The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Optimal Dose of Total Residual Oxidants for Hybrid Tilapia (*Oreochromis mossambicus x O. niloticus*) and Whiteleg Shrimp (*Litopenaeus vannamei*) in Ozone-Treated Sea Water

Hung-Tsai Lee[1], Hung-Jen Lee[2], Jih-Terng Wang[3], Ming-Yih Leu[4,5*] and Pei-Jie Meng[5,6*]

[1] Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica 23-10, Dah-Uen Rd., Jiau Shi, I-lan, 262 Taiwan

[2] Department of Marine Environmental Informatics, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 202-24, Taiwan

[3] Department of Biotechnology, Tajen University, Ping-Tung 907, Taiwan

[4] Department of Aquaculture, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 202-24, Taiwan

[5] National Museum of Marine Biology and Aquarium, 2 Houwan Road, Checheng, Pingtung 944, Taiwan

[6] Graduate Institute of Marine Biodiversity and Evolutionary Biology, National Dong Hwa University, 2 Houwan Road, Checheng, Pingtung 944, Taiwan

(Received 7.4.09, Accepted 17.5.09)

Key words: ozone, total residual oxidants (TRO), oxidation-reduction potential (ORP), hybrid tilapia (*Oreochromis mossambicus x O. niloticus*), whiteleg shrimp (*Litopenaeus vannamei*)

Abstract

The purpose of this study was to use total residual oxidants (TRO) as an indicator for determining the optimal ozone dosage needed to control water quality and thereby enhance survival of cultivated aquatic organisms. When the TRO concentration was maintained at 0.16 mg/l for two hours, the total bacteria plate count dropped from 7.7 x 10^3 CFU/ml in the untreated sea water to less than 10 CFU/ml in the ozone-treated sea water. The TRO concentration in the ozone-treated water was well below the 96-h LC_{50} for hybrid tilapia (*Oreochromis mossambicus x O. niloticus*) and whiteleg shrimp (*Litopenaeus vannamei*) determined in this study. Hence, adjustment of the ozone concentration in aquacultural sea water is a viable option that simultaneously kills the majority of harmful bacteria in the water and enhances survival of cultivated aquatic organisms.

* Corresponding author. Fax: +886-8-8825066, e-mail: pjmeng@nmmba.gov.tw, myl@nmmba.gov.tw

Introduction

Ozone (O3) was discovered in 1785 by Van Marum who utilized air and high voltage discharge equipment to obtain this gas. Ozone, a strong oxidizing agent with a special odor, has many useful applications such as reducing virus populations and pharmaceutical sterilization (Sugita et al., 1992a; Price et al., 1993; Labonne, 1995; Chang et al., 1998a; Meunpol et al., 2003). It is widely used in the treatment of drinking water and the food industry. The use of ozone to improve the quality of sea water in aquaculture is certainly not a new practice (Reid and Arnold, 1992; Chang et al., 1998a; Meunpol et al., 2003).

Ozone has typically been used as a disinfectant, bleaching agent, deodorant, water purifier, and anti-bacterial agent (Labonne, 1995; Sugita et al., 1996, 1997; Chambers et al., 2006; Glatman et al., 2006). Ozone effectively destroys the cell wall and membrane of bacteria, causing the cell to lose activation. In fact, the main effect of ozone is that it changes the protein structure of the cell membrane of the virus, resulting in the inactivation of the virus.

Ozone has been widely used to treat aquarium and cultivating water (Labonne, 1995; Sugita et al., 1996; Chang et al., 1998a). However, the mechanism of seawater treatment using ozone is complicated, contrary to what was previously thought. In contrast to its performance in freshwater applications, ozone undergoes many changes in chemical form when applied to sea water. Free ions within sea water (e.g., dissolved chlorine, bromide, iodine, and ammonium) react with the ozone to produce a series of byproducts such as Cl_2, $HClO^-$, ClO^-, $HBrO^-$, BrO^-, and other halide derivatives (Wong and Davidson, 1977; Wong, 1982).

