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Part 4

UTILIZATION OF PRIMARY PRODUCTS
BY PLANKTONIC AND BENTHIC BACTERIA

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Abstract.

Structural and functional modifications induced by eutrophication of coastal ecosystems are examined from the point of view of planktonic microbial activity.

It is suggested that increasing input of organic matter tends to favour r-strategists among the heterotrophic organisms utilizing organic matter. This implies namely the prevalence of microorganisms food chains and the occurence of special adaptations of microorganisms in their transport system for substrates.

It is also shown that a high bacterial heterotrophic activity is not necessary linked to a high rate of ammonium regeneration, depending on the C/N ratio of the organic matter used.

Increasing flux of organic material depositing on the bottom results in the establishment of more reduced conditions in the sediments, which affects strongly both the form and the efficiency of benthic nitrogen recycling.

Introduction

As stressed in other paper of this volume (Bouquegneau et al.; Daro et al.), one of the most striking features in the ecological working of coastal ecosystems, and of the Belgian coastal zone in particular, is the importance of the role of bacteria, both in the planktonic and in the benthic phases, in the utilization of primary production. This paper discuss the mechanisms and the control of bacterial activity in carbon and nitrogen cycles of coastal ecosystems. We shall suggest that the importance of bacterial activity versus macroorganisms activity is a characteristic of enriched, or concentrated media, -i.e.

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media where the production or input of organic matter is high per unit volume-, versus oligotrophic, diluted environments.

Therefore, the whole paper can be read as a discussion of the effect of increasing organic matter input or stimulation of primary production in coastal ecosystems on the structure of their carbon and nitrogen cycle. The underlying question is of course: How do the structure and the working of our coastal zone react in response to progressive eutrophication. We think that the understanding of bacterial activity is a most important key for answering this question.

1. ROLE OF MICROORGANISMS IN CARBON CYCLE

1.1. Microorganisms-macroorganisms competition

A classical concept in general ecology is the distinction between two kind of organisms according to the strategy they adopt in ther inter-specific competiton for living resources:

r-strategists, on the one hand, devote most efforts in colonizing as fast as possible all potential niches. These are fast reproducing, fast disseminating organisms.

K-strategists, on the other hand, reproduce and disseminate themselves more slowly, but develop complex structures allowing a deeper utilization of the resources.

The symbol r- and K- refer to the parameters of the logistic growth curve of population, r representing the specific capacity for growth, K, the carrying capacity of the environment for the population.

What characterizes microorganisms (bacteria in particular) with respect to higher organisms (table 1) is:
- their low generation time;
- their high growth yield;
- their rather passive nutritional behaviour. While higher organisms have developed complex structure for actively chasing their food, bacteria generally do not dispose of such structures and depend to a great extend on passive diffusion of dissolved substances near to their cells.

As a group. microorganisms can therefore be considered as r-strategists with respect to higher organisms, displaying in general a more K-oriented strategy.

What is observed (see fig. 1) as well in sedimentary than in open water systems, is that microorganisms dominate in the use of organic matter in environments like coastal waters (where an

intense primary production is concentrated in a shallow water column) or a fortiori like coastal sediments (where an important part of the primary produciton of the whole water column is deposited and therefore concentrated in a 10 cm layer). On the contrary, higher organisms prevail in the use of the scarce resources of poor, diluted environments like oceanic waters or deep-sediments, receiving only a very small flux of orgaic matter. In other words, if "richness" is defined as the total flux of organic matter through the system, r-strategy dominates in rich, an K-strategy in pooor environments.

This suggests that a general increase of the level of primary production in our coastal zone, as a result of increasing nutrient input, does not necessarily lead to an increase of pelagic or demersal fish production, but could induce a modification of the food web resulting in enhencement of microorganisms activity at the expense of long trophic chains dominated by macroorganisms.

1.2. Mechanisms of organic matter utilization by bacteria

Most of the organic matter primarily supplied by primary production in marine systems consists in macromolecules (polysaccharides, proteins, lipids), or small organic acids are directly produced (Billen, 1982). Even the process of phytoplankton excretion, at least in the Belgian coastal zone, leads to liberation of high molecular weight material (see Lancelot et al., this volume).

Such macromolecular compounds, either dissolved or particulate, cannot be directly taken up by bacteria, and have first to be hydrolized into smaller units, probably mainly through the action of exoenzymes. Once produced, these low molecular weight, directly usable compounds are rapidly taken up and metabolized by heterotrophic bacteria.

Organic matter utilization through the bacterial food chain therefore involves two successive stages:

- exoenzymatic hydrolysis of macromolecules;
- uptake of small, directly usable substrates.

We shall examine them in turn.

1.2.1. Exoenzymatic activity in sea water

Due to the lack of a convenient and sensitive method, little information is available concerning the occurence and activity of exoenzymes in natural waters. By use of insoluble synthetic

TABLE 1.: Ecological characteristics of microorganisms versus macroorganisms

	Bacteria	Higher organisms	
		Zooplankton Meiobenthos	Fish Macrobenthos
Generation time	1 day	1 month	1 year
Growth Yield	30 - 50%	25%	10%
Strategy in food capture	Passive	Active	Active
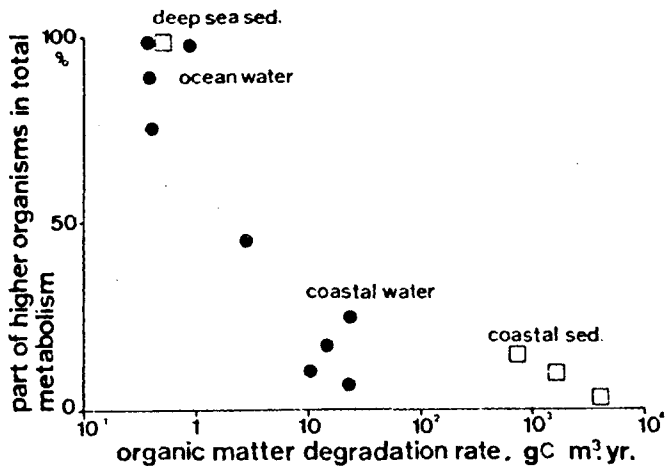
Fig. 1: Relative part of higher organisms versus microorganisms in the use of organic matter in planktonic and sedimentary systems of various "richness" ("richness" being defined by the total flux of organic matter through the system).
(Data from Billen et al., 1976; Jannasch and Wiersen, 1972 and Lancelot, 1982).

