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# Pond Enclosure Evaluations of Microbial Products and Chemical Algicides Used in Lake Management

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## ABSTRACT

In the fall of 1998 and the spring of 1999, pond enclosure studies were conducted to quantify the effects of the commercially available bacterial inocula Aqua-5™, BactaPur™, a 1998 formulation of LakePak™ WSP® and the algicides copper sulfate and diquat on phytoplankton, macroalgae, submersed macrophytes, zooplankton, bacterioplankton and sediment bacteria. One day after treatment, bacterioplankton numbers in the enclosures treated with the microbial product, Aqua-5™ were significantly augmented relative to the non-treated control, 9,300 and 2,200 cells $ml^{-1}$, respectively. Three days after treatment, bacterioplankton numbers increased in the diquat treatments to 78,000 cells $ml^{-1}$ and submersed macrophytes appeared necrotic and showed signs of decomposition. Copper sulfate and diquat treatments significantly affected phytoplankton, macroalgae, submersed macrophytes and zooplankton, but applications of the microbial products Aqua-5™, BactaPur™, LakePak™ WSP® at recommended rates did not significantly affect those water quality variables. Sediment bacteria were not significantly affected by any of the treatments. Under these experimental conditions, bacterial augmentation with the products Aqua-5™, BactaPur™ and LakePak™ WSP® did not significantly reduce planktonic algae growth. These results provide no indication that inoculations of lakes and ponds with commercial preparations of the bacteria tested reduce algal growth.


*Key words:* algae control, bacteria, bacterioplankton, bacterial augmentation, biological control, phytoplankton, zooplankton.

## INTRODUCTION

Planktonic bacteria and phytoplankton are important components in the cycling of inorganic nutrients and organic matter in aquatic ecosystems. Studies have shown that bacteria and phytoplankton may affect each other positively or negatively, depending on the nutrient concentrations of their environment (Azam et al. 1983, Wang and Priscu 1994, Kamjunke et al. 1997). Because bacteria have a high surface area to volume ratio and a high uptake rate for nutrients, it has been suggested that bacteria should be superior competitors with phytoplankton for nitrogen and phosphorus (Currie and Kalff 1984, Elser et al. 1995). In 1972, Rhee demonstrated that the growth of the alga *Scenedesmus* sp. could be suppressed by the presence of the bacterium *Pseudomonas* sp. in mixed cultures. Brett et al. (1999) have shown that phytoplankton and bacterioplankton are each limited by inorganic nutrients and that this nutrient limitation explains the commonly observed positive correlation between phytoplankton and bacterioplankton. These authors have concluded that competition for nutrients may be a critical aspect of phytoplankton-bacterioplankton interactions.

Interactions between bacteria and phytoplankton in lakes and other aquatic ecosystems are however more complicated than simple competition for nutrients (Kirchman 1994). Brett et al. (1999) suggest that the underlying mechanisms behind the positive correlation between bacterioplankton and phytoplankton are tangled in complex interactions between factors such as inorganic nutrient concentrations, organic nutrient availability, protozoan bactivory, availability of physical substrates, as well as light and temperature. Such complications could prevent augmented bacterial populations from having significant effects on phytoplankton. In recent mesocosm experiments by Cottingham et al. (1997), bacteria did not buffer phytoplankton responses to nutrient enrichment.

Despite complex microbial community interactions and our current incomplete resolution of the factors that control natural microbial rates and processes (Karl 1994), a practice of treating lakes with bacteria has been developed and commercially marketed. This relatively new practice, derived from the wastewater treatment industry (Nesbitt 1995) is based on the simple idea that bacteria added to a lake will out-compete algae for nitrogen and phosphorus. Commercial preparations of bacteria and enzymes, called "microbial products", are now used in lake management as biological alternatives to conventional algicides. There are many microbial products now being used for water quality management in aquaculture ponds (Moriarty 1997) and lakes. The practice, called bioaugmentation or biostimulation, is often aimed at preventing or reducing nuisance algae growth. Microbial product treatments are supposed to add enzymes and bacteria in order to stimulate or augment existing populations of bacteria and result in the consumption of organic debris and dissolved nutrients (King 1996).

Not surprisingly, the number of microbial products is increasing at a time when the use of traditional chemical algicides is becoming more restricted (Nesbitt 1995). Microbial

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products are purported to be environmentally friendly alternatives to copper sulfate and other commonly used algicides. Copper sulfate, used since the nineteenth century to control nuisance algae (Murphy and Barrett 1990), has caused recent concerns over fish toxicity and sediment contamination, making its use less popular or even prohibited in many lakes. Because of these and other environmental concerns, microbial products are currently in high demand and new firms are continually introducing their own version of a "biological water quality enhancer" to this growing market. Despite the increased marketing of microbial products, their role in applied limnology has not been scientifically evaluated.

