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Zeolites and diatom growth

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Abstract

Zeolites are widely used in some Asiatic countries, as they are believed to improve water quality and selectively enhance diatom growth through silicon leaching. Culture experiments with the diatom *Chaetoceros* sp. in medium f, with no silicate, and enriched with two natural and three artificial zeolitic products, showed an enhancement of between 0.2 and 0.6 cell duplications, which cannot be as a result of higher silicate availability because solubility experiments showed that natural zeolites do not leach any silicon, and that the amounts released by the artificial products are too small to improve diatom growth.

Keywords: Chaetoceros, growth, zeolites, zeolific silicon

Introduction

The potential of zeolites for water quality management is well documented in the case of freshwater aquaculture and refers, mainly, to the possibility of removing nitrogenous wastes from fish ponds and recirculating water systems, owing to their cation exchange capabilities (Leonard 1980; Liao & Lin 1981; Konstantinov & Pelipenko 1983; López Ruiz & Gomez Garrudo 1994, among others), whereas it has been shown that in brackish water, and in seawater, this property is severely affected by the high concentrations of sodium, calcium, magnesium and other mono- and divalent cations (Chiayvareesajja & Boyd 1993; Boyd 1995).

Despite this, zeolites are used widely by shrimp farmers in several Asiatic countries, at monthly rates of $200\,kg\,ha^{-1}$, adding more than 100 dollars $ha^{-1}\,month^{-1}$ to the overall operating costs of the farms (Briggs & Funge-Smith 1996), in which they are promoted by suppliers under the claim that they stabilize water and bottom quality, and that they promote diatom growth as a result of zeolitic silicon leaching into seawater (Chien 1992).

While there is extensive experimental proof that the ammonium exchange capability of zeolites is reduced in marine waters (Boyd 1995; Briggs & Funge-Smith 1996), the possibility of their effectiveness in improving diatom growth owing to their silicon content has never been tested experimentally, although Boyd (1995) argued that the silicon of the zeolitic structure is not water-soluble and, therefore, that it is unavailable to diatoms.

In view of the fact that zeolites are starting to be promoted also in Latin America, with the same claims used by Asiatic suppliers, and as it has been shown that some zeolitic products improve the yield of microalgae cultures in nutrient-enriched (including silicon), seawater-based growth media (López Ruiz, Garcia Garcia & Ferreiro Almeda 1995; Voltolina, Nieves & López Ruiz 1997; Nieves, Voltolina, López Ruiz, Cisneros & Piña 2000); the purpose of this work was to prove that this effect is not as a result of silicon leaching but, to some other, as yet unexplained, intrinsic property of these products.

Materials and methods

The working hypothesis was tested using two separate experimental approaches. With the first, we grew the high silicon-demanding diatom *Chaetoceros*

sp. (Clone CH-X-1) of the Centro de Investigación Científica y de Educación Superior de Ensenada Culture Collection (Trujillo Valle 1993) in f medium (Guillard & Ryther 1962), prepared without Si enrichment and added with an amount of five zeolitic products similar to that used by Asiatic shrimp farmers (20 mg l^{-1}; Boyd 1995). The growth of these cultures was compared with those obtained in parallel cultures in the same medium (without zeolites), with no silicon added or enriched with 15, 30 and 60 mg l^{-1} of $Na_2SiO_3 9H_2O$, equivalent to 0%, 25%, 50% and 100% of the original f formulation (FSI/0, FSI/4, FSI/2 and F respectively).

The experiments were performed in quadruplicate and were run three times, starting in all cases with an initial concentration of 50 000 cells ml^{-1} of new inoculum, prepared in f medium. Lighting was continuous, with six white fluorescent lamps, and stirring was provided by 1-μm-filtered compressed air without CO_2. Cell concentrations were evaluated daily with a haemacytometer on samples drawn individually from each culture, and the data were used to calculate the mean number of cell divisions from the day of the initial inoculum, using the equation $\Sigma\mu = \log_2 (N_t/N_0)$, in which $\Sigma\mu$ is the total number of cell divisions and N_t and N_0 are the cell concentrations evaluated at any given sampling date, and at the beginning of the experiment respectively (Nieves, Voltolina & Barreras 1998), to compare the maximum number of cell divisions obtained with the zeolitic products with those of the cultures maintained in the Si concentration gradient established using different enrichments of this nutrient.

The second approach consisted of the direct measurement of the silica concentration of natural seawater, 48 h after addition of 10 times the amount of the zeolitic products used in the growth experiments (200 mg l^{-1}), to facilitate the detection of any zeolitic silicon leaching from the five products tested. This experiment was run in triplicate flasks for each product with a control (seawater with no zeolites), which served to evaluate the possible effect of silica polymerization or depolymerization, which are known to occur in seawater (Horne 1969).

All the experiments were run in transparent 3 l polycarbonate flasks, to avoid silica leaching from the walls of glass culture containers, and all were run with the same batch of seawater with an initial SiO_2 concentration of 0.546 mg l^{-1} (mean of five subsamples analysed in triplicate with the low range heteropoly blue method #8186: Hach 1997; SD, 0.012).

