Aquacult Int (2014) 22:653–659
DOI 10.1007/s10499-013-9694-x

Short communication: Acute toxicity of hydrogen peroxide in juvenile white shrimp *Litopenaeus vannamei* reared in biofloc technology systems

Plínio S. Furtado · Fabiane P. Serra · Luis H. Poersch · Wilson Wasielesky Jr.

Received: 6 March 2013 / Accepted: 13 August 2013 / Published online: 28 August 2013

Abstract Biofloc technology (BFT) has been used to rear white shrimp, *Litopenaeus vannamei*. In this culturing system, the absence of aeration causes a rapid drop in dissolved oxygen levels, and hydrogen peroxide (H_2O_2) can be used as an emergency source of oxygen. This study aimed to determine the lethal concentration and safe level of H_2O_2 applied as a source of oxygen for juvenile white shrimp *L. vannamei* in a BFT system. Juveniles (1.39 ± 0.37 g) were exposed for 2 h to different concentrations of H_2O_2 [29 (100), 58 (200), 116 (400), 174 (600), 232 (800), 290 (1,000) and 348 (1,200) μL H_2O_2/L (ppm H_2O_2-29 %/L)] in addition to a control group without addition of H_2O_2, and the survival rates were monitored for 96 h. The LC_{50} values and 95 % confidence intervals at 24, 48, 72 and 96 h were 235.5 (207–268), 199.1 (172–229), 171.1 (146–198) and 143.3 (120–170) μL H_2O_2/L, respectively. The safe level was 14.3 μL H_2O_2/L, and the highest concentration with survival rates similar to the control group (NOAEC) was 29 μL H_2O_2/L. In these concentrations, H_2O_2 can be used as a safe source of oxygen for *L. vannamei* reared in BFT systems.

Keywords Biofloc · Dissolved oxygen · Hydrogen peroxide · *Litopenaeus vannamei* · Toxicity

Introduction

Biofloc technology (BFT) refers to the rearing of aquatic organisms in high stocking densities without water renewal, using strong aeration and predominantly aerobic and heterotrophic biota that form microbial flocs (bacteria, protozoa, rotifers and microalgae, among others) (Avnimelech 2007). This system maintains the water quality and also

P. S. Furtado · F. P. Serra · L. H. Poersch · W. Wasielesky Jr. (✉)
Marine Station of Aquaculture, Institute of Oceanography, Federal University of Rio Grande, C. P. 474, Rio Grande, RS CEP 96201-900, Brazil
e-mail: manow@mikrus.com.br

produces an additional food source for the farmed organisms (De Schryver et al. 2008). The absence of aeration in *Litopenaeus vannamei* ponds with BFT systems critically reduces the levels of dissolved oxygen (DO) in approximately 30 min (Vinatea et al. 2009). As stated by Lima et al. (2012), adding hydrogen peroxide (H_2O_2) at 5 ppm promotes an effective increase in DO levels, although this effect lasts for a short time (2–3 h). In the natural environment, the H_2O_2 half-life varies from a few minutes (eutrophic environments) to several days (oligotrophic environments) (Herut et al. 1998). This process is accelerated by the presence of organic matter in the water column, light intensity, temperature, enzymes (catalase) and metals (iron and manganese), among other factors (Taylor and Ross 1988; Pedersen et al. 2006; Burridge et al. 2010). In addition, H_2O_2 is considered environmentally friendly because it decomposes into water and oxygen gas.

The process of hypoxia and reoxygenation results in high levels of reactive oxygen species and promotes oxidative stress in white shrimp (Zenteno-Savín et al. 2006). An increase in DO levels (hyperoxia) also induces oxidative stress in aquatic vertebrate and invertebrate organisms (Lushchak 2011). The effects of lethal concentrations of H_2O_2 have mainly been reported in fish (Arndt and Wagner 1997; Avendaño-Herrera et al. 2006; Roque et al. 2010) and a few crustaceans (Abele-Oeschger et al. 1997). This study aimed to determine the lethal concentration and the safe level of H_2O_2 during the culture of juvenile white shrimp *L. vannamei* in BFT systems. The results of this study allow researchers and shrimp producers working with BFT systems to use H_2O_2 as a safe source of oxygen.

