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Theoretical performance of countercurrent reactors for production of enantiopure compounds

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ABSTRACT

Irreversible reactions are being applied in enzymatic kinetic resolution to obtain enantiomerically pure compounds from racemic mixtures. Using model calculations for situations without mass transfer limitation, we show that reversible reactions might also be useful for enzymatic kinetic resolution, provided that countercurrent systems are used rather than batch or cocurrent systems. The required reaction time or enzyme amount in a countercurrent system is much lower than in an analogous cocurrent system or its batch equivalent. More importantly, often the calculated yield and enantiomeric excess are better in countercurrent systems. Racemization can also be favorably used in countercurrent systems. Consequently, to achieve with a reversible reaction a particular enantiomeric excess and yield, a countercurrent system needs less dilution or activated co-reactant and less enantioselective enzyme than a cocurrent system.

1. Introduction

According to a recent survey 70–90% of all chemical processes on industrial scale is performed in a catalytic way (Gadler et al., 2006). One type of catalytic processes applied in industry is kinetic resolution (Sheldon, 1993), for which many varieties have been developed. Kinetic resolution of racemates is a useful method for the production of enantiomerically pure compounds. As shown in Table 1 for Type 1 reaction, an enantioselective conversion of enantiomer A^R can lead to a mixture enriched in C^R or A^S. The reaction has to be stopped before the slower reacting enantiomer A^S is converted too much. However, equilibrium limited reactions are not useful for kinetic resolution in batch systems, because the undesired reactant enantiomer A^R is not fully converted. Then, the desired remaining reactant enantiomer will not become enantiopure (Chen et al., 1987). Besides, equilibrium limitations lead to low yields in case of kinetic resolutions that aim at enantiopure products (Chen et al., 1987).

Nevertheless, this work will deal with the production of enantiopure compounds applying kinetic resolution processes for such equilibrium limited reactions. Cases involving racemization will also be investigated. A racemization reaction can be used to keep the proportion of two enantiomers equal, which facilitates the progress of the faster of the two parallel reactions in the kinetic resolution (Sheldon, 1993). An overview of all reaction schemes analyzed is presented in Table 1. In previous studies, reversibility of reaction was usually minimized by diluting (for example, Berendsen et al., 2006; van Tol et al., 1995) or by using activated co-reactant (for example Huber et al., 1996; Janssen et al., 1991; Suan and Sarmidi, 2004), but these methods lead to significant additional costs.

The goal of the current work is to investigate theoretically the potential of a countercurrent reactor for kinetic resolution of equilibrium limited reactions. Reactors in which one or two reactants are introduced and enzymatically converted into two products will be studied. The results will also apply to uncatalyzed or chemically catalyzed reactions. Like shown in Table 1, one of the reactants and in most cases one of the products is chiral.

The countercurrent system that we consider (Fig. 1) contains two immiscible phases that flow in a countercurrent fashion (Takashi and Silveston, 2005). Usually, one is an aqueous reaction phase and the other is a water-immiscible solvent, a vapor, or an adsorbent phase. In this work a water-immiscible solvent is assumed as the second phase (called "auxiliary phase" subsequently). The enzyme can be immobilized or dissolved. If the chiral reactant in the countercurrent reactor would be introduced with one of the phases at one of the ends of the column, much of this reactant would directly leave the column at the same end with the other phase and the extent of conversion would be low (van der Wielen et al., 1996). Therefore, the reactants are introduced at an intermediate position in the column, so that they may be converted almost completely into two products. One of the products should preferably partition to the reaction phase and one to the auxiliary phase. In this way the reaction products will be

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Table 1
Schematic overview of reaction schemes that are investigated. The inclusion of B is optional. Dashed arrows indicate the slower reaction. Boxes indicate target enantiomers.

Type	Scheme of reaction	Purpose
1	$A^R+B \rightleftharpoons \boxed{C^R}+D$ $\boxed{A^S}+B \dashrightarrow C^S+D$	Kinetic resolution for (a) obtaining enantiopure product C^R (b) obtaining enantiopure remaining reactant A^S
2	$A^R+B \rightleftharpoons \boxed{C^R}+D$ $A^S+B \dashrightarrow C^S+D$	Racemization of reactant for obtaining enantiopure product C^R (dynamic kinetic resolution)
3	$A^R+B \rightleftharpoons C^R+D$ $\boxed{A^S}+B \dashrightarrow C^S+D$	Racemization of product for obtaining enantiopure remaining reactant A^S
4	$A^R+B \rightleftharpoons C+D$ $\boxed{A^S}+B \dashrightarrow C+D$	Kinetic resolution for obtaining enantiopure remaining reactant A^S with formation of achiral product

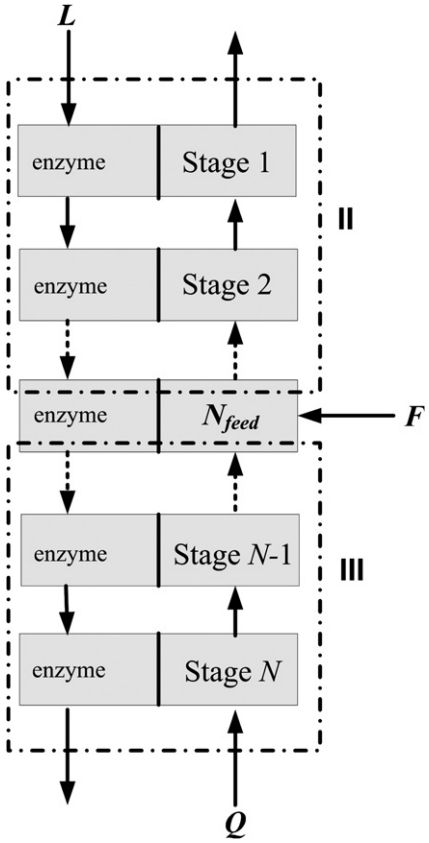

Fig. 1. A fractionating system with two countercurrent sections. The reactant phase L is entering section II and auxiliary phase Q is entering section III. Feed can be dissolved either in L or Q.

transported in opposite directions with the two phases and directly be separated from each other and the raw materials, so that the reverse reaction is minimized.