The zeolitic products used for this work were the Cuban (NZ) and Mexican (MINOR) natural zeolites (Nieves 2000) and the artificial ones were ZESTEC 56 (ZT56), ZESEP 56 (ZS56) and ZEBEN 56 (ZB56), prepared and activated by the University of Cadiz (Spain) Research Group on Zeolites and Aquaculture (López Ruiz 1998). All products were sieved to obtain particles < 50 μm in diameter and they were kept in suspension, in both types of experiments, by vigorous stirring with air bubbling.

The results of both types of experiment were compared using one-way parametric ANOVA tests as, in both cases, the data were normal and homoscedastic (Lilliefors and Bartlett tests respectively), and the significant differences among the mean values were identified with Student Neuman Keuls multiple comparison tests (Zar 1996).

Results

The highest cell concentrations in the complete medium were obtained between the fourth and the fifth day in the three experiments with the complete medium, and varied between 1.2 and 1.8×10^6 cells ml^{-1}, showing that the growth started to be limited with a Si concentration equal to 50% of that of the original formulation of the f medium, with which we obtained, at best, close to 0.5×10^6 cells ml^{-1} after 1 to 2 days, and that it was even poorer in the rest of the media (Fig. 1).

However, the results obtained with the different treatments show that the media added with zeolites gave consistently better results than the medium without any silicate addition. With the complete f medium, the maximum number of cell duplications was 4.6, obtained on the fourth day. With FSI/2 and FSI/4, the number of cell duplications was slightly higher than 3.0 and close to 2.5, after 2 days and 1 day respectively. This last result was close to that of the cultures in the medium added with ZESEP 56 (close to 2.3 duplications), followed by the two other enrichments with PZN and by NZ and MINOR, in this order. All these results were significantly better than those of the control without any silicate, which indicates that all the products tested had a positive effect on diatom growth, though it is not definite proof that this is the result of increased silicate availability (Table 1).

The results of the solubility experiment showed an increase of SiO_2 concentrations in all treatments, including the controls, which might be as a result

of depolymerization of more complex Si compounds. They also confirm that the slight but significant increase in cell duplications and in cell yields obtained with $20\,mg\,l^{-1}$ of the natural zeolites cannot be the result of silica leached from the natural zeolites, as the concentrations of SiO_2, measured 48 h after continuous stirring of 10 times the amount used in the cultures, were not significantly higher than those of the control, whereas the artificial products, and especially ZEBEN 56, added significant amounts of silica to the original $0.546\,mg\,l^{-1}$ of the natural seawater (Table 2).

However, the amount of silica leached in 48 h in our growth experiments, in which we added only 20 mg l of zeolitic products, would have been only one tenth of the values reported in Table 2, equivalent to between 0.8% and 1.5% of the close to $12.68\,mg\,l^{-1}$ of silica ($60\,mg\,l^{-1}$ of $Na_2SiO_3 9H_2O$) of the f medium.

The results of the growth experiments, plotted as percentages of the maximum number of cell duplications obtained within the gradient of Si concentrations, in comparison with those of the complete f medium, lie in an almost straight line that indicates a direct relationship between the silicate content of the medium and *Chaetoceros* sp. reproduction.

The number of cell divisions with 25% of the normal concentration of Si of the f medium gave slightly more than 50% of the number of cell divisions of the f medium, close to those obtained with ZESEP 56, which had less than 5% of the silicon, including the original content of seawater and that leached from the product itself as shown in Fig. 2, in which all the results obtained with zeolitic products

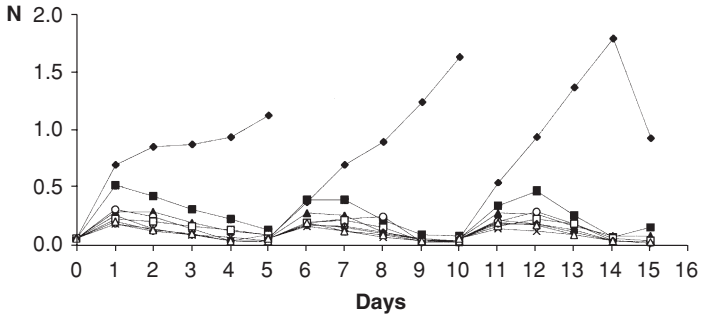

Figure 1 Mean cell concentrations (N, in 10^6 cell ml^{-1}) in complete f medium (◆), or with 50%, 25% and no silicate enrichment (■, y and ▲, x, respectively), or without silicates and with $20\,mg\,l^{-1}$ of the zeolitic products ZESTEC 56 (*), ZESEP 56 (○), ZEBEN 56 (□), MINOR (◇) and NZ (△). Day 5, end of the first experiment, and beginning of the second; day 10, end of the second experiment and beginning of the third.

