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Photosynthetic production of ammonia*

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Introduction

The conversion of solar energy into suitable redox energy through photosynthesis of the water-splitting type carried out by intact or reconstituted systems is of great interest and significance, and a major effort is presently under way to use this process to provide 'biofuels' on a renewable basis. The fact that some photosynthetically generated metabolites are highly reduced compounds of immediate practical interest has, however, been overlooked until now. Such is the case of ammonia, a compound generated by green cells from oxidized inorganic nitrogenous substrates in light-driven reactions, and that, in itself, is a very valuable derivative, both as a fuel and as a fertilizer[1,2].

Ammonia represents the primary source of the nitrogen fertilizers presently used in agriculture to improve crop productivity, which is usually limited by the availability of nitrogen in the soil. The manufacture of fertilizers requires vast inputs of energy, and it should be appreciated that nitrogen fertilizers often contribute up to 50% of the energy input in modern agriculture[3]. Ammonia for fertilizers is made through a catalytic reduction of atmospheric nitrogen with molecular hydrogen in a reaction which requires high pressures and temperatures (Haber-Bosch process). The world's industrial production of nitrogen fertilizers in 1974–75 reached about 42 megatons N with an energy cost of about 2 million barrels of oil per day[4,5]. These values represent more than a 10-fold increase since 1950, and it has been estimated that the world's annual requirement for nitrogen fertilizers will rise to 150–200 megatons N by the end of this century[4].

In addition to its value as a fertilizer ammonia is an excellent and powerful fuel and has been prominently mentioned as a constituent of various types of mixtures both for internal combustion engines and for jet propulsion[1,6]. It can react with molecular oxygen and be oxidized either to dinitrogen or to nitrate, according to the following highly exergonic equations:

$$NH_3 + {}^3/_4\, O_2 \xrightarrow{3e} {}^1/_2\, N_2 + {}^3/_2\, H_2O$$

($\Delta E'_o$, pH 7 $= +1.10$ V; $\Delta G'_o = -318$ kJ · mole^{-1})

$$NH_3 + 2\, O_2 \xrightarrow{8e} HNO_3 + H_2O$$

($\Delta E'_o$, pH 7 $= +0.47$ V; $\Delta G'_o = -360$ kJ · mole^{-1})

These reactions reveal that 1 atom of nitrogen reduced to the state of ammonia can supply either 3 electrons at the potential level of -0.28 V and be oxidized to molecular nitrogen, or 8 electrons at the potential level of $+0.35$ V and be oxidized to nitrate.

In addition, ammonia can undergo thermal dissociation in the presence of certain catalysts to yield molecular nitrogen and molecular hydrogen (E'_o, pH 7, -0.42 V). Decomposition into its elements can also be effected by photochemical means and by passing a silent electrical discharge through the gas. The reaction is weakly endergonic, as shown by the following equation:

$$2\, NH_3 \xrightarrow{6e} N_2 + 3\, H_2$$

($\Delta E'_o$, pH 7 $= -0.14$ V; $\Delta G'_o = +80$ kJ · mole^{-1})

Here, 2 moles of ammonia yield 3 moles of hydrogen. The hydrogen resulting from the lysis of ammonia can be utilized, for example, in the oxy-hydrogen blowpipe for producing intensely hot flame for welding metals and special steels, taking advantage of the strong exergonicity of its reaction with oxygen:

$$H_2 + {}^1/_2\, O_2 \xrightarrow{2e} H_2O$$

($\Delta E'_o$, pH 7 $= +1.24$ V; $\Delta G'_o = -238$ kJ · mole^{-1})

For the above reasons, liquid ammonia can be used for storing and transporting hydrogen in a convenient and compact way.

Aside from its industrial synthesis, a much more significant production of ammonia is being carried out by the plant kingdom. The extent of this biological synthesis of ammonia is as much as 2×10^4 megatons N per year, about 1% of this figure corresponding to the process of dinitrogen fixation, and the rest being accounted for by the assimilatory reduction of nitrate[7–9]. The energy demand for both processes is ultimately supplied by the sun via photosynthesis.

