How-To-Do-It

Using Commercial Fertilizers as Algal Media

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A very constructive experience for a beginning biology student could be experimentation with, or observations of, algae grown in the laboratory. Such algae can be used in exercises dealing with growth rates, pigment analyses or the effect of pollutants on aquatic systems (Darley 1982; Anonymous 1969). These photosynthetic microorganisms are found in local streams, ponds or lakes at all seasons, but often not in sufficient numbers to provide the instructor with possibilities for interesting laboratory experiences.

There are numerous formulations for defined media that would support growth of algae living in such habitats. However, preparation of defined freshwater media, e.g. Bristol's medium, could require considerable time or resources that were not available. Could a higher plant be used as a substitute? Hershey (1990) listed the components of two plant fertilizers (one of which was Miracle Gro® and the well-balanced higher plant nutrient solution, Hoagland. From this data it is apparent that Miracle Gro® was not formulated as a complete nutrient solution, but rather as an additive, for it has no calcium, magnesium or sulfur. Trainor, Wallett & Grochowski (1991) recently reported favorable results when Miracle Gro® was used for short-term culture of both marine and freshwater microalgae. Thus we felt that other plant fertilizers, which are readily available (even in many homes), easy to use and inexpensive, might also prove to be useful. This paper is a report of the successful use of such fertilizers.

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Methods

We experimented with Peters® 30-10-10 fertilizer (Milpitas, CA), Peters® 20-20-20 (Fogelsville, PA), Dyna Gro® (San Pablo, CA) and Miracle Gro® (Port Washington, NY), but again encourage experimentation with any convenient and successful higher plant fertilizer. Media for the growth of the algae were prepared by dissolving 100 mg of the individual fertilizer in a liter of distilled water. Dyna Gro®, available as a solution, was diluted so that the levels of nitrogen, phosphorus and potassium were similar to those of the other fertilizers (Table 1); the recommended dilution is 1:3000. We used full-strength Bristol's medium®, a defined algal medium (Egan & Trainor 1989), as one control (Table 2). Sterile solutions were prepared by autoclaving all flasks with media at one atmosphere (kg cm^{-2}) steam pressure for 20 minutes, which raised the temperature to 121° C. Two other choices seem reasonable: Either prepare the sterile media in a pressure cooker or use the solutions immediately after preparation without sterilization.

Algal growth was detected in two local bodies of water, Mirror Lake and Swan Lake, University of Connecticut, Storrs, CT. The cell number and a list of the genera present in the lakes are given in Table 3. Water from these lakes was used as inocula, as follows:

A series of replicate 50^{-} ml flasks with stainless steel caps, each with 20 ml of a particular medium, was inoculated with 1 ml of Swan Lake water. (Initial cell density for these flasks was 89 cells per ml.) Mirror Lake water was used for the inoculum in a second series. (Initial cell density was 313 cells per ml.) Duplicate flasks containing water with the resident population of organisms, but with no additives, from both Mirror Lake (6563 cells per ml) and Swan Lake (1875 cells per ml) were also used as controls (Table 3).

All flasks were incubated in fluorescent illumination at 100 μmol PAR m^{-2} s^{-1} at 25° C in a 15-hour light, 9-hour dark cycle. We determined the level of growth by using optical density (Sorokin 1973), i.e. absorbance at a spectrophotometer reading of 560 nm, as well as by direct cell enumeration. These absorbance measurements enabled us to detect increases in biomass within individual flasks, for a doubling of the figure for absorbance would indicate a two-fold increase in biomass.

Some Suggestions

Sufficient light intensity can be obtained by growing organisms in vessels positioned 9–36 cm from two (cool white or daylight) fluorescent tubes. Avoid placing flasks in direct sunlight or too close to the room heat source; temperatures above 35° C can be lethal. If constant temperature is not available, attempt to maintain cultures between 25–30° C. Organisms kept at very cool temperatures will merely grow more slowly.

Results

Growth occurred in all flasks except one control. Results (Figures 1 and 2) are presented as doublings of cell number from the initial cell densities, or the number of divisions necessary for the 89 (or 313) cells to achieve a population size of more than a million cells per ml. Organisms in control flasks of Swan Lake water, to which there were no additives, died within 2 weeks (Figure 2).

