ELSEVIER

Aquaculture 207 (2002) 213–224

Aquaculture

www.elsevier.com/locate/aqua-online

Disinfection of seawater for hatchery aquaculture systems using electrolytic water treatment

Milko A. Jorquera [a], Gustavo Valencia [a], Mitsuru Eguchi [b], Masahiko Katayose [c], Carlos Riquelme [a,*]

[a] *Laboratorio de Ecología Microbiana, Facultad de Recursos del Mar, Universidad de Antofagasta, Avda. Angamos 601, Antofagasta, Chile*
[b] *Department of Fisheries, Kinki University, Nara 631-8505, Japan*
[c] *Hoshizaki Electric Co., Japan*

Received 29 April 2001; received in revised form 1 August 2001; accepted 3 August 2001

Abstract

A recently marketed electrolytic water treatment system (Hoshizaki) was evaluated for disinfection of seawater used in disease-prone high-intensity aquaculture systems. Bacterial plate counts (CFU), direct bacterial total counts using 4′,6′ diamidino-2-phenylindole (DAPI) staining, and viable bacterial total counts using 6-carboxy fluorescein diacetate (6CFDA) showed complete inactivation of bacterial populations at an intensity of ≥ 1.3 amp (≥ 2.13 mg Cl l^{-1}). This included disinfection of seawater experimentally dosed with the known scallop pathogen *Vibrio anguillarum*. Experimental use of electrolysis between 1.0 and 4.0 A was able to disinfect cultures of the (food) microalga *Isochrysis galbana* without deleterious effects on its growth rate. When this technique was applied on a commercial scale in a scallop hatchery, higher microalgal growth rates were achieved compared to those of traditionally autoclaved seawater, or seawater treated with germicidal ultraviolet light (UV). Results suggested that disinfection of hatchery culture waters could be achieved using electrolytic release of very low levels of active Cl^- ion, providing an effective and economically attractive alternative to currently used methods in these culture systems.

Keywords: Disinfection; Hatchery aquaculture system; Electrolytic water treatment

1. Introduction

Water quality is an important factor in the production process and expansion of intensive aquaculture systems (Bullock et al., 1997; Summerfelt et al., 1997; Singh et

* Corresponding author. Tel.: +56-55-637447; fax: +56-55-637804.
E-mail address: criquelme@uantof.cl (C. Riquelme).

0044-8486/02/$ - see front matter
PII: S0044-8486(01)00766-9

al., 1999). Current methods for the disinfection of seawater, which reduce bacterial loading in water supplies and/or avoid blooms of potentially pathogenic microorganisms, include treatments with antibiotics, ozone, filtration, heat, and UV irradiation (Whipple and Rohovec, 1994; Pascho et al., 1995; Chang et al., 1998; Liltved and Cripps, 1999; Munro et al., 1999; Frerichs et al., 2000). However, each of these treatments has specific disadvantages such as high cost, need for sophisticated equipment, production of toxic residues for the cultured organisms, or the appearance of resistant microorganism strains (Liltved et al., 1995; Bullock et al., 1997; Reilly and Käferstein, 1997).

Chlorine has a high efficiency as a disinfectant, and is readily availability at low cost, and in its various forms has been widely used for microbiological control in seawater and for surface sanitation in some intensive aquaculture facilities (White, 1992; Pascho et al., 1995). Laboratory and field studies have demonstrated the high biocidal efficiency of chlorine against viruses and bacterial pathogens (Sako et al., 1988; Inouye et al., 1990; Frerichs, 1990; Pascho et al., 1995; Arimoto et al., 1996; Chang et al., 1998). At allowable levels, chlorine does not affect flora or fauna. If an excess amount of chlorine is released by accident into aquatic environments, it may harm aquatic plants and animals until it is diluted to a harmless level (Anonymous, 1999). Some studies suggest that reactions between chlorine and organic nitrogen in water may produce residues which are toxic for marine organisms (Capuzzo, 1977; Breisch et al., 1984; Stauber, 1998; Rajagopal et al., 1997).

Electrolytic methods, studied in recent decades in the US, Japan and Russia, have shown promise as a method of disinfection whereby low levels of free chlorine, sodium hypochlorite, or hypochlorous acid may be produced in situ in NaCl-containing solutions. These methods have shown promise in destruction of microorganisms in medical and dental environments, and in the food industry (Nikulin, 1977; Wilk et al., 1987; Iwasawa and Nakamura, 1996; Tanaka et al., 1999; Kim et al., 2000). Similarly, electrolytic disinfection of seawater may be beneficial in fish culture, increasing survival and also the numbers of fishes which may be maintained in given volumes of water. It has also been able to reduce the need for (and thus costs) of antibiotics, as well as reducing costs of maintenance of pumping systems (Anonymous, 2000). Other studies have described reductions of over 99% in microalgae, bacteria, and viruses in water by means of electrolytic processes (Tsuzuki et al., 1999; Yoshimizu et al., 1998). The objective of the present study was to evaluate the effects of electrolysis on bacterial loading in seawater used in intensive aquaculture systems (hatcheries), and in the possible prophylactic use of this method in massive cultures of microalgae used for feeding cultured marine organisms.

