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# Economic Evaluation of the Hydrolysis of Lactose Using Immobilized β-Galactosidase

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## ABSTRACT

A computer program for preliminary cost estimates of free and immobilized enzyme systems has been developed. The cost for the hydrolysis of lactose by β-galactosidase from *Aspergillus oryzae* has been calculated for a batch tank reactor, with free (BTRF) and immobilized (BTRI) enzymes, a continuously stirred tank reactor (CSTR) and a plug-flow tubular reactor (PFTR), considering the mass transfer behavior and deactivation of the enzyme.

Enzyme immobilization is economically feasible, compared with a system with free enzymes, despite a very high cost for the enzyme attachment. At a half-life time of 80 d, the PFTR gives the lowest cost (0.48 SEK/kg lactose), but the cost for the BTRI is just slightly higher (0.66 SEK/kg lactose) and still much lower than the BTRF (2.10 SEK/kg lactose).

**Index Entries:** Lactose; hydrolysis; economy; immobilized enzymes.

**Abbreviations Used:** Bi, Biot number = $k_{ext} \cdot R/D_{es}$ (−); c, dimensionless concentration of lactose; $D_{es}$, effective diffusion coefficient for lactose ($m^2/s$); $D_{ep}$, effective diffusion coefficient for galactose ($m^2/s$); E, enzyme concentration ($kg/m^3$); I, inhibitor (galactose) concentration ($kg/m^3$); $K_m$, Michaelis-Menten constant (kg lactose/$m^3$); $K_I$, inhibitor constant (kg galactose/$m^3$); k, rate constant (kg lactose/kg/s); $k_{ext}$, mass transfer coefficient (m/s); R, radius of bead (m); $r_s$, reaction rate rate ($kg/m^3/s$); r, dimensionless radial distance within bead (−); S, concentration of lactose ($kg/m^3$); t, time (s); subscript i, interface between bead and external liquid; δ, quotient = $D_{es}/D_{ep}$; φ,

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Thiele module = $(k \cdot E \cdot R^2/D_{es}/k_m)^{0.5}$; $\gamma_1$, dimensionless constant = $S_b/K_m$; $\gamma_2$, dimensionless constant = $I_b/K_m$; $\gamma_3$, dimensionless constant = $S_b/K_i$; $\eta$, effectiveness factor, defined by Eq. (5).

## INTRODUCTION

The main advantages of enzyme immobilization are reuse of enzymes, enzyme-free products, and the possibility of using continuous processes. Also, a wide variety of reactor types, such as batch-tank reactor, continuously stirred tank reactor (CSTR), and fixed-bed and fluidized-bed reactors can be used. Much research has been performed during the last 20 years within the field of immobilization of biocatalysts. An extensive review on this subject has been presented by Mosbach (*1*). Despite the vast literature on this subject, large scale continuous processes with immobilized enzymes are not very frequent, maybe partly owing to problems with contamination in continuous processes. A few processes, however, such as the production of high fructose syrup using immobilized glucose isomerase, have been commercially very successful. The final criteria whether to use immobilized or free enzymes, however, is the overall process economy. A computer program for preliminary cost estimates of free and immobilzied enzyme systems has been developed. It can be used as a tool for determining the economic feasibility and identifying problem areas in potential immobilized enzyme systems.

The hydrolysis of lactose by β-galactosidase has been chosen as a model system. Immobilized β-galactosidase is used commercially for the production of lactose-hydrolyzed milk. It is also of interest in the conversion of lactose from whey to fermentable sugars for the production of yeast (*2*), ethanol (*3,4*), or other chemicals (*5*). In this paper, the cost for the hydrolysis of lactose by β-galactosidase from *Aspergilus oryzae* has been calculated for three reactor types. For each reactor type, the optimal volume and operating time, considering the deactivation of the enzyme, is calculated. The influence on the cost of enzyme loading of the beads, the loss of enzyme in the immobilization procedure, and changes in the enzyme half-life time, is also studied.

## MATERIALS AND METHODS

### Materials

The β-galactosidase (EC 3.2.1.23) from *Aspergillus Oryzae* was a generous gift from Miles KaliChemie GmbH & Co., KG, Hanover, West Germany (Takamine® Brand Fungal Lactase). The activity was 17 ONPG U/mg enzyme with one unit defined as the enzyme activity that will hydrolyze

1 μmol *o*-nitrophenyl-β-D-galactopyranoside (ONPG)/min at 37°C and pH 4.9. Sodium alginate was of the type Manugel GMB. The coupling chemicals *N*-hydroxy-succinimide (NHS, molar weight 115.1 g/mol) and 1-ethyl-3-(3-dimethyl-amino-propyl-1)-carbodiimide hydrochloride (EDC, molar weight 191.7 g/mol) were purchased from Sigma Chemical Co., St. Louis, MO. Other chemicals were of reagent grade and purchased from other commercial sources.

