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Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico

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Abstract

Several aspects of bacterial glucose assimilation and bacterial production (BP) were investigated over 2 d in the upper 300 m of the Gulf of Mexico. Glucose concentrations ranged from 2 to 15 nM in surface seawater; glucose utilization rates were 1–3 nM d^{-1} in the upper 150 m of the water column and 0.02–0.8 nM d^{-1} in deeper water (150–300 m). Turnover of glucose and bacteria were similar and were in the range of 0.01–0.4 d^{-1}. Measured glucose assimilation rates imply that glucose supports ~5–10% of the observed BP in surface waters. The limiting factors for bacterial glucose utilization varied with depth. Glucose assimilation seemed to be limited by availability of inorganic N in the chlorophyll maximum. Additions of nitrate or ammonium had similar effects on glucose assimilation. BP was limited by C below and above the chlorophyll maximum. Additions of high-molecular-weight (HMW) dissolved organic matter (DOM) decreased bacterial glucose uptake rates, indicating that substances competing with glucose assimilation were derived from HMW DOM extracellularly. Thus, the measurement of free glucose assimilation appears to integrate bacterial utilization of combined glucose and indicates that many of the free sugars in seawater may be derived from polymers. The higher glucose assimilation rates achieved with inorganic nutrient additions indicate that bacterial degradation of C-rich organic matter (OM) could vary between ocean regions with varying inorganic nutrient concentrations.

OM in seawater consists of dissolved, colloidal, and particulate components. Of these, the dissolved reservoir is the largest and is considered the major source supporting heterotrophic bacterial metabolism in the ocean (e.g., Azam et al. 1983; Kirchman 1990; Kirchman et al. 1991). However, the reactivity of relatively few individual components of DOM has been investigated because of analytical difficulties in determining very dilute concentrations of organic compounds in seawater.

Recent studies indicate that dissolved carbohydrates are important bacterial substrates in seawater (Billen and Fontigny 1987; Jørgensen et al. 1993; Rich et al. 1996). Large changes in total combined carbohydrate concentrations with depth indicate high reactivity (Benner et al. 1992; Pakulski and Benner 1994; Skoog and Benner 1997), pointing to a potentially important role of carbohydrates in bacterial growth and respiration. A highly sensitive technique for molecular-level determinations of neutral aldose concentrations (Rocklin and Pohl 1983; Johnson and LaCourse 1990) has recently been adapted for seawater samples (Mopper et al. 1992), making direct, sensitive determinations of free and combined aldoses possible in marine samples. Glucose has been shown to be the most abundant free neutral aldose in seawater (Rich et al. 1996; Skoog and Benner 1997).

DOM in general, and HMW DOM in particular, have been found to have elevated C : N ratios in the surface ocean (Benner et al. 1997), whereas DOM ideal for bacterial growth would have a much lower C : N ratio. It has been suggested that inorganic nutrients can influence heterotrophic bacterial activity (e.g., Wheeler and Kirchman 1986; Horrigan et al. 1988; Kirchman 1994). Bacterial utilization of inorganic N during assimilation and growth on C-rich DOM suggests that the availability of inorganic nutrients in the surface ocean can have a profound influence on DOM cycling.

Some physiological constraints regulate bacterial uptake of DOM. The molecular weight cutoff for transport across a bacterial membrane is assumed to be 500–1,000 dalton (Saier and Stiles 1975). It is known that 60–80% of organic material present in seawater is <1,000 dalton (Benner et al. 1992, 1997), making direct transport across the bacterial membrane possible for a large fraction of oceanic organic compounds. However, to utilize DOM > 1,000 dalton, degradation of HMW DOM is required. Extracellular degradation utilizes exoenzymes. The ubiquitous presence of β-glucosidase, an enzyme that degrades glucose polymers with β-bonds, has been suggested as an indicator of heterotrophic glucose utilization in seawater (Christian and Karl 1995). If extracellular degradation of HMW DOM is important in bacterial nutrition, additions of HMW DOM should result in production of low-molecular-weight (LMW) DOM that could be assimilated by bacteria through LMW DOM transport systems.

In this study, we investigated several factors influencing bacterial glucose assimilation. We determined turnover and assimilation rates of glucose in order to assess the importance of glucose in sustaining BP in ocean surface waters. We also investigated the vertical variation in glucose assimilation rates and the factors limiting glucose assimilation and

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BP. In addition, we studied the effect of HMW DOM additions on glucose assimilation and BP.

Methods

Sampling—All samples were collected from the Gulf of Mexico at 26°16′N, 93°59′W during 23–28 May 1996. The total water-column depth was ca. 2,500 m. The surface mixed layer was ca. 22 m deep, and the chlorophyll maximum was centered at 115 m. A broad oxygen minimum zone with oxygen concentrations in the range of 100–105 μmol kg^{-1} was found between 140 and 500 m.

Niskin-type samplers with Teflon-coated springs were used for water collection. Samples for carbohydrate analyses were carefully drawn directly from the Niskin bottles with gloved hands into combusted 20-ml glass scintillation vials with Teflon-lined caps and immediately frozen. Samples for bacterial incubations were collected in 1-liter acid-washed polycarbonate bottles. Incubation experiments were conducted at sea within 20 min of collection. Samples were kept at in situ temperatures during sample handling and incubations.

