The function of enzymes in brewing

Technical Summary 9

By Tim O'Rourke

Continuing this series of technical summaries for the Institute & Guild's AME candidates.

The nature of enzymes

Enzymes are proteins with a special structure capable of accelerating the breakdown of different substrates. They act as catalysts to increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are not used up in the reaction or appear as reaction products. The basic enzymatic reaction can be represented as follows:

Substrate + Enzyme ↔ Product(s) + Enzyme.

Energy is required for chemical reactions to proceed. The energy is called *the energy of activation*. It is the magnitude of the activation energy that determines just how fast the reaction will proceed (See Fig 1).

How enzymes work

Enzymes bind temporarily to substrate of the product they catalyse. In doing so, they lower the amount of activation energy needed enabling the reaction to proceed at more quickly at lower temperatures (See Fig 2).

In order to do its work, an enzyme must combine – even if ever so briefly – with at least one of the reactants. In most cases, the forces that hold the enzyme and its substrate are non-covalent, being an assortment of:

- Hydrogen bonds
- Ionic bonds
- Hydrophobic interactions.

Most of these interactions are weak and successful binding of enzyme and substrate requires that the two molecules are able to approach each other closely over a broad surface. The substrate molecule binds to the enzyme like a key in a lock.

This means that the structure of the substrate has to match the shape of the enzyme and explains the remarkable specificity of most enzymes. The necessity for a close fit between enzyme and substrate explains how the enzyme can be inhibited by molecules with a similar structure.

Many enzymes require the presence of an additional, non-protein, co-factor.

- Some of these are metal ions such as Zn_2+ (the co-factor for alcohol dehydrogenase), Cu_2+, Mn_2+, K+, and Na+.
- Some co-factors are small organic molecules called co-enzymes – for example the B vitamins.

Temperature sensitivity

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. (A 10°C rise in temperature will increase the activity of most enzymes by 50 to 100%). Variations in

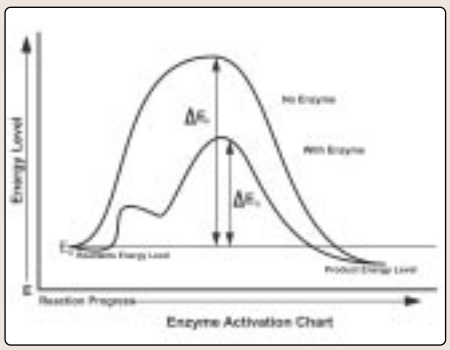

Figure 1. Free Energy diagram showing how enzymes reduce the energy of activation of a reaction.

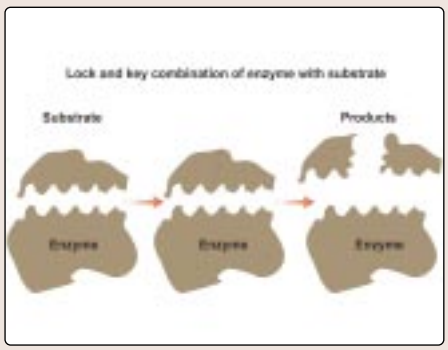

Figure 2 – Schematic of the mechanism for an enzyme binding with a substrate.

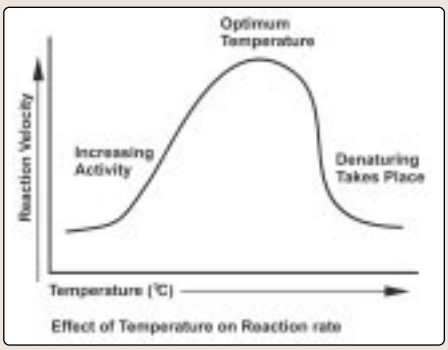

Figure 3. Temperature Sensitivity of Enzymes.