## Kinetics of Lactose Hydrolysis

The hydrolysis rate of lactose with free enzyme was measured at a temperature of 30°C in an acetate buffer solution (0.1*M*, pH 4.5). The enzyme concentration used in the kinetic experiments was 0.25 g/L, whereas the initial lactose concentration varied between 9 and 75 g/L. The reaction was carried out in a stirred batch reactor for 120 min while samples were withdrawn for analysis. The samples were filtered with a 0.25 μm filter and immediately heat-treated in a microwave oven for 12 s to stop the enzymatic activity. The analysis of lactose, glucose, galactose, and oligosaccharides were performed on a HPLC, as described elsewhere (*3*).

The hydrolysis rate of lactose up to a lactose concn. of 75 g/L can be accurately described by the Michaelis-Menten kinetics with product inhibition

$$r_s = dS / dt = - k \cdot E \cdot S / S + K_m \cdot (1 + I/K_I) \quad (1)$$

At higher lactose concentrations, the formation of oligosaccharides has to be incorporated into the kinetic model.

The kinetic constants ($k = 0.1824$; $K_m = 42.75$; $K_I = 1.26$) were determined by least-squares fitting to the data obtained from the kinetic experiments. A good fit was obtained for the lactose concentrations studied. In Fig. 1, the fitted curves, together with the experimental data points, are shown for initial lactose concn. up to 75 g/L.

Hydrolysis is strongly inhibited by the galactose formed. β-galactosidase from other sources, like *A. Niger*, are even more inhibited by galactose (*6*). In some complementary experiments, the hydrolysis rate of sweet deproteinized whey was studied in the same way. The kinetic relationship, according to Eq. (1), was shown to hold also for this kind of whey in the concentration range studied above.

## Deactivation Kinetics

The enzyme is reported to be very stable (*6–8*) especially when it is used at 30°C, whereas the activity is highest at 55°C. As the possibilities of coimmobilizing the enzyme with yeast, working at 30°C, were studied in another investigation (*3,7,9*); the lower temperature was chosen despite its lower activity.

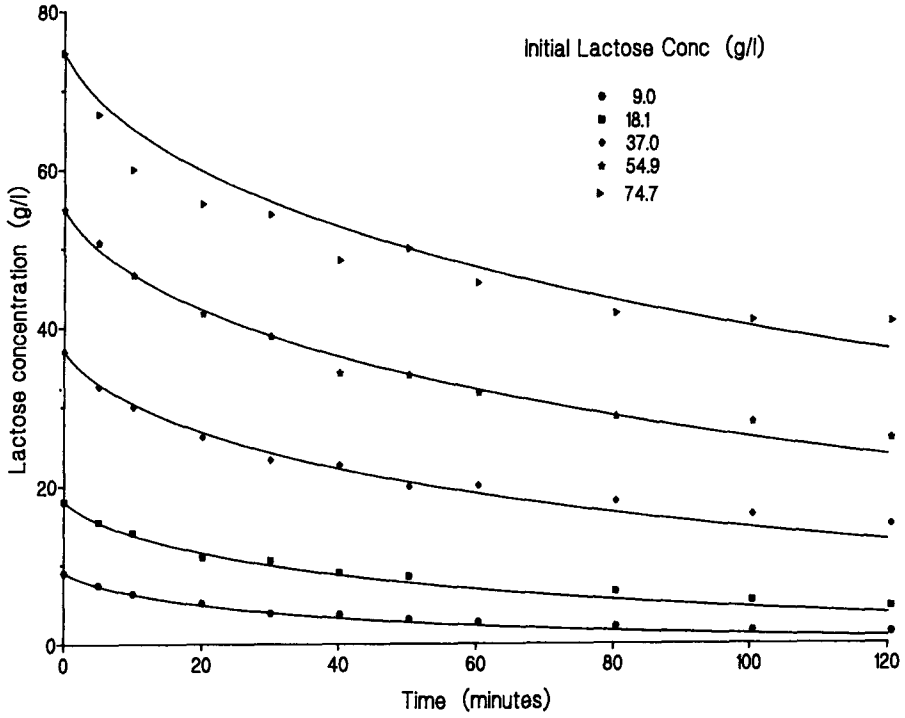


Fig. 1. Kinetic model and experimental data for lactose hydrolysis.

In small-scale experiments, it was shown (*7*) that the activity was unaltered for 60 d during continuous hydrolysis of lactose (50 g/L) at pH = 4.5 and 30°C. On a larger scale, the half-life time probably is shorter owing to fouling of the biocatalyst. Prenosil (*10*) reported a half-life time for the enzyme of 55 d at 30°C. A half-life time of 80 d has been used in the calculations, following a first-order deactivation kinetic relationship.

## Effective Diffusion Coefficients in the Carrier

Calculation of the true hydrolysis rate requires a knowledge of the diffusional behavior of the reactants and products within the carrier. In a previous study (*11*), the effective diffusion coefficients in alginate gel beads were determined to $4.5 \times 10^{-10}$ m²/s for lactose and $6.4 \times 10^{-10}$ m²/s for glucose and galactose. These values are about 90% of their respective value in pure water. The values are independent of the alginate content up to 4 wt% alginate in the gel.

