HETEROTROPHIC ACTIVITY IN THE SEA

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HETEROTROPHIC UTILIZATION AND REGENERATION OF NITROGEN

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INTRODUCTION

Because organic nitrogen compounds represent both a nitrogen and an energy source for heterotrophic microorganisms in the sea, a discussion of the processes of their utilization and mineralization can bring insights either into specific aspects of the nitrogen cycle or into general mechanisms of organic matter metabolism in the sea. This paper will be mainly devoted to the latter aspect. There are indeed some technical advantages in focusing on nitrogen instead of on carbon for studying organic matter utilization in the sea, owing to the greater sensitivity of analytical methods for organic nitrogen than for organic carbon. However, parallelism or lack of parallelism between nitrogen and carbon utilization processes will be underlined.

Many examples discussed in this paper originate from data obtained in the Southern Bight of the North Sea and in the English Channel. This area is dominated by a flow of Atlantic water directed to the north east. However, due to the influence of the Scheldt estuary there is a zone of longer residence time of the water masses, of lower salinity, and of higher turbidity just in front of the Belgian coast. This zone receives important nutrient imputs from the land, so that the whole area offers a complete spectrum of situations from highly eutrophic in the Scheldt estuary to almost as oligotrophic as Atlantic water in the central English Channel (Fig. 1).

Existing data and concepts related to heterotrophic utilization and regeneration of nitrogen will be summarized under four main topics:

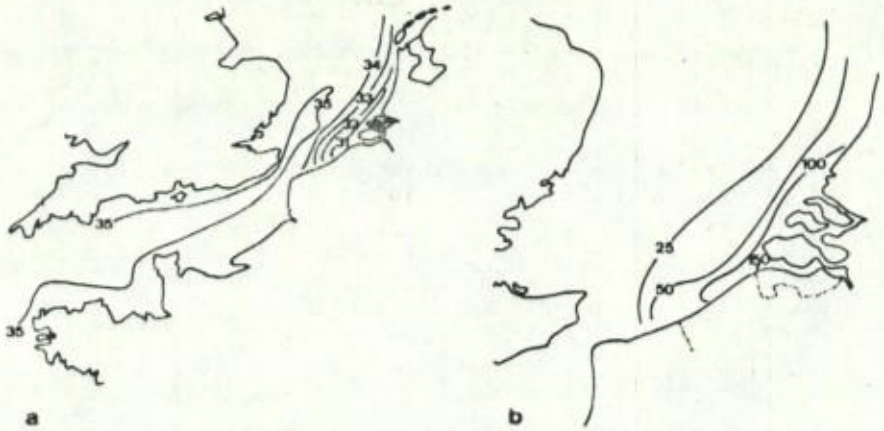

Figure 1. a. Spring distribution of salinity in the English Channel and the Southern Bight of the North Sea (Pichot 1980). b. Total nitrogen concentrations in the Belgian coastal waters (Nihoul and Boelen 1976; Hagel et al. 1973).

(1) Forms and production processes of organic nitrogen compounds in the sea.
(2) Hydrolysis of macromolecular organic nitrogen.
(3) Uptake mechanisms of direct nitrogenous substrates and competition.
(4) Nitrogen mineralization by heterotrophic microorganisms.

FORMS AND PRODUCTION PROCESSES OF ORGANIC NITROGEN COMPOUNDS IN SEA WATER

Dissolved Organic Nitrogen in Sea Water

During the last decade, intensive work has been devoted to the characterization of dissolved organic compounds in sea water, and to the geographical and seasonal variations of their concentration. Although most information available is based on organic carbon analysis, the same major features also apply to organic nitrogen:

- Most organic matter in the sea exists in the dissolved rather in the particulate phase.
- An important fraction of dissolved organic matter exists as high molecular weight compounds (Degens 1970; Ogura 1974; Wheeler 1976; Hama and Handa 1980).
- A significant part of dissolved organic matter is refractory to microbial attack and is defined as humic compounds.
- Direct organic substrates for microbial growth make up only a

very small part of total dissolved organic matter.

Total Organic and Inorganic Nitrogen

Total nitrogen concentration in sea water reflects the general level of nitrogen enrichment and varies widely from estuarine and coastal to open sea environments (see Table 1 and Fig. 1). Obviously, in most situations, total nitrogen concentration does not display seasonal variations (Fig. 2).

Dissolved organic nitrogen makes up 25 to 80% of total nitrogen according to the season; its percentage is at a maximum in late summer and at a minimum in the winter.

Characterization of Dissolved Organic Nitrogen

Numerous authors (Williams 1971, 1975) have pointed out that only a small fraction (10 to 30%) of the total dissolved organic carbon in natural waters can be identified as well defined compounds. As seen in Table 1 and Table 2, the same holds for total dissolved organic nitrogen. Only a small fraction of the dissolved organic nitrogen is compounds, such as free amino acids, that are directly usable by microbes. A much more important part (16-50%, Tuschall and Brezonik 1980) of organic nitrogen is made up by dissolved proteins and polypeptides ("combined hydrolyzable amino acids") of high molecular weight. The remaining fraction is probably made of humic compounds refractory to microbial attack (Thomas et al. 1971). Several hypotheses concerning the origin of these compounds have been suggested including reaction of phenolic compounds with proteinaceous material (Sieburth and Jensen 1969) or interaction between urea or amino acids and metallic ions (Degens 1970).

Regional and Seasonal Variations

While total organic nitrogen varies a great deal from estuarine and coastal to open sea environments, no large variations are found in the concentration of easily usable organic nitrogen compounds such as free amino acids. What is observed for regional variations seems also true for seasonal variations. Distinct seasonal variations in total dissolved organic nitrogen have been reported (Butler et al. 1979) which show accumulation of organic nitrogen compounds following the period of intense phytoplanktonic activities. On the other hand, most authors who have studied the annual cycle of total free amino acids concentration did not find very clear evidence of important seasonal change (Andrews and Williams 1971; Riley and Seagar 1970; Crawford et al. 1974; Billen et al. 1980). Andrews and Williams (1971) concluded that if such changes did occur, they were either small or short lived.

Table 1. Typical values reported for total nitrogen and total dissolved organic nitrogen in various marine environments (in μg-at N $liter^{-1}$).

Environments	Total Nitrogen	Total Dissolved Organic Nitrogen	Reference
Estuaries			
Rhine	420	105	Van Bennekom 1975
Scheldt	800	200	Van Bennekom 1975
Coastal Areas			
Nearshore North Sea	100	70	Nihoul and Boelen 1976
Offshore North Sea	50	20	Nihoul and Boelen 1976
English Channel		4.6	Banoub and Williams 1973
English Channel	12	6	Butler et al. 1979
Open Ocean (upper layers)			
North Atlantic		6	Holm-Hansen et al. 1966
Pacific		8.2	Thomas et al. 1971
Indian Ocean		7.5	Fraga 1966
Mediterranean		5.2	Banoub and Williams 1972
Open Ocean (deep layers)			
Pacific	35	4	Armstrong et al. 1966
Pacific		3.5	Holm-Hansen et al. 1966
Pacific		6.9	Thomas et al. 1971
Indian Ocean		5	Fraga 1966
Mediterranean		3.3	Banoub and Williams 1972

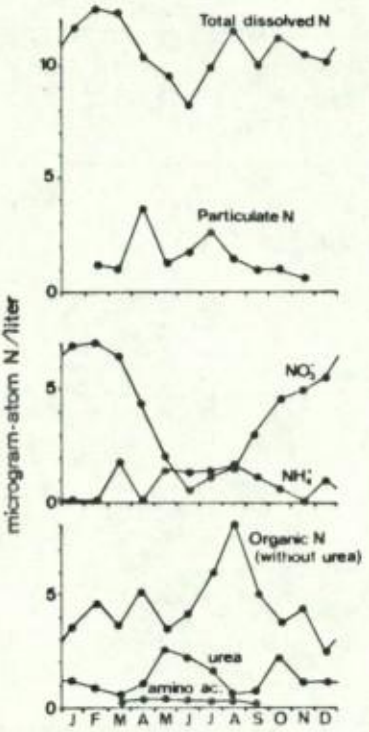

Figure 2. Seasonal variation of the concentration of various classes of nitrogenous compounds in the water of the Western English Channel (composite data from Butler et al. 1979; Banoub and Williams 1973; Andrews and Williams 1971).

