International Journal of Scientific Research in Chemistry (IJSRCH)
© 2020 IJSRCH | Volume 5 | Issue 5 | ISSN : 2456-8457

Mass Transfer Effects and Performance of Immobilized Enzymes

Akshay R.Yadav*, Dr. Shrinivas K. Mohite

Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon, Dist- Sangli, Maharashtra, India

*Corresponding author E-mail:- akshayyadav24197@gmail.com

Article Info
Volume 5, Issue 5
Page Number: 51-56
Publication Issue :
September-October-2020

Article History
Accepted : 18 Oct 2020
Published : 30 Oct 2020

ABSTRACT

Immobilized enzymes are currently the object of considerable interest. This is due to the expected benefits over soluble enzymes or alternative technologies. The number of applications of immobilized enzymes is increasing steadily, however, experimental investigations have produced unexpected results such as a significant reduction or even an increase in activity compared with soluble enzymes. Thus, cross-linked crystals of subtilisin showed 27 times less activity in the aqueous hydrolysis of an amino acid ester compared to equal amounts of soluble enzyme. On the other hand, in the application of lipoprotein lipase in the solvent-mediated synthesis of esters there was a 40-fold increase in activity using immobilized or otherwise modified enzyme preparations as compared to enzyme powder.

Keywords: Immobilized enzymes, Cross-linked crystals, Mass transfer, Performance, Modified enzyme, Enzyme powder.

I. INTRODUCTION

Immobilization of enzymes means a deliberate restriction of the mobility of the enzyme, which can also affect mobility of the solutes. The various phenomena, referred to as mass transfer effects, can lead to a reduced reaction rate, in other words to a reduced efficiency as compared to the soluble enzyme[1]. A reduced reaction rate may result from external diffusional restrictions onthe surface of carrier materials. In stirred tanks external diffusion plays a minor role as long as the reaction liquid is stirred sufficiently[2]. Further, partition effects can lead to different solubilities inside and outside the carriers. Partition has to be taken into account when ionic or adsorptive forces of low concentrated solutes interact with carrier materials[3].

MASS TRANSFER EFFECTS

Porous Diffusion

Considerable importance is given in the literature to describe theoretical and practical aspects of mass transfer effects on immobilized enzymes. All reactions of immobilized enzymes must obey the physicochemical laws of mass transfer and their interplay with enzyme catalysis. The question is, therefore, what are the reasons for restrictions caused by mass transfer, and how can they be avoided if necessary? The mathematical description of diffusional limitations of enzyme kinetics in the combined action with mass transfer has for years been well established. The presentation of these

interactions is very complex, in particular when terms for product inhibition, proton-generation or enzyme deactivation are incorporated in addition to comparably simple Michaelis–Menten kinetics[4]. For Michaelis–Menten based enzyme kinetics the extent of mass transfer control is usually expressed by the efficiency coefficient or effectiveness factor *h*, expressed as:

$$\eta = \frac{v'\,(substrate\ conversion\ rate\ of\ immobilized\ enzyme)}{v\,(substrate\ conversion\ rate\ of\ free\ enzyme)}$$

Numerically calculated values of *h* are available when only substrate diffusion in Michaelis–Menten type kinetics is considered. They can be presented in graphical form, expressing *h* as a function of the Thiele modulus *F*R and of a dimensionless substrate concentration or occasionally in its reciprocal form as a dimensionless Michaelis constant *b* according to Lee and Tsao[5].

$$\eta = f(\Phi_R, \beta)$$

$$\Phi_R = R \times \sqrt{\frac{V_m}{D_{eff} \times K_M}}$$

$$\beta = \frac{S}{K_M}$$

Reaction (Dynamic) and Support-Generated (Static) Proton Gradients

Cross-linked crystals from subtilisin exhibited 27 times less activity than soluble subtilisin in the hydrolysis of benzoyl-L-phenylalanine ethyl ester. Denaturation of the enzyme and restrictions from substrate-dependent internal diffusion were ruled out.A shift in the pH-dependence of the maximum activity to higher pH-values was observed which was explained by intermolecular electrostatic interactions increasing the pKs of the catalytic residues of the active centers[6].

The observed pH-shifts in activity may indeed also be caused by:

1. Static proton or substrate gradients caused by partition of charged groups near surfaces with stationary charges.
2. Dynamic proton gradients due to hydrolytic reactions with liberation of protons as has frequently been observed for immobilized enzymes.

