ACTIONS CONCERTEES EN OCEANOGRAPHIE

RAPPORT D'ACTIVITE

1978

ANNEXE N° 11

Concentration and microbiological utilization of small organic molecules in the Scheldt estuary, the belgian coast zone of the North sea and the english channel

CONCENTRATION AND MICROBIOLOGICAL UTILIZATION OF SMALL ORGANIC MOLECULES IN THE SCHELDT ESTUARY, THE BELGIAN COASTAL ZONE OF THE NORTH SEA AND THE ENGLISH CHANNEL. (1)

Gilles BILLEN (2)
Claude JOIRIS (3)
Jan WIJNANT (3)
Gérard GILLAIN (4)

~~55479~~

55480

SUMMARY

Concentration and utilization rate of alanine, aspartate, lysine, glucose, acetic and lactic acids have been determined at six occasions at least, during a whole seasonal cycle in the water column of three stations (Scheldt estuary, coastal North Sea and English Channel) greatly differing in biological characteristics.

No significant differences in substrate concentration could be detected between the three stations, although the rate of utilization of all substrates greatly differed, decreasing in the order estuarine > coastal > open sea environment.

(1) - Contribution nr 1 of the "Organic matter" group of the "Actions de Recherche Concertées en Océanographie", Ministère belge de la Politique Scientifique.
(2) - Laboratoire d'Océanographie, Université de Bruxelles (Belgiu
(3) - Laboratorium voor Ekologie en Systematiek, Universiteit van Brussel (Belgium)
(4) - Laboratoire d'Océanographie, Université de Liège (Belgium)

This apparent paradoxe is explained by aid of a very simple model showing that the steady state concentration of a particular substrate is independant on its rate of production (and thus of consumption) but only depends on purely physiological characteristics of the bacteria. When using published data for the pertinent physiological parameters of marine bacteria, the model accounts for at least the order of magnitude of substrates concentration observed, and the lack of important seasonal variations.

INTRODUCTION.

Growing evidences show the quantitative importance of planctonic bacterial heterotrophic activity in the carbon cycle of marine ecosystems: Although it has often been assumed in the past that most of the produced organic matter flow s through the zooplankton-fish trophic chain, it now becomes evident that planktonic bacterial activity constitutes, at least in some coastal marine environments, a very important by-pass. (JOIRIS, 1977)

Yet, too few research efforts have been devoted to the basic relationships between organic matter and bacterial heterotrophic activity. In most ecological models it is simply assumed that heterotrophic activity is directly proportional to "total organic load", determined as BOD, COD or TOC.

Most of this organic load (75-99%) in sea water is known to exist as dissolved organic matter (Sharp, 1973). However, dialysis or ultrafiltration reveals that the most important part of dissolved organic matter in sea water is made of macromolecules with molecular weight higher than 400-500 (See table I).

The penetration of an organic molecule across a bacterial cell wall is an active process occuring with the intervention of specific enzymes called permeases. Only low molecular weight organic molecules (monomers or small polymers) can therefore be taken up. Only through the production of exoenzymes can particles or high molecular weight molecules be ultimately absorbed by bacteria. (Rogers, 1961).

Phagocytosis of particles is not developped by bacteria. Therefore the pool of directly usable organic matter is made of the pool of low molecular weight organic molecules. This pool is alimented either by direct excretion of phytoplancton or by exoenzymatic hydrolysis of particles and macromolecules.

A promising approach for showing the relationship between organic matter and heterotrophic activity in aquatic ecosystems consists therefore in determining the in situ concentration and utilization rates of directly usable low molecular weight organic molecules.

This paper presents the results of such determinations performed comparatively in three marine environments greatly differing in their overall biological characteristics ; an estuarine, a coastal and an open sea environment. Among the very long list of possible organic substrates, we selected those which seemed to us the most representative : amino acids (Asparte, Alanine and Lysine beeing selected as the representative examples of acid, neutral and basic aminoacids for utilization rate measurements), glucose (as a biologically representative carbohydrate monomer), glycollic acid (which is oftèn considered as one of the most important excretion product of phytoplancton (Fogg, 1962) , lactate and acetate (which are important fermentation products).

The results will than be interpreted with the aid of a very simple model intended to show the -not always trivial- link between the basic physiological laws of the microbiological processes involved and their overall ecological manifestations as observed in situ.

BIOTOPES and METHODS

1. ENVIRONMENTS STUDIED

Fig. 1 shows the three stations considered in this study. The estuarine station, Hansweert, is located on the Scheldt estuary, at 35 km from the sea. Depending on the tides, salinity varies between 19 and 27‰.
The station "Ostend" (51°24'00"N, 2°48'00"E), is located in the belgian coastal zone of the North Sea.
Salinity in this zone ranges from 30 to 34‰ and the mean depth is 15 m.
The station "Channel" (50°57'30"N, 1°23'30"E), located on the eastern part of the English Channel, is characterized by Atlantic waters with salinities higher than 34‰.

These three stations were visited 6 times between juli 1977 and juli 1978, generally at noon. In addition, some parameters were measured several times at closer intervals at the coastal and Channel stations during special cruises in october 1977 and april 1978.

2. COLLECTION, TREATMENT AND CONSERVATION OF THE SAMPLES.

Samples were collected at 3 m depth with polyethylene Nyskin's bottles.
Biological incubations were immediately initiated.
Samples for chemical analysis were filtered through GF/C Whatmann glass fiber filter.

For amino-acid, glucose, lactate and acetate determinations, the filtrated samples were immediately deep-frozen in poly-ethylene flasks.

Adsorption on alumina for glycollate determination occured immediately; alumina was then kept deep-frozen until analysis.

3. CHEMICAL DETERMINATION.

For amino-acids determination, desalting of the sample is achieved on ligand-exchange resin (Chelex 100, Bio-Rad) converted to the Cuform (Siegel & Degens ; 1966 ; Andrews and Williams , 1971 ; Clark , 1971).
Two liters of sample are passed through the column at a flow rate of 3 ml/min. After washing with distilled water, the amino-acids are eluted together with the copper with 160 ml 3M/NH_4OH. Cu is eliminated by further passing the solution on a Chelex 100-NH_4^+ column. After evaporation, the amino acids are redis-solved with 3 M HCl and a final desalting is carried out on Dowex 50 (H^+ form). The column is then rinsed with distilled water and eluted with 80 ml of IM NH_4OH. The ammoniacal eluate is com-pletely evaporated to remove the ammonia. The amino acids are then dissolved in I ml of citrate buffer for automatic high pres-sion ion exchange chromatography (BECKMAN, model 120).

Blank test were made with ultra-violet irradiated seawater following the same procedure and using the same volumes as for the analysis of the seawater samples.
The contamination from distilled water or from the resins is not significant : serine and glycine give a blank equivalent to 0.007 µM/1. For each of the remaining amino acids, the blank is less than 0.001 µM/1.
The recovery of the whole procedure was tested with standard solution of amino acids in artificial sea water. The recovery of all amino acids was found in the range 80 to 107 %, except for glutamine (33 %) and methionine (56 %). This percentage recovery is used to correct the results from natural seawater samples.

For glycellic acid, a quite specific colorimetry has been described by Calkins (1943), based on the reactions of formaldehyde, formed by oxidative decarboxilation of glycollic acid in concentrated H_2SO_4, with 2.7 dihydroxynaphtalene. Concentration of glycollate from natural samples can be achieved by adsorption on alumina, followed by desorption with concentrated acid as proposed by Shah and Fogg (1973) and by Shah and Wright (1974). The procedure of the latter authors has been adopted with only minor modifications.

For glucose, acetate and lactate determinations, enzymatic methods were developped by modification of the methods used in food analysis (Boehringer-Manheim, 1976). Optimalisation of sample and reagents volumes, and use of fluorimetry instead of spectrophotometry allow to determine the three substances down to a concentration of 0.1µM. The detailled procedures used are summarized in table I. Dawson and Gocke (1977) have stressed the point that trace metal complexation may serve to render an organic substrate unavailable to microorganisms. The use of enzymatic processes, performed with minimal treatment of the sample guarantees that the measured concentration of organic substrates is representative of the stock actually available for biological utilization.

