ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

LABORATORY NOTES:

Phenolphthalein/NaOH agar cube experiment to model the effect of surface area to volume ratio on rate of diffusion in cells

The impact of cell size on diffusion can be modelled with a simple experiment using different size cubes of agar containing a pH indicator. The agar cubes represent biological cells. The volume of the cube correlates to the cytoplasm and the surface area of each cube to the cell membrane.

All living cells are dependent on diffusion processes for survival. These processes transport materials across the cell membrane into and out of the cell. As the volume of the cell increases, the surface area per unit volume decreases. Knowledge of the relationship between the size (volume) of cells and their surface area helps explain the process of diffusion.

Agar blocks and cells with the largest surface area to volume ratio (the smaller cubes) have the highest diffusion rates.

The following method gives reliable results.

Phenolphthalein – sodium hydroxide agar

Materials

- Plain agar powder 20g
- Distilled/deionised water 900mL
- Phenolphthalein indicator 2.0% w/v solution in 60% ethanol (See instructions below)
- 100mL of 1M Sodium hydroxide solution (4g NaOH dissolved in 100mL distilled/deionised water)
- Magnetic stirring/heating platform and magnetic stirring bar
- Straight-sided tray for setting gel. Suitable trays are the ‘Dabco Unitrays’, preferably without the divider, or other suitably sized trays such as ice cube trays.
- 1mL pipette

For student use

- Plastic spoon
- 250mL beakers
- Scalpel/flat blade knife
- Diffusing solution: 0.1M hydrochloric acid or 0.1M sulfuric acid
- Timer
- Clear plastic ruler that measures in mm
- Disposable nitrile gloves
- Paper towel

Method:

- Add 20g of plain agar powder slowly to 880/890mL of distilled/deionised water whilst stirring constantly. (This is best done by stirring the water using a magnetic stirring bar on a magnetic heater/stirrer and slowly adding the agar to the water. Bring to the boil, and then simmer for a few minutes until completely dissolved. Remove off the heating platform and place on a heatproof mat. N.B keep covered with foil to prevent a skin forming.)
- When the agar has cooled to approximately 60°C add 100mL of 1M sodium hydroxide solution, stirring constantly (stir using the magnetic heater/stirrer again without the heat). The final concentration of sodium hydroxide is 0.1M. **Make sure that you wait until the agar has cooled to just below 60°C (and well prior to setting which is about 40°C) before adding the sodium hydroxide solution.**
- Add 10-20mL of 2.0 % Phenolphthalein indicator quickly whilst stirring constantly, **until the agar is a deep pink colour.** Note it may be necessary to add more Phenolphthalein indicator depending on the intensity of colour required. Be aware, however, that the ethanol may affect the firmness of the agar.
- Pour into a shallow tray to a depth of >30 mm and allow to set.
- Cut the agar into 1, 2 and 3cm cubes using a scalpel blade and/or flat blade knife. 1 cube of each per group.
- Add cubes to a beaker containing enough diffusing solution of either 0.1M hydrochloric or 0.1M sulfuric acid to completely cover all the agar cubes.
- Periodically turn the cubes over and stir with the plastic spoon while timing how long it takes for the different size blocks to decolourise. The change from pink to colourless indicates the extent to which the acid (hydrogen ions) has diffused into the agar cubes.
- Determine the surface to volume ratio for each cube and relate to the time taken for each block to decolourise.
- Alternatively: when the first agar block clears completely remove all the blocks from the acid with the spoon, rinse the agar blocks quickly with water and pat dry with paper towel. Working quickly cut the blocks in half and measure the depth of the clear layer in millimetres in each block. This is the depth that the acid has penetrated each block.
- Determine the surface to volume ratio for each cube and relate to the time taken for each block to partially decolourise.

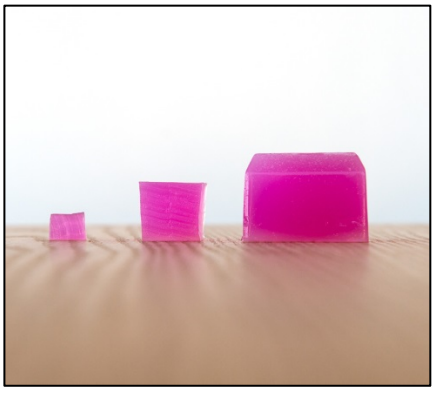

Image attribution: Exploratorium Teacher Institute

Additional tips:

- Increasing the amount of agar from 20g/L to 25g/L achieves a firmer gel.
- When completely cool, agar can be covered with cling wrap and stored in the fridge for several days before use.

Safety considerations:

- Consult safety data sheets for all chemicals used.
- Wear appropriate Personal Protective Equipment (PPE) – safety glasses and nitrile disposable gloves.
- Agar that contains sodium hydroxide is alkaline and has a soapy feel. Wear disposable gloves when handling.
- Sodium hydroxide and acid solutions are corrosive. Wear safety glasses to protect the eyes and disposable gloves to protect the skin.
- Phenolphthalein solution is a flammable liquid and should be kept away from heat and sources of ignition preferably in a flammable liquids cabinet.
- Phenolphthalein indicator can be prepared in house taking into account the safety considerations below. Alternatively, it can be purchased ready made from various scientific suppliers.