This lack of change is illustrated by data on amino acids in the Southern Bight of the North Sea. Although differences of two orders of magnitude exists at some seasons in the relative rate of heterotrophic amino acid utilization from the Scheldt estuary and the Belgian coastal zone to the English Channel (Fig. 3), no significant differences are found in the pool size of amino acids in these three regions (Table 2). Thus the difference between eutrophic and oligotrophic waters lies in the concentration of high molecular weight organic nitrogen rather than in the concentration of easily usable substrates like amino acids.

As far as can be judged from the few data available, urea behaves more like an inorganic nutrient than like an organic substrate as it shows important regional (Table 2) and seasonal (Fig. 2) variations.

Table 2. Typical values reported for different classes of organic nitrogen compounds dissolved in the water of various marine environments (in µg-at N·liter^{-1}).

Environments	Total Amino Acids*	Primary Amines**	Free Amino Acids	Reference
Estuaries				
Scheldt		1.6	0.48	Lancelot, pers. comm.
Scheldt			0.2	Billen et al. 1980
Pamlico River			0.2	Crawford et al. 1974
York River			0.4	Hobbie et al. 1968
Coastal Areas				
North Sea		2.4		Lancelot, pers. comm.
North Sea			0.51	Billen et al. 1980
English Channel		2.6		Lancelot, pers. comm.
English Channel			0.26	Andrews & Williams 1971
English Channel			0.72	Billen et al. 1980
Irish Sea	0.4		0.15	Riley & Segar 1970
Baltic Sea			0.26	Dawson & Gocke 1978
Buzzards Bay	2.2		0.61	Siegel & Degens 1966
Open Ocean (upper layer)				
Atlantic	0.18		0.025	Lee & Bada 1977
Atlantic			0.26	Pocklington 1971
Atlantic		0.67		Liebezeit et al. 1980
Pacific	0.5		0.045	Lee & Bada 1975
Pacific			0.072	Williams et al. 1976
Pacific		0.7		North 1975
Pacific		0.41		Hollibaugh et al. 1980
Open Ocean (deep layer)				
Atlantic	0.1		0.025	Lee & Bada 1977
Atlantic			0.17	Pocklington 1971
Atlantic		0.59		Liebezeit et al. 1980

Environment	Total Nucleic Acid	Reference
Bombay Harbour Bay	0.4	Pillai & Ganguly 1970

Environments	Urea	Creatin	Reference
Estuaries			
Savannah River	6.8		Remsen et al. 1972
Coastal Areas			
English Channel	1.4		Butler et al. 1979
Coastal N. Atlantic	1.29		Remsen 1971
Peru upwelling	3.5		Remsen 1971
Peru upwelling		0.1	Whitledge & Dugdale 1972
Japan Sea	2.8		Mitamura & Saijo 1975
Open Ocean (upper layer)			
Atlantic	0.45		Remsen 1971
Pacific	0.24		McCarthy 1972
Pacific	1.8		Remsen 1971
Open Ocean (lower layer)			
Atlantic	0.5		Remsen 1971

*Total amino acids (free and combined)
** Primary amines (free a.a. and small pept.)

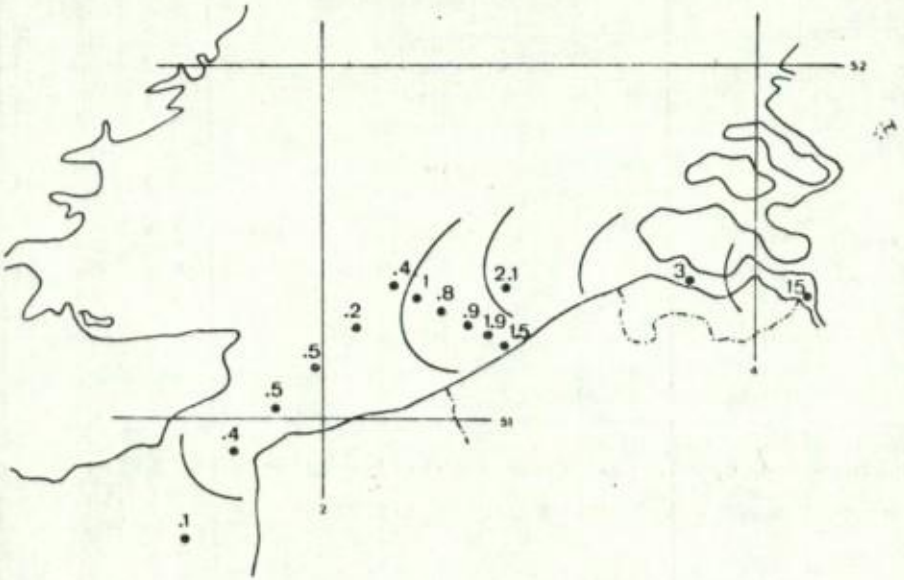

Figure 3. Utilization rate of amino acids (protein hydrolysate) in $\%\cdot h^{-1}$ in the Southern North Sea (April 1981).

Supply of Organic Nitrogen in the Sea

Except in local near-shore situations, primary production is by far the most important source of organic matter in the sea. Moreover, although rivers can carry large amounts of organic matter, most of it is degraded or sedimented in estuarine systems; thus only a small fraction of the organic matter, mostly made of compounds refractory to bacterial attack, is discharged into coastal zones (see e.g., Wollast and Billen 1981).

The three main processes to be discussed with regard to production of dissolved organic nitrogen in the sea are:

- extracellular release of dissolved organic matter by phytoplankton;
- spontaneous lysis or zooplankton spillage from phytoplanktonic cells;
- excretion by zooplankton.

Excretion of Organic Compounds by Phytoplankton

Studies of extracellular release of organic matter by natural phytoplanktonic populations are numerous [see for instance the reviews by Hellebust (1974), Sharp (1977) and the more recent papers by Larsson and Hagström (1979), and Lancelot (1979)]. Although little agreement exists on the relative importance of this process

with respect to total primary productivity (estimations varying from a few to 70%), it is certainly true that it represents a significant source of dissolved organic matter available for bacteria, especially in oligotrophic waters.

The chemical composition of this excreted material varies from species to species (Hellebust 1965). In the Southern Bight of the North Sea, during the spring phytoplankton bloom dominated by the species Phaeocystis poucheti, Lancelot (1982) demonstrated by ultrafiltration of the ^{14}C-labelled excreted material that organic compounds of molecular weight higher than 500 d. represent 60-91% (mean 82%) of the total exudate. Nalewajko et al. (1976) report somewhat different figures for fresh water phytoplankton: one third of the excreta was made of compounds with molecular weight higher than 5000 d. Wiebe and Smith (1977), working in an Australian estuary, reported that 95% of the excreted material is of low molecular weight (i.e., lower than 3500 d.).

The high molecular weight fraction of excreted material is probably mostly polysaccharides but proteins would also be an important part. In the low molecular weight fraction, on the other hand, free amino acids are present but they were found by Juttner and Matuschek (1978) to make up only about 10% of the total. Most authors also found glycolate, Krebs cycle acids and carbohydrates (i.e., non-nitrogenous compounds) as dominant constituents of extracellularly produced organic matter. Only nitrogen fixing blue-green algae were observed to excrete important amounts of free amino acids (Stewart 1963; Jones and Stewart 1969). All these data suggest that organic nitrogen excreted by natural phytoplankton communities in the sea mainly consists of proteins and peptides, while only a small amount of free amino acid is directly produced.

Death and Lysis of Phytoplanktonic Cells

Although it was thought in the past that the normal fate of phytoplankton cells in the sea is to be grazed by zooplankton and that only few simply die (e.g., Harvey 1945), it becomes more and more evident that in many aquatic systems, particularly coastal marine ones, zooplankton grazing cannot explain the variations observed in phytoplankton biomass and that an important spontaneous phytoplankton mortality occurs (Jassby and Goldman 1974; Joiris 1977).