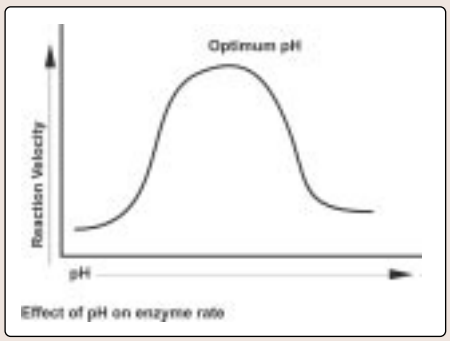

Figure 4. pH Sensitivity of Enzymes.

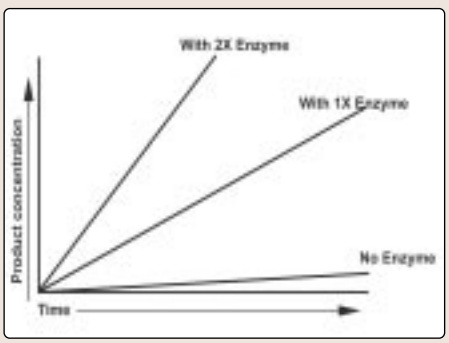

Figure 5. Effect of increasing enzyme concentration on the rate of reaction with unlimited substrate.

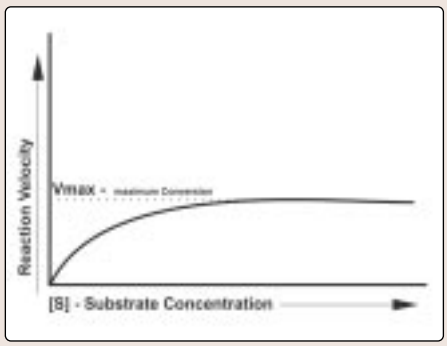

Figure 6. The effect on limited enzyme concentration on the rate of reaction

reaction temperature as small as 1 – 2° may introduce changes of 10 to 20% in the results.
In the case of enzymatic reactions, this is complicated by the fact that high temperatures adversely affect many enzymes. The reaction rate increases with temperature to a maximum level, then abruptly falls off with further increase of temperature. Many enzymes start to become denatured at temperatures above 40°C (See Fig 3).

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable.

pH sensitivity

Enzymes are affected by changes in pH. The most favourable pH value – the point where the enzyme is most active – is known as the optimum pH (See Fig 4).

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes, as with activity, for each enzyme there is also a region of pH optimal stability.
The optimum pH value will vary greatly from one enzyme to another. Most of the brewing enzymes have an optimum pH in the range 4.5 to 6.0 which is the operating range of most brewing process.

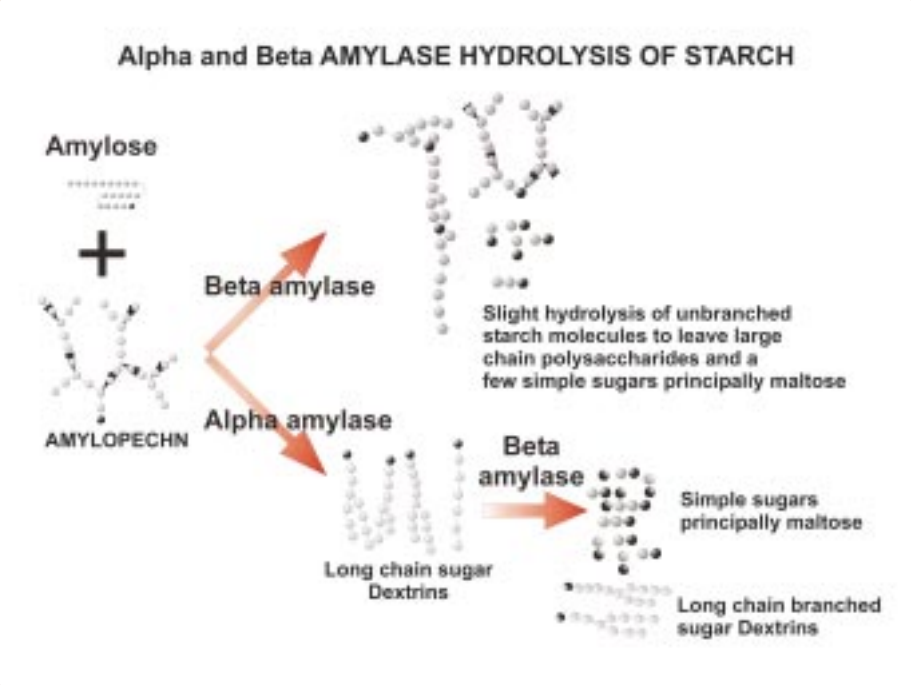

Figure 7: Schematic showing the action of alpha- and beta -amylase in starch hydrolysis.

