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On the control of HAB species using low biosurfactant concentrations

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ABSTRACT

Biosurfactants have been suggested as a method to control harmful algal blooms (HABs), but warrant further and more in-depth investigation. Here we have investigated the algicidal effect of a biosurfactant produced by the bacterium *Pseudomonas aeruginosa* on five diverse marine and freshwater HAB species that have not been tested previously. These include *Alexandrium minutum* (Dinophycaee), *Karenia brevis* (Dinophyceae), *Pseudonitzschia* sp. (Bacillariophyceae), in marine ecosystems, and *Gonyostomum semen* (Raphidophyceae) and *Microcystis aeruginosa* (Cyanophyecae) in freshwater. We examined not only lethal but also sub-lethal effects of the biosurfactant. In addition, the effect of the biosurfactant on *Daphnia* was tested. Our conclusions were that very low biosurfactant concentrations (5 $\mu g\ mL^{-1}$) decreased both the photosynthesis efficiency and the cell viability and that higher concentrations (50 $\mu g\ mL^{-1}$) had lethal effects in four of the five HAB species tested. The low concentrations employed in this study and the diversity of HAB genera tested suggest that biosurfactants may be used to either control initial algal blooms without causing negative side effect to the ecosystem, or to provoke lethal effects when necessary.

1. Introduction

Due to the severe effect of harmful algal blooms (HABs) on fisheries, aquaculture, drinking-water, tourism, and human health, a number of methods of bloom control have been investigated. These methods include chemical (e.g. copper sulphate, ozone) and biological control (e.g. bacteria) as well as physical control using clay/algal flocculation (for review see Sengco, 2008). Although several of these methods show promise, the direct control of HABs is a sensitive issue (e.g. Anderson, 1997), as there is always the risk of negatively impacting other parts of the aquatic ecosystem. The need of newly environment friendly methods to mitigate and control HABs is thus indisputable.

Recent studies indicate the potential of a group of substances, biosurfactants, as potential agents to manage HABs (Sun et al., 2004; Baek et al., 2003; Ahn et al., 2003; Wang et al., 2005). Biosurfactants are complex molecules produced by various groups of microorganisms and comprise a wide variety of chemical structures, including glycolipids and lipopeptides (Singh et al., 2007). The greatest advantage of biosurfactants when compared to synthetic surfactants is that they are easily biodegraded, making them environmentally acceptable in contrast to some synthetic algicides (Mulligan, 2005). Considering the deleterious effect of HABs on the environment and the opportunity to avoid the secondary pollution problem encountered with many other control strategies, the use of biosurfactants is a promising opportunity to mitigate HABs.

Previous studies on biosurfactants as potential HAB mitigation agents have focused on the marine dinoflagellates *Alexandrium tamarense*, *Heterosigma akashiwo*, *Cochlodinium polykrikoides* and *Prorocentrum dentatum*. These species were all observed to be sensitive, though to different extent, to the glycolipid biosurfactants sophorolipid and rhamnolipid (Sun et al., 2004; Wang et al., 2005).

In the present study, we tested the effect of a rhamnolipid biosurfactant produced by the bacterium *Pseudomonas aeruginosa,* on five HAB species from four different algal classes. The species included in this study are the toxin-producing marine species *Alexandrium minutum*, *Karenia brevis*, and *Pseudonitzschia,* and the freshwater species *Gonyostomum semen* and *Microcystis aeruginosa*. Each of these species is discussed below.

Alexandrium minutum Halim (Dinophyceae) has been repeatedly recorded during the last decade in different Mediterranean areas, and blooms have been related to PSP toxin production and paralytic shellfish poisoning (PSP) along the Spanish (Delgado et al., 1990; Giacobbe et al., 1996; Vila et al., 2001) and French coasts (Nezan and Piclet, 1991) and in the Adriatic Sea (Honsell, 1993; Honsell et al., 1996). *Karenia brevis* (Davis) G. Hansen and

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Moestrup is a dinoflagellate that occurs along the eastern Gulf of Mexico, USA, and at times, in parts of the southeast Atlantic coast of the US (Steidinger et al. 1998, Magaña et al., 2003). *Karenia brevis* produces brevetoxins that may kill fish and birds, and that may also cause health problems in humans through neurotoxic shellfish poisoning (NSP) (Steidinger et al. 1998). Brevetoxins can also irritate eyes and respiratory systems when the toxins become airborne in sea spray (Cheng et al., 2005). *Pseudonitzschia* (Peragallo) is a widely distributed genus of marine planktonic diatoms (Hasle, 1965, 1972; Hallegraeff, 1994; Fryxell et al., 1997). Species of the diatom genus *Pseudonitzschia* are common in the marine phytoplankton world-wide. Some species of this genus have been proved to be source of domoic acid (DA), a powerful toxin causing amnesic shellfish poisoning (ASP) in humans and probably mass mortality in sea birds and mammals (Steidinger et al., 1998).

