Factors Affecting the Efficacy of Biological Nutritional Products for Turfgrass Management Kevin Jackson* and Cale Bigelow

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Backaround and Objectives: Increasing pressure to reduce synthetic inputs in recreational turf areas has increased the interest in using biological products among turf managers. These products are intended to feed the turf with minimal amounts of nutrients while also promoting overall soil health by stimulating beneficial microbes. Numerous commercially available products exist in the marketplace which vary in composition and concentration. Turf managers do express interest in using biological products in their management programs, however, field performance and effectiveness has been mixed (Fig. 1) and most would like to better understand why. Depending on the water source used for tank mixing these products prior to application (Fig. 2), several factors may potentially decrease the efficacy of biological products including the possibility of mixing with chlorinated municipal water. In this evaluation, several products were mixed with ultrapure sterile water and ultrapure sterile chlorinated water to determine (1) the variability of bacterial colony forming units (CFUs) between products, and (2) whether the presence of chlorine at a two parts per million concentration (2ppm) reduced the amount of bacterial CFUs.



Materials and Methods:

<u>Product Selection</u>: A range of products were selected for evaluation based on current product trends and popularity within the turf industry. A total of six products (Fig. 4) were evaluated, including three commercial biological products, a fresh vermiextract, a bioextract, and a fresh compost tea. The compost tea and vermiextract were made in house and were one week old prior to plating. The compost tea was made using a commercial plant-based compost, 15mL probiotic, and with 10mL unsulphured molasses in 7.5L of water. The product was refrigerated prior to use. Vermiextract was made using a 1:10 ratio of worm castings to water in a one liter container. The commercial products consisted of a compost extract, kelp/fish tankage extract, and soil-based humic extract and were used as packaged from the vendor.

<u>Water</u>: Two 600mL flasks of ultrapure water (pH 7) were sterilized via autoclave at 250°C. Following sterilization, calcium hypochlorite was added to one flask of water to produce a 2ppm total chlorine concentration. Total chlorine and free chlorine concentrations were verified using test strips (Fig. 5).

<u>Media</u>: Bacteria were grown using a plate count agar (PCA) media consisting of 0.5% peptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar w/v at neutral pH¹ (Fig. 3). <u>Product Dilution</u>: Each product was serially diluted in a 15mL conical flask (Fig. 6), beginning with 1mL product:9mL water or chlorinated water. Dilutions were prescreened to determine the optimal dilution ranges for obtaining optimal CFUs for each producting.

<u>Plating and Plate Counts</u>: Once dilutions were prepared, 100uL of diluent was pipetted and spread on to plates and replicated three times. Samples were vortexed prior to plating to ensure homogenous distribution of product. Once plated, samples were incubated on the lab bench for 10 days at 21°C and total CFUs were enumerated. Log values of CFUs were subject to analysis of variance (ANOVA) by the SAS statistical package.



Fig. 3- Bacterial growth on PCA plates



Table 1. Mean populations of total CFUs per product in ultrapure, sterile water and chlorinated ultrapure sterile water.

log mean CFU/mL	
Ultrapure, Sterile Water	Chlorinated ultrapure, sterile water
7.887 a	3.799 bcd
7.33 ab	6.983 ab
6.797 ab	5.058 ab
5.363 abcd	5.46 abcd
6.405 ab	6.243 ab
5.592 abcd	2.093 abcd
2.454 cd (water)	2.00 cd (chlorinated water)
	Ultrapure, Sterile Water 7.887 a 7.33 ab 6.797 ab 5.363 abcd 6.405 ab 5.592 abcd

Factor Sig Type I SS Mean Square F Value Pr > FNS 13.0474172 6.5237086 1.18 0.323 Ren 0.055 22.2622289 22.262229 Water 0.05 4.03 < 0.05 100.461232 16.743539 3.03 0.022 Product Water*Product 26.2538405 4.3756401 0.79 0.584

<u>Results and Discussion</u>: The popularity of biological products in the marketplace will likely continue to grow as turf managers are asked to use less synthetic products for management. Relatively little research is present in the literature regarding the effect of chlorinated water on biological products in the market today. Many publications mention "gassing off" chlorine prior to mixing, however, this may not always occur. The EPA has set a maximum residual disinfectant level (MRDL) of 4ppm (mg/L) for chlorine. In our research, significant differences (\$0.05) were observed (Table 1) among product types and water (chlorinated at 2ppm vs unchlorinated). No significant interaction resulted between water and product. Turf managers should be aware of disinfectant levels present in municipal water sources. Levels at or above 2ppm (mg/L) may affect product efficacy unless steps are taken to reduce chlorine levels prior to tank mixing.

<u>Conclusions and Next Steps</u>: Future research may be focused on exactly which bacteria are present on growth media via PCR techniques. Various water samples may also be obtained from different sources, including municipal water, to determine exact total chlorine content and the effect on these products. Another investigation could include enumeration of bacterial count prior to tank mixing versus output levels.

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References: ¹Atlas, R.M. (2004). Handbook of Microbiological Media. London: CRC Press p. 1390.