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Effect of CO_2, O_2, DCMU, FCCP, and DL-glyceraldehyde on the nitrogenase activity of *Synechococcus* RF-1

Nathanaël Grobbelaar[1], Wing-Ming Chou, Tan-Chi Huang
Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 115, Republic of China

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Abstract. Elevated atmospheric CO_2 concentrations drastically inhibit nitrogenase activity of the unicellular *Synechococcus* RF-1 but stimulate photosynthetic CO_2 assimilation. The inhibitory effect on nitrogenase activity is stronger in the light than in the dark. During three hours, 1% CO_2 in air can reduce nitrogenase activity in the light by about 50% compared to that in unenriched air. The inhibitory effect of elevated CO_2 concentrations on nitrogenase activity persists for many hours after the organism has been returned to air not enriched with CO_2. The nitrogenase activity of heterocystous cyanobacteria, generally, does not appear to be affected by 5% CO_2 in the air. DCMU strongly enhanced nitrogenase activity and inhibited the assimilation of CO_2 by *Synechococcus* RF-1 in the light, and elevated atmospheric O_2 concentrations reduced the nitrogenase activity, especially in the dark. DL-glyceraldehyde at a concentration of 19.4 mM strongly inhibited nitrogenase activity, dark respiration, and photosynthesis. FCCP had no effect on dark respiration but depressed nitrogenase activity and photosynthesis of *Synechococcus* RF-1. The inhibitory effect of FCCP on nitrogenase activity was stronger in the dark than in the light.

Key words: Carbon dioxide; Nitrogenase activity; Photorespiration; Photosynthesis; Respiration; *Synechococcus*.

Introduction

Synechococcus RF-1 is a unicellular nitrogen-fixing cyanobacterium (Huang and Chow, 1986). When exposed to L/D, it fixes nitrogen almost exclusively during the dark periods. This rhythmic pattern is maintained for several days after the culture is transferred to suitable diurnal L/L conditions (Grobbelaar *et al.*, 1986). When it is maintained in L/L, the culture eventually fixes nitrogen continuously at an unpredictable and relatively low rate, (Huang and Chow, 1986; Chou *et al.*, 1989).

CO_2 at a concentration of 5% (v/v) significantly reduces nitrogenase activity of *Synechococcus* RF-1 (Grobbelaar *et al.*, 1987a). Although air, enriched with 5% CO_2 is commonly bubbled through cyanobacterial cultures to enhance their growth (Smith and Evans, 1971), Gallon *et al.* (1972) reported that attempts to grow *Gloeocapsa* in the presence of 5% CO_2 failed. The latter authors also found that CO_2 concentrations greater than 0.01 atm. (1% v/v) inhibited nitrogenase preparations and that this could not be ascribed to a pH effect.

This paper describes several experiments which were conducted in an attempt to unravel the inhibitory effect of elevated CO_2 concentrations on the nitrogenase activity of *Synechococcus* RF-1 cultures.

Materials and Methods

Organisms and Growth Conditions

[1]Permanent address: Department of Botany, University of Pretoria, Pretoria 0002, Republic of South Africa.

Abbreviations: L/L = Continuous light; L/D = light/dark.

Synechococcus RF-1 (PCC 8801) was cultured, and the cell density of the cultures was determined as described previously (Huang *et al.*, 1990) in either L/L or 12h L / 12h D (L/D). White light of about 35 $\mu E \cdot m^{-2} \cdot s^{-1}$ from fluorescent lamps (Toshiba FL20D/18, Taiwan Fluorescent Lamp Co., Taiwan) was used for illumination. The dark periods of the L/D cycles were always from 08h00 to 20h00. When samples of a L/D culture were used, they were always taken during the dark phase and incubated in either light or darkness. If a culture was illuminated during its dark phase, it was exposed to light during the whole dark phase even if the nitrogenase activity was monitored only at the middle of the dark phase. The temperature in all cases was 28°C.

The axenic filamentous cyanobacteria listed in Table 11 were all drawn from the axenic collection of one of the authors (T.-C. Huang). The *Anabaena* CH-3 was originally obtained from Dr. Pei-Chung Chen, Department of Botany, National Chung Hsing University, Taichung, Taiwan, Republic of China. The *Mastigocladus laminosus* was from Dr. D. L. Balkwill, Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306, U.S.A.. The *Nostoc* WR-103 culture was isolated from a cycad coralloid root (Grobbelaar *et al.*, 1987b). *Anabaena* HA-101, *Calothrix* HC-101 and HC-605, *Cylindrospermum* A and 101, *Hapalosiphon* D and 101, *Nostoc* HN-101, and *Scytonema* A and D were all isolated by one of the authors (T.-C. Huang). All the filamentous cyanobacteria were culture in L/L under the same conditions and by the same methods used for the *Synechococcus* RF-1 culture.