In order to optimize productivity and reduce the loss of catalyst or product it is advisable to minimize the effects of reaction-generated dynamic pH-gradients. Whenever practicable and useful this may be achieved in several ways:

- By reducing enzyme density and/or particle size as indicated for substrate mediated diffusional control.
- By using buffers with sufficient capacity (> 0.05 M) that minimize the dynamic pH-gradients. Occasionally the substrates or products themselves provide such properties so that only the optimal external pH-value has to be adapted. It should not be lower than the pK-value of the weak acid and not lower than the optimum pH of the enzyme.
- By operating at an external pH higher than the optimum pH of the enzyme.
- By co-immobilization of a proton-consuming enzyme. This was successfully done with urease, where *in situ* formed ammonia neutralized the generated protons in an extraordinarily effective manner. Proton-consuming reactions such as the urease reaction are likely to show all the diffusion-related pH-shifts in the opposite direction. Obviously, they may be treated in an analogous manner[7-10].

Temperature Dependence

In a diffusion-free enzyme reaction the reaction rate increases up to a certain critical value exponentially and is described by the Arrhenius equation. In diffusion-controlled reactions the reaction rate is a matter of the efficiency factor *h*. In more detail, the maximum reaction rate is expressed within the root of. Conclusively, the temperature dependence is a function of the square root of the enzyme activity. In

practice, immobilized enzymes are much less temperature dependent when their reaction rate is diffusion controlled[11].

Stability Assessment

It is useful to differentiate between storage stability and operational stability. Storage stability is provided by an appropriate formulation. Mostly, several additives protect the enzyme from denaturation under recommended storage conditions. By these means, shipment and distribution are supported. The operational stability is a matter of working conditions and of detrimental effects from the selected reaction conditions, such as pH, temperature, solvents, impurities, and other factors that contribute to protein denaturation or modification of functional groups and thus enhance inactivation. The operational stability determines the enzyme's performance and determines cost benefits as discussed above. For diffusional-free reactions time-dependent inactivation can proceed along various inactivation rates, such as exponential activity decay according to a first-order reaction. In this case, the rate of inactivation can be described in a similar manner to that given for temperature dependence by the Arrhenius equation. In strictly diffusion-controlled reactions, i.e. at low efficiencies, simply spoken, only a small fraction of the total immobilized enzyme quantity is working. If in the course of operation actively working enzyme is destroyed, some other previously "resting" fraction may substitute to some extent. This is one reason why immobilized enzymes may erroneously appear to be more stable than free enzymes. To avoid such misinterpretation requires the assay of the enzyme activity in the absence of diffusional limitations. For industrial applications it is more useful to consider the enzyme's performance as indicated instead of just assessing its half-life[12].

Other Contributions

Unfortunately, most enzymes do not obey simple Michaelis–Menten kinetics. Substrate and product inhibition, presence of more than one substrate and product,or coupled enzyme reactions in multi-enzyme systems require much more complicated rate equations. Gaseous or solid substrates or enzymes bound in immobilized cells need additional transport barriers to be taken into consideration. Instead of porous spherical particles, other geometries of catalyst particles can be applied in stirred tanks, plug-flow reactors and others which need some modified treatment of diffusional restrictions and reaction technology[13].

PERFORMANCE OF IMMOBILIZED ENZYMES:

Enzyme Formulation and Activity

For reactions in water-immiscible organic solvents the simplest method of immobilization is to use dried enzyme powders and to suspend them in the solvent. They can be removed by filtration or centrifugation and reused afterwards. However, even this simple method can cause trouble. A powder of lipoprotein lipase (LPL) esterified an organic substrate in toluene at a rather poor reaction rate (Table 4), which was to some extent explained by adhesion of the sticky enzyme powder to the surface of the reaction vessel[14]. When polyethylene glycol (PEG) was bound covalently to LPL and this modified enzyme was dissolved in toluene, approximately 3.5 U mg–1 of enzyme protein were assayed. After simple addition of PEG to the reaction mixture together with LPL powder, the same poor reaction rate of the enzyme powder alone was observed. On the other hand, when LPL powder was lyophilized together with PEG the resultant preparation had an activity of 1.8 U mg^{-1}. In this case, the enzyme powder was highly dispersed in the organic medium and could easily be separated by centrifugation. Adsorption of LPL on a carrier material (Celite®) yielded a reaction rate of 2.3 U mg^{-1}. These experimental findings indicate the importance of proper formulation, even if these results are not necessarily merely a matter of distribution, since water activity, enzyme conformation and stability also affect the assayed activity. Improper storage conditions and formulations of powders not specially developed for

