Georgia Journal of Science

Volume 62 *No. 2 Scholarly Contributions from the Membership and Others* — Article 10

2004

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ABSTRACT

Ozone (O_3) is a strong oxidizing agent that is routinely used as a disinfectant in water treatment for bacteria, viral infections, and algae. Brine shrimp are used in many aquaculture environments as fish food. Typically the brine shrimp eggs are hatched on site and fed to the fish stock after maturation. Brine shrimp are capable of transporting microbes into the fish stock. In order to eliminate the detrimental effects of infections from this process we ran a series of mortality tests by applying ozone to the brine shrimp. The time durations of ozone ranged from 5 minutes to 24 hours. A second set of experiments expose the brine shrimp directly with ozone over their life cycle (2 months). Our results demonstrate that ozone can be applied directly to the brine shrimp increasing their survivability.

Key words: brine shrimp, ozone, aquaculture.

INTRODUCTION

Ozone (O_3) is a strong oxidizing agent that is produced commercially by corona discharge systems from oxygen (1, 2). Ozone, which is a gas with a pungent odor, is widely used for different water treatment applications such as the reduction of color, degradation of organic pollutants, increase in dissolved oxygen levels and the oxidation of ammonia to nitrite and nitrate. In the gas phase, ozone can be lethal at high concentrations to humans and animals. When bubbled through water, the polar molecule has a maximum solubility in the 3-5 parts per million range, depending on water temperature and ionic strength.

Ozone is unstable and decomposes slowly (minutes) at ambient temperatures and rapidly (< 1 sec) at higher temperatures. Because of this instability, it must be manufactured on-site for industrial applications. Current ozone production is carried out by several techniques. One approach utilizes electrochemical techniques. These systems suffer from high electrical consumption and the cells produce chemicals that can be toxic or difficult to dispose.

The second general area involves the use of high-energy methods such as UV light, beta rays, and lasers to convert the oxygen to ozone but these means of converting oxygen to ozone have found no large-scale commercial application. The third general approach utilizes electrical discharges or plasmas. In this approach, pure oxygen or air in the gas phase is passed through an electric field generated by putting a high voltage across an anode and a cathode. These systems typically suffer from high electrical consumption and relatively low conversion efficiency (1-15%) of oxygen to ozone.

Like fluorine, chlorine, and hydrogen peroxide, ozone is a strong oxidizing agent.

$$O_2 + 4H_3O^+ + 4e^- \rightarrow 6H_2O \quad E^\circ = +1.23\ V \qquad [1]$$

$$Cl_2 + 2e^- \rightarrow 2Cl^- \quad E^\circ = +1.36\ V \qquad [2]$$

$$HClO + H_3O^+ + e^- \rightarrow 2H_2O + 1/2Cl_2(g) \quad E^\circ = +\ 1.63\ V \qquad [3]$$

$$HClO_2 + 2H_3O^+ + 2e^- \rightarrow HClO + 2H_2O \quad E^\circ = +\ 1.63\ V \qquad [4]$$

$$H_2O_2 + 2H_3O^+ + 2e^- \rightarrow 4H_2O \quad E^\circ = +1.77\ V \qquad [5]$$

$$O_3(g) + 2H^+ + 2e^- \rightarrow O_2(g) + H_2O \quad E^\circ = +2.07\ V \qquad [6]$$

$$F_2 + 2e^- \rightarrow 2F^- \quad E^\circ = +2.87\ V \qquad [7]$$

$$2H^+ + 2e- \rightarrow H_2(g) \quad E^\circ = 0.0\ V \qquad [8]$$

Ozone has some well-known advantages over other strong oxidizing agents. For example, $HClO_2$ and HClO leave behind a chlorine-based residue and fluorine gas (F_2) is highly corrosive. The kinetics of ozone decomposing a variety of organic compounds have been measured (see Table I) and shown to be quite favorable compared to other strong oxidizing agents.

