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Laboratory and Greenhouse Studies of Microbial Products Used to Biologically Control Algae

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ABSTRACT

Outdoor, greenhouse and laboratory studies were conducted in 1997 and 1998 to evaluate the efficacy of several commercially available blends of bacterial inocula, often called microbial products, used to biologically control algae in lakes and ponds. Results were similar among outdoor plastic barrels treated with the microbial products Aqua-5™, a 1997 formulation of LakePak™ WSP®, Algae-Tron™, Biorestoration Formula-2™, Clear Pond™ and non-treated controls. However, barrels treated with Hydrothol 191™ showed dramatic and highly significant ($P < 0.0001$) reductions of chlorophyll *a* concentrations relative to the non-treated controls. Results from a greenhouse study also did not suggest that microbial products are efficacious as biological control for algae.

Key words: algae, bacteria, biological control, lake management.

INTRODUCTION

In recent years, commercial blends of bacteria and enzymes, often called microbial products, have become popular alternatives to algicides used in lake and pond management. The literature suggests that bacteria could be superior competitors for nutrients and in turn determine the abundance of algae (Rhee 1972, Currie and Kalff 1984). However, because microbial products are not regulated as algicides, efficacy data that might otherwise be required by regulatory agencies, especially state agencies, are not available. On the other hand, reports on the successful use of microbial products are common. Unfortunately, these reports are consistently anecdotal and subjective. Three studies were conducted in 1997 and 1998 preliminary to a larger pond-enclosure study that is also presented in this issue. The goal of these studies was to gain basic information about microbial products and investigate the hypothesis that concentrated blends of bacteria can be applied to lakes and ponds in order to clear green water, inhibit algae growth or improve water clarity or quality.

MATERIALS AND METHODS

In the first study, water from an urban lake in Davis, CA was collected from 10 cm below the surface and transferred to 35 separate 60 liter plastic barrels located on a outdoor concrete pad. The water in this lake contained a bloom population of the green alga, *Chlorella* sp. Chlorophyll *a* concentrations in this lake were measured several times in 1997 and were consistently in the range of 100 to 250 mg m^{-3}. Using methods described in APHA Standard Methods (1980), Burnison (1980), and Spencer and Ksander (1987), chlorophyll *a* concentrations from the lake and from the experimental barrels were determined from 25 ml samples collected in the top 10 cm of water. These samples were filtered on Whatman™ glass fiber filters, and the filters were placed in 10 ml of dimethyl sulfoxide for chlorophyll extraction. The optical densities of extracts were measured on a Beckman DU-64 spectrophotometer and used to calculate chlorophyll *a* concentrations in the water using the trichromatic method for chlorophyll as described in APHA Standard Methods (1980).

Five replicates were used for each of the seven treatments: Aqua-5™, a 1997 formulation of LakePak™ WSP®, Algae-Tron™, Biorestoration Formula-2™, Clear Pond™, Hydrothol 191™ [mono (N, N-dimethylalkylamine) salt of endothall] and the non-treated control. The water in the barrels was allowed to acclimate overnight and pre-treatment chlorophyll *a* concentrations were determined. The five microbial products were then applied at the rate of 1.1 mg L^{-1} and the Hydrothol 191™ was applied at 4.3 µl L^{-1}. Post-treatment chlorophyll *a* concentrations were determined at 5 hours, 24 hours, 3 days, 5 days, 10 days, 15 days and 30 days. A maintenance dosage of 0.37 mg L^{-1} every two weeks is recommended for most of the microbial products tested and was applied for all of them on the 14th day of the study. A maintenance dosage of the Hydrothol 191™ was not applied. The pH of the lake and all the barrels remained high, from 9.0 to 9.4 throughout the study. The temperature fluctuated in the barrels from a maximum of 32C to a minimum of 14C while the temperature of the lake fluctuated less and remained around 22C.

Following this first study, a greenhouse study was conducted to address the concern of declining chlorophyll levels and determine if a lower pH and higher application rates would produce different results. Water for this study was prepared by lowering the pH of domestic tap water to 7.0 with 15 ml of 5N HCL in 70 liters of tap water and then removing chlorine from the water with sodium thiosulfate. Small plastic pots

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were used to combine 670 ml of the modified tap water and 140 ml of water collected from an outdoor aquatic plant vault which contained a bloom of algae dominated by *Chlamydomonas* sp. A final volume of 710 ml and final pH of 7.25 resulted from the addition of the algal inoculum to the modified tap water.