4. DETERMINATION OF THE UTILIZATION RATE.

The rate of individual organic substrates incorporation on the one hand, and respiration on the other hand, were determined by use of high specific activity ^{14}C labelled substrates.

The following amount of radioactivity were added to 250 ml of freshly collected sea water :

0.1µCi Alanine	(sp.act.	100	µCi/ mole)
0.1µCi Aspartate	("	215	")
0.1µCi Lysine	("	340	")
0.1µCi Glycollate	("	10	")
0.1µCi Glucose	("	240	")

1μCi Acetate (sp. act. 50 μCi/μ mole)
1μCi Lactate (" 30 "),

so that the amount of substrates added can be considered negligible with respect to natural concentrations. The samples are then incubated in the dark at in situ temperature. At intervals during 4 hours, subsamples of 20 ml are collected and filtered through 0.2μ membrane filters. The radioactivity retained on the filter is counted by liquid scintillation and provides an estimation of substrate incorporation by microorganisms. The filtrate is collected in 2N NaOH; on return to the laboratory, it is acidified and bubbled for extracting the CO_2 which is then trapped (Fig. 2) in a mixing of Carbo-sorb (Packard) and Lipoluma (Lumac) (1:4) for radioactivity counting. This provides an estimation of substrate respiration by microorganisms. An eventual fermentation of the substrate into another organic molecule would not be detected by this method.

Examples of these measurements are shown on Fig. 3. As seen, the incubation was usualy prolonged during 4 hours, but sub-samples were collected and treated every 15 or 20 minutes during the first hour, in order to obtain an acurate picture of the initial trends of incorporation and respiration. The curves obtained as a function of time are sometimes linear from the beginning of the experiment. Most often, however, the incorporation curve presents a small convexity, and the respiration curve, a small concavity during about the first hour of incubation, before becoming linear

Hobbie and Crawford (1969) and Williams (1970) report that they did not observed any lag in the respiration of the organic substrates. To our knowledge, however, they did not use incubation times as short as ours, so that such a phenomena could have escaped notice. The existence of this lag in the respiration curve can be interpreted by considering that the production of $^{14}CO_2$ by respiration is under the dependance of the specific radio - activity of the intra-cellular pool of the substrate considered, which requires some time to reach a stable value. Conversely, the incorporation curve, in its initial part represents the total uptake of the substrate, while its further linear part represents the net uptake, used for biosynthetic purposes.
If this interpretation is correct, the slope of the tangent to the incorporation curve at initial time must be equal to the sum of the rate of biosynthetic incorporation (given by the slope of the curve in its linear part) plus the rate of respiration (given by the slope of the respiration curve in its linear part).
In a few situations, the data for this comparizon are available; table II shows that the accordance is quite good, giving support to our interpretation.
Table III summmarizes for the different substrates the ratio between incorporation and total uptake (=incorporation plus respiration) that we measured in this study.

For most substrates, this ratio (called "growth yield" by Williams 1970) ranges between .30 and .40, indicating a mineralization of about 2/3 and a metabolisation of about 1/3 of the substrate taken up. Only for glycollate is the growth yield far lower, indicating that this substrate is not a good biosynthetic substrate for heterotrophs. Although these figures are quite reasonable in the light of general physiological knowledge (Forrest, 1969), they are considerably lower than most similar data reported in the literature. Most authors reported growth yields of about .66-.87 for amino acids, .51-.86 for glucose, .36-.39 for glycollate and .67 for acetate (Hobbie and Crawford, 1969; Williams, 1970; Wright and Shah , 1975; Williams et al, 1976; Stanley and Staley, 1977; Dawson and Gocke, 1978.

As will be indicated the mean values of the growth yield we measured, shown in table III , have been used for calculating total utilization rates in the situations where only incorporation data were available.

RESULTS .

1. CONCENTRATION OF SUBSTRATES

Table IV a and b presents the values of amino acids, glycollate, glucose, acetate and lactate concentration measured in the three stations considered, over an annual cycle. As can be seen, no significant seasonal variations appears from these values, which

fluctuate, apparently at random, whithin a rather narrow range: 0.01 - 0.2 μmoles/l for the main amino acids, 1 - 5 μmoles/l for glycollic acid, 0.01 - 0.1 μmole/l for glucose, 0.2 - 5 μmole/l for acetic and lactic acids. Surprisingly, these ranges are the same for the 3 environments under study, in spite of the great differences in ecological characterististics between then. Only amino acids at the station "Channel" display significantly higher values than at the 2 other stations.

These values are comparable with those found by various authors, either in open sea, coastal, estuarine- or freshwater environments. Thus , individual amino acid concentrations are generally found in the range 0 - 0.2 μmole/l (see Williams, 1975; Hobbie et al, 1968; Chau and Riley, 1966; Dawson and Gocke, 1978;).

Glycollate concentrations in the range 0 - 2 μmole/l are reported by Anitia et al (1963), Wright and Shah (1975), Al Hasan (1975) Shah and Wright (1974).

Glucose is generally presents on concentrations between 0.005 and -2 μmole/l (Andrews and Williams, 1971, Degens et al 1964; Josefson, 1970).

Acetate concentration between .1 and 10 μmole have been reported by Koyano et al (1959) and Stanley and Staley (1977).

The lack of systematic important differences in the concentration of small organic substrates in all natural waters is a quite remarkable fact.

2. RATE OF UTILIZATION.

Table V presents the values of total utilization rates (incorporation plus respiration) of the 7 substrates as determined in the 3 stations considered and expressed as a percentage of added radioactivity used per hour. This can be used as a measure of the turnover rate of the substrate.

Seasonal variations are not very evident, although maximum values in the late spring or the summer are often observed. Geographical variations, on the other hand are quite clear. All substrates are much more rapidly used in the estuarine than in the coastal station. In the open sea, the utilization rates are still smaller and often lie close to the detection limit of the method.

Substrate to substrate comparizon shows that amino acids have high, and similar turnover rates. Glycollate has considerably lower turnover rates. However, concentration being far higher, the absolute rate of utilization is most often of the same order of magnitude as this of the glucose or aminoacids. Glycollate being often considered as one of the main excretion products of phytoplancton, this is important for assessing the role of phytoplanctonic excretion as a source of directly usable organic substrates for heterotrophic bacteria.

Acetate and lactate turnover rates are quite similar to these of amino acids and glucose. As far as is known, these acids are not important excretion products of algae (Fogg, 1962), but are important products of fermentative matabolisms. Their presence and utilization at high turnover rates in oxygenated sea water are therefore an indication of the occurence of fermentation metabolisms in these environments.

DISCUSSION.

The most important conclusion of this investigation results from the lack of correlation observed between the trends of geographical variations of substrates concentration on the one hand, and of utilization rates on the other hand : substrates concentrations are not very different in the 3 stations considered, while utilization rates differs greatly in the order estuarine > coastal > open sea stations.

This might seem parodoxal at first look because - both implicitely in the mind of most microbiologists and explicitely in numerous models-, concentration of substrates is generally considered as directly determining the rate of heterotrophic activity.

In his general review of literature data concerning concentrations and utilization rates of dissolved organic compounds in sea water, Williams (1975) already stressed the similarity in free amino acids concentrations in estuarine, coastal and oceanic waters, in spite of great differences in their rate of uptake by microorganisms in these three environments. He also indicated that increases of free amino acids concentrations, as a result of planctonic activities can occur, but do not persist for longer than a few days, and are not likely to be observed, unless very intensive sampling programs are performed.

All these facts suggest a close regulation of individual substrates concentration by microbial activity. The following idealized model is intended to investigate the possible mechanisms and properties of this regulation.

1. MODEL OF SUBSTRATE UPTAKE.

Let us consider a particular directly usable substrate S, produced at a rate P either by exoenzymatic hydrolysis of macromolecules or by phytoplanctonic excretion, and taken up by the present bacterial population at a rate determined by substrate concentration S and by bacterial density B (Fig.4.). Let us use a Michaelis-Menten relation between individual uptake by a single bacterium and substrate concentration.

