

## Modelling of Continuous L-Malic Acid Production by Porcine Heart Fumarase and Fumarase in Yeast Cells

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*Dedicated to the memory of Professor Dr. Valentin Koloini*

Continuous production of L-malic acid will be presented in this paper. The fumarase isolated from porcine heart, fumarase in the permeabilized non-growing cells of baker's yeast and *Saccharomyces bayanus* (UVAFERM BC) were used as biocatalysts. In the production of L-malic acid with fumarase isolated from porcine hearts, there was no enzyme deactivation for a period of two days. At the average residence time of 4 hours, the conversion of about 80 % was achieved. Inactivation of the enzyme was observed using permeabilized cells. This inactivation is described as a reversible process. Conversion of about 50 % was achieved with the remaining enzyme activity. A mathematical model that describes the production of L-malic acid, which contains the enzyme inactivation rate, was developed. Based on simulations, the used biocatalysts were compared. The results show that in the continuous production of L-malic acid, one milligram of purified enzyme corresponds to 68 g (wet weight) cells of *Saccharomyces bayanus* or 120 g (wet weight) cells of baker's yeast.

*Key words:*

L-malic acid, fumarase, baker's yeast, permeabilization, modeling

### Introduction

Malic acid has a wide range of applications and is a "bulk" chemical, which has estimated consumption of 40,000 t annually. It is used in the food, animal food, pharmaceutical, cosmetic and building industry.<sup>1</sup> This is the second largest acidulant used in the food industry after citric acid, and holds about 10 % of the market.<sup>2</sup>

L-Malic acid can be prepared by extraction from natural fruit juices, but this process is not economical, because fruit juices contain less than 1 % of L-malic acid.<sup>1</sup> Commercial chemical synthesis based on the hydration of fumaric or maleic acid, carried out at high pressure and high temperature gives racemic mixture of D- and L-malic acid.<sup>3</sup> Industrially, L-isomer of malic acid is produced by an enzymatic process that transforms the fumaric acid to L-malic acid and this biotransformation is catalyzed by enzyme fumarase.<sup>4-5</sup>

Fumarase enzyme as a biocatalyst can be used as whole cells<sup>1,5,6</sup> or as a purified enzyme.<sup>7</sup> Using whole cells, expensive and time-consuming enzyme purification operations are avoided. In this way, the enzyme is in its natural environment and is protected from inactivation during subsequent use in the continuous system.<sup>8</sup> Major limitations that need

to be addressed when using such cells are the diffusion of substrates and products through the cell wall and side reactions due to the presence of other enzymes in the cell. These problems can be overcome by using permeabilized cells as a source of enzymes. Permeabilization of cells removes barriers to free diffusion of the substrates/products across the cell membrane and permeabilized cells can release most of the low molecular weight cofactors, etc., which reduces the side reactions.<sup>9</sup> This fact is very important for L-malic production with whole cells, because both side reactions with which malic acid and fumaric acid are involved in the Krebs cycle for production of oxalic acid or succinic acid, require the coenzymes. Several commercial strains of yeast were tested as a source of fumarase in our previous work.<sup>10</sup> To achieve higher activity of the cells, cell permeabilization with CTAB (hexadecyltrimethylammonium bromide) was performed before the cells were used in the process. Because of this treatment, production of by-products such as succinic acid was eliminated. For further investigation, two yeasts were selected: wine yeast *Saccharomyces bayanus* (UVAFERM BC), whose cells showed the highest activity of fumarase and baker's yeast. The baker's yeast was selected due to its availability and low price. For fumarase in both yeast cells and isolated fumarase from porcine heart, kinetic parameters were estimated and were

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further confirmed in experiments that were carried out in the batch reactor.<sup>10,11</sup>

The aim of this study was to investigate continuous processes and to develop valid mathematical models thereof. These models should allow prediction of the production of L-malic acid in different operating conditions, and could help evaluate the advantages or disadvantages of the process.<sup>12,13</sup> Reactor with ultrafiltration or microfiltration membrane at the exit was used to design an efficient production process in a continuous mode. Membrane serves to keep the large components such as cells and enzymes within the reactor, while allowing small molecules to pass through it.<sup>14,15</sup>

## Experimental part

### Materials and microorganism

Fumaric acid, L-malic acid, hexadecyltrimethylammonium bromide (CTAB) and  $\text{KH}_2\text{PO}_4$  were purchased from Fluka Chemie (Switzerland) and perchloric acid was from Merck (Germany).  $\text{Na}_2\text{HPO}_4$  and  $\text{K}_2\text{HPO}_4$  were purchased from Kemika (Croatia). Fumarase from porcine heart (E.C. 4.2.1.2) was from SIGMA-ALDRICH (Germany). Wine yeast (*Saccharomyces bayanus* – UVAFERM BC) was from Danstar ferment AG (Denmark) and fresh baker's yeast was purchased from KVASAC d.o.o (Croatia).

