Evaluation of algaecide effectiveness of five different oxidants applied on harmful phytoplankton

Javier Moreno-Andrés[1§*], Leonardo Romero-Martínez[1§], Sergio Seoane[2,3], Asunción Acevedo-Merino[1], Ignacio Moreno-Garrido[4] and Enrique Nebot[1]

[1] Department of Environmental Technologies, Faculty of Marine and Environmental Sciences. INMAR - Marine Research Institute, CEIMAR - International Campus of Excellence of the Sea. University of Cadiz. Spain
[2] Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country UPV/EHU, Leioa, 48940, Spain.
[3] Research Centre for Experimental Marine Biology and Biotechnology (Plentzia Marine Station, PiE- UPV/EHU), Plentzia, 48620, Spain.
[4] Institute of Marine Sciences of Andalusia (CSIC), Campus Río San Pedro, s/n 11510, Puerto Real, Cádiz, Spain.

Supplementary Material

Table S.1. Main characteristics for each chemical investigated in this study. *(V vs. SHE; pH 7)

Chemical Reagent	Redox potential, E^{0*}	Life-time	Possible major action mechanism	Target Concentration	Main References
H_2O_2	1.8	Hours to days	*Extracellular:* Passive diffusion trough cell membrane porins. Lipid peroxidation. *Intracellular:* Inhibition of peroxidase activity, metabolic activity, and photosynthesis. Internal Fenton process	0 – 10 mg·L^{-1} (0-0.29 mM)	[1–4]
$C_2H_4O_3$ (PAA)	1.4	Minutes to hours	*Extracellular:* PAA easily adsorb onto cell membrane (alkyl moiety which provides hydrophobic characteristics). Passive diffusion and oxidize sulfhydryl (−SH) and sulfur bonds (S−S). Damage on outer membrane lipoproteins. Lipid peroxidation. *Intracellular:* Inhibition of catalases, denaturalization of DNA. Internal Fenton process.	0 – 2 mg·L^{-1} (0-0.026 mM)	[1,5–8]
$S_2O_8^{2-}$ (PDS)	2.1	Days to months	*Extracellular:* Cell membrane	0 – 192.13 mg·L^{-1} (0-1 mM)	[1,9]
HSO_5^- (PMS)	1.82	Hours	*Extracellular:* Changes in the proteins in cell membranes. Fast reaction with dissolved anions	0 – 2 mg·L^{-1} (0-0.018 mM)	[10]
NaClO	1.4	Hours to days	*Extracellular:* Disrupt cell membrane integrity *Intracellular:* chlorophyll autofluorescence, intracellular esterase activity, primary productivity	0 – 4 mg·L^{-1} (0-0.05 mM)	[1,11,12]

Supplementary Material. *Evaluation of algaecide effectiveness of five different oxidants applied on harmful phytoplankton*

Table S.2. Growth rates calculated for both *P. parvum* and *H. akashiwo* upon exposure to different concentration of reagents tested (H_2O_2, PAA, PMS, PDS, NaClO). The growth rate has been calculated during the exponential growth of each microalgae, which, in general, has been up to day 14: *P. parvum* and day 9: *H. akashiwo*.