This hypothesis is made for the sake of clarity of the foregoing calculations, but another hypothesis would not qualitatively alter the conclusions.

The rate of production P will be treated in this very simple model as a driving parameter.

The rate of variation of substrate concentration can therefore be written :

$$\frac{dS}{dt} = P - \frac{Vm\ S}{Km+S} B \qquad (1)$$

If it is supposed that the bacteria grow with a constant yield factor Y relative to their uptake of the substrate, and that their mortality is random, i.e. proportional to their density, the variation rate of the bacterial population can be written :

$$\frac{dB}{dt} = Y \frac{Vm\ S}{Km+S} B - kB \qquad (2)$$

When a stationary state is reached, the differential equations (1) and (2) are reduced to the algebric system

$$P \frac{Vm\ S}{Km+S} B = 0 \qquad (3)$$

$$Y \frac{Vm\ S}{Km+S} B - kB = 0 \qquad (4)$$

of which the solution is

$$B = Y/k \quad P \qquad (3)$$

$$S = \frac{Km}{\frac{Vm\ Y}{k} - 1} \qquad (4)$$

It is seen that at stationary state, substrate concentration is independant on its rate of production. It only depends on purely physiological characteristics of the bacteria. The density of bacteria on the other hand, is directly affected by the rate of substrate production and this alone explains that total utilization rate is equal to the production rate of the substrate.

2. VALIDITY OF THE STEADY STATE ASSUMPTION.

The time evolution of the system before a stationary state is reached is illustrated for a few situations in Fig. 5 , 6 and 7 . Use of reduced variables allows to describe this evolution without hypothesis about the numerical value of the physiological parameters.

Fig. 5 shows the evolution of substrate concentration and number of bacteria from the stationary state after a sudden increase of the production rate. After a period of time corresponding to one turnover time of the bacterial population, the concentration of the substrate reaches a maximum.

It decreases then very rapidly down to the steady state value, independant, as has been stated, on the new production rate. The number of bacteria increases, during two turnover time, and then decreases again down to its new steady state, depending on the production rate.

Fig. 6a presents the evolution of substrate concentration and bacterial density after a sudden increase of substrate concentration, without modification of the production rate P. This is for instance the case of an instantaneous dumping of the substrate in a system previously at steady state. It is seen from Fig.6a that substrate concentration decreases to low values in a period of time corresponding to about one turnover time of the bacterial population, independently of the value of the initial concentration increase. Similar results have been obtained both experimentally and theoretically by Williams & Gray (1970). They stressed the point that the absolute rate at which added amino acids or glucose were respired was proportional to the quantity of substrate initially added.
Fig. 6b shows indeed that the maximum bacterial density - and therefore the maximum uptake rate- reached after a sudden increase of substrate concentration is proportional to this increase.

Fig. 7 shows the effect of sinusoïdal variations of substrate production : this would be the situation if the production rate of the substrates was under direct and immediate influence of the photosynthetic activity (light-dependent phytoplanctonic excretion, for instance).

The results of fog. 7 shows that under this circomstances, daily variations occur both in number of bacteria and in substrate concentration around the value of the stationnary state corresponding to the daily mean production rate. These variations, however, are rather limited (less than 30 %) as long as the maen turnover rate of the substrate is less than 6 $\%h^{-1}$ (case of fig. 6a and b). They are only likely to be observed in environement supporting very high rates of organic substrates production (and consumption), where the turnover rates exceeds 10 $\%h^{-1}$(case of fog. 6c).

In the frame of this model, one can conclude that substrate concentration and bacterial density in natural environment can be considered in most situations as being in steady state with respect to substrate production rate : after a sudden perturbation, steady state conditions are restored within about one day (one turnover time of the bacterial population); when periodic fluctuations occur in the production rate, even with a period equal to the turnover time of the bacteria, substrate concentration and bacterial density do not vary a lot around their steady state value.

3. QUANTITATIVE PREDICTIONS OF THE MODEL.

The model must now be checked on a quantitative basis. Relation (4) expresses the stationary state value of substrate concentration as a function of physiological characteristics of bacteria.

In order to compare this expression with our own observations, we have to discuss the numerical value of these physiological parameters.

Km values for different substrates have been determined in sea water by numerous authors (see for instance the review of Seepers, 1977). Most measurements lie in the following ranges :

Amino-acids	: 0.03 - 1	µmole/l	(Seepers,1977)
Glucose	: 0.01 - 0.3		(Seepers,1977)
Glycollate	: 0.6 - 3.0		(Wright & Shah, 1975)
Acetate	: 0.1 - 5		(Seepers, 1977; Stanley & Staley, 1977)

Y, the yield coefficient, expressing the number of bacteria formed per mole of substrate utilized can be calculated as :

$\frac{\alpha}{C}$ (where α represents the part of total substrate taken up used for biosynthetic purposes, and C the carbon content of a single bacterial cell).

α is generally about 0.3 to 0.4 (see table III), while Q is about 0.25 - 0.5 10^{-8} µmoleC/$_{\text{bact.}}$ (Stanier et al, 1970)

Y is therefore about 10^{8}bact/$_{\mu\text{moleC}}$.

Vm, the maximum uptake rate per bacterium has been determined for marine strains in a few cases.

White (1969), working with Southampton water, and Hobbie and Wright (1965) in Lake Erken, both report values about 0.1 10^{-8} µmole C/bact.h

Gocke (1975), working in the German Bight of the North Sea, estimated it as 0.03 and 0.07 10^{-8} µmole $C/_{bact.h.}$ for glucose and acetate respectively.

On the other hand, Jannasch (1967 and 1968) has measured the maximum growth constant of marine bacteria when incubated with glucose or lactate as the sole substrate. His measurements are in range 0.15 - 0.8 h^{-1}. Taking into account the value of Y just discussed, the corresponding maximum rates of uptake are in the range 0.15 10^{-8} - 0.8 10^{-8} µmole $C/_{bact.h.}$

As far as we know, very few data exist in the literature concerning the mortality of bacteria. An estimation of mortality constant can however be obtained from the rate of decrease of bacterial density in environmental situations where growth is stopped. The measurements of Joiris (1973) in a closed marine lagoon for instance allow a mortality constant of about 0.12 h^{-1} to be calculated. As this probably represents mortality in adverse conditions, the value of $k = 0.05\ h^{-1}$ has been chosen here for the mortality constant in natural conditions.

With these values of the physiological parameters (Y about 10^8 bact/$_{\mu mole\ C}$; Vm about 0.2 10^{-8} μmole C/$_{bact.h.}$, k about 0.05 h^{-1}) a reasonnable estimate of the steady state value of substrate concentration can be made according to relation (4)

$$S = \frac{Km}{\frac{Vm\ Y}{k} - 1} \simeq \frac{Km}{3}$$

which provides the following results :

Amino acids	:	0.01 - 0.3	μmole/l
Glucose	:	0.003- 0.1	
Glycollate	:	0.2 - 1	
Acetate	:	0.03 - 2	

These ranges are indeed quite compatible with the measurements reported here (see table IV) and in the literature.

The very simple model discussed here explains thus both qualitatively and quantitatively our observations :

- Qualitatively, it explains why substrates concentrations are not very different in estuarine environments, characterized by high heterotropic activities and in open sea, with only very low heterotrophic activity, by showing that the steady state concentration of a substrate is independant of its rate of production.

- Quantitatively, it accounts for at least the order of magnitude of substrates concentration observed, as well in this study as in most other investigations published in the literature.

Aknowledgments.

Part of the experimental work was done on board of the German R.V. "Friedrich Heincke" (7 to 10 october 1977, 7 to 18 april 1978), the rest on board of the R.V. "Mechelen".

We are pleased to aknowledge the technical assistance of R. VANTHOMME and R. SWAELENS, Vrije Universiteit Brussel.

TABLE I Ultrafiltration or dialysis of dissolved organic matter.