Glucose assimilation—Incubations were conducted in glass Environmental Protection Agency vials (30 ml) for 30 min in the dark at in situ temperatures. [^{3}H]-glucose (15 Ci $mmol^{-1}$) was added at a final concentration of 1 nM. Incubations were terminated by passing the sample through 0.2-μm pore-size MF Nuclepore membrane filters, followed by a 10-ml rinse with ice-cold filtered (0.2 μm) seawater. One formaldehyde (4% final concentration)-killed control was run corresponding to every three live incubations. Filters were transferred to 7-ml scintillation vials and frozen until further processing onshore. Filters were dissolved by adding 0.6 ml Solvable (New England Nuclear) and heated in heating block at 50°C, and the solution was neutralized with 0.1 ml 5 M HCl (Amon and Benner 1998) before 5 ml Scintiverse II scintillation liquid was added. Sample activity was determined in a liquid scintillation counter (Beckman).

Bacterial production—BP was estimated from rates of protein synthesis with [^{3}H]leucine (Kirchman et al. 1985). Triplicate 10-ml water samples were incubated in the dark at in situ temperatures for 30 min with 10 nM of leucine (2.52×10^{12} Bq $mmol^{-1}$). One formaldehyde (4% final concentration)-killed control was run corresponding to every three live incubations. Incubations were terminated by filtration through 0.2-μm pore-size MF Nuclepore membrane filters and extraction in 3 ml of ice-cold 5% trichloroacetic acid (TCA) for 3 min. This treatment was followed by a 3-ml rinse with ice-cold 5% TCA and an additional rinse with 3 ml of ice-cold distilled water. The filters were stored in scintillation vials and refrigerated until further processing onshore. Filter dissolution and determination of sample activity were carried out in the same way as for glucose utilization incubations. Rates of [^{3}H]leucine assimilation were linear over 60 min. Killed controls accounted for 0.5–6% of the radiolabel found in live samples. Rates of [^{3}H]leucine incorporation were converted to bacterial C production values with the conversion factor of 2.3 kg C produced mol^{-1} leucine incorporated (Biddanda et al. 1994). This conversion factor assumes a 1.5-fold internal isotope dilution (Simon and Azam 1989).

Nutrient addition experiments—Samples from 15 and 115 m (chlorophyll maximum) were incubated in triplicate for determination of leucine and glucose assimilation (*see above*). Seawater samples were incubated at ambient nutrient concentrations and with the addition of $NaNO_3$ (final concentration = 30 μM), NH_4Cl (final concentration = 1 μM), Na_2HPO_4 (final concentration = 1 μM), and Na_2HPO_4 + NH_4Cl (final concentration = 1 μM each). These nutrient concentrations were chosen to enhance the ambient level 2- to 10-fold. Three killed controls were run with Na_2HPO_4 + NH_4Cl (final concentration = 1 μM each). All nutrients were cell-culture grade (Sigma Chemical).

HMW addition experiments—The HMW fraction of DOM in the size range of 1–200 nm was concentrated from the chlorophyll maximum at 115 m by ultrafiltration (Benner et al. 1997). A 10-liter seawater sample was concentrated to 1 liter. The DOC concentration of the HMW DOM fraction was 347 μM C and comprised 46% of DOC.

Samples for incubation were collected from 15 and 150 m. From each depth, ten 30-ml samples were poured and divided into two sets of five. Unlabeled glucose was added to each set to final concentrations of 10, 25, 40, 75, and 90 nM. HMW DOM was added to one set to final concentrations of ca. 10 μM DOC (15% of ambient DOC level). [^{3}H]-glucose was added to a final concentration of 1 nM. Samples were incubated for 20 min and terminated as described above.

Determination of free neutral aldoses—Immediately before analysis, samples were run through a mixed bed of anion (AG 2-X8, 20–50 mesh, Biorad) and cation (AG 50W-X8, 100–200 mesh, Biorad) exchange resins. Equal resin volumes were mixed, and ca. 3 ml was added to a glass extraction funnel. The resin bed was rinsed three times with milli-UV$^+$-water and then with ca. 1 ml of sample, which was discarded. A sample volume (2–3 ml) barely covering the resin was then added for deionization. The sample–resin mixture was stirred until CO_2 stopped evolving (ca. 5 min) and was drained into sample vials under vacuum. The samples were degassed with He for 1 min before injection in the chromatography system. Aldoses were separated with an isocratic 24-mM NaOH elution using a PA-1 column in a Dionex 500 Ion Chromatography system with pulsed amperometric detection (PAD) (Rocklin and Pohl 1983; Dionex technical note 20; Johnson and LaCourse 1990) employing a gold working electrode and an Ag/AgCl reference electrode. Procedural blanks were run using Milli-UV+ water. Relative standard deviations were in the range of 5–30%. For details of the chromatographic separation, *see* Skoog and Benner (1997).

Bacterial abundance—Samples (10 ml) for bacterial abundance (BA) were preserved with filtered formaldehyde (2% final concentration) and stored at 4°C. All samples were analyzed within 1 month of collection. BAs were determined

