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SOME PECULIARITIES OF *Rhodomonas lens* PASH IN SEA WATER INFLUENCED BY OUTFALLS

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ABSTRACT:

The accomodation capacity of Rhodomonas lens to develop its life cycle in sea water influenced by wastes is presented. The wastes additioned to sea water ranging within 1-20 ml % had satisfied nutritional requests of the species.The chlorophyll a and cellular density were rather at the same level in the control and experimented variants,too. The growth of both heterotrophic bacteria and Rhodomonas as well as the speed or the selectivity of different nitrogen compounds absorbtion are discussed.

The Romanian inshore waters undergoes to outfalls mainly constituted by domestic wastes waters. The first consequences of these outfalls are the modification of physical and chemical features. The concentration level of trophic ions and dissolved organic matter have a special position within them.

Some previous studies have shown how the physical and chemical modifications are reflected on phytoplanktonic communities (15, 16, 17, 18, 19, 20).Besides qualitative phytoplanktonic communities changes the occurence of new taxa was observed. They came from freshwater and developed their life cycle due to the

great adaptative capacity these algae had. Rhodomonas lens belongs to this category. Its presence was observed throughout 1981-1982 and is first mentioned for the Black Sea*. As the frequence of this alga was related to domestic outfalls, the present study was performed to explain Rhodomonas lens accommodation to sea water influenced by them.

MATERIAL AND METHODS

Rhodomonas lens has been isolated from inshore waters in August 1982, and was maintained both on agarized and liquid medium, pH = 7-7.5, temperature 19-21°C, 4,000 lx light intensity in a 16:8 hours light: dark cycle.

The capacity of trophic compounds utilization from sea water influenced by outfalls was studied in four variants of sea water additioned with domestic wastes in following percentages: 1, 5, 10 and 20. Results were compared with control, represented by common medium used for the Black Sea unicellular algae (17).

The sea water was collected from an area 10 Nm far from coast, were the outfalls influence was smaller than in the near-shore waters.

After filtration the sea water was pasteurized and was used for both experimented variants and control.

The domestic wastes source was a treating plant equiped only with mechanical step; wastes were brought into laboratory 1-2 hours before to be used.

The species Rhodomonas lens was precultivated in sea water without nutritive compounds throughout a week before experiment; than it was separated by sedimentation.

The experiment was performed in a 4,000 lx light intensity, 16:8 hours light: dark cycle, 19-21°C temperature, in static conditions with continuous flux air bubbling. Special device to avoid evaporation and algal culture contamination was used.

Throughout the experiment were observed:

1. cellular density - using Fux-Rhosenthal Chamber;

* life cycle, spreed and its association will be presented in other paper

2. division rate $K = \ln \frac{Ct}{Co} \left(\frac{1}{t \ln_2} \right)$ (14);

3. the chlorophyll a content (23);

4. heterotrophic bacteria density: 1 ml of 10^{-1} - 10^{-4} dilution of algal suspension was inoculated on nutritive agar and exposed to 20°C over 24 hours (27, 29);

5. $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$ (25), $N\text{-}NH_4$ (11) and N-urea (21);

6. salinity (25);

7. dissolved organic matter and BOD_5 (9).

RESULTS

The Rhodomonas lens population density (as cell μl^{-1}) increased during the experiment, showing a high correlation with wastes percentage addition (Table 1).

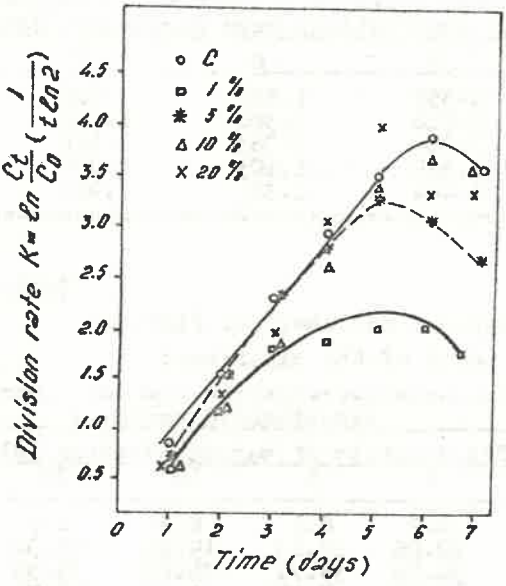

Fig.1 - The division rate of Rhodomonas lens

The division rate demonstrates a strong metabolic activity within first and third or third and fifth day of experiment depending on the proportion of wastes (Fig.1).