Biotope	Fraction	%	Authors
Sea water			
Tokio bay	< 500	27	Ogura, 1974
Georgia coastal waters	< 1000	12	Wheeler, 1976
Sea water	< 400	10	Degens, 1968
Ucha reservoir (USSR)	< 400	10	Brger, 1968
Ucha reservoir (USSR)	< 300	8	Mirkina, 1977
Black sea	< 1500	69	Mirkina, 1977
Surface film (Black sea)	< 200	38	Starikova & al, 1977

TABLE II : Comparizon between the total rate of utilization (in %/hour) of some organic substrates estimated:

a) by the sum of incorporation rate (estimated from the slope of the incorporation curve in its linear part) plus respiration rate (estimated from the slope of the respiration curve in its linear part).

b) from the slope of the tangent of incorporation curve at initial time.

(see text for explanation).

STATION	SUBSTRATE	a			b
		INCORPORATION	RESPIRATION	TOTAL UTILIZATION	TOTAL UPTAKE
Ostende 18.7.77	Glycollate	.096	1.03	1.13	1.12
Hansweert 19.7.77	Glycollate	.32	1.8	2.12	2.0
Hansweert 21.10.77	Glucose	1.23	4.6	5.83	4.6
Ostende 18.7.77	Acetate	2.81	1.75	4.56	4.85
Hansweert 21.10.77	Acetate	2.1	7.9	10.0	5.07
Ostende 18.7.77	Lactate	0.68	0.92	1.60	1.63

TABLE III : mean value and range observed for the ratio of incorporation rate to total utilization rate(growth yield) of various organic substrates.

SUBSTRATE	MEAN	RANGE	NUMBER OF DETERMINATION
Amino acids (1)	.32	.16-.50	17
Glycollate	.15	.05-.25	8
Glucose	.35	.20-.50	7
Acetate	.41	.20-.60	12
Lactate	.38	.15-.60	7

(1) measured with Alanine, Apartate and Lysine.

TABLE IVa : Concentration of organic substrates in the water of the three station under study (in μmole/l)

HANSWEERT

	Ala.	Asp.	Lys.	Glyc.	Gluc.	Acet.	Lact.
19.7.77	0.084	0.033	0.021	2.0	-	0.2	0.2
21.10.77	0.042	0.013	0.014	-	0.06	0.2	0.2
7.4.78	0.044	0.024	0.010	4.5	0.08	1.5	-
21.4.78	0.020	0.015	0.010	4.5	0.08	3.3	-
19.5.78	0.054	0.010	0.010		0.05	0.2	-

OSTENDE

	Ala.	Asp.	Lys.	Glyc.	Gluc.	Acet.	Lact.
18.7.77	0.050	0.019	0.036	1.8	-	-	1.6
18.10.77	0.021	0.031	0.030	-	-	0.2	4.9
5.4.78	0.015	0.028	0.010	3.0	0.02	2.4	
19.4.78	0.010	0.020	0.010	3.1	0.02	1.3	
16.5.78 12h	0.049	0.019	0.015		0.05	0.2	0.2
24h							0.2
11.7.78					0.04		

CALAIS

	Ala.	Asp.	Lys.	Glyc.	Gluc.	Acet.	Lact.
26.7.77	0.141	0.065	0.028	0.9			
19.10.77	0.176	0.100	0.034	-	0.04	4.0	
4.4.78	0.036	0.020	0.031	2.3	0.01	0.2	
18.4.78 12h	0.036	0.010	0.010	2.3	0.005	0.2	
24h	0.010	0.015	0.020		0.005		
17.5.78	0.015	0.012	0.020		0.01	0.2	1.6
12.7.78							0.6

	Asp	Thr	Scr	GluNH$_2$	Pro	Gly	Ala	Val	Cys	Met	Isolev	Lev	Tyr	Phe	Lys	His	TOTAL
Hansweert																	
19.07.77	.033	.040	.240	.036	.052	.243	.084	.030	<.010	<.010	.015	.018	.022	.014	.021	.013	.881
21.10.77	.013	.079	.080	.030	.034	.133	.042	.030	<.010	<.010	.020	.022	.014	.010	.014	.015	.536
07.04.78	.024	.012	.124	<.010	<.010	.096	.044	.025	.013	<.010	.015	.018	<.010	<.010	.010	.010	.420
21.04.78	.015	.050	.070	.022	.014	.062	.020	.015	.010	.010	<.010	<.010	<.010	<.010	<.010	<.010	.300
19.05.78	.010	.020	.017	.010	.010	.052	.054	.010	.010	.010	.010	.012	.015	.015	.010	.010	.275
Ostende																	
18.07.77	.019	.020	.140	.060	.21	.060	.050	.020	<.010	<.010	.018	.012	.020	.020	.036	.029	.545
18.10.77	.031	.030	.123	.079	.029	.046	.021	.028	<.010	<.010	.020	.018	.020	.025	.030	.021	.540
05.04.78	.028	.015	.030	.010	.016	.160	.015	<.010	.010	<.010	<.010	.010	.010	.010	.010	.010	.330
19.04.78	.020	.016	.030	<.010	<.010	.160	.010	<.010	<.010	<.010	<.010	<.010	<.010	<.010	<.010	<.010	.230
16.05.78																	
12h	.019	.016	.090	.154	.041	.383	.049	.019	<.010	<.010	.010	.010	<.010	<.010	.015	.076	.922
Calais																	
26.07.77	.065	.066	.392	.030	.047	.350	.141	.048	<.010	<.010	.028	.034	.023	.014	.028	.028	1.314
19.10.77	.100	.084	.508	.085	.186	.426	.176	.024	<.010	<.010	.034	.044	.052	.040	.034	.030	1.843
04.04.78	.020	.052	.040	.030	<.010	.100	.036	<.010	<.010	<.010	.020	.024	.018	.015	.031	.032	.450
18.04.78																	
12h	.010	.025	.010	<.010	<.010	.030	.036	<.010	<.010	<.010	.025	.010	.012	.016	.010	.016	.300
23h	.015	.025	.010	<.010	<.010	.412	.010	<.010	<.010	<.010	.025	.020	.015	.010	.020	.024	.586
17.05.78																	
12h	.012	.010	.032	.010	.010	.213	.015	.010	<.010	<.010	.010	.010	.010	.010	.020	.010	.402
24h	.010	.010	.020	.010	.015	.015	.012	.010	<.010	<.010	.010	.010	.020	.013	.015	.013	.203

Table IVb : Amino Acids concentrations in the water of the three stations under study (in µmole/1)

TABLE V : Relative rate of total utilization (incorporation plus respiration) of organic substrates (in %/hour)

HANSWEERT

	ALA	ASP	LYS	GLYC.	GLUC.	ACET	LACT.
19.7.77	-	-	-	1.8 (o) 2 (+)	-	27 (o)	5.4(o)
21.10.77	4.9 (+)	5.1 (+)	7.6 (+)	0 (o)	5.8 (o) 4.6 (+)	10 (o) 5.1(+)	.67(+)
7.4.78 10h	2.3 (&)	1.2 (+)	3.2 (+)	1.6 (+)	3.1 (&)	5.1(+)	.85(+)
21.4.78 10h	4.1 (&)	4.4 (+)	3.6 (+)	3.0 (&)	2.7 (&)	10.2(+)	1.3(&)
19.5.78 10h	7.2 (&)	0 (+)	0 (+)	2.0 (&)	6.5 (+)	11.8(&)	1.5(&)
in	4.6	2.7	3.6	1.7	4.4	12.3	1.9

