Master's Thesis

CONTROL OF *COCHLODINIUM POLYKRIKOIDES*, A RED TIDE DINOFLAGELLATE USING CHEMICAL DISINFECTANTS

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(Environmental Science and Engineering)

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Approved by

Advisor

Changha Lee

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ABSTRACT

The harmful algal blooms (HABs) often involve serious economic loss of marine resources and contribute to pollution of coastal areas by causing the mass mortality of natural and aquaculture fish and shellfish. While spraying activated clay is a common practice to mitigate damage from red tide, it is not strong enough to completely control the red tide bloom. The use of chemical oxidants, frequently employed for disinfection in water treatment processes, is a potential method to reduce the risk from red tide for inland fish farms. In this study, chemical disinfectants were investigated as alternative technologies to control red tide in terms of the removal efficiency of *C. polykrikoides*, formation of byproduct, and toxicity to fish.

The first part of this study investigated the removal of *C. polykrikoides* by various oxidants including ozone (O_3), permanganate (MnO_4^-), chlorine (Cl_2) and hydrogen peroxide (H_2O_2) according to cell density and oxidant dose. O_3 showed a much higher efficiency for the *C. polykrikoides* removal than the other oxidants under identical experimental conditions. Disinfectants effectively inactivate the *C. polykrikoides* in the order of $O_3 > MnO_4^- > Cl_2 > H_2O_2$. TRO produced from O_3 and Cl_2 mostly converted into HOBr due to the presence of bromide ion (Br^-) in seawater. The seawater in the presence of *C. polykrikoides* decayed faster than in the absence of *C. polykrikoides*, indicating that TRO decay and HOBr production can be affected by the characteristics of water quality. Ozonation results in a much higher formation of bromate (BrO_3^-) than treatments with Cl_2, MnO_4^-, and H_2O_2. The acute toxicity of MnO_4^-, Cl_2, and H_2O_2 to juvenile red sea bream was reached at 72-h LC_{50} values of 0.39, 1.32, and 102.61 ppm, respectively.

Secondly, the aim of this study to evaluate the removal efficiency of *C. polykrikoides* by O_3 according to various factors affecting the efficiency such as cell density, oxidant dose, humic acid concentration, carbonate concentration, pH, and temperature. The removal of *C. polykrikoides* by O_3 was rapid, and appeared to be caused by initial ozonation, and then slow reaction, in which Br^- was oxidized to HOBr. Low pH and temperature increased the removal efficiency. Thus, the *C. polykrikoides* removal efficiency with a low pH, and low temperature, and a high O_3 dose increased as the in exposure to O_3 increased. The BrO_3^- concentration of less than 10.0 ppb (WHO, 2011) can only be maintained at the O_3 dose of less than 1.0 ppm.

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I. Control of red tide in inland fish farms

The number of red tide cases has gradually increased over the past few decades. The harmful algal blooms (HABs) often involve serious economic loss of marine resources and contribute to pollution of coastal areas by causing the mass mortality of natural and aquaculture fish and shellfish (Kim *et al*., 2010). The *Cochlodinium polykrikoides* has become a major red tide species causing high mortality of fish and shellfish in Korea. In 1995, the killing of aquaculture fish caused economic loss of USD $60 million (Figure 1). While spraying activated clay is a common practice to mitigate damage from red tide, it is not strong enough to completely control the red tide bloom. Particularly, due to the use of seawater to support the growth of fish, the operation of inland fish farms has been directly damaged from red tide.

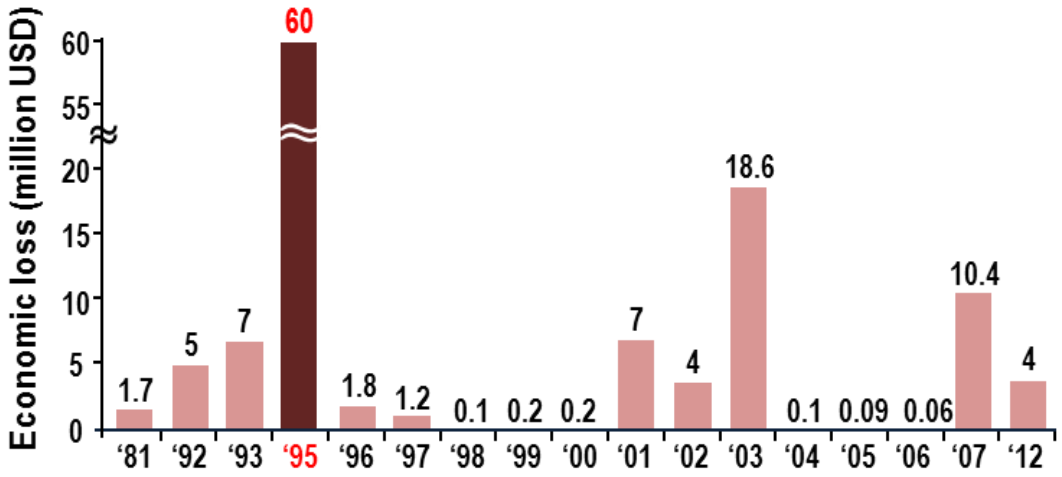

Figure 1. Economic losses in the aquaculture industry by HABs in Korea

(Source: Park *et al*., 2013)

Many potential control technologies have been used for the mitigation and control of red tide outbreaks. The use of chemical oxidants, frequently employed for disinfection in water treatment processes, is a potential method to reduce the risk from red tide for inland fish farms. In this study, we focus on chemical disinfectants. Chemical disinfectants, such as ozone (O_3), permanganate (MnO_4^-), chlorine (Cl_2), and hydrogen peroxide (H_2O_2) have been widely applied for effective disinfection of

drinking water and wastewater (Letterman, 1999). Especially, ozonation has been proved to be an effective oxidation treatment process for the oxidation of certain organic micropollutants in water (Lee *et al*., 2007). O_3 can either react directly with organic compounds, or decompose to produce hydroxyl radicals ($^{\bullet}OH$), which also react with the target compounds (von Gunten, 2003). MnO_4^- is also largely utilized in water treatment processes, and it effectively removes natural organic matter (NOM) and algae with less formation of byproduct (Owen *et al*., 1995). Cl_2 is one of the most generally used disinfectants for drinking water and wastewater treatment (Letterman, 1999). While, H_2O_2 has been used as an environmentally friendly oxidant.

Various red tide control technologies, including ozonation, MnO_4^-, Cl_2, and H_2O_2 treatments have been evaluated in many studies. Studies have shown that the removal efficiency of red tide dinoflagellate by O_3 was much higher than that of other oxidants (MnO_4^-, Cl_2, and H_2O_2). Although studies regarding the removal of red tide dinoflagellate by chemical oxidants carried out, as shown in Table 1, no study has been performed in which the various oxidants are compared.

Table 1. Removal of red tide dinoflagellate by chemical disinfectants

Oxidants	Algal species	Time (min)	Conditions	Removal efficiency	Reference
Ozone (O_3)	*Heterosigma akashiwo* *Heterocapsa triquetra* *Cochlodinium polykrikoides* *Karenia mikimotoi*	1 2 5 5	O_2 gas flow rate = 11 L/min 1.0×10^5 cells/mL	100%	Honjo *et al*., 2002
	Karenia brevis	1	O_2 gas flow rate = 0.5 L/min 5.7×10^7 cells/mL	100%	Schneider *et al*., 2003
	Gymnodinium breve	1	O_2 gas flow rate = 0.5 L/min 5.7×10^7 cells/mL	100%	Schneider *et al*., 2003
	Alexandrium tamarense	5	O_3 conc. = 5.0 mg/L 5.5×10^5 cells/mL	84%	Kang *et al*., 2001
	Scripsiella trochoidea	4	O_3 conc. = 190 mg/h 1.0×10^4 cells/mL	100%	Yang *et al*., 2015
Permanganate (MnO_4^-)	*Karlodinium micrum* *Prorocentrum minimum*	120	$KMnO_4$ conc. = 4.0 ppm $3.5 \times 10^4 \sim 4 \times 10^4$ cells/mL	60% 75%	Deeds *et al*., 2002
Chlorine (Cl_2)	*Cochlodinium polykrikoides*	180	ClO_2 conc. = 3.0 mg/L 6.0×10^3 cells/mL	20%	Ryu *et al*., 1998
	Phaeocystis globosa	96 (h)	ClO_2 conc. = 2.96×10^2 mmol/L 2.35×10^9 cells/mL	100%	Zhang *et al*., 2003
Hydrogen peroxide (H_2O_2)	*Cochlodinium polykrikoides*	180	H_2O_2 conc. = 28 ppm 6.0×10^3 cells/mL	10%	Ryu *et al*., 1998

II. Formation of byproducts

Ozonation has been applied as a disinfectant in drinking water treatment as well as waste water treatment (von Gunten, 2003; Lee and von Gungen, 2010). Although O_3 is a powerful oxidizing agent as a disinfectant, it reacts with bromide ion (Br^-) to produce byproduct. The seawater contains a high concentration of Br^-, which reacts with oxidants to form stable oxidants such as bromine (hypobromous acid and hypobromite, HOBr/OBr^-). The total residual oxidant (TRO) can include residual oxidants consisting of bromine, O_3, and $^{\bullet}OH$ or a combination of all three (Perrins *et al*., 2006), and is active in inactivating marine organisms. In seawater ozonation, O_3 reacts with Br^- to form OBr^- (Haang and Hoigné, 1983; von Gunten and Hoigné, 1994; reaction 1). The OBr^- formed by reaction (1) will immediately reach equilibrium with HOBr; pK_a = 8.8 at 20°C. The concentrations of HOBr and OBr^- generally depend on the pH conditions of the seawater solution used (seawater: pH = 8.0).

$$O_3 + Br^- \rightarrow OBr^- + O_2,\ k_1 = 160\ M^{-1}s^{-1} \quad (1)$$

$$HOBr \leftrightarrow OBr^- + H^+,\ pK_a = 8.8 \quad (2)$$

The proportion of OBr^- is an important intermediate for bromate (BrO_3^-) formation, since the reaction rate of HOBr is much slower than that of OBr- during ozonation. BrO_2^- (bromite) is formed directly by the oxidation of HOBr/OBr^- by O_3, and is quickly oxidized to BrO_3^- by O_3 (Haang and Hoigné, 1983; von Gunten and Hoigné, 1994; reactions 3 and 4).

$$O_3 + OBr^- \rightarrow BrO_2^- + O_2,\ k_2 = 100\ M^{-1}s^{-1} \quad (3)$$

$$O_3 + BrO_2^- \rightarrow BrO_3^-,\ k_3 > 10^5\ M^{-1}s^{-1} \quad (4)$$

The International Agency for the Research on Cancer (IARC) classified BrO_3^- as a potentially carcinogenic substance as it can potentially affect the health of fish in aquaculture and marine ecosystems (Tango and Gagnon, 2003). The World Health Organization (WHO) recommended that a provisional guideline value for BrO_3^- concentration is 10 ppb in drinking water World Health Organization (WHO, 2011). Therefore, it is necessary to minimize the production of any harmful byproducts, such as those resulting from the application of chemical technologies in seawater treatment.

Likewise, Cl_2 can be converted into bromine in water that contain Br^-, such as seawater (Bousher *et al*., 1986). When Cl_2 is dissolved in water, a rapid hydrolysis reaction occurs (Viswanathan and Tilak, 1984; reaction 5). Hypochlorous acid (HOCl) can therefore be converted into HOBr by reaction (6) (Farkas *et al*., 1949).

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad (5)$$

$$HOCl + Br^- \rightarrow HOBr + Cl^-, k_4 = 2.95 \times 10^3\ M^{-1}s^{-1} \quad (6)$$

III. Assessment of acute toxicity of chemicals to fish

Inland fish farms generally operate by intaking seawater. When red tide outbreaks in seawater, chemical control technologies are employed such as treatment using disinfectants. Chemical disinfectants can control red tide dinoflagellate and can disinfect pathogenic bacteria. However, the application of chemical disinfectants can lead to serious health problems for fish and the destruction of ecosystems.

Chemical technologies such as ozonation and treatment with H_2O_2 have recently been used to reduce the risk from red tide in inland fish farms. Despite the use of these chemical treatments, few studies have been carried out on their toxicity to fish or on the relationship between their safe concentration and aquaculture fish, including the following: the toxicity of the ten commercial disinfectants to flounder, black rock fish, and black sea bream (Park *et al*., 2008), the toxicity of O_3 to Japanese charr (Kenji *et al*., 1992), the toxicity of O_3 produced oxidants to juvenile turbot (Reiser *et al*., 2010), the toxicity of copper (Cu) to red sea bream and toy shrimp (Mochida *et al*., 2006), and the toxicity of nickel (Ni) and copper (Cu) to fish gills (Meyer *et al*., 1999).

The toxicity of chemical substances is often presented as a lethal concentration 50 (LC_{50}). LC_{50} is the concentration of a substance that is lethal to 50% of organisms in a toxicity test. LC_{50} can be determined for any exposure time, and the common exposure durations are 24, 48, 72, and 96 h. It is a useful tool because it can predict the effects of a potential toxin in an aquaculture system. LC_{50} data can also help define the maximum allowable toxicant concentrations.

IV. Objectives of the study

Two specific objectives of the present research are as follows:

1. To investigate the removal of *Cochlodinium polykrikoides*, a red tide dinoflagellate using chemical disinfectants and then to examine the effect of toxicity on the fish species through an acute toxicity test:

For this purpose, O_3, MnO_4^-, Cl_2, and H_2O_2 were selected as chemical disinfectants, and the removal of *C. polykrikoides* was studied according to cell density and oxidant dose. The decay of total residual oxidants and formation of BrO_3^- after oxidant treatment were also investigated. The 72-h median lethal concentration (LC_{50}) of various oxidants including MnO_4^-, Cl_2 and H_2O_2 were then determined for red sea bream.

2. To evaluate the control of *Cochlodinium polykrikoides* by O_3:

The removal efficiency of *C. polykrikoides* by O_3 was evaluated. For this purpose, the removal of *C. polykrikoides* during ozonation was examined according to varying factors affecting the removal efficiency, to would provide insight into assessing the strategies for red tide dinoflagellate control in inland fish farms. In addition, the potential of the formation of toxic byproduct (BrO_3^-) in water containing Br^- after ozonation is investigated.

Chapter 2. Materials and Methods

I. Cultivation of *C. polykrikoides*

Red tide dinoflagellate species, *C. polykrikoides* was obtained from the National Fisheries Research and Development Institute (NFRDI), Busan city, Korea. The culture was enriched in Guillard's f/2 medium (Guillard and Ryther, 1962; Guillard, 1975). The cells were maintained at 24°C under a 12 : 12 h light : dark cycle with a light intensity of ~40 μmol $m^{-2}s^{-1}$ and counted using a Sedgwick-Rafter chamber with an optical microscope. The cell concentrations used for this experiment were 0.5×10^3, 1.0×10^3, 2.0×10^3, and 3.0×10^3 cells/mL.

II. Experimental methods

All inactivation and oxidation experiments were performed in a batch system using 10 mL solutions (in 25 mL Pyrex flasks) open to the atmosphere at room temperature (22 ± 2°C). The solution pH was initially adjusted using 1 N $HClO_4$ and 1 N NaOH solutions, and the pH variations during reaction for experiments were less than 0.2 units. The O_3 stock solutions were produced by spaging O_3-containing oxygen (generated by an O_3 generator, Ozonetec Co, Korea) through deionized (DI) water (18 MΩ·cm Milli-Q water from a Millipore system) in an ice bath. The concentration of O_3 stock solution was typically 30 ppm, as spectrophotometrically determined by measuring the absorbance at 260 nm (3000 $M^{-1}cm^{-1}$) (Hoigné and Bader, 1994). Chlorination experiments were performed using sodium hypochlorite solution (NaOCl, Sigma-Aldrich, USA). Stock solutions of Cl_2 were prepared in DI water at 100 ppm and verified for accuracy on the day of testing. Oxidation experiments of MnO_4^- and H_2O_2 were performed using potassium permanganate ($KMnO_4$, Sigma-Aldrich, USA) and 30% (v/w) stock solution (H_2O_2, Sigma-Aldrich, USA), respectively. The reactions were initiated by injecting an aliquot of the stock solutions. Samples were withdrawn at a predetermined time after the initiation of reaction and were immediately filtered with a 0.45-μm hydrophilic polytetrafluorethylene syringe filter.

BrO_3^- was analyzed in experiments to determine the oxidation byproduct formation. Water samples were prepared by spiking the DI water into each sample at a concentration of Br^- of 65 ppm. In addition, the pH solution was adjusted to 7.9 and the O_3 was injected into the 100 mL Pyrex flask at the various oxidant doses.

