The effect of nitrogen deprivation on the frequency of heterocyst occurrence in *Anabaena cylindrica* (cover pic)

Introduction

Anabaena are cyanobacteria which are capable of nitrogen fixation. They grow in long filaments of vegetative cells (Fig. 1). Irregularly spaced between the normal photosynthetic cells are colourless cells called heterocysts (Fig. 2). These heterocysts are able to carry out nitrogen fixation. The functions of photosynthesis and nitrogen fixation have to be kept separate due to the instability of the nitrogen-fixing enzyme, nitrogenase, in the presence of oxygen (a photosynthetic product).

Figure 1 - *Anaebena cylindrica* filaments in normal medium (x400 magnification). Note the absence of heterocysts.

In times of low environmental nitrogen levels, approximately 10% of normal cells will differentiate into heterocysts and thus lose the ability to photosynthesise. These heterocysts then supply neighbouring cells with fixed nitrogen in return for carbohydrate which they themselves are no longer able to produce.

To prevent entry of oxygen into the heterocysts, they have the ability to build three additional layers around their cell wall, giving them the appearance of being enlarged and rounded. (Fig 2).

Figure 2 - *Anaebena* species. The arrow is pointing to a heterocyst. From: http://botit.botany.wisc.edu/images/130/Bacteria/Cyanobacteria/Anabaena/heterocysts_dic.html

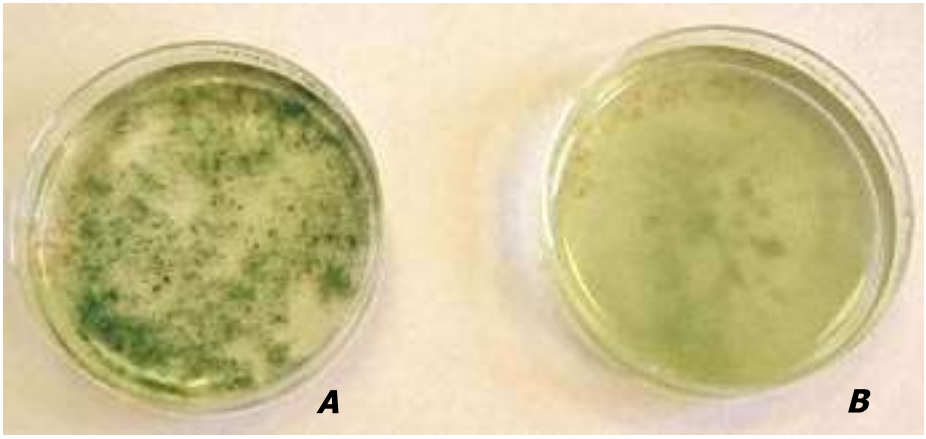


Figure 3a - Cultures of *Anaebena* left on a windowsill for three weeks.
A - Normal medium ***B*** - Nitrogen-deficient medium