Nitrogenase Activity

The acetylene reduction method (Hardy *et al.*, 1973) was used. To 0.5 ml of culture in a 14 ml capped test tube, 1.4 ml commercial acetylene was added. Samples (0.2 ml) of the incubation gas mixture were analyzed for ethylene at 60°C using a Shimadzu 3BF gas chromatograph fitted with a flame ionization detector and a 2 m stainless steel column (internal diameter 3 mm) packed with Poropak N (80-100 mesh, Waters Associates Inc., U.S.A.).

When the air in the incubation test tubes was enriched with either CO_2 or O_2, the calculated volume of the relevant gas was introduced into the tube by means of a syringe. After mixing the gases, the same volume of gas mixture was withdrawn from the test tube before acetylene was added.

Dark Respiration and Net Photosynthesis

A 1.5 ml culture sample was placed in the chamber of a Clark oxygen electrode (Rank Brothers, Bottisham, Cambridge, England) to measure the rate of oxygen uptake in the dark and production in the light. Unless stated otherwise, air was always bubbled through the culture sample for two minutes before it was used.

DCMU, FCCP and DL-Glyceraldehyde

Unless stated otherwise, the following stock solutions were used at the respective dilutions: 2 μl of a 5×10^{-2} mol $\cdot 1^{-1}$ 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU) solution in 75% ethanol per ml culture; 3 μl of a 0.1 mol $\cdot 1^{-1}$ solution of carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) in ethanol per ml culture; 62.5 μl of a 0.33 mol $\cdot 1^{-1}$ DL-glyceraldehyde solution in water per ml culture.

$^{14}CO_2$-Experiment

Eight 2.5 ml glass vials containing 0.3 ml *Synechococcus* RF-1 culture were placed on each of four glass plates. Four of the vials on each plate contained L/L cultures, and the other four vials contained L/D cultures. DCMU was added to two of the L/L and two of the L/D vials on each plate, and the same volume of 75% ethanol was added to the other vials. The vials of each plate were covered with a glass bell jar of about 650 ml capacity. A tube with quartz sand was also placed underneath each bell jar to ensure that the final gas volume enclosed by the bell jar was exactly 619 ml.

The bell jars were sealed with vaseline and held firmly in position by two rubber bands. Two of the bell jars were illuminated and the other two were kept in darkness. To one of the bell jars in the light and the dark, 12.6 ml CO_2 was added through a rubber septum in the injection port. After the CO_2 mixed with the air, 12.6 ml of the gas mixture was withdrawn from the bell jar.

To 0.021g $Ba^{14}CO_3$ (specific activity: 8.18 MBq $\cdot$ mg^{-1}, Amersham International plc) in a vial with a gas volume of 12.225 ml, 3 ml 10% (v/v) lactic acid was added by syringe. After the evolution of $^{14}CO_2$ subsided, 1 ml of the $^{14}CO_2$-enriched air from the vial was injected into each of the four bell jars.

After three hours the vials with the *Synechococcus* cultures were removed from the bell jars and a 0.1 ml sample of each vial was vacuum filtered through a 0.45 μm nitrocellulose membrane (Millipore). The cells on the membrane were washed four times with 0.1 ml water. The membrane with the cells was suspended in a 10 ml Merck 15386 scintillation cocktail and the radioactivity of the sample determined with a scintillation counter (Beckman LS 3801).

Effect of 5% CO_2 on the Nitrogenase Activity of Heterocystous Cyanobacteria

Five milliliters of a relatively dilute suspension of the heterocystous cyanobacterium was placed in each of two matched 60 ml conical flasks which were sealed with rubber caps. Three milliliters air and 5 ml acetylene were added to one flask, and 3 ml CO_2 and 5 ml acetylene were added to the other flask. The flasks were incubated in light at 28°C.