Photosynthesis is usually defined as the light-driven formation of carbohydrates and oxygen from CO_2 and water. This formulation ignores, however, the basic fact that in the photosynthetic process not only CO_2, but also the oxidized forms of other primordial bioelements are reduced and incorporated into cell material. Actually, photosynthesis drives several biosynthetic pathways involved in the assimilation of inorganic carbon, nitrogen, and sulfur. At the expense of sunlight energy, unstable products – cell material and oxygen – are synthesized from fully oxidized substrates with no useful chemical potential, namely water, carbon dioxide, nitrate or dinitrogen, sulfate and phosphate[1,2,9].

Ammonia is an obligate intermediate in the assimilation of oxidized inorganic nitrogen, either nitrate or dinitrogen, since these compounds have to be reduced and converted to ammonia prior to the incorporation

of N into cell material. Whereas practically all of the phototrophs, either prokaryotes or eukaryotes, are able to use nitrate as a nitrogen source, the ability to photoassimilate dinitrogen is restricted to some groups of the photosynthetic prokaryotes, including representatives of both photosynthetic bacteria and cyanobacteria.

The photosynthetic reduction of nitrate to ammonia does not require ATP and involves 8 reducing equivalents of photosynthetic origin. The process takes place in 2 steps: nitrate is first reduced to nitrite in a 2-electron reaction catalyzed by the molybdoprotein nitrate reductase, and then nitrite is reduced to ammonia in a 6-electron reaction catalyzed by the iron-protein nitrite reductase[7-9].

$$NO_3^- \xrightarrow[\text{nitrate reductase}]{2e} NO_2^- \xrightarrow[\text{nitrite reductase}]{6e} NH_3$$

The process of nitrate reduction as it takes place in the blue-green algae, with reduced ferredoxin acting as the immediate electron donor for both partial reactions, is one of the simplest examples of photosynthesis[7-10].

Some photosynthetic bacteria and cyanobacteria (blue-green algae) are also able to carry out the fixation of dinitrogen, in a process which converts N_2 to ammonia. This reduction of molecular nitrogen involves a 6-electron reaction that requires in addition the input of a large quantity of energy in the form of ATP, at least 12 molecules of ATP being hydrolyzed per molecule of dinitrogen reduced:

$$N_2 \xrightarrow[\text{nitrogenase}]{6e,\ \geqslant 12 \sim P} 2\,NH_3$$

The reaction is catalyzed by the oxygen-labile enzyme complex nitrogenase, which is composed of 2 different iron-sulfur proteins, one of them containing molybdenum in addition to non-heme iron[11]. The assimilatory power for this reaction in phototrophic prokaryotes is ultimately provided by photosynthesis.

Assimilatory nitrate reduction and dinitrogen fixation by photosynthetic organisms are thus processes which result in the conversion of solar energy into redox energy and its storage in the molecule of ammonia, as shown by the following exergonic reactions corresponding to the photosynthetic reduction of nitrate and N_2 with water as the electron donor:

$$HNO_3 + 4\,H_2O \xrightarrow{\text{light}} NH_3 + 3\,H_2O + 2\,O_2$$

($\Delta E'_o$, pH 7 = −0.47 V; $\Delta G'_o$ = +360 kJ · mole^{-1})

$$2\,N_2 + 3\,H_2O \longrightarrow 2\,NH_3 + {}^3/_2\,O_2$$

($\Delta E'_o$, pH 7 = −1.10 V; $\Delta G'_o$ = +318 kJ · mole^{-1})

Two potentially valuable systems wherein ammonia is photoproduced are worthy of attention:

1. The type where preparations of active water-photolysing photosynthetic membranes are able to generate the assimilatory power required for the reduction of nitrate or dinitrogen. These preparations should contain, or be supplemented with, the required enzymes and cofactors, and they might in turn be replaced by other biological or synthetic systems able to transduce light energy into suitable redox and chemical energy. This type of system presents several problems, especially in the case of dinitrogen as substrate.

