POTENTIAL HYDROPONIC APPLICATION OF AN ORGANIC N-HALAMINE DISINFECTANT

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INTRODUCTION

As industrial development and expanding population result in increased water recycling in Georgia, effective water treatment is becoming a higher priority for many communities. Although chlorination procedures are commonly used for disinfecting potable water, the free chlorine, hydantoins, and isocyanurates employed have brief useful lifetimes in water and can react with organic impurities to produce trihalomethanes which have been linked to cancer in laboratory animals. Chlorine dioxide and ozone do not provide long-term residuals and being strong oxiding agents, could react with organic matter in water to produce byproducts of unkown health risks. Many of the organic and inorganic chloramines lack stability in solid form or in aqueous solutions. (Tsao et al., 1991) Iodination procedures, which are proven water treatments, depend on compounds which prove toxic to plants thus limiting the recycling capabilites for waste water. Ideally a disinfectant will be stable in solid and aqueous forms, nontoxic, noncorrosive, tasteless, odorless, and effective against a broad spectrum of organisms (Barnela et al., 196). These qualities are present in the organic N-halamines described below.

INITIAL DISINFECTANTS

Initial research focused on 3-chloro-4, 4-dimethyl -2-oxazolidinone (compound I) and 3-bromo-4, 4-dimethyl- -2-oxazolidinone (compound IB). A recent review of N-halamine water disinfectants provides stability and biocidal efficacy data for these compounds as a function of pH, temperature, and water quality (Worley and Williams, 1988). While compound I tends to be slow in the eradication of many water-borne organisms, it is more efficacious than free chlorine in the eradication of Giardia lamblia. Compound IB is probably not sufficiently stable for use as a general purpose biocide. No N-bromamine will be as stable as the N-chloramine analogue due to the longer, weaker N-Br bond compared with the N-Cl bond. The key to the stability of compound I is the presence of the methyl substituents at the 4 position of the oxazolidinone ring.

Understanding the stability of compound I lead to the development of 1,3-dichloro-4,4,5,5-trameth yl-2-imidazolidinone(compoundA), 1,3-dibromo-4,4,5,5-tetramethyl-- 2-imidazolidinone (compound AB), and 1-bromo-3-chloro- -4,4,5,5-tetramethyl-2-imidazolidinone (compound ABC). Compound A is the most stable N-halamine in water or dry storage, compound AB is the most stable N-bromamine ever reported, and compound ABC is an ideal disinfectant with the bromine moiety providing rapid disinfection and the chlorine moiety providing long-term disinfection. Some limitations in the use of the compound A series come as a result of the cost and difficulty in synthesis of the 4,4,5,5-tetramethyl-2-imidazolidinone precursor.

Alterations in the synthetic process lead to the development of compound MC (1-chloro-2, 2, 5, 5-tetramethyl-4- -imidazolidinone), compound DC (1, 3-dichloro-2, 2, 5, 5-- tetramethyl-4-imidazolidinone), compound DB (1, 3-dibromo-2, 2, 5, 5-tetramethyl-4-imidazolidinone), and compound DBC (1-bromo-3-chloro-2, 2, 5, 5-tetramethyl-4 -imidazolidinone). These isomers are inexpensive to synthesize, reasonably stable in aqueous and dry storage, and more actively biocidal than the A series analogues (Tsao et al., 1991).

RECENT DISINFECTANTS

The organic N-halamine research undertaken in the past ten years has proven these compounds to be effective against a broad spectrum of potentially pathogenicorganisms including Enterobacter, Escherichia, Klebsiella, Legionella, Pseudomonas, Salmonella, Serratia, Sphaerotilus, and Staphylococcus as well as Giardia (Elder et al., 1986; Williams et al., 1985; Worley et al., 1985; Worley et al., 1983; Worley et al, 1981). All studies undertaken have utilized the compounds in concentrations of 10 mg/L or less. Combining the broad spectrum with other characteristics such as being stable, noncorrosive, nontoxic, tasteless, and odorless, make these compounds particularly promising biocides.

CURRENT APPLICATION

While previous research has primarily utilized organic N-halamines as water disinfectants, other potential applications exist. Studies in water disinfection indicated the compounds were not detrimental to algal growth. Combining this idea with the impact of iodine on plant growth lead to this study with compound MC in hydroponics. The study included determining the efficacy as a surface steriliant for seeds, impact on germination, efficacy in controlling bacterial growth in the nutrient fluid, and impact on the maturing plants. Compound MC was selected for its stability and ease of synthesis. Radishes were selected based on rapid germination and potential food use.