Gonyostomum semen (Ehrenb.) is the most common freshwater member of the raphidophyceans, and often forms intensive and long-lasting blooms (Pithart et al., 1997; Willén, 2003). *Gonyostomum semen* may dominate the phytoplankton community by as much as 98% for extended period (Le Cohu et al., 1989). This alga adversely affects lakes used for recreation, as it discharges mucilaginous strands upon contact, thereby covering bathers with a slimy layer causing itching and other allergic reactions (Cronberg et al., 1988). Moreover, during the past decades *G. semen* blooms have increased in frequency and intensity in Scandinavian, Finnish, and Estonian lakes (Cronberg, 2005; Rakko et al., 2008; Bloch et al., unpublished data). Finally, the effect of the *Pseudomonas* biosurfactant on *Microcystis aeruginosa* (Kützing) (Cyanophyecae) was tested. This species is the most common bloom-forming cyanobacterium in freshwater and produces a number of hepatoxins known as microcystins. Microcystins may cause illness and death in domestic animals and humans, when contaminated waters are used for drinking (Craig and Holmes, 2000). Previous research has shown that *M. aeruginosa* is sensitive to surfactin, a cyclic lipopeptide biosurfactant (Ahn et al., 2003).

The effect of the biosurfactant was determined through studies of motility (dinoflagellates and raphidophyte), photosynthetic capacity, and cell viability. In addition, the effect of the biosurfactant on *Daphnia magna* and *Daphnia pulex* survival was assessed in order to investigate the effect of the biosurfactant on higher aquatic organisms.

2. Materials and methods

2.1. Bacterial strain

The biosurfactant-producing strain *Pseudomonas aeruginosa* 165 was isolated from a hydroponic plant cultivation system as described previously (Hultberg et al., 2008). Stock cultures of the strain were kept in 40% glycerol (v/v) at −80 °C.

2.2. Biosurfactant production and characterisation

The strain was pre-cultured on nutrient agar for 48 h at room temperature. A single colony from the nutrient agar was transferred to 200 mL of PPGAS medium (Zhang and Miller, 1992) in a 1 L Erlenmeyer flask and incubated on a rotary shaker (180 rpm) for 72 h at room temperature. Biosurfactant was recovered from the culture supernatant after removal of the bacterial cells by centrifugation at 8000 × *g*, at 4 °C for 15 min (Avanti J-20, Beckman Coulter, CA, USA). The biosurfactant was precipitated by acidification of the supernatant to pH 2.0 and collected by centrifuged at 12,000 × *g* for 20 min. The pellet was washed once with distilled water at pH 2 and extracted three times with ethyl acetate. The organic solvent was collected and evaporated on a rotor evaporator. The yellowish residue was dissolved in a 0.02 M $NaHCO_3$ buffer, pH 7.5. The critical micelle concentration (CMC) of the biosurfactant was determined by measurements with a SITA DynoTester (SITA, Messtechnik, GmbH) in distilled water at 22 °C. The surface tension was plotted as a function of surfactant concentration and CMC was considered the point where the slope of the curve abruptly changes. The biosurfactant was analyzed by matrix-assisted desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) using a 4700 TOF/TOF instrument (Applied Biosystems, Framingham, MA, USA) in positive ion reflector mode using dried droplet preparations with 4-hydroxy-α-cyanocinnamic acid as matrix. It was compared with the known rhamnolipid sample JBR425 (Jeneil Biosurfactant Company, Saukville, WI, USA), an aqueous solution of rhamnolipids at 25% concentration, and four identical signals were observed.