Usually countercurrent systems consist of four countercurrent sections, two (section II and III) for reaction and two (section I and IV) for regeneration of the phases (Lode et al., 2001). This work deals with the reactive sections II and III only, shown in Fig. 1.

When a reactant consists of two enantiomers, the systems should be designed such that the fast reacting enantiomer is converted almost completely while the slow reacting enantiomer leaves the system almost unconverted.

Note that one may use countercurrent enantioselective extraction or another way for enantioselective transport to an auxiliary phase (van der Ent et al., 2002) instead of countercurrent enantioselective catalysis. Here we focus on countercurrent enantioselective catalysis.

The possibility of obtaining both enantiopure product and enantiopure remaining reactant for an equilibrium limited reversible reaction has been theoretically described for chemocatalytic (*R*)-propylene glycol production from racemic propylene oxide in a continuous reactive distillation column (Okasinski and Doherty, 2003). The slowly reacting enantiomer was almost inert. The current study will show that in the field of production of enantiopure compounds the scope of countercurrent systems is much wider. The results obtained for countercurrent systems will be compared to results calculated for biphasic cocurrent systems (comparable to biphasic batch systems when the number of stages in a cocurrent system becomes infinite) and some general conclusions of the system behavior will be drawn. Using a simple criterion (Martinek et al., 1981) it was checked beforehand that batch biphasic systems are superior to batch monophasic systems for the model reactions.

The quality of the product of a kinetic resolution is characterized by its enantiomeric excess. For most applications, an enantiomeric excess of $>95\%$ is required (Sheldon, 1993). We will focus on 96%. For a given enzyme, the yield of remaining reactant or formed product with this high enantiopurity should be maximized by reaction and reactor engineering.

The most relevant enzyme property is the enantiomeric ratio (E), which indicates its selectivity for the fast reacting enantiomer relative to the slowly reacting enantiomer (Sheldon, 1993). For irreversible reactions in a batch system 96% enantiomeric excess of product can be obtained only in case of $E>50$, so this is the usual range for commercial enantioselective enzymes. Reversibility will not change the maximum possible enantiomeric excess of product, but due to incomplete conversion the maximum yield of enantiopure product will decrease (Chen et al., 1987). Increasing of the enantiopure product yield by countercurrently separating it from the second, non-chiral product will be investigated in this work.

A high enantiomeric excess of remaining reactant for *irreversible* reactions can be obtained in a batch system even using an enzyme having a low enantiomeric ratio, although the yield may be low. If a reaction is *reversible*, it is no longer possible to obtain a high enantiomeric excess of reactant, due to incomplete conversion of the undesired reactant enantiomer (Straathof and Jongejan, 1997). This is also the case when the enantiomeric ratio is very high. The possibility of increasing the yield of enantiopure reactant by countercurrent separation of the products will be examined.

Kinetic resolution of racemic mixtures suffers from drawbacks that the maximum yield is only 50% of the chiral starting material and that laborious separation of enantiomerically pure reactant and product may be required (Spelberg et al., 2004). This limitation can be overcome by including racemization of the chiral reactant in a so-called dynamic kinetic resolution (Type 2 in Table 1). The impact of the countercurrent system on this reaction type will be also considered. In some kinetic resolution systems, spontaneous racemization of chiral product occurs. In the current study, racemization of chiral product (Type 3 in Table 1) will be investigated and its influence on the enantiomeric excess of the chiral reactant. García Palacios et al. (2009) conceptually combined racemization with countercurrent adsorption, using continuous chiral chromatography, but the present work deals with different concepts where the auxiliary phases will not preferentially adsorb

or extract one enantiomer of a racemate. The enantioselectivity stems from the enzyme.

A mathematical model will be developed to compare system performances for kinetic resolution with and without racemization. Results obtained with this model will be discussed.

2. Mathematical model

2.1. Model description

Mathematical models were developed for countercurrent and for cocurrent continuous systems with the following assumptions:

1. The systems consist of a number of equal theoretical stages, each containing a volume of reaction and auxiliary phase (Figs. 1 and 2).
2. The fed reactants are dissolved in auxiliary phase and introduced in stage N_{feed}, which is the central stage for the countercurrent system.
3. The volume ratio between reaction and auxiliary phase in a stage is assumed to be equal to their flow ratio. This implies that for the countercurrent system the auxiliary phase hold-up ε is different on either side of the feed stage.
4. For simplicity, it is assumed that the enzymatic reaction occurs only in the reaction phase in which enzyme is distributed homogeneously. Enzyme flowing out is substituted by an equal amount flowing in. No enzyme partitions to the auxiliary phase and it does not inactivate.
5. The stages are ideal mixers in which mass transfer limitation is absent. Using this assumption we will overestimate the performance of the systems, but clearly see the limitations introduced due to the reversibility of the reaction
6. The solutions are thermodynamically ideal
7. The processes are isothermal and show no volume change upon reaction, so that volume balances apply in line with Figs. 1 and 2
8. An excess of the co-reactant B will be assumed, so that its role will be trivial, as will be explained at the end of this section.

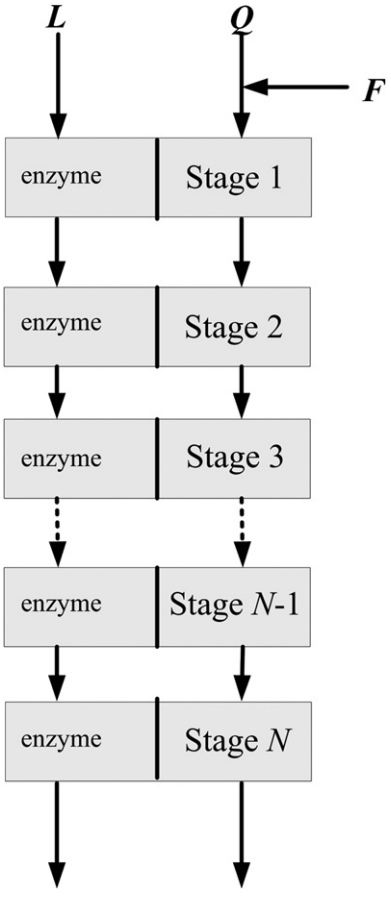

Fig. 2. A cocurrent system: The reactant phase L and the auxiliary phase Q are entering the system from the same side. Feed can be dissolved either in L or Q.