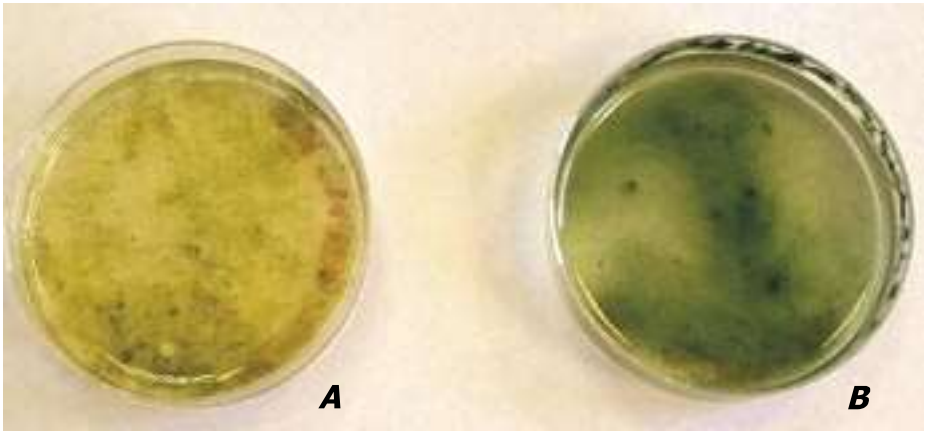


Figure 3b - Cultures of *Anaebena* left under a lightbank for three weeks.
A - Normal medium ***B*** - Nitrogen-deficient medium
Note that the *Anaebena* grown in normal culture medium (***A***) and left under the lightbank did not give particularly viable filaments after approximately three weeks growth.

The activity described below makes use of this easily observed difference between normal vegetative cells and heterocysts to compare heterocyst frequency in *Anaebena* grown in medium of normal nitrogen levels with that of *Anaebena* grown in nitrogen-deficient medium.

Preparing for the Activity

A culture of *Anabaena cylindrica* was obtained from Sciento[1]. The culture media were made up using Sach's tablets, one medium complete and one lacking nitrogen[2]. Ten cm^3 of each medium were made up, according to the accompanying instructions, and added to small plastic Petri dishes. Each dish was inoculated with 0.5 cm^3 of *Anaebena* using a sterile plastic pipette. These were than left either in direct sunlight on a windowsill or under a lightbank which was left on 24 hours a day[3]. Once a week, 1 cm^3 of the appropriate fresh medium was added to each culture (Figs. 3a & 3b). This ensured that the normal medium, in particular, did not become depleted of nitrogen.

Samples of the *Anaebena* from both cultures were observed at x400 magnification and the frequency of heterocysts per filament in normal and nitrogen-deficient medium was calculated.

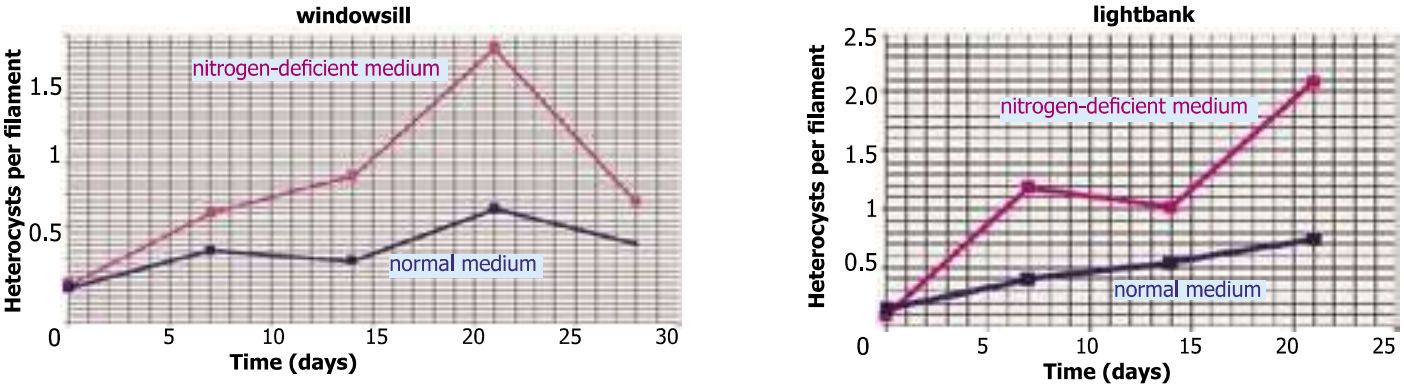
Results

As can be seen from the graphs in Figs. 4a and b the number of heterocysts per filament increased significantly in both normal medium and nitrogen-

