PREPRINT

Manipulation of microbial ecosystems to control the aquatic plant Eurasian water milfoil

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Eurasian water milfoil (*Myriophyllum spicatum L.*) is a particularly aggressive plant that can out-compete most plant species. The extensive growth of this plant, in many regions of the world, is a serious problem that interferes with many uses of waterbodies[1-2]. To date, no widely applicable and especially non-toxic method has been found to control this plant[3-6]. The over abundance of vascular aquatic plants or algae is a symptom due to excessive nutrients[7]. Bioaugmentation or bacterial augmentation is the seeding of communities of water purifying microorganisms. This approach has been used in wastewater treatment plants[8-10] and aquacultural systems[11] to increase rates of nutrient removal. Two 4-month trials demonstrated repeatedly that bioaugmentation reduced the growth and regeneration of Eurasian water milfoil, inspite of continuous pollutional loading. The results show that it cannot be assumed that natural populations of bacteria are always adequate, that beneficial manipulation of microbial ecosystems is both possible and practical, and furthermore that reversal or at least slowing down of the eutrophication process may be achievable.

Efficient water purification requires balanced microbial communities due to the genetic simplicity of individual strains[13]. Accordingly, absence of appropriate parts of the community can be expected to slow the purification process or accelerate eutrophication. The ubiquity principle states that bacteria can be found anywhere; it does not state that all bacteria may be founded in any specific environment all the time[9]. The peak occurrences of lipase and protease, in a lake receiving aquacultural wastes, do not necessarily occur at the same time of year[13]. Clearly, lack of either of these activities will decrease water purification efficiency contributing to deterioration of water quality, accumulation of organic compounds, growth of algae and/or macrophytes and to general eutrophication.

Eurasian water milfoil is a nuisance species. It is a perennial submergent aquatic plant originally endemic to Europe, Asia and parts of Africa[14-15]. Its more recent geographical range has been described as "cosmopolitan" as indicated by its worldwide geographic distribution[2].

Eurasian water milfoil is most common in hard, nutrient rich, alkaline waters and possess a number of adaptations, which allow it to proliferate: rapid vegetative propagation, an opportunistic nature for obtaining nutrients, a life cycle that favors cool weather, and a number of mechanisms which enhance photosynthetic efficiency[17]. The ability of this plant to tolerate a broad range of environmental conditions also contributes to its competitive advantage and high nuisance potential. Eurasian water milfoil creates problems by its abundance and not by its presence. Uncontrolled growth allows it to out-compete more desirable native aquatic plants[1-2].

Until now, attempts to control Eurasian water milfoil have met with only partial success and usually are accomplished at considerable expense. Methods of control have included herbicide treatments[3,4,18], mechanical harvesting[1,5], and manipulation of habitats such as by varying water levels[2]. Biological controls such as white amur, insects, snails, crayfish and plant competitors have also been tried, but they have been shown to have limited potential[2,4]. None of these methods have attacked the cause of the problem, excess nutrients but only the symptom plant growth.

Experimentation on the effects of bioaugmentation on the growth of Eurasian water milfoil have been conducted over the past two years. A commercially available bacterial suspension (Bacta-Pur™) was used. This product contains eight natural strains of heterotrophic and lithotrophic bacteria: *Aerobacter aerogenes*, *Bacillus subtilis*, *Cellulomonas biazotea*, *Pseudomonas denitrificans*, *P. stutzeri*, *Nitrosomonas* sp. and *Nitrobacter winogradskyi*. Heterotrophic bacteria in the seeding solution are $5\text{-}8 \times 10^6$ cells/strain/ml; total plate count is 5×10^7 cells/ml[9]. Initially, we tested different concentrations of bioaugmentation in a range between 20-300 ppm. The results (not presented here) showed that bacteria reduced the growth of Eurasian water milfoil proportionally to the concentration, but these concentrations are too high to be of any practical value for economic reasons.

Bacterial augmentation, at a rate of 9.76 litres of Bacta-Pur™/ha/wk, reduced growth of Eurasian water milfoil by 90 and 60% by the end of the two experimental periods, respectively (Fig.1). The lower value for the second experiment is probably explained by its shorter duration. At the end of this experiment, plants receiving bacterial augmentation were necrosed, while the control plants were green. A die back as observed in the first experiment was inevitable. Eleven new regenerative shoots were present in the control microcosm, at the end of the second studies, but none were observed in the one receiving the bacterial augmentation. A major nuisance aspect of Eurasian water milfoil is its ability to grow to the surface by late summer where it interferes with boating and swimming[1-2]. The growth reduction caused by bacterial augmentation could clearly diminish this nuisance aspect of the plant.

Sediments are the most important source of nitrogen and phosphorus for Eurasian water milfoil[19-21]. Bioaugmentation has been shown to be able to reduce accumulation of organic sediments and nutrients[8,10,11]. Apparently, bioaugmentation decreases the plant's growth by reducing nutrient availability and/or by transforming these nutrients into inaccessible forms for the plant. We are investigating these possibilities.

