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INCLUSION OF GLUCOSE INTO CYANOBACTERIA GROWING MEDIA AND ITS EFFECT ON GROWTH BIOMASS, NITROGEN FIXATION ABILITY AND PIGMENTS CONTENT

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ABSTRACT

The use of glucose in the cyanobacteria culture media increased significantly the biomass production of the cyanobacteria strains in comparison with the control treatment. *Anabaena. oryzae* gave its highest biomass dry weight with the use of 6000 ppm glucose at 21-day incubation period. The highest biomass dry weight of 2.440, 2.850 and 3.530 gl^{-1} medium were corresponding to *N. calcicola, M. tenera* and *C. muscicola* at 21-day old when any of the culture medium was supplied with 6000 ppm glucose, respectively. The presence of 6000 ppm glucose in the cyanobacteria culture media (up to 15-day incubation period) achieved the highest fixed nitrogen amounts for *N. calcicola* and *M. tenera* in respective to 136.800 and 148.010 mg Nl^{-1} medium against 108.690 and 186.300 mg Nl^{-1} medium in respective to *A. oryzae* and *C. muscicola* at 21-day old. The use of glucose in the cyanobacteria culture media increased significantly both of chlorophyll a content and C-phycocyanin content for all tested algae strains.

Keywords: Inclusion, Glucose, Cyanobacteria, Biomass, Nitrogen fixation, Pigments.

INTRODUCTION

The economic and heavy use of chemical N fertilizers in agriculture is a global concern. Sustainability considerations mandate that alternative to N fertilizers must be urgently sought. Biological nitrogen fixation (BNF), a microbiological process that converts atmospheric nitrogen into plant –usable form, offers this alternative. Nitrogen – fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources.

Symbiotic systems such as legumes and *Rhizobium* can be a major source of N in most cropping systems and of *Azolla* and Anabaena can be of particular value to flooded rice crop. Nitrogen fixation by free-living microorganisms like cyanobacteria can also be important. This paper mainly deals with cyanobacteria commonly applied as nitrogen source can partially meet nitrogen requirements in rice cultivation (Bahlool *et al.*, 1992).

A number of cyanobacteria does fix atmospheric N_2 and contributes to the fertility of rice field. In 1939, De suggested their use as biofertilizer in rice production. Since then, numerous investigations have been conducted to enhance N_2-fixation in wetland rice field inoculation with cyanobacteria (Venkataraman, 1972; Roger and Klusooirya, 1980; Reddy and Roger, 1988; Roger *et al.*, 1993).

Unfortunately, the agricultural significance of cyanobacteria as biofertilizers has largely impaired by surrounded environmental factors such as salinity some other nutrient occurrence or limitation like organic compounds. Although cyanobacteria are basically photo synthetic organisms, some can also utilize added organic compounds, either by heterotrophic or photo trophic. Reports on this topics are numerous (Rippika *et al.*, 1979). In most cases, heterotrophy was tested only on a limited number of organic substances, mainly sugars such as glucose fructose, ribose, sucrose as well as glycerol (Rippika *et al.*, 1979 and Radwan and AL-Hasan, 2000).

This work is an attempt to investigate the ability of four cyanobacteria strains (*Anabaena oryzae, Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola.*) to grow glucose as carbon source in their growing culture habitat.

MATERIALS AND METHODS

Four cyanobacteria strains were kindly supplied by the Agricultural Microbiology Department, Soils, Water and Environment Institute, Giza, Egypt and being used in this study. They are namely. *Anabaena oryzae, Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola.*

Cyanobacteria strains were purified using Yeast Extract Agar medium (Oxoid, 1965) and propagated by inoculation into 500 ml conical flasks containing 100 ml sterilized BG11medium (Rippika *et al;* 1979).

Four concentration levels of glucose as 0, 2000, 4ooo and 6000 ppm were introduced to 500 ml round flasks containing 100 ml sterilized BG11 medium and inculcated with a homogenized 10 ml cyanobacteria inoculum at incubation periods of 7, 15 and 21 days under continuous white illumination (3000 Lux) at 28-32°C. Flasks were arranged in complete randomized block design in three replicates according to Gomez and Gomez (1984). At the end of each incubation period the flasks were filtered for obtaining the cyanobacteria biomass which was then subjected to determine the dry cyanobacteria biomass (oven dried at 70°C for 24 h) as g l^{-1} medium, extra and intera-cellular–N in cyanobacteria filaments and filtrate (mg N l^{-1} medium), total fixed –N (mg l^{-1} medium), chlorophyll a (µg Chlor.ml^{-1}cyanobacteriasuspension), C-phycocyanin (mg ml^{-1} cyanobacteria suspension) and C-phycocyanin / chlorophyll a ratio.