2. The type where whole photosynthetic organisms, ideally microorganisms, carry out photosynthesis of the water-splitting type and which, after chemical or genetic manipulation, become suited for the continuous production and release of a significant fraction of the ammonia resulting from the reduction of either nitrate or dinitrogen. The achievement of this goal rests on effectively impeding both the incorporation of ammonia into carbon skeletons and the expression of any of the several antagonistic effects of ammonia on inorganic nitrogen metabolism.

The present status and future prospects of this novel field of biological ammonia photoproduction are described below.

Photoproduction of ammonia from nitrate

The photosynthetic nature of the process of nitrate reduction in blue-green algae was conclusively demonstrated in 1976 by Candau et al.[10]. These authors reported the ability of unsupplemented membrane preparations from the unicellular blue-green alga *Anacystis nidulans* to carry out the light-dependent steady reduction of nitrate to ammonia coupled to the evolution of molecular oxygen. The process took place under aerobic or anaerobic conditions alike, and the activity of the system was linear for at least 90 min, at a rate of ammonia production of about 2 µmoles per mg chlorophyll and h. A reconstituted aerobic system including ferredoxin and the nitrate-reducing enzymes of *Anacystis* together with spinach grana was also able to photoreduce nitrate to ammonia at a rate of about 40 µmoles per mg chlorophyll and h[2,12].

The stability problems inherent in the in vitro photosynthetic systems impose at present a serious limitation to their use on a continuous basis. For this reason, whole photosynthetic microorganisms, when conveniently manipulated, are in the short run better candidates as solar energy converters for achieving a prolonged photoproduction of ammonia.

The steady production of ammonia from nitrate with whole living cells requires the prevention of both ammonia assimilation and the negative effects which ammonia exerts on nitrate utilization, namely inhibition of nitrate uptake, inactivation of nitrate reductase and repression of the 2 enzymes of the nitrate-reducing pathway[7-9]. This seemed at first a difficult task to achieve, but recent evidence obtained in our own and in other laboratories[7-9] has shown that many

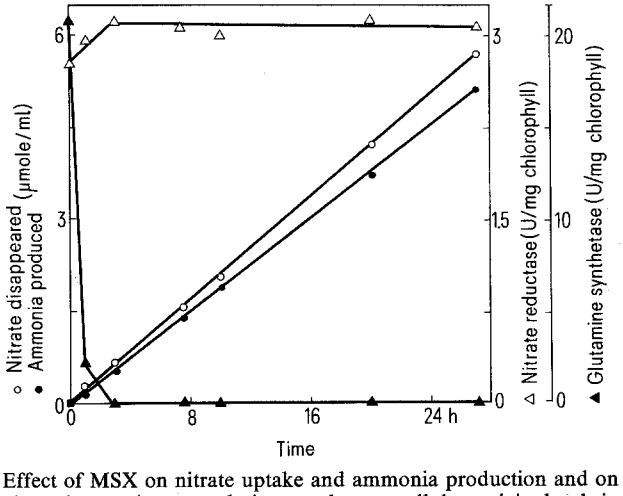
(if not all) of these antagonistic effects of ammonia are in fact not caused by ammonia itself, but rather result from its metabolism. This means that prevention of ammonia assimilation should allow, at least to a certain extent, the production of ammonia from nitrate by whole cells without further interference by the accumulated ammonia on the process.

A series of previous observations found in the literature indicated the viability of such an approach for achieving the production and excretion of ammonia. They corresponded in general to experiments carried out with illuminated suspensions of green algal cells that, under conditions in which the generation of the carbon skeletons required to incorporate ammonia is impeded, i.e. in the absence of CO_2 or another utilizable carbon source, can reduce nitrate to ammonia and export it to the medium[13-17]. These experiments did not try to achieve ammonia photoproduction but were designed to study physiological aspects of nitrate reduction by green algae. The ability of such systems to continuously produce ammonia for long periods of time under the established conditions of prolonged carbon starvation could, however, be questioned, particularly since an essential for nitrate utilization by many species of green and blue-green algae is a carbon source[8].