### Continuous production of L-malic acid catalyzed by fumarase purified from porcine heart

Continuous production of L-malic acid catalyzed by the purified enzyme was carried out in enzyme membrane reactor (EMR – Figure 1A).<sup>12,15,16</sup>

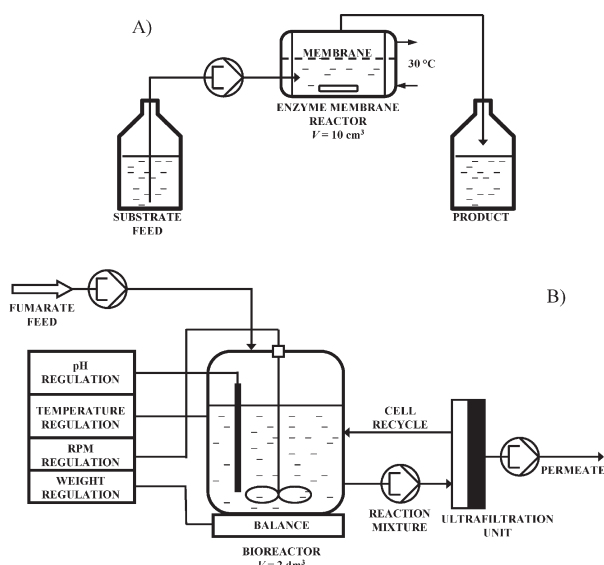


Fig. 1 – Experimental set-up for the continuous L-malic acid production by: (A) isolated fumarase; (B) permeabilized yeast cells

Reactor volume was 10 cm<sup>3</sup>. The reactor was kept at constant temperature of 30 °C. Reaction was initiated with the addition of fumarase by injection through the septum. Polymer membrane (Amicon XM50, Milipore, USA) was used to keep the enzyme in the reactor. Fumarase concentrations were 4.48, 9.80 and 12.82 mg dm<sup>-3</sup>, average residence times were set at 53 and 240 min with a piston pump (M160, RCT, Germany) and fumaric acid concentration in the feed was 63 mmol dm<sup>-3</sup>.

### Continuous production of L-malic acid with permeabilized yeast cells

For continuous production of L-malic acid by yeast cells, two types of yeast were used: a wine yeast – *Saccharomyces bayanus* and baker's yeast – *Saccharomyces sp.* Cells were permeabilized according to the procedure described elsewhere<sup>10,11</sup> and were used for the biotransformation of fumaric to L-malic acid as a non-growing cell.

Experiments were carried out in a 2 dm<sup>3</sup> bioreactor (Biostat MD, B. Braun, Germany, Figure 1B). Volume of reaction mixture was one dm<sup>3</sup>. It was maintained constant by regulation of outlet flow, while inlet flow was kept constant. The solution was stirred at 200 min<sup>-1</sup> and kept at a constant temperature of 30 °C. Ultrafiltration unit (UF-30-E-3,  $A = 0.07$  m<sup>2</sup>,  $cut\ off = 30$  kDa, Sempas Membrantechnik GmbH, Germany) was used to keep the permeabilized yeast cells inside the reactor. For the continuous production by baker's yeast cells, 50 g<sub>ww</sub> dm<sup>-3</sup> of permeabilized cells were used. Average residence time was 914 min and fumaric acid concentration in the feed was 220 mmol dm<sup>-3</sup>. In the experiment, catalyzed by fumarase in the cells of a wine yeast *Saccharomyces bayanus*, permeabilized cell concentration was 36 g (wet weight) dm<sup>-3</sup>. Average residence time was 887 min and the concentration of fumarate in the inlet flow was 200 mmol dm<sup>-3</sup>. Fumaric acid was dissolved in a phosphate buffer (0.1 mol dm<sup>-3</sup>), and pH was set to 7.

