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The rate of utilization of nitrate-nitrite by natural phytoplankton populations in a reactor

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Abstract

Nutrient uptake rates of natural phytoplankton taken from the Belgian coastal area have been assessed. On the one hand, we used a perturbation technique, this provided a drastic modification of the external substrate concentration. On the other hand, it was done by a dynamic method, which allowed a gradual and slow change of the external substrate concentration.

The obtained uptake rate versus substrate concentration (nitrate-nitrite) curves show that the maximum uptake rates range from 8.6×10^{-3} h^{-1} to 18×10^{-3} h^{-1}. From these curves can also be deduced that nitrogen (as $NO_3^- - NO_2^-$) can be a limiting nutrient in our coastal area.

Introduction

Owing to the monthly surveys of the national monitoring program, a quite detailed picture of the spatial-temporal nutrient distributions in the Belgian coastal zone, has been obtained. The twenty sampling stations as well as the subdivision of the coastal area in four sectors are shown in figure 1. Considering the results of nitrate-nitrite in 1978 (figure 2), we observe strong seasonal variations in the four sectors, which are not due to external inputs but to local endogeneous biological activity in the watercolumn and the sediments (Mommaerts *et al.*, 1979). In that context fundamental questions, concerning the dynamics of our coastal ecosystem, raised. Is there a limiting nutrient ? How does it limit planktonic production ? As is already concluded in a previous paper (Mommaerts *et al.*, 1979), we were unable to define the exact nature of the most probable

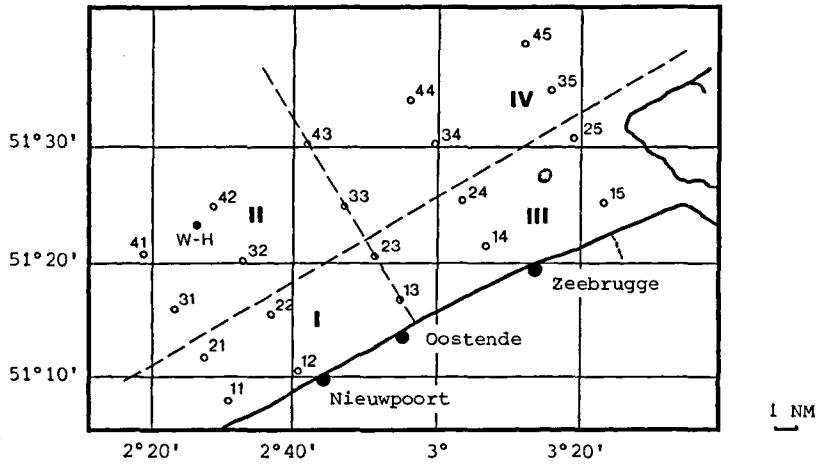

fig. 1.
Sampling stations and subdivision in four sectors of the Belgian coastal area

limiting element on the basis of in situ measurements, without help of a more direct approach : enrichment experiments conducted at sea, kinetic uptake studies in a reactor.

Our goal was therefore to establish the kinetic curves which describe the overall substrate uptake regulation for natural phytoplankton populations of the North Sea. The first phase of this study includes no other limiting nutrients than nitrate-nitrite.

Sampling

The Management Unit of the North Sea and Scheldt Esturarium, Mathematical Models, Ministry of Public Health and Environment, took care of the sampling and the transport of the samples to the laboratory. Seawater samples were collected at point 23 or at the West-Hinder (51°23'N-02°26'E) near point 42 (see figure 1). Fifty litres of seawater were collected with a rotational pump at a depth of 3 m and were then stored in two polyethylene containers of 25 ℓ each. The samples were transferred to the laboratory as quickly as possible (the transport time ranges from four