The analyse of chlorophyll a made evident an active photosynthesis (Table 2). The chlorophyll a concentration ($\mu g \times 10^6 cel^{-1}$) is very close to control excepting some situations. Higher values of chlorophyll a corresponded either to the most intense photosynthesis (e.g. variants with 1 ml% addition within third and fifth day of experiment), or to the high density of algal suspension, which limited the quantity of penetrating light. Compensation had

Table 1

Cell density ($\mu g l^{-1}$) in sea water with 1-20 ml% wastes addition versus the control

Experimented variant	The time within observations were performed (days) 0	3	5	6	7
Control	13.8	122	273	371	295
1 ml% addition	12.8	71	84	84	69
5 ml% addition	14.2	120	274	238	179
10 ml% addition	22.3	124	375	454	413
20 ml% addition	21.9	137	577	376	380

Table 2

Chlorophyll a content at different time of the experiment (as $\mu g \times 10^6 cells^{-1}$)

Experimented variant	The time within observations were performed (days) 0	5	6	7
Control	0.977	1.352	1.459	1.626
1 ml% addition	1.226	1.718	1.360	1.312
5 ml% addition	1.211	1.069	1.283	1.382
10 ml% addition	0.863	1.325	1.103	1.875
20 ml% addition	0.792	1.444	1.311	1.927

Table 3

The level of some physical and chemical factors at the starting point of the experiment

The determination	Experimented variants: Control	The quantity of wastes addition (ml%) 1	5	10	20
pH	8.0	8.2	8.1	8.2	8.2
Salinity (g ‰)	20.32	20.06	20.13	19.94	17.32
Organic matter	25.30	34.76	35.71	36.02	39.50
BOD_5	4.40	4.98	5.10	5.16	8.79
ΣN (μg-at l^{-1})	43.69	70.69	106.83	135.90	184.33
$P\text{-}PO_4$ (μg-at l^{-1})	43.33	2.44	6.97	12.66	23.91
Ratio N:P	1.00	28.97	13.89	10.77	7.70

occured by a greater synthesis of this pigment (e.g. control and variants with 10-20 ml% addition, after seven days of observations.

The dynamics of trophic substrate utilization have revealed some peculiarities correlated with different level of their concentration (Table 3). The differences between the variants with wastes addition and control were: (1) a lower salinity; (2) a higher concentration of dissolved organic matter and a corresponding value of BOD_5; (3) the quantity of nitrogen is over the control: the bigger proportion of wastes addition the greater the value of their concentration; (4) the phosphorus is much lower than the control for all variants; (5) the ratio between N and P is deeply modified (the advantage of N being obviously).

The kinetics of nutritive compounds had a greater speed in the presence of wastes: their influx was 221-581.4% over the control (Tables 4-6).

Table 4

The nitrogen balance (as NO_2, NO_3, NH_4, N-urea) after five days of the experiment (analyses performed only on the medium; in μg-at l^{-1})

Experimented variant	ΣNT_o *	ΣNT_5 **	$\Sigma NT_o - \Sigma NT_5$	The absorbtion (as %) versus the starting point	The absorbtion magnitude versus the control (as %) ***
Control	39.09	30.09	9.00	23.02	
1 ml% addition	71.26	27.51	43.75	61.39	167
5 ml% addition	106.83	43.95	62.88	58.85	156
10 ml% addition	135.90	50.27	85.63	63.00	174
20 ml% addition	184.19	91.52	92.67	50.31	118

* T_o = the starting point of experiment

** T_5 = the fifth day of experiment

*** The nitrogen quantity (ΣN) on the fifth day of observations = 13.6 μg-at l^{-1} was considered as 100 %.

Table 5

The nitrogen kinetics on the fifth days of the experiment
(analyses performed only on the medium; in μg-at l^{-1})

Experimented variant	$N-NO_2$			$N-NO_3$			$N-NH_4$			N-urea		
	T_o	T_5	T_o-T_5	T_o	T_5	T_o-T_5	T_o	T_5	T_o-T_5	T_o	T_5	T_o-T_5
Control	2.07	12.20	+10.13*	37.19	15.50	21.69	2.62	1.4	1.22	1.81	1.99	0.82
1 ml % addition	0.41	1.27	+ 0.86	50.83	21.73	39.00	7.72	3.2	4.52	2.40	1.31	1.09
5 ml % addition	0.99	0.78	0.21	65.74	33.79	31.95	36.02	6.8	29.22	4.08	2.58	1.50
10 ml % addition	4.49	0.34	4.15	80.68	45.46	35.22	42.02	0.9	41.12	8.71	3.57	5.24
20 ml % addition	8.45	1.40	7.05	104.54	83.80	20.74	54.52	1.8	52.72	16.68	4.52	12.16

* With plus was marked the increase of the concentration

Table 6

The ratio between heterotrophic bacteria number and algal number throughout the experiment

