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Short Communication

Marine Microalgae Culture: *Chaetoceros gracilis* with Zeolitic Product ZESTEC-56 and a Commercial Fertilizer as a Nutrient

José L. López-Ruiz, Rocío García García & Maria Soledad Ferreiro Almeda

Group of Investigation 'Zeolites-aquaculture', EUIT Navales, University of Cadiz, Complejo Ciencias del Mar, 11510 Puerto Real (Cadiz), Spain

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ABSTRACT

In this study the employment of zeolitic products on microalgae culture is continued. In this case some new products are tested, especially ZESTEC-56. All of which have been prepared in our laboratory. The diatom Chaetoceros gracilis *is used. A commercial fertilizer is experimented on as a nutrient. The optimum dose of this fertilizer has been calculated. The best yield (g diatom/litre) of the culture concern the zeolitic products called ZEBEN-56 and ZECER-56, although a dose of 5 mg/liter of ZESTEC-56 produces important cellular growth, particularly when associated to a silicate solution.*

INTRODUCTION

Lately we have been studying the involvement of zeolitic products in microbial processes, owing to their important superficial properties. Thus, we have been able to observe their influence on nitrogen transformations ($NH_4^+/NO_2^-/NO_3^-$); for instance, the ammonium production in the decomposition of fish feed. If zeolite is present in sufficient quantities, a realistic reduction of the ammonium production is achieved. This could be explained by ion-exchange taking place on the surface of

the zeolite. Maenwhile a small amount of zeolite only stimulates bacterial action (López-Ruiz & Gómez Garrudo, 1993).

In order to determine the possible microbial influence of zeolitic products we have verified a control of Pseudomonas and Micrococcus. As a result we have found some correlations between the type of zeolite and the type of the bacterium (Thiebaut Estrada *et al.*, 1992).

We also studied in other work the establishment of four different bacteria in an artificial marine system with zeolite. We could appreciate differences, depending on the type of zeolite on the species and number of bacterial colonies produced (Chaves Sanz & López-Ruiz, 1991).

From this we noted the influence of zeolite on some biological processes, and have opted to study the zeolitic action on marine microalgae culture.

To this effect we have carried out some other research in which we have used zeolitic products, especially on diatoms culture (Asorey Rial & López-Ruiz, 1991; Gordo Montalbán & López-Ruiz, 1993; Vidaurreta Campillo and López-Ruiz, 1992).

The results gave us an insight into possible areas of study, e.g. zeolite type, microalgae species, nutrient and experimental conditions.

In the present work we decided to use the microalga *Chaetoceros gracilis* as a nutrient, a commercial fertilizer recommended for gardening, and ZESTEC-56 (zeolite product).

MATERIALS AND METHODS

The culture experiments have been carried out on artificial sea water composed of the following: 24 g of NaCl, 0·7 g of KCl, 1·1 g of $CaCl_2$, 11·0 g of $MgCl_2$, 4·0 g of Na_2SO_4 and 0·2 g of $NaHCO_3$. This composition is dissolved in distilled water and made up to 1 litre. Four millilitres of Tris buffer solution and 2 ml of sodium silicate solution were added. The Tris solution is made up of 125 g of Tris(hydroxymethyl)aminomethane ($C_4H_{11}O_3N.HCl$)/litre, and the silicate solution is made up of 40 ml of sodium silicate ($d = 1{\cdot}365$ g/ml; 10·71% SiO_2) and 1 litre of water.

Nutrients were provided by a commercial fertilizer recommended for gardening called Plantavic (Rickilt and Coleman 5A) (Nutrient P), which is composed of: 5% nitrogen (2% ammonium and 3% nitrate), 1% of P_2O_5 soluble in water, 5·5% of K_2O (nitrate). The microelements composition is: 0·0135% of B, 0·001% of Cu, 0·0029% of Fe, 0·0099% of Mn and 0·0016% of Ni.

For the algae culture we added 100 ml of *Chaetoceros gracilis* inoculum to 900 ml of the medium as well as different amounts of

Nutrient P and zeolite products (especially ZESTEC-56) which we prepare in our laboratory (López-Ruiz & Pérez Sánchez, 1991).

The cultures are mantained at 25°C with constant air stream provided by a compressor, with either continuous lighting or 12 hr day-length using two Sylvania F2OW/I5ARS day-light lamps.

The yield of the culture-cellular concentration (g/litre) was mantained by spectrophotometry, measuring the absorbances at 560 nm, with Philips PU 8620 spectrophotometer and converting the reading to g/litre of dry cellular mass at 60°C.

EXPERIMENTAL RESULTS

Table 1 shows the results obtained by culturing the diatom *Chaetoceros gracilis* using different amounts of Nutrient P over the course of 7 days, indicating that Nutrient P appears to be suitable for culturing this microalga. The increasing quantities of Nutrient P cause increasing cloudiness in the culture but a level of 2 ml/litre Nutrient P produces the highest cell concentration, with only slight turbidity.