Table I. Data that show the kinetics of reaction of ozone on certain organic compounds compared to other oxidizing agents. (11, 12)

Compound	Chlorine	Permanganate	Ozone
Acetophenone	26 days	43 days	25 minutes
Benzaldehyde	>3.2 days	36 minutes	28 minutes
Camphor	>3 days	>5.8 days	12 minutes
p-nitrophenol	2.1 hours	1.1 days	2 minutes
Borneol	1.4 days	7 days	53 minutes
Methyl-m-toluate	>20 days	22 days	5.5 minutes
Diacetone-L-sorbose	100 days	14 days	2.8 minutes

The following thermodynamic considerations are fundamental to understanding the efficient and economic production of ozone from oxygen. First, the production of ozone from oxygen is an endothermic reaction:

$$3O_2(g) \rightarrow 2O_3(g) \quad \Delta H = +286 \text{ kJ/mol} \qquad [9]$$

The discharge provides the energy needed to convert oxygen to ozone. Second, the decomposition of ozone to oxygen is thermodynamically favored:

$$2O_3(g) \rightarrow 3O_2(g) \quad \Delta G = -326 \text{ kJ/mol} \qquad [10]$$

Lowering the temperature of the system can slow this decomposition. If the discharge energy is too low [Eq. 9], the formation of ozone from oxygen will not take place. If the discharge energy is not dissipated rapidly [Eq. 10] the decomposition of ozone will be accelerated.

Although ozone can be toxic to living organisms, if used correctly it can be a highly effective disinfectant in aquaculture systems (3). When applied to aquaculture systems it has the ability to eliminate harmful bacteria, deactivate viruses, remove color, and improve water quality (4). Ozone has been successfully demonstrated in closed loop aquaculture systems, such as fish hatcheries. The ability to eliminate nitrogenous wastes and bacteria produced by aquatic animals is a challenge that must be constantly met by the people that control the closed aquaculture systems (5, 6).

Studies have been conducted on the impact of ozone on different aquatic animals. One such experiment utilized shrimp to see if ozone had any effect on the shrimp pathogens *vibrio* and *Fusarium solani*. The results of the experiment showed that 99.9% of these organisms are killed when they are exposed to ozone for 5 minutes or less (7). Atlantic salmon were treated with 13g of ozone for one hour per day. Water clarity was maintained with the help of ozone. When the use of ozone was terminated there was an accumulation of humic compounds and there was a decrease in water quality. The ozone generator was accidentally left running for 15 hours and there were many fish mortalities observed (8). Other toxins that have been proven to be deactivated by ozone treatment are *Clostridium botulinum* (botulism) and *Gymnodinium breve* that is one of the red tide toxins (9). Ozone has also been used in another separate experiment to compare how fish and shrimp react to being exposed to ozone. The shrimp showed a higher tolerance to ozone than the fish due to their protective shell. Although the fish were not as tolerant as the shrimp were to the ozone, positive results were found from the use of ozone (10).

Brine shrimp are primarily used for food in many fish cultures. Brine shrimp can be a source of bacteria when introduced into these systems when they are used as fish food. Previous studies have shown that brine shrimp also benefit from the use of ozone in their environments (2). The purpose of this project is to see if ozone has any effect on the survival rate of brine shrimp over a period of 96 hours. This information is helpful because the data will provide the maximum amount of ozone brine shrimp can handle and still survive long enough to be used in other mediums such as nourishment for fish.

EXPERIMENTAL

The first set of experiments consisted of separate hatchings of brine shrimp that were subjected to different amounts of ozone exposure (Table II, times from five minutes to six hours). Different quantities of brine shrimp were hatched in 2-liter bottles to measure any correlation between different densities of brine shrimp and their survival rates when exposed to varying time allotments of ozone in brackish water made from "Instant Ocean." The average salinity was 38ppt, the average pH was 8.64, and the average temperature was 21 degrees Celsius. The brine shrimp took approximately 24 hours to hatch. Once the brine shrimp hatched they were fed, and approximately 24 hours after they hatched they were exposed to ozone for the specific time duration. The different time amounts of ozone exposure were 5 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours. The ozone was applied to the brine shrimp by attaching air stones to the ends of the plastic tubing that exited the ozone generator. The water was saturated with ozone which has an accepted concentration of 5 parts per million. The brine shrimp were then observed over 96 hours to measure mortality rates.

Table II. Different quantities of brine shrimp eggs were allowed to hatch in 2-liters of water. Each container was then ozonated for a set amount of time (5 minutes, 30 minutes, etc.). The brine shrimp were then visually observed for 96 hours. Alive indicates some shrimp were observed. Dead indicates no living shrimp were observed.