2.3. Algal cultures

The toxic effect of the biosurfactant was screened against five different HAB species using three different concentrations: 1, 5 and 50 μg mL^{-1}. The strains employed in this study are shown in Table 1. Cultures of *Alexandrium minutum*, *Karenia brevis* and *Pseudonitzschia* spp. were grown at 20 °C, approximately 90 μmol photons $m^{-2}s^{-1}$ with a photoperiod cycle of 12:12 h L:D (light:-dark). Culture stocks were maintained in Erlenmeyer flasks filled with 50 mL of L1 medium (Guillard and Hargraves, 1993) without the addition of silica, prepared using Atlantic seawater adjusted to a salinity of 31 by the addition of sterile double-distilled water. *Gonyostomum semen* culture was grown in modified WC culture medium (MWC) (Guillard and Lorenzen, 1972) and maintained at 15 °C at 30 μmol photons m^{-2} s^{-1} and with a photoperiod cycle of 12:12 h L:D. The microcystin-producing *Microcystis aeruginosa* PCC 7806 was cultivated in Z8 medium (NIVA, 1990) at 20 °C with a photoperiod cycle of 12:12 h L:D and a light intensity of 15–20 μmol photons m^{-2} s^{-1}. The cells used in the experiment were in the exponential phase of the growth curve.

2.4. Experimental design

The effect of the biosurfactants on photosynthetic capacity was studied by using a fluorescence-based method (Section 2.4.1). For each algal culture, aliquots of 200 μL culture were transferred to a total of 24 wells in a 96-well dark microwell plate (Greiner Bio-one Inc., Tokyo, Japan). The four treatments were: control (no addition of surfactant), 1, 5, and 50 μg mL^{-1} (final concentration) of the

Table 1
History of the strains used in this study.

Species	Strain name	Source	Year of isolation	Culture origin	Clonal
Alexandrium minutum	VGO 651	Port Saint Hubert (UK)	2003	Bloom	Yes
Karenia brevis	CCMP 2281	Florida (USA)	1999	Bloom sample	?
Gonyostomum semen	GSTV1	Tvigölen (Sweden)	2006	Bloom	Yes
Pseudonitzschia sp.	098-4	Vigo (Spain)	2007	Water	Yes
Microcystis aeruginosa	PCC 7806	Braakman Reservoir (The Netherlands)	1978	?	?

surfactant. There were six replicates per treatment. Prior to the addition of the biosurfactant, the cells in the dark plates were allowed to acclimate for 24 h at the light intensity used for cultivation of the specific algae species. This was done to reduce any potential negative effect of the dark plates on the algae. According to the scheme above, the five different HAB species were also transferred to transparent 96 wells microwell plates (Nunc, Roskilde, Denmark) to study the effect of the biosurfactant on motility and cell viability. The transparent plates were also acclimated 24 h prior to the addition of the biosurfactant.

2.4.1. Assays of effects on HAB species

2.4.1.1. Motility. The effect of the biosurfactant on motility of *A. minutum*, *K. brevis* and *G. semen* was studied using an inverted light microscope (Nikon Eclipse TS 100) at 400× magnification. Percentage of immobile cells of the total number of cells was estimated after 1, 2, 5 and 24 h for the different treatments. Changes in swimming patterns were also noted.

2.4.1.2. Fluorescence. Change in photosynthetic capacity was measured as a ratio of fluorescence before and after addition of DMCU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Roy and Legendre, 1979; Warwick, 1980). DCMU is a very specific and sensitive inhibitor of photosynthesis (Roy and Legendre, 1979; Warwick, 1980). The fluorescence was measured spectrophotometrically (FLUOstar Optima) after the dark plates had been incubated in darkness for 15 min (F_0) and then again directly after an addition of 2 μl of 1 mM DCMU (F_d), corresponding to a final concentration of 10 μM. The ratio (F_d/F_0) between the values was then calculated. This procedure was repeated after 1, 2, 5, and 24 h after addition of biosurfactant on different plates.

2.4.1.3. Cell viability. After 24 h the presence and numbers of dead cells was determined using the fluorochrome SYTOX (Invitrogen Ltd., Paisley, UK). SYTOX can only penetrate cells with damaged plasma membranes and has a high affinity for nucleic acids, which are stained green in dead cells. Prior to the cell viability assay, tests were carried out on live and dead cells (treated with heat) to determine the optimal time of incubation and concentration of SYTOX. For all five species a concentration of 0.5 μM SYTOX green (freshly diluted from a 50 μM stock solution) was used. The cells were incubated for 20 min and then counted in epifluorescence microscope (Nikon Eclipse TS 100) by switching between fluorescent and bright modes of the microscope. Photographs were taken using a digital camera (Olympus DP11).

