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Some Ozone Applications in Seafood

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The use of ozonized seawater to reduce and eliminate bacterial pathogens in mariculture facilities and to extend shelf life of marine food products is demonstrated. Consequent benefits of this treatment are also discussed. Laboratory and pilot experiments were conducted using ozone gas to reduce disease-producing Vibrio sp. *bacteria at a shrimp (*Litopenaeus vannameii*) hatchery in Ecuador, South America. Pacific Ocean seawater was treated in a 1,540 L capacity fiberglass contact tower (5–7 min retention) with an ozone oxidant residual of 0.07 mg/L. Prior to ozone treatment,* Vibrio *determined by TCBS plating was too numerous to count, causing shrimp to die of disease (30 tanks of 13,000 L each). After treatment,* Vibrio *counts and shrimp disease were eliminated, ozonized seawater decreased the time required for normal molting of shrimp and the total growth cycle was reduced by three days versus control water. From June 1991 until September 1992, survival rates of larval shrimp were robust, routine antibiotic addition was reduced, and one additional growth cycle was realized. Ozonized ice (fresh water) was prepared in the Milford Laboratory CT, USA; Gloucester Food Tech Lab, MA, USA; and a field station (brine water) for sockeye salmon (Oncorhynchus nerka) in Homer, AK. USA. In these studies, squid (*Loligo pealei*) and commercially captured salmon demonstrated a reduction in spoilage and extension of shelf life of 3 to 5 days' time using ozonized ice. Bacteria associated with commercial ice-producing machines were reduced by 4 logs using ozone treatment. In addition, no flavor aberration was noted using ozonized ice.*

Keywords Ozone, Mariculture, Seafood Preservation, Shrimp, Salmon, Squid, *Vibrio*

INTRODUCTION

Disease in shellfish and crustacea may be introduced through both environmental and nutritional routes and is especially prevalent in situations where stock is held in close proximity, such as in culture operations. One of the most common causes of disease in seafood in both natural and culture settings is the bacterial genus *Vibrio* (Evelyn, 1971; Brown and Losee, 1978; Brown, 1981; Colwell, 1984). Results of a *Vibriosis* infection can range from a mild reduction in living stock to a complete die-off in a hatchery situation. The common and immediate response to such an infection in an enclosed aquaculture facility is the use of antibiotics.

Although initially effective, the long-term use of these agents has led to increasingly resistant strains of *Vibrio* and other bacteria pathogenic to marine stock. This has resulted in the use of ever-increasing doses and stronger antibiotics. The concentration of these agents in the environment and in the food chain are undesirable outcomes of such widespread and sometimes, imprudent use. An alternate method of suppressing and controlling the causative agents of bacterial disease is necessary to reduce the use of antibiotic treatment for shellfish diseases.

The utility of employing ozone to disinfect and depurate contaminated seafood has been demonstrated for many years (Blogoslawski and Stewart, 1977b; Fauvel et al., 1982; Blogoslawski and Stewart, 1983). The use of this oxidant to prevent disease situations is also known through laboratory and large-scale applications. The difficulties inherent in the ozone treatment of aquatic stock are lessened as research and experience increase in this area. These difficulties include balancing the appropriate doses and residuals that prove effective in preventing or eliminating disease situations with the fragile larval stages of aquatic animals and in determining the actual compounds formed when ozone is introduced to culture waters, particularly seawater. This article addresses some experiences with ozone in combating and controlling *Vibrio* disease situations in aquaculture and will relate some successes in the ozone preservation of seafood. Specifically, the successful use of ozone to combat a *Vibrio* disease situation at a commercial shrimp hatchery in Ecuador and to preserve salmon in Alaska, USA and squid in Massachusetts, USA are examined.

Commercial Shrimp Hatchery Disease. In response to a decreased production situation at a commercial hatchery in Ecuador raising the shrimp, *Litopenaeus vannameii,* examination of shrimp larvae, food, and water revealed that

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a *Vibrio sp.* bacterial pathogen was the source of the issue. As antibiotics were already a standard part of the rearing cycle at this facility, an alternate method to eliminate the pathogen was needed. Ozone was selected in light of its proven ability to reduce bacterial counts in seawater (Blogoslawski et al., 1975). An additional benefit sought was the reduction or elimination of antibiotics as part of the growth cycle of this food product.