Total nitrogen in cyanobacteria filaments and \ or extra-cellular nitrogen filtrate was determined using the micro-Kjeldahl method according to Jackson (1962).

Chlorophyll a content of the filamentous cyanobacterial was estimated according to the method described by Metzener *et al.* (1965) and Chlorophyll a concentration was then calculated according the following equation:

Chlorophyll a concentration = $10.3E_{663} - 0.918E_{644}$ = µg. chlor. ml^{-1}

Phycocyanin pigment was extracted by the method of Chapman (1988). The phycocyanin concentration was then calculated according to the formula of Bryant et *al.* (1979). This formula is:

$$\text{Concentration of phycocyanin (mg ml}^{-1}) = \frac{E\ (620nm) - 0.72\ E\ (650nm)}{6.29}$$

RESULTS AND DISCUSSION

Data in Table (1) indicate the effect of different levels of glucose on the growth and nitrogen fixation of the tested cyanobacteria strains as well as their Pigments content Fig. (1).

Inclusion of glucose in the cyanobacteria culture medium obviously increased the biomass production of the tested cyanobacteria strains. *Anabaena* culture supplemented with different glucose levels of 2000, 4000 and 6000 ppm had given higher dry weight in comparison with the control treatment without glucose. The highest dry weight of *A .oryzae* (1.500 gl^{-1} medium) was achieved with use of 6000 ppm glucose at 21-day incubation period.

However, increasing both glucose concentration and incubation period led to increase the dry biomass of the tested cyanobacteria strains.

The highest dry biomass values t of other cyanobacteria strains were 2.440, 2.850 and 3.530 g l^{-1} medium corresponding to *N. calcicola*, *M. tenera* and *C. muscicola* at 21-day incubation period when any of the culture medium was supplied with 6000 ppm glucose, respectively.

The addition of glucose to the cyanobacteria culture media had stimulated the extra- and intra-cellular nitrogen production and subsequently the total fixed nitrogen amount. Same as in dry weight biomass production, increasing glucose concentration up to 6000 ppm over the control treatment had exceeded significantly the production of extra- and intra-cellular nitrogen for the tested cyanobacteria strains.

Concerning the extra-cellular nitrogen, each of *A. oryzae N. calcicola* and *C. muscicola* produced their higher extra-cellular amount at 21-day old with respective values of 20.390, 70.500 and 63.200 mg N l^{-1} medium with the use of 6000 ppm glucose in their culture media.

On the other hand, *M. tenera* gave its highest extera-cellular-N amount (59.900 mg l^{-1} medium) at 7-day old with the applying of 4000 ppm glucose. Regardless the amount of intracellular nitrogen, both *A. oryzae* and *C. muscicola* gave their highest amount at 21-day old with the use of 6000 ppm glucose. Their respective intra-nitrogen amounts were 88.30 and 123.100 mg N l^{-1} medium.

On the contrary, both of *N. calcicola* and *M. tenera* gave their maximum intra-nitrogen amounts at 15-day old with the use of different glucose levels. The respective values were 78.100 and 113.510 mg N l^{-1} medium corresponded to 4000 and 6000 ppm glucose, respectively.

Table (1): Effect of different concentrations of Glucose on dry weight, Extracellular-N, Intracellular-N and Total fixed-N of different cyanobacterial strains