For our purpose we have selected the approach of interrupting the early stages of ammonia assimilation by using specific inhibitors of the incorporation of ammonia into carbon skeletons, allowing carbon assimilation to proceed unimpaired. Recent experiments carried out with *Anacystis* have shown that the prevention of ammonia assimilation through inactivation of glutamine synthetase with L-methionine-D,L-sulfoximine (MSX)[18] abolish the inhibitory effects of ammonia on both nitrate uptake[19] and nitrate reductase synthesis[20]. Glutamine synthetase is the first enzyme involved in the main route of ammonium assimilation in *Anacystis*[21] as is also the case for many other photosynthetic organisms where ammonia incorporation into carbon skeletons rests primarily on the operation of the glutamine synthetase/glutamate synthase pathway[22]. MSX-treated *Anacystis* cells are able to take up and reduce nitrate at rates much higher than those in normal untreated cells, a significant fraction of the ammonia resulting from nitrate reduction being exported to the medium.

We have characterized the effects of MSX on *Anacystis* cells using nitrate in order to maximize the extent and the duration of the process of ammonia production, and with the aim of using these MSX-treated cell suspensions as effective ammonia photoproducing systems on a continuous basis. For these experiments, unless otherwise indicated, cell suspensions containing about 10 µg chlorophyll per ml culture medium were illuminated with white light (25 $W \cdot m^{-2}$). Some of the main events following the addition of 10 µM MSX to such a suspension of *A. nidulans* cells are shown in the figure. After addition of the inhibitor, cellular glutamine synthetase is rapidly and completely inactivated, and ammonia appears on the medium concomitantly with this activity loss. Cell growth is prevented, and the rates of nitrate uptake and reduction increase 2- to 3-fold. Ammonia accumulates in the medium at a constant rate of about 25 µmoles per mg chlorophyll and h, which corresponds to about 90% of the rate of nitrate consumption by the cells. The process keeps going on for about 30 h without further additions provided that nitrate is not limiting, and nitrate reductase activity remains at its original high level during this period[23]. The rate of ammonia photoproduction by MSX-treated *Anacystis* cells can be improved by increasing either the pH of the medium, the cell density or the intensity of illumination. Thus, at pH 9.5 the rate of the process is about 1.5-fold higher than at pH 7.0. By using a cell density equivalent to 15 µg chlorophyll per ml medium and a light intensity of 200 $W \cdot m^{-2}$, under conditions otherwise similar to those described in the figure, a rate of ammonia production of 60 µmoles per mg chlorophyll and h has been achieved[24].

With 10 µM MSX (the optimal initial concentration of this compound), the effective period of ammonia production lasts for about 30 h, but its effectiveness is lost thereafter. Readdition of 10 µM MSX to the cell suspension every 24 h allows the initial rate of ammonia production to remain constant for at least 3 days[24]. At this stage the addition of MSX no longer results in the recovery of the process. Reinitiation of ammonia production can, however, be achieved if a pulse of



Effect of MSX on nitrate uptake and ammonia production and on glutamine synthetase and nitrate reductase cellular activity levels in *Anacystis nidulans*[25]. Nitrate-grown *A. nidulans* cells were harvested, washed and suspended in normal growth medium[19] containing 20 mM KNO_3 up to a cell density of 8 µg chlorophyll per ml. MSX to reach a final concentration of 10 µM was added at t = 0, and the cell suspension was illuminated (25 $W \cdot m^{-2}$, white light) at 40 °C and sparged with a mixture air:CO_2 (97:3, v/v). Aliquots were withdrawn at the times indicated, and nitrate and ammonia, in the cell-free supernatants, and nitrate reductase and glutamine synthetase, in the cells, were estimated after centrifugation[19,34].

glutamine is added together with MSX. In fact, after 3 days of active production the MSX-treated cells exhibit negligible levels of glutamine and low levels of glutamate. The metabolic situation which leads to the cessation of ammonia production seems therefore to correspond to glutamine starvation rather than to a generalized nitrogen depletion; this is corroborated by the otherwise normal content of phycobiliproteins, good indicators of the general nitrogen status in cyanobacteria. An alternative and more practical way of prolonging the active phase of ammonia production by MSX-treated cells is to allow them to recover from the glutamine depletion by keeping them recurrently for 8 h in the absence of MSX after every 48-h period of production in the presence of the inhibitor. With this procedure the effective period of ammonia photoproduction by *Anacystis* can be extended for at least 10 days, the rate of the process remaining constant throughout this period of time[24,25].

