Removal of epiphytes of the kelp *Macrocystis pyrifera* (L.) Agardh using different biocides

Remoción de epifitas de *Macrocystis pyrifera* (L.) Agardh con diferentes biocidas

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ABSTRACT

The aim of this work was to evaluate the effectiveness of some biocides for the removal of epiphytes from the blades of the kelp *Macrocystis pyrifera*. The lowest initial removal was with 1% $KMnO_4$ and with the water purifier Fit®, but both gave better results in the long term. The treatments with distilled and tap water, which avoid the use of biocides, gave a better than 75% reduction of the epiphytes and had a long-lasting effect. In addition, the photosynthetic activity of the controls was similar to that of these two treatments. This confirms that a simple immersion in freshwater may achieve a good initial removal of epiphytic diatoms and prevent their subsequent growth.

Keywords: Epiphytes removal, *Macrocystis pyrifera, Navicula incerta.*

RESUMEN

En este trabajo se determinó la mejor eficacia de diferentes biocidas para la eliminación de epifitas de las frondas de *Macrocystis pyrifera*. La menor remoción inicial fue con 1% $KMnO_4$ y con el purificador de agua Fit®, pero ambos tuvieron el mejor efecto a largo plazo. Los tratamientos con agua destilada y agua de uso doméstico, que evitan el uso de biocidas, dieron una reducción inicial superior al 75% y su efecto fue a largo plazo. La actividad fotosintética de la macroalga después de estos tratamientos resultó similar a la del tratamiento control. Estos resultados confirman que una simple inmersión en agua dulce puede lograr una buena remoción de diatomeas epifitas y prevenir su crecimiento después del tratamiento.

Palabras clave: Remoción de epifitas, *Macrocystis pyrifera, Navicula incerta*.

INTRODUCTION

The giant kelp *Macrocystis pyrifera* ranges from Alaska to Baja California, Mexico, and grows from outside the surf area to depths of 50 meters. Its young blades have been harvested and processed for alginate production for the food industry as well as for medical applications since 1910, with an estimated worldwide generated income of close to 250 million US $ (Vásquez, 1999).

In more recent times it has also been cultured and used as food for several grazers such as abalone, sea urchins, and sea cucumbers (Hahn, 1989; Fallu, 1991) or as a supplement for animal diets, mainly in the aquaculture industry (Cruz-Suárez *et al*., 2000; Plana *et al*., 2007).

However, in our studies on its dietary value for abalone it became evident that, because of the high specific richness of its epiphytic diatoms, such as *Cocconeis* cf. *britannica* Naegeli ex Kützing, *C. speciosa* Gregory, *Gonphonemopsis pseudoexigua* (Simon-Sen) Medlin, *Climacosphenia moniligera* Ehrenberg, *and Navicula* spp. (Siqueiros-Beltrones *et al*., 2002), it was necessary to separate the relative contribution of kelp and epiphytes to abalone diets (Siqueiros-Beltrones *et al.,* 2005).

The information on the effectiveness of the techniques used for the removal of epiphytic diatoms from macroalgae blades is scarce and mentions the use of scrubs with a brush in freshwater or of germanium dioxide (GeO_2) which prevents diatom growth (Lewin, 1966; Shea & Thierry, 2007), although none of these treatments is completely effective and long-lasting (Gledhill *et al*., 1998). In addition, scrubbing tends to damage the macroalgae tissues (Lafferty, 2001) and, although the effective dosage of GeO_2 (0.1 to 0.5 mg/L) is within the range described as nephrotoxic to rats (Furst, 1987), there are no specific criteria for the aquatic environment or for invertebrate diets.

In this paper, we examine the effectiveness of some treatments that were tested to achieve a lasting removal of epiphytic diatoms from the kelp blades that were being evaluated as diets for juvenile red abalone *Haliotis rufescens* Swainson.

MATERIALS AND METHODS

Sixty six disks with a diameter of 2.1 cm and a total surface area (considering both sides) of 6.92 cm^2 were cut with a cork-borer from the apical portion of young *M. pyrifera* blades freshly harvested from a natural bed off the coast of northern Baja California (31º 51′ 30″ N, 116º 38′ 38″ W).

Since the discs had few epiphytes, they were left standing for two hours in individual Petri dishes with a suspension (5 x 10^5 cell/mL) of a 4-days old culture of *Navicula incerta* Grunow, 1880, grown in non-axenic batch cultures of progressively increasing volumes (10, 150, 900 mL, and 10 L) in f/2 medium (Guillard, 1975) with double silicate concentration, at a constant temperature of 16 °C and with 100 $\mu E/m^2/s$ continuous photoflux.