When phytoplankton cells are disrupted or lyse, particulate detritus and dissolved organic matter are produced. Table 3 summarizes some data on detailed nitrogen composition of phytoplankton cells. It can be inferred from these figures that mostly macromolecular nitrogen compounds are primarily produced on lysis of phytoplankton. Although data on the pool of free amino acids in algae is rather scarce, this pool appears to be very small so that

Table 3. Detailed composition of phytoplankton nitrogen (calculated from data of Mayzaud and Martin 1975, and Reisner et al. 1960).

	% dry weight	% of total nitrogen
Total nitrogen	4.3	100
Protein nitrogen	3.65	85
Non protein nitrogen	0.69	15
Free amino acids	0.23 - 0.35	5 - 8

only minute amounts of free amino acids are released by simple disruption of algal cells.

Excretion of Organic Nitrogen by Zooplankton

A controversy exists in the literature concerning the forms of dissolved nitrogen excreted by zooplankton. Johannes and Webb (1965) and Webb and Johannes (1967) using high concentration of mixed zooplankton found that considerable amounts of free amino acids were released, while Corner et al. (1965) and Corber and Newell (1967) concluded from experiments with much lower zooplankton concentrations that no significant amounts of nitrogenous substances other than ammonia were excreted. Jawed (1969) measured the following composition of soluble excretion products of zooplankton: 76-82% ammonia-nitrogen, 13-18% amino nitrogen, and about 1% urea. Eppley et al. (1973) found much higher urea excretion (about 50%).

Soluble nitrogen excretion by fishes was also measured by Whiteledge and Dugdale (1972) on Peruvian anchoveta and consisted of about 50% ammonia, 25% creatine, 18% urea.

EXOENZYMATIC HYDROLYSIS OF MACROMOLECULAR ORGANIC NITROGEN

From the preceding section, it is apparent that an important part of both the stocks and of the fluxes of organic nitrogen in the sea is in the form of high molecular weight, polymeric material (proteins or peptides), either particulate or dissolved. Such material normally cannot be directly taken up by bacteria and can only be ultimately absorbed after exoenzymatic hydrolysis (Rogers 1961).

Exoenzymes, and exproteases in particular, can therefore be inferred to play an important role in aquatic ecosystems. Unfortunately, there is little information on their occurence and activity in the sea.

Occurence of Exoproteases in Aquatic Environments

Hydrolytic enzymes are present inside and outside the cell. Exoenzymes, found outside the cell, may be bound to the surfaces, such as a cell, or may be free (Pollock 1962). It is not easy to establish whether an enzymatic activity is intracellular or cell-surface bound, but free exoenzymic activity can be demonstrated after separation of the cells by gentle filtration. It is often difficult, however, to decide whether enzymes found free in the external medium have been liberated by healthy cells or have been liberated by cell disruption or autolysis.

Use of synthetic protein-dye (Azocoll, Calbiochem) or chitin-dye (Chitin-Red, Calbiochem) as substrates allowed some authors to demonstrate the occurence of free proteolytic and chitiniasic exo-enzymic activities in lake water, sea water and interstitial waters of marine sediments (Reichardt et al. 1967; Kim and Zobell 1974). Another technique is to use aminoacyl derivatives of (Beta)-naphthyl-amine. These give rise to fluorescent products upon enzymatic hydrolysis (Roth 1965) and have been found by Somville and Billen (1983) to be a very convenient and sensitive tool for studying exopeptidases in estuarine and marine waters. Some results obtained with this method in the English Channel, the North Sea and the Scheldt estuary are presented in Table 4. A measurable proteasic activity is present in all freshly collected unfiltered samples. In all cases, autoclaved samples lose their activity. Results of proteasic activity determination with or without prior filtration of the samples through 0.2 and 0.8 µm membranes show that some exoproteases may exist as free enzymes in the eutrophic Belgian coastal zone, while in the more oligotrophic waters of the Channel, most of them are bound to particles between 0.8 and 0.2 µm. In the last case, exoenzymes are probably linked to the external surface of bacterial cells, as demonstrated in some instances by Christison and Martin (1971).

Control of Exoenyzymes Production by Environmental Factors

Numerous studies of pure bacterial cultures have been concerned with the physiological regulation of exoenzymes synthesis (see the review by Glenn 1976). The production of many exoproteases can be induced by the presence of peptides or proteins in the external medium; end production inhibition by free amino acids and catabolic repression (e.g., by glucose) has also been reported for most exoprotein producing bacteria. Unfortunately, it is not known to what extent these pure culture observations can be extrapolated to

natural aquatic environments, and at which levels of substrate concentrations these physiological controls are operative.

Moreover, besides this "physiological regulation", some kind of "sociological regulation" (Wuhrmann 1968) must be taken into account when dealing with natural aquatic communities of microorganisms. Martin and Bianchi (Martin 1980; Martin and Bianchi 1980; Bianchi 1980), studying the specific composition of bacterial communities in various aquatic environments, showed that strains which produced exoenzymes are selected when polymeric detritus is the most important

Table 4. Proteolytic activity of filtered and unfiltered water from various marine environments (Billen and Somville, unpublished).

Environments	Sample	Proteolytic activity + 10^{-8} moles·liter^{-1}·min^{-1})
Belgian coastal zone (West Hinder, June 1981)	unfiltered	0.43
	filtered through 0.2 µm	0.38
	autoclaved	0
English Channel (Off Boulogne, June 1981)	unfiltered	0.66
	filtered through 0.8 µm	0.48
	filtered through 0.2 µm	0.18
	autoclaved	0
Scheldt estuary (Doel, May 1981)	unfiltered	1.82
	filtered through 0.2 µm	0.27
	autoclaved	0
	filtered + autoclaved suspended matter	0.27

+ Amount of B-Naphthylamide produced from L Leucyl B-Naphthylamide per minute of incubation time (Roth 1965).

substrate available such as in senescent phytoplankton cultures, sediments, etc.

Kinetics of Detritus Hydrolysis

Although no direct data are available concerning the kinetics of exoenzymes action in sea water, indirect information can be obtained from experiments on bacterial degradation of dead algal cells or on extracts of algal cultures, since it can be thought that exoenzymic hydrolysis is the first limiting step in the decomposition of these complex materials.

Most authors found or assumed first order kinetics for the degradation of detritus, the value of the kinetic constant depending on the nature of the material being degraded and on the environmental conditions. Table 5 shows the range of values reported for the

Table 5. First order constant of the degradation of various classes of complex organic nitrogen in aquatic environments.

Nature of the Material	$k(day^{-1})$	Reference
Dead phytoplankton cells		
- as a whole	0.04 - 0.1	Golterman 1972
	0.038 - 0.056	Von Brand et al. 1937
- soluble fraction (25% of total N)	1 - 0.2	Otsuki and Hanya 1972
- insoluble labile fraction (42% of total N)	0.056	Otsuki and Hanya 1972
- refractory fraction (33% of total N)	0.005	Otsuki and Hanya 1972
Dissolved macromolecular organic nitrogen		
- labile fraction	0.011	Otsuki and Hanya 1972
- dissolved proteins (in the presence of natural suspended matter)	0.015	Kailov and Finenko 1970

degradation of several classes of particulate or dissolved organic nitrogen. Otsuki and Hanya (1972) in one of the more complete studies, suggested that nitrogen compounds in green algae may be divided into three fractions according to their resistance to decomposition: a first fraction (about 25% of total N) is made of soluble, very easily degraded compounds (decomposition constant as high as 1-0.2 day^{-1}); a second fraction (about 42%) comprises labile material with a decomposition constant of about 0.06 day^{-1}; a third fraction (33%) is refractory to degradation (0.05 day^{-1} or less). In comparison to these studies of detritus, the decomposition of dissolved proteinaceous matter is much more subject to variations from one environment to the other. Kailov and Finenko (1970) have found that detritus enhanced the degradation of dissolved protein.

The rather low values of the rate constant of degradation of polymeric nitrogeneous material and the fact that, as shown in the preceding section, most of the organic nitrogen is supplied as macromolecules, imply that bacterial acitivity is buffered to a certain extent against short term variations in phytoplanktonic production by the large pool of nitrogen polymers. Whether such a buffer effect also exists for all other classes of organic material is not clear. Some authors demonstrated a very close coupling between phytoplankton excretion and bacterial activity, the turnover time of excreted products being sometimes as short as a few hours (Nalewajko et al. 1976; Wiebe and Smith 1977; Iturriaga and Hoppe 1977; Larsson and Hagström 1979). As shown above, however, those very rapidly used excretory products are not likely to contain nitrogenous compounds, but rather organic acids and monosaccharides.