## Immobilization Procedure

The enzyme is covalently coupled to calcium alginate by using the coupling chemicals NHS and EDC according to the following mechanism. The carboxylic groups of the alginate polymer are activated through the

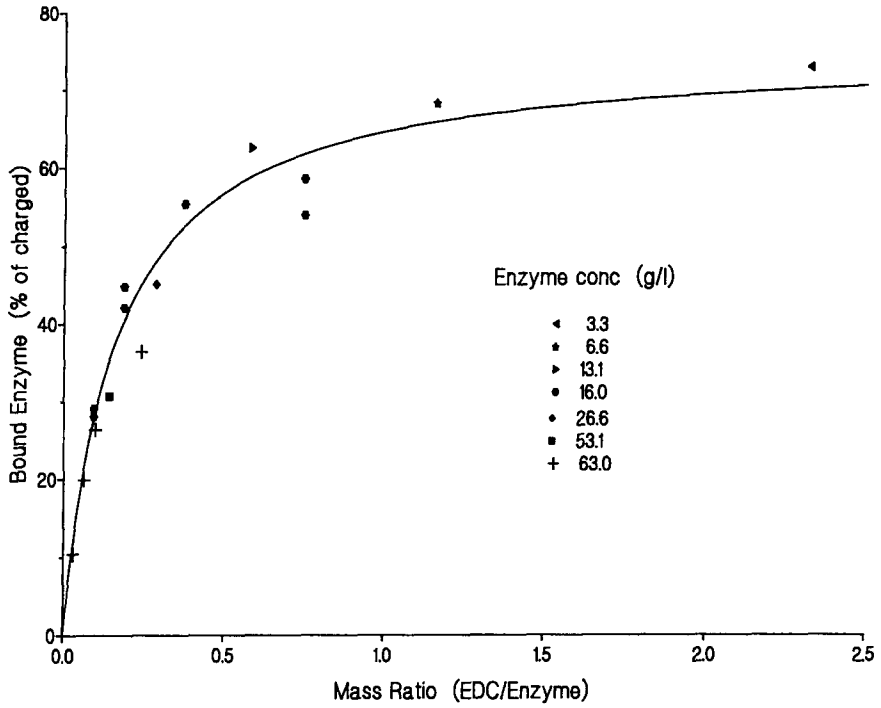


Fig. 2. Percent bound enzyme as a function of EDC/charged enzyme.

transformation into active *N*-hydroxysuccinimide esters by the NHS and EDC. The amino groups of the enzyme bind to the active esters, forming peptide bonds. The procedure is described in detail elsewhere (*3,7,12*). In these studies, it was shown that the most efficient binding was obtained at an EDC/NHS molar ratio of 2/5. The data from these studies are plotted in Fig. 2, which gives the amount of bound enzyme as a function of the amount of EDC. The solid line is obtained by a least-squares fit.

When the enzyme has been coupled to the sodium alginate, the alginate solution, with an alginate content of 0.024 kg/kg solution, is pressed through a nozzle consisting of several syringes into a calcium chloride solution (0.1*M*) where gel beads are precipitated. Curing in a 0.01*M* $CaCl_2$ solution typically results in beads of a diameter of 3 mm. The subsequent shrinkage of the gel results in about 0.6 kg gel/kg initial alginate solution.

Sodium alginate is a commercially available and an inexpensive polymer containing carboxylic groups suitable for the covalent binding of enzymes. It is water soluble and enables the enzyme immobilization to be carried out in homogeneous solutions. The covalent immobilization gives no leakage of enzymes from the immobilized preparation. The covalent immobilization method using NHS and EDC was chosen primarily because it is relatively uncomplicated and can be performed in a water solution without any intermediate separation step, and furthermore, it was shown (*7*) not to alter the specific enzymatic activity by the immobilization procedure.

## MODELS

### Internal Mass Transfer Model

The following assumptions are made:

1. The enzyme activity is supposed to be constant throughout the spherical gel bead.
2. The beads are uniform and of equal size.
3. The effective diffusivities are independent of the concentration and, therefore, are constant since the system is considered to be isothermal.
4. The intrinsic enzyme kinetics are the same as for the free enzyme.

A steady-state intrapartice mass balance for the lactose gives the following differential equation in dimensionless form

$$\frac{d^2c}{dr^2} + \frac{2}{r}\cdot\frac{dc}{dr} = \phi^2\cdot\frac{c}{1 + \gamma_1\cdot c + \gamma_3\cdot\delta\cdot(1-c) + \gamma_2} \qquad (2)$$

The effect of the internal mass transfer limitations on the reactor can be accounted for by introducing the effectiveness factor ($\eta$), i.e., the ratio of the observed or apparent reaction rate to the rate when no mass transfer limitations exist. The effectiveness factor can be calculated from the concentration profile that is obtained by solving Eq. (2) with the following boundary conditions

$$\frac{dc}{dr} = 0 \quad \text{at the center (for } r=0) \qquad (3)$$

and

$$\frac{dc}{dr} = Bi\cdot(1 - c_i) \quad \text{at the surface (for } r=1) \qquad (4)$$

The effectiveness factor is given by the following expression

$$\eta = \frac{3}{\phi^2}\cdot\left(\frac{dc}{dr}\right)_{r=1}\cdot(1+\gamma_1+\gamma_2) \qquad (5)$$

where all the dimensionless constants are given in "Abbreviations Used."

