A PLASTIC CONTAINER FOR ALGAL GROWTH POTENTIAL TESTS

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Abstract. The substitution of a six ounce plastic cup with snap-cap lid as a growth vessel for a standard algal growth potential test 125 ml glass Erlenmeyer flask has been evaluated. Included are a discussion of desired growth vessel characteristics and the relationship of seven day plastic cup algal growth to maximum (twelve to fourteen day) flask.

1. Introduction

The developing problem of humanly induced eutrophication has led to the conception of an algal-based test to establish the quantitative and qualitative responses to the major algal nutrients (EPA, 1978). The Algal Growth Potential Test (AGPT) was developed over 20 years ago to provide a simplified standard method measuring enrichment of aquatic environments. The test was designed to demonstrate that the extent of growth of test algae in a water-based medium is limited by the nutrient which is in least supply, a modified Liebigs law of the minimum (EPA, 1978). Nitrogen and phosphorus are the growth elements most commonly found to be limiting to algal growth (Shiroyama et al., 1971). When used in conjunction with other physical and chemical measurements the AGPT can increase our knowledge of lake conditions (Shiroyama et al., 1971).

The AGPT has been a standard method of measuring enrichment in fresh and marine water bodies in EPA Region IV for many years (Raschke and Schultz, 1987). It has provided a rapid and simple method of testing suspect waters (Rehnberg et al., 1982). Information obtained from the AGPT has been used by the federal, state and local governments as a basis for permits and discharge regulations as well as measuring trophic water conditions and usage within the Clean Water Act (USEPA, 1978).

The amount of time involved in completing an AGPT, approximately two weeks, has been a limiting factor in its usefulness (Schulz et al., in press). The length of time required to complete a standard growth test has been reduced to one week by utilizing a linear regression comparison of the seven-day and maximum algal dry weights to develop a maximum dry weight computation equation (Schultz et al., in press). The cleaning of glassware, including culture flasks, consumes a considerable part of the time required to process an AGPT. In our laboratory, limited resources for glassware cleaning has encouraged us to explore the option of utilizing recyclable plastic growth vessels for the glass 125 ml erlenmeyer culture flasks. This paper will discuss the utilization of plastic cups as growth chambers.
2. Methods

The Environmental Services Division, EPA Region IV performs routine AGPT for state and federal agencies within the region. The AGPT and related quality control procedures are conducted in accordance with Standard Methodology (APHA, 1992). Accordingly, samples are autoclaved, cooled, and filtered through a 0.45 μm membrane filter. Separate replicates of the samples in flasks and in plastic cups were untreated, or separately treated with measured amounts of nitrogen and phosphorus. All treatments are inoculated with the test algal cells. We have varied our procedure from Standard Methods in that we inoculated the growth containers with 3000 cells rather than 1000 cells per ml. The growth containers recommended in Standard Methods are erlenmeyer flasks of good quality borosilicate glass such as Kimax or Pyrex. Some of the culture vessel physical requirements are clarity, lack of toxicity to algae, smooth inner walls, and a shape and size to allow for abundant placement within an incubator chamber. The selected substitute containers were six ounce slope sided, clear juice glasses with semi-opaque snap-cap lids obtained from Plastics Inc., St. Paul, Minnesota. Since sample pH below 8.5 is critical to algal carbon dioxide availability, sample to volume flask ratios in continuously shaken (100 rpm) should not exceed 50% of the container volume (EPA 1978, EPA, 1971). Ventilation of the growth cups was accomplished by punching holes in the cup lid. The hole sizing was varied to determine an adequate size for carbon dioxide exchange. Testing of varied sizes and numbers of holes indicated two holes of 1 ml size provided adequate carbon dioxide to duplicate the algal growth in glass flasks.

The fresh water used in evaluating the use of plastic cups for AGPT came from lake and river samples analyzed in 1993 and frozen for this later use. The varied algal dry weights from these tests came from a wide range of nutrient-rich water bodies. A linear regression comparison was conducted on each samples day seven and maximum dry weights (D.W.). The plots of the untransformed data suggested a simple linear increase of the covariates, seven-day D.W. as the independent variable (x) and maximum D.W. as the dependent variable (y), the slope coefficient (m) and intercept (b) were determined for predicting maximum D.W. (y), and standard error (S.E.) within the data range (Ott, 1988) according to the following equation:

\[ y = mx + b \pm S.E. \]

The regression equation, correlation (r), and graphics were developed using the STATISTICA® program.

3. Results and Discussion

The short term (seven-day) AGPT plastic cups dry weight biomass of 100 data sets ranged in the maximum dry weight from 0.80 to 110.87 mg/l. Within the range