protein-dye, releasing a soluble color upon enzymatic hydrolysis, some authors (Kim and Zobell, 1974; Little et al., 1979; Meyer-Riel, 1981) demonstrated the occurence of free exoproteasic activity in lake- or sea- water samples. This method, however, is not sensitive enough for rapid measurements and requires either very long incubation times (a few days) or preconcentration of the samples by dialysis of ultrafiltration.

Somville and Billen (in press) have reported a very sensitive and reliable method allowing determination of exoproteasic activity in a few minutes without any concentration of the sample even in oligotrophic waters.

The method, adapted from the procedure of Roth (1965) in clinical analysis, is based on the use, as substrate for proteolytic exoenzymes, of amino-acyl derivatives of β-Naphtylamine, which give rise to a fluorescent product after hydrolysis of their peptidic-like bond.

The specificity of the method toward exoproteases was indirectly tested by studying the effect of varying concentrations of serum-albumine on the rate of hydrolysis of LLβN (fig. 2). A strong competitive inhibition was found. The inhibition constant of albumin (which in the case of competitive inhibition is equal to its Km for the enzymes) is about 50 μmoles 1^{-1}.
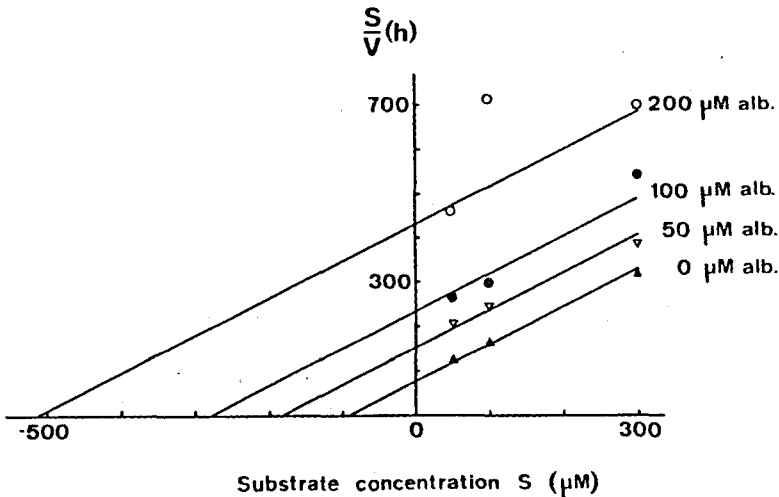

Fig. 2: Inhibitory effect of serumalbumine on the hydrolysis of L-Leuctl-β-Naphtylamide (reciprocal plot).

This indicates that the enzymes responsible for LIβ N hydrolysis have a higher but similar affinity for proteins. On the other hand, free amino acids have only a smaller inhibitory effect on $LL\beta N_1$ hydrolysis, the Ki of L-Leucine being about $200-300 \mu$moles 1^{-1}.

Up to now, about 50 determinations of potential exoproteasic activity are available in various aquatic environments, from rather oligotrophic, as in the English Channel, to hypereutrophic, as in the heavily polluted Scheldt estuary. In some cases, the relative rate of amino-acid utilization has been simultaneously determined on a parallel sample, by means of a ^{14}C labelled amino acid mixture (protein hydrolysate, Amersham) (Williams et al., 1976).

As will be shown below, this relative rate can be considered as a good measurement of the total rate of amino acids heterotrophic utilization. Figure 3 shows that a good correlation is obtained between potential exoproteasic activity and the rate of amino acid uptake.this observation confirms the obligate role of exoenzymatic activity in the bacterial utilization of organic nitrogen in aquatic environments.
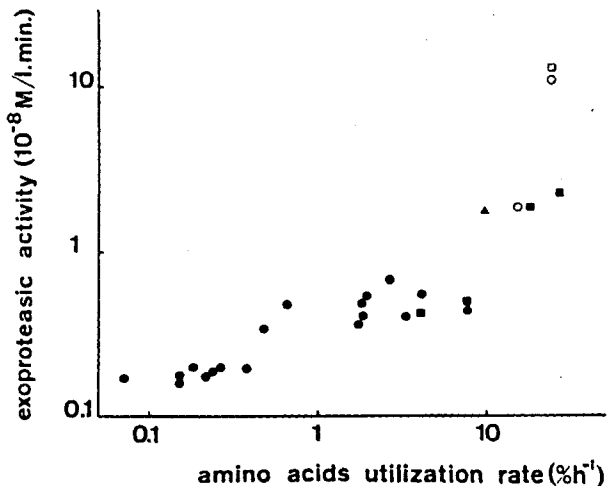

Fig. 3: Relation between exoproteasic activity and relative amino acids utilization rate in various aquatic environments.
(●) coastal seas, (▲) eutrophic pond- Bois de la Cambre-Brussels, (■) Oise river - France, (0) Scheldt estuary and (□) Rupel river-Belgium.

With the method described, proteasic activity can be determined after gentle filtration of the sample through 0.8 or 0.2 μmembranes. The former have been shown by numerous studies in seawater (Derenbach and Williams, 1974; Azam and Hodson, 1977) to retain most phytoplanktonic organisms while most free living bacteria pass them; the latter retain all microorganisms. By this procedure, it is therefore possible to assess to which fraction of the natural microbial community exoenzymes are associated. The data obtained with seawater collected in June 1981 in the eutrophic Belgian coastal zone of the North Sea, show that most oligotrophic waters from the Eastern English Channel at the same period, most of them appear to be bound to particles between 0.8 and 0.2μ. In the last case, exoproteases are probably linked to the external surface of bacterial cells, as demonstrated in some instance by Christison and Martin (1971).