Experimented variant	Time when observations were done (days)				
	0	1	2	4	7
Control	76.85	255.93	15.37	7.55	1.71
1 ml% addition	87.95	383.20	17.12	10.58	0.78
5 ml% addition	88.37	523.20	17.08	4.62	1.23
10 ml% addition	192.47	633.08	9.08	4.22	2.84
20 ml% addition	211.27	612.40	6.09	3.75	2.29

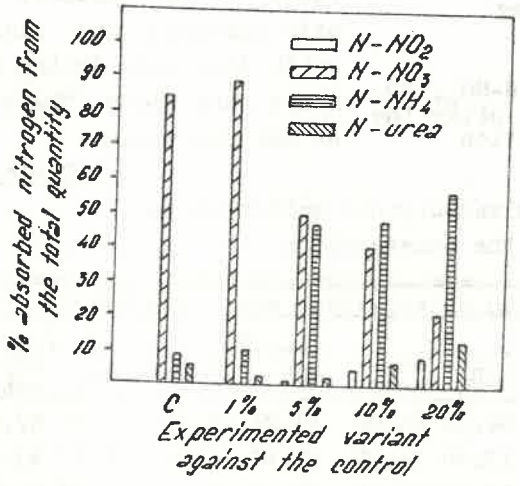

Fig.2 - The absorbtion of different forms of nitrogen

The nitrogen absorbtion depends upon the species of this element which are available in the medium, as well as of their concentration levels (Table 5, Fig.2). The oxydated states of nitrogen (NO_2,NO_3) are mainly absorbed into the control and in the variant with 1 ml% wastes addition. One can suppose the existance of an enzymatic activity specific for oxydated nitrogen states absorbtion while an increase of $N\text{-}NO_2$ concentration was noted in the medium. The percentage of $N\text{-}NO_2$, NO_3 absorbtion decreased in the variants with wastes addition over 1 ml%. A $N\text{-}NH_4$ and N-urea absorbtion was characteristic for the mentioned variants (Fig.3).

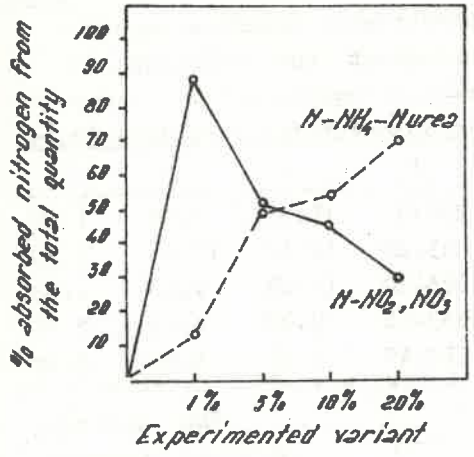

Fig.3 - The absorbtion of $\Sigma N\text{-}NO_2$, NO_3 and $\Sigma N\text{-}NH_4$, N-urea after the fifth day of observation

The heterotrophic bacteria density was obtained as a ratio between their number and Rhodomonas lens density (no. cells x ml^{-1})for each moment of harvesting (Table 6). This ratio had increased over first 24 hours. Its value was dependent of the quantity of wastes addition. The ratio between heterotrophic bacteria and algal cells decreased during 24 hours throughout the end of the experiment.

Table 7

The BOD_5 and dissolved organic matter content during the observations

Experimented variant	Time when observations were done (days) 0		5		6		7	
	BOD_5	org. mat.	BOD_5	org. mat.	BOD_5	org. mat.	BOD_5	org. mat.
Control	4.40	25.30	34.98	85.00	38.28	77.10	48.84	67.31
1 ml% addition	4.98	34.76	17.20	39.50	19.40	41.08	23.00	43.61
5 ml% addition	5.10	35.71	22.80	56.25	36.20	56.88	21.80	44.87
10 ml% addition	5.16	36.02	15.00	52.77	11.20	52.77	29.80	57.51
20 ml% addition	8.79	39.50	23.20	45.19	11.40	48.03	33.40	54.67

The quantity of dissolved organic matter and BOD_5 values have increased in concentration throughout all observation time, both in the control and in the variants with wastes waters addition (Table 7).

DISCUSSION

The variation of division rate depended on the experimented variant and duration of observation. In other words it was a matter of the concentration level of trophic ions (Tables 8,9).