2. Materials and methods

2.1. Effect of electrolysis on bacterial content of seawater

Seawater filtered to 10 μm (sand filter, Jacuzzi) was submitted to electrolysis using a prototype seawater electrolyzer with a nonmembrane electrolytic cell from Hoshizaki

Electric (Japan). This equipment was designed to dissociate the NaCl molecule in seawater to liberate hypochlorite ion (OCl^-) and a sodium ion (Na+). Disinfection treatments included: (i) passing the seawater through the electrolyzer at a rate of 4 l min^{-1} at 11 different current intensities, increasing between 0.1 and 2.0 A (1.9–2.1 V); and ii) passing the seawater through an UV irradiation system (Rainbow Lifegard, USA; 40 W) at a constant flow rate of 4 l min^{-1}. Control observations were made on parallel water samples which had not been exposed to either treatment. Free chlorine in waters that passed through the electrolyzer was analyzed by an ion-specific meter (free and total chlorine)(Hanna Instrument USA, Model HI93734), pH by an pH-meter Orion Model 410A, and salinity by a Hand Refractometer (Atago, Model S/Mill).

2.1.1. Bacteriological evaluations

Treated and control seawater samples were seeded onto tryptone soy agar supplemented with 2% NaCl (TSA2, Oxoid) using standard culture methodology to enumerate culturable heterotrophic bacteria, which were recorded as colony forming units (CFU). Direct counts of total bacteria (DTC) and direct counts of viable bacteria (DVC) were carried out on: (i) water subjected to electrolytic treatments at 0.3, 1.3, and 2.0 A; (ii) UV irradiation-treated water, and (iii) an untreated control water. DTC was carried out using the DNA-specific fluorochrome DAPI which is excited under UV light and DVC was carried out using a modification of the 6CFDA staining methods described by Yamaguchi et al. (1997). The fluorescein diacetate (FDA) contained in 6CFDA compound is normally a nonfluorescent, nonpolar compound that readily penetrates cell membranes. Intracellular FDA is hydrolyzed by nonspecific esterases, if present and active, resulting in the release of fluorescein, which fluoresces brilliant green when irradiated with blue light. 6CFDA is a modification of FDA allows better cell retention, and thus stains a wider variety of bacteria. 6CFDA was chosen for the detection of esterase active bacteria in this study. Water samples (0.8 ml) were mixed with 0.4 ml CFDA buffer (0.3 M phosphate buffer pH 8.5; 1.5 mM EDTA). Subsequently, a stock solution of 6CFDA (Sigma; 10 mg ml^{-1} in acetone) and DAPI (Sigma; 10 μg ml^{-1}) were applied to the samples to give a final concentration of 150 μg ml^{-1} (6CFDA) and 1 μg ml^{-1} (DAPI). The samples were incubated for 30 min at ambient temperature in the dark, and then the cells were filtered off onto black polycarbonate filters (Poretics Products; 0.2-μm porosity). The filters were placed on microscope slides and the stained bacteria were enumerated under UV light (DAPI) and blue light (6CFDA) excitation by epifluorescence microscopy using Olympus BH-2 microscope.

2.2. Effect of electrolysis on seawater dosed with the pathogen Vibrio anguillarum

This experiment was carried out using a pure culture of the scallop pathogen *V. anguillarum* (Riquelme et al., 1995). Cells obtained from an overnight culture on TSA2 medium were resuspended in nine-salts solution (Malmcrona-Friberg et al., 1990), counted using simple crystal-violet staining (Gerhardt et al., 1994) and inoculated into polyethylene bags containing 600 l of 10 μm-filtered seawater at a final density of 1.5×10^5 cells ml^{-1}. Immediately after inoculation, water containing

the pathogen was passed through the electrolytic disinfection device at a constant flow of 4 l min^{-1} and a current intensity of 1.3 A (2.1 V). Counts of CFU were made as described above on TSA2 medium, and also thiosulfate–citrate–bile salts–sucrose agar (TCBS, selective medium for vibrios genus). DTC and DVC were also carried out as described above.

2.3. Use of electrolysis-treated seawater in microalgal cultures

Water samples submitted to electrolysis at 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 A were collected in 250-ml glass bottles (Schott-Duran). Free chlorine measured in these waters was neutralized by the addition of sodium thiosulfate at a ratio of 5 mol thiosulfate for every 8 mol NaOCl in the water. Following neutralization, the seawater was filtered through 0.2-μm cellulose nitrate filters (Micro Filtration Systems), and aliquots of 100 ml were distributed into sterile 250-ml Erlenmeyer flasks.