Methods of measuring the amount of dissolved ozone in sea water have been compared (Buchan et al., 2005). One important consideration in the treatment of sea water with ozone is the total residual oxidant (TRO) concentration (Sugita et al., 1992b). At excess levels, these chemical compounds are toxic to aquatic organisms and can reduce their survival rate (Chang et al., 1998a; Douillet et al., 1999; Meunpol et al., 2003). For example, mortality of red sea bream (*Pagrus major*) was 50% within one day when the TRO concentration reached 0.16 mg/l (Kawahara et al., 1997).

Seawater characteristics, including organic content and ammonia, affect the amount of ozone required to achieve a desired TRO level. The rate of TRO decay has been reported (Perrins et al., 2006; Wang et al., 2008). Further, total organic carbon (TOC) has a significant reducing effect on ozone availability (e.g., Wong, 1977; Allonier, et al., 1999; Nebot et al., 2006).

The quality assurance and quality control (QA/QC) system of the Taiwan government, set up by the Environmental Protection Administration in 1987, was established in our laboratory, which maintains high standards of laboratory practices for environmental monitoring. The procedures and frequency used for periodic calibration of monitoring equipment must be specific. Calibration practices include initial calibration, routine calibration, and specific calibration.

The purpose of this study was to evaluate the effectiveness of ozone for controlling the quality of culture water. Adjustment of the ozone concentration can result in the elimination of the majority of harmful bacteria in the water and the enhancement of survival of cultivated aquatic organisms. Consequently, if we can determine the relationship between oxidation-reduction potential (ORP) and TRO, we will be able to estimate the TRO concentration according to the ORP value, thus replacing the need to determine TRO.

Materials and Methods

Prior to ozone treatment, the sea water was sampled and salinity, biological oxygen demand (BOD_5), ammonia, nitrite, and TOC were monitored. The sea water had the following characteristics: pH 8.0-8.3, salinity 33-35 practical salinity units (psu), and TOC 0.05-1.00 mg/l.

A coaxial hydraulic pressure mixing device (Coaxial Liquid Pressurized Mixer, AirTree Ozone Technology Co., Taiwan) was used to thoroughly mix ozone with one ton of sea water. The coaxial hydraulic pressure mixing device was equipped with an oxidation-reduction potential (ORP)

detector (WTW pH330 ORP electrode) that was calibrated before measurements. Redox-Buffer Solution 468mv (pH 0.1; Lot 1M125B; Mettler Toledo Co.) was used.

The ozone generator (M-Twins ozone generator, Model M260M04P50, AirTree Ozone Technology Co., Taiwan) has an ozone production of about 20 g/h. The ozone production rate in this experiment was 10%, with a real output of approximately 1.08 g/h. After the ozone treatment system was initiated, ORP data were recorded at sampling times, when values were stable. Water was treated with ozone for 120 min. Water samples were analyzed for total plate counts (TPC) and TRO.

Details of the TRO detection method can be found in Sugita et al. (1992b). In this study, 1 ml of 0.2 M acetate buffer (pH 4.0) was mixed with 10 ml of the water sample, then added to 0.4 ml of 10% KCl solution. The absorbance at 325 nm was determined with a UV-VIS spectrophotometer (Hitachi model U-2000) after 40 min of incubation. The iodine colorimetric standard was prepared by dissolving 6.4 g KI and 1.2692 g I_2 in 1 liter of milli-Q water and preserved in a brown bottle. Potassium chloride in the concentration of 3.3% was used as the seawater blank.

Treatments included the following six TRO levels: 0.05 (control), 0.07, 0.11, 0.19, 0.47, and 0.90 mg/l. Two 324-l tanks (120 x 60 x 45 cm) for each treatment were filled with ozone-treated (except for the control) sea water and 100 tilapia (30.0 g) plus 100 whiteleg shrimp (9.2 g). The tanks were divided into two sections: one for the tilapia and the other for the shrimp. The tilapia had been acclimated in salt water (33 psu), and were confirmed to be healthy. The tilapia and shrimp were unfed. Mortality was recorded at 1, 3, 6, 12, 24, 48, and 96 h. The lethal concentrations for 50% mortality (LC_{50}) of the tilapia and whiteleg shrimp were determined at each of these times. Toxicity data were analyzed using probit analysis (Collett, 1991).

