# HABITABILITY OF THE EARLY EARTH: CLUES FROM THE PHYSIOLOGY OF NITROGEN FIXATION AND PHOTOSYNTHESIS

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**Abstract.** In the absence of direct evidence concerning the nature of the early Earth environments, it is acceptable under the uniformitarian principle to attempt to define primitive habitats from modern procaryotic physiology. Combining the rock and fossil record with present phylogenetic reconstuctions, application of this paleoecological approach to the evolutionary biochemistry and physiology of nitrogen fixation and photosynthesis leads to several inferences about the nature of Archean environments:

1. To stimulate nitrogenase evolution and avoid its repression, the activity of the $NH_4^+$ ion was less than $10^{-3}$, and probably lower.
2. To be consistent with a moderately protective ozone screen, while not also repressing nitrogenase activity, incursions of abiotic dissolved oxygen at levels in the range $10^{-1.2}$–$10^{-3.5}$ PAL would have been acceptable.
3. To induce the formation and activity of RuBP carboxylase, the $pCO_2$ was less than 100 PAL.
4. To support Photosystem I activity, sulfide concentrations of at least $10^{-4}$ M were present in the photic zone.
5. To avoid a too-rapid oxidation of sulfide, the pH was probably between 6–7, where $H_2S$ exceeds $HS^-$.

Evolutionary 'pressure' to stimulate the later development of oxygenic photosynthesis (Photosystem II), would require several subsequent habitat modifications:

1. Lowering the sulfide to $< 10^{-4}$ M to inhibit Photosystem I.
2. Raising the pH above neutral ($HS^- > H_2S$), to mediate more rapid oxidation of $HS^-$.
3. Maintaining either an illumination below 300–400 lux (to avoid photosynthetic $O_2$ self-repression of nitrogen fixation), or an adequate local source of combined nitrogen ($aNH_4^+ > 10^{-4}$) to repress nitrogen fixation entirely.

## 1. Introduction

Today when we speak of global habitability, we think in terms of a modern environment suitable for the balanced coexistence of an enormous variety of organisms, ranging from the most primitive procaryotes to Man. This habitability has, of course, developed and changed over the long history of the Earth. What was it like for the Earth's earliest microbiotas? Under what environmental conditions did these early organisms live and evolve? How did they act to alter the early environment toward its present condition?

Guided by the uniformitarian principle, students of Earth history, especially paleoecologists and paleobiologists, study the behavior of modern organisms, hoping to reconstruct how earlier, extinct but presumably related organisms may have acted. For the early Precambrian, there is little evidence to go on and we can only assume that the various primitive procaryotic organisms had many of the same physiological requirements as their modern, yet still primitive descendents.

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The purpose of the present paper is to apply this paleoecological approach to two major aspects of early procaryotic physiology and biochemistry: nitrogen fixation and photosynthesis. In this way, perhaps some boundary conditions can be placed on the habitability of the early Earth. These limits, then, could be factored into models of early global habitability derived from purely geochemical or other non-biological considerations.

## 2. Physiology of Nitrogen Fixation

Nitrogen fixation is the utilization of molecular nitrogen and its conversion to nitrogen compounds at the redox level of ammonia. It occurs in the most primitive of the procaryotes and was phylogenetically among the first of the major metabolic innovations to appear (Figure 1). Because of the similarity of the nitrogenase enzymes in all procaryotes that fix nitrogen, it is probably monophyletic (Broda and Peschek, 1983). Therefore, with the procaryotic fossil record now as old as 3500 Myr. (Schopf, 1978), its early evolution seems unavoidable. The physiology of the nitrogen fixation process is therefore of considerable evolutionary significance and can be of importance to deciphering probable conditions of habitability during its early evolution.

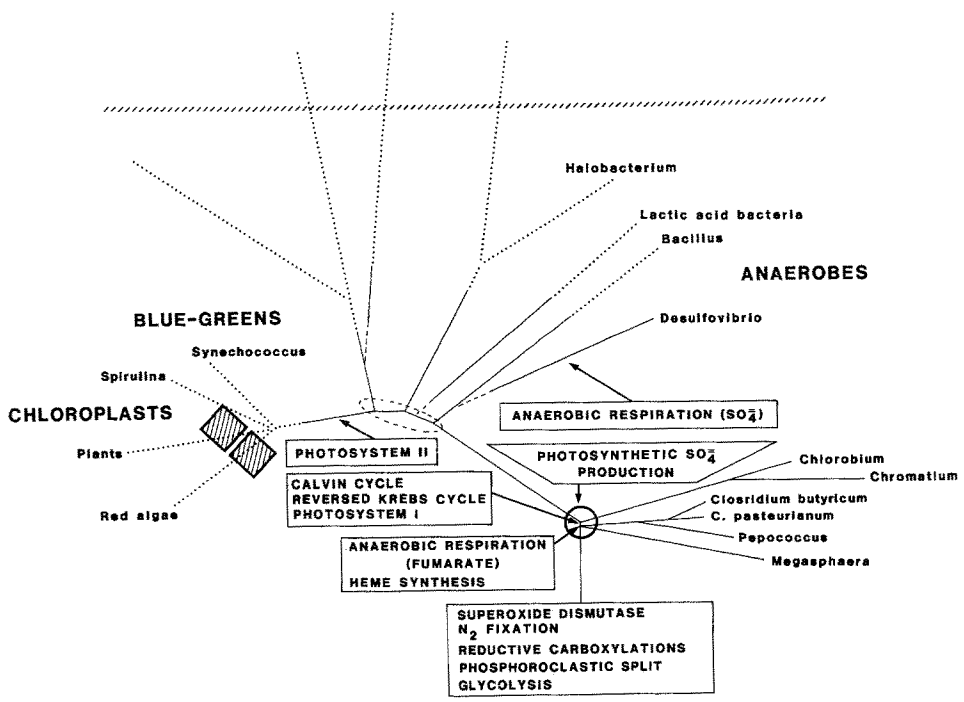


Fig. 1. Lower portion of the phylogenetic tree of Barnabas *et al.* (1982) showing the sequence of metabolic innovations over evolutionary time. Note the presence of superoxide dismutase, nitrogen fixation, and sulfide-utilizing photosynthesis at, or near the base of the tree.

