Stabilization and Modification of Enzymes for Industrial Use

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Practicality of industrial enzymes

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In the late 1960s and early 1970s 'enzyme technology' was viewed with the optimism and enthusiasm currently devoted to 'biotechnology'. With one or two spectacularly successful exceptions, industry as a whole has not welcomed enzyme technology with open arms. Consideration of successful and less-successful processes indicates what industry wants and what it has been able to achieve; consideration of the patent literature indicates the processes that may be employed industrially and how the relevant enzymes may have to be improved to make these processes operate economically.

To be of value on an industrial scale an enzyme system must have the following attributes:

1. It must do the job required. This may not be the conversion which that enzyme does normally in Nature, e.g. most glucose isomerases are truly xylose isomerases, or it may be a conversion of materials that do not occur widely in Nature, e.g. pesticides, xenobiotics in general.

In certain cases the enzyme may be required to operate in 'non-physiological' conditions, for instance in organic solvents, because the substrate or product, or both, are sparingly soluble in water or because use of such a solvent allows manipulation of the equilibrium reaction mixture.

2. It must be simple to use. An industrial enzyme must be stable to changes of temperature, pH and, ideally, microbial contamination. Immobilized enzymes must be robust: neither the enzyme nor the support material must find its way into the final product. Although sophisticated control equipment is available, 'Murphy's Law' (anything that can go wrong will go wrong) will always apply, and simple systems offer the least scope for problems.

3. It must be cheap. 'Cheap' is a relative term: penicillin acylases are much more expensive than glucose isomerases, but that fact is tolerable to the antibiotic manufacturer because of the high value of his product. A frequently voiced criticism, usually by chemical engineers, of enzyme processes is that they are 'killed' by heating at 140°C briefly, to disrupt the starch grains, and is then held at 90°C for up to 1 h, when a D.E. value of 12–20 has been attained. For some purposes the enzyme must be 'killed' by heating at 140°C briefly. This enzyme seems well-nigh perfect: there is no point in immobilizing it for this use, it operates at a high substrate concentration (up to 40%, w/w) and is very cheap (about £2.50 per tonne of starch hydrolysed). If anything it is too stable!

Glucose and fructose syrups. Probably the most successful large-scale use of enzymes in an industrial process is in the production of glucose and fructose syrups from starch. 'Glucose syrup' is a term used to describe starch hydrolysates of various compositions that are refined and used in the food industry (Bucke, 1979). The extent of hydrolysis is described by the 'dextrose equivalent' (D.E.), which is the percentage of theoretical maximum hydrolysis that has actually been attained. Glucose has approx. 70% the sweetness of sucrose but fructose has about 130%: this conversion of glucose into fructose greatly increases its desirability (and therefore commercial value) as a foodstuff. The enzymic hydrolysis of starch to glucose and the partial isomerization of glucose to fructose are the basis of an industry producing 4000000 tonnes of material per annum.

Three enzymes are involved: bacterial a-amylase (EC 3.2.1.1), glucoamylase (EC 3.2.1.3) and glucose isomerase (EC 5.3.1.5). Bacterial (usually Bacillus sp.) a-amylase initiates the hydrolysis of starch. It is added to a slurry of starch, which is then heated at 105–110°C briefly, to disrupt the starch grains, and is then held at 90°C for up to 1 h, when a D.E. value of 12–20 has been attained. For some purposes the enzyme must be 'killed' by heating at 140°C briefly. This enzyme seems well-nigh perfect: there is no point in immobilizing it for this use, it operates at a high substrate concentration (up to 40%, w/w) and is very cheap (about £2.50 per tonne of starch hydrolysed). If anything it is too stable!

Glucoamylase is a fungal (Aspergillus or Rhizopus sp.) exo-glucanase that is used to hydrolyse the 12–20 D.E. material to monomeric glucose. The substrate is cooled to 55–60°C, and acidified to pH 4.5 before addition of the enzyme. Conventionally, soluble enzyme is used in vast reactor tanks and allowed to work for 48–72 h, when 97 D.E. is attained. The enzyme remains active at the completion of the hydrolysis and is removed during the refining steps. Immobilized glucoamylases...
are available, but have not been used extensively because diffusion limitations mean that the maximum D.E. values attainable are lower, and therefore unattractive for commercial use. Glucoamylase is a very cheap enzyme, costing about £2 per tonne of 97 D.E. material produced.

Glucoisomerases used commercially are produced by Bacillus sp., Streptomyces sp., Actinoplanes sp. etc. etc. (Antrim et al., 1979). The enzymes are produced intracellularly, and most commercial preparations consist of the enzyme immobilized within the cells that produced them. The immobilization process usually involves heat treatment which inactivates unwanted enzymes but, fortunately, barely alters the activity of the glucoisomerase.