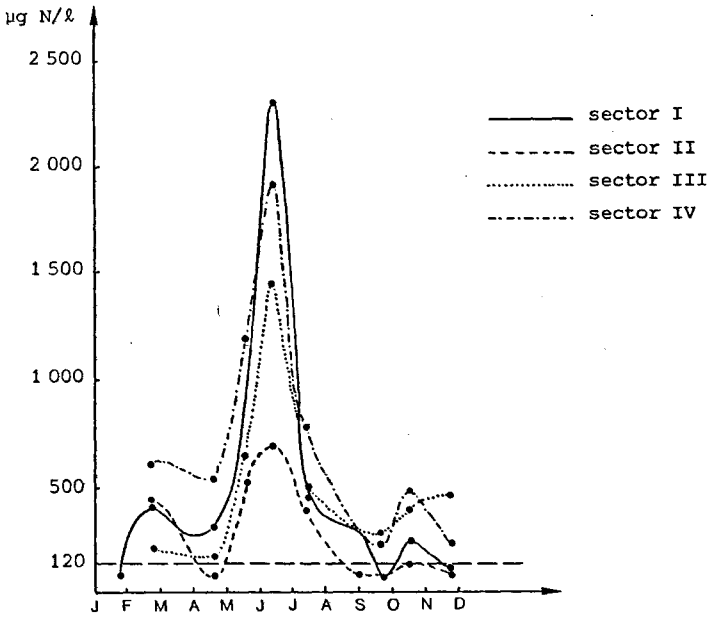

fig. 2.

Seasonal variations of nitrate - nitrite in the four sectors in 1978

to six hours). There they were immediately, or after preconcentration (this takes about three hours), taken to the reactors and thermostatized at 10°C.

Methods and materials

The analysing methods for nutrients (ammonium, nitrate-nitrite, phosphate and silicate) have been described in a previous paper (Mommaerts *et al.*, 1979). Chlorophyll a was measured, using the method of Strickland and Parsons and SCOR-UNESCO (Strickland and Parsons, 1968). Phaeopigments were determined, according to the method of Lorenzen (Lorenzen, 1967). On the basis of these results, we estimated the phytoplankton biomass.

The nutrient concentrations were measured in the natural samples, the preconcentrated samples, if any, and the filtrate (0.22 µm pore-size Millipore filter). At the beginning of the experiment, all nutrient concen-

trations, except nitrate-nitrite, were brought to a non-limiting level. The chlorophyll a and phaeopigment concentrations were determined in the natural and preconcentrated samples.

The reactor is a double-wall plexiglass container, with an inner content of 4.75 . A scheme of the apparatus is given in figure 3. A mechanical stirrer provides complete mixing of the sample, while the temperature is maintained constant by a LAUDA compact refrigerated thermostat type RC20. The light intensity was 13 300 lux and the photoperiod was 12 hours light-12 hours dark.

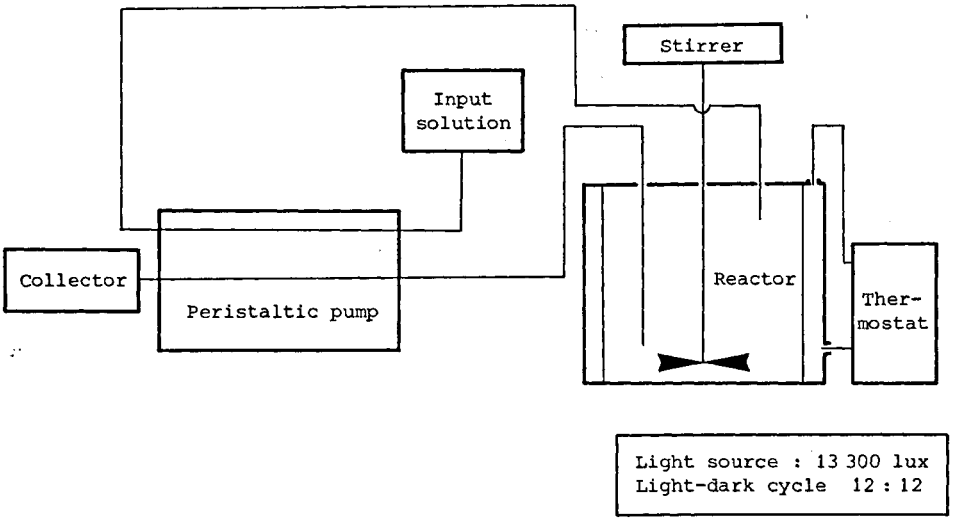

fig. 3.
Scheme of the apparatus

Sampling of reactor solution can be carried out manually with a syringe or automatically with a peristaltic Techniconpump. This pump regulates the input and output flows of the reactor (in these experiments both flows are taken identical). The output tube is connected with a Gilson fraction collector. Thus, time integrated samples are obtained (the period is adjustable). The collected samples were finally analysed by an automated analyser. In this way, the evolution of the nitrate-nitrite concentrations can be