strains	*Anabaena oryzae*			*Nostoc calcicola*			*Microchaete tenera*			*Cylindrospermum muscicola*		
Glucose	Period days											
ppm	7	15	21	7	15	21	7	15	21	7	15	21
	Dry weight (gl^{-1} medium)											
0	0.620	0.680	0.750	0.670	1.180	1.650	0.670	1.380	2.250	1.070	1.160	1.540
2000	0.650	0.760	0.810	1.030	1.540	2.060	1.110	1.980	2.490	1.070	1.200	1.990
4000	0.670	1.220	1.240	1.360	1.590	2.370	1.210	2.010	2.710	1.190	1.300	2.290
6000	0.710	1.320	1.500	1.430	1.740	2.440	1.910	2.800	2.850	1.530	1.680	3.530
	Extracellular-N (mg l^{-1}medium)											
0	3.150	3.770	5.390	4.300	12.200	16.200	17.300	12.300	6.600	9.400	13.200	7.000
2000	4.140	4.930	6.120	11.500	13.800	18.500	18.700	11.100	3.600	36.100	14.500	20.800
4000	8.290	5.210	8.450	27.000	34.000	40.000	59.900	15.100	12.900	26.400	28.100	46.100
6000	18.600	9.460	20.390	42.000	66.000	70.500	59.200	34.500	30.900	30.000	31.200	63.200
	Intracellular-N (mg l^{-1}medium)											
0	20.350	28.010	30.470	9.970	63.360	69.600	45.600	83.300	94.900	52.000	97.500	100.700
2000	20.800	64.700	76.900	28.400	74.400	72.540	50.300	116.100	120.300	50.500	105.500	113.400
4000	28.500	78.800	83.100	42.800	78.100	68.820	51.900	113.500	111.400	41.100	109.100	116.300
6000	28.860	81.600	88.300	39.100	70.800	61.380	52.100	113.510	112.200	66.400	112.800	123.100
	Total fixed-N (mg l^{-1}medium)											
0	23.500	31.780	35.860	14.270	75.560	85.800	62.900	95.600	101.500	61.400	110.700	107.700
2000	24.940	69.630	83.020	39.900	88.200	91.040	69.000	127.200	123.900	86.600	120.000	134.200
4000	36.790	84.010	91.550	69.800	112.100	108.820	111.800	128.600	124.300	67.500	137.200	162.400
6000	47.460	91.060	108.690	81.100	136.800	131.880	111.300	148.010	143.100	96.400	144.000	186.300

LSD values for the aforementioned parameters.

	dry weight		Extracellular-N		Intracellular-N		Total fixed-N	
	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
A	0.042	0.055	1.570	2.080	5.990	7.960	2.200	2.920
B	0.042	0.055	1.570	2.080	5.990	7.960	2.200	2.920
C	0.048	0.062	1.810	2.400	6.920	9.190	2.540	3.380
AB	0.021	0.028	0.780	1.040	2.990	3.980	1.100	1.460

