Bio Factsheet

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Nitrogen Fixation

Every living organism needs nitrogen to make nucleic acids and proteins. But before atmospheric nitrogen can be used, the strong triple bond between the two nitrogen atoms has to be broken. In nature, there are two things that can do this – lightning and certain bacteria. This Factsheet summarises:
- how bacteria do this
- the biological implications of this process
- the way in which this topic is examined

First, test your understanding:

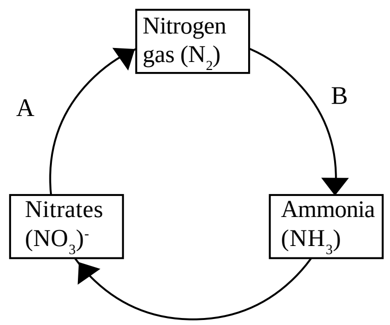
Which of these statements are true?
- Nitrifying bacteria are involved in nitrogen fixation
- Ammonification is the same as fixation
- Nitrogen fixation only occurs in root nodules
- Nitrogen fixation requires ATP so nitrogenase works best when there is plenty of oxygen
- Nitrogen fixation involved the conversion of “nitrogen in lightning” to nitrates
- Fixation is the conversion of nitrogen to proteins

All false, and all mentioned by Chief Examiners in recent Exam reports as being common misconceptions. If you knew they were all false, you are already a nitrogenous expert, otherwise, read on…….

Nitrogen fixation is the conversion of gaseous nitrogen into ammonia

Fig 1 shows part of the nitrogen cycle.

Fig 1



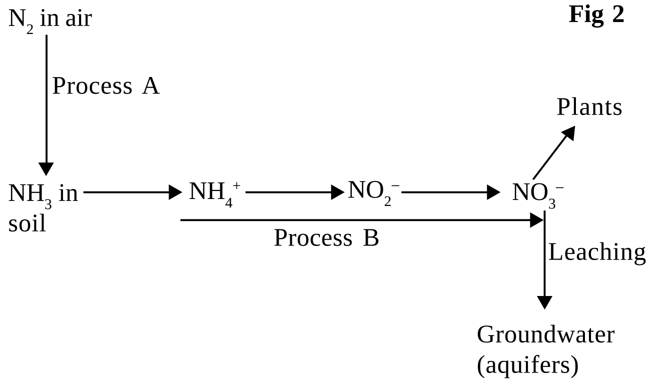
A is denitrification and B is fixation. This is a surprisingly common exam question!

Extract: Chief Examiner’s report
More candidates were able to identify process A as denitrification than were able to identify process B as nitrogen fixation. Nitrification was a common wrong answer, as was ammonification. This process refers to the conversion of nitrogen in **organic compounds** to ammonia.

Annually, 2×10^8 tonnes of nitrogen gas is turned into ammonia. But 3×10^9 tonnes of ammonia are converted to nitrate. Where does the extra ammonia come from? The decay and decomposition of proteins, DNA and urea.

Fig 2 shows a bit more of the nitrogen cycle.

Fig 2



- Learn the chemical symbols of each of the forms of nitrogen.
- Nitrogen (N_2) gas is converted into ammonia (NH_3) by bacteria
- The ammonia is converted into ammonium ions (NH_4^+)
- The ammonium ions are converted into nitrite ions by bacteria such as ***Nitrosomonas***
- The nitrite ions are converted into nitrate ions by bacteria such as ***Nitrobacter***

These last two conversions are called **nitrification**. Note that the ammonium ions have been oxidized (hydrogen has been removed, oxygen added). Plant root hairs can absorb nitrate ions and the plant can then use them to make amino acids, hence proteins.

Nitrogen fixing bacteria

Many types of bacteria can fix nitrogen (Table 1).

Table 1. Nitrogen fixing bacteria

Free living		Symbiotic with plants	
Aerobic	**Anaerobic**	**Legumes**	**Other plants**
Azotobacter *Beijerinckia* *Klebsiella* (some) Some cyanobacteria	*Clostridium* (some) *Desulfovibrio* Purple sulphur bacteria Purple non-sulphur bacteria Green sulphur bacteria	*Rhizobium*	Frankia Azospirillum

All nitrogen fixers have one thing in common –they use the enzyme nitrogenase. Nitrogenase is composed of two proteins. This enzyme is inactivated by oxygen because this reacts with the iron component of one of these two proteins so the bacteria have to ensure that oxygen is excluded. This is achieved in different ways in different organisms.

