Designation: D3978 – 21a

Standard Practice for Algal Growth Potential Testing with *Pseudokirchneriella subcapitata*[1,2]

This standard is issued under the fixed designation D3978; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 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 alga. 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**)[3].

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 *Pseudokirchnereilla subcapitata* (also known as *Selenastrum capricornutum* and *Raphidocelis subcapitata*), 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 *Pseudokirchnereilla subcapitata*, 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 E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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[2] Renamed by Gunnar Nygaard, Jirf Komárek, Jørgen Kristiansen and Olav M. Skulberg, 1986. Taxonomic designations of the bioassay alga NIVA-CHL1 ("Selenastrum capricornutum") and some related strains. Opera Botanica 90:5-46.

[3] The boldface numbers in parentheses refer to the references at the end of this practice.

1. Scope*

1.1 This practice measures, by *Pseudokirchnereilla subcapitata* 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, *Pseudokirchnereilla subcapitata* 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 concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For a specific precautionary statement, see Section 16.

1.3 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate

E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E1733 Guide for Use of Lighting in Laboratory Testing

SI10–16 IEEE/ASTM SI-10 American National Standard for Metric Practice

[4] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3. Terminology

3.1 *Definitions:*

3.1.1 The words "must", "should", "may", "can", and "might" have very specific meanings in this guide. "Must" is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. "Must" is used only in connection with factors that directly relate to required test procedures (see Section 14). "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "is desirable," "is often desirable," and "might be desirable" are used in connection with less important factors. "May" is used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus, the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

3.1.2 For definitions of other terms used in this guide, refer to Terminologies D1129 and E943 and Guide E729. For an explanation of units and symbols, refer to SI10–16.

3.2 *Definitions of Terms Specific to This Standard:*