A, Strain; B, concentration and C,period

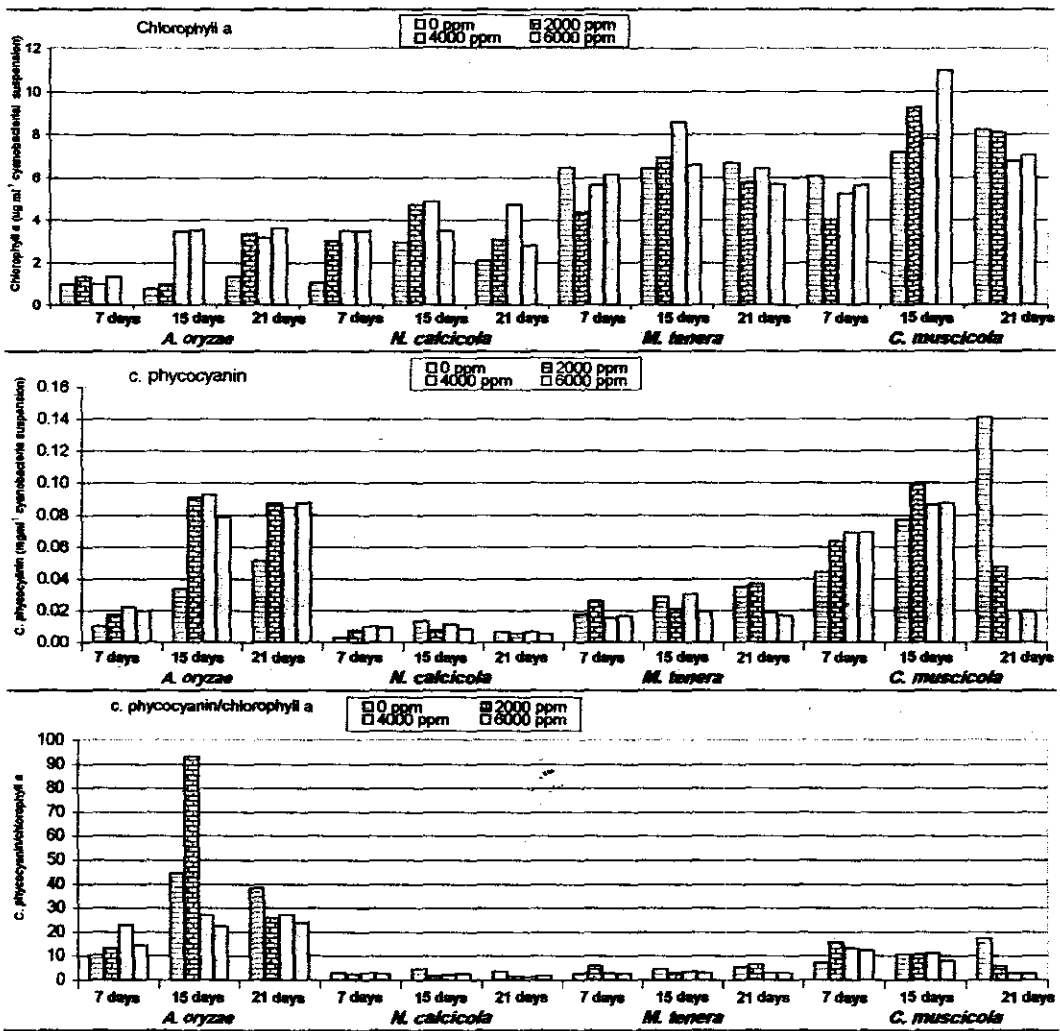

Fig.(1): Effect of different concentrations of Glucose on cyanobacteria pigments.

Relatively, the total fixed nitrogen amount had increased in response to glucose application in the cyanobacterial culture media at all levels and/or incubation periods. However, *N. calcicola* and *M. tenera* gave their highest amounts 136.800 and 198.010 mg N l^{-1} medium at 15-day old with the use of 6000 ppm glucose, respectively. While any of *A. oryzae, M. tenera* and *C. muscicola* gave their highest nitrogen fixed amounts with the use of 6000 ppm glucose at 21-day old. The corresponding values were 108.690, 148.010 and 186.300 mg N l^{-1} medium.

Figure (1) explain the effect of glucose application with different glucose levels of 2000, 4000 and 6000 ppm in the culture media of *A. oryzae, N. calcicola, M .tenera* and *C. muscicola* on their chlorophyll a and/or C-phycocyanin content as well as the C-phycocyanin/chlorophyll a ratio.

Generally, the use of glucose in the cyanobacterial culture medium had increased the amount of chlorophyll a content compared with the control treatments. Only *A. oryzae* gave its highest chlorophyll a amount (3.623 µg ml^{-1} cyanobacteria suspension) with the use of 6000 ppm glucose at 21-day incubation period, while the other tested cyanobacteria strains gave maximum chlorophyll a content at 15-day incubation period with different glucose levels application. The corresponding values were 4.870 and 8.586 µg ml^{-1} cyanobacteria suspension at 15-day old with the use of 4000 ppm glucose for *N. calcicola* and *M. tenera*, respectively. *C. muscicola* gave its highest chlorophyll a content (10.973 µg ml^{-1} cyanobacteria suspension) at 15-day old with use of 6000 ppm glucose. Irrespective of incubation period or glucose concentrations, *C. muscicola* had recorded the highest chlorophyll a content (10.973 µg ml^{-1} cyanobacteria suspension) among the tested cyanobacterial strains.

The use of glucose in *A. oryzae* culture medium resulted in increasing the C-phycocyanin content with increasing the incubation period up to 21-day. *A. oryzae* achieved its highest C-phycocyanin content (0.093 mg ml^{-1} cyanobacteria suspension) at 15-day old.

M. tenera recorded its highest C-phycocyanin content (0.030 mg ml^{-1} cyanobacteria suspension) after 15-day incubation period with the use of 4000 ppm glucose. The highest C-phycocyanin content for *C. muscicola* was (0.099 mg ml^{-1} cyanobacteria suspension) when glucose supplied to the culture medium in the rate of 2000 ppm in 15-day age old.

However, *N. calcicola* exhibited different behaviors, the other algal strains, hence the use of glucose increased the C-phycocyanin content than the control treatment only up to 7-day incubation period after which the C-phycocyanin content started to decline to be less than those of the control treatment up 21-day incubation period. The highest value of phycocyanin content for *N. calcicola* (0.013 mg ml^{-1} cyanobacteria suspension) had recorded at 15-day age old with the control treatment.

The C-phycocyanin/chlorophyll a ratio had fluctuated according to the algal content of both pigments under the effect of glucose application in their culture medium. *A. oryzae* gave the highest ratio of 93.333 at 15-day old (2000 ppm glucose) followed by *C. muscicola* (15.786 at 7-day old with 2000 ppm glucose), *M. tenera* (6.394 at 21-day with 2000 ppm glucose) and finally

N. calcicola which recorded 2.869 with the use of 4000 ppm glucose at 7-day incubation period.

Glucose supplementation up 6000 ppm to the cyanobacteria culture medium increased both biomass production and N_2-fixation over the control treatment. The highest obtained dry weight biomass were 1.500 $g1^{-1}$ medium (*A. oryzae*), 2.440 $g1^{-1}$ medium (*N. calcicola*), 2.850 g^{-1} medium. *M. tenera* and 3.530 g^{-1} medium (*C. muscicola*) all at 21 day incubation periods.