Viable cell counts were performed on *Vibrio*-selective TCBS agar (4.5% Difco TCBS agar, 0.9% marine broth 2216, 0.45% NaCl, and 1.2% Difco Bacto agar). Plates were incubated for 16 h at 37°C, and the numbers, types, and colors of fungus colonies were recorded. The operations in each group were triplicated.

Results

ORP values increased to 740 mv when the rate of the generated ozone reached 37.5 mg/h (Fig. 1). The ozone-generated rate was linearly correlated with the TRO (Fig. 2). The LC_{50} value after 96 h (96-h LC_{50}) for tilapia was approximately 0.39 mg/l (Fig. 3). For whiteleg shrimp it was 0.80 mg/l (Fig. 4). *Vibrio* sp. dropped to the detection level when the TRO concentration reached 0.16 mg/l (Fig. 5). Total plate counts of microorganisms completely vanished when the TRO concentration reached 0.33 mg/l (Fig. 6).

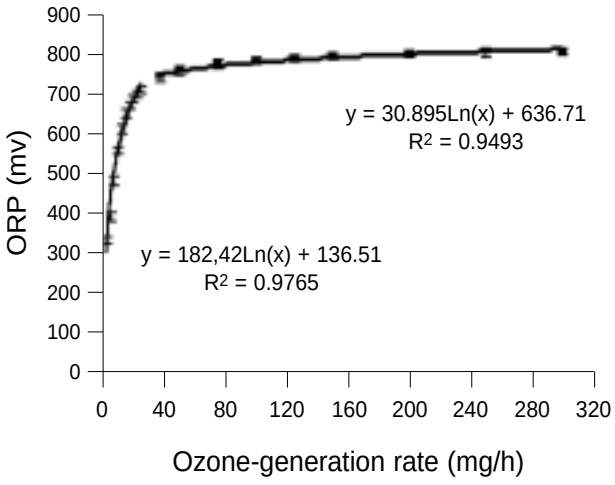

Fig. 1. Oxidation-reduction potential (ORP) as a function of the ozone-generation rate.

Discussion

The chemical reaction of ozone used to treat sea water is quite complicated. For example, the most important reaction is the oxidation

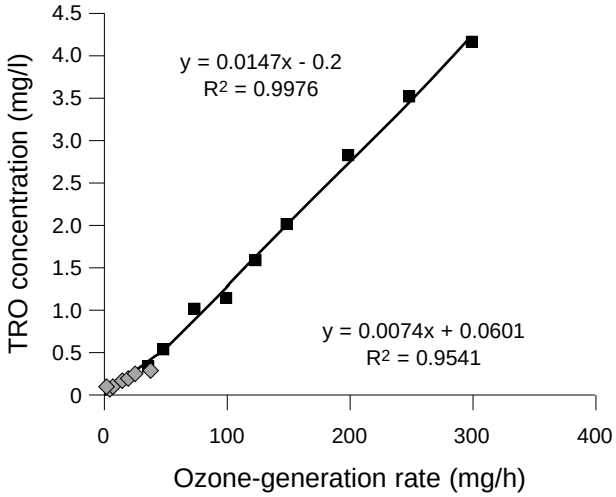

Fig. 2. Relationship between the ozone-generation rate and the total residual oxidant (TRO) concentration in the cultivated water.

of bromide ions (Br^-) to form hypobromite ions (OBr^-) that can then be reduced back to Br^- or further oxidized to form bromate ions, BrO_3^- (Grguric et al., 1994). Bromide ions can also react with ozone and cause oxidation, which ultimately decreases the ozone concentration. Consequently, the oxidation-reduction potential (ORP) theoretically increases with ozone dosage. In this study, we found that the ORP value reached 740 mv when the rate of generated ozone reached 37.5 mg/h.