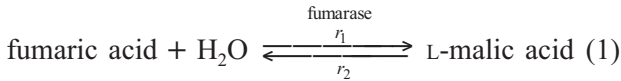
### HPLC analysis

The fumaric and L-malic acid were analyzed with HPLC (LC-20 AT, Shimadzu, Japan) using the reverse phase C<sub>18</sub> column (LiChrosorb® RP-18, 5 μm, 125 x 4 mm, Merck, Germany) and the UV detector at 210 nm. Mobile phase was water (pH 2.10–2.15 adjusted with perchloric acid) at a flow rate of 0.7 cm<sup>3</sup> min<sup>-1</sup>. The analysis was carried out at a temperature 30 °C.

### Mathematical model and data processing

A mathematical model for continuous L-malic acid production includes kinetic and reactor model.

The kinetic experiments for the reaction of L-malic acid production from fumaric acid catalyzed by the isolated fumarase and the fumarase from whole yeast cells were reported previously.<sup>10,11</sup>

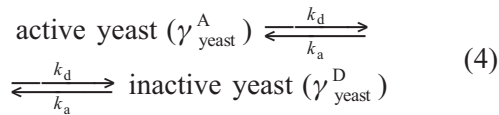


Since the reaction is reversible (eq. 1) the kinetic model consisted of the equation for the fumaric acid hydration (reaction 1), and the equation for the reverse reaction of L-malic acid dehydration (reaction 2). For purified fumarase from porcine heart, kinetics of both reactions were described by Michaelis-Menten equation with competitive product inhibition (eqs. 2–3).

$$r_1 = \frac{V_{\text{ml}} \cdot \gamma_{\text{fumarase}} \cdot c_{\text{fumaric acid}}}{K_{\text{ml}} (1 + c_{\text{malic acid}} / K_i^{\text{malic acid}}) + c_{\text{fumaric acid}}} \quad (2)$$

$$r_2 = \frac{V_{\text{m2}} \cdot \gamma_{\text{fumarase}} \cdot c_{\text{malic acid}}}{K_{\text{m2}} (1 + c_{\text{fumaric acid}} / K_i^{\text{fumaric acid}}) + c_{\text{malic acid}}} \quad (3)$$

Biocatalyst inactivation was observed during experiments in which yeast cell were used. Inactivation mechanism is described by eq. 4, which includes active and inactive form of enzyme in cell of yeast that are in balance with each other.<sup>17,18</sup>



In the case of permeabilized yeast cells, kinetics of fumaric acid hydration was also described with the Michaelis-Menten equation with competi-

tive product inhibition (eq. 5), while the reverse reaction kinetics of L-malic acid dehydration is described with Michaelis-Menten kinetics (eq. 6).

$$r_1 = \frac{V_{\text{ml}} \cdot \gamma_{\text{yeast}}^{\text{A}} \cdot c_{\text{fumaric acid}}}{K_{\text{ml}} (1 + c_{\text{malic acid}} / K_i^{\text{malic acid}}) + c_{\text{fumaric acid}}} \quad (5)$$

$$r_2 = \frac{V_{\text{m2}} \cdot \gamma_{\text{yeast}}^{\text{A}} \cdot c_{\text{malic acid}}}{K_{\text{m2}} + c_{\text{malic acid}}} \quad (6)$$

Kinetic parameters were estimated previously by non-linear regression analysis,<sup>10,11</sup> and are given in Table 1.

The balance equations for the yeast with active, as well as for the yeast with inactive enzyme are given by eqs. 7–8.

$$\frac{d\gamma_{\text{yeast}}^{\text{A}}}{dt} = -k_d \cdot \gamma_{\text{yeast}}^{\text{A}} + k_a \cdot \gamma_{\text{yeast}}^{\text{D}} \quad (7)$$

$$\frac{d\gamma_{\text{yeast}}^{\text{D}}}{dt} = k_d \cdot \gamma_{\text{yeast}}^{\text{A}} - k_a \cdot \gamma_{\text{yeast}}^{\text{D}} \quad (8)$$

The reactor model is based on mass balance equations (eqs. 9–10) for an ideal continuous stirred tank reactor, assuming that there is no diffusion limitation through membrane.<sup>19</sup>

$$\frac{dc_{\text{fumaric acid}}}{dt} = \frac{c_{0, \text{fumaric acid}} - c_{\text{fumaric acid}}}{\tau} + r_2 - r_1 \quad (9)$$

$$\frac{dc_{\text{malic acid}}}{dt} = -\frac{c_{\text{malic acid}}}{\tau} + r_1 - r_2 \quad (10)$$