This suggests the existence of two strategies in exoenzymatic hydrolysis of macromolecules: the first one consists for the bacteria to produce free dissolved exoenzymes and wait until the hydrolysis products diffuse again to the cell. The second one consists in keeping the exoenzymes linked to the cell membranes so that the hydrolysis products have more chance to be taken up.

Is it probably significant that this last, more active strategy, in which the bacteria "chase" in a sense the macromolecules, prevails in oligotrophic situations, while the former dominates in the summer in the eutrophicated Belgian coastal zone.

1.2.2. Uptake of direct substrates

Following the work of Wright and Hobbie (1966), numerous measurements of the uptake kinetics of various substrates by intact natural communities of aquatic microorganisms have been determined. In most situations, the uptake was found to obey a Michaelis-Menten-Monod relationship:

$$V = \sum_i v_i \qquad \frac{V_{max} S}{S + K_t} \qquad (1)$$

where V is the total rate of uptake
        v_i is the rate of uptake by population i in the microbial community
        V_{max} is the maximum total rate of uptake
        S is the substrate concentration
        K_t is the half-saturation constant of uptake

The validity of this approach when dealing with heterogeneous microbial communities has been discussed in detail by Williams (1973).

The validity of relation (1) indicates either that one single microbial strain dominates all the others in the utilization of the substrate, or that all the strain utilizing the substrate have a very similar value of K_t. Therefore, the K_t value obtained from this kind of measurement with natural communities characterizes the affinity toward the substrate of the dominant microbial populations.

When comparing a whole range of aquatic environments, a relationship is found between the K_t value of the microbial community and its natural rate of substrate utilization: the lower the flux of the substrate, the lower the K_t value (fig. 4 and 5) (Billen, in preparation). As the rate of utilization of a substrate rather than its concentration is the best index of the "richness" of an environement in this substrate, this trend can be interpreted as reflecting the competition between microorganisms for their substrates: the lower the availability of the substrate, the higher the selective pressure for developing sophisticated and expensive permease systems with great affinity for this substrate.
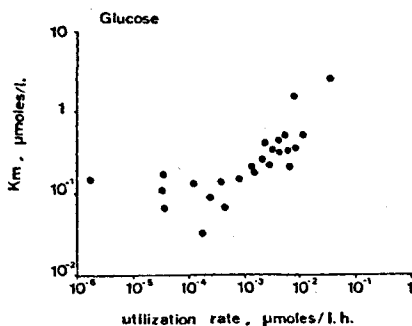

Fig. 4: Half-saturation constant for glucose uptake measured in various aquatic environments, plotted against the total flux of glucose utilization. (Data from Japanese marine and brackish environments, and from canadian lakes, Seki et al, 1975, 1980 a, 1980 b).

Note that these high affinity permeases require substantial energy costs and result therefore in lower growth rates (fig. 6).
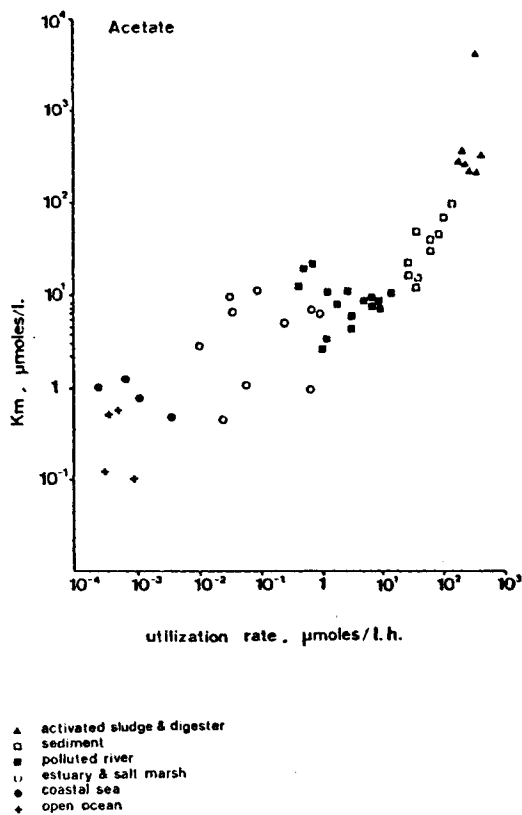
Fig. 5: Half-saturation constant for acetate uptake measured
in various aquatic environments, plotted against the
total flux of acetate utilization.
(Data from Seki et al, 1974, 1980; Strayer and Tiedje,
1977; Stanley et Staley, 1977; Russel and Baldwin, 1979;
De Staercke, 1980; Billen et al, 1980; Billen unpublished).

Again, in the competition between microorganisms for direct substrates, r- and K-strategies are possible: the K-strategy consists in developing high affinity transport systems, at the expense of growth rate, while the r-strategy consists in maximizing the growth rate at the expense of affinity for the subtrates. Figure 4 and 5 show that r-strategy prevails in rich environments.