Bacteria of the genus ***Rhizobium*** secrete substances into the soil that stimulate roots of leguminous plants (eg peas, beans, clover) to form nodules. The bacteria enter the nodules which oxygen cannot enter or enters very slowly. The bacteria can then fix nitrogen. In return, they are able to use some of the plant's sugars. The bacteria do need a source of oxygen – they have to aerobically respire – but they do this by binding oxygen very tightly to leghaemoglobin, which has a huge affinity for the gas. Interestingly, leghaemoglobin is found only in the nodules and is not produced by either the bacterium or the plant when grown alone.

Azotobacter species protect their nitrogenase by having extremely high rates of metabolic activity. This might protect the enzyme by maintaining a very low level of oxygen in their cells. *Azotobacter* species also produce large quantities of an extracellular polysaccharide slime layer. This slows down the diffusion of oxygen into the cells.

The aquatic fern *Azolla* is the only fern that can fix nitrogen. It can do so because a prokaryote – Anabaena – lives inside its leaves (Fig 3)

Termites require a large amount of protein in their diets, but they feed on wood which is low in protein. However, they benefit from the presence of mutualistic, spirochaete bacteria which live in their guts. These bacteria are able to fix nitrogen, taking carbon from the termites in return.

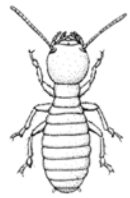
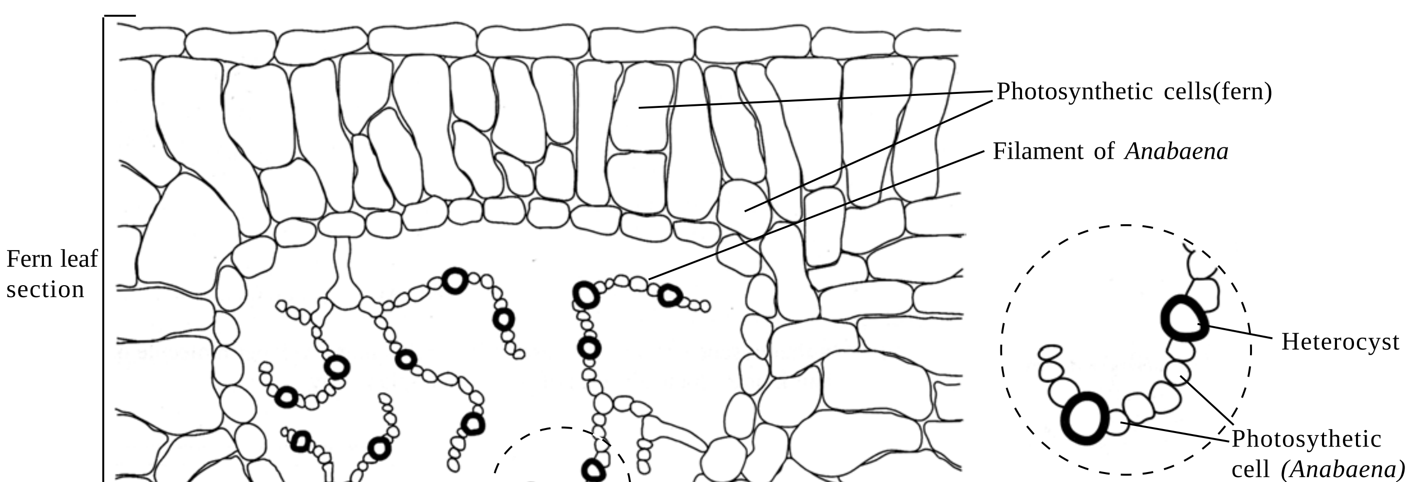


Fig 3. Anabaena in fern leaf



Nitrogen fixation occurs in special cells (heterocysts). Heterocysts only possess photosystem I (which just generates ATP) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water in order to supply H_2 for reducing carbon dioxide).

Significance of fixation

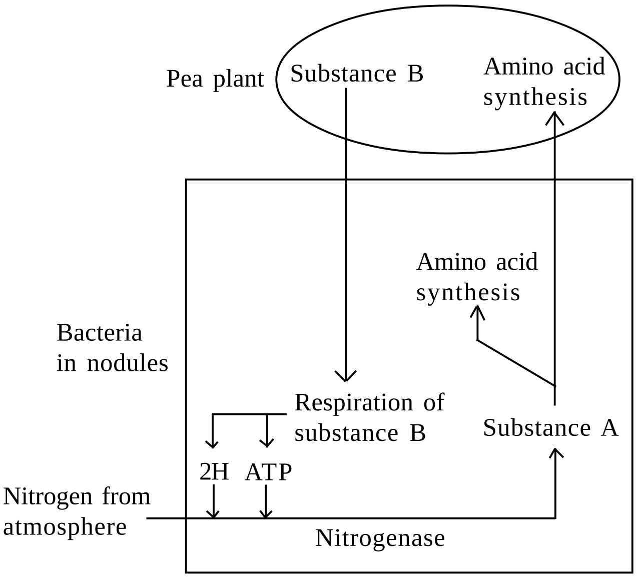
It is commonly believed that all legume crops are a GOOD THING because they fix nitrogen. Then, when we plough the crop residue back in, the net effect is that we have added nitrogen to the soil. This is not always the case. Even soyabeans may be net removers of nitrogen. It has been estimated that a good soyabean crop which yields 2.5t per hectare will incorporate in its grain, straw and pods, about 200kgN per hectare. But it will have fixed only 90kg N per hectare. The rest has to come from the soil, either from natural stores or from fertilizers.

