Increase and Stabilization of Photoproduction of Hydrogen in *Nostoc muscorum* by Photosynthetic Electron Transport Inhibitors

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Z. Naturforsch. **33 c**, 541–547 (1978) ; received May 24, 1978

Cyanobacteria, Water Photolysis, Nitrogenase, Uptake Hydrogenase, Photoproduction of Hydrogen, Photosynthetic Electron Transport Inhibitors

Nitrogen-fixing cells of *Nostoc muscorum* grown under nitrogen or in the presence of nitrate exhibit substantial light-induced hydrogen production for over 15 hours in the presence of electron transport inhibitors. Rates attain levels of 12 μmol H_2 evolved/ml packed cell volume and hour. The ATP-dependent nitrogenase, not a hydrogenase, is responsible for hydrogen production. This is indicated by poor sensitivity to CO and inhibition of the reaction by uncouplers, acetylene, and N_2. An active uptake hydrogenase minimizes light-induced H_2 production. Although nitrogenase activity is somewhat decreased by several photosynthetic electron transport inhibitors, hydrogen production is markedly increased. This is due to lowering the partial pressure of oxygen in the cell, preventing oxidative hydrogen consumption.

Introduction

Hydrogen gas formed from biological material using solar energy is increasingly considered as a possible candidate for an alternative fuel source [1–4]. Photoproduction of H_2 gas following current opinion may occur in algae in two ways: a) Photoevolution of H_2 by green algae or also cyanobacteria mediated by the enzyme hydrogenase which is rather sensitive to carbon monoxide and oxygen labile [5], b) as a side reaction of nitrogenase. Contrary to a) this H_2 evolution is ATP-consuming and insensitive to carbon monoxide [3, 4, 6].

Recent reports on the presence of H_2-producing hydrogenase in cyanobacteria have been controversial [6, 8, 9]. Whereas Tel Or *et al.* [8] have demonstrated soluble and membranebound hydrogenase with activities related to H_2 production and H_2 consumption in crude cell-free preparations of *Nostoc muscorum*, Bothe *et al.* [6] have shown that the simultaneous addition of acetylene and carbon monoxide to intact filaments of *Anabaena cylindrica* blocks the uptake hydrogenase and at the same time reveals the hydrogen-producing capacity of nitrogenase which is quite sensitive to the presence of uncouplers.

In order to clarify the conditions under which photoevolution of hydrogen occurs in blue-green algae, the purpose of this investigation was threefold: Firstly to increase H_2 production using the inherent capacity of the organism without adding reductant and to evaluate several effects of growth conditions, secondly to study the effect of inhibitors of photosystem II on hydrogen evolution, and thirdly to clarify whether H_2 production can be attributed to a hydrogenase or to nitrogenase.

Materials and Methods

Nostoc muscorum (strain No. 7119, orginally from R. Y. Stanier), a generous gift from Dr. H. Tsujimoto, Berkeley, was grown axenically on a shaker in 1.2 l batches in 2 l Fernbach flasks [10] in a medium according to [11] at 21–23 °C and continuously illuminated by 5 J/m² × sec fluorescent white light. The cultures were bubbled with air or nitrogen, both gases enriched by 5% CO_2 (v/v) as indicated. Cultures with KNO_3 or NH_4Cl as nitrogen source were gassed with air/CO_2.

Cultures with maximum heterocyst frequency were obtained under N_2/CO_2, those with reduced heterocyst frequency by supplying the medium with 20 mM KNO_3. Substitution of this nitrogen source by 2 mM NH_4Cl yielded heterocyst-free algae, which was also reported for *Anabaena* [6]. Gassing N_2-

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Abbreviations: atrazine, 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; bentazon, 3-isopropyl-2,1,3-benzothiadizin-4-one-2,2-dioxide; DCMU, N-(3,4-dichlorophenyl)-N′,N′-dimethylurea; Cl-CCP, m-chloro-carbonyl-cyanide-phenylhydrazone; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; Chl, chlorophyll; DBMIB, 2,4-dibromo-3-methyl-6-isopropyl-benzoquinone; metribuzin, 4-amino-6-isopropyl-3-methylthio-1,2,4-triazine-5-one; simazine, 2-chloro-4,6-diethylamino-1,3,5-triazine; pcv, packed cell volume.

fixing cultures with N_2/CO_2 had the effect of establishing almost anaerobic conditions during cultivation.