The knowledge of the Km for direct substrates is important because, as we shall now see, its value controls the in situ concentration of the substrate.
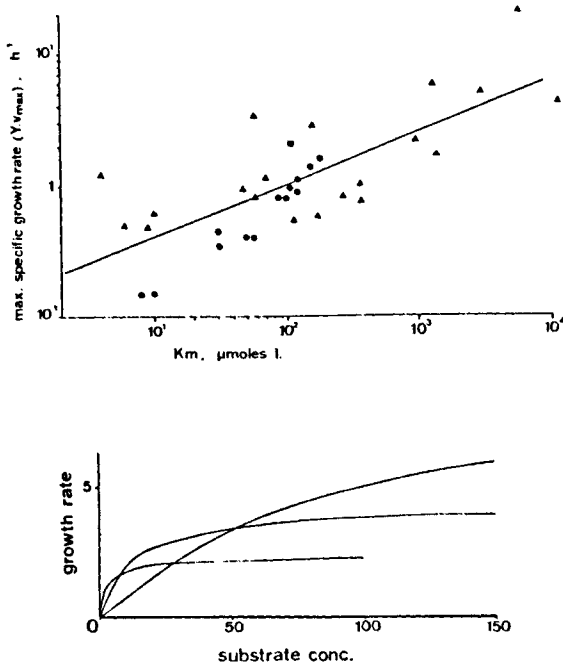

Fig. 6: a. Relation between the maximal specific rate of bacterial growth on a single substrate and their affinity for this substrate(Data from Jannasch, 1968; Herbert et al, 1956; Russel & Baldwin, 1979).

b. Illustration of the various strategies during the competition of bacteria for a direct substrate.

The concentration of a particular substrate results from the balance between the production rate of this substrate (e.g. by phytoplanktonic excretion, exoenzymatic hydrolysis, etc...) and the rate of uptake by the dominant microorganisms populations.

If this population is limited by the substrate, and is able to grow fast enough for maintaining a stationary state between substrate production and uptake, the concentration of the substrate is independent of its rate of production and depends only on physiological characteristics of the microorganisms (Billen et al., 1980).

This can be shown in the following way. The rate of change of substrate concentration (S) can be written:

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

where P is the rate of production of the substrate
      B is the mass of organisms utilizing S
   Vmax is the maximum rate of uptake per organism
    K_t is the transport constant of S by the organism.

On the other hand, the rate of change of organisms biomass can be written:

$$\frac{dB}{dt} = \frac{Y\ Vmax\ S}{S + K_t}\ B - k_d\ B \qquad (2)$$

where   Y is the yield constant, i.e. the mass of
        organisms formed by unit substrate taken up
    k_d is a first order mortality constant.

At stationary state the solution of (1) and (2) is:

$$S = \frac{K_t}{\dfrac{Y\ Vmax - 1}{k_d}} \qquad (3)$$

$$B = \frac{Y}{k_d}\ P \qquad (4)$$

showing that at stationary state only the biomass of microorganisms is affected by the rate of production of the

substrate. The concentration of the substrate depends only on the transport constant, and on the ratio between the maximum growth rate (μmax = YVmax) and the death rate of organisms.

The question is of course to know how closely a stationary state is approached by natural aquatic systems. It has been shown by very simple simulations (Billen et al. 1980) that the time required for reaching a stationary state or for restoring it after a sudden perturbation, is about $\frac{1}{k_d}$, i.e. is of the order of the generation time of the microorganisms at steady state. Thus bacteria, having smaller generation times, can be thought effectively to control the concentration of the direct substrates they use predominantly. On the contrary, algae, having longer generation times (and moreover, being limited by other factors like light intensity) are not always able to maintain the concentration of their direct substrates at a stationary state.

The validity of relation (3) for organic substrates predominantly used by bacteria can be tested with experimental concentration and K_t data for glucose and acetate, reported in the literature for various aquatic environments (fig. 7). Both sets of data agree to relation (3), showing that a steady state is not far from being reached, and that the control of substrate concentration by microorganisms uptake is effective. The relation obtained is in both cases:

$$S \qquad \frac{K_t}{3}$$

suggesting that in all the environements considered the ratio $\frac{Vmax}{k_d}$ is about 4.

As can be see in figures 4 and 5, which compare an extreme range of environmental situations, Km values vary much less from one environment to another than the rate of uptake of the substrates. This explain why no significant variations exist in the concentration of direct substrates between environments of similar, although different, richness. This is the case, e.g. for amino-acids and glucose concentration in the Scheldt estuary, the Belgian coastal zone and the Eastern English Channel, in spite of 1 order of magnitude difference in the rate of uptake of these substrates between these three environments (Billen et al., 1980).

This is the basis of our method for estimating heterotrophic bacterial activity. Knowing the mean concentration of all classes of direct substrates, which is stable in time and space, and measuring accurately the relative rate of utilization which varies a lot both in time and space, it is possible to calculate by multiplication the total rate of organic matter utilization
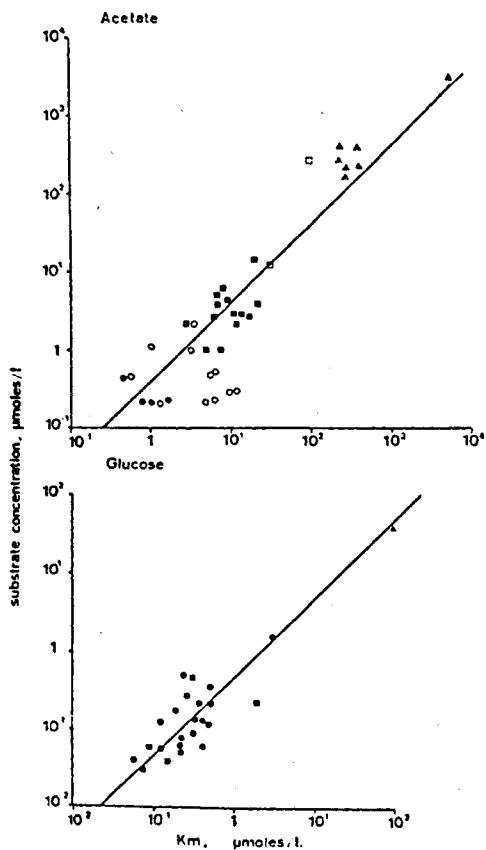
Fig. 7: Relation between the naturel concentration of glucose
and acetate and their half-saturation constant of uptake
in various aquatic environments.
(Data from Seki et al, 1974, 1980; Walher and Monk, 1971;
Russel and Baldwin, 1979; Stanley and Staley, 1977;
Kaspar and Wuhrman, 1978; De Staercke, 1980; Billen et
al, 1980).

by bacteria. This approach allowed us to estimate heterotrophic bacterial activity independently of phytoplankton endogenous respiration.

