

ESAT

Biology

Enzymes and respiration

Free lesson notes

Covers	B8.1 to B8.4 and B9.1
Format	Lesson, worked examples, self-check
Price	Free

About this lesson

Every living cell is a tiny chemical factory. In a single cell thousands of reactions run side by side, at body temperature and in water, and each of them must happen at the right place and the right speed. Two ideas explain how: *enzymes*, the protein catalysts that make the reactions fast enough to matter, and *respiration*, the set of reactions that transfers energy from food so that everything else in the cell can happen.

This lesson builds both from scratch. We start with what a catalyst is, then look at the shape of an enzyme and why its shape explains specificity. We then study what changes the rate of an enzyme-controlled reaction (temperature, pH and concentration), meet the three digestive enzymes you need to know (amylase, protease, lipase), and see how enzyme experiments are done and how their results are read. The second half is about respiration: aerobic respiration, anaerobic respiration in muscles and in yeast, what happens to the body during exercise, and how respiration is measured. Graph reading and experiment design appear throughout, because the ESAT likes to test them.

How to use it. Cover each worked example and try it yourself before reading the answer. The real ESAT has no calculator, so numbers are friendly. Finish with the self-check, then try the practice set. Specification codes covered: B8.1 (enzymes as protein catalysts), B8.2 (active site, specificity), B8.3 (effects of temperature and pH), B8.4 (amylase, protease, lipase) and B9.1 (aerobic and anaerobic respiration).

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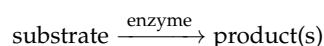
1 Enzymes: biological catalysts (B8.1)

A **catalyst** is a substance that speeds up a chemical reaction without being used up. After the reaction it is unchanged and can be used again, so a tiny amount of catalyst can deal with a huge amount of reactant. A catalyst does not make a reaction possible that could not happen at all, and it does not change how much product is made in the end: it only makes the reaction reach that point sooner.

Enzymes are the catalysts of living things. They are *proteins*. A protein is a long chain of amino acids that has folded up into a particular three-dimensional shape; that shape is the key to everything that follows.

Why does a cell need catalysts? The reactions of life (breaking down food, building new molecules, releasing energy) would be far too slow at 37°C without help. We cannot simply heat the cell up to make them faster, because high temperatures damage cells. Enzymes solve this: they let reactions run quickly at body temperature.

Substrate and product. The molecule an enzyme acts on is its **substrate**; what comes out is the **product** (or products). Enzymes can break a large substrate into smaller products (as in digestion) or join small substrates into a bigger product (as in building proteins). Written in words,



The enzyme is written over the arrow because it is not used up. Enzyme names usually end in *-ase*, and often tell you the substrate: *lipase* acts on *lipids*, *protease* on *proteins*, *amylase* on starch (*amylum* is Latin for starch).

Key ideas

- An enzyme is a **biological catalyst**: a protein that speeds up a reaction and is not used up.
- The molecule it acts on is the **substrate**; the molecules made are the **products**.
- Enzymes work at body temperature, so cells do not need to be heated to react.
- A catalyst does not change the amount of product finally made, only how quickly it is made.

Common mistakes

- Saying enzymes are “alive”. They are molecules (proteins), so they are *denatured*, not “killed”.
- Saying the enzyme is “used up” or “turned into” the product. The enzyme is released unchanged, ready to be

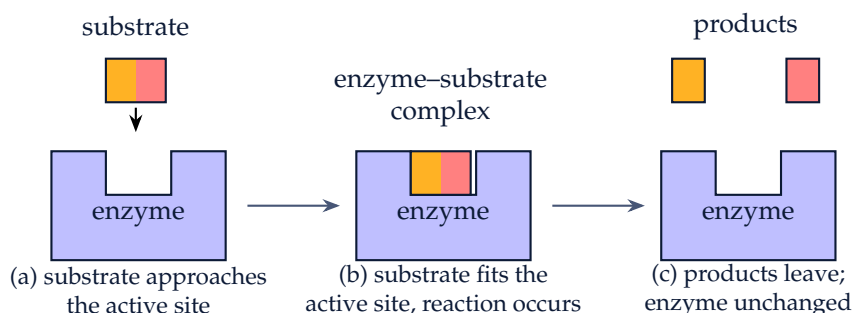
used again.