There were several obstacles to overcome in the installation of an effective ozone system due to the remote location of the hatchery and the limited power supply available. Initially, three tanks of shrimp were exposed to various concentrations of ozonized seawater to determine the best method of supplying ozone to the tanks and to work out a concentration that was effective in removal of the pathogen without harming the delicate larval shrimp. The success of the three-tank trial led to the installation of ozone throughout the entire commercial hatchery. In addition to eliminating the bacterial pathogen, other benefits of ozone use included the reduced use of antibiotics and acceleration in the growth and maturation process of the shrimp.

Laboratory Trials for Preservation of Seafood. The preservation of freshly caught fish on large commercial fishing boats is of great value to fisheries. A higher price is commanded for product with a fresh appearance and consequent flavor. The fishers were accustomed to receiving a higher price for product caught closer to the end of the fishing voyage and a lower price for product caught early on. The earlier caught product, often dumped on the deck of a barge from the fishing boat and left out in the open, did not retain the appearance sought by fish vendors and often would be sold for canning at a reduced price. Because of ozone's history as an outstanding disinfectant, it was thought that the introduction of ozone to fresh water ice to preserve freshly caught seafood would enhance the ability of the seafood product to retain its fresh-caught properties for a longer period than product stored in non-ozonized ice (which may surprise one to know, often contains high bacterial counts).

Accordingly, small-scale studies were set up at marine laboratories in Milford, CT and Gloucester, MA USA to examine the ability of ozonized ice to extend the shelf life of squid *(Loligo pealei).* A similar study was conducted in Homer, Alaska, USA, for the valuable commercial product, Alaskan salmon *(Oncorhynchus nerka).* These successful studies opened the industry to using ozonized ice for enhanced seafood preservation.

MATERIALS AND METHODS

Ozone Equipment in Commercial Shrimp Hatchery

Shrimp Maturation System. The circular 5,000-Liter tanks where adult shrimp are kept for breeding purposes are made of concrete covered with epoxy paint. Flowrate to the tanks was 60 L/minute and the tanks were in dark quiet rooms. During the experiment, each tank contained approximately 45 mature shrimp, water passed through a UV system, and shrimp food that was composed of squid, artificial food pellets and bloodworms. Ozone, produced by a 3.0 g/day on air DEL Industries (San Luis Obispo, California, USA) ozone-UV generator (Model No. Z0920), was bubbled through an airstone placed at the bottom of each tank.

Shrimp Larval System. Initial studies used a corona discharge generator constructed from a plate from Griffin Technics (Lodi, New Jersey, USA -now Ozonia, N.A., Elmwood Park, New Jersey, USA) that was capable of producing 20 g ozone/day on oxygen. The voltage was unknown as the transformer was taken from a light pole at the hatchery, that being the only source of sufficient amperage to produce ozone. It was later determined that the transformer produced 7,400 VAC. The transformer setting selected produced 8.06 mg/Liter ozone using the Griffin plate. All subsequent studies used a 5.0 lbs/day (on air) Griffin ozone generator (Model GTC-5B). Dry air (−60°F dew point) was supplied to the generator by a Griffin-supplied Air Preparation Unit (AU-3). All piping exposed to dry ozone gas was made of type 304 stainless steel.

Ozone Contacting. Raw seawater was pumped to a main holding area where it was UV-treated. The water was then pumped to the ozone treatment site at a rate of 40–60 gallons per minute. A fiberglass contacting tower with a volume of 1,540 Liters with three large airstones at the base allowed ozone to be introduced. Flow rate varied according to the demands of the hatchery system and complete turnover in the tower was between five and seven minutes. Ozonized seawater at an average dose of 0.07–0.08 ppm was then sent to all larval tanks.

Ozone Residual. In seawater, the residual measured is that of an ozone produced oxidant (OPO) due to the association of ozone with various chemical species in seawater (Haag and Hoigné, 1983; Stewart et al., 1979). Water samples were obtained for residual measurement by immersing a 250 mL graduated cylinder in the water source to be measured. Residual determinations were made using a modification of the 2% KI-sodium thiosulfate titration method (Standard Methods, 1971).

Larval System

The tanks containing larvae are rectangular, constructed of conrete and have a total volume of 13,000 Liters. They are maintained in warm bright rooms. Water temperature in the tanks ranged from 27–31 °C. Water exchanges with ozonized seawater were accomplished on a regular schedule by partially draining the tanks and then replenishing them with freshly ozone-treated seawater. Larval shrimp (nauplii) of *Litopenaeus vannameii* were obtained from the wild or from the maturation tanks at the hatchery. Nauplii were seeded into separate tanks dependent upon their origin (wild or cultured).