### 2.1. Effects of Ammonia-Nitrogen

There is a singularly important characteristic of all nitrogenases: both their synthesis and activity are inhibited, and thus regulated by ammonia and the ammonium ion (see: Fogg, 1974; Yates, 1977; Postgate, 1982). This characteristic stems from the fact that nitrogen fixation is an energetically expensive process (Broda, 1975) requiring large amounts of ATP; up to 20 mols of ATP per mol of $N_2$ reduced (Daesch and Mortenson, 1968). Therefore, since it requires more energy to fix $N_2$ than to assimilate preformed $NH_3$, this inhibition or repression of the nitrogen fixation enzymes has been interpreted as a functional adaptation to avoid the energy-expensive and thereby wasteful production of endogenous $NH_3$ (Postgate, 1982). The organism is thus protected from wastefully manufacturing this substance endogenously.

Experimental evidence shows that small amounts ($10^{-6}$ M) of $NH_4^+$ are already partially effective at inhibition, but a concentration of $10^{-3}$ M will completely repress both the synthesis (Drozd *et al.*, 1972) and the activity (Hamadi and Gallon, 1981) of nitrogenase. Furthermore, in their efforts to avoid the large energy expenditure of nitrogen fixation, microorganisms have adapted to assimilate and transport exogenous ammonium ions at very low ambient concentrations in the micromolar range (Koike *et al.*, 1983). As an example, the half-saturation $K_m$ ($NH_4^+$) for the $N_2$-fixing obligate anaerobe *Clostridium pasteurianum* was reported at $10^{-5.05}$ M.

These levels of $NH_4^+$ are equal to, or are orders of magnitude below either the prebiotic equilibrium values of Bada and Miller (1968) for amino acid stability, or those calculated by Wigley and Brimblecombe (1981) for a range of primitive $CO_2$–$NH_3$ atmospheres. Therefore, because of its high equilibrium solubility, the substantial depletion of ammonium in seawater to micromolar levels under a long-persisting ammonia atmosphere would seem difficult.

A dilemma quickly arises for the evolution of $N_2$-fixation in a reducing environment beneath a world-wide reducing atmosphere. If ammonia inhibits both the synthesis and activity of the nitrogenase enzymes, and small amounts of exogenous dissolved ammonia can be easily assimilated and transported, how could this complex bio-energetic machinery have evolved under an ammonia atmosphere? In other words, why should an organism have 'invented' an energetically expensive and complex mechanism to 'manufacture' $NH_3$ if easily useable and continuously replaceable supplies were available in the surrounding milieu?

It would seem, then, that the principal problem is not how to maintain a persistent source of dissolved ammonia for interim nitrogen needs (Broda and Peschek, 1983), but rather, how to deplete environmental ammonia to the sufficiently low levels in the micromolar range required to (1) inhibit $NH_4^+$-assimilation and (2) avoid the self-destructive repression of the nitrogenase enzyme during its evolution. What appear needed on the early Earth during the period of experimentation with nitrogenase evolution are areas of locally oligotrophic conditions, perhaps similar to those that exist in parts of the modern ocean (North Pacific Gyre; Sargasso Sea) where the levels of ammonium nitrogen are exceedingly low ($< 3 \times 10^{-8}$ M), and where adaptive

$NH_4^+$-assimilation and $N_2$-fixation are found taking place (Eppley *et al.*, 1977; Martinez *et al.*, 1983). Easily visualized under redox-neutral or weakly oxidizing atmospheres, such environments are hard to imagine on an early Earth surrounded by a generally reducing atmosphere.

### 2.2. Effects of Molecular Oxygen on Nitrogen Fixation

A better-known characteristic of the nitrogenase enzymes than their ammonia sensitivity is their sensitivity to molecular oxygen. Nitrogenases are rapidly and irreversibly destroyed by exposure to free oxygen (Postgate, 1982). Clearly, this well-known $O_2$-sensitivity is good evidence that the enzyme must have evolved in an aqueous, generally anoxic environment, but it does not automatically follow that the *atmosphere* above this aqueous environment was also reducing. This is immediately obvious from the fact that strongly reducing environments exist today beneath a clearly oxidizing atmosphere. Thus, the almost legendary oxygen sensitivity of nitrogen fixation is no more an indicator of an anoxygenic *atmosphere* in the early Precambrian than it is an indicator of an anoxygenic atmosphere today.