Algae were harvested during the early log-phase (3 – 5 days after inoculation) by centrifugation at $4000 \times g$ for 5 minutes and resuspended in fresh medium at the cell density required.

Experiments were carried out using 38 ml cylindrical allglass reaction vessels suspended at a 30° angle in the waterbath of a conventional Warburg apparatus at 26 °C. Light was provided from below by 6×150 W bulbs filtered through a 25 cm layer of water, part of the heat was dissipated by fans. Illumination intensity could be adjusted, ranging from 0 – 310 J/m² × sec. Each vessel contained 10 ml of algae with a density equivalent to 10 – 12 µl packed cell volume (pcv)/ml. pcv was chosen as a reference in preference to chlorophyll since the latter showed greater variation during growth. It can be assumed that during the early log phase of growth values of µg Chl/ml and µl pcv/ml suspension are numerically about the same. The pcv was determined in graduated micro centrifuge tubes of 80 µl capacity; a 2 ml aliquot of algal suspension was centrifuged for 5 min at $3000 \times g$. The reaction vessels, closed gas-tight with Suba Seal turnover stoppers (Freeman Company, Barnsley, England), were evacuated and flushed with argon for 15 minutes. Any gases or dissolved reagents added prior to incubation were injected through the seals with gas-tight or liquidtype Hamilton syringes. During the experiments, aliquots of the gas phase (100 µl for C_2H_2; 250 µl for H_2 determination) were removed with syringes (Precision Sampling) and analyzed by gas chromatography.

Acetylene reduction was followed by injecting the sample into a Perkin-Elmer PE 22 gas chromatograph fitted with a flame ionization detector and a Poropak R column (100 – 120 mesh, 1 m, 1/8 inch). The amount of hydrogen was determined by using the same model fitted with a thermal conductivity detector and a molecular sieve (5 A, 60 – 80 mesh, 1/8 inch, 2.5 m).

Quantitative results were obtained by relating the peak heights to a standard calibration curve, and by employing a Hewlett Packard HP 3285 A integrator system.

The inhibitors added were dissolved in methanol except for atrazine and simazine which were dissolved in dimethylsulfoxide. The volume of solvent added never exceeded 20 – 50 µl per 12 ml. The influence of solvent was negligible. DCMU was purchased from Riedel-de Haen (Hannover). Metribuzin was a gift rom Bayer AG (Leverkusen), atrazine and simazine from Ciba-Geigy, Basle. All other reagents were of highest purity from Merck AG (Darmstadt).

Results

Hydrogen evolution in *Azotobacter* and in *Anabaena*, respectively, can be increased considerably by adding acetylene and carbon monoxide [6, 12]. This effect is due to inhibition of uptake hydrogenase accompanied by an only partial inhibition of nitrogenase. Since the oxygen-dependent uptake

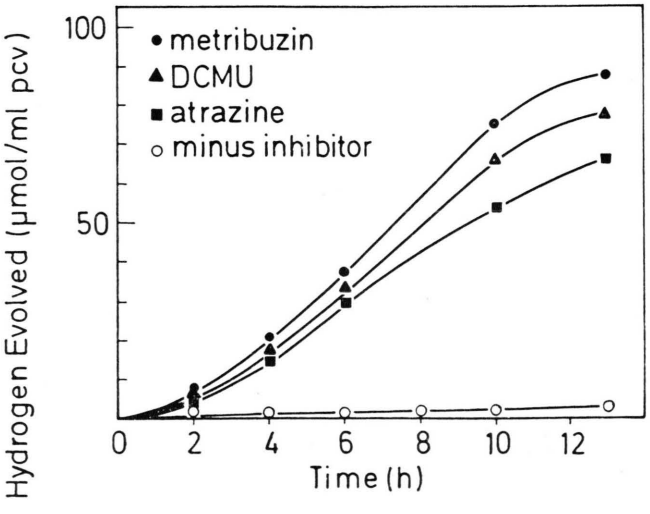

Fig. 1. Photoproduction of hydrogen in the presence of inhibitors. Reaction was carried out in 38 ml flasks under argon with 12 ml (cell density equivalent to 12 µl pcv) of *Nostoc muscorum* filaments grown under air nitrogen/CO_2. Illumination 20 J/m² × sec, temperature 26 °C. Inhibitor 2×10^{-6} M. ● Metribuzin, ▲ DCMU, ■ atrazine, ○ minus inhibitor.