UPTAKE MECHANISMS OF NITROGENOUS SUBSTRATES AND COMPETITION BETWEEN MICROBIAL SPECIES FOR THESE SUBSTRATES

Physiological Mechanisms of Nitrogen Uptake

All heterotrophic microorganisms need nitrogen compounds (either mineral or organic) for biosynthetic purposes. In addition, most of them can use organic nitrogen compounds as substrates for energy. Algae use ammonium or nitrate as nitrogen source, but many species have been demonstrated to be capable of autotrophic growth with amino acids or urea as the sole nitrogen source. Finally, ammonium is an energy source for the chemolithotrophic nitrifying bacteria.

Mineral Nitrogen Assimilation by Heterotrophic Bacteria and Algae

At low concentration, ammonium is generally assimilated as well in bacteria as in algae by the glutamine synthetase (GS)/glutamine-oxoglutarate amino transferase (GOGAT) system, involving ATP dependent synthesis of glutamine from glutamate and NH_3 and NADPH-linked synthesis of two glutamates from glutamine and oxaglutarate (Tempest

et al. 1970; Brown et al. 1972; Falkowski and Rivkin 1977; Miflin and Lea 1977). This system is much more efficient than glutamate deshydrogenase (GDH), which catalyses the direct glutamate synthesis from NH_3 and oxoglutarate at high ammonium concentrations.

When used as nitrogen source, nitrates are first converted into ammonium via nitrite before being assimilated by the same pathway. In algae, nitrate reductase (and perhaps the nitrate permease system) is generally suppressed by ammonium concentrations higher than about 0.5-1 $\mu moles \cdot liter^{-1}$ unless the cells are severely nitrogen depleted (Eppley and Solorzano 1969; Morris 1974; Conway 1977). This last regulation mechanism has not been demonstrated in marine bacteria (Brown et al. 1972), although it has often been observed that ammonium is preferentially taken up by heterotrophic microorganisms grown on both nitrate and ammonium (Alexander 1977).

In 12 strains of marine pseudomonads, Brown et al. have shown that the GS/GOGAT system is repressed by the presence of amino acids, indicating that amino acids, when available in sufficient amounts, are a preferred source of nitrogen for marine bacteria. When used also as source of carbon, amino acids are generally converted to their respective oxoacids by transamination with oxoglutarate yielding glutamate which is then deaminated by GDH (Brown et al. 1972).

Active Transport Systems

Active transport systems (permeases) for both mineral and organic nitrogen compounds have been demonstrated in bacteria and algae. These system in algae for ammonium and nitrate are very efficient in terms of the half-saturation constant (Table 6). Falkowski (1975) has suggested that nitrate uptake by marine phytoplanktonic algae is related to a NO_3^- activated ATPase associated with the cell membrane. Fewer studies exist on mineral nitrogen permeases in heterotrophic microorganisms, although two distinct ammonium uptake systems have been demonstrated in *Saccharomyces cerevisiae* (Roon et al. 1975; Dubois and Grenson 1979).

Permeases systems for amino acids have been much more intensively studied. In most microorganisms investigated (e.g., *Escherichia coli* (Piperno and Oxender 1968; Brown 1971; Rahmanian et al. 1973; Guardiola et al. 1974) *Pseudomonas aeruginosa* (Kay and Gronlund 1969, 1971) *Saccharomyces cerevisiae* (Grenson et al. 1966, 1970; Grenson 1966; Gits and Grenson 1967) several amino acid transport systems with high affinity and high substrate specificity were demonstrated, each responsible for the uptake of only single or closely related amino acids. Often, multiple transport systems exist for a single or a group of amino acids.

In some cases, besides these specific very efficient permease systems, an additional system with wider specificity was

Table 6. Transport constant (K_t) for amino acids, urea and mineral nitrogen (μmoles/l) of different species and strains of heterotrophic bacteria, phytoplanktonic algae and nitrifying bacteria. (+ Values in brackets refer to a second transport system with lower affinity.)

Organism	Transport Constant for Amino Acid		Urea	NH_4^+	NO_3^-
Heterotrophic microorganisms					
Saccharomyces cerevisiae[a]				1-(20)+	
Pseudomonas aeruginosa[b]	Phe	0.4			
	Tyr	0.5			
	Try	1.4			
Alteromonas haloplanktis[c]	Ala	4.6			
Heterotrophic strain RP 303[d]	Pro	1.8			
Oligotrophic strain 486[d]	Pro	0.2			
Phytoplanktonic algae					
Skeletonema costatum[e]			4.25		
				0.5-0.4	3.6-0.8
Ditylum brightwellii[f]			0.20	0.6	1.1
Dunaliella tertiolecta[f]				1.4	0.1
Platymonas subcordiformis[g]	Gly	5			
Melosira nummuloides[h]	Arg	7.7			
	Val				
Navicula pavillardi[i]	Glu	18			
Coccolithus huxleyi[f]			0.1	0.1	0.1
Cyclotella nana[f]				0.3, 0.7	0.4
			0.21		
Nitrifying bacteria (Apparent transport constants based on oxidation rates by culture of nitrifying bacteria)					
Mixed culture[j]				500	
Nitrocystis oceanus[k]				150	
Nitrosomonas europaea[l]				86-715	
Enrichment culture from the Scheldt estuary[m]				250	

[a] Dubois and Grenson 1979; [b] Kay and Gronlund 1971; [c] Fein and MacLeod 1975; [d] Akaji and Tage 1980; [e] Carpenter et al. 1972; [f] Eppley et al. 1969; [g] North and Stephens 1967; [h] Hellebust 1970; [i] Lewin and Hellebust 1975; [j] Knowles et al. 1965; [k] Carlucci and Strickland 1968; [l] Painter 1977; [m] Somville 1980

The affinity of transport systems of different strains and species of aquatic microorganisms for amino acids, urea, NH_4^+ and NO_3^- are given in Table 6. From these data, it can be seen that heterotrophic bacteria have developed amino acid transport systems with higher affinity for substrates than have phytoplanktonic algae. The reverse seems to be true for nitrite and ammonium, although few data are available for mineral nitrogen uptake by bacteria. The K_t values of phytoplanktonic algae for urea are of the same order of magnitude as for mineral nitrogen. In contrast, the K_t values of nitrifying bacteria for ammonium are at least two order of magnitude higher than those of algae. When comparing these figures however, it must be remembered that for bacteria, considerable difference can exist between the value of K_t of an organism in natural water and the value measured after isolation and cultivation of this organism. For example, Jannasch (1968) showed that the half saturation constant of growth of several bacteria increased 2 to 10 fold during repeated transfers in media enriched in organic substrates.

In situ Determination of Half-Saturation Constant for the Uptake of Nitrogenous Compounds

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 reported. In most situations, the uptake was found to obey a Michaelis-Menten-Monod relationship:

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

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, and 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). He showed that the validity of relation (1) is better when the community becomes less diverse (in terms of the values of K_{ti} of the various populations). Indeed, some cases of departure from Michaelis-Menten-Monod kinetics have been reported for the uptake of some substrates by natural population of microorganisms (Vaccaro and Jannasch 1966; Hamilton and Preslan 1970; Barvenik and Malloy 1979); they concern generally very oligotrophic environments where it is known (Martin and Bianchi 1980) that microbial diversity is more important. In all the other cases 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 strains 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.

For substrates used purely for energy, like glucose and acetate, there appears to be a relationship 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). Since the rate of utilization of a substrate (rather than its concentration) is the best index of the "richness" of an environment in terms of this substrate, this trend can be interpreted as reflecting the competition between microorganisms for their substrates; the lower the availability of a substrate, the higher the selective pressure for developing sophisticated and expensive permease systems with great affinity for this substrate.

The same relationship holds for mineral nitrogen substrates as shown by the data of McIsaac and Dugdale (1969) and Paasche and Kristiansen (1982) from oligotrophic and eutrophic marine areas (Fig. 6). These data show K_t values less than 0.2 $\mu moles \cdot liter^{-1}$ in oligotrophic waters and higher than 0.5 $\mu moles \cdot liter^{-1}$ in eutrophic waters.

