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A quantitative method for determining the efficacy of algicides in industrial cooling towers

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SUMMARY

Quantitative sampling of periphyton from natural substrates is difficult and uncommon due to the non-homogenous and irregular nature of most natural substrates. This paper describes an experimentally verified method for quantitative sampling of periphyton directly from the relatively homogenous and regular upper deck of a cooling tower.

INTRODUCTION

The problems associated with excessive algae growth in cooling towers have been long recognized by tower operators [11]. Removing this growth by manually scraping algae from the tower deck is a labor intensive undertaking and thus cooling water treatment programs frequently include chemical additions to control algae.

Current practice is to judge the efficacy of a chemical treatment program by non-quantitative means [4,15]. A quantitative sampling method and its use in developing an industrial water treatment product is reported.

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MATERIALS AND METHODS

The cooling tower for the first trial was located in full sun on the roof of a hospital in Chicago. The 10 000 gallon air conditioning system was maintained at five to six cycles of concentration by supplying make-up water at a rate of 70 000 to 80 000 gallons per day.

The cooling tower for the second trial served a textile factory in the Southeastern United States. The 240 000 gallon system which cooled factory machinery was maintained at 5 cycles of concentration by suppling 201 600 gallons of make-up water daily.

Approximately one-eighth of the total deck surface was scraped clean of algae and scrubbed with a stiff-bristled brush on the first day of sampling the Trial 1 tower. A separate area was similarly cleaned

immediately after the proprietary experimental antimicrobial feed began. These two 'cleaned' surfaces and the 'fouled' surface constituted three separate areas of the tower deck, each sampled independently. Samples were taken weekly for the first three weeks and twice-a-week for the four following weeks. The entire tower deck was then scraped clean (but not brushed) and the trial continued.

For Trial 2 one section of the tower deck was cleaned and brushed as described above one week prior to beginning treatment with the experimental compound.

The biofilm was sampled as follows: A Pyrex® glass flared pipe connector (10 cm i.d., 30 cm long) was placed on the biofilm and twisted slightly to seat on the tower deck material. Water inside the pipe was removed with a bulb pipet without disturbing the biofilm. The enclosed biofilm was then dislodged with a nylon bristle brush and the slurry transferred to a 1 l plastic bottle with rinsings of cooling tower water using the bulb pipet. Variations in the amount of biofilm across the sampled area were minimized by pooling two, four, or six brushings for each sample. Samples from any one area contained the same number of brushings and approximately the same total volume per sample. Samples were packed on ice, transported to the lab, and frozen upon receipt, usually within six hours of sampling.

All samples were kept frozen until analyzed. Frozen samples were thawed for 30 min in a 50°C water bath. Large clumps of algae were broken up by blending for ten seconds at lowest speed in a Hamilton-Beach blender (Model 680-1). Two 5.0 ml aliquots were immediately withdrawn and placed on ice for duplicate chlorophyll analyses. The total sample volume was then determined with a 1 l graduated cylinder and corrected for the aliquots already removed. The sample was then shaken and a 20 ml aliquot homogenized in a tissue grinder for additional analyses. The homogenization step was necessary to prevent the lumpy nature of the blended sample from interfering with accuracy and reproducibility of analyses requiring small aliquots. The remaining blended sample was refrozen. After completing all analyses, those samples from Trial 1 were thawed and blended as above. Aliquots of ten milliliters each were arranged chronologically by sample area and photographed as a qualitative measure of algal biofilm growth.

Five analyses were performed on each sample from Trial 1. Dry weight and ash-free dry weight (AFDW) were determined on 1 to 5 ml portions of the homogenate according to Standard Methods (American Public Health Association, *Standard Methods For The Examination of Water And Wastewater,* 15th ed., Method 1002 H.4., 1980, p. 955). Triplicate determinations were averaged and expressed as mass per unit surface area of tower deck (mg/sq cm). The coefficient of variation (C.V.) for dry weight was less than fifteen percent for all but two of fifty-eight samples measured from Trial 1; the C.V. for these two samples was less than 25%. The C.V. for the AFDW was less than 21% for all but seven of the same set of fifty-eight samples; three had very low average AFDW values (<0.2 mg/sq cm) and the remaining four had relatively low AFDW values (<4 mg/sq cm), two of which correspond to samples with a high C.V. for dry weight.

Chlorophyll analyses were performed according to Standard Methods (American Public Health Association, *Standard Methods For The Examination of Water And Wastewater,* 15th ed., Method 1002 G.1., 1980, p. 950–952) with the following modifications: Chlorophyll concentration was determined by measuring the absorbance at 663 nm of an acetone extract (90% acetone + 10% water V/V, 70% extraction efficiency). Standard working curves for chlorophyll concentration were prepared with spinach chlorophyll a (Sigma Chemical Co.). Duplicate analyses were performed on all but those taken on the first four sampling dates (eight samples) for which only single measurements were made. The C.V. was less than 16% for all but three of the fifty samples measured in duplicate from Trial 1. Two of these three had very low average values (<0.5 μg/sq cm).