This diatom was chosen as the dominant epiphyte, because several *Navicula* species have been described as common on the *M. pyrifera* blades used as food when abalone juveniles are weaned from a microalgae to a macroalgae diet (Siqueiros-Beltrones *et al*., 2002, 2005), and because it was one of the common diatoms in the gut content of juvenile *H. rufescens* (Siqueiros-Beltrones & Voltolina, 2000).

After this enrichment, all disks were individually rinsed with 1-µm filtered seawater and placed in clean Petri dishes with fresh, sterile seawater. Six received no treatment and served as controls. The remaining 60 were treated for 10 minutes, in groups of six disks for each treatment, with seven treatments common in aquaculture, used at the concentrations suggested by Lázaro-Chávez (1985), as well as with three biocides (Biopur® = 0.35% Ag_2SO_4; Microdin® = 0.35% Ag_2SO_4; Fit® = $CH_3(CH_2)_{11}OSO_3Na$ and $Na_3PO_4{\cdot}12H_2O$), in the concentrations used for vegetable and fruit disinfection for human consumption (Biopur and Microdin: 1 drop/L; Fit: 4.5 g/L: Table 1). After treatment, the disks were rinsed with sterile seawater to remove any residue of the cleaning agents.

Both sides of three disks of each treatment were individually scraped under a microscope with a soft brush to remove all epiphytic diatoms. These were counted with a haemocytometer after ultrasonic treatment to achieve their thorough dispersion for ease and precision of counting (Voltolina, 1991). The results were used to evaluate the initial efficiency of removal as the mean percentage of epiphytic diatoms (original community and *N. incerta*) found on the three disks of each treatment, in comparison to the mean value of the three control disks.

Physiological damage was evaluated comparing the photosynthetic activity and the chlorophyll content of the disks treated to that of the controls. The maximum photosynthetic O_2 evolution (Pmax) of each disk was measured under a light gradient of 0 to 1000 $mE/m^2/s$ in a 7 ml acrylic chamber, using a Clark-type Yellow Spring Instruments 5221 O_2 electrode (Mercado *et al*., 2004). Chlorophyll was determined as in Strickland & Parsons (1972).

The long-term effectiveness of each treatment was evaluated as positive or negative diatom growth on the remaining disks, incubated at 18 °C for 4 days in f/2 medium, using the equation:

$$Gi = \log_2 (Nti / Nto),$$

where Nti = number of live diatoms on disk i after four days; Nto = mean number of live cells on the three disks used for the evaluation of initial efficiency after treatment and Gi = total num-

Table 1. Mean *Macrocystis pyrifera* epiphyte concentration (10^3cells/cm^2), percent removal after treatment, epiphytes present after 4 days and mean number of total cell duplications (G). Negative G values indicate the number of 50% cell reductions. Different letters indicate significant differences (one-way ANOVA, $\alpha = 0.05$; a<b<c<d<e<f<g<h<i).

Treatment	Day 0 10^3 cells/cm^2	% Removal	Day 4 10^3 cells/cm^2	G
Control	3.24 (0.16)	0.0 (5.0) a	8.71 (0.55)	1.43 (0.15) h
Distilled H_2O	0.73 (0.15)	77.4 (4.4) cd	0.79 (0.23)	0.11 (0.09) e
Tap H_2O	0.76 (0.13)	76.6 (9.3) cd	0.78 (0.20)	0.03 (0.01) d
150 ppm NaClO	0.46 (0.13)	85.7 (3.9) def	0.34 (0.08)	-0.43 (0.09) c
30 ppm iodine	0.21 (0.04)	93.6 (1.1) ef	0.15 (0.06)	-0.47 (0.06) c
1.0 % $C_2H_4O_2$	0.11 (0.03)	96.6 (0.8) f	2.06 (0.31)	4.26 (0.05) i
1.0 % $CuSO_4$	0.68 (0.13)	78.9 (4.2) cde	0.51 (0.09)	-0.42 (0.05) c
1.0 % $KMnO_4$	1.26 (0.11)	61.1 (3.5) b	0.43 (0.06)	-1.56 (0.12) b
0.015 ppm Biopur®	0.39 (0.12)	87.9 (3.6) def	0.73 (0.13)	0.91 (0.01) g
0.028 ppm Microdin®	0.34 (0.09)	89.4 (2.8) def	0.48 (0.14)	0.48 (0.05) f
6.0 % Fit®	1.16 (0.09)	64.2 (2.8) bc	0.15 (0.02)	-2.89 (0.08) a

ber of positive or negative duplications of the cell number on disk i (one negative duplication = 50% reduction of the cell number in comparison to Nto; Gómez-Villa *et al*., 2002).

