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Aerobic hydrogen production and dinitrogen fixation in the marine cyanobacterium *Trichodesmium erythraeum* IMS101

Abstract—Rates of hydrogen production and acetylene reduction were measured in aerobic cultures of the marine cyanobacterium *Trichodesmium erythraeum* IMS101 grown in a supplemented seawater medium low in combined inorganic nitrogen. Both hydrogen production and acetylene reduction conformed to a circadian pattern with maximum activity in the afternoon. Normalized hydrogen production rates ranged from 0.06 to 0.71 nmol H_2 μg chlorophyll *a* (Chl *a*)$^{-1}$ h^{-1}, while nitrogen fixation rates, calculated using an assumed ratio of 4 mol acetylene to 1 mol dinitrogen, ranged from 0.12 to 4.71 nmol N_2 Chl a^{-1} h^{-1}. Yields of hydrogen from nitrogen fixation were in the range 0.15–0.48 mol H_2 evolved per mol N_2 reduced. A comparatively low net rate of H_2 uptake was observed in one culture during the dark period (-0.01 nmol H_2 μg Chl a^{-1} h^{-1}). These measurements suggest that *T. erythraeum* is potentially a net source of hydrogen to the low-latitude surface ocean.

Atmospheric hydrogen (H_2) indirectly affects the Earth's climate by regulating the concentrations of tropospheric methane and carbon monoxide and by influencing the stratospheric heat budget through the formation of water vapor (Ehhalt and Prather 2001). Observations dating back almost 30 yr have shown that the low-latitude surface ocean is typically supersaturated with molecular hydrogen (Conrad and Seiler 1988), but this phenomenon remains largely unexplained. Three documented in situ sources of hydrogen in the upper ocean are (1) fermentation by anaerobic bacteria occupying low-oxygen microenvironments (Schropp et al. 1987), (2) abiotic photochemical processes in coastal waters (Punshon and Moore 2008), and (3) nitrogen fixation by the marine cyanobacterium *Trichodesmium* (Scranton 1983). The distribution of nitrogen-fixing *Trichodesmium* is generally restricted to oligotrophic waters with a temperature greater than 20°C (LaRoche and Breitbarth 2005). For these regions, nitrogen fixation is potentially the most important production mechanism for excess hydrogen, since the observed profiles and diurnal cycles of dissolved H_2 are generally consistent with a photobiological source. *Trichodesmium* plays a key role in the ocean nitrogen cycle, providing an estimated 80 Tg N yr^{-1} to the oligotrophic ocean (Capone et al. 1997), about 70% of annual marine nitrogen fixation (Gruber and Sarmiento 1997). Colonies of *Trichodesmium thiebautii* have been shown to evolve hydrogen during incubation experiments, but at rates generally too low to represent a significant ocean source (Scranton et al. 1987). However, net yields of hydrogen from nitrogen fixation (mol H_2 produced : mol N_2 reduced) may be influenced by two poorly understood loss processes. First, a fraction of the evolved hydrogen may be consumed by the chemolithotrophic H_2-oxidizing bacterial community likely to be present in natural seawater samples (Nishihara et al. 1991). Second, diazotrophic cyanobacteria have the capacity to respire some of the hydrogen released during nitrogen fixation by means of uptake hydrogenase enzymes (Tamagnini et al. 2002). Respiratory hydrogen oxidation is thought to protect O_2-sensitive nitrogenase from deactivation in aerobic environments as well as to provide a source of energy (Robson and Postgate 1980). These loss processes may be influenced by taxonomy or morphology, i.e., whether the *Trichodesmium* species is in the form of free trichomes or puff or tuft type colonies. In recent years there has been a great deal of interest in the *Trichodesmium erythraeum* strain IMS101 isolated from warm waters off the eastern coast of the United States (Chen et al. 1996). A number of studies have investigated factors regulating growth and nitrogen fixation in this isolate under controlled laboratory conditions (Mulholland and Bernhardt 2005). This study examines the relationship between aerobic hydrogen production and nitrogen fixation in *T. erythraeum* IMS101 to determine whether *T. erythraeum* is a potential net marine source of dissolved hydrogen.