In order for bacteria to be effectively used in the prevention or reduction of nuisance algal growth, the bacterial uptake of nutrients would have to be stimulated to a point where ambient nutrient concentrations are reduced below that which would be limiting to the nuisance algae. This could be achieved by increasing the number of the bacterioplankton in the system. However, simply increasing the total number of bacterial cells may not be enough to reduce algal growth if bacteria are not taking up sufficient nutrients to limit supplies for algae. Planktonic bacteria can be grouped into two sub populations according to their activity. They can exist in a lake either as dormant, or dead, cells or as metabolically active cells (Mason et al. 1986). In regard to augmenting algal-bacterial competition, a reduction in algal growth could occur only if the number or activity of cells in the active subpopulation increased. Research is needed to establish what effects microbial products have on the active and dormant bacterial sub-populations in lakes.

Recent studies suggest that there is significant, natural regulation of the total number and activity of aquatic bacteria (Pace and Cole 1994). Poor environmental conditions, depleted inorganic and organic nutrients, competition, selective grazing of bacterivores on active bacteria, lytic virus infection and cell inactivation are all possible mechanisms regulating bacterial abundance, activity and biomass in aquatic ecosystems. However, controversy surrounds what little is known about the balance between direct and indirect factors regulating aquatic bacteria and whether bacterial communities are controlled by limiting resources or bacterivorous consumers (Pace and Cole 1996, Cottingham et al. 1997, Zohary and Robarts 1998). Regardless of this controversy, if bacterial activity is low it is because biological, chemical or physical factors are preventing an increase in the number or activity of bacteria, not because of a shortage of reproductive bacterial cells (Boyd 1995).

Bacterial augmentation products have been investigated for the wastewater treatment industry. The use of bacteria in the cleanup of sewage and environmentally toxic pollutants is well-established (Madigan et al. 1997). Specialized cultures of bacteria and blends of enzymes have been available to the wastewater treatment industry for fifty years (Horsfall 1979). Microorganisms, selected from naturally occurring populations or created through genetic modification, have been successfully used to enhance the removal of specific pollutants such as aromatic hydrocarbons, halogenated aliphatics and heavy metals in wastewater treatment processes (Chin et al. 1996, Madigan et al. 1997). Specific bacteria are marketed in the wastewater treatment industry for specific purposes, although some products are purported to provide general benefits such as enhanced treatment stability, increased efficiency and improved performance (Hyde 1981). Hung et al. (1986) report that bioconversion of accumulated sludge during the period of January to August, 1984, was enhanced with bacterial augmentation with the product Liquid Live Micro-Organisms (LLMO®), when compared to the treatment-plant performance during the period of January to December, 1983. Hung et al. (1986) also report general improvements such as odor reduction and energy savings but do not explain the mechanism responsible for these observed benefits. Critics note the lack of controlled experiments to evaluate the general benefits of these products (Hyde 1981). Generally, bacterial augmentation products have been shrouded in proprietary mystery, as to both their composition and their activity in the treatment facility (Horsfall 1979) and the value of this practice has not been established (Boyd 1995).

More recently, microbial products were introduced for the management of aquaculture ponds. Termed "probiotics" in aquaculture, Jory (1998) defines them as, "cultures (single or mixed) of selected strains of bacteria that are used in culture and production systems (tanks, ponds and others) to modify or manipulate the microbial communities in the water and sediment, reduce or eliminate selected pathogenic species of microorganisms, and generally improve growth and survival of the targeted species". In several aquaculture studies, the addition of microbial products did not significantly affect inorganic nitrogen, total phosphorus, soil respiration, chlorophyll *a* or the numbers of bacteria and phytoplankton (Chiayvareesajja and Boyd 1993, Boyd and Pippopinyo 1994, Boyd et al. 1984). Queiroz and Boyd (1998) similarly report few significant differences in water and bottom soil quality in channel catfish ponds but do report significant increases in survival and net production of fish. However, Queiroz and Boyd (1998) do not suggest a mechanism for these increases and the level of confidence in their determination of significance is questionable as they used only three treatment ponds and three control ponds together with a 0.1 probability level. The use of microbial products in lake and pond management is an area requiring much research on the potential for using these products (Jory 1998).

Objective studies are needed to evaluate the efficacy of microbial products for algae control, establish their role in applied limnology and examine the effects that these unregulated products have on non-target organisms. One goal of this study was to clarify the question of efficacy surrounding microbial products. By obtaining data from carefully controlled experiments, one can determine whether microbial products are useful in reducing algal growth in lakes and if they are efficacious alternatives to commonly used chemical algicides. The objective of this study was to quantify the effects of microbial products and chemical algicides on naturally occurring populations of phytoplankton, macroalgae, submersed macrophytes, zooplankton, bacterioplankton and sediment bacteria.

## MATERIALS AND METHODS

Laboratory and pond experiments were conducted between June 1998 and July 1999 at the USDA/ARS Exotic and Invasive Weed Research Laboratory at the University of Cali-

fornia, Davis, California. Table 1 describes the water quality of the experimental ponds and the well that supplies water to the laboratory and ponds.

*Sources of microbial products.* LakePak™ WSP® (a 1998 formulation), manufactured by Becker-Underwood Inc., Ames, Iowa, and Aqua-5™, manufactured by Environmental Alternatives Inc., Ventura, California, were obtained from agricultural chemical suppliers in northern California. Bacta-Pur™, manufactured by International Ecological Technologies Inc., North Hatley, Quebec, Canada, was purchased from an aquaculture supply catalog and shipped from Florida. Copper sulfate and the aquatic herbicide diquat, [6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide] (trade name Reward®), manufactured by Zeneca Inc., Wilmington, Delaware, were available from research supplies at the USDA Laboratory.