Enzyme concentration

With an excess concentration of substrate,

such as starch in a brewers wort, there is a linear effect of increasing the enzyme concentration upon the reaction rate (See Fig 5).

Hence the if all other factors are kept constant, malts with higher enzymic power will break down the starch faster.
The amount of enzyme present in a reaction is measured by the activity it catalyses.

Substrate concentration

It has been shown that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.

It is thought that when this maximum velocity had been reached, all of the available enzyme has been converted to the enzyme substrate complex. This point on the graph is designated Vmax. This information can be used to calculate enzyme activity in terms of rate of reaction (See Fig 6).

In addition to temperature and pH there are other factors, such as ionic strength, which can affect the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimised in order for an enzymatic reaction to be accurate and reproducible.

Biochemical changes during brewing

Enzymes are essential in catalyzing the biochemical changes, which occur in the brewing process. There are two principal processes of interest to the brewer:

- The break down the carbohydrate, principally starch in malted barley to sugars.
- The fermentation of sugars and other nutrients under anaerobic conditions by yeast to release energy and producing ethanol as a metabolic by-product.

These biological reactions are catalyzed by enzymes from the barley and yeast respectively. Every organism is obliged to produce all the enzymes needed to break down its component molecules.

Thus barley is able to produce all the enzymes needed to degrade starch, beta-glucan, pentosans, lipids and proteins, which are the major compounds of concern to the brewer. It follows that it must be possible to produce malting and mashing profiles to allow all these reactions to take place.

Table 1 shows how enzymes work best between specific pH and temperature ranges.

Table 1

Enzyme	Action	Optimum temp °C	Optimum pH
Alpha amylase	Random hydrolysis of starch	67	5.2
Beta amylase	Hydrolysis of pairs of sugars from the non-reducing end	62	5.5
Proteases (various)	Solubilises proteins and polypeptides	52 (some higher)	5.5
Malt beta glucanase	Breaks gum cell walls, reduces viscosity	56	6.0

Breaking down the carbohydrates

Although malting and mashing are physically separate processes and usually take place in different locations, malting has a profound influence on the subsequent release of sugars during mashing.

During malting the barley corn is allowed to germinate where it produces enzymes which break down the cell walls in the corn and produces enzymes capable of releasing the energy stored as starch in the endosperm.

The starch, which is laid down in concentric granules surrounded by a protein matrix, has to be broken down during mashing before the starch hydrolysing enzymes, Amylases, can gain access to the enclosed starch store. There are three principal enzymic reactions in malt involved in the mashing process, which are listed below along the upper limits of their thermal activity.

Table 2. Effect of mash temperature on wort fermentability (all malt mash)

Mash temp °C	% Extract	% Fermentability	Attenuation limit for a 1040 wort (10°P)
60	75.6	76.2	2.7
65.5	74.2	69.7	3.2
68.3	74.0	65.3	3.7

Starch hydrolysis

The principal enzyme reaction involved in mashing is the hydrolysis of starch to sugars by alpha- and beta-amylase. Before enzyme hydrolysis can occur it is necessary to exceed the starch gelatinisation temperature of malt. Therefore it is necessary to select the optimum conditions for the saccharifying enzymes to operate. This is achieved by stabilising the enzymes in a number of ways:

- Optimising pH at mashing (usually between pH5 and 6)
- Adding calcium ions to stabilise the enzyme
- Using thick mash (high concentration substrate to insulate the enzymes against denaturing)
- Optimising temperature to favour the activity of both the alpha and beta amylase.

