

Consumption of nutrients from sewage effluents by the Green Alga *Enteromorpha prolifera* (Mull.) J. AG.

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Introduction : *Enteromorpha prolifera* was selected in the present investigation for its ability to withstand wide salinity variations. Experiments were conducted to evaluate its quantitative role in the removal of inorganic nutrient salts (nitrogen and phosphorus) from diluted sewage effluents and production of protein rich algal biomass.

Materials and Methods : The alga was cultured outdoors in glass jars. A series of 15 liters culture media were prepared by mixing clear sewage effluents and seawater to obtain dilutions of 20, 40 & 60% respectively. About 15 gm of fresh fronds of *Enteromorpha* were inoculated in each medium. The culture media with 20 & 40% sewage effluent were changed twice during three successive incubation periods of 13, 12 & 7 days (in October-November, 1989), but with the same algal fronds. The 60% culture medium extended for 21 days with the original medium. Ammonia, nitrate, nitrite and phosphorus were determined at the beginning and by the end of the 3 periods. The increase in fresh and dry weights and protein content of the seaweeds were measured by the end of the each period as indices of growth.

Results and Discussion : The average water temperature of the culture media was 26.8°C and the day length was 11.5 hours. The water salinities were respectively 30.2‰, 23.4‰, and 16.6‰ in cultures with 20, 40 and 60% sewage effluents. The initial and final concentrations of the total inorganic nitrogen and phosphorus in the different culture media during the successive incubation periods are illustrated in table (1). Of all culture experiments performed, *Enteromorpha* showed better utilization of inorganic nutrients at 20% concentration. The rate of algal growth in 20% culture medium reached 1.85 gm freshweight/day and that of 40% sustained growth rate of about 1.2 times higher than that recorded for 20% during the first incubation period, but decreased again to lower values comparable to that obtained in the former dilution in the other 2 periods (table 2). The total nitrogen, built up through protein synthesis in *Enteromorpha* exceeded the total inorganic N present in the original media. Such high utilization rate can be sufficed by excess inorganic nitrogen, produced through bacterial breakdown of organic materials present in the effluents. Waite and Mitchell (1972a), found that the carbon assimilation by *Ulva lactuca* was inhibited by ammonia at concentrations higher than 60 µM. In the current study, the growth of *Enteromorpha* was maintained well with ammonia as high as 6.9 mg NH₃-N/liter.

The protein content in algal fronds increased by about 82% and 66% of the original values by the end of the 32 days in culture media with 20 and 40% sewage effluent respectively.

Results indicate that tertiary treatment of domestic wastes by photosynthetic algal growth appears to be successfully achieved at dilutions of about 20% sewage effluent. Concentrations higher than 40% appear to reduce algal growth as well as protein synthesis.

Table (1) : Initial & final concentrations of inorganic nitrogen & phosphorus in different culture media of 15 liters exposed *in situ* with 15 gm fresh wt. algal fronds.

%Sewage effluent	Duration (days)	Conc.	mg/15 liters				
			NH ₄ -N	NO ₂ -N	NO ₃ -N	Total inorg. N	Total inorg. P
20%	13	Initial	40.50	0.24	5.10	45.84	31.40
		Final	0.01	0.00	0.00	0.01	3.60
	12	Initial	69.00	2.04	2.80	73.84	39.60
		Final	1.65	0.00	0.00	1.65	6.60
	7	Initial	61.95	0.17	1.70	63.82	30.10
		Final	12.30	0.00	0.00	12.30	11.30
40%	13	Initial	93.00	0.18	0.80	93.98	60.20
		Final	2.40	0.00	0.00	2.40	5.90
	12	Initial	108.00	1.53	2.10	111.63	60.90
		Final	43.50	0.00	0.00	43.50	11.10
	7	Initial	123.80	0.13	1.20	125.13	58.10
		Final	41.55	0.00	0.00	41.55	24.90
60%	21	Initial	117.20	0.12	1.40	118.72	91.10
		Final	39.90	0.00	0.00	39.90	23.70

Table (2) : Daily yield of 15 gm fresh algae (fresh and dry weights) grown in 15 liters culture media with 20, 40 & 60% sewage effluents. The percentage of protein content in algal dry weights with initial concentration of 22.6% after the different exposure periods is also illustrated.

% Sewage	Duration in days	Fresh Wt mg	Dry Wt mg	% Protein
20%	13	1850	140	28.0
	12	580	30	33.8
	7	1430	80	41.3
40%	13	2310	160	28.0
	12	580	30	35.6
	7	1140	50	37.6
60%	21	860	50	24.4

References

Waite, I. and Mitchell, R., 1972a. The effect of nutrient fertilization on the benthic *Ulva lactuca*. Bot. Mar., 15 (3), 151 : 167.

