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Enzyme Reaction Engineering

Christian Wandrey*

1. Introduction

Engineering aspects may become decisive in enzyme technology if enzymatically catalyzed enzyme reactions reach a preparative or productive scale. With the increasing number of enzymes or microorganisms available for biotransformations it is not sufficient to prepare useful biocatalysts. Additionally, methods of reaction engineering have to be employed in order to design a process competitive [1–11].

2. Reaction Conditions

First of all, thermodynamics of a given reaction system have to be analyzed. Next, suitable reaction conditions must be specified with respect to pH, temperature, substrate concentration and enzyme concentration. These reaction conditions have to be suitable for the biocatalyst involved. Important parameters are mechanical fragility, activity, and stability of the catalyst. Reaction conditions may also influence the achievable selectivity and enantioselectivity. For instance a high catalyst concentration (and correspondingly a short residence time) can discriminate non-desired parallel or consecutive reactions. After reaction conditions have been set, kinetic parameters can be identified. Using this information the final reactor design may follow to predict a suitable residence time, achievable conversion, space-time-yield and product specific enzyme consumption (*Fig. 1*).

3. Kinetics

Kinetic measurements have to be carried out under initial rate conditions in order to achieve information independent of product concentrations. Furthermore, one has to follow the reaction progress along the entire range of conversion. This is of special importance if several enzymes are analyzed since reactants for one enzyme may be inhibitors for another enzyme. Parameter estimation should be

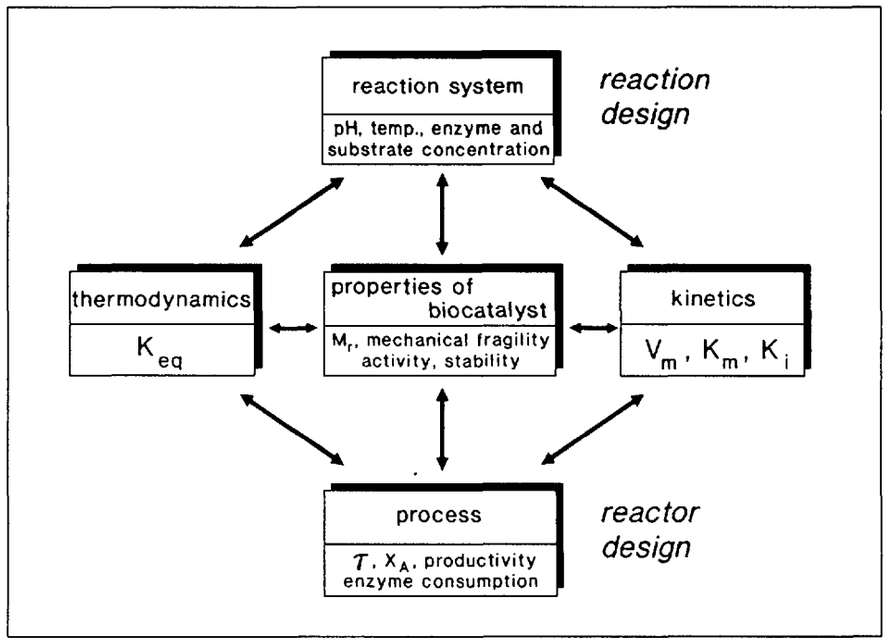

Fig. 1. *Influence of reaction conditions on reaction design and reactor design*

**Correspondence*: Prof. Dr. C. Wandrey
Institute of Biotechnology of the Research Centre Jülich
P.O. Box 19 13
D–5170 Jülich, Germany

carried out by nonlinear regression. Linearisation of kinetic expressions (*Lineweaver-Burk*-Method) leads to a non-optimal use of kinetic measurements. The kinetic information is then used in the appropriate mass balances to simulate relevant operating points (*Fig. 2*).

It can be shown that most of the kinetic models for enzymes represent a subgroup of classical heterogeneous kinetic models (*Fig. 3*). It is shown that for instance the random bi-uni model only takes in account specific adsorbtion sites for reactant A and reactant B while in classical heterogeneous kinetics all theoretically possible adsorbtion cases are formulated [12].