The flasks were enriched with sterile Fritz f/2 algae food (Aquaculture Massachusetts, USA) and inoculated with axenic log-phase cultures of the microalga *Isochrysis galbana* (CCMP 1323) at 4×10^5 cells ml^{-1}. Control cultures were carried out in autoclaved seawater containing the same nutrients as the treatments. Algal densities were determined for experimental and control flasks by quantitative cell counting in a Neubauer chamber every 24 h post-inoculation for a period of 6 days. Microalgal growth rates were calculated using the Guillard method (Stein, 1979). Results of determination of microalgae growth rates among treatments were compared by analysis of variance ($P = 0.05$) using Statgraphics software (version 2.1 for Windows, Statistical Graphics). Also, comparative factors which might demonstrate significant differences between treated and untreated water were evaluated using a multiple range comparison least significant differences (LSD) test. Bacteriological analyses included DTC by means of DAPI (Porter and Feig, 1980) and CFU counts on TSA2 at 0 and 144 h.

This experiment was carried out twice, and in each instance, each treatment was replicated fivefold. Controls consisting of microalgal cultures with electrolytically treated seawater and omission of the neutralization step were not carried out, as preliminary testing showed poor growth of the microalgae under these conditions.

2.4. Use of electrolytically treated seawater in commercial-scale microalgal culture

This step was carried out within the algal culture unit of the scallop hatchery of Cultivos Marinos Internacionales (27°45′ S). The microalga utilized was clone TISO (nonaxenic) of *I. galbana* (CCMP 1324) which is routinely used in feeding protocols in the culture of larval *Argopecten purpuratus*. Seawater used in mass algae cultures was subjected to three different treatments for comparative evaluation of disinfection of the culture water and effect on growth of the microalgae. Seawater treatments included : (i) electrolysis of the water at 4.0 A, followed by thiosulfate neutralization as described above. Water was enriched using a sterile solution of Fritz f/2 algae culture nutrients as described in the previous section; (ii) UV irradiation of seawater and addition of sterile Fritz f/2 algae nutrients; (iii) autoclaving of seawater to which had

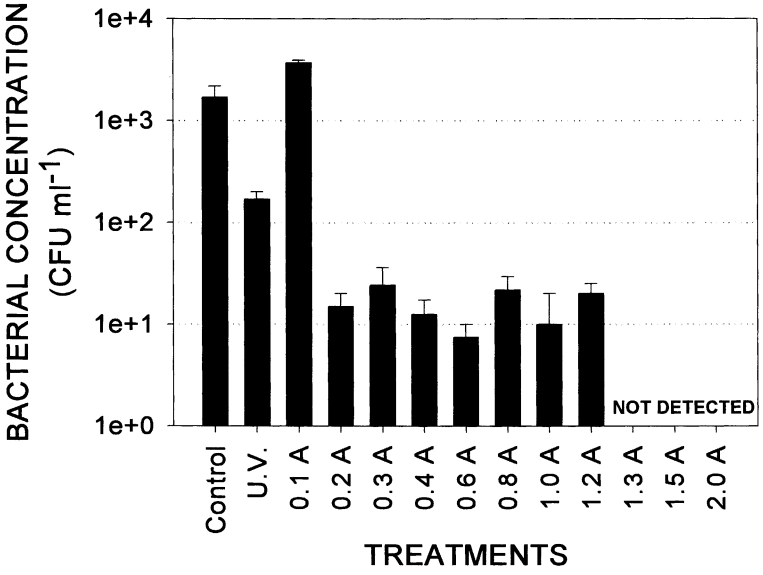

Fig. 1. Counts of culturable bacterial heterotrophs on TSA2 medium from seawater treated with different electrolytic intensities.

previously been added the Fritz f/2 algae nutrients. All treatments were replicated fourfold in 20-l polycarbonate algae culture bottles (Van Leer) containing 18 l of 1 μm-filtered seawater from each of the three treatments. The culture bottles were inoculated with log-phase TISO to give starter concentrations in the bottles of 1.5×10^5 cells ml^{-1}. Counts of microalgal density were carried out on all bottles of all replicates on the 4th day of culture prior to their use in the feeding of larval scallops.

Table 1
Bacterial counts in incoming seawater at the shellfish hatchery of Antofagasta University

Treatments	Total cells[a] (cells ml^{-1})	Active cells[b] (cells ml^{-1})	Percentage of active bacteria (%)
Control	3.96×10^5	3.12×10^4	7.88
UV	2.83×10^5	3.52×10^4	12.46
0.3 A	2.16×10^5	ND	ND
1.3 A	1.90×10^3	ND	ND
2.0 A	3.26×10^2	ND	ND

Control = untreated seawater; UV = ultraviolet irradiated seawater; A = ampere intensity of electrolytic treatment of seawater; ND = not detected.