- Thinking an enzyme changes the final amount of product. It changes only the speed, if everything else stays the same.

2 The active site and specificity (B8.2)

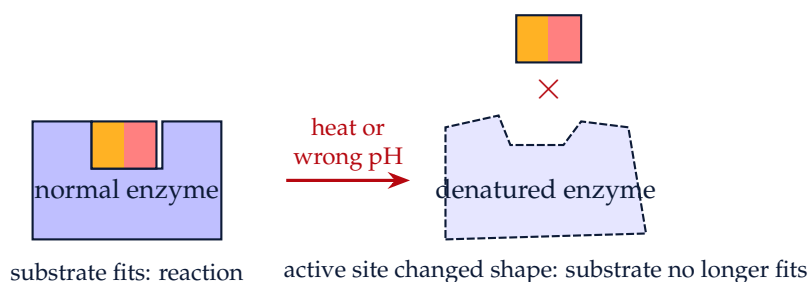
Every enzyme has a small region on its surface, the **active site**. The active site is a pocket or groove whose shape is set by how the protein chain folds. The substrate molecule binds to the active site and the reaction takes place there.

Lock and key. Each enzyme has a single, particular shape of active site, and it fits only substrates of a matching shape, like a key that fits only one lock. This is the **lock-and-key** model: the substrate fits the active site, forming an **enzyme–substrate complex**; the reaction happens; the products leave, and the enzyme is free to take another substrate molecule.



We say each enzyme is **specific** for its substrate. This matters because a cell contains thousands of different molecules and reactions: because each enzyme fits only its own substrate, each reaction can be controlled separately. An amylase molecule will digest starch but cannot digest protein, because protein does not fit its active site. (A more refined picture, the *induced-fit* model, says the active site changes shape a little as the substrate binds, to grip it more tightly, but the main idea, a specific match between shape of substrate and active site, is the same.)

Denaturation. The shape of the active site depends on the shape of the whole protein, and the protein is held in shape by weak attractions between different parts of the chain. If the temperature is too high, or the pH is far from the enzyme's optimum, these weak attractions are broken, the protein unfolds and changes shape, and the active site is distorted. The substrate no longer fits, the enzyme stops working, and we say it is **denatured**. Denaturation is usually permanent: a boiled egg does not become raw again when it cools.



Worked example: explaining why an enzyme stops working

A student heats a solution of amylase to 90°C for five minutes, cools it, and adds it to starch. No starch is digested. Explain why.

The high temperature broke the weak attractions that hold the amylase in its folded shape. The protein unfolded and the shape of its active site changed (it was *denatured*). On cooling the original shape does not come back, so starch no longer fits the active site and no enzyme–substrate complex forms.

Exam tip

For a "why does the enzyme stop working?" question, always give the chain: *change in temperature or pH* ⇒ *shape of the active site changes* ⇒ *substrate no longer fits* ⇒ *no enzyme–substrate complexes*. Say *active site*, not just "the enzyme".

is damaged”.

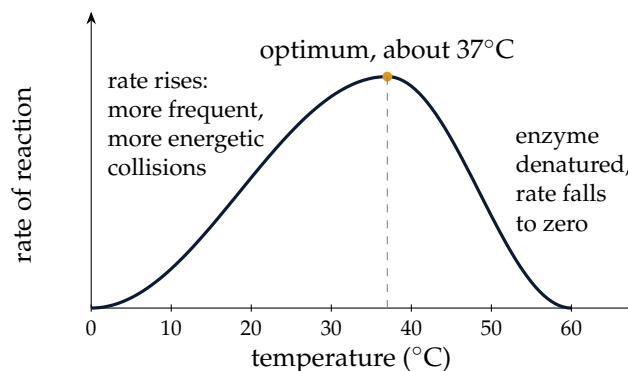
Common mistakes

- Saying the *substrate* is denatured. Only the enzyme (a protein) is denatured.
- Saying the enzyme “dies” or is “killed” by heat.
- Saying a cold enzyme is denatured. Cold slows the reaction but does not change the shape of the enzyme, so it works again when warmed.

