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Zeolitic products as enrichment for cultures of a marine microalga

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Abstract

The cell yields of batch and semicontinuous cultures in f/2 medium of the marine diatom *Chaetoceros* sp., widely used in Mexican mariculture, may be improved by more than 30% by the addition of 10 mg l^{-1} of experimental zeolitic products to the medium. Over 3 days, the best yields are obtained with ZESTEC 56 and ZESEP 56, but ZEBEN 06 gives better results after 5 days, or in semicontinuous cultures. © 1997 Elsevier Science B.V.

Keywords: Microalgae cultures; *Chaetoceros* sp.; Cell yield; Non-conventional enrichments; Zeolitic products

1. Introduction

Clinoptilolites, more commonly known as zeolites, are natural hydrous Al silicates with one or more alkaline or terrous-alkaline metals, easily exchanged for other cations. They have been used for water softening, and their use has been advocated for several years in aquaculture for their beneficial effects on water

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quality. Fast ammonia removal by zeolites is a well known process (Sand and Mumpton, 1977) and it has been shown more recently that some experimental zeolitic products play an important role in nitrification and in several other biological processes (López Ruiz et al., 1995a). Some results suggest, for instance, that yields of microalgae cultures, which are the main diet for the larvae of most of the important commercial mariculture species, might be improved by these products (López Ruiz et al., 1995b). However, these results were obtained with experiments not consistent with commercial practices. Cultures were grown in a strongly buffered simplified artificial seawater, nutrient enrichments were with a garden fertilizer and, most of all, cultures were allowed to grow undisturbed (other than for daily sampling) for times ranging from 7 to 10 days. In a commercial hatchery, this would mean an undue increase in cost, given the slow turnover of the cultures, with consequent increases in ancillary costs of space, lighting fixtures and culture vessels. In addition, it would mean harvesting cultures in their stationary phase of growth, which may be associated with a declining dietary value of the biomass (Brown et al., 1989) and it would also increase the risks of contamination by bacteria or by other exogenous contaminants.

In this paper we give the results of some experiments run with a microalga used in several Mexican hatcheries for feeding bivalve and penaeid larvae, which we grew and maintained with the two techniques most commonly used for commercial purposes.

2. Materials and methods

The microalga used for these experiments is a local unicellular *Chaetoceros* which, under the strain designation CH-X-1, has become as popular in Mexican commercial hatcheries as the clone CHGRA, commonly known as *Chaetoceros gracilis* (actually, *C. muelleri* Lemmerman) which it closely resembles in morphology and in growth characteristics (Trujillo Valle, 1993).

For these experiments CH-X-1 was first grown for at least ten generations in four 18 l carboys, one with 10 l of medium f/2 (Guillard and Ryther, 1962) and the others with equal volumes of the same medium, each one enriched with 10 mg l^{-1} of each of three experimental zeolitic products (ZESTEC 56, ZESEP 56, and ZEBEN 06), manufactured by the Zeolite Research Group of the University of Cadiz (López Ruiz et al., 1995a).

Appropriate volumes of these cultures were used to inoculate three carboys for each of the media, to start all cultures with $0{\cdot}2 \times 10^6$ cells ml^{-1}. These were allowed to grow for 5 days, cell concentrations being checked on Days 3 and 5 with a haemocytometer.

Cultures were non-axenic and media were prepared with 5 μm filtered natural seawater sterilized by the addition of 3 ml l^{-1} of commercial 5% bleach, which was neutralized with 150 mg l^{-1} of sodium thiosulphate before nutrient (including silicate) addition.

Stirring was by continuous air bubbling without CO_2 enrichment; lighting was

also continuous, provided by Daylight fluorescent lamps, giving an approximate irradiance of 200 μmol m^{-2} s^{-1} at the center of the culture vessels. The water temperature was 20 ± 1°C.