Table 1 – Kinetic parameters of isolated fumarase and fumarase in permeabilized yeast cells estimated previously

Parameters	Fumarase isolated from porcine heart*	Fumarase in permeabilized yeast cells	
		<i>Saccharomyces bayanus</i> **	baker's yeast <i>Saccharomyces sp.</i> *
$V_{\text{ml}}/$ U mg <sup>-1</sup> U g <sup>-1</sup> (wet weight)	607.214 ± 17.220	264.995 ± 8.183	94.057 ± 1.215
$K_{\text{ml}}$ mmol dm <sup>-3</sup>	3.999 ± 0.364	18.812 ± 1.618	4.379 ± 0.317
$V_{\text{m2}}/$ U mg <sup>-1</sup> U g <sup>-1</sup> (wet weight)	179.456 ± 2.607	84.075 ± 0.986	26.379 ± 0.724
$K_{\text{m2}}/$ mmol dm <sup>-3</sup>	4.286 ± 0.199	19.360 ± 0.683	13.912 ± 1.297
$K_i^{\text{fumaric acid}}/$ mmol dm <sup>-3</sup>	0.279 ± 0.031	33.078 ± 2.870	5.217 ± 0.429
$K_i^{\text{malic acid}}/$ mmol dm <sup>-3</sup>	1.807 ± 0.093	–	–

\* Presečki, A. V., Zelić, B., Vasić-Rački, Đ., *Enzym. Microb. Technol.* **41** (2007) 605.

\*\* Presečki, A. V., Vasić-Rački, Đ., *Biotech. Lett.* **27** (2005) 1835.

Constants in eqs. 7–8 (inactivation constant,  $k_d$  and the activation constant,  $k_a$ ) were estimated by non-linear regression analysis using the data of experiments in the reactor. Simplex method and the method of least squares implemented in commercial software (SCIENTIST) were used<sup>20</sup> to estimate values of parameters. Parameters values were calculated by fitting a mathematical model to experimental data. Calculated data were compared with experimental data, recalculated in the optimization routine and were evaluated again until the minimum error between the experimental and calculated value was realized. The optimal parameters were used to simulate the model equations. The Episode algorithm<sup>20</sup> for stiff systems of differential equations, also included in the SCIENTIST software, was used for the simulation of eqs. 7–10.

## Results and discussion

### Continuous L-malic acid production catalyzed by isolated fumarase

Parameters of the kinetic model for isolated fumarase (eqs. 2–3) were estimated by independent measurements in our previous work<sup>11</sup> (Table 1). Mathematical model (eqs. 2–3, 9–10) was validated in continuous enzyme membrane reactor for the L-malic acid production for three enzyme concentrations and two residence times (Fig. 2).

It can be seen that the mathematical model agrees with experimental data in all conditions (Fig. 2). Fumaric acid conversion was changed, depending on the residence time and enzyme concentration (Table 2). At the lowest concentration of enzyme ( $\gamma_{\text{fumarase}} = 4.48 \text{ mg dm}^{-3}$ ) and the average residence time of 53 min, steady-state conversion of fumaric acid was 55 %. At the same residence time and two times higher concentration of enzyme ( $\gamma_{\text{fumarase}} = 9.80 \text{ mg dm}^{-3}$ ) steady-state conversion was 68 %. Approximately the same steady-state conversion as the conversion in the batch reactor<sup>11</sup> ( $X \approx 80 \%$ ), was achieved with average residence time of 240 min. At this residence time, the experiment was