The ozone-generated rate was linearly correlated with TRO. However, two linear segments were needed to fully demonstrate the relationship between TRO and the rate of generated ozone so as to more accurately control fluctuations of culture water quality. In Fig. 2 the slopes are divided into two segments with correlation coefficients of 0.9976 and 0.9541.

Organisms have different tolerances for TRO concentration. The LC_{50} value after 96 hours (96-h LC_{50}) was approximately 0.39 mg/l for tilapia and 0.80 mg/l for whiteleg shrimp, suggesting that whiteleg shrimp are more tolerant to ozone than tilapia. When estimated from the equations obtained from Figs. 3 and 4, the safe TRO concentrations for tilapia and whiteleg shrimp were 0.08 and 0.12 mg/l, respectively. Applying the equation obtained from Fig. 2, the ozone-generated rates that produce safe levels of TRO were 2.69 for tilapia and 8.09 mg/h for whiteleg shrimp.

Analysis of biochemical and enzyme characteristics showed that *Vibrio* spp. were the most important colonies in the water. Yellow colonies were identified as *Vibrio splendidus* and *V. carchariae*, whereas green colonies were *V. parahaemolyticus* (Themptander, 2005). *Vibrio* sp. dropped to its detection limit when the TRO concentration was above 0.16 mg/l, a concentration that can be obtained at the ozone-generation rate of 15.0 mg/h. The microorganisms completely vanished at a TRO concentration of 0.33 mg/l, which can be achieved by an ozone generation rate of 37.5 mg/h.

Sea water ozonized at a rate of 15.0 mg/h will kill most of the bacteria. The TRO concentration to achieve this purpose is about 0.16 mg/l, which is well below the 96-h LC_{50} of tilapia and whiteleg shrimp determined in this study. Had the rate of ozone generation been 17.5 mg/h, the

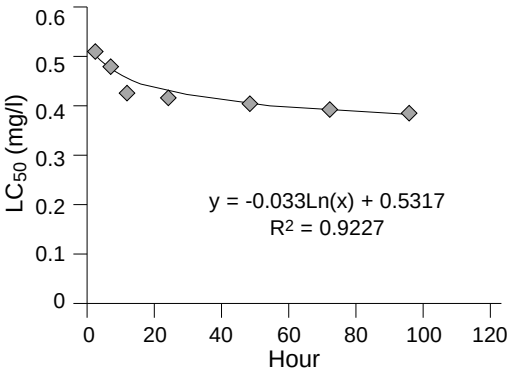

Fig. 3. Relationship between the total residual oxidant (TRO) lethal concentration (LC_{50}) of hybrid tilapia (*Oreochromis mossambicus x O. niloticus*) and time.

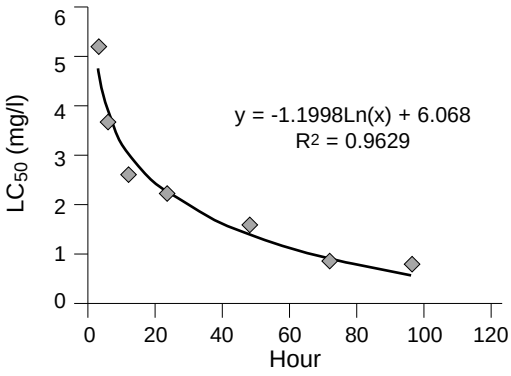

Fig. 4. Relationship between the total residual oxidant (TRO) lethal concentration (LC_{50}) of white-leg shrimp (*Penaeus vannamei*) and time.

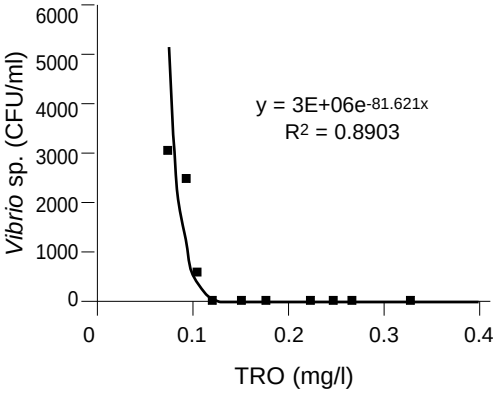

Fig. 5. *Vibrio* sp. counts in water containing various concentrations of total residual oxidants (TRO).