hydrogenase seems to be present in *Nostoc* [8], our approach to increased hydrogen production was to suppress continuous regeneration of photosynthetic oxygen by applying inhibitors of photosystem II. Fig. 1 demonstrates that hydrogen photoproduction is increased by DCMU, metribuzin, and atrazine from values smaller than 1 μmol to over 8 μmol H_2/ml pcv $\times$ h, and that this activity can be maintained at a slightly reduced rate for at least 15 h. Algal material was used from the early phase of growth after induction of nitrogenase (3 – 5 days after inoculation) grown at low light intensity of 5 J/m^2 $\times$ sec, with values of both chlorophyll μg/ml and pcv μl/ml of 3 – 4. The control (minus inhibitor) shows only small amounts of H_2 being released under these conditions. Concurrent assays with the oxygen electrode demonstrated that under the same conditions rather small amounts of oxygen are evolved in the light (comp. [13] for general inhibition details) which generally do not reach the level of dark oxygen consumption in the presence of inhibitor (4×10^{-6} M). Thus, H_2 production proceeds under microaerobic conditions.

Since H_2 production in this organism is a light-dependent process, it was important to know the degree of dependence on light and to check whether there were differences in algal material grown under nitrogen/CO_2, air nitrogen/CO_2 or NO_3^-/air/CO_2. For comparison, data of uninhibited light-induced acetylene reduction are given, thus presenting maximum rates of both reactions. Table I shows that with increasing light intensity algae grown under N_2/CO_2 exhibit a dramatic increase of H_2 production and acetylene reduction in the presence of DCMU. The rates increase from 3.5 – 7 – 10 μmol H_2/ml pcv $\times$ h and similarly from 12.8 – 25.6 – 51.2 μmol acetylene reduced/ml pcv $\times$ h. Usually, rates from algae grown under air nitrogen/CO_2 are somewhat lower, especially in C_2H_2 reduction which may indicate partial inactivation of nitrogenase by the higher level of oxygen during growth as compared to the culture bubbled with N_2/CO_2 only. Maximum evolution of H_2 in both samples approaches 10 μmol/ml pcv $\times$ h. Net hydrogen evolving activity in cultures derived from nitrate cultures is absent at low light and comparatively small at medium and high light intensities.

Table I. Effect of light intensity on H_2 production and C_2H_2 reduction in *Nostoc muscorum*.

Nitrogen source and gas phase during growth	H_2 production in the presence of DCMU, light intensity in J/m^2 $\times$ sec			C_2H_2 reduction in the absence of DCMU *, light intensity in J/m^2 $\times$ sec		
	5	20	50	5	20	50
N_2	3.5	7	10.6	12.8	25.6	51.2
air nitrogen	2.1	8.5	9.1	7	14	25
NO_3^-/air	—	0.9	1.5	—	5	10

12 ml of algal filaments with a packed cell volume of 10 – 12 μl/ml culture suspension (corresponding to 10 – 20 μg Chl/ml) were incubated under argon in 38 ml flasks at 26 °C for 5 – 10 hours. Activity is given in μmol H_2 evolved or C_2H_4 formed/ml pcv $\times$ h. Rates were calculated from the straight portion of the time course (10 h). Dark rates (0.5 for H_2, 0.5 for C_2H_4) were subtracted from the light rates given. DCMU: 2 μM.

* C_2H_2 reduction at low and medium light intensity is decreased by 20% in the presence of DCMU. Maximum production of H_2 proceeds in the presence, maximum reduction of C_2H_2 in the absence of DCMU.

Acetylene reduction rates in the presence of DCMU, atrazine and metribuzin at 2×10^{-6} M were decreased by approximately 30% in all three types of algal cultures (see also Table IV).