[a] Total direct count with DAPI.

[b] Direct count of active bacteria with 6CFDA.

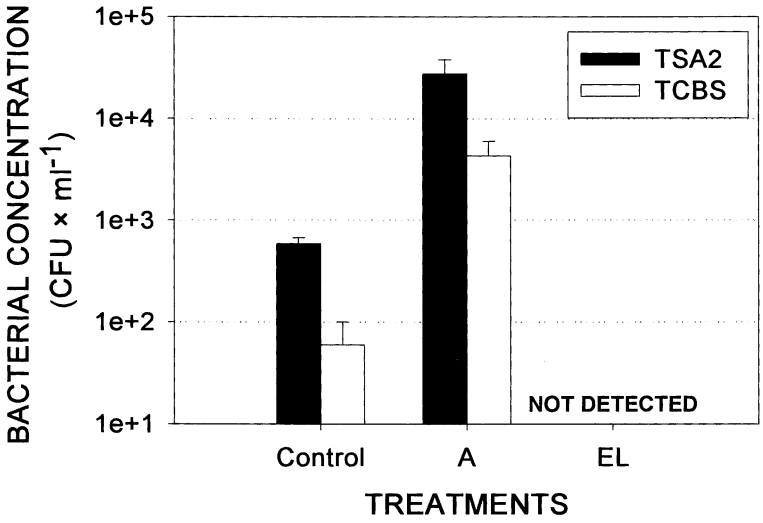

Fig. 2. Counts of culturable bacterial heterotrophs (TSA2) and culturable vibrios (TCBS) in seawater experimentally dosed with *V. anguillarum*. Control = seawater without pathogen; A = pathogen-dosed seawater, prior to electrolytic treatment; EL = pathogen-dosed seawater after electrolytic treatment.

Calculation of algal growth rates in the bottles and statistical comparisons of results were carried out as described for laboratory cultures in the previous section.

3. Results

The values for free chlorine in electrolytically treated seawater ranged from 0.39 to 2.13 mg Cl^- l^{-1} (0.3 and 1.3 A), and 2.95 to 6.5 mg Cl^- l^{-1} l^{-1}(1.5 and 2.0 A). The pH remained between 8.24 and 8.27 and salinity between 34‰ and 35‰, showing no apparent fluctuations.

A mean reduction of 3.43 $\log_{10}$ CFU ml^{-1} (99.4% inactivation, Fig. 1) in counts of culturable bacteria was obtained in seawater treated with electrolytic current intensities between 0.2 and 1.2 A. Complete inactivation was obtained at intensities equal to or

Table 2
Bacterial counts in seawater experimentally dosed with *V. anguillarum* and treated by electrolysis

Treatments	Total cells (cells ml^{-1})	Active cells (cells ml^{-1})	Percentage of active bacteria (%)
Control	6.25×10^5	5.79×10^4	9.26
A	9.30×10^5	1.03×10^5	11.08
B	4.92×10^3	ND	ND

Control = seawater without addition of bacteria; A = bacterially dosed seawater; B = bacterially dosed seawater, electrolytically treated; ND = not detected.

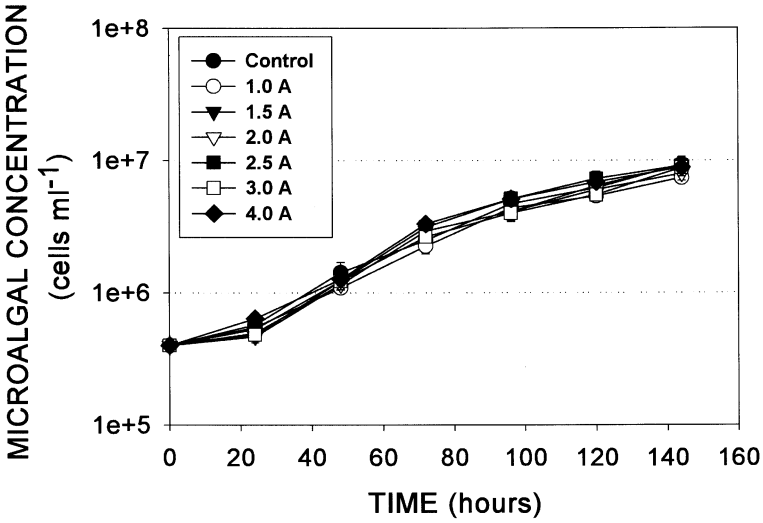

Fig. 3. Growth of the microalga *I. galbana* (CCMP 1323) in seawater treated at different electrolytic intensities then neutralized with sodium thiosulfate. Bar = standard error.

greater than 1.3 A. Irradiation with UV produced a CFU reduction of 93.6% (3.4 $\log_{10}$ CFU ml^{-1}). Electrolytic treatment of seawater at 0.3, 1.3, and 2.0 A produced complete absence of physiologically active bacteria in DVC determinations, whereas 7–12% of