Nevertheless, any dissolved oxygen would have been important. What then might the oxygen tolerance have been for an evolving procaryote faced with a shortage of environmental ammonia? Experimental evidence with living procaryotes indicates that the concentration of dissolved oxygen needed to cause repression of the synthesis of nitrogenase ranges from about 16 $\mu$M to 0.1 $\mu$M-$O_2$, or from $10^{-1.2}$ to $10^{-3.4}$ PAL (present atmospheric level) (Klucas, 1972; Hill *et al.*, 1981; Hamadi and Gallon, 1981; Bergersen *et al.*, 1982). If only the lowest level (i.e., $10^{-3.4}$ PAL) is used as a paleoecological guide, then all early habitats could certainly have encountered $pO_2$ levels at least as high a $10^{-3.5}$ PAL without anywhere significantly affecting the evolution of this important enzyme. On the other hand, the range of values fits very well with the conclusions that levels ranging from $10^{-3}$ to $10^{-1}$ PAL would be required to sustain a biologically effective ozone screen (Berkner and Marshall, 1965; Ratner and Walker, 1972; Hesstvedt *et al.*, 1974; Blake and Carver, 1977; Levine *et al.*, 1979; Kasting and Donahue, 1980).

The ecological importance of an effective ozone screen should not be underestimated. According to Jerlov (1950), clear ocean water is very transparent to short wavelength solar UV. And, a study of the effects of UV on a wide variety of aquatic organisms, (Calkins and Thordardottir, 1980) concluded that UV-tolerance is adaptive, with little reserve capacity to cope with increased exposure. Thus, it is difficult to visualize the global spread of shallow-water, light-requiring stromatolitic or planktic communities living under the searing influence of ultraviolet radiation in the absence of an ozone screen (Towe, 1981). It is unreasonable to suppose that photoautotrophy (not to mention DNA-RNA) evolved and diversified with an increasing demand for solar energy over hundreds of millions of years in the presence of unattenuated UV-radiation. Yet this is precisely the situation with all anoxic atmosphere scenarios. The standard geochemical and atmospheric modelling studies which conclude that the early atmospheric oxygen mixing ratios were in the $10^{-12}$ to $10^{-15}$ range leave the biosphere without ozone screen protection.

Rambler and Margulis (1980) have shown that the survival of modern anaerobic organisms to unattenuated UV-radiation is poor. In their experiments, the survival of five species was reduced by 99.9% in times ranging from 11 to 62 minutes. Even with massive levels of nitrate protection (0.01 M nitrate is unlikely for the Archean), survival was only 3.5–4.5 hours. Therefore, with the paleontological evidence of shallow-water, periodically-dessicated stromatolitic mats now extending as far back as 3,500 Myr ago (Lowe, 1980; Walter *et al.*, 1980), the 'standard' models which emphasize geochemical or atmospheric effects, but which predict uninhabitable shallow-waters, are clearly in need of revision. This would be especially troublesome when the early Sun was producing 4 times the present UV-flux at 3500 Myr ago (Gaustad and Vogel, 1982; Canuto *et al.*, 1982).

An early atmosphere containing small amounts of abiotically-formed free oxygen has other advantages for the evolution of nitrogen metabolism. The oxygen can help to produce nitrite and nitrate which, although unstable in reducing environments (Zohner and Broda, 1979), would serve to provide evolutionary 'pressure' to stimulate their $NH_3$-competitive assimilatory reduction (Broda, 1975) and their ultimate use in denitrification (nitrate respiration). In this context, it may be no coincidence that in the modern oceans denitrification is favored in oxygen-deficient waters, seldom occurring when the $O_2$-content exceeds 10 $\mu$M ($< 1\ \% O_2$) (Fogg, 1982). In fact, the idea that nitrate respiration appeared before oxygen respiration (e.g. Egami, 1974, 1976; Hall, 1973) deserves serious reconsideration. Additional support comes from the theoretical studies by Yung and McElroy (1979) on the possibility of nitrate formation in the primitive atmosphere. And there are the recent experimental results of Schrauzer *et al.* (1983) showing that $Fe^{3+}$-$TiO_2$-rich desert sands can catalyze the photoreduction of $N_2$ to form free $O_2$ and oxidized forms of nitrogen, in addition to $NH_3$. If this had happened in the early Precambrian, the $NH_3$ might have rapidly photodecomposed, the $O_2$ might have been added to the atmosphere, and the nitrogen oxides left behind for biochemical experimentation.

As long as the levels of ammonia nitrogen and molecular oxygen remained low, there would have been few problems for organisms using the process of nitrogen fixation. On the other hand, the later evolutionary development of oxygenic photosynthesis by the cyanobacteria would have presented an immediate threat. How could an organism have survived and continued to rely on a process that is rapidly inactivated by a substance ($O_2$) that it was suddenly able to produce within its own cell walls?

Three evolutionary approaches may have been used. The organisms could have:

1. Dispensed with the nitrogen fixation process by remaining in microhabitats where external sources of ammonia-nitrogen were available. The kinetic studies of Issack and Eady (*in* Yates, 1977) have shown that $NH_4^+$ reacts before $O_2$ to repress nitrogenase (Figure 2). Therefore, with no nitrogenase being synthesized there would be none to inactivate.