A production of ammonia lasting for several hours in response to the addition of 1 mM MSX has also been reported to occur in nitrate-utilizing glutamate-grown cells of *Cyanidium caldarium,* a thermophilic red alga[26]. The presence of MSX prevented the inactivation of nitrate reductase which otherwise took place in the presence of ammonia, indicating that a product of ammonia metabolism, but not ammonia itself, is responsible for this effect in *Cyanidium.* On the other hand, Florencio et al.[27] have reported that the addition of MSX to cell suspensions of the green alga *Chlamydomonas reinhardii* results in a transient accumulation of ammonia in the medium. This accumulated ammonia causes the inactivation of nitrate reductase even in the presence of MSX, supporting the idea that ammonia plays a direct role in the reversible inactivation of nitrate reductase in *Chlamydomonas*[7-9].

Photoproduction of ammonia from dinitrogen

Effective in vitro photobiological systems for the generation of ammonia from dinitrogen have, to the best of our knowledge, not yet been reported. In addition to being inherently unstable, such systems would require strict anaerobic conditions to be operative since the nitrogenase complex is extremely sensitive to molecular oxygen. The limitations on the effective operation of these types of systems are comparable to those currently encountered in the photoproduction of hydrogen through 'water biophotolysis' with in vitro systems[28].

The in vivo photoproduction of ammonia from N_2 by cells of photosynthetic diazotrophs offers, however, much better perspectives. Anaerobic or microaerophilic conditions are still required for in vivo N_2-fixation by photosynthetic bacteria and a range of blue-green algae. Contrary to the cyanobacteria, the N_2-fixing photosynthetic bacteria cannot use water as the electron donor for photosynthesis, but they require substrates with a more negative potential, usually reduced inorganic compounds. Certain blue-green algae, the filamentous cyanobacteria able to differentiate heterocysts, are unique in that they can fix N_2 to ammonia under aerobic conditions with light as the unique source of energy, and water as the source of reductant. Moreover, they (as other cyanobacteria) are also able to carry out anoxygenic photosynthesis similar to that found in the photosynthetic bacteria, being thus able to develop in various habitats.

As is the case with nitrate photoreduction, ammonia behaves as an antagonist of photosynthetic N_2-fixation, leading to repression of nitrogenase in photosynthetic bacteria and cyanobacteria and to a reversible inactivation of nitrogenase activity in the former group of organisms. Both of these negative effects of ammonia on N_2-fixation have been shown to require ammonia metabolism, indicating again an indirect involvement of this compound. In fact, the accumulation of ammonia is harmless for N_2-fixation provided that its further metabolism is prevented[29-31].

Following the demonstration by Gordon and Brill[32] with the chemotrophic bacterium *Azotobacter* that the inhibition of ammonia assimilation by certain glutamate analogs caused derepression of N_2-fixation and excretion of ammonia to the medium, a similar behavior was reported for the photosynthetic diazotrophs, both cyanobacteria[29] and photosynthetic bacteria[30], in response to treatments with the glutamine synthetase inactivator MSX. With the filamentous blue-green algae *Anabaena cylindrica,* Stewart and Rowell[29] presented evidence that MSX alleviated the inhibitory effect of exogenous ammonia on heterocyst production and nitrogenase synthesis. In the presence of 1 µM MSX, illuminated *Anabaena* cell suspensions excreted ammonia at a rate of about 3 µmoles per mg chlorophyll and h. Subsequently, Weare and Shanmugam[30] presented similar results for the photosynthetic bacterium *Rhodospirillum rubrum.* The production of ammonia by the bacterial suspensions was significatively increased when a fixed nitrogen source was added to the cells together with MSX. In the presence of 55 mM MSX and 5 mM glutamate, a net production of 11 mM ammonia after 6 days with a maximal rate of 0.7 µmoles per mg cell protein per h was attained.