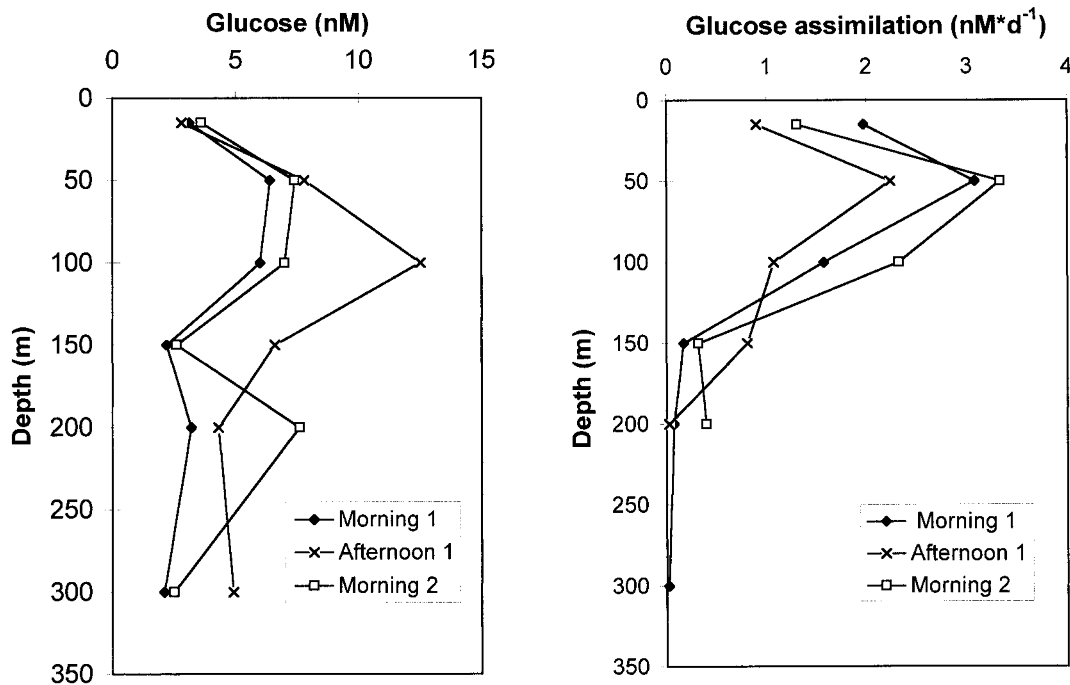

Fig. 1. Glucose concentrations and assimilation in the upper 300 m. Morning 1 and afternoon 1 are samples from the same day, and morning 2 are samples from the following day.

by epifluorescence microscopy at ×1,260 magnification of 4′,6-diamidino-2-phenylindole (DAPI)-stained samples collected on 0.2-μm black Nuclepore filters (Porter and Feig 1980). Duplicate filters were prepared for each sample, and 25–50 fields of view were examined. Bacterial C content was estimated using a standard conversion factor of 20 fg C cell^{-1} (Lee and Fuhrman 1987).

DOC determinations—Seawater samples for DOC analysis were passed through muffled (450°C, 4 h) Whatman GF/F glass-fiber filters. The filtrate was collected directly in acid-cleaned and muffled glass scintillation vials, sealed with Teflon-lined caps, and stored frozen until analysis. DOC concentrations were determined by high temperature catalytic oxidation with a Shimadzu 5000 C analyzer (e.g., Benner et al. 1992, 1997) after HCl acidification (pH < 4) and 10-min sparging.

Results

Glucose concentration—Glucose was the most abundant free neutral aldose and constituted 50–100% of detectable free aldoses. Mannose and xylose were also detected at low concentrations. We did not determine fructose concentrations, and no free galactose was detected. The free glucose concentration varied between 1.5 and 13 nM in the upper 300 m of the water column (Fig. 1).

Free glucose concentrations had maxima in the region of the chlorophyll maximum at 115 m (Fig. 1). In addition, the square of the Pearson correlation coefficient (Table 1) indicates that 60% of variations in glucose concentrations can be explained by variations in chlorophyll fluorescence. High glucose concentrations were observed in the afternoon of day 1 (Fig. 1) compared with lower glucose concentrations in the mornings of days 1 and 2. Free glucose concentrations

Table 1. Correlation matrix showing R^2 (the square of the Pearson correlation coefficient) for bacterial production and parameters relating to DOC.

	BP	[Glucose]	Glucose assim.	[DOC]	[Chlorophyll]	Glucose turnover	Bacterial turnover	[Oxygen]
BP	1	0.08	0.51	0.94	0.09	0.85	0.74	0.75
[Glucose]	0.08	1	0.43	0.18	0.59	0.07	0.13	0-.47
Glucose assimilation	0.51	0.43	1	0.73	0.11	0.73	0.46	0.85
[DOC]	0.94	0.18	0.73	1	0.14	0.92	0.91	0.89
[Chlorophyll]	0.09	0.59	0.11	0.14	1	0.01	0.17	0.32
Glucose turnover	0.85	0.07	0.73	0.92	0.01	1	0.74	0.73
Bacterial turnover	0.74	0.13	0.46	0.91	0.17	0.74	1	0.76
[Oxygen]	0.75	0.47	0.85	0.89	0.32	0.73	0.76	1

