Topic 20: Working with enzymes

This Topic (dated 2016) is new. It has been added because of the greater range of enzymes now available to schools and the current lack of straightforward, relevant publications for schools.

20.1 Introduction

Practical work with enzymes forms an essential part of the science and/or biology curriculum, and the current post-16 school biology specifications in England (and elsewhere in the UK) place emphasis on quantitative investigations of enzyme activity.

Thirty years ago, very few enzymes were commonly available to schools. Those that were included amylases from human saliva or germinating barley and products such as porcine or bovine trypsin from school laboratory suppliers. Pectinase preparations sold for amateur wine-making or rennet, used for making cheese, were sometimes available from high street stores. Occasionally schools made use of solutions of enzyme-containing 'biological' washing powders. Many of these enzymes had short shelf-lives and unpredictable activity.

Today, there are at least 20 different commercial enzyme preparations that are regularly used in schools alongside more 'traditional' enzymes. In addition, enzymes for DNA modification and amplification are used on a very small (microlitre) scale.

20.2 Enzyme names and classification

Enzyme nomenclature is generally a chaotic area, but since the early 1960s the International Union of Biochemistry and Molecular Biology (IUBMB) has tried to classify enzymes, allocating them systematic names that reflect their action[1]. The main groupings are given in Table 1. For example, lipases are classified as EC 3.1.1.3 (EC here stands for 'Enzyme Commission') with the systematic name 'triacylglycerol acylhydrolase'. The names of individual enzymes may be found at the Enzyme Nomenclature Database: **http://enzyme.expasy.org**

Unsurprisingly, shorter and less systematic ('trivial') names have gained common acceptance, and these may be listed as synonyms of the systematic names by the IUBMB. Trivial names are predominantly used in school biology (this contrasts with the situation in chemistry, where efforts are made to use the systematic names for chemicals recommended by the IUPAC).

Trivial names can be highly problematic, however. Sometimes the same name has been given to several different enzymes, or a single enzyme has been given different names by different researchers.

Many trivial names (such as 'catalase' and 'papain') convey nothing about the reaction being catalysed. And even when a name appears to give information about the reaction, this can be misleading. For example, an α-amylase will hydrolyse α-1,4 glycosidic linkages in amylose and amylopectin, the two components of starch, producing dextrins and oligosaccharides. So-called β-amylases will also hydrolyse the same α-1,4 bonds in starch, but here the resulting product is β-maltose. Thus there is no direct relationship between the 'alpha' and 'beta' in the names of the enzymes and the configuration of the glycoside bonds in the substrates they are acting on.

Furthermore, some enzyme preparations that are given a single trivial name are in fact mixtures of several different enzymes: 'pectinase', for example, is usually a mixture of polygalacturonase, pectin lyase and other pectin-degrading enzymes. Similarly, 'rennet' is a crude extract containing bovine chymosin, pepsin and lipase, and 'diastase' contains a mixture of several different amylases.

Table 1: Enzyme classification (adapted from 'Enzymes at work' [2]).

Enzyme Commission class	Examples	Reaction catalysed
EC 1. Oxidoreductases	Catalase; Glucose oxidase	Oxidation and reduction reactions involving the transfer of electrons from one molecule to another.
EC 2. Transferases	Phosphorylase	These enzymes transfer a group of atoms from one molecule to another or from one position in a molecule to other positions on the same molecule.
EC 3. Hydrolases	Amylase; Cellulase; Lipase; Protease; Polygalacturonase (the main component of most 'pectinases').	These enzymes break up (hydrolyse) larger molecules to smaller ones with the addition of water. Due to their availability and ease of use and detection of the products of the reactions, this group of enzymes is the most likely to be used in schools. Unfortunately, they can give students the impression that all enzymes break down or 'digest' their substrates.
EC 4. Lyases	Pectate lyase (a component of most 'pectinases')	Lyases catalyse the addition of groups to double bonds or the formation of double bonds by the removal of groups.
EC 5. Isomerases	Glucose isomerase	Isomerases catalyse the rearrangement of atoms within the same molecule e.g., glucose isomerase converts glucose to fructose.
EC 6. Ligases	T4 DNA ligase	Ligases join molecules together requiring the simultaneous input of energy from ATP. This requirement means that they are seldom used commercially, but DNA ligases are essential tools in molecular biology.

The trade names of commercial enzyme preparations are usually chosen for marketing purposes. It might be a challenge to guess, for example, that *Viscozyme*® is a blend of several different plant tissue-degrading enzymes, or that *Stainzyme*® is an α-amylase preparation.

From an educational perspective, it would seem appropriate to ensure that at least 16–19 year-old students understand that there are systematic names for enzymes (even if they are not expected to know the details) and that many of the enzyme preparations they are dealing with are in fact mixtures, not a single enzyme.

20.3 What's in the bottle?

Throughout this Topic, enzymes are referred to as 'enzyme preparations'. This is because the products supplied to schools often include other ingredients, such as stabilisers, in addition to the enzyme.

Dried (lyophilised) enzymes in powdered form may contain 90–100% protein. The drying process can destroy much of the activity of the enzyme, however.

