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## Urine helps plants grow

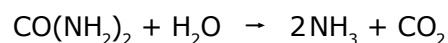
Urease from soya beans and the nitrogen cycle

### Aim

An inexpensive qualitative demonstration of urease activity.

### Introduction

In this investigation, the enzyme urease, from soya beans (*Glycine max*), breaks down urea to ammonia and carbon dioxide:



Ammonia solution has a high pH, which can be detected using a simple pH indicator, such as that obtained from red cabbage. The ammonia produced by the reaction can also be smelt. It is of course necessary to compare the colour of the indicator mixed with urea solution and soya bean extract with the colour produced by 'controls' which contain only urea or soya bean extract, so this procedure could also provide an introduction to experimental design.

This practical activity can be used to support teaching about the:

- nitrogen cycle — how the nitrogen from mammalian urine can be used by other organisms;
- influence of organisms on their environment — how organisms make their environment more suitable for them (*e.g.*, the bacterium *Helicobacter pylori*, responsible for stomach ulcers, raises pH of its host's stomach juice by excreting urease);
- adaptation of animals to different diets — in a ruminant's stomach, bacterial urease helps the ruminant to use nitrogen from urea;
- general nature of enzymes — proteins catalysing biochemical reactions and the factors influencing enzyme activity (*e.g.*, temperature, pH, the enzyme, substrate and product concentrations).



*Soya plant*

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## Equipment and materials

Needed by each person or group

Equipment

- Pasteur pipettes or plastic drinking straws, 4, for transferring the solutions
- Test tubes, 6 and test tube rack; or Petri dishes, 6
- Glass rod or plastic drinking straw, for mixing the solutions
- Water-resistant marker pen

Materials

- 10 % solution of urea fertilizer, 20 mL
- 10 % solution of citric acid, 5 mL (a low pH solution)
- 10 % solution of sodium bicarbonate ( $\text{NaHCO}_3$ ), 5 mL (a high pH solution)
- 40 mL of red cabbage indicator, prepared as described below
- 10 mL of soya bean extract, prepared as described below

### Soya bean extract (containing urease)

Because it is difficult to blend small volumes of beans, prepare the suspension for the entire class. This can be done entirely in advance by the teacher, or the final blending and filtering can be done as a demonstration in front of the class, or by one group of students. Pre-soak the beans in water for at least an hour (or preferably, overnight) before you prepare the extract. The quantities given here are enough for 30 students working in pairs.

Figure 1.

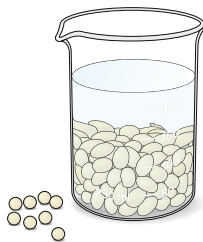
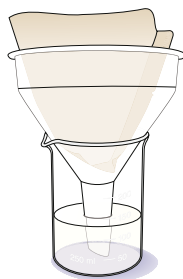


Figure 2.



Figure 3.



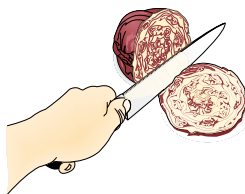
1. Soak ~ 20 g of soya beans in water. Fig. 1.
2. Put the soaked soya beans in a food blender with ~200 mL of water (the water from soaking the beans can be used). Fig. 2.
3. Blend for ~5 minutes or until the mixture is smooth.
4. Place a coffee filter paper in a funnel. Fig. 3.
5. Filter the soya puree — collect and keep the filtrate (this will contain the urease).

*Note: If the soya extract is prepared by students themselves, then provide about 6 pre-soaked beans for each student or group — the beans can be ground using a mortar and pestle, then filtered.*

### Red cabbage indicator

This can be made in advance by the teacher if desired or by individual students or groups. The quantities given here are enough for 30 students working in pairs.

Figure 4.



1. Cut three large red cabbage leaves to strips (10–20 mm wide). Fig. 4.
2. Place the leaves in a 1 litre beaker or other heat-resistant container and pour on 600 mL of freshly-boiled water. Ensure that the leaves are soaked completely. Fig. 5.
3. After 5 minutes decant the liquid into another beaker and leave it to cool.

Figure 5.



*Note: If the extract is prepared by students themselves, then provide one small red cabbage leaf and ~100 mL of hot water for each student or group.*

### Procedure

Depending on the age of participants, the time available etc. the solutions, red cabbage indicator and soya bean extract can be prepared by the students themselves or in advance by the teacher or technician. The volumes given below are for standard (~10 ml) glass test tubes. When using other containers, please adjust the volumes and try the experiment in advance.

Figure 6.



