Algicidal effects of Bioionix's electro-chemical technology on *Alexandrium catenella*: laboratory and field experiments

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Introduction

The development of salmon farming in the southern regions of Chile has made it necessary to transport salmon in wellboats, from farming areas to processing plants. One of the problems with this means of transport is the transfer of vegetative cells of *Alexandrium catenella* from areas of greater risk, especially in spring and summer which is the period of greatest occurrence of Harmful Algal Bloom (HAB). The main objective of this work was to evaluate the effect of Bioionix's electro-chemical technology on the vegetative cell of *A. catenella* viability.

Materials and Methods

Laboratory: In June 2016 an experiment was carried out under controlled conditions (see experimental design in Fig.1), where *A. catenella* (2000 cells mL^{-1}) was subjected to four doses: 0.13, 0.33, 0.79, 1.59 Ampere L^{-1}min^{-1}, using Bioionix electrochemical technology (Fig.2). **Field**: In February 2018, given the presence of a bloom of *A. catenella* in the region of Aysén, Chile; an experiment was carried out to test three doses: 0.79, 1.59 and 3.18 A L^{-1} min^{-1}, under field conditions, in Seno Canalad (44° 35' 26''S, 73° 18' 51''W). To carry out the test, a Bioionix electrochemical reactor was installed on the Patagon IV wellboat (Figs. 3 and 4) from the company Patagonia Wellboat. Once the dose was established in the reactor, seawater with *A. catenella* cells was passed through the reactor, samples were collected before (control) and after the reactor (treatment) (Figs 4, C and D) . Five replications were made for each treatment, and for each of them, 10 liters of water were filtered with 85-65-20 µm mesh size sieves. The fraction retained in 20 µm mesh size sieve placed in Erlenmeyer flask, which volume was completed to 100 mL with L1 culture medium. Samples were transported to the Lab to evaluated morphological changes and growth rates using Sedgewick-rafter chamber and inverted microscope during 11 days. All the samples were observed in vivo, which allowed us to identify morphological changes such as the formation of temporary cysts, cellular mobility, levels of cellular pigmentation, integrity of the cell wall, and discoloration levels.

Fig.1. Experimental Design

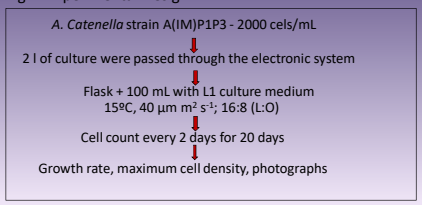


Fig.2. Continuous Catalytic Electrochemical Reactor System Bioionix 6500, June 2016

Fig.3. Patagon IV wellboat from the company Patagonia Wellboat

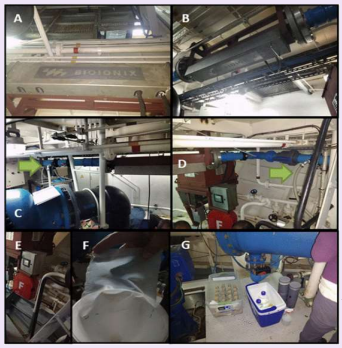


Fig.4. (A and B) Electrochemical reactor installed in wellboat. (C and D) The green arrow shows the hoses from where the samples were taken, before (control) and after (treatment) passing through the reactor. (E) Electrochemical reactor programming module. (F) 20 µm filter with retained *A. catenella* cells. (G) Flasks with samples of *A. catenella.*

Results

Laboratory :

After 21 days of culture of *A. catenella*, the growth rates were negative for the doses of 0.79 and 1.59 A L^{-1}min^{-1} (Figs. 5 and 6).