A total of 60 pots were placed in the greenhouse. One third of these pots received a high rate nutrient addition (11 mg L^{-1} N and 3.0 mg L^{-1} P), one third received a low rate of nutrients (1.1 mg L^{-1} N and 0.3 mg L^{-1} P) and the final one third did not receive any additional nutrients. Each of the three nutrient concentrations then received either a 100× labeled rate (150 mg per pot), 10× labeled rate (15 mg per pot), maximum labeled rate (approximately 2 mg L^{-1}) or a zero rate (non-treated) application of a 1997 formulation LakePak™ WSP®.

Three days after treatment, 50 ml water samples were taken from the middle of the pots. After fifteen days, all pots were mixed with a magnetic stir bar prior to collecting water samples in order to homogenize and suspend filamentous algae that had developed on the surface of some pots. Chlorophyll *a* concentrations were determined using the aforementioned methods.

Laboratory studies were conducted to quantify the viability of bacterial cells in several microbial water treatment products. Viable cell counts or CFU (colony forming units) were obtained using the indirect method of serial dilution and agar plate spread as described in APHA Standard Methods (1980). The average number of CFU from six microbial products: Aqua-5™, a new (1998) formulation of LakePak™ WSP®, the old (1997) formulation of LakePak™ WSP®, Algae-Tron™, Biorestoration Formula-2™, and Biozyme™ were then compared. All six of the microbial products tested were in the form of a freeze-dried powder and are applied to lakes and ponds at a rate of between one and two mg L^{-1}. For each of the products, two milligrams of the powder was added to one liter of sterile distilled water and stirred for five minutes before serially diluting and plating the suspension. The CFU from water containing two mg L^{-1} of the microbial products were then compared to CFU from untreated pond water and pond sediments.

Different types of media were used to quantify viable cells in microbial products. Plate Count Agar (per liter: 9.0 g agar, 5.0 g pancreatic digest of casein, 2.5 g yeast extract, and 1.0 g glucose), Tryptic Soy Agar (per liter: 15.0 g agar, 15.0 g pancreatic digest of casein, 5.0 g papaic digest of soybean meal, and 5.0 g NaCl) and Beef Extract Agar (15.0 g agar, 5.0 g peptone, and 3.0 g beef extract) are three media commonly used for the culture of a wide range of microorganisms from the water (Atlas 1995). These media were also compared in order to determine the best medium for the culture of both microbial products and naturally occurring pond bacteria in subsequent studies.

RESULTS AND DISCUSSION

The first study, using lake water in 60 liter barrels, resulted in little or no difference among the microbial products and the non-treated controls (data not shown). Chlorophyll *a* concentrations in the lake where the water was collected declined from 170 mg m^{-3} on September 13 to 110 mg m^{-3} on September 24. A similar decline from 170 mg m^{-3} to 75 mg m^{-3} over the same time period was observed in all the experimental barrels treated with microbial products and the non-treated controls. The somewhat larger chlorophyll decline in the barrels was likely due to container effects. However, those barrels treated with Hydrothol 191™ showed a dramatic and highly significant ($P < 0.0001$) reduction of chlorophyll, relative to the non-treated controls. Chlorophyll concentrations in the Hydrothol 191™ barrels declined from 170 mg m^{-3} to 25 mg m^{-3} five hours after treatment and 24 hours after Hydrothol 191™ treatment chlorophyll concentrations were essentially zero.

The declining concentration of chlorophyll in all treatments, including the controls, could have complicated the identification of microbial product treatment effects. Thus these conditions may not have been the optimal conditions for testing algal control via bacterial competition for nutrient. However, decisions to treat a lake for algae control are often made only after algal populations reach their highest and most noxious levels and begin to decline. This study shows how the placebo effect in a lake treatment or the container effect in a bottle test could lead to testimonials or anecdotal reports of successful algal control following a microbial product treatment. The results of this study suggest that the microbial products tested may not clear green water or inhibit algal growth as effectively as algicides such as Hydrothol 191™.

Additionally, results from the greenhouse study did not support the hypothesis that added bacteria will reduce algal growth. After three days, chlorophyll *a* concentrations in both the pots with added nutrients and the pots with high rates of the microbial product significantly increased, ($P < 0.05$), relative to the non-treated controls (Figure 1). After 15 days, the pots with the high rate nutrient amendments maintained a significantly higher ($P < 0.01$) concentration of chlorophyll relative to the pots with no added nutrients. In the high nutrient pots, those that received the 150 mg L^{-1} microbial product treatment were significantly higher ($P < 0.01$) in chlorophyll *a* concentrations after 15 days when compared to the zero rate microbial treatments.