Liquid (aqueous) enzyme preparations are generally more convenient to handle and are often more active than the dried equivalent. They usually contain only a small proportion of enzyme (sometimes less than 1% protein, but as much as 30%, depending upon the product), with the bulk of the solution being water and often glycerol to stabilise the protein. Other components such as calcium chloride or sodium chloride may also be added to further stabilise the enzyme, which can be a problem when immobilising the enzyme using sodium alginate. The calcium ions cause the alginate solution to gel when the enzyme is mixed with it (see Section 20.11). Sometimes enzyme preparations also contain a preservative to prolong their shelf-lives.

Some dried enzymes, particularly those used in washing powders, are supplied as 'prills'. These are small, crush-proof granules of cellulose with a small amount of dried enzyme at the centre — you can easily identify the spherical prills in biological washing powders by examining the product under a low-power binocular microscope or hand lens. Such granules prevent the release of enzyme dust, which caused health problems for some factory workers when 'biological' washing powders were first introduced in the late 1960s. When added to the washing water, the prills break up, releasing the enzyme. The cellulose is insoluble however, so the impression may be gained that the enzyme has not dissolved. Similarly, enzymes in tablet form (such as those obtained from health food shops) may contain insoluble or low-solubility bulking agents.

Reducing sugars

A common concern for schools is whether certain enzyme preparations contain 'reducing sugars', as these may react with Benedict's or dinitrosalycylic acid (DNSA) reagent when trying to detect the product of the reaction. Sugars may be present in an enzyme preparation, as it is costly to remove them from enzyme preparations of microbial or plant origin. For example, even if *Termamyl*® or AMG are diluted 100-fold for use in the reaction, the amount of sugar present will still give a positive result with Benedict's qualitative reagent. If necessary, an appropriate 'control' with a *quantitative* Benedict's or DNSA test will enable a correction to be made for the presence of reducing sugars in the enzyme preparation.

20.4 'Concentration' and activity

There is frequent confusion over the 'concentration' of enzymes and unfortunately some school suppliers do not help by describing their liquid products in vague terms such as 'full strength' or 'working concentration'.

It follows from section 20.3 that it is difficult, if not impossible, to be sure of the amount of enzyme in a given preparation (especially when in liquid or tablet form). This makes safety assessments based on the concentration of the enzyme being used problematic or indeed meaningless.

What matters with an enzyme is its *activity*, and this will be affected by the pH and temperature of the reaction, the nature and concentration of the substrate, the presence or absence of cofactors and/or inhibitors and so on. Consequently the definition of enzyme activity has to include a description of the conditions of the reaction.

The SI unit of enzyme activity is the katal (abbreviated to kat). It officially replaced the older measure of *enzyme units* (U) in 1999, although some suppliers still specify the activity of their products in U. The katal is defined as the enzyme activity that transforms one mole of substrate per second under specified conditions (of pH, temperature, etc);1 kat = 1 mol s^{-1}. One katal of trypsin for example, is the amount of trypsin that breaks one mole of peptide bonds per second under specified conditions. The katal is generally too large a unit for practical use, so enzyme activities are expressed as micro-, nano- or even picokatals (μkat, nkat or pkat).

In practice, few enzyme suppliers use the katal, preferring instead units that reflect the historical preferences of their customers. For example, bakers may be familiar with amylase activity expressed in 'SKB units', whereas brewers traditionally refer to amylase activity (which they call diastatic activity) in 'Degrees Lintner'. To obtain these various measures of enzyme activity, different laboratory assay methods have been used, and conversion between them is seldom possible.

There is not necessarily any relationship between the declared activity of the product and its activity in a given practical application, because laboratory assays will seldom correspond with the conditions in which the enzyme is used in the classroom (or in industry or research).

It is therefore difficult if not impossible to provide precise recommendations regarding the amount of an enzyme preparation that should be used. Preliminary experiments may need to be carried out in which the enzyme concentration is varied. As a rule of thumb, the concentration of the enzyme preparation could be varied from 0.01% up to 1% (v/v or w/v) in the solution where the reaction catalysed by the enzyme is occurring.

20.5 Hazards and control methods

There are two principal hazards from enzymes. Firstly, *some* enzymes may directly affect human tissues. For example, a protease, splashed into the eye or entering the skin through a cut or scratch may break down proteins and cause serious damage or irritation. Secondly, *all* enzymes have the potential to trigger an allergic reaction in susceptible individuals, usually through inhalation of enzyme-containing dust or an aerosol.

If enzymes are extracted from plant, animal or microbial sources, such as proteases from kiwi fruit (*Actinidia* spp.), urease from Jack beans (*Canavalia ensiformis*), invertase from baker's yeast or catalase from liver, these source materials and/or the extraction process may present hazards.

Often, enzymes are used with buffer solutions or reagents to test their action and these may also present hazards. These, and appropriate control measures are summarised in Table 2.

Table 2: Hazards and control measures associated with using some enzymes.