Food sources added to the tanks were the alga, *Chaetoceros gracilis* and the brine shrimp, *Artemia sp.* Prior to the full-scale use of ozone, artificial foods, EDTA, copper, formalin, trifluralin, and antibiotics were also added to the tanks.

Examination of Total and Pathogenic *Vibrio* Bacteria

Water samples for bacterial counts were obtained by immersing a 12 × 75 mm sterile tube into the tank to be examined, capping the tube immediately upon removal from the water. The water was then plated on OZR media for total bacterial counts and on TCBS agar, a media selective for *Vibrio sp.* Plating was done by placing 0.1 mL of water by sterile pipette onto each plate and spreading the water in a across the plate.

A second set of plates was then inoculated with 10 μLiters of water using a sterile loop to spread in a three-quadrant style. A last set of plates was inoculated with 0.1 μLiter of water by sterile loop. All plates were incubated at ambient RT and read after approximately 18 hours. Bacterial studies were conducted on: Water from the maturation tanks; Water from the larval rearing tanks; shrimp nauplii from the wild and from the maturation tanks; larval foods to include algae, *Artemia,* and commercially prepared foods; and various environmental cultures of the water system and hatchery physical plant.

Salmon And Squid Preservation

Salmon. Freshly caught adult salmon *(Oncorhynchus nerka)* weighing 4–6 pounds each were packaged separately in large plastic bags that contained ozonized brine ice. The ice was made by bubbling ozone into brine water contained in 5 gallon PVC buckets. The ozonized water was then frozen at −10 °C. Ozone was applied at 2 ppm to make a 0.5 ppm residual in the ice. Ozone was generated by a Crane PA, USA (Lab 70) 20 g/hour unit on oxygen.

Some of the salmon were left intact and others were completely dressed out (gutted). The salmon were then left at room temperature (15–18 °C) in the bags with ozonized brine ice for six days. The melted ice was poured off every day and each fish was examined for signs of spoilage before being repackaged. Controls consisted of whole and dressed-out salmon separately packaged in non-ozonized brine ice that were left under the same room temperature conditions for 6 days. The controls underwent the same pour-off and examination as the salmon packaged in ozonized ice.

Squid. Fresh squid *(Loligo pealei)* were stored in ozonized ice for up to 13 days. Ozone was made from oxygen using a Welsbach Corp., Philadelphia, PA, USA (Model T-816) generator and bubbled into flasks containing fresh water. The ozonized water was then frozen. Control batches of squid were held in the same conditions but were stored in conventional ice. The squid were examined daily for signs of deterioration using visual examination, total bacterial plate counts, and trimethylamine (an indicator of spoilage) values. The experiment was conducted in marine laboratories located in Milford, CT USA and in Gloucester, MA USA.

RESULTS AND DISCUSSION

Commercial Shrimp Hatchery: Experimental Results

Maturation tanks. During experimental studies, two tanks were provided ozone treatment to reduce or eliminate Vibriosis in the maturation system. Prior to ozone treatment, the tanks had *Vibrio* sp. counts of >200 colonies at a concentration of 10^{-2}. Vibrio counts were reduced by half every 3 hours after ozone treatment commenced, reaching a count of zero between 3–6 hours after the start of treatment. The ozone treatment did not appear to affect the mature shrimp adversely as judged by the normal movement of the animals in the tank throughout and following the treatment. No mortality of shrimp was experienced during the treatment or 24 hours post-treatment.

Foods. Of the six artificial commercial foods used to rear larval shrimp, one revealed significant levels of *Vibrio sp.* Algae consistently had zero plate counts on the agar specific for *Vibrio sp.* (TCBS agar). *Vibrio sp.* counts in the brine shrimp, *Artemia,* were 350 at a concentration of 10^{-1} prior to treatment. After 50 min of ozone treatment, *Vibrio* counts in the *Artemia* were reduced to three colonies at a concentration of 10^{-1}.

Shrimp nauplii. Unwashed shrimp nauplii from the wild contained high *Vibrio sp.* counts.

Larval tanks. Ozone-treated tanks showed reduction in *Vibrio* counts, as opposed to non-treated tanks, with water and algae in the tanks revealing zero plate counts for *Vibrio sp.* With the addition of wild nauplii, a commercial food, and/or *Artemia,* all known to contain high amounts of *Vibrio sp.,* the *Vibrio* count increased. After treatment with ozonized water, *Vibrio* plate counts decreased and after a full exchange of the tank with ozonized water, plate counts were zero for *Vibrio sp.* The ozone-produced residuals in the tanks were generally adequate to reduce *Vibrio* counts. The tanks without ozone treatment, however, required the addition of several antibiotics and disinfectants to combat the *Vibrio* sufficiently to permit larval survival.