Materials and methods

Post-larvae of *L. vannamei* (PL_{20}) were reared in April 2012 at the Marine Aquaculture Station hatchery, Federal University of Rio Grande, Southern Brazil. Subsequently, they were stocked in nursery tanks (1,500 PL/m^2) with BFT systems. Juveniles shrimp (1.39 ± 0.37 g) was acclimated for 48 h to the experimental conditions to reduce the stress from handling. The shrimp was fed twice per day with commercial feed (Guabi®, 38 % crude protein and 8 % ether extract), and the feeding amount was set at 8 % of the total biomass of each experimental unit. The photoperiod was set at 12 L:12D with a luminous intensity of 500 lux and temperature of 28 °C.

The experiment was performed in 4-L plastic tanks (effective volume = 3 L) filled with sea water (salinity 32 ‰ and alkalinity 125 mg $CaCO_3$/L) and 534 mg/L of bioflocs from the shrimp culture tank, with air diffusers to maintain aeration. The experimental system was static without water renewal, and feeding was suspended during the test. The trial consisted of 3 shrimps per experimental unit (tank) (1 juvenile/L), 15 tanks per treatment ($n = 45$ shrimp/treatment) and eight treatments ($n = 360$ juveniles). The treatments included different concentrations of H_2O_2 [29 (100), 58 (200), 116 (400), 174 (600), 232 (800), 290 (1,000) and 348 (1,200) µL H_2O_2/L (ppm H_2O_2-29 %/L)] and a control group (without addition of H_2O_2).

The concentration of H_2O_2 (29 % H_2O_2, Synth, Brazil) was equivalent to 100 % of H_2O_2, and the dosages were nominal. After the acclimatization period, the aeration was interrupted until the DO levels reached 2.5 mg/L (approximately 30 min), simulating a power failure. Hydrogen peroxide was applied, and after 2 h of exposure, the aeration was re-established in each test unit. The shrimp behavior was recorded for 12 h after the exposure to H_2O_2, and mortality was verified every 24 h. Animals were considered dead when they were still and did not respond to mechanical stimuli with a glass cane (Lin and Chen 2003) and were immediately removed from the tank.

Temperature, pH and oxygen levels were measured daily in the morning and afternoon with a pH meter (model 100, Yellow Springs Instruments, USA) and oximeter (model 55, YSI). The salinity was checked at the beginning and end of the test with an optical refractometer (Atago, Japan). The concentrations of total ammonia nitrogen (TAN) ($NH_3 + NH_4^+$) and nitrite ($N–NO_2^-$) were measured daily, according to UNESCO (1983) and Bendschneider and Robinson (1952), respectively.

Cumulative mortality at 24, 48, 72 and 96 h was used to estimate the median lethal concentrations (LC_{50}) using the software code for the Trimmed–Spearman–Karber method (Hamilton et al. 1977). The safe level was calculated using the 0.1 factor suggested by Sprague (1971). The NOAEC or the highest concentration with survival rates similar to the control group (EPA 2002) was calculated by comparing the number of survivors in each treatment at the end of the test. The data were subjected to analyses of variance (one-way ANOVA) followed by Tukey's test ($p < 0.05$).

Results

The water quality was negatively affected by the increasing H_2O_2 concentrations. The total ammonia nitrogen level significantly differed ($p < 0.05$) among the control, 29 μL H_2O_2/L treatment and the other treatments. The mean nitrite concentrations were significantly lower ($p < 0.05$) in the treatment with 29 μL H_2O_2/L, followed by the 58 μL H_2O_2/L, control, 116, 174, 232, 290 and 348 μL H_2O_2/L treatments. No significant differences ($p > 0.05$) related to salinity, temperature, DO or pH were observed among the treatments. The water quality parameters are presented in Table 1.