### Reactor Equations

The batch reactor with free enzyme is considered to be completely mixed. In the immobilized enzyme reactors (IME), a Biot-number of 50 is used. This means that the external mass transfer hindrance is very small,

and about 5% of the total concentration difference between the bulk liquid and the liquid in the center of the bead is located to the film outside the bead. In the plug-flow reactor, there is no dispersion.

### Numerical Methods

The concentration profiles in the beads were calculated by solving Eqs (2)-(4). The differential Eq. (2) was first reduced to a set of nonlinear algebraic equations by orthogonal collocation (*13*). The nonlinear equation system was then solved using a Newton-Raphson procedure. The effectiveness factor was calculated with Eq. (5) using the slope dc/dr at $r=1$ obtained from the calculated concentration profile. The number of collocation points may be varied in the program. Four points were found to give sufficient accuracy for Thiele modulus up to 50.

A time step of 1 h was used for the integration of the reactor equations. The effectiveness factor was recalculated every third time step. For the batch reactor and PFTR, a mean value for the effectiveness factor was used based on the logarithmic mean value of the substrate concentrations. The initial and final concentrations were used for the batch reactor, whereas the inlet and outlet concentrations were used for the PFTR. This procedure is time-saving and, for the system investigated, found to give only a few percent deviation from a more rigorous procedure. This has also been shown by Lee and Tsao (*14*).

## ECONOMICS

As a basis for the calculations, a capacity of 5000 tons lactose/yr was used. This corresponds to a large-size dairy in Sweden. The required reactor volume, obtained from the computer simulation, is increased by 25% to get the real reactor tank volume. The cost for a 127 $m^3$ reactor is 950,000 SEK, calculated from Maiorella (*15*), with an exchange rate of 6.50 SEK/dollar and updated to conditions of 1988. This was scaled to the actual reactor size required by simply applying a capacity ratio exponent of 0.55. As the reactor volume is maximized to 100 $m^3$, the true scale-up factor will, in reality, be close to 1.0 when many reactors are used.

The fixed capital cost was estimated by multiplying the equipment cost by 3.55 (*16*). This figure reflects the additional costs of installation, process piping, instrumentation, buildings, engineering, construction, and contingency. The total capital costs were converted to equivalent fractions per year by multiplying by an annuity present-worth factor. A factor of 0.31 was used in this study based on an effective interest rate of 16% and an interest period of 5 yr.

The hydrolysis plant is supposed to be run by one operator with additional man-hours for exchange of batches and immobilized enzymes. For every exchange of a batch, two hours are required to fill and empty each

reactor. For every exchange of immobilized enzyme, eight hours are required to empty, sanitize, and fill each reactor. The labor costs can then be calculated considering that five shifts are required and that the wages/operator was 170,000 SEK/yr in Sweden in 1988. Indirect labor costs are calculated as 40% of the direct labor costs. Maintenance was calculated as 3% of the capital costs and 25% of the direct labor costs (*17*).

The chemicals prices, in SEK/kg, are; enzyme 1120, EDC 15,000, NHS 1000, and alginate 80. The prices for EDC and NHS are given for quantities of 500 kg and was obtained from Sigma Chemical Co. The price for β-galactosidase is valid for quantities of 1 ton and was obtained from Miles KaliChemie GmbH & Co.

## RESULTS AND DISCUSSION

The economic evaluation is performed for a plant processing 5000 tons lactose/yr, with a lactose concentration in the feed of 50 g/L. The lactose concentration corresponds to the concentration obtained in whey permeate. It was shown in a previous investigation (*18*) that preconcentration was uneconomical when the hydrolysate was to be used for fermentation to ethanol.

A 70% conversion of lactose is assumed. This was found by Scott et al. (*2*) to be the optimal lactose conversion in an immobilized lactase reactor when the hydrolysate was used for production of baker's yeast. For higher overall conversions, it was more economical to separate and recirculate nonhydrolyzed lactose than to increase the conversion in the hydrolysis step.

Two main operation strategies can be used to reduce the fluctuations in capacity owing to enzyme deactivation (*19*). One strategy is to raise the temperature to compensate for activity loss and maintain the original production rate and conversion level. This strategy was investigated by Suga et al. (*20*) and Scott et al. (*21*). The other strategy is to perform the reaction at isothermal conditions using a multiple-reactor system.

In this study, hydrolysis is assumed to be performed isothermally at 30°C and at constant conversion, taking into account the capacity fluctuation. The primary aim is to compare the immobilized enzyme reactors with a conventional batch reactor, using free enzymes. Since the consumption of lactose, for a fixed conversion, is the same in all the alternatives, this is not incorporated in the economic analysis.

Figure 3 shows the production cost for the alternative using a batch reactor with free enzymes. The cost for the enzyme increases with increasing enzyme loadings, whereas the capital cost decreases. The optimal enzyme loading is around 0.06 kg/m$^3$ reactor. The total cost is very high, 2.1 SEK/kg lactose, of which the cost for the enzyme constitutes about 30%. The total reactor volume required is 590 m$^3$ and the active batch time is 36 h.