Such a relationship, however, cannot be demonstrated for amino acids. The K_t values found in a whole range of environmental situations, from oligotrophic ocean area to organic rich sediments, always are very low, ranging from 0.02 to 1.5 $\mu moles \cdot liter^{-1}$ (Fig. 7). Perhaps the double nature of amino acids as both a carbon and a nitrogen

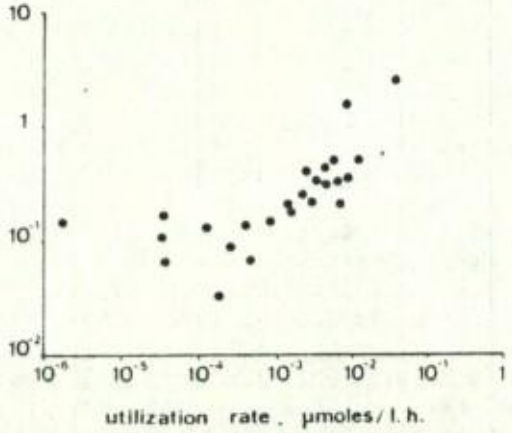

Figure 4. Half saturation constant for glucose uptake measured in various aquatic environments plotted against the glucose utilization rate. (Data from Japanese marine and brackish environments, and from Canadian lakes, Seki et al. 1975, 1980a,b).

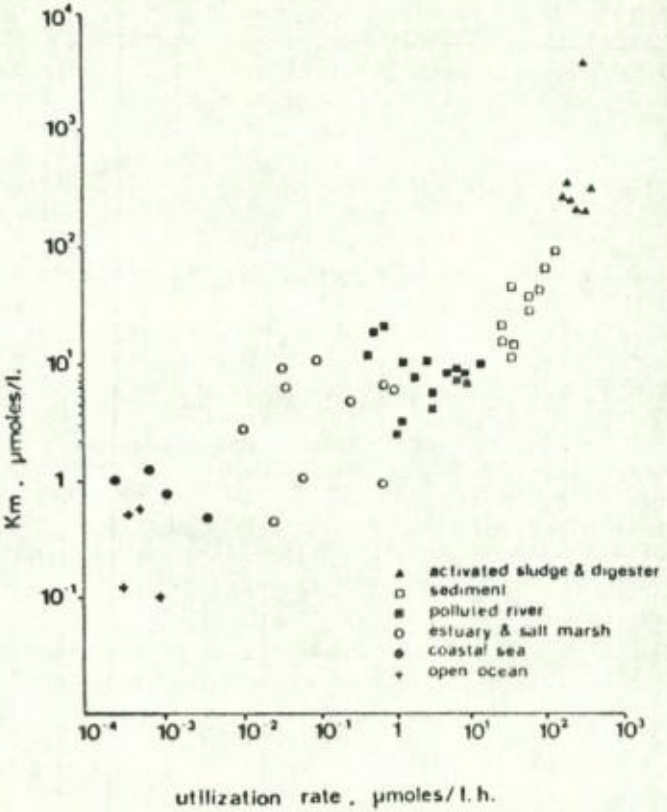

Figure 5. Half-saturation constant for acetate uptake measured in various aquatic environments plotted against the acetate utilization rate. (Data from Seki et al. 1974, 1980; Strayer and Tiedje 1977; Stanley and Staley 1977; Russel and Baldwin 1979; Billen et al. 1980; Billen, unpublished.)

source for microorganisms explains the fact that competition for them is always very severe even when their total flux is important.

Comparing the range of the K_t values found in situ for natural mixed microbiological communities (Fig. 6 and 7) with those found for pure species of microorganisms (Table 6), suggests the following conclusions concerning the competition conditions of the various microorganisms for each class of organic nitrogen compounds.

(i) Amino acids uptake seems to be dominated by bacteria rather than by algae. This conclusion is supported by the work of Williams (1970) and Derenbach and Williams (1974) showing by differential filtration following incubation with labelled amino acids that most of the radioactivity incorporated was associated with particles

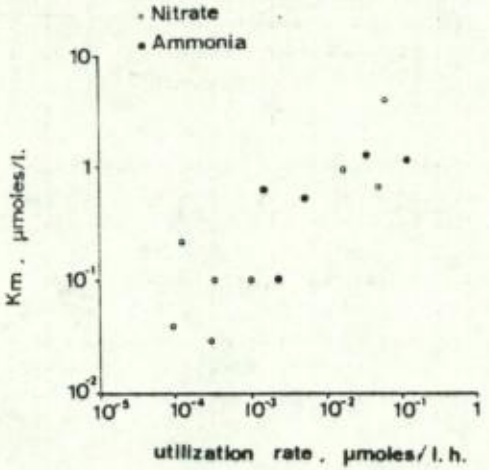

Figure 6. Half-saturation constant for nitrate and ammonia uptake in oligotrophic and eutrophic marine systems plotted against the total rate of nitrate or ammonia utilization. (Data from McIssaac and Dugdale 1969; Paasche and Kristiansen 1982.)

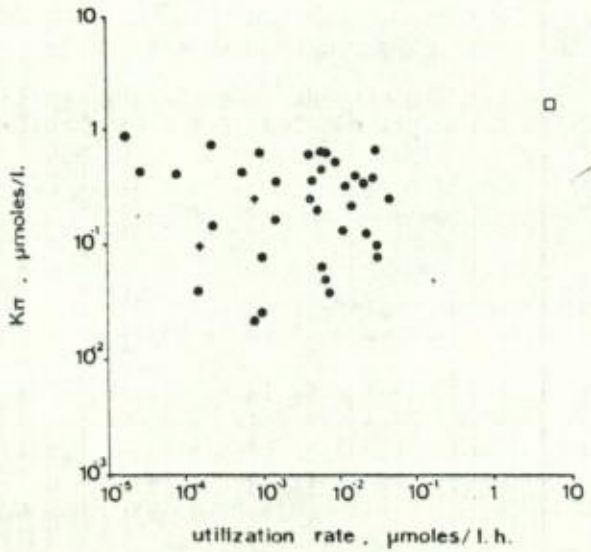

Figure 7. Half-saturation constant for alanine uptake in various aquatic environments plotted against the alanine utilization rate. (Data from Seki et al. 1974, 1980; Crawford et al. 1974; Christensen and Blackburn 1980.)

smaller than 8 m. These works were criticized however by Wheeler et al. (1977) who emphasized the possibility of artifact due to postfiltration; using differential filtration prior to incubation and autoradiography these authors found that phytoplankton were sometimes responsible for up to 50% of glycine uptake. On the other hand, Schell (1974) using ^{15}N-labelled glycine and glutamate, showed that these amino acids only contribute a very small part of the nitrogen requirements of phytoplankton.

The situation is different for urea which has been shown to contribute appreciably to nitrogen uptake by phytoplankton, both by ^{15}N studies (McCarthy 1972) and by differential filtration methods (Remsen et al. 1972; Mitamura and Saijo 1975). The same studies indicate that the contribution of bacteria to urea decomposition is minor compared to that of phytoplankton.

(ii) Mineral nitrogen uptake in open water is probably dominated by phytoplankton, although no data are available concerning a possible ammonium uptake by heterotrophic bacteria. In most situations in the photic layer, autotrophic nitrifying bacteria are unable to compete with algae for NH_4^+ uptake. These bacteria are only active in situations where, owing to intense ammonium production or reduced phytoplankton uptake, ammonia accumulates. Accordingly, active nitrification is found in sediments (Billen 1976; Henriksen 1980), in the water column below the photic layer (Gundersen and Mountain 1973), or in polluted estuaries (Billen 1975; Somville 1978) but no convincing evidence of active nitrification in the photic layer of unpolluted seas has been obtained so far.

Control of Substrate Concentration by Rapidly Growing Microorganisms

The purpose of this section is to investigate the relations existing in natural waters between the uptake characteristics of microorganisms discussed in the preceding sections and the concentration of directly usable substrates, such as amino acids, urea or inorganic nitrogen species.