LakePak™ WSP® and Aqua-5™ were provided as powder-like products. BactaPur™ was provided as a two-part product consisting of a nutrient powder and liquid suspension of bacteria. Recommended application rates vary between products and often the individual labels offer a range of treatment rates depending on water temperature and the degree of algal infestation. A recommended application rate of between one and two mg $L^{-1}$ is common for most products. To ensure consistency in the comparison of products, equal weights of each product were used in these studies. The rate for Bacta-Pur™ was the only exception where a volumetric rate specific for the liquid suspension of bacteria was used.

## Laboratory Studies

*Viable cell counts and nutrient concentrations of microbial products.* Because very few data are available for microbial products, the viability of bacterial cells and nutrient concentrations in the products Aqua-5™ and LakePak™ WSP® were examined. CFU (colony forming units) counts and nutrient analyses were not conducted for the product BactaPur™ because the product was not purchased until after the preliminary laboratory studies were completed. Using standard serial dilution and agar plate spread techniques, viable cells were counted as CFU on agar plates. The carbon (C) and nitrogen (N) contents of the products were determined using a Perkin-Elmer model 2400 CHN analyzer with acetanilide used as a standard. Phosphorus concentrations measured as free orthophosphate ($PO_4$3-) were determined colorimetrically using a Beckman DU-64 spectrophotometer and the ascorbic acid method described in APHA Standard Methods (1980).

Several media commonly used for the culture of a wide range of microorganisms (Atlas 1995) were also examined to determine the best for the culture of the bacteria in microbial products. Those media included: 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), Trypticase® 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). Premixed agar preparations were used for Plate Count Agar and Trypticase® Soy Agar. A premix was not available for the Beef Extract Agar. All of the agar media were purchased from UC Davis Veterinary Medical Supply. Agar plates were prepared in the laboratory using aseptic techniques. In one-liter Erlenmeyer flasks, 700 ml of the aforementioned media suspensions were autoclaved for 30 minutes. Approximately 25 ml of molten agar was poured into pre-sterilized 100 mm by 15 mm disposable polystyrene petri dishes.

Agar plates were inoculated by spreading 0.1 ml aliquots from dilutions of the microbial product suspensions, untreated pond water and untreated suspensions of pond sediment. The plates were then incubated aerobically at 37C for 24 h. Preliminary results showed that cooler incubations for longer periods of time were problematic because actinomycete colonies would spread across large portions of the plates and interfered with colony development and counting. Similarly, with longer incubation times, some of the microbial product treatments would produce fast-growing bacterial colonies that spread over other colonies and obscured resolution of individual colonies. The microbial product and sediment suspensions were prepared by adding two mg of each product to a separate one liter Erlenmeyer flask containing 1000 ml of sterile distilled water. Each flask was then mixed for five minutes before serially diluting and plating the suspensions. On each of the aforementioned media, mean CFU counts for the microbial products were then compared with untreated pond water and sediment samples. Aseptic technique was confirmed by inoculating one in ten plates with only 0.1 ml of sterile saline buffer used for the serial dilutions.

## Pond Studies

*Experimental design.* Enclosure experiments were conducted in four 61,000 L ponds (11 m by 11 m) in the fall of 1998 and spring of 1999. In the center of each pond, open-ended Lexan™ tubes (38 cm diameter and 122 cm height) were pushed in to the clay bottom to form water-tight, 100 L experimental enclosures (1 m deep). Each of the four ponds

TABLE 1. PHYSICAL AND CHEMICAL CONDITIONS OF WELL WATER AND PONDS USED IN THE EXPERIMENTS.

| Location | DO mg $L^{-1}$ | Temp. fall[a] C | Temp. spring[b] C | pH | Hardness mg $L^{-1}$ | TKN mg $L^{-1}$ | $NH_4$-N mg $L^{-1}$ | $NO_3$-N mg $L^{-1}$ | P mg $L^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Well | 1.8 | 24 | 24 | 7.96 | 130 | ** | ** | 30 | 0.2 |
| Ponds | 13.6[c] | 16.2 | 24.3 | 8.95 | 150 | 1.3 | 0.13 | <0.05 | 0.06 |

[a]30 day mean water temperature during fall 1998 study.
[b]30 day mean water temperature during spring 1999 study.
[c]11:30 AM at 0.5 m depth and 17C.

received 24 of the enclosures and functioned as a randomized complete block. In each block, three microbial treatments (Aqua-5™, LakePak™ WSP® and BactaPur™), two chemical treatments (copper sulfate and diquat) and one non-treated control were replicated four times. With four complete blocks, treatments were replicated 16 times so that natural variability could be accounted for in evaluating algal growth suppression.

The microcosms enclosed the naturally occurring aquatic communities present in the ponds. The water was mixed among the four ponds to make the planktonic communities more uniform prior to the setting of the tubes. Macrophytes were manually removed from the ponds before the enclosures were set but the naturally occurring seed bank, vegetative propagules and the benthic fauna remained. Aeration streams were used for daily mixing of each microcosm. A timer was set to operate the aeration streams for three hours just prior to sunrise.