1.3. Degradation of organic matter in sediments

The importance of the benthos in carbon cycling is another important characteristics of shallow coastal systems. The factors affecting the flux of organic matter sedimenting and the consequences of increased input of organic matter on the physicochemical conditions of the benthos will be briefly examined.

1.3.1. Flux of organic material to the sediment

From a compilation of sediment trap data from various sites, Suess and Muller (1980) showed a clear relationship with depth of the flux of sedimenting organc carbon expressed in percentage of primary production. In shallow coastal seas, such as the Belgian shelf, up to 50% of net primary production is deposited on the benthos. Fecal pelets and zooplankton corpses can only make up a small fraction of this sedimentation flux. It is therefore likely that phytoplanktonic cells and phytoplanktonic-derived detritus constitute the bulk of the organic matter deposited on the sediment. Direct confirmation of this has been obtained by analysis of the vertical profile of chlorophyl pigments and particulate nitrogen in sediment cores (Joiris et al., 1982).

The local distribution of the flux of sedimenting organic material in the Belgian coastal zone, as revealed by the spatial distribution of organic matter content of the uppermost layer of the sediment, can be explained on basis of a hydrodynamical model of the tidal circulation, by considering the role in the sedimentation-erosion process of both the energy available for the erosion of the sediment and the bottom stress indicating whether or not suspended sediments are taken away by the flow (Adam et al., 1981). Some places like the mud accumulation zone in front of Zeebrugge(with low energy and low bottom stress) appears to act as traps for organic material produced in the whole coastal zone. The annual flux of organic carbon deposition there has been estimated to 390 gC/m^2y, while the mean value for the Belgian coastal zone as a whole is only 160 gC/m^2y and is 70 gC/m^2y in the offshore zone.
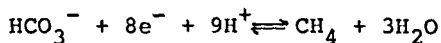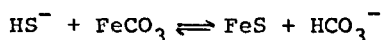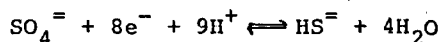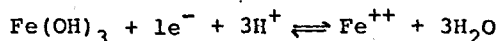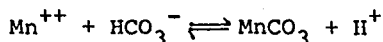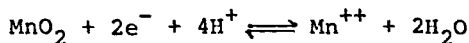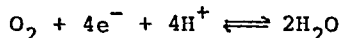
1.3.2. Redox state of the sediment as a result of organic matter degradation

Microbiological organic matter degradation involves the consumption of an equivalent amount of mineral oxidants, either directly in the case of respiratory metabolisms, or indirectly in the case of fermentative metabolisms, the reduced products of which (organic acids, alcohols, H_2) have to be further oxidized by respirative organisms. Oxidants succeptible to be used in microbial metabolisms are oxygen, manganese oxides, nitrate and nitrite, ferric oxides, sulfate and carbon dioxide. Organic matter degradation within the sedimentary column causes a depletion of these oxidants (X_i) and an accumulation of the corresponding reduced species (Y_i).

The concept and measurement of redox potential in natural environments have been discussed by several authors (Bass Becking et al., 1960; Stumm, 1966; Thorstenson, 1970; Billen, 1978b). It has been stated that the concept of redox potential in natural environments is meaningful owing to the fact that an internal thermodynamic equilibrium is reasonably approached within the subsystem formed by the main mineral redox species (X_i, Y_i) involved in energy yielding metabolisms of microorganisms. The redox potential (Eh) is defined with respect to this subsystem only and does not take into account the presence of highly reduced organic matter: it only characterizes the availability of oxidants succeptible to use by microbial respirations. Direct measurements of Eh in sediments with a platinum electrode must be interpreted with caution, but can provide valuable relative indications (Whitfield, 1969; Bågander and Niemislö, 1978).

Defined as above, the redox potential in sediments can be viewed as the result of microbial metabolisms. Organotrophic metabolisms generate a flux of electrons to the subsystem formed by mineral redox couples, while chemolithotrophic metabolisms tend to restore the internal thermodynamic equilibrium by oxidizing reduced mineral species at the expense of oxidized ones, when thermodynamically possible. Knowing the intensity and distribution of organotrophic processes to which all oxidants and their reduced forms are subject it is possible to calculate the vertical profiles of all oxidants and of redox potential. This is the principle of a general idealized redox model of marine sediments proposed by Billen and Verbeustel (1980). Their model, however, was based on complete internal termodynamic equilibrium, including nitrogen species, which is rather unrealistic. Moreover, only one mixing coefficient for both solid and dissolved species was considered. An improved version of this redox model, based on data collected in the sandy sediments of the North Sea, has been presented by Billen (1982).

It considers the following equilibria:

$$O_2 + 4e^- + 4H^+ \rightleftharpoons 2H_2O$$

$$MnO_2 + 2e^- + 4H^+ \rightleftharpoons Mn^{++} + 2H_2O$$

$$Mn^{++} + HCO_3^- \rightleftharpoons MnCO_3 + H^+$$

$$Fe(OH)_3 + 1e^- + 3H^+ \rightleftharpoons Fe^{++} + 3H_2O$$

$$Fe^{++} + HCO_3^- \rightleftharpoons FeCO_3 \quad H^+$$

$$SO_4^= + 8e^- + 9H^+ \rightleftharpoons HS^= + 4H_2O$$

$$HS^- + FeCO_3 \rightleftharpoons FeS + HCO_3^-$$

$$HCO_3^- + 8e^- + 9H^+ \rightleftharpoons CH_4 + 3H_2O$$
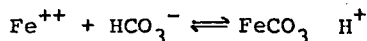
Nitrate is not considered to be at equilibrium with respect to the other redox couples. It is assumed to be produced from ammonium through nitrification above a critical value of redox potential (Billen, 1975) and to be reduced into dinitrogen through denitrification below this potential. Organic material is assumed to be degraded according to first order (one G) kinetics and the resulting organotrophic acitivity causes a electron flux which is absorbed by the various oxidants according to the reactions listed above. In this model, the flux of depositing organic matter is taken as the independent variable, so that it is possible to relate the input of organic material in the sediment to its redox state.