Filamentous algae formed mats at the surface of the pots that received the zero, 2 mg L^{-1}, and 15 mg L^{-1} LakePak™ WSP® treatments. However, filamentous algae were nearly absent from the pots treated with the high rate of 150 mg L^{-1} LakePak™ WSP®. Filamentous algae did not grow in the pots that had the highest chlorophyll concentrations, those pots with both the high rate nutrient amendments and the high rate LakePak™ WSP® treatments. Rapid phytoplankton growth may have prevented development of filamentous algae or something in the LakePak™ WSP® might have inhibited filamentous algae growth at the 150 mg L^{-1} treatment rate. Metallic fragments were observed sticking to the magnetic stir bars used to mix the water in the pots, especially in the 150 mg L^{-1} LakePak™ WSP® treatments. Further investigation revealed a high number of these metallic fragments in the old formulation of LakePak™ WSP®. Results from this study suggested that macronutrients such as nitrogen and phosphorus or metals such as iron copper and zinc contained in microbial products could have a stimulatory or inhibitory effect on one or more types of algae, especially if the

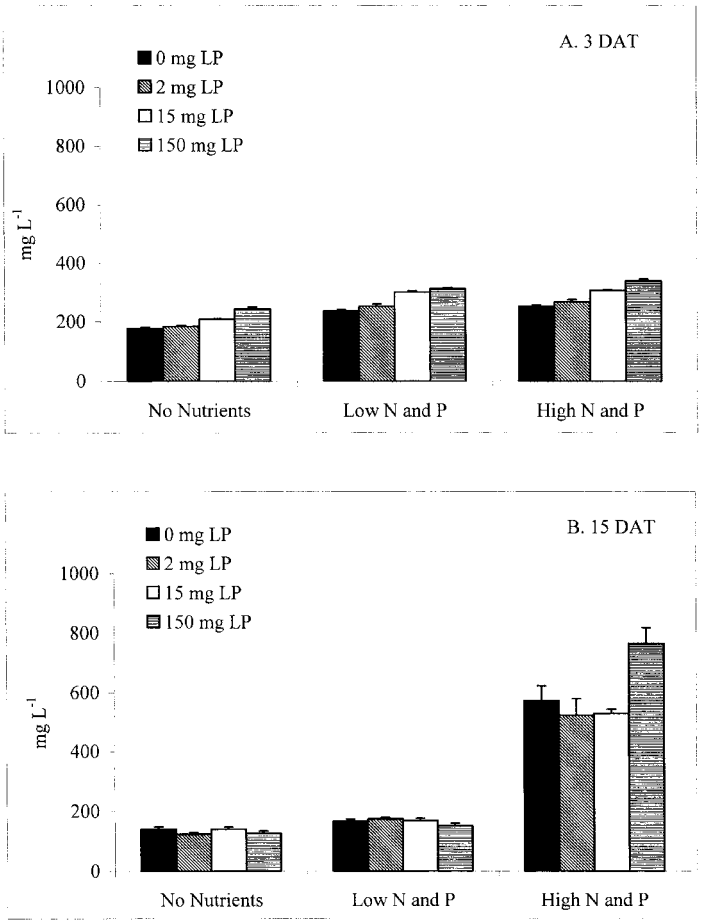

Figure 1. Mean chlorophyll *a* concentrations plus standard errors, N = 5, in greenhouse pots, (A) 3 days after treatment and (B) 15 days after treatment. Each pot received a zero rate, low rate (0.3 mg L^{-1} P and 1.1 mg L^{-1} N) or high rate (3 mg L^{-1} P and 11 mg L^{-1} N) fertilizer amendment and a zero, 2, 15 or 150 mg L^{-1} treatment of LP (LakePak™ WSP®). Subsequent studies have shown that this formulation of LakePak™ WSP®, purchased in 1997, contained few viable bacteria.

microbial products are applied at high rates. The source of these metallic fragments is not known and could be a component of formulation or a contaminant of some sort.

Manufacturers of microbial products often guarantee that their products will contain a minimum bacterial count. These counts are usually on the order of a billion per gram or a trillion per pound. One of the products tested, LakePak™ WSP®, offers a guaranteed bacterial count of four billion CFU g^{-1}, which is equivalent to 8,000 CFU ml^{-1} in a 2.0 mg L^{-1} suspension. Results of laboratory plate counts are presented in Table 1. The CFU count of untreated pond water ranged from 940 CFU ml^{-1} on Plate Count Agar to 4660 CFU ml^{-1} on Beef Extract Agar. The CFU count of untreated pond sediment ranged from 230,000 CFU ml^{-1} on Plate Count Agar to 1,300,000 CFU ml^{-1} on Beef Extract Agar. Viable cell counts for the products tested ranged from zero to 30,130 CFU ml^{-1} in a 2.0 mg L^{-1} suspension. All of the products tested produced CFU counts on the order of 10^3 ml^{-1} in a 2.0 mg L^{-1} suspension with the exception of Aqua-5™ and the old formulation of LakePak™ WSP®. Aqua-5™ produced the highest average CFU count of 30,130 on Tryptic Soy Agar. The old formulation of LakePak™ WSP®, which was used in the greenhouse study before these CFU counts were done, consistently produced zero colonies at dilutions of 10^{-1} and 10^{-2} and only a small number of CFU could be detected in undiluted suspensions of 2.0 mg L^{-1}. The minute number of viable cells in the old formulation of LakePak™ WSP® is one explanation for why a reduction in algal growth was not observed in the greenhouse study and why the manufacturer might have changed the formulation. The CFU count for the new formulation of LakePak™ WSP® ranged from 1700 CFU ml^{-1} in a 2.0 mg L^{-1} suspension on Nutrient Agar to 6090 CFU ml^{-1} in a 2.0 mg L^{-1} suspension on Tryptic Soy Agar. These counts are close to the guaranteed four billion CFU g^{-1} at but still short of the expected 8000 CFU ml^{-1} in a 2.0 mg L^{-1} suspension.