2.5. Effects on Daphnia

The effect of the biosurfactant on *Daphnia magna* and *Daphnia pulex* was studied during 48 h. For each species, two individuals were placed in a 60 mL beaker containing 50 mL at concentrations of 0, 1, 5 and 50 μg mL^{-1} of the biosurfactant. Six replicates were performed for each concentration. Survival and motility were checked after 1, 2, 4, 24, and 48 h.

2.6. Statistics

ANOVA followed by Tukey's multiple comparison test was used to determine differences in flurorescence between treatments after 24 h. As no difference was detected between the results after 24 h and the other time points, these data are not shown or analyzed. Statistical analyzes were performed with SPSS (Version 10 for Macintosh; SPSS 2000).

3. Results

3.1. Surface tension

The surface tension of distilled water went down to a minimum of 26.2 mN m^{-1} and the CMC of the biosurfactant was 110 μg mL^{-1}. The surface tension of the media used for cultivation of the algal species was unaffected by addition of biosurfactant in the concentrations of 1.0 and 5.0 μg mL^{-1}. Addition of biosurfactant to a concentration of 50.0 μg mL^{-1} slightly decreased the surface tension of the media.

3.2. Effect on motility, fluorescence and cell viability

Control (optimal) fluorescence ratio differed among species (Fig. 1). Accordingly, the values implying negative effects on photosynthesis were also different. However, the fluorescence ratio obtained from 100% dead cells was more comparable. Below, the results for each species are reported separately.

3.2.1. Alexandrium minutum

3.2.1.1. Fluorescence. Exposure to 5 and 50 μg mL^{-1} of the biosurfactant had a negative effect on fluorescence (ANOVA, $F_3 = 27.79$, $p < 0.001$), while cells exposed to the lowest concentration showed no impairment of photosynthetic activity (Fig. 1).

3.2.1.2. Motility. A concentration of 1 μg mL^{-1} of biosurfactant caused immobility in half of the cells after 1 and 2 h, but the effect levelled off and after 5 h all cells had recovered and showed normal motility pattern. In 5 μg mL^{-1} of biosurfactant less than 10% were moving after 1 h. All cells were immobilized after 2 h and no recovery of the motility was observed. In the 50 μg mL^{-1} treatment all cells were immobilized after 1 h (Table 2).

3.2.1.3. Algicidal effects. Treatments with 5 and 50 μg mL^{-1} of biosurfactant led to a 100% mortality for *A. minutum* (Fig. 2). The lowest concentration however, had no effect on cell viability (Table 2).

Table 2
Percent immobilized and dead cells in the five tested algal species after 24 h exposure to 1, 5 and 50 μg mL^{-1} of the biosurfactant.

Biosurfactant concentration	1 μg mL^{-1}		5 μg mL^{-1}		50 μg mL^{-1}	
Effect after 24 h on motility and mortality	Percent immobilized cells	Percent dead cells	Percent immobilized cells	Percent dead cells	Percent immobilized cells	Percent dead cells
Alexandrium minutum	0	0	100	100	100	100
Karenia brevis	50*	0	100	100	100	100
Pseudonitzschia sp.	n.a.	<5	n.a.	<5	n.a.	100
Gonyostomum semen	50*	50*	100	100	100	100
Microcystis aeruginosa	n.a.	0	n.a.	0	n.a.	0

n.a. = not applicable.
* Semi-quantitative percentages since accurate counting of the total number of cells (live plus dead) was impossible in *K. brevis* due to swimming cells, and in *G. semen* due to complete lysis and disappearance of some cells.