For the experiments, seawater was obtained from Busan city, Korea. The quality parameters of the seawater samples are summarized in Table 2. The seawater samples were filtered with a 0.45-μm filter within 24 h after sampling and stored at 4 ^{o}C until use.

Table 2. Water-quality parameters of seawater (Busan, Korea)

Parameters		Seawater
pH		7.9
Conductivity (mS/cm)		50.1
Alkalinity (mg/L as $CaCO_3$)		115.4
Turbidity (NTU)		0.05
DOC (mg/L)		1.5
A_{254} (cm^{-1})		0.0166
Anion (mg/L)	Cl^-	17,385
	SO_4^{2-}	1,929
Cation (mg/L)	Na^+	10,244
	K^+	501
	Mg^{2+}	1,350
	Ca^{2+}	392

III. Analytical methods

The cells were counted using an optical microscope (Axio Scope, Zeiss, Germany) with a Sedgwick-Rafter chamber (50 × 20 × 1 mm). After sampling, the samples were spread over the Sedgwick-Rafter chamber. The results were averaged, and the counts were converted to the unit of cells/mL. Viable cells were considered as active cells, and the total number of cells was calculated by adding the number of active and inactivate cells that had maintain their cell structure during the treatment.

The total dissolved O_3 residual in the reaction solution was determined by Indigo method (Bader and Hoigné, 1981). K MnO_4 was measured using the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) method after filtering with a 0.2 μm syringe membrane filter. The titanium sulfate method was used to measure the concentration of H_2O_2 (ε_{405} = 730 $M^{-1}s^{-1}$; Eisenberg, 1943).

The total residual oxidant (TRO) was measured by the DPD colorimetric method using a S-3100 UV-Vis spectrophotometer (Sinco Co.) at 515 nm. The concentration of TRO was presented as mg/L

as Cl_2. The concentration of HOBr was determined after quenching the reaction by adding 0.5 M phenol at pH 3 and scavenged by phenol forming 2- and 4-bromophenol. These species were stable and were analyzed by High performance liquid chromatography (HPLC) with UV absorbance detection at 225 nm. For the HPLC analyses, separation was performed on a 150 mm × 4.6 mm, 35 µm C18 column (Eclipse XRD-C18, Agilent) using 0.1% (w/w) aqueous solution of phosphoric acid and neat acetonitrile as the eluent at a flow rate of 1.0 mL/min.

The concentration of BrO_3^- in water containing Br^- was determined using ion chromatography (IC; Dionex ICS 3000, Thermo Fisher Scientific Inc., USA) with the AS9-SC column. A solution of 9.0 mM Na_2CO_3 (at a flow rate of 1.0 mL/min) was used as eluents for the analyses of BrO_3^-. To allow the determination at BrO_3^- concentration below 1 µg/L, spectrophotometric detection has previously been used after a post-column reaction of 3-3' dimethoxybenzidine, *o*-dianisidine (ODA) (Wagner *et al*., 2002; Almendral *et al*., 2009). The reaction is based on the formation of Br_2 in the presence of excess Br^- and the ODA bromination reaction, generating a product that absorbs at 450 nm.

To investigate the relationship of the solution pH and O_3 dose as well as the temperature and O_3 dose, on *C. polykrikoides* removal efficiency, response surface methodology (RSM) was employed to generate the design of the experiment. RSM is a useful statistical technique to determine the combined effects of complex processes and is widely used for the design of experiment in order to examine the statistical models through the untested points with a minimal number of experimental trials. Experimental data were analyzed using the trial version of Design-Expert 7.1.6 software (Stat-Ease, Minneapolits, USA). The design considered nine experimental runs with the center point being triplicated. The ranges explored of pH, temperature, and O_3 dose were set at 8.0±1.5, 24±5°C, and 0.5±2 mg/L, respectively.

IV. Fish acute toxicity test

To determine the effect of various oxidants on toxicity, acute toxicity studies were conducted. Juvenile red sea bream of *Pagrus major* (average weight 5.8 g and average length 8.3 cm) were provided by the Southeast Sea Fisheries Research Center (Namhae, Korea). The fish were acclimatized for a few days in 150 L fiber reinforced plastic (FRP) tanks at 18 to 20°C within a natural photoperiod. The fish were fed a controlled diet once a day until the start of the toxicity test. Tests were conducted in an aerated tank containing 100 L of filtered seawater with 30 fish per tank. Red sea bream were exposed to Cl_2, MnO_4^-, and H_2O_2 at a range of concentrations and fish toxicity tests were repeated at least twice. Mortality and seawater quality parameters (DO, pH, and temperature) were monitored daily. The fish were not fed and seawater was not circulated during the 72-h tests. Toxicity data were calculated at a standard LC_{50} value.

Chapter 3. Results and Discussion

I. Removal of *C. polykrikoides*, a red tide dinoflagellate using chemical disinfectants and fish acute toxicity test

1.1. Removal of *C. polykrikoides* by chemical disinfectants

C. polykrikoides has become a major fish and shellfish killing red tide species in inland fish farms in Korea. Figure 2 shows the optical microscopy images of *C. polykrikoides*. The average length of the *C. polykrikoides* cells is 33.6 μm and single ellipsoidal cells were observed (Figure 2a). The cells form chains consisting of two or four cells (Figures 2b and 2c); eight-cell chains were rarely observed (Figure 2d).

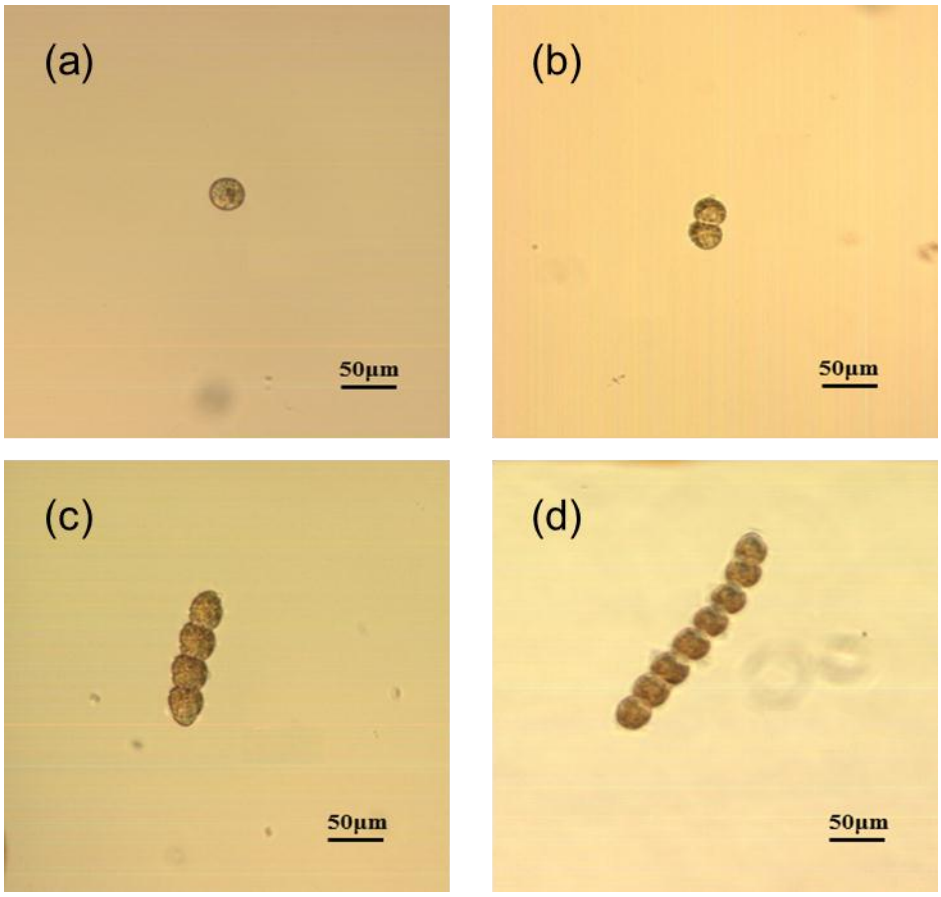

Figure 2. Optical microscopy images of *C. polykrikoides*. Scale bar: 50 μm

The removal rate and observed rate constant for the inactivation of *C. polykrikoides* by various oxidants were examined (Figure 3). Viable cells were considered as active cells, and total number of cells was calculated by adding the number of active and inactivated cells that had maintained their cell structure during the treatment. For an initial cell density of 1.0×10^3 cells/mL, the results of the removal and inactivation rates of algal cells according to the various oxidant doses are presented in Figure 3. The removal and inactivation rates of *C. polykrikoides* increased with the increasing dose of oxidants. The cells were inactivated by 99% after 10 min exposure to an O_3 dose of greater than 1.0 ppm. When injecting MnO_4^- and Cl_2 at doses exceeding 1.0 ppm, the removal efficiency was enhanced by 99% after 180 min. In addition, treatment with 10.0 ppm H_2O_2 resulted in 99% removal of viable cells and 91% removal of total cells after 180 min. For 1.0×10^3 cells/mL, the treatment of O_3 dose above 1.0 ppm resulted in 99% removal of viable cells, whereas 10.0 ppm H_2O_2 was required for a comparable level of inactivation. Also, for 0.5×10^3, 1.0×10^3, 2.0×10^3, and 3.0×10^3 cells/mL, the removal of *C. polykrikoides* by O_3, MnO_4^-, Cl_2, and H_2O_2 was examined as shown in Figures 16, 4, 5, and 6, respectively. This result demonstrates that O_3 showed much higher removal efficiency for the *C. polykrikoides* than other oxidants under identical experimental conditions. Disinfectants effectively inactivate the *C. polykrikoides* in the order of $O_3 > MnO_4^- > Cl_2 > H_2O_2$. The results demonstrate the potential of chemical disinfectants as alternative technologies for the removal of red tide.

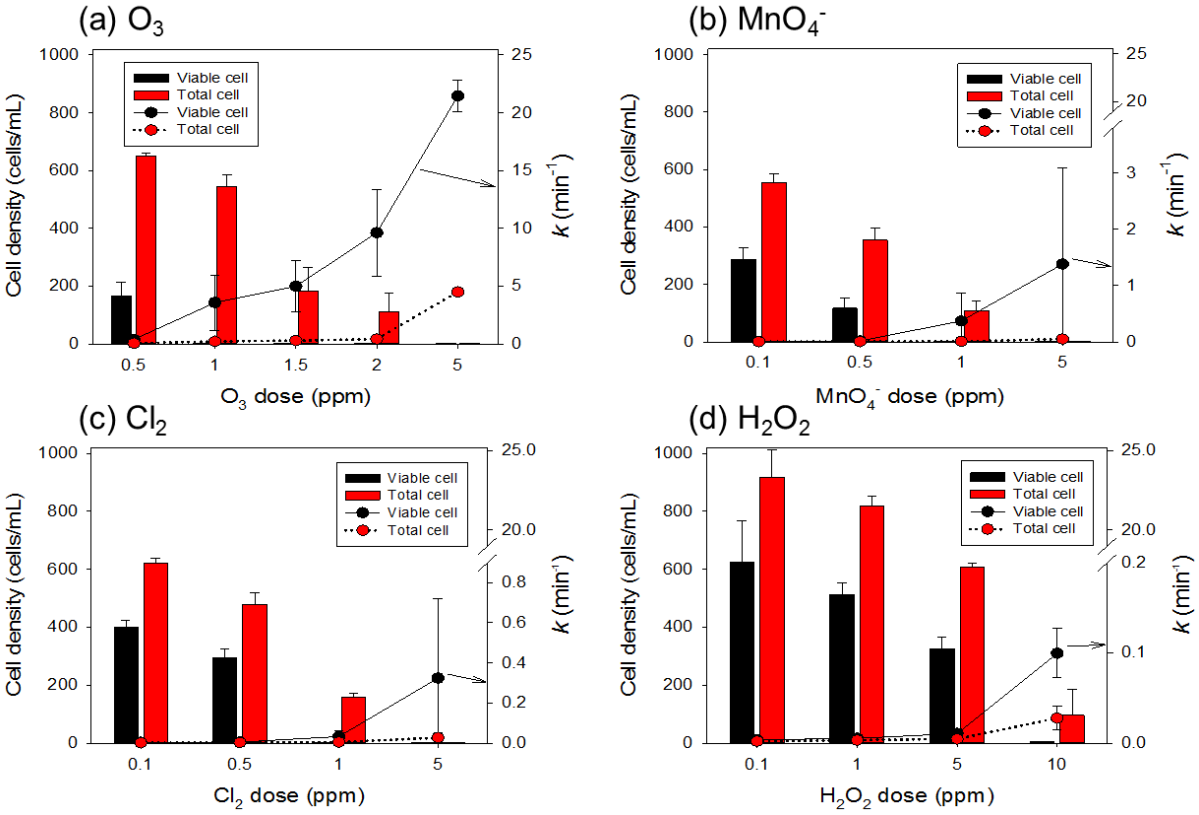

Figure 3. Removal and observed rate constant for inactivation of *C. polykrikoides* by various oxidants ((a) O_3, (b) MnO_4^-, (c) Cl_2, (d) H_2O_2)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; $[MnO_4^-]_0 = [Cl_2]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; $[H_2O_2]_0$ = 0.1, 1.0, 5.0, 10.0 ppm; pH_i = 7.9; Reaction time = 10 min (a), 180 min (b, c, d)

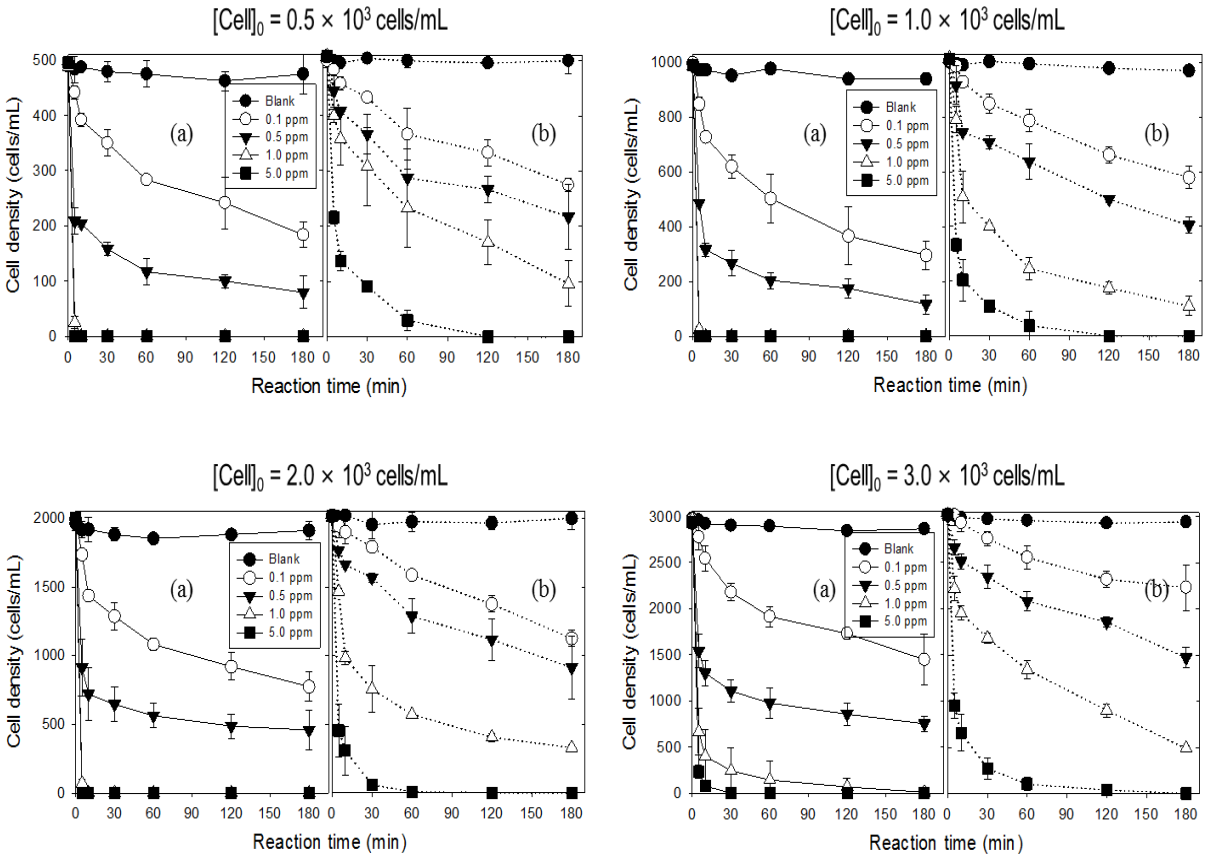

Figure 4. Removal of *C. polykrikoides* by MnO_4^- ((a) viable cell, (b) total cell)