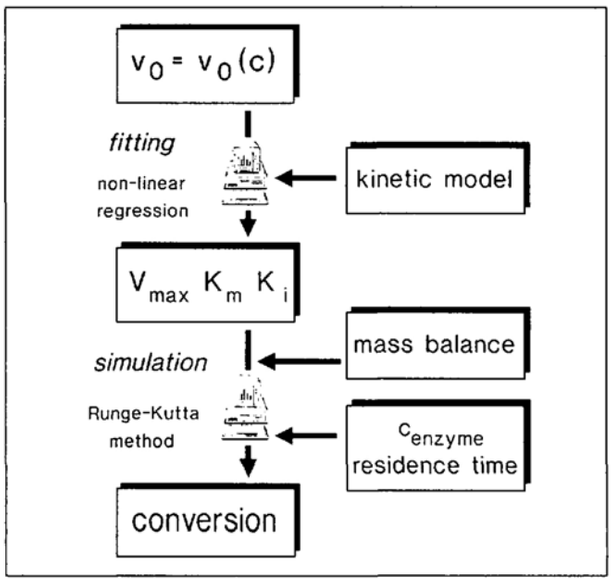

Fig. 2. *Kinetics: Calculation of operating points*

Heterogeneous Kinetics – Enzyme Kinetics

$$-RA = \frac{\frac{k}{K_A * K_B} * P_A * P_B}{1 + \frac{P_A}{K_A} + \frac{P_B}{K_B} + \frac{P_A * P_B}{K_A * K_B} + \frac{P_A}{K_A} + \frac{P_B}{K_B} + \frac{P_A * P_B}{K_A * K_B} + \frac{P_A^2}{K_A^2} + \frac{P_B^2}{K_B^2}}$$

<– enzyme kinetics –>

Thus, it becomes clear that due to the often more specific information kinetic expressions for enzymes can significantly be simplier in comparison to classical heterogeneous kinetics.

4. Yield

Up till now yield and selectivity are mixed up rather often. Yield and selectivity are only equal at quantitative conversion of the key component. Product yield may be more important than product concentration in spite of the fact that in the literature very often the final achieved concentration is given. Especially for fine chemicals it may be an alternative to operate (for kinetic reasons) at a medium level of initial substrate concentration followed by a downstream concentration *via* reverse osmosis. This may be economically decisive for reaction systems where thermodynamically a high conversion is attainable only at low substrate concentration. Substrate yield can be an interesting aspect in stereospecific ester hydrolysis. It is not only possible to aim for a high yield with respect to an optically pure product but also for a high yield of the remaining substrate (in optical pure form). In other words the object could be a (*R*)-product or a (*S*)-substrate in high yield and in high optical purity.

5. Space-time Yield

Prejudices still exist that biotransformations can only be performed at low space-time yield. Meanwhile there are a number of cases showing that remarkably high values may be achieved. For instance enzymatic peptide synthesis can be performed with a value of 25 kg-product/(l x d). Here enzyme membrane reactors are of special use since a high concentration of a soluble enzyme retained by an ultrafiltration membrane can be used in a continuously operated reactor. Thus, mass transfer limitations may be avoided.

6. Catalyst Consumption

The costs of enzyme and coenzyme (for multi enzyme systems) should be calculated with respect to unit weight of product. Such values are only meaningful at well specified conversion. Wherever possible data should be given as function of conversion. So-called half-life-times of enzymes can be misleading if such values are calculated from the time course of decreasing conversion not taking into account that rate changes with conversion. For instance the decrease of conversion

