Diffusion in a gel

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Most teachers will be familiar with the common experiment for showing diffusion in a 'solid' where a coloured crystal such as copper(ii) sulfate is placed on some gelatine. The diffusion is slow, the material has to be left overnight to achieve a notable effect. In the spring of 2001, I was able to benefit from a Science and Plants for Schools (SAPS) Schoolteacher Fellowship at Robinson College Cambridge. I carried out practical research in the Department of Plant Sciences into the transformation of cyanobacteria, in an attempt to transfer herbicide resistance. As part of this activity I investigated methods of selecting cyanobacteria using an agar growth medium. The intention was to add the selective agent (herbicide) to agar in the base of a Petri dish and then add an established culture of cyanobacteria on a disc of agar on top of this. To investigate the efficiency of diffusion, I carried out a trial using agar plus acid in the bottom layer and overlaid this with a disc of agar containing bromocresol green. I was surprised at the speed of diffusion. A clear colour change was visible after 5 minutes.

Method

**Bottom layer:** 1.5% agar in water, to which was added 1 cm$^3$ of 1 mol dm$^{-3}$ hydrochloric acid per 20 cm$^3$ of dissolved agar (see technical note below).

**Top layer:** 1.5% agar in water to which bromocresol green solution had been added at a ratio of 1 cm$^3$ per 20 cm$^3$ agar.

The bromocresol green solution was 50 mg per 20 cm$^3$ water with 2 drops of 1 mol dm$^{-3}$ sodium hydroxide solution added to produce a blue colour.

The different layers were poured into separate Petri dishes, either 20 cm$^3$ per 9 cm dish or 10 cm$^3$ per 6 cm dish, and allowed to set. Once set, a spatula was used to carefully lift the blue agar out of one dish and place it on top of the acidified agar in the other dish.

A change of colour from blue to green was visible almost immediately and a yellow colour was quite obvious within 5 minutes. If there are areas where contact between the two discs of agar is not close then these change later, but the decrease in size of the original coloured area is easily observable in a short time span. These concentrations look very dark (when viewed using an OHP), and the changes are more difficult to follow compared with observing the agar above a white surface. A lower concentration of bromocresol green might give better results with the OHP.

Looking at the agar from the side will show the vertical diffusion.

Other investigators have had success with Universal Indicator and bromophenol blue, and other indicators may work well, but the quantities of indicator stated above may need to be adjusted to give a colour intensity which works well.

**Safety**

*Bromocresol green:* solid bromocresol green carries the following safety warning:

**Harmful if swallowed or inhaled, may cause irritation to skin, eyes, and respiratory tract.**

If this is done as a class experiment, the amounts used by each pupil group would be very small. Particular care should be taken when weighing out the powder and using a stock solution.

The amounts of sodium hydroxide and hydrochloric acid used are also small but suitable eye protection should be worn by those making up the gels and students should use eye protection when carrying out the experiment.

If the agar solution is made using a microwave care should be taken to avoid the contents boiling over. Extreme care must be taken when removing the container from the microwave in case superheating causes the contents to boil over whilst being removed.

**Technical note**

The agar will not set if the acid is added to the agar before the agar is dissolved by boiling. Best results are obtained by cooling the dissolved agar to about 50 °C and then adding the acid, just before pouring into the Petri dish.

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