3 Temperature, pH and concentration (B8.3)

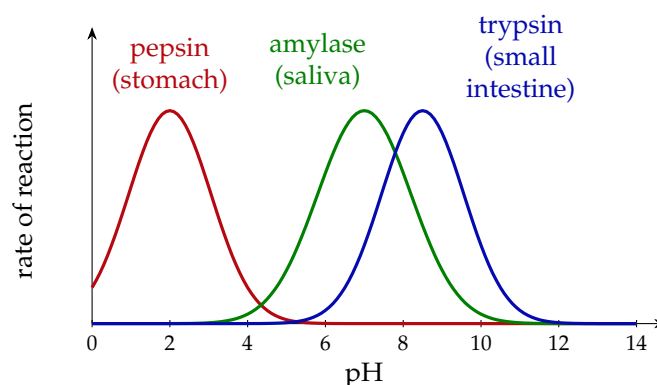
The rate of an enzyme-catalysed reaction is how quickly substrate is used up or product is made. Four things change it.

(a) Temperature. For a reaction to occur, enzyme and substrate molecules must collide, with the substrate entering the active site. As temperature rises, the molecules move faster, so they collide more often and with more energy: the rate rises. But above a certain temperature the enzyme begins to denature, so fewer active sites have the right shape, and the rate falls, quickly reaching zero. The temperature giving the highest rate is the **optimum temperature**. For most human enzymes it is about 37°C.



Notice the asymmetry: the rise is gradual, but the fall is steep, because once the enzyme has begun to denature, more and more of it is damaged for every further degree. Below the optimum the enzyme is merely slow (and recovers on warming); above it, it is destroyed.

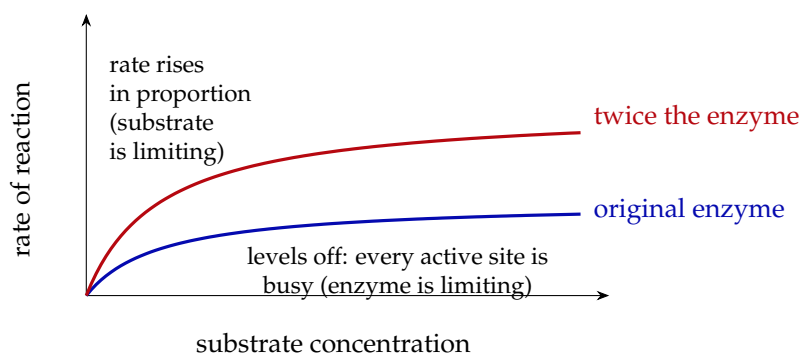
(b) pH. The pH measures how acidic or alkaline a solution is: below 7 is acidic, above 7 alkaline, and 7 neutral. Extreme pH values break the attractions holding the enzyme in shape, so each enzyme has an **optimum pH**, and the rate falls on both sides of it. Different enzymes have different optima, matching where they work: pepsin works in the stomach (very acidic, about pH 2), salivary amylase in the mouth (about neutral) and the protease trypsin in the small intestine (slightly alkaline, about pH 8).



So the stomach’s acid is not harmful to pepsin; it is exactly what pepsin needs. But the same acid denatures salivary amylase, which is one reason starch digestion by saliva stops once food reaches the stomach.

(c) **Substrate concentration.** With a fixed amount of enzyme, increasing the substrate concentration first increases the rate in proportion, because collisions between enzyme and substrate become more frequent. But then the graph levels off. At that point every active site is occupied all the time, so the enzyme is working at its maximum rate, and more substrate cannot make it faster. The *enzyme*, not the substrate, is now the limiting factor.

(d) **Enzyme concentration.** With plenty of substrate, doubling the enzyme concentration doubles the number of active sites and so doubles the rate (the top curve in the graph below). When substrate runs out, though, extra enzyme makes no difference to the *total* product, only to how quickly it is made.



Worked example: reading rate from a graph of product against time

In an experiment the mass of product is measured at intervals: 0 g at 0 min, 6 g at 2 min, 10 g at 4 min, 12 g at 6 min and 12 g at 8 min. Find the rate in each interval and explain the pattern.

Rate = $\frac{\text{change in product}}{\text{time}}$. First 2 min: $6 \div 2 = 3$ g/min. Next 2 min: $(10 - 6) \div 2 = 2$ g/min. Next: $(12 - 10) \div 2 = 1$ g/min. Last: 0. The rate falls because the substrate is being used up, so there are fewer substrate molecules to collide with enzymes; at the end the substrate has run out and the graph is flat.