Results

Nitrogenase Activity of ***Synechococcus***

Effect of CO_2. The nitrogenase activity of a L/L culture (Table 1) was drastically reduced by CO_2 concentrations of 1% and higher in the light. In the dark, 4% and 5% CO_2 also inhibited nitrogenase activity whereas 1% to 3% CO_2 had a stimulatory effect. CO_2 concentrations of 10% to 30% all had drastic inhibitory effects on nitrogenase activity of L/D cultures both in the light and the dark, although the effects in the light were much stronger than in the dark (Result not shown). In all cases, nitrogenase activity increased with time of incubation as described previously (Grobbelaar and Huang, 1992). The inhibitory effect of an elevated

Table 1. *The effect of CO_2 concentration on the nitrogenase activity of a L/L* ***Synechcococcus*** *RF-1 culture in light and dark*

Each figure is the mean of three values.

Incub. period (h)	C_2H_2 reduced (nmoles • 10^{-8} cells) in the presence of the following % CO_2 (v/v) in:											
	Light						Dark					
	0[a]	1	2	3	4	5	0[a]	1	2	3	4	5
1	10	9	8	7	5	3	8	8	9	8	7	9
3	31	16	16	11	4	3	35	47	48	42	31	28
7	109	32	30	15	5	2	145	217	221	200	127	92

[a]Unaltered air containing about 0.03% CO_2 was used.

Table 2. *Effect of a 5% CO_2 pretreatment on the nitrogenase activity of a L/L* ***Synechococcus*** *RF-1 culture in the light and the dark*

Each figure is the mean of four values.

Time (h)	Acetylene reduced (nmoles • 10^{-8} cells) in			
	Light		Dark	
	Pretreated in light with		Pretreated in dark with	
	Air	5% CO_2	Air	5% CO_2
	After 1 h preincubation			
1	21	1	31	28
5	259	70	520	493
	After 4 h preincubation			
1	37	3	77	46
5	256	84	517	450

Table 3. *The effect of CO_2 and DCMU on the nitrogenase activity on* ***Synechococcus*** *RF-1*

A L/L and a L/D culture were each tested in the light and the dark. The L/D culture was tested in the light and the dark during the same dark phase. Air enriched with 2% (v/v) CO_2 and 2 μl of a 5 × 10^{-2} M DCMU solution in 75% ethanol was used per ml culture in some treatments. Each figure is the mean of three values.

Incub. period (h)	Acetylene reduced (nmoles • 10^{-8} cells) in							
	Light				Dark			
	Air		2% CO_2		Air		2% CO_2	
	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU
	L/L CULTURE							
1	68	78	59	74	41	52	48	55
8	900	1645	446	1566	984	1188	1170	1136
	L/D CULTURE							
1	0	3	1	3	15	17	16	15
8	1255	1731	413	1343	660	716	698	717

CO_2 concentration on nitrogenase activity of a L/L culture in the light persisted for several hours after the culture was returned to air not enriched with CO_2 (Table 2). When the experiment was carried out in the dark, the elevated CO_2 pretreatment did not have a significant effect on the subsequent nitrogenase activity of the culture.

DCMU stimulated nitrogenase activity of both L/L and L/D cultures in the light (Table 3). The stimulatory effect of DCMU was much stronger in the presence of an elevated CO_2 concentration. In the dark, DCMU did not have a significant effect on nitrogenase activity of either culture.

Oxygen concentrations in excess of 20% drasically inhibited nitrogenase activity of a L/D culture both in the light and the dark (Table 4). The deleterious effect was much stronger in the dark than in the light. In the light (Table 5), the stimulatory effect of DCMU on the nitrogenase activity was not significantly affected by the O_2 concentration used. In the dark, however, DCMU suppressed nitrogenase activity of L/D cultures at all O_2 concentrations tested.

DL - glyceraldehyde markedly inhibited nitrogenase activity of L/L cultures in the light (Table 6). Of the DL-glyceraldehyde concentrations tested, 19.4 mM gave the most complete inhibition during a short incubation period. Consequently, this DL-glyceraldehyde concentration was used in all later studies involving this inhibitor. It inhibited nitrogenase activity of a L/L as well as a L/D culture both in the light and the dark (Table 7).

FCCP inhibited nitrogenase activity of L/L cultures both in the light and the dark (Table 8). The effect was much more pronounced in the dark than in the light. With a L/D culture, FCCP had no effect in the light although it did inhibit nitrogenase activity in the dark.