Although growth in Bristol's medium was usually superior, the plant fertilizers supported excellent growth (Figures 1 and 2). Flasks with either Peters formulation appeared greener

Table 1. The levels of nitrogen, phosphorus and potassium (mg l^{-1}) in four plant fertilizers when solutions contain 100 mg per liter of the commercial fertilizer. Levels for these three elements in Bristol's medium® are also given.

	mg/l		
Medium	*Nitrogen*	*Phosphorus*	*Potassium*
Miracle Gro®	15	13	12
Peters 30-10-10®	30	4.4	8.3
Peters 20-20-20®	20	8.7	17
Dyna Gro®	35	20	21
Bristol's®	41	53	34

Table 2. The levels in mg l^{-1} of the components of Bristol's medium®.

nutrient	*mg · l^{-1}*
$NaNO_3$	250.0
K_2HPO_4	75.0
KH_2PO_4	175.0
$MgSO_4 \cdot 7H_2O$	75.0
$CaCl_2 \cdot 2H_2O$	26.5
$FeCl_3 \cdot 6H_2O$	5.0
$MnCl_2 \cdot 4H_2O$	0.3
$CoCl_2 \cdot 6H_2O$	0.02
$CuSO_4$	0.01
$ZnSO_4 \cdot 7H_2O$	0.04
$Na_2MoO_4 \cdot 2H_2O$	0.02
$Na_2EDTA \cdot 2H_2O$	6.9

Table 3. Composition of the algal flora in Mirror and Swan Lakes, University of Connecticut, Storrs, in July 1992.

MIRROR LAKE Cell density = 6563 cells/ml^{-1}	
Genera Present	*Percentage*
Scenedesmus (3 species)	30
Anabaena	30
Trachelomonas	2
Microcystis	20
Ceratium	<1
Kirchneriella	<1
Ankistrodesmus	3
Dictyosphaerium	<1
Pediastrum	6
Rhodomonas	1
Various unicells	6
SWAN LAKE Cell density = 1875 cells/ml^{-1}	
Genera Present	*Percentage*
Ceratium	6
Pandorina	32
Trachelomonas	5
Anabaena	50
Nitzschia	4
Various unicells	4

for the first 2 or 3 weeks; Dyna Gro® and Miracle Gro® flasks continued to show increases in growth for an additional week or two (Figures 1, 2). However, an analysis of variance for the duration of each experiment showed no significant differences among the fertilizers.

Microcystis and *Anabaena*, two dominant organisms in Mirror Lake, as well as *Pandorina* and *Anabaena*, dominant in Swan Lake (Table 3), did not grow in culture. However, several *Scenedesmus* species (Table 3) were dominant in many flasks, along with several other unicellular or colonial green algae. Diatoms were also observed.

Discussion

The plant fertilizers proved to be useful substitutes for a defined algal medium. When Swan Lake water was used as an inoculum, adequate growth occurred in all flasks, but Bristol's medium clearly out-performed the fertilizers (Figure 2). Bristol's medium® has been extensively modified over the years and is now widely used in laboratory culture of freshwater algae. This well-balanced formulation provides all essential elements needed for the typical alga. When compared to the other solutions used, it has the highest levels of nitrogen, phosphorus and potassium (Table 1), and thus supports excellent growth. However,

