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Standard Practice for Algal Growth Potential Testing with *Selenastrum Capricornutum*¹

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INTRODUCTION

Algae are natural inhabitants of surface waters and are found in almost every water environment that is exposed to sunlight. The algae contribute to self purification (both organic and inorganic) of streams and lakes and are necessary as food for fish and fish food organisms. When large amounts of nutrients are available, excessive growths referred to as “blooms” can occur. Some algal blooms release substances toxic to fish, birds, domestic animals, and other algae. When nutrients are exhausted, the growth of algae and production of oxygen by photosynthesis decreases. The respiration of bacteria decomposing the large quantity of algal cells can deplete dissolved oxygen to the extent that fish and other oxygen consumers die. Both the abundance and composition of algae are related to water quality, with algal growth primarily influenced by the availability of nutrients.

The presence of indigenous algae in a water sample suggests that they are the most fit to survive in the environment from which the sample was taken. The indigenous algae should produce biomass until limited from further growth by some essential nutrient. If the indigenous algal production is limited from further growth by an essential nutrient, the laboratory test alga cultured in a noncompetitive environment and responding to the same limiting nutrient will produce parallel maximum yield growth responses. Generally, indigenous phytoplankton bioassays are not necessary unless there is strong evidence of the presence of long-term sublethal toxicants to which indigenous populations might have developed tolerance (1)².

A single-indigenous algal species, dominant at the time of sampling, may not be more indicative of natural conditions than a laboratory species that is not indigenous to the system. The dynamics of natural phytoplankton blooms, in which the dominant algal species changes throughout the growth season, makes it quite certain that even if the indigenous algal isolate was dominant at the time of collection, many other species will dominate the standing crop as the season progresses.

When comparing algal growth potentials from a number of widely different water sources there are advantages in using a single species of alga. The alga to be used must be readily available and its growth measured easily and accurately. It must also respond to growth substances uniformly. Because some algae are capable of concentrating certain nutrients in excess of their present need when they are grown in media with surplus nutrients, this factor must be taken into account in selecting the culture media and in determining the type and amount of algae to use. (2) showed that a blue-green algae *Microcystis aeruginosa*, cultured in a low-nitrogen concentration medium, would not grow when transferred to medium lacking nitrogen. However, when the alga was cultured in medium containing four times as much nitrogen it was able to increase growth two-fold after transfer into nitrogen-free medium. A green alga *Selenastrum capricornutum*, gave a similar response. In an analogous experiment with phosphorus, both organisms increased four-fold when transferred to medium lacking phosphorus. However, if algae are cultured in relatively dilute medium as recommended in the Algal Assay Procedure: Bottle Test (3) for culturing *Selenastrum capricornutum*, disclosed no significant further growth in medium lacking nitrogen or phosphorus when these were transferred from the initial medium over a wide range of inoculum sizes (4).

There are several methods available for determining algal growth. Measurements of optical density, oxygen production, carbon dioxide uptake, microscopical cell counts, and gravimetric cell mass determinations have been used, but often lack sensitivity when the number of cells is low. Measurement of the uptake of carbon-14 in the form of bicarbonate is a sensitive method but can also

be time-consuming. *In vivo* fluorescence of algal chlorophyll has been used with many types of algae and has proved particularly useful with indigenous algae or filamentous forms not easily measured at low concentrations by other methods. The method is sensitive and measurements can be quickly performed. However, chlorophyll to cell mass ratio may vary significantly with growth in water samples of different chemical composition (5). The electronic particle counter has been used for counting and sizing nonfilamentous unialgal species (6,7). Shiroyama, Miller, and Greene (8) have developed a procedure for using an electronic particle counter to count and size *Anabaena flos-aquae* filaments cultured in natural waters.

The need for standardization of techniques for measuring the potential for algal growth was recognized by the Joint Industry/Government Task Force on Eutrophication (9). Thereafter, the Environmental Protection Agency developed, in association with industrial and university cooperation, a Bottle Test for assaying algal growth potential in natural water samples (3). An expanded and improved version of the Bottle Test was published in 1978 (10). It is this work on which the following test is based.

¹ This practice is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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² The boldface numbers in parentheses refer to the references at the end of this practice.

1. Scope

1.1 This practice measures by *Selenastrum capricornutum* growth response, the biological availability of nutrients, as contrasted with chemical analysis of the components of the sample. This practice is useful for assessing the impact of nutrients, and changes in their loading, upon freshwater algal productivity. Other laboratory or indigenous algae can be used with this practice. However, *Selenastrum capricornutum* must be cultured as a reference alga along with the alternative algal species.

1.2 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For a specific precautionary statement, see Section 15.

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water³

D 1193 Specification for Reagent Water³

D 3370 Practices for Sampling Water³

3. Summary of Practice

3.1 A water sample is filtered or autoclaved and filtered, placed in a covered Erlenmeyer flask, inoculated with the test algal species, and incubated under constant temperature and light intensity until the increase in biomass is less than 5 % per day (generally between day 7 and 14). Nutrients may also be added to aliquots of the sample to determine growth controlling nutrients.

4. Significance and Use

4.1 The significance of measuring algal growth potential in water samples is that differentiation can be made between the

nutrients of a sample determined by chemical analysis and the nutrients that are actually available for algal growth. The addition of nutrients (usually nitrogen and phosphorus singly or in combination) to the sample can give an indication of which nutrient(s) is (are) limiting for algal growth (1,10,11,12,13,14).

5. Interferences

5.1 Autoclaving may cause precipitation of certain constituents in the sample and elevate the pH. These precipitates are not necessarily irreversible or unavailable as nutrients. The sample may often be clarified by equilibrating it in a CO₂ atmosphere followed by equilibration in air to its original pH.

5.2 Toxic substances in the sample may affect the growth response of the algae.

6. Apparatus

6.1 *Water Sampler*, nonmetallic.

6.2 *Sample Container*—Linear polyethylene bottles.

6.3 *Centrifuge*.

6.4 *Environmental Chamber*, with temperature control (24 ± 2°C) and illumination (cool white fluorescent) that provides 4300 lm/m² ± 10 %, or equivalent.

6.5 *Shaker*, rotary, capable of 100 to 120 rpm.

6.6 *Flasks*, Erlenmeyer, 250-mL.

NOTE 1—Other sizes are acceptable as long as the liquid does not exceed 50 % of the total flask volume.

6.7 *Flask Covers, Beakers, or Foam Plugs*—Some foam plugs, upon autoclaving, may release substances toxic to the test algae. Each laboratory, when changing its source of supply, must determine whether the new closures have a significant effect on the maximum standing crop.

6.8 *Tubes*, graduated centrifuge.

6.9 *Pipets*, Eppendorf or equivalent, with disposable tips, 0.1 or 1.0 mL.

6.10 *Filtration Apparatus*, nonmetallic, with vacuum or pressure source.

³ Annual Book of ASTM Standards, Vol 11.01.