Worked example: the limiting factor

At low substrate concentration, doubling the enzyme concentration has little effect on a particular reaction; at high substrate concentration it doubles the rate. What is the limiting factor in each case?

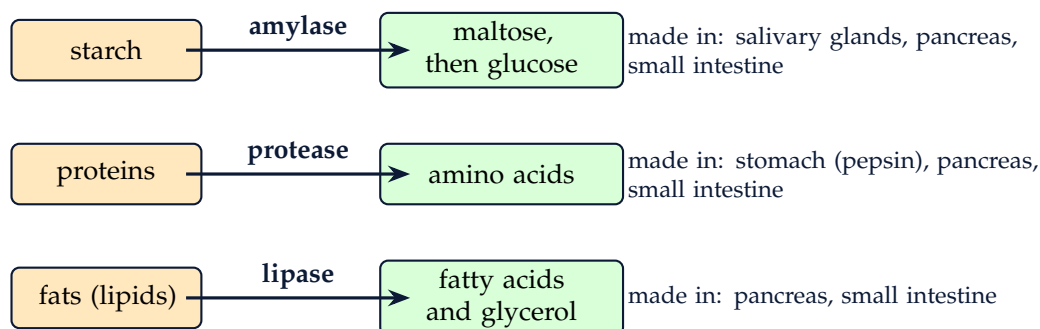
At low substrate concentration there are few substrate molecules, so active sites often stand empty: *substrate* is limiting and extra enzyme can do nothing useful. At high concentration, every active site is already busy: *enzyme* is limiting, so more enzyme means more active sites and a proportionally faster rate.

Common mistakes

- Writing that the enzyme is “denatured” at low temperatures. It is merely slowed.
- Believing every enzyme has an optimum pH of 7, or an optimum temperature of 37°C. Pepsin’s optimum pH is about 2; enzymes of heat-loving bacteria work well at over 70°C.
- Explaining the plateau as “the substrate is used up”. In a plateau at high concentration, the enzyme active sites are all occupied; the substrate has *not* run out.

4 The digestive enzymes (B8.4)

Food contains large molecules (starch, proteins, fats) that are too big to cross the wall of the gut and enter the blood. **Digestion** breaks them into small soluble molecules that can be absorbed, and enzymes do the breaking. You need three groups.



- **Amylase** breaks down *starch* (a carbohydrate) into the sugar maltose, which another enzyme then splits into glucose. Salivary glands make it (it begins starch digestion in the mouth), and so do the pancreas and the small intestine.
- **Protease** breaks down *proteins* into *amino acids*. The stomach makes the protease pepsin, which works at acidic pH; the pancreas and small intestine make proteases that work in the slightly alkaline small intestine.
- **Lipase** breaks down *lipids* (fats and oils) into *fatty acids and glycerol*. It is made by the pancreas and the small intestine.

Bile. Bile is made by the liver, stored in the gallbladder and released into the small intestine. It is *not* an enzyme: it contains no protein catalyst. It does two jobs. It is alkaline, neutralising the acid from the stomach so that the enzymes of the small intestine have a suitable pH, and it *emulsifies* fats, breaking large fat droplets into tiny ones. Tiny droplets have a much bigger total surface area, so lipase can reach more fat at once and digestion is faster.

Why does digestion matter? Glucose (from starch) is used for respiration, amino acids are rebuilt into the body's own proteins, and fatty acids and glycerol are used to build cell membranes and to store energy.

Worked example: surface area and bile

A fat droplet is a cube of side 2 mm. Bile breaks it into 8 cubes of side 1 mm. By what factor does the total surface area, which lipase can attack, increase?

One big cube: area = $6 \times 2^2 = 24 \text{ mm}^2$. Eight small cubes: $8 \times 6 \times 1^2 = 48 \text{ mm}^2$. The area doubles, so the rate of fat digestion by lipase roughly doubles. (Splitting into much smaller droplets gives a much bigger increase.)

Common mistakes

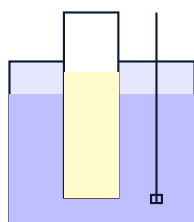
- Saying bile contains enzymes or digests fat chemically. It *emulsifies* fat; lipase does the digesting.
- Mixing up substrate and product: amylase → sugars, protease → amino acids, lipase → fatty acids *and* glycerol.
- Forgetting that digestive enzymes work *outside* cells, in the gut cavity, even though cells make them.