$[Cell]_0$ = 0.5 × 10^3, 1.0 × 10^3, 2.0 × 10^3, 3.0 × 10^3; $[MnO_4^-]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; pH_i = 7.9; Reaction time = 180 min

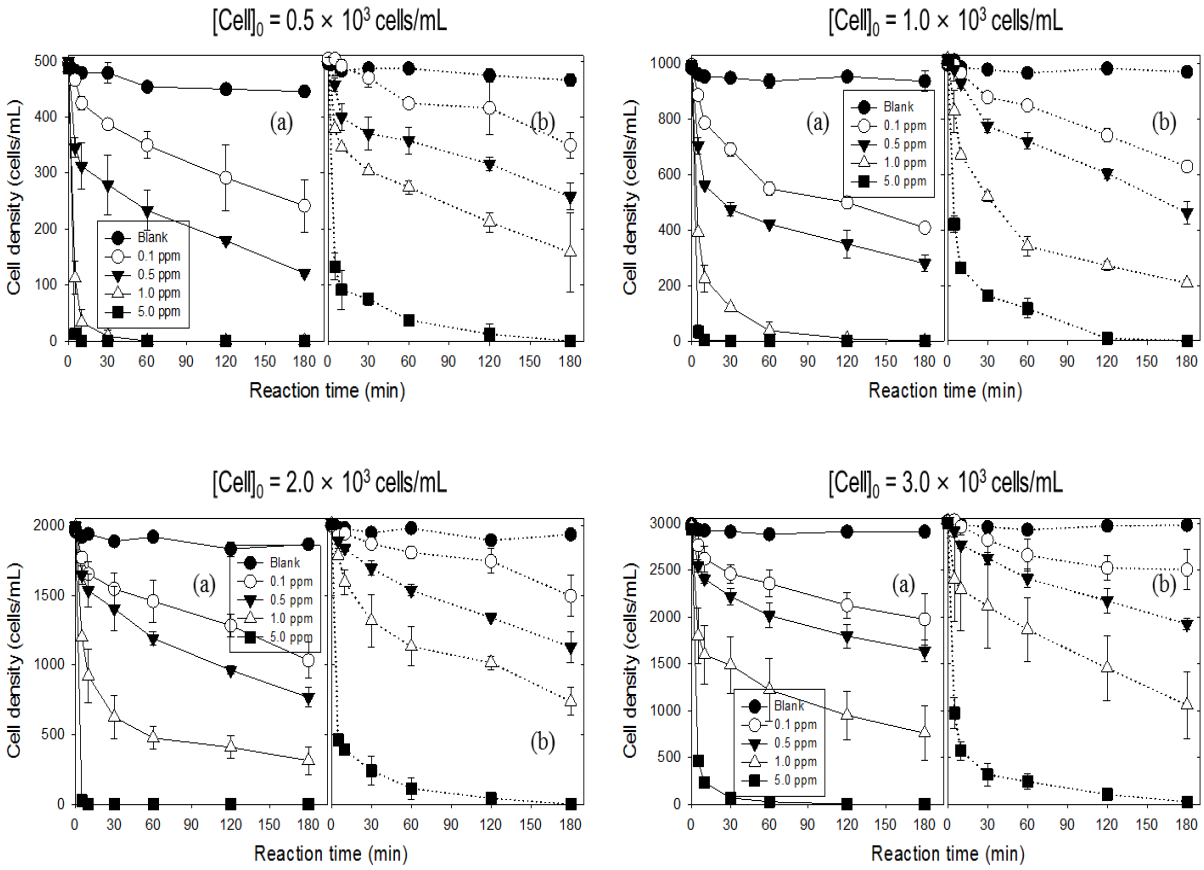

Figure 5. Removal of *C. polykrikoides* by Cl_2 ((a) viable cell, (b) total cell)

$[Cell]_0$ = 0.5 × 10^3, 1.0 × 10^3, 2.0 × 10^3, 3.0 × 10^3; $[Cl_2]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; pH_i = 7.9;

Reaction time = 180 min

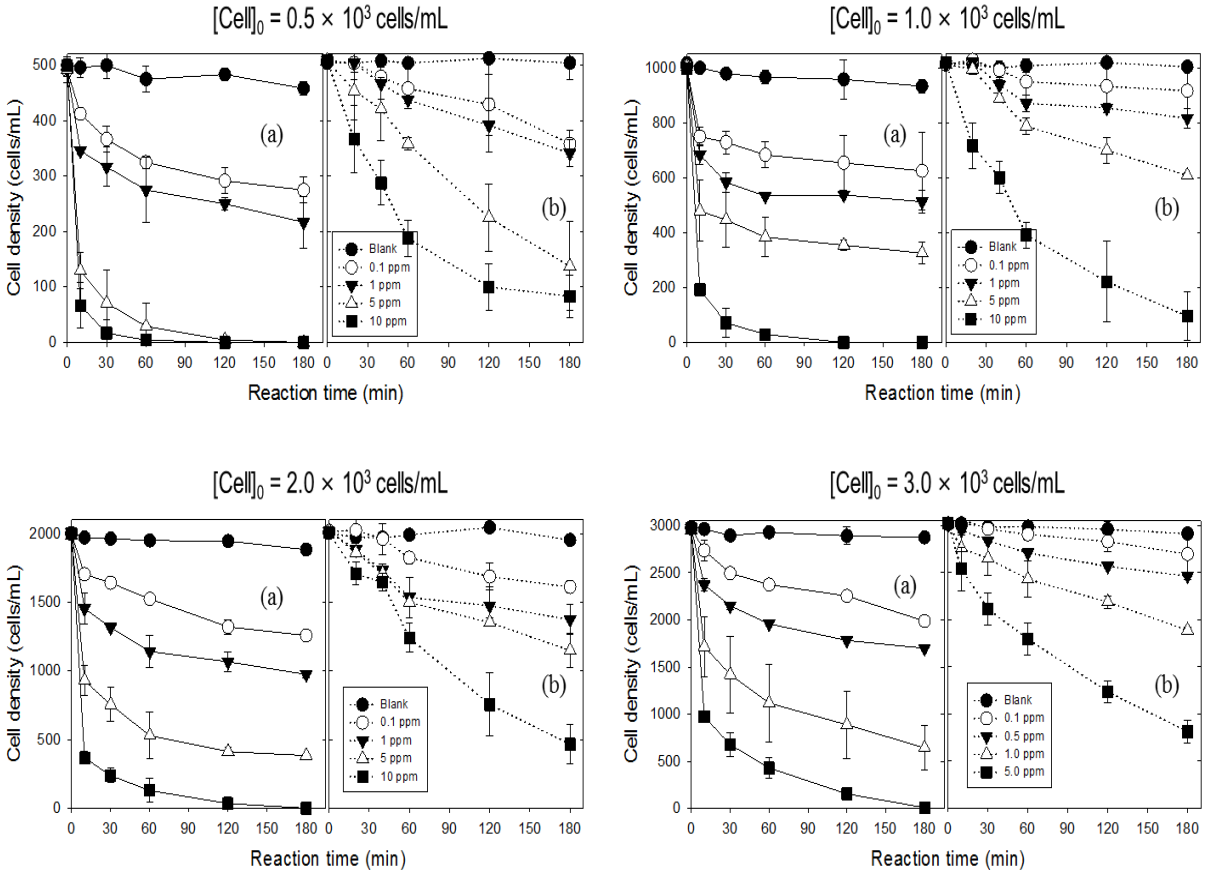

Figure 6. Removal of *C. polykrikoides* by H_2O_2 ((a) viable cell, (b) total cell)

$[Cell]_0$ = 0.5 × 10^3, 1.0 × 10^3, 2.0 × 10^3, 3.0 × 10^3; $[H_2O_2]_0$ = 0.1, 1.0, 5.0, 10.0 ppm; pH_i = 7.9; Reaction time = 180 min

The observed rate constant for the inactivation of *C. polykrikoides* by O_3, MnO_4^-, Cl_2, and H_2O_2 was examined (Figure 7). The experiment was conducted to compare the observed rate constants among O_3, MnO_4^-, Cl_2 and H_2O_2 for the inactivation of *C. polykrikoides* as a function of cell density. The cell densities used for this experiment were 0.5×10^3, 1.0×10^3, 2.0×10^3, and 3.0×10^3 cells/mL and the concentration of oxidants was 1.0 ppm. The inactivation of *C. polykrikoides* was inhibited more by O_3 than by H_2O_2; note that the inhibitory effect was much greater for viable cells than for total cells when cell density decreased. The observed rate constant for the inactivation of *C. polykrikoides* by the various chemical disinfectants of O_3, MnO_4^-, Cl_2 and H_2O_2 as a function of oxidant dose are shown in Figures 17, 8, 9 and 10, respectively. The observed rate constant for the inactivation of *C. polykrikoides* was in the order of $O_3 > MnO_4^- > Cl_2 > H_2O_2$. The inactivation rate of *C. polykrikoides* by O_3 was found to be approximately 7 times higher than that by MnO_4^- relative to the experimental conditions. This result shows that ozonation is a stronger treatment method than the other oxidant treatments for the control of red tide dinoflagellate.

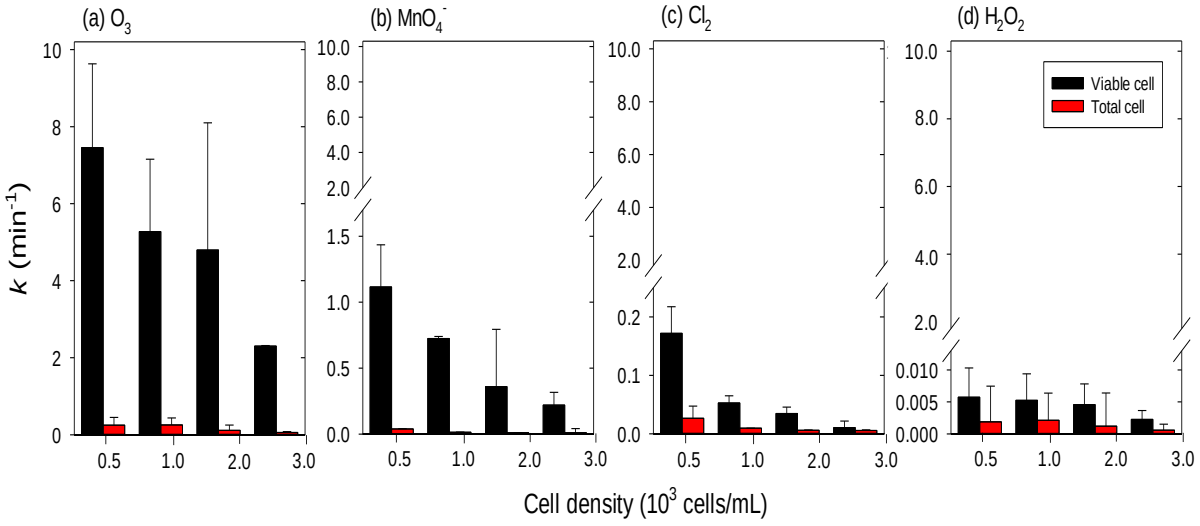

Figure 7. Observed rate constant for inactivation of *C. polykrikoides* by various oxidants ((a) O_3, (b) MnO_4^-, (c) Cl_2, (d) H_2O_2)

$[Cell]_0 = 0.5 \times 10^3$, 1.0×10^3, 2.0×10^3, 3.0×10^3 cells/mL; $[Oxidants]_0 = 1.0$ ppm; $pH_i = 7.9$; Reaction time = 10 min (a), 180 min (b, c, d)

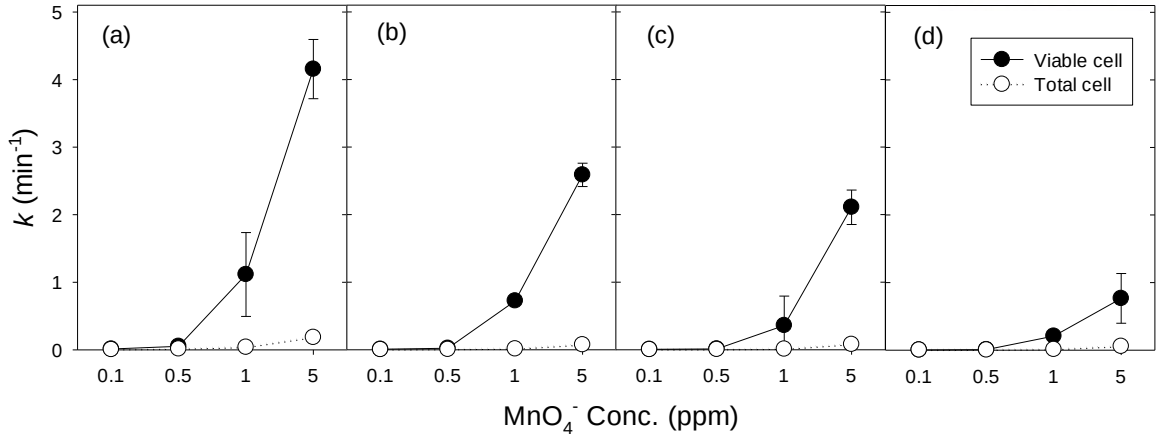

Figure 8. Observed rate constant for removal of *C. polykrikoides* by MnO_4^-

((a) $[Cell]_0 = 0.5 \times 10^3$ cells/mL, (b) 1.0×10^3 cells/mL (c) 2.0×10^3 cells/mL, (d) 3.0×10^3 cells/mL)

$[Cell]_0 = 0.5 \times 10^3, 1.0 \times 10^3, 2.0 \times 10^3, 3.0 \times 10^3$; $[MnO_4^-]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; pH_i = 7.9; Reaction time = 180 min

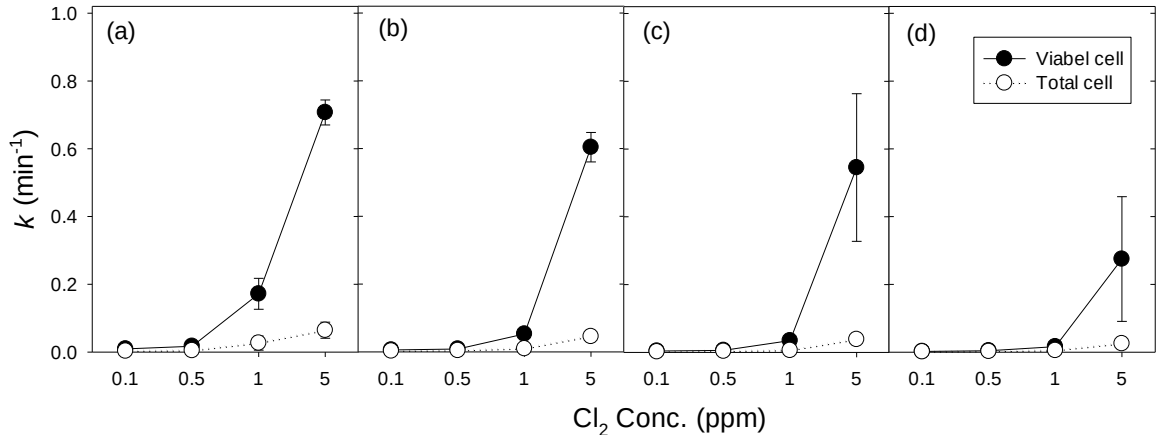

Figure 9. Observed rate constant for removal of *C. polykrikoides* by Cl_2

((a) $[Cell]_0 = 0.5 \times 10^3$ cells/mL, (b) 1.0×10^3 cells/mL (c) 2.0×10^3 cells/mL, (d) 3.0×10^3 cells/mL)

$[Cell]_0 = 0.5 \times 10^3, 1.0 \times 10^3, 2.0 \times 10^3, 3.0 \times 10^3$; $[Cl_2]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; pH_i = 7.9; Reaction time = 180 min

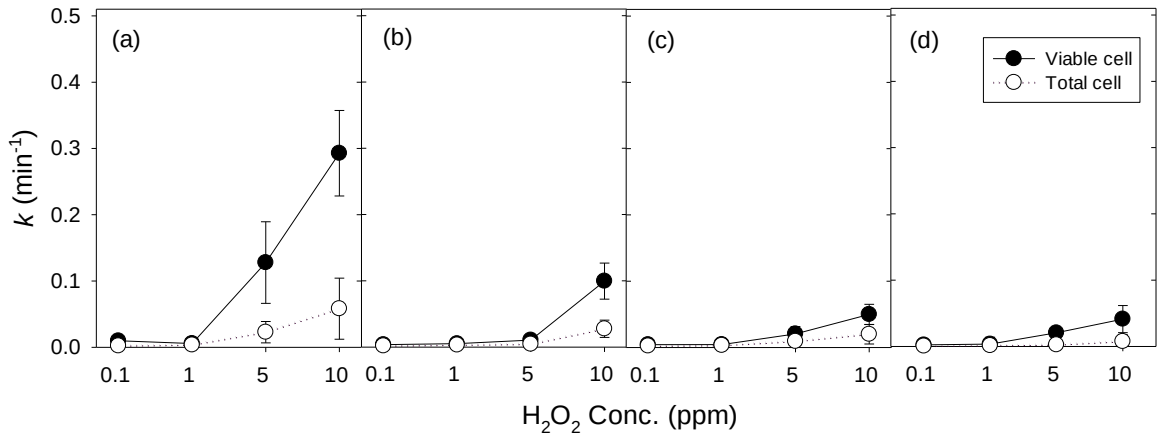

Figure 10. Observed rate constant for removal of *C. polykrikoides* by H_2O_2

((a) $[Cell]_0 = 0.5 \times 10^3$ cells/mL, (b) 1.0×10^3 cells/mL (c) 2.0×10^3 cells/mL, (d) 3.0×10^3 cells/mL)

$[Cell]_0 = 0.5 \times 10^3, 1.0 \times 10^3, 2.0 \times 10^3, 3.0 \times 10^3$; $[H_2O_2]_0$ = 0.1, 1.0, 5.0, 10.0 ppm; pH_i = 7.9; Reaction time = 180 min

1.2. Decay of total residual oxidants

In this study, the depletion of residual oxidants was investigated to determine the effect of the treatment on the absence and presence of *C. polykrikoides*. The control experiment indicates that O_3 consumption was very rapid and the decomposition of MnO_4^- and H_2O_2 did not affect the decrease of concentration during the experimental course (data not shown). Figure 11 shows the depletion of residual oxidant as a function of oxidant dose in the presence of *C. polykrikoides*. For 1.0×10^3 cells/mL, the O_3 was completely decomposed in under 10 min, and the residual O_3 was not detected. The depletion reactions of the oxidant were observed as a function of reaction time. Moreover, as the applied oxidant dose increased, the time required for the residual oxidant to be consumed also increased. When dose of 1.0 ppm of MnO_4^- and H_2O_2 were applied, the oxidant was consumed within 180 min. When the concentration of 5.0 ppm of MnO_4^- and H_2O_2 were applied, 20% and 23% residue were observed after 180 min, respectively.