Preliminary bacterial experiments indicated that *Vibrio sp.* was responsible for the mortality experienced at the hatchery. The source of the Vibriosis was found to be from nauplii from the wild as well as from the brine shrimp, *Artemia,* used as food for the larvae and from artificial commercial food given to the larvae. Ozone-treated seawater was successful in reducing and in several cases eliminating measurable *Vibrio sp.* concentrations in the tanks.

Oxidant Residual. When the oxidant residual exceeded 0.1 ppm, the larval shrimp exhibited behavior different from normal. They sank to the bottom of the tank and, when observed under a microscope, had damaged appendages. After a few hours, however, those larvae were observed to molt and exhibit normal behavior, rising to the surface, feeding and swimming actively. This led to the observation that in addition to controlling the *Vibrio* concentration, careful management of the oxidant residual could induce molting, reducing the larval growth period.

Commercial Production Results

The success of the experimental studies led to the ozone treatment of all thirty larval rearing tanks at the hatchery. In addition to supplying ozone-treated water for the tanks, ozone washes were also used for the wild nauplii and *Artemia* that were supplied to the tanks, those two additions being sources of high *Vibrio* counts prior to ozone water treatment. The ozone-produced residual was regulated to be between 0.066 to 0.250 ppm throughout the growth cycle to provide maximum control of *Vibrio sp.* ranging from 680 colonies at 10^{-2} to 0 following a water exchange with freshly ozonized water.

The use of ozonized seawater with residuals >0.1 ppm consistently occasioned early molting in the larvae, reducing the growth cycle by 3 days. This reduction allowed one additional rearing cycle per year. The percent survival of larvae with ozone use was between 60.0–99.1%, indicating that the use of ozone has not negatively impacted larval survival. Finally, the use of ozone eliminated the use of several additives formerly employed before ozone and reduced the use of antibiotics by 67%.

Salmon Preservation

The salmon iced with the non-ozonized ice were completely spoiled 4 days after capture. In contrast, the salmon preserved on ozonized ice did not start to spoil until the sixth day. The dressed-out salmon preserved on ozonized ice remained fresh for eight days. A commercial-sized study was conducted after this experimental effort. This larger study, using a Howe Baker Escozone generator, was conducted by Bob Marshall of Howe Baker and Dr. Richard Neve of the University of Alaska at Seward. The tests were conducted at the Alaska Ocean Product Ice House in Kenai, Alaska. The results from this later study confirmed our earlier observations as they noted that ozonized ice was able to preserve salmon 33-50% longer than conventional ice.

Squid Preservation

The squid stored on ozonized ice displayed significantly lower (2 logs lower) aerobic bacterial plate counts and had a 12% increase in shelf life over the squid stored on conventional flake ice alone with no ozone treatment after 13 days. Typical results are shown in Figure 1. A taste panel did not detect any difference in flavor when comparing squid stored on ozonized ice with squid stored on conventional flake ice. (Blogoslawski et al., 1983).

CONCLUSIONS

Commercial Hatchery Operations

From the experience gained at the commercial shrimp hatchery in Ecuador, it can be concluded that ozone treatment of seawater used to grow shrimp in useful production numbers is extremely beneficial. Bacterial pathogens were reduced to a manageable concentration or eliminated as determined by plate count technique and microscopic examination of larval shrimp. The threat of a pathogenic bacterial infection in a hatchery is extremely serious and can result in anything from reduction in production to the complete loss of the stock, causing economic distress that can end in the closure of the hatchery and of the business.

In a time when it is increasingly apparent that wild marine stock is not sufficient to meet the demands of an increasing population due to overfishing, pollution, and natural disasters, it is all the more important that viable means of producing nutritious and popular species by means of aquaculture and mariculture be explored and implemented. The primary threat to a seafood hatchery is from viral and bacterial disease that can be introduced from several sources and that can spread rapidly in hatchery conditions where stock is held in containers that provide close quarters. The use of antibiotics to contain disease situations in hatcheries is not only expensive in monetary terms but extracts an expense in the bioconcentration of these drugs in our food and through their release into the environment from hatchery effluent, giving rise to ever resistant strains of pathogens.

The use of a disinfectant that is efficient, cost-effective, and does not provide a harmful residual is ideal. Ozone has been proven to be that necessary agent. We are now past the days of saying that this oxidant shows great promise for future commercial application. We have demonstrated its efficacy in a large-scale commercial shrimp hatchery under less than ideal conditions. While it is more expensive than antibiotic use in the short term, it is more cost efficient over time and does not harm the environment as does the release of antibiotic-containing effluent. In addition to reducing the threat of a bacterial disease that can close a hatchery, ozone treated water has provided unanticipated benefits at this hatchery such as increased production and the elimination of supplemental disinfectants as it does not select for the most resistant bacteria.