Table 4. *The effect of enriching the air with oxygen on the nitrogenase activity of a L/D* ***Synechococcus*** *RF-1 culture*

The culture was experimented on in light and dark during the same dark phase. Each figure is the mean of three values.

Incub. period (h)	Acetylene reduced (nmoles • 10^{-8} cells) in Light at an oxygen conc. of				Dark at an oxygen conc. of			
	20%	30%	40%	50%	20%	30%	40%	50%
3	403	186	91	51	338	113	6	4
6	1365	651	247	128	646	196	17	11

Respiration and Photosynthesis of ***Synechococcus***

FCCP at concentrations up to 4×10^{-4} M did not have any effect on the dark respiration rate of a L/L culture (Table 9). It did, however, increasingly inhibit the photosynthetic rate as the concentration of FCCP was increased to 4×10^{-4} M.

DL-glyceraldehyde at a concentration of 19.4 mM inhibited both dark respiration and photosynthesis of a L/L culture (Table 9). The effect on photosynthesis was much stronger than on respiration.

Table 5. *The effect of DCMU and oxygen-enriched air on the nitrogenase activity of* ***Synechococcus*** *RF-1*

The experiment was performed on a L/L culture in light and on a L/D culture in light and dark during the dark phase. When DCMU was applied, 2 μl of a 5×10^{-2} M solution in 75% ethanol was used per ml culture. Each figure is the mean of three values.

Incub. period (h)	C_2H_2 reduced (nmoles • 10^{-8} cells) in O_2 concs. of 20%		30%		40%		50%	
	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU
L/L CULTURE IN LIGHT								
3	268	560	136	281	74	86	30	21
6	701	1435	366	848	185	362	75	118
L/D CULTURE IN LIGHT								
3	675	1278	493	678	239	324	117	121
6	2076	3772	1316	2086	484	881	171	329
L/D CULTURE IN DARK								
3	389	306	162	111	37	18	4	2
6	705	529	282	206	88	45	8	0

Table 6. *The effect of different DL-glyceraldehyde concentrations on the nitrogenase activity of a L/L* ***Synechococcus*** *RF-1 culture in the light*

Each figure is the mean of two values.

Incub. period (h)	Acetylene reduced (nmoles • 10^{-8} cells) in presence of the following DL-glyceraldehyde concentrations (mM)			
	0.00	4.85	9.70	19.40
2	67	37	14	4
4	152	55	8	0

Table 7. *The effect of 19.4 mM DL-glyceraldehyde (Gald) on the nitrogenase activity of a L/L and a L/D* ***Synechococcus*** *RF-1 culture in the light and the dark. The L/D culture was treated in light and dark during the same dark phase*

Each figure is the mean of two values.

Incub. period	Acetylene reduced (nmoles • 10^{-8} cells) in Light		Dark	
(h)	-Gald	+Gald	-Gald	+Gald
	L/L CULTURE			
2	91	39	94	6
4	251	44	328	5
	L/D CULTURE			
2	140	17	345	29
4	597	21	797	21

Table 8. *The effect of FCCP on the nitrogenase activity of* ***Synechococcus*** *RF-1. A L/L and a L/D culture were each tested in the light and in the dark. The L/D culture was tested in the light and the dark during the same dark phase. Three µl of a 0.1 M FCCP solution in ethanol was used per ml culture*

Each figure is the mean of two values.

Incub. period	Acetylene reduced (nmoles • 10^{-8} cells) in Light		Dark	
(h)	−FCCP	+FCCP	−FCCP	+FCCP
	L/L CULTURE			
2	75	57	91	39
4	223	158	329	43
	L/D CULTURE			
2	173	171	355	249
4	662	600	824	489

Table 9. *The effect of FCCP and DL-glyceraldehyde (Gald) on the dark respiration rate and photosynthetic rate of a L/L* ***Synechococcus*** *RF-1 culture. The FCCP (0.1 M solution in ethanol) and Gald (0.33 M solution) were added to the culture samples immediately before recordings were made*

Each figure is the mean of two values.

Treatment	nmole O_2 • $(10^8$ cells$)^{-1}$ • min^{-1}	
	taken up in dark	produced in light (net)
	EXPERIMENT 1	
Control	6.9	34.1
+ 5 × 10^{-5} M FCCP[a]	6.6	20.3
+ 1 × 10^{-4} M FCCP[a]	7.0	15.8
+ 2 × 10^{-4} M FCCP[a]	7.2	6.7
+ 4 × 10^{-4} M FCCP[a]	6.8	4.0
+ 4 µl ethanol	7.0	32.3
	EXPERIMENT 2	
Control	10.8	26.9
+ 19.4 mM Gald	6.2	1.4

[a] The FCCP added was dissolved in 4 µl ethanol.