Type and source of hazard	**Nature of the hazard**	**Type of activity in which risks may arise**	**Means of limiting risks (control measures)**
Detergents and proteases in washing powders/liquids	May cause allergy or asthma symptoms or breathing difficulties if inhaled (Cat. 1). May cause serious eye irritation (Cat. 2). Can be harmful to aquatic life with long lasting effects (Cat. 3).	Using biological washing powders or liquids, or adding enzymes to a non-biological detergent base to make DIY biological washing products.	Avoid raising or inhaling dust or aerosols. Use a fume cupboard and gloves where appropriate. Rinse any spills promptly with water. Use small volumes of dilute solutions only. *Note: Typically, one would add up to 2% by mass of these enzyme preparations to a liquid detergent product, then about 8 g of this liquid would be used per litre of water in a typical European wash. These dosages will result in an enzyme protein concentration of between 0.1 and 1 mg per litre in the wash. As the molecular weights of the enzymes vary from approximately 27,000 to about 55,000 g per mole, the enzyme concentration during a wash is in the nanomolar range.*
Enzymes	**All enzymes:** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **Proteases:** Irritation to eyes and skin / Risk of serious damage to eyes.	Spills and splashes of enzymes; formation of aerosols.	Use dilute solutions of enzymes. Avoid the formation of aerosols. Do not spray enzymes. Rinse spills/splashes of enzymes with water and wipe up promptly to prevent the formation of enzyme dust.
Bacteria and/or fungi from incubated plates	Infection; Inhalation of spores.	Demonstration of the production of extra-cellular enzymes on plates of microbiological media.	Use non-pathogenic strains and good microbiology laboratory practice. If plates are opened to flood with iodine or other indicators, this must be done safely. Do not open plates containing *sporulating* cultures e.g., *Aspergillus oryzae* used to demonstrate amylase production. Destroy all cultures by autoclaving after use.

20.6 Labels and hazard warnings

Since June 2015, when Regulation (EC) No. 1272/2008 [CLP] came fully into force, the labels on nearly all enzyme products sold by laboratory suppliers are likely to include the words:

'DANGER. May cause allergy or asthma symptoms or breathing difficulties if inhaled' plus the 'Serious health hazard' ('exploding chest') hazard pictogram (GSH08).

While this may appear alarming, there is only an allergy hazard from the product if it is inhaled. This is extremely unlikely to occur in an educational context where small volumes of enzyme are used, often in a very dilute form, or mixed with a larger volume of substrate solution. Smelling the product is unlikely to be hazardous.

The hazard warning is more relevant in an industrial context, where large volumes of enzyme may be used and workers may be exposed repeatedly to aerosols of the enzyme preparation, which could be breathed in.

Most enzymes are classed as skin, eye and respiratory irritants. The majority are respiratory sensitisers at a concentration of 1% or more, so care should be taken to avoid raising dust or creating aerosols. A few are respiratory sensitisers (category 1A) at 0.1% or above and need extra care. The Safety Data Sheet provided with the enzyme will tell you whether the product has been classified as Resp. Sens. 1 or Resp. Sens. 1A.

Although uncommon, some individuals may be *sensitised* to particular types of enzymes. It is important to note that for anyone already sensitised to any of these enzymes, a much lower concentration will be sufficient to trigger a response, typically 1/10 of the sensitising dose so 0.1% or even 0.01% for the Category 1A ones.

However, as long as enzymes and their solutions are handled carefully, by avoiding raising dust, preventing aerosol formation, using dilute solutions and wiping up spills promptly, there should be no health issues.

That said, it is wise to ensure that enzyme dust or aerosols are not produced when handling enzyme products (see section 20.7). Technicians producing enzyme solutions from powder may find it safest to do this in a fume cupboard with the fan switched off.

Additional risk from proteases

In addition, *some* proteases present extra concerns as they can cause eye or skin irritation or damage. When handled in bulk, the proteases added to washing powders or liquids are of particular concern here, although the quantities present in *dilute* washing solutions, which are in the nanomolar range, do not present a safety concern.

20.7 Handling enzymes

Storage

Dried enzymes can often be stored in a dry, cool place without refrigeration: the supplier's advice (from the product's Safety Data Sheet) should be followed. Dried enzymes stored in the fridge should be in air-tight containers to avoid them becoming damp.

Liquid preparations are best stored in a fridge at 3–5 °C, although many are very stable and will lose little of their activity if kept at room temperature. Liquid enzyme preparations should not usually be frozen: exceptions are solutions of DNA-modifying enzymes that must be stored frozen at –18 °C (and in some cases at –80 °C). Such enzymes are usually kept on ice while they are being used in the lab. Again, the supplier's advice should be followed.

Once a dilute solution of enzyme has been prepared, it should be used as quickly as possible. Note that solutions of some dried enzymes can be stored frozen in measured quantities (aliquots) at –18

°C. Solutions of some enzymes (such as pepsin and trypsin) should be stored frozen to prevent autolysis (self digestion). Repeated cycles of freezing and thawing may lead to a reduction in enzyme activity, however.

Dispensing powdered enzymes

When weighing out powders, especially proteases, it is advisable to use a fume cupboard without the fan running. If a spill occurs clean it up and then the fan can be used to draw away any remaining dust. Since enzymes are water-soluble, any spilt powder can be washed with water and wiped up with paper towels that can be placed in a plastic bag, tied and disposed of in the normal waste. Disposable gloves should be worn when wiping up spills of enzymes that are particularly hazardous, e.g., skin sensitiser.

Dealing with spills and splashes of solutions

If enzyme solutions are allowed to dry up, there is a risk of dust formation. In susceptible people the repeated inhalation of dust may provoke asthma or hayfever-like symptoms. That is why any spill should be rinsed with water straight away. Paper towels used to mop up the liquid can be put into a plastic bag, tied and disposed of in the normal waste. Disposable gloves should be worn when wiping up spills of enzymes that are particularly hazardous, eg. skin sensitiser.