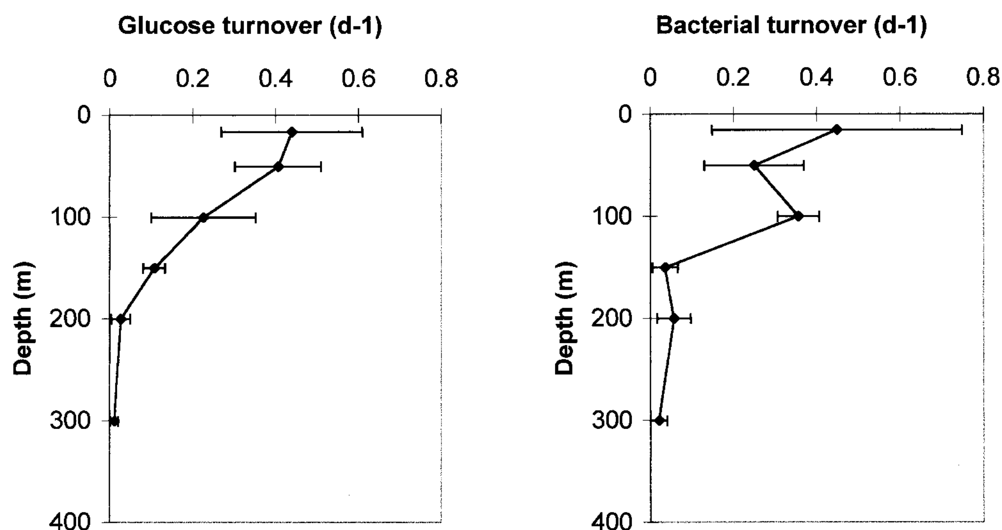

Fig. 2. Fractional turnover of glucose and bacteria. Turnover was calculated as an average of three sampling times. Error bars represent 1 SD.

were 50% higher in midafternoon compared with early-morning concentrations.

Assimilation and fractional turnover of glucose—Glucose incorporation rates were high (1 and 3 nM d^{-1}) above 115 m, with lower rates (<1 nM d^{-1}) below 115 m (Fig. 1). The chlorophyll maximum (115 m) had the highest glucose concentrations, while highest glucose assimilation rates were found at 50 m. The mean fractional turnover of glucose was 0.2–0.5 d^{-1} down to 115 m (Fig. 2). Below 150 m, the mean fractional turnover for glucose decreased to ca. 0.012 d^{-1}.

Table 2. BA and BP and DOC concentrations. Days 1 and 2 are consecutive days. DOC determinations were made on one sample set and are assumed to be constant over the 2 d of the experiment.

Sampling time	Depth (m)	BA ($\times 10^8$ liter^{-1})	BP (μM C d^{-1})	DOC (μM C)
Day 1, morning	15	5.97	0.276	80
Day 1, morning	50	6.75	0.170	76
Day 1, morning	100	3.16	0.18	74
Day 1, morning	150	2.99	0.006	57
Day 1, morning	200	2.19	0.012	53
Day 1, morning	300	0.82	0.006	53
Day 1, afternoon	15	5.08	0.676	
Day 1, afternoon	50	4.85	0.312	
Day 1, afternoon	100	4.8	0.334	
Day 1, afternoon	150	3.18	0.014	
Day 1, afternoon	200	2.98	0.012	
Day 1, afternoon	300	2.46	0.004	
Day 2, morning	15	4.34	0.194	
Day 2, morning	50	5.45	0.192	
Day 2, morning	100	4.32	0.222	
Day 2, morning	150	2.47	0.028	
Day 2, morning	200	1.92	0.036	
Day 2, morning	300	1.6	0.002	

BA, BP, and turnover time—BA was about 5×10^8 liter^{-1} above 150 m and decreased from about 3×10^8 liter^{-1} at 150 m to about 2×10^8 liter^{-1} at 300 m (Table 2). Maximal rates of BP occurred in surface waters (Table 2). BP correlated well with DOC concentrations (Tables 1, 2) but showed poor correlation with chlorophyll and glucose concentrations (Table 1). High correlations were found between BP and glucose assimilation rates and turnover (Table 1). About 85% of the variation in glucose turnover can be attributed to variations in BP.

Effect of nutrient additions on bacterial glucose assimilation—No statistically significant effect of NO_3, NH_4, or PO_4 additions on glucose assimilation was observed ($P >$ 0.1) in samples from 15-m depth (Fig. 4). In contrast, in incubations with samples from the chlorophyll maximum at 115 m, glucose assimilation was equally stimulated by addition of NO_3 and NH_4 ($P < 0.1$ when comparing nutrient additions with controls, Fig. 4).

Effects of HMW DOM on BP—BP increased 2.4-fold after addition of HMW DOM to samples from 50 m (Fig. 5). At 150 m, BP increased 24-fold after HMW DOM addition.

Effects of HMW DOM on glucose assimilation rates—HMW DOM was added to samples at concentrations that increased the amount of HMW DOM by about 30%. Addition of HMW DOM did not appear to affect glucose assimilation rates in surface waters (Fig. 6). However, glucose assimilation in incubations with water from 150 m was inhibited by HMW DOM (Fig. 6), as can be seen from the statistically significant (t-test, $P < 0.05$) positive shift in the slope of the line in a Lineweaver–Burke plot when HMW DOM was added.

Discussion

Concentration ranges and accuracy of glucose concentration determinations—Free glucose concentrations found in

this study are low compared to free glucose concentrations that have been found in surface waters of the equatorial Pacific using similar methods. Rich et al. (1996) found concentrations in the approximate range of 5–110 nM, with averages of 15 nM in late summer and 38 nM in winter.

In addition to chemical determinations, we calculated glucose concentrations using a microbiological approach. Results from incubation experiments with varied glucose concentrations were used to calculate $K_t + S_n$ by applying the Michaelis–Menten equation. K_t is the concentration where V is equal to $0.5 \times V_{max}$, and S_n is the glucose concentration. The sum of these two parameters gives an approximate upper possible ambient concentration for glucose in the incubation. We calculated $K_t + S_n$ for samples from 50 and 150 m, and the results agree well with the chemically determined concentrations of glucose. $K_t + S_n$ at 50 and 150 m were 9.1 and 4.4 nM, respectively. The corresponding chemically determined concentrations were 7.2 and 3.8 nM, respectively. Literature values for $K_t + S_n$ for free glucose range between 0.6 and 1,600 nM (Hoppe 1978). Likewise, Fuhrman and Ferguson (1986) found that calculated values of $K_t + S_n$ for amino acids agreed well with chemically determined concentrations. The close agreement between chemically and microbiologically estimated concentrations in the present study indicates that the reported concentrations have good accuracy.