followed in real time, allowing a modification of the parameter conditions, at any moment if necessary. The input solution is generally filtrated natural sample. Depending on the evolution of the limiting nutrient concentration in the reactor, its concentration in the inlet solution will be increased or filtrate of aged seawater, exhausted in nitrate-nitrite, will be used.

The uptake kinetics are assessed in an automated manner. As a general rule, the limiting nutrient is measured every hour during the light period. This frequency however is adapted whenever very fast or slow concentration changes are observed. The uptake rate of nitrate-nitrite can be derived using the law of mass conservation. At time t, we can write :

Change of $NO_3^- - NO_2^-$ in reactor = ingoing mass - outgoing mass + uptake

$$\frac{d(VC)}{dt} = Q \times C_{in} - Q \times C + U_r \qquad (1)$$

where V is the sample volume in reactor at time t; C is the nitrate-nitrite concentration in the reactor at time t which is, as a consequence of the complete mixing of the reactor solution, equal to its concentration in the outlet; Q is the input-output flow rate; C_{in} is the nitrate-nitrite concentration in the inlet solution; U_r is the decrease of nitrate-nitrite in the reactor at time t due to assimilation by the living organisms. The uptake rate U_r, expressed in mass of nutrient per unit time, can thus be determined at any moment. When we divide this value by the reactor volume and the biomass at time t, we get the commonly used uptake rate in h^{-1}.

All other nutrient concentrations as well as phytoplankton biomass are measured at the beginning and at the end of the light period. These latter analyses require 50 mℓ sample, which are manually withdrawn from the reactor. This causes a corresponding volume decrease every 12 hours.

Results and discussion

Steady-state experiments, such as described by Droop (1968, 1974), enable the assessment of nutrient uptake rates by selected algal species, versus a broad spectrum of substrate concentrations. In our case however, these experiments are not utilizable, because they run over several weeks. It is obvious that the population composition will strongly change during this time.

Using the perturbation technique of Caperon and Meyer (1972), Harrison and Davis (1977) were able to measure the nutrient uptake rates of the natural population versus a broad range of substrate concentrations in a short time. During the first phase they let the nutrient concentrations decrease until one of them reached zero. Then, at the beginning of the second phase, they injected a known amount of the limiting nutrient, while all other nutrient concentrations were brought to a non-limiting level. We wanted to test the feasibility of their method for our purposes. The initial physico-chemical conditions as well as parameter conditions of our sample are shown in table 1.

Table 1

Initial conditions of the sample :

Sampling time Situation	Temperature	Biomass µg chlor a/ℓ	$NO_3^- + NO_2^-$ µg N/ℓ	NO_2^- µg N/ℓ	PO_4^{---} mg P/ℓ	Si mg SiO_2/ℓ
26-07-78 11.00 a.m. Point 23	14 °C	4.8	418	12	0.35	1.6

Phytoplankton was concentrated 4-fold using a reverse flow filter system (filter diameter is 142 mm; filter poresize is 1.2 µm). The efficiency of the concentration is estimated from measurements of chlorophyll a and phaeopigments before and after concentration (Table 2). The loss was due to cells which stuck to the filter. On 27-06-78 at 9 a.m. (beginning of the first light period) 50 µmoles of phosphate and silicate were added to the concentrated population. This provided a non-limiting phosphate and silicate concentration of respectively 1.9 ppm P and 3.0 ppm SiO_2. During the first phase nutrient levels were

Table 2

Concentrations of chlorophyll *a* and phaeopigments before and after preconcentration

Method		Before µg chlor *a*/ℓ	After µg chlor *a*/ℓ	Concentration efficiency (%)
Strickland-Parsons		4.9	19.6	75
SCOR - UNESCO		4.8	19.1	75
Lorenzen	chlorophyll	3.3	15.0	86
	phaeopigments	2.6	7.6	55

followed by manual sampling, until the nitrogen (NO_3^- + NO_2^-) concentration approached zero. This occured near the end of the fourth light period. Therefore the second phase of the pertubation experiment started at the beginning of the light period of 01-07-78. Table 3 represents nutrient and chlorophyll a concentrations at the beginning of the second phase and of two light periods later.