Glucoisomerase is an example of an enzyme being used to transform a substrate other than its 'normal' substrate. Most of the glucoisomerases are truly xylose isomerases: for full activity with xylose, Mn2+ ions are required, if the Mn2+ is replaced by Co2+ glucose isomerization is fully efficient.

Industrial glucoisomerase is invariably used as the immobilized enzyme in columns or beds. The enzymes are fully stable at 60°C; their Km values for glucose are high (about 0.1m), and enzyme manufacturers recommend using glucose substrate at 50% (w/w) for maximum enzyme activity and longevity. Immobilized preparations now have half-lives of about 6 weeks, so the cost of isomerization is not high (around 26£ per tonne of product).

How will the processes be improved? Glucoamylase with a greater stability to temperature would allow shorter incubation periods, with some savings on the capital costs of reactors. There is not great scope for this, because very high temperatures would result in glucose loss and colour formation. A more temperature-stable glucoamylase would be valuable if an efficient immobilization system were developed for it.

Similarly, a glucoisomerase with greater thermal stability would be valuable, not because it would be used at higher temperatures but because it should last longer, when immobilized, at the present process temperature. Glucose isomerase is inhibited by Ca2+ ions: consequently substrates must be refined expensively before this enzyme is used. Modification to overcome this inhibition and allow all the refining to be done at the end of the process would be valuable. It might then be possible to use glucoamylase and glucose isomerase in the same reactor.

Co-immobilization of enzymes. There are very few examples of the successful use of pairs of enzymes immobilized together to conduct a single process. Pairs that might be employed in this manner include: glucoamylase and glucose isomerase; ß-galactosidase and glucose isomerase; glucoamylase and pullulanase; glucooxidase and peroxidase.

Problems arise because of incompatibilities: glucoamylase and glucose isomerase have similar stabilities but different pH optima (4.5 and 8.0 respectively); ß-galactosidases are, usually, less heat-stable than glucose isomerase and have different pH optima (4.5). Glucoamylase action is speeded by the presence of pullulanase (E.C. 3.2.1.-), but pullulanase is much less heat-stable (Ram & Venkataramanan, 1982). In short, there is room for the modification of one or both enzymes where pairs might be used co-immobilized, so as to increase their compatibility and practicability.

Future process that may require enzyme modification

Fructose production. Although glucose isomerase is used very successfully, it produces an equilibrium reaction mixture containing only 55% fructose: it is most unlikely that modification of the enzyme might result in an alteration of this equilibrium. A process that would convert glucose quantitatively and cheaply into fructose would be most desirable. Such a process has been outlined in the patent literature: various basidiomycetes produce a glucose 2-oxidase that oxidizes glucose to glucosone, which, on catalytic hydrogenation, produces fructose. A particularly elegant aspect of this process is to employ the H202 by-product of glucosone formation (Cetus Corporation, 1979):

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\begin{align*}
\text{Glucose} + O_2 & \rightarrow H_2O_2 + \text{glucose} \\
\text{Gluconose} + H_2 & \rightarrow \text{fructose}
\end{align*}
\]

to oxidize olefins to epoxides and glycols, e.g.:

- Ethylene + HCl + H2O2 \rightarrow 2-chloroethanol
- 2-Chloroethanol \rightarrow ethylene epoxide
- Ethylene epoxide \rightarrow ethylene glycol

So far all these steps have been done only at low temperatures in dilute solution: there is plentiful scope for stabilizing these enzymes so that they are able to operate at high substrate concentrations, at higher temperatures and without being inhibited by the various reaction products.

Lactose hydrolysis. Whey is produced in abundance as a by-product of cheese manufacture. It contains around 5% (w/w) of lactose, which causes problems because it is not digestible by 70% of the world's adult population and because it is a relatively insoluble sugar crystallizing from 16% (w/v) solution at 25°C. This makes the transport of concentrated solutions difficult. Hydrolysis of the lactose overcomes these problems because glucose and galactose are digestible and freely soluble (Nijpels, 1980). Many microbial ß-galactosidases are available, and are used successfully when the hydrolysed lactose is to be employed as high-value human food. Otherwise the enzymes are too expensive when the product is to be used as animal feed or a fermentation substrate. The primary problem is the instability of the enzyme to heat, which means that its half-life when immobilized is uneconomically short. There is scope for stabilizing ß-galactosidases for industrial use.

Conclusions

Enzymes are used industrially if they are very effective and cheap or if they are effective and produce an expensive material. Industry would welcome many new enzymes, either improvements on existing industrial preparations, or cheaper versions of known but excessively expensive enzymes or enzymes that will perform completely novel tasks. 'Cheapness' in this sense means the cost attributable to the enzyme per unit of product: thus an initially costly enzyme will be perfectly acceptable if its active life on immobilization is sufficient.

It would seem sensible for those aiming to study the basics of enzyme stabilization and modification to concentrate on enzymes of current and potential commercial value.


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