The direct kinetic response to increased light intensity of hydrogen and oxygen levels in intact filaments originally grown under air nitrogen/CO_2 is shown in Fig. 2. In the dark, these algae produce only small amounts of H_2 (trace c). At 3 J/m^2 $\times$ sec the hydrogen evolution rate of the light sample without DCMU (trace b) is only a little smaller than the rate of the sample plus DCMU (trace a), indicating that some H_2 is consumed, whereas traces of O_2 are beyond detection (trace d). Upon increasing illumination by a factor of at least 6, the H_2 production rate of the sample minus inhibitor is decreased to < 1 μmol/ml pcv $\times$ h (trace b), whereas the DCMU sample is *increased* from 2 to 6 μmol H_2/ml pcv $\times$ h (trace a). No net oxygen evolution can be detected in the DCMU sample but a considerable O_2 evolution rate is observed in the sample minus inhibitor under these conditions (30 μmol/ml pcv $\times$ h; trace d). Thus, the time course presents an inverse relationship between H_2 evolved in inhibited and O_2 evolved in non-inhibited samples when switching to higher light intensity.

Data in Table II from experiments at medium light intensity in all 3 types of algal cultures demonstrate that *Nostoc* may well produce substantial amounts of H_2 *in vivo* provided the oxygen level is kept low. In algae grown under pure N_2/CO_2 there is considerable hydrogen production of 3.8 μmol/ml

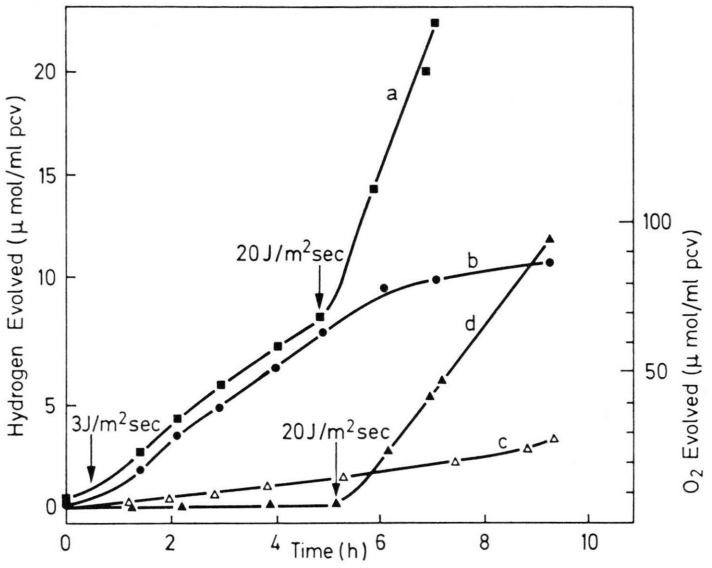

Fig. 2. Light dependence of photoproduction of hydrogen in *Nostoc muscorum* grown under air nitrogen/CO_2. Conditions as in Fig. 1. Gas phase argon. Traces a—d: a) ■ H_2 production plus DCMU (2 μM), light; b) ● H_2 production minus inhibitor, light; c) △ H_2 production minus inhibitor, dark; d) ▲ O_2 production minus inhibitor, light.

Table II. H_2 production and C_2H_2 reduction by *Nostoc muscorum* grown under varying conditions.

Nitrogen source and gas phase for growth	Hydrogen production Additions			Acetylene reduction Additions		
	None	Metribuzin	Cl-CCP	None	Metribuzin	Cl-CCP
Nitrogen/5% CO_2	3.8	9	0.2	34	26	0
Air nitrogen/5% CO_2	0.25	10.6	0	14	12.6	0
NO_3^-/air/5% CO_2	1.0	1.3	0	5	4.2	0

Reaction conditions and rates obtained from time course measurements as given in Fig. 1, inhibitors (metribuzin 2 μM, Cl-CCP 20 μM) added before incubation. Dark rate subtracted from the data obtained.

pcv × h with a fully intact photosystem II. Even algal material grown on nitrate produces small, though measurable amounts of H_2. Addition of the uncoupler Cl-CCP almost completely abolishes this production in contrast to other reports [8]. This uncoupling effect is strikingly similar in acetylene reduction regardless of the presence of photosystem II inhibitor (Table II). Using other uncouplers, such as FCCP or salicylaldoxime (data not shown) similar effects were found. CO as inhibitor of hydrogenase [6] at 0.15% (v/v) resulted only in partial inhibition of hydrogen production (up to 30%) in experiments carried out over 5 – 6 hours (data not shown) which again is in contrast to other reports [8]. Since results presented above suggested a similar pattern of inhibition for both H_2 production and C_2H_2 reduction we investigated the concentration dependence of inhibition using metribuzin (Fig. 3). H_2 production exhibits a maximum with metribuzin at 2 – 5 μM but is inhibited by higher concentrations. On the other hand acetylene reduction is gradually inhibited with metribuzin by all concentrations used, almost approaching 50% inhibition.