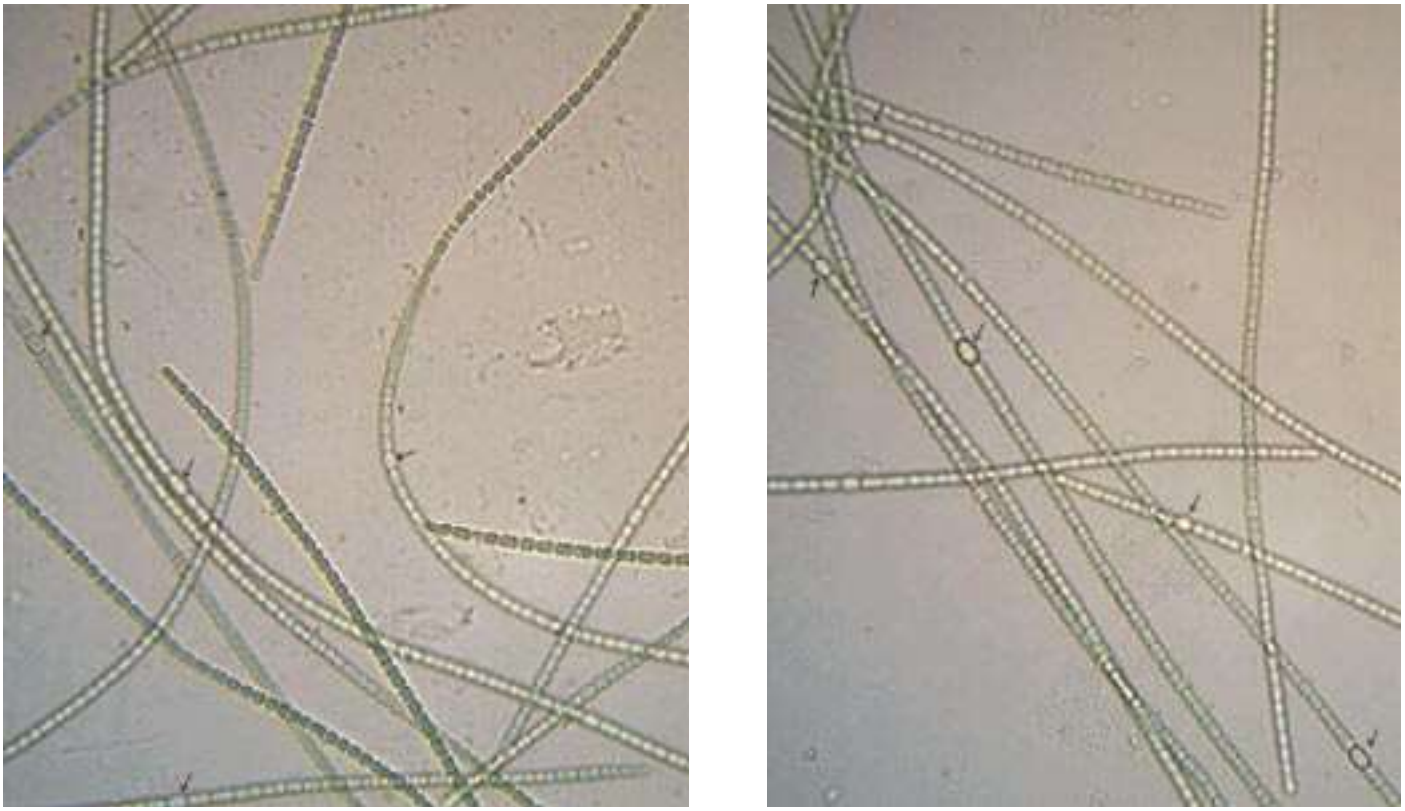
1 Sciento, 61 Bury Old Road, Whitefield, Manchester M45 6TB. Tel/Fax 01607736338 Email: sales@sciento.co.uk. Product No A490 £6.20

2 Supplied by Timstar Laboratory Suppliers Ltd, Timstar House, Marshfield Bank, Crewe, Cheshire CWL 8UY. Tel:01270 250459, Fax: 01270250601 Email: sales@timstar.co.uk. Product No PL3555, £19.50 per set

3 Light Bank versus windowsill? It was found that leaving the cultures on the windowsill provided viable cultures which lasted for longer (See Figs. 3a and 3b).