The treatments were applied according to the manufacturers' labels. Aqua-5™ and LakePak™ WSP® were applied at the rate of 2.0 mg $L^{-1}$. BactaPur™, a two-part product was applied at the rate of 136 mg $L^{-1}$ for the NutrientPak™ followed by 118 µl $L^{-1}$ of the bacterial suspension, N3000™. The two chemical treatments, copper sulfate and diquat, were applied at the rate of 1.0 mg Cu as copper sulfate $L^{-1}$ (15.7 µmol Cu) and 2.7 µl diquat $L^{-1}$ (0.37 ppm diquat, ai) respectively. Three days after treatment a nutrient solution was added at a rate of 1.1 ppm N, and 0.3 ppm P to stimulate algal growth.

*Bacteria.* Heterotrophic bacterioplankton populations were monitored by the indirect method of serial dilution and plate count. An agar-based counting method was used instead of more sensitive microscopy procedures that can accurately determine total cell numbers. The goal was to compare the number of viable bacteria in water samples treated with microbial products with non-treated control and confirm that the number of viable bacteria in the water were augmented to a degree consistent with product labeling. Additionally, preliminary studies had shown that distinct colonies resulting from microbial product treatments were recognizable on agar plates.

Five hundred-ml water samples taken from ten centimeters below the water surface were collected four days before treatment, one hour after treatment, and three, nine and 16 days after treatment. Samples were serially diluted in an osmotic buffer and 0.1 ml aliquots from dilutions of $10^{-1}$, $10^{-2}$ and $10^{-3}$ were then spread on Trypticase® Soy Agar plates. Fresh samples were maintained at ambient temperatures and processed within one hour of sample collection. Colonies were counted manually on a New Brunswick Scientific plate counter after a 24 h aerobic incubation at 37C.

Sediment samples were collected with a glass tube (122 cm long by 8 mm diameter) from the top centimeter of sediment. Twenty-ml samples were taken three days before treatment and four and 19 days after treatment. Dilutions of $10^{-4}$, $10^{-5}$ and $10^{-6}$ were used for plating sediment samples. Populations of sediment bacteria were otherwise monitored using the same indirect methods of serial dilution and agar plate spread as those used for the bacterioplankton.

*Phytoplankton.* Phytoplankton populations were monitored as the chlorophyll *a* concentration in water samples. Water samples were collected two days before treatment, and one, three, nine, 11, 16 and 18 days after treatment. Five hundred-ml samples were collected from ten centimeters below the surface. Phytoplankton were vacuum-filtered onto Whatman 42.5-mm diameter glass microfiber filters. Chlorophyll pigments were extracted from phytoplankton by placing the loosely rolled filter papers in 10 ml dimethyl sulfoxide and heated at 65C for 10 minutes as described by Burnison (1980) and Spencer and Ksander (1987). Chlorophyll *a* and pheophytin *a* concentrations were determined using a Beckman DU-64 spectrophotometer and methods described in APHA Standard Methods (1980).

*Zooplankton.* Zooplankton were homogeneously mixed at the beginning of the experiments before the enclosures were set. Samples were collected 30 days after treatment in the fall 1998 study. Samples were collected again after the winter interim, i.e. before the spring 1999 treatment and 28 days after treatment in the spring 1999 study. Twelve liters of water were sampled by pulling a 12.75-cm diameter zooplankton net up the mixed, one-meter water column enclosed by each Lexan™ tube. Samples were preserved with a 1% final concentration of acid Lugol solution for the later enumeration of cladocerans, copepods, nauplii and larger rotifers. These rotifers resembled *Euchlanis dilatata, Brachionus* spp. and *Keratella quadrata* but were not positively identified by an expert.

*Macrophytes.* Filamentous algae and higher aquatic plants were harvested from the enclosures at the end of the fall 1998 study, after the winter interim and at the end of the spring 1999 study. Filamentous algae growing at the surface of the enclosures were easily harvested with a small net. However, a 30-cm blade attached to the end of a two-meter pole was required to first cut the sessile macrophytes from the bottom before they could be harvested with a hand held net. After separating the algae from the vascular plants, which were predominantly *Zannichellia* sp. and *Eleocharis* sp., the macrophytes' fresh and dry weights were recorded.

*Statistical treatments.* Dunnett's least significant difference method was used to compare the control with each of the other treatments at a 95% simultaneous confidence level. All significant differences reported were determined using Dunnett's LSD method. Because the pond enclosure studies were first time field trials, a more liberal analysis, the Fisher's protected least significant difference was also used for comparison and to avoid rejecting significant results that may be obscured by highly variable field conditions.