Table 2 gives the performance (g of microalga/litre) of three different cultures. The first is carried out with the culture medium only, without nutrient. The second with 5 ml/litre of Nutrient P, and the third without nutrient but with 5 mg/litre of ZESTEC-56. The results were run over the course of 10 days, with the highest cell concentrations occurring on the eighth day in treatment 2. Using ZESTEC-56 alone, 65% of this yield is achieved.

TABLE 1
Yield of the Culture (g/litre) with Different Quantities of the Nutrient P (ml/litre) over 7 Days

Quantity of nutrient	*Days*						
	0	*2*	*3*	*4*	*5*	*6*	*7*
0	0·005	0·01	0·02	0·03	0·04	0·04	0·03
0·5	0·005	0·01	0·02	0·03	0·04	0·05	0·05
1	0·005	0·03	0·05	0·06	0·07	0·07	0·07
2	0·005	0·11	0·12	0·14	0·15	0·15	0·15
3	0·005	0·08	0·09	0·10	0·10	0·10	0·10
4	0·005	0·03	0·04	0·05	0·06	0·06	0·06

TABLE 2
Yields of the Culture (g/litre), First with the Medium Only (I), Secondly with 5 ml of Nutrient P/L (II) and, Finally, with 5 mg of ZESTEC-56 (III) Over 10 Days

Test	*Days*										
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
I	0·01	0·02	0·03	0·03	0·04	0·05	0·06	0·06	0·06	0·06	0·06
II	0·01	0·03	0·07	0·08	0·09	0·10	0·10	0·11	0·12	0·12	0·12
III	0·01	0·03	0·04	0·05	0·06	0·07	0·08	0·07	0·07	0·07	0·07

TABLE 3
Values of pH in the Culture of *Caetoceros gracilis*, under Different Conditions, Over 7 Days

Conditions	*Days*							
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Medium only	8·04	8·05	8·05	0·07	8·07	8·08	0·10	8·13
Nutrient P	7·60	7·60	7·59	7·60	7·61	7·61	7·63	7·68
ZESTEC-56	8·04	8·06	8·09	8·11	8·12	8·15	8·18	8·20

TABLE 4
Yields of Culture (g/litre) with 10 mg/l of Different Zeolite Products Over 7 Days, using Nutrient P (2 ml/litre in all Cases)

Products	*Days*							
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*
ZECER-56	0·01	0·04	0·05	0·06	0·08	0·13	0·14	0·14
ZESTEC-56	0·01	0·06	0·07	0·07	0·08	0·09	0·10	0·10
ZEBEN-56	0·01	0·05	0·08	0·08	0·09	0·10	0·15	0·16
ALSI-R.10	0·01	0·04	0·07	0·07	0·08	0·10	0·10	0·10
ZESEP-56	0·01	0·06	0·10	0·12	—	0·16	0·18	0·19
None	0·10	0·04	0·06	0·07	—	0·08	0·10	0·10

Table 3 gives the pH values of Table 2. The values in the culture with Nutrient P are slightly smaller with little increase during the course of the experiment.

Table 5 shows the results of test carried out to compare the performances of three cultures. In Test I, 1 ml/litre of dilute sodium silicate was

TABLE 5
Yields (g/l) of Three Cultures on which 1 ml Silicate/litre (I), 1 ml Silicate/litre and 10 mg ZESTEC-56/litre (II), and 10 mg ZESTEC-56 are Added, Respectively

Test	*Days*										
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
I	0·02	0·03	0·04	0·07	0·10	0·11	0·13	0·14	0·15	0·16	0·17
II	0·04	0·06	0·08	0·10	0·14	0·15	0·16	0·17	0·18	0·19	0·20
III	0·03	0·05	0·06	0·09	0·12	0·13	0·15	0·16	0·17	0·18	0·19

added to the medium (see Materials and Methods). In Test II, 1 ml of dilute sodium silicate and 10 mg/litre of ZESTEC-56 was added. In Test III only 10 mg/litre of ZESTEC-56 was added. We can appreciate (Table 5) that the combined addition of silicate and ZESTEC-56 (Test II) provides the best performance, but using ZESTEC-56 is superior to using silicate only. However, it should be remembered that the culture medium described in Materials and Methods already contained silicate. Therefore in Test III the zeolitic product seems to augment the silicate action.

CONCLUSIONS

From these results we can deduce that the best dose, under these conditions, is 2 ml/litre of Nutrient P (Table 1). Also the addition of 5 mg/litre of the zeolitic product ZESTEC-56 only (Table 2), provides substantial growth.

On the other hand, testing the different zeolitic products prepared in our laboratory has shown that ZEBEN-56 and ZECER-56 produce larger yields than ZESTEC-56, which was the main purpose of this work. The final result is therefore satisfactory (Table 5).

We finally point out (Table 5) that the combined action of ZESTEC-56 and silicate provides better performances than using silicate alone.

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