Figs 4a & b - Graphs showing the development of heterocysts in *Anaebena* cultured in Petri dishes kept on a windowsill (a) and a lightbank (b).



Figs. 5a & b - Anaebena filaments growing in normal medium from: (a) a one day old culture (left graphic) and (b) a 2 week old culture (right graphic). Arrows indicate heterocysts.

deficient medium, regardless of which light source was used. However, in both cases, *Anaebena* cultured in the nitrogen-deficient medium showed a far higher heterocyst to filament ratio than that in the normal medium after two to three weeks.

To make a one-off comparison of heterocyst frequencies in the two media, we would recommend the use of cultures which have undergone three weeks of incubation (Figs 5-6). After this time, the number of heterocysts per filament decreased in both types of media in the culture kept on the windowsill. As outlined earlier[3] the cultures kept under the light bank were not viable beyond the three-week incubation period and so no data was obtained for this time. When viewed under the microscope, the filaments often appeared very fragmented, and the heterocysts became difficult to identify.

A possible procedure for use in the classroom is as follows:

Pupil Procedures

Pupils could work individually on this activity if there are enough microscopes available.

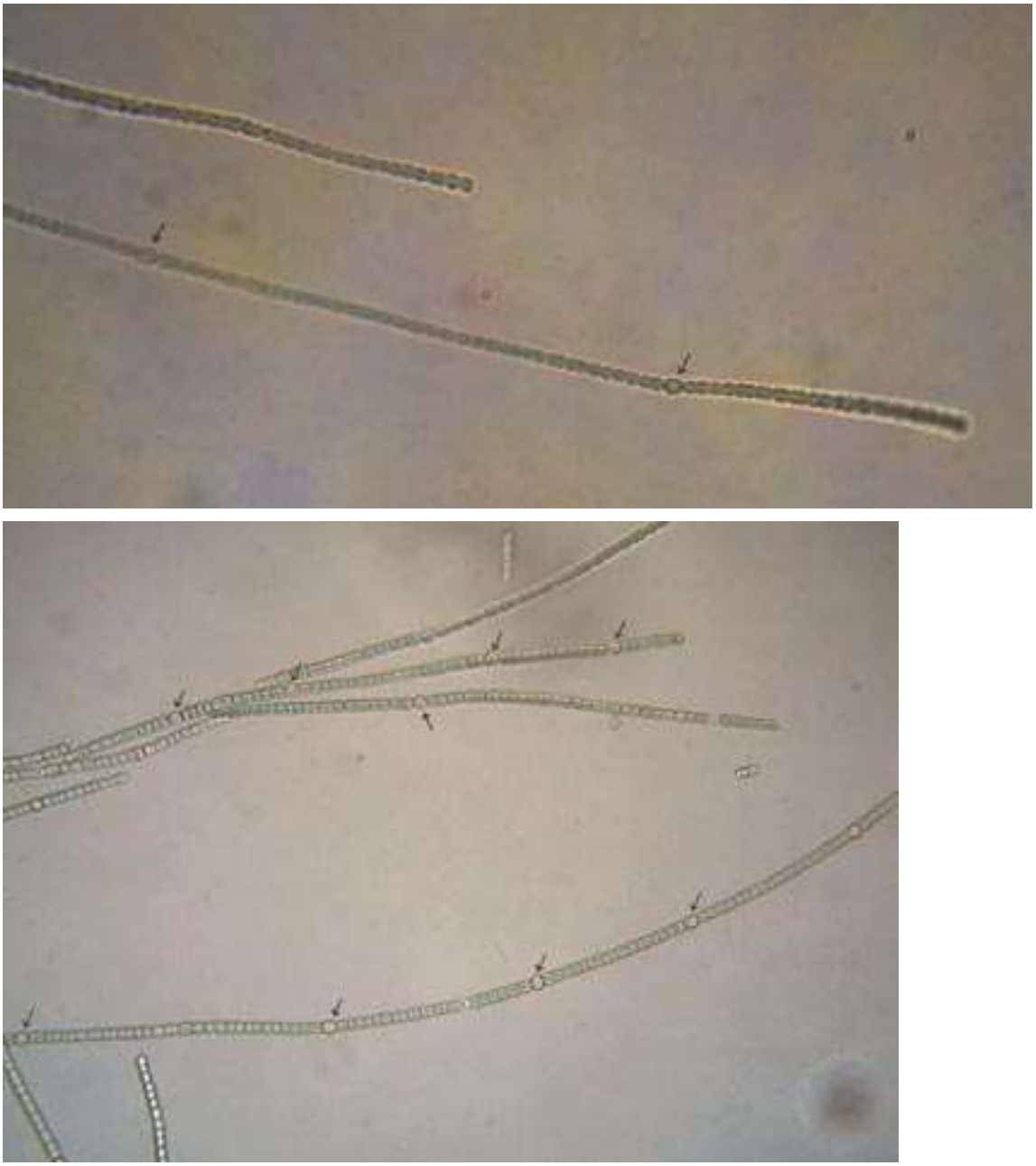
Materials for each pupil/group

2 microscope slides
2 coverslips
2 sample Petri dishes containing cultures of *Anabaena*, labelled ***A*** and ***B*** (one has been cultured with nitrogen in the medium, the other has no nitrogen - omit to tell pupils which is which so that they can work it out)
2 plastic pipettes

microscope (any microscope which has a x40 lens would be suitable)

Method

1. Take a drop of *Anabaena* from culture ***A*** using a pipette and place on a microscope slide.
2. Place a coverslip on top and view with a microscope using x400 total magnification.
3. Draw and label a typical filament, making sure that you can tell the difference between a normal cell and a heterocyst.