2. Separated the nitrogen-fixing and oxygen-producing machineries so that one could not readily affect the other. This is the adaptation in filamentous algae known as heterocyst formation. A specialized cellular structure is developed (provided that

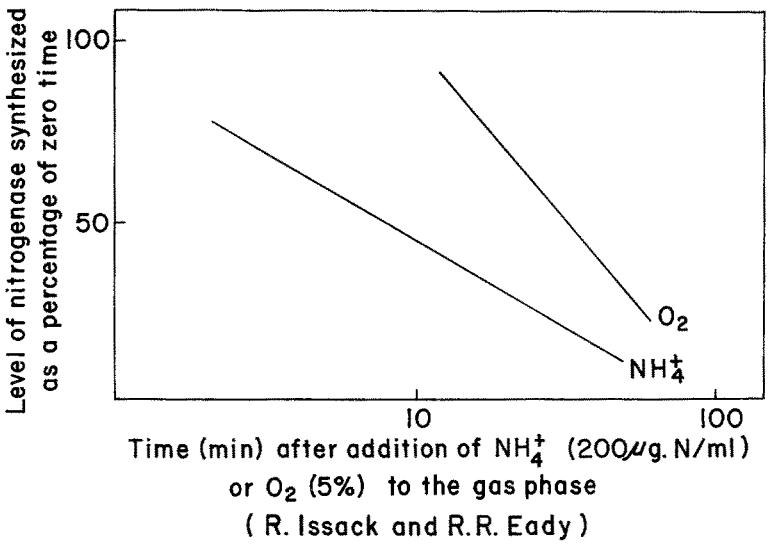


Fig. 2. Kinetics of the repression of nitrogenase in *Klebsiella pneumoniae* showing the priority of repression exhibited by ammonia over oxygen.

ammonia-nitrogen is unavailable) in which nitrogenase is unaccompanied by certain Photosystem II pigments and the necessary levels of manganese required for photosynthetic oxygen production (see Stewart, 1977). To prevent the oxygen being produced by the neighboring cells from feed-back interference, the heterocyst also develops a thicker wall than the other cells.

3. Occupied habitats where the illumination remained very low so as to avoid high intracellular $O_2$ production. Experiments with non-heterocystous, nitrogen-fixing cyanobacteria have shown that photosynthetically produced oxygen will 'short-circuit' $N_2$-fixation (Hamadi and Gallon, 1981), unless the illumination is kept below 300–400 lux to avoid $O_2$-buildup (Kalininskaya *et al.*, 1981).

Only two of these evolutionary 'strategies' may have been an option for the earliest cyanobacteria, because the heterocyst is so obviously adaptive, it must have come later.

## 3. Early Habitability and Nitrogen Fixation: Conclusions

If the foregoing discussion can be used to infer something about the conditions in the early Archean, then the following environmental constraints would seem to be required when and where the process of nitrogen fixation was being evolved:

1. The activity of the ammonium ion was no greater than $10^{-3}$, and was probably lower. Because of the high equilibrium solubility of ammonia in water, this would mean that there could have been little, if any ammonia in the atmosphere. And for this reason, if there had ever been significant amounts of ammonia in the Earth's early atmosphere, it would have to have been rapidly and quantitatively removed (cf. Kuhn and Atreya, 1979; Kasting, 1982) to provide the conditions under which the energy-expensive and

complicated process of nitrogen fixation could have evolved. This would be especially true if nitrogen fixation was one of the earliest of the major metabolic innovations to appear (Figure 1).

2. Atmospheric oxygen levels at least as high as $10^{-3.5}$ PAL (perhaps even as high as $10^{-2}$ PAL) could have existed, worldwide, without significantly affecting the evolution of a nitrogenase enzyme system. Indeed, the presence of abiotically-produced free atmospheric oxygen, so important to ozone screen formation, may also have contributed to the early shortage of ammonia that then stimulated the early biosphere faced with a reduced-nitrogen crisis to begin its evolutionary search for a substitute.

## 4. Physiology of Photosynthesis

According to the phylogenetic tree erected by Barnabas *et al.* (1982), the earliest development of photosynthesis (bacterial-type, Photosystem I) soon followed the evolution of nitrogen fixation; the development of Photosystem II came later (Figure 1). This position on the tree is consistent with ideas that photoautotrophs evolved soon after heterotrophs (Broda, 1970) or chemolithotrophs (Woese, 1977) encountered an 'energy crisis'. The process of bacterial photosynthesis, utilizing carbon dioxide and hydrogen sulfides, is fundamentally anaerobic. Therefore, like the process of nitrogen fixation, its evolution has also been linked to the presence of an early reducing atmosphere. Yet, while it too clearly indicates an evolutionary habitat that was aqueous and generally reducing, it need not require an *atmosphere* above the aqueous environment that was also reducing, or even anoxic. The widespread presence of anaerobic bacterial photosynthetic organisms on the modern Earth shows that this must be true.

### 4.1. Effects of Carbon Dioxide on Photosynthesis

Carbon dioxide is the ultimate source of carbon fixed in the normal process of photosynthesis. In the bacterial-type, Photosystem I-based process, hydrogen sulfides serve as electron donors with sulfur being the common 'waste' product.

The enzyme that is responsible for catalyzing the incorporation of $CO_2$ into the Calvin cycle is RuBP carboxylase. Three points are worthy of note:

1. $CO_2$ is the only species that can be used by this enzyme; $HCO_3^-$ or $CO_3^=$ cannot be used directly (Cooper *et al.*, 1969).

2. In the modern world where the $pCO_2$ is low, carbon dioxide can often be limiting.

3. The affinity of RuBP carboxylase for $CO_2$ varies with the species, and in general, appears to have adapted in the direction of a more efficient utilization of $CO_2$ (Jordan and Ogren, 1981).