The redox profiles, theoretically predicted by this model for two different values of the input of fresh organic matter to marine sediments, are shown in Figure 8a and 8b. Figure 8a corresponds to the situation termed "suboxic diagenesis" by Froelich et al. (1979), in which the organic matter input to the sediment is low enough with respect to the diffusion flux of oxidants that only oxygen consumption, manganese-reduction, denitrification and ferrireduction are involved in organic matter degradation. This is the case im most sandy sediments of the Southern Bight of the North Sea, which does not receive important amounts of organic matter.

Figure 8b, on the other hand, shows a situation of "anoxic diagenesis", where oxygen, manganese oxide, nitrate and ferric oxides are rapidly exhausted and sulfate reduction dominates organotrophic activity. This is the case of the mud accumulation zone.
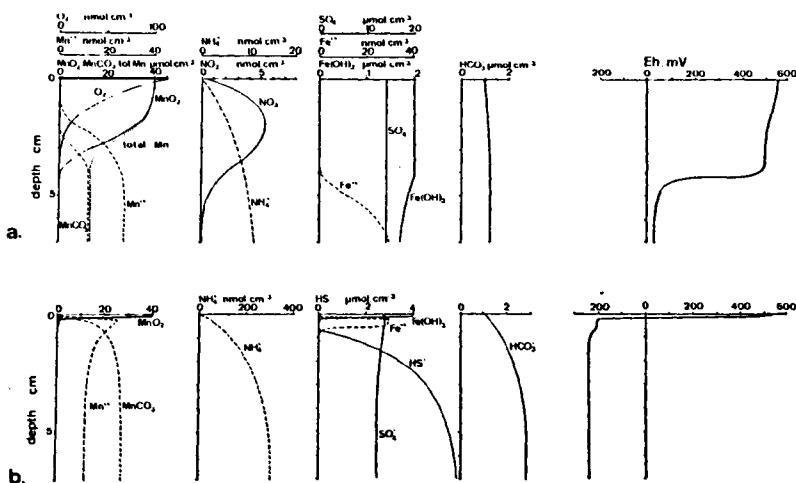
Fig. 8: Calculated vertical profiles of mineral redox species in
sediments according to thermodynamic equilibrium model
(Billen, in press) for two different values of the flux
of organic matter depositing on the sediment surface.
($Di = 10^{-4}$ cm^2sec^{-1}; $Ds = 10^{-7}cm^2sec^{-1}$; $k = 3$ $10^{-8}sec^{-1}$).

a. "sub-oxic diagenesis": case of a marine sediment with
a flux of organic matter of 8 10^{-9} mmoles $cm^{-2}sec^{-1}$(30 gC
$cm^{-2}year^{-1}$)(overlying water containing 230 mM oxygen,
0 nitrate, 28 mM sulfate and 2 mM bicarbonate; upper sediment
containing 40 μmole cm^{-3} manganese and 200 μmole cm^{-3} reactive
iron - porosity: 0.5).

b. "anoxic diagenesis": case of a marine sediment with a
flux of organic matter of 10^{-7} mmoles $cm^{-2}sec^{-1}$ (378 gC m^{-2}
$year^{-1}$) (some overlying water and sediment composition).

The vertical profiles calculated by this equilibrium model are of course idealized. Moreover, they depend strongly on the values chosen for the various parameters (D_i, D_s, k). Nevertheless, they display the same general trends as numerous experimental observations.

Pearson and Stanley (1979) have recently used the measurement of redox potential in the sediments of a sea loch as a means of assessing the effect of organic pollution by a paper mill. They experimentally related the Eh reached in depth in the sedimentary column to the input of organic material to the sediments. Such a relation can be theoretically deduced from the redox model discussed above, as shown in figure 9 for a set of value of the mixing coefficients, D_i and D_s.
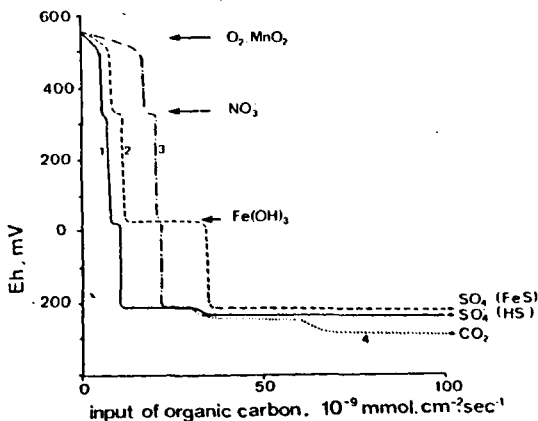

Fig. 9: Calculated relationship between the minimum redox potential reached in depth in sediments and the input of organic carbon depositing, according to a thermodynamic equilibrium model, for different values of the mixing coefficients.

1. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 10^{-7} cm^2 sec^{-1}$, sea water
2. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 5 \cdot 10^{-6} cm^2 sec^{-1}$, sea water
3. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 10^{-8} cm^2 sec^{-1}$, sea water
4. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 10^{-7} cm^2 sec^{-1}$, fresh water

These curves show the "buffering capacity" of the various redox couples present in sediment toward "titration" by depositing organic matter. For oxidants present in the solid phase this buffering capacity is closely dependent on the value of D_s, both because the mixing coefficient directly determines the "availability" of these oxidants within the sediment and because it determines the penetration of organic material down into the sediment and thus the depth distribution of organotrophic activity, for a given flux of depositing organic matter.