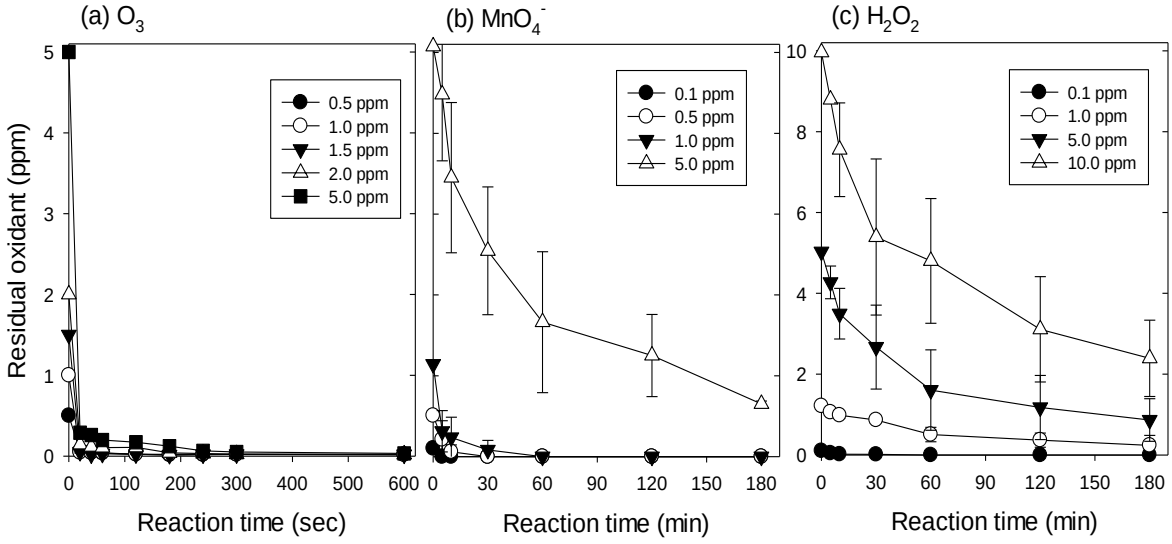

Figure 11. Depletion of residual oxidant as a function of oxidant dose in the presence of *C. polykrikoides* ((a) O_3, (b) MnO_4^-, (c) H_2O_2)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[Oxidants]_0$ = 1.0, 5.0 ppm; pH_i = 7.9;

Reaction time = 10 min (a), 180 min (b, c)

These oxidants (MnO_4^- and H_2O_2) either were completely consumed or remained as residual oxidants. Seawater contains high concentrations of Br^-, which reacts with oxidants to form bromine (hypobromous acid and hypobromite, HOBr/OBr^-). The stable oxidants such as bromine are called total residual oxidant (TRO), and can contain chlorine, bromine, $^{\bullet}OH$, and so on (Perrins *et al*., 2006), which are active in inactivating marine organisms. In seawater, the primary brominated compound formed by O_3 is hypobromous acid (HOBr), which is in equilibrium with hypobromite (OBr^-). The first reaction shows that, in seawater, O_3 oxidizes Br^- to OBr^-. The OBr^- hydrolyzes into HOBr, which is a weak acid with a pK_a of 8.8. Cl_2 could be converted to HOBr by reactions. However, the MnO_4^- reacts with Br^- to generate HOBr in acid medium (Lawani and Sutter, 1973) and H_2O_2 does not promote the production of HOBr through reaction with Br^-.

As mentioned above, the oxidant treatment in seawater generates bromine, which is expressed as TRO (unit; TRO as Cl_2 μM). During oxidant treatment in seawater, the concentration of TRO was observed to slowly decrease, while HOBr sharply increased by 5 min and then decreased as a function of reaction time (data not shown). The depletion of TRO and production of HOBr as a function of oxidant dose were examined in the presence of *C. polykrikoides* (Figure 12). The concentration of TRO and HOBr increased as the oxidant dose increased and then decreased as the reaction time increased. For 1.0×10^3 cells/mL, the rapid decay of TRO by O_3 and Cl_2 occurred in the initial reaction and TRO was mostly decomposed after 180 min. A slow rate of decay of TRO by MnO_4^- was observed, which remained approximately at 13% at the concentration of 5.0 ppm after 180 min. The concentration of TRO by H_2O_2 was much lower than that of other oxidants. During ozonation and chlorination, 10 μM and 3 μM of HOBr was produced after 5 min, respectively. On the other hand, MnO_4^- and H_2O_2 do not promote the HOBr production of brominated byproducts. The study compared the TRO decay and HOBr production in the absence and presence of *C. polykrikoides*. It was found that the TRO in the presence of *C. polykrikoides* was shown to decay faster than in the absence of *C. polykrikoides*, and it is known that TRO decay and HOBr production can be affected by the water quality characteristics.

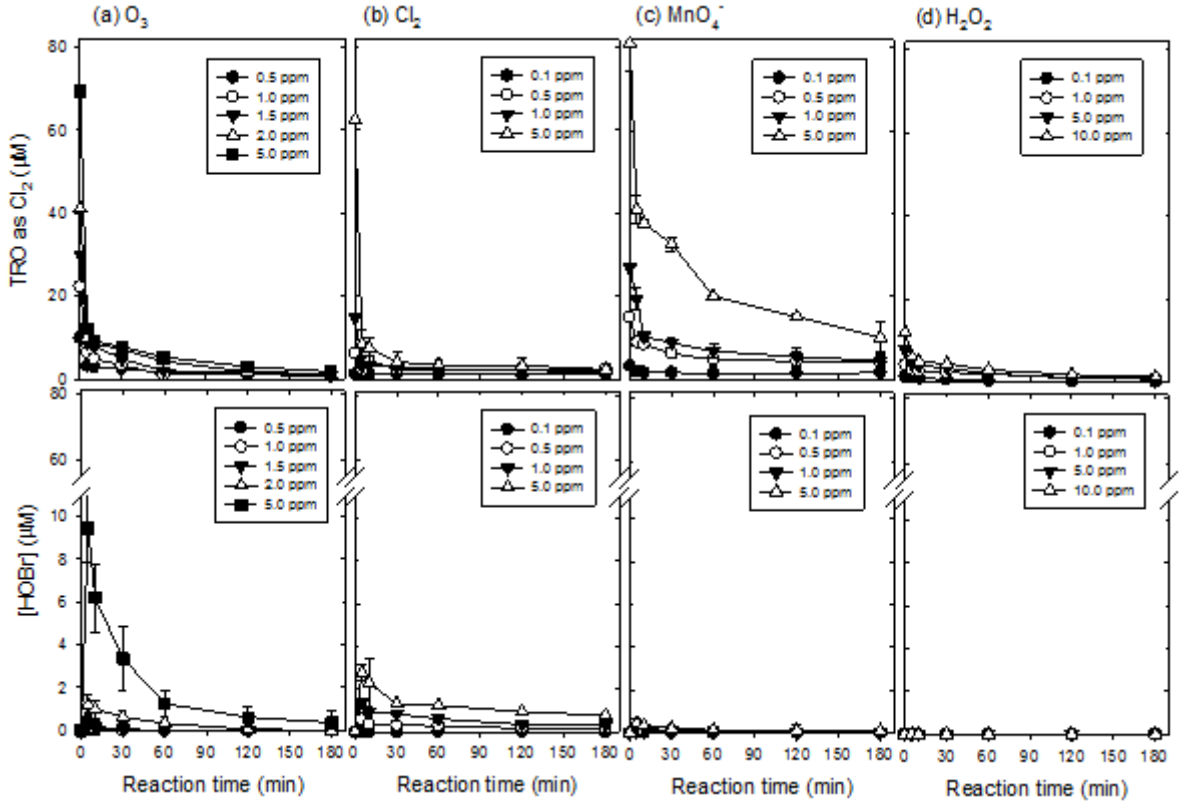

Figure 12. Depletion of TRO and production of HOBr as a function of oxidant dose in the presence of *C. polykrikoides* ((a) O_3, (b) Cl_2, (c) MnO_4^-, (d) H_2O_2)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; $[MnO_4^-]_0 = [Cl_2]_0$ = 0.1, 0.5, 1.0, 5.0 ppm; $[H_2O_2]_0$ = 0.1, 1.0, 5.0, 10.0 ppm; pH_i = 7.9; Reaction time = 180 min

1.3. Formation of BrO_3^-

In the ozonation of water containing Br^- containing water, it is an important to monitor BrO_3^- as an inorganic byproduct because of its risk to the ecosystem and human health. The seawater generally contained 65 ppm of Br^-, which is much higher than the concentration of Br^- in fresh water. The formation of BrO_3^- after chemical disinfectant treatment was examined (Figure 13). Water samples were prepared by spiking each sample with DI water at a Br^- concentration of 65 ppm. The pH of solution was then adjusted to 7.9. The concentration of BrO_3^- increased with increasing oxidant dose. At dose of Cl_2 and O_3 of 0.5 ppm, the concentrations of BrO_3^- were detected to be 0.12 ppb and 0.75 ppb, respectively. Especially, the O_3 dose of 5.0 ppm resulted in formation of 278 ppb BrO_3^-. However, the production of BrO_3^- by MnO_4^- and H_2O_2 was not detected until the oxidant dose of 5.0 ppm. O_3 resulted in a much higher formation of BrO_3^- than Cl_2, MnO_4^-, and H_2O_2. Ozonation generates important oxidants such as bromine ($HOBr/OBr^-$), which reacts with molecular O_3 and then terminates in BrO_3^- formation. Therefore, the ozonation conditions need to be optimized in order to minimize the formation of BrO_3^- and other byproducts.

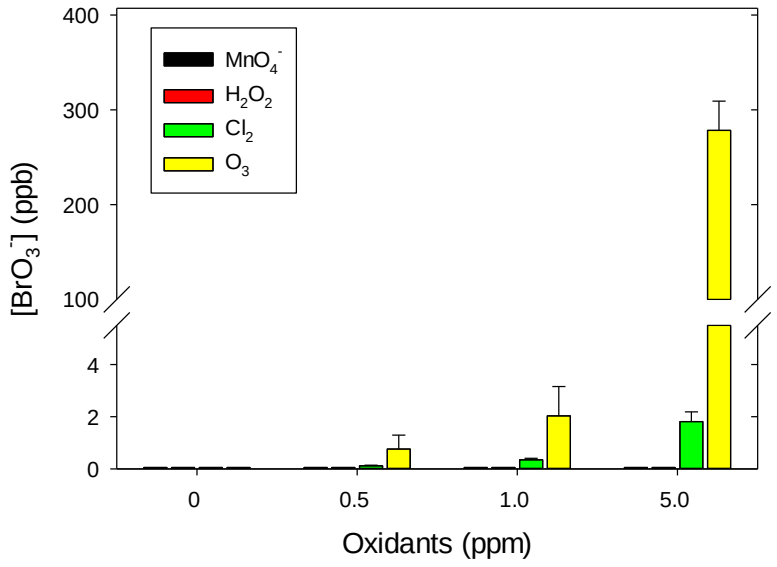

Figure 13. BrO_3^- formation as a function of oxidant dose

$[Br^-]_0$ = 65 ppm; $[Oxidants]_0$ = 0.5, 1.0, 5.0 ppm; pH_i = 7.9; Reaction time = 180 min

1.4. Fish acute toxicity test

The acute toxicity of farmed fish should be assessed to determine the safe concentrations of oxidants for fish by confirming the level of toxicity to fish of MnO_4^-, Cl_2, and H_2O_2 treatments. The fish selected for the acute toxicity test was juvenile red sea bream (*Pagrus major*), the concentration of which are commonly damaged from red tide in Korea. The seawater sample used for this study was collected from Namhae, Korea. Table 3 summarizes the water quality data of the seawater. The average pH and conductivity of seawater were 7.9 and 47.8 mS/cm, respectively. The chloride ion concentration of the sampled seawater was 17,451 ppm.

Table 3. Water-quality parameters of seawater (Namhae, Korea)

Parameters		Seawater
pH		7.9
Conductivity (mS/cm)		47.8
Alkalinity (mg/L as $CaCO_3$)		117.2
Turbidity (NTU)		0.05
DOC (mg/L)		2.38
A_{254} (cm^{-1})		0.01
Anion (mg/L)	Cl^-	17,451
	SO_4^{2-}	1,821
Cation (mg/L)	Na^+	11,071
	K^+	511
	Mg^{2+}	1,136
	Ca^{2+}	391

A pre-test was performed using 30 fish in a tank in order to monitor the mortality of juvenile red sea bream, *Pagrus major* when exposed to three oxidants at various concentrations. The MnO_4^- and Cl_2 concentrations of 5.0 ppm and 10.0 ppm, respectively, resulted in the mortality of 30 fish in 5 min. When the concentration of H_2O_2 was 500 ppm, most of the fish perished after 30 min. The concentrations of oxidants were as follows: MnO_4^- = 0.1, 0.2, and 0.5 ppm; Cl_2 = 0.5, 1.0, and 1.5 ppm; and H_2O_2 = 50, 100, and 200 ppm.

Juvenile red sea bream were exposed to MnO_4^-, Cl_2, and H_2O_2 at a range of concentrations, and their survival was investigated, similar to the fish acute toxicity test described above. Control tests were performed without any chemical disinfectants to confirm the appropriate concentrations of oxidants for fish survival during the experiment. When the concentration of oxidants was increased, the survival of juvenile red sea bream decreased. Figure 14a reveals that the MnO_4^- concentration of 0.1 ppm did not affect the toxicity to fish and at a dose of 0.2 ppm, 95% of fish survived after 72 h exposure. Especially, fish survival was affected by 0.5 ppm MnO_4^- within 12 h of the initial exposure. As shown in Figure 14b, the fish survived the effect of Cl_2 treatment at lower doses of MnO_4^-; 0.5 ppm and 1.0 ppm Cl_2 resulted in 95% and 90%, respectively, of survival during 72 h. However, the Cl_2 strongly effected fish survival as the concentration increased to more than 1.5 ppm. In the H_2O_2 test (Figure 14c), almost all of the juvenile red sea bream survived at a dose of 50 ppm of H_2O_2 concentration. The survival decreased gradually with exposure time, with 0% survival after 72 h exposure at the H_2O_2 concentration of 200 ppm.