The standard plate count method was appropriate for detecting the ability of microbial products to augment natural populations of bacterioplankton. However, direct counting of bacteria by epifluorescence microscopy is considered clearly the best method available for making total counts of bacteria in water (Austin 1988). Using epifluorescence, total bacterioplankton numbers are generally reported to be on the order of 10^6 ml^{-1} in a variety of surface waters (Salonen et al. 1994, Pace and Cole 1996, Tuomi et al. 1997, Ooms-Wilms 1997). Using the standard plate count method, bacterioplankton counts in untreated pond water were on the order of 10^3. At the recommended application rate of about two mg L^{-1}, microbial products add on the order of 10^3 bacteria ml^{-1} to the water. Therefore, the epifluorescence method which routinely produces bacterial counts on the order of 10^6 bacteria ml^{-1} is probably too sensitive to detect significant changes in bacterial numbers after the application of microbial products. The standard plate count method allows confirmation that the numbers of viable bacteria in the water are augmented to a degree consistent with product labeling. Using the plate count method also allowed for the identification of distinct colonies that resulted from microbial product treatments. Bacterial counts from these studies are consistent with those from a similar study by Queiroz and Boyd (1998) who also used the standard plate count method to determine bacterial abundance in catfish ponds treated with a microbial product.

There are several possible explanations for the observed lack of microbial product efficacy in the barrel and greenhouse studies. As mentioned, the experimental conditions might not have been optimal for identifying treatment effects of algal control via bacterial competition for nutrients. However, effects from the Hydrothol 191™ treatment were obvious in the barrel study. Additionally, there was a clear and consistent trend of increasing chlorophyll concentrations with increased nutrient supply and increased application rate of a microbial product in the greenhouse study (Figure 1). Other possible explanations might be related to the lack of regulation seen in the pesticide industry that provides some assurance of product efficacy and consistency. The shelf-life of some microbial products might not be very long. Poor viability or survivability of the freeze dried bacterial cells in the products following application to lake water is another possibility. Bacterial populations in natural waters may be largely unaffected by the addition of more bacteria because bacteria

TABLE 1. VIABLE CELLS COUNTED AS CFUS (COLONY FORMING UNITS) IN 2 MG L^{-1} SUSPENSIONS OF THE MICROBIAL PRODUCTS ALGAE-TRON, AQUA-5, BIOZYME, BIORESTORATION FORMULA-2, NEW LAKEPAK, OLD LAKEPAK, 1.0 ML OF UNTREATED POND WATER AND 1.0 ML OF SEDIMENT ON THE FOUR MEDIA BEEF EXTRACT AGAR (BX), PLATE COUNT AGAR (PC), TRYPTIC SOY AGAR (TSA) AND NUTRIENT AGAR (NA). A 0.1 ML INOCULUM WAS SPREAD ON EACH PLATE AND THEN INCUBATED AT 37C FOR 24 HOURS.

Microbial Product	(BX)	(PC)	(TSA)	(NA)
	No. cells per ml			
Algae-Tron[a]	—	—	600	490
Aqua-5	13730	15680	30130	10250
Biozyme	—	—	4300	1000
Formula-2[a]	—	—	1000	950
new LakePak	1930	3320	6090	1700
old LakePak	0	0	26	0
pond water	4660	940	1800	—
pond sediment	1.3×10^6	0.23×10^6	0.36×10^6	—

[a]Product samples over three years old.
"—" = Not tested.

are limited by chemical, physical and biological factors of the environment and not simply by a lack of bacterial propagules (Boyd 1995). These studies were limited but provided some basic information about microbial products that was subsequently used in a more rigorous pond enclosure evaluation, the result of which are also published in this issue.

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