Proceedings of International Commemorative Symposium 70th Anniversary of The Japanese Society of Fisheries Science,
Fish. Sci. 2002; 68 Supplement I: 821-824. October 1-5, 2001. Yokohama, Japan

DISINFECTION OF WATER FOR AQUACULTURE

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SUMMARY: Disinfection of water for aquaculture is critical for preventing the introduction and spread of infectious disease. A pathogen-free water source is essential for success in aquaculture. Typical treatment systems make use of high efficiency sand filters to clarify the water before treatment with ultraviolet (UV) light or ozonization. Fish pathogens are divided into two groups based on their sensitivity to UV and total residual oxidants (TROs) produced by ozonization of seawater. Hypochlorite produced by electrolysis of seawater (salt water) showed bactericidal and viricidal effects. This method can easily treat large volumes of water, and is suitable for disinfecting wastewater before discharging.

***KEY WORDS*: disinfection, aquaculture, fish pathogen, UV, ozone, oxidant, electrolization**

INTRODUCTION

Water supplies for seed production and aquaculture often provide an efficient means for the introduction and spread of infectious diseases. A pathogen-free water source is essential for success in aquaculture. Surface waters commonly used in aquaculture come from coastal waters or rivers and may contain some fish pathogens and such open water supplies should not be used without treatment. Disinfection of wastewater before discharging is necessary to avoid the pathogen contamination in the environment. In this study, we examine the cidal effect of ultraviolet (UV), oxidant produced by ozonization of seawater and hypochlorite produced by electrolization of seawater. Additionally the disinfectant effects of the three methods for a hatchery water supply and wastewater were compared and the survival rate of cultured fish that were reared using water treated with these methods was assessed.

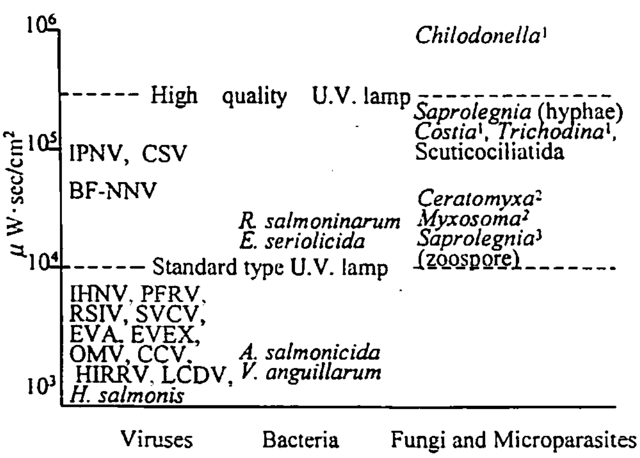

Fig. 1. U.V. susceptibility of fish pathogens.
[1] Vlasenko, M. I. (1969) [2] Hoffman, G. L. (1974)
[3] Normandeau, D. A. (1968)

UV susceptibility of fish pathogens and the effects of UV treatment on hatchery water

The disinfectant effects of UV irradiation were examined on cell suspensions of 4 species of fish pathogenic bacteria and *Escherichia coli*. using a punched agar medium disk covered with 10 strains of aquatic fungi and 14 strains of cell free fish pathogenic viruses. Of the viable bacterial cells of Gram negative bacteria and Gram positive bacteria, 99.9% or more were killed by UV irradiation at the dose of 4.0×10^3 and 2.0×10^4 μ W · sec/cm^2, respectively.[4] The hyphae of aquatic fungi showed relatively lower susceptibility to UV irradiation, levels that inhibited the growth of hyphae were 1.5×10^5 to 2.5×10^5 μ W · sec/cm^2.[5] Six fish rhabdoviruses, three fish herpesviruses and two fish iridovirus were found to be sensitive to UV irradiation. The dose that resulted in a 99 % or more infectivity decrease (ID_{99}) was observed at the dose of 1.0×10^3 to 3.0×10^3 μ W·sec/cm^2. Susceptibility of two birnaviruses, a fish reovirus and a fish nodavirus was found to be low, ID_{99} measured 1.5×10^5 to 2.5×10^5 μ W·sec/cm^2 (Fig.1).[6]