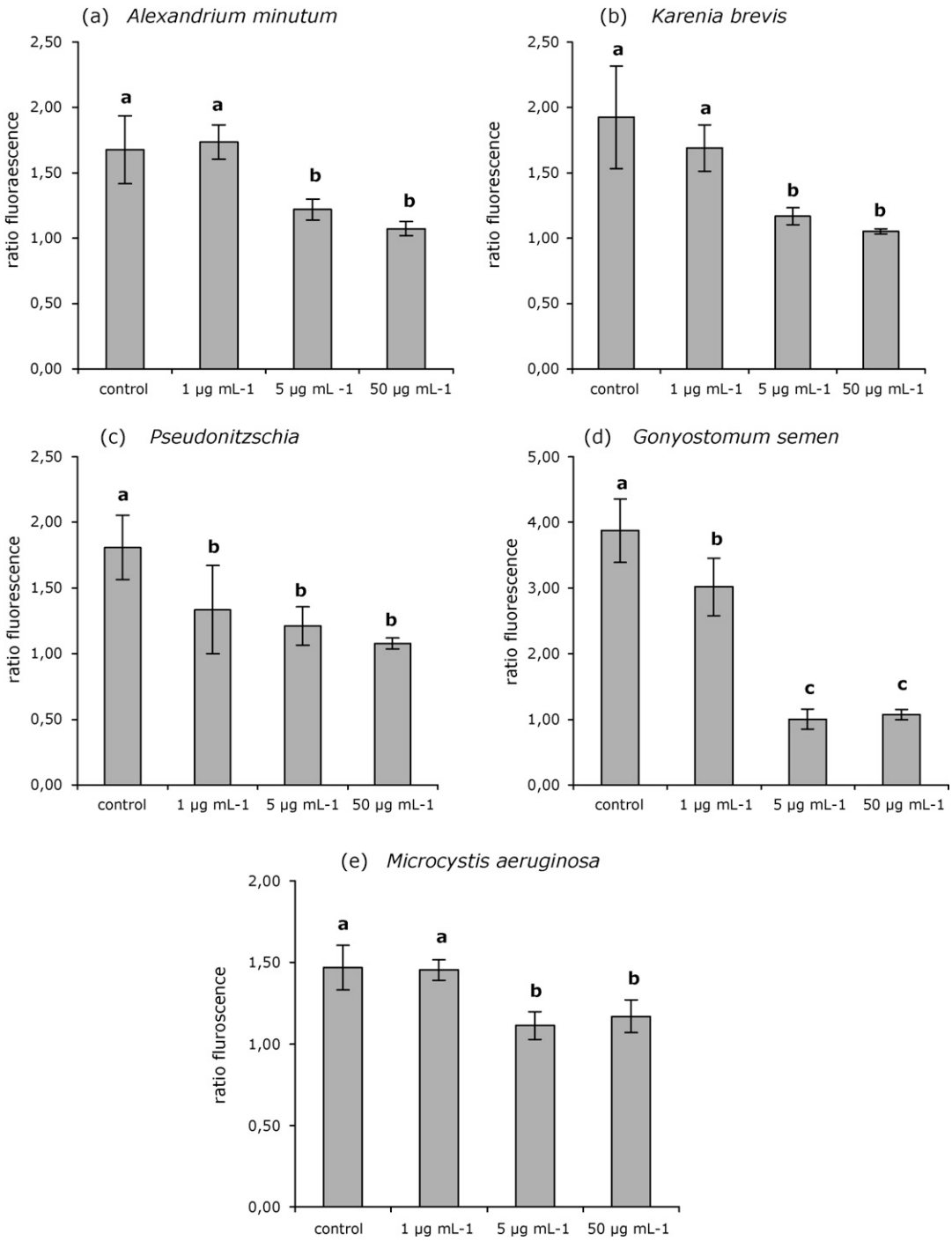

Fig. 1. The ratio of fluorescence calculated from fluorescence values before and after addition of DCMU for *Alexandrium minutum*, *Karenia brevis*, *Pseudonitzschia* sp., *Gonyostomum semen* and *Microcystis aeruginosa* after 24 h, $n = 6$. Different lowercase letters indicate significantly different means (Tukey's test: $p < 0.05$). Error bars denote standard deviation.

3.2.2. *Karenia brevis*

3.2.2.1. Fluorescence. The biosurfactant had a significantly negative effect on photosynthetic activity in the two highest concentrations (ANOVA, $F_3 = 21.67$, $p < 0.001$). The negative effect increased between 1 and 5 $\mu g\,mL^{-1}$ of biosurfactant but not between the 5 and 50 $\mu g\,mL^{-1}$ treatments (Fig. 1).

3.2.2.2. Motility. Approximately one third of the cells were immobilized after 1 h when exposed to 1 $\mu g\,mL^{-1}$ of biosurfactant, and the remaining cells moved slower compared to cells in the control. After 2 h half of the cells were immobilized but the moving cells had recovered and showed a normal motility pattern. The same effect was seen after 5 and 24 h. The higher concentrations cause 100% immobility of the cells (Table 2).