General Theory

The concentration of a particular substrate results from the balance between the rate production of this substrate (e.g., by phytoplanktonic excretion, exoenzymatic hydrolysis, etc.) and the rate of uptake by the dominant microorganisms population. If this population is limited by the substrate and is able to grow fast enough to maintain a steady 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:

$$dS/dt = P - V_{max}S/(S + K_t)B \quad (2)$$

Where P is the rate of production of the substrate, B is the mass of organisms utilizing S, V_{max} is the maximum rate of uptake per organism, and K_t is the transport constant of S by the organism.

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

$$dB/dt = B(YV_{max}\ S)/(S + K_t) - k_dB \quad (3)$$

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

At stationary state the solution (2) and (3) is

$$S = K_t/((YV_{max}/k_d)-1) \quad (4)$$

$$B = (Y/k_d)P \quad (5)$$

showing that at steady 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 ($\mu_{max} - YV_{max}$) and the death rate of the organisms.

The question is of course to know how closely a steady 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 steady state, or for restoring it after a sudden perturbation, is about $1/k_d$, that is, of the order of the generation time of the microorganisms at steady state. Thus bacteria, having short generation times, effectively control the concentration of the substrates they use predominantly. In contrast, algae, having relatively long generation times (and moreover, being limited by other factors like light intensity) are not always able to maintain the concentration of their substrates at a stationary state.

The validity of equation (4) 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. 8). Both sets of data agree well for these substrates, showing that these systems are close to being in a steady state, and that the control of substrate concentration by the uptake of microorganisms is effective. The relation obtained in both cases is $S \simeq Km/3$ which suggests that in all the environments considered the ratio μ_{max}/k_d is about 4.

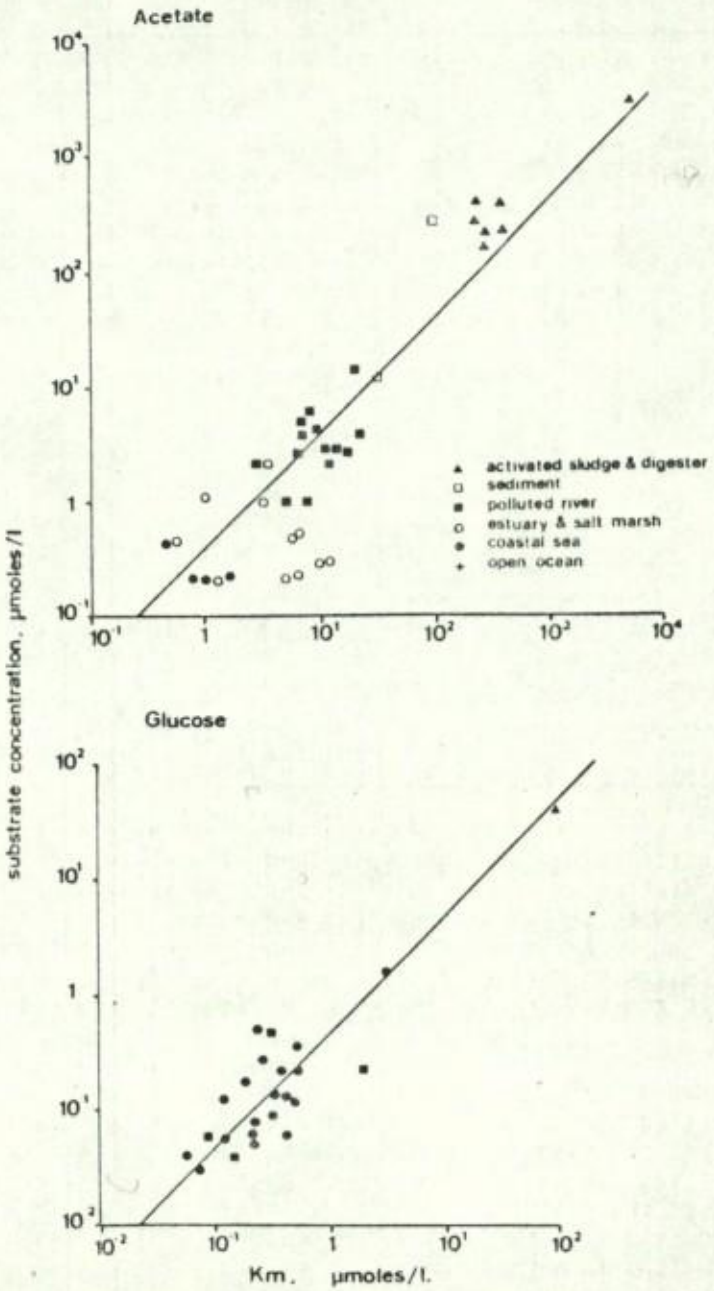

Figure 8. Relation between the natural concentration of glucose and acetate and the half-saturation constant of uptake in various aquatic environments. (Data from Seki et al. 1974, 1980; Walker and Monk 1971; Russel and Baldwin 1979; Stanley and Staley 1977; Kaspar and Wuhrmann 1978; Billen et al. 1980.)

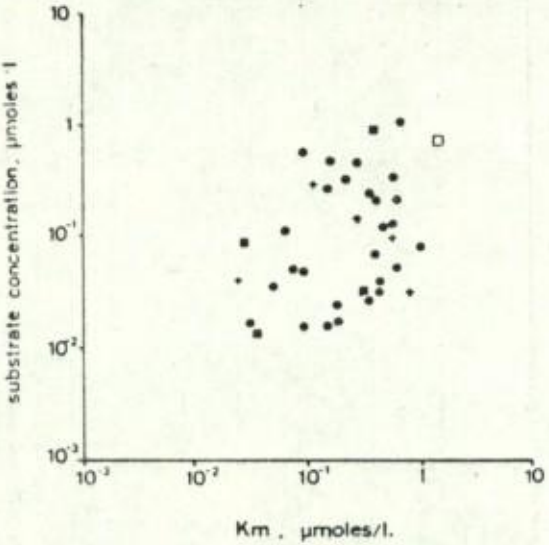

Figure 9. Relation between the natural concentration of alanine and its half-saturation constant of uptake in various aquatic environments. (Data from Seki et al. 1974, 1980; Crawford et al. 1974; Christensen and Blackburn 1980.)

Application to the Case of Nitrogenous Substrates

Based on the information given above, it appears that amino acids are predominantly taken up by bacteria while urea and mineral nitrogen species are mostly used by phytoplankton. This division is consistent with the ideas presented above that the rapidly growing microbes can actively control the concentration of substrates. Thus, amino acid concentrations do not change significantly over the seasons in contrast to the wide fluctuations of urea and inorganic nitrogen.

Figure 9 shows, as an example, the relation observed between concentration and the half-saturation for uptake of alanine. The theoretical relationship (equation 4) does not fit the data as well as it did for glucose and acetate (Fig. 8). This can be explained by the fact that no microorganism is likely to be limited by a single individual amino acid. Nevertheless individual amino acid concentrations in natural water are well within the range of experimentally determined transport constants.

NITROGEN MINERALIZATION BY HETEROTROPHIC MICROORGANISMS

In the preceding part of this paper, the gross utilization of

organic nitrogen compounds by heterotrophic microorganisms has been discussed without concern for the fate of the compounds. After uptake by the microbes, organic nitrogen compounds can be either incorporated into biomass or excreted as ammonia. In the classical conception, 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 possible importance of the former process, claiming that the bacteria account directly for only a minor fraction of nutrient regeneration and may even compete with algae for mineral nitrogen. This question of organic nitrogen incorporation versus mineralization can now be reexamined in the light of recent physiological and ecological data.

Mineralization of Individual Organic Nitrogen Substrates

Most of the information available on microbiological mineralization of individual organic compounds has been obtained by ^{14}C tracer studies and therefore strictly refers to carbon mineralization even when organic nitrogen compounds are involved. By simultaneously measuring respiration and incorporation of labelled compounds, it is possible to define a growth yield ratio (ratio of incorporation to total uptake) or its complement the C-mineralization ratio (ratio of respiration to total uptake). For amino acids, the latter ratio varies from 0.15 to 0.60. This variability is much lower, however, when considering a single amino acid.