Disposal

Aqueous (liquid) enzyme preparations can be disposed of down the sink

Powdered enzymes should be dissolved in water and then disposed of down the sink.

Avoid the formation of aerosols

If enzyme-containing aerosols are formed, there is a risk of inhalation of the enzyme. In susceptible people the repeated inhalation of an aerosol may provoke asthma or hayfever-like symptoms. That is why enzyme solutions ***should never be sprayed***.

Skin and eye contact

If, by accident, you get liquid enzyme on your skin or in your eyes, the remedy is to rinse quickly with plenty of tap water. Enzymes are soluble in water, so contaminated clothing can be washed in water as usual.

This action will generally prove sufficient, but if symptoms develop in the respiratory passages, on the skin or in the eyes, consult a doctor immediately, showing them the Safety Data Sheet that was supplied with the enzyme.

20.8 DIY enzymes

Salivary amylase

It is sometimes said that the use of salivary amylase has been banned in schools. However, the collection of saliva has never in fact been nationally prohibited in UK schools but it was banned in Northern Ireland. In addition, some educational employers also banned it under the general heading of collecting bodily fluids. In all cases, such prohibitions have been rescinded. Provided sensible precautions are taken, salivary amylase can be used safely in schools and can have several advantages over commercial supplies of α-amylase:

- unlike most microbial or plant amylases, it is activated by chloride ions
- it is readily denatured by boiling (in contrast to products such as *Termamyl*®)
- it is free-of-charge and its use can be highly motivating to many students

Because saliva may contain pathogenic bacteria and viruses, students should collect and work only with their own saliva samples. Good hygiene should be observed when the saliva is handled, and the formation of aerosols, which may carry infection, should be avoided. After use, all test tubes,

spotting tiles, syringes *etc* that have been in contact with the saliva should be immersed in a 1% (w/v) *Virkon*® solution or 5% (v/v) *Biocleanse*® solution for at least 30 minutes before washing them in the usual way.

After work with saliva, wipe benches with disinfectant and ensure that students wash their hands.

Note: schools in Scotland use the following, slightly different, sampling procedure:

Rinse mouth with water using disposable cup. Hold a sip of water in the mouth for a few moments. Collect the water and saliva in a clean boiling test-tube or disposable cup and proceed with the experiment.

Catalase from liver

If liver or similar animal-derived material is used as a source of catalase, only materials sold as food should be used. The material should be fresh and must be disposed of appropriately after use.

Plant enzyme extracts

A wide variety of plant materials can be used as sources of enzymes. Examples include proteases from kiwi fruit, pineapples and papaya, urease from soya or Jack beans (*Canavalia ensiformis*), amylase from germinated barley or sweet potatoes, polyphenol oxidase from fruit [6] and potato phosphorylase in the synthesis of starch [3, 4].

Both the source material and crude enzyme extracts may contain allergens or toxins and they must therefore be handled appropriately, or avoided by susceptible individuals. For example, proteases can be extracted from kiwi fruit. Reports of allergies to kiwi fruit are increasingly common and reactions to even small amounts can be severe, especially in children [7].

Microbial enzymes

A common method of demonstrating extracellular enzyme production by microorganisms in schools is to grow the microorganism and to show the breakdown of the substrate in the growth medium. Sometimes this can be seen as clear zones in the growth medium around the colonies of microorganisms. On other occasions it may be necessary to pour an indicator solution, such as iodine dissolved in potassium iodide, onto the incubated plate (see Table 3).

Good microbiology laboratory practice *must* be followed when carrying out this work. If the microorganism's identity is known and it is safe, the lid of the plate can be lifted slightly so that the indicator solution can be poured onto the plate. If the plate is thought to be contaminated with an unknown species, or an unidentified species has been used, the plate should not be opened. (Refer to Chapter 15 of *Topics in Safety*.)

Table 3. Production of enzymes by microorganisms

Organism(s)	Enzyme(s) produced	Growth medium	Indication of enzyme action
Aspergillus oryzae	Amylase	Starch agar	Clear zones when agar is stained with iodine in potassium iodide solution. *Do not open plates containing sporulating cultures.*
	Protease	Milk agar	Clarification of agar. No need to open plates or to stain medium. *Do not open plates containing sporulating cultures.*
Bacillus subtilis	Amylase	Starch agar	Clear zones after staining with iodine in potassium iodide solution (see above).
	Lipase	Tributyrin agar	Clear zones where the tributyrin has been broken down are visible around colonies. No need to stain the medium.
	Protease	Milk agar	Clarification of agar. No need to open plates or to stain medium.
Pectobacterium carotovorum (= *Erwinia carotovora*)	Lipase	Egg yolk agar	Opalescent zones visible around colonies. No need to open plate.
Saccharomyces cerevisiae	Lipase	Tributyrin agar	Clear zones visible around colonies (see *Bacillus*, above).