OSTENDE

	ALA	ASP	LYS	GLYC.	GLUC.	ACET	LACT.
18.7.77				1.1(+)(o)	-	4.56(o) 4.85(+)	1.60(o) 1.63(+)
7.8.10.77	0 (+)	.39(+)	0 (+)	.44(+)	.46(+)	0 (+)	0 (+)
18.10.77	0 (+)	.93(&)	1.8 (&)	0 (+)	1.7 (&)	.24(&)	.24(&)
5.4.78	.75 (+)	.72(&)	3.3 (+)	.18(+)	1.1 (+)	.15(+)	.11(+)
2.19.4.78	-	-	-	-	-	-	.20(+)
19.4.78	5.0 (+)	5.7 (+)	.65(&)	.20(&)	1.3 (&)	.17(&)	.03(&)
16.5.78 12h	1.9 (&)	3.9 (&)	7.1 (&)	0 (+)	12.3 (+)	1.7 (&)	4.8 (&)
24h	-	-	-	-	-	-	-
11.7.78	0. (+)	0 (+)	.26(+)	0 (+)	0 (+)	.04(+)	0 (+)
in	1.2	1.5	2.2	.27	2.8	1.0	.87

CALAIS

	ALA	ASP	LYS	GLYC.	GLUC.	ACET	LACT.
26.7.77	-	-	-	0 (+)	-	.07(+)	.05(o)
9.10.10.77	2.4 (&)	.62(+)	1.5 (&)	.46(+)	.21(+)	.16(+)	.13(&)
19.10.77	0	0 (+)	.22(&)	0 (+)	0 (+)	.04(+)	.04(&)
4.4.78	.44 (&)	0 (+)	1.1 (+)	0 (+)	.83(&)	.07(+)	.01(&)
3-18.4.78	.50 (&)	-	-	-	-	-	.05(&)
18.4.78 12h	0 (+)	0 (+)	.19(&)	0. (+)	0 (+)	0 (+)	.01(&)
24h	.12 (+)	0 (+)	.56(+)	0 (+)	0 (+)	.10(+)	.03(&)
17.5.78	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)
12.7.78	0 (+)	-	4.8 (&)	0 (+)	.49(&)	-	.03(&)
in	.43	.10	1.2	.06	.22	.06	.04

(o) sum of incorporation and respiration measured separately
(+) total uptake estimated from the slope of the tangent to the incorporation curve at initial time
(&) total utilization estimated from the value of incorporation and the mean ratio incorporation/total utilization of table III.

REFERENCES

ANDREWS,P & WILLIAMS, P.S.leB, 1971.- Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of the oxidation rate and concentration of glucose and amino acids in sea water. J.Mar.Biol.Ass.U.K. 51, 11-125.

BOEHRINGER-MANHEIM,1977. Methods of Enzymatic Food Analysis. Boehringer Manheim GmbH, Biochemica.

CALKINS V.P., 1943.- Microdetermination of Glycolic and oxalic acids. Ind.Eng.Chem. 15, 762-763.

CHAU Y.K. & RILEY, J.P., 1966.- The determination of amino-acids in sea water. Deep-sea Res. 13, 1115-1124.

CLARK , 1971

DAWSON R. and GOCKE,K., 1978.- Heterotrophic activity in comparison to the free amino acid concentrations in Baltic sea water samples.Oceanologica Acta 1, 45-54.

DEGENS E.T. REUTER, J.H. and SHAW K.N.F., 1964.- Biochemical compounds in offshore California sediments and sea water. Geochim. Cosmochim. Acta 28, 45-66.

FORREST, W.W, 1969.- Energetic aspects of microbial growth. In Microbial growth, 19th Symp. Soc.Gen.Microbial pp 65-86. Cambridge University Press.

FOGG 1962.- Extracellular products. In physiology and Biochemistry of algae.Lewin R.A.(ed) pp 475-489.Acad.P.4

GOCKE,K,1975.- Studies on short-term variations of heterotrophic Activity in the Kiel Fjord. Mar.Biol. 33,49-55.

HELLEBUST,J.A., 1974.- Extracellular products. In Algal physiology and biochemistry.Stewart W.D.P.(éd) pp 838-863 Blackwell scientific publications.

HOBBIE J.E., CRAWFORD C.C., WEBB K.L. 1968.- Amino acid flux in an estuary. Science 159, 1463-1464.

HOBBIE J.E. & WRIGHT,R.T. 1965.- Competition between planktonic bacteria and algae for organic solutes pp 175-185 In C.R. Goldman(ed.) Primary productivity in Aquatic Environments. Mem. Ist. Ital.Idrobial.,18 Suppl. Univ. California Press, Berkeley.

HOBBIE J.E.& CRAWFORD C.C., 1969.- Limnol Oceanogr. 14, 528-

JAUNASCH H.W., 1967.- Growth of marine bacteria at limiting concentrations of organic carbon in sea water. Limnol. Oceanogr.12, 264-271.

JAUNASCH H.W. 1968. - Growth characteristics of heterotrophic bacteria in sea water. J. Bact. 95, 722-723.

JOIRIS, C. 1973.- Evolution des populations bactériennes planctoniques dans le Bassin de Chasse d'Ostende (Belgique) en 1971 et 1972. Hydrobiol. Bull. 7, 54-59.

JOIRIS,C,1977.- On the role of heterotrophic bacteria in marine ecosystems : Some problems. Helgoländer.wiss. Meeresunters. 30, 611-621.

JOSEFSON, 1970.- Determination of soluble carbohydrates in sea water by partition chromotography after desalting by ion-exchange membrane electrodialysis. Anal. Chim. Acta 52, 65-73.

KOYAMA,T & THOMSON,T.G.,1959.- Organic acids in sea water. In Preprints Int.Oceanogr. Congr. 825-926.

MIRKINA S.D. 1977.- Separation and biological characteristics of dissolved organic matter of natural waters. Oceanology 17, 412-417.

OGURA,N.1974.- Molecular weight fractionation of dissolved organic matter in coastal seawater by ultrafiltration. Mar.Biol.24, 305-312.

ROGERS,H.J. 1961.- The dissimilation of high molecular weight organic compounds.
In The Bacteria, GUNSALUS & STANIER (ed.) Vol.II.

SEPERS,A.B.S.,1977.-The utilization of dissolved organic compounds in aquatic environments. Hydrobiol.52,39-54.

SHAH N.M.& FOGG G.E.,1973.- The determination of Glycollic acid in sea water. J. Mar. Biol.Ass. U.K. 53, 321-324.

SHAH N.M. & WRIGHT R.T.,1974.- The occurence of Glycollic acid in coastal sea water. Mar.Biol.24, 121-124.

STANLEY P.M. & STALEY J.T.,1977.- Acetate uptake by aquatic bacterial communities measured by autoradiography and filterable radioactivity. Limnol. Oceanogr.22,26-37.

SHARP J.H.,1973.- Size classes of organic carbon in sea water. Limnol.Oceanogr.18, 441-447.

STARIKOVA N.D.,YABLOKOVA O.G. & KORZHIKOVA,L.I.,1977.- Determining the molecular composition of dissolved organic matter by gel filtration. Oceanology 16,571-575.

SIEGEL,A. & DEGENS E.T. 1966.- Concentration of dissolved amino acids from saline waters by ligand exchange chromatography. Science 151, 1098-1101.

STANIER R.Y. DOUDOROFF M. & ADELBERG E.A. 1970.- General Microbiology. 3nd Edition. Macmillan Press Ltd.

WHEELER J.R., 1976.- Fractionation by molecular weight of organic substances in Georgia coastal water. Limnol. & Oceanogr. 21, 846-852.

WILLIAMS P.J. leB, 1970.- Heterotrophic utilization of dissolved organic compounds in the sea
I. Size distribution of population and relationship between respiration and incorporation of growth substrates. J. Mar. Biol. Ass. U.K. 50, 859-870.

WILLIAMS P.J. leB & GRAY, 1970.- Heterotrophic utilization of dissolved organic compounds in the sea. II. Observations in the responses of heterotrophic marine populations to abrupt increases in amino acid concentration. J.Mar.Biol.Ass.U.K. 50, 871-881.

WILLIAMS P.J. leB, BERMAN T., HOLM-HANSEN O.,1976.- Amino acid uptake and respiration by marine heterotrophs. Mar.Biol. 35, 41-47.

WILLIAMS P.J. leB, 1975.- Aspects of dissolved organic material in sea water. In Chemical Oceanography. Riley J.P. & Skirroco G. (ed.) Chapter 12, pp 301-363. Academic Press.