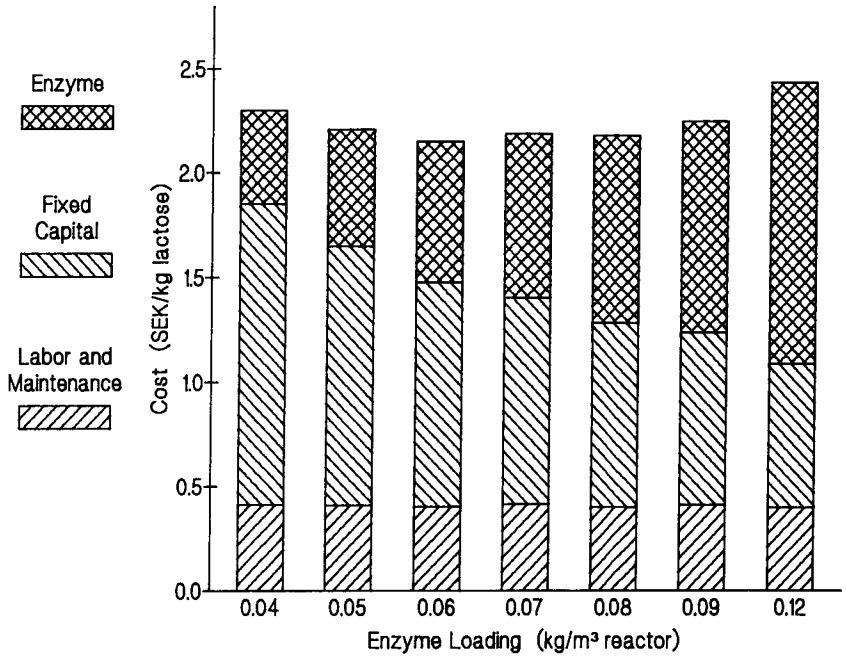


Fig. 3. Hydrolysis cost for batch hydrolysis with free enzymes.

To calculate the cost of the reactor alternatives with immobilized enzymes, the ratio between the amount of EDC and enzyme used in the immobilization step must be chosen. This determines the amount of charged enzyme that is bound during the immobilization, according to the correlation (solid line) shown in Fig. 2. Figure 4 shows the total hydrolysis cost, per kilogram lactose, obtained for the PFTR. For all three enzyme loadings shown, the total cost has a minimum at a bound-to-charged enzyme ratio of about 30%. This corresponds to an EDC-to-enzyme ratio of 0.12. This optimum was also found to be valid for the other two reactor types. Thus, these values were used in all subsequent calculations.

Figures 5–7 show the production costs for the alternatives with immobilized enzymes, i.e., the batch tank reactor, the CSTR, and the PFTR, with varying enzyme loadings. The total cost has been broken down into costs for labor and maintenance, capital, enzyme, and immobilization. The cost for immobilization consists mainly of the cost for the coupling chemical EDC.

For the batch tank reactor, the total cost decreases very slowly with increasing loadings up to about 2 kg enzyme/$m^3$ and then increases. For an enzyme loading of 2 kg/$m^3$ beads, the mean batch time is 4.5 h, and the total vol is 73 $m^3$. The lowest total cost is about 0.66 SEK/kg lactose.

Also, for the CSTR, the total cost decreases with increasing loading and reaches a minimum for an enzyme loading of 5 kg/$m^3$ beads. The cost is somewhat higher than for the batch reactor, 0.72 SEK/kg lactose. The mean residence time is 2.6 h, and the total reactor vol is 43 $m^3$. The cost