Protein measurements on the homogenate were made with a commercial kit based on a modified Lowry method in which bovine serum albumin served as the standard (Sigma Diagnostics, Proce-

dure No. 690). Duplicate analyses were performed on all but ten samples for which only single measurements were made. The C.V. was less than 40% for all but seven of forty-eight samples measured in duplicate. Six of these had low average values (0.3 mg/sq cm or less).

The optical density at 260 nm was determined with a Hitachi model 150-20 spectrophotometer on known dilutions of the homogenate in phosphate buffered water and corrected to a dilution of 1:100 by applying Beer's Law. Heterocyclic compounds are known to absorb strongly at this wavelength and nucleic acids contribute to the absorbance [9]. Thus, the OD260 can serve as a rough indicator of nucleic acid content. Measurements were made on forty-six of the fifty eight samples. Duplicate analyses were performed on all but those taken on the first four sampling dates (eight analyses) for which only single measurements were made. The C.V. was less than 45% for all but four of thirty-eight samples measured in duplicate. These four had very low average optical density values (<0.04).

The relatively high C.V. for the few cases described did not alter data interpretation and no data was discarded in the data analyses.

Samples from Trial 2 were analyzed for dry weight, AFDW, and chlorophyll *a* as described above.

RESULTS

Figs. 1 and 2 show quantitative measurements of biofilm samples plotted against time for Trial 1 and 2 respectively. Qualitative observations and descriptions of major events are outlined in the captions.

Quantitative data from Trial 1 was pooled and grouped by tower area to measure the correlation between quantitative parameters. Each analysis method was paired with every other method in turn and the correlation coefficient calculated. The significance of the correlation coefficient (r) between quantitative measurements was tested at the 95% level using a two-tailed t-test with $n - 1$ degrees of freedom. All correlations were significant at the 95% level. Fig. 3 shows 10 ml aliquots of the biofilm samples taken during Trial 1, grouped by area and arranged chronologically. The relative volume of sediment in each tube is a qualitative measure of the amount of biofilm present on the tower deck. Area

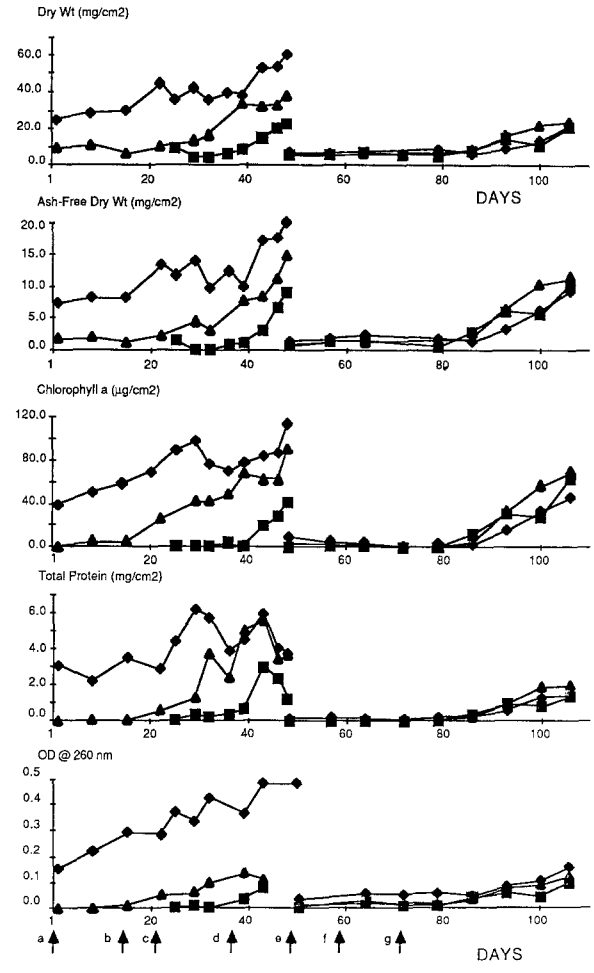

Fig. 1. Quantitative analyses from Trial 1. Plots follow indicated parameters for the fouled area (diamonds), Area 2 cleaned on day 1 (triangles), and Area 3 cleaned on day 21 (squares). Continuous bromochlorodimethylhydantoin (BCDMH) feed sufficient to maintain 0.5 to 1.0 ppm halogen as Cl_2 plus 0.25 to 0.75 ppm methylene bisthiocyanate (MBT) was maintained for the first two weeks of sampling. Arrows indicate major events and observations: a. Area 2 cleaned. b. Discontinue MBT. A 10 mm biofilm layer on Area 2 reported. c. Discontinue BCDMH. Begin feeding experimental antimicrobial and clean Area 3. Area 2 had a 25 mm thick layer of biofilm. d. Low antimicrobial concentrations first reported. Noticeable biofilm regrowth occurs. e. Upper deck manually cleaned. f. Begin 2X/day MBT doses. g. Discontinue MBT and begin feeding experimental antimicrobial.