applications in solvents are likely to be responsible for low activities, for example, when air humidity or reaction-generated water make hygroscopic lyophilisates sticky. In conclusion, proper formulation of lyophilisatesor of carrier-fixed preparations will result in appreciable activities. In contrast to the poor reaction rates reported elsewhere [109], crude powder of lipase from *Pseudomonas cepacia* (PSL) acylates secondary phenylethyl alcoholat reasonable reaction rates. Based on active enzyme protein, the highest reaction rates were observed for carrier-fixed enzymes. This is also true for lipase from *Candida antarctica* (CAL-B). PSL crystals catalyzed with comparable activity when they had been pretreated with surfactants[15].

Stability

Increased operational stability of immobilized enzymes is essential in order toachieve the cost benefits already mentioned. Enzyme stability can be controlled by assaying the activity decay over time until half-life activity is reached[16]. This is a useful means of control when thermal inactivation takes place according to first-order kinetics. Complications occur when more or less stable mixtures of different enzyme species are present, either as a result of more or less tight binding or differing intrinsic stabilities. The role of mass transfer effects, whether occurring accidentally or by design, is ambivalent, causing Trevan to ask the question "Diffusion limitation – friend or foe?". Lower activity as a result of low efficiency indicates that only a minor portion of enzyme is active during operation. The other unused portion may, in simple terms, replace the enzyme as it is inactivated step by step[17-20]. In other words, mass transfer controlled reactions appear to be much less sensitive to decay of enzyme activity, thus falsely creating an impression of stabilization. Under harsh reaction conditions it may be advantageous to operate under these conditions to keep the reaction rate constant until the diffusion limitation disappear[21-25]. With regard to such effects it is advisable not only to determine the operational stability by tracing the time course of activity but to follow its productivity, or vice versa its consumption, related to the formed product[26-28]. Carrier-fixed penicillin G amidase in the multi-ton hydrolysis of penicillin G is a useful example to illustrate enzyme consumption. The enzyme is applied in stirred tanks with sieve plates at the bottom to retain the enzyme particles when the product solution is drained off. The pH-value is kept constant by controlled feed of ammonia solution. Fresh substrate solution is refilled about a thousand times or more. The consumption of enzyme in such a process is below10 mg kg^{-1} (0.2 kU kg^{-1}) of isolated 6-APA when the enzyme activity is determined with penicillin G solutions at 28° C and pH 8.0. Under identical conditions the consumption of soluble enzyme for each tank filling would be beyond all reasonable cost. Cross-linked crystals of lipase from *Candida rugosa* (CRL) were applied in the resolution of racemic ketoprofen chloroethyl ester. In batch-wise operation, the half-life of the catalyst was reached after about 18 cycles or, in terms of enzyme consumption, about 5.6 g of enzyme protein were consumed to prepare1 kg of (*S*)-ketoprofen. CRL suffers from a low specific activity towards this poorly water-soluble substrate which may explain the high enzyme input. When cross-linked crystals of the rmolysin were applied in peptide synthesisin ethyl acetate, they were stable for several hundred hours at amazingly low enzyme consumption, whereas a soluble enzyme preparation became inactive within a short period of time. Again it is worthwhile to consider the quality of the soluble enzyme preparation[29].When soluble the rmolysin was stored in mixed aqueous–organic solutions, it lost about 50% of its activity within the first day of incubation only to be then quite stable for the next 15 days. It is possible that the initial inactivation was caused by an unstable fraction of thermolysin and that crystals of thermolysin no longer contained this unstable fraction. Productivity comparable to that of crystals was achieved with thermolysin adsorbed on Amberlite® XAD-7 resin which was employed in

continuous plug flow reactors with *tert*-amyl alcohol as solvent With regard to purity, quality, and formulation, and hence to cost considerations, it can be useful to define "productivity" as the fermentation volume required to prepare the immobilized enzyme activity needed to synthesize a certain amount of product. This is useful when the overall performance of an immobilized enzyme catalyzed process has to be competitive with other technologies, such as preparation of the desired product by fermentation or by whole cell biotransformations[30-31].