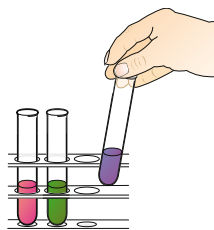
### Step 1: Demonstration of pH indicator from red cabbage

1. Label 3 tubes for A(cid), B(asic) and N(eutral) solutions with labels or a water-resistant marker.
2. Add 3 mL of red cabbage indicator to each tube. Fig. 6 and 7.
3. Add 2 drops of citric acid solution to test tube A, mix and observe the colour change.
4. Add 2 drops of sodium bicarbonate solution to test tube B, mix and observe the colour change.
5. Add 2 drops of distilled water to test tube N, mix and observe the colour change.
6. Compare and note the colours of the solutions in all three test tubes. Put the tubes to one side to act as a reference. Fig.8.

Figure 7.



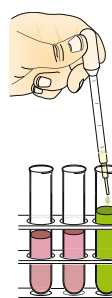
Figure 8.



### Step 2: Effect of urease from soya beans on urea

1. Label 3 tubes U(rea), S(oya bean urease), U+S (urea and soya bean urease) with labels or water-resistant marker.
2. Add 2 mL of red cabbage extract to each tube. Fig 9.
3. Mix 2 mL of urea solution into each of the tubes marked U and U+S. *Notice the colour – what is the approximate pH of the urea solution?*
4. Mix 2 mL of the soya suspension into each of the test tubes marked S and U+S.
5. Compare the colour and odour of the mixtures in tubes marked U, S and U+S.
6. After 3 minutes compare tubes U, S and U+S with test tubes A, B and N from the Step 1. Approximately what pH is the liquid in each of the tubes?

Figure 9.





### **Safety guidelines**

Approximately 8% of children may be allergic to soya bean extract. Teachers are advised to check that none of their students are affected before attempting this protocol.

### **Preparation and timing**

The experiment can be completed in an hour. We suggest that students work in pairs or small groups.

The soya bean suspension and red cabbage extract can be prepared by the teacher in advance or their preparation demonstrated during the lesson. This takes only 10 minutes, but note that the soya beans must be soaked in water for at least 1 hour before the lesson.

Step 1: Red cabbage extract as a pH indicator: 10 minutes.  
Step 2: Reaction between urease from soya seeds and urea: 10 minutes.

### **Troubleshooting**

The pH indicator does not change colour

- The soya beans were heat-treated (*e.g.*, dried in hot air). Try soya beans from another source.
- The pH was out of pH indicator's range. We recommend red cabbage extract as a pH indicator, because it will work at a wide range of pHs.

The pH indicator changes colour very slowly

- Too little enzyme was available — soya wasn't mashed enough or too little of the soya suspension was used.

The pH indicator changes colour too rapidly

This can raise pH of the fertilizer enough to turn the colour of the pH indicator. Try lowering the pH of the urea solution with citric acid (or use an alternative pH indicator).

## Open-ended investigations

- Try adding 2–3 unsquashed (but soaked) soya beans to a 2 mL of urea solution mixed with an equal volume of red cabbage extract. Wait minimum 20 minutes. Does the the colour of the mixture change?
- How does the pH of the solution influence urease activity?
- Does high temperature treatment of the soya beans (*e.g.*, boiling them) influence enzyme activity? Try to run the experiment with cooked soya beans (few minutes cooking is enough).
- Try other urea sources (urine, animal fertilizer)
- Test whether the seeds of other plants can also be used as urease sources. Try other legumes or jack beans (*Canavalia ensiformis*). Pumpkin seeds also contain urease, but these should be dried at room temperature before use.
- Try other pH indicators, with different sensitivity ranges or measure the pH with an electronic pH probe.

## Suppliers

Soya beans, red cabbage, citric acid and sodium bicarbonate (baking soda) should all be available from a supermarket. Urea fertilizer is sold in garden centres and is inexpensive or at chemical suppliers as karbamid.

## Storage of materials

Soaked soya beans can be stored in room temperature overnight or for 24 hours in a fridge at 3–5 °C. Red cabbage extract can be stored for 2–3 days in the fridge and for least 2 months in a domestic freezer (-18 °C). Red cabbage leaves may also be frozen.

## Waste and recycling of materials

In the version of the experiment described above, all the waste material is safe and can be discarded in the normal waste or liquids can be washed down a toilet or sink.

## Remarks and alternative materials

### Soya

The soya beans must be soaked before the experiment. When soaking, the seeds change their shape from round to oval. The soya beans must be raw — commercially available seeds are not usually heat-treated, but we recommend that seeds from a given batch are checked before trying this experiment with a class. Instead of soya beans, one can use jack beans, *Canavalia ensiformes*. Soaked seeds can be ground with a fork and bowl instead of blender, but this process is laborious.

## pH indicators

Red cabbage extract can also be prepared from frozen cabbage (2–3 leaves can be stored in a freezer for spring and early summer use when red cabbage is not readily available). Liquid from pickled red cabbage is also an effective pH indicator. In this case, as it is acidic (red in colour), the solution drained from the cabbage should be neutralised before use with sodium bicarbonate solution until it is blue-violet. Laboratory pH indicators such as phenolphthalein, bromothymol blue or litmus may also be used. Phenolphthalein's colour transition point is pH 8.2–10; bromothymol blue changes colour at pH 6.2–7.6.