Temperature - Initiation factor of Red Tide Bloom in the Kastela Bay (Adriatic Sea, Yugoslavia)

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Year-to-year recurrence of red tides by the *Gonyaulax polyedra* in the same area (eastern part of the Kaštela Bay) led us to suspect that cysts or "seed population" were involved. The fact that sea water samples from this area very often contain resting cysts of *G. polyedra* supports this hypothesis to a certain extent. Vegetative cells are typically present from April to November but not observed during the winter season when a massive diatom bloom often occurs. During April, and thereafter, dinoflagellates become more and more important within the phytoplankton community. During July, a monospecific bloom of *G. polyedra* extending through August and September vary in intensity over short time scales. In order to study mechanism initiating and supporting red tide occurrences in the Kaštela Bay a monitoring was undertaken during the summer 1988 and 1989. All standard oceanographic parameters (T, S, σ_t , O₂, pH, transparency, nutrients, density of phytoplankton cells) were sampled on a weekly basis.

The analysis of temperature data pointed to the fact that red tide bloom in the Kaštela Bay is always associated with the increased sea water temperature exceeding 20°C. When surface temperature attains 20°C the bloom begins to develop reaching its peak intensity not earlier than when bottom layers attain the same temperature. The bloom persists until the surface temperature drops below 20°C (Table 1).

Table 1. Sea water temperature, existence of *G. polyedra* red tide and number of *G. polyedra* cells in the eastern part of the Kaštela Bay

Period	T (°C)	Existences of R.T. + or -	N _o of <i>G. polyedra</i> cells
July, 1983.	23,3	+	1,0 x 10 ⁶
June, 1984.	21,2	+	1,2 x 10 ⁶
July, 1984.	23,2	+	1,0 x 10 ⁷
August, 1984.	22,1	+	1,1 x 10 ⁷
May, 1985.	19,2	-	9,2 x 10 ⁴
June, 1986.	20,7	+	1,3 x 10 ⁶
April, 1988.	15,9	-	0
June, 1988.	24,1	+	3,5 x 10 ⁶
July, 1988.	26,0	+	3,8 x 10 ⁶
August, 1988.	26,9	+	4,0 x 10 ⁶
September, 1988.	23,7	+	3,2 x 10 ⁷
June, 1989.	19,7	-	6,0 x 10 ⁴
July, 1989.	23,9	+	4,3 x 10 ⁷

Even though the bloom of *G. polyedra* takes place in the surface layer, temperature of the bottom layer is also of importance for its development, that is the temperature which makes possible the excystment of *G. polyedra*. As shown by our results, temperature at which the excystment of *G. polyedra* starts at about 20°C. Upon excystment, vegetative cells of *G. polyedra* swim actively to the sea surface concentrating in large quantities. Red tide bloom terminates with the cooling of surface layer (temperature drops below 20°C). This is due to the fact that the bloom is limited to the surface layer since *G. polyedra* is markedly photophilous requiring high light intensity (ANDERSON *et al.*, 1987).

Red tide spreading all over the bay (during September, 1988 and July, 1989) may also be related to the heating of deeper layers. Data on temperatures in summer 1988 and 1989 point to the fact that spreading of red tide all over the bay came as a consequence of thermocline descending between 10 and 20 m depth (Table 2). At that time the bottom layer of a large part of the bay attained 20°C temperature causing thus the excystment of a large number of *G. polyedra* cells.

Table 2. Sea water temperature (°C) in the deepest part of the Kaštela Bay during the period of investigation (1988 and 1989)

Depth (m)	V	VI	VII	VIII	IX	X
0	20,00	22,68	24,20	26,20	23,21	-
5	18,02	19,02	24,20	21,38	22,98	-
10 1988	15,32	18,40	17,32	19,04	22,00	-
20	14,70	17,12	15,30	15,54	16,49	-
35	13,99	14,90	14,18	14,06	14,78	-
0	14,69	19,60	24,64	22,23	-	16,80
5	14,70	18,80	24,56	22,18	19,85	16,80
10 1989	14,65	18,24	21,10	21,87	19,60	16,83
20	14,50	16,60	17,38	18,66	18,90	17,41
35	14,28	15,62	14,94	14,60	17,19	16,60

Our analyses indicate that the temperature determines initiation and termination of *G. polyedra* blooms in the Kaštela Bay. The limiting temperature is found to be around 20°C.

REFERENCES

ANDERSON, D.M., C.D. TAYLOR and E.V. ARMBURST, 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.*, 32(2): 340-351.