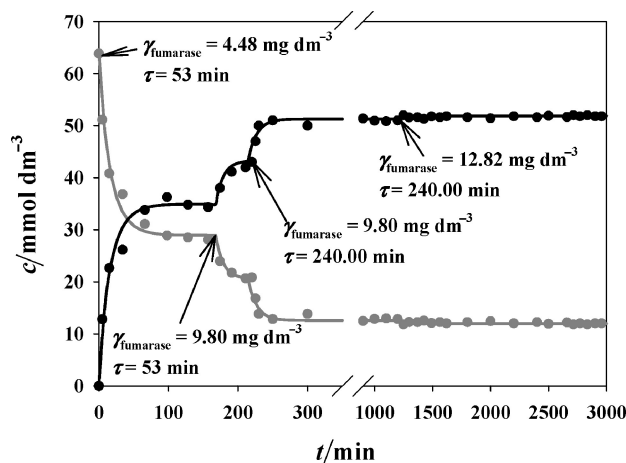


Fig. 2 – Production of L-malic acid by isolated fumarase in the continuously operated EMR ( $0.1 \text{ mmol dm}^{-3}$  phosphate buffer pH 7;  $T = 30 \text{ }^\circ\text{C}$ ;  $V_{\text{reactor}} = 10 \text{ cm}^3$ ;  $c_{0,\text{fumaric acid}} = 63 \text{ mmol dm}^{-3}$ ), (circles – experiment, line – model; grey – fumaric acid, black – L-malic acid)

performed at two enzyme concentrations, but the difference in steady-state conversion (Table 2) was negligible. However, deactivation of enzyme was not observed in the experiment during 50 hours and the enzyme membrane reactor seemed to be a good choice for continuous L-malic acid production catalyzed by isolated fumarase.

The calculated values of volumetric productivity ( $Q_P$ ), biocatalyst productivity ( $Q_{BP}$ ) and biocatalyst consumption ( $BC$ ) are shown in Table 2. The highest volumetric productivity was achieved at lower residence time for the fumarase concentration of  $9.80 \text{ mg dm}^{-3}$ . With an enzyme concentration of  $4.48 \text{ mg dm}^{-3}$  and average residence time of 53 min biocatalyst productivity was the highest, and the biocatalyst consumption (Table 2) the lowest. In the case of L-malic acid production catalyzed by isolated fumarase, the price of biocatalyst was probably the decisive factor, because fumaric acid is inexpensive and it is easily separated from the reaction mixture.<sup>6,22</sup> Thus, the biocatalyst productivity and biocatalyst consumption are very important in this case.

Table 2 – Volumetric productivity<sup>21</sup> (malic acid produced daily for the volume of reaction mixture), biocatalyst productivity<sup>21</sup> (malic acid produced daily per amount of enzyme) and biocatalyst consumption (the amount of enzyme used for daily production of one mmol of L-malic acid) at different conditions during the L-malic acid continuous production catalyzed by isolated fumarase

$\tau/\text{min}$	$\gamma_{\text{fumarase}}/\text{mg dm}^{-3}$	$X/\%$	$Q_P/\text{mmol dm}^{-3} \text{ d}^{-1}$	$Q_{BP}/\text{mmol d}^{-1} \text{ mg}^{-1}$	$BC/\text{mg mmol}^{-1} \text{ d}^{-1}$
53	4.48	0.55	943.67	<b>210.74</b>	<b>0.005</b>
53	9.80	0.68	<b>1164.93</b>	118.83	0.008
240.0	9.80	0.80	307.57	31.37	0.032
240.0	12.82	0.81	311.16	24.27	0.041

### Continuous L-malic acid production catalyzed by fumarase in permeabilized yeast cells

The permeabilization of yeast cells appears to be very useful in the case of enzyme fumarase. The activity of permeabilized cells in hydration of fumaric acid was significantly higher than that of the non-permeabilized ones.<sup>10,11</sup> A mathematical model for continuous production of L-malic acid was composed of the kinetic equations (eqs. 5–6) and mass balance equations (eqs. 9–10). Kinetic parameters of this model were estimated earlier<sup>10,11</sup> (Table 1) and the model was confirmed in a continuous reactor with cell retention. The results are shown in Fig. 3.

During the continuous L-malic acid production by both yeasts, enzyme inactivation was observed, but was not complete. It seems that the enzyme activity became constant after 83 hours in the process with the permeabilized cells of *Saccharomyces bayanus* (Fig. 3A) and after 50 hours in the process with permeabilized cells of baker's yeast (Fig. 3B).