After 2 h of exposure to H_2O_2, the DO concentration was 6 mg/L in the control treatment and 13 mg/L in the treatment with 29 μL H_2O_2/L, and it exceeded the oximeter detection limit of 20 mg/L in the other treatments. Gas bubbles were observed on all of the tank walls of the treatments with 174, 232, 290 and 348 μL H_2O_2/L before aeration was reactivated (Fig. 1a). The shrimp in these treatments behaved differently from in the control, including erratic swimming and loss of balance. Small bubbles appeared between the carapace and muscle of individuals from the treatments with 290 and 348 μL H_2O_2/L.

Table 1 Water quality parameters (mean ± SD) measured during a 96 h test with different concentrations of H_2O_2 in tanks with juveniles *L. vannamei*

μL H_2O_2/L	Salinity (‰)	Temperature (°C)	DO (mg/L)	pH	TA-N (mg/L)	NO_2^-–N (mg/L)
0	32.5 ± 0.5	26.5 ± 1.0	6.1 ± 0.4	8.0 ± 0.2	0.14 ± 0.1^a	0.15 ± 0.03^a
29	32.3 ± 0.3	26.3 ± 1.4	5.9 ± 0.3	8.0 ± 0.2	0.42 ± 0.3^{ab}	0.08 ± 0.03^b
58	32.5 ± 0.5	26.8 ± 1.2	6.0 ± 0.2	8.0 ± 0.1	0.58 ± 0.4^b	0.12 ± 0.09^{ab}
116	32.4 ± 0.6	26.5 ± 0.9	6.0 ± 0.3	8.1 ± 0.1	1.72 ± 1.2^c	0.18 ± 0.05^a
174	32.6 ± 0.3	26.6 ± 1.1	6.1 ± 0.4	8.1 ± 0.1	1.55 ± 0.9^c	0.18 ± 0.06^a
232	32.6 ± 0.5	26.3 ± 1.0	6.3 ± 0.2	8.1 ± 0.1	1.80 ± 1.3^c	0.15 ± 0.03^a
290	32.4 ± 0.6	26.7 ± 1.0	6.3 ± 0.3	8.0 ± 0.2	1.65 ± 1.0^c	0.21 ± 0.06^a
348	32.0 ± 0.0	26.0 ± 0.0	6.2 ± 0.1	8.2 ± 0.0	1.82 ± 1.1^c	0.20 ± 0.05^a

Different letters after the values in each column indicate a significant difference ($p < 0.05$) according one-way ANOVA followed by Tukey's test

DO dissolved oxygen; *TA-N* total ammonium nitrogen; *NO_2^-–N* nitrite

After 48 h of exposure, melanization was observed in the carapace and gills of the shrimp exposed to 116, 174, 232 and 290 μL H_2O_2/L (Fig. 1b). One hundred percent mortality was observed in the treatment with 348 μL H_2O_2/L after 16 h of the experiment, while the treatment with 290 μL H_2O_2/L showed 53.38 % mortality in the first 24 h. Survival rates over the 96 h of the experiment are shown in Fig. 2. The LC_{50}-96 h was 143.3 μL H_2O_2/L equivalent to 493 ppm (with a 95 % confidence interval of 120–170 μL H_2O_2/L). The values of LC_{50} at 24, 48, 72 and 96 h are shown in Table 2. The safe level was 14.3 μL H_2O_2/L, approximately 50 % of the largest applied dose that caused no mortality (NOAEC), which was 29 μL H_2O_2/L.