WRIGHT R.T. & SHAH N.M., 1975.- The trophic role of glycolic acid in coastal sea water. I. Heterotrophic metabolism in sea water and bacterial cultures. Mar.Biol. 33, 175-183.

Figure 1. Position of the stations visited and sampled in the present study.

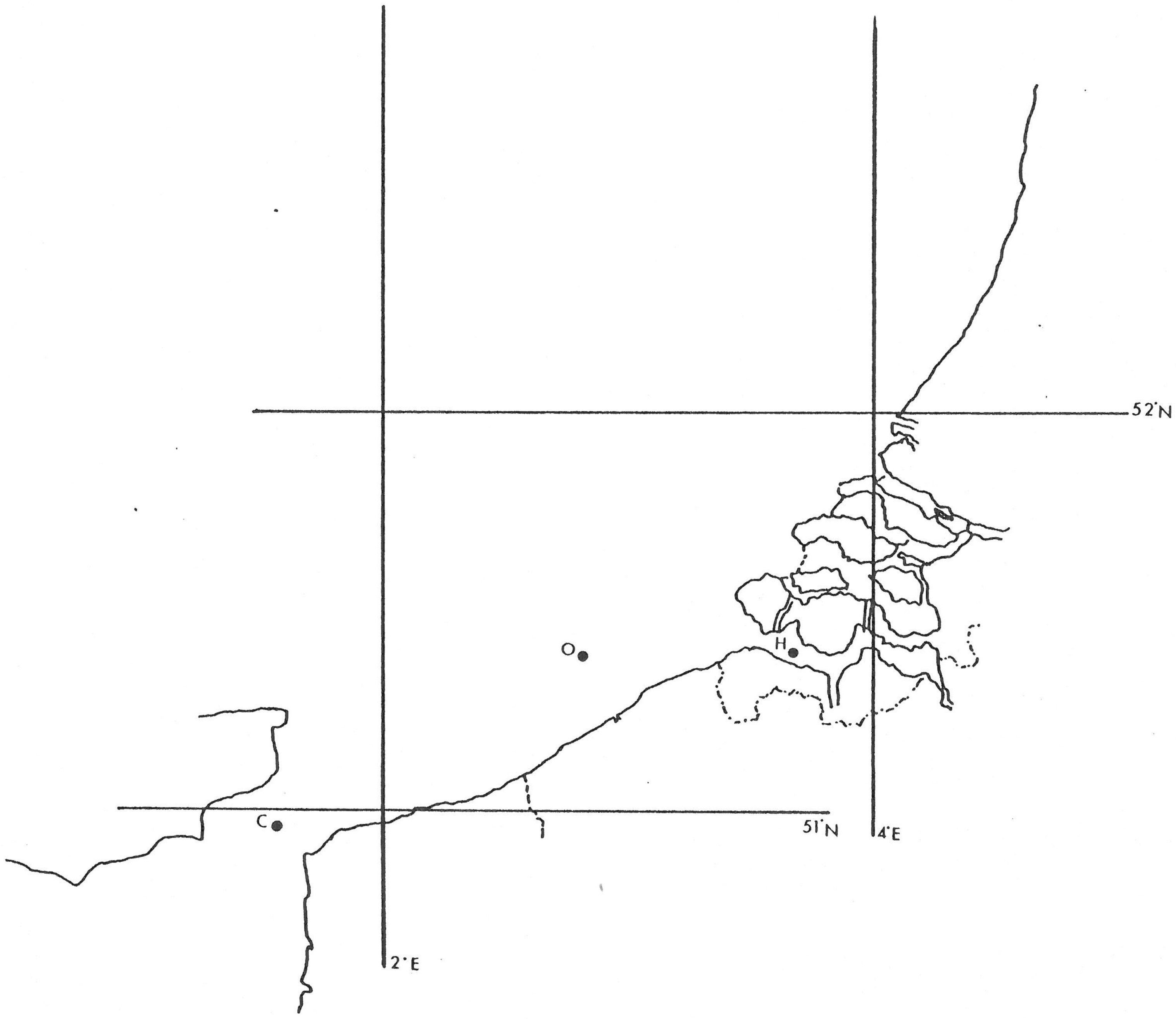
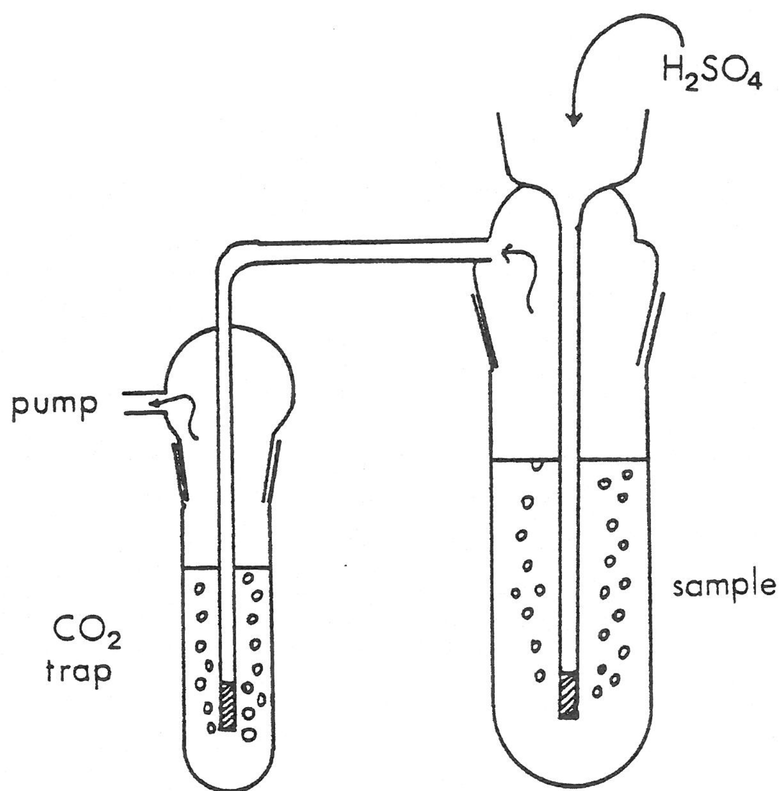


Figure 2. Device for trapping the CO_2 produced by respiration of labelled substrates before radioactive counting.