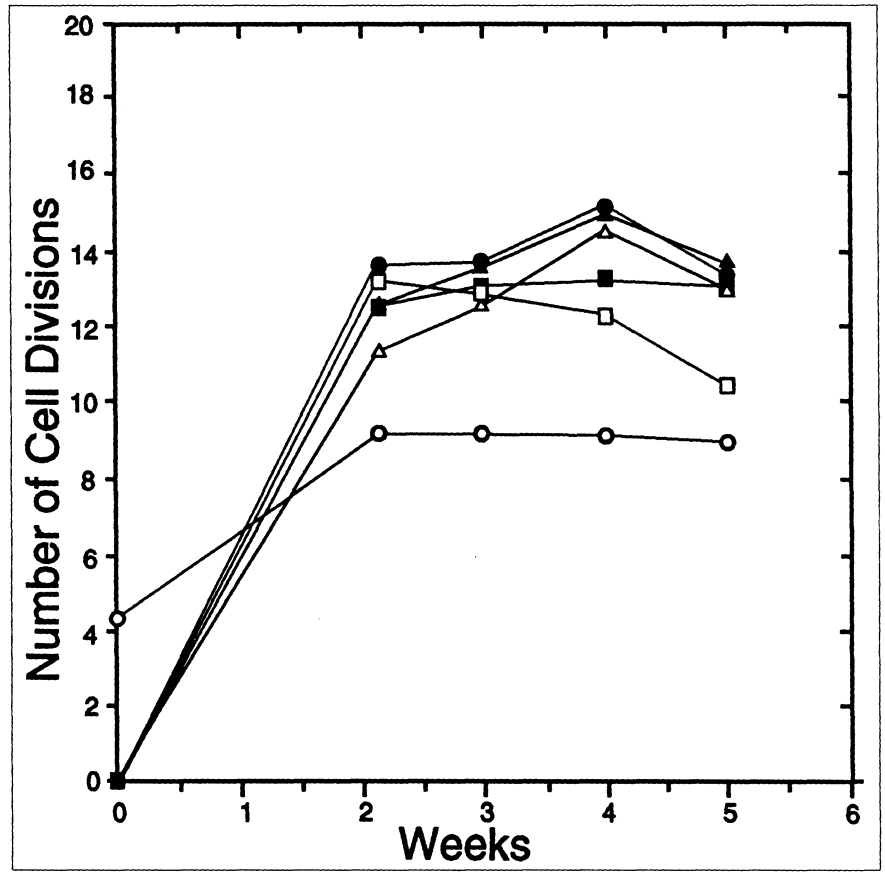

Figure 1. Mirror Lake algal growth in several media, including fertilizer solutions and Bristol's medium®, over 5 weeks. Duplicate flasks were inoculated with 313 cells per ml; cells in the inoculum are given in Table 2. Mirror Lake water alone (open circles), with the endemic population of 6563 cells per ml, served as a control. Bristol's medium®—closed circles; Peters 30-10-10®—open squares; Peters 20-20-20®—closed squares; Miracle Gro®—open triangles; Dyna-Gro®—closed triangles. [# cells = $2^x \times 313$, when x = # cell divisions.]