The weekly addition of small amounts of bacterial seed stock (approx. 2.5×10^7 cells/150 liter microcosm), into the sediments, resulted in higher bacterial counts in both the water column and the sediments as compared to the control (Fig. 2). The bacterial additions on a strictly cell count basis clearly cannot explain the observed increases, certainly not the approximately two fold augmentation found in the sediments. There are synergistic effects involved. Microbiological water purification is based on communities of bacteria working together where processes started by one strain are continued by others[12]. Lack or insufficiency of one or more members of the necessary community, whether caused by differential growth rates under suboptimal conditions and/or selective predation, can limit total bacterial numbers[22]. Bioaugmentation has been shown to be able to increase efficiency of water purification and removal of organic sediments[8-11] and appears to have potential for reversing or reducing rates of eutrophication.

The exact mechanisms producing the observed growth repression of Eurasian water milfoil are not yet clearly understood, but the efficacy of the microbial manipulation raises fundamental questions about microbial processes in aquatic ecosystems as well as about the direction of research concerning ecological microbial productivity. The manipulation of food chains to improve overall ecological efficiency is an important area for further research[23]. The time is passed of trying to define bacterial water purification processes merely in terms of total bacteria; it is often assumed either that all bacteria are the same, as all mammals are the same, or that all necessary bacteria are always present. It is time to face the challenges and opportunities of manipulating of microbial communities in nature.

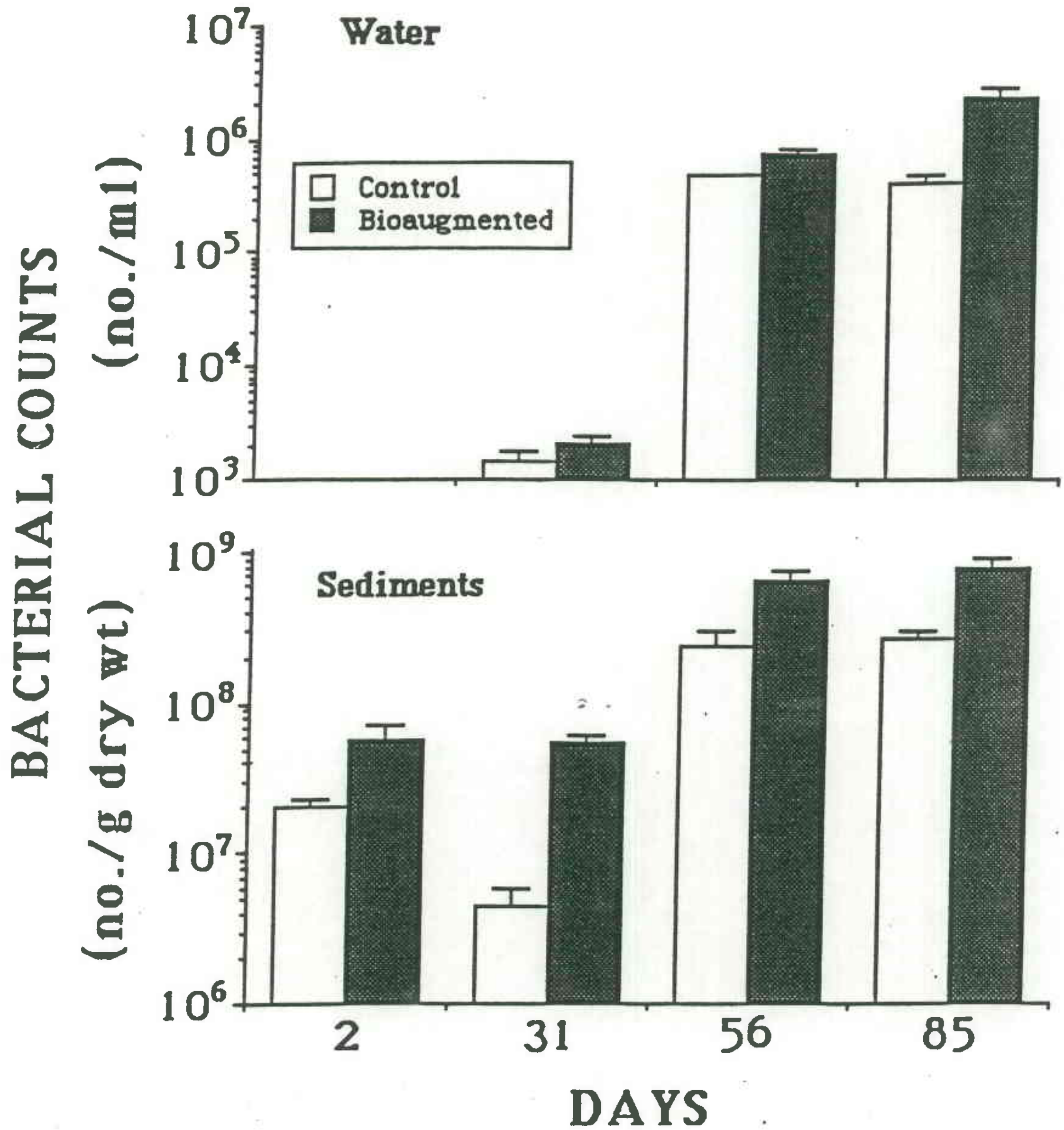

Fig. 2 Bacterial counts in water and sediments. Bacteria were counted, using epifluorescence microscopy after staining with acridine orange based on techniques of Moriarty[24]. This was conducted four times, over 85 days during the second experiment. Values are mean ± s.d., n=3.