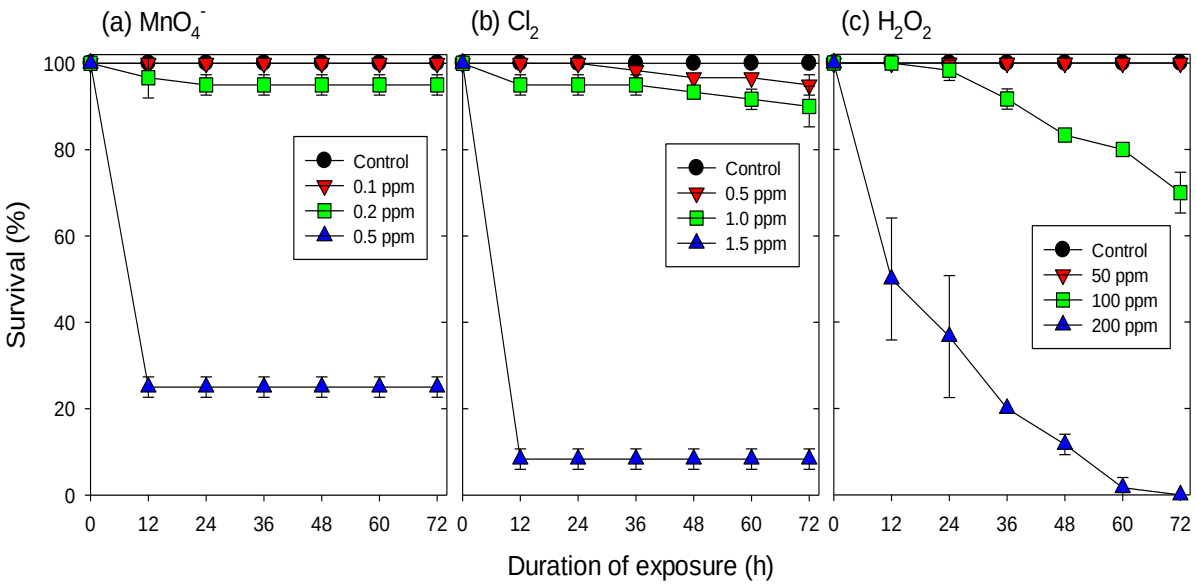

Figure 14. Time course change in survival of juvenile red sea bream (*Pagrus major*) exposed to various oxidants for 72-h

$[MnO_4^-]_0$ = 0.1, 0.2, 0.5 ppm; $[Cl_2]_0$ = 0.5, 1.0, 1.5 ppm; $[H_2O_2]_0$ = 50, 100, 200 ppm;
Exposure time = 72 h

The log concentration and mortality (%) regression for juvenile red sea bream are shown in Figure 15. The LC_{50} values were calculated by fitting the sigmoid regression model between the log concentration and mortality. When the concentration of oxidants was increased, the mortality of juvenile red sea bream (calculated from the slopes of the mortality curves shown in Figures 15a, 15b, and 15c) increased.

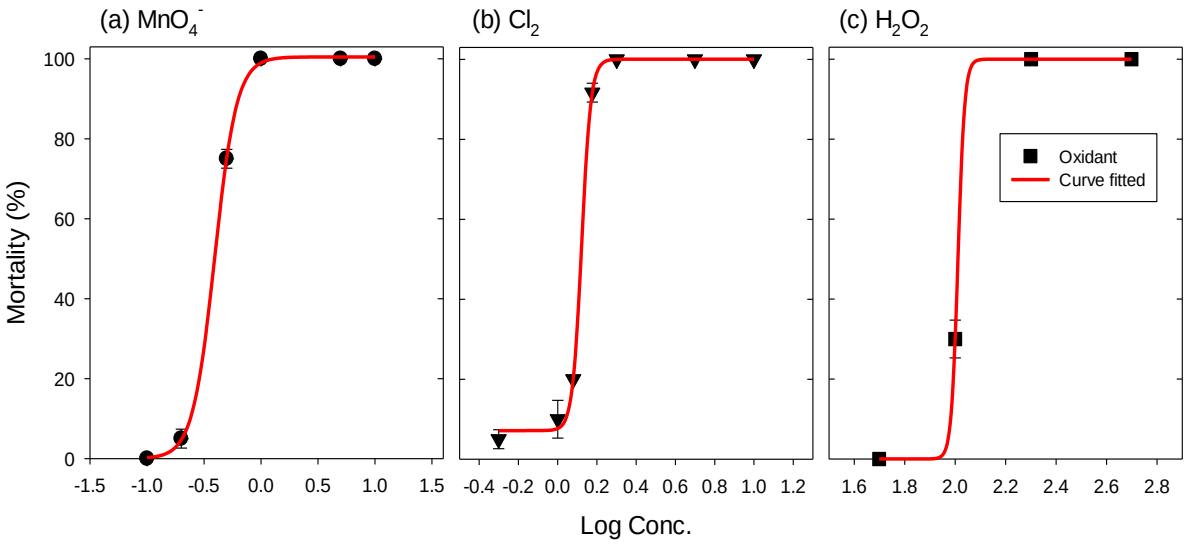

Figure 15. Log concentration and mortality regression for juvenile red sea bream (*Pagrus major*) by various oxidants for 72-h

$[MnO_4^-]_0$ = 0.1, 0.2, 0.5, 1.0, 5.0, 10.0 ppm; $[Cl_2]_0$ = 0.5, 1.0, 1.2, 1.5, 2.0, 5.0, 10.0 ppm; $[H_2O_2]_0$ = 50, 100, 200, 500 ppm; Exposure time = 72 h

The LC_{50} value is the chemical concentration at which 50% of the test animals or fish are killed. In general, the smaller the LC_{50} value, the more toxic the chemical, while the larger the LC_{50} value, the lower the toxicity. The LC_{50} values of 72-h acute toxicity with the three disinfectants (MnO_4^-, Cl_2, and H_2O_2) tested on juvenile red sea bream (Table 4) showed that MnO_4^- was the most toxic among the three compounds on organism. The acute toxicity of MnO_4^-, Cl_2, and H_2O_2 to juvenile red sea bream was reached at 72-h with LC_{50} values of 0.39, 1.32, and 102.61 ppm, respectively. Concentration of MnO_4^- lower than 1.0 ppm showed a very strong effect on toxicity to fish, while H_2O_2 showed a lower toxicity than other oxidants. The LC_{50} values of juvenile red sea bream by various oxidants for 72-h were shown to be higher than the residual oxidant concentration during oxidant treatment. This result indicates that the use of chemical oxidants does not affect the aquaculture fish and simultaneously removes red tide dinoflagellate.

Table 4. LC_{50} value of juvenile red sea bream (*Pagrus major*) by various oxidants for 72-h

Oxidants	MnO_4^-	Cl_2	H_2O_2
LC_{50} value (ppm)	0.39	1.32	102.61

2. Control of *C. polykrikoides* by O_3

2.1. Removal of *C. polykrikoides* by O_3

Further experiments were conducted to examine the removal efficiency of *C. polykrikoides* by O_3 (Figure 16). Viable cells were considered as active cells, and the total number of cells was calculated by adding the number of active and inactivate cells that had maintained their cell structure during the treatment. The results were averaged, and the counts were converted to units of cells/mL. All experiments were performed in a batch system and the cell densities used for this experiment were 0.5×10^3, 1.0×10^3, 2.0×10^3, and 3.0×10^3 cells/mL. The *C. polykrikoides* was exposed to 0.5, 1.0, 1.5, 2.0, and 5.0 ppm of O_3. The removal of *C. polykrikoides* by O_3 increased with increasing O_3 dose. The *C. polykrikoides* cells were rapidly inactivated by 99% after 10 min exposure to an O_3 dose of more than 1.0 ppm. At an O_3 dose of 5.0 ppm, most of the cells were ruptured. Before O_3 injection, the motility of the active cells of *C. polykrikoides* was monitored, but exposure to O_3 resulted in inactivate cells. The *C. polykrikoides* cells treated with the O_3 system exhibited significant disruption of their cell membrane during continuous O_3 exposure. Therefore, the removal of red tide dinoflagellate by O_3 is demonstrated as an effective cell disruption method compared to spraying with activated clay which merely results in flocculation or sedimentation of the algal cells and does not remove the species.

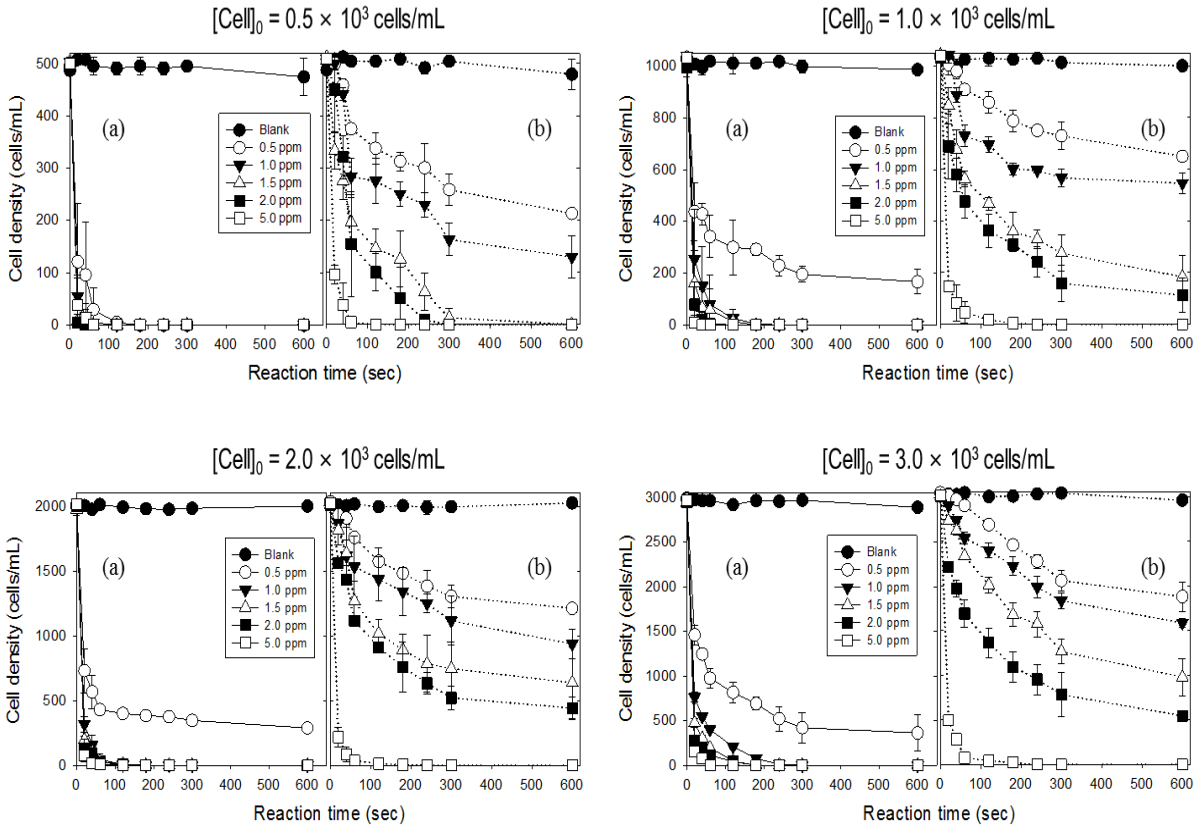

Figure 16. Removal of *C. polykrikoides* by O_3 ((a) viable cell, (b) total cell)

$[Cell]_0$ = 0.5 × 10^3, 1.0 × 10^3, 2.0 × 10^3, 3.0 × 10^3; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; pH_i = 7.9; Reaction time = 600 sec

Figure 17 shows the inactivation kinetics of *C. polykrikoides* by O_3. The experiment was conducted to compare the observed rate constant for the inactivation of *C. polykrikoides* as a function of cell density and O_3 dose. The removal rate was faster with the cell density of 0.5 × 10^3 cells/mL, than with the high cell density, whereas the cell densities of 1.0 × 10^3 and 2.0 × 10^3 cells/mL showed similar rate constant results. The removal rate was the slowest with the high cell density. In addition, a constant increase of the removal rate was shown as the O_3 dose increased, and a decrease of the removal rate was shown with a higher density of *C. polykirkoides*. For 0.5 × 10^3 cells/mL, the inactivation rate of *C. polykrikoides* by O_3 was found to be approximately 2 times higher than that relative to the experimental conditions on 3.0 × 10^3 cells/mL.

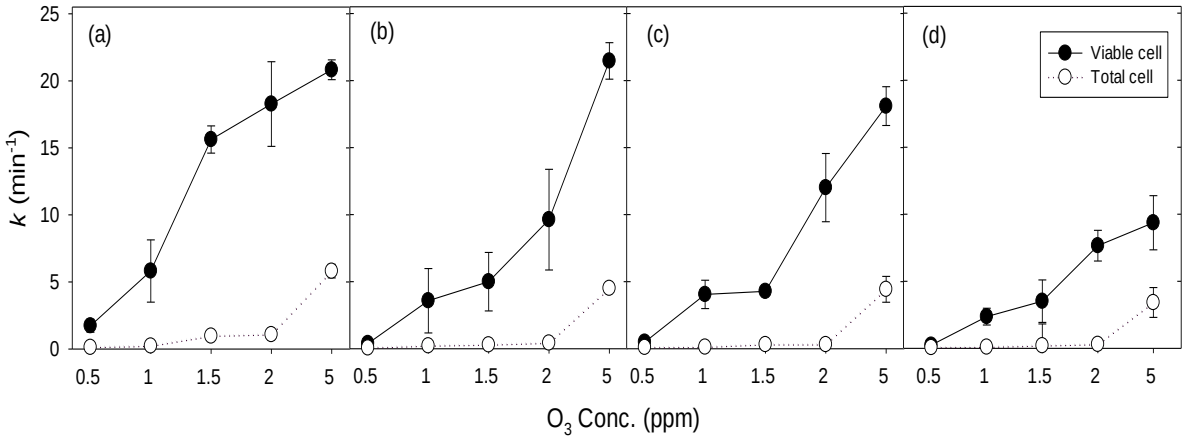

Figure 17. Observed rate constant for removal of *C. polykrikoides* by O_3
((a) $[Cell]_0 = 0.5 \times 10^3$ cells/mL, (b) 1.0×10^3 cells/mL (c) 2.0×10^3 cells/mL, (d) 3.0×10^3 cells/mL)

$[Cell]_0 = 0.5 \times 10^3$, 1.0×10^3, 2.0×10^3, 3.0×10^3; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; pH_i = 7.9;
Reaction time = 600 sec

2.2 Oxidation for *C. polykrikoides*

To further verify the characteristics of oxidation for *C. polykrikoides* removal, experiments were conducted for quantification of the oxidants by varying the O_3 dose. Fig. 18 shows the depletion of residual O_3 and production of HOBr as a function of O_3 dose in the presence of *C. polykrikoides*. For 1.0×10^3 cells/mL, a very rapid O_3 decrease during the initial phase of the ozonation can be observed. The second step of O_3 demand is the slow decrease in O_3 occurring during the 5 minutes after the initial step. The *C. polykrikoides* were then completely consumed after 10 min (Fig. 18a). Moreover, as the applied O_3 dose increases, the time required for the residual O_3 to be consumed also increases. The decomposition of O_3 in seawater can be characterized by two reaction steps. In the first step, O_3 is rapidly consumed to produce $^{\bullet}OH$, which is caused by the instantaneous O_3 demand (IOD). The second step occurs slowly or moderately. IOD increases as the natural organic matter in water increases in general (Cho et al., 2003). These results demonstrate that the decay rate of O_3 in the presence of *C. polykrikoides* was much higher, but only a small amount of $^{\bullet}OH$ was produced from O_3 decomposition in the presence of *C. polykrikoides*.

As shown in Fig. 18b, the concentration of HOBr sharply increased at 5 min and then decrease as a function of reaction time during ozonation in the presence of *C. polykrikoides*.

As previously mentioned, seawater contains high levels of Br^-, which reacts with O_3 to generate stable oxidants such as bromine (hypobromous acid and hypobromite, HOBr/ OBr^-). These oxidants are stable and can be active in inactivating marine organisms. In seawater, the primary brominated compound formed by O_3 is HOBr, which is in equilibrium with OBr^-. The first reaction shows that O_3 in seawater oxidizes the Br^- to OBr^-. The OBr^- hydrolyzes into HOBr, which is a weak acid with a pK_a of 8.8 (von Gunten and Hoigné, 1994). The main disinfection oxidant in seawater after ozonation is HOBr, which is known as a weak oxidant but a strong disinfectant (Juretić et al., 2011).

Thus, the O_3 showed the effective removal efficiency of *C. polykrikoides* with two distinct stages. The first stage of inactivation, which occurred rapidly within 1 min reaction time, was mainly achieved by the direct oxidation of molecular O_3. The second stage, which occurred by HOBr reactions, showed a slower inactivation than that at the first stage. In other words, the removal of *C. polykrikoides* by O_3 was rapid, and appeared to be caused by initial ozonation, and then slow reaction, in which Br^- was oxidized to HOBr.