Methods—Culture conditions: Batch cultures of *Trichodesmium* IMS101 were grown in 2-liter square PETE media bottles (Nalgene) or 4-liter glass bottles (Kimax). The growth medium consisted of natural seawater, low in combined inorganic nitrogen, supplemented with phosphate, trace metals, and vitamins as for the YBC-II medium of Chen et al. (1996). Cultures were maintained at 20°C in a 12 : 12 light : dark cycle under a bank of cool white fluorescent tubes providing around 60–80 μmol quanta m^{-2} s^{-1}. Although the parent culture was not axenic, bacterial contamination was minimized by maintaining the cultures in an exponential growth phase using the transfer procedure of Chen et al. (1996). Trichomes were separated from old culture medium by filtration with a 20-μm pore-size nylon mesh, rinsed with sterile seawater, then backwashed into fresh medium. Transfers were performed at about 3–4-week intervals, and the cultures were routinely examined under low magnification for evidence of severe bacterial contamination. Chlorophyll *a* (Chl *a*) concentrations were used as an indicator of *Trichodesmium* biomass and measured at about 3-d intervals. Triplicate 10-mL aliquots of culture were filtered

onto Whatman GF/F filters and the chlorophyll extracted in 95% ethanol for 3 h (Wasmund et al. 2006) at laboratory temperature. Chl *a* was determined by fluorometry according to the method of Welschmeyer (1994). *Trichodesmium* growth rates were calculated from the increase in Chl *a* with time.

Hydrogen production: Hydrogen production rates in the *Trichodesmium* cultures were measured using a headspace equilibration method. Cultures or control samples were transferred to calibrated glass 1- or 2-liter flasks fitted with septum ports and gently purged for 1 h with zero air at a flow rate of about 100 mL min^{-1} to reduce the level of background hydrogen. This purging protocol was based on convenience and certainty of its effectiveness. In retrospect it would have been better to evaluate the minimum purge time required to remove excess hydrogen since the disturbance caused by bubbling may adversely affect the behavior of *Trichodesmium*. No comparative studies of purged and nonpurged cultures were made to establish its effects. At the end of the purging procedure, subsamples of the *Trichodesmium* culture were withdrawn for nitrogen fixation measurements and the volume of liquid adjusted in each flask to leave a 200-mL headspace. The flasks were then sealed with septa and placed on a shaking table under culture growth light and temperature. Two milliliters of headspace was withdrawn from each flask at around 1-h intervals and injected into the sample loop of a gas analyzer fitted with a reducing compound photometer (Peak Laboratories). Gas extracted from the flask headspace was replaced by an equal volume of hydrogen-free zero air to maintain atmospheric pressure. Hydrogen was separated isothermally at 104°C on a 2-m column packed with 13× molecular sieve. The analytical precision for repeated injections of a standard gas was typically about ±1%. Calibration curves were made from dilutions of a primary standard consisting of 4.6 Pa H_2 in zero air.

Four H_2 production experiments were conducted with *Trichodesmium* IMS101 cultures having Chl *a* concentrations in the range 9–48 $\mu g\ L^{-1}$. These experiments began shortly after the onset of daily illumination and ran for several hours. Experiment 4 was continued into the evening dark period. The first two experiments included control flasks with 0.2-μm filtered culture medium to test for photochemical H_2 production. Experiments 3 and 4 had control samples consisting of *Trichodesmium* culture that had been gently filtered with a 5-μm pore-size membrane to remove trichomes. These controls were used to correct for net H_2 production or consumption by other cells that could be present in the culture material.

Dark hydrogen uptake: One further experiment using the same culture material used in experiment 4 investigated net hydrogen uptake in *Trichodesmium* IMS101 during the dark period. Three 1-liter glass headspace flasks contained (1) The *Trichodesmium* culture, (2) the same culture filtered through a 5-μm pore-size membrane to correct for H_2 uptake by small unicellular H_2-oxidizing bacteria, and (3) filter-sterilized culture medium to correct for diffusive loss of H_2. Each sample was purged for 1-h with zero air at a flow rate of 100 mL min^{-1} then sealed and spiked with around 20 nmol H_2 shortly after the start of the 12-h night period. The flasks were then placed on a shaker table in a dark room at 20°C. Headspace measurements were made at hourly intervals as before over a period of more than 6 h.