The infectivity of infectious hematopoietic necrosis virus (IHNV), in virus-contaminated river water and pond water, as measured by the molecular filtration method was 0.56 and 5.6 $TCID_{50}/l$, respectively. UV treatment of river water with 10^4 μ W·sec/cm^2 UV dose prevented an IHN outbreak.[7] Furthermore, UV treatment of the hatchery water supply also decreased the viable bacterial counts and fungal infection rates of salmonid eggs.[8]

Table 1. Effect of total residual oxidants (TROs) concentrations produced by ozonization of seawater on infectivities of fish pathogens

Fish pathogens		TROs concentration (mg/l)	Treatment time (sec)	Reduction rate (%)	Initial number (Log.)
Yellowtail ascites virus (YAV)		0.5	60	>99	4.3[1]
hirame rhabdovirus (HIRRV)		0.5	15	>99	5.6[1]
Infectious pancreatic necrosis virus (IPNV)		0.5	60	>99	4.1[1]
Infectious haematopoietic necrosis virus (IHNV)		0.5	15	>99	4.1[1]
Oncorhynchus masou virus (OMV)		0.5	15	>99	3.1[1]
Chum salmon virus (CSV)		0.5	60	>99	4.1[1]
Vibrio anguillarum	NCMB6	0.5	15	>99.9	5.6[2]
Enterococcus seriolicida	538	0.5	15	>99.9	5.8[2]
Aeromonas salmonicida	ATCC 14174	0.5	15	>99.9	5.1[2]
Aeromonas hydrophila	IAM 1018	0.5	15	>99.9	4.6[2]
Scuticociliatida	BR-9001	0.8	30	>99.9	5.5[3]

[1] Initial viral infectivity ($TCID_{50}$/ml). [2] Initial viable bacterial number (CFU/ml). [3] Initial viable number.

Disinfectant effect of oxidant produced by ozonization of sea water on fish pathogens

Treatment of natural seawater with ozone produced oxidant that showed a disinfectant effect. Total residual oxidant (TROs) produced in seawater were stable for 1 h or more. Disinfectant effect of TROs against fish pathogenic organisms was observed at a dose of 0.5 mg/l for 15 to 30 s or 0.1 mg/l for 60 s, and killed more than 99.9 % of bacterial cells of *Vibrio anguillarum, Enterococcus serioricida, Aeromonas salmonicida, A. hydrophila and E. coli,* and inactivated 99 % or more of IHNV, hirame rhabdovirus (HIRRV) and *Oncorhynchus masou* virus (OMV). To inactivate or kill more than 99 % of yellowtail ascites virus (YAV), infectious pancreatic necrosis virus (IPNV), chum salmon virus (CSV), and a Scuticociliatida (ciliata), higher doses of 0.5 to 1.0 mg/l for 1 min were required (Table 1).[9,10]

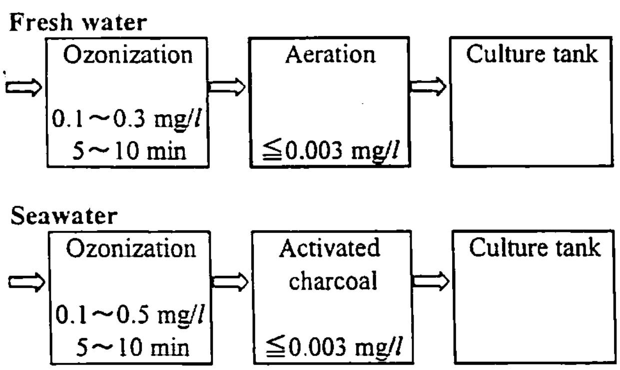

Fig. 2. Ozonization of water for aquaculture.