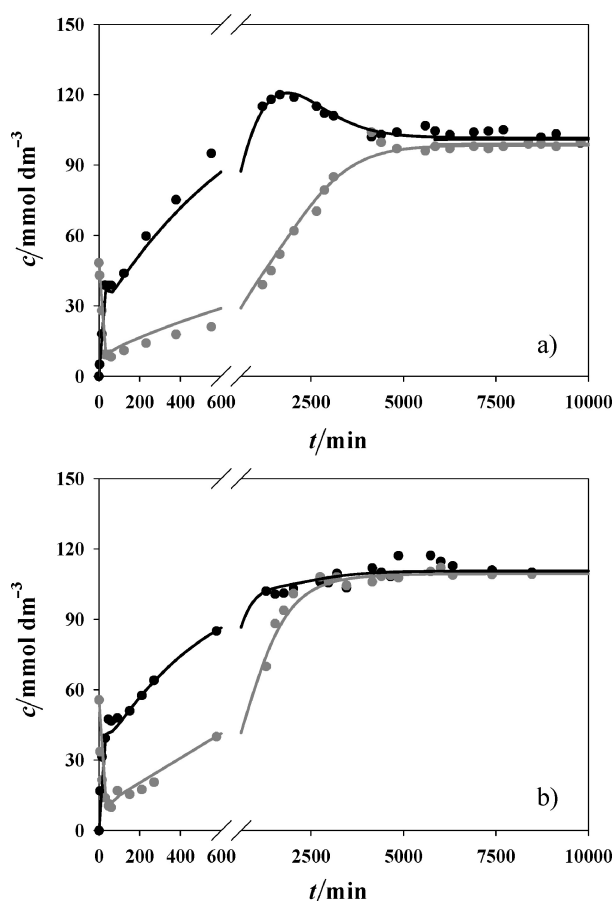


Fig. 3 – Production of L-malic acid by permeabilized yeast cells in the continuously operated reactor with cell retention ( $0.1 \text{ mmol dm}^{-3}$  phosphate buffer pH 7;  $T = 30 \text{ }^\circ\text{C}$ ;  $V_{\text{reactor}} = 1 \text{ dm}^3$ ) (A) *Saccharomyces bayanus* cells ( $c_{0,\text{fumaric acid}} = 200 \text{ mmol dm}^{-3}$ ,  $\gamma_{\text{yeast}} = 36 \text{ g (wet weight) dm}^{-3}$ ;  $\tau = 887 \text{ min}$ ) (B) baker's yeast cells – *Saccharomyces sp* ( $c_{0,\text{fumaric acid}} = 220 \text{ mmol dm}^{-3}$ ,  $\gamma_{\text{yeast}} = 50 \text{ g (wet weight) dm}^{-3}$ ;  $\tau = 914 \text{ min}$ ) (circles – experiment, line – model; grey – fumaric acid, black – L-malic acid)

Decrease of enzyme activity was described by enzyme reverse inactivation scheme presented in eq. 4. In addition, the model was upgraded by eqs. 7–8, and these are the balance equations for the active yeast and inactive yeast. Parameters of the model (eqs. 7–8) were estimated from experimental data, and are shown in Table 3.

Table 3 – Estimated inactivation and activation constants of fumarase in permeabilized yeast cells

Parameters	<i>Saccharomyces bayanus</i>	Baker's yeast <i>Saccharomyces sp.</i>
$k_d/\text{min}^{-1}$	$1.36 \cdot 10^{-3} \pm 5.95 \cdot 10^{-5}$	$2.38 \cdot 10^{-3} \pm 1.98 \cdot 10^{-4}$
$k_a/\text{min}^{-1}$	$5.67 \cdot 10^{-5} \pm 3.34 \cdot 10^{-6}$	$2.40 \cdot 10^{-4} \pm 2.27 \cdot 10^{-5}$

Baker's yeast cells have a higher inactivation constant, as well as the activation constant than *S. bayanus* cells. This is in accordance with the experimental results because the constant activity of fumarase in *Saccharomyces bayanus* cells was achieved around 30 hours later. Simulation of the model gives more information on the activities of enzymes in yeast cells (Fig. 4). It is obvious that almost 90 % of the initial enzyme activity in the cells of baker's yeast was lost, while in the cells of *Saccharomyces bayanus* this loss was 96 %.