The following mass balances were developed for the countercurrent system with stages numbered $j=1\ldots N$ (Fig. 1) for component i=A, C, D (per enantiomer if applicable):

Reaction phase:

$$M_j(1-\varepsilon_j)\frac{dc_{i,j}}{dt} = L_{j-1}c_{i,j-1} - L_j c_{i,j} - M_j(1-\varepsilon_j)\left[r_{r,i,j} + r_{rac,i,j}\right] + M_j(1-\varepsilon_j)r_{mt,i,j} \quad (1)$$

Auxiliary phase:

$$M_j\varepsilon_j\frac{dq_{i,j}}{dt} = Q_{j+1}q_{i,j+1} - Q_j q_{i,j} + F_j f_{i,j} - M_j(1-\varepsilon_j)r_{mt,i,j} \quad (2)$$

M_j is the mass in stage j; c_i, q_i and f_i are the concentrations of component i in the reaction phase, auxiliary phase, and feed, respectively, where $f_{i,j}$ is zero for $j \neq N_{feed}$; L, Q and F are the flows of reaction phase, auxiliary phase and feed, respectively; r_r is the reaction rate, r_{rac} is the racemization rate (if applicable) and r_{mt} is the mass transfer rate of the extraction. These rates are expressed per amount of reaction phase.

In the case of the cocurrent system (Fig. 2) the mass balance equation is different for the auxiliary phase:

$$M_j\varepsilon_j\frac{dq_{i,j}}{dt} = Q_{j-1}q_{i,j-1} - Q_j q_{i,j} + F_j f_{i,j} - M_j(1-\varepsilon_j)r_{mt,i,j} \quad (3)$$

The mass transfer rate of component i is defined by the following equation:

$$r_{mt,i} = k_{mt}a\left(\frac{q_i}{K_{mt,i}} - c_i\right) \quad (4)$$

k_{mt} is the mass transfer coefficient, a is the contact area per amount of reaction phase, and $K_{mt,i}$ the partition coefficient of component i between auxiliary and reaction phase.

For simplicity, the reversible reaction rates are taken first order in each of the compounds involved, in a typical mass action law form. For example, for R-enantiomer in Type 1–3 reactions the equation is

$$r^R_{r,i} = k^R_r c_E\left(c_{A^R}c_B - \frac{c_{C^R}c_D}{K_{eq}}\right) \quad (5)$$

where k^R_r is the reaction rate constant of R-enantiomer and c_E is the enzyme concentration. For S-enantiomer the R-superscripts are replaced by S-superscripts.

In the rate equations the reaction equilibrium constant is defined by

$$K_r = \left(\frac{c_{C^R}c_D}{c_{A^R}c_B}\right)_{eq} = \left(\frac{c_{C^S}c_D}{c_{A^S}c_B}\right)_{eq} \quad (6)$$

For Type 4 reactions, product C is not chiral and has no R- or S-superscripts in Eqs. 5 and 6.

The enantiomeric ratio is defined by

$$E = \frac{k^R_r}{k^S_r} \quad (7)$$

In the case of racemization of reactant (Kitamura et al., 1993), the racemization rate of A^S to A^R is defined by the following equation:

$$r_{rac,A^S} = k_{rac}(c_{A^S} - c_{A^R}) \quad (8)$$

where k_{rac} is the racemization rate constant. This definition assumes racemization to occur only in the reaction phase. Racemization may be spontaneous, but if a racemization catalyst is involved,

it is assumed that its concentration is constant and can be incorporated in k_{rac} (cf. assumption 4 for the enzyme).

An analogous equation applies to racemization of product.

The yield of a reaction (Y) is defined as the sum of the molar amounts of the chiral product leaving the system with the two phases divided by the molar amount of the chiral reactant introduced. For a cocurrent system this definition becomes

$$Y = \frac{Q_N(q_{C^R,N}+q_{C^S,N})+L_N(c_{C^R,N}+c_{C^S,N})}{F(f_{A^R}+f_{A^S})} \tag{9}$$

For a countercurrent system

$$Y = \frac{Q_1(q_{C^R,1}+q_{C^S,1})+L_N(c_{C^R,N}+c_{C^S,N})}{F(f_{A^R}+f_{A^S})} \tag{10}$$

Likewise, yields are defined for the remaining chiral reactant. This equals 1 minus the extent of conversion, but the term "yield" is retained here when unconverted reactant is the target.

The average enantiomeric excesses of the remaining chiral reactant and the product are calculated for both systems. The average enantiomeric excess is defined as the ratio between the difference between the total mole amount of enantiomers in both phases at the system outlet and the sum of the total mole amount of enantiomers in both phases at the system outlet. For the cocurrent system, this is defined as

$$ee_A = \frac{A^S_{L,N}-A^R_{L,N}+A^S_{Q,N}-A^R_{Q,N}}{A^S_{L,N}+A^R_{L,N}+A^S_{Q,N}+A^R_{Q,N}} \times 100\% \tag{11}$$

$$ee_C = \frac{C^R_{L,N}-C^S_{L,N}+C^R_{Q,N}-C^S_{Q,N}}{C^S_{L,N}+C^R_{L,N}+C^S_{Q,N}+C^R_{Q,N}} \times 100\% \tag{12}$$

For the countercurrent system the average enantiomeric excess is

$$ee_A = \frac{A^S_{L,N}-A^R_{L,N}+A^S_{Q,1}-A^R_{Q,1}}{A^S_{L,N}+A^R_{L,N}+A^S_{Q,1}+A^R_{Q,1}} \times 100\% \tag{13}$$

$$ee_C = \frac{C^R_{L,N}-C^S_{L,N}+C^R_{Q,1}-C^S_{Q,1}}{C^S_{L,N}+C^R_{L,N}+C^S_{Q,1}+C^R_{Q,1}} \times 100\% \tag{14}$$

By using the aforementioned definitions of yield and enantiomeric excess, information about the system's performance is given in a condensed way, for reasons of brevity. Yield and enantiomeric excess will not be specified per exit flow. Such information will become critical when focusing on product recovery, but here we limit our focus on the overall reaction performance. To condense information further, the variable Y_{96} will be used, which is the yield of target compound when its enantiomeric excess is at the target value of 96%.