5 Investigating enzymes

You may be asked to design an experiment, or to interpret one. The logic is always the same: change *one* thing (the **independent variable**), measure the effect on the rate (the **dependent variable**), and keep everything else the same (the **control variables**).

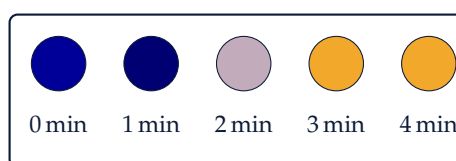
An amylase and starch experiment. Starch gives a *blue-black* colour with iodine solution; when starch has been digested to sugar, the iodine stays orange-brown. So we mix starch and amylase in a tube in a water bath, take a sample every minute and drop it into iodine on a spotting tile. The time at which the iodine first stays orange-brown tells us how long it took to digest all the starch.

tube: starch + amylase



water bath at 37°C

spotting tile: iodine added to a sample each minute



blue-black = starch present; orange-brown = no starch left

Turning a time into a rate. The faster the reaction, the *shorter* the time taken, so the rate is proportional to $\frac{1}{\text{time}}$. Doubling the speed halves the time. That is why we use

$$\text{rate} = \frac{1}{\text{time taken}} \quad \text{or} \quad \text{rate} = \frac{\text{amount of product (or substrate used)}}{\text{time}}$$

To investigate temperature, we would repeat with water baths at several temperatures; to investigate pH, we add buffer solutions of different pH. **Controls:** the volumes and concentrations of starch and amylase, the temperature (when testing pH) and the pH (when testing temperature). A good test also has a *negative control*: for example a tube with boiled amylase, which should show no digestion, proving that the enzyme and not something else caused the change.

The lipase experiment. Lipase breaks fat into fatty acids, which lower the pH. Mix milk (which contains fat), a little sodium carbonate solution (alkaline) and the indicator phenolphthalein (pink in alkali, colourless below about pH 8–9): the mixture is pink. Add lipase: as fatty acids form they neutralise the alkali, so the pink fades. The time to go colourless shows the rate. Adding bile salts speeds it up by emulsifying the fat.

Worked example: rate as 1/time

Starch takes 120 s to be digested at 20°C and 40 s at 30°C. Compare the rates.

Rate = 1/time: $\frac{1}{120}$ and $\frac{1}{40}$ per second. The ratio is $\frac{1/40}{1/120} = 3$. The reaction is three times as fast at 30°C. (Do not say “it took 3 times as long”: the *time* fell to a third.)

Worked example: doubling per ten degrees

Suppose the rate of a reaction doubles for each 10°C rise (true over part of the range below the optimum). It takes 160 s at 20°C. How long at 40°C?

From 20 to 40°C is two steps of 10°C, so the rate doubles twice: $\times 4$. The time falls to a quarter: $160 \div 4 = 40$ s.

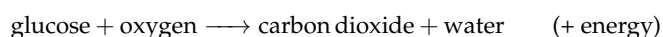
Common mistakes

- Concluding that a certain pH or temperature is the exact optimum when you only tested a few values: you can only say the optimum is *near* the best one tested.
- Saying a faster reaction has a *longer* time. Rate and time are inversely related.
- Changing two variables at once (e.g. different pH *and* different volumes), so that the results cannot be attributed to one cause.

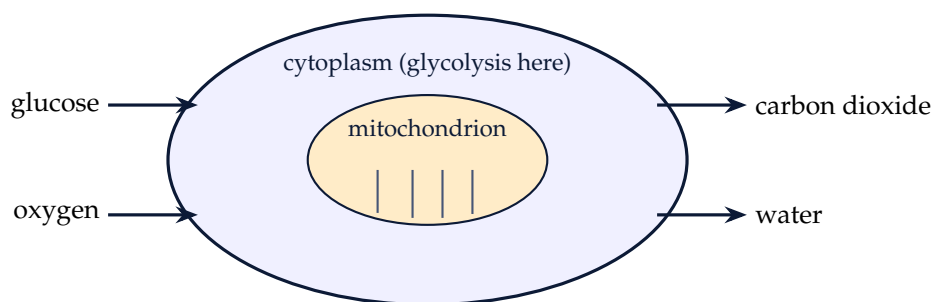
6 Aerobic respiration (B9.1)

Cells need a constant supply of energy: to contract muscles, build proteins, move substances across membranes against a concentration gradient, make heat to keep the body warm, and so on. **Respiration** is the chemical reaction in cells that releases energy from nutrient molecules such as glucose. It is an *exothermic* reaction (it gives out energy). It is not the same as breathing (*ventilation*), which only moves air in and out of the lungs. Respiration goes on in every living cell, all the time, in plants and animals alike.