Table 1 Mean number of cell divisions of *Chaetoceros* sp. (± standard deviation; $n = 12$ in all cases) grown in three experiments with the standard f medium (F) or in the same medium with 50% and 25% of the normal silicate concentration (FSI/2 and FSI/4 respectively) or with no silicate added (FSI/0)

Medium	Day 1	Day 2	Day 3	Day 4	Day 5
F	3.342 ± 0.436	4.010 ± 0.323	4.330 ± 0.420	4.634 ± 0.535[h]	4.461 ± 0.732
FSI/2	2.976 ± 0.461	3.032 ± 0.368[g]	2.183 ± 0.847	1.066 ± 0.872	1.091 ± 0.603
FSI/4	2.467 ± 0.095[f]	2.377 ± 0.237	1.419 ± 0.895	0.680 ± 0.979	0.375 ± 0.949
FSI/0	1.643 ± 0.192[a]	1.311 ± 0.206	0.606 ± 0.314	−0.338 ± 0.550	−0.853 ± 0.437
Minor	1.803 ± 0.035[b]	1.564 ± 0.298	0.947 ± 0.230	−0.578 ± 0.245	−1.260 ± 0.606
NZ	1.943 ± 0.061[c]	1.405 ± 0.243	0.794 ± 0.119	−0.607 ± 0.202	−1.134 ± 0.372
ZB56	1.920 ± 0.283	2.085 ± 0.103[d]	1.689 ± 0.145	0.102 ± 1.268	−0.414 ± 0.927
ZT56	2.072 ± 0.212[d]	1.609 ± 0.226	1.009 ± 0.402	−0.540 ± 0.331	−1.036 ± 0.274
ZS56	2.175 ± 0.302	2.299 ± 0.187[e]	1.863 ± 0.370	−0.796 ± 0.320	−0.723 ± 1.117

Minor, NZ, ZB56, ZT56 and ZS56: medium FSI/0 with $20\,mg\,l^{-1}$ of the respective zeolitic products. Different letters indicate significant differences among the highest values obtained with each treatment with $a < b < c$ (one-way ANOVA and SNK multiple comparison tests; $\alpha = 0.05$).

Table 2 Differences between initial and final concentrations of reactive silicates (in $mg\,l^{-1}$ of SiO_2, initial value 0.546 ± 0.012) after 48 h from the addition of $200\,mg\,l^{-1}$ of four artificial (PZN) and two natural zeolites

	Flasks			
	1	2	3	$\bar{X} \pm S$
PZN				
ZB 56	1.827	1.847	1.881	1.852 ± 0.0269^{c}
ZS 56	1.027	0.921	1.057	1.002 ± 0.0718^{b}
ZT 56	1.114	0.727	1.191	1.011 ± 0.2484^{b}
Zeolites				
NZ	0.211	0.107	0.124	0.147 ± 0.0555^{a}
Minor	0.047	−0.003	−0.006	0.013 ± 0.0299^{a}
Control	0.171	0.034	0.017	0.074 ± 0.0841^{a}

Equal and different letters indicate differences, with $a < b < c$ (one-way ANOVA and SNK multiple comparison tests; $\alpha = 0.05$).

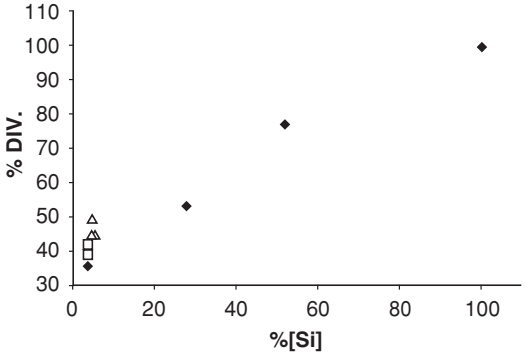

Figure 2 Relationship between number of cell duplications and Si concentrations, expressed as percentage of the results obtained in complete f medium. Full symbols, medium f with 100%, 50%, 25% and 0% addition of silicates; squares, with addition of $20\,mg\,l^{-1}$ of two natural zeolites; triangles, with $20\,mg\,l^{-1}$ of three artificial zeolites. Percentages of Si concentrations are corrected for initial Si contents of seawater and for Si leaching of zeolites.

lie above the line of direct response of *Chaetoceros* sp. to Si concentrations.

Discussion

Although there is experimental evidence that some artificial zeolites may enhance microalgae production in monospecific, non-axenic cultures (López Ruiz *et al.* 1995; Voltolina *et al.* 1997), including non-silicon-demanding flagellates (Nieves *et al.* 2000), the claim that they may have a similar effect in shrimp ponds (Chien 1992; Troy 1994) has not been substantiated by the investigations of Briggs & Funge-Smith (1996). That they may be effective specifically on diatom growth has been questioned by Boyd (1995), because of their virtual insolubility in water that would, therefore, prevent silicon leaching, as claimed by Chien (1992).

Our results show that all the zeolitic products have a significant effect on the number of cell divisions of the diatom *Chaetoceros* sp. but that this is not because of silicon, given the fact that the two natural zeolites did not leach any silicate and that the amounts released by the artificial ones, although measurable, are not large enough to explain the increased reproduction of this microalgae, in comparison with that of the cultures without any silicate addition, demonstrating that silicon leaching from zeolites, is not the variable involved in the enhancement of microalgae production, be it in cultures or in shrimp ponds.

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