The model was programmed in Matlab (The MathWorks, Natick, Massachusetts). The model developed here is a transient model. The results of the model, for the cases when no reaction is present and when an irreversible first order reaction is present, were consistent with the analytical solutions for these cases. The data presented in this work are obtained from the steady states obtained after dynamic simulation. Steady states were assumed when there was less than 0.01% change in the composition of outlet streams upon continuing simulations.

2.2. Model settings

The values of parameters used in the simulations are presented in Table 2 (2nd column). The flow rates were selected on the basis of literature recommendations for non-enantioselective reactions (den Hollander et al., 2004) where it was concluded that the highest conversion is obtained when the reactant has no tendency to move with either phase. The following inequality (den Hollander et al., 2004) is obeyed:

$$K_{mt,D} < \frac{Q_{II}}{L_{II}} < K_{mt,A} < \frac{Q_{III}}{L_{III}} < K_{mt,C} \tag{15}$$

Indexes II and III represent the two countercurrent sections of system on either side of the feeding point. Simulations to check if this design criterion is also valid for the current systems gave positive results. The implicit requirement $K_{mt,D} < K_{mt,A} < K_{mt,C}$ is fulfilled in many cases, for example when partitioning between the countercurrent phases is based on hydrophobicity, because reactant A will be split into molecules C and D with usually a hydrophobicity higher and lower, respectively, than A. Examples are conversions of chiral alcohols into alkenes+H_2O, and conversion of aspartic acid into fumaric acid+ammonia.

For simplicity, the ratio of reaction phase hold-up to auxiliary phase hold-up in a stage was assumed to be equal to the reaction to auxiliary flow rate ratio.

To identify the full potential of the countercurrent systems, ideal countercurrent conditions were chosen. In the simulations the number of stages was set at 80. The yield of reaction for either countercurrent or cocurrent system did not change noticeably with a further increase of the number of stages. Also results for the cocurrent system with $N=80$ were comparable with those for a batch system ($N\to\infty$). To avoid mass transfer limitations, which will be unfavorable, $k_{mt}a$ was given a much larger value than the reaction kinetic parameters $k_r^S c_E$ and k_{rac}.

In the simulations the feed concentration of the second reactant f_B was given a much larger value than f_A. So B will hardly be consumed and the concentration of B can be assumed to be fixed at $c_B=f_B$. Such an excess will apply, for example, when B is water while using an aqueous phase. In this way there is no need to evaluate the influence of the value of parameter z_B separately from the other parameters, and the simulation results can also be used for reaction schemes in which B is not involved.

To obtain 96% enantiomeric excess of product a high enantiomeric ratio is required. In the present simulations the default value was $E=100$.

Table 2
Parameter values used in the simulations. For parameter units see the Nomenclature. n.a.=not applicable.

Parameter	Default value	For HMN case	For ibuprofen case
N	80	80	80
N_{feed}	40	40	40
$f^R_A=f^S_A$	1	6	0.1
f_B	50	n.a.	0.8
f_C	0	0	0
f_D	0	0	2.5×10^{-4}
$K_{mt,A}$	5	14	0.1
$K_{mt,B}$	1×10^{-3}	n.a.	1×10^{-3}
$K_{mt,C}$	100	21	0.01
$K_{mt,D}$	0.01	2.6	0.3
F	0.5	0.1	0.5
$Q_{entry\ stage}$	0.5	1	10
$L_{entry\ stage}$	5	14	0.5
$k_{mt}a$	100	100	100
$k_r^S c_E$	10^{-7}	10^{-6}	0.43
E	100	Several	n.a.
K_r	10^{-4}	0.05	3.9×10^{-3}
k_{rac}	1	n.a.	n.a.

3. Results and discussion

3.1. Conversions in cocurrent and countercurrent systems

In the simulations, different steady states were obtained by changing the total mass in the system, so that the residence time

changed. (Alternatively, these steady states might have been achieved by changing merely the enzyme amount.) For a typical case (Table 1, Type 1), Fig. 3 shows that the obtained yields in the countercurrent system are much higher than in analogous cocurrent systems. It can be concluded that in a countercurrent system a shorter residence time or a lower enzyme amount is required to obtain a particular yield. This confirms earlier results (den Hollander et al., 2004).

The subsequent part of this paper does not focus on residence time but on the relation between enantiomeric excess and yield, while the residence time was varied via the mass in the system to achieve different enantiomeric excess and yield values.

3.2. Kinetic resolution for obtaining enantiopure product (reaction type 1a)

The relation between yield of enantiopure product and the reaction equilibrium constant for type 1a reactions for different feed concentrations of racemic A and a fixed enantiomeric excess of product P of 96% is presented in Fig. 4. In line with Section 3.1, residence times varied, but only yields obtained in simulations giving 96% enantiomeric excess are reported.

From Fig. 4 it can be concluded that there is a maximal value of the equilibrium constant for which the countercurrent system is the preferable option. This maximal value increases if the feed concentration of racemic reactant is increased.

The enantiomeric excess vs. yield values were plotted for a feed concentration of racemic reactant of 1 mol kg^{-1} per enantiomer and are presented in Fig. 5. The values giving 96% enantiomeric

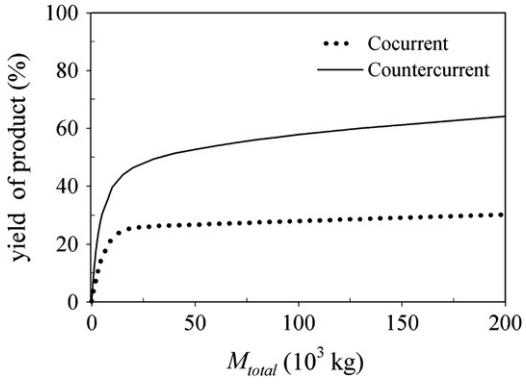

Fig. 3. The yield of product vs. the total reactor size for cocurrent and countercurrent systems for Type 1a reaction in Table 1. For parameter values see Table 2.