20.9 Enzymes from high street stores

Several enzymes can be purchased from high street stores such as health food shops, usually as powders or tablets. These include bromelain, papain and lactase tablets, proteolytic meat tenderizer (often to be found in the herbs and spices section of supermarkets), rennet essence and pectinases sold for wine-making. Biological washing powders can contain several different enzymes: they always contain proteases, sometimes with the addition of amylases and lipases, and very occasionally, cellulases (the claims made for the product can indicate which enzymes are present if they are not listed on the packaging). Some contact lens cleaning solutions contain proteases. With the exception of pectinase and rennet essence, most of these products have limited activity however, and they can be an expensive way of obtaining enzymes. Unless the products are being used for a specific practical investigation, schools might be better advised to obtain enzymes from conventional school suppliers.

20.10 Enzymes from laboratory suppliers

Dried enzymes from scientific suppliers tend to be very pure, but can as a consequence be costly. In addition, they may not have a long shelf-life.

Liquid enzyme preparations made for industrial use tend to be cheaper, more stable and reliable. Their stability can be an issue however, if teachers wish to demonstrate denaturation of the enzyme by changing the pH or by heating. Most enzymes can be denatured by boiling them for 5 minutes or so (an exception being the α-amylase *Termamyl*®, which is used at high temperatures for desizing fabric). At the risk of stating the obvious, it is important to denature enzymes *before* adding them to the substrate.

Although many commercial enzymes are produced for use in the food industry, they may not have been re-packaged aseptically by school laboratory suppliers. Therefore if enzyme preparations are to be used for making foodstuffs that will be tasted or eaten, it is important to check with the supplier that they are safe to use. Remember, food must never be prepared in a laboratory; use only a suitable food preparation area. To obtain results in a typical school lesson period, the quantity of enzyme used may be far greater than would be used in a food product. For example, when using pectinase to enhance the yield of juice from apples, students might add several millilitres of enzyme solution to the pulp from one apple. In industry, by contrast, approximately 130 millilitres of the same enzyme would be added to a tonne of apples. It is essential that students do not overdose with enzymes any food products that are to be consumed.

20.11 Enzyme immobilisation

There are several methods of immobilising enzymes. The procedure most commonly used in schools is to mix the enzyme preparation with a solution of sodium alginate, then to add it dropwise from a syringe into calcium chloride solution. On contact with the calcium ions, links are formed between the alginate molecules, trapping the enzyme in a porous matrix of calcium alginate. If the alginate/enzyme mixture is dropped from ~12 cm above the calcium chloride perfect spheres of immobilised enzyme will be formed. If the beads are dropped from too low a height 'tadpoles' with long tails will be produced instead. The enzyme-containing beads benefit from being left in the calcium chloride solution for 5–10 minutes to harden before being filtered from the liquid and rinsed with distilled water before use.

While this method can work well, the concentration and type (roughly speaking, the physical strength or viscosity) of alginate can make an important difference. If the alginate is too strong, the beads may be insufficiently porous to allow the substrate to enter and come into contact with the enzyme. Conversely, if the alginate is physically weak, or the enzyme molecule too small (a molecular weight of <300,000), the bead may be unable to retain the enzyme, and much of it will leach out. Depending upon the type of alginate used, an alginate concentration of 2% (w/v) and a calcium chloride concentration of 1.5% (w/v) will usually be effective (this assumes that $CaCl_2.2H_2O$ is being used). The enzyme preparation should usually be mixed with an equal volume of alginate solution.

To prevent premature gelling of the alginate, the alginate and enzyme solution must be made up using distilled or deionised water. Note that some enzyme preparations e.g., *Termamyl*® contain calcium ions and are unsuited to this method. Sodium alginate is not readily soluble, and requires both warm water and stirring to dissolve it (greater success may be achieved by leaving the alginate to dissolve overnight). Once made up, the alginate solution may be kept for many months in a fridge, but the enzyme-containing beads have only a limited storage life and should be used within 48 hours of preparing them — again, they should be stored in a fridge.

20.12 Enzymes for DNA modification and amplification

A limited range of enzymes are used in schools and colleges for DNA modification and amplification. These include restriction enzymes, DNA ligase, and *Taq* DNA polymerase used in the PCR (polymerase chain reaction). Only microlitre quantities of the enzymes are involved, and they present no hazard. Like all enzymes they are completely biodegradable, and will break down rapidly after use.

When they are supplied in dry form, these enzymes should be stored dry and at room temperature. They are usually supplied in a foil pouch with a desiccant sachet. The pouch should be re-sealed firmly to prevent moisture from entering. Liquid enzymes must be stored frozen at –18 or –80 °C, following the suppliers' instructions.

20.13 References

1. *Enzyme Nomenclature.* International Union of Biochemistry and Molecular Biology (1992) Academic Press, San Diego. ISBN 0 12 227165 3. This publication has been updated regularly since 1992 and is available online at: **http://www.chem.qmul.ac.uk/iubmb/enzyme/**

2. *Enzymes at work* Novozymes A/S. **http://www.novozymes.com**

3. Practical Biology. Enzyme catalyzed synthesis. **http://www.nuffieldfoundation.org/practical-biology/enzyme-catalysed-synthesis**

4. Phosphorylase. Practical protocol at: http://www.mystrica.com/Enzymes/phosphorylase

5. Microscale investigations with catalase. http://www.saps.org.uk/secondary/teaching-resources/293-student-sheet-24-microscale-investigations-with-catalase

6. Polyphenol oxidase assay (and related documents) **http://www.saps.org.uk/secondary/teaching-resources/176-polyphenoloxidase-catechol-oxidase-assay**