Table 8

The nitrogen kinetics within the fifth and the seventh day of the experiment (analyses performed only on the medium; in μg-at l^{-1})

Experimented variant	Chemical state/concentration level/ absorbed quantity (in μg-at l^{-1})							
	$N-NO_2$		$N-NO_3$		$N-NH_4$		N-urea	
	T_7	T_5-T_7	T_7	T_5-T_7	T_7	T_5-T_7	T_7	T_5-T_7
Control	6.55	5.65	17.35	+1.85	2.20	+0.80	1.78	+0.79
1 ml% addition	0.95	0.32	15.50	6.23	5.75	+2.75	7.49	+6.18
5 ml% addition	0.79	+0.01	3.66	30.13	3.10	3.70	2.88	+0.30
10 ml% addition	0.54	+0.20	3.46	42.00	2.50	+1.60	1.43	2.14
20 ml% addition	0.43	0.97	2.66	81.14	3.25	+1.45	1.52	3.00

A difference between division rate in 1 ml% addition variant and the other addition variants was obvious even in the first 24 hours of experimentation. In one percent wastes addition variant the division was going only up to the fifth day due to the trophic ions that were limitative.The bigger the volume of wastes addition, the longer and higher the division rate.

Table 9

The kinetics of $P-PO_4$ throughout the experimentation (in μg-at l^{-1})

Experimented variant	Time/concentration level/absorbed quantity (in μg-at l^{-1})				
	T_o	T_5	T_o-T_5	T_7	T_5-T_7
Control	43.44	33.75	9.69	20.62	13.13
1 ml% addition	2.44	0.62	1.81	1.04	+0.41
5 ml% addition	6.97	0.81	6.16	1.45	0.64
10 ml% addition	12.66	1.06	11.60	2.35	+1.29
20 ml% addition	23.91	8.53	15.38	4.17	4.36

The chlorophyll a concentration proved a normal photosynthesis state even though the division rate decreased or stopped. Exceptional high value of this pigment occurred after

seven days of observations and corresponded to control and to variants with 10 and 20 ml% wastes addition. In the latter cases cell density reached high values (Control: 295 x 10^6 cell x l^{-1}; 10 ml% addition: 413 x 10^6 cell x l^{-1}; 20 ml% addition: 380 x 10^6 cell x l^{-1}, in table no.1), implying a decrease of penetrating light. This fact requested to intensify the chlorophyll *a* synthesis.

The kinetics of different nitrogen compounds focalized the attention on some aspects:

a. the preferential utilization of $N\text{-}NH_4$ and N-urea in variants with a bigger concentration of these compounds;

b. a higher speed of total nitrogen absorbtion in variants with wastes addition.

Many evidences on unicellular algae utilizing N-urea are available (7, 8, 12, 22). A test performed on 26 unicellular algae showed that 88% of them grew better on N-urea than other nitrogen compounds (1). $N\text{-}NH_4$ is a good source of nitrogen, too, being successfuly utilized by some algae (10, 13, 16). The explanation of $N\text{-}NH_4$ and N-urea preferential absorbtion could be the modification of algal enzimatic equipment when these nitrogen sources are present. $N\text{-}NH_4$ and N-urea inhibit the specific enzimes for oxydated state of nitrogen (26).

A heavy content of dissolved organic matter characterized all variants. It supported the heterotrophic bacteria development up to 24 hours (Tables 6, 7). A cessation of heterotrophic bacteria increase took place after 24 hours, even if the level of dissolved organic matter had not decreased. One can suppose that the development of Rhodomonas lens could clarify these events.

Many references on algae bacteria relationship are known. BERLAND, BONIN and MAESTRINI (4, 5) have shown that bacteria utilize dissolved organic matter excreted by dead or alive algae. In turn, bacteria supply algae with some stimulant products (28). Some unicellular marine algae have the potency to synthetize antibiotics (2, 3, 6, 24). This antibacterial activity against a variety of marine isolates takes an important place in natural relationship between the two categories of organisms.

In the present study it seems that the two latter sorts of relationship acted: (1) algal division began only when hetero-

trophic bacteria density was reached; (2) the algal development reduced heterotrophic germ number, even if excreted organic matter was enough during all experimentation time.

ECOLOGICAL IMPLICATIONS

In natural environment, wastes are mixed with sea water at experimented percentages. In the case of large volume outfalls, which rate is 1 to 5 m x s^{-1}, the experimented percentages are located as follows*:

- 20 ml% close to the outfall;
- 10 ml% 1 Km far from the outfall;
- 1 ml % over 2 Km, farther from the outfall.

The values cited could vary due to the physical factors. The presence of these concentrations in the natural environment explains the appearance and development of a freshwater species in this area. The lower salinity as well as the existence of trophic compounds requested by Rhodomonas lens have favoured its accomodation to sea water.

CONCLUSIONS

1. The experiment has proved the potency of Rhodomonas lens to develop in sea water additioned with domestic wastes.

2. The division rate and the speed of trophic ions absorbtion are stimulated by domestic wastes.

3. The stimulants are mainly supposed to be $N\text{-}NH_4$ and N-urea.

4. The heterotrophic germs seem to have a close relationship with Rhodomonas lens as they have shown to be implied in triggering its cellular division.

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