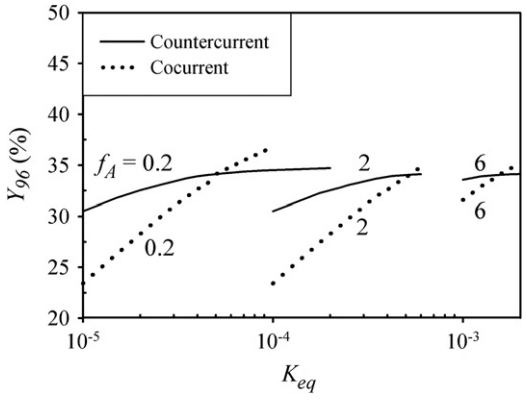

Fig. 4. Kinetic resolution for obtaining product of ee_C=96%. Numbers inside the figure indicate the feed concentration of racemic reactant per enantiomer in mol kg^{-1}. For other parameter values see Table 2.

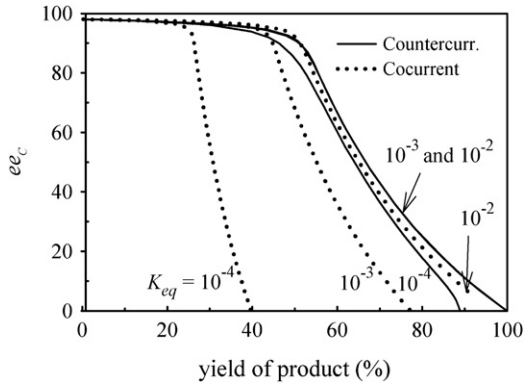

Fig. 5. Kinetic resolution for obtaining enantiopure product: Enantiomeric excess of product vs. yield for assumed different reaction equilibrium constants. For other parameter values see Table 2. The countercurrent curves for K_r=10^{-2} and 10^{-3} overlap.

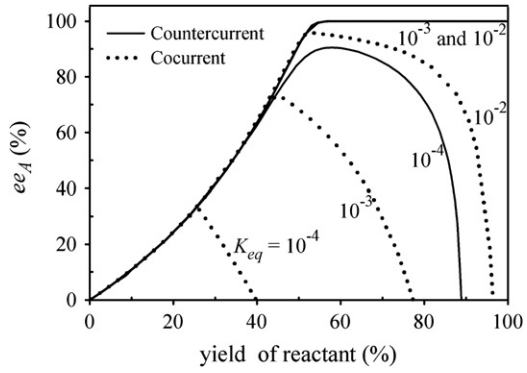

Fig. 6. Kinetic resolution for obtaining enantiopure reactant: Enantiomeric excess of reactant vs. yield for assumed different reaction equilibrium constants. For other parameter values see Table 2. The countercurrent curves for K_r=10^{-2} and 10^{-3} overlap.

excess in the figure correspond to values given in Fig. 4. The cocurrent curves in Fig. 5 are in agreement with calculations for batch systems (Chen et al., 1987). Fig. 5 indicates that for type 1a reaction countercurrent and cocurrent curves almost overlap for K_r=10^{-2}, but that for K_r=10^{-3} and especially for K_r=10^{-4} the countercurrent system show a superior yield. Thus, the worse the equilibrium, the more useful the countercurrent system is. The value of K_r does not matter anymore when it is sufficiently high. Then the performance of the system is determined by the value of E only.

3.3. Kinetic resolution for obtaining enantiopure remaining reactant (reaction type 1b)

Kinetic resolution for obtaining enantiopure remaining reactant (Type 1b in Table 1) was also considered. The enantiomeric excess vs. yield values of enantiopure reactant are presented in Fig. 6. From Fig. 6 it can be noticed that for some values of K_r it is not possible to obtain 96% enantiomeric excess with the cocurrent system. Then one is bound to use a countercurrent system.

For the used excess of co-reactant B, dilution should increase the achievable yield of the equilibrium reactions, according to Le Chatelier's principle. Fig. 7 shows that for f_A^R=f_A^S=1 mol kg^{-1} one needs $K_r > 0.015$ to achieve enantiomeric excess of 96% in the cocurrent system while in the countercurrent system this enantiomeric excess can be obtained with $K_r > 0.008$. For higher f_A values both K_r values increase. Comparison of these numbers with

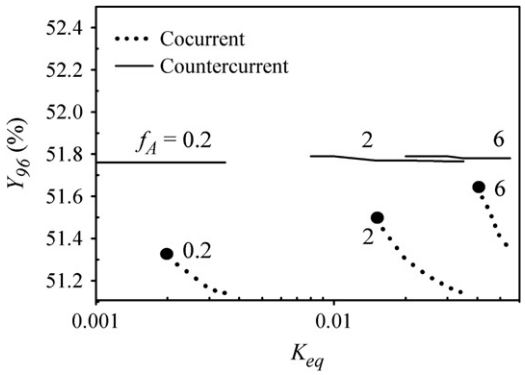

Fig. 7. Kinetic resolution for obtaining reactant of ee_A=96%. Numbers inside the figure indicate the feed concentration of racemic mixture per reactant in mol kg^{-1}. For other parameter values see Table 2. The large dots indicate the lower limits of K_r for achieving ee_A=96% in the cocurrent system.

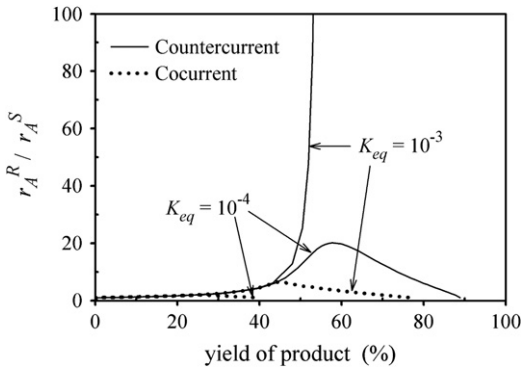

Fig. 8. Ratio of reactant consumption rates at different product yield values for different reaction equilibrium constant values. For other parameter values see Table 2.