## RESULTS AND DISCUSSION

Using the indirect method of serial dilution and agar plate spread to determine bacterial populations, it was confirmed that the microbial products Aqua-5™ and 1998 LakePak™ WSP® contained viable bacteria in concentrations sufficient to significantly augment total bacterioplankton populations (see Table 1 in the previous article, page 98). CFU counts were generally in agreement with advertised claims and contained viable bacteria concentrations on the order of a billion per gram or a trillion per pound. The label information included with the product, LakePak™ WSP®, guaranteed a minimum bacterial count of four billion CFU per gram. This count is equivalent to 8,000 CFU $ml^{-1}$ in a two

mg $L^{-1}$ suspension. The product 1998 LakePak™ WSP® produced a mean CFU count of 6090 (n = 12) from two mg $L^{-1}$ suspensions, however, one of the replicated plates was above the guaranteed 8,000 CFU count. The product Aqua-5™ produced the highest mean colony counts, above 10,000 CFU. Naturally occurring bacteria from both the sediment and water column were most effectively cultured on Beef Extract Agar. Tryptic Soy Agar produced the highest CFU counts for the microbial products examined and naturally occurring bacteria grew reasonably well on this medium. Therefore, Tryptic Soy Agar was used exclusively for samples from the pond-enclosure experiments.

Bacterial populations were significantly augmented, compared to the non-treated control ($P < 0.0001$), by the product Aqua-5™ on the first post-treatment sampling date in both the fall 1998 (Figure 1A) and spring 1999 (Figure 1B) studies. Those augmented populations quickly declined in the fall 1998 study and by the second post-treatment sampling date, three days after treatment, no longer differed significantly from the non-treated control. The Aqua-5™ treatments had a longer lasting effect in the spring 1999 study when bacterioplankton populations remained significantly higher than the control ($P < 0.0001$) through the second post-treatment sampling date, also three days after treatment. Agar plates from the Aqua-5™ treatments had many colonies that were much larger and faster growing than colonies from all the other treatments (data not shown). Using Dunnett's LSD analysis there were no significant increases in the number of bacterioplankton, relative to the control ($P > 0.016$), measured as CFU, on any sampling date due to the products LakePak™ WSP® and BactaPur™. However, using the more liberal Fisher's PLSD analysis, significant increases in bacterioplankton numbers, ($P < 0.05$) relative to the control, were detected following the LakePak™ WSP® treatments. In both the fall 1998 and spring 1999 studies, significant increases in bacterioplankton were seen the diquat treatments by the third day post-treatment compared to the control ($P < 0.0001$). These increased bacterial numbers preceded the nutrient additions. In the diquat treatments, significantly higher bacterial populations were present for several days before the start of significant increases in phytoplankton growth (Figures 1A and 1B and Figures 2A and 2B, respectively). Sediment bacteria were not significantly affected by any of the microbial product treatments relative to the non treated control on any sam-

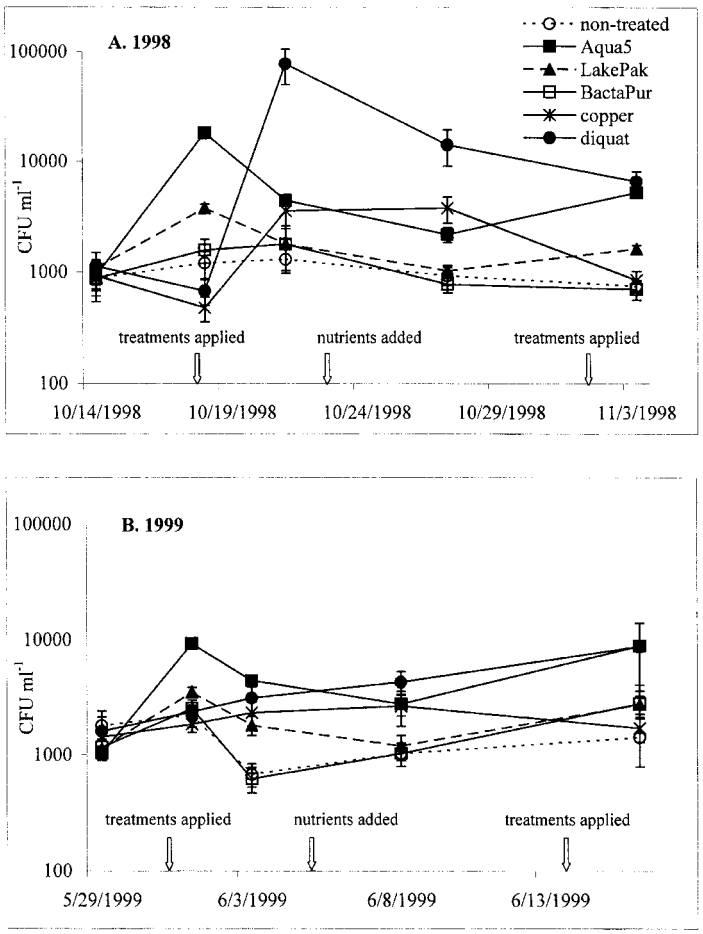


Figure 1. Mean bacterioplankton in 100-L enclosures measured as CFU (colony forming units) in (A) fall 1998 and (B) spring 1999 following application of Aqua-5™, BactaPur™, LakePak™ WSP®, copper sulfate and Reward® (diquat). Bars indicate standard errors of the mean, N = 16.