Discussion

The water quality parameters remained within the range considered adequate for the development of juveniles *L. vannamei* (Ponce-Palafox et al. 1997; Van Wyk and Scarpa 1999; Lin and Chen 2001, 2003). Although the TAN levels rose with increasing

Fig. 1 **a** Gas bubbles and erratic swimming were observed on all of the tank walls in the treatments with 174, 232, 290 and 348 μL H_2O_2/L before aeration was reactivated. **b** Melanization observed in the carapace and gills of the shrimp exposed to 116, 174, 232 and 290 μL H_2O_2/L

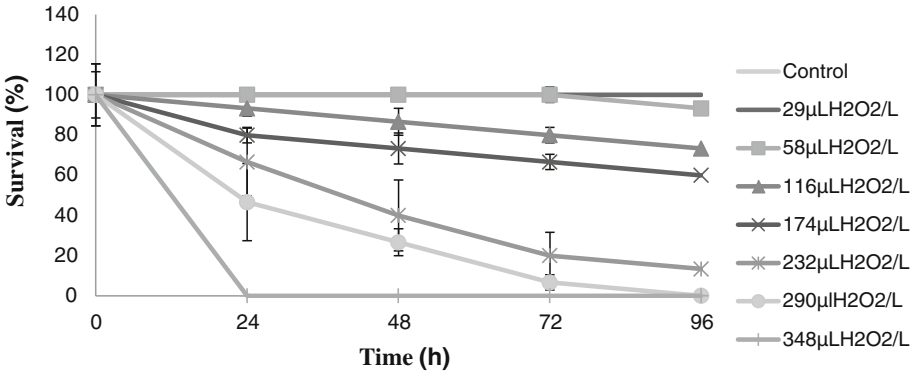

Fig. 2 Survival (mean ± SD) during a 96 h test after 2 h of exposure to different levels of H_2O_2

Table 2 Median LC_{50} of H_2O_2 given to juvenile white shrimp *L. vannamei*. The data in brackets represent the 95 % confidence interval

Time (h)	LC_{50} H_2O_2 (µL/L)
24	235.5 (207–268)[a]
48	199.1 (172–229)[ab]
72	171.1 (146–198)[bc]
96	143.3 (120–170)[c]

Values followed by different letters significantly differed ($p < 0.05$) based on a one-way ANOVA followed by Tukey's test

concentrations of H_2O_2, they did not affect the survival of the shrimp (Lin and Chen 2001). However, H_2O_2 may have negatively impacted the microorganisms. According to Schwartz et al. (2000), removal of ammonia in a biofilter is reduced by 80 % with doses of 100 mg H_2O_2/L in fish recirculation systems. The nitrite concentrations were significantly reduced ($p < 0.05$) in the treatment with 29 µL H_2O_2/L.

Pedersen and Pedersen (2012) verified that the bacterial communities that colonize biofilters may not collapse completely depending on the amount of organic matter present in the biofilter and the dosage of H_2O_2 that is applied to the system. Therefore, the high TAN levels in the treatments with higher doses of H_2O_2 were due to the excreta of shrimp and the toxic effect on microorganisms from the bioflocs (heterotrophic nitrifying bacteria and protozoa, rotifers and microalgae, among others).

H_2O_2 is a neutral molecule that passes easily through cell membranes by diffusion. Within the cells, reactions catalyzed by transition metals or other cellular reducers release hydroxyl ($H_2O_2 + e^- \rightarrow OH^* + OH^-$), which can induce peroxidation of lipids and membrane proteins at elevated concentrations, generating a transmembrane ion-transport disorder that affects cellular integrity (Abele-Oeschger et al. 1997). The decomposition of H_2O_2 into water and oxygen gas ($2H_2O_{2(aq)} \rightarrow 2H_2O_{(l)} + O_{2(g)}$) can be accelerated or slowed by several processes. Pedersen et al. (2006) observed that the oxygen release occurred immediately and showed a peak after 1 h, a prolonged release period when compared to the decomposition of H_2O_2. The DO levels in the present study were above the oximeter detection limit (20 mg/L) after 2 h of exposure, except in the control group and the treatment with 29 µL H_2O_2/L. These data are corroborated by those of Pedersen

et al. (2006), who observed an increase from 99 to 128 % saturation in systems with low organic matter and from 93 to 140 % in those high levels of organic matter.