Dissolved free amino acids (DFAA) and neutral sugars (DFNS) are both assumed to be major contributors to bacterial growth. For individual amino acids, $K_t + S_n$ has been found to be in the range of 0.2–9 nM (Ferguson and Sunda 1984; Fuhrman and Ferguson 1986; Fuhrman 1987; Suttle et al. 1991). Suttle et al. (1991) found concentrations in the range of 0.1–11 nM for individual amino acids in surface waters of the Sargasso Sea. Pomeroy et al. (1995) determined free amino acid concentrations in surface waters of the Gulf of Mexico, where leucine concentrations ranged from 14 to 31 nM. Thus, concentration ranges of the most abundant DFAA (leucine) and DFNS (glucose) seem to be similar.

Water-column sources for free glucose—A diel pattern (Fig. 1) and a strong correlation between glucose concentrations and chlorophyll fluorescence (Table 1) were observed in the present study. Previous studies of dissolved carbohydrates in surface ocean waters have also noted a diel pattern with higher concentrations during daylight hours (Burney et al. 1982; Burney 1986; Hama and Handa 1992). Studies have shown that algae exude large amounts of carbohydrates (e.g., Mopper et al. 1995; Biddanda and Benner 1997 and references therein), and herbivorous grazing releases dissolved carbohydrates (Strom et al. 1997). These observations suggest a relationship between water-column free glucose concentrations and primary production. The correspondence of high glucose and chlorophyll fluorescence suggests that phytoplankton and/or herbivorous grazers are a source of dissolved glucose.

Fractional turnover of glucose—Mean fractional glucose turnover was 0.2–0.5 d^{-1} down to 115 m (Fig. 2), a result that agrees well with other studies (e.g., Williams and Askew 1968; Gocke 1977; Rich et al. 1996). When comparing glucose turnover with amino acid turnover (1.3 d^{-1}) in central Gulf of Mexico surface water (Ferguson and Sunda 1984), we found that amino acid turnover was faster than glucose turnover. Since the amino acid pool is larger than the neutral sugar pool in the Gulf of Mexico, higher turnover of amino acids indicates a much higher amino acid assimilation rate than neutral sugar assimilation rate. For comparison, Cherrier et al. (1996) made estimates of turnover of labile DOM in Eastern North Pacific surface water (0.17–0.5 d^{-1}) that agree well with glucose turnover estimates in the present study.

Estimates of glucose pool turnover times based on utilization rates in this study (0.2–0.5 d^{-1}) adequately explain the observed diurnal changes in glucose concentrations (about twofold) in the water column. The high fraction of glucose turnover variation explained by variations in BP (Table 1) indicates the central role bacteria play in glucose cycling and also, the importance of glucose in bacterial growth and respiration. However, the weak correlation between glucose concentrations and BP indicates that an additional factor is necessary to stimulate BP.

Assimilation of glucose—Glucose assimilation rates found in this study (<1–3 nM d^{-1}) fall in the lower range of the only other data set available for comparison. In a study from the tropical Pacific, Rich et al. (1996) measured glucose assimilation rates of 3–148 nM d^{-1}. In our study, glucose assimilation supported 5–10% of BP, except immediately below the chlorophyll maximum. At 150 m, glucose supported an average of 20% of BP (Fig. 3), with a maximum of 30% during the day. The high fraction of BP supported by glucose at 150 m is possibly a result of particulate flux from the chlorophyll maximum situated at 115 m. For comparison, Rich et al. (1996) found that as much as 40% of BP in surface waters could be supported by glucose assimilation in the Equatorial Pacific.

The fraction of BP accounted for by sum amino acid and neutral sugar assimilation can be estimated. Suttle et al. (1991) made investigations on amino acid assimilation in the Sargasso Sea, an oligotrophic open-ocean region. The production supported by free amino acids was 20–30% of C demand (values averaged for individual amino acids), with individual amino acids accounting for 5–20% in winter and 1–10% in summer (Suttle et al. 1991). Glucose supported 5–30% of BP in the Gulf of Mexico. If the proportion of BP supported by glucose in other ocean regions is comparable to the Gulf of Mexico, BP supported by the sum of amino acids and neutral aldoses appears to be high (~50%). Amino acids and neutral aldoses play important roles in heterotrophic growth and respiration in the ocean.

Glucose assimilation rate maxima did not coincide with glucose concentration maxima. The chlorophyll maximum (115 m) had the highest glucose concentrations, while highest glucose assimilation rates were found at 50 m. The reason for the relatively low glucose assimilation in the chlorophyll maximum was found to be nutrient limitation (Fig. 4). Several studies have suggested that inorganic nutrients can influence heterotrophic bacterial activity (e.g., Wheeler and Kirchman 1986; Horrigan et al. 1988; Kirchman 1994).