3.2.2.3. Algicidal effect. Exposure to 5 and 50 $\mu g\,mL^{-1}$ biosurfactant killed all the *Karenia brevis* cells (Fig. 2). The lowest concentration had no effect on cell viability. 5 $\mu g\,mL^{-1}$ caused complete lysis of the cells, however this was not the case in the

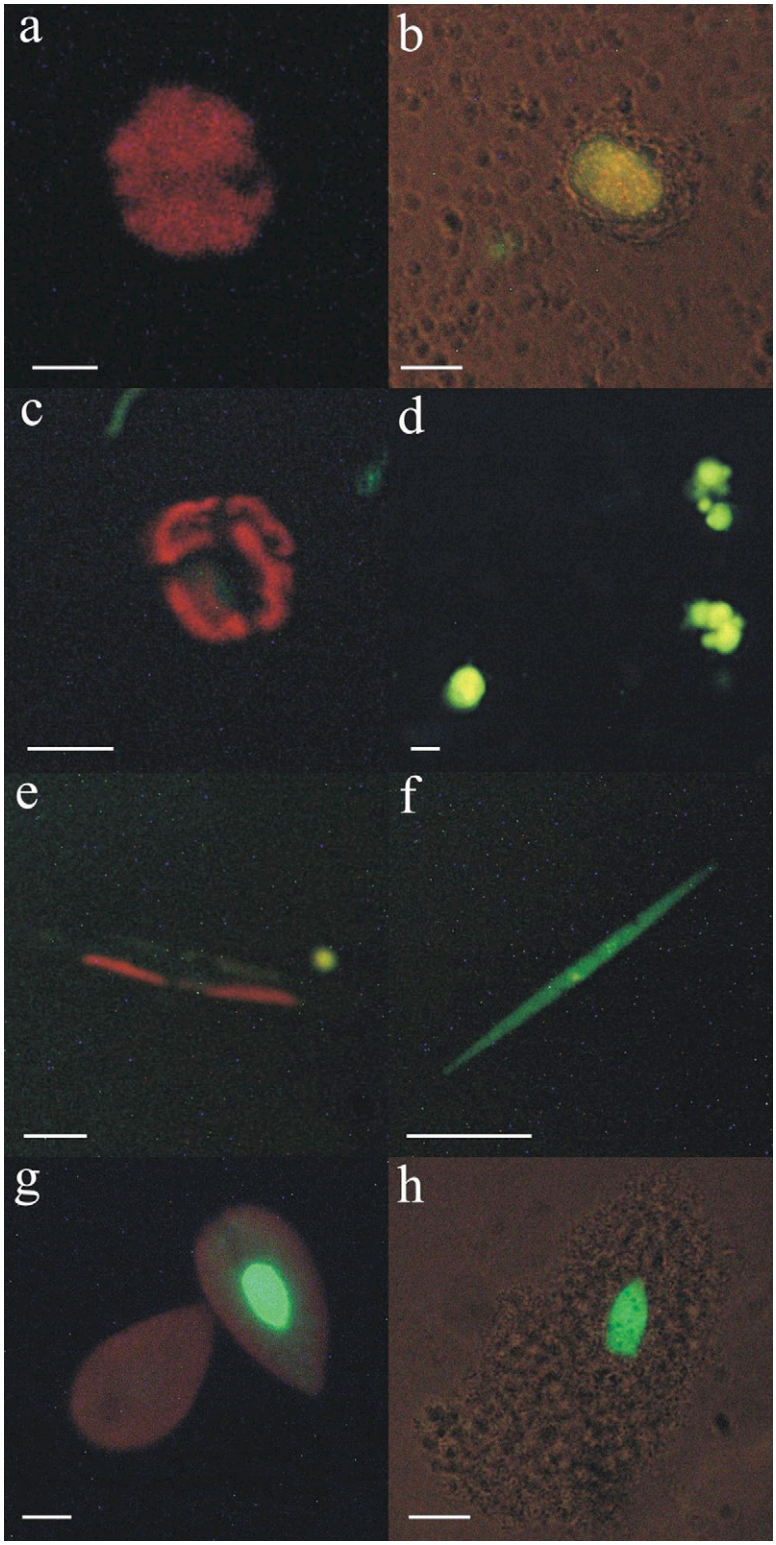

Fig. 2. Live (left) and dead cells showing sytox staining (right) of *Alexandrium minutum* (a and b); *Karenia brevis* (c and d); *Pseudonitzschia* spp. (e and f); *Gonyostomum semen* (g and h). Scale bars 10 μm.

concentration of 50 μg mL^{-1} where the cells were killed but not completely lysed (Table 2).