Nitrogen fixation: Nitrogen fixation rates for the *Trichodesmium* cultures used in experiments 1 to 4 were determined by acetylene reduction assays (Capone 1993) concurrent with the hydrogen measurements. No measurements of N fixation were made during the dark uptake experiment. Duplicate 8-mL subsamples of the purged *Trichodesmium* culture were sealed in 10-mL septum vials and spiked with 20% acetylene (C_2H_2). Two similar vials contained control samples: one with the *Trichodesmium* culture but without C_2H_2, and one with 0.2-μm filtered culture and C_2H_2. The first control was used to correct for potential biological production of ethylene (C_2H_4), whereas the second provided reference measurements of C_2H_4 present as a contaminant in the acetylene. All samples were incubated under growth light and temperature conditions. Immediately after spiking with acetylene and subsequently at around 1-h intervals, 100 μL of headspace was drawn from each vial and analyzed for C_2H_4 by on-column injection with a SRI 310 gas chromatograph equipped with a flame ionization detector. C_2 gases were separated at 65°C on a 2-m column packed with HayeSep A (Hayes Separations). Calibration curves were constructed from 0–100 μL injections of a standard gas mixture (Scott Specialty Gases) consisting of 10.1 Pa C_2H_4 in ultra high purity (UHP) nitrogen, and sample sets were routinely bracketed by standard injections to monitor instrument stability. Ethylene production rates were converted to nitrogen fixation rates by assuming a ratio of 4 mol C_2H_4 produced per mol N_2 reduced (Capone 1993; Mulholland et al. 2004).

Results—Trichodesmium growth rates: Growth in the *Trichodesmium* cultures, determined from Chl *a* measurements, was generally exponential for at least 4 weeks, with growth rates ranging from 0.06 d^{-1} to 0.08 d^{-1} in experiments 1, 3, and 4. The culture used in experiment 2 was in an early stage of growth and may have been in a prelog phase. These growth rates are at the low end of the range of reported growth rates for nutrient-replete laboratory cultures of *Trichodesmium* IMS101 (Mulholland and Bernhardt 2005), probably due to the growth temperature (20°C) being suboptimal (LaRoche and Breitbarth 2005; Brietbarth et al. 2006). The *Trichodesmium* cells remained in the form of free trichomes throughout the exponential growth phase, and no puff or tuft type colonies were visible in any of the cultures used in the experiments. At Chl *a* concentrations greater than around 50 $\mu g\ L^{-1}$, which generally occurred in cultures older than about 1 month, the trichomes began to twist together to form lacelike structures and eventually tuft colonies. The formation of tufts invariably heralded a rapid decline in the health of the culture followed by cell lysis.

Hydrogen production: Hydrogen production in experiments 1 to 4 followed a circadian pattern. The onset of net

Table 1. Normalized daytime hydrogen production rates, N_2 reduction rates, and molar H_2 yields from nitrogen fixation ($H_2:N_2$) in four cultures of *Trichodesmium* IMS101 grown in a seawater medium low in combined inorganic nitrogen. The N_2 reduction rates are given as the mean of duplicate measurements followed by the range.

Experiment	Chl *a* (μg L^{-1})	H_2 production rate (nmol H_2 μg Chl a^{-1} h^{-1})	N_2 reduction rate (nmol N_2 μg Chl a^{-1} h^{-1})	$H_2:N_2$
1	48	0.70	4.71 (4.29–5.13)	0.15
2	9	0.06	0.12 (0.11–0.14)	0.48
3	33	0.40	2.25 (1.65–2.85)	0.19
4	17	0.23	0.59 (0.57–0.62)	0.39

H_2 production in each experiment occurred 4 to 6 h after the start of irradiation and reached a maximum linear rate for about 3 h in the afternoon. Net H_2 production rates were calculated from concentration data obtained during this afternoon period. Maximum normalized rates of hydrogen production ranged from 0.06 to 0.71 nmol H_2 μg Chl a^{-1} h^{-1} (Table 1). In experiments 1 and 4, where measurements continued for >9 h, net H_2 production declined in the evening then ceased at the beginning of the dark period (Fig. 1). A single measurement of the headspace in experiment 1 at the end of 12 h of darkness showed that the H_2 concentration was almost unchanged from the previous evening (data point not shown), suggesting that hydrogen production and loss rates were near zero or close to equilibrium during the night. No evidence of hydrogen production was seen in the filtered control samples.