Aerobic respiration uses *oxygen*. Glucose is broken down completely to carbon dioxide and water:



In symbols, $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \longrightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$. (Check that it balances: 6 carbon atoms on each side; 12 hydrogen atoms on each side; $6 + 12 = 18$ oxygen atoms on the left, $12 + 6 = 18$ on the right.) The first stage takes place in the cytoplasm, and the later stages, which release most of the energy, take place in the **mitochondria**. This is why cells with a high energy demand, such as muscle cells and sperm cells, contain very large numbers of mitochondria.



ATP. The energy released is not used directly. It is used to build a small molecule called **ATP** from ADP and phosphate. ATP is the cell's energy currency: it is cheap to make, carries energy to where it is needed and releases it when it breaks down back into ADP and phosphate. Respiration and ATP are two halves of a cycle.



Uses of the energy. (i) Muscle contraction; (ii) building large molecules such as proteins from amino acids; (iii) active transport, moving substances against a concentration gradient; (iv) keeping a constant body temperature in mammals and birds; and, in plants, building sugars and proteins and absorbing minerals.

Gases. Aerobic respiration takes in oxygen and gives out carbon dioxide. So exhaled air contains less oxygen and more carbon dioxide than inhaled air (roughly 16% oxygen rather than 21%, and about 4% carbon dioxide instead of 0.04%).

Worked example: how many oxygen molecules?

Using $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \longrightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$, how many molecules of carbon dioxide are produced when 50 molecules of glucose are respired aerobically, and how many molecules of oxygen are needed? Each glucose gives 6 CO_2 and needs 6 O_2 . So 50 glucose give $50 \times 6 = 300$ CO_2 and need 300 O_2 . Aerobic respiration takes in as many oxygen molecules as it gives out carbon dioxide molecules.

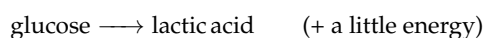
Common mistakes

- Writing "respiration = breathing". Breathing is a mechanical process; respiration is chemistry inside cells.
- Thinking plants do not respire, or respire only at night. Plants respire all the time; in daylight, photosynthesis hides it.
- Saying ATP *is* energy. It is a molecule that carries energy and releases it when broken down, like a charged battery.
- Putting the equation backwards: oxygen is a reactant (used) and carbon dioxide and water are products.

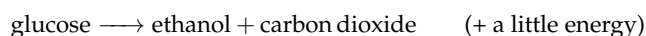
7 Anaerobic respiration and exercise (B9.1)

Anaerobic respiration is respiration without oxygen. The glucose is only partly broken down, so *much less energy* is released per glucose molecule: about 2 molecules of ATP, compared with about 30 for aerobic respiration. It happens entirely in the cytoplasm.

In muscles (animals). During hard exercise the heart and lungs cannot deliver oxygen to the muscles as fast as they use it. The muscle cells then supplement their energy supply by respiring anaerobically:

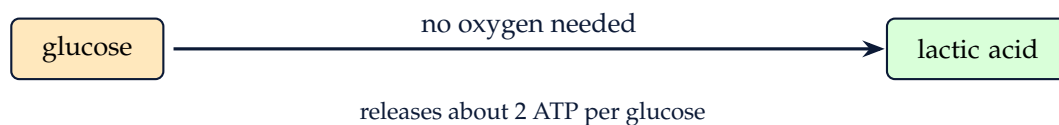


In yeast (fermentation). Yeast is a single-celled fungus. Without oxygen it makes *ethanol* and carbon dioxide:

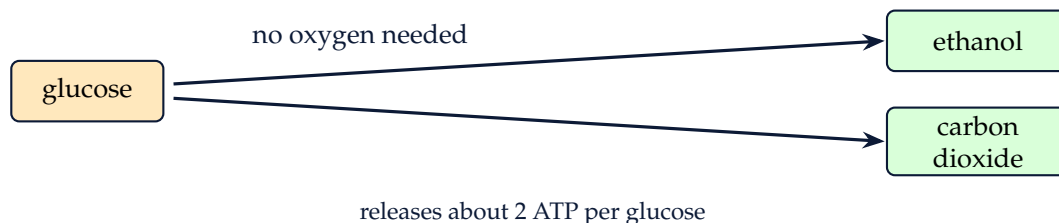


This is the basis of brewing and bread-making: in beer the alcohol is wanted, in bread the carbon dioxide is wanted (it forms bubbles that make the dough rise, and the ethanol evaporates as the bread bakes).

(a) in muscle cells (animals)

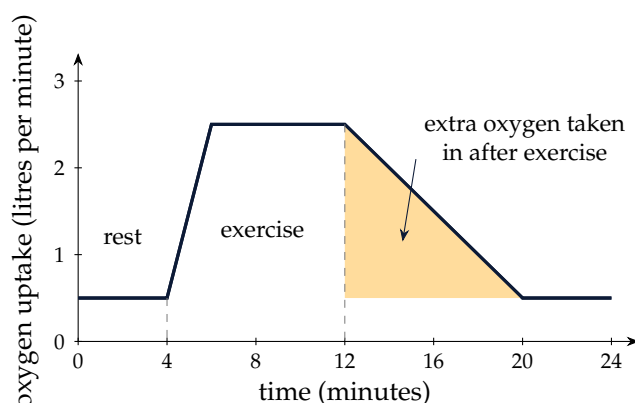


(b) in yeast (fermentation)



Plants too can respire anaerobically (for example roots in waterlogged soil) and the products are ethanol and carbon dioxide, as in yeast: in this respect plants resemble yeast, not animals.

Oxygen debt. Lactic acid builds up in muscles during hard exercise; it is harmful if it accumulates (it causes the muscles to fatigue and ache). It is carried in the blood to the liver, where it is converted back to glucose, or broken down using oxygen. After the exercise stops you therefore keep breathing hard and your heart rate stays high for a while: you are taking in extra oxygen to break down the lactic acid. The extra oxygen needed to do this is called the **oxygen debt**.



Exercise also changes the body in other ways: heart rate and breathing rate increase, to supply more oxygen and glucose to the muscles and to remove more carbon dioxide. Anaerobic respiration is a short-term solution only: it gives a quick but small amount of energy, and the lactic acid limits how long it can last.

Worked example: how much extra glucose without oxygen?

A cell needs 120 units of ATP. Assume aerobic respiration yields 30 ATP per glucose molecule and anaerobic respiration yields 2. How many glucose molecules are needed in each case?

Aerobic: $120 \div 30 = 4$ glucose. Anaerobic: $120 \div 2 = 60$ glucose. So the anaerobic route needs 15 times as much glucose, 56 extra molecules. This is why anaerobic respiration is wasteful, and why it is only used when oxygen is short.

Worked example: reading an oxygen-uptake graph

In the oxygen-uptake graph, at rest 0.5 litres of oxygen are used per minute. After the exercise stops at 12 min the uptake falls steadily back to the resting value at 20 min, starting from 2.5 litres per minute. How much *extra* oxygen is taken in during recovery?

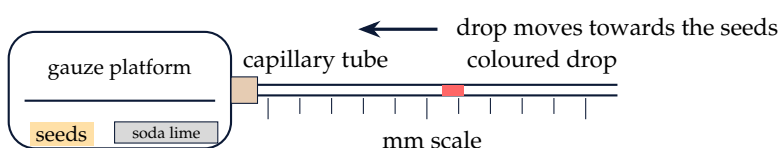
The extra oxygen is the area between the graph and the resting level. It is a triangle with base $20 - 12 = 8$ minutes and height $2.5 - 0.5 = 2.0$ litres per minute. Area = $\frac{1}{2} \times 8 \times 2.0 = 8$ L. (The mistake is to measure the height from zero, which counts the resting uptake as well.)

Common mistakes

- Writing that lactic acid is made in yeast. Yeast makes ethanol and carbon dioxide; muscles make lactic acid only.
- Saying anaerobic respiration releases *no* energy. It releases a small amount.
- Saying the muscles produce lactic acid “because they have run out of glucose”. It is the lack of *oxygen* that matters.
- Believing that oxygen debt is repaid before the exercise stops: it is repaid afterwards.