2.- ROLE OF MICROORGANISMS IN NITROGEN CYCLING

Following the introduction of the concept of the "Redfield molecule", it has often been assumed that organic matter from phytoplanktonic origin has a constant composition so that both the uptake of nutrients by phytoplankon and the release of nutrient upon organic matter mineralization obey a very simple and constant stoechiometry. In the scope of this paradigm, nitrogen cycling could be deduced from carbon measurements, if the adequate C/N ratio is known.

The paper by Lancelot in this volume, has shown that the composition of primary produced organic material is not constant at all and widely varies according to nutrient concentrations in the surrounding medium.

We will show that the relative amount of nitrogen released as a result of organic matter mineralization by planktonic and benthic bacteria also depends on environmental factors which can lead to important lack of parallelisms between carbon and nitrogen cycling, particularly in eutrophicated environments.

2.1. Ammonification

According to their biosynthetic or energetic fate in microbial metabolism, organic nitrogen compounds can be either incorporated into biomass or excreted as ammonia. In classical conceptions the latter process is thought to be the most important, and microorganisms are viewed as direct mineralizers of organic matter. Some authors however (Rittenberg, 1963; Johannes, 1968) have stressed the posssible importance of the former process, claiming that the bacteria account directly for only a minor fraction of nutrient regeneration or even compete with algae for mineral nitrogen. This question of organic nitrogen immobilization versus mineralization can now be reexamined in the light of recent physiological and ecological data.

The balance between N-mineralization and immobilization depends on the ratio carbon:nitrogen $(\frac{C}{N})_S$ of the total organic matter utilized. The amount of ammonia released (ΔNH_4) per unit carbon taken up (ΔC) is given by the following relation:

$$\frac{\Delta NH_4}{\Delta C} = \frac{1}{(\frac{C}{N})_S} - \frac{Y}{(\frac{C}{N})_B} \qquad (5)$$

where Y   is the growth yield ratio,
 and $(\frac{C}{N})_B$ is the carbon:nitrogen ratio of bacterial biomass

The experiments of Hollibaugh (1978), Somville (1980) and Billen (unpublished), who supplemented natural sea water with mixtures of organic subsrates and followed the consumption of the substrates and the release of ammonia, permit to test the validity of relation (5) (fig. 10). Although the data come from two different environments and were obtained with different organic substrates, a very good fit is obtained with $\frac{Y}{(\frac{C}{N})_B} = 0.1$ gN/gC.

If 4 gC/gN is taken as a reasonable estimate for $(\frac{C}{N})_B$, Y can be evaluated as 0.4 in good agreement with the values cited in the literature.

Relation (5) and figure 10 also show the lack of parallelism between the role of bacteria in carbon nitrogen cycling. When the ($\frac{C}{N}$) ratio of the organic matter used by bacteria increases, ammonium release decreases and, for $(\frac{C}{N})_S$ higher than 10 gC/gN, uptake instead of release can even occur during organic matter degradation. The role of bacteria as nitrogen mineralizers thus not necessaily parallels their role as carbon mineralizers. A striking example is provided by data obtained by Joiris et al. (1982) and Billen (unpublished) in the Belgian coastal zone, the English Channel and the Scheldt estuary, where total heterotrophic activity has been estimated by measuring over a whole year cycle the concentration and the relative rate of bacterial utilization of the three main classes of direct organic substrates (free amino acids, monosaccharides and glycollate) (Table 2). From these data, ammonium remineralization can be calculated according to relation (5). As seen, the most important ammonium release occurs in the Western Channel, where heterotrophic carbon utilization is the lowest. In the Belgian coastal zone, the relative importance, as substrates for heterotrophic activity, of carbohydrates, part of which coming from the mucopolysaccharides excreted by Phaeocystis poucheti (Lancelot, 1982), severely limits ammonium regeneration by bacteria. In the heavily polluted Scheldt estuary, net ammonium uptake occurs, due to the high ratio of the terrigenous organic material being degraded.
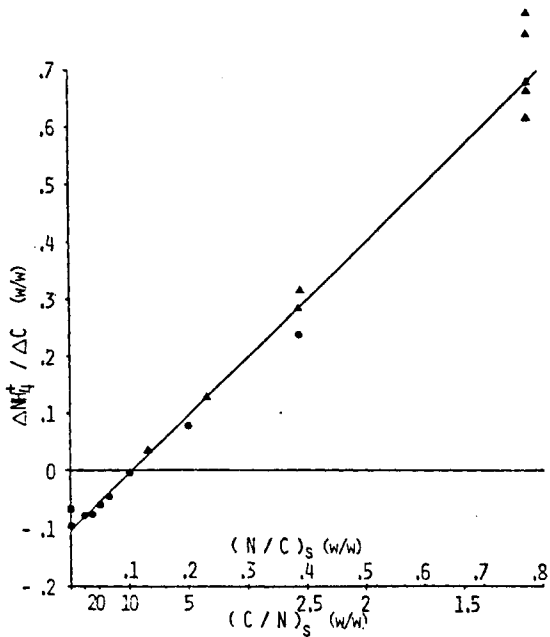
Fig. 10: Release or uptake of ammonia per unit carbon
taken up by microbiological communities of
marine environments, supplemented with mixed
substrates of various C/N ratio.

(Data from Hollibaugh (1978) (▲),
 Somville (1980) (■), and Billen. unpublished
 (●)).

TABLE 2: Annual means of rates or organic substrates uptake and calculated rates of ammonium release in three marine environments.

	Eastern Channel (off Boulogne)	Belgian coastal zone (off Ostend)	Scheldt estuary (Hansweert)
Heterotrophic activity			
Amino acids uptake(mgC/l.y)	3.1	2.4	6.5
Monosaccharides uptake(")	2.6	4.4	16.3
Glycollate uptake (")	0.3	1.2	15
Total (mgC/l.y)	6	8	37.8
$\frac{C}{N}$ of organic matter taken up	6	10	17
NH_4 release (mgC/l.y)	0.4	0.08	- 0.115

2.2. Nitrogen recycling in Sediments

Another cause of non parallelism between carbon and nitrogen behaviour during organic matter mineralization results from the occurence of microbial transformations of nitrogen after the stage of ammonification. This primarily concerns benthic mineralization.