Dark hydrogen uptake: In the single dark experiment, the unfiltered *Trichodesmium* culture showed a small but significant net loss of hydrogen over the 6-h incubation (Table 2). No significant changes in H_2 concentrations were seen in either of the control flasks, indicating that losses due to physical diffusion, and production or consumption by other free-living bacteria present in the culture medium, were not important. It is not possible to conclude with certainty that the net loss of hydrogen in the unfiltered sample was due to uptake by *Trichodesmium cells*, since the presence of attached unicellular hydrogen-metabolizing bacteria cannot be ruled out. In either case, the net rate of hydrogen loss in the unfiltered dark sample was small compared with the net H_2 production rate measured in similar culture material during the light period (experiment 4).

Nitrogen fixation: Nitrogen fixation rates followed a similar daily pattern to H_2 production with an initial induction period followed by a rapid switch to maximum activity in the afternoon (Fig. 1). Nitrogen fixation was not measured at night except in experiment 4, where three sets of duplicate measurements were taken over a 2-h period after the lights were switched off. No ethylene accumulation was seen in these samples, indicating that nitrogen fixation had ended (Fig. 1B). The reported nitrogen fixation rates were calculated from C_2H_4 concentration

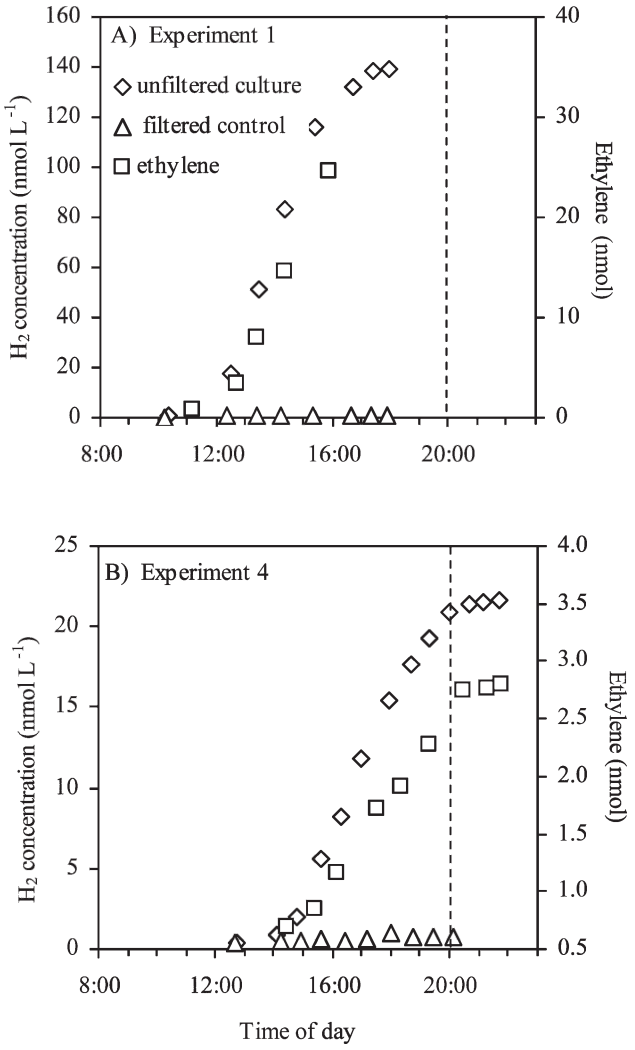

Fig. 1. Data from (A) experiment 1 and (B), experiment 4 showing dissolved hydrogen concentrations in incubated *Trichodesmium* cultures, and control samples where the trichomes had been removed by filtration. Also shown are measurements of ethylene in 8-mL culture samples incubated with a 20% acetylene headspace. The ends of the light periods are indicated by vertical dashed lines. Chl *a* concentrations were 48 μg L^{-1} in experiment 1 and 17 μg L^{-1} in experiment 4.