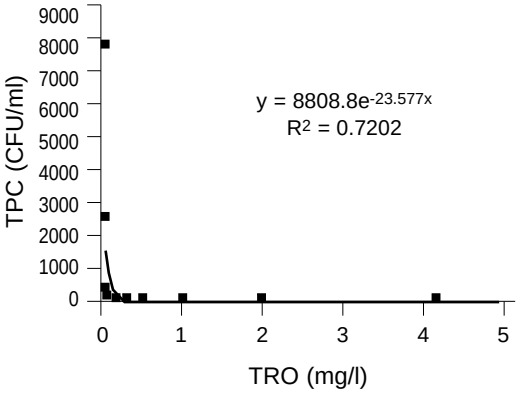

Fig. 6. Total plate counts (TPC) in water containing various concentrations of total residual oxidants (TRO).

expected TRO concentration would be approximately 0.18 mg/l and the *Vibrio* would be essentially eliminated or reduced to less than 20 CFU/ml. This indicates that ozone treatment of sea water is a viable option for the cultivation of most aquatic organisms.

Although this experiment was not designed to test virus treatments, a flow rate of 0.5 or 0.8 mg/l for 10 min is expected to reduce infection from white spot bacilliform virus (WSBV; Chang et al., 1998b, 1998c). A similar result can be achieved by treatment with TRO at a concentration of 0.16 mg/l in excess of 10 min.

Some scientists have used activated carbon to eliminate TRO (Kawahara et al., 1997). An important reason to eliminate TRO is that the time required for ozone addition is usually extensive, leading to the production of high TRO concentrations. By using the relationship between

the rate of ozone generated and TRO as shown above, it is now possible to calculate the amount of ozone necessary for a given water volume to achieve the desired TRO concentration. Thus, we can simply monitor the ORP sensor to achieve the desired parameters. Ozone can be used effectively as mentioned above, and is cheaper than using activated carbon. Additionally, ozone can be a very effective disinfectant for water when TRO is well controlled.

In short, it is extremely difficult to control the ozone dosage when using ozone to treat common cultivated water. However, the amount of ozone to be added may be effectively assessed by measuring the TRO concentration.

Acknowledgements

We are truly grateful to the AirTree Ozone Technology Co. for assisting with the installation of the ozone experimental facilities, subsequently refitting the ozone machine to adapt to the micrometer, and correcting the ozone machine, which helped the experiments run more smoothly. This study was financially supported by the Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan.

References

Allonier A.S., Khalanski M., Camel V. and A. Bermond, 1999. Characterization of chlorination by-product in cooling effluents of coastal nuclear power stations. *Mar. Pollut. Bull.*, 38:1232-1241.

Buchan K.A.H., Martin-Robichaud D.J. and T.J. Benfey, 2005. Measurement of dissolved ozone in sea water: a comparison of methods. *Aquac. Eng.*, 33:225-231.

Chambers L.D., Stokes K.R., Walsh F.C. and R.J.K. Wood, 2006. Modern approaches to marine antifouling coatings. *Surf. Coat. Tech.*, 201:3642-3652.

Chang P.S., Chen L.K. and Y.C. Wang, 1998a. The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus (WSBV). *Aquaculture*, 166:1-17.

Chang P.S., Chen H.C. and Y.C. Wang, 1998b. Detection of white spot syndrome baculovirus (WSBV) in experimentally infected wild shrimps, crabs and lobsters by *in situ* hybridization. *Aquaculture*, 164:233-242.

Chang P.S., Tasi D.H. and Y.C. Wang, 1998c. Development and evaluation of a dot blot analysis for the detection of white spot syndrome baculovirus (WSBV) in *Penaeus monodon*. *Fish Pathol.*, 33:45-52.