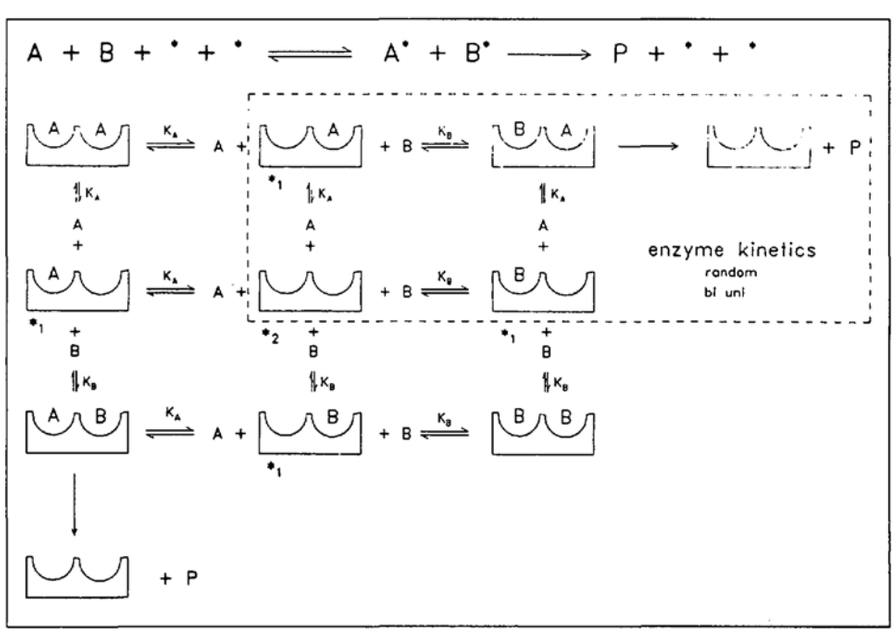

Fig. 3. *Enzyme kinetics as subgroup of heterogeneous kinetics*

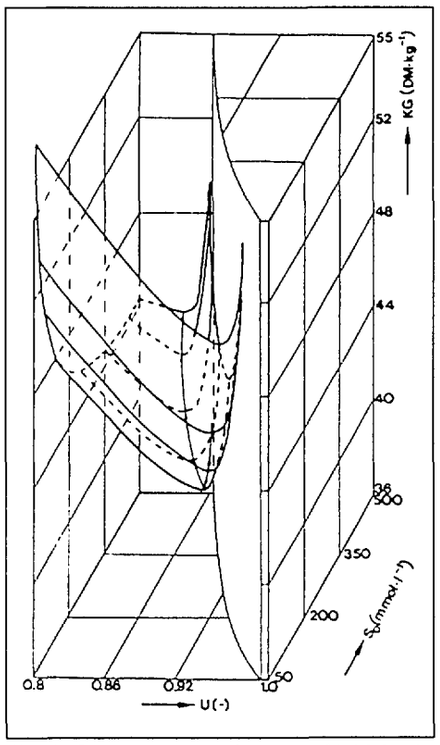

Fig. 4. *Cost optimum as function of initial substrate concentration and conversion* (racemic resolution of amino acids *via* the acylase method as example)

from 99 to 98% which results in a doubling of remaining substrate concentration can lead to a doubled activity. In other words 1% decrease in conversion can relate to a 50% decrease in activity. To avoid these problems catalyst consumption should be measured at constant conversion what may be achieved by adjusting the residence time (for immobilized enzymes) or the concentration of the active enzyme (for soluble enzymes in a membrane reactor).

7. Reactor Concepts

Reactor design is determined by the kinetics. All classical batch and continuously operated reactors are used [13]. With respect to back mixing the entire range from well mixed conditions until plug flow conditions can be found in praxis. At first glance there is no difference with respect to classical chemical reaction engineering. The aspect of catalyst size introduces new possibilities. Enzymes in native form may be used in membrane reactors [14]. Biocatalysts bound to micro carriers can be applied in slurry reactors, while enzymes on macro carriers are found in fixed bed reactors and fluidized bed reactors. For coenzyme depending reactions where coenzymes have to serve as transport metabolites only the membrane reactor concept is the method of choice. More recently, reactor concepts have been developed for non-aqueous or multiphase reaction systems (solid supported liquid crystals [15], membranes for the separation of aqueous/organic phases).

8. Control

Modern control techniques offer the possibility to maintain a given conversion, space-time yield or even selectivity and enantioselectivity. With the progress in bioprocess analytics (in-line or on-line) there is an increasing number of practical cases using such a control strategy.

9. Scale Up

Scale up has not been a deciding problem in the past. Since the enthalpy change of most reactions is small, an appropriate heat transfer can easily be carried out. Mass transfer may become a problem if a very high enzyme activity is immobilized in particles with too great diameter. But this problem may be overcome by macroporous carriers. Mixing might be a problem if for instance pH has to be adjusted by titration (what may be better than using a buffer). Once again classical principles can be employed (loop reactors with static mixers in the loop or segmental plug flow reactors with intermediate adjustment of pH).

10. Cost Optimization

For cost optimization substrate costs, enzyme costs, coenzyme costs, and reactor costs as well as the costs for downstream processing have to be taken into account simultaneously. Rather often no sufficient attention is payed to the influence of down stream processing. A typical example for cost optimization is shown in *Fig. 4*.