II. CONCLUSION

Enzymes have achieved acceptance as catalysts in the synthesis of chemical compounds, particularly in the fine chemicals industry for the manufacture of enantiopure compounds. Immobilization of enzymes is a useful tool to meet cost targets and to achieve technological advantages. Immobilization enables repetitive use of enzymes and hence significant cost savings. From the technological point of view, immobilized enzymes can easily be separated from the reaction liquid and make laborious separation steps unnecessary. Additional benefits arise from stabilization against harsh reaction conditions which are deleterious to soluble enzyme preparations. Due to the wide variation in the properties of the individual enzyme species and due to the varying requirements of reaction technology for the target compounds it is advisable to exploit fully the wealth of methods and techniques of immobilization. Which of the available methods is the best in the end will be decided by both the specific technical requirements and the overall business framework.

III. REFERENCES

[1]. Gloger M, Tischer W (1983) In: Bergmeyer HU, Bergmeyer J, Graßl M (eds) Methods of enzymatic analysis, 3rd edn.VCH, Weinheim, vol 1, pp 142–163

[2]. Tischer W (1995) In: Drauz K,Waldmann H (eds) Enzyme catalysis in organic synthesis. VCH, Weinheim, pp 73–87.

[3]. Lee YY, Tsao GT (1974) J Food Sci 39:667.

[4]. Yamane T (1981) J Ferment Technol 59:37.

[5]. Kasche V (1983) Enzyme Microb Technol 5:2.

[6]. Schlothauer RC (1996) Thesis TU Hamburg-Harburg Engasser J-M, Horvath C (1974) Biochemistry 13:3845.

[7]. Horvath C, Engasser J-M (1974) Biotechnol Bioeng 16:909.

[8]. Carleysmith SW, Dunnill P, Lilly MD (1980) Biotechnol Bioeng 22:735

[9]. Horvath C, Engasser J-M (1973) Ind Eng Chem Fundam 12:229.

[10]. McLAren AD, Packer L (1970) Adv Enzymol 33:245.

[11]. Goldstein L, Levin Y, Katchalski E (1964) Biochemistry 3:1913.

[12]. Trevan MD (1980) Immobilized enzymes – an introduction and applications in biotechnology. Wiley, New York, pp 11–55

[13]. Halwachs W, Wandrey C, Schügerl K (1978) Biotechnol Bioeng 20:541.

[14]. Ruckenstein E, Sasidhar V (1984) Chem Eng Sci 39:1185

[15]. Kasche V, Bergwall M (1977) In: Salmona M, Saranio M, Garattini S (eds) Insolubilized enzymes. Raven Press,New York, pp 77–86.

[16]. Rolinson GN (1988) J Antimicrob Chemother 22:5.

[17]. Liou JK, Rousseau I (1986) Biotechnol Bioeng 28:1582.

[18]. Bailey JE, Chow MTC (1974) Biotechnol Bioeng 16:1345.

[19]. Ruckenstein E, Rajora P (1985) Biotechnol Bioeng 27:807.

[20]. Dickinson M, Fletcher PDI (1988) Enzyme Microb Technol 11:55

[21]. Klibanov AM (1997) Trends Biotechnol 15:97

[22]. Carrea G, Ottolina G, Riva S (1995) Tibtech 13:63.

[23]. Tsai S-W,Dordick JS (1996) Biotechnol Bioeng 52:296.
[24]. Zacharis E, Moore B, Halling PJ (1997) J Am Chem Soc 119:12396.
[25]. Tischer W, Giesecke U, Lang G, Röder A, Wedekind F (1992) Ann N Y Acad Sci 672:502
[26]. Schmid RD, Verger R (1998) Angew Chem 110:1694.
[27]. Naik SS, Karanth NG (1978) J Appl Chem Biotechnol 28:569.
[28]. Lalonde JJ, Govardhan CP, Khalaf N, Martinez AG, Visuri KL, Margolin A (1995) J Am Chem Soc 117:6845.
[29]. Persichetti RA, Clair NLS, Griffith JP, Navia AL, Margolin AL (1995) J Am Chem Soc 117:2732.
[30]. Nagayasu T, Miyanaga M, Tanaka T, Sakiyama T, Nakanishi K (1997) Biotechnol Bioeng 43:1118

Cite this article as :

Akshay R.Yadav, Dr. Shrinivas K. Mohite, "Mass Transfer Effects and Performance of Immobilized Enzymes", International Journal of Scientific Research in Chemistry (IJSRCH), ISSN : 2456-8457, Volume 5 Issue 5, pp. 51-56, September-October 2020. URL : http://ijsrch.com/IJSRCH205615