These three points are consistent with the idea that environmental $pCO_2$ was higher in the distant past than it is today. For the early Precambrian, atmospheric modelling studies have suggested levels of $pCO_2$ as high as 1000 PAL (Hart, 1978; Owen *et al.*, 1979).

If it is true that the $pCO_2$ in the early Precambrian was orders of magnitude greater

than today, then it is obvious that the evolutionary development of RuBP carboxylase must have taken place under these higher partial pressures. The available biochemical-physiological evidence may allow us to place an upper limit on the $pCO_2$ during this time.

Among the most primitive anaerobic procaryotes living today is the purple sulfur bacterium *Chromatium vinosum* (see Figure 1). As it is capable of both heterotrophic and autotrophic growth, it would seem a plausible 'holdover' candidate – a 'living fossil' that was able to adapt from its presumed primitive heterotrophic existence to its derived autotrophic existence. The biosynthesis of RuBP carboxylase in *C. vinosum* has been intensively studied by Kobayashi and Akazawa (1982). These authors were able to show in heterotrophically-grown cells, that during autotrophic culture, if the $CO_2$ concentration exceeded $10^3$ $\mu$m (100 PAL) the inducible formation and activity of this enzyme was dramatically suppressed. The data are shown in Figure 3, together with a stippled bar indicating a range of highest values taken from the literature for the half-saturation $K_m(CO_2)$ of RuBP carboxylases from a variety of organisms ranging from cyanobacteria to higher plants.

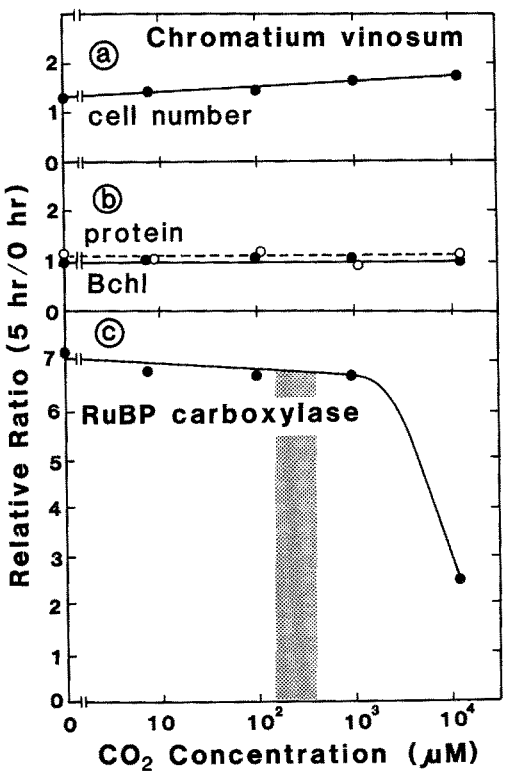


Fig. 3. Effects of $CO_2$ concentration during the autotrophic culture of *Chromatium vinosum* (after Kobayashi and Akazawa, 1982). Note the repression of RuBP carboxylase at $CO_2$ concentrations above $10^3$ $\mu$M (or 100 PAL). See text for explanation of stippled bar.

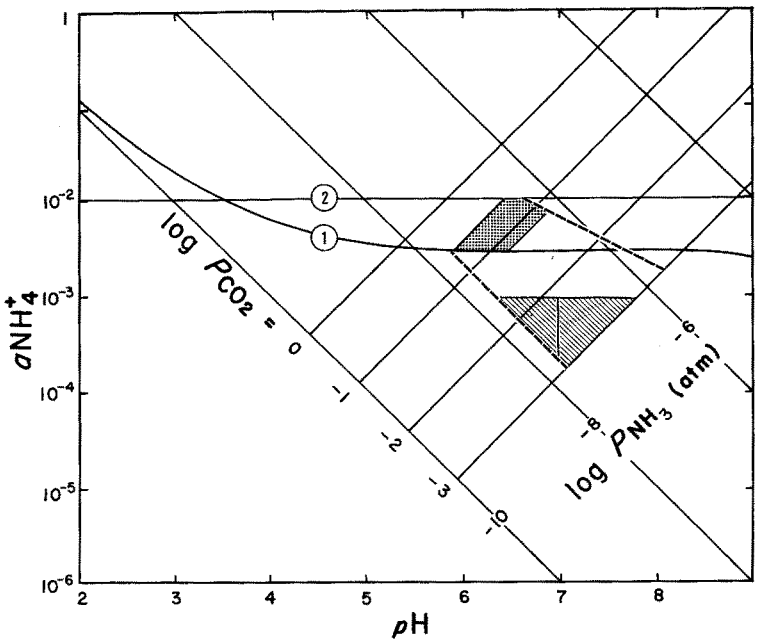


Fig. 4. Limits of a habitability zone (hashed region) for the early Precambrian defined in terms of pH, $pCO_2$, and $pNH_3$. (See text for sources and details.)

Placing these observations into a paleoecological context, it is permissible to conclude that during the early Archean evolution of the principal enzyme of bacterial photosynthesis, the concentration of $CO_2$ in the habitat was likely to have been greater than at present, but probably less than 100 PAL. The data are not consistent with global values as high as 1000 PAL during this period of time.

If this evidence from photosynthesis is combined with the previous evidence from nitrogen fixation, a habitability zone for life in the early Precambrian can be outlined (hashed region, Figure 4). The base for this figure is taken from the work of Wigley and Brimblecombe (1981) and is a modification of their Figure 1. Curves 1 and 2, (based on the amino acid stability presumed necessary by Bada and Miller (1968) for life to evolve) define the approximate lower and upper boundaries for the ammonium ion activity as a function of pH. The stippled region is Wigley and Brimblecome's 'life zone' for the period between 3500 and 4500 Myr ago. The dashed lines on Figure 1 define limits of pH and $pCO_2$ determined by Walker (1983).