Since oxygen may have an effect on both nitrogenase and uptake hydrogenase reactions we checked the balance of H_2 at optimum hydrogen evolving capacity in the presence of DCMU in a series of experiments with algal cultures grown on N_2/CO_2 by

Table III. Light-induced H_2 evolution (+) or uptake (−) in *Nostoc muscorum*.

Conditions	μmol H_2/ml pcv × h
Light	+5.9
Light + H_2 (1%)	+6.54
Light + DCMU	+7.6
Light + DCMU + H_2 (1%)	+6.8
Light + DCMU + H_2 (1%) + O_2 (2%)	−5.3
Light + DCMU + H_2 (1%) + O_2 (10%)	−5.9
Dark	+1.1

Reaction conditions as in Table I. Additions were made before incubation.

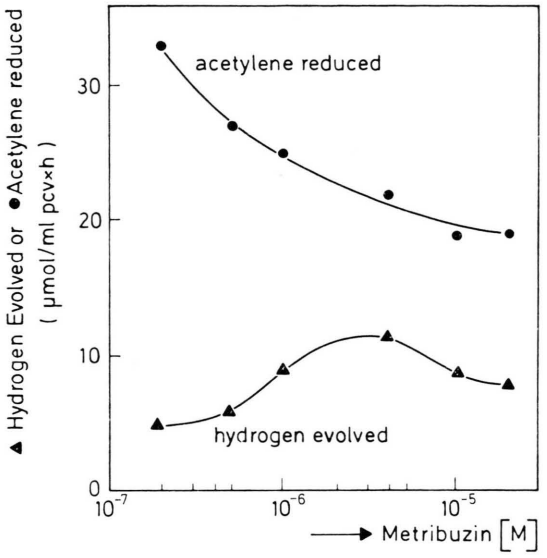

Fig. 3. Effect of metribuzin concentration on light-induced hydrogen production and acetylene reduction in *Nostoc muscorum* filaments grown under N_2/CO_2. Light intensity 15 J/m²×sec. Other conditions as in Fig. 1. Rates calculated from the straight portion of the respective time course as shown in Fig. 1 (3rd to 7th hour), dark value subtracted. ● Acetylene reduced, ▲ hydrogen evolved.

adding H_2 and/or O_2 (Table III). In the control (line 1) H_2 is produced in substantial amounts, but addition of 1% of hydrogen results in H_2 production over the amount present at the start of the experiment (line 2). The rate with DCMU (10^{-6} M) is not significantly altered by further addition of H_2 (line 4). On the other hand O_2 in increasing amounts diminishes the amounts of H_2 present (lines 5, 6), also indicating that 2% O_2 are sufficient to consume any H_2 available. These data demonstrate an uptake hydrogenase in active cells. The optimum curve in Fig. 3 can thus be explained by the inhibition of an uptake hydrogenase which by increasing amounts of electron transport inhibitor is deprived of its substrate O_2.

In order to further resolve the question of whether nitrogenase or hydrogenase is responsible for H_2 production we applied a number of inhibitors for both enzymes in intact filaments of *Nostoc* with high H_2-evolving activities. Data in Table IV demonstrate a drastic increase of H_2 production by other well known photosystem-II inhibitors, with a slightly smaller increase by bentazon. DBMIB on the other hand inhibits H_2 production (40%) which is reduced to <5% by all uncouplers employed which corresponds to the results of acetylene reduction. CN^- and CO (not shown) only partially block H_2 evolution, whereas N_3^- completely inhibits this reaction. The rate still remaining with CN^- can be attributed to the well known cyanide-resistant respiration in blue-green algae. As expected, acetylene reduction is only partially affected by photosystem-II inhibitors, but to a greater degree by DBMIB, uncouplers and alternate substrates for nitrogenase, such as cyanide or azide (Table IV, column 2). These inhibition studies thus support the concept of nitrogenase-catalyzed hydrogen evolution.