Reagent	**Species**	**Growth rate μ (d^{-1}) ± S.D.**				
		Control	**2.5 mg/L**	**5 mg/L**	**7.5 mg/L**	**10 mg/L**
H_2O_2	*P. parvum*	0.25 ± 0.02	0.28± 0.01	0.20± 0.03	0.15± 0.02	-0.06± 0.04
	H. akashiwo	0.18 ± 0.01	0.18± 0.01	0.18± 0.01	0.12± 0.02	-0.15± 0.01
		Control	**0.1 mg/L**	**0.5 mg/L**	**1 mg/L**	**2 mg/L**
PAA	*P. parvum*	0.27 ± 0.01	0.26± 0.01	-0.10± 0.01	-0.07± 0.01	--
	H. akashiwo	0.16 ± 0.01	0.13± 0.02	0.08± 0.03	0.06± 0.05	0.06± 0.01
		Control	**0.1 mg/L**	**0.5 mg/L**	**1 mg/L**	**2 mg/L**
PMS	*P. parvum*	0.26 ± 0.01	0.25± 0.02	0.20± 0.03	-0.12± 0.05	-0.08± 0.10
	H. akashiwo	0.18 ± 0.01	0.17± 0.01	0.22± 0.03	0.18± 0.01	0.12± 0.02
		Control	**19.21 mg/L**	**48 mg/L**	**144 mg/L**	**192 mg/L**
PDS	*P. parvum*	0.25 ± 0.01	0.27± 0.01	0.25± 0.01	0.25± 0.01	0.25± 0.01
	H. akashiwo	0.15 ± 0.01	0.12± 0.02	0.14± 0.01	0.13± 0.02	0.12± 0.01
		Control	**0.5 mg/L**	**1 mg/L**	**2 mg/L**	**4 mg/L**
NaClO	*P. parvum*	0.28 ± 0.01	0.26± 0.01	0.25± 0.01	0.01± 0.02	--
	H. akashiwo	0.18 ± 0.01	0.18± 0.01	0.17± 0.01	0.16± 0.02	0.13± 0.01

Supplementary Material. *Evaluation of algaecide effectiveness of five different oxidants applied on harmful phytoplankton*

Table S.3a. Fitted parameters obtained from modelling the concentration-response curves represented on Figures 1-5. Each model parameter has been obtained from Eq. 3 and for each reagent (H_2O_2, PAA, PMS, NaClO) at specific incubation days (from day 2 to day 14) and for both target microalgae: *Prymnesium parvum* (Table S3a) and *Heterosigma akashiwo* (Table S3b).

Species	Reagent	Integration time (d)	f ± SE (p-value)	x_0 ± SE (p-value) (ppm)	b ± SE (p-value)	R^2
P. parvum	H_2O_2	4	0.00 ± 0.47 (1.000)	12.82 ± 13.29 (0.367)	0.99 ± 2.67 (0.722)	0.737
		7	0.00 ± 0.26 (1.000)	7.09 ± 1.27 (0.001)	1.72 ± 0.66 (0.035)	0.768
		9	0.00 ± 0.12 (1.000)	6.71 ± 0.83 (0.000)	2.46 ± 1.18 (0.075)	0.830
		11	0.00 ± 0.04 (1.000)	7.12 ± 0.43 (0.000)	4.01 ± 1.28 (0.016)	0.921
		14	0.00 ± 0.04 (1.000)	6.45 ± 0.36 (0.000)	3.52 ± 0.80 (0.003)	0.958
P. parvum	PAA	4	1.56 ± 2.88 (0.604)	0.34 ± 0.08 (0.004)	1.93 ± 0.40 (0.002)	0.948
		7	0.31 ± 1.16 (0.795)	0.32 ± 0.27 (0.281)	4.20 ± 7.69 (0.602)	0.967
		9	0.02 ± 0.72 (0.981)	0.32 ± 0.63 (0.625)	5.40 ± 23.75 (0.827)	0.985
		11	0.46 ± 0.50 (0.386)	0.33 ± 1.67 (0.848)	6.90 ± 82.40 (0.936)	0.991
		14	0.50 ± 0.31 (0.156)	0.34 ± 6.94 (0.963)	8.75 ± 443.96 (0.985)	0.996
P. parvum	PMS	4	3.22 ± 1.72 (0.103)	0.51 ± 0.05 (0.000)	2.11 ± 0.29 (0.000)	0.975
		7	0.97 ± 1.27 (0.469)	0.40 ± 0.05 (0.000)	2.16 ± 0.38 (0.001)	0.977
		9	0.01 ± 0.64 (0.988)	0.40 ± 0.04 (0.000)	2.34 ± 0.52 (0.003)	0.983
		11	0.00 ± 0.56 (1.000)	0.39 ± 0.04 (0.000)	2.50 ± 0.60 (0.004)	0.985
		14	0.00 ± 0.59 (1.000)	0.44 ± 0.04 (0.000)	3.86 ± 2.06 (0.103)	0.975
P. parvum	NaClO	4	0.38 ± 0.20 (0.104)	1.47 ± 0.09 (0.000)	3.18 ± 0.52 (0.001)	0.957
		7	0.21 ± 0.15 (0.217)	1.28 ± 0.07 (0.000)	3.78 ± 0.66 (0.001)	0.970
		9	0.00 ± 0.11 (1.000)	1.23 ± 0.06 (0.000)	4.35 ± 0.79 (0.001)	0.977
		11	0.00 ± 0.14 (1.000)	1.17 ± 0.06 (0.000)	4.30 ± 1.02 (0.004)	0.970
		14	0.00 ± 0.21 (1.000)	1.10 ± 0.08 (0.000)	3.64 ± 1.07 (0.011)	0.957