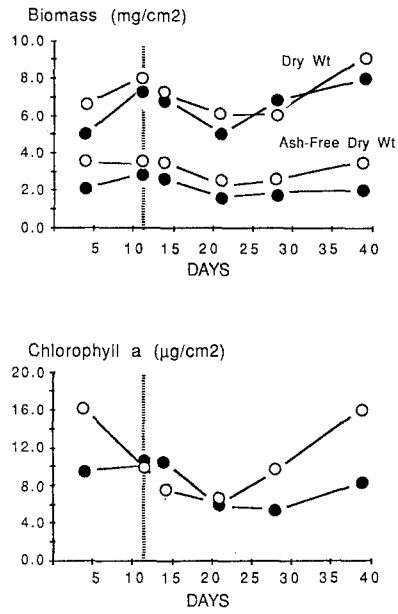

Fig. 2. Quantitative analyses from Trial 2. Plots follow indicated parameters for the fouled area (open circles) and the cleaned area (closed circles). Treated initially with BCDMH. BCDMH discontinued and experimental antimicrobial began at day 11 as indicated.

1 contains six brushings per sample. Areas 2 and 3 each contain 2 brushings per sample and are directly comparable.

DISCUSSION

Currently, no published method exists for routine sampling of algal biofilms from cooling towers. With a few exceptions [8] methods which have been commonly used for monitoring algal growth provide no quantitative data for evaluating or comparing treatment programs.

Methods for sampling periphyton with artificial substrate samples as is common in limnological applications [1,2,6,7,10,12–14,16,17] are not well suited to cooling water applications. Methods employing artificial substrates require a conditioning and colonization period of weeks to months. Algae treatment programs are usually modified after the biofilm has been well established and an algae related problem has occurred, a time when close monitoring is essential and a delay in monitoring to allow for installation and colonization of a sampler is unacceptable.

No installation or colonization delay is necessary to sample the tower deck itself. Worldwide, the deck material is most often wood submerged by a few centimeters of flowing water and as such presents a substrate which is defined, reproducible, homogenous, and accessible, although immobile. Limnological methods for sampling similar surfaces that were transportable have been described by several investigators [3,5,17]. The method described in this paper most closely resembles that of Douglas for sampling periphyton from small rocks collected from a stream.

The sampling method is validated by the agreement between qualitative evaluations of biofilm growth, major events (cleanings, change of antimicrobial), and quantitative measurements made on samples returned to the laboratory (Fig. 1). The high correlation between quantitative measurements indicate the sampling method did not select for or against any measured parameter.

Throughout Trial 1, quantitative measurements follow trends seen in biofilm volume as shown in Fig. 3. Low sediment volumes for Area 2 on Days 1 through 15, Area 3 on days 24 through 38, and all areas on Days 49 through 85 correspond to low values for quantitative measurements on those dates. Increases in sediment volume on Days 38–49 and Days 85–105 for all areas are paralleled by similar trends in quantitative measurements. Samples from Areas 2 and 3 each contain two brushings per sample and are thus directly comparable. Aliquots from Area 2 contain a volume of sediment greater than Area 3 at each sampling date prior to Day 49 when the deck was manually cleaned. Aliquots from all three areas contain comparable volumes of sediment for each sampling date after Day 49. These relationships between sample areas hold for each of the quantitative measurements also.

The quantitative sampling method refined and verified during Trial 1 was used to monitor the effectiveness of the experimental compound in Trial 2. Comparison of the 'fouled' area to the 'cleaned'

Fig. 3. Relative biofilm levels, Trial 1.

area on the first sampling day show that the level of fouling present during the period prior to treatment with the experimental compound is low compared to a well developed biofilm such as that in Trial 1 (Fig. 2). Changes in values for quantitative measurements were small, and the values did not exceed initial levels during the course of the experiment. This suggests that the treatment maintained control of the biofilm during the trial, consistent with visual observations.

In summary, the method described above provides a simple means to quantitatively sample biofilms growing on the upper deck of cooling towers. The biofilm thus sampled can be quantified using any of several common analysis techniques and the results used to monitor the effectiveness of antimicrobial treatment programs.

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