Generally though legumes are valuable. Beans, peanuts and chickpeas do not usually produce a net N loss – they fix more than is removed at harvest. It is also the case that ploughing in legume residues reduces the need for artificial nitrogen fertilizers that are energy intensive to produce. It also adds nitrogen in a complex form which is less likely to leach than, for example, nitrogen fertilizers, thus reducing problems such as eutrophication.

In China, ferns containing the nitrogen – fixing Anabaena are cultivated and ploughed back into the fields to act as an organic fertilizer. The residues are broken down by decomposers. Proteins, amino acids and other nitrogen – containing organic compounds are broken down, slowly releasing nitrates back into the soil. These are then available for next season's crop.

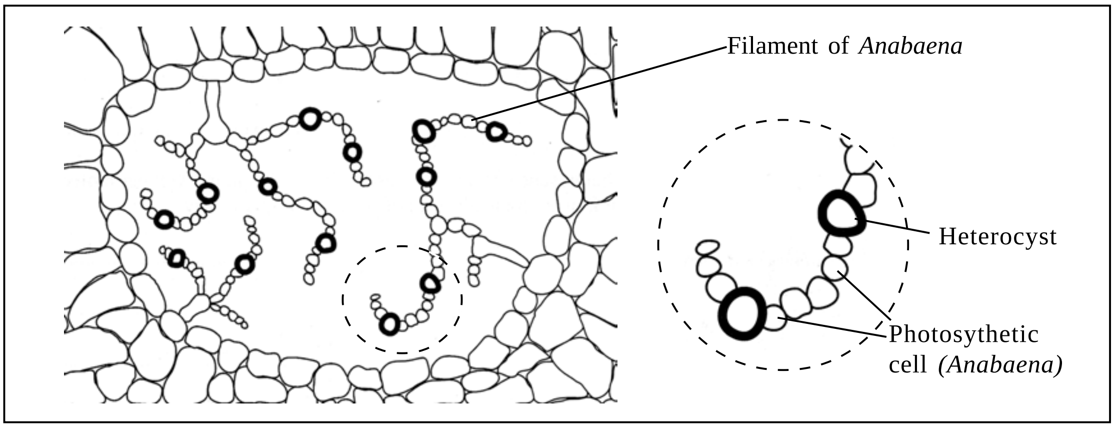
Typical exam Questions

1. The diagram shows some of the processes involved in nitrogen fixation by bacteria inside root nodules of a leguminous plant.



(a) Name substances A and B
(b) It is hoped that, someday, nitrogen – fixing genes can be transferred into crop plants. Outline the potential benefits that this would bring.
(c) Sodium ions act as a non-competitive inhibitor of the enzyme nitrogenase. Explain how sodium ions would affect the activity of nitrogenase.

2. The diagram shows Anabaena, a prokaryotic nitrogen – fixing organism living inside the leaves of ***Azolla***, a fern which is found on the surface of water in ponds or slow-running streams.



(a) What is the significance of each of the following:
(i) heterocysts are thick walled and have no chloroplasts
(ii) *Azolla* can grow in nutrient-deficient water
(b) Suggest why nitrogen – fixing plants are often found in nitrogen – deficient soils but are less commonly found in fertile soils

Answers

1. (a) A = ammonia B = glucose
(b) Greater food production;
Crop growth in nitrogen –deficient soils;
Less need for N fertilisers/less pollution/eutrophication/ ref to Haber process;
(c) Sodium ions bind to enzyme at a site other than active site;
this changes the shape of the active site;
the shape of the active site is no longer complementary to the substrate/nitrogen;
nitrogen cannot bind to the nitrogenase;
enzyme-substrate complexes no longer formed;
fixation stops;

2. (a) (i) Nitrogen fixation occurs inside the heterocysts;
Thick walls stop oxygen entering;
Oxygen inactivates nitrogenase;
Chloroplasts would photosynthesise/release oxygen;
(ii) Fern can obtain nitrogen from Anabaena;
(b) It takes a lot of ATP to fix nitrogen;
In nitrogen –deficient soils there is a competitive advantage in using ATP in this way;
But not in fertile soils;

Acknowledgements:
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