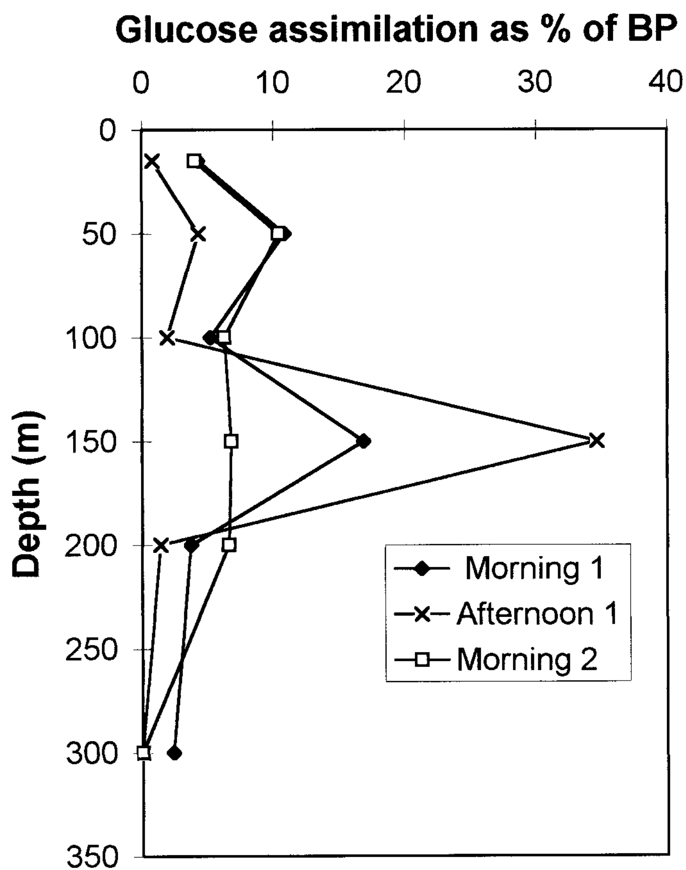

Fig. 3. Glucose assimilation as percentage of BP with depth.

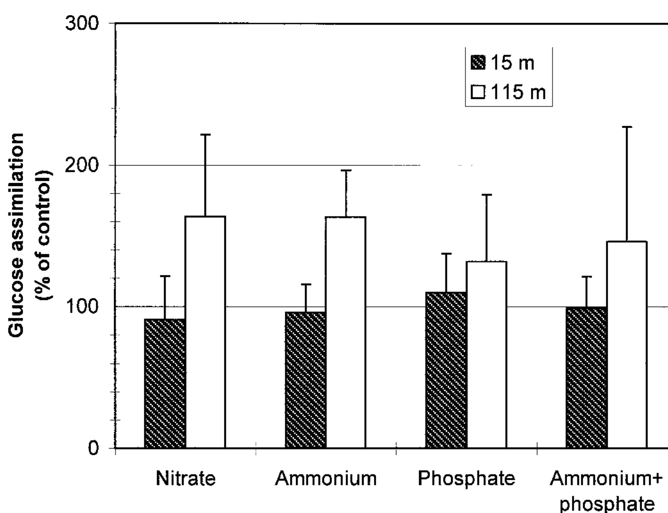

Fig. 4. Results from incubations evaluating the effect of nutrient additions on glucose assimilation. Nutrients were added to final concentrations of 30 μM nitrate, 1 μM ammonium, and 1 μM phosphate.

In incubations with samples from the chlorophyll maximum at 115 m, glucose assimilation was equally stimulated by addition of NO_3 and NH_4 ($P < 0.1$ when comparing nutrient additions with controls, Fig. 4), indicating N limitation. The chlorophyll maximum is also the site of the glucose concentration maximum, suggesting glucose accumulation could result from N limitation of glucose utilization. To assimilate C-rich photosynthetic products, heterotrophic bacteria may therefore compete with phytoplankton for inorganic nutrients in the chlorophyll maximum.

In contrast, no statistically significant effect of NO_3, NH_4, or PO_4 additions on glucose assimilation was observed ($P > 0.1$) in samples from 15-m depth (Fig. 4), indicating that heterotrophic C assimilation in surface water was not N or P limited. Pomeroy et al. (1995) found that BP was P limited in surface waters of the Gulf of Mexico during summer. In addition, the same study found high concentrations of free amino acids in surface waters, suggesting that bacterial N demand may have been satisfied by assimilation of organic N. The insignificant effect of nutrient additions in surface-water samples in the present study suggests that BP in Gulf of Mexico surface water was C limited.

Inhibition of substrate assimilation can be evaluated in plots based on Michaelis–Menten kinetics, e.g., a Lineweaver–Burke plot (Figs. 5, 6). In our study, a constant amount of HMW DOM was added to samples with varying concentrations of glucose. In a Lineweaver–Burke plot (Fig. 6), where the inverse of the initial uptake rate is plotted vs. inverse of glucose concentration, a substance that inhibits glucose assimilation gives a positive slope shift. Glucose assimilation in incubations with water from 150 m was inhibited by HMW DOM additions (Fig. 6), indicating that bacteria derive LMW neutral aldoses by extracellular hydrolysis of HMW DOM material. Glucose released from HMW DOM by exoenzymes would compete with assimilation of radiolabeled glucose, resulting in inhibition of labeled glucose assimilation. The apparent inhibition could have two different causes (Saier 1985): (1) heterotrophic bacteria at 150 m assimilated a compound from HMW DOM via the