Crawford et al. (1974) and Wright (1974) have shown that amino acids can be divided into three groups: the first (including Glu, Asp, Asn, Pro, Arg) has high ratios for mineralization (often equal or higher than 0.50), the second (including Leu, Isoleu, Lys, Phe, Val) has low values for the mineralization ratio (about 0.15), and the third (Ala, Ser, Tyr) has intermediate values for the mineralization ratio (0.20-0.50). Amino acids of the first group are those which are the most directly metabolized into Krebs' tricarboxylic acid cycle. They are also those for which the maximum rates of uptake are the higher (Wright 1974).

The values just discussed of the C-mineralization ratio are for short term (2-5 hr) experiments. They probably do not reflect the natural steady state metabolism of amino acids by bacteria. In long-term incubation experiments (about 100 hr) Williams and Gray (1970) and Hollibaugh (1978) found much higher values of the C-mineralization ratio, from 0.65 to 0.70.

Because amino acids can be very easily deaminated at early stages of their metabolism in microorganisms, the fate of nitrogen after amino acid uptake is not necessarily similar to that of the carbon skeleton. Using parallel ^{15}N and ^{14}C labelling experiments, Schell (1974) demonstrated that the nitrogen of glycine was

preferentially incorporated with respect to carbon (C uptake/N uptake ratio = 0.6) while the reverse was true with glutamate (C uptake/N uptake = 2.6). The mean ratio of ammonium release to total uptake of amino N was 0.74 in long-term incubation experiments with added amino acids (Hollibaugh 1978).

Mineralization of Complex Substrates

In natural situations, heterotrophic microbial populations do not assimilate a single individual compound but rather a mixture of several substrates. The balance between N-mineralization and incorporation will then depend on the C:N ratio 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:

$$\Delta NH_4/\Delta C = 1/\left(\frac{C}{N}\right)_S - Y/\left(\frac{C}{N}\right)_B \qquad (6)$$

where Y is the growth yield ratio, $(C/N)_S$ is the C:N in the substrate, and $(C/N)_B$ is the C:N of bacterial biomass.

The experiments of Hollibaugh (1978), Somville (1980) and Billen (unpublished), who supplemented natural sea water with mixtures of organic substrates and followed the consumption of the substrates and the release of ammonia, permit a test of the validity of relation (6) (Fig. 10). Although the data come from two different environments and were obtained with different organic substrates, a very good fit is obtained, with $\frac{Y}{\left(\frac{C}{N}\right)_B} = 0.1$ gN/gC.

If 4 gC/gN is taken as a reasonable estimate for $(C/N)_B$, Y can be evaluated as 0.4 in good agreement with the values cited above for C-assimilation ratio in long-term experiments.

Relation (6) and Fig. 10 also show the lack of parallelism between the role of bacteria in carbon and nitrogen cycling. When the (C/N) ratio of the organic matter used by bacteria increases, ammonium release decreases and, for $(C/N)_S$ higher than 10 gC/gN, uptake instead of release can even occur during organic matter degradation. The role of bacteria as nitrogen mineralizers thus does not necessarily parallel 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 (Table 7). Total heterotrophic activity throughout the year was estimated by measuring the concentration and the relative rate of bacterial utilization of the three main classes of direct organic substrates (free amino acids, monosaccharides, and glycollate). From these data, ammonium remineralization can be calculated according to equation (4). As seen, the most important

Table 7. Annual means of rate of organic substrate uptake and calculated rate of ammonium release in three marine environments.

Heterotrophic Activity	Eastern Channel (off Boulogne)	Belgian Coastal Zone (off Ostend)	Scheldt Estuary (Hansweert)
Amino acid uptake*	3.1	2.4	6.5
Monosaccharide uptake*	2.6	4.4	16.3
Glycollate uptake*	0.3	1.2	15
Total*	6	8	37.8
C/N of organic matter taken up	6	10	17
NH_4 release**	0.4	0.08	-0.115

* mg $C \cdot liter^{-1} \cdot yr^{-1}$
** mg $N \cdot liter^{-1} \cdot yr^{-1}$

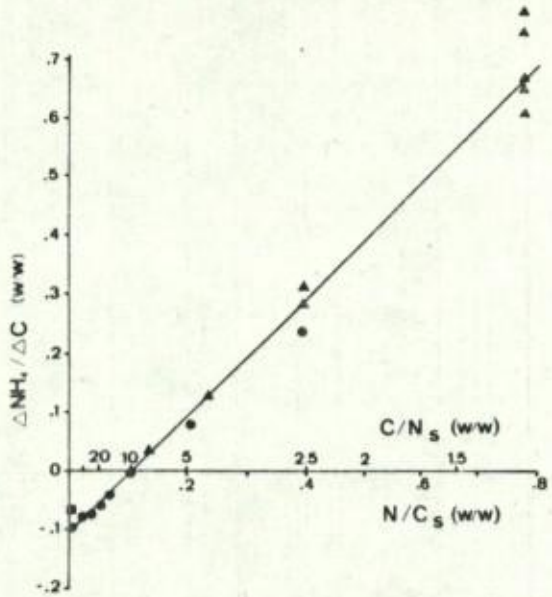

Figure 10. Release or uptake of ammonia per unit carbon taken up by microbiological communities of marine environments supplemented with mixed substrates of various C/N ratio. (Data from Hollibaugh 1978 (▲), Somville 1980 (■), Billen, unpublished (●)).

ammonium release occurs in the Western Channel, where heterotrophic carbon utilization is the lowest. In the Belgian coastal zone, the ammonium regeneration by bacteria is limited because the most important substrates for heterotrophic activity are carbohydrates which in part come from the mucopolysaccharides excreted by Phaeocystis poucheti (Lancelot 1982). In the heavily polluted Scheldt estuary there is actually net ammonium uptake due to the high C/N ratio of the terrigenous organic material being degraded.

Relative Role of Bacteria and Zooplankton in Nitrogen Mineralization in the Sea

There are a few recent studies which indicate the relative role of bacteria, net plankton, and microplankton in providing nitrogen for phytoplankton (Table 8). Net zooplankton excretion has been measured by various authors after concentration by filtration through 180 or 100 μm mesh net. The results suggest a clearcut difference between oceanic environments, where net zooplankton excretion accounts for 36-100% of N-requirements of primary producers, and coastal (and upwelling) environments, where zooplankton excretion only represents a small fraction (less than 20% and often less than 10%) of phytoplankton nitrogen uptake.

Direct measurements by a ^{15}N isotope dilution technique of

Table 8. Relative role of various mineral nitrogen sources in meeting the requirements of primary producers in different marine environments.

Environments	Period	N requirement for net primary production (mg-at $N \cdot m^{-2} \cdot day$)	(Net)* zooplankton excretion (% of phytoplanktonic N requirements)	(Micro) zooplankton excretion	Bacterial mineralization
Coastal Areas					
Washington coast[a]	June-July	(4.6)**	3		
Southern California Bight[b]	March	2.4	7.5		101
Saanich Inlet[b] (CEPEX enclosure)	September	16.6	11	(33)	(91)
North Sea Southern Bight					
- Belgian coastal zone[c]	Annual budget	(5.2)	(20)		
- offshore zone[c]	Annual budget	(5.6)	(18)		
Open Ocean Areas					
Pacific off Oregon[a]	June-July	(1.6)	36		
Sub-tropical Pacific central gyre					
- station 1 (0-75 M)[d]	November	1.8	40		
- station 2 (0-75 M)[d]	Novepber	3.0	110		

[a] Jawed 1973; [b] Harrison 1978; [c] Billen 1978; [d] Eppley 1973

* Separated by filtration through 180 - 100 µm mesh net according to the authors.

** Values in brackets are indirect estimations based on C measurements. The other data result from direct nitrogen flux measurements.

nitrogen remineralization in coastal areas have been reported by Harrison (1978). By differential filtration, he showed that only 10% of the total ammonium regeneration activity was associated with particles greater than 35 µm, 40% passed a 1 µm filter, and the remaining 50% fell between 1-35 µm. Free bacteria are probably responsible for ammonium production in the < 1 µm fraction and attached bacteria for a part of the activity in the other fractions. In the latter, however, other organisms could have a significant role.