Table 3

Nutrient and chlorophyll *a* concentrations during the second phase of the perturbation experiment

Date	NO_3^- + NO_2^- µg N/ℓ	NO_2^- µg N/ℓ	PO_4^{---} mg P/ℓ	Si mg SiO_2/ℓ	Biomass SC. UN. µg chlor *a*/ℓ
01-07-78 9.00 a.m.	4.0 [1] 286 [2]	1.8	1.7	1.4	36.6
02-07-78 8.40 p.m.	4.4	0.9	1.7	0.3	51.8

(1) Before spiking with NO_3^-
(2) After spiking with NO_3^-

The disappearance of nutrients was measured by automated sampling (each sample is a 25 minutes averaged sample) during the light period. From the perturbation results (figure 4) we concluded that

$V_{max} = 8.6 \times 10^{-3}\ h^{-1}$

and that

$K_s = 43$ µg N/ℓ .

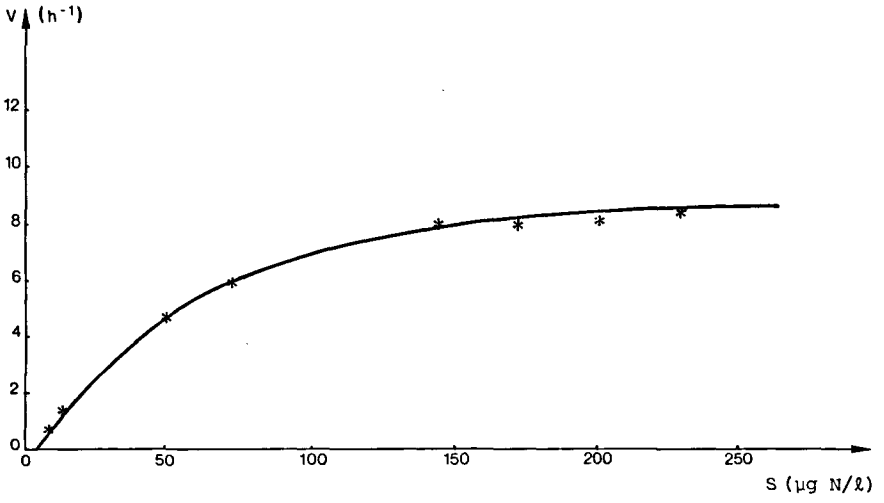

fig. 4.

Uptake rate versus substrate concentration
obtained with the perturbation technique

Though this method gave quite good results, we abandonned the perturbation concept because we can never ensure that the uptake proceeds fast enough to complete the experiment in a reasonable period of time. According to Mommaerts (personal communication, 1978), the experiment should be carried out in maximum three days to avoid unacceptable diversity changes of the population.

We thought it more realistic to start the assessment of the uptake rates, as soon as the sample was transferred to the reactor.

As we are able to adjust the input mass rate of the limiting nutrient, only gradual and relatively slow changes of the external substrate concentration are induced. In addition, such procedure has the advantage that :

(1) the biological system will anyhow be less disturbed than could possibly occur by a large injection of the limiting substrate;

(2) and that the uptake kinetics of any nutrient can be studied.

According to this new approach, nitrate-nitrite uptake rates of natural phytoplankton populations have been studied on four samples, which were collected in the period April-May 1979. The initial physico-

chemical conditions as well as parameter concentrations are summarized in table 4.