METHODS

All glassware and water used during the experiment were chlorine demand free. For glassware the procedure involves Alconox washing, rinses with tap and distilled water, a 24 hour soak in a distilled deionized water bath supplemented with chlorine bleach. The glassware was dried for 24 hours then sterilized by autoclaving at 121° C and 15 psi for 15 minutes. For chlorine demand free water, chlorine bleach was added to distilled deionized water and allowed to stand for 24 hours. The water was then exposed to sunlight for 18 to 24 hours to remove the residual chlorine.

Commercially available radish seeds were soaked, at room temperature, in 10.0 mg/L Cl+ solutions of compound MC or in sterile chlorine demand free water for 1 hour. To ensure contact of the seeds with the fluid, the flasks were rotated at 160 rpm. To determine the efficacy as a surface sterilant, part of the seeds from the soaking solutions were transferred to nutrient agar plates which were incubated at 37° C for 24 hours. Bacterial growth was recorded as percentage contamination. The remaining seeds were transferred to petri dishes lined with sterile filter paper and moistened with the 10 mg/L Cl+ biocide solutions or sterile chlorine demand freewater. The plates were incubated, at 24° C in the dark and checked daily to observe germination. After 4 days the percentage of seeds germinated was calculated.

The seedlings were transferred to the hydroponic growth systems, consisting of sterile plastic bags lined with sterile filter paper, with 10 bags for each set of conditions. After inserting 1 seedling into each bag, each bag/filter paper was moistened with 20 ml of liquid fertilizer diluted in 1.25, 2.5, 5.0, or 10 mg/L Cl+ biocide solutions or in sterile chlorine demand free water. The bags were sealed around the seedling stems and placed in a 21° C temperature controlled growth chamber with a 12 hour light/dark cycle.

The plants were monitored daily to maintain the volume of nutrient solution and to visually assess any changes in the conditions of the plants. To determine the presence of bacterial contamination, 1 ml samples were collected as each system was placed in the growth chamber and every other day for the duration of the experiment. The samples were mixed with equal volumes of sterile sodium thiosulfate to quench the disinfectant. Serial dilutions were spot plated on nutrient agar and incubated at 37° C. Counts were made at 24 and 48 hours to allow for the growth of injured cells.

RESULTS AND CONCLUSIONS

Compound MC proved to be an effective surface sterilant. As indicated in Table 1, nearly three times as many seeds soaked in chlorine demand free water supported bacterial and fungal growth as did the seeds soaked in compound MC.

In both the 10 mg/L Cl+ solution of compound MC and in the chlorine demand free water, germination was at least 99.7 %, there were no significant differences present. Results from the bacteriological sampling of the nutrient solutions are presented in Table 2. The results shown are averages of the 90 counts (3 spots/dilution with 10 replicates/set of conditions and 3 trials).

All the nutrient solutions were capable of supporting the growth of the radishes. No visible differences were present between the plants grown in the varying concentrations of compound MC or in the chlorine demand free water.

Based on the experiments undertaken, compound MC proved to be an effective additive for hydroponic gardening. The compounds functioned very efficiently in surface sterilization, in germination, and in growth of the radishes. Future directions for this research will include utilization of other plants with potential food applications, utiliazation of other biocides, monitoring any uptake of the biocides by the plants, and development of less cumbersome systems.

Table 1. Pre-germination Soaking

Trial	Soaking Agent	Percentage Contaminated
1	CDF	44.83
	10 mg/L MC	15.79
2	CDF	43.12
	10 mg/L MC	14.23
3	CDF	45.50
	10 mg/L MC	15.65

Table 2. Bacterial Load of Nutrient Solutions (cfu/ml).

Day	Concentration of Compound MC (mg/L Cl^+) 0	1.25	2.5	5.0	10.0
0	0	0	0	0	0
2	2.34×10^2	1.23×10^1	1.06×10^1	1.22×10^0	1.03×10^0
4	1.94×10^3	1.49×10^1	1.13×10^1	2.70×10^0	1.97×10^0
6	1.70×10^4	7.98×10^1	4.33×10^1	3.60×10^1	2.65×10^0
8	2.84×10^4	3.60×10^2	2.30×10^2	7.93×10^1	8.54×10^0
10	4.24×10^4	1.20×10^3	4.52×10^2	3.23×10^2	2.43×10^1
12	1.23×10^6	1.60×10^3	1.32×10^3	3.60×10^2	4.67×10^1
14	1.62×10^6	2.79×10^3	2.45×10^3	3.77×10^2	5.19×10^1
16	2.87×10^6	6.23×10^3	5.20×10^3	3.45×10^2	5.27×10^1
18	1.22×10^7	7.84×10^3	7.70×10^3	2.78×10^2	1.94×10^2
20	1.84×10^7	1.22×10^4	1.07×10^4	3.01×10^2	1.87×10^2

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