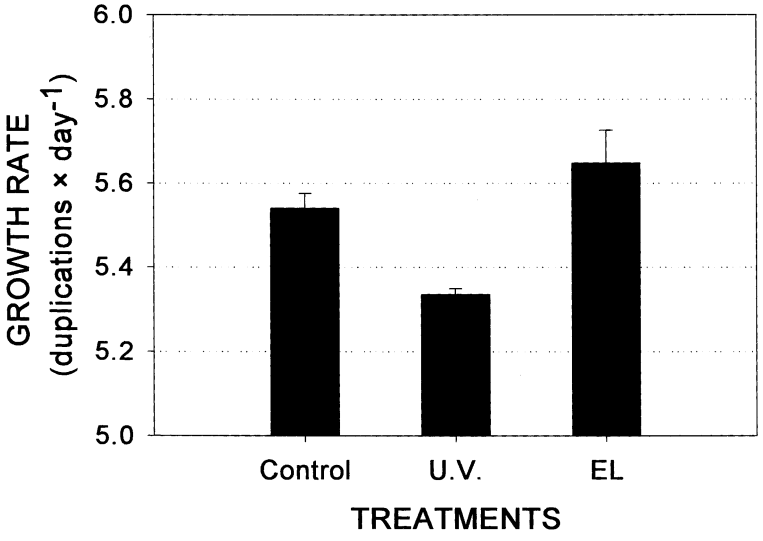

Fig. 4. Growth rates of *I. galbana* TISO (CCMP 1324) in mass culture. Control = autoclaved seawater; U.V. = UV-irradiated seawater; EL = seawater treated by electrolysis at 4.0 A, and then neutralized with sodium thiosulfate. Bar = standard error.

the total bacterial population remained active in control and UV treated waters (Table 1). Also, it is important to indicate that bacteriolysis began to be observed at intensities ≥ 1.3 A, and became highly significant ($P > 0.05$) at an intensity of 2.0 A.

Bactericidal effects of electrolysis similar to the preceding ones were observed in assays of seawater dosed with *V. anguillarum*. Counts of culturable bacteria were reduced significantly ($P>0.05$) by 4.44 $\log_{10}$ CFU ml^{-1} on TSA2 and 3.63 $\log_{10}$ CFU ml^{-1} on TCBS when treated with 1.3 A (Fig. 2). After electrolytic treatment, the DVC determinations showed complete bacterial inactivation (Table 2).

Fig. 3 shows that electrolytically treated, neutralized seawater had no deleterious effect on the growth of the microalga *I. galbana*. Statistical analyses among electrolytic treatments showed no significant differences ($P < 0.05$) between microalgal growth in treated and control groups. The axenic condition of these cultures was not altered during these experiments, as confirmed by bacterial culture and direct observational methods.

Mass cultures of *I. galbana* TISO showed significantly higher growth rates ($P>0.05$) in seawater subjected to electrolytic treatment when compared with growth in UV-irradiated and autoclaved seawater (Fig. 4).

4. Discussion

Bactericidal electrochemical treatment has been proposed for a variety of applications, including: (i) destruction of microorganisms in medical/dental environments; (ii) disinfection of human water supplies; (iii) sanitization in public facilities such as restaurants and toilets; (iv) agronomy; (v) aquaculture; and (vi) horticulture (Matsunaga et al., 1992; Pol et al., 2000; Frerichs et al., 2000; Anonymous, 2000). Diverse studies have described bactericidal and cytotoxic properties of electrolyzed solutions (Tanaka et al., 1999; Okubo et al., 1999; Len et al., 2000), with inactivation of bacterial and viral pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Salmonella enteriditis*, *Listeria monocytogenes*, hepatitis B virus (HBV) and acquired immunodeficiency virus (HIV)(Iwasawa and Nakamura, 1996; Horiba et al., 1999; Venkitanarayanan et al., 1999; Kim et al., 2000; Morita et al., 2000). Results of the present study showed the effective action of electrolysis for reducing seawater bacterial counts to undetectable levels of both natural populations and artificially produced populations of the pathogen *V. anguillarum*. Studies on the disinfection of seawater by electrolysis have shown mortality of greater than 99.9% of *V. anguillarum*, *Aeromonas salmonicida*, etiologic agents of vibriosis, and furunculosis in fishes using treatments of 0.1 mg Cl^- l^{-1} at 0.1 A; yellowtail ascites virus and hirame rhadovirus (HIRRV) have been reduced >99.9% with treatments of 0.58 mg Cl^- l^{-1} (Yoshimizu et al., 1998). In the cited study, treatments of 1.0 to 1.3 mg Cl^- l^{-1} at 2 to 2.5 A reduced bacterial counts in entering and exiting hatchery seawater by greater than 99.99%. This effectiveness observed over a wide range of microorganisms was supported by the observations of Wilk et al. (1987), who indicated that electrolytically treated solutions could be much more effective against microorganisms than comparable solutions produced by the addition of sodium hypochlorite. The high percentage of microorganism cells damaged by electrolytic treatments of above 2.0 A

may be due to the liberation of hypochlorite ion (OCl^-), which is a powerful oxidizing agent that may attack cell walls and damage membrane functions (Beer, 2000; Keith, 2000).