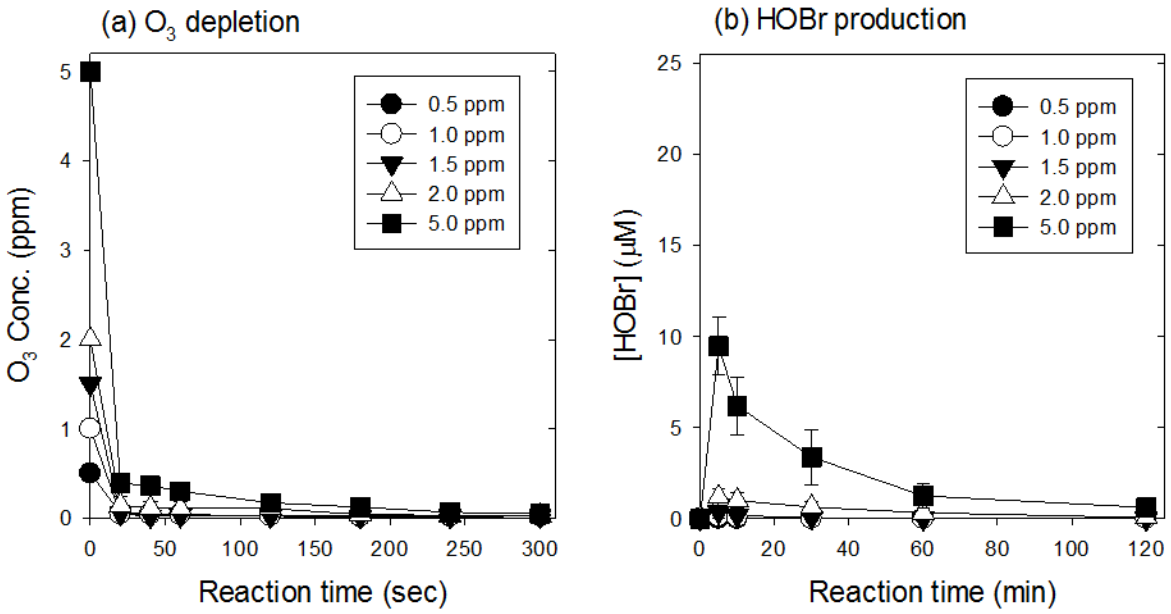

Figure 18. Depletion of O_3 (a) and production of HOBr (b) as a function of O_3 dose

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; pH_i = 7.9;

Reaction time = 300 sec (a), 120 min (b)

2.3 Role of the O_3 decomposition promoter and inhibitor

Molecular O_3 is capable of including the formation of perhydroxyl ion (HO_2^-), which then reacts with other O_3 to form ozonide ion ($^{\bullet}O_3^-$) and superoxide radical anion ($^{\bullet}O_2^-$) (Staehelin and Hoigné, 1985). Promoters such as humic substances, formic acid, glyoxylic acid and primary alcohols are responsible for the regeneration of the $^{\bullet}O_2^-$ from the $^{\bullet}OH$. Figure 19 shows the effect of humic acid concentration on *C. polykrikoides* removal efficiency. Humic acid plays the role of a promoter for O_3 decomposition. As shown in Figure 19, humic acid expressed a relatively low level of removal efficiency on the cells; 2.0 ppm humic acid and 0.7 ppm O_3 doses resulted in 67% and 35% of viable and total cells inactivation, respectively, of *C. polykrikoides* within 10 min. However, a greater effect was achieved when humic acid concentration was decreased from 2.0 to 0.5 ppm. The removal efficiency of *C. polykrikoides* decreased with increasing concentration of humic acid. This effect can be explained by the consumption of O_3 and $^{\bullet}OH$ by the humic acid.

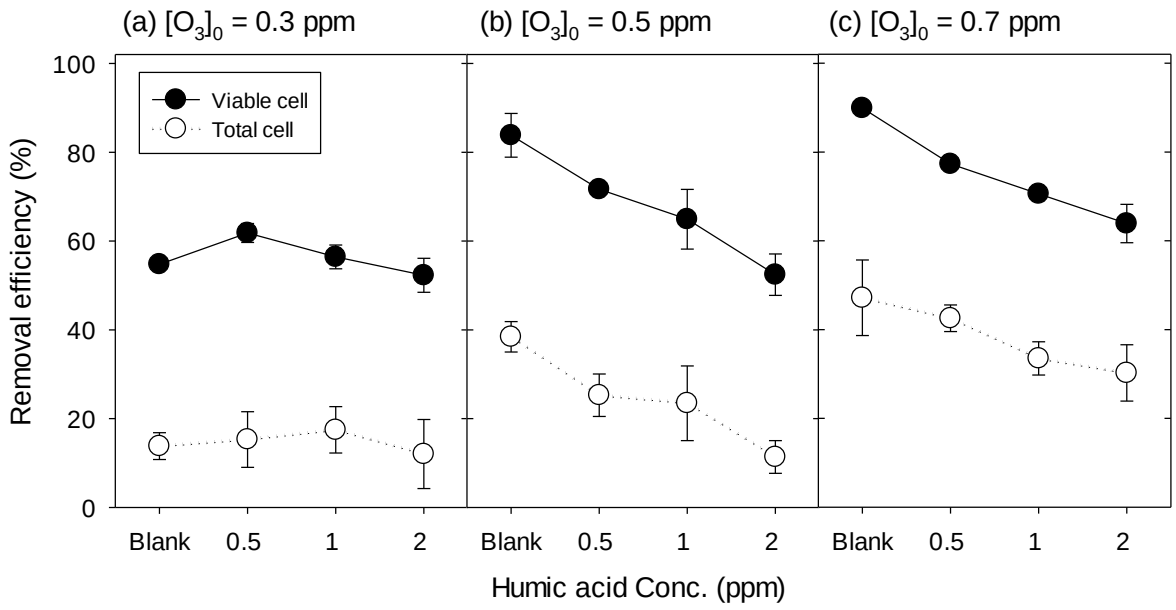

Figure 19. Effect of humic acid concentration on *C. polykrikoides* removal efficiency ((a) $[O_3]_0$ = 0.3 ppm, (b) 0.5 ppm, (c) 0.7 ppm)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.3, 0.5, 0.7 ppm; $[humic\ acid]_0$ = 0.5, 1.0, 2.0 ppm; pH_i = 7.9; Reaction time = 600 sec

In addition, the role of the O_3 decomposition inhibitor is shown in Figure 20. The inhibitors are compounds capable of consuming $^{\bullet}OH$ without regeneration of the superoxide anion. Carbonate, bicarbonate, alkyl groups, and tertiary alcohols are some of the common inhibitors. Carbonate acts as an inhibitor of the radical-type chain reaction that accelerates the decomposition of O_3. In contrast, humic acid was shown to have a remarkable effect on *C. polykrikoides* removal efficiency, as shown in Figure 20. Figure 20 shows the limited inactivation of carbonate concentration; the removal of less than 50% of total cells was obtained with 1.0 mM carbonate after 10 min. When the carbonate concentration increased over the range of the experiment from 0.1 to 5.0 mM, no significant alteration was found in the removal effect, in which approximately 88% viable cell removal efficiency was achieved after 10 min when the carbonate and 0.7 ppm O_3 dose were not applied. However, removal efficiency of about 90% of viable cells was steadily maintained despite the elevation of carbonate concentration from 0.1 to 5.0 mM. The test result indicated that the removal efficiency of *C. polykrikoides* total cells was only slightly increased by changing the carbonate concentration. On the other hand, the removal efficiency of *C. polykrikoides* viable cells was increased as a function of carbonate concentration. The results indicate that the removal of *C. polykrikoides* by O_3 was increased more with increasing O_3 exposure than by $^{\bullet}OH$.

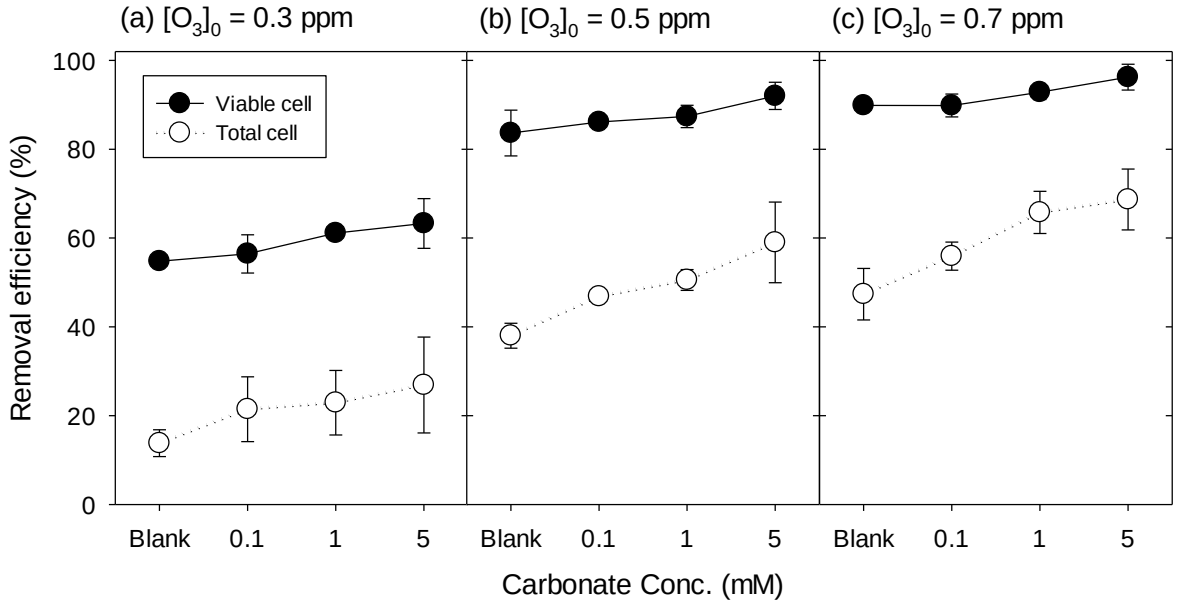

Figure 20. Effect of carbonate concentration on *C. polykrikoides* removal efficiency ((a) $[O_3]_0$ = 0.3 ppm, (b) 0.5 ppm, (c) 0.7 ppm)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.3, 0.5, 0.7 ppm; $[carbonate]_0$ = 0.1, 1.0, 5.0 mM; pH_i = 7.9; Reaction time = 600 sec

2.4. Effect of solution pH and temperature

The response surface method (RSM) is a useful statistical technique for the optimization of complex processes and is widely used for the design of experiment (Myers and Montgomery, 2002). The most important factors affecting the O_3 decomposition are pH and temperature. RSM was employed in this study to investigate the relationship between the solution pH, temperature, and O_3 dose.

The effect of pH on *C. polykrikoides* inactivation activity of O_3 was investigated by conducting experiments at three different pH of 6.5, 8.0, and 9.5, the results of which are presented in Figure 21. Figure 21 shows a response surface plot of the effect of pH and O_3 dose on *C. polykrikoides* removal efficiency. This result reveals a good consistency in inactivation activity of O_3 over the pH range; for all O_3 dose conditions, the efficiency of *C. polykrikoides* removal increased when pH decreased to 6.5. Stronger removal efficiency was achieved at lower pH condition with the higher level of O_3 used. By decreasing the initial pH and increasing O_3 dose, the *C. polykrikoides* removal efficiency increased. Table 5 shows the analysis of variance (ANOVA) for the regression equation used to investigate the relationship between the pH of solution and O_3 dose. The experimental data showed a determination coefficient (R^2) of 0.97 and 0.98 for the viable cell and total cell, respectively. Also, the calculated model showed no significant lack of fit at $p > 0.05$, showing it is possible to estimate the pure error. The results indicated that the model used showed a significant fit between the response variables and the interaction (pH of solution and O_3 dose model terms) ($p < 0.05$).

The pH of the solution is an important factor affecting the O_3 decomposition because hydroxide ions (OH^-) initiate the O_3 decomposition. At a low pH, O_3 decomposition is relatively slow; however, as an initiator product for the $^{\bullet}OH$, hydroxide ion has a high pH. The products of the $^{\bullet}OH$ reactions might therefore generate $HO_2^{\cdot}/O_2^{\cdot-}$, which further promotes radical-type chain reactions (Elovitz *et al.*, 2000). Accordingly, O_3 decomposition occurs faster in a higher pH solution. This indicates that the high removal efficiency of *C. polykrikoides* in this study is mainly due to direct O_3 reaction, and that the effect of pH has a more significant influence on red tide dinoflagellate removal.

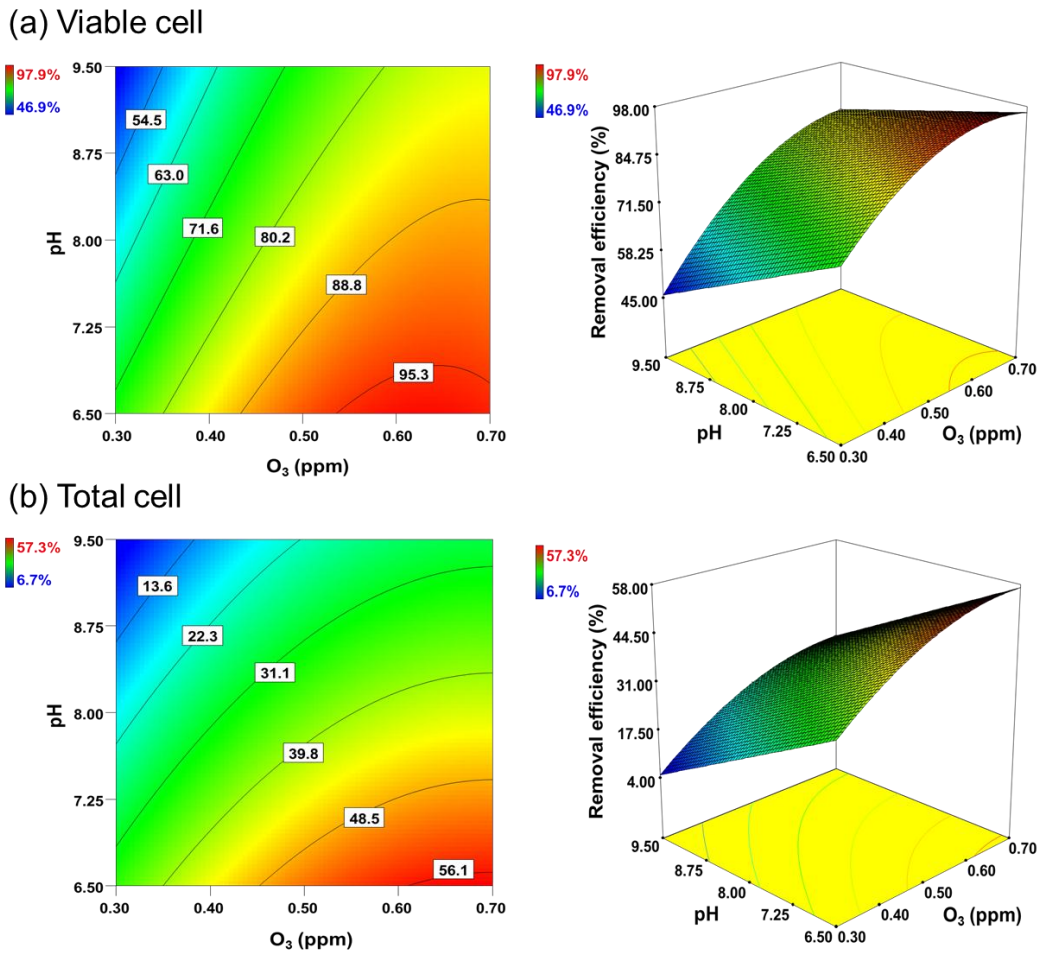

Figure 21. Effect of pH and O_3 dose on *C. polykrikoides* removal efficiency ((a) viable cell, (b) total cell)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.3, 0.5, 0.7 ppm; pH_i = 6.5. 8.0. 9.5; Reaction time = 600 sec

Table 5. Statistical significance of estimated response surface model coefficients (X_1: O_3, X_2: pH; Removal efficiency of *C. polykrikoides*)

Terms	Viable cell			Total cell		
	Coefficient	**F-value**	***p*-value**	**Coefficient**	**F-value**	***p*-value**
Intercept	83.55			37.09		
X_1	15.27	103.29	<0.0001	11.68	106.44	<0.0001
X_2	-10.02	44.43	0.0006	-14.48	163.58	<0.0001
X_1X_2	3.81	4.28	0.0841	0.28	0.041	0.8456
X_1^2	-8.59	14.85	0.0084	-5.75	11.73	0.0141
Model	***p*-value**	**R^2**	**Adequate precision**	***p*-value**	**R^2**	**Adequate precision**
Regression	0.0002	0.9653	20.380	<0.0001	0.9792	27.984
Lack-of-fit	0.5259			0.1650		

Two-dimensional contour and three-dimensional response surface plots are presented in Figure 22; the figure shows the results of ozonation experiments with seawater cultured *C. polykirkoides* at temperatures of 19, 24, and 29°C. With the decrease of temperature and increase of O_3 dose, the removal efficiency increased. The ANOVA of the solution temperature and O_3 dose showed that the results had an R^2 of above 0.98 (viable cell and total cell) (Table 6). The ANOVA for the lack of fit was not significant ($p > 0.05$) for the model. Moreover, the model terms showed significance ($p < 0.05$), indicating that the model could adequately fit the experiment data.