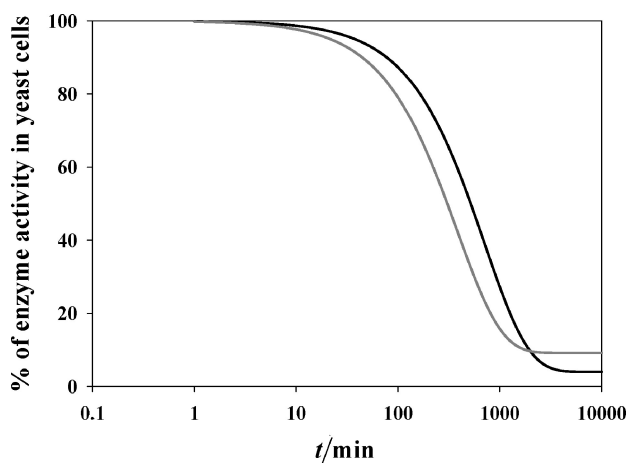


Fig. 4 – Decrease of enzyme activity in yeast cells – simulated by model (black *Saccharomyces bayanus* cells, grey – baker's yeast cells – *Saccharomyces sp*)

Regardless of the initial concentration of yeast, the steady-state conversion was about 50 % in the continuous L-malic acid production by both yeasts.

It could be seen from the data in Table 4 ( $X_{\text{fumaric acid}}$ ,  $Q_P$ ,  $Q_{BP}$  and  $BC$ ) that both biocatalysts were almost equally efficient in the continuous production of L-malic acid. Baker's yeast cells gave somewhat higher volumetric productivity due to higher concentration of fumaric acid in the feed. The

Table 4 – Volumetric productivity, biocatalyst productivity and biocatalyst consumption in the L-malic acid continuous production catalyzed by permeabilized yeast cells

Yeast	<i>Saccharomyces bayanus</i>	Baker's yeast
$\tau/\text{min}$	887	914
$\gamma_{\text{yeast}}/\text{g (wet weight) dm}^{-3}$	36	50
$X_{\text{fumaric acid}}/-$	0.51	0.50
$Q_{\text{p}}/\text{mmol dm}^{-3} \text{ d}^{-1}$	165.00	174.26
$Q_{\text{BP}}/\text{mmol g}^{-1} \text{ (wet weight) d}^{-1}$	4.58	3.49
$BC/\text{g (wet weight) mmol}^{-1} \text{ d}^{-1}$	0.22	0.29

low initial enzyme activity in baker's yeast cells affects lower biocatalyst productivity and higher biocatalyst consumption. Steady-state conversion of fumaric acid and volumetric productivity are more important than the amount of biocatalyst used, because the price of biocatalyst is very low when using permeabilized yeast cells. Therefore, these biocatalysts are useful for the L-malic acid production.

### Comparison of biocatalysts in the continuous production of L-malic acid

The proposed mathematical models were validated in a continuous type reactor, which has enabled the simulation of L-malic acid production in different initial conditions. In order to compare the three biocatalysts that have been used, the same initial value for residence time ( $\tau = 900$  min) and the fumaric acid concentration in input flow ( $c_{0,\text{fumaric acid}} = 250$  mmol dm<sup>-3</sup>) were set. For each biocatalyst the L-malic acid concentration in the steady state conditions was calculated. Dependence of steady-state conversion on biocatalyst concentrations is shown in Fig. 5.

Steady-state conversion of 70 % was chosen as a reference because it was achieved in the industrial production of L-malic acid by immobilized cells of *Brevibacterium flavum*.<sup>6,23</sup> Table 5 shows the biocatalyst concentration, and calculated biocatalyst productivity and biocatalyst consumption in steady-state conversion of 70 %.

It can be concluded that the use of one mg of purified fumarase in continuous production of L-malic acids, corresponds to the use of enzyme in 68 g (wet weight) cells of *S. bayanus* or in 120 g (wet weight) cells of baker's yeast (Table 5). Therefore, the use of purified enzyme in L-malic acid production is uneconomical because whole cells are cheaper. However, with the large quantities of cells that are required, it is very difficult to handle in an industrial reactor. For example, the same productivity as in industrial production in Japan (30 t/month of L-malic acid in a 1000 dm<sup>3</sup> reactor at a flow rate