Table 4

Initial conditions of the samples

Sample	Situation and date	Temperature °C	Biomass µg chl a/ℓ S.P.	$NO_3^- + NO_2^-$ µg N/ℓ	PO_4^{---} µg P/ℓ	Si mg SiO_2/ℓ
I	W-H 19-04-79 06.00	7.5	8.48	50	27	0.4
II	W-H 25-04-79 04.00	8	7.27	35.8	23.5	0.29
III	W-H 08-05-79 04.00	8	7.48	15	24.3	0.56
IV	W-H 17-05-79 04.00	9	9.48	1.4	13.3	0.21

Because of the relatively high biomass contents, preconcentration of these samples was not necessary. As an example, the evolution of the nitrate-nitrite concentration versus the time for the experiments IV-A and IV-B, carried out respectively on the original sample IV and on a 1:1 dilution with its filtrate (0.22 µ) are shown in figure 5. The input mass rate of the limiting nutrient is sometimes higher than the uptake rate; this explains why the overall nitrate-nitrite concentration profile increases in the time.

Figure 6 gives a global picture of the various uptake rates in function of the substrate concentration, obtained for the four samples. A synopsis of reactor conditions (temperature, flows, light,...), initial parameter concentrations in the reactor, and obtained results for each experiment are given in table 5. From these results it appears that :

(a) the ratio final biomass : initial biomass ranges from 1.29 to 1.76;

(b) the evolution of the nitrate-nitrite concentration is minimum 1 µg N/ℓh and maximum 15 µg N/ℓh.

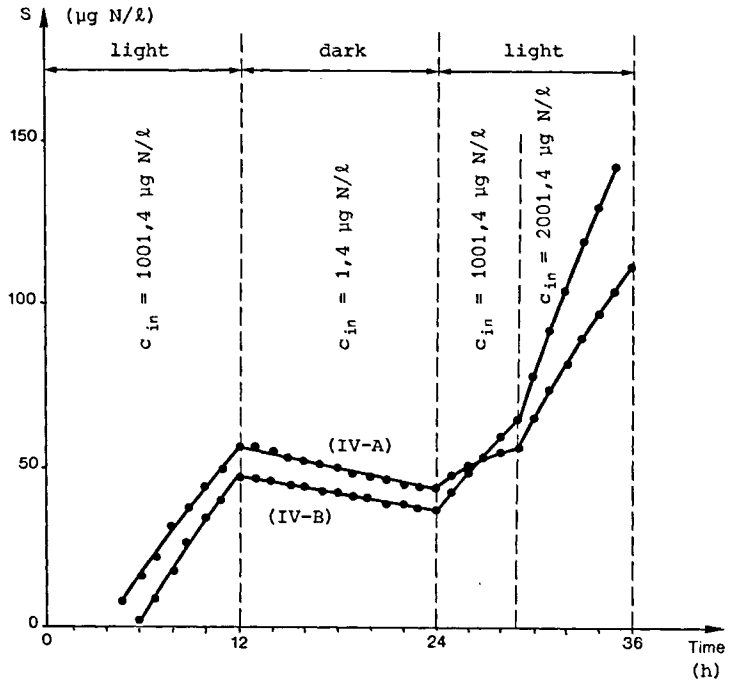

fig. 5.

Substrate concentration versus time
Experiment IV-A and IV-B

With respect to the non-perturbating simulation of nitrate-nitrite assimilation by natural phytoplankton species in a reactor, we see that the applied experimental procedure is very satisfactory. The concentrations of the two main parameters change in a gradual, moderate way, leaving at the biological system the time to adapt itself continuously.

For reasons which are explained above, most of the experiments do not cover a broad substrate concentration range. Therefore, all obtained results are brought together in one figure (figure 6), showing the relation between uptake rates and substrate concentrations. As those determinations are carried out on different samples or under different experimental conditions of nitrate-nitrite nutrition or/and phytoplankton biomass, we expected a rather large dispersion of the results. It is therefore