As in depuration facilities (Fauvel et al., 1982), the use of ozone in a hatchery environment ensures the provision of a food product that is safe for consumers. Work has also shown that ozone is effective in detoxification of harmful marine algal blooms that can affect marine food products (Blogoslawski and Stewart, 1977a). The adoption of this technology is spreading (Steffen and Rice, 2010; Rice and Wrenn, 2010) and it is incumbent upon those of us who work

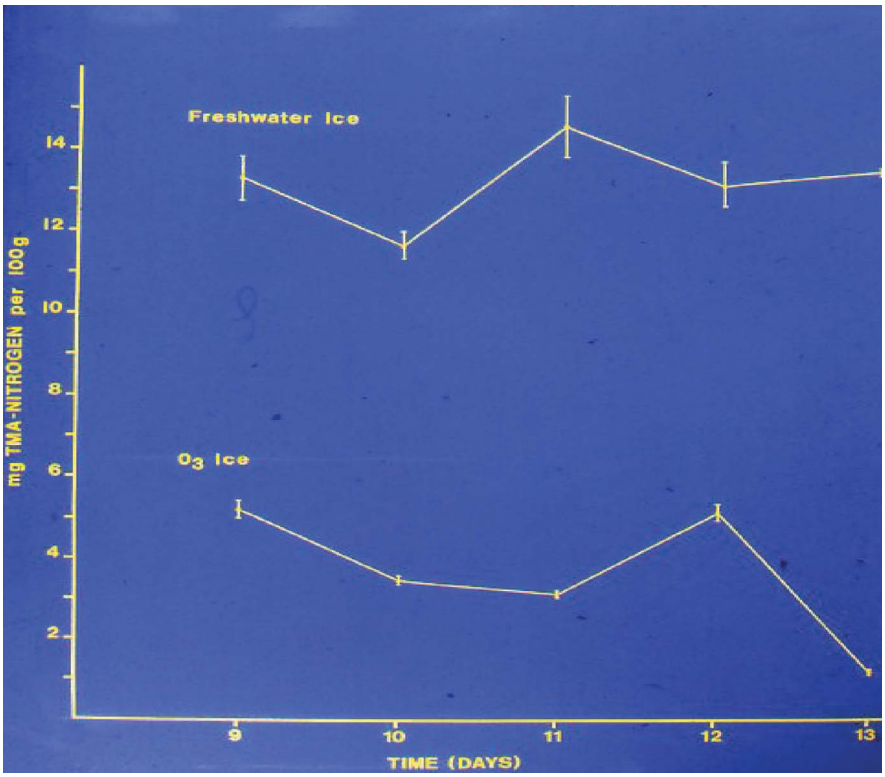

FIGURE 1. TMA (trimethylamine) levels in squid treated with ozonated vs. conventional ice (color figure available online).

with seafood to advance the incorporation of this effective agent in aquaculture and mariculture operations.

Seafood Preservation with Ozonized Ice

Salmon and squid are important fisheries in the United States and abroad. A desire to increase the shelf life of commercially important fish led to work with ozonized ice to determine whether this could be a better preservative for freshly caught fish. Early work by this author showed that plastic milk bottles filled with triple distilled, super-ozonized (2–4 mg ozone/mL water) fresh water and frozen at −80 °C could retain a residual of 1–2 mg of ozone for up to six months. It was interesting to note that as the bottles were melted in hot potassium iodide solution they issued a characteristic ozone off-gassing odor.

Using this information, it was thought that as ozone is known to be an extremely effective disinfectant, ozonized ice could be of great value to the fishing industry in the preservation of freshly caught fish during transport and processing. The results of the experiments with salmon in Alaska and with squid in Milford, CT and Gloucester, MA laboratories indicate the utility of ozone for this function. Ozone yields additional benefits beyond increasing shelf life in that it does not affect the taste of the product and it assures a safe food item for consumers.

It has been interesting and gratifying to see the experimental work adapted to commercial scale. Many different applications for ozone in food disinfection and preservation are currently employed (Steffen and Rice, 2010; Rice and Wrenn, 2010; North Carolina Sea Grant, 2002). In a time when safe protein resources are vitally needed for our world, the utility of ozone to assist in the provision of these resources is of great benefit.

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