### 4.2. Effects of Sulfides on Photosynthesis

Photosynthesis of the bacterial type, using Photosystem I, requires a habitat where both light and dissolved sulfides are available. Studies on the sulfide utilization of primitive photosynthetic bacteria (e.g. *Chlorobium*) have established that the half-saturation $K_m$ value for sulfide uptake in laboratory cultures is 0.65 mM (Bergstein and

Cavari, 1983); a value consistent with their field observations that *Chlorobium* 'blooms' occurred only after the sulfide concentration in the thermocline reached 0.03 mM. Their cell yields increased as sulfide reached 3–5 mM, and dropped off until sulfide toxicity stopped growth at 7 mM.

If representative, these data as applied to the early Precambrian, would suggest that sulfide concentrations in the range of 0.02–0.2 mM (but $< 7$–10 mM) would be required in the photic zone to support the evolution of a Photosystem I, bacterial-type photosynthesis.

In most discussions of early Precambrian habitats, the availability of sulfides is usually taken for granted under the widespread reducing conditions that are postulated. Sulfides are presumed to have been plentiful, perhaps even in amounts known to be toxic to photosynthetic bacteria. However, sulfde concentrations sufficient to cause such toxicity would have been mediated by the insolubility of pyrite (or other iron sulfides), which would act in concert with the supply of ferrous iron to keep available sulfide low (Drever, 1974). Indeed, because of this effect, it is possible to infer that sulfide-based photosynthesis might have first evolved in habitats where shallow-water hydrothermal vents/rifts may have maintained a steady supply of dissolved sulfide in excess of ferrous iron. On the other hand, the requirement for at least some ozone screen UV-protection during the evolutionary experimentation with photoautotrophy would presuppose at least small amounts of atmospheric free oxygen. This oxygen, diffusing into the hydrosphere would tend to oxidize ferrous iron and sulfides. As a potential electron donor for Photosystem I, $HS^-$ would be especially susceptible (see Stewart and Pearson, 1970, p. 305). However, at the lower pHs (6–7) expected as a result of high $pCO_2$ in the early Precambrian, the less rapidly oxidized $H_2S$ was probably the dominant species. Nevertheless, colonization of other photic habitats would have been at the mercy of fluctuations in sulfide availability, and even sulfide depletion due to sulfate formation without sulfate recycling. This lack of recycling stems from the fact that the inorganic, low-temperature reduction of sulfate is a very sluggish reaction; too slow to be of significance in sedimentary locales. Thus, in addition to the pyrite 'sink', the absence (during the early Archean) of the sulfate-reducing bacteria, that are the catalytic resuppliers of sulfide in modern anaerobic habitats, would have made global dissolved sulfide a dwindling commodity. The global biological availability of sulfides in the photic zone would have been slowly reduced. And, as rock weathering reactions continued to lower the $pCO_2$, the pH would rise thereby increasing the $HS^-/H_2S$ ratio, in turn allowing for the more rapid oxidation of sulfide to sulfate. This would signal a shift in the habitability zone of Figure 4 to the right of the vertical line. What is more, for every mole of $CO_2$ reduced to '$CH_2O$', four moles of $HS^-$ are required instead of two moles of $H_2S$. Thus, with a rising pH, less sulfur would be available as more sulfur was being required for photosynthetic reactions.

Except for the areas around shallow-water hydrothermal vent/rift systems, this shift in the habitability zone toward global reduction in the the biological availability of sulfides would place strong evolutionary 'pressure' on the photosynthetic bacteria.

Hydrogen sulfide as an electron donor would have to be replaced by water, even at the demand of more light energy. Thus, oxygenic photosynthesis with Photosystem II was the next major evolutionary metabolic step (Figure 1), heralding the appearance of the cyanobacteria, or blue-green algae.

The cyanobacteria would be capable of using either hydrogen sulfide (with Photosystem I) or water (with Photosystems I & II), if sulfides were lacking. The evidence from physiological studies of modern cyanobacteria can be used, in the paleoecological sense, to infer what the levels of habitat sulfide may have been during this important period.

Garlick *et al.* (1977) have studied the levels of sulfide that would support Photosystem I activity in 11 strains of cyanobacteria. They found that anoxygenic photosynthetic $CO_2$ assimilation, similarly to that in the photosynthetic bacteria, was first detected at levels of sulfide ranging from 0.05 to 1.0 mM (mean, 0.49 mM). The optimum values supporting this Photosystem I activity ranged from 0.12 to 3.5 mM (mean, 0.82 mM) sulfide. These values may be read to indicate that for evolutionary pressure to have been exerted on Archean photosynthetic bacteria to 'invent' Photosystem II, the habitat sulfide levels would have to have stayed consistently below 0.5 mM. They may well have been below 0.1 mM since the levels of sulfide that will begin to block and poison Photosystem II activity in extant cyanobacteria (Figure 5)