The transition from hypoxia to hyperoxia (reoxygenation) in *L. vannamei* was marked by significant changes in the activity of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) in the muscle and hepatopancreas (Parrilla-Taylor and Zenteno-Savín 2011). Li et al. (2006) reported that the consumption of DO in juvenile *Fenneropenaeus chinensis* was significantly higher under hyperoxic conditions (10–18 mg/L). Supersaturation reduced the enzyme activity of superoxide dismutase and hemolysin but increased the activity of peroxidase. Abele-Oeschger et al. (1997) exposed *Crangon crangon* to 20 μM H_2O_2/L for 5 h and verified a reduction in the metabolic rate of 26 % in the animal and 60 % in the exposed muscle as well as a significant decrease in the intracellular pH. Therefore, the shrimp exposed to the process of reoxygenation and the hyperoxic conditions generated by the decomposition of H_2O_2 and release of oxygen in this study may have been damaged by oxidative stress and metabolic changes.

Dicentrarchus labrax juveniles exposed to 50 μL H_2O_2/L for 1 h showed significant changes in plasma ions, such as sodium, magnesium and calcium. These changes can represent a disorder in acid–base equilibrium, the transport of oxygen or the transport of ions, and these combined changes may cause a modification in the exchange of gases in the gills (Roque et al. 2010). *Scophthalmus maximus* exposed for 30 min to 240 and 480 μL H_2O_2/L showed signs of stress, such as increased ventilation rate, erratic swimming and escape from the tank (Avendaño-Herrera et al. 2006). The erratic swimming, loss of balance and small bubbles between the carapace and the muscle observed in the shrimp exposed to levels of 174, 232, 290 and 348 μL H_2O_2/L are similar to those described by Lightner et al. (1974). These authors described gas-bubble disease in juvenile *Penaeus aztecus*, which was caused by the supersaturation of dissolved gases in the water.

Data concerning the lethal concentration of H_2O_2 for shrimp are scarce. According to Reichwaldt et al. (2012), the LC_{50} of H_2O_2 for two zooplankton species (*Moina* spp. and *Daphnia carinata*) after 48 h of exposure were 2.0 and 5.6 mg H_2O_2/L, respectively, while the NOAEC was 1.5 and 3.0 mg H_2O_2/L, respectively. Arndt and Wagner (1997) exposed *Oncorhynchus clarkii* and *Oncorhynchus mykiss* for 2 h to concentrations from 0 to 540 μL H_2O_2/L and found an LC_{50} of 189–280 μL H_2O_2/L. An LC_{50} at 96 h of 143.3 (120–170) μL H_2O_2/L was obtained for juvenile *L. vannamei*, which are more resistant to H_2O_2 than zooplankton species (*Moina* spp. and *D. carinata*) and less resistant than juvenile rainbow trout.

Hydrogen peroxide at concentrations below −29 μL/L (NOAEC) and at the safe level of 14.3 μL H_2O_2/L can be applied as a source of oxygen for *L. vannamei* reared with bioflocs without causing mortality of the shrimp or negative impacts on the water quality. However, new studies should assess oxidative stress and the long-term effects of H_2O_2 on shrimp and bioflocs.

Acknowledgments The authors are grateful for the financial support provided by the National Council for Scientific and Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). W. J. Wasielesky and L. H. Poersch are research fellows of CNPq.

References

Abele-Oeschger D, Sartoris FJ, Portner HO (1997) Hydrogen peroxide causes a decrease in aerobic metabolic rate and in intracellular pH in the shrimp *Crangon crangon*. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 117:123–129

Arndt RE, Wagner EJ (1997) The toxicity of hydrogen peroxide to rainbow trout *Oncorhynchus mykiss* and cutthroat trout *Oncorhynchus clarki* fry and fingerlings. J World Aquac Soc 28:150–157

Avendaño-Herrera R, Magariños B, Irgang R, Toranzo AE (2006) Use of hydrogen peroxide against the fish pathogen *Tenacibaculum maritimum* and its effect on infected turbot (*Scophtalamus maximus*). Aquaculture 257:104–110