Table 5

Summary of the four experiments

Experiment	Start day : hour / End day : hour	Biomass µg chlor a/ℓ Initial	Biomass µg chlor a/ℓ Final	Nutrient concentrations Initial NO_3^-, NO_2^- µg N/ℓ	Initial PO_4^{3-} µg P/ℓ	Initial Si µg SiO_2/ℓ	Final NO_3^-, NO_2^- µg N/ℓ	Final PO_4^{3-} µg P/ℓ	Final Si µg SiO_2/ℓ	Input output flow rate	Input concentration NO_3^- µg N/ℓ	Concentration range	Uptake rate $\times 10^{-9} h^{-1}$
I	10.04:12.00 / 11.04:12.00	8.5	10.9	50	27	400	26.2	1500	3300	0.0096	50	25-50	5.07
II A	25.04:11.00 / 26.04:15.00	7.2	11.8	35.8	23.5	295	56	1470	3290	0.0096	1095	35-55	6.62
II B	25.04:11.00 / 26.04:19.00	7.3	9.6	35.8	23.5	295	41.2	1520	3540	0.0096	606	35-42	5.22
III A	08.05:12.00 / 10.05:11.00	7.5	11.3	15	24.3	560	*	1540	3350	0.0096	1000	15- ?	5.32
III B	08.05:12.00 / 10.05:11.00	7.5	7.5	15	24.3	560	*	1520	3380	0.0096	500	15- ?	3.0
IV A	17.05:12.00 / 18.05:19.00	9.5	16.2	1.4	13.3	210	125	1500	3100	0.0427	1001.4	8-16	2.98
												16-23	3.49
												23-30	3.99
												30-37	4.46
												37-43	4.91
												43-49	5.38
												49-55	5.82
											1.4	55-42	
											1001.4	42-46	7.15
												46-50	8.33
												50-52	9.48
												52-54	10.61
												54-55	11.14
											2001.4	55-64	12.85
												64-73	12.90
												73-81	13.11
												81-89	13.36
												89-96	13.58
												96-103	13.81
												103-110	14.04
IV B	17.05:12.00 / 18.05:19.00	4.7	8.3	1.4	13.3	210	150	1490	3050	0.0439	1001.4	2-9	2.79
												9-18	4.24
												18-26	5.69
												26-33.5	7.04
												33.5-40	8.40
												40-46.5	10.28
											1.4	46.5-36	
											1001.4	36-42	8.55
												42-48	9.45
												48-53	10.37
												53-58.5	11.24
												58.5-63	11.74
											2001.4	63-77	10.53
												77-91	11.32
												91-104	12.14
												104-117	12.94
												117-130	13.70
												130-142	14.78

* Non detectable

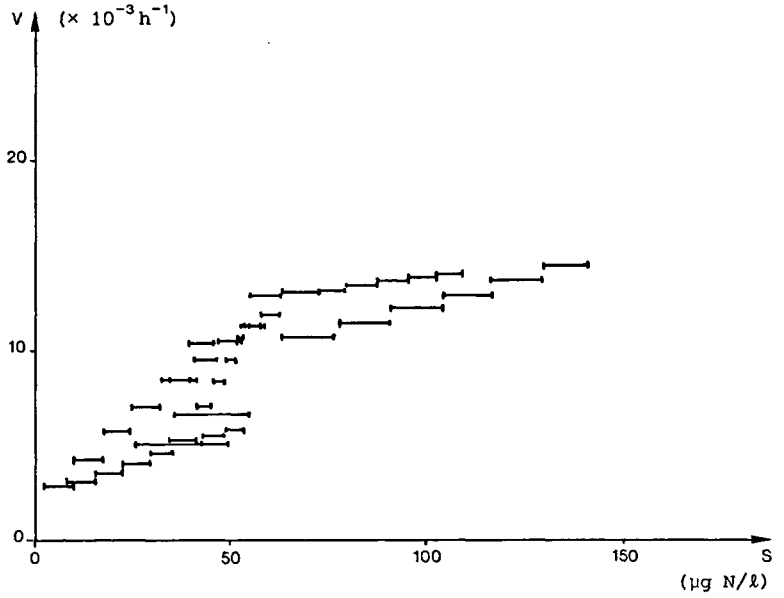

fig. 6.

Uptake rate versus substrate concentration for the four samples
(Period April-May 1979)

very surprising to notice that on the one hand the relation tends asymptotically to a maximum and that on the other hand the uptake rates are of a comparable magnitude at any choosen substrate concentration. At this moment, however, we are not able to answer the question whether such behaviour is due to

(1°) the fact that all samples were taken in the spring bloom period and hence are qualitatively quite similar or

(2°) the fact that the uptake kinetics of phytoplankton from the Belgian coastal zone obey all one and the same equation.