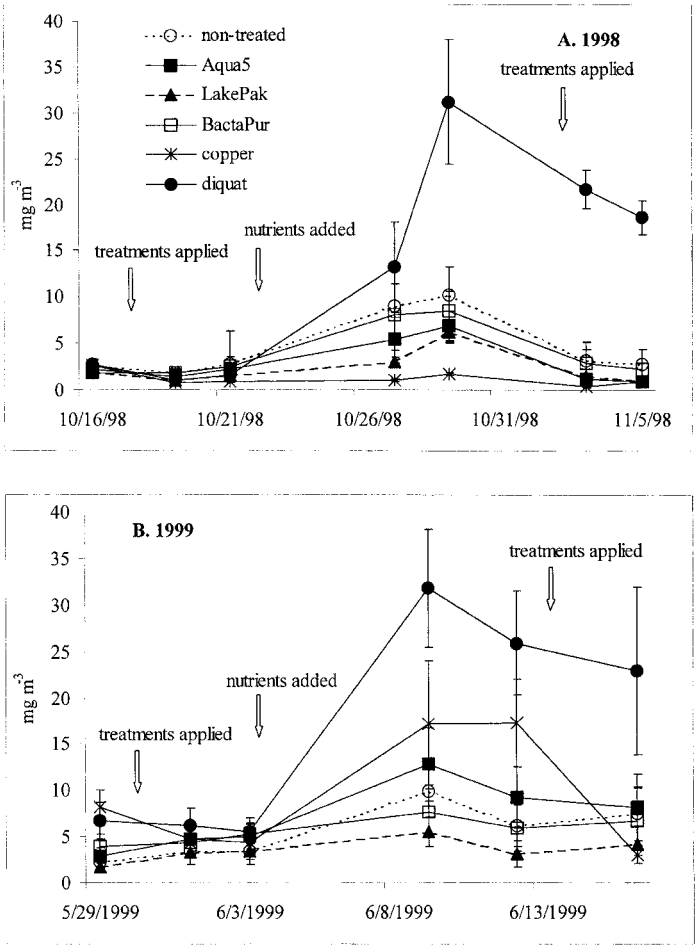


Figure 2. Mean chlorophyll *a* concentration in 100-L enclosures in (A) fall 1998 and (B) spring 1999 following application of Aqua-5™, BactaPur™, LakePak™ WSP®, copper sulfate and Reward® (diquat). Bars indicate standard errors of the mean, N = 16.

pling dates (data not shown). However, three weeks after treatment, sediment bacteria in the diquat treatments significantly increased relative to the control ($P < 0.05$).

In both studies and on all sampling dates, there were no significant decreases ($P > 0.1$) in phytoplankton growth due to the microbial products compared to the non treated control (Figures 2A and 2B). There were significant reductions in phytoplankton growth from the copper treatment in the fall 1998 study compared to the control ($P < 0.01$). The following spring however, pre-treatment chlorophyll concentrations in the copper treatment were significantly higher than the control and all three microbial treatments ($P < 0.02$). The copper treatments suppressed algal growth in the fall 1998 study but by the third application, which occurred at the beginning of the spring 1999 study, appeared to be no longer effective in preventing algal growth. The phytoplankton in the copper treatments might have become acclimated to higher copper levels or selection for copper resistant species could have occurred. Following the nutrient additions in the spring 1999 study, algal growth was significantly higher in the copper treatment than in the control ($P < 0.05$). Several days after treatment, the phytoplankton bloomed in the diquat treatments and was dramatically higher than the control ($P < 0.0001$). These algae blooms occurred in the treatments despite the significantly larger populations of bacterioplankton present in the enclosures prior to the nutrient additions.

The microbial products did not significantly affect cladocerans, copepods, nauplii or rotifers compared to the control (Figures 3A, 3B, and 3C). However, the fall 1998 applications of copper sulfate and diquat essentially eliminated all zooplankton from the enclosures. By the next spring, zooplankton populations had recovered from the fall chemical treatments and populations were no longer significantly lower than populations in the control. The spring chemical treatments produced less dramatic, but significant ($P < 0.01$) reductions in zooplankton populations relative to the control.

Filamentous algae and aquatic vascular plants were not significantly affected by any of the microbial products compared to the control (Figures 4A, 4B and 4C). In both studies, the copper sulfate and diquat treatments significantly reduced filamentous algae biomass compared to control (0.001). Copper sulfate also had a less dramatic, but significant effect on vascular plant biomass in both studies ($P < 0.02$). On all sampling dates, vascular aquatic plant biomass was significantly reduced by the aquatic herbicide diquat ($P < 0.0002$).

Variability of results in field experiments and variations in water quality among similar ponds are inherently high (Boyd et al. 1979, Horne and Goldman 1994). Such variability was expected for the pond-enclosure studies, so 16 replicated enclosures were used for each treatment in a randomized complete block design. Under these experimental conditions, none of the apparent differences in algal growth due to the microbial products were significant, even with a liberal statistical interpretation. Despite the high variability in the pond studies (coefficients of variability of 50 to 90%), significant decreases in algal growth were observed in the fall 1998 copper sulfate treatments. Additionally, significant reductions of vascular aquatic plant biomass and significant increases of phytoplankton were seen with the diquat herbicide treatments in both studies.