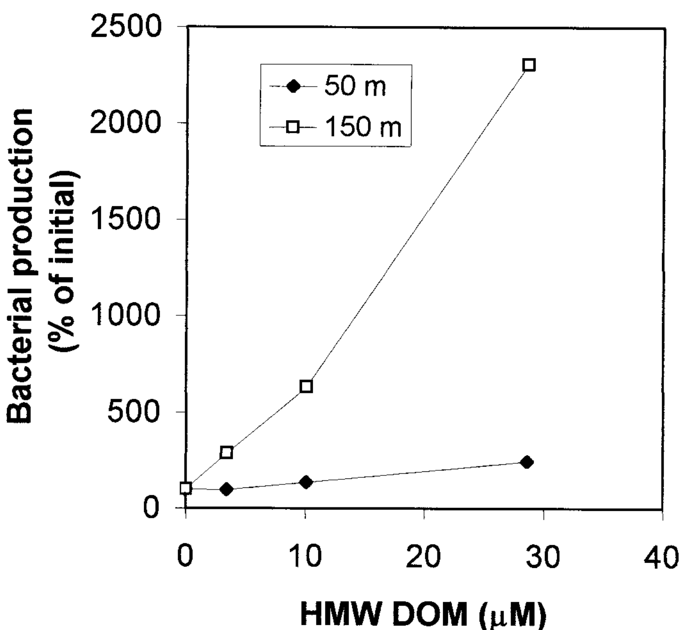

Fig. 5. Effect of addition of HMW DOM on BP. HMW DOM was concentrated by ultrafiltration from 100-m depth at the investigated station at the time of the study.

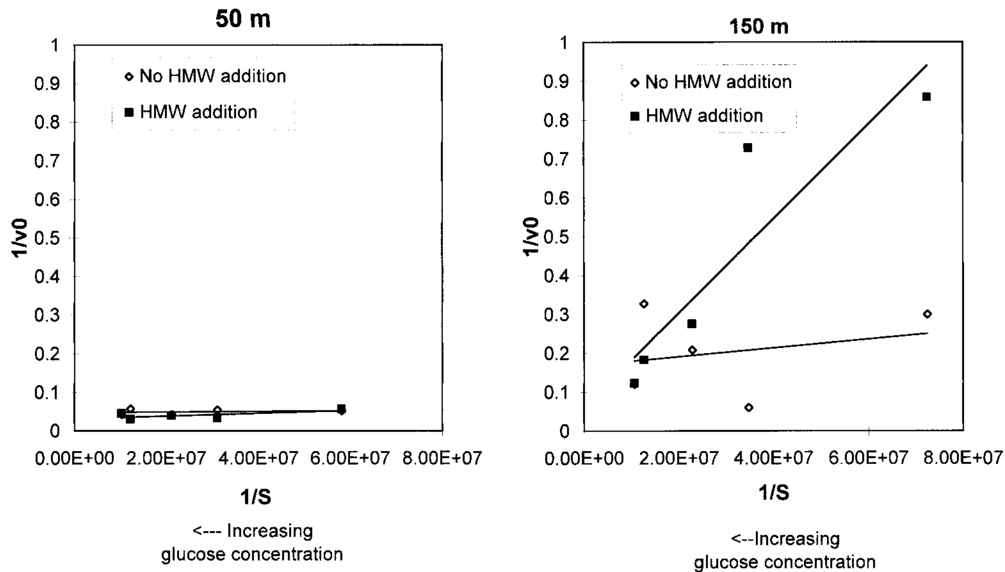

Fig. 6. Lineweaver–Burke plots evaluating the effect of 10-μM HMW DOM additions on glucose assimilation. V_0 is the initial assimilation rate of glucose, and S is substrate concentration—in this case, glucose concentration. An increase in slope of the line after HMW DOM addition indicates inhibition of glucose assimilation. Slopes of the lines in the right panel are statistically different (t-test, $P < 0.05$).

glucose transport system, resulting in an apparent decrease of glucose assimilation; and (2) bacteria assimilated a compound from HMW DOM through another transport system, and accumulating concentrations inside the cell inhibited glucose transport. Since HMW DOM is >1,000 dalton and can not be directly transported into the cell by bacteria (Saier and Stiles 1975), bacteria would have to hydrolyze HMW DOM to LMW substances (Fig. 7).

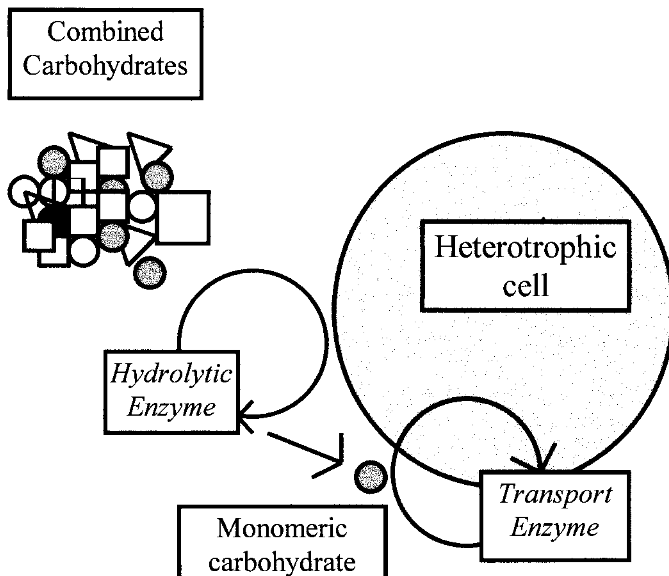

Fig. 7. Conceptual figure of bacterial degradation of HMW DOM in seawater.