Avnimelech Y (2007) Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140–147

Bendschneider K, Robinson RJ (1952) A new spectrophotometric method for the determination of nitrite in sea water. J Mar Res 11:87–96

Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306:7–23

De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W (2008) The basics of bio-flocs technology: the added value for aquaculture. Aquaculture 277:125–137

Environmental Protection Agency (EPA) (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. US EPA, Washington, DC

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations on toxicity bioassays. Environ Sci Technol 11(7):714–719

Herut B, Shoham-Frider E, Kress N, Fiedler U, Angel DL (1998) Hydrogen peroxide production rates in clean and polluted coastal marine waters of the Mediterranean, Red, and Baltic Seas. Mar Pollut Bull 36:994–1003

Li Y, Li J, Wang Q (2006) The effects of dissolved oxygen concentration and stocking density on growth and non-specific immunity factors in Chinese shrimp, *Fenneropenaeus chinensis*. Aquaculture 256:608–616

Lightner DV, Salser BR, Wheeler RS (1974) Gas-bubble disease in the brown shrimp, *Penaeus aztecus*. Aquaculture 4:81–84

Lima JPV, Brito LO, Costa WM, Costa WM, Gálvez AO (2012) The use of hydrogen peroxide (H_2O_2) to increasing dissolved oxygen in *Litopenaeus vannamei* (Boone, 1931) culture. Pesq Agropec Per 17:73–77

Lin YC, Chen JC (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. J Exp Mar Biol Ecol 259:109–119

Lin YC, Chen JC (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. Aquaculture 224:193–201

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101:13–30

Parrilla-Taylor DP, Zenteno-Savín T (2011) Antioxidant enzyme activities in pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation. Aquaculture 318:379–383

Pedersen LF, Pedersen PB (2012) Hydrogen peroxide application to a commercial recirculating aquaculture system. Aquac Eng 46:40–46

Pedersen LF, Pedersen PB, Sortkjaer O (2006) Dose-dependent decomposition rate constants of hydrogen peroxide in small-scale biofilters. Aquac Eng 34:8–15

Ponce-Palafox J, Martinez-Palacios CA, Ross LG (1997) The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture 157:107–115

Reichwaldt ES, Zheng L, Barrington DJ, Ghadouani A (2012) Acute toxicological response of *Daphnia* and *Moina* to hydrogen peroxide. J Environ Eng 138:607–611

Roque A, Yildiz HY, Carazo I, Duncan N (2010) Physiological stress responses of sea bass (*Dicentrarchus labrax*) to hydrogen peroxide (H_2O_2) exposure. Aquaculture 304:104–107

Schwartz MF, Bullock GL, Hankins JA, Summerfelt ST, Mathias JA (2000) Effects of selected chemotherapeutants on nitrification in fluidized-sand biofilters for coldwater fish production. IJRA 1:61–81

Sprague JB (1971) Measurement of pollutant toxicity to fish—III. Sublethal effects and "safe" concentrations. Water Res 5:245–266

Taylor NI, Ross LG (1988) The use of hydrogen peroxide as a source of oxygen for the transportation of live fish. Aquaculture 70:183–192

UNESCO (1983) Chemical methods for use in marine environmental monitoring. Manual and guides 12. Intergovernmental Oceanographic Commission, Paris

Van Wyk P, Scarpa J (1999) Water quality and management. In: Van Wyk P et al (eds) Farming marine shrimp in recirculating freshwater systems. Florida department of agriculture and consumer services, Tallahassee, pp 128–138

Vinatea L, Gálvez AO, Venero J, Leffler J, Browdy C (2009) Oxygen consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero water exchange. Pesq Agropec Bras 44(5):534–538

Zenteno-Savín T, Saldierna R, Ahuejote-Sandoval M (2006) Superoxide radical production in response to environmental hypoxia in cultured shrimp. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 142:301–308