Although chloration treatment of seawater is known to produce significant toxic effects on microalgae such as *Nitszchia closterium*, *Dunaliella tertiolecta*, and *Microcystsis* sp. (Stauber, 1998; Tsuzuki et al., 1999), neutralization of hypochlorite ion using sodium thiosulfate allowed the growth of *I. galbana* with no negative effects. This species of microalga is one of the main food sources used in the culture of larval marine invertebrates (Albentosa et al., 1996; Reitan et al., 1998; Fidalgo et al., 1998). Since food cultures are a serious source of contamination in culture systems (Elston, 1989), control of bacterial numbers and types in these systems is of prime importance.

As mentioned in the Introduction, several methods of water treatment have been available for reducing bacterial loading in culture systems, among which ozonization and UV irradiation may be effective in the control and prevention of microbial infections in cultures (Sugita et al., 1992; Liltved et al., 1995; Bullock et al., 1997; Chang et al., 1998). However, several studies have shown the presence in seawater of residues which are products of high doses of ozone and are toxic for fish and bivalves. Certain systems have been proposed for removing ozone from the water prior to use in culture tanks (DeManche et al., 1975; Wedemeyer et al., 1979; Bullock et al. 1997). Although UV irradiation may produce residual peroxides which may irritate some marine larvae, technical difficulties encountered in the use of this method are more important. Severe reduction in the efficiency of UV systems may occur due to turbidity of the water which protects microorganisms from the UV by particle shading. Glass interfaces between the UV source and the treated water may accumulate films of organic and inorganic matter which may significantly reduce the efficiency of UV systems. Since UV systems require regular cleaning, prefiltration of water to 1 μm, and extension of exposure time of the water to achieve their best effectiveness, they may not be very effective against viral infections, are less efficient in treating large volumes of water, and may involve higher costs of maintenance relative to other methods (Blogoslawski and Stewart, 1983; Liltved et al., 1995; Chang et al., 1998; Frerichs et al., 2000). Yoshimizu et al. (1998) suggested that electrolytic treatment in the disinfection of seawater was as efficient as that of ozonization and UV treatment, but had the advantage of being able to disinfect larger volumes of hatchery effluents compared with the other two methods.

5. Conclusions

Electrolytic treatment of seawater at >1.3 A (>2.13 mg Cl^- l^{-1}) produced inactivation of bacterial populations to undetectable levels. Similar observations were obtained when using the same electrolytic current intensity (1.3 A) to treat seawater experimentally dosed with the pathogen *V. anguillarum*.

Mass cultures of *I. galbana* TISO showed better growth in electrolytic-treated seawater (after thiosulfate neutralization of Cl^-) than in autoclaved or UV-irradiated seawater.

Results obtained suggested the feasibility of seawater electrolysis as an efficient and comparatively low-cost alternative method for the control of opportunistic pathogens both in hatchery culture systems and for massive culture of microalgae.

Acknowledgements

The authors wish to thank the Hoshizaki Electric (Japan) for donation of the electrolysis equipment used in the present study. We thank the Chilean National Fund (FONDEF) for financial support of this project, #D97I2033. The authors also extend thanks to Ernesto Retamales and Leonel Gonzales from Microalgae Laboratory (Antofagasta University) and Dr. L. Di Salvo for his critical review of early draft; and special thanks to the management of the Cultivos Marinos Internacionales for extending the use of their scallop hatchery facilities for these studies.

References

Albentosa, M., Perez-Camacho, A., Labarta, U., Fernández-Reiriz, M.J., 1996. Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus* using phycological and biochemical parameters. Aquaculture 148, 11–23.

Anonymous, 1999. Chlorine Effects on Health and Environment. 3rd edn. The Chlorine Institute, Washington, DC, 8 pp.

Anonymous, 2000. 5 December 2000, revision date. [Online] Aquatechnology Website http://www.aquatechnology.net/electrolyzed.html (11 July 2001, last date accessed).

Arimoto, M., Sato, J., Maruyama, K., Mimura, G., Furusawa, I., 1996. Effect of chemical and physical treatments on the inactivation of striped jack nervous necrosis virus (SJNNV). Aquaculture 143, 15–22.

Beer, C., 2000. Recreational water disinfection by Copper/silver ions. 5 December 2000, revision date. [Online] http://www.clearwaterpoolsystems.com/waterdis.htm (13 December 2000, last date accesed).