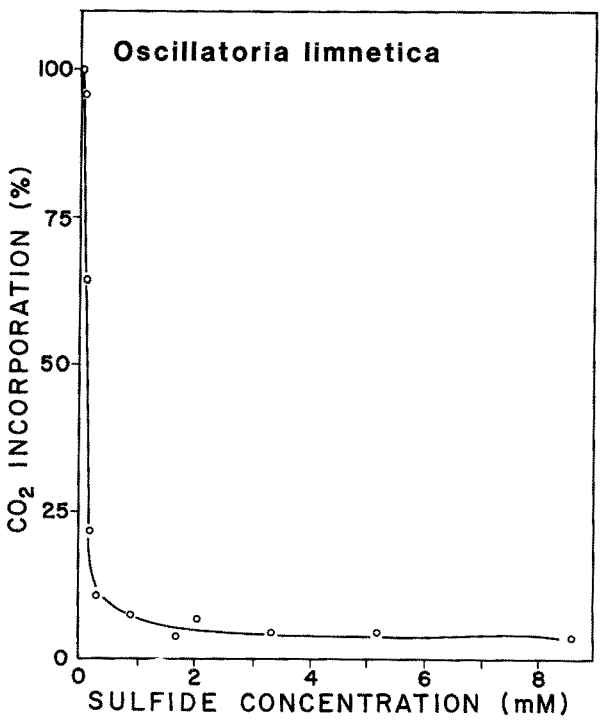


Fig. 5. Dramatic influence of even very low levels of sulfide on the aerobic photoassimilation of $CO_2$ by a blue-green alga using Photosystem II. (Modified from Oren and Padan, 1978)

are very low indeed (Oren and Padan, 1978; Howsley and Pearson, 1979; Jørgensen *et al.*, 1979; Oren *et al.*, 1979).

Looking at the origin of Photosystem II from an evolutionary perspective, the habitat problem would appear to be one of maintaining a minimum level of sulfide to support Photosystem I activity and avoid its extinction, yet not be enough to destroy any experiments leading to oxygenic photosynthesis. The evidence from modern microbiotas would place that level somewhere around 0.1 mM sulfide. But clearly, for oxygenic photosynthesis to operate efficiently in a sustained fashion (i.e., take over in global importance), photic zone dissolved sulfide would have to remain essentially absent – in the low micromolar range, or below. The ability of the cyanobacteria to switch back and forth from $H_2S$ to $H_2O$ would have been a great advantage in environments fluctuating between oxidizing and reducing conditions (Towe, 1978).

### 4.3. Importance of Oxygen to Photosynthesis

Similarly to the process of nitrogen fixation, bacterial-type photosynthesis is sensitive to free oxygen, although unlike nitrogen fixation, the process is not clearly related to an immediate and irreversible effect of exposure to oxygen on a specific enzyme. Nevertheless, all of the organisms that depend solely on Photosystem I for photoautotrophic growth are obligate anaerobes and the *sustained* presence of free oxygen or oxidizing conditions will eliminate them. Because many of these organisms are phylogenetically primitive (Figure 1), their ancestors having appeared early in Earth history, it is commonly assumed, or even taken as proof, that their oxygen sensitivity must have demanded an equally anoxic atmosphere. These microorganisms do not demand an anoxic *atmosphere* today, and there is no reason to expect that such a demand should have been required in the past. Indeed, there is evidence to the contrary.

Apart from the arguments in favor of an ozone screen, biochemical support for the presence of free oxygen on the early Earth before the invention of oxygenic photosynthesis comes in the form of two enzymes that are phylogenetically primitive and occur in obligate anaerobes.

Superoxide dismutases (SODs) occur at the base of the phylogenetic tree of metabolic innovations, together with nitrogen fixation (Figure 1). These enzymes have the key role of protecting an organism from oxygen toxicity (Halliwell, 1978). Their presence in primitive anaerobes, whose Archean ancestors antedated the cyanobacteria must mean that there was at least some free oxygen from somewhere, and of sufficient duration to allow for the evolution of SOD function (Lumsden and Hall, 1975; Towe, 1978). Anaerobic organisms endowed with superoxide dismutase activity, have a substantial tolerance to oxygen. Rolfe *et al.* (1978) subjected 13 strains of anaerobic bacteria to 21 % oxygen. The most intolerant strains survived for less than 45 minutes, but seven strains survived from 8 to more than 72 hours of exposure. Tolerance to lower amounts of oxygen ($< 1$ % oxygen) increases substantially (Loesche, 1969; Tally *et al.*, 1975), with many strains not only surviving, but actually experiencing maximal growth!

RuBP oxygenase is the other half, so to speak, of the principal enzyme of

photosynthesis. The oxygenase activity is well-known in higher plants as being responsible for the inefficient process of photorespiration. It affects net photosynthesis because the oxygenase and carboxylase activities compete directly with one another for $O_2$ and $CO_2$ respectively. The presence and function of this same oxygenase in anaerobic photosynthetic bacteria is a puzzle and argues for its early evolution under conditions where at least some oxygen was present. As suggested earlier (Towe, 1978), perhaps it acted as another oxygen detoxifier in the early history of photosynthesis where, in a $CO_2$-rich environment, competition for $CO_2$ would not have been a problem (Smith, 1976).

The influence of oxygen on the activity of RuBP carboxylase in the anaerobic photosynthetic bacterium *Chromatium vinosum* has been investigated by Kobayashi and Akazawa (1982). Although they found that $O_2$ markedly reduced enzyme activity, after 5 hours exposure to 21 % oxygen, the enzyme still retained 50 % of its activity as compared to its activity in the absence of oxygen. Even after 5 hours exposure to 100 % oxygen, 20 % of the enzyme activity was retained, along with 90 % of its bacteriochlorophyll content. It should be clear from these experiments that this anaerobe has considerable photosynthetic tolerance to free oxygen. What it is less tolerant to, as discussed above, is the absence of sulfide, and it is probably more the need for sulfide than the direct presence of oxygen that makes this organism an obligate anaerobe.