A cost optimum is shown for a biotransformation where substrate costs, enzyme costs, and costs for downstream processing are taken into account. The cost per unit weight of product is given as function of conversion and initial substrate concentration. This example represents the well known racemical solution of L-amino acids starting from *N*-acetyl D,L-amino acids stereospecifically hydrolyzed by acylase. The costs drop with increasing conversion (reduced substrate costs) until the cost minimum is passed due to the increasing influence of the catalyst costs. Close to the maximal achievable conversion the costs rise sharply due to the decreasing catalyst activity. This effect depends very much on the initial substrate concentration since quantitative conversion can only be reached at initial substrate concentration zero. With decreasing initial substrate concentration the optimal costs decrease, since this system exhibits a substrat surplus inhibition. Simultaneously one can operate under higher and higher conversion (for thermodynamic reasons). Finally, the costs will increase again due to down stream processing where the product has to be recovered from a more and more diluted solution.

11. Conclusion

- selectivity, activity, stability = f (reaction conditions)
- modelling of complex reactions possible
- yield may strongly influence selectivity
- space-time yield of up to 20 kg product/(l x d)
- product specific enzyme / coenzyme consumption sensible only at given conversion
- continuous coenzyme regeneration of up to 600.000 cycles
- cost optimization for reaction and downstream processing
- growing importance of enzyme reaction engineering

The mentioned aspects will be exemplified by means of processes which have reached preparative or even productive scale (racemic resolution of amino acids, amino acids from prochiral precursors [16], enzymatic peptide synthesis [17], enzyme catalyzed C–C-bound formation [18]).

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How to Select a Useful Biocatalyst

Klaus Kieslich*

Early euphoria in biotransformations has declined in recent years to have a more realistic assessment of the uses in chemical syntheses. Reservations concerning this sector can be summarized as follows:

a) Finding a biological system suitable for the intended enzymatical reaction is mainly *empirical.*

b) When using living material biological deviations of unknown origin severly hamper the *reproducibility.*

c) The multivarious parameters and factors present, when investigating variables for growth and propagation of cells, formation and activity of required enzymes, and for the technical performance of the transformation reaction, demand *time consuming experimental series.*

d) Whereas initially *low yields*, due to *incomplete transformation*, can be partially increased by the results of these studies, the improvement of low yields, due to *undesired side reactions*, or *further degradations* by selective inhibition, requires some knowledge of the properties of the different enzymes or broad mutation programs.

e) The *purification of the metabolite*, with the separation of components of the nutrient media or of products formed in parallel by biosynthesis, is sometimes laborious.

f) In order to achieve economic production, the *yield* of a biotransformation, in relation to *volume and time*, requires high educt concentration, short conversion time and high level of the formed product using cheap nutrient media and low cost methods of recovery and purification.

g) Some of these problems can be decreased or eliminated by using *cell-free enzymes*, preferably in immobilized form for repeated use. This technique, however, is *limited* to stable enzymes without a cofactor requirement or with cofactors which can be regenerated in economic systems.

h) In general, the application of cell-free enzymes could eliminate a further problem, namely the *health risk*, using microorganisms which are virtually harmless or fungi forming spores.

i) A significant improvement in the process is achieveable by using gene transfer of a single usable enzyme from any cell, perhaps containing several undesired enzymes, into one host cell having the best properties for cultivation and increased enzyme production. However, this excellent method, needs special laboratory conditions required by the regulations *governing the use of rDNA-strains.*

k) Finally, the controlled *synthesis of an enzyme*, with all desired specifities and high activity is thought likely in the future taking the protein design path. This strategy requires either knowledge or cast-iron predictions of the active side and the tertiary structure. However, this condition is fulfilled in only a few cases and only in two examples in the field of the numerous P450-enzymes catalyzing multifold reactions of various important educts. *Plenty of time and patience* is apparently necessary for enlarging on the first successes with relatively simply regulated enzymes.

Some of these problems would be eliminated by the selection and use of a good biocatalyst.

There are various routes to this objective, all of unknown efficiency.

1. Search for Novel Biocatalytic Systems

The cultivation of mammalian and plant cells in submerged culture is made more difficult: by the exclusion of various cell types; by the requirement for expensive media; by the amount of time required for propagation; and by the sensitivity of higher cells against shear forces. Therefore, the search for novel biocatalytic systems is focussed on testing new isolated microorganisms.

Such screening programs (*Cheetham* 1987) are generally oriented towards finding the concrete enzymatic conversion of one substrate (educt) into one single de-

**Correspondence*: Prof. Dr. K. Kieslich
Gesellschaft für Biotechnologische Forschung mbH
D–3300 Braunschweig, Germany

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**Correspondence*: Prof. Dr. K. Kieslich
Gesellschaft für Biotechnologische Forschung mbH
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