7. Anaphylaxis campaign. **http://www.anaphylaxis.org.uk**

8. Timmerman, A. P. (2012) The isolation of invertase from baker's yeast. In: Protein Purification, by Rizwan Ahmad [Ed] pp 29–51. Intech. ISBN: 978 953 307 831 1. Available from: **http://www.intechopen.com/books/protein-purification/the-isolation-of-invertase-from-baker-s-yeast-an-introduction-to-protein-purification-strategies**

9. *Practical microbiology and biotechnology for schools* by Paul Wymer (1987) Macdonald, London. ISBN: 0 356 11566 6. *Note: Several of the practical investigations in this publication have been re-published in: Practical Microbiology for Secondary Schools by John Grainger and Janet Hurst [Eds] (2014) Society for General Microbiology/Microbiology in Schools Advisory Committee. ISBN: 095368 382 6. This can be downloaded free-of-charge from:* **http://www.microbiologyonline.org.uk/media/transfer/doc/sgm_practical_microbiology_for_secondary_schools_2.pdf**

10. Practical Biology. Investigating the effect of temperature on the activity of lipase.

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-activity-lipase

11. Practical Biology. Quantitative food test. Protein content of powdered milk. **http://www.nuffieldfoundation.org/practical-biology/quantitative-food-test-protein-content-powdered-milk**

Note: it is essential to use a fresh supply of dried skimmed milk for this test: the contents of packets can deteriorate within a month of opening.

12. *In a jam and out of juice* by Dean Madden (2000) NCBE, University of Reading. ISBN: 0 7049 1373 9. Available from the NCBE web site: **http://www.ncbe.reading.ac.uk**

Appendix 20A. Some enzymes commonly used in schools

Enzyme [Trade names of products in square brackets]	Sources	Substrate/ Product(s)	Notes / Typical school use
Actinidin	Green kiwi fruit (*Actinidia* spp.)	Hydrolyses proteins to peptides and amino acids.	Fresh kiwi fruit is sometimes used as a meat tenderiser. Actinidin and three other kiwi fruit proteins are known allergens and may trigger an allergic reaction in susceptible individuals [7].
α-amylase [*Termamyl*®; *Stainzyme*®]	Human saliva; Mealworm (*Tenebrio molitor*) salivary glands; Commercial α-amylases come from microorganisms e.g., *Bacillus* spp.	Hydrolyses the α-1,4 bonds in amylose and amylopectin in starch, producing glucose and dextrins.	Animal α-amylases (but NOT microbial ones*) require chloride ions to activate them. When using human saliva as a source, follow the guidelines in section 20.7. Commercially-produced microbial α-amylases may remain active at high temperatures e.g., *Termamyl*® exhibits optimum activity at about 90 °C. This makes it ideal for producing a temperature/activity graph but poor for demonstrating heat denaturation. *Termamyl*® also contains calcium ions to help stabilise the protein. 'Diastase' is sometimes used as a synonym for α-amylase, but it is in fact a crude mixture of α-, β- and γ-amylase. * an exception to this is an α-amylase produced by the unusual marine microorganism *Pseudoalteromonas haloplanktis*.
β-amylase	Germinated barley; sweet potato; various microorganisms.	β-amylase catalyses the hydrolysis of alternate α-1,4 bonds in amylose, cleaving off two glucose units at a time, producing maltose.	It is easy to extract β-amylases from germinated barley* or sweet potato by grinding then filtering and centrifuging the extract. Animals do not produce β-amylases. * The amylase in ungerminated seeds is inactive.
Amyloglucosidase (also called AMG or glucoamylase)	*Aspergillus niger*.	Completely converts starch to glucose, hydrolysing both α-1,4 and α-1,6 bonds in amylose and amylopectin.	AMG can be used in place of conventional α-amylases. It has the advantage that it will break down starch completely to glucose. AMG is more heat-labile than *Termamyl*® but it still requires heating to 80 °C for about 5 minutes to denature it (but holding AMG at 60 °C for an hour will NOT denature it). Note: AMG preparations may contain reducing sugars, so a 'control' quantitative test for them is advisable. They may also contain calcium ions, so cannot be immobilised in alginate.
Bromelain (sometimes called ananase)	All parts of the pineapple plant (*Ananas comosus*), but usually obtained from waste pineapple stems.	Hydrolyses proteins to peptides, cleaving the protein after any lysine, alanine or tyrosine residue.	Fresh pineapple is sometimes used as a meat tenderizer. 'Bromelain' can be a mixture of two different proteases, 'stem bromelain' and 'fruit bromelain'. 'Bromelain' is sometimes sold in health food shops in tablet form as an aid to digestion, although there is no scientific evidence to support this use.