In the experiments IV A and IV B uptake rates are determined for a wide range of substrate concentrations. Through the set of experimental data points, the following linear transformations of the Michaëlis-Menten relation have been fitted (Mahler and Cordes, 1969; Lehninger, 1970; Falkowski, 1975) :

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_s}{V_{max}} + \frac{1}{S} \qquad \text{(Lineweaver-Burk)}$$

$$\frac{S}{V} = \frac{K_s}{V_{max}} + \frac{1}{V_{max}} \times S \qquad \text{(Woolf)}$$

$$V = V_{max} - K_s \times \frac{V}{S} \qquad \text{(Eadie-Hofstee)}$$

None of these three relations fits well with the experimental points of experiment IV A. Indeed the uptake rate versus substrate concentration curve, shown in figure 7, indicates that the curve tends to a constant value for increasing substrate concentration values. For the lower substrate concentration values, it does not follow a Michaëlis-Menten relation. Nevertheless in a graphical way, an estimation can be made for :

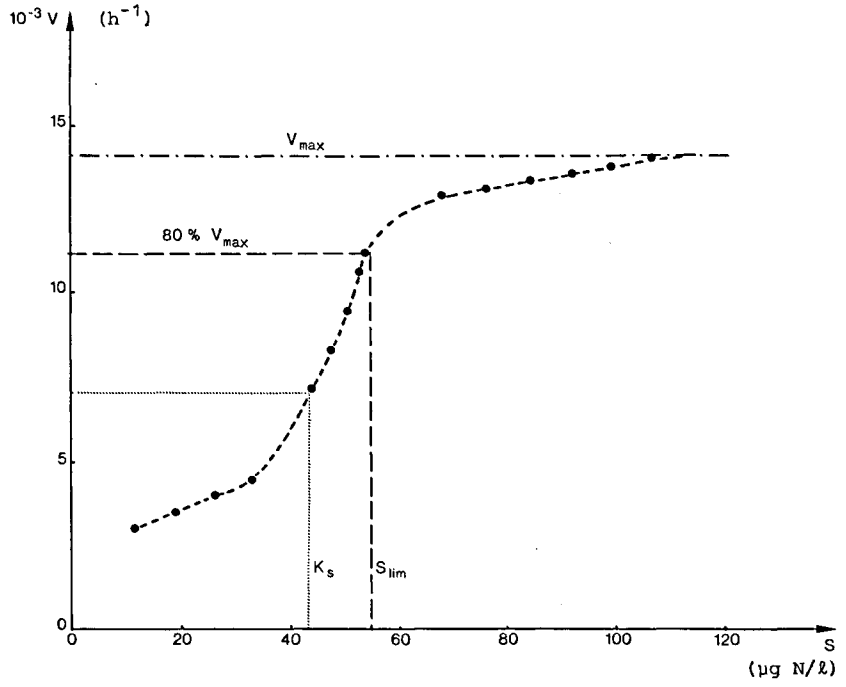

fig. 7.

Uptake rate versus substrate concentration
Experiment IV A

$V_{max} = 14.2 \times 10^{-3}\ h^{-1}$

and

$K_s = 44\ \mu g\ N/\ell$.

The uptake rate profile (figure 8), determined on half the original amount of biomass (experiment IV B), can much better be described by a Michaëlis-Menten relation.