Microzooplankton (either ciliates or flagellates), which graze on bacteria, are known as efficient mineralizers with high specific ammonia excretion rates. Fenchel (1980), however, showed that bacterivorous ciliates cannot play an important role as grazers at the low bacterial densities normally encountered in marine environments. Available estimates (Harrison 1978) of NH_4 production by microzooplankton indicates that they produce a maximum of 1/3 of the total ammonia.

Phytoplankton ammonium excretion is probably of minor importance, although there is some evidence supporting the occurrence of the process (Prochazkova et al. 1970; Schell 1974).

Most of the planktonic mineralization, at least in coastal areas, is thus the result of bacterial activity. Direct mineralization of amino acids have been shown by Hollibaugh (1980) to account for about 60% of the observed flux of ammonia production.

CONCLUSION

According to the preceding discussion, nitrogen cycling in the photic layer can be viewed as represented in Fig. 11. Primary production requires mainly mineral nitrogen (or urea), part of which comes from the subphotic layer or the benthos ("new production", Dugdale and Goering 1967), and the other part comes from remineralization mechanisms in the photic layer ("regenerated production"). There are three fates of the phytoplanktonic material produced. One fraction sinks to the subphotic layer or the benthos. A second fraction is grazed by zooplankton, initiating the food chain leading to fish. The third fraction (comprising all dissolved material produced and part of the particulates produced) is available for bacterial utilization. This involves two steps: (i) exoenzymatic hydrolysis of the macromolecules which form the bulk of the organic nitrogen; (ii) rapid uptake of the hydrolysis products (mainly amino acids), the concentration of which are maintained at a constant level. The organic nitrogen taken up is partly remineralized as ammonium and partly incorporated into bacterial biomass, according to the C/N ratio of the organic matter taken up.

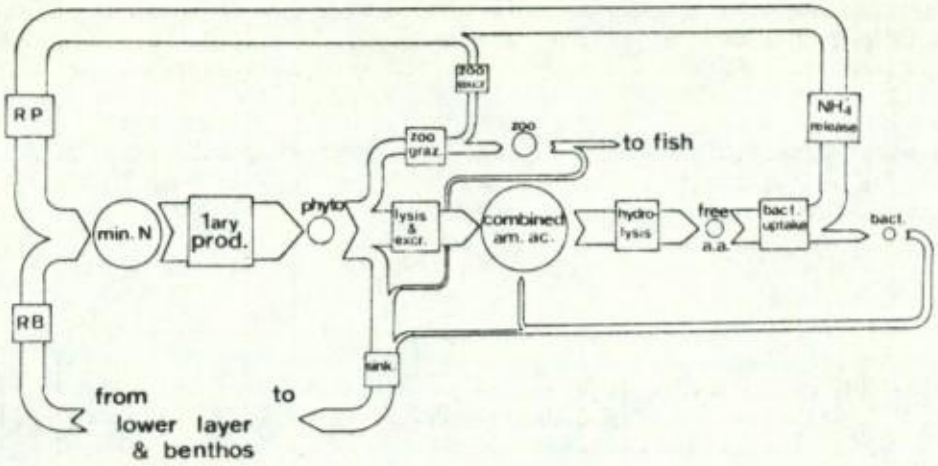

Figure 11. Schematic representation of nitrogen cycling in the upper layer of the ocean. (RP is N regeneration in the photic layer (corresponding to "regenerated production") RB is regeneration in the lower layer and the benthos (corresponding to "new production")).

Table 9. Estimation of planktonic nitrogen regeneration in the photic zone by water type of the world ocean. Data from Eppley and Peterson (1979).

	Primary Production ($g\ C \cdot m^{-2} \cdot yr^{-1}$)	% New Production (%)	Planktonic N Regeneration in the Photic Layer[+] ($g\ N \cdot m^{-2} \cdot yr^{-1}$)
Oligotrophic waters from anticyclonic eddies	25.6	6	4.1
Transitional waters	51	13	7.7
Equatorial divergence and subpolar zones	73	18	10.5
Inshore waters	124	30	15.1
Neritic waters	365	46	34.6

[+]Calculated from the Redfield ratio for primary production.

Bearing in mind this picture of the nitrogen cycle in sea water, it is possible to examine the coherency of the various experimental measurements of nitrogen related microbiological activities in the world ocean. Eppley and Peterson (1979), summarizing the data available on new and regenerated production from ^{15}N measurements, proposed an estimate of global new primary production. From their data, planktonic nitrogen regeneration in the photic zone can be calculated by water types from the world ocean (Table 9).

Table 10. Measurements of the rate of utilization of amino acids by heterotrophic microbial communities in the photic layer of various marine environments.

Environment	Reference	Turnover time (hours)
Pamlico River estuary	Crawford et al. 1974	35
Scheldt estuary	Billen, unpublished	32
Tokyo Bay	Seki et al. 1975	13
Shimoda Bay	Seki et al. 1975	28
Belgian coastal zone	Joiris et al. 1982	66 - 50
Florida Strait	Williams 1975	24 - 50
Eastern English Channel	Billen, unpublished	100
Coastal Eastern Pacific	Williams 1975	60 - 2400
Pacific off California	Williams et al. 1976	40 - 70
Mediterranean	Willaims 1975	96 - 80
Kuroshio current	Seki et al. 1972	3100
Subarctic Pacific	Seki et al. 1972	3000
NW Pacific central waters	Seki et al. 1972	9000
NW Pacific central waters	Seki et al. 1974	4000

Another type of data, measurements of the annual mean turnover time of total free amino acids in different marine environments, is also available (Table 10). As discussed earlier, total free amino acid concentration in each of these water types is maintained near to steady state by the microbial population and does not vary a lot. From the data of Table 2, the concentration can be grossly estimated as 0.5 μg-at N·liter^{-1} in coastal and neritic waters and 0.05–0.025 μg-at N·liter^{-1} in oligotrophic oceanic areas. Combining these estimations with those of Table 10, it is possible to calculate the rate of amino acid utilization by water types in the ocean (Table 11).

The data in Tables 9 and 11 can be used for further calculations by taking a mean value of 0.75 for the nitrogen mineralization ratio of amino acids (Hollibaugh 1978) (although this ratio has been shown in Fig. 10 to depend on the C/N ratio of the organic substrates taken up by bacteria). The calculations clearly show that bacterial mineralization of amino acids is responsible for most of the planktonic nitrogen regeneration in the photic zone in the neritic and coastal zones, but that in oligotrophic waters zooplankton excretion must play the dominant role. This is in agreement with the data of Table 7 and confirms the idea of Joiris (1977) and Joiris et al. (1982) that a fundamental difference exists between planktonic food

Table 11. Amino acid utilization by bacterial communities by water type of the world ocean.

	Turnover Time (hr)	Concentration (μg-at N·liter^{-1})	Utilization Rate^{+} (g N·m^{-2}·yr^{-1})
Oligotrophic waters from anticyclonic eddies	4000 - 9000	0.05	0.06 - 0.15
Transitional waters	1000 - 3000	0.25	1 - 3
Equatorial divergence and subpolar zones	3000	0.25	1
Inshore waters	100 - 150	0.5	20 - 30
Neritic waters	25 - 100	0.5	30 - 122

$^{+}$ Calculated by considering a photic zone of 50 m for neritic and inshore waters and of 100 m for oceanic waters.

chains in oceanic systems dominated by grazing and coastal systems dominated by the activity of microorganisms.

The amino acid utilization data in Table 11 also represent amino acid production through macromolecular organic nitrogen hydrolysis. In neritic waters, where the mean concentration of total proteins and peptides probably ranges between 2.5 and 5 μg-at N·liter^{-1} (Table 2), this implies a first order rate constant of degradation of about 0.2-0.02 day^{-1}. This is in reasonable agreement with the range found experimentally for fresh, phytoplankton-derived, organic nitrogen (Table 5). In oligotrophic areas, on the other hand, the concentration of dissolved, combined amino acids is about 0.3-0.5 μg-at N·liter^{-1} (Table 2), implying a first order degradation constant smaller than 0.0015 or at least two order of magnitude lower than the values found for phytoplankton-derived organic nitrogen. This suggests that an important part of combined amino acids dissolved in these waters consists of refractory compounds.

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