Table 2. Dark hydrogen uptake in a culture of *Trichodesmium* IMS101, a sample of culture filtered through a 5-μm pore-size membrane to selectively remove the trichomes, and a sample of filter-sterilized seawater growth medium.

Sample	Rate of [H_2] change in culture (nmol h^{-1})	Normalized rate of change (nmol H_2 μg Chl a^{-1} h^{-1})	*p* value
Unfiltered culture	−0.21 ± 0.07	−0.012	$0.05 > p > 0.01$
Filtrate (5 μm)	+0.01 ± 0.04	+0.001	$p > 0.05$
Filtered medium (0.2 μm)	−0.05 ± 0.04	−0.002	$p > 0.05$

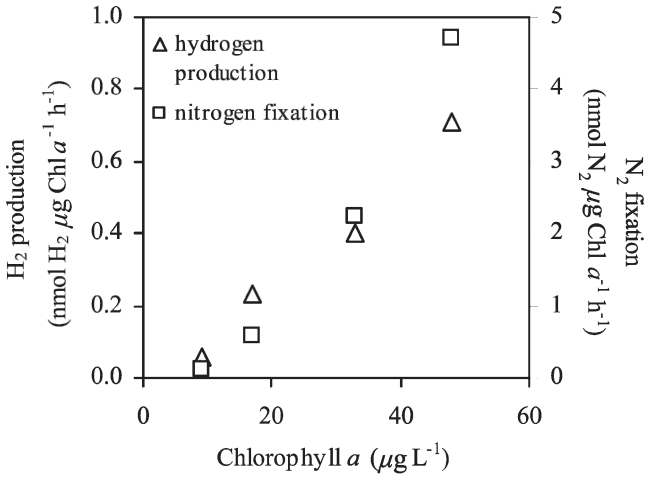

Fig. 2. Normalized rates of hydrogen production and nitrogen fixation for experiments 1–4 plotted against the Chl *a* concentration of each culture.

data obtained during the afternoons as for H_2 production. Normalized N_2 fixation rates ranged from 0.59 to 4.71 nmol N_2 μg Chl a^{-1} h^{-1} (Table 1). Normalized N_2 fixation and H_2 production rates increased nonlinearly with Chl *a* concentration and hence age of the cultures (Fig. 2). The compiled data from the four experiments showed a good correlation between H_2 production and N_2 fixation rates, with an R^2 of 0.97. The H_2 yield ranged from 0.15 to 0.48 mol H_2 produced : mol N_2 reduced and showed a decreasing trend with increasing chlorophyll concentration (Table 1).

Discussion—The emerging pattern of hydrogen evolution and nitrogen fixation in these experiments conformed to a diurnal periodicity with both activities commencing about halfway through the light period and reaching maximum rates in the afternoon. This pattern was rather different from the circadian cycles of nitrogen-fixing activity typically seen in cultures of this *Trichodesmium* strain (Chen et al. 1996; Holl and Montoya 2005), where maximum N-fixation rates occurred at the middle of the photoperiod. The reason for this is not yet clear. Although nitrogen fixation rates were generally not made after dark, the absence of hydrogen accumulation during the night periods in the case of experiment 1 and the H_2 uptake experiment suggest that N fixation was not occurring at night. The relatively wide range of normalized H_2 production and nitrogen reduction rates in experiments 1 to 4 (10-fold and 40-fold respectively) is interesting and may be related to the growth stages of the cultures. The culture used for experiment 2 was in an early stage of growth, whereas that used for experiment 1 was approaching the end of the logarithmic growth phase. Nitrogen availability could also have played a role in controlling N-fixation rates, so that the rate of N fixation increased as ambient combined inorganic nitrogen was depleted. In contrast to measurements using natural colonies of *T. thiebautii* from the Caribbean (Scranton 1983; Scranton et al. 1987), N_2 fixation and hydrogen production rates from the four experiments were very well correlated. In addition, the ratio of H_2 production : N_2 reduction was at least 10 times higher than in *T. thiebautii* colonies (Scranton et al. 1987).