3.2.3. *Pseudonitzschia sp.*

3.2.3.1. Fluorescence. Exposure to the biosurfactant had a negative effect on photosynthetic activity and the effect was high in all three treatments with 1, 5 and 50 μg mL^{-1} biosurfactant (ANOVA, $F_3 = 21.67$, $p < 0.001$) (Fig. 1).

3.2.3.2. Algicidal effect. The highest concentration of the biosurfactant caused a 100% algicidal effect of the cells (Fig. 2) while the two lower concentrations had a limited (<5%) cell lysis effect on the cells (Table 2).

3.2.4. Gonyostomum semen

3.2.4.1. Fluorescence. The biosurfactant had a negative effect on photosynthetic capacity at all concentrations compared to the control (ANOVA, $F_3 = 107.69$, $p < 0.001$). 1 μg mL^{-1} of the biosurfactant was enough to cause a decrease in fluorescence and at 5 μg mL^{-1} the negative effect was more pronounced while the highest concentration caused no additional negative effect (Fig. 1).

3.2.4.2. Motility. Approximately half of the cells were dead at the lowest concentration after 1 h, while the motility of living cells was unaffected by the biosurfactant. Accurate counting was impossible due to completely lysis of some of the dead cells. In the two highest concentrations all cells were dead after 1 h (Table 2).

3.2.4.3. Algicidal effect. A concentration of 1 μg mL^{-1} biosurfactant killed approximately 50% of the cells while exposure to the two highest concentrations resulted in 100% mortality (Fig. 2). At a concentration of 5 μg mL^{-1} the entire cell was lysed but at a concentration of 50 μg mL^{-1} the cell damage was less pronounced (Table 2).

3.2.5. Microcystis aeruginosa

3.2.5.1. Fluorescence. Exposure to the biosurfactant had a negative effect on photosynthetic at the two highest concentrations, 5 and 50 μg mL^{-1}, while no negative effect was observed at the lowest concentration (ANOVA, $F_3 = 20.73$, $p < 0.001$) (Fig. 1).

3.2.5.2. Algicidal effect. The highest concentration of the biosurfactant had no algicidal effect on *M. aeruginosa* after 24 h (Table 2).

3.3. Effects on Daphnia

The biosurfactant had no negative effect on survival or motility in any concentration after 48 h.

4. Discussion

Here we have investigated the sub-lethal and lethal effects of a biosurfactant, a potential HAB mitigation agent, on five different freshwater and marine HAB species. Our results clearly showed that low concentrations of a biosurfactant produced by *P. aeruginosa* severely reduce the efficiency of the photosynthesis and motility in important HAB species. In addition, higher concentrations of the biosurfactant proved to be lethal to the cells.

Previous studies on biosurfactants as HAB mitigation agents have focused on the lytic effect of the biosurfactants (e.g. Sun et al., 2004; Wang et al., 2005). Here, on the other hand, we also showed a negative effect of biosurfactants on photosynthesis, which implies that cell viability and growing capability can be reduced drastically even when cell lysis is not observed. This would likely lead to a reduction in the competitive ability of susceptible species. In addition, the biosurfactant had a negative effect on the motility of the motile species, which could make these algae more prone to predation. Consequently, these effects could yield a decline of these populations in the field. Taken together, both impaired photosynthesis and reduced motility at the lowest concentration of the tested biosurfactant, implies a potential use of low biosurfactant levels to control initial bloom stages.