$^{14}CO_2$ Assimilation

In the absence of DCMU increasing the CO_2 concentration from 0.056% to 2.047% more or less doubled the rate of CO_2 assimilation in the light by both the L/L and L/D cultures (Table 10). In the presence of DCMU, only trace amounts of CO_2 were assimilated by both cultures in the light. In the dark, virtually no CO_2 was assimilated by either culture in either the absence or presence of DCMU.

Effect of 5% CO_2 on Nitrogenase Activity of Heterocystous Cyanobacteria

The nitrogenase activity of only 2 of the 13 heterocystous cyanobacteria tested was significantly affected by increasing the CO_2 concentration to 5% (Table 11). The nitrogenase activity of the *Cylindrospermum* A culture was boosted by about 22% but the activity of the *Scytonema* A culture was suppressed by almost 42%.

Discussion

Biological nitrogen fixation and photosynthetic CO_2 assimilation both require ATP and reducing

Table 10. *The effect of CO_2 concentration and the presence of 0.1 mM DCMU in the light and dark on the assimilation of CO_2 by a L/L and a L/D culture of* ***Synechococcus*** *RF-1. The light and dark treatments were applied to the L/D culture during the same dark phase*

Each figure is the mean of two values.

Culture	Relative amount of CO_2 assimilated during 3 h in							
	Light with air containing				Dark with air containing			
	0.056% CO_2		2.047% CO_2		0.056% CO_2		2.047% CO_2	
	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU	−DCMU	+DCMU
L/L	1018	12	2380	25	8	7	24	21
L/D	936	16	1810	30	7	6	26	14

Table 11. *The effect of 5% CO_2 in the air, on the nitrogenase activity of 13 cyanobacterial isolates in L/L. The relative nitrogenase activity was measured every half hour for four hours and the mean of the eight rates was calculated*

Cyanobacterial isolate	Relative amount of C_2H_2 reduced per hour (GC reading / 0.2 ml gas sample injected)	
	Unenriched air	CO_2-enriched air
Anabaena CH-3	24	23
Anabaena HA-101	35	39
Calothrix HC-101	38	38
Calothrix HC-605	55	56
Cylindrospermum A	59	72
Cylindrospermum 101	30	34
Hapalosiphon D	34	33
Hapalosiphon 101	16	17
Mastigocladus laminosus	26	24
Nostoc HN-101	19	22
Nostoc WR-103	35	33
Scytonema A	53	31
Scytonema B	31	34

power. It is therefore conceivable that in the light these two processes may compete for at least whatever ATP is available in the cell. Increasing the CO_2 concentration has been shown to increase the rate of CO_2 assimilation (Table 10) and to significantly decrease nitrogenase activity of *Synechococcus* RF-1 (Table 1) in the light. These results therefore support the hypothesis that the two processes compete for available ATP in the cell. DCMU which inhibits photosynthetic CO_2 assimilation by blocking photosystem II (Codd and Cossar, 1978) should have the reverse effect on the nitrogenase activity of *Synechococcus* RF-1 in the light. This was indeed found to be the case (Tables 3 & 5). Because DCMU does not prevent ATP formation by photosystem I of the light phase of photosynthesis, this source of ATP would be available for the nitrogen-fixing process without competition from the photosynthetic CO_2 assimilation process.

The protracted after-effect of a 5% CO_2 treatment in the light on nitrogenase activity of *Synechococcus* RF-1 (Table 2) is, however, difficult to reconcile with the explanation above. Indeed, the results seem to suggest that nitrogenase is irreversibly inactivated by exposure to elevated CO_2 levels.