Glucose added to the culture medium of the tested cyanobacteria had also significantly increased the amount of fixed nitrogen. The highest fixed nitrogen amounts were for *C. musicicola* (186.300 mg Nl^{-1} medium) followed by 143.100 mg Nl^{-1} medium *(M. tenera),* 136.8 mg Nl^{-1} medium (*N. Calcicola*) and 108.690 mg Nl^{-1} (*A. oryzae*) When they compared either to each others or to the control treatment.

These results had been confirmed by Ghazal, (1980) who found that cyanobacteria propagation and N_2-fixation were increased at 4000 ppm glucose supplemented to cultural medium. Kumar *et al.* (1994) declared that exogenous supplementation of glucose (0.1%) and fructose (1.3%) simulated) light dependent and nitrogenase activity. Although cyanobacteria are basically oxygenic photosynthetic organisms, some can also utilize added organic compounds, either by heterotrophy or phototrophy. In most cases heterotrophy was tested only on a limited number of organic substrates, namely sugars such as glucose, fructose, ribose, sucrose as well as glycerol (Whitton and Potts, 2000). There is evidence for the cyanobacterial glucose metabolism via the oxidative pentose- phosphate (Raboy and Pandau, 1978).

Generally it could be claimed that some cyanobacteria can survive in presence of glucose as carbon source in their growing environment habitat and thus their growth can be accelerated and consequently increase their capacity to fix atmospheric nitrogen.

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وجود الجلوكوز فى بيئة تنمية السيانوبكتريا وأثره على نموهـا وقدرتـها علـى تثبيت النيتروجين ومحتواها من الصبغات

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فى هذه الدراسة أجريت تجربة في المعمل باستخدام أربعة سلالات من السيانوبكتريا كل على حدة وكانت هذه السلالات هي:

Anabaena oryzae, Nostoc calcicola, Microshaete tenera, and Cylindrospermum muscicola

حيث نميت هذه السلالات على بيئةBG11 عند درجة حرارة من ٢٨ -٣٢°م فى وجود تركيزات مختلفة من الجلوكوز هى صفر و ٢٠٠٠و٤٠٠٠و٦٠٠٠ جزء فى المليـون و حضنـت تحـت الاضاءة المستمرة على فترات ٧و١٥و ٢١ يوما.

ويمكن تلخيص التجارب والنتائج المتحصل عليها كما يلى:

١--لقد أدى بوضوح إضافة أى من مستويات الجلوكوز إلى بيئة تنمية الطحالب إلى زيادة معنويـة في وزنها الجاف بالمقارنة مع معاملة الكنترول .

٢- لقد أعطت سلالات *A.oryzae, N.calcicola, C.muscicola* أعلى وزن جاف له عنـد ٢١ يوم عند تركيز ٦٠٠٠ جزء من المليون جلوكوز بينما أعطت سلالة M.tenera أعلـى وزن جاف لها عند تركيز ٤٠٠٠ جزء في المليون جلوكوز عند نفس العمر .

٣- لقد أعطت سلالة *N .calcicola* و *M. tenera* أعلى كمية نيتروجين مثبتة فـي وجـود ٦٠٠٠ جزء في المليون جلوكوز عند عمر ١٥ يوم ، بينما تحقق هذا لسلالتى *A. oryzae* و *C. muscicola* حيث سجلا أعلى كمية نيتروجين مثبته في وجود ٦٠٠٠ جزء في المليون جلوكوز عند ٢١ يوم وقد كـانت كميـات النيـتروجين المثبتـة هـي (١٣٦,٨٠ و ١٤٨,٠١ و ١٠٨,٦٩ و ١٨٦,٣٠) مليجرام نيتروجين/ لتر بيئة على التوالي .

٤- لقد أدى إضافة الجلوكوز إلى بيئة تنمية الطحالب إلى زيادة كل مـن محتـوى الكلوروفيـل والفيكوسيانين عند جميع فترات النمو .

٥- كان من الواضح ان زيادة تركيز الجلوكوز فى بيئة نمو السيانوبكتريا أدى الى زيادة قدرتـها على تثبيت النيتروجين وكذا محتواها من كل من الكلوروفيل والفيكوسيانين .