A concentration of 5 μg mL^{-1} biosurfactant was enough to cause a 100% algicidal effect on *A. minutum*, *K. brevis*, and *G. semen*, while 50 μg mL^{-1} of the biosurfactant was needed to kill *Pseudonitzschia* sp. However, the biosurfactant did not cause mortality in the cyanobacteria *Microcystis aeruginosa* and neither was the reduction in photosynthetic efficiency as pronounced as for the other species. Different cell wall structures of this prokaryote species compared to the other eukaryotic algae may be responsible for the different effects of the biosurfactants. It is known that certain cell wall compositions have been reported to protect the cell from the surfactant attack to different extents (Lang and Wagner, 1993). Species lacking cell wall, such as *Karenia brevis* and *Gonyostomum semen* were easily lysed. Faster lysis of naked dinoflagellate species has previously been reported by both Sun et al. (2004) and Wang et al. (2005) using approximately the same concentration of biosurfactant. Additionally, the thecate species *Alexandrium minutum* formed ecdysal stages before dying, which in this and other dinoflagellates species have been considered as a defence mechanism against sudden adverse changes in the environmental conditions (Fistarol et al., 2004; Figueroa et al., 2007). However, increasing the concentration ten-fold did not result in lysis of *Alexandrium minutum* cells despite that staining of the cells indicated that they were dead. This has not been reported in previous studies, in which the trend has been that increasing the concentration of biosurfactant increased the amount and speed of the cell lysis (Wang et al., 2005). A possible practical application of this result is that the release of toxins from damaged but intact cells may be lower compared to completely lysed cells. Several compounds have been reported to have cytotoxic effects on phytoplankton through an alteration in the membrane permeability. Similarly, free fatty acids may cause cell damage and total inhibition of photosynthesis, which however, can be non-lethal (Wu et al., 2006). Moreover, compounds exuded by *Karenia brevis* damage their competitors' cell membranes, and it has been hypothesized that *K. brevis* may be able to maintain almost monospecific blooms by producing these allelopathic compounds (Prince et al., 2008). Additionally, these authors remarked that damaged but not dead cells were susceptible of SYTOX green staining. Although we have not observed swimming stained cells in our experiment, the lethal effect may simply be delayed given that living stained cells are probably damaged enough to finally die (Prince et al., 2008). However, further research is needed to elucidate the possible environmental advantages and disadvantages of using higher or lower concentrations of biosurfactant.

The primary site for cellular damage caused by surfactants is on the cytoplasmic membranes (Nielsen et al., 2005). Thus, a likely explanation for the algicidal effect is that biosurfactants cause changes in the membrane and affect the membrane rigidity. For instance, Wang et al. (2005) showed that the plasma membrane of *H. akashiwo* was partly disintegrated after exposure to biosurfactant. However, the cell wall also plays a role in keeping the membrane integrity (Sun et al., 2004). This could help explain the low sensitivity of *M. aeruginosa* to the biosurfactant. *Microcystis aeruginosa* is a gram-negative species and the complex cell wall structure of gram-negative bacteria has recently been shown to be less affected by biosurfactants produced by *P. aeruginosa* (Sotirova et al., 2008). It is possible that other types of biosurfactants, i.e. not glycolipids, are more efficient against cyanobacteria. For example, surfactin, which is a cyclic lipopeptides type of biosurfactant, could selectively control *M. aeruginosa* (Ahn et al., 2003). One strategy is either to add a cocktail of different types of biosurfactants to blooms or use cyanobacteria-specific biosurfactants to specifically control cyanobacteria.

In a recent study, a combination of biosurfactants and clay (Lee et al., 2008) was suggested as a method to mitigate HAB blooms. It should, however, be clear that additions of foreign substances, biosurfactants, clay or others, may have adverse secondary effects on other aquatic organisms. Regardless of the method, the lowest concentration possible of the added substance must be used to avoid detrimental environmental effects. When the biosurfactant used in the present study was tested against two different species

of *Daphnia*, no effects were observed even at the highest concentration. Additionally, the surface tension was unaffected when low concentrations of biosurfactants were used. This suggests that the risk for unwanted side effects on other biota in aquatic ecosystems is low using a concentration of 5 $\mu g\ mL^{-1}$ of the *P. aeruginosa* biosurfactant. Nevertheless, further testing on other types of aquatic invertebrates as well as vertebrates is necessary to rule out potential toxic effects.

Given the environmental considerations for the marine and limnic ecosystems, many promising HAB mitigation agents prove difficult to use. Our results show that even very low concentration of biosurfactants cause impaired photosynthesis and reduced motility of several HAB species. In addition, no effect was noted on the crustacean *Daphnia*, a common species for limnic toxicity tests. Thus, this study further supports the idea that the use of biosurfactants is a possible approach to mitigate HABs with a low impact on the ecosystem. However, more research is needed to identify specific biosurfactants, HAB species interactions, and effects on the entire aquatic food web. This is crucial in order to develop efficient biological control systems for HABs consisting of different species and population adaptations to different geographic locations.

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