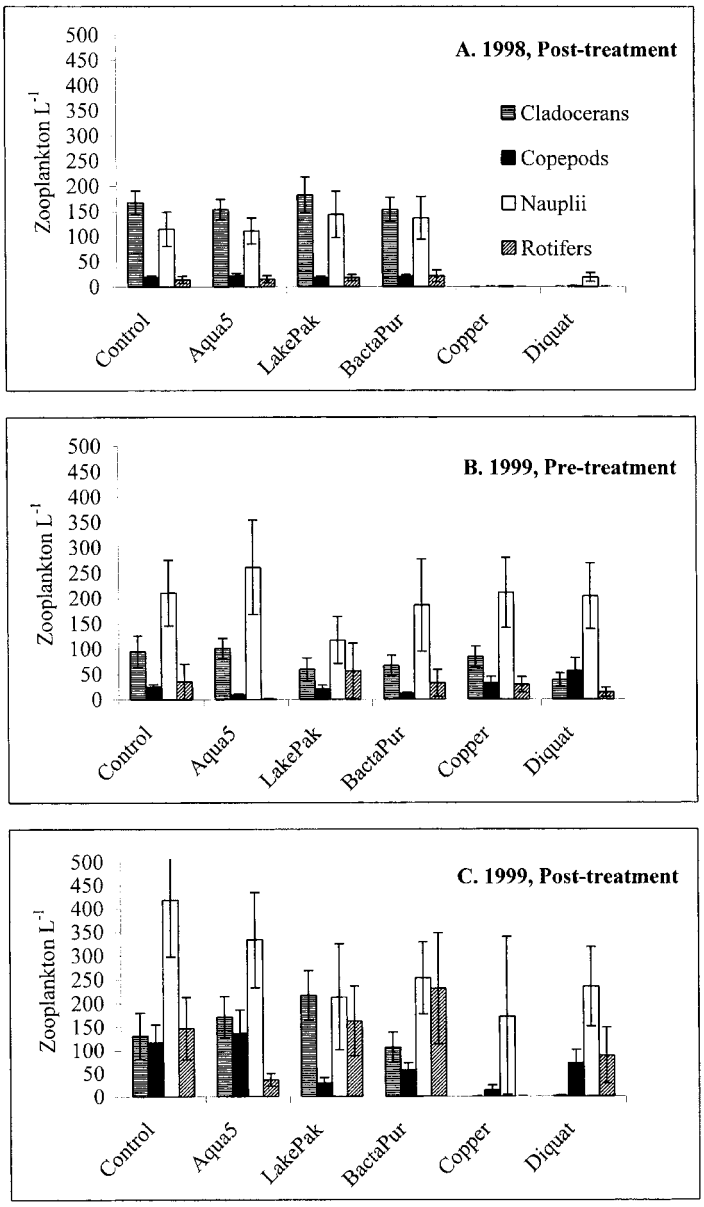


Figure 3. Mean zooplankton abundance in 100-L enclosures (A) one month post-treatment fall 1998, (B) pre-treatment spring 1999 and (C) one month post-treatment spring 1999. Bars indicate standard errors of the mean, N = 16.

The use of microbial products to control nuisance algae or otherwise improve water quality has not been established. The results of this study are consistent with similar studies conducted in aquaculture (Boyd et al. 1984, Chiayvareesajja and Boyd 1993, Boyd and Pippopinyo 1994, Queiroz and Boyd 1998) in that there are no indications that microbial products reduce chlorophyll concentrations or control algal growth. These results suggest that a degree of skepticism is warranted when considering microbial products for lake management. In contrast to the studies of Boyd et al. (1984) and Queiroz and Boyd (1998), who did not detect significant differences in bacterial numbers following the application of microbial products, significant increases in bacterial populations were detected in our studies with some of the treatments.