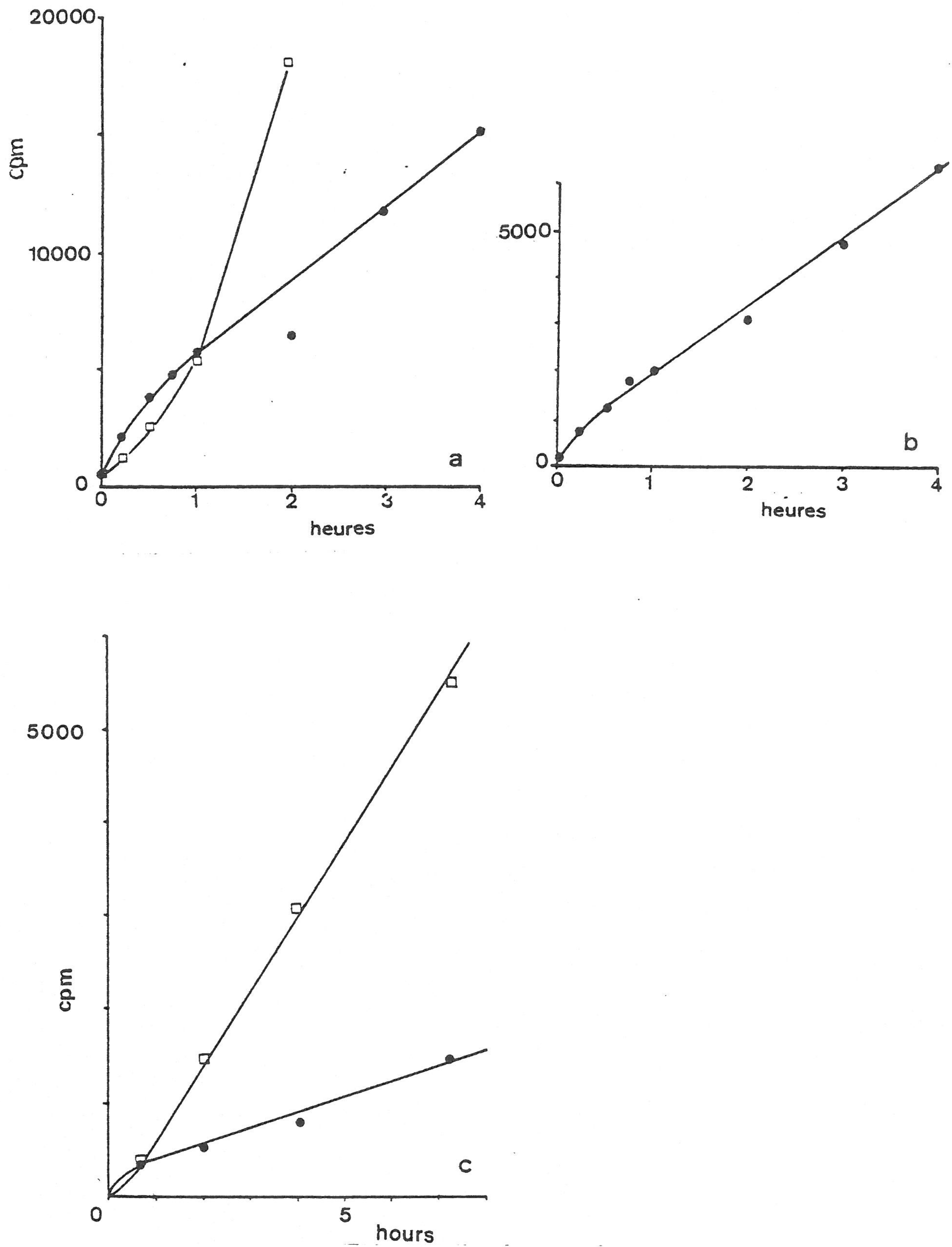


Figure 3. Examples of measurements of incorporation and respiration of labelled substrates. a. Incorporation (●) and respiration (□) of acetate by a sample of Scheldt water (Hansweert, oct. 1977). b. Incorporation of lactate by sample of Scheldt water (Hansweert, octobre 1977). c. Incorporation (●) and respiration (□) of glycollate by a sample of Scheldt water (juillet 1977).

Figure 4: Schematic representation of the model of bacterial utilization of directly usable substrates devel opped in the text.

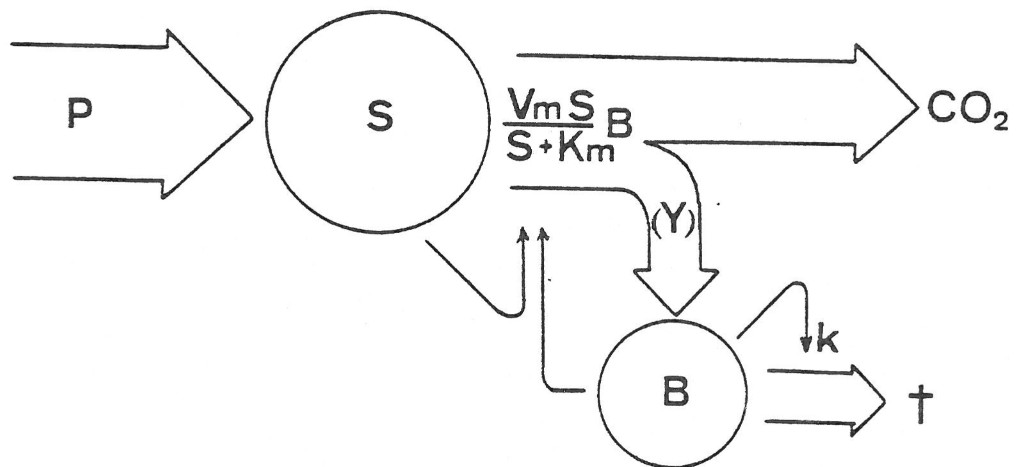


Figure 5. Evolution of bacterial density and substrate concentration after a sudden increase of the rate of production of the substrate.

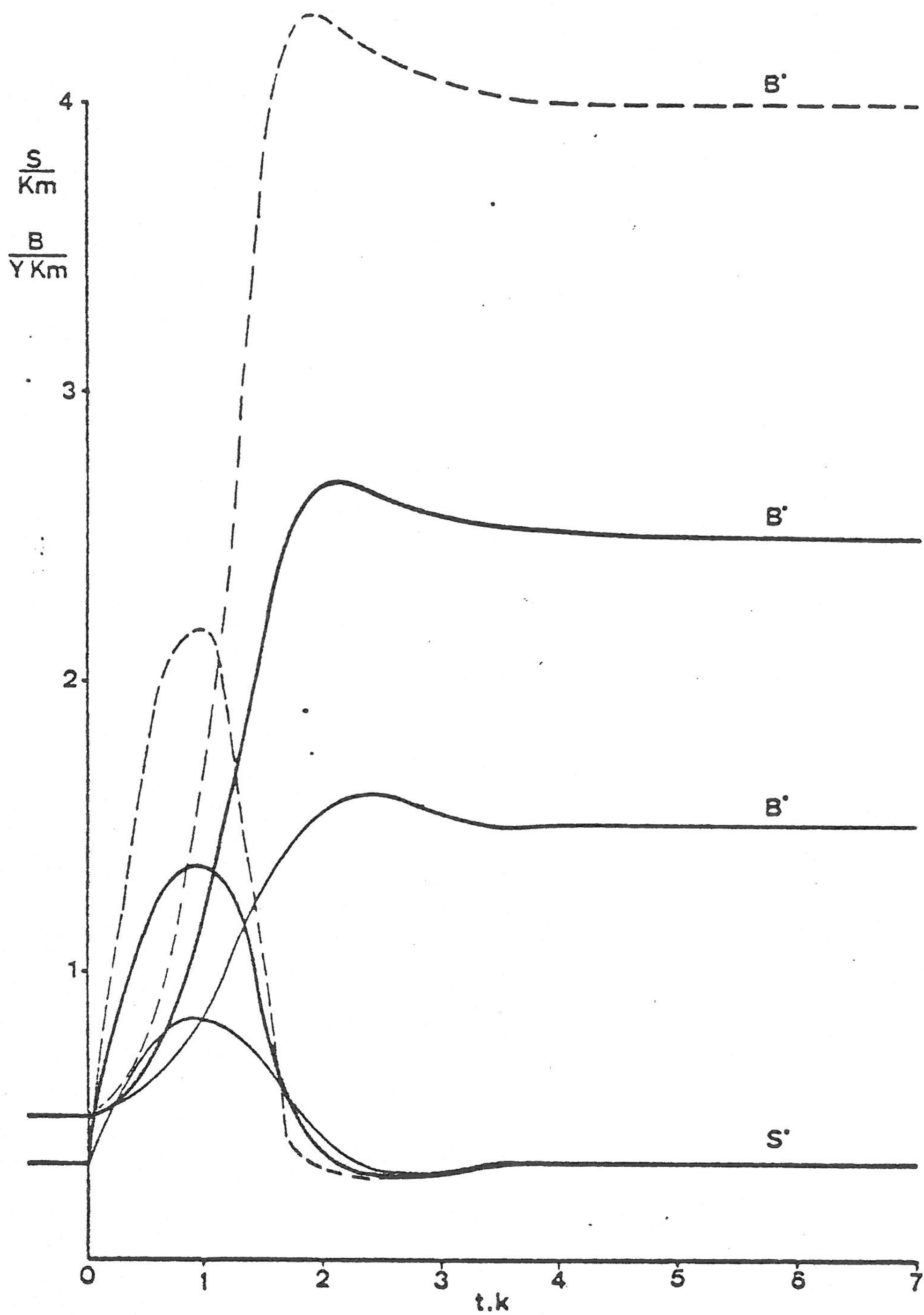


Figure 6.a. Evolution of bacterial density and substrate concentration after a sudden increase of substrate concentration.

b. Relation between the maximum bacterial density reached and the initial increase of substrate concentration.