Table 3. Temperature of beta glucan enzyme activity

Enzyme	pH Optimum	Optimum Temp.	Temp. Inactive
Endo beta 1-4 glucanase	4.5-4.8	37-45°C	55°C
Endo barley beta glucanase	4.7-5.0	40°C	63°C
beta glucan solubilase	6.3	60°C	73°C

Table 4. Effect of mashing temperatures on the release of beta glucan.

Temp °C	Wort beta glucan mg/l	Wort viscosity cp	Wort filtration rate volume in 30 mins
45	1.2	1.20	277
55	2.8	1.35	178
65	6.3	1.63	133
75	9.7	2.01	78

Table 5. Effect of adding exogenous beta glucanase on the filterability of wort

Beta glucanse as % of grist weight	All malt mash		60% barley + 40% malt	
	Wort filtration rate vol in 30 mins	Wort viscosity cp	Wort filtration rate vol in 30 mins	Wort viscosity cp
0	260	1.73	200	1.82
0.01	283	1.73	218	1.81
0.05	288	1.60	236	1.65
0.1	325	1.43	290	1.43
0.2	325	1.43	290	1.44

The amylase enzymes are able to break the alpha-1,4 links in amylose and amylopectin to give a mixture of glucose, maltose, maltotriose and higher sugars called dextrins, which are unfermentable, to give a wort (malt derived sugar solution) which is about 70% fermentable.

- alpha-amylase produces random

hydrolyses of starch to dextrins.

- beta-amylase attacks the starch and dextrins from the reducing end, stripping off pairs of sugars molecules (maltose) as shown in Figure 7.

By varying the mashing temperature it is possible to preferentially favour one enzyme reaction over the other and hence influence the fermentability of the wort, with the lower temperatures giving higher fermentable worts as shown in Table 2.

Beta-glucan breakdown

As well as starch there are a number of non-starch barley polysaccharides. The most significant non-starch polysaccharide in barley and malt is beta-glucan which makes up more than 75% of the cell wall. The molecule has a distinctive linear structure in with roughly 70% beta-1,4 linkages and 30% beta-1,3 linkages.

Most beta-glucan is water soluble, but a proportion is bound co-valently to cell wall proteins. If there is insufficient degradation of the cell walls, then enzymic access to the protein and starch will be restricted, and the extract from the malt reduced.

Although much of the necessary beta-glucanase activity occurs during malting, there is inevitably some survival of cell wall material (even in the most fully modified malt).

This will be exacerbated if adjuncts such as barley and wheat are also used. Consequently it is necessary to ensure the continued activity of beta-glucanase during mashing, since the release of beta-glucan will continue through the activity of beta-glucan solubilase which is more heat stable than the malt beta-glucanase which breaks down the beta-glucan structure. (See Table 3).

The results of the different optimum temperature can have an effect on the viscosity and hence the filterability of wort and beer (See table 4).

If the large viscous beta-glucan molecules are not broken down during malting or mashing other process problems can also occur:

- Reduced extract recovery
- High wort viscosit
- Poor run off performance
- Beer filtration problems
- Beer haze problems

The high molecular weight beta-glucans released by beta-glucan solublilase contribute to wort viscosity and poorer extract recovery.

Most brewers are very careful in selecting malt with low beta-glucan levels, and beta-glucan degradation occurs during malting. However most initial mash temperatures are at or above the maximum stability temperature of the malt beta glucanase enzymes, and it is common practice in many breweries to add exogenous beta-glucanase to decrease wort and beer viscosity and to improve filterability.

The effects of enzyme addition on the breakdown of beta-glucan can be shown by an increasing in filter flow rate and decrease in wort viscosity. The effect will be more noticeable with higher concentrations of unbroken beta-glucans when using for example raw (un-malted) barley adjunct (See Table 5).

Table 6: Effect of mashing temperature after 1/2 hour stand on protein hydrolysis.