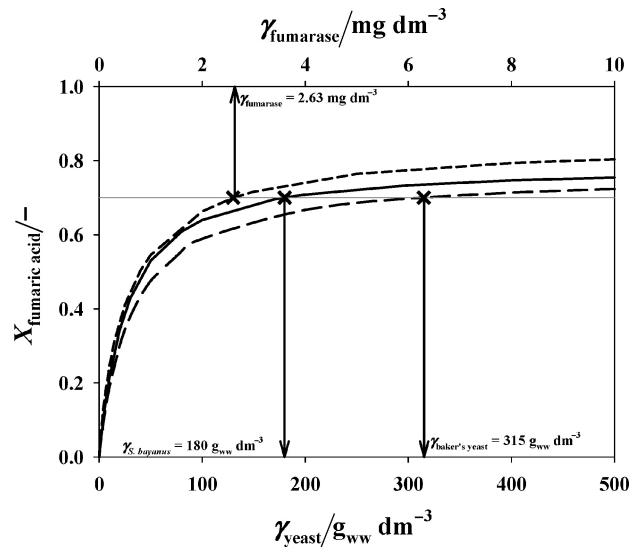


Fig. 5 – The dependence of steady-state conversion on initial biocatalyst concentration in the L-malic acid continuous production at  $\tau = 900$  min and  $c_{0,\text{fumaric acid}} = 250$  mmol dm<sup>-3</sup> – simulated by model (short dash line – isolated fumarase, solid line – *Saccharomyces bayanus* cells, long dash line – baker's yeast cells – *Saccharomyces sp*)

Table 5 – Biocatalyst concentration, biocatalyst productivity and biocatalyst consumption in the L-malic acid continuous production at  $\tau = 900$  min and  $c_{0,\text{fumaric acid}} = 250$  mmol dm<sup>-3</sup> and  $X_{\text{fumaric acid}} = 0.7$

Parameters	Isolated fumarase	<i>Saccharomyces bayanus</i>	Baker's yeast
$\gamma/$ mg dm <sup>-3</sup>	2.63		
$\gamma/$ g (wet weight) dm <sup>-3</sup>		180	315
$Q_{\text{BP}}/$ mmol mg <sup>-1</sup> d <sup>-1</sup>	106.49		
$Q_{\text{BP}}/$ mmol g <sup>-1</sup> (wet weight) d <sup>-1</sup>		1.56	0.89
$BC/$ mg mmol <sup>-1</sup> d <sup>-1</sup>	0.0094		
$BC/$ g (wet weight) mmol <sup>-1</sup> d <sup>-1</sup>		0.64	1.12

of 450 dm<sup>3</sup> h<sup>-1</sup> of 1 mol dm<sup>-3</sup> fumarate)<sup>6,23</sup> in our case could be achieved with a 5000 kg *Saccharomyces bayanus* cells. This is because fumarase loses about 96 % of its initial activity in the cells due to enzyme inactivation. Therefore, further studies are needed to find a method to stabilize the enzymes in the cells.

### Conclusion

Continuous production of L-malic acid catalyzed by fumarase purified from porcine heart and fumarase in cells of *Saccharomyces bayanus* or baker's yeast has been successfully performed. High biocatalyst consumption, which was obtained in the case of whole cell biocatalyst, is due to inactivation of enzymes in the cells. Although yeast is a

very inexpensive biocatalyst, large quantities needed for an interesting industrial production neutralize the effectiveness of industrial applications.

#### ACKNOWLEDGEMENT

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#### List of symbols

$BC$	– biocatalyst consumption, $\text{mg mmol}^{-1} \text{d}^{-1}$ , $\text{g (wet weight) mmol}^{-1} \text{d}^{-1}$
$c$	– molar concentration, $\text{mmol dm}^{-3}$
$k_a$	– activation constant, $\text{min}^{-1}$
$k_d$	– inactivation constant, $\text{min}^{-1}$
$K_i$	– inhibition constant, $\text{mmol dm}^{-3}$
$K_m$	– Michaelis-Menten constant, $\text{mmol dm}^{-3}$
$Q_{BP}$	– biocatalyst productivity, $\text{mmol mg}^{-1} \text{d}^{-1}$ , $\text{mmol g}^{-1} \text{(wet weight) d}^{-1}$
$Q_P$	– volumetric productivity, $\text{mmol dm}^{-3} \text{d}^{-1}$
$r$	– reaction rate, $\text{U cm}^{-3}$
$T$	– temperature, $^{\circ}\text{C}$
$t$	– time, $\text{min}$
$V$	– volume, $\text{cm}^3$ , $\text{dm}^3$
$V_m$	– maximal reaction rate, $\text{U mg}^{-1}$ , $\text{U g}^{-1}$ (wet weight)
$X$	– conversion, –
$\gamma$	– mass concentration, $\text{g (wet weight) dm}^{-3}$ , $\text{mg dm}^{-3}$
$\tau$	– average residence time, $\text{min}$

#### References

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