4. Count the number of filaments in the field of view. Then count the number of heterocyst cells in the same field of view.
5. Do this for at least five fields of view, noting down your results for each one in a table (or combine class results).
6. Repeat steps 1-5 for culture ***B***.



Figs. 6a & b - *Anaebena* filaments growing in nitrogen-deficient medium from: (a) a one day old culture (top graphic) and (b) a 2 week old culture (bottom graphic). Arrows indicate heterocysts.

7. Calculate the number of heterocysts per filament for each culture. Repeat this for the combined class results.
8. Decide which of the two cultures has been kept in nitrogen-deficient medium.

Application

The development of heterocysts in *Anaebena* under different environmental conditions can be easily studied in the classroom and may be of use in supporting the following areas of the Advanced Higher Biology curriculum:

- Environmental Biology Unit - The nitrogen cycle
- Cell and Molecular Biology Unit - Differentiation of cells
- Biotechnology Unit (optional) - Enhancing nitrogen fixing

It may also be used as an Advanced Higher investigation. Some suggestions for investigations are as follows:

- What is the time course for heterocyst development in nitrogen-rich compared with nitrogen-deficient media?
- Do deficiencies of other nutrients have similar effects?
- Is there a level of nitrogen which triggers off heterocyst production?

Use of this protocol may also stimulate class discussion on the following:

1. The mechanism by which the cells are differentiated into heterocysts (allowing revision of Higher work on cell differentiation and the switching on and off of genes)
2. How this ability may be of future potential use in agriculture (see references below).

References

http://www-biol.paisley.ac.uk/bioref/Eubacteria/Anabaena.html

http://microbewiki.kenyon.edu/index.php/Anaebena

Cover pic reproduced with kind permission of Mike Clayton, Department of Botany, University of Wisconsin - Madison

http://botit.botany.wisc.edu/images/130/Bacteria/Cyanobacteria/Anabaena/heterocysts_dic.html