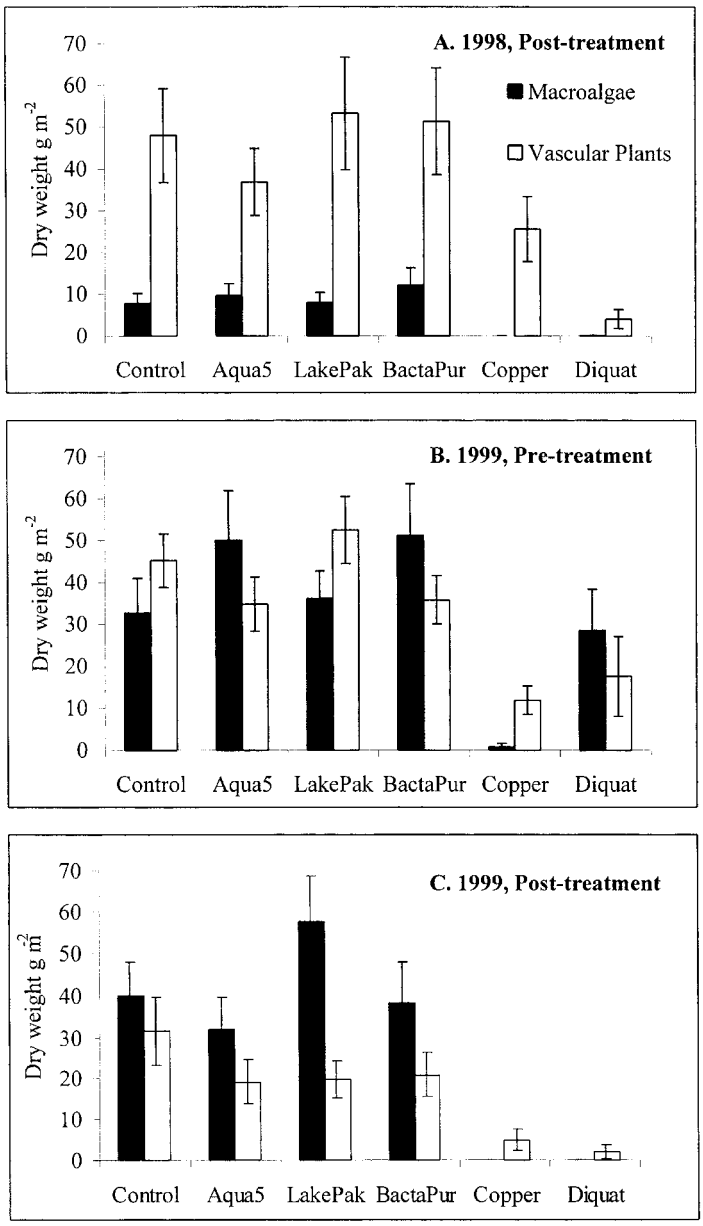


Figure 4. Biomass in grams dry weight $m^{-2}$ of macroalgae and vascular aquatic plants in 100-L enclosures (A) one month post-treatment fall 1998, (B) pre-treatment spring 1999 and (C) one month post-treatment spring 1999. Bars indicate standard errors of the mean, N = 16.

Some successes have been reported with the use of microbial products in wastewater treatment, especially in the targeted bioremediation of manmade pollutants (Horsefall 1979, Hyde 1981, Hung 1986, Chin 1996, Madigan et al. 1997). Thus, it is not surprising that microbial products used in lake management have been developed from similar bacterial products used in the wastewater treatment industry. It should be noted that adding bacteria to highly concentrated wastewater liquor before or during the treatment process is quite different than adding bacteria to natural aquatic ecosystems. Wastewater may have an insufficient population of bacteria needed to breakdown a specific manmade pollutant in the time it takes to complete the treatment process. The microbial environment of sewage, with high organic matter and biochemical oxygen demand is very different than conditions in lakes in which bacteria and phytoplankton are in quasi equilibrium. Enhancing the treatment of manmade pollutants and high-strength sewage is quite different from attempts to indirectly reduce phytoplankton growth with bacterial augmentation in natural ecosystems. There is still controversy over the role of microbial products in wastewater treatment and it is unclear how applicable these processes are to lake management. As a result, the direct transfer of bacterial augmentation technology from wastewater treatment to lake management may not be possible.

The products Aqua-5™ and LakePak™ WSP® significantly increased bacterioplankton populations (measured as CFU on agar plates) and therefore the observed lack of efficacy in reducing planktonic algae was not due to the failure of the products to significantly increase bacterial populations. The products augmented bacterial populations as claimed and supplied the advertised number of CFU. One explanation for why the products did not reduce algal growth is that the total bacterial uptake of nutrients, or the bacterial activity, may not have been effective even though the number of bacteria increased. If bacterial activity, or growth, was stimulated by the microbial product treatments, then bacterial populations should have remained large or even increased relative to the control. While the products did increase the number of bacteria for a short time, augmented populations quickly declined (Figures 1A and 1B). These results suggest that increases in bacterial numbers do not necessarily result in increased bacterial competition with phytoplankton for nutrients. Additional research is needed to better understand the natural mechanisms controlling the proportion of metabolically active and dormant cells in aquatic ecosystems. The lack of microbial product efficacy observed in these experiments is most likely the result of microbial products having been developed on an incorrect or over-simplified assumption that adding bacteria to an aquatic ecosystem will control algae through competition for nutrients.

Microbial products are generally used by lake managers and pond owners to control nuisance algae. However, microbial products are technically not considered algicides. The anti-algal claim is only implied because microbial product advertisements and labeling information are worded in a way that avoids making claims of algae control. The products are generally purported to provide biological enhancement through the supply of beneficial bacteria which out-compete algae for nitrogen and phosphorus. This seemingly trivial technicality is important because it means that state agencies and the United States Environmental Protection Agency do not regulate microbial products as algicides. Therefore, efficacy data and information on the effects of microbial products on non-target organisms that would otherwise be required for the registration of an algicide are not available.

While these experiments did not provide evidence that microbial products are effective in reducing algal growth, microbial products do appear capable, at least in the short term, of significantly increasing bacterioplankton populations. Additional studies are needed to investigate the effects of microbial products on nutrient flow in aquatic ecosystems. More detailed experiments using microbial products or oth-

er bacterial treatments to manipulate bacterial numbers or metabolic activities would also provide insight into microbial trophic interactions and how the management of aquatic bacteria might be effectively integrated with current algae and aquatic weed control tactics.

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