Supplementary Material. *Evaluation of algaecide effectiveness of five different oxidants applied on harmful phytoplankton*

Table S.3b. Fitted parameters obtained from modelling the concentration-response curves represented on Figures 1-5. Each model parameter has been obtained from Eq. 3 and for each reagent (H_2O_2, PAA, PMS, NaClO) at specific incubation days (from day 2 to day 14) and for both target microalgae: *Prymnesium parvum* (Table S3a) and *Heterosigma akashiwo* (Table S3b).

Species	Reagent	Integration time (d)	f ± SE (p-value)	x_0 ± SE (p-value) (ppm)	b ± SE (p-value)	R^2
H. akashiwo	H_2O_2	4	0.00 ± 0.01 (1.000)	6.82 ± 0.13 (0.000)	4.64 ± 0.45 (0.000)	0.993
		7	0.00 ± 0.01 (1.000)	6.67 ± 0.15 (0.000)	5.86 ± 0.77 (0.000)	0.990
		9	0.00 ± 0.01 (1.000)	6.81 ± 0.16 (0.000)	6.11 ± 0.98 (0.000)	0.989
		11	0.00 ± 0.01 (1.000)	6.96 ± 0.17 (0.000)	6.17 ± 1.10 (0.001)	0.986
		14	0.00 ± 0.01 (1.000)	7.19 ± 0.20 (0.000)	6.18 ± 1.31 (0.002)	0.979
H. akashiwo	PAA	4	0.92 ± 0.34 (0.031)	2.93 ± 0.42 (0.000)	2.15 ± 0.35 (0.000)	0.895
		7	0.51 ± 0.28 (0.112)	2.70 ± 0.35 (0.000)	2.16 ± 0.45 (0.002)	0.902
		9	0.43 ± 0.31 (0.203)	2.71 ± 0.38 (0.000)	2.03 ± 0.44 (0.003)	0.906
		11	0.27 ± 0.25 (0.317)	2.88 ± 0.45 (0.000)	2.00 ± 0.53 (0.007)	0.918
		14	0.88 ± 0.54 (0.147)	5.64 ± 1.99 (0.025)	1.43 ± 0.14 (0.000)	0.918
H. akashiwo	PMS	4	0.00 ± 0.00 (0.295)	1.95 ± 0.31 (0.002)	1.79 ± 1.15 (0.181)	0.835
		7	0.00 ± 0.01 (1.000)	1.99 ± 0.11 (0.000)	9.65 ± 0.84 (0.763)	0.803
		9	0.00 ± 0.15 (1.000)	2.01 ± 0.10 (0.000)	6.05 ± 16.26 (0.721)	0.795
		11	0.00 ± 0.13 (1.000)	2.10 ± 1.00 (0.075)	7.87 ± 81.95 (0.926)	0.852
		14	0.00 ± 0.12 (1.000)	2.17 ± 2.74 (0.455)	8.62 ± 139.20 (0.952)	0.891
H. akashiwo	NaClO	4	0.01 ± 0.04 (0.879)	3.85 ± 0.36 (0.000)	7.87 ± 19.44 (0.698)	0.960
		7	0.07 ± 0.06 (0.316)	3.20 ± 0.21 (0.000)	4.15 ± 0.94 (0.003)	0.945
		9	0.10 ± 0.06 (0.143)	3.13 ± 0.17 (0.000)	3.96 ± 0.68 (0.001)	0.958
		11	0.07 ± 0.05 (0.224)	3.23 ± 0.16 (0.000)	3.81 ± 0.65 (0.001)	0.967
		14	0.05 ± 0.04 (0.281)	3.48 ± 0.13 (0.000)	3.43 ± 0.56 (0.001)	0.977