TROs showed toxicity for fish. Barfin flounder *Verasper moseri* and herring *Clupea pallasii* died after 16 and 2 h exposure to TROs of 0.1 and 0.5 mg/l, respectively. However, Japanese flounder could be cultured in ozonized seawater after the TRO were removed by charcoal (Fig.2), resulting in survival rates similar to fish cultured in UV treated or non-treated seawater.[11]

Disinfectant effects of electrolyzed salt water on fish pathogenic bacteria and viruses

The bactericidal and viricidal effects of hypochlorite produced by electrolysis of salt water were examined against pathogenic bacteria and viruses of fish.

Table 2. The chlorine concentration produced by electrolysis of salt water and treatment time required to reduce the viability of bacteria and the infectivity of viruses by 99.9 %

Pathogens		Chlorine concentration (mg/l)	Treatment time (min)	Initial number (Log.)	Reduction rate (%)
Vibrio anguillarum	NCMB6	0.07	1	6.7[1]	>99.99
Aeromonas salmonicida	ATCC 14174	0.06	1	6.6[1]	99.96
Escherichia coli	O-26	0.14	1	6.6[1]	99.98
Yellowtail ascites virus (YAV)		0.45	1	4.5[2]	99.92
hirame rhabdovirus (HIRRV)		0.34	1	4.5[2]	99.97

[1] Initial viable bacterial number (CFU/ml). [2] Initial viral infectivity ($TCID_{50}$/ml).

Table 3. Effects of U.V. irradiation, ozonization and electrolyzation on the viability of bacteria in hatchery waste-seawater

Methods	Flow rate (m^3/h)	Treatment	Viable counts (CFU/m*l*)	Reduction rate (%)
Non-treated	2.0	-	4.7×10^4	-
U.V. irradiation	2.0	1.0×10^5 μW·sec/cm^2	1.3×10^0	99.99
Ozonization	2.0	TROs 0.5 mg/*l*, 1 min	3.0×10^0	99.99
Electrolization	2.0	Chlorine 0.6 mg/*l*, 1 min	4.1×10^2	99.13

Sodium chloride solutions, ranging from 0.5 to 3 % were electrolyzed and the concentration of chlorine produced was measured. Similar concentrations of chlorine were produced when 1.0 % or higher NaCl solution and seawater were electrolyzed.[12] A 3 % solution of sodium chloride containing pathogenic bacteria or virus was electrolyzed and the organisms were exposed to chlorine. Greater than 99.9 % of *V. anguillarum* and *A. salmonicida* cells were killed when the bacteria were exposed to 0.1 mg/*l* chlorine for 1 min. YAV and HIRRV were 99.9 % or greater inactivated after treatment with 0.45 mg/*l* chlorine for 1 min (Table 2).[13]

The bactericidal and viricidal effects of hypochlorite produced by electrolysis (3.5 m^3/h, 0.1 A) were greater than that of the chemical reagent. The purity of the sodium chloride used influenced the effects of production of hypochlorite. Sodium chloride obtained as a super grade chemical reagent was more effective than food-grade sodium chloride. However, a sufficient disinfectant effect was observed in electrolyzed seawater, a treatment which may have an application in aquaculture. To use electrolyzed seawater for culture, the chlorine has to be removed with charcoal because of its toxicity.