The theoretical yield of hydrogen from nitrogen fixation is usually given as 1 mol of H_2 evolved per mol of N_2 reduced according to the simplified reaction

$$N_2 + 8H^+ + 8e^- + 16\ ATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16\ ADP + 16\ Pi \quad (1)$$

Net yields of hydrogen evolution below this 1 : 1 ratio may be at least partially explained by H_2 respiration by the cyanobacterium. Although the net rate of dark H_2 uptake by the *Trichodesmium* culture used in this study was low relative to net daytime production rates, higher rates of uptake occurring during the light period, concurrent with peaks in nitrogenase activity, may occur. Correlations between nitrogenase activity and uptake hydrogenase activity have already been seen in some filamentous heterocystous cyanobacteria (Tamagnini et al. 2002). Thus, variability in the efficiency of H_2 recycling among the cultures tested, as well as in the efficiency of H_2 production, would affect the net hydrogen yield from N_2 fixation. The H_2 yields determined by Scranton et al. (1987) in wild colonies of *T. thiebautii* were at least an order of magnitude lower than seen here, but it is possible that H_2 recycling is more efficient in the advanced colonial stage of the *Trichodesmium* life cycle than in free-living trichomes. Alternatively, *Trichodesmium* puff or tuft type colonies may provide a protected and substrate-replete environment for hydrogen-metabolizing bacteria, which could act to reduce the apparent net H_2 yield. It is interesting that there was an apparent decreasing trend in H_2 : N_2 ratios with increasing biomass of the cultures tested here, which could indicate a progressive improvement in H_2 recycling efficiency with the age of the culture. It must, however, be recognized that the calculated H_2 yield is also sensitive to the ratio chosen for converting acetylene reduction rates to N_2-fixation rates. Scranton et al. (1987) observed a ratio of 4 mol C_2H_2 reduced : 1 mol N_2 fixed for colonies of *T. thiebautii*, and Mulholland et al. (2004) concluded that the same conversion ratio was appropriate for total N_2 fixation in *T. erythraeum* IMS101. On the other hand, while Mulholland and Bernhardt (2005) used a C_2H_2 : N_2 ratio of 3 : 1 to routinely estimate N_2 fixation rates in *T. erythraeum* IMS101, they also observed ratios in the range 3–22 : 1 during the course of their study. So while it seems reasonable to adopt a C_2H_2 : N_2 ratio of 4 : 1 for this study, a downward drift in the C_2H_2 : N_2 ratio during the lifetime of the cultures could also account for the apparent decline in the H_2 yield.

Information on hydrogen yields from nitrogen-fixing cyanobacteria has potential use in at least two ways. One application concerns the global H_2 budget. The net ocean to atmosphere hydrogen flux is estimated at 3 ± 2 Tg yr^{-1}, about 4% of total global H_2 sources (Novelli et al. 1999). The uncertainty of this estimate reflects the very limited extent of sea-surface H_2 concentration data together with a poor understanding of marine H_2 production and con-

sumption processes. Knowledge of H_2 yields for important diazotrophic taxa could enable the hydrogen source from marine nitrogen fixation to be better constrained. For example, the genus *Trichodesmium* is thought to reduce around 80 Tg N_2 yr^{-1} (Capone et al. 1997). Applying the H_2 yields observed in this study gives a mean upper source limit for *Trichodesmium* sp. of around 1.7 (range: 0.9–2.7) Tg H_2 yr^{-1}.

A second potential application lies in the study of ocean nitrogen fixation. Marine nitrogen fixation is of central importance to carbon cycling in the oligotrophic ocean, but field studies of marine nitrogen fixation are currently hampered by limitations in the methods of rate determination. The ^{15}N assay requires samples to be returned to shore-based laboratories for analysis, preventing real-time data acquisition. The acetylene reduction assay generally requires sample preconcentration to improve sensitivity, and the sample incubation period introduces a delay in the availability of data. For both methods, sample processing constraints limit spatial resolution. It is clear from this study that *T. erythraeum* IMS101 can be, at least in the form of free trichomes, a substantial net source of dissolved hydrogen. If this strain is typical of other *Trichodesmium* species, then dissolved hydrogen could be a sensitive indicator of marine nitrogen-fixing activity. Measurements of dissolved hydrogen concentration can be performed rapidly at sea, perhaps allowing real-time high-resolution mapping of nitrogen-fixing activity in the surface ocean.