Fig. 4 shows that in the case of the enantiopure remaining reactant the values of the reaction equilibrium constants for which countercurrent system becomes the more interesting option are one order of magnitude higher than the limiting values to obtain enantiopure product (in the case of the $f_A^R = f_A^S = 1$ mol kg^{-1}, for ee_A=96% $K_r < 1.5 \times 10^{-2}$ while for ee_C=96% $K_r < 5.3 \times 10^{-4}$, which is a more severe constraint). Thus it might be easier to find suitable countercurrent reaction systems if the focus is on enantiopure remaining reactant.

In order to understand the system behavior the ratio of enantiomers of remaining reactant and ratio of formed enantiomers for different yields were plotted (Figs. 8 and 9).

During the reaction, so while product yield increases, three consecutive regimes can be distinguished:

1. The fast and slow reactions are not at equilibrium. This regime is presented for the ascending part of the curves in Fig. 8.
2. The fast reaction is close to equilibrium but the slow reaction not (maximum of the curves in Fig. 8).
3. The fast reaction is at equilibrium and the slow reaction is close to equilibrium (descending part of the curves in Fig. 8).

For some parameter values the third regime is not pronounced (see Fig. 8 for $K_r = 10^{-3}$ in the countercurrent system) because even though the slow reaction is far from equilibrium and the fast one not, the *R*/*S* reaction rate ratio of the remaining reactant is still high.

From Fig. 9 it can be noticed that the initial ratio of product formation rates is higher for the cocurrent than for the countercurrent system. From this it can be concluded that in the first

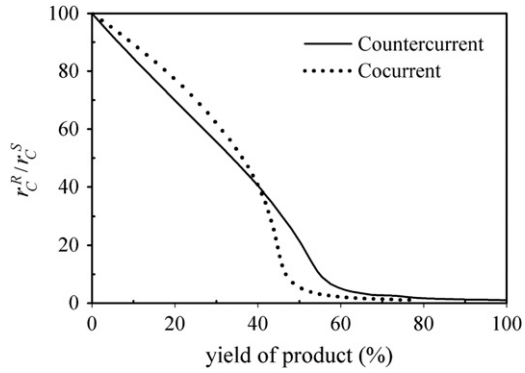

Fig. 9. Kinetic resolution: Ratio of product formation rates for different yields of reaction for $K_r = 10^{-3}$. For other parameter values see Table 2.

regime, where both reactions are far from equilibrium, the countercurrent product separation speeds up the slow reaction more than on fast reaction. As a consequence the reaction ratio of the formed enantiomers in the countercurrent system is lower than in the cocurrent system. For short residence times (conditions leading to low yields) this will have a negative influence on the enantiomeric excess of formed enantiomers, but no noticeable effect on the enantiomeric excess of remaining reactant because the extent of conversion is still low. However, it is known that in the countercurrent system, due to countercurrent separation of products, the equilibrium position is improved and the higher yields can be obtained (Lode et al., 2001). For longer residence times (higher yields) the cocurrent system will faster approach the second regime where the fast reaction is close to equilibrium but the slow reaction not. In the countercurrent system both reactions will still be far from equilibrium. As a consequence for longer residence times, the reaction ratio between either formed enantiomers or remaining reactant enantiomers becomes lower in the cocurrent than in the countercurrent system (Figs. 8 and 9). This will have a positive influence on the enantiomeric excess. From this it can be concluded that the countercurrent system will become the system of interest in cases in which the fast reaction is close to equilibrium for the cocurrent system.

As shown in Fig. 5 the highest enantiomeric excess of product is obtained initially. For the highest values of the reaction equilibrium constant these are situations still far from reaction equilibrium. Because of the distance from the equilibrium and the fact that in countercurrent systems countercurrent separation has more influence on the slow reacting enantiomer, the countercurrent system will not be the preferable option for systems with high values of the reaction equilibrium constant. When the feed concentration of racemic reactant is increased or the value of the reaction equilibrium constant is decreased, the reaction equilibrium is reached at low yields of reaction and for these cases the countercurrent system will become interesting.

For the case of obtaining enantiopure reactant the situation is obvious. The enantiomeric excess is at its maximum value when the fast reaction is close to equilibrium. Due to this it is expected that for the equilibrium limited reactions the enantiomeric excess of remaining reactant can always be improved in the countercurrent system.

3.4. Racemization of reactant (reaction type 2)

For this case, the enantiomeric excess of product vs. yield of product was plotted for different values of the reaction equilibrium constant (Fig. 10). From the figure it can be concluded that for a fixed enantiomeric excess of product at low values of the reaction equilibrium constant ($K_r = 10^{-4}, 10^{-3}$) higher yields of enantiopure

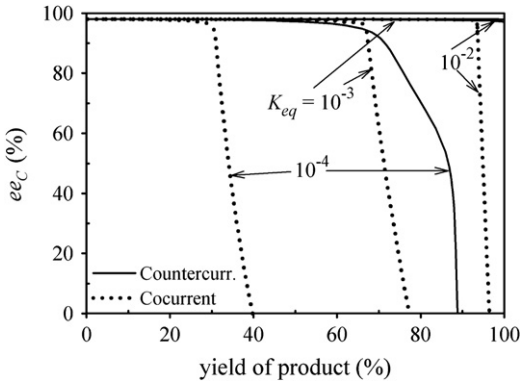

Fig. 10. Racemization of reactant: enantiomeric excess of reactant vs. yield of product. For parameter values see Table 2. The countercurrent curves for $K_r = 10^{-2}$ and 10^{-3} overlap.