Blogoslawski, W.J., Stewart, M.E., 1983. Depuration and public health. J. World Maric. Soc. 14, 535–545.

Breisch, L.L., Wright, D.A., Powell, D.M., 1984. Chlorine and the Chesapeake Bay: A Review of Research Literature. Maryland Sea Grant Association Publication, UM-SG-TS-84-02, Maryland, USA, 176 p.

Bullock, G.L., Summerfelt, S.T., Noble, A.C., Weber, A.L., Durant, M.D., Hankins, J.A., 1997. Ozonation of a recirculating rainbow trout culture system: I. Effects on bacterial gill disease and heterotrophic bacteria. Aquaculture 158, 43–55.

Capuzzo, J.M., 1977. The effects of free chlorine and chloramine on growth and respiration rates of larval lobsters (*Homarus americanus*). Water Res. 11, 1021–1024.

Chang, P.-S., Chen, L.-J., Wang, Y.-C., 1998. The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus. Aquaculture 166, 1–17.

DeManche, J.M., Dinaghay, P.L., Breese, W.P., Small, L.F., 1975. Residual toxicity of ozonized seawater to oyster larvae. ORESU-T-003. Sea Grant College Program, Oregon State University, Corvallis. 11–18 pp.

Elston, R., 1989. Bacteriological methods for diseased shellfish. In: Austin, B., Austin, D.A. (Eds.), Methods for Microbiological Examination of Fish and Shellfish. Ellis Horwood, Chichester, UK, pp. 187–215.

Fidalgo, J.P., Cid, A., Torres, E., Sukenik, A., Herrero, C., 1998. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. Aquaculture 166, 105–116.

Frerichs, G.N., 1990. Efficacy of chemical disinfectants against snakehead rhadovirus. J. Appl. Ichthyol. 6, 117–123.

Frerichs, G.N., Tweedie, A., Starkey, W.G., Richards, R.H., 2000. Temperature, pH and electrolyte sensitivity, and heat, UV and disinfectant inactivation of sea bass (*Dicentrarchus labrax*) neuropathy nodavirus. Aquaculture 185, 13–24.

Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R., 1994. Methods for General and Molecular Bacteriology. America Society for Microbiology, Washington, DC.

Horiba, N., Hiratsuka, K., Onoe, T., Yoshida, T., Suzuki, K., Matsumoto, T., Nakamura, H., 1999. Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals. Oral Surg., Oral Med., Oral Pathol., Oral Radiol. Endod. 87, 83–87.

Inouye, K., Ikeya, F., Yamazaki, T., Hara, T., 1990. Virucidal activities of various germicidal to infectious pancreatic necrosis virus (IPNV). Fish Pathol. 25, 81–86.

Iwasawa, A., Nakamura, Y., 1996. Bactericidal effect of acidic electrolyzed water—comparison of chemical acidic sodium hypochloride (NaOCl) solution [Japanese]. Kansenshogaku Zasshi 70, 915–922.

Keith, A.C., 2000. Cloro (Spanish). Chlorine Chemistry Council Home. 13 August 2000, revision date. [Online] http://www.c3.org/chlorine_knowledge_center/whitepapers10-98.html. 11 July 2001, last date accessed).

Kim, C., Hung, Y.C., Brackett, R.E., 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. J. Food Microbiol. 61, 199–207.

Len, S.V., Hung, Y.C., Erickson, M., Kim, C., 2000. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. J. Food Prot. 63, 1534–1537.

Liltved, H., Cripps, S.J., 1999. Removal of particle-associated bacteria by prefiltration and ultraviolet irradiation. Aquacult. Res. 30, 445–450.

Liltved, H., Hektoen, H., Efraimsen, H., 1995. Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. Aquacult. Eng. 14, 107–122.

Malmcrona-Friberg, K., Goodman, A., Kjelleberg, S., 1990. Chemotactic responses of marine *Vibrio* sp. strain S14 (CCUG 15956) to low-molecular-weight substances under starvation and recovery conditions. Appl. Environ. Microbiol. 56, 3699–3704.

Matsunaga, T., Nakasono, S., Takamuku, T., Burgess, J.G., Nakamura, N., Sode, K., 1992. Disinfection of drinking water by using a novel electrochemical reactor employing carbon-cloth electrodes. Appl. Environ. Microbiol. 58, 686–689.

Morita, C., Sano, K., Morimatsu, S., Kiura, H., Goto, T., Cono, T., Hong, W., Miyoshi, H., Iwasawa, A., Nakamura, Y., Tagawa, M., Yokosuka, O., Saisho, H., Maeda, T., Katsuoka, Y., 2000. J. Virol. Methods 85, 163–174.

Munro, P.D., Henderson, R.J., Barbour, A., Birkbeck, T.H., 1999. Partial descontamination of rotifers with ultraviolet radiation: the effect of changes in the internal bacterial load and flora of rotifers on mortalities in start-feeding larval turbot. Aquaculture 170, 229–244.