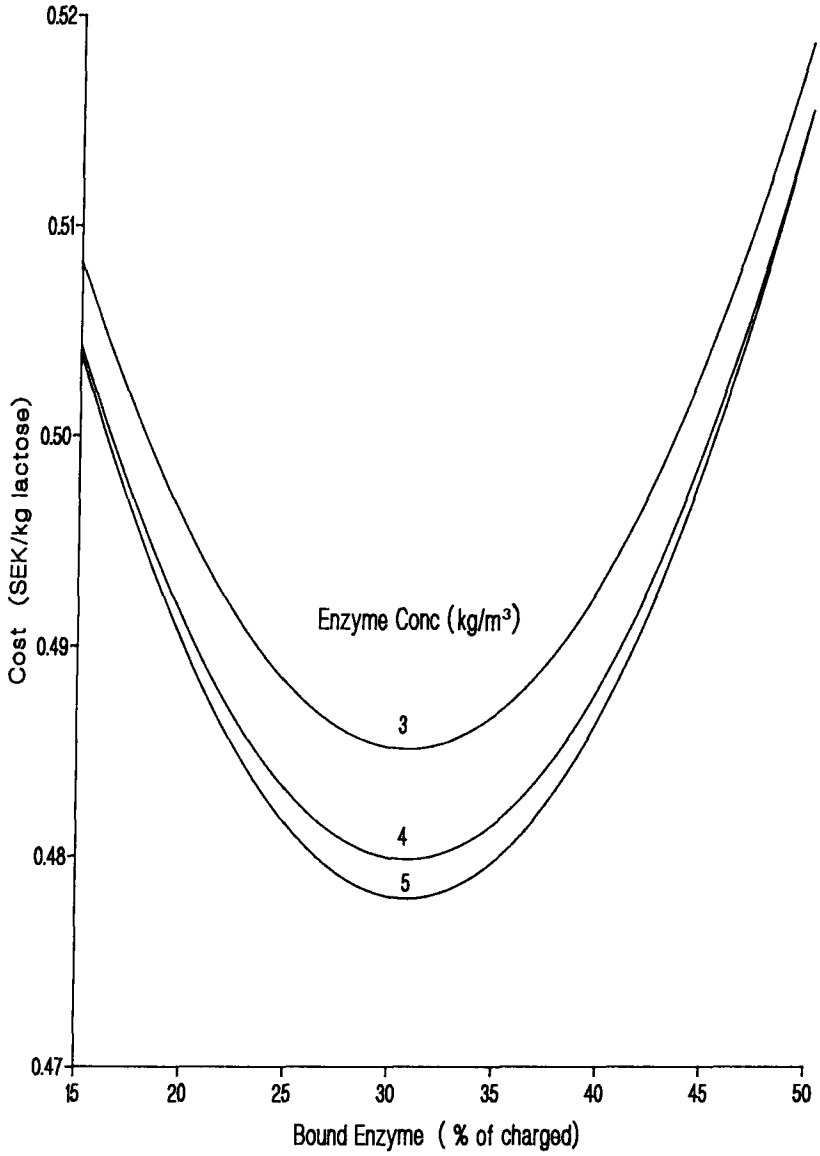


Fig. 4. Hydrolysis cost for the PFTR as function of percent bound enzyme.

for the enzyme is only 17% of the total, whereas the cost for the immobilization constitutes almost 30%.

The PFTR (Fig. 7) gives the lowest cost of about 0.48 SEK/kg lactose, obtained for an enzyme loading of 5 kg/m$^3$. The mean residence time is 1 h, and the reactor vol is about 17 m$^3$. The main difference, when compared to the batch-tank reactor, is the labor cost, which is higher for the batch operation. The cost for the enzyme is about the same for the two alternatives and constitutes only about 6 and 10% of the total cost for the batch and PFTR, respectively.

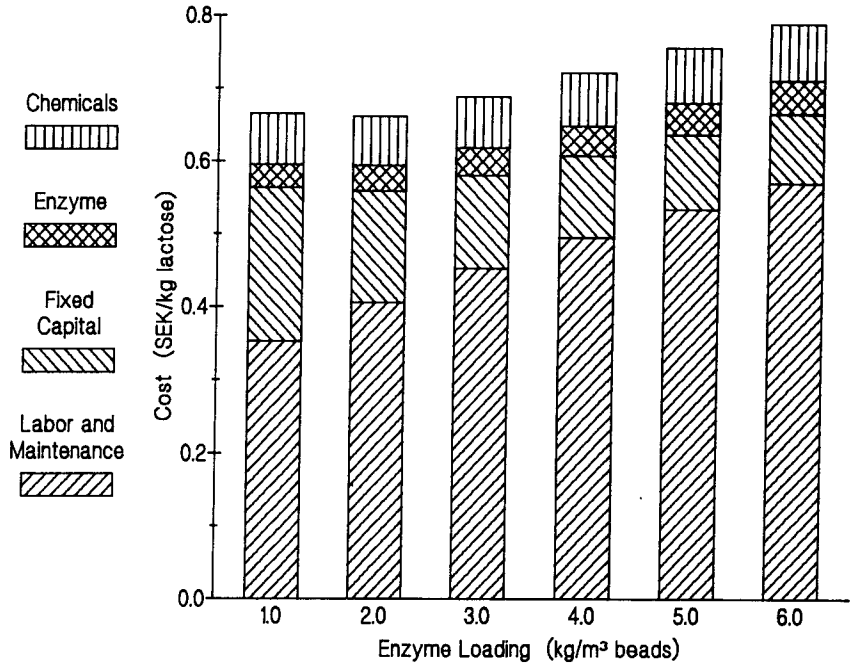


Fig. 5. Hydrolysis cost for batch hydrolysis with immobilized enzymes.