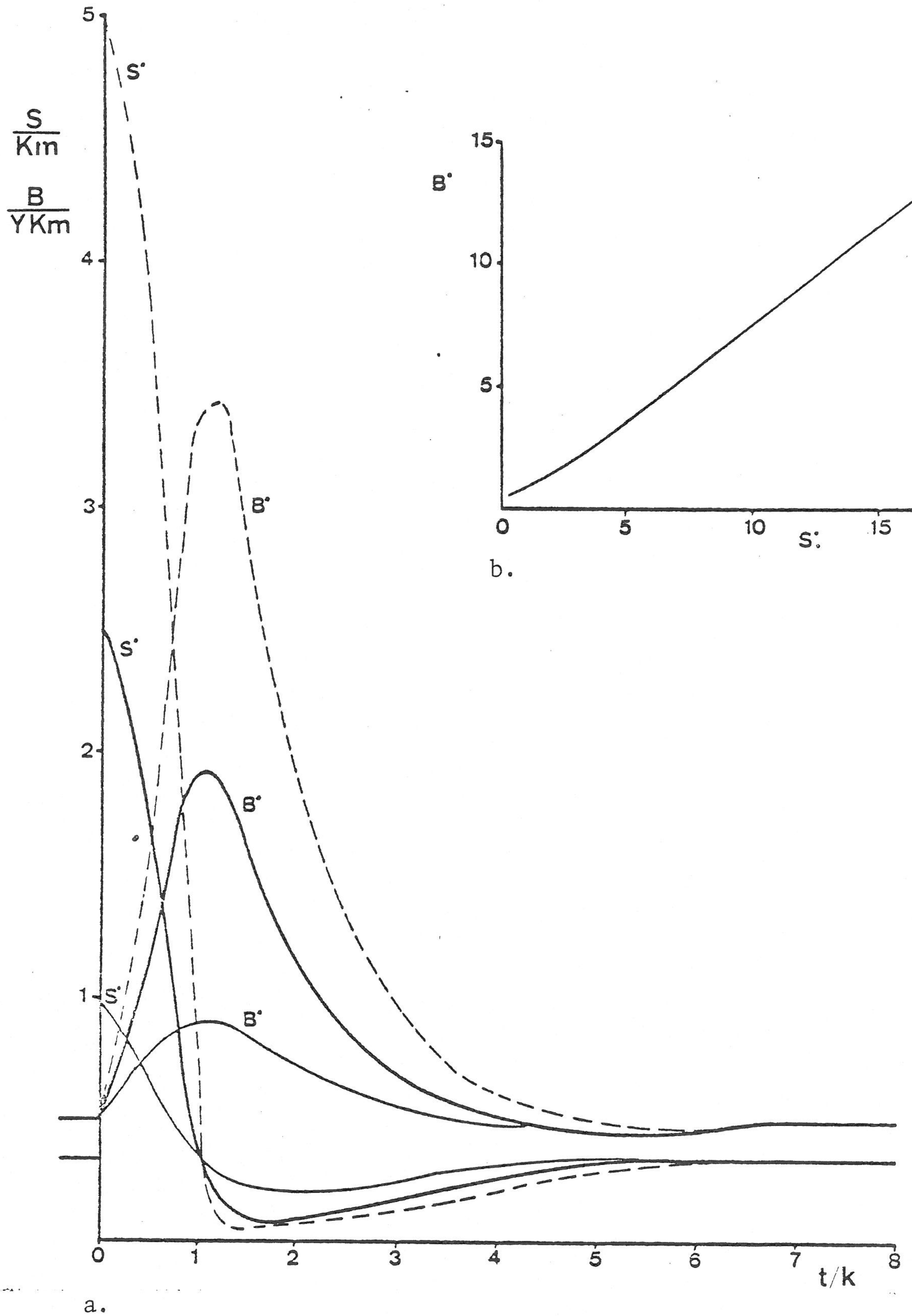
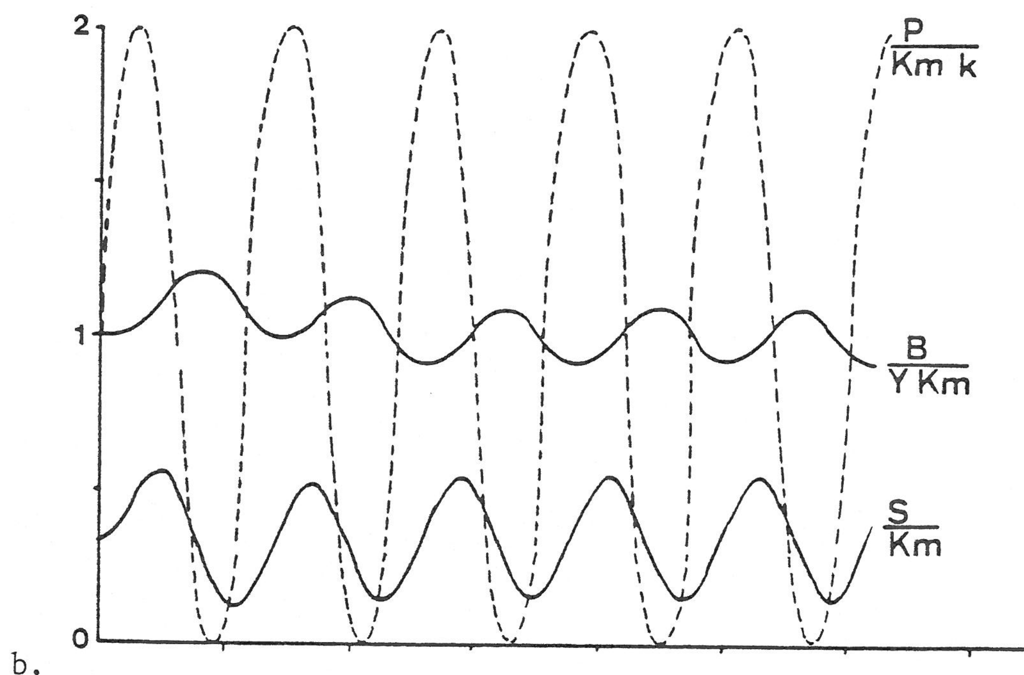


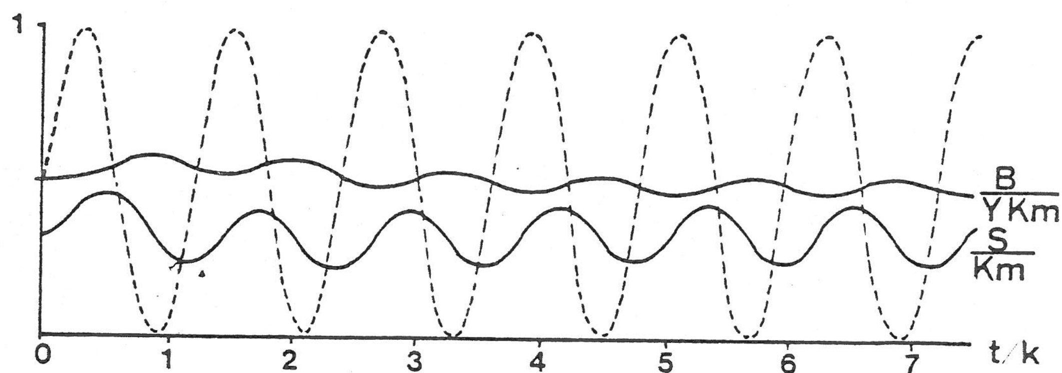
Figure 7. Evolution of bacterial density and substrate concentration when the production rate of the substrate varies sinusoïdally with a period of one day.

a. Mean turnover rate of the substrate: 6 % h^{-1}

b. Mean turnover rate of the substrate: 12 % h^{-1}



b.



a.