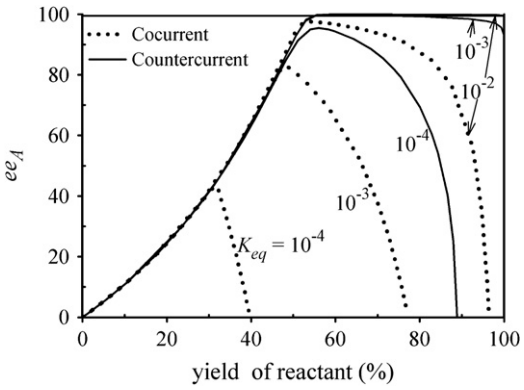

Fig. 11. Racemization of product: Enantiomeric excess of reactant vs. yield for different reaction equilibrium constants. For other parameter values see Table 2.

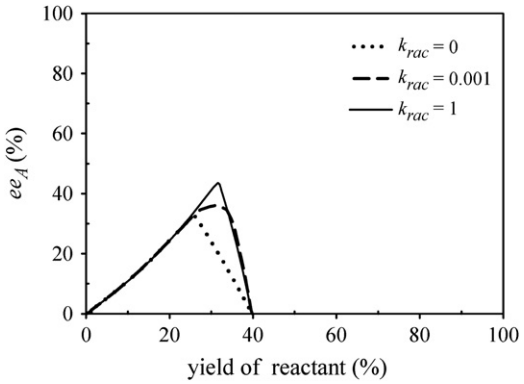

Fig. 12. Racemization of product: Enantiomeric excess of reactant vs. yield in cocurrent system for different racemization rate constants. For other parameter values see Table 2.

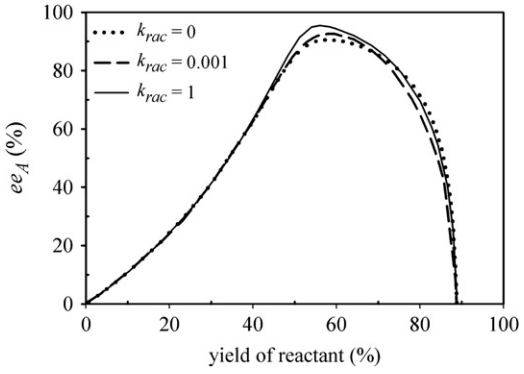

Fig. 13. Racemization of product: Enantiomeric excess of reactant vs. yield in countercurrent system for different racemization rate constants. For other parameter values see Table 2.

product can be obtained in the countercurrent system than in the cocurrent system. For a high value of the reaction equilibrium constant ($K_r > 10^{-2}$) almost the same yields can be obtained for the two systems but the countercurrent one may be preferable due a decreased residence time or enzyme amount requirement.

The same results (not shown) were obtained for $k_{rac} = 1$ but for $k_{rac} = 0.01$ the racemization is not at dynamic equilibrium and slightly lower enantiomeric excess values were obtained.

3.5. Racemization of product (reaction type 3)

Kinetic resolution with in-situ product racemization was also investigated. In this way the racemization will pull the equilibrium of the fast reaction further to the product side. The compound of interest will be the remaining slowly reacting enantiomer.

In Fig. 11 its enantiomeric excess is plotted vs. the yield. From the figure it can be concluded that the achievable enantiomeric excess of remaining reactant is higher in a countercurrent system than in an analogous cocurrent system. This situation with in-situ racemization should be compared to Fig. 6 where no racemization is applied.

For $K_r = 10^{-4}$, such a comparison is presented in Figs. 12 and 13. These figures also include lines for an intermediate situation in which the racemization is not at dynamic equilibrium ($k_{rac} = 0.001$).

For $k_{rac} > 1$ the dependence of ee_A on yield was the same as for $k_{rac} = 1$, so in all these cases racemization is at dynamic equilibrium.

Product racemization increases enantiomeric excess of remaining reactant. For the cocurrent system that is more pronounced (Fig. 12) than for the countercurrent system (Fig. 13). However, only for the countercurrent system the enantiomeric excess of reactant is raised to the value of industrial interest (96%) for the parameter values chosen here. This demonstrates that a countercurrent system is still preferable for this reaction type.

3.6. Kinetic resolution—obtaining achiral product (reaction type 4)

This case is analogous to the previous one. The reaction equilibrium constant for this case is defined as

$$K_r = \left(\frac{c_C c_D}{c_{A^R} c_B}\right)_{eq} = \left(\frac{c_C c_D}{c_{A^S} c_B}\right)_{eq} \tag{16}$$

In the case of product racemization, the reaction equilibrium constant was defined by Eq. (6). From these definitions and the reaction stochiometry it can be expected that the same ee_A vs. yields curves for obtaining achiral product can be obtained when the feed concentration of racemic reactant is half of the value in the previous case (racemization of product). For $f_A^R = f_A^S = 0.5$ mol kg^{-1}, Fig. 10 was indeed obtained again.

3.7. Case studies

3.7.1. Production of (S)-4-hydroxymandelonitrile

In the previous sections it was concluded that with the countercurrent system the enantiomeric excess of reactant can be increased compared to a cocurrent system. In order to demonstrate this for an actual enzyme reaction the production of (*S*)-4-hydroxymandelonitrile from its racemate was simulated. *Prunus amygalus R*-selective hydroxynitrile lyase might be used for kinetic resolution of racemic

4-hydroxymandelonitrile (HMN) (Willeman et al., 2002). This enzyme has a very high E value and (*S*)-4-hydroxymandelonitrile is converted only by a spontaneous background reaction. However, for simplicity this system will be modeled as an enzymatic reaction with limited selectivity ($E=10$–1000) without background reaction. The system is described by the following reaction scheme which is type 4 in Table 1:

Fast: (*R*)-HMN ↔ 4-hydroxybenzaldehyde + HCN

Slow: (*S*)-HMN ↔ 4-hydroxybenzaldehyde + HCN

The partition coefficients and reaction equilibrium constant have been determined experimentally at 20 °C (Willeman et al., 2002). The values of phase flows used in the model were set at values for which the countercurrent and cocurrent system might work, on the basis of recommendations from the literature (den Hollander et al., 2004; Willeman et al., 2002) and are presented with other model parameters in Table 2 (3rd column).

In Fig. 14 the calculated enantiomeric excess of remaining reactant vs. yield is given for different values of the enantiomeric ratio. For the parameter values used, the desired enantiomeric excess values can only be obtained with the countercurrent system.