Catalase [*Catazyme*®]	Found in nearly all aerobic cells; Commercial sources are usually microbial e.g., *Aspergillus niger*.	Breaks down hydrogen peroxide to oxygen and water.	Some catalases (but NOT the microbial ones) are inhibited by Cu^{2+} ions. The microbial enzyme is inhibited by ethanol, however. Consider micro-scale investigations to reduce the hazard from hydrogen peroxide[5].
Cellulase [*Celluclast*®; *Denimax*®]	Microbial e.g., *Cellulomonas* sp. and *Trichoderma reesei*.	Breaks down cellulose to glucose, cellobiose and dextrins.	Sometimes used in biological washing powders to prevent 'pilling' of cotton fabrics. Also used in the biological 'abrasion' of denim fabrics. In the school laboratory, its activity can be measured by adding it to a solution of carboxymethylcellulose (CMC) and measuring the change in viscosity. This is done by timing how long the CMC solution takes to run through a syringe barrel.
Chymosin (also called rennin) [*Maxiren*®; *Chy-Max*®]	Genetically-modified microorganisms (*Kluyveromyces lactis* or *Aspergillus niger*).	The enzyme cuts casein molecules between phenylalanine and methionine residues, and between any two adjacent hydrophobic amino acids.	Chymosin partly degrades casein. In the presence of calcium ions, aggregates of calcium paracaseinate then form, making cheese 'curds'. When the curd is cut, liquid whey is released. The majority of cheese sold in the UK is made using chymosin from genetically-modified *K. lactis*. The effect of calcium ion concentration on the coagulation of milk proteins can be studied by adding calcium chloride to milk. Several other school investigations, studying the effect of temperature, pH etc. have been described.
Glucose isomerase [*Sweetzyme*®]	*Streptomyces murinus*	Converts glucose to fructose.	This enzyme is used, immobilised, on an industrial scale to produce glucose-fructose syrup (sometimes called high fructose syrup).
Invertase [*Maxinvert*®; *Bioinvert*®]	*Saccharomyces cerevisiae* (Baker's yeast)	Hydrolyses sucrose to glucose and fructose.	Invertase was used by Michaelis and Menten to investigate basic enzyme kinetics. It has been used commercially since the 1930s in the manufacture of soft-centred after dinner mints and liqueur chocolates. A common use today is making invert sugar syrups e.g., for beekeeping. Invertase can easily be extracted from fresh or dried yeast. The dried yeasts sold for use with bread machines produce 2–3 times more invertase than conventional dried yeasts [8]. Note: 'Sucrase', an enzyme from animals, performs the same function as invertase, but by a slightly different mechanism. Sucrase and invertase are NOT the same enzyme.
Lactase (β-galactosidase) [*Lactozym*®; *Saphera*®; *Maxilact*®; *Lactaid*®]	*Kluyveromyces lactis* (a dairy yeast), *Bifidobacterium bifidum* (a probiotic bacterium) or *Aspergillus oryzae*. Also produced by lac+ strains of *E. coli*.	Hydrolyses lactose to glucose and galactose.	Lactase is made in the small intestine of juvenile mammals and in adult humans with a lactase persistence trait. It is also produced by lac+ strains of *E. coli* when lactose is present in the growth medium. Commercially, it is used either immobilised or in free form for producing lactose-reduced dairy products, including lactose-free milk for cats. Most commercial lactases have optimum activity at pH 6.5–7, but *Saphera*®, a new

			product from *Bifidobacterium*, works in acidic conditions, so it can be used directly in the manufacture of lactose-reduced yoghurt. *Lactaid*® tablets have a similar low-pH optimum.
Lipase [*Lipex*®]	Pig pancreas; a variety of microbes, including *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Pectobacterium carotovorum* (= *Erwinia carotovora*) and commercially, genetically-modified microorganisms e.g., genes taken from *Humicola* sp. and transferred to a production strain of *Aspergillus*.	Breaks down fats to fatty acids and glycerol.	In 1991 a commercial lipase called *Lipolase*® was the first enzyme from a genetically-modified source to be used in a consumer product (it was added to some biological washing powders). Common methods of assaying the activity of lipases in schools are to use tributyrin or egg yolk agar (similar to the method used for proteases)[9], or to measure the change in pH using phenolphthalein when the enzyme is added to milk[10] or a solution of non-dairy creamer (the sort sold for adding to coffee). The latter method is effective, but will be affected by the pH of the water used to dissolve the creamer, so check the pH of your distilled water.
Alkaline proteases (sometimes called 'subtilisin' as such proteases were originally obtained from *B. subtilis*). [e.g., *Alcalase*®; *Savinase*®]	*Bacillus licheniformis.*	Hydrolyses most peptide bonds in proteins, producing peptides and amino acids.	Since the late 1960s, such proteases have been used in biological washing powders. They are active in the alkaline conditions (~pH 8–11) typically found in washing machines. A common method of assaying their activity used to involve using gelatin-based photographic film, but such films are now difficult or impossible to obtain. An alternative is to cast gelatin-based jelly, made up with half the volume of water specified on the packet, in a Petri dish. The protease solution is added to a well cut in the jelly using a cork borer. After one or more days, the action of the enzyme can be judged by measuring the diameter of the well.
Neutral protease [*Neutrase*®]	*Bacillus amyloliquefaciens.*	Hydrolyses proteins to peptides.	Commercially this enzyme has a variety of uses, from removing the 'stickwater' around fish roe to softening wheat gluten. In schools, *Neutrase*® is often used in the extraction of DNA from plant tissues e.g., onions. It can also be used as an alternative to trypsin to clarify skimmed milk[11]. Note: It is essential to use a new stock of dried skimmed milk for this practical, as dried milk deteriorates rapidly after opening.
Papain	Sap of *Carica papaya* (papaya or paw paw). Similar proteases are made by a wide range of microbes, plants and mammals.	Hydrolyses protein to peptides, cutting after arginine or lysine residues where they are preceded by the hydrophobic amino acid and followed by any other amino acid except valine.	Papain is sometimes sold in health food shops in tablet form as an aid to digestion. These products may contain other ingredients e.g., sweeteners, flavouring, α-amylase, other proteases and bulking agents, so may not be a good source of the enzyme. Papain is also sold as a meat tenderiser, which may prove to be a better source.
'Pectinase' or 'Pectic enzyme' [*Pectinex*®; *Pectolase*]	Microbial e.g., *Pectobacterium carotovorum* (= *Erwinia carotovora*)	Breaks down pectin in several ways[11].	'Pectinase' preparations are generally a mixture of enzymes, the chief components being polygalacturonase and pectin lyase. A classic use in schools is enhancing the yield