Supplementary Material. *Evaluation of algaecide effectiveness of five different oxidants applied on harmful phytoplankton*

References:

[1] G. Farinelli, M. Giagnorio, F. Ricceri, S. Giannakis, A. Tiraferri, Evaluation of the Effectiveness, Safety, and Feasibility of 9 Potential Biocides to Disinfect Acidic Landfill Leachate from Algae and Bacteria, Water Res. 191 (2021) 116801. https://doi.org/10.1016/j.watres.2020.116801.

[2] H. Zhang, R. Zong, H. He, T. Huang, Effects of hydrogen peroxide on Scenedesmus obliquus: Cell growth, antioxidant enzyme activity and intracellular protein fingerprinting, Chemosphere. 287 (2022) 132185. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132185.

[3] E. Arvin, L.F. Pedersen, Hydrogen peroxide decomposition kinetics in aquaculture water, Aquac. Eng. 64 (2015) 1–7. https://doi.org/10.1016/j.aquaeng.2014.12.004.

[4] M. Drábková, H.C.P. Matthijs, W. Admiraal, B. Maršálek, Selective effects of H2O2 on cyanobacterial photosynthesis, Photosynthetica. 45 (2007) 363–369. https://doi.org/10.1007/s11099-007-0062-9.

[5] X. Ao, J. Eloranta, C.H. Huang, D. Santoro, W. jun Sun, Z. dong Lu, C. Li, Peracetic acid-based advanced oxidation processes for decontamination and disinfection of water: A review, Water Res. 188 (2021) 116479. https://doi.org/10.1016/j.watres.2020.116479.

[6] L. Pedersen, C. Lazado, Decay of peracetic acid in seawater and implications for its chemotherapeutic potential in aquaculture, Aquac. Environ. Interact. 12 (2020) 153–165. https://doi.org/10.3354/aei00354.

[7] M. Kitis, Disinfection of wastewater with peracetic acid: a review, Environ. Int. 30 (2004) 47–55. https://doi.org/10.1016/S0160-4120(03)00147-8.

[8] P. Sun, T. Zhang, B. Mejia-Tickner, R. Zhang, M. Cai, C.H. Huang, Rapid Disinfection by Peracetic Acid Combined with UV Irradiation, Environ. Sci. Technol. Lett. 5 (2018) 400–404. https://doi.org/10.1021/acs.estlett.8b00249.

[9] S. Ahn, T.D. Peterson, J. Righter, D.M. Miles, P.G. Tratnyek, Disinfection of ballast water with iron activated persulfate, Environ. Sci. Technol. 47 (2013) 11717–11725. https://doi.org/10.1021/es402508k.

[10] Z. Chen, J. Li, M. Chen, K.Y. Koh, Z. Du, K.Y.H. Gin, Y. He, C.N. Ong, J.P. Chen, Microcystis aeruginosa removal by peroxides of hydrogen peroxide, peroxymonosulfate and peroxydisulfate without additional activators, Water Res. 201 (2021) 117263. https://doi.org/10.1016/J.WATRES.2021.117263.

[11] X. Li, S. Chen, J. Zeng, K. Chabi, W. Song, X. Xian, X. Yu, Impact of chlorination on cell activation, toxin release and degradation of cyanobacteria of development and maintenance stage, Chem. Eng. J. 397 (2020) 125378. https://doi.org/10.1016/j.cej.2020.125378.

[12] V. Ebenezer, Y.V. Nancharaiah, V.P. Venugopalan, Chlorination-induced cellular damage and recovery in marine microalga, Chlorella salina, Chemosphere. 89 (2012) 1042–1047. https://doi.org/10.1016/J.CHEMOSPHERE.2012.05.067.