3.7.2. *Production of (S)-ibuprofen by esterification*

Esterifications are equilibrium reactions, and can be used to convert aryl-propionic acids, for example. The anti-inflammatory and analgesic effects of the aryl-propionic acids, also known as profens, are attributed almost exclusively to the *S*-enantiomers (Chang and Hsu, 2005; Chang and Tsai, 1997; Tsai et al., 1997; Tsai and Wei, 1994). One way of producing profens is via kinetic resolution of racemates in an esterification reaction. Accumulated water from the esterification influences the enantiomeric excess due to the esterification-hydrolysis equilibrium of the esterified enantiomer.

The potential of a countercurrent system was investigated for (*S*)-ibuprofen production using kinetic resolution catalyzed by *Candida antarctica* lipases (Duan and Ching, 1998; Tsai et al., 1997; Zhang et al., 2005). In this case the enzyme is highly enantioselective towards the *R*-enantiomer so the conversion of the *S*-enantiomer can be neglected (Duan and Ching, 1998). This reaction scheme is equal to type 1b in Table 1. An enantiomeric excess of remaining reactant of 93% was obtained by gram scale experiments carried out in the monophasic batch mode (Duan and Ching, 1998). For a biphasic system an adsorbent was assumed as second, auxiliary phase with favorable hypothetical partition coefficients as shown in Table 2 (4th column).

For this case the monophasic batch, cocurrent and countercurrent calculations were performed using initial concentrations from the preparative scale separation (Duan and Ching, 1998). The reaction rate equation has the same shape as Eq. 5 but includes inhibition terms. The equation and its parameters were taken from the literature (Duan and Ching, 1998).

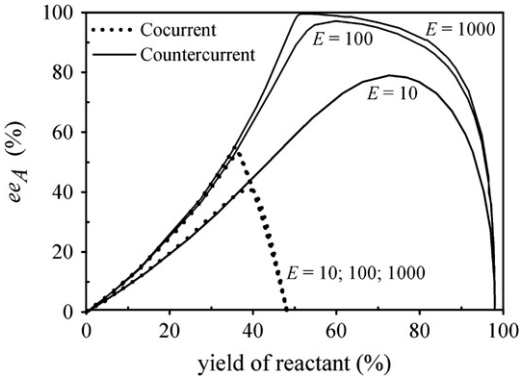

Fig. 14. Kinetic resolution of hydroxymandelonitrile: Comparison of enantiomeric excess of reactant vs. yield for different enantiomeric ratios. For other parameter values see Table 2. The three curves of the cocurrent system initially overlap with the corresponding countercurrent curve and later overlap with each other.

From the results obtained with model simulations the enantiomeric excess was increased from $ee_A=93.4\%$ in a monophasic batch system analogous to a monophasic plug flow system to 96.8% in a cocurrent system and finally to 100% in a countercurrent system.

For both case studies the results agree with the results of the general cases. Therefore, future work should focus on experimental demonstration of the principle for these reactions and the other reaction types listed in Table 1. Practical issues that have to be solved are to select a suitable conversion/partitioning combination and to establish the required countercurrent flow while maintaining enzymatic activity in the reaction phase and avoiding mass transfer limitation (cf. den Hollander et al., 2002). Theoretical issues are the incorporation of real hydrodynamics and real reaction and mass transfer kinetics (including non-ideality) in the model.

4. Conclusions

According to model simulations a novel field of application for countercurrent systems has been identified. For reversible kinetic resolutions, countercurrent systems often enable a better yield of reaction than analogous cocurrent systems. Besides, the countercurrent systems decrease the required residence time or enzyme amount. The maximum equilibrium constant for which the countercurrent system is the preferable option strongly depends on the feed concentration of racemate and it is shifted to higher values for more concentrated systems. For kinetic resolution this maximum value is higher for obtaining enantiopure remaining reactant than for obtaining enantiopure product. In-situ racemization of product or reactant, respectively, is favorable in these cases. All conclusions apply in case of favorable but potentially unrealistic model settings, where reaction rates and not mass transfer rates are limiting.

After this model-based evaluation of potentially attractive systems, detailed studies will be required for experimental evidence.

Nomenclature

a	specific mass transfer area, $m^2\ kg^{-1}$ reaction phase
A	amount of reactant A, mol
C	amount of product C, mol
c	concentration in the reaction phase, mol kg^{-1}
c_E	concentration of enzyme, U kg^{-1} reaction phase
E	enantiomeric ratio, 1
ee	enantiomeric excess, 1
F	feed flow rate, kg s^{-1}
f	feed concentration, mol kg^{-1}
K_{mt}	partition coefficient, mol kg^{-1} (mol kg^{-1})$^{-1}$
K_r	reaction equilibrium constant, 1 or kg mol^{-1}
k_{mt}	mass transfer coefficient, kg s^{-1} m^{-2}
k_r	reaction rate constant, kg^2 (mol s U)$^{-1}$ or kg (s U)$^{-1}$
k_{rac}	racemization rate constant, s^{-1}
L	flow rate of reaction phase, kg s^{-1}
M	mass of the stage, kg
M_{total}	mass of all stages (total reactor), kg
N	number of stages
N_{feed}	feed stage
Q	flow rate of auxiliary phase, kg s^{-1}
q	concentration in auxiliary phase, mol kg^{-1}
r_r	enzymatic reaction rate, mol kg^{-1} s^{-1}
r_{rac}	racemization rate, mol kg^{-1} s^{-1}

r_{mt} rate of mass transfer, mol kg^{-1} s^{-1}
t time, s
V_{react} volume of the system, m^3
Y molar yield of target compound, %
Y_{96} yield of target compound when its enantiomeric excess equals 96%, %

Greek letters

ε hold-up of the auxiliary phase, 1

Subscripts and superscripts

1 in 1st stage
A of reactant A
B of reactant B
C of product C
D of product D
eq at equilibrium
i of (enantiomer of) component i=A, C, D
j in stage number $j=1\ldots N$
L in reaction phase
N in last stage
Q in auxiliary phase
R for *R*-enantiomer
S for *S*-enantiomer

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