HMW DOM effects on bacterial growth—Experiments were conducted to determine the influence of HMW DOM on BP. HMW DOM in surface waters is abundant and rich in carbohydrates relative to deep waters (Benner et al. 1992; McCarthy et al. 1996; Skoog and Benner 1997). Ultrafiltered HMW DOM isolated from surface ocean waters has a C : N ratio of approximately 16 (Benner et al. 1997). In the present study, BP increased 2.4-fold after HMW DOM addition to samples from 50 m, indicating that BP was C limited (Fig. 5). At this depth, the glucose assimilation rate was the highest. The indication that BP is C limited at 50 m agrees with results from experiments in the surface mixed layer at 15 m, showing that inorganic nutrient additions had no effect on BP. C limitation of BP in oceanic surface waters has been noted in other studies (e.g., Kirchman 1990; Kirchman and Rich 1997). At 150 m, BP increased 24-fold after HMW DOM addition. The sample from 150 m came from the nutrient-rich water below the surface mixed layer, suggesting inorganic N and P concentrations were sufficient to support utilization of HMW DOM and that significant uptake of inorganic N and P may accompany utilization of HMW DOM at depth.

Vertical variation in factors limiting BP—The decoupling between maximum glucose assimilation and maximum glucose concentrations in a vertical profile (Fig. 1; Table 2) indicates that glucose concentrations alone do not determine glucose assimilation rates. In addition, our nutrient addition results indicate that factors limiting BP and DOC utilization show vertical variation. Above the chlorophyll maximum, glucose (Fig. 1) and inorganic N have low concentrations. Inorganic nutrient additions did not increase glucose assimilation at 15 m (Fig. 4). Samples from 50 m showed a 2.4-

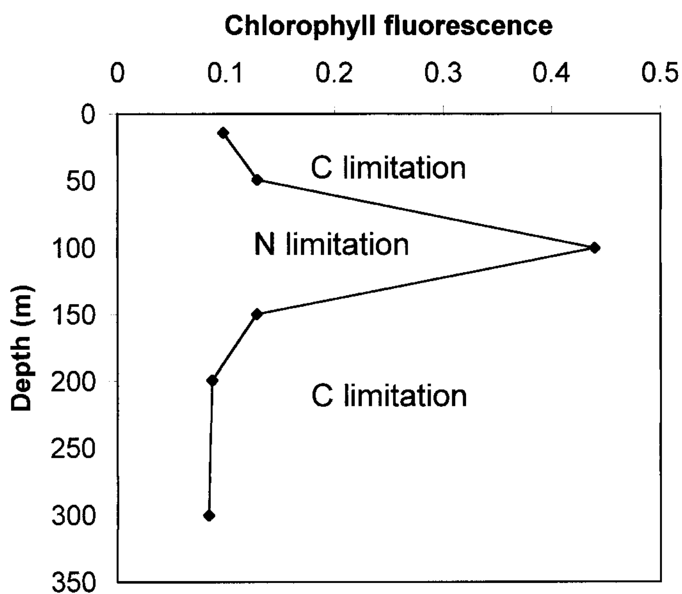

Fig. 8. Vertical variation in chemical factors limiting BP in the water column.

fold increase in BP after addition of C-rich HMW DOM. Both experiments indicated that BP and DOM utilization were C limited above the chlorophyll maximum. In contrast, in the glucose concentration maximum (chlorophyll maximum, 115 m; Fig. 1), glucose assimilation increased after inorganic nutrient additions (Fig. 4), indicating that DOC utilization was N limited at this depth. Below the chlorophyll maximum, DOC concentrations are low (Table 1). Here, BP increased 24-fold after addition of HMW DOM (Fig. 5), indicating BP was severely limited by C availability. Thus, DOC utilization in the chlorophyll maximum was nutrient limited, but BP was C limited above and below the chlorophyll maximum (Fig. 8). Could such depth-wise variability in factors limiting bacterial growth be a general phenomenon in the sea?

Implications for DOM residence times and C fluxes—The large proportion of bacterial C demand apparently supported by amino acids and neutral sugars suggests that only a very small DOM fraction, on the order of 1–10% of the DOM pool in surface waters, is cycled by heterotrophic bacteria on the order of days. Several studies have addressed the variable reactivity of DOM components (e.g., Carlson and Ducklow 1995). In addition, evidence of varying residence times for DOM has been presented in several publications employing radioisotope content for DOM average age approximations (e.g., Williams and Druffel 1987; Santschi et al. 1995). The microbiological and isotope-based studies both suggest a large difference in residence times between labile and refractory pools of marine DOM. The large differences in residence time will have an impact on the fluxes through the DOM pool. DOM flux calculated using the average DOM age of 1,000 yr would be much smaller than DOM flux achieved when the two different pools and their respective average apparent ages are recognized (Mantoura and Woodward 1983).

Implications for cycling of C-rich material—It has been shown that marine DOM in general and surface HMW DOM especially are rich in C. The C : N ratio of HMW DOM in the Pacific and Atlantic Oceans is on average = 16, and a large fraction of HMW DOM is carbohydrate (Benner et al. 1997). We also showed that HMW DOM seemed to supply glucose or a glucose-like substance that was rapidly assimilated by bacteria. These experiments indicated that polysaccharides from HMW DOM were enzymatically hydrolyzed to compounds that competed with glucose for transport and assimilation by bacteria. Thus, the measurement of free glucose assimilation appears to integrate bacterial utilization of combined glucose and indicates that many of the free sugars in seawater may be derived from polymers.

Increased assimilation of glucose after inorganic nutrient additions indicates that variations in inorganic N availability may cause variations in C assimilation in the surface ocean. We speculate that ocean regions with inorganic N available will have higher C assimilation than ocean regions with low inorganic N availability. In addition, seasonal variations in inorganic nutrient concentrations could cause seasonal variations in heterotrophic C assimilation.

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