The cultures were run simultaneously with the four media and the above procedure was followed twice. At the end of the second run, all cultures were kept with the semicontinuous technique, first with 30% and later with 50% daily dilutions. In each case the cultures were allowed to reach steady-state conditions, verified by daily cell counts, after which the mean cell concentrations obtained during four consecutive days with each dilution rate were used for statistical analysis.

Data were normal and homoscedastic (Lilliefors' and Bartlett's tests, respectively) only in the cases of the 3 and 5 day-old batch cultures, which were consequently compared using one-way ANOVA. Those of the semicontinuous cultures failed to pass the Bartlett's test of equal variances and were compared with the nonparametric Kruskal Wallis test. In all cases, the data were finally compared using the a posteriori SNK multiple comparison test with $\alpha = 0.05$.

3. Results

After 3 days, the batch cultures with the zeolitic products ZESTEC 56 and ZESEP 56 gave significantly higher cell yields than those in medium f alone, or enriched with ZEBEN 06. With the latter, however, growth continued at a high rate during the following 2 days, so that, on the 5th day from the start of the experiments, the control cultures had the lowest concentrations. Those with the two enrichments which gave the best results on Day 3 were only slightly higher, and the highest cell numbers were those of the ZEBEN 06-enriched cultures (Table 1).

Further proof of the beneficial effects of zeolitic products on microalgae growth is given by the results of the semicontinuous cultures. With both dilution routines, the lowest yields were with medium f/2 and the highest were with ZEBEN 06. It is

Table 1
Mean cell concentrations in cells $\times 10^6$ ml^{-1} (standard deviations in brackets) obtained at Days 3 and 5 in two experiments with *Chaetoceros* sp. grown in triplicate batch cultures with f/2 medium alone, or enriched with 10 mg l^{-1} of the experimental zeolitic products indicated. Letters indicate the results of the SKN multiple comparison test among mean data for each date: $a < b < c$ ($\alpha = 0.05$)

Day	Medium			
	f/2	ZESTEC 56	ZESEP 56	ZEBEN 06
3	1·80 (0·11)[a]	2·55 (0·22)[b]	2·34 (0·05)[b]	1·98 (0·09)[a]
5	2·57 (0·11)[a]	2·83 (0·05)[b]	2·77 (0·06)[b]	3·41 (0·08)[c]

Table 2
Mean daily cell concentration in cell $\times 10^6$ ml^{-1} (standard deviations in brackets) obtained during 4 days of semicontinuous cultures of *Chaetoceros* sp. kept at 30 and 50% daily dilutions. Letters indicate the results of the SNK multiple comparison test among data of the same dilution rate: a < b < c < d ($\alpha = 0.05$)

Dilution	Medium			
	f/2	ZESTEC 56	ZESEP 56	ZEBEN 06
30%	2·67 (0·07)[a]	2·79 (0·12)[b]	2·98 (0·08)[c]	3·67 (0·21)[d]
50%	2·58 (0·08)[a]	2·60 (0·26)[a]	3·32 (0·21)[b]	3·50 (0·40)[d]

also worth mentioning that, contrary to expectations, the cell yields with ZESEP 56 increased with the higher dilution and did not differ from those obtained with ZEBEN 06 (Table 2).

4. Discussion and conclusions

At this stage, the reason for the effect of zeolites on growth of the diatom CH-X-1 in unclear. Previous results suggest some effect on silicon utilization (López Ruiz et al., 1995b). On the other hand, the organic compounds released as extracellular products by microalgae might be recycled through the enhancement of bacterial activities associated with the use of zeolites (López Ruiz and Gómez Garrudo, 1994), increasing the availability of inorganic carbon and nitrogen sources for microalgae utilization.

Finally, their active site might act in lieu of the organic ligands (e.g. EDTA, citrate) which are not usually added, as in our case, to the culture media used for commercial production, thus regulating the concentrations and activities of heavy metal species or other growth-retarding compounds.

All these possibilities are worth exploring in depth. For the time being, it is clear that a slight increase in the cost of production due to the addition of a small amount of a fairly inexpensive compound, may greatly increase the daily output of a microalgal production unit, which was the first objective of this study.

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