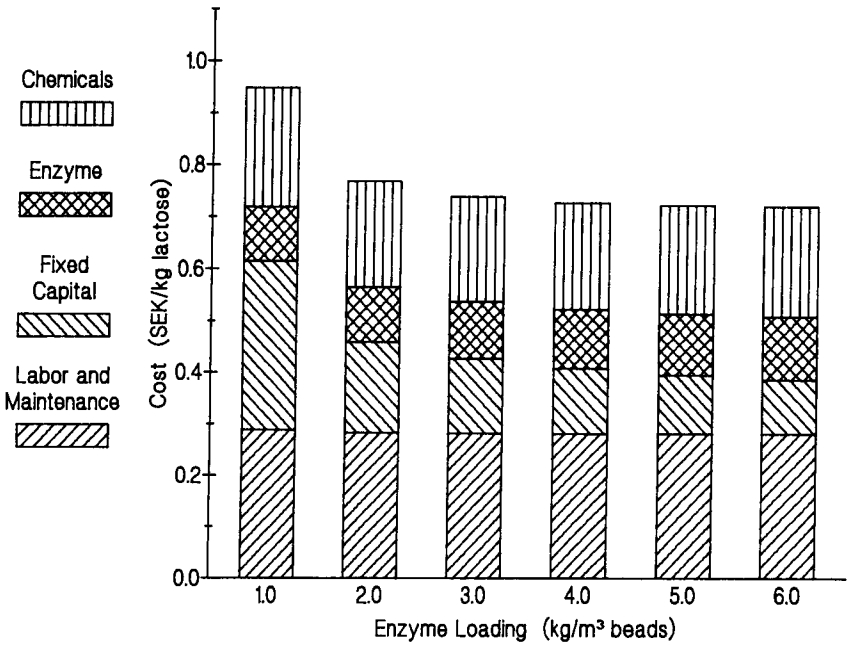


Fig. 6. Hydrolysis cost for the CSTR alternative.

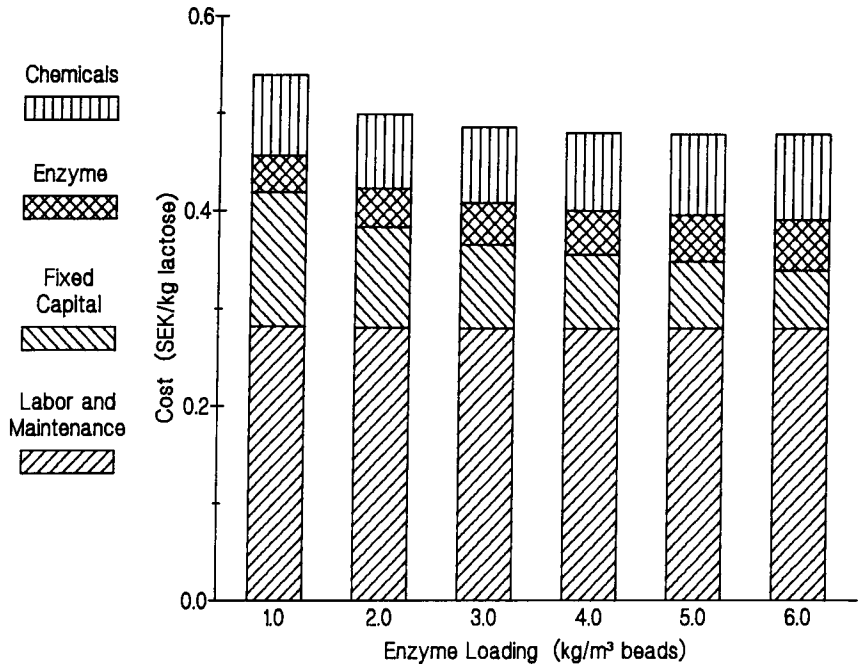


Fig. 7. Hydrolysis cost for the PFTR alternative.

The effectiveness factor varied between 0.48 and 0.97. The highest value is for the CSTR at low enzyme loadings, and the lowest value is for the PFTR and batch tank reactor with high enzyme loadings. As expected for a product-inhibited system, the alternatives with the PFTR and batch-tank reactor have lower capital cost and cost for enzyme than the alternative with the CSTR, despite its higher effectiveness factor.

A decrease in the enzyme half-life time increases the enzyme and immobilization costs. For the PFTR with a half-life time of 40 d, the optimal enzyme loading changes to 4 kg/m$^3$, and the cost increases to 0.60 SEK/kg. Even with this change, the PFTR still remains the most economical choice.

The optimum conversion is dependent of the purpose of the hydrolysis, as well as of the raw material cost. Plants for sugar sweetening are run at conversions up to 85%. When the hydrolysate is to be used for production of ethanol or other chemicals, even higher conversions may be optimal. The effect of the conversion on the cost is shown in Fig. 8 for the PFTR, with varying raw material prices. The cost is related to the amount of product since it varies when the conversion is varied. When the cost for the raw material is set to zero, the optimum in conversion is about 70–75%. With increasing raw material costs, the optimum moves to a higher conversion.

With varying conversion, the PFTR still remains the most economical choice although the difference between the reactor alternatives may alter, as can be seen in Fig. 9, where the cost for 70 and 90% conversion are