In the oxidized upper layer of the sediments, ammonium is generally actively oxidized to nitrate by nitrifying bacteria. Direct measurements in the sediments of the North Sea have shown that nitrification rates are closely correlated with ammonification rate and amount to 80% of it (Billen, 1982). The depth of the layer where nitrification is possible is therefore a major factor in determining under which form (ammonium or nitrate) nitrogen is released to the water column. Moreover, nitrates formed in this oxidized layer can diffuse into the reduced layer and be reduced into dinitrogen which is far less accessible for primary pproduction. The extend of this loss of nitrogen is also determined, in a complex way, by the depth of the oxidized layer.

A general, although idealized, model of nitrogen recycling in sediments based on data collected in the sandy sediments of the North Sea has been presented by Billen (1982). It relates the flux of organic material deposited on the sediments to the release of ammonium- and nitrate-nitrogen to the overlying
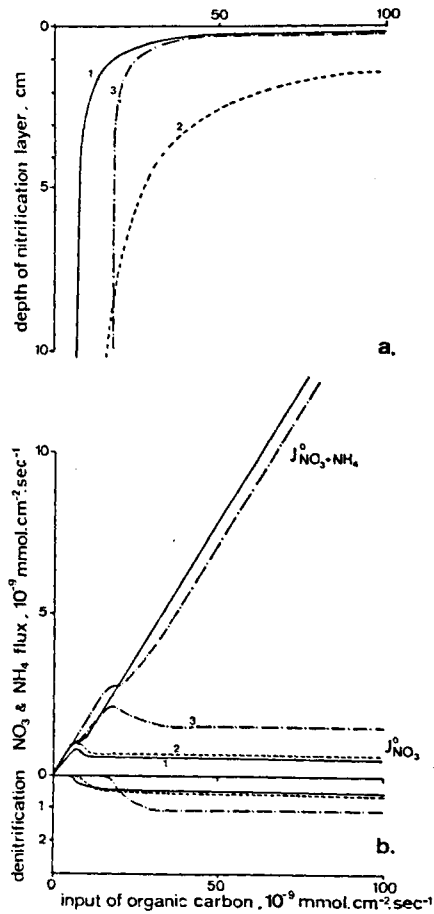
Fig. 11 : Model of nitrogen recycling in marine sediments as a
function of the input of organic material, for different
values of the mixing coefficients.
1. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 10^{-7} cm^2 sec^{-1}$
2. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 5 \ 10^{-6} cm^2 sec^{-1}$
3. $Di = 10^{-4} cm^2 sec^{-1}$, $Ds = 10^{-8} cm^2 sec^{-1}$
        C/N ratio = β = 6
a. Depth of the nitrification layer
b. Fluxes of nitrate and total mineral nitrogen across the
water sediment interface and integrated rate of denitrifi-
cation.

water. The results of this model are shown in Figure 11 for the same values of the mixing coefficients as those used in the redox model of Figure 8. It is seen that at low input of organic material to the sediments (what can be considered as a "low" flux of organic material depends on the mixing conditions prevailing in the sediment) most of the nitrogen recycling occurs as nitrate. With increasing input of organic matter nitrifying activity is restricted to a shallower and shallower upper layer (fig. 11a), vertically integrated nitrification reaches a maximum and ammonium release becomes more important. The last process prevails at high organic input (fig. 11b). Denitrification, being dependent on nitrates formed in the nitrification layer, reaches a plateau above a certain input of organic ,material. Paradoxically it implies that the relative value of denitrification in the overall nitrogen cycle is maximum at an intermediate input of depositing material and decrease at higher inputs.
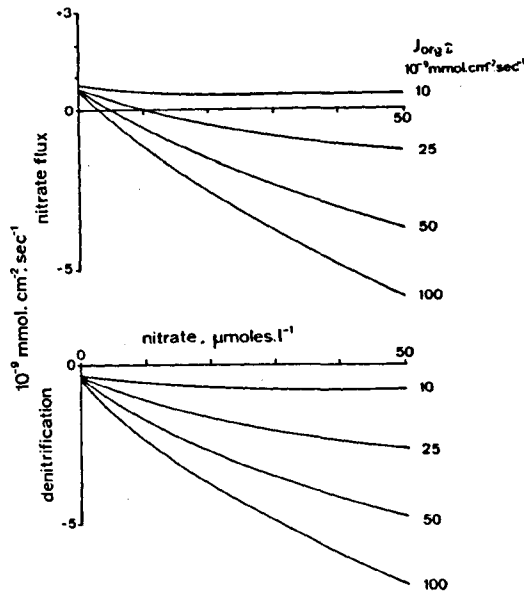

Fig. 12: Effect of nitrate concentration in the overlying water on
the flux of nitrate across the sediment-water interface
and on the rate of denitrification, calculated with the
model described for the same values of the parameters as
in fig. 6, and for various values of the flux of depositing
organic material ($J_{org}C$), indicated in 10^{-9} mmoles $cm^{-2}sec^{-1}$

Because denitrification mostly results in producing of flux of N_2 to the water colomn (although, some N_2O and NH_4^{+2} can also be produced, it can be considered as causing a net loss of nitrogen from the ecosystem. In the absence of nitrate in the overlying water, this loss is not expected to concern more than about 30% of the flux of nitrogen remineralized. Only when high nitrate concentrations exist in the overlying water, more important denitification rates can occur, with the sediment acting as a sink for nitrates from the water column (fig. 12). This effect of high nitrate concentration in the overlying water on the rate of denitrification in the sediment is much pronounced, however, at high organic content of the sediment (i.e. at high organic flux to the sediment) than for organic poor sediments (fig. 12).

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