The cyanobacteria behave similarly to oxygen. Although their photosynthetic efficiency can be impaired under high oxygen levels (21 %, Weller *et al.*, 1975; > 10 %, Stewart and Pearson, 1970), they too can survive large concentrations of this gas. Stewart and Pearson (1970) noted that after a 6-hour exposure to 60 % oxygen there was full recovery within 5 hours return to 20 %! Under normal field conditions Jørgensen *et al.* (1979) observed that oxygen concentrations, due to local photosynthesis, commonly reach levels close to 1 atmosphere. Even the non-heterocystous nitrogen-fixing cyanobacteria whose internal $O_2$ production can be devastating at light levels of about 400 lux, can survive external oxygen concentrations in the medium of up to 5 % (Kalininskaya *et al.*, 1981).

## 5. Early Habitability and Photosynthesis: Conclusions

The evolutionary development of bacterial photosynthesis (Photosystem I) resulting from the 'energy crisis' presented to the early heterotrophs or chemolithotrophs, required an electron donor of which hydrogen sulfide was the choice. Modern procaryotic behavior suggests that in order to support this photoautotrophic process, habitat sulfide concentrations of at least $10^{-4}$ M would have been required.

Although carbon dioxide was the readily available source for organic carbon, in primitive anaerobes the modern behavior of the principal enzyme used in photosynthesis suggests that global $pCO_2$ probably was below 100 PAL during the time the enzyme was being evolved.

Evolutionary 'pressure' on the sulfur-based photoautotrophs to find an alternate electron donor ($H_2O$) would come in the form of a sulfide 'crisis' due to either the

formation of pyrite or the more rapid oxidation of sulfide to sulfate (irreversible in the absence of the yet-to-have-evolved sulfate-reducing bacteria), at the higher pHs (where $HS^- > H_2S$) appearing as a result of weathering reactions lowering global $pCO_2$. Although this indirect effect of atmospheric oxygen would have strongly affected bacterial photosynthesis, the direct effects on Photosystem I would not have been a problem at the minimum levels necessary for an effective ozone screen (about $10^{-2}$ PAL). Modern behavior of anaerobic procaryotes reveals that most can survive extensive exposure to *external* free oxygen, even at levels above atmospheric ($> 21$ %). This ability to tolerate encounters with free oxygen is closely related to the presence of enzymes such as superoxide dismutase, whose occurrence in phylogenetically primitive anaerobes is testimony to their ancestors' own encounters with this toxic substance. The *internal* production of free oxygen by the first photoautotrophs, even with SOD activity, would have been a problem unless they had found or remained in cryptic habitats where the illumination was low ($< 400$ lux).

## 6. Some Closing Remarks

The paleoecological approach used in this study places some *biological* limitations on the amounts of ammonia, carbon dioxide and oxygen that could have been present in the Archean atmosphere. A 'classical' reducing atmosphere is very difficult to reconcile with evolutionary biological needs, and an atmosphere containing more than 100 PAL of carbon dioxide is also unlikely by the time the first photoautotrophs evolved.

It should be clear from all of the evidence and the experiments described herein that the presence of small amounts of atmospheric free oxygen during the early history of the Earth would probably have posed little or no direct danger to early life processes taking place in the hydrosphere. The viewpoint that early life could not have evolved or survived under an *atmosphere* containing free oxygen is poorly founded and is not supported by either the phylogenetic data or the laboratory and field experiments on procaryotes. It seems preferable to argue that in spite of its toxicity, early atmospheric oxygen would have been a necessary 'hazard', if not simply to prepare organisms for the inevitable 'shock' of their own internal production of the substance, then surely for the creation of the ozone screen necessary for their protection against the devastation of short wavelength ultraviolet radiation.

In the past whenever a choice had to be made between the potentially detrimental effects of atmospheric oxygen or solar UV, it was the oxygen that was singled out. The primary reason for this attitude was the overwhelming importance that was attached to the chemical requirements imposed by the prebiotic experiments to make the building blocks for life; experiments supposedly simulating early atmospheric conditions, and that simply would not work in the presence of even small amounts of free oxygen. But with the evidence now increasing that those atmospheres were probably unlikely on the early Earth, and that most of the building blocks, plus a few more, could be found in the carbonaceous meteorites, and that these meteorites (or cometary nuclei) could provide the organic matter for prebiotic experiments, the need for eliminating free oxygen from

the early atmosphere is substantially lessened (cf. Towe, 1981). This evidence (including newer geological-geochemical evidence) continues to grow. With it comes a realization that the need to have an *atmosphere* without oxygen is no longer mandatory; certainly not so important that it can continue to overrule the potentially devastating impact to life of the unattenuated solar UV that would accompany such conditions. An acceptance of this realization reverses the priority usually given to these two potential hazards in models for the early Earth. A willingness to reject major amounts of solar UV and accept small amounts of atmospheric oxygen (rather than the other way around) makes early evolutionary continuity easier. Life facing a need for solar energy is not only able to survive it, but is able to take full advantage of it, and then evolve further from a life that does not produce oxygen, but is already adapted to tolerate it, to life that not only produces it, but learns to take advantage of it!

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