Shrimp in grams	5 mins Ozone	30 mins Ozone	1 hr Ozone	3 hrs Ozone	6 hrs Ozone	24 hrs Ozone
0.15	Alive	Alive	Alive	Alive	Dead	Dead
0.3	Alive	Alive	Alive	Alive	Alive	Dead
0.45	Alive	Alive	Alive	Alive	Alive	Dead
0.6	Alive	Alive	Alive	Alive	Dead	Dead
0.75	Alive	Alive	Alive	Alive	Dead	Dead
0.9	Alive	Alive	Alive	Alive	Dead	Dead
1.05	Alive	Alive	Alive	Alive	Dead	Dead
1.2	Alive	Alive	Alive	Alive	Dead	Dead
1.35	Alive	Alive	Alive	Alive	Dead	Dead
1.5	Alive	Alive	Alive	Alive	Dead	Dead

In a second set of experiments, six 50-liter tanks were used to measure the periodic application of ozone and its impact on brine shrimp survival. Table III shows the dosages per week to each tank over an eight-week cycle. Typically marine organisms, such as shrimp, when living in an unfiltered environment will have high mortality rates due to the accumulation of ammonia, bacterial infections derived from feces and discarded body armor due to molting, and viral infections. All tanks were continuously aerated (control and o-zone) throughout the 8-week experiment. The saturated levels of ozone in pure water (3-5 ppm) were achieved rapidly in small (<10 seconds) and large containers (<30 seconds). Attempting to measure the oxidizing agent concentration $[O_3]$ in a system with a range of living organisms (i.e. diatoms, algae, shrimp), various suspended organic (i.e. undigested food) and inorganic matter (shrimps shells, ammonia, etc.) give inconsistent results. We assume it was at saturated levels for the duration of the ozonation.

Table III. Tank #1 and 2 represent controls for the experiment (no ozone applied). Tanks #3-6 represent different levels of ozone application to the 50-liter solutions. The experiments were started on 1/6/03 and ended 2/26/03. Each tank was sampled ten times by removing 310 mL of water and counting the number of brine shrimp. (Tank #3 and 4, ozonated 3X per week each for 10 minutes; tanks #5 and 6, O_3 applied 1X per week, 10 minutes each)

Dip #	Tank 1 # (No O_3)	Tank 2 # (No O_3)	Tank 3 #	Tank 4 #	Tank 5 #	Tank 6 #
1	0	0	1	5	4	6
2	0	0	1	5	6	13
3	0	0	0	5	4	9
4	0	0	2	8	3	11
5	0	0	1	7	3	12
6	0	0	2	6	4	12
7	0	0	1	3	7	3
8	0	0	1	2	3	7
9	1	0	1	5	8	13
10	0	0	5	3	4	8

RESULTS AND DISCUSSION

Table II provides the average results of three sets of mortality tests. All results were recorded after 96 hours of observation. In trials #1-4 all shrimp remained alive after exposure to ozone. During the observation in trial #5, many of the shrimp showed a severe decrease in movement. The brine shrimp that received 24 hours of exposure to ozone were all found dead when the ozone was removed from the soda bottles. The soda bottles had the pH, DO, and ORP measured before and after receiving treatment with ozone. The use of ozone did not affect the long term levels of pH, DO, and ORP levels in any of the experiments. Once this set of results was completed, a series of grow outs were conducted to study the impact that ozone had on the long-term survival of the brine shrimp. Table III provides the results after the eight-week grow-outs were complete. In these experiments there was a control in which brine shrimp were allowed to grow at a relatively high density (10 grams of eggs per 50 liters of solution) with only aeration. As the data set indicates, simple aeration was not enough to maintain a level of water quality needed for the shrimp to survive for the average duration of a brine shrimp life cycle. We found that over-ozonation (i.e. 7 times per week, 30 minutes each) quickly killed the shrimp. As the data demonstrate, ozonation for 1-3 times per week for duration of 10 minutes each increased the survival ability of the organisms.