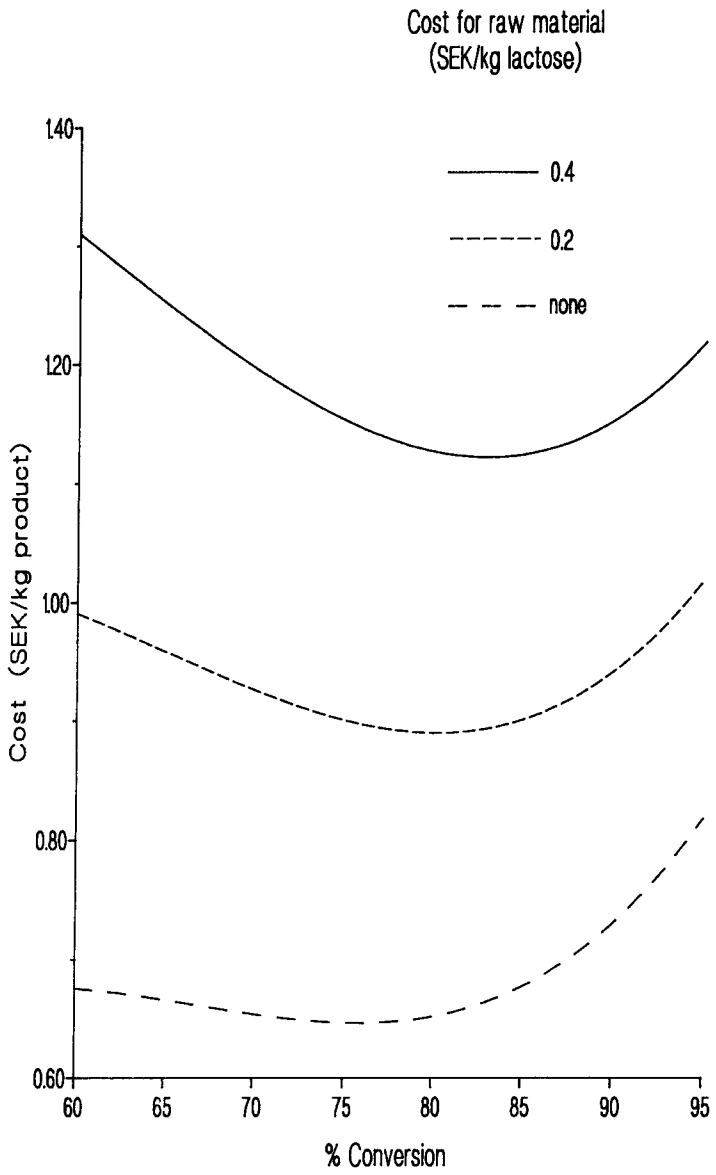


Fig. 8. Influence of conversion and raw material cost on the product cost for the PFTR.

given. The optimum enzyme loading is used in each alternative, and the cost for raw material is set to zero. At 90% conversion, the cost is increased for all the alternatives although the batch reactor with immobilized enzymes is least influenced.

The use of immobilized enzymes do not automatically imply continuous operation. From Fig. 9, it can be seen that a batch immobilized process gives a price just slightly higher than the continuous process, with

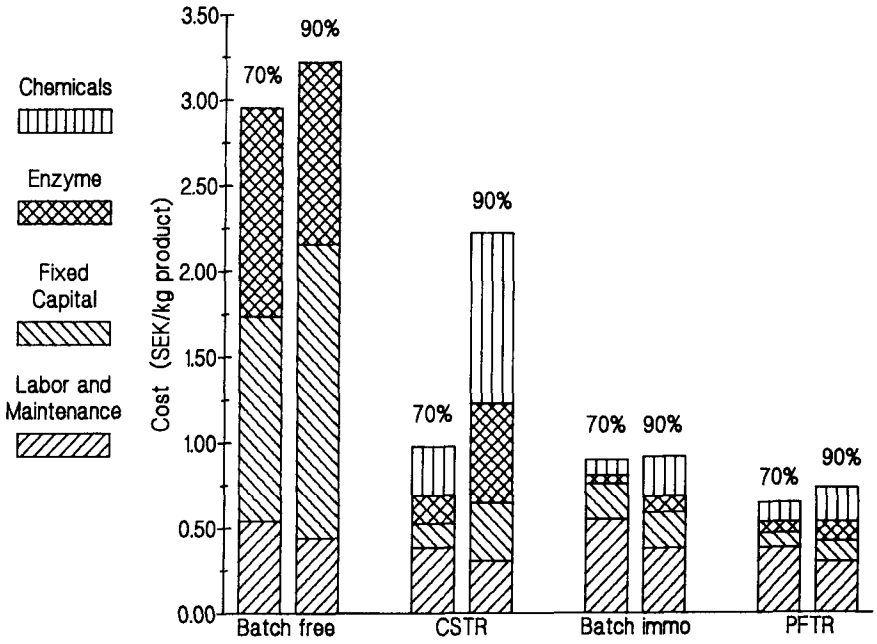


Fig. 9. Influence of the conversion on the cost.

the PFTR especially at a higher conversion. Since the capital and labor costs vary between different countries, it could be of interest to notice that an increase in these costs both will enhance the conclusions made.

## CONCLUSION

The results show that the enzyme immobilization is economically feasible for the hydrolysis of lactose, compared with a system with free enzymes, despite the very high cost for enzyme attachment. The price of the coupling chemicals is 15 times the price of the enzyme and causes the cost for the immobilization to be twice as high as the cost for the enzyme itself. This really indicates an area for improvements.

The lowest cost is obtained for the PFTR. A highly automatized batch process, with decreased labor costs, however, can be a competitive alternative to the continuous process especially when the risk for contamination is considered.

In our ongoing work, we are studying how the use of multiple reactor systems, such as CSTRs in series and PFTRs in parallel to reduce the capacity fluctuation, will influence the economy of the process. This will be compared to a nonisothermal process with optimal temperature increase. The program will also be used to investigate the economy for a process with coimmobilized $\beta$-galactosidase and yeast for production of ethanol from whey.

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