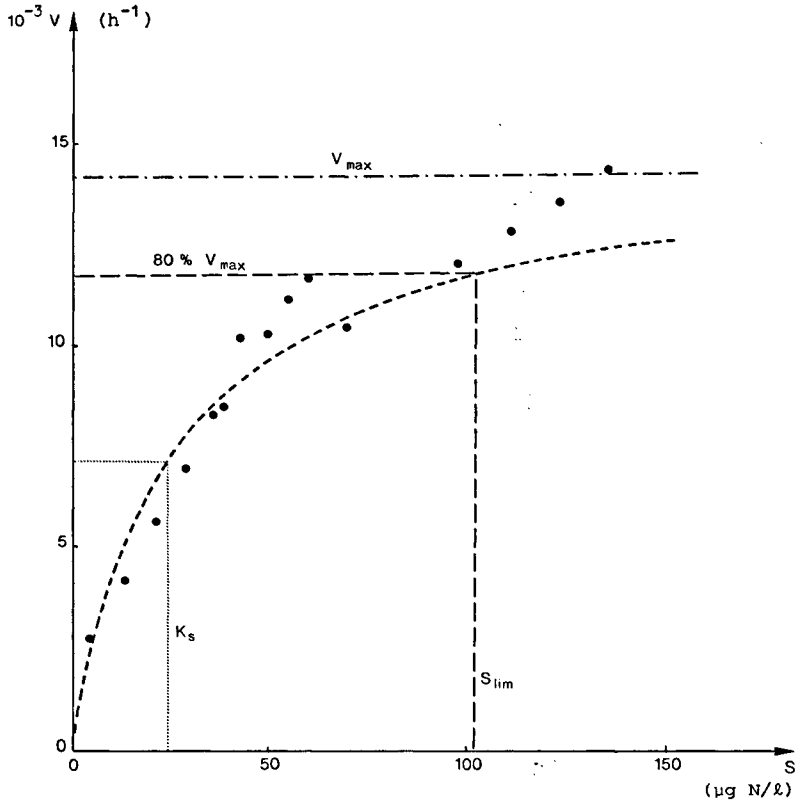

fig. 8.

Uptake rate versus substrate concentration
Experiment IV B

To determine the equation parameters V_{max} and K_s a least-squares method has been applied. Table 6 shows that the best correlations are obtained with equations (1) and (2). V_{max} ranges from 14.8 to 18×10^{-3} h^{-1} and K_s from 26.3 to 40.9 µg N/ℓ depending on the kind of equation used.

Table 6

Calculation of V_{max} and K_s
Experiment IV B

Linear transformation	Equation	Corr. coëff.	V_{max} (h^{-1})	K_s (µg N/ℓ)
(1) Lineweaver-Burk	$y = 67.53 + 1776.3\ x$	0.95	14.8×10^{-3}	26.3
(2) Woolf	$y = 2267.9 + 55.5\ x$	0.97	18×10^{-3}	40.9
(3) Eadie-Hofstee	$y = 16.20 \times 10^{-3} - 31.15\ x$	0.81	16.2×10^{-3}	31.2

Comparison of the obtained values for V_{max} and K_s are in good agreement with values for a similar eutrophic marine system, found in the literature (Eppley *et al.*, 1969; Carpenter and Guillard, 1971; Falkowski, 1975).

Finally, it is very interesting to verify if nitrate-nitrite occurs as limiting nutrient in our coastal area. Assuming there is a limitation at a substrate concentration corresponding to 80 % of V_{max}, nitrate-nitrite may become limiting at concentrations smaller than 120 µg N/ℓ. This happened for 1978 (see figure 2) :

- during the months April-May and September-October in sector II;
- during the months September-October in sector I;
- and not at all in sectors III and IV.

Conclusion

Our newly developped method seems more suitable than the perturbation technique for the assessment of the uptake kinetics of natural phytoplankton populations. Still, however, there is the uncertainty about which type of function these uptake kinetics obey. Uptake rate versus substrate concentration curves, determined in one single experiment as well as the overall uptake pattern, which is obtained from the total set of experiments

(figure 6), do not exclude a Michaëlis-Menten relation. An increased number of experiments, allowing a more elaborated statistical treatment, could possibly clarify this problem.

It is also clear that not only the period of April-May has to be considered. To perform uptake kinetics on North Sea samples, September and October seem to be favourable months as well.

Moreover, until now we only considered $NO_3^- - NO_2^-$ as limiting nutrient. However, with our dynamic procedures we are able to study the uptake kinetics of any substrate. For the study of the uptake kinetics for other substrates and for other periods of the year, a more suitable biomass determination should be developped, allowing us a higher frequency of biomass determination.

Acknowledgment

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