This result was primarily due to the increase of O_3 decomposition rate with increasing temperature. In other words, as temperature increases, O_3 causes more rapid decomposition, and then becomes less stable in water, leading to the consumption of O_3 and $^{\bullet}OH$. Thus, the *C. polykrikoides* removal efficiency at a low pH, low temperature, and high O_3 dose is increased with the increase in exposure to O_3.

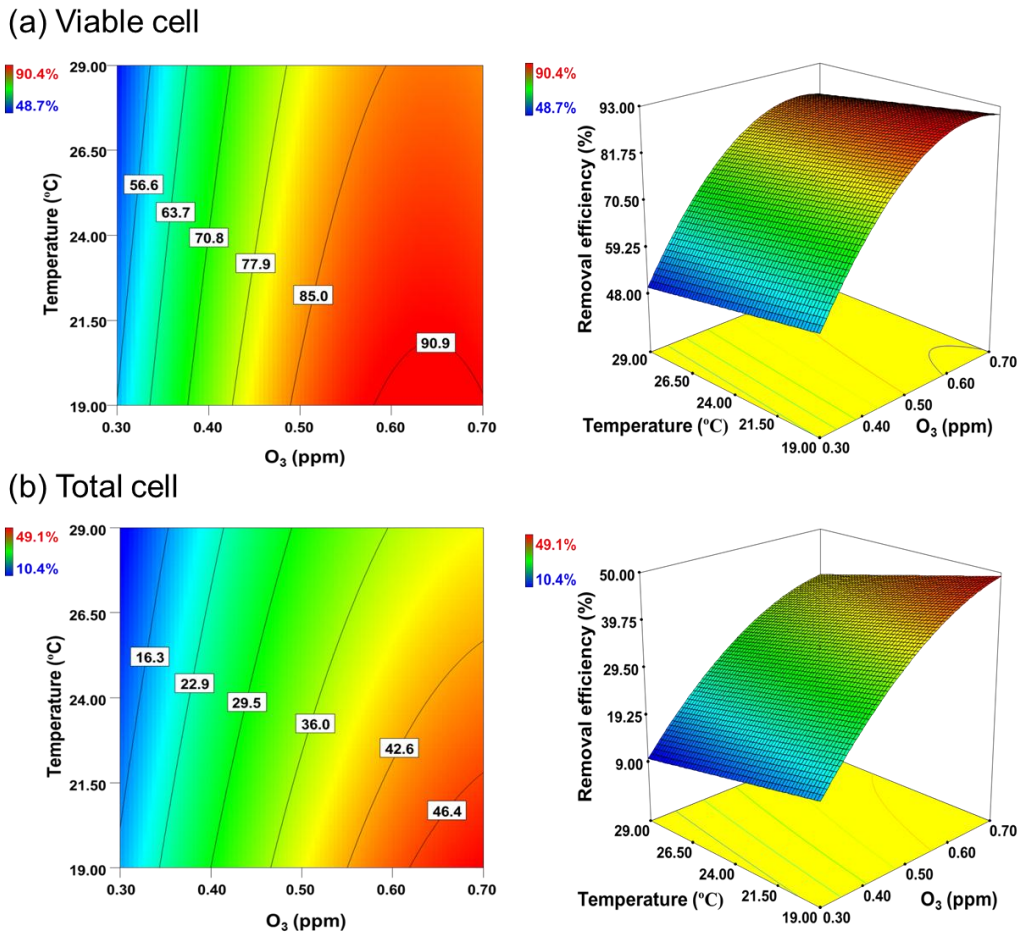

Figure 22. Effect of temperature and O_3 dose on *C. polykrikoides* removal efficiency ((a) viable cell, (b) total cell)

$[Cell]_0 = 1.0 \times 10^3$ cells/mL; $[O_3]_0$ = 0.3, 0.5, 0.7 ppm; Temp. = 19, 24, 29°C; Reaction time = 600 sec

Table 6. Statistical significance of estimated response surface model coefficients (X_1: O_3, X_2: pH; Removal efficiency of *C. polykrikoides*)

Terms	Viable cell			Total cell		
	Coefficient	**F-value**	***p*-value**	**Coefficient**	**F-value**	***p*-value**
Intercept	82.57			34.65		
X_1	17.45	229.80	<0.0001	15.42	247.74	<0.0001
X_2	-3.32	8.31	0.0345	-4.33	19.54	0.0069
X_1X_2	0.29	0.043	0.8436	-0.60	0.25	0.6370
X_1^2	-11.96	43.18	0.0012	-5.79	13.96	0.0135
Model	***p*-value**	**R^2**	**Adequate precision**	***p*-value**	**R^2**	**Adequate precision**
Regression	0.0001	0.9825	20.833	0.0001	0.9825	23.280
Lack-of-fit	0.9295			0.9390		

2.5. Formation of BrO_3^-

O_3 reacts with Br^- to create toxic byproducts such as BrO_3^-. The concentration of Br^- in seawater is determined to be 65 ppm, which is much higher than the Br^- concentration in fresh water. The BrO_3^- formation was examined during ozonation in water containing Br^-. Water samples were prepared by spiking each sample with DI water at a concentration of Br^- of 65 ppm. In addition, the pH of the solution was adjusted to 7.9 and the O_3 was injected into 100 mL Pyrex flasks at an O_3 dose from 0.5 to 5.0 ppm. Figure 23 shows the formation of BrO_3^- as a function of O_3 dose. The concentration of BrO_3^- increased with increasing O_3 dose. The concentration of BrO_3^- of 0.75 ppb was detected at an O_3 dose of 0.5 ppm and gradually increased as a function of O_3 dose. An O_3 dose of 5.0 ppm resulted in the formation of a BrO_3^- concentration of 278 ppb. The concentration of BrO_3^- can only be maintained at less than 10 ppb (WHO, 2011) at the O_3 dose of less than 1.0 ppm. Therefore, to determine the optimal O_3 dose, the formation of BrO_3^- needs to be minimized.

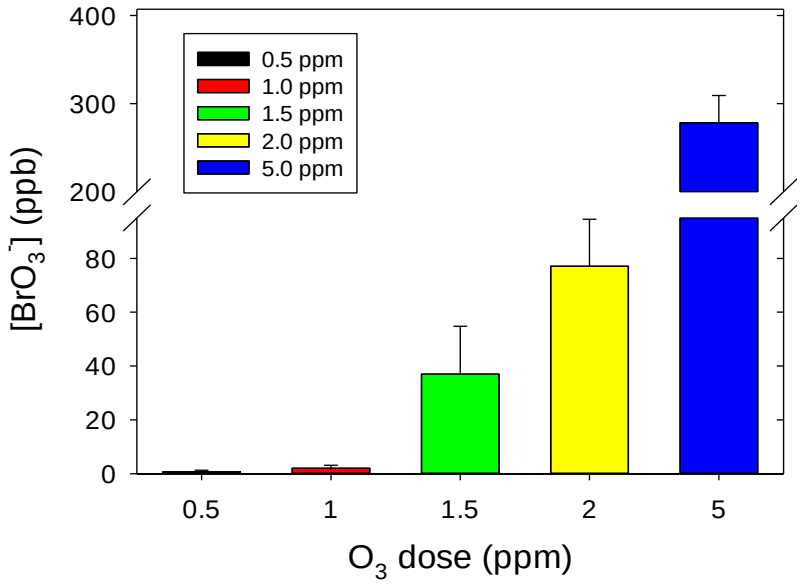

Figure 23. Formation of BrO_3^- as a function of the O_3 dose

$[Br^-]_0$ = 65 ppm; $[O_3]_0$ = 0.5, 1.0, 1.5, 2.0, 5.0 ppm; pH_i = 7.9; Reaction time = 180 min

2.6. Prediction model for full scale application of the O_3 system

In this study, the treatment efficiency of O_3 when introduced as a pretreatment process for intake seawater to inland fish farms is predicted on the basis of the results obtained from the removal of *C. polykrikoides* (Figure 24). The flow rate of the O_3 generator and the concentration of the O_3 influent gas are assumed to be 2 L/min and 830 mg/L, respectively. The tank has a capacity of 120 m^3 (W = 10 m; L = 10 m; H = 1.2 m), and the flow rate of seawater into the tank is 120 m^3/h, which is the control used for inland fish farms. In this process, the concentration of O_3 flowing through the intake pipe was calculated from the flow rate of seawater and the concentration of O_3 influent gas. In addition, considering that the hydraulic retention time (HRT) is the main factor in defining the intake pipe volume, the treatment efficiency of the O_3 process was examined based on the HRT with intake pipes of various lengths. For a given set of operating conditions, the applied O_3 concentration flowing through the intake pipe from the O_3 generator was 0.83 ppm and when the length of the intake pipe was 20 and 40 m, the HRT was calculated as 117.8 and 235.6 sec, respectively.

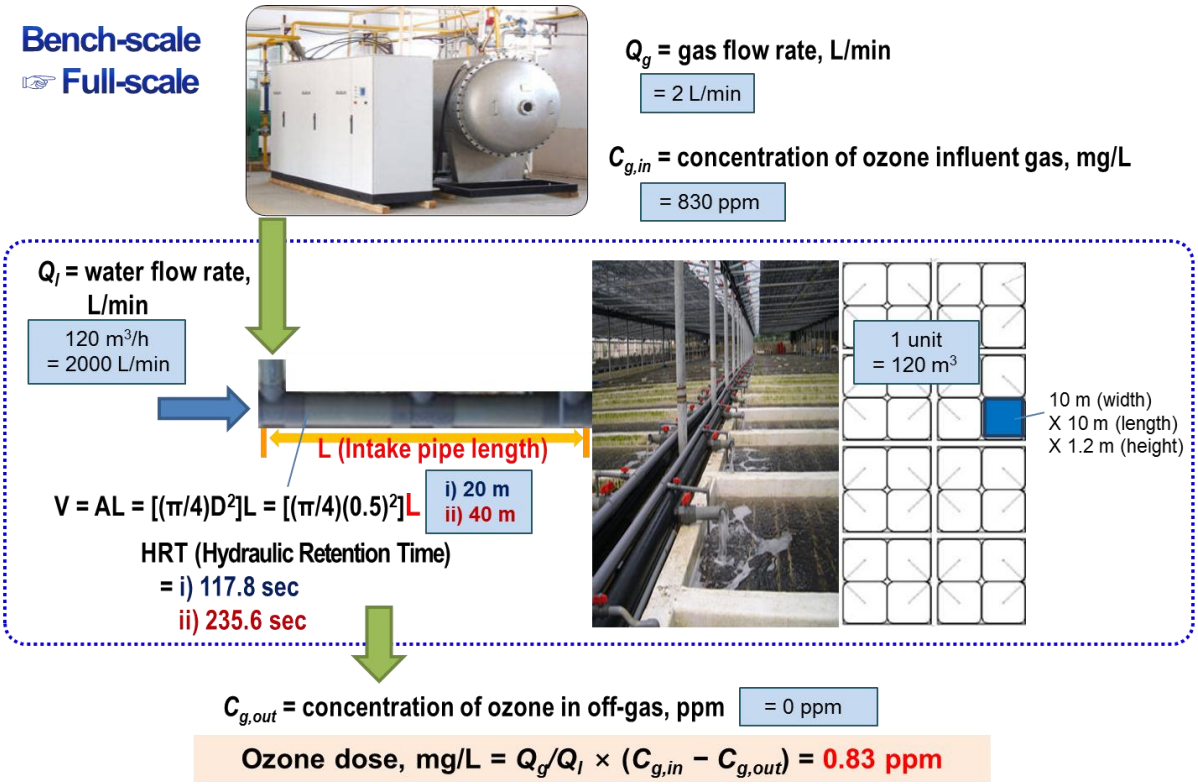

Figure 24. Prediction model for full scale application of the O_3 system

Figure 25 shows the prediction results of the removal efficiency of *C. polykrikoides* at the O_3 doses of 0.5, 1.0, and 1.5 ppm and the reaction times of 60, 180, and 300 sec by applying the response surface method (RSM). The removal efficiency of *C. polykrikoides* increased with increasing O_3 dose and longer reaction time (Figure 25). This result, obtained from a statistical modeling equation using RSM, demonstrates that the removal efficiency of *C. polykrikoides* can be examined based on the HRT and concentration of O_3 influent from the intake pipe to the tank of inland fish farms.

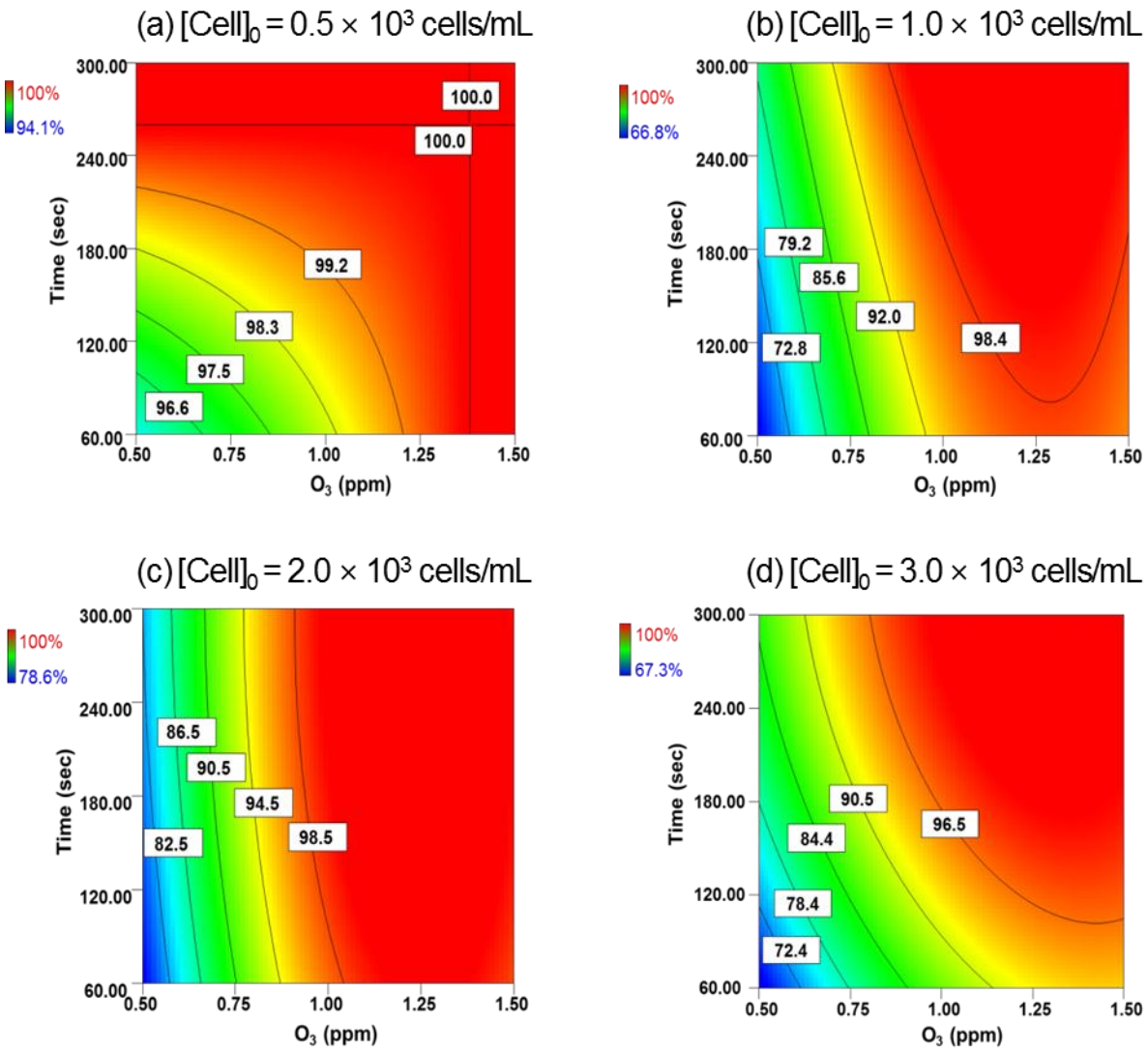

Figure 25. Effect of reaction time and O_3 dose on *C. polykrikoides* removal efficiency ((a) $[Cell]_0$ = 0.5 × 10^3 cells/mL, (b) 1.0 × 10^3 cells/mL (c) 2.0 × 10^3 cells/mL, (d) 3.0 × 10^3 cells/mL)

$[Cell]_0$ = 0.5 × 10^3, 1.0 × 10^3, 2.0 × 10^3, 3.0 × 10^3 cells/mL; $[O_3]_0$ = 0.5, 1.0, 1.5 ppm; reaction time = 60, 180, 300 sec

The results of RSM applied to the model equation could be used to evaluate the removal efficiency of *C. polykrikoides* according to the cell density. It should be noted that the forecasting of red tide blooming in Korea involves four steps: red tide appearance, red tide attention, red tide alert, and warning lift. The attention and alert notices are issued when the cell density of fish killing species (*C. polykrikoides*) exceeds 100 and 1,000 cells/mL, respectively. We studied the effect of the intake pipe length (L = 20, 40 m) and hydraulic retention time (HRT = 177.8, 235.6 sec) using the RSM and model equation at an applied O_3 dose of 0.83 ppm. The effect of HRT on the performance of the process was highly significant: The removal efficiency was found to be up to 99.9% and increased when the cell density decreased. In addition, the inhibitory effect was greater with increasing HRT; it was concluded from these results that a longer HRT is more efficient in achieving a high removal efficiency of the red tide dinoflagellate (*C. polykrikoides*) for the applied O_3 dose.