Collett D., 1991. *Modelling Binary Data*. Chapman and Hall/CRC.

Douillet P.A and P.L. Pickering, 1999. Seawater treatment for larval culture of the fish *Sciaenops ocellatus* Linnaeus (red drum). *Aquaculture*, 170:113-126.

Glatman L., Sachs O., Khanin Y., Drabkin V. and A. Gelman, 2006. Ozone action on survival and storage life of live and chilled tilapia. *Isr. J. Aquac. - Bamidgeh*, 58(3):147-156.

Grguric G., Trefry J.H. and J.J. Keaffaber, 1994. Ozoneation products of bromine and chlorine in seawater aquaria. *Water Res.*, 28:1087-1094.

Kawahara H., Horike H., Yotsumoto H., Ozawa T., Sasaki T. and S. Nakayama, 1997. Ozone treatment of seawater. pp. 139-142. In: *Proc. 4th. Int. Aquar. Cong.*, Tokyo.

Labonne D.L., 1995. Survey of ozone system design and chemical application of major facilities in North America. pp. 347-359. In: *Proc. 3rd Int. Aquar. Cong.*

Meunpol O., Lopinyosiri K. and P. Menasveta, 2003. The effects of ozone and probiotics on the survival of black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 220:437-448.

Nebot E., Casanueva J.F., Casanueva T., Fernandez-Bastom M.M. and D. Sales, 2006. *In situ* experimental study for the optimization of chlorine dosage in seawater cooling systems. *Appl. Therm. Eng.*, 26:1893-1900.

Perrins J.C., Cooper W.J., Leeuwen J.V. and R.P. Herwig, 2006. Ozoneation of seawater

from different locations: formation and decay of total residual oxidant - implications for ballast water treatment. *Mar. Pollut. Bull.,* 52:1023-1033.

Price M.L., Bailey R.W., Enos A.K., Hook M. and S.W. Hermanowicz, 1993. Evaluation of ozone/biological treatment for disinfection byproducts control and biologically stable water. *Ozone Sci. Eng.*, 15:95-130.

Reid B. and C.R. Arnold, 1992. The intensive culture of penaeid shrimp *Penaeus vannamei* Boone in a recirculating raceway system. *J. World Aquac. Soc.*, 23:146-153.

Sugita H., Asai T., Hayashi K., Mitsuya T., Amanuma K., Maruyama C. and Y. Deguchi, 1992a. Application of ozone disinfection to remove *Enterococcus seriolicida*, *Pasteurella piscicida*, and *Vibrio anguillarum*. *Appl. Environ. Microbiol.*, 58(12):4072-4075.

Sugita H., Hayashi K., Asai T., Mitsuya T., Amanuma K., Maruyama C. and Y. Deguchi, 1992b. Spectrophotometric method for determination of total residual oxidants in seawater. *Suisanzoshoku*, 40:45-49.

Sugita H., Mita J. and Y. Deguchi, 1996. Effect of ozone treatment on amylases in seawater. *Aquaculture*, 141:77-82.

Sugita H., Mita J. and Y. Deguchi, 1997. Disinfection and purification of seawater by ozone treatment. pp. 321-324. In: *Proc. 4th Int. Aquar. Cong.*, Tokyo.

Themptander K.S., 2005. *Detection and Characterisation of Vibrio harveyi Isolates.* School Biol. Sci., Dublin Inst. Technol. Press. 63 pp.

Wang J.T., Chen M.H., Lee H.J., Chang W.B., Chen C.C., Pai S.C. and P.J. Meng, 2008. A model to predict total chlorine residue in the cooling seawater of a power plant using an iodine colorimetric method. *Int. J. Mol. Sci.*, 9:542-553.

Wong G.T.F., 1982. Factor affecting the amperometric determination of trace quantities of total residual chlorine in seawater. *Environ. Sci. Tech.*, 16:785-790.

Wong G.T.F. and J.A. Davidson, 1977. The fate of chlorine in seawater. *Water Res.*, 11:971-978.