Table IV. Effect of inhibitors on the rates of light-induced H_2 evolution and acetylene reduction in *Nostoc muscorum* grown under nitrogen.

Inhibitor	H_2 evolution % activity	C_2H_2 reduction % activity
Control, (−) inhibitor	100	100
Atrazine (2×10^{-6} M)	145	65
Bentazo (2×10^{-5} M)	140	81
DCMU (2×10^{-6} M)	190	76
Metribuzin (2×10^{-6} M)	210	71
Simazine (2×10^{-6} M)	185	100
DBMIB (5×10^{-6} M)	40	7
FCCP (10^{-5} M)	5	5
Cl-CCP (2×10^{-5} M)	5	5
KCN (2×10^{-4} M)	35	37
NaN_3 (10^{-3} M)	0	38

Experiments were performed with 12 ml algal suspension of 12 μl pcv/ml suspension in 38 ml flasks. Gas phase argon, 26 °C, illumination 20 J/m²×sec. Rates determined after 5–6 h. Control rates: 5 μmol H_2 evolved/ml pcv×h, 12 μmol C_2H_4 formed/ml pcv×h; dark rate subtracted.

Discussion

Considering the output of H_2 in green algae and cyanobacteria two major problems have been encountered: low rates of light-induced H_2 production or even its absence in whole cells and the absolute dependence of H_2 production with cell fragments on external supply of artificial reductant and mediator.

Several authors have demonstrated the presence of a highly active *uptake* hydrogenase in some cyanobacteria which may at least in part account for low net H_2 production (Peterson and Burris [4]; Tel Or *et al.* [8]; Bothe *et al.* [14]). Whereas some authors have used CO/C_2H_2 to suppress uptake hydrogenase [14, 15], our approach to increase net H_2 output was to exclude possible alternate electron acceptors other than H^+ in intact cells, such as CO_2, NO_3^-, N_2 (Mitsui, personal communication) and to remove O_2 as a substrate for the uptake hydrogenase

reaction by applying inhibitors of photosynthetic electron transport of the DCMU type, preventing generation of oxygen. In this way, undesirable side effects on general cell metabolism by relatively high concentrations of C_2H_2 and/or CO can be avoided. In accordance with earlier observations that there is only small enhancement of acetylene reduction by photosystem II (see [16] for review), the drastic increase in H_2 production upon application of photosynthetic electron transport inhibitors as demonstrated in this paper shows that hydrogen formation is by and large independent of photosystem II. Thus a direct coupling to H_2O oxidation appears to be unlikely. Although optimum concentration of metribuzin (3×10^{-6} M) for H_2 generation does suppress evolution of O_2 as measured in the oxygen electrode under the same conditions, the observed residual rate (1.5 μmol O_2 per mg Chl $\times$ h) is by a factor of 10 smaller than the concurrent H_2 evolution. This consideration and the observation of a steady high production of H_2 by intact cells ranging between 10 and 15 μmol/ml pcv $\times$ h over a period up to 15 h rather suggest that possibly organic donors are fed into photosystem I depleting the cells of endogenous reserves, which is quite in agreement with other investigators [17, 18].

Strict dependence on illumination power of H_2 production is another prominent feature of intact *Nostoc* cells (Fig. 2, Table I). The parallelism of H_2 production in the presence of inhibitor and acetylene reduction seems to be striking. Furthermore, in samples minus photosystem-II inhibitor, higher light intensities lead to a decline of net H_2 production, while O_2 output still increases (not shown) which is accompanied by a bleaching of the cell material. Cells originally grown under low light thus seem to deteriorate under a sudden increase of the oxygen partial pressure. NO_3^--grown cell material tested in the absence of inhibitor can sustain light intensities up to 20 J/m^2 $\times$ sec without showing major damage to the pigment system or a decrease of the H_2-evolution rate. We observed a higher phycobilin content in NO_3^--grown cells as compared to N_2-grown material. This may explain the greater stability of hydrogen production when nitrate-grown cells are exposed to higher light intensities.