Using a marine strain of the filamentous blue-green alga *Anabaena* (*Anabaena* sp. strain ATCC 33047), we have recently achieved an efficient and continued photoproduction of ammonia from N_2[33]. The addition of 35 µM MSX to suspensions (about 10 µg chlorophyll per ml medium) of this cyanobacterium causes a rapid inhibition of cellular glutamine synthetase and an increase in nitrogenase activity of about 2-fold over the normal derepressed activity level found in

the absence of MSX. About 90% of the N_2 fixed by the cells, which do not grow any longer, is exported to the medium as ammonia at a rate of about 25 µmoles per mg chlorophyll and h (equivalent to 1.4 µmoles per mg cell protein and h) when illumination is 25 $W \cdot m^{-2}$ (white light). With successive additions of 35 µM MSX every 20 h, and intercalating 8-h periods in the absence of the inhibitor every 40 h, a steady generation of ammonia lasting for 5 days with a net production of 20 mM ammonia has been achieved. Increasing conveniently the cell density and the light intensity allows the achievement of higher production rates: 60 µmoles per mg chlorophyll and h for a cell density of 11 µg chlorophyll per ml and a light density of 200 $W \cdot m^{-2}$ [25,33,34].

A further step in optimizing conditions for an effective photoproduction of ammonia from N_2 is reached by the use of mutant strains of photosynthetic diazotrophs blocked in the early stages of ammonia assimilation and derepressed for nitrogenase. In this context, Weare[35] has reported the isolation of a mutant of *Rhodospirillum* with low glutamate synthase activity and significant derepression of nitrogenase which exports ammonia to the medium at a rate of about 0.02 µmoles per mg cell protein per h. More recently, Wall and Gest[36] have isolated glutamine auxotrophs of *Rhodopseudomonas capsulata,* another photosynthetic bacterium. These mutant strains have negligible levels of glutamine synthetase activity and exhibit derepressed nitrogenase activity, thus allowing a continued production of ammonia from N_2 leading to a net ammonia production of about 15 mM after 4 days. Mutants of filamentous blue-green algae similar to those described above are currently being sought in our laboratory. The interest in obtaining such mutant strains is manifest since they represent solar energy converters which, by themselves, would generate ammonia under aerobic conditions with ubiquitous substrates (air and water) by drawing energy from an unlimited energy source (sunlight).

Concluding remarks

Research on the biochemical, physiological and genetic aspects of inorganic nitrogen metabolism in microorganisms has provided relevant information on the mechanism and the regulation of the uptake and the reduction of oxidized inorganic nitrogenous substrates and the incorporation of the resulting ammonia into carbon skeletons. The information discussed here indicates the feasibility of using photosynthetic organisms, preferably microalgae, with chemically or genetically deregulated inorganic nitrogen metabolism, for the steady photoproduction of ammonia in significant amounts. In its presently most relevant version, using whole cells of N_2-fixing filamentous blue-green algae, the valuable biofuel ammonia is generated at the expense of only light, air and water in a remarkable example of biological solar energy conversion. The state of the art in this field is still in its infancy, but very interesting perspectives can be envisaged after further improvement of the in vivo biological systems considered here or the development of other in vitro or artificial systems. Certainly much more investigation is needed for the installment of practical ammonia-generating systems, and emphasis should also be laid on both environmental and bioengineering aspects.