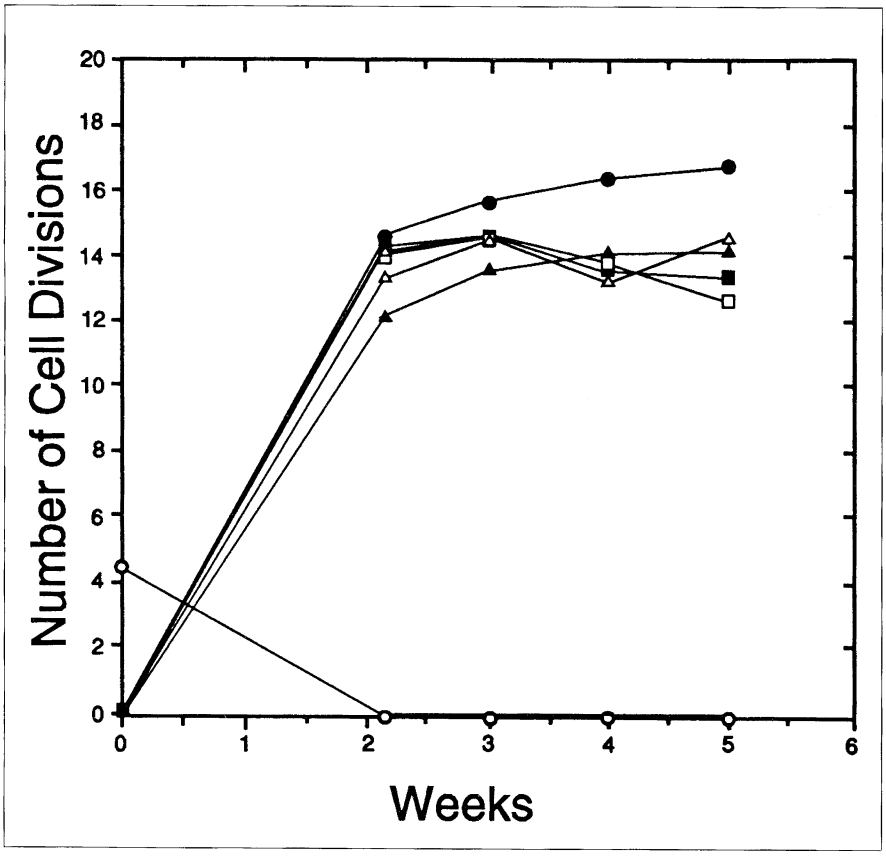

Figure 2. Swan Lake algal growth in several media, including plant fertilizers and Bristol's medium®, over 5 weeks. Duplicate flasks were inoculated with 89 cells per ml; cells in the inoculum are given in Table 2. [# cells = $2^x \times 89$, when x = # cell divisions.] Swan Lake water alone (open circles), with the endemic population of 1875 cells per ml, was one control. Symbols as in Figure 1.

all of the ingredients listed might not be available in the average laboratory.

Trainor et al. (1991) pointed out that the carrying capacity (maximum density of cells) of Miracle Gro® cultures could be increased by providing 500 mg per liter, rather than just 100 mg. The instructor could experiment with nutrient concentration in order to achieve the desired results. We used just 100 mg of plant fertilizer so that we might avoid overwhelming organisms with excess nutrients, for more nutrients would simulate eutrophic conditions. Also, if a pH meter were available, the system could be used to monitor changes in pH in each of the cultures over time (Hershey 1990).

Some organisms may be quite sensitive to changes in nutrient concentration as well as light intensity. For example, Swan Lake algae incubated in flasks of lake water in the laboratory, one of our controls, died. We would point out that, in stationary batch cultures, organisms benefited when we supplied additional nutrients (Figure 2) to the lake water. In nature individual nutrients are available in adequate concentrations because of nutrient cycling; thus, the same organisms survive in the lake.

Unfortunately certain of the dominant organisms in the lakes did not grow in any of our cultures. Although there is no guarantee that a particular organism will survive in a plant fertilizer solution, or even in a defined medium, sufficient organisms do grow that various fertilizers could be very useful as laboratory media. *Anabaena*, *Pandorina* and *Microcystis* have been grown in culture in the past and perhaps would grow under other circumstances, e.g. with other competitors, at a different temperature or light intensity. Inasmuch as they are all large enough to be manipulated under the dissecting microscope, they could be isolated and inoculated individually into an appropriate fertilizer solution. This is clearly an experiment that could be carried out with a minimum of additional materials.

We have not attempted to use plant foods for long-term maintenance of cultures. Without an essential element such as calcium (Hershey 1990) or with nitrogen supplied as urea rather than nitrate, some higher plant fertilizers might possibly be of limited value for such purposes. One rule of thumb: If the solution could not be used for a hydroponics system, it would not be a good medium for **long-term** algal culture. However, in short term experiments, when some essential elements are carried over in the cells and/or water of the inoculum, plant fertilizers can be effectively used. We predict that those products with a more complete formulation would be most useful for long-range experimentation.

Conclusions

Media for the growth of a variety of microalgae can be easily prepared using any of several commercial fertilizers. Organisms grow quickly and within 2 weeks reach population densities sufficient for use in several kinds of laboratory experiments.

Acknowledgment

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References

Anonymous (1969). *Provisional algal assay procedure*. New York: Joint Government/ Industry Task Force on Eutrophication.

Darley, W.M. (1982). *Algal biology: A physiological approach*. Oxford: Blackwell Scientific Publication.

Egan, P. & Trainor, F. (1989). The role of unicells in the polymorphic *Scenedesmus armatus* (Chlorophyceae). *Journal of Phycology*, 25, 65–70.

Hershey, D.R. (1990). Pardon me, but your roots are showing. *The Science Teacher*, 57(2), 42–45.

Sorokin, C. (1973). Dry weight, packed cell volume and optical density. In J. Stein, (Ed.), *Handbook of phycological methods. Culture methods and growth measurements* (pp. 321–345). Cambridge, MA: Cambridge University Press.

Trainor, F., Wallett, S. & Grochowski, J. (1991). A useful algal growth medium. *Journal of Phycology*, 27, 460–461.