Temperature °C	Nitrogen mg/100 ml	Head Retention Rudin (sec)	Shelf life (weeks)
62.8	43	88	12
65.5	40	100	10
68.3	37	99	8

Table 7: Common enzymes used in syrup manufacture

Type of enzyme	Action	Principal sugars produced
Heat stable alpha amylase	Endo-1,4 alpha bonds	Reduces viscosity – Maltodextrins
Alpha amylase and glucoamylase	Alpha-1,4 bonds and alpha-1,4 & 1,6 bonds	Glucose syrup
Alpha amylase and beta amylase	Alpha-1,4 bonds	Principally maltose syrup
Alpha amylase and pullulanase	Alpha-1,4 bonds Alpha-1,6 bonds	Principally very high maltose syrup

Based on information supplied by ABM – Rhone-Poulenc

The first aid kit

Location	Symptom	Remedy
Cereal Cooker	Glutinous starch	Heat stable alpha-amylase
	Retrograded starch	
Mash mixer	Enzyme deficient malt	Bacterial alpha-amylase
	Starch in wort	
	Set mash – will not filter	Heat stable beta-glucanase
	Adjunct brewing – wheat or barley	Alpha-amylase, protease & betaglucanse
	Low wort nitrogen	Neutral protease
Fermentation	Poor wort fermentability	Fungal alpha-amylase
	Starch in fermenting wort	
	High beer attenuation	Amyloglucosidase or pullulanase + beta-amylase
	Rapid diacetyl removal	Alpha-acetolacate decarboxylase
Maturation and filtration	Low sweetness	Amyloglucosidase
	Promote secondary fermentation	Amyloglucosidase
	Chill haze protection	Papain
	Poor filterability	Fungal alpha-amylase or beta-glucanase
	Haze from starch or glucans	Fungal alpha-amylase or beta glucanase
Bottling	Resistance to oxidation or oxygen barrier	Immobilised glucose oxidase in crown liner

Hydrolysis of Proteins and Polypeptides

While about 95% of the starch from malt is solubilised by the end of mashing, only about 35 – 40% of the malt protein (TN – total nitrogen) is solubilised. This is referred to as the TSN (total soluble nitrogen) in an unboiled wort.

The permanently soluble nitrogen (PSN) is the nitrogenous material which remains in the wort after wort boiling (i.e. is not precipitated as break). The PSN is usually calculated as TSN x 0.94.

The principal groups of enzymes involved in the breakdown of malt proteins are Endoproteases which break the large protein molecules into relatively large polpeptide chains, and the Exopeptidases which attack the polypeptides from a specific end stripping off small units to produce amino acids.

Endopeptidases

They have a relatively low optimum temperature and hence with high temperature mashing (e.g. 65oC isothermal mashing) most of the protein breakdown will have taken place during the malting process, and randomly attack the protein chain

Optimum conditions

pH	**3.9-5.5**
Temperature	**45-50°C**
Inactivation temperature	**70°C**

Exopeptidases
They are able to withstand higher temperatures and release the amino acids from the prolypeptide chains.

There are two principal groups of Exopeptidase enzymes:

- *Carboxypeptidase* which attacks the proteins from the carbonyl end. This enzyme is not present in raw barley, but is rapidly produced during steeping and is active at normal mash pH.

Optimum conditions

pH	**3.9-5.5**
Temperature	**45-50°C**
Inactivation temperature	**70°C.**

- *Aminopeptidase*, which attacks the proteins from the amino end, is much less active at mash pH and does not play a significant role in protein breakdown during mashing.

Optimum conditions

pH	**4.8-5.2**
Temperature	**50°C**
Inactivation temperature	**>70°C**

Most of the proteolysis occurs during malting. It is impossible to completely compensate for a nitrogen deficiency in malt by introducing a prolonged mash stand at < 50°C without adding exogenous enzymes.

Nitrogenous materials account for 5-6% of wort solids, which is equivalent to around 30-40% of the total nitrogen in malt. Good yeast growth and rapid fermentation requires 160mg/l of free amino nitrogen (at 12°P wort) – depending on the yeast strain.

Carboxypeptidases can release amino acids in mashing provided that the endopeptidase has broken down the protein substrate during the malting process. The optimum temperature to produce free amino nitrogen production is 50°C.

