.
11. *Job, C., Blass, E.,* Chem. Eng. J. Biochem. Eng. J. **56** (1994) B1.
12. *Caşcaval, D., Oniscu, C., Caşcaval, C.,* Rev. Roum. Chim. **44** (1999) 403.
13. *Katikaneni, S. P. R., Cheryan, M.,* Ind. Eng. Chem. Res. **41** (2002) 2745.
14. *Monteiro, T. I. R. C., Porto, T. S., Carneiro-Leão, A. M. A., Silva, M. P. C., Carneiro-Da-Cunha, M. G.,* Biochem. Eng. J. **24** (2005) 87.
15. *Vijayakumar, J., Aravindan, R., Viruthagiric, T.,* Chem. Biochem. Eng. Q. **22** (2008) 245.
16. *Kang, C. D., Sim, S. J.,* Biotechnol. Lett. **30** (2008) 441.