CONCLUSIONS

Past work in this lab with ozone has centered on fundamental and applied studies associated with its generation[11-13] as well as its potential applications in nanotechnology[14,15]. This work has given us opportunities to understand the potential of the environmental friendly (provided it is applied correctly) oxidizing agent in a number of chemical systems. Because of its favorable standard reduction potential (>2.0 V), it is easy to over use in many applications. In this application, if ozone is applied continuously, it will kill the shrimp rapidly. For this work, we found that ozonating between 1-3 times per week for 10-20 minutes per time increased the survivability of the shrimp when compared to the control. We did monitor parameters such as pH, DO, and salinity but found no correlation of these chemical parameters with survivability. The lack of ozone may have allowed ammonia levels to increase to toxic levels and/or bacterial and viral species that are harmful to the shrimp to multiply uncontrollably.

This work shows that applying ozone directly to brine shrimp can increase their survival rate if it is done in a controlled fashioned. Past work with ozone and shrimp has always applied ozone to the water off-line (i.e. a holding tank) and then returned the treated water to the runway. This adds complexity to the aquaculture process and increases the capital costs. All shrimp share a similar attribute in that their hard outer exoskeleton, composed of chitin, proteins, and some calcium carbonate, protects them from the oxidizing effects of ozone. Using ozone directly in a shrimp runway may be a method to increase the efficiency and lower the cost of raising shrimp in an aquaculture setting.

ACKNOWLEDGMENTS

This work was supported in-part by an SBIR grant from National Oceanic and Atmospheric Administration (NOAA) to MIC Systems, Inc of Valdosta, GA. Valdosta State University is also thanked for use of labs and equipment. We'd also like to thank Dr. Jim Nienow, Dr. Linda Chamberlin, Dr. Dave Bechler and Dr. John Elder of the VSU biology department and Mr. Jack Rudloe of the Gulf Specimen Marine Lab (Panacea, FL) for discussions related to this work. Jason Ames and Jerry Purcell built much of the apparatus used here and these efforts are greatly appreciated.

REFERENCES

1. Owsley D: Ozone for disinfecting rearing water. American Fisheries Society Symposium 10: 417-420, 1991.
2. Hochheimer J and Summerfelt S: Review of ozone processes and applications as an oxidizing agent in aquaculture. The Progressive Fish-Culturist 59: 94-105, 1997.
3. Theisen D, Stansell D and Woods LC: Disinfection of Nauplii of Artemia franciscana by ozonation. The Progressive Fish-Culturist 60: 149-151, 1998.
4. Singh S and Wheaton F: Ozone application in aquaculture. Aquaculture Magazine January/Feburuary: 58-63, 1999.
5. Summerfelt ST, Hankins JA, Weber AL and Durant MD: Ozonation of a recirculating rainbow trout culture system 1 II. Effects on microscreen filtration and water quality. Aquaculture 158: 57-67, 1997.
6. Suantika G et al: The use of ozone in a high density recircultaion system for rotifers. Aquaculture 201: 35-49, 2001.
7. Danald DA, Ure J and Lightner DV: Preliminary results of ozone disinfection of sea water containing the potential shrimp pathogens Vibrio species and Fusarium solani. Ozone: Science & Engineering 1: 329-334, 1980.
8. Williams RC, Hughes SG and Rumsey GL: Use of ozone in a water reuse system for salmonids. Progressive Fish-Culturist 44: 102-105, 1982.
9. Rosenthal H: Ozonation and sterilization. World Symposium on Aquaculture in Heated Effluents and Recirculation Systems May 28-30, 1980. 1: 219-274, 1981.
10. Reid B and Arnold C: Use of ozone for water treatment in recirculating-water raceway systems. Progressive Fish-Culturist 56: 47-50, 1994.
11. Manning TJ: Production of ozone in an electrical discharge using inert gases as catalysts. Ozone: Science & Engineering 22: 53-64, 2000.
12. Manning TJ and Hedden, J: Gas mixtures and ozone production in an electrical discharge. Ozone: Science & Engineering 23: 95-103, 2001.
13. Manning TJ: Apparatus and method for generating ozone. United States Patent #6,022,456
14. Manning TJ, Olsen K, Hardin L, Purcell J, Ayers TM, Duncan MA and Phillips D: Extensive ozonation of C_{60}: Degradation or polymerization? Submitted Ozone: Science and Engineering.
15. Manning TJ, Olsen K, Hardin L, Purcell J, Ayers TM, Duncan MA and Phillips D: Degradation of carbon nanotubes by ozone and nitric acid. Submitted Ozone: Science and Engineering.