## Solutions with different pHs

Citric acid and baking soda can be replaced with others solutions with pH lower than 6 and higher than 8 (*e.g.*, vinegar may be used as the acidic solution). If strong acids or alkalis are to be used, ensure that the appropriate safety procedures are followed.



## Acknowledgement

This practical protocol was adapted for the Volvox project, which is funded under the Sixth Framework Programme of the European Commission.

## Additional information

### What is urea?

Every organism decomposes nucleic acids and proteins. This process generates nitrogenous waste, because nucleic acids and proteins contain nitrogen. Mammals, amphibians and some invertebrates excrete nitrogenous waste as urea. Urea is produced in the liver and is water-soluble. Human urine contains 2% urea.

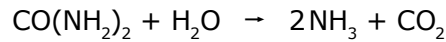
### Urea — the first organic compound ever synthesized

In 1828 Friedrich Wöhler synthesized urea from organic compounds. This was a landmark achievement, because until then it was thought that only living organisms could produce organic compounds, and that these compounds were special and required a 'vital force' to make them. Wöhler bridged the divide between the living and non-living worlds.

He didn't receive a Nobel Prize for his discovery, because the Nobel Prize did not exist at this time! Today urea is synthesized in vast quantities: it is used to make plastics and as a cheap nitrogenous fertiliser.

### What is urease?

Urease is an enzyme (I.U.B 3.2.1.81 in international enzyme classification), which breaks up urea to ammonia and carbon dioxide:



### What happens in the experiment?

Urease from soya seeds breaks up urea to ammonia and carbon dioxide. Ammonia has a specific smell and raises pH of the solution. This change in pH causes the pH indicator to change colour.

### Is urease in soya beans harmful to humans?

Urease is not harmful. However, raw soya beans contain other compounds which are unhealthy. For example, there is a protein inhibitor in raw soya beans that prevents the digestive enzyme trypsin from working and makes raw soya beans inedible. The presence of the inhibitor is not easy to detect, but by chance it is susceptible to inactivation by heating, exactly as urease is. Therefore, to ensure that the inhibitor is inactivated, commercial preparations of soya beans (soya flour or foods that contain soya, like tempe, tofu *etc.*) are tested for urease activity — in just the same way as the test described here.

### Why is there urease in soya beans?

That's not clear at the moment. Soya leaves also contain urease, but at a much lower concentration (100–1 000 times less) than soya beans. Leaf urease helps to recycle nitrogen from proteins (the proteins are broken down to urea); urease in the beans does the same when the beans germinate. Ammonia in the plant cells may also protect them from pathogens.

### Urease — the first enzyme ever crystallised

Urease from jack beans (*Canavalia ensiformis*) was the first enzyme ever purified and crystallised. James B. Sumner did this in 1926, at a time when most scientists believed that it was impossible to crystallise enzymes. For this achievement Sumner received the Nobel Prize for Chemistry in 1946. Today, crystallisation of proteins helps scientists to discover their structure and to determine how they work. This permits the design of substances which interfere with enzyme action, such as the anti-AIDS drugs which inhibit the action of HIV's enzymes.

### How does urease help to make our urine useful?

Nitrogen is a crucial element for plant growth. Most plants can only use nitrogen in the form of ammonium or nitrate. Only legumes (thanks to the bacteria they live in symbiosis with) and cyanobacteria can use nitrogen from the air.

Many animals excrete urea. Soil microorganisms produce urease and transform urea from urine to ammonia, which is readily-accessible to plants. This is part of the nitrogen cycle, the process by which the nitrogen from proteins and other compounds is constantly recycled.

#### Where else can be urease found?

Many species of bacteria produce urease, including *Helicobacter pylori*, the bacterium responsible for stomach ulcers. By doing this, *Helicobacter* raises the pH of the gastric juice from pH ~3 to pH 7, the optimal pH for its growth. A commercially-available test for *H. pylori* tests for the presence of urease in the breath. Cellulose-digesting bacteria, which live in the first compartment of the stomachs of ruminants (e.g., cows and sheep) also excrete urease. Ruminants excrete urea into this part of the stomach, which makes an excellent source of nitrogen for bacterial growth. The animal digests the bacterial mass. Many other bacteria excrete urease — that's why fresh urine, which is more-or-less odourless, becomes smelly after a while.

#### Red cabbage extract – great pH indicator

Red cabbage extract contains anthocyanins. These compounds structure are pH sensitive and then their colour in solution can change colour. At pH 7 the solution is violet/blue; in the acidic range it turns red. When the pH raises above 7 and the solution is more alkaline and the extract turns green. These colour changes are reversible — just check what happens when you add citric acid and baking soda one after another.