Proteins in the mash dissolve at these low temperatures and then precipitate at 65°C, which can inhibit lautering.

Excessive proteolysis in malting and mashing will reduce foam stability and the pH of a normal mash is not optimal for proteolysis (See Table 6).

Typical types of protein material found in wort

Proteins
Large molecules with a unique identity. Much of the surplus protein is left behind in the spent grains, but when oxidised can form a protein "scum" which causes run off problems. Some of the soluble proteins play an essential role as enzymes catalysing the reactions described above.

Polypeptides
Long chain sequences of relatively high molecular weight amino acids, with two important groups in brewing, hydrophobic polypeptides which make up beer foam and acidic polypeptides which can combine with polyphenols to produce hot and cold break, and if not removed, these contribute to colloidal instability in beer.
This group of compounds are also probably important in contributing to the texture and mouthfeel of the beer.

Peptides
These are short chain sequences of amino acids usually 2 to 10 units long, and probably have a minor effect on body and mouthfeel.

Amino Acids
These make up 10 to 15% of the TSN and are an essential source of nutrient for yeast growth. The usual concentration of soluble free amino nitrogen (FAN) in wort is required to be above 160 mg/l; lower levels can lead to a defective fermentation.

In addition to role of amino acids in yeast growth, they are also involved in a number of metabolic pathways, producing significant flavour active compounds, which contribute to the final flavour of the beer.

The activity of proteolytic enzymes are effected by temperature of mashing, which in turn will effect the total nitrogen, amino nitrogen, head retention and shelf life stability.

Fermentation

Most living organisms respire aerobically, converting sugars to carbon dioxide and water releasing the energy bound by photosynthesis in the carbohydrate (sugars) molecules.

However some micro-organisms, including yeast, are able to respire anaerobically, but under anaerobic conditions they can only partially break down the sugar molecules to ethanol to release energy in the form of ATP (adenosine triphosphate).

The role of yeast in the fermentation is that of a living catalyst, effecting the reaction without becoming part of the finished product. During the course of the fermentation the yeast cells grow and replicate up to 5 times.

Although the yeast gains its energy from the sugar, which it converts to alcohol it can only utilise simple sugars. The sugars are taken up in a specific order, with the monosaccharides, glucose and fructose used first, together with sucrose. Although the latter is a disaccharide, it behaves like a monosaccharide since it is broken down to glucose and fructose outside the cell through the action of the yeast enzyme invertase.

Once the wort glucose level falls, the yeast starts to use the disaccharide, maltose, which is usually the most abundant sugar in brewers wort. Maltose has to be transported into the cell, where it is broken down to glucose. Lastly most yeast strains can utilise the trisaccharide, maltotriose, but only slowly.

Brewing strains of yeast cannot generally ferment the longer chained or branched sugars (called dextrins) which persist in to the finished beer as unfermentable extract to give the beer body and mouthfeel.

As well as sugars, yeast requires nitrogen, which in wort comes from the malt in the form of soluble amino nitrogen. A healthy fermentation yeast requires more than 160 mg/l of soluble nitrogen.

If there is insufficient soluble nitrogen, for example when high cereal or sugar adjunct are used, then additional nitrogen may be required in the form of simple ammonium salts.

Syrup manufacture

A number of brewers use brewing syrups which are manufactured from hydrolysed starch solution. Since the starch is not malted, microbial exogenous enzymes have to be used and by selecting different enzyme combinations the syrup producer can control the composition and fermentability of the syrup. (See Table 7).

A brewers first aid kit

It is the objective of most brewers to avoid the use of external enzymes and rely on the naturally produced enzymes from the malt and the activity of the yeast alone to produce their beer (See table on previous page for some first aid). ■

Further Reading

O'Rourke "Mashing" in *Brewing Science and Technology Series III* (in print) published by the Institute of Brewing

O'Rourke "Brewing" chapter 2.6 from *Industrial Enzymology Ed 2* edited by Godfrey and West Macmillan 1996.

O'Rourke "Mashing" *Brewers Guardian* December 1999