Field:

On the third day, after conducting the experiment in Aysén, a morphological evaluation and determination of the density of *A. catenella* was made. In control samples normal vegetative cells with integrated cell wall, brown-green pigmentation, and cell movement were observed. In addition to this species, the presence of the *Dinophysis acuta*, *Protoceratium reticulatum*, *Polykrikos sp.*, *Protoperidinium sp.*, and *Thalassiosira sp*. was observed. The dinoflagellates and diatoms presented normal morphology, ie, integrated cell walls and characteristic pigmentation (Fig.7), while the dinoflagellates also showed specific movement. In lower dose (0.79 A L^{-1}min^{-1}), a pigmentation gradient of *A. catenella cells* was observed, which varied from the presence of empty theca and discolored cells, to the presence of cells normal. For the dose of 1.59 A L^{-1}min^{-1} an increase in the presence of empty theca cells and discoloration of *A. catenella* cells was observed, although the presence of normal cells was also observed. Regarding the other species of dinoflagellates and diatoms, these generally showed discolored cells(Fig.8) . Finally, at the highest dose of 3.18 A L^{-1}min^{-1}, evident effects on the cells of *A. catenella* were observed, showing a severe discoloration and, in many cases, only empty theca were observed (Fig.9).

On the ninth day of culture, the decrease in the density of *A. catenella* was evident, both in the control and in the dose of 0.79 and 1.59 A L^{-1}min^{-1} . Regarding the control, normal cells were observed in the process of encysting, and others showed the formation of an intracellular sphere (Fig. 10). Additionally, the appearance of grayish-white structures that had not been previously identified was observed. These same characteristics were repeated in the samples of the doses of 0.79 and 1.59 A L^{-1} min^{-1}. For the 3.18 A L^{-1} min^{-1} dose, the presence of empty *A. catenella* thecae were observed as well as for the other dinoflagellates and diatoms present in the flasks.

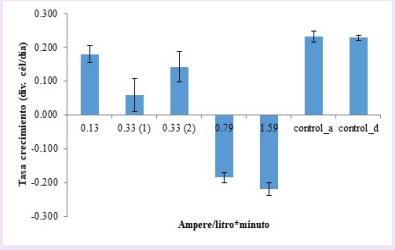


Fig.5. Growth rate (div. day^{-1})

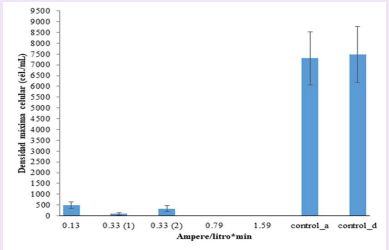
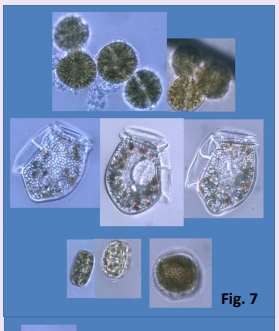
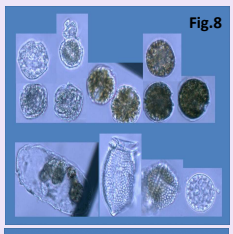
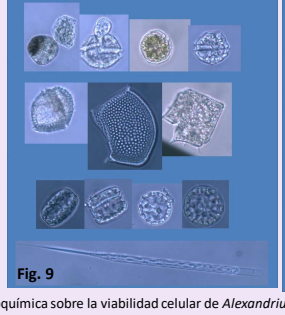
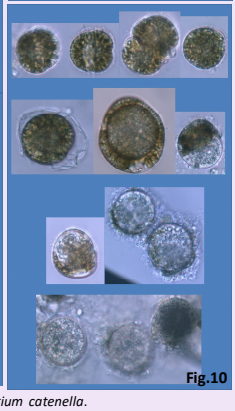


Fig.6. Maximum cell density (cels/mL)









Conclusions

- Results of lab tests suggests that intensities 0.79 and 1.59 A L^{-1}min^{-1} are capable of producing non-viable cells of *A. catenella*, ie, cell mortality.
- The consistent presence of empty thecae of *A. catenella* cells in the culture flasks from field tests, throughout all the samplings, and of empty thecae of other dinoflagellates and diatoms, added to the significant effect of the doses on the morphology, show that the dose of 3.18 A L^{-1}min^{-1} had a negative effect on the viability of the entire population of vegetative cells of *A. catenella* (and other phytoplankton species) that passed through the electrochemical reactor.
- Despite the dose of 0.79 A L^{-1}min^{-1} generated a high mortality of vegetative cells, and that these were affected by parasitism (Alacid et al., 2016), on the 21st day vegetative cells were observed in some flasks, which shows that the dose didn't generate the mortality of the entire vegetative population of *A. catenella* that passed through the electrochemical reactor.

References

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Acknowledgments

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