With regard to the in vivo systems, the microalgae seem the most interesting organisms, with obvious advantages over the photosynthetic bacteria, which carry the extra requirement for reduced compounds and also for anaerobic or microaerobic conditions. Till adequate mutant strains of these algae are available, chemical manipulation with specific inhibitors, especially MSX, seems to be the preferred procedure for inducing excretion of a significant fraction of the biologically generated ammonia. It has to be pointed out that not every alga would be suitable for the production of ammonia after treatment with MSX. An unavoidable requisite to this end is that ammonia assimilation takes place solely or predominantly through glutamine synthetase, the enzyme target of this inhibitor. Obviously, the cell wall should be permeable to MSX, a condition that is not shared by all algal species. A careful screening of different algal strains is needed to select those exhibiting sustained high rates of ammonia production. In this selection, high activity levels of the corresponding enzymes involved in the generation of ammonia and high rates of photosynthetic electron flow are also important aspects to be taken into account, since these factors ultimately determine the rate of ammonia production. The maximum expected efficiency of light conversion into chemical energy stored in ammonia by whole plant or algal photosynthetic nitrate reduction under 'ideal' conditions is about 13% of the radiant energy involved in the process[1,2]. This corresponds to a maximum expected ammonia production rate of about 2 moles per m^2 and day (120 tons ammonia per ha and year) for a solar energy value of 200 $W \cdot m^{-2}$, when allowance is made for factors such as the fraction of photosynthetic active radiation with respect to total solar radiation, and losses by reflection, absorption and transmission.

In experiments carried out in 50-l containers with 0.1 m^2 surface exposed to white light (500 $W \cdot m^{-2}$) using MSX-treated *Anacystis* cell suspensions (3 µg chlorophyll per ml of nitrate medium), values of net ammonia production of 1.5 mM ammonia in 12 h, corresponding to 0.75 moles ammonia per m^2 and day, have been obtained[25]. From a dual extrapolation of both area and time, a production of 46 tons ammonia per ha and year can be calculated from these values. This represents as much as 30% of the

maximum expected value under our experimental conditions. In fact, we have estimated that the rest of the available electron flow is used for CO_2 fixation, which results in an accumulation of carbohydrates by the MSX-treated cells[24,25]. Should this electron flow to carbon be avoided and channeled to nitrate reduction, the values of ammonia production would be increased by a factor of 3.

Ammonia photoproduction from N_2 might result in even higher yields since the quantum requirement for the production of ammonia by photosynthetic N_2 fixation can be estimated at 12 quanta per molecule of ammonia instead of the 16 quanta which are required with nitrate as the substrate.

In addition to possible applications at the industrial level, the photoproduction of ammonia by appropriate N_2-fixing microorganisms might prove useful for the in situ generation of nitrogen fertilizers in rural areas. If, for example, the process were carried out in small ponds, it might provide, with the help of a simple technology, an ammonia-enriched fluid to be used for direct watering of the field.

The possibility of achieving effective photoproduction of ammonia by in vitro photosynthetic systems depends on the development of stable components. A range of model systems which might in principle also be adequate for ammonia photoproduction can be found in the recent review paper by Bolton and Hall[28]. These authors have also considered the possibility of using artificial systems in solar energy conversion to mimic the photosynthetic systems by the use of synthetic catalysts, and Losada[1] has also stressed the potential of flavin photosystems for practical uses in this field. In this respect it is worth mentioning the use of a deazaflavin photosystem for the reduction of nitrate to nitrite with ferredoxin and nitrate reductase of the blue-green alga *Anacystis*[37]. Since nitrite reductase of this and other photosynthetic organisms uses also reduced ferredoxin as physiological reductant, an enzyme system composed of nitrate reductase, nitrite reductase and ferredoxin may be envisaged that, upon illumination in the presence of a suitable electron donor and catalytic amounts of a flavin, might also generate ammonia from nitrate. The photoproduction of ammonia from N_2 might also be achieved with the help of flavin photosystems. In fact, a deazaflavin mediated photoreduction of acetylene with a reconstituted nitrogenase system from *Azotobacter* has already been reported[38]. The non-enzymatic photochemical reduction of N_2 to ammonia has also been achieved with titanium dioxide catalysts, these systems being particularly effective when illuminated with UV-light[39].

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