Table 7. Effect of O_3 dose and HRT on *C. polykrikoides* removal efficiency using statistic modeling

Red tide forecast	Red tide attention	Red tide alert		
Cell density (cells/mL)	0.5×10^3	1.0×10^3	2.0×10^3	3.0×10^3
Modeling f(t,[O_3]) / HRT (sec)	$91.28+6.38[O_3]+0.04t-0.03[O_3]t$	$10.31+130.32[O_3]+0.08t-0.04[O_3]t-49.48[O_3]^2$	$38.75+94[O_3]+0.04t-0.01[O_3]t-36.63[O_3]^2-5.3*10^{-5}t^2$	$22.13+86.24[O_3]+0.16t-0.02[O_3]t-28.06[O_3]^2-1.96*10^{-4}t^2-0.01[O_3]^2t$
177.8	98.4	89.9	94.5	94.5
235.6	99.9	95.4	96.0	95.6

Chapter 4. Conclusions

The first part of this study investigated the removal of *C. polykrikoides* by various oxidants including ozone (O_3), permanganate (MnO_4^-), chlorine (Cl_2) and hydrogen peroxide (H_2O_2) according to cell density and oxidant dose. The decay of total residual oxidants and formation of bromate (BrO_3^-) after oxidant treatment were examined. In addition, the 72-h LC_{50} value of various oxidants was determined for red sea bream. The focus of this study is on the use of chemical disinfectants (O_3, MnO_4^-, Cl_2, and H_2O_2) as oxidants for water treatment. O_3 showed a much higher efficiency for the *C. polykrikoides* removal than the other oxidants under identical experimental conditions. Disinfectants effectively inactivate the *C. polykrikoides* in the order of $O_3 > MnO_4^- > Cl_2 > H_2O_2$. The results show the potential of chemical disinfectants as alternative technologies to remove the red tide dinoflagellate. The seawater contains a high concentration of bromide ion (Br^-), which reacts with oxidants to form bromine (HOBr/OBr^-). The stable oxidants such as bromine are called total residual oxidants (TRO), and can contain chlorine, bromine, $^{\bullet}OH$, and so on. TRO produced from O_3 and Cl_2 mostly converted into HOBr due to the presence of Br^- in seawater. The depletion of TRO and production of HOBr as a function of oxidant dose were examined in the presence of *C. polykrikoides*. It was found that the seawater in the presence of *C. polykrikoides* decayed faster than in the absence of *C. polykrikoides*, indicating that TRO decay and HOBr production can be affected by the characteristics of water quality. In addition, the concentration of BrO_3^- increased with increasing oxidant dose. Ozonation results in a much higher formation of BrO_3^- than treatments with Cl_2, MnO_4^-, and H_2O_2. The acute toxicity of MnO_4^-, Cl_2, and H_2O_2 to juvenile red sea bream was reached at 72-h LC_{50} values of 0.39, 1.32, and 102.61 ppm, respectively. MnO_4^- concentrations lower than 1.0 ppm showed very strong toxicity to fish, whereas H_2O_2 showed less toxicity than other oxidants. The LC_{50} values of juvenile red sea bream by MnO_4^-, Cl_2, and H_2O_2 oxidants for 72-h were higher than the residual oxidant concentration by oxidant treatment.

Secondly, the control of *C. polykrikoides*, a red tide dinoflagellate by O_3 was examined. The aim of this study to evaluate the removal efficiency of *C. polykrikoides* by O_3 according to various factors affecting the efficiency such as cell density, oxidant dose, humic acid concentration, carbonate concentration, pH, and temperature. In addition, the formation of BrO_3^- after ozonation was examined. The removal efficiency of *C. polykrikoides* increased with increasing the dose of O_3. The *C. polykrikoides* cells were inactivated by 99% after 10 min exposure to an O_3 dose of more than 1.0 ppm, while most of the cells ruptured at a 5.0 ppm O_3 dose. The removal rate of cells with a high density was the slowest. The removal rate showed a constant increase as the O_3 dose increased, and then reduced with a higher density of *C. polykirkoides*. The O_3 showed the effective removal efficiency of *C. polykrikoides* with two distinct stages. The first stage of inactivation, which occurred

rapidly within 1 min reaction time, was mainly achieved by the direct oxidation of molecular O_3. The second stage, which occurred by HOBr reactions, showed a slower inactivation than that at the first stage. In other words, the removal of *C. polykrikoides* by O_3 was rapid, and appeared to be caused by initial ozonation, and then slow reaction, in which Br^- was oxidized to HOBr. Factors such as humic acid concentration, carbonate concentration, pH, and temperature affect the removal efficiency of *C. polykrikoides* by changing the O_3 exposure. Humic acid plays the role of a promoter of O_3 decomposition. The removal efficiency of *C. polykrikoides* decreased with increasing concentration of humic acid. This effect can be explained by the consumption of O_3 and $^{\bullet}OH$ by the humic acid. Also, carbonate acts as an inhibitor for the radical-type chain reaction that accelerates decomposition of the O_3 and the removal efficiency of *C. polykrikoides* increased with the increasing concentration of carbonate. The results indicate that the removal of *C. polykrikoides* by O_3 increased more with increasing O_3 exposure than by $^{\bullet}OH$. Low pH and temperature increased the removal efficiency. Thus, the *C. polykrikoides* removal efficiency with a low pH, and low temperature, and a high O_3 dose increased as the in exposure to O_3 increased. The concentration of BrO_3^- increased with increasing O_3 exposure. The BrO_3^- concentration of less than 10.0 ppb (WHO, 2011) can only be maintained at the O_3 dose of less than 1.0 ppm.

REFERENCES

Almendral, M. J., Alonso, A., & Fuentes, M. S. (2009). Development of new methodologies for on-line determination of the bromate ion in samples of water subjected to ozonation treatment. *Journal of Environmental Monitoring*, 11(7), 1381- 1388.

Bader, H., & Hoigné, J. (1981). Determination of ozone in water by the indigo method. *Water research*, 15(4), 449-456.

Bousher, A., Brimblecombe, P., & Midgley, D. (1986). Rate of hypobromite formation in chlorinated seawater. *Water Research*, 20(7), 865-870.

Cho, M., Kim, H., Cho, S. H., & Yoon, J. (2003). Investigation of ozone reaction in river waters causing instantaneous ozone demand. *Ozone Science & Engineering*, 25(4), 251-259.

Deeds, J. R., Terlizzi, D. E., Adolf, J. E., Stoecker, D. K., & Place, A. R. (2002). Toxic activity from cultures of *Karlodinium micrum* (= *Gyrodinium galatheanum*)(Dinophyceae)—a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. *Harmful Algae*, 1(2), 169-189.

Elovitz, M. S., von Gunten, U., & Kaiser, H. P. (2000). Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. *Ozone: science & engineering*, 22(2), 123-150.

Eisenberg, G. (1943). Colorimetric determination of hydrogen peroxide. *Industrial & Engineering Chemistry Analytical Edition*, 15(5), 327-328.

Farkas, L., Lewin, M., & Bloch, R. (1949). The reaction between hypochlorite and bromides. *Journal of the American Chemical Society*, 71(6), 1988-1991.

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In *Culture of marine invertebrate animals*, 29-60, Springer US

Guillard, R. R., & Ryther, J. H. (1962). Studied of marine planktonic diatoms: I. *Cyclotella nana* hustedt, and detonula confervacea (cleve) gran. *Canadian journal of microbiology*, 8(2), 229-239.

Haag, W. R., & Hoigne, J. (1983). Ozonation of bromide-containing waters: kinetics of formation of hypobromous acid and bromate. *Environmental Science & Technology*, 17(5), 261-267.

Honjo, T., Imada, N., Anraku, Y., Kim, D., Muramatsu, M., & Soshima, Y. (2002). Removal of harmful red tide plankton by ozone treatment. *Harmful Algae*, 525-527.

Juretić, H., Dobrović, S., Ružinski, N., Lovrić, J., Pećarević, M., Mikuš, J., Crnćević, M., Marćelja, E. J., Rajćić, M. M., Sirac, S., Cooper, W. J., Grewell, D., & van Leeuwen, J. (2011). Pilot studies of ozonation for Inactivation of Artemia salina Nauplii in Ballast Water. *Ozone: science & engineering*, 33(1), 3-13.

Kang, S. J., Lim, S. I., & Lee, B. H. (2001). The Removal of red - tide organisms by using microscreen and ozone. *Journal of Korea Technological Society of Water and Waste Water Treatement*, 9(4), 11-17.

Kenji, F., Tetsuya, S., Arita, M., Suzuki, S., Hara, A., Yamauchi, K., Shinriki, N., Ishizaki, K., & Takama, K. (1992). Acute toxicity of ozone against morphology of gill and erythrocytes of Japanese charr (Salvelinus leucomaenis). *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 101(2), 331-336.

Kim, D., Cha, S. –H., Niwano, Y., Kohno, M., Jiang, Z., Yamasaki, Y., Matsuyama, Y., Yamaguch, K. & Oda, T. (2010). Evaluation of the potential biological toxicities of aqueous extracts from red tide phytoplankton cultures in in *vitro* and in *vivo* systems. *The Journal of toxicological sciences*, 35(4), 591-599.

Lawani, S. A., & Sutter, J. R. (1973). Kinetic studies of permanganate oxidation reactions. IV. Reaction with bromide ion. *The Journal of Physical Chemistry*, 77(12), 1547-1551.

Lee, C., Yoon, J., & Von Gunten, U. (2007). Oxidative degradation of N-nitrosodimethylamine by conventional ozonation and the advanced oxidation process ozone/hydrogen peroxide. *Water Research*, 41(3), 581-590.

Lee, Y., & von Gunten, U. (2010). Oxidative transformation of micropollutants during municipal wastewater treatment: comparison of kinetic aspects of selective (chlorine, chlorine dioxide, ferrate VI, and ozone) and non-selective oxidants (hydroxyl radical). *Water Research*, 44(2), 555-566.

Letterman, R. D., & American Water Works Association. (1999). *Water quality and treatment*. McGraw-Hill Professional.

Meyer, J. S., Santore, R. C., Bobbitt, J. P., DeBrey, L. D., Boese, C. J., Paquin, P. R., Allen, H. E., Bergman, H. L., & DiToro, D. M. (1999). Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environmental science & technology*, 33(6), 913-916.

Mochida, K., Ito, K., Harino, H., Kakuno, A., & Fujii, K. (Reise2006). Acute toxicity of pyrithione antifouling biocides and joint toxicity with copper to red sea bream (Pagrus major) and toy shrimp (Heptacarpus futilirostris). *Environmental Toxicology and Chemistry*, 25(11), 3058-3064.

Myer, R. H., & Montgomery, D. C. (2002). Response surface methodology: process and product optimization using designed experiment. *John Wiley and Sons, New York*, 343-350.

Owen, D. M., Amy, G. L., Chowdhury, Z. K., Paode, R., McCoy, G., & Viscosil, K. (1995). NOM: characterization and treatability: Natural organic matter. *Journal-American Water Works Association*, 87(1), 46-63.

Park, K. H., Kim, S. R., Kang, S. Y., Jung, S. J., & Oh, M. J. (2008). Toxicity of Disinfectants in Flounder *Paralichthys olivaceus*, Black Rockfish *Sebastes pachycephalus* and Black Sea Bream *Acanthopagrus schlegelii*. *Journal of Aquaculture*, *21*(1), 7-12

Park, T. G., Lim, W. A., Park, Y. T., Lee, C. K., & Jeong, H. J. (2013). Economic impact, management and mitigation of red tides in Korea. *Harmful algae*, 30, S131-S143.

Perrins, J. C., Cooper, W. J., Van Leeuwen, J. H., & Herwig, R. P. (2006). Ozonation of seawater from different locations: formation and decay of total residual oxidant—implications for ballast water treatment. *Marine pollution bulletin*, 52(9), 1023-1033.

Reiser, S., Schroeder, J. P., Wuertz, S., Kloas, W., & Hanel, R. (2010). Histological and physiological alterations in juvenile turbot (Psetta maxima, L.) exposed to sublethal concentrations of ozone-produced oxidants in ozonated seawater. *Aquaculture*, 307(1), 157-164.

Ryu, H. Y., Shim, J. M., Bang, J. D., & Lee, C. (1988). Experimental chemical treatments for the control of dinoflagellate *Cochlodinium polykrikoides* in the land-based culture of olive flounder *Paralichthys olivaceus*. *The Korean Society of Fisheries and Aquatic Science*, 11(3), 285-294.

Schneider, K. R., Pierce, R. H., & Rodrick, G. E. (2003). The degradation of Karenia brevis toxins utilizing ozonated seawater. *Harmful Algae*, 2(2), 101-107.

Staehelin, J., & Hoigné, J. (1985). Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environmental Science & Technology*, 19(12), 1206-1213.

Tango, M. S., & Gagnon, G. A. (2003). Impact of ozonation on water quality in marine recirculation systems. *Aquacultural Engineering*, 29(3), 125-137.

Viswanathan, K., & Tilak, B. V. (1984). Chemical, electrochemical, and technological aspects of sodium chlorate manufacture. *Journal of The Electrochemical Society*, 131(7), 1551-1559.

von Gunten, U. (2003). Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water research*, 37(7), 1443-1467.

von Gunten, U., & Hoigne, J. (1994). Bromate formation during ozonization of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. *Environmental Science & Technology*, 28(7), 1234-1242.

WHO, G. (2011). Guidelines for drinking-water quality. *World Health Organization*.

Wagner, H. P., Pepich, B. V., Hautman, D. P., & Munch, D. J. (2002). US Environmental Protection Agency Method 326.0, a new method for monitoring inorganic oxyhalides and optimization of the postcolumn derivatization for the selective determination of trace levels of bromate. *Journal of Chromatography A*, 956(1), 93-101.

Yang, Z., Jiang, W., Zhang, Y., & Lim, T. M. (2015). Inactivation of dinoflagellate *Scripsiella trochoidea* in synthetic ballast water by advanced oxidation processes. *Environmental technology*, 36(6), 750-759.

Zhang, H., Yang, W., Gao, J., & Liu, J. (2003). Inhibition and elimination of chlorine dioxide on Phaeocystis globosa. *Ying yong sheng tai xue bao= The journal of applied ecology/Zhongguo sheng tai xue xue hui, Zhongguo ke xue yuan Shenyang ying yong sheng tai yan jiu suo zhu ban*, 14(7), 1173-1176.

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지난 3 년 동안 연구원과 석사과정을 함께 생활한 우리 고도산화 환경기술 연구실(ART) 멤버들, 깊이 있는 연구를 할 수 있도록 조언해주신 이홍신 박사님, 편안하면서도 카리스마 있는 형은언니, 대학원 생활 동안 많은 도움을 주고 늘 응원해준 나의 사수 혜진언니, 믿음직하고 듬직한 도희 아버지 민식, 똑똑하고 배려심 많은 지원, 풍부한 지식을 가진 매력덩어리 학현, 긍정적이며 늘 밝은 모습을 보여준 준영, 4 차원 귀염둥이 기명, 개성있고 씩씩한 태완, 모두 감사합니다. 그리고 함께 대학원 생활하며 서로를 응원해준 민규, 성현, 병문, 학부생 형진, 재민, 원표, 지혜 감사합니다.

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HARD COVER