Disinfection of wastewater

The bactericidal effect of hypochlorite produced by a continuous flow electrolyzer on hatchery wastewater was investigated. The number of viable bacteria in the wastewater was reduced more than 99 % when the water was treated with chlorine at a concentration of 0.6 mg/*l* for 1 min, and over 99.9 % of the bacteria cells were killed when treated with 1.28 mg/*l* for 1min (Table 3). In another experiment, 2.0 m^3/min of hatchery wastewater was electrolyzed and the produced hypochlorite that was mixed with the remaining, 16.5 m^3/min wastewater. Viability of bacteria was reduced greater than 99 % after treatment with 0.5 mg/*l* of chlorite for 1 min. The bactericidal effect of electrolysis was almost the same as that of ultraviolet irradiation (1.0×10^5 μ W·sec/cm^2) or ozonization (TROs 0.5 mg/*l*, 1 min) of seawater. Electrolization can be used to treat a large volume of wastewater compared with the ultraviolet irradiation or ozonization.[14]

Disifection of water for hatchery water supplies and survival rate of cultured fish

The effects of the three disinfection methods on bacteria in the hatchery water supplies are shown in Table 4. All methods resulted in a reduction of 96.6 to 99.8 % after the treatment. Survival rate of Japanese flounder *Paralichthys olivaceus* and barfin flounder

Table 4. Viability of bacteria in hatchery water supply after treatment using different disinfection methods

Method	Treatment time (min)	Viable counts (CFU/m*l*)	Reduction rate (%)
Control	-	2.2×10^2	-
U.V. irradiation	1	7.3×10^0	96.6
Ozonization ²	5	5.0×10^{-1}	99.8
Electrolization ³	1	1.1×10^0	99.5

¹ U.V. dose: 1.0×10^5 μ W·sec/cm^2.
² TROs: 0.5 mg/*l*. ³ Chlorine: 0.5 mg/*l*

Table 5. Survival rate of Japanese flounder cultured in U.V. irradiated, ozonized, electrolyzed or non-treated seaw

Survival rate	U.V. irradiated¹	Ozonized ²	Electrolyzed³	Non-treated
Japanese flounder⁴	71.5	71.8	NT	67.4
Barfin flounder⁵	NT	34.3	32.6	25.7

¹ U.V. dose: 1.0×10^4 μ W·sec/cm^2.
² Ozonization: 1.0 mg/*l* TROs for 8.5 min for Japanese flounder. 0.5 mg/*l* for 5 min for Barfin flounder.
³ Electrolization: 0.5 mg/*l* chlorine for 5 min.
⁴ Tank size: 0.5 t, Number of fish: 2000, Duration: 49 days, Feeding: 1 time/day.
⁵ Tank size: 0.5 t, Number of fish: 6500-7100, Duration: 30 days, Feeding: 2 times/day.

cultured in UV irradiated, ozonized and electrolyzed seawater are shown in Table 5. No statistically significant differences in survival rates were found between the three groups of fish cultured with treated water.

Ozonized and electrolyzed seawater have been demonstrated to be effective for disinfecting equipment used in aquaculture[15,16] and ozonized seawater is effective for disinfecting fertilized barfin flounder eggs contaminated with viral nervous necrosis virus.[17] Therefore, ozonization and electrolization of seawater seem to be effective methods for disinfection of the water for fish culture.

CONCLUSION

Gram negative bacteria and fish rhabdoviruses, herpesviruses and iridoviruses were killed when UV irradiated at the dose of 10^4 μ W·sec/cm^2. Standard, inexpensive UV lamps can irradiate at that dosage and may be suitable for hatcheries or culturing stations that have problems caused by these microorganisms. This would be the best method for disinfection of UV susceptible pathogens. Water contaminated with Gram-positive bacteria, fish birnaviruses, fish reoviruses, fish nodaviruses and aquatic fungi that showed lower susceptibility should be disinfected with ozonization, electrolization or high quality UV lamps.

Disinfection of wastewater is necessary to prevent pathogenic contamination of the environments. Electrolization is easy to scale up and can be used to treat a large volume of water, thus making it a suitable method for disinfecting wastewater.

ACKNOELEDGMENT

This research was supported in part by Grant in Aid for Scientific research (B)(2) No.09556041 and (B)(2) 13556027 under the Ministry of Education, Science, Sports and Technology, Japan.

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