The removal efficiency, the photosynthetic activity and the chlorophyll contents were compared by one-way analysis of variance (ANOVA) or Kruskall-Wallis tests, depending on the results of the Lilliefors' and Bartlett´s tests of normality and equal variances, separating the significant differences with Tukey's or Dunn's tests. All statistical analysis were performed with $\alpha = 0.05$ (Zar, 1996).

RESULTS AND DISCUSSION

The best initial results were with NaClO, iodine, acetic acid (AA = $C_2H_4O_2$), Biopur® and Microdin® and the lower removals were with $KMnO_4$ and Fit ®. However, these two treatments gave the lowest epiphyte concentration after four days, whereas that on the AA-treated disks had rebounded to more than 60% of the initial value, from 0.11 to 2.06 x 10^3 cells/cm^2, for a total of 4.26 cell duplications during the four days of incubation after treatment. Fit ® gave the best final result, with the highest mean value (-2.89) of negative cell duplications (Table 1).

There was also a greater than 75% initial removal of the epiphytes present on the disks with the two types of freshwater, which also gave negligible growth after treatment (Table 1). Although significantly lower than most treatments, this seems of particular interest because it avoids the use of biocides. In addition, these treatments did not affect the consistency or texture of the blades because the cell membranes of this species are permeable to solutes but not to water (Bold & Wynne, 1985).

$CuSO_4$ and iodine stained the disks, indicating their presence in the blade tissues. In spite of this, all disks were readily consumed by abalone and for this reason the use of these biocides should be avoided if kelp is intended as food for grazers.

According to Gledhill *et al.* (1998), the total elimination of epiphytes from the blade surface of *Fucus vesiculosus* may be achieved using a combination of chemical (ethanol, ascorbic acid, and sodium hypochlorite) and physical treatments (brushing or scrubbing).

However, although there were no significant differences between the photosynthetic activity of the controls and those of any other treatment, the mean chlorophyll content of the disks treated with acetic acid, copper sulphate and Biopur® were significantly lower than the controls (Table 2), and in addition our previous experience with *M. pyrifera* showed that scrubbing caused cell damage and excessive exopolysaccharide production (Simental, 2005).

In contrast, a simple immersion in freshwater removes most epiphytic diatoms, prevents their subsequent growth and does not cause any significant textural or physiological damage to the blades. Our results show that this cheap and environmentally friendly treatment (Table 3), which is commonly used in abalone hatcheries to eliminate potential pathogenic organisms (Lafferty, 2001), is also effective for the removal of epiphytes from the kelp blades. This should be taken into consideration, because this treatment may affect the dietary quality of macroalgae, because it removes epiphytic diatoms, which have a high nutritional value (Simental *et al.*, 2004).

Table 2. Mean chlorophyll *a* content (mg/cm^2) and Pmax (µmol O_2/cm^2/min) of *Macrocystis pyrifera* after different treatments. Different letters indicate significant differences (one-way ANOVA, $\alpha = 0.05$; a<b<c).

Treatment	Chl *a*	Pmax
Control	7.31 (0.39) c	0.498 (0.113) ab
Distilled H_2O	7.11 (0.18) c	0.386 (0.004) ab
Tap H_2O	7.16 (0.19) c	0.106 (0.005) a
150 ppm NaClO	6.33 (0.32) c	0.188 (0.091) a
30 ppm Iodine	6.29 (0.60) c	0.327 (0.096) ab
1.0 % $C_2H_4O_2$	5.61 (0.47) b	0.170 (0.002) a
1.0 % $CuSO_4$	5.61 (0.16) b	0.154 (0.014) a
1.0 % $KMnO_4$	7.18 (0.10) c	0.164 (0.035) a
0.15 ppm Biopur®	4.17 (0.34) a	0.489 (0.290) ab
0.028 ppm Microdin®	7.18 (0.34) c	0.726 (0.053) b
6.0 % Fit®	6.86 (0.19) c	0.378 (0.292) ab

Table 3. Cost of the treatments ($/m^3) of the biocides used for epiphyte removal.

Treatment	Cost US $
Control	0.00
Distilled H_2O	50.00
Tap H_2O	0.72
150 ppm NaClO	0.01
30 ppm Iodine	1.00
1.0 % $C_2H_4O_2$	2.00
1.0 % $CuSO_4$	3.00
1.0 % $KMnO_4$	3.00
0.15 ppm Biopur®	0.10
0.028 ppm Microdin®	0.10
6.0 % Fit®	0.10

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