Because an elevated CO_2 concentration has been shown to stimulate the photosynthesis of *Synechococcus* RF-1, an oxygen-generating process, and because oxygen inhibits nitrogenase, it is conceivable that the inhibitory effect of an elevated CO_2 concentration on the nitrogenase activity of *Synechococcus* RF-1 can be explained by the following alternative hypothesis: In the light the elevated CO_2 concentration stimulates oxygen

production in the cells which inhibits its nitrogenase activity. Because DCMU prevents photosynthetic oxygen production, it will counteract the detrimental effect of photosynthetic CO_2 assimilation on nitrogenase activity. Therefore, this stimulatory effect of DCMU and the deleterious effect of an elevated CO_2 concentration should manifest itself only in the light. This was indeed found to be the case. To test this hypothesis, it is necessary to establish that loss of nitrogenase activity in the presence of an elevated CO_2 concentration in the light is, in fact, due to an irreversible inactivation of nitrogenase. Also it must be shown that the protracted after-effect of a CO_2 treatment in the light (Table 2) is consistent with the time required by the cell to synthesize new nitrogenase.

In an earlier paper, Grobbelaar *et al.* (1987a) claimed that DCMU did not have a significant effect on nitrogenase activity of *Synechococcus* RF-1 in the light or the dark. In those experiments the cultures were incubated for one hour only. It has become evident that the stimulatory effect of DCMU in the light takes some time to become significant (Table 3). Working with *Gloeothece* sp. which is quite similar to *Synechococcus* RF-1, Maryan *et al.* (1986) found that at saturating light intensities DCMU stimulated nitrogenase activity twofold.

Altering the O_2:CO_2 ratio of the air could influence the internal O_2 concentration of the cell and, therefore, influence the rates of photorespiration and photosynthetic CO_2 assimilation. The nitrogenase of *Synechococcus* RF-1 was found to be more resistant to elevated O_2 concentrations in the light than in the dark (Table 4). This could be due to an increased rate of photorespiration brought about by the increased ratio of O_2/CO_2.

The much stronger inhibitory effect of FCCP on nitrogenase activity in the dark than in the light (Table 8) suggests that when ATP production is uncoupled from respiration by FCCP in the light, the ATP requirement of the nitrogenase activity can largely be satisfied by ATP derived from the light phase of photosynthesis. The inhibitory effect of FCCP on net oxygen production in the light (Table 9) could be due to an inhibition of photosystem II caused by an accumulation of reduced NADP as a result of a deficiency of ATP to drive the Calvin cycle.

DL-glyceraldehyde has been reported to inhibit the Calvin cycle (Stokes and Walker, 1972; Bamberger and Avron, 1975). Therefore, it will presumably also inhibit the pentose phosphate respiratory pathway thought to be the main respiratory pathway of the cyanobacteria (Stewart, 1980; Smith, 1982). The detrimental effect of DL-glyceraldehyde on the nitrogenase activity of *Synechococcus* RF-1 both in the light and the dark (Tables 6 & 7) can, therefore, be ascribed to its inhibitory effect on respiration, apparently the main provider of ATP and reducing power for nitrogenase activity. In the light, inhibition of the Calvin cycle by DL-glyceraldehyde should, therefore, counteract the effect on the nitrogenase activity resulting from the inhibition of respiration by DL-glyceraldehyde. Some evidence for this appears to exist in the results provided in Table 7.

From the above, it is clear that additional work needs to be done to fully explain the inhibitory effect of elevated CO_2 concentrations on the nitrogenase activity of *Synechococcus* RF-1. The results in Table 11, furthermore indicate that this phenomenon is not common among the cyanobacteria although it also does not appear to be unique to *Synechococcus* RF-1.

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二氧化碳，氧氣，抑制劑 DCMU，抗偶合劑 FCCP 和甘油醛對聚球藻 RF-1 固氮活性的影響

N. Grobbelaar　周文敏　黃檀溪

中央研究院　植物研究所

提高空氣中二氧化碳的濃度明顯地抑制聚球藻 RF-1 品系的固氮活性，但能促進二氧化碳的固定作用。CO_2對固氮活性的抑制效果在照光時比黑暗時大，空氣中二氧化碳提高到 1%，三小時後照光下 RF-1 的固氮活性會減至一半。而且其抑制效果在細胞回到正常空氣中仍可持續數小時。對於具異形細胞之藍綠藻，其固氮活性通常不受 5%二氧化碳的影響。在照光下，DCMU 能顯著地提高固氮活性，抑制二氧化碳之固定。提高空氣中氧濃度會使固氮活性減小，特別是在黑暗中。19.4 mM 甘油醛強烈抑制固氮活性、暗呼吸作用和光合作用，然而 FCCP 對暗呼吸作用沒有影響，但可抑制固氮活性和光合作用。FCCP 對固氮活性的抑制效果在黑暗時比光照時大。