Nikulin, V.A., 1977. Use of an electrolyzed sodium chloride solution for disinfection in therapeutic and prophylactic institutions [Russian]. Sov. Med. 12, 105–108.

Okubo, K., Urakami, H., Tamura, A., 1999. Cytotoxicity and microbicidal activity of electrolyzed strong acid water and acidic hypochlorite solution under isotonic conditions. Kansenshogaku Zasshi 73, 1025–1031.

Pascho, R.J., Landolt, M.L., Ongerth, J.E., 1995. Inactivation of *Renibacterium salmoninarum* by free chlorine. Aquaculture 131, 165–175.

Pol, I.E., Mastwijk, H.C., Bartels, P.V., Smid, E.J., 2000. Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. Appl. Environ. Microbiol. 66, 428–430.

Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948.

Rajagopal, S., Nair, K.V.K., der Velde, G., Jenner, H.A., 1997. Response of mussel *Brachidontes striatulus* to chlorination: an experimental study. Aquat. Toxicol. 39, 135–149.

Reilly, A., Käferstein, F., 1997. Food safety hazard and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquacult. Res. 28, 735–752.

Reitan, K., Raimuzzo, J., Øie, G., Olsen, Y., 1998. Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus* L.) larvae. Aquaculture 118, 257–275.

Riquelme, C., Hayashida, G., Toranzo, A.E., Vilches, A., Chavez, P., 1995. Pathogenicity studies of a *Vibrio anguillarum*-related (VAR) strain causing an epizootic in *Argopecten purpuratus* larvae cultured in Chile. Dis. Aquat. Org. 22, 135–141.

Sako, H., Yshida, N., Maeno, Y., Sorimachi, M., 1988. Bactericidal activities of five disinfectants on *Aeromonas salmonicida*, *Vibrio anguillarum*, and *V. ordalli*. Fish Pathol. 23, 219–229.

Singh, S., Ebeling, J., Wheaton, F., 1999. Water quality trials in four recirculating aquacultural system configurations. Aquacult. Eng. 20, 75–84.

Stauber, J.L., 1998. Toxicity of chlorate to marine microalgae. Aquat. Toxicol. 41, 213–227.

Stein, J., 1979. Handbook of Phycological Methods, Culture and Growth Measurements. Cambridge Univ. Press, Cambridge.

Sugita, H., Asai, T., Hayashi, K., Mitsuya, T., Amanuma, K., Maruyama, C., Deguchi, Y., 1992. Application of ozone disinfection to remove *Enterococcus seriolicida, Pasteurella piscicida*, and *Vibrio anguillarum* from seawater. Appl. Environ. Microbiol. 58, 4072–4075.

Summerfelt, S.T., Hankins, J.A., Weber, A.L., Duran, M.D., 1997. Ozonation of a recirculating rainbow trout culture system II. Effects on microscreen filtration and water quality. Aquaculture 158, 57–67.

Tanaka, N., Fujisawa, T., Daimon, T., Fujiwara, K., Yamamoto, M., Abe, T., 1999. The cleaning and disinfecting of hemodialysis equipment using electrolyzed strong acid aqueous solution. Artif. Organs 23, 303–309.

Tsuzuki, K., Tanaka, T., Takagi, T., Shimomura, M., 1999. Effects of electrochemical treatment on *Microcystis* extinction [Japanese]. J. Water Environ. Soc. 22, 228–231.

Venkitanarayanan, K.S., Ezeike, G.O., Hung, Y.-C., Doyle, M.P., 1999. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteriditis*, and *Listeria monocytogenes*. Appl. Environ. Microbiol. 65, 4276–4279.

Wedemeyer, G.A., Nelson, N.C., Yasutake, W.T., 1979. Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36, 605–614.

Whipple, M.J., Rohovec, J.S., 1994. The effects of heat and low pH on selected viral and bacterial fish pathogens. Aquaculture 123, 179–189.

White, G.C., 1992. The Handbook of Chlorination and Alternative Disifectants, 3rd edn. Van Nostrand-Reinhold, New York.

Wilk, I.J., Altmann, R.S., Berg, J.D., 1987. Antimicrobial activity of electrolyzed saline solution. Sci. Total Environ. 63, 191–197.

Yamaguchi, N., Kenzaka, T., Nasu, M., 1997. Rapid in situ enumeration of physiologically active bacteria in river water using fluorescent probes. Microb. Environ. 12, 1–8.

Yoshimizu, M., Ishikawa, A., Hori, Y., Yoshinaka, T., Ezura, Y., Watanabe, K., Uenishi, T., 1998. Disinfection of water system for aquaculture by electrolyzation of sea water [Japanese]. Functional Water Symposium '98. Functional Water Foundation, Tokyo, Japan, pp. 82–83.