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References

Breitbarth, E., A. Oschlies, and J. LaRoche. 2006. Physiological constraints on the global distribution of Trichodesmium—effect of temperature on diazotrophy. Biogeosci. Discuss. **3:** 779–801.

Capone, D. G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, p. 621–631. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Current methods in aquatic microbiology. Lewis.

———, J. P. Zehr, H. W. Paerl, B. Bergman, and E. J. Carpenter. 1997. Trichodesmium, a globally significant marine cyanobacterium. Science **276:** 1221–1229.

Chen, Y.-B., J. P. Zehr, and M. Mellon. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: Evidence for a circadian rhythm. J. Phycol. **32:** 916–923.

Conrad, R., and W. Seiler. 1988. Methane and hydrogen in seawater (Atlantic Ocean). Deep-Sea Res. **35:** 1903–1917.

Ehhalt, D., and M. Prather. 2001. Atmospheric chemistry and greenhouse gases, p. 239–287. *In* J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Da, K. Maskell and C. A. Johnson [eds.], Climate change 2001: The scientific basis. Cambridge Univ. Scientific Press.

Gruber, N., and J. L. Sarmiento. 1997. Global patterns of marine nitrogen fixation and denitrification. Glob. Biogeochem. Cycles **11:** 235–266.

Holl, C. M., and J. P. Montoya. 2005. Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (cyanobacteria). J. Phycol. **41:** 1178–1183.

LaRoche, J., and E. Breitbarth. 2005. Importance of the diazotrophs as a source of new nitrogen in the ocean. J. Sea Res. **53:** 67–91.

Mulholland, M. R., and P. W. Bernhardt. 2005. The effect of growth rate, phosphorous concentration, and temperature on N_2 fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101. Limnol. Oceanogr. **50:** 839–849.

———, D. A. Bronk, and D. G. Capone. 2004. Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101. Aquat. Microb. Ecol. **37:** 85–94.

Nishihara, H., Y. Igarashi, and T. Kodama. 1991. *Hydrogenovibrio marinus* gen. nov., sp. nov., a marine obligately chemolithoautotrophic hydrogen-oxidizing bacterium. Int. J. Syst. Bacteriol. **41:** 130–133.

Novelli, P. C., P. M. Lang, K. L. Masarie, D. F. Hurst, R. Myers, and J. W. Elkins. 1999. Molecular hydrogen in the troposphere: Global distribution and budget. J. Geophys. Res. **104:** 30427–30444.

Punshon, S., and R. M. Moore. 2008. Photochemical production of molecular hydrogen in lake water and coastal seawater. Mar. Chem. **108:** 215–220.

Robson, R. L., and J. R. Postgate. 1980. Oxygen and hydrogen in biological nitrogen fixation. Annu. Rev. Microbiol. **34:** 183–207.

Schropp, S. J., M. I. Scranton, and J. R. Schwarz. 1987. Dissolved hydrogen, facultatively anaerobic, hydrogen-producing bacteria, and potential hydrogen production rates in the western North Atlantic Ocean and Gulf of Mexico. Limnol. Oceanogr. **32:** 396–402.

Scranton, M. I. 1983. The role of the cyanobacterium *Oscillatoria* (Trichodesmium) *thiebautii* in the marine hydrogen cycle. Mar. Ecol. Prog. Ser. **11:** 79–87.

———, P. C. Novelli, A. Michaels, S. C. Horrigan, and E. J. Carpenter. 1987. Hydrogen production and nitrogen fixation by *Oscillatoria thiebautii* during in situ incubations. Limnol. Oceanogr. **32:** 998–1006.

Tamagnini, P., R. Axelsson, P. Lindberg, F. Oxelfelt, R. Wünschiers, and P. Lindblad. 2002. Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol. Biol. R. **66:** 1–20.

Wasmund, N., I. Topp, and D. Schories. 2006. Optimising the storage and extraction of chlorophyll samples. Oceanologia **48:** 125–144.

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. **39:** 1985–1992.

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