8 Measuring respiration and putting it together

Measuring the rate of aerobic respiration. A *respirometer* measures how quickly living material takes in oxygen. Germinating seeds (or small invertebrates) sit in a sealed tube above a pellet of soda lime (or a small dish of sodium hydroxide) that absorbs the carbon dioxide they produce. So the only gas change is that oxygen is used up and the pressure falls. A drop of coloured liquid in a capillary tube is drawn towards the tube, and the distance it moves in a given time is a measure of the oxygen used.



The capillary tube is a thin cylinder, so **volume of oxygen used** = $\pi r^2 \times \text{distance moved}$, where r is the internal radius of the capillary. Then the rate is this volume divided by time (and, to compare different organisms fairly, by their mass). **Controls:** a second tube with dead (boiled) seeds or glass beads, and a water bath to hold the temperature constant, because temperature changes the volume of gas as well as the rate of respiration.

Worked example: a respirometer calculation

The capillary tube of a respirometer has an internal diameter of 2 mm. The drop moves 24 mm in 8 minutes. Find the rate of oxygen use, in mm^3/min , leaving π in your answer.
 Radius = 1 mm. Volume = $\pi \times 1^2 \times 24 = 24\pi \text{ mm}^3$. Rate = $24\pi \div 8 = 3\pi \text{ mm}^3/\text{min}$ (about 9.4). Beware: using the diameter as the radius would give four times too much.

Putting it together: a checklist for the ESAT. (1) For an enzyme question, identify substrate, product and the shape of the active site, and say what has changed (temperature or pH, or concentration of something). (2) For a graph, name what the axes are, decide which part of the curve is being asked about, and say what is limiting. (3) For respiration, begin with the word equation, decide whether oxygen is present, and give the correct products. (4) For rates, use $1/\text{time}$ or $\text{change} \div \text{time}$, and watch units.

Exam tip

If a question says “*explain*”, give the cause in terms of molecules: collisions, active sites, shape, oxygen supply. A bare statement of the trend is only half the marks.

Common mistakes

- Using the diameter in πr^2 in a respirometer calculation.
- Leaving out the control (boiled enzyme or dead seeds), so that the change cannot be linked to the living material.
- Confusing the substance absorbed in the respirometer: soda lime absorbs carbon dioxide, so the movement shows *oxygen* used.

Check yourself

Try these without looking back, then compare with the answers on the next page.

1. What is an enzyme, and what happens to it during a reaction?
2. Explain why amylase cannot digest protein.
3. Why does the rate of an enzyme reaction fall to zero at 80°C?
4. A graph of rate against substrate concentration levels off. Explain.
5. Name the products of digestion by amylase, protease and lipase.
6. Reaction A takes 50 s and reaction B takes 200 s. How many times faster is A?
7. Write the word equation for aerobic respiration, and state where most of the energy is released.
8. Why do you keep breathing hard after you have stopped sprinting?

Answers to the self-check

1. A protein that acts as a biological catalyst, speeding up a reaction. It is not used up: it is released unchanged at the end of the reaction.
2. The active site of amylase has a shape that fits starch; protein has a different shape and does not fit, so no enzyme–substrate complex forms. The enzyme is specific.
3. The enzyme is denatured: the shape of the active site has changed so the substrate no longer fits.
4. All the active sites are occupied at the same time, so the enzyme is working at its maximum rate and the enzyme concentration is now the limiting factor.
5. Sugars (maltose, then glucose); amino acids; fatty acids and glycerol.
6. $\text{Rate} = 1/\text{time}$, so the ratio is $200 \div 50 = 4$: A is four times faster.
7. $\text{glucose} + \text{oxygen} \rightarrow \text{carbon dioxide} + \text{water} (+ \text{energy})$. Most is released in the mitochondria.
8. To take in extra oxygen to break down the lactic acid made in anaerobic respiration (repay the oxygen debt).

Next step

Heurisco's *ESAT Biology: Enzymes and Respiration Practice Set* has twenty original exam-style questions on this lesson, with graphs and apparatus diagrams, in order of difficulty, with a full worked solution, a common-trap note and the specification code for every question.