	and *Aspergillus aculeatus*.		of juice from chopped or puréed apples[12].
Pectin esterase (Pectin methyl esterase) [*Novoshape*®; *Rapidase CME*®; *Klercidre*®]	Often produced by genetically-modified microorganisms e.g., the gene encoding pectinesterase is taken from *Aspergillus aculeatus* and added to a production strain of *A. oryzae*.	Strips methyl groups from methylated pectin, enabling Ca^{2+} ions to form cross-links between the pectin molecules, causing the pectin to gel.	Used in the 'keeving' process in traditional sweet cider making, especially in Normandy. Here, the pectin is precipitated, removing essential yeast nutrients with it. This prevents the fermentation proceeding to completion, so the cider contains more sugar. Pectin esterase is also used commercially to produce firmer fruit pieces in pies etc.[12].
Pepsin	Gastric mucosa of pigs, cattle or sheep. Commercial supplies usually come from pigs.	Hydrolyses proteins to peptides, cleaving the bonds between any two adjacent hydrophobic amino acid residues.	Pepsin solution should be stored in the freezer at –18 °C to prevent autolysis. Pepsin requires an acidic environment (pH of 1.5–2.5) to function. A convenient way of measuring the activity is to add the enzyme to a suspension of egg white (~50 g of egg white in 500 ml of water) and to record the time taken for the suspension to clear. This can be done at a range of temperatures and pH values.
Polyphenol oxidase (Catechol oxidase)	Bananas; Mushrooms; Apples.	In the presence of air, colourless catechol in fruit is converted to brown-coloured melanins.	SAPS has produced several practical protocols involving this enzyme, which can be inhibited by lead ethanoate[6].
Phosphorylase	Present in a wide variety of plants. A common source in schools are potatoes.	Breaks down starch or glycogen to glucose-1-phosphate or catalyses the reverse reaction.	A useful practical exercise because unlike most enzymes used in schools, phosphorylase can be used to synthesise a product. Catalases the (reversible) synthesis of starch[3]. Glucose-1-phosphate is expensive, but some school methods use very little[4].
Rennet (essence)	Stomachs of slaughtered, unweaned calves.	The chymosin in rennet partly degrades milk proteins, principally casein. In the presence of Ca^{2+} ions aggregates of calcium paracaseinate are formed making cheese 'curds'. When the curd is cut, liquid whey is released.	'Rennet' is a crude enzyme preparation obtained from calf stomachs and is traditionally used for cheese-making. It contains a mixture of chymosin (70–80%), pepsin (10–20%) and a small amount of lipase. A litre of rennet essence contains approximately 0.7 g of enzymes – the rest is water, salt and sometimes sodium benzoate (0.5% –1.0%) as a preservative. Calcium ions are needed to form the curd, and additional calcium may be added to the milk as calcium chloride.
Rennin	See Chymosin.		
'Rennin', fungal (sometimes called 'vegetarian rennet') [*Fromase*®]	Microorganisms e.g., *Rhizomucor miehei.*	Milk proteins are partly broken down (see notes on rennet).	Although fungal 'rennins' are completely different proteases to animal rennins, they perform a similar function. Fungal 'rennins' are is generally less heat-labile than true rennin, so manufacturers may deliberately alter the thermal stability of the fungal enzyme so that it offers similar performance to the animal product. In addition, all *Rhizomucor miehei* 'rennins' are inhibited by a compound present in raw milk. This

			compound can be destroyed by heating the milk to 68 °C.
Trypsin	Pig or cow pancreas	Hydrolyses proteins to peptides, cutting after any arginine or lysine residue.	Trypsin solution should be stored in a freezer, at –18 °C to prevent autolysis. Trypsin is often used in schools to clarify skimmed milk. The skimmed milk must be from a fresh supply of dried milk (dried milk deteriorates rapidly once the container has been opened). *Neutrase*® is a suitable alternative for this practical exercise[11].
Urease	Jack beans; Soya beans.	Urease breaks down urea to ammonia and carbon dioxide.	Two ureases exist in soya bean plants, a highly active enzyme present only in the embryo (seed) and another urease of much lower activity that is found throughout the plant. Urease is easily extracted from soya beans, by soaking them in water, then blending them and filtering the slurry through a paper coffee filter. The urease is in the filtrate.
Viscozyme®	*Aspergillus* sp.	Multi-enzyme complex containing a range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase and xylanase.	Used commercially to break down cell walls of when extracting useful components from plant materials. In the school laboratory, it can be used to produce plant cell protoplasts or to macerate plant tissue so that different cell types may be studied microscopically.