The markedly different response of acetylene reduction and hydrogen evolution towards rising concentrations of metribuzin (Fig. 3) raises two points. It can be assumed that the presence of an electron transport inhibitor such as DCMU [18] prevents the build-up of (an) endogenous donor(s) for the nitrogenase. This may explain the decrease of acetylene reduction with rising concentration of metribuzin, the same holds for hydrogen production. On the other hand, the optimum curve found for hydrogen produced apparently represents a superimposed effect of the oxygen evolved by photosystem II. Metribuzin will prevent the oxygen-hydrogen reaction of the uptake hydrogenase by depriving the reaction of oxygen. However, both hydrogen production and acetylene reduction require ATP. It was reported that nitrogenase obtains ATP by cyclic photophosphorylation mediated by photosystem I in the heterocysts [20]. Arnon and Chain [19] demonstrated that increasing DCMU concentrations inhibit cyclic photophosphorylation in photosystem I by totally restricting the small linear electron flow necessary for cyclic photophosphorylation thus unpoising the system. Therefore, besides the exhaustion of endogenous donor(s) the decreased activity of the nitrogenase reactions may be due to the reduced supply of ATP.

Although other authors [8] could not detect H_2 evolution in intact filaments of Nostoc they found rates ranging from 0.5 – 5 μmol H_2 evolved/mg Chl $\times$ h in cell fragments and broken heterocyst. Our results differ in two ways: Firstly, nitrogenase activity of intact cell material was an order of magnitude higher, secondly, hydrogen is evolved without adding inhibitor by cells grown under N_2/CO_2 (3.8 μmol/ml pcv $\times$ h) and air nitrogen/CO_2 (0.25 μmol/ml pcv $\times$ h). By addition of a photosystem-II inhibitor H_2 output can be increased by a factor up to 40. Carbon monoxide at 0.15% exerts an inhibition of only 25% for the initial 4 hours. The uncouplers FCCP or Cl-CCP are effective regardless of the presence of an electron transport inhibitor. Moreover, it should be noted that in the presence of a nitrogen atmosphere only minimum rates of hydrogen evolution ($\ll$ 1 μmol/ml pcv $\times$ h) can be observed, and in the presence of acetylene it is virtually absent. On the assumption that a distinction is possible between hydrogenase- and nitrogenase-catalyzed H_2 production [4], by ATP dependence, C_2H_2 sensitivity and a relatively small effect of CO for nitrogenase, our data rather support the findings of Bothe *et al.* [6] and Weissmann and Benemann [9] that H_2 production in *Nostoc* is largely due to nitrogenase.

In contrast to the effects of atrazine, simazine and metribuzin DBMIB inhibits acetylene reduction to more than 90% and hydrogen evolution only partially. In agreement with other investigators [17, 20] we suggest that for both reactions electrons are introduced at plastoquinone by (endogenous) organic donors. However, we cannot explain yet why acetylene reduction is more strongly inhibited by DBMIB than hydrogen production. Whereas inhibition of acetylene reduction by both azide and CN^- give about the same value, inhibition of hydrogen production by azide and cyanide is different. The partial inhibition of H_2 production by CN^- (65%) can be explained by the blocking of uptake hydrogenase.

Bothe *et al.* [6] reported a substantial increase in H_2 evolution by application of CO/C_2H_2 (see also [15]). Although *Nostoc muscorum* exhibits H_2 uptake properties, our material did not show an increase of H_2 evolution over the control (under argon) in the presence of C_2H_2/CO. This discrepancy may stem from different species, from growth conditions or different pretreatment of the alga or from other factors which will be subject to further study.

The results of this investigation open up an alternative for biological large-scale production of H_2 from cyanobacteria: Instead of producing oxygen and hydrogen simultaneously, both processes, which have been proved to interfere with one another on the cellular level, may be separated. The cells may accumulate large endogenous reserves during a period of photoautotrophic growth, after which the culture is transferred to nitrogenase-inducing conditions. Then, switching on production of hydrogen under non-growing conditions can be initiated by application of suitable electron transport inhibitors.

This study was conducted within the "Energieforschungszentrum" of this University. The authors thank Miss Silvia Binder for accurately taking care of the algae cultivation and Mr. G. Bookjans for helpful discussions.

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