Waste disposal

Rinse the agar cubes with water to dilute and remove residual acid or alkali and dispose of into the rubbish bin.

Neutralise the acid diffusion solution, using a base such as sodium carbonate, and dispose of to waste water.

Preparation of phenolphthalein indicator

- When preparing indicators, such as phenolphthalein, it is essential to observe safe handling practices to minimise exposure to any dust.
    - Wear PPE: safety glasses, gloves, laboratory coat and closed in shoes. Wear a dust mask, or work in a fume cupboard that is not turned on, with the sash lowered, to minimise exposure to any dust. Position an electronic balance in the fume cupboard. If working outside a fume cupboard, make sure you work in a draft free area.
    - Carry out any transfers of the powder in a shallow tray in the fume cupboard. The tray will contain any spills of the powder.
    - After the solution has been prepared, switch the fume cupboard on. With damp paper towel, wipe down any surfaces which may be contaminated with the powder
- When preparing the phenolphthalein indicator, it is best to dissolve the solid phenolphthalein in the ethanol first due to its low solubility in water and then make up to the required volume.
- For this activity a solution of 2% phenolphthalein in 60% ethanol is recommended to enable a deep pink colour. i.e. 2g phenolphthalein dissolved in 60mL of ethanol and then made up to 100mL with distilled water.
- This stock solution could be further diluted to produce a general-purpose indicator of 0.1% phenolphthalein in 60% ethanol, by taking 5mL and diluting up to 100mL with 60% ethanol. [or 5mL of 2% phenolphthalein in 60% ethanol + 57 ml ethanol and 38 mL distilled water]

Alternative methods:

There are variations in the literature for cell diffusion experiments, however Science ASSIST has not trialled them. Here we provide links from two reputable sources for those who wish to trial a different method.

- 'Effect of size on uptake by diffusion', Nuffield Foundation website, http://www.nuffieldfoundation.org/print/3128 (Accessed November 2018)

  (**Note**: *This method uses universal indicator in place of phenolphthalein*)

- Flinn Scientific. 2018. *Diffusion in Agar Cells*. Flinn Scientific website, https://www.flinnsci.com/api/library/Download/7ab4bee9010448c4a2732ef34c570c6b

  (**Note**: *This method uses sodium hydroxide as the diffusing solution)*

References:

'Agar Cell Diffusion: Biology and Chemistry Science Activity', Exploratorium website. https://www.exploratorium.edu/snacks/agar-cell-diffusion (Accessed November 2018)

Chem-supply. 2016. *AGAR 1000 Gel*, Safety Data Sheet, Chem-supply website, https://www.chemsupply.com.au/documents/AL0271CH0M.pdf

Chem-supply. 2016. *Phenolphthalein*, Safety Data Sheet, Safe Chem-Supply website, https://www.chemsupply.com.au/documents/PA0331CH52.pdf

Chem-supply. 2016. *Phenolphthalein Solution 0.5%*, Safety Data Sheet, Chem-supply website, https://www.chemsupply.com.au/phenolphthalein-0-5-solution-in-50-ethanol-lr-pl101-500m

Chem-supply. 2017. *Hydrochloric acid 0.1 - <10.0%,* Safety Data Sheet, Chem-supply website, https://www.chemsupply.com.au/documents/HL0141CHC1.pdf

Chem-supply. 2018. *Sodium hydroxide 2-4% Solutions*, Safety Data Sheet, Chem-Supply website, https://www.chemsupply.com.au/documents/SL1321CHBZ.pdf

Dungey, Barbara. 2006. *The Laboratory: a science reference and preparation manual for schools* (Rev. ed), National Library of Australia: Traralgon, Vic.

'Effect of size on uptake by diffusion', Nuffield Foundation website, http://www.nuffieldfoundation.org/print/3128 (Accessed November 2018)

Flinn Scientific. 2018. *Diffusion in Agar Cells*. Flinn Scientific website, https://www.flinnsci.com/api/library/Download/7ab4bee9010448c4a2732ef34c570c6b

Labchem. 2013. *Sulfuric Acid, 0.2N (0.1M)*, Safety Data Sheet, Labchem website, http://www.labchem.com/tools/msds/msds/LC25700.pdf

Scharlau. 2013. *Phenolphthalein, solution 1% in ethanol, indicator*, Safety Data Sheet, Chem-supply website, https://www.chemsupply.com.au/documents/FE0496_AU.pdf

Scharlau. 2013. *Sodium hydroxide, solution 0,1 mol/l (0,1 N)*, Safety Data Sheet, Chem-Supply website, https://www.chemsupply.com.au/documents/SO0443_AU.pdf