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The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner, included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daudenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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THE USE OF ALGAL CULTURES IN EXPERIMENTS CONCERNED WITH WATER SUPPLY PROBLEMS

By C. MERVIN PALMER AND THOMAS E. MALONEY*

Rapid and routine methods for isolating and culturing algae are essential for laboratory studies of those algae which create problems in water supplies and for the development of methods for their control. Just as the bacteriologist must isolate the bacteria in order to study their actions under various conditions, so too, the applied phyco-ologist must now follow essentially the same procedure with the algae. Matheson (1952) has emphasized this need in his recent report to the 1952 Congress of the International Water Supply Association.

Carefully controlled experiments involving algae can be accomplished only when pure algal cultures, free of all other organisms, are available. In general it is not as easy to obtain a pure culture of algae as it is to obtain a pure culture of bacteria because it is difficult to separate contaminants from algal cells and the algal cultures grow slowly and must be kept longer. Once such a culture is obtained a number of pertinent investigations can be made such as screening tests for algicides, determining the effect on algae of changes in their physical and chemical environment, the determination of growth characteristics in standard media, determination of growth rates, the selection of indicator algae in polluted water, and experiments involving the testing of products of algal growth for tastes, odors, and toxicity.

The initial step in isolating algae to obtain pure cultures frequently involves dilution and plating out on a semi-solid medium. A number of diatoms, green and blue-green algae will develop as colonies on liquefiable solid media. These colonies are then transferred to sterile media. Since contaminants are difficult to separate from algal cells, one or more of the following additional methods may be required to obtain pure cultures: selective media, exposure to ultra-violet, utilization of antibiotics (Pappas and Hoffman, 1952), centri-

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fuging, repetition of plating out procedures, and single thallus isolation by means of a micro-pipette. The standard thioglycollate and mycophil broths are recommended in testing for contamination by molds and bacteria. In addition to these organisms, the green alga *Chlorella*, some of the amoeboid protozoa, and yeasts are among the other contaminants which almost invariably have to be eliminated. Several of the flagellate algae generally considered as taste and odor producers, do not form recognizable colonies on semi-solid media. These must be isolated by the more tedious method of the micro-pipette.

In the culturing of various genera and species of algae, it is desirable to select a few general purpose media, each of which permits the active growth of a number of types of algae. This procedure makes possible a direct comparison of the several algae in a common type of culture medium. Gerloff's modification of Chu's #10 medium (Gerloff, Fitzgerald, and Skoog, 1950; Chu, 1942) has been found to be excellent for many blue-green algae and, in addition, for several green algae and a few diatoms. It is a synthetic medium, contains carbonate in addition to the usual nutrients and has a relatively high pH, approximating 8.5.

A low pH medium may be required for certain of the phytoflagellates. A formula furnished by Dr. L. Provasoli of the Haslins Laboratory, which calls for the use of peat extract as one of the ingredients is now being tested. The medium has a pH of 5 and has been used successfully by Dr. Provasoli for the culture of *Synura*, *Pandorina*, and *Peridinium*.

A third medium should be one approximating pH 7 which would be suitable for isolating and culturing some of the many species of diatoms, desmids, golden brown and filamentous green algae which seldom are found growing in the low and high pH media already being used. Rodhe (1948) has developed a medium which comes close to meeting this need in water supply research. He reported his medium VIII to be "very suitable for unlimited cultivation of about forty species and forms belonging to Chlorococcales, Volvocales, Conjugatae, Heterokontae, and diatoms."

The incubation room for algal cultures at the Environmental Health Center has been described in a previous paper (Palmer, 1952).

This room which has continuous illumination, with fluorescent tubes at an intensity of 140 foot candles, and is provided with an air conditioner to hold the temperature at 22° C, has been found very satisfactory for the experiments conducted to date. New cultures inoculated with one part of an active liquid culture to twenty-five parts of sterile medium, will generally develop visible growth within three days in this culture room. In many of the cultures growth often continues for one to four weeks.

When more adequate provisions become available in the new laboratory of the Environmental Health Center, it is planned to test the growth of algal cultures under various combinations of light intensities and temperatures. It is quite probable that the optimum conditions may be found to differ from those now in use.

For simple screening tests, a very satisfactory receptacle for individual cultures is the 25 ml Erlenmeyer flask. Its small size is economical of space and its flat bottom furnishes a surface over which the algae may grow. Tests are now being made with the still smaller 10 ml flask which would be even more efficient in the use of space.

For the continuous growth of algae in pure cultures, a separatory funnel has been found convenient. A special ground glass stopper can be made for the top to provide openings for entrance and exit of gas and for the addition of liquid nutrients. The opening at the bottom permits easy drainage of any or all of the medium in which the algae have been growing. By placing a fluffy mass of sterile glass wool in the funnel, most of the algae will attach themselves to the wool rather than to the surface of the funnel. This permits the extraction of the liquid medium from the funnel without disturbing the mass of algal growth. Since the glass wool tends to float, gas can be bubbled through the entire algal growth. This would not be possible if the algae were permitted to develop on the surface of the funnel.

Rejuvenation of old cultures of algae which have turned brown to yellow with age can often be accomplished by the addition of supplementary nitrate solution. Some algae respond within a period of a few hours while others may require a week or more. However, it may be more satisfactory to store stock cultures in a semi-dormant state in a refrigerator than to depend on the rejuvenation of aged cul-

tures. The storage refrigerator used for this purpose at the Environmental Health Center is furnished with fluorescent light tubes and it can be used also as an incubator for some of the psychrophilic algae.

The above techniques have been used to conduct under controlled environmental conditions numerous serial dilution tests with potential algicides on a considerable number of algae. Other experiments are being conducted to determine the effect of particular environmental changes on algal growth. Although much remains to be done in the development of techniques for the routine culturing of algae it is now planned to undertake direct laboratory studies on the production of tastes and odors by algae. While many procedures for the culturing of particular kinds of algae have been described in the literature, what is now required in the Biology Laboratory of the Environmental Health Center is a procedure for comparative research with many kinds of algae under uniform conditions. At the present time, more than fifty uni-algal cultures representing approximately thirty-five species and varieties are being used and others are being prepared for addition to this list. Forms under study at present are those which are most readily cultivated and include *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Ankistrodesmus*, *Oocystis*, *Phormidium*, *Calothrix*, *Achnanthes*, and *Nitzschia*. Modification and improvement of techniques are required to allow the rapid isolation and continuous cultivation of many other algae of importance in the provision of satisfactory water supplies.

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